Supplementary Data

Mechanistic Studies of the Bypass of a Bulky Single-Base Lesion Catalyzed by a Y-Family DNA Polymerase

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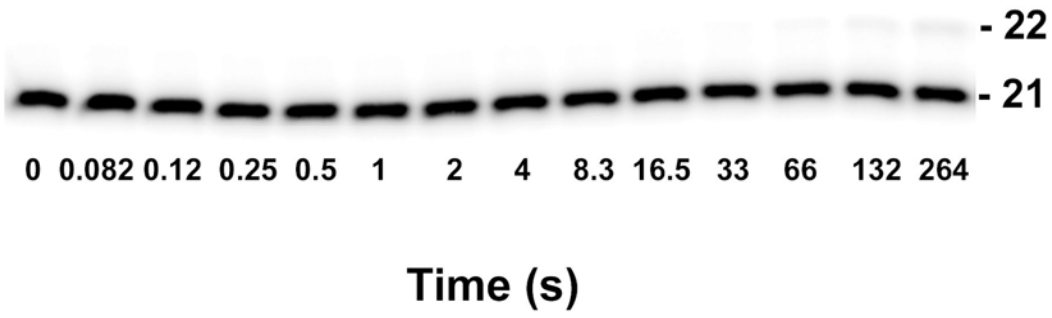
Supplementary Table 1. Kinetic parameters of dNTP incorporation into undamaged DNA

	$K_{d,\,dNTP}$ (μM)	k_p (s^{-1})	$k_p/K_{d,\,dNTP}$ ($\mu M^{-1}s^{-1}$)	Fidelity[a]
19/26-mer				
dGTP	183 ± 54	4.6 ± 0.4	2.5×10^{-2}	-
dATP	731 ± 175	$(1.0 \pm 0.1) \times 10^{-2}$	1.4×10^{-5}	5.6×10^{-4}
dCTP	350 ± 107	$(5.0 \pm 0.5) \times 10^{-2}$	1.4×10^{-4}	5.6×10^{-3}
dTTP	1440 ± 305	$(1.3 \pm 0.2) \times 10^{-2}$	8.9×10^{-6}	3.6×10^{-4}
20/26-mer				
dCTP	205 ± 64	11.6 ± 0.9	5.7×10^{-2}	-
dATP	631 ± 136	$(1.2 \pm 0.1) \times 10^{-2}$	1.9×10^{-5}	3.3×10^{-4}
dGTP	77 ± 16	$(3.5 \pm 0.2) \times 10^{-3}$	4.5×10^{-5}	7.9×10^{-4}
dTTP	489 ± 52	$(4.0 \pm 0.2) \times 10^{-2}$	8.3×10^{-5}	1.5×10^{-3}
21/26-mer				
dGTP	437 ± 19	1.62 ± 0.03	3.7×10^{-3}	-
dATP	859 ± 168	$(2.0 \pm 0.2) \times 10^{-3}$	2.4×10^{-6}	6.5×10^{-4}
dCTP	701 ± 30	$(2.14 \pm 0.04) \times 10^{-2}$	3.1×10^{-5}	8.3×10^{-3}
dTTP	1180 ± 144	$(2.1 \pm 0.2) \times 10^{-3}$	1.8×10^{-6}	4.9×10^{-4}
22/26-mer				
dCTP	129 ± 19	4.4 ± 0.2	3.4×10^{-2}	-
dATP	1313 ± 154	$(5.5 \pm 0.4) \times 10^{-3}$	4.2×10^{-6}	1.2×10^{-4}
dGTP	567 ± 95	$(4.8 \pm 0.4) \times 10^{-3}$	8.5×10^{-6}	2.5×10^{-4}
dTTP	1340 ± 454	$(1.5 \pm 0.3) \times 10^{-2}$	1.1×10^{-5}	3.2×10^{-4}
23/26-mer				
dGTP	116 ± 24	2.8 ± 0.1	2.4×10^{-2}	-
dATP	431 ± 25	$(6.6 \pm 0.1) \times 10^{-3}$	1.5×10^{-5}	6.2×10^{-4}
dCTP	918 ± 102	$(1.5 \pm 0.1) \times 10^{-3}$	1.6×10^{-6}	6.7×10^{-5}
dTTP	1220 ± 59	$(3.8 \pm 0.1) \times 10^{-3}$	3.1×10^{-6}	1.3×10^{-4}

[a]Calculated as $(k_p/K_{d,\,dNTP})_{incorrect}/[(k_p/K_{d,\,dNTP})_{correct} + (k_p/K_{d,\,dNTP})_{incorrect}]$.



Supplementary Fig. 1. Effectiveness of the DNA trap for biphasic kinetic assays. A preincubated solution of Dpo4 (120 nM), 5′-[^{32}P]-labeled 21/26-mer-dG^{AP} (30 nM) and DNA trap (5 μM, 21/41-mer D-1) was rapidly mixed with dGTP (1.2 mM) and quenched at various time intervals with 0.37 M EDTA. The products were resolved on a 17% polyacrylamide gel with 8 M urea. The autoradiographed gel image revealed minimal product formation (22-mer) after 264 s. Thus, a molar ratio of 167:1 for the D-1 DNA trap to the radiolabeled damaged DNA substrate was effective at sequestering free Dpo4 that dissociated from the 5′-[^{32}P]-labeled 21/26-mer-dG^{AP}.