Multifrequency Electron Paramagnetic Resonance Characterization of PpoA, a CYP450 Fusion Protein that Catalyses Fatty Acid Dioxygenation

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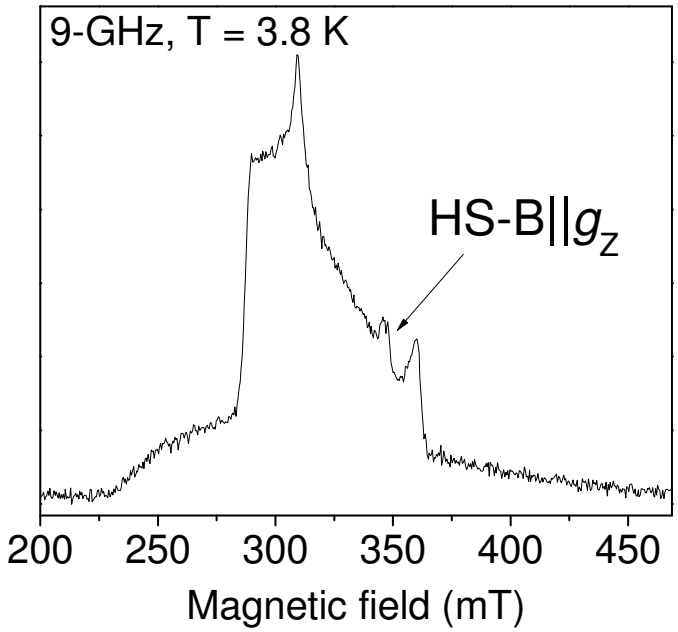
(Supporting Information)

Table S1. Magnetic Parameters for Heme EPR and ENDOR spectra

Enzyme	g_1^{eff}	g_2^{eff}	g_3^{eff}	^{14}N *Ligand*	A_1 *(MHz)*	A_2 *(MHz)*	A_3 *(MHz)*	Q_1 *(MHz)*	Q_2 *(MHz)*	Q_3 *(MHz)*	*Ref*
met-myoglobin (Mb)	5.85	5.95	1.99	His N_ε	8.33	8.08	11.55	0.31	0.81	-1.12	[1]
				Por. N	9.86	6.89	7.11	-0.77	1.04	-0.27	
				His N_ε	8.30	8.03	11.5	0.06	1.21	-1.28	[2]
				Por. N1	10.0	6.9	7.4	-0.75	1.08	-0.33	
				Por. N2	9.6	6.7	7.1	-0.78	1.01	-0.23	
Hemin	6	6	1.99	Por. N1	7.7	6.1	6.8	-0.87	1.14	-0.29	[3]
				Por. N2	8.6	6.7	6.7	-0.88	1.15	-0.29	
Cytochrome d	6	6	2	Por. N1	8.2	6.5	7.0	-0.97	1.3	-0.33	[3]
				Por. N2	9.3	7.1	7.1	-1.01	1.33	-0.33	
PGHS-1	6.6	5.3	2	His N_ε							[4]
				Por. N							
$P450_{CAM}$ [a]	6	6	2								[5]
$P450_{CAM}$ [b]	5.9	5.9	2								[6]
P450 model	5.67	5.67	2	Por. N							[7]
CcmE	6.1	6.1	2	Por. N			8.1			0.25	[8]
	6.7	4.9	2								
CcmE	3.2/2.96	2.27	1.52	His N_ε	-	-	5.1	-	-	0.8	[8]
				Por. N	-	-	5.8	-	-	0.4	
Mouse Neuroglobin	2.79	2.12	1.36	His N_ε1	5.25	5.65	4.9	0.16	0.64	-0.8	[9]
				His N_ε2	5.25	5.65	4.9	0.3	0.55	-0.85	
				Por. N	4.2	4.2	5.7	-0.43	-0.52	0.95	
Cytochrome b559	2.98	2.25	1.52	His N_ε	-5.6	-6.2	-5.1	0	-0.8	0.8	[10]
				Por. N	-4.9	-4.7	-5.8	0.4	0.53	-0.95	
$(COP)Fe(Im)_2$+	2.92	2.28	1.56	His N_ε	5.12	-	-	0.78	-	-	[11]
				Por. N	6.07	-	-	0.45	-	-	
$(TPP)Fe(Im)_2$+	2.93	2.28	1.54	His N_ε	-	-	-	-	-	-	[11]
				Por. N	6.18	-	-	0.42	-	-	
MbImidazole	2.9	2.26	1.52	His N_ε	-	-	-	-	-	-	[11]
				Por. N	6.11	-	-	0.5	-	-	
MbOH	2.55	2.14	1.83	His N_ε	-5.5	-5.5	-4.2	0.52	0.63	-1.15	[12]
				Por. N	-4.9	-5.1	-5.3	-0.5	-0.6	1.10	
$FeTPP(4\text{-}MeIm)_2$	2.9	2.285	1.57	His N_ε	-5.7	-6.2	-5.3	0.34	0.51	-0.85	[13]
				Por. N	-4.8	-4.8	-5.8	-0.42	-0.51	0.93	
PGHS-1	2.99	2.19	1.56	His N_ε1							[4]
				His N_ε2							
				Por. N							
$P450_{CAM}$	2.45	2.25	1.91	Por. N	5.7	-	-	0.3	-	-	[14]
PpoA [c]	**6.0**	**6.0**	**1.99**	**His N_ε**	**-**	**-**	**|11.9|**	**-**	**-**	**|0.9|**	**this work**
				Por. N1	**-**	**-**	**|7.9|**	**-**	**-**	**|0.3|**	
				Por. N2	**-**	**-**	**|8.1|**	**-**	**-**	**|0.2|**	
PpoA	**2.43**	**2.25**	**1.91**								**this work**

[a] On reaction with sulfhydryl reagents. [b] On reaction with peroxy acetic acid. [c] Values of A_1, A_2, Q_1 and Q_2 used in the second order approximation formula for the simulations of the PpoA heme spectra were assumed to be of similar magnitude to that of heme model systems.[1] As reported in the main text, the deviation from first order resonance frequencies was found less than ~0.2 MHz.

Heme ENDOR



9-GHz Echo-detected spectrum of PpoA 0.3 mM in 50 mM HEPES/H_2O solution pH 7.4: 50 % glycerol solution. The experimental parameters: $\pi/2$ = 48 ns, τ = 224 ns, π = 96 ns; shot repetition time = 50 ms; shots/point = 60; 1024 points; scans = 40; T = 3.8 K.

Figure S1

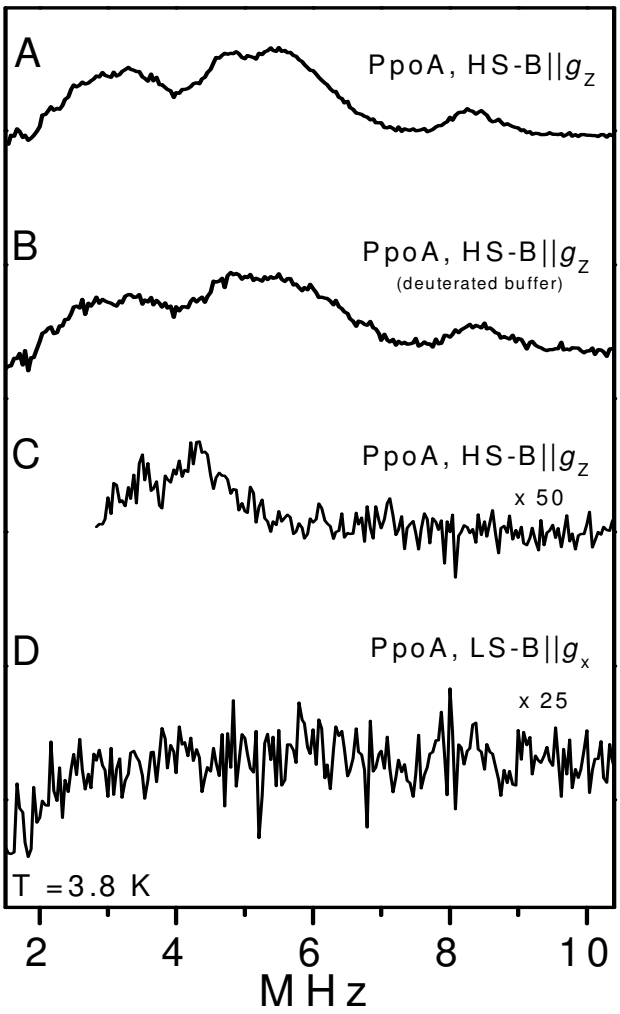


Figure S2. 9-GHz Davies-ENDOR spectra recorded at 3.8 K. Experimental parameters: $\pi/2$ = 24 ns; π = 48 ns; τ = 240 ns. shot repetition time = 2.5 ms; shots/point = 60; RF pulse = 2 μs. (A) PpoA 0.3 mM in 50 mM HEPES/H_2O solution pH 7.4: 50 % glycerol solution at 347.2 mT; 888 scans. (B) PpoA 0.4 mM in 50 mM HEPES/D_2O solution pD 7.8: 50 % glycerol-d8 solution at 347.2 mT, 200 scans. Contributions of weakly coupled deuterium nuclei around their Larmor frequency are not visible in Davies ENDOR with a hard inversion pulse. (C) PpoA 0.3 mM in 50 mM HEPES/H_2O solution pH 7.4: 50 % glycerol solution at 347.2 mT, shots/point = 10, shot repetition time = 50 ms, 236 scans. Pulses were optimized for LS. Spectrum cut from < 2.5 MHz to remove artifact. The spectrum shows that at this field position with pulses optimized for LS heme the features of spectrum (a) disappear. Therefore, spectrum (a) must be contributed by the HS heme. (D) PpoA 0.3 mM in 50 mM HEPES/H_2O solution pH 7.4: 50 % glycerol solution at 361.1 mT, 144 scans. Pulses were optimized for HS. This second

control shows again that it is not possible to detect ENDOR signals from the LS heme with parameters optimized for the HS heme. The conclusion is the same as under (C).

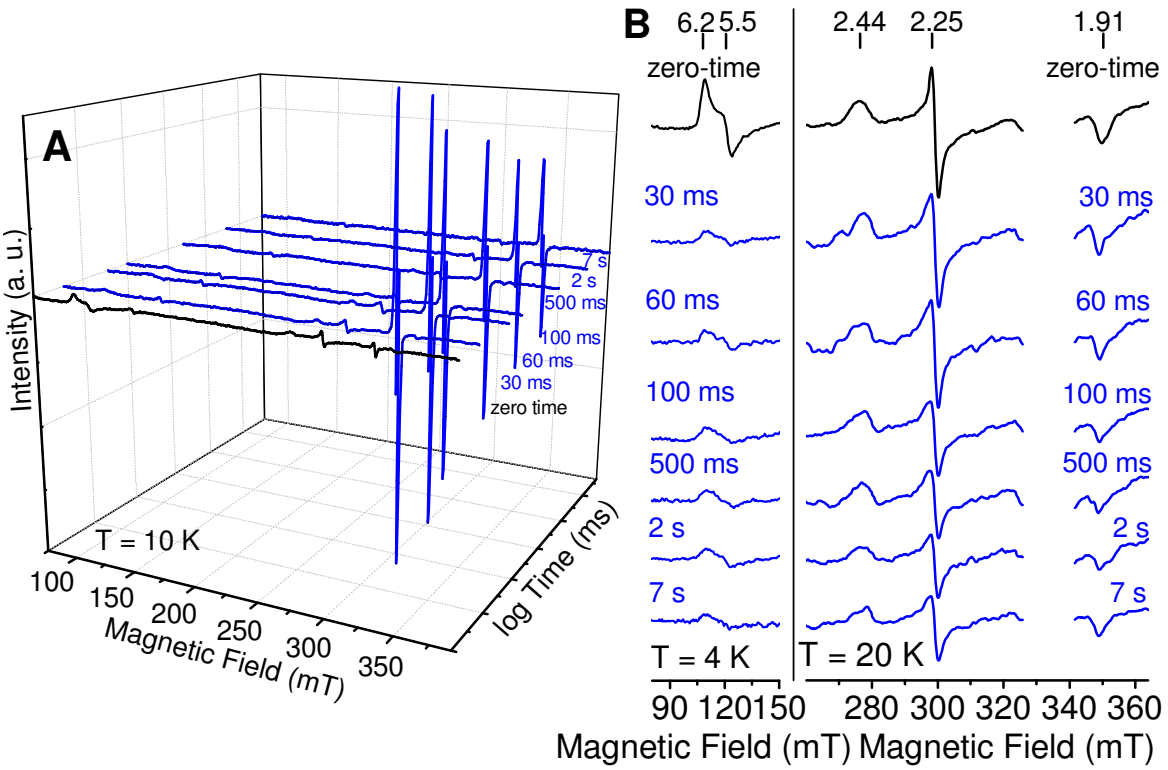


Figure S3. 9-GHz CW EPR spectra of the reaction between PpoA (25 μM) and 160-fold (8*R*)-HPODE in HEPES buffer pH 7.4 using aging times of 30 ms, 60 ms, 100 ms, 2 s and 7 s (blue). Zero time is PpoA mixed with buffer under identical conditions using an aging time of 30 ms. (A) Spectra were recorded at 10 K, MA = 4 G, 0.2 mW microwave power, 100 kHz, 6 scans. (B) Spectra were recorded at 4 K, MA = 4 G, P = 1.0 mW, and at 20 K, MA = 7 G, 0.2 mW microwave power, 100 kHz, 10 scans. *g*-Values are marked. Radical is removed for clarity (20 K).

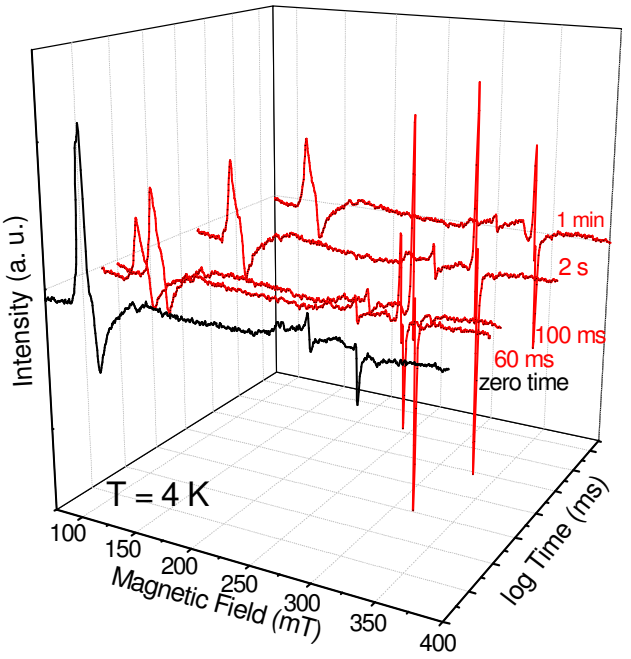


Figure S4. 9-GHz CW EPR spectra of the reaction between PpoA (25 μM) and 160-fold LA in HEPES buffer pH 7.4 using aging times of 60 ms, 100 ms, 2 s and 1 minute (red). PpoA mixed with buffer (25 μM) using an aging time of 60 ms shown in black. Spectra were recorded at 4 K, MA = 4 G, P = 1 mW, 100 kHz, 6 scans.

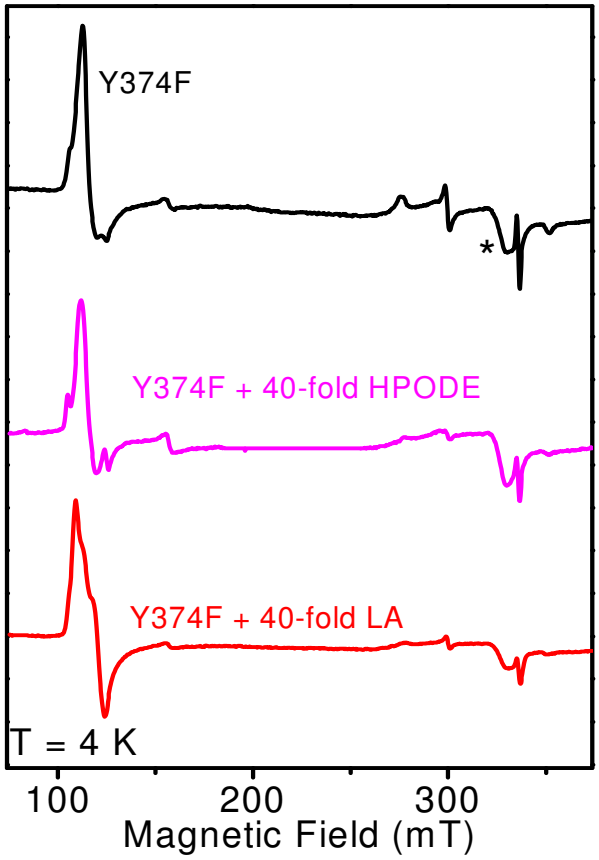


Figure S5. 9-GHz CW EPR spectra at 4 K of Y374F (25 μM, black) and after reaction with 160-fold (8*R*)-HPODE (pink) and 160-fold LA (red) for 3 s in HEPES pH 7.4. Spectra were recorded at 4 K, MA = 4 G, P = 1 mW, 100 kHz, 4 scans. * Cavity background.

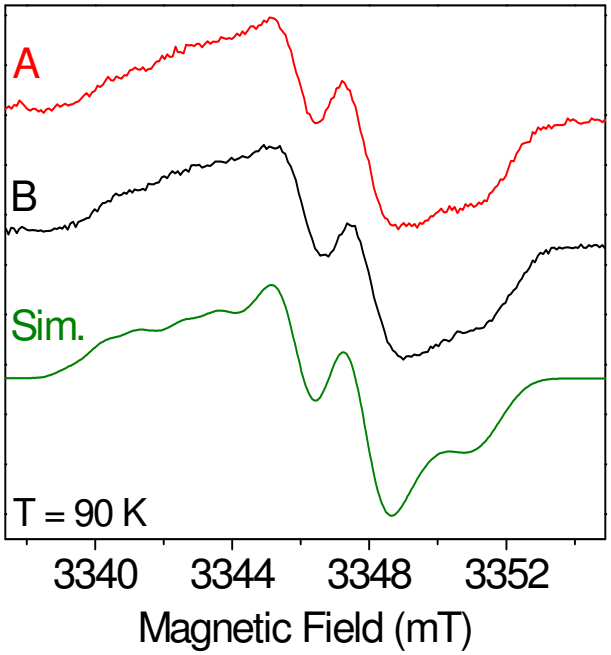


Figure S6. (A) 94-GHz CW spectrum of the amino-acid based radical after reaction with 160-fold excess LA for 3 s (red). (B) 94-GHz CW spectrum CW spectrum of the amino-acid based radical after reaction with 160-fold excess LA for 2 minutes (black). Spectra were recorded at 90 K, MA = 2 G, P = 0.05 mW, 100 kHz, 20 scans. Simulation is shown in green.

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