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Fluorescence enhancement of CdTe quantum dots by HBcAb-HRP for sensitive detection of H_2O_2 in human serum

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ABSTRACT

A simple, high selective, ultra-sensitive and stable biosensor based on hepatitis B core antibody labeled with horseradish peroxidase (HBcAb-HRP) induced fluorescent enhancement of CdTe QDs for recognition of H_2O_2 have been constructed. In this assay, sulfurs in HBcAb-HRP, which possess a strong affinity towards Cd^{2+}, can improve greatly the recombination fluorescence of CdTe QDs by creating more radiative centers at CdTe/Cd-SR complex. Then, H_2O_2 oxidizes Cd-S bonds in CdTe QDs to organic disulfide product (RS-SR), causing thioglycolic acid (TGA) and HBcAb-HRP detach from surface of CdTe QDs and thus leading to fluorescence quenching. Just with the addition of HBcAb-HRP, sensitivity of the new biosensor has been improved by near one order of magnitude as compared with CdTe QDs probe. Detection limit of HBcAb-HRP-CdTe QDs biosensor for determination of H_2O_2 was 6.9×10^{-8} mol L^{-1} (3σ/slope), and the excellent linear range was 1.0×10^{-7}~1.5×10^{-4} mol L^{-1}. By using sodium diethyldithiocarbamate (DDTC) and NH_4OH as masking agents of Ag^+, Hg^{2+} and Cu^{2+}, H_2O_2 can be selectively detected in coexistence with Ag^+, Hg^{2+} and Cu^{2+}, and the biosensor has been used to detect H_2O_2 in human serum with satisfactory results. The superior properties of this biosensor showed great potential usage in more chemical and biological researches.

1. Introduction

In recent years, H_2O_2 has been attracting much attentions in biochemical fields since it is an important intermediate product of pathological processes and involves in molecular mechanisms underlying the development and progression of various disease, such as Alzheimer's disease, atherosclerosis, Parkinson's, myocardial infarction, cancer, etc (Costas-Mora et al., 2015; Bai and Jiang, 2013). However, reactivity of H_2O_2 is high and its concentration is usually low in biofluid. Therefore, establishing an effective and simple method for sensitive and selective detection of H_2O_2 is significant.

Analytical methods including fluorescence (Lim et al., 2016; Liang et al., 2016), chemiluminescence (Yu et al., 2016), electrochemistry (Wang et al., 2015), and colorimetry (Lu et al., 2016) have been used for H_2O_2 detection. Among these methods, fluorescence methods have been received increasing attentions of analytical scientists owing to the advantages of simplicity, rapid analysis, high sensitivity, little damage to sample and economy (Song et al., 2016a).

In fluorescence technique, selection of an appropriate fluorescence probe is an important strategy, and various fluorescence probes (Liang et al., 2016; Zhao et al., 2016) have been performed for detection of H_2O_2. Liang et al. (2016) first designed a fluorescent nanoplatform to achieve in situ, real-time and accurate determination of H_2O_2 released from cancer cells. Zhao et al. (2016) presented a rational design of a dual-emission and two-photon graphene quantum dot (GQDs) probe for imaging of H_2O_2 in living cells, the fluorescence response toward H_2O_2 is rapid and extremely specific.

Recently, the interactions between QDs containing Cd^{2+} and functional groups of amino (Wang et al., 2012), phosphate (Liu et al., 2012) and thiol (Gui et al., 2012), which can cause further increase in fluorescence intensity, have been studied. The fluorescence enhancement phenomenon can be explained by formation of covalent bonds between donor atoms of sulfur, oxygen, nitrogen and Cd^{2+} ions on surface of QDs (Rodrigues et al., 2014). Therefore, in this work, to improve fluorescence intensity of CdTe QDs, hepatitis B core antibody labeled with horseradish peroxidase (HBcAb-HRP), which contains donor atoms of sulfur, oxygen, nitrogen, were selected and tested. Hepatitis B core antibody (HBcAb) is an immunoglobulin (IgG), and horseradish peroxidase (HRP) is a monomeric heme-containing redox enzymes. For the first time, a new biosensor based on HBcAb-HRP-

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induced fluorescent of CdTe QDs (HBcAb-HRP-CdTe QDs) for high performance of H_2O_2 analysis was investigated.

2. Experimental

2.1. Materials and instruments

Preparation and characterization of CdTe QDs were as our previous work (Gong et al., 2016). Analytical grade of K_2TeO_3 (Aladdin), $Cd(CH_3COO)_2{\cdot}H_2O$ and $NaBH_4$ (Tianjin Kermel Chemical Reagent Company), thioglycolic acid (TGA) (Alfa Aesar), sodium diethyldithiocarbamate (DDTC) (Shanghai Zhanyun Chemical Co. Ltd.), H_2O_2 (Sinopharm Chemical Reagent Co. Ltd), HBcAb (Hubei Center for Clinical Laboratory), HRP (Sigma), HBcAb-HRP (Diagnostic kit for antibody to hepatitis B core antigen, InTec (Xiamen) Technology Co., Ltd.), quality control serum (Hubei Center for Clinical Laboratory), a series of Britton-Robinson (B-R) buffer solutions containing H_3PO_4, CH_3COOH and H_3BO_3 each with concentrations at 40 mmol L^{-1} and double distilled water (DDW) were used. HBcAb-HRP is hepatitis B virus core antibody detection kit with horseradish peroxidase-labeled mouse monoclonal IgG antibody.

The pH measurements were performed by a PB-10 exact digital pH meter (Sartorius, Germany). The fluorescence measurements were recorded on a LS45 fluorescence spectrofluorometer (Perkin Elmer, USA). UV–vis absorption spectra was conducted on a U-3010 spectrophotometer (Hitachi, Japan). Size and morphology of CdTe QDs were observed using a transmission electron microscope (TEM, JEOL JEM-2100, Japan).

2.2. Fluorescence assay for H_2O_2 detection by HBcAb-HRP-CdTe QDs biosensor

25 μL CdTe QDs, 500 μL 40 mmol L^{-1} B-R buffer (pH 7.0), 20 μL HBcAb-HRP were mixed at room temperature. A series concentration of H_2O_2 were added into the mixture and diluted to 5.0 mL with DDW. After 30 min, the resulting solution was monitored by fluorescence spectrophotometer (λ_{ex}=458 nm, λ_{em}=560 nm).

2.3. Sample pretreatment

The tested human serum samples from the Clinical Laboratory in the Second Hospital of Huangshi were provided by volunteers of the patients. Human sera from patients at random, which had been centrifuged high-speedly (3500 r min^{-1}) to extract serum from blood. Thus, human sera and quality control serum without any pretreatment were directly analyzed according to procedure in Section 2.2.

3. Results and discussion

3.1. Establishing and optimization HBcAb-HRP-CdTe QDs biosensor

3.1.1. Effect of HBcAb-HRP, HBcAb and HRP on the fluorescence intensity of CdTe QDs

To obtain the maximum fluorescence enhancement effect and to investigate mechanism between CdTe QDs and HBcAb-HRP/HBcAb/HRP, fluorescence intensity of HBcAb-HRP, HRP and HBcAb themselves and effects of HBcAb-HRP, HRP and HBcAb on fluorescence intensity of CdTe QDs were tested, respectively, and the results are shown in Fig. 1A. As seen, firstly, the fluorescence characteristics of CdTe QDs itself (λ_{ex}=458 nm, λ_{em}=560 nm), which is entirely different from those of HRP (λ_{ex}=350 nm, λ_{em}=436 nm), HBcAb (λ_{ex}=275 nm, λ_{em}=345 nm) and HBcAb-HRP (λ_{ex}=275 nm, λ_{em}=345 nm), had no obvious changes with the addition of HBcAb-HRP, HRP and HBcAb, respectively; secondly, HBcAb and HBcAb-HRP can both improve remarkably fluorescence intensity of CdTe QDs at 560 nm, while HRP just has a little enhancement, and HBcAb-HRP has the maximum fluorescence enhancement effect for CdTe QDs. The mechanism is attributed to sulfurs in HBcAb-HRP and HBcAb, which possess a strong affinity towards Cd^{2+}, can improve greatly recombination fluorescence of CdTe QDs by creating more radiative centers at CdTe/Cd-SR complex (Gong et al., 2016; Khantaw et al., 2013). While, for HRP, active center of the heme group (Wang et al., 2009) can passivate surface of CdTe QDs (Hu et al., 2008), leading to improving slightly fluorescence intensity of CdTe QDs.

To investigate further the possible reason why it is HBcAb-HRP, which shows the best fluorescence enhancement effect, effect of HRP on fluorescence of CdSe QDs and HBcAb was tested, and results in Fig. S1 display that the fluorescence intensity shows an overall downward trend. These may be due to existence of HRP, which can inhibit the sufficient interaction of sulfur in HBcAb and Cd^{2+} on surface of CdTe QDs. However, HBcAb-HRP can significantly enhance the fluorescence intensity of CdTe QDs, probably because of the synergistic effect of HBcAb and HRP in HBcAb-HRP (curve g in Fig. 1A). As for the optimum volume of HBcAb-HRP, Fig. S2, shows that 20 μL is the best.

In order to more fully explain the interaction of HBcAb-HRP and CdTe QDs, Fig. S3 shows the TEM images of CdTe QDs (A,B), HRP +CdTe QDs (C,D), HBcAb+CdTe QDs (E,F), HBcAb-HRP+CdTe QDs (G, H) at different magnifications, respectively. As seen, CdTe QDs are spherical in morphology and separated from each other with the addition of HRP, however, not monodisperse spherical in presence of HBcAb or HBcAb-HRP, indicating HRP has no obvious effect on the morphology of CdTe QDs, and HBcAb especially HBcAb-HRP was successfully modified on the surface of CdTe QDs owning to the sufficient interaction of sulfur in HBcAb and Cd^{2+} on surface of CdTe QDs.

3.1.2. Optimization of pH

H_2O_2 was selected as the researching target since it can readily oxidize Cd-S to an organic disulfide product (RS-SR), causing TGA and HBcAb-HRP detach from the surface of CdTe QDs and thus leading to fluorescence quenching (Meng et al., 2013). Jin et al. (2016) also suggested that H_2O_2 was oxidized to O_2, which occupied electron-hole traps on surface of CdTe QDs, acted as a good electron acceptor by forming non-fluorescent CdTe QDs anion and leaded to fluorescence quenching. Hence, according to the sizes of CdTe QDs (about 3.0 nm, TEM image in Fig. S3-A) and HBcAb-HRP (over 14×10×4 nm, Droz et al., 1994), an-alogue process of fluorescence enhancing of CdTe QDs by HBcAb-HRP and subsequent fluorescence quenching biosensor of HBcAb-HRP-CdTe QDs in presence of H_2O_2 is shown in Fig. 1B.

In order to achieve the best performance, we first thoroughly investigated effect of pH on the biosensor in absence and presence of H_2O_2. Results in Fig. S4 demonstrate that, in absence of H_2O_2, fluorescence intensity of the biosensor increases rapidly from pH 4.0–7.0, and reaches maximum at pH 7.0. While, in presence of 4.0×10^{-5} mol L^{-1} H_2O_2, fluorescence intensity of the biosensor increases quickly from pH 4.0–6.0, then decreases slightly when pH is over 6.0. By comparing the two curves, presence of H_2O_2 can obviously decrease fluorescence intensity of the biosensor, and a remarkable response corresponding to the fluorescent quenching efficiency was obtained at pH 7.0. Thus, pH 7.0 was used for this biosensor.

The reason, why fluorescence intensity of the biosensor both decreases quickly at pH $<$ 6.0, keeps stable at pH 6.0–11.0 and a little decreases at pH $>$ 11.0 whether there is H_2O_2 or no, is that highly acidic or alkaline surroundings would cause protonation-deprotonation of CdTe QDs, leading to fluorescence intensity declines (Wang and Ding, 2014).

3.1.3. Effect of CdTe QDs volume on the fluorescence intensity of HBcAb-HRP-CdTe QDs

Usually, the bigger volume of a fluorescence probe, the stronger of fluorescence intensity. However, to obtain the optimum sensitivity, volume of CdTe QDs is still needed to be tested. Hence, effect of CdTe

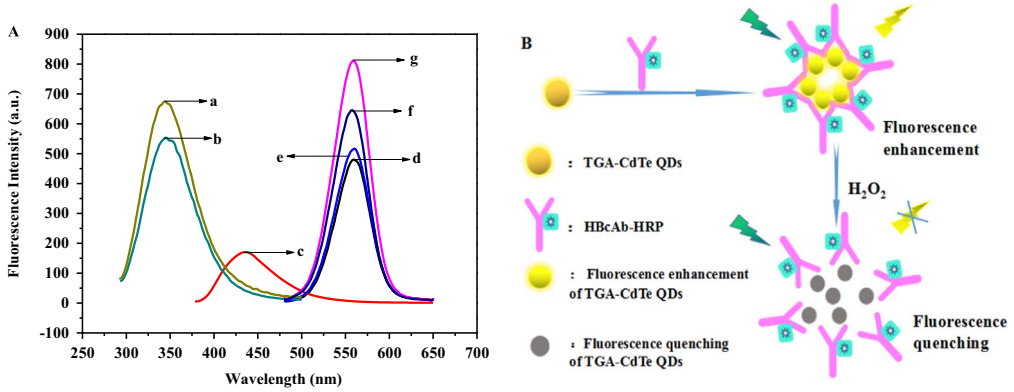

Fig. 1. (A) Fluorescence emission spectrum of (a): HBcAb; (b): HBcAb-HRP; (c): HRP; (d): CdTe QDs; (e): HRP + CdTe QDs; (f): HBcAb+CdTe QDs; (g): HBcAb-HRP+CdTe QDs. HRP: 1.0 μg mL^{-1}; HBcAb-HRP: 20 μL; HBcAb: 2.0×10^{-3} U mL^{-1}; CdTe QDs: 40 μL. (B) Schematic principle for the detection of H_2O_2.

QDs volume (15–35 μL) on the biosensor was studied in absence and presence of H_2O_2, and the results are shown in Fig. S5. As seen, although fluorescence intensity of the biosensor both increased with the volume of CdTe QDs whether there is H_2O_2 or not, the best quenching degree, i.e., the maximum fluorescence intensity difference between the biosensor without H_2O_2 and with H_2O_2, is obtained with 25 μL CdTe QDs. Thus, 25 μL CdTe QDs was selected.

3.1.4. Effect of incubation time between HBcAb-HRP-CdTe QDs and H_2O_2

Fig. S6 shows effects of incubation times (5–310 min) on fluorescence intensity of the biosensor with the addition of H_2O_2. As seen, fluorescence intensity has little changes from 30 to 310 min except for a little decrease from 5 to 30 min, indicating the biosensor is stable and HRP almost does not catalyze the breakdown of H_2O_2 under this experimental conditions. Thus, 30 min was used.

3.2. Selectivity of the biosensor

Selectivity is an important parameter for accurate detecting H_2O_2 by the biosensor. Effects of some coexisting substances (cysteine (Cys), glycine (Gly), cystine (Cys-Cys), tyrosine (Tyr), tryptophan (Try), aspartic acid (Asp), histidine (His), glucose (Glu), bovine serum albumin (BSA), ascorbic acid (AA), Cr^{6+}, Cr^{3+}, Pb^{2+}, Cd^{2+}, Ag^{+}, Fe^{3+}, Hg^{2+}, Cu^{2+}, K^{+}, Zn^{2+}, Ca^{2+}, Mg^{2+} and Al^{3+}) in blood samples on fluorescence intensity of the biosensor were investigated, respectively, and the results are listed in Fig. 2A. F_0 represents fluorescence intensity of the initial biosensor, and F represents the fluorescence intensity after addition of the above component into the initial biosensor. It is clear that all of these 23 substances except for Ag^{+}, Hg^{2+}, Cu^{2+} have no

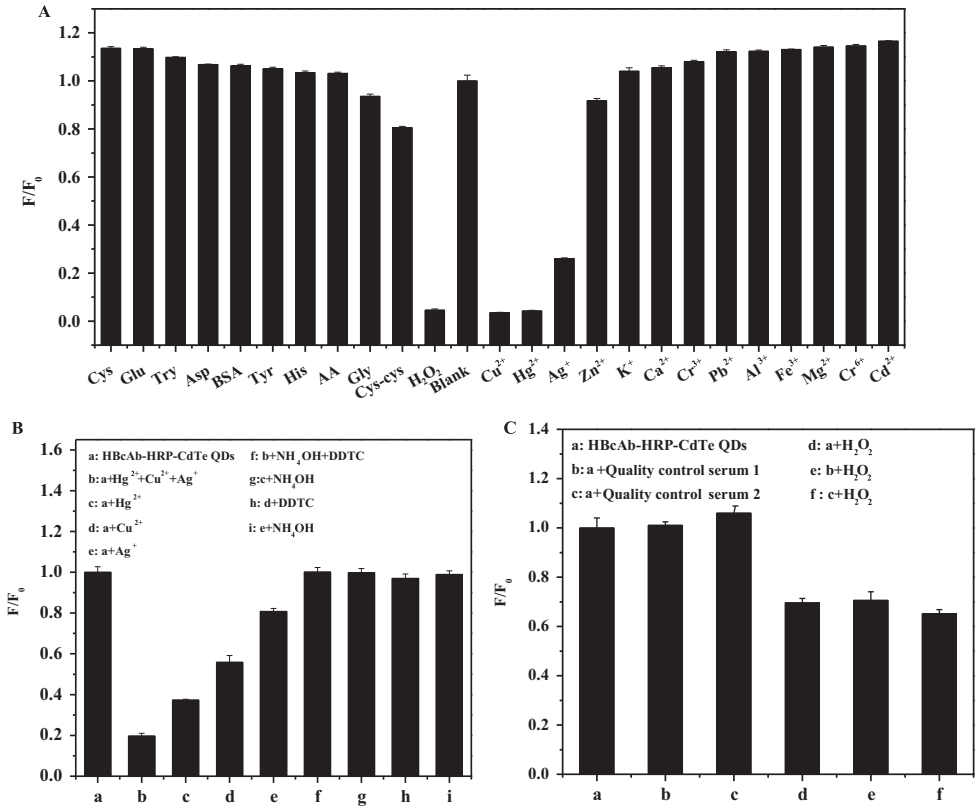

Fig. 2. (A) Effect of various substances on the fluorescence intensity of HBcAb-HRP-CdTe QDs. CdTe QDs: 25 μL; Amino acids, glucose, BSA, AA and H_2O_2: 2.0×10^{-4} mol L^{-1}; All metal ions: 40 ng mL^{-1}. (B) Relative fluorescence intensity of HBcAb-HRP-CdTe QDs. Ag^{+}, Hg^{2+}, Cu^{2+}: 1.0×10^{-7} mol L^{-1}; DDTC: 2.0×10^{-6} mol L^{-1}; NH_4OH: 6.0×10^{-5} mol L^{-1}. (C) Effect of quality control serum on detection of H_2O_2 by HBcAb-HRP-CdTe QDs biosensor·H_2O_2: 2.5×10^{-5} mol L^{-1}; HBcAb-HRP: 20 μL.

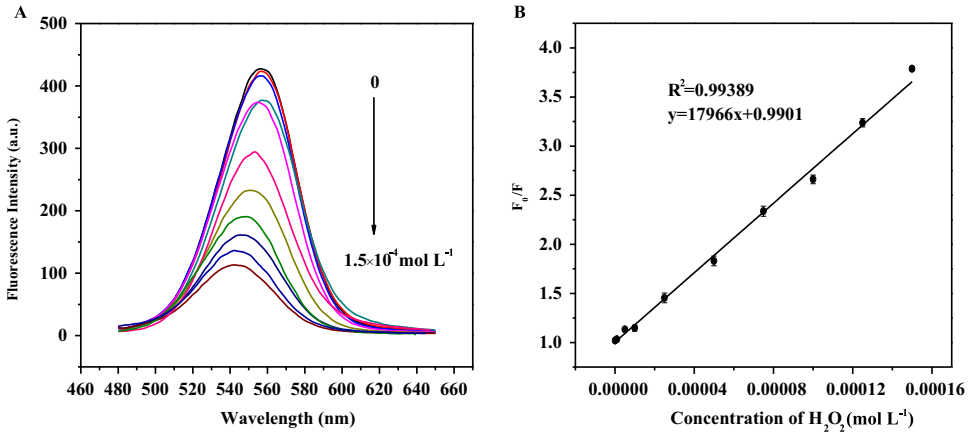

Fig. 3. (A) Fluorescent spectra of the biosensor in the presence of different concentrations of H_2O_2 (0–1.5×10^{-4} mol L^{-1}). (B) Plot of F_0/F versus the corresponding concentrations of H_2O_2.

obvious interference. To eliminate interference of Ag^+, Hg^{2+} and Cu^{2+}, effects of masking agents of DDTC and NH_4OH on every ion and mixture of Ag^+, Hg^{2+} and Cu^{2+} have been tested, respectively. Results in Fig. 2B display that the interference can be eliminated well by using masking agent of NH_4OH for Ag^+, Hg^{2+}, DDTC for Cu^{2+} and mixture of DDTC and NH_4OH for Ag^+, Hg^{2+} and Cu^{2+}, respectively. It is attribute to Cu^{2+} can coordinate favorably and strongly with sulphide bond of DDTC, NH_4OH is able to capture Hg^{2+} and Ag^+ ions to form metal chelates, respectively (Wang et al., 2011). These also indicate the biosensor has an excellent selectivity toward H_2O_2 by using DDTC and NH_4OH as mixture masking agents of Ag^+, Hg^{2+} and Cu^{2+}.

To study further the interference of main components in serum, two kinds of quality control sera were tested based on this biosensor. 20 main components in serum and their contents of each in the two sera are listed in Table S1. They are both higher than the actual levels of a healthy human, and most of the component in quality control serum 2 is higher than that in quality control serum 1. Results in Fig. 2C show that all components even at higher levels in the quality control serum have no obvious interference. All results indicate this biosensor exhibit excellent anti-interference ability towards both inorganic and organic compounds.

3.3. Fluorescence assay of H_2O_2 by HBcAb-HRP-CdTe QDs

To study sensitivity of the biosensor, various contents of H_2O_2 were added to the system for 30 min, respectively, and changes of fluorescence intensity are recorded in Fig. 3. As seen, fluorescence quenching levels of the biosensor increase with H_2O_2 contents (Fig. 3A) and calibration curve is established by quenching ratio F_0/F versus concentration of H_2O_2 (Fig. 3B). Analytical characteristic data of this work are summarized in Table S2. A wide and excellent linear range (1.0×10^{-7}~1.5×10^{-4} mol L^{-1}) of H_2O_2 is obtained, and the limit of detection (LOD), calculated as the concentration of analyte that give rise to peak areas with three times of S/N, is 6.9×10^{-8} mol L^{-1}.

In addition, fluorescence quenching degrees of CdTe QDs in absence of HBcAb-HRP versus H_2O_2 concentration were researched, and the results are also listed in Table S2. As seen, detection sensitivity of H_2O_2 based on HBcAb-HRP-CdTe QDs biosensor has been improved by near one order of magnitude (from 3.7×10^{-7} to 6.9×10^{-8} mol L^{-1}) as compared with CdTe QDs probe.

Table S3 compares the analytical characteristics of different methods reported for determination of H_2O_2 from real-world samples. As seen, as the same kind of fluorescent method based on application different probes in detection of H_2O_2, LOD of this work is lower than those of studies (Costas-Mora et al., 2015; Wang et al., 2014; Molaabasi et al., 2015; Abdelhamid and Wu, 2015; Yuan et al., 2015; Lin et al., 2013), close to works (Song et al., 2016b; Ge et al., 2015), but higher than literature (Zhou et al., 2016). However, as compared with other kinds of methods, including fluorescence resonance energy transfer (FRET) (Huang et al., 2013; Zhang et al., 2015), electrochemical (Bai and Jiang, 2013) and colorimetry (Lin et al., 2015; Tang et al., 2016) and photoelectrochemical (Li et al., 2015; Wang et al., 2016), this LOD is lower than or comparable to most of them, only higher than study (Wang et al., 2016). This new fluorescence enhancement system, just based on the addition of HBcAb-HRP, is not only sensitive, but also simpler and more convenient.

3.4. Real sample analysis

To evaluate feasibility of the biosensor, we tested content of H_2O_2 in human serum under the optimum conditions. Samples and their spiked recoveries were treated as in Section experimental, and results are summarized in Table 1. Data of recovery is between 91.3% and 107.2%, confirming this biosensor is acceptable and suitable for detection H_2O_2 in human serum.

4. Conclusions

A novel H_2O_2 biosensor based on HBcAb-HRP-CdTe QDs has been presented. In this paper, sulfurs in HBcAb-HRP, which possess a strong affinity towards Cd^{2+}, can improve greatly recombination fluorescence of CdTe QDs by creating more radiative centers at CdTe/Cd-SR complex. Then, H_2O_2 oxidizes Cd-S bonds in CdTe QDs

Table 1
Analytical results of H_2O_2 in human serum.

Sample	Added (μmol L^{-1})	Found[a] (μmol L^{-1})	RSD (%)	Recovery (%)
Human serum 1	0	3.01	0.92	–
	10	13.11	1.29	101.0
	25	28.35	1.01	101.4
Human serum 2	0	6.71	0.56	–
	10	16.83	0.50	101.2
	25	30.05	0.72	93.4
Human serum 3	0	3.59	0.83	–
	10	13.50	2.12	99.1
	25	30.38	1.18	107.2
Human serum 4	0	ND[b]	0.48	–
	10	9.13	0.95	91.3
	25	25.67	0.20	102.7

[a] For three determinations.
[b] Not detected.

to organic disulfide product (RS-SR), causing TGA and HBcAb-HRP detach from surface of CdTe QDs and thus leading to fluorescence quenching. Just with the addition of HBcAb-HRP, the sensitivity is improved near one order of magnitude as compared with CdTe QDs probe, and also superior to most of the reported works (Table S3). Although it is interfered with Ag^{+}, Hg^{2+} and Cu^{2+}, masking agents of DDTC and NH_4OH can eliminate well. The test of quality control serum, including 20 main components in serum, certified the biosensor showed excellent anti-interference ability, and presages more possibility for the study of biological system in future applications.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2017.01.048.

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