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Electronic Supplementary Information (ESI)

A Target-activated Autocatalytic DNAzyme Amplification Strategy for the Assay of Base Excision Repair Enzyme Activity

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1. Experimental Section

Reagents and Apparatus

The DNA oligonucleiotides used in this work (sequences shown in Table S1) were synthesized by Takara Biotechnology Co., Ltd. (Dalian). uracil–DNA glycosylase, endonuclease Ⅳ, T4 DNA ligase, Bst DNA polymerase (large fragment), dNTPs and UDG inhibitor (UGI) were obtained from New England Biolabs (Ipswich, MA). All other chemicals were of analytical grade and were used as received. The solutions were prepared using ultrapure water which was obtained through a Millipore Milli-Q water purification system (Billerica, MA) and had an electric resistance >18.2 MΩ.

Fluorescence measurements were performed using a Hitachi F-7000 fluorescence spectrometer (Hitachi. Ltd., Japan) at 37 ℃. The excitation wavelength was 494 nm and the fluorescence intensity was recorded at 519 nm with both excitation and emission slits of 5 nm.

Table S1. Synthesized Probes (5'→3') Used in This Work

MB Probe	FAM-CCACCACAATGTTATACAGGTACTAT*rA*GGAAGTT GAGTTACGAGGCGGTGGTGG-BHQ1
P1	CTCAACTTCAGCGATCCGGAACGGCACCCATGTTAGTA CCT
P2	AUCGCUGAAGUUGAG
P3	ATCGCTGAAGTTGAG
P4	CUGAAGUUGAG
P5	CGCUGAAGUUGAG
P6	GGAUCGCUGAAGUUGAG
P7	CCGGAUCGCUGAAGUUGAG
Ligation Probe	CCAAGTGAATACAGCATCAGAACCUTACGTACAGAAA AAA*dd*C
Ligation Probe 2	CCAAGTGAATACAGCATCAGAACCTTACGTACAGAAAA AA*dd*C
Padlock Probe	PO_3-GATGCTGTATTCACTTGGAGGTACTAACATGGGTGC CGTTCCGGATCGCTGAAGTTGAGTCTGTACGTAAGGTT CT

rA at the middle of MB probe is adenine ribonucleotide. *dd* at the 3' terminus of ligation probe and ligation probe 2 is dideoxynucleotide modification. PO_3 at the 5' terminus of padlock probe is phosphorylation modification. U in P2, P4, P5, P6, P7 and ligation probe denote the uracil deoxyribonucleotide modification.

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Assay of UDG Activity and Inhibition using Double-stranded Substrate

To prepare the double-stranded substrates, each single strand probe (P1, P2) was dissolved in 1×NEB UDG reaction buffer (20 mM Tris–HCl, 1 mM EDTA, 1 mM DTT, pH 8.0). Then two solutions were mixed together and diluted to the desired concentration. The mixture of two single strand DNA probes was denatured at 95 ℃ for 10 min and cooled slowly to room temperature. The detection of UDG activity was carried out in 75 μL of an enzyme reaction mixture containing 1×NEB UDG reaction buffer, 200 nM denatured double-stranded substrate, 10 mM Mg^{2+}, 300 nM DNAzyme substrate (MB) and various concentration of UDG. The solutions of UDG were diluted with a storage buffer consisting of 50% glycerol, 20 mM Tris–HCl (pH 8.0), 50 mM KCl, 1 mM EDTA, 1 mM DTT and 0.1 mg/mL BSA. The fluorescence-time curve of each sample was recorded immediately after the addition of UDG to the solution at 37 ℃.

For the assay of UGI inhibition on the activity of UDG, 1.5 μL UGI was first added into the enzyme reaction mixture containing 1×NEB UDG reaction buffer, 200 nM denatured double-stranded substrate, 10 mM Mg^{2+} and 300 nM MB probe. Then 1.5 μL UDG was added and fluorescence-time curve was recorded immediately. To investigate the effect of UGI on the activity of E6 DNAzyme, P1 probe was used instead of double-stranded substrate as a control experiment. Briefly, 200 nM P1 probe and 300 nM MB probe were first mixed with or without 20 U/mL UGI in 70 μL of 1×NEB UDG reaction buffer. Then the fluorescence-time curve was recorded immediately after the addition of 5 μL Mg^{2+} (150 mM, final concentration was 10 mM) to the solution at 37 ℃.

Assay of UDG Activity using RCA-assisted Strategy

To prepare the circular DNA substrate, 10 μL padlock probe (10 μM), 10 μL ligation probe (10 μM), 2.5 μL 10×NEB T4 DNA ligase buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP, pH 7.5) and 2 μL H_2O were mixed together. The ligation mixture was denatured at 70 ℃ for 30 min and cooled slowly to room temperature. After annealing, 0.5 μL T4 DNA ligase was added into the mixture and incubated at 20 ℃ for 2 h. For the UDG assay using a RCA-assisted strategy, 60 nM prepared circular probes, 250 U/mL endonuclease Ⅳ and various concentration of UDG were incubated at 37 ℃ for 1 h in 40 μL 1×NEB UDG reaction buffer. Then, 5 μL 10×NEB ThermoPol reaction buffer (200 mM Tris-HCl, 100 mM KCl, 100 mM $(NH4)_2SO_4$, 20 mM $MgSO_4$ and 1% Triton X-100, pH 8.8), 3 μL of 10 mM/each dNTPs mixture, and 2 μL Bst DNA polymerase (large fragment, 8 U/μL) were added. The RCA reaction started at 65 ℃ for 5 h and finally terminated by heating at 85 ℃ for 20 min to inactivate the Bst polymerase. The fluorescence-time curve was recorded immediately at 37 ℃ after the addition of MB probe and Mg^{2+} (final concentration were 300 nM and 10 mM, respectively) to the solution.

2. Optimization of Detection Conditions

Mg^{2+} acts as a cofactor of the E6 DNAzyme, and the hybridization efficiencies are also influenced by the concentration of Mg^{2+}. So the effect of Mg^{2+} concentration on the assay was investigated. As shown in Fig. S1A, with the increasing of Mg^{2+}

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concentration from 2.5 to 10 mM, the ratio of signal to background increased. And the highest value was obtained at 10 mM. When the Mg^{2+} concentration further increased, the ratio decreased. This effect might due to the higher concentration of Mg^{2+} influencing the dissociation of the MB probe. Therefore, 10 mM was used as the optimal concentration of Mg^{2+} in this work.

The concentration of MB probe also has great effect on the fluorescence signal intensity. The influence of the MB concentration on the assay performance was also tested. From Fig. S1B, one can observe that the largest enhancement was obtained at 300 nM MB and the enhancement decreased with increasing MB probe from 300 to 500 nM. This effect might due to the higher concentration of MB probe resulting in higher background signal. In order to obtain the best signal-to-background ratio, 300 nM was chosen as the optimum concentration of MB probe.

In our design, we used a block DNA to restrain the activity of DNAzyme. The length of block sequence was important in the assay. We explored the influence of the length of block sequence on the background fluorescence intensity. As shown in Fig. S1C, the background fluorescence intensity decreased with increasing the length of block sequence up to 15mer, and reached a plateau after 15mer. To obtain lower background fluorescence intensity, P2 probe, a 15mer oligonucleotide, was selected to form double-stranded UDG substrate with E6 DNAzyme.

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3. Supplementary Figures

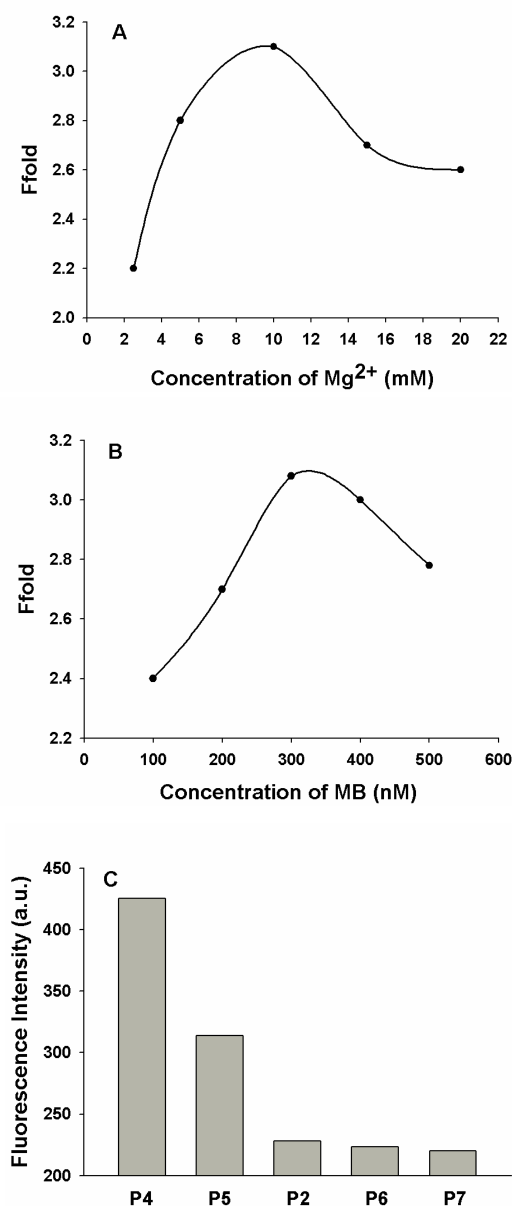

Fig. S1. (A) The effect of Mg^{2+} concentration on the assay. The concentrations of UDG and MB were 10 U/mL and 300 nM, respectively. (B) The effect of the MB concentration on the assay performance in the presence of 10 U/mL UDG. (C) Influence of the length of block sequence on the background fluorescence intensity. The length of P2, P4, P5, P6, P7 probes were 15, 11, 13, 17 and 19 mer, respectively.

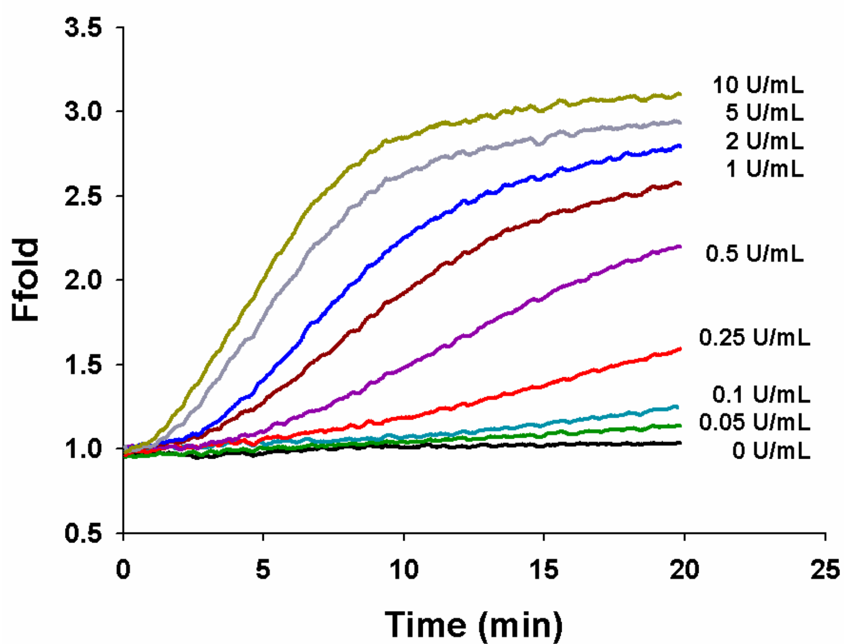

Fig. S2. Time-dependent fluorescence response over background fluorescence with varying concentrations of UDG. using double-stranded substrates. The concentrations of double-stranded substrate, MB probe and Mg^{2+} were 200 nM, 300 nM and 10 mM, respectively.

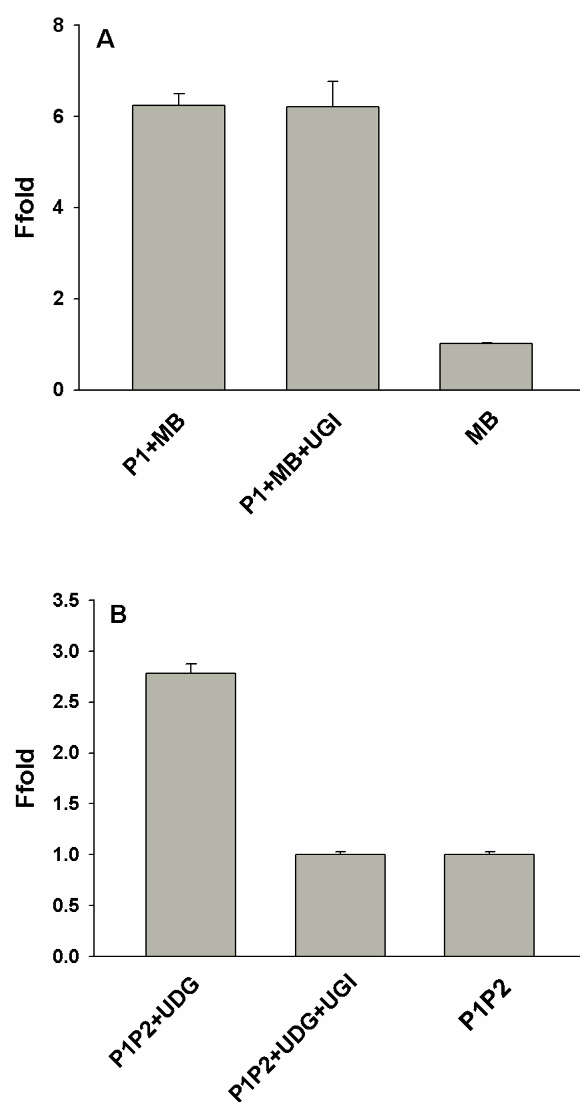

Fig. S3. (A) Influence of UGI on the activity of E6 DNAzyme. The concentration of UGI was 20 U/mL. (B) Influence of UGI on the activity of UDG. The concentrations of UGI and UDG were both 2 U/mL. In the absence of UGI, UGI storage buffer (50% glycerol, 10 mM Tris–HCl, pH 7.4, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT and 0.2 mg/mL BSA) was used instead of UGI.

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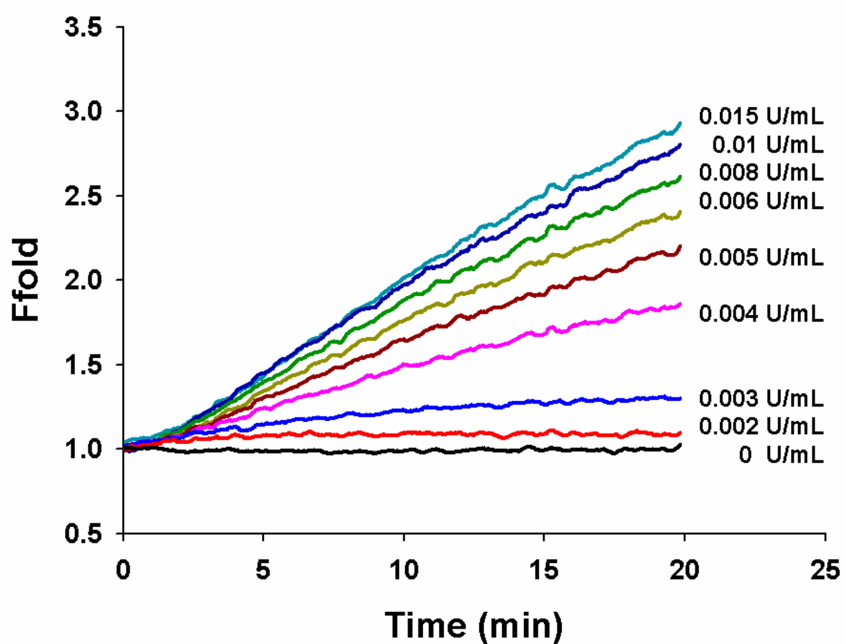

Fig. S4. Time-dependent fluorescence response over background fluorescence with varying concentrations of UDG using RCA-assist strategy. The concentrations of circular probe and Endo Ⅳ were 60 nM, and 250 U/mL, respectively.