

Supporting Information

EPR-ENDOR Characterization of (^{17}O , ^1H , ^2H) Water in Manganese Catalase and Its Relevance to the Oxygen-Evolving Complex of Photosystem II

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Running Title: Isotopically Labeled Water in Manganese Catalase and Photosystem II

Keywords: ENDOR, Manganese Catalase, OEC, PSII, electronic structure

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Protocols A and B for Preparation of Concentrated PSII Highly Enriched in

Exchangeable H₂¹⁷O

- Protocol **A** shows the preparation of EPR or ENDOR tubes containing highly concentrated PS II membranes. 60 μ l 2 mg [Chl]/ml of PSII membranes were loaded into the EPR or ENDOR tube, and then the supernatant was removed after centrifugation by 20,817 g for 12 min, as depicted in steps (a) to (b). The same amount of PSII membranes was then loaded on top of the pre-existing pellet, homogenized with teflon tubing gently, and then centrifuged again followed by removal of supernatant, steps (b) to (c). Steps (a) to (c) were repeated another 5 times until the height of PSII membranes in the tube came to 1 cm. The final [Chl] concentration of PS II membranes in the tube was estimated to be 10-20 mg/ml. The initial PSII membranes had an O₂-evolution activity of 500 [μ mol of O₂/ (mg of Chl)/h].
- Protocol **B** shows the preparation of highly concentrated PSII membranes enriched with H₂¹⁷O: The tube with highly concentrated PSII in panel (A) was treated with 10 μ l of H₂¹⁷O (84 % atomic enrichment in ¹⁷O, Isotec.) and homogenized with teflon tubing by steps (1) to (2). The supernatant was removed after centrifugation at 20,817 g for 12 min, as shown in steps (2) to (3). Steps (1) through (3) were repeated twice, and for dark adaptation of the S₁ state, the tube was incubated in the dark for 24 hours at 4 °C. After the third iteration the supernatant had approximately 74% enrichment in ¹⁷O. This approximation was based on the ¹⁸O gas chromatography-mass spectrometry (GC-MS) analysis done after performing the same exchange preparation with H₂¹⁸O, instead of H₂¹⁷O, to measure the enrichment in ¹⁸O in the supernatant.

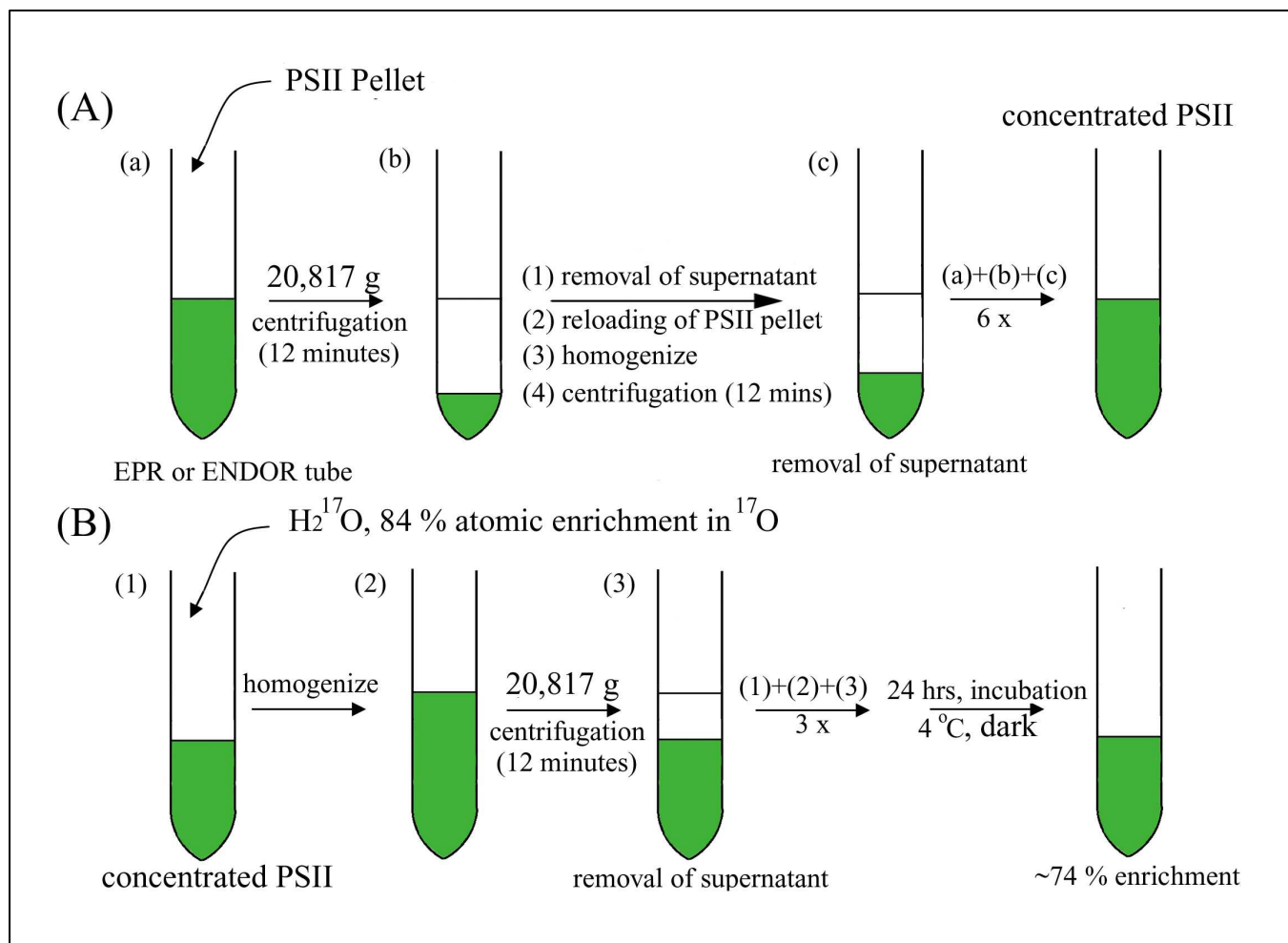


Figure 1S. Protocols A and B for Preparation of Concentrated PSII Highly Enriched in Exchangeable H_2^{17}O

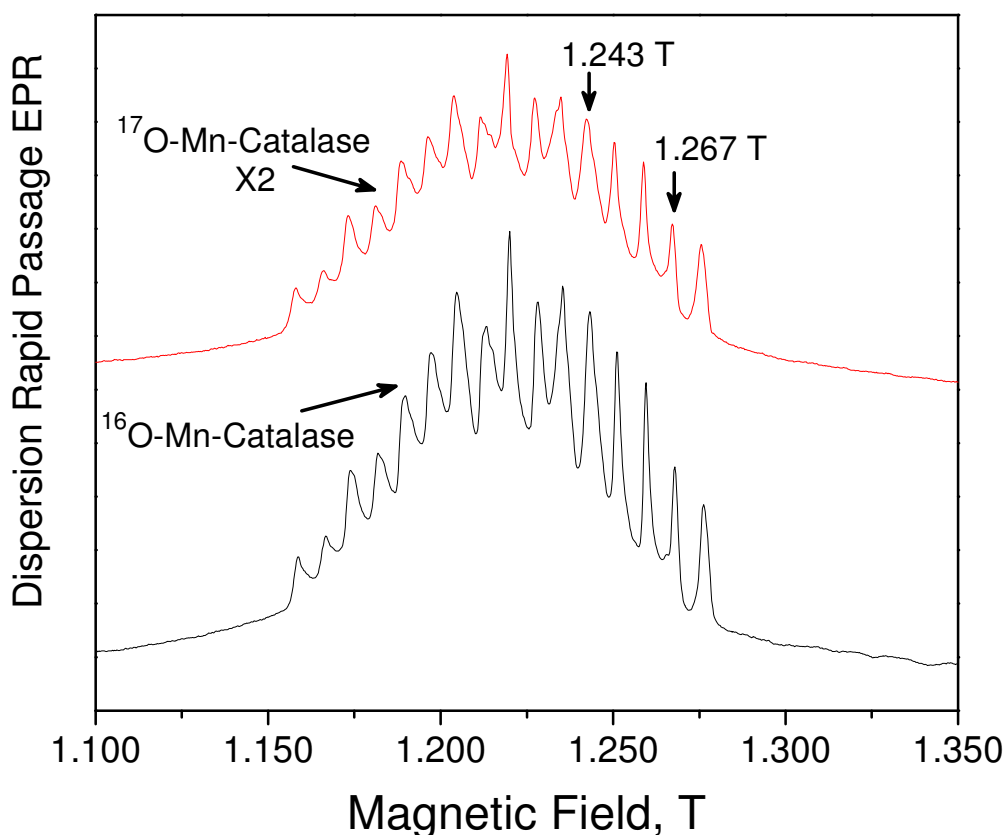


Figure 2S. Comparison of Q-band rapid passage EPR signals from the MnCat prepared with ^{17}O -water and with ^{16}O -water. Conditions were adiabatic rapid passage, $T = 2.1\text{ K}$, microwave power = $0.22\text{ }\mu\text{W}$, 100 kHz field modulation = 5 G p.t.p., a system time constant = 89 ms, Q-band EPR frequency of 34.10 GHz. The arrow at 1.243 T was where most of the ENDOR data were taken. ENDOR data shown in **Figure 3S** were also taken for comparison at 1.267 T.

It was determined that we could obtain a better passage signal and better ENDOR if we made a 50:50 glycerol glass. There was no evidence from any Mn^{II} signal that we had reductively degraded the $\text{Mn}^{\text{III}}\text{-Mn}^{\text{IV}}$ center in the MnCat. The rapid passage signal above in **Figure 2S**, which is desirable for field-modulated CW ENDOR, gives the appearance of an absorption signal and when integrated gives us an estimate of the relative number of spins in the sample.

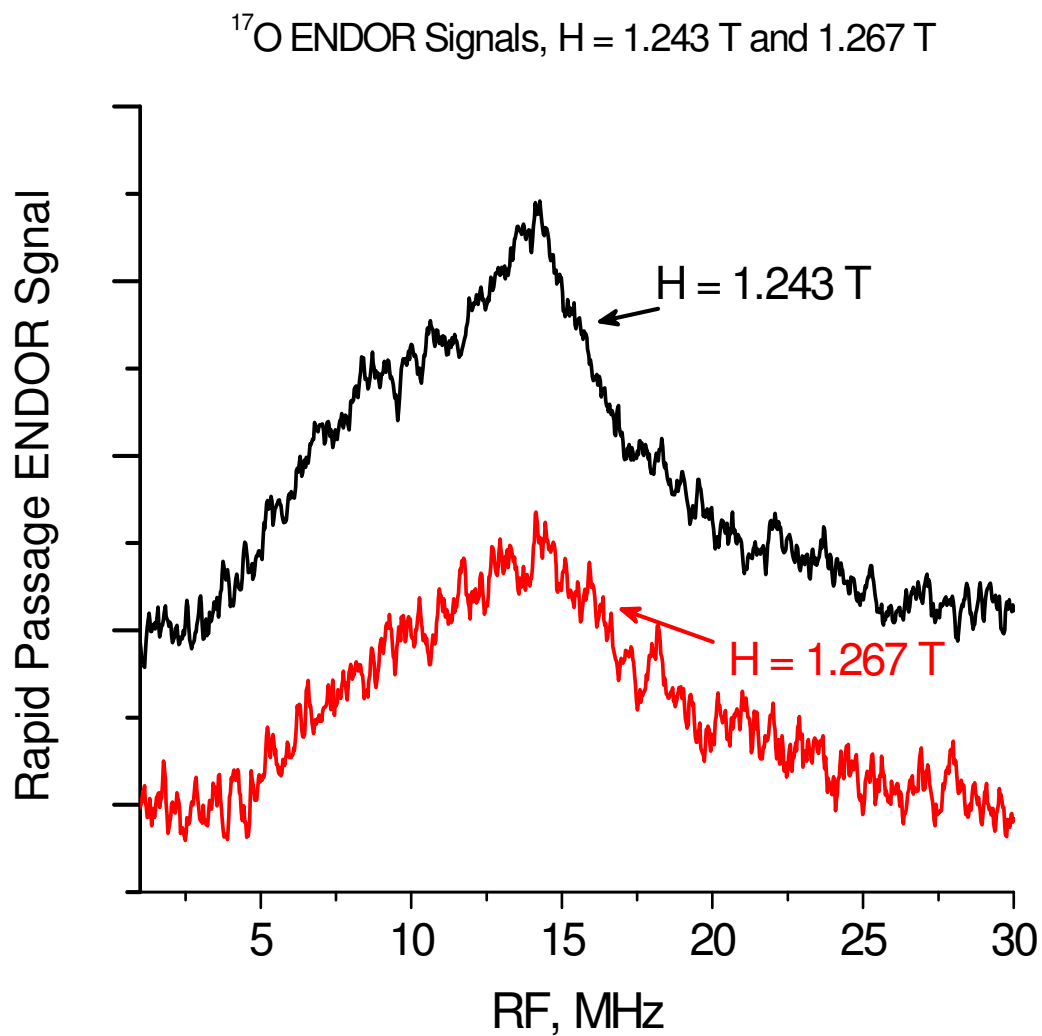


Figure 3S. This figure compares ENDOR spectra obtained at 1.243 and 1.267 T under the conditions of **Figure 3A** in the text from a MnCat sample prepared with a two hour exchange of ¹⁷O-water.

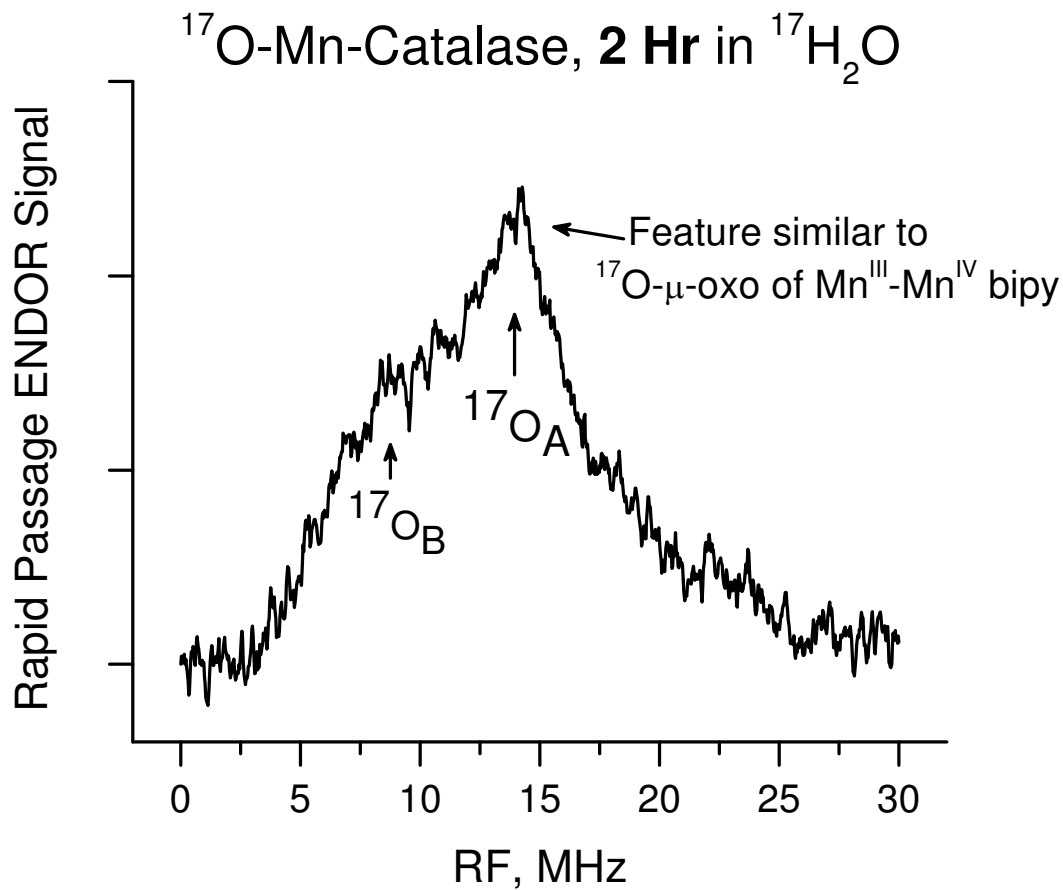


Figure 4S. This figure provides the CW Q-band ENDOR of MnCat exchanged in ^{17}O -water for 2 hours. The conditions for this figure are identical to those in **Figure 3A** except that a two-fold higher RF power was used. The feature labeled $^{17}\text{O}_A$ is assigned to a μ -oxo oxygen and the features in the vicinity of $^{17}\text{O}_B$ are assigned to a terminal water.

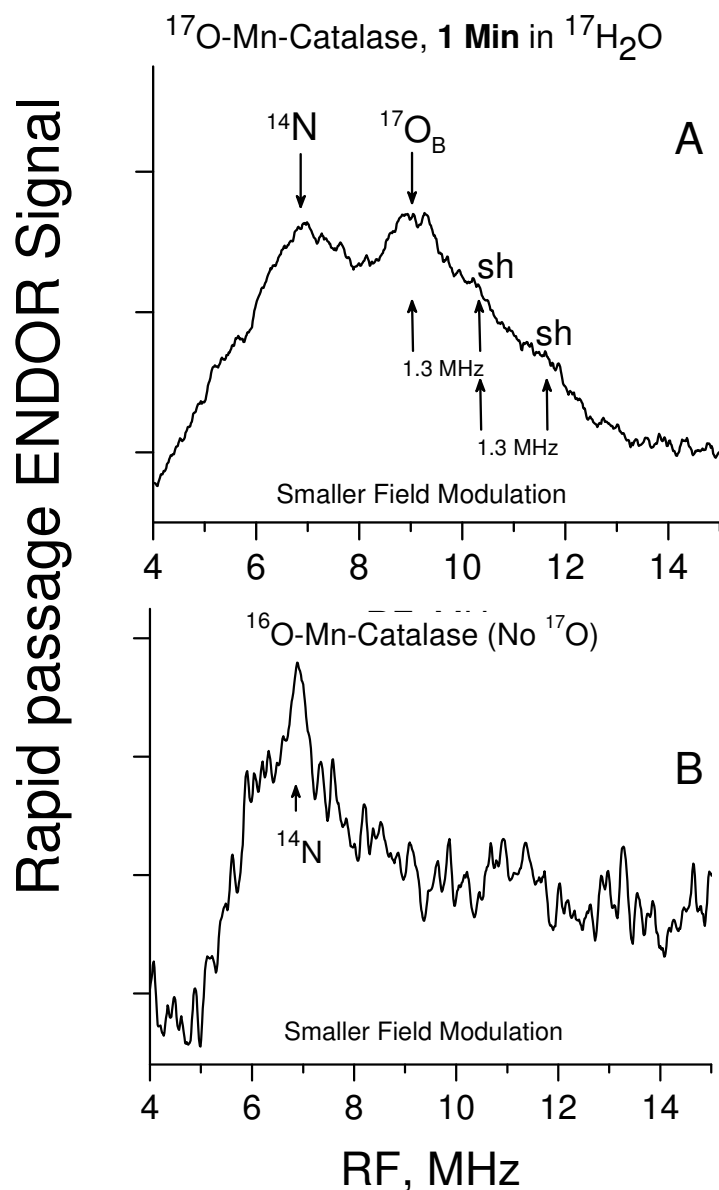


Figure 5S. Spectrum **A** shows the ENDOR of MnCat exchanged in ^{17}O -water for 1 minute, which is the same spectrum as the inset to **Figure 3B** obtained with 2.5 G field modulation. The spectrum was the result of 1100 5 s sweeps. Spectrum **B** was obtained from the MnCat sample exchanged with standard H_2^{16}O and with the same experimental conditions, except that this signal was obtained from 600 sweeps. The instrument stability was unfortunately sub-par in the acquisition of control spectrum **B**, but the signal attributed to ^{17}O is clearly not present in the ^{14}N , H_2^{16}O spectrum.

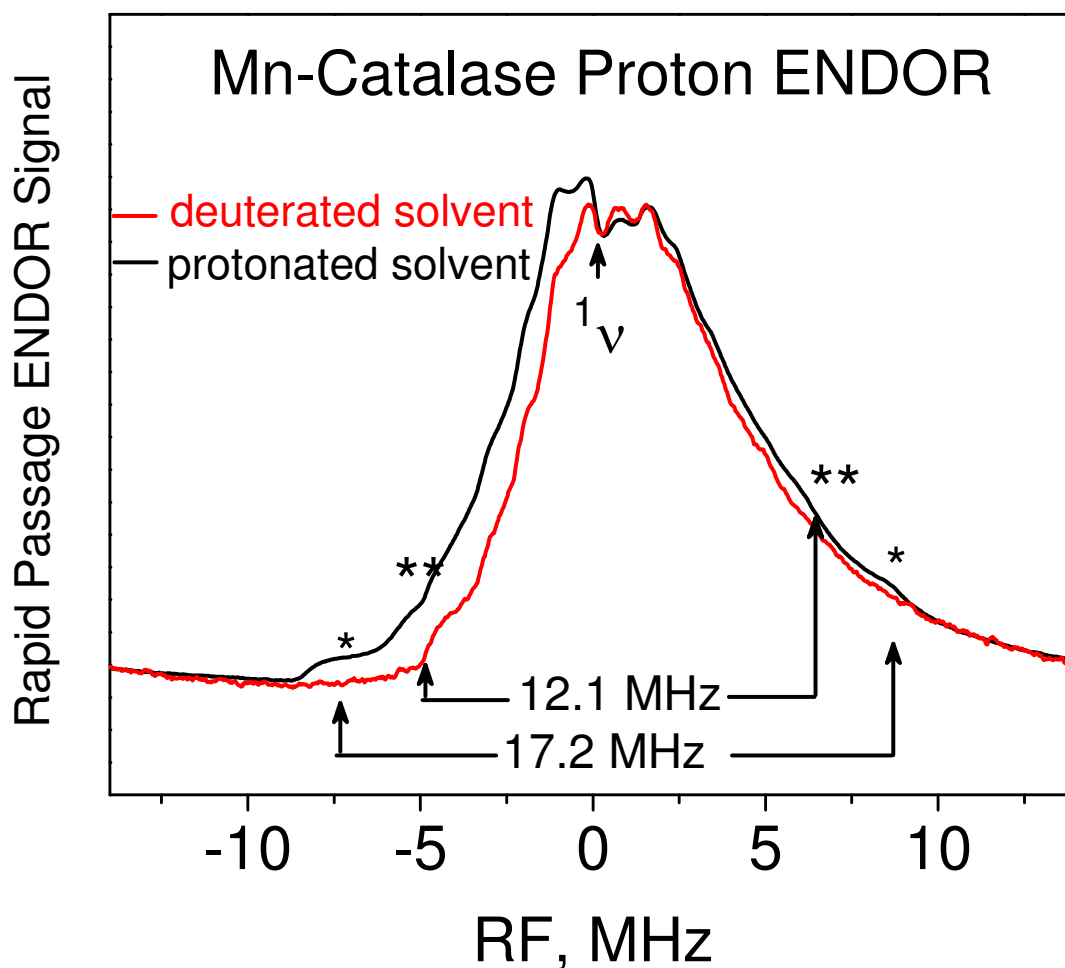


Figure 6S. These are the rapid passage absorption-like ENDOR signals of MnCat in protonated and deuterated solvent. The conditions are as described for proton ENDOR in the legend to **Figure 4** in the text. These spectra are centered at the free proton frequency, ν_{H} . The derivative spectra in **Figure 4** were obtained from these spectra by taking a numerical first derivative using Origin 7.0.

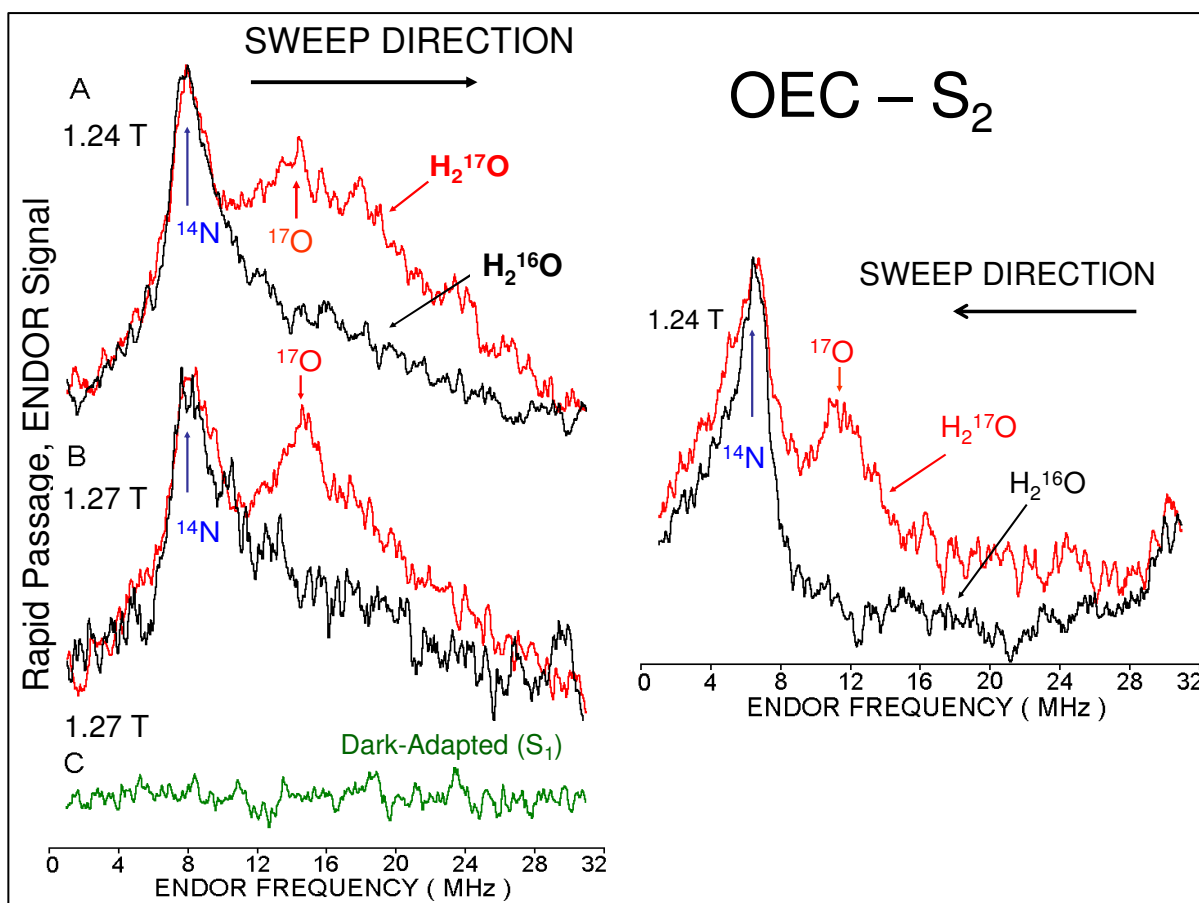
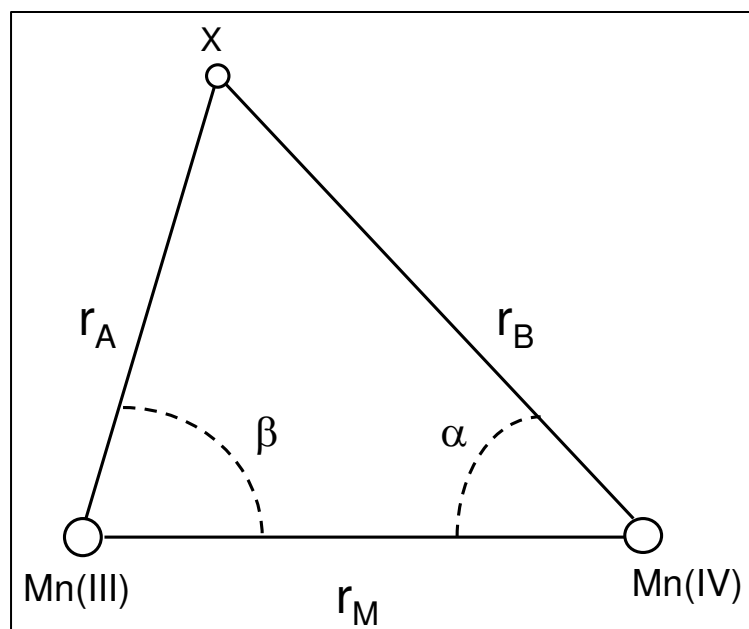


Figure 7S. This provides a comparison of the ENDOR spectra of OEC – S₂ with frequency sweeps in the upward and the downward directions. The average frequency for the ¹⁷O feature was 12.8 MHz. The average frequency of the ¹⁴N feature was 7.3 MHz. The ENDOR frequencies of ENDOR features are slightly increased in the direction of the sweep.

Extended Dipole Interaction

We recapitulate the details for computing the point dipolar contribution to the anisotropic hyperfine interaction for a nucleus such as a ^1H , ^2H , or ^{17}O in the vicinity of the antiferromagnetically coupled Mn(III)-Mn(IV) pair. We largely follow the methodology presented by Randall et al.,¹ originally used to explain the large anisotropic hyperfine coupling to terminal water protons ligated to Mn(III). We use this methodology in our work here to calculate dipolar contributions for terminal water protons and deuterons and to calculate the approximate point dipolar contribution to the ^{17}O $\mu\text{-oxo}$ oxygen.



Scheme 1S. The geometrical parameters are shown for the extended point-dipole model used to estimate proton and ^{17}O anisotropic hyperfine couplings.

Randall et al.¹ diagonalized the combined dipolar Hamiltonian arising from the interaction of a nuclear moment with spin on both the Mn(III) and Mn(IV). The result is a rhombic hyperfine tensor, whose components are:

$$A_{xxdip} = \frac{1}{2}(2T_A - T_B - 3 \mathring{A}); A_{yydip} = -(2T_A - T_B); A_{zzdip} = \frac{1}{2}(2T_A - T_B + 3 \mathring{A}) \quad (S.1)$$

$$\mathring{A} = [4T_A^2 + T_B^2 - 4T_A T_B \cos(2\mathring{\alpha} + 2\mathring{\beta})]^{1/2} \quad (S.1a)$$

$$T_A = (g_e \mathring{g}_e g_n \mathring{g}_n) / (hr_A^3); T_B = (g_e \mathring{g}_e g_n \mathring{g}_n) / (hr_B^3) \quad (S.2)$$

Where $(g_e \mathring{g}_e g_n \mathring{g}_n) / h = 79.3 \text{ MHz} \cdot \text{\AA}^3$ for ^1H and $(g_e \mathring{g}_e g_n \mathring{g}_n) / h = -10.8 \text{ MHz} \cdot \text{\AA}^3$ for ^{17}O .

r_B and $\mathring{\alpha}$ are given as

$$r_B = [r_A^2 + r_{Mn}^2 - 2 r_A r_{Mn} \cos \mathring{\alpha}]^{1/2}; \mathring{\alpha} = \arcsin[r_A \sin \mathring{\beta} / r_B] \quad (S.3)$$

For the $\mathring{\alpha}$ -oxo ^{17}O ($^{17}\text{O}_A$) of MnCat, with $r_M = 2.72 \text{ \AA}$, $r_A = r_B = 1.82 \text{ \AA}$, $\mathring{\alpha} = \mathring{\beta} = 42^\circ$, we calculate the anisotropic dipolar character to the ^{17}O hyperfine tensor $^{17}\mathbf{A}_{dip}$ as $^{17}A_{xxdip}$, $^{17}A_{yydip}$, and $^{17}A_{zzdip} = +7.1, +1.8, -8.9 \text{ MHz}$

For the terminal ^{17}O -water ($^{17}\text{O}_B$) of MnCat a dipolar calculation of the hyperfine coupling of the ^{17}O -water ($^{17}\text{O}_B$) can be performed, assuming the 2.72 \AA Mn-Mn distance for Mn(III)-Mn(IV) di- $\mathring{\alpha}$ -oxo complexes² and the average 2.09 \AA Mn(III)-O bond length and $\mathring{\alpha} = 102.8^\circ$ from the *L. plantarum* crystal structure, 1JKU.³ The purely dipolar coupling to the oxygen atom from ^{17}O -water ($^{17}\text{O}_B$) would, thus, be $^{17}A_{xxdip}$, $^{17}A_{yydip}$, $^{17}A_{zzdip} = 2.19, 2.18, -4.37 \text{ MHz}$, numbers comparable with the coupling measured by ENDOR from $^{17}\text{O}_B$ in **Figures 3B and 5S**.

For the terminal water proton of MnCat oriented in a position to hydrogen bond to the carboxylate of Glu178, $r_M = 2.72 \text{ \AA}$, $r_A = 2.72 \text{ \AA}$, $r_B = 3.95 \text{ \AA}$, $\mathring{\alpha} = 93.8^\circ$, $\mathring{\beta} = 43.3^\circ$. The dipolar coupling to this proton is $^1A_{xxdip}$, $^1A_{yydip}$, $^1A_{zzdip} = -8.6, -6.6, +15.2 \text{ MHz}$.

^{17}O -Mn-Catalase, **2 Hr** in $^{17}\text{H}_2\text{O}$

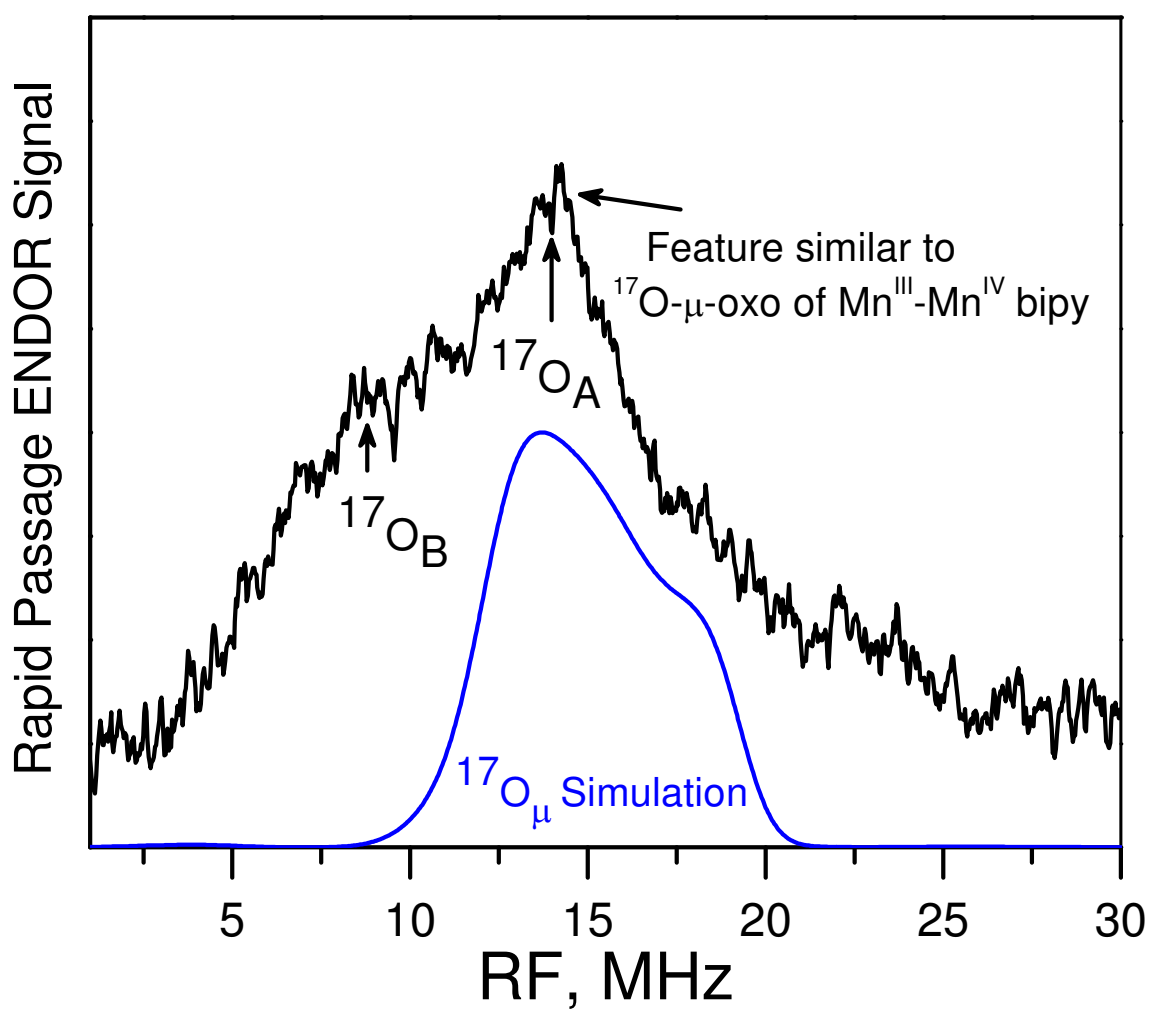


Figure 8S. This figure compares experimental spectrum of ^{17}O -enriched MnCat from **Figure 3**, where the protein was subject to a several hour exchange of ^{17}O -water, to the simulated ^{17}O - δ -oxo ENDOR spectrum in blue from δ -oxo ^{17}O . This simulation was performed using comprehensive software package of EasySpin 3.1.6,⁴ with details provided on the next page.

The purpose of the ^{17}O - δ -oxo ENDOR simulation in **Figure 8S** was to demonstrate the overall broad ENDOR line shape due to the expected anisotropic hyperfine coupling described on pp S11&S12. The simulation of **Figure 8S** was based on an overall ^{17}O hyperfine tensor of $^{17}\text{A}_{xx} = -8.5$ MHz, $^{17}\text{A}_{yy} = -13.8$ MHz, and $^{17}\text{A}_{zz} = -24.5$ MHz which has a powder pattern peak near 14 MHz like the experimentally measured ^{17}O ENDOR pattern. (Such a hyperfine tensor would arise from the combination of the calculated anisotropic dipolar tensor from p. S12 of $^{17}\text{A}_{xx\text{dip}}, ^{17}\text{A}_{yy\text{dip}}, ^{17}\text{A}_{zz\text{dip}} = +7.1, +1.8, -8.9$ MHz and an isotropic hyperfine interaction of $^{17}\text{A}_{\text{iso}} = -15.6$ MHz.) The value of $^{17}\text{A}_{\text{iso}}$ is a very rough estimate. Minor broadening of the line shape can be affected by the ^{17}O quadrupole coupling, which is not known at this time for δ -oxo ^{17}O . The quadrupole couplings were arbitrarily chosen here to be those of water.

Specific Details of ^{17}O - δ -oxo ENDOR Simulation

1) The underlying EPR spectrum was first fit by the following set of EPR parameters from Schäfer *et al.*⁵ for oxidized MnCat from *Thermus.thermophilus* :

$$g_x, g_y, g_z = 2.0048, 2.004, 1.9876$$

$$\text{for Mn(III): } A_{xx}, A_{yy}, A_{zz} = -434, -412, -300 \text{ (MHz)}$$

$$P_{xx}, P_{yy}, P_{zz} = -0.0619, -0.0756, +0.1375 \text{ (MHz)}$$

$$\text{for Mn(IV): } A_{xx}, A_{yy}, A_{zz} = 224, 232, 255 \text{ (MHz)}$$

$$P_{xx}, P_{yy}, P_{zz} = -0.0813, -0.0438, +0.125 \text{ (MHz)}$$

$\lambda_{\text{EPR}} = 34.087 \text{ GHz}$, Field = 1241.3 mT, EPR Linewidth, 1.5 mT, FWHM

2) The ^{17}O ENDOR simulation was based the following ^{17}O hyperfine information:

^{17}O δ -oxo hyperfine coupling $^{17}A_{xx}$, $^{17}A_{yy}$, $^{17}A_{zz} = -8.5, -13.8, -24.5$ (MHz). (This hyperfine tensor arises from the combination of an isotropic hyperfine interaction of $^{17}A_{\text{iso}} = -15.6$ MHz and an anisotropic dipolar tensor of $^{17}A_{xx\text{dip}}$, $^{17}A_{yy\text{dip}}$, $^{17}A_{zz\text{dip}} = +7.1, +1.8, -8.9$ MHz.)

^{17}O quadrupole tensor of $^{17}P_{xx}$, $^{17}P_{yy}$, $^{17}P_{zz} = 0.33 -0.33, 0$ (MHz). An isotropic ENDOR line width of 1.75 MHz FWHM.

References

1. Randall, D. W.; Gelasco, A.; Caudle, M. T.; Pecoraro, V. L.; Britt, R. D., ESE-ENDOR and ESEEM Characterization of Water and Methanol Ligation to a Dinuclear Mn(III)Mn(IV) Complex. *J. Am. Chem. Soc.* **1997**, 119, 4481-4491.
2. Larson, E.; Lah, M. S.; Li, X.; Bonadies, J. A.; Pecoraro, V. L., Manganese-manganese separations in oxide- and alkoxide-bridged complexes: correlation of structure with ligand type and number. *Inorg. Chem.* **1992**, 31, 373-378.
3. Barynin, V. V.; Whittaker, M. M.; Antonyuk, S. V.; Lamzin, V. S.; Harrison, P. M.; Artymiuk, P. J.; Whittaker, J. W., Crystal structure of manganese catalase from *Lactobacillus plantarum*. *Structure* **2001**, 9, 725-738.
4. Stoll, S.; Schweiger, A., EasySpin, a comprehensive software package for spectral simulation and analysis in EPR. *J Magn Reson* **2006**, 178, 42-55.
5. Schäfer, K.-O.; Bittl, R.; Lenzian, F.; Barynin, V.; Weyhermüller, T.; Wieghardt, K.; Lubitz, W., Multifrequency EPR Investigation of DimanganeseCatalase and Related Mn(III)Mn(IV) Complexes. *J Phys Chem B* **2003**, 107, 1242-1250.
6. Glotfelty, H. W., A ^{17}O study of cobalt and manganese hydrated complexes of some double nitrates. Ph.D., University of Kansas, Lawrence, KS, 1978.

