
(Supporing Information)

S1. Transient absorbance change at 410 nm (pH 7 buffer) of a 4 µM solution of RuC7MbFe3+ upon pulsed excitation at 480 nm. Top trace is the residual from the fit of back electron transfer (Ru3+Fe2+ → Ru2+Fe3+; \( k = 1.7 \times 10^7 \text{ sec}^{-1} \)). The Ru3+Fe2+ state is formed at >108 s^{-1}. In the transient difference spectra (not shown), the absorbance increase due to formation of RuC7MbFe2+ and the corresponding bleach due to loss of RuC7MbFe3+ both occur within 10 ns.

**pH Dependence of Ferryl Formation**

Kinetic traces at 425 nm

difference spectra
S2. Kinetics traces (425 nm) and transient difference spectra after 1 ms for oxidative flash-quench experiments at variable pH. Note that while the rate of porphyrin cation radical (P•+) reduction increases dramatically, the overall yield of ferryl (as measured by the rise over baseline after 1 ms) does not significantly increase with rising pH. This suggests that the competitive reduction by protein residues is likewise pH dependent.

![Graph showing log k vs pH](image)

S3. pH dependence of log k for P•+ reduction (monitored at 425 nm) in RuC7Mb. The slope of the line is ca. 0.5, much less than that predicted for simple ferryl formation.

![Graph showing transient spectra](image)

S4. Comparative transient difference spectra recorded 1 ms after flash-quench (pH 7) for RuC7Mb in the presence and absence of NaF, demonstrating the F⁻ inhibition of ferryl formation.

![Graph showing EPR spectra](image)

S5. Subtraction of the EPR signal in Figure 4B (see main text) from the signal in Figure 4A (taking into account that thawing/refreezing reduces the intensity of the first signal to about 20% of its original value) gives a spectrum (blue trace) with a hyperfine pattern that is strongly reminiscent of a tyrosyl radical. However, this procedure gives only qualitative results, as the actual intensity of the "narrow" signal prior to thawing/refreezing and the contribution of W14' are unknown.