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Fluorescent Detection of Specific DNA Sequences Related to *Toxoplasma gondii* Based on Magnetic Fluorescent Nanoparticles Fe_3O_4/CdTe Biosensor

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Authors' contributions

This work was carried out in collaboration between all authors. Author SX designed the study, wrote the protocol and supervised the work. Authors LN and XZ carried out all laboratories work and performed the statistical analysis. Author CZ managed the analyses of the study. Author LH wrote the first draft of the manuscript. Author RL managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Magnetic fluorescent nanoparticles (Fe_3O_4/CdTe) were prepared in this work and applied for *Toxoplasma gondii* DNA detection. First, CdTe quantum dots were synthesized with 3-mercaptopropionic (MPA) capped. Fe_3O_4 magnetic particles were prepared by hydrothermal method with NaOH as precipitator, and they were surfacely modified with silane coupling agent (KH550). After then, the MPA-capped CdTe QDs were immobilized on the Fe_3O_4 particles surface via electrostatic interaction, and the Fe_3O_4/CdTe particles were prepared with the average size of 10 nm. The DNA sensing probe was fabricated through labeling a stem-loop *Toxoplasma gondii* DNA oligonucleotides with Fe_3O_4/CdTe (donor) at the 5′ end and BHQ2 (acceptor) at 3′ end,

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respectively. The assembly prosess was verified by UV-Vis, TEM, IR, XRD etc. The sensitivity characterization of the molecular beacon probe was performed by fluorescence spectrum (FS) with a detection limit of $8.339x10^{-9}$M. This chemical strategy can be further applied to prepare the magnetic nanoparticles for DNA detection.

Keywords: Fe_3O_4/CdTe nanoparticles; molecular beacon probe; fluorescence resonance energy transfer; Toxoplasma gondii DNA detection.

1. INTRODUCTION

Magnetic nanoparticles have been intensively studied due to the fact that they can be used to many areas such as magnetic storage devices, optical magnetic materials, magnetic separation, and DNA targeted diagnose. The remarkable potential of Fe_3O_4 nanoparticles has stimulated the development of synthesis technology such as organism or inorganism. However, the traditional synthesis route often need protecting gas (e.g. N_2), time consuming, and high temperature [1]. In this work, we adopted an aqueous-phase synthesis method to prepare high-quality Fe_3O_4 magnetic nanoparticles, which was at facile temperature, eco-friendly, and low toxicity.

Quantum dots (QDs) are becoming outstanding fluorophores for biosensing, bioimaging, and the detection of heavy metal ion [2-4]. QDs are more photostable and higher brightness than the traditional organic fluorophores [5,6]. The broad absorption spectra and narrow emission peak make QDs have some excellent optical properties. Moreover, their physical properties can be transformed by the size [7]. By combining Fe_3O_4 and quantum dots together, magnetic quantum dots show magnetic and optical properties. Therefore, they show great potential to reveal some novel applications in biomedical, bio-detection, and magnetic functional materials fields. There are many reports on the synthesis and characterization of magnetic quantum dots: Wang et al. [8] prepared luminescent Fe_3O_4/CdTe nanocomposites with excellent optical and magnetic properties. But Wang and our group used different modifiers to react with Fe_3O_4. Wang chose tetramethylammonium hydroxide and we chose KH550. In addition, the reaction time, temperature and some agents were also different in the experiment. Jie et al. [9] synthesized Fe_3O_4@CdSe composite quantum dots for detecting thrombin by multiple DNA cycle amplification strategy molecular beacons. Lan et al. [10] synthesized Fe_3O_4 nanowires which were decorated by CdTe quantum dots, and can be used in fabrication of nanoscale magneto-optics devices.

Molecular beacons, have been providing a variety of exciting opportunities in DNA/RNA studies [11-13]. Based on the principle of fluorescence resonance energy transfer (FRET), the molecular beacons have a stem-loop structure, the loop region is a single-stranded probe sequence and the stem is formed of two short complementary sequences unrelated to the target [11,14], with a fluorophore and a quencher linked to the two ends. Systems that utilize the exquisite molecular recognition capability of biomolecules such as DNA to guide the assembly of nanoparticles have been reported previously [15-17]. For example, DNA-labeled gold [18] and CdSe nanoparticles [19] possessing unique colorimetric and fluorescent properties have been used as biosensors of DNA hybridization.

The *Toxoplasmosis* is a zoonosis caused by *Toxoplasma gondii*, an obligate intracellular protozoan that infects warm-blooded animals. In the previous report [20], *toxoplasmosis* may be life-threatening to the immunocompromised patients, and infection of pregnant women with *Toxoplasma gondii* may lead to congenital defects. In consequence, the detection of *Toxoplasma gondii* is important for medical workers and common people. This paper presents the efforts made in the preparation of sensing probe, with magnetic-fluorescent composite nanoparticles Fe_3O_4/CdTe conjugated to synthetic *Toxoplasma gondii* oligonucleotides that may revealed some excellent magnetic-optical properties. As the results, the Fe_3O_4/CdTe sensing probe detected the *Toxoplasma gondii* DNA successfully, and the limit of detection was 8.339 nM. This high sensitive and specific sensing probe may be used to wide applications such as DNA targeted diagnosis and biological molecular detection.

2. EXPERIMENTAL

2.1 Materials

All the chemicals used in the current research are of analytical grade without further purification. Ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous chloride

($FeCl_2 \cdot 6H_2O$) and sodium hydroxide (NaOH) were purchased from Tianjin KRS Fine Chemical CO.LTD, silane coupling agent (KH550) was obtained from Tianjin Chemical Reagents Factory. *Toxoplasma gondii* DNA (Table 1), and 1-ethyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) were obtained from Shanghai Yingjun Biotech Co. Ltd (PRC). Ultrapure water was used throughout experiments.

2.2 Methods

2.2.1 Preparation of Fe_3O_4/CdTe nanoparticles

Fe_3O_4 nanoparticles were prepared by co-precipitation method with a ferrous complex using NaOH as a precipitation agent: a mixture of ferrous and ferric chlorides (1: 2, molar ratio) were reacted in aqueous phase with NaOH as precipitator at 50ºC for 30min. The reaction mixture was separated with a common magnet and washed several times with water, finally dried in a vacuum oven at 70ºC.

CdTe QDs aqueous solution modified by 3-mercaptopropionic (MPA) was firstly synthesized as previously reported [21]. Magnetic nanoparticles Fe_3O_4 were surfacely modified with silane coupling agent (KH550), then CdTe was added with molar ratio 3:1(CdTe excess). The reactant solution was refluxed at 45°C for 30 min, and Fe_3O_4 /CdTe magnetic QDs were obtained.

2.2.2 Construction of Fe_3O_4 /CdTe conjugated molecular beacon probe

50 μL of Fe_3O_4 /CdTe QDs aqueous solution, 20 μL of BHQ2 modified DNA and 10 mole times more than DNA of 1-ethyl-3-(dimethy1aminopmpy1) carbodi-imide hydmchl0ride (EDC) were mixed in 700 μL of 20 mM PBS (pH 8.0), and the Fe_3O_4 /CdTe conjugated molecular beacons were prepared after 24 h at ambient temperature.

The Fe_3O_4 /CdTe particle size can be quantitatively evaluated from the XRD data using the Scherrer equation:

$$D = \frac{0.89\lambda}{\beta \cdot \cos\theta}$$

Where D is the average particle size, λ is the wavelength (0.15406 nm) of the X-rays, β is the integral half-height width (in radians), and θ is the Bragg angle. In addition, the characterization of specificity of prepared sensing probe was investigated by the fluorescence spectrum.

2.2.3 Hybridization of target DNA

100μL target DNA was added into sensing probe system containing Tris-HCl buffer. The hybridization was performed at 37ºC for 2 h. The results were detected with fluorescence spectrum (FS), and the fluorescence spectrum was also chosen to characterize the quantitative detection of target DNA.

2.2.4 Characterization

An infrared spectroscopy (IR) (FTIR-650, made by Tianjin Gangdong Scientific and Technical Development CO., Ltd, PRC) was used to determine information about the chemical structure of Fe_3O_4. The particle size and morphology analyses were performed using transmission electron microscopy (TEM, Tecnai G2 20, FEI company, USA). X-ray diffraction (XRD) patterns were recorded by using a D/max-IIA (RIGAKU, JAPAN) powder diffractometer, with CuKα line (40 kv, 50 mA). All fluorescence spectrum were conducted on a WGY-380 fluorospectrophotometer (Tianjin Gangdong Scientific and Technical Development CO., Ltd, PRC).

Table 1. The DNA sequences in experiments

Nomenclature	Sequence
Molecular beacon probe	5'-NH_2-$(CH_2)_6$-AGCTATTATAAACTCGTTGGATGCATAGCT-3'-BHQ2
Mismatch sequence	5'-TGCATCCAATGAGTTTATAA-3
complementary sequences	5'-TGCATCCAACGAGTTTATAA-3

3. RESULTS AND DISCUSSION

3.1 Optical Property and Structural Characterization of MPA-CdTe NPs

The fluorescence spectra shown in Fig. 1A indicates that the synthesized CdTe QDs has excellent optical properties. All experiments are under the same conditions and the concentration of every sample is unified. A red-shift emission spectra of MPA-CdTe was observed from 525nm to 565nm when the time increased from 1h to 6h. Moreover, the Fluorescence Intensity (FI) of QDs greatly enhanced with the time increasing. The Full Width Half Maximum (FWHM) was broadened from 42.6nm to 61.2nm, which indicated the narrow size distribution of CdTe QDs. In this research, 4h-CdTe (shown in Fig. 1B) was used through all experiments. The particle sizes of 4h-CdTe QDs were determined according to Eq. (1) and the values were around 2.1 nm, corresponding with the first absorption maximum from UV (Fig. 2 B).

$$D = (9.8127\times10^{-7})\lambda^3 - (1.7147\times10^{-3})\lambda^2 + 1.0064\lambda - 194.84 \quad (1)$$

The concentration of prepared CdTe QDs was obtained from the UV spectrum by using Lambert–Beer's law (Eq. (2)) [22]:

$$A=\varepsilon\cdot C\cdot l \quad (2)$$

In Eq. (2), *A* is the absorbance of first excitonic absorption peak for CdTe. *C* is the concentration of CdTe QDs. *l* is the path length of the radiation beam used, and *ε* is the molar extinction coefficient of CdTe QDs at the first excitonic absorption peak, which could be obtained from the formula ε = 10043(D) $^{2.12}$, where *D* is the particle size of the prepared QDs. Based on Eq. (2), the concentration of the prepared CdTe QDs is 1.724×10^{-5} M.

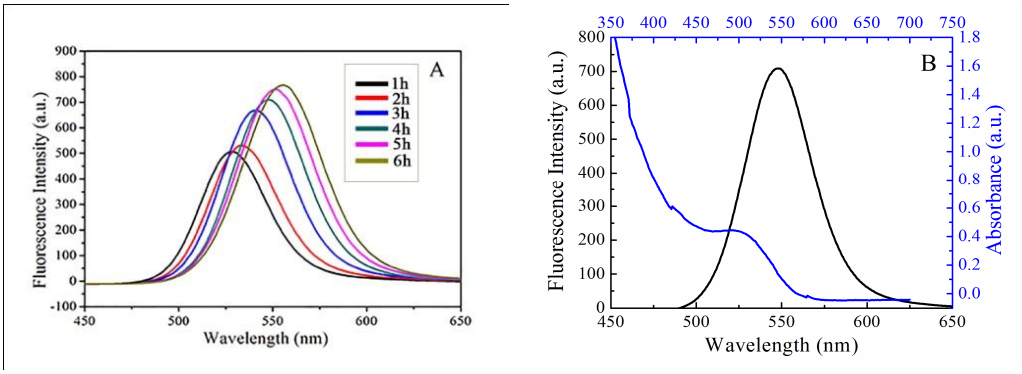

Fig. 1. Optical spectra of CdTe NPs with MPA as stabilizer: A- fluorescence spectra from 1h to 6h; B- UV (blue) and fluorescence spectra (black) of 4h-CdTe

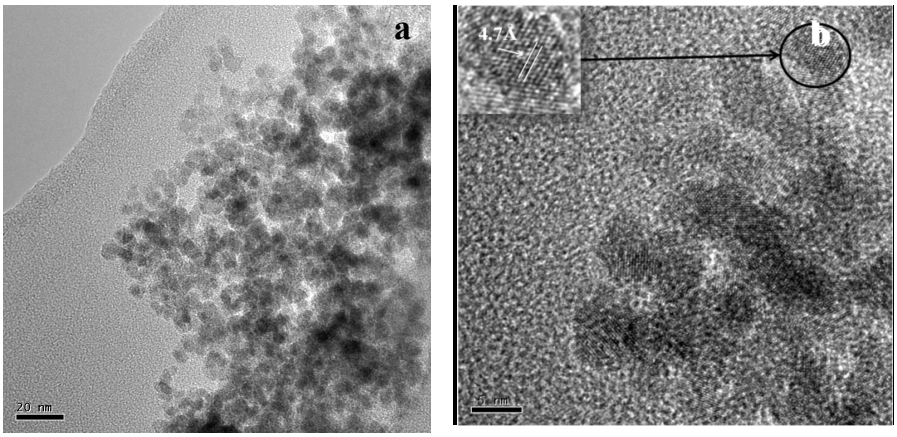

Fig. 2. TEM (a) and corresponding HRTEM (b) images of MPA-CdTe nanoparticles

TEM and HR-TEM images for MPA-capped CdTe are shown in Fig. 2. Fig. 2a shows that MPA capped CdTe QDs are spherical with an average size of 2.5nm, which is corresponding to the previous theoretical calculating value in Eq. 1. Fig. 2b presents the HRTEM image of CdTe. The lattice planes confirmed the crystallinity of MPA capped CdTe QDs. Therefore, it showed that MPA-capped CdTe QDs were synthesized successfully.

The IR spectrum of naked Fe_3O_4 and Fe_3O_4-KH550 nanoparticles is shown in Figs. 3 (a and b). The peak around 3409 cm^{-1} observed in Fig. 3a relates to the O-H stretching vibration. The peak at 1627 cm^{-1} (Fig. 3a) and 1637 cm^{-1} (Fig. 3b) was due to the bending vibration of the O-H, Which also proved the existence of O-H. If the sample obtained crystallizes completely, the products would not include -OH, conversely, the final products would comprise -OH [23]. For the naked Fe_3O_4 (Fig. 3a), the peak at 575 cm^{-1} relates to the vibrations of Fe-O-Fe, which is consistent with the reported IR spectra for spinel Fe_3O_4 [24]. For the IR spectrum of Fe_3O_4-KH550 nanoparticles (Fig. 3b), the peaks around 3430 cm^{-1} should be ascribed to the stretching vibration of the N–H, which may overlap with stretching vibration peak of O-H group. The peaks around 2920 and 2858 cm^{-1} assignable to the asymmetric and symmetric stretching vibration of $(CH_2)_n$ can be obviously found. In addition, there is an absorption peak at 1005 cm^{-1}, which is the characteristic stretching vibration peak of Si-O, and a new sharp peak 574 cm^{-1} relates to Fe–O group appeared. KH550 was hydrolyzed into silanol in aqueous solution, and a dehydration reaction was carried out between hydroxyl of Fe_3O_4 and silanol, so KH550 molecules are chemically bonded to the surface of the nanoparticles. Because the surface of iron oxide with negative charges (Fig. 4a) has an affinity toward KH550, protonated KH550 (Fig. 4b) could coat the magnetite nanoparticles by the electrostatic interaction.

3.2 Characterization of Fe_3O_4-KH550 Magnetic Nanoparticles

In our experiment, XRD is used to analysis the structure of Fe_3O_4 and confirm whether the Fe_3O_4/CdTe nanoparticles had been synthesized. Fig. 5 shows the XRD patterns for the naked Fe_3O_4 and Fe_3O_4/CdTe nanoparticles (a and b). As metallic iron itself has fluorescent effect, in our study we used Cu target as X-ray excitation light source, when the rays hit the iron nanoparticles, iron will be launched as a secondary X-ray excitation source, making XRD chart with a high basement and covering up the weak diffraction peaks of Fe_3O_4. Four main characteristic peaks for Fe_3O_4 marked by their indices ((2 2 0), (3 1 1), (2 2 2), (4 0 0)) were observed. These peaks basically consistent with the standard data for Fe_3O_4 structure (JCPDS.72-2303) which can be indexed as the spinel structure with a lattice parameter *a*=0.840nm. Fig. 5b showed XRD pattern of Fe_3O_4/CdTe magnetic QDs, the decreased intensity of Fe_3O_4 (311) was detected, this result indicated the formation of CdTe on surface of Fe_3O_4. It is also explained that the coating process did not result in the phase change of Fe_3O_4. The broad nature of the diffraction bands in the pattern below is an indication of small particle size. According to Scherrer's formula, the average crystallite size of the powder samples were calculated using the strongest phases of the diffraction plane (311) in Fig. 5, and the obtained particle size of Fe_3O_4 nanoparticles is 5.24 nm.

The TEM image of Fe_3O_4/CdTe nanoparticles is shown in Fig. 6. It demonstrates that the samples particle size is about 10 nm. There are three possible types of interactions among the nanoparticles: London-Van der Waalsforce, magnetic force, and the reacting force of the double electronic layer. The preceding two affinities among the particles must be counteracted by the later repulsive force to make the particles stable in the solvent. However, this kind of repulsive force is very small. Generally, to make the particles disperse well, the surface of nanoparticles should be modified to make them absorb one layer of polymer on the surface. Thus, the space hindrance repulsive force from the polymer can conquer the affinities between the particles. Meanwhile, the rim angle and the wetting property of the nanoparticles with other mediums can be improved by the surface modification of the particles, and then the dispersal property will be better. In our experiment, KH550 was used as the surfactant to make the Fe_3O_4 nanoparticles disperse well. The reason for adopting KH550 as dispersant agent is to take advantage of its absorption on the surface of Fe_3O_4 particles by dehydration reaction between hydroxyl. It forms a layer of molecular membrane to hinder inter-contact between the particles and reduce surface tension. Moreover, it also has an effect of steric hindrance to some extent. Therefore, the sample is a uniform distribution of spherical particles with no obvious aggregation.

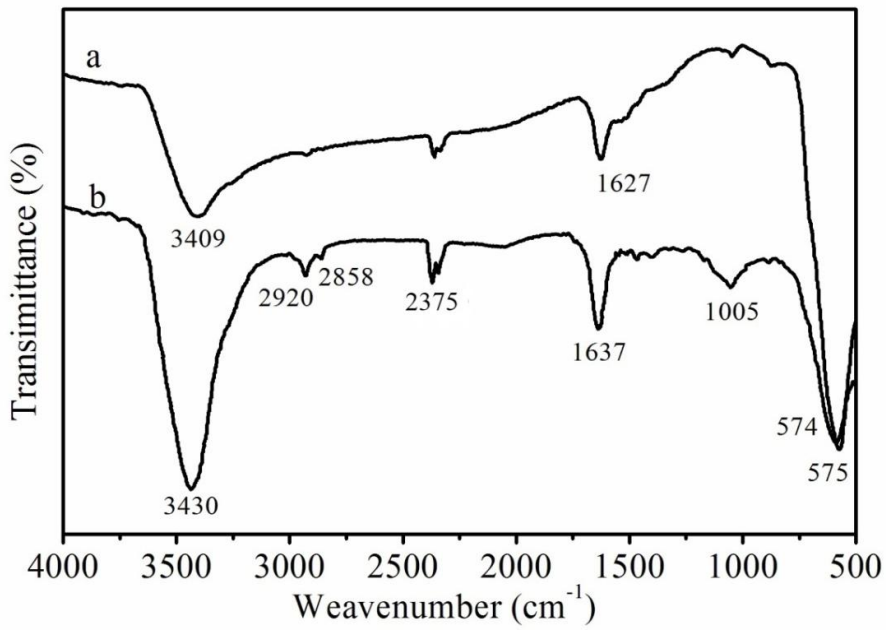

Fig. 3. FT-IR spectrum of the naked Fe_3O_4 (a) and Fe_3O_4-KH550 nanoparticles (b)

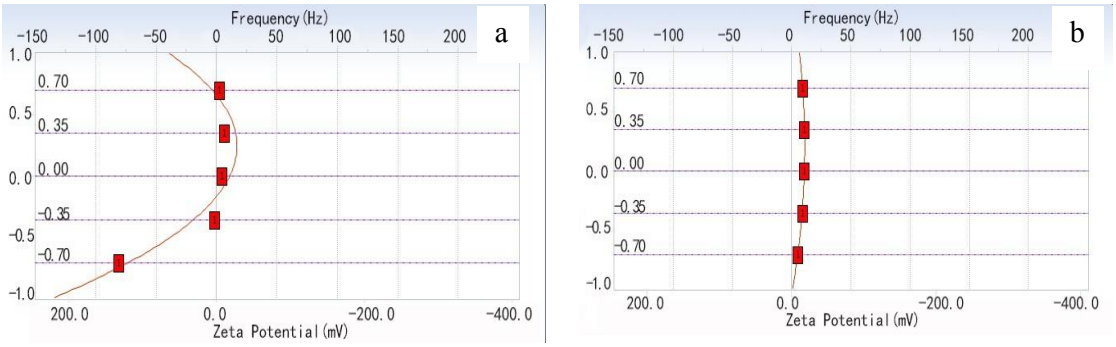

Fig. 4. Zeta-potential of the naked Fe_3O_4 (a) and Fe_3O_4-KH550 nanoparticles (b)

Characterization of Fe_3O_4/CdTe magnetic nanoparticles

Molecular beacons are single-stranded oligonucleotides that possess a stem-loop structure. The loop portion of the molecular beacons can recognize the specific complementary nucleic acid. The stem has five to seven base pairs which are complementary. A fluorophore and a quencher are linked to the two ends of the stem. In this conformation of the probe, the fluorophore is in close proximity of the quencher and thus the fluorescence emitted by the fluorophore in response to an excitation light is adsorbed by the quencher and the probe does not fluoresce. When the probe encounters a target DNA molecule, the molecular beacon undergoes a conformational change, and the fluorophore and quencher are separated by a distance equal to the stretch of nucleotide sequence between them, leading to the restoration of fluorescence (illustrated in Fig. 7. In our research, the Fe_3O_4/CdTe -magnetic DNA molecular beacon probe with Fe_3O_4/CdTe as the fluorophore and BHQ2 as the quencher, based on the principle of fluorescence resonance energy transfer (FRET).

In Fig. 8A. There is an obvious difference in the fluorescence enhancement when different DNA molecules hybridize with the molecular beacon: the BHO2-ssDNA-Fe_3O_4/CdTe sensing probe (a), Fe_3O_4/CdTe solution (b), hybridization with complement target DNA (c), and one-base mismatch DNA (d). The highest fluorescence signal was observed with the Fe_3O_4/CdTe solution alone because there was no quencher in

solution. After adding the target DNA into mixture, the complement ssDNA on the probe started to hybridize with them, and stem-loop structure became to line structure, the distance between BHQ2 and Fe_3O_4/CdTe exceeded Foster radius (10 nm) [25,26]. Therefore, the fluorescence intensity of line c is approximate to line b. When the mismatch DNA was added in to the solution (Fig. 8A (d)), the probe ssDNA didn't hybridize with them adequately, and fluorescence didn't recover to the original intensity.

3.3 Mechanism and Characterization of Molecular Beacons

Fig. 8B showed the linear relationship between fluorescence intensity change (ΔI_F) and target DNA concentrations (c). A linear regression equation, ΔI_F =1.805c+10.804, with a correlation coefficient R = 0.9833, can be obtained. The limit of detection (LOD) was calculated to 8.339 nM.

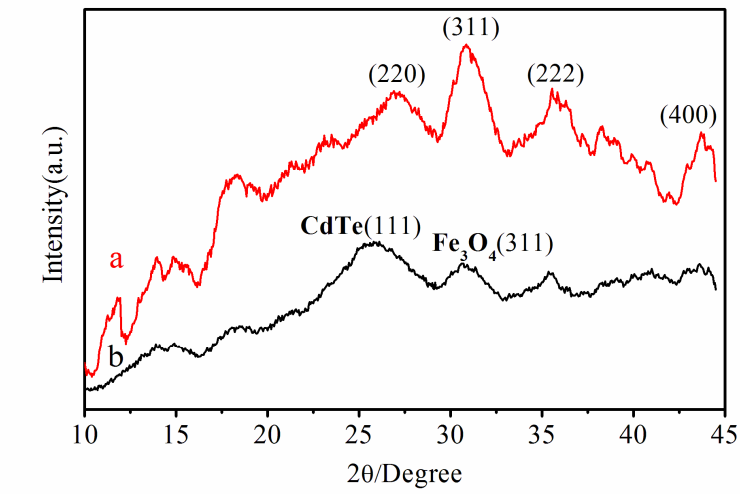

Fig. 5. XRD pattern for the naked Fe_3O_4 (a) and Fe_3O_4/CdTe nanoparticles (b)

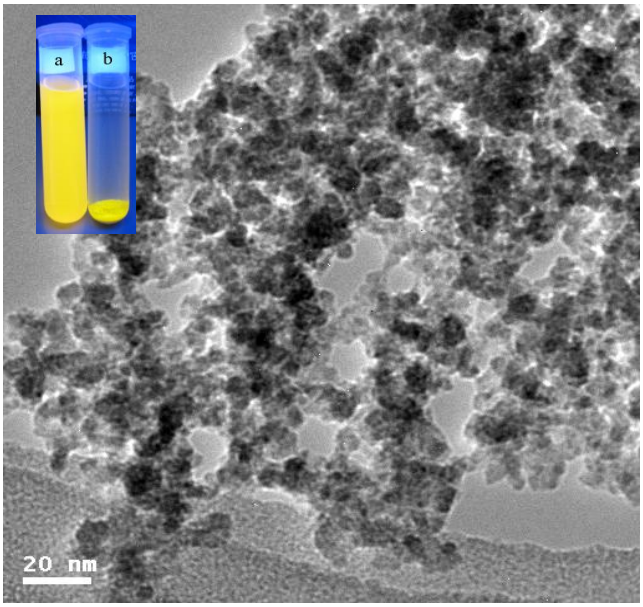

Fig. 6. TEM of Fe_3O_4/CdTe nanoparticles (Inset: a-Fe_3O_4/CdTe nanoparticles suspension; b-After magnet separating)

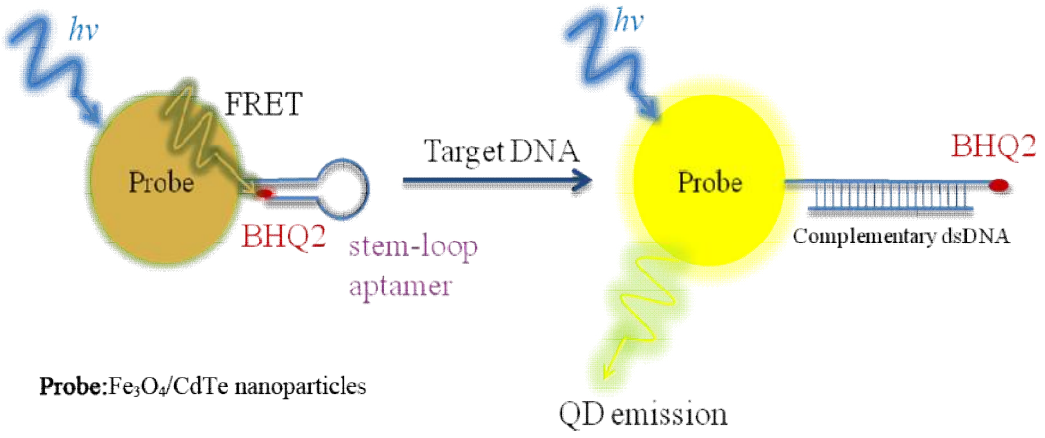

Fig. 7. Schematic illustration of the Fe_3O_4/CdTe molecular beacon and its detection mechanism

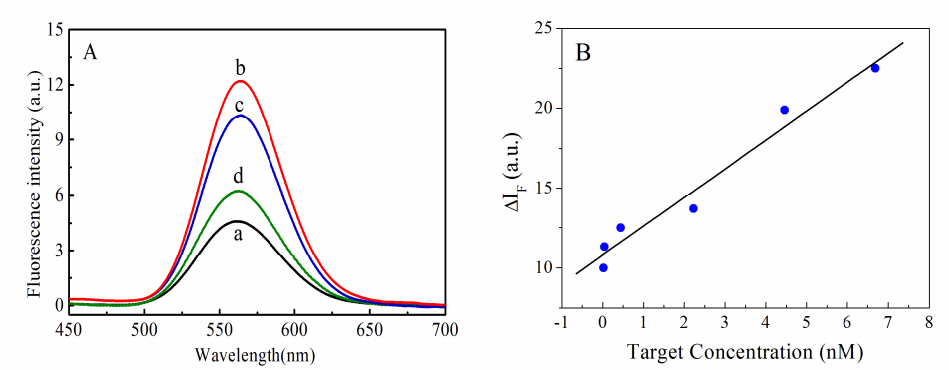

Fig. 8. Fluorescence spectrum Characterizations of prepared Fe_3O_4/CdTe sensor: specificity (A) and quantitative detection of target DNA (B)

4. CONCLUSION

In this paper, using the quenching of fluorescence of Fe_3O_4/CdTe, a novel method for the detection of specific DNA sequences related to *Toxoplasma gondii* has been fabricated based on FRET mechanism. In detail, MPA-capped CdTe QDs, of which the average size was 2.5 nm and the concentration was 1.724×10^{-5}M, supported by UV-Vis absorption, fluorescence spectroscopy and TEM images, bound with Fe_3O_4-KH550, which can be judged from IR spectrum, XRD pattern, and TEM image. Above all, fluorescence spectrum characterization of prepared Fe_3O_4/CdTe sensor showed that it has a good selectivity and sensitivity, a linear regression equation was obtained and LOD was confirmed to 8.339×10^{-9}M. The excellent capability of studying biological process in real time and *in vivo*, can avoid the inconvenience caused by using DNA intercalation reagents or by labeling the target molecules or using competitive assays.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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