

Supplementary Information

Exchangeable oxygens in the vicinity of the molybdenum center of the high-pH form of sulfite oxidase and sulfite dehydrogenase.

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Figure S1 shows the effect of ^{17}O in the X-band EPR spectrum of *hpH* cSO. Similar spectra are obtained for SDH.

Figure S2 shows the assignment of various fundamental and combination lines in two-pulse ESEEM spectra of ^{17}O -oxo ligand. This Figure is intended as an explanation for Fig. 4 of the paper.

Figures S3 through S6 show representative ESEEM spectra of *wt* and Y236F SDH.

Figure S7 shows an example of a normalized τ -integrated three-pulse ESEEM trace.

Figures S8 through S10 show the unphased and phased (using a linear phase correction to account for the dead time $t_d = 150$ ns) spectra of τ -integrated three-pulse ESEEM of *hpH* cSO. The purpose of these figures is to demonstrate that (1) the low-frequency fundamental line of the equatorial ^{17}O (indicated by a diamond in all figures) is observable in the unphased spectra (black traces), but (2) at g_x it is obscured by the side lobes of other lines in the phased spectra (red spectra), which prevents accurate determination of the line maximum positions. For this reason, the spectra shown in Fig. 6 of the manuscript were obtained from the ESEEM traces extrapolated into the dead time (using the Fourier transform slicing algorithm, by A.V. Astashkin and A. Kawamori, *J. Magn. Reson. A*, 1995, **112**, 24-29).

Figure S1. X-band EPR spectra of *wt hpH* cSO in a buffer with H_2^{16}O (black traces) and in a buffer with $\sim 50\%$ H_2^{17}O (red traces). Top traces, experimental; bottom traces, simulated. Experimental conditions: mw frequency, 9.465 GHz; mw power, 2 mW; modulation amplitude, 0.1 mT; temperature, 77 K. Simulation parameters: $(g_z, g_y, g_x) = (1.989, 1.966, 1.955)$; The ^{17}O *hfi* tensor for the red trace is $(A_z, A_y, A_x) = (32, 39.5, 39.5)$ [MHz]. The simulation is not perfect because it does not account for the magnetic isotopes of Mo ($\sim 25\%$ of $^{95}\text{Mo} + ^{97}\text{Mo}$, $I = 5/2$), broadening induced by the ^{17}O -oxo ligand, statistical distribution of the *hfi*, and the forbidden transitions with $\Delta m_1 \neq 1$. Still the hyperfine structure at g_z is qualitatively reproduced.

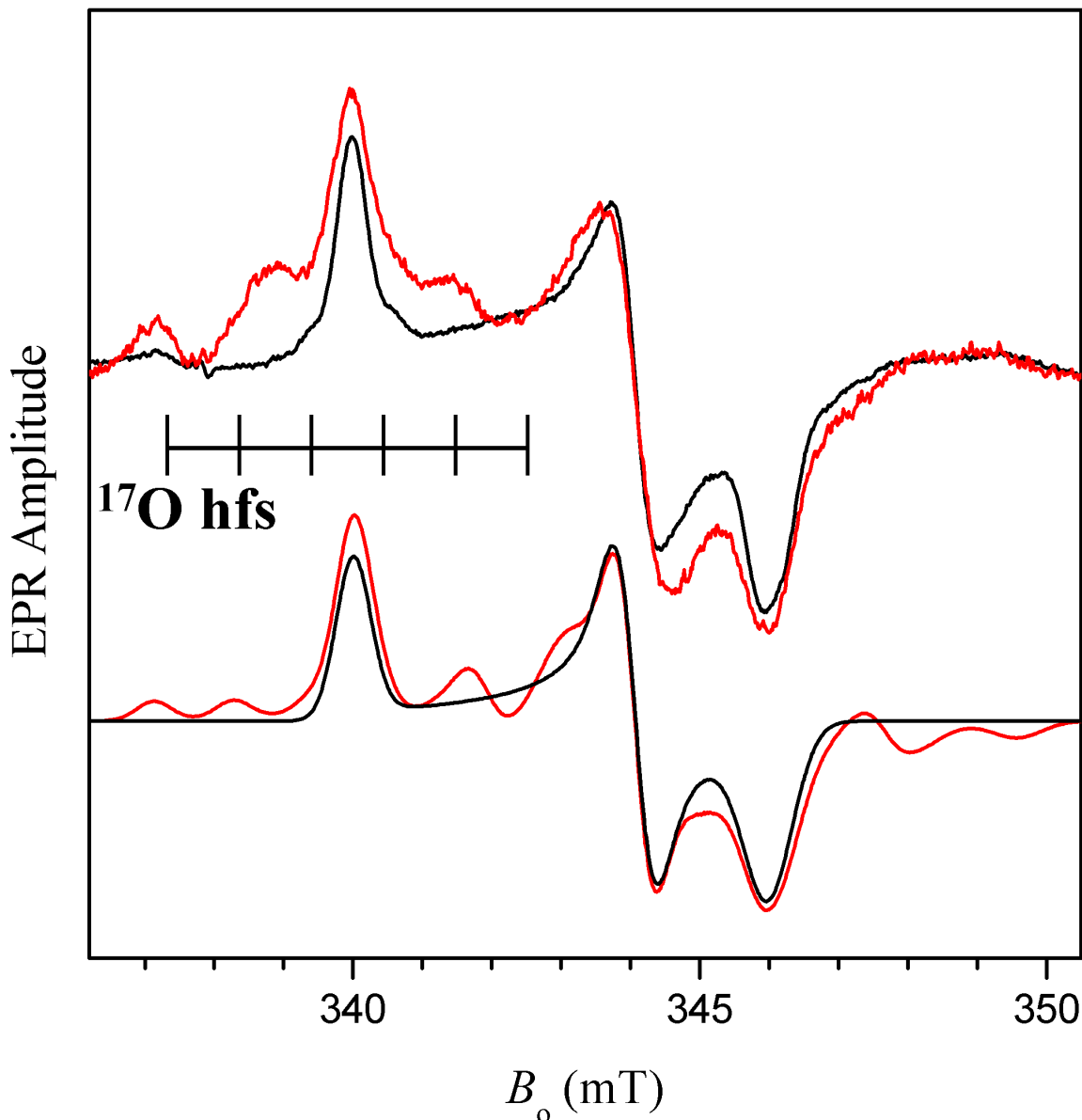


Figure S2. Primary ESEEM spectra (cosine FT) simulated for the g_x position ($B_0 = 1071.5$ mT) of the EPR spectrum of *wt hpH cSO*. The ^{17}O simulation parameters are the same as in Fig. 4 of the paper: $a_{\text{iso}} = 6.3$ MHz; $(T_{11}, T_{22}, T_{33}) = (2.4, 1, -3.4)$ MHz; $(\varphi_h, \theta_h, \psi_h) = (55^\circ, 20^\circ, -10^\circ)$; $e^2Qq/h = 1.7$ MHz; $\eta = 1$; $(\varphi_q, \theta_q, \psi_q) = (70^\circ, 30^\circ, 0^\circ)$. The black trace is for zero dead time ($t_d = 0$) and very long duration in time domain: $\Delta\tau = 20$ μs (no dead time distortions and a high resolution). The red trace is for $t_d = 160$ ns and $\Delta\tau = 20$ μs (dead time distortions are seen, but frequency resolution is high). The green trace is for $t_d = 160$ ns and $\Delta\tau = 2.5$ μs (dead time distortions are seen and the resolution is low: approximates the experimental situation). This trace reproduces trace 5 of Fig. 4 in the paper. The assignments of several fundamental and combination lines are shown. The subscripts at the frequency symbols indicate the electron spin manifold. The superscripts indicate Δm_I .

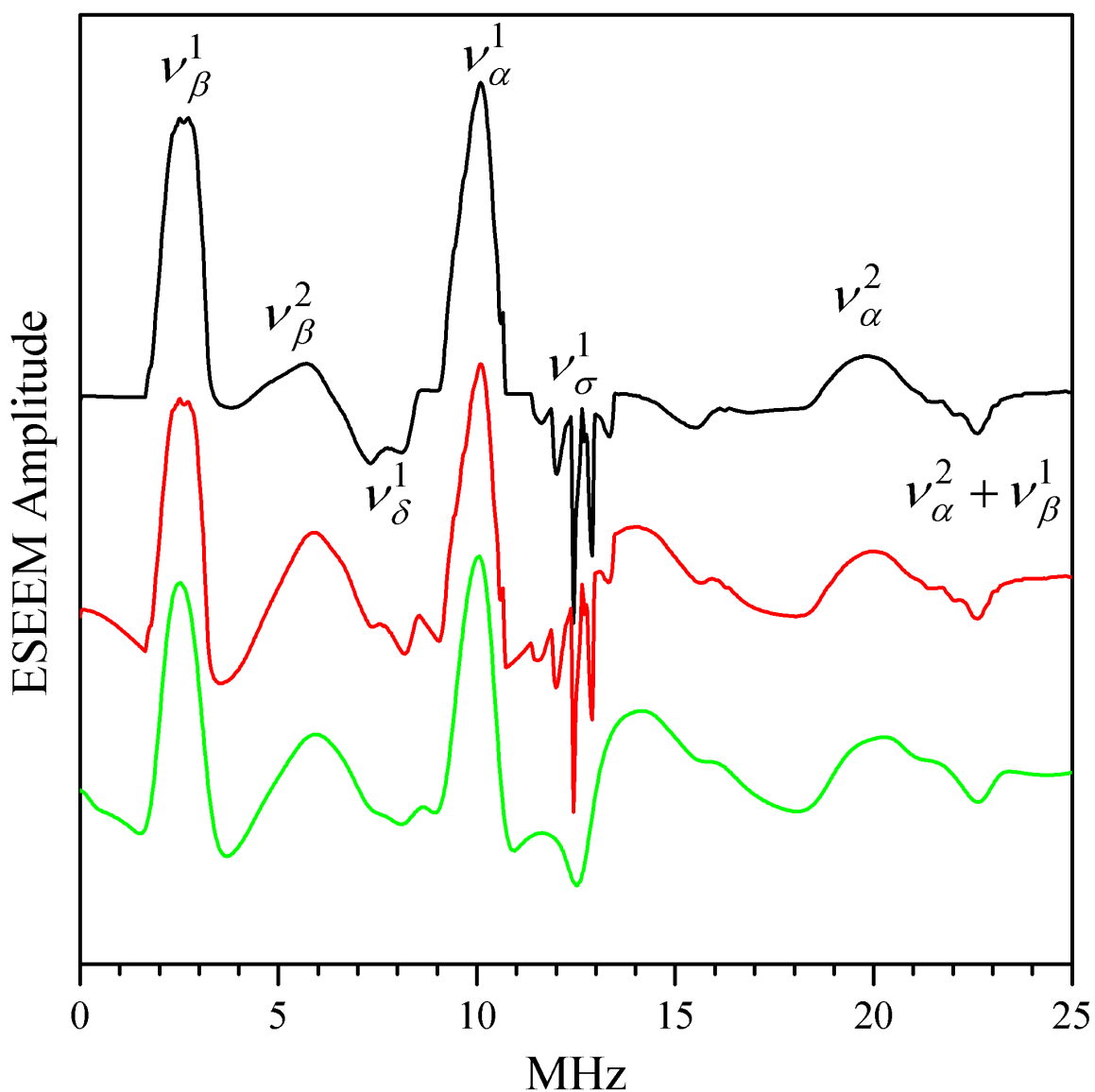


Figure S3. Two-pulse ESEEM spectra (cosine FT) of *hpH wt* SDH incubated for 3 hrs (black) and 2 min (red) in H_2^{17}O buffer. Asterisks, a diamond, and triangles indicate the $\Delta m_I = 1$ fundamental lines of axial oxo- ^{17}O , equatorial ^{17}O (from the OH ligand), and unidentified weakly coupled ^{17}O , respectively. Experimental conditions: mw frequency, 29.810 GHz; $B_0 = 1083.3$ mT (g_Y); mw pulses, 2×15 ns; temperature, 21 K. The line indicated by a diamond is weak and is obscured by the side lobes from other lines. It is seen much more clearly in the spectra of τ -integrated stimulated ESEEM (see Fig. S5).

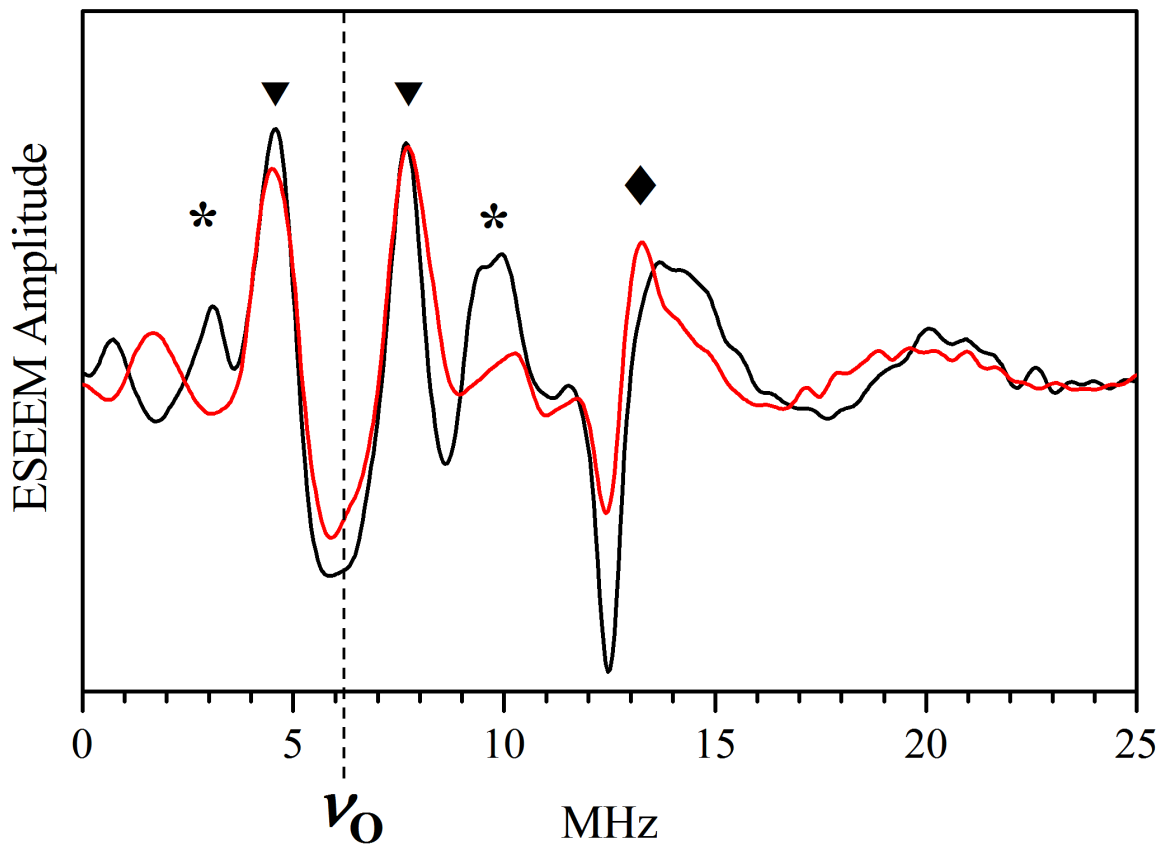


Figure S4. Two-pulse ESEEM spectra (cosine FT) of *hpH* Y236F SDH incubated for 3 hrs (black) and 2 min (red) in H_2^{17}O buffer. Asterisks and a diamond indicate the $\Delta m_I = 1$ fundamental lines of axial oxo- ^{17}O and equatorial ^{17}O , respectively. Experimental conditions: mw frequency, 29.634 GHz; $B_0 = 1077$ mT (g_Y); mw pulses, 2×15 ns; temperature, 21 K. The line indicated by a diamond is weak and is obscured by the side lobes from other lines. It is seen much more clearly in the spectra of τ -integrated stimulated ESEEM (see Fig. S6).

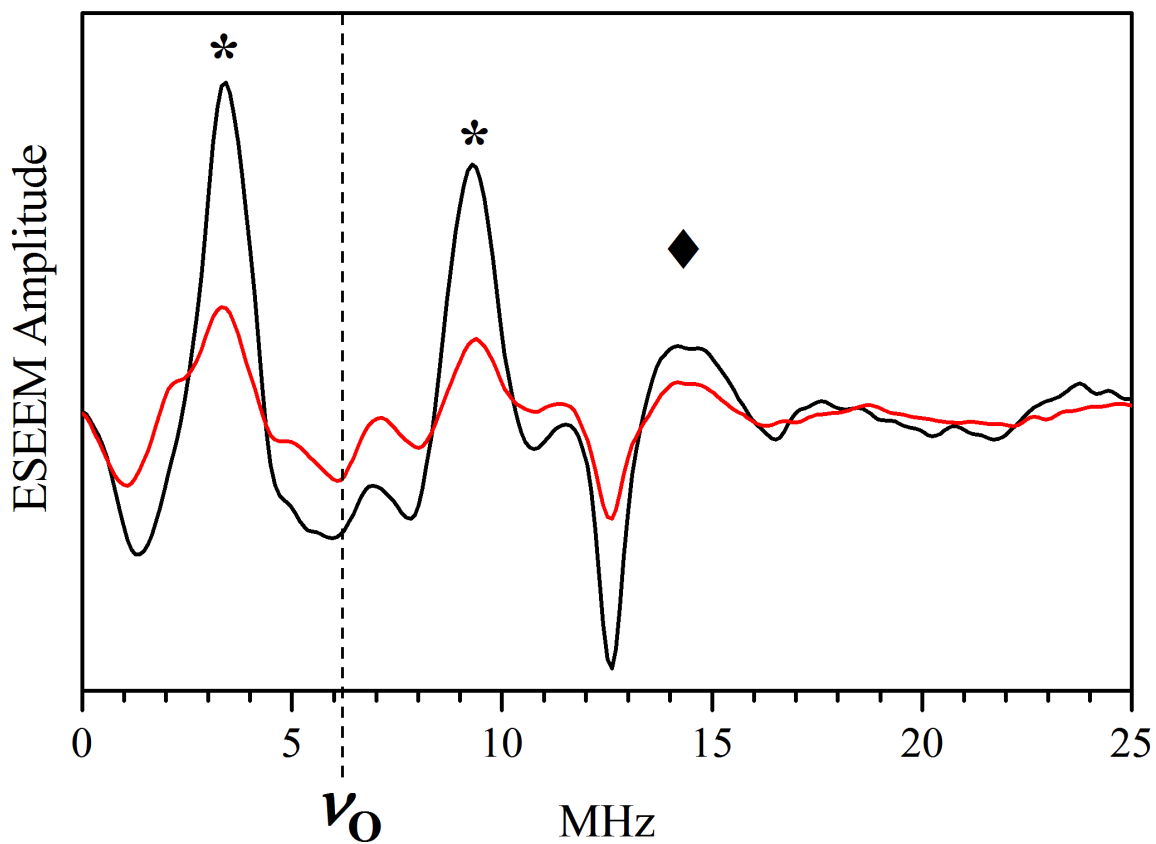


Figure S5. τ -integrated three-pulse ESEEM spectra (cosine FT) of *hpH wt* SDH incubated for 2 min (short incubation) in H_2^{17}O buffer. Asterisks, a diamond, and triangles indicate the $\Delta m_I = 1$ fundamental lines of axial oxo- ^{17}O (residual lines), equatorial ^{17}O (from the OH ligand), and unidentified weakly coupled ^{17}O , respectively. Experimental conditions: mw frequency, 29.810 GHz; $B_0 = 1083.3$ mT (g_Y); mw pulses, 3×13 ns; temperature, 21 K.

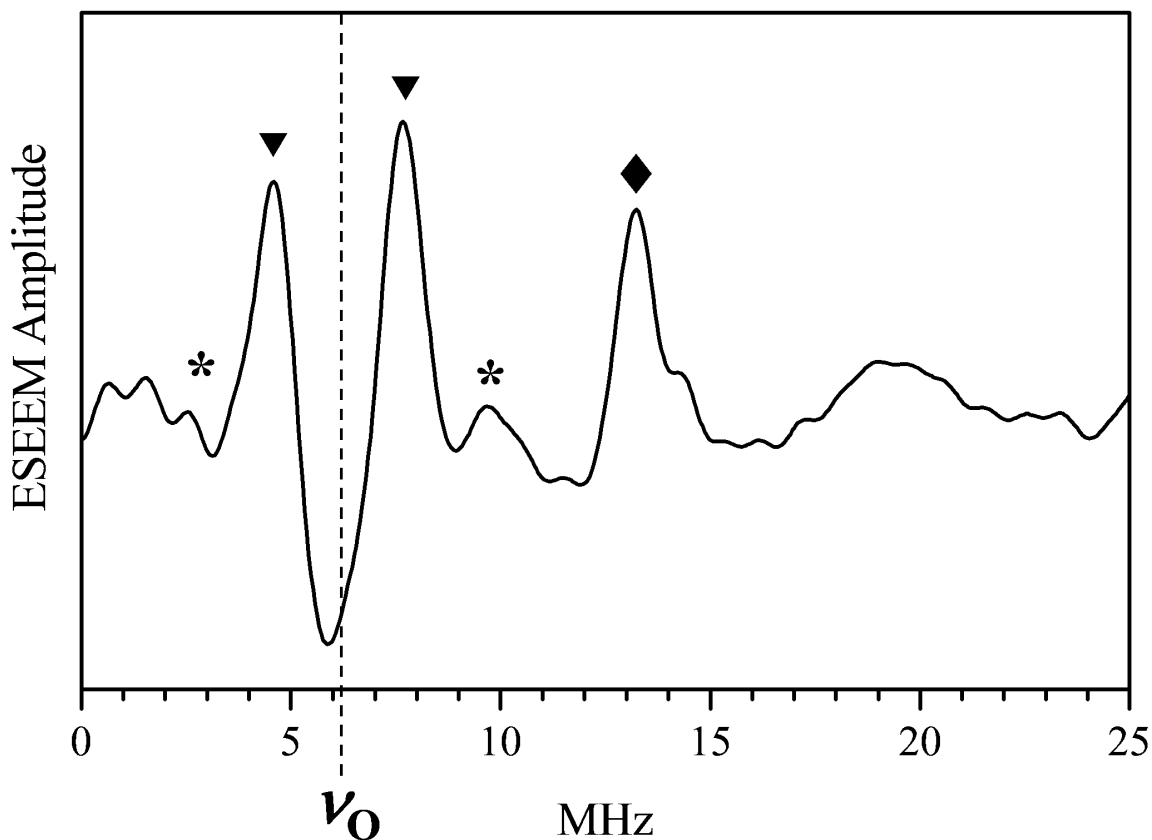


Figure S6. τ -integrated three-pulse ESEEM spectra (cosine FT) of *hpH* Y236F SDH incubated for 2 min (short incubation) in H_2^{17}O buffer. Asterisks and a diamond indicate the $\Delta m_I = 1$ fundamental lines of axial oxo- ^{17}O (residual lines) and equatorial ^{17}O (from the OH ligand), respectively. Experimental conditions: mw frequency, 30.047 GHz; $B_0 = 1092$ mT (g_Y); mw pulses, 3×13 ns; temperature, 21 K.

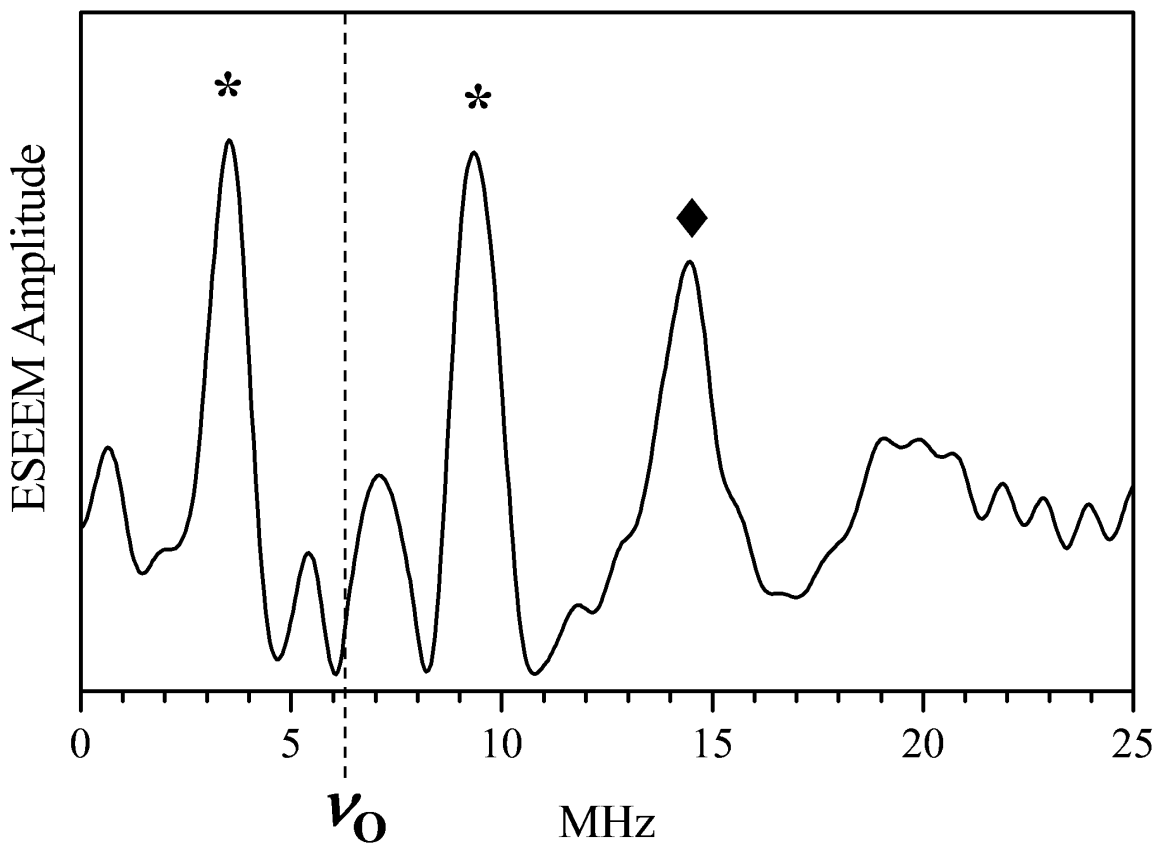


Figure S7. τ -integrated three-pulse ESEEM spectra (cosine FT) of *hpH* cSO incubated for 2 min (short incubation) in H_2^{17}O buffer. Experimental conditions: mw frequency, 29.810 GHz; $B_0 = 1071.8$ mT (g_z); mw pulses, 3×13 ns; temperature, 21 K.

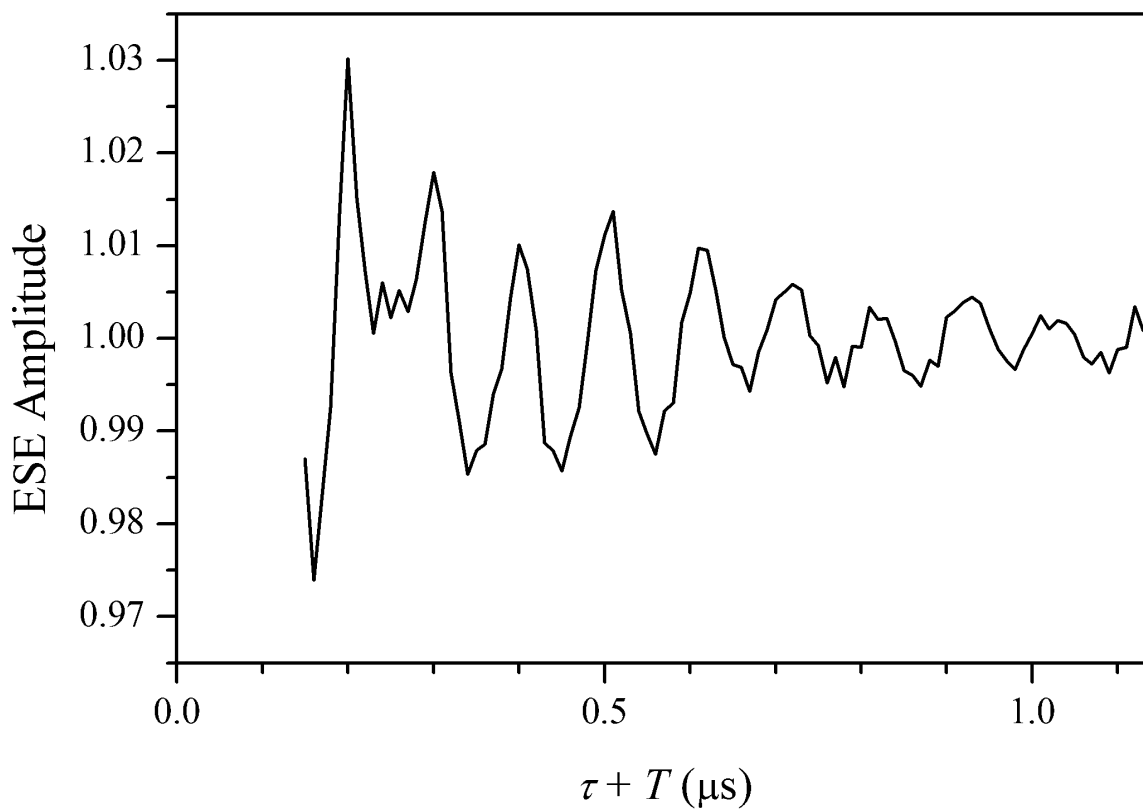


Figure S8. τ -integrated three-pulse ESEEM spectra (cosine FT) of *hpH* cSO incubated for 2 min (short incubation) in H_2^{17}O buffer. Black trace, without phasing to zero time origin. Red trace, after linear phase correction to zero time origin. A diamond and triangles indicate the $\Delta m_1 = 1$ fundamental lines of equatorial ^{17}O (from the OH ligand) and unidentified weakly coupled ^{17}O , respectively. Experimental conditions: mw frequency, 29.810 GHz; $B_0 = 1071.8$ mT (g_z); mw pulses, 3×13 ns; temperature, 21 K. The $\Delta m_1 = 1, 2$ and 3 labels indicate various fundamental lines of equatorial ^{17}O .

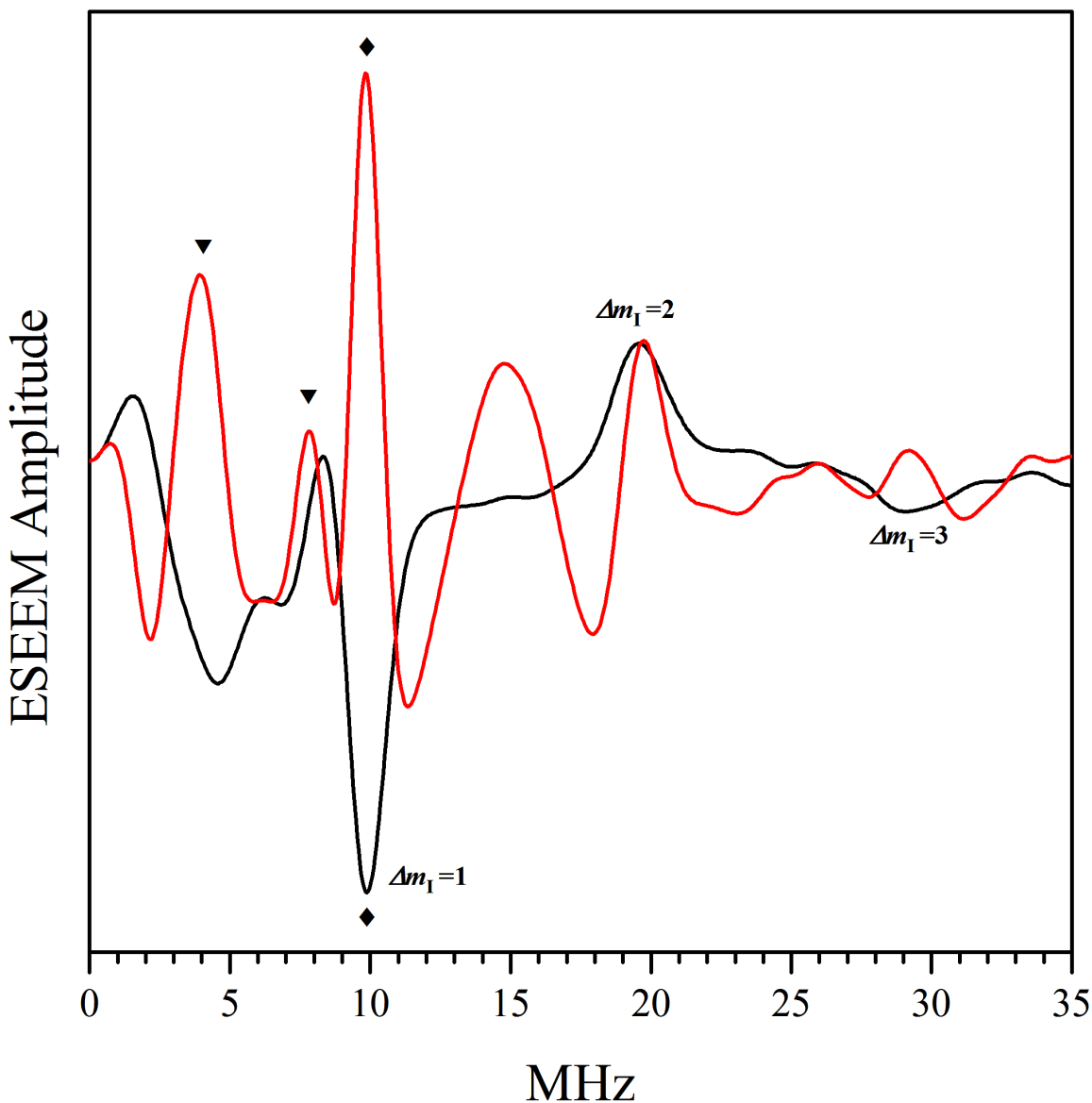


Figure S9. τ -integrated three-pulse ESEEM spectra (cosine FT) of *hpH* cSO incubated for 2 min (short incubation) in H_2^{17}O buffer. Black trace, without phasing to zero time origin. Red trace, after linear phase correction to zero time origin. A diamond and triangles indicate the $\Delta m_1 = 1$ fundamental lines of equatorial ^{17}O (from the OH ligand) and unidentified weakly coupled ^{17}O , respectively. Experimental conditions: mw frequency, 29.810 GHz; $B_0 = 1083.4$ mT (g_Y); mw pulses, 3×13 ns; temperature, 21 K. The $\Delta m_1 = 1$ and 2 labels indicate various fundamental lines of equatorial ^{17}O .

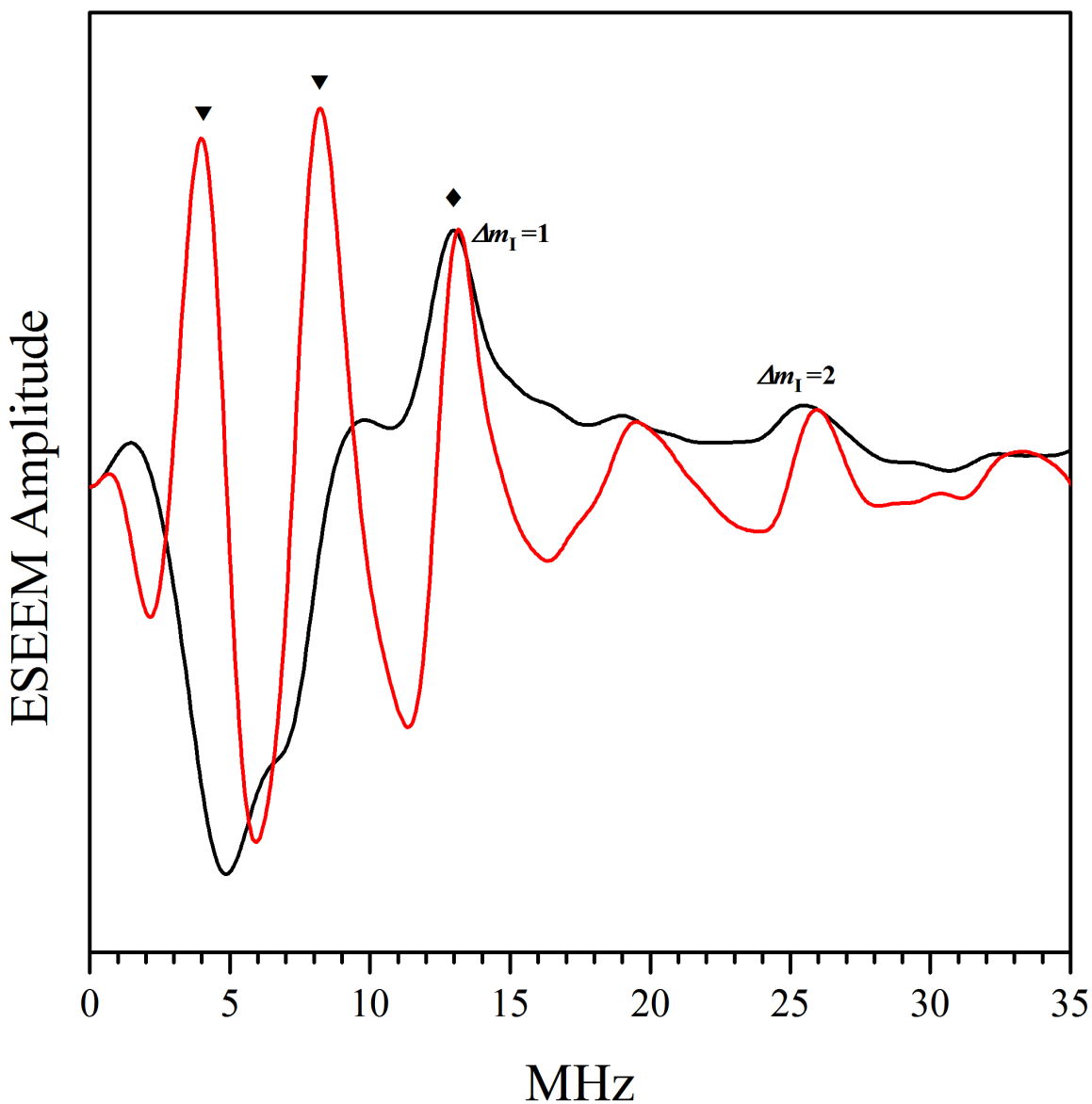


Figure S10. τ -integrated three-pulse ESEEM spectra (cosine FT) of *hpH* cSO incubated for 2 min (short incubation) in H_2^{17}O buffer. Black trace, without phasing to zero time origin. Red trace, after linear phase correction to zero time origin. A diamond and triangles indicate the $\Delta m_1 = 1$ fundamental lines of equatorial ^{17}O (from the OH ligand) and unidentified weakly coupled ^{17}O , respectively. Experimental conditions: mw frequency, 29.810 GHz; $B_0 = 1088.6$ mT (g_X); mw pulses, 3×13 ns; temperature, 21 K. The $\Delta m_1 = 1$ label indicates the only visible fundamental line of equatorial ^{17}O .

