Supplemental Data

Mechanism of Abasic Lesion Bypass Catalyzed by a Y-Family DNA Polymerase[†]

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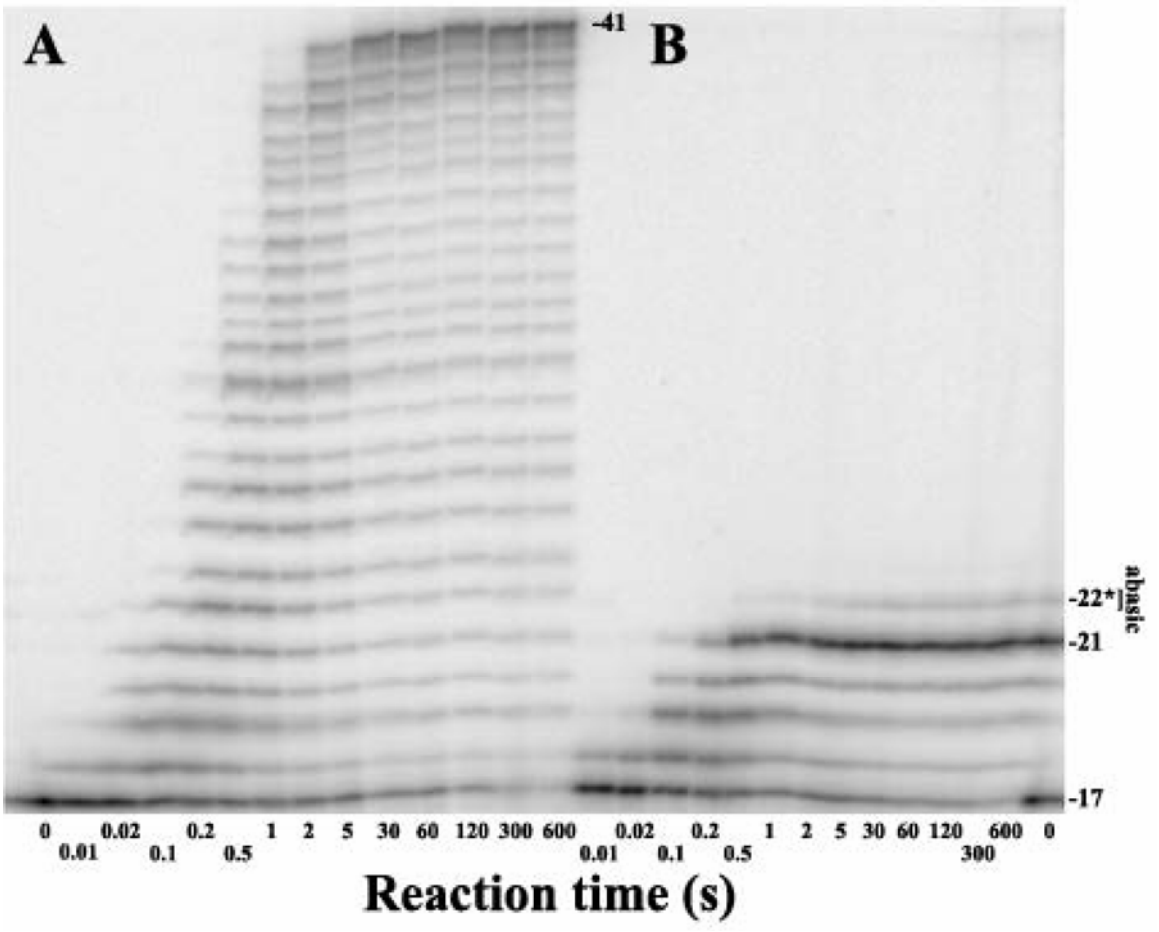
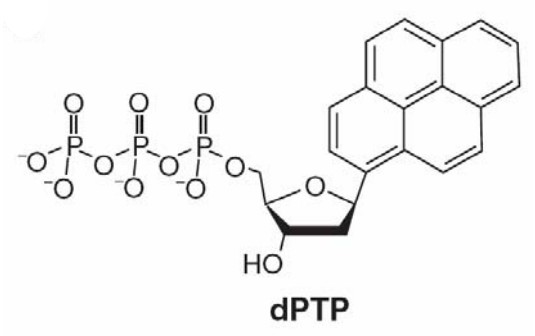
Running Title: Pre-steady state kinetic studies of abasic lesion bypass

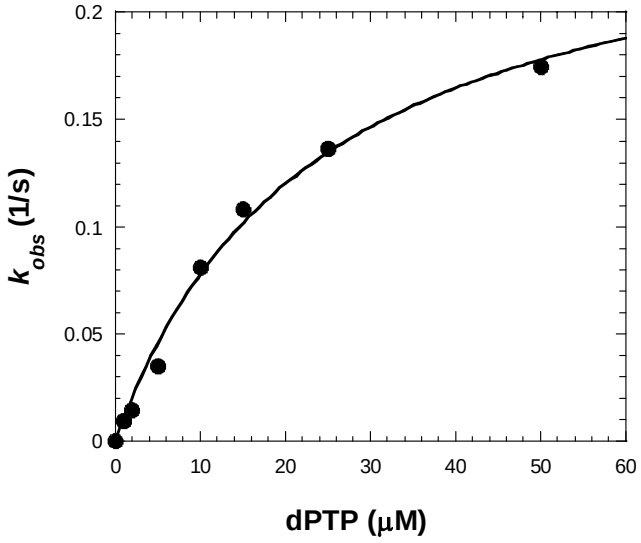


Sup 1 Figure 1 Running start nucleotide incorporation assay. A preincubated solution of *S. solfataricus* replicative DNA polymerase PolB1 (100 nM) and 17-mer/41-mer (100 nM) were mixed with all four dNTPs (200 μM each) for various reaction times before quenching with EDTA. The product lengths were labeled and the AP was designated. (A) Reaction with the 17-mer/41CTL substrate, (B) reaction with the 17-mer/41AP substrate.

A



B



Sup Figure 2 Concentration dependence of observed dPTP incorporation rate constant. (A) the chemical structure of dPTP; (B) The extracted observed rate constants were plotted as a function of dPTP concentration and fit to eq 2 (Materials and methods) to obtain a k_p of 0.26 ± 0.02 s^{-1} and a K_d of 24 ± 4 μM.

Sup Table I Kinetic parameters for each nucleotide incorporation into damaged DNA downstream of the pause sites

dNTP	K_d (μM)	k_p (s^{-1})	k_p/K_d ($\mu M^{-1}s^{-1}$)	Incorporation%[a]
Damaged 23-A-mer/41AP				
dGTP	368 ± 104	1.2 ± 0.1	3.4 x $\mathbf{10^{-3}}$	98.6%
dATP	827 ± 275	0.007 ± 0.001	8.1 x $\mathbf{10^{-6}}$	0.2%
dCTP	173 ± 36	0.0038 ± 0.0002	2.2 x $\mathbf{10^{-5}}$	0.6%
dTTP	1454 ± 566	0.026 ± 0.006	1.8 x $\mathbf{10^{-5}}$	0.5%
Damaged 24-A-mer/41AP				
dTTP	447 ± 119	2.2 ± 0.2	4.8 x $\mathbf{10^{-3}}$	99.7%
dATP	496 ± 58	0.00097 ± 0.00004	2.0 x $\mathbf{10^{-6}}$	0.1%
dCTP	612 ± 196	0.008 ± 0.001	1.4 x $\mathbf{10^{-5}}$	0.3%
dGTP	334 ± 37	0.00039 ± 0.00001	1.2 x $\mathbf{10^{-6}}$	0.0%
Damaged 26-A-mer/41AP				
dGTP	412 ± 48	2.8 ± 0.1	6.7 x $\mathbf{10^{-3}}$	99.9%
dATP	1499 ± 377	0.004 ± 0.001	3.0 x $\mathbf{10^{-6}}$	0.0%
dCTP	1436 ± 151	0.0016 ± 0.0001	1.1 x $\mathbf{10^{-6}}$	0.0%
dTTP	1303 ± 489	0.005 ± 0.001	3.8 x $\mathbf{10^{-6}}$	0.1%
Damaged 27-A-mer/41AP				
dATP	260 ± 45	8.5 ± 0.5	3.3 x $\mathbf{10^{-2}}$	99.9%
dCTP	524 ± 81	0.0018 ± 0.0001	3.4 x $\mathbf{10^{-6}}$	0.0%
dGTP	578 ± 110	0.0083 ± 0.0006	1.4 x $\mathbf{10^{-5}}$	0.1%
dTTP	329 ± 34	0.0029 ± 0.0001	8.9 x $\mathbf{10^{-6}}$	0.0%

[a]Incorporation% = $(k_p/K_d)_{dNTP}/\Sigma(k_p/K_d)_{dNTP}$

Sup Table II Kinetic parameters for each nucleotide incorporation into damaged DNA downstream of the pause sites

dNTP	K_d (μM)	k_p (s^{-1})	k_p/K_d ($\mu M^{-1}s^{-1}$)	Incorporation%[a]
Damaged 23-C-mer/41AP				
dTTP	267 ± 51	0.68 ± 0.05	2.6 x $\mathbf{10^{-3}}$	98.5%
dATP	253 ± 23	0.0029 ± 0.0001	1.1 x $\mathbf{10^{-5}}$	0.4%
dCTP	398 ± 58	0.0077 ± 0.0004	1.9 x $\mathbf{10^{-5}}$	0.8%
dGTP	575 ± 145	0.0047 ± 0.0005	8.2 x $\mathbf{10^{-6}}$	0.3%
Damaged 24-C-mer/41AP				
dCTP	156 ± 44	1.6 ± 0.1	1.0 x $\mathbf{10^{-2}}$	99.9%
dATP	472 ± 47	0.00056 ± 0.00002	1.2 x $\mathbf{10^{-6}}$	0.0%
dGTP	531 ± 198	0.0006 ± 0.0001	1.2 x $\mathbf{10^{-6}}$	0.0%
dTTP	1076 ± 234	0.008 ± 0.001	7.7 x $\mathbf{10^{-6}}$	0.1%
Damaged 25-C-mer/41AP				
dGTP	232 ± 93	2.9 ± 0.4	1.3 x $\mathbf{10^{-2}}$	99.9%
dATP	339 ± 72	0.0024 ± 0.0002	7.2 x $\mathbf{10^{-6}}$	0.1%
dCTP	1087 ± 141	0.0014 ± 0.0001	1.3 x $\mathbf{10^{-6}}$	0.0%
dTTP	608 ± 126	0.0018 ± 0.0001	3.0 x $\mathbf{10^{-6}}$	0.0%
Damaged 26-C-mer/41AP				
dATP	129 ± 41	16.3 ± 1.5	1.3 x $\mathbf{10^{-1}}$	99.8%
dCTP	607 ± 128	0.025 ± 0.002	4.2 x $\mathbf{10^{-5}}$	0.0%
dGTP	817 ± 184	0.15 ± 0.02	1.9 x $\mathbf{10^{-4}}$	0.1%
dTTP	786 ± 125	0.052 ± 0.004	6.6 x $\mathbf{10^{-5}}$	0.1%

[a]Incorporation% = $(k_p/K_d)_{dNTP}/\Sigma(k_p/K_d)_{dNTP}$

Sup Table III Kinetic parameters for each nucleotide incorporation into damaged DNA substrates AP-1 and AP-8

dNTP	K_d (μM)	k_p (s^{-1})	k_p/K_d ($\mu M^{-1}s^{-1}$)	Incorporation%[a]
AP-1[b]				
dATP	567 ± 201	0.044 ± 0.006	7.7 x $\mathbf{10^{-5}}$	48.0%
dCTP	1266 ± 155	0.0058 ± 0.0004	4.6 x $\mathbf{10^{-6}}$	2.9%
dGTP	680 ± 182	0.0034 ± 0.0004	5.0 x $\mathbf{10^{-6}}$	3.1%
dTTP	583 ± 132	0.043 ± 0.004	7.4 x $\mathbf{10^{-5}}$	46.0%
AP-8[b]				
dATP	623 ± 96	0.045 ± 0.003	7.3 x $\mathbf{10^{-5}}$	63.4%
dCTP	1373 ± 304	0.0043 ± 0.0005	3.2 x $\mathbf{10^{-6}}$	2.8%
dGTP	885 ± 129	0.018 ± 0.001	2.0 x $\mathbf{10^{-5}}$	17.3%
dTTP	853 ± 120	0.016 ± 0.001	1.9 x $\mathbf{10^{-5}}$	16.5%

[a]Incorporation% = $(k_p/K_d)_{dNTP}/\Sigma(k_p/K_d)_{dNTP}$

[b]AP-1 and AP-8 are both modified 21-mer/41-AP substrates possessing a template adenine or cytosine, respectively, 5′ to the AP site

Sup Table IV Kinetic parameters for each nucleotide incorporation into damaged DNA substrates 20-mer/41AP

dNTP	K_d (μM)	k_p (s^{-1})	k_p/K_d ($\mu M^{-1}s^{-1}$)	Fidelity[a]
20-mer/41AP				
dATP	283 ± 73	7.7 ± 0.6	2.7 x $\mathbf{10^{-2}}$	
dCTP	1547 ± 218	0.014 ± 0.001	9.0 x $\mathbf{10^{-6}}$	3.3 x $\mathbf{10^{-4}}$
dGTP	658 ± 69	0.101 ± 0.005	1.5 x $\mathbf{10^{-4}}$	5.6 x $\mathbf{10^{-3}}$
dTTP	1796 ± 999	0.10 ± 0.03	5.6 x $\mathbf{10^{-5}}$	2.0 x $\mathbf{10^{-3}}$

[a]Fidelity = $(k_p/K_d)_{incorrect}/[(k_p/K_d)_{incorrect} + (k_p/K_d)_{correct}]$