Supplemental Information

The substrate binding interface of alkylpurine DNA glycosylase AlkD

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Supplemental Tables

Table S1. DNA binding by wild-type and mutant AlkD [a]

Enzyme	K_d					
	G•C-DNA (μM)	Relative to WT	G•T-DNA (μM)	Relative to WT	THF•C-DNA (μM)	Relative to WT
WT	1.3 ± 0.2	1.0	1.4 ± 0.3	1.0	2.8 ± 0.8	1.0
Y27A	1.5 ± 0.3	1.1	3.0 ± 0.5	2.1	4.6 ± 0.6	1.6
Y27F	1.5 ± 0.1	1.1	1.4 ± 0.3	1.0	2.9 ± 0.6	1.0
Q38E	3.0 ± 0.3	2.3	3.8 ± 0.3	2.7	7.6 ± 1.2	2.7
T39D	6.4 ± 1.1	4.9	3.1 ± 0.7	2.2	11.4 ± 2.8	4.1
R43E	12.8 ± 0.2	9.8	14.7 ± 2.0	10.5	13.5 ± 4.1	4.8
W109A	3.7 ± 1.2	2.8	2.2 ± 0.1	1.6	4.3 ± 0.6	1.5
D113N	0.7 ± 0.1	0.5	1.0 ± 0.1	0.7	1.3 ± 0.1	0.5
R148A	4.6 ± 0.8	3.5	3.9 ± 0.6	2.8	6.9 ± 3.0	2.5
F179A/F180A	2.4 ± 0.5	1.8	2.3 ± 0.9	1.6	4.7 ± 1.1	1.7
W187A	3.6 ± 0.7	2.8	4.7 ± 0.5	3.4	7.6 ± 0.9	2.7
R190A	0.8 ± 0.1	0.6	1.1 ± 0.2	0.8	1.3 ± 0.1	0.5

[a] Dissociation constants (K_d) for AlkD binding to 25-mer oligonucleotide duplexes containing the specified modification as measured by fluorescence anisotropy. Binding was performed at 25°C, pH 6.5, and 120 mM ionic strength. Values represent the averages and standard deviations from three experiments.

Table S2. Excision of 7mG from oligonucleotide DNA by wild-type and mutant AlkD [a]

Enzyme	k_{obs} ($M^{-1} s^{-1}$)	Relative to WT
WT	$(1.2 \pm 0.2) \times 10^{3}$	1.0×10^{0}
Y27A	$(1.5 \pm 0.1) \times 10^{2}$	1.3×10^{-1}
Y27F	$(4.6 \pm 1.8) \times 10^{2}$	3.8×10^{-1}
Q38E	$(3.3 \pm 1.1) \times 10^{2}$	2.8×10^{-1}
T39D	$(1.1 \pm 0.6) \times 10^{1}$	9.2×10^{-3}
R43E	$(3.4 \pm 0.6) \times 10^{1}$	2.8×10^{-2}
W109A	$(4.1 \pm 1.2) \times 10^{0}$	3.4×10^{-3}
D113N	$(5.8 \pm 0.2) \times 10^{1}$	4.8×10^{-2}
D113N (25°C)[b]	$(1.3 \pm 4.3) \times 10^{0}$	1.1×10^{-3}
R148A	$(1.0 \pm 0.1) \times 10^{1}$	9.2×10^{-3}
F179A/F180A	$(3.2 \pm 0.1) \times 10^{1}$	2.7×10^{-2}
W187A	$(1.6 \pm 0.1) \times 10^{1}$	1.3×10^{-2}
R190A	$(3.4 \pm 0.5) \times 10^{0}$	2.8×10^{-3}
No enzyme	$(1.4 \pm 0.6) \times 10^{-1}$	1.2×10^{-4}

[a] Second-order rate constants for excision of 7mG from a 25-mer oligonucleotide duplex. Reactions were performed at 37°C (unless otherwise noted), pH 7.5, and 150 mM ionic strength. Values represent the averages and standard deviations from three experiments.
[b] Reactions were performed at 25°C.

Table S3. Excision of 3mA and 7mG from genomic DNA by wild-type and mutant AlkD [a]

Enzyme	Adduct released			
	3mA (pmol)	Relative to WT	7mG (pmol)	Relative to WT
HCl	66.9 ± 8.0	0.96	352 ± 40	1.32
WT	69.7 ± 3.3	1.00	266 ± 3	1.00
Y27A	66.3 ± 3.4	0.95	190 ± 9	0.71
Y27F	71.0 ± 2.1	1.02	277± 5	1.04
Q38E	67.5 ± 1.0	0.97	230 ± 3	0.86
T39D	66.5 ± 3.2	0.95	107 ± 4	0.40
R43E	65.3 ± 2.3	0.94	179 ± 4	0.67
W109A	28.4 ± 1.2	0.41	33.0 ± 0.7	0.12
D113N	30.5 ± 1.8	0.44	21.0 ± 0.2	0.08
D113N (25°C)[b]	19.2 ± 1.1	0.28	12.3 ± 0.3	0.05
R148A	45.6 ± 1.0	0.65	14.6 ± 0.1	0.05
F179A/F180A	66.3 ± 2.2	0.95	81.7 ± 0.6	0.31
W187A	53.9 ± 0.2	0.77	24.3 ± 0.3	0.09
R190A	62.9 ± 1.2	0.90	57.2 ± 0.4	0.22
No enzyme	1.90 ± 0.18	0.03	10.1 ± 0.1	0.04

[a] Adducts released from 10 µg methylated calf thymus DNA by 5 µM enzyme, 5 N HCl, or glycosylase buffer. Reactions were carried out at 37°C (unless otherwise noted), pH 7.5, and 150 mM ionic strength. Values represent the averages and standard deviations from three experiments.

[b] Reactions were carried out at 25°C.

Supplemental Figures

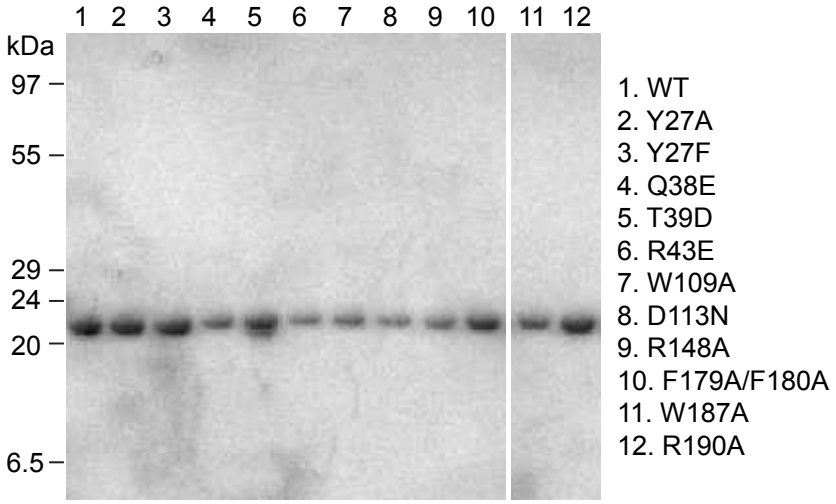
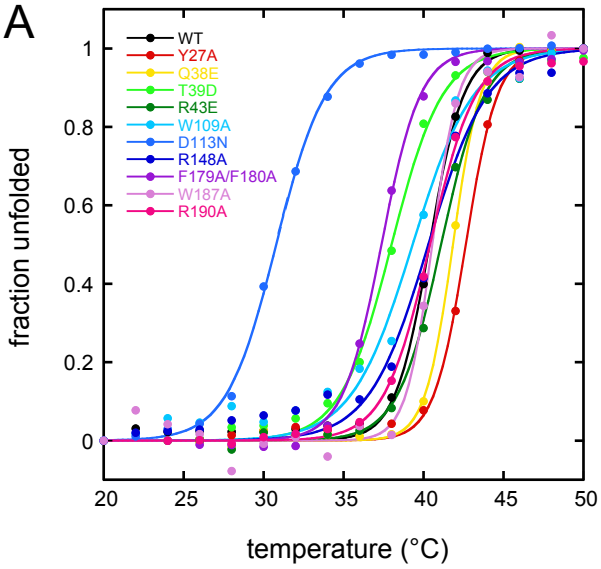


Figure S1. SDS-PAGE analysis of purified wild-type and mutant AlkD.

A



B

Enzyme	T_m (°C)
WT	40.4
Y27A	42.6
Y27F	ND*
Q38E	41.8
T39D	38.0
R43E	41.1
W109A	39.3
D113N	30.7
R148A	40.3
F179A/F180A	37.4
W187A	40.5
R190A	40.4

*not determined

Figure S2. Thermal denaturation of wild-type and mutant AlkD. (A) Thermal melting profiles. Protein unfolding was monitored by changes in molar ellipticity at 222 nm during heating. (B) Melting temperatures (T_m) derived from panel A.

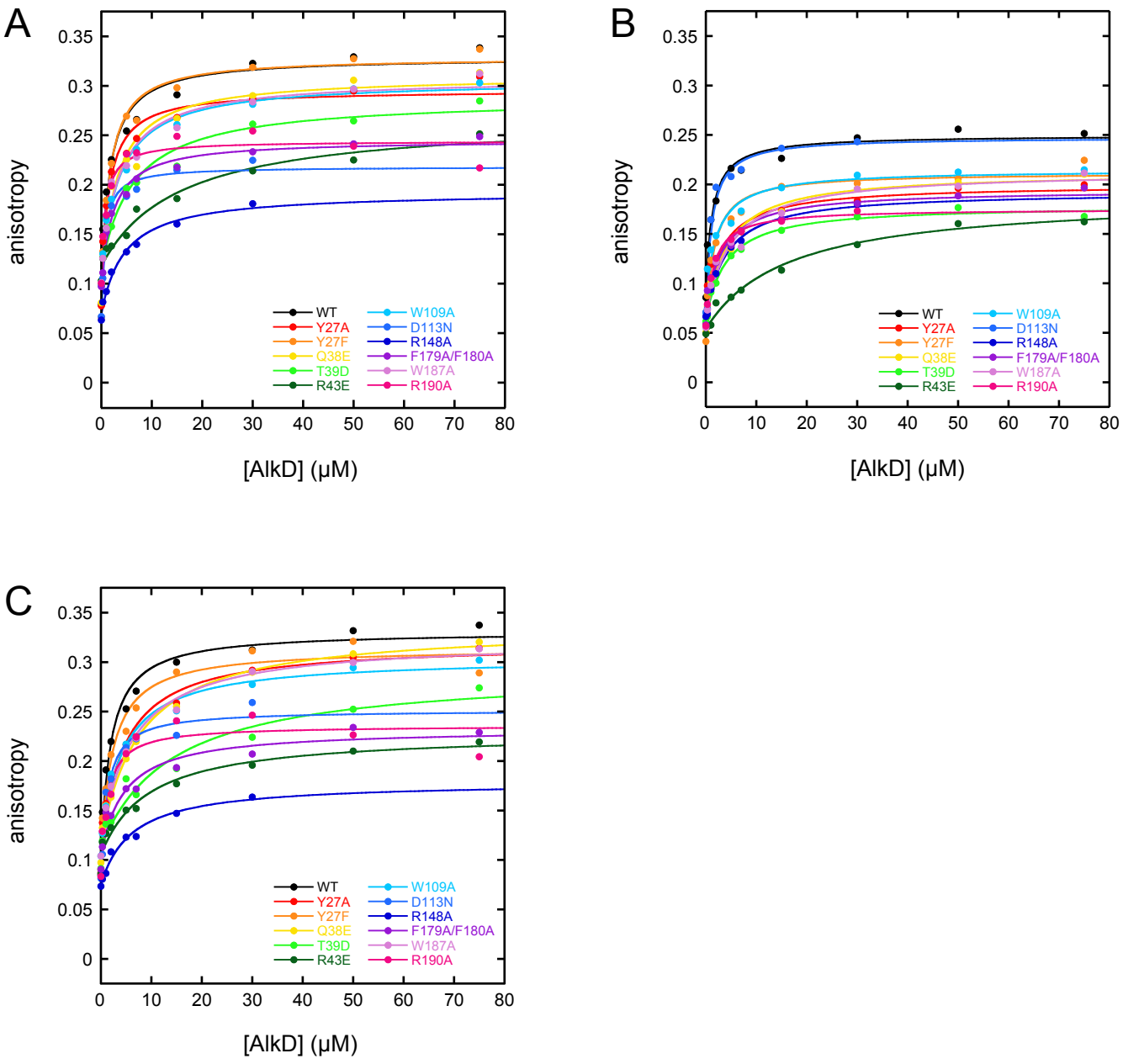


Figure S3. DNA binding by wild-type and mutant AlkD. Binding of FAM-labeled DNA by wild-type and mutant AlkD was measured using fluorescence anisotropy. 25-mer oligonucleotides contained a centrally located (A) G•C, (B) G•T, or (C) THF•C. Equilibrium dissociation constants are provided in Table S1.

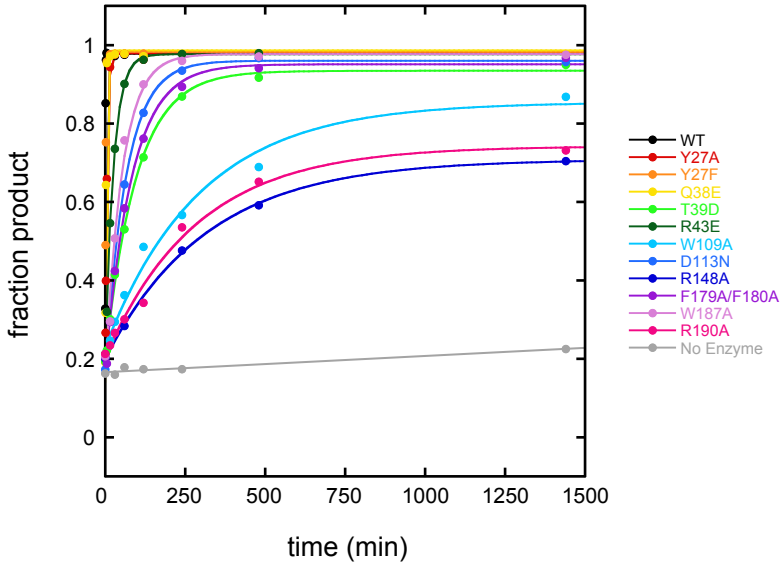


Figure S4. 7mG excision from oligonucleotide DNA by wild-type and mutant AlkD. Second-order rate constants are provided in Table S2.

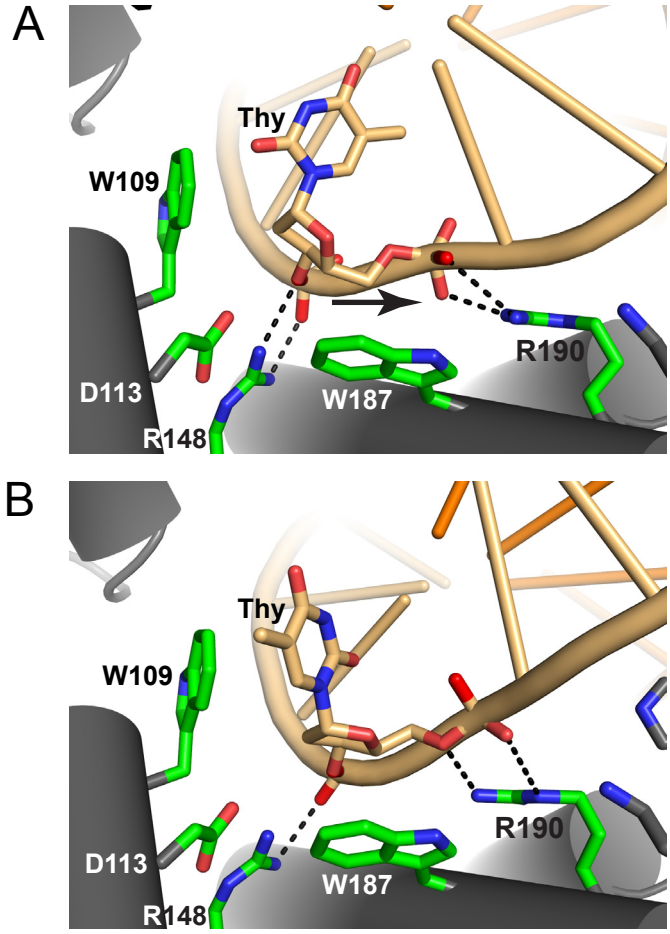


Figure S5. Changes in AlkD-DNA contacts to accommodate the distorted THF-DNA conformation. (A) Substrate-like complex containing 3-deaza-*N*3-methyladenine (3d3mA). (B) Product-like complex containing tetrahydrofuran (THF). The thymine (Thy) opposite the lesion and side chains lining the concave binding surface (green) are shown as sticks. The black arrow denotes the movement of the thymine backbone necessary to attain the conformation observed in the product-like complex.