

Sensitive fluorometric sensor for Ag⁺ based on the hybridization chain reaction coupled with glucose oxidase dual-signal amplification strategy

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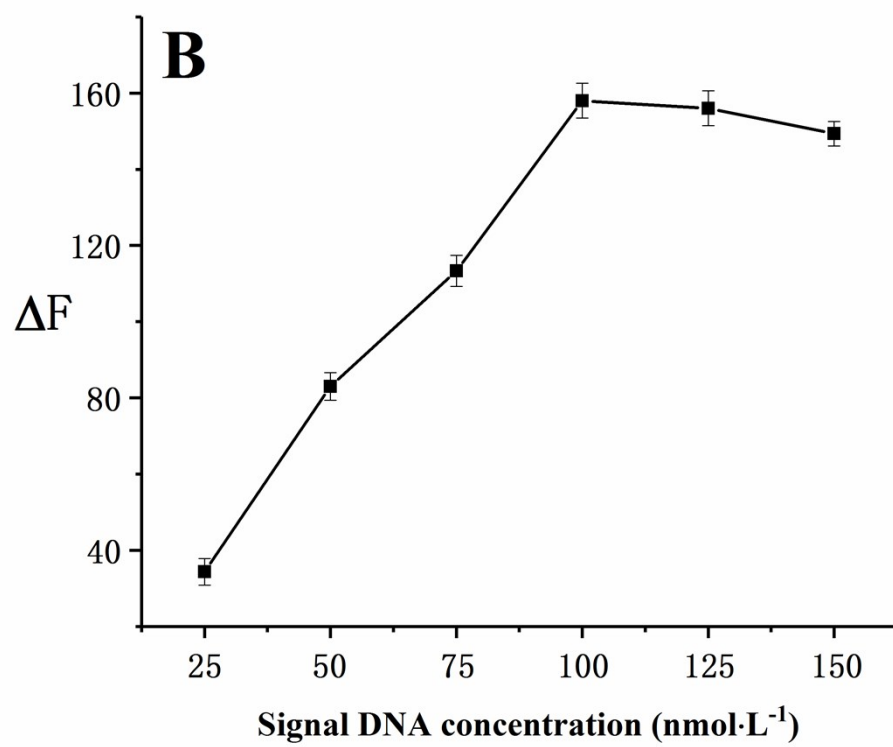
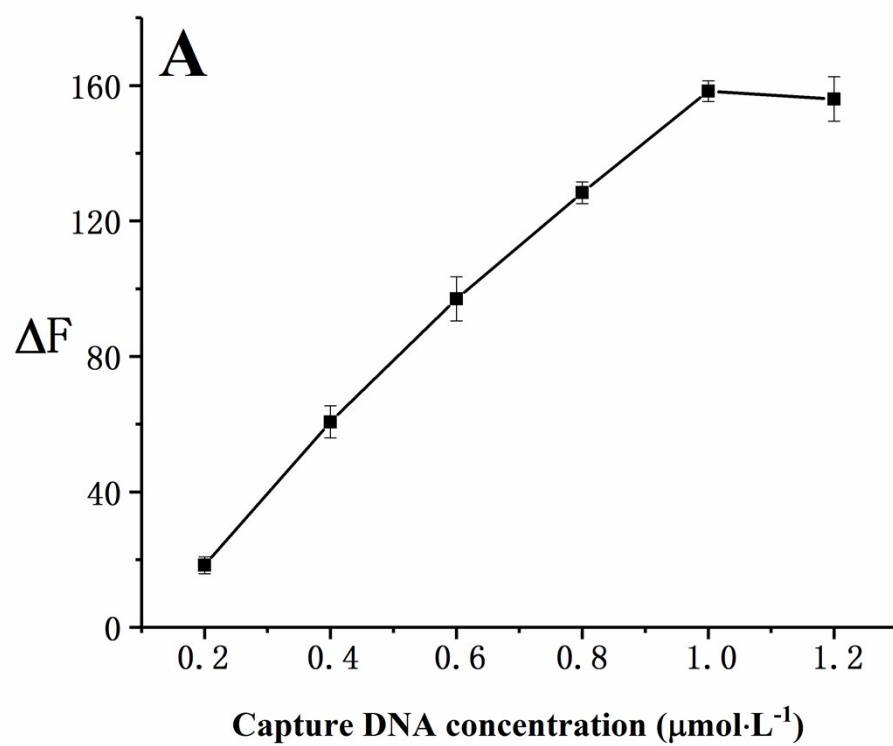
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Table 1 Sequences of oligonucleotides

Name	Sequence
Capture DNA	5'-SH-(CH ₂) ₁₀ -CCA ACC ACA CCA ACC-3'
Signal DNA	5'- AGT CTA GGA TTC GGC GTG GGT TAA (T) ₁₅ GGT TGG TGT GGT TGG-3'
Hairpin DNA 1 (HP1)	5'- Biotin – TTA ACC <u>CAC GCC GAA TCC TAG ACT</u> CAA AGT <u>AGT CTA GGA TTC GGC GTG</u> -3'
Hairpin DNA 2 (HP2)	5'- Biotin - <u>AGT CTA GGA TTC GGC GTG</u> GGT TAA <u>CAC GCC</u> <u>GAA TCC TAG ACT</u> ACT TTG -3'

Optimization of the experimental condition



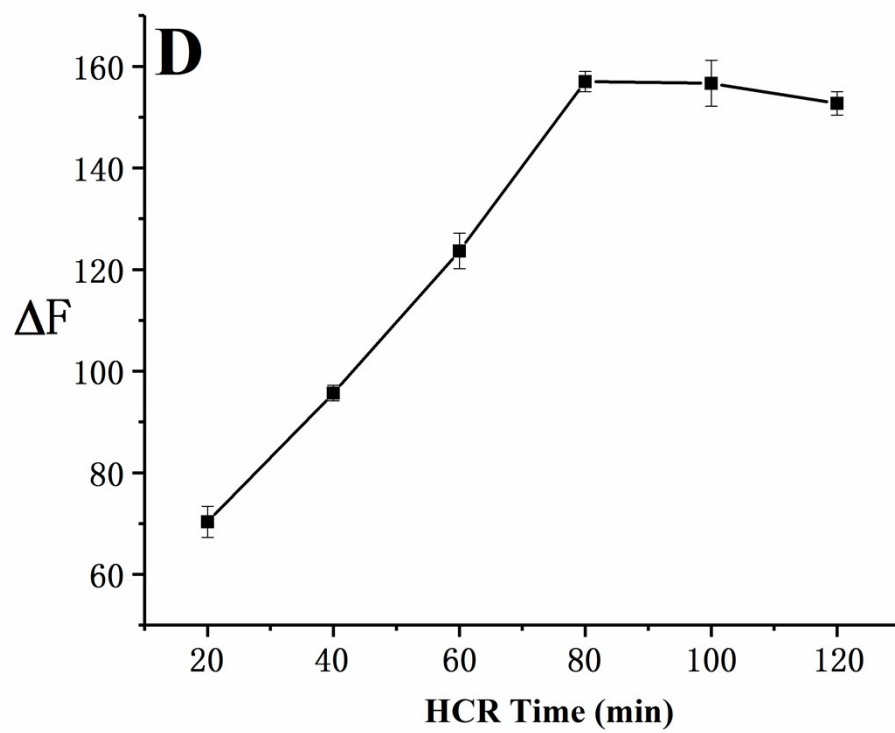
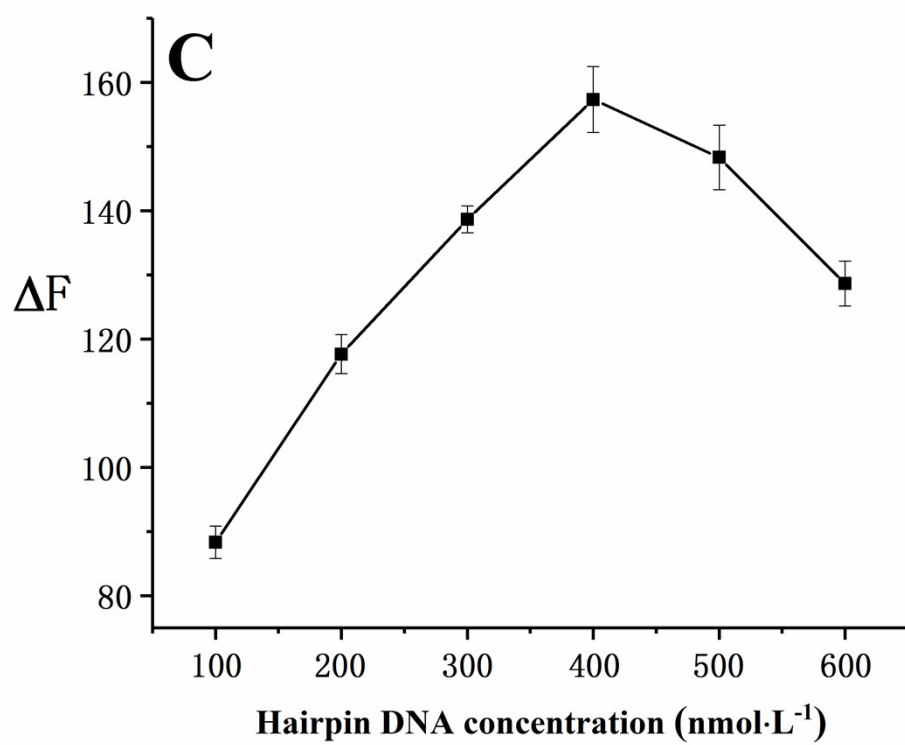


Fig. s1 Optimization of experimental conditions: (A) Effect of capture DNA concentration; (B) Effect of signal DNA concentration; (C) Effect of hairpin DNA concentration; (D) Effect of the incubation time of HCR. Experimental conditions: $1.0 \mu\text{mol}\cdot\text{L}^{-1}$ capture DNA, $100 \text{ nmol}\cdot\text{L}^{-1}$ signal DNA, $400 \text{ nmol}\cdot\text{L}^{-1}$ H1, $400 \text{ nmol}\cdot\text{L}^{-1}$ H2, $2 \mu\text{g}\cdot\text{mL}^{-1}$ SA, $50 \mu\text{g}\cdot\text{mL}^{-1}$ GOx-biotin, $50 \text{ mmol}\cdot\text{L}^{-1}$ glucose, $50 \mu\text{mol}\cdot\text{L}^{-1}$ HPPA and $0.5 \mu\text{g}\cdot\text{mL}^{-1}$ HRP. Error bars represent the standard deviation of three independent experiments.

In order to maximize the response signal of the proposed method, various conditions were optimized. A key concept to our success is the HCR process. Therefore, the following factors such as the concentration of capture DNA, the concentration of signal DNA, the concentration of H1 and H2, the incubation time of HCR play major roles, and control the fluorescence signal.

As shown in Fig.s1A, as the concentration of capture DNA was increased to $1.0 \mu\text{mol}\cdot\text{L}^{-1}$, ΔF reached to the maximum and remained a steady value. Thus, the capture DNA concentration was set at $1.0 \mu\text{mol}\cdot\text{L}^{-1}$ for subsequent experiments. Furthermore, the signal DNA can trigger the HCR, thus the effect of the concentration of signal DNA was investigated. As exhibited in Fig.3B, the optimal concentration of signal DNA was set as $100 \text{ nmol}\cdot\text{L}^{-1}$. Similarly, as shown in Fig.3C, the optimal concentrations of hairpin DNA (H1 and H2) were set at $400 \text{ nmol}\cdot\text{L}^{-1}$ for subsequent experiments. As displayed in Fig.3D, ΔF reaches a maximum when the HCR time was 80 min, and almost remained stable with a longer reaction time. Therefore, in this work, 80 min was selected as the incubation time for the HCR amplification processes.