Electronic Supplementary Information

A new mode to light up adjacent DNA-scaffolded silver probe pair and its application for specific DNA detection

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1. Experiment section

Reagents. Oligonucleotides were synthesized and purified by Sangon Inc. (Shanghai, China). The sequences of these oligonucleotides are listed in Table S1. *E.coli* exonuclease III (Exo III) was purchased from Fermentas Inc. (Vilnius, Lithuania). Silver nitrates ($AgNO_3$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium borohydride ($NaBH_4$) was obtained from Tianlian Fine Chemical Co., Ltd. (Shanghai, China). Other chemicals were analytical grade and directly used without additional purification, purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solutions were prepared with ultrapure water (18.2 MΩ·cm) from a Millipore Milli-Q system (Bedford, MA, USA). The reaction buffers used in DNA/AgNCs synthesis were as follows: 2× MOPS (100 mM $NaNO_3$, 40 mM MOPS, pH 7.0), 2× HEPES (20 mM HEPES, 200 mM $NaNO_3$, pH 7.4), 2× Na_3PO_4 (40 mM Na_3PO_4, pH 6.6), 10× Tris (100 mM Tris, pH 7.9).

Table S1 The sequence information of DNA oligonucleotides used in this work.

Name	Sequence* (5'→ 3')
Probe 1	CCCTAACTCCCCAGCACATCTGATAGTTCTATGCT
Probe 2	GAACTATCAGATGTGCTCCTCCTTCCTCC
Probe 3	CCCTTAATCCCCAGCACATCTGATAGTTCTATGCT
Probe 4	GAACTATCAGATGTGCTCCCTAACTCCCC
Probe 5	GAACTATCAGATGTGCTGGGTGGGGTGGGGTGGGG
Probe 6	CCCTAACTCCCCGTGCGTGTTATCAGATCAAACACGCACCTCAA
Probe 7	ATCTGATAACACGCACCCTCCTTCCTCC
Probe 8	CCCTAACTCCCCTGTGCGTGTATCAGATCAAACACGCACCTCAA
Probe 9	ATCTGATACACGCACACCTCCTTCCTCC
Probe 10	CCCTAACTCCCCTGTGCGTGAATCAGATCAAACACGCACCTCAA
Probe 11	ATCTGATTCACGCACACCTCCTTCCTCC
T1	TTGAGGTGCGTGTTTGTGCC
T2	TTGAGGTGCATGTTTGTGCC
T3	TTGACGTGCATGTTTGTGCC
T4	TTGACGTGCATGTTTATGCC

* The bases in blue color represent the AgNCs-nucleation sequences used as templates to synthesize DNA/AgNCs. The bases in brown color represent G-rich DNA sequence. The bases in red color represent the stem sequences in hairpin-shaped probes. The bases in green color represent the Exo III-resistant 3'-protruding sequences in hairpin-shaped probes. The bases in purple color represent the mutation sites in tested DNA targets compared to target T1.

Instrumentation. Fluorescence spectra were recorded by a fluorescence microplate reader Synergy™ Mx (Bio Tek Instruments, Inc., Winooski, USA) using a black 384-well microplate (Greiner, Germany). Transmission electron microscope (TEM) measurements were performed on Jeol JEM-2100 instrument (JEOL Ltd., Japan).

Synthesis of DNA/AgNC solution. The aqueous DNA/AgNC solution were prepared according to the reported protocols developed by Petty and co-workers [1] with minor modification. Briefly, a reaction buffer was sequentially added 1 μM DNA template and 6 μM $AgNO_3$, and then the mixture was kept in the dark at room temperature for 20 min. Subsequently, 6 μM $NaBH_4$ was added to the above mixture following by vigorous shaking for 5 s. Finally, the mixture was kept in the dark at room temperature for 1 hr before use.

Procedures for Exo III-assisted specific DNA detection. First, a reaction solution containing 100 nM Probe A was heated at 90°C for 5 min, and slowly cooled to and kept at 37°C for 20 min to form the stable hairpin structure. Exo III and target DNA were added to the reaction solution and incubated at 37°C for 100 min. After the incubation step, the mixture were heated at 90°C for 5 min to inactivate Exo III, and slowly cooled to room temperature. Then 6 μM $AgNO_3$ in reaction buffer was added, and kept in the dark at room temperature for 20 min. Subsequently, 6 μM $NaBH_4$ was added to the mixture following by vigorous shaking for 5 s and keeping in the dark at room temperature for 1 hr. After that, a prepared Probe B/AgNCs solution was added to the above mixture and kept in the dark at room temperature for 80 min. Finally, the resultant mixture was transferred to a black 384-well microplate and scanned using the fluorescence microplate reader Synergy™ Mx from 580 nm to 750 nm under excitation at 560 nm. Unless noted otherwise, all experiments in this work were repeated three times.

2. Optimization of the reaction buffer for DNA/AgNC synthesis

It is well-known that Ag^+ ion easily forms an insoluble product with some general anions, such as Cl^-, SO_4^{2-}, a process that breaks the formation of AgNC. In this work, four reaction buffers were selected for DNA/AgNC synthesis, including 2× MOPS (100 mM $NaNO_3$, 40 mM MOPS, pH 7.0),

2× HEPES (20 mM HEPES, 200 mM $NaNO_3$, pH 7.4), 2× Na_3PO_4 (40 mM Na_3PO_4, pH 6.6), 2× Tris (20 mM Tris, pH 7.9). According to the procedures of DNA/AgNC synthesis as described in experimental section, a series of Probe 1/AgNC solution were made by adding $AgNO_3$ to the Probe 1 solution in the tested reaction buffers, followed by reduction with $NaBH_4$, respectively. Similarly, a series of Probe 2/AgNC solutions were synthesized under the identical condition. Then Probe 1/AgNC solution and Probe 2/AgNC solution were mixed and kept in the dark at room temperature for 2 hr before fluorescence measurement. As shown in Fig. S1, MOPS buffer provided the best fluorescence signal, thus we chose it as reaction buffer for DNA/AgNC synthesis in this work.

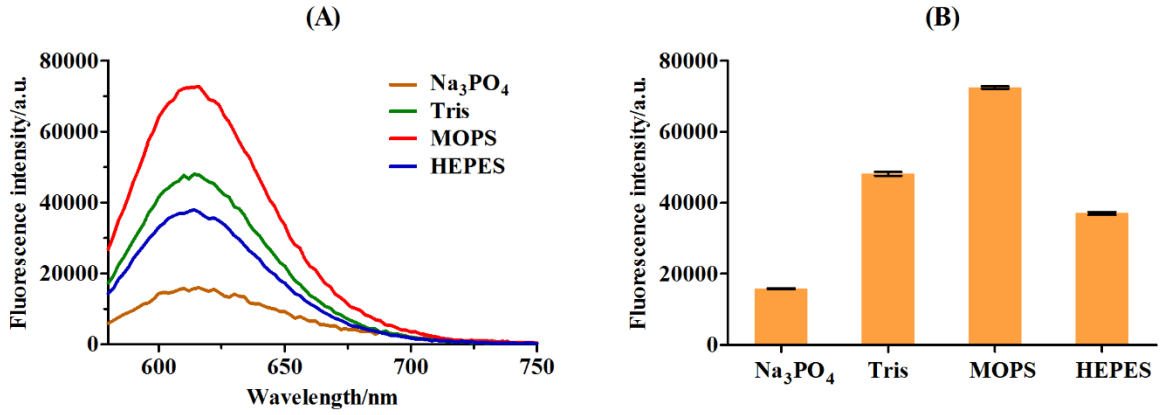

Fig. S1. Selection of reaction buffer for DNA/AgNC synthesis by comparison of fluorescence intensity among four tested reaction buffers.

3. Kinetic behavior of fluorescence enhancement of DNA/AgNC in proximity to G-rich overhang and DNA/AgNC

We investigated the kinetic behavior of fluorescence enhancement of DNA/AgNC in proximity to G-rich overhang and DNA/AgNC by monitoring the fluorescence intensity as a function of time. Following the procedures of DNA/AgNC synthesis as described in experimental section, Probe 1/AgNC solution and Probe 2/AgNC solution were prepared, respectively. Then, fluorescence microplate reader Synergy™ Mx was employed to monitor the kinetic behavior of fluorescence enhancement of Probe 1/AgNC in the presence of Probe 2/AgNC and Probe 5, respectively. As shown in Fig. S2, the fluorescence intensity rapidly increased in Probe 1/AgNC solution when adding the Probe 2/AgNC, and reached a plateau around 80 min. The fluorescence in the mixture of Probe 1/AgNC and Probe 5 increased for approximately 60 min before reaching a plateau.

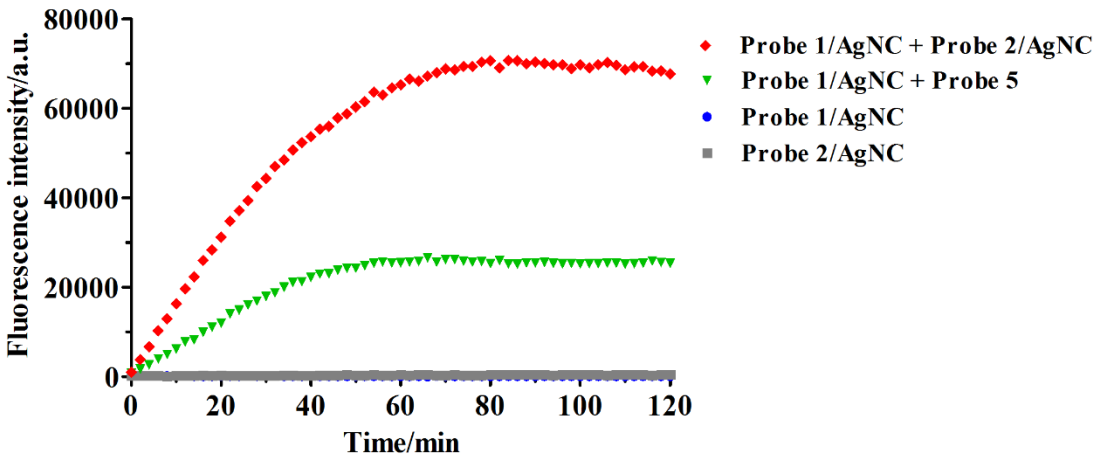

Fig. S2 The kinetics of fluorescence enhancement of Probe 1/AgNC upon the addition of Probe 2/AgNC and Probe 5.

4. Characteristic of DNA/AgNC and proximity-dependent DNA/AgNC probe pair

The Probe 1/AgNC and proximity-dependent DNA/AgNC probe pair (Probe 1/AgNC and Probe 2/AgNC) were characterized by TEM measurement, which were performed on Jeol JEM-2100 instrument operated at an accelerating voltage of 120 kV. Samples for TEM experiment were prepared by placing a drop of silver colloidal solution on a copper grid. The films on the TEM grids were allowed to dry for 2 min following that the extra solution was removed using a blotting paper. As shown in Fig. S3, silver colloidal were highly dispersed and uniform in aqueous solution, and the average size of DNA/AgNC probe pair made from Probe 1/AgNC and Probe 2/AgNC (Fig. S3B) was obviously larger than Probe 1/AgNC alone (Fig. S3A).

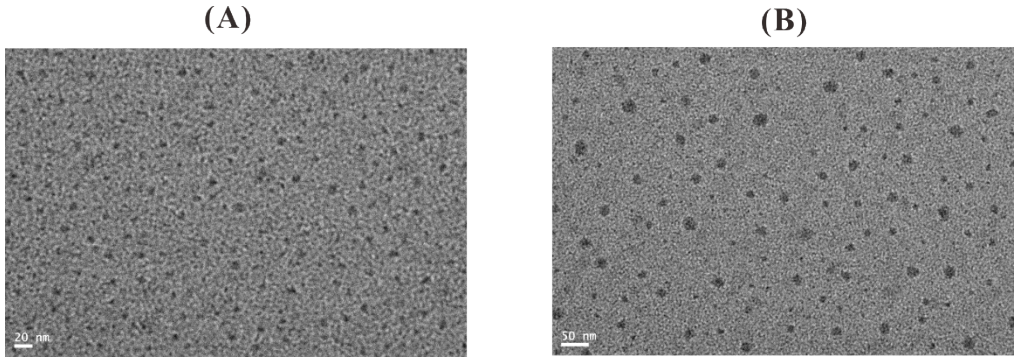

Fig. S3 TEM micrographs of (A) Probe 1/AgNC, (B) proximity-dependent DNA/AgNC probe pair made from Probe 1/AgNC and Probe 2/AgNC.

5. Investigation of the stem length in hairpin structure in probe design

In order to find the best stem length in hairpin structure in probe design, three probes (named as Probe 6, Probe 8, and Probe 10) acted as Probe A were rationally designed (see Table S1). The stem lengths in Probe 6, Probe 8, and Probe 10 were 9 nt, 8 nt, and 7 nt, respectively. According to the procedures for Exo III-assisted specific DNA detection as described in experimental section, three tested probes (Probe 6, Probe 8, and Probe 10) and their corresponding probes (Probe 7, Probe 9, and Probe 11) acted as Probe B were used to detect 100 nM target T1 in the presence of 10 U Exo III, respectively. As shown in Fig. S4, the Probe 6 with 9-bp stem length provided the best performance, which might be ascribed to its best stability.

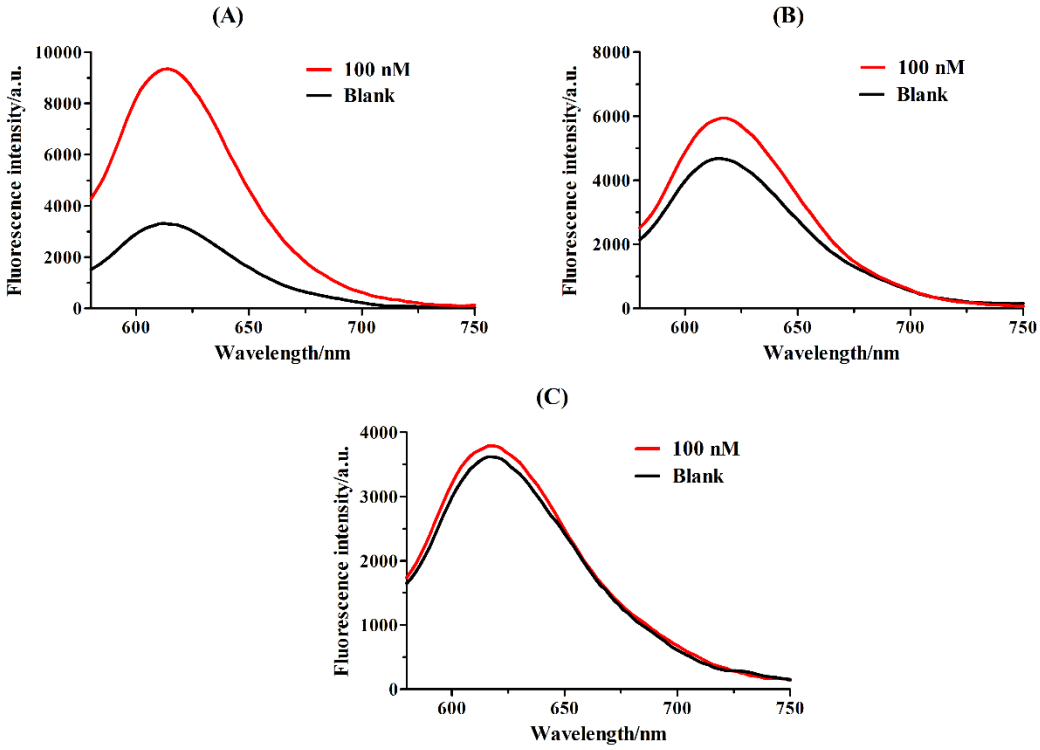

Fig. S4 Investigation of the stem length in hairpin structure in probe design. (A) The reaction were performed with Probe 6 and Probe 7. (B) The reaction were performed with Probe 8 and Probe 9. (C) The reaction were performed with Probe 10 and Probe 11.

6. Optimization of the amount of Exo III

We performed the experiments to select the optimal amount of Exo III. According to the procedures for Exo III-assisted specific DNA detection as described in experimental section, six amounts of Exo III (5, 10, 20, 30, 40, and 50 U) were used to detect 100 nM target T1, respectively. As shown in Fig. S5, the amount of 10 U Exo III provided the highest fluorescence signal. Thus, the amount of 10 U Exo III was chosen in the specific DNA detection experiments.

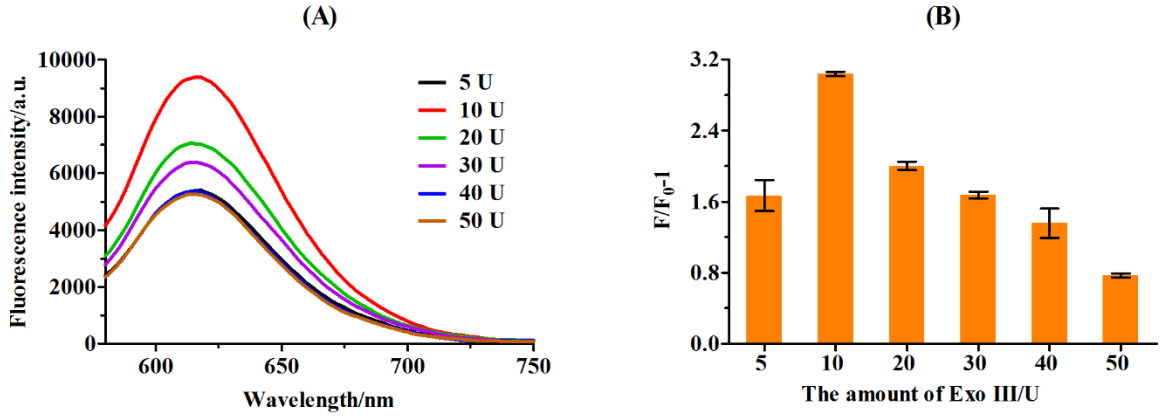

Fig. S5 Optimization of the amount of Exo III. Six concentrations were tested: 5, 10, 20, 30, 40, and 50 U. The reaction system consisted 100 nM Probe 6, 100 nM Probe 7, 100 nM T1, and Exo III.

7. Investigation of the optimal reaction time for Exo III-mediated probe digestion

We investigated the optimal reaction time for Exo III-mediated probe digestion by testing the reaction system in different incubation time in the presence and absence of 10 U Exo III. According to the procedures of specific DNA detection described above, a series of reaction solutions containing100 nM Probe 6 and 10 U Exo III were prepared, incubated at 37°C at different times, and then formed the proximity-dependent DNA/AgNC probe pair upon the addition of 100 nM Probe B/AgNC. As shown in Fig. S6, the Exo III-assisted Probe 6 digestion could be finished within 100 min. Thus, we selected 100 min as incubation time of Exo III-mediated probe digestion.

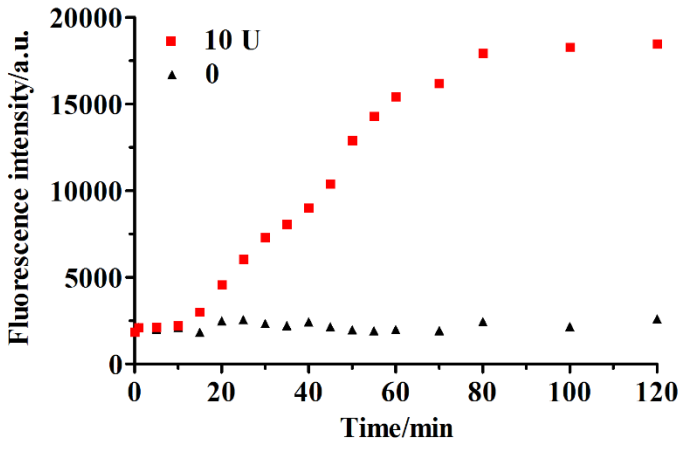

Fig. S6 Real-time analysis of Exo III- kinetic reaction by monitoring the fluorescence intensity as a function of time. The reaction system consisted 100 nM Probe 6, 100 nM Probe 7, 100 nM T1, and 10 U Exo III.

8. Determination of spiked T1 at different concentration

The spiked T1 samples at four concentrations (50, 100, 150 and 175 nM) were prepared and quantitatively detected according to the procedures for Exo III-assisted specific DNA detection. The experiments were repeated five times. The result is listed in Table S2.

Table S2. Recovery results of spiked T1 target at different concentration

Spiked amount (nM)	Detected amount (nM)	Recovery (%)	CV (%)
50.00	52.46	104.92	10.16
100.00	97.02	97.02	5.74
150.00	149.28	99.52	6.50
175.00	178.54	102.02	6.08

Reference

1 C. M. Ritchie, K. R. Johnsen, J. R. Kiser, Y. Antoku, R. M. Dickson, J. T. Petty, *J. Phys. Chem. C*, 2007, **111**, 175.