Supplemental Information

Table S1

		Arrhenius Analysis[a]		Global Analysis
Enzyme	**dNTP**	**ΔH (kcal mol^{-1})**	**1/K_1 (μM)**	**1/K_1 (μM)**
Wt HIV RT	TTP	-13.8 ± 6.8	368 ± 248	310 ± 10
	AZT-TP	-10.6 ± 5.2	115 ± 31	110 ± 10
TAMs HIV RT	TTP	-6.5 ± 1.3	897 ± 886	1350 ± 30
	AZT-TP	-10.0 ± 0.8	445 ± 418	470 ± 25

[a]Arrhenius analysis was performed by collecting experimental data at lower temperatures then extrapolating to expected values for 37°C. K_1 was obtained by fitting the experimental data to the equation $k_{obs} = (K_1k_2[S])/(1+K_1[S])$. Changes in enthalpy (ΔH) were obtained by fitting the K_1 values using the Van't Hoff equation where the slope of the linear line is equal to $-\Delta H/R$.

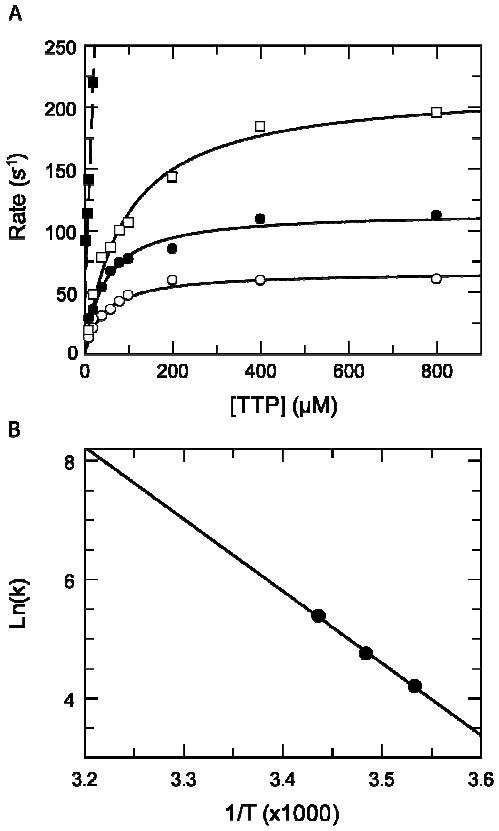

Figure S1. *Temperature Dependence of the HIV RT-MDCC Conformational Change Rate Upon TTP Binding*. (A) The experiment shown in Figure 1B was repeated at various temperatures. A pre-incubated complex of 200 nM HIV RT-MDCC and 300 nM 25/45 DNA was rapidly mixed with various concentrations of TTP (10, 20, 40, 60, 80, 100, 200, 400, and 800 µM) at 10°C (○), 14°C (●), 18°C (□), and 37°C (■). The concentration dependence of the fast decrease of fluorescence (corresponding to conformational closing) was fit to a hyperbolic equation to obtain the maximum rate of the conformational change (k_2) at each temperature resulting in rates of $66\pm2s^{-1}$, $115\pm4s^{-1}$, and $216\pm6s^{-1}$, respectively. The data corresponding to the conformational change rate at 37°C were fit to a linear equation because insufficient data were available to constrain the maximum rate at high concentrations of nucleotide due to dead time limitations of the instruments and the fast rate of reaction. (B) The temperature dependence of k_2 was analyzed by Arrhenius plot to estimate a maximum rate for k_2 of $2700\pm100s^{-1}$ at 37°C (values are expressed in absolute temperature). The value was then fixed to constrain data fitting by global analysis. For S1A and S1B error measurements are not shown on the figure because they fall within the size of the point.

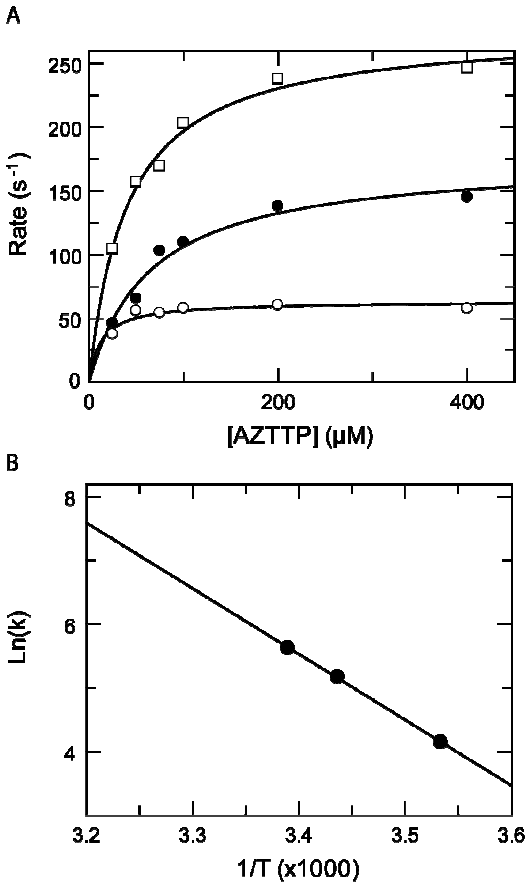

Figure S2. *Temperature Dependence of the HIV RT-MDCC Conformational Change Rate Upon AZTTP Binding*. (A) The experiment shown in figure 1E was repeated at various temperatures. A pre-incubated complex of 200 nM HIV RT-MDCC and 300 nM 25/45 DNA was rapidly mixed with various concentrations of AZTTP (25, 50, 75, 100, 200, and 400 µM) at 10°C (○), 18°C (●), and 22°C (□). The concentration dependence of the fast decrease of fluorescence (corresponding to conformational closing) was fit to a hyperbolic equation to obtain the maximum rate of the conformational change (k_2) at each temperature resulting in rates of 63±3 s^{-1}, 175±6 s^{-1}, and 277±9 s^{-1}, respectively. (B) The temperature dependence of k_2 was analyzed by Arrhenius plot to estimate a maximum rate for k_2 of 1500±100 s^{-1} at 37°C (values are expressed in absolute temperature). The value was then fixed to constrain data fitting by global analysis. For S2A and S2B error measurements are not shown on the figure because they fall within the size of the point.

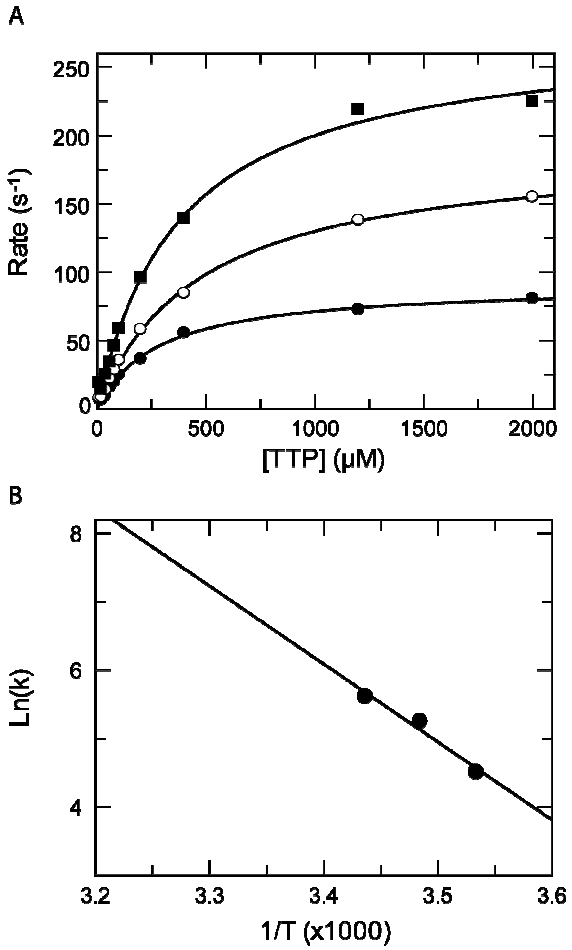

Figure S3. *Temperature Dependence of the TAMs HIV RT-MDCC Conformational Change Rate Upon TTP Binding*. (A) The experiment shown in figure 2B was repeated at various temperatures. A pre-incubated complex of 200 nM TAMs HIV RT-MDCC and 300 nM 25/45 DNA was rapidly mixed with various concentrations of TTP (10, 20, 40, 60, 80, 100, 200, 400, 1200, and 2000 µM) at 10°C (○), 14°C (●), and 18°C (□). The concentration dependence of the fast decrease of fluorescence (corresponding to conformational closing) was fit to a hyperbolic equation to obtain the maximum rate of the conformational change (k_2) at each temperature resulting in rates of 91±2 s^{-1}, 191±3 s^{-1}, and 276±9 s^{-1}, respectively. (B) The temperature dependence of k_2 was analyzed by Arrhenius plot to estimate a maximum rate for k_2 of 3250±150 s^{-1} at 37°C (values are expressed in absolute temperature). The value was then fixed to constrain data fitting by global analysis. For S3A and S3B error measurements are not shown on the figure because they fall within the size of the point.

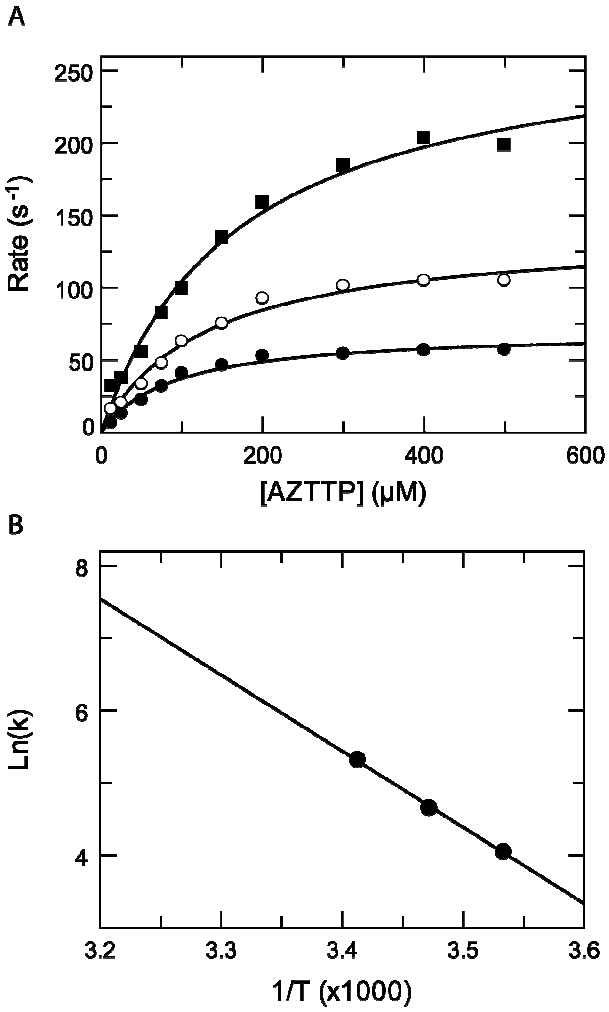

Figure S4. *Temperature Dependence of the TAMs HIV RT-MDCC Conformational Change Rate Upon AZTTP Binding*. (A) The experiment shown in figure 2E was repeated at various temperatures. A pre-incubated complex of 200 nM TAMs HIV RT-MDCC and 300 nM 25/45 DNA was rapidly mixed with various concentrations of AZTTP (12, 25, 50, 75, 100, 150, 200, 300, 400, and 500 µM) at 10°C (○), 15°C (●), and 20°C (□). The concentration dependence of the fast decrease of fluorescence (corresponding to conformational closing) was fit to a hyperbolic equation to obtain the maximum rate of the conformational change (k_2) at each temperature resulting in rates of 70±3 s^{-1}, 139±6 s^{-1}, and 279±10 s^{-1}, respectively. (B) The temperature dependence of k_2 was analyzed by Arrhenius plot to estimate a maximum rate for k_2 of 1500±100 s^{-1} at 37°C (values are expressed in absolute temperature). The value was then fixed to constrain data fitting by global analysis. For S4A and S4B error measurements are not shown on the figure because they fall within the size of the point.