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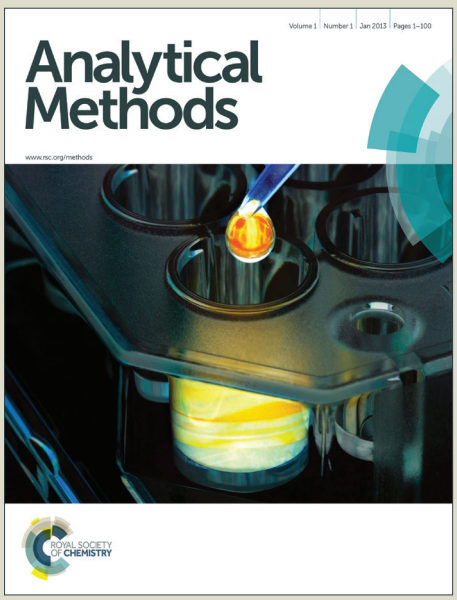

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High-sensitive aptasensor for oxytetracycline based on upconversion and magnetic nanoparticles

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Abstract

A novel sensitive aptasensor was developed for the quantification of oxytetracycline (OTC) in this study. Artificial aptamer-modified magnetic nanoparticles (aptamer-MNPs) were employed as capture probes, and complementary oligonucleotides modified upconversion nanoparticles (cDNA-UCNPs) were used as signal probes. Then, the probes were hybridized to form the poly-network structure MNPs-UCNPs signal probes. Finally, when the target was introduced, the aptamer combine with the target in priority and the signal probe was replaced. The proposed method achieved a linear range between 0.05 to 100 ng mL^{-1}, and the limits of detection (LOD) was as low as 0.036 ng mL^{-1}, benefiting largely from UCNPs labeling, aptamer affinity and magnetic separation. Then, we successfully applied the method to measure OTC in milk samples and validated it by a commercially available enzyme-linked immunosorbent assay (ELISA) method. The results demonstrated that the method possessed great sensitivity and good selectivity for the determination of OTC and is applicable to the determination of OTC in food samples.

Keywords: Aptamer; Oxytetracycline; Magnetic Nanoparticles; Upconversion Nanoparticles

1 Introduction

With a unique upconversion mechanism that enables the conversion of low-energy photons (near infrared photons) into high-energy photons (visible to ultraviolet photons) via multiphoton processes, lanthanide doped upconversion nanoparticles (UCNPs) have attracted enormous attention in the recent years [1-3]. Upconversion nanoparticles possess tremendous advantages in biological applications over other types of fluorescent materials (e.g., organic dyes, fluorescent proteins, gold nanoparticles, quantum dots, and luminescent transition metal complexes) [4], including low toxicity, large Stokes shifts, high quantum yields, high resistance to photo bleaching, blinking, photochemical stability and the lack of both auto-luminescence and a light scattering background, which consequently results in detection with high sensitivity and signal-to-noise ratio [5-10]. Meanwhile, magnetic nanoparticles (MNPs) with good biocompatibility and rapid separation from the substrate solution have been extensively implemented in the fields of biological detection [11-13].

Tetracyclines (TCs) are broad-spectrum antibiotics that include oxytetracycline, which is likely the most widely used antibacterial in aquaculture [14]. The presence of tetracycline residue especially oxytetracycline (OTC) from animal-derived foods poses a great risk towards human health, such as allergic reactions, toxic effects and the

development of resistance of microorganisms to antibiotics [15, 16]. Therefore, researchers have made significant efforts to develop methods to identify and quantify OTC, such as HPLC–DAD and LC–MS/MS [17, 18]. Though sensitive and accurate, these methods demand expensive equipment, tedious sample extraction procedures and technical skills [19]. Similarly, with wide range of application and low testing costs, immunochemical methods using antibody usually lack specificity and sensitivity due to the high similarity in structure of tetracycline derivatives. In addition, the methods are unstable because the antibody is sensitive to pH, temperature and other physicochemical environments in biological samples [20, 21]. Therefore, it is still in demand to develop sensitive and specific methods to detect OTC.

Aptamers are DNA or RNA molecules, which can adopt specific three-dimensional conformations to combine with target analytes. Compared with antibodies, aptamers are more stable, are easier to synthesize, can be modified in bulk and have many other advantages [22, 23]. To the author's knowledge, a series of aptasensors for OTC have been developed [24, 30]. Though the reported electrochemical, colorimetric light scattering and microcantilever methods should be highly sensitive, they are of limited utility because of either high background signal or instability in biological samples due to the inherent characteristics of the nanoparticles used, and can be difficult to utilize for on-site detection

because of the tedious immobilization steps of the aptamers [31]. Therefore, it is meaningful to overcome the above limitations and to search for a simple and sensitive aptamer-based detection method for OTC.

Herein, we present an aptasensor for OTC based on upconversion and magnetic nanoparticles, possessing excellent sensitivity and selectivity, lacking in interference from auto-luminescence of other biomolecules. Artificial aptamer-modified magnetic nanoparticles (aptamer-MNPs) were employed as capture probes, and complementary strand-modified upconversion nanoparticles (cDNA-UCNPs) were used as signal probes. Then, the probes were hybridized to form the poly-network structure MNPs-UCNPs signal probes. Finally, when the target was introduced, the aptamer combined with the target in priority and the signal probe was replaced. Thus, a novel analytical method has been successfully applied to the detection of OTC relying on UCNP labeling, aptamer selectivity and magnetic separation. Additionally, the detection limit was as low as 0.036 ng·mL^{-1}. To our knowledge, this is the first report to detect OTC using UCNPs and aptamer. Furthermore, as UNCPs can be doped with lanthanide ions, such as Er3+, Ho3+ and Tm3+, this proposed method has great potential in the detection of structurally similar tetracycline derivatives based on multicolor UCNPs.

2 Experiments

2.1 Materials and chemicals

The rare earth chloride and nitrates used in this work, including $YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$, $ErCl_3 \cdot 6H_2O$, Y $(NO_3)_3 \cdot 6H_2O$, Yb $(NO_3)_3 \cdot 5H_2O$, and Er $(NO_3)_3 \cdot 5H_2O$, were of 99.99% purity and were purchased from Aladdin Industrial Inc. (Shanghai, China). Oleic acid, octadecene (ODE), cyclohexane, 25% ammonia, 25% glutaraldehyde ($OHC(CH_2)_3CHO$), Iron trichloride ($FeCl_3 \cdot 6H_2O$) ,tetraethyl orthosilicate (TEOS) , 1,6-hexanediamine and ethanol were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd.(Shanghai, China). IGEPAL CO-520, 98% 3-aminopropyltrimethoxysilane (APTES) was purchased from Alfa Assar (USA). OTC aptamer (reported by Javed H. Niazi. et al. [22]) and its partially complementary strand were synthesized by Shanghai Sangon Biological Science & Technology Company (Shanghai, China). The sequence of the OTA aptamer was 5'-NH_2-GGAATTCGCTAGCACGTTGACGCTGGTGCCCGGTTGTGG TGCGAGTGTTGTGTGGATCCGAGCTCCACGTG-3'(aptamer), the sequence of its partially complementary strand was 5'-NH_2-CGGATCCACACAACA-3'(cDNA). Oxytetracycline (OTC), tetracycline (TET), and doxycycline (DOX) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China).

2.2 Apparatus

The size and morphology of nanoparticles were observed on a JEM-2100HR transmission electron microscope (TEM, JEOL Ltd.,

Japan), using a 200 kV accelerating voltage 200 kV. X-ray diffraction (XRD) measurements were performed using a D8-advance instrument (Bruker AXS Ltd., Germany) with graphite-mono-chromatized Cu-Kα radiation (λ=0.15406 nm). The luminescence spectra of UCNPs were measured on an F-7000 luminescence spectrophotometer (Hitachi Co., Japan) attached to an external 980 nm laser (Beijing Hi-Tech Optoelectronic Co., China) instead of the internal excitation source. The maximum power of the laser was 1300 mW. FTIR spectra of the amino-modified NPs were measured with a Nicolet Nexus 470 Fourier transform infrared spectrophotometer (Thermo Electron Co., USA) using the KBr method. Ultraviolet-visible (UV-vis) absorption spectra were recorded using a Shimadzu UV-2300 UV-vis spectrophotometer (Shimadzu, Japan) and concentration of oligonucleotides was measured using the One Drop OD-1000 Spectrophotometer (OneDrop Technologies, Inc., USA).

2.3 Synthesis and surface modification of rare-earth-doped $NaYF_4$: Yb, Er upconversion nanoparticles

$NaYF_4$: 18% Yb, 2% Er UCNPs were synthesized by method described by Zhengquan Li and Yong Zhang with some modifications [32]. All of the doping ratios of Ln^{3+} ions are molar in our experiments. Briefly, YCl_3, $YbCl_3$ and $ErCl_3$ were mixed with 6 mL of oleic acid and 17 mL of octadecene in a 50 mL flask and heated to 160 °C to form a homogeneous

solution, and then cooled to room temperature. A 10 mL methanol solution containing NaOH and NH_4F was slowly added into the flask. The solution was stirred for 30 min, and the temperature was raised to evaporate the methanol; then, the solution was degassed at 100 °C for 10 min, before being heated to 300 °C and maintained for 1 h under argon atmosphere. After the solution was cooled, nanocrystals were precipitated from the solution with ethanol and washed with ethanol/water (1:1 v/v) three times.

Surface modification of $NaYF_4$: Yb, Er UCNPs was completed using a microemulsion method to cap silica onto the surface of the UCNPs [33]. Then, 500 µl of CO-520 and 10 ml of cyclohexane containing $NaYF_4$ nanospheres were mixed and stirred for 10 min, then ammonia was added and the solution was sonicated for 20 min until a transparent emulsion was formed. TEOS and APTES were added to the solution, and the solution was rotated for two days at a speed of 600 rpm. Silica/$NaYF_4$ nanospheres were precipitated by adding acetone, and the nanospheres were washed with ethanol/water.

2.4 Preparation of amino-modified Fe_3O_4 magnetic nanoparticles (MNPs)

To prepare MNPs, a one-pot synthetic method was adopted [34]. For ~25 nm magnetic nanoparticles, a solution of 1, 6-hexanediamine (6.5 g),

anhydrous sodium acetate (2.0 g) and $FeCl_3 \cdot 6H_2O$ (1.0 g) as a ferric source in glycol (30 mL) was stirred vigorously to give a transparent solution. This solution was then transferred into a Teflon-lined autoclave and reacted at 198 °C for 6 h. The magnetic nanoparticles were then rinsed with water and ethanol (2 or 3 times) to effectively remove the solvent and unbound 1, 6-hexanediamine and then dried before characterization and application. During each rinsing step, the nanoparticles were separated from the supernatant by using magnetic force.

2.5 Preparation of signal probes and capture probes

The procedure for the preparation of oligonucleotides conjugated MNPs and UCNPs was adapted from the classical glutaraldehyde method [35]. 10 mg of the amino-modified nanoparticles was dispersed in 5 mL of phosphate buffer solution (PBS) at pH 7.4 by ultrasonication for 15 min, and then 0.2 mL of 25% glutaraldehyde was added into the mixture. The mixture was shaken slowly on a shaking table at room temperature for 2 h. After incubation, the MNPs were separated with an external magnetic field and the UCNPs were separated by centrifugation. The nanoparticles were washed with phosphate buffer solution three times and dispersed in 5 mL of PBS buffer solution.

Then, amino-modified aptamer was subsequently added to the glutaraldehyde modified MNPs and amino-modified cDNA was added to

the glutaraldehyde modified UCNPs. The mixture was then incubated at 37°C for another 2 h. After removal of the supernatant and washing, the resulting solution was resuspended in fresh STE buffer (10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA) and stored at 4 °C.

2.6 Procedure for the detection of OTC

A total of 100 μL of aptamers-MNPs was hybridized in STE buffer with the optimized cDNA-UCNPs at 37 °C for 2 h to obtain UCNPs-MNPs signal probes. The UCNPs-MNPs probes were separated by a magnetic field and were resuspended in PBS buffer. Then various concentrations of OTC standard were added to the mixture and further incubated at 37 °C for 2 h. The remaining UCNPs-MNPs were then separated and washed three times, and the luminescence intensity was measured with a 980 nm excitation laser.

2.7 Method comparison with ELISA analysis in food samples and recovery experiment

The accuracy of OTC detection in food samples was evaluated by determining the recovery of OTC (Recovery ratio = (Detected Concentration - Background Content) / Added Concentration). Briefly, a series of known quantities of OTC standard were added into the milk samples. And the milk samples were pretreated with centrifugation separation (10 °C, 3500 $r \cdot min^{-1}$) for 10 min. Next, the supernatant was collected and diluted with PBS buffer proportionately (1:10 v/v). Finally,

the OTA content of the resulting solution was measured with the developed aptasensor by the above mentioned procedure. Furthermore, the commercially available ELISA method was also applied to detect the OTC in the same milk samples.

3 Results and discussion

3.1 Detection principle

The luminescence bioassay platform for the detection of OTC based on upconversion and magnetic nanoparticles was illustrated in Fig. 1. Specifically, amino-modified MNPs were conjugated with amino-modified aptamer via the classical glutaraldehyde method [35] to form the capture probes (aptamer-MNPs), and amino-modified NaYF4: Yb, Er UCNPs were conjugated with amino-modified cDNA to form the signal probes (cDNA-UCNPs). Then, the probes were hybridized to form the MNPs-UCNPs signal probes.

After magnetic separation with an external magnet, the intensity of the emission peak at 544 nm was at a maximum in the absence of OTC, because of the abundance of the MNPs-UCNPs signal probes. Subsequently, OTC was added to the system, and aptamer preferentially bound to OTC caused the dissociation of some cDNA, thereby liberating some cDNA-UCNPs. Finally, the intensity of the emission peak at 544 nm decreased as a result of the reduced concentration of MNPs-UCNPs signal probes.

3.2 Design of complementary DNA sequences

In this study, we experiment with a sequence of the partially complementary strand of the aptamer 5'-NH2-CGGATCCACACAACA-3'. The basic principle of the cDNA design was that aptamers could form a defined conformation when conjugating to the targets and were also able to hybridize to the cDNA to form a duplex structure [36-37]. While designing oligonucleotides strand of cDNA, proper care was taken to avoid excessive adhesive strength with the aptamer. When the targets and the complementary oligonucleotides were introduced, the aptamers preferentially bound to the targets, resulting in the specific recognition of the targets [38-39].

3.3 Preparation and characterization of UCNPs

The UCNPs were synthesized via a solvothermal method using chloride. Transmission electron microscopy (TEM) images of the nanocrystals in Fig. 2 (a-b) show the size and morphology of the oleic acid-capped UCNPs. The NPs were then coated by the microemulsion method and characterized by the TEM at the same time.

In this work, uniform $NaYF_4$ nanospheres with strong upconversion luminescence were produced, with uniform silica coating on the surface. The microemulsion method has been one of the most commonly used methods for coating silica on nanocrystals. However, it is quite challenging to coat individual nanoparticles with very thin shells. Thus,

we have optimized the microemulsion method by adding different proportions of UCNPs and TEOS, and finally resulted in the thickness of silica shell with approximately 2 nm (Fig. 2 c-d). By shortening the reaction time and increasing the UCNPs concentration, it is possible to create somewhat thinner layers of silica. After silica coating, the nanocrystals were dispersible in water and demonstrated good chemical and photochemical stability. Fig. 3 shows the luminescence intensity is only slightly reduced with the thin silica shell on the surface.

$NaYF_4$: Yb, Er nanocrystals have been shown to be the best NIR-to-visible upconversion luminescence materials and hexagonal-phase nanocrystals have higher upconversion efficiency than cubic-phase nanocrystals [32]. Fig. 4 gives the XRD patterns of $NaYF_4$: Yb, Er UCNPs, revealing the prepared UCNPs are pure hexagonal-phase (JCPDS no. 16-0334).

In addition to fabricating thin and uniform silica shells, the UCNPs were furthermore functionalized with APTES to form an amino-terminated surface. The functional groups on the surfaces of the amino-modified NaYF4: Yb, Er UCNPs were identified by FT-IR spectra in Fig. 5. The wide absorption peak at 3412 cm^{-1} in Fig. 5 a is the stretching vibration of the hydroxyl group. Two peaks found at 1560 and 1458 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of the carboxylic group (COO–). In addition, bands at 2921

and 2853 cm^{-1} are assigned to the asymmetric and symmetric stretching vibrations of the methylene group, respectively, caused by the long chain alkyl from oleic acid molecules. The results verified the UCNPs before modifying were coated by oleic acid molecules. In contrast, the peaks mentioned above disappeared together in Fig. 5 b, and the characteristic peaks corresponding to amino groups appeared instead. The hydroxyl stretching vibration band of a silanol group (Si–OH) appears in the region approximately 3402 cm^{-1}, and the band at 1068 cm^{-1} is attributed to the stretching vibration of the Si–O bond. The spectrum at 1625 cm^{-1} is the stretching and bending vibration bands of the amino group indicating that the silica-coated UCNPs have been successfully functionalized with amino groups.

3.4 Preparation and characterization of MNPs

The amino-modified Fe_3O_4 MNPs applied here were prepared by a one-pot synthesis. TEM (Fig. 6 a) and FT-IR (Fig. 6 b) are used to characterize the synthesized MNPs. It can be observed that the MNPs have an average size of approximately 25 nm. FT-IR spectroscopy shows a strong IR band at 583 cm^{-1}, which is characteristic of the Fe–O vibrations. The transmissions at approximately 1634, 1396, and 1054 cm^{-1} from the amino-modified nanoparticles matched well with those from free 1,6-hexanediamine, indicating the existence of the free $–NH_2$ group on the amino-modified nanomaterials. The results from FT-IR

revealed that the MNPs have been functionalized with amino groups in the synthetic process.

3.5 Characterization of amino-modified nanoparticles conjugated with amino-modified oligonucleotides

We applied UV-vis spectrophotometry to validate successfully coupling of amino-modified oligonucleotides to amino-modified nanoparticles. As is shown in Fig. 7, the strong absorbance of oligonucleotides can be seen at 260 nm before conjugation to nanoparticles. After incubation of amino-modified nanoparticles and amino-modified oligonucleotides, the supernatant was collected by magnetic separation and centrifugation. The absorbance of the supernatant liquor was weaker at 260 nm with the decrease of peak intensity since part of the oligonucleotides has been combined with amino-modified nanoparticles.

We further determined the number of oligonucleotides conjugated on nanoparticles applying the One Drop OD-1000 Spectrophotometer. Initially, the concentration of aptamer was 179 ng μL^{-1}, and the concentration of aptamer in the supernatant liquor collected after incubating with the MNPs (2mg ml^{-1}) decreased to 35 ng μL^{-1}, indicating the conjugation yield between aptamer and MNPs was 77000 ng $mg^{-1.}$ Subsequently, the concentration of cDNA decreased from 104 ng μL^{-1} to 6 ng $\mu L^{-1,}$ indicating the conjugation yield between cDNA and UCNPs

was 49000 ng mg^{-1}.

3.6 Optimize the dosage of probes

To identify the optimal dosages of cDNA-UCNPs and aptamer-MNPs, a comparative study was performed to reach a maximum background luminescence of UCNPs-MNPs probes. A total of 100 μL of aptamer-MNPs and various volumes of cDNA-UCNPs solution was hybridized together. As shown in Fig. 8, the luminescence intensity increased with the dose of cDNA-UCNs solution increasing, and the luminescence intensity reach maximum with the addition of 800 μL of cDNA-UCNPs solution. Initially, only a small quantity of UCNPs-MNPs was fabricated as the low concentration of cDNA-UCNPs led to the abundance of Apt-MNPs not having any cDNA-UCNPs to bind. When the concentration of cDNA-UCNPs and the concentration of Apt-MNPs were matched, the luminescence intensity reached a maximum. However, if the quantity of cDNA-UCNPs was in excess, the luminescence intensity of the UCNPs-MNPs decreased because the cDNA-UCNPs were unable to combine with more aptamer-MNPs. The MNPs-UCNPs obtained at the optimal dosages formed relatively stable suspension in buffer as is shown in Fig. 9 a. And Fig. 9 b shows the MNPs-UCNPs suspension emits green luminescence when excited by a 980 nm excitation laser.

Furthermore, we measured the luminescence intensity of the

cDNA-UCNPs to determine the conjugation yield between Apt-MNPs and cDNA-UCNPs. The black line in Fig. 10 was the luminescence Spector of the 800 μL of cDNA-UCNPs solution before hybridizing with 100 μL Apt-MNPs together. After hybridization of Apt-MNPs and cDNA-UCNPs, the supernatant was collected by magnetic separation and was measured with a 980 nm excitation laser. As is shown in Fig. 10, the intensity of the emission peak at 544 nm was reduced by about 41%, since part of the cDNA-UCNPs has been combined with amino-modified nanoparticles.

3.7 Optimize the washing time

After complete reaction between OTC and UCNPs-MNPs, the cDNA-UCNPs dissociated from UCNPs-MNPs should be washed thoroughly to prevent the interference. Therefore, we optimize the washing time to reach the best washing effect. The UCNPs-MNPs probes were exposed to OTC (10 ng mL^{-1}) and only buffer without OTC respectively. The remaining UCNPs-MNPs were then separated an external magnetic field and washed three times and the luminescence intensity was measured with a 980 nm excitation laser. The results are shown in Fig. 11, the luminescence intensity decreased with each washing time increasing, and the remained constant when the washing time was 15 s.

3.8 Determination of OTC

In this experiment, the luminescence intensity was at a maximum before the OTC was in absence. When the target molecule was introduced, the luminescence intensity decreased gradually as the aptamers preferentially bound to OTC and caused the dissociation of some cDNA-UCNPs from UCNPs-MNPs. Various intensities of luminescence spectra obtained in the presence of different concentrations of OTC are shown in Fig. 12 a. Fig. 12 b shows the linear relationship between the intensity of the upconversion luminescence and the concentration of OTC. The LOD of the aptasensor for OTC is as low as 0.036 ng mL^{-1} (calculated by the function of 3s/S, where s is the standard deviation of the blank solution and S is the slope of the linear relationship). The precision expressed by the relative standard deviation (RSD) of OTC detection is 3.71% (10 ng mL^{-1}, n = 10). Table 1 presents some of the latest detection methods reported in recent years for OTC, which suggests that the proposed method is more sensitivity than most of those previously described.

Furthermore, we applied TEM to observe the morphology of MNPs-UCNPs before and after target treating. Fig. 13 a shows the morphology of MNPs-UCNPs before target treating. After OTC was added to the system, aptamer preferentially bound to OTC causing the dissociation of some cDNA, thereby liberating some cDNA-UCNPs. And Fig. 13 b shows the morphology of MNPs-UCNPs after target treating.

3.9 Specificity

Three structurally similar tetracycline derivatives, including OTC, DOX, and TET were used to verify the good selectivity of this method. The aptasensor were exposed to the derivatives, with the same concentration (10 ng mL^{-1}). The results are shown in Fig. 14, OTC caused a dramatic luminescence change as is respected, while the others failed. The good specificity of this method was attributed to the inherent specificity of aptamer toward OTC.

3.10 Analytical application

The accuracy of OTC detection in food samples was evaluated by determining the recovery of OTC by adding a series of known quantities of OTC into the milk samples. As shown in Table 2, the recoveries were between 98.60 % and 119.00 %, and there is no significant difference ($P<0.0001$) between the results obtained by the aptasensor and ELISA method in Fig. 15, indicating the proposed aptamer-based bioassay can be applied for OTC detection in food samples.

4 Conclusions

In this study, a high-sensitive aptasensor for the rapid, and specific detection of OTC based on upconversion and magnetic nanoparticles was successfully developed and evaluated. Typically, the use of UCNPs avoided the auto luminescence originating from the biomolecules possibly contained in the solution entirely as the UCNPs were excited by

infrared 980 nm laser. Furthermore, the magnetic separation simplified the experimental processes as the magnetic nanoparticles can concentrate and separate targets from the food solution easily and rapidly. Lastly, the aptamers were stable compared to traditional antibodies and highly specific to the target OTC. In summary, the aptasensor offers a new approach of convenience, sensitivity, specificity, and stability to detect OTC in food samples.

Acknowledgments

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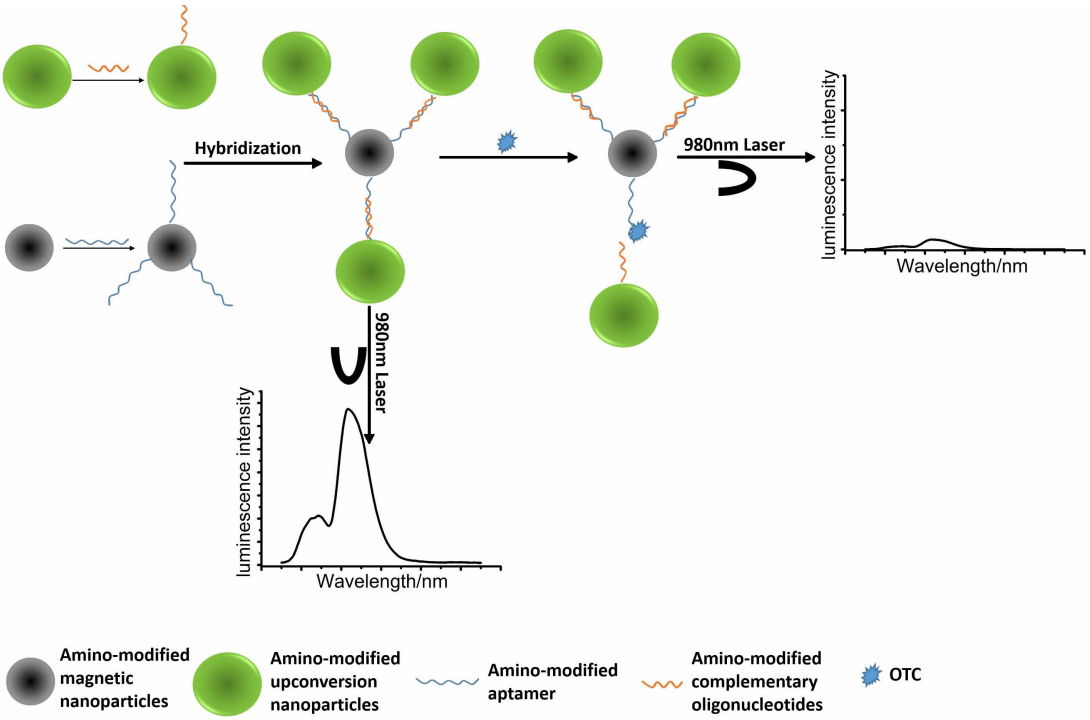


Fig. 1 Schematic illustration of the highly sensitive aptasensor for oxytetracycline based on upconversion and magnetic nanoparticles

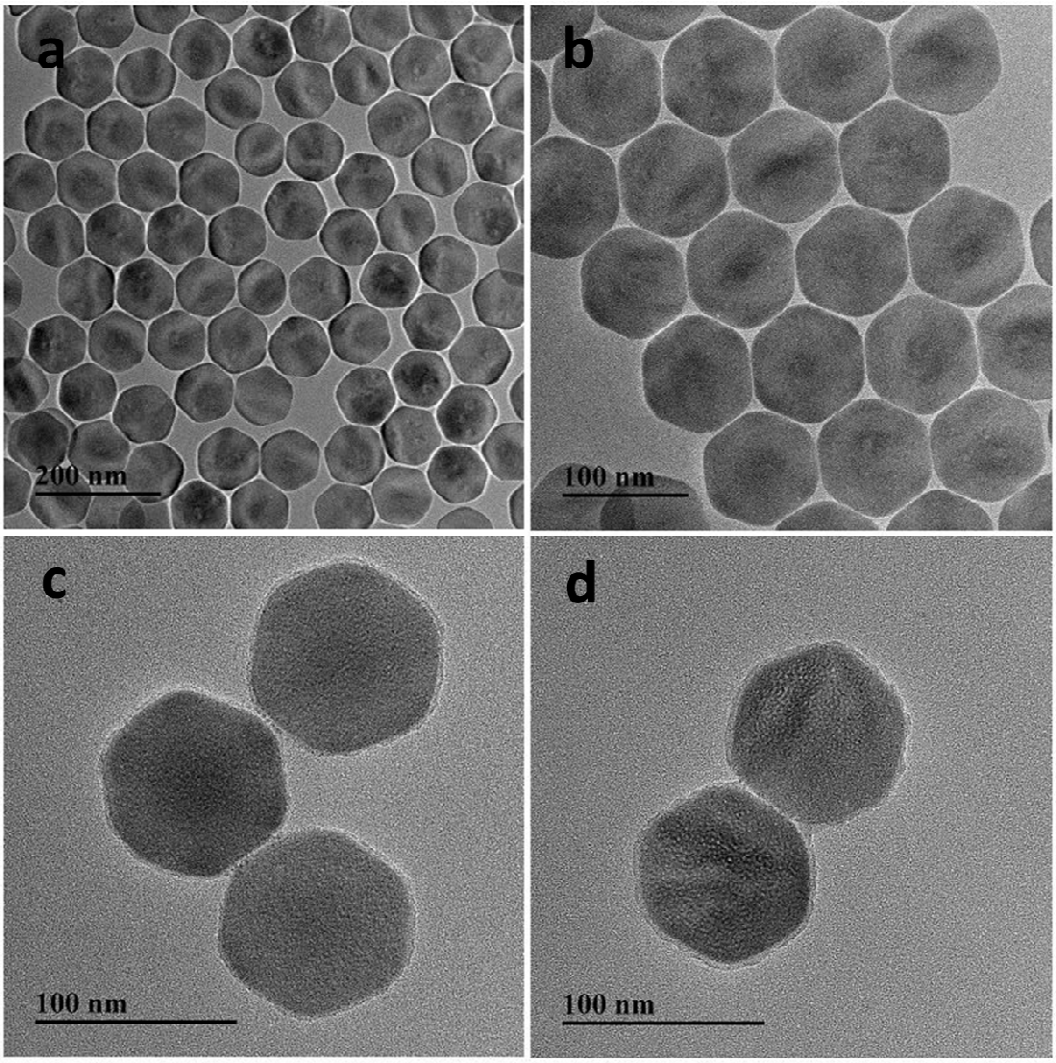


Fig. 2 TEM images of $NaY_{0.78}F_4$: $Yb_{0.20}$, $Er_{0.02}$ UCNPs before coating silica (a-b) and after coating silica (c-d)

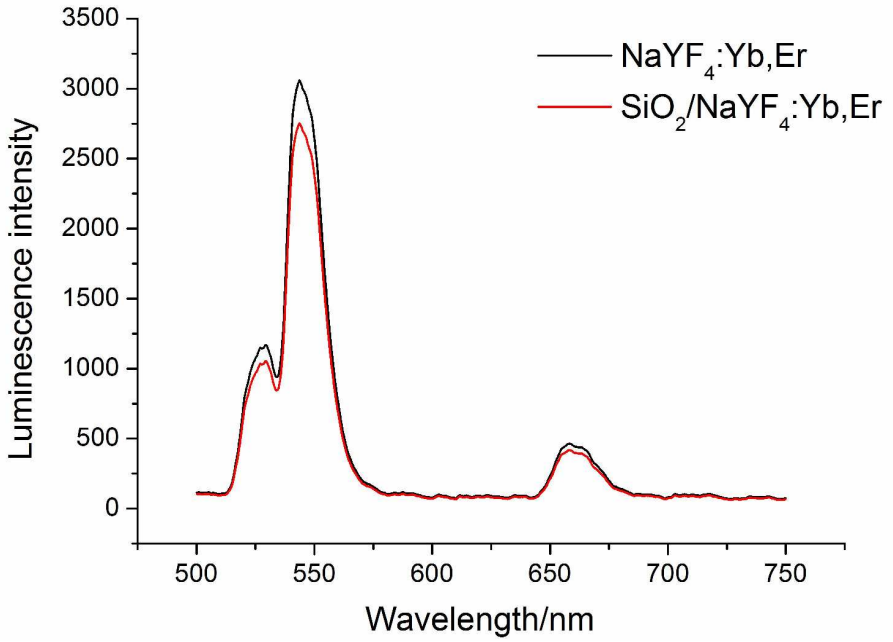


Fig. 3 Luminescence spectra of NaYF4: Yb, Er nanospheres, with and without silica coating

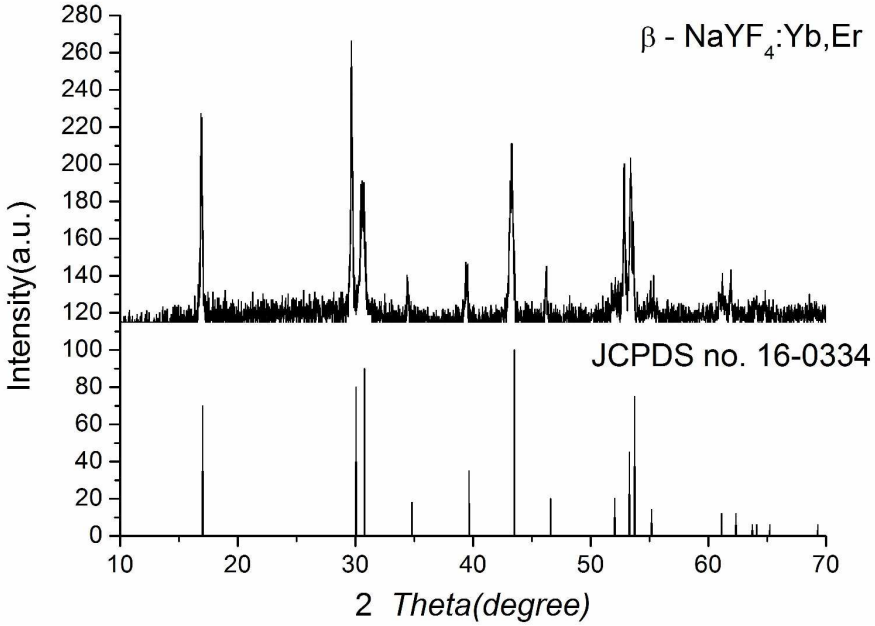


Fig. 4 XRD patterns of $NaYF_4$: Yb, Er UCNPs synthesized via method 1 (a) and method 2 (b)

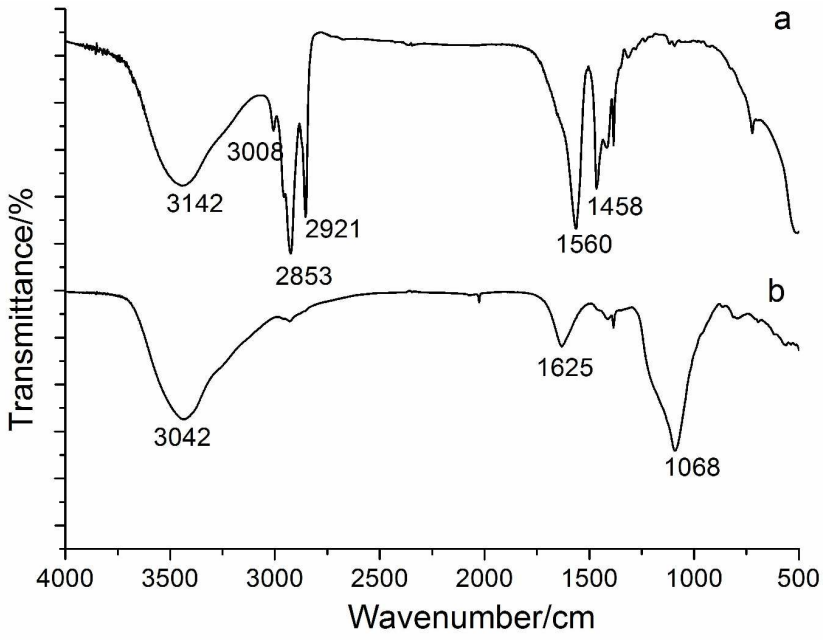


Fig. 5 FT-IR spectra without silica shells (a) and with silica shells (b)

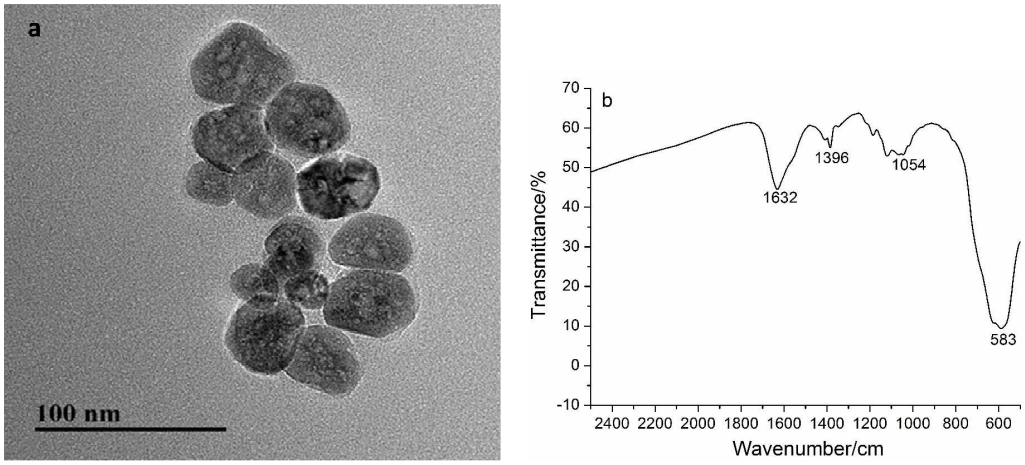


Fig. 6 TEM image (a) and FT-IR spectrum (b) of the amino-modified Fe_3O_4 nanoparticles

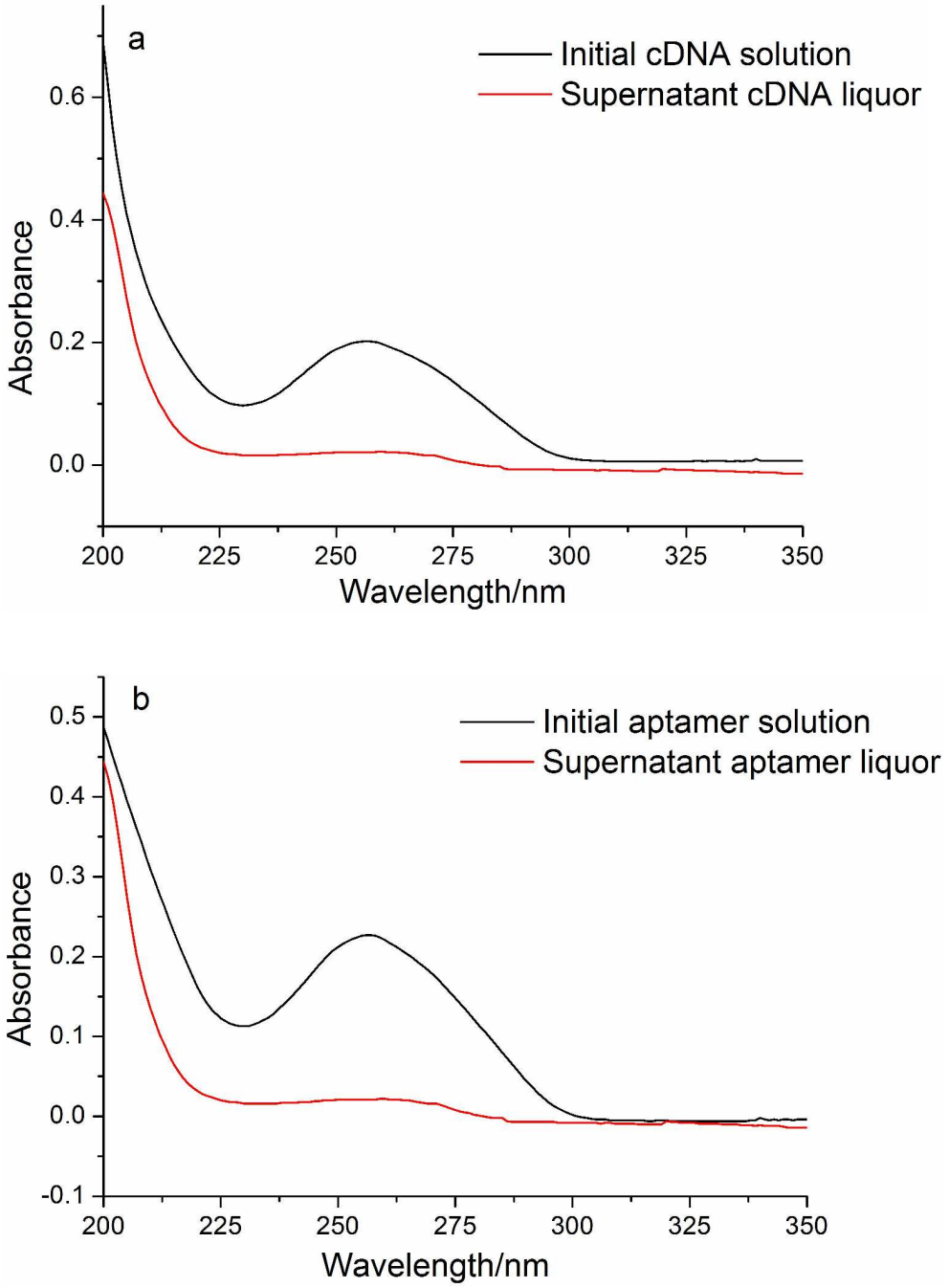


Fig. 7 UV−vis absorption spectra of cDNA solution (a) and aptamer solution (b) before and after conjugating with amino-modified nanoparticles (2mg ml^{-1})

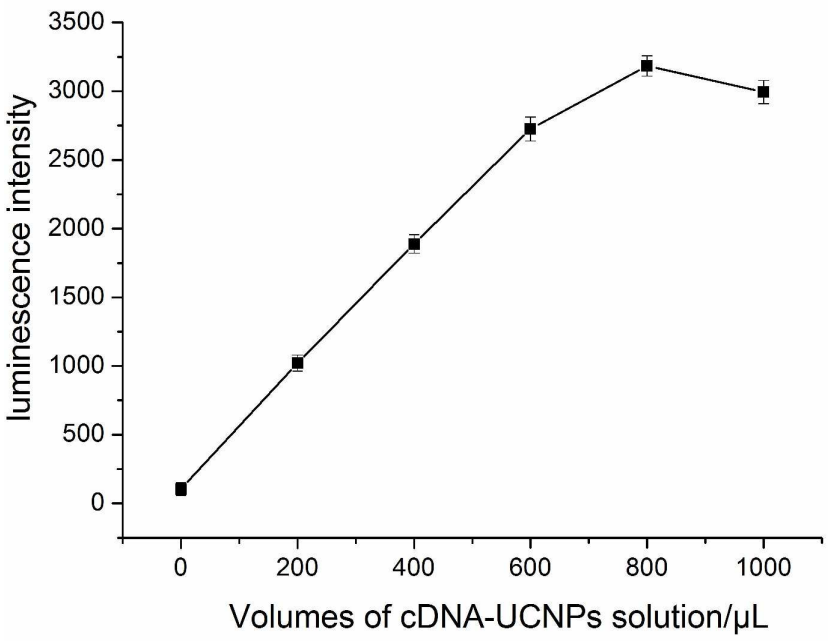


Fig. 8 Luminescence intensity of various volumes of cDNA-UCNPs (2 mg ml^{-1}) incubated with 100 μL of aptamer-MNPs (2 mg ml^{-1})

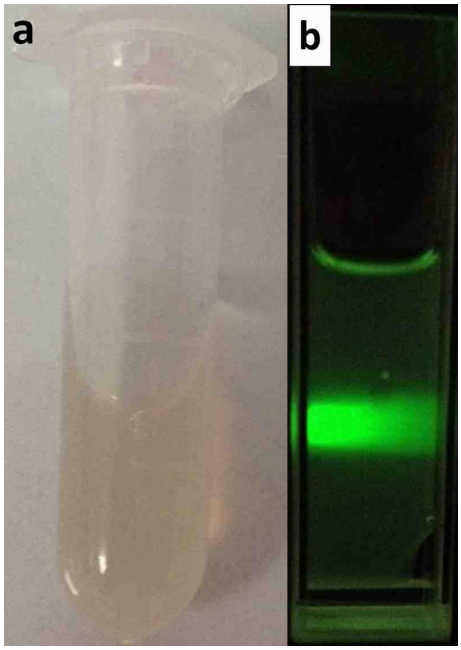


Fig. 9 The photographs of MNPs-UCNPs suspension (a) and the luminescence of the suspension excited by a 980 nm excitation laser (b)

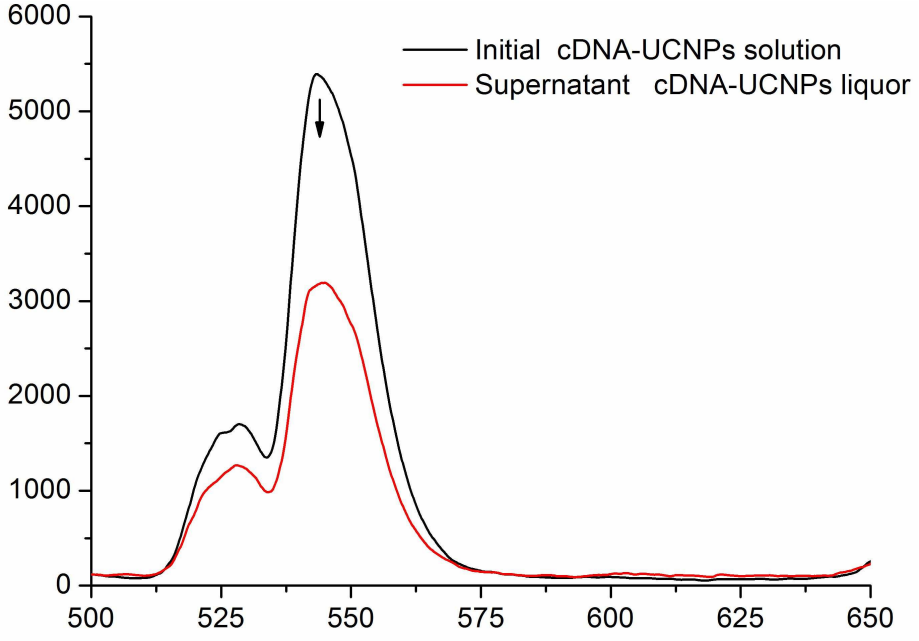


Fig. 10 Luminescence spectra of cDNA-UCNPs nanospheres (2 mg ml^{-1}) before and after hybridizing with 100 μL of Apt-MNPs (2 mg ml^{-1})

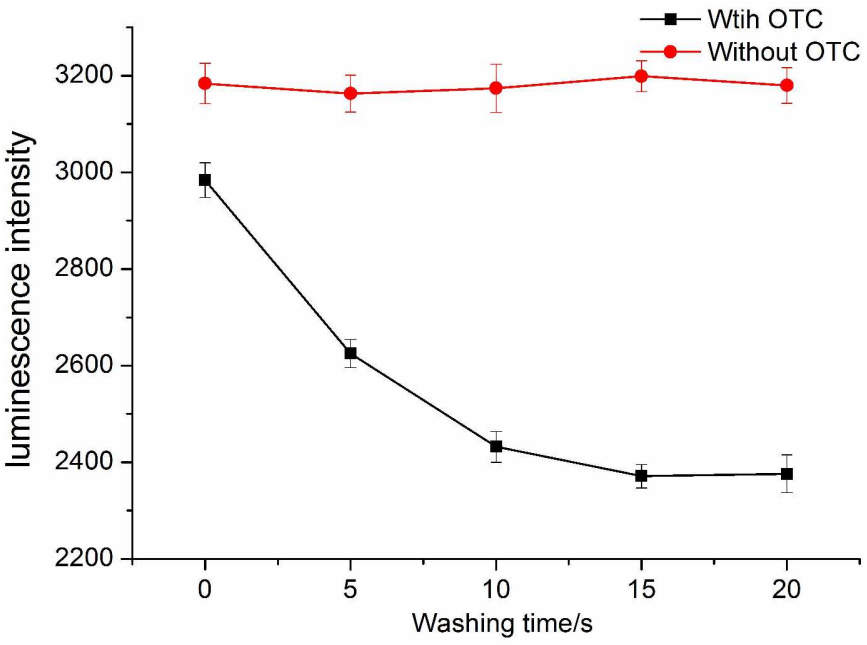


Fig. 11 Luminescence intensity of various washing time with OTC (10 ng ml^{-1}) and without OTC

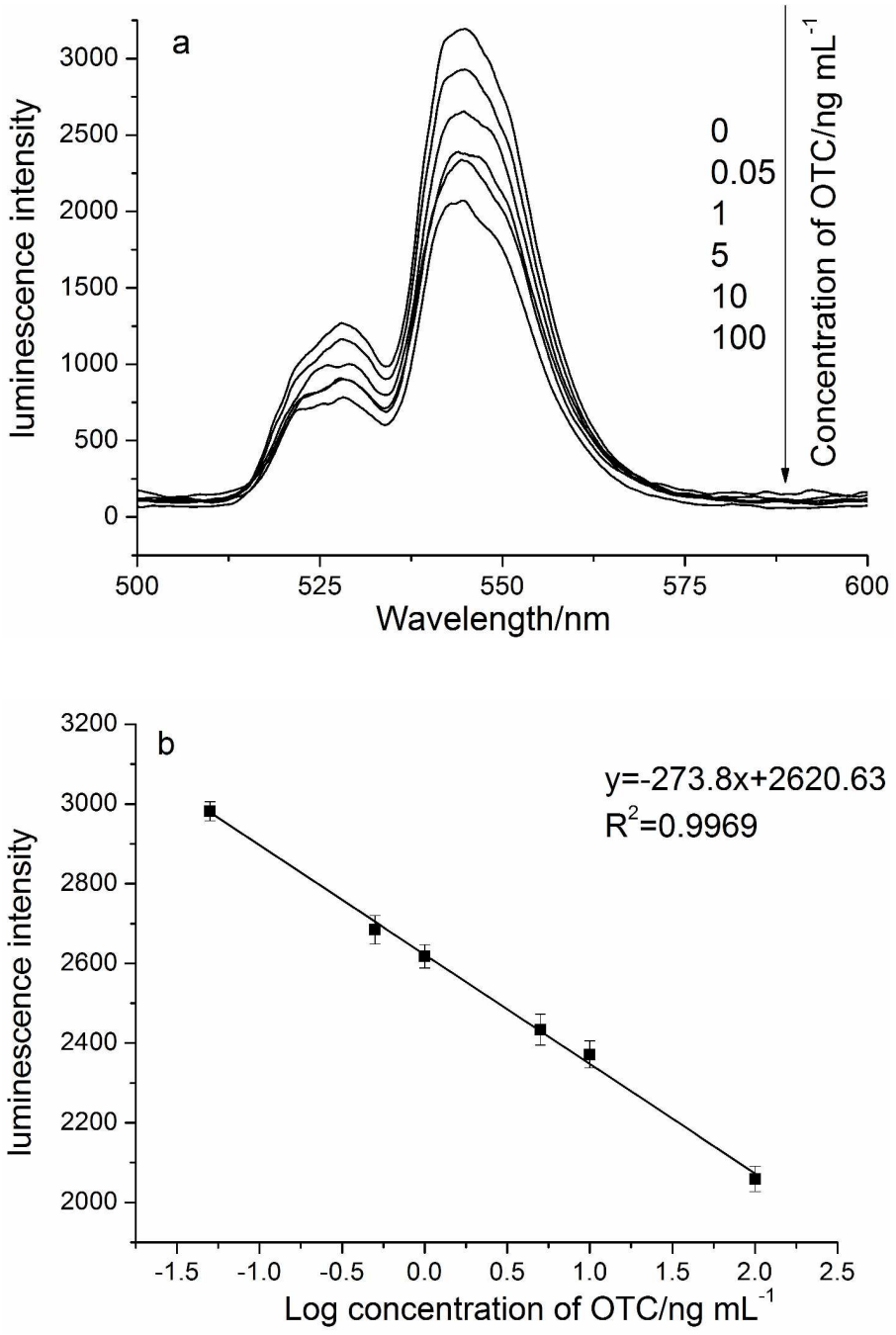


Fig.12 Typical recording output for the detection of different concentrations of OTC (a). Standard curve of the related upconversion luminescence intensity versus the concentrations of OTC (b)

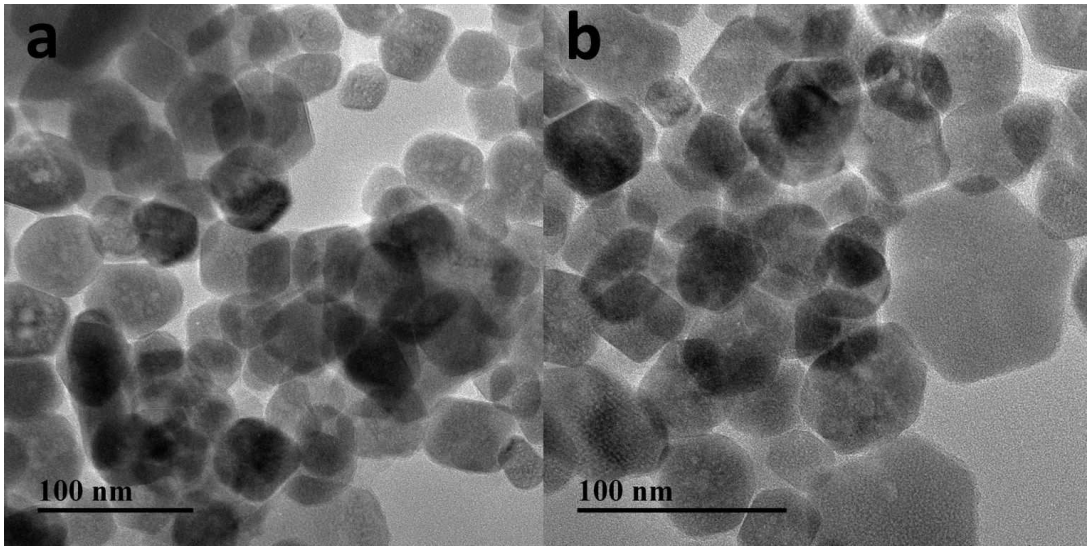


Fig. 13 TEM images of MNPs-UCNPs before (a) and after (b) target treating.

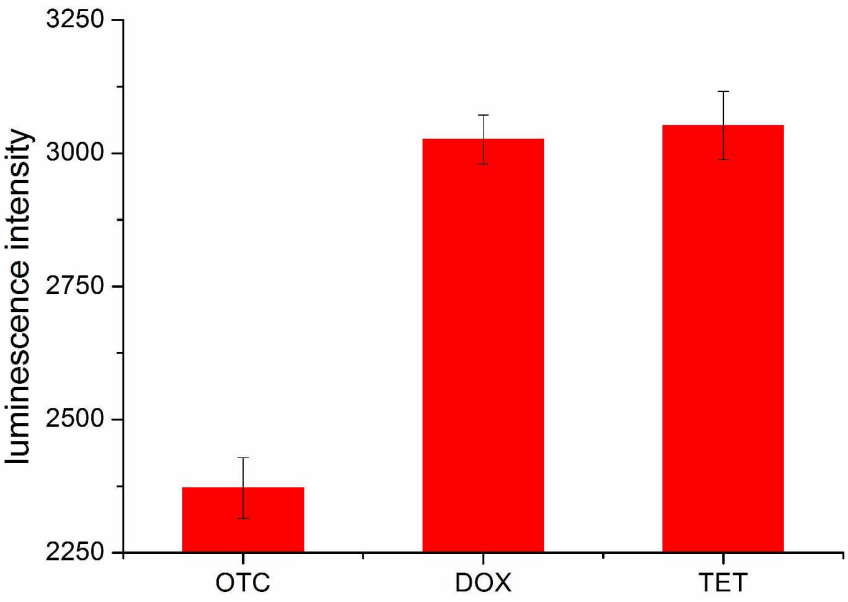


Fig. 14 Differential luminescence response of the aptasensor at 544 nm to OTC, DOX, and TET at the same concentration (10 ng mL^{-1})

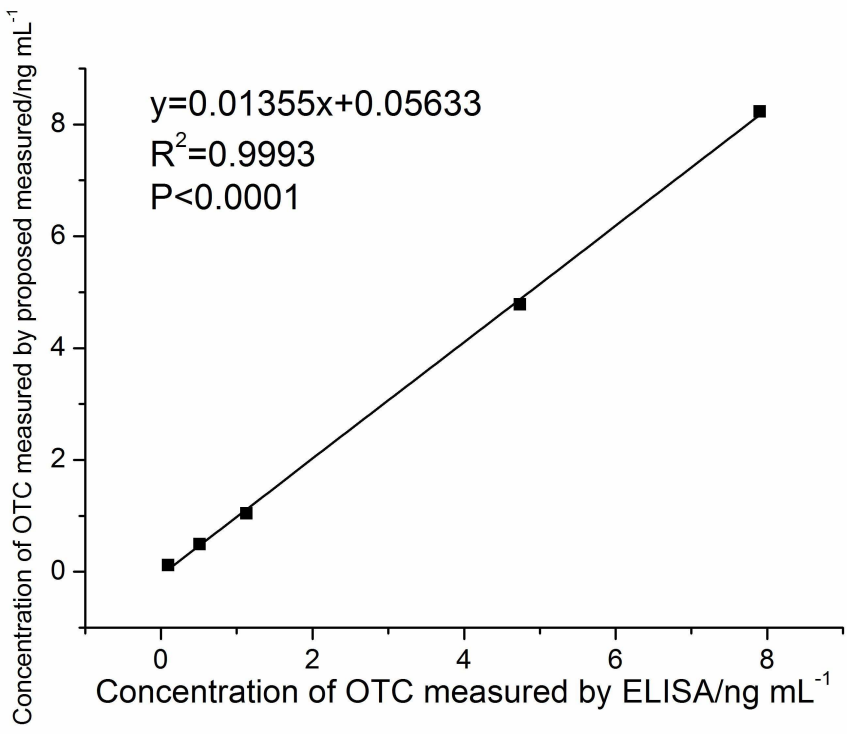


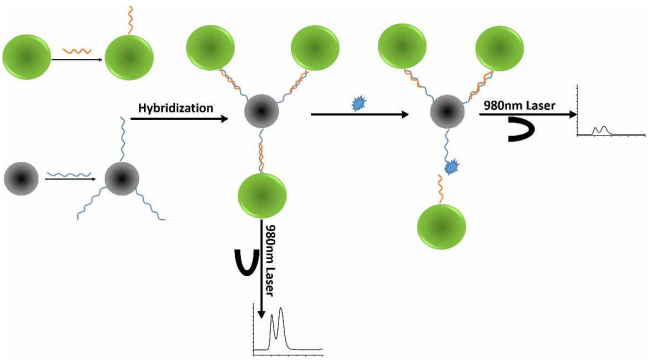
Fig. 15 Relationship between the proposed method and ELISA method for OTC detection

Table 1 The reported detection methods for OTA and the limit of detection

Methods	LOD	References
UHPLC method	0.2 / 0.003 ng ml^{-1}	19
Method based on indirect competitive ELAA	12.3 ng ml^{-1}	20
ELISA	7.01 ng ml^{-1}	21
Electrochemical determination	0.1 μM	24
An electrochemical biosensor	9.8 ng ml^{-}	27
AuNP-based colorimetric assay	0.1 nM	29
Cantilever Array Sensors	0.2 nM	35
Fluorescent assay	10 nM	36

Table 2 Recovery results for the added standard OTC from milk samples obtained by the developed method

Sample	Background Content (ng ml^{-1})	Added Concentration (ng ml^{-1})	Detected Concentration (ng ml^{-1})		Recovery Ratio (%)
			ELISA	aptasensor	
1	0	0.1	0.093	0.119	119.00
2	0	0.5	0.512	0.493	98.60
3	0	1	1.127	1.042	104.20
4	0	5	4.738	4.782	95.64
5	0	8	7.901	8.232	102.75



Schematic illustration of the highly sensitive aptasensor for oxytetracycline based on upconversion and magnetic nanoparticles