Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information for

Endonuclease IV discriminates mismatches next to the apurinic/apyrimidinic site in DNA strands: Constructing DNA sensing platforms with extremely high selectivity

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Materials and methods

1. Materials

Endonuclease IV was purchased from Fermentas, Canada. Lambda exonuclease and Uracil DNA Glycocasylase was purchased from New England Biolabas, USA. All DNA strands were purchased from Sangon (Shanghai, China, purified by HPLC).

2. Sequences used

Table S1. Sequences used for Figure 1, Figure S1a and Figure S1b, Column 1-4

Probe Name	Sequence (5'-3')
Uracil containing probe-5'C and 3' A	5'FAM-TATCTGCACUAGATGCACCTT-3'BHQ1
3' A:C	CGTAAGGTGCATCCAGTGCAGATAGC
3' A:G	CGTAAGGTGCATCGAGTGCAGATAGC
3' A:A	CGTAAGGTGCATCAAGTGCAGATAGC
(=5'C:G &3'A:A)	
5' C:A &3'A:A	CGTAAGGTGCATCAAATGCAGATAGC
5' C:T &3'A:A	CGTAAGGTGCATCAATTGCAGATAGC
5' C:C &3'A:A	CGTAAGGTGCATCAACTGCAGATAGC

Table S2. Sequences used for Figure S1b Column 5-8

Probe Name	Sequence (5'-3')
Uracil containing probe-5' T and 3' A	5'FAM-TATCTGCATUAGATGCACCTT-3'BHQ1
5'T:A &3'A:A	CGTAAGGTGCATCAAATGCAGATAGC
5'T:T &3'A:A	CGTAAGGTGCATCAATTGCAGATAGC
5'T:C &3'A:A	CGTAAGGTGCATCAACTGCAGATAGC
5'T:G &3'A:A	CGTAAGGTGCATCAAGTGCAGATAGC

Table S3. Sequences used for Figure S1b Column 9-12

Probe Name	Sequence (5'-3')
Uracil containing probe-5'A and 3' A	5'FAM-TATCTGCAAUAGATGCACCTT-3'BHQ1
5' A:T &3'A:A	CGTAAGGTGCATCAATTGCAGATAGC
5' A:A &3'A:A	CGTAAGGTGCATCAAATGCAGATAGC
5' A:C &3'A:A	CGTAAGGTGCATCAACTGCAGATAGC
5' A:G &3'A:A	CGTAAGGTGCATCAAGTGCAGATAGC

3. Determination of the cleavage rates of different types of duplexes by Endo IV

To a 50-μL PCR tube, 5 μL of 10×ThermolPol buffer (New England Biolabs, USA), 5 pmol of uracil-containing probe and 1.0 unit of UDG were added. After reaction for 1 min at room temperature, 5 pmol of certain kind of target strand and 0.1 unit of Endo IV were added and the solution was brought up to a total volume of 50 μL. The reactions were performed on a Rotor-Gene Q 5plex HRM Instrument (QIAGEN, Hilden, Germany). The excitation and emission wavelengths were set to 470 nm and 510 nm, respectively. Fluorescence intensity was measured once a cycle with each cycle lasting for 5 s (gain level of the detector: 9.33). The total detection time is 500 s. The rate of fluorescence increase is determined by the slope of the 0-150 s of the linear portion of the time curve.

4. Differentiation of target strands immersed in a large background of interfering strands

To the AP-probe solutions obtained as described above, 20 pmol of mixed target strands at different 3' mismatch to 5',3' mismatch ratios (10%, 5.0%, 1.0%, 0.5%, 0.1%, 0.05%, 0.02%, 0.01% and 0%) and 0.1 unit of Endo IV were added. The solution was brought up to a total volume of 50 μL. The detection was then performed in the same way as described above. The total detection time is 20 min.

5. Genotyping 5'C:C & 3'A:A and 3'A:A after PCR

Two solutions were prepared for PCR experiments. Solution 1 contained 10 ng of PCR-template-5'C:G & 3'A:A and its complementary strand. Solution 2 contained 10 ng of PCR-template-5'C:C & 3'A:A and its complementary strand. To each of the solution, 15 pmol of forward primer, 15 pmol of reverse primer, 2.0 mM dNTP and 1.0 unit of Taq polymerase were added. After 20 cycles of PCR, the PCR products were treated with 1.5 units of λ exonuclease for 5 min. Then the solutions were heated to 80ºC for 10 min to inactivate λ exonuclease. Finally, 5 pmol of uracil containing probes and 0.1 units of Endo IV were added to each of the solution and the fluorescence intensity was measured at 52.5 ºC.

Supplementary results

1. Investigation of the generality of the discrimination capability of Endo IV.

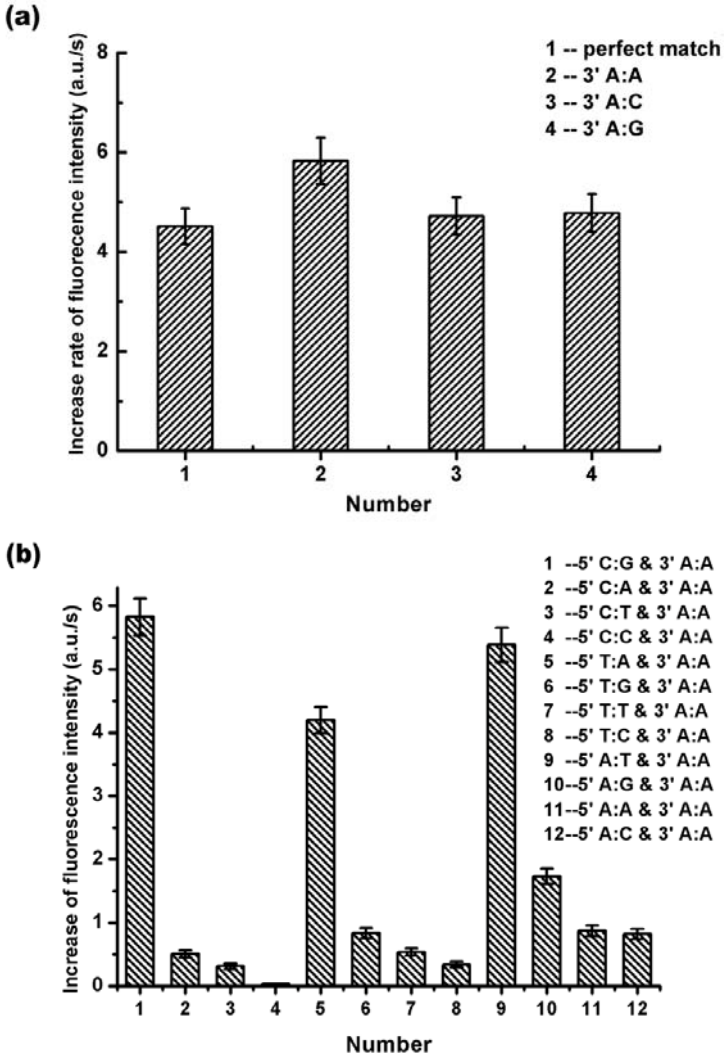

Figure S1. Comparison of the increase of fluorescence intensity per second (increase rate) of sixteen solutions containing different types of (a) 3' mismatch targets and (b) 5', 3' mismatch targets. The numbers and base pairs listed in the legends indicate the positions and types of mismatch bases.

2. Detection of target DNA strands in a large background of single-base different interfering strands

(1) Uracil containing probe-5'C and 3' A; Target strand: 3' A:A; Interfering strand: 5'C:T & 3'A:A

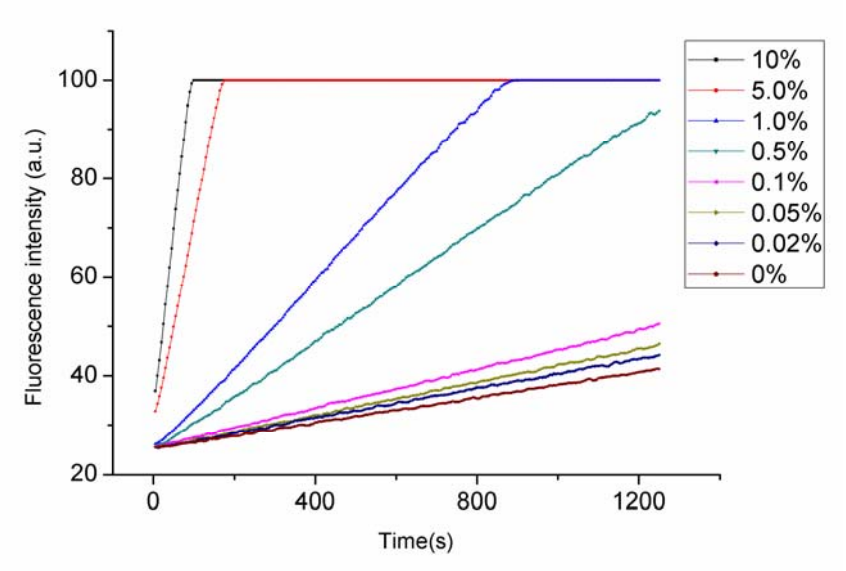

Figure S2. Detection of 3' A:A targets immersed in a large background of 5'C:T & 3'A:A interfering strands.

(2) Uracil containing probe-5'C and 3' A; Target strand: 3' A:A; Interfering strand: 5'C:A & 3'A:A

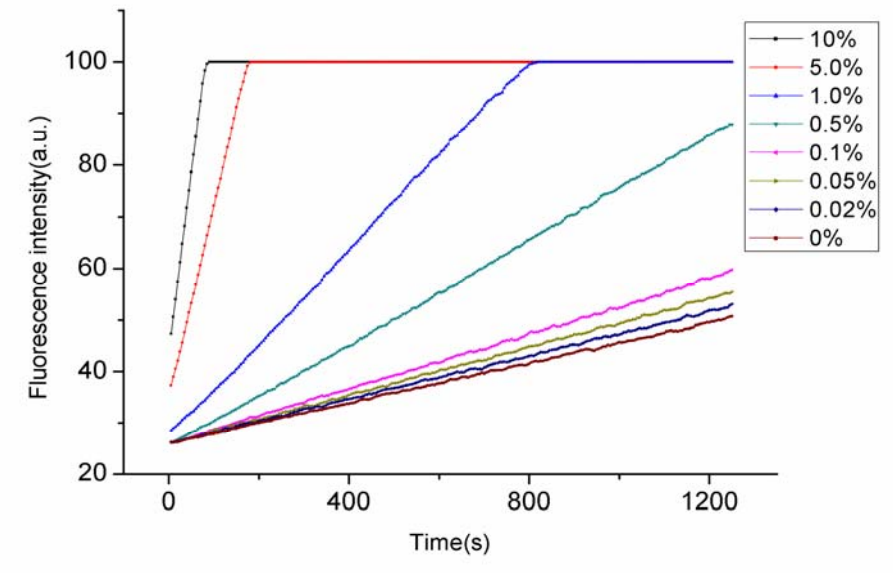

Figure S3. Detection of 3' A:A targets immersed in a large background of 5'C:A& 3'A:A interfering strands.

(3) Uracil containing probe-5’ T and 3’ A; Target strand: 5’T:A& 3’ A:A;
Interfering strand: 5’ T:C& 3’A:A

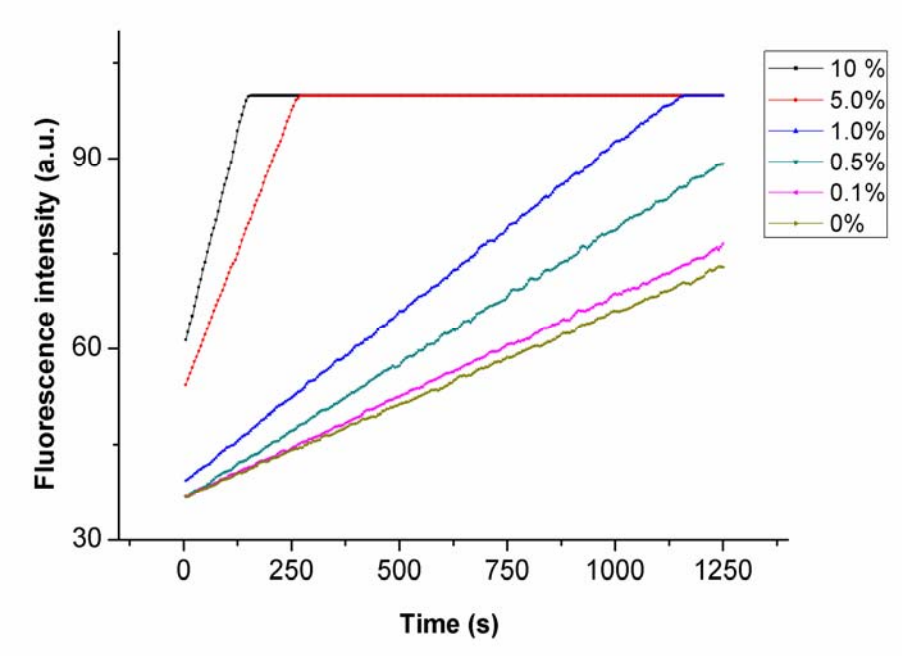

Figure S4. Detection of 5’T:A&3’ A:A targets immersed in a large background of 5’T:C& 3’A:A interfering strands.

(4) Uracil containing probe-5’ T and 3’A; Target strand: 5’T:A& 3’ A:A;
Interfering strand: 5’ T:T& 3’A:A

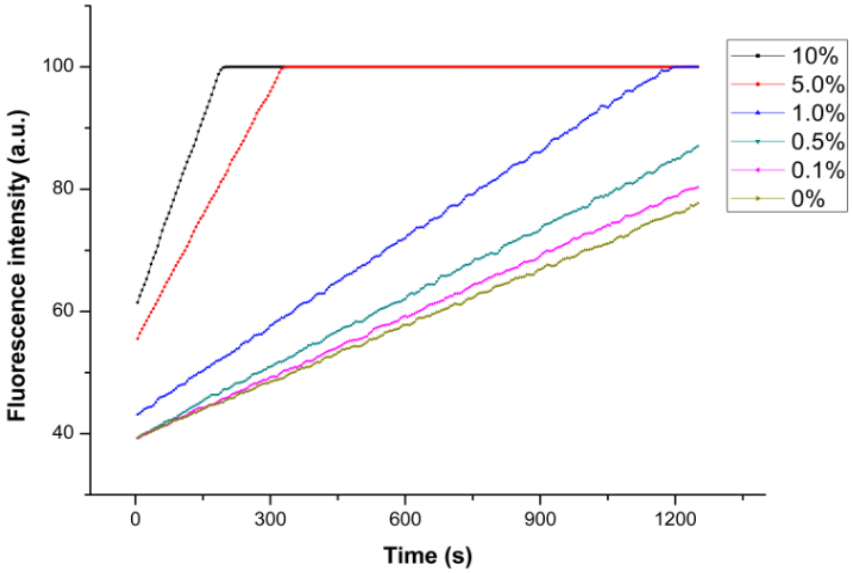

Figure S5. Detection of 5’T:A&3’ A:A targets immersed in a large background of 5’T:T& 3’A:A interfering strands.

(5) Uracil containing probe-5'A and 3' A; Target strand: 5'A:T& 3' A:A;
Interfering strand: 5'A:C& 3'A:A

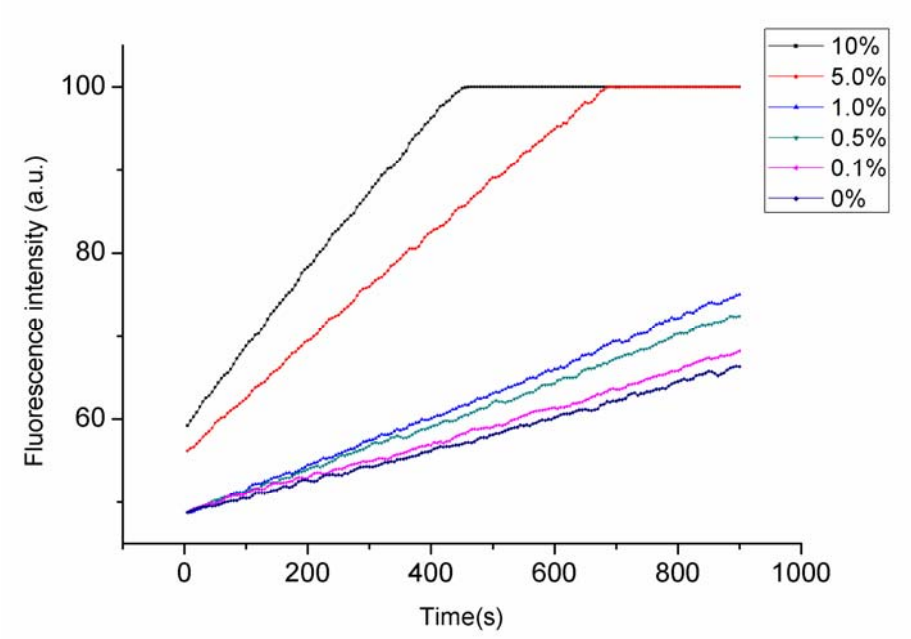

Figure S6. Detection of 5'A:T&3' A:A targets immersed in a large background of 5'A:C& 3'A:A interfering strands.

3. Post-PCR Genotyping

PCR template-5'C:G&3'A:A

CTTAATTTTTAATGGAACTGACAAAACGTAAGGTGCATCAAGTGCAGATACCAAAAAGA
CTATCCCTGAGTAAAACC

PCR template-5'C:C& 3'A:A

CTTAATTTTTAATGGAACTGACAAAACGTAAGGTGCATCAACTGCAGATACCAAAAAGA
CTATCCCTGAGTAAAACC

Foward primer: CTTAATTTTTAATGGAACTGAC
Reverse primer: 5'-PO_4-GGTTTTACTCAGGGATAGTCT
3'A:A target sequence: CGTAAGGTGCATCAAGTGCAGATAGC
5'C:A&3'A:A target sequence: CGTAAGGTGCATCAAATGCAGATAGC
Uracil containing probe-5'C and 3'A: 5'FAM-TATCTGCACUAGATGCACCTT-3'BHQ1

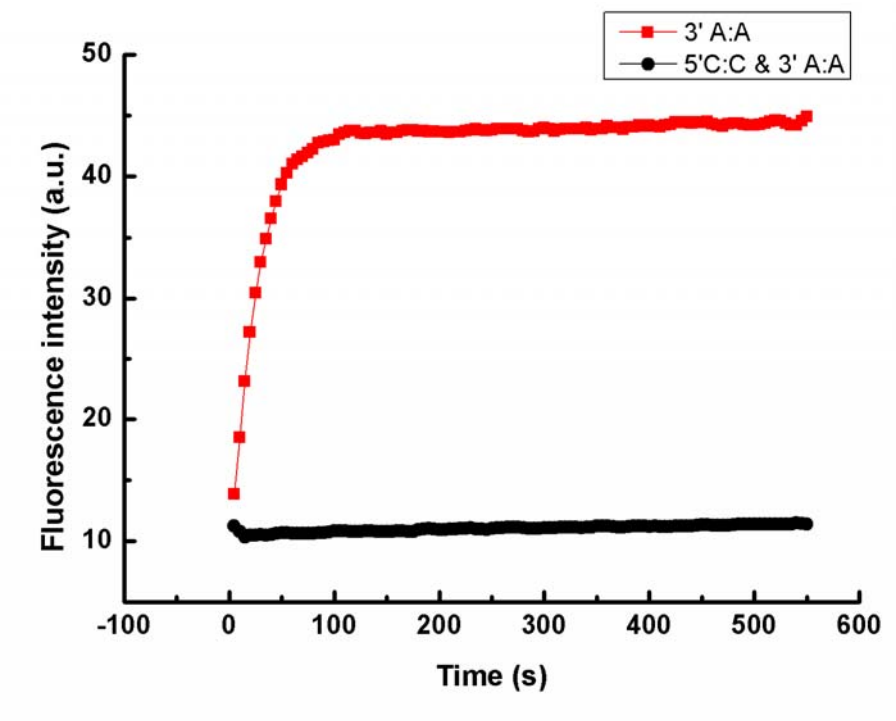

Figure S7. Genotyping 3'A:A (i.e. 5’C:G & 3’ A:A) strand and 5'C:C&3'A:A strand after PCR.