Electronic Supplementary Information (ESI)

Sensitive and label-free fluorescent detection of apurinic/apyrimidinic endonuclease 1 activity based on isothermal amplified-generation of G-quadruplex

Yufeng Huang, Yefei Ma, Yina Li, Mei Xiong, Xuejun Li, Liangliang Zhang* and Shulin Zhao

State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, School of Chemistry and Pharmaceutical Sciences, Guangxi Normal University, Guilin 541004, PR China

Experimental

1. Reagents

Klenow fragment (KF) polymerase (3'→5' exo$^-$), apurinic/apyrimidinic endonuclease 1 (APE1), deoxyribonucleoside triphosphates (dNTPs), Nt.BbvCI endonuclease (Nt.BbvCI), Dam methyltransferase (Dam MTase), M.SssI methyltransfer (M.SssI), uracil-DNA glycosylase (UDG) and 10×NEB buffer 4 were purchased from New England Biolabs, Ltd. (NEB, Beijing, China). HPLC-purified DNA oligonucleotides, DNA maker, bovine serum albumin (BSA), and ethidium bromide (EB) were obtained by Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of DNA probes employed in this work were shown in Table S1. N-Methyl mesoporphyrin IX (NMM) was bought from J&K Scientific Ltd. (Beijing, China). All other reagents used in this work were of analytical grade. Water was purified by a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA) and used throughout the work.

The storage concentration of APE1 obtained from NEB was 10 U μL^{-1} (1 U mL^{-1} $\approx$ 32.9 pM, the molecular weight is 35554 Daltons). According to NEB's definition, one unit is defined as the amount of APE1 required to cleave 20 pmol of a 34 mer oligonucleotide duplex containing a single AP site in a total reaction volume of 10 μL in 1 hour at 37°C.

2. Detection of APE1 activity

AP site-contained HP was first heated at 95 ℃ for 10 min and allowed to cool naturally to room temperature. After that, a volume of 100 μL mixture containing 100

nM HP, 1×NEB buffer 4 (20 mM Tris-acetate, pH 7.9, 50 mM potassium acetate, 10 mM magnesium acetate and 1 mM dithiothreitol), 5 U KF polymerase, 10 U Nt.BbvCI endonuclease, 100 μM dNTPs, 0.5 μM NMM and various concentrations of APE1 was incubated at 37 °C for 90 min. The fluorescence spectra were then measured by using a Cary Eclipse fluorescence spectrophotometer (Agilent, USA) at the excitation wavelength of 399 nm. The spectra were recorded in the range from 570 to 650 nm. Slit widths for the excitation and emission were both set at 10 nm. The fluorescence emission intensity was recorded at 610 nm.

3. Gel electrophoresis analysis

In the gel electrophoresis assay, 12 μL of samples were loaded into the notches of the freshly prepared agarose gel (4 %), and electrophoresis was performed at 100 V for 90 min in 1× TBE buffer (90 mM Tris, 90 mM boricacid, 10 mM EDTA, pH 8.3). After staining with ethidium bromide, the electrophoresis image was taken by a digital camera under UV light.

Supplementary Tables

Table S1 Sequences of oligonucleotides used in this work.

Probes	Sequences
HP	ACGATGCTAAACCCTXAGACTGCACCCAAAACCCAAAACCCAAAACCCGCTGAGGTGCAGTCTAAGGGTTTAGCATCGT
GP	TCAGCGGGTTTTGGGTTTTGGGTTTTGGGTGCAGTCT

X in HP is tetrahydrofuran modification, which is a AP site analog.

Table S2 The comparison of this method with other reported approaches for the detection of APE1.

Methods	Modification	Time	Linear range	LOD	Ref.
Nickel hexacyanoferrates nanoparticle-decorated Au nanochains and alkaline phosphatase amplified electrochemical detection	Complicated surface modification with nanomaterial, enzyme and antibody	>12 h	0.01~100 pg mL^{-1}	3.9 fg mL^{-1}	1
Ionic liquid doped Au nanoparticle/ graphene and alkaline phosphatase amplified electrochemical detection	Complicated surface modification with nanomaterial, enzyme and antibody	>8 h	0.1~80 pg mL^{-1}	0.04 pg mL^{-1}	2
On-bead fluorescent DNA nanoprobes	Dual-modification with Cy3 and biotin in DNA substrate	> 120 min	0.10～5.0 U	0.02 U	3
Unimolecular chemically modified DNA probe	Dual-modification with ROX and BHQ_2 in DNA substrate	~20 min	0.10～5.0 U mL^{-1}	0.1 U mL^{-1}	4
Double-stranded DNA based fluorescent probe	Dual-modification with TAMRA and DABYCL in DNA substrate	~9 min	0.024～2.0 U mL^{-1}	0.024 U mL^{-1}	5
Label-free detection based on isothermal amplified-generation of G-quadruplex	Label-free without modification	90 min	0.01～1.0 U mL^{-1}	0.006 U mL^{-1} (0.197 pM, 7.0 pg mL^{-1})	This work

1. J. Han, Y. Zhuo, Y. Chai, Y. Xiang, R. Yuan, Y. Yuan, N. Liao, *Biosens. Bioelectron.*, 2013, 42, 116-122.
2. Z. Zhong, M. Li, Y. Qing, N. Dai, W. Guan, W. Liang, D. Wang, *Analyst*, 2014, 139, 6563-6568.

3. G. Gines, C. Saint-Pierre and D. Gasparutto, *Anal. Chim. Acta*, 2014, 812, 168-175

4. S. Fang, L. Chen and M. Zhao, *Anal. Chem.*, 2015, 87, 11952-11956.

5. B. Liu and L. Peng, *Anal. Methods*, 2016, 4, 862-868.

Supplementary Figures

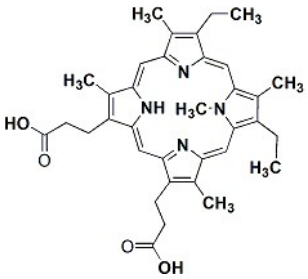

Fig. S1 The molecular structure of NMM.

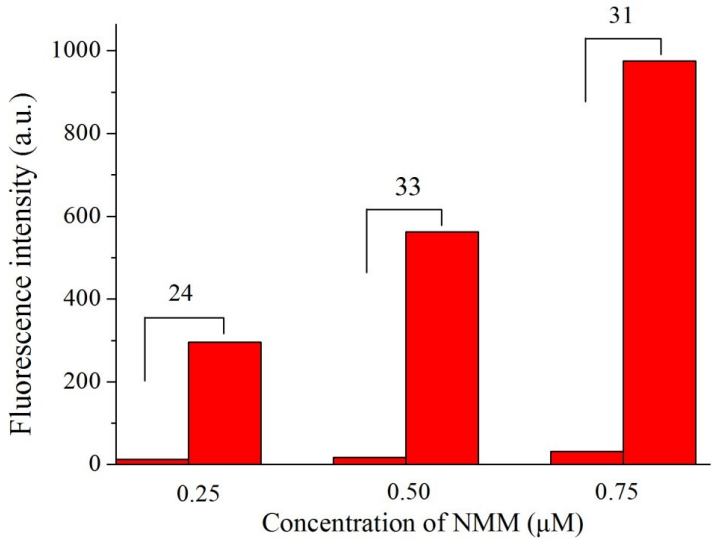

Fig. S2 Signal-to-background ratio at different concentrations of NMM. The concentrations of HP, dNTPs, KF, Nt.BbvCI and APE1 were 100 nM, 100 μM, 5.0 U, 10.0 U and 20 U mL^{-1}, respectively.

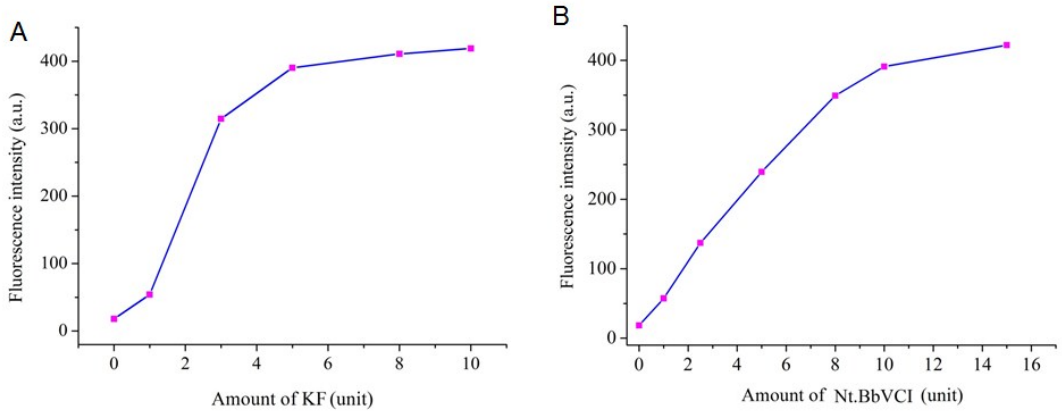

Fig. S3 (A) The effect of the KF polymerase amounts on the assay. The concentrations of HP, dNTPs, Nt.BbvCI and APE1 were 100 nM, 100 μM, 10.0 U and 1 U mL^{-1}, respectively. (B) The effect of the Nt.BbvCI endonuclease amounts on the assay. The concentrations of HP, dNTPs, KF and APE1 were 100 nM, 100 μM, 5.0 U and 1 U mL^{-1}, respectively.

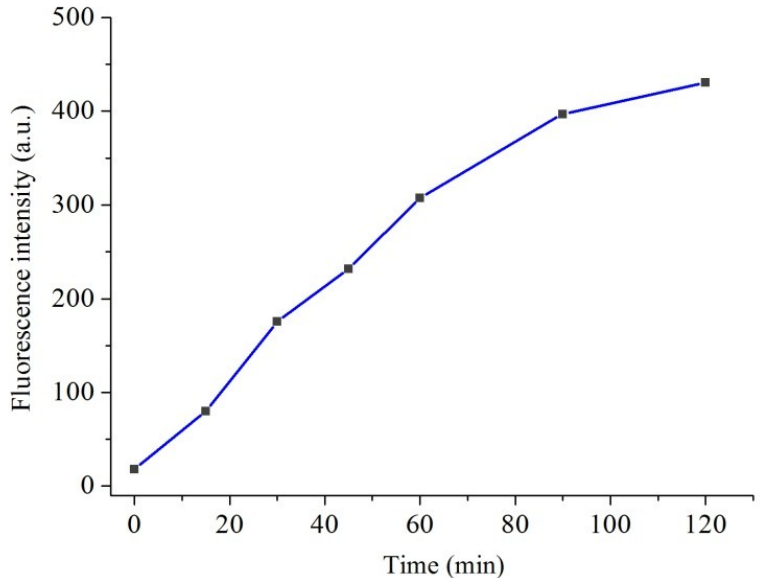

Fig. S4 Optimization of the reaction time. The concentrations of HP, dNTPs, KF, Nt.BbvCI, NMM and APE1 were 100 nM, 100 μM, 5.0 U, 10.0 U, 0.5 μM and 1 U mL^{-1}.

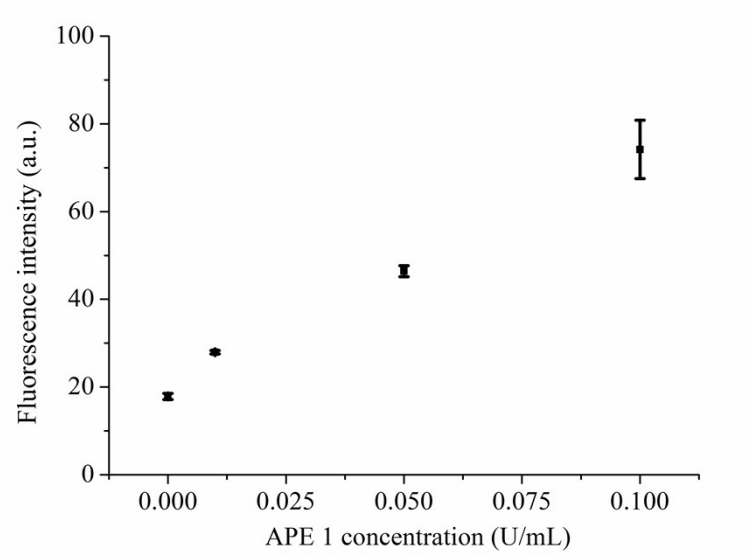

Fig. S5 The relationship between fluorescence intensity and APE1 concentration at low concentration range. Error bars were estimated from three replicate measurements.