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Supporting Information

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Direct Fluorescence Monitoring of DNA Base Excision Repair**

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Materials and reagents. The nucleoside phosphoramidites (A, U, T, S) and 3'-phosphate CPG column were purchased from Glen Research. Solvents and reagents were purchased from Fisher Scientific, Aldrich or ACROS unless otherwise noted. UDG, UDG inhibitor (UGI), *Afu* UDG, 10x UDG reaction buffer (200 mM Tris-HCl, 10 mM EDTA, 10 mM dithiothreitol), 10x ThermoPol II (Mg-free) reaction buffer (200 mM Tris-HCl, 100 mM $(NH_4)_2SO_4$, 100 mM KCl, 1 % Triton X-100) were purchased from New England Biolabs.

Oligodeoxynucleotide synthesis. 5'-DMT 3'-cyanoethyl α-pyrene phosphoramidite (**Y**) was prepared according to published methods.[1, 2] Probe **1**-**9** and reference compound **10** was synthesized on a Applied Biosystems 394 DNA/RNA synthesizer, using 3'-phosphate CPG column on 1 μmol scale with DMT-off method. Coupling of each monomer used standard 3' to 5' cyanoethyl phosphoramidite chemistry with extended coupling time (999 s). Oligomers were deprotected by saturated NH_4OH for 17 hour at 55 °C. Purification was carried out utilizing a Shimadzu Series HPLC with an Alltech C5 column with acetonitrile and TEAA buffer (100 mM, pH 7.4) as eluents. The identities were confirmed by MALDI-TOF-MASS. The concentrations of oligonucleotides were determined by pyrene UV absorbance at 342 nm (ε = 47000 cm^{-1} M^{-1}).[3]

Quantum yield measurements. Fluorescence studies were performed on a Jobin Yvon-Spex Fluorolog 3 spectrophotometer equipped with a thermostat accurate to 0.1 °C. The emission spectra were obtained by exciting the samples at 340 nm and scanning the emission from 350 to 500 nm with a step of 1 nm. Quinine sulfate was used as a fluorescence quantum yield standard.[4] Quenching efficiency was determined by dividing the fluorescence intensity of the each probe by the fluorescence intensity of the reference compound **10**, multiplying the result by 100 and then subtracting the result from 100.

In vitro enzymatic fluorescence assay. The probes were dissolved in water to prepare a stock solution of 10 uM. The samples were incubated at 37.0 °C for UDG in 1x UDG reaction buffer and at 65.0 °C for *Afu* UDG in 1x ThermoPol II (Mg-free) reaction buffer. When the fluorescence intensity was stable, the UDG or *Afu* UDG was added to the solution. After mixing rapidly, the fluorescence spectra or the time curves of the fluorescence intensity of solutions were recorded.

Bacterial culture and imaging. An E. coli strain lacking the UDG enzyme (BW310DE3) was transformed with pET28a-afung plasmid using standard procedures (Sambrook J, Maniatis T,

1989, Molecular Clong, A Laboratory Manual, Second Ed., Cold Spring Harbor Press, Cold Spring Harbor). Growth medium (Luria-Bertani) and glass containers were sterized by autoclaving at 120 °C for 20 min. *E. coli* BW310DE3 (pET28a-afung) inoculated into LB medium containing 34 mg/ml kanamycin (LB-kan) and was grown 12-16 hours at 37 °C as a starter culture. The starter culture was diluted 1:100 with fresh LB-kan medium and was grown at 37 °C until OD_{600} of the culture reached 0.6. Isopropyl-1-thio-β-D-galactopyranoside (IPTG) was then added to a final concentration of 1 mM, and the culture was incubated for an additional 5 h at 30 °C. Aliquots (10 – 20 mL) were centrifuged and the supernatant decanted. Pellets were washed with water and resuspended in 1x ThermoPol II (Mg-free) reaction buffer (20 mM Tris-HCl, 10 mM$(NH_4)_2SO_4$, 10 mM KCl, 0.1% Triton X-100) (+ IPTG bacteria). The cells without addition of IPTG was also harvested and resuspended in 1x ThermoPol II (Mg-free) reaction buffer buffer (- IPTG bacteria). *Escherichia coli* (BW(310)DE3udg(-)) cells were cultured with LB medium, and harvested and resuspended in 1x ThermoPol II (Mg-free) reaction buffer. These bacteria suspensions were used as a stock solution. Probes were added (final concentration: [probe] = 5 μM, [bacteria, OD_{600}] = 0.31) and the samples were incubated at 20 °C or 65 °C without shaking and protected from light. Aliquots of incubated bacteria were spotted on cover slides without washing or fixation. Samples were imaged under a Nikon Eclipse 80i epifluorescence microscope equipped with 100X objective. Images were captured using a QIclick digital CCD camera and QCapture Pro 7 Imaging software with excitation 330–380 nm and emission > 420 nm.

References

(1) R. X.-F. Ren, N. C. Chaudhuri, P. L. Paris, S. R. IV and E. T. Kool, *J. Am. Chem. Soc.*, **1996**, *118*, 7671-7678.
(2) J. Gao, S. Watanabe and E. T. Kool, *J. Am. Chem. Soc.*, **2004**, *126*, 12748-12749.
(3) J. Gao, C. Strassler, D. Tahmassebi and E. T. Kool, *J. Am. Chem. Soc.*, **2002**, *124*, 11590-11591
(4) J. R. Lakowicz, Principles of Fluorescence Spectroscopy, 3rd ed., Springer, New York, **2006**.

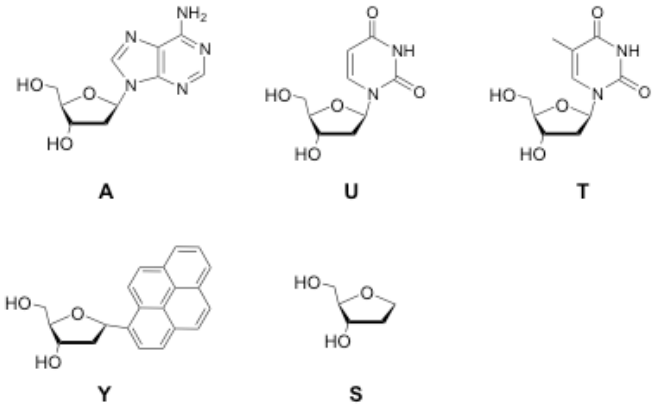
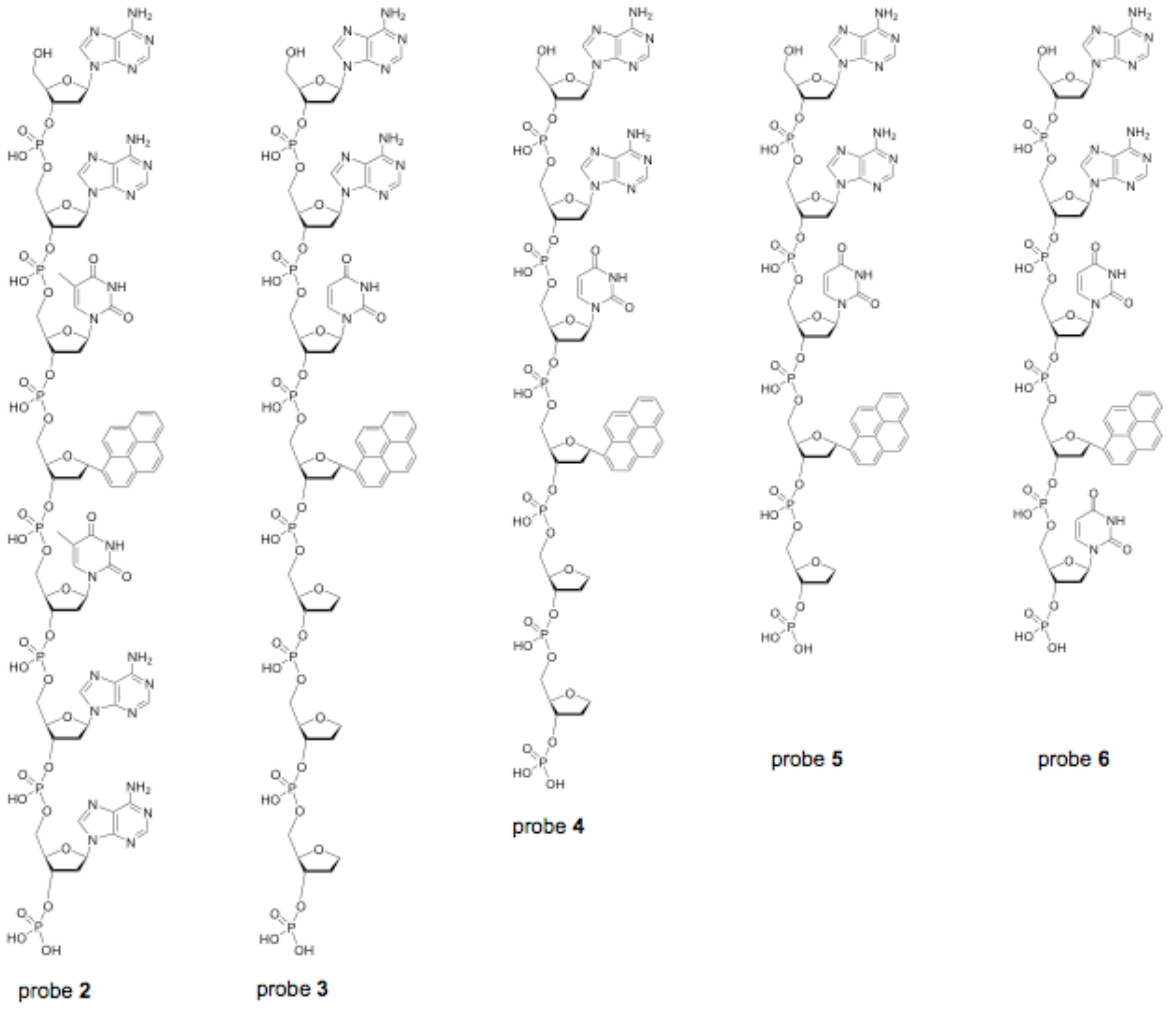


Figure S1. Natural and non-natural monomers employed as components of the probe sequences.



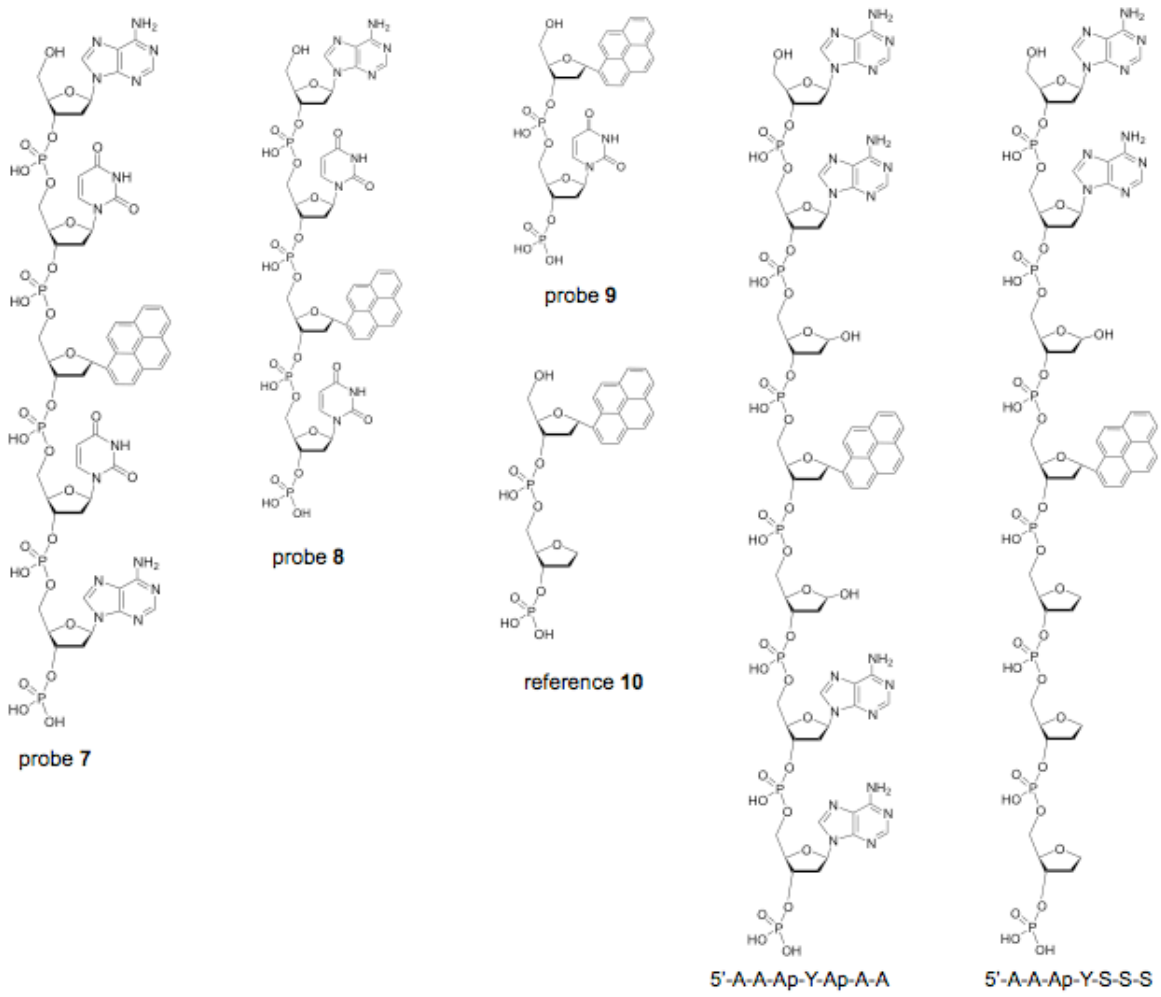
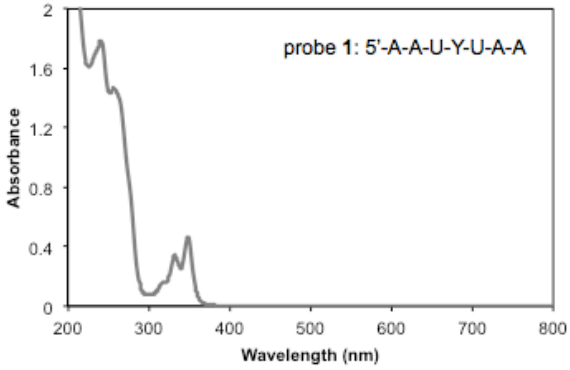


Figure S2. Chemical Structure of probes **1**-**9**, reference **10**, as well as enzymatic reaction products 5'-A-A-Ap-Y-Ap-A-A and 5'-A-A-Ap-Y-S-S-S.

Table S1. MALDI-TOF mass characterization of probes **1**-**9** and reference **10**.

Oligomer	Sequence	Calc'd Mass	Found
probe **1**	5'-A-A-U-Y-U-A-A	2231.5	2231.6
probe **2**	5'-A-A-T-Y-T-A-A	2258.9	2258.9
probe **3**	5'-A-A-U-Y-S-S-S	1855.2	1854.2
probe **4**	5'-A-A-U-Y-S-S	1675.1	1675.6
probe **5**	5'-A-A-U-Y-S	1495.0	1495.5
probe **6**	5'-A-A-U-Y-U	1605.1	1606.9
probe **7**	5'-A-U-Y-U-A	1605.1	1606.7
probe **8**	5'-A-U-Y-U	1292.0	1291.2
probe **9**	5'-Y-U	688.5	688.3
reference **10**	5'-Y-S	578.4	578.7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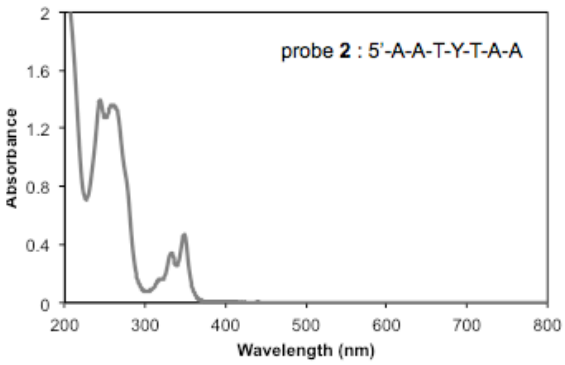
Absorbance
Wavelength (nm)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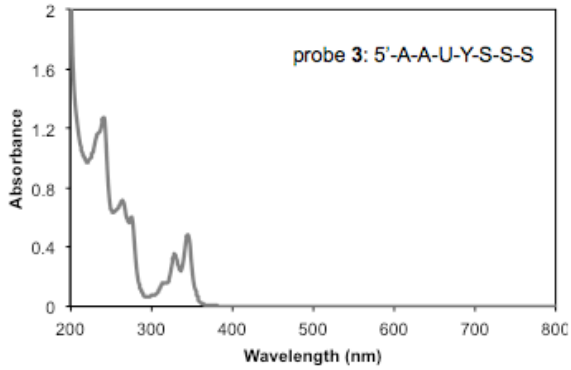
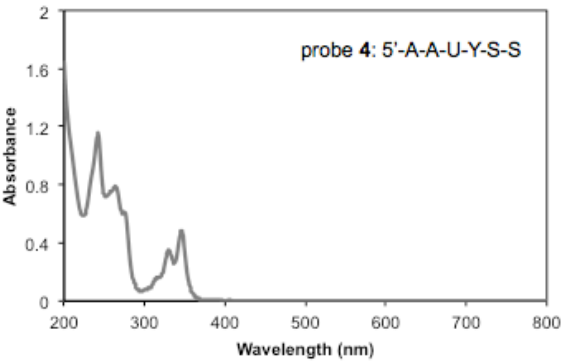
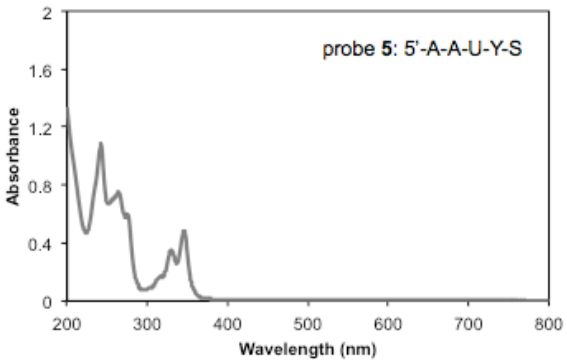
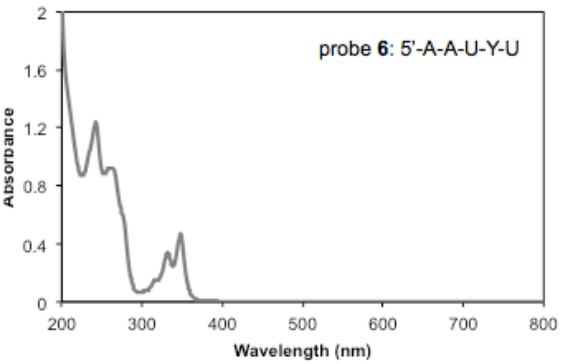
Absorbance
Wavelength (nm)


probe 3: 5'-A-A-U-Y-S-S-S
Absorbance
Wavelength (nm)


probe 4: 5'-A-A-U-Y-S-S
Absorbance
Wavelength (nm)


probe 5: 5'-A-A-U-Y-S
Absorbance
Wavelength (nm)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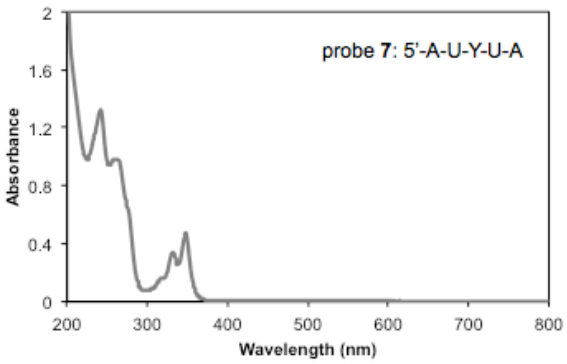
Absorbance
Wavelength (nm)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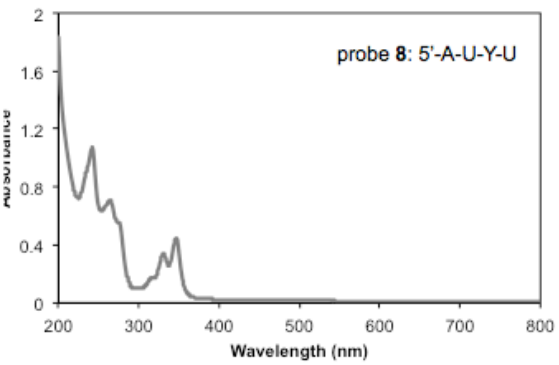
Absorbance
Wavelength (nm)


probe 8: 5'-A-U-Y-U
Wavelength (nm)

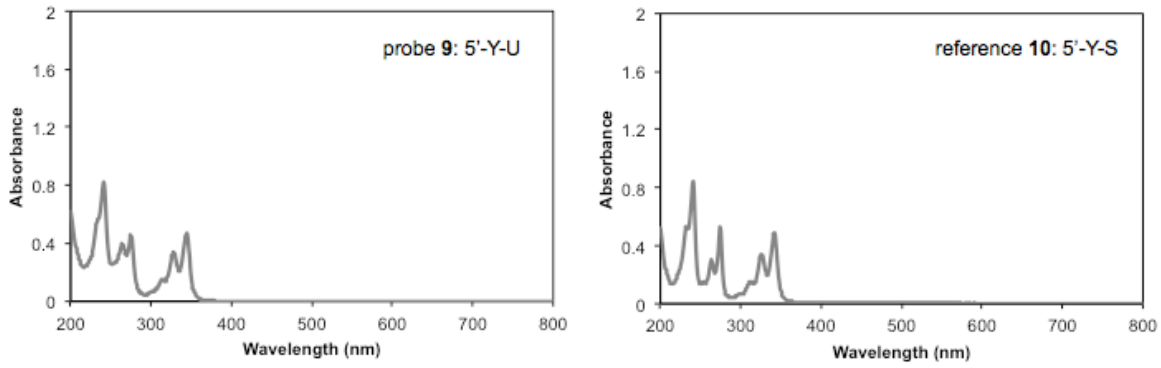


Figure S3. UV-vis absorption spectra of probes **1**-**9** and reference **10**. [probe] = 10 μM, in water

Table S2. Quantum yields (QY) and quenching effect of **1**-**9** and reference **10** in water.

Compound	Sequence	QY	Quench Effect
probe **1**	5'-A-A-U-Y-U-A-A	0.004	98.7 %
probe **2**	5'-A-A-T-Y-T-A-A	0.006	98.0 %
probe **3**	5'-A-A-U-Y-S-S-S	0.030	90.3 %
probe **4**	5'-A-A-U-Y-S-S	0.032	89.7 %
probe **5**	5'-A-A-U-Y-S	0.032	89.7 %
probe **6**	5'-A-A-U-Y-U	0.004	98.7 %
probe **7**	5'-A-U-Y-U-A	0.004	98.7 %
probe **8**	5'-A-U-Y-U	0.005	98.4 %
probe **9**	5'-Y-U	0.013	95.8 %
reference **10**	5'-Y-S	0.310	---

Table S3. Apparent relative rates of enzymatic cleavage ($k_{app, rel}$) from initial velocities of probes. [probe] = 400 nM; [UDG] = 1 unit/mL at 37 °C.

Compound	Sequence	k_{app}
probe **1**	5'-A-A-U-Y-U-A-A	258
probe **3**	5'-A-A-U-Y-S-S-S	480
probe **4**	5'-A-A-U-Y-S-S	536
probe **5**	5'-A-A-U-Y-S	294
probe **6**	5'-A-A-U-Y-U	2
probe **7**	5'-A-U-Y-U-A	33
probe **8**	5'-A-U-Y-U	1

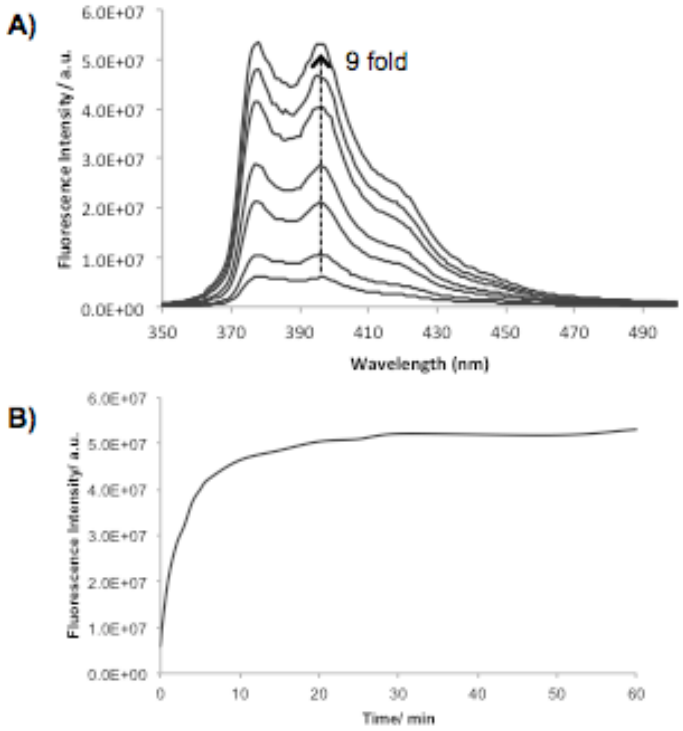


Figure S4. In vitro enzymatic assay of probe **5** incubated with *E. coli* UDG. (A) Fluorescence spectra over a 60-min timecourse (excitation 340 nm). (B) Increase in emission over time at 395 nm. [probe **5**] = 400 nM; [UDG] = 1 unit/mL at pH 8.0, 37 °C.

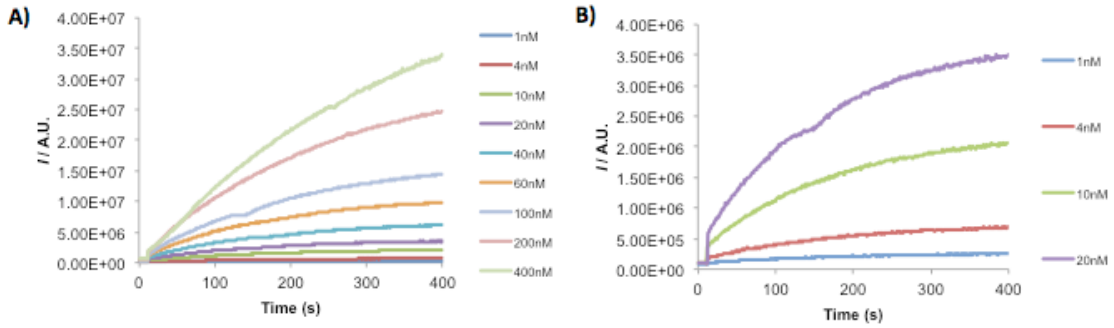


Figure S5. In vitro enzymatic assay of probe **1** incubated with *E. coli* UDG. (A, B) Fluorescence spectra probe **1** (1 to 400 nM) with UDG 1 unit/mL at pH 8.0, 37 °C

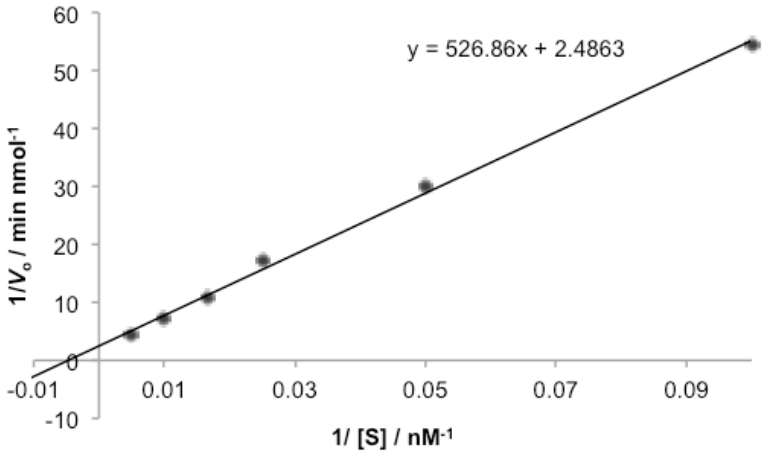


Figure S6. Lineweaver–Burk plot of reciprocals of initial rates (V_o) versus probe **1** concentration for the determination of kinetic parameters K_M and V_{max} of UDG assay. From the linear fit of the data, we determined K_M = 212 nM, V_{max}= 0.40 nmol min^{-1}, k_{cat} = 2062 min^{-1}, k_{cat}/K_M = 9.7 nM^{-1} min^{-1}, y = 528.86x + 2.4863. Reaction conditions: [probe **1**] = 10, 20, 40, 60, 100, 200 nM, [UDG] = 1 unit/mL at 37 °C.

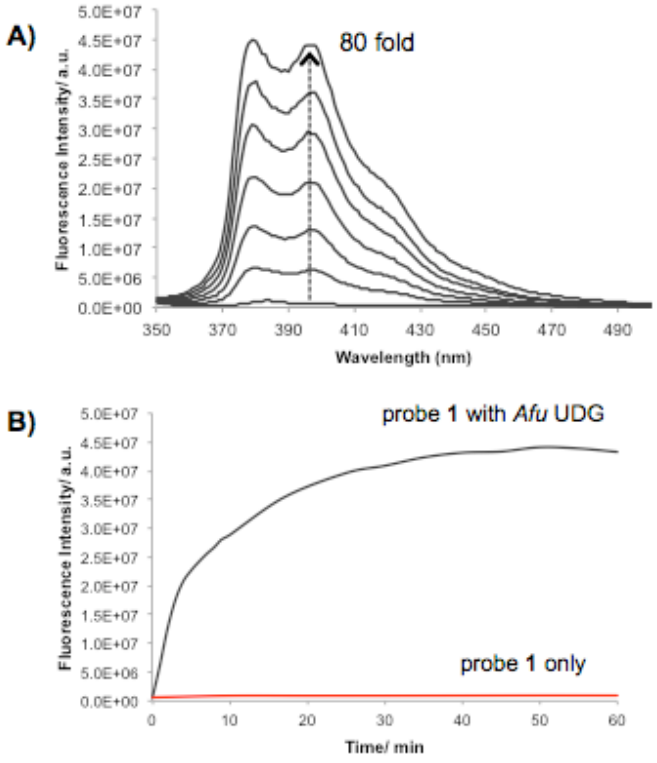


Figure S7. In vitro enzymatic assay of probe **1** incubated with *E. coli Afu* UDG. (A) Fluorescence spectra over a 60-min timecourse (excitation 340 nm). (B) Increase in emission over time at 395 nm. [probe **1**] = 400 nM; [*Afu* UDG] = 1 unit/mL at 65 °C (pH 7.4 @ 25 °C).

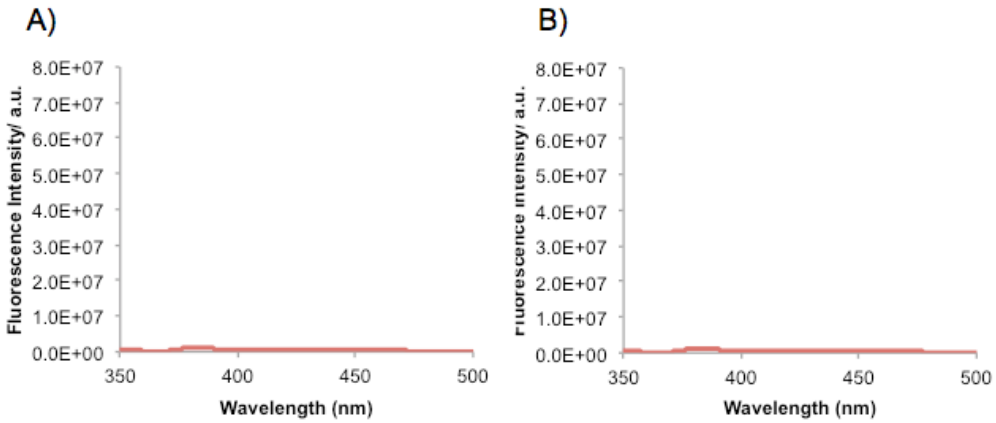


Figure S8. (A,B) Control experiments: Spectra of bacterial solutions diluted tenfold (excitation 340 nm). (A) Probe **1** with bacteria expressing *Afu* UDG (+ IPTG), incubated with probe for 1 h (20 °C) showing low signal at short times and low temperature; (B) Control probe **2** with bacteria under the same conditions.

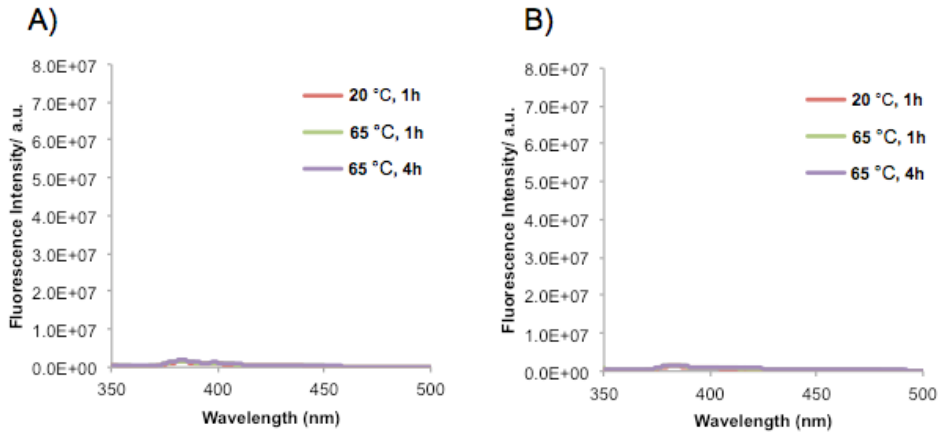


Figure S9. (A,B) Control experiments: Spectra of bacterial solutions diluted tenfold (excitation 340 nm). (A) Probe **1** with control Ec with UDG gene deleted, incubated with probe for 1- 4 h (20 or 65 °C); (B) Control probe **2** with same bacteria under the same conditions.