Supporting Information

One-Step Fluorescent Method for Sensitive Detection of APOBEC3A Activity by using FRET DNA Probe

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1. Materials and chemicals

All chemicals were purchased from Beijing Innochem Sci. & Tech. Co. Ltd. (Beijing, China) unless mentioned otherwise. All of the DNA oligonucleotides were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). All of the enzyme were purchased from New England Biolabs (NEB) Inc. pH was measured with Mettler Toledo, FE20-Five Easy™ pH (Mettler Toledo, Switzerland). DNA concentration was quantified by NanoDrop 2000c (Thermo Scientific, USA). One-photo Fluorescent emission spectra were acquired with PerkinElmer LS 55 (PerkinElmer, USA). Gel Imaging was monitored with Pharos FX Molecular imager (Bio-Rad, USA). HTS was monitored with Cytation3 microplate reader (BioTek, USA). The nucleic acid stains YeaRed Nucleic Acid Gel Stain (NO.: 10202ES76) was purchased from YEASEN Biotechnology Co. Ltd., (Shanghai, China). The Hela and HEK 293T cells were obtained from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China), and culture medium was purchased from Cyagen Biosciences (China).

2. In vitro experimental procedure

Multi-step: the deamination reaction was carried out in a 10 μL solution with 1 μL of 250 mM HEPES (pH 6.5), 1 μL of 10 μM DNA substrate (DNA-C or DNA-G, 10 pmol) and varied concentration of APOBEC3A enzyme at 37 ℃ for 2 h. Deamination reaction was terminated by incubation of the mixture at 90 ℃ for 10 min. [1] Then added 1 μL USER Enzyme (1 U/μL) to the solution. Further added to the mixture was 2 μL CutSmart® Buffer and 7 μL of water to a final volume of 20 μL, which was then reacted at 37 ℃for 8 h. Finally, added 30 μL of water to solution and detected by fluorescence spectrometer.

One-step: the deamination reaction and cleavage reaction was conducted at a time. The reaction system containing 1 μL of 10 μM DNA substrate (DNA-C or DNA-G, 10 pmol), 1 μL of 250 mM HEPES (pH 6.5), 1 μL USER Enzyme (1 U/μL), 2 μL CutSmart® Buffer, varied concentration of APOBEC3A enzyme and water to a final volume of 20 μL. Then this reaction system was incubated at 37 ℃for 8 h. Finally, added 30 μL of water to solution and detected by fluorescence spectrometer.

3. Denaturing polyacrylamide gel electrophoresis assay

After the completion of reaction, added 1 μL or 2 μL reaction system to deionized formamide to a final volume of 10 μL. Then the mixture was vortexed and loaded to the polymerized and pre-ran gel. The gel was allowed to run at 150 V for about 0.5 h. Finally, the results were monitored with Super GelRed (NO. S-2001, Suzhou, China) using Molecular Imager® ChemiDocTM XRS+ Imaging System (Bio-Rad).

4. Selectivity assay

DNA selectivity: to investigate the selectivity of APOBEC3A, a DNA containing G (DNA-G) was chose as a model. A mixture containing 1 μL of 10 μM DNA substrate (DNA-G, 10 pmol), 1 μL of 250 mM HEPES (pH 6.5), 1 μL USER Enzyme (1 U/ μL), 2 μL CutSmart® Buffer, 2 μL of APOBEC3A (2.5 μM) and 13 μL water. And DNA-C1 as a control to make sure the reaction was completed. The reaction system was incubated at 37 ℃for 8 h. When reaction was completed, 30 μL of water added to the system, and detected by fluorescence spectrometer.

Protein selectivity: to investigate the influence of protein to the DNA-C1, the effect of three other proteins including bovine serum albumin (BSA), tyrosinase and klenow polymerase were checked. The different amounts of protein were added to the mixture containing 1 μL of 10 μM DNA substrate (DNA-C1, 10 pmol), 1 μL of 250 mM HEPES (pH 6.5), 1 μL of USER Enzyme (1 U/μL), 2 μL CutSmart® Buffer, and water to a final volume of 20 μL. The reaction system was incubated at 37 ℃for 8 h. When reaction was completed, 30 μL of water added to the system, and detected by fluorescence spectrometer.

5. Inhibition APOBEC3A activity by MN1

First, incubated the APOBEC3A (0.5 μM) with different concentration of MN1 in 10 μL solution at 37 ℃ for 1 h. Then the DNA substrate (DNA-C1, 10 pmol) was added to the mixture. Further added to the mixture was 1 μL of 250 mM HEPES (pH 6.5), 2 μL CutSmart® Buffer, 1 μL of USER Enzyme (1 U/μL) and water to a final volume of 20 μL, which was then reacted at 37 ℃ for 8 h. Then 30 μL of water added to the solution, and detected by fluorescence spectrometer. IC50 values were determined using GraphPad Prism.

6. The details of high-throughput screen

First, the same concentration (1 mM in DMSO) of compound 1 to 5 was prepared. Then incubated 1 μL of different compounds with APOBEC3A (0.5 μM) at 37 ℃ for 1 h in 384-well black plates. The next, added 1 μL of 10 μM DNA substrate (DNA-C1, 10pmol), 3 μL of 250 mM HEPES (pH 6.5), 1 μL of USER Enzyme (1 U/μL), and 5 μL CutSmart® Buffer to the solution. After added water to a final volume of 50 μL, the system was reacted at 37 ℃for 6 h. The procedures of the control group were the same. Finally, Fluorescence was quantified using a microplate reader with excitation at 490 nm and emission at 520 nm. For one-step of high-throughput screening inhibitors of APOBEC3A, added all of the materials at a time into 384-well black plates. And pipetted the mix several times. Then incubated the solution at 37 ℃for 6 h.

7. Preparation of Hela and HEK 293T cell lysates

Hela and HEK 293T cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented Page S3 with 10% fetal bovine serum and maintained at 37 ℃ 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. Then cells were lysed in 25 mM HEPES (pH 7.9), 10% glycerol, 150 mM NaCl, 0.5% Triton X-100, 1 mM EDTA, 1 mM $MgCl_2$, RNase A and 1 mM $ZnCl_2$, and protease inhibitors. [2] The cell lysates were revolved on vortex for 1 min and then incubated at 4 ℃ for 4 min. And did the circle for 6 times. Then centrifuged 30 min at 12,000 × g at 4℃. The concentration of cell lysates was determined by BCA protein assay kit.

8. DNA deamination with cell extracts

The cell extracts (2 μg) was incubated with UGI in 1X UDG Reaction Buffer at 37 ℃ for 1 h. Then added 2 μL of DNA-C1 (10pmol), 3 μL of USER Enzyme (1 U/μL), 10 μL of CutSmart® Buffer and water to a final volume of 100 μL. After reacted at 37 ℃ for 4 h, the fluorescence was detected at 4 ℃. [3]

9. Supplementary figures and tables

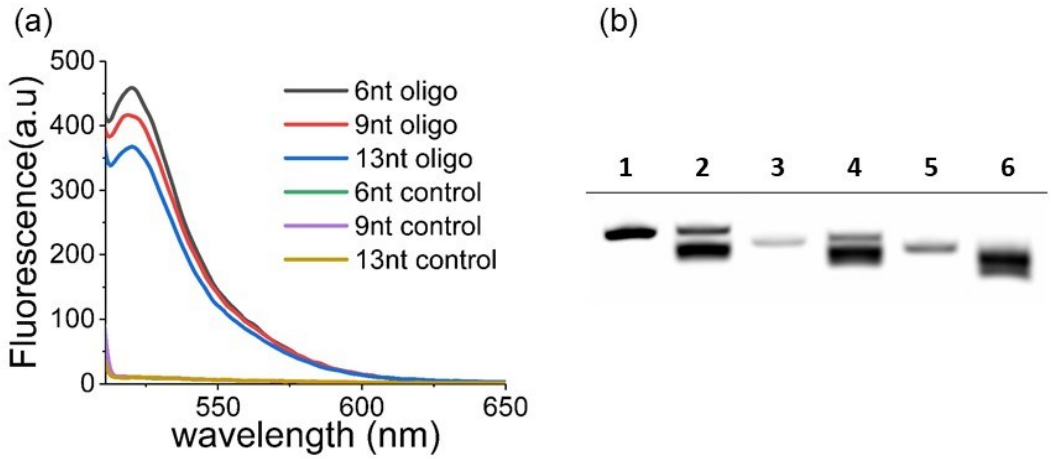


Figure S1. The fluorescence spectra and PAGE analysis of different length of DNA. The 1 μM DNA substrate (6nt, 9nt, 13nt), 0.5 μM APOBEC3A, 25 mM HEPES (pH 6.5),

USER Enzyme (1 U/μL), and 1X CutSmart® Buffer were added together and reacted at 37 ℃ for overnight. Then detected the APOBEC3A activity by fluorescence and PAGE. (a) Fluorescence spectra of different length of DNA. (b)PAGE analysis of different length of DNA: (lane 1) 13nt DNA only; (lane 2) 13nt DNA after reacted; (lane 3) 9nt DNA only; (lane 4) 9nt DNA after reacted; (lane 5) 6nt DNA only; (lane 6) 6nt DNA after reacted.

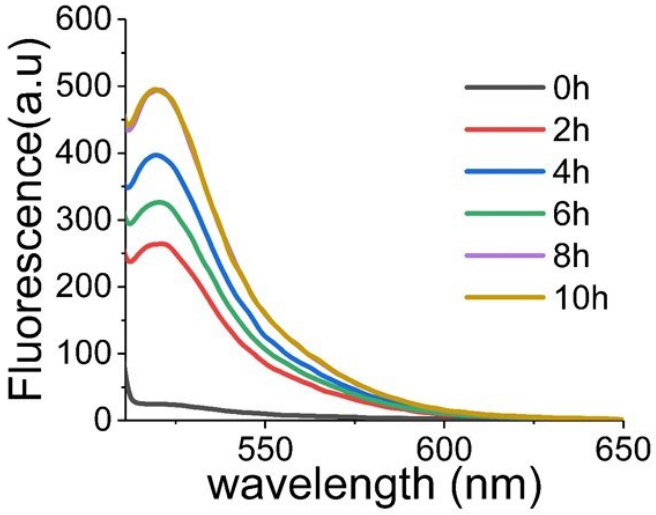
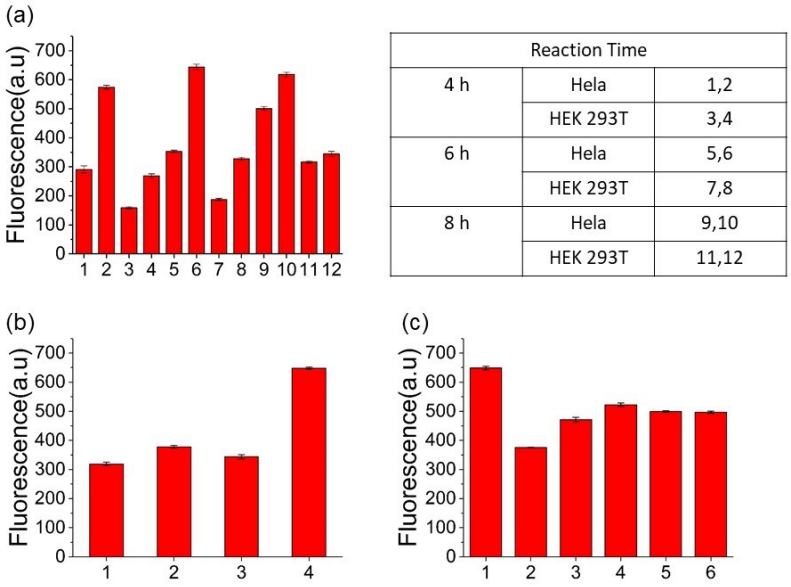


Figure S2. The fluorescence spectra of DNA-C with different time. The 1 μM DNA substrate (DNA-C), 0.5 μM APOBEC3A, 25 mM HEPES (pH 6.5), USER Enzyme (1 U/μL), and 1X CutSmart® Buffer were added together and reacted at 37 ℃ for different time. Then detected fluorescence of different system.



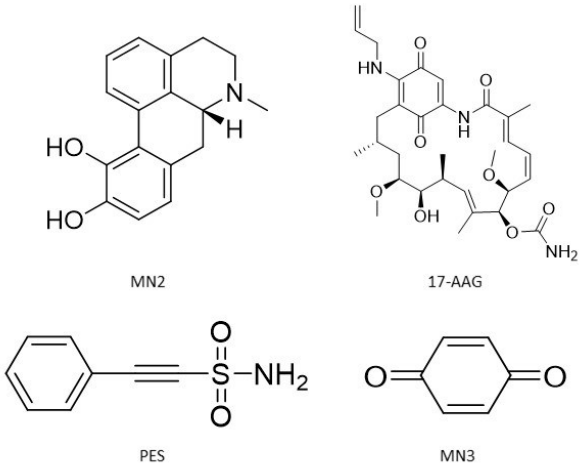
Reaction Time		
4 h	Hela	1,2
	HEK 293T	3,4
6 h	Hela	5,6
	HEK 293T	7,8
8 h	Hela	9,10
	HEK 293T	11,12

Figure S3. Cell lysates. Cell lysates was extracted from Hela and HEK 293T. (a) added cell lysates (2 μg) and UGI (1 U) to reaction system and reacted for different times. With UGI (1,3,5,7,9,11) and without UGI (2,4,6,8,10,12). (b) the cell lysates with different amounts of UGI: (1) 1 U, (2) 2 U, (3) 4 U, (4) without UGI. (c) used USER Enzyme to cleavage DNA-C1 after

treatment with UGI: (1) cell lysates, (2) cell lysates with 1 U UGI. Cell lysates with 1 U UGI and different amounts of USER: (3) 1 U, (4) 2 U, (5) 3 U, (6) 4 U.

Table S1. The sequence of DNA substrate used in experiments.

Name	Sequence
DNA-C1	5'-(F
DNA-C2	5'-(F
DNA-C3	5'-(F
DNA-G	5'-(F



Scheme S1. The structure of compounds used in HTS.

References

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