Electronic Supplementary Information

Target-induced quenching for highly sensitive detection of nucleic acids based on lable-free luminescent supersandwich DNA/silver nanoclusters

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1. Experimental parts

Materials:

Silver nitrate ($AgNO_3$), 99.99%, and sodium borohydride ($NaBH_4$), 98%, were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. All DNA oligonucleotides were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China) and their names and sequences were listed in Table S1. The stock solution of DNAs were prepared in 25 mM phosphate buffer (PBS, pH=7.4) and accurately quantified using UV-vis absorption spectroscopy with the following extinction coefficients ($\varepsilon_{260\ nm}$, $M^{-1}\ cm^{-1}$) for each nucleotide (A = 15400, T = 8700, G = 11500, C = 7400).

Apparatus

UV-vis absorbance measurements were performed on a UV-vis spectrophotometer (Hitachi, Japan) equipped with a quartz cell (1 cm × 0.33 cm cross-section). Fluorescence emission spectra were recorded on F-4500 fluorescence spectrophotometer (Hitachi, Japan).

Synthesis of DNA/AgNCs:

A 170 μM solution of $AgNO_3$ was added into a 10 μM solutions of S1 in 25 mM PBS buffer (pH 7.4), followed by vigorous shaking for 30 s at room temperature. After 30 min the freshly prepared 170 μM $NaBH_4$ was added into the above mixture, followed by vigorous shaking for 30 s. The solutions were kept in the dark and allowed to react for at least 2.0 h reaction at room temperature.

The purification of the DNA/AgNCs:

The synthesized DNA/AgNCs were centrifuged by using Nanosep Centrifugal Devices (30K, molecular weighy cutoffs) with 5000 rpm for 5 min. The obtained

solution containing DNA/AgNCs was prepared for further application.

Synthesis of supersandwich DNA/AgNCs:

For the target DNA detection assay, we added various concentrations of S2 (0.01 μM to 1.2 μM) or a fixed amount of S3, T1, T2, T3 and T4 (9.0 μM) to the S1 (10 μM) and incubated for at least 15 h at 25℃ to form supersandwich structure. Then, freshly prepared $AgNO_3$ (170 μM) was added to the above solutions, followed by vigorous shaking for 30 s and incubated for 30 min in the dark at room temperature. The freshly prepared $NaBH_4$ (170 μM) were added to the S1/DNA mixtures to a final volume (150 μL), followed by vigorous shaking for 30 s. All the DNA/AgNCs were incubated for 2 h in the dark at room temperature and diluted with 850 μL of ultrapure water before measurement. The supersandwich DNA/AgNCs was excited at wavelengths of 580 nm.

Name	Sequences (5'-3')
S0	CCCCCCCCCCCC
S1	CCCCCCCCCCCC-AGCTTGCAT-CGGTCAGAG
S2	ATGCAAGCT-CTCTGACCG
S3	CTCTGACCG-ATGCAAGCA
T1	ATGCATGCT-CTCTGACCG
T2	ATGCATGCT-CTGTGACCG
T3	ATGCATGCA-CTGTGACCG
T4	CTAGGAGTC-AGTGCCTGA

Table S1 Names and sequences of the oligonucleotides

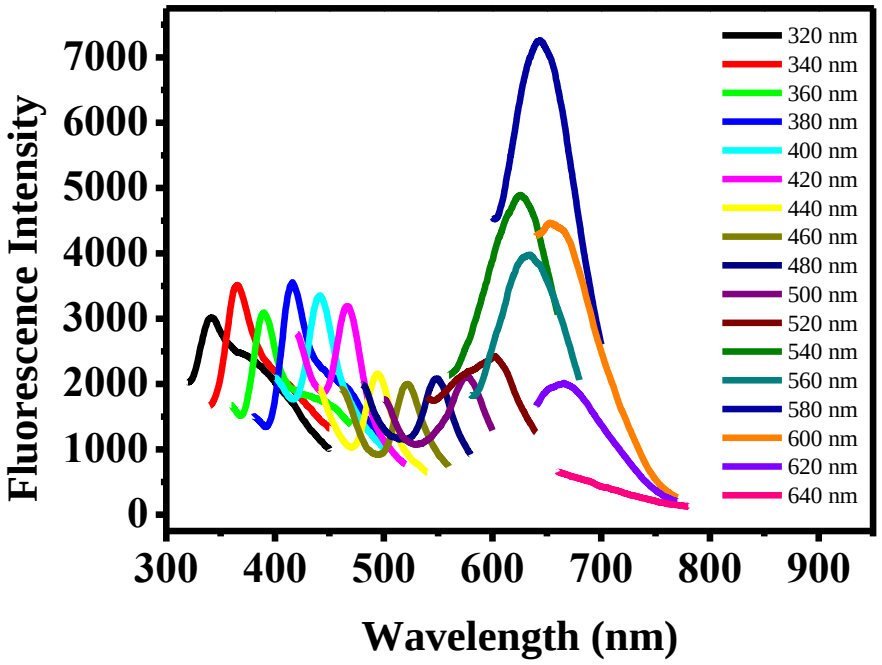
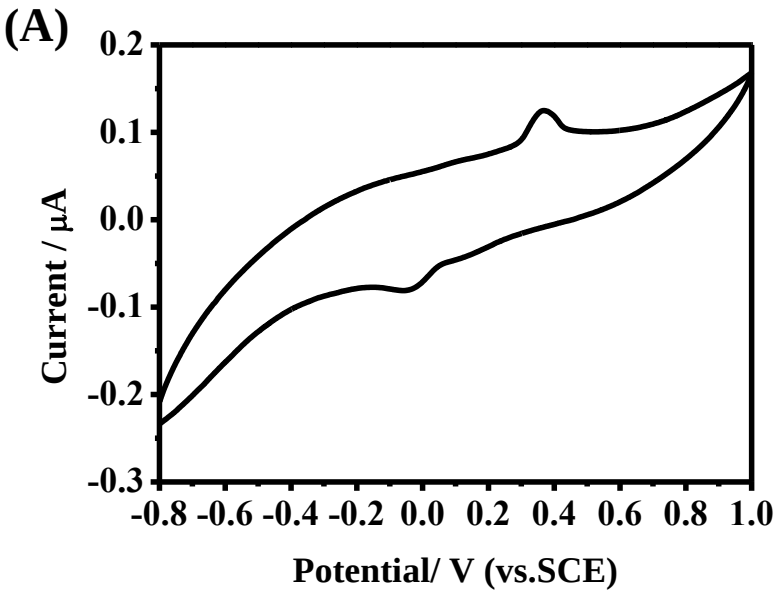
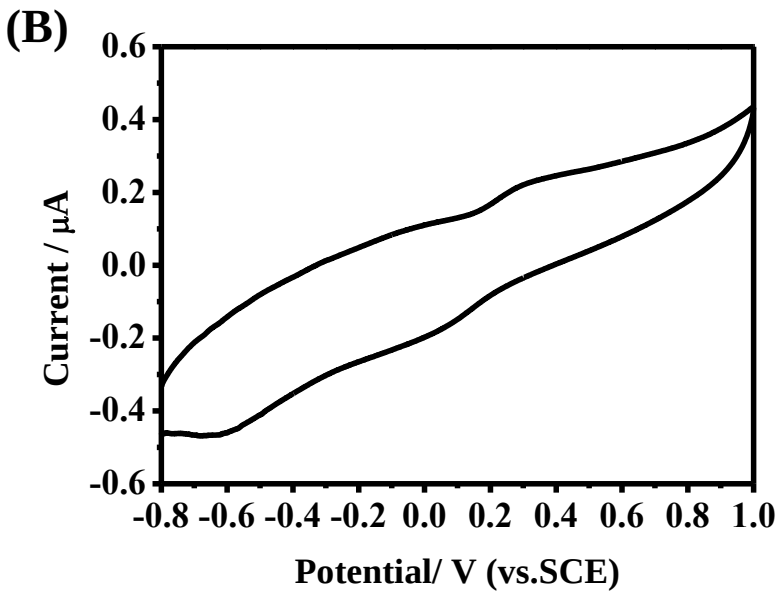
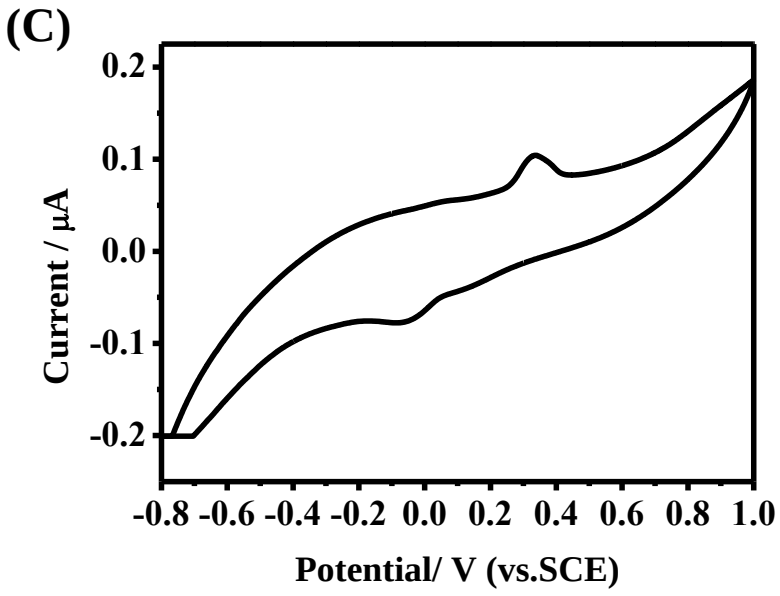


Fig. S1 Emission spectra of 10 μM S1 probe, 2 hour after preparation, as a function of excitation wavelength (from 320 to 640 nm, in 20 nm step size)







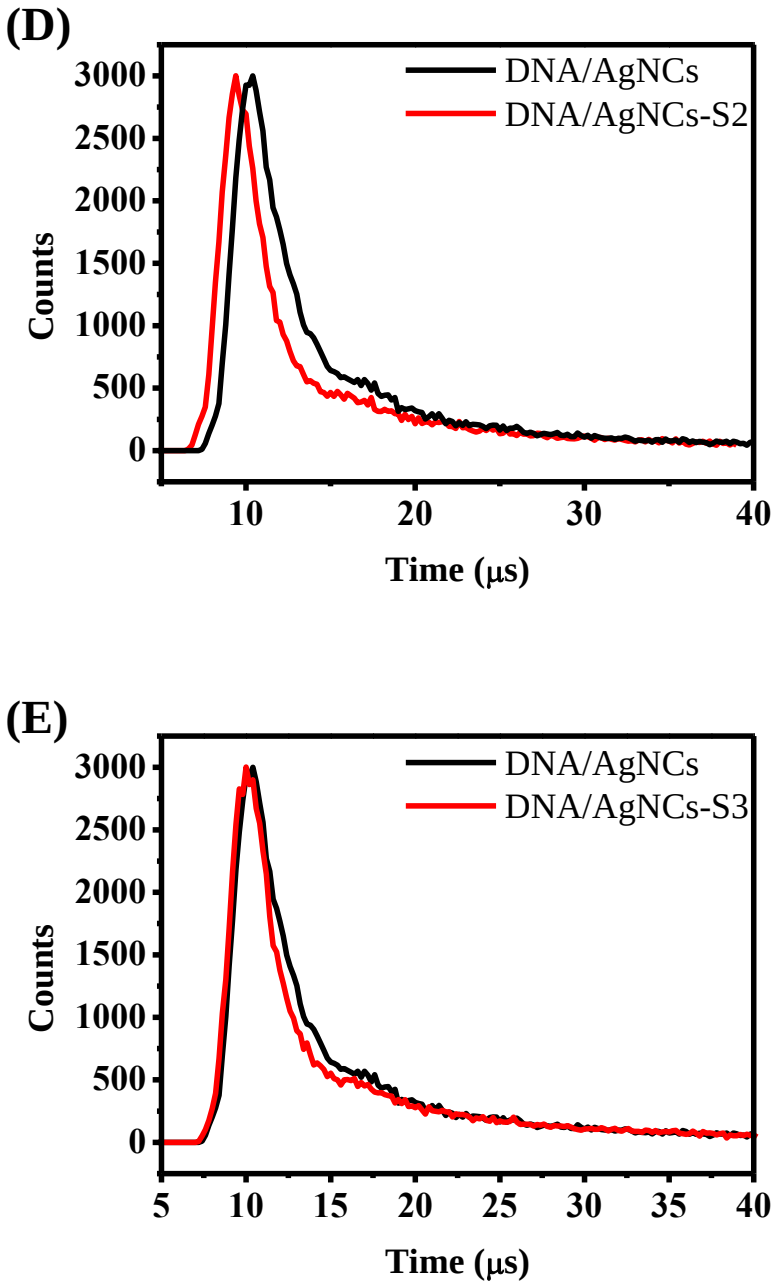


Fig. S2 (A) Cyclic voltammogram of DNA/AgNCs in phosphate buffer solution on the glassy carbon electrode; (B) Cyclic voltammogram of 900 nM S2 in phosphate buffer solution on the glassy carbon electrode, the scan rate is 50 mV/s; (C) Cyclic voltammogram of 900 nM S3 in phosphate buffer solution on the glassy carbon electrode, the scan rate is 50 mV/s; (D) Fluorescence decay of DNA/AgNCs in the (black) absence and (red) presence of S2;

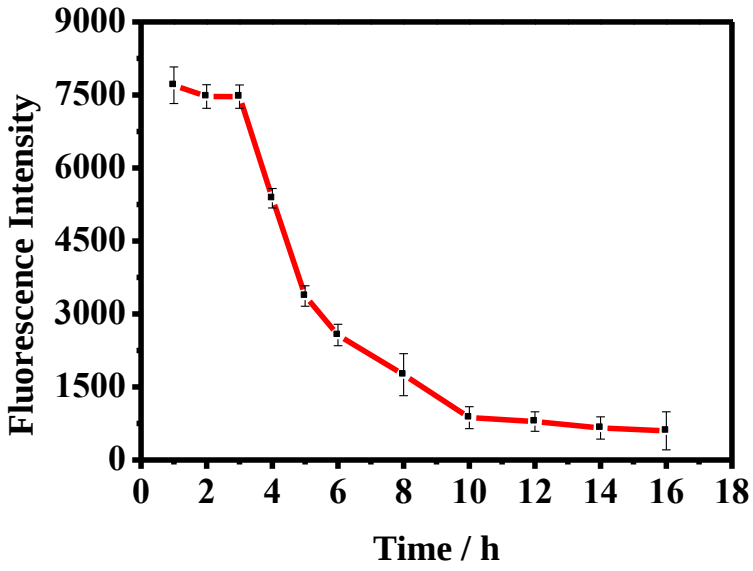


Fig. S3 Time revolution of the fluorescence intensity of DNA/AgNCs probe

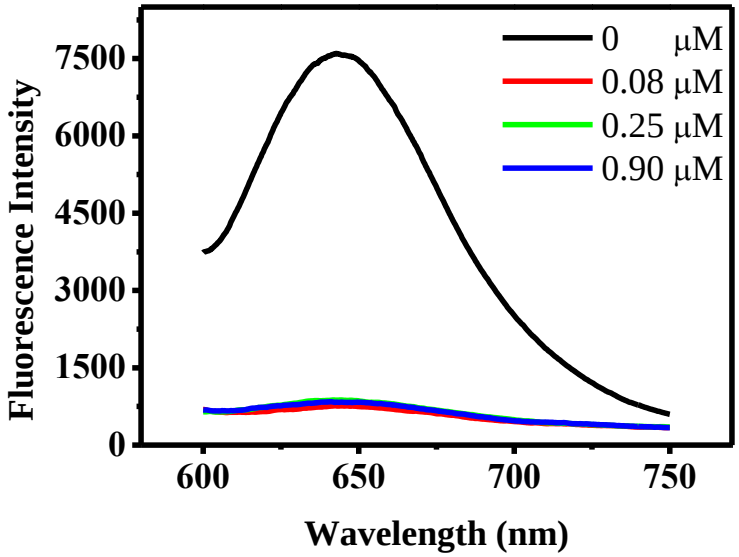


Fig. S4 Emission spectra of 10 μM S1 probe, 2 hours after addition of $AgNO_3$ and $NaBH_4$ (black curve). After this spectrum was recorded, S2 target (final concentration 0.08 μM, 0.25 μM, 0.9 μM) was added to the S1 probe solution. A new spectrum was recorded 10 hours later (red, green, and blue curve).

In order to obtain the highest quenching efficiency of the designed supersandwich DNA/AgNCs sensor, we have investigated various experimental conditions. It was observed in Fig. S5A that the pH value of the detection solution is an important

parameter. Fig. S5A shows the effect of pH value of the detection solution on the current response of the sensor in PBS containing 10 μM DNA/AgNCs. The current change was increased the increment of pH value from pH 3.4 to 7.4 and then decreased. The maximum fluorescence intensity was achieved at pH 7.4. In addition, the effect of the incubation time for S1 hybridized with S2 to form supersandwich DNA/AgNCs was also investigated. With an increasing incubation temperature from 10 ℃ to 25 ℃ (Fig. S5B), the DNA/AgNCs sensor shows a maximum fluorescence response at 25 ℃ in pH 7.4 PBS buffer. Hence, 25 ℃ was chosen as the optimal temperature for the determination of S2 target in the following experiments. Fig. S5C shows the time-dependent fluorescence intensity response of supersandwich DNA/AgNCs. It was observed that the fluorescence intensity response decreased with the increasing in the incubation time from 2 h to 15 h, and it reached a plateau after 15 h. Therefore, 15 h was chosen as the optimal incubation time.

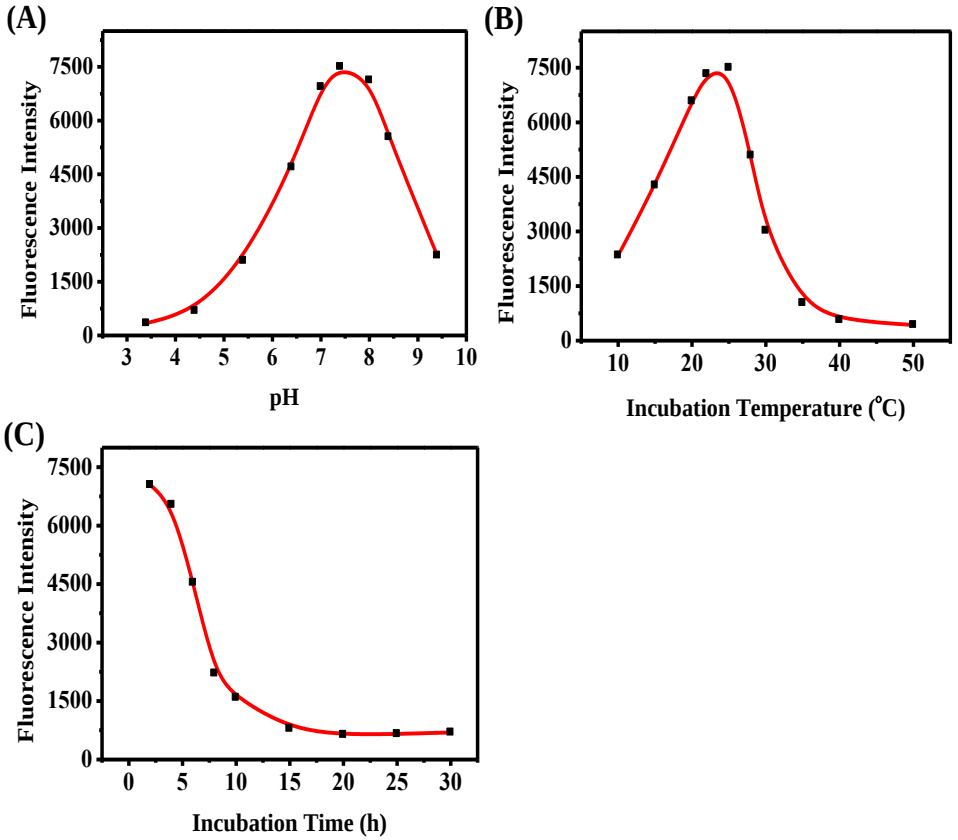


Fig. S5 Effects of (A) pH of phosphate buffer, (B) incubation temperature of supersandwich DNA/AgNCs, and (C) incubation time on fluorescence response of the supersandwich DNA/AgNCs sensor toward 900 nM S2 under optimal conditions.

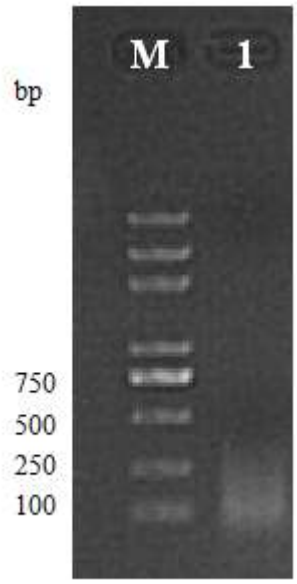
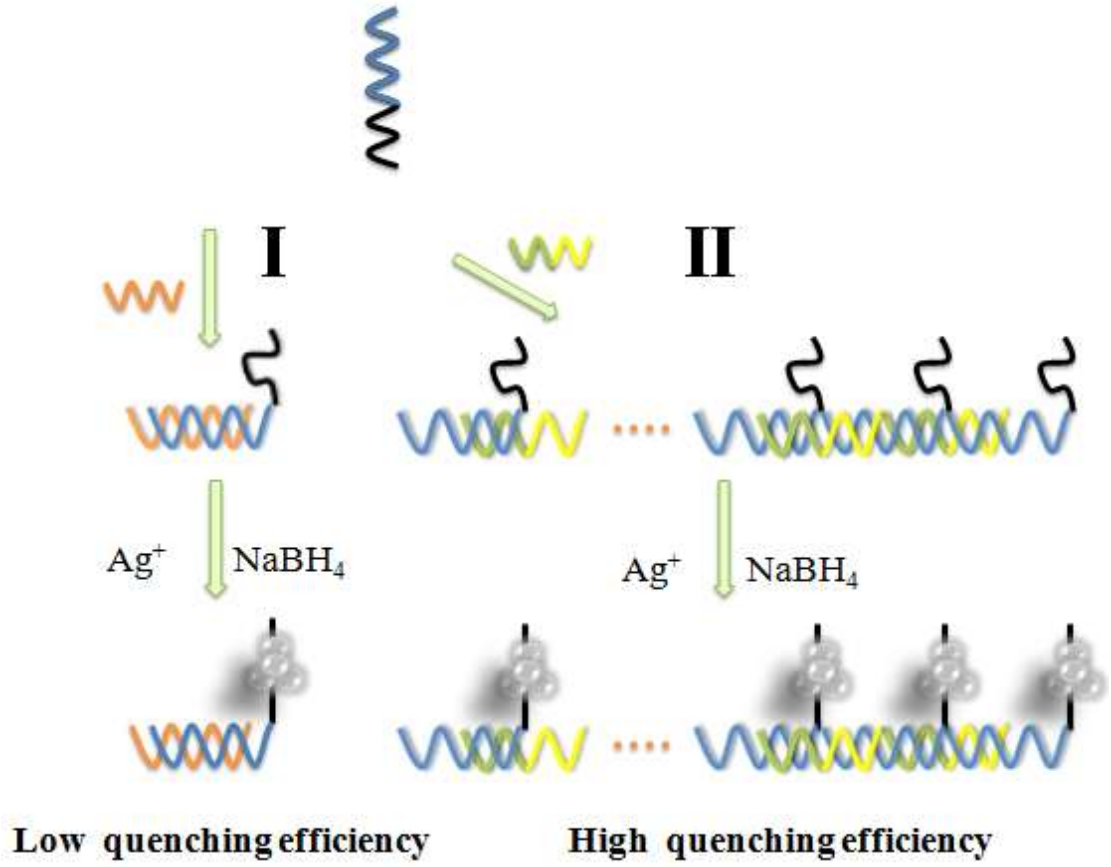


Fig. S6 The gel-electrophoresis results of the supersandwich DNA/AgNCs.

The gel-electrophoresis results confirm the formation of the supersandwich DNA/AgNCs structure. As we can see, it produces a ladder of different lengths of the supersandwich structure, with the maximum in the range 250 to 500 base pairs.



Scheme S1 The fabrication of typical sandwich and supersandwich DNA/AgNCs luminescent sensor for detecting nucleic acids.