Supporting Information

Photochemical Generation of a Tryptophan Radical within the Subunit Interface of 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; [Re^I], methylpyridyl rhenium(I) tricarbonyl phenanthroline phosphorushexafluorate complex; photoβ_2, $C_{268}S/C_{305}S/S_{355}C$-$\beta_2$ appended with [Re^I]; W-photoβ_2, $C_{268}S/C_{305}S/S_{355}C/Y_{356}W$-$\beta_2$ appended with the [Re^I] complex; TA, transient absorption; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; MLCT, metal-to-ligand charge transfer; HU, hydroxyurea; ATP, adenosine 5′-triphosphate; CDP, cytidine 5′-diphosphate; [^{3}H]-CDP, 5-tritiated cytidine 5′-diphosphate sodium salt hydrate; HEPES, 4-(2-hydroxyethyl)-piperazin-1-ylethanesulphonic acid; TR, thioredoxin; TRR, thioredoxin reductase; AP, calf alkaline phosphatase.

Materials

Generation of $C_{268}S/C_{305}S/S_{355}C/Y_{356}W$-pBAD-*nrdB* and $Y_{356}W$-pBAD-*nrdB* was achieved by site-directed mutagenesis using 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′

$S_{355}C/Y_{356}W$ (f): 5′–GGAAGTGGAAGTCAGTTGTTGGCTGGTCGGGCAGATTG–3′
$S_{355}C/Y_{356}W$ (r): 5′– CAATCTGCCCGACCAGCCAACAACTGACTTCCACTTCC–3′

$Y_{356}W$ (f): 5′– GGAAGTGGAAGTCAGTTCTTGGCTGGTCGGGCAG –3′
$Y_{356}W$ (r): 5′– CTGCCCGACCAGCCAAGAACTGACTTCCACTTCC –3′

Experimental methods

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 and MADLI-TOF analysis, activity was determined by the spectrophotometric assay (described briefly below).[1]

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

General 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 HEPES, 15 mM $MgSO_4$ and 1 mM EDTA adjusted to pH 7.6.

Absorption spectra in the UV and visible region were collected on a Varian Cary 5000 UV-vis-NIR spectrophotometer on samples as indicated. Temperature control was maintained with a Lauda RE106 circulating water bath. DNA sequencing was performed at the MIT Biopolymers Laboratory.

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 (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 (1 μM) and NADPH (0.2 mM) and the change in A_{340} was monitored for 1 min.

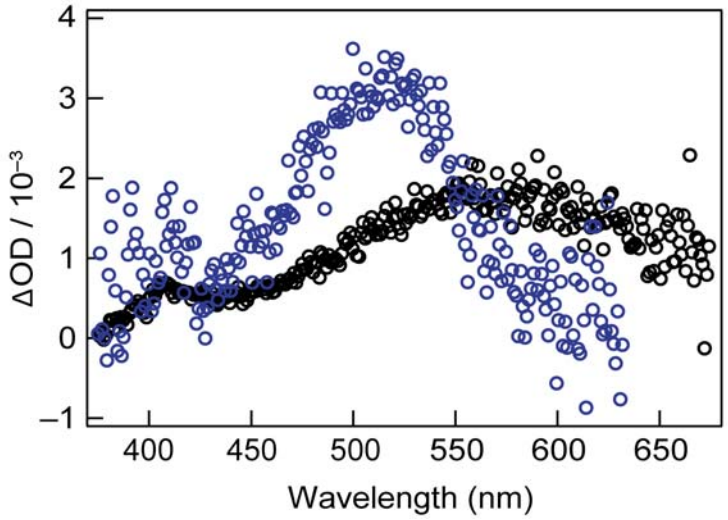

Figure S1. TA spectra of $Y_{356}W$- and $Y_{356}F$-photoβ_2 collected in the absence of α_2 8 μs after the 355 nm excitation pulse. The displayed spectra are averages of 3 independently prepared samples, each containing either 50 μM $Y_{356}W$-photoβ_2 or 30 μM $Y_{356}F$-photoβ_2 (blue and black, respectively). Samples also contained 1 mM CDP, 3 mM ATP, and 10 mM $Ru(NH_3)_6Cl_3$, in assay buffer at pH 7.6.

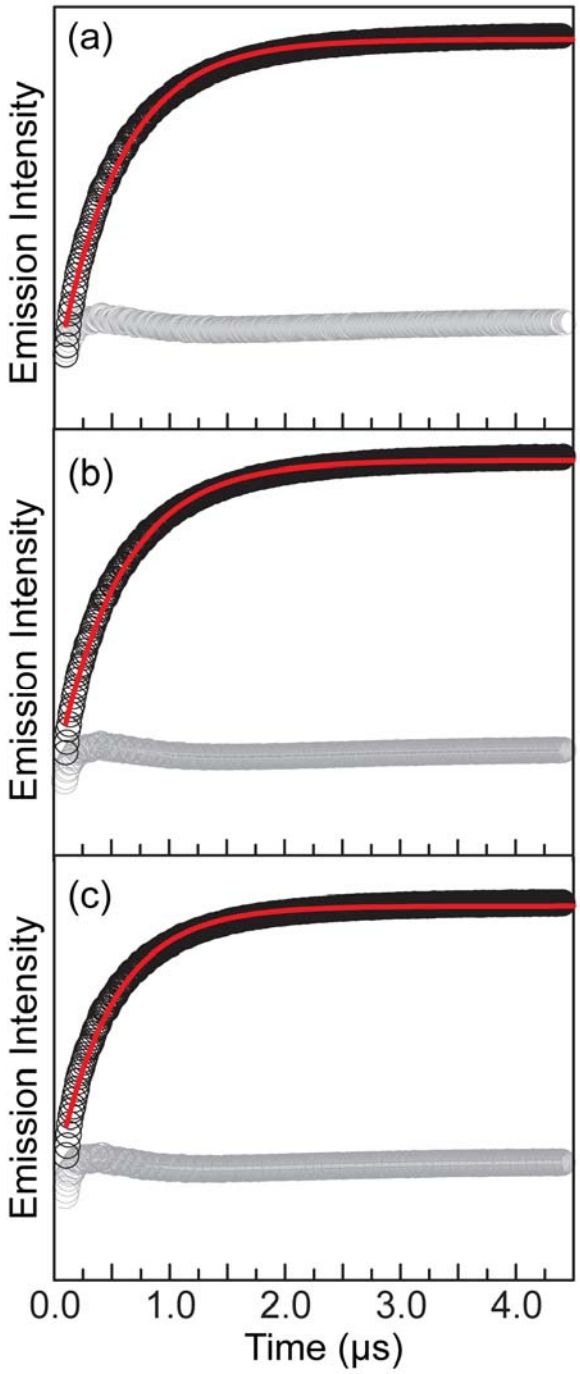

Figure S2. Representative emission quenching data (black circles), monoexponential fits to the data (red lines) and residuals (gray) for W-photoβ_2 in the presence of wt-α_2 (A), $Y_{731}F$-α_2 (B), and in the absence of α_2 (C), with CDP and ATP in assay buffer at pH 7.6 with λ_{exc} = 355 nm and λ_{det} = 600 nm.