Electronic Supplementary Material (ESI) for Chemical Communications


# Supporting Information

## Endonuclease IV cleaves apurinic/apyrimidinic sites in single-stranded DNA and its application for biosensing

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**Table 1. Sequences of all DNA molecules used in this research[a]**

| Name | Sequences(5'-3') |
|---|---|
| Linear probe | CCATGCAGTCAGTGAGTGNCAATGGCG ddC |
| Control probe | CCATGCAGTCAGTGAGTGTCAATGGCG ddC |
| Padlock probe | p-TGTCTTCGTCTGCACTCACTGACTATACAATCTACTACCTCAACTCTCGCTCTAC |
| Linear probe-C | CCATGCAGTCAGTGAGTCNCAATGGCG ddC |
| Linear probe-A | CCATGCAGTCAGTGAGTANCAATGGCG ddC |
| Linear probe-T | CCATGCAGTCAGTGAGTTNCAATGGCG ddC |
| Padlock probe-C | p-TGTCTTCGTCTGGACTCACTGACTATACAATCTACTACCTCAACTCTCGCTCTAC |
| Padlock probe-A | p-TGTCTTCGTCTGTACTCACTGACTATACAATCTACTACCTCAACTCTCGCTCTAC |
| Padlock probe-T | p-TGTCTTCGTCTGAACTCACTGACTATACAATCTACTACCTCAACTCTCGCTCTAC |
| Ligation probe | CAGACGAAGACAGTAGAGCGAGA |
| Complementary probe | GCGCCATTGACACTCACT |
| Biotin-DNA | CAT GCA GTC AGT GAG TGN CTT TAC TTT TTT TTT TTT TT-biotin |
| Signal probe | ATACAAT(FAM)CTACT(Dabcyl)ACCTCAAC |

[a] N is the tetrahydrofuran abasic site mimic, ddC is 2',3'-dideoxycytidine. The signal probes were the fluorescence-quenched probe with 4-(4-dimethylaminophenyl) diazenylbenzoic acid (Dabcyl) quencher and fluorescein (FAM) fluorophore.

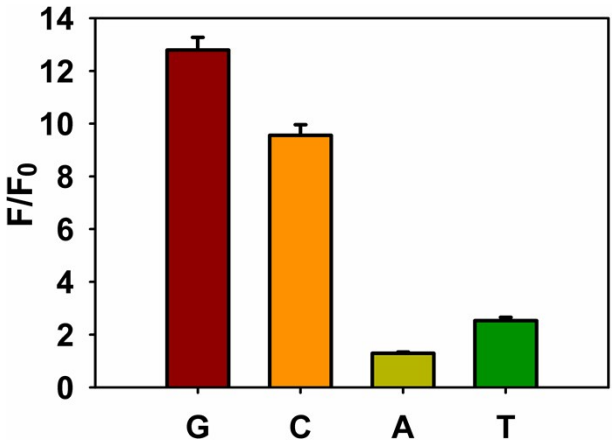


**Fig. S1.** Comparison of the fluorescence intensity ratio of the sensing system to different DNA bases at the 5' side of the AP site. The concentration of Endo IV was fixed at 5 U/mL.

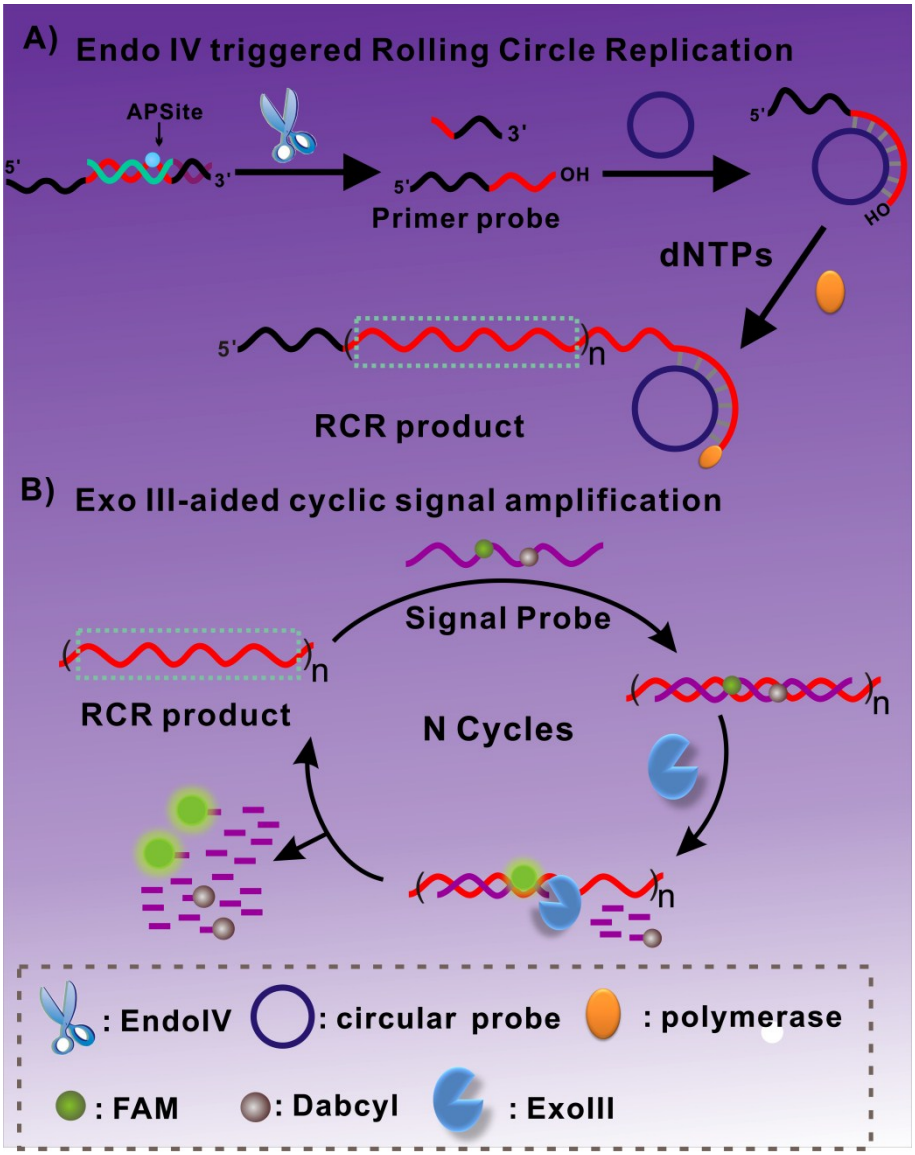


**Fig. S2.** Schematic illustration of Exo III-aided RCR dual signal amplification reaction for Endo IV activity assay to AP sites in dsDNA. A) Endo IV cleaves AP site in the formed AP:A base pair and releases primer probe containing 3' hydroxyl to initiate RCR reaction. B) Exo III–aided cyclic signal amplification reaction.

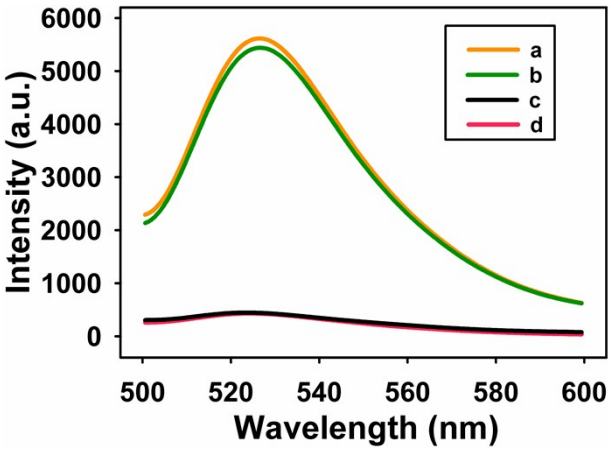


**Fig. S3.** Fluorescence spectra responses of Endo IV cleaving AP sites activity in ssDNA and dsDNA using Exo III-aided RCR dual signal amplification strategy. Curve a, dsDNA (25 nM)+Endo IV(5 U/mL); curve b, ssDNA (25 nM)+Endo IV(5 U/mL); curve c, dsDNA (25 nM); curve d, ssDNA (25 nM).

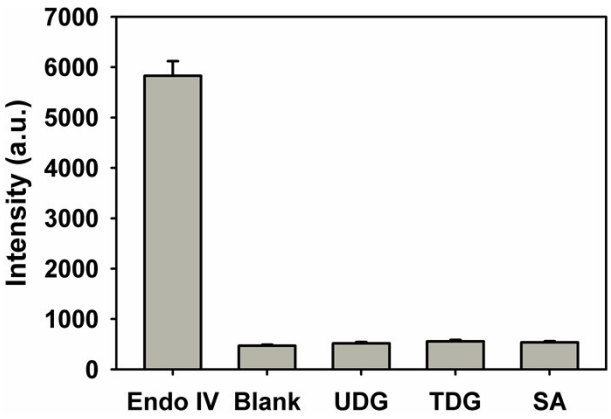


**Fig. S4** Specificity evaluation of the strategy for Endo IV detection. Conditions: Endo IV, 5 U/mL; SA, 0.05 μM; TDG and UDG, 10 U/mL. Error bars are standard deviation of three repetitive experiments.

**Table S2. Recovery detection of Endo IV activity in diluted HeLa cell lysate.[a]**

| Samples | added(U/mL) | found(U/mL) | recovery | RSD(n=3) |
|---|---|---|---|---|
| 1 | 0. 1 | 0.105 | 105% | 3.2% |
| 2 | 0.3 | 0.306 | 102% | 4.5% |
| 3 | 0. 5 | 0. 476 | 95.2% | 4.9% |
| 4 | 0.8 | 0.793 | 99.1% | 3.1% |

[a]The concentration of the cell line was $1 \times 10^4$ cells/mL.

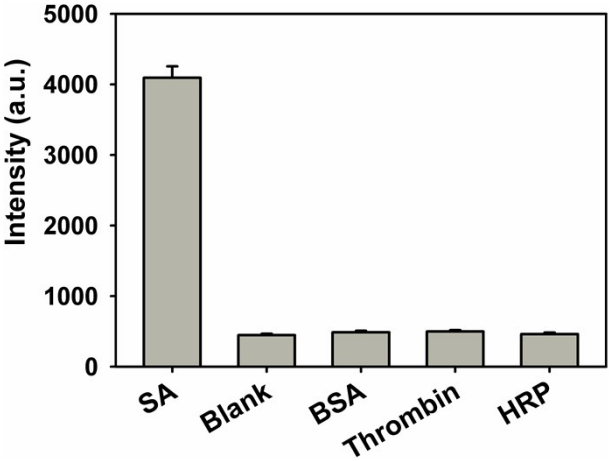


**Fig. S5** Specificity evaluation of the strategy for SA detection. Conditions: SA, 1 nM; BSA, Thrombin and HRP, 10 nM. Error bars are standard deviation of three repetitive experiments.

**Table S3. Comparison of different analytical methods for SA detection sensitivity by our strategy and those reported in literature**

| Analytical Method | Detection Limit (pM) | Ref. |
|---|---|---|
| Label-free fluorescent method using SYBR Green I without amplification | 100 | 1 |
| Poly T templated Cu NPs based fluorescent biosensor without amplification | 100 | 2 |
| Dual-color fluorescent biosensor without amplification | 138 | 3 |
| Solid-state Ag/AgCl process based amplified electrochemical assay | 10 | 4 |
| Endo IV-mediated signal amplification coupled with rolling circle replication | 2.5 | This work |

**Table S4. Recovery detection of SA in 2% human sera samples**

| Samples | added(nM) | found(nM) | recovery | RSD(n=3) |
|---|---|---|---|---|
| 1 | 0.01 | 0.093 | 93% | 3.7% |
| 2 | 0.05 | 0.052 | 104% | 2.9% |
| 3 | 0.1 | 0.097 | 97% | 4.6% |
| 4 | 0.5 | 0.497 | 99.4% | 3.2% |

References


1. Z. Wu, H. Q. Wang, M. Gou, L. J. Tang,, R. Q. Yu and J. H. Jiang, *Anal. Chem.*, 2011, **83**, 3104-3111.
2. H. B. Wang, H. D. Zhang, Y. Chen and Y. M. Liu, *Biosens.Bioelectron.*, 2015, **74**, 581-586.
3. C. H. Chen, X. Xiang, Y. F. Liu, G. H. Zhou, X. H. Ji and Z. K. He, *Biosens.Bioelectron.*, 2014, **58**, 205-208.
4. Q. Wang, B. Y. Jiang, J. Xu, J. Q. Xie, Y. Xiang, R. Yuan and Y. Q. Chai, *Biosens.Bioelectron.*, 2013, 43, 19-24.