Electronic Supplementary Information

Transcriptionally amplified synthesis of fluorogenic RNA aptamers for label-free DNA glycosylase assay

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EXPERIMENTAL SECTION

Chemicals and Materials

All oligonucleotides (Table S1) were synthesized by Sangong Biotechnology (Shanghai, China). Uracil-DNA glycosylase (UDG), endonuclease IV (Endo IV), 10× NEBuffer 3 (1000 mM sodium chloride (NaCl), 500 mM trizma hydrochloride (Tris-HCl), 100 mM magnesium chloride ($MgCl_2$), 10 mM DL-Dithiothreitol (DTT), pH 7.9), uracil glycosylase inhibitor (UGI), 10× UDG reaction buffer (200 mM Tris-HCl, 10 mM DTT, 10 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0), T7 RNA Polymerase, 10× RNAPol reaction buffer (400 mM Tris-HCl, 60 mM $MgCl_2$, 10 mM DTT, 20 mM spermidine, pH 7.9), and ribonucleotide solution mix (rNTP Mix) were obtained from New England Biolabs (Beijing, China). DFHBI was obtained from Sigma Aldrich (St Louis, MO, USA). Diethylpyrocarbonate (DEPC) water used in all experiments was obtained from Sangong Biotechnology (Shanghai, China). Human cervical carcinoma cell line (HeLa cells) and human embryonic kidney 293 cell line (HEK-293T cells) were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China).

Table S1 Sequences of the Oligonucleotides

NOTE	Sequence (5′to 3′)
Probe-dU	CTAGGGAGCTCACACTCTACTCAACAGCGCGAACGCTGGACCCGTCCTTC TCCCTAGCCCTATAGTGAGTCGTATTA TTTTTAATACGACUCACTATAGGG
Probe-rU	CTAGGGAGCTCACACTCTACTCAACAGCGCGAACGCTGGACCCGTCCTTC TCCCTAGCCCTATAGTGAGTCGTATTA TTTTTAATACGAC/rU/CACTATAGGG
Probe-T	CTAGGGAGCTCACACTCTACTCAACAGCGCGAACGCTGGACCCGTCCTTC

	TCCCTAGCCCTATAGTGAGTCGTATTA TTTTTAATACGACTCACTATAGGG

Preparation of Stock Solutions.

The probes were diluted to 4 μM in annealing buffer (1.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.0) at 95 °C for 5 min, followed by slowly cooling to room temperature to perfectly fold into a hairpin structure. The obtained probes were stored at 4 °C for further use.

UDG Assay

2.5 μL of probes was added into the excision reaction system (20 μL) containing different-concentration UDG, 2 U of Endo IV, and 2 μL of 10× NEBuffer 3, followed by incubation at 37 °C for 40 min for UDG-actuated excision repair. Then the cleavage products were added to 30 μL of solution containing 3 μL of 10× RNAPol reaction buffer, 30 U of T7 RNA Polymerase, 500 μM rNTPs at 37 °C for 2 h to perform the transcription reaction.

Fluorescence Measurement

The products were mixed with 0.6 μL of fluorescent dye DFHBI (500 μM) and then subjected to fluorescence measurement using a FLS1000 fluorescence spectrophotometer (Edinburgh Instruments, UK). The fluorescence emission spectrum of 480—650 nm was obtained at the excitation wavelength of 468 nm, and the fluorescence intensity at 508 nm was used for data analysis.

Gel Electrophoresis

The reaction products were analyzed by a 12% nondenaturating polyacrylamide gel (PAGE) in 1× TBE buffer (9 mM Tris-HCl, 9 mM boric acid, 0.2 mM EDTA, pH 7.9) at a 110 V constant voltage for 60 min at room temperature. After being stained by SYBR Gold, the gel was imaged

by a Bio-Rad ChemiDoc MP Imaging System (Hercules, CA). For silver staining assay, the products were analyzed by 15% PAGE in 1× TBE buffer (9 mM Tris-HCl, 9 mM boric acid, 0.2 mM EDTA, pH 7.9) at a 110 V constant voltage for 40 min at room temperature. The gel was stained with a silver staining kit (81104-1000, Tiandz Inc., Beijing, China) and visualized by a Kodak Image Station 4000 MM (Rochester, NY, U.S.A.).

Cell Culture and Preparation of Cell Extracts

Human cervical carcinoma cell line (HeLa cells) and human embryonic kidney 293 cell line (HEK-293T cells) were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen, USA) and 1% penicillin-streptomycin in 5% CO_2 incubator at 37°C. The number of cells was measured by Countstar cell counter. The nuclear cell extracts were prepared by using a Nuclear Extract kit (it consists of protease inhibitor cocktail, lysis buffer AM1, PBS, phosphatase Inhibitors, hypotonic buffer, and detergent, ActiveMotif, Carlsbad, CA). The supernatant was transferred into a fresh tube and stored at -80 °C. Countstar cell counter (Ruiyu biotech, China) was used to determine the number of cells. The 1×10^6 cells in single tube were prepared and then stepwisely diluted to indicated numbers required for further study.

SUPPLEMENTARY RESULTS

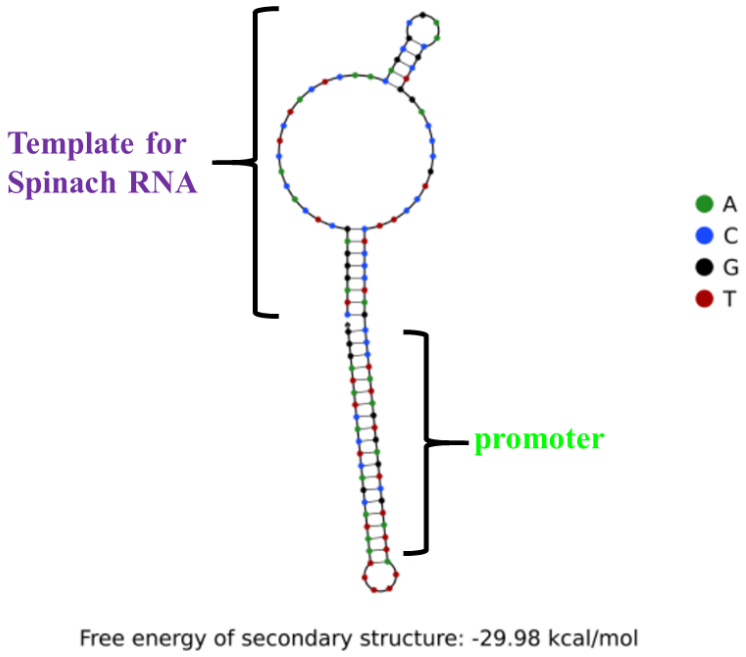

Fig. S1 Predicated secondary structure of detection probe using NUPACK software.

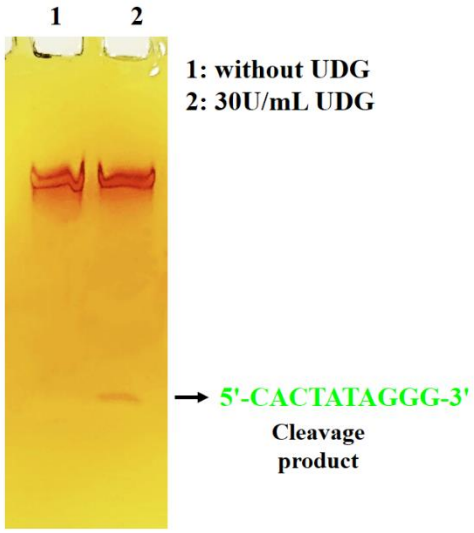

Fig. S2 PAGE analysis of cleavage of probe-dU in the absence (lane 1) and presence of UDG (lane 2), respectively.

We employed 15% PAGE to analyze UDG-mediated cleavage of probe-dU. Because the cleavage product (5'-CACTATAGGG-3') is too short to be clearly visualized, the silver staining is

conducted after PAGE. As shown in Fig. S2, a distinct cleavage product band is observed in the presence of UDG (Fig. S2, lane 2), while this band disappears when UDG is absent (Fig. S2, lane 1). These results clearly demonstrate that the UDG can induce the cleavage of probe-dU.

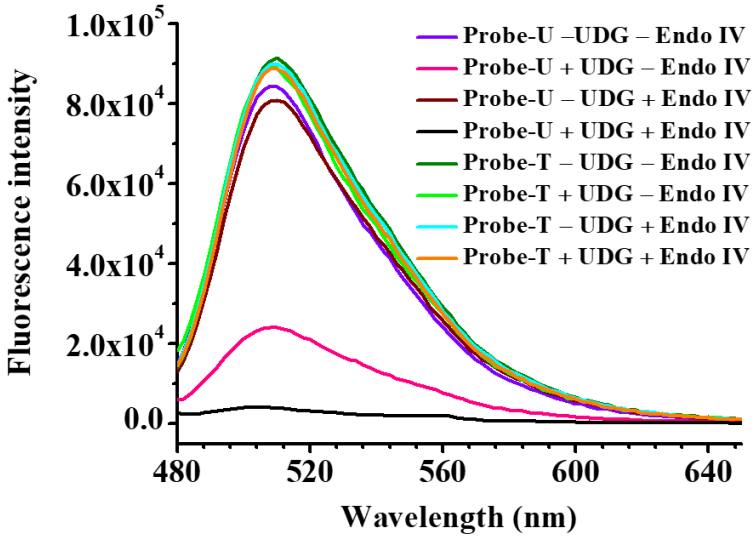

Fig. S3 Fluorescence emission spectra in response to different experimental conditions.

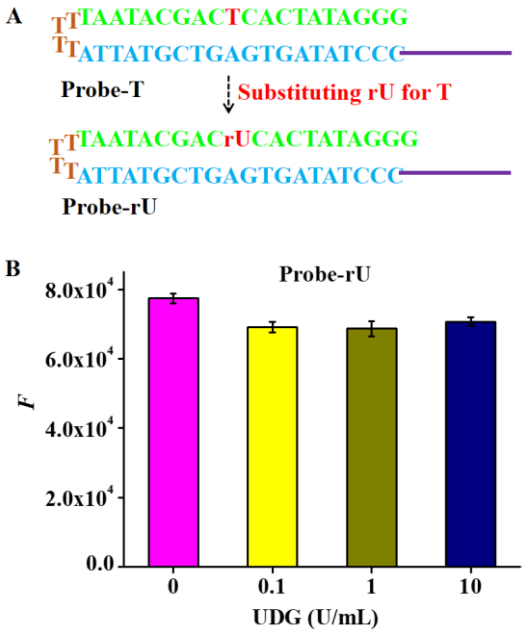

Fig. S4 (A) Design of probe-rU. (B) Fluorescence signal of probe-rU in response to

different-concentration UDG. Error bars show the standard deviation of three experiments.

Probe-rU with ribouracil (rU) substitution (Fig. S4A) is used to investigate the specificity of the biosensor. As shown in Fig. S4B, in the absence of UDG, a distinct fluorescent signal is detected, indicating probe-rU can be used to transcribe fluorescent Spinach RNA. However, no significate signal changes are observed in the presence of 0.1, 1, and 10 U/mL UDG, suggesting that probe-rU cannot serve as the efficient substrate for UDG sensing. These results clearly demonstrate that only probe-dU rather than probe-T or probe-rU can be used for UDG biosensing.

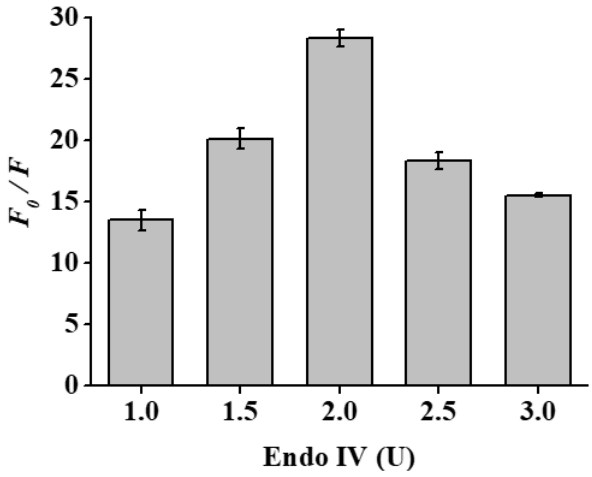

Fig. S5 Variance of F_0/F value in response to different-amount Endo IV. The optimized amount of Endo IV is 2.0 U. Error bars represent the standard deviation from three independent experiments.

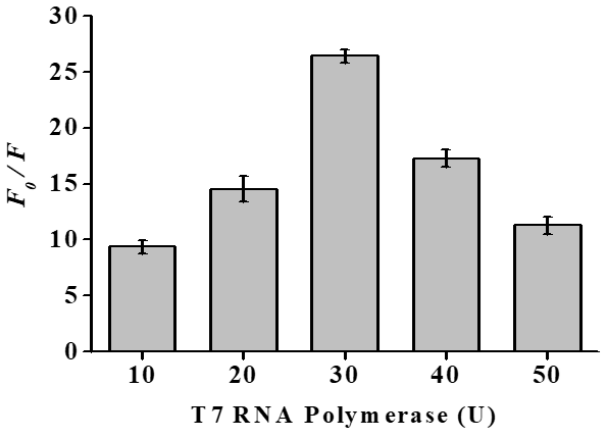

Fig. S6 Variance of F_0/F value in response to different-amount T7 RNA polymerase. The optimized amount of T7 RNA polymerase is 30 U. Error bars represent the standard deviation from three independent experiments.

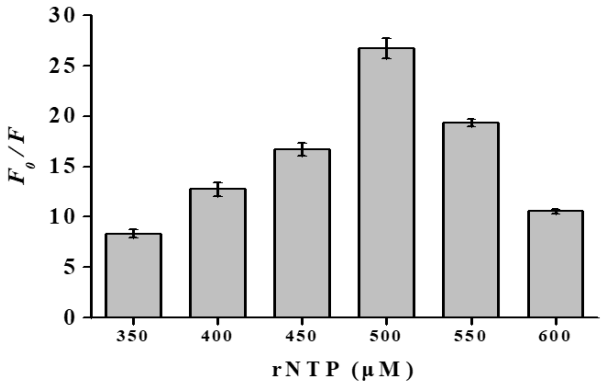

Fig. S7 Variance of F_0/F value in response to different-concentration rNTP. The optimized concentration of NTP is 500 μM. Error bars represent the standard deviation from three independent experiments.

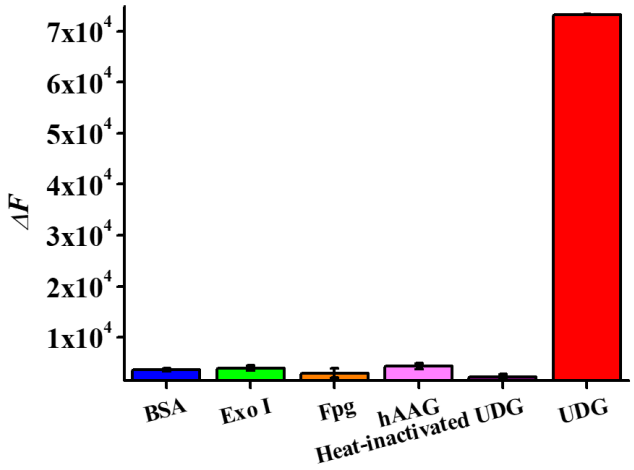

Fig. S8 Measurement of ΔF in response to 0.1g/L BSA (blue column), 30 U/mL Exo I (green column), 30 U/mL Fpg (orange column), 30 U/mL hAAG (pink column), 30 U/mL heat-inactivated UDG (black column), and 30 U/mL UDG (red column), respectively. Error bars show the standard deviation of three experiments.

We used three DNA-modifying enzymes including exonuclease I (Exo I), formamidopyrimidine [fapy]-DNA glycosylase (FpG), human alkyladenine DNA glycosylase (hAAG) to investigate the specificity of the proposed UDG biosensor. Bovine serum albumin (BSA) is used as the negative control. Exo I removes nucleotides from 3' end of ssDNA.[1] FpG repairs damaged purine bases.[2] The hAAG repairs alkylated and oxidized purine bases.[3] Theoretically, none of these interferences can destroy the promoter of the detection probe to affect the synthesis of fluorescent Spinach RNA. As expected, a significant signal change is produced by target UDG (Fig. S8, red column), while no significant fluorescent signal change is observed in response to exo I (Fig. S8, green column), FpG (Fig. S8, orange column), and hAAG (Fig. S8, pink column), identical to that in response to BSA (Fig. S8, blue column). Moreover, when UDG is inactivated by heat treatment at 95 ℃ for 10 min (Fig. S8, black column), the signal decreases

to the same level of that of interferences. These results demonstrate the good specificity of the proposed biosensor toward UDG.

Table S2 Compassion of the proposed biosensor with the reported amplification-based fluorescent methods for UDG assay.

Amplification Strategy	Number of probes required	Fluorescent Labels	LOD (U/mL)	Ref
Transcription reaction	1 (probe-dU)	no	6.3×10^{-6}	This work
RNase H-coupled exponential amplification	3 (substrate, template, signal probe)	FAM, BHQ1	1×10^{-4}	4
CRISPR/Cas12a-coupled strand displacement amplifcation	4 (UDG-primer, template, crRNA-template, cleavage reoprter)	HEX, BHQ1	3.1×10^{-5}	5
loop-mediated isothermal amplification	5 (HP, H1, H2, FP, BP)	no	6.8×10^{-4}	6
Terminal deoxynucleotidyl transferase combined CRISPR-Cas12a amplification	(substrate, crRNA, F-Q probe)	FAM, BHQ1	5×10^{-6}	7
T7 exonuclease-assisted amplification	2 (DS-U substrate, poly-dT)	FAM, BHQ1	1.5×10^{-4}	8

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