Supporting Information

Defining the Role of Nucleotide Flipping in Enzyme Specificity using ^{19}F NMR

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Table S1. Thymine excision activity of TDG$^{82\text{-}308}$ at 37 °C

Substrate	k_{max} (min^{-1})	k_{max} relative to G·TGG
G·TGG	1.36 ± 0.09	-
G·TGA	0.70 ± 0.02	1/ 1.9
G·TGC	0.98 ± 0.04	1/ 1.4
G·TGT	0.72 ± 0.03	1/ 1.9
G·TAG	0.283 ± 0.006	1/ 4.8
G·TAA	0.056 ± 0.002	1/ 24
G·TAC	0.062 ± 0.007	1/ 22
G·TAT	0.092 ± 0.001	1/ 15
G·TCG	0.095 ± 0.001	1/ 14
G·TCA	0.0118 ± 0.0002	1/ 115
G·TCC	0.0117 ± 0.0003	1/ 116
G·TCT	0.054 ± 0.001	1/ 25
G·TTG	0.0169 ± 0.0004	1/ 80
G·TTA	0.0060 ± 0.0002	1/ 227
G·TTC	0.0046 ± 0.0001	1/ 296
G·TTT	0.0109 ± 0.0002	1/ 125

k_{max} values represent the mean and standard deviation for at least three independent single turnover kinetics experiments. Data fitting is shown in Supporting Information Figure S1.

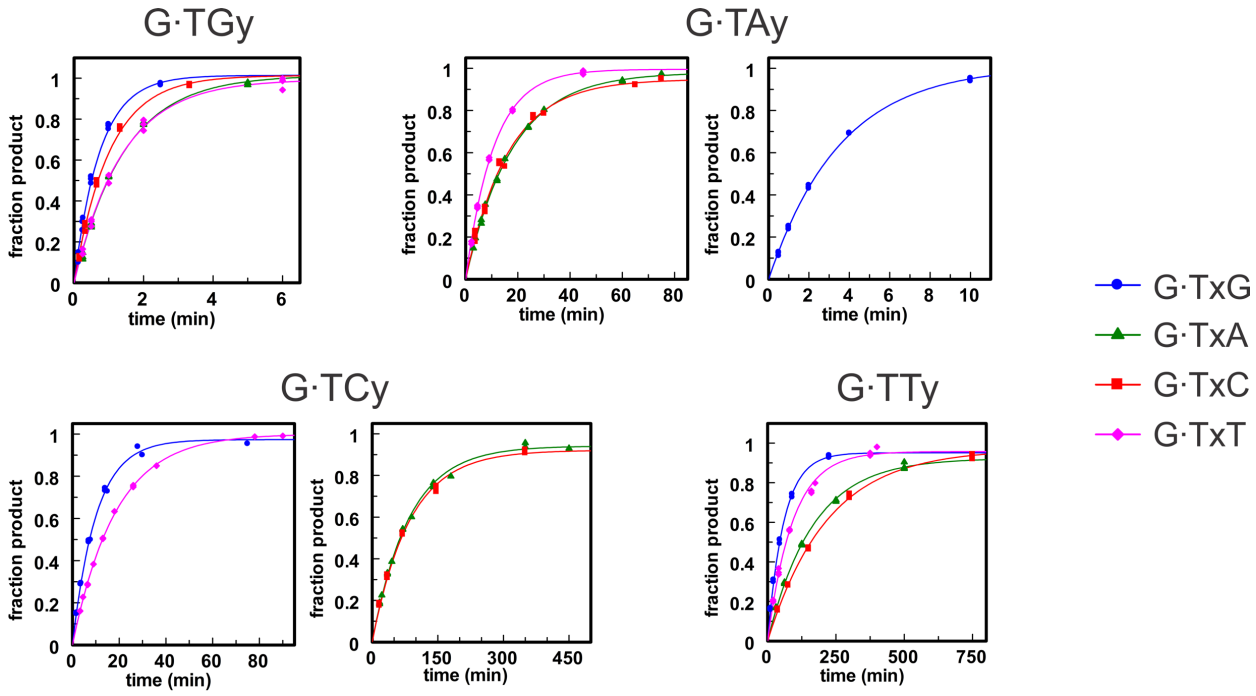

Figure S1. Thymine excision activity (k_{max}) of TDG$^{82\text{-}308}$ for G·Txy substrates. Single turnover kinetics experiments were performed at 37 °C using a saturating enzyme concentration. Data were fitted to a single exponential equation (eq. 2) and the resulting rate constants (k_{max}) are shown in Supporting Information Table S1. The panes are grouped according to the +1 base, and the fitted curves and data points are colored according to the base at the +2 site of the DNA, as shown in the key. For example, blue is used for the four G·TxG substrates (G·TGG, G·TAG, G·TCG, G·TTG).

Table S2. Thymine excision activity of TDG$^{82\text{-}308}$ and two variants at 37 °C

Enzyme	Substrate	k_{max} (min^{-1})	k_{max} relative to G·TG (or G·UG)
TDG	G·TG	0.72 ± 0.03	1
TDG	G·TA	0.092 ± 0.001	1/ 7.8
TDG	G·TC	0.054 ± 0.001	1/ 13
TDG	G·TT	0.0109 ± 0.0002	1/ 65
A145G-TDG	G·TG	9.0 ± 0.3	1
A145G-TDG	G·TA	1.16 ± 0.04	1/ 7.8
A145G-TDG	G·TC	0.69 ± 0.04	1/ 13
A145G-TDG	G·TT	0.144 ± 0.004	1/ 64
Q278A-TDG	G·TG	0.31 ± 0.05	-
Q278A-TDG	G·TA	0.164 ± 0.007	1/ 1.9
Q278A-TDG	G·TC	0.050 ± 0.002	1/ 6.2
Q278A-TDG	G·TT	0.0234 ± 0.0006	1/ 14
TDG	G·UG	6.1 ± 0.6	1
TDG	G·UA	2.42 ± 0.06	1/ 2.5
TDG	G·UC	2.64 ± 0.09	1/ 2.3
TDG	G·UT	0.50 ± 0.03	1/ 12

k_{max} values represent the mean and standard deviation for at least three independent single turnover kinetics experiments. The G·Tx and G·Ux substrates have a T at the +2 site (G·TxT, G·UxT). Data fitting is shown in Supporting Information Figure S2 (G·Tx) and S4 Figure (G·Ux).

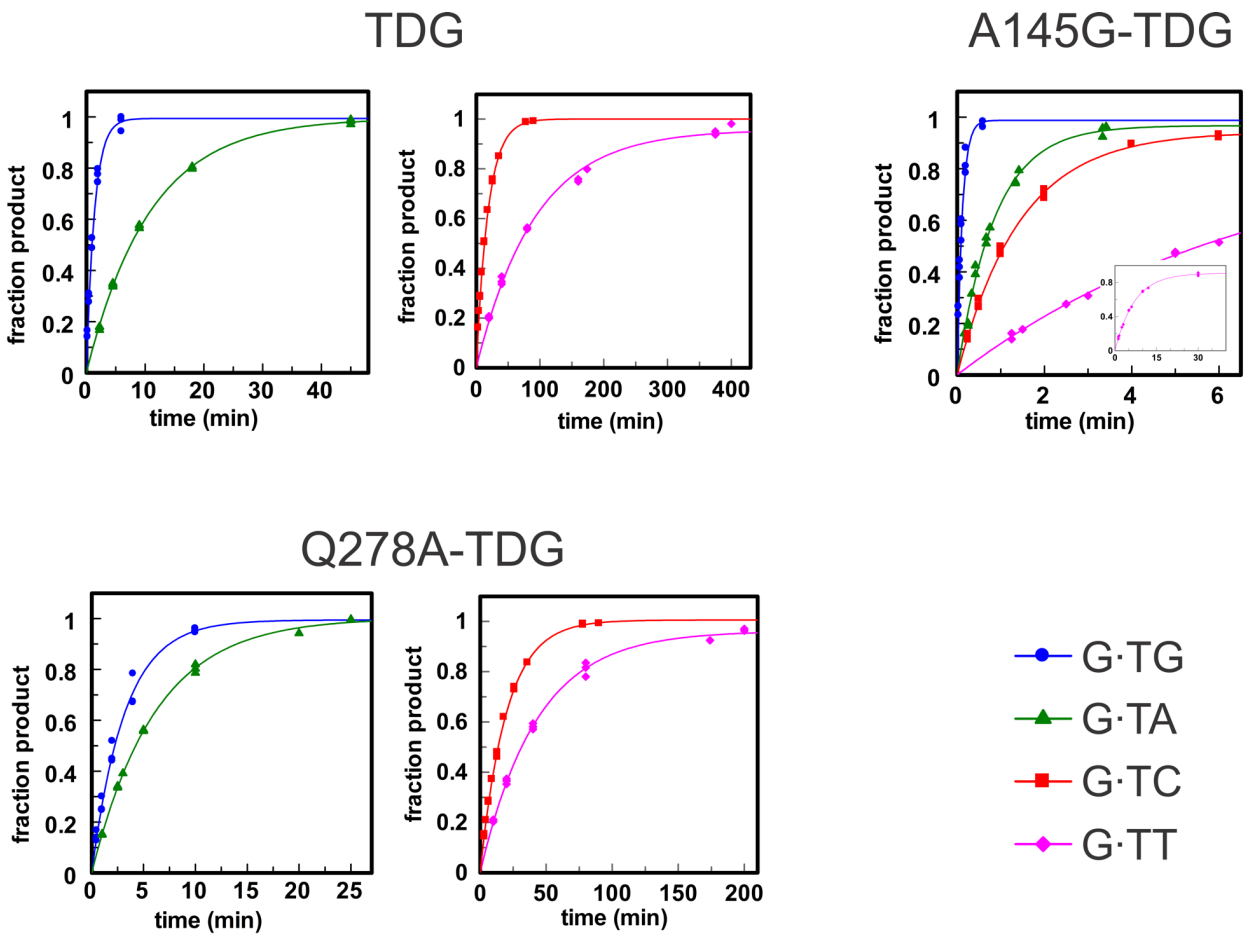

Figure S2. Thymine excision activity (k_{max}) of TDG$^{82\text{-}308}$ and two variants for G·Tx substrates. Single turnover kinetics experiments were performed at 37 °C using a saturating enzyme concentration. Data were fitted to a single exponential equation (eq. 2) and the resulting rate constants (k_{max}) are shown in Supporting Information Table S2. The panes are grouped according to enzyme and the fitted curves and data points are colored according to the +1 base of substrate as shown in the key.

Table S3. k_{max} and K_{flip} for TDG$^{82\text{-}308}$ and A145G variant at 18 °C

Enzyme	Substrate	k_{max} (min^{-1})	K_{flip}
WT	G·TGG	0.130 ± 0.004	2.1
WT	G·TGC	0.11 ± 0.01	2.1
WT	G·TGT	0.067 ± 0.002	1.4
WT	G·TAG	0.0226 ± 0.0004	0.45
WT	G·TAT	0.0073 ± 0.0004	0.14
A145G	G·TGG	2.4 ± 0.1	30
A145G	G·TGT	1.4 ± 0.1	12
A145G	G·TAT	0.128 ± 0.002	2.0
A145G	G·TCT	0.0542 ± 0.0005	0.46
A145G	G·TTT	0.0089 ± 0.0008	0.57

k_{max} values represent the mean and standard deviation for at least three independent kinetics experiments. Data fitting is shown in Supporting Information Figure S3.

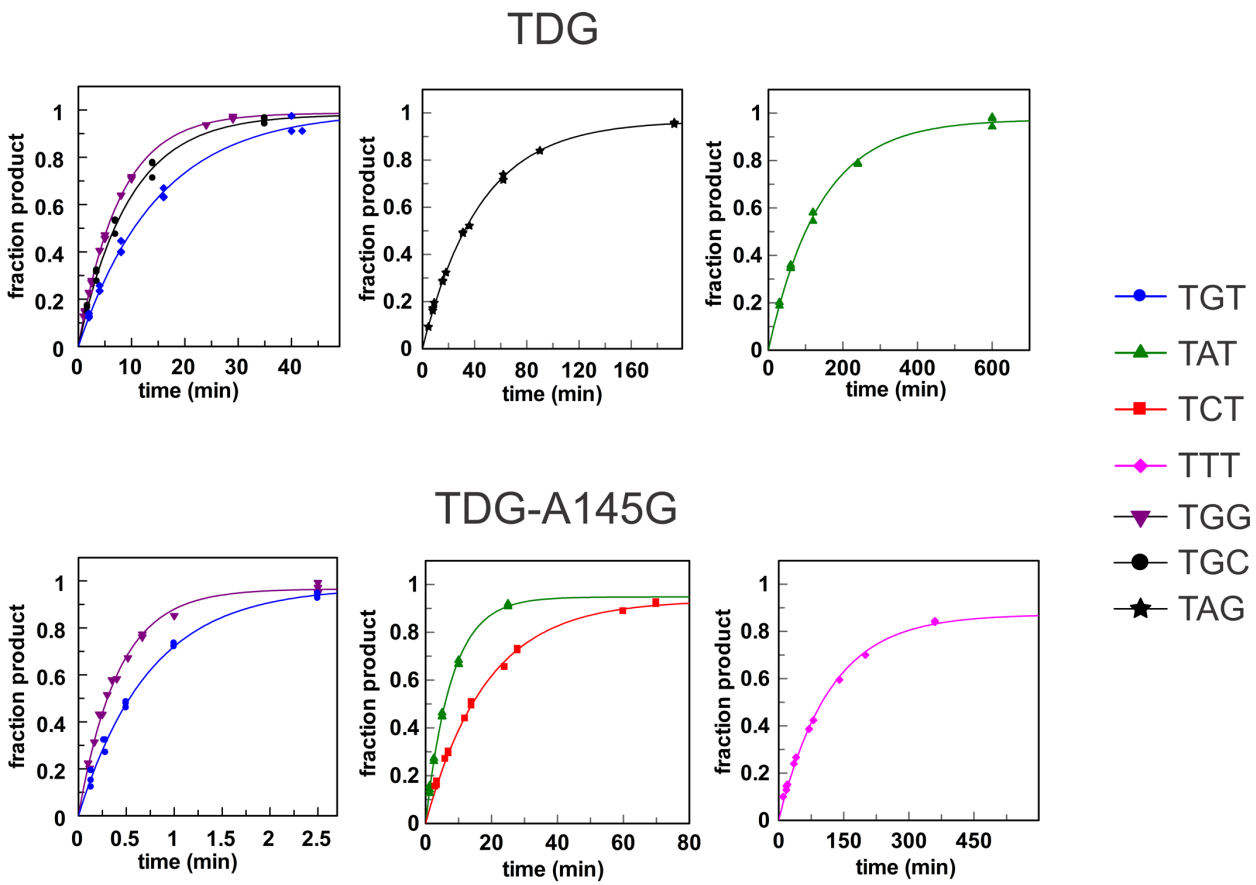

Figure S3. Thymine excision activity (k_{max}) of TDG$^{82\text{-}308}$ and the A145G variant for G·Txy substrates. Single turnover kineics experiments were performed at 18 °C using a saturating enzyme concentration and the data was fitted to a single eponential equation (eq. 2), with rate constants (k_{max}) provided in Supporting Information Table S3. The panes are grouped according to enzyme and the fitted curves and data points are colored by substrate as indicated in the key.

Table S4. Glycosylase activity of TDG$^{82\text{-}308}$ at 18 °C and 37 °C

Substrate	k_{max} 18 °C (min^{-1})	k_{max} 37 °C (min^{-1})	k_{max}^{37C}/ k_{max}^{18C}
G·TG	0.067 ± 0.002	0.72 ± 0.03	11
G·TA	0.0073 ± 0.0004	0.092 ± 0.001	13
G·TC	0.0016 ± 0.0001	0.054 ± 0.001	34
G·TT	0.00025 ± 0.00001	0.0109 ± 0.0002	44
G·UG	1.40 ± 0.08	6.1 ± 0.6	4
G·UA	0.54 ± 0.01	2.42 ± 0.06	5
G·UC	0.52 ± 0.02	2.64 ± 0.09	5
G·UT	0.070 ± 0.002	0.50 ± 0.03	7

k_{max} values represent the mean and standard deviation for at least three independent single turnover kinetics experiments. These G·Tx and G·Ux substrates all contained T at the +2 site (G·TxT, G·UxT). For some substrates the same k_{max} values are shown in another table above. Data fitting for G·Ux substrates is shown in Supporting Information Figure S4.

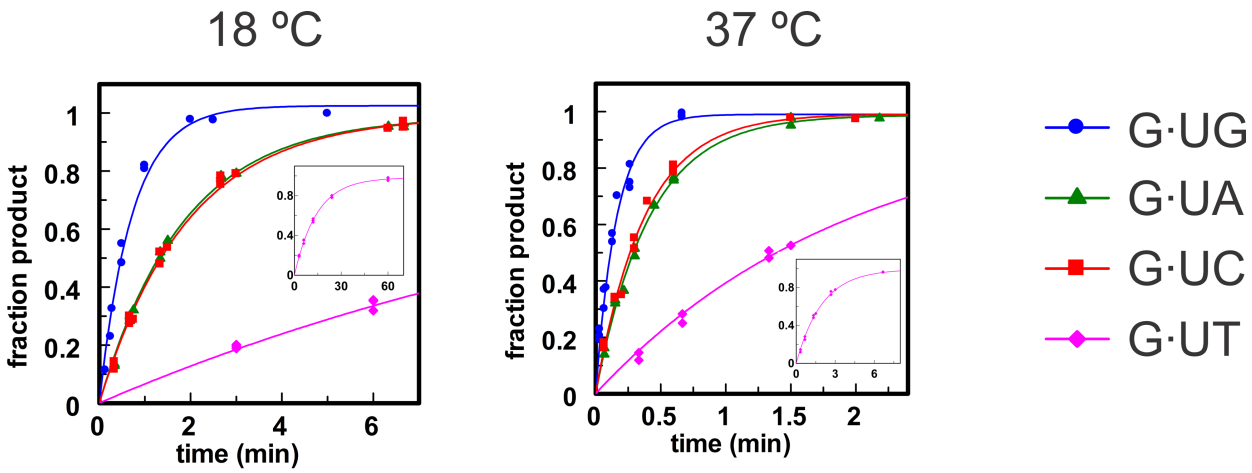

Figure S4. Uracil excision activity (k_{max}) of TDG$^{82-308}$ for G·Ux substrates. Single turnover kinetics experiments were performed at 18 °C or 37 °C using a saturating enzyme concentration and the data was fitted to a single exponential equation (eq. 2), with rate constants (k_{max}) provided in Supporting Information Table S4. Fitted curves and data points are colored by substrate as shown in the key.