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# Quick Review of Chemistry Related to the [Fe]-Hydrogenases

**Nasser Thallaj**

***Abstract:*** ***This review provides a comprehensive overview of the synthesis, reactivity, and electrochemistry of chemical models of active site structures in [Fe] hydrogenase, an enzyme that catalyzes the reversible reduction of protons to submergence. Related literature on the structure and functions of the [Fe] hydrogenase active site, H cluster, is discussed with an emphasis on the di-iron organosome. In addition, various methods for the preparation and characterization of model complexes are discussed, as well as reactivity studies focusing on the oxidation and reduction of model compounds and their interaction with small molecules such as hydrogen and carbon monoxide. Finally, the electrochemical behavior of the model compounds is discussed in relation to their performance in electrocatalytic applications. This review provides a comprehensive analysis of the chemistry and reactivity of chemical models of the active site of [Fe] hydrogenase, which can be a valuable reference for further research.***

***Key words:*** ***Fe-hydrogenase, H-cluster, Fe4S4 Cubane, Cysteinyl Bridge, Di-Iron Organometallic Subsite, Dithiolate, Iron-Sulfur Proteins.***

## I. INTRODUCTION

The science of iron-sulfur proteins is fascinating and has been the subject of much research in recent years. Iron-sulfur proteins are metalloenzymes that contain iron-sulfur complexes known as prosthetic groups and are responsible for the catalysis of various biochemical reactions. One of the most important of these enzymes is [Fe] hydrogenase, which catalyzes the reversible reduction of protons to dip. This enzyme is notable for its unusual active site "H-cluster" consisting of a {Fe4 S4 }-cube cysteinyl-bridged to a di-iron organometallic site and linked with dithiolate, CO, and CN groups. The review focuses on the synthesis, reactivity, and electrochemistry of chemical models of [Fe] hydrogenase active site structures. The purpose of the review is to provide an overview of the state of the art in [Fe] hydrogenase research and to discuss the current understanding of the reaction chemistry and its implications for the development of new catalytic materials. The review begins with an overview of the biology and chemistry of iron-s. [1]

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## II. INORGANIC CHEMISTRY OF BIOLOGICAL SYSTEMS

Metals are an integral part of living systems, and the energy-generating reactions for these systems are usually inorganic (eg, photosynthesis and respiration). It is estimated that at least one third of proteins and enzymes require metal ions as cofactors for biological activity. These "biological" metals, including magnesium, calcium, and members of the first transition series (excluding scandium, titanium, and chromium), as well as molybdenum, tungsten, cadmium, and mercury, are usually bound to polypeptide bones by endogenous ligands. of amino acid side chains. Iron, which is ubiquitous on Earth (about 5% in the earth's crust) and readily available, is one such metal that is essential for living organisms and participates in essential biological mechanisms.

### A. Roles of iron in metalloproteins

Nature has used metal ions to perform many functions related to life processes. Substrate-binding metalloproteins are capable of catalyzing redox reactions, even if the overall reaction does not involve a net change in oxidation state. Iron metalloproteins are mainly used to regulate dioxygen and its metabolic byproducts and electron transfer. Iron proteins can be classified into three categories: heme proteins, non-heme proteins, and iron-sulfur proteins. The term heme is derived from a Greek root meaning "blood", referring to a porphyrin macrocycle that has the ability to bind to a protein covalently or by coordinating two axial positions above and below the macrocycle. It is the most common group of prostheses in the human body. [2]

*a.* *a. Dioxygen uptake, transport, and storage*

The reversible binding of dioxygen molecules does not depend on the enzymatic activity of proteins, but is facilitated by three known systems involving metalloproteins. These include hemoglobin (Hb), which has a hemiprosthetic group, and two other proteins that contain octahedrally coordinated iron centers linked by µ-oxo and two bridging carboxylate groups, namely hemerythrin (Hr) and myohemerythrin (myo Hr). This bond enables the retention of O2 in a tissue such as muscle, facilitated by a metal core structure analogous to transport proteins. [3]

*a.* *b. Dioxygen activation*

Iron porphyrin proteins are responsible for many catalytic biochemical activities. The number of oxygen atoms added to C-H or C-C bonds determines whether these enzymes are called dioxygenases (eg, catechol dioxygenase) or monooxygenases (eg, cytochrome P-450 and methane oxygenase).

*a. c. Electron transfer*

Organisms use cytochromes to facilitate the transfer of electrons in both the respiratory chain and photosynthesis. These heme proteins are classified according to their structures and physical properties, where the heme group is either covalently bound to the protein (cytochromes b and c) or bound to the protein through non-covalent interactions, such as hemoglobin. Hemiiron ligands can be histidine, methionine, cysteine or lysine, which control the redox potential of the FeIII/FeII couple and can vary from +260 mV for cytochrome c to +20 mV for cytochrome b5 compared to a standard hydrogen electrode. Some cytochromes may also contain multiple heme units, such as cytochrome c or nitrite reductase. [4]

## III. IRON-SULFUR PROTEINS

About 1% of mammalian iron is found in the form of iron-sulfur proteins. Most of these proteins are involved in electron transfer, while some have enzymatic, structural, and sensory functions. These proteins are thought to have played an influential role in early evolution due to their ease of formation, thermal stability, and abundance in most organisms. Compared to other electron transfer proteins such as cytochromes and flavoproteins, the active sites of iron-sulfur proteins are iron and inorganic sulfur, which were abundant on the early Earth. [5]

### A. Iron-sulfur cluster biosynthesis

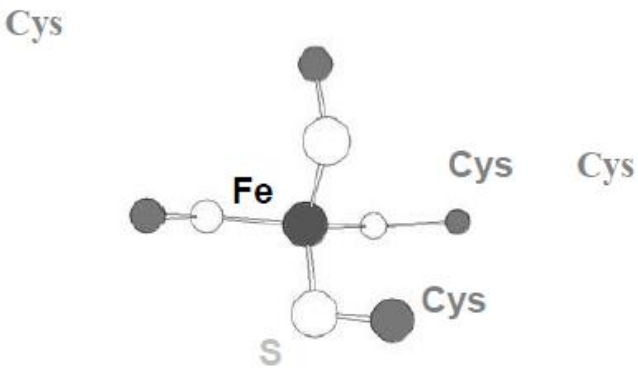
Iron-sulfur clusters are collections of iron and sulfur atoms found in various proteins. Over the last four decades, more than a hundred proteins containing these clusters have been identified. Studies have shown that these clusters are involved in several biological processes such as nitrogen fixation, photosynthesis and respiration. As a result, scientists began to investigate the mechanisms by which these systems are formed. The processes underlying the biosynthesis of iron-sulfur clusters are not fully understood, but recent studies have made significant progress in understanding the internal pathways controlling metal transport. Iron-sulfur clusters can be built in vitro with apoproteins from the necessary components and under the right conditions. However, this is not possible in life because there is no free metal inside the cell. To avoid the dangerous effects of freely available metals, such as free radical-generating Fenton reactions, certain proteins known as metallochaperones can transport metals to a specific apoprotein. Three different pathways have been identified for the biosynthesis of iron-sulfur clusters in bacteria: ISC, NIF and SUF. ISC is responsible for cluster synthesis, while NIF is only involved in nitrogen maturation. SUF is a minor iron and sulfur biosynthetic pathway.

Homologs of ISC proteins have been identified in many fully sequenced genomes, from bacteria to plants and humans, and are considered to be an important pathway for the biosynthesis of iron-sulfur clusters. Of the ISC proteins, IscU and IscS appear to be the most critical; IscU binds the iron-sulfur cluster, which is then transferred to the desired protein, while IscS delivers the sulfur to the IscU protein to produce the cluster. [6]

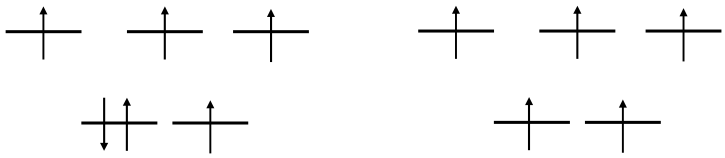
*a. a. Classes of Fe-S clusters*

The iron-sulfur centers can be classified into four sites: rubredoxin (Rb) [Fe(S-Cys) ] and three different sites for ferredoxin proteins (rhombic [Fe2S2(S-Cys) ], cubic [Fe3S (S-) ) ) Cys )3] and cubane [Fe2 S3 (S-Cys) ]). The oxidation states of the iron atoms in these centers are mostly FeII and/or FeIII in the roughly tetrahedral FeS coordination unit. [6]

*a. b. Rubredoxins (Rb)*




Proteins consist of a single iron center surrounded by a sequence of four cysteine amino acids with redox capability. X-ray crystallography showed that the distances between the iron and sulfur atoms remain almost constant (about 2.3 angstroms) when comparing the reduced and oxidized forms, although a slight distortion of the tetrahedral geometry is observed at the iron site. Spectroscopic studies, including electron paramagnetic resonance (EPR) and Mössbauer spectroscopy, have shown that ferrous iron (FeII) has a high spin state and resides in a tetrahedral environment (as depicted in Scheme I-1).



$Fe^{II}$ high-spin $Fe^{III}$ high-spin

**Scheme I-1. High-spin electronic distribution for Fe(II) and Fe(III).**

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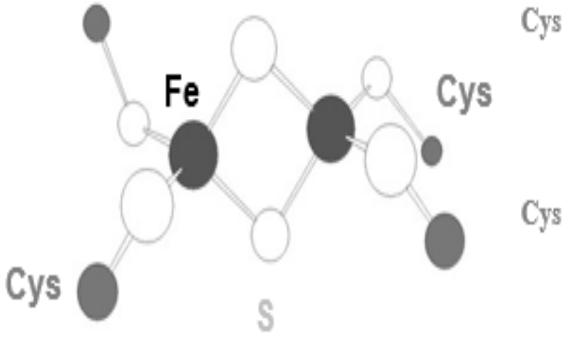
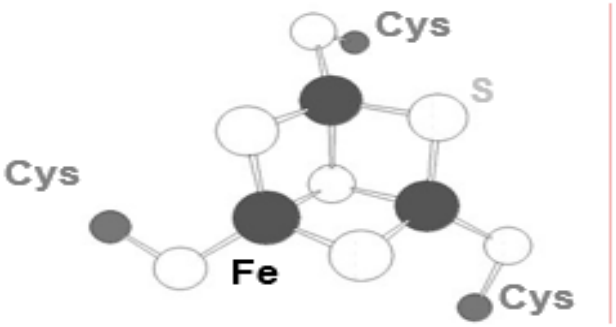
This indicates the fact that the electronic interaction between iron and sulfur is strong enough to produce specific spectroscopic and redox properties in different Fe-S centers. This effect is illustrated by the strong red color resulting from a ligand-metal charge transition (LMCT) from the σ- and π-orbitals of the thiolate ligands (electron-rich) to oxidized FeIII (electron-poor). . the protein is in an oxidized state. The reduction potential of waste redoxins is typically between -50 and+ 50 millivolts relative to a standard hydrogen electrode (SHE). [7]

*a. c. β {Fe2S2}-centres (2-Iron ferredoxin)*



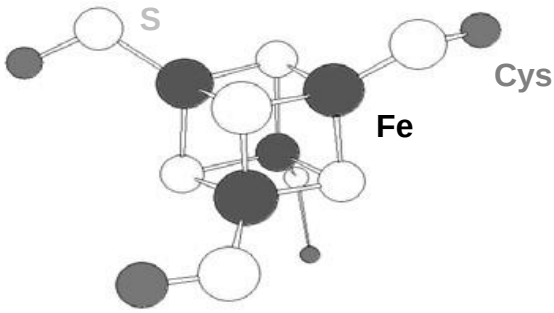

A type of protein with two inorganic sulfurs connecting two irons can have a {Fe2S2} center with an Fe-Fe distance of 2.7 Å. This protein can be studied by EPR methods because the metal center in the oxidized form is diamagnetic (S = 0, EPR silent) and becomes paramagnetic after one-electron reduction (S = ½). The one-electron transfer reaction of the protein involves the reduction of the oxidized form Fdox {Fe2S2}2 (FeIII/FeIII) to the mixed-valent form Fdred {Fe2S2}+ (FeII/FeIII). The reduction potential of such centers is typically between - 50 and -150 mV (vs. SHE). However, some Rieske centers were found to have unusually high redox potentials (-100 to +400 mV) due to asymmetric coordination involving two histidines instead of two cysteines {(Cys)2Fe(μS)2Fe(His)2}.

*a. d. γ {Fe3S4}-centres (3-Iron ferredoxin)*

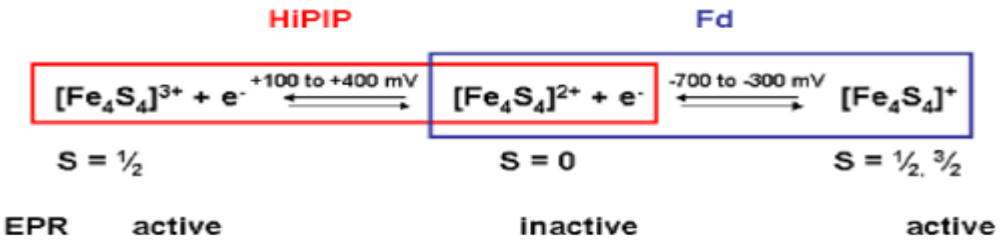



A single-vacancy cubic structure has been identified in several Fe-S proteins, the inactive form of aconitase, and [Ni-Fe] hydrogenase, which facilitates electron transfer after dihydrogen oxidation. The reduction potential of the [Fe3S4 ]+/[Fe3S4 ]0 couple varies from - 25 mV in Azobacter vinelandii ferredoxin I to -70 mV in Desulfovibrio gigas [NiFe] hydrogenase (compared to a standard hydrogen electrode) and is also affected by changes in pH. [8]

*a. e. δ {Fe4S4}-centres (4-Iron ferredoxin)*




The most common type of iron-sulfur centers found in biology is the {Fe4 S4 }- type, which has a distorted cubic crystal structure with alternating Fe and S angles and Fe-Fe and S-S distances of 2.75 and 3.55 Å. . This type of center has three possible oxidation states ([Fe4 S4 ]$_3$+, [Fe4 S4 ]$_2$+, [Fe4 S4 ]+) and is usually linked to the protein by four cysteine amino acids. Ferredoxin proteins can be divided into two subclasses based on the redox couple they use: Fd proteins, which use the [Fe4 S4 ]$_2$+/[F4e S4 ]+ couple (-700 to -300 mV vs. SHE), and HiPIP- proteins (high-potential). iron). proteins) using the pair [Fe4 S4 ]3 / [Fe4 S4 ]2 (+100-+400 mV vs. SHE). The potential of these pairs is affected by pH, ionic strength, and temperature. [9]




**Scheme I-2. Redox Potentials for HiPIP and Ferredoxin Proteins.**

The comparable structures of Fd and HiPIP Fe-S clusters do not take into account their distinct redox potentials, which instead arise from the local environment of the clusters. X-ray crystallographic studies show that the Fe-S center of Fd is closer to the protein surface than that of HiPIP, making it more accessible to aqueous solution and possible hydrogen bonding. In contrast, HiPIP contains a buried darker Fe-S center with a more restricted environment. [2,6,10]

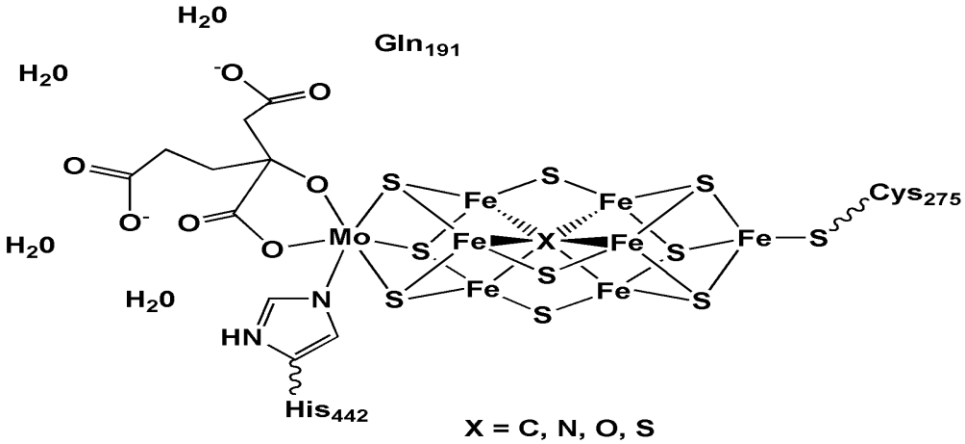
## IV. I.4. COMPLEX IRON-SULFUR PROTEINS

### A. Nitrogenase

Molybdenum iron protein is able to catalyze the conversion of dinitrogen to ammonia (Equation I-1) and thus plays an important role in the biological nitrogen cycle. [7,8]

$$N_2 + 8H^+ + 16MgATP + 8e^- \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i \quad \textbf{(I-1)}$$

The enzyme is composed of an iron-protein with a {Fe4S4(S•Cys)4} cluster and a molybdenum-iron protein, the cofactor of which is composed of two cuboidal fragments, one containing four iron atoms and the other containing a molybdenum atom and three iron atoms (Scheme I-3). This cofactor is dissociable from the protein, and the {Fe4S4} cluster of the iron protein serves as the ultimate electron donor to the MoFe protein for the reduction of dinitrogen. The exact location of the dinitrogen binding site is not yet known. [11]




**Scheme I-3. Fe Moco of Nitrogenase with An Unknown Ligand X located in its centre. The Immediate Protein Environment Surrounding the Homocitrate is Also Shown.**

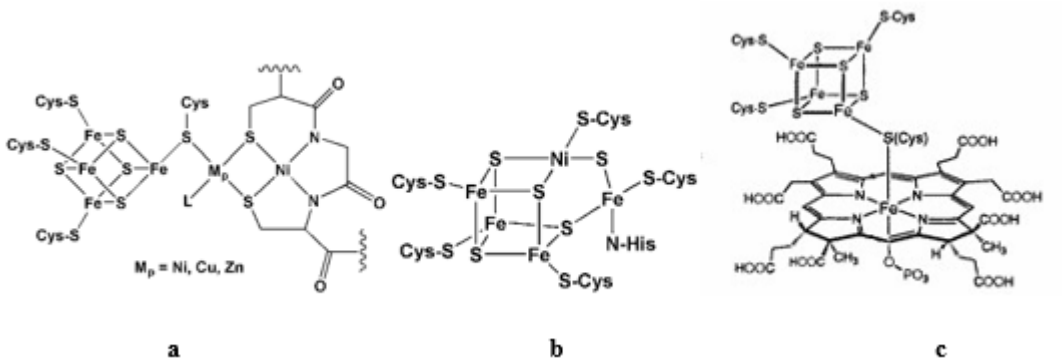
The enzyme's ability to produce H2 in the absence of dinitrogen has been demonstrated.

*a. a. Other iron-sulfur enzymes*

Fe-S cluster cross-linked assemblies, such as sulfite reductase, acetyl coenzyme A synthase (ACS), and carbon monoxide dehydrogenase (CODH) active sites, consist of two separate moieties linked by one or more covalent bonds (Scheme I-4). These assemblies are responsible for catalysing reactions such as

$SO_3^{2-} + 7H^+ + 6e^- \leftrightarrow HS^- + 3H_2O$,

$CH_3$-Cobalt(III)alamin + CO + HSCoA ↔ $CH_3COSCoA$ + Cobalt(I)alamin, and $CO_2 + 2H^+ + 2e^- \leftrightarrow CO + H_2O$, respectively.




**Scheme I-4.** Schematic representation of **a)** the "A-cluster" of ACS from *Moorella thermoacetica*. $M_p$ has been identified as Ni, Cu, or Zn; L is an unknown ligand; **b)** the "C-cluster" of reduced CODH from *C. hydrogenoformans*; **c)** the *E. coli* sulfite reductase hemoprotein active site in the phosphate-bound form.

## V. HYDROGENASES

Hydrogenases catalyze the reversible oxidation of dihydrogen and are essential for microbial energy metabolism (Equation I-2).

$$2H^+ + 2e^- \leftrightarrow H_2 \qquad \textbf{(I-2)}$$

This enzyme is named after Stephenson and Stickland showed that bacteria can release H2 during growth and use H2 but not N2 to reduce artificial and physiological substrates. [8,12-15]

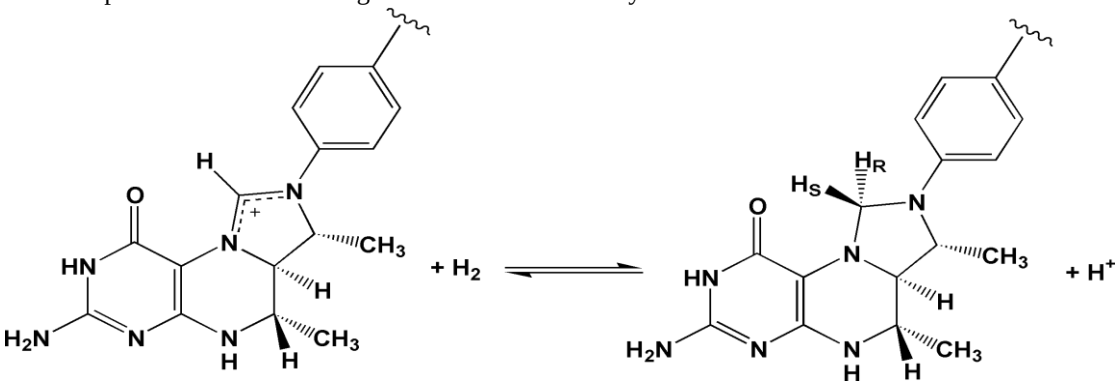
Most of these enzymes are found in archaea and bacteria. Three classes of hydrogenases are known: [Ni-Fe] hydrogenases; [Fe] hydrogenases; and iron-sulfur free hydrogenases. Most of the known hydrogenases belong to the first two classes, and more than 100 of these enzymes have been genetically and/or biochemically characterized. [9,15] Although most hydrogenases can catalyze proton reduction in both directions in vitro, they usually catalyze either hydrogen absorption or development . in vivo (Table I-1).

**Table I-1. Catalytic activities (measured as molecule of dihydrogen per second per hydrogenase molecule at 30°C) of purified [Ni-Fe]- and [Fe]hydrogenases. [10]**

| | [Ni-Fe]-hydrogenase | [Fe]-hydrogenase |
|---|---|---|
| **$H_2$ production** | 700 | 6 000 - 9 000 |
| **$H_2$ consumption** | 700 | 28 000 |

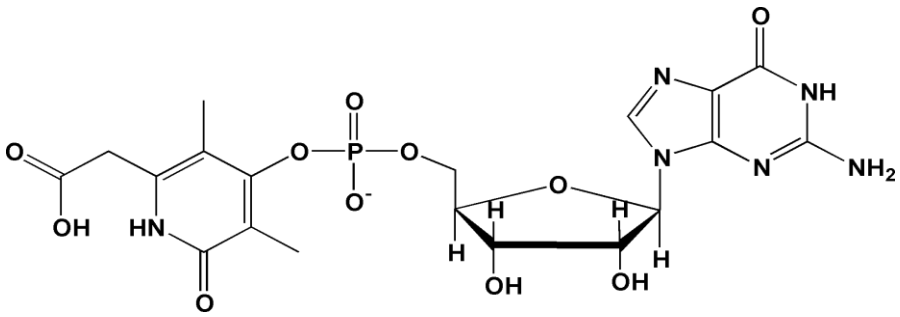
### A. Iron-Sulfur Cluster-Free Hydrogenase

The H2-generating enzyme methylenetetrahydromethanopterin dehydrogenase (Hmd) is present in many methanogenic archaea that use H2 and CO2 [11] for growth. This enzyme catalyzes the reversible reduction of methylenetetrahydromethanopterin (methylene-H MPT+) and H2 to form methylenetetrahydromethanopterin (methylene-H MPT). This process involves a stereospecific transfer of a hydride to the pro-R site of methylene-H MPT (Scheme I-5), and Hmd does not promote H2/H+ exchange in the absence of methylene-H MPT+.



**Scheme I-5. Reaction Catalysed by Hmd.**

Since its discovery more than a decade ago, it was assumed that this enzyme has no metal components. [11,12] The proposed explanation for its dihydrogen activation was similar to the chemical reactions of alkanes in highly acidic solutions.1 , [14,15] However, recently functional iron. and a related cofactor that binds iron has been identified in the enzyme [16,18]. The inactivated cofactor was extracted and analyzed by nuclear magnetic resonance (NMR) and mass spectrometry (MS) (Scheme I-6). [17,19]



**Scheme I-6. Structure proposed for the inactive cofactor of Hmd.**

The exact crystal structure of the enzyme is not yet known, but a pyridone derivative is likely to be the iron coordination site. Infrared spectroscopy showed that two carbonyl ligands [18,20] were attached to the iron and no cyanide ligand was observed. This was further confirmed by inhibition of the enzyme in the presence of CN molecules. Mössbauer spectroscopy showed that the iron was in the low-spin Fe(II) state. Higher resolution analysis is needed to better understand iron coordination.

*a. a. [Ni-Fe]-Hydrogenase*

[Ni-Fe] hydrogenases catalyze the oxidation of dihydrogen to protons and electrons[19,22]. These enzymes usually exist as heterodimers containing a large (60 kDa) and a small (30 kDa) subunit. X-ray crystallography revealed a bimetallic nickel-iron center in the active site of the large subunit and up to three Fe-S clusters in the small subunit that carry electrons from the active site to the physiological electron acceptor. In addition, crystallographic analysis of xenon binding identified hydrophobic channels in the lumen that allow H2 diffusion within the enzyme. [20-23]

The active site of the enzyme contains two π-accepting ligands, carbonyl and cyanide (Figure I-1), which stabilize the low oxidation state of iron (+2, diamagnetic). Two cysteines of the enzyme backbone form a bridge between the two metals. When the enzyme is in its oxidized, inactive form, an additional bridging ligand is present, which is thought to be oxo or hydrox. That ligand is a hydride in its active form.

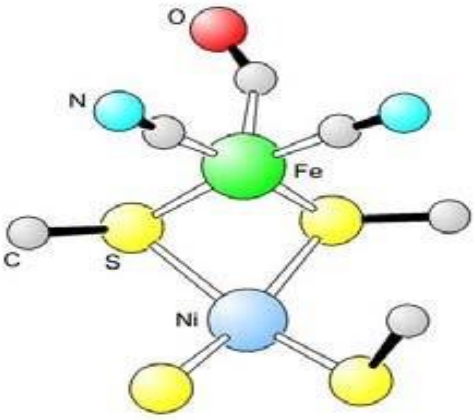



**Figure I-1. Active site of [Ni-Fe]-hydrogenase.**

The nickel atom has different oxidation states (+1,+ 2 and +3) and is coordinated by two other cysteines. One terminal cysteine can be replaced by a selenocysteine, as found in the Desulfomicrobium baculatum enzyme. The mechanism of hydrogen uptake has not been fully explained and the binding site of H2 has not yet been determined. [22-25]

*a. b. [Fe]-Hydrogenase*

*I.5.c.α Physiological Role*

The absence of nickel in hydrogenase enzymes was demonstrated in the periplasmic [Fe] hydrogenase of Desulfovibrio vulgaris in 1984 , [21-26] and this class of hydrogenases generally plays a role in the development of divers. The location of hydrogenases in the bacterial cell reflects the function of the enzyme.23 Periplasmic Desulfovibrio desulfuricans [Fe]-hydrogenase (DdH) participates in the absorption of hydrogen. Protons from the oxidation of dihydrogen produced by [Fe] hydrogenases of Desulfovibrio species create a transmembrane gradient that is thought to be linked to ATP synthesis in the cytoplasm. Clostridium pasteurianum [Fe] hydrogenase I (Cp1) is a cytoplasmic enzyme that accepts electrons from ferredoxin and produces dihydrogen with protons as electron acceptors. This reaction allows the regeneration of oxidized ferredoxin.Molecular masses of hydrogenases can vary from 45 to 130 kDa. Unlike [Ni-Fe]hydrogenases, [Fe]-hydrogenases are mainly monomeric (in the cytoplasm), but dimeric, trimeric and tetrameric enzymes are also known (in the periplasm).

*a. c. β The catalytic subunit*

In the 1980s, Electron Paramagnetic Resonance (EPR) spectroscopy determined the presence of a 6Fe cluster (the "H-cluster") in addition to the two commonly observed {Fe4S4}-clusters (the "F-clusters") in [Fe]-hydrogenases. [22-28] X-ray crystallographic studies carried out in 1998 by two separate groups in Grenoble (France) and Logan (Utah, USA) provided further insight into the structure of this unusual "H-cluster" (crystal structures from DdH25 and Cp126). The active site of these enzymes is located deep within the protein, and a continuous hydrophobic channel has been noted between the surface and the "H-cluster", which is conserved in the two [Fe]-hydrogenases studied to date. This suggests that the same pathway is used by dihydrogen to access or exit the active site, despite the DdH and Cp1 enzymes being involved in different reactions (dihydrogen uptake for DdH and dihydrogen evolution for Cp1). The “H-cluster” is composed of a {Fe4S4}-cluster attached to a {2Fe2S}-subsite via a cysteinyl residue (Figure I-2). The {Fe4S4}-cluster is stabilized by three cysteines. The binuclear metal centre is linked by a dithiolate ligand, which can be either 1,3propanedithiolate, di(thiomethyl)amine or di(thiomethyl)oxo. The amino bridge is largely due to the potential for hydrogen-bonding with amino acids from the protein, as well as its ability to facilitate proton transfer to the active site. However, to date, no X-ray data has been obtained to accurately distinguish between the CH2, NH or O ligands. [24-29] Biological studies have revealed the presence of unusual carbonyl and cyanide ligands that coordinate the two irons in Cp1 and DdH [Fe]-hydrogenases. FTIR studies point to the presence of a bridging CO in the oxidised state of D. vulgaris [Fe]-hydrogenase. Meanwhile, X-ray structures suggest that the active site in DdH is more reduced than that in Cp1. Additionally, the distal iron atom in Cp1 is bound to a low-affinity ligand (H2O), which is missing in the active site of DdH. This indicates that the unoccupied site may have a role in catalysis, possibly as a binding site for hydride or dihydrogen molecules. When large amounts of CO are added to the enzyme under turnover conditions, it leads to permanent inhibition, and the structure of Cp1 reveals the terminal binding of CO on the distal iron.

This supports the possibility of this site playing a major part in the uptake or evolution of dihydrogen.Cysteine

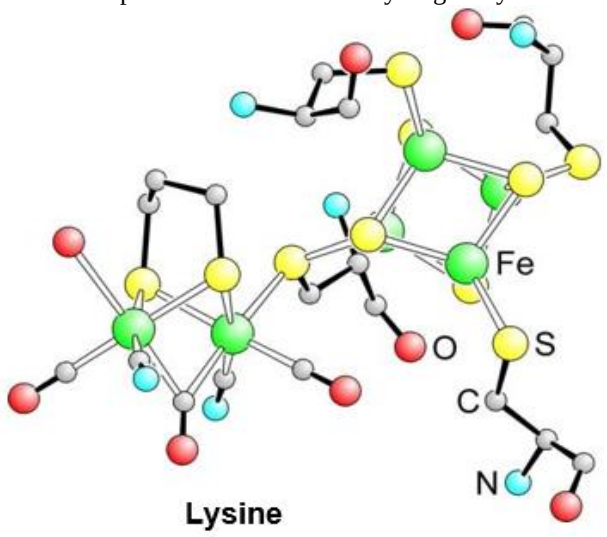
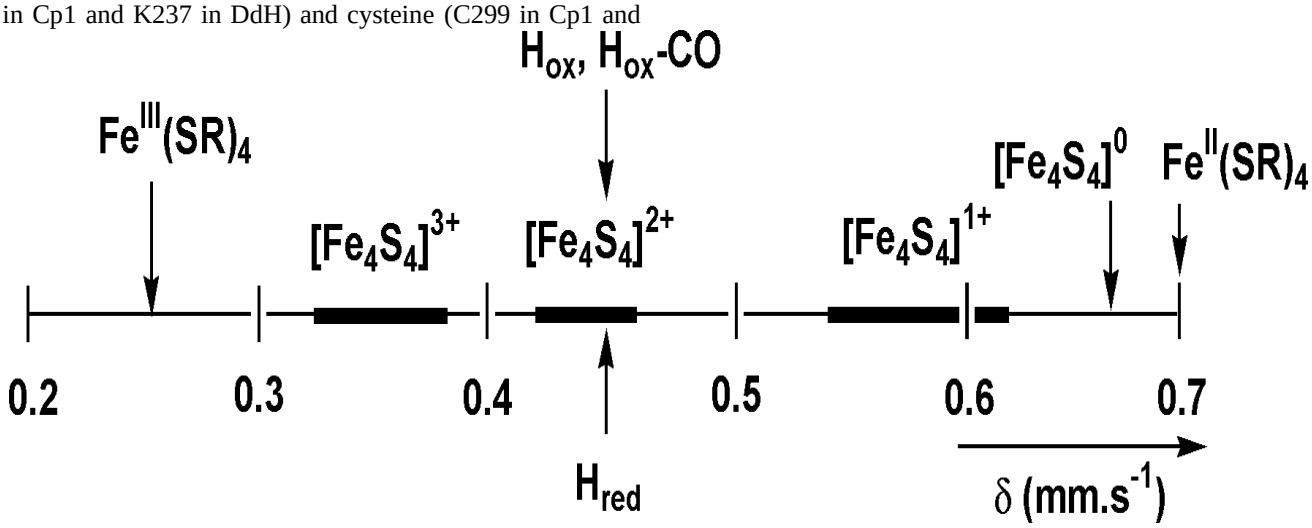



**Figure I-2.** Composite structure of the H-cluster constructed from the crystal structures of Fe-only hydrogenase isolated from DdH[25] (PDB code 1HFE) and Cp1[26] (PDB code 1FEH) and FTIR data [28-29] from *D. vulgaris*. The apical group on the sub-site ligand may possibly be $CH_2$, NH or O. Crystallographic analysis reveals the ability of lysine (K358 in Cp1 and K237 in DdH) and cysteine (C299 in Cp1 and C178 in DdH) amino acids to function as either proton donors or acceptors. Lysine is situated at an approximate distance of 4 Å from the distal iron and forms a hydrogen bond with one of the two cyanides. Cysteine is located roughly 5 Å from the distal iron and is linked to the terminal water molecule through a hydrogen bond. [23-29]

a. *d. γ Spectroscopic features and oxidation states of the "H-cluster"*

Spectroscopic studies have indicated that the "H-cluster" of proteins from different organisms is largely the same. [24-31] The EPR (electron paramagnetic resonance) and Mössbauer spectroscopy studies have revealed a covalent link between the {Fe4S4}-cluster and the {2Fe2S}-subsite of the "H-cluster".[30] Additionally, isomer shifts, which indicate the s-electron density at the 57Fe nucleus, have been found to be related to the oxidation state (os) at both 4.2 and 77 K for iron-sulfur clusters. This linear relationship is expressed by equations I-3 and I-4.$\delta = 1.51 - 0.41 \times os$ (4.2 K) (I-3) $\delta = 1.43 - 0.40 \times os$ (77 K) (I-4) The isomer shifts of the model complexes were compared to those of the "H-cluster". The results indicated that the {Fe4S4} cluster of the "H-cluster" was in a +2 state in both its oxidised ($\delta ave = 0.44 \pm 0.02$ mm.s-1) and reduced forms ($\delta ave = 0.44 \pm 0.02$ mm.s-1) (Scheme I-7).




**Scheme I-7. Isomer shifts δ for {Fe4S4}-cluster (adapted from reference [31]).**

Analysis of Mössbauer data shows that there are two low-spin ferrous iron sites in the {2Fe2S}-subsite of Hred, with one ferrous and one ferric iron in the Hox-active state[30-33]. The presence of strong CO and CN ligands makes it difficult to use the isomer shift parameter to assign the oxidation state of the {2Fe2S}-subsite. EPR data indicates that the proximal iron in Hox-active is Fe(II) due to the weak magnetic coupling of the paramagnetic {Fe4S4}-cluster to the {2Fe2S}-subsite. Furthermore, EPR signals for the Hox-active-CO (CO inhibited form of the active oxidised state) are consistent with a low-spin ferrous for the distal iron. However, FTIR 13CO labelling studies of DdH suggest an Fe(I) oxidation state for the distal iron in the Hox-active-CO state. [29]

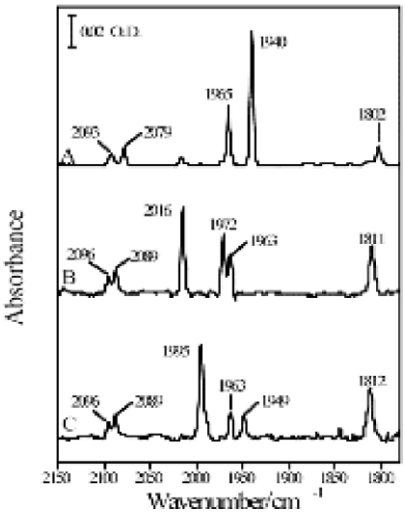


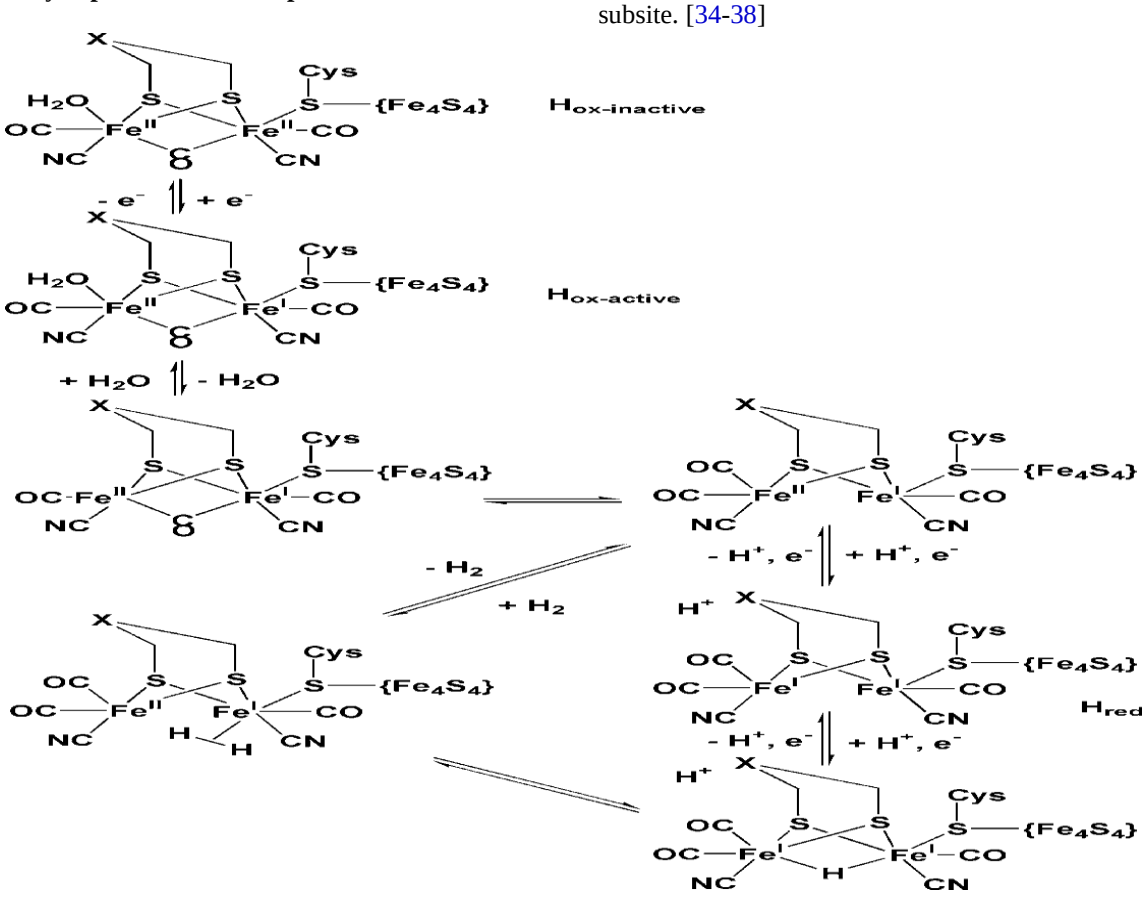

**Figure I-3. FTIR spectra of DdH: (A) $H_{ox\text{-}active}$; (B) $H_{ox\text{-}active}$-CO; (C) $H_{ox\text{-}active}$-13 CO (taken from reference [29]).**

If the oxidation state of the distal iron **were** higher than that of the proximal iron, the **donation of** π-electron density to the CO and CN ligands would be **reduced, leading to** higher **band frequencies.** The different frequencies of the **groups** indicated that the oxidation state of the distal iron was lower than that of the proximal iron, **suggesting** that the **active** state **of Hox** should be **Fe(I) - Fe(II).** These results were **confirmed** by **experiments with Cp1.** [30-33].

The results of DFT studies indicate that the inactive, fully oxidized state of the Hox-inactive cluster is more likely to consist of two Fe(II) atoms, while the active, partially reduced state of the Hox-active is likely to be Fe(I)) -Fe(II). Furthermore, the calculations show that the formation of Fe(II)-Fe(III) and Fe(III)-Fe(III) species is not compatible with the FTIR data. This is also supported by the replacement of the propanethiolate ligand with di(thiomethyl)amine. Furthermore, the analysis showed that the distal iron is more electrophilic than the proximal iron in the mixed-valent form μ-CO Fe(II)-Fe(I), while the opposite is observed for the CO-terminal isomers, suggesting that H2 is likely to bind to the nearby iron as CO- in terminal isomers . [31-36] .

*a. e. δ Catalytic mechanism*

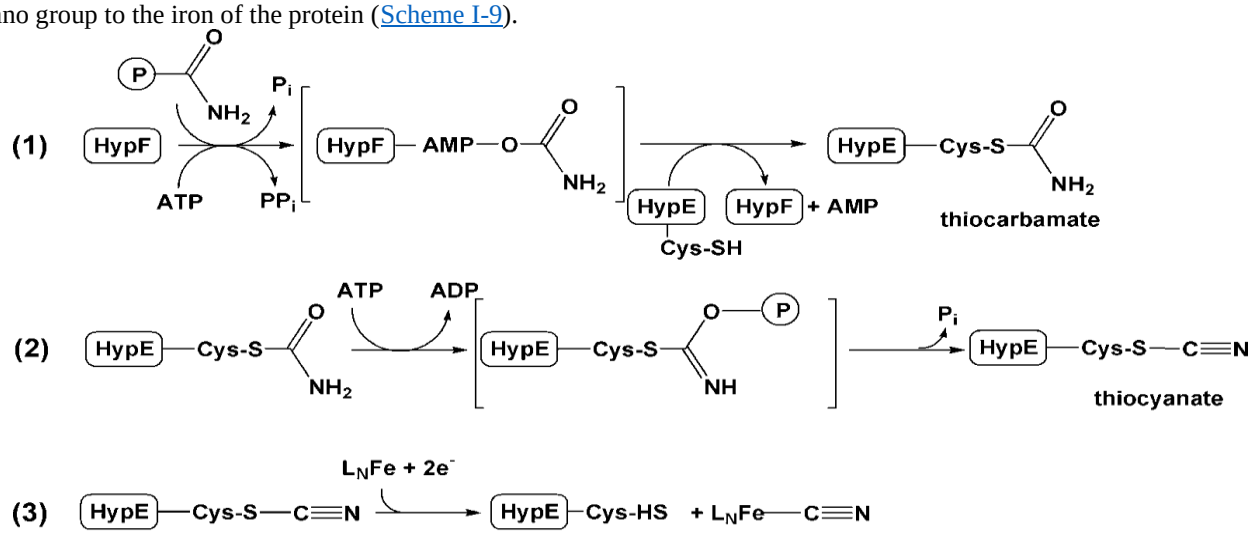
The catalytic activity of [Fe]-hydrogenases is higher than that of [NiFe]-hydrogenases, as evidenced by numerous studies. However, most of the physicochemical investigations have been conducted on [NiFe]-hydrogenases. FTIR studies and DFT analyses (Scheme I-8) have enabled the development of a proposed catalytic mechanism, but many of the key steps remain speculative. 21Important questions remain to be answered such as the binding site for dihydrogen, the presence or absence of a bridging hydride, the location of the proton donor site and the electron transfer mechanism from the {Fe4S4}-cluster to the {2Fe2S}-subsite. [34-38]




**Scheme I-8. Proposed mechanism for $H_2$ uptake / evolution of [Fe]hydrogenases (adapted from ref [21]).**

a. *f. ε Biosynthesis of the H-cluster and origin of the CO and CN ligands*

It is speculated that the same method is used to form [Fe] hydrogenases, although no direct evidence has yet been provided. The additional Fe-S centers of hydrogenases probably accumulate in the same way as conventional iron-sulfur proteins. Most genomic studies have been performed on Ni-Fe hydrogenases, and although Ni-Fe and Fe hydrogenases are not genetically related, similarities have been observed between the two enzymes in their CO and CN ligands, dinuclear metal center, and their ability to det. take up and evolve hydrogen. Many genes are required for the biosynthesis of these complex enzymes, and the first report of the required genes, hydEF and hydG, for the maturation of active Fe hydrogenase was described in a study on H2 photoproduction in Chlamydomonas reinhardtii. [37] These genes are essential for the formation of active Fe hydrogenase and are conserved among organisms that contain Fe hydrogenase enzymes. The binding of CO and CN ligands to the active sites of Ni-Fe and Fe hydrogenases is an interesting question, because these two ligands are toxic in their free state and unprecedented in biology. [37-39] A study by Böck and colleagues showed , that the formation of cyanide ligands requires the hydrogenase maturation proteins HypF and HypE two activities for the synthesis of [Ni-Fe] hydrogenases and fixation of the metal center. [38] The HypF protein accepts carbamoyl phosphate and carries its cysteine. HypE, which leads to the formation of the enzyme thiocarbamate. After scarbamoyl is dehydrated, the enzyme thiocyanate then transfers the cyano group to the iron of the protein (Scheme I-9).




**Scheme I-9. Biosynthesis of the cyanide ligand. (1) Formation of the enzymethiocarbamate. (2) Dehydration followed by dephosphorylation. (3) Transfer to the iron (adapted from ref [38]).**

The efficiency of this system was evaluated against chemical models, showing that the comparison between biochemistry and organometallic chemistry can be useful for understanding a complex biochemical process. Although no evidence has yet been provided to explain the origin of CO, the idea that deamination of the carbamoyl group can lead to the formation of the carbonyl ligand has been rejected, as evidence indicates that CO biosynthesis differs from CN biosynthesis. [39] Another hypothesis suggests that acetate or one of its derivatives may be a CO precursor, but more evidence is needed to confirm that idea.I.6.

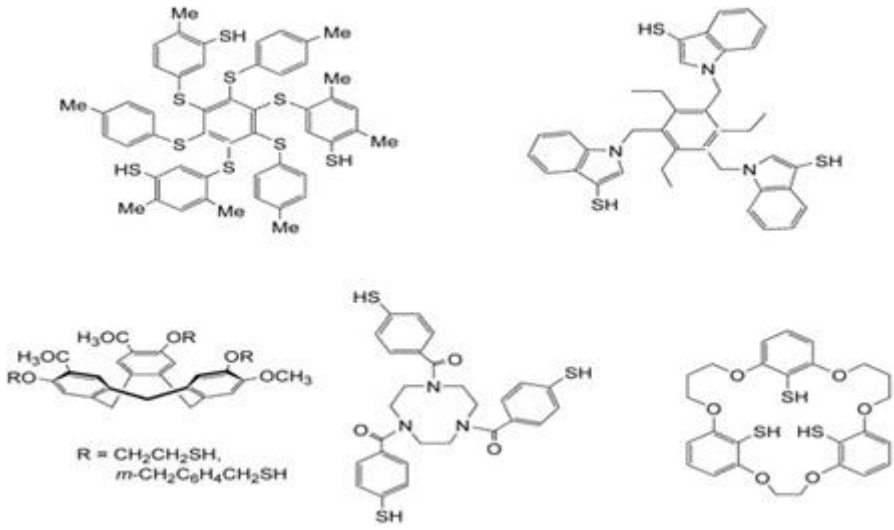
**Synthetic models of the active sites of iron-sulfur proteins**

*a. g. Analogues of rubredoxin and ferredoxin sites*

The synthesis of iron-sulfur active sites began in the early 1970s after the X-ray crystallographic determination of iron-sulfur proteins. [31] The first analogue, {Fe4S4}-centres, was a cubane-type structure $[Fe4S4(SCH2Ph)4]^{2-}$ [40] and was synthesized using ferric chloride, sodium methoxide, sodium hydrosulfide, and the relevant alkyl mercaptan. This {Fe4S4}core is a distorted cube, with iron and sulfur atoms at alternating vertices and is closely linked to the active site structures of HiPIP and ferredoxin proteins. From 1975 [41] to 1995 [42], the chemistry of iron-sulfur complexes has been thoroughly explored, leading to a greater understanding of these assemblies and their electronic properties. [31] Utilizing a range of spectroscopic and magnetic techniques, from the first rubredoxin analogues [Fe(SR)4]2- to cuboidal [Fe3S4(SR)3]3-, synthetic analogues and protein-bound iron-sulfur centres have been studied. Mössbauer spectroscopy has proved to be a useful tool in determining the oxidation state and magnetic coupling, as well as elucidating the electronic ground state.

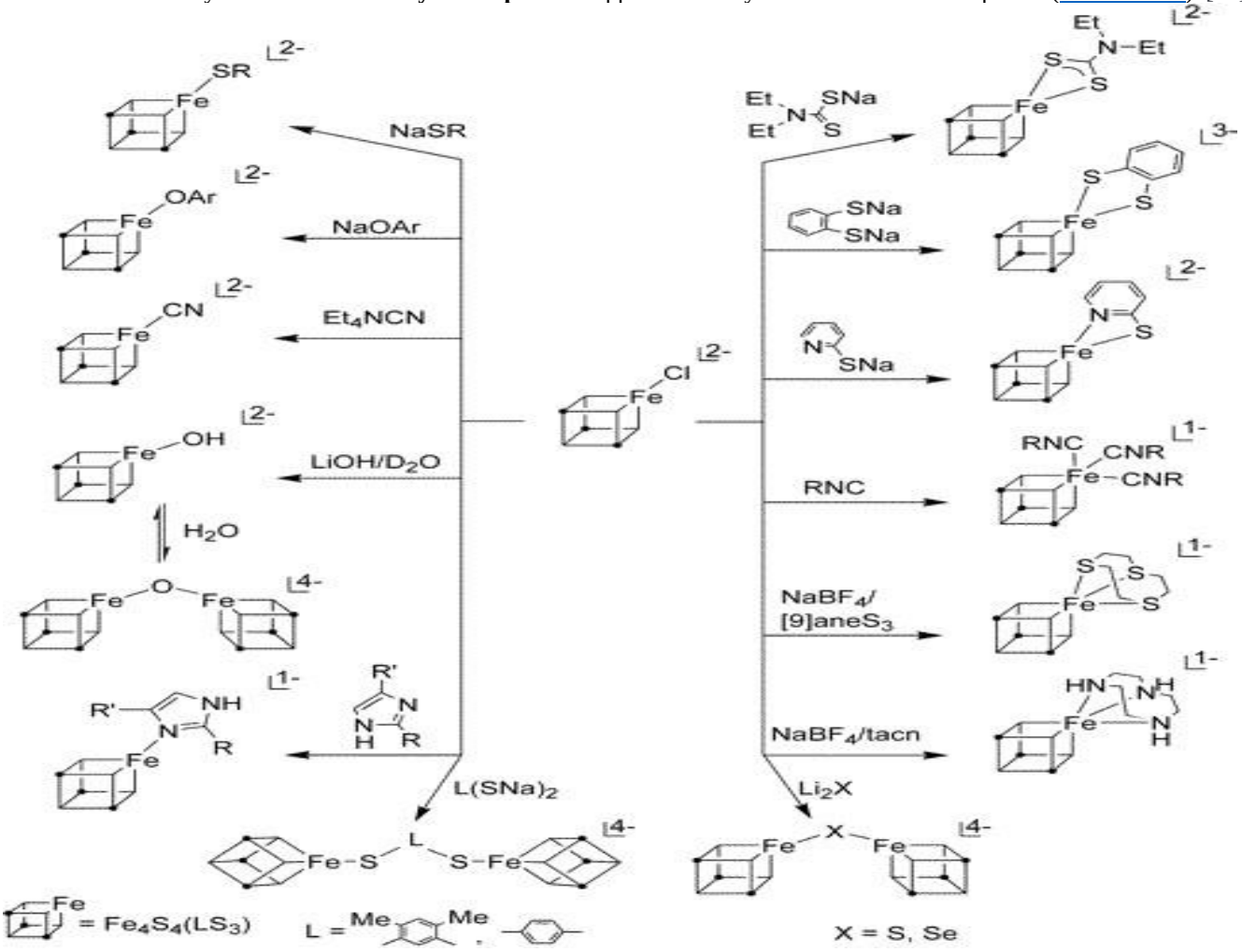
*a. h. Site-differentiated clusters*

The Fe atoms in {Fe4S4}-centres of proteins are not identical, and can either facilitate catalysis by the enzyme (e.g. aconitase) or can bind other metal cofactors (see sections I.4 and I.5). Consequently, research has been conducted on the synthesis of a polydentate ligand that could differentiate irons with a 3:1 ratio. Five trithiol ligands have been synthesised (Scheme I-10), and are able to coordinate {Fe4S4}- or {Fe3S4}-clusters. [42-45]




**Scheme I-10. Trithiols whose deprotonated forms stabilize 3:1 sitedifferentiated {$Fe_4S_4$}-clusters. The trianions bind clusters in a trigonally symmetric arrangement except the crown ethers trithiol, which have mirror symmetry (taken from ref [31]).**

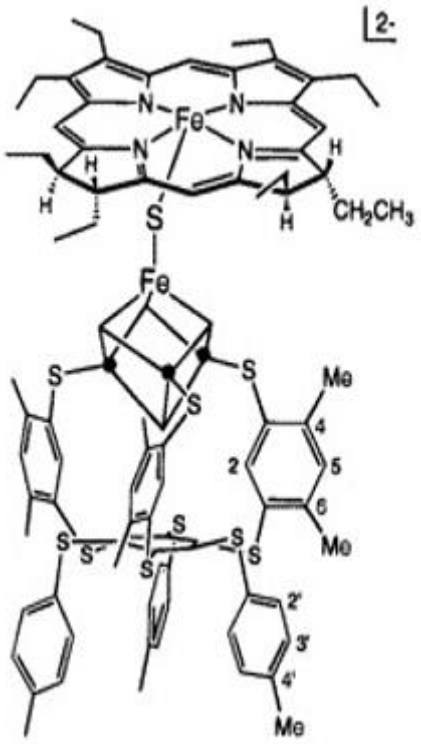
**LS3 cluster** chemistry has been **extensively developed and** applied to the synthesis of **several** complexes (Scheme I-11). [31]




**Scheme I-11. Selected site-specific reactions of $[Fe_4S_4(LS_3)\ Cl]^{2-}$ that afford synthesis of a variety of product clusters, including single cubanes with four-, five-, and six-coordinate unique sites and bridged double cubanes (taken from reference [31]).**

*a. i. Synthetic analogues of iron-sulfur complexes*

The complexity of iron-sulfur active sites has been a difficult challenge in organometallic synthesis. Thus, seven metal-sulfur complexes (sulfitoreductase, CODH C-cluster, acetyl coenzyme A synthase cluster, nitrogen P and FeMo co-groups, [Ni-Fe] and [Fe] hydrogenases) are in the spotlight. in the last decade as structural studies revealed their active sites. However, connecting different subsites of the active sites to form iron-sulfur clusters proved problematic. For example, attempts to attach the heme group to the [Fe4 S4 ] cluster with a thiolate bridge to reproduce the active site of sulfite reductase did not yield the desired products because the closest analogs have a sulfide bridge. cysteine bridge (Scheme I-12).[43-47]



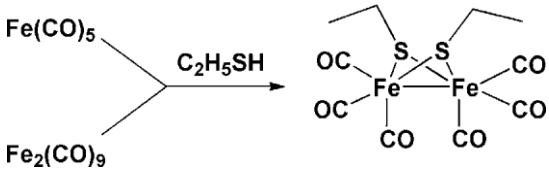
**Scheme I-12. The bridged assembly $[Fe_4S_4(LS_3)$-S-Fe(OEiBC)$]^{2-}$.**

Incorporation of nickel complexes into the construction of A-cluster, C-cluster, or [Ni-Fe] hydrogenase active site analogs is under investigation; however, the models are far from functionally and structurally robust. [48-50] More than two decades of research have not yielded a successful synthesis of analogs of nitrogen clusters. [49-51] However, synthetic systems based on {MoFe3S4 } cuboidal clusters are able to reduce; hydrazine to ammonia, but no satisfactory analogues have been found. , which would mimic the reduction of ditipone to ammonia. Recently, the high-resolution crystal structure (1.16 Å) of the FeMo cofactor revealed the presence of an interstitial atom (carbon, nitrogen, or oxygen atom). [50-52] This discovery prompted the use of organometallic chemistry to explain the nature of its nature. . substance this ligand and the most likely suggestion are nitrate complexes. [53]

## VI. SYNTHETIC MODELS OF THE DI-IRON SUBSITE OF [FE]-HYDROGENASE

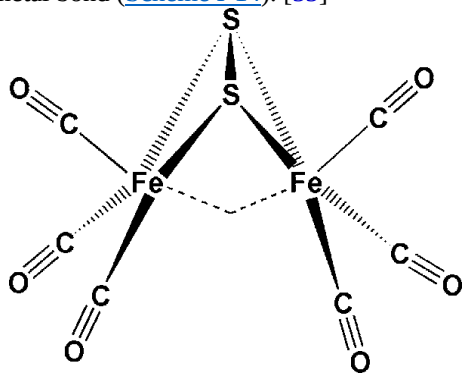
### A. Early {2Fe2S}-complexes

In 1929, Reihlen et al. reported the synthesis of Fe2(CO)6(SEt)2 (Scheme I-13), [53] and the striking similarity to the {2Fe2S} subsite paved the way for the synthesis of analogues in the H cluster.".




**Scheme I-13. Synthesis of $Fe_2(CO)_6(SEt)_2$. [53]**

Based on this type of compounds, a large number of iron-hexacarbonyl complexes have been synthesized. The volatile Fe2(CO)6S2 complex resulting from the "stinking" reaction between Fe(CO)5, KOH, and polysulfide has been extensively studied since 1958. [54] The Fe-Fe bond length of 2.55 Å is very close to the distance of 2.6 . Å observed in the "H-cluster" {2Fe2S} subregion. [24,25] The complex is butterfly-shaped (distorted Fe2S2 tetrahedron) with a bent metal-metal bond (Scheme I-14). [55]




**Scheme I-14. Schematic representation of the $Fe_2(CO)_6S_2$ complex. The dashed line represents the bent metal-metal bond.**

Sulfur atoms can also be connected with organic bonds, and carbonyl ligands can be replaced by phosphines and phosphites. [56]

*a. a. {2Fe2S}-frameworks*

Determination of the X-ray crystal structure of [Fe]-hydrogenase encouraged the organometallic community to reconsider the chemistry of these $[Fe_2(\mu\text{-}SR)_2(CO)_6]$ systems. The synthesis of the propanedithiolate (pdt) bridged diironhexacarbonyl [57] was the first step through the synthesis of the first models of the {2Fe2S}-subsite. Soon after the publication of the structure of the "H-cluster", three independent groups published the structure of the dianion $[Fe_2(pdt)(CO)_4(CN)_2]^{2-}$ (Figure **I-4**). [58-60]

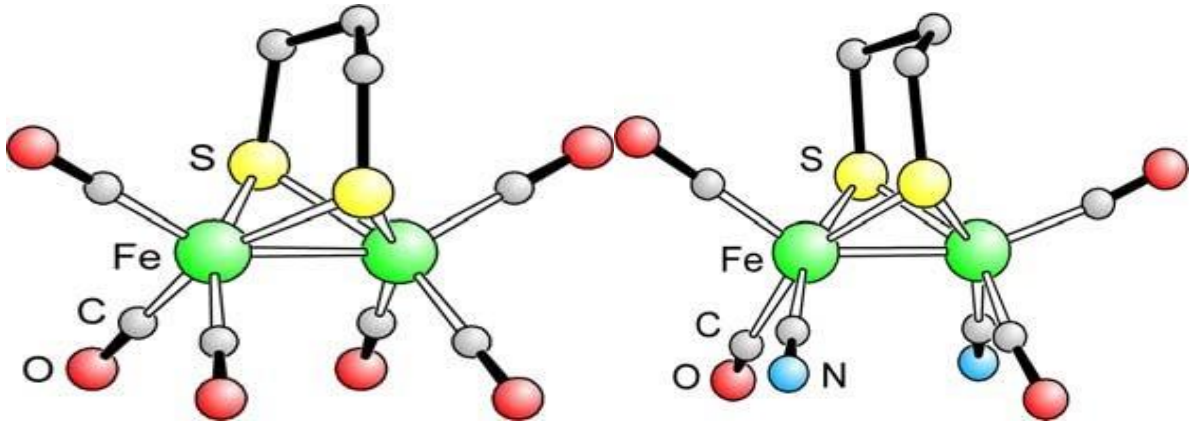
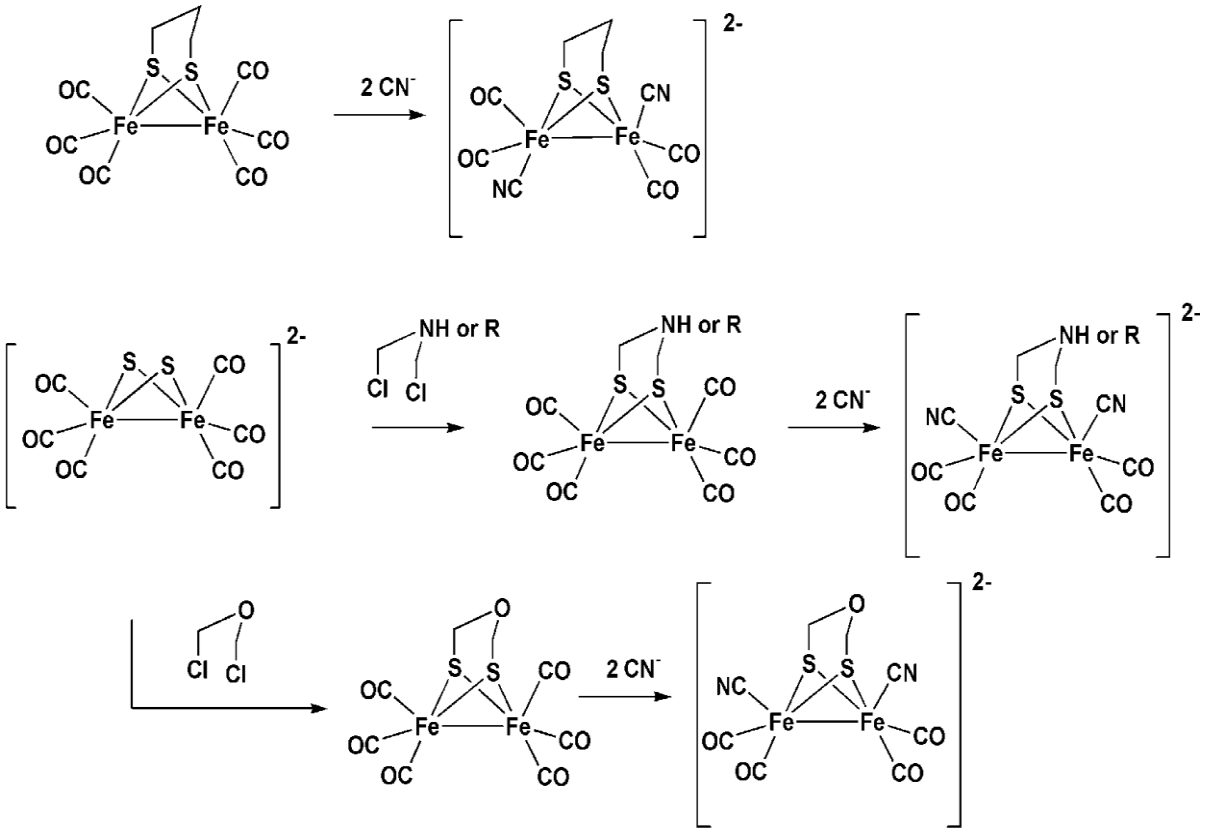



**Figure I-4. Crystal structures of analogues of the {2Fe2S}-subsite.**

$[Fe_2(pdt)(CO)_6]$ (left); $[Fe_2(pdt)(CO)_4(CN)_2]^{2-}$ (right).

Propanedithiolate is one possibility for the bridging dithiolate ligand between the two metals of the subsite, so the amino [51] and oxo [52] derivatives were also synthesised (Scheme **I-15**).

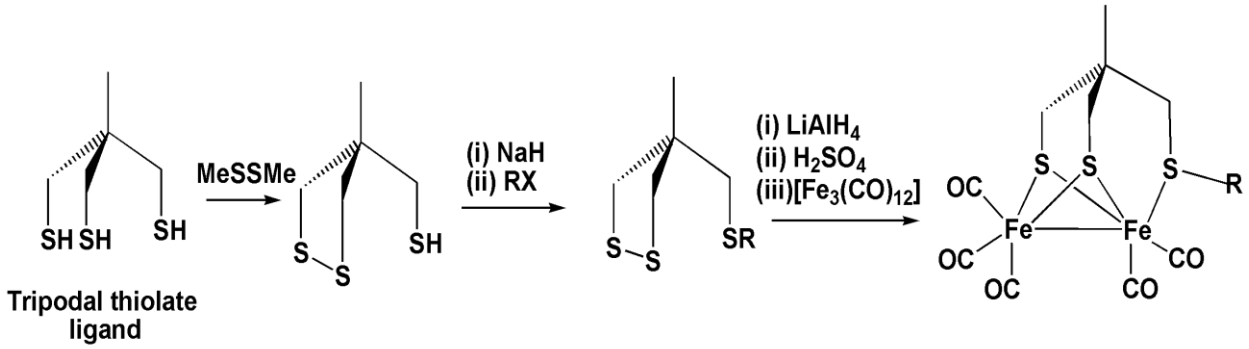



**Scheme I-15. Synthesis of {2Fe2S}-subsite analogues. [58-60]**

The butterfly arrangement of the dithiolate ligand in these complexes is very similar to that of the "H-cluster" and therefore the subsite is modeled in the CO-inhibited form Hox-CO. Due to the presence of a bridging cysteine between the {Fe4 S4 }-cluster and the {2Fe2S}-subsite of the H-cluster, these model complexes do not contain a third coordinating sulfur.

*a. b.{2Fe3S}-framework*

Synthesis of the {2Fe3S} core with carbonyl and cyanide ligands was achieved by synthesizing propanedithiolate ligands bearing a thioether group capable of introducing additional functions to the nearby iron. [56-59] This framework is structurally very close to the substructure. [Fe]-hydrogenase (Scheme I-16).




**Scheme I-16. Synthesis of {2Fe3S}-complexes (R = Me or $CH_2Ph$).**

Cyanization of the pentacarbonyl complex provides a stable monocyanide at the distal iron, and further cyanation can provide a moderately stable intermediate. It has a bridging carbonyl ligand and one CN on each iron, which are the main structural components of the CO-inhibiting form of the "H-cluster".[60] A transient {Fe(I)Fe(II)}-bridging carbonyl a group species can also electrochemically and was characterized by FTIR and suspension methods. It has a very similar spectrum to Hox-CO.29. This supports the low oxidation state of the iron in the subsection.

*a. c. Hydride complexes*

The presence of hydride species during the catalytic process of hydrogen evolution and assimilation of hydrogenases was not detected spectroscopically. However, the formation of HD during cycling under D2 [24] indicates that metal hydride formation is possible. This theory is also supported by DTF studies. [32,54] Research into the production of hydride complexes with {2Fe2S} nuclei led to the synthesis of a hydride species [58,59] with CO and phosphine ligands. Furthermore, by photolytic activation of Ru2(CO)4(PCy3)2(pdt) in a dihydrogen atmosphere, a complex is formed with ruthenium atoms instead of iron with both bridging and terminal hydrides. [59-60] This experiment also showed that HCl can react. with dihydrogen to form the product.

*a. d. Hydrogen evolution and model complexes*

Alternative energy to fossil fuels and reduction of greenhouse gases are important goals for the beginning of this century. Molecular hydrogen would be an ideal fuel because it does not cause pollution when it is burned or oxidized in a fuel cell, and it releases more energy per gram than any other fuel. [47] One of the many problems associated with the "hydrogen economy" concerns the production and consumption of hydrogen. Four main technologies are currently used to produce hydrogen: hydrocarbon reforming; ammonia cracking; pyrolysis (breakdown of hydrocarbons in a waterless and airless environment into water and carbon); and water reforming. [48] Unfortunately, all these methods use unsustainable hydrocarbon raw materials and emit greenhouse gases. Platinum-based metals are currently the best electrocatalysts for hydrogen uptake in fuel cells. This has proven to be economically impractical in the long term due to the limited availability of this metal, and the US Department of Energy has also identified strategic supply issues. [49]

A major challenge in modeling the active site of [Fe]-hydrogenase is to find a complex capable of reversibly catalyzing proton reduction. A first attempt to react Fe2(CO)4 (CN)2(pdt) with two equivalents of acid resulted in an insoluble polymeric material, but in the co-production of H2. [60], electrochemistry is the preferred method to monitor the various materials. . complexes for the catalytic reduction of protons (see Chapter III) and almost all {2Fe2S} systems have shown this ability with more or less efficiency compared to the enzyme. [56-60] Ligand modifications (CO, CN, phosphines) Dithiolate ligands (CH2 , NH or O ) or iron-centered oxidation facilities did not offer the possibility to produce efficient catalysts. However, this type of iron sulfide complex was clearly shown to be capable of catalyzing proton reduction.

## VII. CONCLUSION

This review has provided an overview of the synthesis, reactivity and electrochemistry of chemical models of the active site structures found in [Fe]-hydrogenase. The H-cluster found in this enzyme is an interesting and complex site, with its combination of an {Fe4S4}cubane and an organometallic di-iron subsite. Through the synthesis of various chemical models, researchers have been able to better understand the structure and reactivity of this active site. Furthermore, the development of electrochemical techniques has enabled the investigation of the electrochemical properties of these systems, which could be useful for the design of artificial catalysts. In conclusion, the development of chemical models of the [Fe]-hydrogenase active site has not only helped to further our understanding of this enzyme, but could also lead to the development of new applications in catalysis.

## DECLARATION


| | |
|---|---|
| Funding/ Grants/ Financial Support | No, I did not receive. |
| Conflicts of Interest/ Competing Interests | No conflicts of interest to the best of our knowledge. |

| | |
|---|---|
| Ethical Approval and Consent to Participate | No, the article does not require ethical approval and consent to participate with evidence. |
| Availability of Data and Material/ Data Access Statement | Not relevant. |
| Authors Contributions | I am only the sole author of the article. |

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