Electronic Supplementary Information

A cascade signal-amplified fluorescent biosensor combining APE1 enzyme cleavage-assisted target cycling with rolling circle amplification

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1. Experimental section

1.1 Reagents and chemicals

The DNA and RNA sequences purified by high-performance liquid chromatography (HPLC) (see Table S1.) were purchased from Sangon Biotech Biotechnology Co. Ltd. (Shanghai, China). Sulfur T (AR) was also purchased from Sangon Biotech Biotechnology Co. Ltd. (Shanghai, China). Purchase of sodium dihydrogen phosphate (AR) and disodium hydrogen phosphate (AR) from Shanghai Hushi Chemical Co., Ltd. Sodium acetate (AR), tri-(hydroxymethyl) aminomethane (99.99%), tetramethylethylenediamine (99.5%), and ammonium persulfate (98%) were purchased from Shanghai Aladdin Biochemical Technology Co. Ltd. Magnesium Chloride (98%) was purchased from Tianjin Guangfu Technology Development Co. Ltd. Magnesium acetate (98%) was purchased from McLean Biochemical Technology Co. Ltd. Both Bst polymerase and Apurinic/apyrimidinic endonuclease I (APE1) were obtained from NEB. All solutions were prepared using double-distilled ultrapure water purified by a Milli-Q system (Eco-S30, Shanghai Hetai Instrument Co. Ltd., China).

1.2 Apparatus and measurements

The UV visible absorption spectrum was measured using an ultramicro nucleic acid protein analyzer (NanoDrop One, ThermoFisher) with wavelengths ranging from 200 to 800 nm. The fluorescence spectrum was recorded using an F-7000 fluorescence spectrophotometer (Hitachi) at room temperature, with an excitation wavelength of 440 nm and a fluorescence collection range of 460-640 nm.

2. Optimizing experimental conditions

2.1 Optimizing APE1 enzyme concentration and reaction time

The reaction products obtained by optimizing APE1 enzyme concentration and APE1 enzyme reaction time were characterized by 8% polyacrylamide gel

electrophoresis. Polyacrylamide gel electrophoresis experiment was performed at 100 V constant voltage for 70 min in 0.5xTBE buffer at room temperature. Then, the gel was put into GelRed dye and dyed for 30 min before gel imaging in Genosens 2100 imaging system (Clinx Science Instruments Co., China.).

To optimize the concentration of APE1 enzyme and the enzyme cleavage time, we conducted experiments using the following conditions: HP and miRNA-21 were each set at a concentration of 1 μM, and the reaction was carried out in 1× Bst reaction buffer with a total reaction volume of 10 μl. The reaction was maintained at 37 ℃ for 2 hours to ensure complete hybridization between miRNA-21 and HP. Subsequently, APE1 enzyme was introduced at three different concentrations: 0.05 U/μl, 0.1 U/μl, and 0.15 U/μl, respectively. The reaction was allowed to proceed at 37 ℃ for 1 hour. Finally, the reaction mixture was heat-treated at 65 ℃ for 20 minutes to deactivate the APE1 enzyme.

To optimize the APE1 reaction time, we utilized the optimal enzyme concentration condition of 0.05 U/μl, and then the enzyme cleavage reactions were carried out at 37 ℃ for varying durations, specifically, 0.5 hours, 1 hour, 1.5 hours, and 2 hours, respectively. Following each reaction, the samples were subsequently treated at 65 ℃ for 20 minutes to deactivate the APE1 enzyme.

2.2 Optimizing the rolling circle amplification reaction conditions

The second part of this experiment involves hybridization of HP1 with a dumbbell circular template DP, followed by a rolling circle amplification reaction to obtain DNA long chains products containing G quadruplex. We conducted optimization of the rolling circle amplification (RCA) reaction parameters as follows: (1) Optimization of Bst Polymerase Concentration: We varied the concentration of Bst polymerase in the reaction system, testing it at 0.1 U/μl, 0.2 U/μl, 0.3 U/μl, and 0.4 U/μl. (2) Optimization of dNTPs Concentration: We experimented with different concentrations of dNTPs in the reaction system, ranging from 0.25 mM, 0.5 mM, 0.75 mM, to 1 mM. (3) Optimization of Reaction Time for Rolling Circle Amplification: The reaction time was systematically adjusted to 0.5 h, 1 h, 2 h, 3 h, and 4 h, respectively. After obtaining the

RCA products containing G quadruplex, 10 mM of KCl and 10 μM of ThT were added into the reaction system, and then the fluorescence signal intensity was detected using a fluorescence spectrophotometer (HITACHIF 7000, Ex=440nm)

2.3 Optimizing the concentration of ThT and KCl

In the third part of this experiment, KCl was added into RCA products to form the G quadruplex structure, and then the added ThT would insert into the G quadruplex to achieve fluorescence signal enhancement. We optimized the concentration of ThT and KCl: (1) Optimize KCl concentration: We varied the KCl concentration in the reaction system, testing it at 5 mM, 10 mM, 20 mM, and 40 mM; (2) Optimize ThT concentration: The ThT concentrations in the reaction system were adjusted to 5 μM, 10 μM, 20 μM, 40 μM, and 50 μM, respectively. Subsequently, fluorescence signal intensity was measured using a fluorescence spectrophotometer (Ex=440 nm).

Table S1. The sequences for the nucleic acids used in this work.

Name	Sequence（5'-3'）
DP	PCGCGTTCCCAACCCGCCCTACCCAACGCGGTGGATGT TGACTAGTTCCGAATCCAC
HP	TCGGAACTAGTCAACATCXGTCTGATAAGCTATGACT AGACCTTCCGAATAGCTT
miRN-21	UAGCUUAUCAGACUGAUGUUGA

Note: X represents the AP site recognized by APE1, and P represents the Phosphate.

Table S2. Comparison of this biosensor with the previously reported strategies for miRNAs detection.

Method	Linear range	Detection limit	Reaction time	Nanomaterials sample	type of microRNA	amplification strategy	In vitro detection /in vivo imaging	With APE1	References
Fluorescence	2-60 nM	680 pM	2 h	ZIF-8	microRNA21	No	In vivo imaging	No	1
Fluorescence	0-16 nM	47 pM	30 min	GO	microRNA21	CHA	In vitro detection	No	2
Fluorescence	0.2-30 nM	100 pM	3 h	DNA-AgNCs	microRNA155	No	In vitro detection	No	3
Fluorescence	0.15-37.5 nM	130 pM	2 h	DNA tetrahedron	microRNA21	No	In vitro detection and imaging	No	4
Fluorescence	2-10 nM	1000 pM	30 min	QD	microRNA21	No	In vitro detection	No	5
Fluorescence	0.1-10 nM	80 pM	4 h	DNA triangular prism	microRNA21	CHA	In vivo imaging	No	6
Fluorescence	0.2-100nM	200pM	20 min	—	microRNA21	SDR	In vivo imaging	Yes	7
Fluorescence	0-10nM	5.6pM	110 min	Streptavidin protein	microRNA21	CHA	In vivo imaging	Yes	8
Fluorescence	0.2-6nM	22.9 pM	—	gold nanorod	microRNA21	DNAzyme	In vivo imaging	Yes	9
Fluorescence	0-100 nM	3.33 pM	2 h	—	microRNA21	RCA	In vitro detection	Yes	This work

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