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Supporting Information

Protein-DNA interactions: A novel approach to improve the fluorescent stability of DNA/Ag nanoclusters

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Experimental Section

Materials

Oligonucleotides with specific sequences (the sequences are listed in Scheme 1B) were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The concentrations of oligonucleotides were determined using the 260 nm UV absorbance with the corresponding extinction coefficient. *E. coli* Single-Stranded DNA Binding Protein (SSB) (4470 μg/mL) was purchased from Promega Corporation. Other reagents were of analytical grade and used as received. All aqueous solutions were prepared with ultrapure water (＞18 MΩ) from a Milli-Q Plus system (Millipore).

Characterization

The fluorescence emission spectra were collected using a Fluoromax-4 Spectrofluorometer (HORIBA Jobin Yvon, Inc., NJ, USA) at room temperature. The decay curves for N-DNA/Ag NCs and N-DNA/SSB/Ag NCs are monitored three hours after the initiation of NC nucleation, and the decay curves for R-DNA/Ag NCs and R-DNA/SSB/Ag NCs are monitored nine hours after the initiation of NC nucleation. The decay curve at 80 °C was also monitored by the Fluoromax-4 Spectrofluorometer with temperature held at 80 °C.

UV-vis absorption spectra were recorded by a CARY 500 UV/vis-near-IR Varian spectrophotometer.

Synthesis of Ag NCs with Different DNA Templates

In a typical synthesis experiment, 10 μL 1 mM $AgNO_3$ was added into the ssDNA templates dissolved in PBS-Buffer (20 mM phosphate, pH 7.4, 1 mM magnesium acetate). After the mixtures were stirred for 30 s, they were reduced with 10 μL 1 mM $NaBH_4$. The final volume is 400 μL with $[Ag^+]$=6 μM, $[BH_4^-]$=6 μM, [ssDNA]=1 μM. For ssDNA/SSB template, SSB (44.7 μg/mL, ~0.59 μM) was mixed with ssDNA (1 μM) and allowed to react for ten minutes. Other steps were the same as the preparation of ssDNA-directed Ag NCs described above.

For the experiment that SSB is introduced after the formation of N-DNA-stabilized Ag NCs,

DNA-templated Ag NCs were first prepared as described above, then SSB (44.7 μg/mL) was added immediately (Fig. 2A, curve a) or ten hours later (Fig. 2A, curve d).

Native Polyacrylamide Gel Electrophoresis

The DNA solution (N-DNA, N-DNA/Ag NCs and N-DNA/SSB/Ag NCs) mixed with 6×loading buffer was analyzed in 15% native polyacrylamide gel. The electrophoresis was conducted in 1×TBE (pH 8.2) at constant voltage of 110 V for 1 h. The gels were scanned by a UV transilluminator after staining with Gel-Red.

Analysis of Hg^{2+} with N-DNA/SSB-stabilized Ag NCs as probes In a typical procedure, N-DNA/SSB-stabilized Ag NCs were prepared as descried above, and were then mixed with aliquots of Hg^{2+} (0-0.5 μM). The fluorescence of the mixtures was then measured at room temperature.

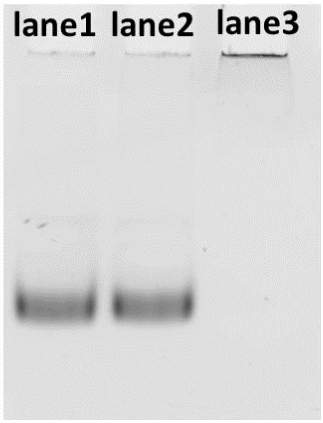

Fig. S1 Native PAGE analysis of the binding of SSB to N-DNA/Ag NCs：lane 1: N-DNA; lane 2: N-DNA/Ag NCs; lane 3: N-DNA/SSB/Ag NCs.

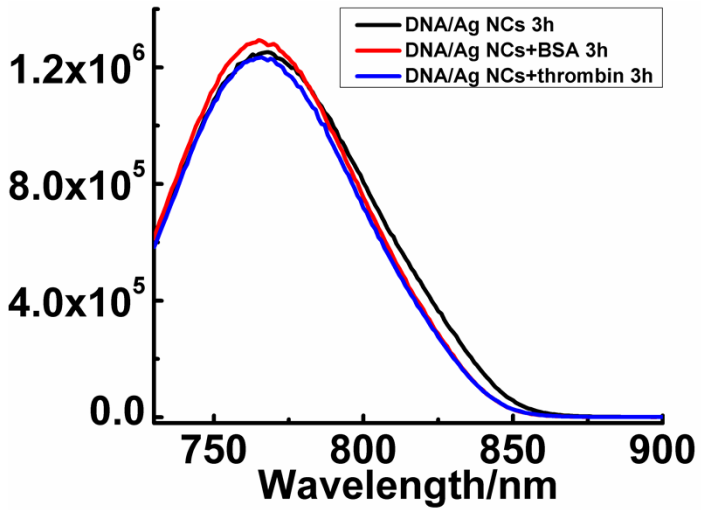

Fig. S2 Fluorescence spectra of N-DNA-stabilized Ag NCs (black line); N-DNA-stabilized Ag NCs in the presence of BSA (red line); N-DNA-stabilized Ag NCs in the presence of thrombin (blue line). The spectra were collected three hours after the initiation of NC nucleation. BSA or thrombin itself shows no quenching effect on the fluorescence of Ag NCs.

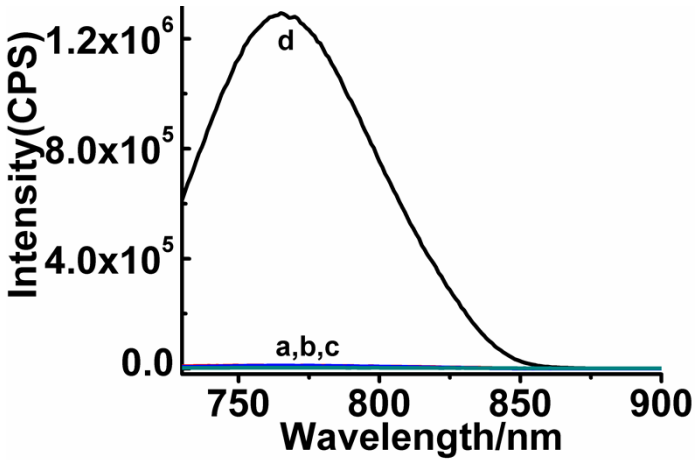

Fig. S3 Fluorescent spectra of various solutions: synthesis Ag NCs with a) SSB; b) G-DNA; c) G-DNA/SSB complex; d) N-DNA. The spectra were collected three hours after the initiation of NC nucleation.

Table S1 Investigation of the stability of N-DNA/SSB/Ag NCs in cell culture media.

10×N-DNA/SSB/Ag NCs (μL)	PBS-Buffer (μL)	DMEM (μL)	F_{DMEM}/ $F_{PBS\text{-}Buffer}$
50	**100**	**100**	**96%**
50	**200**	**200**	**92%**
50	**300**	**300**	**85%**
50	**450**	**450**	**77%**

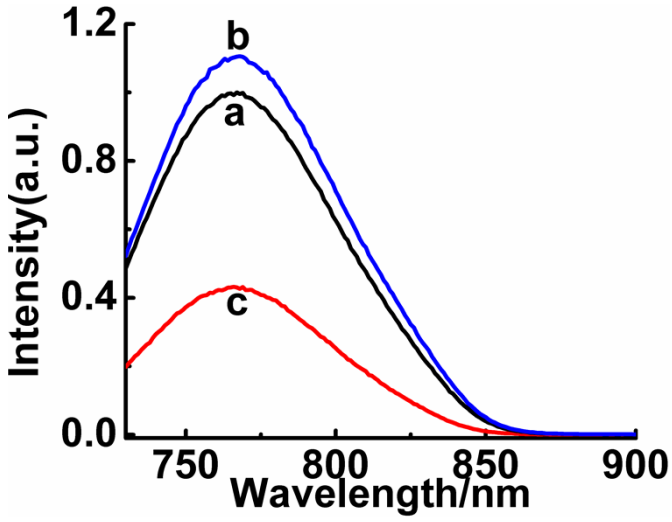

Fig. S4 Fluorescent spectra of various solutions: a) N-DNA/SSB/Ag NCs; b) addition of $NaNO_3$ (0.5 M) to (a); c) addition of NaCl (0.5 M) to (a).

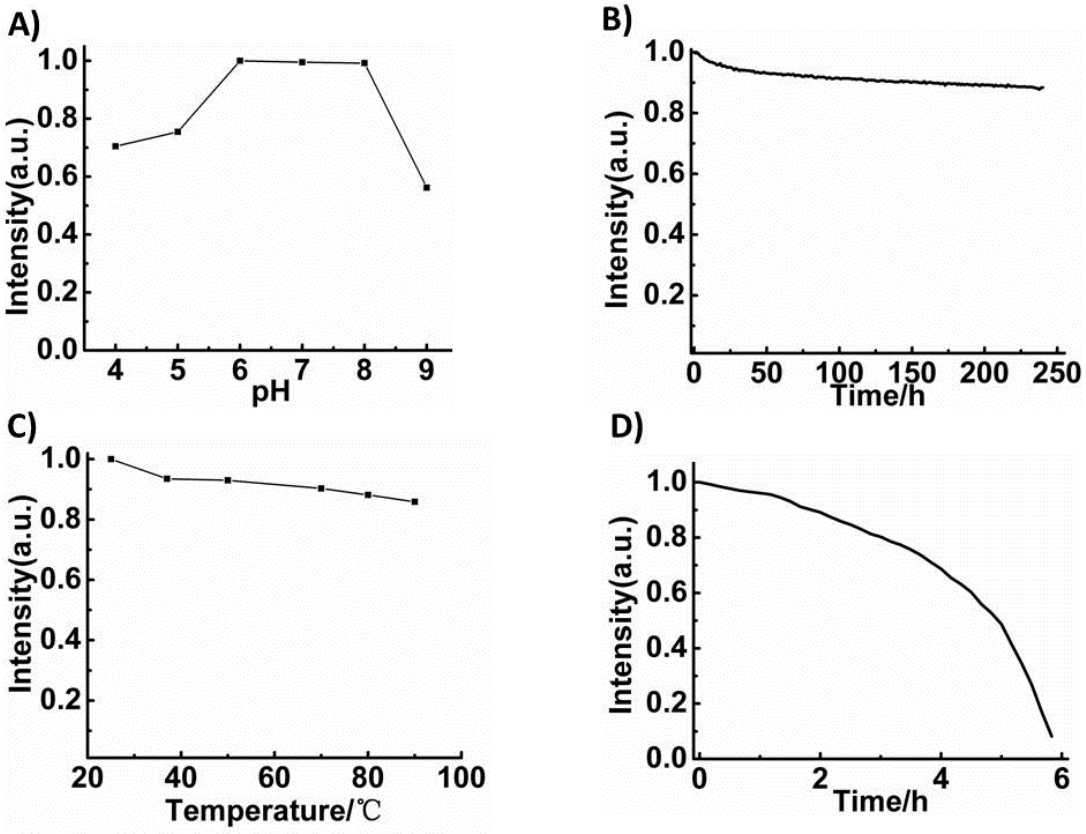

Fig. S5 (A) The influences of pH values on the fluorescence of N-DNA/SSB/Ag NCs. (B) The decay curve of N-DNA/SSB/Ag NCs at pH 5.0. (C) The influences of temperature on the fluorescence of N-DNA/SSB/Ag NCs. (D) The decay curve of N-DNA/SSB/Ag NCs monitored at 80 ◦C.