Supplementary Material

The Effect of Different Divalent Cations on the Kinetics and Fidelity of RB69 DNA Polymerase.

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Supplemental Figures S1-S6.

Supporting Information

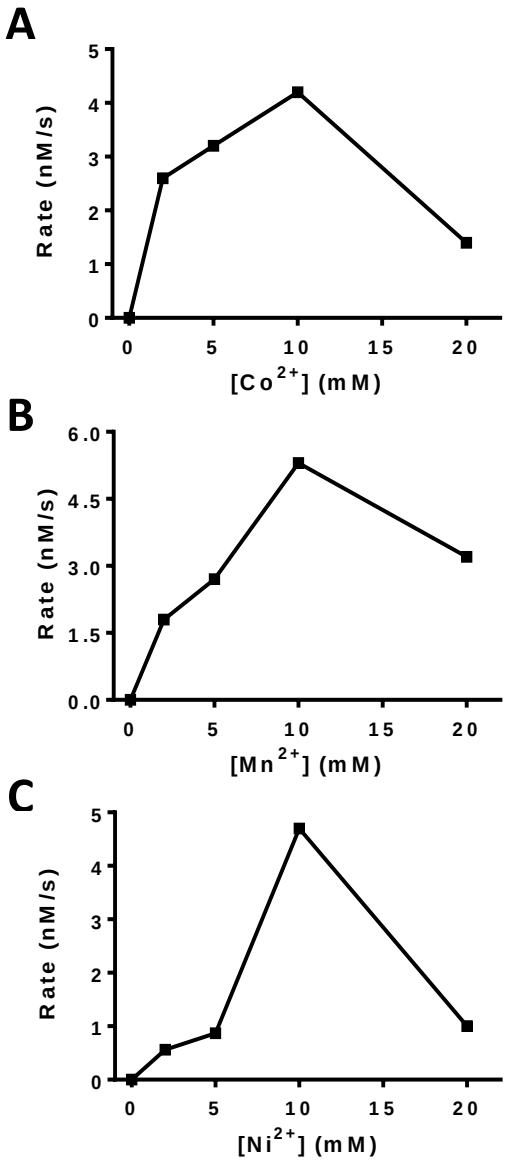

Figure S1. Determination of optimum divalent cation concentrations. RB69pol (40 nM) was pre-incubated with DNA_{13A} (100 nM) in reaction buffer containing increasing concentrations of the divalent cation (Co^{2+}, Mn^{2+}, or Ni^{2+}) [0, 2, 5, 10, and 20 mM]. Reactions were initiated by adding 500 µM dTTP and subsequently quenched with 0.5 M EDTA (pH 8.0) at various times ranging from 10-100 s. All data were obtained at 23 °C. **(A)** Plot of the rate of DNA product formation as a function of [Co^{2+}]. **(B)** Plot of the rate of DNA product formation as a function of [Mn^{2+}]. **(C)** Plot of the rate of DNA product formation as a function of [Ni^{2+}].

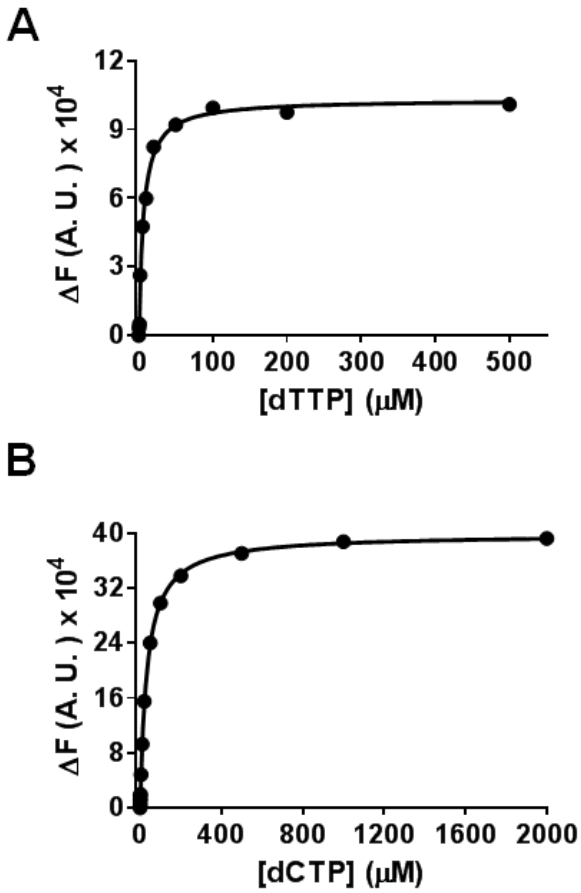

Figure S2. Equilibrium fluorescence titration plots of the RB69pol-ddP/T complex fluorescence quenching with increasing [dTTP] (or [dCTP]). The concentration of DNA_{Pdd} was 200 nM and that of RB69pol was 1 μM. **(A)** Plot showing the change in fluorescence quenching as a function of [dTTP] in the presence of Co^{2+}. The concentrations of dTTP used were 0, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, and 500 μM. Fluorescence intensities at 365 nm were fitted to a hyperbolic equation. Titration of dTTP vs. 2AP in the presence of 10 mM Co^{2+} gives a $K_{d,g}$ = 5.8 ± 0.7 μM. **(B)** Plot showing the change in fluorescence quenching as a function of [dCTP] in the presence of Mn^{2+}. The concentrations of dCTP used were 0, 0.02, 0.04, 0.1, 0.25, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, and 2000 μM. Fluorescence intensities at 365 nm were fitted to a hyperbolic equation to obtain a $K_{d,g}$ = 33 ± 6 μM. (ΔF) represents the change in fluorescence in the direction of quenching and ΔF increases with an increase in [dTTP] (or [dCTP]).

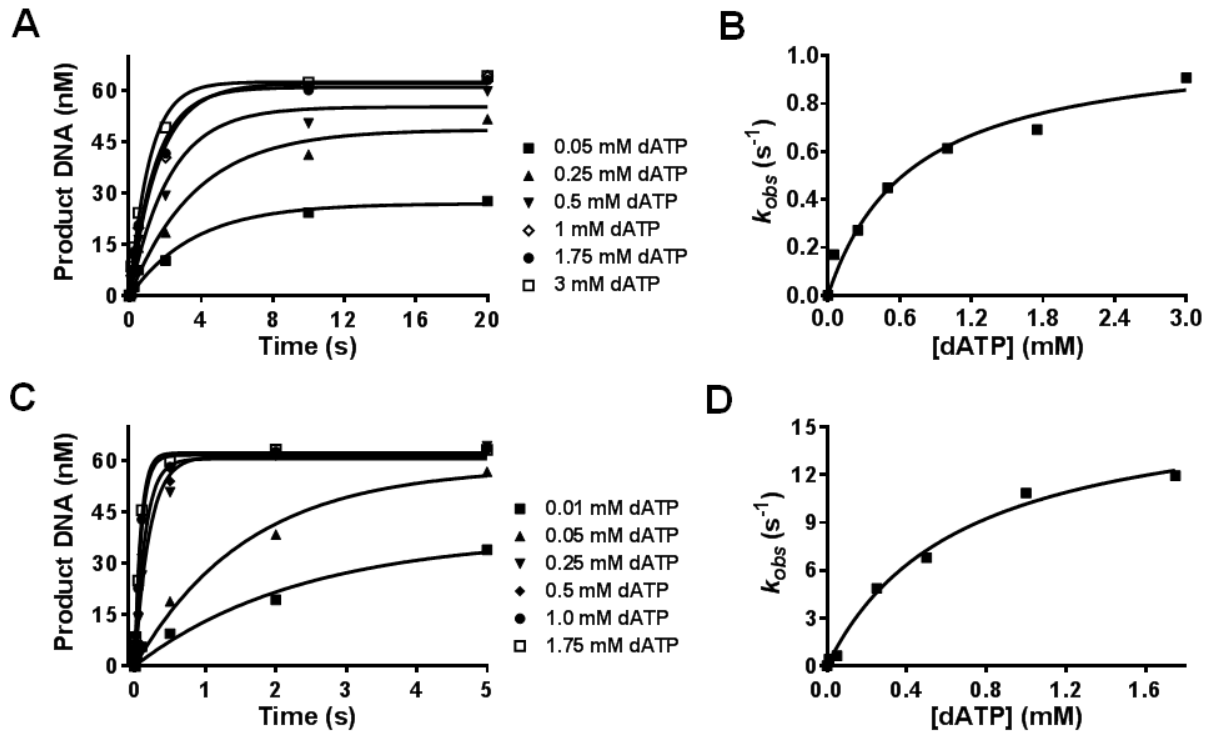

Figure S3. Concentration dependence of the rate of dATP incorporation opposite dT past DNA containing dA/dC mismatch at the primer terminus. RB69pol (1 μM) was pre-incubated with DNA_{ACMM} (80 nM) in reaction buffer and was mixed with increasing concentrations of dATP [0.05, 0.25, 0.5, 1, 1.75, and 3 mM] containing 10 mM Mg^{2+}. Reactions were quenched with 0.5 M EDTA (pH 8.0) at various times ranging from 0.1-20 s. All data were obtained at 23 °C. **(A)** Plots of the amount of extended DNA product obtained as a function of time at various [dATP] in 10 mM Mg^{2+}. Points are experimental, while curves are based on a fit of the data to Eq.1. **(B)** The single exponential rates obtained were plotted as a function of [dATP] and fitted to Eq. 2 to obtain a k_{pol} of 1.1 ± 0.1 s^{-1} and a $K_{d,app}$ of 0.8 ± 0.2 mM. RB69pol (1 μM) was pre-incubated with DNA_{ACMM} (80 nM) in reaction buffer and was mixed with increasing concentrations of dATP [0.01, 0.05, 0.25, 0.5, 1.0, and 1.75 mM] containing 10 mM Mn^{2+}. Reactions were quenched with 0.5 M EDTA (pH 8.0) at various times ranging from 0.04-5 s. **(C)** Plots of the amount of extended DNA product obtained as a function of time at various [dATP] in 10 mM Mn^{2+}. Points are experimental, while curves are based on a fit of the data to Eq.1. **(D)** The single exponential rates obtained were plotted as a function of [dATP] and fitted to Eq. 2 to obtain a k_{pol} of 17 ± 1 s^{-1} and a $K_{d,app}$ of 0.6 ± 0.1 mM.

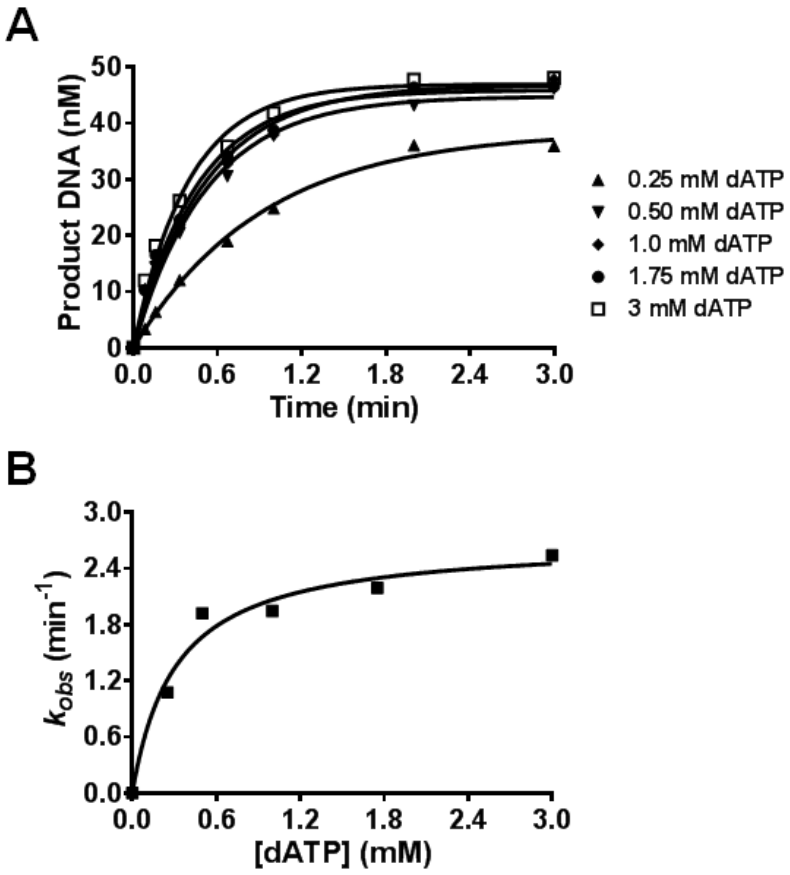

Figure S4. Concentration dependence of the rate of dATP incorporation opposite dT past DNA containing dA/dG mismatch at the primer terminus. RB69pol (1 μM) was pre-incubated with DNA_{AGMM} (80 nM) in reaction buffer and was mixed with increasing concentrations of dATP [0.25, 0.5, 1, 1.75, and 3 mM] containing 10 mM Co^{2+}. Reactions were quenched with 0.5 M EDTA (pH 8.0) at various times ranging from 0.08-3 min. All data were obtained at 23 °C. **(A)** Plots of the amount of extended DNA product obtained as a function of time at various [dATP] in 10 mM Co^{2+}. Points are experimental, while curves are based on a fit of the data to Eq. 1. **(B)** The single exponential rates obtained were plotted as a function of [dATP] and fitted to Eq. 2 to obtain a k_{pol} of 0.05 ± 0.005 s^{-1} and a $K_{d,app}$ of 0.31 ± 0.08 mM.

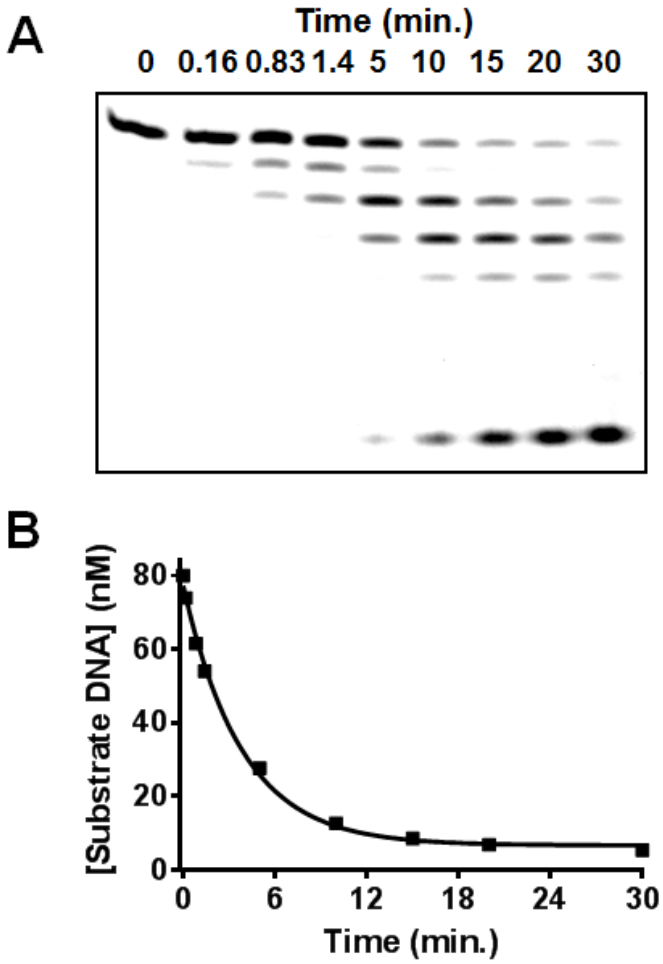

Figure S5. Exonuclease activity of RB69pol using Ni^{2+}. The reaction mixture contained 80 nM 5′-fluorescein labeled 13/18 mer substrate (DNA_{13A}), 1 μM RB69pol and 10 mM Ni^{2+}. **(A)** Digestion patterns visualized using FUJI scanner with fluorescein as the probe. **(B)** Plot of [Substrate DNA] remaining versus time fit to Eq. 4 to extract a k_{exo} of 0.24 ± 0.02 s^{-1}.

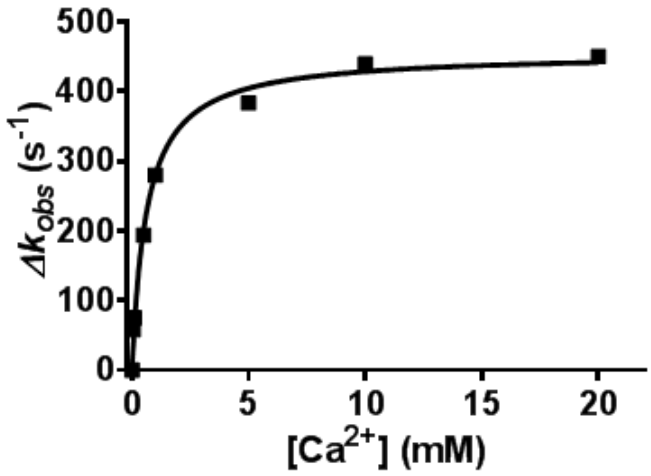

Figure S6. Competition between Ca^{2+} and Mg^{2+} for metal ions binding site. RB69pol (1 μM) was pre-incubated with DNA_{13A} (80 nM) in reaction buffer and was mixed with dTTP (1 mM) containing Mg^{2+} (10 mM), and varying [Ca^{2+}] (0.05-20 mM). Reactions were quenched with 0.5 M EDTA (pH 8.0) at various times ranging from 4-100 ms. All data were obtained at 23 °C. Plot of Δk_{obs} as a function of [Ca^{2+}]. Points are experimental, while the curve is based on a fit of the data to Eq. 7 to obtain a $K_{d,Ca}$ of 630 ± 72 μM. (Δk_{obs}) represents change in the rate of reaction as a function of increasing [Ca^{2+}] and Δk_{obs} increases with an increase in [Ca^{2+}].