



# A molecular beacon-like Ag nanocluster fluorescence probe for nucleic acid detection

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## Abstract

We have developed a type of low-cost, label-free silver nanocluster molecular beacon-like fluorescence sensor with a DNA template. To detect target DNA with this probe, we use a hairpin DNA sequence based on a “turn-on” strategy. The transformation of hairpin DNA would visibly influence the formation of Ag nanoclusters, such that the stronger fluorescence will be measured with the solution containing target nucleic acids than that without targets nucleic acids. There is a good liner relationship between the fluorescence and the target DNA concentrations, ranging from 1 to 750 nmol L<sup>-1</sup>. Importantly, the detection sensing platform allows down to 1 nmol L<sup>-1</sup>, which is much lower than other studies.

**Keywords** Nucleic acid detection · Ag nanoclusters · Molecular beacon-like · Fluorescent biosensor

## Introduction

Molecular beacons (MBs) are a class of oligonucleotide probe with a hairpin stem-loop structure invented in 1996 [1]. MBs have double labels at both of two terminals, which are a fluorophore and a quencher, respectively [2]. In the presence of target DNA or RNA, hybridization happened in the loop domain result in fluorescence recovery because hairpin conformation is destroyed, while the fluorophore and quencher would keep away in space [3]. Based on the fluorescence resonance energy transfer, MBs are like a switch, that enable it to be widely used in biochemical analysis [4]. However, conventional MBs still have plenty of limitations, such as numerous expending on DNA modification, tedious preparation, fluorescent stability and toxicity of the fluorescent dye [5]. To solve the above problems, researchers have

concentrated on label-free MB, which could be labeled by metal nanoclusters with unique superiorities, such as lower cost, more stable structure, timesaver and so on.

Fluorescent metal nanoclusters (MNCs) are composed of several to tens of metal atoms, which approximates the Fermi wavelength of electrons, and MNCs are a new kind of materials with ultra-small sizes (< 2 nm) [6]. Due to remarkable fluorescent properties of MNCs, such as excellent biocompatibility, ultrafine size, lower toxicity, large Stokes shifts, high photostability and convenient surface modification, MNCs have a series of applications, for instance, biosensing, bioimaging, therapeutic, detection of DNA, RNA, protein, ions, small molecule and enzyme activity assay [7, 8]. Ag nanoclusters (AgNCs) are one of the MNCs have been studied by many scientists because of its excellent performance. AgNCs are a transition state between atoms and nanoparticles, which exhibit unique optical, electrical and chemical properties. There are many methods for synthesizing AgNCs: for example, chemical methods, a photolysis method, radiolysis and sonochemistry methods and a tremendous amount of ligands could be used to prepare AgNCs. Recently, DNA-templated AgNCs have obtained broad attention owing to their naturally water-soluble, low toxic and biocompatible [9]. Guo et al. synthesized silver nanoclusters with hairpin DNA under different conditions of hairpin-AgNC, first. They then found the percentage of the GC base pair and stem length could affect the property of hairpin-AgNCs [10].

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Chen et al. proposed a multifunctional template for AgNCs or CuNCs, which is synthesized on two hairpin loops and an adenine–thymine-rich double-helical stem. [11].

A vital matter plays a role that cannot be ignored in all living individuals is nucleic acid. Nucleic acid has extremely versatile biological functions which are mainly concerned with storing message, encoding and transmitting [12, 13]. In addition to the above mentioned-effects, nucleic acid also associates with many therapeutics of multiple gene mutation illnesses; hence, nucleic detection is worthy of study [14]. Especially the novel coronavirus disease (COVID-19) outbreak in 2019 is closely related to nucleic acid detection [15]. Specific nucleic acid is usually detected by the hybridization of complementary base-pairs [16]. Exactly as the Ja Yeon Song study group build up a novel method for nucleic acid detection based on self-priming hairpin-utilized [17–19].

In this study, we successfully constructed a label-free, low consumption and facile hairpin probe based on DNA-templated AgNCs to detect the concentrations of target DNA. This probe will close its loop region because of no target DNA, and lead to weak fluorescence. Once target DNA is added to the probe solution, the target DNA will specifically be binding with recognition of the sequence on the loop region. At the same time, a hairpin probe opened, and a notable fluorescent enhancement can be observed. We could detect nucleic acid by the variety of fluorescence.

## Experimental

### Apparatus

UV–Vis absorption spectroscopy was performed on a TU-1810 UV–Vis spectrophotometer (General Instrument Co. Ltd. Beijing China). Fluorescence emission spectra were recorded at room temperature on a F97XP spectrophotometer (Lengguang Tech. Co. Ltd. Shanghai China) executed by irradiating at 466 nm. The synthesis of Ag nanoclusters occurred in SHA-C reciprocating water bath oscillators. Transmission electron microscopy (TEM) was carried out using a FEI Tecnai G<sup>2</sup> F20 S-TWIN instrument. Calibrating the buffer's pH value is actualized by a Sartorius PB-10 pH meter (Sartorius Instruments Co. Ltd. Beijing, China).

### Reagents

DNA samples mentioned in the article, which is used to compose AgNCs, were purchased from Sangon Biotechnology Co. Ltd. Shanghai, China, and their sequences are as follows:

Template DNA probe (5'–3'): GAG ATT TTCC CAC CCA CCC TCC CAA GTC AGT GTG GAA AAT CTC TAG C

Target DNA HIV (5'–3'): GCT AGA GAT TTT CCA CAC TGA CT

Analogues1 CAM1 (5'–3'): GCT TGA GAT TTT CCA CAC TGA CT

Analogues2 CAM2 (5'–3'): GCT TGA GAT TTT CCA CTC TGA CT

Analogues3 CAM4 (5'–3'): GCT TGA GAT ATT CGA CTC TGA CT

Silver nitrate (AgNO<sub>3</sub>) and sodium borohydride (NaBH<sub>4</sub>) were purchased from National Pharmaceutical Group Chemical Reagent Co. Ltd. Tris–HNO<sub>3</sub> buffer, Mg(NO<sub>3</sub>)<sub>2</sub> and NaNO<sub>3</sub> were dissolved in 250 mL of ultra-pure water to obtain a 100 mmol L<sup>-1</sup> Tris–HNO<sub>3</sub> buffer solution with 5 mmol L<sup>-1</sup> Mg<sup>2+</sup> and 20 mmol L<sup>-1</sup> Na<sup>+</sup>. Other reagents involved in the experiment were all of analytical reagent grade. Ultra-pure water from Milli-Q Reagent Water System (Millipore, Bedford, MA, USA) was used throughout.

### Preparation of fluorescent silver nanoclusters (AgNCs)

According to a previous report, [5] first, we add 10 μL 30 μmol L<sup>-1</sup> hairpin template DNA into 50 μL Tris–HNO<sub>3</sub> buffer (100 mmol L<sup>-1</sup> Tris, 5 mmol L<sup>-1</sup> Mg(NO<sub>3</sub>)<sub>2</sub>, 20 mmol L<sup>-1</sup> NaNO<sub>3</sub>, pH = 7.4) and 40 μL water, then shake gentle at room temperature for 2 h. In addition, 100 μL of Tris–HNO<sub>3</sub> buffer and 80 μL water were fixed into a mixture. At last, 10 μL of 180 μmol L<sup>-1</sup> AgNO<sub>3</sub> and 10 μL of 180 μmol L<sup>-1</sup> NaBH<sub>4</sub> were solved in a mixed solution, respectively. Mixtures were all reacted in a 25 °C water bath for 6 h to synthesize AgNCs. We then optimized the experimental conditions for synthesizing AgNCs with different temperature, reaction time and molar ratio of DNA to AgNO<sub>3</sub>.

### Detection of target DNA

In this study, the detection of Target DNA (HIV) was accomplished by prepared 6 μmol L<sup>-1</sup> AgNCs. Different concentrations of Target DNA were fixed into Tris–HNO<sub>3</sub> with the probe, and the mixture was incubated for 2 h. The AgNCs were prepared according to the above methods. To verify the selectivity of the sensing system, three analogs were also investigated. All experiments were repeated three times.

### Actual sample test

The verification of the probes performance in real samples was tested via fetal bovine serum. We recorded three

concentrations of the sample in fetal bovine serum, which was used to simulate the physiological environment.

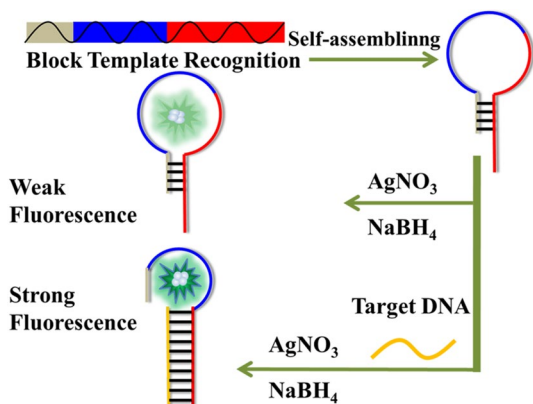
## Results and discussion

### Mechanism of detection system

Figure 1 shows the principle of detecting HIV genes by the molecular beacon-like Ag nanoclusters fluorescence probe. There is a DNA sequence as a molecular beacon to form a hairpin shape. In this sequence, C3AC3AC3TC3A acts as template to create silver clusters with an I-Motif folding drawing on the experience of an earlier report [20]. The HIV sequence is complementary to a part of the loop and stem domain of the hairpin probe. A specialty is a difference from other studies in that identified sequences not only exit in the loop, but also exit in the stem. In the absence of Target DNA, the DNA probe forms a loop configuration, and AgNC template cannot transform to an I-Motif folding structure by a steric hindrance, which destroys the formation of silver clusters. While Target DNA were added into the probe solution, the hairpin molecule beacon changes its structure to open the loop of the probe, which results in an obvious fluorescence enhancement of the Ag nanoclusters because of being AgNCs template free. The sensing platform of nucleic acid detection is based on the above facts.

### Feasibility analysis

We investigated the UV–Vis spectrum of Template DNA only, and Template DNA with Target DNA. Compared to the probe only, there is an obvious absorption peak at 466 nm (Fig. 2A). This result can demonstrate that the Target DNA-specific binding to the loop region hairpin structure of DNA leads to an environment for forming silver clusters.



**Fig. 1** Schematic representation Ag nanocluster molecular beacon-like probe for the detection of Target DNA

DNA–Ag NCs show the maximum emission at 545 nm when excited at 466 nm (Fig. 2B). This blue-green emitting that observed may be caused by lower concentrations of the DNA solution [21].

We monitored the fluorescent spectra under different conditions to further verify the feasibility of the analytic procedure. As shown in Fig. 3A, in the absence of target DNA, the probe emerges as a weak fluorescence, caused by the leaking of AgNCs. When Target DNA exists, a complementary section of the probe would hybridize to form a stable double helix-structure, which is accompanied by a significant increase in fluorescence for the formation of DNA–AgNCs. Based on the change of the fluorescence intensity, DNA sequences could be detected sensitively.

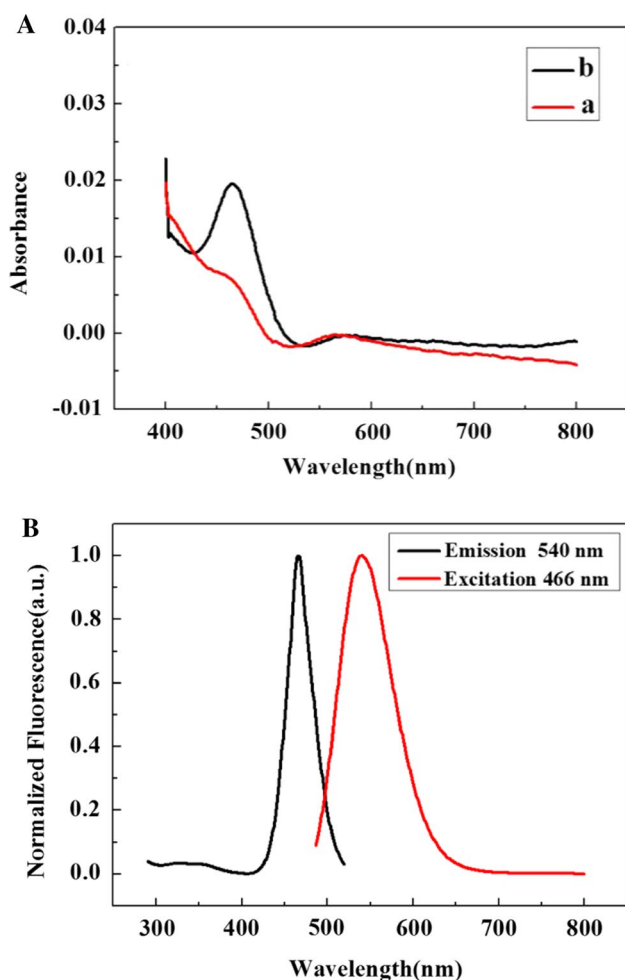
In the meantime, we obtain TEM images of the DNA–Ag nanoclusters. As shown in Fig. 3B, Ag nanoclusters uniformly dispersed in solution with sizes of about 2–3 nm. It is hard to find formed AgNCs in the absence of target DNA by TEM images.

### Optimization of the experimental conditions

The experimental conditions have certain effects on the synthesis of silver nanoclusters. First, we synthesized silver clusters at two different temperatures of 4 °C and 25 °C, which are common temperatures for the synthesis of silver nanoclusters. According to Fig. 4A, the fluorescence intensity changes of silver clusters synthesized at 25 °C is better than that at 4 °C, and 25 °C is also easily available. Hence, we selected 25 °C as the synthesis temperature. Then the effects of reaction time were studied. In Fig. 4B, when the time is 6 h, the fluorescence intensity reaches the peak, so 6 h is selected as the synthesis time. Finally, the molar ratio of DNA to AgNO<sub>3</sub> was also researched for the synthesis of silver nanoclusters. As shown in Fig. 4C, the changes of the fluorescence intensity reach the highest value with the molar ratio of DNA to AgNO<sub>3</sub> for 1: 6, and which is slightly decreases with the molar ratio of DNA to AgNO<sub>3</sub> increasing. Therefore, we choose 1:6 for the molar ratio of DNA to AgNO<sub>3</sub>.

### Detection of target DNA

To detect the concentration of Target DNA, different concentrations of Target DNA (HIV) sequences are added into a hairpin template DNA solution. Specific samples of different concentrations of Target DNA are a–j severally. The fluorescence intensity increases gradually, based on growing Target DNA addition from a to i, which could be attributed to the change of the structure in DNA impacting on the yields of AgNCs. Furthermore, there is a good linearity relationship between the AgNC fluorescence and the Target DNA concentrations, the corresponding

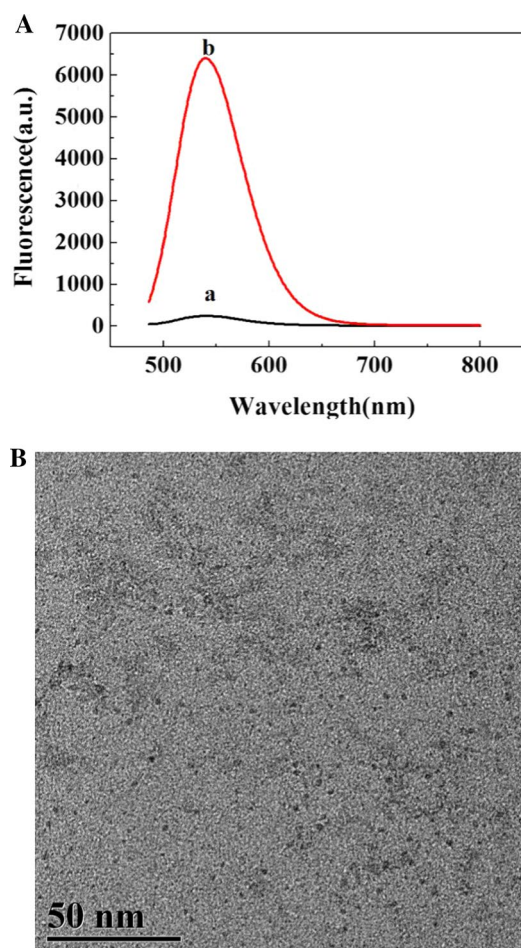


**Fig. 2** **A** UV-Vis spectra of (a) template DNA and (b) template DNA + Target DNA. **B** Fluorescence excitation (curve a) and emission (curve b) spectra of AgNCs synthesized with template DNA + target DNA (HIV)

equation is  $F = 358.63 + 8.29C_{\text{HIV}}$  (the concentration ranged from 1 to 750  $\text{nmol L}^{-1}$ ,  $R^2 = 0.9971$ ). We think that the high sensitivity is probably because the identification area of the designed probe does not only exist in the loop domain, but also is included in a part of stem domain (Fig. 5).

### Selectivity

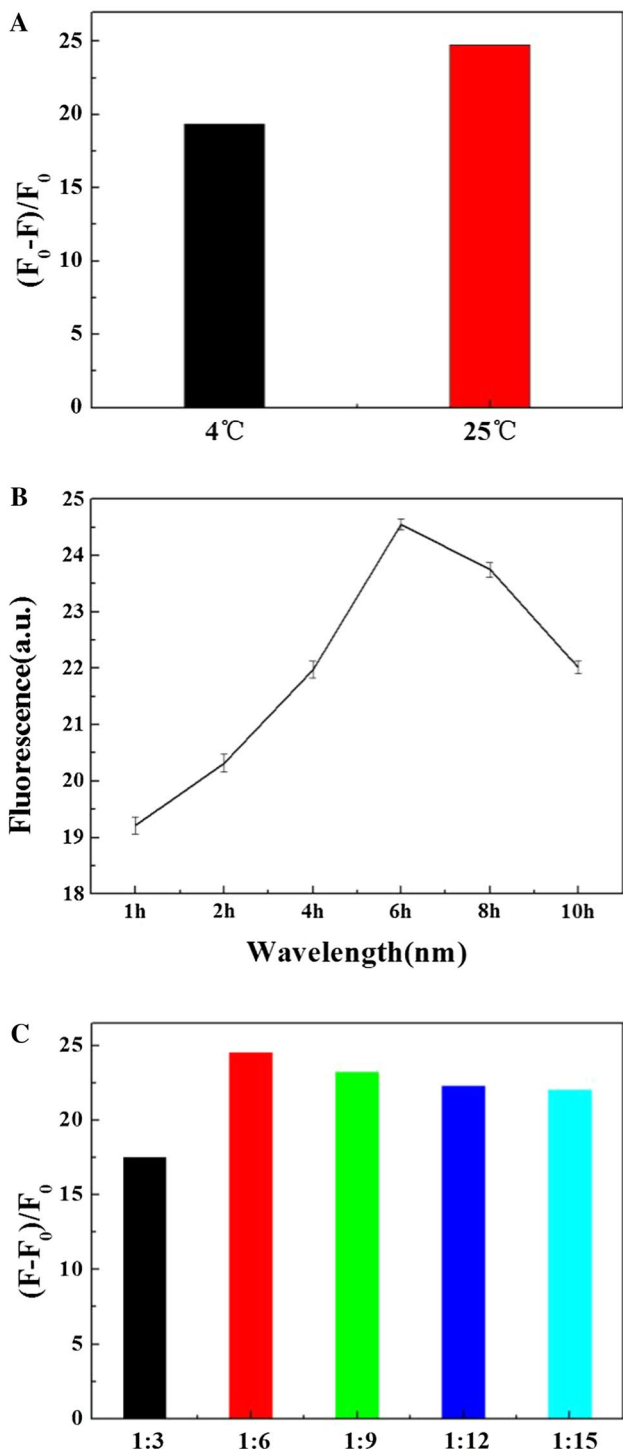
The selectivity is also a kind of indicator of our biosensing system, so that we replace a target DNA sequence with three similar DNA sequences to verify the selectivity. The results are shown in Fig. 6A; target DNA HIV has a significantly higher response than the other three, exactly illustrating the selectivity of our probe. The CAM1 and HIV sequences are just the same, except for a base. However, CAM2 has two bases that are differ to



**Fig. 3** **A** Fluorescence spectra under different conditions (a) template DNA only, (b) template DNA + Target DNA; **B** TEM images of the DNA-Ag nanoclusters

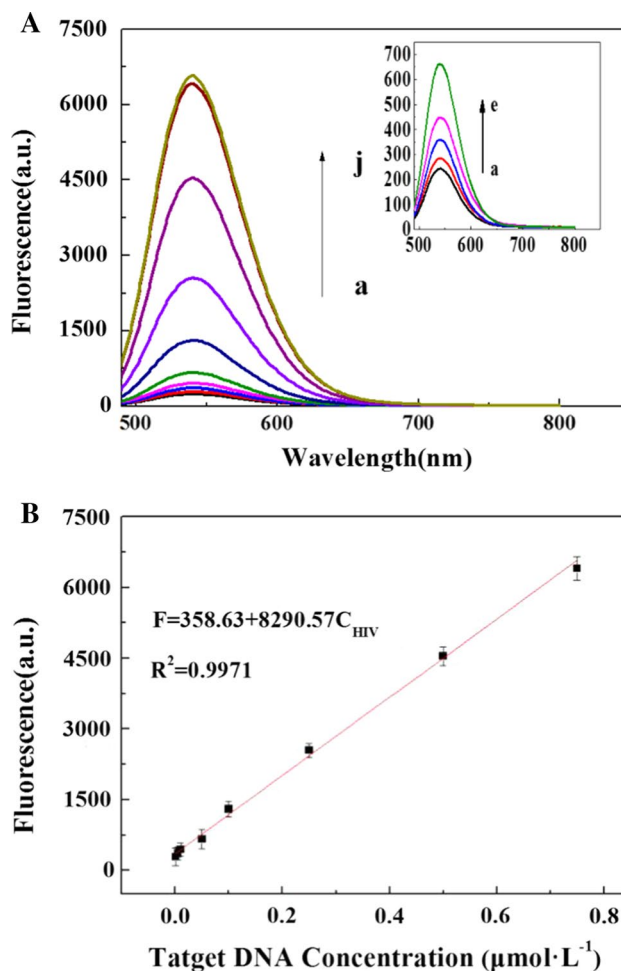
the HIV sequence. Maybe this is why the response of CAM2 is more than double compared to CAM1. Moreover, CAM4 means there are four mismatch bases compare with HIV. The fluorescence will decrease as the number of mismatched bases increases; then, the selectivity of our probe performs relatively better. ( $F_0$ , fluorescence of templated DNA only;  $F$ , fluorescence of templated DNA and target DNA or analogs such as CAM1.)

Finally, the experiment temperature effects of the selectivity are studied. As shown in Fig. 6B, there is good selectivity at 4 °C and 25 °C for the formation of a stable hairpin-like DNA probe. Then, only complete complementary target DNA could easily open the molecular beacon-like structure, leading to a high fluorescence enhancement. However, when the temperatures are increased to 37 °C and 50 °C, the hairpin-like DNA probe became flexible. The Ag nanoclusters are hardly synthesized and similar DNA sequences of target DNA can also



**Fig. 4** Optimization of the experimental conditions for synthesizing Ag nanoclusters with different **A** temperature, **B** reaction time, **C** molar ratio of DNA to AgNO<sub>3</sub>

cleave the hairpin structure, which cause decreasing sensitivity and selectivity.



**Fig. 5** **A** Fluorescence responses of DNA–AgNC probes with varying concentrations of target DNA were (from a to j) 0 nmol L<sup>-1</sup>, 1 nmol L<sup>-1</sup>, 5 nmol L<sup>-1</sup>, 10 nmol L<sup>-1</sup>, 50 nmol L<sup>-1</sup>, 100 nmol L<sup>-1</sup>, 250 nmol L<sup>-1</sup>, 500 nmol L<sup>-1</sup>, 750 nmol L<sup>-1</sup>, 1000 nmol L<sup>-1</sup>. The insert is the fluorescence responses of DNA–AgNC probes with varying concentrations of target DNA from a to e. **B** The linear relationship between the fluorescence intensity and the concentration of HIV target sequence ranges from b to i (1 nmol L<sup>-1</sup> ~ 750 nmol L<sup>-1</sup>). Each data is the mean of three replicates (*N* = 3), and the error bars represent the standard deviations of the measurements

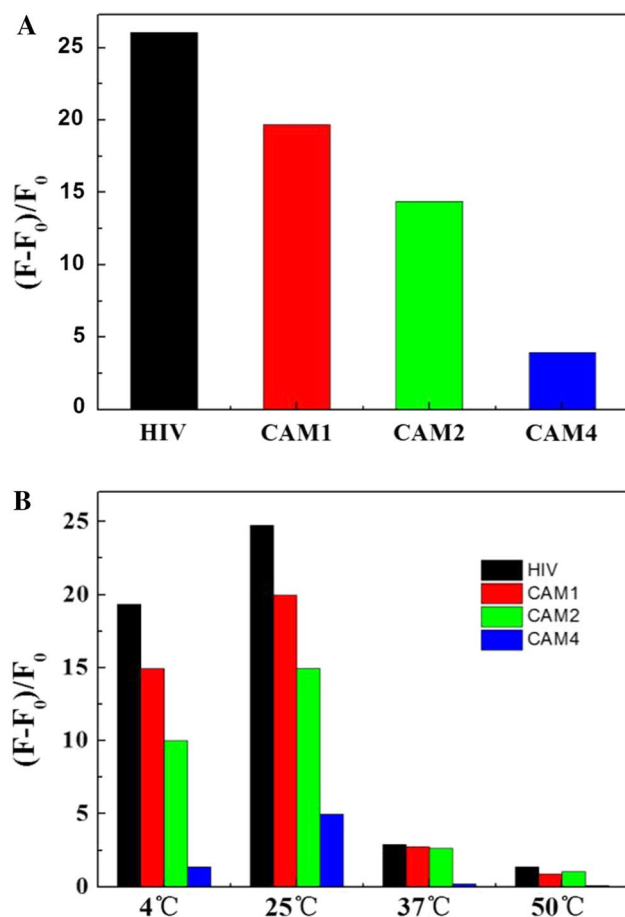
**Actual sample test**

The detected result of real sample in fetal bovine serum is shown in Table 1. The recovery is 97–104% which illustrates our probe could be applied for detecting in real sample.

**Conclusions**

A label-free, cost-effective and highly sensitive molecular beacon was successful designed based on DNA-templated AgNCs. Based on the shape transition of molecular beacon-like DNA probe, we could detect a target DNA sequence





**Fig. 6** **A** Selectivity and interference of the sensing system in Target DNA detection. HIV sequence is replaced by analogs with the same concentration (e.g., CAM1, CAM2, CAM4) in the detection. **B** Selectivity of probe in different temperature

**Table 1** Detected target DNA in fetal bovine serum

Sample	Added amount/ nmol L <sup>-1</sup>	Detect amount/ nmol L <sup>-1</sup>	Recovery, %
Fetal bovine Serum	50	52	104
	100	97	97
	200	203	101.5

with a good liner range. Our sensing system shows a

high selectivity and sensitivity to detecting a target DNA sequence. By replacing the DNA sequence of the identification region, the probe would have a wider application prospect in biosensing.

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