Supporting Information for

A hot oxidant, 3-NO_2Y_{122} radical, unmasking conformational gating in ribonucleotide reductase

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Table of Contents

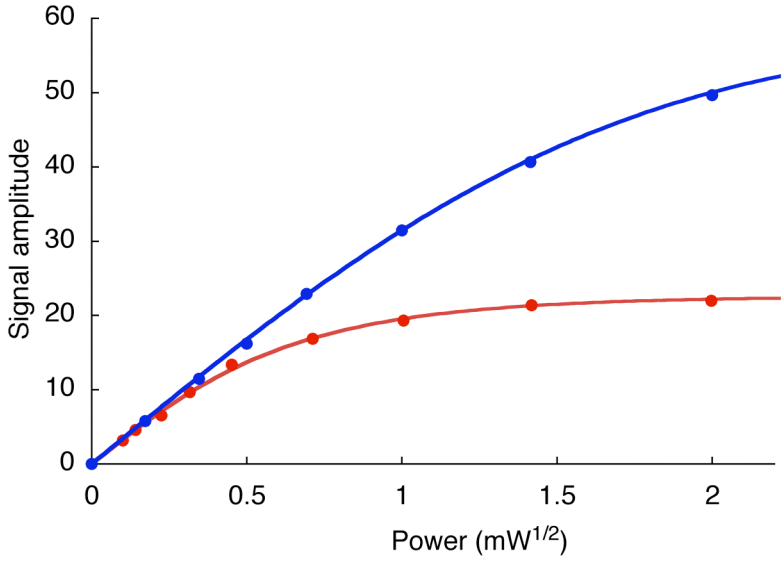

Figure S1. Microwave power saturation behavior of NO_2Y• (blue) and the new radical (red) signals. The lines are fits to Eq. 1 and yielded $P_{1/2}$ of 11.4 ± 0.5 mW (b = 1.2 ± 0.3) and 0.54 ± 0.08 mW (b = 1.1 ± 0.2) for NO_2Y_{122}• (blue) and the new Y• (red), respectively.

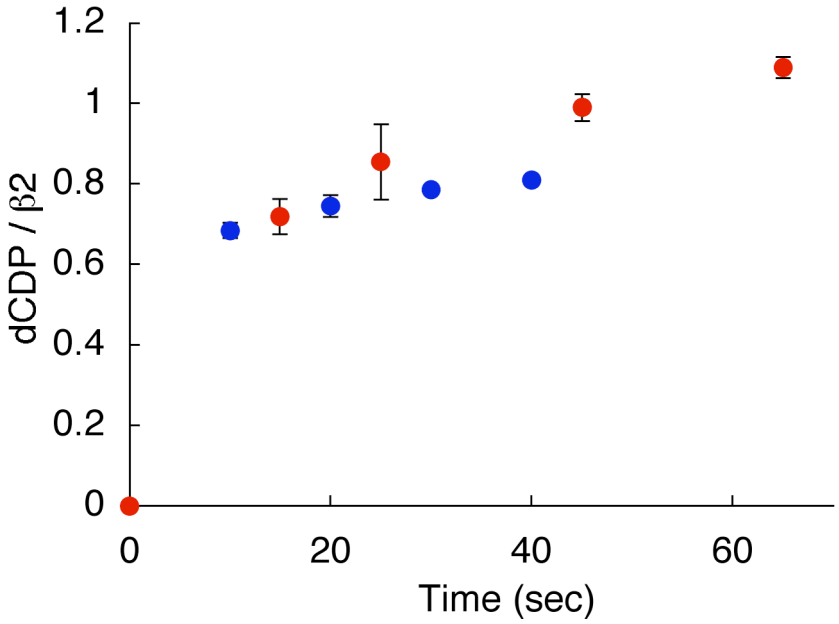

Figure S2. dCDP formation by [NO_2Y•]-β2 (0.95±0.05 NO_2Y•/β2) incubated with α2, ATP and CDP under steady state conditions using TR/TRR/NADPH as reductant (red circles) or under single turnover conditions with no reductant (blue circles). The slow phase after 10 s in the steady-state assay corresponds to 6.9 nmol/min/mg, 0.1% that of wt-β2, consistent with our earlier characterization of dCDP formation by contaminating wt-β2.[1]

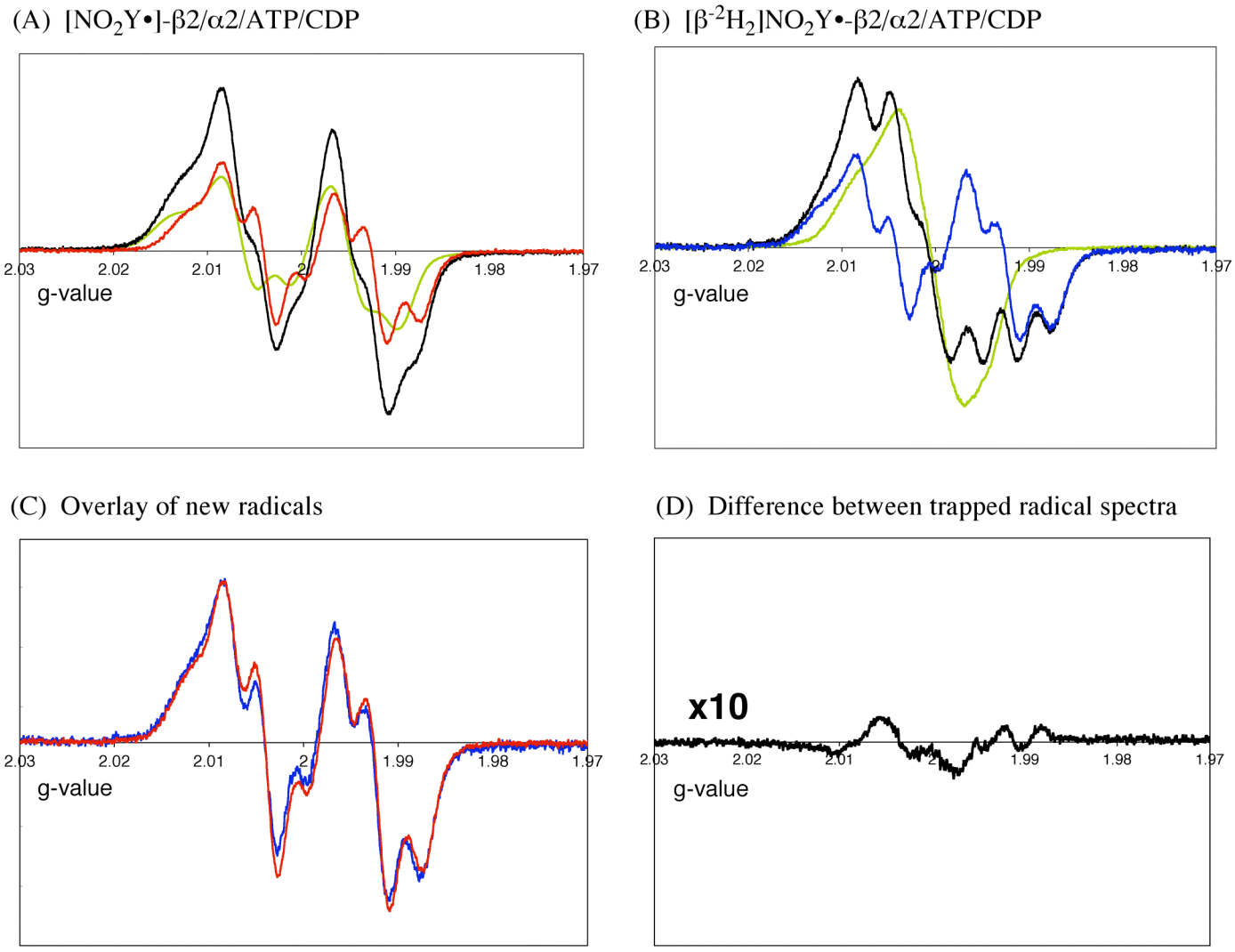

Figure S3. EPR spectra of (A) $Y_{122}NO_2Y$-β2 or (B) $Y_{122}[\beta\text{-}^2H_2]NO_2Y$-β2 incubated with wt-α2, ATP, and CDP freeze-quenched at 120 ms (black traces). Fifty percent of the spectrum of the NO_2Y• (A) or $[\beta\text{-}^2H_2]NO_2Y$• (B) (green) was subtracted to give the spectrum of new radical species (red and blue traces in (A) and (B), respectively). (C) Overlay of the red and blue spectra from (A) and (B). (D) Difference spectrum of the new radical species, red and blue traces in (C). The spectrum is magnified 10 folds.

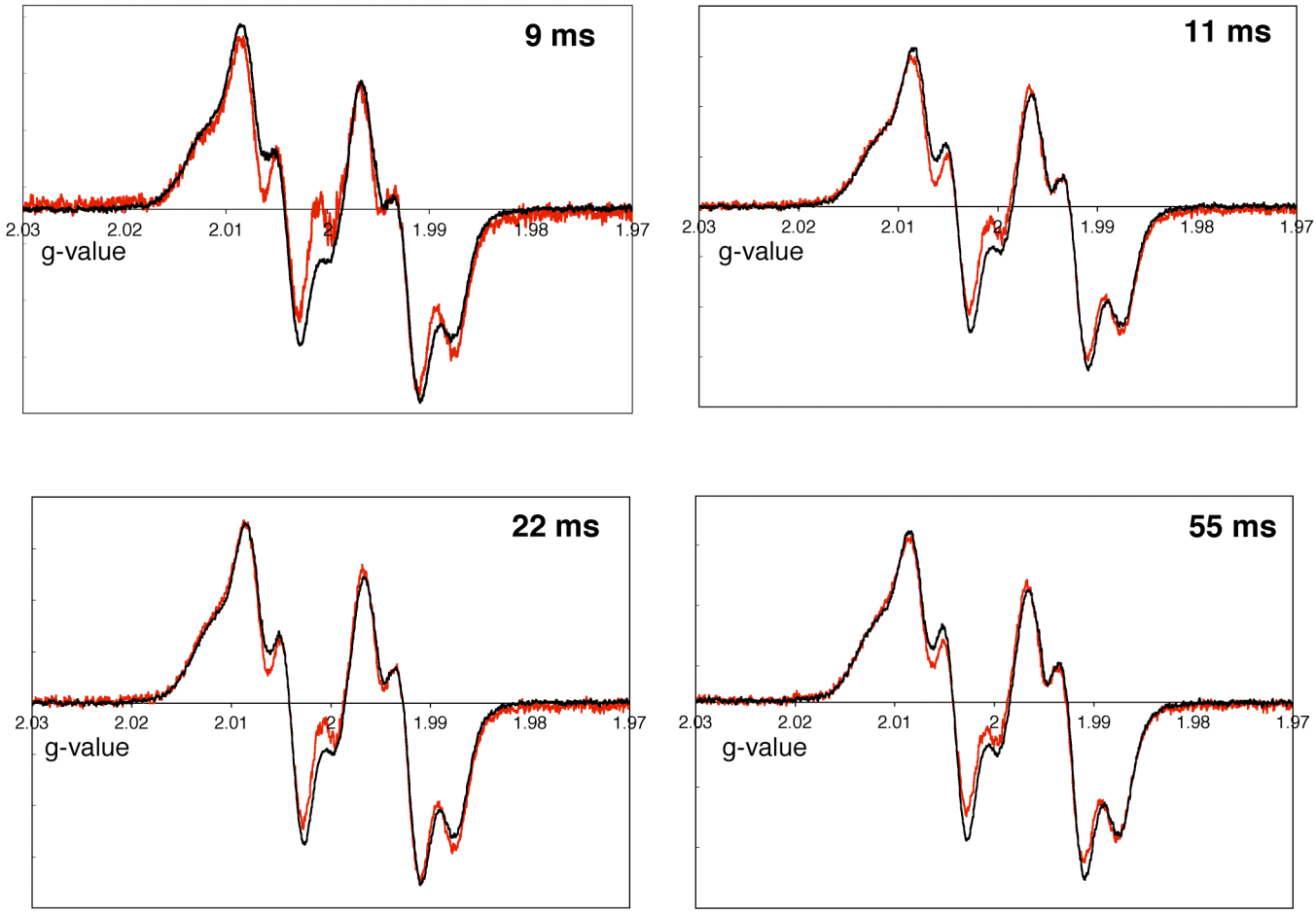

Figure S4. EPR spectra of the new radical acquired at the indicated time points. The spectrum of the new radical obtained as described in Figure S3 with $Y_{122}NO_2Y$-β2 (black trace) is overlaid with that obtained from $Y_{122}[\beta\text{-}^2H_2]NO_2Y$-β2 (red trace) at the same time point.

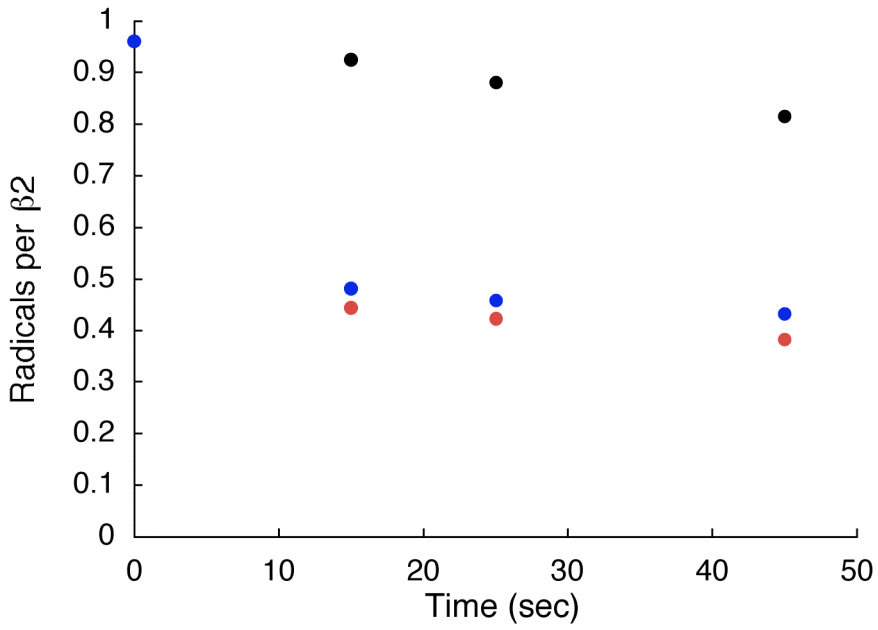

Figure S5. The stability of the new radical and NO_2Y•. $Fe(II)_2$-$Y_{122}NO_2Y$-β2 was manually mixed at 25 °C with O_2-saturated reconstitution buffer containing CDP and incubated for 10 s to generate 0.95 NO_2Y•/β2 (t = 0 time point). This mixture was then incubated with α2 and ATP. The reaction was quenched by hand in liquid N_2. Black circles (total radical at each time point); blue circles (NO_2Y•) and red circles (new radical).

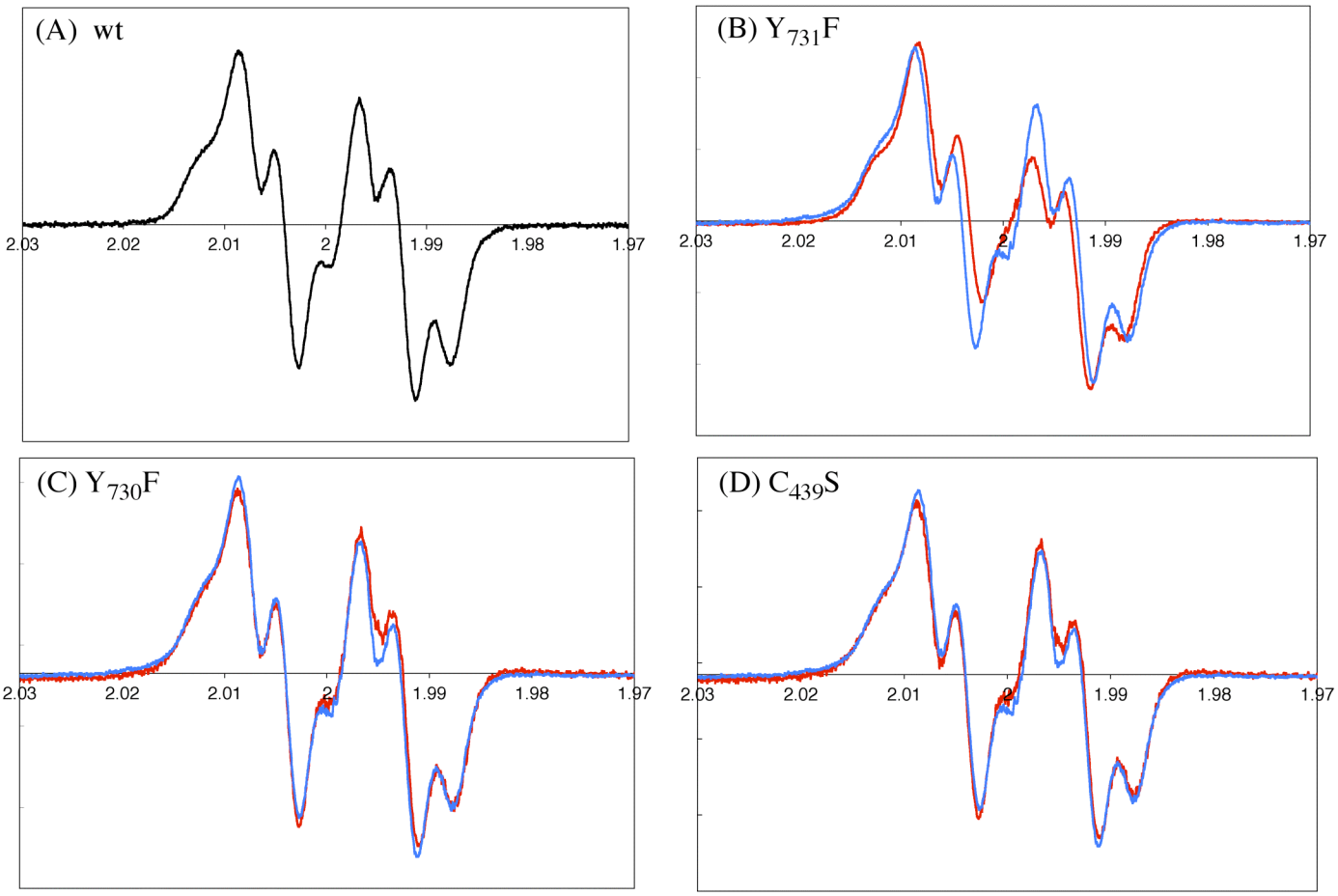

Figure S6. EPR spectra of the new radical observed in (A) wt-α2 (B) $Y_{731}F$-α2, (C) $Y_{730}F$-α2 and (D) $C_{439}S$-α2 after incubation with $Y_{122}NO_2Y$-β2, ATP, and CDP. The EPR spectrum of NO_2Y• was subtracted from each spectrum as described in Methods. The new radical spectrum observed in wt-α2 (blue traces) was overlaid with the spectrum observed in each mutant (red traces).

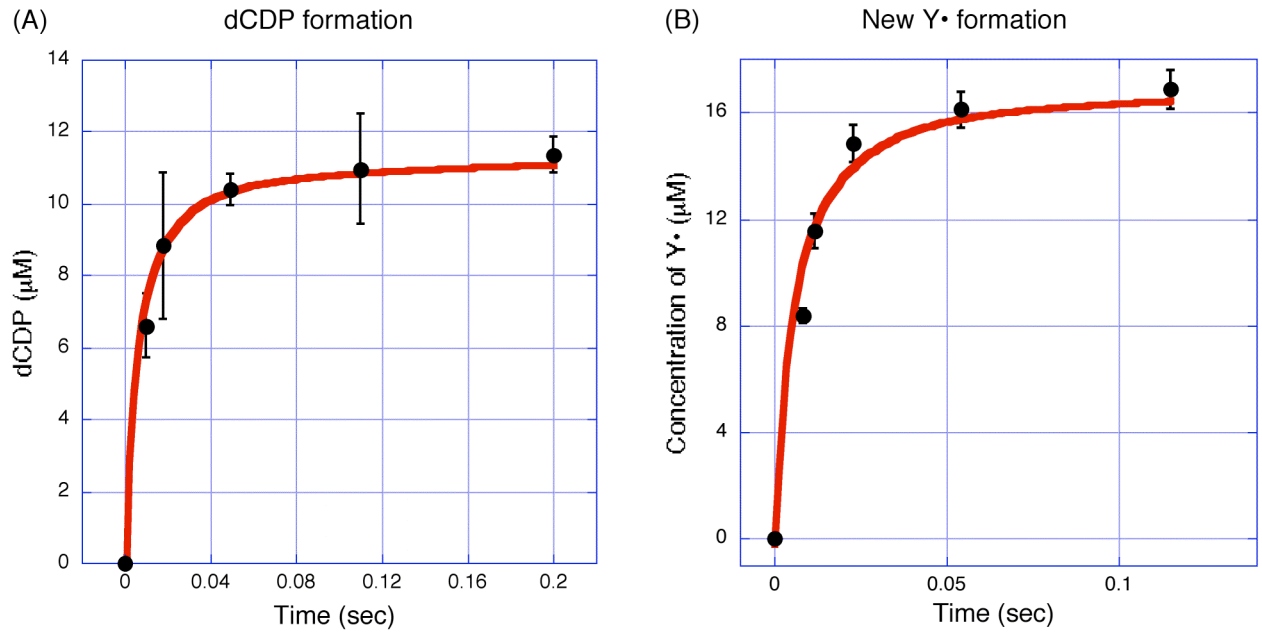

Figure S7. Refitting of experimental data for dCDP formation measured by RCQ (Figure 8) and for new radical formation by RFQ EPR (Figure 11) with tri-exponential fitting using the parameters obtained from the SF experiments measuring phenolate formation (Figure 9B, Table 2). The rate constants (k1 = 283; k2 = 65; and k3 = 10) and amplitudes (A1, 48%; A2, 43%; A3, 9%) are fixed.. The red line is the result of fitting with (A) 11.2 μM and (B) 16.9 μM for total dCDP and Y• formation, respectively.

Reference

(1) Yokoyama, K.; Uhlin, U.; Stubbe, J. *J. Am. Chem. Soc.* **2010**, *132*, 8385-8397.