Electronic Supplementary Information

Fluorescent G-quadruplex probe for the assay of base excision repair enzyme activity

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Experimental Section

Materials: All oligonucleotides used in the present study were synthesized by Genotech Co. (Daejeon, South Korea) and purified with HPLC.[1, 2] The sequences of the DNA oligonucleotides are listed in Table S1. Uracil DNA glycosylase (UDG), human alkyladenine DNA glycosylase (hAAG), 8-oxoguanine DNA glycosylase (hOGG1), exonuclease I (Exo I) and uracil DNA glycosylase inhibitor (UGI) were purchased from New England Biolabs Inc. (Beverly, MA, USA).[3] Magnesium chloride ($MgCl_2$) and potassium chloride (KCl) were purchased from Sigma-Aldrich. All other chemicals were of analytical grade and used without any further purification. Ultrapure DNase/RNase-free distilled water obtained from Bioneer® (Daejeon, South Korea) was used throughout the experiments.

Procedures to detect UDG activity: The SP containing 2-AP (5 μM) and the UP containing several uracil bases (5 μM) in Scheme 1 were first added to the reaction buffers (20 mM Tris-HCl, 1 mM DTT, 1 mM EDTA, 10 mM $MgCl_2$, pH 8.0). The solutions were then heated to 95 °C for 10 min, cooled slowly to 37 °C (0.1 °C/s), and incubated for 20 min, allowing the formation of the duplex substrate (SP1-UP3 duplex). After the incubation, KCl (100 mM) and UDG at varying concentrations were sequentially added to the solutions. The UDG reaction was then conducted at 37 °C for 60 min, followed by fluorescence measurement at room temperature. The fluorescence emission spectra were measured in a range of 340 - 480 nm at an excitation wavelength of 310 nm. The final concentrations of the SP1-UP3 duplex and KCl are 500 nM and 10 mM, respectively.

UDG Inhibition assay: All the procedures are the same as those to detect the UDG activity, except that UGI at varying concentrations was added before the addition of UDG (5 U/ml).

Melting curve analysis: SP1A or SP1 (5 μM), UP3 (5 μM), and EvaGreen™ (20X, Seoul, Korea) were first added to the reaction buffers (20 mM Tris-HCl, 1 mM DTT, 1 mM EDTA, 10 mM $MgCl_2$, pH 8.0). The solutions were then heated to 90 °C for 5 min, cooled slowly to

20 °C (0.1 °C/s), and incubated for 15 min. The resulting fluorescence signal was measured on a C1000^{TM} thermal cycler (Bio-Rad, CA, USA) as the temperature was increased from 20 °C to 90 °C with an increment of 0.5 °C. The first derivative plot [-d(RFU)/dT] was used to determine the melting temperature. The final concentrations of the SP1A-UP3 duplex, SP1-UP3 duplex, and EvaGreen^{TM} are 500 nM, 500 nM, and 1X, respectively.

Gel electrophoresis analysis of the UDG reaction products: The UDG reaction products were resolved on 15% polyacrylamide gel using 1X TBE as the running buffer at a constant voltage of 100 V for 60 min. The visualization of the reaction products was conducted with ethidium bromide (EtBr) staining.

CD measurement: SP1 (100 μM) and UP3 or TP3 (100 μM) were first added to the reaction buffers (20 mM Tris-HCl, 1 mM DTT, 1 mM EDTA, 10 mM $MgCl_2$, pH 8.0). The solutions were then heated to 95 °C for 10 min, cooled slowly to 37 °C (0.1 °C/s), and incubated for 20 min, allowing the formation of the duplex substrate. After the incubation, KCl (100 mM) and UDG (200 U/ml) were sequentially added to the solutions. The UDG reaction was then conducted at 37 °C for 60 min, followed by CD measurement at room temperature. The final concentrations of the SP1-UP3 duplex, SP1-TP3 duplex, KCl, and UDG are 4 μM, 4 μM, 10 mM, and 20 U/ml, respectively. CD spectra were recorded on a Jasco-815 spectropolarimeter (Tokyo, Japan) in the range of 200 - 320 nm in 0.5 mm pathlength cuvettes, using a scanning speed of 100 nm/min, a response time of 1 s, a bandwidth of 1 nm, and an accumulation of 10 scans.

Instrumentation: Fluorescence intensities were measured with a Tecan Infinite M200 pro microplate reader (Mnnedorf, Switzerland) and black, 384-well Greiner Bio-One microplates (ref: 781077, Courtaboeuf, France) at an excitation wavelength of 310 nm.[4, 5] The resulting image of the gel electrophoresis was obtained with the Gel Doc EZ Imager (Bio-rad, Hercules, USA).

Scheme 1 Schematic illustration of the UDG activity assay based on the excision induced G-quadruplex formation with significant fluorescence enhancement of 2-AP.

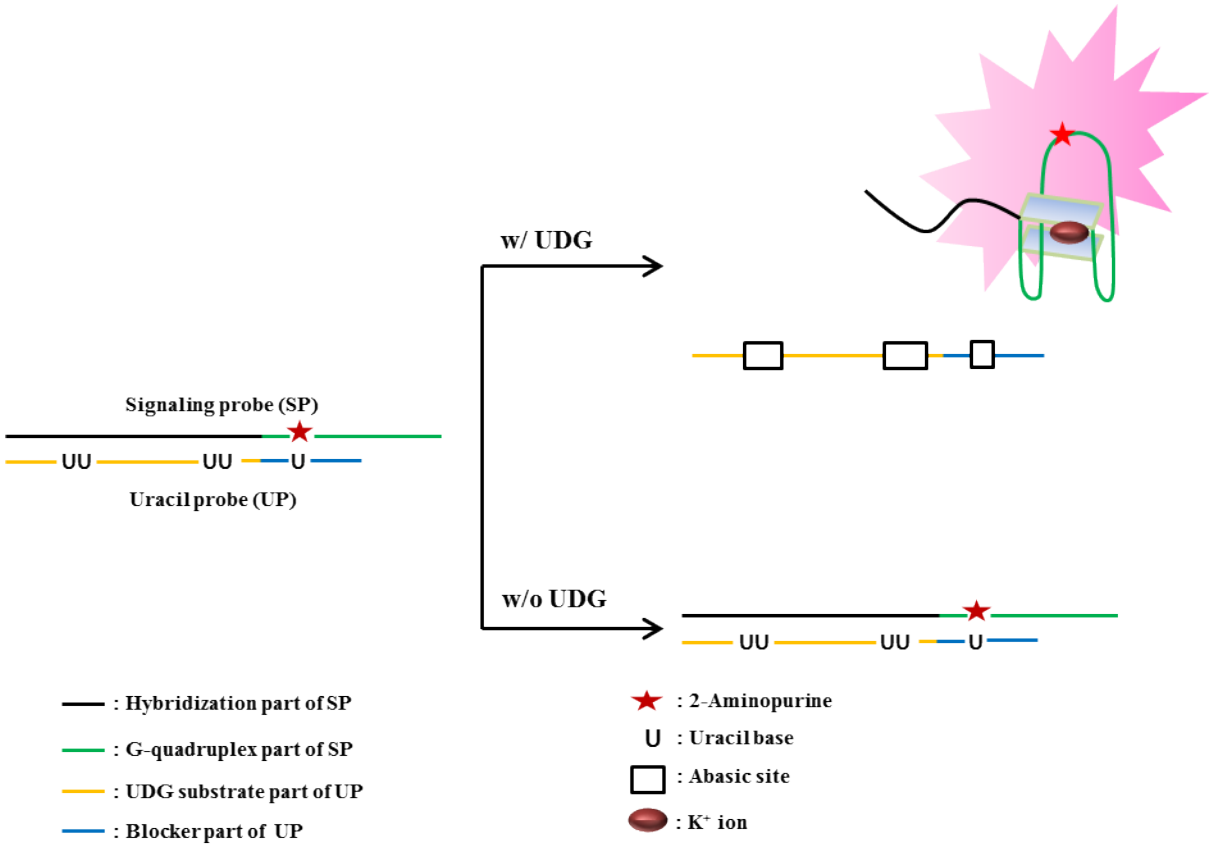


Fig. 1 UDG activity assay based on the excision induced G-quadruplex formation. (a) Fluorescence emission spectra of 2-AP in the UDG activity assay (1: SP1-UP3 duplex without UDG, 2: SP1-UP3 duplex with UDG, 3: SP1-TP3 duplex with UDG). (b) Polyacrylamide gel electrophoresis image of the resulting products after UDG treatment (lane M: DNA size marker, 1: SP1-UP3 duplex without UDG, 2: SP1-UP3 duplex with UDG, 3: SP1-TP3 duplex with UDG). The final concentrations of the SP1-UP3 duplex, SP1-TP3 duplex, and UDG are 500 nM, 500 nM, and 5 U/ml, respectively.

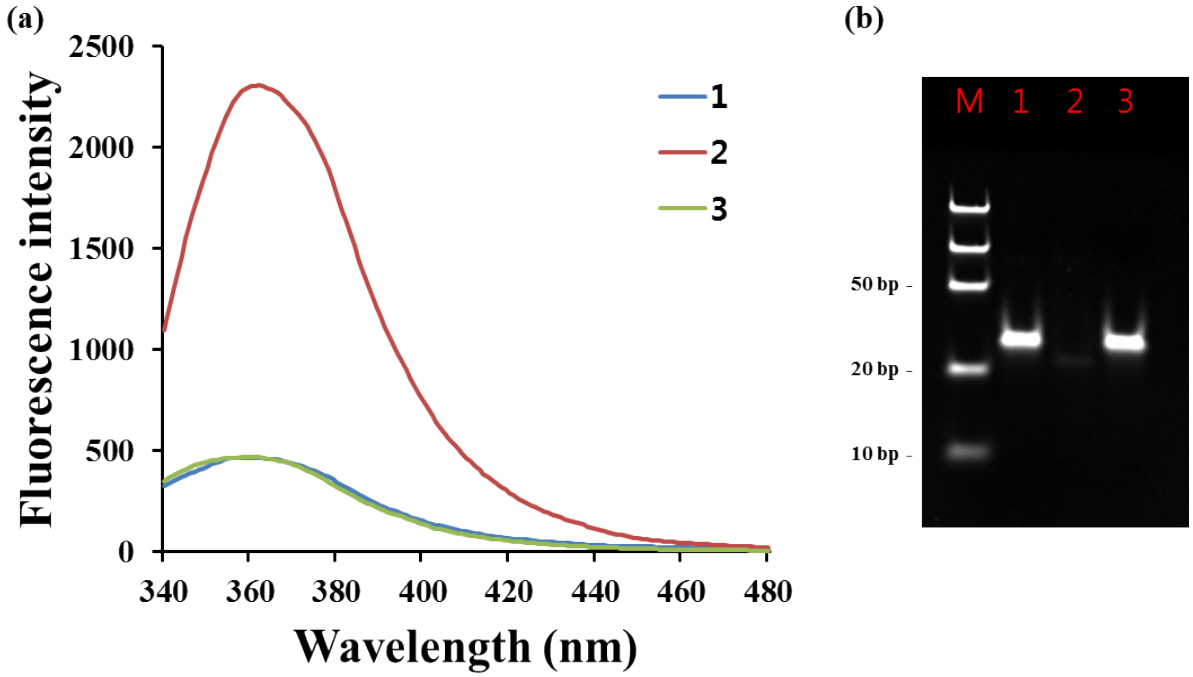


Fig. 2 Sensitivity of the UDG activity assay. (a) Fluorescence emission spectra from 2-AP after treatment with UDG at varying concentrations. (b) Fluorescence intensities at 360 nm from 2-AP in the presence of UDG at varying concentrations. Inset: linear relationship between fluorescence intensity and UDG concentration. The final concentration of SP1-UP3 duplex is 500 nM.

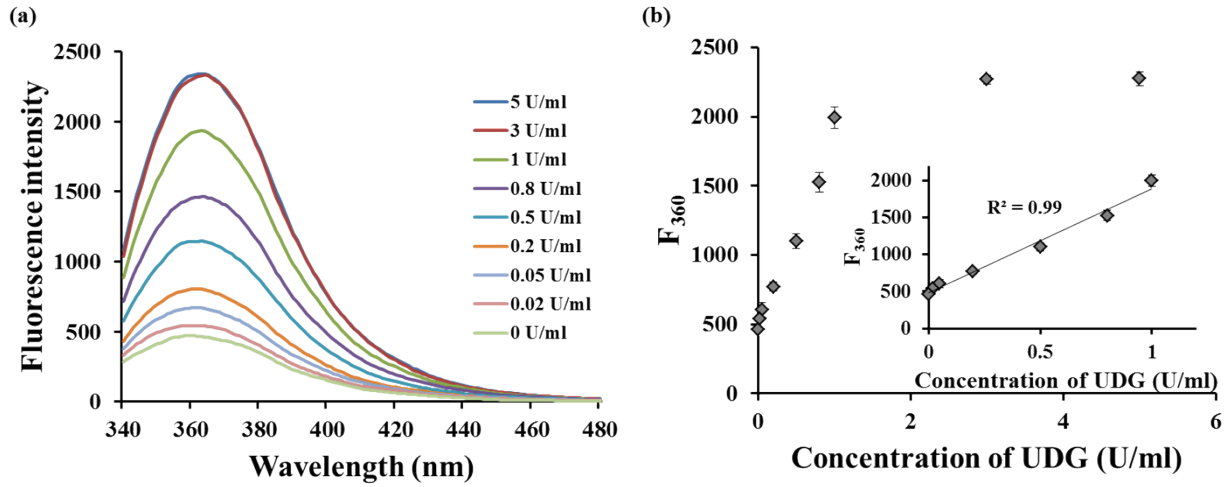


Fig. 3 Specificity of the UDG activity assay. Fluorescence responses after treatment with UDG (5 U/ml), other DNA repair enzymes (50 U/ml), and nuclease (50 U/ml). The final concentration of the SP1-UP3 duplex is 500 nM.

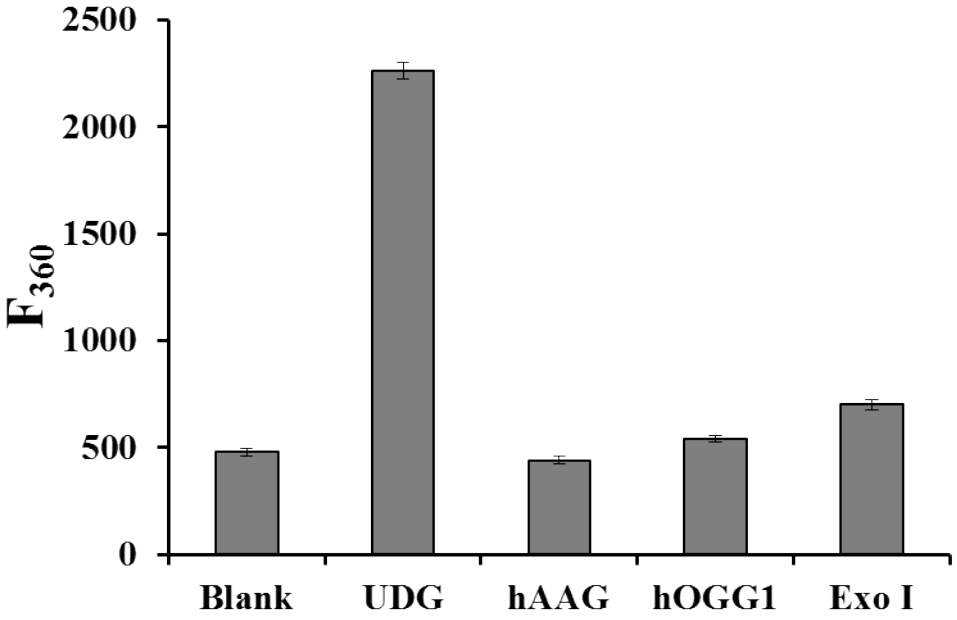


Fig. 4 Screening assay for the UDG inhibitor. Fluorescence intensities at 360 nm from 2-AP in the presence of UGI at increasing concentrations (0, 0.15, 0.25, 0.35, 0.5, 0.75, 1, 2, and 3 U/ml). The final concentrations of the SP1-UP3 duplex and UDG are 500 nM and 5 U/ml, respectively.

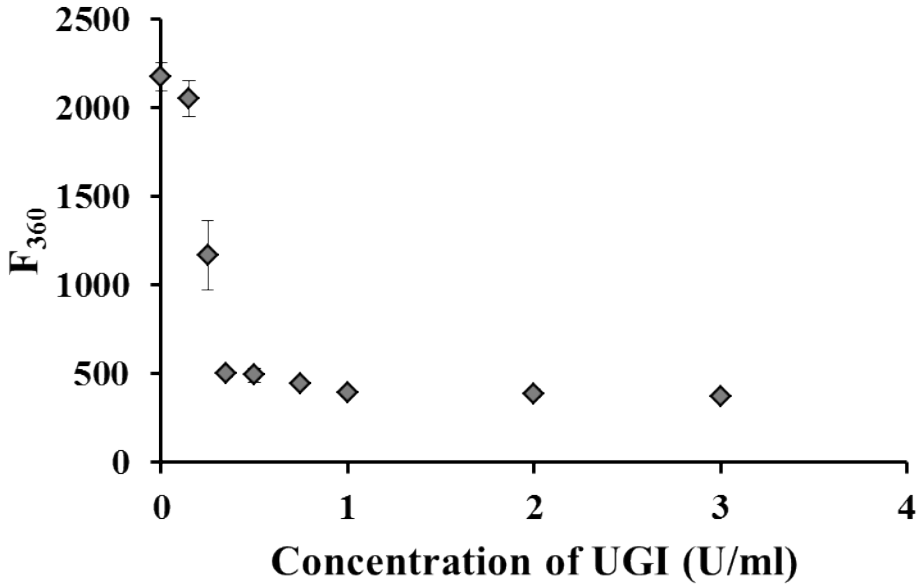


Table S1 DNA sequences studied in this work.

Strand name	DNA sequence (5' → 3')	Length of blocker
SP1	GAA ATT GTT AAG T GGG 2APGG GTG GGT GGG	
SP1A	GAA ATT GTT AAG T GGG AGG GTG GGT GGG	
TP3	CCC TCC CAC TTA ACA ATT TC	7
UP1	CUC CCA CUU AAC AAU UTC	5
UP2	CCU CCC ACU UAA CAA UUT C	6
UP3	CCC UCC CAC UUA ACA AUU TC	7
UP4	ACC CUC CCA CUU AAC AAU UTC	8
UP5	CAC CCU CCC ACU UAA CAA UUT C	9
UP6	CCA CCC UCC CAC UUA ACA AUU TC	10

G-quadruplex sequence is in green, blocker sequence is in blue, and 2-AP is in red.

Fig. S1 Time dependent fluorescence intensities at 360 nm from 2-AP of SP1 in the absence (blue) and presence (red) of K^+ ions. The final concentrations of SP1 and K^+ ions are 500 nM and 10 mM, respectively.

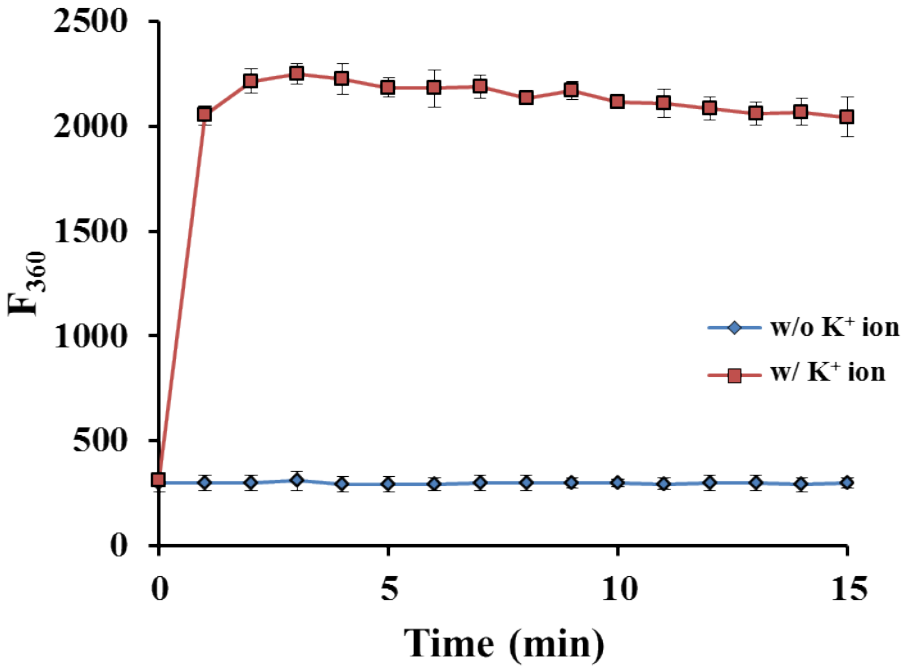
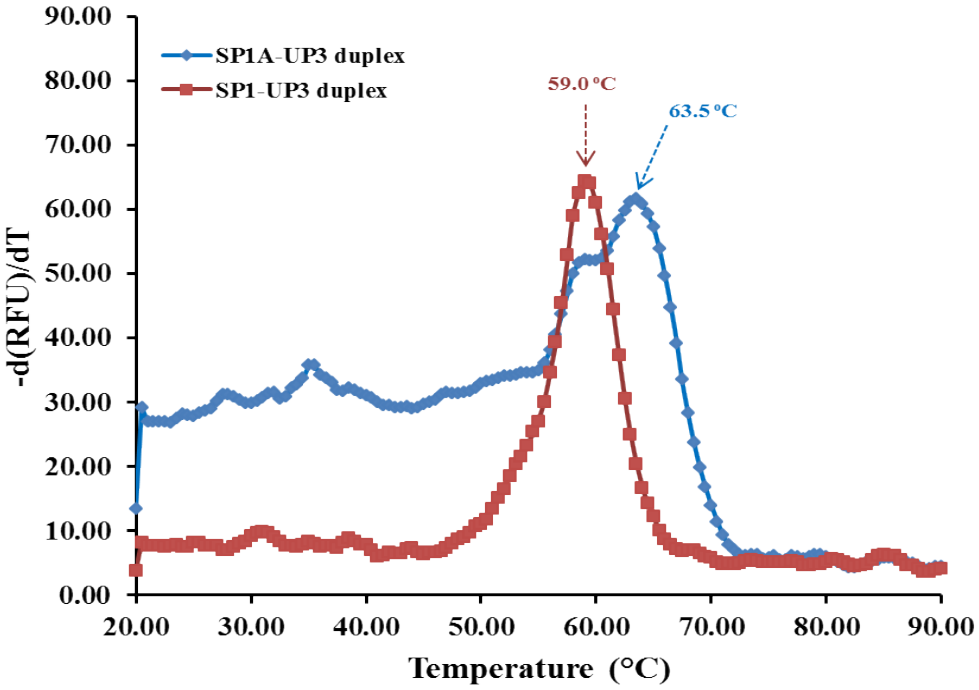
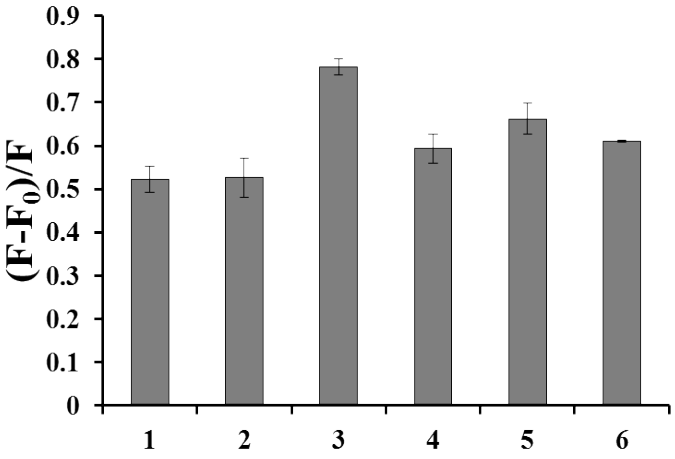


Fig. S2 Melting curve analysis of SP1A-UP3 duplex and SP1-UP3 duplex. The final concentrations of the SP1A-UP3 duplex and SP1-UP3 duplex are 500 nM and 500 nM, respectively.



The data in Fig. S2 shows that SP1-UP3 duplex containing 2-AP is less stable than SP1A-UP3 duplex without 2-AP, indicating that modification of 2-AP slightly reduces the stability of G-quadruplex probe.[6] However, the degree of destabilization is not significant and the lowered stability would not deteriorate the reliability of our strategy, considering that the assay is conducted at 37 °C.

Fig. S3 Optimization of the blocker length to achieve the lowest background signal. The degree of fluorescence quenching is defined as $(F-F_0)/F$, where F_0 and F are the fluorescence emission intensities at 360 nm from the 2-AP of the SP1-UP# duplex and SP1, respectively. The final concentrations of the SP1-UP# duplex, SP1, and K^+ ions are 500 nM, 500 nM, and 10 mM, respectively.



Number	SP1-UP# duplex	Length of blocker
1	SP1-UP1 duplex	5
2	SP1-UP2 duplex	6
3	SP1-UP3 duplex	7
4	SP1-UP4 duplex	8
5	SP1-UP5 duplex	9
6	SP1-UP6 duplex	10

Fig. S4 Optimization of the concentration of K^+ ions for the efficient formation of G-quadruplex. (a) Fluorescence emission intensities at 360 nm from the 2-AP of the SP1-UP3 duplex (blue bar) and SP1 (red bar) at varying concentrations of K^+ ions. (b) $(F-F_0)/F_0$ is defined as the degree of signal enhancement at varying concentrations of K^+ ions, where F_0 and F are the fluorescence emission intensities at 360 nm from the 2-AP of the SP1-UP3 duplex and SP1, respectively. The final concentrations of the SP1-UP3 duplex and SP1 are 500 nM and 500 nM, respectively.

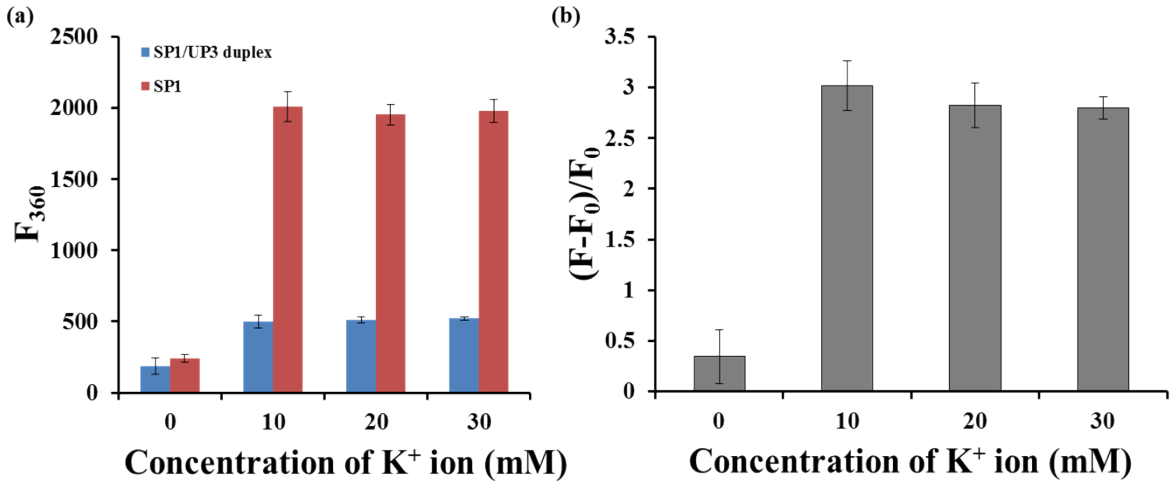
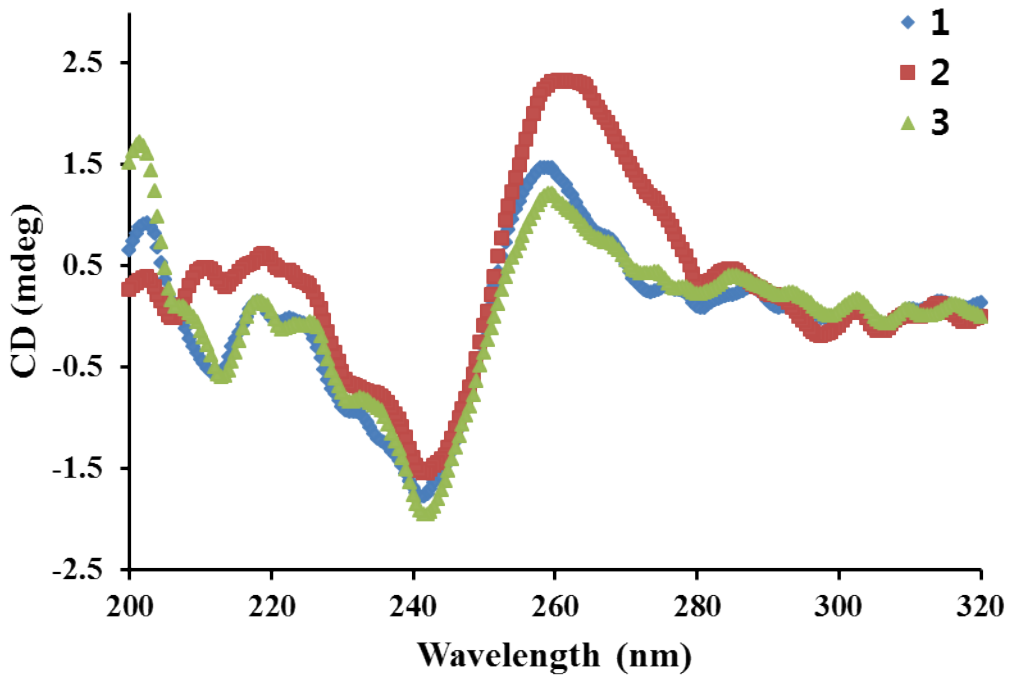
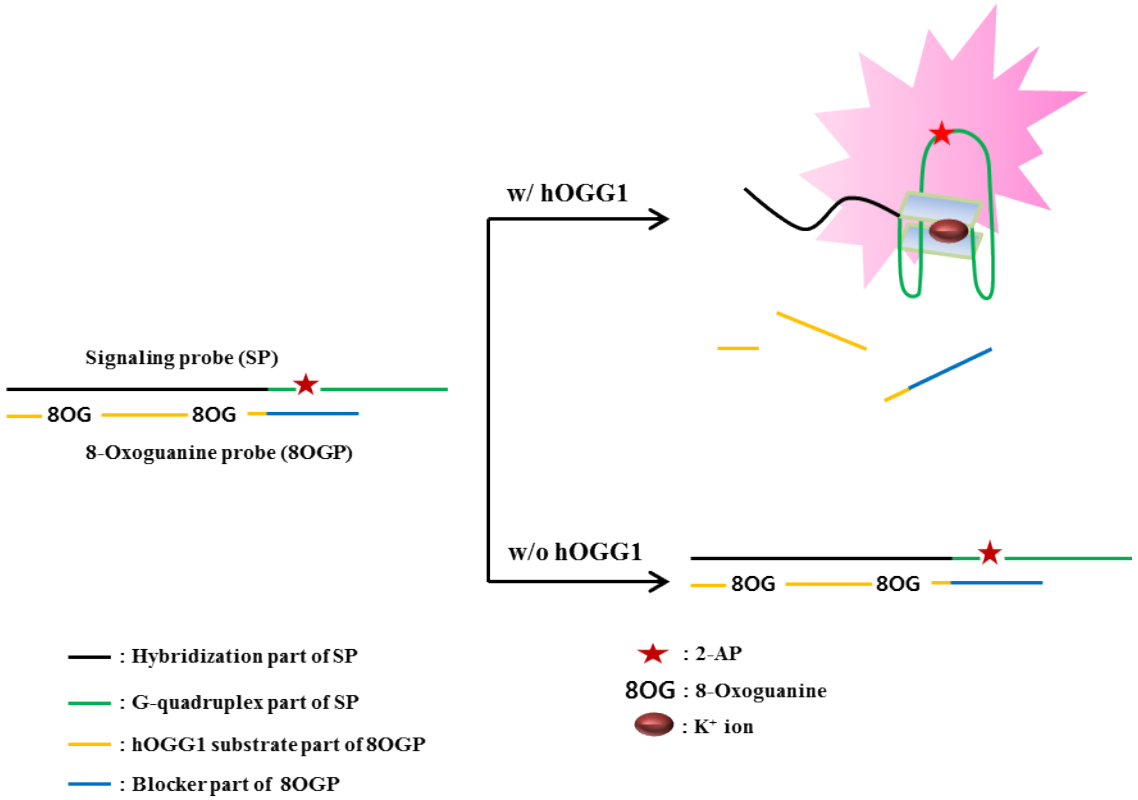


Fig. S5 CD spectra of G-quadruplex probes after treatment with UDG (1: SP1-UP3 duplex without UDG, 2: SP1-UP3 duplex with UDG, 3: SP1-TP3 duplex with UDG). The final concentrations of the SP1-UP3 duplex, SP1-TP3 duplex, and UDG are 4 μM, 4 μM, and 20 U/ml, respectively.



UDG treatment led to the dissociation of SP1-UP3 duplex and subsequent formation of G-quadruplex, consequently producing a strong positive peak at near 265 nm which is characteristic of a parallel G-quadruplex structure together with a negative peak at near 240 nm.[7] In contrast, a weak peak near 265 nm is observed from the same SP1-UP3 duplex without UDG treatment and SP1-TP3 duplex with UDG treatment, confirming that the formation of G-quadruplex structure is induced exclusively through the specific catalytic activity of UDG.[8]

Fig. S6 Schematic illustration of the hOGG1 activity assay based on the excision induced G-quadruplex formation with significant fluorescence enhancement of 2-AP.



To support the universal applicability of our strategy, we herein describe the assay for the activity of another BER enzyme, human 8-hydroxyguanine glycosylase (hOGG1), which specifically recognizes and cuts off 8-oxoguanine (8OG), an oxidative damage product, in duplex DNA. As illustrated in Fig. S6, the duplex DNA is composed of signaling probe (SP) and 8-oxoguanine probe (8OGP) that are rationally designed to achieve sensitive assay for the hOGG1 activity. The SP consists of a hybridization part (black region) complementary to the hOGG1 substrate part of the 8OGP and the G-quadruplex part (green region with red star) containing the 2-AP in a G-rich sequence. The 8OGP consists of the hOGG1 substrate part (yellow region) containing two 8OG residues and the blocker part (blue region) which partially binds to the G-quadruplex part in the SP and prevents the formation of the G-quadruplex. In the absence of hOGG1, the G-rich sequence of the SP remains hybridized with the 8OGP, and its formation of the G-quadruplex is blocked, consequently resulting in a weak

fluorescence emission from 2-AP. However, in the presence of hOGG1, it catalyzes the removal of 8OG bases and cleavage of resulting abasic sites in the 8OGP, leading to the dissociation of the duplex DNA by reducing the stability of the double-stranded state. The blocker part of the 8OGP can no longer cage the G-quadruplex part of the SP, thus facilitating the formation of the G-quadruplex upon interaction with K^+ ions. As a consequence, 2-AP in the G-quadruplex generates a remarkably enhanced fluorescence signal. Based on the enhanced fluorescence, the activity of hOGG1 can be determined.

References

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