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Electronic Supplementary Information

Application of a Cation-exchange Reaction of CuS Nanoparticles and Fluorescent Copper Nanoparticles in a DNA Biosensor

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EXPERIMENTAL SECTION

Materials. DNA oligonucleotides synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) were listed in Table S1 of support information. Strepavidin modified magnetic beads (MBs, 1.0~2.0 μm) were purchased from Tianjin Baseline ChromTech Research Centre (China). Exonuclease III (10000 U/mL) was ordered from New England Biolabs Inc. Silver nitrate ($AgNO_3$) was obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 3-Mercaptopropionic acid (3-MPA) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma. 3-(N-Morpholino) propanesulfonic acid (MOPS) was obtained from Beijing Solarbio Science□Technology Co.,Ltd. Sodium L-ascorbate (SA) was purchased from J&K Technology Co., Ltd. (Beijing, China). All other reagents were of analytical reagent grade and were used without further purification. All solutions were prepared with deionized water.

MCF-7 cell was supplied by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and human serum samples were kindly provided by the Qingdao Center Hospital (Qingdao, China).

Table S1 DNA sequence used in the experiments

Oligonucleotides name	Sequence (5'-3')
Target DNA (TD)	GCTGGCTTATAACCGTTACGTTCTATTACG
DNA1	Biotin-CCGTTACGTTCTCCATTGCATCGGTTTTTT
DNA2	NH_2-AGAACGTAACGGTTATAAGCCAGC
DNA3	Biotin-CCATTGCATCGGTTTTTT
DNA4	NH_2-CCGATGCAATGGAGAACGTAACGG
Single-base mismatched DNA (1MT)	GCTGCCTTATAACCGTTACGTTCTATTACG
Single-base mismatched DNA (1'MT)	GCTGGCTTATAACTGTTACGTTCTATTACG
Two-base mismatched DNA(2MT)	GCTGCCTTATAACCGTTACGGTCTATTACG
Non-complementary target DNA (NC)	ACCCTTGTGCTAAGACAGTCCTAACTCCCC
Poly T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

TTTTT

Preparation of CuS NPs[1-3]

Mercaptopropionic acid-stabilized CuS NPs were synthesized as follows. Briefly, 15 μL of 3-MPA was added to 50 mL of $Cu(NO_3)_2$ (0.4 mM) solution. Then NaOH solution (0.5 M) was added to adjust the pH to 7.0. After bubbled with N_2 for 30 min, 50 mL of Na_2S solution (1.34 mM) was added dropwisely and bubbled with N_2 at 30°C for 24 h until the color of solution turned to dark-green. The obtained CuS colloid was dialyzed against distilled water using a dialysis membrane (MWCO 7000) for 72 h, and then stored at 4 °C.

Coupling DNA with CuS NPs

2 mL of amino group capped **DNA 2** (2.0 μM) and 200 μL of imidazole solution (0.1 M, pH 6.8) were mixed. After stirring for 30 min, 100 μL EDC (0.1 M) and 3.0 mL prepared CuS NPs were added. The mixture was stirred at room temperature for 12 h and then centrifuged at 10 000 rpm for 30 min to remove any free oligonucleotides. The precipitate was washed three times, re-suspended in 3.0 mL of deionized water and stored at 4 °C before use.

Preparation of MB-DNA-CuS conjugates

First, 10 μL of streptavidin modified magnetic microbeads were washed three times with 20 μL 0.01 M PBS (pH 7.4) and dispersed in 10 μL of 0.01 M PBS (pH 7.4). Then, 10 μL of biotin modified DNA 1 (1.0×10^{-6} M) was added. The mixture was vibrated at 37°C for 30 min. After magnetic separation and washing three times with PBS, the resulting MB-DNA conjugates were resuspended in 10 μL of 0.01 M PBS.

For the preparation of the MB-DNA-CuS conjugates, 100 μL of DNA2-CuS was added to 10 μL DNA1-MB and incubated at 37°C for 2 h. After washing three times with PBS and the magnetic separation, the resulting MB-DNA1/DNA2-CuS conjugates were resuspended in 10 μL of 0.01 M PBS. Conjugates MB-DNA3/DNA4-CuS were prepared in the same manner.

Exo III-assisted cation-exchange reaction and the formation of fluorescent Cu NPs

Different concentrations of the target DNA (10 μL) and 10 μL of the MB-DNA1/DNA2-CuS conjugates were added to 0.28 μL Exo III (100 units/μL), and the mixture was incubated at 37°C

for 48 min with gentle shaking. After washing and magnetic separation, 25 μL of 8.0×10^{-4} M $AgNO_3$ was added to the supernatant and the mixture was incubated at 25 °C for 10 min. Then, 35 μL of MOPS buffer (10 mM MOPS, 150 mM $NaNO_3$, pH 7.5), 10 μL of 9.0×10^{-6} M poly T and 10 μL of 2.0×10^{-2} M SA were added. The mixture was incubated at room temperature for 10 min before the fluorescence test.

Cascade recycling amplification of the cation-exchange reaction and the formation of fluorescent Cu NPs

Different concentrations of the target DNA (10 μL), 10 μL of MB-DNA1/DNA2-CuS conjugates and 10 μL of MB-DNA3/DNA4-CuS conjugates were added to 0.5 μL of Exo III (100 units/μL). The mixture was incubated at 37°C for 90 min with gentle shaking. After washing and magnetic separation, 25 μL of 8.0×10^{-4} M $AgNO_3$ was added to the supernatant, which was incubated at 25 °C for 10 min. Then, 25 μL of MOPS buffer (10 mM MOPS, 150 mM $NaNO_3$, pH 7.5), 10 μL of 9.0×10^{-6} M poly T and 10 μL of 2.0×10^{-2} M SA were added. The mixture was incubated at room temperature for 10 min before the fluorescence test (E_x 340 nm, E_m 500-660 nm).

RESULTS AND DISCUSSION

Conditions for the preparation of fluorescent copper nanoparticles

DNA poly T was used to act as a template for CuNPs formation. It has been reported that the fluorescence intensity caused by poly T of less than 15 bases was negligible and longer poly T sequence could produce brighter fluorescence. [4] Refer to previous work,[5] poly T40 was selected in this work. Then reaction conditions for the formation of CuNPs, including the concentration of poly T40, concentration of SA, buffer pH and reaction time were optimized to get stronger fluorescence intensity. From the results shown in Fig, S1 of supporting information, 900 nM poly T40, 2.0 mM SA, pH 7.5 and reduction 10 min were selected as preference conditions for the formation of fluorescence CuNPs. It should be noted that the reduction time for in situ preparation of fluorescence CuNPs was very short, which demonstrated this method could be well applied in the field of biosensor.

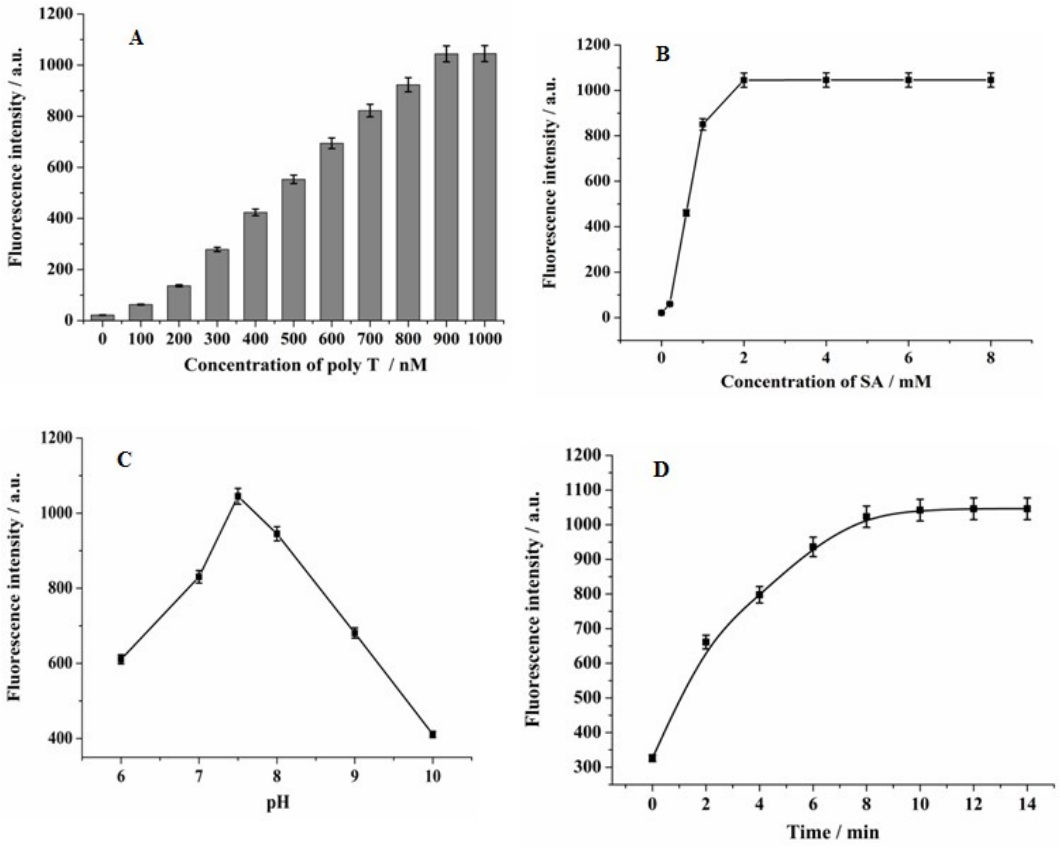

Fig. S1. The reaction conditions for the preparation of CuNPs. (A) The relationship between different concentrations of poly T and fluorescence intensity. Condition: concentrations of Cu^{2+} and SA were 40 μM and 2.0 mM，respectively; (B) The relationship between the concentration of sodium ascorbate and the fluorescence intensity. Condition: concentrations of Cu^{2+} and poly T40 were 40 μM and 90 nM, respectively; (C) The effect pH to fluorescence intensity; (D) The relationship between the reduction time for fluorescent copper nanoparticles and fluorescence intensity.

Optimization of the detection conditions in cycle I

To achieve the best performance, the reaction time for this small cycle and the amount of Exo III was optimized using 1.0×10^{-6} M target DNA. From the results shown in Figure S2, we can see that reaction time can be chosen as 48 min and the amount of Exo III can be selected as 28 units.

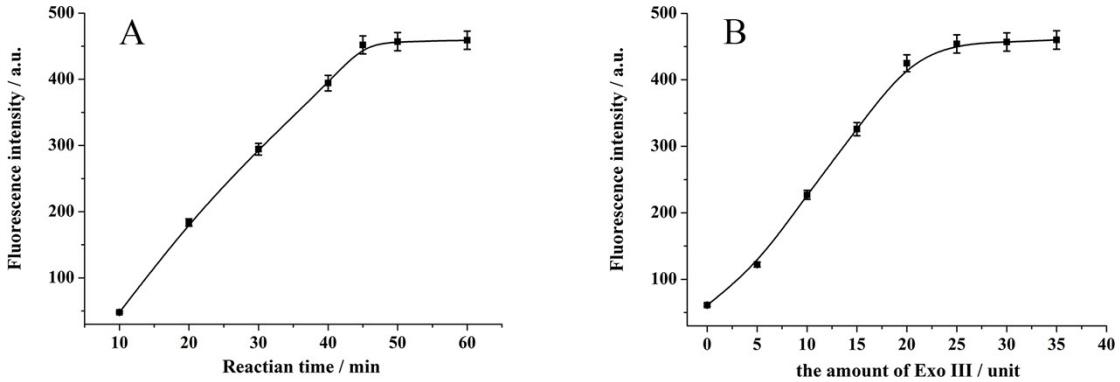

Fig. S2 Influence of reaction time (A) and the amount of Exo III (B) on the FL signal responding to1.0×10^{-6} M target DNA for cycle I process.

Under optimum detection condition, different concentrations of target DNA were measured. As shown in Fig. S3 of support information, the FL intensity increased with the increasing target DNA concentration in the range from 1.0×10^{-9} M to 1.0×10^{-6} M. The linear regression equation was F=112.35 lg *C* + 1113.27 (F represents FL intensity; C is the concentration of target DNA) with a coefficient of R^2=0.9902. A detection limit of 8.0×10^{-10} M was estimated using 3σ.

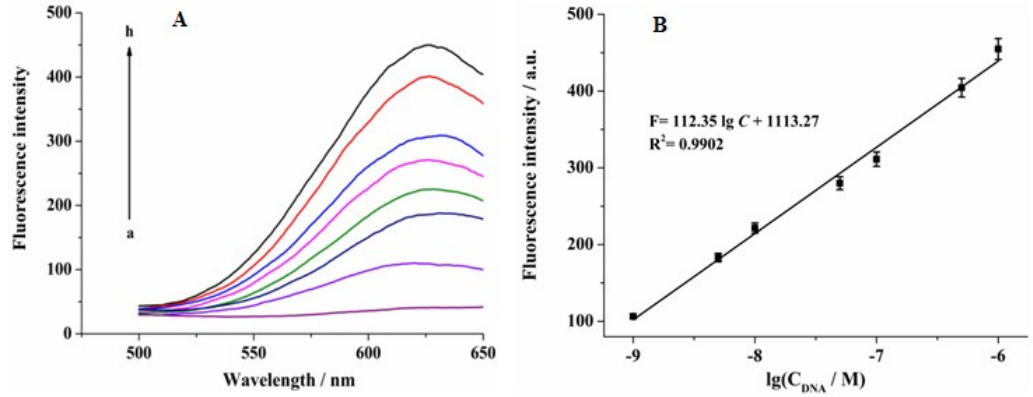

Fig. S3 Fluorescent signal in response to different concentrations of target DNA: (a) 0; (b) 1.0×10^{-9} M; (c) 5.0×10^{-9} M; (d) 1.0×10^{-8} M; (e) 5.0×10^{-8} M; (f) 1.0×10^{-7} M; (g) 5.0×10^{-7} M; (h) 1.0×10^{-6} M. (B) Linear relation-ship between the fluorescent intensity and the DNA concentrations. Error bars are standard deviation of three repetitive measurements.

Optimization of the detection conditions for cycle I+ cycle II process

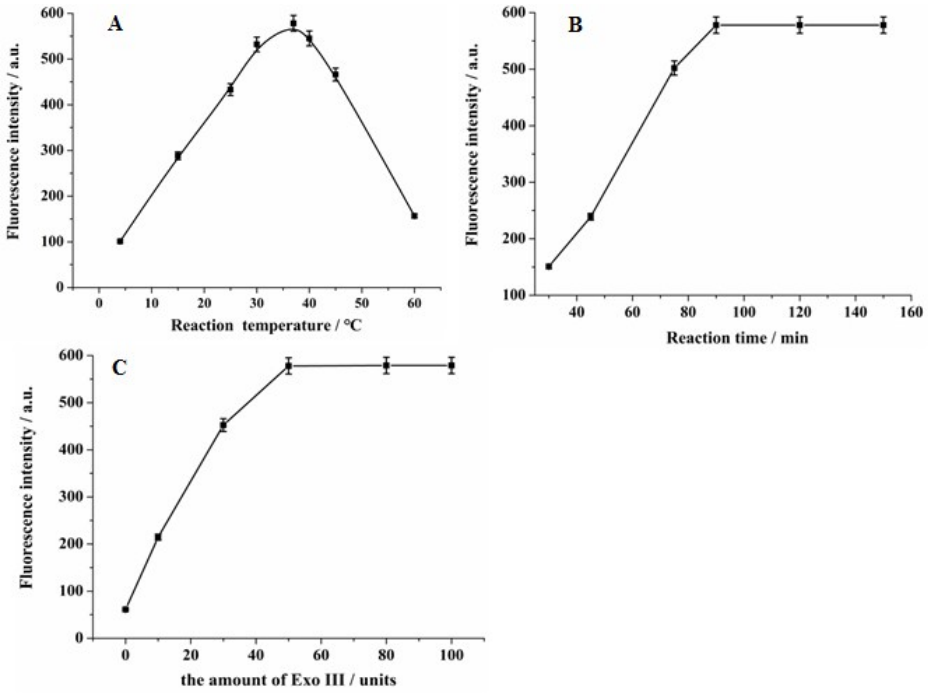

Fig. S4 Influence of reaction temperature (A) and reaction time (B) the amount of Exo III on the FL signal responding to1.0×10^{-8} M target DNA for cycle I+ cycle II process.

To test the effect of SNP position on the selectivity of the developed sensor, other 1MT DNA with different position was also tried. The result shown in Fig. S5 suggested that the mutations located in the center of binding domain (1' MT) present better selectivity than those at the ends (1 MT). This means that central mutations result in much weaker hybridization.

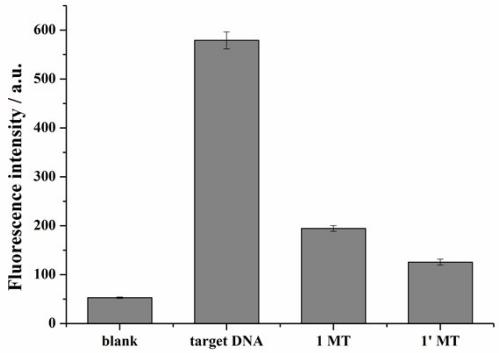

Fig. S5 The effect of SNP position on the selectivity of the developed sensor.

qRT-PCR Procedure for DNA Analysis.

Three samples with different concentrations of target DNAs dissolved in MCF-7 cell lysate were measured using SYBR Green RT-qPCR assays (Vazyme). The threshold cycle (CT) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. First, the Ct values were plotted against the logarithmic starting concentration of target DNA in a standard curve. Then, three samples were tested and the results shown in Fig. S6 suggested an acceptable agreement for the developed method with those obtained by RT-qPCR. Experiments for each data point were run in triplicate.

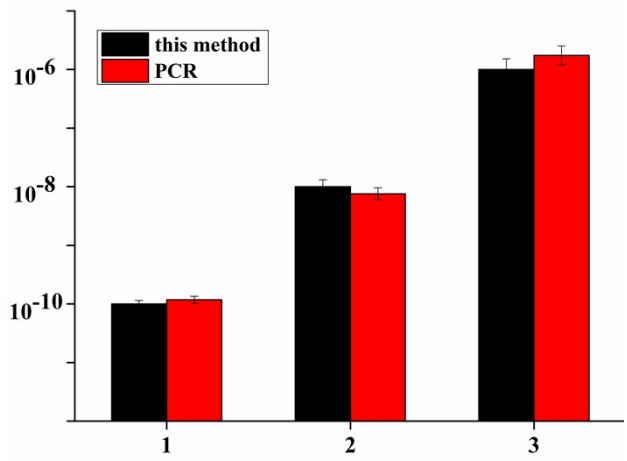

Fig. S6 Determination the concentration of target DNA in MCF-7 cell lysate using the developed method and the RT-qPCR method.

References

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