Design enzyme-interfaced DNA logic operations (AND, OR and INHIBIT) with an assaying application for single-base mismatch

Long Ma,* Aipo Diao

Experimental Section

1. AND, OR and INHIBIT logic gates

EcoP15I and BSA (bovine serum albumin) was purchased from New England Biolabs. All chemicals used were analytical grade or better. All DNA were chemically synthesised and purified by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. The sequences of oligonucleotides are listed in Tab. S1.

Each sample was buffered with 50 mM Tris–HCl, 20 mM sodium chloride, 10 mM magnesium chloride, pH 7.5. For each gate, the two inputs were pre-mixed at heated at 60°C, cooled down to ambient for use. 500 μL samples of 200 nM input oligonucleotides plus 2.5 μM EcoP15I enzyme was incubated for 30 min at 25°C prior to measurement. For the input contained two types of oligonucleotides, they were put in 1:1 equivalent (100 nM for each). Steady-state fluorescence spectra were measured using a fluorescence spectrophotometer F-7000 (Hitachi) with a slit of 10 nm for both excitation (315 nm) and emission (370 nm) as previously described. All fluorescence intensities were normalised for graph plotting.

2. Application of AND, OR logic gates for single-mismatch detection

Each sample was buffered with 100 mM Tris–HCl, 20 mM sodium chloride, 20 mM magnesium chloride, pH 7.8. For each gate, the input(s) were pre-mixed at heated at 60°C, cooled down to ambient temperature for use. 500 μL samples of 300 nM input oligonucleotides plus 2.5 μM EcoP15I enzyme, 1 μM BSA and 50 nM other four types of non-relevant oligonucleotides were incubated for 30 min at 25°C prior to measurement. For the input contained two types of oligonucleotides, they were put in 1:1 equivalent (150 nM for each). Steady-state fluorescence spectra were measured using a fluorescence spectrophotometer F-7000 (Hitachi) with a slit of 10 nm for both excitation (315 nm) and emission (370 nm) as previously described. All fluorescence intensities were normalised for graph plotting.

ANDin 1 (ssDNA)	(5′–3′)TAGGTCAGAATTCAGC2GACCCTAAGTAGCC
ANDin 2 (ssDNA)	(5′–3′)GGCTACTTAGGGTCTGCTGAATTCTGACCTA
ORin 1 (dsDNA)	(5′–3′)TAGGTCAGAATTCAGC2GACCCTAAGTAGCC (3′–5′)ATCCAGTCTTAAGTCGTCTGGGATTCATCGG
ORin 2 (dsDNA)	(5′–3′)GAACGTCGTGATGTGCCAGC2GAGGCGGGATGAA (3′–5′)CTTGCAGCACTACACGGTCGTCTCCGCCCTACTT
INHin 1 (ssDNA)	5′-TCGCCTCTGCTGAGCATGAAGTCGATGACTTCATGCTCAGC2GAGGCGAGAAAATTT-3′
INHin 2 (ssDNA)	3′-AGCGGAGACGACTCGTACTTCAGCTACTGAAGTACGACGCCGCTCCGCTCTTTTAAA-5′
AS1 sequence	(5′–3′)GCAGCTTAACAGC2GTTCCACGGA (3′–5′)CGTCGAATTGTCGTCAAGGTGCCT
AS2 sequence	(5′–3′)GCAGCTTAACAGC2GTTCCACGGA (3′–5′)CGTCGAATTGTCGTCAAGGTGCCT
AS3 sequence	(5′–3′)GCAGCCCGACAGC2GCATCCACGGA (3′–5′)CGTCGGGCTGTCGTCGTAGGTGCCT
AS4 sequence	(5′–3′)CAGCCCAGATCAGC2GAACATCCAC (3′–5′)GTCGGGTCTAGTCGTCTTGTAGGTG
AS5 sequence	(5′–3′)GCAGCTTAACAGC2GTTCCACGGA (3′–5′)CGTCGAATTGTGGTCAAGGTGCCT
AS6 sequence	(5′–3′)GCAGCTTAACAGC2GTTCCACGGA (3′–5′)CGTCGAATTGTCATCAAGGTGCCT

Tab. S1 The sequences of oligonucleotides used in this study. The underlined sequence represents the recognition site for EcoP15I; 2 denotes 2AP base.

Input	**0,0**	**0,1**	**1,0**	**1,1**
Output	0	0	0.11	1

Tab. S2 The normalised fluorescence intensities for AND logic gate.

Input	**0,0**	**0,1**	**1,0**	**1,1**
Output	0	1	0.72	0.91

Tab. S3 The normalised fluorescence intensities for OR logic gate.

Input	**0,0**	**0,1**	**1,0**	**1,1**
Output	0	0	1	0.12

Tab. S4 The normalised fluorescence intensities for INHIBIT logic gate.

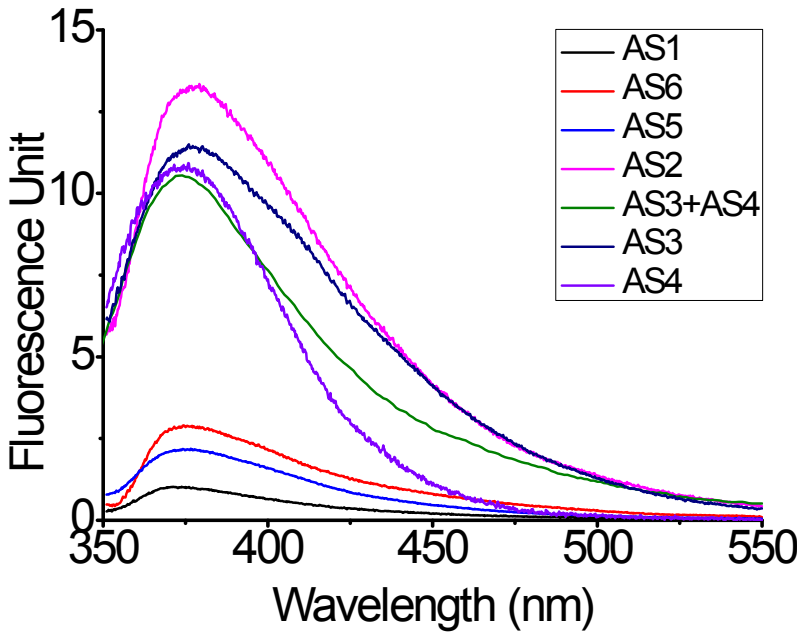

Fig. S1 The Fluorescence emission spectra for Fig. 5.