Electronic Supporting information (ESI)

Hairpin DNA Probes Based on Target-induced *in situ* Generation of Luminescent Silver Nanoclusters

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Experimental details.

Reagents: All DNA strands were supplied by Sangon Biotechnology Co., Ltd. (Shanghai, China). The human α-thrombin was purchased from Haematologic Technologies Inc. (Essex Junction, VT, USA). Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Amino acids were from Ru-Ji Biotechnology Co., Ltd. (Shanghai, China). The rest of the chemical reagents were commercially available and at least analytical grade. All aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore, 18.2 MΩ resistivity).

Synthesis of Ag nanoclusters: In a typical protocol, 2.5 μM DNA solutions in 20 mM phosphate buffer (5.0 mM Mg^{2+}, pH 7.0) was heated at 85 ℃ for 15 min, and gradually cooled to room temperature. Then, $AgNO_3$ in a molar ratio of 6:1 was added to the DNA solutions. After incubated for 15 minutes at room temperature, freshly prepared sodium borohydride (15 μM) was added to the mixture and quickly vortexed for one minute. The obtained solution was left in the dark for 4 h at 4 ℃ to form Ag NCs.

Sensing of HBV gene: In the hybridization procedure, different concentrations of HBV gene were individually introduced to 0.3 μM probe DNA in 20 mM phosphate buffer (5.0 mM Mg^{2+}, pH 7.0). The DNA solutions were incubated at room temperature for 1.5 h with gentle shaking. Then, 1.8 μM $AgNO_3$ was added to the DNA duplexes solutions. After incubated for 15 minutes at room

temperature, this mixture reduced with freshly prepared sodium borohydride (1.8 μM). The reaction was kept in the dark for 1 h at 4 ℃ before fluorescence measurement. A single-base mismatched DNA chain and a non-complementary DNA chain were added in place of the target DNA with the same experimental conditions to test the specificity of the probe.

Detection of HBV gene in diluted serum: Human serum was obtained from Zhongnan hospital (Wuhan University). They were first diluted 50 times with 20 mM phosphate buffer (5.0 mM Mg^{2+}, pH 7.0). Then the diluted serum were loaded into centrifugal filtration devices (MWCO = 50,000 Da, Millipore) and subjected to centrifugation (6,000 rpm, 20 min).[1] For the standard addition experiments, different concentrations of HBV gene were spiked into pretreated human serum and the identical assay procedure as in the aqueous solution was followed.

Sensing of thrombin: A 1 μM probe DNA solution in 20 mM phosphate buffer (5.0 mM Mg^{2+}, pH 7.0) was heated at 85 ℃ for 15 min, and gradually cooled to room temperature. Then, the DNA solution was incubated with various concentrations of thrombin in 20 mM Tris–HAc buffer (100 mM NaAc, 10 mM KAc, 2 mM $Mg(Ac)_2$, pH 7.4) at 37 ℃ with gentle shaking for 1 h. After gradually cooled to room temperture, the mixture was incubated with 6 μM $AgNO_3$ for 15 minutes and reduced with 6 μM $NaHB_4$ for 2 h to generate Ag NCs. Thereafter, the mixtures were diluted to 500 μL with Tris–HAc buffer for subsequent fluorescence measurements. To examine the specificity of the probe towards thrombin, some other biomolecules were added in place of thrombin with the same experimental conditions and procedures.

Gel electrophoresis: The 16% polyacrylamide gel electrophoresis (PAGE) analysis of the DNAs was carried out in 1×Tris-Borate-EDTA at a constant voltage of 120 V for about 2 h. After ethidium bromide staining, the gel was scanned using a Pharos FX Molecular Imager (Bio-Rad, USA).

Instrumentation: The size and morphology of Ag nanoclusters were characterized by a JEM-2010 transmission electron microscope with an acceleration voltage of 200 kV. The UV-vis absorption spectra were acquired using a UV-2550 UV-vis spectrometer (Shimadzu, Japan). The fluorescence measurements were performed with a RF-5301 PC fluorometry (Shimadzu, Japan).

Supplementary Tables and Figures

Table S1. Sequences of the oligonucleotides used in the experiments.

Names		Sequence (5'-3')
DNA-a		CCCTTAATCCCC
DNA-b		CCCTT
Probe for HBV gene	Probe **1**	CCCTTAATCCCCTACCACATCATCCATATAACTG AAAGCCAAGGGGATT
	Probe **2**	CCCTTAATCCCCTACCACATCATCCATATAACTG AAAGCCAAGGGGA
	Probe **3**	CCCTTAATCCCCTACCACATCATCCATATAACTG AAAGCCAAGGGGATTAAG
HBV gene	Target **1**	TTGGCTTTCAGTTATATGGATGATGTGGTA
	Target **2**	TTGGCTTTCAGTTATATGGATGATG
	Target **3**	TTGGCTTTCAGTTATATGGA
The single-base mismatched (SBM) strand*		TTGGCTTTCAGTTATATTGATGATGTGGTA
The non-complementary (NC) strand		ACTAAGGACTACAAGTACATTTCGAATTCT
Probe for thrombin		CCCTTAATCCCCTTTTTTTTTGGTTGGTGTGGTTG GGAT

*The mismatched base is underlined.

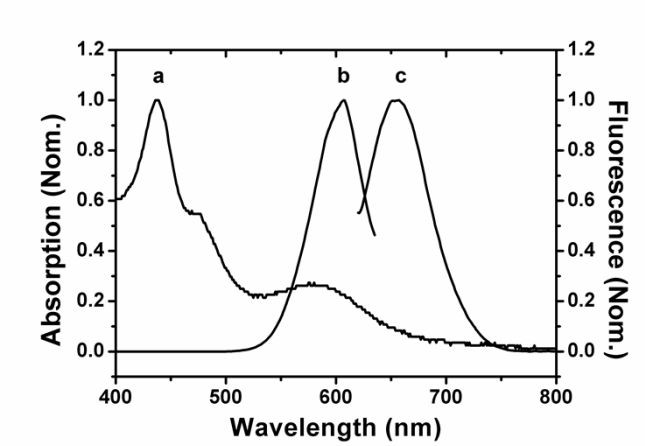

Figure S1. Normalized UV-vis absorption (a) and fluorescence excitation (b) and emission (c) spectra of the silver species produced with the sequence DNA-a in Table S1 as the template.

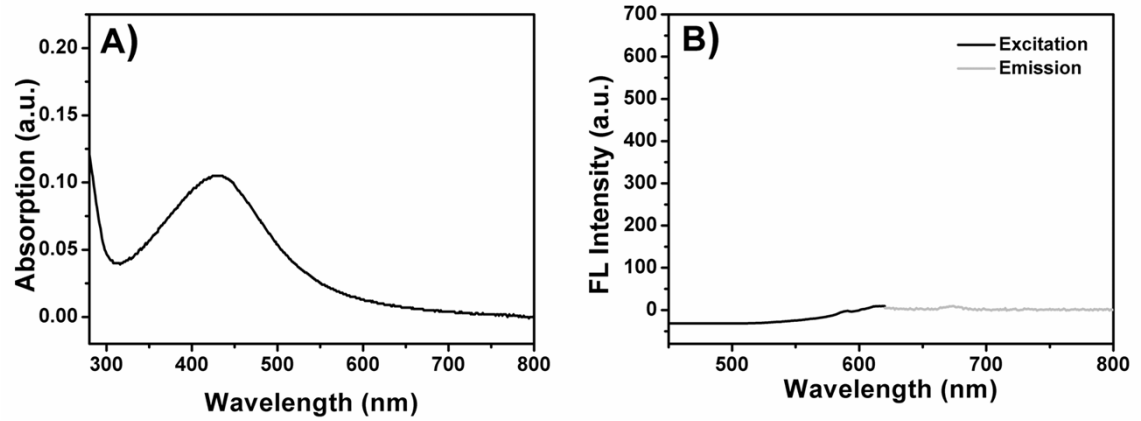

Figure S2. (A) UV-vis absorption and (B) fluorescence excitation (black curve, λ_{em} = 655 nm) and emission (gray curve, λ_{ex} = 600 nm) spectra of the silver species produced with the sequence DNA-b in Table S1 as the template.

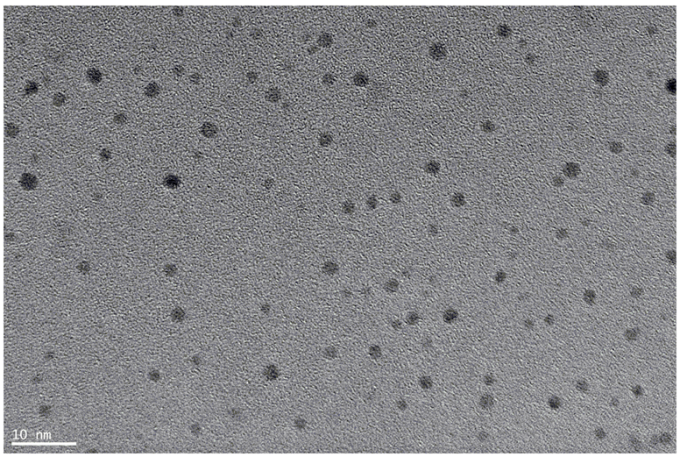

Figure S3. TEM image of the as-synthesized DNA-Ag NCs

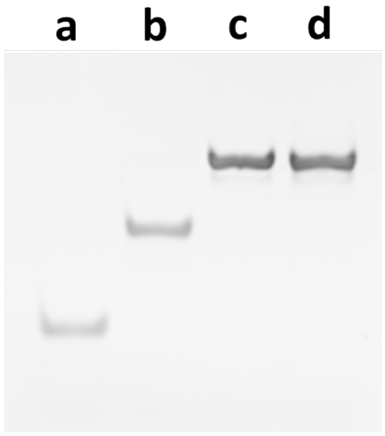

Figure S4. The polyacrylamide gel electrophoresis analysis of (a) HBV gene, (b) Probe, (c) Probe-HBV gene duplex and (d) Probe-HBV gene duplex after the generation of Ag NCs. Concentrations for each DNA strand in PAGE are all 2 μM.

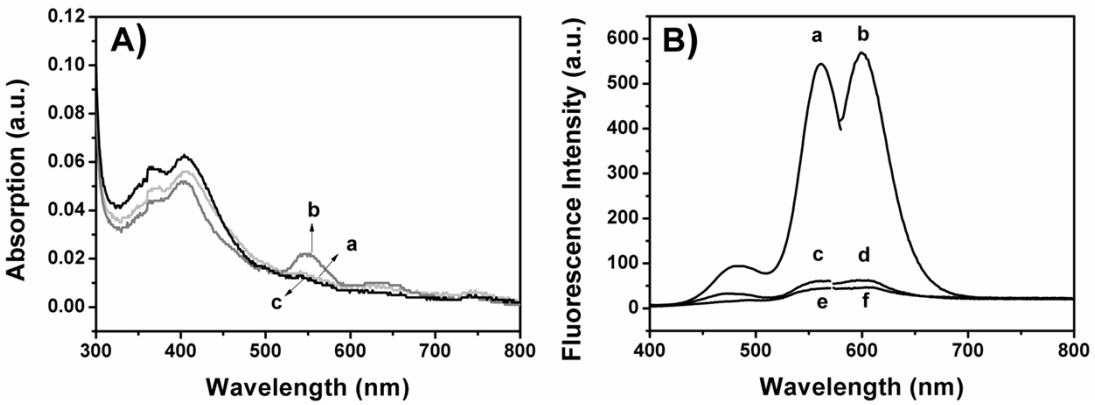

Figure S5. (A) UV-vis absorption spectra of the silver species synthesized with the locked probe **1** (a), probe **2** (b) and probe **3** (c). (B) Fluorescence excitation and emission spectra of silver species produced with the locked probes (a and b: probe **2**; c and d: probe **1**; e and f: probe **3**).

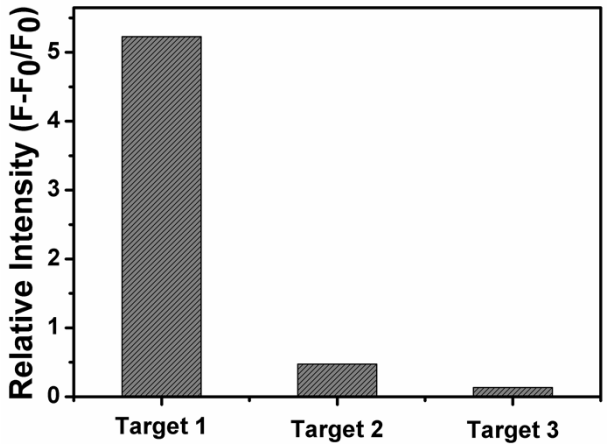

Figure S6. Relative fluorescence intensity ($(F-F_0)/F_0$, where F and F_0 represent the emission intensity of Ag NCs in the presence and in the absence of the tested species, respectively) of the HBV gene probe in the presence of different targets.

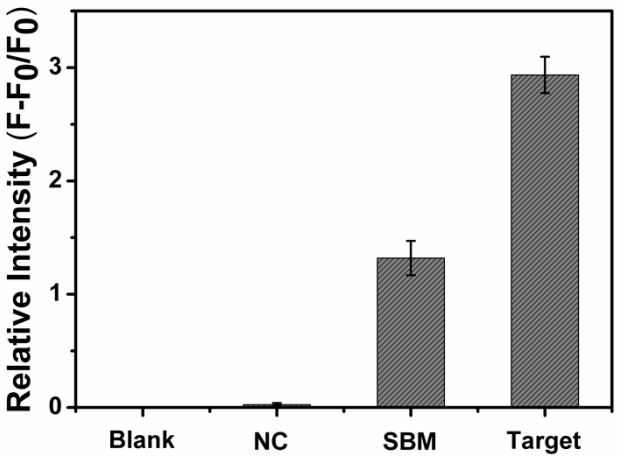

Figure S7. Relative fluorescence intensity ($(F-F_0)/F_0$, where F and F_0 represent the emission intensity of Ag NCs in the presence and in the absence of the tested species, respectively) of the HBV gene probe in the presence of different substances. The concentration of all tested species was 80 nM.

Table S2. Recovery of HBV gene in spiked serum samples.

Samples	Added (nM)	Found (nM)	Recovery(%)	RSD(%) n=3
1	40	38.2	96	7.3
2	80	78.9	99	4.2
3	120	124.0	103	4.0

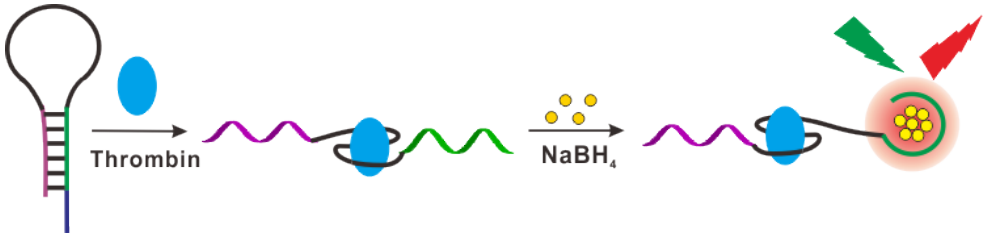

Figure S8. Schematic illustration of the analysis of thrombin using the hairpin DNA probe.

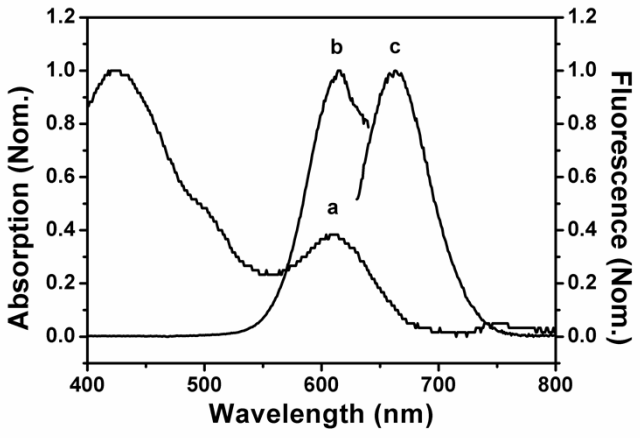

Figure S9. Normalized UV-vis absorption (a) and fluorescence excitation (b) and emission (c) spectra of Ag NCs generated on the scaffold of thrombin probe.

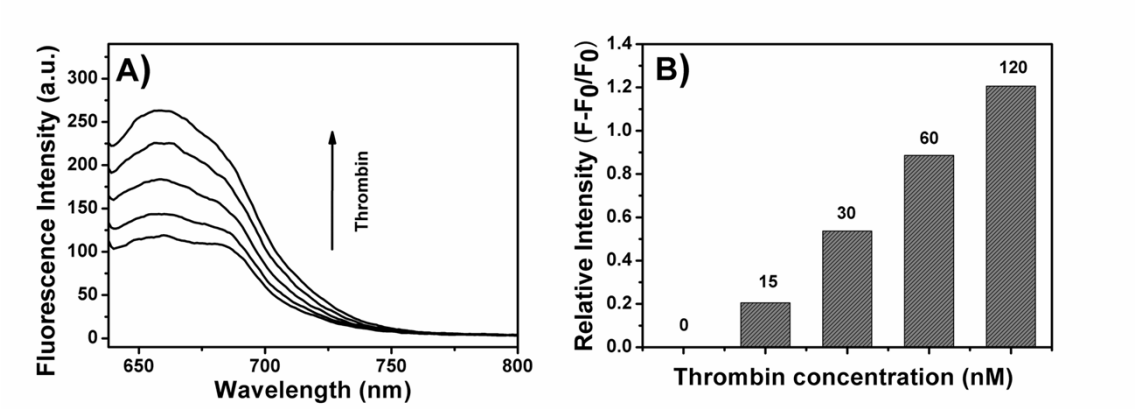

Figure S10. (A) The fluorescence emission of DNA-Ag NCs in the presence of varying concentration (0, 15, 30, 60, 120 nM) of thrombin. (B) Relative fluorescence intensity of Ag NCs in the presence of different concentrations of thrombin. Excitation wavelength: 600 nm.

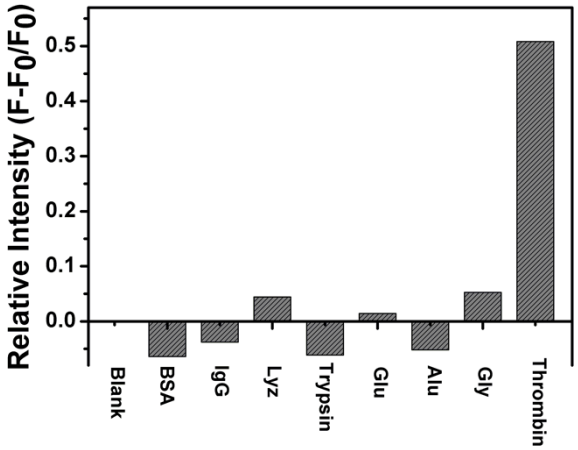

Figure S11. Relative fluorescence intensity ($(F-F_0)/F_0$, where F and F_0 represent the emission intensity of Ag NCs in the presence and in the absence of the tested species, respectively) of the thrombin probe in the presence of different substances. The concentration of thrombin was 30 nM, and the concentration of other species was 1 μM.

1. J. Li, X. Zhong, H. Zhang, X. C. Le and J. J. Zhu, *Anal. Chem.* 2012, **84**, 5170.