Supplementary Information

Importance of isothermal titration calorimetry for the detection of the direct binding of metal ions to mismatched base pairs in duplex DNA

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Table S1. Melting temperatures (T_m) of 1 μM duplex DNA [F21**X**:R21**Y** (**X-Y**=A-A, A-C, A-G, A-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G and T-T)] at pH 6.8 in 10 mM sodium cacodylate-cacodylic acid and 100 mM $NaClO_4$ without or with 1 or 2 μM $AgNO_3$, obtained from UV melting.

X-Y	T_m (-Ag^+) (°C)	T_m (+Ag^+) (°C)	ΔT_m (°C)[a]	T_m (+2Ag^+) (°C)	ΔT_{m2} (°C)[b]
A-A	59.5 ± 0.3	60.7 ± 0.7	1.2	61.9 ± 0.2	2.4
A-C	60.9 ± 0.3	62.6 ± 0.4	1.7	64.5 ± 0.7	3.6
A-G	64.6 ± 0.4	65.4 ± 0.6	0.8	65.8 ± 0.7	1.2
A-T	67.6 ± 0.4	68.3 ± 0.1	0.7	70.1 ± 0.6	2.5
G-A	62.4 ± 0.4	64.1 ± 0.8	1.7	66.1 ± 0.4	3.7
G-C	71.2 ± 0.5	72.8 ± 0.4	1.6	73.9 ± 0.9	2.7
G-G	64.8 ± 0.3	65.7 ± 0.4	0.9	66.0 ± 0.7	1.2
G-T	64.9 ± 0.3	65.9 ± 0.2	1.0	67.6 ± 0.9	2.7
T-A	65.8 ± 0.4	66.7 ± 0.6	0.9	68.9 ± 0.5	3.1
T-C	58.3 ± 0.3	59.9 ± 0.4	1.6	62.5 ± 0.7	4.2
T-G	62.2 ± 0.3	63.0 ± 0.1	0.8	63.7 ± 0.3	1.5
T-T	60.1 ± 0.2	61.3 ± 0.6	1.2	62.2 ± 0.4	2.1

[a] $\Delta T_m = T_m$ (+Ag^+) - T_m (-Ag^+). [b] $\Delta T_{m2} = T_m$ (+2Ag^+) - T_m (-Ag^+).

Table S2: Melting temperatures (T_m) of 1 μM duplex DNA [F21**X**:R21**Y** (**X-Y**=A-A, A-C, A-G, A-T, C-A, C-C, C-G, C-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G and T-T)] at pH 6.8 in 10 mM sodium cacodylate-cacodylic acid and 100 mM $NaClO_4$ without or with 2 μM $Mn(NO_3)_2$, obtained from UV melting.

X-Y	T_m (-Mn^{2+}) (°C)	T_m (+2Mn^{2+}) (°C)	ΔT_m (°C)[a]
A-A	59.5 ± 0.3	59.5 ± 0.1	0.0
A-C	60.9 ± 0.3	61.6 ± 0.1	0.7
A-G	64.6 ± 0.4	64.5 ± 0.8	-0.1
A-T	67.6 ± 0.4	67.8 ± 0.6	0.2
C-A	59.2 ± 0.1	58.9 ± 0.2	-0.3
C-C	57.5 ± 0.7	57.6 ± 0.1	0.1
C-G	68.9 ± 0.8	68.6 ± 0.1	-0.3
C-T	58.7 ± 0.6	59.1 ± 0.4	0.4
G-A	62.4 ± 0.4	62.2 ± 0.7	-0.2
G-C	71.2 ± 0.5	71.3 ± 0.8	0.1
G-G	64.8 ± 0.3	64.4 ± 0.2	-0.4
G-T	64.9 ± 0.3	64.9 ± 0.3	0.0
T-A	65.8 ± 0.4	65.9 ± 0.9	0.1
T-C	58.3 ± 0.3	58.8 ± 0.7	0.5
T-G	62.2 ± 0.3	62.0 ± 0.3	-0.2
T-T	60.1 ± 0.2	60.4 ± 0.2	0.3

[a] $\Delta T_m = T_m$ (+2Mn^{2+}) - T_m (-Mn^{2+}).

Table S3: Melting temperatures (T_m) of 1 μM duplex DNA [F21**X**:R21**Y** (**X-Y**=A-A, A-C, A-G, A-T, C-A, C-C, C-G, C-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G and T-T)] at pH 6.8 in 10 mM sodium cacodylate-cacodylic acid and 100 mM $NaClO_4$ without or with 2 μM $Co(NO_3)_2$, obtained from UV melting.

X-Y	T_m ($-Co^{2+}$) (°C)	T_m ($+2Co^{2+}$) (°C)	ΔT_m (°C)[a]
A-A	59.5 ± 0.3	58.9 ± 0.5	-0.6
A-C	60.9 ± 0.3	60.9 ± 0.5	0.0
A-G	64.6 ± 0.4	64.5 ± 0.7	-0.1
A-T	67.6 ± 0.4	68.1 ± 0.8	0.5
C-A	59.2 ± 0.1	58.9 ± 0.3	-0.3
C-C	57.5 ± 0.7	57.8 ± 0.5	0.3
C-G	68.9 ± 0.8	69.0 ± 0.4	0.1
C-T	58.7 ± 0.6	58.8 ± 0.2	0.1
G-A	62.4 ± 0.4	62.1 ± 0.3	-0.3
G-C	71.2 ± 0.5	72.1 ± 0.5	0.9
G-G	64.8 ± 0.3	64.3 ± 0.5	-0.5
G-T	64.9 ± 0.3	64.8 ± 0.3	-0.1
T-A	65.8 ± 0.4	65.7 ± 0.6	-0.1
T-C	58.3 ± 0.3	58.7 ± 0.5	0.4
T-G	62.2 ± 0.3	61.7 ± 0.7	-0.5
T-T	60.1 ± 0.2	60.0 ± 0.7	-0.1

[a] $\Delta T_m = T_m$ ($+2Co^{2+}$) - T_m ($-Co^{2+}$).

Table S4: Melting temperatures (T_m) of 1 μM duplex DNA [F21**X**:R21**Y** (**X-Y**=A-A, A-C, A-G, A-T, C-A, C-C, C-G, C-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G and T-T)] at pH 6.8 in 10 mM sodium cacodylate-cacodylic acid and 100 mM $NaClO_4$ without or with 2 μM $Ni(NO_3)_2$, obtained from UV melting.

X-Y	T_m (-Ni^{2+}) (^oC)	T_m (+2Ni^{2+}) (^oC)	ΔT_m (^oC)[a]
A-A	59.5 ± 0.3	59.3 ± 0.7	-0.2
A-C	60.9 ± 0.3	61.1 ± 0.7	0.2
A-G	64.6 ± 0.4	64.2 ± 0.4	-0.4
A-T	67.6 ± 0.4	67.6 ± 0.5	0.0
C-A	59.2 ± 0.1	59.0 ± 0.6	-0.2
C-C	57.5 ± 0.7	57.6 ± 0.8	0.1
C-G	68.9 ± 0.8	68.8 ± 0.7	-0.1
C-T	58.7 ± 0.6	58.8 ± 0.3	0.1
G-A	62.4 ± 0.4	61.8 ± 0.3	-0.6
G-C	71.2 ± 0.5	70.8 ± 0.2	-0.4
G-G	64.8 ± 0.3	64.2 ± 0.5	-0.6
G-T	64.9 ± 0.3	64.8 ± 0.4	-0.1
T-A	65.8 ± 0.4	65.2 ± 0.7	-0.6
T-C	58.3 ± 0.3	58.2 ± 0.7	-0.1
T-G	62.2 ± 0.3	61.7 ± 0.5	-0.5
T-T	60.1 ± 0.2	60.2 ± 0.4	0.1

[a] $\Delta T_m = T_m$ (+2Ni^{2+}) - T_m (-Ni^{2+}).

Table S5: Melting temperatures (T_m) of 1 μM duplex DNA [F21**X**:R21**Y** (**X-Y**=A-A, A-C, A-G, A-T, C-A, C-C, C-G, C-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G and T-T)] at pH 6.8 in 10 mM sodium cacodylate-cacodylic acid and 100 mM $NaClO_4$ without or with 2 μM $Zn(NO_3)_2$, obtained from UV melting.

X-Y	T_m (-Zn^{2+}) (°C)	T_m (+2Zn^{2+}) (°C)	ΔT_m (°C)[a]
A-A	59.5 ± 0.3	59.6 ± 0.4	0.1
A-C	60.9 ± 0.3	61.5 ± 0.2	0.6
A-G	64.6 ± 0.4	64.5 ± 0.5	-0.1
A-T	67.6 ± 0.4	68.0 ± 0.6	0.4
C-A	59.2 ± 0.1	59.1 ± 0.3	-0.1
C-C	57.5 ± 0.7	57.9 ± 0.4	0.4
C-G	68.9 ± 0.8	68.7 ± 0.4	-0.2
C-T	58.7 ± 0.6	58.6 ± 0.1	-0.1
G-A	62.4 ± 0.4	62.2 ± 0.4	-0.2
G-C	71.2 ± 0.5	71.7 ± 0.6	0.5
G-G	64.8 ± 0.3	64.8 ± 0.7	0.0
G-T	64.9 ± 0.3	65.3 ± 0.6	0.4
T-A	65.8 ± 0.4	65.8 ± 0.6	0.0
T-C	58.3 ± 0.3	58.7 ± 0.4	0.4
T-G	62.2 ± 0.3	62.0 ± 0.3	-0.2
T-T	60.1 ± 0.2	60.3 ± 0.1	0.2

[a] $\Delta T_m = T_m$ (+2Zn^{2+}) - T_m (-Zn^{2+}).

Table S6: Melting temperatures (T_m) of 1 μM duplex DNA [F21**X**:R21**Y** (**X-Y**=A-A, A-C, A-G, A-T, C-A, C-C, C-G, C-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G and T-T)] at pH 6.8 in 10 mM sodium cacodylate-cacodylic acid and 100 mM $NaClO_4$ without or with 2 μM $Cd(NO_3)_2$, obtained from UV melting.

X-Y	T_m ($-Cd^{2+}$) (°C)	T_m ($+2Cd^{2+}$) (°C)	ΔT_m (°C)[a]
A-A	59.5 ± 0.3	59.5 ± 0.4	0.0
A-C	60.9 ± 0.3	61.2 ± 0.3	0.3
A-G	64.6 ± 0.4	64.6 ± 0.5	0.0
A-T	67.6 ± 0.4	68.0 ± 0.5	0.4
C-A	59.2 ± 0.1	58.8 ± 0.1	-0.4
C-C	57.5 ± 0.7	57.8 ± 0.2	0.3
C-G	68.9 ± 0.8	69.5 ± 0.6	0.6
C-T	58.7 ± 0.6	59.2 ± 0.5	0.5
G-A	62.4 ± 0.4	61.9 ± 0.1	-0.5
G-C	71.2 ± 0.5	71.2 ± 0.1	0.0
G-G	64.8 ± 0.3	64.5 ± 0.4	-0.3
G-T	64.9 ± 0.3	64.9 ± 0.3	0.0
T-A	65.8 ± 0.4	65.7 ± 0.6	-0.1
T-C	58.3 ± 0.3	58.8 ± 0.5	0.5
T-G	62.2 ± 0.3	61.8 ± 0.2	-0.4
T-T	60.1 ± 0.2	60.2 ± 0.1	0.1

[a] $\Delta T_m = T_m$ ($+2Cd^{2+}$) - T_m ($-Cd^{2+}$).

Table S7: Melting temperatures (T_m) of 1 μM duplex DNA [F21**X**:R21**Y** (**X-Y**=A-A, A-C, A-G, A-T, C-A, C-C, C-G, C-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G and T-T)] at pH 6.8 in 10 mM sodium cacodylate-cacodylic acid and 100 mM $NaClO_4$ without or with 2 μM $TlNO_3$, obtained from UV melting.

X-Y	T_m (-Tl^+) (°C)	T_m (+2Tl^+) (°C)	ΔT_m (°C)[a]
A-A	59.5 ± 0.3	58.8 ± 0.2	-0.7
A-C	60.9 ± 0.3	60.6 ± 0.3	-0.3
A-G	64.6 ± 0.4	64.0 ± 0.2	-0.6
A-T	67.6 ± 0.4	67.1 ± 0.2	-0.5
C-A	59.2 ± 0.1	58.6 ± 0.3	-0.6
C-C	57.5 ± 0.7	57.2 ± 0.2	-0.3
C-G	68.9 ± 0.8	68.7 ± 0.5	-0.2
C-T	58.7 ± 0.6	58.4 ± 0.3	-0.3
G-A	62.4 ± 0.4	62.1 ± 0.6	-0.3
G-C	71.2 ± 0.5	70.9 ± 0.4	-0.3
G-G	64.8 ± 0.3	64.6 ± 0.4	-0.2
G-T	64.9 ± 0.3	64.7 ± 0.6	-0.2
T-A	65.8 ± 0.4	65.5 ± 0.7	-0.3
T-C	58.3 ± 0.3	57.9 ± 0.3	-0.4
T-G	62.2 ± 0.3	61.6 ± 0.1	-0.6
T-T	60.1 ± 0.2	59.5 ± 0.2	-0.6

[a] $\Delta T_m = T_m$ (+2Tl^+) - T_m (-Tl^+).

Table S8: Melting temperatures (T_m) of 1 μM duplex DNA [F21**X**:R21**Y** (**X-Y**=A-A, A-C, A-G, A-T, C-A, C-C, C-G, C-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G and T-T)] at pH 6.8 in 10 mM sodium cacodylate-cacodylic acid and 100 mM $NaClO_4$ without or with 2 μM $Pb(NO_3)_2$, obtained from UV melting.

X-Y	T_m (-Pb^{2+}) (^oC)	T_m (+2Pb^{2+}) (^oC)	ΔT_m (^oC)[a]
A-A	59.5 ± 0.3	58.9 ± 0.4	-0.6
A-C	60.9 ± 0.3	60.6 ± 0.1	-0.3
A-G	64.6 ± 0.4	63.8 ± 0.2	-0.8
A-T	67.6 ± 0.4	66.7 ± 0.1	-0.9
C-A	59.2 ± 0.1	58.4 ± 0.2	-0.8
C-C	57.5 ± 0.7	57.0 ± 0.2	-0.5
C-G	68.9 ± 0.8	68.1 ± 0.2	-0.8
C-T	58.7 ± 0.6	57.9 ± 0.1	-0.8
G-A	62.4 ± 0.4	61.7 ± 0.3	-0.7
G-C	71.2 ± 0.5	70.9 ± 0.5	-0.3
G-G	64.8 ± 0.3	64.5 ± 0.4	-0.3
G-T	64.9 ± 0.3	64.4 ± 0.4	-0.5
T-A	65.8 ± 0.4	64.9 ± 0.3	-0.9
T-C	58.3 ± 0.3	57.7 ± 0.3	-0.6
T-G	62.2 ± 0.3	61.7 ± 0.4	-0.5
T-T	60.1 ± 0.2	59.6 ± 0.2	-0.5

[a] $\Delta T_m = T_m$ (+2Pb^{2+}) - T_m (-Pb^{2+}).

Table S9: Melting temperatures (T_m) of 1 μM duplex DNA [F25**Z**:R25**W** (**Z-W**=A-A, A-C, A-G, A-T, C-A, C-C, C-G, C-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G, and T-T)] at pH 6.8 in 10 mM sodium cacodylate-cacodylic acid and 100 mM $NaClO_4$ without or with 1 or 2 μM $AgNO_3$, obtained from UV melting.

Z-W	T_m (-Ag^+) (°C)	T_m (+Ag^+) (°C)	ΔT_m (°C)[a]	T_m (+2Ag^+) (°C)	ΔT_{m2} (°C)[b]
A-A	67.2 ± 0.1	68.5 ± 0.7	1.3	68.8 ± 0.2	1.6
A-C	68.4 ± 0.5	70.2 ± 0.4	1.8	70.8 ± 0.7	2.4
A-G	71.7 ± 0.4	72.6 ± 0.6	0.9	72.5 ± 0.7	0.8
A-T	73.6 ± 0.2	74.4 ± 0.1	0.8	75.3 ± 0.6	1.7
C-A	66.9 ± 0.3	68.4 ± 0.8	1.5	69.5 ± 0.5	2.6
C-C	66.5 ± 0.8	69.5 ± 0.8	3.0	70.2 ± 0.3	3.7
C-G	74.7 ± 0.5	75.9 ± 0.3	1.2	75.9 ± 0.7	1.2
C-T	67.9 ± 0.3	69.4 ± 0.7	1.5	70.7 ± 0.5	2.8
G-A	69.8 ± 0.6	71.6 ± 0.8	1.8	72.3 ± 0.4	2.5
G-C	76.6 ± 0.8	78.3 ± 0.4	1.7	78.4 ± 1.0	1.8
G-G	72.1 ± 0.2	73.1 ± 0.4	1.0	72.9 ± 0.7	0.8
G-T	72.1 ± 0.1	73.2 ± 0.2	1.1	73.9 ± 0.9	1.8
T-A	71.7 ± 0.0	72.7 ± 0.6	1.0	73.8 ± 0.5	2.1
T-C	66.7 ± 0.7	68.4 ± 0.4	1.7	69.5 ± 0.7	2.8
T-G	69.7 ± 0.5	70.6 ± 0.1	0.9	70.7 ± 0.3	1.0
T-T	68.4 ± 0.3	69.7 ± 0.6	1.3	69.8 ± 0.4	1.4

[a] $\Delta T_m = T_m$ (+Ag^+) - T_m (-Ag^+). [b] $\Delta T_{m2} = T_m$ (+2Ag^+) - T_m (-Ag^+).

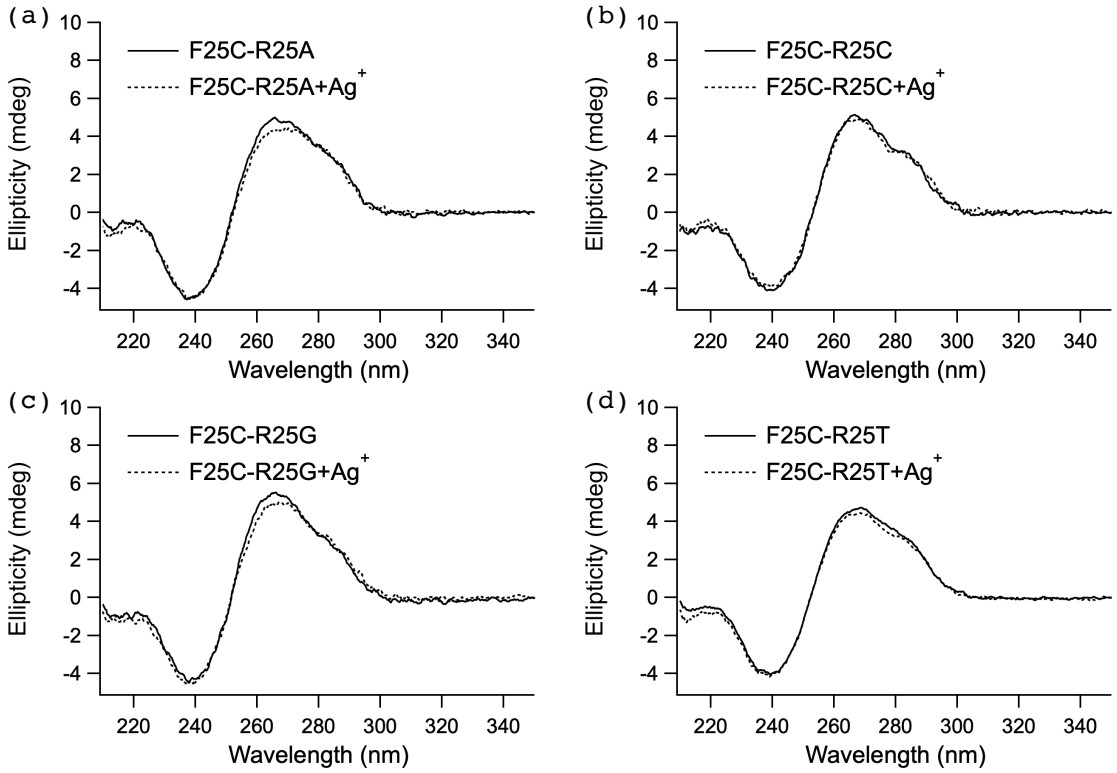

Figure S1: CD spectra of the duplex DNA, F25C-R25A (a), F25C-R25C (b), F25C-R25G (c), and F25C-R25T (d), without or with $AgNO_3$. Duplex DNA (1 μM) at 25°C and pH 6.8 in buffer A (see *UV melting*) without or with 1 μM $AgNO_3$ were measured at a wavelength of 210-350 nm. The cell path length was 1 cm.

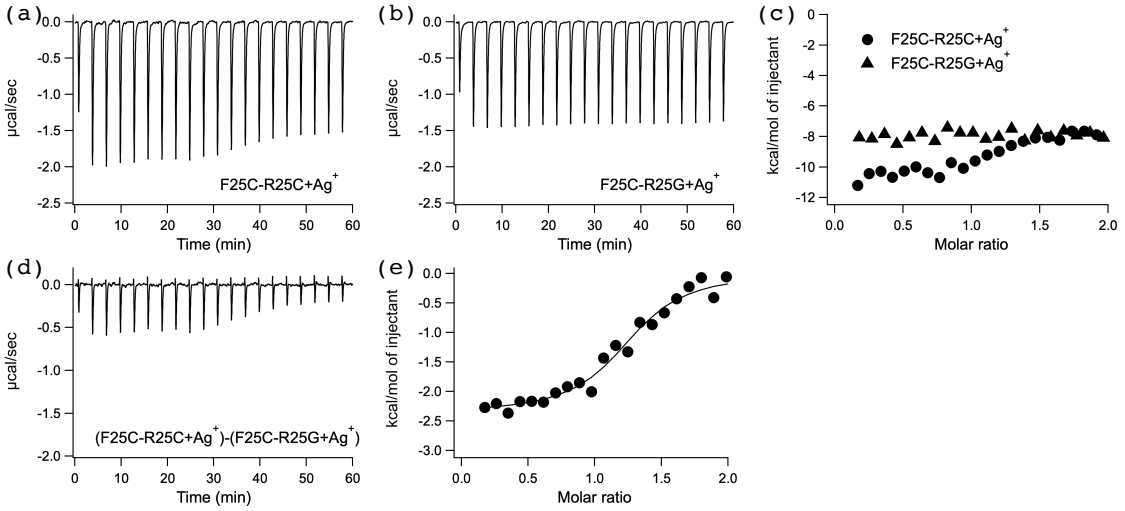

Figure S2: Thermodynamic analyses of the interaction between Ag^+ and each of the C-C mismatches (F25C-R25C) and the perfectly matched duplex DNA (F25C-R25G). (a, b) Typical ITC profile for the interaction between $AgNO_3$ and F25C-R25C (a) and F25C-R25G (b) at 25°C and pH 6.8 in buffer A (see *UV melting*). $AgNO_3$ solution (1 mM in buffer A) was injected 20 times in 5 μl increments into each of F25C-R25C (a) and F25C-R25G (b) solution (40 μM in buffer A). Injections were administered over 12 s at 3 min intervals. (c) Titration plots against the molar ratio of $[Ag^+]$/[duplex DNA], obtained from the ITC profiles in (a) and (b). (d) ITC profile for the binding between Ag^+ and the C-C mismatched base pair, obtained by subtracting the ITC profile observed for F25C-R25G in (b) from that observed for F25C-R25C in (a). (e) Titration plot against the molar ratio of $[Ag^+]$/[duplex DNA], obtained from the ITC profile in (d). The data were fitted by a nonlinear least-squares method.