Supporting Information for

# Addition of Oxygen to the Diiron(II/II) Cluster is the Slowest Step in Formation of the Tyrosyl Radical in the W103Y Variant of Ribonucleotide Reductase Protein R2 from Mouse

Danny Yun, Lana Saleh, Ricardo García-Serres, Brandon M. Chicalese, Young H. An, Boi Hanh Huynh, J. Martin Bollinger, Jr.

**Table S1**: Total "spin" equivalencies and fractions and equivalencies of **X** and Y177• determined through the reconstruction analysis of the EPR spectra (Figure 4).

| reaction time (s) | equiv spin | fraction X | equiv X | fraction Y177• | equiv Y177• | corrrected[a] equiv Y177• |
|---|---|---|---|---|---|---|
| 0.017 | 0.11 | 0.40 | 0.042 | 0.60 | 0.063 | 0.01 |
| 0.074 | 0.18 | 0.49 | 0.089 | 0.51 | 0.092 | 0.02 |
| 0.15 | 0.25 | 0.33 | 0.084 | 0.67 | 0.17 | 0.11 |
| 0.50 | 0.47 | 0.12 | 0.056 | 0.88 | 0.41 | 0.35 |
| 1.0 | 0.59 | 0.05 | 0.029 | 0.95 | 0.56 | 0.50 |
| 5.0 | 0.81 | 0.02 | 0.016 | 0.98 | 0.79 | 0.73 |
| 100 | 1.0 | ~ 0 | ~ 0 | 1.0 | 1.0 | 0.94 |

[a]The [Y177•] in the starting material, which is attributable to a low level of contaminating $O_2$, was subtracted from the actual [Y177•] in each sample.

**Figure S1:** Determination of the drop-line corrected molar absorptivity, $\varepsilon_{416} - (\varepsilon_{410} + \varepsilon_{422})/2$, for Y177• in mouse R2-W103Y. The concentrations of Y177• in samples of R2-W103Y reconstituted to different extents were determined by EPR, as previously described (*1*). The values of the drop-line corrected absorbance $[A_{416} - (A_{410} + A_{422})/2]$ are plotted against [Y177•] for the same samples. The slope of the linear fit gives $\varepsilon_{416} - (\varepsilon_{410} + \varepsilon_{422})/2 = (247 \pm 20)\ M^{-1}cm^{-1}$. This value depends markedly on the resolution of the spectrometer and was found to be (180 ± 15) on the diode array spectrophotometer associated with the stopped-flow apparatus (not shown).

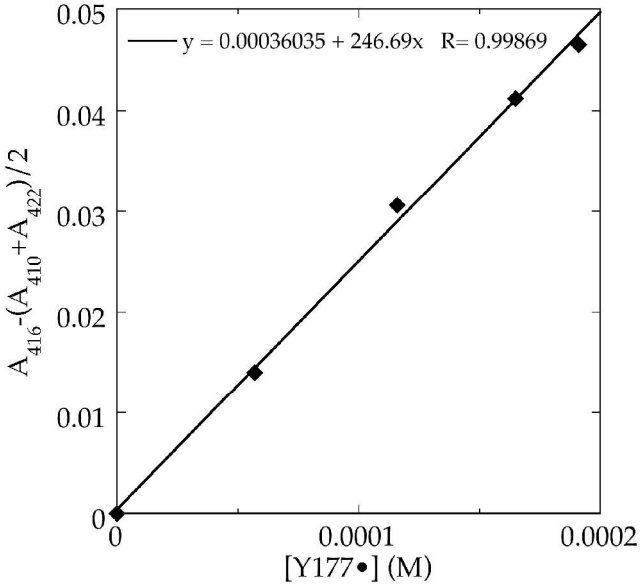
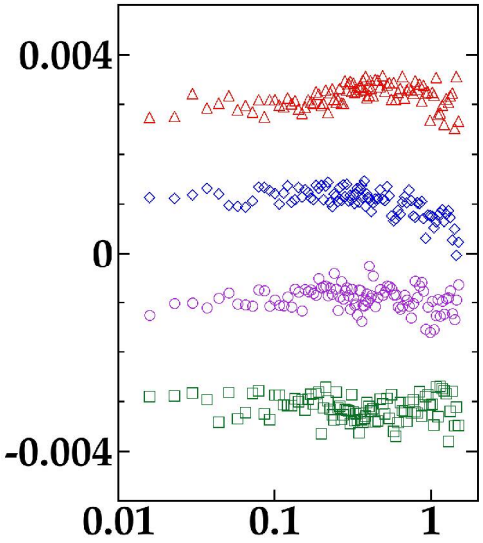


**Figure S2:** Residual errors of the simulations of the inset to Figure 3. The symbol- and color-coding are the same as in Figure 3. Each trace has been offset from zero for ease of visualization.




**REFERENCE**

1. Bollinger, J. M., Jr., Tong, W. H., Ravi, N., Huynh, B. H., Edmondson, D. E., and Stubbe, J. (1994) Mechanism of assembly of the tyrosyl-diiron(III) cofactor of *E. coli* ribonucleotide reductase. 2. Kinetics of the excess $Fe^{2+}$ reaction by optical, EPR, and Mössbauer spectroscopies. *J. Am. Chem. Soc. 116*, 8015-8023.