A Rapid Label- and Enzyme-free G-Quadruplex-based Fluorescence Strategy for Highly-Sensitive Detection of HIV DNA

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Optimization of Experimental Conditions

To obtain optimal sensing performance of the fluorescence platform for target detection, various parameters were optimized. The concentration of helper A and B is an important factor which affects the sensing performance. Therefore, the influence of different concentrations of helper A and B (10.0, 25.0, 35.0, 50.0, 75.0, 85.0 nM) was investigated firstly. Different concentrations of helper A and B were incubated with 50.0 nM of H1, 50.0 nM target DNA, 50.0 mM K⁺ and 2.0 μM THT at 37 °C for 60 min. As shown in Fig. S2, the biggest fluorescence change $F-F_0$ was observed when the concentrations of helper A and B was 25.0 nM. Thus, the helper probe concentration of 25.0 nM was used for subsequent experiments.
K$^+$ is a cationic ligand, which can stabilize G-quadruplex. Thus, the dosage of K$^+$ must be optimized. 50.0 nM target, 50.0 nM H1, 25.0 nM helper A and B, 2.0 µM THT were mixed in buffers containing different concentrations of K$^+$ ranged from 5.0 mM to 60.0 mM at 37 °C for 60 min. The highest signal to noise ratio $F/F_0$ in fluorescence intensity was observed when the concentration of K$^+$ was 50.0 mM (Fig. S3). Thus, 50.0 mM K$^+$ was chosen for the subsequent experiments.

THT has a weak fluorescence by itself but exhibits a dramatic fluorescence in the presence of the G-quadruplexes. 50.0 nM target, 50.0 nM H1, different concentrations of THT, and K$^+$ (50.0 mM) were added to the mixture of helper A and B solutions (25.0 nM). Then the mixture was incubated at 37 °C for 60 min. Fig. S4 showed that the biggest fluorescence intensity ratio $F/F_0$ was observed when the THT concentration was 2.0 µM. Therefore, the concentration of THT was chosen at 2.0 µM.

Furthermore, under the optimized experimental conditions, the effects of hybridization temperature and reaction time on the amplification process were also investigated. H1 (50.0 nM), helper A and B (25.0 nM), K$^+$ (50.0 mM), 2.0 µM THT and target (50.0 nM) were mixed and incubated at 25, 30, 37, 40, 45 and 50 °C for 60 min, respectively. Fig. S5 showed that the highest fluorescence signal change was obtained when the temperature was 37 °C. The incubation time has an effect on the detection procedure. It can be seen from Fig. S6 that the fluorescence signal value increases significantly with the incubation time and has a stable value at 40 min, indicating that the amplification reaction has been completed. Thus, 40 min was chosen as the optimum incubation time.

**Interference of Mg$^{2+}$**

Mg$^{2+}$ is an ion that promotes chain binding. Considering the interference of Mg$^{2+}$ in
this sensing platform, five concentrations of Mg$^{2+}$ (0, 5, 10, 20, 30) was selected for experiments. 50.0 nM H1, 50.0 nM target, 25.0 nM helper A and B, 2.0 µM THT were mixed in buffers containing different concentrations of Mg$^{2+}$ ranged from 0 mM to 30.0 mM at 37 °C for 40 min. The results are shown in Fig. S7. It can be seen that the $F/F_0$ obtained in the environment with different concentrations of Mg$^{2+}$ is basically the same, indicating that the interference of Mg$^{2+}$ to the experiment can be neglected.
Fig. S1. Polyacrylamide gel electrophoresis (PAGE) Analysis. Lane 1: H1; Lane 2: helper A + helper B; Lane 3: target; Lane 4: H1 + target + A + B; Lane 5: H1 + target. The concentration of H1, helper probes, K⁺, time were 50.0 nM, 25.0 nM, 40.0 mM, 40min, respectively.

Fig. S2. Concentrations optimization of helper probes. The concentration of H1 was 50.0 nM. And the concentration of helper probes varied from 10.0 nM to 85.0 nM.
Fig. S3. Concentrations optimization of K⁺. The concentration of H1 and helper probes were 50.0 nM, 25.0 nM, respectively, and the concentration of K⁺ varied from 5.0 mM to 60.0 mM.

Fig. S4. Concentrations optimization of THT. The concentration of H1, helper probes, K⁺ were 50.0 nM, 25.0 nM, 40.0 mM, respectively, and the concentration of THT
varied from 0.5 μM to 60.0 μM.

**Fig. S5.** Optimization of the hybridization temperature

**Fig. S6.** Optimization of the amplification reaction time
Fig. S7. Interference of Mg$^{2+}$. The concentration of H1, helper probes, K$^+$, time were 50.0 nM, 25.0 nM, 40.0 mM, 40min, respectively, and the concentration of Mg$^{2+}$ varied from 0 mM to 30.0 mM.