Supporting Information

Subunit Interaction Dynamics of the *E. coli* Class Ia Ribonucleotide Reductase: In Search of a Robust Assay

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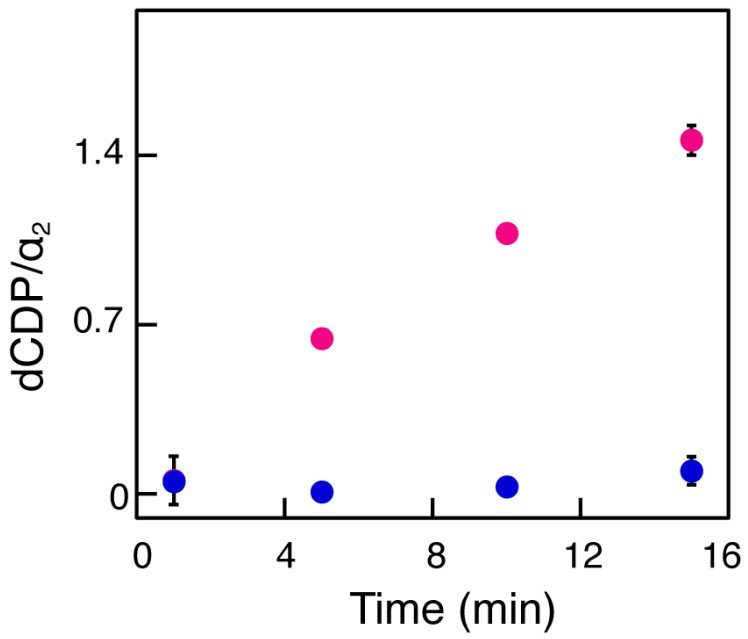

Figure S1. dCDP formation for inactive variants: $Y_{356}F$-β_2 (●) and $Y_{731}F$-α_2 (●). Reactions contained in a total volume of 170 µL, 2 µM each of α_2 and β_2 (or variants), 0.5 mM [5-^{3}H]-CDP (7000 cpm/nmol), and 3 mM ATP in assay buffer.

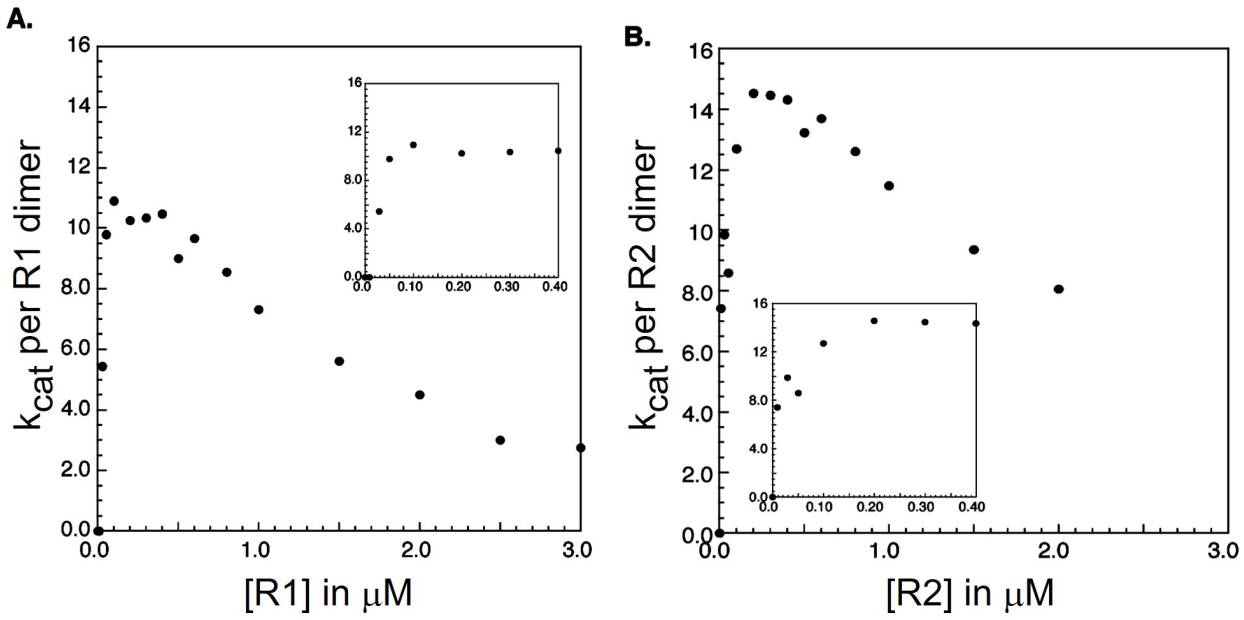

Figure S2. Turnover kinetics of *E. coli* RNR in the presence of TR/TRR/NADPH. Figure reproduced from reference 14 in the text.

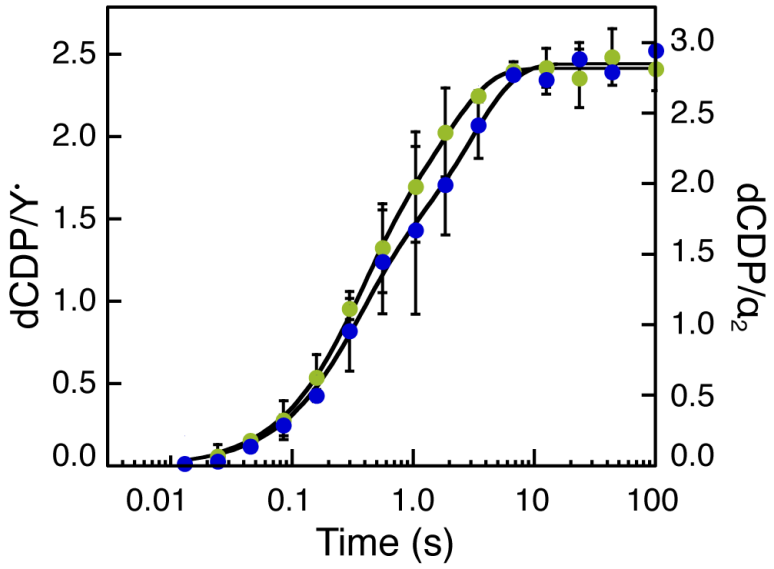

Figure S3. Pre-steady state single turnover kinetics of dCDP formation at 1:1 α_2:β_2 with 1 μM (●), and 10 μM (●) of each subunit, respectively. Points and error bars represent the two independent trials. Traces represent fits to Eq. 1; $y = A_1(1 - e^{-k_1 t}) + A_2(1 - e^{-k_2 t})$ and produced rate constants and amplitudes listed in Table 1 of the main text.

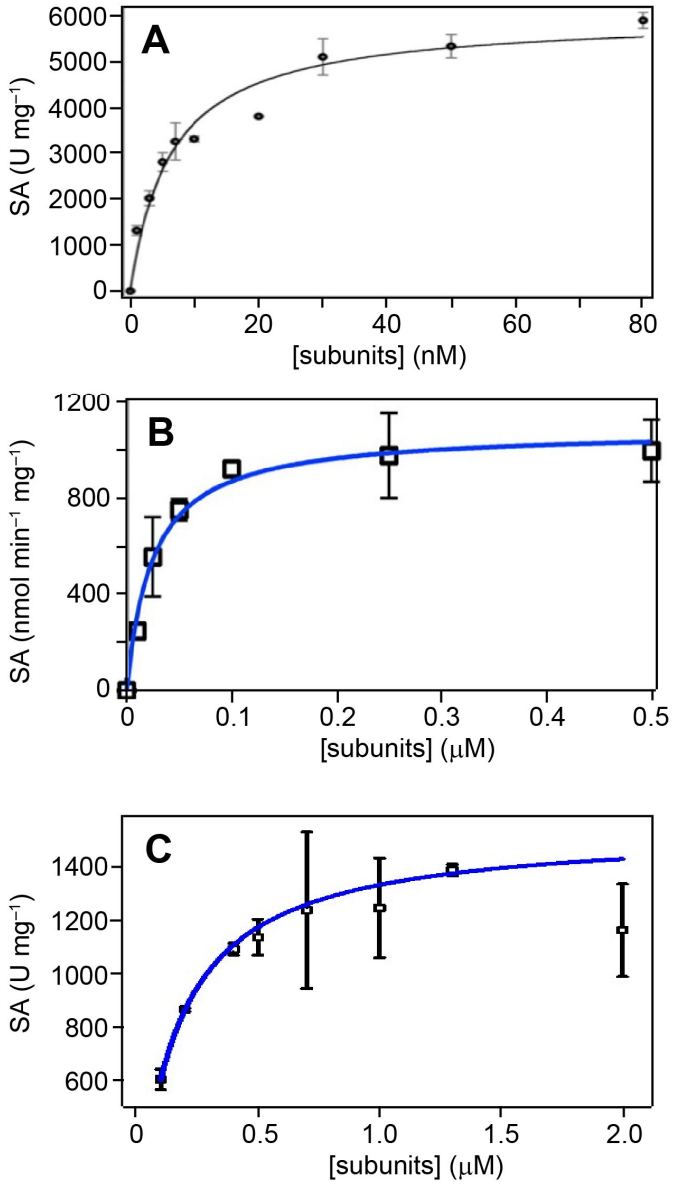

Figure S4. RNR assays in the presence of optimized endogenous reductant with α:β (1:1) at increasing concentrations. (A) Activity assay of *S. sanguinis* NrdF and NrdE in the presence of 0.1 mM dATP, 10 μM NrdH (endogenous reductant), and 20 mM DTT. V_{max} = 6000 nmol min^{-1} mg^{-1} and K_m = 6.4 nM. Figure reproduced from reference 46. (B) Activity assay of *B. subtilis* NrdF2 and full length NrdE in the presence of 1 mM CDP, 3 mM ATP, and optimized concentrations of TrxA/TrxB/NADPH. V_{max} = 1081 ± 36 nmol min^{-1} mg^{-1} and K_m = 0.025 ± 0.003 μM. Figure reproduced from reference 47. (C) Activity assay of *M. tuberculosis* NrdF2 and NrdE using a 1:1 ratio of subunits, 1 mM CDP, 3 mM ATP 3 μM NrdH and 20 mM DTT in 50 mM Hepes pH 7.6, 15 mM $MgSO_4$ and 1 mM EDTA at 37°C. The error bars are for two measurements. The V_{max} = 1545 nmol min^{-1} mg^{-1} and K_m = 0.10 μM. When the enzyme was assayed in the presence of a five-fold excess of NrdF2, the activity increased to 2300 nmol min^{-1} mg^{-1}.