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Sensitive fluorescence detection of DNA using isothermal exponential amplification coupled quantum dots coated silica nanospheres as label†

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A new strategy to combine isothermal exponential amplification (IEA) with CdTe quantum dots (QDs) functionalized silica nanospheres as a label was designed for highly sensitive fluorescent detection of target DNA. A well plate was used as a support to immobilize a molecular beacon (MB) as a recognition probe and perform the IEA procedure. After the MB recognized target DNA and opened its cycle, the stem part could hybridize with a primer to initiate the polymerization of the DNA strand, which led to the release of target to open another MB molecule and start the next cycle of strand-replacement polymerization. Meanwhile, the formed double-stranded DNA was recognized by nicking endonuclease, leading to an endonuclease-based strand-replacement polymerization, which produced a DNA trigger to open more MB. The opened MB molecules were finally bound to the label by biotin–streptavidin coupling. Upon a dissolving process, the released cadmium cation could sensitize the fluorescent emission of Rhod-5N to achieve cascade signal amplification. The proposed method could detect target DNA ranging from 10^{-17} to 10^{-11} mol L^{-1} with a detection limit down to ~50 copies. It also showed high selectivity. This highly sensitive and specific assay has potential to become a promising DNA quantification method in biomedical research and clinical diagnosis.

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Introduction

Sequence-specific DNA detection has attracted significant attention due to the possible applications in various fields ranging from molecular diagnostics to environmental monitoring.[1] Many approaches have been developed for highly sensitive detection of DNA, including fluorescence,[2] electrochemistry,[3] surface-enhanced Raman spectroscopy,[4] electric signal,[5] chemiluminescence[6] and colorimetric detection.[7] Traditionally, the biosensing-type sequence detection of DNA is based on simple hybridization recognition between target and probe DNA, in which each target strand hybridizes with only one probe. To achieve highly sensitive detection, target recycling has recently been developed to allow a single DNA target molecule to interact with multiple nucleic acid-based signaling probes. Typically, the target DNA cycling procedure can be performed *via* nucleases *e.g.* endonuclease,[8] exonuclease[9] and polymerase.[10] These target DNA recycling amplification methods have been widely employed to amplify the electrochemical, optical and visual signals.[11] Furthermore, these strategies can be integrated in a one-pot amplification procedure. For example, a so-called isothermal exponential amplification (IEA) coupling polymerase strand extension and single-strand nicking[12] has emerged as a powerful amplification technique for the detection of DNA. Compared with other amplification methods, the IEA method has the distinct advantages of its isothermal nature, high amplification efficiency (10^6–10^9 fold),[13] and rapid amplification kinetics.[14] This work introduced this technique into molecular beacon (MB)-based biosensing and combined the exponential amplification with a quantum dots functionalized silica nanosphere (QDs/SiNS) label to design a strategy for cascade signal amplification.

QDs are a popular kind of fluorescent material and possess numerous advantageous features, such as broad excitation and narrowband emission spectra, resistance against photobleaching, and feasibility for surface modification.[15] However, the direct utilization of QDs fluorescence for detection of biomolecules suffers from the problems such as biotoxicity of QDs, low quantum yield, and chemical and colloidal instabilities under harsh chemical environments.[16] Fortunately, the sensitizing effects of cadmic cation on fluorescence dyes such as Fluo-4 and Rhod-5N have been reported, which makes the non- or weak fluorescence of dye molecules become highly fluorescent. Thus by dissolving a Cd^{2+}-containing QD label to sensitize the fluorescent signal of

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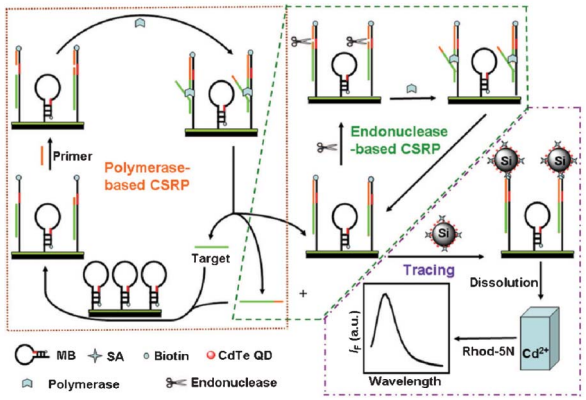

Scheme 1 Schematic illustration of cascade signal amplification by IEA with quantum dot coated silica nanospheres as a label for fluorescent detection of DNA.

these dyes, some methods for fluorescent bioassays have been developed.[17] Here, Cd^{2+}-sensitized fluorescence was used for detection of the IEA product, which was associated with the target DNA sequence and the specific recognition of MB, leading to a highly sensitive and selective method for fast detection of target DNA.

The MB as recognition probe was firstly covalently immobilized on the chitosan-coated inner wall of a well. The biosensing system included two steps: the target recycling amplification process of IEA and the coupling of SA/QD/SiNS for Cd^{2+}-sensitized fluorescence detection (Scheme 1). After the MB recognized the target DNA, its cycle was opened, and the stem part hybridized with a primer to initiate the polymerization of the DNA strand in the presence of polymerase, which led to the release of the target and then another cycle of recognition, polymerization and strand replacement on the MB immobilized surface. Once the duplex beacon was produced, the DNA recognition sequence became a suitable substrate for endonuclease nicking. Thus an endonuclease-based circular strand-replacement polymerization (CSRP) was synchronously initiated. The latter could produce the DNA trigger for opening more MB. The IEA procedure exposed large numbers of biotin groups labeled at the 3′-end of MB for binding QDs/SiNS by biotin–streptavidin (SA) coupling. Sequentially, through the sensitizing effect of Cd^{2+} on the fluorescence of Rhod-5N dye, the highly sensitive detection of DNA was achieved with a detection limit down to the attomolar level. The cascade signal amplification with IEA on support surface and metal cation sensitized fluorescence emission not only provided a sensitive detection strategy in bioanalysis and molecular diagnosis but also promoted the practical applications of IEA.

Experimental

Materials and reagents

Nb.BbvCI nicking endonuclease and Bst large fragment DNA polymerase were obtained from New England Biolabs. Deoxyribonucleotides (dNTPs) were obtained from Fermentas Biotechnology Co. Ltd (Canada). Cadmium chloride ($CdCl_2 \cdot 2.5H_2O$) and mercaptopropionic acid (MPA) were purchased from Alfa Aesar China Ltd. Chitosan (CS, M.W. 100 000–300 000, deacetylation degree $\geqslant$ 95%), glutaraldehyde (GA, 25%), SA, (3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), tetraethoxysilane (TEOS), (3-aminopropyl)triethoxysilane (APTS), polyethylene glycol sorbitan monolaurate (Tween-20), and tris(hydroxymethy)aminomethane (tris) were purchased from Sigma-Aldrich (USA). Rhod-5N was purchased from Invitrogen (USA). DNA hybridization buffer was phosphate-buffered saline (137 mmol L^{-1} NaCl, 2.5 mmol L^{-1} Mg^{2+}, 10 mmol L^{-1} Na_2HPO_4, and 2.0 mmol L^{-1} KH_2PO_4, pH 7.4). DNA storage solution was prepared with Tris-HCl (10 mmol L^{-1}, pH 8.0) containing 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA). Phosphate-buffered salines (PBS, 0.01 mol L^{-1}) of various pHs were prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. The washing buffer was PBS (0.01 mol L^{-1}, pH 7.4) containing 0.05% (w/v) Tween-20. Ultrapure water obtained from a Millipore water purification system (18 MΩ cm, Milli-Q, Millipore) was used in all experiments.

The oligonucleotides were purchased from Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. Their sequences were as follows:[12b]

Target: 5′-CTCT*TCCAGCCTTCCTTCCT*AA-3′.

NH_2-modified MB: 5′-$NH_2(CH_2)_6$GCCGTCG*AGGAAGGAAGG-CTGGA*CCTCAGCGACGGC-$(CH_2)_6$-biotin-3′.

Primer: 5′-GCCGTCGC-3′.

Single-base mismatch: 5′-CTCTTCCAGCGTTCCTTCCTAA-3′.

Non-complementary: 5′-ACACCAAGTAAGGAACCAACGG-3′.

Instrumentation

Photoluminescence spectra were recorded with a F900 fluorescence spectrometer (Edinburgh Instruments Ltd., UK). The transmission electron microscope (TEM) image was observed on a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). X-ray photoelectron spectroscopy (XPS) measurements were performed with an ESCALAB 250 spectrometer (Thermo-VG Scientific, USA) with an ultrahigh vacuum.

Preparation of monodispersed SiNS

Synthesis of monodispersed SiNS was carried out according to the reported seed-growth methods.[18] First, 80 mL of ethanol, 4.8 mL of H_2O, and 3.6 mL of $NH_3 \cdot H_2O$ were added into a 250 mL flask, and heated gradually to 55 °C under constant vigorous stirring. A mixed solution of 3.1 mL of TEOS and 8 mL of ethanol was added to the solution quickly. After maintaining solution temperature at 55 °C for 5 h, the colloidal suspension of SiNS was obtained.

Preparation of QDs coated SiNS (QDs/SiNS)

The water-soluble CdTe QDs were prepared using MPA as stabilizing agent according to a method reported previously.[19] The obtained QDs solution was subjected to ultrafiltration using a Vivaspin concentrator (Sartorius, 10 000 MW) at 10 000*g* for 10 min to remove excess MPA. The upper phase

was washed twice with water and diluted to a certain concentration with pH 7.4 PBS.

For the preparation of QDs coated SiNS, 0.02 g of SiNS was first dispersed in 2 mL of ethanol and treated with 0.4 mL of APTS. After stirring for 6 h, the suspension was centrifuged and washed with ethanol repeatedly for four times, and the amino-functionalized SiNS were obtained. Then, the amino-functionalized SiNS was dispersed in a mixture of 1 mL of QDs (5 mg mL^{-1}) stabilized with NHS (10 mg mL^{-1}) and 1 mL of EDC (20 mg mL^{-1}). The mixed suspension was stirred at 4 °C for 12 h. Unbound QDs were removed by successive centrifugation and washing with water several times. Finally, the obtained QDs/SiNS were dispersed in water to a final volume of 1 mL. To generate the SA/QDs/SiNS label, 1 mL of the above QDs/SiNS suspension was mixed with 1 mL of SA solution (20 μg mL^{-1}). Subsequently, 100 μL of freshly prepared EDC (20 mg mL^{-1}) and 100 μL of NHS (10 mg mL^{-1}) were added. After incubation at room temperature for 2 h, free SA was removed by centrifugation and washed with 0.01 mol L^{-1} PBS for several times to obtain the SA/QDs/SiNS label.

Immobilization of MB

15 μL of 0.25 mg mL^{-1} CS was dropped in a microwell at room temperature. After activating with 2.5% GA for 2 h and washing with water, 15 μL MB (1 μmol L^{-1}) was dropped on the GA-modified microwell for another 2 h to covalently immobilize 5-NH_2 modified MB.[10c] Afterwards, the well was washed with 10 mmol L^{-1} PBS buffer solution.

IEA reaction

50 μL duplication solution containing 10 μL target DNA, 5 μL of primer (1.0 μmol L^{-1}), 3.0 U polymerase, 4.0 U nicking endonuclease and dNTPs (2 mmol L^{-1} for each component) in buffer was dropped in the microwell. The duplication process was allowed to proceed for a certain time at 37 °C, and terminated through washing thoroughly. Then, 15 μL SA/QD/SiNS solution was dropped in the microwell to incubate at room temperature for 1 h, which was terminated through washing with PBS.

Measurement procedure

The captured SA/QD/SiNS on the surface of microwell wall was dissolved with 0.05 mL 0.1 mol L^{-1} HNO_3. The HNO_3-treated mixture was diluted to 0.4 mL with PBS and centrifuged at 1000 g for 5 min to obtain a supernatant. After adjusting the pH of the supernatant to 7.4 using 0.1 mol L^{-1} NaOH, 0.3 mL of the resulting Cd^{2+} solution was mixed with 2 μL 3 μmol L^{-1} Rhod-5N. The fluorescence signal was then detected with a F900 fluorescence spectrometer at room temperature at the excitation wavelength of 540 nm. The emission spectrum from 550 to 650 nm was collected with a slit width of 3 nm.

Gel electrophoresis

A 20% polyacrylamide gel electrophoresis (PAGE) analysis was carried out in 1 × Tris-Borate-EDTA (pH = 8.3) at 100 V constant voltage for about 2 h. After ethidium bromide staining, gels were scanned using a Molecular Imager Gel Doc XR (BIO-RAD, USA).

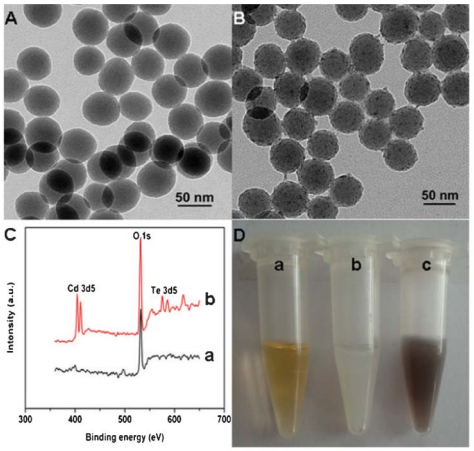

Fig. 1 TEM images of (A) SiNS and (B) QDs/SiNS, (C) XPS spectra of SiNS (a) and QDs/SiNS (b), and (D) photos of CdTe QDs (a), SiNS (b) and QDs/SiNS (c) solution.

Results and discussion

Characterization of SA/QDs/SiNS label

SiNS was synthesized with a seed-growth method reported previously.[18a] The TEM image of the as-prepared SiNS showed a chemically clean and homogeneous structure with a diameter of 50 ± 3.0 nm (Fig. 1A). After APTS was coupled to the hydroxyl group on the SiNS surface, CdTe QDs were covalently immobilized onto the surface of the SiNS through acylamide binding in the presence of EDC and NHS as an activator. The TEM image of the formed QDs/SiNS showed numerous QDs islands on the surface of SiNS (Fig. 1B). Compared with SiNS, its XPS showed two new strong peaks at 405.45 and 573.1 eV (Fig. 1C), which correspond to Cd3d5 and Te3d5, respectively, confirming the assembly of CdTe QDs on the SiNS surface. The coating of CdTe QDs on SiNS was also demonstrated by the color change of SiNS from white to brown under sunlight illumination (Fig. 1D), which could be easily monitored by the naked eye. All these results confirmed that CdTe QDs were successfully attached on the surface of the SiNS. The homogeneous surface structure and the good dispersion of QDs/SiNS in water were favorable for its labeling to protein molecules.

SA/QDs/SiNS conjugates were simply prepared by incubating a mixture of SA and QDs/SiNS solutions in the presence of EDC and NHS as activating reagents. Upon coating SA, the amount of carbon elemental composition increased from 24.14% to 33.93%, while the amount of cadmium, tellurium and sulfur decreased from 1.93%, 0.52% and 0.72% to 0.42%, 0.19% and 0.18%, respectively, which confirmed the successful coupling of SA on QDs/SiNS.

Verification of IEA product and its fluorescent behavior

After target DNA initiated the IEA procedure in the MB-coated well and the IEA product was bound to the SA/QDs/SiNS label, the attached QDs were dissolved from the inner wall. Since the QDs after being dissolved by HNO_3 did no show any fluorescence (ESI†, Fig. S1), it should not interfere with the

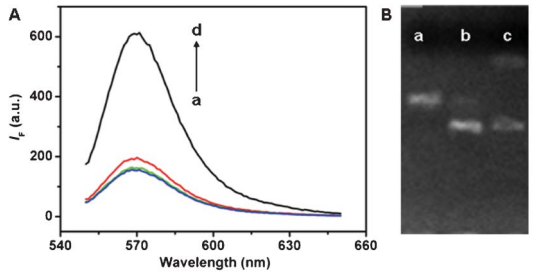

Fig. 2 (A) Fluorescence spectra of Rhod-5N triggered by the solution dissolved from the wall after IEA in blank (a), polymerase/endonuclease (b), 10 fmol L^{-1} target (c) and 10 fmol L^{-1} target + polymerase/endonuclease (d), and (B) PAGE analysis of 0.1 μmol L^{-1} MB (a), 0.1 μmol L^{-1} MB + 0.05 μmol L^{-1} primer + 0.05 μmol L^{-1} target in absence (b) and presence (c) of polymerase/endonuclease. IEA time: 80 min at 37 °C.

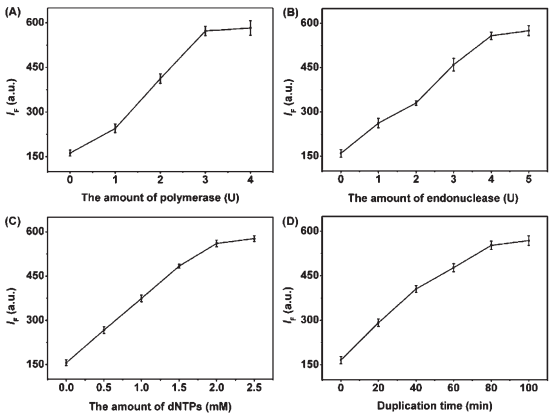

Fig. 3 Dependence of fluorescence intensity for 10 fmol L^{-1} target DNA on the amount of (A) polymerase, (B) endonuclease, (C) dNTPs, and (D) duplication time. When one parameter changes the others are under their optimal conditions (n = 3).

fluorescent detection. The dissolved cadmic cation reporter was then used to trigger the fluorescence of Rhod-5N. As shown in Fig. 2A, the polymerase/endonuclease solution without target did not show an obvious change in fluorescence signal (curves a and b), and the target DNA slightly enhanced the fluorescent peak intensity (curve c). The slight increase resulted from the recognition of the immobilized MB to target DNA, which opened the MB and exposed the biotin group labeled at 3′-end of MB to bind QDs/SiNS. In the presence of polymerase/endonuclease, the addition of target to the MB immobilized well led to an increase of fluorescence intensity by about 3.2 times (curve d). This result should be attributed to the recognition of the immobilized MB to target DNA, which initiated the polymerization of the DNA strand and sequential IEA amplification to expose a large number of biotin groups to bind QDs/SiNS, thus greatly increasing the concentration of cadmic cation reporter in the dissolved solution and thus improved the sensitivity of fluorescence detection.

The IEA reaction was further confirmed with PAGE analysis (Fig. 2B). The MB showed only one band (lane a) at the position different from the mixture of MB, primer and target (lane b). The difference resulted from the hybridization of MB with target and then with primer in the mixture. The formed dsDNA produced a new band. In the presence of polymerase and endonuclease, the mixture of MB, target and primer showed two bands: one was similar to lane b from the formed dsDNA, and another showed faster migration rate (lane c). The latter could be attributed to the DNA trigger produced in the cycling of IEA *via* the polymerization and strand-scission process.

Optimization of IEA conditions

As shown in Fig. 3A, B and C, the fluorescence intensity increased with the increasing amount of polymerase, endonuclease and dNTPs, and then tended a maximum value, indicating a saturated amplification. The optimal amount of polymerase, endonuclease and dNTPs were selected at 3.0 U, 4.0 U and 2.0 mmol L^{-1}, respectively.

After the target was added to the optimal mixture containing polymerase, endonuclease and dNTPs, the fluorescence intensity increased with the increasing reaction time. After the reaction time of 80 min (Fig. 3D), the signal did not increase obviously. Therefore, 80 min was chosen as the reaction duration for IEA.

Target DNA detection

In view of the outstanding ability for signal amplification, the dynamic range of the designed method was examined for the detection of target DNA. Under optimal IEA conditions, the fluorescent intensity of Rhod-5N at 570 nm increased with the increasing concentration of target DNA (Fig. 4A). The plot of fluorescence intensity *vs.* the logarithm of target DNA concentration showed a good linearity in the range from 1.0 × 10^{-17} to 1.0 × 10^{-11} mol L^{-1} (Fig. 4B). The correlation equation was I_F (a.u.) = 125.5 log c (mol L^{-1}) + 2341 (R^2 = 0.9955). The corresponding detection limit for the target was calculated to be 8.5 amol L^{-1} at 3σ. This detection limit was lower than those of many amplification assays, such as nanoparticle-based (10 pmol L^{-1}),[20] enzyme-based (1.0 fmol L^{-1})[21] and target cycling-based amplification (1.0 fmol L^{-1}),[10a] and was close to the detection limit in PCR amplification (30 amol L^{-1}).[22] Considering the fact that the volume of incubation solution was only 10 μL, the designed strategy

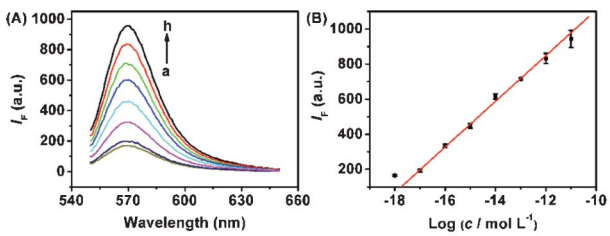

Fig. 4 (A) Fluorescence spectra of Rhod-5N triggered by cadmic cation responding to target concentrations from 10^{-18} to 10^{-11} mol L^{-1} (from a to h) with IEA for signal amplification, and (B) plot of fluorescence intensity *vs.* logarithm of target concentration (n = 3).

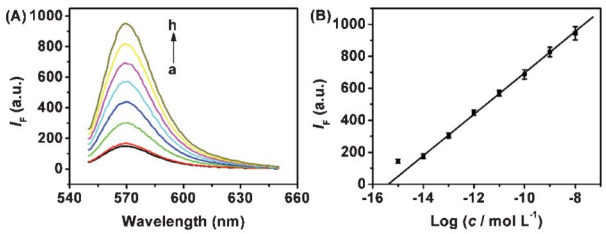

Fig. 5 (A) Fluorescence spectra of Rhod-5N triggered by cadmic cation responding to target concentrations from 10^{-15} to 10^{-8} mol L^{-1} (from a to h) without IEA for signal amplification, and (B) plot of fluorescence intensity *vs.* logarithm of target concentration (n = 3).

achieved the limit of ~50 copies. The low detection limit was attributed to the cascade signal amplification.

In order to confirm the contribution of the IEA amplification to the high sensitivity, a control experiment was carried out in the absence of polymerase/endonuclease. As shown in Fig. 5, the fluorescent intensity increased with the increasing concentration of target in the range from 1.0×10^{-14} to 1.0×10^{-8} mol L^{-1}. The correlation equation was I_F (a.u.) = 128 log c (mol L^{-1}) + 1975 (R^2 = 0.9993). The detection limit was 9.2 fmol L^{-1}, which was about 1000 times higher than that obtained in the presence of polymerase/endonuclease. Thus the high sensitivity of this method was mainly attributed to the amplification of IEA process, which increased the number of QDs/SiNS on the surface of MB modified well.

Selectivity

The selectivity of the proposed fluorescence method was studied using three kinds of DNA sequence including perfectly complementary target, single-base mismatched strand and non-complementary strand at a concentration of 10 fmol L^{-1}. The comparison of the three responses and background is shown in Fig. 6. The single-base mismatch sequence showed a response 3.5 times lower than that of the perfectly complementary target, while the responses to the non-complementary strand and the background were close to that of the single-base mismatch sequence, indicating good selectivity for sequence detection of target DNA. This high specificity arose from the conformational constraint of the stem-loop structure of MB,[23] and the intrinsic reactions of polymerase and endonuclease, which led to great potential for single nucleotide polymorphism analysis.

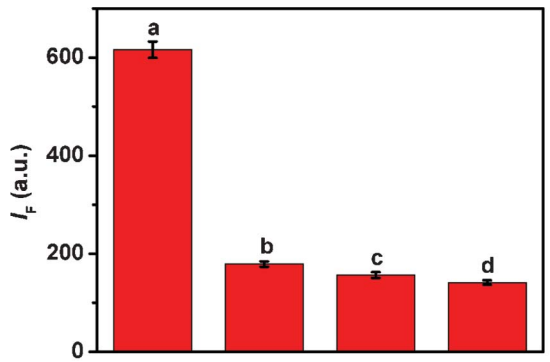

Fig. 6 Histograms of fluorescence intensity for 10 fmol L^{-1} complementary (a), single-base mismatch (b), non-complementary sequences (c), and blank (d).

Conclusions

A versatile method was successfully developed for highly sensitive and selective detection of DNA by combination of IEA reaction in a MB-immobilized well with a CdTe QDs functionalized silica nanospheres label for signal amplification. The amplified signal could be read out with the Cd^{2+}-sensitized fluorescence of Rhod-5N. The SA/QDs/SiNS label showed a homogeneous surface structure and good dispersion in aqueous solution. The IEA procedure led to an about 1000 times lower detection limit. The proposed method could detect target DNA down to the attomolar level and could discriminate mismatched DNA from perfectly matched target DNA with high selectivity. Although the detecting system is relatively complex, the IEA reaction can be carried out in one pot, which is simpler than some recently published procedures.[24] Moreover, this work uses routine reagents for DNA sensing, thus the cost is also lower. This technique does not rely on the intrinsic fluorescence property of the QDs, and thus has high flexibility to select an effective supporter for improving the loading of signal probe. Therefore, the method presented here provides a versatile tool for detection of DNA in biomedical and bioanalytical applications.

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Notes and references

1 (*a*) J. G. Lee, K. H. Cheong, N. Huh, S. Kim, J. W. Choi and C. Ko, *Lab Chip*, 2006, **6**, 886–895; (*b*) S. W. Yeung, T. M. H. Lee, H. Cai and I. M. Hsing, *Nucleic Acids Res.*, 2006, **34**, e118; (*c*) J. H. Chen, J. Zhang, J. Li, F. F. Fu, H. H. Yang and G. N. Chen, *Chem. Commun.*, 2010, **46**, 5939–5941; (*d*) I. Palchetti and M. Mascini, *Analyst*, 2008, **133**, 846–854.

2 (*a*) Y. Li, Y. T. H. Cu and D. Luo, *Nat. Biotechnol.*, 2005, **23**, 885–889; (*b*) R. L. Stoermer, K. B. Cederquist, S. K. McFarland, M. Y. Sha, S. G. Penn and C. D. Keating, *J. Am. Chem. Soc.*, 2006, **128**, 16892–16903; (*c*) H. F. Dong, W. C. Gao, F. Yan, H. X. Ji and H. X. Ju, *Anal. Chem.*, 2010, **82**, 5511–5517.

3 (*a*) J. Wang, D. K. Xu, A. N. Kawde and R. Polsky, *Anal. Chem.*, 2001, **73**, 5576–5581; (*b*) J. Xu, B. Y. Jiang, J. Su, Y. Xiang, R. Yuan and Y. Q. Chai, *Chem. Commun.*, 2012, **48**, 3309–3311.

4 (*a*) Y. W. C. Cao, R. Jin and C. A. Mirkin, *Science*, 2002, **297**, 1536–1540; (*b*) J. Hu and C. Y. Zhang, *Anal. Chem.*, 2010, **82**, 8991–8997; (*c*) Y. He, S. Su, T. T. Xu, Y. L. Zhong, J.

A. Zapien, J. Li, C. H. Fan and S. T. Lee, *Nano Today*, 2011, **6**, 122–130.
5 S. Basuray, S. Senapati, A. Aijian, A. R. Mahon and H. C. Chang, *ACS Nano*, 2009, **3**, 1823–1830.
6 M. Luo, X. Chen, G. H. Zhou, X. Xiang, L. Chen, X. H. Ji and Z. K. He, *Chem. Commun.*, 2012, **48**, 1126–1128.
7 (*a*) Y. C. Cao, R. Jin, C. S. Thaxton and C. A. Mirkin, *Talanta*, 2005, **67**, 449–455; (*b*) M. G. Deng, D. Zhang, Y. Y. Zhou and X. Zhou, *J. Am. Chem. Soc.*, 2008, **130**, 13095–13102; (*c*) L. H. Tang, Y. Liu, M. M. Ali, D. K. Kang, W. A. Zhao and J. H. Li, *Anal. Chem.*, 2012, **84**, 4711–4717.
8 (*a*) J. H. Chen, J. Zhang, Y. Guo, J. Li, F. F. Fu, H. H. Yang and G. N. Chen, *Chem. Commun.*, 2011, **47**, 8004–8006; (*b*) W. Xu, X. J. Xue, T. H. Li, H. Q. Zeng and X. G. Liu, *Angew. Chem., Int. Ed.*, 2009, **48**, 6849–6852.
9 X. L. Zuo, F. Xia, Y. Xiao and K. W. Plaxco, *J. Am. Chem. Soc.*, 2010, **132**, 1816–1818.
10 (*a*) Q. P. Guo, X. H. Yang, K. M. Wang, W. H. Tan, W. Li, H. X. Tang and H. M. Li, *Nucleic Acids Res.*, 2009, **37**, e20; (*b*) R. Ren, C. C. Leng and S. S. Zhang, *Chem. Commun.*, 2010, **46**, 5758–5760; (*c*) F. L. Gao, Z. Zhu, J. P. Lei, Y. Geng and H. X. Ju, *Biosens. Bioelectron.*, 2013, **39**, 199–203.
11 H. X. Ju, *J. Biochips Tissue Chips*, 2012, **2**, 1000e14.
12 (*a*) J. Van Ness, L. K. Vanness and D. J. Galas, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 4504–4509; (*b*) A. R. Connolly and M. Trau, *Angew. Chem., Int. Ed.*, 2010, **49**, 2720–2723.
13 E. Tan, B. Erwin, S. Dames, K. Voelkerding and A. Niemz, *Clin. Chem.*, 2007, **53**, 2017–2020.
14 Y. Zhang and C. Y. Zhang, *Anal. Chem.*, 2012, **84**, 224–231.
15 (*a*) H. Peng, L. Zhang, T. H. M. Kjallman, C. Soeller and J. Travas-Sejdic, *J. Am. Chem. Soc.*, 2007, **129**, 3048–3049; (*b*) W. R. Algar and U. J. Krull, *Anal. Chem.*, 2009, **81**, 4113–4120; (*c*) R. Gill, M. Zayats and I. Willner, *Angew. Chem., Int. Ed.*, 2008, **47**, 7602–7625; (*d*) P. Reiss, M. Protiere and L. Li, *Small*, 2009, **5**, 154–168.
16 (*a*) I. L. Medintz, L. Berti, T. Pons, A. F. Grimes, D. S. English, A. Alessandrini, P. Facci and H. Mattoussi, *Nano Lett.*, 2007, **7**, 1741–1748; (*b*) M. Bruchez, M. Moronne, P. Gin, S. Weiss and A. P. Alivisatos, *Science*, 1998, **281**, 2013–2016; (*c*) D. Gerion, F. Pinaud, S. C. Williams, W. J. Parak, D. Zanchet, S. Weiss and A. P. Alivisatos, *J. Phys. Chem. B*, 2001, **105**, 8861–8871.
17 (*a*) J. S. Li, T. R. Zhang, J. P. Ge, Y. D. Yin and W. W. Zhong, *Angew. Chem., Int. Ed.*, 2009, **48**, 1588–1591; (*b*) E. Han, L. Ding and H. X. Ju, *Anal. Chem.*, 2011, **83**, 7006–7012.
18 (*a*) G. S. Lai, J. Wu, C. Leng, H. X. Ju and F. Yan, *Biosens. Bioelectron.*, 2011, **26**, 3782–3787; (*b*) L. Y. Chen, C. L. Chen, R. N. Li, Y. Li and S. Q. Liu, *Chem. Commun.*, 2009, 2670–2672.
19 D. Zhou, M. Lin, Z. L. Chen, H. Z. Sun, H. Zhang, H. C. Sun and B. Yang, *Chem. Mater.*, 2011, **23**, 4857–486.
20 Z. Gao, S. Rafea and L. H. Lim, *Adv. Mater.*, 2007, **19**, 602–606.
21 A. Numnuam, K. Y. Chumbimunitorres, Y. Xiang, R. Bash, T. P. Havarungkul, P. Kanatharana, E. Pretsch, J. Wang and E. Bakker, *J. Am. Chem. Soc.*, 2008, **130**, 410–411.
22 W. P. Halford, *Nat. Biotechnol.*, 1999, **17**, 835–835.
23 G. Bonnet, S. Tyagi, A. Libchaber and F. R. Kramer, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 6171–6176.
24 B. J. Zou, Y. J. Ma, H. P. Wu and G. H. Zhou, *Angew. Chem., Int. Ed.*, 2011, **50**, 7395–7398.

