# Supporting Information

# [FeFe]-hydrogenase oxygen inactivation is initiated at the H cluster 2Fe subcluster

Kevin D. Swanson,[§†] Michael W. Ratzloff,[§‡] David W. Mulder,[‡] Jacob H. Artz,[†] Shourjo Ghose,[†] Andrew Hoffman,[†] Spencer White,[†] Oleg A. Zadvornyy,[†] Joan B. Broderick,[†] Brian Bothner,[†] Paul W. King*,[‡] and John W. Peters*,[†]

†Department of Chemistry and Biochemistry, Montana State University, Bozeman, Montana 59717, United States
‡Biosciences Center, National Renewable Energy Laboratory, Golden, Colorado 80401, United States

**Figure s1.** FTIR spectra of CrHydA1 $H_{ox}$-CO before (a), and after (b) 2 h exposure to 24.2% $O_2$ at 4 °C. CrHydA1 concentration 70 $mg^{-1}$ $ml^{-1}$. The principle *v*CO peaks are colored as Hox-CO (black), $H_{ox}$ (red), $O_2$ damaged (purple), unassigned (cyan).

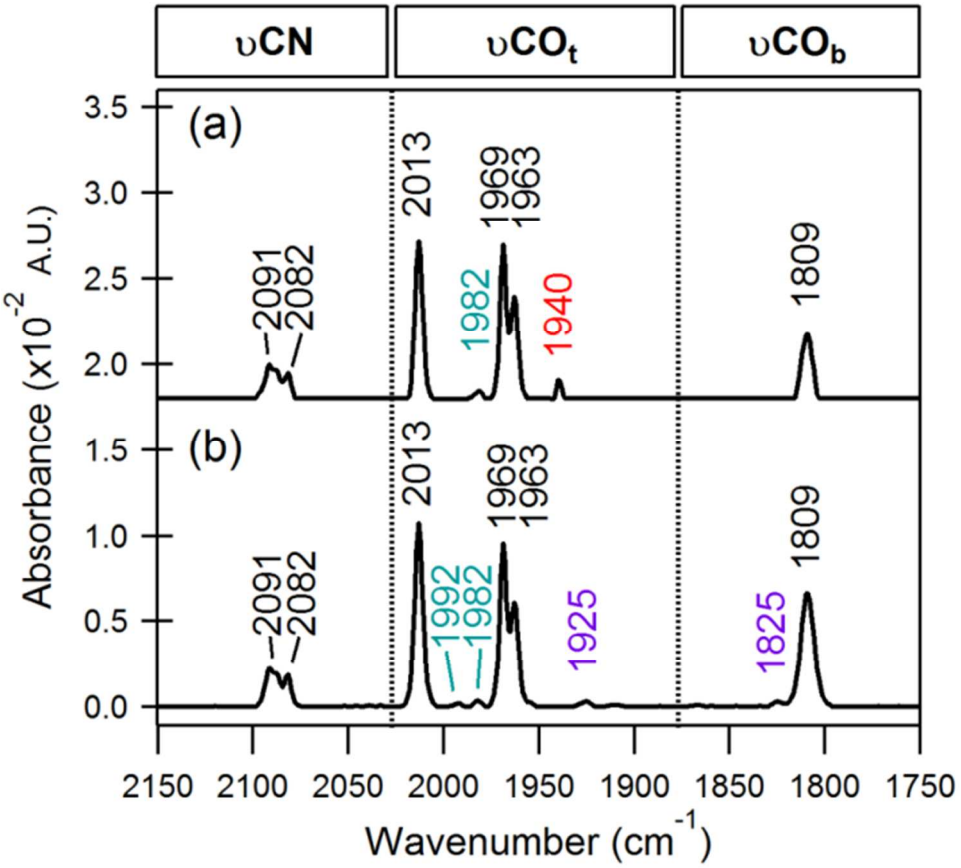


**Figure S2**. FTIR spectra of $O_2$ titration of NaDT-treated CrHydA1. (a) After 2 h of exposure to 0.002% $O_2$. (b) After 2 h exposure to 2% $O_2$. While there are additional features that may be a result of the interaction of NaDT and $O_2$, similar trends are observed here as seen in $H_{ox}$ experiments. The growth of $H_{ox}$-CO and of signals assigned to $O_2$ damage, (1825 and/or 2025 $cm^{-1}$) are seen here. The *ν*CO and *ν*CN peaks are color-coded as; $H_{ox}$-CO (black), $H_{ox}$ (red), $H_{red}$-NaDT (magenta) $O_2$ damaged (purple), unassigned (cyan).

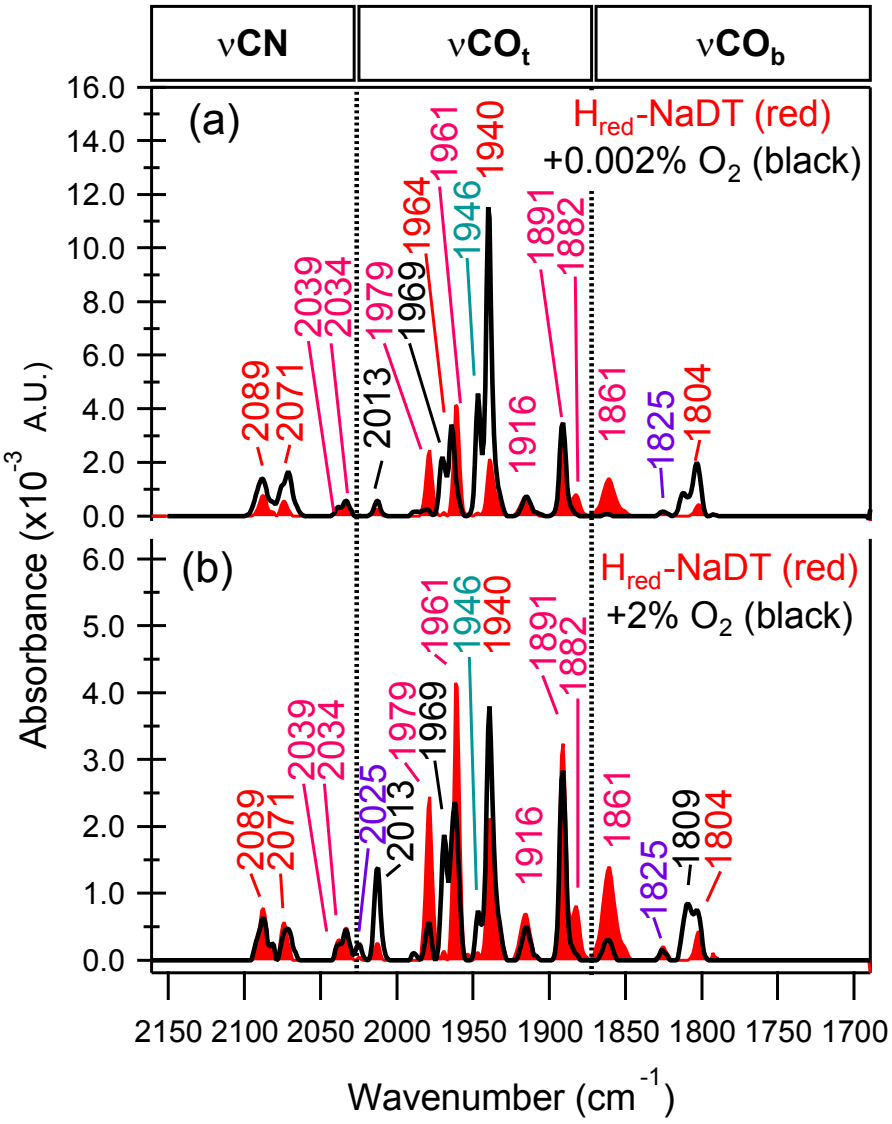


**Figure S3**. FTIR time-course of non-$O_2$ treated CrHydA1 under N2 atmosphere. CrHydA1 (59 mg $mL^{-1}$) was buffer exchanged by G-25 into NaDT-free buffer as described in the Experimental Section, but minus the $O_2$ injection. Time (min) is indicated on the right of the spectra. The increase in the $H_{ox}$ specific 1940 $cm^{-1}$ *ν*CO peak along with the decrease in the $H_{red}$ *ν*CO peaks at 1891 and 1933 $cm^{-1}$ indicates auto-oxidation of a small fraction of residual $H_{red}$.

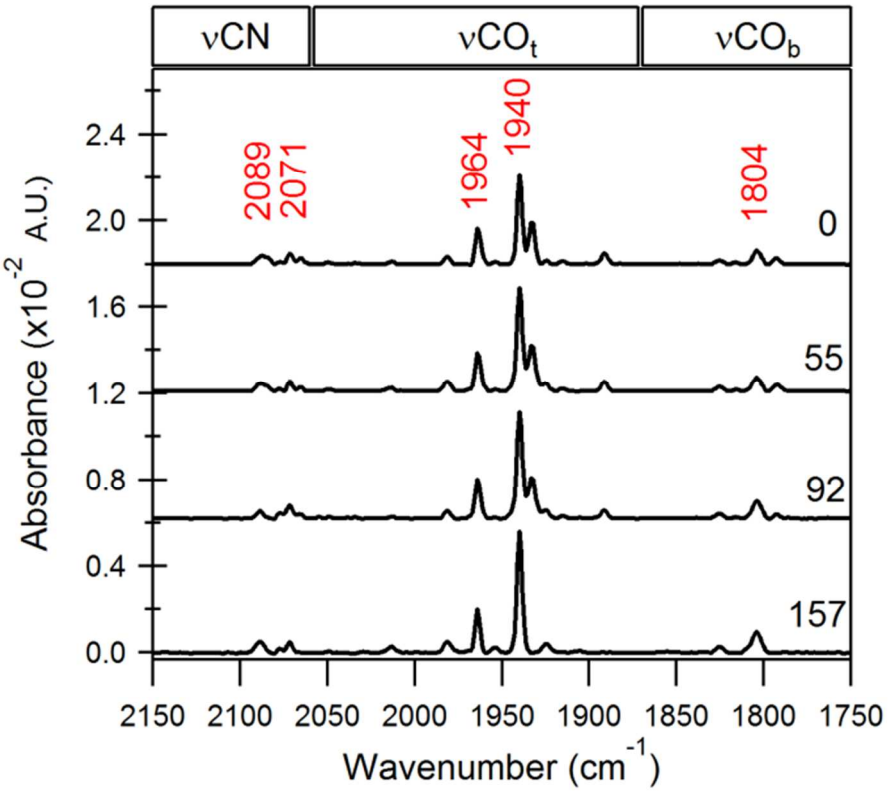


**Figure S4.** FTIR spectra of $O_2$ titration of $H_{ox}$ CrHydA1. (a) $H_{ox}$ CrHydA1 at 45 mg $mL^{-1}$ (red) and after 133 min exposure to 0.01% $O_2$ (black), (b) $H_{ox}$ CrHydA1 at 40 mg $mL^{-1}$ (red) and after 120 min exposure to 1% $O_2$ (black), (c) $H_{ox}$ CrHydA1 at 40 mg $mL^{-1}$ (red) and after 120 min exposure to 10% $O_2$ (black).

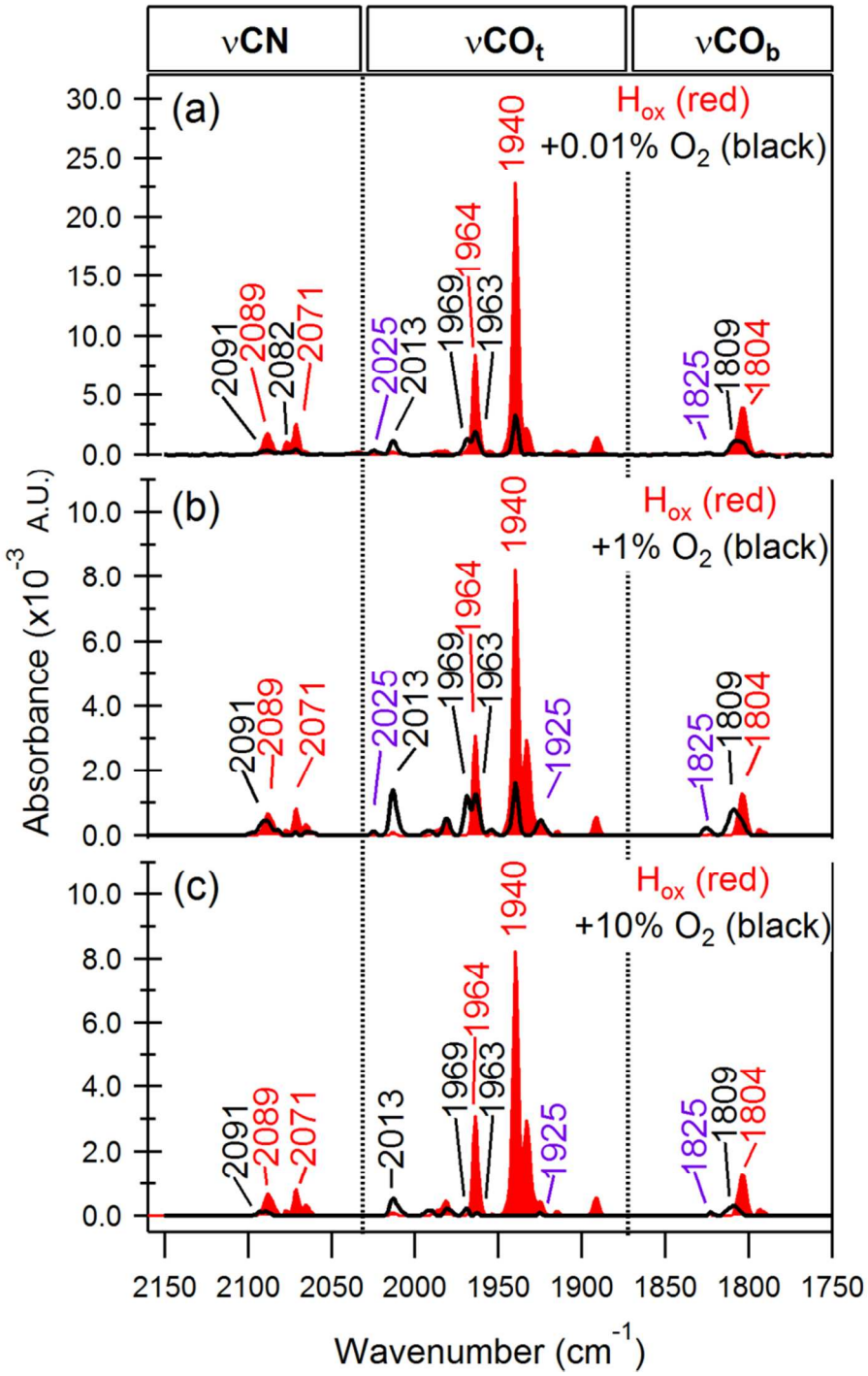


**Figure S5.** FTIR spectral time course of the 2Fe subcluster in CaI that was exposed to 0.28% $O_2$. CaI was prepared in the $H_{ox}$ state and exposed to 0.28% $O_2$ at 4 °C with magnetic stirring. Aliquots were removed at the time intervals indicated on the right (in min, with increasing time from bottom to top), and analyzed by FTIR. The red dashed lines and arrows indicate the appearance of *ν*CO peaks assigned to the $H_{ox}$-CO state of CaI (2016 and 1807 $cm^{-1}$).

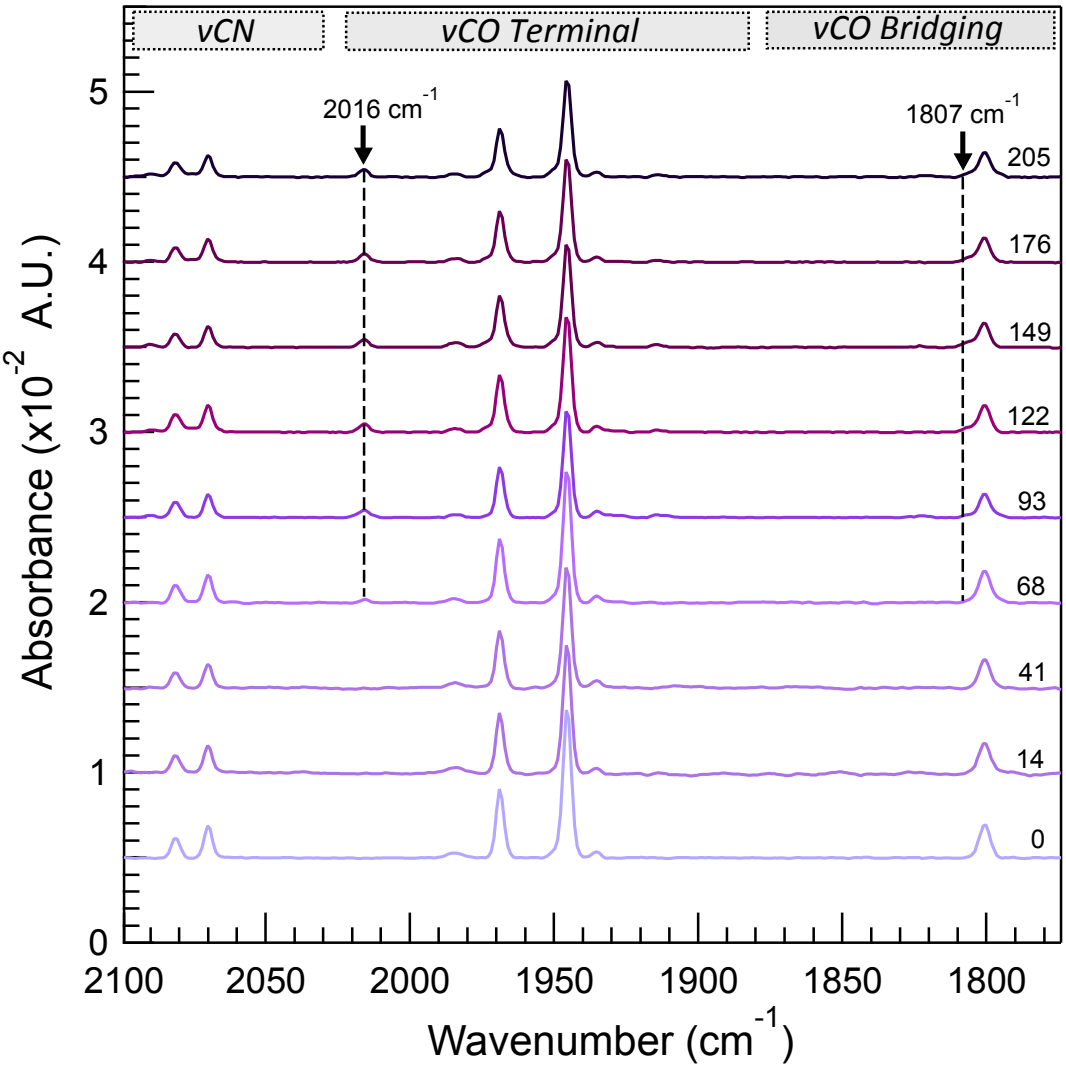
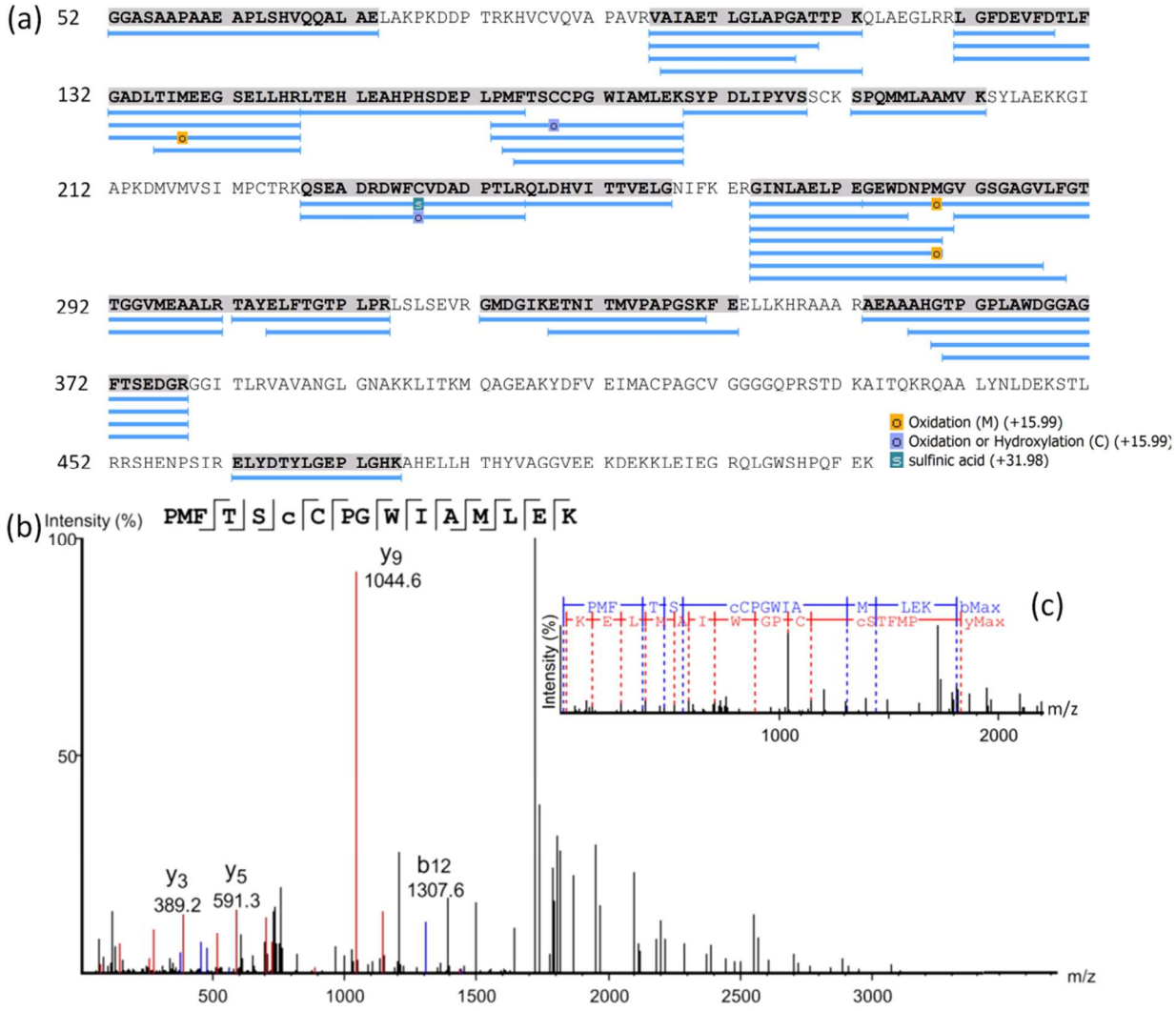


**Figure S6** CrHydA1 (10 mg $mL^{-1}$) was exposed to ~0.1% $O_2$ for 2 h. The enzyme activity was monitored by gas chromatography, after the activity decayed to 50 µmol $min^{-1}$ $mg^{-1}$ the sample was allowed to equilibrate in an anaerobic Coy chamber for 30 min. The enzyme was then subjected to a trypsin digest and analyzed by mass spectrometry as described in the Experimental Section of the manuscript. Peptides were searched for by nonspecific cleavage, which increased coverage, likely due to fragmentation during HPLC and ionization.[1] No peptides were identified when using a nonspecific cleavage search against a non-CrHydA1 protein sequence. (a) Sequence coverage of CrHydA1 (60%) with modifications indicated at the bottom right of the coverage map. (b) Mass spectra of the peptide fragment that corresponds to sulfenic acid and site 169. (c) Annotated spectrum with alignment.




(1) König S., Zeller M., Peter-Katalinic J., Roth J., Sorg C., Vogl T. *J. Am. Chem. Soc. Mass Spectrom.* **2001**, 12, 1180

**Figure s7.** Titration of $O_2$ inactivated of CrHydA1 with $HydF^{EG}$ showing restoration of activity of activation of oxygen inactivated CrHydA1. Excess of $HydF^{EG}$ to CrHydA1 is required to reach high levels of activation presumably due to the low occupancy of the activating element (2Fe sublcuster) on HydF when HydF is heterologously expressed in *E. coli* as previously described (McGlynn, S. E.; Shepard, E. M.; Winslow, M. A.; Naumov, A. V.; Duschene, K. S.; Posewitz, M. C.; Broderick, W. E.; Broderick, J. B.; Peters, J. W., *FEBS Lett.* **2008,** *582* (15), 2183-2187.).

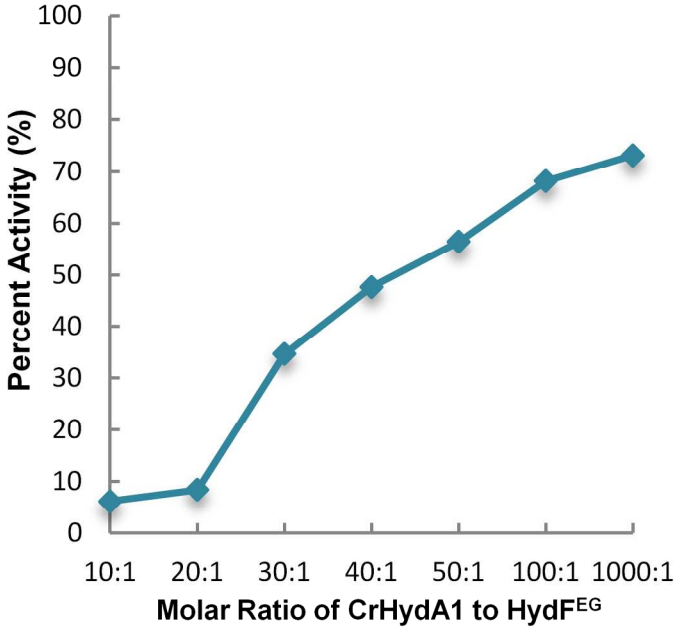


**Table S1. Summary of $O_2$ concentrations and O2:CrHydA1 molar ratios for FTIR experiments.**

| Treatment | Mole ratio ($O_2$:CrHydA1) | Final O2 Headspace (%, SD = +/- 0.15*%) | Time (min) | Figure | Experiment |
|---|---|---|---|---|---|
| $H_{ox}$ | 0.06 | 0.01 | 133 | Figures 2a and 3 | FTIR |
| | 5.07 | 1.02 | 120 | Figure S4 | FTIR |
| | 50.43 | 10.15 | 120 | Figure S4 | FTIR |
| $H_{ox}$-CO | 39.3 | 2.47 | 120 | Figure 2b | FTIR |
| | 392.99 | 24.69 | 120 | Figure S1 | FTIR |
| $H_{red}$-$H_2$ | 1.25 | 0.02 | 120 | Figure 2c | FTIR |
| $H_{red}$-DT | 0.06 | 0.002 | 120 | Figure S2 | FTIR |
| | 1.25 | 0.05 | 120 | Figure 2d | FTIR |
| | 51.05 | 2.06 | 120 | Figure S2 | FTIR |
| $H_{ox}$ | 0.06 | 0.001 | 120 | Figures 5b and 5c | UV-Vis |
| | 1000 | 17 | 120 | Figures 5b and 5c | UV-Vis |
| | 17,647 | 300 | 180 | Figures 5b and 5c | UV-Vis |

**Table S2. Average and standard deviation values of $\%O_2$ used for O2 treatments.**

| Vial | Injection volume (µL) | [a]$O_2$ (nmol) | [b]Molarity ($mol^{-1}$ $L^{-1}$ $O_2$) | [c]$pO_2$ (atm) | [d]$O_2$ (%) |
|---|---|---|---|---|---|
| 1 | 50 | 14 | 3 E-04 | 0.0069 | 0.84 |
| 2 | 50 | 15 | 3 E-04 | 0.0072 | 0.88 |
| 3 | 50 | 12 | 2.4 E-04 | 0.0059 | 0.71 |
| 4 | 50 | 15 | 3 E-04 | 0.0070 | 0.85 |
| 5 | 50 | 19 | 4 E-04 | 0.0090 | 1.10 |
| Average ± SD | | 15 ± 2 | | | 0.88 ± 0.14 |

[a] The $O_2$ peak area from GC was converted to nmol O2 based on a $O_2$ standard curve.
[b] Molarity=O2 (nmol)/injection volume (µL).
[c] $p$O2=$n$RT/V, where $n$/V is "molarity", T=298K.
[d] %O2=$p$O2/barometric pressure. Barometric pressure was 0.82 atm in Golden, CO.

**Table S3. FTIR time-course fits and experimental conditions used to calculate O2 induced transitions in CaI and CrHydA1.**

| Enzyme | T (K) | Barometric Pressure (atm) | [a]$[O_2]$ | | [b]$k$ ($min^{-1}$) | | $k_{Hox}$ ($s^{-1}$ µM $O_2^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | | % | µM | Δ1945 $cm^{-1}$ | Δ1940 $cm^{-1}$ | |
| CaI | 277 | 0.82 | 0.28 | 3.2 | 2.2 x $10^{-3}$ | | 1.1 x $10^{-5}$ |
| CrHydA1 | 277 | 0.82 | 0.010 | 0.11 | | 2.0 x $10^{-3}$ | 3.0 x $10^{-4}$ |
| CrHydA1 | 277 | 0.82 | 0.13 | 1.49 | | 2.1 x $10^{-2}$ | 2.4 x $10^{-4}$ |

[a] molar concentration = [% $O_2$*barometric pressure (atm)/(T(K)*0.082056)]*0.03181
[b] $k$ ($s^{-1}$) = -[ln($A$)/$A_o$)]/$t$ (s). "$A$" is taken as the $H_{ox}$ specific peak at 1945 $cm^{-1}$ for CaI, and at 1940 $cm^{-1}$ for CrHydA1.

**Table S4. Rate constants for $O_2$ induced changes in CrHydA1 and CaI $H_{ox}$ *ν*CO peak intensities, and $H_2$ evolution activities.**

| Enzyme | [1] $k_{Hox}$ $\Delta H_{ox}$ *ν*CO ($s^{-1}\,\mu M^{-1}\,O_2$) | [1] $k_{inact}$ $H_2$ evolution activity ($s^{-1}\,\mu M^{-1}\,O_2$) | [2] Ref $k_{inact}$ $H_2$ evolution activity ($s^{-1}\,\mu M^{-1}\,O_2$) | [2] pH | [2] Temp (K) |
|---|---|---|---|---|---|
| CrHydA1 | ($\Delta$1940 $cm^{-1}$) $3.0 \times 10^{-4}$ | $2.0 \times 10^{-4}$ | $4.3 \times 10^{-4}$ (a)<br>$2.2 \times 10^{-3}$ (b) | This study, 8<br>Ref. a, 6<br>Ref. b, 6 | This study, 277<br>Ref. a, 283<br>Ref. b, 298 |
| CaI | ($\Delta$1945 $cm^{-1}$) $1.1 \times 10^{-5}$ | $6.4 \times 10^{-6}$ | $5.1 \times 10^{-6}$ (b)<br>$4 \times 10^{-5}$ (c) | This study, 8<br>Ref. b, 6<br>Ref. c, 7 | This study, 277<br>Ref. b, 298<br>Ref. c, 303 |

[1] $k_{Hox} = -[\ln(A/A_o)]/t/[O_2]$ ($s^{-1}\,\mu M^{-1}\,O_2$). Fits and other parameters used to calculate $k_{Hox}$ are listed in Table S3. "*A*" is the $H_{ox}$ absorption signal assigned to the 1940 or 1945 $cm^{-1}$ *ν*CO peak for CrHydA1 and CaI, respectively. Reaction conditions; pH 8, 277K.

[2] (a) Goldet, G., et al. 2009. *J. Am. Chem Soc*. 131:14979. Reaction conditions; pH 6, 283K.
(b) Stripp, S.T., et al. 2009. *P. Natl. Acad. Sci. U.S.A*. 106(41):17331. Reaction conditions; pH 6, 298K.
(c) Baffert et al. *Ang. Chem. Int. Ed*. 47(11):2052. Reaction conditions; pH 7, 303K.

**Table S5.** Data collection and refinement statistics for O2 exposed CrHydA1

| **Data Collection** | **CrHydA1** |
|---|---|
| Wavelength (Å) | 1.734 |
| Unit cell parameters<br>a, b, c (Å)<br>α, β, γ (°) | <br>77.6, 71.0, 94.7<br>90.0, 91.9, 90.0 |
| Space group | P 1 21 1 |
| Resolution range (Å) | 34.73-2.29<br>(2.241-2.29*) |
| Total reflections | 288694 |
| Unique reflections | 45032 |
| R-merge(%) | 11 (40*) |
| I/σ (I) | 6.6 (3.1*) |
| Completeness (%) | 96 (87*) |
| Redundancy | 6.4 (5.9*) |

*Refinement*

| | |
|---|---|
| Resolution limits (Å) | 35-2.3 |
| No of used reflections | 39011 |
| No of protein atoms | 6282 |
| R factor (%) | 20.9 |
| R free (%) | 25.2 |
| Ramachandran Favorite regions (%) | 95.9 |
| Ramachandran Allowed regions (%) | 4.1 |
| Ramachandran Outliers (%) | 0.0 |
| R.m.s. deviations from ideal values, bond lengths of refined atoms(Å) | 0.019 |
| Angels (Å) | 2.09 |
| Average B, all atoms ($A^2$) | 49.48 |
| Wilson B-factor ($A^2$) | 25.6 |

* Values in parentheses are for the highest resolution shell.