Supporting Material

for

Probing the oxyferrous and catalytically active ferryl states of *Amphitrite ornata* dehaloperoxidase by cryoreduction and EPR/ENDOR spectroscopy. Detection of Compound I.

Roman Davydov,[†,§] Robert L. Osborne,[‡,§] Muralidharan, Shanmugam [†] Jing Du,[‡] John H. Dawson[‡,¶*], and Brian M. Hoffman[†,*]

[†]Department of Chemistry, Northwestern University, Evanston, Illinois 60208
[‡]Department of Chemistry and Biochemistry and [¶]School of Medicine
University of South Carolina, Columbia, South Carolina 29208
[§]These authors contributed equally.

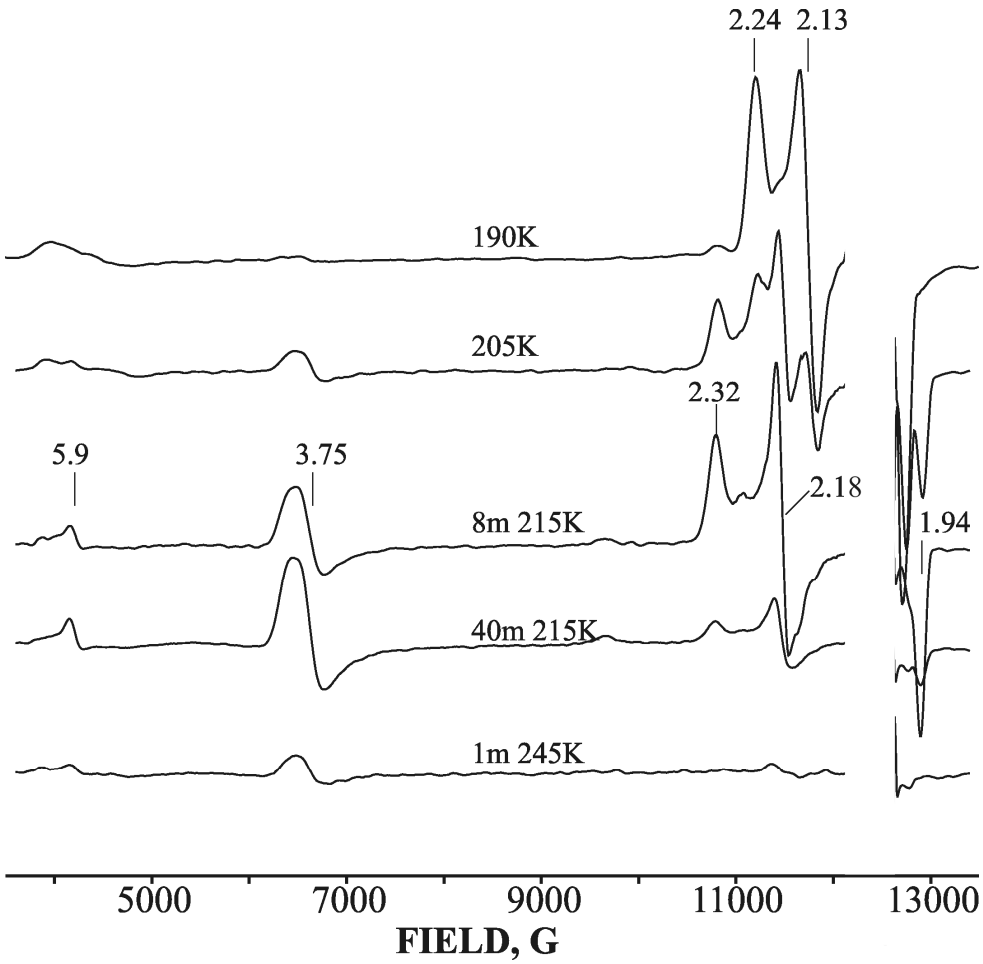

Figure S1. 2 K Q-band EPR spectra of cryoreduced oxyferrous DHP in 20% ethylene glycol/ 0.05 M Tris buffer, pH 9, annealed at indicated temperatures and times. Instrument conditions: T = 2 K; modulation amplitude = 1G; rf power = 20 db; microwave frequency = 35.03 GHz.

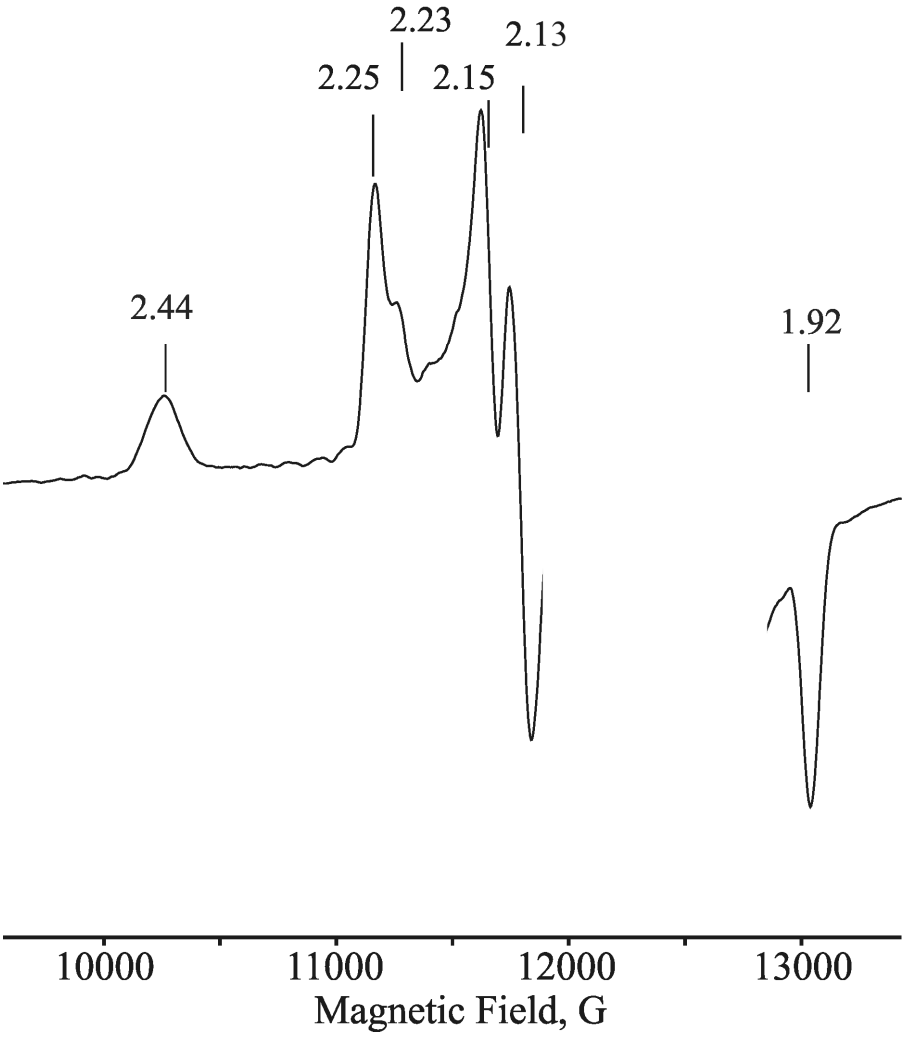

Figure S2. EPR spectrum of cryoreduced oxyferrous DHP in 20% glycerol/0.05 M Tris buffer, pH 9, annealed at 250 K and irradiated at 77 K with a dose of 2.6 Mr. Instrument conditions are the same as described in Figure S1.

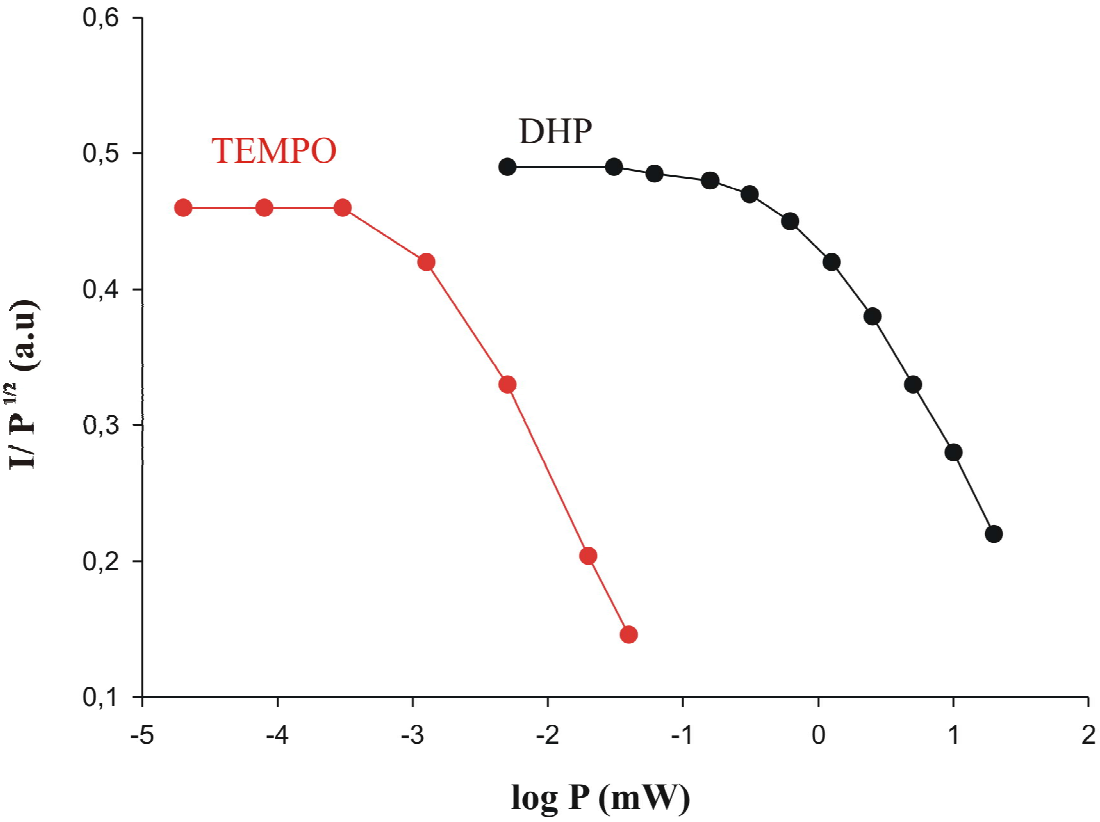

Figure S3. Power dependences of intensity of EPR signals of TEMPO and of the radical intermediate trapped during rapid freeze-quench of reaction between ferric DHP and 2.5 mM H_2O_2 (see Figure 4) at 18 K.

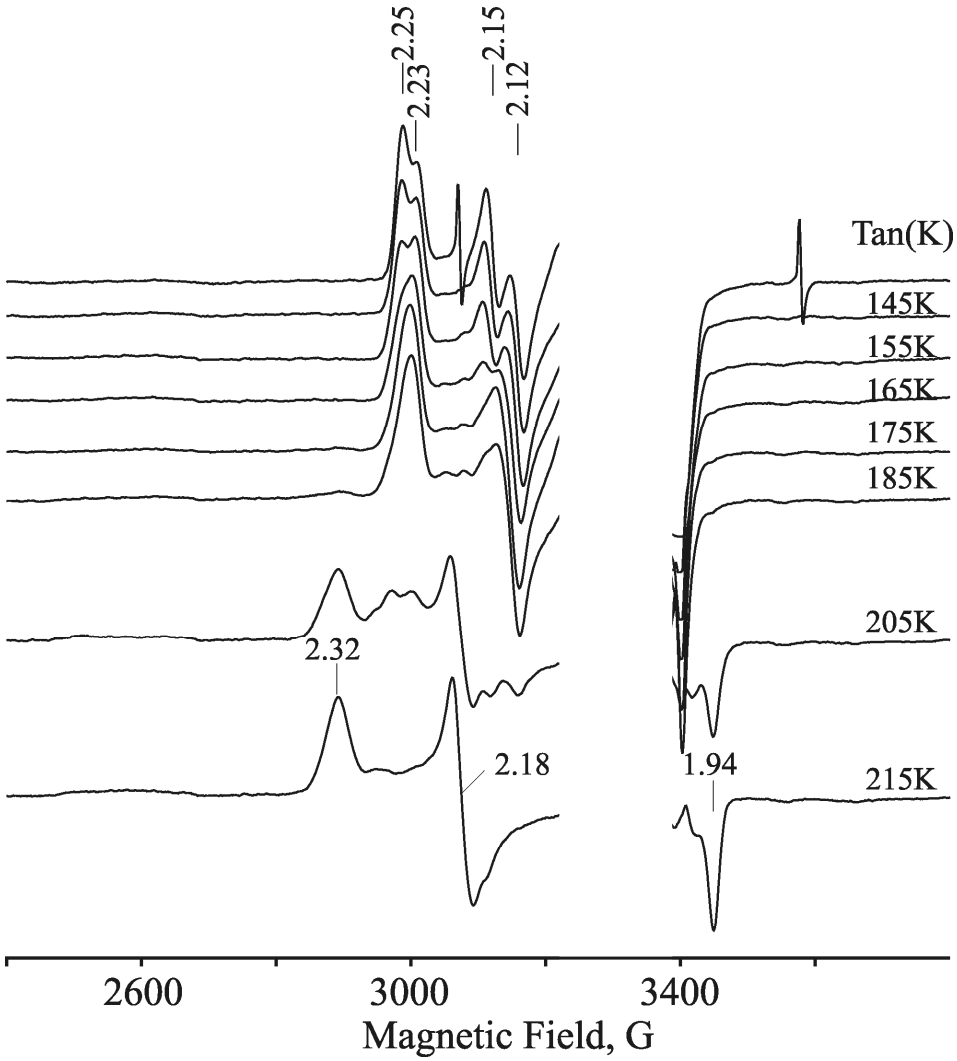

Figure S4. EPR spectra of cryoreduced oxyferrous DHP in the presence of 20 mM F_3PhOH in 16% glycerol/0.05 M Tris buffer, pH 9, annealed at indicated temperatures. Instrument conditions: T = 28 K; modulation amplitude = 5 G; rf power = 10 mW; microwave frequency = 9.378 GHz.

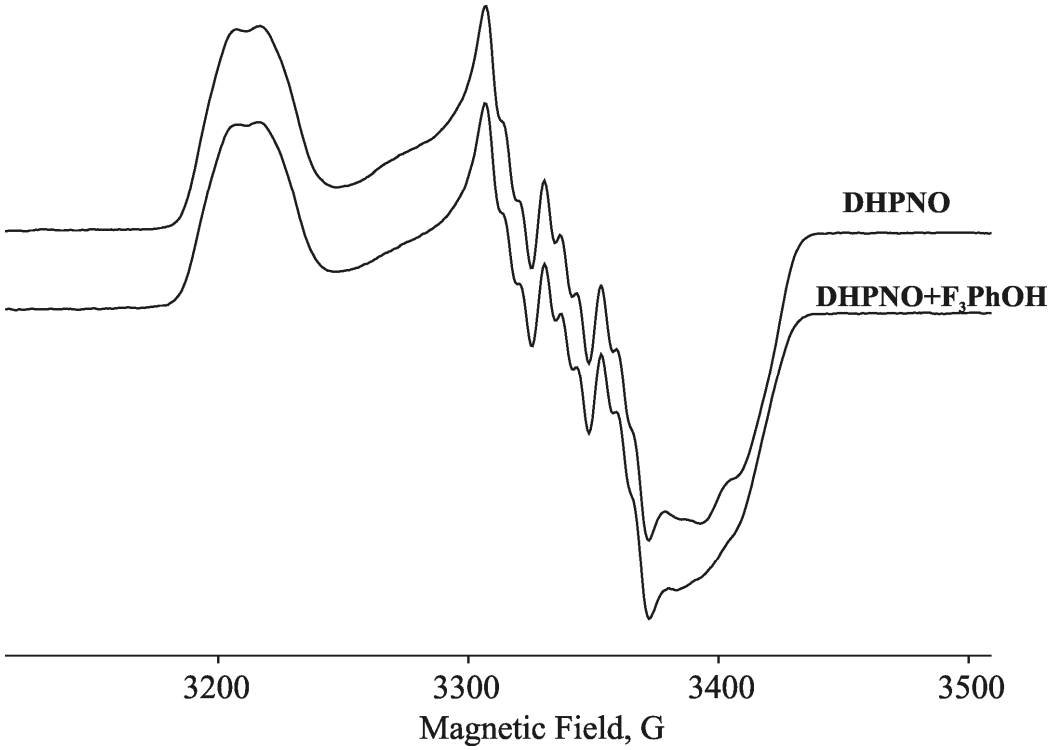

Figure S5. Effect of 20 mM F_3PhOH on EPR spectra of the ferrous-NO DHP complex in 16% glycerol/0.1 M potassium phosphate buffer, pH 7.5. Instrument conditions: T = 34 K; modulation amplitude = 2.5 G; rf power = 10 mW; microwave frequency = 9.375 GHz.

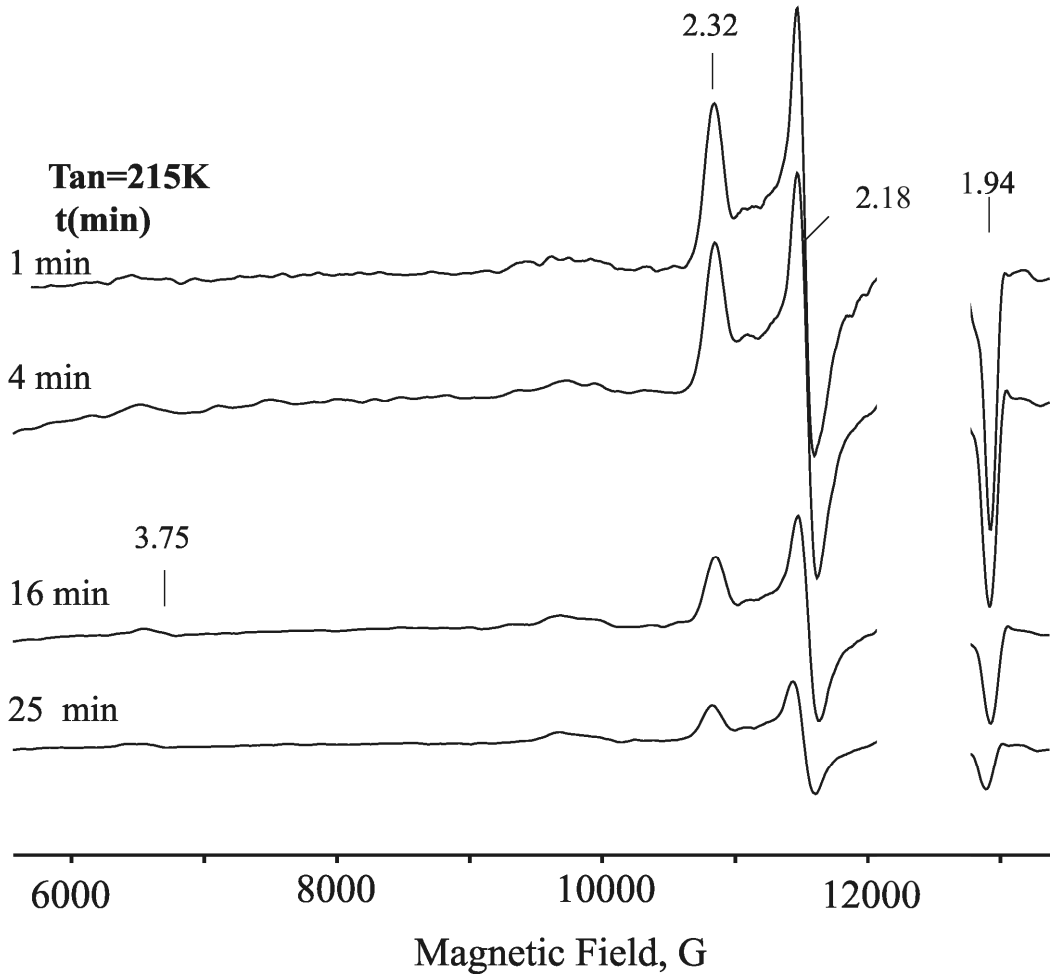

Figure S6. EPR spectra of cryoreduced oxyferrous DHP in the presence of 20 mM F_3PhOH in 16% glycerol/0.05 M Tris buffer, pH 9, taken during progressive annealing at 215 K. Instrument conditions: T = 2 K; rf power = 20 μW; modulation amplitude = 4 G; microwave frequency = 35.08 GHz.

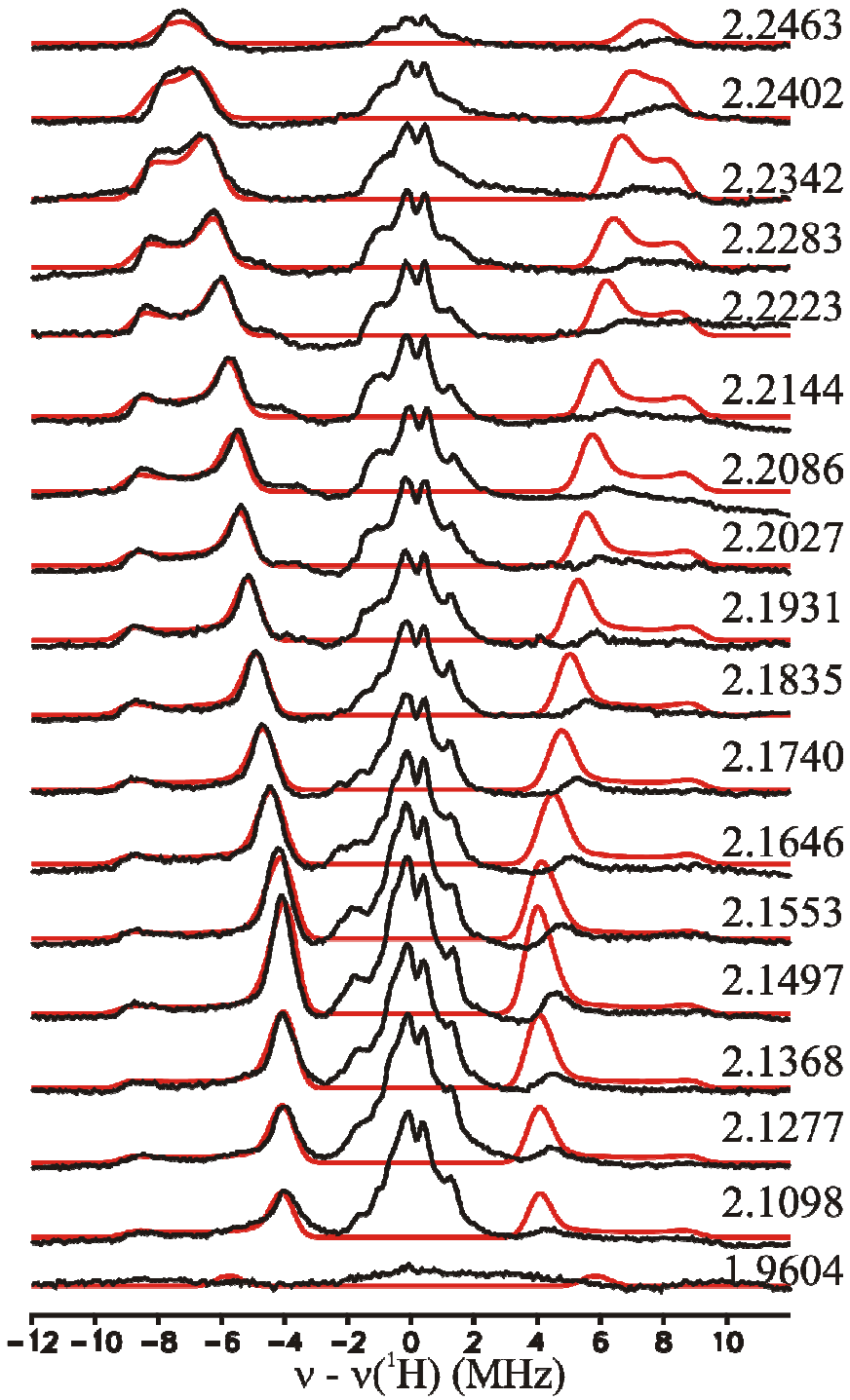

Figure S7. Simulation of 2D frequency/field ^{1}H CW ENDOR spectra of peroxoferric DHP species A presented in Figure 5. Simulation parameters: g = [2.25, 2.15, 1.97]; A = [8.22, 7.6, 18.55] MHz; $\alpha = 0$, $\beta = 55$, $\gamma = 0$.

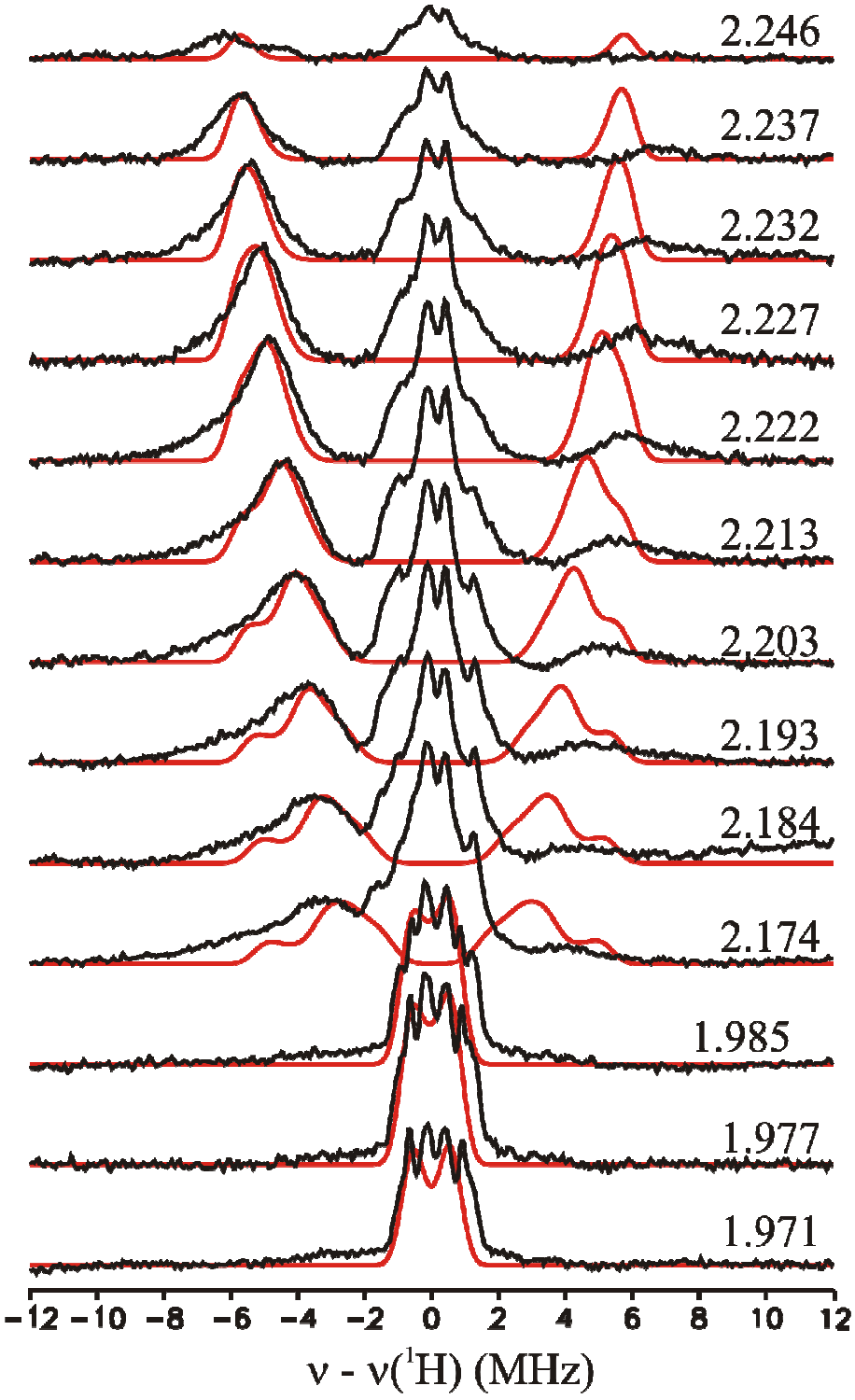

Figure S8. Simulation of 2D frequency/field ^{1}H CW ENDOR spectra of peroxoferric DHP species B presented in Figure 6. Simulation parameters: g = [2.24, 2.13, 1.97]; A = [-1.45, -1.45, 12.06] MHz; α = 0, β = 80, γ = 25.

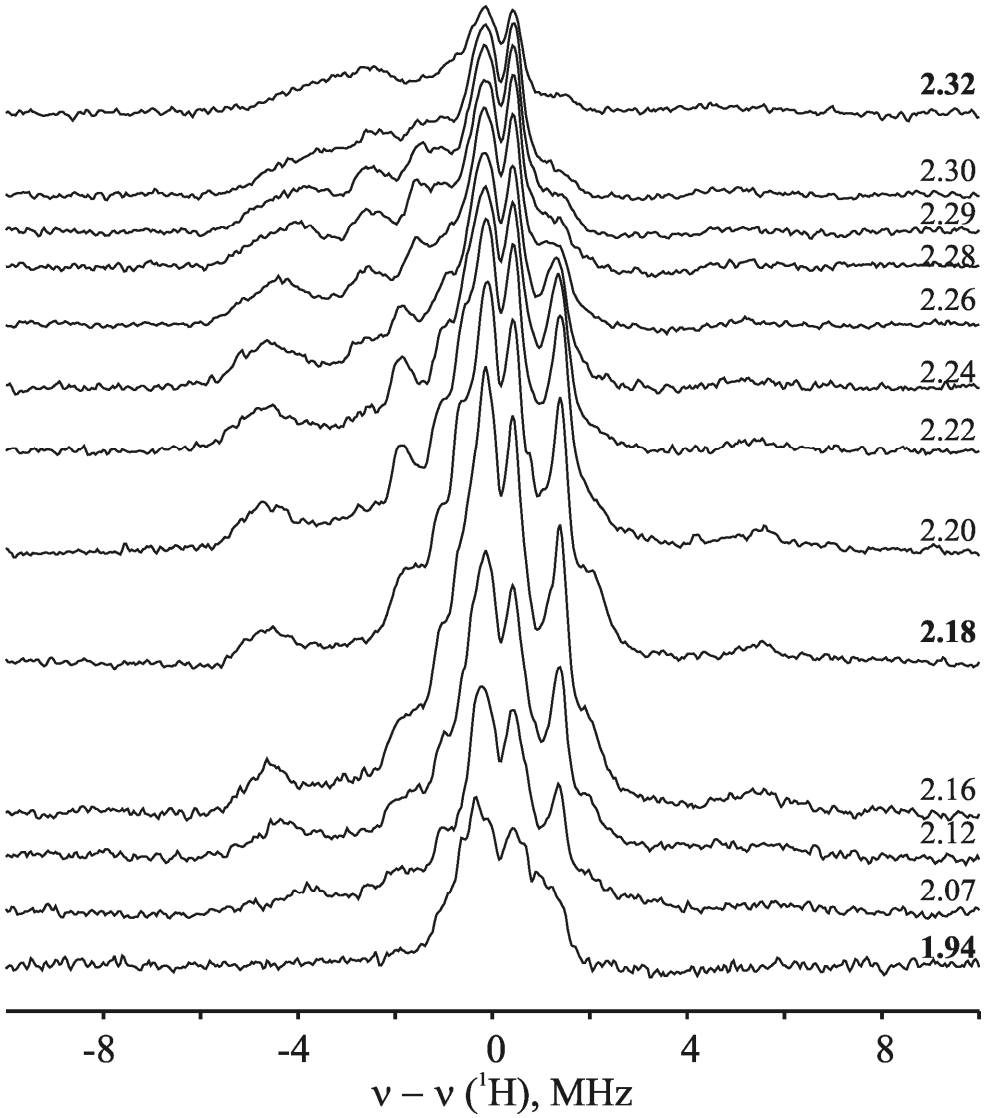

Figure S9. 2D frequency/field ^{1}H CW ENDOR spectra of the hydroperoxoferric DHP intermediate generated by annealing the cryoreduced oxyferrous DHP complex in 0.05 M Tris buffer, pH 9, at 215 K for 1 min. Instrument conditions: T = 2 K; microwave frequency 34.99 MHz; microwave power = 20 μW; modulation amplitude = 2 G; scan rate = 0.5 MHz/s; rf power = 7 W; frequency bandwidth = 60 kHz.

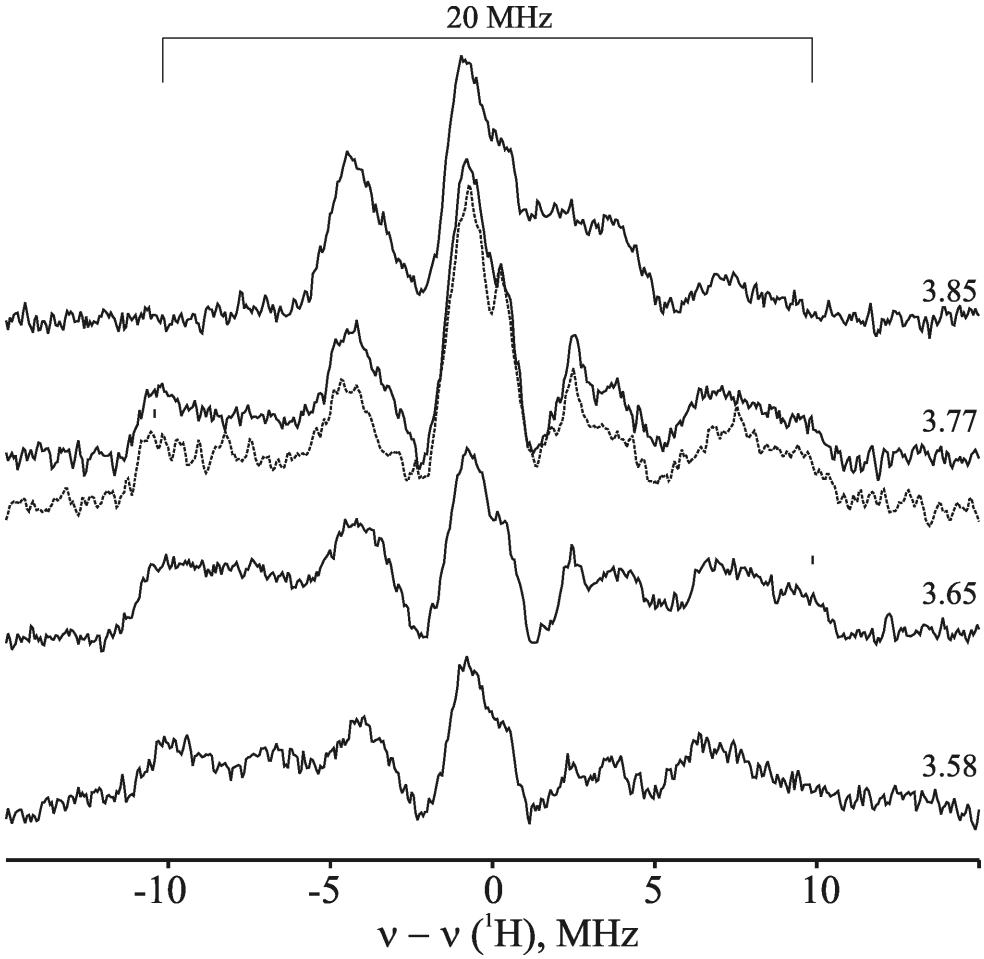

Figure S10. 2D frequency/field ^{1}H CW ENDOR spectra of g 3.75 intermediate arising during annealing of cryoreduced oxyferrous DHP in 0.05 M Tris buffer, pH 9, at 217 K for 2 min in H_2O (solid line) and in D_2O (dotted line). Instrument conditions are the same as described in Figure S9.

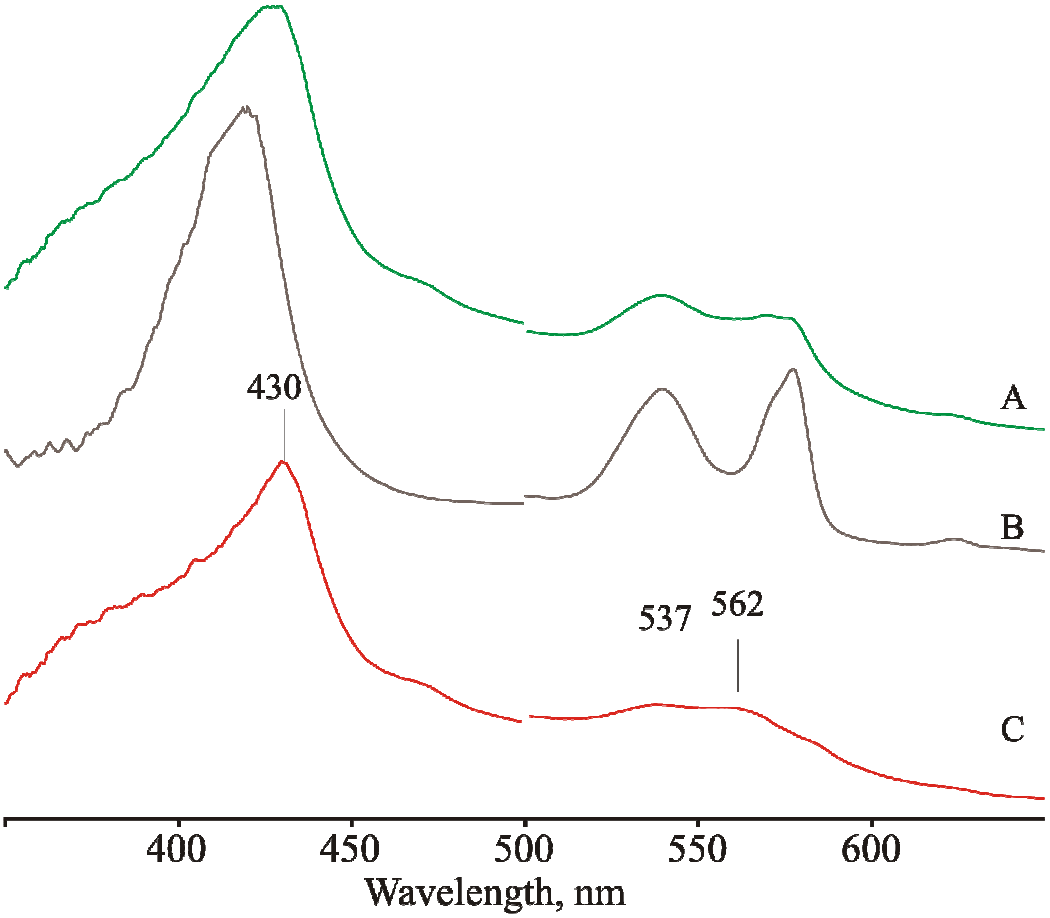

Figure S11. UV-Visible absorption spectra at 77 K of 0.1 mM oxyferrous DHP in 60% glycerol/0.05 M Tris buffer, pH 7 (B) and after γ-irradiation of this sample with a dose of 5 Mr at 77 K (A). The difference spectrum, C = A - B, of cryoreduced oxyferrous DHP.

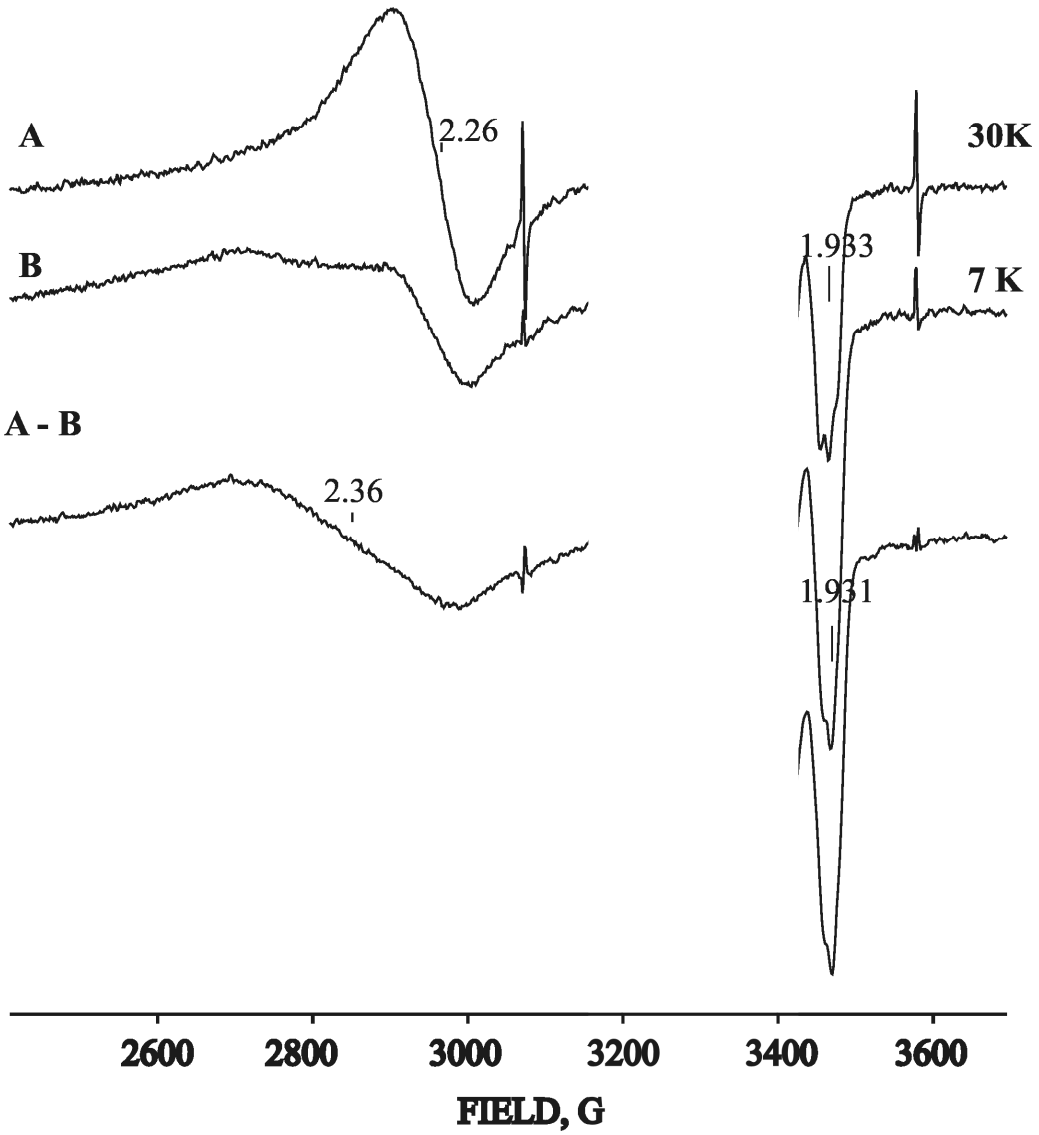

Figure S12. X-band EPR spectra of cryoreduced ferrous DHP taken at (A) 30 K and (B) 7 K. EPR spectrum of fast relaxing species obtained by subtraction of the scaled spectrum A from spectrum B. Instrument conditions: modulation amplitude = 10 G, microwave power = 1 mW, microwave frequency = 9.38 GHz.

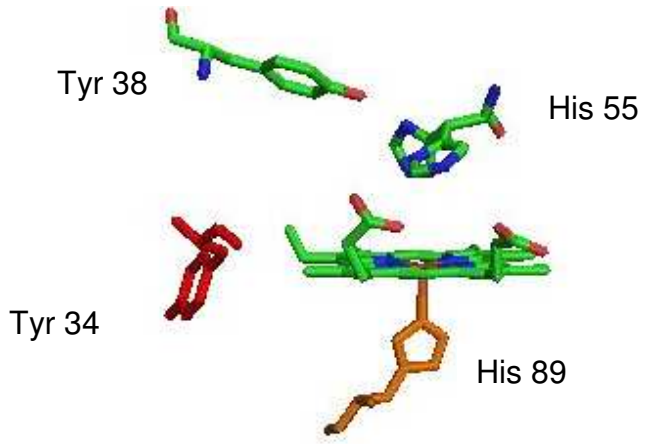

Figure S13. Heme active site of *A. ornata* dehaloperoxidase recreated from PDB: 1EWA.[2] The distal histidine (His55) and tyrosine (Tyr38) are shown. The tyrosine mutated in this study is shown in red (Tyr34). The proximal histidine (His89) is shown in orange to easily differentiate the distal and proximal sides of the heme active site.