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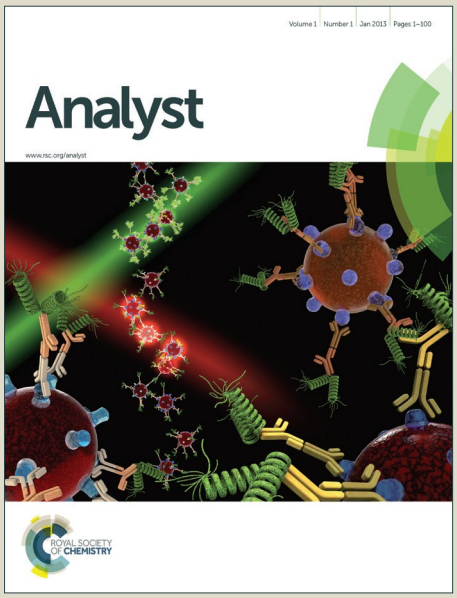

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Detection of glutathione with an "off-on" fluorescent biosensor based on N-acetyl-L-cysteine capped CdTe quantum dots

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Abstract This paper reported a quantum dots (QDs)-based "off-on" fluorescent biosensor specifically for the determination of glutathione (GSH) with high sensitivity. The biosensor was based on the following two properties. Firstly, the high fluorescence of N-acetyl-L-cysteine (NALC) capped CdTe QDs could be effectively quenched by Hg^{2+} due to the binding of Hg^{2+} to the NALC on the surface of the QDs and the electron transfer from the photoexcited NALC-capped CdTe QDs to Hg^{2+}. Secondly, in the presence of GSH, the fluorescence intensity of NALC-capped CdTe QDs was found to be efficiently recovered. Under some optimized conditions, the relatively restored fluorescence intensity was proportional to the concentration of GSH in the range of 4-64 $\mu g \cdot mL^{-1}$, with a correlation coefficient of 0.9980 and a limit of detection of 2.49 $ng \cdot mL^{-1}$. In addition, the established method shows a high selectivity for some other amino acids except cysteine. Moreover, to further investigate its performance, the biosensor was applied to the determination of GSH in human serum samples through standard addition method and determination of normal GSH concentration in original human serum samples with satisfactory results.

1 Introduction

Glutathione (GSH) is one of tripetide which contains γ-amido bond and sulfydryl. It is composed of glutamic acid, cysteine and glycine, and plays a critical role in a variety of important cellular functions, such as detoxifation and metabolism. As an antioxidant, GSH can prevent free radicals and peroxides from damaging important cellular components [1]. The ratio of GSH to glutathione disulfide is a key indicator for monitoring the cellular oxidative stress [2-5]. GSH has many other biological functions like deoxication, signal transduction and transportation/elimination of metal ions since thiol is involved in most of biological processes [6]. Medical studies show that the level of GSH in plasma is related to diseases such as HIV, Alzheimer's disease, and Parkinson's disease [7]. Therefore, sensitive and selective techniques for GSH

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detection are critically important.

So far, a number of chemical and instrumental techniques for the determination of GSH in biological samples have been reported in a number of fields, including surface enhanced Raman scattering (SERS) [8, 9], electrochemical analysis [10-12], high performance liquid chromatography (HPLC) [13, 14], capillary electrophoresis [15], enzyme linked immunosorbent assay (ELISA) [16], and etc. Although these conventional strategies exhibit promising results for the determination of GSH, they still have some shortcomings. For example, HPLC provides a high degree of specificity, but is expensive, time-consuming, and less sensitive. ELISA is laborious and complicated, so that they are limited and not suitable for routine analysis. In recent years, fluorescence spectrometry has been emerging as a promising tool for GSH detection due to its advantages such as sensitivity, simplicity and low cost. However, most fluorescent probes are based on organic dyes and their chemical reactions with the –SH group of thiols, which makes this technique less selective. And most of organic dyes have poor photostability, narrow excitation spectra, and broad emission bands with red tailing, which have intrinsically confined their further applications. Recently, fluorescent nanomaterials have attracted tons of attentions of GSH detection such as quantum dots (QDs) [17], silver and gold nanoparticles [18, 19], upconversion nanoparticles [20], and graphitic-C_3N_4 [21].

Quantum dots (QDs) are colloidal semiconductor nanocrystals formed from group 2-16, 13-15, or 14-16 materials, such as those made of CdSe and CdTe. They can be prepared readily in aqueous media or made water soluble by appropriate capping with hydrophilic ligands. In the aqueous synthesis, QDs are stabilized by some functional ligands, such as mercaptopropionic acid (MAP) and glutathione, among others, and exhibit some unique properties such as lower cost, less toxicity, water solubility, and biocompatibility. Researches on QDs have increased fast in the past few decades. The main attractions for the use of fluorescent QDs in bioanalysis and bioimaging are their optical and chemical properties such as superior photostability, excellent resistance to chemical and photochemical degradation, wide absorption spectra, narrow photoluminescence spectrum and good fluorescence quantum yield [22-24]. In the past, a large number of researchers have been focusing on the exploration of QDs utilized as fluorescent “turn-off” sensors. Recently, some studies about QDs-based fluorescent “turn off-on” sensors are reported [25-28]. Since a variety of factors rather than analytes can induce the ultimate fluorescence “off” state, a fluorescent “turn off-on” model is designed to over come this problem and effectively lessen the chance of false judgment. Meanwhile, this “turn off-on” pattern is amenable to comply with situations, for instance, the simultaneous exclusively detections of sorts analytes [29]. In this study, we had realized a new fluorescent sensor for the determination of GSH based on the reversible “off-on” fluorescence change of N-acetyl-L-cysteine capped CdTe QDs. Hg^{2+} was utilized as it could efficiently quench the highfluorescence of QDs, then fluorescence intensity of this system could selectively recovered by GSH as a result of high Hg^{2+}-GSH interaction, as shown in Fig 1.

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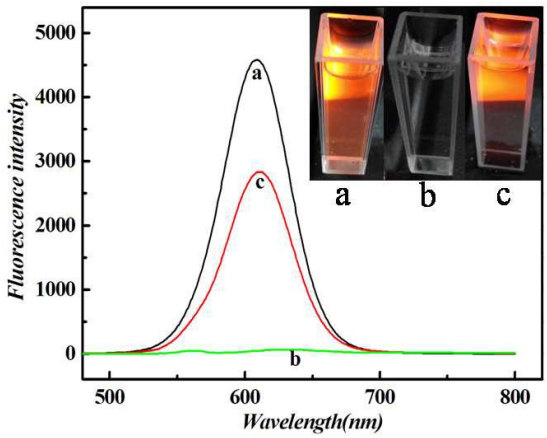

Fig 1 The fluorescence spectra of NALC-capped CdTe QDs (*curve a*), NALC-capped CdTe QDs-Hg^{2+} system (*curve b*) and NALC-capped CdTe QDs-Hg^{2+} system in the presence of GSH (*curve c*)； the inset is the photos under UV illumination, NALC-capped CdTe QDs (a), NALC-capped CdTe QDs-Hg^{2+} before (b) and after adding GSH (c). (NALC-capped CdTe QDs: 1.425×10^{-4} mol·L^{-1}; $HgCl_2$: 30 μg·mL^{-1}; GSH: 64 μg·mL^{-1}; 0.5 mL Tris-HCl buffer solution (7.4)).

Herin, a novel biosensing method for GSH detection was developed based on QDs based fluorescent "turn off-on" biosensor. As it will be demonstrated in this study, the new optical biosensor has many advantages such as high sensitivity with fast response in the detection of GSH. The sensor can detect very low concentration of GSH without any pre-concentration steps. In addition, we also discussed the selectivity of the fluorescence turn-on sensor for other biologically relevant chemical species. Moreover, this new sensor had been applied to the determination of GSH in blood sample with satisfactory results.

2 Experimental

2.1 Reagents and chemicals

All chemicals and reagents were available with the highest degree of purity and doubly distilled water was used in the experiments, including $CdCl_2·2.5H_2O$ (Shanghai Chemicals Reagent Co., Shanghai, China), Te powder (Sinopharm Chemical Reagent Co., Shanghai, China), $NaBH_4$ (Tianjin Huanwei Fine Chemical Co., Tianjin, China), N-acetyl-L-cysteine (NALC, Sinopharm Checal Reagent Co., Shanghai, China), glutathione (GSH, Sinopharm Chemical Reagent Co., Shanghai, China). Tris-HCl buffer solutions with different pH were prepared by mixing 0.2 mol·L^{-1} Tris and 0.1mol·L^{-1} HCl in different proportions.

A stock solution of 200 μg·mL^{-1} GSH were prepared by dissolving an appropriate amount of GSH in water into a 100 mL standard flask, and so for the stock solution of 2 mg·mL^{-1} $HgCl_2$. Lower GSH and $HgCl_2$ concentrations were prepared by sequential dilution of the stock solution.

2.2 Apparatus and measurements

A Hitachi F-2500 spectrofluorophotometer (Hitachi Company, Tokyo, Japan) was used to record the fluorescence spectra and resonance Rayleigh scattering (RRS) spectra. A UV-2450 spectrophotometer (Tianmei Corporation, Shanghai, China) was

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applied to record the absorption spectra. Hitachi-600 transmission electron microscopy (TEM, Hitachi Company, Japan) were adopted to examine the appearance and size of nanoparticles. A PHS-3C pH meter (Leici, Shanghai, China) was used to adjust the pH values of the aqueous solutions.

2.3 Preparation of water-soluble N-acetyl-L-cysteine capped CdTe QDs

Synthesis of water soluble N-acetyl-L-cysteine (NALC) capped CdTe QDs was performed according to the method described before [30-32] with modification. Under N_2 atmosphere and magnetic stirring, Te power (0.0255 g) was reacted with excessive sodium borohydride in deionized water to produce the colorless solution of sodium hydrogen telluride (NaHTe). $CdCl_2 \cdot 2.5\ H_2O$ (0.0488 g) and NALC (0.0574 g) were dissolved in 150 mL deionized water. With the slow N_2 flow and vigorous stirring, the pH of the mixture was adjusted to 11.19 by using the dropwise attention of NaOH solution (1.0 $mol \cdot L^{-1}$). The solution was deaerated by N_2 bubbling for about 30 min. Under stirring, H_2Te gas generated by the reaction of the solution of NaHTe with diluted H_2SO_4 (0.5 $mol \cdot L^{-1}$) was passed through the oxygen-free Cd^{2+} solution together with the slow N_2 flow. Then the resulting solution mixture was heated to 369 K and refluexed for one hour under N_2 atmosphere. The salmon pink CdTe solution was obtained. Finally, the concentration of CdTe QDs was 1.425×10^{-3} $mol \cdot L^{-1}$ (determined by Cd^{2+} concentration).

2.4 Fluorescence assays

For the determination of GSH, a freshly prepared mixture containing 1.425×10^{-4} $mol \cdot L^{-1}$ of NALC-capped CdTe QDs, 30 $\mu g \cdot mL^{-1}$ $HgCl_2$, and an appropriate volume of the sample solution were mixted and buffer with 0.5 mL Tris-HCl buffer solution (pH 7.4). Then, the fluorescence intensity of the solution was recorded at an exciation wavelength of 278 nm and the emission spectrum was recorded in the range of 470-750 nm. The fluorescence spectrum was recorded using an excitation slit of 10.0 nm and emission slit of 10.0 nm. The response function F_1 values of the biosensor were obtained with different concentration of GSH, where F_1 is the recovered fluorescence intensity of NALC-capped CdTe QDs in the presence of Hg^{2+}.

2.5 Sample preparation

Human blood plasma were filtered to remove any articles and then mixed thoroughly with trichloroacetic acid, centrifuged at 5000 rpm for 5 min. Finally using the supernatant fluid and 100 times were diluted before analysis. Standard addition method was used for the determination of GSH. An appropriate amount of standard GSH solution was spiked into the human serum. After its filtration, 0.5 mL of the spiked serum sample was added to 4.5 mL of the sensing solution (containing 1.425×10^{-4} $mol \cdot L^{-1}$ of NALC-capped CdTe QDs, 30 $\mu g \cdot mL^{-1}$ $HgCl_2$ and 0.5 mL Tris-HCl buffer solution, pH 7.4). Then, the fluorescence spectrum was recorded at 470-750 nm upon excitation at 278 nm.

3 Results and discussion

3.1 Principle of the operation

Modifying the outer surface of QDs with sulfhydryl compounds is one of the most commonly used methods for dispersing QDs in aqueous solution [33]. For this purpose, NALC was used. At adequtly basic pH, electronstatic repulsion between

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QDs affords a stable colloidal suspension. TEM image of NALC-capped CdTe QDs (Fig S1A) shows monodisperse in the shape and the sizes were about 2-3 nm. Fig S1B shows the absorbance and emission (fluorescence) spectra of the QDs, As shown in the spectrum, the fluorescence spectrum is appeared between 470-750 nm.

3.2 Hg^{2+} as an excellent quencher to NALC-capped CdTe QDs: "turn-off" process

Under the optimum conditions, the fluorescence emission spectra of NALC-capped CdTe QDs was recorded in the absence and presence of $HgCl_2$ with varying concentration (0-40 $\mu g \cdot mL^{-1}$), as shown in Fig 2. Obviously, the fluorescence intensity of NALC-capped CdTe QDs was greatly quenched in the presence of Hg^{2+}. Moreover, the quenching was accompanied with a shift of the wavelength emission maxmum λmax (a red shift, from 609 to 630 nm) in the NALC-capped CdTe QDs spectrum. And for the red shift, it may be reasonably attributed to an increased in polarity in the vicinity of the NALC-capped CdTe QDs as a result of the formation of a QDs-Hg^{2+} complex [34]. In other words, this quenching may be result from the coordination of Hg^{2+} to the ethanoyl group of the capping molecule on the surface of CdTe QDs [35]. Therefore, the fluorescence quenching by Hg^{2+} may stem from the facilitation of the recombination of nonradiative e^-/h^+ recombination on the surface of NALC-capped CdTe QDs through an effective electron transfer process between surface ethanoyl groups and Hg^{2+}, as shown in Fig 3.

As a quenching reaction, there are usually two quenching mechanisms for fluorescence emission from QDs, which are either dynamic quenching or static quenching. To our knowledge, the well-known Stern-Volmer equation is the best way to demonstrate the quenching behavior of a quencher on the fluorescence of QDs [36].

$$F_0/F=1+K_{SV}[Q] \quad (1)$$

In the expression, F_0 and F are the fluorescence intensity of NALC-capped CdTe QDs in the absence and presence of a quencher (Hg^{2+}), respectively; [Q] is the concentration of the quencher; K_{SV} is the Stern-Volumer quenching constant, which defines the quenching efficiency of the quencher. As shown in Fig S2, the linear plot for the NALC-capped CdTe QDs-Hg^{2+} solution system at three different temperatures (277K, 298K and 308K) were investigated. The K_{SV} value of the NALC-capped CdTe QDs-Hg^{2+} solution system at three different temperatures were calculated according to eqn(1) and are listed in Table 1. It can be seen that the quenching constants decreased with the rise of temperature, which indicated that the probable quenching mechanism is the formation of NALC-capped CdTe QDs-Hg^{2+} complex (static quenching) rather than by dynamic quenching [37].

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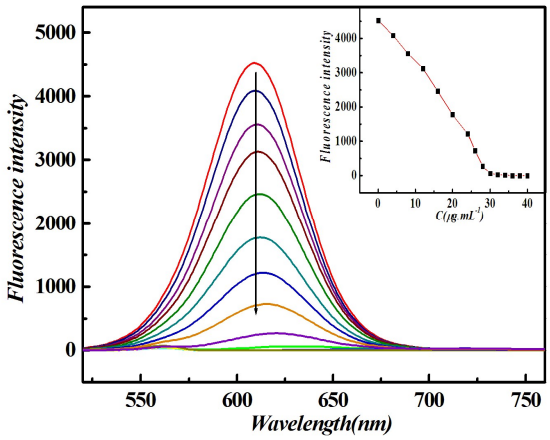

Fig 2 Fluorescenc spectra of NALC-capped CdTe QDs in the presence of Hg^{2+} in 0.5 mL Tris-HCl buffer solution at pH=7.4; the inset shows the linear of the quenched fluorescence inensity of QDs against the concentration of $HgCl_2$. (NALC-capped CdTe QDs: 1.425×10^{-4} mol·L^{-1}; 0.5 mL Tris-HCl buffer solution (7.4)).

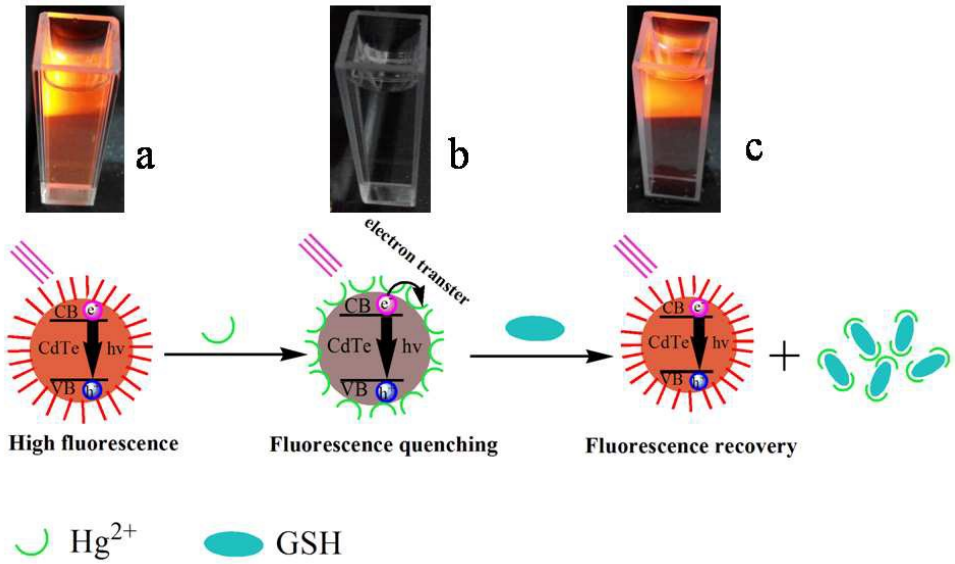

Fig 3 The mechanism of the proposed "off-on" fluorescence biosensor.

Table.1 Stern-Volmer quenching constants for the interaction of NALC-capped CdTe QDs with Hg^{2+} at three different temperatures.

Temperature(K)	Sterm-Volmerlinear equation	K_{SV}(L·mol^{-1})	R[a]	S.D.[b]
308	F0/F=0.8457+1.699×10^5[Q]	1.699×10^5	0.9990	0.01158
298	F0/F=0.8479+1.810×10^5[Q]	1.810×10^5	0.9984	0.01652
277	F0/F=0.8253+2.053×10^5[Q]	2.053×10^5	0.9989	0.01443

[a] R is the correlation cofficient.

[b] S.D.is the standard deviation for the K_{sv} values.

On the other hand, in dynamic quenching, charge transfer occurs and the

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fluorescence is quenched when the electron acceptor collides with the excited fluorophore. The collision between the quencher and the fluorescence affects only the excited state of the fluorophore, on this account, no changes in the absorption spectra are expected. In contrast, in static quenching, the absorption spectra of the fluorophore will be perturbed during the formation of ground-state complex [38]. To further confirm our speculation, UV-vis absorption spectra of NALC-capped CdTe QDs-Hg^{2+} solution system were studied and the results were presented in Fig 4. In comparison with NALC-capped CdTe QDs, the absorption spectrum of QDs in the presence of Hg^{2+} was higher, a bit flatter and with an observed red shift of 4 nm was appeared, which implies changes in size of surface properties of the QDs. Namely, the quenching type is static quenching.

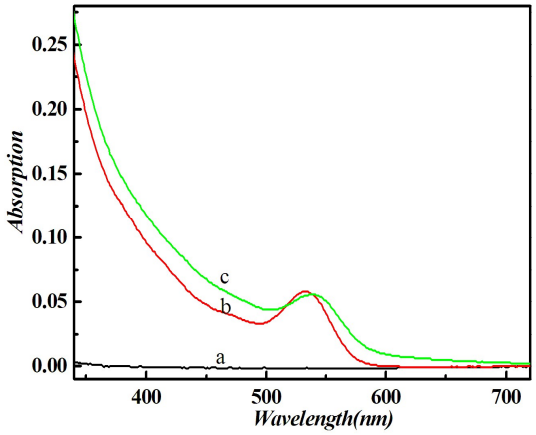

Fig.4 UV-vis absorption spectra of (a) Hg^{2+} (with distilled water as the reference), (b) NALC-CdTe QDs, (c) mixture solution system (NALC-CdTe QDs and Hg^{2+}).

In order to further prove our conjecture, the resonance Rayleigh scattering (RRS) spectra of the NALC-capped CdTe QDs-Hg^{2+} system were studied. As shown in Fig 5, the RRS intensity of NALC-capped CdTe QDs and Hg^{2+} (*curves 1 and 2*) are very weak, whereas, when the QDs and Hg^{2+} were mixed together, the RRS intensity of the system significantly enhanced and the maximum peak was located at 371 nm. Then, we investigated the effect of ionic strength on the RRS intensity of NALC-capped CdTe QDs–Hg^{2+} system by adding NaCl solution. The results shown that with the increase of the concentration of NaCl, the RRS intensity of the system increased. Therefore, the electrostatic attraction played an important role in the reaction of NALC-capped CdTe QDs and Hg^{2+}.

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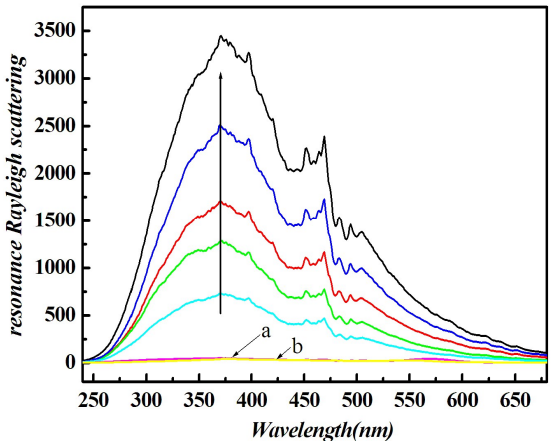

Fig 5 RRS spectra of NALC-capped CdTe QDs in the presence of Hg^{2+} in pH 7.4 Tris-HCl buffer solution, the concentration of $HgCl_2$ (from bottom to top): 2.0, 4.0, 6.0, 10.0 and 14.0 μg·mL^{-1}; NALC-capped CdTe QDs: 1.425×10^{-4} mol·L^{-1}.(curve a: 1.425×10^{-4} mol·L^{-1} NALC-capped CdTe QDs; curve b: 14 μg·mL^{-1} $HgCl_2$).

3.3 Effect of analytical conditions

In comparison with the fluorescent "turn-on" sensors, the fluorescent "turn-off" modes seem to be more preferable due to the reduction of the chance of false positives. Namely, the fluorescence "turn-on" sensing systems have better selectivity than "turn-off" sensors [39, 40]. Before quantitative analytisis of GSH, we optimized the "turn-on" detection conditions, including pH value, concentration of NALC-capped CdTe QDs, amount of Hg^{2+}, incubation time and NaCl concentration.

Effect of sample solution pH. The relationship between the fluorescent "off-on" system and the solution pH was investigated. At first, the pH of the solution strongly affected the fluorescence quenching of Hg^{2+} toward the NALC-capped CdTe QDs. Then, the pH of the solution could also have an effect on the fluorescence restoration ability of GSH. During this experiment, we used Tris-HCl as the buffer solution. As shown in Fig 6, the effect of pH value of Tris-HCl buffer solution on the fluorescence intensity of the "off-on" system was investigated from 6.8 to 8.2. From Fig 6A, we could find that the maximum value of F_0-F was obtained when the pH value was 7.4 (F_0 and F were the fluorescence intensities of the aqueous CdTe QDs without and with a given concentration of Hg^{2+}). Therefore, the optimal pH of QDs-Hg^{2+} system was 7.4. And Fig 6B, we can see that the maximum fluorescence recovery F_1-F (where F_1 is the fluorescenc intensity of NALC-capped CdTe QDs-Hg^{2+} system with a given concentration of GSH) of NALC-capped CdTe QD-Hg^{2+} system after the addition of GSH was obtained at pH 7.4, which fitted well with the optimum pH (7.4) of QDs-Hg^{2+} system. Hence, we chose Tris-HCl (7.4) as the buffer solution in this experiment.

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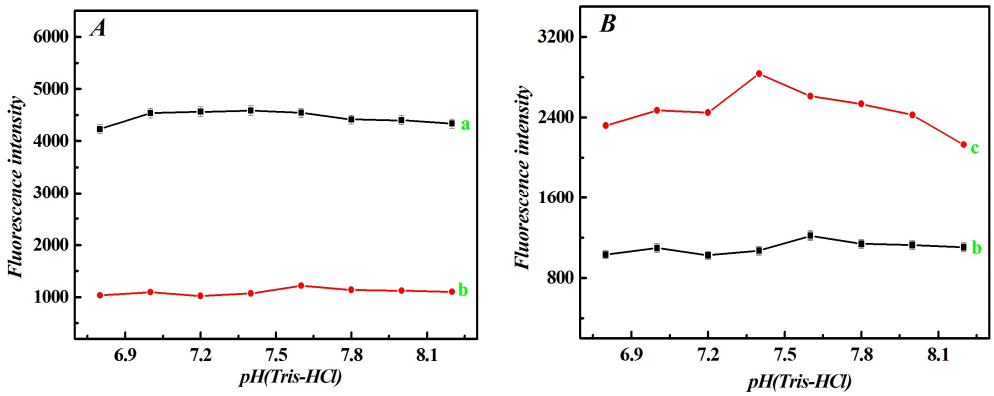

Fig 6 (A) Effect of acidity on the decrease of fluorescence intensity of NALC-capped CdTe QDs after the addition of Hg^{2+} in the Tris-HCl buffer solution; (B) Effect of acidity on the recovery of fluorescence intensity of NALC-capped CdTe QDs and Hg^{2+} system after the addition of GSH in the Tris-HCl buffer solution (NALC-capped CdTe QDs: 1.425×10^{-4} mol·L^{-1}; $HgCl_2$: 24 μg·mL^{-1}; GSH: 64 μg·mL^{-1}; 0.5 mL Tris-HCl buffer solution (7.4)).

Effect of NALC-capped CdTe QDs. It was found that the concentration of NALC-capped CdTe QDs affect the method sensitivity. As shown in Fig 7, where the concentration of NALC-capped CdTe QDs increased in the presence of 30 μg·mL^{-1} Hg^{2+} at pH 7.4 Tris-HCl buffer solution, the sensitivity increased considerably. In addition, if the concentration was too low, the fluorescence intensity was also very low, which may sacrifice the linear calibration range. Based on the obtained results, 1.425×10^{-4} mol·L^{-1} NALC-capped CdTe QDs was selected as an optimum concentration of QDs for further study.

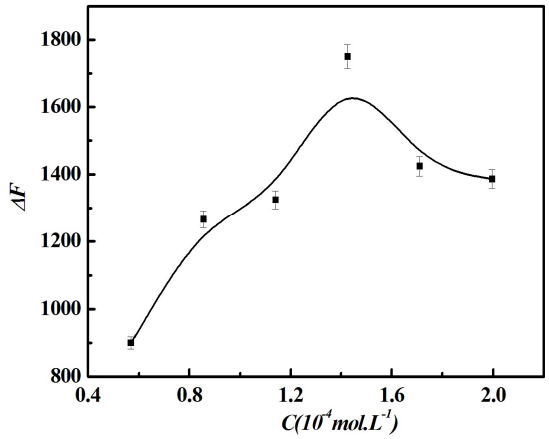

Fig 7 The effect of the concentration of NALC-capped CdTe QDs on the NALC-capped CdTe QDs-Hg^{2+} system in 0.5 mL Tris-HCl buffer solution (pH=7.4).

Effect of Hg^{2+}. At first, according to previous reports, large number of metal ions could quench the fluorescence intensity of QDs. In this work, in order to find the best metal ion for the fluorescence "off-on" biosensor for the determination of GSH, we have discussed the response of some other metal ions to this "off-on" system. As shown in Fig 8A, upon the addition of the same concentration of Fe^{3+}, Co^{2+}, Pd^{2+},

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Cu^{2+}, Ce^{4+}, Pb^{2+} and Hg^{2+}, all of those metal ions could quench the fluorescence intensity of QDs, except Fe^{3+} and Co^{2+}, other metal ions have the same ability for the quenching of NALC-capped CdTe QDs. Therefore, we also studied the fluorescence recovery of those QDs-metal ions system with the addition of GSH, and the results are shown in Fig 8B. Compared Fig 8A and Fig 8B, we could find that, upon the addition of the same concentration of GSH, the maximum recovered fluorescence intensity of QDs-meatl ions is Hg^{2+}, so in this work, we chose Hg^{2+} to modulate the fluorescence intensity of QDs. In addition, we also discussed the effect of the amount of Hg^{2+} on the ternary system. To our knowledge, if the amount of Hg^{2+} in the solution were excessive, some GSH would react with free Hg^{2+}. Therefore, the remainder GSH reacting with Hg^{2+} on the surface of QDs would decrease. On the other hand, if the solution contained an insufficient amount of Hg^{2+}, the working range and sensitivity of the sensor would be limited. As shown in Fig 2, with the increase of the concentration of Hg^{2+}, the fluorescence intensity of QDs-Hg^{2+} system decreased. The inset of Fig 2 shown that when the Hg^{2+} was at a lower concentration, the fluorescence intensity decreases greatly, but with the increase of the concentration of Hg^{2+}, the extent of the decrease in fluorescence intensity dimished. Thus, considering the above discussion, we chose 30 $\mu g \cdot mL^{-1}$ $HgCl_2$ as the optimum concentration in the follow experiment.

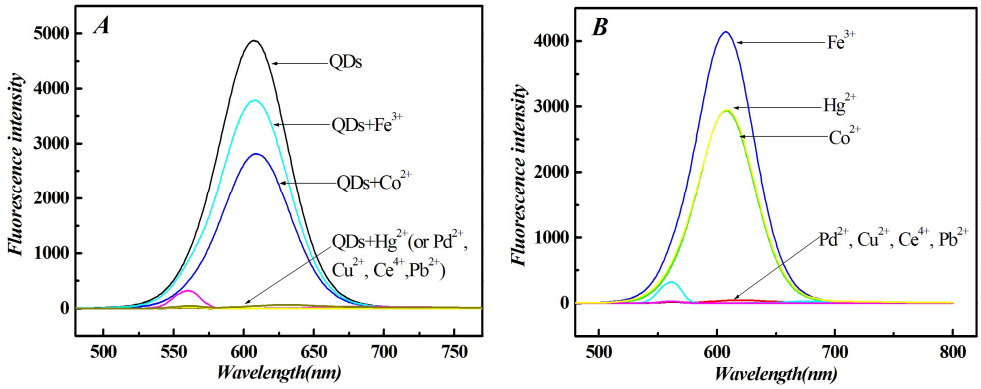

Fig 8 (A) Fluorescence intensity change of NALC-capped CdTe QDs upon addition of $HgCl_2$ (30 $\mu g \cdot mL^{-1}$) and the same concentration of other metal ions (Fe^{3+}, Co^{2+}, Pd^{2+}, Cu^{2+}, Ce^{4+}, and Pb^{2+}) in 0.5 mL Tris-HCl buffer solution (pH=7.4); (B) Fluorescence spectra of QDs-metal ions upon addition of GSH in 0.5 mL Tris-HCl buffer solution (pH=7.4).

Effect of reaction time. At first, the effect of reaction time on the fluorescence intensity of NALC-capped CdTe QDs by Hg^{2+} was investigated. Preliminary experiments demonstrated that the fluorescence quenching of NALC-capped CdTe QDs by Hg^{2+} was completed within 5 min and the fluorescence signals remained constant for more than 90 min (as shown in FigS3A), indicating that the NALC-capped CdTe QDs-Hg^{2+} system exhibited good stability. Thus, the fluorescence intensity of NALC-capped CdTe QDs-Hg^{2+} system was recorded after the addition of Hg^{2+} for 5 min. Then, the effect of reaction time on the fluorescence of NALC-capped CdTe QDs-Hg^{2+} system by GSH was also studied. The results indicated that the fluorescence reaction of Hg^{2+} modulated NALC-capped CdTe QDs

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by adding GSH was completed within 10 min and lasted for more than 2 h (as shown in Fig S3B), which indicated that the "off-on" fluorescence biosensor exhibited higher stability. Therefore, the fluorescence intensity of NALC-capped CdTe QDs-Hg^{2+}-GSH system was measured after adding GSH for 10 min.

Effect of NaCl concentration. The ionic strength of QDs-Hg^{2+} system may affect the quenching and recovery efficiency. Fig S4 shows the relationship between the NaCl concentration and the recovery or quenching efficiency. We can see that when NaCl concentration was less than 0.14 $mol \cdot L^{-1}$, the fluorescence intensity of QDs-Hg^{2+} system, and QDs-Hg^{2+}-GSH system reached a platean which is the maximum value, and their fluorescence intensity slightly decreased with the increasing NaCl concentration from 0.14 $mol \cdot L^{-1}$. Thus, in the following experiment, we should controlled the ionic strength and did not over 0.14 $mol \cdot L^{-1}$ NaCl.

3.4 Determination of GSH based on fluorescence recovery

According to the primary analysis above, the fluorescence intensity of NALC-capped CdTe QDs quenched greatly after adding Hg^{2+}. But, the fluorescence of Hg^{2+} modulated NALC-capped CdTe QDs was switched "on" after the addition of GSH. The fluorescence spectra of NALC-capped CdTe QDs-Hg^{2+} system with different concentration of GSH are shown in Fig 9A. Under the optimum conditions, the fluorescence intensity restoration of NALC-capped CdTe QDs-Hg^{2+} system was proportional to the concentration of GSH in the range of 4-64 $\mu g \cdot mL^{-1}$ with a correlation coefficient of 0.9980. The linear relationship between the fluorescence intensity of NALC-capped CdTe QDs-Hg^{2+}-GSH system and GSH concentration in that range is shown in Fig 9B. The linear regression equation was F_1=43.66C+77.91 (F_1 is the fluorescence intensity of NALC-capped CdTe QDs-Hg^{2+}-GSH; C is the concentration of GSH). The detection limit of 2.49 $ng \cdot mL^{-1}$ for GSH was determined by using 3σ/K, where σ was the standard deviation of eleven replicate measurements of the fluorescence intensity of using this fluorescence sensing system for GSH determination in aqueous solution. In Fig 3, we can see the light observed from three different systems: (a) NALC-capped QDs; (b) NALC-capped CdTe QDs-Hg^{2+} and (c) NALC-capped CdTe QDs-Hg^{2+}-GSH. The original QDs emitted the fluorescence light under UV illumination, which was turned off upon adding Hg^{2+}. After adding GSH into the NALC-capped CdTe QDs-Hg^{2+} solution, the emission light could be turned on again with a more shallow color compared with the original QDs. This observation demonstrated that the partial recovery of the spectrum from that of the original QDs obtained by the spectrofluorometer. For the partial fluorescence recovery, in section 3.2, we have discussed the quenching mechanism of Hg^{2+} to NALC-capped CdTe QDs may be the coordination of Hg^{2+} to the ethanoyl group of the capping molecule on the surface of CdTe QDs. But, on the other hand, the incorporation of Hg^{2+} into the quantum dots results in the surface decfects of QDs, leading to a nonradiative recombination of the excitons and fluorescence quenching, which can be described as the mechanism of cation-exchange [41]. Accordingly, these metals (such as HgS, CuS,

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Ag_2S and PbS) have a lower Ksp value which can display the QDs cations via cation-exchange. For Hg^{2+} ions, cation-exchange also made some contribution to the quenching mechanism in fluorescence intensity. Therefore, in the work, some Hg^{2+} may replaced the Cd in the QDs, which has a lower energy level than that of pure CdTe QDs. With the presence of GSH, the fluorescence intensity of the system was recovered as the results of the competed interaction between NALC and GSH with Hg^{2+} that coordinated with the ethanoyl group of the capping molecule on the surface of CdTe QDs. But, may be GSH could not react with the Hg^{2+} that replaced the Cd in the QDs. Thus, from Fig 8, we can see that the fluorescence recovery is about 60%.

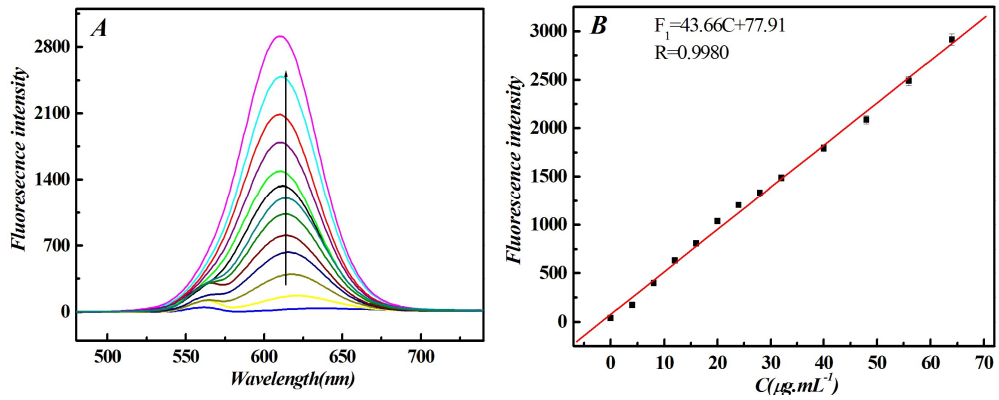

Fig 9 (A) Fluorescence spectra of NALC-capped CdTe QDs with the addition of 30 $\mu g \cdot mL^{-1}$ of Hg^{2+} recover due to introducing GSH in the concentration range of 0-64 $\mu g \cdot mL^{-1}$; (B) The linear calibration plot of the fluorescence intensity against the concentration of GSH. 20(Concentration of NALC-capped CdTe QDs: 1.425×10^{-4} $mol \cdot L^{-1}$)

In order to investigated the interaction of GSH with the proposed "off-on" fluorescence sensor, we have investigated the reaction of GSH with NALC-capped CdTe QDs. As shown in Fig 10A, the fluorescence emission of NALC-capped CdTe QDs was not influenced by the addition of GSH, which indicated that GSH had no obvious influence on the fluorescence intensity of NALC-capped CdTe QDs, and illustrated that the recovered fluorescence of NALC-capped CdTe QDs-Hg^{2+}-GSH system caused by the interaction of GSH with Hg^{2+}. To further prove this hypothesis, the absosption spectrums NALC-capped CdTe QDs (a), NALC-capped CdTe QDs –Hg2+ (b) and NALC-capped CdTe QDs-Hg^{2+}-GSH (c) (shown in Fig 10B) were measured in 0.5 mL Tris-HCl buffer solution at pH=7.4. From the absorption spectrum, we could see that the addition of Hg^{2+} induced the characteristic absorption peak of QDs at 530 nm had a red-shift behavior, which we have discussed before as having been caused by the formation of NALC-capped CdTe QDs-Hg^{2+} complex. What is more important is that after adding GSH in NALC-capped CdTe QDs-Hg^{2+} system, a hypochromatic shift appeared. Compared with the range of the red-shift, the blue-shift was shorter. These results corresponds well with the partial recovery of the fluorescence "off-on" sensor. Therefore, it could say that the fluorescence recovery of NALC-capped CdTe QDs-Hg^{2+}-GSH system was caused by the interaction of Hg^{2+}

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with GSH. And according to the literatures reported before [42], Hg^{2+} could reacted with GSH through chelation. In this experiment, the added GSH chelated with Hg^{2+} on the surface of QDs and lead to the fluorescence recovery of QDs, whose fluorescence was quenched by Hg^{2+}. And the designed "off-on" fluorescent biosensor worked in principle as exhibited in Fig 3.

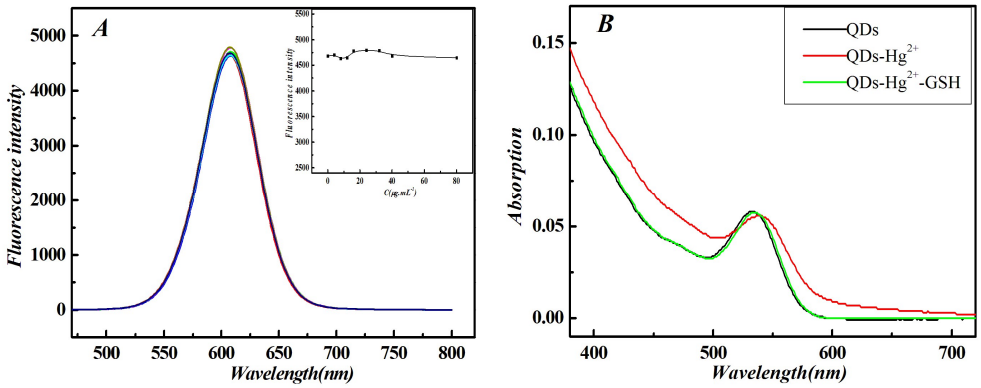

Fig 10 (A) The effect of GSH on the fluorescence intensity of NALC-capped CdTe QDs in 0.5 mL Tris-HCl buffer solution at pH=7.4 (Concentration of NALC-capped CdTe QDs: 1.425×10^{-4} mol·L^{-1}; GSH, 0, 4, 8, 12, 16, 24, 32, 40 and 80 μg·mL^{-1}); (B) UV-vis absorption spectra of NALC-capped CdTe QDs,QDs-Hg^{2+} and QDs-Hg^{2+}-GSH system.

3.5 Selectivity of the proposed "off-on" fluorescence biosensor

To evaluate the selectivity of the present "off-on" fluorescence biosensor, GSH, Cys and other 11 kinds of amino acids at the same concentration were investigated. Fig 11 shows the efficiencies of NALC-capped CdTe QDs-Hg^{2+} probe with GSH, Cys and other kinds of amino acids. Fluorescence intensity of NALC-capped CdTe QDs was defined as 100 % and NALC-capped CdTe QDs-Hg^{2+} "off-on" probe is zero. Form Fig 11, we can see that GSH exhibited significant fluorescence enhancing effect on the proposed "off-on" biosensor. And at the same time, Cysteine could also recover the fluorescence of NALC-capped CdTe QDs-Hg^{2+} system. But compared with GSH, the recovered efficiency of Cys is strikingly lower. For the other kinds amino acids, they recovered efficiencies are very low. In other words, aminos acids could not restore the fluorescence of NALC-cappde CdTe QDs-Hg^{2+} "off-on" biosensor. Therefore, this proposed method had high selectivity for GSH and can be used for the determination of GSH.

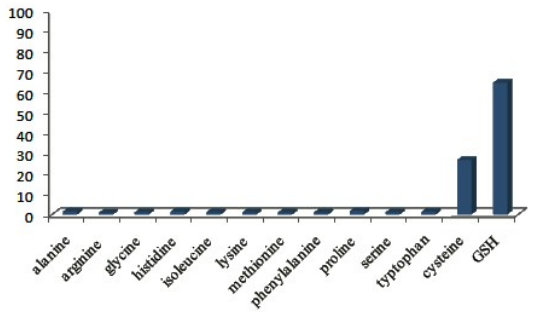

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Fig 10 The selectivity of the "off-on" fluorescence sensor to various amino acids.

3.6 Application of the proposed "off-on" fluorescence biosensor.

3.6.1 Determination of GSH in fresh human serum samples through standard addition method.

To evaluate the feasibility of the proposed method for the determination of real samples, the applicability of the biosensor to real sample was tested in fresh human serum samples. The standard addition method was used for the determination of GSH in the serum samples. The results are given in Table 2, it showed that the recovery was acceptable. The results revealed that the proposed "off-on" fluorescence biosensor was an applicable method for GSH analysis in real samples.

Table 2 Results for the determination of GSH in fresh human serum samples through standard addition method, n=5.

Sample	Found (n=5, μg·mL^{-1})	Added (μg·mL^{-1})	Found (μg·mL^{-1})	Recovry (n=5, %)	R.S.D (n=5, %)
Serum 1	ND[c]	20.0	20.5	102.5	3.5
Serum 2	ND[c]	30.0	29.6	98.7	1.9
Serum 3	ND[c]	50.0	49.7	99.4	2.8

[c] Not detected

3.6.2 Determination of normal concentration of GSH in serum human.

Commonly, the blood of human contain certain amount of GSH, and according to the literature reported before [43], in blood plasma, the normal GSH concentration is about 0.50±0.10 μg·mL^{-1}. Fortunately, the assay proposed in this work had a detection limit of 2.49 ng·mL^{-1}, which was lower than the normal GSH concentration in human. Therefore, we could use this method to detect the normal GSH concentration in human. The human serum samples was obtained through section 2.5 and without diluted. Then used the method developed in this work to detect the concentration of GSH in the original serum samples. The results are shown in Table 3, the results indicated that the normal concentration of GSH in serum sample is about 0.48 μg·mL^{-1}, which was corresponding well with the results in the literature (0.50± 0.10 μg·mL^{-1}).

Table 3 Results of the determination of normal concentration of GSH in serum of human, n=5.

Sample	Found (n=5, μg·mL^{-1})	R.S.D (n=5, %)	Average value (μg·mL^{-1})	HPLC (μg·mL^{-1})[43]
Serum 1	0.46, 0.48, 0.45, 0.47, 0.49	3.4		
Serum 2	0.46, 0.49, 0.47, 0.50, 0.49	3.4	0.48	0.50±0.10
Serum 3	0.48, 0.51, 0.50, 0.46, 0.50	4.1		

4 Conclusion

GSH is an important pharmaceutical compounds with an antioxidative effect. Development of a sensitive, rapid and accurate method for measuring GSH is essential. Here a novel fluorescence biosensor is introduced for quantitative analysis of GSH using Hg^{2+} added NALC-capped CdTe QDs. At first, the initial fluorescence intensity of NALC-capped CdTe QDs was markly quenched, which was ascribed to

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the electrostatic association of Hg^{2+} on the surface of CdTe QDs resulting in the occurrence of photoinduced electron transfer process. Then, with the addition of GSH, the fluorescence enhancement of Hg^{2+} modulated QDs was observed, which was attributed to GSH induced Hg^{2+} to be dissociated from the surface of QDs, and then Hg^{2+} chelated with GSH to form stable complex. This method is simple, rapid and specific. In addition, low detection limit (2.49 $ng \cdot mL^{-1}$), high sensitivity and selectivity are the most important advantages of the present "off-on" fluorescence biosensor. Finally, the proposed optical biosensor has been applied successfully to analysis of GSH in real samples with satisfactory results.

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