Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2023

Electronic Supplementary Information

Target-initiated fluorescent aptasensor based on multisite strand displacement amplification for label-free detection of ochratoxin A

Dandan Zhang,[a] Xiangyue Cai,[a] Qian Zhang,[b] and Chunyang Zhang *[c]

[a] College of Materials and Chemistry & Chemical Engineering, Chengdu University of Technology, Chengdu 610059, Sichuan, China.

[b] College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014, China.

[c] School of Chemistry and Chemical Engineering, Southeast University, Nanjing, 211189, China.

[*] Corresponding author. E-mail: zhangcy@seu.edu.cn

EXPERIMENTAL SECTION

Reagents and materials

TdT, Klenow fragment (3′→5′ exo-) polymerase, dATPs, dTTPs, Exo III were provided by New England Biolabs. Ltd. (Hitchin, UK). SYBR Green I, SYBR Gold and DNA marke were purchased from Thermo Fisher Scientific (MA, USA). Ochratoxin A was purchased from LGC Standards (U.K). Aflatoxin B1 (AFB1), Zearalenone (ZEN), Deoxynivalenol (DON) were purchased from Pribolab Pte. Ltd. (Qingdao, China). The red wine purchased from a local supermarket was diluted 10 times with water and filtered through an ultrafiltration membrane. The OTA solid is dissolved in methanol to obtain standard solution with various concentrations. Other reagents were all of analytical grade. The buffer solution was prepared with ultrapure water (18.2 M/.cm^{-1}, Millipore System Inc). All oligonucleotides were synthesized by Sangong Biotechnology Co., Ltd. (Shanghai, China). Sequences of the oligonucleotides are listed as follows.

(1) Aptamer: 5′-GATCGGGTGTGGGTGGCGTAAACCGAGCAT CGGACA-SH-3′

(2) cDNA: 5′- CCCACACCCGATC-3′

(3) T_{20}: 5′-TTTTTTTTTTTTTTTTTTTT-3′

Conversion of OTA signal to DNA signal

The aptamer/cDNA duplexes were prepared by incubating OTA aptamer with cDNA in annealing buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 8.0) at 95 °C for 5 min, followed by slowly cooling to room temperature. Then, different concentrations of OTA and binding buffer (10 mM Tris,120 mM NaCl, 5 mM KCl, 20 mM $CaCl_2$) were added into above solution, followed by reacting at 37 °C for 30 min. Afterwards, Exo III (100 U/μL) and 10× NEB buffer 1 (100 mM Bis-Tris, 100 mM $MgCl_2$, 10 mM DTT) were added in the solution for the digestion reaction. The digestion re-

action was carried out at 37 °C for 30 min, and terminated by heating at 75 °C for 30 min to obtain 20 μL of solution I.

TdT-catalyzed extension reaction

For the TdT-catalyzed extension reaction, the obtained solution I was added into 30 μL of mixture containing 500 nM T_{20}, 10 mM dATP, 10 U of TdT, 0.25 mM $CoCl_2$ and 1× TdT buffer (50 mM KAc, 20 mM Tris-Ac, 10 mM $Mg(Ac)_2$, pH 7.9). The extension reaction was incubated at 37 °C for 30 min, and terminated by heating at 80 °C for 5 min to obtain solution II.

Multisite strand displacement amplification reaction

For the multisite strand displacement amplification reaction, the prepared solution II was added into 50 μL of reaction mixture containing 10 mM dTTP (0.1 μL of 100 mM), 10 U of KF polymerase, 1× NEB buffer 2 (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9). The reaction mixture was incubated at 37 °C for 60 min to prepare solution III.

Recovery assay in red wine-spiked samples

The 10 μL of solution containing 10% red wine, target OTA, 100 nM aptamer/cDNA duplexes, 1× binding buffer (10 mM Tris, 120 mM NaCl, 5 mM KCl, 20 mM $CaCl_2$) was incubated at 37 ℃ for 30 min. The subsequent measurement was performed with same protocols described above.

Gel glectrophoresis

To analyze the reaction products, 12% nondenaturing polyacrylamide gel electrophoresis (PAGE) was performed in 1× TBE buffer (9 mM boric acid, 9 mM Tris-Hcl, 0.2 mM EDTA, pH 7.9) at 110 V constant voltage for 50 min at room temperature.

Measurement of fluorescence spectra

The obtained solution III was mixed with 10 μL of 20× SYBR Green I dye and diluted to a final

volume of 200 μL with 1× buffer (50 mM NaCl, 10 mM Tris-HCl, pH 7.4). All fluorescence spectra were measured by a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan). The excitation wavelength was 497 nm, and the emission spectra were recorded between 505 and 590 nm.

SUPPLEMENTARY RESULTS

Optimization of the reaction conditions

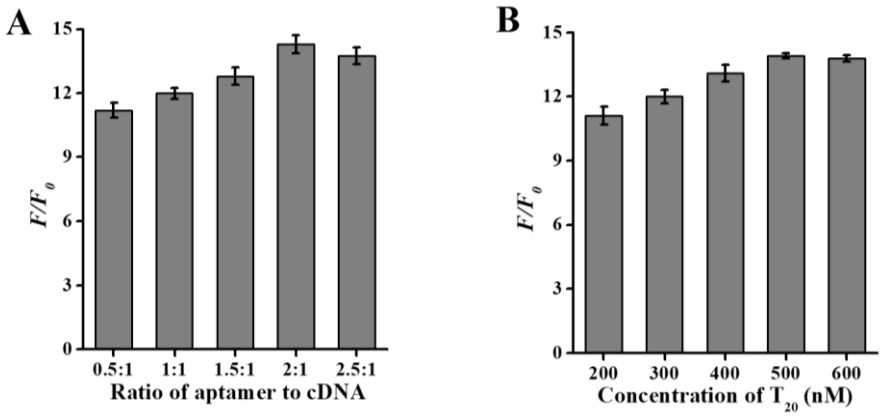

Fig. S1 (A) Variance of F/F_0 value with the ratio of aptamer to cDNA. (B) Variance of F/F_0 value with the concentration of T_{20}. Error bars show the standard deviations of three experiments.

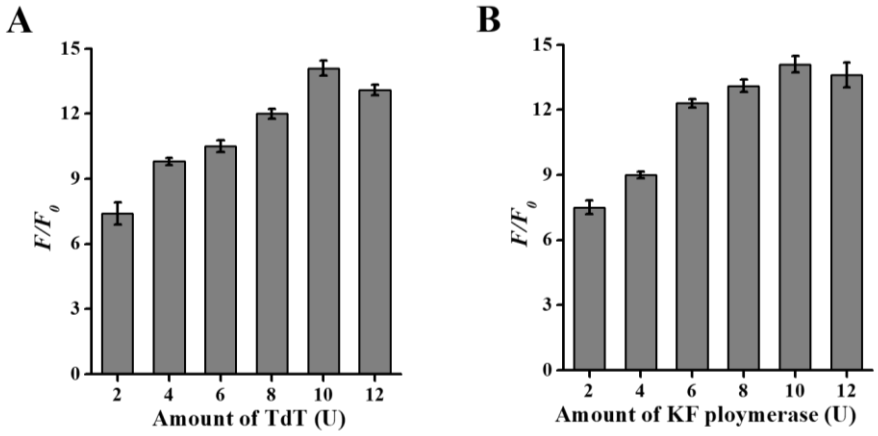

Fig. S2 (A) Variance of F/F_0 value with different amounts of TdT. (B) Variance of F/F_0 value with different amounts of KF polymerase. Error bars show the standard deviations of three experiments.

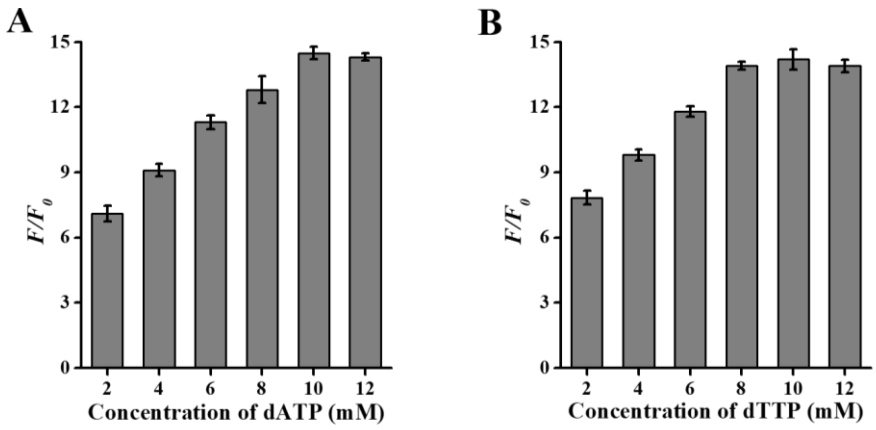

Fig. S3 (A) Variance of F/F_0 value with different concentrations of dATP. (B) Variance of F/F_0 value with different concentrations of dTTP. Error bars show the standard deviations of three experiments.

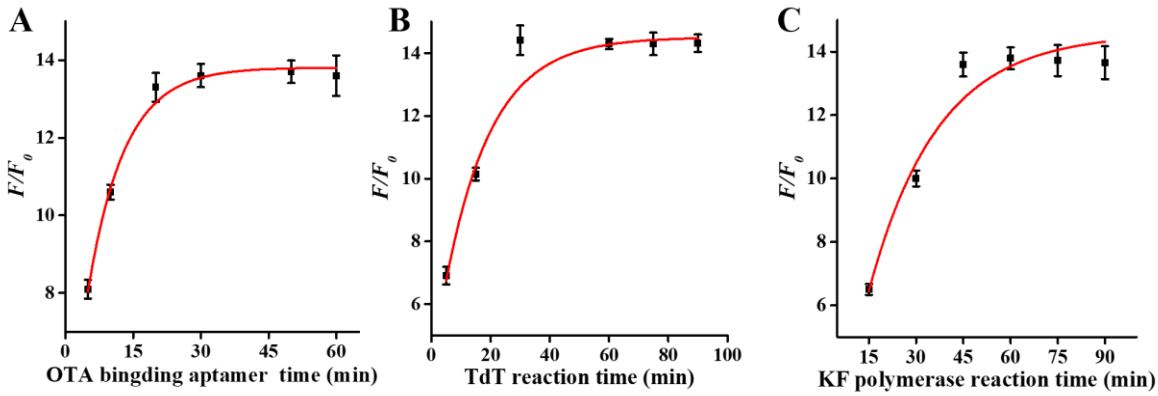

Fig. S4 (A) Variance of F/F_0 value with the incubation time of aptamer/cDNA duplex with OTA. (B) Variance of F/F_0 value with TdT reaction time. (C) Variance of F/F_0 value with KF polymerase reaction time. Error bars show the standard deviations of three experiments.