

Supplementary Material
for
**The Structure of Formaldehyde-Inhibited Xanthine Oxidase Determined
by 35 GHz ^2H ENDOR Spectroscopy**

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Fig. S1

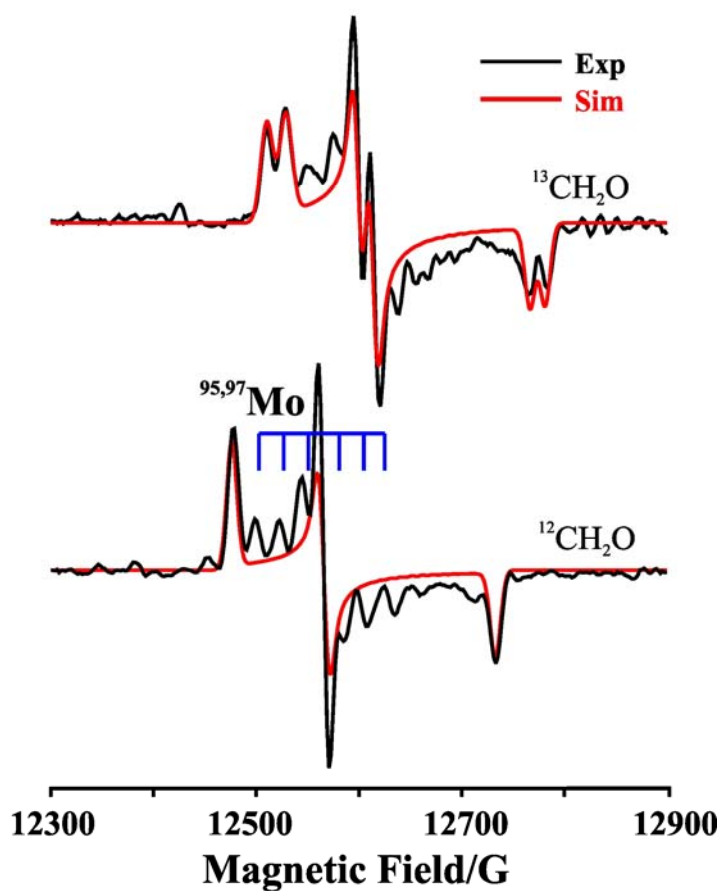


Fig S1: Echo detected 35 GHz EPR spectra of $\text{I}(^{12,13}\text{CH}_2\text{O})$ in H_2O and its simulations (red solid lines); $\mathbf{g} = [1.988, 1.974, 1.948]$, $\mathbf{A}(^{13}\text{C}) = [51.5, 40.0, 40.0]$ MHz, line widths = [12, 9, 10] G. *Conditions:* Two-pulse echo, π -pulse = 80 ns, $\tau = 600$ ns, repetition time = 50 ms, 34.78 GHz, $T = 2$ K. The blue horizontal bar at g_2 shows $^{95,97}\text{Mo}$ hyperfine couplings.

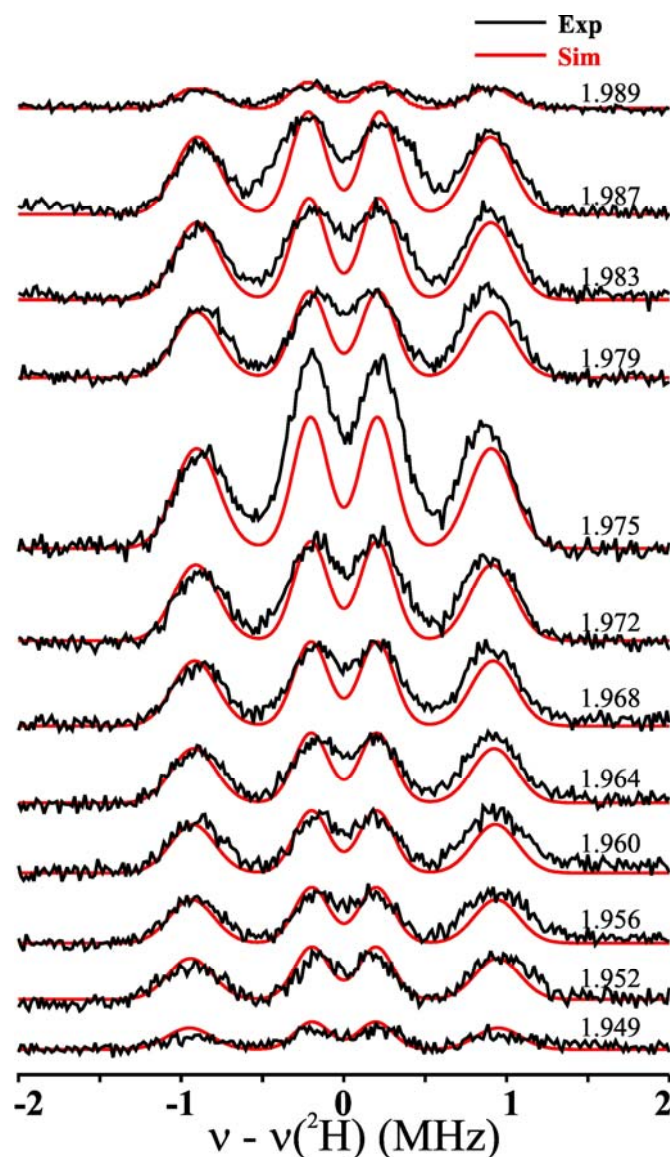


Fig S2: Simulated (red) and experimental (black) 2-D field-frequency plot of Mims ^2H -ENDOR spectra of $\text{I}(\text{C}^2\text{H}_2\text{O})$ in H_2O ; $\mathbf{g} = [1.988, 1.974, 1.948]$, $\mathbf{A}(^2\text{H}_\text{A}) = [1.8, 1.8, 1.9]$ MHz; $\mathbf{A}(^2\text{H}_\text{B}) = [0.44, 0.4, 0.39]$ MHz, ENDOR line width = 0.29 MHz, EPR line width = 30 MHz; The intensities of $^2\text{H}_\text{A}$ and $^2\text{H}_\text{B}$ signals were added (1:1) and the result was scaled to the maximum of the experiment. *Conditions:* $\pi/2$ pulse = 50 ns, $\tau = 800$ ns, repetition time = 50 ms, 34.87 GHz, $T = 2$ K.

DFT Calculation Methods:

Density functional theory (DFT) calculations were performed using Amsterdam Density Functional (ADF) software with the BLYP functional in a spin unrestricted formalism, and converged to ADF default criteria.[ADF version 2008.01, SCM, Theoretical Chemistry, Vrije Universiteit, Amsterdam, The Netherlands.] The initial coordination geometry of the Mo center was obtained from the AOR crystal structure (pdb: 3FAH), replacing the pyranopterin moiety with terminal methyl groups. Geometry optimization calculations were performed for each model using the triple- ζ TZ2P basis set with a small core potential for all atoms except Mo, for which the ZORA/TZ2P basis set was used instead. The quality of the functional and basis sets is typical of DFT calculations on these molybdo-enzyme model systems. Hyperfine values were calculated using the basis set above, but with no core potential. For comparison, additional hyperfine values were calculated with the hybrid B3LYP exchange-correlation functional as shown in the table **S1** below. The accuracy for numerical integrations was set to 5, and diffuse functions were included in all calculations employing this hybrid functional. These calculations were also converged to ADF default criteria. The calculations of the hyperfine values with the two functionals give only modestly different values, with BLYP being closer to experiment, and exhibit the same trends. Most importantly for the text discussion, for both functionals the isotropic ^{13}C coupling for model **A** is close to the experimental value, while that for **C** is not, with the ratio of the isotropic coupling being the same for the two functionals, $a_{\text{iso}}\mathbf{A}(^{13}\text{C})/a_{\text{iso}}\mathbf{C}(^{13}\text{C}) \sim 3/1$.

Table S1: Experimental and theoretical hyperfine tensor of ^{13}C of the CH_2O in various models present in **Scheme 1**.

		Model A		Model C	
	Exp	BLYP	B3LYP	BLYP	B3LYP
A_1	51.5	44.6	37.8	11.7	8.55
A_2	40.0	45.4	38.7	13.4	11.5
A_3	40.0	53.6	46.8	23.2	19.2
a_{iso}	43.83	47.8	41.1	16.1	13.1