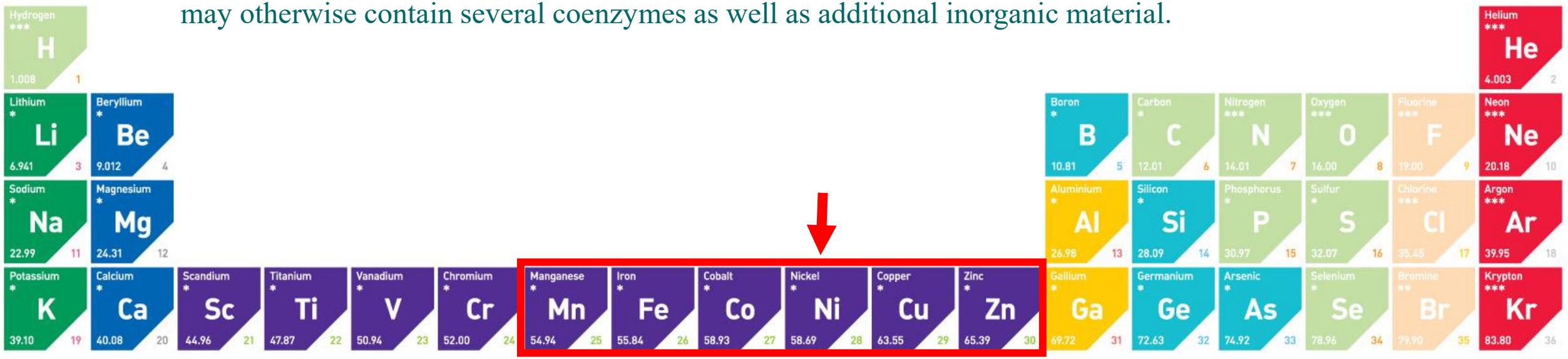
Nickel-containing Enzymes

For a long time, nickel was the only element of the "late" 3d transition metals for which a biological role could not be definitely established. This changed only in 1975, when B. Zerner discovered that urease is a nickel enzyme.

Reasons for this "oversight":

- nickel ions do not exhibit a very characteristic light absorption (color) in the presence of physiologically relevant ligands. Mössbauer effects are not easily accessible for nickel isotopes, and even paramagnetic Ni(I) (d9) or Ni(III) (d7) cannot always be unambiguously detected by electron paramagnetic resonance (EPR) spectroscopy due to the lack of metal isotope hyperfine coupling (the natural abundance of ^{61}Ni with I = 3/2 is only 1.25%).
- it has now been shown that Ni is often only one of several components of complex enzymes, which may otherwise contain several coenzymes as well as additional inorganic material.



Ni-dependent proteins	Organisms
urease	archaea, bacteria, eukaryotes
[NiFe] hydrogenases	archaea, bacteria
carbon monoxide dehydrogenase (CODH)	archaea, bacteria
acetyl-*CoA* synthase/decarbonylase (ACS)	archaea, bacteria
methyl-coenzyme M reductase (MCR) (including the F_{430} cofactor)	archaea
superoxide dismutase (Ni-SOD)	bacteria

Nickel is not particularly rare in the lithosphere or in sea water, where it is soluble as Ni^{2+}. In view of its very low potential requirement as an ultratrace element, no real deficiency symptoms have been reported for human beings.

Ni^{2+}-specific antibodies are responsible for the not uncommon "nickel allergy".

The bioinorganic chemistry of nickel is unique mainly in view of the organometallic species that occur in a number of biological processes catalyzed by nickel enzymes.

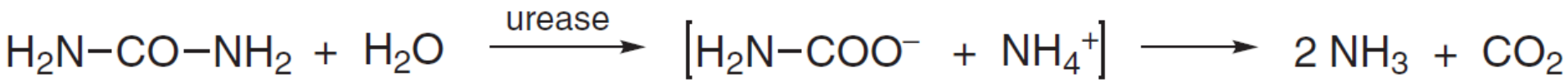
Many of these "**organometallic" transformations**, occurring exclusively in archaea and bacteria, involve the consumption or **production of CH_4 (from CO_2),** and this biological "C1" chemistry has a large impact on the global C cycle.

Urease

Urease enzymes, which may be isolated for example from bacteria or plant products such as jack beans (*Canavalia ensiformis*), have an interesting history. Contrary to the opinion of R.Willstatter, they were the first enzyme to be prepared in pure crystalline form. However, their nickel content wasn't determined until about 50 years later.

Urea is a very stable molecule, which normally hydrolyzes very slowly to give isocyanic acid and ammonia, the half-life value of the uncatalyzed reaction being 3.6 years at 38°C.

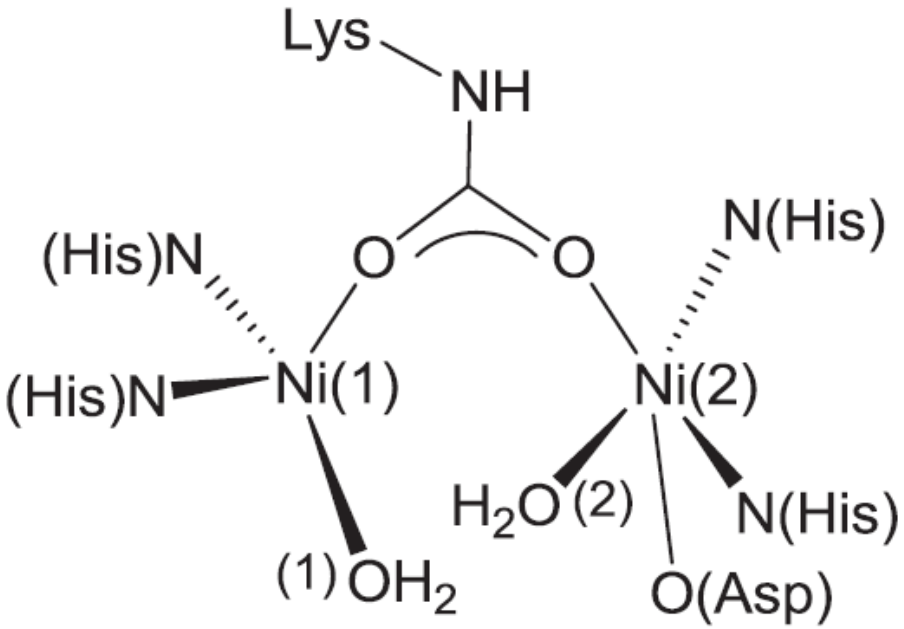
The "classical" urease (urea amidohydrolase) catalyzes the degradation of urea to carbon dioxide and ammonia



The catalytic activity of the enzyme increases the rate of complete hydrolysis by a factor of about 10^{14}.

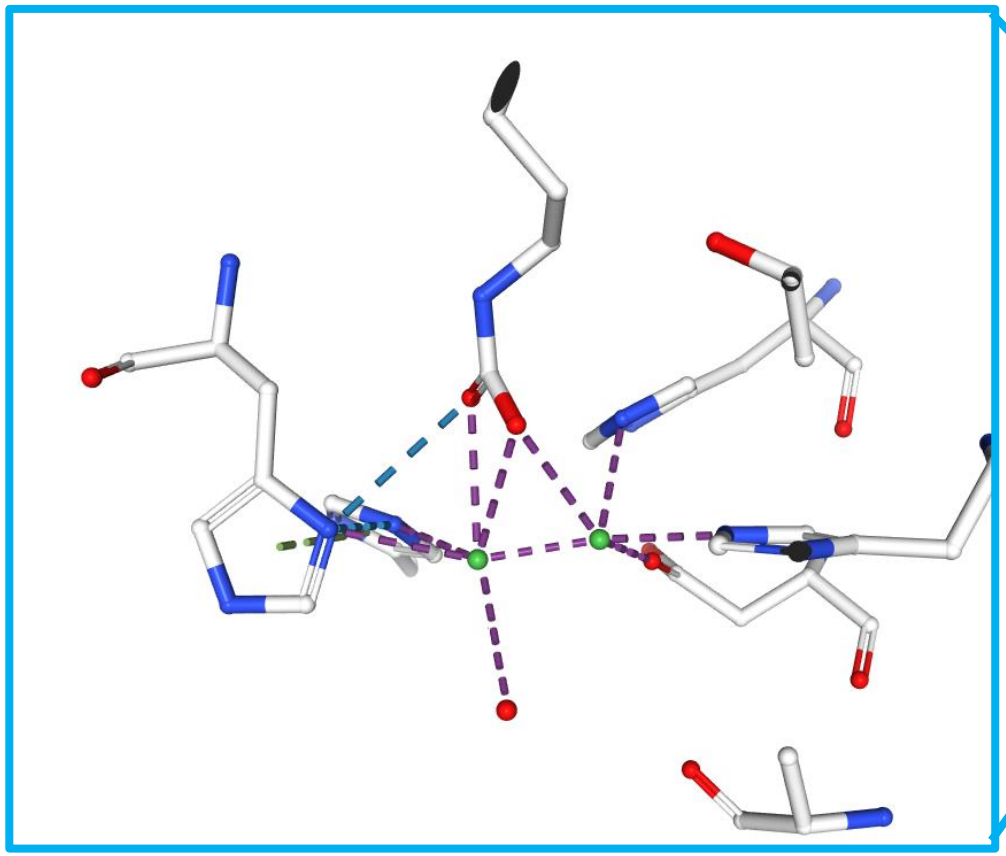
Change in the reaction mechanism.
While the uncatalyzed reaction involves a direct elimination of ammonia, the enzyme presumably catalyzes a hydrolysis reaction with carbamate, $H_2N–COO^-$, as the first intermediate. A metal-to-substrate binding would facilitate this latter reaction mechanism. Additional support for substrate binding to the metal comes from the fact that phosphate derivatives binding strongly to nickel inhibit the activity of the enzyme.

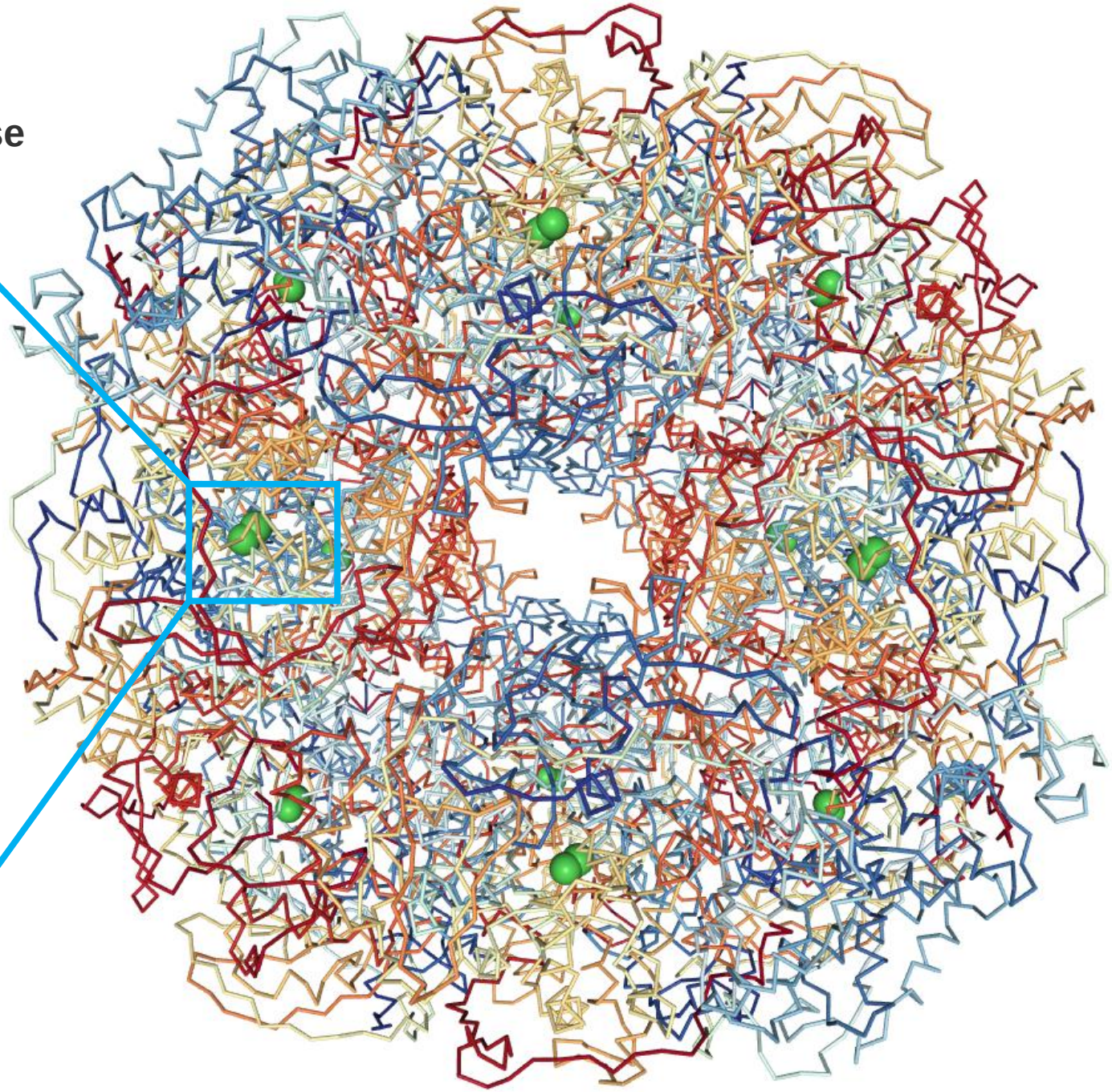
Main structural features of the reaction center:
two Ni(II) atoms are separated by 3.5 Å and bridged by a carbamylated lysine residue;
one Ni(II) atom is five-coordinate (NiN_2O_3), while the other is described as "pseudotetrahedral" (NiN_2O_2), with a weakly bound ligand -This ligand may be a water molecule-



PDB = 1E9Z

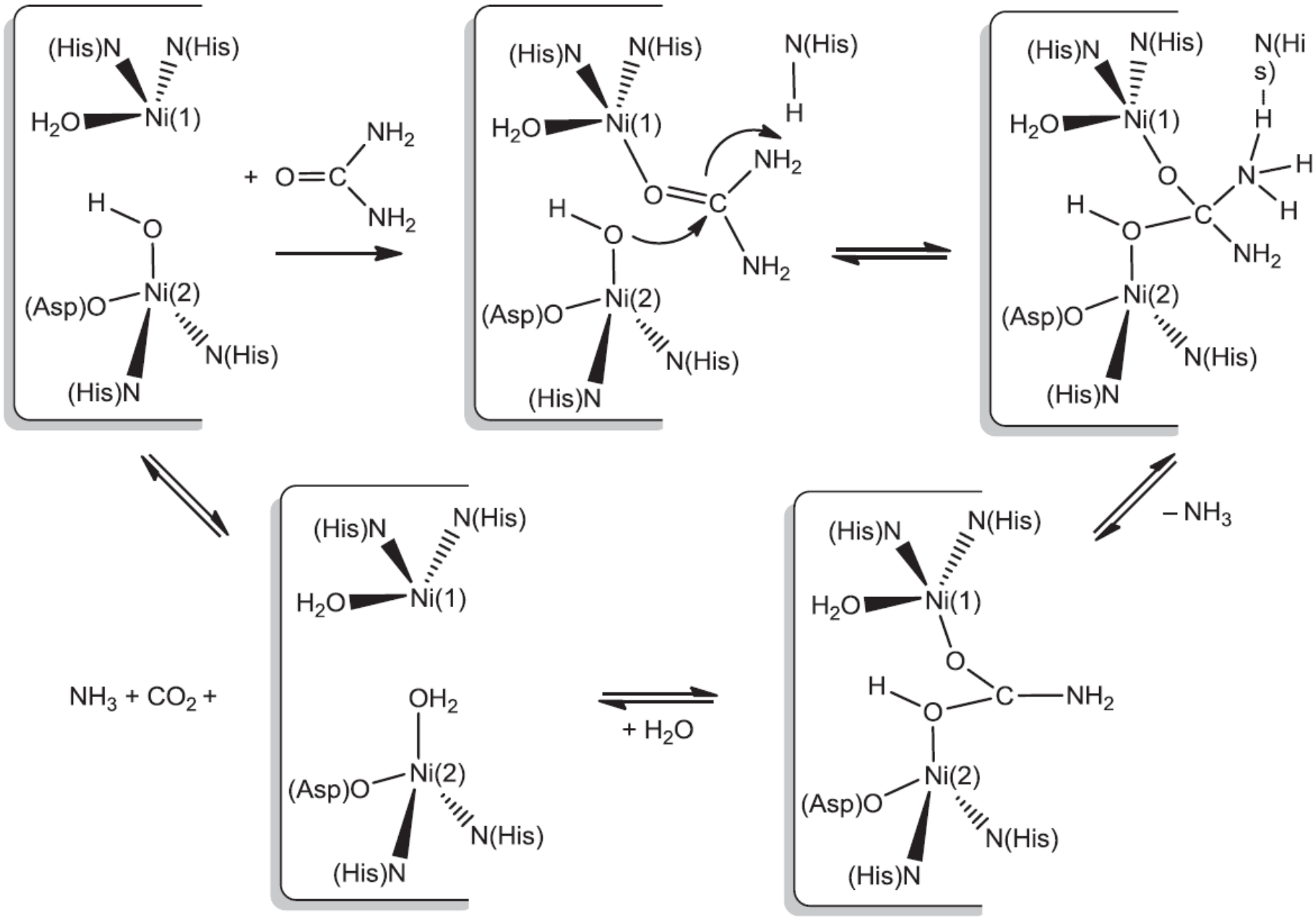
Crystal structure of Helicobacter pylori urease



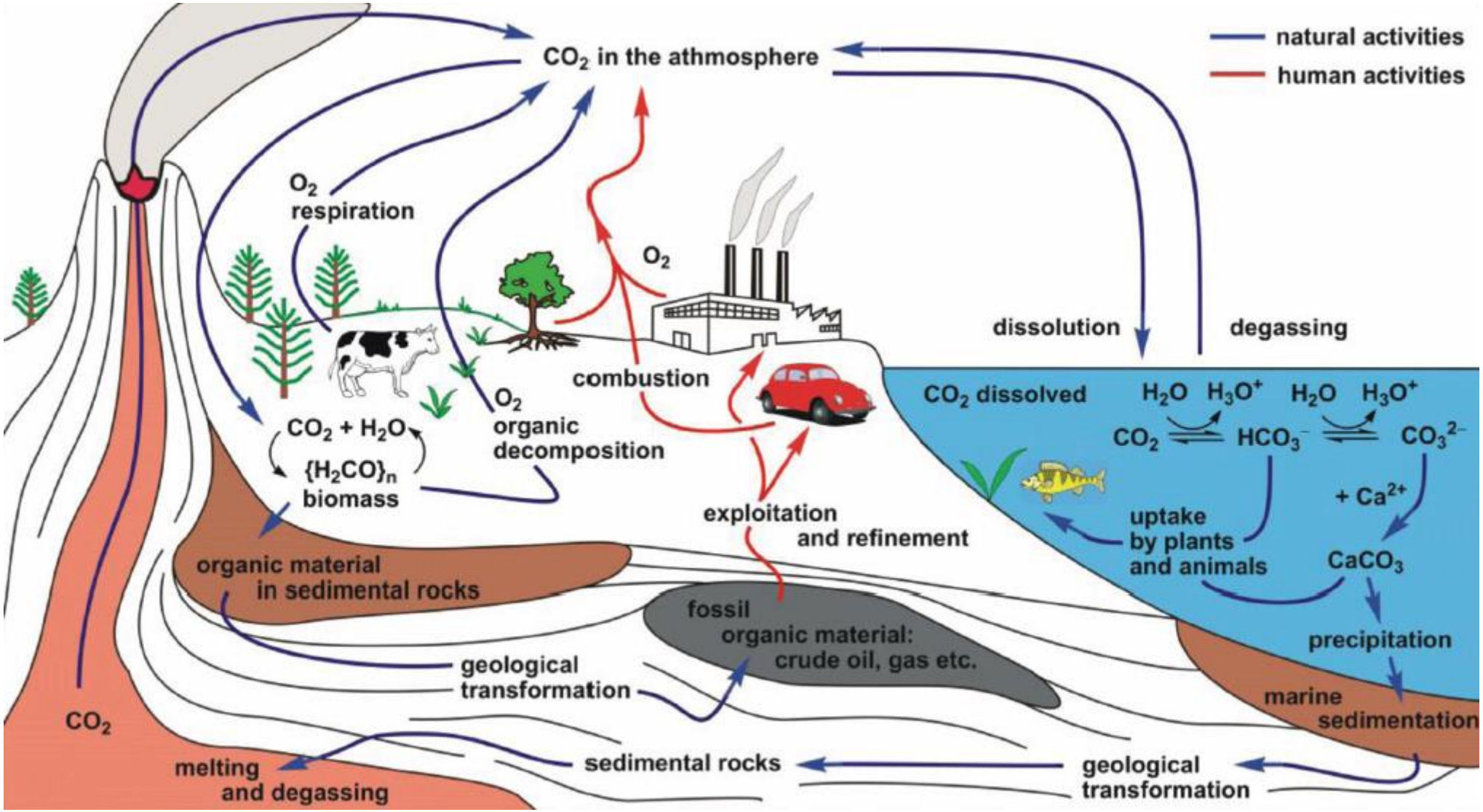


Reaction mechanism

Electrophilic attack of one of the nickel centers on the carbonyl oxygen atom and **nucleophilic attack** of a nickel hydroxo species on the carbonyl carbon center (**push–pull mechanism**)



The Global C Cycle and the Marine Inorganic C Cycle



Methane has also a key role in the global carbon cycle.

It is increasingly important as energy source but it is also contributing greatly to the greenhouse effect.
In fact **CH_4 is 25 times more effective than CO_2 in absorbing thermal IR radiation.**

The main anthropogenic source of methane are coal and oil extraction, hydraulic fracturing, leaking gas pipes and the burning of bio-mass.

Natural sources can be **biogenic** or **non-biogenic**

Methanogen bacteria
(e.g. in the stomach of ruminant)

Serpentinization

Serpentinite reactions

Serpentinization is a geological low-temperature metamorphic process involving heat and water in which low-silica mafic and ultramafic rocks are oxidized (anaerobic oxidation of Fe^{2+} by the protons of water leading to the formation of H_2) and hydrolyzed with water into serpentinite.

Serpentinite is formed from olivine via several reactions, some of which are complementary. Olivine is a solid solution between the magnesium-endmember forsterite and the iron-endmember fayalite.

In the presence of carbon dioxide, however, serpentinitization may form either magnesite ($MgCO_3$) or generate methane (CH_4). It is thought that some hydrocarbon gases may be produced by serpentinite reactions within the oceanic crust.

Olivine water carbon dioxide serpentine magnetite methane

$$(Fe,Mg)_2SiO_4 + n\cdot H_2O + CO_2 \rightarrow Mg_3Si_2O_5(OH)_4 + Fe_3O_4 + CH_4$$

or, in balanced form:

$$18\ Mg_2SiO_4 + 6\ Fe_2SiO_4 + 26\ H_2O + CO_2 \rightarrow 12\ Mg_3Si_2O_5(OH)_4 + 4\ Fe_3O_4 + CH_4$$

Methanogenesis

Conversion of inorganic and organic carbon to CH_4

Complex process involving functional centers in enzymes which contain iron, molybdenum, cobalt and nickel.

Biomass (e.g. cellulose) is broken down by bacteria protozoa and fungi to acetate, lactate, propionate, butyrate and ethanol and further fermented by bacteria to formate, CO_2 and H2

Substrate in methanogenesis

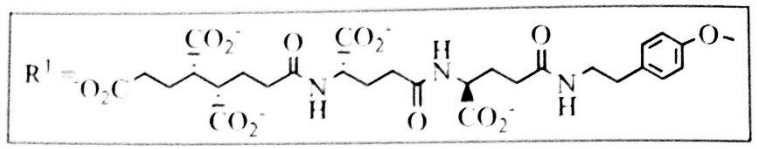
$$CH_3CO_2H \rightarrow CH_4 + CO_2$$

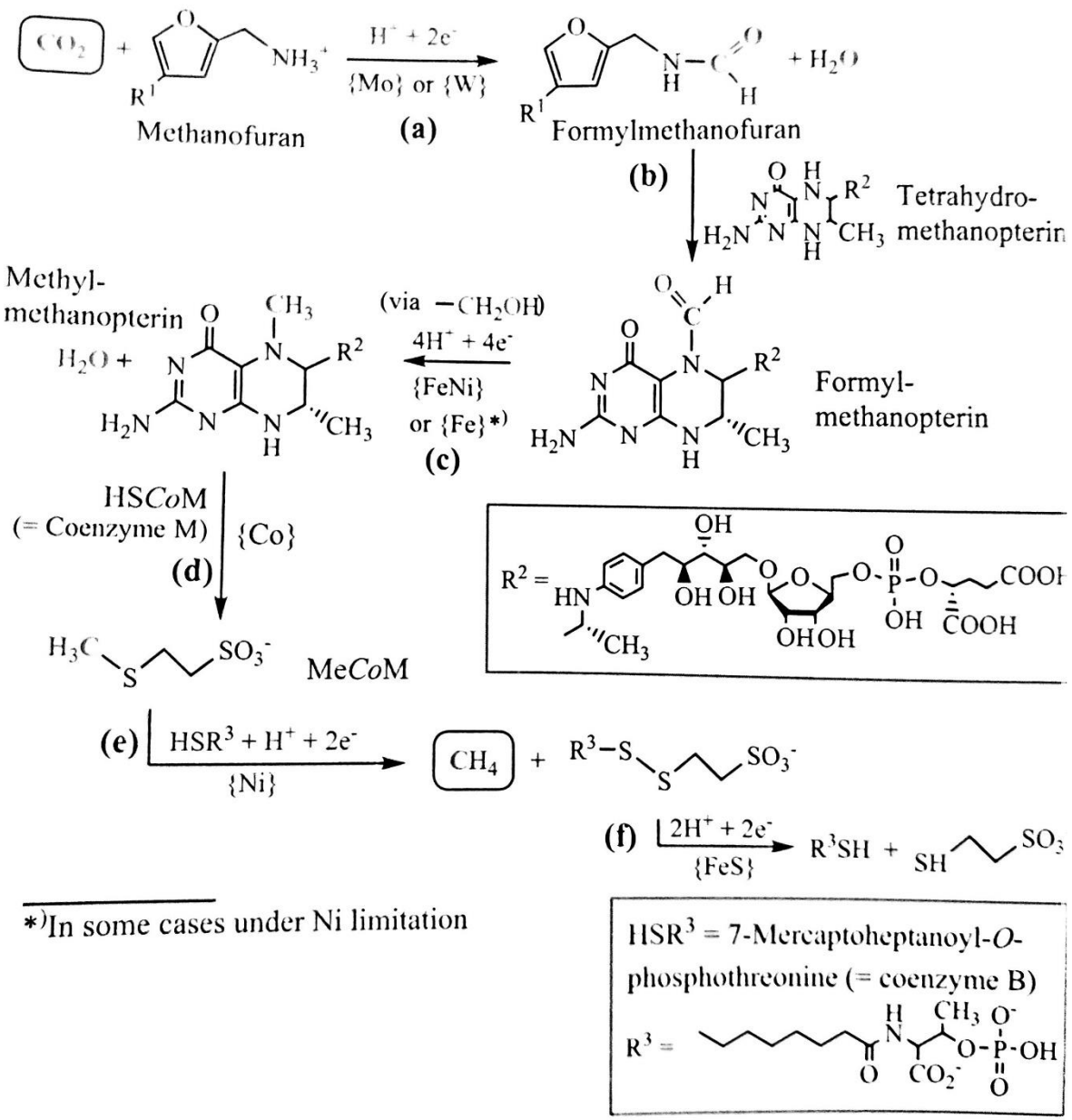
$$CO_2 + 8[H] \rightarrow CH_4 + 2H_2O$$

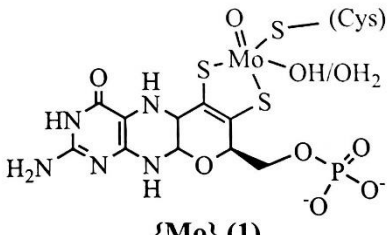
$$HCO_2^- \rightarrow CO_2 + [H] + e^-$$

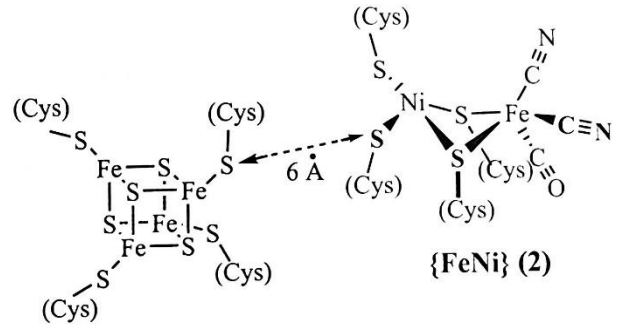
$$HCO_2^- + H_2O \rightarrow HCO_3^- + H_2$$

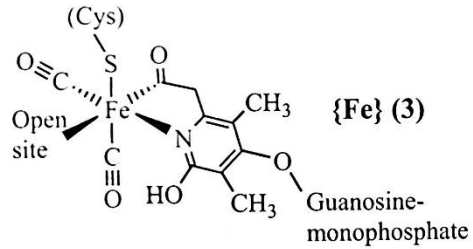
Overview of the reductive conversion of CO_2 to CH_4

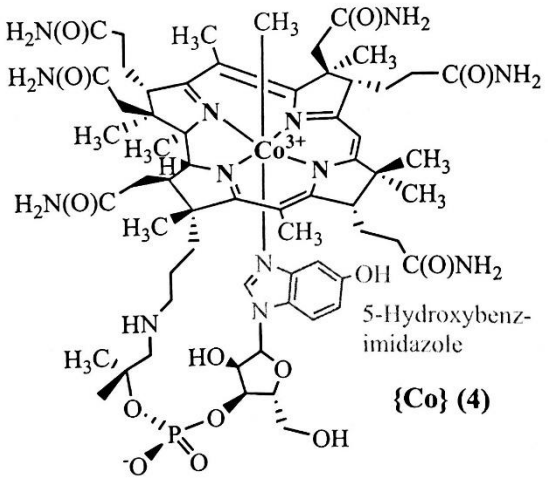


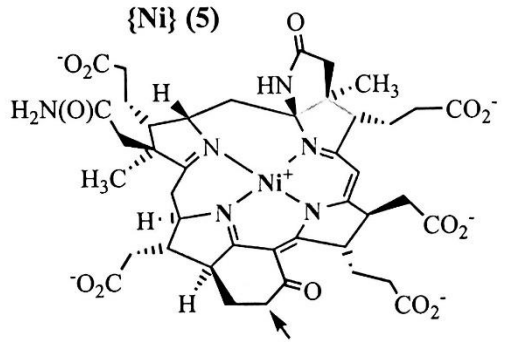



{Mo} (1)


{FeNi} (2)


{Fe} (3)

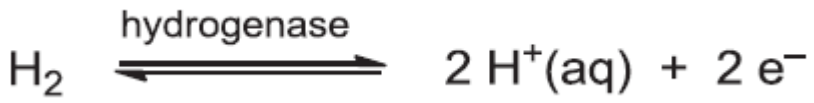

5-Hydroxybenz-imidazole
{Co} (4)


{Ni} (5)

Active centers of involved enzymes

Hydrogenases

Hydrogenases ("H_2ases") are enzymes which catalyze the reversible two-electron oxidation of molecular hydrogen ("dihydrogen").



This reaction plays a major role in the course of **dinitrogen "fixation",** in **microbial phosphorylation** and in the **fermentation of biological substances to methane**, among other things.

H_2 can either serve as an energy source instead of NADH or occur as the product of reductive processes

Nature's concept of hydrogen conversion or the reverse process of hydrogen generation is based on heterolytic splitting of dihydrogen ($H_2 = H^+ + H^-$) at catalytic centers.
The acidity of H_2, which is extremely low (p*K*a = 35), is dramatically increased on binding to a metal. Thus, for reaction, a two-electron transfer is feasible under physiological conditions at about −0.3V.
The simple one electron reduction of a proton to give a hydrogen atom would require much more negative potentials of less than −2V and thus completely unphysiological conditions.

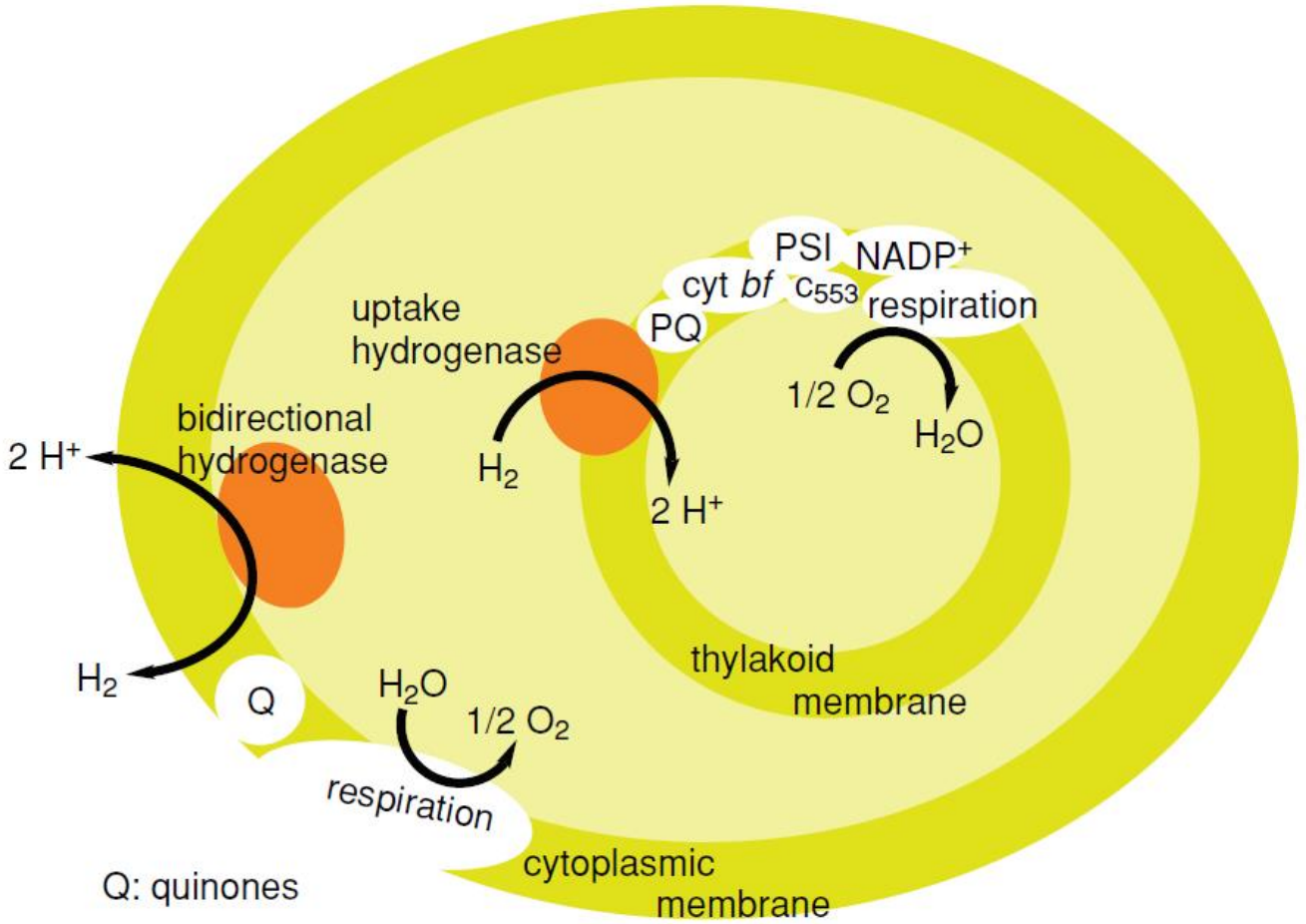
The inorganic components of hydrogenases serve as electron reservoirs and, presumably, as catalytic centers.

Three phylogenically distinct classes of hydrogenase are know:

- [NiFe]-hydrogenases (including [Ni/Fe/Se]-hydrogenases) -the most common form-
- [FeFe]-hydrogenases, structurally similar to the [NiFe]-hydrogenases
- [Fe]-hydrogenases, which contain mononuclear iron next to a special organic cofactor

The **hydrogenases are mostly of medium size** (40–100 kDa) and are, in principle, reversibly functioning enzymes. However, the potentials of electron-transferring Fe/S cluster and flavin components are often such that catalysis proceeds preferentially in one direction under physiological conditions

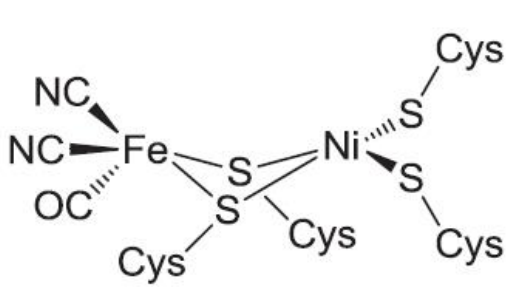
In some organisms, membrane-bound hydrogenases are found in addition to those which are soluble in the cytoplasm. In these cases, the soluble form catalyzes the reduction of NAD^+ by H_2, whereas **the electrons generated via the membrane-localized oxidation of dihydrogen are inserted into the respiratory chain** and serve in the production of high-energy phosphates

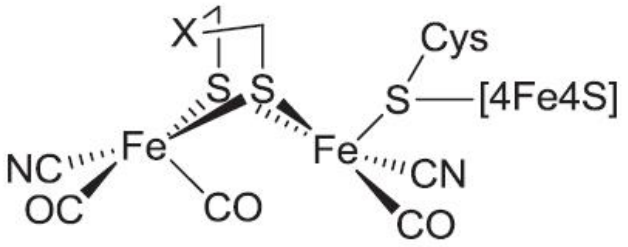


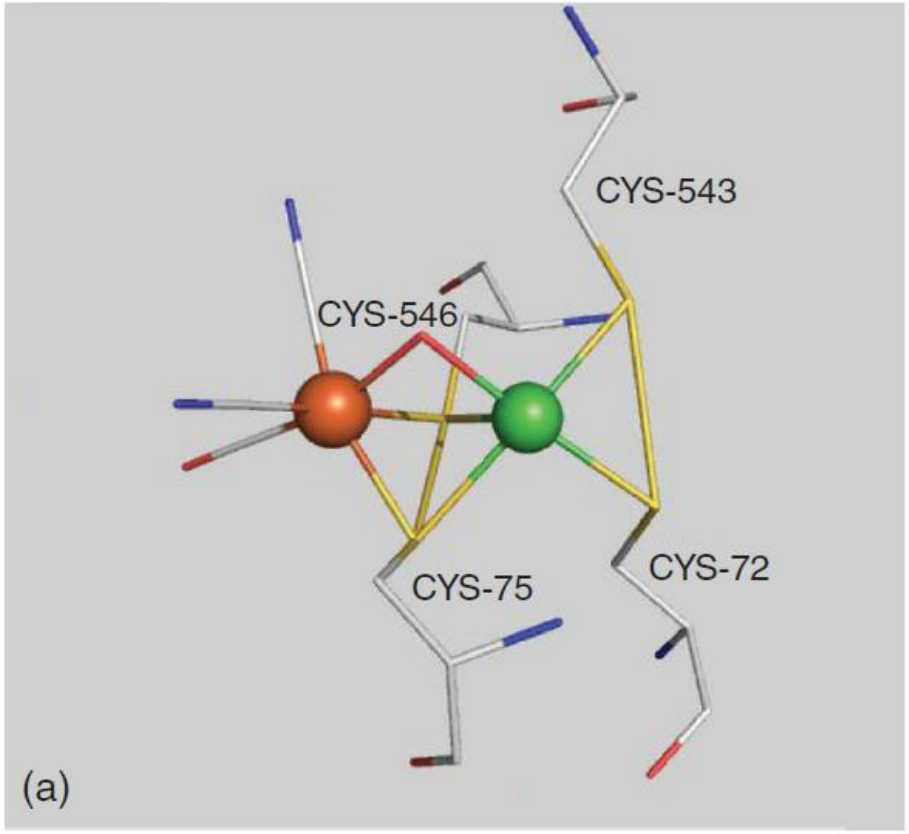
Both the [FeFe]- and the [NiFe]-hydrogenases contain
bimetallic active centers featuring
the biologically very unusual COandCN- ligands

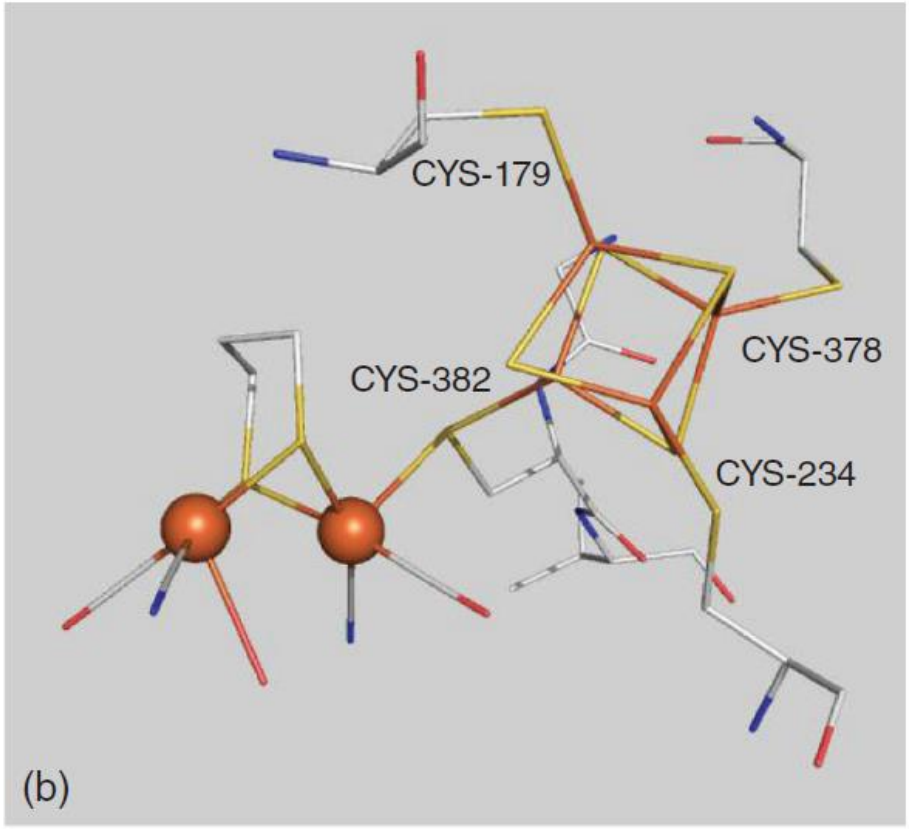
The [NiFeSe]-enzymes
incorporate a selenocysteine coordinated to the Ni in place of
one of the terminal cysteine
residues of the standard [NiFe]-enzymes.

For [NiFe]-hydrogenases, a number of different
redox states through which the system shuttles during catalytic
activity have been found,
including the oxidation states Ni(III) and Ni(I).







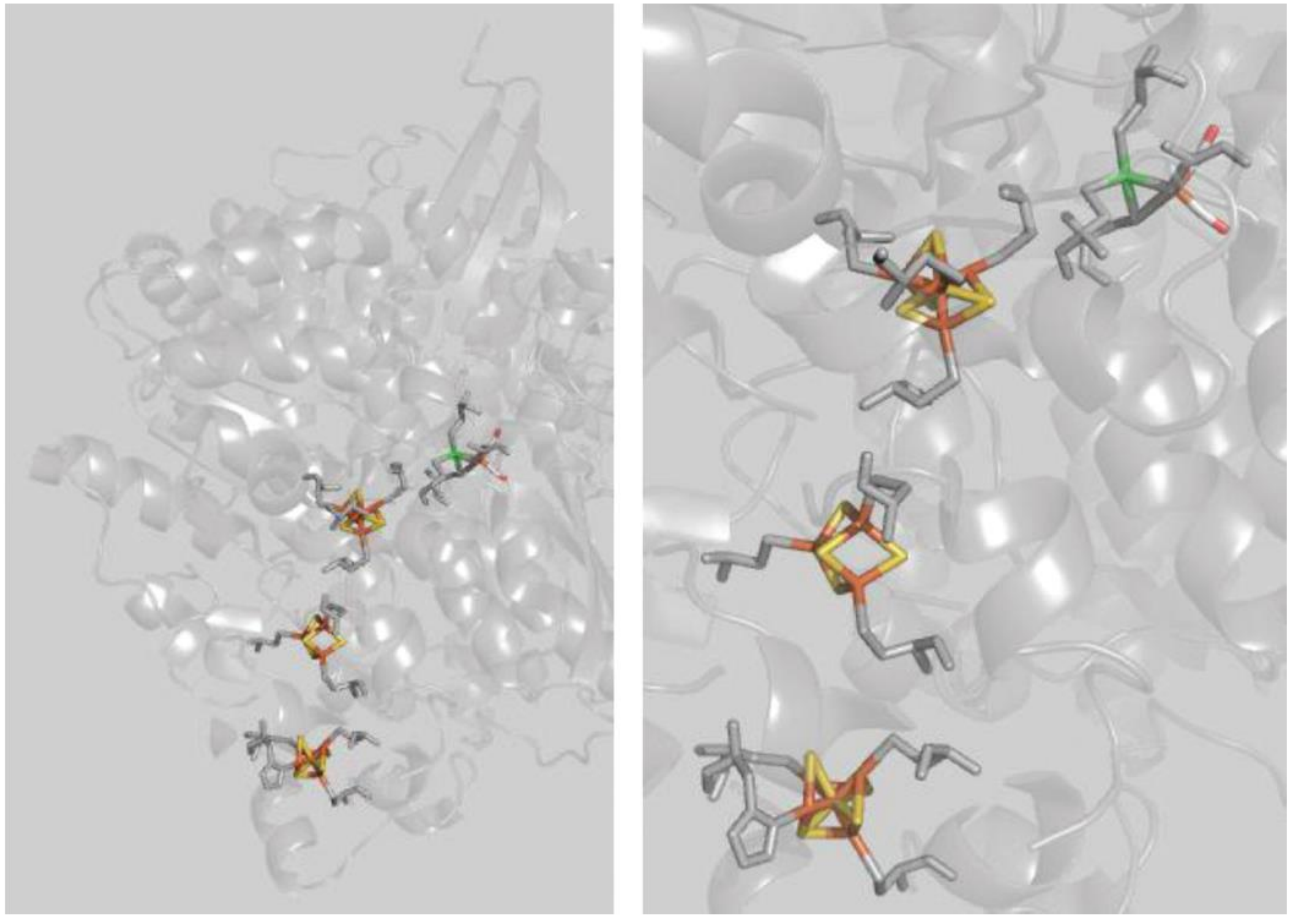


(a) *Desulfovibrio gigas* [NiFe]-hydrogenase (PDB code 1YRQ) and (b) *Desulfovibrio desulfuricans* [FeFe]-hydrogenase (PDB code 1HFE)

The mechanism of catalysis by [NiFe]-hydrogenases and the nature of the participating species and oxidation states are still controversial!

Considering that CO, CN− and acetylene (ethyne) reversibly inhibit hydrogenases, there is a great deal of evidence that H_2 is split in the active center, while hydrophobic tunnels help to pass H_2 quickly through the protein to the Ni site.

Additionally, **the electrons are transported through the enzyme molecule by a special relay system comprising Fe/S clusters**

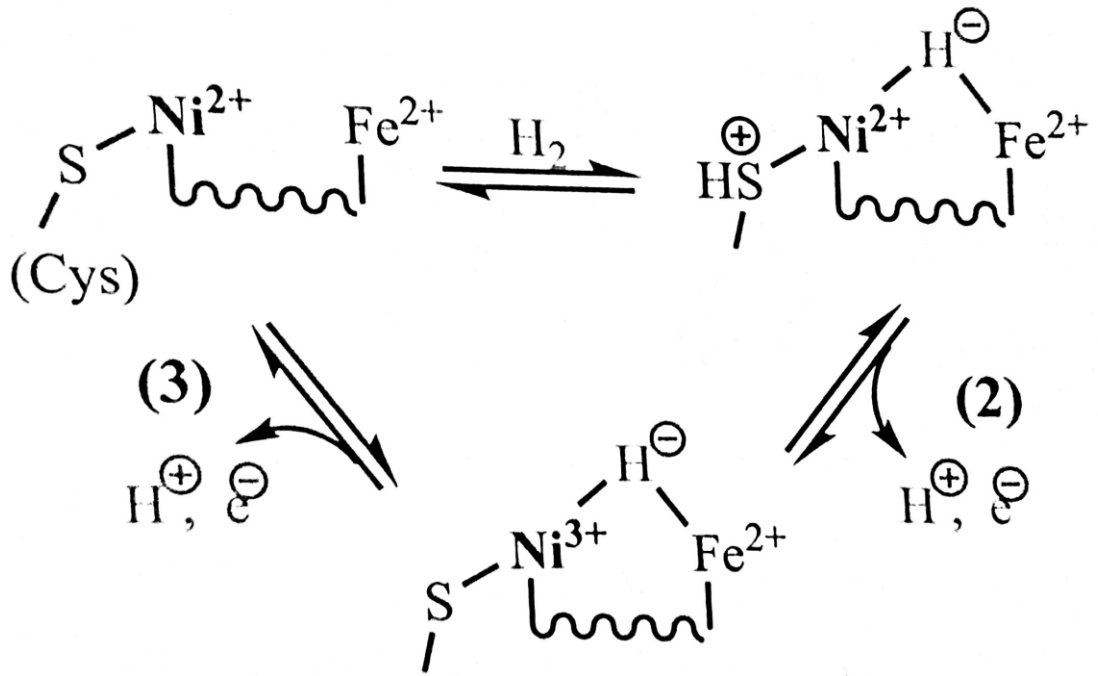


Structure of the [NiFe]-hydrogenase of *Desulfovibrio vulgaris* at 1.35 °A resolution, representing the reduced active enzyme. (PDB = 1UBU)

Corresponding models postulate the heterolytic cleavage of H_2 (as already outlined) where the hydridic hydrogen atom (H^-) remains at the metal while the protic component (H^+) binds to a metal-coordinated sulfide, to hydroperoxo centers or, as the reaction proceeds, to other basic sites within the protein.

The nature of the assumed bond between hydrogen and the metal (Fe or Ni) is of particular interest. More than Fe–H species, the hydrides of nickel are well known from organometallic chemistry.

For the **primary approach and binding of H_2** to the active site of the enzyme, the possibility **of side-on (η^2) coordination of dihydrogen** to Ni (or Fe) is discussed, since this binding mode has now been found in many complexes

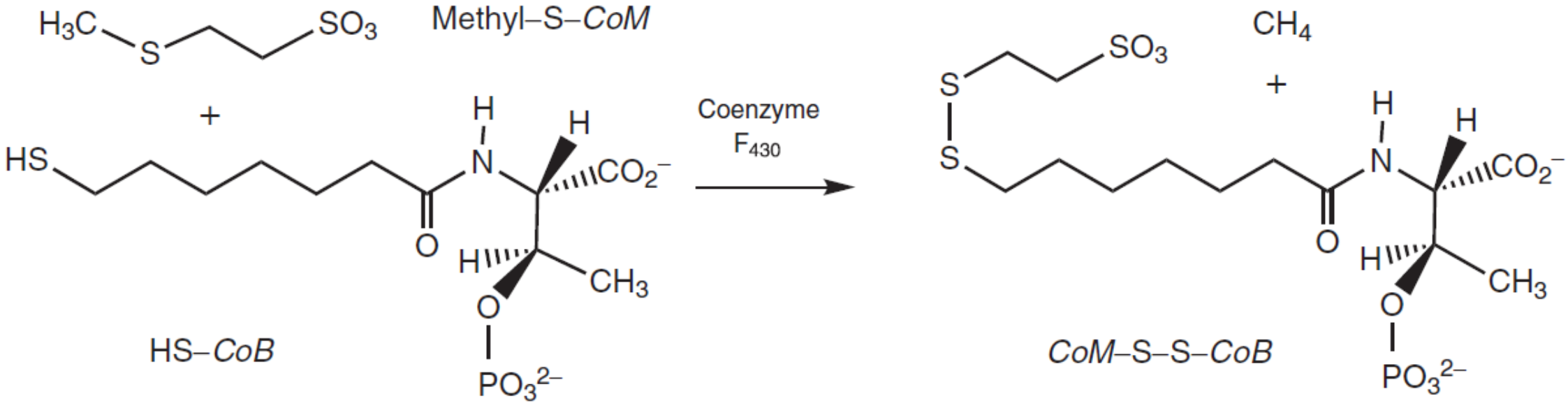


Methyl-coenzyme M Reductase

Methyl-coenzyme M reductase (MCR) serves methanogenic bacteria in the eventual formation and liberation of methane by catalyzing the reduction of methyl-*CoM* (2-methylthioethanesulfonate)

last step in the energy-producing synthesis of CH_4 from CO_2 by autotrophic archaebacteria such as *Methanobacterium thermoautotrophicum*.

The driving force of this reaction is the disulfide formation between *CoM* and the additional component HS–*CoB* (thioheptanoyl threonine phosphate)

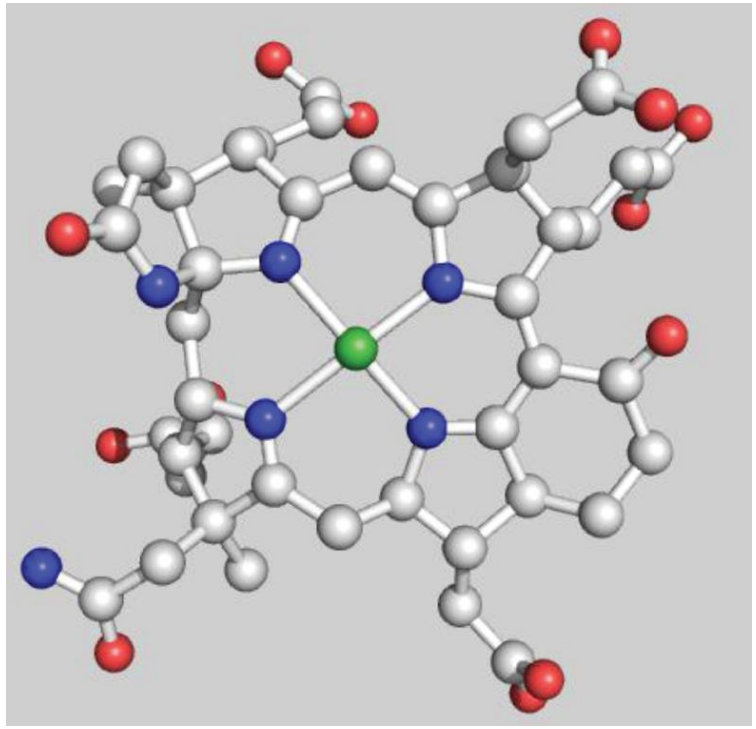


Several coenzymes are essential for the entire CO_2 to CH_4 conversion process, which requires a total of eight electrons.

Methanofuran serves in the uptake of CO_2 and for the transformation of the carboxylic function during the first 2e_ reduction. The resulting formyl group, -CHO, is transferred to tetrahydromethanopterin, which functionally resembles tetrahydrofolic acid of eukaryotes. After two more two-electron-transfer steps (formyl → hydroxymethyl → methyl), the resulting methyl group is transferred to *CoM*, which releases methane in a reaction catalyzed by MCR.
The electrons required for the various reduction steps are obtained through the oxidation of molecular hydrogen, which is catalyzed by various hydrogenases.

MCR is a very sensitive and complex enzyme. The dimeric protein has a molecular mass of approximately 300 kDa and is composed of three subunits of 68, 47 and 38 kDa each.

A yellow, low-molecular-weight substance which contains nickel and shows an intense absorption maximum at 430 nm has been isolated from the enzyme: the factor F_{430}



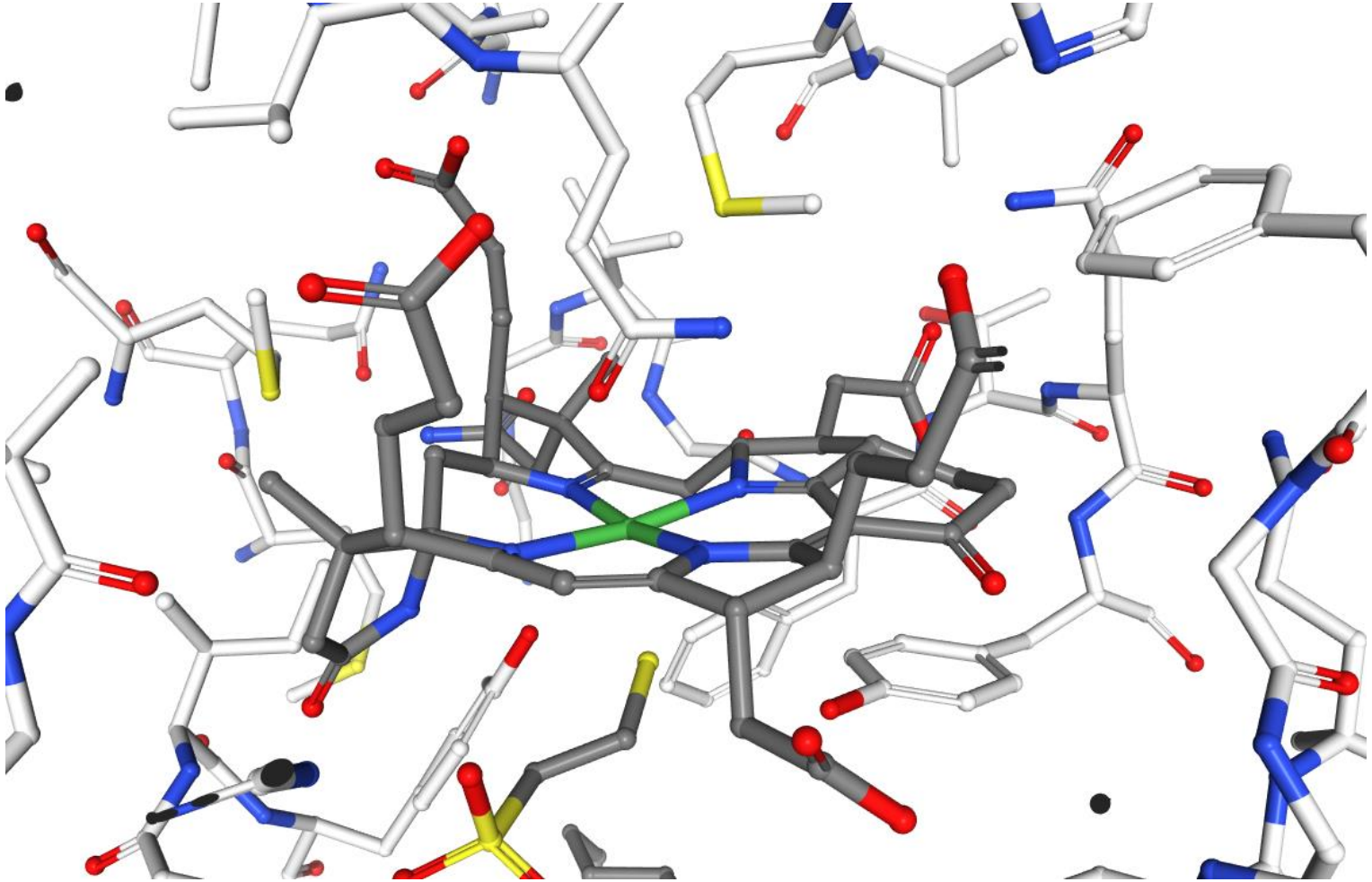
Structure of the F_{430} cofactor

It features a **highly hydrogenated porphin system with anellated lactam and cyclohexanone rings** in which the chromophore only extends over three of the four nitrogen atoms. The underlying ring structure has been named "**hydrocorphin**" in order to highlight the relationships with both porphyrins and the not-cyclically-conjugated corrins.

(PDB code 1HBN).

The partially saturated character of the macrocycle and the anellation of the additional saturated rings allow a marked structural flexibility, especially with regard to a folding of the tetrapyrrole ring in the direction of an S4-distortion (**saddle-like**). As an important consequence, both the spin crossover to low-spin Ni(II) (*S*=0) and the transition to the d^9-configurated Ni(I) state are facilitated for the otherwise not axially activated high-spin nickel(II) center (*S* = 1).

A **low-spin d^8 configuration** (i.e. spin-pairing) only results after **strong distortion of the octahedral ligand field**; the degeneracy of the eg orbitals, which would disfavor spin pairing, has to be effectively lifted.

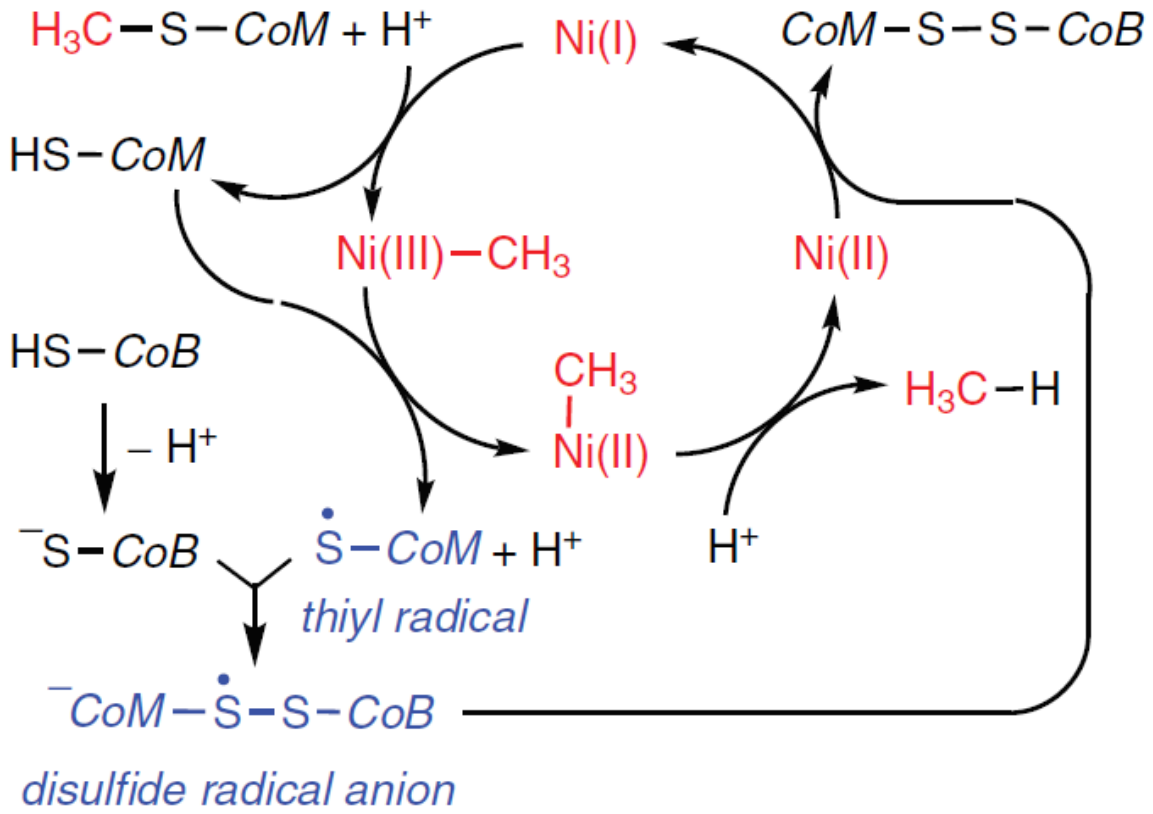


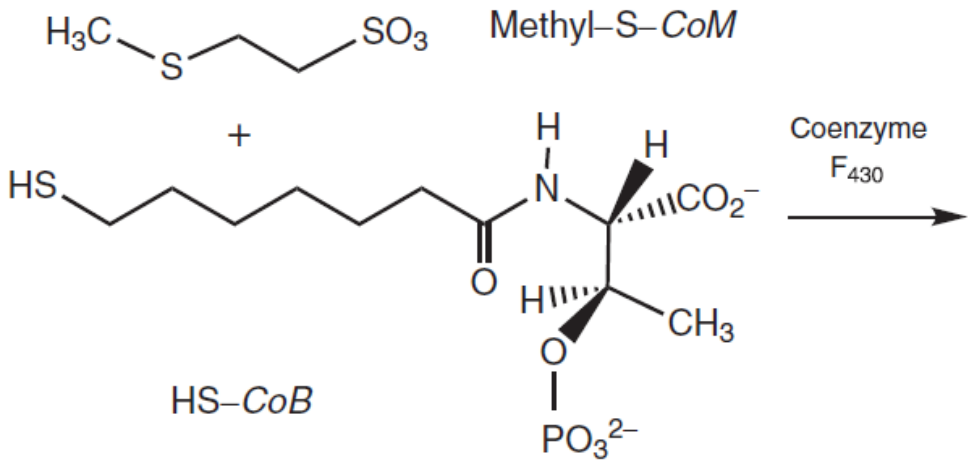
Two different mechanisms are under discussion.

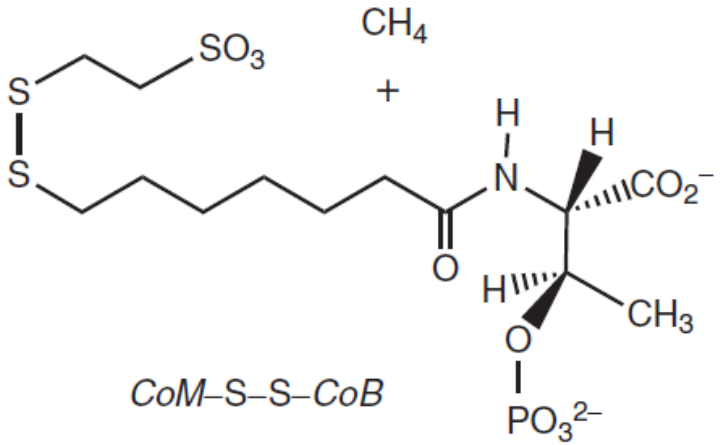
Catalytic mechanism I postulates as the key step a nucleophilic attack of the Ni(I) (reduced state) of F_{430} at the methyl group of CH_3–S*CoM*.

Subsequently withdraws an electron from *CoM* thiolate to form CH_3–Ni(II) and a *CoM* thiyl radical. Correspondingly,CH_3–Ni(II) becomes protonated, producing Ni(II) andCH_4.

The *CoM* thiyl radical reacts with HS–*CoB* to give a heterodisulfide anion radical, and finally the excess electron returns to nickel, converting Ni(II) to Ni(I).

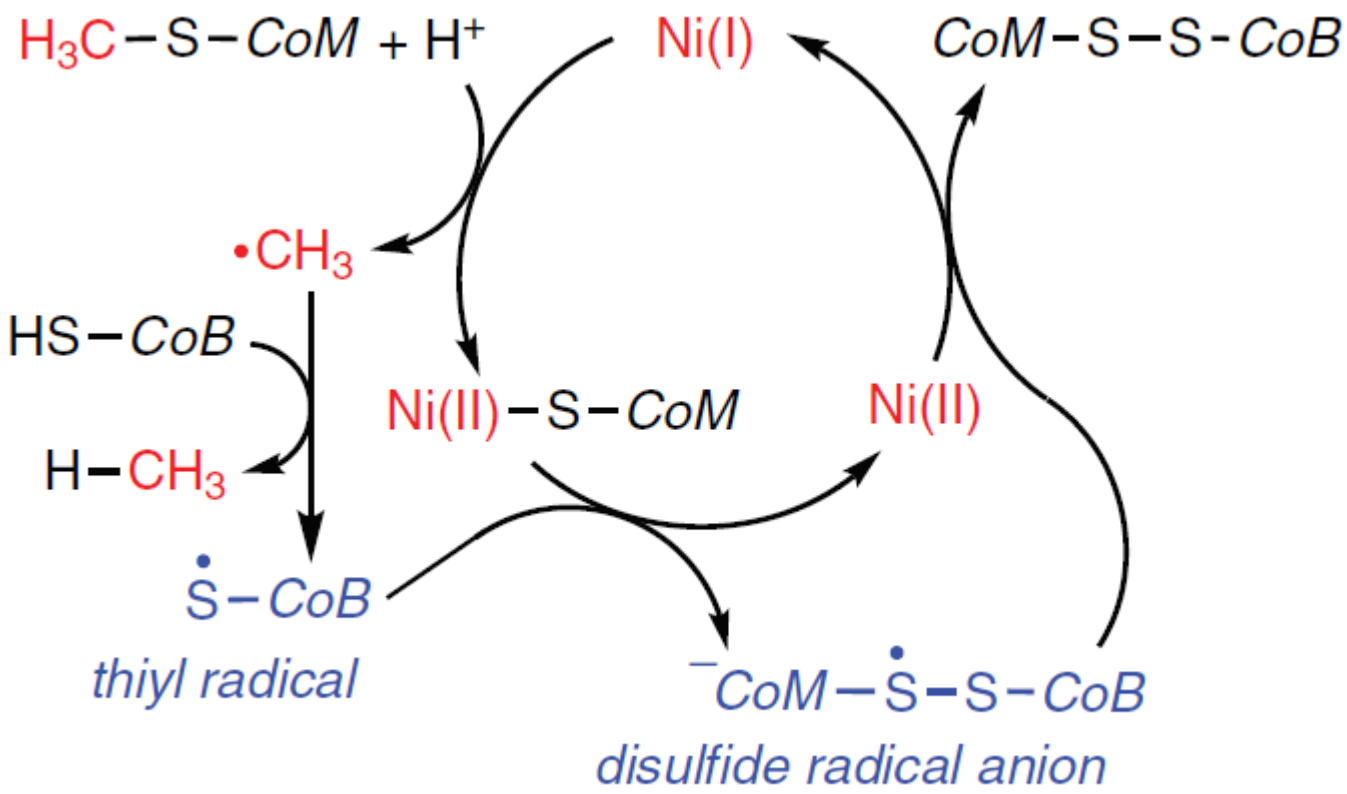






Mechanism II includes in its key step an attack of Ni(I) at the thioether sulfur of CH_3–S*CoM*, generating a free methyl radical (also highly reactive and thus not directly detected), which is converted to CH_4 by withdrawing a hydrogen atom from the sulfhydryl group of HS–*CoB*. The thiyl

radical form of *CoB* thus produced combines with *CoM* thiolate to form the heterodisulfide radical anion, whereas the excess electron is returned to the nickel.



CO Dehydrogenase = CO Oxidoreductase = Acetyl-*CoA* Synthase

Many methanogenic and acetogenic (i.e. methane and acetic acid-producing) bacteria contain a "CODH" enzyme which catalyzes the oxidation of CO to CO_2.

N.B. In biochemistry, oxidation is often equated with dehydrogenation; however, CO does not contain any hydrogen atoms and the term "CO oxidoreductase" instead of "CO dehydrogenase" would thus be more appropriate.

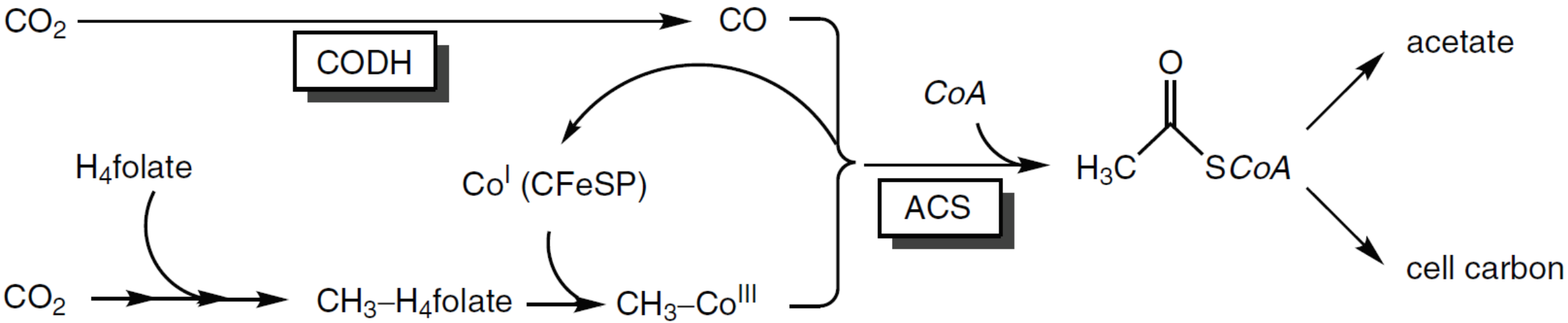
$$CO + H_2O \rightleftharpoons CO_2 + 2\,H^+ + 2\,e^-$$

The other biological function of the enzyme is to catalyze the reversible formation of acetyl-*CoA* in combination with *CoA* itself and a methyl source

$$H_3C{-}[Co] + CO + HS\mathit{CoA} \rightleftharpoons CH_3C(O)S{-}\mathit{CoA} + H^+ + [Co]^-$$

In methanogenic bacteria, the further degradation of acetic acid to CO2 and CH4 presumably proceeds via CO as an intermediate.

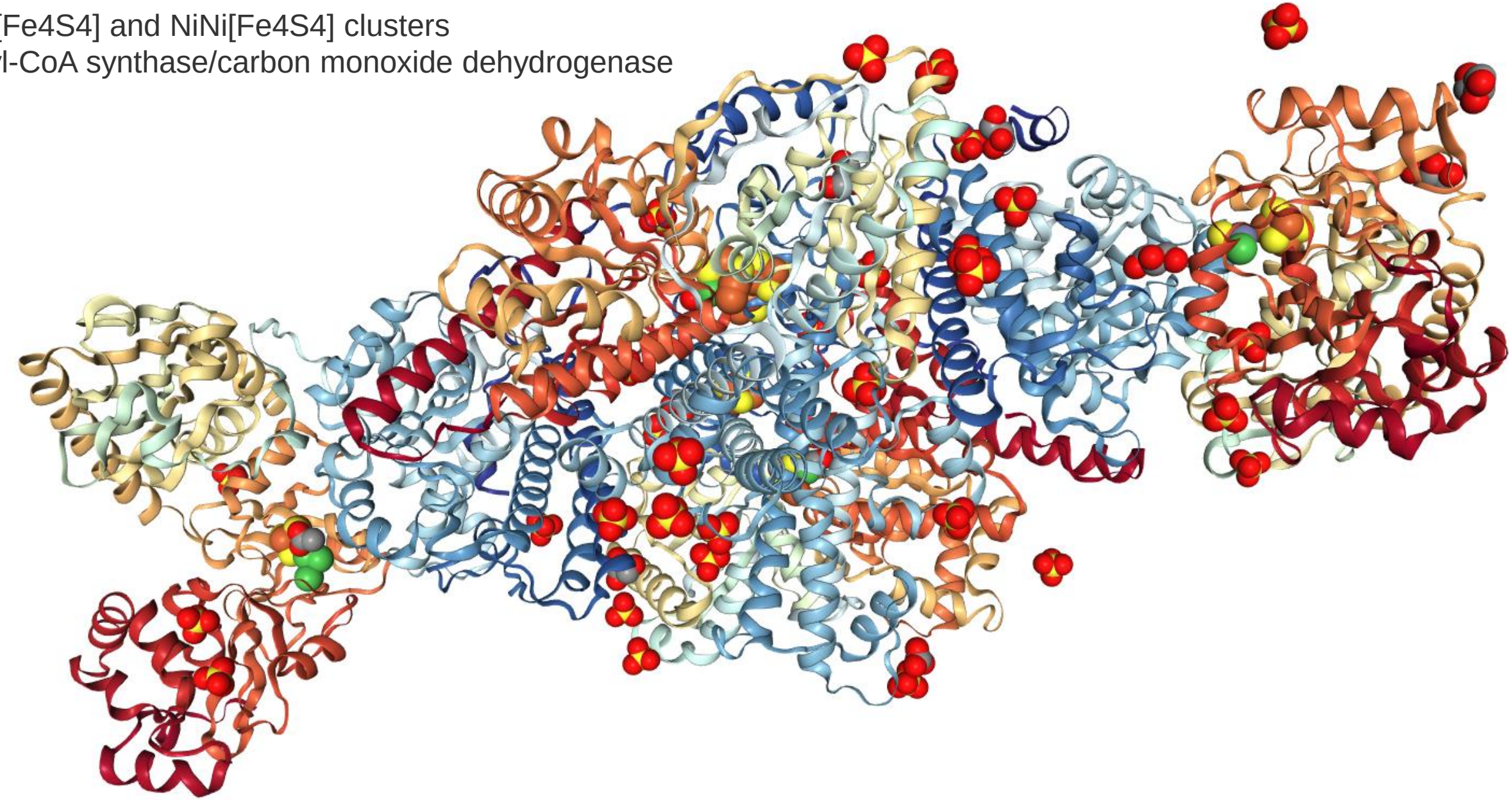
Acetyl- *CoA* synthase (ACS), together with CODH, is a key player in the metabolism of anaerobic microorganisms via the Wood–Ljungdahl pathway (Figure 9.4) and a major component of the global carbon cycle (see bottom scheme).



PDB = 1OAO

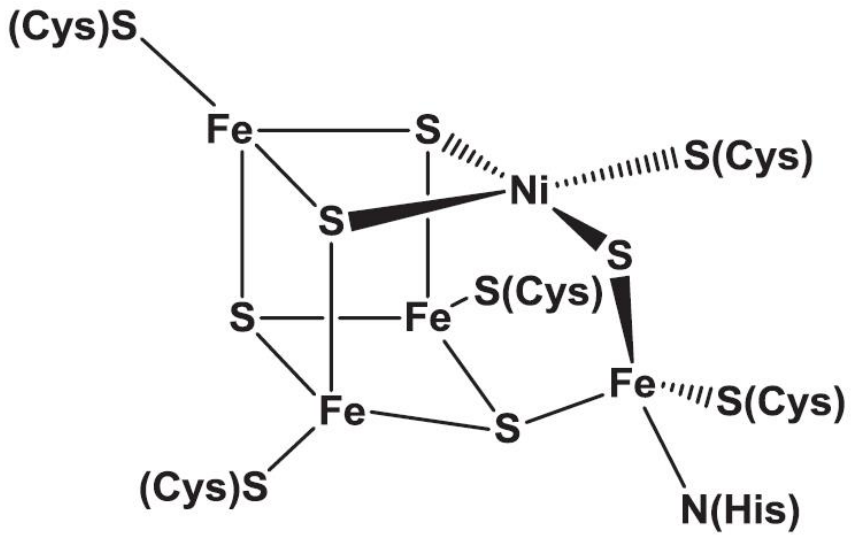
NiZn[Fe4S4] and NiNi[Fe4S4] clusters

acetyl-CoA synthase/carbon monoxide dehydrogenase

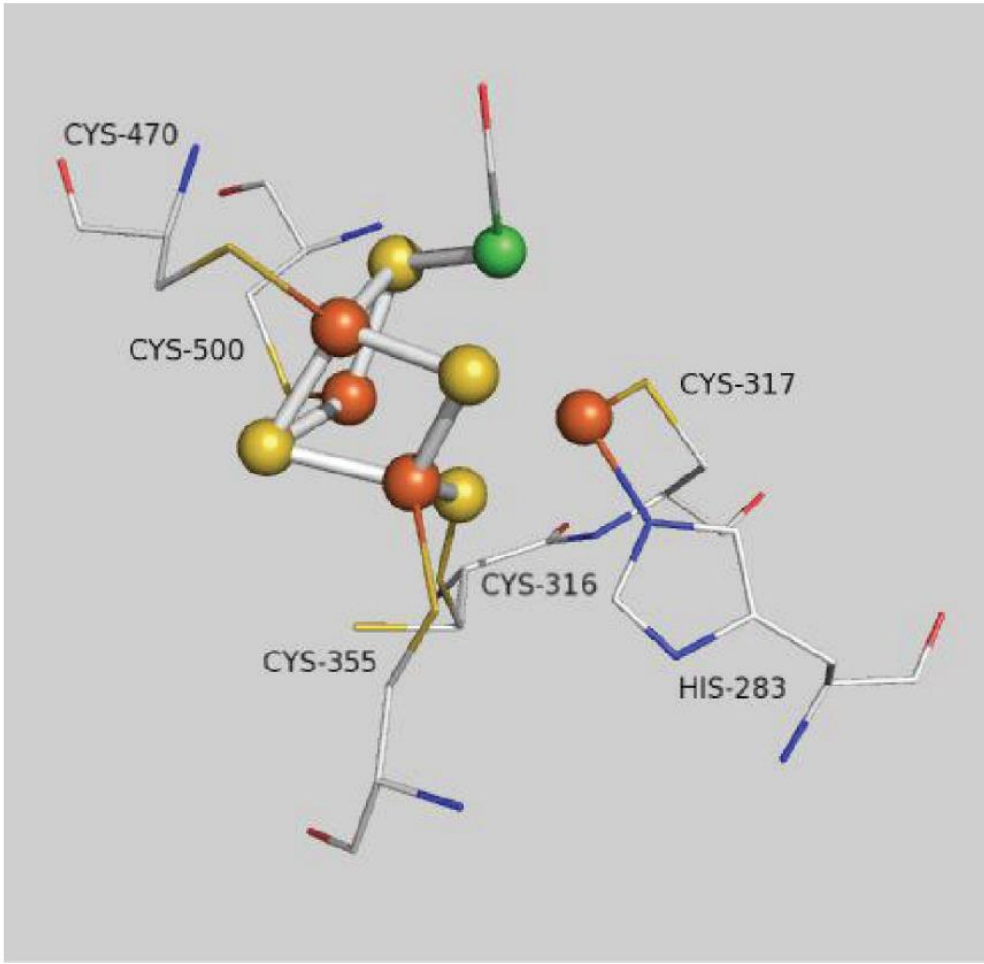


It has been shown that the CODH of all anaerobic bacteria contains nickel, while aerobic species require molybdopterin.

Several crystal structures have been obtained which show that the enzyme is a mushroom-shaped homodimer **containing metal clusters. The cluster "C" is a [3Fe-4S] cluster bridged to a heterodinuclear [NiFe] site**, the catalytic center for CO oxidation. **Clusters B and D are [4Fe-4S]$^{2+/1+}$ clusters that transfer electrons between the C cluster and external redox proteins**.



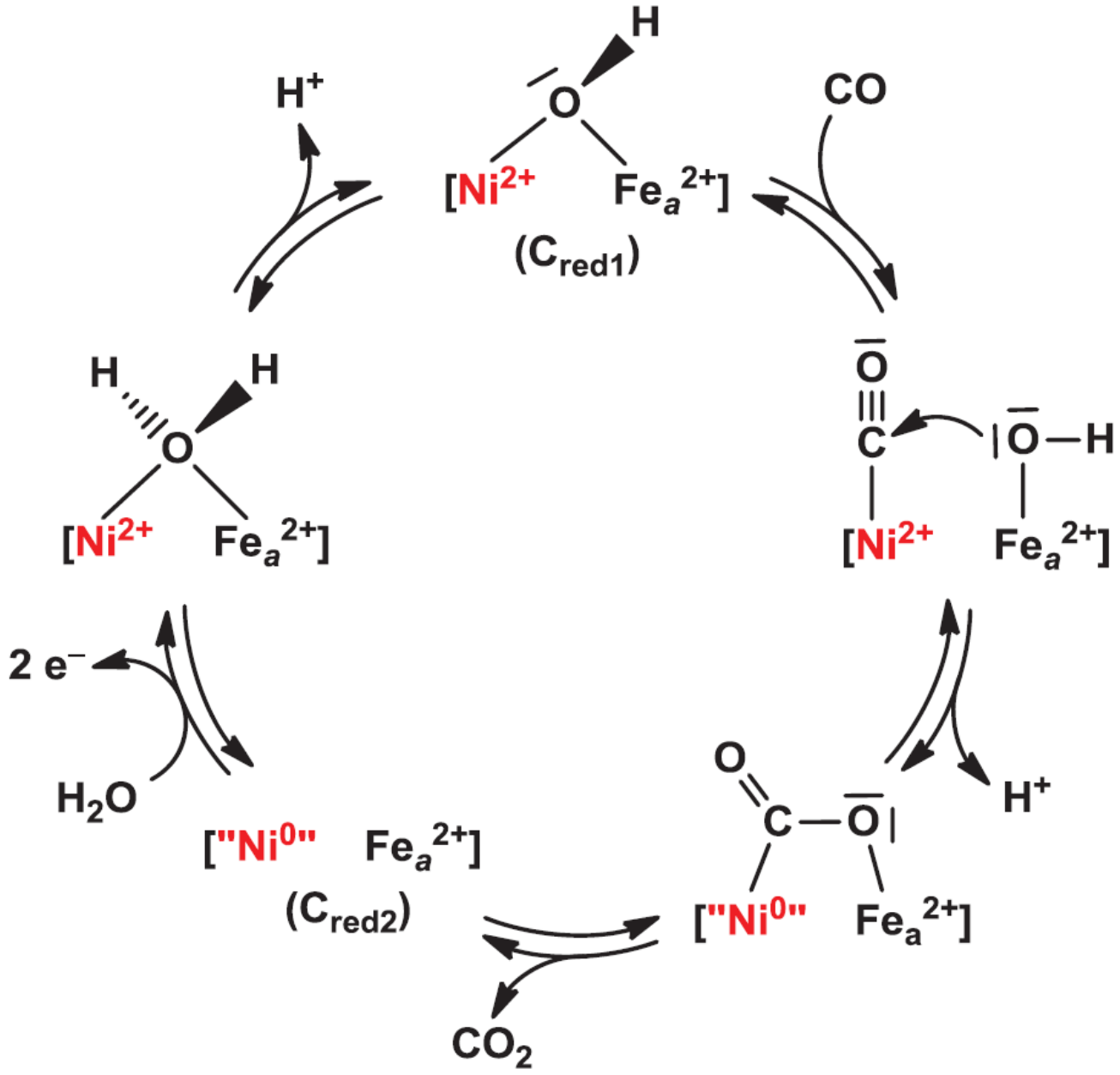
C cluster of CODH (PDB code 1OAO)



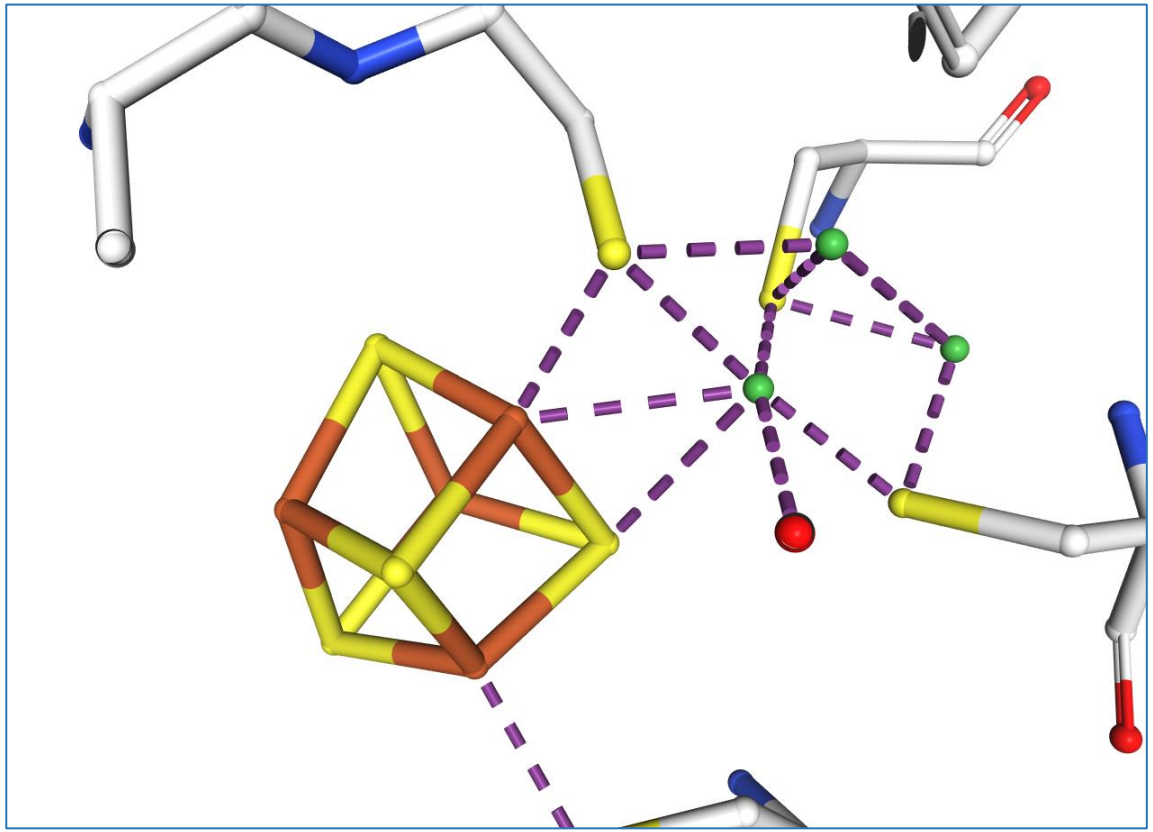
Mechanism

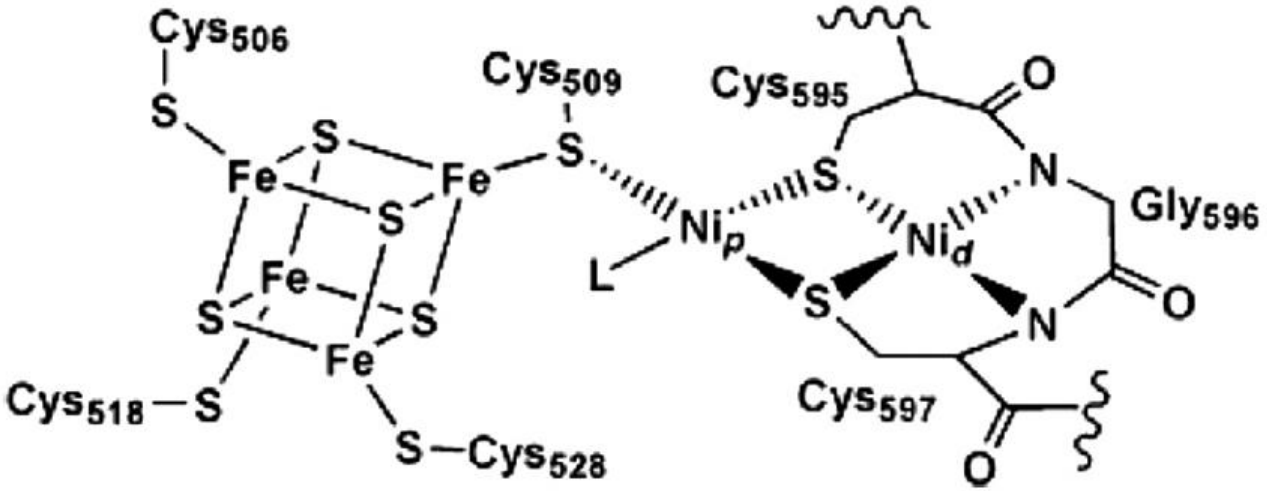
H_2O is bound to the C cluster in the bridging Fe. . . Ni position, losing a proton (C_{red1}). When CO is bound (presumably to Ni), the Fe-bound OH− is able to perform a nucleophilic attack on CO.

The Ni–CO(OH) intermediate (not shown) is deprotonated and CO_2 is formed, while two electrons are transferred on to the C cluster ($C_{red1} \rightarrow C_{red2}$). The two electrons are subsequently transferred to the B cluster, then the D cluster, and finally to external electron acceptors.



The ACS active site (the "A cluster") consists of a [4Fe-4S] cluster, which is CysS-bridged to a proximal Ni site (Ni*p*), which is thiolate-bridged to the so-called "distal Ni ion" (Ni*d*), which has a square-planar thiolato- and carboxamido-type N_2S_2 coordination environment. There is a similarity between this arrangement and the hydrogenases. The coordination sphere of Ni*p* is completed by an exogenous ligand L of so far unknown identity.





What is generally agreed is that the crystal structures of CODH/ACS reveal the presence of a hydrophobic channel connecting the CODH active site (C cluster) to the ACS A cluster. This gas channel opens at the metal proximal to the [4Fe-4S] cluster, suggesting that Ni*p* is the site of CO binding.

The depicted radical mechanism for the **transmetallation reaction requires a one-electron input**, and since no external electron transfer has been found for ACS, the electron may be redonated internally.