Supporting Information

Differential Temperature Dependent Multimeric Assemblies of Replication and Repair Polymerases on DNA Increase Processivity

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Running head: B and Y-Family DNA Polymerase Equilibria

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Simulation of polymerase binding to DNA: The kinetic scheme in Figure 6A or 6B was used to develop a model to fit the individual binding equilibria (K_1 and K_2) to populations of monomeric or trimeric Dpo1 or monomer and dimeric Dpo4. The following sequential binding scheme which includes cooperative binding of the second and third molecule of Dpo1 or just sequential single binding of a second Dpo4 was simulated using Berkeley Madonna (University of California, Berkeley, CA) using our cumulative binding data according to the following differential equations:

<u>For sequential and cooperative Dpo1 binding to DNA:</u>

Total Dpo1 $[P]_T = [P] + [PD] + 3[P_3D]$ (S1)

Total DNA $[D]_T = [D] + [PD] + [P_3D]$ (S2)

$$K_1 = \frac{k_a}{k_b} \quad \text{(S3)}$$

$$K_2 = \frac{k_c}{k_d} \quad \text{(S4)}$$

$$\frac{d}{dx}(PD) = k_a([D]_T - [PD] - [P_3D])([P]_T - [PD] - 3[P_3D])$$

$$-k_b[PD] - k_c([P]_T - [PD] - 3[P_3D])^2[PD] + k_d[P_3D] \quad \text{(S5)}$$

$$\frac{d}{dx}(P_3D) = k_c[PD]([P]_T - [PD] - 3[P_3D])^2 - k_d[P_3D] \quad \text{(S6)}$$

For sequential Dpo4 binding to DNA:

Total Dpo4 $[P]_T = [P] + [PD] + 2[P_2D]$ (S7)

Total DNA $[D]_T = [D] + [PD] + [P_2D]$ (S8)

$$K_1 = \frac{k_a}{k_b} \quad \text{(S9)}$$

$$K_2 = \frac{k_c}{k_d} \quad \text{(S10)}$$

$$\frac{d}{dx}(PD) = k_a([D]_T - [PD] - [P_2D])([P]_T - [PD] - 2[P_2D])$$

$$-k_b[PD] - k_c([P]_T - [PD] - 2[P_2D])[PD] + k_d[P_2D] \quad \text{(S11)}$$

$$\frac{d}{dx}(P_2D) = k_c[PD]([P]_T - [PD] - 2[P_2D]) - k_d[P_2D] \quad \text{(S12)}$$

P is free protein; PD represents monomer Dpo1 or Dpo4 bound to DNA; P_2D represents dimeric Dpo4 bound to DNA; and P_3D represents trimeric Dpo1 bound to DNA. Individual parameters from the fits of normalized anisotropy binding curves at each temperature were modeled according to Equations S1-S6 or S7-S12 to give the relative populations of each species as shown in Figure 6.

SUPPORTING TABLES

Table S1: Thermodynamic parameters for DNA binding by Dpo1

	Monomeric Dpo1			Trimeric Dpo1		
Temp (^oC)	ΔG^o (kcal mol^{-1})[a]	ΔH^o (kcal/mol)[c]	$T\Delta S^o$ (kcal mol^{-1})[c]	ΔG^o (kcal mol^{-1})[b]	ΔH^o (kcal/mol)[c]	$T\Delta S^o$ (kcal mol^{-1})[c]
6.8	-8.3 ± 0.1	12.3	20.7	-20.5 ± 0.1	49.1	69.8
12.0	-8.7 ± 0.2	10.1	18.9	-22.1 ± 0.4	41.6	63.5
17.0	-9.0 ± 0.1	8.0	17.0	-23.0 ± 0.1	34.4	57.3
22.2	-9.4 ± 0.1	5.8	15.1	-23.9 ± 0.3	26.8	50.7
27.4	-9.6 ± 0.1	3.6	13.2	-24.6 ± 0.1	19.3	44.0
32.8	-9.8 ± 0.1	1.3	11.1	-25.5 ± 0.1	11.5	36.9
38.0	-10.0 ± 0.2	-0.9	9.0	-26.0 ± 0.2	4.0	30.0
43.3	-9.9 ± 0.1	-3.2	6.9	-26.1 ± 0.1	-3.7	22.7
48.8	-10.1 ± 0.1	-5.5	4.7	-27.0 ± 0.3	-11.7	15.1
53.9	-10.4 ± 0.2	-7.7	2.6	-27.3 ± 0.6	-19.1	7.9
58.9	-10.2 ± 0.1	-9.8	0.5	-26.9 ± 0.2	-26.3	0.7
63.7	-10.4 ± 0.1	-11.8	-1.6	-26.9 ± 0.1	-33.2	-6.3

[a]Calculated from the Gibbs free energy Equation 6, $\Delta G^o = -RT\ln K_1$ for monomeric Dpo1 binding. [b]Calculated from the Gibbs free energy Equation 7, $\Delta G^o = -RT\ln K_1 - 2RT\ln K_2$ for formation of the trimeric Dpo1-DNA complex. [c]The predicted enthalpy (ΔH^o) and entropy ($T\Delta S^o$) components are calculated from the fit to the Gibbs-Helmholtz Equations 8-10.

Table S2: Thermodynamic parameters for DNA binding by Dpo4

	Monomeric Dpo4			Dimeric Dpo4		
Temp (^oC)	ΔG^o (kcal mol^{-1})[a]	ΔH^o (kcal/mol)[c]	$T\Delta S^o$ (kcal mol^{-1})[c]	ΔG^o (kcal mol^{-1})[b]	ΔH^o (kcal/mol)[c]	$T\Delta S^o$ (kcal mol^{-1})[c]
6.8	-8.2 ± 0.2	18.6	26.6	-14.6 ± 0.1	35.4	49.9
12.0	-8.5 ± 0.2	15.0	23.5	-15.4 ± 0.2	29.0	44.4
17.0	-8.9 ± 0.1	11.6	20.5	-16.0 ± 0.1	22.9	39.0
22.1	-9.1 ± 0.1	8.2	17.4	-16.5 ± 0.1	16.7	33.4
27.3	-9.3 ± 0.1	4.6	14.1	-17.0 ± 0.1	10.3	27.5
32.9	-9.7 ± 0.1	0.8	10.5	-17.4 ± 0.1	3.5	21.1
37.8	-9.8 ± 0.3	-2.5	7.3	-17.9 ± 0.2	-2.5	15.4
43.2	-10.0 ± 0.1	-6.2	3.8	-18.3 ± 0.1	-9.1	9.0
48.8	-10.2 ± 0.3	-10.0	-0.02	-18.6 ± 0.5	-16.0	2.3
53.8	-10.3 ± 0.3	-13.4	-3.4	-18.6 ± 0.2	-22.1	-3.8
58.9	-9.7 ± 0.2	-17.4	-7.6	-18.0 ± 0.2	-29.3	-11.2
65.2	-9.6 ± 0.1	-21.2	-11.5	-17.6 ± 0.1	-36.2	-18.3

[a]Calculated from the Gibbs free energy Equation 6, $\Delta G^o = -RT\ln K_1$ for monomeric Dpo4 binding. [b]Calculated from the Gibbs free energy Equation 7, $\Delta G^o = -RT\ln K_1 - RT\ln K_2$ for formation of the dimeric Dpo4-DNA complex. [c]The predicted enthalpy (ΔH^o) and entropy ($T\Delta S^o$) components are calculated from the fit to the Gibbs-Helmholtz Equations 8-10.

SUPPORTING FIGURES

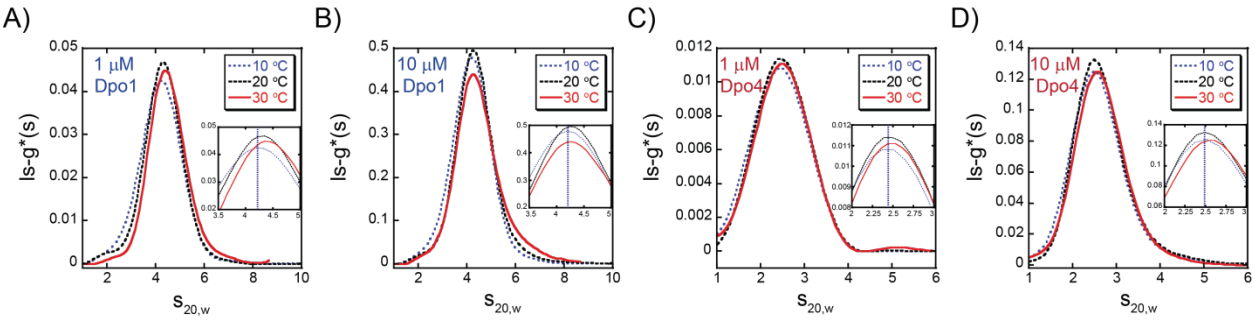

Figure S1: Analytical ultracentrifugation (AUC) velocity absorbance experiments of Dpo1 and Dpo4 alone. Shown are the *ls-g*(s)* distribution profiles for A) 1 μM or B) 10 μM Dpo1 or C) 1 μM or D) 10 μM Dpo4 alone at 10 (blue), 20 (black), or 30 °C (red). The inset highlights the region of the weigh average $s_{20,w}$ values, and the vertical blue line indicates the weight average $s_{20,w}$ value at 10 °C.

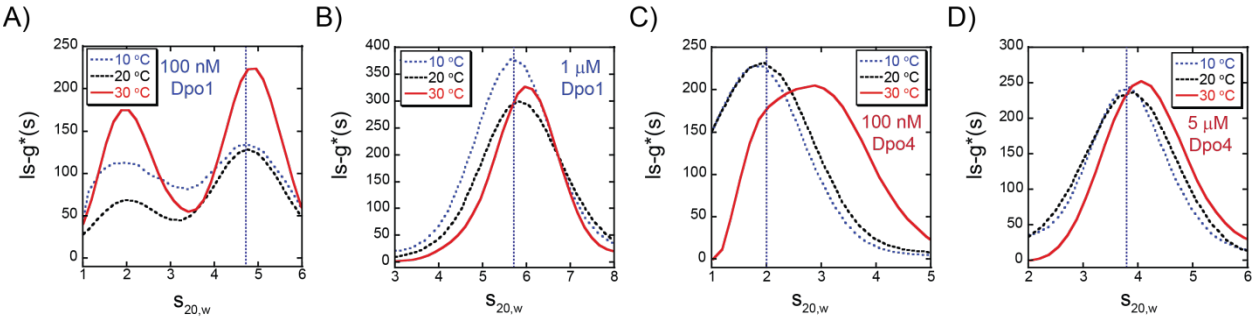

Figure S2: Analytical ultracentrifugation velocity fluorescent experiments (AU-FDS) of Dpo1 and Dpo4 bound to DNA. Shown are the *ls-g*(s)* distribution profiles for 50 nM fluorescent DNA hairpin primer-template and Dpo1 at A) 100 nM and B) 1 μM or Dpo4 at C) 100 nM and D) 5 μM as a function of temperature [10 (blue dotted) , 20 (black dotted), and 30 °C (red solid)]. Data was analyzed as described in Materials and Methods. The vertical dotted blue line indicates the position of the weight average $s_{20,w}$ value at 10 °C.

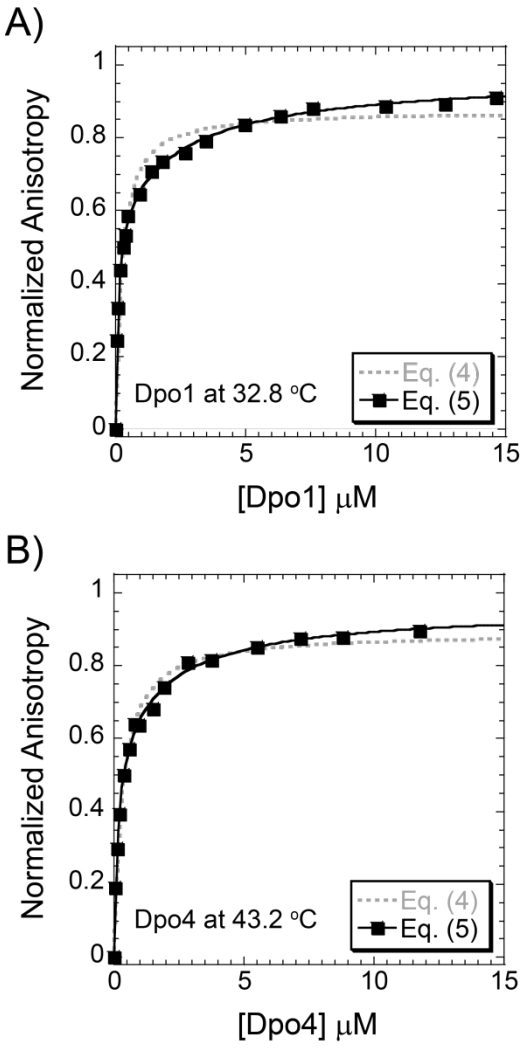

Figure S3: Representative equilibrium fluorescence anisotropy titrations. A) Dpo1 at 32.8 ^{o}C binding to a 5' Cy3 labeled DNA hairpin. The dashed line (χ^2 = 0.0365) show the fit for single binding (Dpo1 $K_{d,app}$ = 0.213 ± 0.023) (Equation 4). The solid line (χ^2 = 0.0017) shows the fits for a sequential binding mode (Dpo4 K_{d1} = 0.078 ± 0.024 μM and K_{d2} = 2.53 ± 0.46 μM) (Equation 5). B) Dpo4 at 43.2 ^{o}C binding to a 5' Cy3 labeled DNA hairpin. The dashed line (χ^2 = 0.0163) shows the fit for single binding (Dpo4 $K_{d,app}$ = 0.299 ± 0.025) (Equation 4). The solid line (χ^2 = 0.0021) shows the fits for a sequential binding mode (Dpo4 K_{d1} = 0.136 ± 0.024 μM and K_{d2} = 1.92 ± 0.36 μM) (Equation 5).

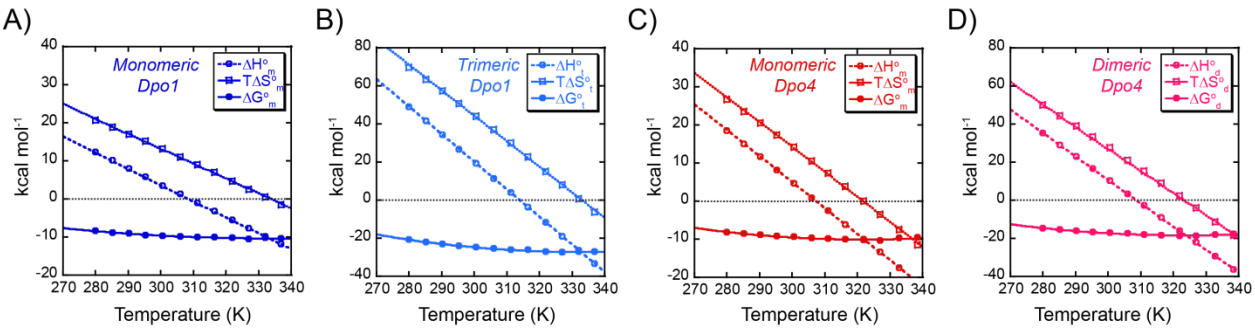

Figure S4: Fitted thermodyanamic parameters ΔH^o (dashed -○-) , $T\Delta S^o$ (dotted -□-), and ΔG^o (solid -●-), for A) monomeric Dpo1 (dark blue) B) trimeric Dpo1 (light blue), C) monomeric Dpo4 (red), or D) dimeric Dpo4 (pink) assemblies on DNA primer template plotted from values in Tables 3 and 4

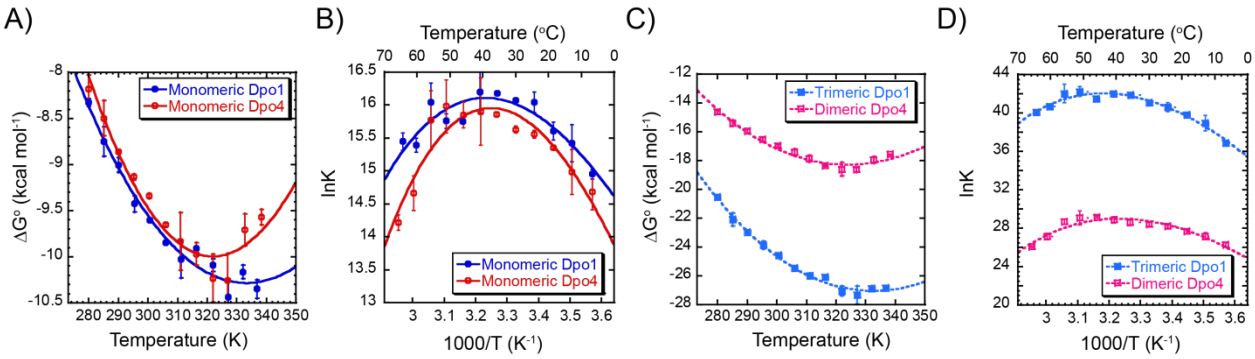

Figure S5: Thermodynamic differences between Dpo1 and Dpo4 binding to DNA. A) Gibbs-Helmholtz or B) van't Hoff plot comparison of the free energy of binding (ΔG^o) for monomeric Dpo1 (solid -●-, blue) or Dpo4 (solid -○-, red) as a function of temperature. C) Gibbs-Helmholtz or D) van't Hoff plot comparison of the free energy (ΔG^o) for formation of trimeric Dpo1 (dashed -■-, light blue) or dimeric Dpo4 (dashed -□-, pink) as a function of temperature. Error bars represent the standard error from multiple experiments at each point. Lines in the Gibbs-Helmholtz plots show the fits of the data to Equations 8-10. Lines in the van't Hoff plots show the fits to Equation 11.

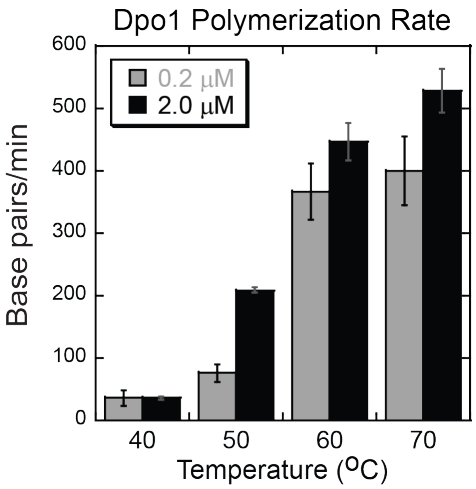

Figure S6: Quantification of the average rate (bp/min) for 0.2 μM (grey) and 2.0 μM (black) Dpo1 at 40, 50, 60, and 70 ^{o}C from alkaline agarose gels. DNA length values were obtained compared to DNA size markers and calculated using ImageQuant software. Error bars represent the standard error from at least three independent kinetic experiments. Kinetic experiments were quenched after 4 minutes for all temperatures.

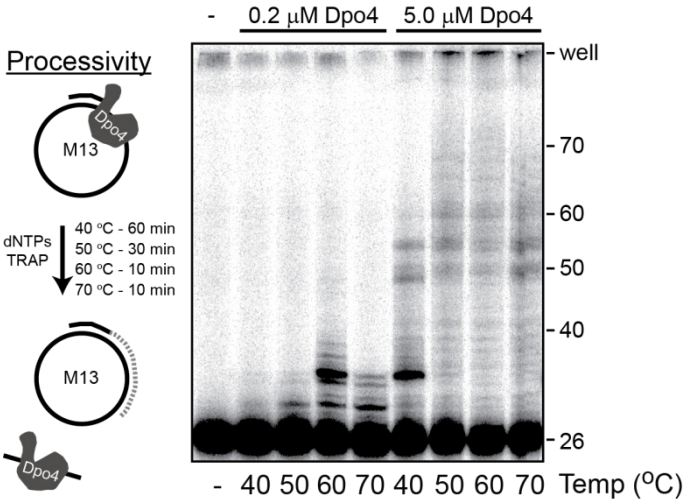

Figure S7: Dpo4 processivity assays were performed at 40, 50, 60, and 70 ^{o}C representing monomer (0.2 μM) (left panel) or dimer (5.0 μM) (right panel) concentrations and separated on a denaturing acrylamide gel as described in the Materials and Methods. The inset cartoon describes the experimental protocol for processivity experiments. Longer reaction times were used for lower temperatures to compensate for slower polymerase rates.