Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information

Cu^{II}-mediated stabilisation of DNA duplexes bearing consecutive ethenoadenine lesions and its application to a metal-responsive DNAzyme

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1. Experimental methods

1-1. DNA synthesis

DNA strands containing 1,N^6-ethenoadenosine (**εA**) were chemically synthesised on an automated DNA synthesiser (NTS M-4-MX DNA/RNA synthesiser). DNA synthesis was carried out on a 1-μmol scale in a DMTr-on mode with ultra-mild deprotection phosphoramidites. All the reagents including **εA** phosphoramidites were purchased from Glen Research. The synthesis was performed following the standard protocol except for the extended coupling time (15 min) for **εA** nucleoside. The products were cleaved from the solid supports and deprotected using 25% NH_3 aqueous solution at room temperature for 2.5 h. The cleaved oligonucleotides were loaded on a Poly-Pak II or a Glen-Pak cartridge (Glen Research), detritylated on column using 2% aqueous TFA and then purified roughly. Further purification was performed using reverse phase HPLC (Waters XBridge C18 column, buffers: A = MeCN, B = 0.1 M TEAA (pH 7.0) + 2% MeCN, flow rate: 0.5 mL min^{-1}, temperature: 60 °C). The purified DNA strands were characterised by ESI-TOF or MALDI-TOF mass spectrometry. ESI-MS was measured on Waters Micromass LCT premier. MALDI-MS was measured on Bruker Autoflex III using a mixture of 3-hydroxypicolinic acid (3-HPA) and ammonium citrate as a matrix. The concentration of DNA strands was determined based on the UV absorbance at 260 nm. The molar extinction coefficients (ε_{260}) of the DNA strands were calculated by the nearest neighbour method. The ε_{260} value of **εA** nucleotides was assumed as 5.0×10^3 M^{-1} cm^{-1}.[1] The unmodified oligonucleotides and the 6-carboxyfluorescein (FAM)-labelled substrates containing a riboadenosine (rA) for the DNAzyme experiments were purchased from Japan Bio Service Co., Ltd (Saitama, Japan) at HPLC purification grade. The DNA oligonucleotides synthesised in this study are as follows:

ODN1. 5′-CAC ATT **εA**GT TGT A-3′. HPLC retention time: 24.2 min (gradient: 4%A (0 min), 7%A (40 min)). ESI-MS: *m/z* calcd for $[C_{130}H_{162}N_{46}O_{77}P_{12} - 3H]^{3-}$: 1322.89; found: 1322.88.
ODN2. 5′-TAC AAC **εA**AA TGT G-3′. HPLC retention time: 22.1 min (gradient: 4%A (0 min), 7%A (40 min)). ESI-MS: *m/z* calcd for $[C_{130}H_{160}N_{52}O_{73}P_{12} - 3H]^{3-}$: 1328.90; found: 1328.90.
ODN3. 5′-CAC ATT **εAεA**G TTG TA-3′. HPLC retention time: 27.8 min (gradient: 4%A (0 min), 7%A (40 min)). ESI-MS: *m/z* calcd for $[C_{142}H_{174}N_{51}O_{82}P_{13} - 3H]^{3-}$: 1435.58; found: 1435.55.

ODN4. 5′-TAC AAC **εAεA**A ATG TG-3′. HPLC retention time: 26.4 min (gradient: 4%A (0 min), 7%A (40 min)). ESI-MS: *m/z* calcd for $[C_{142}H_{172}N_{57}O_{78}P_{13} - 3H]^{3-}$: 1441.25; found: 1441.32.

ODN5. 5′-CAC ATT **εAεAεA** GTT GTA-3′. HPLC retention time: 24.2 min (gradient: 3%A (0 min), 8%A (40 min)). ESI-MS: *m/z* calcd for $[C_{154}H_{186}N_{56}O_{87}P_{14} - 3H]^{3-}$: 1547.93; found: 1547.94.

ODN6. 5′-TAC AAC **εAεAεA** AAT GTG-3′. HPLC retention time: 36.2 min (gradient: 4%A (0 min), 7%A (40 min)). ESI-MS: *m/z* calcd for $[C_{154}H_{184}N_{62}O_{83}P_{14} - 3H]^{3-}$: 1553.94; found: 1553.91.

ODN7. 5′-CAC ATT **εAεAεA εA**GT TGT A-3′. HPLC retention time: 25.2 min (gradient: 5%A (40 min), 8%A (40 min)). ESI-MS: *m/z* calcd for $[C_{166}H_{198}N_{61}O_{92}P_{15} - 3H]^{3-}$: 1660.28; found: 1660.37.

ODN8. 5′-TAC AAC **εAεAεA εA**AA TGT G-3′. HPLC retention time: 22.3 min (gradient: 5%A (40 min), 8%A (40 min)). ESI-MS: *m/z* calcd for $[C_{166}H_{196}N_{67}O_{88}P_{15} - 3H]^{3-}$: 1666.29; found: 1666.41.

εA-DNAzyme-1. 5′-GCG GTA CCA GGT CAA AGG TGG GTG AGG G**εAεA εA**TC T-3′. HPLC retention time: 26.2 min (gradient: 6%A (0 min), 8%A (40 min)). MALDI MS: *m/z* calcd for $[C_{341}H_{415}N_{142}O_{199}P_{33} - H]^{-}$: 10705.81; found: 10705.10.

εA-DNAzyme-2. 5′-AGA **εAεAεA** CCC GCG GTT AGA TAG AG-3′. HPLC retention time: 26.5 min (gradient: 6%A (0 min), 8%A (40 min)). MALDI MS: *m/z* calcd for $[C_{232}H_{280}N_{98}O_{130}P_{22} - H]^{-}$: 7201.76; found: 7202.75.

1-2. Spectroscopic analysis

The metal-binding affinity of the DNA oligonucleotides bearing **εA** was evaluated using various spectroscopic measurements such as duplex melting analysis, CD spectroscopy, fluorescence spectroscopy and ESI mass spectrometry.

Metal sources. Metal sources were purchased from Fujifilm Wako Pure Chemical Corporation ($MnCl_2 \cdot 4H_2O$ (99% purity), $NiCl_2 \cdot 6H_2O$ (99.9%), $CuSO_4 \cdot 5H_2O$ (99.9%)), Soekawa Chemical Co. ($ZnSO_4 \cdot 7H_2O$ (99.9%)) and Tokyo Chemical Industry (K_2PtCl_4 (98%)).

Duplex melting analysis. All samples for the spectroscopic studies were prepared by mixing the DNA strands (2.0 or 20 μM) in 10 mM HEPES buffer (pH 7.0) containing 100 mM NaCl. After

the addition of the metal ions, the solutions were heated to 60 °C and cooled slowly to 4 °C at the rate of –1.0 °C min^{-1}. Absorbance at 260 nm was monitored by a UV-1900 spectrophotometer (Shimadzu) equipped with a TMSPC-8 temperature controller while the temperature was raised from 4 °C to 60 °C at the rate of 0.2 °C min^{-1}. A cell with path length of 1.0 cm or 0.1 cm was used depending on the concentration of DNA samples. A drop of mineral oil was laid on the samples to prevent evaporation. Normalised absorbance shown in the Figures were calculated as follows:

$$Normalised\ \Delta A_{260} = \{A_{260}(t\ °C) - A_{260}(4\ °C)\}/\{A_{260}(60\ °C) - A_{260}(4\ °C)\} \times 100.$$

The melting temperature (T_m) was determined from the inflection point of a melting curve using a T_m analysis software LabSolutions (Shimadzu) with a 17-point adaptive smoothing program. Average T_m values of at least 3 independent runs were calculated.

CD spectroscopy. CD spectra were recorded at 5 °C on a JASCO J-820 spectropolarimeter with 10-time accumulation using a cell with path length of 0.3 cm. The spectra were smoothed using a simple moving average smoothing program.

Fluorescence spectroscopy. Fluorescence spectra were recorded on a JASCO FP-8300 spectrofluorometer using a cell with path length of 0.3 cm at 5 °C.

ESI-TOF mass spectrometry. Electrospray ionisation-time-of-flight (ESI-TOF) mass spectra were recorded on a Waters Micromass LCT premier. The samples were prepared in 20 mM NH_4OAc buffer (pH 7.0) and annealed just before the measurements (from 60 °C to 4 °C, –1.0 °C min^{-1}). The spectra were smoothed using a mean smoothing program.

1-3. RNA-cleaving reaction by DNAzymes

The **εA**-modified DNAzyme strands were mixed in a reaction buffer (10 mM HEPES (pH 7.0), 0.1 M NaCl) in the absence or presence of $CuSO_4$ (3.0 equiv.) and the mixture was annealed prior to the reaction (from 60 °C to 4 °C, 1.0 °C min^{-1}). The RNA-cleaving reaction was initiated by adding a FAM-labelled substrate strand. The final concentration of each component was as follows: [DNAzyme] = 10 μM, [substrate] = 10 μM, [$CuSO_4$] = 0 or 30 μM. After incubated at 25 °C, an aliquot of the sample solution was taken at the designated time points. The reaction was stopped by adding a loading buffer (5.25 M urea, 1.25 mM EDTA and 7.5% (v/v) glycerol). The

cleavage of the substrate was analysed by denaturing polyacrylamide gel electrophoresis (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 initial velocity was calculated at the time points when F was less than 20%. The initial reaction velocity was estimated to be 0.0157 h^{-1} and 0.0833 h^{-1} in the absence and the presence of Cu^{II}, respectively, and the on–off ratio was determined to be 5.3.

2. Supplementary figures and tables

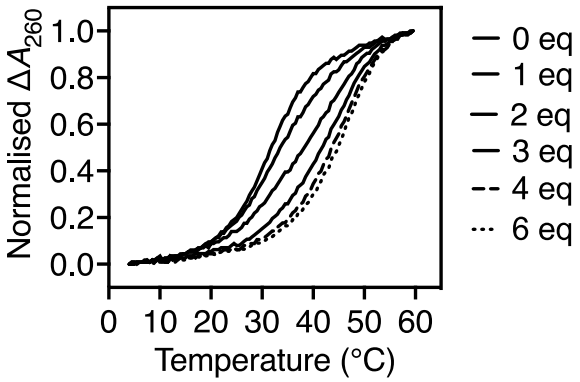

Fig. S1. Melting curves of DNA duplex **5**·**6** containing three **εA**–**εA** mismatch pairs in the presence of different concentrations of Cu^{II} ions at lower duplex concentration. [duplex] = 2.0 μM, [Cu^{II}]/[duplex] = 0, 1, 2, 3 (solid lines), 4 and 6 (dashed lines) in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl, 0.2 °C min^{-1}.

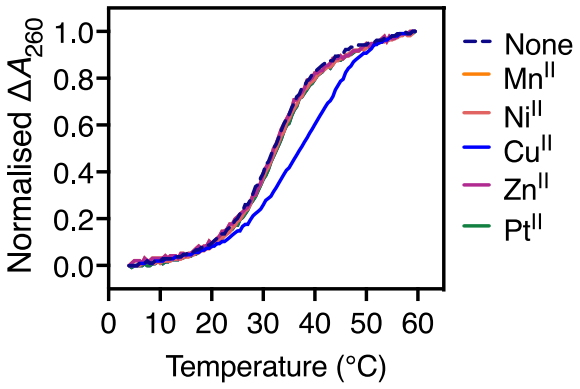

Fig. S2. Melting curves of DNA duplex **5**·**6** containing three **εA**–**εA** mismatch pairs in the presence of various metal ions. [duplex] = 2.0 μM, [metal ion]/[duplex] = 0 (dashed line) or 3.0 (solid lines) in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl, 0.2 °C min^{-1}.

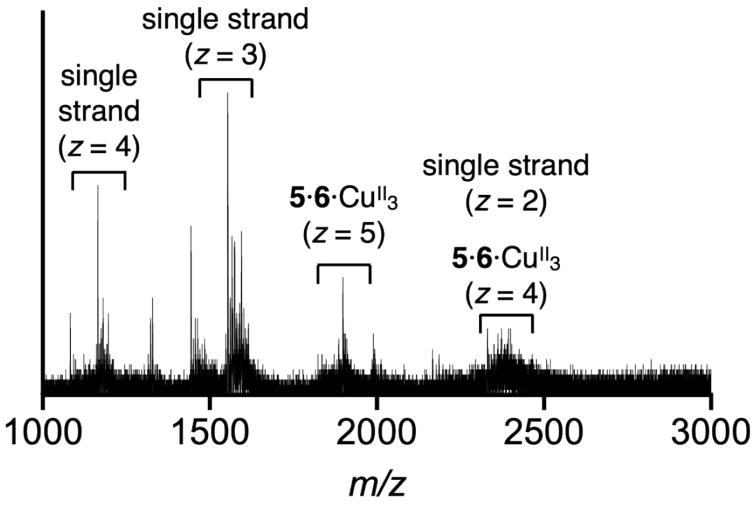

Fig. S3. ESI-TOF mass spectrum of duplex **5**·**6** with 3 equiv. of Cu^{II} ions. **5**·**6**·Cu^{II}_3 = $C_{308}H_{364}N_{118}O_{170}P_{28}Cu_3$ (found: 1898.07 (z = –5); calcd for [**5**·**6**·Cu^{II}_3–5H]$^{5-}$: 1898.28). [duplex] = 100 μM, [Cu^{II}] = 300 μM in 20 mM NH_4OAc (pH 7.0). Negative mode. A magnified spectrum is shown in Fig. 2b.

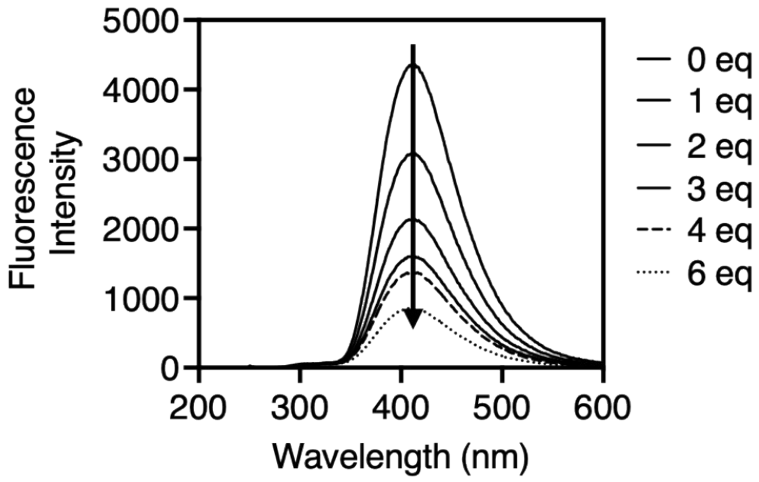

Fig. S4. Fluorescence spectra of duplex **5**·**6** in the presence of varying concentrations of Cu^{II} ions. [duplex] = 20 μM, [Cu^{II}]/[duplex] = 0, 1, 2, 3, 4 and 6 in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl. λ_{ex} = 232 nm, l = 0.3 cm, 5 °C. Samples were annealed before the measurement.

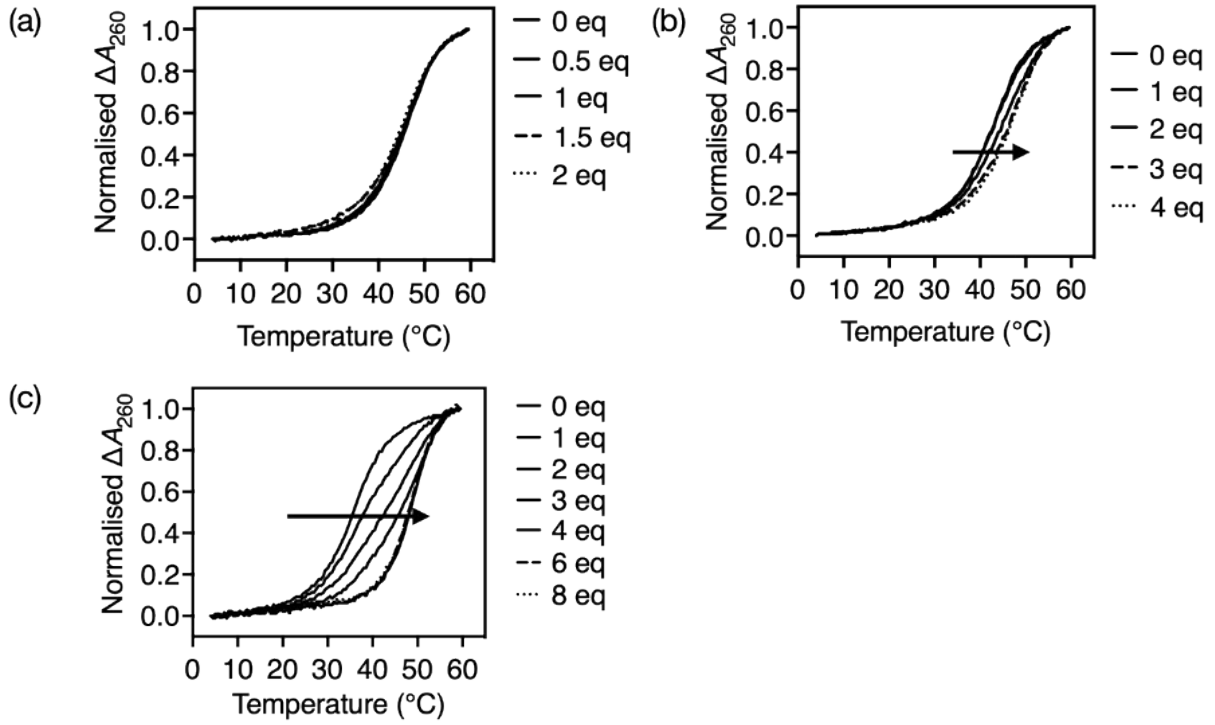

Fig. S5. Melting curves of DNA duplexes, (a) **1**·**2**, (b) **3**·**4** and (c) **7**·**8**, containing one, two and four **εA**–**εA** pairs, respectively, in the presence of different concentrations of Cu^{II} ions. [duplex] = 20 μM in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl, 0.2 °C min^{-1}. (a) [Cu^{II}]/[duplex] = 0, 0.5, 1 (solid lines), 1.5 and 2 (dashed lines), (b) [Cu^{II}]/[duplex] = 0, 1, 2 (solid lines), 3 and 4 (dashed lines), (c) [Cu^{II}]/[duplex] = 0, 1, 2, 3, 4 (solid lines), 6 and 8 (dashed lines).

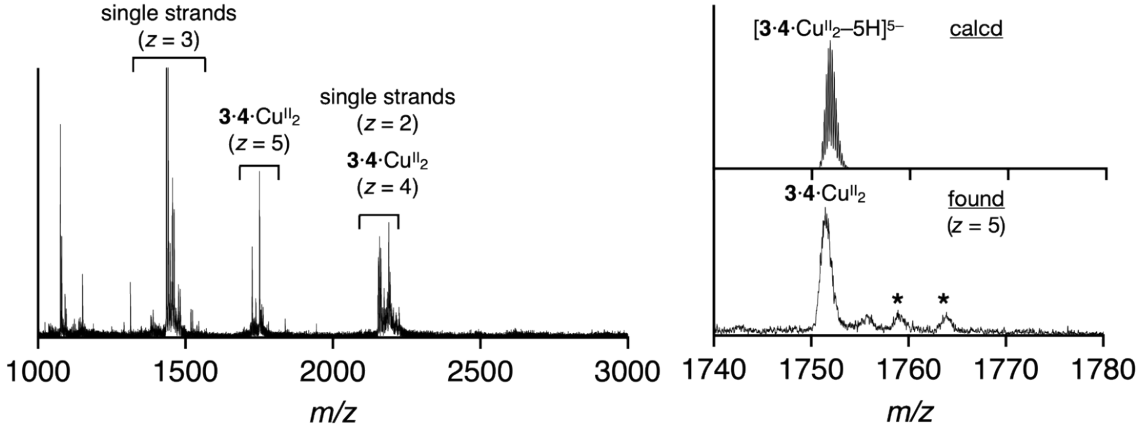

Fig. S6. ESI-TOF mass spectrum of duplex **3**·**4** with 2 equiv. of Cu^{II} ions. **3**·**4**·Cu^{II}_2 = $C_{284}H_{342}N_{108}O_{160}P_{26}Cu_2$ (found: 1751.37 (z = –5); calcd for [**3**·**4**·Cu^{II}_2–5H]$^{5-}$: 1751.07). [duplex] = 100 μM, [Cu^{II}] = 200 μM in 20 mM NH_4OAc (pH 7.0). Negative mode. Signals ascribed to sodium, potassium and/or ammonium adducts are shown with asterisk (*).

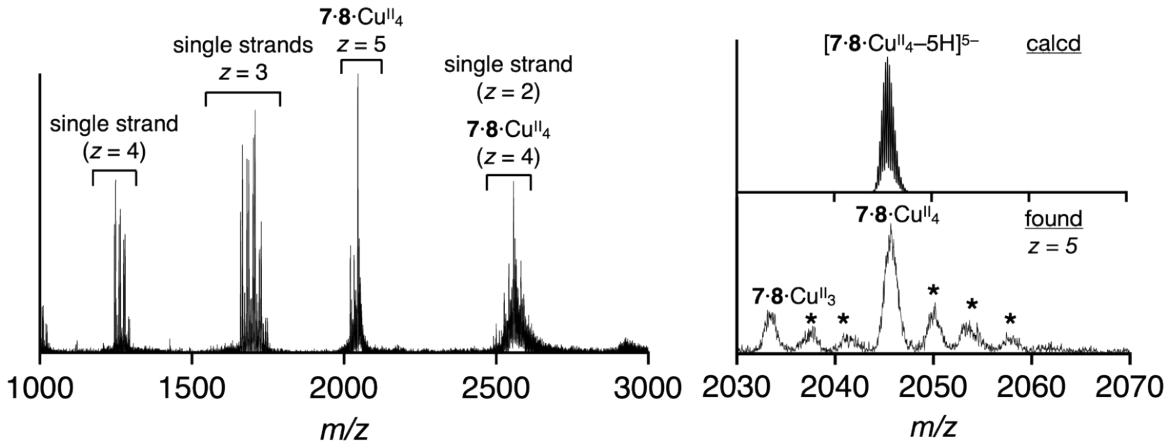

Fig. S7. ESI-TOF mass spectrum of duplex **7**·**8** with 3 equiv. of Cu^{II} ions. **7**·**8**·Cu^{II}_4 = $C_{332}H_{386}N_{128}O_{180}P_{30}Cu_4$ (found: 2045.55 (z = –5); calcd for [**7**·**8**·Cu^{II}_4–5H]$^{5–}$: 2045.48). [duplex] = 100 μM, [Cu^{II}] = 400 μM in 20 mM NH_4OAc (pH 7.0). Negative mode. Signals ascribed to sodium, potassium and/or ammonium adducts are shown with asterisk (*).

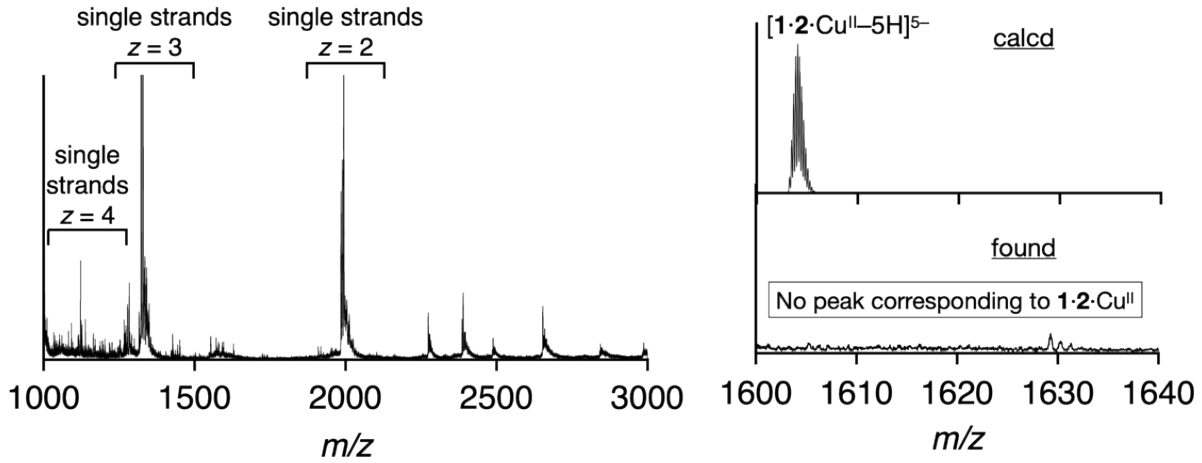

Fig. S8. ESI-TOF mass spectrum of duplex **1**·**2** with 1 equiv. of Cu^{II} ions. **1**·**2**·Cu^{II} = $C_{260}H_{320}N_{98}O_{150}P_{24}Cu$ (calcd for [**1**·**2**·Cu^{II}–5H] $^{5–}$: 1603.87). [duplex] = 100 μM, [Cu^{II}] = 100 μM in 20 mM NH_4OAc (pH 7.0). Negative mode. Signal ascribed to **1**·**2**·Cu^{II} was not observed.

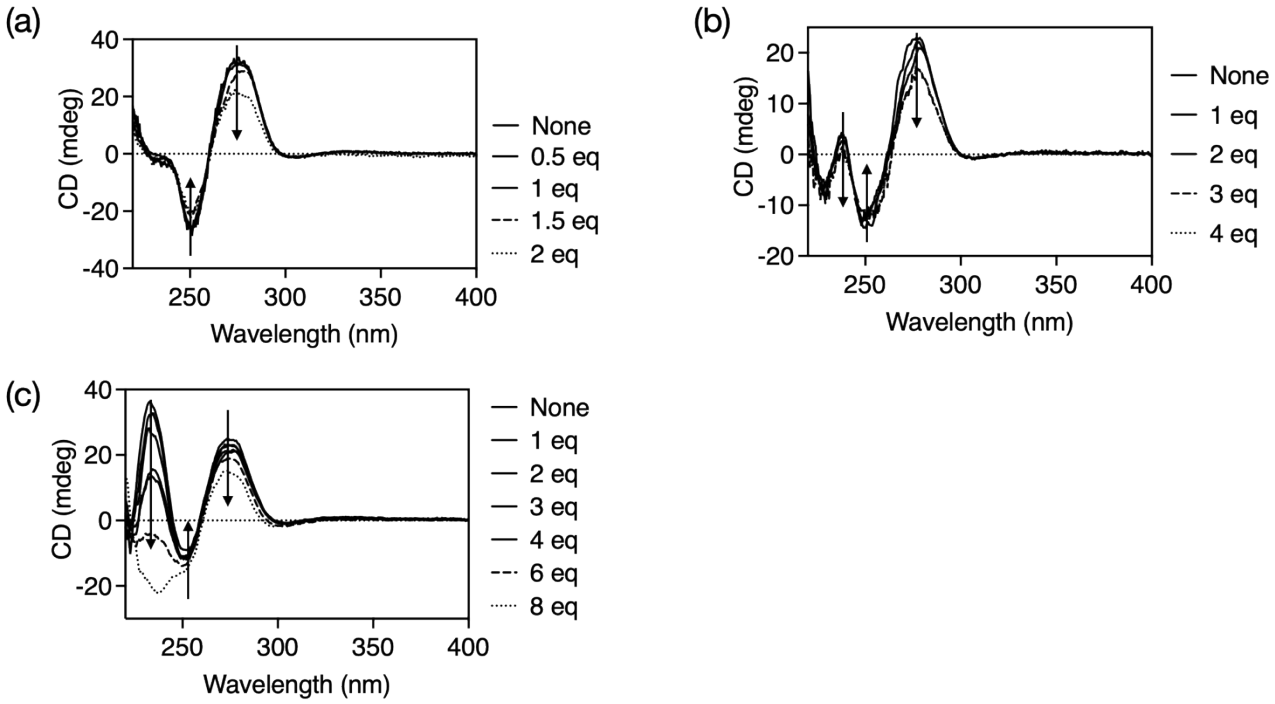

Fig. S9. CD spectra of duplex **1**·**2**, **3**·**4** and **7**·**8** with one, two and four **εA**–**εA** pairs, respectively, in the presence of different concentration of Cu^{II} ions. [duplex] = 20 μM in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl. *l* = 0.3 cm, 5 °C. (a) [Cu^{II}]/[duplex] = 0, 0.5, 1 (solid lines), 1.5 and 2 (dashed lines), (b) [Cu^{II}]/[duplex] = 0, 1, 2 (solid lines), 3 and 4 (dashed lines), (c) [Cu^{II}]/[duplex] = 0, 1, 2, 3, 4 (solid lines), 6 and 8 (dashed lines). Samples were annealed before the measurement.

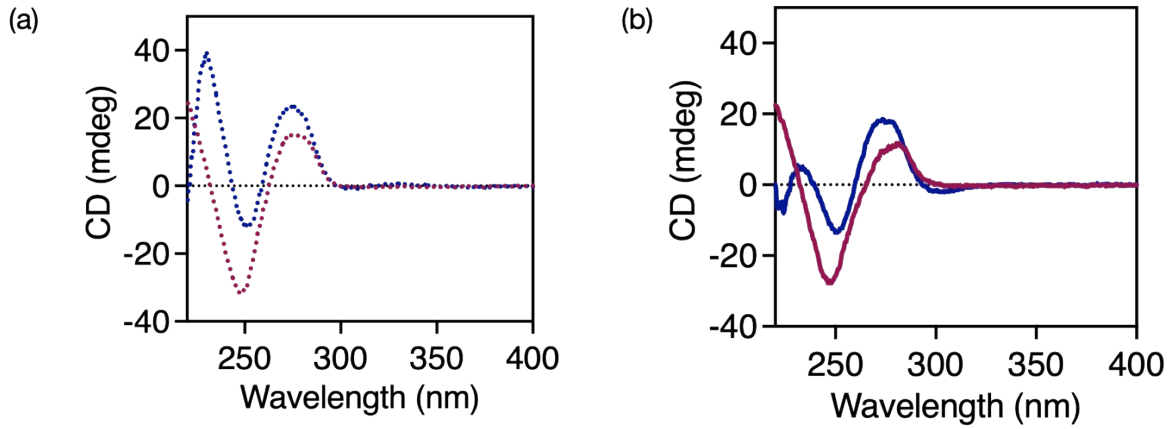

Fig. S10. CD spectra of natural full-match 15-mer duplex **5A**·**6T** (red) and duplex **5**·**6** with three **εA**–**εA** pairs (blue) in the absence (a) and presence of Cu^{II} ions (b). [duplex] = 20 μM, [Cu^{II}]/[duplex] = 0 (a) and 3 (b) in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl. *l* = 0.3 cm, 5 °C. Samples were annealed before the measurement.

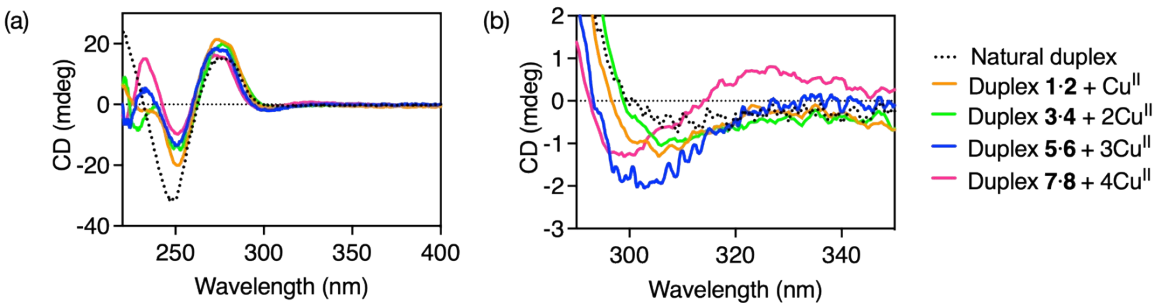

Fig. S11. (a) CD spectra of natural full-match duplex **5A·6T** (dashed line) and duplexes **1·2**, **3·4**, **5·6** and **7·8** with one, two, three and four **εA**–**εA** pairs, respectively, in the presence of stoichiometric amount of Cu^{II} ions. (b) Magnified spectra to show the new signal around 320 nm. [duplex] = 20 μM, [Cu^{II}]/[**εA**–**εA**] = 1.0 in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl. *l* = 0.3 cm, 5 °C. Samples were annealed before the measurement.

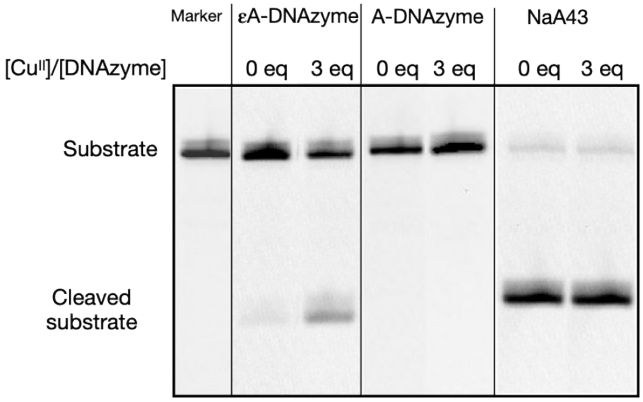

Fig. S12. Denaturing PAGE analysis of the RNA-cleaving reaction by DNAzymes. [DNAzyme] = 10 μM, [substrate] = 10 μM, [Cu^{II}]/[DNAzyme] = 0 or 3.0 in 10 mM HEPES (pH 7.0), 0.1 M NaCl, 25 °C, 6 h. FAM detection. The results with a positive control (NaA43 DNAzyme) and a negative control (A-DNAzyme) are also shown.

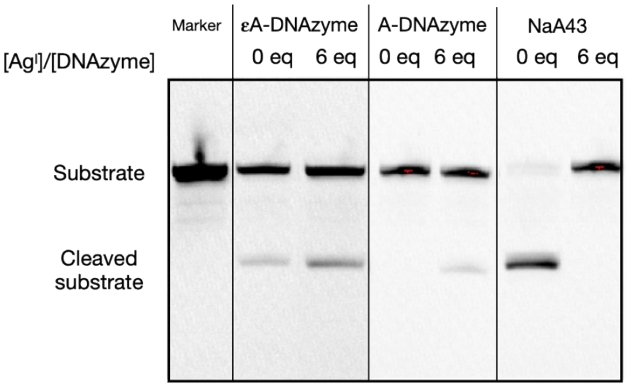

Fig. S13. Denaturing PAGE analysis of the RNA-cleaving reaction by DNAzymes. [DNAzyme] = 10 μM, [substrate] = 10 μM, [Ag^{I}]/[DNAzyme] = 0 or 6.0 in 10 mM HEPES (pH 7.0), 0.1 M $NaNO_3$, 25 °C, 6 h. FAM detection. The results with a positive control (NaA43 DNAzyme) and a negative control (A-DNAzyme) are also shown. Note that the activity of the unmodified NaA43 DNAzyme was greatly suppressed upon Ag^{I} addition, presumably due to the unintended binding of Ag^{I} ions to the natural nucleobases.

Table S1. Base sequences of DNA strands used in this study.

Name	Sequence (5′→3′)
ODN1	CAC ATT **εA**GT TGT A
ODN2	TAC AAC **εA**AA TGT G
ODN3	CAC ATT **εAεA**G TTG TA
ODN4	TAC AAC **εAεA**A ATG TG
ODN5	CAC ATT **εAεAεA** GTT GT A
ODN6	TAC AAC **εAεAεA** AAT GTG
ODN7	CAC ATT **εAεAεA εA**GT TGT A
ODN8	TAC AAC **εAεAεA εA**AA TGT G
ODN5A	CAC ATT **AAA** GTT GTA
ODN6A	TAC AAC **AAA** AAT GTG
ODN6T	TAC AAC **TTT** AAT GTG
εA-DNAzyme-1	GCG GTA CCA GGT CAA AGG TGG GTG AGG G**εAεA εA**TC T
εA-DNAzyme-2	AGA **εAεAεA** CCC GCG GTT AGA TAG AG
substrate	FAM-CTC TAT CTA T(**rA**)G GAA GTA CCG C
NaA43 DNAzyme	GCG GTA CCA GGT CAA AGG TGG GTG AGG GGA CGC CAA GAG TCC CCG CGG TTA GAT AGA G
A-DNAzyme-1	GCG GTA CCA GGT CAA AGG TGG GTG AGG G**AA A**TC T
A-DNAzyme-2	AGA **AAA** CCC GCG GTT AGA TAG AG

3. Reference

1. S. Mandal, C. Wang, R. K. Prajapati, J. Kösters, S. Verma, L. Chi and J. Müller**,** *Inorg. Chem.,* 2016, **55**, 7041–7050.