Biosensors and Bioelectronics 61 (2014) 351–356

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Ultrasensitive fluorescence detection of nucleic acids using exonuclease III-induced cascade two-stage isothermal amplification-mediated zinc (II)-protoporphyrin IX/G-quadruplex supramolecular fluorescent nanotags

Qingwang Xue [a], Yanqin Lv [a], Yuanfu Zhang [a], Shulin Xu [a], Rui Li [a], Qiaoli Yue [a], Haibo Li [a], Lei Wang [a], Xiaohong Gu [b], Shuqiu Zhang [b], Jifeng Liu [a,*]

[a] Department of Chemistry, Liaocheng University, Liaocheng, 252059, Shandong, China
[b] Shandong Provincial Key Lab of Test Technology on Food Quality and Safety, Shandong Academy of Agricultural Sciences, Jinan 250100, China

ARTICLE INFO

Article history:
Received 6 March 2014
Received in revised form
8 May 2014
Accepted 22 May 2014
Available online 27 May 2014

Keywords:
Fluorescence detection
ZnPPIX/G-quadruplex structure
Magnetic nanoparticles
Exonuclease III
Biosensor
Two-stage isothermal amplification

ABSTRACT

A cascadic sensing system was developed for detection of DNA target at ultralow concentration by a combination of magnetic nanoparticles (MNPs) and exonuclease III (Exo III)-induced cascade two-stage isothermal amplification in the study. An ingeniously designed capture hairpin probe (CHP) that integrates target-binding and signal transduction sequences within one multifunctional design was assembled on MNPs. Upon sensing of the analyte nucleic acid, the hairpin probe on MNPs could be opened and stepwise removed by Exo III accompanied by the releasing of target DNA for the successive hybridization and cleavage process and the generation of bare signal transduction sequences of CHP as a new trigger for next circular reaction. The new DNA triggers initiate hybridizing with hairpin DNA probe that contains a partially "caged" G-quadruplex sequence (GHP), forming a duplex structure and liberating the active G-quadruplex structure. Then, Exo III digests the resulting duplex domain, leading to the recycling of new DNA trigger and simultaneously generating numerous ZnPPIX/G-quadruplex supramolecular complexes with the help of the zinc (II)-protoporphyrin IX (ZnPPIX), as an optical label for amplified fluorescence sensing event. Finally, numerous liberated cascade ZnPPIX/G-quadruplex supramolecular complexes give a remarkable fluorescence response. Because of two-stage autocatalytic recycling amplification and the specifically catalyzed formation of ZnPPIX/G-quadruplex supramolecular complexes, this newly designed protocol provides a high sensitivity with a detection limit of 0.75 fM, can discriminate mismatched DNA from perfectly matched target DNA, and gives low matrix effect due to using MNPs as the separation and amplification elements in the real samples. Therefore, it holds great potential for early diagnosis in gene-related diseases.

1. Introduction

Highly sensitive and selective detection of ultra-low concentrations of specific DNA is extremely important in gene therapy, mutation analysis and clinical diagnosis. Detecting and quantifying trace amounts of specific DNA sequences have relied heavily upon both various signal amplification methods and target amplification (Goodrich et al., 2004; Z. Li et al., 2008; Zhao et al., 2003). Fluorescence detection techniques are one of the most popular detection techniques due to the features of stability, robustness and detection sensitivity. In signal amplification, multi-labeling strategy is the most commonly used technique, such as core–shell structured labeling based on an idea of holding multiple signal reporters in a larger container and the assembly of nano- or micro-architectures on surfaces with dendritic-type signal reporters by virtue of bridge molecules (Chai et al., 2010). Although the protocols based on multi-labeling strategies as amplifying labels can achieve very high sensitivity for target assays, these methods usually involve multiple assay steps and require the addition of many exogenous reagents, and, the doped reagents readily leak in core–shell structured labeling. These problems limit the practical applications of these protocols. Thus, it is highly desired to exploit a new strategy that is sensitive, selective and low-cost with a simple analytical process, which is of great value in practical applications for DNA assays.

* Corresponding author. Tel./fax: +86 635 8239001.
E-mail address: liujifeng111@gmail.com (J. Liu).

http://dx.doi.org/10.1016/j.bios.2014.05.047
0956-5663/

Recently, many DNA amplification techniques based on various principles have been reported, such as polymerase chain reaction (PCR) (Barany, 1991), ligase chain reaction (LCR) (Saiki et al., 1988), strand displacement amplification (SDA) (Zhou et al., 2013) and rolling circle amplification (RCA) (Zhao et al., 2008). Although a high sensitivity can be achieved, these amplification protocols share the drawbacks of complex handling procedures, vulnerability to contamination and tedious polymerase replication process (Zhao et al., 2011). Recently, nicking endonuclease signal amplification (NESA) is a newly developed method that is promising for sensitive DNA detection (J.J. Li et al., 2008). Although promising, one of the common drawbacks is the need of specific target sequence for enzyme recognition. To overcome this limitation, exonuclease III (Exo III) which did not require any specific enzymatic recognition sequence and could catalyze the stepwise removal of mononucleotides of duplex DNA was advocated. Contrary to the nicking endonuclease-based target recycling amplification which requires specific target sequence, Exo-III assisted target recycling amplification does not require any specific recognition sequence, making it an ideal candidate for the development of universal detection platforms. Exo III-assisted target recycling has been applied for sensitive analysis of DNA in combination with molecular beacons (Zuo et al., 2010), semiconductor quantum dots (Freeman et al., 2011), and graphene or carbon nanotube (Chen et al., 2012; Peng et al., 2012). These methods mainly relied on the fluorescence resonance energy transfer (FRET) process. Nearly all of these reported studies were involved in fluorophore-label probes. The fluorescently labeled probes bring about complexity and high cost which may limit its practical use. Moreover, the labeled fluorophore might not be completely quenched, which increased the background. Recently, the use of functional nucleic acid nanostructures that act as optical labels or amplifying optical labels for sensing is extensively studied, which avoid the tedious process of fluorescently labeled probes and amplify the signal intensity (Zhang et al., 2012). The G-quadruplex is one of the most extensively studied nucleic acid nanostructures acting as host for molecular fluorophores or catalysts that lead to supramolecular complexes that provide optical labels for sensing events (Juskowiak, 2006; Kosman and Juskowiak, 2011). Recently, a supramolecular structure, zinc(II)-protoporphyrin IX/G-quadruplex, as an optical label was reported for the amplified fluorescence detection of DNA (Zhang et al., 2012). However, it shows poor sensitivity. Consequently, the development of a novel, label-free and signal-amplified approach for further improving sensitivity is highly desirable.

In the present study, we developed a label-free, isothermal, and ultrasensitive fluorescence DNA cascadic sensing system based on the combination of the designed hairpin probe functionalized magnetic nanoparticles (MNPs) and exonuclease III (Exo III)-assisted quadratic amplification strategy responsive cascade ZnPPIX/G-quadruplex supramolecular fluorescent nanotags as the sensing element. In the sensing system, an ingeniously designed capture hairpin probe (CHP) that integrates target-binding and signal transduction sequences within one multifunctional design was assembled on MNPs for target DNA recognition. MNPs can provide larger immobilization area than the planer structures, leading to higher loading of biomolecules. Importantly, using MNPs as the separation and amplification elements could effectively reduce the background signal and the interference from the real samples. A hairpin DNA probe that contains a partially "caged" G-quadruplex sequence (GHP) was engineered for target DNA detection probe, which can effectively improve the signal-to-noise (S/N) ratio. The zinc (II)-protoporphyrin IX (ZnPPIX) fluorophore can incorporate into the G-quadruplex nanostructure and use the resulting supramolecular structure, ZnPPIX/G-quadruplex, as an optical label for amplified fluorescence detection of DNA. The sensing system takes advantage of Exo III-induced cascade two-stage autocatalytic recycling amplification to generate numerous ZnPPIX/G-quadruplex supramolecular complexes to achieve quadratic signal amplification. This designed protocol provides an ultrasensitive fluorescence detection of DNA down to the 0.75 fM level, can discriminate mismatched DNA from perfectly matched target DNA, and has a low matrix effect in the real biological sample analysis. Therefore, this approach provides a potential platform for highly sensitive fluorescence detection of various biomarkers for early disease diagnostic applications.

2. Experimental section

2.1. Chemicals and materials

All the HPLC-purified oligonucleotide sequences were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and listed below: the capture hairpin DNA probe (CHP), 5′-biotin-$(CH_2)_3$-TTTTTTGCGCGCGCAATA GGGTT GATAGACTGATGACCG-TACCGCACCCTATTGCGCGCGCTATTA -3′; the "caged" inactive G-quadruplex hairpin DNA probe (GHP), 5′-**GGG TTG GGC GGG ATG GG TT *TCTA TCA ACC C TATTG***-3′; the 35-base target DNA (t-DNA), 5′-TAA TAG CGC GCG CAA TAG GCA GCG GTA CGC GCA GT-3′; one-base mismatched DNA, 5′-TAA TAG CGC **C**CG CAA TAG GCA GCG GTA CGC GCA GT-3′; two-bases mismatched DNA, 5′-TAA TAG CGC **CA**G CAA TAG GCA GCG GTA CGC GCA GT-3′; non-complementary target DNA, 5′-CAT GTA CGT GCC GGA CGT TAC AGT CGT AGC GCA GT-3′. Streptavidin-MNPs (830 nm diameter, aqueous suspension containing 100 mM borate, 0.1% BSA, 0.05% Tween-20, and 10 mM EDTA at a concentration of 1.527×10^{10} beads mL^{-1}) from Bangs Laboratories Inc. (Fishers, IN). Protoporphyrin IX zinc (II) (ZnPPIX) was purchased from Sigma-Aldrich and used without further purification. Exonuclease III (Exo III) were purchased from New England BioLabs (Ipswich, MA, USA). Other chemicals (analytical grade) were obtained from standard reagent suppliers. Water ($\geq$ 18.2 MΩ) was used and sterilized throughout the experiments.

2.2. Apparatus

All the fluorescence measurements were performed on a Hitachi F-7000 spectrofluorimeter (Hitachi, Japan). The excitation wavelength was 416 nm, and the spectra are recorded between 570 and 660 nm. The fluorescence emission intensity was measured at 592 nm.

2.3. Functionalization of MNPs with CHP DNA probe

Before functionalization of MNPs, 2 μL (10 mg/mL) streptavidin-MNPs were firstly washed three times with 500 μL of TTL buffer to remove surfactants. Then, 90 μL of PBS buffer was added. Subsequently, biotin-CHP was conjugated to the streptavidin-MNPs by adding 10 μL (20 μM) and gently shaking for 120 min at 37 °C. The nonspecific CHP probe was removed with 0.15 mol/L NaOH solution. Then, the CHP-MNBs were washed twice with 100 μL of PBS buffer. All washing steps in this work were performed under a magnetic field.

2.4. Exo III-induced cascade two-stage isothermal amplification detection of nucleic acids

The modified MNPs with CHP probe was then incubated into 50 μL of 10 mM Tris–HCl solution (pH 8.0, 50 mM NaCl, 10 mM $MgCl_2$) containing 1 unit/μL Exo III and different concentrations of target DNA for 90 min at 37 °C. Then, the new DNA trigger (bare signal transduction sequences on the surface of MNPs) was first thoroughly rinsed by using an external magnet and then incubated with 50 μL of 20 mM HEPES buffer (pH 8.0, 50 mM KCl, 150 mM

NaCl) containing 1 unit/μL Exo III and 2.5×10^{-5} mol/L GHP probe for 90 min at 37 °C. Subsequently, the new DNA trigger was removed quickly by magnetic separation and 10 μM zinc porphyrin solution was added to the remainder sample making the final volume of the solution 80 μL. Finally, the mixture solution was incubated at room temperature for 10 min. The fluorescence intensity of the mixture solution was measured at room temperature.

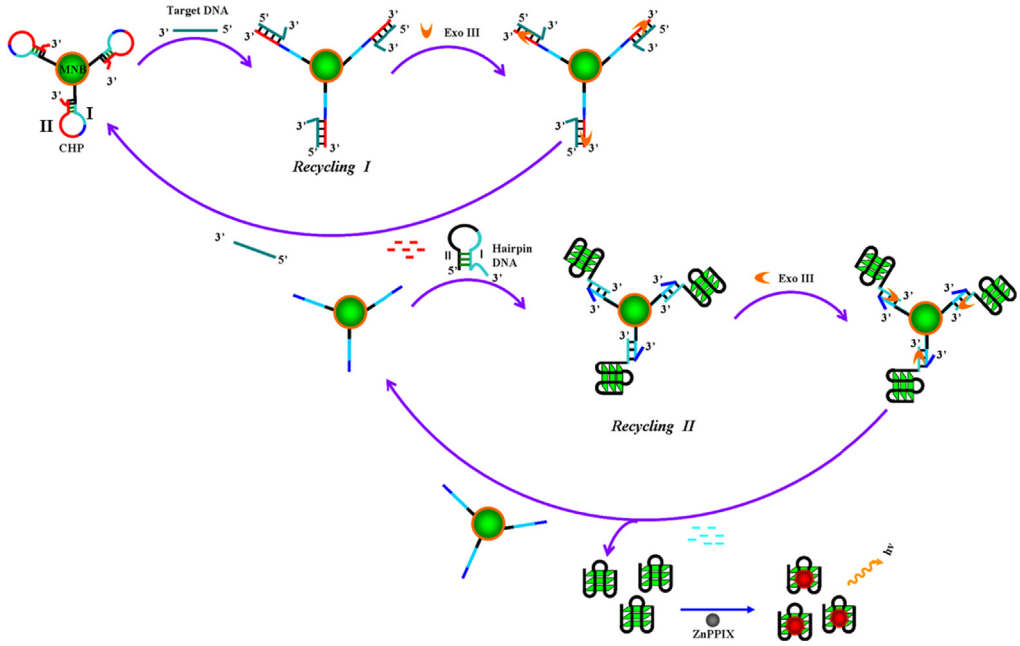
3. Results and discussion

3.1. Design of the proposed sensing system

The designed sensing system consists primarily of a CHP DNA probe assembled on MNPs, a hairpin DNA probe that contains a partially "caged" G-quadruplex sequence (GHP) and Exo III-assisted autocatalytic recycling module, which induced the catalyzed formation of ZnPPIX/G-quadruplex supramolecular complexes for the generation of amplified fluorescence signal. The CHP probe that integrates signal transduction sequences (a region I) and target-binding sequences (a region II) within one multifunctional design was immobilized on the MNPs by streptavidin–biotin interaction. The region I of CHP is complementary to the domain I (blue part) of the sequence of GHP. The region II of CHP is complementary to the sequence of the target DNA. The GHP includes the recognition sequence for the region I of the CHP, domain I (blue part), and a G-quadruplex-forming oligomer which is partially "caged" in the stem region and extends to the loop region of the hairpin, domain II (black part). The caged structure of the G-quadruplex region prohibits the formation of the active G-quadruplex structure. This CHP probe is ingeniously designed with the protruding 3′ end and can not be digested by Exo III which specifically cleaves duplex DNA from blunt or recessed 3′ termini. When the CHP probe is challenged with target DNA, the recognition and hybridization of target DNA with the region II of CHP probe leads the CHP probe to have a blunt 3′-terminus. Exo III then can catalyze the stepwise removal of mononucleotides from this terminus, releasing the target DNA. The released target DNA is free to bind to the region II of another CHP to trigger a new cleavage process, which constitutes the cycle I in Scheme 1. At the same time, the region I of CHP on the surface on MNPs is also bared during each cleavage process, which can be functionalized as a new trigger to activate the following successive cleavage process. The new trigger initiates hybridizing with the domain I of GHP, liberating the active G-quadruplex structure. The new trigger has a longer base sequence than the region I of GHP. After its recognition and hybridization with the domain I of GHP, the second Exo III can also catalyze the cleavage of duplex DNA probe, releasing again the new trigger for the next cleavage cycle and liberating the active G-quadruplex structure. The numerous liberated G-quadruplexes are folded into ZnPPIX/G-quadruplex supramolecular complexes with the help of the ZnPPIX, as an optical label for amplified fluorescence sensing event. This constituted the cycle II in Scheme 1. Based on the autonomous cleavage in cycle I and II, the target DNA trigger can be exponentially produced. Therefore, the current autocatalytic recycling amplification strategy by only a simple Exo III assisted double cleavage process is hopeful to offer an ultrahigh sensitivity for the assay of nucleic acid.

3.2. The verification of the designed sensing system

To investigate the viability of our designed sensing system, Fig. 1 shows the fluorescence emission spectra of the ZnPPIX/G-quadruplex supramolecular fluorescent labels under different conditions. Fig. 1, curve a shows low fluorescence signal intensity of free ZnPPIX in the presence of GHP probe. The fluorescence of ZnPPIX was scarcely influenced by the addition of Eox III (Fig. 1, curve b) because the "caged" inactive G-quadruplex sequence can not been liberated. The caged structure of the G-quadruplex region prohibits the formation of the fluorescence ZnPPIX/G-quadruplex nanostructure. In the presence of only target DNA, the fluorescence of ZnPPIX had a tender enhancement (Fig. 1, curve c), which can be ascribed to the region I of the opened CHP probe triggering the hybridization with the domain I of GHP, liberating the active G-quadruplex structure. However, the hybridization rate is influenced by steric hindrance, leading to the low fluorescence signal intensity. However, the fluorescence of ZnPPIX had a remarkable enhancement upon simultaneous addition of target DNA and Eox III (Fig. 1, curve d), which can be ascribed to the released bare signal transduction sequences of CHP as new trigger in Cycle I.



Scheme 1. Schematic Illustration of the cascadic sensing system based on the designed hairpin probe functionalized magnetic nanoparticles (MNPs) and Exo III-induced cascade two-stage autocatalytic recycling amplification Strategy for Highly Sensitive and Selectivity Detection of Nucleic Acids. (For interpretation of the references to color in this scheme, the reader is referred to the web version of this article.)

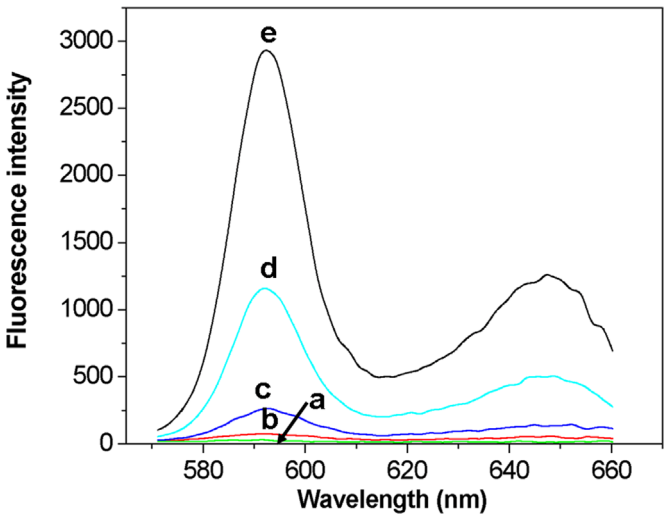


Fig. 1. Fluorescence-emission spectra of ZnPPIX/G-quadruplex supramolecular fluorescent labels under different conditions: (a) MNP-CHP+GHP+ZnPPIX, (b) MNP-CHP+Exo III+GHP+ZnPPIX, (c) MNP-CHP+t-DNA+GHP+ZnPPIX, (d) MNP-CHP+t-DNA+Exo III+GHP+ZnPPIX, (e) MNP-CHP+t-DNA+Exo III+GHP+Exo III+ZnPPIX.

The released bare signal transduction sequences of CHP as new trigger initiate hybridizing with the with the domain I of GHP, liberating the "caged" G-quadruplex sequence in the GHP probe. After further treatment with Eox III again, the fluorescence of ZnPPIX had a dramatically significant increase (Fig. 1, curve e), which can be related to the second Eox III cleavage in cycle II (Scheme 1), the target DNA trigger can be exponentially produced. Although the introduction of Exo III resulted in an increased background fluorescence (Fig. 1, curve b), compared with that in the absence of Exo III (Fig. 1, curve a), fortunately, it also resulted in a much larger signal to background ratio, assuring a higher sensitivity for detection of target. By introducing exonuclease quadratic amplification, we observed a dramatically significant signal increase in the cycle I and II, respectively (in this cases, a target concentration of 10 pM was used). Therefore, the signal amplification of the sensing system is obvious.

To further verify the feasibility of the designed sensing system, differential fluorescence image of MNPs obtained upon analyzing the target DNA in the presence of Exo III and those obtained in a series of control experiments are depicted in Fig. S1. As shown in Fig. S1A, a well-defined fluorescent image of MNPs can be observed in the presence of target DNA and the first Exo III. This fluorescent image can be contributed to the bare signal transduction sequences of CHP functionalized as a new trigger initiating hybridization with the GHP probe. The GHP probe opens to yield the fluorescent ZnPPIX/G-quadruplex structure in the surface of MNPs with the help of ZnPPIX fluorophore. After further treatment with the second Exo III, no bright spots was observed (Fig. S1B), suggesting that the second Exo III can also only catalyze the cleavage of duplex DNA probe, releasing again the new trigger and the ZnPPIX/G-quadruplex structure. However, as a control experiment, in the absence of target DNA, no bright spots can be observed (Fig. S1C), which was almost the same as with that of CHP probe modified MNPs treated only by GHP probe and ZnPPIX (Fig. S1E). In the absence of the first Exo III, dimmed and small spots can be observed (Fig. S1D), which can be ascribed to the region I of the opened CHP probe triggering the hybridization with the domain I of GHP, liberating the active G-quadruplex structure. However, the hybridization efficiency is influenced by steric hindrance, leading to the dimmed and small spots. A statistical analysis on the fluorescence intensity of spot in Fig. S1A and D by investigating 100 bright spots was made. The result is shown in Fig. S1F. These strongly indicated that only target DNA can trigger the cleavage process by the first Exo III to release bare signal transduction sequences of CHP as a new trigger for initiating hybridization with the GHP, opening to yield the fluorescent ZnPPIX/G-quadruplex structure in the surface of MNBs with the help of ZnPPIX fluorophore.

3.3. Optimization of experimental parameter

The assembly density of CHP probe on the MNPs surface would have an important effect on the performance of fluorescence DNA biosensor. The immobilization concentration of CHP probe was first examined on the basis of the fluorescence response toward target DNA detection. It could be found that the immobilization concentration of duplex DNA probe of 2×10^{-5} mol/L could achieve the better fluorescence response than that at other concentrations (Fig. S2). The higher immobilization concentration of CHP probe can increase the assembly density on the MNBs surface, but it may restrict the hybridization efficiency of target DNA to the CHP probe on the surface of MNPs owing to the steric hindrance effect. Also, the densely packed CHP probe will not be favorable for the effective folding of G-quadruplex forming oligomer. These two factors may contribute to the relatively low fluorescence response at high concentration of duplex DNA probe. Thus, the concentration of 2×10^{-5} mol/L CHP probe was chosen as the optimized immobilization concentration for the following DNA detection.

To further verify the signal amplification mechanism of the designed sensing system, a GHP probe concentration-course experiment was conducted. The CHP probe modified MNBs was incubated into a mixture containing 10 pM target DNA and 1 units/μL Exo III in Tris–HCl buffer (pH 8.0, 50 mM NaCl, 10 mM $MgCl_2$) at 37 °C for 90 min. Then, the released new trigger (bare signal transduction sequences on the surface of MNBs) was incubated with 50 μL of HEPES buffer (20 mM HEPES, pH 7.0, 50 mM KCl, 150 mM NaCl) containing 1 unit/μL Exo III and different concentrations of GHP probe from 1 μM to 50 μM for 90 min at 37 °C. After that, the new DNA trigger was removed quickly by a magnetic separation and 10 μM zinc porphyrin solution was added to the remainder sample; the fluorescence signal was measured and plotted against the concentrations of GHP. As shown in Fig. S3, the fluorescence signal increased with bigger concentrations of GHP probe of incubation before reaching saturation after 25 μM. The continuously increasing signal indicates that the designed Exo III-induced cascade two-stage isothermal amplification was indeed taking place, while the signal saturation at 25 μM GHP probe suggests the signal transduction sequence of CHP probes was almost liberated from CHP probe for triggering hybridization with GHP, opening to liberate G-quadruplex structure. The surface coverage of CHP probe on magnetic particles under the optimum condition had also been determined on the basis of measuring the concentration of CHP probe solution added and washed down after magnetic separation, which was determined by the absorbance at 260 nm using ultraviolet spectrophotometry. It was estimated to be 6.75 $(\pm0.19)\times10^{-12}$ mol/cm^2 based on three independent experiments.

One of the key factors that affects the assay performance of the proposed method is exonuclease reaction time. In order to achieve desirable analytical characteristics, the exonuclease reaction time was optimized in cycle I and II. The effect of the reaction time on the signal output of the proposed method was investigated by monitoring the fluorescence intensity response at a time interval of 15 min from 15 to 120 min. As displayed in Fig. S4, the fluorescence intensity increases rapidly with increasing reaction time in the range from 15 to 90 min and reaches a plateau thereafter. To ensure complete quadratic reactions, a reaction time

of 90 min was selected for subsequent experiments, indicating saturation of the reaction time due to the inactivation of Exo III.

3.4. Detection performance of the assay

The sensitivity of the fabricated fluorescence DNA biosensor based on Exo III-induced cascade two-stage autocatalytic recycling amplification strategy was investigated using different concentrations target DNA. As shown in Fig. 2A, the fluorescence intensity increased when the concentration of target DNA was raised from 0 to 10 pM, indicating that the liberation of the "caged" inactive G-quadruplex structure in the GHP is highly dependent on the concentration of target DNA. This confirms the working principle that the target DNA is hybridized with the CHP probe on the surface of MNB, triggering the Exo III digestion, and finally liberating the "caged G-quadruplex structure in the GHP probe. Fig. 2B shows the fluorescence signal intensity of ZnPPIX/G-quadruplex nanostructure supramolecular as a function of the concentration of target DNA. Under the optimal conditions, the average fluorescence signal intensity showed a good linear correlation with the concentrations of the target DNA ranging from 1 fM to 100 fM. The detection limit of this method was calculated to be 0.75 fM according to the responses of the blank tests plus 3 times the standard deviation (3σ), providing superior detection sensitivity compared with those reported fluorescence methods that involve in the use of nucleases. Reproducibility and accuracy of the fabricated fluorescence DNA biosensor were further examined. The relative standard deviation (RSD) of fluorescence signal to 1 pM target DNA is 3.7% for five parallel measurements, indicating the good precision and desirable reproducibility for DNA detection. The ultrahigh sensitivity and the broad dynamic range were attributed to the following: (i) Exo III-induced cascade two-stage autocatalytic recycling amplification and (ii) MNPs can provide larger immobilization, leading to higher loading of biomolecules effectively reducing the background signal. The results demonstrated that this signal amplification method was efficient for ultrasensitive fluorescence detection of DNA hybridization.

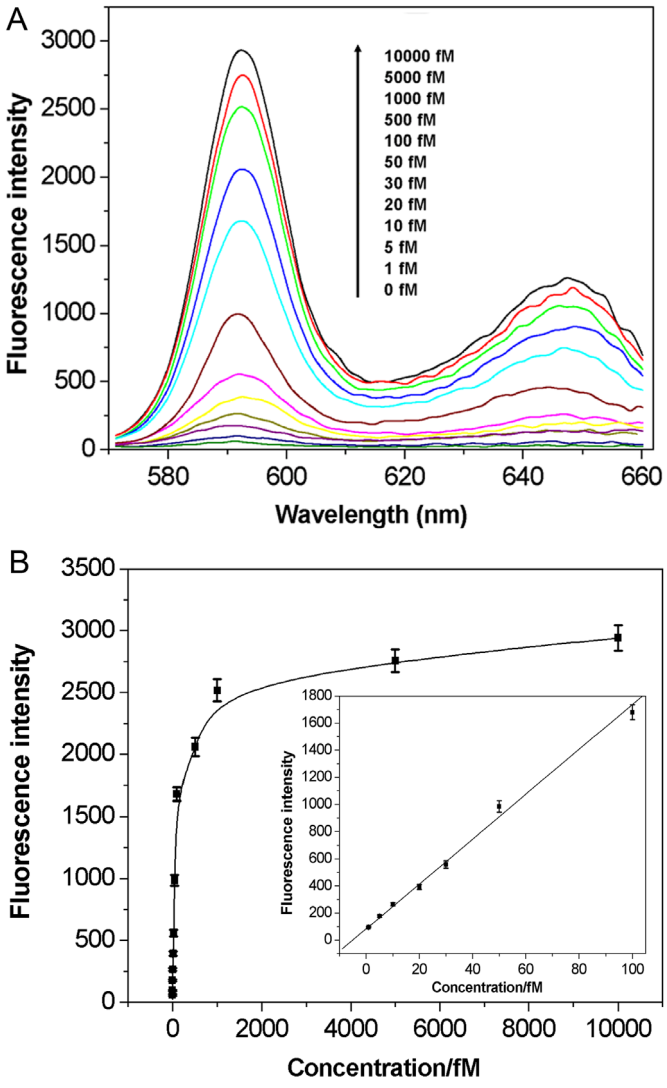


Fig. 2. (A) Fluorescence emission spectra obtained in the quadratic amplification strategy for detection of target DNA with varying concentrations from 0 to 10 pM. (B) Calibration curve of fluorescence intensity changes at 592 nm as a function of the concentration of target DNA. Each data point represents an average of 3 measurements (each error bar indicates the standard deviation).

3.5. Selectivity of the assay

The specificity of the proposed sensing system was further investigated by exposing the immobilized CHP probe to four kinds of DNA sequences, including perfect complementary target DNA, single-base mismatched DNA, two-bases mismatched DNA, and non-complementary DNA at the same concentration of 10 pM. As shown in Fig. 3, the fluorescence signal intensity for one-base mismatched DNA and two bases mismatched DNA was only about 29.2% and 15.4% of that for perfect target, respectively. The non-complementary target DNA shows almost the same response with the blank solution. This high specificity could be attributed to the impaired hybridization ability of mismatched DNA with CHP probe and the limited Exo III digestion property for the mismatched DNA duplex. Thus, this method exhibited a good performance to discriminate perfect complementary target and the mismatched targets and great potential for single nucleotide polymorphism analysis.

The feasibility of the designed sensing system for practical applications was also investigated by analyzing complex biological fluids: 50% cell culture fluid and 50% human serum. In the 50% biological fluids, different concentrations of target DNA were detected. The results in Fig. 4 showed that the sensing platform was not affected when target DNA existed in these biological fluids. This could be attributed to the separation by MNBs which

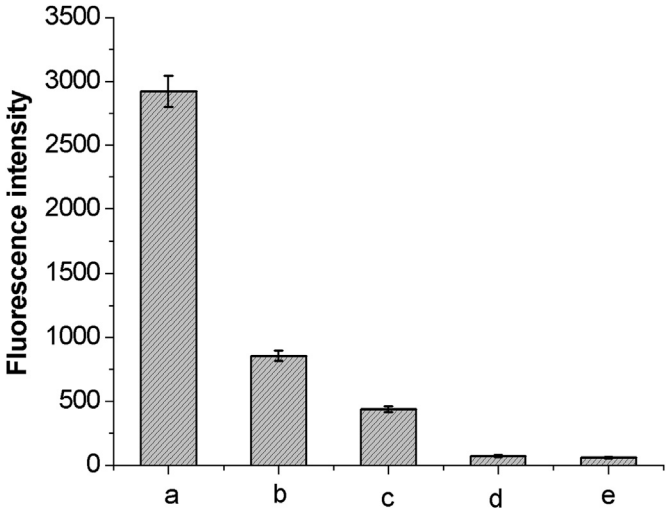


Fig. 3. Fluorescence intensity responses at 10 pM of complementary target DNA (a), single-base mismatched DNA (b), two-bases mismatched DNA (c), non-complementary target DNA (d), and the absence of DNA (e), Each data point represents an average of 3 measurements (each error bar indicates the standard deviation).

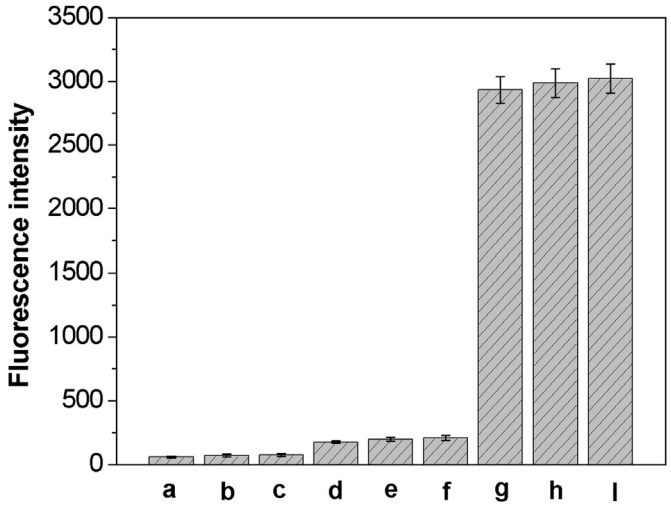


Fig. 4. Feasibility of the approach in complex biological matrices: (a) binding buffer, (b) 50% cell culture fluid, (c) 50% human serum, (d) 5 fM target in binding buffer, (e) 5 fM target in 50% cell culture fluid, (f) 5 fM target in 50% human serum, (g) 10 pM target in binding buffer, (h) 10 pM target in 50% cell culture fluid, (i) 10 pM target in 50% human serum. Conditions: Each data point represents an average of 3 measurements (each error bar indicates the standard deviation).

may reduce the serum-induced optical background. The results also definitely illuminated the potential application of this sensing system in practical samples. In addition, we determined that the proposed fluorescence DNA biosensor can also be developed as a versatile approach for the sensing of any DNA sequences (see Fig. S5, ESI†).

The stability for the CHP probe modified MNPs has also been examined. Five independent experiments demonstrated that the CHP probe modified MNPs could retain about 95% of its initial response toward target DNA after its storage in the refrigerator at 4 °C over one week, showing a relatively robust stability of the CHP probe modified MNBs.

4. Conclusions

In summary, we have developed a label-free fluorescence cascadic sensing system for ultrasensitive DNA detection using magnetic nanoparticles (MNPs) and Exo III-induced cascade two-stage isothermal amplification. The sensing system possesses several excellent features. First, it provides an ultrasensitive fluorescence detection of DNA down to the 0.75 fM level only within approximately 3 h. Besides, the developed method also exhibits high selectivity toward target DNA against other interfering molecules and great potential for single nucleotide polymorphism analysis. Second, the current protocol is really simple and avoids complex procedures for the achievement of target DNA quadratic amplification. It only requires one exonuclease to realize quadratic amplification for sensitive detection DNA without the need for multiple enzymes required in common signal amplification assays. In addition, using different target-binding sequences specifically designed for individual targets, on the MNBs surface the sensing system could be directly expanded for multiplex DNA detection. Furthermore, it demonstrated that the developed method can be used as a versatile approach for the sensing of any DNA sequences, and gives low matrix effect due to using MNPs as the separation and amplification elements in the real samples. Therefore, the proposed sensing system holds a great potential for early diagnosis in gene-related diseases

Acknowledgments

This project was supported by the National Natural Science Foundation of China for Funding (21305058, 21005036, 21075058, 21127006, 21105041). This work was also supported by Natural Science Foundation (ZR2010BZ004, JQ201106). This work was also supported by Shandong Academy of Agricultural Sciences (SDAAS201301).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.flora.2014.02.009.

References

Barany, F., 1991. Proc. Natl. Acad. Sci. USA 88 (1), 189–193.
Chai, Y., Tian, D., Wang, W., Cui, H., 2010. Chem. Commun. 46, 7560–7562.
Chen, H., Wang, J., Liang, G., Zhang, P., Kong, J., 2012. Chem. Commun. 48, 269–271.
Freeman, R., Liu, X., Willner, I., 2011. Nano Lett. 11, 4456–4461.
Goodrich, T.T., Lee, H.J., Corn, R.M., 2004. J. Am. Chem. Soc. 126, 4086–4087.
Juskowiak, B., 2006. Anal. Chim. Acta 568, 171–180.
Kosman, J., Juskowiak, B., 2011. Anal. Chim. Acta 707, 7–17.
Li, J.J., Chu, Y., Lee, B.Y., Xie, X.S., 2008a. Nucleic Acids Res. 36, e36.
Li, Z., Hayman, R.B., Walt, D.R., 2008b. J. Am. Chem. Soc. 130, 12622–12623.
Peng, L., Zhu, Z., Chen, Y., Han, D., Tan, W., 2012. Biosens. Bioelectron. 35, 475–478.
Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A., 1988. Science 239, 487–491.
Zhang, Z., Sharon, E., Freeman, R., Liu, X., Willner, I., 2012. Anal. Chem. 84, 4789–4797.
Zhao, C., Wu, L., Ren, J., Qu, X., 2011. Chem. Commun. 47, 5461–5463.
Zhao, W., Ali, M.M., Brook, M.A., Li, Y., 2008. Angew. Chem. Int. Ed. 47, 6330–6337.
Zhao, X., Tapec-Dytioco, R., Tan, W., 2003. J. Am. Chem. Soc. 125, 11474–11475.
Zhou, H., Xie, S.J., Zhang, S.B., Shen, G.L., Yu, R.Q., Wu, Z.S., 2013. Chem. Commun. 49, 2448–2450.
Zuo, X., Xia, F., Xiao, Y., Plaxco, K.W., 2010. J. Am. Chem. Soc. 132, 1816–1818.