

Supporting Information for

Sensitive and Selective Amplified Visual Detection of Cytokines Based on Exonuclease III-Aided Target Recycling

Kai Zhang,^{*,a,†} Tianli Ren,^{b,†} Ke Wang,^a Xue Zhu,^a Hao Wu^a and Minhao Xie^{*,a}

^aKey Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine. Wuxi, Jiangsu 214063, China.

^bDepartment of Immunology and Rheumatology, The Second People's Hospital of Wuxi, Wuxi, Jiangsu 214000, China.

Experimental section

Materials and reagents: bovine serum albumin (BSA), α -fetoprotein (AFP) and carcino-embryonic antigen (CEA) were purchased from Sigma-Aldrich (St. Louis, MO). Exonuclease III (Exo III) with 10×NEBuffer was purchased from New England Biolabs (Beijing, China). IFN- γ and DNA marker were obtained from Takara (Dalian, China). ABTS (2, 2-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid), Hemin, H₂O₂, and HEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt) were purchased from Aladdin Reagents (Shanghai, China) and used as supplied. The HPLC-

purified oligonucleotide sequences were ordered from Nanjing Genscript Co., Ltd (Nanjing, China) and the sequences were listed in Figure S1.

All chemicals were of analytical grade and all solutions were prepared with ultrapure water, which was purified with Milli-Q purification system (Branstead) to a specific resistivity of $> 18.2 \text{ M}\Omega \text{ cm}$. Hemin stock solution (1 mM) was prepared in dimethylsulfoxide (DMSO) and stored at $-20 \text{ }^\circ\text{C}$. ABTS²⁻ and H₂O₂ solution were freshly prepared before used.

Amplification reaction: Firstly, the mixture of IFN- γ aptamer (10 μM , 4 μL), DNA1 (10 μM , 4 μL) and DNA2 (10 μM , 4 μL) in 14 μL 10 mM phosphate buffer solution (PBS, 0.1 M NaCl, pH 7.4) in a microtest tube and the solution of DNA3 in another tube were heated to $90 \text{ }^\circ\text{C}$ for 10 min and allowed to cool to room temperature for about 4 h to form the partially complementary dsDNA strands and the stem-loop structure DNA, respectively. Then the DNA3 (10 μM , 4 μL) solution was added into the mixture solution of DNA1 and DNA2. Subsequently, the solution was incubated with 30 μL of $10\times$ NEBuffer solution containing various concentrations of IFN- γ and 10 U Exo III at $37 \text{ }^\circ\text{C}$ for 90 min. Finally, the resulting mixtures were heated at $90 \text{ }^\circ\text{C}$ for 10 min to deactivate the Exo III. For control experiment, the BSA, AFP, and CEA were used.

Colorimetric Measurement: For high-throughput detection, the above mentioned solutions were moved to 96-well microtiter plates. 4 μL of hemin (10 μM) and 116 μL

of HEPES buffer (25 mM HEPES, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100, and 1% DMSO, pH 7.4) were then added, and the mixture was incubated for 60 min at room temperature. ABTS²⁻ and H₂O₂ (20 μL in total) were added to the mixture to reach final concentrations of 6 mM and 2 mM, respectively, at room temperature, and photographs of the solutions were taken after 5 min of color development.

Instrumentation: The fluorescence spectra were recorded by a SpectraMax M5 Multi-Mode Microplate Readers (Molecular Devices, USA), using a Costar 96 well microtiter plate (No. 3599, Corning, New York, USA).

Gel electrophoresis: Agarose gel (4%) electrophoresis was carried out in 1× TBE buffer at a 120 V constant voltage for 45 min. Before amplification reaction, the mixture of IFN-γ aptamer, DNA1 and DNA2 and the solution of DNA3 were heated to 90 °C for 10 min and allowed to cool to room temperature for about 4 h to form the partially complementary dsDNA strands and the stem-loop structure DNA, respectively. Samples needed digestion were initiated by addition of Exo III and maintained at 37 °C (reaction mixtures contained 50 U of Exo III). After 90 min, digestions were stopped by heating at 90 °C for 10 min. The gel was taken photograph under UV light after staining with EtBr. The concentration of each DNA in agarose gel is 1 μM. IFN-γ is added at a concentration of 10 μM.

DNA sequences

The system mainly consists of IFN- γ aptamer, DNA1, DNA2, and DNA3. The sequences of the oligonucleotides are listed in Figure S1. DNA1 includes two domains: a sequence that is complementary to part of DNA2 and target DNA (5'-ACACAACAC-3') which is complementary to part of IFN- γ aptamer. DNA2 also includes three domains: a sequence that is complementary to IFN- γ aptamer, a sequence that is complementary to part of DNA1 and four bases (5'-AAAA-3'). DNA3 includes three domains: the classical G-rich sequence which can form a G-quadruplex in the presence of hemin and K⁺, the sequence that is complementary to part of G-rich sequence and the sequence that is complementary to target DNA.

IFN- γ aptamer: 5'-GGGGTTGGTTGTGTTGGGTGTTGTGT-3'

DNA1: 5'-ACACAACACATACATTCTGAGAGC-3'

DNA2: 5'-CCAACACAACCAACCCCGCTCTCAGAATGTATAAAA-3'

DNA3: 5'-TTTGGGTAGGGCGGGTTGGGTACCCAAAGTGGTTGTGT-3'

Figure S1. DNA oligonucleotides sequence used in this strategy. The colors of the sequences are the same as given in Scheme 1.

Optimization of quadratic reaction time and catalytic oxidization time

One important factor of the assay is the quadratic reaction time. In order to achieve optimal assay conditions, the quadratic reaction time was optimized. For this purpose,

the effect of the reaction time on the signal output of the proposed method was investigated by monitoring the UV-vis absorption intensity of the mixture DNA solution with the presence of IFN- γ (1000 pM), and Exo III (10 U) at a time interval of 15 min from 15 to 150 min. As displayed in Figure S2A, the absorption intensity of the mixture increase rapidly with increasing reaction time in the range from 15 to 90 min and reaches a plateau thereafter. To ensure complete quadratic reactions, the reaction time of 90 min was selected for subsequent experiments. In addition, time-dependent absorbance intensities arising from the assay of the IFN- γ after addition of the ABTS²⁻ and H₂O₂ were measured. The absorbance intensity of the respective concentrations increases after addition of 0.1 and 100000 pM IFN- γ with as the catalytic reaction proceeds. As shown in Figure S2B, after about 5 min, the catalytic oxidization of ABTS²⁻ reaches saturation for both samples. So a time interval of 5 min was fixed for the UV-vis measuring.

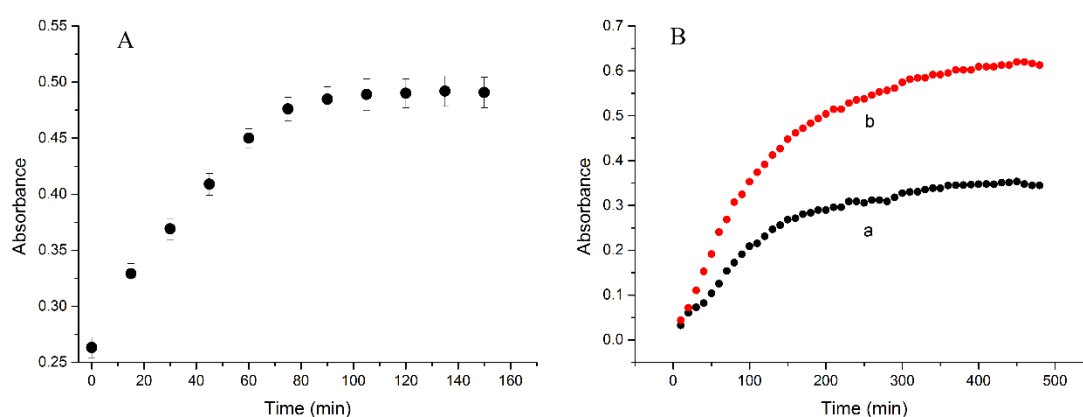


Figure S2. (A) When performed at 37 °C, the effect of the quadratic reaction time on the UV-vis absorption intensity. (B) UV-vis absorption intensity after the addition of ABTS²⁻ and H₂O₂ versus the time in the presence of 0.1 pM and 100000 pM IFN- γ .