Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Spectroscopic Sensing and Quantification of AP-Endonucleases Using Fluorescence-Enhancement by Cis-trans Isomerization of Cyanine Dyes

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1. DNA sequence table

DNA	Sequence
dsDNA-FE top ssDNA	5′Bio - GGT AAG ATG AAG AGA ACA GTG CGT CGG AGA GGA GAG AGA G/idSp/AG GAA GTG TG - Bio3′
dsDNA-FE bottom ssDNA	5′Bio - CAC ACT TCC TTC TCT CTC TCC TC/AmMC6T/ CCG ACG CAC TGT TCT CTT CAT CTT ACC - Bio3′
AP DNA top ssDNA	5′ - CGG AAG CCT AGT ATC GTT AGT CAT CGC CAT G – 3′
AP DNA bottom ssDNA	5′ - AmMC6/ GCG ATG ACT AAC G /idSp/ TA CTA GGC TTC CGA GCC – 3′
Mid-AP top ssDNA	5′Bio - GGT AAG ATG AAG AGA ACA GTG CGT C/idSp/GA GAG GAG AGA GAG AAG GAA GTG TG - Bio3′
No-AP top ssDNA	5′Bio - GGT AAG ATG AAG AGA ACA GTG CGT CGG AGA GGA GAG AGA GAA GGA AGT GTG - Bio3′
One-sided Cy3 bottom ssDNA	5′Bio - CAC ACT TCC TTC TCT CTC TCC TCT CCG ACG CAC TGT TCT CTT CAT C/AmMC6T/T ACC - Bio3′

Table 1.

Cy3, a fluorescent dye, was labelled at amine-modified sites denoted by /AmMO/ or /AmMC6/.

2. Supplementary Figures

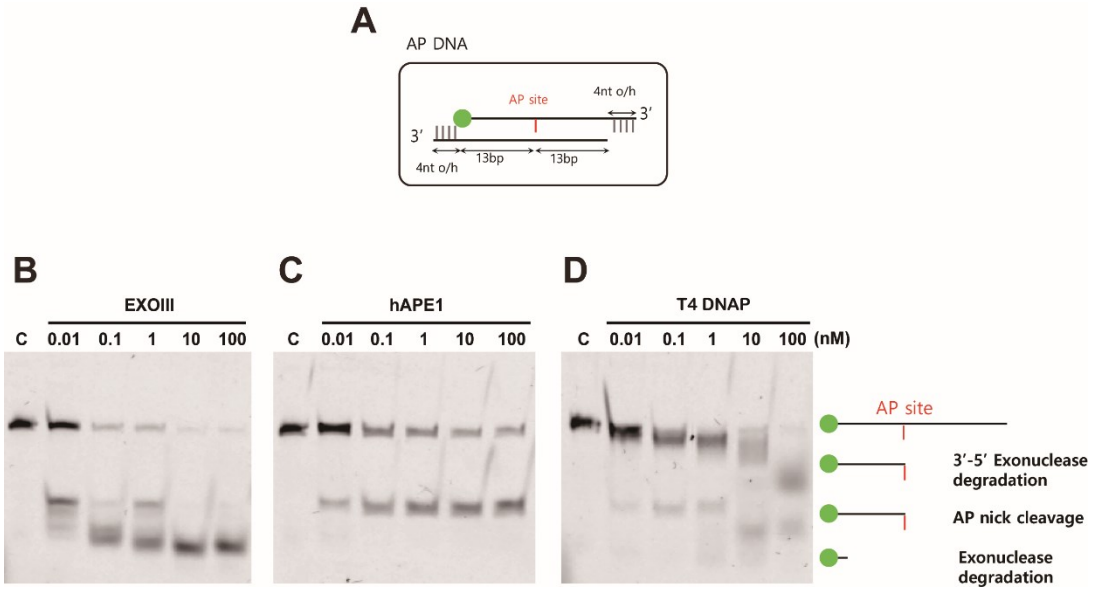

Figure S1. Degradation gel assay showing the activity of three enzymes. (A) Schematic illustration of the dsDNA substrate, in which the 3′ overhang strands prevents AP endonucleases (e.g., ExoIII and hAPE1) from initiating exonucleolytic degradation in the 3′→5′ direction since ExoIII and hAPE1 cannot bind to ssDNA. (B) Degradation assay for ExoIII. (C) Degradation assay for hAPE1. (D) Degradation assay for T4 DNAP, in which the domain of 3′→5′ exonuclease preferentially digests a 3′ single stranded overhang, unlike ExoIII and hAPE1.

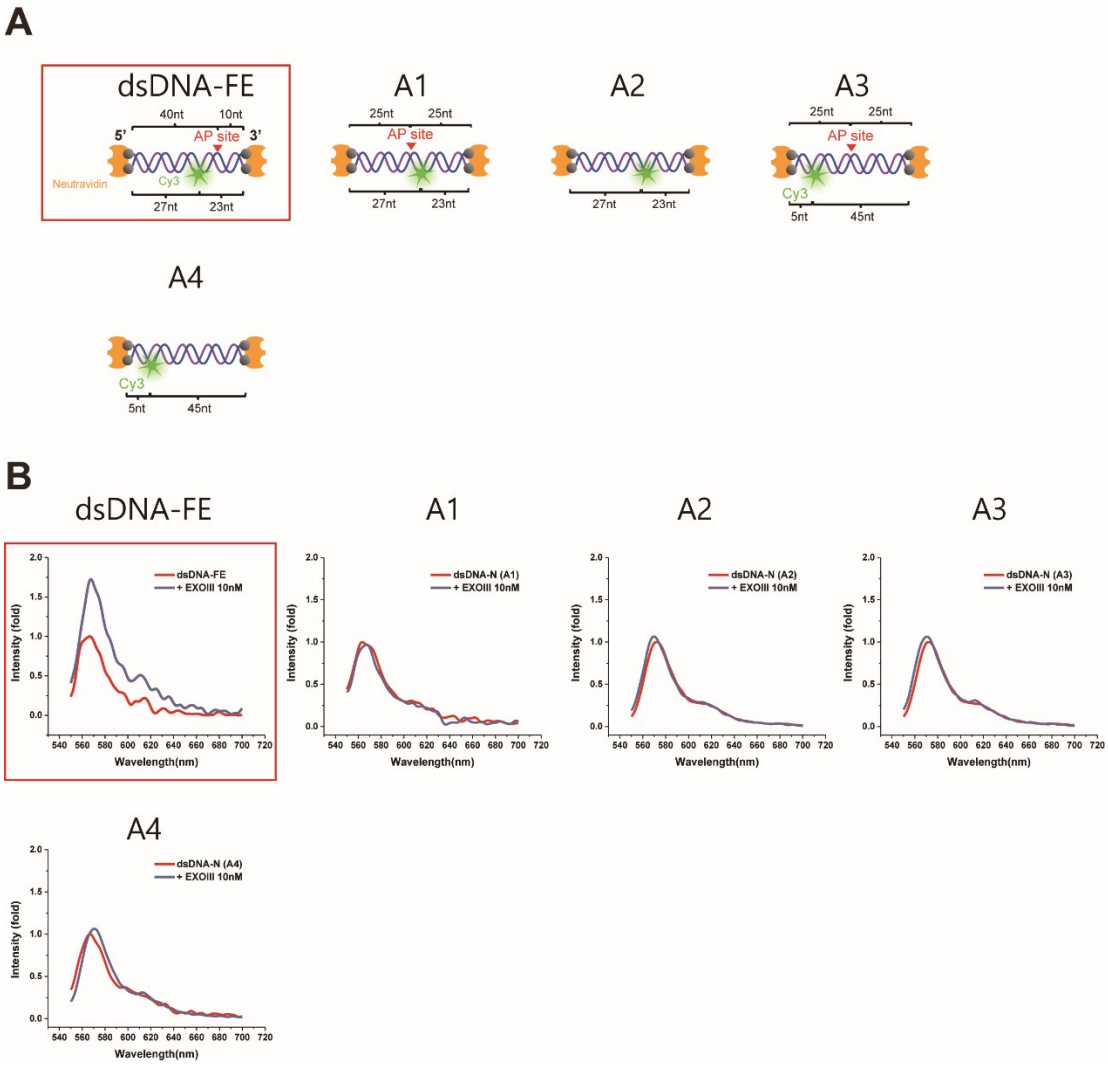

Figure S2. Screening for various DNA probes to attain a stable FE signal, even after the reaction is completed by the activity of AP endonucleases. (A, B) dsDNA-FE, a stable FE since the Cy3-carrying strand becomes ssDNA after the reaction; A1, no FE because the region around Cy3 is not degraded and remains on dsDNA after the reaction; A2, no FE due to the lack of an AP site, which is a binding site for AP endonucleases for degradation (also see the main Figure 3); A3, no FE because AP endonuclease could not digest the region of Cy3, presumably due to steric hindrance from the terminal NeutrAvidin and the enzyme footprint; A4, the same as in A2.

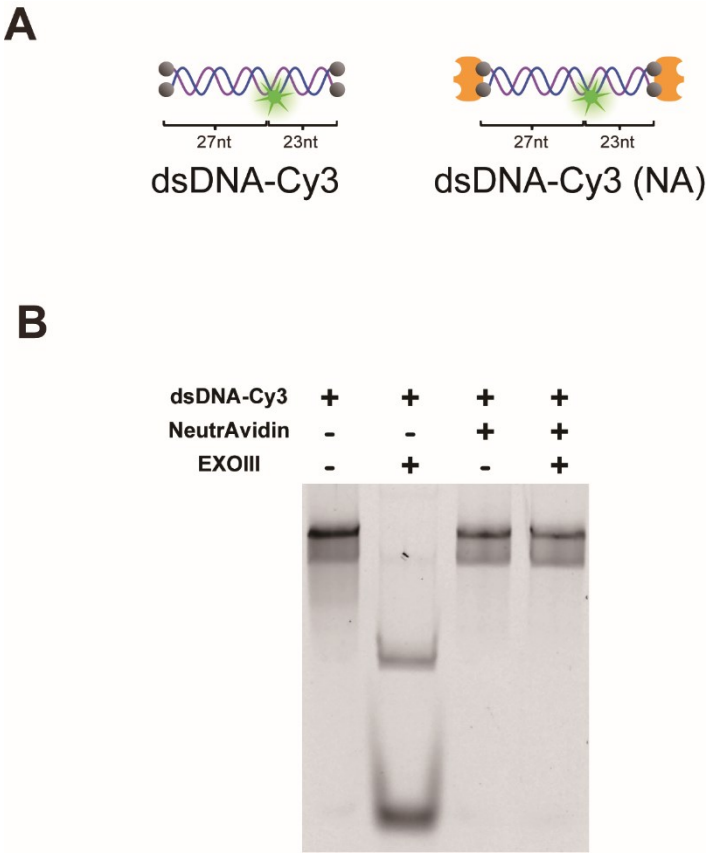

Figure S3. (A-B) dsDNA-FE probe is protected from both 3′→5′ or 5′→3′ exonucleases since it is not digested by exonucleases due to the NeutrAvidin caps present at both ends of dsDNA.

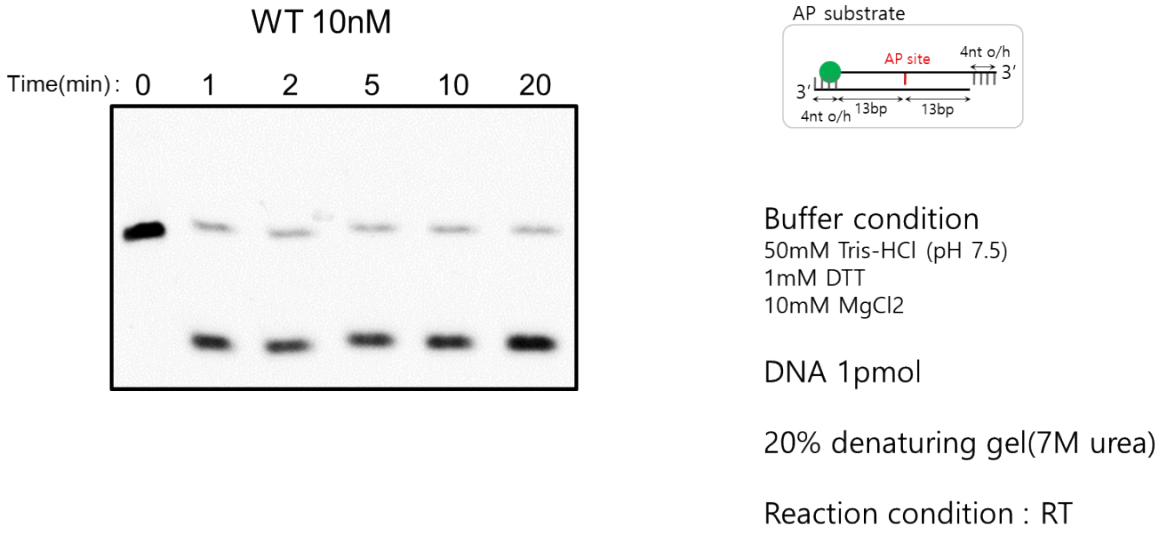

Figure S4. Enzymatic kinetics of hAPE1. 1 min after the reaction (below) is enough to degrade all the substrate at 10 nM hAPE1. We performed the enzymatic fluorescent reaction for 10 min, so that all the reaction was done and we measured the fluorescence signals.