Supporting Information

Entropy beacon: a hairpin-free DNA amplification strategy for efficient detection of nucleic acids

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Abstract

This Supporting Information provides additional information about the Ebeacon system, including fluorescence kinetics and electrophoresis characterization data and a table containing sequences used in this experiment.

3-hour fluorescence kinetics in low target strand concentrations

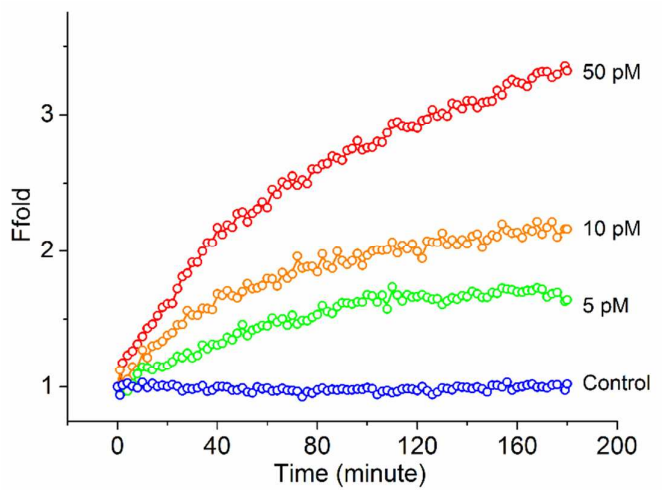

Figure S-1. A 3-hour fluorescence response over background fluorescence at low target strand concentrations. As low as 5 pM target could be distinguished from the background (control, blue dots). The control signal was kept at a stable level during the 3-hour experiment, which further demonstrated the zero-background of the Ebeacon system.

The effect of toehold length on reaction rate

We studied the relationship between toehold length and reaction rate. Previous reports have shown that the kinetics of strand displacement can be accurately modeled and predicted from the length and sequence of the toehold domain. In detail, characterization of the kinetics of strand displacement reactions showed an exponential dependence of kinetics on the length of the toehold. The entropy beacon presented here was also toehold-mediated, allowing us to study the relationship between toehold length and reaction rate. To accomplish this, synthesized target sequences with different toehold lengths, range from 0 nt to 6 nt, were used to initiate the Ebeacon system. Results showed that the reaction rate increases as toehold length progresses from 0 to 6. For toehold length less than 4 nt, the reaction rate was too low to be observed (Figure S2).

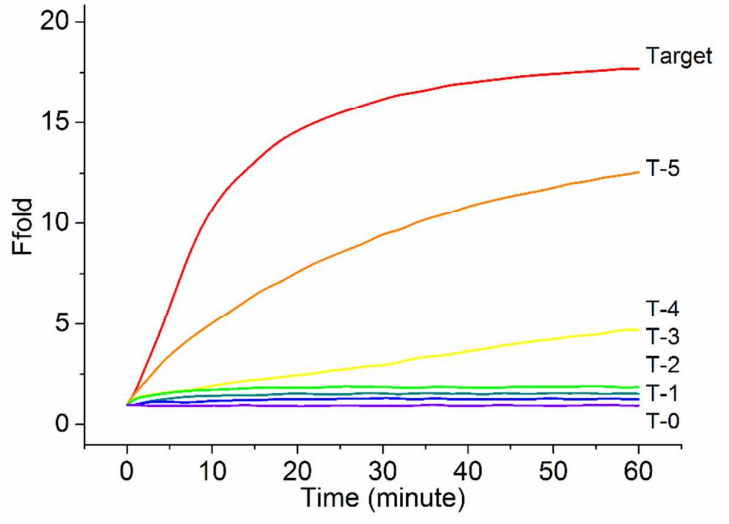

Figure S-2. Fluorescence kinetics of Ebeacon with different toehold lengths. The reaction rate decreased rapidly as toehold length decreased from 6 to 0. For reactions of toehold length shorter than 4 nt, the reaction rate was negligible.

The effect of fuel strand concentration on reaction rate

To clearly show the effect of fuel strand concentration on reaction rate, we chose a 10 nM Ebeacon system with 0.1× target strand (1 nM) and different concentrations of fuel strand (Figure S3). Results showed that the concentration of fuel strand could change the reaction rate over a wide range, indicating the potential tunability of Ebeacon. For systems with fuel strand concentration less than 18 nM (from 0× to 1.8×, compared with beacon complex), the reaction rate was demonstrated to have a positive correlation with fuel strand concentration (Figure S3a). In contrast, for systems with fuel strand concentration more than 18 nM (over 1.8×, compared with beacon complex), excess fuel strand concentration seemed to hinder the catalytic reaction (Figure S3b).

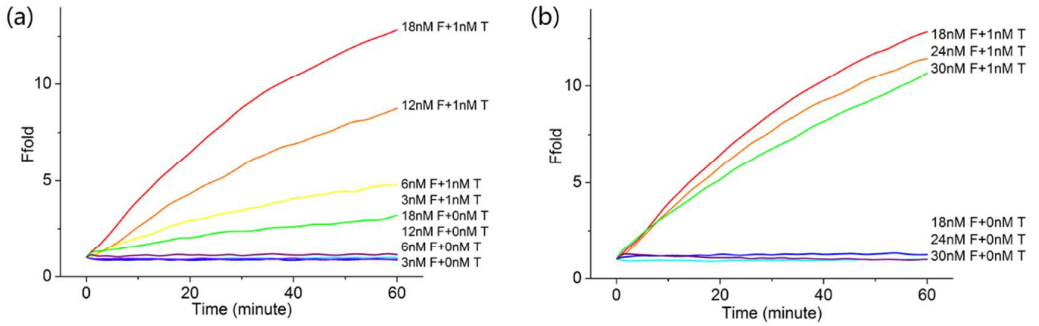

Figure S-3. The effect of fuel strand concentration on reaction rate. a) Ebeacon system with fuel strand concentration less than 18 nM. b) Ebeacon system with fuel strand concentration more than 18 nM. Results showed that the concentration of fuel strand could change the reaction rate over a wide range, indicating the potential tunability of Ebeacon. For systems with fuel strand concentration less than 18nM (From 0× to 1.8×, compared with beacon complex), the reaction rate was demonstrated to have a positive correlation with fuel strand concentration. For systems with fuel strand concentration more than 18nM (over 1.8×, compared with beacon complex), excess fuel strand concentration was shown to restrain catalytic reaction.

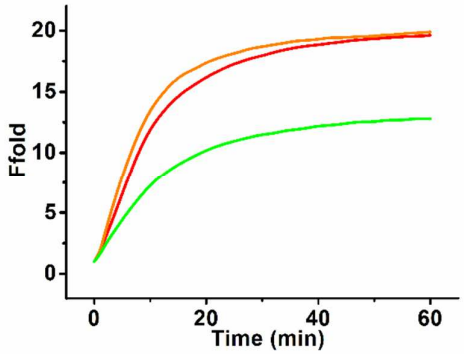

Figure S-4. Fluorescence kinetics of Ebeacon system at different temperatures: 25 °C (red line), 35 °C (orange line), 50 °C (green line). The obviously restrained reaction rate shown in green line is probably due to the accelerated dissociation reaction rate at toehold domain at 50 °C.

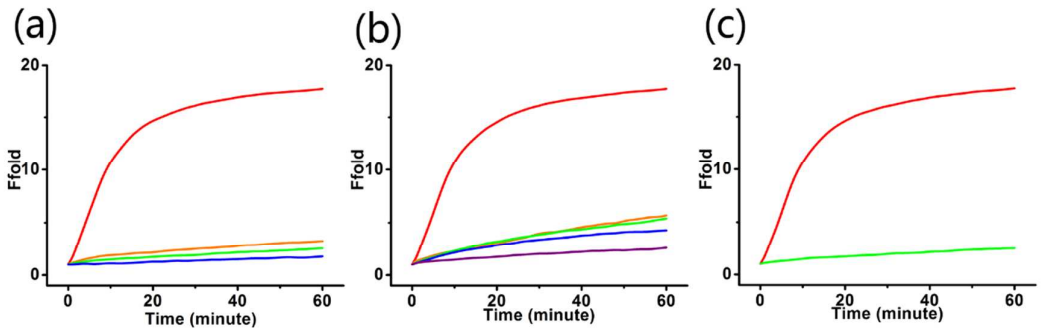

Figure S-5. Fluorescence kinetics of Ebeacon system in the presence of matched, mismatched, deleted, and inserted targets. a) Selectivity toward single base mismatch. Red line: target; orange line: mG; green line: mT; blue line: mC. b) Selectivity toward single base insertion. Red line: target; orange line iT; green line: iA; blue line: iC; purple line: iG. c) Selectivity toward single base deletion. Red line: target; green line: dA.

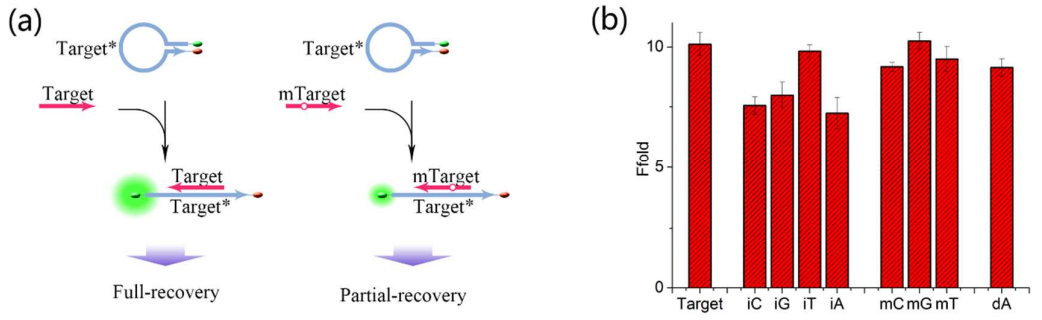

Figure S-6. SNP detection of target strand using molecular beacon. (a) Scheme of SNP detection using molecular beacon. (b) The fluorescence experiment showed that molecular beacon-based detection could not effectively distinguish SNP.

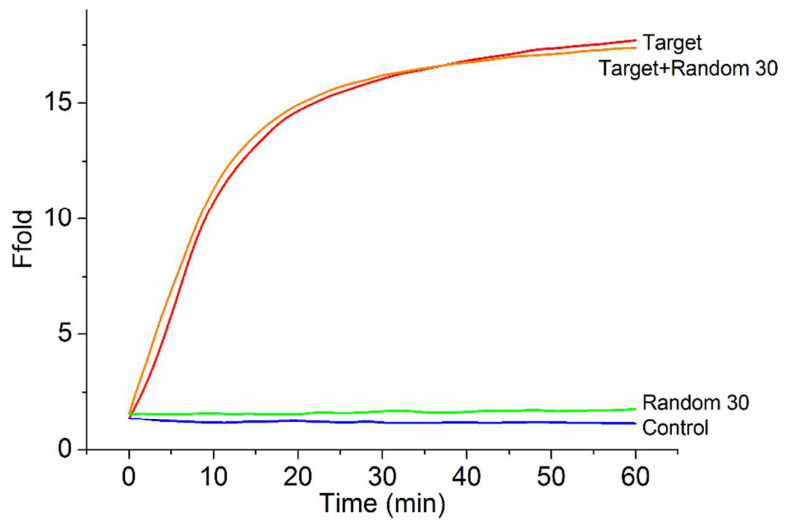

Figure S-7. Ebeacon system in a complex environment consisting of 10-fold excess of 30 nt DNA library. Red line: 10 nM beacon complex + 12 nM fuel strand + 10 nM target strand. Orange line: 10 nM beacon complex + 12 nM fuel strand + 10 nM target strand + 100 nM 30 nt random strand. Green line: 10 nM beacon complex + 12 nM fuel strand + 100 nM 30 nt random strand. Blue line: 10 nM beacon complex + 12 nM fuel strand.

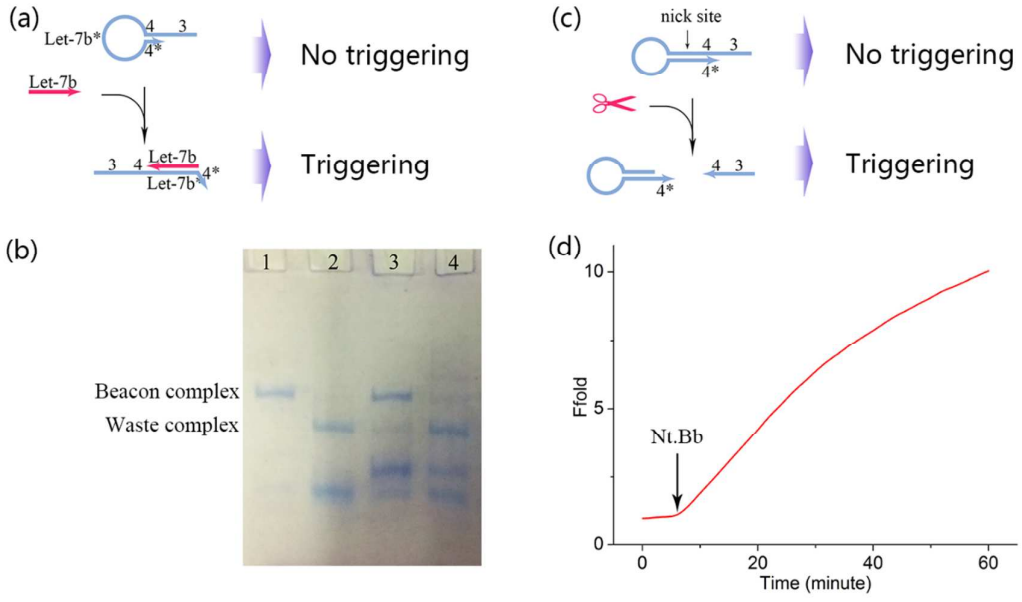

Figure S-8. Two kinds of triggering mechanisms: hybridization and hydrolysis. (a) Scheme of microRNA-triggered signal amplification. MicroRNA let7b was chosen as target sequence in this experiment. (b) Lane 1: beacon complex + fuel strand. Lane 2: beacon complex + fuel strand +

target strand. Lane 3: beacon complex + fuel strand + hp-let7b. Lane 4: beacon complex + fuel strand + hp-let7b + let7b. (c) Scheme of nick enzyme-triggered signal amplification. (d) Fluorescence kinetics monitoring the nick enzyme-triggered signal amplification.

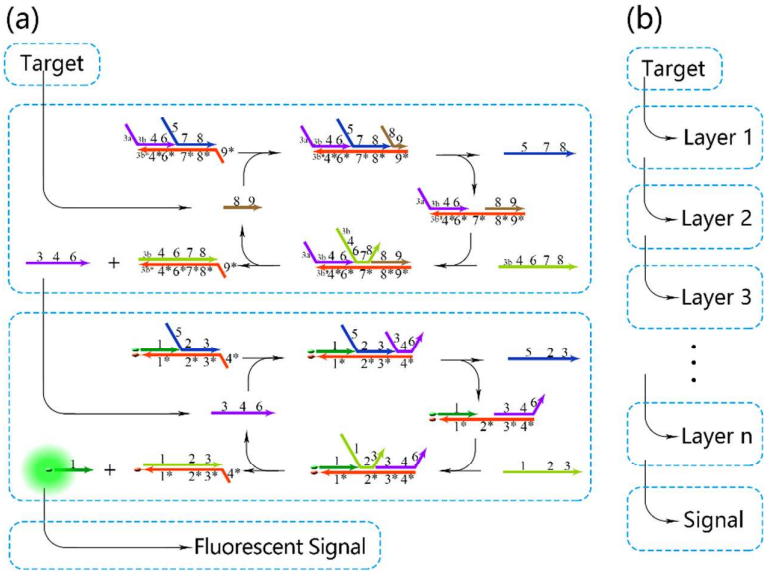

Figure S-9. Potential of constructing cascading amplification platform. (a) An example of two-layer cascading Ebeacon system. (b) A theoretical n-layer cascading Ebeacon system.

Table S-1. Different DNA hybridization and catalysis methods for nucleic acids detection.

Amplification Method	Detection Limit	Reaction Time	Ref
DNA kissing hairpin complex	200 pM	2 h	24
CHA-based bimolecular beacons	10 pM	2 h	26
Autonomous assembly of polymers consisting of DNAzyme wires	10 fM	12 h	28
Target-catalyzed dynamic assembly-based pyrene excimer switching	10 pM	3 h	29
Nonlinear hybridization chain reaction	150 pM	30 min	37
Zn^{2+}-ligation DNAzyme-driven enzymatic and nonenzymatic cascades	10 pM	5 h	39
Autonomous ligation DNAzyme machinery	10 pM	3 h	40
Zn^{2+}-dependent DNAzyme based amplified biosensing platforms	20 pM	30 min	41
Entropy beacon	5 pM	Less than 3h	Present study

Table S-2. Sequences used in the experiment.

Name	Sequence
R	5’-FAM-CCTACGTCTCCAACTAACTTACGG-3’
Q	5’-TGGAGA_CGTAGGGTATTGAATG_AGGG_CCGTAAGTTAGTTGGAGA CGTAGG-Dabcyl-3’
P	5’-TTTTTTTTTTTTTTTT_CCCT_CATTCAATACCCTACG-3‘
F	5’-CCTACGTCTCCAACTAACTTACGG_CCCT_CATTCAATACCCTACG-3’
T	5’-CATTCAATACCCTACG_TCTCCA-3’
T-5	5’-CATTCAATACCCTACG_TCTCC-3’
T-4	5’-CATTCAATACCCTACG_TCTC-3’
T-3	5’-CATTCAATACCCTACG_TCT-3’
T-2	5’-CATTCAATACCCTACG_TC-3’
T-1	5’-CATTCAATACCCTACG_T-3’
T-0	5’-CATTCAATACCCTACG-3’
M-T	5’-CATTCATTACCCTACG_TCTCCA-3’
M-C	5’-CATTCACTACCCTACG_TCTCCA-3’
M-G	5’-CATTCAGTACCCTACG_TCTCCA-3’
I-T	5’-CATTCATATACCCTACG_TCTCCA-3’
I-A	5’-CATTCAAATACCCTACG_TCTCCA-3’
I-C	5’-CATTCACATACCCTACG_TCTCCA-3’
I-G	5’-CATTCAGATACCCTACG_TCTCCA-3’
D-A	5’-CATTCATACCCTACG_TCTCCA-3’
S	5’-TGGAGA_CGTAGGGTATTGAATG-FAM-3‘
C	5‘-Dabcyl-CATTCAATAC_CCTACG-3‘
I	5’-CATTCAATACCCTACG_TCTCCA-3’
mI	5’-CATTCACTACCCTACG_TCTCCA-3’
mC	5‘-Dabcyl-CATTCTATAC_CCTACG-3‘
Let 7b	5’-UGAGGUAGUAGGUUGUGUGGUU-3’
Hp-Let 7b	5’-CATTCAATACCCTACG_TCTCCA AACCACACAACCTACTACCTCAT TGGAGACGT-3’

Hp-nick	5’-CATTCAATACCCTACG_TCTCCA CCTCAGC CAC_TTTTTTTTTTTTTTT_GTG GCTGAGG TGGAGA CG-3’
MB for Target	5’-Dabcyl-GGTTG_TGGAGACGTAGGGTATTGAATG_CAACC-FAM-3’

Mutant bases are marked in red. Domains are separated with underline.