Hydrogenase

Chem 489

April 20, 2010

Philip Duttweiler

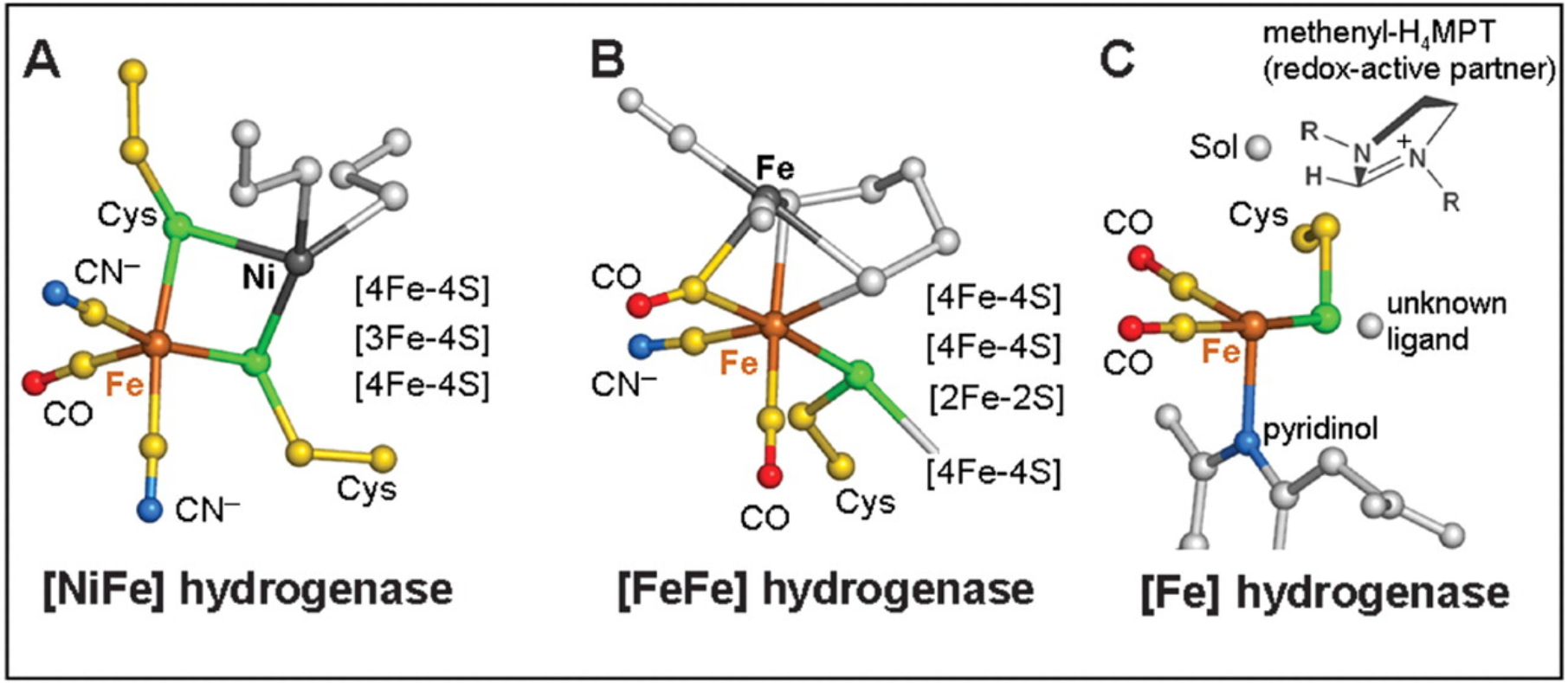
Hydrogenase Sophistication

Richard Cammack, (*Nature*, *Vol. 397*, 1999)

"The [FeFe]-hydrogenases are highly evolved catalysts. Under optimum conditions, each molecule of the *D. desulfuricans* enzymes can produce 9,000 molecules of hydrogen per second at 30°C; for *C. pasteurianum* hydrogenase the figure is 6,000 s^{-1}. Extrapolation suggests that 1 mole of hydrogenase could produce enough hydrogen to fill the airship *Graf Zeppelin* in ten minutes, or the main liquid-hydrogen tank of the Space Shuttle in two hours (this fanciful calculation assumes a sufficient supply of reductant and protons, and disregards the time required to transfer hydrogen from solution to the gas phase)."

But, 1 mole = 90 kD = ca. 200 lbs.

Active Sites of Three Types of Hydrogenase

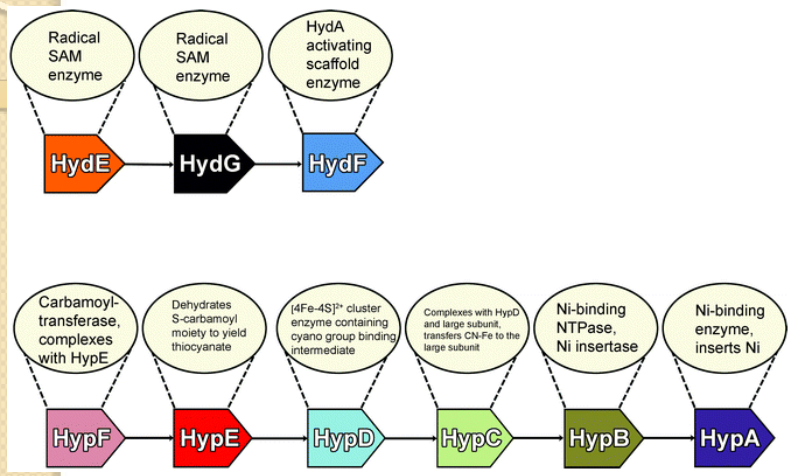


Shima, Seigo; Pilak, Oliver; Vogt, Sonja; Schick, Michael; Stagni, Marco S.; Meyer-Klaucke, Wolfram; Warkentin, Eberhard; Thauer, Rudolf K.; Ermler, Ulrich. Science (Washington, DC, United States) (2008), 321(5888), 572-575

[NiFe]- and [FeFe]-Hydrogenases: Some important events and people

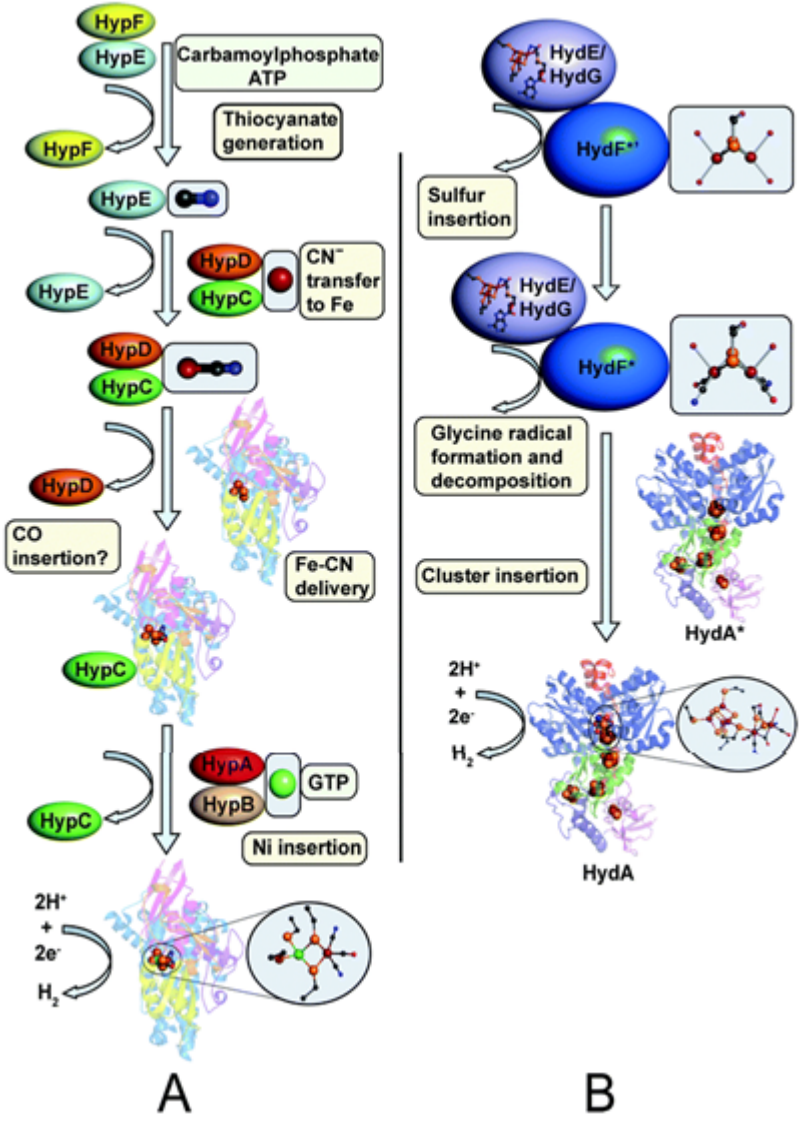
- Interesting obituary of discoverer: Marjory Stephenson, 1885-1948. Biochem. 1950, vol. 46, p. 25.
- Discovery, 1950's. She also proved H_2ases were bidirectional.
- Understanding the copious iron is in form of FeS clusters, 1950's
- Discovery that 80% or so H_2ases also contained Ni, 1980
- Studies connect activity of ([NiFe]-H_2ases with spectroscopic (epr) signals of nickel
- Discovery of diatomic ligands, CO and CN^- in [NiFe] active site: (Kim Bagley, Simon Albracht and W. Woodruff, 1994).
- First protein crystal structure ([NiFe]-H_2ase) (Anne Volbeda, Michel Frey and Juan Fontecilla Camps, 1995)
- Protein crystal structures of [FeFe]-H_2ase) (John Peters (1) and Juan Fontecilla Camps, 1999 and 2000)
- Maturation and other studies establish the three classes, [Fe]-, [NiFe]- and [FeFe]-H_2ases are phylogenetically distinct: Convergent evolution. (Bock; Friedrich 1990's and forward.)

Natural synthesis



A) Ni-Fe Hydrogenase

B) Fe-Fe Hydrogenase



McGlynn, S. E.; Mulder, D.W.; Shepard, E. M.; Broderick, J. B.; Peters, J.W. Hydrogenase cluster biosynthesis: organometallic chemistry nature's way. *Dalton Transactions* ***2009, 4274-4285.***

Hmd = H_2-forming methylene-H_4MPT dehydrogenase: Rolf Thauer's „Hydrogenase (Doesn't do typical H_2ase chemistry)

A general overview:

- Contains no FeS clusters for electron transport
- A single active iron in active site; very air and light sensitive
- A single redox level Two cofactors extracted from holoenzyme; one the inorganic or organometallic active site; the second an organic cofactor, a Guanylylpyridone (GP)
- Crystal structure as of 2007 has not defined the single iron active site. Good IR data however indicates diatomic ligands.
- Very limited biochemical role in nature.
- Promotes H/D scrambling (D_2/H_2O) mixtures only in presence of substrate
- Does double exchange (makes H_2) with rates equal to HD production

Hydrogenases (H_2ase)

$$2H^+ + 2e = H_2$$

Present in diverse organisms; 200 million tons per year

[NiFe]H_2ase: H_2 uptake

[FeFe]H_2ase: H_2 production

[Fe]H_2ase: Fe-S cluster free, methanogenic archaea.

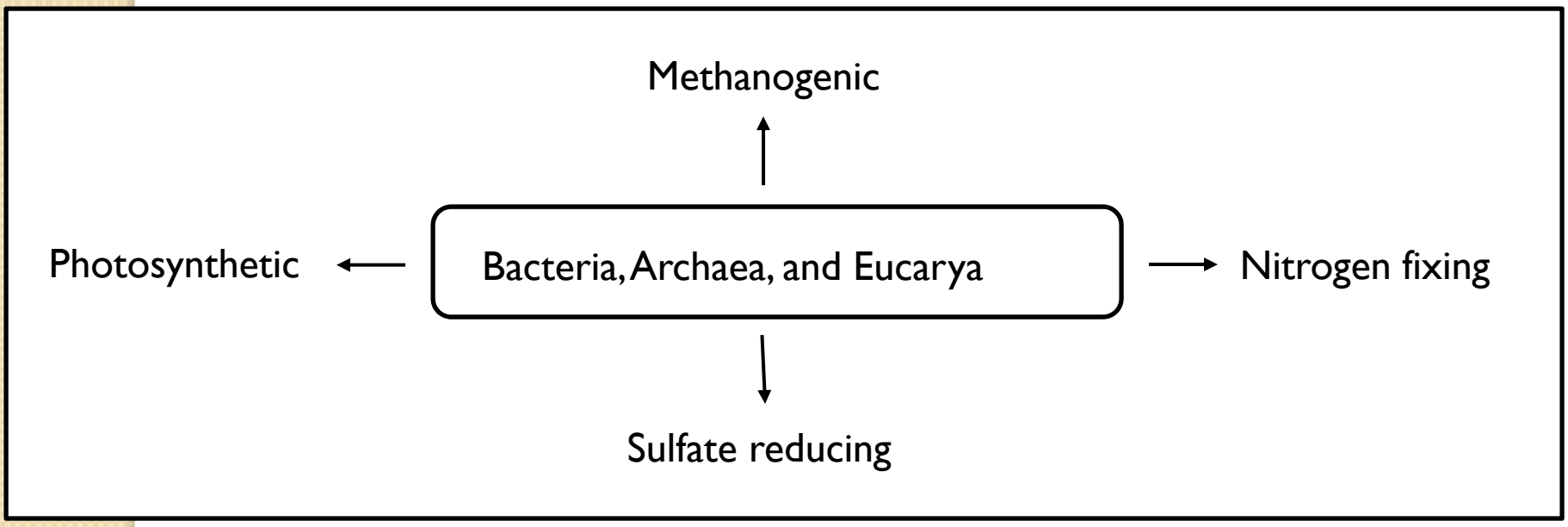
Coordination chemistry review, 249 (2005) 1609-1619
Dalton Trans., 2003, 4030-4038
Chem. Soc. Rev. 2003, 32, 268-275
PNAS, 2003, 100, 3683-3688

Hydrogenases

Ubiquitous; 100's known

Reaction catalyzed: $H_2 \rightleftharpoons 2\,H^+ + 2\,e^-$

Heterolytic spitting: $H_2 \rightleftharpoons H^+ + H^-$



Classification: 4Fe4S Cluster-Containing

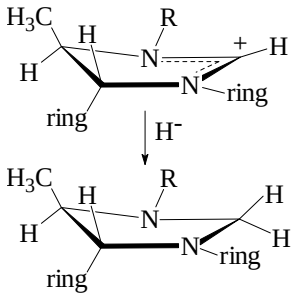
[FeFe]-H_2ase

- H_2 production
- Activity 10 – 100 x [NiFe]H_2ase
- Most O_2 sensitive H_2ase

[NiFe]- H_2ase

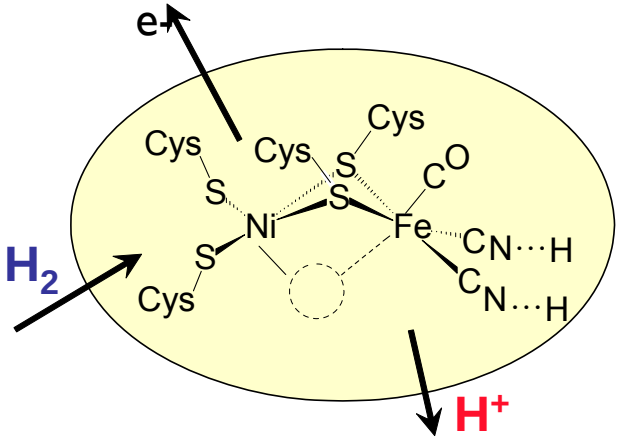
- H_2 consumption
- Majority of H_2ases
- H_2 affinity 100 x [Fe]H_2ase

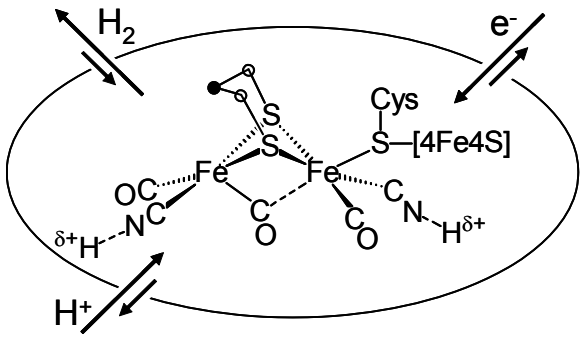
Hmd (single Fe)



Thauer

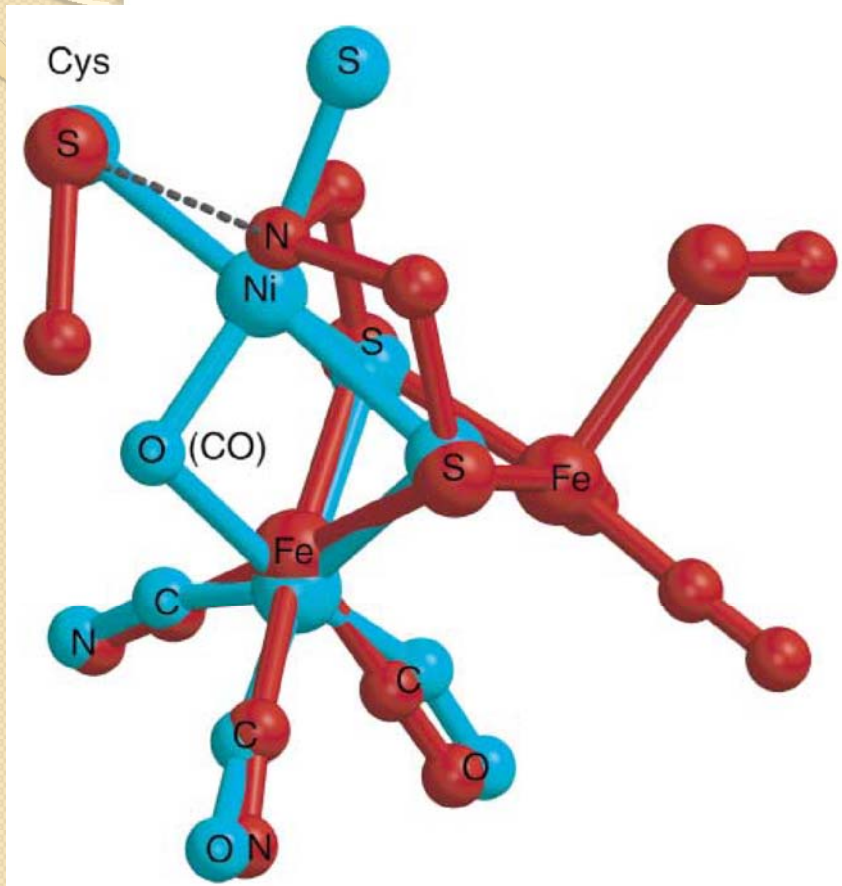
Comparison of [NiFe] and [FeFe] H_2ase Active Sites

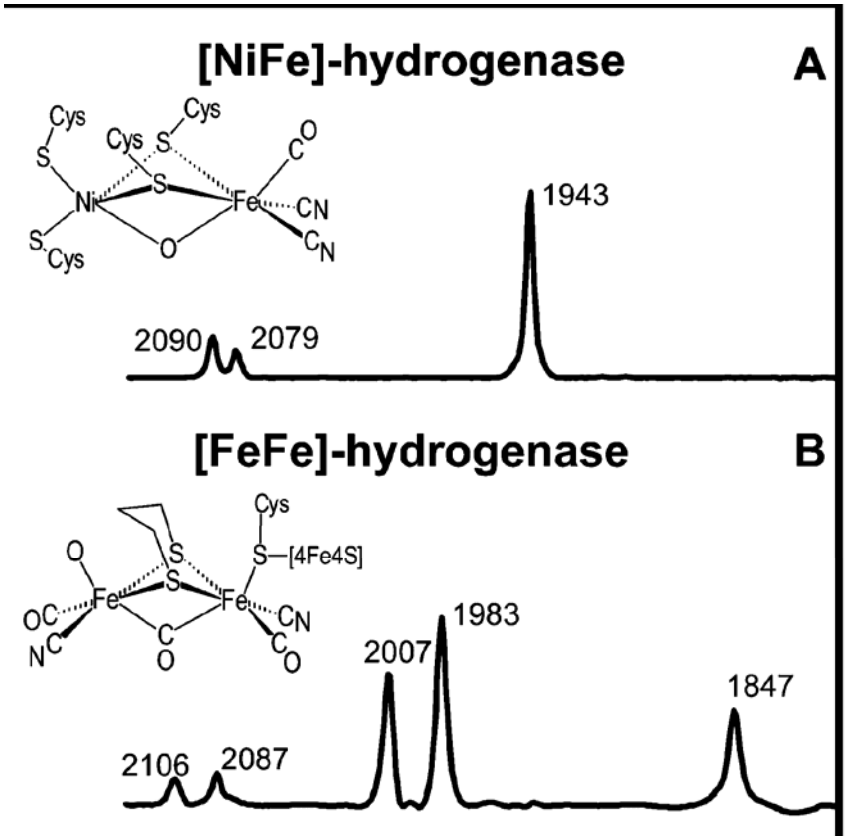




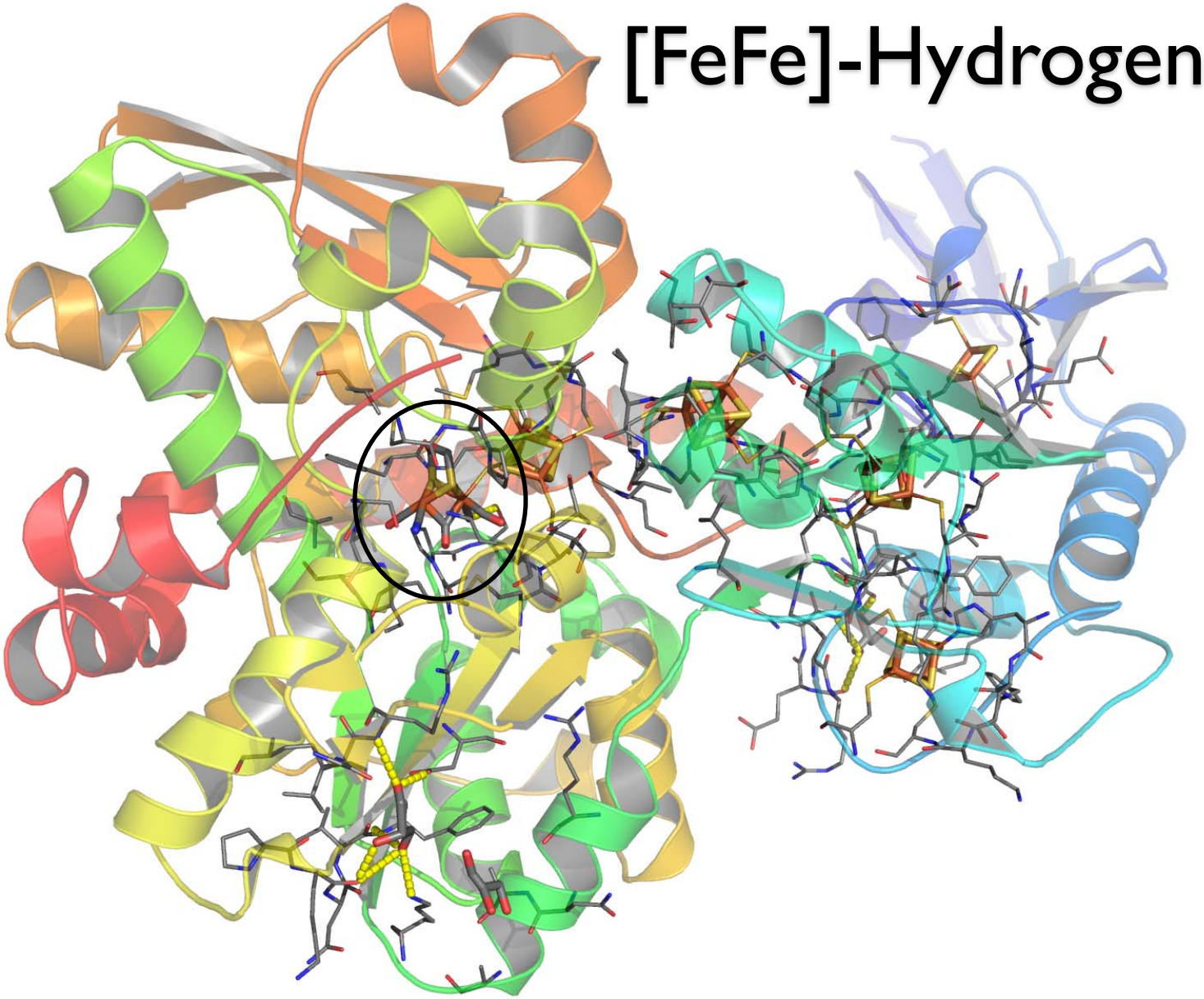
cores	M_2S_2 butterfly	Fe_2S_2 butterfly
M···M	2.7, 2.8 Å	2.6 Å
S-bridges	2 Cysteinates, RS^-	bidentate $^-SCH_2CH_2CH_2S^-$ or $^-SCH_2NHCH_2S^-$
Observed Redox levels	Ni^IFe^{II} $Ni^{II}Fe^{II}$ $Ni^{III}Fe^{II}$	Fe^IFe^I reduced $Fe^{II}Fe^I$ as isolated, Hox $Fe^{II}Fe^{II}$ oxidized
CO/CN Ligands	on Fe	on Fe
Open Site	on Fe	on Fe
Exogeneous CO binds	on Ni side	on Fe

Overlay of [NiFe] and [FeFe]H_2ase





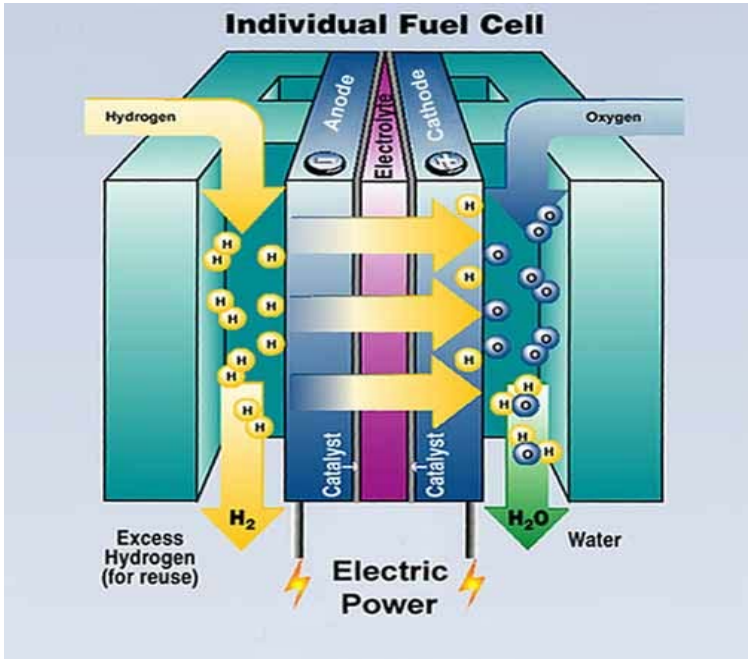
[FeFe]-Hydrogenase



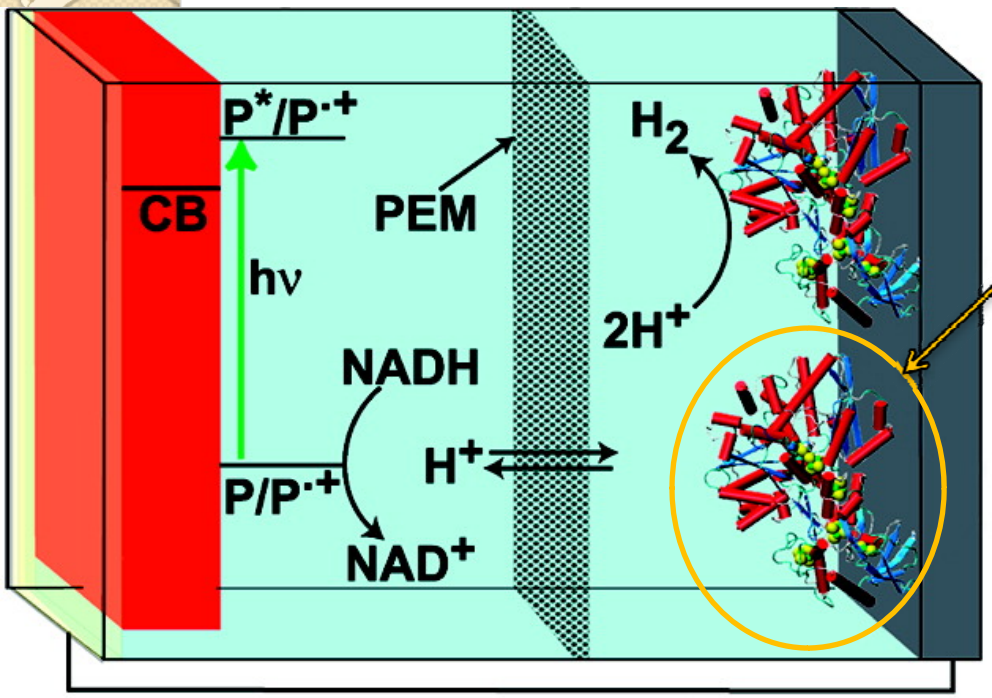
Pandey, A. S. ; Harris, T.V.; Giles, L. J.; Peters, J. W. Szilagyi, R. K. *J. Am. Chem. Soc.* **2008,** *130*, 4533

[FeFe]-Hydrogenase: The Importance

- Hydrogen Fuel Cells
  - Catalyst
- Platinum
  - Limited Supply
    - 0.003 ppb in crust
  - Poisoned by S, CO



Enzyme Effectiveness



Hydrogenase enzyme

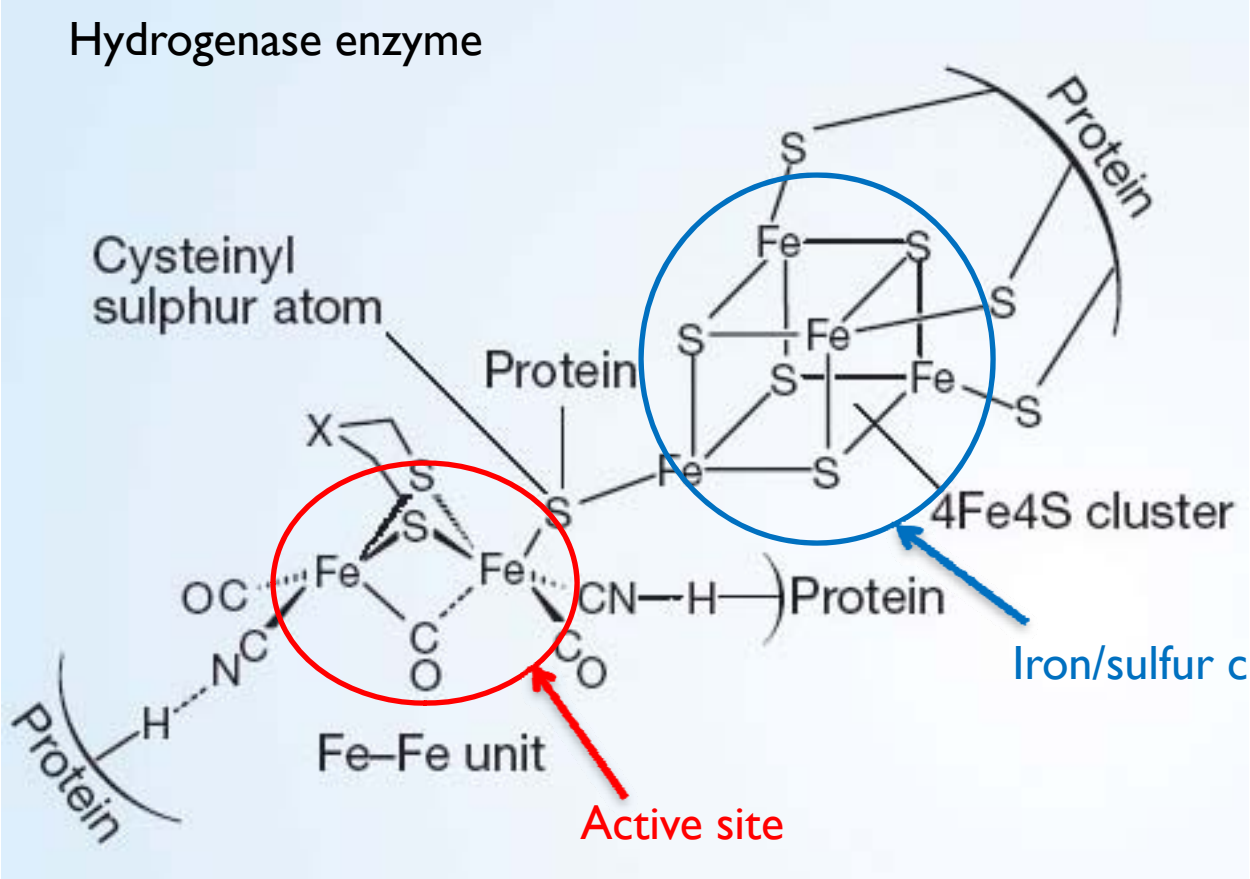
Photoelectrochemical Biofuel Cell

Had effectiveness of about 40% platinum
Without structural organization

J. Am. Chem. Soc. **2008,** *130*, 2015-2022.

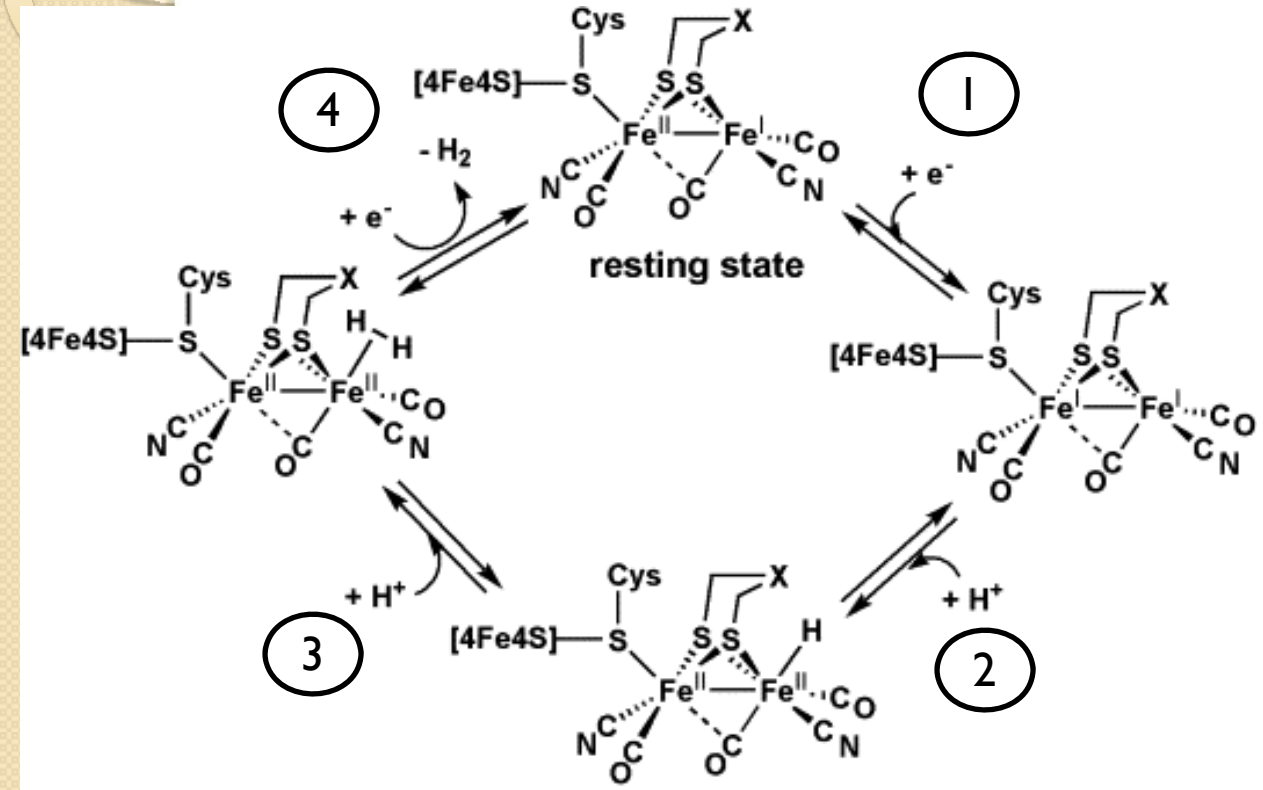
Hambourger, M.; Gervaldo, M.; Svedruzic, D.; King, P.W.; Gust, D.; Ghirardi, M.; Moore, A. L.; Moore, T.A. FeFe -Hydrogenase-Catalyzed H-2 production in a photoelectrochemical biofuel cell. *J. Am. Chem. Soc.* **2008,** *130*, 2015-2022.

[Fe Fe]H_2ase Structure



Nature **2005**, *433*, 589-591.

Reaction Sequence For [Fe Fe] Hydrogenase

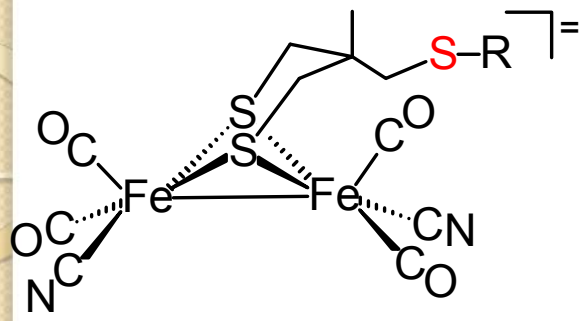


1. Addition of e^-
2. Addition of 1st proton
3. Addition of 2nd proton
4. Addition of e^- and removal of H_2

J. Inorg. Biochem. **2007,** *101*, 1752-1757.

Thomas, C. M.; Darensbourg, M.Y.; 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,* 1752-1757.

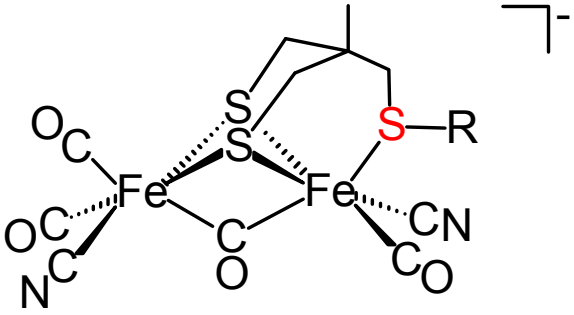
FTIR Spectroelectrochemical evidence for a Fe(I)(μ-CO)Fe(II) complex*



Fe(I)Fe(I)

ν(CN): 2075 cm^{-1}

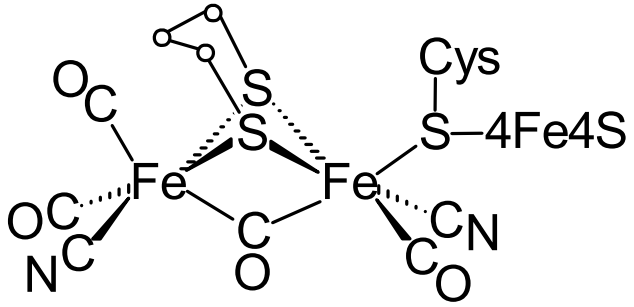
ν(CO): 1995, 1964, 1921, 1885 cm^{-1}



Fe(I)Fe(II)

ν(CN): 2107, 2083 cm^{-1}

ν(CO): 2030, 1978, 1945, 1790 cm^{-1}



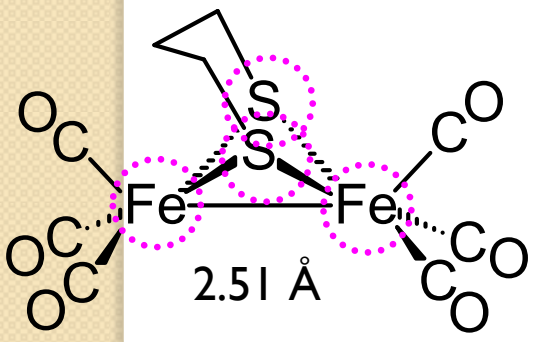
H_{ox}-CO

ν(CN): 2096, 2089 cm^{-1}

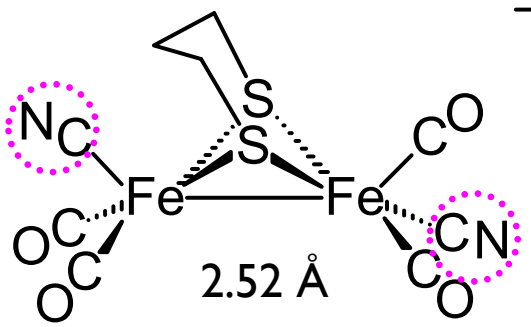
ν(CO): 2016, 1972, 1965, 1811 cm^{-1}

* M. Razavet, S. J. Borg, S. J. George, S. P. Best, S. A. Fairhurst, C. J. Pickett, *Chem. Commun.*, **2002**, 700.

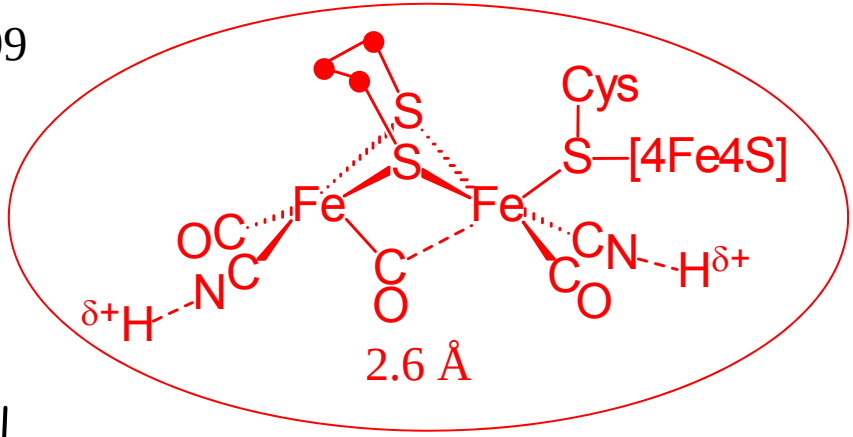
Models of [FeFe] Hydrogenase Active Site

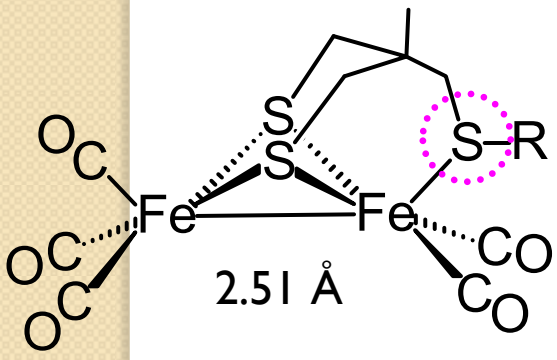


Darensbourg, 1999

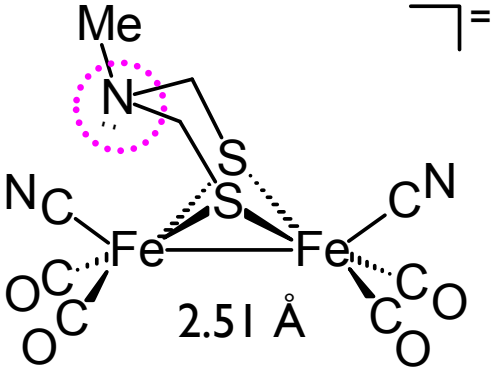


Rauchfuss, 1999
Pickett, 1999
Darensbourg, 1999



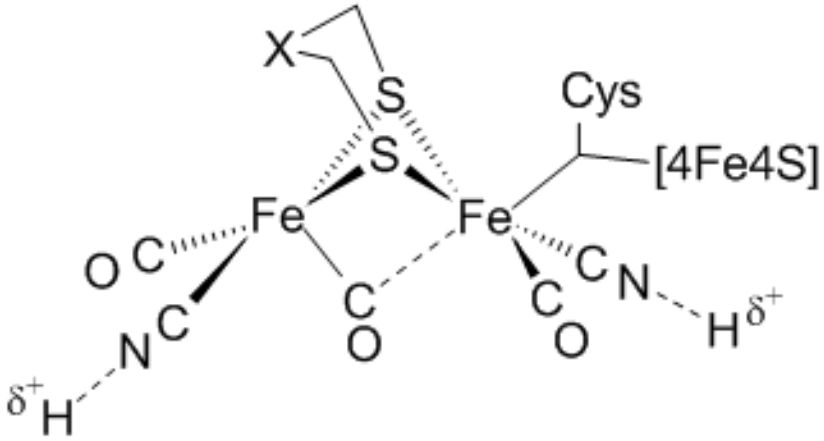


Pickett, 2001



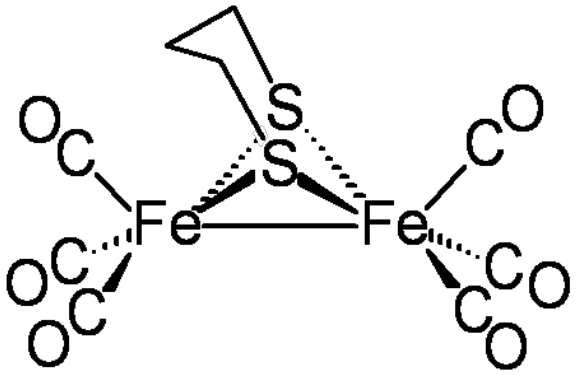
Rauchfuss, 2001

Active Site vs. Models



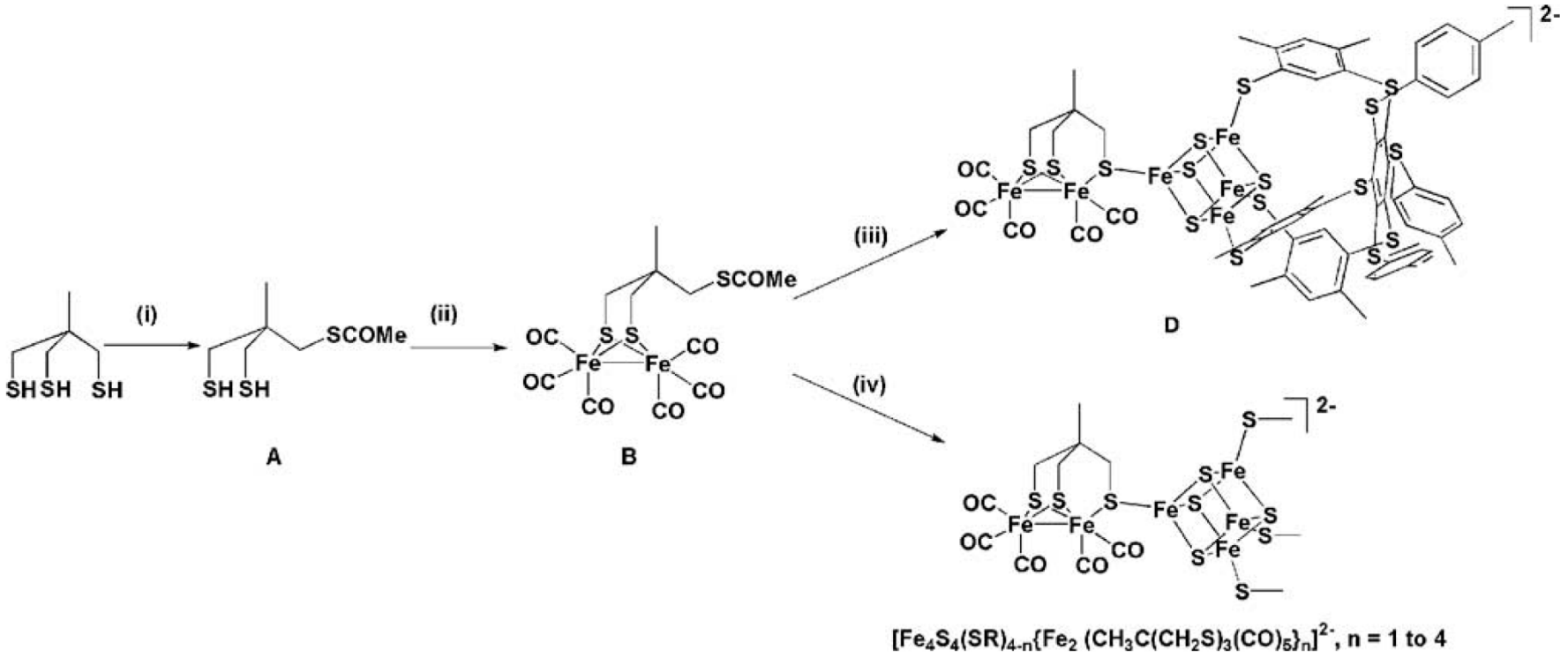
X= CH_2, NH, O

- $Fe^{I}Fe^{II}$
- CO/CN ligands
- Bridging CO / "Rotated" geometry
- Open Site



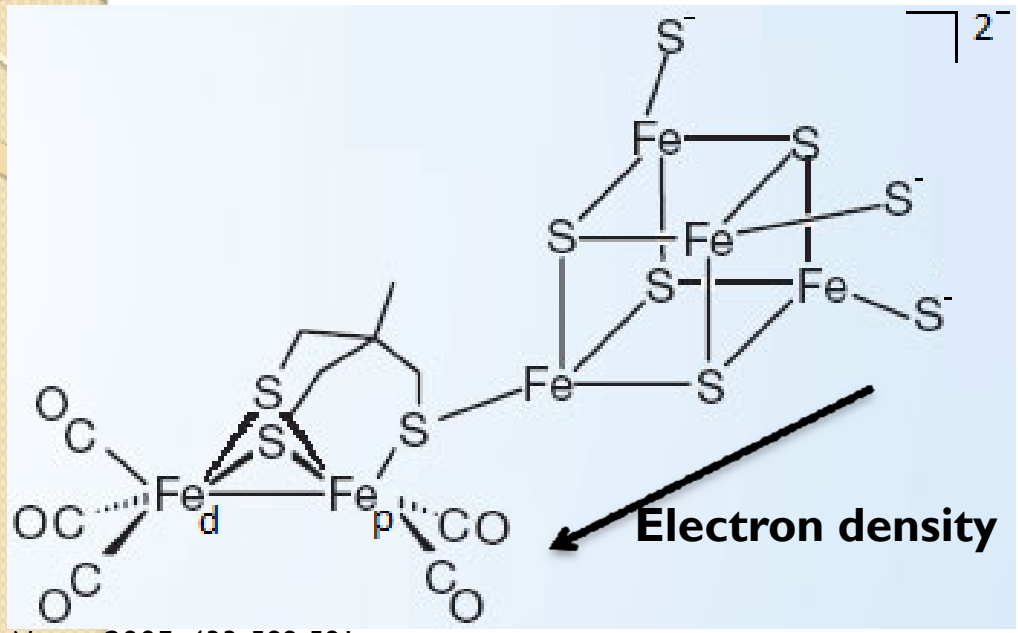
- $Fe^{I}Fe^{I}$
- All CO
- All terminal CO / "Unrotated" geometry

[FeFe]H_2ase model

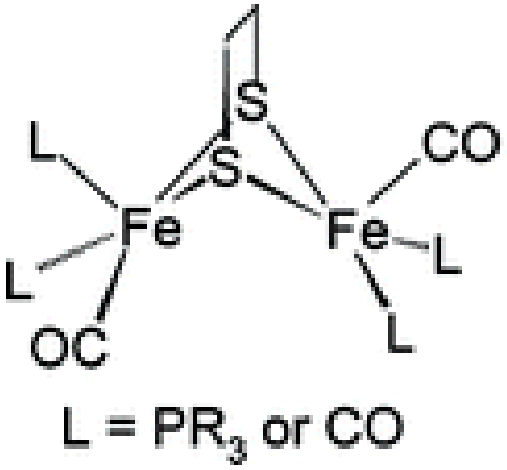


Nature, 2005, 433, 610-613

Stereochemistry of Active Site



Nature **2005**, *433*, 589-591.



JACS **2009**, *131*, 10909-10917.

Iron-iron active site drains electron density

Use of phosphine or cyanide as better electron donors than carbon monoxide

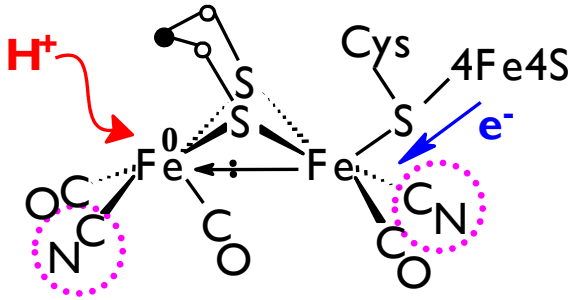
Wang, Y. W.; Li, Z. M.; Zeng, X. H.; Wang, X. F.; Zhan, C. X.; Liu, Y. Q.; Zeng, X. R.; Luo, Q. Y.; Liu, X. M. Synthesis and characterisation of three diiron tetracarbonyl complexes related to the diiron centre of FeFe -hydrogenase and their protonating, electrochemical investigations. *New J. Chem.* **2009,** *33,* 1780-1789.

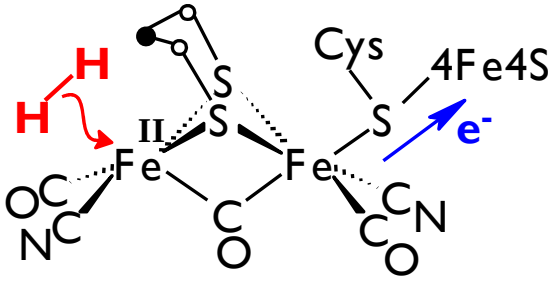
Zampella, G.; Fantucci, P.; De Gioia, L. Unveiling How Stereoelectronic Factors Affect Kinetics and Thermodynamics of Protonation Regiochemistry in FeFe Hydrogenase Synthetic Models: A DFT Investigation. *J. Am. Chem. Soc.* **2009**, *131,* 10909-10917.

Design of Models for H_2 Activation

$2H^+ + 2e^- \rightarrow H_2$ $\quad$ $H_2 \rightarrow 2H^+ + 2e^-$

Hydrogenase



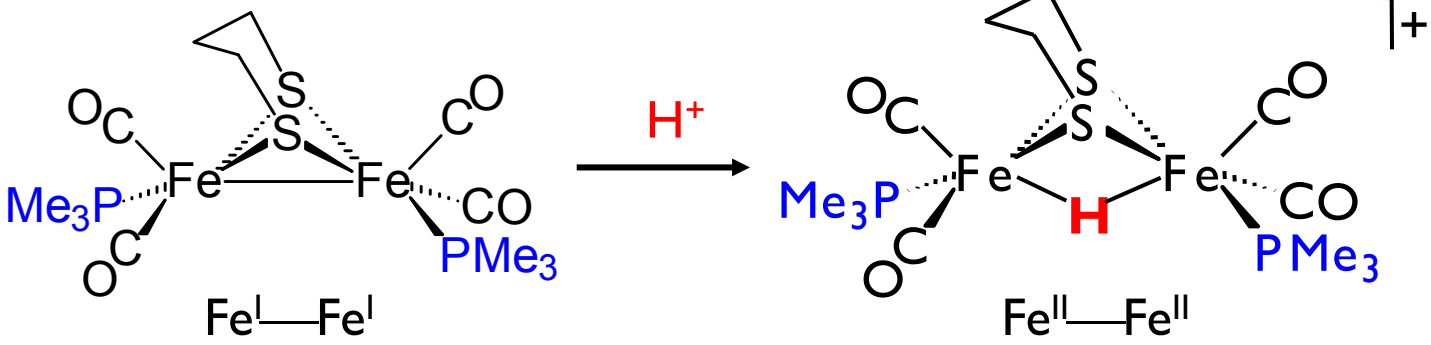


Low oxidation state
for H^+ uptake and H_2 production

High oxidation state
for H_2 binding

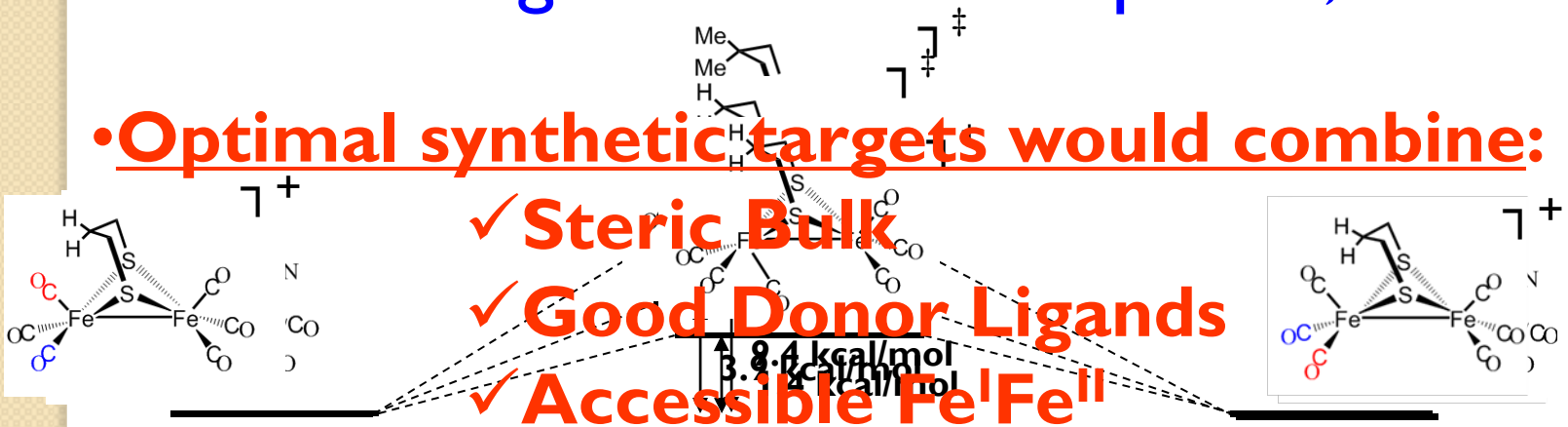
e^- rich:
$PMe_3 \sim CN^-$

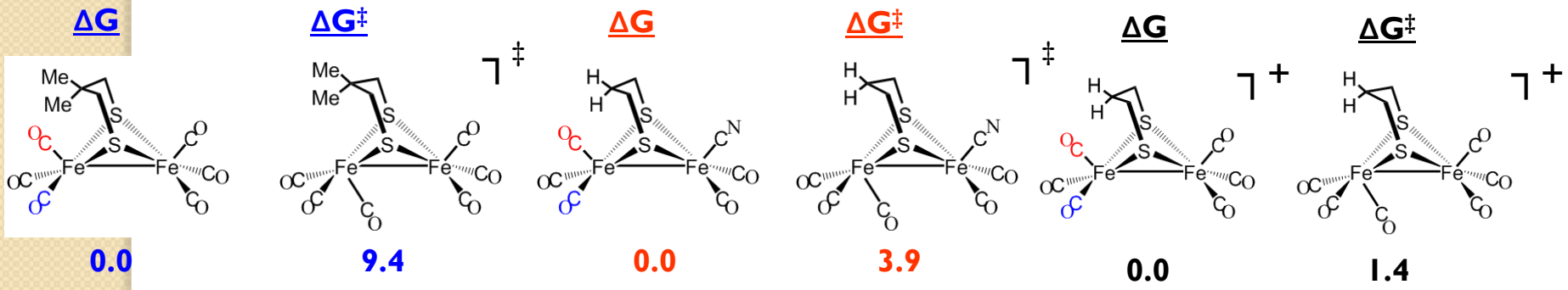
Models



DeNovo Design of Model Complexes, $Fe^{I}Fe^{I}$

- **Optimal synthetic targets would combine:**
  - ✓ **Steric Bulk**
  - ✓ **Good Donor Ligands**
  - ✓ **Accessible $Fe^{I}Fe^{II}$**

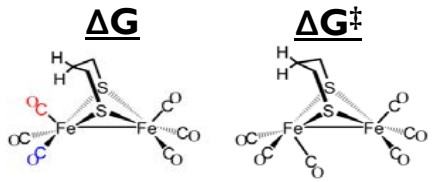




Additional Steric Bulk

Stronger Donor Ligands

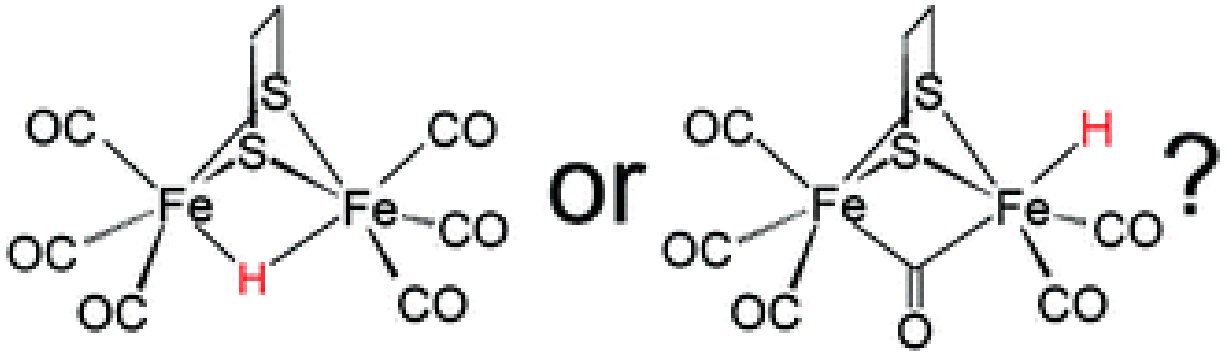
Oxidation



Tye, J. W.; Darensbourg, M.Y.; Hall, M.B. Inorg. Chem., 2006, 45, 1552-1559

Bridging Inhibition

μ-H > μ-C=O

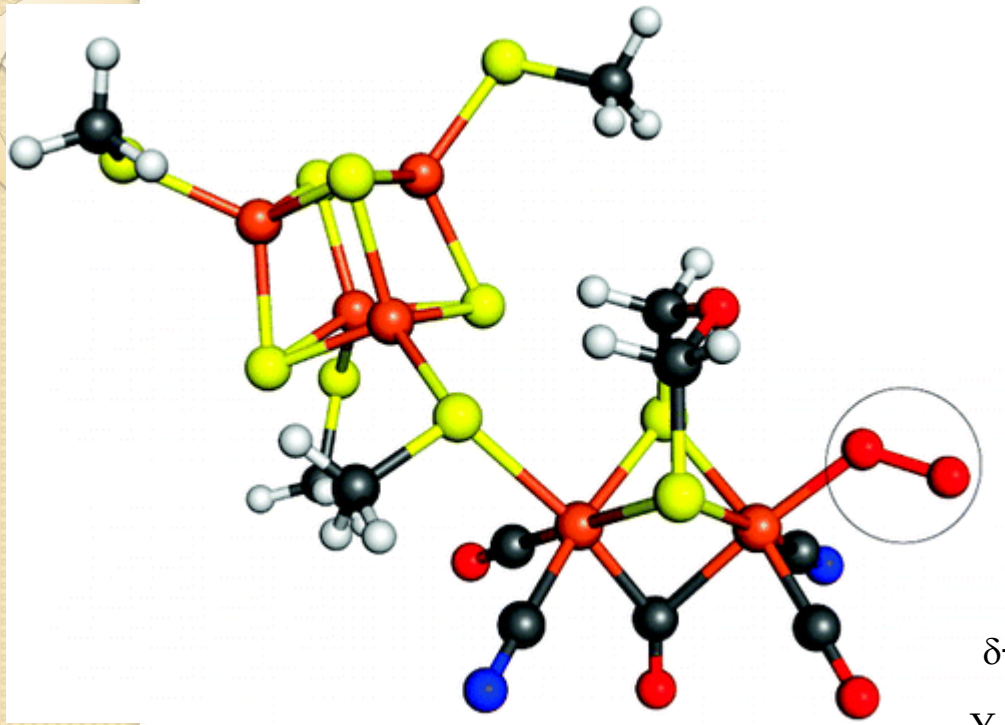


JACS **2009**, *131*, 10909-10917.

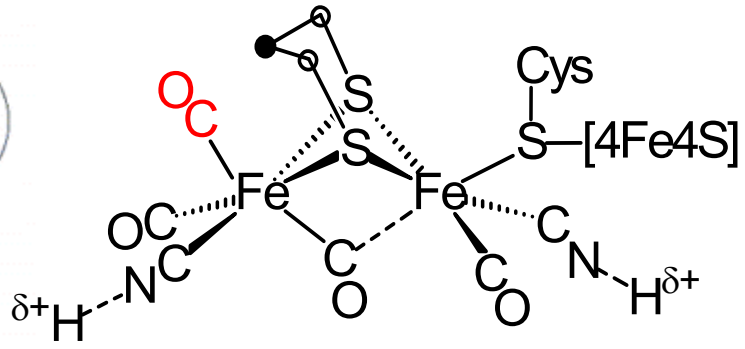
Because Hydrogen s orbitals have better overlap it is more stable in the bridging position than CO

Song, L. C.; Gai, B.; Wang, H.T.; Hu, Q. M. Synthesis, characterization and electrocatalysis of diiron propanediselenolate derivatives as the active site models of FeFe -hydrogenases. *J. Inorg. Biochem.* **2009**, *103*, 805-812.

CO and O_2 Inhibition

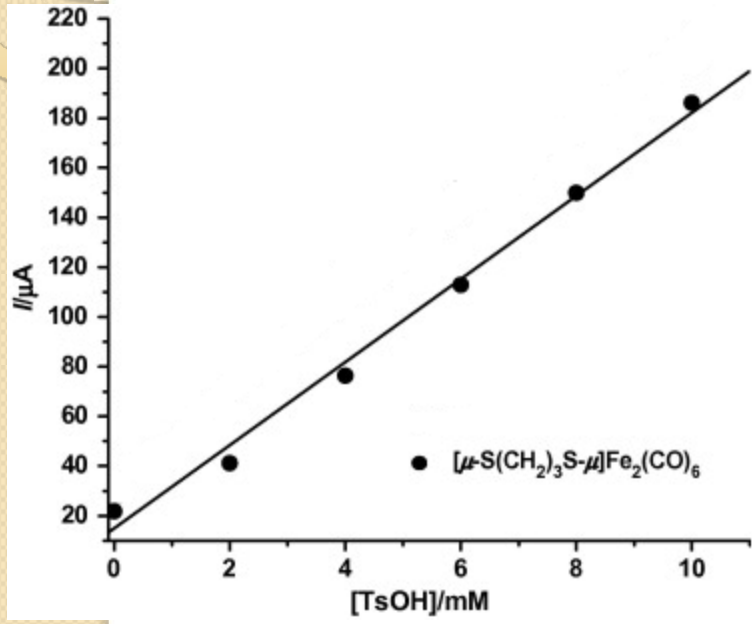


Stiebritz, M. T.; Reiher, M. Theoretical Study of Dioxygen Induced Inhibition of [FeFe]-Hydrogenase. *Inorganic Chemistry* ***2009, 48, 7127-7140.***



Y. Nicolet, et al., TIBS, **2000**, 138.

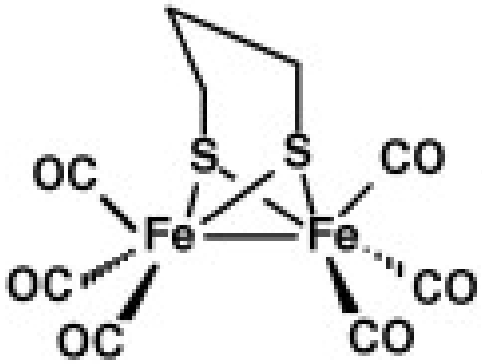
Catalytic Effectiveness



J. Inorg. Biochem. **2009**, *103*, 805-812.

Goal: 9000 H_2 molecules per second

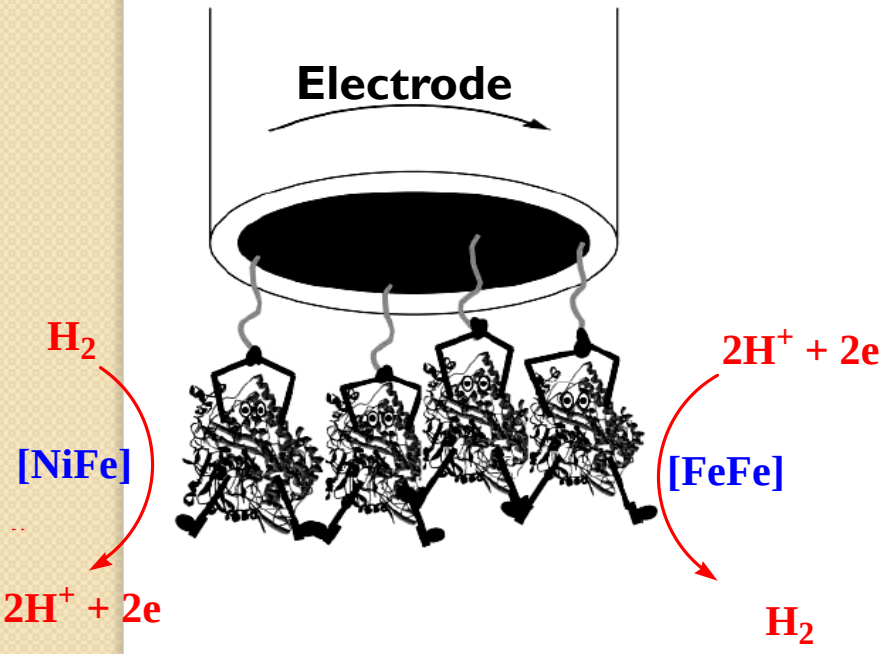
Actual: ~12 H_2 molecules per second



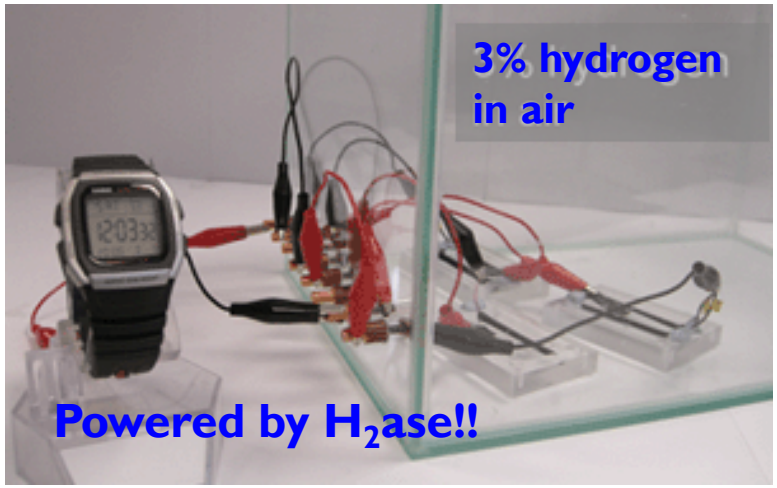
J. Inorg. Biochem. ***2008,*** *102*, 1973-1979

Sinfelt, J. H. The turnover frequency of methylcyclohexane dehydrogenation to toluene on a Pt reforming catalyst. *J. Mol. Catal. A-Chem.* **2000**, *163*, 123-128

In Vitro Applications of Hydrogenases



F. A. Armstrong Group



Downsides:
a. Difficult to produce large quantities
b. Lack of long-term stability due to air sensitivity

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