Electronic Supplementary Information for

A label-free and sensitive fluorescent method for the detection of uracil-DNA glycosylase activity

Jing Tao[a], Panshu Song,[b] Yusuke Sato,[c] Seiichi Nishizawa,[c] Norio Teramae,[c] Aijun Tong[a] and Yu Xiang*[a]

[a] Department of Chemistry, Beijing Key Laboratory for Microanalytical Methods and Instrumentation, Key Laboratory of Bioorganic Phosphorus Chemistry and Chemical Biology (Ministry of Education), Tsinghua University, Beijing 100084, China
Email: xiang-yu@tsinghua.edu.cn
[b] National Institute of Metrology, Beijing 100029, China
[c] Department of Chemistry, Graduate School of Science, Tohoku University, Sendai 980-8578, Japan

Table of Contents

Experimental Section

1. Materials

Recombinant uracil-DNA-glycosylase (UDG) from *E. Coli* was purchased from Sigma-Aldrich (St. Louis, USA) and New England Biolabs (Beverly, USA). The stock solutions of UDG were diluted with a storage buffer consisting of 50% (v/v) glycerol, 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1 mM EDTA, and 1 mM DTT. Uracil glycosylase inhibitor protein (UGI) was obtained from New England Biolabs (Beverly, USA). The BCA protein assay kit and cell lysis buffer were purchased from Beyotime Institute of Biotechnology (Haimen, China). The cell lysis buffer contains 20 mM Tris (pH 7.5), 150 mM NaCl, 1% Triton X-100, sodium pyrophosphate, β-glycerophosphate, EDTA, Na_3VO_4 and leupeptin. Other chemicals were purchased from Alfa Aesar (Tianjin, China). All solutions were prepared using the water from a Milli-Q water system. The following oligonucleotides (from left to right: 5' to 3') were synthesized by Integrated DNA Technologies (Coralville, USA), where the underlined **U** = deoxyuridine and **X** = dSpacer.

DNA-U: TAT ACT C**U**C TAG ATC

DNA-X: GAT CTA G**X**G AGT ATA

DNA-C: GAT CTA GCG AGT ATA

DNA-Hairpin: GAG TAT AAA AAT ATA CTC **U**CT AGA TCA AAA GAT CTA G

DNA-Vac1: TAA TAC TCT ATA CTC **U**CA TGA TCC TAG ACT

DNA-Vac2: AGT CTA GGA TCA TG

DNA-Vac3: GAG TAT AGA GTA TTA

Reaction Buffer: 50 mM NaCl, 0.5 mM EDTA and 50 mM Tris-HCl at pH 7.0

2. Fluorescence measurements

Fluorescence spectra were measured on a JASCO FP-6500 spectrofluorophotometer equipped with a thermoelectrically temperature-controlled cell holder (Japan Spectroscopic Co. Ltd., Tokyo, Japan) using a 6 × 50 mm borosilicate glass culture tube (VWR International LLC., Radnor, USA). Excitation wavelength for ATMND was set at 358 nm.

3. Detection of UDG activity in buffer solution

UDG detection in buffer solution was carried out in a total volume of 300 μL reaction buffer containing 0.5 μM ATMND, 1 μM DNA-U, 1 μM DNA-X and different concentrations of UDG. The mixture was incubated at 37 ^{o}C for 10 min before fluorescence spectra were recorded at 5 ^{o}C.

4. Preparation of MCF-7 and HeLa cell lysates

MCF-7 and HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented

with 10% fetal bovine serum and maintained at 37 °C in an atmosphere of 5% CO_2. Approximately 1×10^6 cells were collected and dispensed in a 1.5 mL centrifuge tube, washed twice with PBS buffer and centrifuged at 2000 rpm for 3 min to discard the buffer. A volume of 100 μL lysis buffer was added into the cell residues and incubated on ice for 30 min, with vortex for 15 s every 5 min. The resulting mixture was centrifuged at 12000 rpm for 20 min at 5 °C. The supernatant could be used immediately for UDG activity assay or frozen at −80 °C for long-term storage.

5. Detection of UDG activity in cell lysates

A total volume of 300 μL reaction buffer containing different volumes of cell lysates (and known amounts of UDG in the standard addition method) was incubated with 0.5 μM ATMND, 1 μM DNA-U and 1 μM DNA-X at 37 °C for 10 min before fluorescence spectra were recorded at 5 °C. As for the inhibition of UDG in the cell lysates, 0.2 U mL^{-1} UGI was incubated with 10 μL cell extract or buffer at 37 °C for 10 min, then 0.5 μM ATMND, 1 μM DNA-U and 1 μM DNA-X were added and the solution was incubated at 37 °C for another 10 min before fluorescence measurement at 5 °C.

6. Detection of UGI Activity

The reaction buffer contains 0.1 U mL^{-1} UDG, 0.2 U mL^{-1} UGI, 0.5 μM ATMND was incubated at 37 °C for 10 min, then 1 μM DNA-U and 1 μM DNA-C were added to the solution to reach a final volume of 300 μL. The mixture was incubated at 37 °C for another 10 min before fluorescence spectra at 5 °C.

7. Detection of gentamicin in UDG inhibition

A total volume of 300 μL reaction buffer containing 0.1 U mL^{-1} UDG and different concentrations of gentamicin was incubated at 37 °C for 10 min. Then 0.5 μM ATMND, 1 μM DNA-U and 1 μM DNA-C were added and the mixture was kept at 37 °C for 10 min before fluorescence spectra were recorded at 5 °C.

Additional Figures

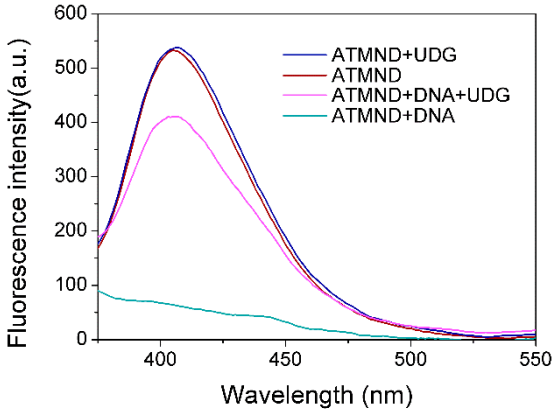

Figure S1. The fluorescence spectra of ATMND alone, ATMND with UDG, ATMND with DNA-U/DNA-X, and ATMND with UDG and DNA-U/DNA-X. Concentrations: ATMND, 0.5 μM; UDG, 0.1 U mL^{-1} UDG; DNA, 1 μM.

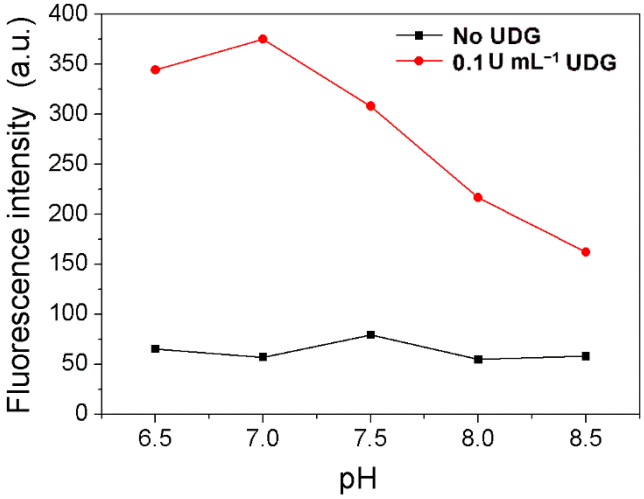

Figure S2. The effect of pH on the fluorescence enhancement of 0.5 μM ATMND and 1 μM DNA-U/DNA-X in the absence and presence of 0.1 U mL^{-1} UDG.

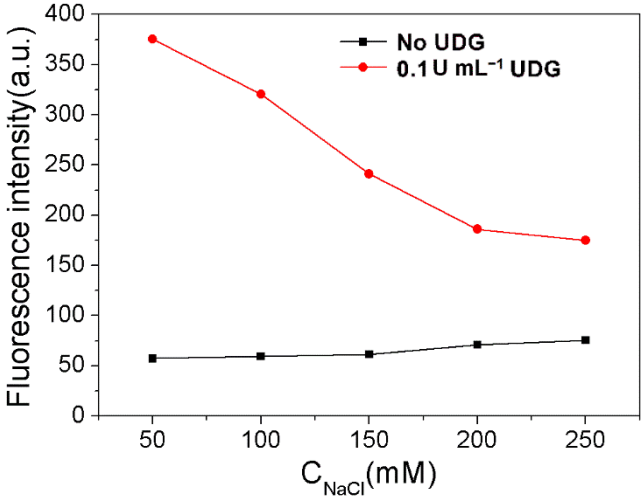

Figure S3. The effect of ionic strength on the fluorescence enhancement of 0.5 μM ATMND and 1 μM DNA-U/DNA-X in the absence and presence of 0.1 U mL^{-1} UDG.

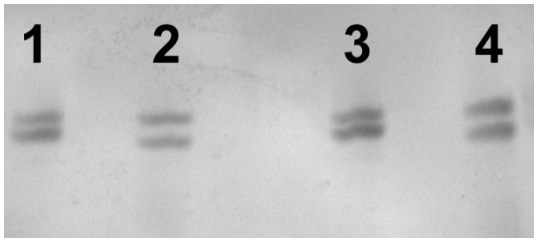

Figure S4. PAGE gel for DNA-U/DNA-X in the absence and presence of 1 U mL^{-1} UDG for 1 h. Lanes 1 and 3: DNA-U/DNA-X. Lanes 2 and 4: DNA-U/DNA-X and UDG. The upper band in each lane is DNA-X (4507.0 Da), which underwent no change in the presence of UDG, while the lower band is DNA-U (4488.0 Da) or DNA-AP (4393.9 Da, product of UDA-U after processed by UDG). DNA-AP (Lane 2 and 4) shows a larger shift than DNA-U (Lane 1 and 3) because of a lower molecular weight (−94.1 Da). The result suggested the complete removal of the uracil in DNA-U by UNG.

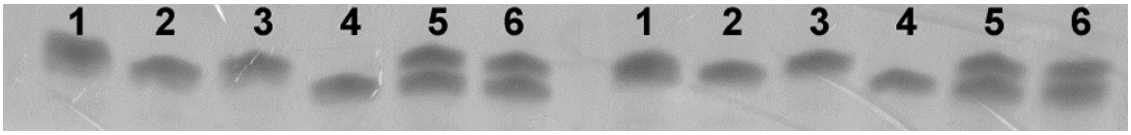

Figure S5. PAGE gels (two repeats) showing: Lane 1, DNA-U and DNA-X; Lane 2, DNA-U; Lane 3, DNA-X; Lane 4, DNA-U and UDG; Lane 5, DNA-U, DNA-X and UDG; Lane 6, DNA-U, DNA-X, ATMND and UDG. The almost identical lowest band in lanes 4~6 suggested that the removal of dU from DNA-U by UDG was not affect by ATMND or whether the DNA is ssDNA or dsDNA. The concentrations of DNA, ATMND and UDG used for the reaction are 1 μM, 0.5 μM and 1 U mL^{-1} UDG, respectively.

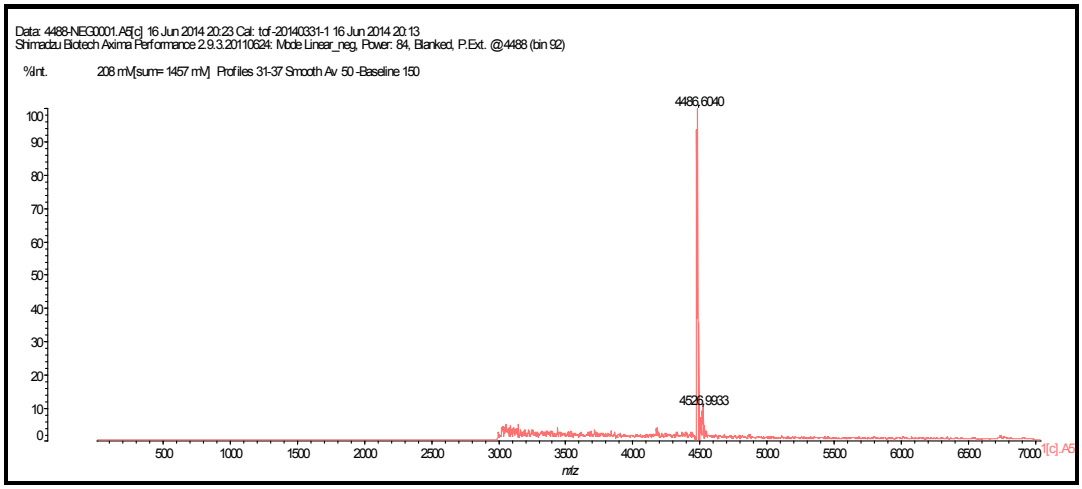

Figure S6A. MALDI-TOF MS of DNA-U in the absence of UDG for 1 h. Detected: 4486.6 Da; Calculated: 4488.0 Da.

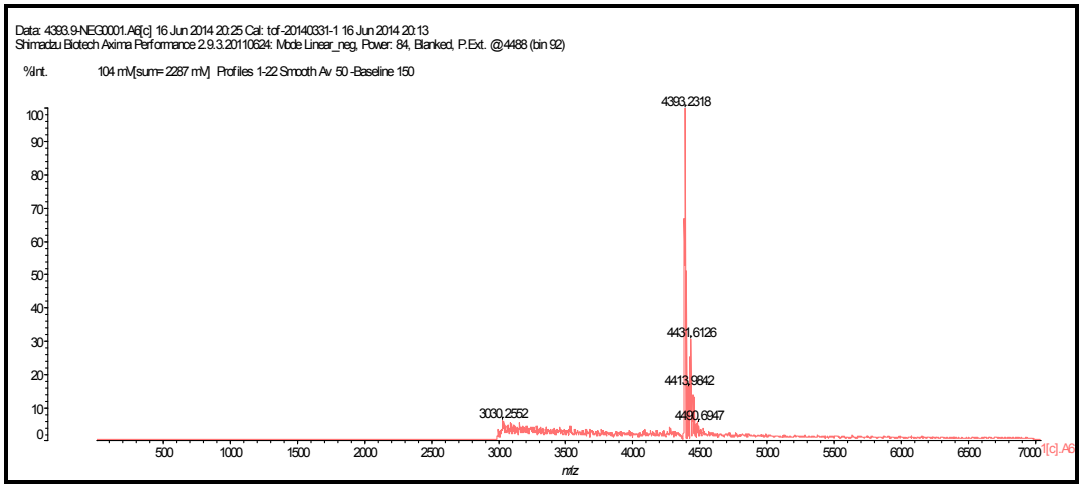

Figure S6B. MALDI-TOF MS of DNA-U in the presence of 1 U mL^{-1} UDG for 1 h. Detected: 4393.2 Da; Calculated: 4393.9 Da.

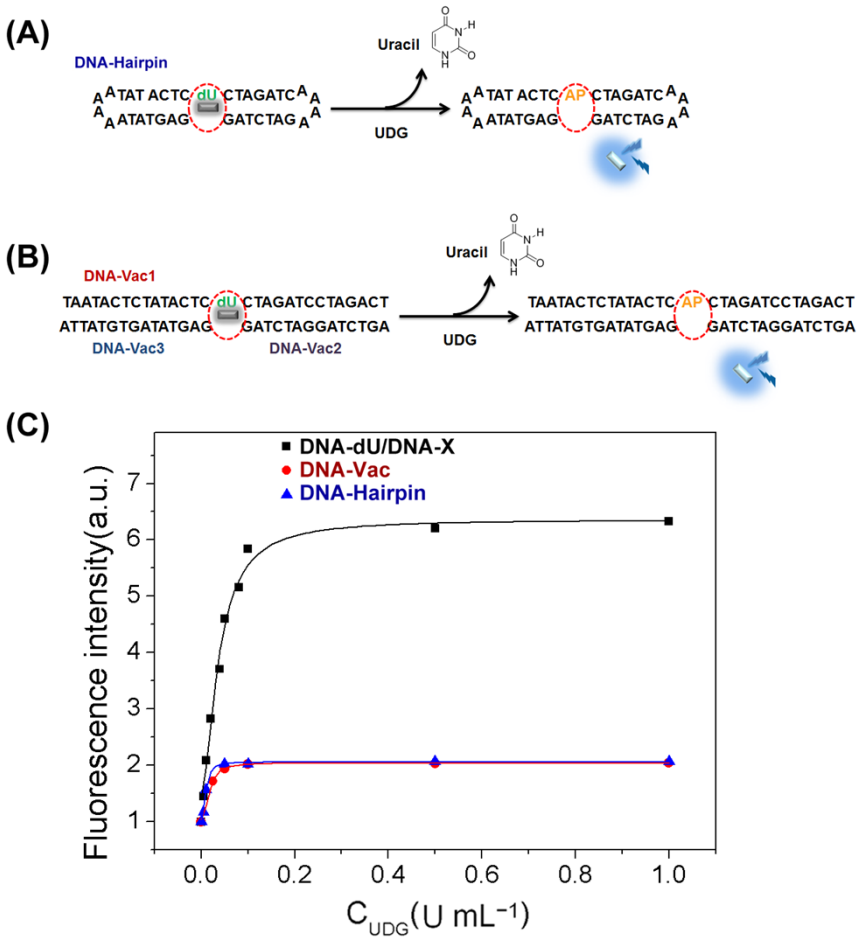

Figure S7. Design of the vacant-site-containing DNA substrates without dSpacer for UDG detection (See the following paragraph for discussion). (A) DNA-Hairpin design. (B) DNA-Vac design. (C) Comparison of UDG detection performance using different dU-containing DNA substrates. Black square, DNA-dU/ DNA-X (Figure 1A). Blue triangle, DNA-Hairpin (Figure S5A). Red circle, DNA-Vac1/DNA-Vac2/DNA-Vac3 (Figure S5B). Condition: 0.5 μM ATMND, 1 μM DNA substrate (each component), 100 mM NaCl, 0.5 mM EDTA and 50 mM HEPES at pH 7.0. Under this condition, DNA-dU/DNA-X, DNA-Hairpin and DNA-Vac showed 6-fold, 2-fold and 2-fold fluorescence enhancement, respectively.

As displayed in Figure S7A and S7B, the two designs (DNA-Hairpin and DNA-Vac) were tested to replace a dSpacer. Here the vacant site (ref 32 of the article) is the position of a missing nucleotide, which provide the space for ATMND binding to the opposite uracil. Under the same condition (0.5 μM ATMND, 1 μM each DNA component, 100 mM NaCl, 0.5 mM EDTA and 50 mM HEPES at pH 7.0), they each showed a UDG-dependent fluorescence enhancement response (Figure S7C), suggesting their capability for UDG detection. Although the DNA-Hairpin and DNA-Vac are composed of all natural DNA bases without any dSpacer, their performance is compromised, with only 2-fold enhancement compared with 6-fold for dSpacer-containing DNA-dU/DNA-X (Figure S7C). The reason for this result is most likely because of the weaker binding of ATMND to the vacant site compared with the dSpacer, thus leaving more ATMND fluorescent in solution to give a stronger background fluorescence and thus a lower fold of enhancement.