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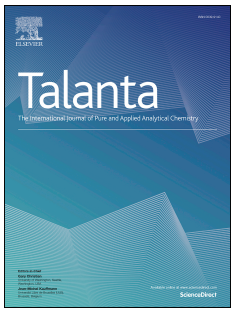

A highly sensitive and selective fluorescence biosensor for hepatitis C virus DNA detection based on δ-FeOOH and exonuclease III-assisted signal amplification

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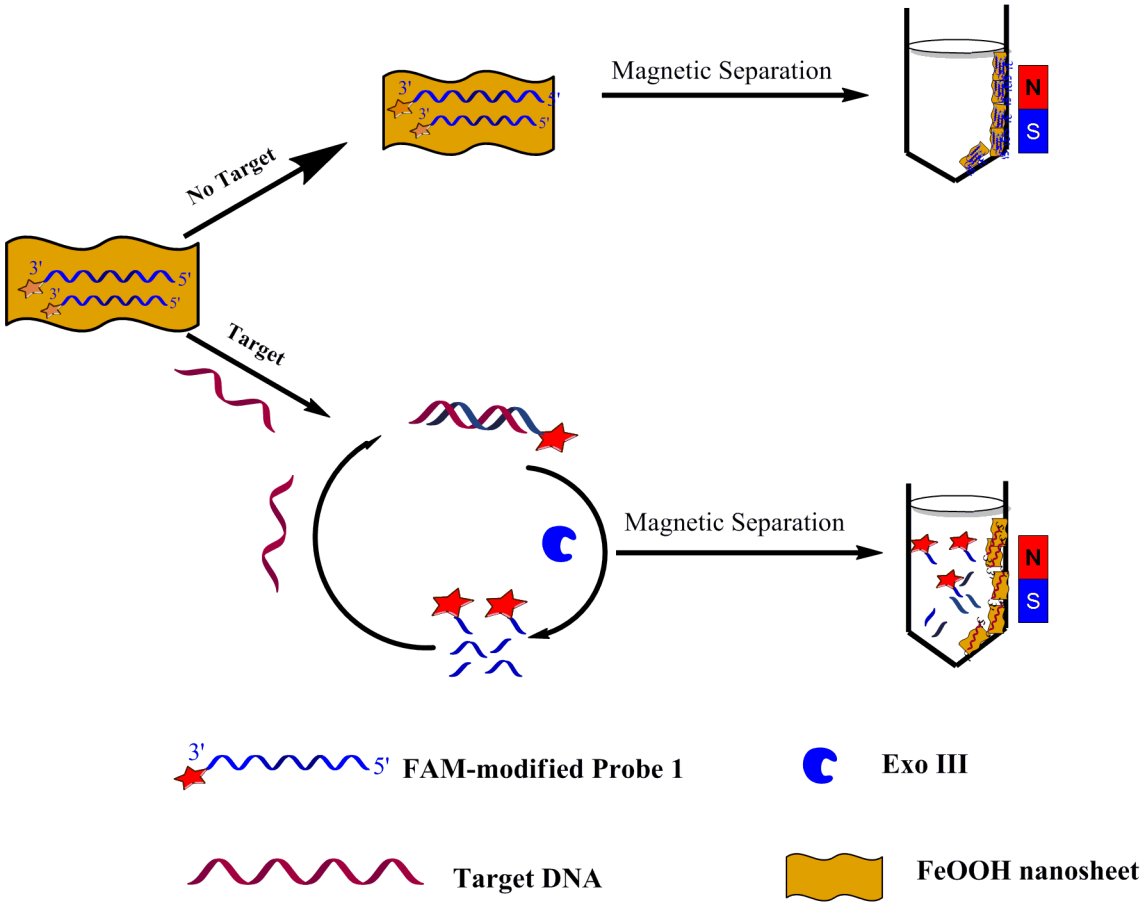
CRediT authorship contribution statement

Tian Wu: Conceptualization, Data curation, Investigation, Formal analysis, Writing - original draft. Xiang Li: Conceptualization, Data curation, Investigation, Visualization, Funding acquisition, Project administration, Writing - review & editing. Yuanqi Fu: Formal analysis. Xuelian Ding: Methodology, Formal analysis. Zhongjian Li: Methodology, Resources. Guifen Zhu: Formal analysis, Resources. Jing Fan: Supervision, Validation, Funding acquisition, Project administration, Writing - review & editing.


No Target
Target
Magnetic Separation
Magnetic Separation
N
S
N
S
3'
5'
FAM-modified Probe 1
Exo III
Target DNA
FeOOH nanosheet

A highly sensitive and selective fluorescence biosensor for hepatitis C virus DNA detection based on δ-FeOOH and Exonuclease III-assisted signal amplification

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Abstract

Developing the high selectivity and sensitivity strategy for nucleic acid detection is crucial for early diagnosis and therapy of diseases. In this work, a novel low back-ground fluorescent sensor platform for the detection of nucleic acid has been developed based on δ-FeOOH nanosheets integrating with exonuclease III-assisted target-recycling signal amplification. Because of the strong binding ability between the single-strand DNA (ssDNA) and the δ-FeOOH nanosheets, the dye-labeled ssDNA probe would be quenched by δ-FeOOH nanosheets through fluorescence resonance energy transfer (FRET). By using magnetic separate properties of δ-FeOOH, the background signal was separated from the sensor system, and the low background sensor system was obtained. After adding the target DNA, a double-strand DNA complex (dsDNA) would be formed between the target DNA and dye-labeled ssDNA probe. Then, the dye-labeled ssDNA probe in the dsDNA complex would be stepwise hydrolyzed into short fragments from 3′-terminus by Exonuclease III, and the fluorescence signal was recovered due to the weak bind affinity between the short fragments and δ-FeOOH nanosheets. By using the fluorescence quenching ability of δ-FeOOH nanosheets and enzyme-assisted target-recycling signal amplification, this strategy could show an excellent selectivity toward hepatitis C virus DNA with a low detection limit of 10 pM. By simply changing the dye-labeled ssDNA probe sequence, this sensing platform can be developed as a universal approach for the simple, sensitive, and selective detection of different target DNA.

Key words: δ-FeOOH nanosheets, Enzyme amplification, Hepatitis C virus DNA, Fluorescence nano-biosensor

1. Introduction

Developing rapid, cost-effective methods for the high sensitivity and selectivity detection of nucleic acid have placed a very high value in many areas, such as gene expression profiling, biomedical applications, early diagnosis and the prognosis [1-2]. The conventional methods, such as gel electrophoresis, radioisotopic, polymerase chain reaction (PCR) and DNA microarrays have been performed for the detection of nucleic acid [3-5]. However, these methods usually required the costly apparatus and complicated pretreatment procedures to perform efficiently nucleic acid detection. Fluorescence-based techniques for nucleic acid detection have attracted extensive attention because of their high stability, sensitivity and simplicity [6-8]. Most of those fluorescence sensors for the nucleic acid detection were designed based on the fluorescence resonance energy transfer (FRET) between the fluorophore and the quencher [9-11]. In the past decades, based on the affinity difference of nanomaterials with DNA of different conformation (such ssDNA and dsDNA) and the efficient fluorescence quenching effect of nanomaterials, different types of nanomaterials include zero-dimensional (0D) nanoparticals (such as gold nanoparticles), one-dimensional (1D) nanotubes (such as carbon nanotubes) and two-dimensional (2 D) nanosheets (such as grapheme) have been used as “nano-quencher” to reduce the background signal of sensor system and thus enhance the detection sensitivity [12-16]. Among those nanomaterials, two-dimensional nanosheets have been shown the excellent platforms for the detection of DNA sequence [17, 18]. This is because that the two-dimensional nanomaterials exhibit planar structures and ultra-large surface areas, which show the superior abilities to the low dimensional nanomaterials (such as 0D and 1D) in both the quenching efficiency and hybrid kinetics [19]. Due to these excellent properties, various 2D nanosheets, including transition metal dichalcogenide [20-22], metal oxides [23], and carbon nanomaterials [24, 25], have been developed to construct nano-fluorescence sensor for the DNA detection. However, in those 2D-based nano-fluorescence sensors, the quenching efficiency reach to 90% was generally considered to be ideal “quenching-off” states. However, the residual fluorescence signal remained in the solution, affecting the detection of trace target.

Therefore, seeking for a new approach to design low-background even to background-elimination fluorescent sensors is particularly important for improving trace analytical performances.

As a metastable species in the iron oxide family, feroxyhyte (δ-FeOOH) ultrathin nanosheet is a new type of inorganic graphene analogue with ferromagnetic and semiconducting properties, which has been widely used in the field of catalysis and adsorption [26, 27]. Because of the significant number of hydroxyls on the δ-FeOOH surface, δ-FeOOH shows excellent water solubility, good biocompatibility and large specific surface area, which provides conditions for the application of ferric hydroxide in biological system. More importantly, δ-FeOOH possesses the strong magnetic properties in the room temperature, and it is higher than that of various traditional two-dimensional nanomaterials [28-30]. Thus, the application of δ-FeOOH nanosheets to construct sensor strategy is worthy of investigation. However, using the magnetic properties of δ-FeOOH to construct the biosensor for the DNA detection has not been reported at present.

Herein, we demonstrated that δ-FeOOH nanosheets showed various binding ability not only for ssDNA versus dsDNA, but also had different adsorption effect for different length ssDNA. Moreover, δ-FeOOH nanosheets exhibit wide range absorption spectrum, which shows the high fluorescence quenching ability for various fluorescence dyes. Then, a “turn-on” fluorescence strategy for the high sensitivity detection of hepatitis C virus sequence has been proposed by using ultrathin δ-FeOOH nanosheets as fluorescent sensing platform. By using magnetic separate properties of δ-FeOOH, the background signal was separated from the sensor system, and the low background sensor system was obtained. Through combination of the magnetic separate and enzyme-aided target signal amplification, the proposed sensor can greatly enhance the detection sensitivity and the detection limit of 10 pM was achieved. Additionally, we also applied this amplified sensor for the detection hepatitis C virus sequence DNA in the spiked human serum samples, and satisfactory recoveries were obtained. It can be expected that this δ-FeOOH nanosheets based signal amplification sensor strategy holds greatly potential application in the field of

early clinical diagnosis.

2. Materials and methods

2.1 Regents and material

All oligonucleotides sequences used in this manuscript are listed in Table S1. The oligonucleotides and Exo III were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The 50 mM Tris-HCl (pH 8.0) buffer containing 5 mM $MgCl_2$ was used in this experiment. Other reagents were obtained from Aladdin Chemical Reagent Co., Ltd (Shanghai, China).

2.2 Apparatus

Transmission electron microscopy (TEM) imaging of δ-FeOOH nanosheets was performed using a JEM-2100 transmission electron microscope operating at 200 kV (JEOL). X-ray diffraction (XRD) pattern was characterized on the X'Pert3 Powder X-ray diffractometer (PANalytical B.V.). X-ray photoelectron spectrometry (XPS) spectra were performed on an ESCALAB250Xi X-ray photoelectron spectrometer (Thermo Fisher Scientific). UV-vis spectra were taken using an UV-T6 spectrophotometer (Purkinie). Fluorescence spectra were measured on a Cary Eclipse fluorescence spectrometer (Varian). Each fluorescence measurement is repeated three times and the standard deviation is drawn as an error bar.

2.3 Synthesis of δ-FeOOH nanosheet

δ-FeOOH nanosheets were synthesized by one-pot method according to the reported literature [31]. In brief, dissolving 0.5 g NaOH in 30 mL water-ethylene glycol (EG) mixture (V_{water} : V_{EG} = 5:1) to form solution A. Then, 100 mg $FeSO_4 \cdot 7H_2O$ was dissolved in 6 mL H_2SO_4 (0.01 M) solution to obtain solution B. Next, kept solution A and solution B under continuous nitrogen deoxidation for 1 hour while stirring. After that, solution B was dropwise injected into solution A and the solution color turned from colorless to light green and then to dark green under vigorous stirring for 3 h. Centrifuging and harvesting the precipitates and then washed with ethanol and water for several times. Next, 40 mL hydrogen peroxide solution (3 wt%) was added rapidly, and then the mixture was kept overnight (for 8 h) at room temperature.

Finally, the δ-FeOOH nanosheets were readily collected using magnetic decantation, and the obtained products were dried in vacuum for further characterization.

2.4 The process for the target DNA detection

In a typical DNA detection, different concentrations of target DNA (T1) was added to 1000 μL Tris-HCl buffer containing 20 nM dye-labeled ssDNA probe (P1) and hybridized for 30 min at 37 °C. Then, δ-FeOOH nanosheets (0.5 mg/mL) was added to the hybridization reaction solutions for another 5 mins. After that, the fluorescence emission spectra were recorded with excitation wavelength at 490 nm and the fluorescence intensity was measured at 520 nm (Scheme 1).

The Exo III-assisted signal amplification experiment were carried out using the same reagent as the prior step, different concentration of T1 and 20 nM P1 was first incubated for 30 min at 37 °C, and then 20 U/mL Exo III was added to the solution further incubated for 30 min. The δ-FeOOH nanosheets (0.5 mg/mL) was added and the fluorescence spectra of the reaction solutions were measured after magnetic separation (Scheme 2).

3. Results and discussion

3.1. Characterization of δ-FeOOH

For the purpose of clarifying the morphology and structural information of the δ-FeOOH ultra-thin nanosheets, a series of micro-characterization have been carried. The transmission electron microscopy (TEM) has revealed that the synthesized δ-FeOOH is a kind of graphene-like ultra-thin nanosheets with large area folds and almost transparent (Figure. 1a). The phase and purity of the obtained samples can be determined by the X-ray diffraction (XRD). As shown in Figure. 1b, the sample spectra has diffraction peaks at 35.24°, 39.95°, 54.51°, 63.15°, and these peaks correspond to the crystal plane diffraction (JCPDS No.13-0087) of δ-FeOOH at (100), (002), (102) and (110), respectively. The diagram of the sample may easily be referred to as a hexagonal phase of δ-FeOOH nanosheets. We also investigated the surface chemical composition and elemental distribution of δ-FeOOH nanosheets by X-ray photoelectron spectroscopy. As explained in Figure 1c, there are three peaks in high-resolution XPS spectrum of Fe 2p. The peaks at 710.7 eV pertain to the Fe^{3+}

2p3/2. The binding energy of Fe^{3+} 2p1/2 at 724.3 eV demonstrates the Fe^{3+} state of the δ-FeOOH structure. There is a shake-up satellite at 719.5 eV in the Fe 2p core level spectrum which is consistent with references [32, 33]. As shown in Figure 1d, the chemical environment of oxygen is confirmed by analyzing the energy divided into three peaks of O1s, Fe-O bind (529.59 eV), Fe-O-H bind (531.19 eV) and H-O-H bind (533.04 eV). Especially, because the material is synthesized in aqueous phase and dried in vacuum, water molecules are adsorbed on the surface of the material, the H-O-H bond corresponds to water molecule [34, 35]. The UV-vis absorption spectrum of δ-FeOOH nanosheets were also determined (Fig. 1e). It can be concluded from the test result that the absorption of light covers a wide range of wavelengths from ultraviolet light to visible regions, which indicates that the δ-FeOOH nanosheets can be an ideal energy acceptor for diverse chromophores. Magnetic measurement of the sample was carried out at room temperature using vibrating sample magnetometer. The result is shown in the Figure 1f. The magnetization-magnetic intensity (M-H) curves for the δ-FeOOH nanosheets measured at 300 K shows the coercivity (Hc) reaches up to 195 Oe. Furthermore, the saturation magnetization (Ms) reaches to 7.04 emu/g, gives the evidence that the δ-FeOOH nanosheets have ferromagnetism.

3.2 Fluorescence quenching property of δ-FeOOH nanosheets

To demonstrate the feasibility of δ-FeOOH nanosheets could be used as the fluorescence sensing platform, the fluorescence quenching ability of δ-FeOOH nanosheets to dye-labeled ssDNA probe (P1) was investigated firstly. As showed in Figure 2, when increasing the amount of δ-FeOOH nanosheets, the fluorescence of P1 was quenched. When the concentration of δ-FeOOH nanosheets was high than 20 μg/mL, the quenching efficiency was almost achieved 95%, which indicating the strong interaction between P1 with the δ-FeOOH nanosheets and the high fluorescence quenching efficiency of the δ-FeOOH nanosheets. We also measure the fluorescence quenching ability of δ-FeOOH nanosheets to different bases length of dye-labeled ssDNA. As shown in Figure S1, the fluorescence quenching ability of δ-FeOOH nanosheets to different base number of ssDNA showed significant difference. For the long base ssDNA (25-base DNA), the quenching efficiency is 97%, while for the short base ssDNA (3-base DNA), the quenching efficiency is only 15%.

This huge difference indicates that δ-FeOOH nanosheets show the weaker adsorbing affinity to short ssDNA than that of the long ssDNA. Upon addition of the δ-FeOOH to the dye-labeled dsDNA solutions (21 base) (Figure S2), the quenching efficiency of δ-FeOOH nanosheets to dsDNA was lower compare with the ssDNA (21 base) at the same conditions, which suggested that the weak interactions between dsDNA and δ-FeOOH nanosheets than that between ssDNA and δ-FeOOH nanosheets. The above results were similar with other 2D nanosheets [36-38], which indicateds that the mainly interaction between nucleic acid and δ-FeOOH nanosheet was non-covalent π-stacking interactions.

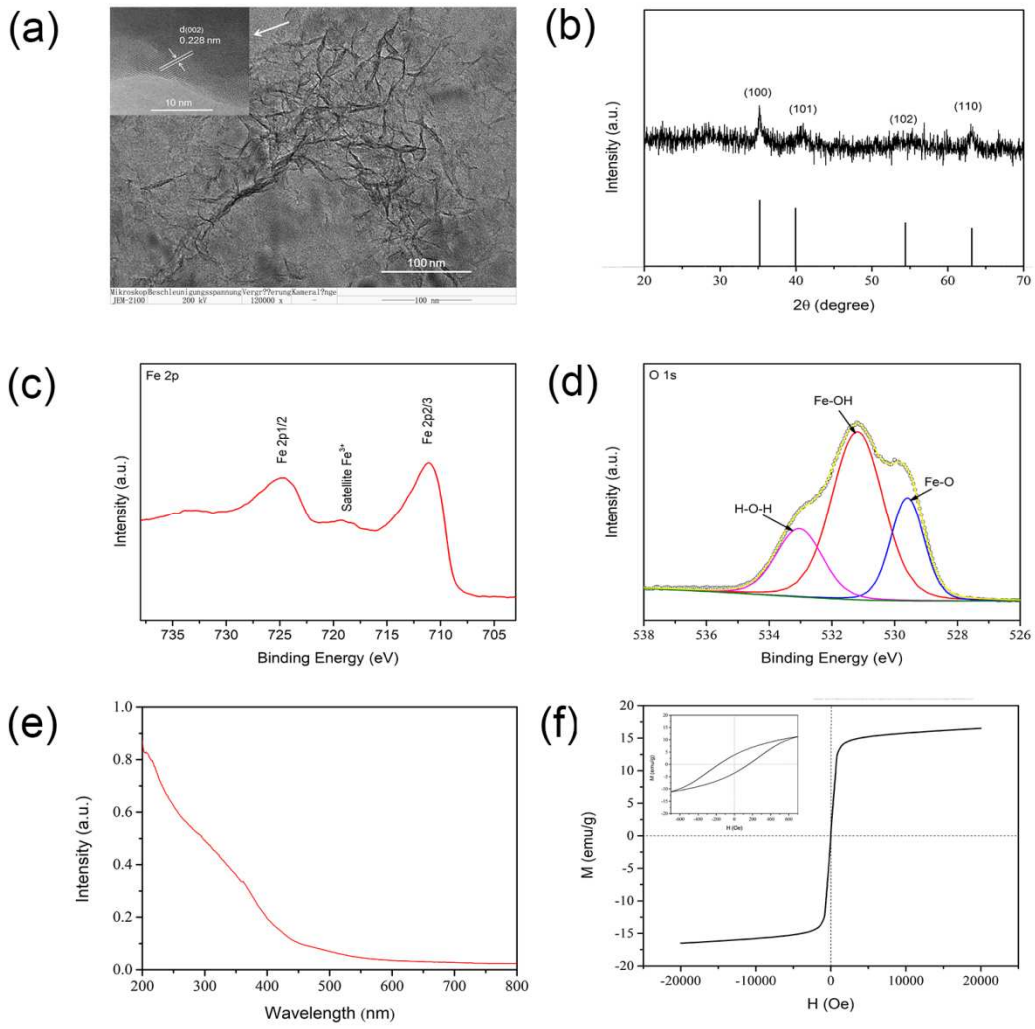


Figure 1 (a) TEM image of δ-FeOOH nanosheets. (b) XRD pattern of δ-FeOOH nanosheets. (c) Fe2p XPS patterns of as-synthesized δ-FeOOH nanosheets. (d) O1s High-resolution XPS spectra. (e) UV-vis absorption spectrum of the δ-FeOOH nanosheets. (f) M-H curves for the δ-FeOOH nanosheets measured at 300K, inset: magnetic hysteresis loop of the δ-FeOOH nanosheets.

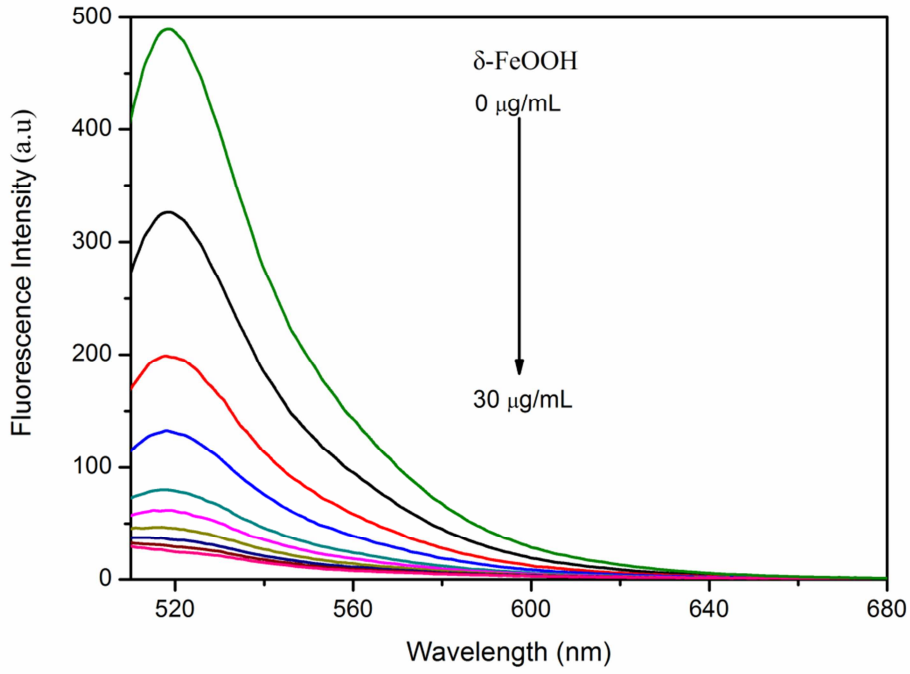
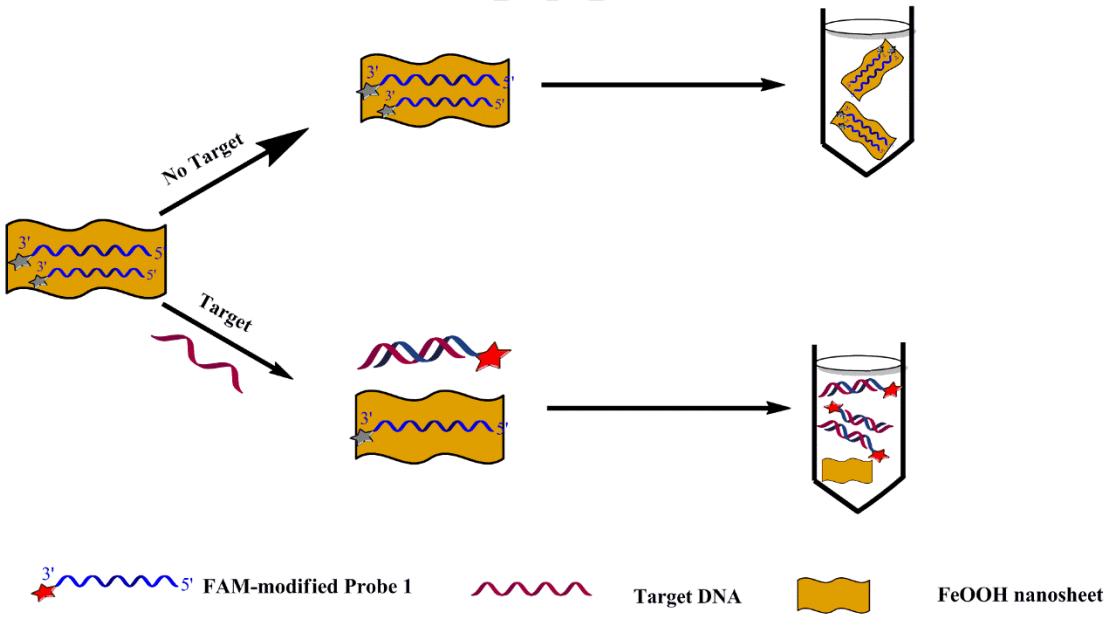


Figure 2. Fluorescence spectra of probe DNA (P1) upon adding different amount of δ-FeOOH nanosheets.

3.3 The sensing mechanism of δ-FeOOH for DNA detection

According to the different fluorescence quenching efficiency of δ-FeOOH nanosheets toward various DNA conformations, δ-FeOOH nanosheets could be used to construct a biosensing strategy for the detection of DNA. The hepatitis C virus sequence (HCV, 5'-TCC AGG CAT TGA GCG GGT TTA-3') were selected as the model [39, 40]. The detection strategy was present in the scheme 1, which illustrates the mechanism for the DNA detection by using δ-FeOOH nanosheets as a sensing platform. The HCV sequence was used as target DNA (T1) and its dye-labeled complementary strand DNA (FAM-5'-TAA ACC CGC TCA ATG CCT GGA-3') was used as the signal probe (P1). Figure 3 shows the fluorescence emission spectra of P1 under different conditions. In the absence of δ-FeOOH nanosheets, the fluorescent probe (P1) showed strong fluorescence emission. However, upon adding the δ-FeOOH nanosheets to the P1 solution, almost 93.5% of fluorescence intensity was quenched, indicating that δ-FeOOH nanosheets could adsorb P1 and quench the fluorescent dye effectively.

Incubation of P1 with target DNA (T1) will cause their efficient hybridization, thus the fluorescence intensity was recovered. Additionally, we also added the single-base mismatched DNA (T2) and random sequence DNA (R1) to the P1/δ-FeOOH solution, respectively (see Figure 3). The fluorescence intensity value of P1/T1/δ-FeOOH was approximately two times higher than that of P1/T2/δ-FeOOH and nine times higher than that of P1/R1/δ-FeOOH, demonstrating δ-FeOOH based fluorescent sensor possesses excellent selectivity. These results illustrate the δ-FeOOH nanosheets could serve as a sensing platform for the detection of DNA. For the sensitivity of HCV detection, different amounts of target DNA (0-200 nM) were added into the P1/δ-FeOOH system, and incubated 30 minutes at room temperature. As shown in Figure S3A, the fluorescence intensity of P1/δ-FeOOH gradually increases with the increase of concentration of target DNA in addition to the system. A linear relationship with T1 concentration in the range of 0-20 nM was observed and a detection limit of 0.3 nM is achieved (Figure S3B).



Scheme 1 The Scheme of the δ-FeOOH-based biosensor for DNA detection.

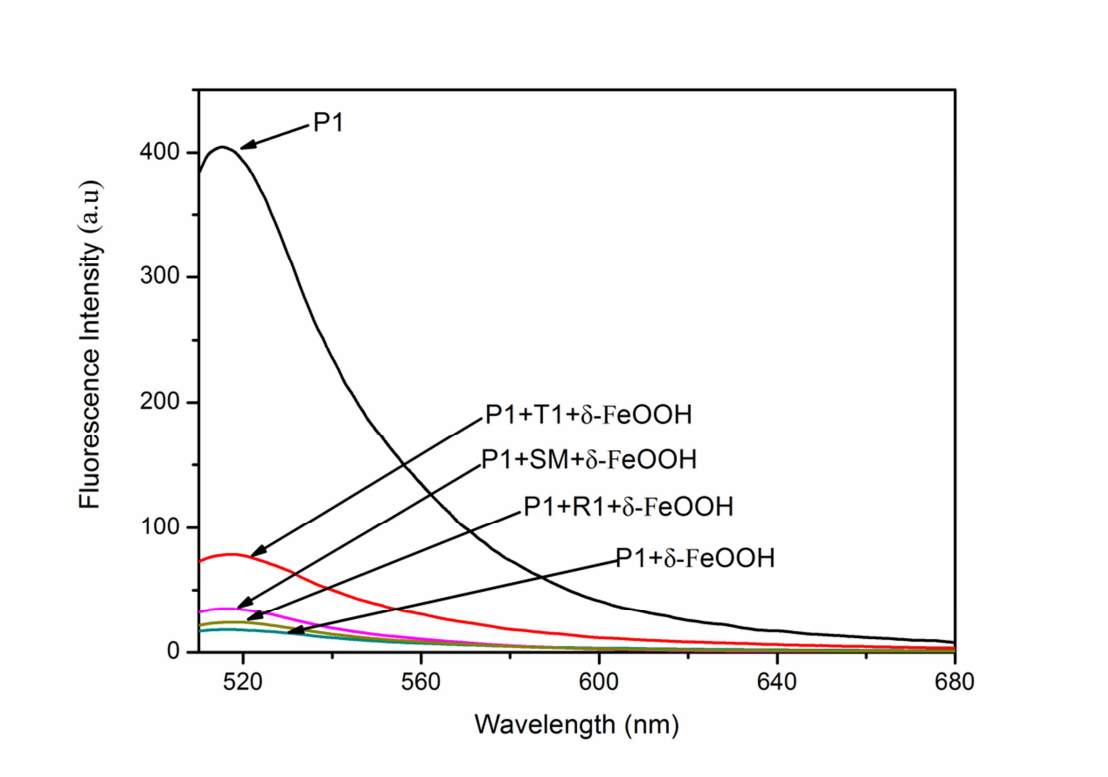
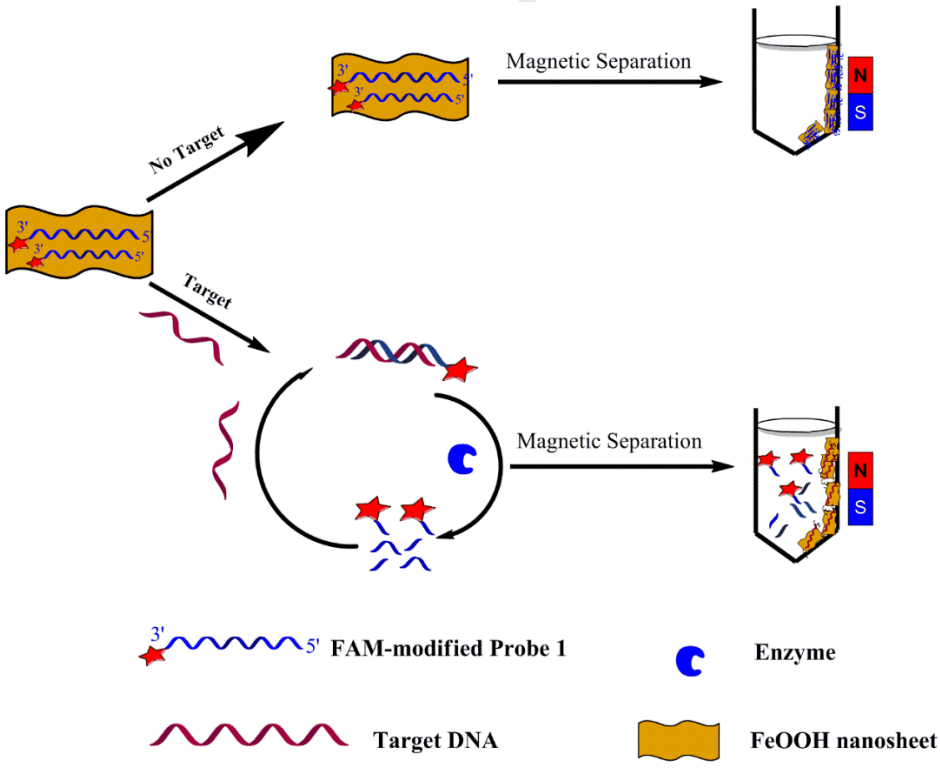


Figure 3. Fluorescence spectra of P1 (20 nM) under different conditions: P1; P1 + T1 (20 nM) + δ-FeOOH; P1 + T2 (20 nM) + δ-FeOOH; P1 + R1 (20 nM) + δ-FeOOH; P1 + δ-FeOOH.

3.4 The principle and feasibility of amplified δ-FeOOH-based strategy for DNA detection

Although the quenching efficiency of P1 by δ-FeOOH nanosheets reaches 95%, the residual fluorescence signals in solution still impact the detection sensitivity. Considering the magnetic separation ability of δ-FeOOH nanosheets, we further design a background eliminated fluorescence sensing platform to improve the detection sensitivity of HCV DNA based on magnetic separation. Additionally, we also use the enzyme-aided target recycle to realize signal amplification. The detection strategy is presented in scheme 2 and the feasibility of the designed background eliminated fluorescence sensing platform for the detection of HCV DNA was also performed (Figure 4). The fluorescence of P1 (curve a) was quenched after mixed with δ-FeOOH nanosheets (curve e). After adding an external magnetic field, the

P1/δ-FeOOH nano-complex were separated from the solution, the residual fluorescence signals from no complete quenched fluorescence can be readily and thoroughly cleared away, which eliminate the background signal in the solution (curve f). In the presence of the target DNA (T1), a duplex DNA with a recessed 3' terminus was formed. The quenched fluorescence was partially recovered because duplex DNA has a weaker affinity with δ-FeOOH nanosheets than P1 (curve d). When introducing Exo III to the hybridization process, the P1 in the duplex DNA would be stepwise hydrolyzed to the mononucleotides by Exo III, resulting in the release of the fluorophore and T1 to the solution. The released T1 would bind with another P1 and then initiate the next round of cleavage (curve b). Hence, the restoration of fluorescence can be applied to the quantitative analysis of the target. These results suggested that the introduction of δ-FeOOH nanosheets and Exo III achieves the combination of low background signals and signal amplification, so that it can be used as an effective low background fluorescent sensing platform for DNA detection.



Scheme 2. The principle for detection of DNA by combination of magnetic separation and enzyme-aided target recycle signal amplification.

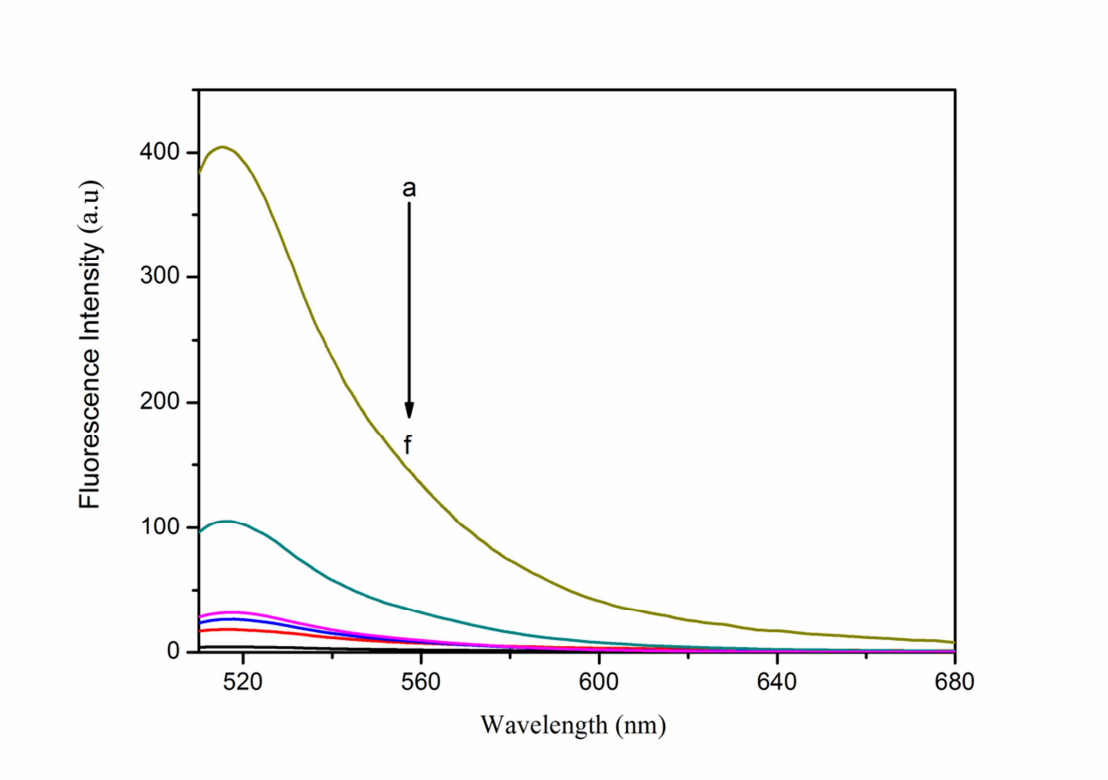


Figure 4. Fluorescence emission spectra of P1 under different conditions: P1(curve a); P1 + δ-FeOOH + magnetic separation + T1 + Exo III (curve b); P1 + δ-FeOOH + magnetic separation + T1 (curve c); P1 + δ-FeOOH +T1 (curve d); P1 + δ-FeOOH (curve e); P1 + δ-FeOOH + magnetic separation (curve f). [P1] = 20 nM, [T1] = 5 nM, [δ-FeOOH] = 20 μg/mL, [Exo III] = 10 U/mL.

3.5 Optimal conditions of amplified the sensor system

The effect of the amounts of δ-FeOOH on the fluorescence quenching of P1 before and after magnetic separation was also investigated. As displayed in Figure 5, with increasing the amount of δ-FeOOH nanosheets, the fluorescence intensity of P1 gradually decreased. This may be due to more δ-FeOOH nanosheets result in more efficient adsorption of ssDNA, which leading the more quenching the fluorescence of P1. When the concentration of δ-FeOOH nanosheets was 20 μg/mL, the quenching efficiency could reach 95%. However, by using an external magnetic field, we found that the background fluorescence signal could be eliminated when the concentration of δ-FeOOH nanosheets 15 μg/mL. Therefore, 15 μg/mL of δ-FeOOH nanosheets was selected in further experiments.

In the fluorescence signal amplification process, the sensitivity has been dramatically

influenced by the amount of Exo III. In order to get the optimal amount of Exo III, the fluorescence intensity enhancement of this sensor system in the presence of T1 was investigated with different amounts of Exo III. As shown in Figure 5B, the signal amplified effect was gradually enhanced when the amount of Exo III increased from 0-20 U/mL, and then the fluorescence enhancement was decreased by further increasing the concentration of Exo III. This may be attributed that the signal probe P1 could be digested by an excess amount of Exo III in the absence of T1 as well, which causes high background noise [41-44]. Therefore, 20 U/mL Exo III was selected for further amplification experiments.

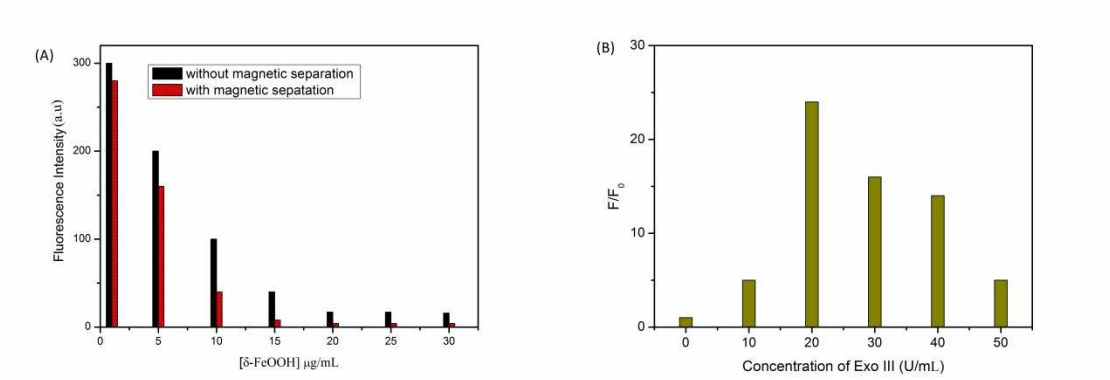


Figure 5. (A) Comparison of fluorescence intensity of P1 quenched by different concentrations of δ-FeOOH nanosheets with or without magnetic separation; (B) The effect of Exo III concentration on the fluorescence enhancement of the sensing system. F_0 and F are the fluorescence intensity of this strategy in the absence and of presence 5 nM of target DNA, respectively. δ-FeOOH nanosheets concentration is 15 μg/mL.

3.6 The sensitivity of the amplified sensor system for the DNA detection

Under the optimized conditions, we recorded the changes of the fluorescent spectra of the biosensor under the different concentrations of T1. As shown in Figure 6A, when the concentration of T1 in the range of 0-20 nM, the fluorescence intensity of the sensor system was significantly increased, suggesting that the fluorescent sensing platform is effective in the sensitive detection of DNA. Based on the calibration curve (Figure 6B), the fluorescence intensity is found to exhibit a linear relationship with the concentration in the range of 0.05-5 nM. The correlation equation was F = 30.072 × [T1] (nM) + 5.524 (R^2=0.998)，where F is the fluorescence intensity. The limit of detection (LOD) was estimated to be as low as 0.01 nM based on 3σ/S (where σ is the

standard deviation of blank measurements and is measured as 0.107 (n = 11), the S is the slope of the linear equation). In comparison with other nano-sensor platforms for DNA detection (Table S2), our platform detection limit is much lower than that of most of the previous reported fluorescence sensors for the DNA detection, even with the amplification efforts. What more, compared with conventional 2D nanosheet, the δ-FeOOH exhibits the distinct advantages of very abundant low cost and straightforward synthesis procedure, which greatly reduced the detection cost.

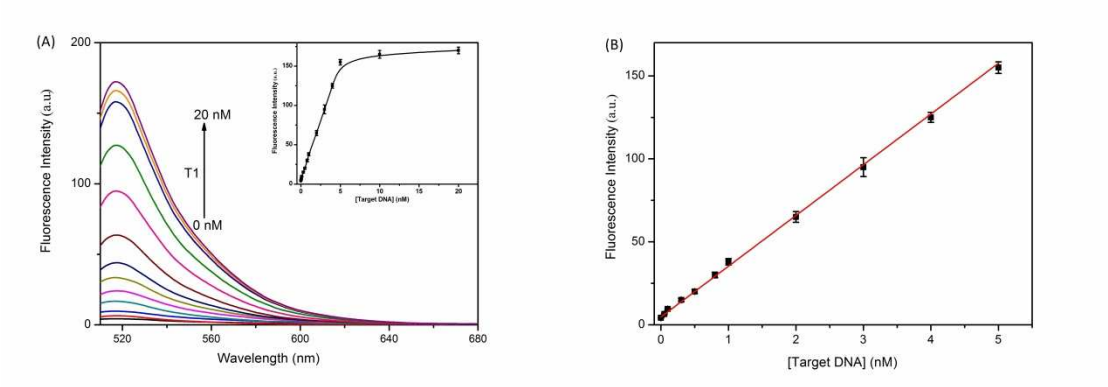


Figure 6 (A) Fluorescence emission spectra of P1/δ-FeOOH incubating with different concentrations of T1 in the presence of Exo III after magnetic separation. (From bottom to top: 0, 0.05, 0.1, 0.3, 0.5, 0.8, 1.0, 2.0, 3.0, 4.0, 5.0, 10.0 and 20.0 nM), the inset shows the relationship between the fluorescence intensity and T1 concentration; (B) the linear relationship in the concentration ranging from 0.05 to 5.0 nM. [P1] = 20 nM, [δ-FeOOH] = 15 μg/mL, [Exo III] = 20 U/mL.

3.7 The selectivity of the sensing system

In order to further assess the selectivity of the detection platform, the fluorescence response of the P1/δ-FeOOH complex for the complementary target DNA (T1), the single-base mismatch target DNA (T2), three-base mismatch target DNA (T3), four-base mismatch target DNA (T4) and random sequence DNA (R1) were detected under the same experimental conditions. As shown in Figure 7, it could be seen that the fluorescence intensity of P1/T1 was much higher than that of the mismatch DNA sequence. These observations demonstrate that this enzyme-aided signal amplification nano-biosensor exhibits superior selectivity for the target DNA, and shows feasible in the actual sample DNA detection.

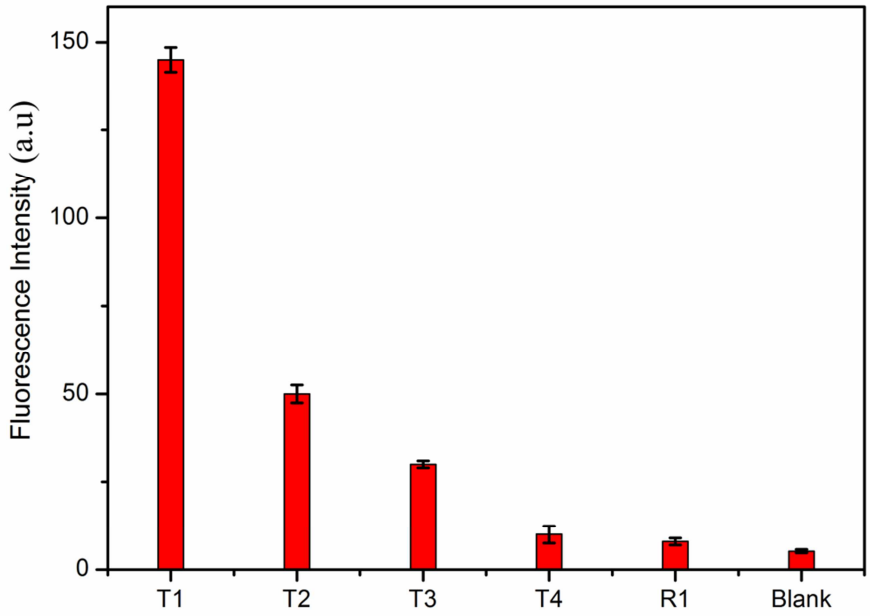


Figure 7 Selectivity of the P1/δ-FeOOH platform detecting target DNA T1 (5.0 nM) over other mismatched DNA (5.0 nM): T2 (single-base mismatched target), T3 (three-base mismatched target), T4 (four base mismatched target), T5 (random sequence DNA). [P1] = 20 nM, [δ-FeOOH] = 15 μg/mL, [Exo III] = 20 U/mL.

3.8 Fluorescent Sensing of HCV DNA in spiked serum samples

To demonstrate the potential applicability of this strategy, the sensing system was applied to measure the concentration of target DNA in spiked human serum samples. The serum samples were obtained from healthy volunteers provided by Xingyang People's Hospital (Xingyang, China). The spiked human serum samples were prepared by adding different concentrations of target DNA to 10-fold diluted human serum samples solution. A standard addition method was used to determine the amount of DNA in these spiked samples. The results were shown in table 1, it can be seen that, the recovery of target DNA detection were between 98.5% and 101.6% and the relative standard deviations (RSD) were all below 3%, which suggests that this sensing strategy has the great potential for the detection of target DNA in human serum samples.

Table 1. Recovery Analysis of HCV DNA in Spiked Human Serum Samples

sample	added (pM)	measured (pM)	Recovery (%)	RSD (%, n=3)
1	100	98.5	98.5	1.26
2	250	252	100.8	2.82
3	500	508	101.6	2.28

Conclusions

In summary, we for the first time demonstrate that δ-FeOOH nanosheets exhibit differential affinity toward ssDNA versus dsDNA and shown the obvious discriminability quenching ability toward dye-labeled ssDNA vis dye-labeled dsDNA. Base on this finding, we have constructed a novel fluorescence biosensor for the DNA detection by using δ-FeOOH nanosheets as the fluorescence quencher. The detective limit was 0.3 nM for the hepatitis C virus DNA detection. Consider the magnetic separation ability of δ-FeOOH nanosheets, a low background fluorescence sensing platform to improve the sensitivity of HCV DNA was designed based on the enzyme-aided signal amplification and magnetic separation for the first time. An enhanced detective sensitivity was achieved with a lower detection limit (10 pM), which is much lower than previously reported. Additionally, this δ-FeOOH nanosheets also possesses good biocompatibility, easy preparation and low cost. Therefore it could be applied in the area of cell imaging, biomedical research, and other biological applications.

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►A novel low background fluorescence assay for high sensitivity and selectivity detection of hepatitis C virus DNA is developed.

► Low-cost δ-FeOOH nanosheet was used as the novel fluorescent quencher.

►By using δ-FeOOH nanosheet integrating with exonuclease III-assisted target-recycling signal amplification, the detection limit was highly improved.

► The limit of detection is as low as 10 pM, which is much lower than most of previous reported fluorescence sensors for hepatitis C virus DNA detection.

The authors have declared no conflict of interest.