Supplemental Information

Conformational Dynamics of *Thermus aquaticus* DNA Polymerase I during Catalysis*

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Table S1. The estimated distance (Å) between the donor Alexa488 labeling site on DNA and acceptor Alexa594 labeling site on the domains of Taq Pol[1].

Domains	Residues[2]	Location	Binary[a] (9th bp[d])	Open ternary[b]	Close ternary[c]	Binary[a] (10th bp[e])
N-Terminal	V256C	Loop	-	-	-	-
Intervening	K346C	Loop	37.8	38.2	38.1	37.0
	R411C	Helix	32.2	32.1	32.1	32.9
Thumb	E524C	Loop	41.0	39.9	39.7	44.1
	E465C	Helix	32.4	31.9	31.8	32.7
Finger	V649C	Loop	47.7	----	50.7	51.4
	S644C	Helix (N)	54.3	54.4	47.3	57.8
Palm	A814C	Loop	49.7	49.1	49.0	52.5
	R801C	Helix	48.0	47.2	47.3	49.1

Note: Distances are estimated based on the Klentaq1 crystal structures of [a]4KTQ, [b]2KTQ, and [c]3KTQ. The reported distance is measured from the alpha carbon of each amino acid to the C5 position of either the [d]9th (dC) or [e]the 10th (dA) base from the primer 3′-end. The distances are not reported here for mutant V256C as no structure of the Full-length Taq Pol in complex with a DNA duplex longer than 8 base pairs is available. The distance for the open ternary complex of V649C is not reported here because the residue is not included in 2KTQ.

[1]Distances do not accurately represent distance between the donor and acceptor fluorophores due to uncertainty in the dye position resulting from the flexibility of the linker between the dye and the attachment site on the protein or DNA

[2]All Taq Pol mutants contain G46D mutation which inactivates the 5'→3' exonuclease activity.

Table S2. The rates of dTTP incorporation into S-1 DNA[a] by Taq Pol and Klentaq1 mutants obtained from ^{32}P-based single-turnover experiments at 20 °C.

Domain	Residue[b]	Location	k_p (s^{-1})	K_d (μM)	k_p/K_d ($\mu M^{-1}s^{-1}$)
Full Length Taq Pol					
wt-Taq Pol	-	-	0.105 ± 0.006	48 ± 11	2.19×10^{-3}
N-terminal	V256C	Loop	0.076 ± 0.004	46 ± 9	1.65×10^{-3}
Intervening	K346C	Loop	0.104 ± 0.005	94 ± 17	1.11×10^{-3}
	R411C	Helix	0.100 ± 0.006	24 ± 18	4.17×10^{-3}
Thumb	E524C	Loop	0.117 ± 0.004	54 ± 8	2.17×10^{-3}
	E465C	Helix	0.101 ± 0.002	57 ± 4	1.77×10^{-3}
Finger	V649C	Loop	0.076 ± 0.004	82 ± 14	0.93×10^{-3}
	S644C	Helix	0.136 ± 0.004	19 ± 3	7.16×10^{-3}
Palm	A814C	Loop	0.136 ± 0.004	47 ± 5	2.89×10^{-3}
	R801C	Helix	0.096 ± 0.003	45 ± 6	1.56×10^{-3}
Klentaq1					
wt-Klentaq1	-	-	0.232±0.007	62±7	3.74×10^{-3}
Intervening	K346C	Loop	0.33±0.02	62±12	5.32×10-3
Finger	V649C	Loop	0.22±0.01	73±11	3.01×10-3
Palm	A814C	Loop	0.27±0.15	60±35	5.67×10-3

[a] Experiments were performed using the unlabeled S-1 DNA substrate which contained the modified dT base.
[b] Mutants are labeled with acceptor fluorophore Alexa594.

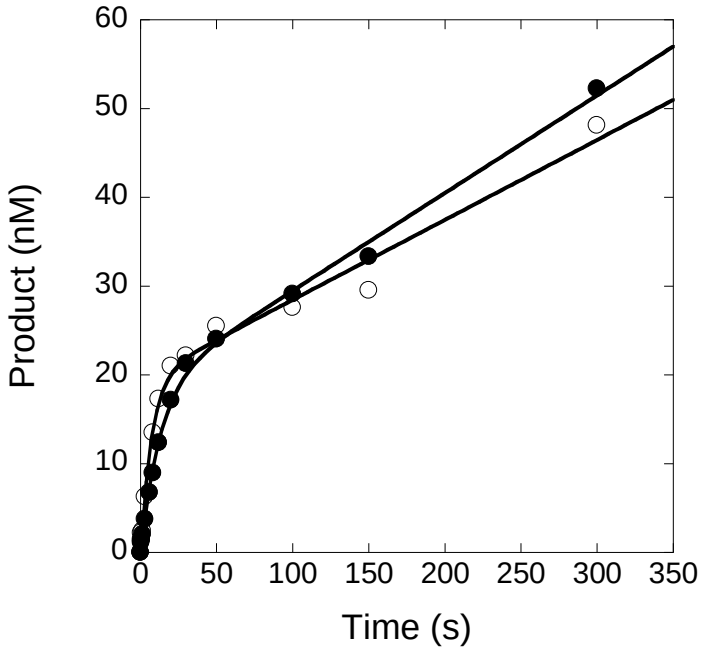

FIGURE S1. Burst kinetics of Taq Pol-catalyzed dTTP incorporation into DNA substrates with or without an Alexa488 fluorescent label. A pre-incubated solution of wild-type Taq Pol (30 nM) and 5′-^{32}P-labeled DNA substrate S-1 (120 nM) either containing (open circles) or lacking (closed circles) the Alexa488 fluorescent label was rapidly mixed with dTTP (500 μM) and the reaction mixture was quenched at various times with 0.37 M EDTA. The data were fit to a biphasic equation ([Product] = A[1 - exp(- k_1t) + k_2t]) to yield the following parameters: k_1 = 0.065 ± 0.003 s^{-1} and k_2 = 0.0059 ± 0.0006 s^{-1} for the unlabeled DNA substrate and k_1 = 0.12 ± 0.02 s^{-1} and k_2 = 0.0056 ± 0.0007 s^{-1} with the Alexa488-labeled DNA substrate.

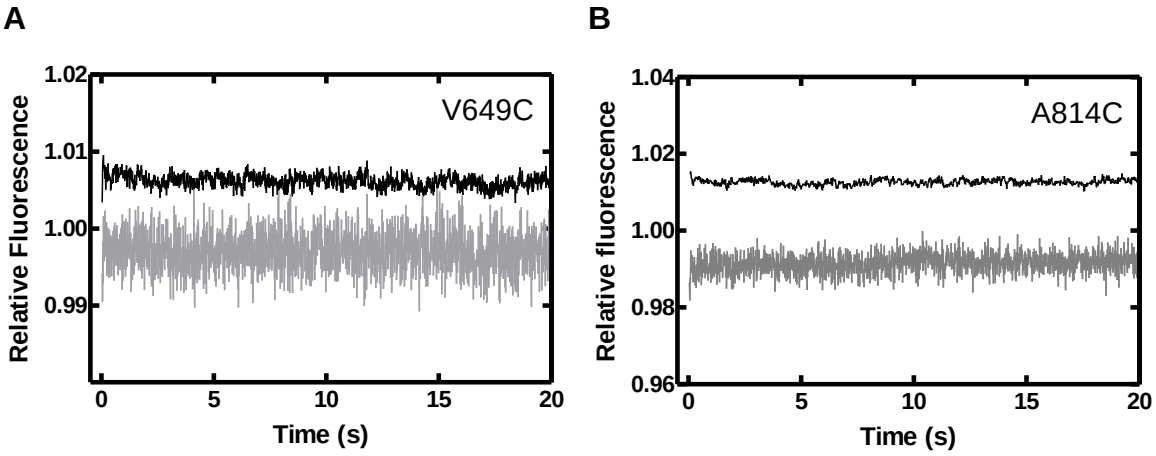

FIGURE S2. Stopped-flow control experiments. A solution containing a Taq Pol mutant (200nM) pre-incubated with DNA substrate S-1 (100nM) was rapidly mixed with buffer R and fluorescence was monitored using a stopped-flow apparatus. Representative donor (black) and acceptor (grey) traces are shown for (A) V649C or (B) A814C.

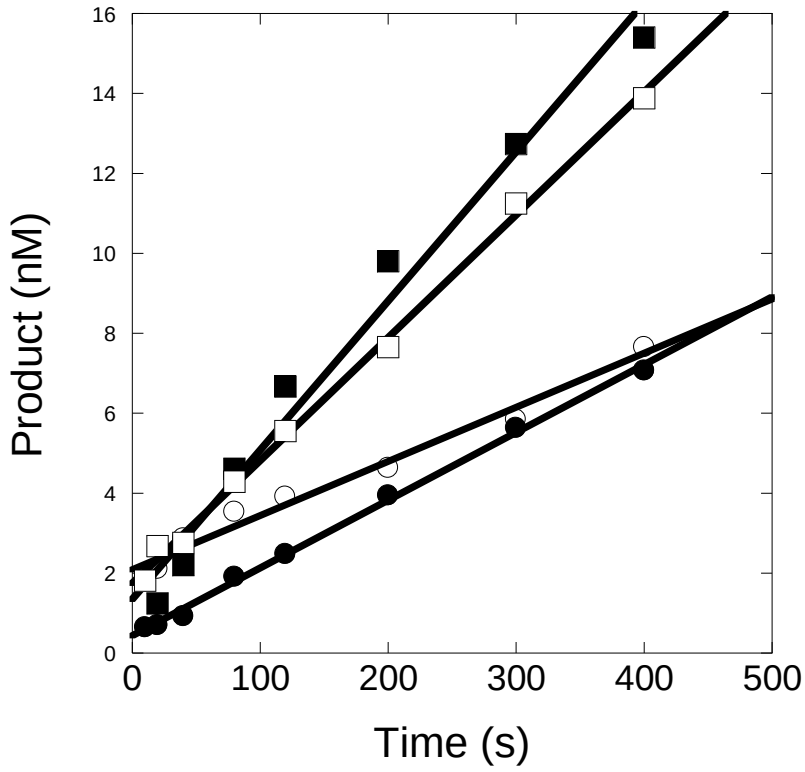

FIGURE S3. Steady-state kinetics of dTTP incorporation catalyzed by Taq Pol or Klentaq1. A pre-incubated solution of 1.5 nM wild-type Taq Pol (filled circles), Taq Pol mutant E524C (open circles), wild-type Klentaq1 (filled squares) or Klentaq1 mutant A814C (open squares) and 5′-^{32}P-labeled DNA substrate S-1 (250 nM) was rapidly mixed with dTTP (500 μM) and the reaction mixture was quenched at various times with 0.37 M EDTA. The steady-state rate for wild-type Taq Pol, Taq Pol mutant E524C, wild-type Klentaq1, and Klentaq1 mutant A814C were determined to be 0.011 ± 0.001 s^{-1}, 0.009 ± 0.002 s^{-1}, 0.023 ± 0.002 s^{-1} and 0.020 ± 0.001 s^{-1} respectively (See Materials and Methods).