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A novel linear molecular beacon based on DNA-scaffolded silver nanocluster for DNA detection via exonuclease III-assisted cyclic amplification

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Developing label-free molecular beacon (MB)-based methods for DNA detection has been of great significance in bioanalysis because of their simplicity, low cost, and specificity. In this work, we have developed a novel DNA-scaffolded silver nanocluster linear molecular beacon (AgNC-LMB)-based strategy for sequence-specific DNA detection via exonuclease III (Exo III)-assisted signal amplification. The proposed method involves two processes: target-mediated digestion by Exo III and the synthesis of AgNC-LMB as a switch for signal output. Upon hybridization of the rationally designed probe with target, Exo III removes nucleotides from 3' terminus of the probe. The resultant fragment acts as a favorable template to form highly fluorescent DNA/AgNCs. The resulting fluorescence enhancement of the AgNCs provides a quantitative readout proportional to the target concentration in the range of 5 to 300 nM with an LOD of ~ 3.2 nM. This method is simple, cost-effective, highly selective, and free of modification or separation.

1. Introduction

Molecular beacons (MB) have developed rapidly as dominant probes for quantitative nucleic acid sequence research, due to their rapid and specific respond to a given target.[1] A traditional MB is a single-stranded oligonucleotide hybridization probe with a representative hairpin structure, including a target recognition site in the center region and complementary domains at the two ends. In comparison, a linear molecular beacon (LMB) is linear with a fluorophore at 3' terminus and quencher located at penultimate nucleotides. The close proximity of fluorophore and quencher ensures efficient quenching with low background signal.[2-3] However, although MBs and LMBs are excellent separation-free probes, some challenging probes exists, primarily because of the required double labelling (F and Q), leading to increase cost and singly-labeled impurities.[4] Therefore, the fabrication of label-free, low cost, and simple MB biosensors is worthy of attention.

Noble metal nanoclusters as a new class of nanomaterials are typically comprised of a few tens of metal atoms with the dimensions up to the Fermi wavelength, thus bridging the size range between metal atoms and nanoparticles. From this size-dependent standpoint, the unique optical and electrical functionalities of nanoclusters have the potential to produce electronic properties compare to much larger nanoparticles owing to the presence of molecule-like discrete energy levels.[5] Among the various types of nanoclusters, silver nanoclusters (AgNCs) have become promising substitutes for organic fluorophores because of the excellent brightness, biocompatibility, photostability, and controllable fluorescence emission of AgNCs.[6] Particularly, the creation of water-soluble DNA-scaffolded AgNCs (DNA/AgNCs) by binding silver ions to bases (mainly cytosine) and adding a reducing agent have attracted considerable attention. Their exhibited switchable luminescence, tunable fluorescence emission, and facile synthesis[7] have made DNA/AgNCs good candidates for biological imaging, fluorescent labelling, and sensor design in recent years.[8-10] DNA/AgNCs as fluorescent biolabels have enabled applications ranging from detection of various analytes, including metal ions,[11-12] nucleic acid,[13-16] and enzyme activity in biocatalysis,[17-18] to design of complex nanostructures[19-20] and logic gates.[21]

Interestingly, it has been demonstrated that the fluorescence emission characteristics of DNA/AgNCs originated from the base arrangement and length of the DNA template.[22-23] The precise formation of AgNCs can be modulated by the AgNC-forming region of the template as well as the nearby domain, thereby leading to accurate secondary structure of the DNA template.[24-25] When the target hybridizes with the recognition site on the probe, fluorescence enhancement and quenching can be achieved due to hybridization-induced structural changes that modulate formation of robust DNA/AgNCs.[13-14, 26-27] Significantly, interaction between the sequence with a guanine-rich (G-rich) overhang and dark DNA/AgNCs can induce a 500-fold fluorescence enhancement ratio.[28] By tailoring the distance between the AgNC-forming template and the G-rich sequence for switchable fluorescence

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probes, a number of turn-on sensing platforms with excellent signal-to-background ratio have been designed.[29-31] Recently, several researchers have found that target-enabled displacement of inhibitor binding to the DNA host template led to the recovery of AgNCs fluorescence, and they applied this "Ag nanocluster-based MB-like probe" for the development of inexpensive label-free biosensors.[13, 32-33] However, the need for accurate, duplex structure formation to quench AgNC fluorescence before performing DNA detection results in complex experimental procedures. Thus, there is still need for the development of a simple MB-like biosensor.

In this work, we used a label-free, AgNC linear molecular beacon (AgNC-LMB)-based strategy for fluorescence turn-on detection of DNA. In previous studies, similar to the fluorescence light-up mode via hybridization of the template for generation of DNA/AgNCs and G-rich DNA activator, we built a new structure to light up two adjacent, dark DNA/AgNCs probes by putting them together to form a AgNCs pair through their complementary sequences.[34] Based on this finding, we found that fluorescence enhancement also occurred when two dark AgNCs were simultaneously located at each end of the same DNA template. It was observed that the DNA/AgNCs also displayed a bright fluorescence emission, resulting in a new form of fluorescent AgNCs. By combining our previous design and Exonuclease III (Exo III), we developed the AgNC-LMB-based strategy reported here. In the proposed method, when the obtained template forming DNA/AgNCs contains target binding site at the 3' end, the highly fluorescent AgNCs are not formed. However, in the presence of target, exonuclease III can recognize the double-stranded structure (resulting from hybridization between target DNA and its complementary domain in the Probe), and cleave the binding site from the 3' terminus, exposing the AgNC-forming region and producing intense fluorescence. In absence of target DNA, the intact Probe remains in the solution, and results in the synthesis of DNA/AgNCs with low fluorescence. The proposed low-cost, label-free method provides a simple AgNCs-based biosensor.

2. Experimental section

2.1. Reagents and materials

Oligonucleotides used in this work were custom-synthesized by Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The sequences of these oligonucleotides are listed in Table 1. Silver nitrate ($AgNO_3$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium borohydride ($NaBH_4$) was purchased from Tianlian Fine Chemical Co., Ltd. (Shanghai, China). Exo III was purchased from Fermentas Inc. (Vilnius, Lithuania). All chemicals used were of analytical reagent, obtained from commercial sources, and directly used without additional purification. The solutions in all experiments were prepared using deionized (DI) water with an electrical resistance of 18.2 MΩ·cm from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA).

Table 1 Sequences of DNA oligonucleotides and probes used in this work.

Name	Sequence* (5'→3')
Probe	**CCCTTAATCCCCTTTTTTTTTTTTTTTCCCTAACTCCCC***ATACAACCTACTACCTCA*
P0	**CCCTTAATCCCCTTTTTTTTTTTTTTTCCCTAACTCCCC**
P1	**CCCTTAATCCCCTTTTTTTTTTTTTTTCCCTAACTCCCC***A*
P2	**CCCTTAATCCCCTTTTTTTTTTTTTTTCCCTAACTCCCC***AT*
P3	**CCCTTAATCCCCTTTTTTTTTTTTTTTCCCTAACTCCCC***ATA*
P4	**CCCTTAATCCCCTTTTTTTTTTTTTTTCCCTAACTCCCC***ATAC*
P5	**CCCTTAATCCCCTTTTTTTTTTTTTTTCCCTAACTCCCC***ATACA*
P6	**CCCTTAATCCCCTTTTTTTTTTTTTTTCCCTAACTCCCC***ATACAA*
R	**CCCTTAATCCCCTTTTTTTTTTTTTTTCCCTAACTCCCC***AACAGCTATAAACAGTCCTG*
T1	TGAGGTAGTAGGTTGTATAGTT
T2	TGAGGTAGTA<u>C</u>GTTGTATAGTT
T3	TGA<u>C</u>GTAGTA<u>C</u>GTTGTATAGTT
T4	T<u>A</u>A<u>TACTG</u><u>CCTGG</u>T<u>AATGA</u><u>TGA</u>

* The bases in bold represent the region for formation of AgNCs. The bases in italics represent various tail domains (including target recognition site and random sequence). The underlined bases in T2, T3, and T4 represent the mutation sites in tested DNA targets compared to target T1.

2.2. Instrumentation

All fluorescence emission scans were acquired with a fluorescence microplate reader (Bio-Tek Instrument, Winooski, USA) using a transparent 96-well microplate and a black 96-well microplate (Corning Inc., NY, USA). Transmission electron microscope (TEM) measurements were collected on a Jeol JEM-2100 instrument (JEOL Ltd., Japan).

2.3. Procedures for Exo III-assisted specific DNA detection based on AgNC-LMB

The detailed procedure for Exo III-assisted specific DNA detection based on AgNC-LMB was as follows. First, 5 μL Probe (500 nM) and target DNA with different concentrations were added to phosphate buffer (3.8 mM NaH_2PO_4, 6.2 mM Na_2HPO_4, 500 μM $MgNO_3$, pH 7.0) in a total volume of 200 μL to prepare the Probe-target duplex, followed by heating the reaction solution at 90°C for 5 min, and slowly cooling to room temperature. A volume of 1 μL Exo III (1 U/μL) was added to the reaction solution and incubated at 37°C for 2 h. After the incubation step, the mixture was heated to 70°C for 10 min to deactivate Exo III, and slowly cooled to room temperature. The reaction mixture were then used as the template to synthesize fluorescent DNA/AgNCs according to the reported literature with minor modification.[35] Briefly, a portion of $AgNO_3$ solution (1 mM, 1.2 μL) was mixed with the reaction solution in a Ag^+-to-Probe molar ratio of 12:1. Then, the mixture was incubated in the dark at room temperature for 20 min. Subsequently, an aliquot of fresh $NaBH_4$ solution (1 mM, 1.2 μL) was added to the above mixture to reduce the silver ions, followed by vigorous shaking for 5 s, and storage in the dark at room temperature for 15 min before use.

2.4. Gel Electrophoresis

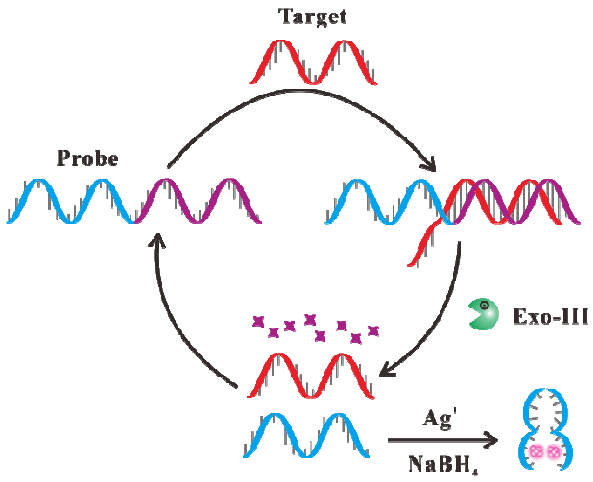
A volume of 20μL reaction mixture containing 500 nM Probe, 1 U/μL Exo III, and 500 nM target DNA in phosphate buffer (3.8 mM NaH_2PO_4, 6.2 mM Na_2HPO_4, 500 μM $MgNO_3$, pH 7.0) before and after incubation at 37 °C for 2 h was loaded into a 20% nondenaturing polyacrylamide gel electrophoresis plate (PAGE). The PAGE was carried out in 1×TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH = 8.3) at 120 V constant voltage for about 2 h at room temperature. After staining with Gold View, the gels were photographed using Gel Image System (Tanon, China).

2.5. Serum samples

Serum samples were loaded into centrifugal filtration devices[31] (Molecular weight cutoff or MWCO 50kDa, Millipore Amico Ultra), and centrifuged at 6,000 rpm for 20 min. Specific DNA targets with different concentrations were then spiked into the pretreated serum.

3. Results and discussion

3.1. Working principle of the proposed method



Scheme 1. Schematic illustration of the proposed AgNC-LMB-based method for label-free detection of sequence-specific DNA via Exo III -aided signal amplification.

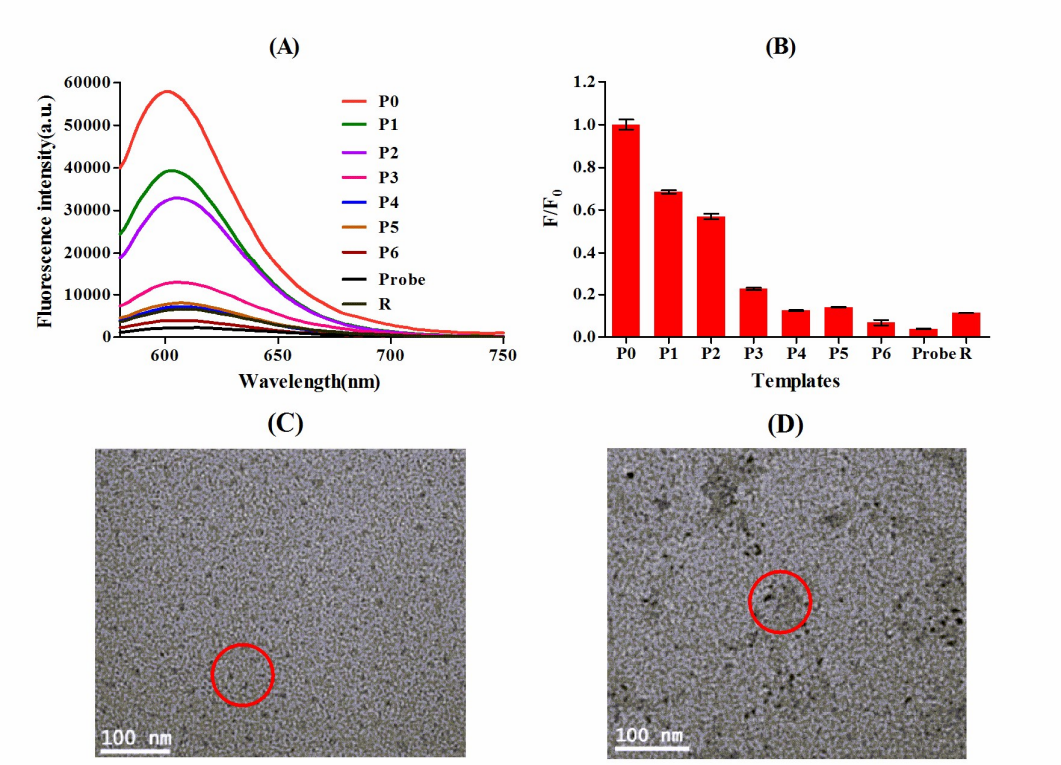


Fig. 1 (A) Comparison of fluorescence intensities of AgNCs stabilized by various templates with and without nucleotide tail. In contrast to P0 without tail, the templates ranging from P1 to P6 possess 1-nt, 2-nt, 3-nt, 4-nt, 5-nt, 6-nt tail, respectively. Probe contains a target recognition site with 18-nt tail. (B) The fluorescence ratio F_0/F values obtained based on date from (A), where F and F_0 are the fluorescence intensities at a peak value of 604 nm in the presence and absence of tail, respectively. TEM images of DNA/AgNCs stabilized by P0 (C) and Probe (D), respectively.

The proposed AgNC-LMB-based assay for label-free specific DNA detection is demonstrated in Scheme 1. The reaction system consists primarily of linear Probe and Exo III. The novel linear Probe is designed with two consecutive domains, the segment (denoted in purple) complementary to the target DNA at 3' terminus and the AgNC-forming template domain (denoted in blue) at 5' terminus. Exo III is a specific exodeoxyribonuclease which can enable the successive cleavage of mononucleotides from the blunt or recessed 3'-hydroxyl terminus of a duplex DNA, while its activity is resistant to duplex DNA with protruding 3' terminus (more than 4 nt) or single-stranded DNAs.[36-37] Due to its unique enzymatic property, Exo III is utilized to manipulate selective enzymatic digestion of probe after hybridization with target DNA to remove the target binding domain and to realize signal readout coupling with target amplification. In the absence of target DNA, Probe remains intact and cannot effectively generate highly fluorescent Probe/AgNCs, since the target binding segment beyond the AgNC domain in the Probe disrupts the proper formation of DNA/AgNCs. Thus, the reaction solution exhibits low fluorescence resulting from the darkish DNA/AgNCs. When target DNA is added, the combination of Probe and target DNA forms a double-stranded structure with a 3'-blunt terminus from Probe and 3'-overhang terminus from the target DNA. Exo III specifically binds to the duplex region, and then selectively digests the Probe from its 3' terminus, releasing the intact target DNA due to protection by a 4-nt single-stranded domain in the target DNA at its 3'-protruding terminus.[36] The released target DNA hybridizes with a new linear Probe to trigger another Probe-digestion cycle. After enzymatic reaction, the resultant products are employed as templates to synthesize DNA/AgNCs, which exhibits a brighter red fluorescence emission in solution. Correspondingly, the resulting fluorescence signal is directly proportional to the amount of target DNA present, allowing quantitative determination of the target DNA of interest.

3.2. Investigation on the performance of AgNC-LMB

Prior to specific DNA detection, the fluorescence intensities of DNA/AgNCs stabilized by the various templates with different tail domains (including partial and whole target recognition site and random sequence) were first investigated. In order to show the results more directly, we designed a series of sequences (P0, P1, P2, P3, P4, P5, P6, Probe, R). P0 without nucleotide tail is the segment after removal of the target binding domain in Probe, while the templates, ranging from P1 to P6 are ones that have 1-nt, 2-nt, 3-nt, 4-nt, 5-nt, 6-nt tails, respectively. Probe has an 18-nt target recognition site, and R

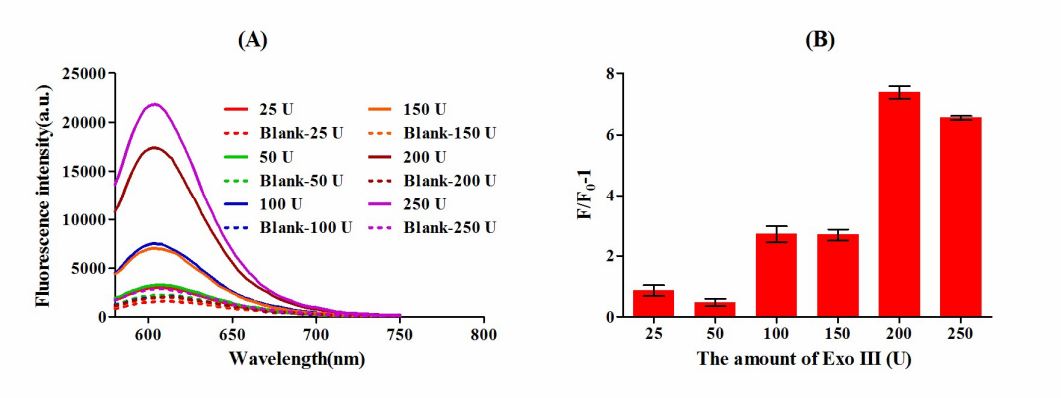


Fig. 2 Selection of the amount of Exo III. (A) Fluorescence emission spectral responses for the different amounts of Exo III (25 U, 50 U, 100 U, 150 U, 200 U, and 250 U) in the absence and the presence of T1. (B) Bar graph of fluorescence ratio (F/F_0-1) responses in the presence of different amounts of Exo III. F and F_0 are the fluorescence intensities at a peak value of 604 nm in the presence and absence of T1, respectively. The error bars were calculated from three independent experiments.

contains a 20-nt random base sequence. The DNA sequences were used as templates to prepare DNA/AgNCs with a molar ratio of DNA/Ag^+/$NaBH_4$ at 1:12:12 according to the reported method with minor revision.[35] As shown in Fig. 1A, the decrease of fluorescence intensity coincides with the increasing number of nucleotides at the 3' terminus. Obviously, the tails with different numbers of nucleotides confine the precise generation of DNA/AgNCs relative closely to the secondary structure of the DNA template. In particular, compared to P0 producing highest fluorescence intensity, Probe shows ~26-fold fluorescence reduction ratio, due to the presence of a tail with the target recognition site. Therefore, it is possible for Exo III to digest the target recognition site in the duplex DNA produced by hybridization of Probe and target to obtain a favorable template for highly fluorescent DNA/AgNCs. TEM images of DNA/AgNCs obtained with Probe and P0 demonstrate further how two templates affect the characteristics of the obtained DNA/AgNCs. As shown in the Fig. 1C and 1D, DNA/AgNCs hosted by P0 template are monodispersive with ~3 nm size, compared with DNA/AgNCs created by Probe, leading to a larger nanoparticle with low fluorescence, which may be attributed to the reason that tail domain combining with silver ions results in change in the secondary structure of DNA template.[24] These images provide further verification that the tails integrated into the template disturb the optimum synthesis of DNA/AgNCs. This phenomenon provides the basis for design of the Exo III-mediated DNA detection assay. In the presence of synthetic target DNA, Probe and Exo III, the resulting reaction mixture can produce the intense fluorescence signal after incubation at 37°C for 2 h. As shown in Fig. S1A, the fluorescence intensity of the obtained DNA/AgNCs increases dramatically after incubation in contrast to that before incubation, because degradation of the target recognition site leads to the template that favors optimum formation of DNA/AgNCs. The gel electrophoresis result (seen in Fig. S1B) verifies that the duplex domain disappears under the operation of Exo III. Based on the above results, we demonstrate the feasibility of the method based on AgNC-LMB coupling with amplification by Exo III for specific DNA detection.

3.3. Optimization of reaction conditions

Prior to performance of the proposed AgNC-LMB-based DNA detection assay via Exo III assistance, the reaction conditions (reaction buffer, reduction time, peak excitation) in DNA/AgNCs synthesis were investigated to acquire robust DNA/AgNCs. By preparing DNA/AgNCs in three buffers (phosphate, Tris, and MOPS) used widely in procedures to make highly fluorescent DNA/AgNCs, as shown in Fig. S2, phosphate buffer offers excellent conditions for obtaining the best fluorescence readout of DNA/AgNCs. Another factor involved in DNA/AgNCs formation is reaction time. As shown in Fig. S3, the fluorescence increases until reaching a plateau at approximately 15 min, which is faster than the conventional procedure with the need of 1 h after adding $NaBH_4$.[35] Based on the excitation spectra of P0/AgNCs performed at 604 nm emission (Fig. S4), the peak excitation occurs at 550 nm. Therefore, the optimum conditions for DNA/AgNCs preparation are as follows: reaction in phosphate buffer, reduction by $NaBH_4$ for 15 min, and excitation at 550 nm.

Based on the optimum conditions for DNA/AgNCs synthesis, we selected a synthetic and specific DNA sequence as a model to optimize the additional experimental conditions. As shown in Fig. 2A, the fluorescence intensity increases as the concentration of Exo III increases from 25 U to 250 U. The fluorescence ratio (F/F_0-1) shows that the best fluorescence enhancement ratio is reached when adding 200 U Exo III (Fig. 2B), where F and F_0 are the fluorescence intensities at a peak value of 604 nm in the presence and absence of T1, respectively. The time-course experiments for the kinetic response upon enzymatic digestion were investigated by monitoring the fluorescent signal of reaction system at

increasing time points (0.5, 1, 1.5, 2.0, and 2.5 h). As shown in Fig. S5, the fluorescent signal reaches the highest value within 2 h. Therefore, the final concentration of Exo III and reaction time was set to 200 U and 2 h for subsequent experiments, respectively.

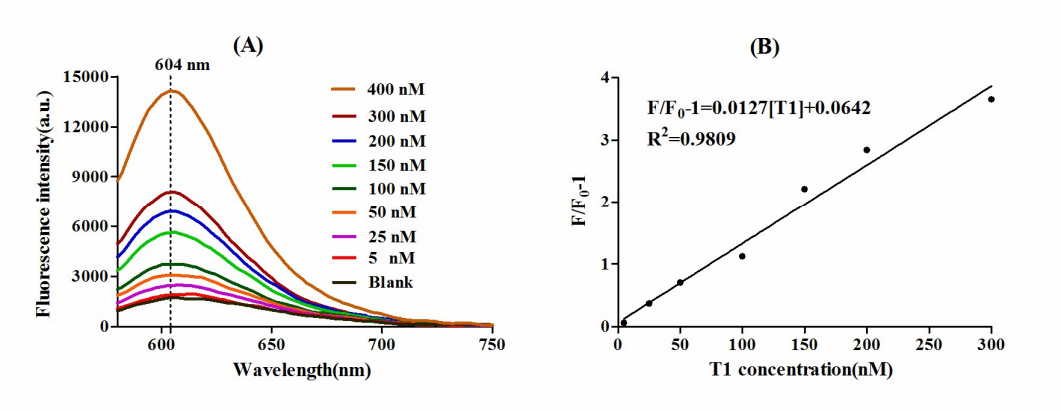


Fig. 3 Sensitivity investigation of the proposed method for specific detection of T1 target. (A) Fluorescence emission spectra for excitation at 550 nm in the presence of T1 at different concentrations (0, 5, 25, 50, 100, 150, 200, 300, and 400 nM) by the proposed method. (B) Plot of the linear relationship between the fluorescence ratio (F/F_0-1) and the concentration of target T1 from 5 nM to 300 nM, where F and F_0 are the fluorescence intensities at 604 nm in the presence and absence of target T1, respectively.

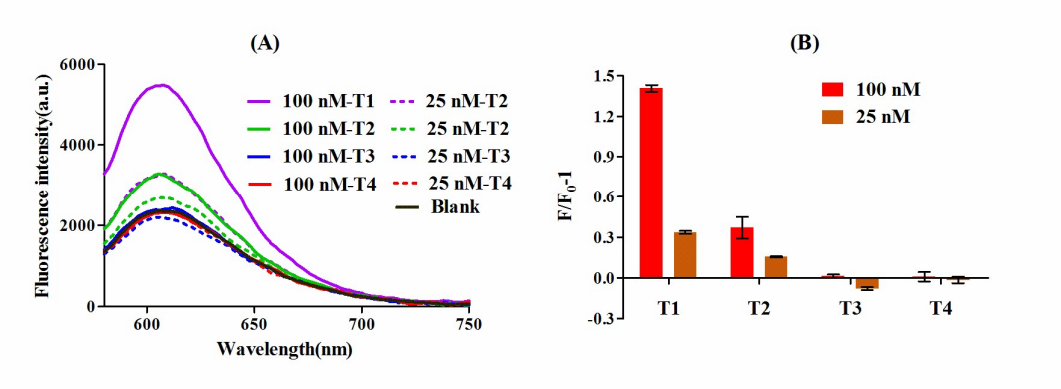


Fig. 4 Selectivity investigation of the proposed method using different target DNAs. (A) Fluorescence emission spectra in the presence of different targets. The solid and dashed lines represent fluorescence spectra in the presence of the 100 nM and 25 nM target DNA, respectively. Ex: 550 nm. (B) The fluorescence ratio (F/F_0-1) values obtained based on date from (A), where F and F_0 are the fluorescence intensities at 604 nm in the presence and absence of tested target, respectively.

3.4. Sensitivity investigation

Using the optimized experimental conditions, we investigated the sensitivity of the proposed AgNC-LMB-based method by detecting T1 with different concentrations. As shown in Fig. 3A, a gradual increase in the fluorescence emission intensity is clearly observed when the concentration of T1 is increased from 5 nM to 400 nM, indicating that the digestion level for Exo III is highly dependent on the amount of target DNA. Fig. 3B illustrates that the fluorescence ratio (F/F_0-1) exhibits a good linear relationship with T1 concentration in the dynamic range of 5 nM and 300 nM, where F and F_0 are the fluorescence intensities at a peak value of 604 nm in the presence and absence of target T1, respectively. The fitting equation is $[F/F_0\text{-}1]=0.0127[T1]+0.0642$, with a correlation coefficient R^2 of 0.9809. The detection limit was estimated to be 3.2 nM ($3\sigma/S$, σ is the standard deviation of the blank solution). These results confirm that our proposed AgNCs-LMB-based method is promising for the quantitative detection of DNA target. In addition, we spiked different amounts of T1 into serum to test the proposed method with practical samples. As shown in Table S1, quantitative detection of specific T1 using the proposed method is achieved with an acceptable recovery ratio. These results indicate that our proposed AgNC-LMB-based DNA detection method can be applied to biological samples.

3.5. Selectivity test

We further investigated the sequence-specificity of the proposed AgNCs-LMB-based method. Four kinds of targets, including the perfect match target (T1), single-base mismatch target (T2), two-base mismatch target (T3), and random target (T4), were used (the detailed sequence information is shown in Table 1) at two concentrations of 25 nM and 100 nM. Fig. 4B exhibits the comparison result of (F/F_0-1) values from the four tested target DNAs, where F and F_0 are the fluorescence intensities at 604 nm in the presence and absence of tested target, respectively. The (F/F_0-1) values for perfect matched target (T1) of 100 nM and 25 nM are ~3.8 and ~2.2 fold higher than those for single-base mismatched DNA with the same concentration, respectively. The two-base mismatch and random target showed almost the same response as the control without any target. These results clearly suggest that the assay developed in this work allows good sequence specificity to discriminate against single nucleotide-mutation target DNA.

4. Conclusions

We have designed a novel label-free LMB probe based on the rapid synthesis of DNA/AgNCs by lighting up two darkish DNA/AgNCs pairs separated by 15-nt thymine in the same template. Compared to a conventional MB, our proposed AgNCs-LMB has the advantage of no specific structural requirement (duplex and hairpin) of a linear probe with a fluorophore and a quencher for proximity-dependent fluorescence quenching. Coupling Exo III-mediated signal amplification and readout, we have successfully applied the AgNC-LMB system for the specific DNA detection with good selectivity and simple manipulation in the range of 5 to 300 nM with an LOD of ~3.2 nM. We believe our combination of an LMB concept and highly fluorescent DNA/AgNCs expand the applications of DNA/AgNCs and are promising for the development of additional label-free fluorescence turn-on sensing platforms.

Acknowledgements

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References

1 X. Fang, X. Liu, S. Schuster and W. Tan, *J. Am. Chem. Soc.*, 1999, **121**, 2921-2922.
2 L. Cui, Z. Zhi, N. Lin, H. Zhang, Z. Guan and C. J. Yang, *Chem. Commun.*, 2014, **50**, 1576-1578.
3 C. J. Yang, L. Cui, J. Huang, L. Yan, X. Lin, C. Wang, W. Y. Zhang and H. Kang, *Biosens. Bioelectron.*, 2011, **27**, 119-124.
4 Z. Zhou, Y. Du, L. Zhang and S. Dong, *Biosens. Bioelectron.*, 2012, **34**, 100-105.
5 J. Zheng, P. R. Nicovich and R. M. Dickson, *Annu. Rev. Phys. Chem.*, 2007, **58**, 409.
6 I. Díez and R. H. Ras, *Nanoscale*, 2011, **3**, 1963-1970.
7 J. M. Obliosca, C. Liu and H. C. Yeh, *Nanoscale*, 2013, **5**, 8443-8461.
8 J. Li, X. Zhong, F. Cheng, J. R. Zhang, L. P. Jiang and J. J. Zhu, *Anal. Chem*., 2012, **84**, 4140-4146.
9 B. Han and E. Wang, *Anal. Bioanal. Chem.*, 2012, **402**, 129-138.
10 W. Guo, J. Yuan and E. Wang, *Chem. Commun.*, 2009, 3395-3397.
11 J. L. MacLean, K. Morishita and J. Liu, *Biosens. Bioelectron.*, 2013, **48**, 82-86.
12 L. Deng, Z. Zhou, J. Li, T. Li and S. Dong, *Chem. Commun.*, 2011, **47**, 11065-11067.
13 J. T. Petty, B. Sengupta, S. P. Story and N. N. Degtyareva, *Anal. Chem*., 2011, **83**, 5957-5964.
14 S. W. Yang and T. Vosch, *Anal. Chem.*, 2011, **83**, 6935-6939.
15 X. Liu, F. Wang, R. Aizen, O. Yehezkeli and I. Willner, *J. Am. Chem. Soc.*, 2013, **135**, 11832-11839.
16 N. Enkin, F. Wang, E. Sharon, H. B. Albada and I. Willner, *ACS Nano*, 2014, **8**, 11666-11673.
17 C. Shen, X. Xia, S. Hu, M. Yang and J. Wang, *Anal. Chem.*, 2015, **87**, 693-698.
18 X. Liu, F. Wang, A. Niazov-Elkan, W. Guo and I. Willner, *Nano Lett.*, **2012**, 13, 309-314.
19 S. M. Copp, D. E. Schultz, S. Swasey and E. G. Gwinn, *ACS Nano*, 2015, **9**, 2303-2310.
20 P. R. O'Neill, K. Young, D. Schiffels and D. K. Fygenson, *Nano Lett.*, 2012, **12**, 5464-5469.
21 T. Li, L. Zhang, J. Ai, S. Dong and E. Wang, *ACS Nano*, 2011, **5**, 6334-6338.
22 E. G. Gwinn, P. O'Neill, A. J. Guerrero, D. Bouwmeester and D. K. Fygenson, *Adv. Mater.*, 2008, **20**, 279-283.
23 C. I. Richards, S. Choi, J. C. Hsiang, Y. Antoku, T. Vosch, A. Bongiorno, Y. L. Tzeng and R. M. Dickson, *J. Am. Chem. Soc.*, 2008, **130**, 5038-5039.
24 P. Shah, A. Rørvig-Lund, S. B. Chaabane, P. W. Thulstrup, H. G. Kjaergaard, E. Fron, J. Hofkens, S. W. Yang and T. Vosch, *ACS Nano*, 2012, **6**, 8803-8814.
25 J. M. Obliosca, M. C. Babin, C. Liu, Y.L. Liu, Y.A. Chen, R. A. Batson, M. Ganguly, J. T. Petty and H.C. Yeh, *ACS Nano*, 2014, **8**, 10150-10160.
26 J. T. Petty, S. P. Story, S. Juarez, S. S. Votto, A. G. Herbst, N. N. Degtyareva and B. Sengupta, *Anal. Chem.*, 2011, **84**, 356-364.
27 W. Guo, J. Yuan, Q. Dong and E. Wang, *J. Am. Chem. Soc.*, 2010, **132**, 932-934.
28 H. C. Yeh, J. Sharma, J. J. Han, J. S. Martinez and J. H. Werner, *Nano Lett.*, 2010, **10**, 3106-3110.
29 H. C. Yeh, J. Sharma, I. M. Shih, D. M. Vu, J. S. Martinez and J. H. Werner, *J. Am. Chem. Soc.*, 2012, **134**, 11550-11558.
30 M. Zhang, S. M. Guo, Y. R. Li, P. Zuo and B. C. Ye, *Chem. Commun.*, 2012, **48**, 5488-5490.
31 J. Li, X. Zhong, H. Zhang, X. C. Le and J. J. Zhu, *Anal. Chem.*, 2012, **84**, 5170-5174.
32 L. Zhang, J. Zhu, Z. Zhou, S. Guo, J. Li, S. Dong and E. Wang, *Chem. Sci.*, 2013, **4**, 4004-4010.
33 L. Zhang, J. Zhu, S. Guo, T. Li, J. Li and E. Wang, *J. Am. Chem. Soc.*, 2013, **135**, 2403-2406.
34 B. C. Yin, J. L. Ma, H. N. Le, S. Wang, Z. Xu and B. C. Ye, *Chem. Commun.*, 2014, **50**, 15991-15994.
35 C. M. Ritchie, K. R. Johnsen, J. R. Kiser, Y. Antoku, R. M. Dickson and J. T. Petty, *J. Phys. Chem. C*, 2007, **111**, 175-181.
36 S. Bi, L. Li and Y. Cui, *Chem. Commun.*, 2012, **48**, 1018-1020.

37 X. Zuo, F. Xia, Y. Xiao and K. W. Plaxco, *J. Am. Chem. Soc.*, 2010, **132**, 1816-1818.