Electronic Supplementary Information

Metal-dependent activity control of a compact-sized 8-17 DNAzyme based on metal-mediated unnatural base pairing

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Table of Contents

1. Experimental methods

Materials and equipment. All the natural DNA strands and 6-carboxyfluorescein (FAM)-labeled substrates containing a riboadenosine (rA) were purchased from Japan Bio Service Co., Ltd. (Saitama, Japan) at HPLC purification grade. A DNAzyme strand (**DzIm**) containing a 4-carboxyimidazole ($\mathbf{Im}^{\mathbf{C}}$) nucleotides was synthesized on an automated DNA synthesizer and purified by reverse-phase HPLC according to the reported procedure.[S1] The **DzIm** strand was characterized by matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry on Autoflex III (Bruker) using a mixture of 3-hydroxypicolinic acid (3-HPA) and ammonium citrate as a matrix. Metal sources ($CuSO_4 \cdot 5H_2O$ (99.5% purity), $Hg(ClO_4)_2 \cdot 3H_2O$ (99%), $AgNO_3$ (99.8%), $Pb(OAc)_2 \cdot 3H_2O$ (99.9%)) were purchased from FUJIFILM Wako Pure Chemical Industries. Denaturing polyacrylamide gel electrophoresis (PAGE) was carried out with 20% polyacrylamide gel containing 7 M urea. The gels were imaged and analyzed using Gel Doc EZ Imager and Image Lab software (Bio-Rad).

RNA-cleaving reaction by $\mathbf{Im}^{\mathbf{C}}$-modified DNAzyme (DzIm). DNAzyme strands were annealed (85 °C → 25 °C, 1.0 °C/min) in a reaction buffer (50 mM HEPES buffer (pH 7.5), 100 mM NaCl, 10 μM $Pb(OAc)_2$) in the absence or presence of $CuSO_4$ (1.0 equiv). The concentrations of Pb^{II} ions were set to 10 μM to reduce unintended interactions between Pb^{II} and $\mathbf{Im}^{\mathbf{C}}$ nucleobases. The DNAzyme reactions were initiated by the addition of a FAM-labeled substrate strand. The final concentration of each component was as follows: [DNAzyme] = 1.0 μM, [substrate] = 10 μM, [$CuSO_4$] = 0 or 1.0 μM. After incubation at 25 °C, an aliquot of the sample solution was taken at the designated time points. The reaction was stopped by the addition of a loading buffer (5.25 M urea, 1.25 mM EDTA, 7.5% glycerol), and the mixtures were stored at –28 °C until the time course was completed. The cleavage of the substrate was analyzed by denaturing PAGE. The fractions of the cleaved substrate (F) were calculated as follows:

$F\ (\%) = I_c / (I_c + I_u) \times 100,$

where I_c and I_u are the band intensities of the cleaved product and the uncleaved substrate, respectively. The apparent first-order rate constants (k_{obs}) were calculated from the initial reaction rates, which were determined from the time points when F was less than 20%.

RNA-cleaving reactions by DNAzymes DzT and DzC. DNAzyme strands were annealed (85 °C → 25 °C, 1.0 °C/min) in a reaction buffer (50 mM HEPES buffer (pH 7.5), 100 mM $NaNO_3$, 10 μM $Pb(OAc)_2$) in the absence or presence of $Hg(ClO_4)_2$ or $AgNO_3$ (1.0 equiv). Note that NaCl

was replaced with $NaNO_3$ in the standard reaction buffer to prevent the precipitation of $HgCl_2$ and AgCl. The following procedures are the same as that for **DzIm**. The final concentration of each component was as follows: [DNAzyme] = 1.0 μM, [substrate] = 10 μM, $[Hg(ClO_4)_2]$ = $[AgNO_3]$ = 0 or 1.0 μM.

Cu^{II} titration experiment with DzIm DNAzyme. The overall procedures are the same as the DNAzyme reactions described above. The final concentration of each component was as follows: [DNAzyme] = 1.0 μM, [substrate] = 10 μM, $[CuSO_4]$ = 0, 0.5, 1.0, 2.0, or 3.0 μM.

CD spectroscopy. The samples were prepared by combining an equimolar amount of the DNAzyme (8-17 DNAzyme or **DzIm**) and an uncleavable DNA substrate that contains a dA nucleotide instead of the scissile rA, and annealed prior to the measurements (85 °C → 4 °C, 1.0 °C/min). The concentrations of each component were as follows: [DNAzyme] = 1.0 μM, [substrate] = 1.0 μM, $[CuSO_4]$ = 0 or 3.0 μM. The CD spectra were recorded on a JASCO J-820 spectropolarimeter with 10-time accumulation using a path length of 0.5 cm at 4 °C. The spectra were smoothed using a simple moving average smoothing program.

2. Supporting figures

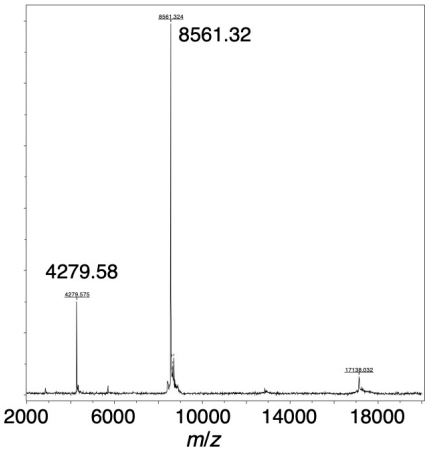

Figure S1. MALDI-TOF mass spectrum of the synthesized Im^C-modified 8-17 DNAzyme (**DzIm**). Negative mode. Observed: 8561.32; calcd for $[M - H]^-$: 8560.33.

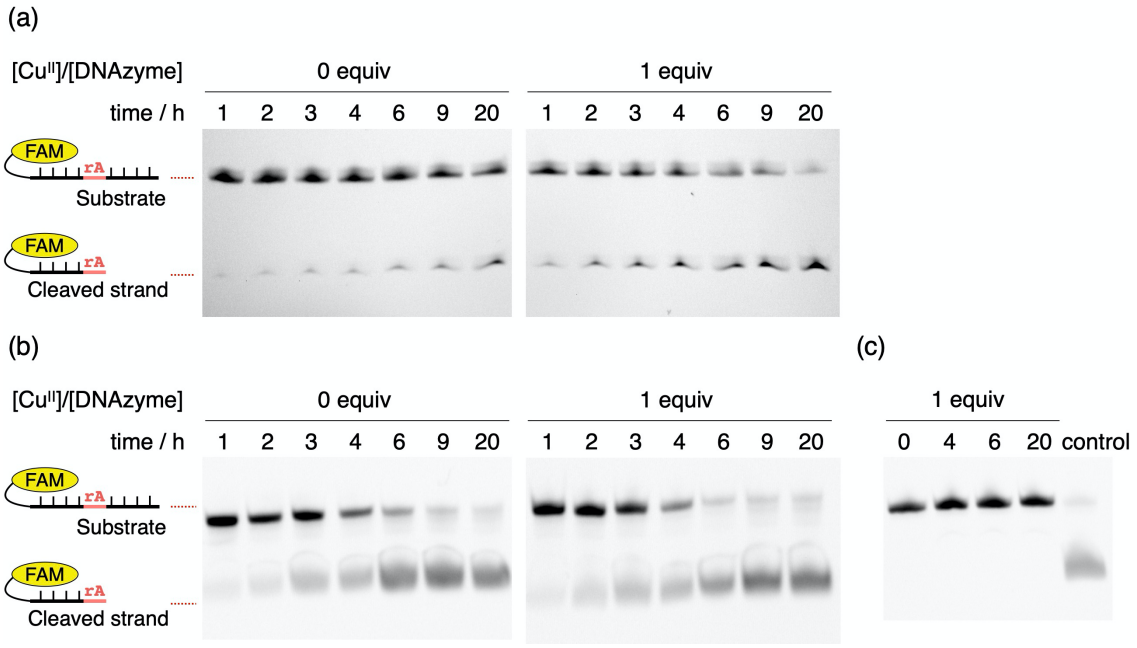

Figure S2. Denaturing polyacrylamide gel electrophoresis (PAGE) of the RNA-cleaving reactions (a) catalyzed by the **Im**^{C}-modified 8-17 DNAzyme (**DzIm**), (b) catalyzed by the original 8-17 DNAzyme, and (c) without DNAzymes. [DNAzyme] = 1.0 μM, [substrate] = 10 μM in 50 mM HEPES (pH 7.5), 100 mM NaCl, 0.01 mM $Pb(OAc)_2$, 25 °C. 20% polyacrylamide gel containing 7 M urea, detected by FAM fluorescence. The results are summarized in Fig. 3 in the main text.

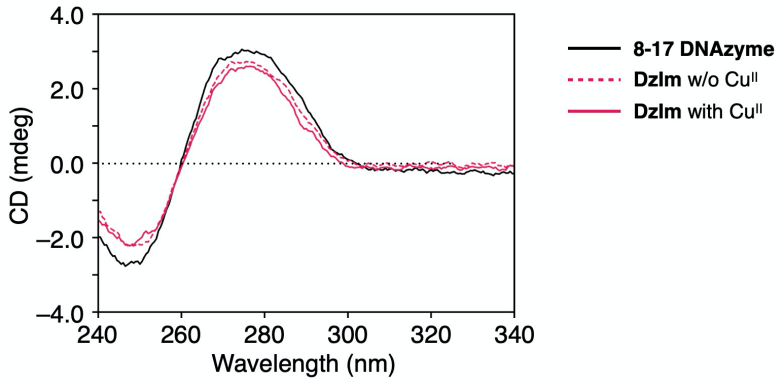

Figure S3. CD spectra of 8-17 DNAzyme and **DzIm** in the presence of an uncleavable DNA substrate containing a dA instead of the scissile rA. [DNAzyme] = 1.0 μM, [DNA substrate] = 1.0 μM, [Cu^{II}] = 0 or 1.0 μM in 50 mM HEPES buffer (pH 7.5), 100 mM NaCl, 0.01 mM $Pb(OAc)_2$, l = 0.5 cm, 4 °C.

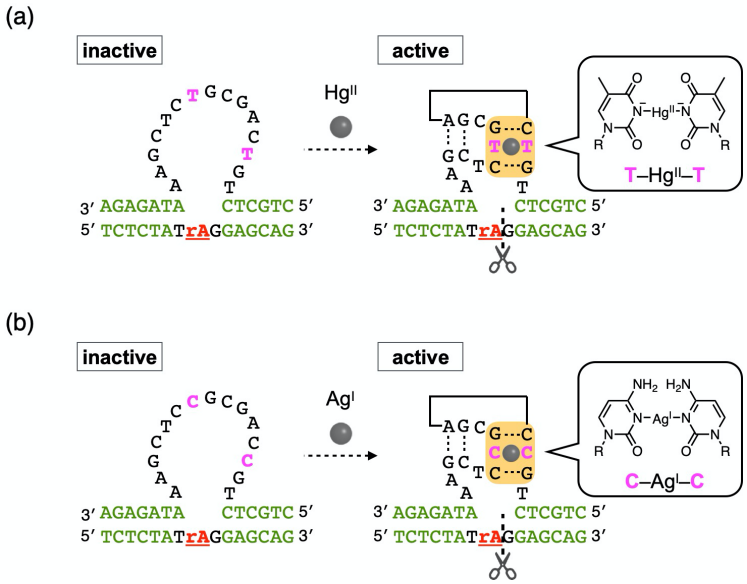

Figure S4. Molecular design of Hg^{II}- and Ag^{I}-responsive 8-17 DNAzymes containing a **T**–Hg^{II}–**T** or a **C**–Ag^{I}–**C** base pair (**DzT** and **TzC**, respectively). **rA** = adenine ribonucleotide at the cleavage site. Plausible secondary structures for both active and inactive states are shown.

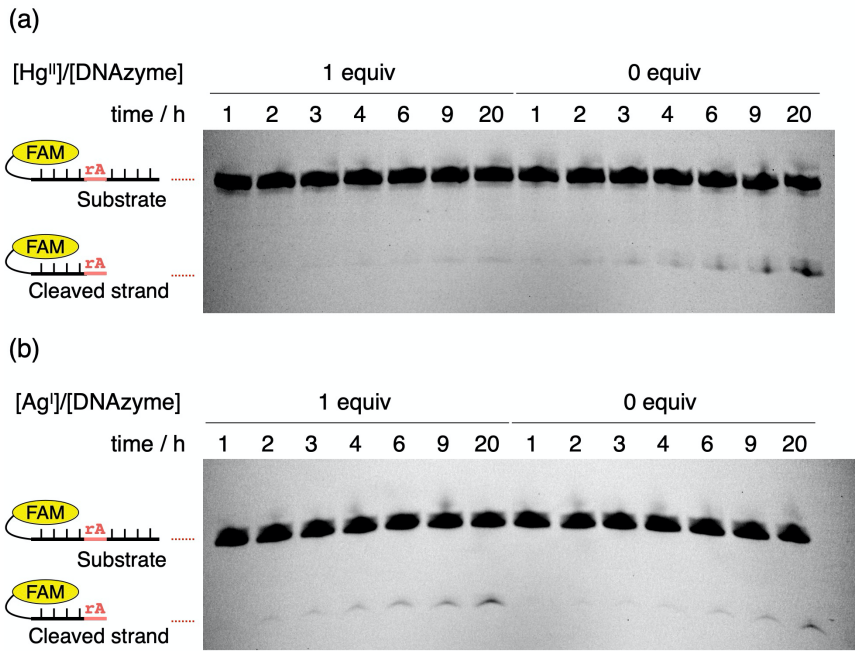

Figure S5. Denaturing polyacrylamide gel electrophoresis (PAGE) of the RNA-cleaving reactions catalyzed by the DNAzymes modified (a) with a T–T base pair (**DzT**) and (b) with a C–C base pair (**DzC**). [DNAzyme] = 1.0 μM, [substrate] = 10 μM in 50 mM HEPES (pH 7.5), 100 mM $NaNO_3$, 0.01 mM $Pb(OAc)_2$, 25 °C. 20% polyacrylamide gel containing 7 M urea, detected by FAM fluorescence. The results are summarized in Fig. 4b in the main text.

3. Reference

S1. Y. Takezawa, L. Hu, T. Nakama and M. Shionoya, *Angew. Chem., Int. Ed.*, 2020, **59**, 21488–21492.