Nicking Enzyme Assisted Amplification Combined with CRISPR-Cas12a System for One-Pot Sensitive Detection of APE1

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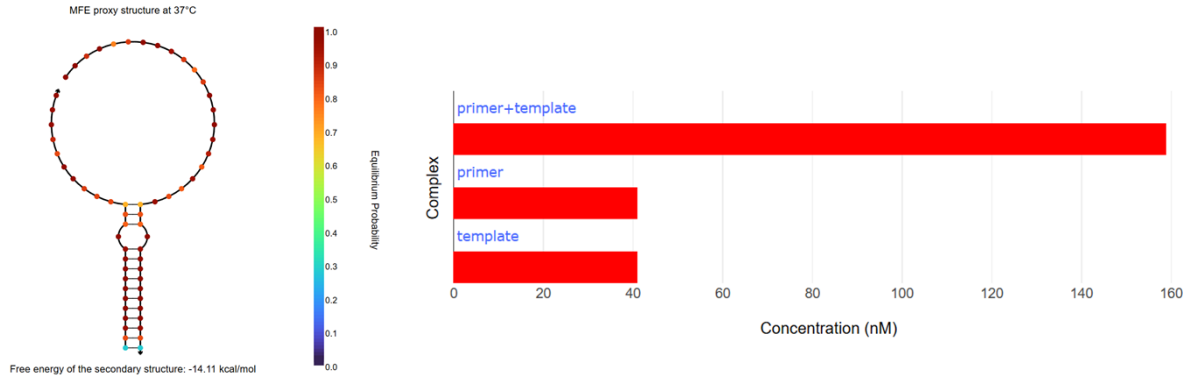
Table S1. Sequences used in the study

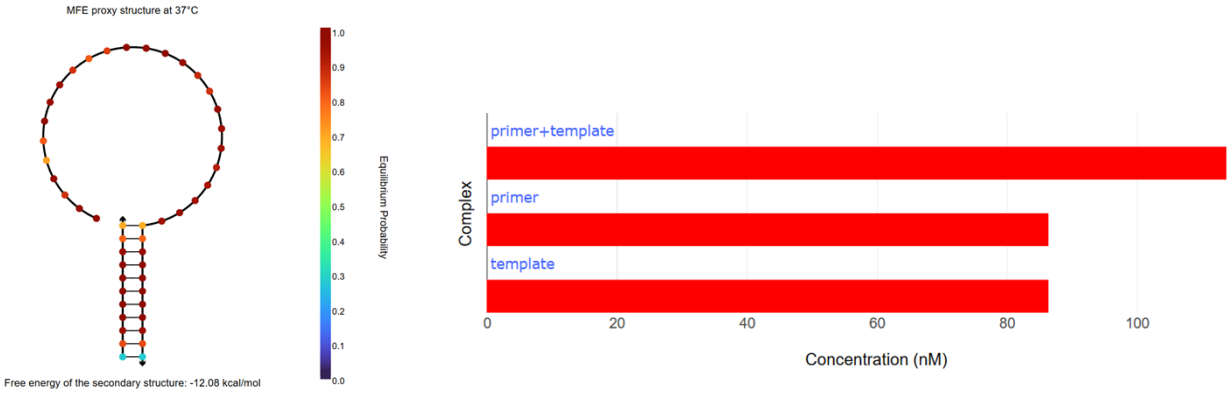
Name	Sequence（5'—>3'）	Role in the study
crRNA	UAAUUUCUACUAAGUGUAGAUC UAUAAUUUUUCGUGCAGUG	Figure 1-5
Primer-1	AAATGACCTAG/**AP**/ACTGACCTCA AAC	Figure 1-3
Template-1	CTATAATTTTTCGTGCAGTGTTAG TTCTAGGTCATTT	Figure 1-3
Reporter Probe	**BHQ-**TTATTATT**-FAM**	Figure 1-5
Primer-2	AAATGACCTAG/**AP**/ACTGACCTTA TA	Figure 3
Primer-3	ACGACCAAATGACCTAG/**AP**/ACTG ACCTTATA**-P**	Figure 3
Template-2	CTATAATTTTTCGTGCAGTGTTAG TTCTAGGTCATTTGGTCGT	Figure 3
Primer-4	ACGACCAAATGACCTAG/**AP**/ACTG ACCTTATA**-C6 spacer**	Figure 3
Template- Primer	CTATAATTTTTCGTGCAGTGTTAG TTCTAGGTCATTTGGTCGT**AAAAA** **A**ACGACCAAATGACCTAG/**AP**/ACT GACCTTATA**-C6 spacer**	Figure 3-5

The modifications on probes were highlighted with bold font.

Figure S1. The structure and concentration distribution of the detection probe and the cleaved detection probe in the system.



A. Detection Probe（Primer-1 and Pemplate-1）.



B. Detection Probe after cleavage （Primer-1 and Pemplate-1）.

Table S2. Comparison of the analytical performance of some reported methods in APE1 detection

Method strategy	Signal	Sensitivity	Operation complexity	Time	Assay temperature	Ref
A sensitive sensing system enables rapid activation of gated HRCA upon intelligent recognition of APE1.	fluorescence	0.0001U/mL	Simple	90min	37℃	[1]
Host-guest interaction between b-CDP and pyrene	fluorescence	0.05 U/mL	moderate	40 min	37 °C	[2]
An integrated electrochemical biosensing system that gives consideration to both immunoassay and enzyme activity analysis	Electrochemical signal	0.00518 U/mL	complex	＞3h	37℃	[3]
closed cycle circuit (CCC)	fluorescence	7.8×10^{-5} U/mL	complex	1h	?	[4]
primer exchange reaction (PER) cascade rolling circle amplification (RCA)/ dimeric G-quadruplex	fluorescence	0.001U/mL	Simple	2h	37℃	[5]

Table S3. Introduction to the enzymes used in the thesis.

Enzymes	1X R2.1 Components	Nature	Activity in r2.1	Optimum reaction temperature
APE1	50 mM NaCl 10 mM Tris-HCl 10 mM MgCl2 100 µg/ml Recombinant Albumin pH 7.9@25°C	DNA AP endonuclease that catalyzes the cleavage of DNA phosphodiester backbone at AP sites via hydrolysis leaving a 1 nucleotide gap with 3'-hydroxyl and 5' deoxyribose phosphate (dRP) termini	50%	37°C
Nb.BtsI		Nb.BtsI is a nicking endonuclease that cleaves only one strand of DNA on a double- stranded DNA substrate.	100%	37°C
Cas12a		Cas12a has been shown to specifically recognize and cleave double-stranded DNA (dsDNA), subsequently releasing single-stranded deoxyribonuclease (ssDNase) activity. This activity enables Cas12a to indiscriminately cleave nearby single-stranded DNA (ssDNA).	100%	37°C
BST 3.0		Bst 3.0 DNA Polymerase contains 5′→3′ DNA polymerase activity with either DNA or RNA templates and strong strand displacement activity, but lacks 5′→3′ and 3′→5′ exonuclease activity.	100%	65°C

Table S4. Reagent Storage Information.

Reagents	Storage Buffer	Storage temperature	Shelf life
Bst 3.0 DNA Polymerase	100 mM KCl 10 mM Tris-HCl 0.1 mM EDTA 1 mM DTT 0.1% Triton® X-100 50% Glycerol pH 7.4 @ 25°C	-20°C	2 years
Nb.BtsI	10 mM Tris-HCl 50 mM NaCl 1 mM DTT 0.1 mM EDTA 200 µg/ml BSA 50% Glycerol pH 7.4 @ 25°C	-20°C	2 years
EnGen® Lba Cas12a (Cpf1)	500 mM NaCl 20 mM sodium acetate 0.1 mM EDTA 0.1 mM TCEP 50% Glycerol pH 6 @ 25°C	-20°C	2 years
10X NEB r2.1 buffer		-20°C	2 years
Detection Probe	TE buffer	-20°C	1 years
Reporter Probe	TE buffer	-20°C	1 years
crRNA	DEPC treated water	-20°C	6 months

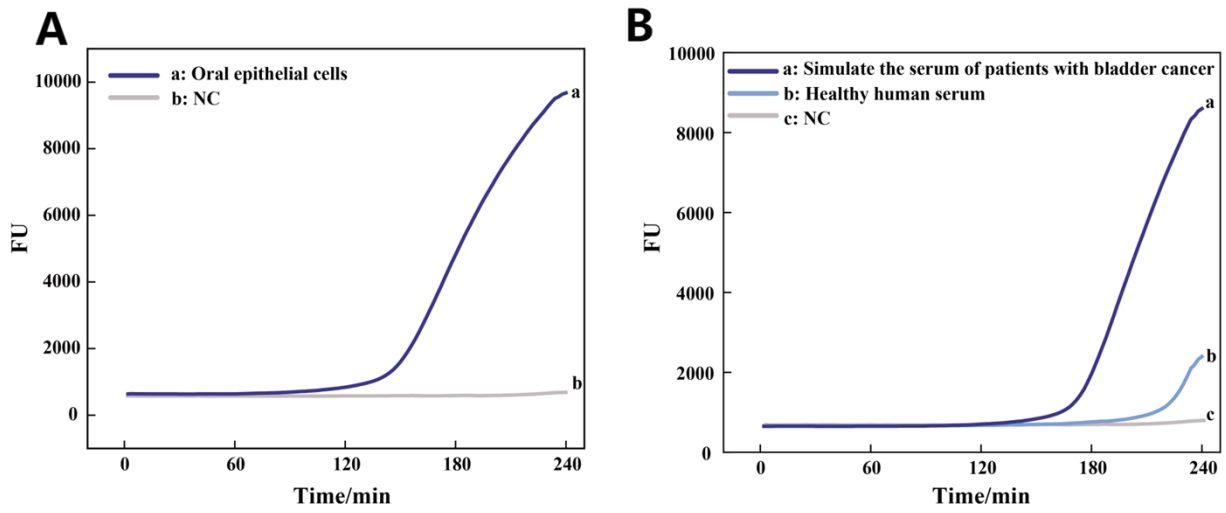


Figure S2. Detection of APE1 in human oral epithelial cells and human serum samples. A. Oral epithelial cell sample. B. Healthy human serum and serum samples from simulated bladder cancer patients.

References

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