Supporting Information for

Characterization of a Synthetic Peroxodiiron(III) Protein Model Complex by Nuclear Resonance Vibrational Spectroscopy


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Experimental

Materials and Methods: Commercial reagents were used as received without further purification. Air-sensitive manipulations were performed using standard Schlenk line techniques or under a nitrogen atmosphere inside an MBraun drybox. Solvents were saturated with argon and purified by passage through two columns of activated alumina. The \([^{57}\text{Fe}_2(N\text{-EtHPTB})(\text{PhCO}_2)](\text{BPh}_4)_2\) complex was synthesized according to a literature procedure.\(^1\) \(^{18}\text{O}\)-Dioxygen (99%) was obtained from ICON isotopes and used as received.

Preparation of \([^{57}\text{Fe}_2(O_2)(N\text{-EtHPTB})(\text{PhCO}_2)](\text{BPh}_4)_2\) for NRVS. A 50 mM THF solution (0.5 mL) of \([^{57}\text{Fe}_2(N\text{-EtHPTB})(\text{PhCO}_2)](\text{BPh}_4)_2\) was prepared inside the glovebox and added to a septum-sealed vessel. The flask was brought outside of the box and cooled to -78 °C using a dry ice/acetone bath. For the \(^1\text{O}_2\) sample, the diiron(II) solution was injected by syringe with 10 mL of \(^{16}\text{O}_2\). For the \(^1\text{O}_2\) sample, \(^{18}\text{O}_2\) gas was directly transferred to the diiron(II) solution on a high-vacuum manifold. After stirring for ~10 min, 150 μL of the deep blue solution was transferred via syringe, pre-cooled to ensure that the oxygenated solution did not warm up above -30 °C, to an NRVS cell and immediately frozen in liquid nitrogen.

Nuclear Resonance Vibrational Spectroscopy (NRVS)

Data Acquisition. Samples were loaded into \(3 \times 7 \times 1\ mm^3\) (interior dimensions) Lucite cuvettes wrapped with Mylar tape. \(^{57}\text{Fe}\) NRVS spectra were recorded using published procedures\(^2\) at the BL09XU beamline at SPring-8, Japan. The flux was on the order of \(~1.2 \times 10^9\) photons/sec in a 0.9 meV bandpass. During NRVS measurements, samples were maintained at low temperatures using a liquid He cryostat (head temperature < 10 K). The real sample temperatures were calculated from the ratio of Stokes to anti-Stokes intensity by the expression\(^3\)

\[
S(-E) = S(E)e^{(-E/kT)}
\]

and determined to be at ~45-60 K. Nuclear fluorescence and delayed Fe K
fluorescence were recorded with an avalanche photodiode (APD) array. Each scan took about 50 min, and all scans were added and normalized to the intensity of the incident beam using the program PHOENIX. Spectra were recorded between 0 and 600 cm\(^{-1}\). The number of scans acquired for each sample is as follows: one for \(\text{I}\), five for \(\text{I} \cdot ^{16}\text{O}_2\), and three for \(\text{I} \cdot ^{18}\text{O}_2\).

**Empirical Force Field Normal Mode Analysis.** Empirical normal mode calculations were performed using the program VIBRATZ\(^5\),\(^6\) which uses the Urey-Bradley force field. Analysis of \(\text{I}\) utilized only the Cartesian coordinates of the \(\{\text{Fe}_2\text{N}_6\text{O}(\mu-\text{PhCO}_2)\}\)\(^{2+}\) core to reduce the complexity of the simulation. Since the Fe–N/O modes are “uncoupled” in this virtual model, differences in the calculated peak intensities compared to those observed for \(\text{I}\) are not meaningful because they depend on the precise stretching force constants and bond angles of all the atoms involved in the vibration. The normal mode calculations for \([\text{Fe}_2(\mu-\text{O}_2)(\text{N}-\text{EtHPTB})(\text{PhCO}_2)]^{2+}\) (\(\text{I} \cdot \text{O}_2\)) employed the Cartesian coordinates of the \(\{\text{Fe}_2\text{N}_6\text{O}(\mu-\text{O}_2)(\mu-\text{PhCO}_2)\}\)\(^{2+}\) core from the X-ray structure of \([\text{Fe}_2(\mu-\text{O}_2)(\text{Ph-bimp})(\text{PhCO}_2)\text{]}^{2+}\) \(\text{II} \cdot \text{O}_2\), Chart 2\)\(^7\) using a \(C_s\) point group. It was necessary to use \(\text{II} \cdot \text{O}_2\) as an approximate model for \(\text{I} \cdot \text{O}_2\) because the structure of \(\text{I} \cdot \text{O}_2\) is not available. The calculated results are intended to provide a qualitative description of the modes that involve significant motion of the \(\{\text{Fe}_2(\text{O}_2)\}\)\(^{4+}\) core, rather than a complete description of the full molecule. Because the simulated frequencies do not take into account the bonding interactions of the \(\text{N}-\text{EtHPTB}\) ligand with the diiron centers, the calculated intensities are not an exact match to those observed in the NRVS of \(\text{I} \cdot \text{O}_2\). Nevertheless, the modes at energies greater than 350 cm\(^{-1}\) can be assigned unequivocally to the \(\{\text{Fe}_2(\text{O}_2)\}\)\(^{4+}\) unit because they are well separated from those in the main Fe–N/O manifold, ranging from \(~150-350\) cm\(^{-1}\).
**Figure S1.** Resonance Raman spectra of \([^{57} \text{Fe}_2(\mu-^{16}\text{O}_2)(\text{N-EtHPTB})(\text{PhCO}_2)]^{2+}\) (1·^{16}\text{O}_2, black) and \([^{57} \text{Fe}_2(\mu-^{18}\text{O}_2)(\text{N-EtHPTB})(\text{PhCO}_2)]^{2+}\) (1·^{18}\text{O}_2, blue) as previously reported.\(^1\) In panel A, the red spectrum represents the 1·^{18}\text{O}_2 sample before a 1·^{16}\text{O}_2 impurity was subtracted. The lower energy region, in panel B, does not exhibit any clear isotopically shifted resonances. Compound 1·^{16}\text{O}_2 has a broad absorption maximum centered at \(~590\) nm. The data were obtained with 647 nm laser excitation at 110 K. The sample undergoes radiative decay when excited at wavelengths below 514 nm.
Figure S2. Difference plots ($^{18}$O$_2$ minus $^{16}$O$_2$) of the experimental (A, top) and simulated (B, bottom) NRVS. The full NRVS of $^{16}$O$_2$ and $^{18}$O$_2$ are shown in Figure 1.
Addition to Fig. 1 caption (main text):

Panel A: Blue spectrum (selected peaks) = 182, 224, 240, 298, and 321 cm$^{-1}$. Red spectrum (selected peaks) = 192, 246, 295 cm$^{-1}$. Panel B: blue spectrum (selected peaks) = 190, 280, 338, 467, 480 cm$^{-1}$; red spectrum (selected peaks) = 176, 225, 264, 325, 471, 579 cm$^{-1}$. Panel C: blue spectrum (selected peaks) = 186, 241, 281, 311, 446, 458 cm$^{-1}$; red spectrum (selected peaks) = 175, 224, 262, 313, 452, 551 cm$^{-1}$.
References

(4) Sturhahn, W. Hyperfine Interact. 2000, 125, 149-172.