

Original Research Article

Fluorescent Detection of specific DNA sequences related to *Toxoplasma gondii* based on Magnetic Fluorescent Nanoparticles Fe₃O₄/CdTe biosensor

Abstract: Magnetic fluorescent nanoparticles (Fe₃O₄/CdTe) were prepared in this work and applied for *Toxoplasma gondii* DNA detection. First, CdTe quantum dots were synthesized with 3-mercaptopropionic (MPA) capped. Fe₃O₄ 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 Fe₃O₄ particles surface via electrostatic interaction, and the Fe₃O₄/CdTe ~~core-shell~~ 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 Fe₃O₄/CdTe (donor) at the 5' end and BHQ2 (acceptor) at 3' end, respectively. The assembly process 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 8.339x10⁻⁹M. This chemical strategy can be further applied to prepare the ~~core-shell~~ magnetic nanoparticles for DNA detection.

Keywords: Fe₃O₄/CdTe nanoparticles; molecular beacon probe; Fluorescence resonance energy transfer; *Toxoplasma gondii* DNA detection

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 Fe₃O₄ nanoparticles has stimulated the development of synthesis technology such as organism or inorganism. However, the traditional synthesis route often need protecting gas (e.g. N₂), time consuming, and high temperature [1]. In this work, we adopted an aqueous-phase synthesis method to prepare high-quality Fe₃O₄ 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 Fe₃O₄ 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 $\text{Fe}_3\text{O}_4/\text{CdTe}$ 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 $\text{Fe}_3\text{O}_4@\text{CdSe}$ 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, 12, and 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, 16, and 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 $\text{Fe}_3\text{O}_4/\text{CdTe}$ conjugated to synthetic *Toxoplasma gondii* oligonucleotides that may revealed some excellent magnetic-optical properties. As the results, the $\text{Fe}_3\text{O}_4/\text{CdTe}$ 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.

Experimental

Materials

All the chemicals used in the current research are of analytical grade without further purification. Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), ferrous chloride ($\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$) 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.

Table 1. The DNA sequences in experiments

Nomenclature	Sequence
molecular beacon probe	5'-NH ₂ -(CH ₂) ₆ -AGCTATTATAAACTCGTTGGAT GCATAGCT-3'-BHQ2
mismatch sequence	5'-TGCATCCAATGAGTTTATAA-3
complementary sequences	5'-TGCATCCAACGAGTTTATAA-3

Methods

Preparation of Fe₃O₄/CdTe nanoparticles

Fe₃O₄ 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 50 °C for 30 min. The Fe₃O₄ nanoparticles were gained after The reaction mixture was separated with a common magnet and washed several times with water, finally dried in a vacuum oven at 70 °C.

CdTe QDs aqueous solution modified by 3-mercaptopropionic (MPA) was firstly synthesized as previously reported [21]. Magnetic nanoparticles Fe₃O₄ 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 Fe₃O₄ /CdTe magnetic QDs were obtained.

Construction of Fe₃O₄ /CdTe conjugated molecular beacon probe

50 μL of Fe₃O₄ /CdTe QDs aqueous solution, 20 μL of BHQ2 modified DNA and 10 mole times more than DNA of 1-ethyl-3-(dimethylaminopmpy1) carbodi-imide hydmmchl0ride (EDC) were mixed in 700 μL of 20 mM PBS(pH 8.0), and the Fe₃O₄ /CdTe conjugated molecular beacons were prepared after 24 h at ambient temperature. The Fe₃O₄ /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.

Hybridization of target DNA

100μL target DNA was added into sensing probe system containing Tris-HCl buffer. The hybridization was performed at 37 °C 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.

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 $\text{CuK}\alpha$ line (40kv, 50mA). All fluorescence spectrum were conducted on a WGY-380 fluorospectrophotometer (Tianjin Gangdong Scientific and Technical Development CO., Ltd, PRC).

Results and Discussion

Optical property and structural characterization of MPA-CdTe NPs

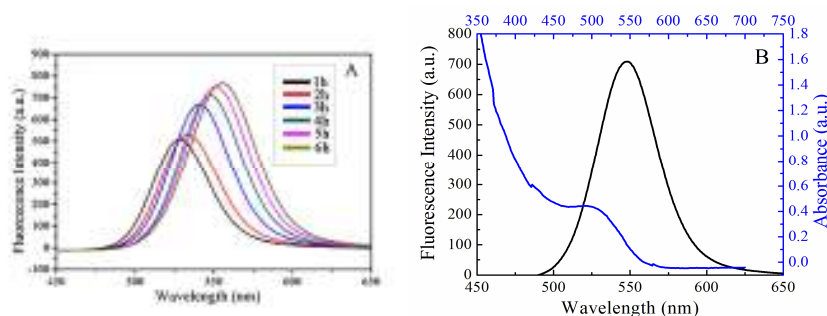


Fig. 1 Optical spectra of CdTe NPs with MPA as stabilizer :A- fluorescence spectra from 1h to 6h; B- UV (blue) and fluorescence spectra (black) of 4h-CdTe.

The fluorescence spectra shown in Figure.1 (A) 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 Figure.1 (B)) 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)).

$$D = (9.8127 \times 10^{-7})\lambda^3 - (1.7147 \times 10^{-3})\lambda^2 + 1.0064\lambda - 194.84 \quad (1)$$

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 $\varepsilon = 10043(D)^{2.12}$, 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.

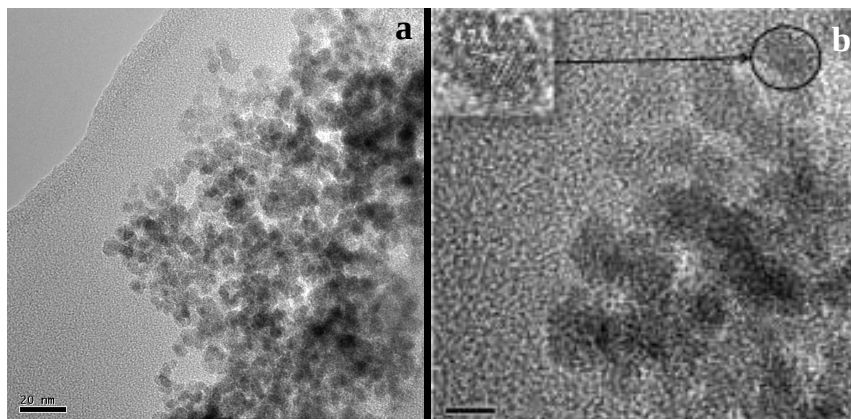


Fig.2 TEM (a) and corresponding HRTEM (b) images of MPA-CdTe nanoparticles.

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.

Characterization of Fe_3O_4 -KH550 magnetic nanoparticles

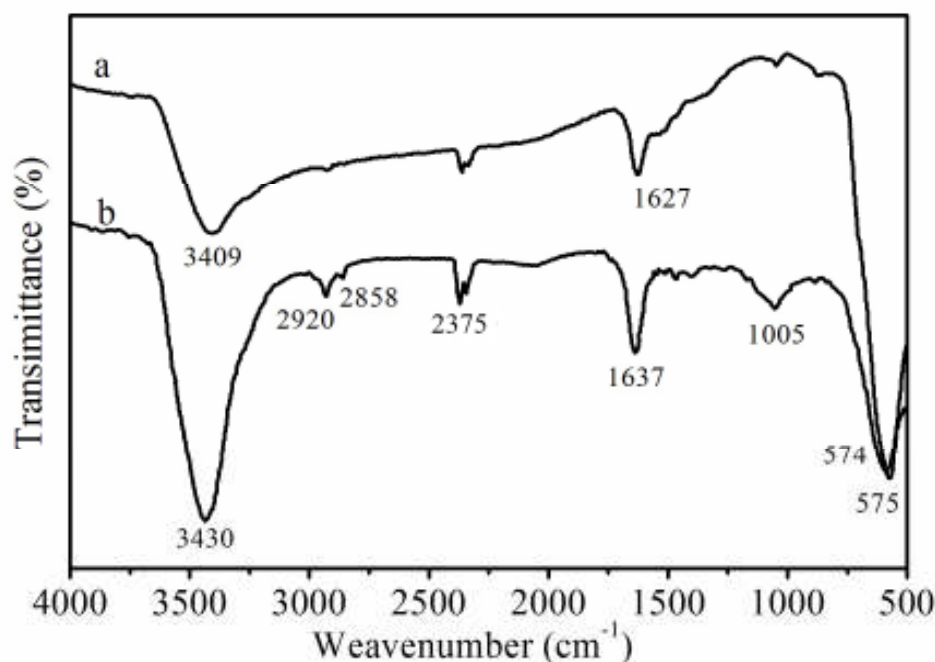


Fig.3 FT-IR spectrum of the naked Fe_3O_4 (a) and Fe_3O_4 -KH550 nanoparticles (b)

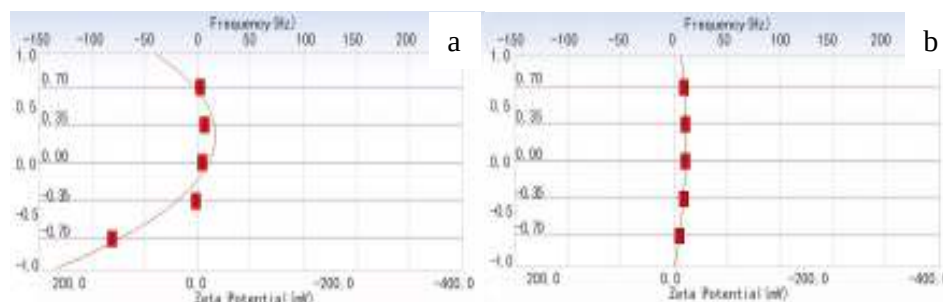


Fig.4 Zeta-potential of the naked Fe_3O_4 (a) and Fe_3O_4 -KH550 nanoparticles (b)

The IR spectrum of naked Fe_3O_4 and Fe_3O_4 -KH550 nanoparticles is shown in Fig. 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 $(\text{CH}_2)_n$ 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.

Characterization of $\text{Fe}_3\text{O}_4/\text{CdTe}$ magnetic nanoparticles

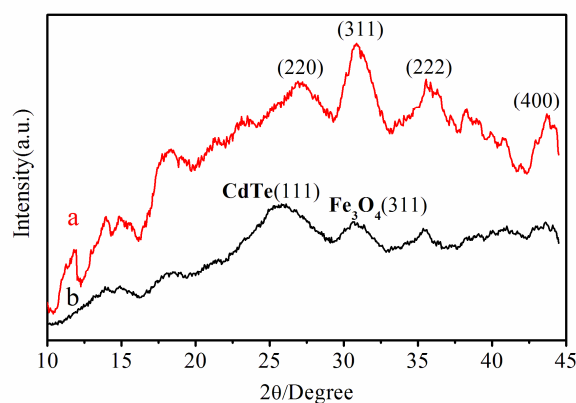
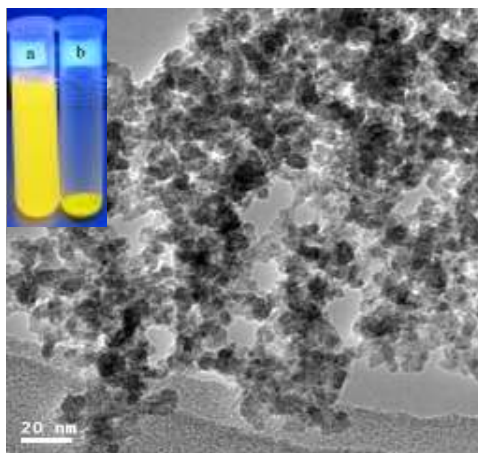


Fig.5 XRD pattern for the naked Fe_3O_4 (a) and $\text{Fe}_3\text{O}_4/\text{CdTe}$ nanoparticles (b)

XRD is another important method for the surface structure studies. In our experiment, XRD is used to analysis the structure of Fe_3O_4 and confirm whether the $\text{Fe}_3\text{O}_4/\text{CdTe}$ nanoparticles had been synthesized. Fig. 5 shows the XRD patterns for the naked Fe_3O_4 and $\text{Fe}_3\text{O}_4/\text{CdTe}$ 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 $a=0.840\text{nm}$. Fig.5b showed XRD pattern of $\text{Fe}_3\text{O}_4/\text{CdTe}$ magnetic QDs, the decreased intensity of Fe_3O_4 (311) was detected, this result indicated the formation of CdTe shell on surface of Fe_3O_4 core. 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. It is

218 interesting to note that the Fe_3O_4 nanoparticles calculated from the XRD pattern is
219 5.24nm, which is considerably smaller than that determined from the TEM images
220 (Fig. 6) suggesting that the spherical Fe_3O_4 particles may contain a number of
221 ultra-small crystals.



222
223 Fig.6 TEM of $\text{Fe}_3\text{O}_4/\text{CdTe}$ nanoparticles (Inset: a- $\text{Fe}_3\text{O}_4/\text{CdTe}$ nanoparticles
224 suspension; b- After magnet separating)
225

226 The TEM image of $\text{Fe}_3\text{O}_4/\text{CdTe}$ nanoparticles is shown in Fig.6. It demonstrates
227 that the samples particle size is about 10 nm. There are three possible types of
228 interactions among the nanoparticles: London-Van der Waalsforce, magnetic force,
229 and the reacting force of the double electronic layer. The preceding two affinities
230 among the particles must be counteracted by the later repulsive force to make the
231 particles stable in the solvent. However, this kind of repulsive force is very small.
232 Generally, to make the particles disperse well, the surface of nanoparticles should be
233 modified to make them absorb one layer of polymer on the surface. Thus, the space
234 hindrance repulsive force from the polymer can conquer the affinities between the
235 particles. Meanwhile, the rim angle and the wetting property of the nanoparticles with
236 other mediums can be improved by the surface modification of the particles, and then
237 the dispersal property will be better. In our experiment, KH550 was used as the
238 surfactant to make the Fe_3O_4 nanoparticles disperse well. The reason for adopting
239 KH550 as dispersant agent is to take advantage of its absorption on the surface of
240 Fe_3O_4 particles by dehydration reaction between hydroxyl. It forms a layer of
241 molecular membrane to hinder inter-contact between the particles and reduce surface
242 tension. Moreover, it also has an effect of steric hindrance to some extent. Therefore,
243 the sample is a uniform distribution of spherical particles with no obvious
244 aggregation.

245
246 ***Mechanism and Characterization of molecular beacons***

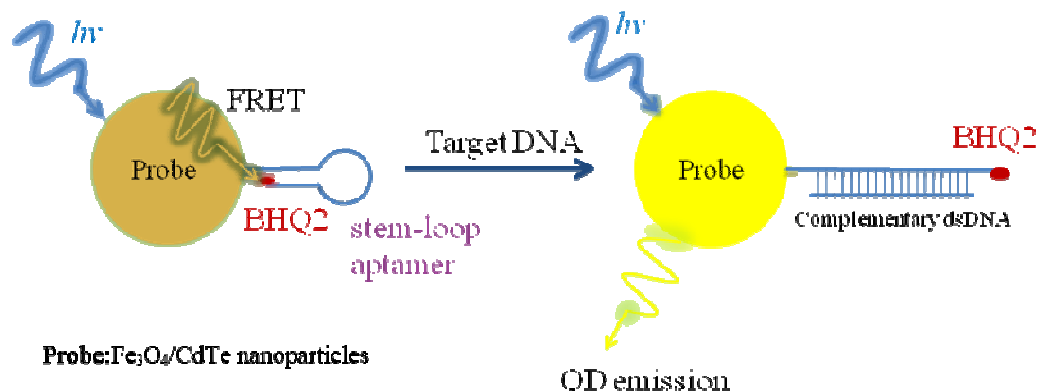


Figure.7 Schematic illustration of the $\text{Fe}_3\text{O}_4/\text{CdTe}$ molecular beacon and its detection mechanism.

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 $\text{Fe}_3\text{O}_4/\text{CdTe}$ -magnetic DNA molecular beacon probe with $\text{Fe}_3\text{O}_4/\text{CdTe}$ as the fluorophore and BHQ2 as the quencher, based on the principle of fluorescence resonance energy transfer (FRET).

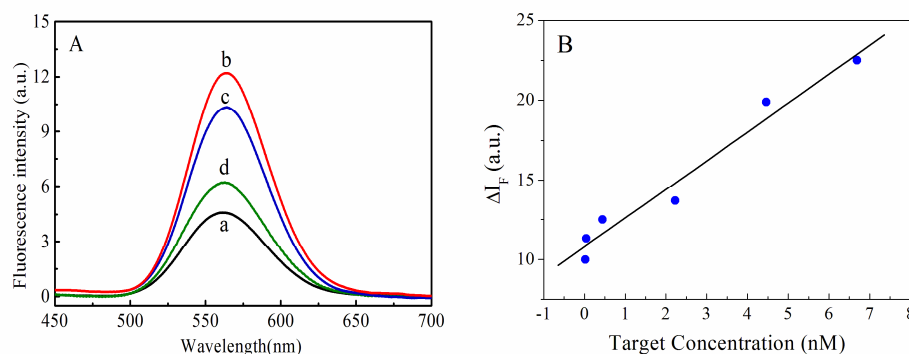


Fig. 8 Fluorescence spectrum Characterizations of prepared $\text{Fe}_3\text{O}_4/\text{CdTe}$ sensor: specificity (A) and quantitative detection of target DNA (B).

The characterization of specificity of prepared sensing probe was investigated by the fluorescence spectrum In Fig.8A. There is an obvious difference in the fluorescence enhancement when different DNA molecules hybridize with the

molecular beacon: the BHO2-ssDNA-Fe₃O₄/CdTe sensing probe (a), Fe₃O₄/CdTe solution(b), hybridization with complement target DNA (c), and one-base mismatch DNA (d). The highest fluorescence signal was observed with the Fe₃O₄/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 Fe₃O₄/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.

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

Conclusion

In this paper, using the quenching of fluorescence of Fe₃O₄/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 $1.724 \times 10^{-5} \text{M}$, supported by UV-Vis absorption, fluorescence spectroscopy and TEM images, bound with Fe₃O₄-KH550, which can be judged from IR spectrum, XRD pattern, and TEM image. Above all, fluorescence spectrum characterization of prepared Fe₃O₄/CdTe sensor showed that it has a good selectivity and sensitivity, a linear regression equation was obtained and LOD was confirmed to $8.339 \times 10^{-9} \text{M}$. 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.

References

1. Huimin Cao, Juchuan Li, Yuhua Shen, Shikuo Li, Fangzhi Huang, Anjian Xie. Green synthesis and surface properties of Fe₃O₄@SA core-shell nanocomposites. Applied Surface Science, 301, 244–249, 2014.
2. Chung-Shieh Wu, Maung Kyaw Khaing Oo, and Xudong Fan. Highly Sensitive Multiplexed Heavy Metal Detection Using Quantum-Dot-Labeled DNAzymes. ACS NANO, 4(10), 5897-5904, 2010.
3. Rolf Koole, Willem J. M. Mulder, Matti M. van Schooneveld, Gustav J. Strijkers, Andries Meijerink and Klaas Nicolay. Magnetic quantum dots for multimodal imaging. WIREs Nanomed Nanobiotechnol, 1, 475-491, 2009.
4. Zhengtao Deng, Yun Zhang, Jiachang Yue, Fangqiong Tang, and Qun Wei. Green and Orange CdTe Quantum Dots as Effective pH-Sensitive Fluorescent Probes for Dual Simultaneous and Independent Detection of Viruses. J. Phys. Chem. B, 111, 12024-12031, 2007.

- 315 5. Liang Li, Huifeng Qian, Nenghu Fang, Jicun Ren. Significant enhancement of the
316 quantum yield of CdTe nanocrystals synthesized in aqueous phase by controlling
317 the pH and concentrations of precursor solutions. *Journal of Luminescence*, 116,
318 59–66, 2006.
- 319 6. Narayan Pradhan, David Goorskey, Jason Thessing, and Xiaogang Peng. An
320 Alternative of CdSe Nanocrystal Emitters: Pure and Tunable Impurity Emissions in
321 ZnSe Nanocrystals. *J. AM. CHEM. SOC.* 127, 17586 -17587, 2005.
- 322 7. Guannan Wang, Xingguang Su, Shijing Yang, Yunhong Jia, Donghui Li. The
323 double-effect mechanism between Fe₃O₄ nanoparticles and MSA-capped CdTe
324 QDs. *Journal of Luminescence*, 132, 2505–2511, 2012.
- 325 8. Xiuling Wang, Lu Wei, Guan hong Tao, Mengqiong Huang. Synthesis and
326 characterization of magnetic and luminescent Fe₃O₄/CdTe nanocomposites using
327 aspartic acid as linker. *Chinese Chemical Letters*, 22 (2), 233-236, 2011.
- 328 9. Guifen Jie and Jinxin Yuan. Novel Magnetic Fe₃O₄@CdSe Composite Quantum
329 Dot-Based Electrochemiluminescence Detection of Thrombin by a Multiple DNA
330 Cycle Amplification Strategy. *Anal. Chem.*, 84, 2811– 2817, 2012.
- 331 10. Xianmei Lan, Xuebo Cao, Wenhui Qian, Weijian Gao, Cui Zhao, Yang Guo. Long
332 Fe₃O₄ nanowires decorated by CdTe quantum dots: Synthesis and magnetic–optical
333 properties. *Journal of Solid State Chemistry*, 180, 2340–2345, 2007.
- 334 11. Chun-Wei Chi, Yeh-Hsing Lao, Yi-Shan Li, Lin-Chi Chen. A quantum
335 dot-aptamer beacon using a DNA intercalating dye as the FRET reporter:
336 Application to label-free thrombin detection. *Biosensors and Bioelectronics*, 26,
337 3346–3352, 2011.
- 338 12. Dong-shan Xiang, Guo-ping Zeng, Zhi-ke He. Magnetic microparticle-based
339 multiplexed DNA detection with biobarcode quantum dot probes. *Biosensors and*
340 *Bioelectronics*, 26, 4405– 4410, 2011.
- 341 13. W. Russ Algar and Ulrich J. Krull. Multiplexed Interfacial Transduction of
342 Nucleic Acid Hybridization Using a Single Color of Immobilized Quantum Dot
343 Donor and Two Acceptors in Fluorescence Resonance Energy Transfer. *Anal.*
344 *Chem.*, 82, 400–405, 2010.
- 345 14. Xi-Peng Liu, Jing-Li Hou, Jian-hua Liu. A novel single nucleotide polymorphism
346 detection of a double-stranded DNA target by a ribonucleotide-carrying molecular
347 beacon and thermostable RNase HII. *Analytical Biochemistry*, 398(1), 83-92, 2010.
- 348 15. W. Russ Algar, Anthony J. Tavares, Ulrich J. Krull. Beyond labels: A review of the
349 application of quantum dots as integrated components of assays, bioprobes, and
350 biosensors utilizing optical transduction. *Analytica Chimica Acta*, 673, 1–25, 2010.
- 351 16. Nathaniel C. Cady, Aaron D. Strickland, Carl A. Batt. Optimized linkage and
352 quenching strategies for quantum dot molecular beacons. *Molecular and Cellular*
353 *Probes*, 21, 116 – 124, 2007.
- 354 17. Kim E. Sapsford, Thomas Pons, Igor L. Medintz, and Hedi Mattoussi. Biosensing
355 with Luminescent Semiconductor Quantum Dots. *Sensors*, 6, 925-953, 2006.
- 356 18. James J. Storhoff, Robert Elghanian, Robert C. Mucic, Chad A. Mirkin, and
357 Robert L. Letsinger. One-Pot Colorimetric Differentiation of Polynucleotides with
358 Single Base Imperfections Using Gold Nanoparticle Probes. *J. Am. Chem. Soc.*,

- 120, 1959-1964, 1998.
19. M.Y. Han, X.H. Gao, J.Z. Su, S. Nie. Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. *Nature Biotechnology*, 19(7), 631-635, 2001.
20. Emily J. Aston, Pedro Mayor, Dwight D. Bowman, Hussni O. Mohammed, Janice L. Liotta, Oliver Kwok, J.P. Dubey. Use of filter papers to determine seroprevalence of *Toxoplasma gondii* among hunted ungulates in remote Peruvian Amazon, *International Journal for Parasitology: Parasites and Wildlife*, 3, 15–19, 2014.
21. Shichao Xu, Jimei Zhang, Caixia Liu, and Xiuyun Song. Synthesis and Characterization of Water Soluble CdTe and CdTe/CdS Core-shell Quantum Dots for Biosensing Applications. *Proc. of SPIE*, 6423, 64235F-64235K, 2007.
22. Wenzhuo Guo, J. Jack Li, Y. Andrew Wang, and Xiaogang Peng. Luminescent CdSe/CdS Core/Shell Nanocrystals in Dendron Boxes: Superior Chemical, Photochemical and Thermal Stability. *J. AM. CHEM. SOC.*, 125, 3901 -3909, 2003.
23. JianHua Meng, GuiQin Yang, LeMei Yan, XiuYu Wang. Synthesis and characterization of magnetic nanometer pigment Fe₃O₄. *Dyes and Pigments*, 66, 109-113, 2005.
24. Liese H.C. An infrared absorption analysis of magnetite. *Am Mineralogist*, 52, 1198–1205, 1967.
25. S. Tyagi , F. R. Kramer. Molecular beacons: probes that fluoresce upon hybridization. *Nature Biotechnology*, 14, 303-308, 1996.
26. S. Tyagi, D. Bratu, F. R. Kramer. Multicolor molecular beacons for allele discrimination. *Nature Biotechnology*, 16, 49-53, 1998.