## Electronic Supplementary Information

# Isothermal double-cycle catalytic system using DNAzyme and RNase H for highly selective one-pot detection of oligonucleotides

Ran An,[a,b,c] Hayato Kawai,[c] Hiroyuki Asanuma,*[c] Makoto Komiyama[a] and Xingguo Liang*[a,b]

[a]College of Food Science and Engineering, Ocean University of China, Qingdao, 266003, P. R. China

[b]Laboratory for Marine Drugs and Bioproducts, Qingdao National Laboratory for Marine Science and Technology, Qingdao, 266235, P. R. China

[c]Graduate School of Engineering, Nagoya University, Nagoya, 464-8603, Japan

* To whom correspondence should be addressed.

Email: liangxg@ouc.edu.cn; asanuma@chembio.nagoya-u.ac.jp

**Tab. S1** Oligonucleotides used in this study

| Name | Sequences (5'→3') | Length |
|---|---|---|
| sl-MB | E-TTCTGCTAG**rArU**TACCTGTAT-QQ[1,2] | 20 nt |
| target DNA | TTACCTGTATTTCTGCTAG[3] | 19 nt |
| target-1mis | TTACCTGTAC<u>T</u>TCTGCTAG[4] | 19 nt |
| target-2mis | TTACCTGT<u>GC</u>TTCTGCTAG[4] | 19 nt |
| target-3mis | TTACCTGT<u>GCC</u>TCTGCTAG[4] | 19 nt |
| target RNA | **rCrUrArGrCrArGrArArArUrArCrArGrGrUrArArCrUrArU**[2] | 23 nt |
| stem-loop primer | GTCGTATCCAGTGCAGGGTCTGACTTATTCGCACTGGATACGACATAGTTACC | 53 nt |
| l-Dz$^{pre6}$ | TACAACGACTAGCA**rGrArArArUrA**CAGGTAAGGCTAGC[2,5] | 34 nt |
| Dz$^{act6}$-I | AATACAGGTAAGGCTAGCTACAACGACTAGCAGA | 34 nt |
| Dz$^{act6}$-II | ATACAGGTAAGGCTAGCTACAACGACTAGCAGAA | 34 nt |
| Dz$^{act6}$-III | TACAGGTAAGGCTAGCTACAACGACTAGCAGAAA | 34 nt |
| Dz$^{act6}$-IV | ACAGGTAAGGCTAGCTACAACGACTAGCAGAAAT | 34 nt |
| Dz$^{act6}$-V | ACAGGTAAGGCTAGCTACAACGACTAGCAGA | 31 nt |
| F-3R | F-TTATGATCCACTACAACCTAGCAGA**rArArU**ACAGGTAAGCTAGACAACC[2,6] | 47 nt |
| F-4R | F-TTATGATCCACTACAACCTAGCAG**rArArArU**ACAGGTAAGCTAGACAACC[2,6] | 47 nt |
| F-5R | F-TTATGATCCACTACAACCTAGCA**rGrArArArU**ACAGGTAAGCTAGACAACC[2,6] | 47 nt |
| F-6R | F-TTATGATCCACTACAACCTAGCA**rGrArArArUrA**CAGGTAAGCTAGACAACC[2,6] | 47 nt |
| F-28 | F-TTATGATCCACTACAACCTAGCAGAAAT[6] | 28 nt |
| F-27 | F-TTATGATCCACTACAACCTAGCAGAAA[6] | 27 nt |
| p-20 | TACAGGTAAGCTAGACAACC[5] | 20 nt |
| p-19 | ACAGGTAAGCTAGACAACC[5] | 19 nt |

[1]E = perylene; Q = anthraquinone. The MB was synthesized by a DNA synthesizer according to previous report. (*Murayama, et al., Chembiochem, 2015, 16, 1298-1301*)

[2]Bold parts are ribonucleotides.

[3]The target sequence RNDzSA was chosen from NS1 gene of Human parvovirus B19.

[4]Mismatching bases to Dz$^{pre6}$ are underlined.

[5]Phosphorylated strands.

[6]F = FAM.

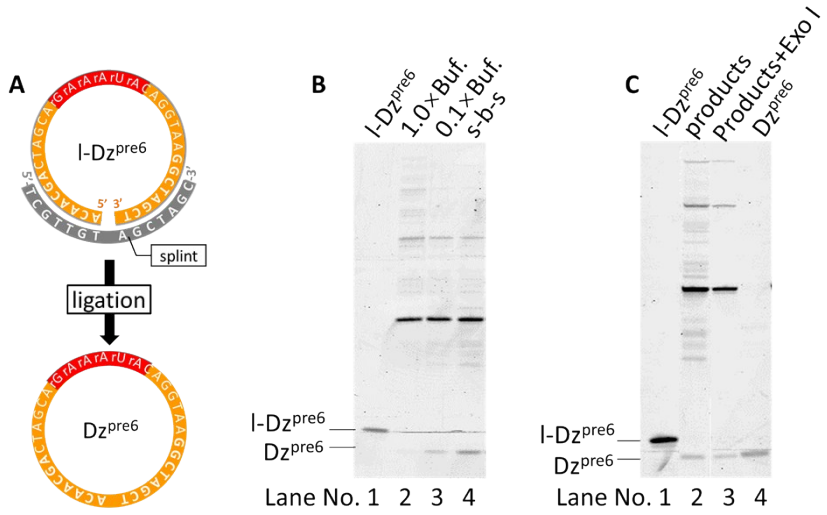


**Fig. S1** Preparation and purification of $Dz^{pre6}$. (A) Sequences of l-$Dz^{pre6}$ (linear form of $Dz^{pre6}$), $Dz^{pre6}$ and splint. (B) Cyclization under conventional conditions of 1× T4 ligase buffer (lane 2), 0.1× T4 ligase buffer (lane 3) and "step by step" method (lane 4), respectively. [l-$Dz^{pre6}$] = 10 μM, [splint] = 20 μM, 60 U T4 DNA ligase in 240 μL, at 25°C. (C) Purified $Dz^{pre6}$ after Exonuclease I digestion (lane 3) and gel extraction (lane 4). We used the "step by step" method according to the previous report (*An, et al., Nucleic Acids Res., 2017, 45, e139*).

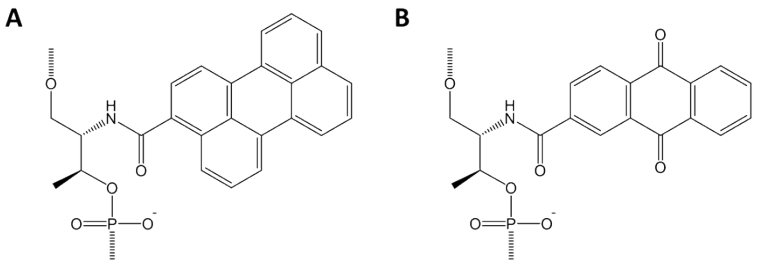


**Fig. S2** Chemical structures of the units involving perylene (E) and anthraquinone (Q). (A) perylene; (B) anthraquinone. The synthesis was carried out according to the previous report (*Murayama, et al., Chembiochem, 2015, 16, 1298-1301*).

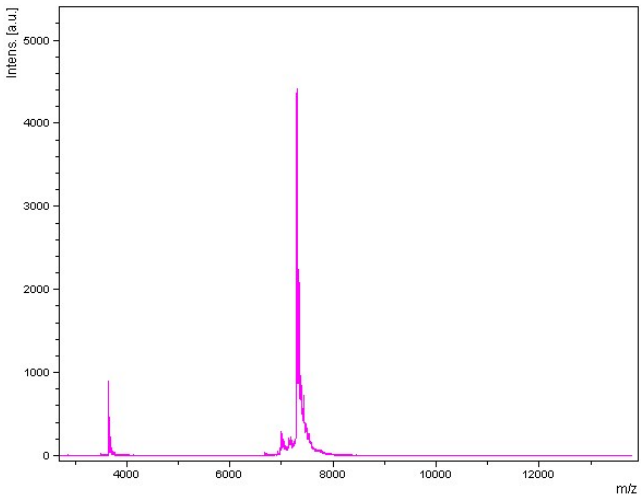


**Fig. S3** Mass spectrum of sl-MB. The sequence of sl-MB is E-TTCTGCTAGrArUTACCTGTAT-QQ, and its molecular weight is 7335.34.

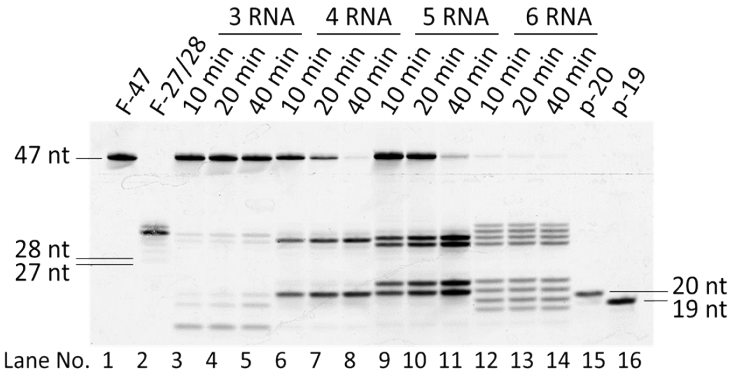


**Fig. S4** SYBR Green II staining analysis of RNase H-digestion of DNA-RNA-DNA strands. In order to detect all the scission products, the results of Fig. 1B were further analyzed by staining with SYBR Green II. Lane 1, 47-nt standard strand with FAM at 5'-end; lane 2, 28-nt and 27-nt standard strands (5'-FAM); lane 15, 20-nt standard strands (5'-phosphorylated); lane 16, 19-nt standard strands (5'-phosphorylated). Lanes 4-14 show the results of RNase H digestion. Lanes 3-5, 3-nt RNA portion (F-3R); lanes 6-8, 4-nt RNA portion (F-4R); lanes 9-11, 5-nt RNA portion (F-5R); lanes 12-14, 6-nt RNA portion (F-6R). [DNA-RNA-DNA] = 1 μM. [DNA-RNA-DNA]:[target DNA] = 1:1 in lanes 3-5, and = 10:1 for the others. Other conditions: 1 U RNase H in 20 μL, at 37°C.

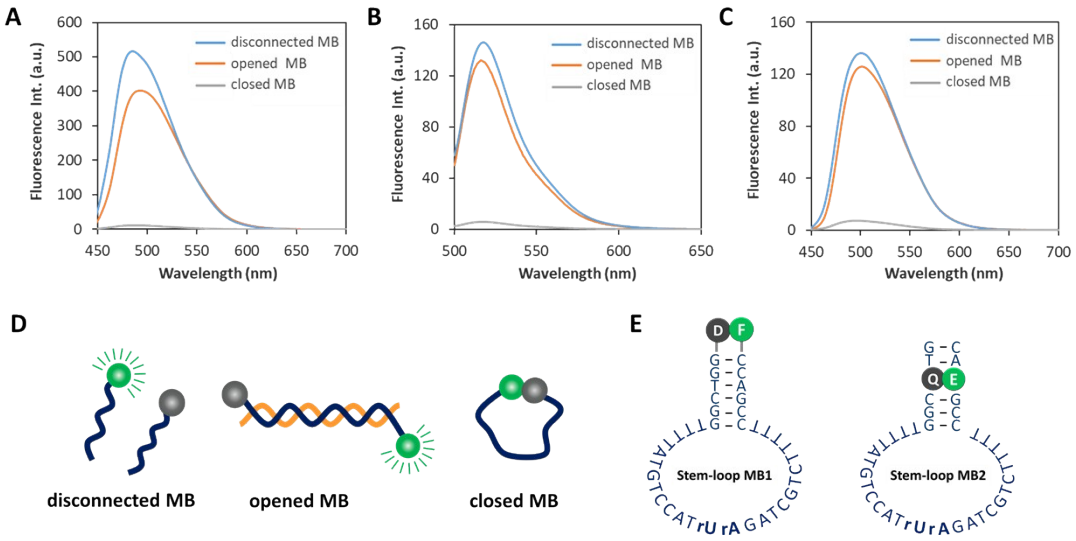


**Fig. S5** Fluorescent features of the stemless MB (A) and two stem-loop MBs (B and C). (A) Emission spectra of the stemless MB (sl-MB), used in the present study in Fig. 2. $\lambda_{ex}$ = 426 nm. (B) Emission spectra of Stem-loop MB1 in (E). $\lambda_{ex}$ = 495 nm. (C) Emission spectra of Stem-loop MB2 in (E). $\lambda_{ex}$ = 426 nm. (D) Structures of "disconnected MB" (blue lines in (A), (B), and (C)), "opened MB" (orange lines), and "closed MB" (grey lines). In "disconnected MB", MBs were disconnected to two fragments by RNase $I_f$ (a single strand specific RNA endonuclease). The "opened MB" was formed by competitive binding of a complementary strand. The "closed MB" was the one in the quenching state. (E) Sequences of the stem-loop MBs (F = FAM, D = Dabcyl; E = perylene, Q = anthraquinone). [MBs] = 1 μM in pH 7 buffer ([NaCl] = 100 mM and [sodium phosphate] = 10 mM). Stem-loop MB2 and sl-MB were synthesized according to Murayama's report (*Murayama, et al., Chembiochem, 2015, 16, 1298-1301*). Stem-loop MB1 was ordered from Integrated DNA Technologies (Coralville, IA, USA).

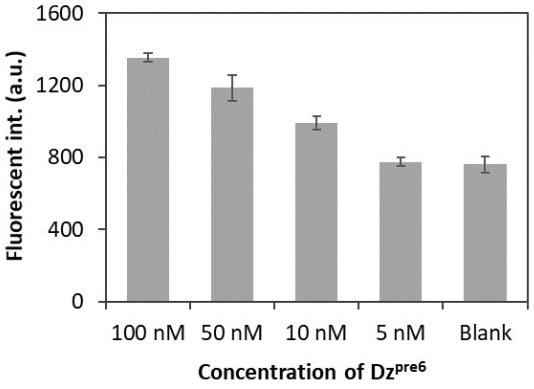


**Fig. S6** Disconnection of sl-MB by various concentrations of $Dz^{pre6}$. [sl-MB] = 200 nM and [$Mg^{2+}$] = 20 mM at 37°C for 1 h. $\lambda_{ex}$ = 426 nm, $\lambda_{em}$ = 500 nm.

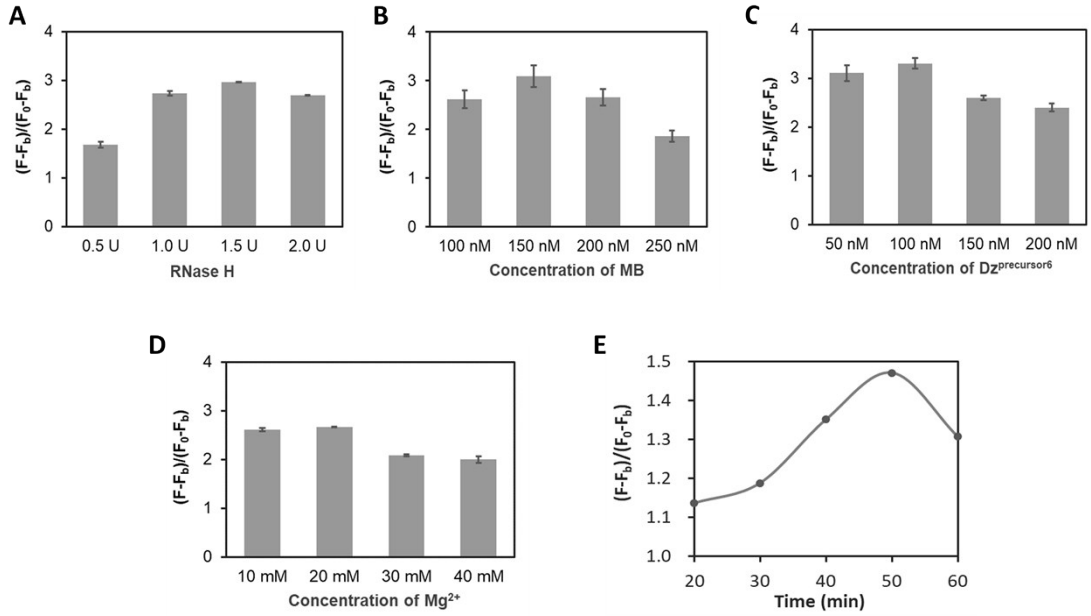


**Fig. S7** RNDzSA reactions under different conditions. (A) Various amounts of RNase H. [target DNA] = 0.5 nM, [sl-MB] = 200 nM, [$Dz^{pre6}$] = 50 nM, [$Mg^{2+}$] = 20 mM, 37°C for 1 h. (B) Various concentrations of sl-MB. [target DNA] = 0.5 nM, [$Dz^{pre6}$] = 50 nM, [$Mg^{2+}$] = 20 mM, 1.5 U RNase H in 50 μL, 37°C for 1 h. (C) Various concentrations of $Dz^{pre6}$. [target DNA] = 0.5 nM, [sl-MB] = 200 nM, [$Mg^{2+}$] = 20 mM, 1.5 U RNase H in 50 μL, 37°C for 1 h. (D) Various concentrations of $Mg^{2+}$. [target DNA] = 0.5 nM, [sl-MB] = 150 nM, [$Dz^{pre}$] = 50 nM, 1.0 U RNase H in 50 μL, 37°C for 1 h. (E) Various reaction times. [target DNA] = 10 pM, [sl-MB] = 200 nM, [$Dz^{pre}$] = 100 nM, [$Mg^{2+}$] = 20 mM, 1.0 U RNase H in 50 μL, 37°C. $\lambda_{ex}$ = 426 nm, $\lambda_{em}$ = 500 nm. Error bars were obtained from three parallel experiments.