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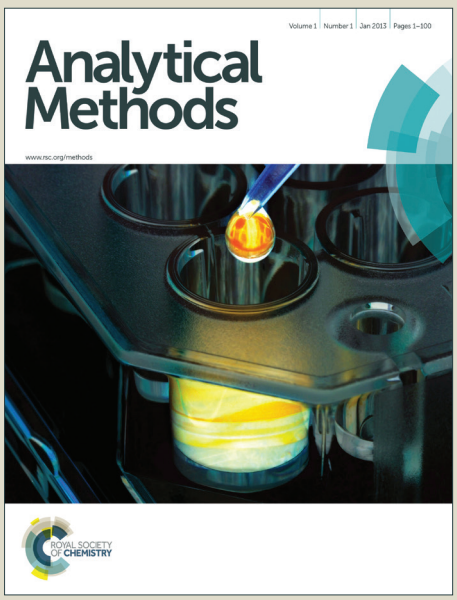

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ARTICLE

Label-free DNA detection based on DNA-silver nanoclusters pair

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We present a simple, selective and label-free sensor for detecting DNA based on the fluorescence of DNA-silver nanoclusters (DNA-Ag NCs). Two kinds of DNA-Ag NCs with different oligonucleotide sequences, can bind together as a DNA-Ag NCs pair by hybridization and show strong fluorescent emission at the wavelength of 624 nm. In the presence of the target DNA, the competing reaction of DNA hybridization occurs, and the formed DNA-Ag NCs pairs decrease, exhibiting weak fluorescnet emission. The fluorescent intensity decreases linearly with the increase of the target DNA concentration in range of 0.20-10.00 uM with a limit of detection of 0.13 uM. The relative standard deviation (RSD) obtained from the same batch of H1N1 target DNA were 1.80%, 0.30% and 2.90% at 2.0×10^{-7} , 4.0×10^{-7} and 6.0×10^{-7} $mol \cdot L^{-1}$, respectively. The coexisting random DNA do not interfere the detection at a concentration of 5.0×10^{-6} $mol \cdot L^{-1}$. With this sensor, we successfully detected H1N1 target DNA and the results indicated that our method was reliable and had the potential for real sample application.

1. Introduction

DNA detection related to disease diagnose[1], gene profiling[2], environmental analysis[3-5] and forensic analysis[6]. Hence, it is of great significance to develop methods for sensitive and selective DNA detection. Nowadays, there are many DNA detection methods, such as chemiluminescence[7-9], electrochemistry[10,11], colorimetry[12,13], fluorescence[14-18] etc. Fluorescent probes are becoming increasingly popular due to their convenience operation, rapid binding kinetics, and ease of automation[19,20]. Therefore, there has been ever-increasing demands to develop simple, sensitive, selective and cost effective label-free fluorescent methods for DNA detection.

With a few to tens of atoms and the molecule-like properties, noble metal nanoclusters have been regarded as promising fluorescent probes[21]. Especially, DNA-silver nanoclusters, DNA-Ag NCs, are one of the important noble metal nanoclusters, which are first synthesized and reported by Dickson et al in 2004 by using DNA as templates [22]. The synthesis of DNA-Ag NCs is very easy, and the fluorescent emission bands can be tuned throughout visible and near infrared range, by simply varying the DNA sequence and length[23]. Compared to organic dyes, DNA-Ag NCs can be brighter and more photostable. Compared to quantum dots, DNA-Ag NCs are smaller, less prone to blinking on long timescales, and do not have a toxic core. The preparation of DNA-Ag NCs is simple and there is no need to remove excess precursors as they are non-fluorescent[20]. Based on this, DNA-Ag NCs are applied as sensors for detecting proteins[18,24], nucleic acids[25-27], small molecules[28-31], metal ions[18,32,33], cells[34] etc. Moreover, DNA-AgNCs are used for microscopic imaging[35,36]. Therefore, the development of new sensors based on DNA-Ag NCs is an interesting and popular area right now. There is a specific phenomenon that two DNA-Ag NCs with little intrinsic fluorescent emission can be lighted up through single stranded DNA hybridization[37]. Moreover, there is a stronger fluorescence enhancement upon placing two darkish DNA-Ag NCs together to form a probe pair through their complementary linkers than that observed by placing darkish DNA-Ag NCs close to the G-rich DNA activator. In theory, the sensor designed based on DNA-Ag NCs pair is more sensitive than the G-rich enhancement based methods. Furthermore, there are a great many researches about G-rich DNA activator, while little researches on the DNA-Ag NCs pair. The DNA-Ag NCs pair based sensor can give a new eye on the property and mechanism of DNA silver nanoclusters. In addition, if a single stranded DNA (ssDNA) is partially complementary to the other two kinds of ssDNAs respectively, DNA sequence competing reaction for hybridization happens, where three kinds of DNAs coexist in a solution. Two of the three ssDNAs, which have greater combining capacity, will bind together through hybridization and produce double stranded DNAs.[38,39]

Herein, we build a new simple and selective fluorescent sensor for DNA detection, based on the above principles. In the present work, we design two kinds of ssDNA probes for DNA-Ag NCs synthesis, and both of them have two regions: a segment used as templates for synthesis of DNA-Ag NCs and a complementary segment for hybridization. After silver nanoclusters nucleation reaction, both synthesized DNA-Ag NCs barely have fluorescent emission. Whereas, they form DNA-Ag NCs pair and show enhanced fluorescence through hybridization. We design the probe DNA complementary to a portion of the target DNA. When adding the target DNA into the solution containing these two DNA-Ag NCs, the bulk solution exhibits a weak fluorescence signal, because the formation of DNA-Ag NCs pair is blocked by the DNA competing reaction. We succeed in detecting the H1N1 target DNA and the DNA-Ag NCs

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pair offers a platform for the potential application in label-free analysis of the target DNA detection. Particularly, compared to the aptamer based DNA-Ag NCs sensors[40,41], our sensor can be easily adjusted to analyze different DNA by simply varying the probe DNA sequence, hardly affacting the DNA hybridization. However, the DNA sequence design may affact the binding of apatmers and there are limits in broadening the application of the apatamer based turn-on sensors for other target detection. Also, turn-off DNA silver nanocluster based sensor[42,43] is a important part, and our sensor is label free and shows high selectivity. To target DNA detection, our label free DNA-Ag NCs pair based sensor is selective, simple and esasily expanded to other DNA detection.

2. Experimental

2.1. Chemicals and materials

Oligonucleotides were synthesized and purified by Sangon Inc. (Beijing, China). The sequences of these oligonucleotides are listed in Table 1. The two kinds of DNA probes are partially complementary. One of the probe DNA sequences is a bifunctional oligonucleotide that both binds the other DNA template and the target DNA strand.

Sodium borohydride ($NaBH_4$) and Silver (I) nitrate (99+%, A.C.S. reagent) were obtained from Alfa Aesar (Shanghai, China). Other chemicals were analytical grade and purchased from BioDee Co. Ltd (Beijing, China). All solutions were prepared with ultrapure water (18.2 MΩ • cm) from a Millipore Milli-Q system. 3-(N-Morpholino)propanesulfonic acid (MOPS) buffer used in synthesizing DNA-Ag NCs: 2 × MOPS (100 mM $NaNO_3$, 40 mM MOPS, pH 7.0)

2.2. Apparatus

Fluorescence spectra were recorded by a multimode plate reader (EnVision, PerkinElmer, Inc., Waltham, USA) using a black 96-well microplate (Greiner, Germany).

Table 1. Sequences of oligonucleotides used in this study.

Name	Sequences (5′-3′)
H1H1	TTTGGGTCTTATTGCTATTTCCGGCTTGAACTTCTTGCTGTATCTTGATGACCCCACAA
V1	CCCTAACTCCCCAGATACAGCAAGAAGTTCAAGCCGGAAATA
V2	AACTTCTTGCTGTATCTCCTCCTTCCTCC
V1.1	CCCTAACTCCCCAGATACAGCAAGAAGTTCAAG
V1.2	CCCTAACTCCCCAGATACAGCAAGAAGTTCAAGCCG
V1.3	CCCTAACTCCCCAGATACAGCAAGAAGTTCAAGCCGGAA
V1.4	CCCTAACTCCCCAGATACAGCAAGAAGTTCAAGCCGGAAATAGCA
V1.5	CCCTAACTCCCCAGATACAGCAAGAAGTTCAAGCCGGAAATAGCAATA
R1	ATGGAGCCAGTAGATCCTAGACTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAAAC
R2	ATTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTTCATAACAAAAGCCTTA
R3	TGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGTCAGACTCATCAAG
R4	AAGCAGTAAGTAGTACATGTAATGCAACCTATACCAATAGTAGCAATAGTAGCATTAGT
R5	TAATAGCAATAGTTGTGTGGTCCATAGTAATCATAGAATATAGGAAAATATTAAGACAA
R6	CAGGTTAATTGATAGACTAATAGAAAGAGCAGAAGACAGTGGCAATGAGAGTGAAGGAG
R7	CTTGTGGAGATGGGGGTGGAGATGGGGCACCATGCTCCTTGGGATGTTGATGATCTGTA

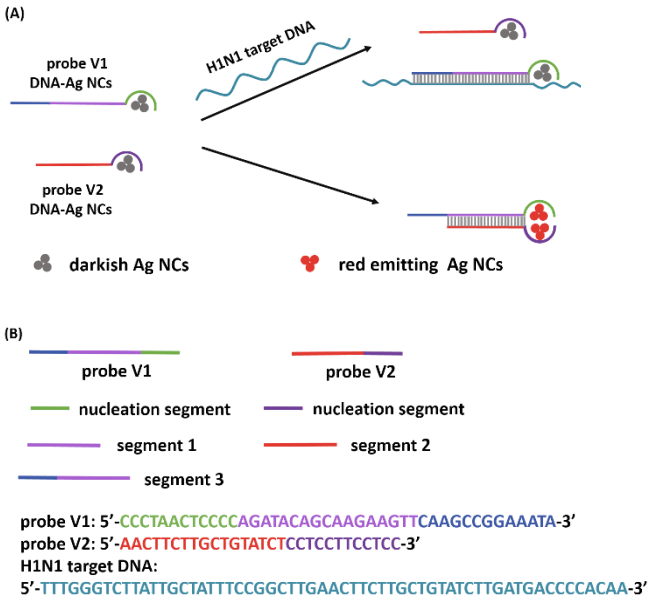

Fig.1: Schematic illustration of the principle of the proposed fluorescent sensor for detecting target DNA.

2.3. DNA-Ag NCs synthesis

The aqueous DNA-Ag NCs solution were prepared according to the reported protocols developed by Petty and Co-workers[22] with minor modification. Briefly, the aqueous DNA Ag NCs solution was prepared by adding $AgNO_3$ (300 uM) to the solution containing Ag NCs nucleation template sequences (50 uM) in MOPS buffer. After incubation for 20 min in the ice bath, added freshly prepared $NaBH_4$ (300 uM) with vigorous shaking. The DNA-to Ag^+- to $NaBH_4$ molar ratio is 1: 6: 6. The as-prepared DNA-Ag NCs were then mixed together and kept in the dark at 0℃ for 1 hr.

2.4. Fluorescence measurement

To determine the fluorescent properties of the DNA-Ag NCs with and without target DNA, probe DNA V1 and V2 were used as templates for the DNA-Ag NCs synthesis. Probe V1 DNA-Ag NCs and probe V2 DNA-Ag NCs were mixed with H1N1 target DNA or MOPS buffer (20 mM, pH 7.0). The probe V1 DNA-Ag NCs- to probe V2 DNA-Ag NCs- to H1N1 target DNA molar ratio was 1: 1: 1. Then the mixtures were assayed by excited at 560 nm, and the fluorescent emission range was 580 to 750 nm.

For the H1N1 target detection, probe V1.3 DNA-Ag NCs and probe V2 DNA-Ag NCs were mixed with H1N1 target DNA with different concentrations or pure MOPS buffer (20 mM, pH 7.0), followed by incubating at 37℃ for 9 min. Then the mixtures were assayed by excited at 566 nm, and the fluorescent emission range was 590 to 750 nm.

To test the selectivity of the DNA Ag NCs for H1N1 target DNA, random DNA samples (R1-R7, 5.0×10^{-6} mol·L^{-1}) were used as controls. To evaluate the tolerance of the sensor for random

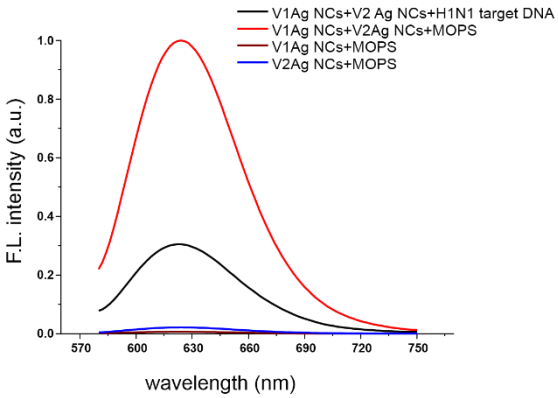

Fig.2: The fluorescent emission (excitation wavelength, 560 nm) from probe V1 and prove V2 templated DNA-Ag NCs mixed with H1N1 target DNA (1 uM) or MOPS buffer.

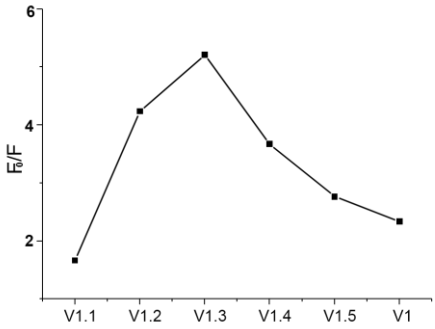

Fig.4: Effects of different lengths of probe sequences. The mixtures were assayed by 566 nm excitation and 624 nm emission.

DNAs in detecting H1N1 target DNA, MOPS solution (20 mM, pH 7.0) containing H1N1 target DNA (5.0×10^{-7}, 6.0×10^{-7}, 7.0×10^{-7} mol·L^{-1}), random DNA R1-R7 (5.0×10^{-7} mol·L^{-1}, respectively) were prepared. The mixtures were then assayed according to the procedure used for the H1N1 target DNA.

3. Results and discussion

3.1. The fluorescent properties of the DNA-Ag NCs with and without target DNA

The schematic illustration of the principle of the proposed fluorescent sensor for detecting target DNA is shown in Fig. 1. Two regions will be engineered: (1) nucleation sequence used as templates for synthesis of Ag NCs: nucleation segment is depicted in Fig. 1(B); (2) hybridization sequence: segment 1 is complementary to segment 2, while H1N1 target DNA is partially complementary to segment 3. Without the target DNA, two kinds of DNA-Ag NCs form a nanocluster pair and exhibit strong fluorescent emission. However, with the target DNA, it will compete for the binding site and the formed Ag NCs pairs decrease, exhibiting weak fluorescent emission.

As shown in Fig. 2, DNA-Ag NCs barely had intrinc fluorescent emission. The prepared DNA-Ag NCs pair mixed with H1N1 target DNA showed very weak fluorescence excitation and emission, while showed a strong fluorescence emission without H1N1 target DNA. The results reveal that the DNA-Ag NCs pair has the potential for detecting target DNA.

The prepared DNA-Ag NCs fluorescent probe was characterized to evaluate the optical properties, and the synthesis could be tested at the same time. We used the posterior optimization probe V1.3 and probe V2 as the templates to synthesize the DNA-Ag NCs. As shown in Fig. 3, with the H1N1 target DNA, the bulk solution showed very weak fluorescence excitation and emission. The maximum excitation and emission wavelength were 566 nm and 624 nm, respectively.

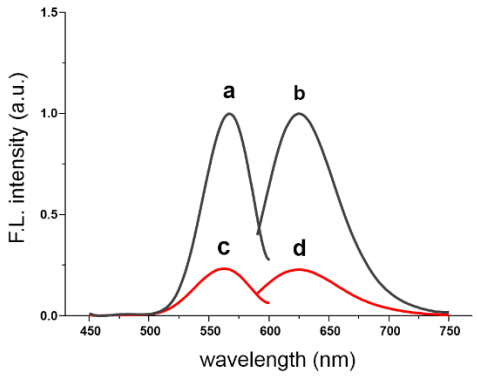

Fig. 3: Fluorescence excitation and emission spectra of DNA-Ag NCs with (c, d) and without (a, b) H1N1 target DNA.

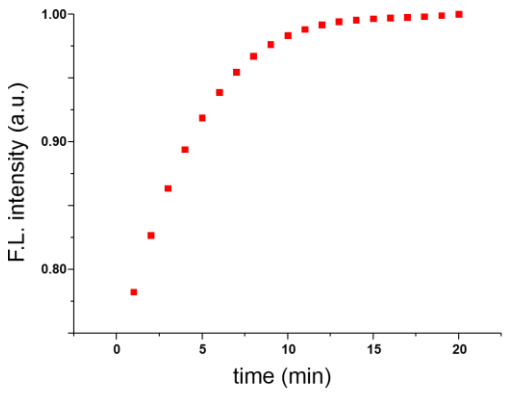

Fig. 5: The dynamic curve of fluorescence of probe V1.3 DNA-Ag NCs and probe V2 DNA-Ag NCs upon the addition of H1N1 target DNA

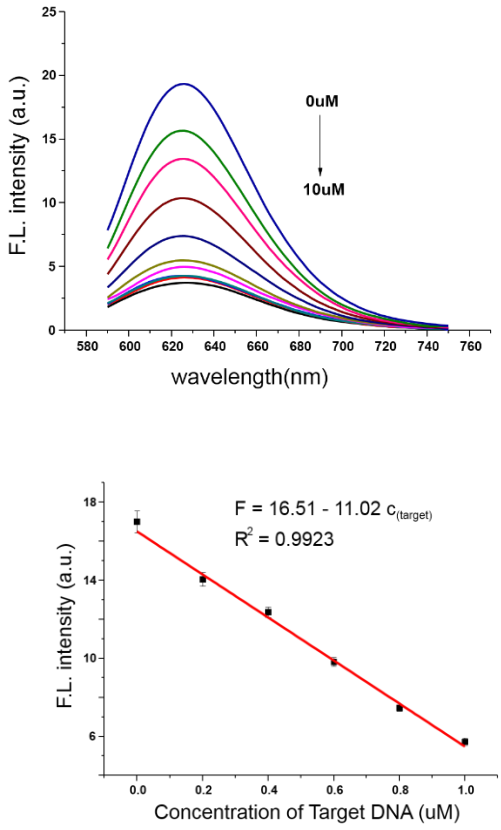

Fig.6: (A) Fluorescence emission spectra of the solution with various concentrations of H1N1 target DNA: 0, 2.0×10^{-7}, 4.0×10^{-7}, 6.0×10^{-7}, 8.0×10^{-7}, 1.0×10^{-6}, 2.0×10^{-6}, 4.0×10^{-6}, 6.0×10^{-6}, 8.0×10^{-6}, 10.0×10^{-6} mol·L^{-1}; (B) Linear relationship between the fluorescence intensity and the concentration of H1N1 target DNA (each error bar indicates the standard deviation)

3.2. Optimization of the DNA length

The lengths of the DNA strands play a significant role in determining the DNA strand competition efficiency, which is a crucial factor for fluorescent intensity. We keep probe V2 invariant, and adjust the probe V1 lengths, with the different sequences listed in Table 1. Probe V1.1~1.5, are partially complementary to probe V2, respectively. Probe V1, probe V1.1~1.5-Ag NCs (with probe V1 and probe V1.1~1.5 as templates) and probe V2-Ag NCs (with probe V2 as templates) were mixed with H1N1 target DNA or MOPS buffer.

We define $E=F_0/F$ to express the efficiency that different probes can analyze the target DNA. F_0 is the fluorescent intensity from the mixtures of DNA-Ag NCs pair without H1N1 target DNA, while F is the fluorescent intensity from the mixtures of DNA-Ag NCs pair with H1N1 target DNA. As shown in Fig. 4, we find that the E value of probe V1.3 group is the biggest and choose probe V1.3 for the following experiments.

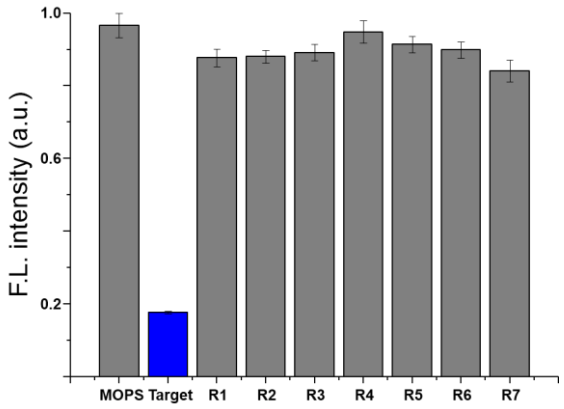

Fig.7: Selectivity of the method for H1N1 target DNA detection (H1N1 Target, Random DNA: 5.0×10^{-6} mol·L^{-1}). The error bars are standard deviations of three repetitive measurements.

3.3. Dynamic curve

To optimize the reaction time, we investigated the dynamic behavior of fluorescent emission of probe V1.3-Ag NCs and probe V2-Ag NCs mixed with H1N1 target DNA, by monitoring the fluorescence intensity as a function of time. We synthesized the DNA-Ag NCs using the probe V1.3 and probe V2 as the templates. Following, the two types of DNA-Ag NCs and the H1N1 target DNA were mixed together, and the fluorescence multimode plat reader was employed to monitor the dynamic behavior. As shown in Fig. 5, the fluorescence intensity rapidly reached a plateau around 9 min. In the following experiments, the DNA-Ag NCs pair and the H1N1 target DNA were kept in 37℃ for 9 min before the fluorescent measurements.

3.4. Analytical characteristics

Under the optimum experimental conditions and according to the experimental procedures aforementioned, we investigated the sensitivity of the proposed method by detecting H1N1 target DNA at different concentrations. As shown in Fig. 6(A), a gradual decrease in the fluorescent emission is clearly observed with an increase in H1N1 target DNA concentration from 0.20 uM to 10.00 uM. Fig.6 (B) illustrates that the fluorescence intensity at a peak value of 624 nm exhibited a good linear relationship with the H1N1 target DNA concentration in the dynamic range from 0.20 uM to 10.00 uM. The fitting equation is $F = 16.51 - 11.02c_{target}$, with a limit of detection (LOD) of 1.3×10^{-7} mol·L^{-1} at a signal- to- noise (S/N) ratio of 3. As the experiment results suggest, the relative standard deviation (RSD) obtained from the same batch were 1.80%, 0.30% and 2.90% at 2.0×10^{-7}, 4.0×10^{-7} , 6.0×10^{-7} mol·L^{-1} H1N1 target DNA, respectively.

3.5. Specificity

To characterize the specificity of this proposed DNA sensor, we

Table 2. Recovery results of spiked H1N1 target DNA at different concentration

Spiked amount (uM)	Detection amount (uM)	Recovery (%)	CV (%)
0.50	0.56	111.70	3.08
0.60	0.61	100.16	1.62
0.70	0.73	104.29	1.73

measured and compared responsive fluorescence signals of H1N1 target DNA and random DNAs with various nucleotide sequences, and MOPS buffer. Fig. 6 displays the selectivity of the DNA-Ag NCs pair probes toward H1N1 target DNA over the random DNA samples R1-R7 (controls) at a concentration of 5.0 × 10^{-6} mol·L^{-1}.

The value of H1N1 target DNA is much lower than the other random DNA samples, suggesting that this analysis strategy holds excellent selectivity for the target DNA.

3.6. Detection of samples

To verify the applicability and reliability of the proposed sensor, the recovery of target DNA in mixed sample was examined. The recoveries of H1N1 target DNA (5.0 × 10^{-7} , 6.0 × 10^{-7} ,7 × 10^{-7} mol·L^{-1}) spiked with R1-R7 random DNAs (5 × 10^{-7} mol·L^{-1}, respectively) were investigated. The results were listed in Table 2. The analytical results indicated that our method was reliable and had the potential for real sample application.

4. Conclusion

In summary, we have developed a simple, selective and label-free sensor for DNA detection. We synthesize two types of DNA-Ag NCs, which are partially complementary. The two DNA-Ag NCs can be lighted up though hybridization and show weak fluorescent emission in the presence of target DNA. We succeed in detecting H1N1 target DNA selectively. This mix-and-detect assay format is simple, and can be achieved by using a common spectrophotometer. Taking into consideration the advantages, the proposed sensor has the potential in clinical application.

Acknowledgements

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