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# Visual fluorescence detection of $H_2O_2$ and glucose based on "molecular beacon"-hosted Hoechst dyes†

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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 regulation 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 medium 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 these 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 fluorescence 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 applied to the non-invasive 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 ends that are internally quenched by fluorescence resonance energy transfer (FRET) due to the proximity between the donor and the acceptor.[1] Traditional MBs have been applied in a wealth of fields but the dual labeling procedure is always expensive and time-consuming. Therefore, it is highly desirable to develop label-free MB-like probes to overcome these drawbacks. Recently, extensive efforts have been devoted to the devising of the label-free and convenient probes for fluorescence sensing of varied target molecules.[2] The label-free and straightforward fluorescent probes may have advantages over the traditional MBs, because of their simple and economical manufacture procedure as well as their good fluorescence 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 groove 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. These sequence-dependent fluorescence-enhancing properties lead 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. In addition, $H_2O_2$ is a focus of the study on 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. Enlightened 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

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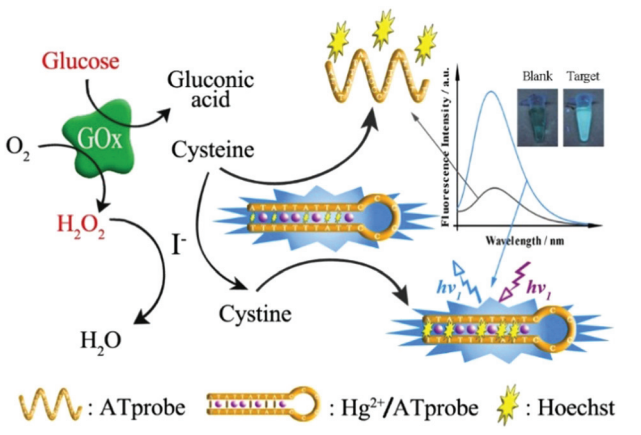
$Hg^{2+}$, forming a T–$Hg^{2+}$–T complex with high selectivity and strong affinity.[7] In this work, the designed AT-rich ssDNA can be directed to form a stem-loop "molecular beacon" with an $Hg^{2+}$-induced T–$Hg^{2+}$–T complex, which can provide a host for enhancing the fluorescence of Hoechst dyes in a label-free format. It is reported that cysteine (Cys) can grab $Hg^{2+}$ from the 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. It is also reported that Cys can be oxidized by $H_2O_2$ in the presence of the iodine ion ($I^-$) as the catalyst.[8] The resulting disulfide reverses the Cys-mediated fluorescence decrease in the "molecular beacon"-hosted Hoechst dyes, which can be applied to the development of a "turn on" fluorescence assay for $H_2O_2$. 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).

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 properties of MB-like probes and the extremely high reacting ability of the detected objects. The selectivity of our proposed method is excellent, which is benefitted from the reaction catalyzed by a 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 using 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.




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

# 2 Experimental

## 2.1 Reagents and materials

The AT-rich DNA (ATprobe: 5′-ATATTATTATCCCCCC-TTATTTTTTT-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 on a fluorescent 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 fluorescence "on" detection of $Hg^{2+}$, an aliquot of the tested $Hg^{2+}$ or Milli-Q water (as the blank sample) was added to the ATprobe/Hoechst system. The final concentrations of the ATprobe and Hoechst dyes were 2 μM and 0.01 mg $mL^{-1}$, 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 fluorescence "off" detection of Cys, an aliquot of the tested Cys or Milli-Q water (as the blank sample) was added to the $Hg^{2+}$/ATprobe/Hoechst system. The final concentrations of $Hg^{2+}$, ATprobe and Hoechst dyes were 6 μM, 2 μM and 0.01 mg $mL^{-1}$, 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 fluorescence "on" detection of $H_2O_2$, an aliquot of the tested $H_2O_2$ or Milli-Q water (as the blank sample) was added to the $Hg^{2+}$/ATprobe/Hoechst–Cys system. The final concentrations of $Hg^{2+}$, ATprobe, Hoechst dyes, Cys and $I^-$ were 6 μM, 2 μM, 0.01 mg $mL^{-1}$, 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 fluorescence "on" detection of glucose, an aliquot of the tested glucose or control samples or Milli-Q water (as the blank sample) or control samples was added to the $Hg^{2+}$/ATprobe/Hoechst–Cys–GOx system. The final concentrations of $Hg^{2+}$, ATprobe, Hoechst dyes, Cys, $I^-$ and GOx were 6 μM, 2 μM, 0.01 mg $mL^{-1}$, 20 μM, 10 nM and 0.1 mg $mL^{-1}$, 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 an MB-like hairpin through the T–$Hg^{2+}$–T complex (*i.e.*, $Hg^{2+}$/ATprobe hairpin). The free Hoechst dyes showed a 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 medium for enhancing the fluorescence of Hoechst dyes, and the 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 the 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 the $Hg^{2+}$/ATprobe hairpin will be destroyed by Cys, resulting in the breaking down of the 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 the Cys catalyzed by $I^-$, the $Hg^{2+}$/ATprobe hairpin will be formed anew to accommodate the Hoechst dyes, causing their enormous fluorescence 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 fluorescence monitoring of glucose in combination with the GOx-involved biocatalytic process.

### 3.2 Preparation of the $Hg^{2+}$/ATprobe/Hoechst–Cys system

The $Hg^{2+}$/ATprobe hairpin can be prepared by the sufficient combination of ATprobe and $Hg^{2+}$ *via* the $Hg^{2+}$-induced T–$Hg^{2+}$–T complex. Upon the addition of an $Hg^{2+}$/ATprobe hairpin, the solution of Hoechst dyes shows a marvelous fluorescence enhancement compared to the sole presence of the ATprobe (Fig. 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 (Fig. 1A, inset), which indicates that 6 μM $Hg^{2+}$ can be suitable to form the $Hg^{2+}$/ATprobe/Hoechst system for the following applications. Unless noted otherwise, the following experiments were all carried out under the optimal conditions (*i.e.*, 6 μM $Hg^{2+}$, 2 μM ATprobe and 0.01 mg $mL^{-1}$ Hoechst dyes used).

Upon 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 unfolds 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 the addition of Cys with increasing concentrations, a gradual fluorescence decrease in the $Hg^{2+}$/ATprobe/Hoechst system was observed (Fig. 1B). Fig. 1B, inset, depicts a dependence of fluorescence

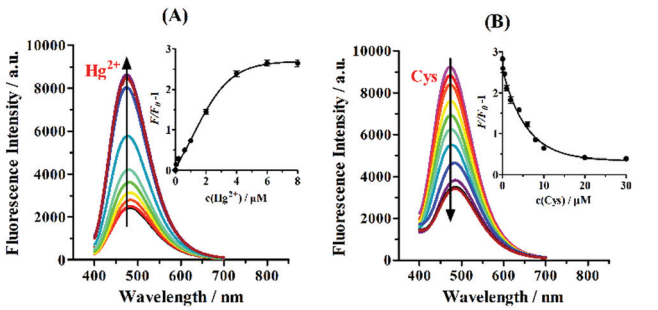



**Fig. 1** (A) Fluorescence responses of the ATprobe/Hoechst system (2 μM ATprobe and 0.01 mg $mL^{-1}$ Hoechst dyes used) to different $Hg^{2+}$ concentrations from 0 to 8 μM. Inset: plot of fluorescence responses of the ATprobe/Hoechst system to the various concentrations of $Hg^{2+}$ indicated. (B)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

decrease on the Cys concentration. When the concentration of Cys is about 20 μM, the $Hg^{2+}$/ATprobe/Hoechst system is completely quenched, showing only a weak fluorescence with the Hoechst dyes (Fig. 1B). Thus, the $Hg^{2+}$/ATprobe/Hoechst–Cys system (including 6 μM $Hg^{2+}$, 2 μM ATprobe, 0.01 mg $mL^{-1}$ 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 presence of $I^-$ yields cystine.[8] Based on this fact, we proposed that the resulting disulfide can reverse the Cys-mediated fluorescence decrease in the $Hg^{2+}$/ATprobe/Hoechst system, which can be exploited to the development of a "turn on" fluorescence assay for $H_2O_2$. As shown in Fig. 2A, upon the 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 using the 365 nm UV lamp excitation. These results were further confirmed by fluorescence spectroscopy. Fig. 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 fluorescence increase in the $Hg^{2+}$/ATprobe/Hoechst–Cys system was observed. From Fig. 2C, the fluorescence 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 Fig. 2C). The limit of detection of $H_2O_2$ using the $Hg^{2+}$/ATprobe/Hoechst–Cys system was approximately 0.1 μM based on $3\sigma$.

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 fluorescence increase of the $Hg^{2+}$/ATprobe/Hoechst–Cys–GOx system toward glucose at a fixed concentration of 100 μM was monitored, and it reached a plateau within 30 min (Fig. S1†).

Fig. 3 depicts the fluorescence responses of the $Hg^{2+}$/ATprobe/Hoechst–Cys–GOx system upon analyzing different concentrations of glucose for a fixed time interval of 30 min. Fig. 3A reveals that after adding glucose with increasing concentrations, a gradual fluorescence 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

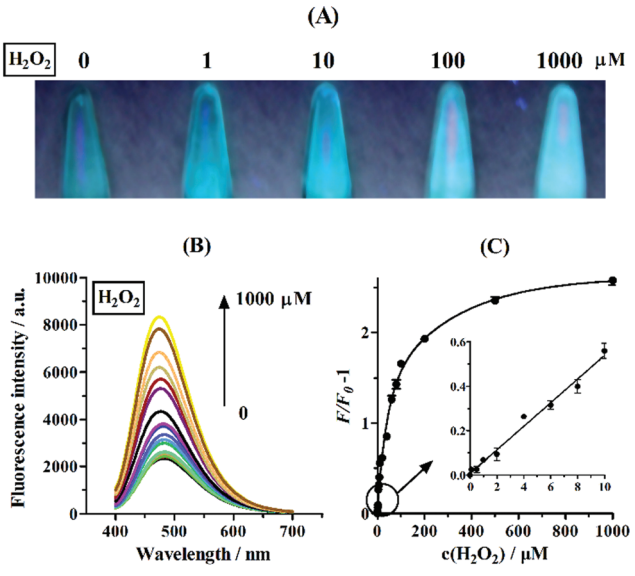



Fig. 2 (A) Visual fluorescence detection of $H_2O_2$ (0, 1, 10, 100 and 1000 μM) based on the $Hg^{2+}$/ATprobe/Hoechst–Cys system. The picture was taken using the 365 nm UV lamp excitation using a digital camera. (B) Fluorescence response of the $Hg^{2+}$/ATprobe/Hoechst–Cys system upon the addition of various $H_2O_2$ concentrations from 0 to 1000 μM. (C) Plot of fluorescence 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.

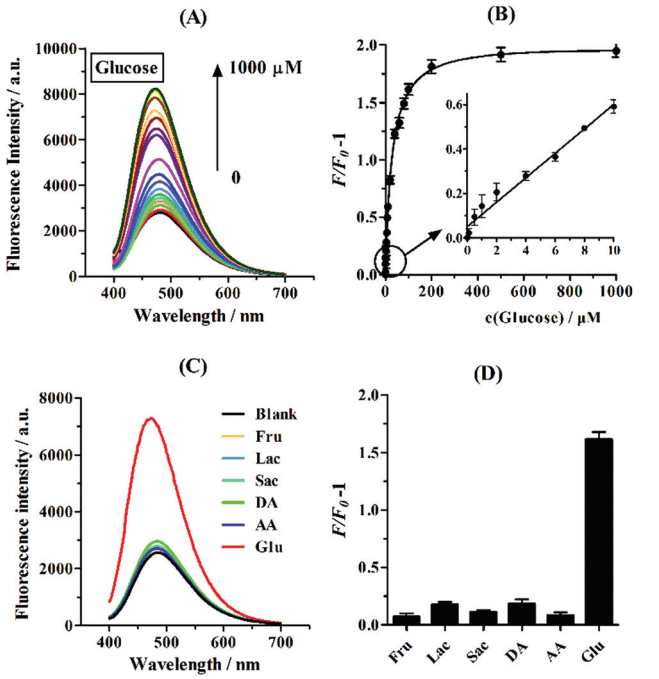



Fig. 3 (A) Fluorescence responses of the $Hg^{2+}$/ATprobe/Hoechst–Cys–GOx system upon the addition of various glucose concentrations from 0 to 1000 μM. (B) Plot of fluorescence responses of the $Hg^{2+}$/ATprobe/Hoechst–Cys–GOx system to the various concentrations of glucose indicated, using the fluorescence 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.

oxidation of Cys to cystine. From Fig. 3B, it can be seen that the fluorescence 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 $3\sigma$. The selectivity to glucose by our proposed method is evaluated by testing its fluorescence responses towards the glucose analogues such as fructose, lactose, and saccharose, as well as some common interferes, such as dopamine (DA) and ascorbic acid (AA). As shown in Fig. 3C and D, 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 fluorescence 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 the fluorescence monitoring of the 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 glucose 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 approach can be possibly applied for non-invasive detection of glucose in biological fluids.

## 4 Conclusions

In summary, we have proposed a label-free fluorescence 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* the $Hg^{2+}$-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. In addition, 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 the $H_2O_2$-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, the need for labels, *etc*.

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## Notes and references

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 and 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, *Biosens. Bioelectron.*, 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 X. G. Qu, *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, 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. Syndr. Obes.: Targets Ther.*, 2012, **5**, 149.

11 V. S. Siu, J. Feng, P. W. Flanigan, G. Tayhas, R. Palmore and 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.

 