Electronic Supplementary Information (ESI)

Stimuli-responsive DNAzyme displaying Boolean logic-gate responses

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Contents:

1. Experimental generals. Unless stated otherwise, all commercial reagents were used as received. Human *O*^{6}-methylguanine-DNA-methyltransferase (MGMT) protein (His tag, Recombinant) was purchased from Antibodies-online GmbH (ABIN805634) through Funakoshi (Japan). MGMT was used without further purifications and its concentration was evaluated on the basis of molar extinction coefficient calculated from the sequence. The unmodified 8-17 DNAzyme (**8-17Dz**), the substrate with a single ribonucleotide at the cleavage site bearing fluorescein (6-FAM) at 5´ end, and the other oligonucleotides were ordered from *Fasmac* Corp. (Kanagawa, Japan). The concentration of the oligonucleotides was determined by absorbance at 260 nm. All water used in the experiments refers to ultra-pure water obtained from a Millipore system having a specific resistance of 18 MΩ•cm. IP RP HPLC was conducted with a Shimadzu Prominence instrument LC-20AD and SPD-20A equipped with a GL Science Inertsil ODS-3 column (150 mm × 4.6 mm I. D., 5 μm) for analysis and GL Science Inertsil ODS-3 column (150 mm × 10 mm I. D. , 5 μm) for purifications in a column oven CTO-20A. Matrix-assisted laser desorption/ionization (MALDI) coupled with time-of-flight (TOF) mass spectra were recorded using a Shimadzu AXIMA-CFR plus mass spectrometer. UV-vis spectra were obtained using a Jasco V-630 or a Shimadzu UV-2450 spectrophotometer.

2. Solid-phase oligonucleotide synthesis. Oligonucleotide synthesis of **8-17Dz^{NB}** was carried out by using an NTS H-6 DNA/RNA synthesizer (Nihon Techno service) using phosphoramidite method (commercially available β-cyano phosphoramidites with synthesized **dG^{NB}** phosphoramidite[1]) at 1 μmol scale. After cleavage from the solid support, deprotection of bases and phosphates was performed in aqueous ammonia (1.0 mL, 28%) at 55 ºC for 12 h. The purification of the "trityl-on" oligonucleotide was carried out on IP RP HPLC {GL Science Inertsil ODS-3 column (150 mm × 10 mm I. D.), linear gradient from A:B = 100:0 (A: 0.1 M TEAA containing 5% acetonitrile, B: acetonitrile) to 50:50 over 30 min was used with a flow rate of 3.0 mL/min, detection wavelength = 260 nm}. The purified "trityl-on" oligonucleotide was treated with 80% CH_3COOH for 15 min at 37 ºC to remove the dimethoxytrityl residues. The detritylated oligomer was purified by IP RP HPLC {GL Science Inertsil ODS-3 column (150 mm × 10 mm I. D.), linear gradient from A:B = 100:0 (A: 0.1 M TEAA containing 5% acetonitrile, B: acetonitrile) to 50:50 over 30 min was used with a flow rate of 3.0 mL/min, detection wavelength = 260 nm}. IP RP HPLC charts were shown in **Figure S1**. The identity of the oligonucleotides **8-17Dz^{NB}** has been established by MALDI-TOF MS {matrix: 3-hydroxypicolinic acid (3-HPA), negative, **Figures S2**}, and the observed molecular weights were in good agreement with their structures: **8-17Dz^{NB}**, Calcd. For $[M - H]^-$: 10234.7 Found: 10220.4. The oligonucleotide concentrations were determined by UV spectroscopy. The molar extinction coefficient (at 260 nm) ratio of **dG^{NB}/dG** (= 1.15) was used.

Table S1. Sequences of oligonucleotides used in this study.

Abbreviations	Sequences (from 5′ to 3′)
8-17Dz[1]	CATCTCTTC**TCCGAGCCGG TCGAA**ATAGTGAGT: 33 nt
8-17Dz^{NB}[1]	CATCTCTTC**TCCGAGCCGG^{NB}TCGAA**ATAGTGAGT: 33 nt
F-Substrate[2]	FAM-ACTCACTATa↓GGAAGAGATG: 20 nt
Product	FAM-ACTCACTATa: 10 nt

[1]Catalytic core is highlighted in bold (stem part is shown in blue color). [2] Small capital in the sequence of substrate represents ribonucleotide. Cleavage site of substrate is shown by an arrow.

3. Characterization of oligonucleotides

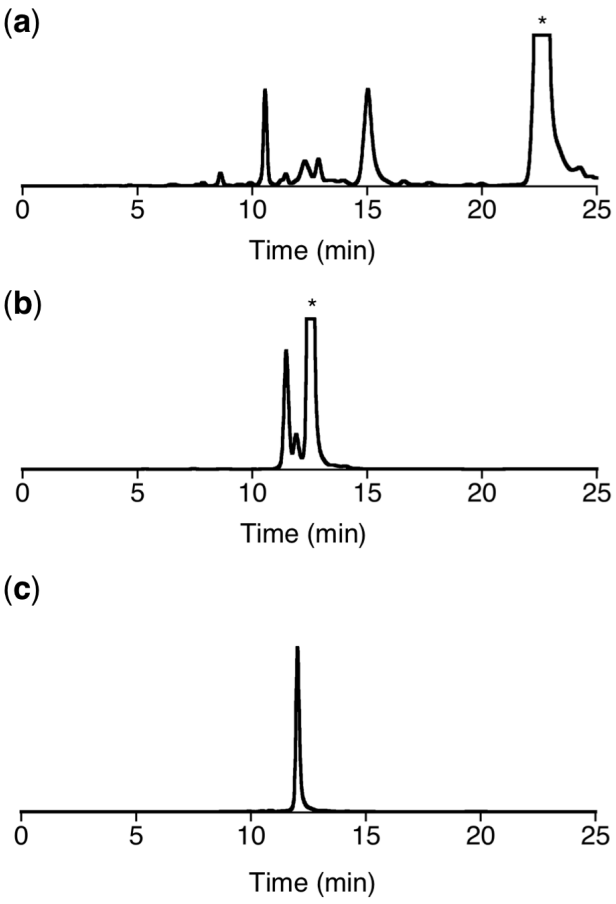

Figure S1. HPLC charts before (**a**: after aqueous ammonia (28%) treatment, **b**: after aqueous 80% CH_3COOH treatment) and after purification (**c**) of **8-17Dz^{NB}**.

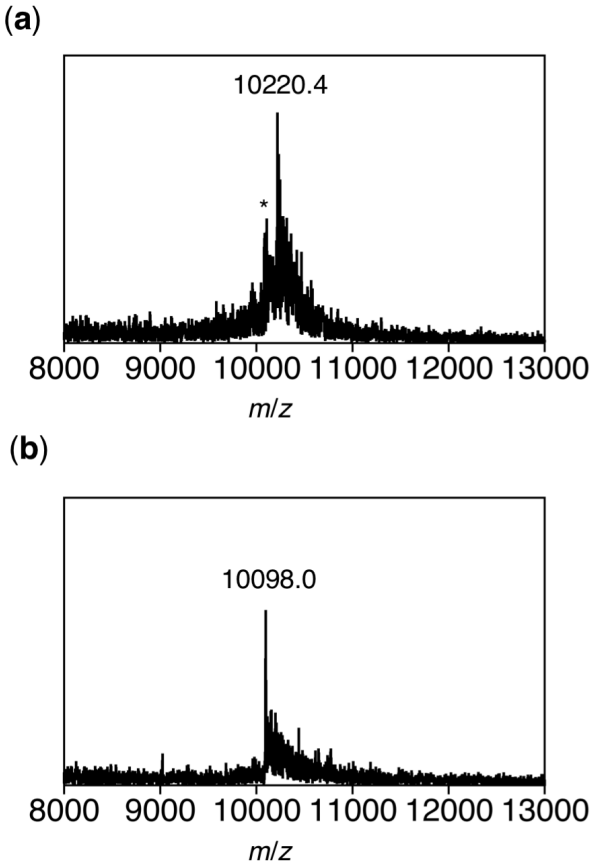

Figure S2. MALDI-TOF MS spectra (3-HPA as matrix, negative mode) of (**a**) **8-17Dz^{NB}** and (**b**) **8-17Dz**. The asterisk in chart (**a**) denotes a peak most probably due to the removal of the 4-nitrobenzyl (NB) group during UV laser-induced ionization.[1]

4. In vitro substrate cleavage assay. The cleavage reactions were performed under single-turnover conditions. The reactions were carried out under the conditions (Reaction mixture: 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [**8-17Dz** or **8-17Dz^{NB}**] = 25 nM, [substrate] = 25 nM, [$Zn(NO_3)_2$] = 50 μM, [$Na_2S_2O_4$] = 2.0 mM, 37 °C). Typically, reaction samples were incubated at 37 ºC and quenched upon the addition of an urea loading buffer (8 M urea, 500 mM EDTA•2Na 10% glycerol). Samples were loaded onto 8 M urea 20% PAGE gels (6 cm × 6 cm compact gels; ATTO Inc., Tokyo, Japan), which were run at 24 W for 30 minutes at room temperature in TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2 mM EDTA•2Na, pH 8.0). The gels were imaged using by ImageQuant LAS 4000 (GE Healthcare) equipped with a blue epi light (460 nm) and Y515 filter (Epi-B set). The cleaved product and uncleaved substrate were quantified and analyzed by Image J software (freely available at https://imagej.nih.gov/ij/index.html).

5. Reduction responsive property

5.1 Monitoring reduction responsive structural change by IP RP HPLC. The reductant ($Na_2S_2O_4$) was added to adjust 2.0 mM as the final concentrations in an aqueous buffer containing **8-17Dz^{NB}** (25 nM) with substrate (25 nM). After designated incubation time, the reaction mixture was immediately desalted to remove of $Na_2S_2O_4$ by a Sep-pak cartridge (Waters) and the eluent was concentrate by approximately 20 folds, which was subjected to IP RP HPLC analysis (GL Science Inertsil ODS-3 column (150 mm × 4.6 mm I. D., 5 μm), linear gradient from A:B = 100:0 (A: 0.1 M TEAA containing 5% acetonitrile, B: acetonitrile) to 50:50 over 30 min was used with a flow rate of 1.0 mL/min, detection wavelength = 260 nm). The identity of the starting oligonucleotides (**8-17Dz^{NB}**) and the desired reduction product (**8-17Dz**) were established by comparing the retention time of authentic standard. Experiment was repeated in at least duplicates.

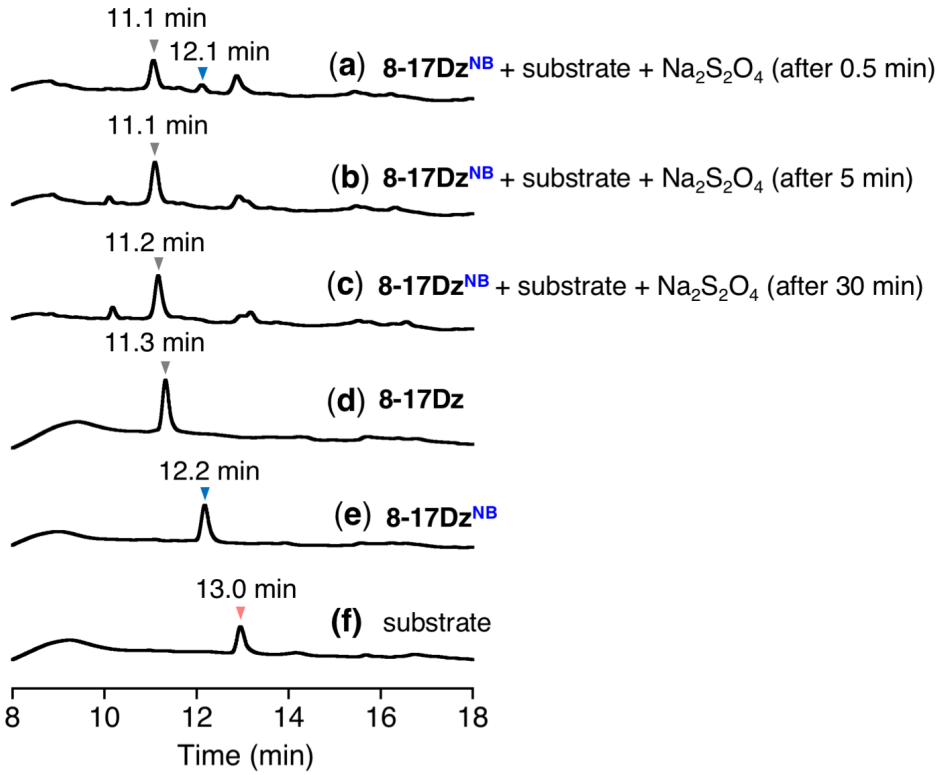

Figure S3. IP RP-HPLC traces (detection wavelength = 260 nm) of **8-17Dz^{NB}** ((**a**) 0.5 min, (**b**) 5 min, and (**c**) 30 min after the addition of $Na_2S_2O_4$) in the presence of substrate, (**d**) **8-17Dz**, (**e**) **8-17Dz^{NB}**, and (**f**) substrate. *Conditions*: 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [**8-17Dz** or **8-17Dz^{NB}**] = 25 nM, [substrate] = 25 nM, [$Zn(NO_3)_2$] = 50 μM, [$Na_2S_2O_4$] = 2.0 mM, 37 °C.

6. $Na_2S_2O_4$ dependent cleavage activity of 8-17Dz^{NB}

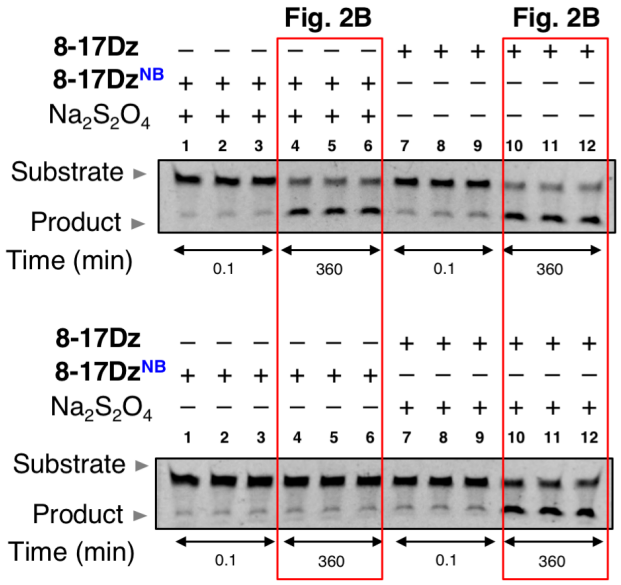

Figure S4. PAGE (20%, denatured) analysis to evaluate substrate cleavage by DNAzymes **8-17Dz** and **8-17Dz^{NB}** with or without the addition of $Na_2S_2O_4$. *Hydrolysis conditions*: 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [**8-17Dz** or **8-17Dz^{NB}**] = 25 nM, [substrate] = 25 nM, [$Zn(NO_3)_2$] = 50 μM, [$Na_2S_2O_4$] = 2.0 mM, 37 °C.

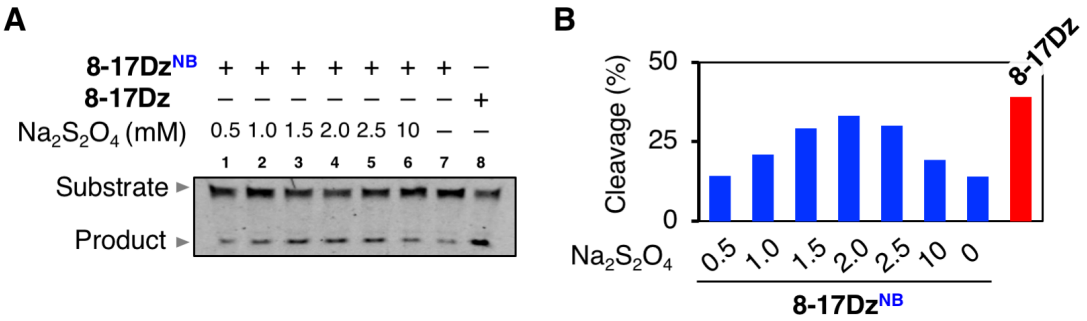

Figure S5. (**A**) PAGE (20%, denatured) analysis (gel image) to evaluate $Na_2S_2O_4$ dependent cleavage of substrate by **8-17Dz^{NB}**. (**B**) $Na_2S_2O_4$ dependent cleavage activity of **8-17Dz^{NB}** (6 h after the addition of $Na_2S_2O_4$). *Hydrolysis conditions*: 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [**8-17Dz** or **8-17Dz^{NB}**] = 25 nM, [substrate] = 25 nM, [$Zn(NO_3)_2$] = 50 μM, 37 °C.

7. Substrate cleavage activity of 8-17Dz

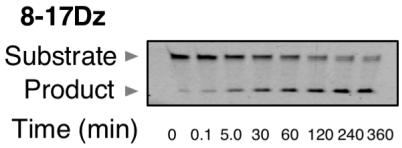

Figure S6. PAGE (20%, denatured) analysis for substrate hydrolysis (single-turnover conditions) by 8-17Dz. See also Fig. 3. *Hydrolysis conditions*: 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [**8-17Dz**] = 25 nM, [substrate] = 25 nM, $[Zn(NO_3)_2]$ = 50 μM, $[Na_2S_2O_4]$ = 2.0 mM, 37 °C.

8. MGMT responsive property and OR logic-gate response

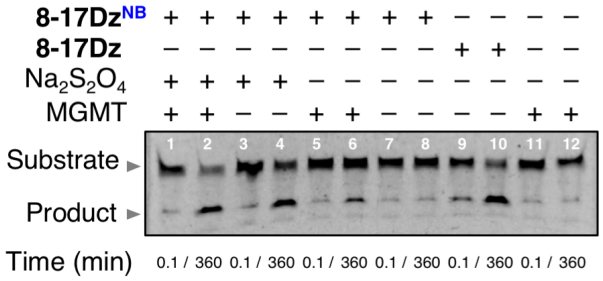

Figure S7. PAGE (20%, denatured) analysis (gel image) to evaluate the cleavage of substrate by the DNAzymes (**8-17Dz** and **8-17Dz^{NB}**) upon the addition of $Na_2S_2O_4$ or MGMT. *Hydrolysis conditions*: 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [**8-17Dz** or **8-17Dz^{NB}**] = 25 nM, [substrate] = 25 nM, $[Zn(NO_3)_2]$ = 50 μM, $[Na_2S_2O_4]$ = 2.0 mM, [MGMT] = 100 nM, 37 °C.

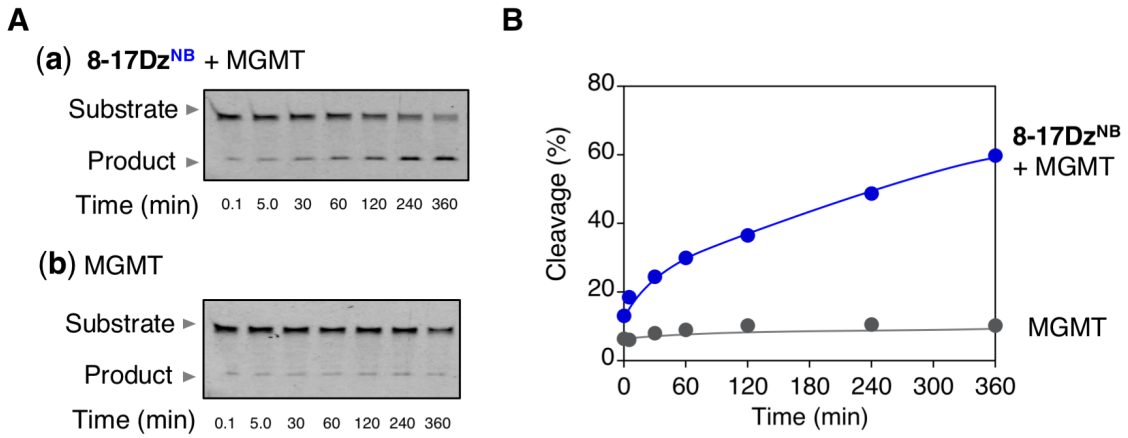

Figure S8. (**A**) PAGE (20%, denatured) analysis (gel images) and (**B**) kinetic plots for hydrolyzing substrate upon the addition of MGMT in the presence (**a**) or absence (**b**) of **8-17Dz^{NB}**. *Hydrolysis conditions*:

50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [**8-17Dz^{NB}**] = 25 nM, [substrate] = 25 nM, [$Zn(NO_3)_2$] = 50 μM, [MGMT] = 100 nM, 37 °C.

9. Zn^{2+} responsive property and AND logic-gate response

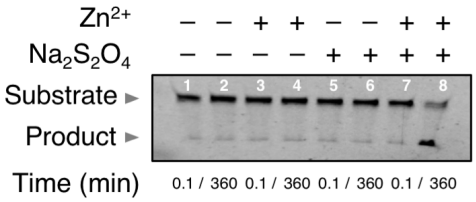

Figure S9. PAGE (20%, denatured) analysis (gel image) to evaluate the cleavage of substrate by the **8-17Dz^{NB}** upon the addition of Zn^{2+} and $Na_2S_2O_4$. *Hydrolysis conditions*: 50 mM Tris-HCl (pH 7.4) containing 100 mM NaCl, [**8-17Dz^{NB}**] = 25 nM, [substrate] = 25 nM, [$Zn(NO_3)_2$] = 50 μM, [$Na_2S_2O_4$] = 2.0 mM, 37 °C.

References

S1. M. Ikeda, M. Kamimura, Y. Hayakawa, A. Shibata and Y. Kitade, *ChemBioChem,* 2016, **17**, 1304–1307.