Electronic Supplementary Material (ESI) for Chemical Science
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Supporting Information for

A universal mismatch-directed signal amplification platform for ultra-selective and sensitive DNA detection under mild isothermal conditions

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Other sequences used in Figure 2b (5' to 3'):

Strand 1, 1-mismatch target (C:T):

GTTTTAAATTATGGAGTATGTGTCTGTGGA**T**ACGAGAGTAAG

Strand 2, 1-mismatch target (C:C):

GTTTTAAATTATGGAGTATGTGTCTGTGGA**C**ACGAGAGTAAG

Strand 6, 1,3- mismatch target (C:A; C:A):

GTTTTAAATTATGGAGTATGTGTCTGTG**A**A**A**ACGAGAGTAAG

Strand 7, 1,3-mismatch target (C:A; C:C):

GTTTTAAATTATGGAGTATGTGTCTGTG**C**A**A**ACGAGAGTAAG

All the mismatched bases are shown in bold and underlined.

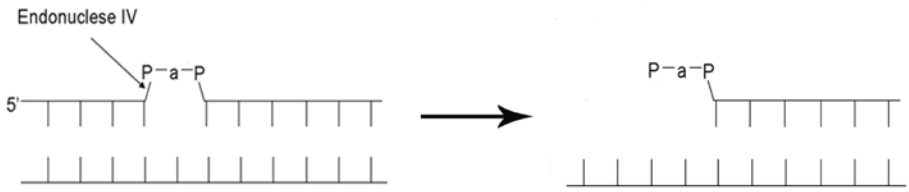

Figure S1. Illustration of the cleavage reaction of mAP-probe by Endo IV. A 5'-phosphate abasic end was produced for further digestion by λ exo.

Comparison of the contributions of Endo IV and λ exo to the enhancement of the signal amplification reactions caused by 1-mismatch

First, we prepared two solutions that contained the same amount (5 pmol) of mAP-probe and target strands (Solution 1 for perfectly matched target and Solution 2 for 1-mismatch target). To the two solutions, 0.1 units of Endo IV and 5 units of λ exo were simultaneously added. In this case, we may directly observe the kinetic curves of the coupling enzymatic reactions without the amplification. As shown in Figure S2a, the mAP-probes in the probe/target duplexes could be cleaved and degraded by the two enzymes within 500 s. This implies that the added Endo IV is capable of cutting all the AP sites of the mAP-probe in the solution in no longer than 500 s.

Based on above results, we prepared another two solutions which contained 5 pmol of mAP-probe and same amount of target strands (Solution 3 for perfectly matched target and Solution 4 for 1-mismatch target), to which 0.1 units of Endo IV was first added and mixed well. After 30 min incubation at 37 °C (much longer than 500 s to ensure the cleavage reactions fully completed), 5 units of λ exo were added to each solution and the fluorescence signals were immediately recorded. The fluorescence intensity responses shown in Figure S2b reflected the kinetics of the degradation reactions catalyzed by λ exo.

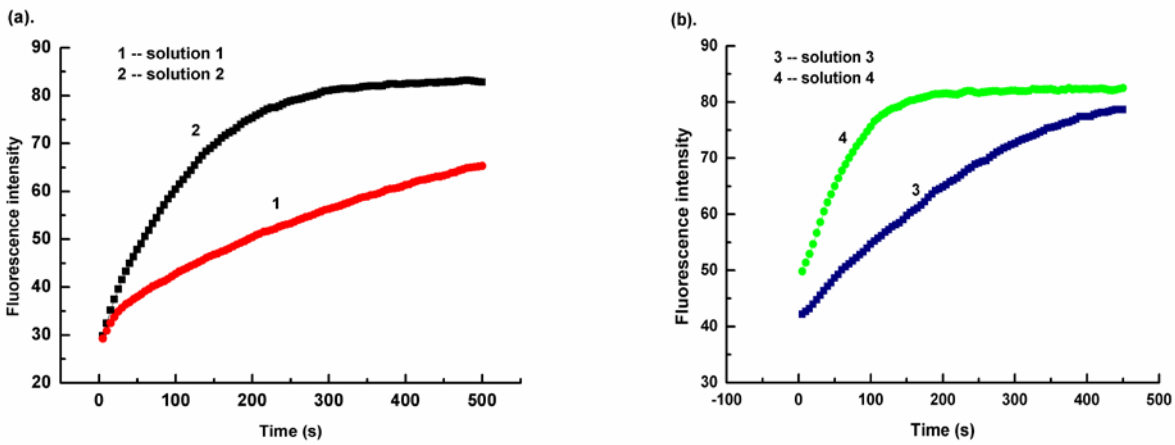

Figure S2. Fluorescence intensity responses of the four different reaction solutions.

We calculated the slopes of the linear sections of the four curves in Figure S2 and the results were represented by K_1, K_2, K_3 and K_4 for Solutions 1 to 4, respectively. The ratios of K_2 to K_1 (K_2/K_1) and K_4 to K_2 (K_4/K_3) were compared in Figure S3.

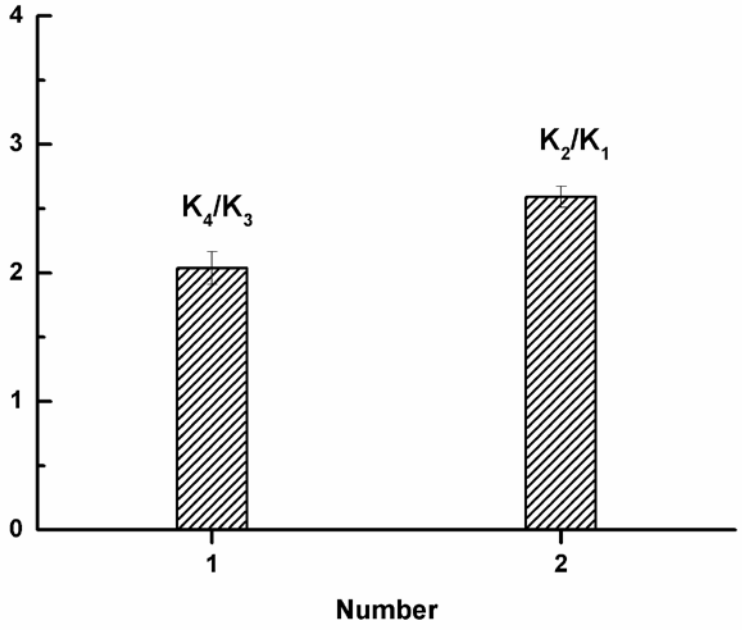

Figure S3. Comparison between K_2/K_1 and K_4/K_3.

K_2/K_1 reflects the total contributions of the two enzymes to the 1-mismatch enhancement effect, while K_4/K_3 reflects the sole contribution of λ exo. The results demonstrate that the contribution of λ exo to the signal enhancement is much greater than that of Endo IV, but the latter plays an essential role in providing the special 5'-end for the λ exo to quickly digest the probe.

Discrimination ability of the method toward 1-mismatch strand (1-C:A), perfectly matched target, and 1,3-mismatch strand (1-C:A, 3-T:T or 3-T:G)

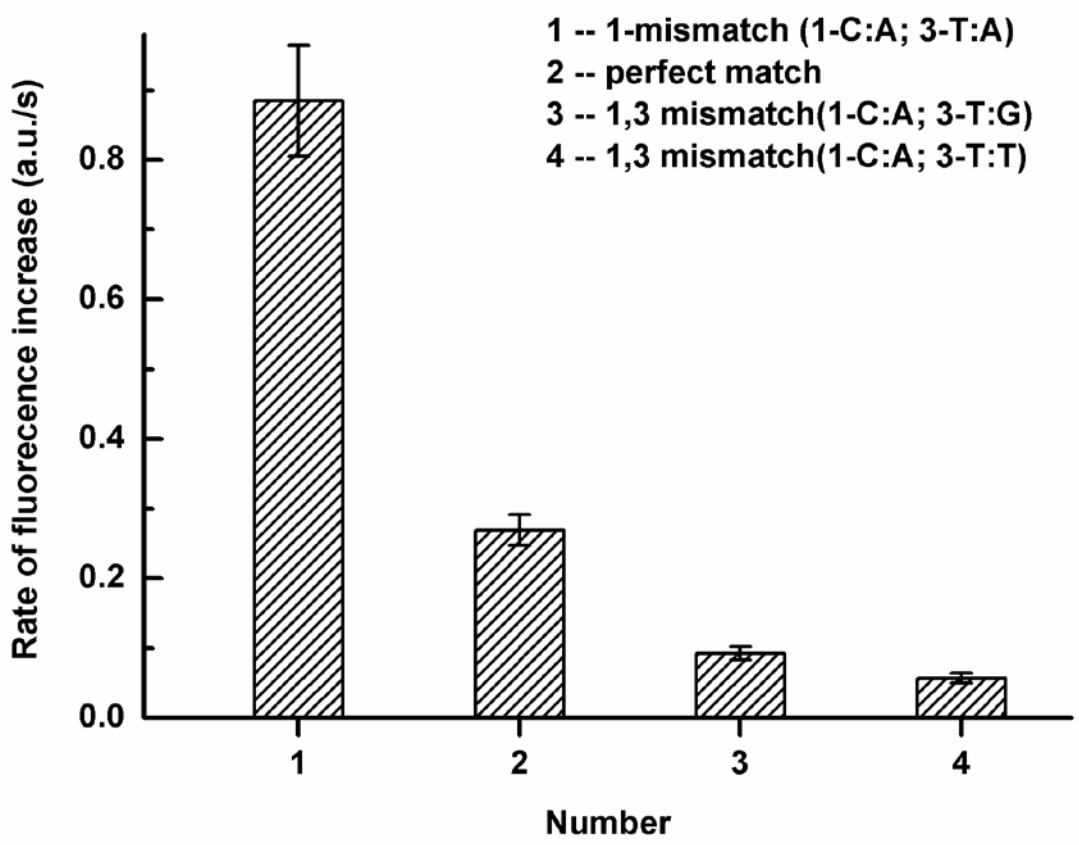

Figure S4. Comparison of the increase rate of fluorescence intensity of four reaction solutions containing different types of target strands. Each solution comprises 5 pmol of mAP-probe, 0.1 units of Endo IV, 5 units of λ exo and 1 pmol of target strand. The numbers and base pairs listed in brackets indicate the positions and types of mismatch bases in 1-mismatch and 1,3-mismatch target strands, respectively.

Sequences used in Figure S4 (5' to 3'):

mAP-probe: TCG□CT(-FAM)TCACAGACACATACTCCA-BHQ1

Column1: GTTTTAAATTATGGAGTATGTGTCTGTGAA**A**ACGAGAGTAAG

Column2: GTTTTAAATTATGGAGTATGTGTCTGTGAAGACGAGAGTAAG

Column3: GTTTTAAATTATGGAGTATGTGTCTGTG**G**A**A**ACGAGAGTAAG

Column4: GTTTTAAATTATGGAGTATGTGTCTGTG**T**A**A**ACGAGAGTAAG

All the mismatched bases are shown in bold and underlined.

Discrimination ability of the method toward 1-mismatch strand (1-C:A), perfectly matched target, and 1,3-mismatch target (1-C:A, 3-G:G or 3-G:A)

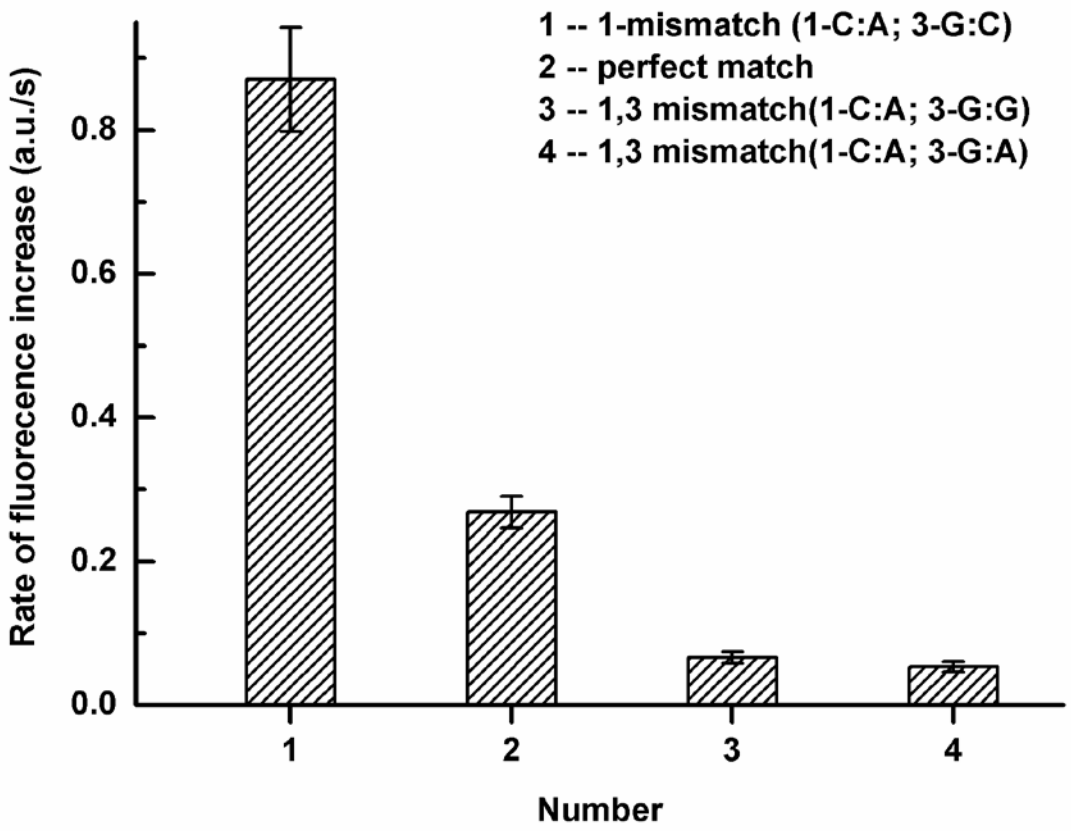

Figure S5. Comparison of the increase of fluorescence intensity per second (increase rate) of four reaction solutions containing different types of target strands. Each solution comprises 5 pmol of mAP-probe, 0.1 units of Endo IV, 5 units of λ exo and 1 pmol of target strand. The numbers and base pairs listed in brackets indicate the positions and types of mismatch bases in 1-mismatch and 1,3-mismatch target strands, respectively.

Sequences used in Figure S5 (5' to 3'):

mAP-probe: TC***A***□CT(-FAM)GCACAGACACATACTCCA-BHQ

Column1: GTTTTAAATTATGGAGTATGTGTCTGTGCA**A**A***T***GAGAGTAAG

Column2: GTTTTAAATTATGGAGTATGTGTCTGTGCAGATGAGAGTAAG

Column3: GTTTTAAATTATGGAGTATGTGTCTGTG**G**A**A**A***T***GAGAGTAAG

Column4: GTTTTAAATTATGGAGTATGTGTCTGTG**A**A**A**A***T***GAGAGTAAG

All the mismatched bases are shown in bold and underlined. The A:T at the left side of AP site are shown in bold and Italic.