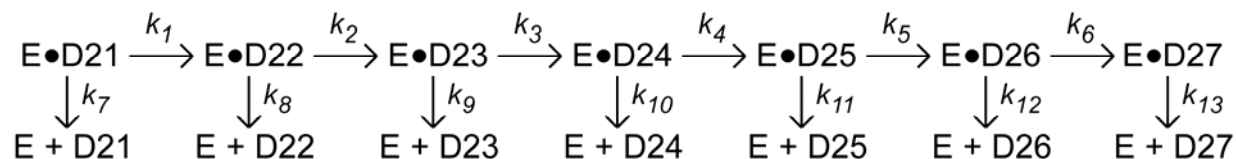


# Supporting Information

## Elucidating the Kinetic Mechanism of DNA Polymerization Catalyzed by *Sulfolobus solfataricus* P2 DNA Polymerase B1

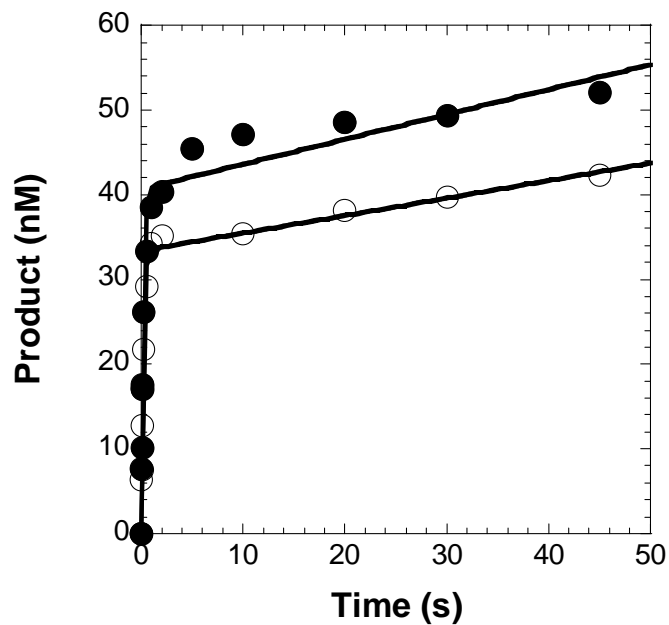
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### Supplementary Figure 1



**Supplementary Figure 1.** Kinsim and Fitsim analysis. The above reaction scheme represents the mechanism entered into Kinsim. There were six consecutive polymerization reactions ( $k_1$ - $k_6$ ) and seven DNA dissociation events ( $k_7$ - $k_{13}$ ). Please note, three nucleotides (dCTP, dGTP, and dTTP) were present during the reaction, although, this mechanism does not explicitly account for them. For the Fitsim analysis, all thirteen rate constants were varied to obtain polymerization rates of  $k_1 = 4.8 \pm 0.2 \text{ s}^{-1}$ ,  $k_2 = 7.5 \pm 0.5 \text{ s}^{-1}$ ,  $k_3 = 12 \pm 1 \text{ s}^{-1}$ ,  $k_4 = 8.2 \pm 0.8 \text{ s}^{-1}$ ,  $k_5 = 4.9 \pm 0.4 \text{ s}^{-1}$ ,  $k_6 = 15 \pm 3 \text{ s}^{-1}$  and DNA dissociation rates of  $k_7 = 0.6 \pm 0.1 \text{ s}^{-1}$ ,  $k_8 = 0.7 \pm 0.1 \text{ s}^{-1}$ ,  $k_9 = 0.7 \pm 0.2 \text{ s}^{-1}$ ,  $k_{10} = 0.4 \pm 0.2 \text{ s}^{-1}$ ,  $k_{11} = 0.2 \pm 0.1 \text{ s}^{-1}$ ,  $k_{12} = 0.7 \pm 0.5 \text{ s}^{-1}$ , and  $k_{13} = 0.8 \pm 0.2 \text{ s}^{-1}$ .

## Supplementary Figure 2



**Supplementary Figure 2.** Measurement of the dissociation rate for the E•DNA•dNTP complex at 35 °C. PolB1 exo- (55 nM) and 5'-[<sup>32</sup>P]-labeled D-1 DNA (60 nM) was rapidly mixed with 100 μM dTTP in the absence (●) or presence of (○) an unlabeled DNA trap (2.5 μM of D-1). An identical burst rate of  $5.5 \pm 0.6 \text{ s}^{-1}$  was determined for both time courses. The burst amplitudes were  $41 \pm 1 \text{ nM}$  and  $34.3 \pm 0.9 \text{ nM}$  in the absence and presence of the DNA trap, respectively. An amplitude reduction of 16% equates to a  $k_7$  of  $1.1 \pm 0.1 \text{ s}^{-1}$ .