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DNA-regulated silver nanoclusters for label-free ratiometric fluorescence detection of DNA†

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Experimental

Materials and reagents. All oligonucleotides were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China) and their sequences are listed in Table S1. Tris−HCl buffer (20 mM, pH 7.5) was used for the stock solutions of oligonucleotides. The stock solutions were accurately quantified by UV-Vis absorption spectroscopy with the extinction coefficients obtained from http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/. $AgNO_3$ was obtained from Shanghai Reagent Co. (Shanghai, China). All other reagents were of analytical grade and used without further purification. Human serum samples were kindly provided by Jiangsu Cancer Hospital (Nanjing, China). A mixture containing equal volumes of human serum sample and DNA hybridization buffer was used for recovery testing. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all assays.

Apparatus. UV-vis absorbance spectroscopy was recorded on a UV-3600 Spectrophotometer from (Shimadzu, Japan). The morphology of Ag nanoclusters (Ag NCs) was examined using a JEM 2100 high-resolution transmission electron microscopic (TEM) (JEOL, Japan).

Fluorescence (FL) spectra were recorded on a F97XP fluorospectrometer (Shanghai LengGuang Technology co., LTD., China).

Table S1. Oligonucleotides employed in this work

Oligonucleotides	**Oligonucleotides Sequence (**5′ - 3′**)**
R-tm target	TCAGCGGGGAGTTTGGGAGTAAAGTTAATA
B-tm target	ACGGTGGGGAGGAAGGGAGTAAAGTTAATA
Probe RA	TATTAACTTTACTCCCTTCCCTTCCTCCCCGCTGA
Probe RT	TATTAACTTTACTCCCTTTCCCTTCCTCCCCGCTGA
Probe RC	TATTAACTTTACTCCCTTCCCCTTCCTCCCCGCTGA
Probe RG	TATTAACTTTACTCCCTTGCCCTTCCTCCCCGCTGA

The red parts of Probe represent the loop for generating Ag NCs1. The mismatch bases are labeled by brown.

Synthesis of DNA-regulated Ag NCs. First of all, the solutions of probes and target DNA in 20 mM Tris-HCl buffer (pH = 7.5) were heated at 92 °C for 10 min, and gradually cooled to room temperature. Then, 1.2 μL $AgNO_3$ solution (1.0 mM) was added into the 197.6 μL resulting solution. After the mixture was vibrated for 15 min and incubated for 1 h at 4 °C, 1.2 μL $NaBH_4$ solution (1.0 mM) was added. The mixture was further kept in the dark at room temperature for 2 h. The final concentrations of probes, $AgNO_3$ and $NaBH_4$ were 1, 6 and 6 μM, respectively.

Ratiometric fluorescence detection of target DNA. To measure fluorescent signals of Ag NCs, the fluorescence spectra were scanned in the range of 570 to 690 nm and 620 to 750 nm at excitation wavelengths of 550 and 600 nm, respectively, and the excitation and emission slits were both set to 10 nm.

Fluorescence property of Ag NCs

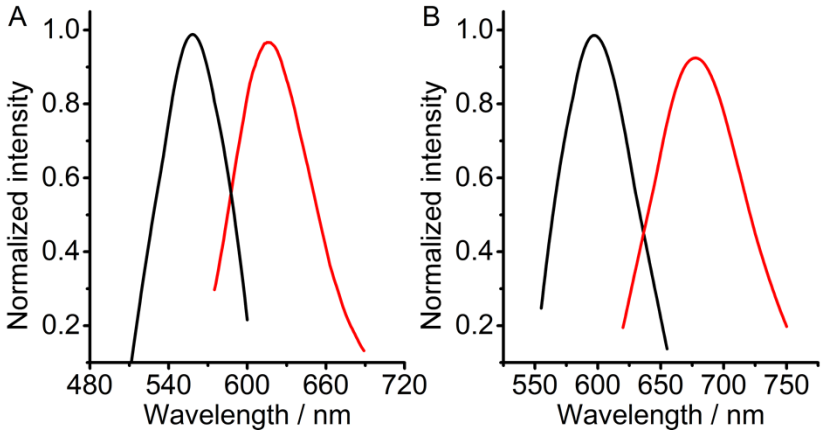

Fig. S1 Excitation (black) and emission (red) spectra of (A) Ag NCs1 (λ_{ex} = 550 nm, λ_{em} = 620 nm) and (B) Ag NCs2 (λ_{ex} = 600 nm, λ_{em} = 675 nm) generated from 1 μM probe 1.

Effect of the sequence of loop on fluorescence intensity of Ag NCs

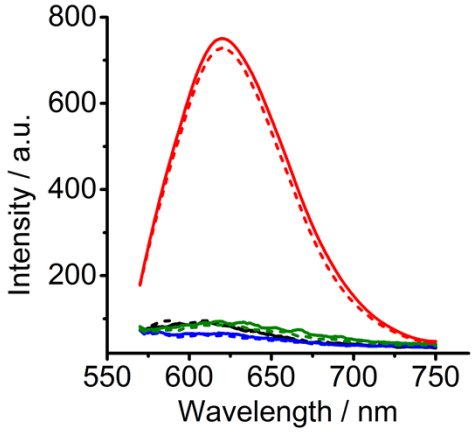

Fig. S2 Fluorescence spectra of Ag NCs generated from 1 μM probe RA (black), probe RT (blue), probe RG (green) and probe RC (red) with (solid) and without (dash) 100 nM target DNA.

Optimization for synthesis of DNA-regulated Ag NCs

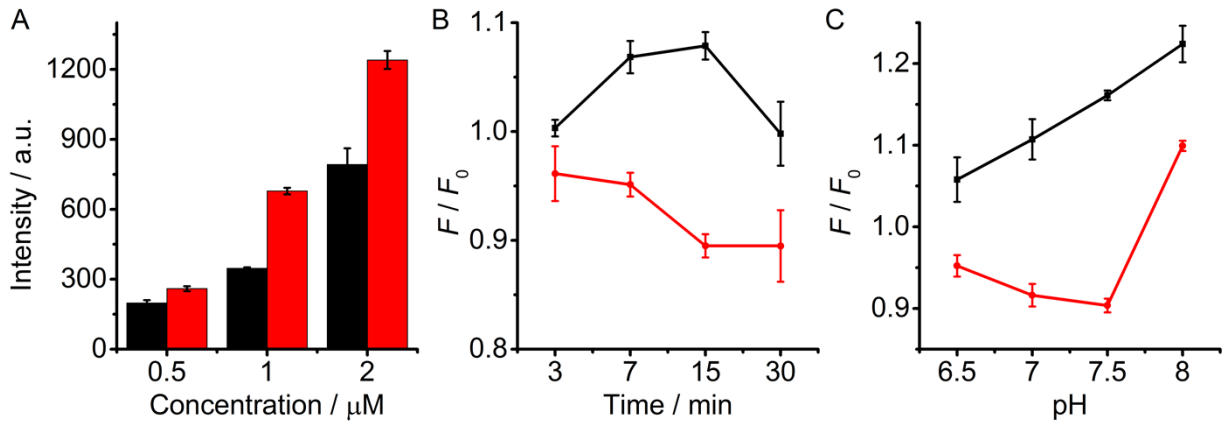

Fig. S3 (A) Fluorescence intensity of Ag NCs1 (black) and Ag NCs2 (red) generated from 0.5, 1 and 2 μM probe 1. Effect of (B) vibration time of $NaBH_4$ and (C) pH during Ag NCs synthesis on the ratio of F/F_0. F and F_0 are the fluorescent intensities of Ag NCs1 (black) and Ag NCs2 (red) generated from 1 μM probe 1 after and before addition of 100 nM target, respectively.

TEM characterization of Ag NCs

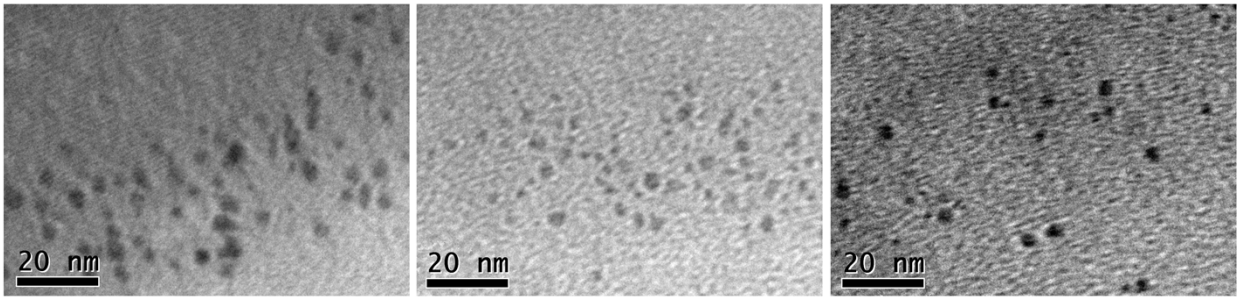

Fig. S4 TEM images of Ag NCs generated from 5 μM probe 1 (A), probe B (B), probe R (C).

Specificity of DNA sensing protocol

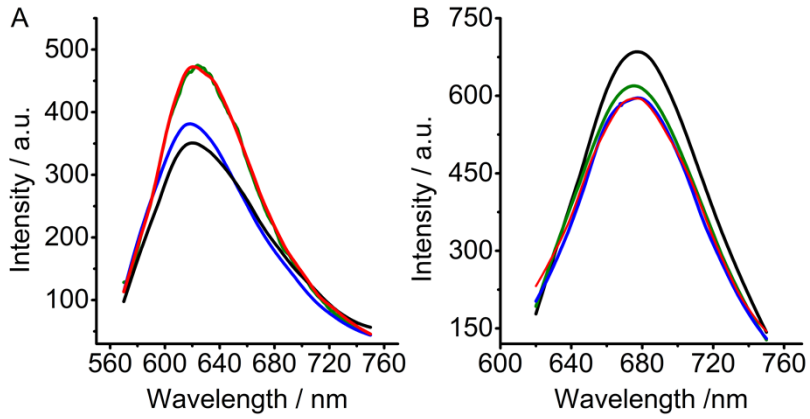

Fig. S5 Fluorescence spectra of 1 μM probe in absence of target (black), and presence of 250 nM complementary target (red line), R-tm (blue) and B-tm mismatched target (green) when excited at (A) 550 nm and (B) 600 nm.