Supporting Information

Kinetics of Hydrogen Atom Abstraction from Substrate by an Active Site Thiyl Radical in Ribonucleotide Reductase

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Abbreviations

RNR, *E. coli* class Ia ribonucleotide reductase; α_2, large subunit of RNR containing substrate and effector binding sites; β_2, small subunit of RNR containing the diiron-tyrosyl radical cofactor; PCET, proton-coupled electron transfer; RT, radical translocation; [Re]-β_2, C_{355}-β appended methylpyridyl rhenium(I) tricarbonyl phenanthroline complex; F_3Y, 2,3,5-trifluorotyrosine; TA, transient absorption; MLCT, metal-to-ligand charge transfer; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; HU, hydroxyurea; ATP, adenosine 5′-triphosphate; CDP, cytidine 5′-diphosphate; [^{3}H]-CDP, 5-tritiated cytidine 5′-diphosphate sodium salt hydrate; C, cytosine; dC, 2'-deoxycytidine; HEPES, 4-(2-hydroxyethyl)-piperazin-1-ylethanesulphonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid, TR, thioredoxin; TRR, thioredoxin reductase; AP, calf alkaline phosphatase.

Experimental methods and instrumentation

Distilled deionized water (ddH_2O) (18 MΩ cm^{-1}) was used for all aqueous solutions and other uses of water. All buffers were adjusted to the noted pH at room temperature. Assay buffer consisted of 50 mM either MES (for pH 5.2–6.8) or HEPES (pH 7.0–8.2), 15 mM $MgSO_4$ and 1 mM EDTA.

Absorption spectra in the UV and visible region were collected on a Varian Cary 5000 UV-vis-NIR spectrophotometer on samples as indicated. High-performance liquid chromatography (HPLC) was performed using a Waters 717/515/2487 instrument with an Alltech Econosil C18 column (10 μm, 250 mm × 4.6 mm). Temperature control was maintained with a Lauda RE106 circulating water bath. DNA sequencing was performed at the MIT Biopolymers Laboratory.

Nucleotide concentrations were calculated from the following extinction coefficients: ε_{259}(ATP) = 15.4 mM^{-1} cm^{-1} and ε_{271}(CDP) = 9.1 mM^{-1} cm^{-1}. The concentrations of α_2 and β_2 were determined using $\varepsilon_{280}(\alpha_2)$ = 189 mM^{-1} cm^{-1}, $\varepsilon_{280}(\beta_2)$ = 131 mM^{-1} cm^{-1}, and ε_{280}([Re]-β_2) = 189 mM^{-1} cm^{-1}. Purity of subunits was determined by SDS-PAGE analysis, activity was determined by the spectrophotometric assay (described briefly below).[1]

RNR activity was monitored through a continuous coupled-enzyme assay in which the consumption of NADPH was determined by monitoring the decrease in absorbance at 340 nm using the subunit to be measured in the presence of a five-fold excess of the other. Subunit 1 (0.5 μM), ATP (3 mM), CDP (1 mM), TR (30 μM), and TRR (0.5 μM) were mixed in assay buffer in a final volume of 300 μL. The assay was initiated by addition of subunit 2 (0.1 μM) and NADPH (0.2 mM) and the change in A_{340} was monitored for 1 min. To assess

[1] Bollinger, J. M. Jr.; Tong, W. H.; Ravi, N.; Huynh, B. H.; Edmondson, D. E.; Stubbe, J. *Meth. Enzymol.* **1995**, *258*, 278–303.

the activity of holo-$S_{355}C$-2,3,5-F_3Y_{356}-β_2, the subunit was used as 1 μM with 5 μM α_2, 300 μM TRR and 5 μM TR with all other concentrations as listed above.

Construction of the plasmid for expression of $C_{268}S$, $C_{305}S$, $S_{355}C$, $Y_{356}Z$-pBAD-*nrdB* was achieved by site-directed mutagenesis using the previously prepared pBAD-*nrdB* as a template and the following forward (f) and reverse (r) primers. Point mutations are in red and the three-letter codon being mutated is underlined.

$C_{268}S$ (f): 5′– CGGAAATTGCCGAAGAGAGTAAGCAGGAGTGCTAT –3′
$C_{268}S$ (r): 5′– ATAGCACTCCTGCTTACTCTCTTCGGCAATTTCCG –3′

$C_{305}S$ (f): 5′– GAATAAAGACATTCTCAGCCAGTACGTTGAATACATC –3′
$C_{305}S$ (r): 5′– GATGTATTCAACGTACTGGCTGAGAATGTCTTTATTC –3′

$Y_{356}Z$ (f): 5′–GAAGTGGAAGTCAGTTCTTAGCTGGTCGGGCAGATTGACTCG–3′
$Y_{356}Z$ (r): 5′–CGAGTCAATCTGCCCGACCAGCTAAGAACTGACTTCCACTTC–3′

$S_{355}C$ (f): 5′–GAAGTGGAAGTCAGTTGTTAGCTG–3′
$S_{355}C$ (r): 5′– AATCTGCCCGACCAGCTAA –3′

Calculation of radical yield

The photochemical yield of trifluorotyrosyl radical ($\phi_{Y\bullet}$), measured in molecules of radical generated per photon, was calculated using the equation:

$$\Phi_{Y\bullet} = \frac{N_A V_{bore}\left(\frac{\Delta OD}{\varepsilon_{Y\bullet}\ell}\right)}{\left(\frac{E_P\lambda}{hc}\right)\left(1-10^{\varepsilon_{355,Re}[Re]\ell}\right)} \quad \text{(S1)}$$

where N_A is Avogadro's number, V_{bore} is the volume of the cuvette in the path length (70 μL), ΔOD is the transient absorption of the radical (2×10^{-3}), $\varepsilon_{\bullet Y}$ is the extinction coefficient of the radical (1,783 M^{-1} cm^{-1}),[1] ℓ is the sample path length (1 cm), E_P is the energy of each laser pulse (2.0 mJ), λ_{obs} = 355 nm, h is Planck's constant, c is the speed of light, $\varepsilon_{355,Re}$ is the extinction coefficient of the rhenium complex (5,300 M^{-1} cm^{-1}) at 355 nm,[2] and [Re] is the concentration of the complex (50 μM). For the radical measured in the presence of wt-α2, $\Phi_{Y\bullet}$ = 0.019, or 1.9%.

[2] Pizano, A. A.; Lutterman, D. A.; Holder, P. G. Teets, T. S.; Stubbe, J.; Nocera, D. G. *Proc. Nat. Acad. Sci.* **2012**, *109*, 39–43.

Table S1. Single wavelength kinetics data

α_2 variant	Substrate	Trial 1			Trial 2			Avg.	
		τ (μs)	Error[a] (μs)	Amp. (mΔOD)	τ (μs)	Error[a] (μs)	Amp. (mΔOD)	τ (μs)	σ[b] (μs)
wt	CDP	17.5	1.1	1.21	18.4	1.4	2.06	17.9	0.87
$Y_{731}F$	CDP	24.2	2.2	1.28	23.5	2.0	1.61	23.9	1.5
$C_{439}S$	CDP	25.4	2.2	1.27	25.0	2.3	1.81	25.2	1.6
wt	[3′-^{2}H]-CDP	25.1	1.6	1.09	26.9	2.3	1.94	25.7	1.3

[a] Represents error associated with the goodness of fit. [b] Absolute standard deviation propagated from weighted average and associated error.

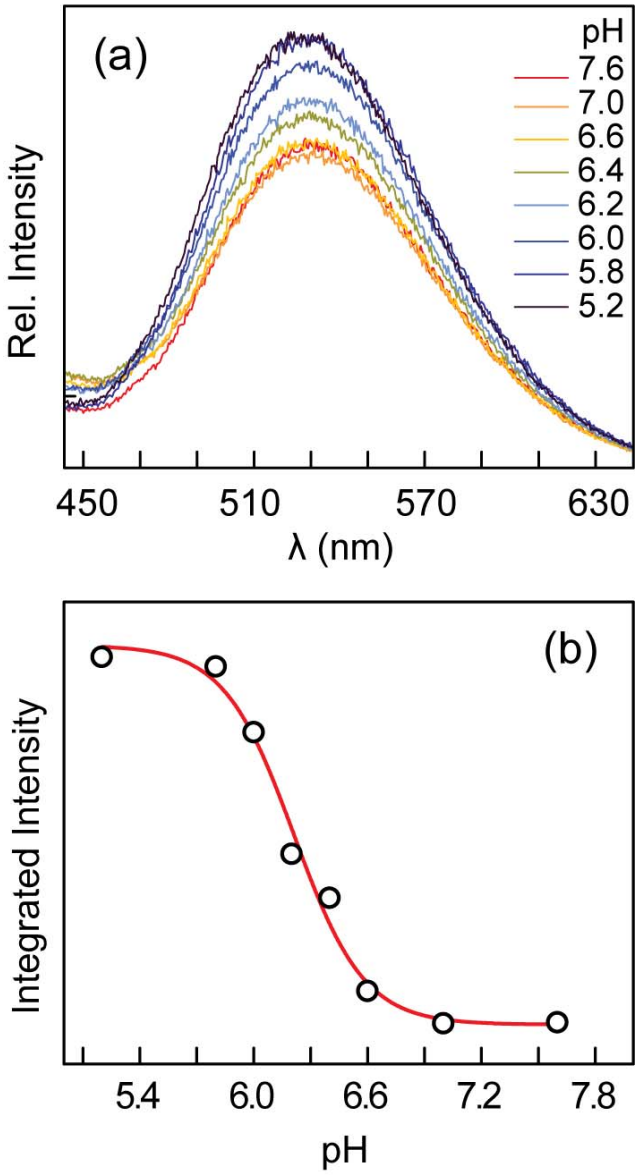

Figure S1. (a) Emission spectra of [Re]-F_3Y_{356}-β_2 (5 μM) and α_2 (20 μM), CDP (1 mM) and ATP (3 mM) in assay buffer of varying pH. (b) Total emission intensity integrated from 450 to 650 nm (o) versus pH with a fit (—) to a monoprotic titration equation.

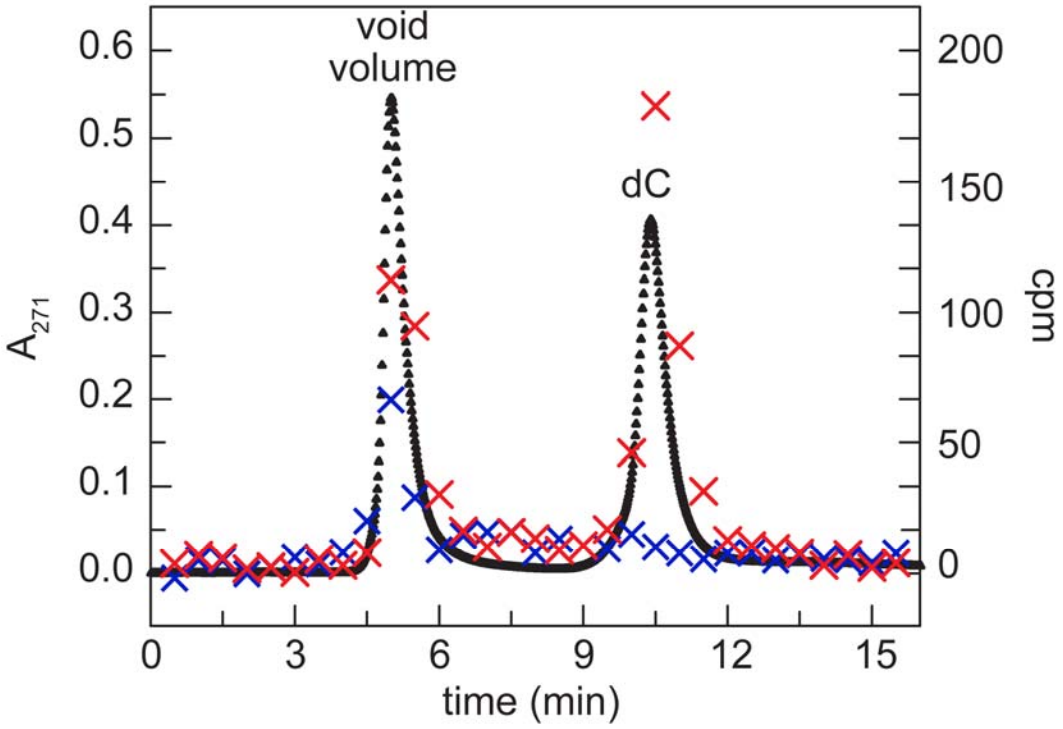

Figure S2. HPLC trace of photoproducts and carrier C collected during photochemical single turnover experiments. The C and dC bases are measured by absorbance at 271 nm (▲) and ^3H_2O and radiolabeled product are measured by cpm (×). Elution with 20 mM KP_i at pH 6.7 leads to the appearance of dephosphorylated 2′-dC at ~10 min, and ^3H_2O and carrier C within the void volume at ~5 min. 90% of photoproducts were recovered. Samples originally contained 10 μM wt-α_2, 0.2 mM [5-^{3}H]-CDP, 3 mM ATP, 10 mM $Ru(NH_3)_6Cl_3$ with (×) or without (×) 10 μM [Re]-F_3Y-β_2. The photochemical products of the reaction are consistent with dC.

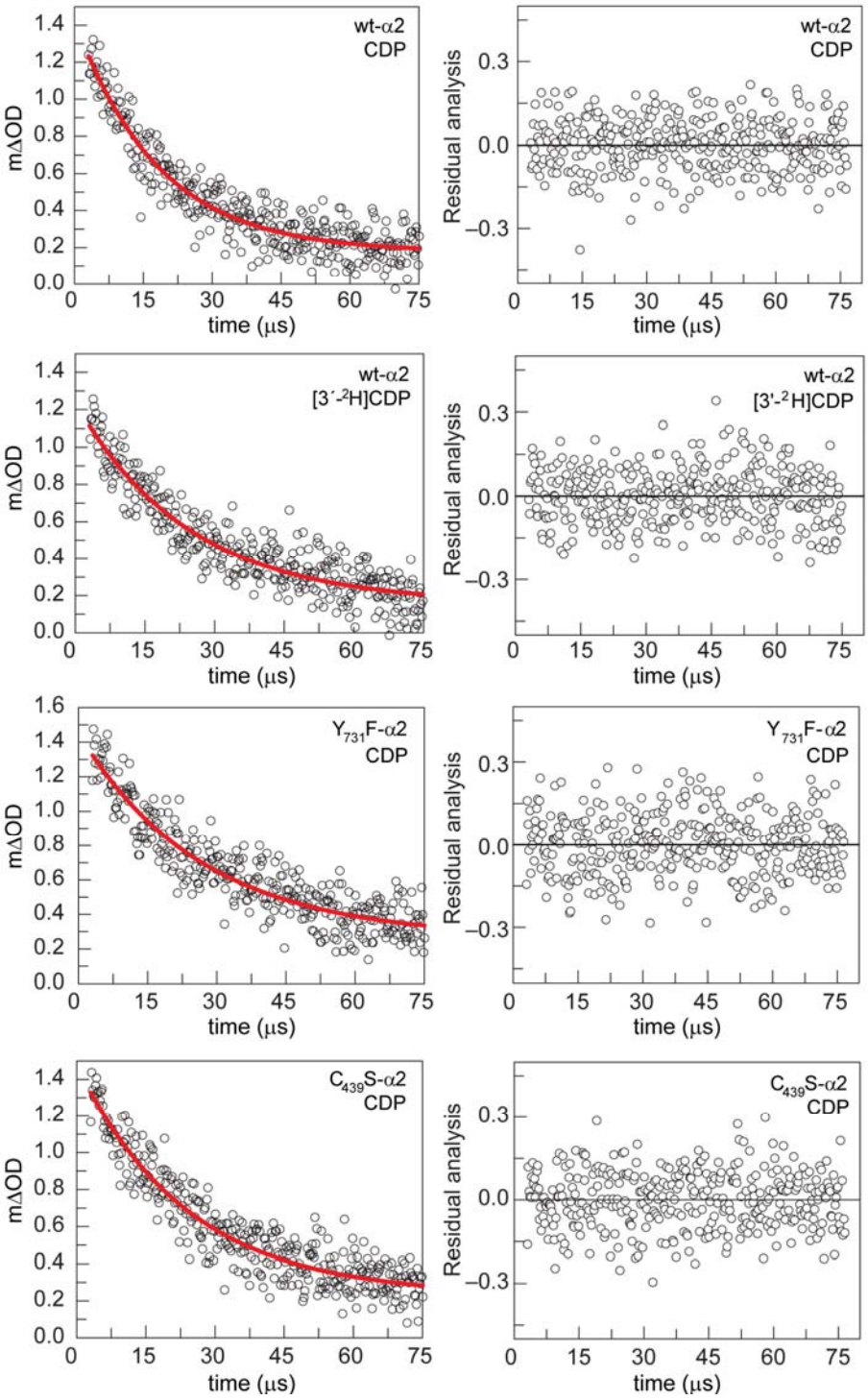

Figure S3. Single wavelength kinetics transients absorbing at 412.5 nm (±4.5 nm) (left, **o**). Monoexponential fits to the data over 3 - 76.5 μs (left, —) and the corresponding residuals analysis of the fit (right). Each trace represents an average of three independent samples containing 50 μM [Re]-F_3Y-β_2, 75 μM α_2 (wt-, $Y_{731}F$-, or $C_{439}S$- as noted), 1 mM CDP or 0.5 mM [3′-²H]CDP, 3 mM ATP, 10 mM $Ru(NH_3)_6Cl_3$, 50 mM HEPES pH 8.2, 15 mM $MgSO_4$, and 1 mM EDTA

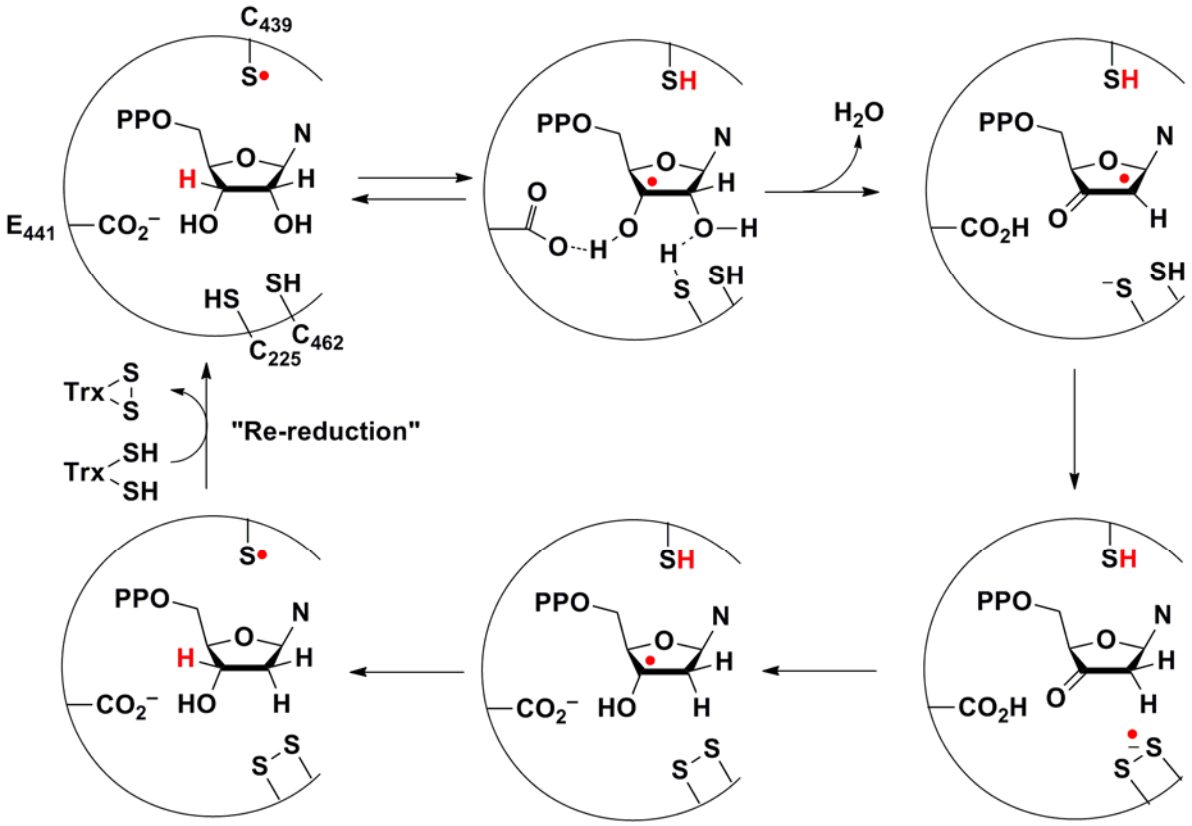

Figure S4. Current model for active site chemical transformations in *E. coli* class Ia RNR. Here Trx stands for thioredoxin.