Supporting Information

Photochemical Rescue of a Conformationally Inactivated Ribonucleotide Reductase

Brandon L. Greene,[a] JoAnne Stubbe[b,c,]* and Daniel G. Nocera[a,]*

[a] *Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA USA 02138.*
[b] *Department of Chemistry and* [c]*Department of Biology, Massachusetts Institute of Technology, Cambridge, MA USA 02139.*

Email: dnocera@fas.harvard.edu; stubbe@mit.edu

Table of Contents *Page*

Materials

$(His)_6$-WT α_2 (specific activity of 2,100 nmol/min/mg), $(His)_6$-$Y_{731}F$ α_2, WT β_2 (1.1 Y•/β_2, specific activity of 5,800 nmol/min/mg), photoβ_2 ($C_{268}S$:$C_{305}S$:$S_{355}C$-β_2, [Re^I]-ligated), and $Y_{356}F$-photoβ_2 ($C_{268}S$:$C_{305}S$:$S_{355}C$:$Y_{356}F$, [Re^I]-ligated) were available from a previous study.[1] Primers 5′-CCGCAG**C**AAGTGGAAGTCAGT-3′ and 5′-CAACGAGGCGTC**G**TTCACCTT-3′ (forward and reverse, respectively, the bold nucleotide represents the site of mutation) were used for all site directed mutagenesis experiments generating the corresponding $E_{350}Q$ mutations in photoβ_2s and were purchased from Integrative DNA Technologies, Inc. Thioredoxin (TR, 40 μmol/min/mg) and thioredoxin reductase (TRR, 1800 μmol/min/mg) were available from a previous study [*1,2*]. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2-(N-morpholino)ethanesulfonic acid (MES), anhydrous magnesium sulfate ($MgSO_4$), ethylenediaminetetraacetic acid (EDTA), adenosine-5′-triphosphate (ATP), cytidine-5′-diphosphate (CDP), hydroxyurea (HU), ruthenium hexamine dichloride, kanamycin (Km), chloramphenicol (Cm), 2xYT media, M9 minimal salts, L-arabinose (ara), β-mercaptoethanol (β-ME), streptomycin sulfate, and pyridoxal phosphate (PLP) were purchased from Sigma-Aldrich. Dithiothreitol (DTT) and isopropyl-β-thiogalacto-pyranoside (IPTG) were purchased from Promega Inc. Tricarbonyl(1,10-phenanthroline)-(4-bromomethylpyridine)rhenium(I) hexafluorophosphate ([Re]-Br) was available from a previous study [*3*].

Methods

Site directed mutagenesis. Genes encoding *Escherichia coli* $E_{350}Q$:$C_{268}S$:$C_{305}S$:$S_{355}C$-*nrdB* ($E_{350}Q$-photoβ_2), $E_{350}Q$:$C_{268}S$:$C_{305}S$:$S_{355}C$:$Y_{356}F$-*nrdB* and $E_{350}Q$:$C_{268}S$:$C_{305}S$:$S_{355}C$:$Y_{356}Z$-nrdB (where Z indicates the amber stop codon) were constructed by site directed mutagenesis using the corresponding pBAD photoβ_2, $Y_{356}F$-photoβ_2, and $Y_{356}Z$-photoβ_2 plasmids as templates. PCR products were transformed into XL10 Gold cells, and twice plated onto 100 μg/mL Ampicillin supplemented LB-agar plates. A single colony was selected for growth and the plasmid was isolated by the Qiagen mini-prep kit and sequenced by Quintara Biosciences.

Protein expression and purification. pBAD *nrdB* plasmids were transformed into Top10 cells, and cell growth, protein purification, and [Re^I]-Br ligation were performed as previously described [*1,3*]. Expression of the $E_{350}Q$:$Y_{356}F_2Y$-photoβ_2 resulted in ~50% F2Y incorporation (suppression) over truncation and was purified to >85% (β_2) as determined by SDS-PAGE relative to the homo- and hetero-truncation products ($\beta`_2$ and $\beta\beta`$ respectively, where β` indicates truncated β at the amber codon). The native Y_{122}• in all photoβ_2 constructs (>0.8 Y•/β_2) was reduced with hydroxyurea prior to all experiments.

3,5-Difluorotyrosine synthesis. 3,5-F_2Y was synthesized enzymatically from 2,6-difluorophenol via tyrosine phenol lyase (TPL) as previously described [*4*].

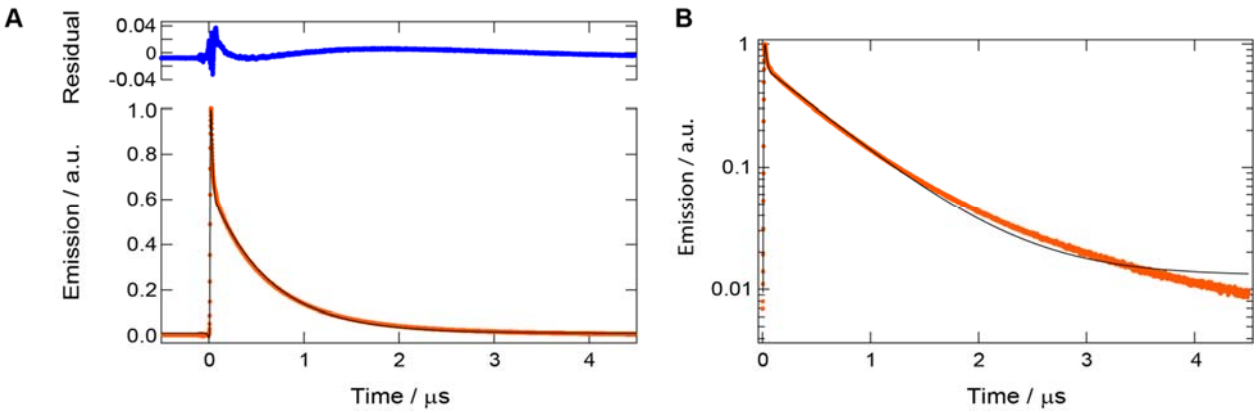

Figure S1 | A Emission trace (orange) for E_{350}Q-photoβ_2 in complex with WT α_2 and associated fit to an instrument response and mono-exponential decay function (black). Samples contain 2 μM E_{350}Q-photoβ_2, 5 μM WT α_2, 1 mM CDP and 3 mM ATP in assay buffer, and data represent the average of 150 individual traces (3 × 50). The associated residual trace (data – fit) shown in upper panel (blue). **B** Data presented in panel A viewed on a logarithmic emission intensity scale.

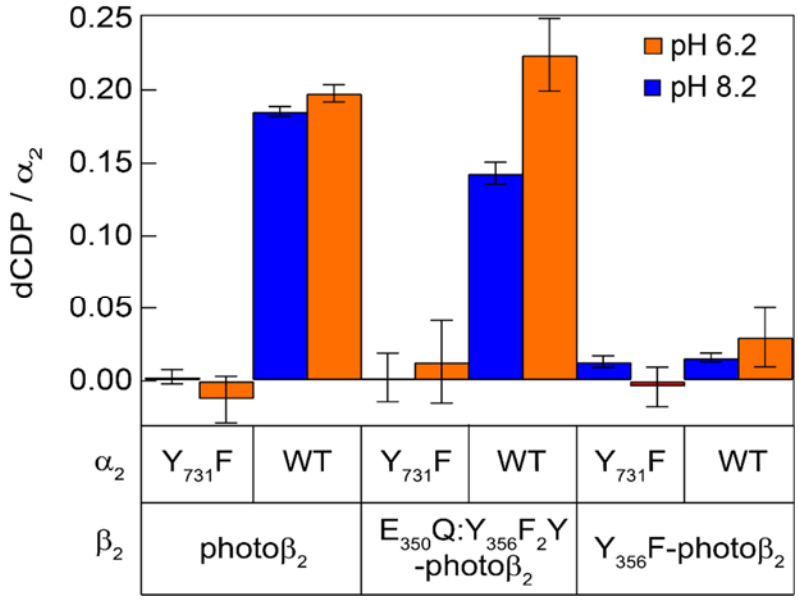

Figure S2. pH dependent photochemical turnover assay of $E_{350}Q:Y_{356}F_2Y$-photoβ_2, photoβ_2 and $Y_{356}F$-photoβ_2. Photochemical turnover assays performed at pH 6.2 (orange) and 8.2 (blue) with 10 µM α_2, 20 µM -photoβ_2, 0.2 mM [^{3}H]-CDP (32,000 cpm/nmol), 3 mM ATP, 10 mM $Ru(NH_3)_6Cl_3$ in 50 mM MOPS, 50 mM HEPES, 15 mM $MgSO_4$, 1 mM EDTA, and 5% glycerol. Samples illuminated by a 150 W Xe arc lamp equipped with a $\lambda > 320$ nm long pass cutoff filter for 4 minutes at room temperature. Error bars represent one standard deviation among triplicate measurements. A dark reaction performed otherwise identically (photoβ_2/WT α_2) served as the background reference.

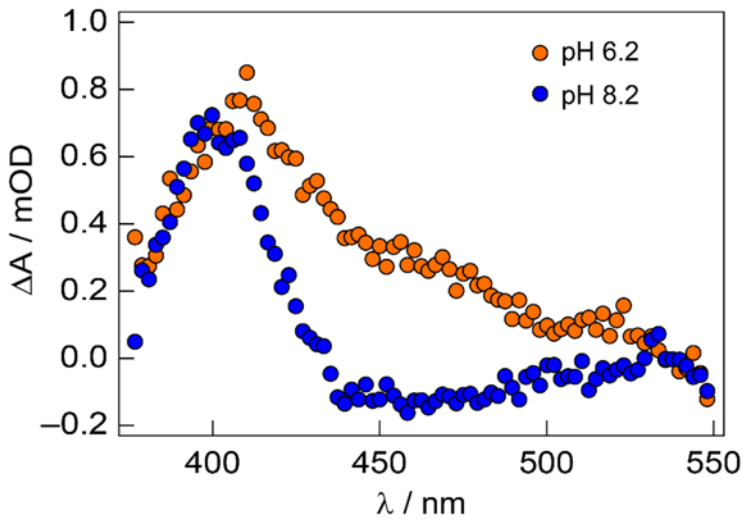

Figure S3. pH dependent transient absorption spectra of $E_{350}Q$:$Y_{356}F_2Y$-photoβ_2. Spectra collected identically as in the main text with the exception of the supporting buffer which was identical to that used in the generation of Figure S2 and adjusted to pH 6.2 (orange) or 8.2 (blue). Spectra represent the average of three independent experiments corresponding to 1000 laser exposures each.

Table S1. $Y_{356}F_2Y$-photoβ_2 and $E_{350}Q$:$Y_{356}F_2Y$-photoβ_2 emission lifetimes. Error represents one standard deviation among three independent measurements. k_{CS} rates are calculated with eq (3), where k_0 is taken as the $Y_{356}F$-photoβ_2/$Y_{731}F$ α_2 complex [Re^I]* emission lifetime of 680(5) ns. $Y_{356}F_2Y$-photoβ_2 was available from a previous study [*5*].

α_2	β_2	τ_{obs} (ns)	k_{CS} (10^5 s^{-1})
WT	$E_{350}Q$:$Y_{356}F_2Y$	558(3)	3.2(1)
$Y_{731}F$	$E_{350}Q$:$Y_{356}F_2Y$	580(8)	2.5(2)
WT	$Y_{356}F_2Y$*	619(6)	1.5(2)
$Y_{731}F$	$Y_{356}F_2Y$*	629(7)	1.2(2)

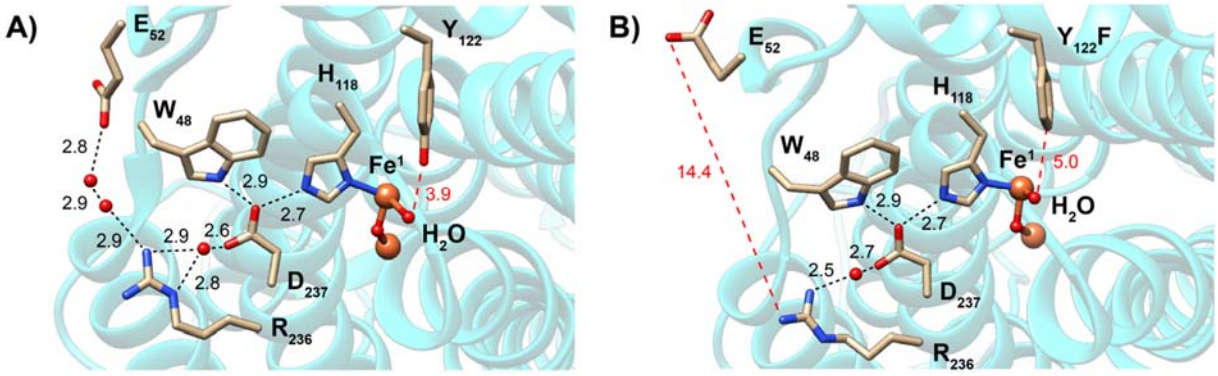

Figure S4. H-bonding network connecting E_{52} to Y_{122} in the β_2 subunit of *E. coli* class 1a RNR in both (A) "in" and (B) "out" conformations. The β ribbon structure is shown in cyan, and essential residue sidechains and the Fe_2O cofactor (with Fe^1 bound H_2O ligand) are colored by element; carbon, tan; nitrogen, blue; oxygen, red; iron, orange. Putative H-bonds shown in dashed black lines with distances reported in ångströms, and other relevant distances shown as dashed red lines. The "in" conformation (PDB 5CI4) displays two conserved water molecules bridging E_{52} to R_{236}, whereas in the "out" conformation (PDB 2AV8, a $Y_{122}F$ mutant β_2) this water network is lost and E_{52} approaches the proposed interface with α_2.

References

[1] Greene, B. L.; Taguchi, A. T.; Stubbe, J.; Nocera, D. G. Conformationally dynamic radical transfer in ribonucleotide reductase. *J. Am. Chem. Soc.* **2017**, *139*, 16657−16665.

[2] Lin, Q.; Parker, M. J.; Taguchi, A. T.; Ravichandran, K.; Kim, A.; Kang, G.; Shao, J.; Drennan, C. L.; Stubbe, J. Glutamate 52-β at the α/β subunit interface of *Escherichia coli* class Ia ribonucleotide reductase is essential for conformational gating of radical transfer. *J. Biol. Chem.* **2017**, *292*, 9229−9239.

[3] Pizano, A. A.; Lutterman, D. A.; Holder, P. G.; Teets, T. S.; Stubbe, J.; Nocera, D. G. Photo-ribonucleotide reductase β_2 by selective cysteine labelling with a radical phototrigger. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 39−43.

[4] Seyedsayamdost, M. R.; Yee, C. S.; Stubbe, J. Site-specific incorporation of fluorotyrosines into the R2 subunit of *E. coli* ribonucleotide reductase by expressed protein ligation. *Nat. Protoc.* **2007**, *2*, 1225−1235.

[5] Olshansky, L.; Stubbe, J.; Nocera, D. G. Charge-transfer dynamics at the α/β subunit interface of a photochemical ribonucleotide reductase. *J. Am. Chem. Soc.* **2016**, *138*, 1196−1205.