

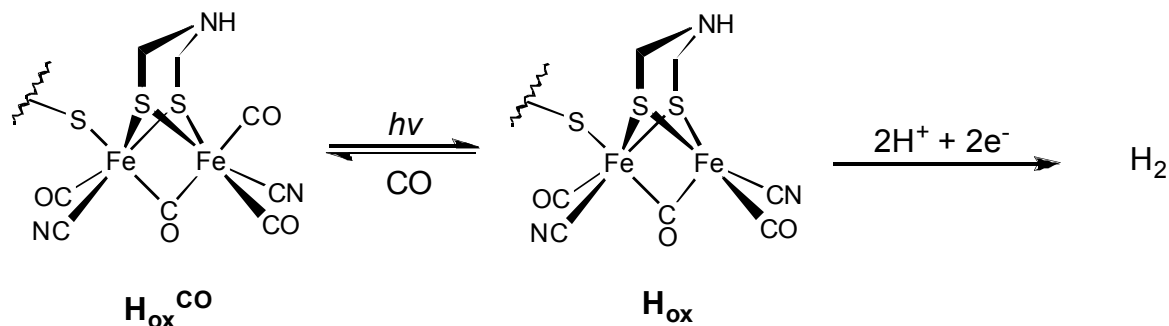
## Structural and Spectroscopic Models of the [FeFe]-Hydrogenase Enzyme

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The [FeFe]-hydrogenase enzymes catalyze the interconversion of hydrogen with protons and electrons. The active site (H-cluster) is composed of a diiron core in a face-shared bi-octahedral structure.<sup>1, 2</sup> The H-cluster is attached to the protein via coordination of a [Fe<sub>4</sub>S<sub>4</sub>(Cys)<sub>4</sub>] cluster to the proximal iron (Fe<sup>P</sup>) center via a cysteinyl thiolate. Each iron is coordinated by a terminal cyanide and terminal carbonyl ligand. The irons are bridged by a carbonyl ligand and a five member light atom bridge, proposed to an azadithiolate bridge (SCH<sub>2</sub>NHCH<sub>2</sub>S).<sup>3</sup> The active state of the enzyme (H<sub>ox</sub>) is characterized by an open coordination site on the distal iron (Fe<sup>D</sup>). It has been shown that the enzyme is inhibited by CO, resulting in H<sub>ox</sub><sup>CO</sup> (Figure 1). The oxidation state of the irons in H<sub>ox</sub> and H<sub>ox</sub><sup>CO</sup> is a subject of a large amount of research.<sup>4, 5</sup> Spectroscopy of the enzyme reveals for both states the diiron core is paramagnetic (S = 1/2). It is proposed that the active state of the enzyme consists of an Fe<sup>II</sup>-Fe<sup>I</sup> species.<sup>6</sup> Research in this area is aimed at elucidating the mechanism of the enzymatic catalysis. A specific goal is the preparation of mixed-valence complexes that are similar in structure and have spectroscopy analogs to the enzyme with the expectation that function will follow form.



**Figure 1.** The H<sub>ox</sub><sup>CO</sup> and H<sub>ox</sub> states of the active site of the [FeFe]-hydrogenase enzyme

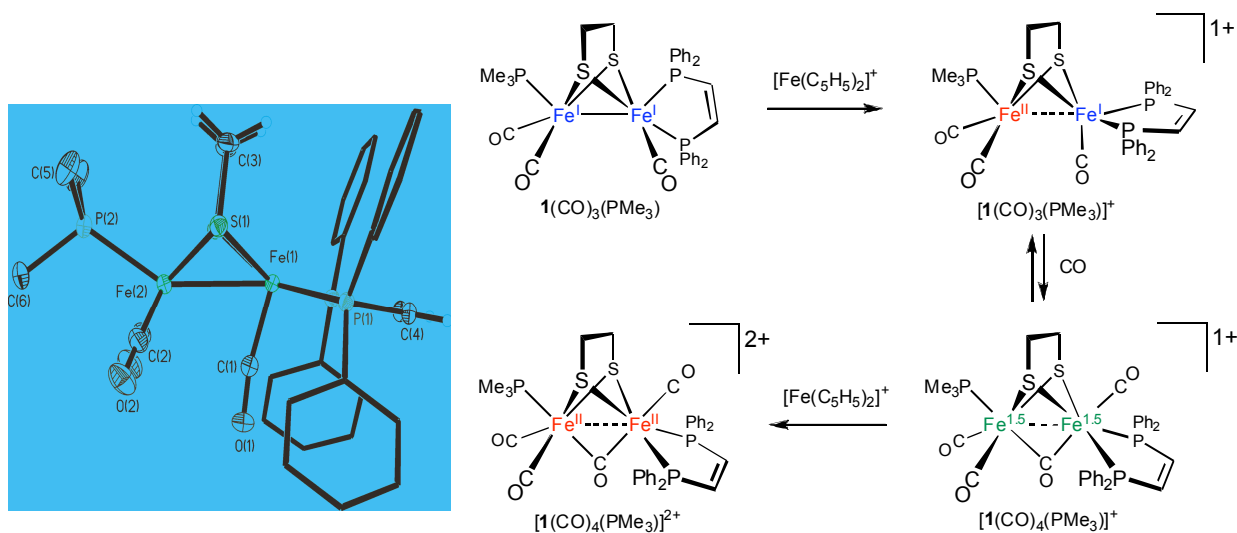
Synthetic model complexes are based on the chelating phosphine cis-1,2-bis(diphenylphosphino)ethylene (dppv).<sup>7, 8</sup> The dppv ligand was chosen because of its preference to chelate to a single iron center, rather than bridge between the two iron centers. The complex Fe<sub>2</sub>(S<sub>2</sub>C<sub>2</sub>H<sub>4</sub>)(CO)<sub>4</sub>(dppv) (**1**(CO)<sub>4</sub>) was prepared in high yield by the decarbonylation of Fe<sub>2</sub>(S<sub>2</sub>C<sub>2</sub>H<sub>4</sub>)(CO)<sub>6</sub> in the presence of dppv. The geometry adopted by the dppv ligand (apical-basal vs. di-basal) is influenced by the nature of the chelating dithiolate. For **1**(CO)<sub>4</sub>, the dppv ligand spans the apical-basal coordination sites, while for the larger 1,3-propanedithiolate (**2**(CO)<sub>4</sub>) the dppv ligand exists in both the apical-basal and di-basal coordination geometries. Complex **1**(CO)<sub>4</sub> was found to readily react with PMe<sub>3</sub> to yield Fe<sub>2</sub>(S<sub>2</sub>C<sub>2</sub>H<sub>4</sub>)(CO)<sub>3</sub>(PMe<sub>3</sub>)(dppv) (**1**(CO)<sub>3</sub>(PMe<sub>3</sub>)). The substitution process was found to proceed through a “rotated” transition state,<sup>9</sup> which is favored by the unsymmetrical ligand set.

Oxidation of **1**(CO)<sub>3</sub>(PMe<sub>3</sub>) in MeCN with two equiv of FcPF<sub>6</sub> yielded the diferrous complex [Fe<sub>2</sub>(S<sub>2</sub>C<sub>2</sub>H<sub>4</sub>)(μ-CO)(CO)<sub>2</sub>(PMe<sub>3</sub>)(dppv)(NCMe)]<sup>2+</sup>.<sup>10</sup> When only one equiv of oxidizing agent was used, a 1:1 mixture of unreacted starting material and the diferrous complex

was observed, with no evidence for a mixed-valence intermediate. Upon changing the solvent to the non-coordinating  $\text{CH}_2\text{Cl}_2$ , very different reactivity was observed. The cyclic voltammogram of  $\mathbf{1}(\text{CO})_3(\text{PMe}_3)$  in  $\text{CH}_2\text{Cl}_2$  under  $\text{N}_2$  revealed a reversible one electron oxidation (+20 mV vs. Ag/AgCl). When the cyclic voltammogram was recorded under a CO atmosphere two separate one electron oxidations were observed (-05 mV and +230 mV vs. Ag/AgCl).

On a preparative scale, the addition of one equiv  $\text{FcBF}_4$  to a  $\text{CH}_2\text{Cl}_2$  solution of  $\mathbf{1}(\text{CO})_3(\text{PMe}_3)$  at  $-45^\circ\text{C}$ , resulted in complete consumption of the starting material and yielded the mixed-valence complex  $[\text{Fe}_2(\text{S}_2\text{C}_2\text{H}_4)(\text{CO})_3(\text{PMe}_3)(\text{dppv})]\text{BF}_4$  ( $[\mathbf{1}(\text{CO})_3(\text{PMe}_3)]\text{BF}_4$ ).<sup>11</sup> The structure of  $[\mathbf{1}(\text{CO})_3(\text{PMe}_3)]^+$  was confirmed crystallographically (Figure 2). The “inverted” structure of  $[\mathbf{1}(\text{CO})_3(\text{PMe}_3)]^+$  approaches that proposed for the  $\text{H}_{\text{ox}}$  state of the active site with a vacant apical coordination site and a bridging carbonyl. The EPR spectrum of  $[\mathbf{1}(\text{CO})_3(\text{PMe}_3)]^+$  is consistent with a  $S = 1/2$  species, with  $g$  values of 2.1384, 2.0280 and 2.0102. Each  $g$  value appeared as a triplet, indicating the unpaired spin ( $\text{Fe}^{\text{I}}$ ) was coupled to two equivalent phosphorus nuclei. From solution EPR data the oxidation state of  $[\mathbf{1}(\text{CO})_3(\text{PMe}_3)]^+$  can be confidently assigned; the  $\text{Fe}(\text{CO})(\text{dppv})$  remains  $\text{Fe}^{\text{I}}$  while the  $\text{Fe}(\text{CO})_2(\text{PMe}_3)$  has been oxidized to  $\text{Fe}^{\text{II}}$ .<sup>12</sup>

The unsaturated character of  $[\mathbf{1}(\text{CO})_3(\text{PMe}_3)]^+$  is indicated by its reactivity with CO, which occurs in seconds.<sup>13</sup> The product;  $[\text{Fe}_2(\text{S}_2\text{C}_2\text{H}_4)(\mu\text{-CO})(\text{CO})_3(\text{PMe}_3)(\text{dppv})]^+$   $[\mathbf{1}(\text{CO})_4(\text{PMe}_3)]^+$  is highly unstable and could only be observed under a CO atmosphere. The EPR spectrum for  $[\mathbf{1}(\text{CO})_4(\text{PMe}_3)]^+$  featured a doublet of triplets, coupled to three phosphorus nuclei with two distinct phosphorus hyperfine couplings ( $A(^{31}\text{P}) = 283, 32$  and  $34$  MHz). Based upon the changes in the EPR, upon binding CO the unpaired spin becomes delocalized over both iron centers resulting in two  $\text{Fe}^{(1.5)}$  metal centers (Figure 2).



**Figure 2.** Left) Structure of the cation in  $[\text{Fe}_2(\text{S}_2\text{C}_2\text{H}_4)(\text{CO})_3(\text{PMe}_3)(\text{dppv})]\text{BF}_4$  Right) Reaction scheme for one electron oxidation chemistry of  $\text{Fe}_2(\text{S}_2\text{C}_2\text{H}_4)(\text{CO})_3(\text{PMe}_3)(\text{dppv})$

## References

- 1) Peters, J. W., Lanzilotta, W. N., Lemon, B. J. and Seefeldt, L. C. X-ray Crystal Structure of the Fe-Only Hydrogenase (*CpI*) from *Clostridium pasteurianum* to 1.8 Å Resolution *Science* **1998**, *282*, 1853-58.
- 2) Nicolet, Y., Piras, C., Legrand, P., Hatchikian, C. E. and Fontecilla-Camps, J. C. *Desulfovibrio desulfuricans* Iron Hydrogenase: the Structure Shows Unusual Coordination to an Active Site Fe Binuclear Center *Structure*, **1999**, *7*, 13-23.
- 3) Nicolet, Y., de Lacey, A. L., Vernede, X., Fernandez, V. M., Hatchikian, E. C. and Fontecilla-Camps, J. C. Crystallographic and FTIR Spectroscopic Evidence of Changes in Fe Coordination Upon Reduction of the Active Site of the Fe-Only Hydrogenase from *Desulfovibrio desulfuricans* *J. Am. Chem. Soc.* **2001**, *123*, 1596-601.
- 4) Roseboom, W., Lacey, A. L., Fernandez, V. M., Hatchikian, E. C. and Albracht, S. P. J. The active site of the [FeFe]-hydrogenase from *DdH* II. Redox properties and CO-exchange as observed by IR spectroscopy *J. Biol. Inorg. Chem.* **2006**, *11*, 102-18.
- 5) Albracht, S. P. J., Roseboom, W. and Hatchikian, E. C. The active site of the [FeFe]-hydrogenase from *DdH* I. Light sensitivity and magnetic hyperfine interactions as observed by electron paramagnetic resonance *J. Biol. Inorg. Chem.* **2006**, *11*, 88-101.
- 6) Silakov, A., Reijerse, E. J., Albracht, S. P. J., Hatchikian, E. C. and Lubitz, W. The Electronic Structure of the H-Cluster in the [FeFe]-Hydrogenase from *Desulfovibrio desulfuricans*: A Q-band <sup>57</sup>Fe-ENDOR and HYSCORE Study *J. Am. Chem. Soc.* **2007**, *129*, 11447-58.
- 7) de Beer, J. A., Haines, R. J., Greatrex, R. and Greenwood, N. N. Stereochemistry of the bis-substituted derivatives of bis-(m-alkyl- and -phenylsulfidotricarbonyliron) *J. Organomet. Chem.* **1971**, *27*, C33-C35.
- 8) de Beer, J. A., Haines, R. J., Greatrex, R. and Greenwood, N. N. Synthesis, infrared spectra, and Moessbauer spectra of some tertiary and ditertiary phosphine, arsine, and stibine derivatives of bis(m-methylsulfido-, -ethylsulfido-, and -phenylsulfidotricarbonyliron) *J. Chem. Soc.* **1971**, 3271-82.
- 9) George, S. J., Cui, Z., Razavet, M. and Pickett, C. J. The Di-iron subsite of all-iron hydrogenase: mechanism of cyanation of a synthetic {2Fe3S} - carbonyl assembly *Chem. - Eur. J.* **2002**, *8*, 4037-46.
- 10) van der Vlugt, J. I., Rauchfuss, T. B. and Wilson, S. R. Electron-Rich Diferrous-Phosphane-Thiolates Relevant to Fe-only Hydrogenase: Is Cyanide "Nature's Trimethylphosphane"? *Chem. Eur. J.* **2005**, *12*, 90-98.
- 11) Liu, T. and Darensbourg, M. Y. A Mixed-Valent, Fe(II)Fe(I), Diiron Complex Reproduces the Unique Rotated State of the [FeFe]Hydrogenase Active Site *J. Am. Chem. Soc.* **2007**, *129*, 7008-9.
- 12) Thomas, C. M., Darensbourg, M. Y. and Hall, M. B. Computational definition of a mixed valent Fe(II)Fe(I) model of the [FeFe]hydrogenase active site resting state *J. Inorg. Biochem.* **2007**, *101*, 1752-57.
- 13) Razavet, M., Borg, S. J., George, S. J., Best, S. P., Fairhurst, S. A. and Pickett, C. J. Transient FTIR Spectroelectrochemical and Stopped-Flow Detection of a Mixed Valence {Fe(I)-Fe(II)} Bridging Carbonyl Intermediate with Structural Elements and Spectroscopic Characteristics of the Di-Iron Sub-Site of All-Iron Hydrogenase *Chem. Commun.* **2002**, 700-01.