

Hydrogenases and Nitrogenases

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Fe-S Proteins

- Complex Fe-S Proteins:
 - Have enzymatic activity and contain additional prosthetic groups
 - Ex: flavins, pterins, siroheme
- Simple Fe-S proteins
 - No enzymatic activity
 - Ex: fubredoxins, ferredoxins, aconitase



Complex Fe-S Proteins Roles

- Electron transfer only
 - Very common
- Catalytic only
 - unknown
- Both
 - Today's discussion



Catalytic and e⁻ Transfer Fe-S Proteins



- Nitrogenases
 - Two types
 - Fe only catalytic center
 - Fe-Mo additional catalytic center
- Hydrogenases
 - Two types
 - Fe only catalytic center
 - Fe-Ni catalytic center

Nitrogenases



- Found in bacteria and archea
- The conversion of N₂ to NH₃
- Essential in the global nitrogen cycle
- Catalytic enzymes help in nitrogen fixation
 - $N_2 + 8H^+ + 16MgATP + 8e^- \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i$
 - Net: $N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$

Nitrogenase Function



- Consists of the Mo-Fe site and/or the Fe site
- Fe binds ATP
- ATP is hydrolyzed producing:
 - Inorganic phosphate
 - ADP
- Conversion of N₂ to NH₃ thermodynamically favored
 - Need ATP to break N₂ triple bond
- H₂ is evolved

Hydrogenases



- Found eukarya, bacteria and archaea
 - Enzymes
 - Discussing Bacteria only
- Catalyzes reversible two-electron oxidation of molecular Hydrogen
 - $2\text{H}^+ + 2\text{e}^- \leftrightarrow \text{H}_2$

Hydrogenase Function

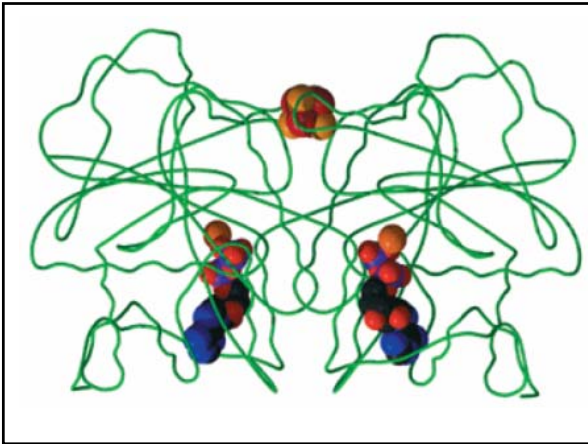


- Provide reducing equivalents for energy production
- Generally coupled with Phosphorylation
 - Ultimately leads to reduction of inorganic substances
 - SO_4^{2-} , NO_3^- , or O_2
- Generates H^+ gradient
 - For other systems (nitrogenases)

Fe Clusters in Nitrogenase



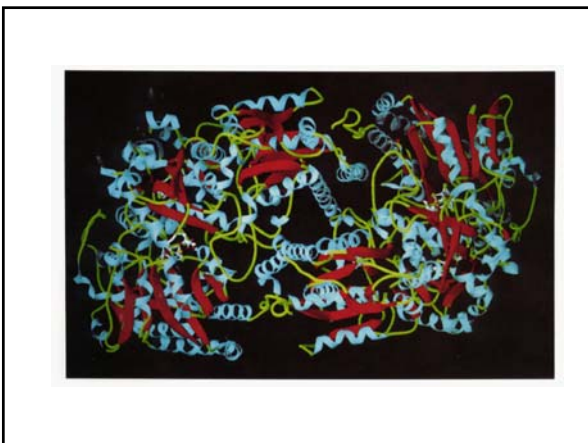
- Clusters consist of $[\text{Fe}_4\text{S}_4(\text{S}\cdot\text{Cys})_4]$
- Cluster is exposed between protein subunits
 - Sensitive to solvent and attack by dioxygen
- $[\text{Fe}_4\text{S}_4]$ cycles between 2^+ and 1^+ states
 - Donates electron to Mo-Fe cluster



Components of Mo-Fe protein



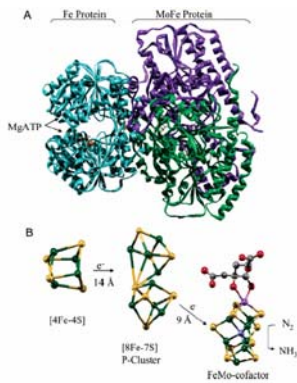
- Heterotetramer, $\alpha_2 \beta_2$,
 - subunit- $\alpha \beta$
- Two clusters
 - P- cluster
 - Provides a docking site for the Fe protein
 - Receives e^- from Fe protein which is then transferred to the M- cluster
 - M- cluster
 - Mo-Fe cofactor clusters



Fe-Mo Cofactor

- Determined through X-ray from *Azotobacter vinelandii* by Reese in 1992
- Composed of two metal sulfur, homocitrate, and N (within homocitrate)
- Undergoes three oxidation states
 $M^{OX} \leftrightarrow M^N \leftrightarrow M^{RED}$
- Relevant to binding





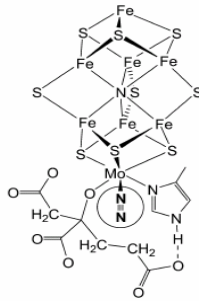
N₂ Binding Site

- The binding site for N₂ is debatable
 - Currently two theories
 - At Mo atom
 - In central region of Fe cluster
 - Problems due to MoFe protein only binding to N₂ during "turnover" conditions



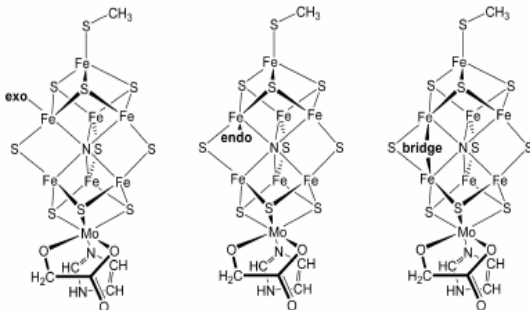
Mo as Substrate Binding Site

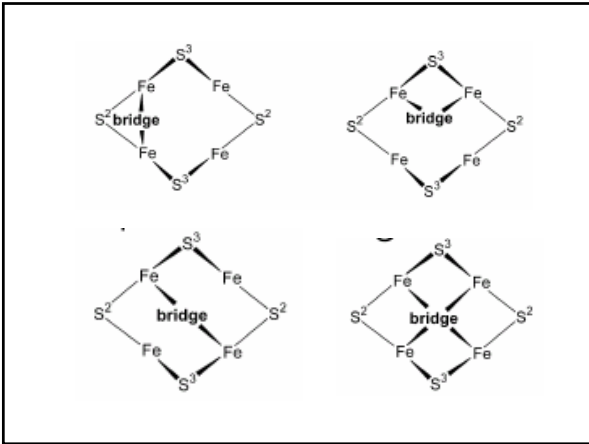
- Chatt Cycle
- 1 or more ligands need to be dissociated to provide an open coordination site
 - Possibly carboxylate O of homocitrate
- Impact N_2 reduction
 - 1- and 2- carboxylate groups
 - Hydroxyl group
 - R- coordination
 - Carbon chain length (4-6)
 - Mo



Central Fe Atom(s) as Substrate Binding Site

- Haber-Bosch process
- Studies with substrates or inhibitors
 - Not N_2
 - EPR shows new signal during 'freeze trapped'
- Does not specify which of the 6 central Fe binding occurs





Fe-only Hydrogenases

Isolated from only a few genera of bacteria

- Most studied: *Clostridium pasteurianum*
- Produces Cpl and CplI

Prior attempts at determining the H-cluster structure

- EPR
- ENDOR
- MCD
- EXAFS
- ESEEM
- RR
- Mössbauer

Cpl



- 20 Fe atoms and 18 S atoms
- 574 amino acids (22 Cys residues)
- 3- Fe_4S_4 (F) clusters, 1- Fe_2S_2 cluster, and a H-cluster

Cpl



CplI



- 14 Fe atoms and 11 S atoms
- 2- Fe_4S_4 clusters and a H-cluster

Cpl and CplI Properties



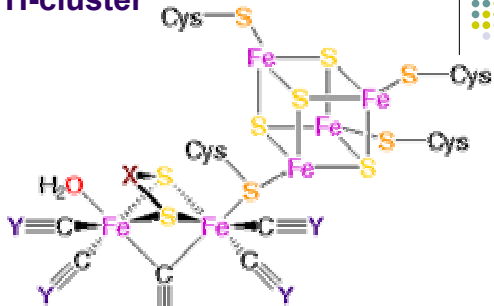
- Both are irreversibly inhibited by CO
- It is proposed that the F clusters are working as intramolecular electron transfer centers

H-cluster in Cpl



- Determined by X-ray crystallography
- 6Fe6S cluster: 2 Fe subunit is cysteine-S bridged to a 4Fe4S cubane

H-cluster



- $[\text{Fe}_2(\text{S}_2\text{X})\text{CY}](\text{CY})_4(\text{H}_2\text{O})[\text{Fe}_4\text{S}_4](\text{S Cys})_4$
- X = H₂O or ?; Y = O or N



Fe-Ni Hydrogenases

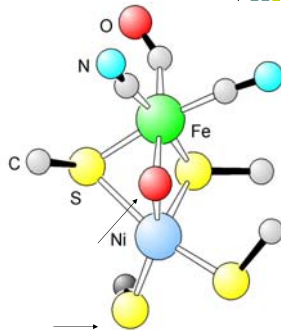


- Obtained from *Desulfovibrio gigas*
 - Commonly studied
- Alpha subunit
 - 63 kDa contains active site [Ni-Fe] metal center
- Beta subunit
 - 26 kDa subunit has two $[\text{Fe}_4\text{S}_4]^{2+/1+}$ and one $[\text{Fe}_3\text{S}_4]^{1+/0+}$ clusters
- Cytochrome C_3 protein: natural e^- acceptor

Alpha Subunit



- Ni-Fe complex
 - Ni is highly distorted square pyramidal
 - Fe is highly distorted octahedral
- Catalytic site
 - OH bridging ligand
 - Arrows indicate hydrogen present

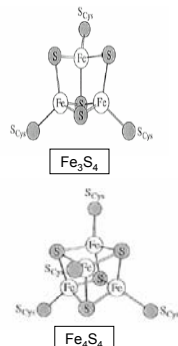


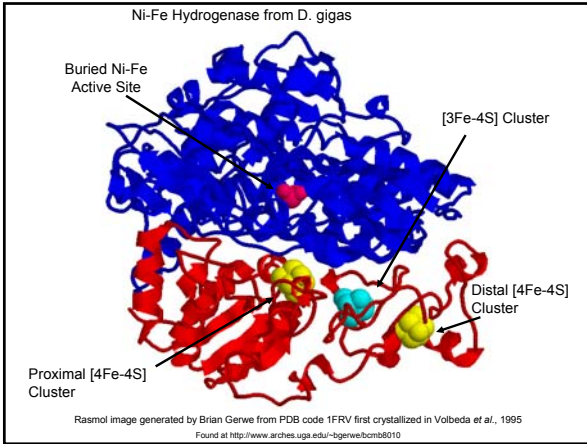
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Found at www.lbl.gov/LBL-Programs/pbd/energy/photosynthesis

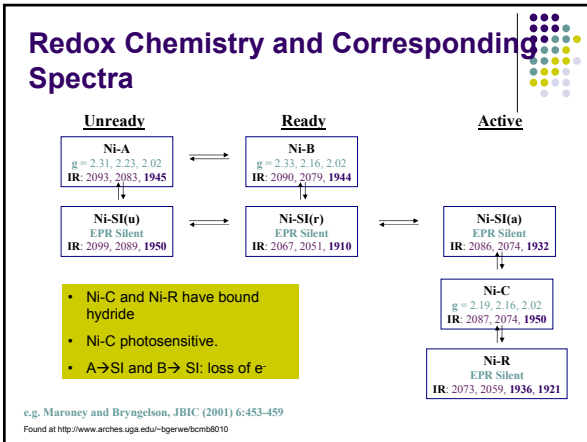
Beta subunit

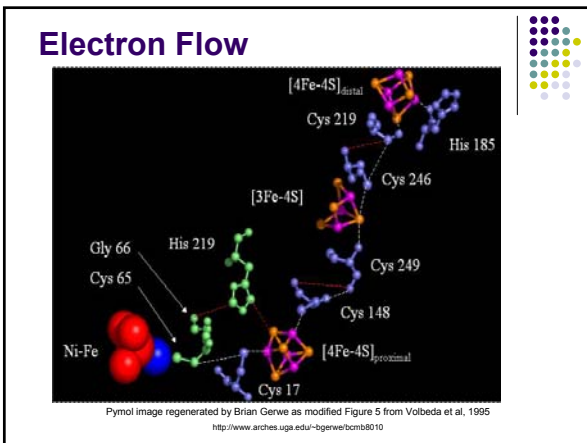


- Arranged linearly
 - $[\text{Fe}_4\text{S}_4] - [\text{Fe}_3\text{S}_4] - [\text{Fe}_4\text{S}_4]$
- Serve to transfer electrons between the Ni center and external redox agents

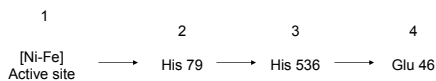








Proton Flow



- 3 → 4 occur using ordered water molecules

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