Supporting Information

Novel specific binding of copper ions to naturally modified base pairs involving 5-fluorouracil in duplex DNA

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Table S1. Melting temperature (T_m) of 1 μM duplex DNA, F25A:R25FdU, in 10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) without or with each of 2 μM $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $CrCl_3$, $MnCl_2$, and $TlNO_3$ obtained from by UV melting.

Metal	T_m (−Metal)(°C)	T_m (+2Metal)(°C)	ΔT_m (+2Metal)(°C)[a]
$Zn(NO_3)_2$	74.1±0.5	73.9±0.4	-0.2
$Cd(NO_3)_2$	74.1±0.5	74.1±0.2	0
$Fe(NO_3)_3$	74.1±0.5	72.9±0.6	-1.2
$Co(NO_3)_2$	74.1±0.5	72.6±0.5	-1.5
$Ni(NO_3)_2$	74.1±0.5	72.9±0.5	-1.2
$Pb(NO_3)_2$	74.1±0.5	72.1±0.0	-2.0
$CrCl_3$	74.1±0.5	73.0±0.4	-1.1
$MnCl_2$	74.1±0.5	72.8±0.5	-1.3
$TlNO_3$	74.1±0.5	72.1±0.2	-2.0

[a] ΔT_m (+2Metal) = T_m (+2Metal) − T_m (−Metal)

Table S2. Melting temperature (T_m) of 1 μM duplex DNA, F25C:R25FdU, in 10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) without or with each of 2 μM $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $CrCl_3$, $MnCl_2$, and $TlNO_3$ obtained from by UV melting.

Metal	T_m (−Metal)(°C)	T_m (+2Metal)(°C)	ΔT_m (+2Metal)(°C)[a]
$Zn(NO_3)_2$	66.4±0.4	67.9±0.1	1.5
$Cd(NO_3)_2$	66.4±0.4	68.0±0.3	1.6
$Fe(NO_3)_3$	66.4±0.4	66.8±0.4	0.4
$Co(NO_3)_2$	66.4±0.4	66.9±0.4	0.5
$Ni(NO_3)_2$	66.4±0.4	67.0±0.5	0.6
$Pb(NO_3)_2$	66.4±0.4	66.6±0.2	0.2
$CrCl_3$	66.4±0.4	67.2±0.4	0.8
$MnCl_2$	66.4±0.4	67.1±0.4	0.7
$TlNO_3$	66.4±0.4	66.5±0.6	0.1

[a] ΔT_m (+2Metal) = T_m (+2Metal) − T_m (−Metal)

Table S3. Melting temperature (T_m) of 1 μM duplex DNA, F25G:R25FdU, in 10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) without or with each of 2 μM $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $CrCl_3$, $MnCl_2$, and $TlNO_3$ obtained from by UV melting.

Metal	T_m (−Metal)(°C)	T_m (+2Metal)(°C)	ΔT_m (+2Metal)(°C)[a]
$Zn(NO_3)_2$	70.9±0.3	71.7±0.3	0.8
$Cd(NO_3)_2$	70.9±0.3	72.3±0.3	1.4
$Fe(NO_3)_3$	70.9±0.3	71.6±0.4	0.7
$Co(NO_3)_2$	70.9±0.3	71.1±0.5	0.2
$Ni(NO_3)_2$	70.9±0.3	70.6±0.4	-0.3
$Pb(NO_3)_2$	70.9±0.3	70.4±0.2	-0.5
$CrCl_3$	70.9±0.3	71.4±0.6	0.5
$MnCl_2$	70.9±0.3	71.1±0.5	0.2
$TlNO_3$	70.9±0.3	70.8±0.4	-0.1

[a] ΔT_m (+2Metal) = T_m (+2Metal) − T_m (−Metal)

Table S4. Melting temperature (T_m) of 1 μM duplex DNA, F25T:R25FdU, in 10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) without or with each of 2 μM $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $CrCl_3$, $MnCl_2$, and $TlNO_3$ obtained from by UV melting.

Metal	T_m (−Metal)(°C)	T_m (+2Metal)(°C)	ΔT_m (+2Metal)(°C)[a]
$Zn(NO_3)_2$	67.3±0.3	68.2±0.2	0.9
$Cd(NO_3)_2$	67.3±0.3	68.5±0.3	1.2
$Fe(NO_3)_3$	67.3±0.3	67.1±0.4	-0.2
$Co(NO_3)_2$	67.3±0.3	66.9±0.4	-0.4
$Ni(NO_3)_2$	67.3±0.3	67.2±0.4	-0.1
$Pb(NO_3)_2$	67.3±0.3	66.8±0.5	-0.5
$CrCl_3$	67.3±0.3	67.4±0.4	0.1
$MnCl_2$	67.3±0.3	67.6±0.5	0.3
$TlNO_3$	67.3±0.3	66.8±0.3	-0.5

[a] ΔT_m (+2Metal) = T_m (+2Metal) − T_m (−Metal)

Table S5. Melting temperature (T_m) of 1 μM duplex DNA, F25FdU:R25A, in 10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) without or with each of 2 μM $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $CrCl_3$, $MnCl_2$, and $TlNO_3$ obtained from by UV melting.

Metal	T_m (−Metal)(°C)	T_m (+2Metal)(°C)	ΔT_m (+2Metal)(°C)[a]
$Zn(NO_3)_2$	72.4±0.4	72.3±0.2	-0.1
$Cd(NO_3)_2$	72.4±0.4	72.5±0.5	0.1
$Fe(NO_3)_3$	72.4±0.4	71.3±0.4	-1.1
$Co(NO_3)_2$	72.4±0.4	71.0±0.0	-1.4
$Ni(NO_3)_2$	72.4±0.4	71.3±0.5	-1.1
$Pb(NO_3)_2$	72.4±0.4	70.5±0.5	-1.9
$CrCl_3$	72.4±0.4	71.4±0.6	-1.0
$MnCl_2$	72.4±0.4	71.2±0.2	-1.2
$TlNO_3$	72.4±0.4	70.5±0.4	-1.9

[a] ΔT_m (+2Metal) = T_m (+2Metal) − T_m (−Metal)

Table S6. Melting temperature (T_m) of 1 μM duplex DNA, F25FdU:R25C, in 10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) without or with each of 2 μM $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $CrCl_3$, $MnCl_2$, and $TlNO_3$ obtained from by UV melting.

Metal	T_m (−Metal)(°C)	T_m (+2Metal)(°C)	ΔT_m (+2Metal)(°C)[a]
$Zn(NO_3)_2$	66.5±0.2	68.3±0.6	1.8
$Cd(NO_3)_2$	66.5±0.2	68.4±0.4	1.9
$Fe(NO_3)_3$	66.5±0.2	67.2±0.4	0.7
$Co(NO_3)_2$	66.5±0.2	67.3±0.2	0.8
$Ni(NO_3)_2$	66.5±0.2	67.4±0.5	0.9
$Pb(NO_3)_2$	66.5±0.2	67.0±0.4	0.5
$CrCl_3$	66.5±0.2	67.6±0.4	1.1
$MnCl_2$	66.5±0.2	67.5±0.3	1.0
$TlNO_3$	66.5±0.2	66.9±0.1	0.4

[a] ΔT_m (+2Metal) = T_m (+2Metal) − T_m (−Metal)

Table S7. Melting temperature (T_m) of 1 μM duplex DNA, F25FdU:R25G, in 10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) without or with each of 2 μM $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $CrCl_3$, $MnCl_2$, and $TlNO_3$ obtained from by UV melting.

Metal	T_m (−Metal)(°C)	T_m (+2Metal)(°C)	ΔT_m (+2Metal)(°C)[a]
$Zn(NO_3)_2$	70.3±0.4	70.9±0.4	0.6
$Cd(NO_3)_2$	70.3±0.4	71.5±0.5	1.2
$Fe(NO_3)_3$	70.3±0.4	70.8±0.6	0.5
$Co(NO_3)_2$	70.3±0.4	70.3±0.2	0
$Ni(NO_3)_2$	70.3±0.4	69.8±0.4	-0.5
$Pb(NO_3)_2$	70.3±0.4	69.6±0.5	-0.7
$CrCl_3$	70.3±0.4	70.6±0.4	0.3
$MnCl_2$	70.3±0.4	70.3±0.3	0
$TlNO_3$	70.3±0.4	70.0±0.3	-0.3

[a] ΔT_m (+2Metal) = T_m (+2Metal) − T_m (−Metal)

Table S8. Melting temperature (T_m) of 1 μM duplex DNA, F25FdU:R25T, in 10 mM sodium cacodylate-cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) without or with each of 2 μM $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $CrCl_3$, $MnCl_2$, and $TlNO_3$ obtained from by UV melting.

Metal	T_m (−Metal)(°C)	T_m (+2Metal)(°C)	ΔT_m (+2Metal)(°C)[a]
$Zn(NO_3)_2$	68.2±0.3	69.4±0.3	1.2
$Cd(NO_3)_2$	68.2±0.3	69.7±0.5	1.5
$Fe(NO_3)_3$	68.2±0.3	68.3±0.4	0.1
$Co(NO_3)_2$	68.2±0.3	68.1±0.5	-0.1
$Ni(NO_3)_2$	68.2±0.3	68.4±0.4	0.2
$Pb(NO_3)_2$	68.2±0.3	68.0±0.4	-0.2
$CrCl_3$	68.2±0.3	68.6±0.4	0.4
$MnCl_2$	68.2±0.3	68.8±0.3	0.6
$TlNO_3$	68.2±0.3	68.0±0.2	-0.2

[a] ΔT_m (+2Metal) = T_m (+2Metal) − T_m (−Metal)

Table S9. Crystal data, statistics of data collection and structural refinements.

Data collection	
Space group	$P4_12_12$
Cell dimensions(Å)	$a = b = 39.5$, c = 74.8
Resolution (Å)	2.50 (2.65 - 2.50)
No. of observations	53,355
No. of unique reflections	3,924
R_{merge}[a] (%)	4.7 (38.5)
$I/\sigma I$	29.7 (5.1)
$CC_{1/2}$[b] (%)	99.8 (99.3)
Completeness (%)	99.9 (100)
Redundancy	13.6 (14.0)
Refinement	
Resolution (Å)	27.96 - 2.50
No. of used reflections	2,277
R_{work}[c] / R_{free}[d] (%)	19.7/21.0
B factors (Å^2)	88.4
r.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.532

Values in parentheses correspond to the highest resolution shell.

[a]$R_{merge} = \Sigma_{hkl}\Sigma_i |I_i(hkl)-\langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i I_i(hkl)$.

[b]$CC_{1/2}$ is the percentage of correlation between intensities from random half-datasets.

[c]$R_{work} = \Sigma_{hkl} ||F_o(hkl)|-|F_c(hkl)|| / \Sigma_{hkl}|F_o(hkl)|$.

[d]R_{free} is the R_{work} calculated for 5% of the data set not included in refinements.

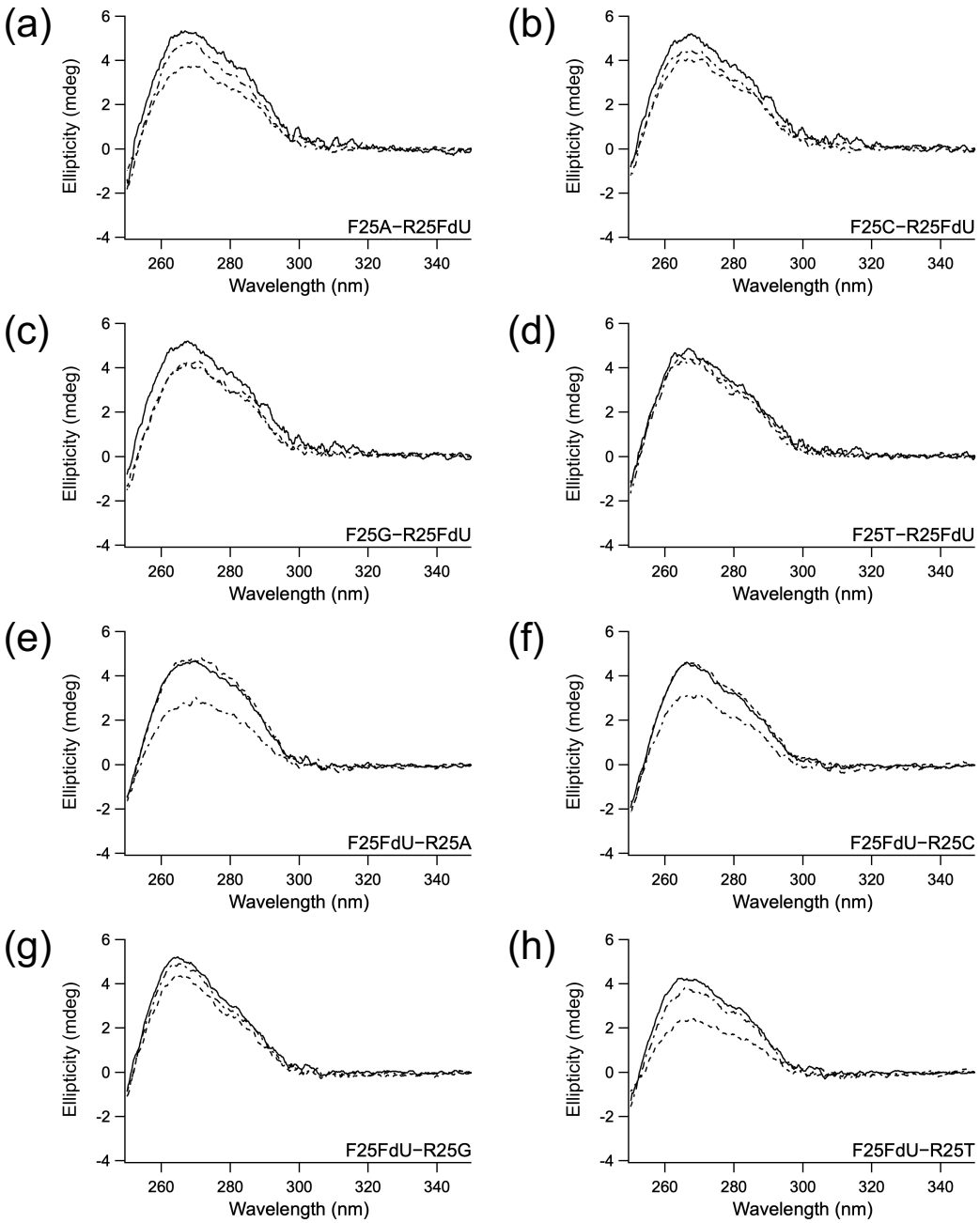

Figure S1. CD spectra of the duplex DNA, (a) F25A–R25FdU, (b) F25C–R25FdU, (c) F25G–R25FdU, (d) F25T–R25FdU, (e) F25FdU–R25A, (f) F25FdU–R25C, (g) F25FdU–R25G, and (h) F25FdU–R25T, with or without $Cu(NO_3)_2$. Duplex DNA (1 μM) at 25 °C in 10 mM sodium cacodylate–cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) without (solid line) or with 1 (dotted line) or 2 (dashed-dotted line) μM $Cu(NO_3)_2$ was measured at 250–350 nm. Path length was 1 cm.

Experimental Section

Oligonucleotide preparation

A pair of 25-mer complementary DNA oligonucleotides, F25**X**: 5'-d(GCCCTGCCTGTC**X**CCCAGATCACTG)-3' (**X**=A, C, G, T, and 5-fluorouracil (FdU)) (Figure 1) and R25**Y**:5'-d(CAGTGATCTGGG**Y**GACAGGCAGGGC)-3' (**Y**= A, C, G, T, and FdU) (Figure 1), and a 12-mer self-complementary DNA oligonucleotide, C5FdU:5'-d[GGACCC(FdU)GGTCC]-3' (Figure 1), were purchased from Japan Bio Service Co., Ltd. (Saitama, Japan). The concentrations of all purchased single-stranded oligonucleotides were determined by UV absorbance considering the molar extinction coefficient of the individual nucleobases within each single-stranded oligonucleotide. Pairs of the complementary strands, F25**X** (**X**=A, C, G, and T) and R25**Y** (**Y**=FdU), F25**X** (**X**=FdU) and R25**Y** (**Y**=A, C, G, and T), as well as the self-complementary strand, C5FdU (Figure 1), were annealed by heating to 90 ℃, followed by gradual cooling to room temperature. The annealed samples were applied to a hydroxyapatite column (BIORAD Inc.) to remove the unpaired single strands. The concentrations of the prepared duplex DNA, F25**X**:R25**Y** [**X**–**Y**=A–FdU, C–FdU, G–FdU, T–FdU, FdU–A, FdU–C, FdU–G, and FdU–T**]** and $(C5FdU)_2$ (Figure 1), were determined by UV absorption considering the DNA concentration ratio of (Absorbance of 1 at 260 nm) = 50 μg/mL. When we change into molar concentration, we use the molecular weight of the individual nucleobases within duplex DNA.

UV melting

UV melting experiments were performed on a DU-640 spectrophotometer (Beckman Inc.) equipped with a Peltier-type cell holder. The cell path length is 1 cm. The UV melting profiles were measured in 10 mM sodium cacodylate–cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) with or without 1 or 2 μM $Cu(NO_3)_2$ or 2 μM $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Fe(NO_3)_3$, $Co(NO_3)_2$, $Ni(NO_3)_2$, $Pb(NO_3)_2$, $CrCl_3$, $MnCl_2$ and $TlNO_3$ at 0.2 ℃/min with detection at 260

nm. The first derivative was calculated using the UV melting profile. The peak temperatures in the derivative curve are designated as T_m. The concentration of the duplex DNA used was 1 μM.

CD spectroscopy

CD spectra were recorded at 25 ℃ in 10 mM sodium cacodylate–cacodylic acid (pH 6.8) and 100 mM $NaNO_3$ (buffer A) with or without 1 or 2 μM $Cu(NO_3)_2$ on a JASCO J-725 spectropolarimeter interfaced with a microcomputer. The cell path length is 1 cm. The concentration of the duplex DNA used was 1 μM.

X-ray crystallography

To reveal the detailed structure of the complex between C-FdU or FdU-C base pair in duplex DNA and copper ion, we crystallized a duplex DNA, $(C5FdU)_2$ (Figure 1), with a self-complementary sequence involving consecutive C-FdU and FdU-C base pairs in the center by the hanging-drop vapor diffusion method under conditions containing $Cu(NO_3)_2$. Crystallization was performed at 20℃ by mixing 1.0 μL of a sample solution containing 2 mM $(C5FdU)_2$ and 4 mM $Cu(NO_3)_2$ solutions and 1.0 μL of crystallization solution containing 50 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS) (pH 7.0), 10% 2-methyl-2,4-pentanediol (MPD), 10 mM $Sr(NO_3)_2$, and 10 mM spermine. Single crystals were scooped from droplets using CryoLoop (Hampton Research) and frozen immediately in liquid nitrogen.

X-ray diffraction datasets were collected at the X06SA (PXI) of the Swiss Light Source. Experimental phase of the $(C5FdU)_2$ duplex DNA with Cu^{2+} was obtained by the single-wavelength anomalous difference (SAD) method using the copper anomalous signal with the *AutoSol* program[1] in the *Phenix* suite.[2] Molecular structures were constructed and manipulated using *Coot*.[3] The atomic parameters of the $(C5FdU)_2$ duplex DNA in complex with Cu^{2+} were refined using *phenix.refine* from the *Phenix* suite.[2] Observations and molecular drawings of the

structures were made using the *PyMOL* software. The data collection and structure refinement statistics are summarized in Table 2. The atomic coordinates and experimental data of the $(C5FdU)_2$ duplex DNA in complex with Cu^{2+} were deposited in the Protein Data Bank with ID code, 9KCC.

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