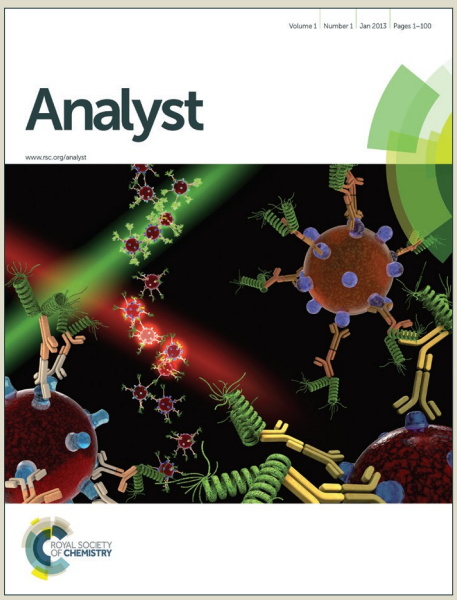
Analyst

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/analyst

Journal Name

RSCPublishing

ARTICLE

Visual fluorescent detection of H_2O_2 and glucose based on "molecular beacon"-hosted Hoechst dyes

Cite this: DOI: 10.1039/xoxxoooooox

Ling-Fei Lu, Yan-Yun Li, Min Zhang,* and Guoyue Shi*

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

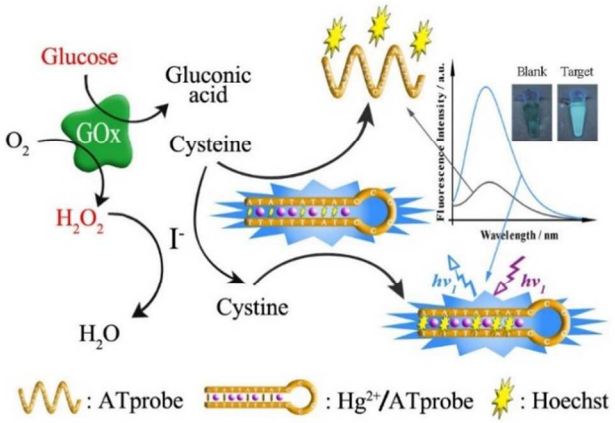
In this work, a label-free molecular beacon (MB)-like biosensor is designed for the determination of H_2O_2 and glucose based on the fluorescence regulating of Hoechst dyes hosted by the designed AT-rich single-stranded DNA (ssDNA), in which Hg^{2+} and cysteine (Cys) act as activators. The designed AT-rich ssDNA (ATprobe) can be directed to form a hairpin with an Hg^{2+}-induced T-Hg^{2+}-T complex, which provides a media for enhancing the fluorescence of Hoechst dyes significantly. On the other hand, Cys can effectively grab Hg^{2+} from the T-Hg^{2+}-T complex by thiol-Hg^{2+} interactions, destructing the hairpin and then switching the Hoechst dyes to the fluorescence "off" state. Combined with this properties, we have demonstrated its application for label-free fluorescence "turn on" detection of H_2O_2. The sensing mechanism is based on the specific reaction between H_2O_2 and Cys catalyzed by I^-, the resulting disulfide reverses the Cys-mediated fluorescent decrease of the MB-hosted Hoechst dyes. The approach achieves a low detection limit of 0.1 μM for H_2O_2. Moreover, this method is further demonstrated to the noninvasive detection of glucose in artificial saliva and urine samples, combining with glucose oxidase (GOx) for the oxidation of glucose and formation of H_2O_2. Compared to traditional methods, the proposed design is cost-effective, simple to prepare and manipulate without fluorescence labeling or chemical modification.

1 Introduction

Molecular beacons (MBs) are dually labeled single-stranded DNA (ssDNA) with a fluorophore (donor) and a quencher (acceptor) at either end that are internally quenched by fluorescence resonance energy transfer (FRET) due to the proximity between donor and acceptor.[1] Traditional MBs have been applied in a wealth of fields but the dual labeling procedure is always expensive and time-wasting. Therefore, it is highly desirable to develop label-free MB-like probes to overcome these shortages. Recently, extensive efforts have been devoted to the devise of the label-free and convenient probes for fluorescent sensing varied target molecules.[2] The label-free and straightforward fluorescent probes may have advantages over the traditional MBs, because of their simple and economy manufacture procedure as well as their good fluorescent characters.[3] Hoechst dyes are part of a family of blue fluorescent dyes used as cell permeable nucleic acid stains. The dyes can bind to the minor of double-stranded DNA (dsDNA) with a preference to the sequences that are abundant in adenine (A) and thymine (T). AT-rich dsDNA can enhance the fluorescence of Hoechst dyes to a huge extent. This sequence-dependent fluorescence-enhancing property leads to the design of label-free MB-like probes, where certain designed functional DNA sequences and their target molecules were incorporated.[3, 4]

Hydrogen peroxide (H_2O_2) has been broadly employed in various applications, e.g. in industrial applications, water treatment, therapeutic use, etc.[5] Researchers have revealed that high concentrations of H_2O_2 can induce cellular damage. Thus, the sensitive and specific recognition of H_2O_2 to detect and control its concentration is important before and after its exposure to the environment. Additionally, H_2O_2 is a focus of study into the molecular mechanisms underlying the development and progression of a disease.[6] H_2O_2 is also the intermediate product of the decomposition reaction of glucose. Enlighten by the above facts, in this work, we have developed a novel detection method for H_2O_2 and glucose utilizing a label-free MB-like probe consisting of a designed AT-rich ssDNA, mercury ions (Hg^{2+}) and Hoechst dyes. Thymine (T) has been shown to be one of the most specific ligands for Hg^{2+}, forming a T-Hg^{2+}-T complex with high selectivity and strong affinity.[7] In this work, the designed AT-rich ssDNA can be directed to form a stem-loop "molecular beacon" with an Hg^{2+}-induced T-Hg^{2+}-T complex, which can provide a host for enhancing the fluorescence of Hoechst dyes in a label-free format. It is reported that cysteine (Cys) can grab Hg^{2+} from T-Hg^{2+}-T complex through thiol–Hg^{2+} interactions.[7a] In this case, a simple and facile strategy can be developed for regulating the fluorescence of Hoechst dyes hosted by the AT-rich "molecular beacon", in which Hg^{2+} and Cys act as activators. And it is also reported that Cys can be oxidized by H_2O_2 in the presence of

iodine ion (I^-) as catalyst.[8] The resulting disulfide reverses the Cys-mediated fluorescent decrease in the "molecular beacon"-hosted Hoechst dyes, which can be applied to the development of a "turn on" fluorescent assay for H_2O_2. And the successful detection of H_2O_2 can be further performed for the analysis of glucose, which is mainly based on the enzymatic conversion of glucose by glucose oxidase (GOx) to form H_2O_2 (Scheme 1).



Scheme 1. Schematic representation of the visual fluorescent sensing of H_2O_2 and glucose based on "molecular beacon"-hosted Hoechst dyes.

Compared with the reported fluorescence-based H_2O_2 detection approaches, our proposed method possesses some notable features. Our method has relatively high sensitivity owing to the property of MB-like probe and the extremely high reacting ability of the detected objects. The selectivity of our proposed method is excellent, which is benefit from the reaction catalyzed by specific catalyst. It is also a highlight of our method that the obvious visual fluorescence makes the detection signal easier to read by the naked eye or can be concisely assessed with fluorescence spectrometry. Most importantly, the present strategy can be realized to follow the biocatalytic process that involve H_2O_2-generating oxidase, such as glucose oxidase (GOx), etc., which allows for facile detection of various targets (e.g. glucose) without increasing the complexity and cost of the probe. Therefore, our proposed method is not only sensitive and selective but also convenient and versatile.

2 Experimental

2.1 Reagents and Materials. The AT-rich DNA (ATprobe: 5'-ATATTATTATCCCCCCTTATTTTTTT-3') was synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). 10×$NaNO_3$–MOPS buffer (500 mM $NaNO_3$ and 200 mM 3-(4-morpholinyl)-1-propanesulfonic acid, pH 7.0) was prepared using metal-free reagents in distilled water purified by a Milli-Q water purification system (Millipore Corp., Bedford, MA) with an electrical resistance of 18.2 MΩ. Cysteine (Cys) and glucose oxidase (GOx) were purchased from Sigma-Aldrich (St. Louis, MO). Glucose was purchased from Qiangsheng Chemical (Jiangsu, China) Co. Ltd. All other reagents were of analytical purity and obtained from Shanghai Chemical Reagent Company (Shanghai, China).

2.2 Instrumentation. Fluorescence spectra were recorded in a fluorescence microplate reader (Infinite M200 pro, TECAN, Switzerland) using a black 384-well microplate (Fluotrac 200, Greiner, Germany). The excitation wavelength used was 360 nm for the emission spectra. Photographs were taken with a digital camera under a 365 nm UV lamp excitation.

2.3 Assays for Hg^{2+} using the ATprobe/Hoechst system. The ATprobe/Hoechst system (ATprobe and Hoechst dyes were used) was prepared in $NaNO_3$–MOPS buffer (pH 7.0), and the mixture was incubated for 10 min at room temperature. For the fluorescent "on" detection of Hg^{2+}, an aliquot of the tested Hg^{2+} or Mill-Q water (as blank sample) was added to the ATprobe/Hoechst system. The final concentration of ATprobe and Hoechst dyes was 2 μM and 0.01 mg/mL, respectively. The mixture was vortexed to mix all the reagents and then incubated for 30 min at room temperature and after that an aliquot of 0.1 mL mixture was placed in the black 384 well microplate to measure the fluorescence intensity.

2.4 Assays for Cys using the Hg^{2+}/ATprobe/Hoechst system. The Hg^{2+}/ATprobe/Hoechst system (Hg^{2+}, ATprobe and Hoechst dyes were used) was prepared in $NaNO_3$–MOPS buffer (pH 7.0), and the mixture was incubated for 10 min at room temperature. For the fluorescent "off" detection of Cys, an aliquot of the tested Cys or Mill-Q water (as blank sample) was added to the Hg^{2+}/ATprobe/Hoechst system. The final concentration of Hg^{2+}, ATprobe and Hoechst dyes was 6 μM, 2 μM and 0.01 mg/mL, respectively. The mixture was vortexed to mix all the reagents and then incubated for 30 min at room temperature and after that an aliquot of 0.1 mL mixture was placed in the black 384 well microplate to measure the fluorescence intensity.

2.5 Assays for H_2O_2 using the Hg^{2+}/ATprobe/Hoechst-Cys system. The Hg^{2+}/ATprobe/Hoechst-Cys system (Hg^{2+}, ATprobe and Hoechst dyes, Cys and I^- were used) was prepared in $NaNO_3$–MOPS buffer (pH 7.0), and the mixture was incubated for 10 min at room temperature. For the fluorescent "on" detection of H_2O_2, an aliquot of the tested H_2O_2 or Mill-Q water (as blank sample) was added to the Hg^{2+}/ATprobe/Hoechst-Cys system. The final concentration of Hg^{2+}, ATprobe, Hoechst dyes, Cys and I^- was 6 μM, 2 μM, 0.01 mg/mL, 20 μM and 10 nM, respectively. The mixture was vortexed to mix all the reagents and then incubated for 30 min at room temperature and after that an aliquot of 0.1 mL mixture was placed in the black 384 well microplate to measure the fluorescence intensity.

2.6 Assays for glucose using the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system. The Hg^{2+}/ATprobe/Hoechst-Cys-GOx system (Hg^{2+}, ATprobe and Hoechst dyes, Cys, I^- and GOx were used) was prepared in $NaNO_3$–MOPS buffer (pH 7.0), and the mixture was incubated for 10 min at room temperature. For the fluorescent "on" detection of glucose, an aliquot of the tested glucose or control samples or Mill-Q water (as blank sample) or control samples was added to the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system. The final concentration of Hg^{2+}, ATprobe, Hoechst dyes, Cys, I^- and GOx was 6 μM, 2 μM, 0.01 mg/mL, 20 μM, 10 nM and 0.1 mg/mL, respectively. The mixture was vortexed to mix all the reagents and then incubated for 30 min at room temperature and after that an aliquot of 0.1 mL mixture was placed in the black 384 well microplate to measure the fluorescence intensity.

3 Results and discussion

3.1 Sensing Scheme. The principle of our designed H_2O_2 and glucose sensor is illustrated in Scheme 1. A designed AT-rich ssDNA (ATprobe: 5'-ATATTATTATCCCCCCTTATTTTTTT-3') was used in our work, which can combine with Hg^{2+} forming a MB-like hairpin

through the T-Hg^{2+}-T complex (i.e., Hg^{2+}/ATprobe hairpin). The free Hoechst dyes show weak fluorescence, but underwent a marked fluorescence enhancement (i.e., the fluorescence "on" state) upon binding to the Hg^{2+}/ATprobe hairpin. The Hg^{2+}/ATprobe hairpin gives a media for enhancing fluorescence of Hoechst dyes, and Hoechst dyes work as a signal indicator in our system. On the other hand, cysteine (Cys) can effectively grab Hg^{2+} from the complex of T-Hg^{2+}-T by thiol-Hg^{2+} interactions, causing the destruction of the hairpin structure and then the Hoechst dyes switch to the fluorescence "off" state again. The Hg^{2+}/Cys-mediated reversible fluorescence changes in the "molecular beacon"-hosted Hoechst dyes sensing system enabled the design of a H_2O_2 sensor. In the absence of H_2O_2, the T-Hg^{2+}-T complex in Hg^{2+}/ATprobe hairpin will be destroyed by Cys, resulting in the breaking down of hairpin-like structure, and the fluorescence of Hoechst dyes will be relatively weak. Upon the addition of H_2O_2, with the specific reaction between H_2O_2 and Cys catalyzed by I^-, the Hg^{2+}/ATprobe hairpin will be formed anew to accommodate Hoechst dyes, causing its enormous fluorescent enhancement. In this case, the Hg^{2+}/ATprobe/Hoechst-Cys system can be used as an indicator for the detection of H_2O_2 in a fluorescence "turn on" format. Furthermore, H_2O_2 can be in situ generated by a mixture of glucose and glucose oxidase (GOx). Therefore, our proposed sensing system could be feasible for the fluorescent monitoring of glucose in combination with GOx-involved biocatalytic process.

3.2 Preparation of the Hg^{2+}/ATprobe/Hoechst-Cys system. The Hg^{2+}/ATprobe hairpin can be prepared through the sufficient combination of ATprobe and Hg^{2+} via Hg^{2+}-induced T-Hg^{2+}-T complex. Upon addition of an Hg^{2+}/ATprobe hairpin, the solution of Hoechst dyes shows a marvelous fluorescent enhancement compared to the sole presence of ATprobe (Figure 1A). A gradual increase in the fluorescence intensity was clearly detected with the addition of an increasing concentration of Hg^{2+} to the ATprobe-Hoechst solution. It can be seen that the fluorescence intensity (FI) value is sensitive to the concentration of Hg^{2+} and in the presence of 6 μM Hg^{2+}, the assay exhibited a nearly saturated signal (Figure 1A, inset), which indicates 6 μM Hg^{2+} can be suitable to form Hg^{2+}/ATprobe/Hoechst system for the following applications. Unless noted otherwise, the following experiments were all carried out under the optimal condition (i.e., 6 μM Hg^{2+}, 2 μM ATprobe and 0.01 mg/mL Hoechst dyes used).

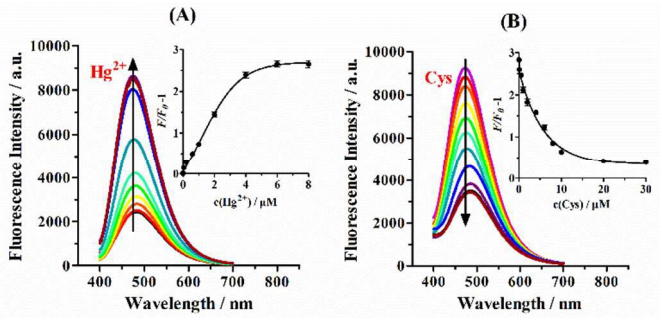


Figure 1. (A) Fluorescent responses of the ATprobe/Hoechst system (2 μM ATprobe and 0.01mg/mL Hoechst dyes used) to different Hg^{2+} concentrations from 0 to 8 μM. Inset: plot of fluorescent responses of the ATprobe/Hoechst system to the various concentrations of Hg^{2+} indicated. (B) Fluorescent responses of the Hg^{2+}/ATprobe/Hoechst system (6 μM Hg^{2+}, 2 μM ATprobe and 0.01mg/mL Hoechst dye used) to different Cys concentrations from 0 to 30 μM. Inset: plot of fluorescent responses of the Hg^{2+}/ATprobe/Hoechst system to the various concentrations of Cys indicated.

Further addition of Cys to the above system, Hg^{2+} is grabbed from the T-Hg^{2+}-T complex and then the Hg^{2+}/ATprobe hairpin unfold to turn off the fluorescence of Hoechst dyes again. The following step is to choose an optimum concentration of Cys to grab Hg^{2+} for destroying the Hg^{2+}/ATprobe hairpin completely. Upon addition of Cys with increasing concentrations, a gradual fluorescent decrease in the Hg^{2+}/ATprobe/Hoechst system was observed (Figure 1B). Figure 1B, inset, depicts a dependence of fluorescent decrease on Cys concentration. When the concentration of Cys is about 20 μM, the Hg^{2+}/ATprobe/Hoechst system will be completely quenched, showing only a weak fluorescence with Hoechst dyes (Figure 1B). Thus, the Hg^{2+}/ATprobe/Hoechst-Cys system (including 6 μM Hg^{2+}, 2 μM ATprobe, 0.01 mg/mL Hoechst dyes and 20 μM Cys) was rationally established for the following applications.

3.3 Assays for H_2O_2 and glucose using the Hg^{2+}/ATprobe/Hoechst-Cys system. The oxidation of Cys by H_2O_2 in the present of I^- yields cysteine.[8] Based on this fact, we proposed that the resulting disulfide can reverse the Cys-mediated fluorescent decrease in the Hg^{2+}/ATprobe/Hoechst system, which can be exploited to the development of a "turn on" fluorescent assay for H_2O_2. As shown in Figure 2A, upon addition of increasing concentrations of H_2O_2 from one stock solution to the Hg^{2+}/ATprobe/Hoechst-Cys system, a visual readout can be obtained under the 365 nm UV lamp excitation. These results were further confirmed by fluorescence spectroscopy. Figure 2B shows the fluorescence spectra of the Hg^{2+}/ATprobe/Hoechst-Cys system to various H_2O_2 concentrations from 0 to 1000 μM. The results reveal that as the concentration of H_2O_2 increases, the extent of oxidation of Cys is enhanced and then a gradual fluorescent increase in the Hg^{2+}/ATprobe/Hoechst-Cys system was observed. From Figure 3C, the fluorescent increase in the Hg^{2+}/ATprobe/Hoechst-Cys system is sensitive to H_2O_2 with increasing concentrations, which could ultimately obtain a novel detection method for H_2O_2. The linear range of the calibration curve was obtained from 0 to 10 μM (inset of Figure 3C). The limit of detection of H_2O_2 using the Hg^{2+}/ATprobe/Hoechst-Cys system was approximately 0.1 μM based on 3σ.

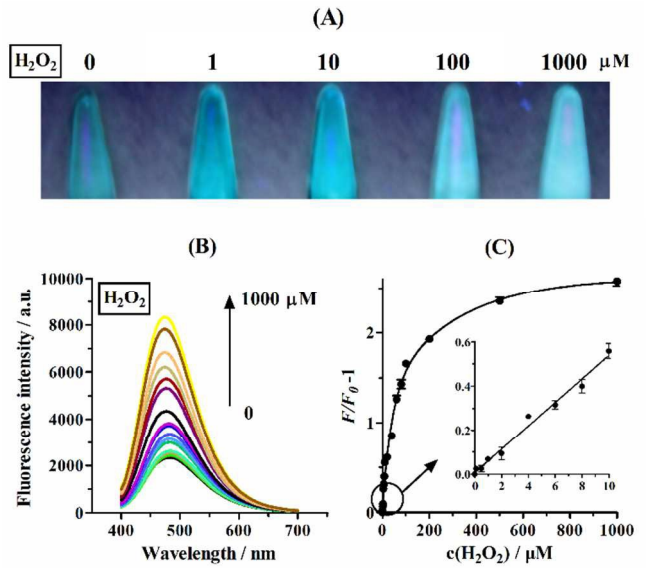


Figure 2. (A) Visual fluorescent detection of H_2O_2 (0, 1, 10, 100 and 1000 μM) based on the Hg^{2+}/ATprobe/Hoechst-Cys system. The picture was taken under the 365 nm UV lamp excitation using a digital camera. (B) Fluorescent response of the Hg^{2+}/ATprobe/Hoechst-Cys system upon addition of various H_2O_2 concentrations from 0 to 1000 μM. (C) Plot of fluorescent responses of the Hg^{2+}/ATprobe/Hoechst-Cys system to the various concentrations of H_2O_2 indicated, using the fluorescence enhancement (F/F_0-1) at 475 nm to monitor the responses.

The successful sensitive detection of H_2O_2 was further extended for the analysis of glucose by using the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system, in which GOx can catalyze the oxidation of glucose to yield H_2O_2. The resulting H_2O_2 oxidizes Cys to cystine, which can turn on the fluorescence of the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system. The time-dependent fluorescent increase of the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system toward glucose at a fixed concentration of 100 μM was monitored, and it reach a plateau within 30 min (Figure S1).

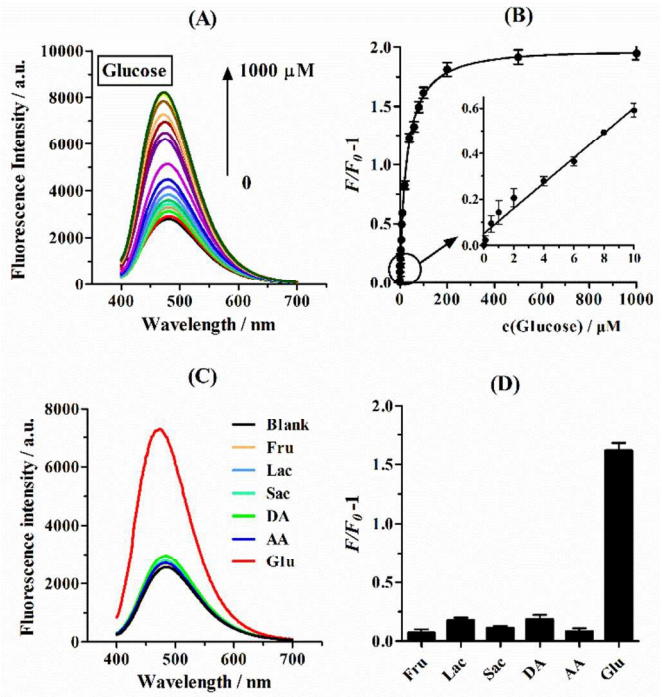


Figure 3. (A) Fluorescent responses of the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system upon addition of various glucose concentrations from 0 to 1000 μM. (B) Plot of fluorescent responses of the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system to the various concentrations of glucose indicated, using the fluorescence enhancement (F/F_0-1) at 475 nm to monitor the responses. (C) Selectivity analysis for glucose detection. The fluorescence emission spectra are shown for fructose, lactose, saccharose, dopamine ascorbic acid and glucose. (D) Bars represent the fluorescence ratios (F/F_0-1) of the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system toward the presence of glucose and common interferes. The final concentrations of the analytes were: fructose (Fru) 20 mM, lactose (Lac) 20 mM, saccharose (Sac) 20 mM, dopamine (DA) 0.1 mM, ascorbic acid (AA) 0.1 mM and glucose (Glu) 0.1 mM.

Figure 3 depicts the fluorescent responses of the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system upon analyzing different concentrations of glucose for a fixed time interval of 30 min. Figure 3A reveals that after adding glucose with increasing concentrations, a gradual fluorescent increase in the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system was observed, consistent with the high content generation of H_2O_2 that mediated the oxidation of Cys to cystine. From Figure 3B, it can be seen that the fluorescent increase in the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system is sensitive to glucose with increasing concentrations, where the linear fitting range is from 0 to 10 μM. The limit of detection of glucose using the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system was approximately 0.1 μM based on 3σ. The selectivity to glucose by our proposed method is evaluated by testing its fluorescent responses towards glucose analogues fructose, lactose, and saccharose, as well as some common interferes, such as dopamine (DA) and ascorbic acid (AA). As shown in Figure 3C and 3D, only the addition of glucose could induce a prominent increase in the fluorescence of the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system. Thus, a highly sensitive and selective fluorescent detection method for glucose was developed by using the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system. The analytical performance of the present glucose

sensing system was compared with some of the reported methods for glucose detection (Table S1). The Hg^{2+}/ATprobe/Hoechst-Cys-GOx system is more sensitive than the reported approaches.[9]

Saliva is known to be one of the most abundant secretions in the human body, and it is easy and non-invasive to collect saliva. Saliva is reported to play a major role in diagnosing and monitoring diabetes.[10] A dependable method for glucose monitoring that uses saliva rather than blood would be a remarkable improvement in managing diabetes.[11] Therefore, it is an interesting challenge to develop novel non-invasive assays of glucose in saliva for diabetes management (concentrations of glucose in saliva are in the range of 8 to 200 μM).[8] We find that the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system is suitable for fluorescent monitoring glucose levels in saliva (Table S2). Moreover, we studied the possible applicability of the Hg^{2+}/ATprobe/Hoechst-Cys-GOx system for the direct measuring of glucose in artificial urine. Urine is a variable biological fluid, both between individuals and in the same individual over time. In this respect, we prepared a stable artificial urine according to the reported literature,[12] which is a reasonable replacement for normal urine in a wealth of applications.[4c,13] A summary of the spiked concentration for each urine sample and the corresponding analysis results are presented in Table S3. The concentrations of histidine in the spiked artificial urine samples determined by the developed method were in good agreement with those of the added glucose. The results suggest that our proposed can be possibly applied for non-invasive detection of glucose in biological fluids.

4 Conclusions

In summary, we has proposed a label-free fluorescent approach for the detection of H_2O_2 and glucose based on the "molecular beacon" (MB)-directed fluorescence of Hoechst dyes. The designed AT-rich DNA (ATprobe) can be directed to form a MB-like hairpin (Hg^{2+}/ATprobe) via Hg^{2+}-induced T-Hg^{2+}-T complex. The Hg^{2+}/ATprobe hairpin provides a harbor to accommodate Hoechst dyes to form the Hg^{2+}/ATprobe/Hoechst system, in which the fluorescence of Hoechst dyes is lit up significantly. However, Cys is readily applicable to grab Hg^{2+} for quenching the fluorescence of the Hg^{2+}/ATprobe/Hoechst system. Additionally, the oxidation of Cys to cystine by H_2O_2 in the presence of I^- can prohibit the Cys-triggered fluorescence quenching of the Hg^{2+}/ATprobe/Hoechst system, which enables the "turn on" analysis of H_2O_2. As the GOx-biocatalyzed oxidation of glucose yields H_2O_2, the biocatalytic process could be probed by the Hg^{2+}/ATprobe/Hoechst-Cys system, which provides a novel sensing platform for the monitoring of glucose in the presence of GOx. The noninvasive detection of glucose in saliva was also demonstrated in our work. The present system would be possibly applied to follow other biocatalytic processes that involve H_2O_2-generating oxidase. This "mix-and-detect" and label-free MB-like probe possesses many advantages, including simplicity of preparation and manipulation compared with other methods that employ specific strategies including tedious procedures, and the need for labels, etc.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (No.21275055, No.21405047) and Project funded by China Postdoctoral Science Foundation (2014M550224).

Notes and references

Department of Chemistry, School of Chemistry and Molecular Engineering, East China Normal University, 500 Dongchuan Road, Shanghai 200241, China. Email: mzhang@chem.ecnu.edu.cn; gyshi@chem.ecnu.edu.cn; Tel&Fax: +86-21-54340043.

Electronic Supplementary Information (ESI) available. See DOI: 10.1039/b000000x/

1 S. Tyagi and F. R. Kramer, *Nat. Biotechnol.*, 1996, **14**, 303.

2 (a) M. Zhang and B. C. Ye, *Analyst*, 2011, **136**, 5139; (b) M. Zhang, S. M. Guo, Y. R. Li, P. Zuo and B. C. Ye, *Chem. Commun.*, 2012, **48**, 2133; (c) M. Zhang, Y. Q. Liu, C. Y. Yu, B. C. Yin and B. C. Ye, *Analyst*, 2013, **138**, 4812; (d) Y. Q. Liu, M. Zhang, B. C. Yin and B. C. Ye, *Anal. Chem.*, 2012, **84**, 5165.

3 M. Zhang, H. N. Le, X. Q. Jiang and B. C. Ye, *Chem. Commun.*, 2013, **49**, 2133.

4 (a) M. Zhang, H. N. Le and B. C. Ye, *ACS Appl. Mater. Interfaces*, 2013, **5**, 8278; (b) X.Q. Jiang, S. M. Guo, M. Zhang, M. Zhou and B. C. Ye, *Analyst*, 2014, **139**, 5682; (c) H. N. Le, X. Q. Jiang, M. Zhang and B. C. Ye, *Anal. Methods*, 2014, **6**, 2028.

5 W. Lei, A. Dürkop, Z. Lin, M. Wu, O. S. Wolfbeis, *Microchim. Acta*, 2003, **143**, 269.

6 G. C. Van de Bittner, E. A. Dubikovskaya, C. R. Bertozzi and C. J. Chang, *Proc. Natl. Acad. Sci. U.S.A.*, 2010, **107**, 21316.

7 (a) M. Zhang, B. C. Yin, W. Tan and B. C. Ye, *Biosensors & Bioelectronics*, 2011, **26**, 3260; (b) G. D. Huy, M. Zhang, P. Zuo and B. C. Ye, *Analyst*, 2011, **136**, 3289.

8 F. Wang, X. Liu, C. H. Lu and I. Willner, *ACS Nano*, 2013, **7**, 7278.

9 (a) C. Xu, J. S. Ren, L. Y. Feng and Qu, X. G. *Chem. Commun.*, 2012, **48**, 3739; (b) A. X. Zheng, Z. X. Cong, J. R. Wang, J. Li, H. H. Yang and G. N. Chen, *Biosens. Bioelectron.*, 2013, **41**, 519; (c) Z. Luo, L. Yuwen, Y. Han, J. Tian, X. Zhu, L. Weng and L. Wang, *Biosens. Bioelectron.*, 2012, **36**, 179; (d) R. Habib and M. R. Rahim, *Biosens. Bioelectron.*, 2013, **41**, 498; (e) Y. H. Yi, J. H. Deng, Y. Y. Zhang, H. T. Li and S. Z. Yao, *Chem. Commun.*, 2013, **49**, 612; (f) P. F. Shen and Y. S. Xia, *Anal. Chem.*, 2014, **86**, 5323; (g) L. Wang, J. Zheng, Y. Li, S. Yang, C. Liu, Y. Xiao and J. Li, C. Zhong and R. Yang, *Anal. Chem.*, 2014, **86**, 12348; (h) L. Zhang, Z. Y. Zhang, R. P. Liang, Y. H. Li and J. D. Qiu, *Anal. Chem.*, 2014, **86**, 4423.

10 P. Abikshyeet, V. Ramesh and N. Oza, *Diabetes Metab. Synd. Obes.*, 2012, **5**, 149.

11 V. S. Siu, J. Feng, P. W. Flanigan, G. Tayhas, R. Palmore, D. Pacifici, *Nanophotonics*, 2014, **3**, 125.

12 T. Brooks and C. W. Keevil, *Lett. Appl. Microbiol.*, 1997, **24**, 203.

13 (a) Y. Zhou, T. Zhou, M. Zhang and G. Shi, *Analyst*, 2014, **139**, 3122; (b) M. Zhang, H. N. Le, X. Q. Jiang, B. C. Yin and B. C. Ye, *Anal. Chem.*, 2013, **85**, 11665.