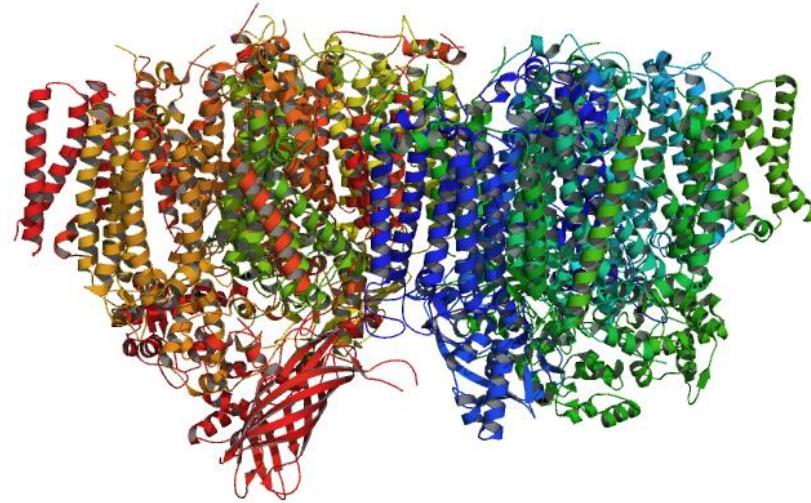
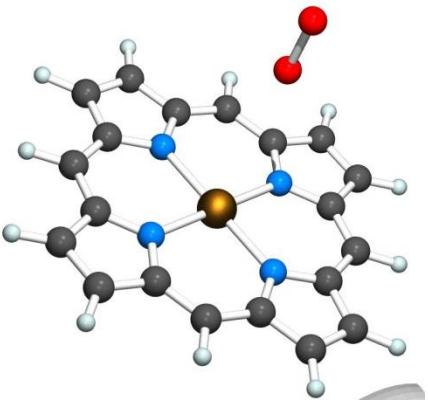
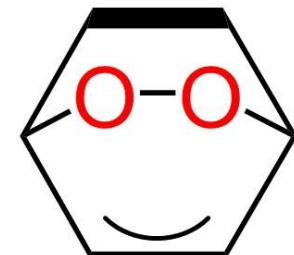


Bioinorganic Chemistry (BIC)

VII. Hydrogen, Carbon & Nitrogen Metabolism



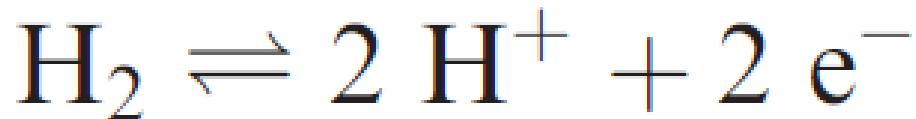
Dr. ($O_6S_4C_4Ar$) Lung Wa CHUNG(钟龙华)
(oscarchung@sustech.edu.cn)
Department of Chemistry



1. Hydrogen Metabolism & Hydrogenases

Hydrogenases (H_2 ases)

- Formation or usage of H_2 gas plays some key physiological roles in some organisms (e.g. anaerobic prokaryotes, eukaryotes & aerobes).



- H_2 can donate 2e^- to reduce some inorganic compounds in some bacteria, e.g. CO_2 , N_2 , NO_3^- & SO_4^{2-} or
- H^+ can be an e^- acceptor
- The enzymes involved in this redox reaction are called **hydrogenases (H_2 ases)**.

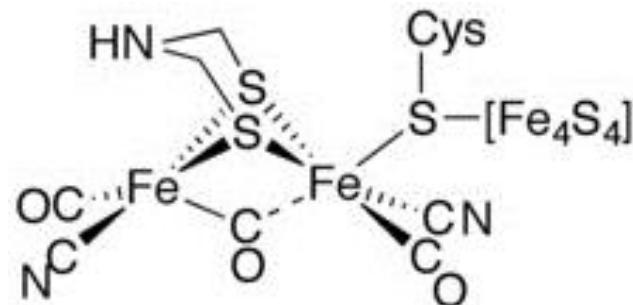
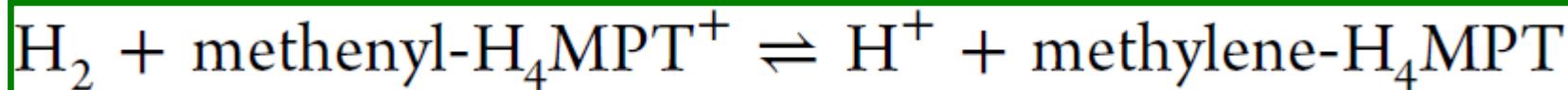
Classifications & Functions

• All known H₂ases are **metalloenzymes** & classified into 3 types (with different number & type of the metal in the active site):

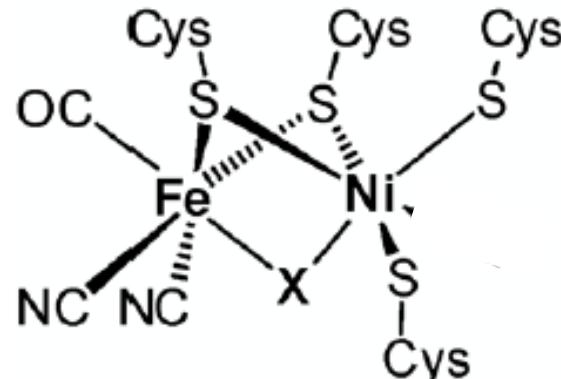
1. **[FeFe] H₂ase**,

2. **[NiFe] H₂ase**,

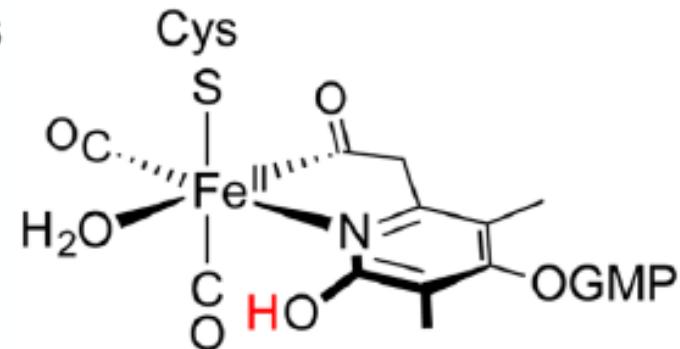
3. **[Fe] H₂ase** (**Hmd**, H₂-forming N^{5,N¹⁰}-methenyl-5,6,7,8-tetrahydromethanopterin dehydrogenase).



[FeFe]H₂ase

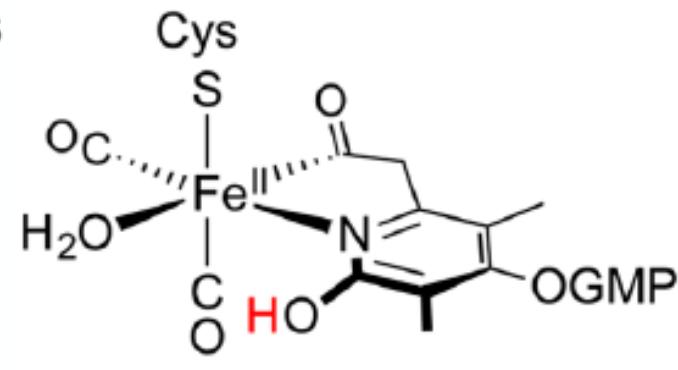
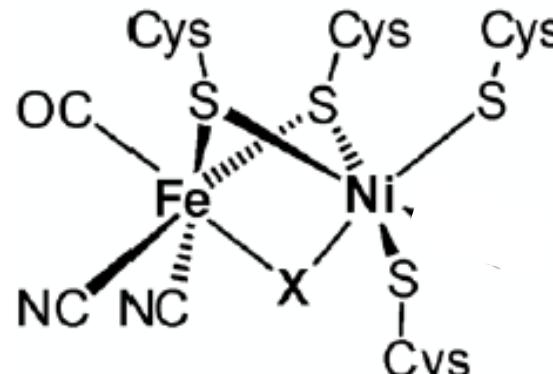
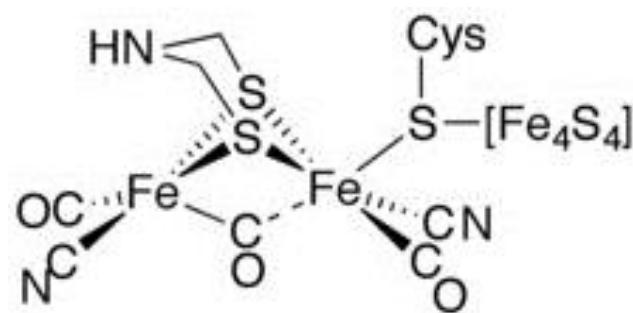


[NiFe]H₂ase



[Fe]H₂ase

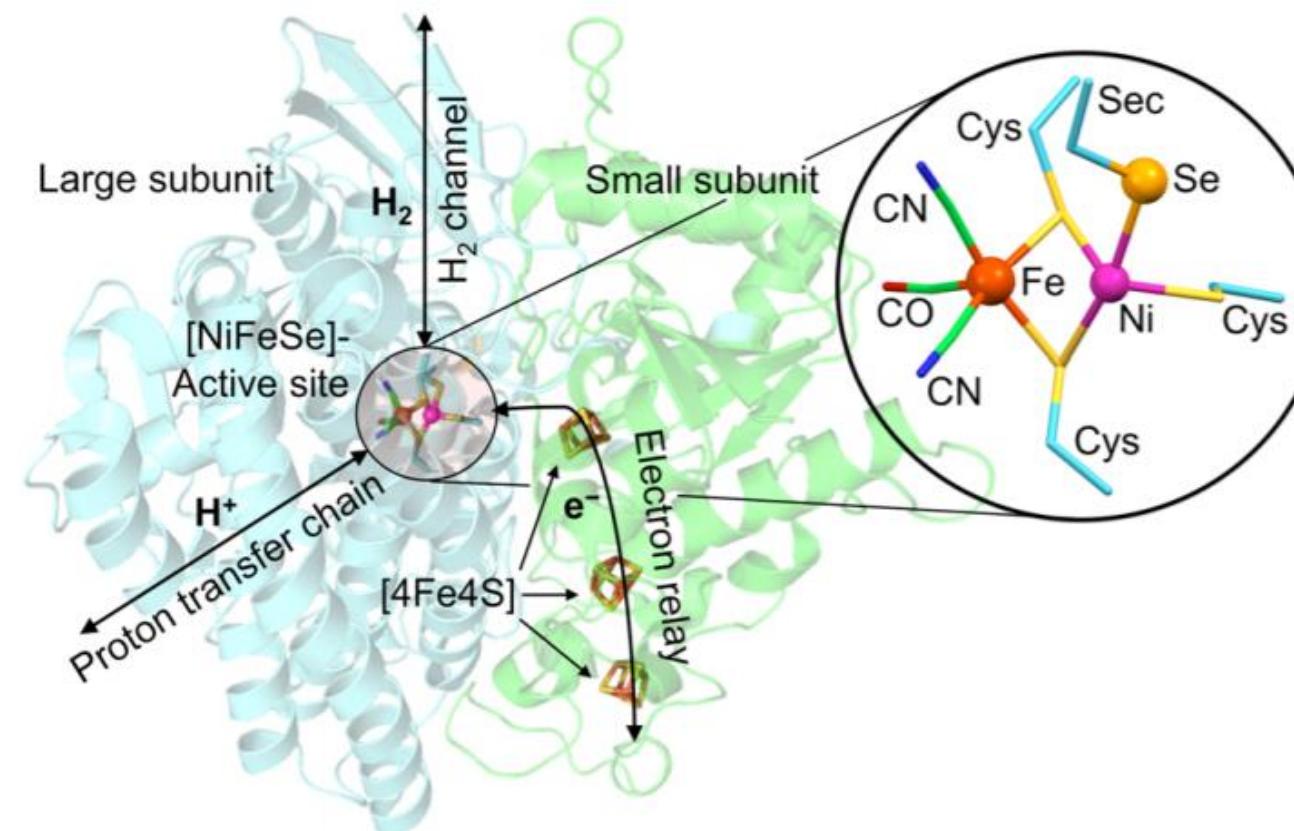
- Uncommon **CO ligands** in the active-site Fe center in all H₂ases, & **CN⁻ ligand** also in [FeFe] & [NiFe] H₂ases: the first example found in biology. **Low-spin Fe**.



- **[FeFe] H₂ases** are generally involved in the **H₂ formation**. They have **high reaction rates** *in-vitro*, but can be rapidly & **irreversibly oxidized/inactivated** by air (**O₂**).



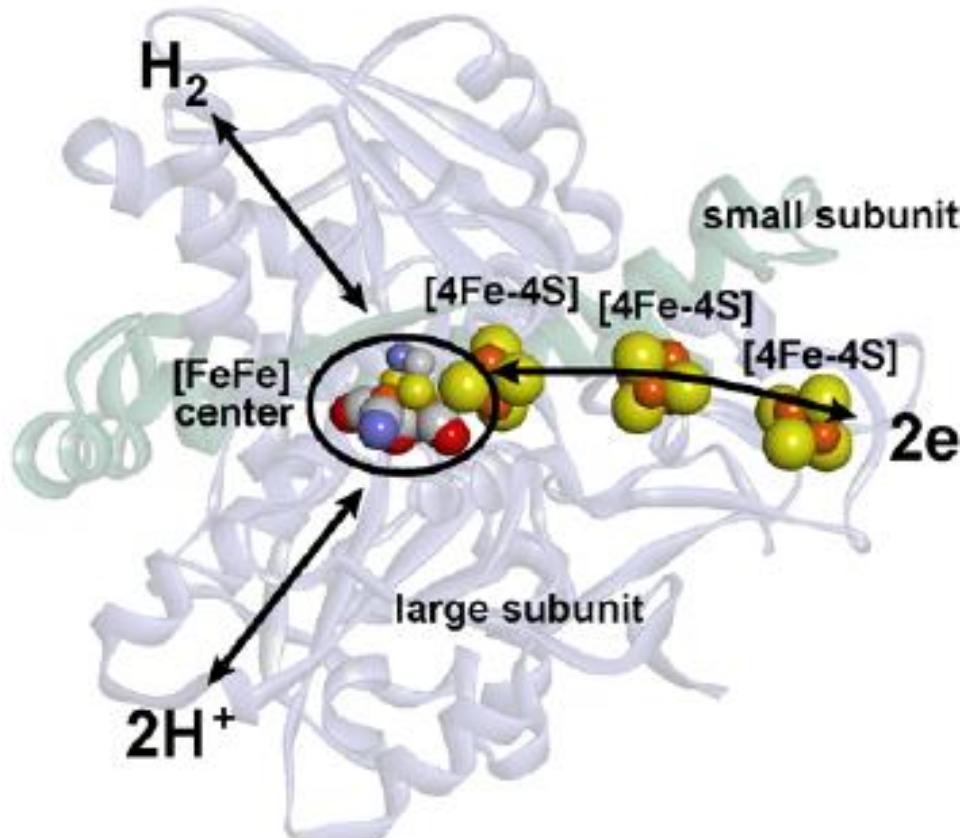
- [NiFe] H₂ases are generally involved in the H₂ oxidation. They are more O₂ tolerant, as they can be reversibly oxidized by air. Their reaction rates are less than ~10% [FeFe] H₂ases, but they have higher substrate affinity & thus can catalyze oxidation at lower H₂ partial pressures.



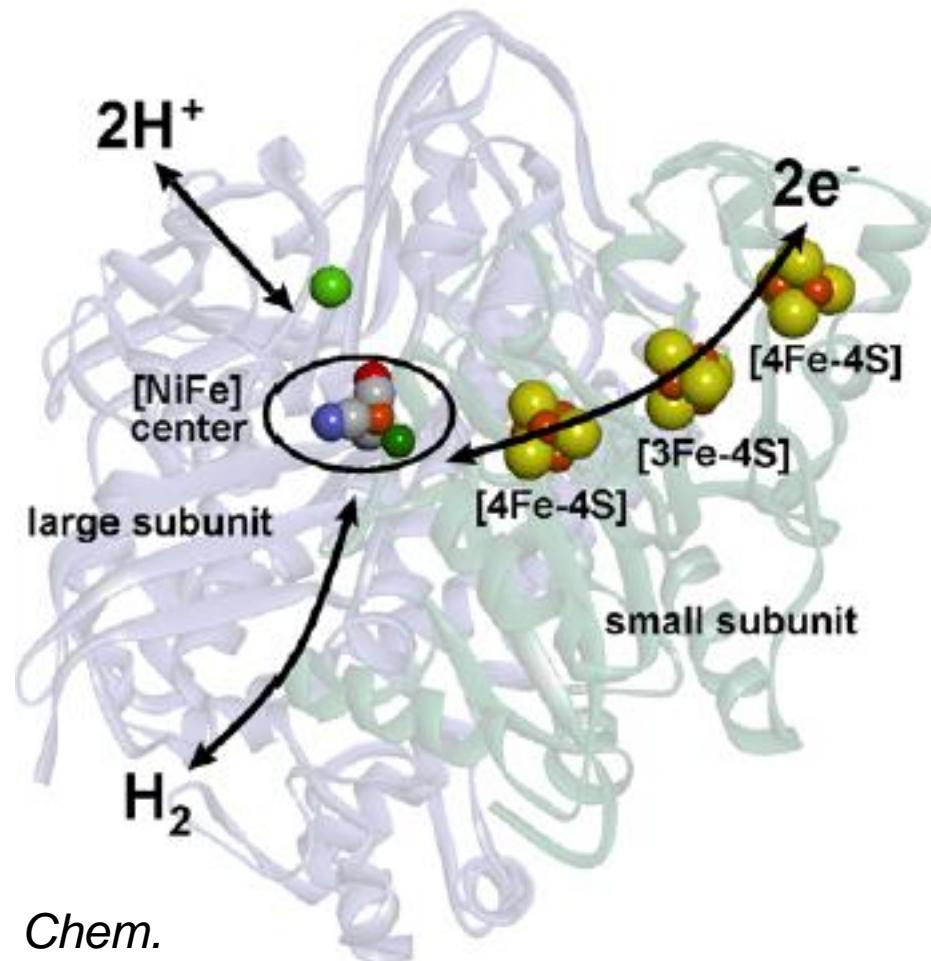
- Some [NiFe] H₂ases use a selenocysteine (SeCys) ligand instead of Cys, so-called [NiFeSe] H₂ases.

- [FeFe] & [NiFe] H₂ases contain Fe-S clusters, but not [Fe] H₂ase.

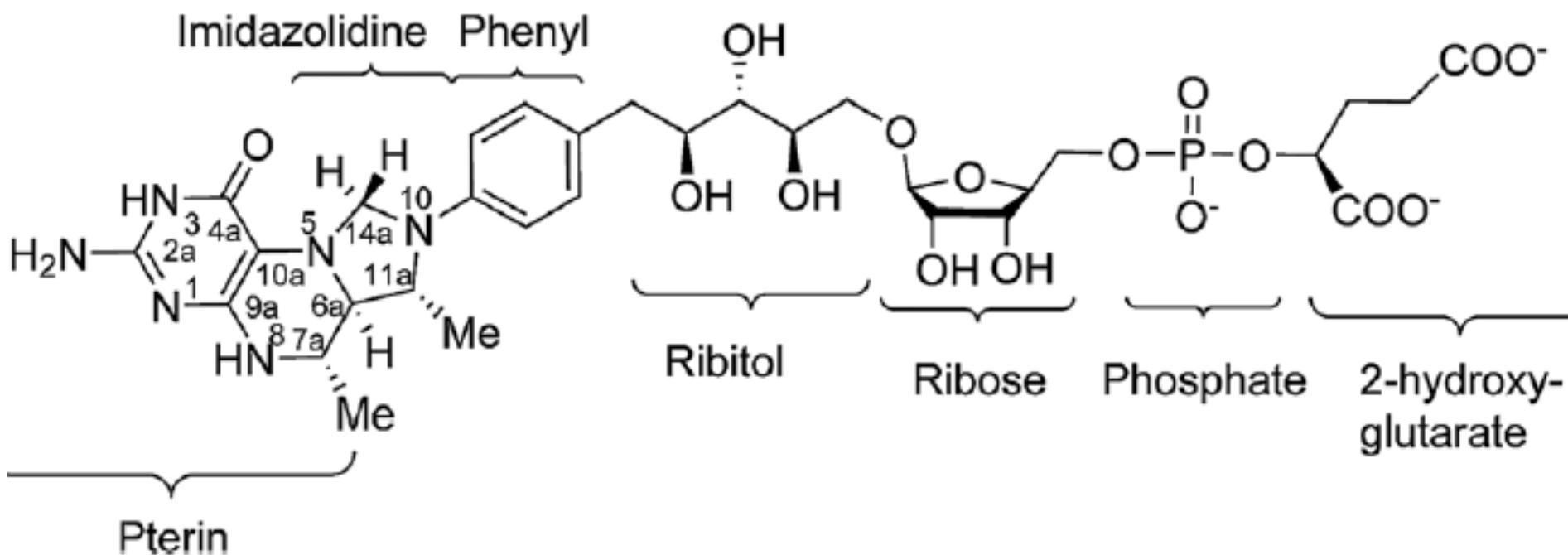
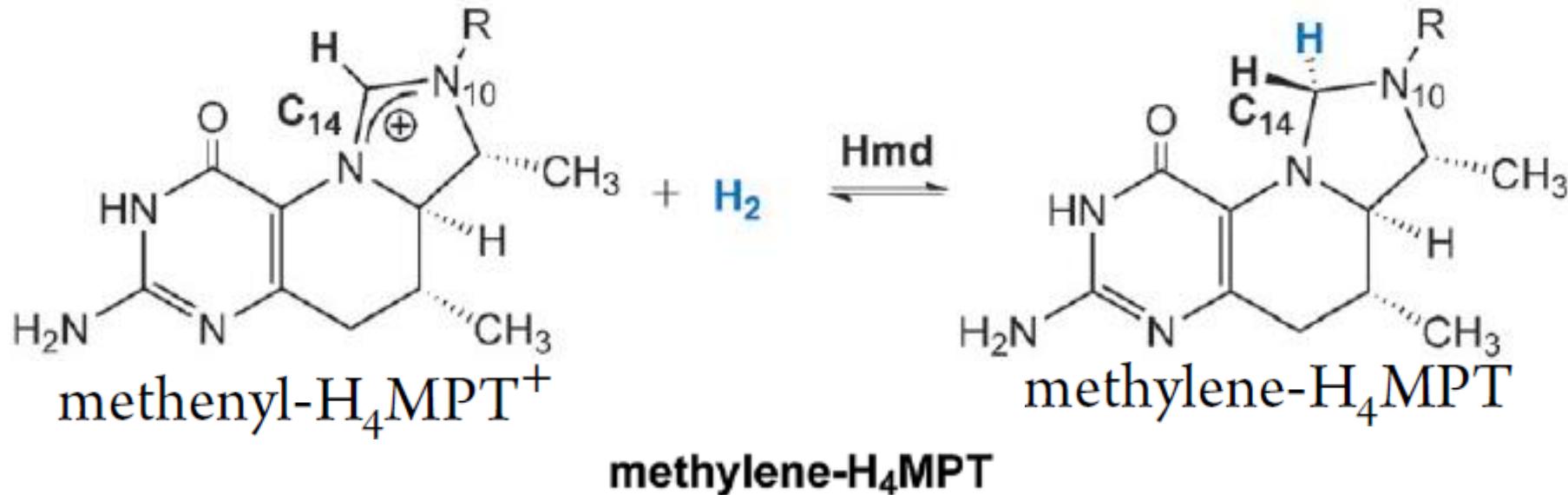
[FeFe] Hydrogenase



[NiFe] Hydrogenase

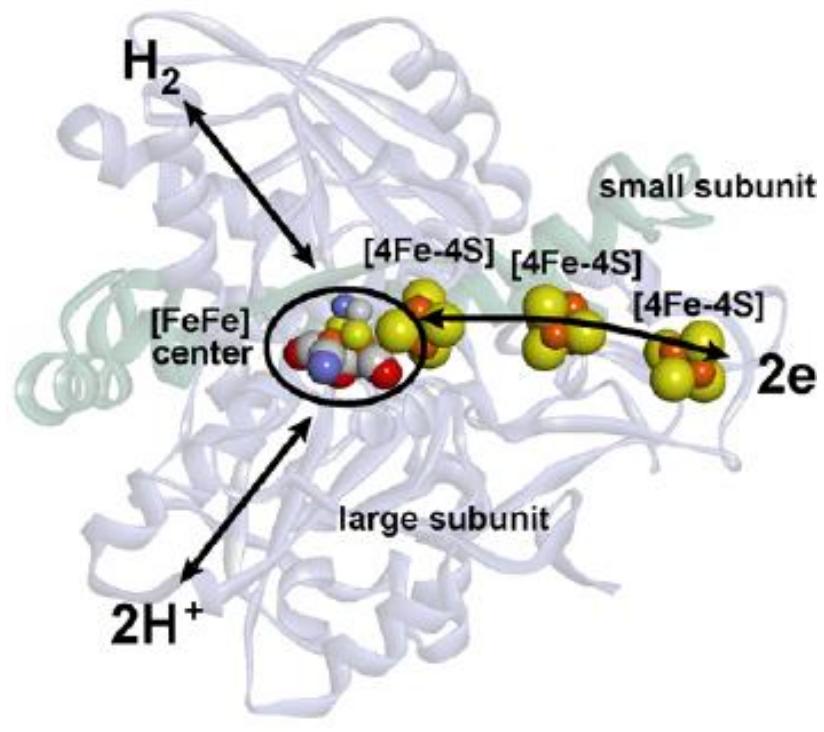
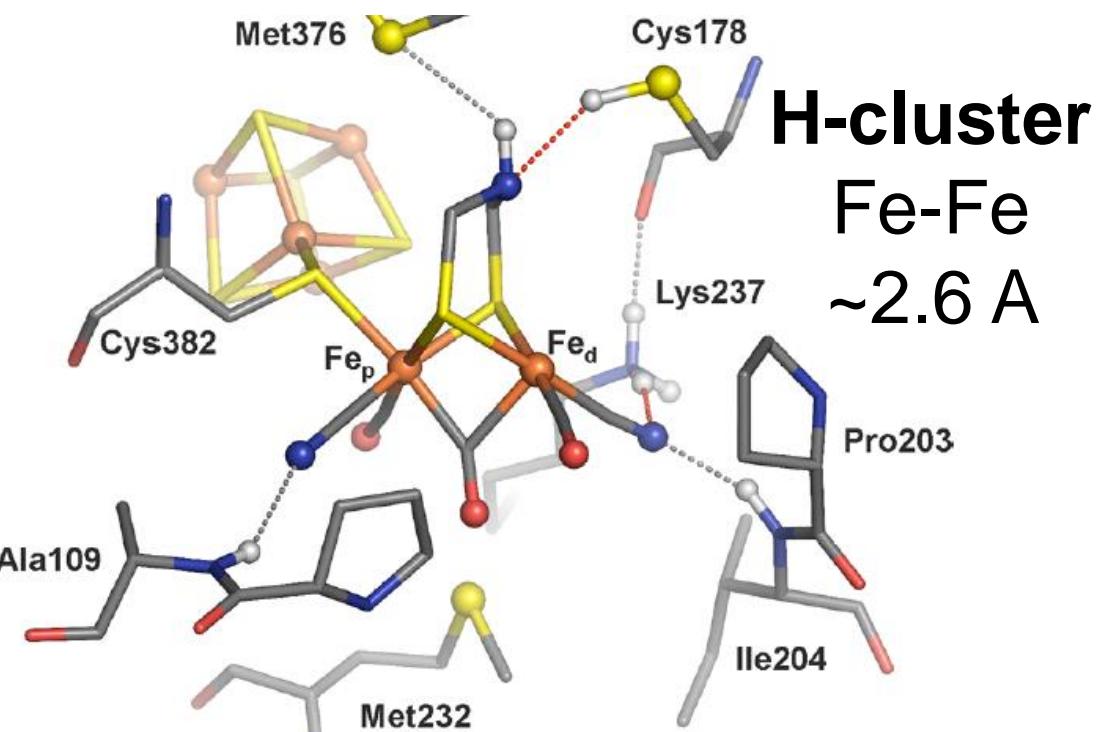


- [Fe] H₂ase uses an **organic cofactor** as a hydride carrier.

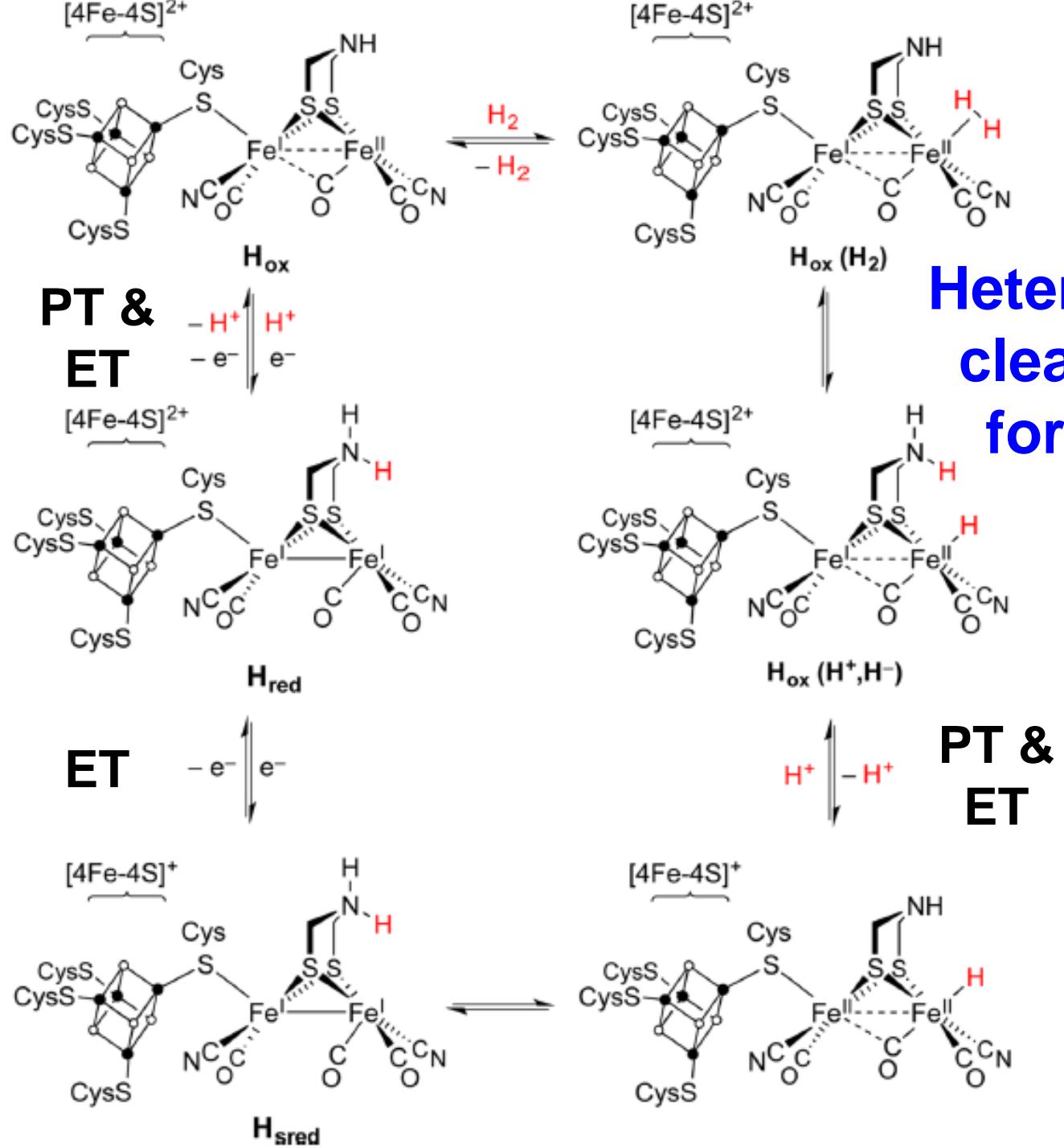


[FeFe] H₂ase

- May contain various Fe-S clusters in *anaerobe C. pasteurianum* (e.g. 3*Fe4S4 clusters, 1*Fe2S2 cluster).
- The **active site** (so-called the **H-cluster**): a **diiron** subcluster & a **Fe4S4** subcluster with a Cys ligation.



Proposed Mechanism

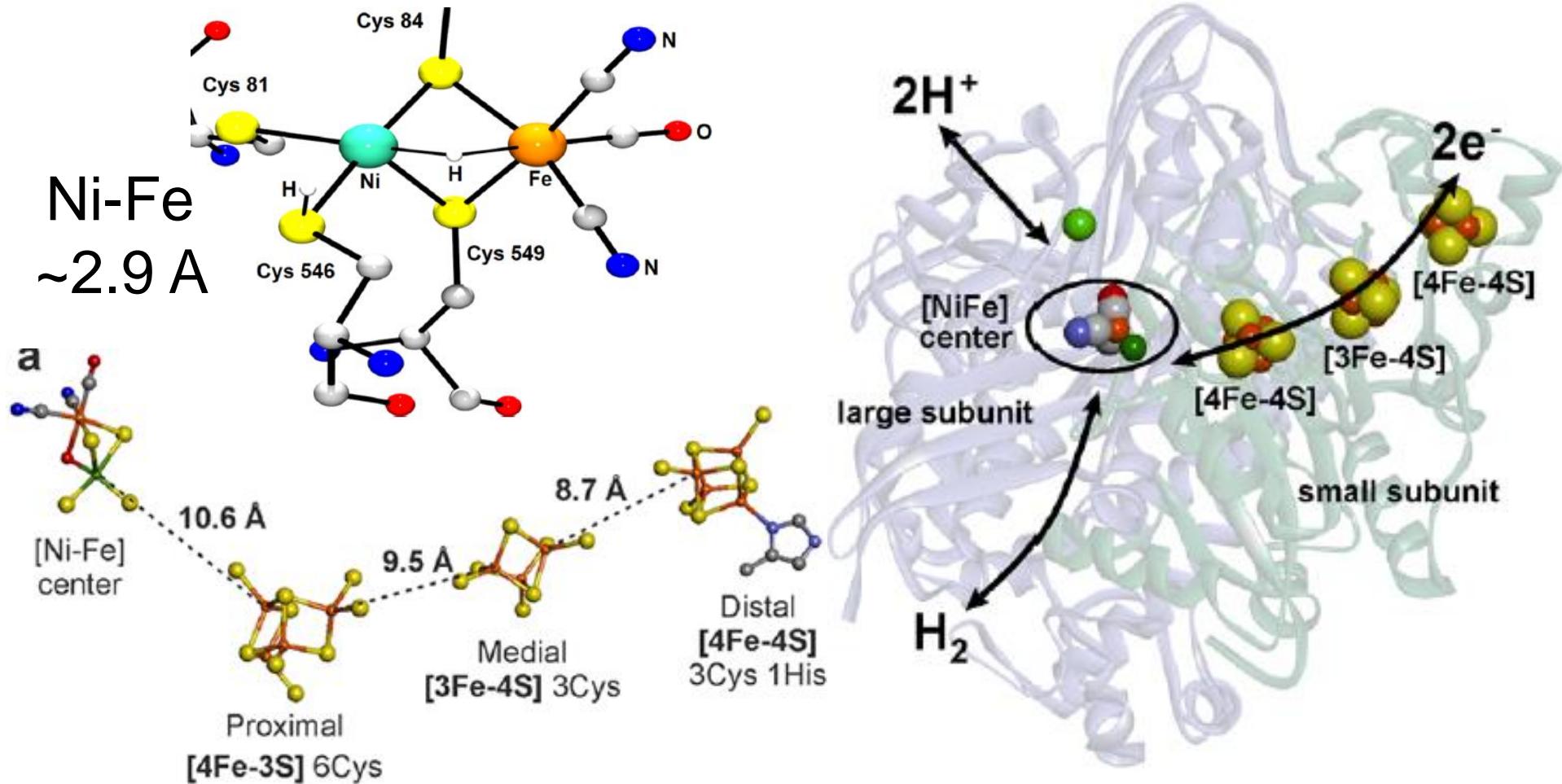


Heterolytic H_2
cleavage or
formation

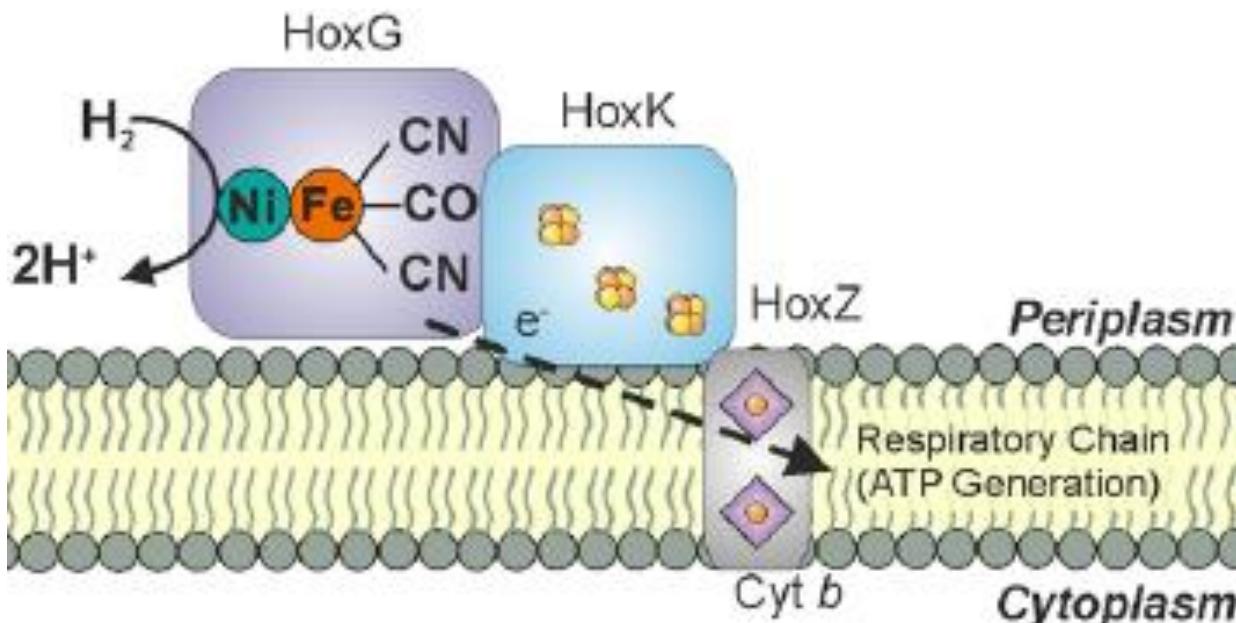
Schilter et al. *Chem. Rev.* 2016, 116, 8693.

[NiFe] H₂ase

- *D. gigas* [NiFe] H₂ase has several Fe-S clusters in the small subunit, & the Ni-Fe active site in the large subunit.
- The distal Fe₄S₄ cluster coordinates to 1 His & 3 Cys.

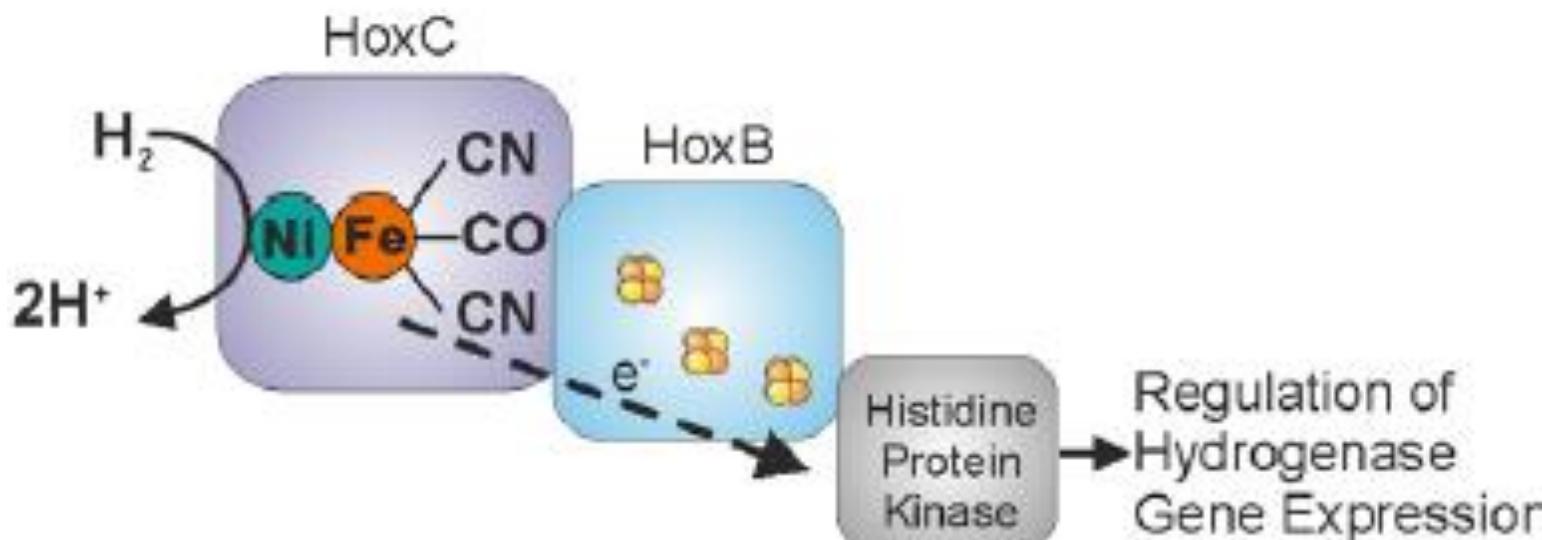


MBH (Membrane Bound Hydrogenase)



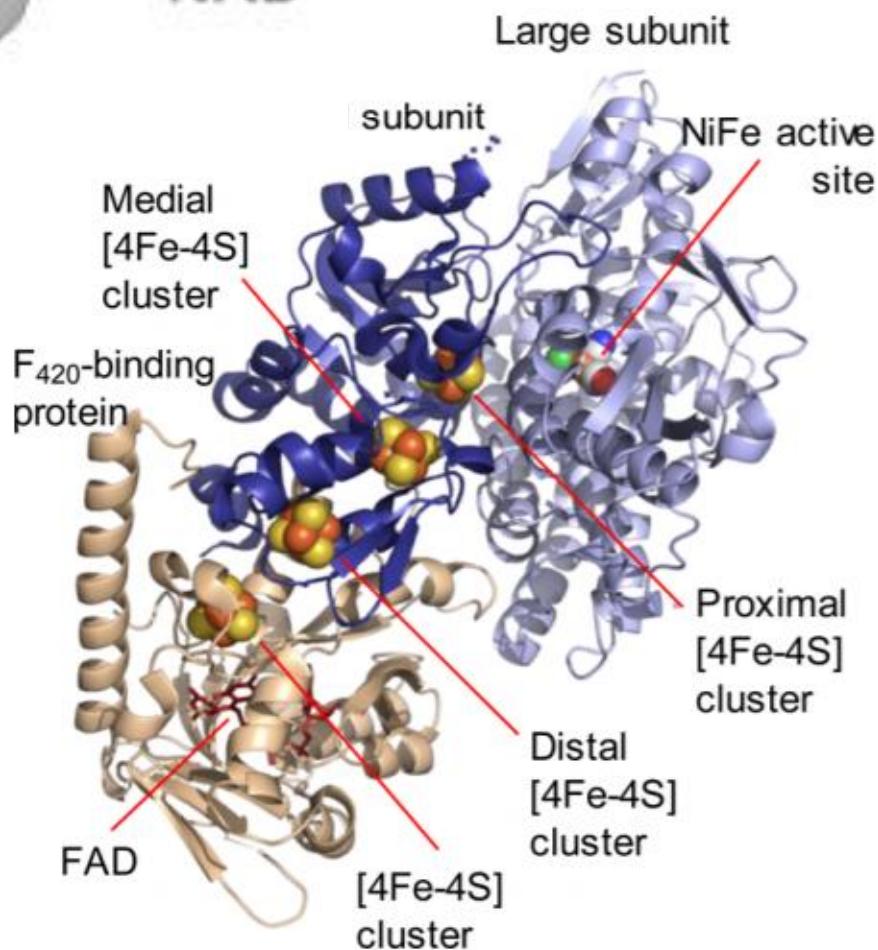
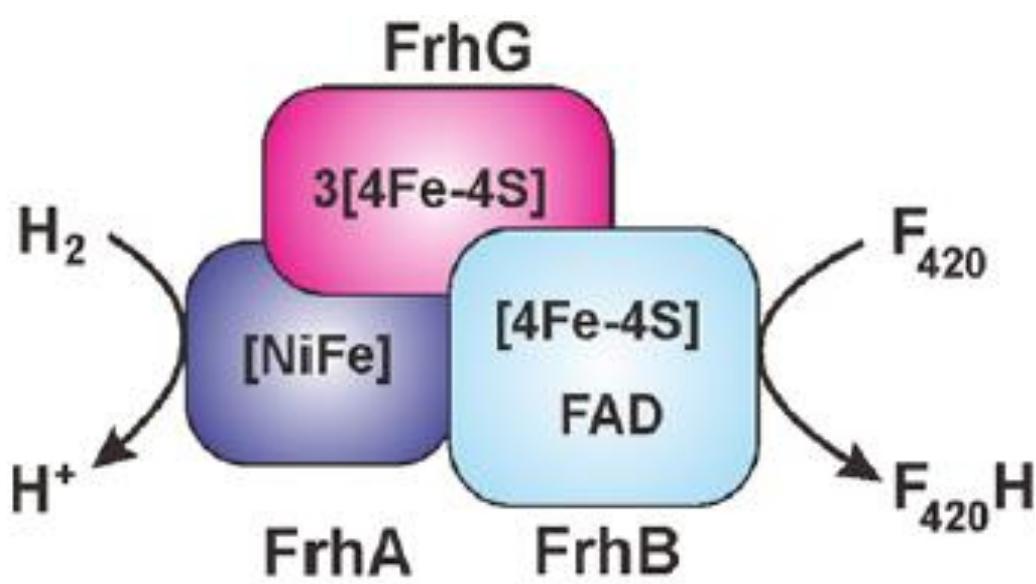
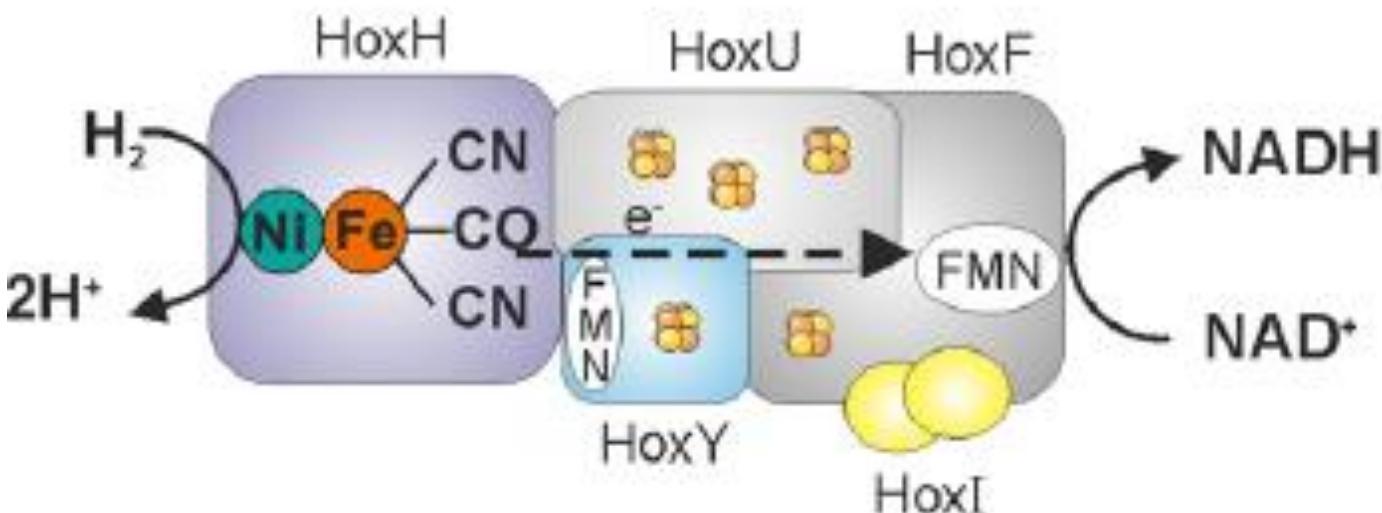
Lubitz, et al. Chem. Rev. 2014, 114, 4081.

RH (Regulatory Hydrogenase)

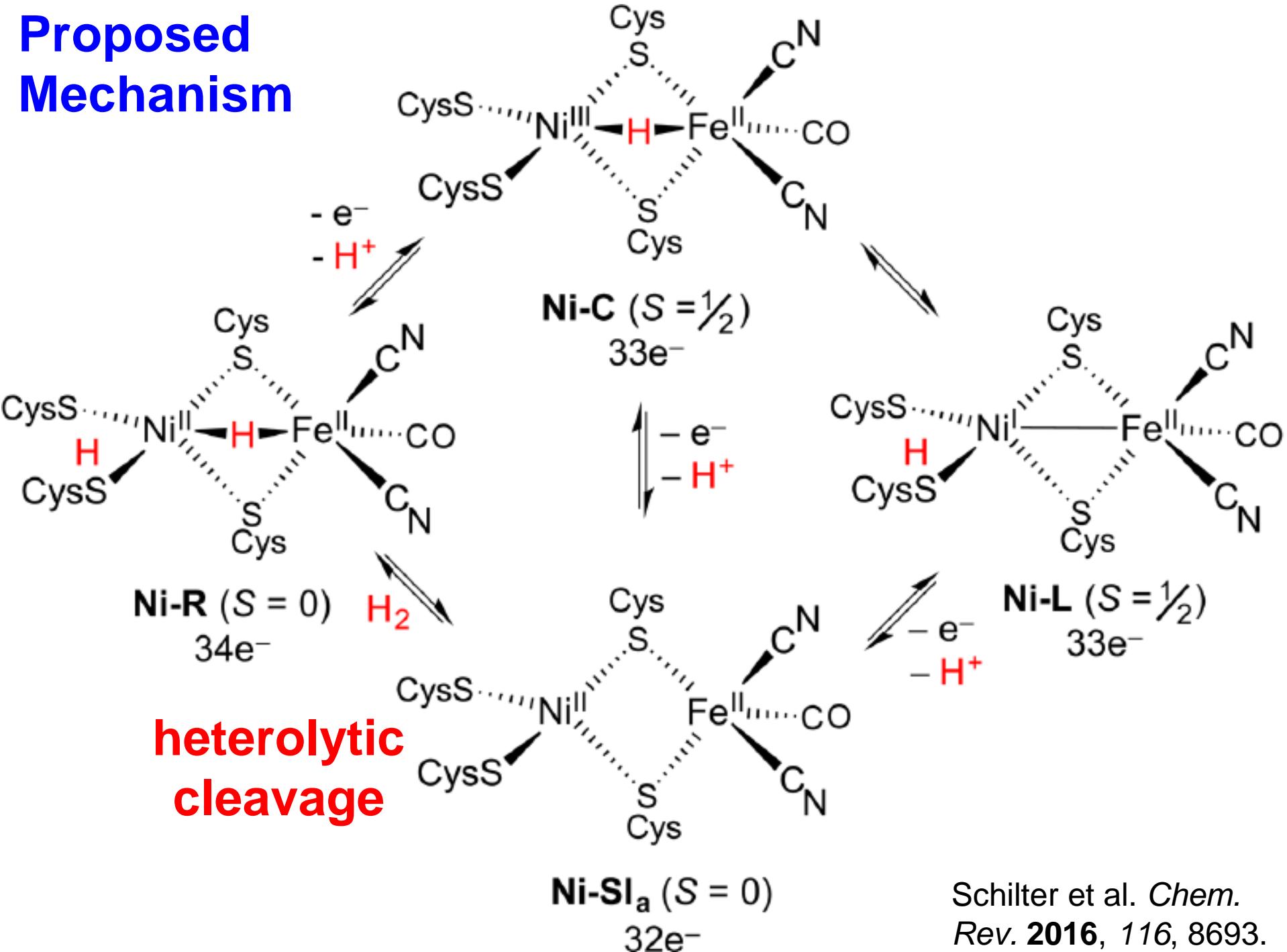


SH (Soluble Hydrogenase)

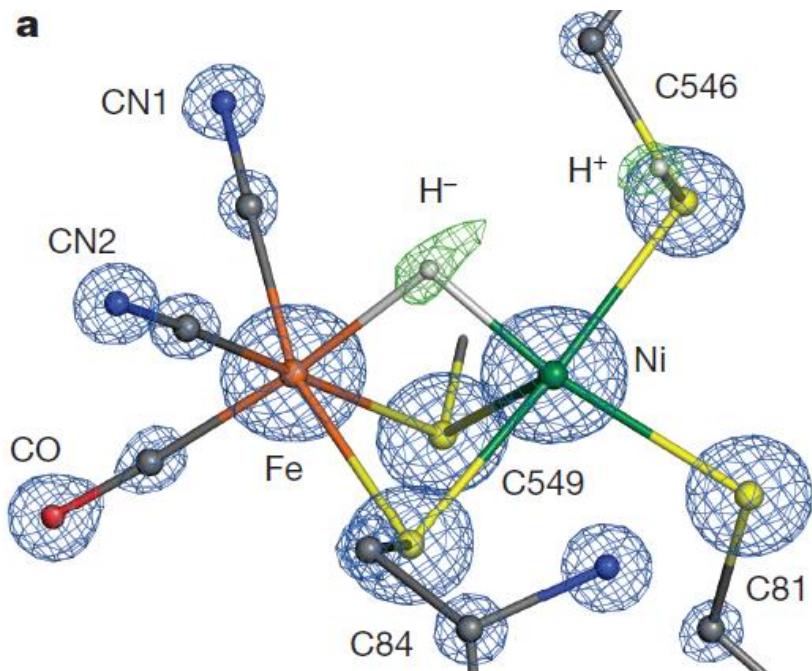
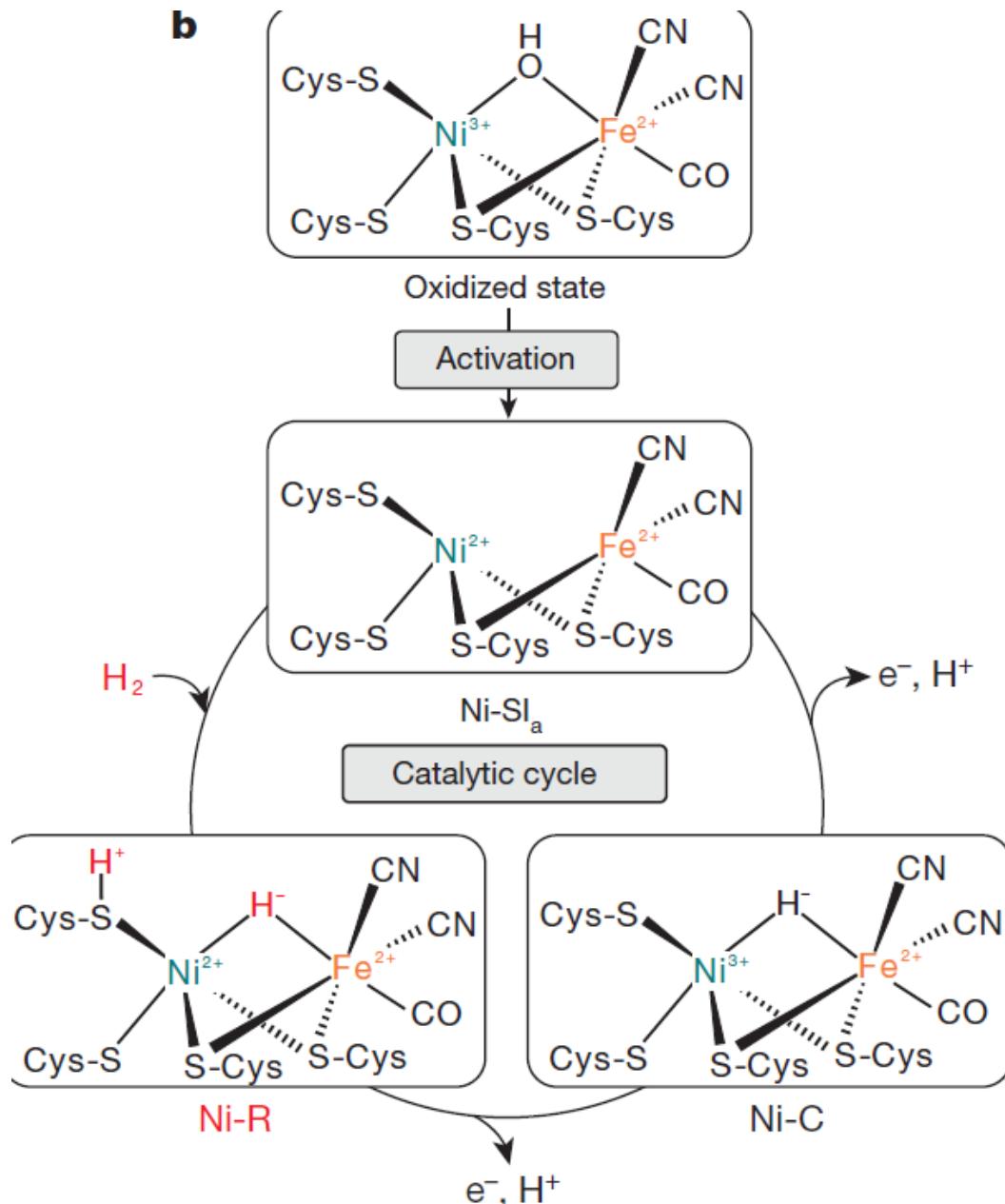
Lubitz, et al. Chem.
Rev. **2014**, *114*, 4081.



Proposed Mechanism



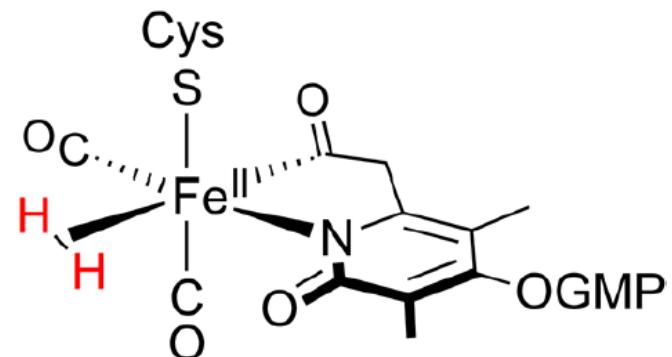
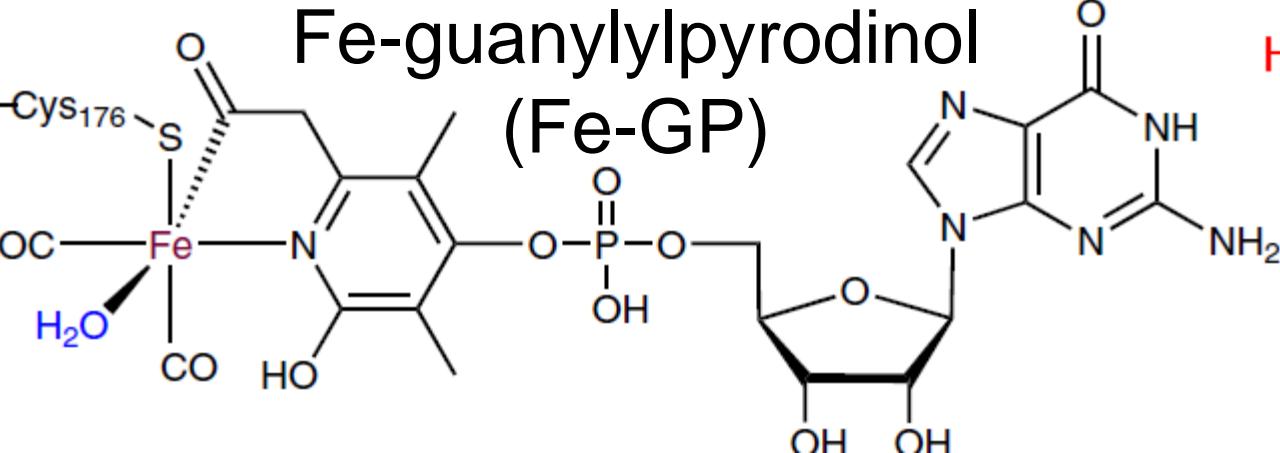
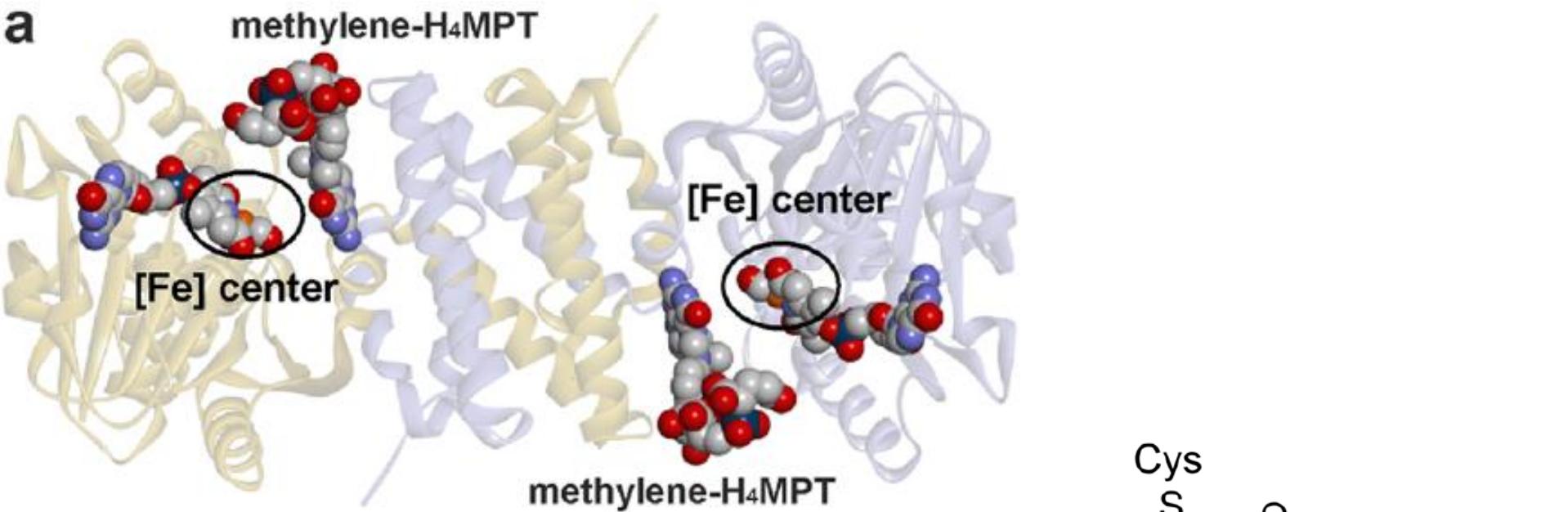
Hydrogens detected in a [NiFe] H₂ase (X-ray)



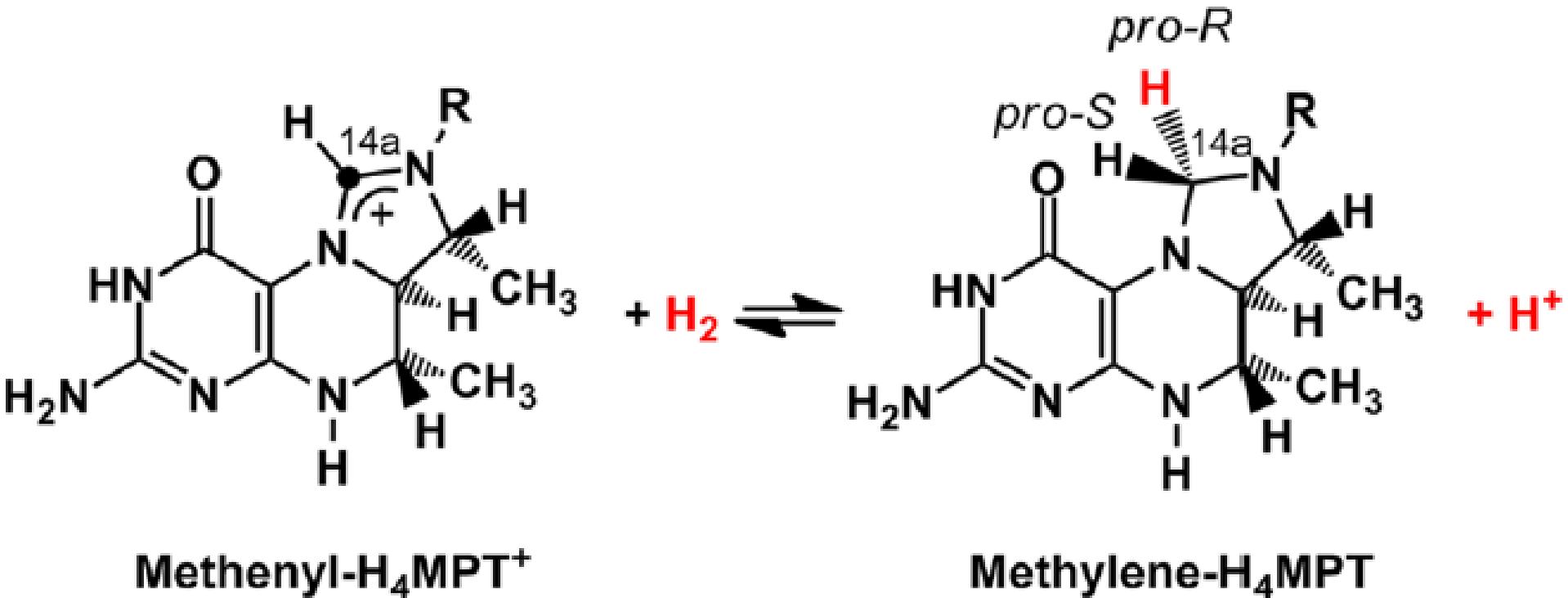
**The active site in
the Ni-R form**

[Fe] H₂ase

- Found only in methanogenic archaea & use a unique cofactor (Fe-GP), but not use Fe-S clusters.

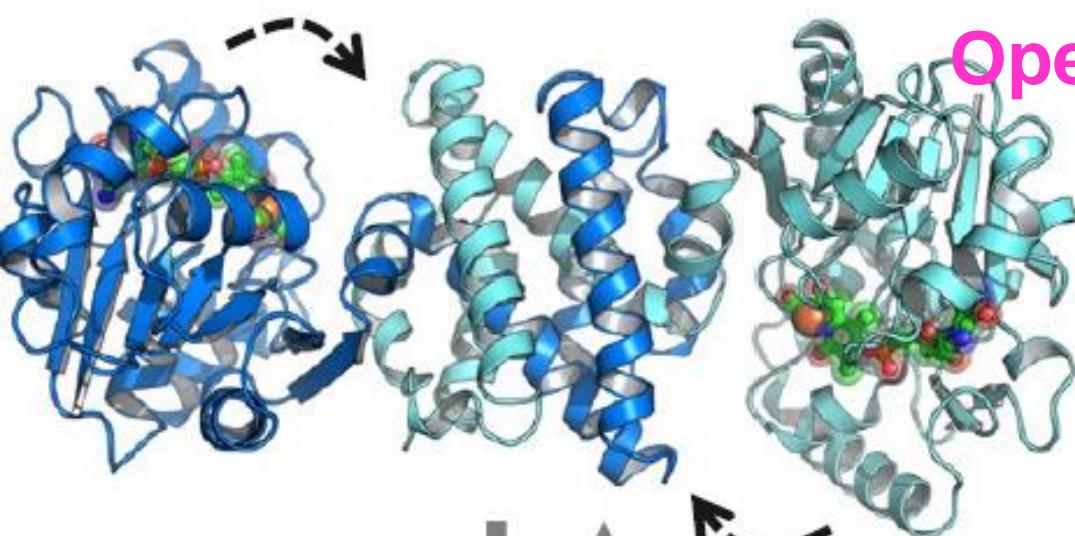


Lubitz, et al. *Chem. Rev.* **2014**, 114, 4081.

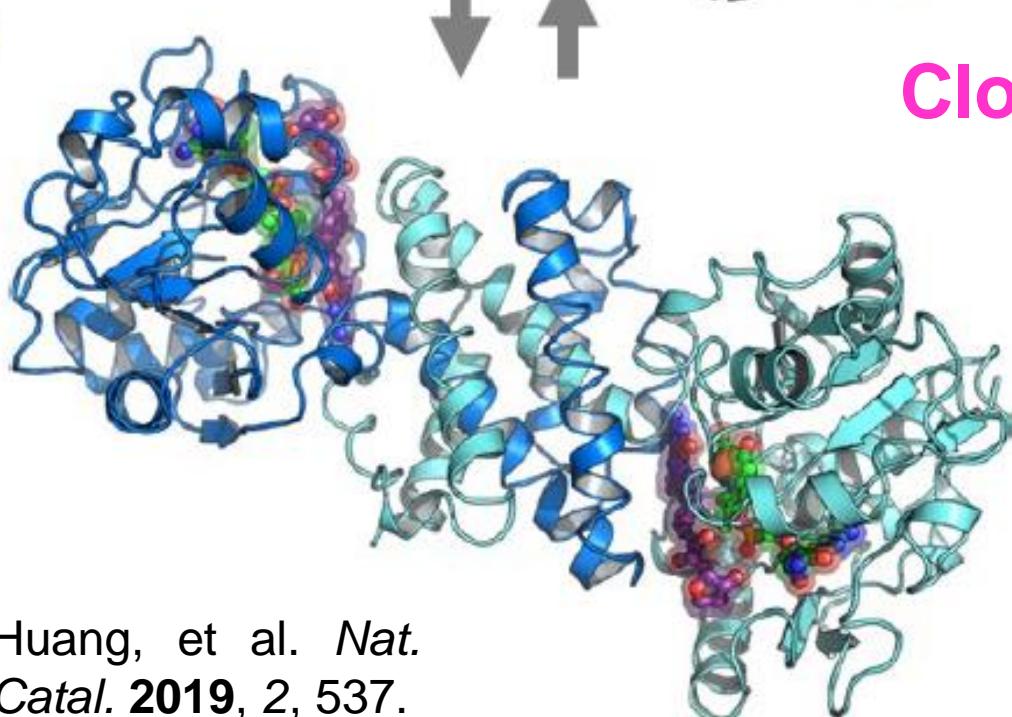
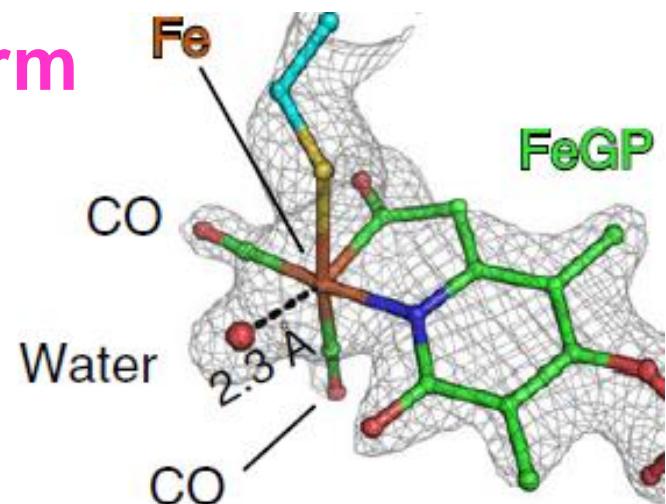


- Catalyzes the **reversible reduction** of methylene- H_4MPT^+ with H_2 to form methylene- H_4MPT & release a H^+ (via hydride transfer to the *pro-R* position).

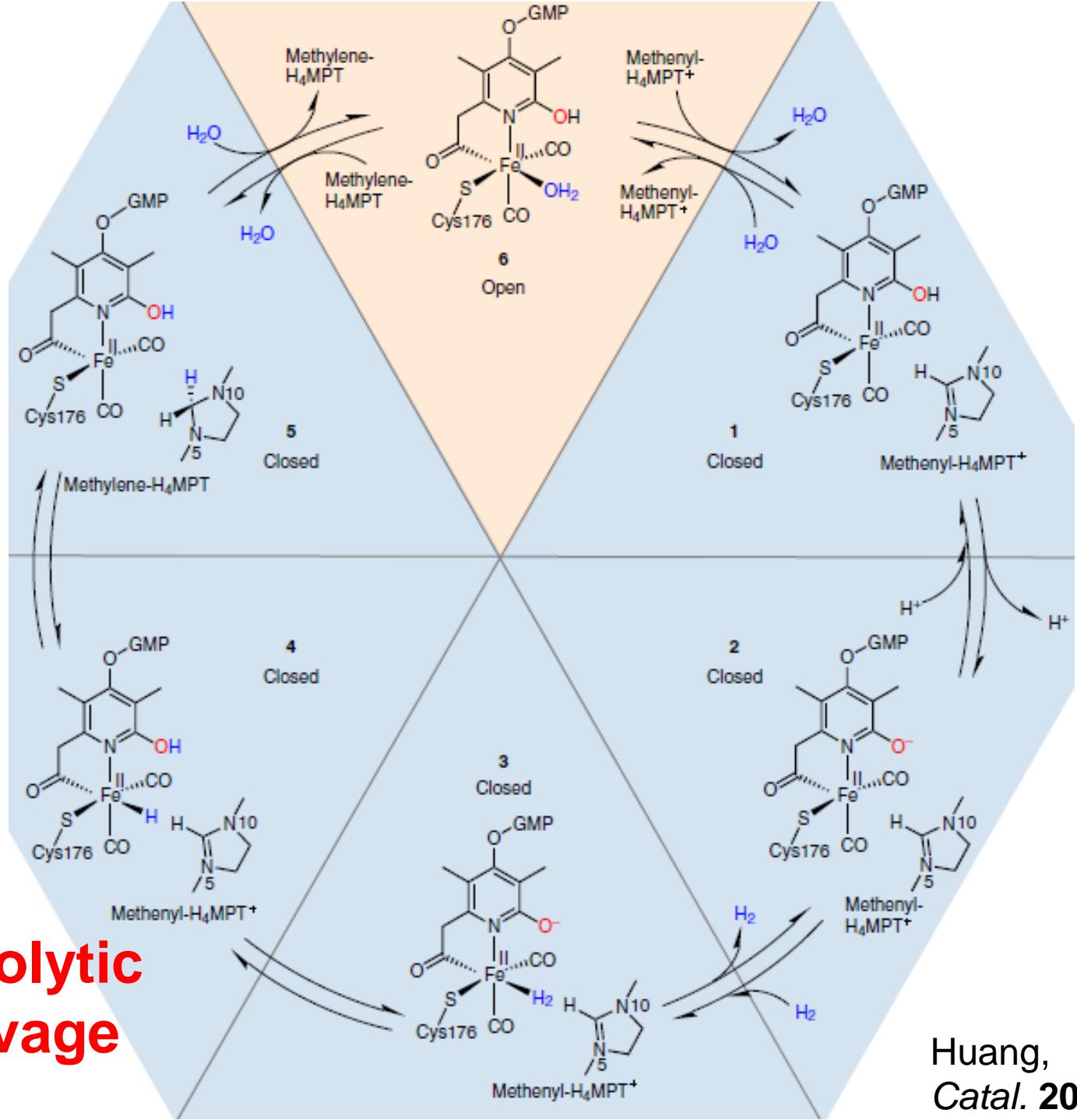
A Recent Structure of Close-form [Fe] H₂ase (X-ray)



Open form



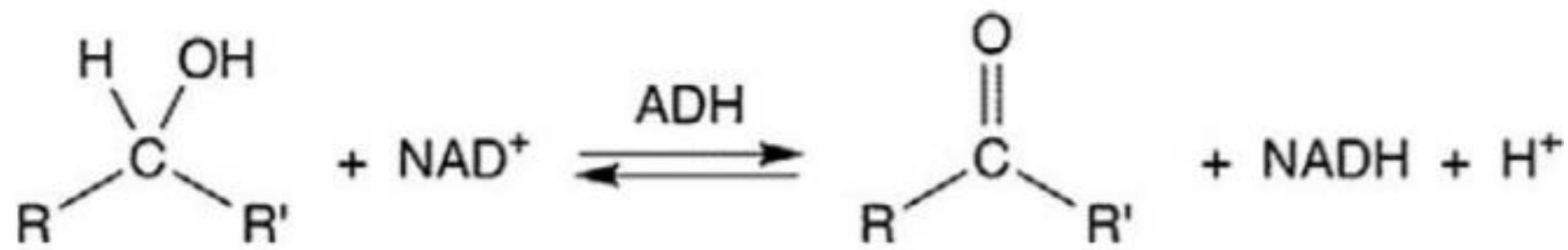
Close form



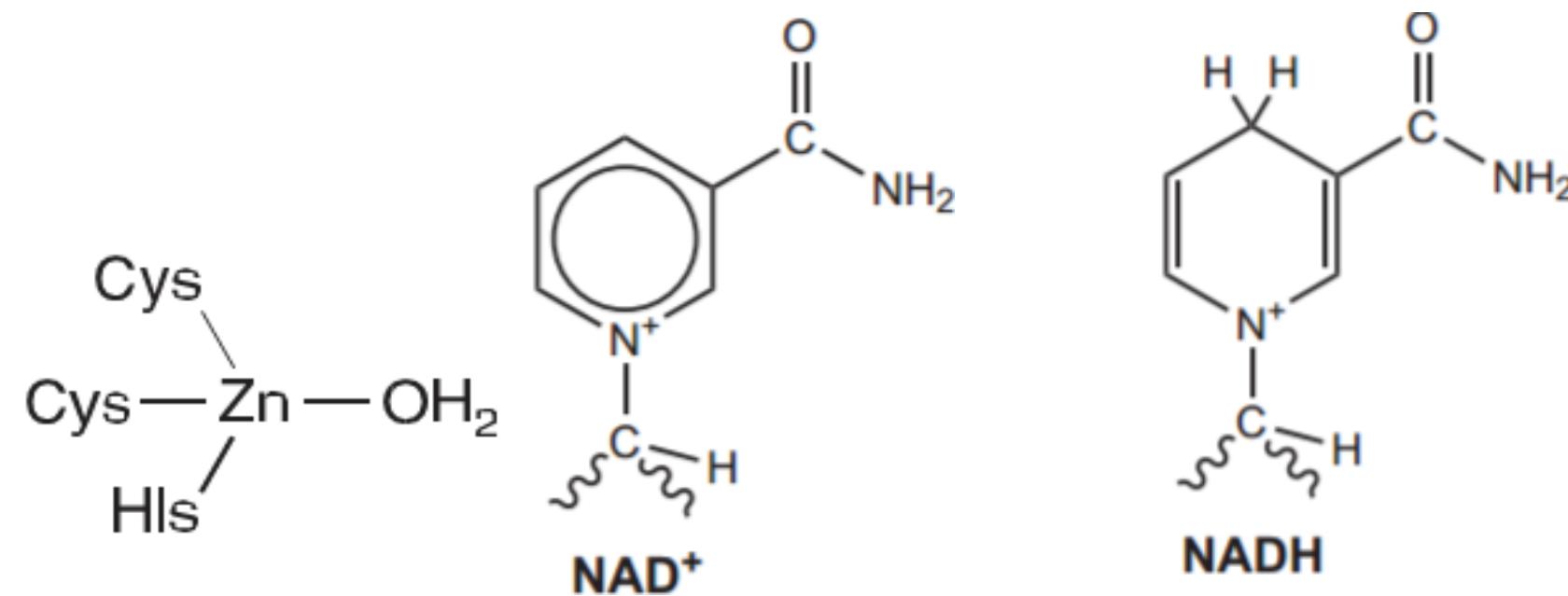
**heterolytic
cleavage**

Huang, et al. *Nat. Catal.* 2019, 2, 537.

Liver Alcohol Dehydrogenase (LADH)

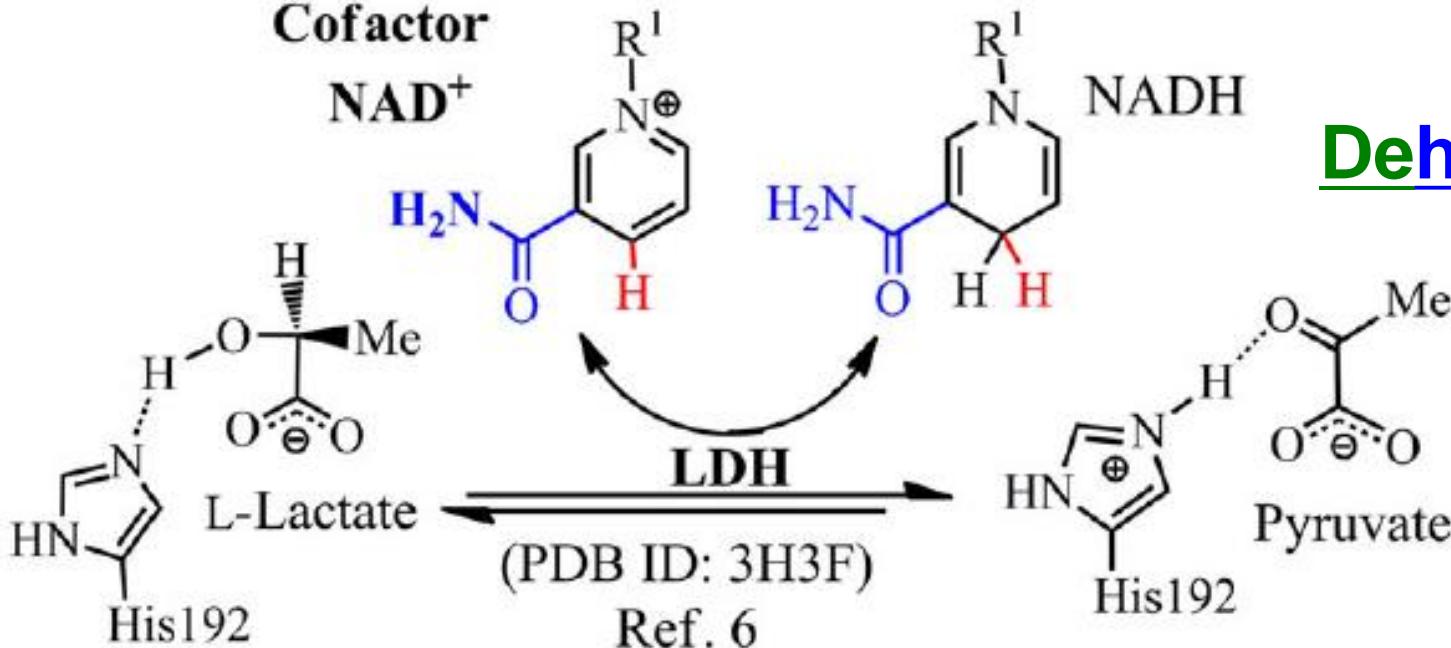


- Formally, LADH catalyzes an oxidation of an alcohol to give an aldehyde or ketone via **hydride transfer to NAD⁺**.

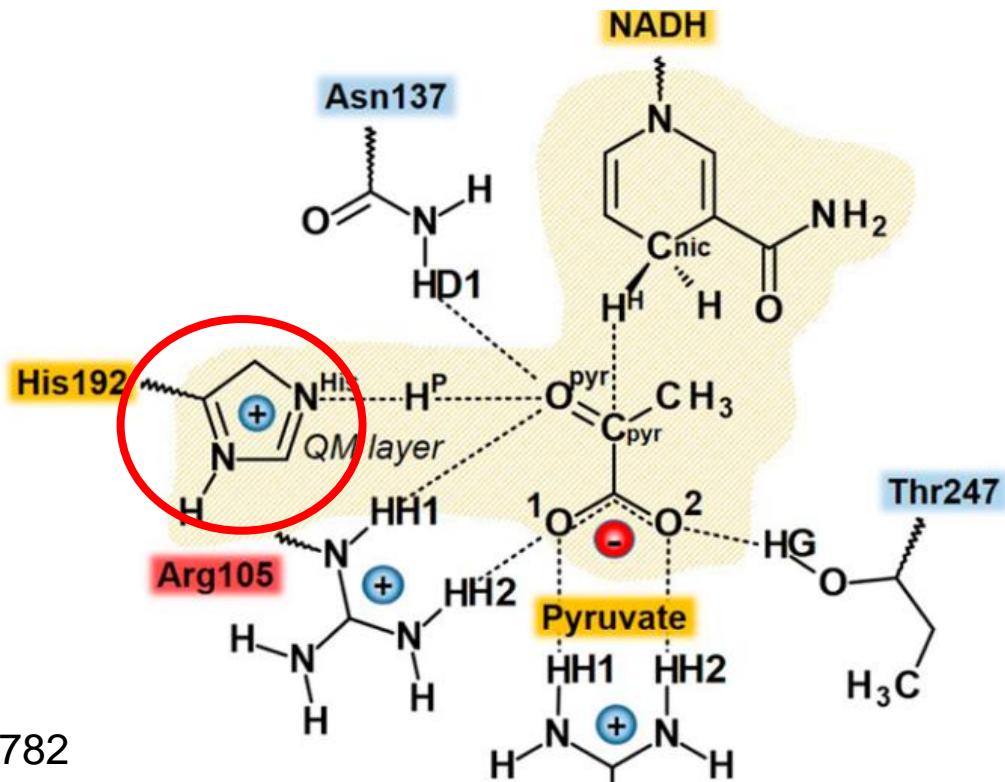


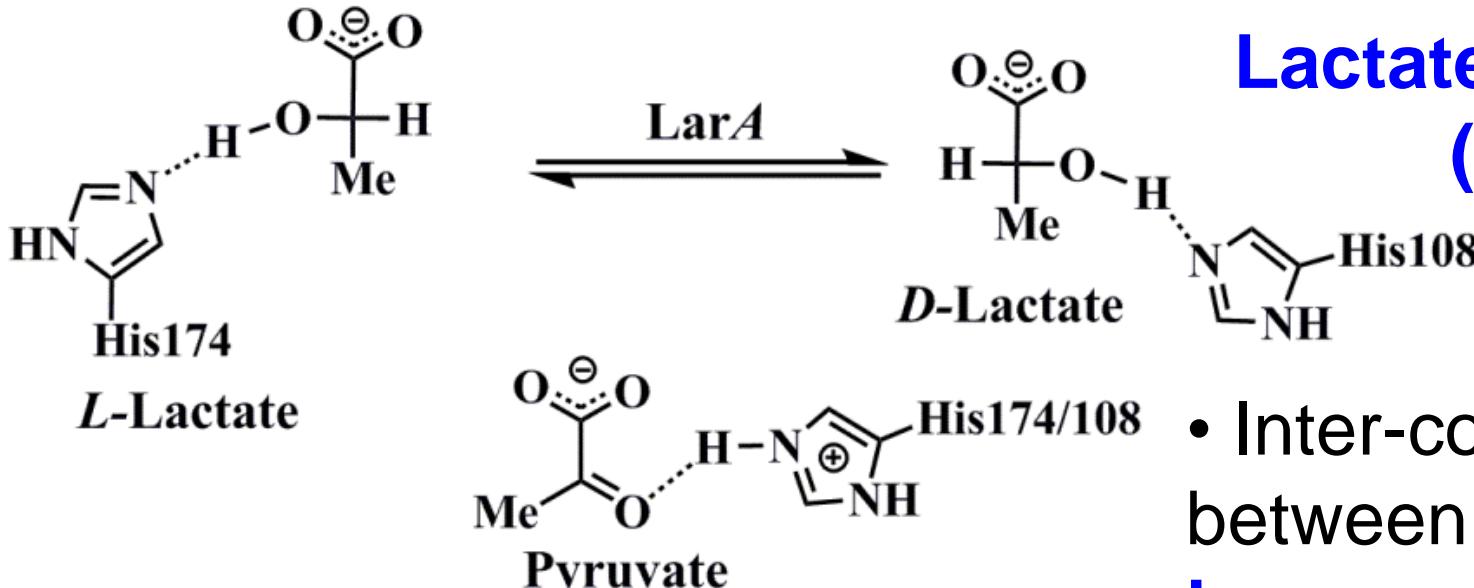
Lactate

Dehydrogenase (LDH)



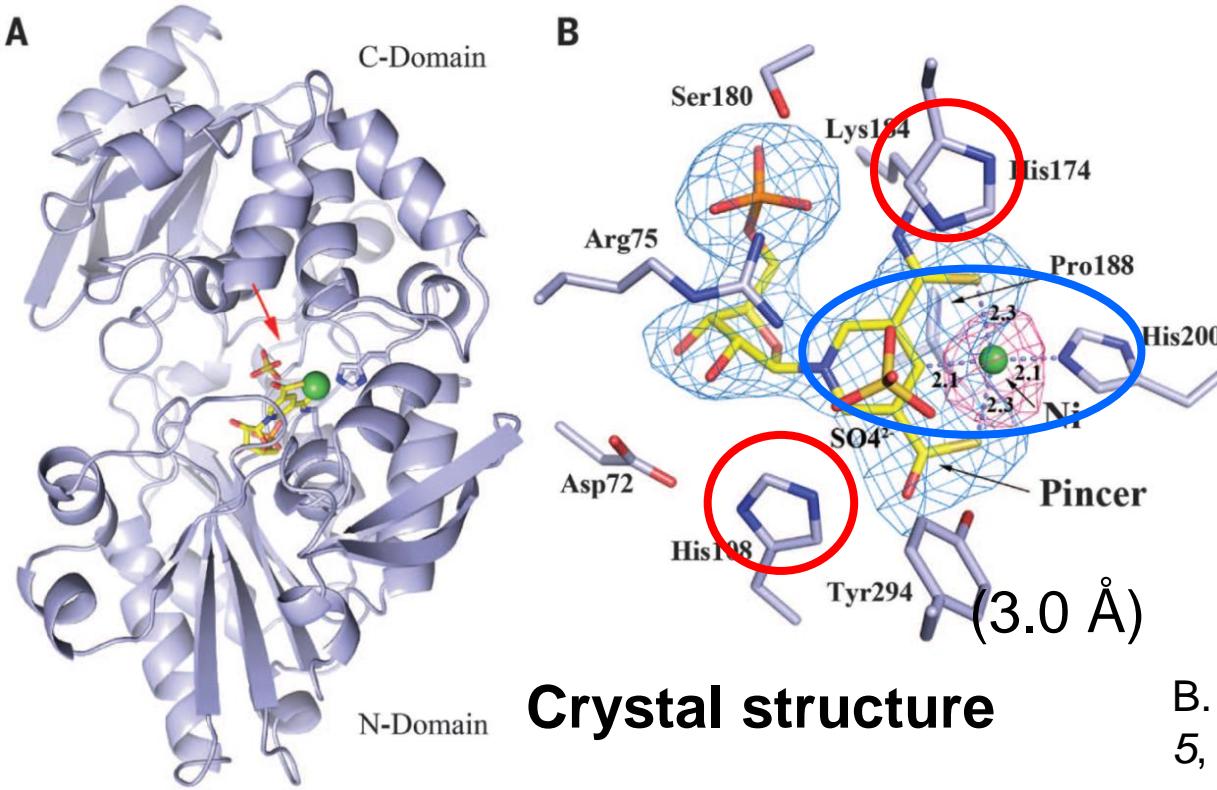
- Interconversion of one lactate stereo-isomer (e.g. L-form) & pyruvate.
- **NAD cofactor & 1 His base.**





Lactate Racemase (LarA)

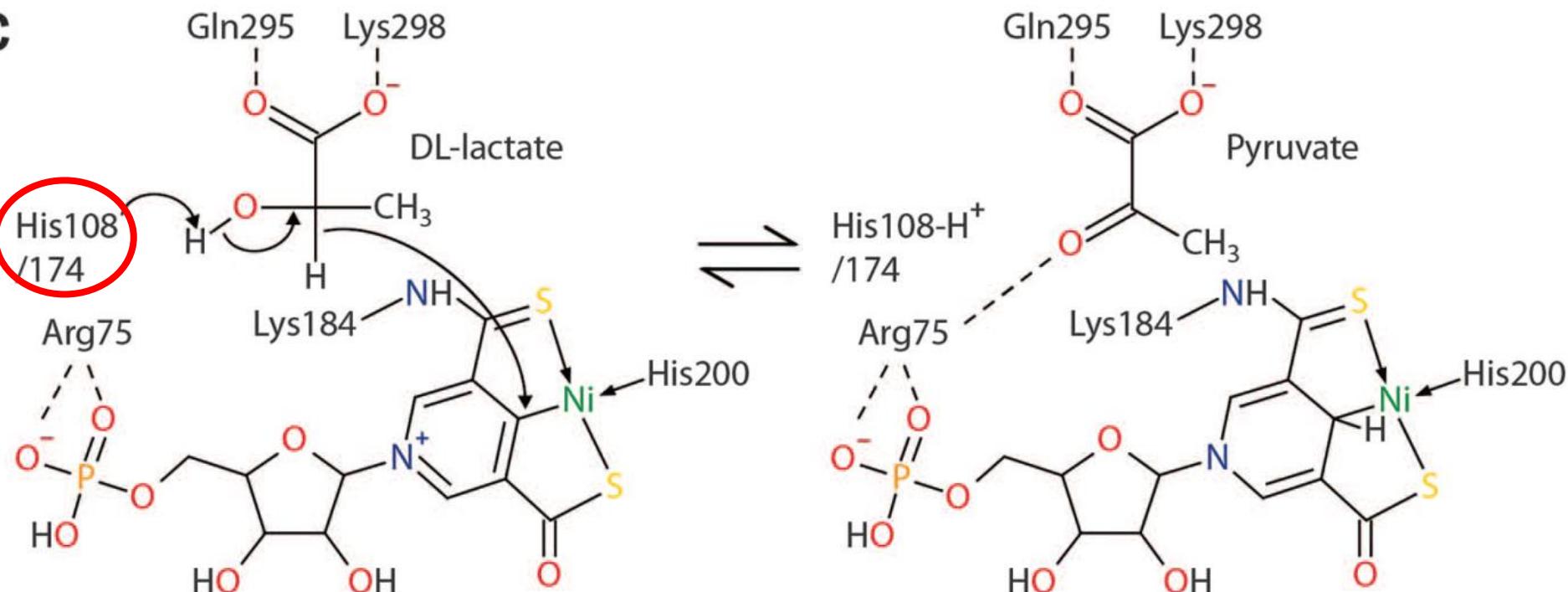
- Inter-conversion between **L-** & **D-isomers** via pyruvate.



- A **new Ni metallo-enzyme** with a **new pincer NAD-like** cofactor + **2** potential His bases.

B. Desguin et al., *Nat. Commun.* 2014, 5, 3615; *Science* 2015, 349, 66.

C

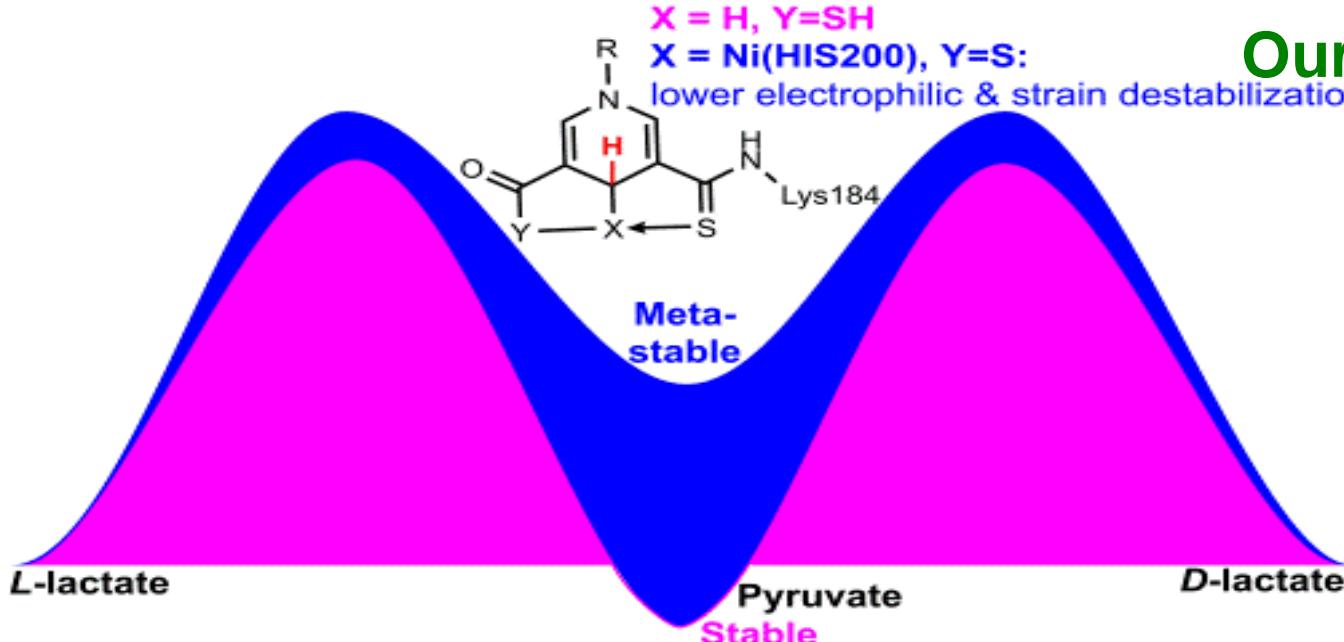


- **Critical His108 & His174** could act as a base.
- Dehydrogenation: outer-sphere proton-coupled hydride transfer.
- Hydride transfer to C & Ni were proposed.
- **Ni(II)** is required for the racemization.

Our Computations

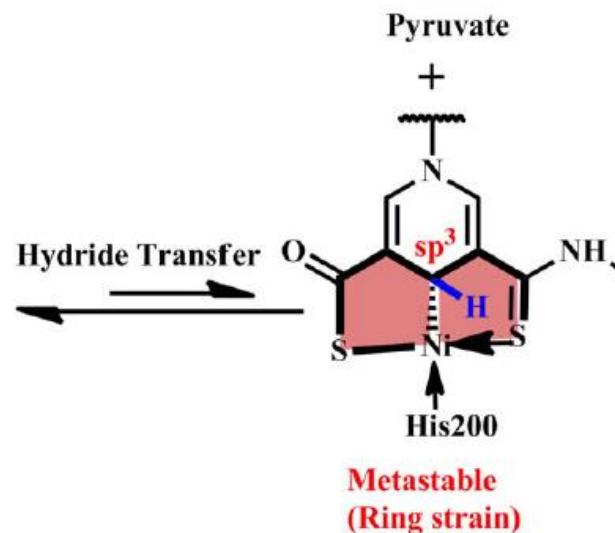
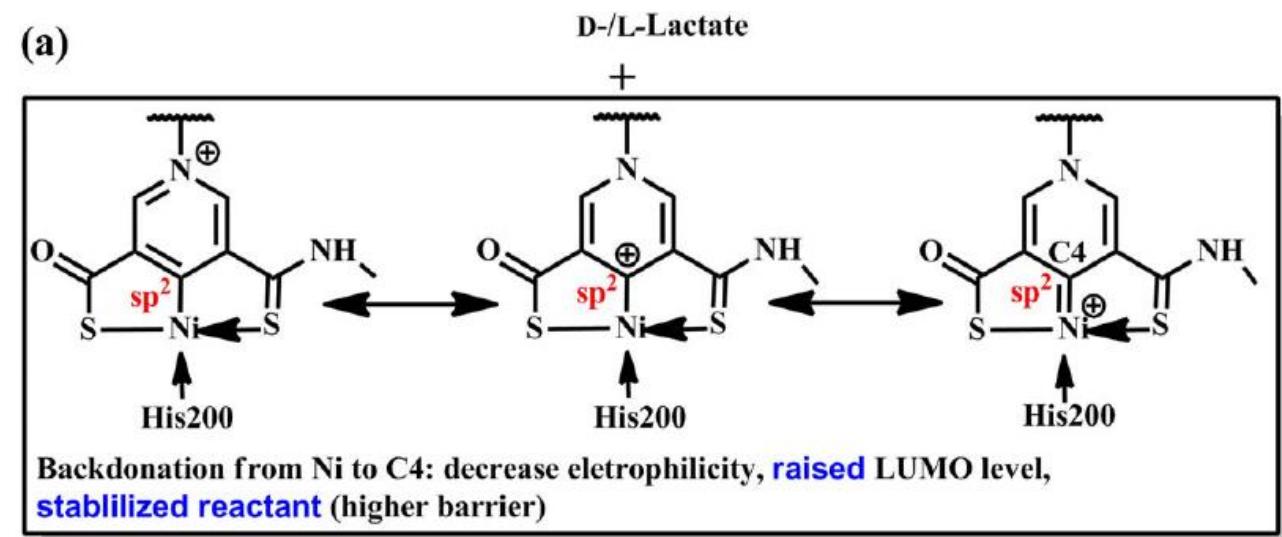
(SCS)Ni:
metastable INT
→ racemization
(LarA),

NOT (LDH)
dehydrogenation

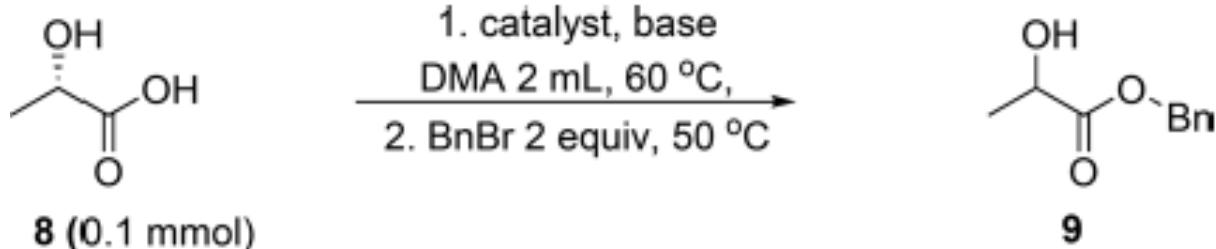
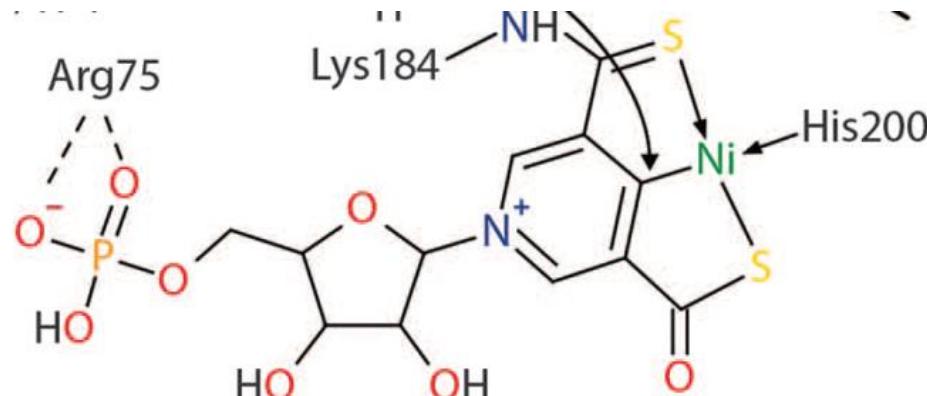
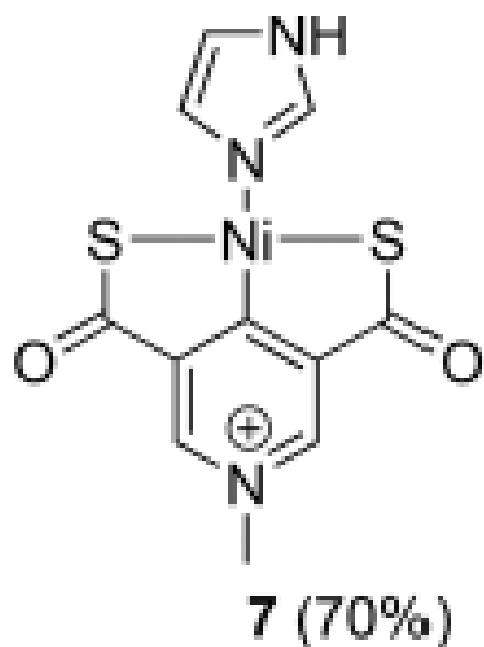
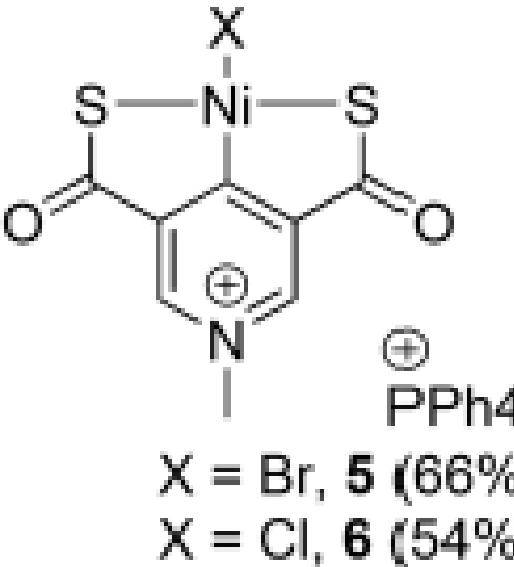


Alternative Mechanistic Strategy for Enzyme Catalysis: INT Destabilization by the Cofactor (vs. TS stabilization, R destabilization)

(a)



A Recent Synthetic Model LarA Ni Complex



Catalyst	t [h]	ee [%]
5 (10 mol %)	12	75
none	12	94
6 (10 mol %)	12	76
7 (10 mol %)	12	72
5 (10 mol %)	36	66
NiCl₂ (10 mol %)	12	93

2. Carbon Metabolism

Reductive CO₂ fixation pathways

- 4 known reductive CO₂ fixation pathways (to the carbon cycle), e.g. the Wood–Ljungdahl pathway & methanogenesis pathway.

The net reaction in the Wood–Ljungdahl pathway:



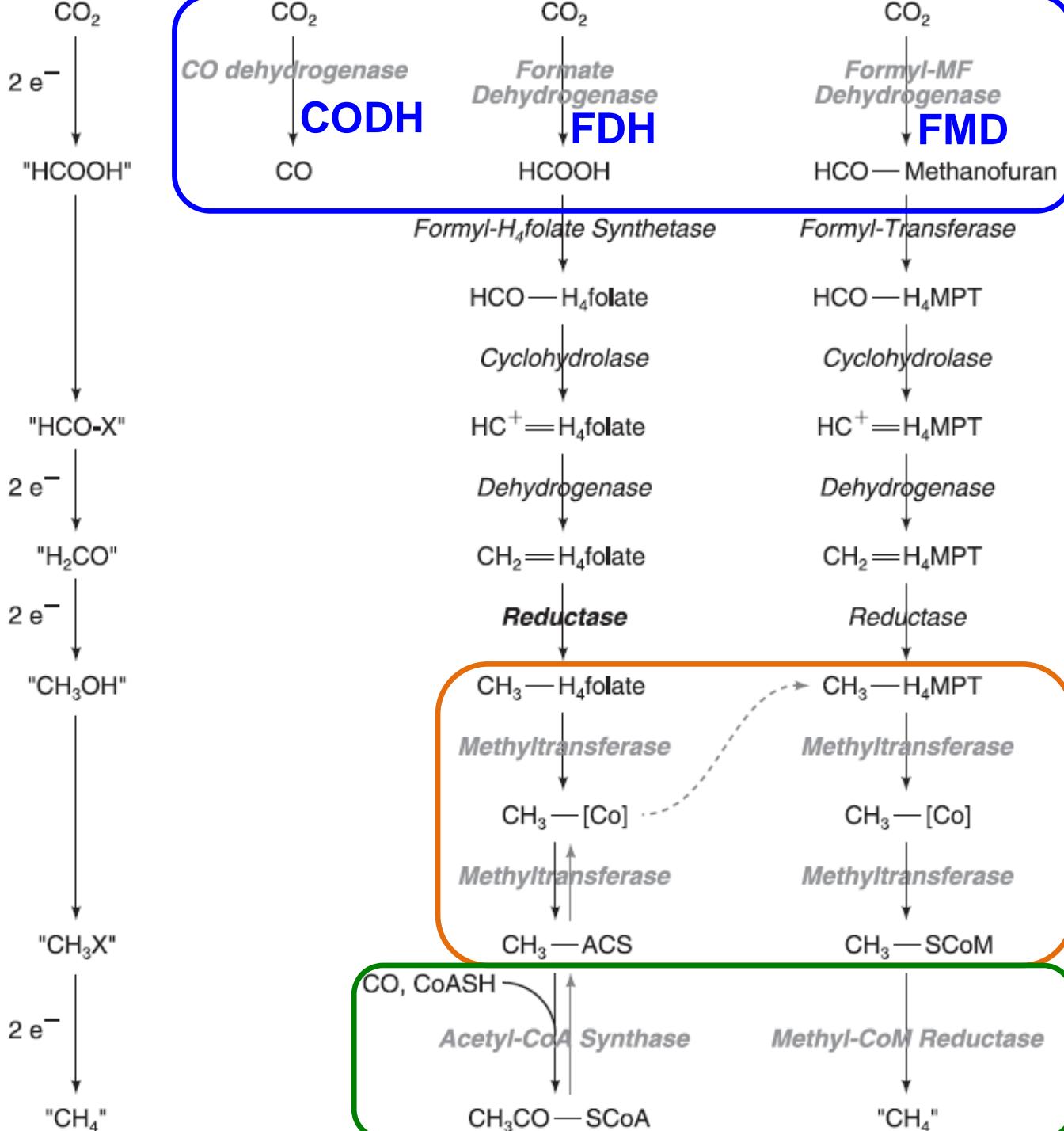
$$\Delta G^\circ = -95 \text{ kJ mol}^{-1}$$

The net reaction in the methanogenesis pathway:

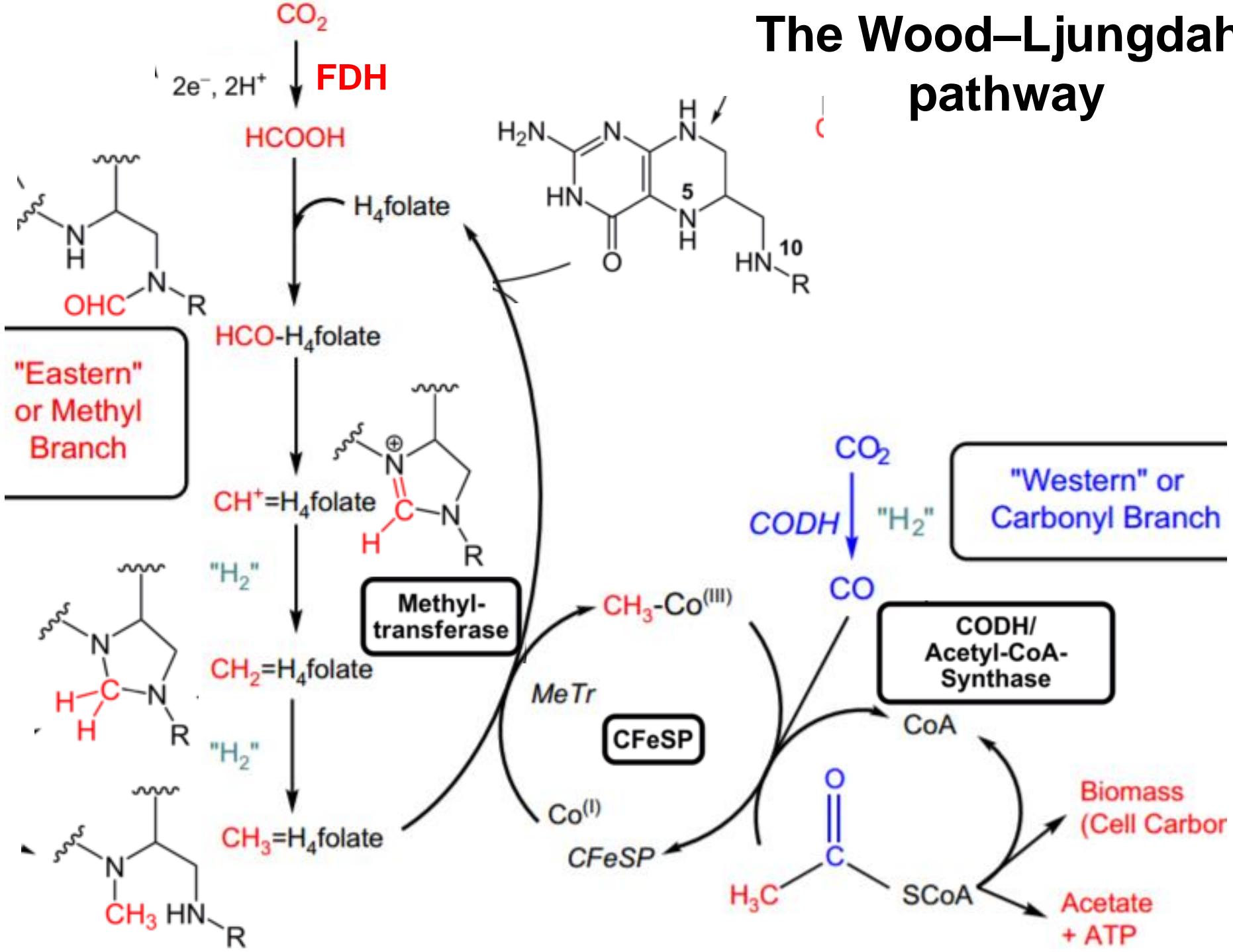


$$\Delta G_0 = -131 \text{ kJ mol}^{-1}$$

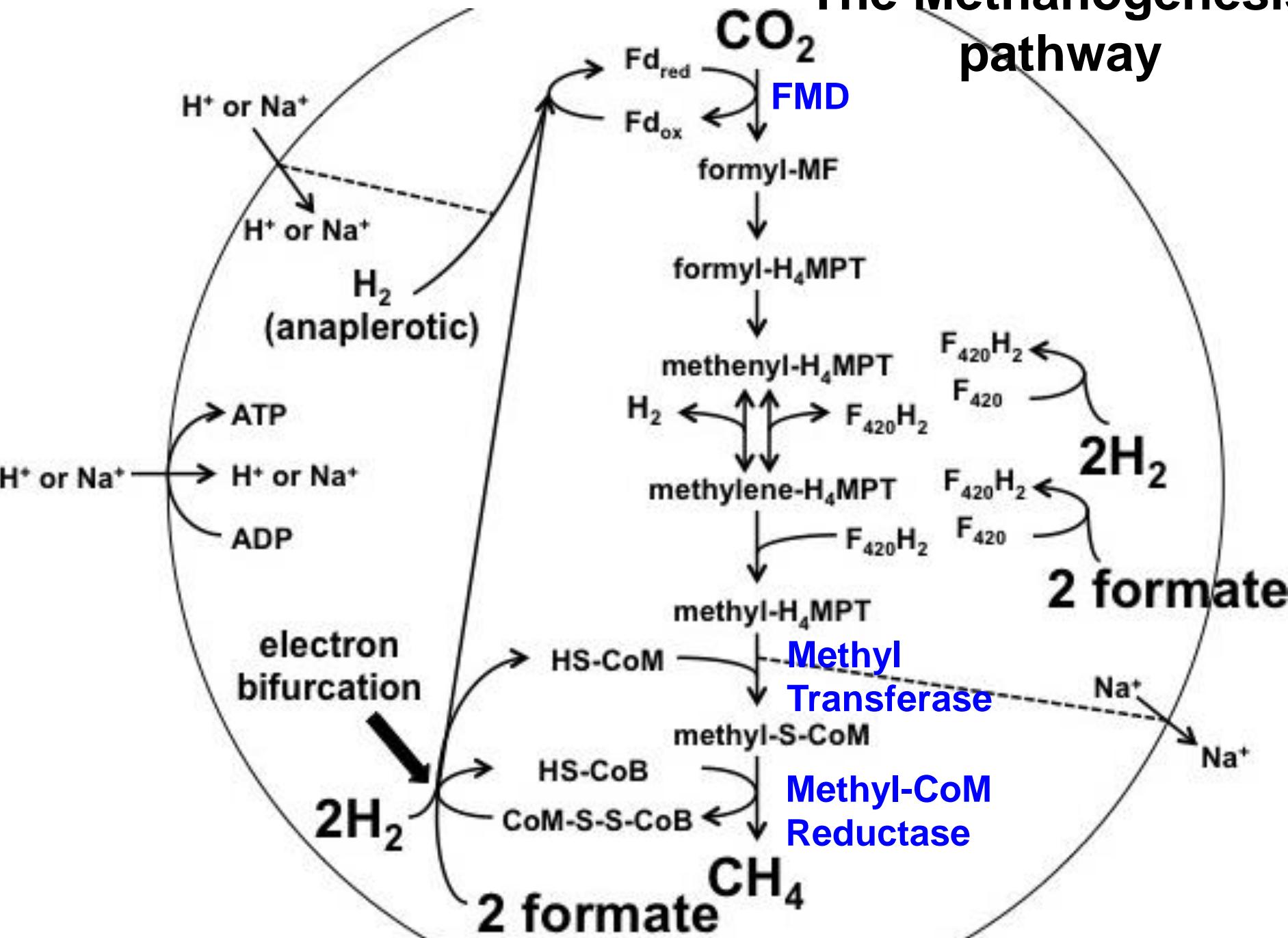
Redox states of C involved



The Wood–Ljungdahl pathway

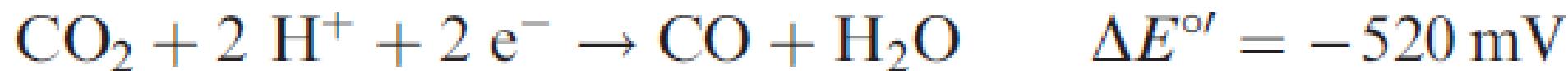


The Methanogenesis pathway



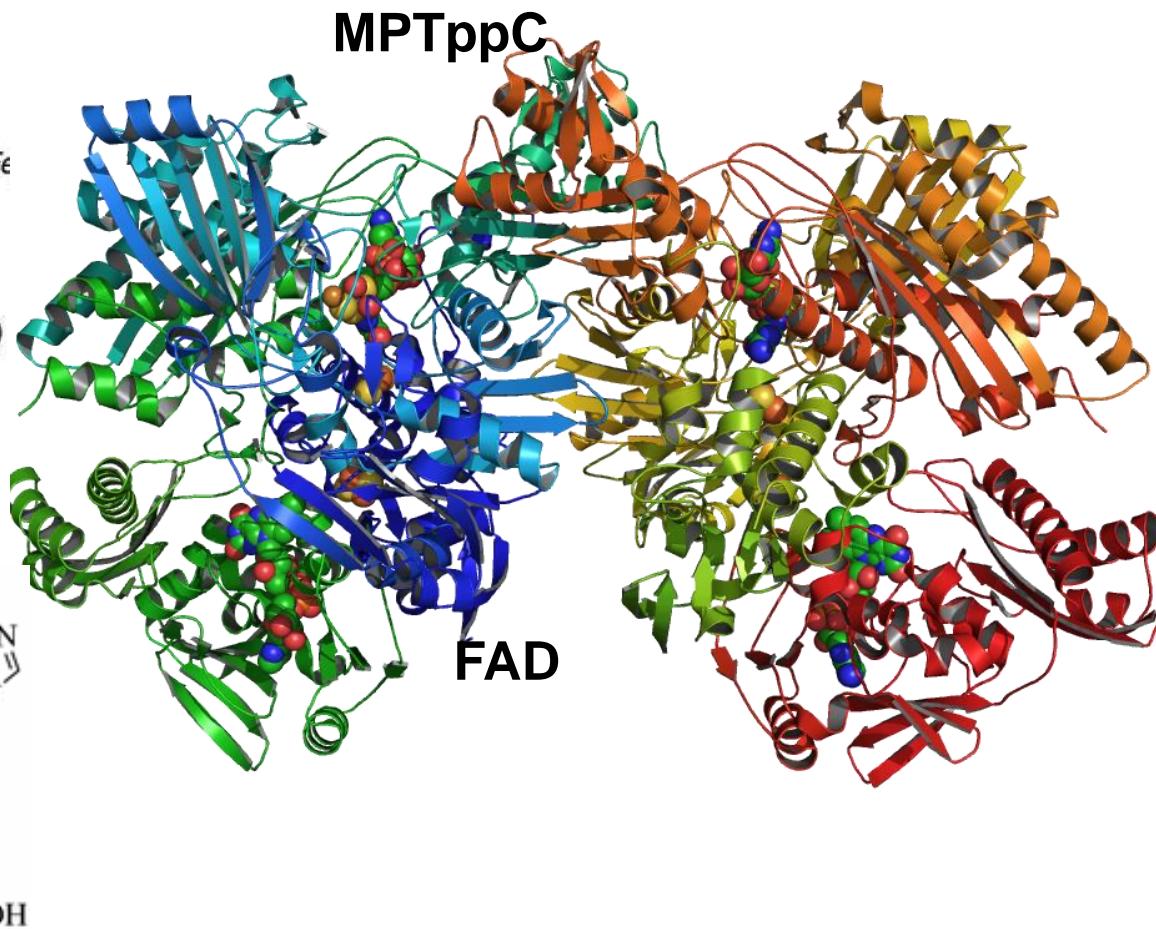
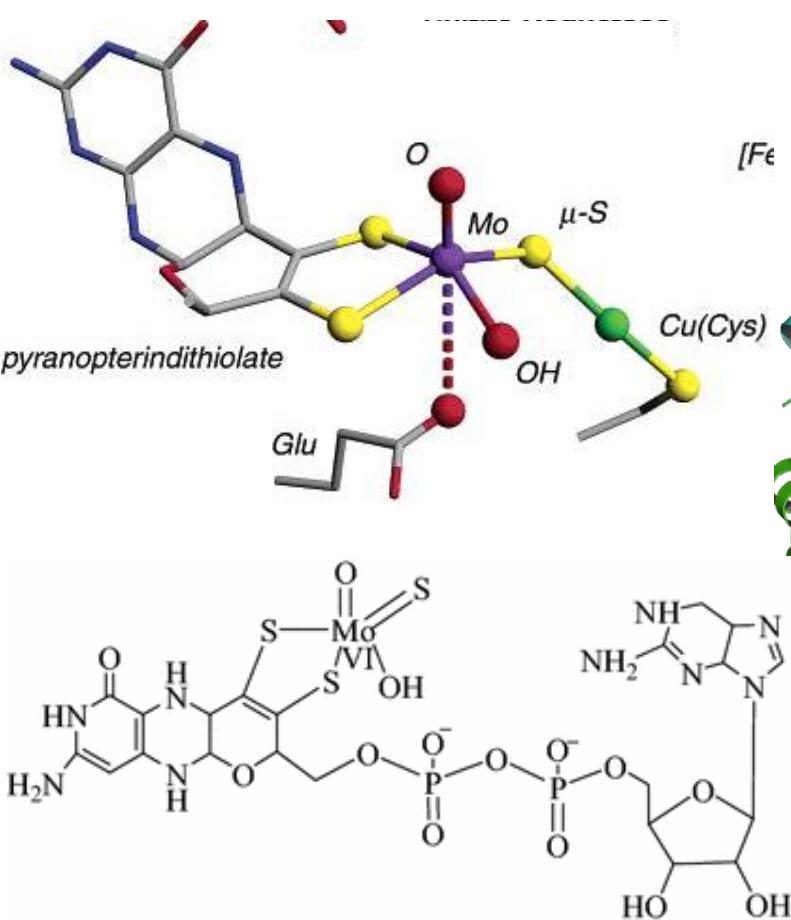
(2A) Carbon Monoxide Dehydrogenase (CODH)

- Some microbes use CO as a carbon & energy source catalyzed by CODH. CODH enzymes can **reversibly oxidize CO to CO₂**. ~108 tons of CO are removed by bacterial oxidation every year.

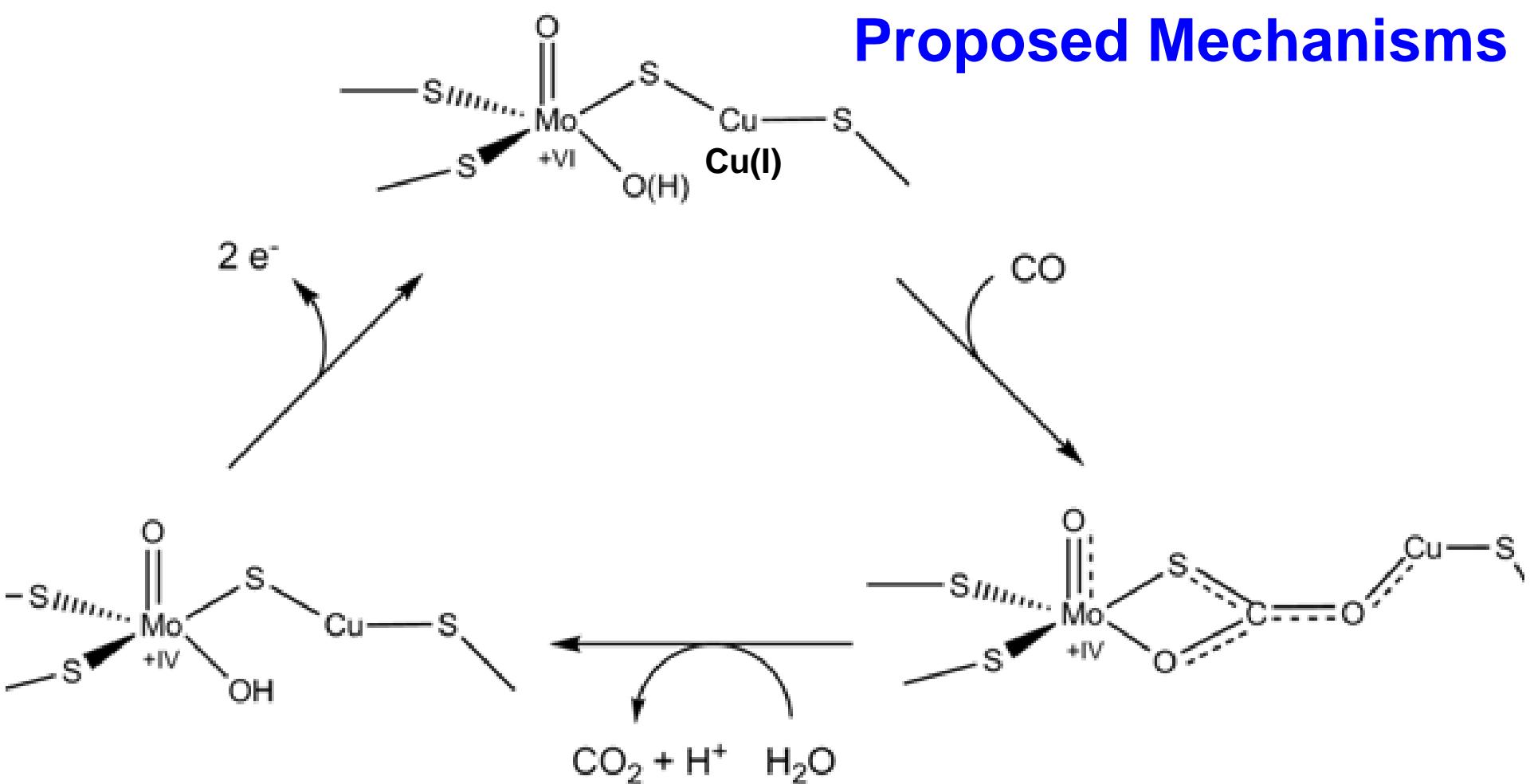


- 3 classes of CODHs:
 1. **Mo-CODH** (with 1 **Cu(I)**),
 2. **Ni-CODH**,
 3. Bifunctional **Ni-CODH/ACS** (Acetyl-CoA Synthase).

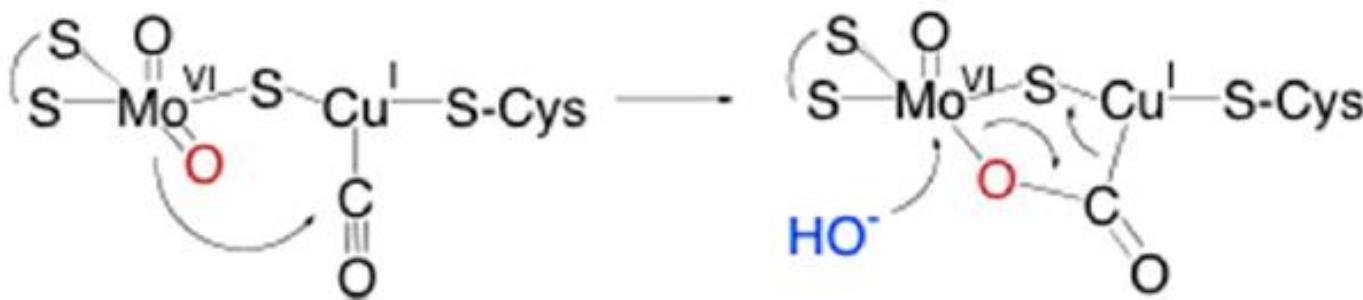
- **Mo-CODH** in aerobic bacteria can oxidizes CO. The electrons transfer from the CO oxidation through an ET chain & finally reduce O₂.
- Contains **FAD**, **[2Fe2S]** clusters, & a **active site with a Cu & molybdopterin cytosine dinucleotide (MPTppC)**.



Proposed Mechanisms



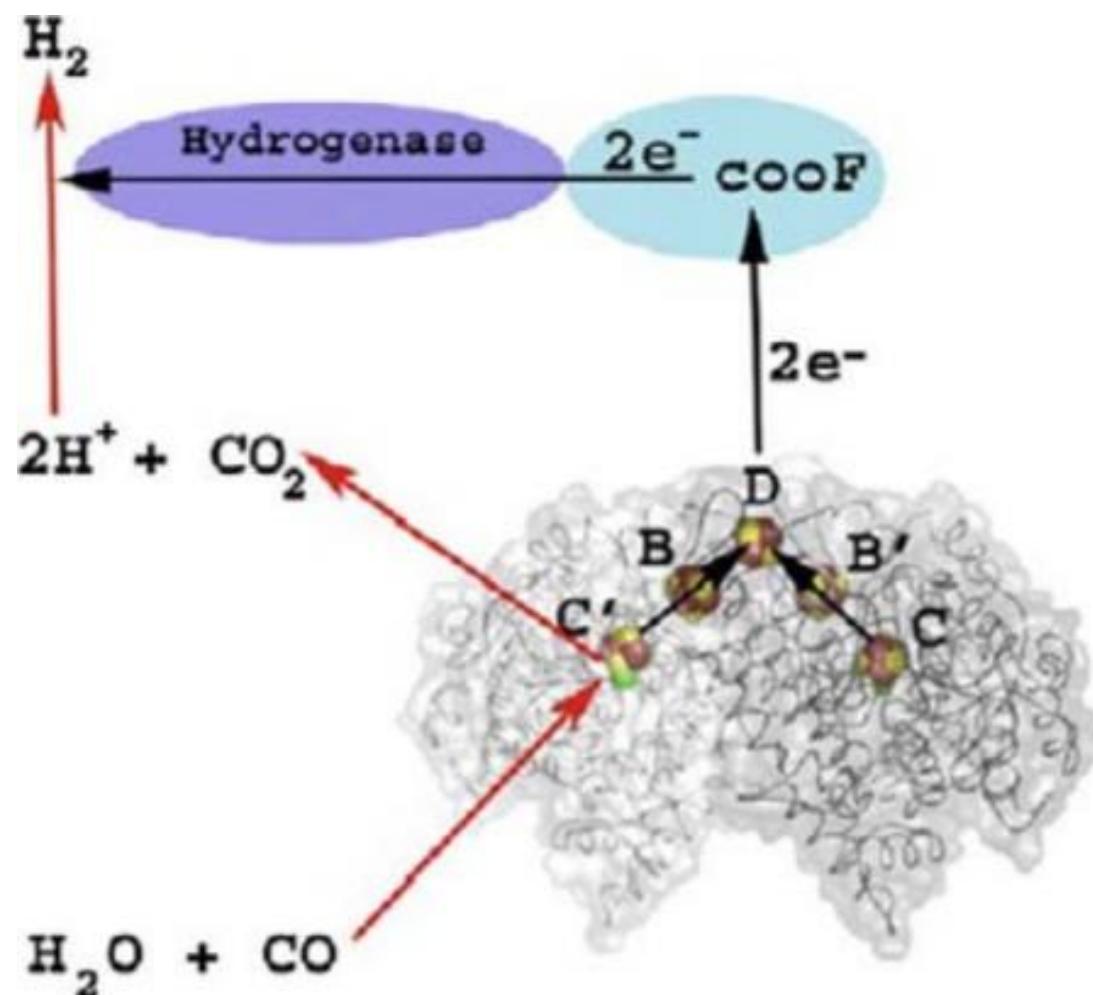
Alternative



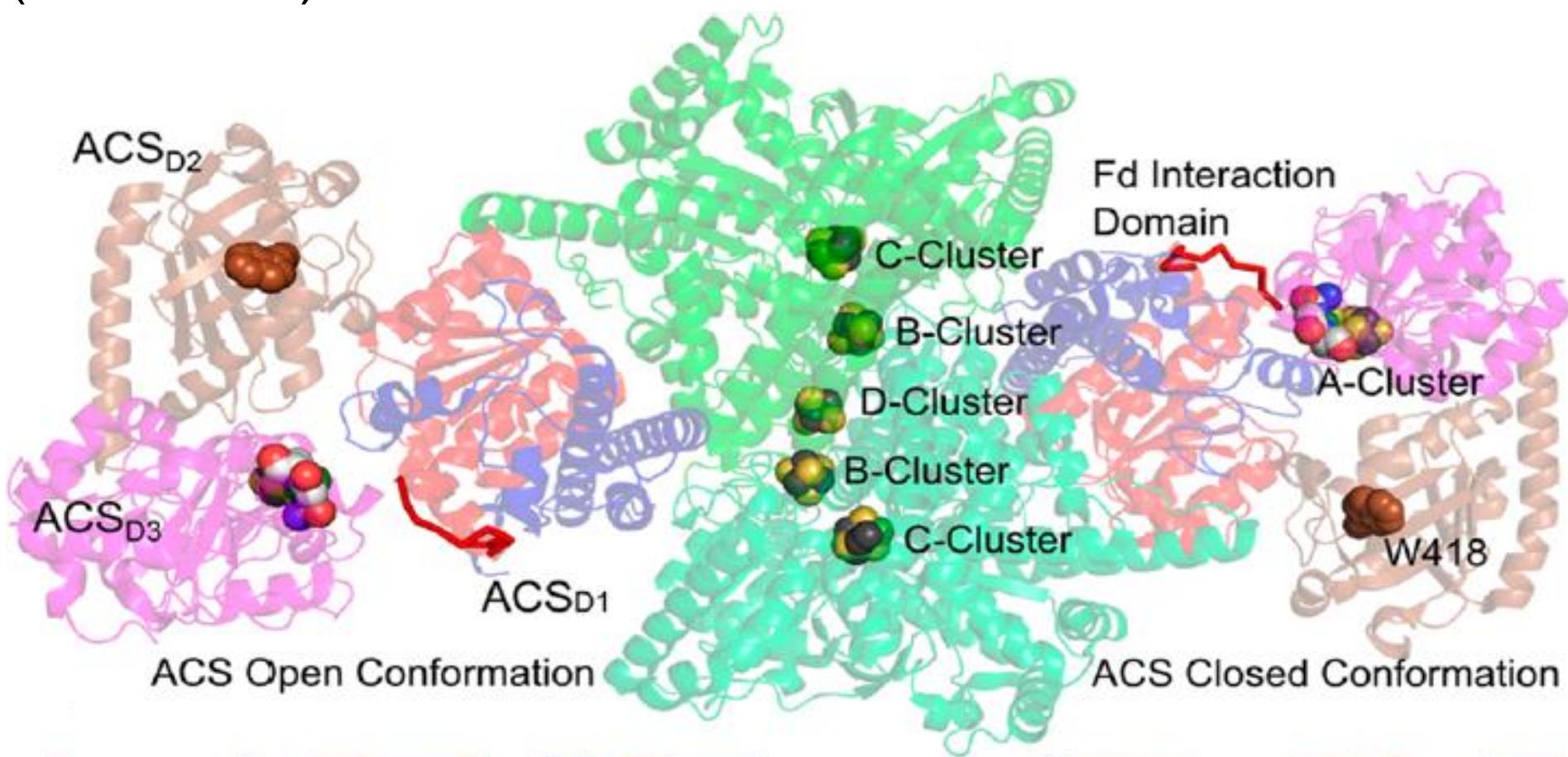
- Ni-CODH together **with hydrogenase** can catalyze the reaction of CO with H₂O to form CO₂ & H₂.



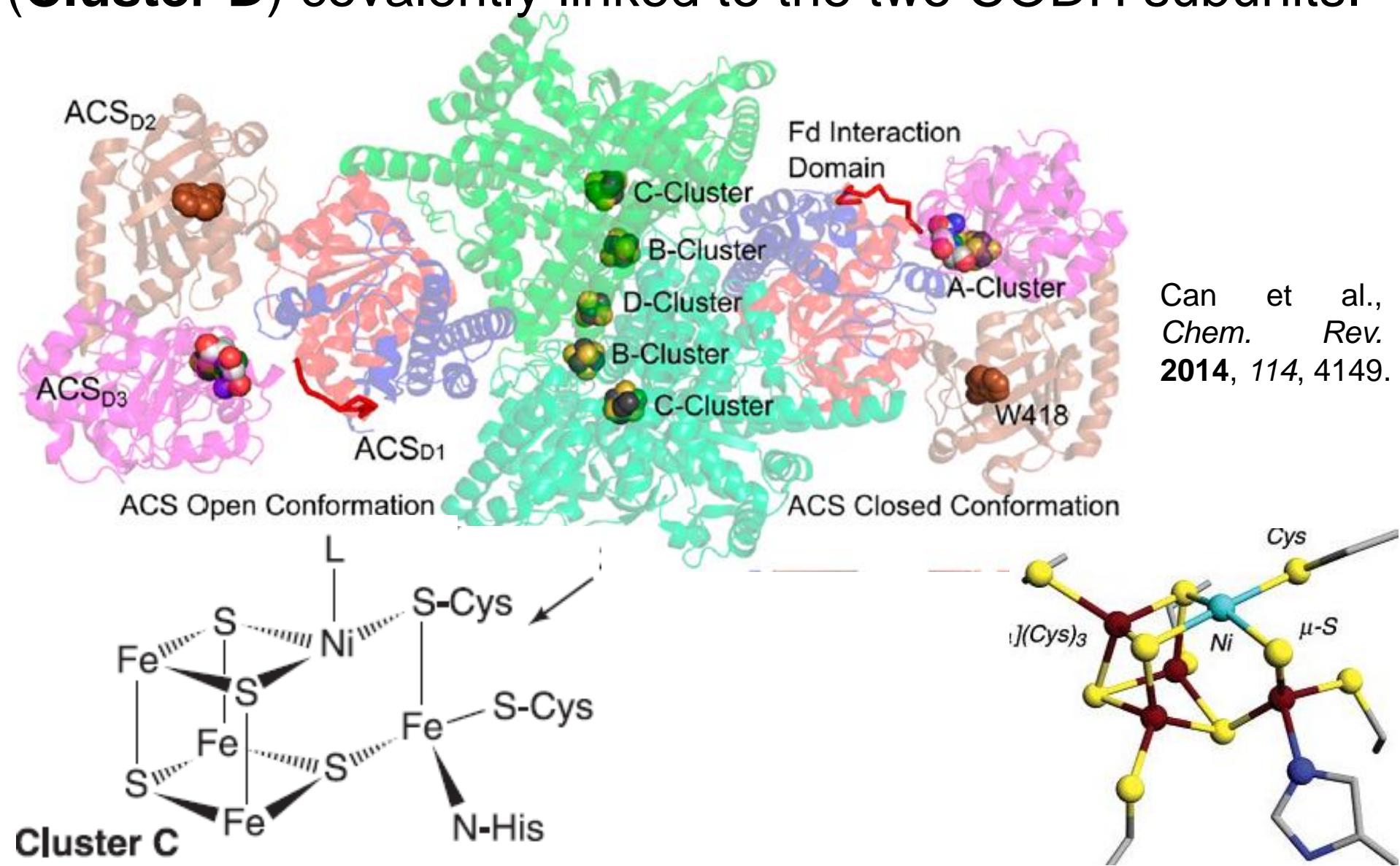
$$\Delta E^{\circ\prime} = -106 \text{ mV}$$

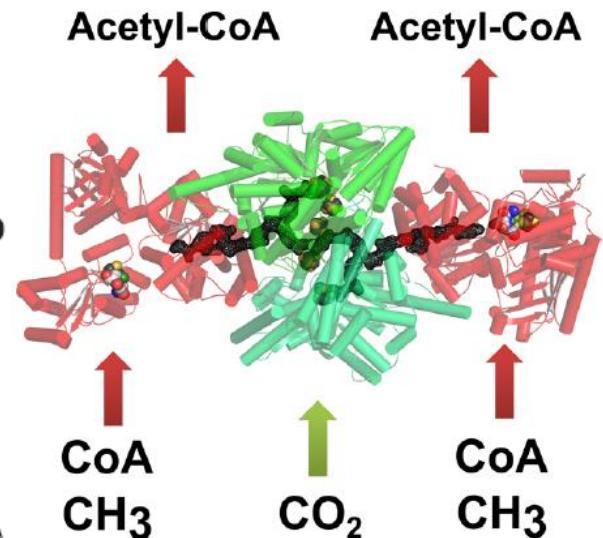
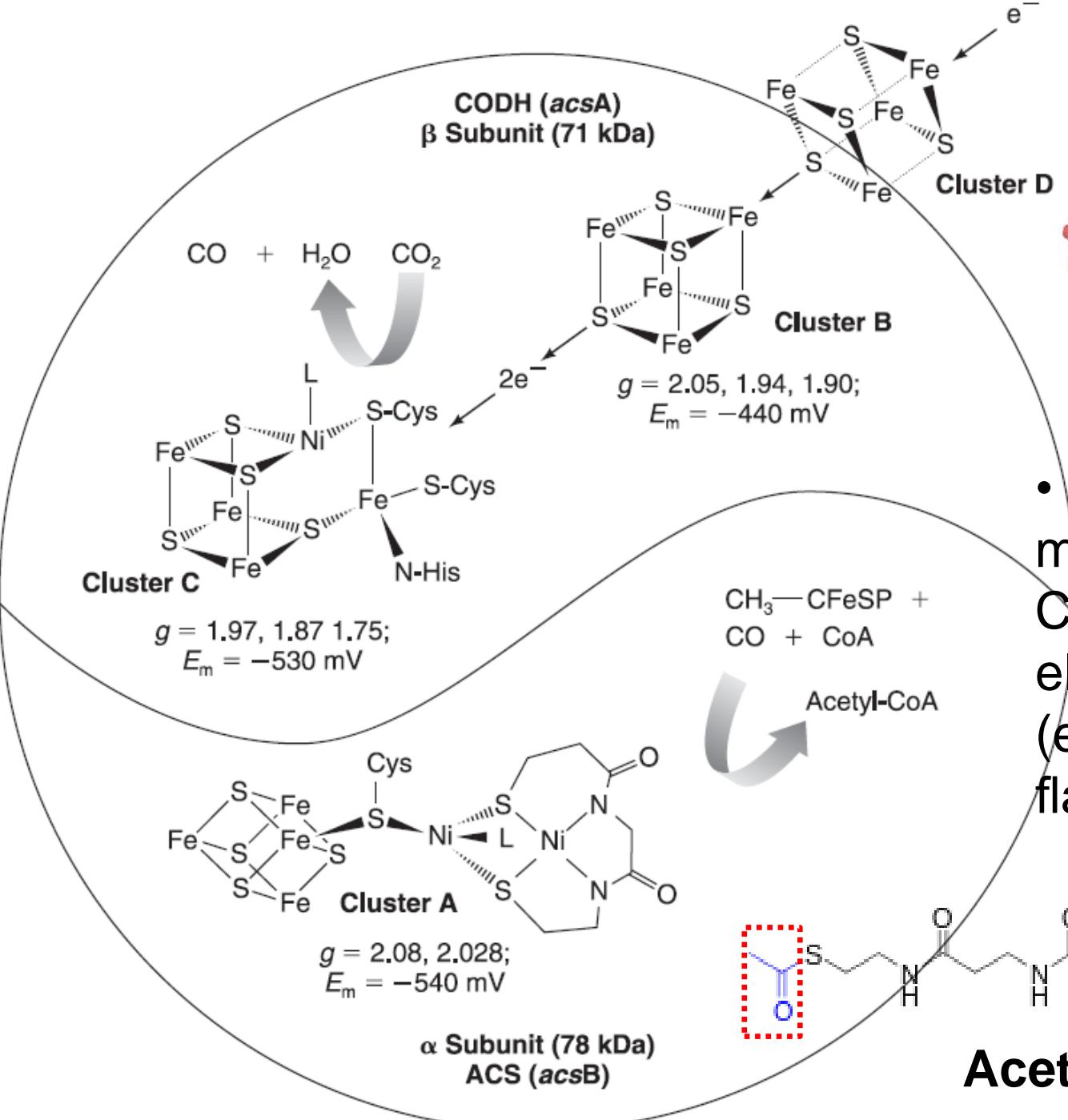


- **Bifunctional CODH/ACS protein** has a 2 separate reaction sites on each subunit.
- In microbes, **CO₂ reduction forms CO** at the CODH subunit (**Cluster C**). CO then reacts a **CH₃** group & **CoA** (Coenzyme A) to **form acetyl-CoA** at the ACS subunit (**Cluster A**).



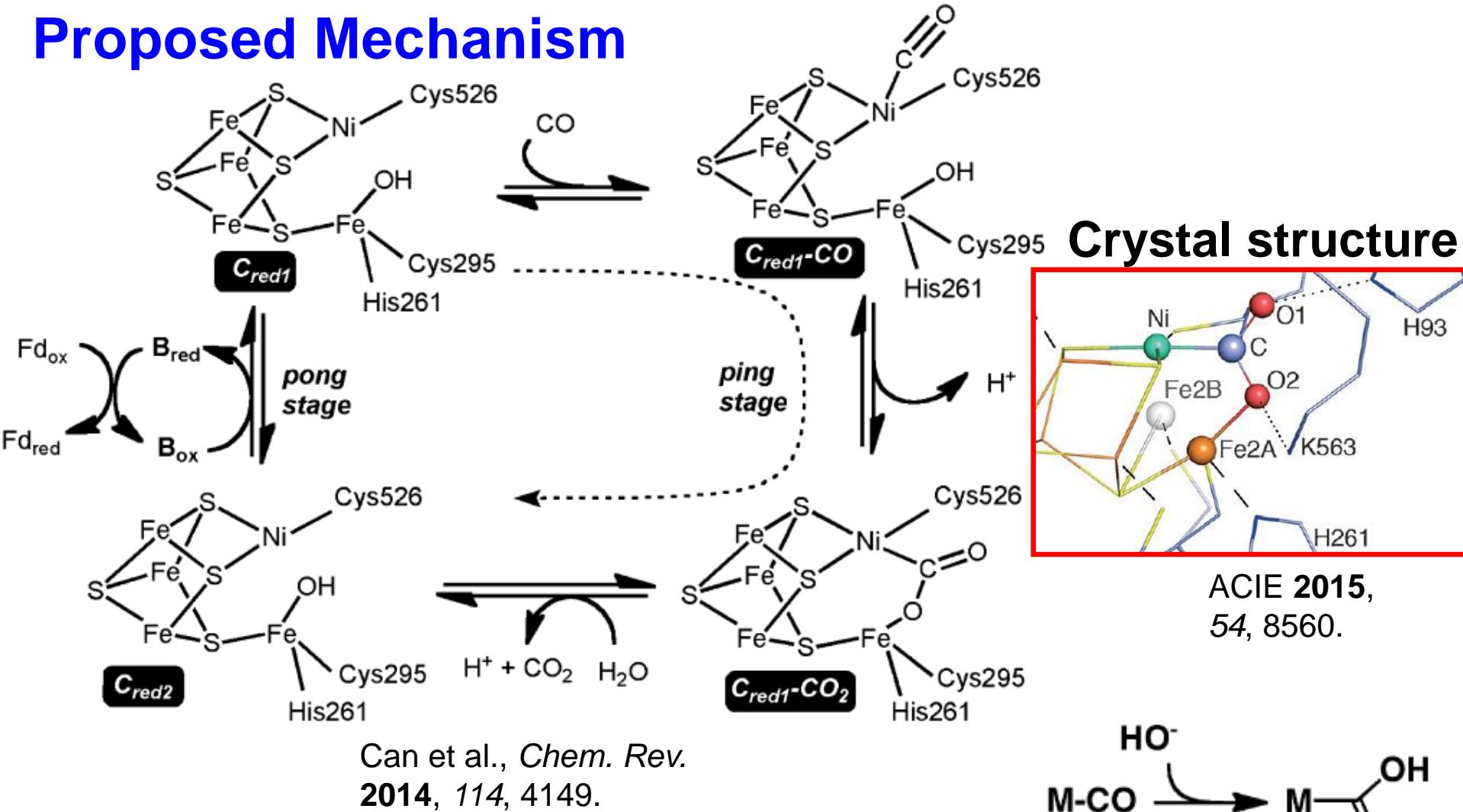
- Each CODH subunit contains a **NiFeS** cluster (**Cluster C**), a **[4Fe4S]** cluster (**Cluster B**) & a **[4Fe4S]^{2+/1+}** cluster (**Cluster D**) covalently linked to the two CODH subunits.



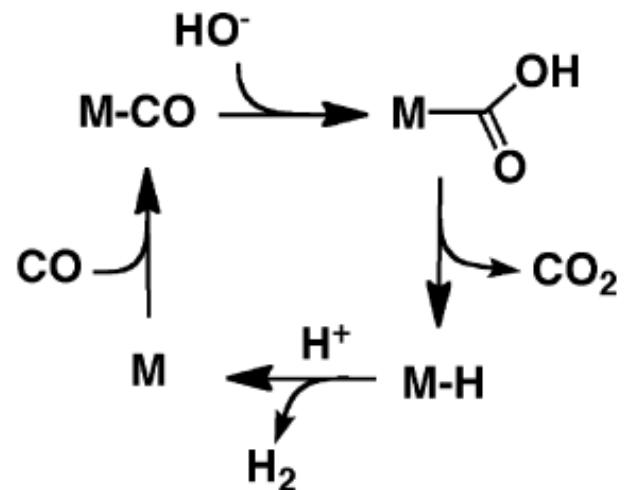


- Cluster D possibly mediates ET between CODH & the terminal electron acceptor (e.g. ferredoxin, flavodoxin).

Proposed Mechanism



ACIE 2015,
54, 8560.



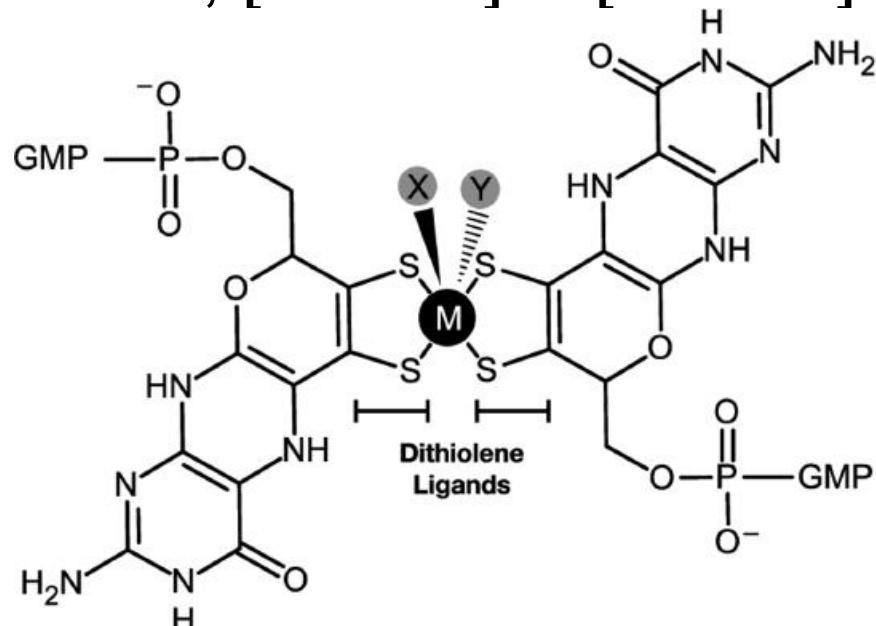
- The mechanism for the Ni-CODH is similar to metal-catalyzed water-gas shift reaction.

Formate Dehydrogenase (FDH)

- FDH (or called CO₂ reductase) usually catalyzes **oxidation of formate** to **form CO₂**.



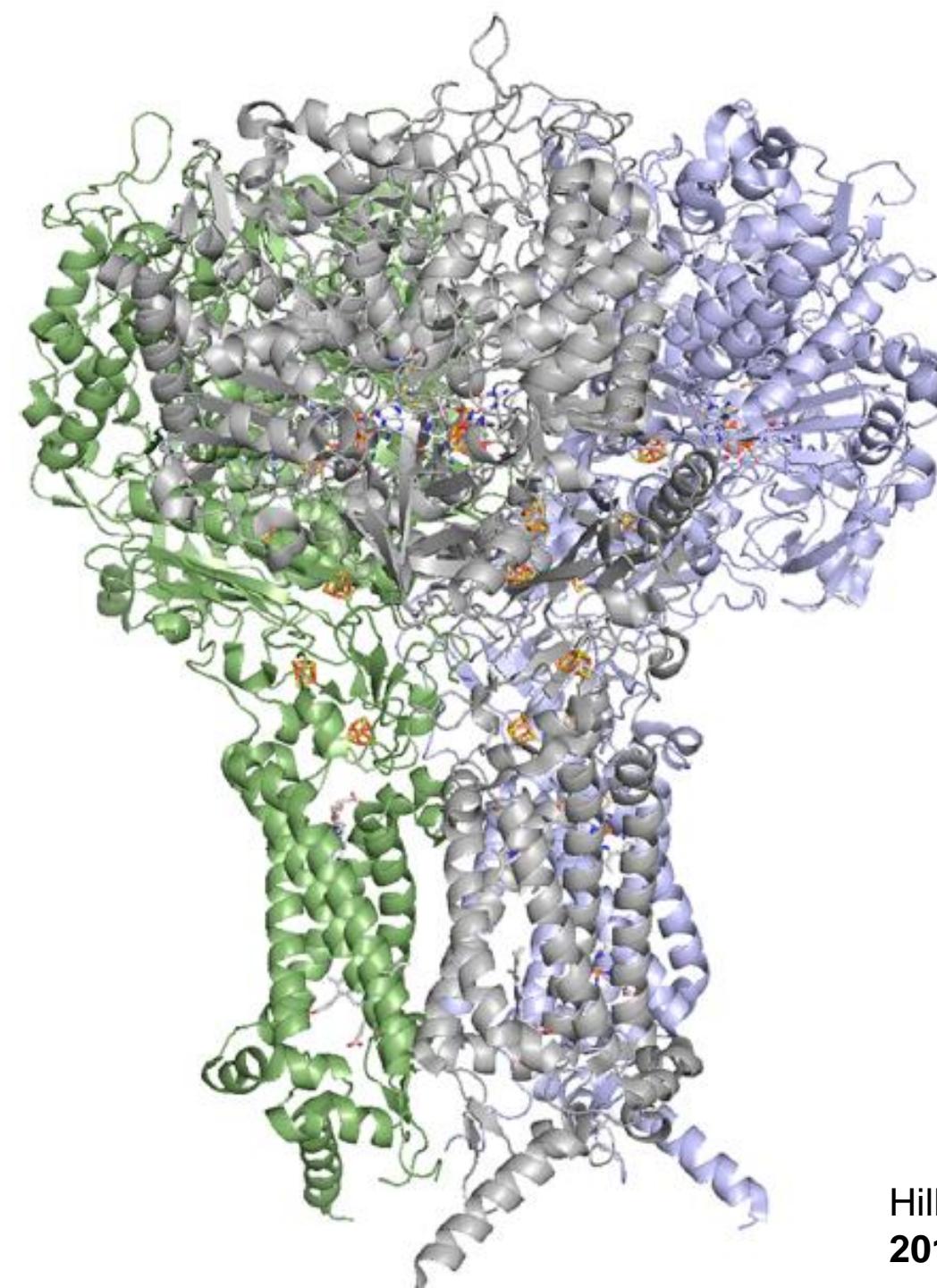
- They have a diverse metal composition & even some lack metals. FDH from *E. coli* contains Mo, Selcys, & Fe-S cluster. Some FDHs contain Mo- or W-dithiolene cofactor, [2Fe2S] & [4Fe4S] clusters.



M = **Mo or W**

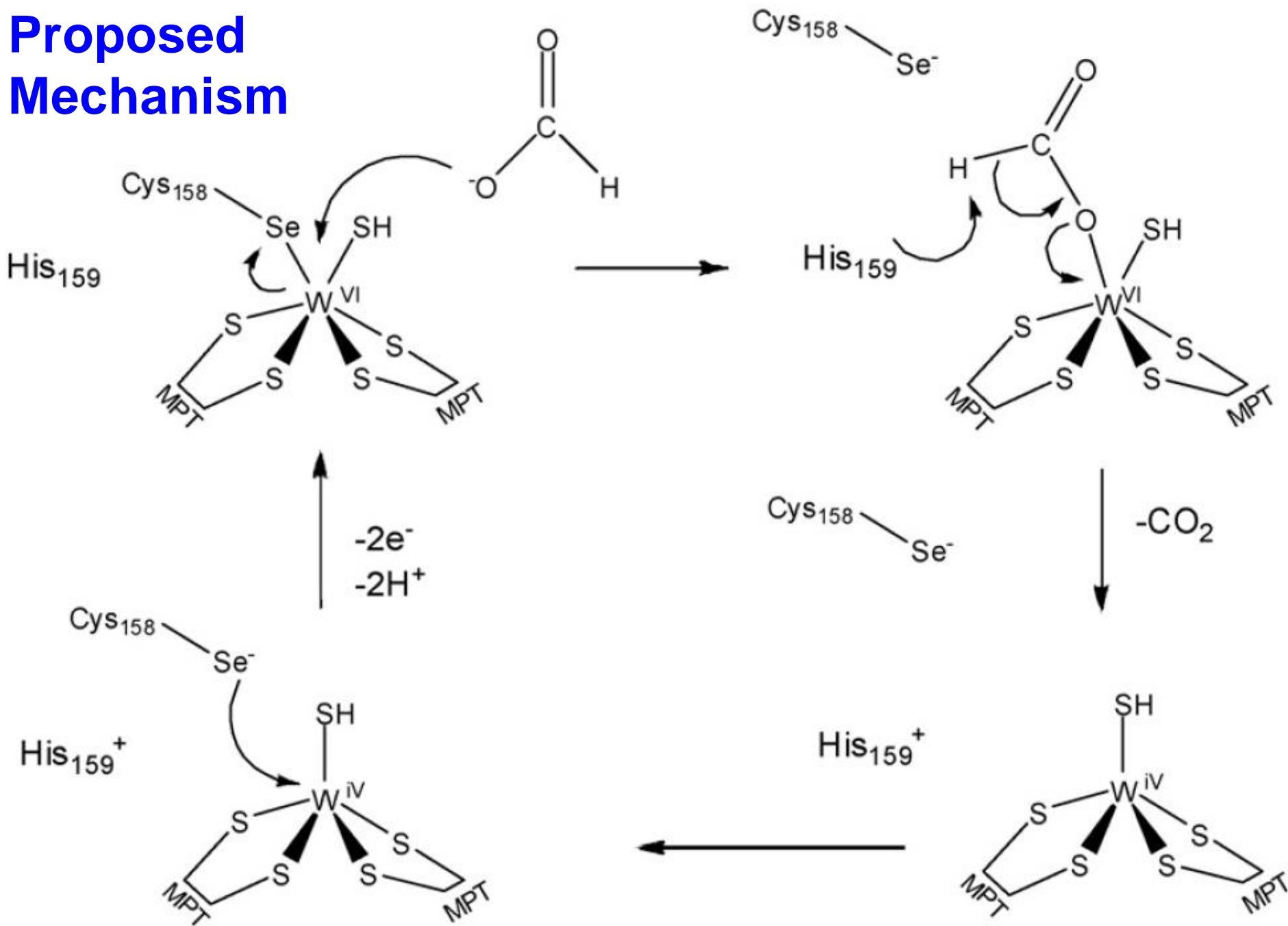
X = Selcys

Y = sulfido (S) or oxo (O)



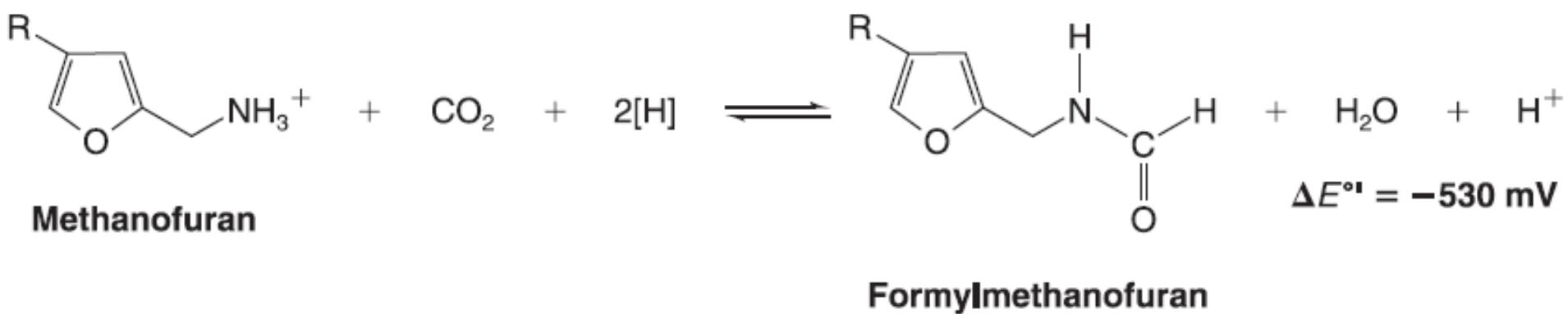
Hille et al., *Chem. Rev.*
2014, 114, 3663.

Proposed Mechanism

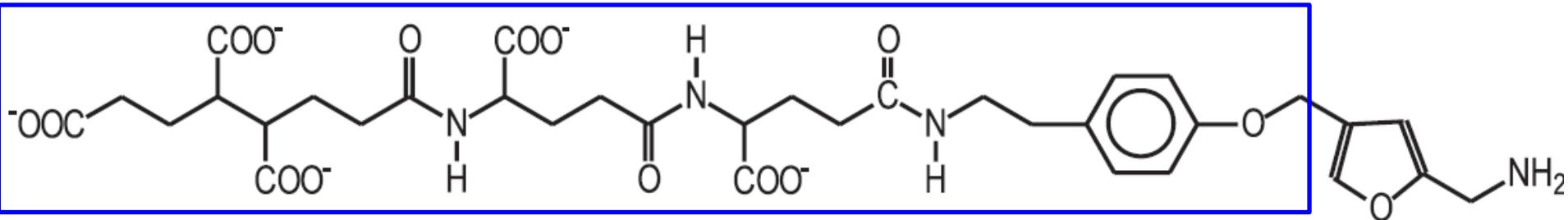


Formyl-Methanofuran Dehydrogenase (FMD)

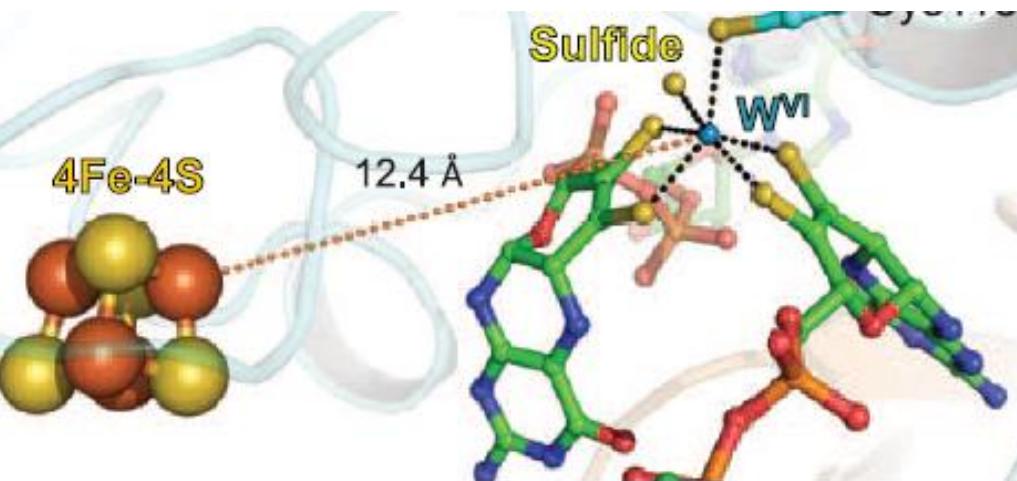
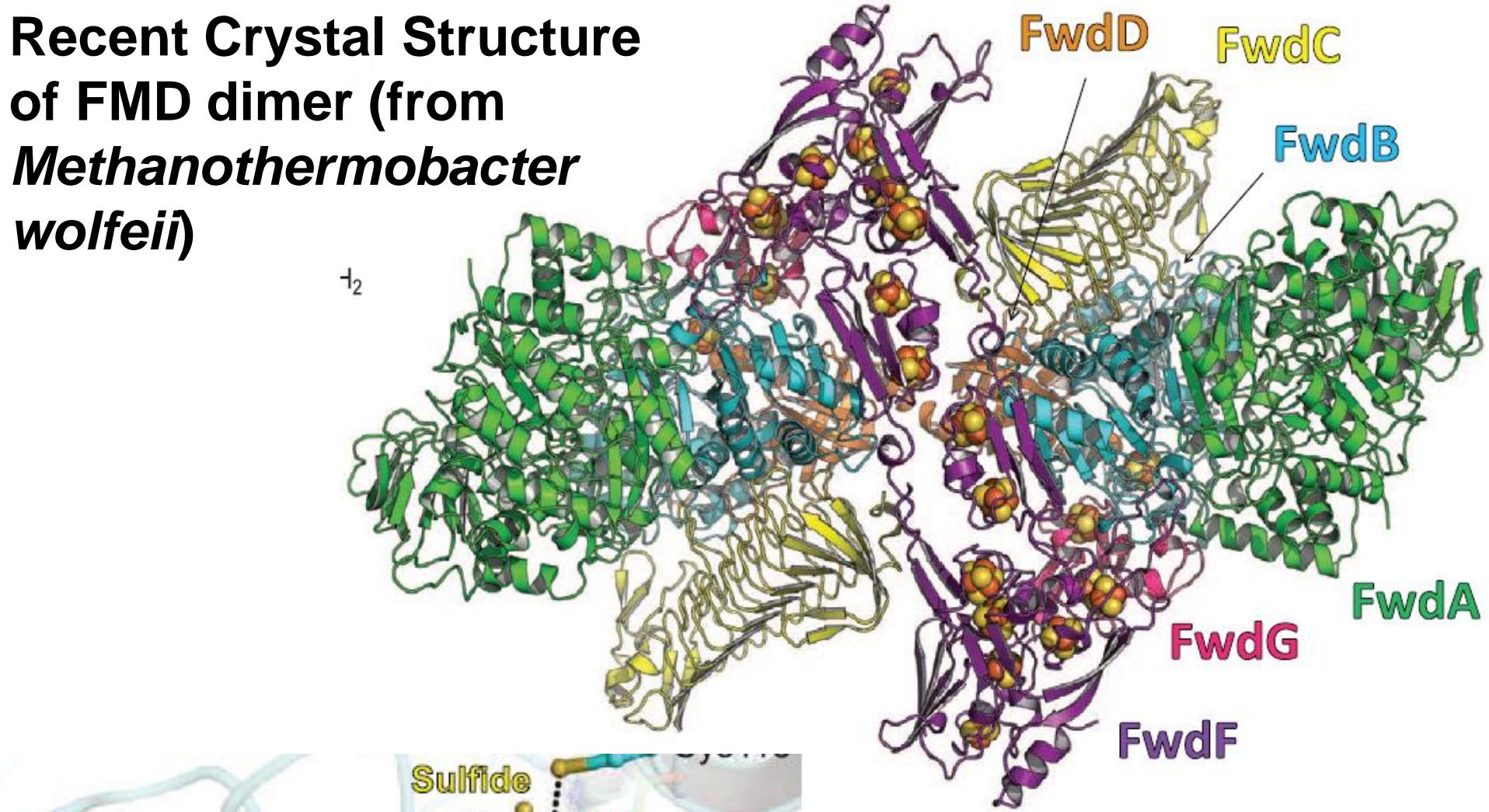
- Similar to FDH, FMD is a **Mo or W Fe-S enzyme** & CO₂ reductase.
 - Catalyzes the reduction of CO₂ with methanofuran to form formylmethanofuran:



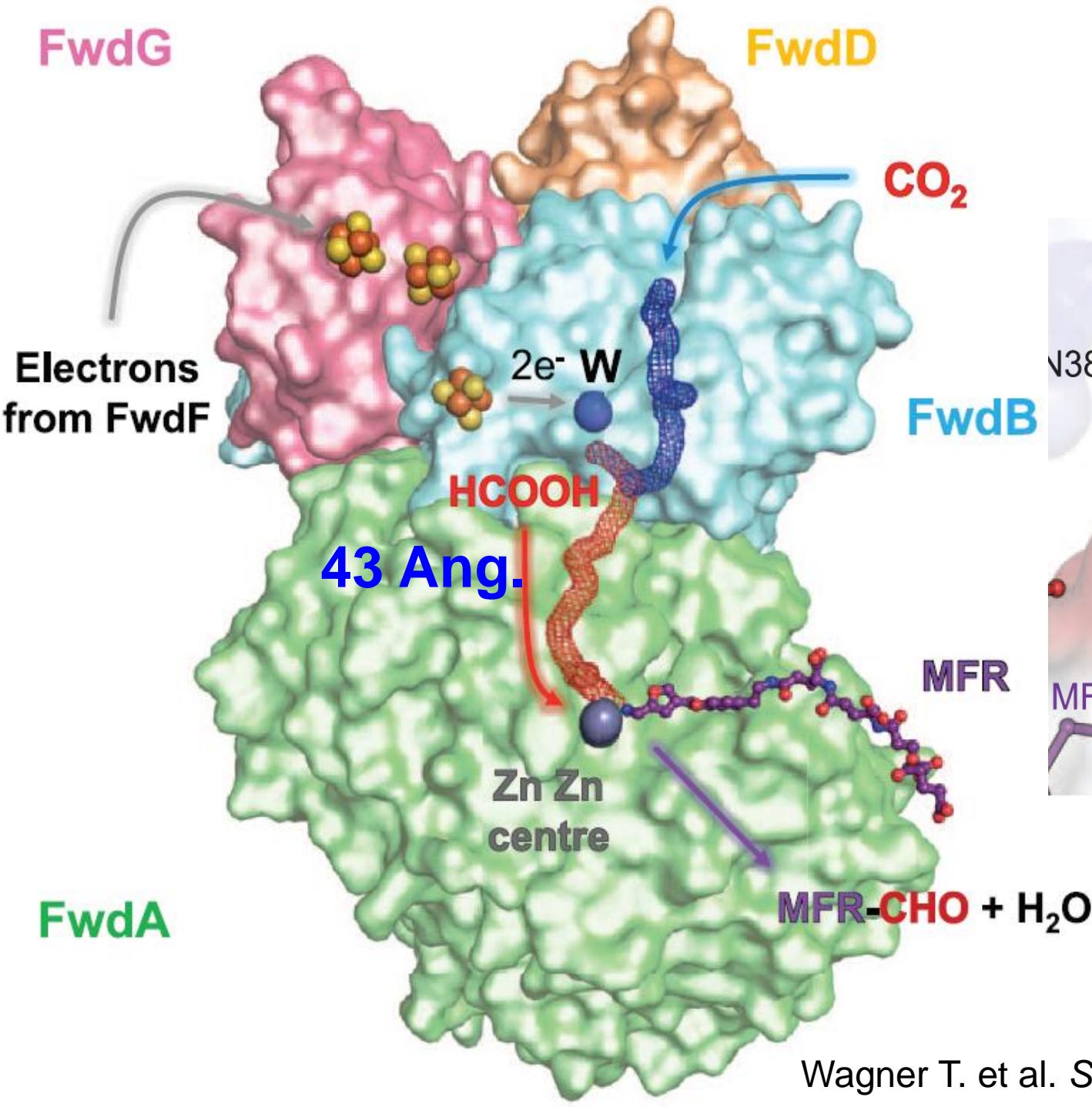
R:



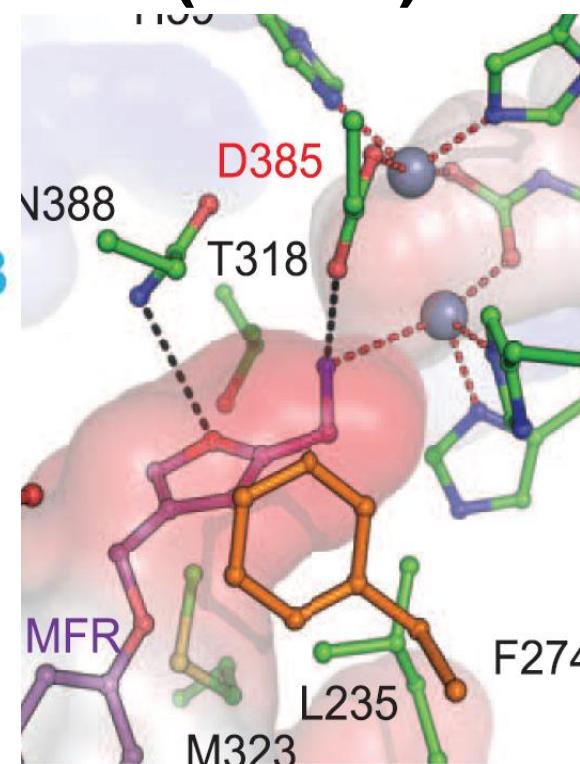
Recent Crystal Structure of FMD dimer (from *Methanothermobacter wolfeii*)



The W active
site (**FwdB**)

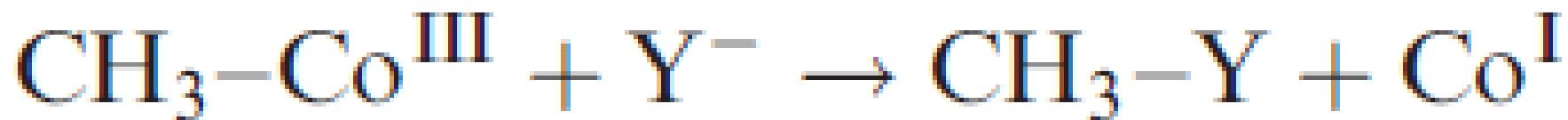


The Zn²⁺ active site (FwdA)

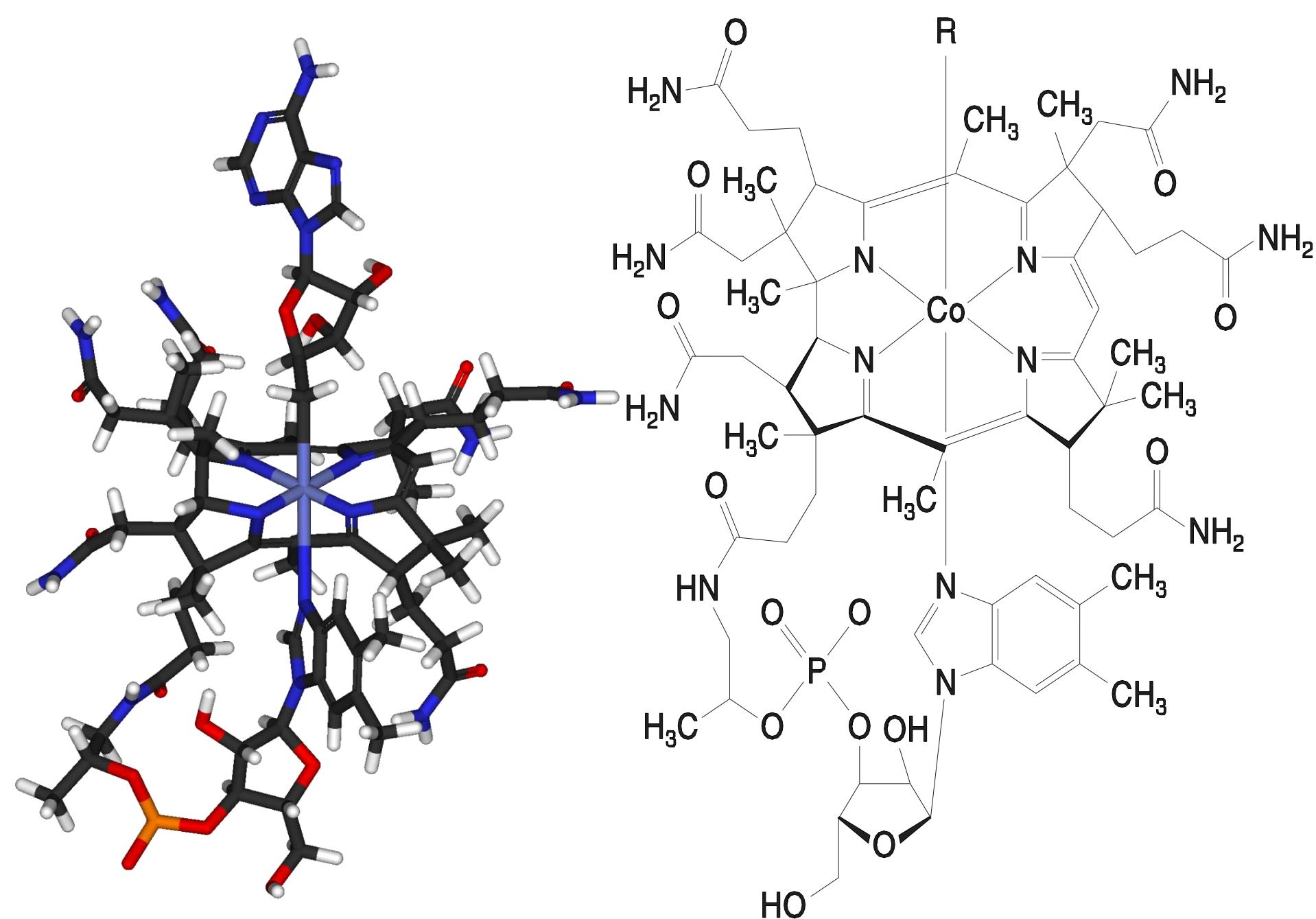


(2B) Methyltransferases

- **Vitamin B12-dependent** methyltransferases play a key role in methyltransferases.

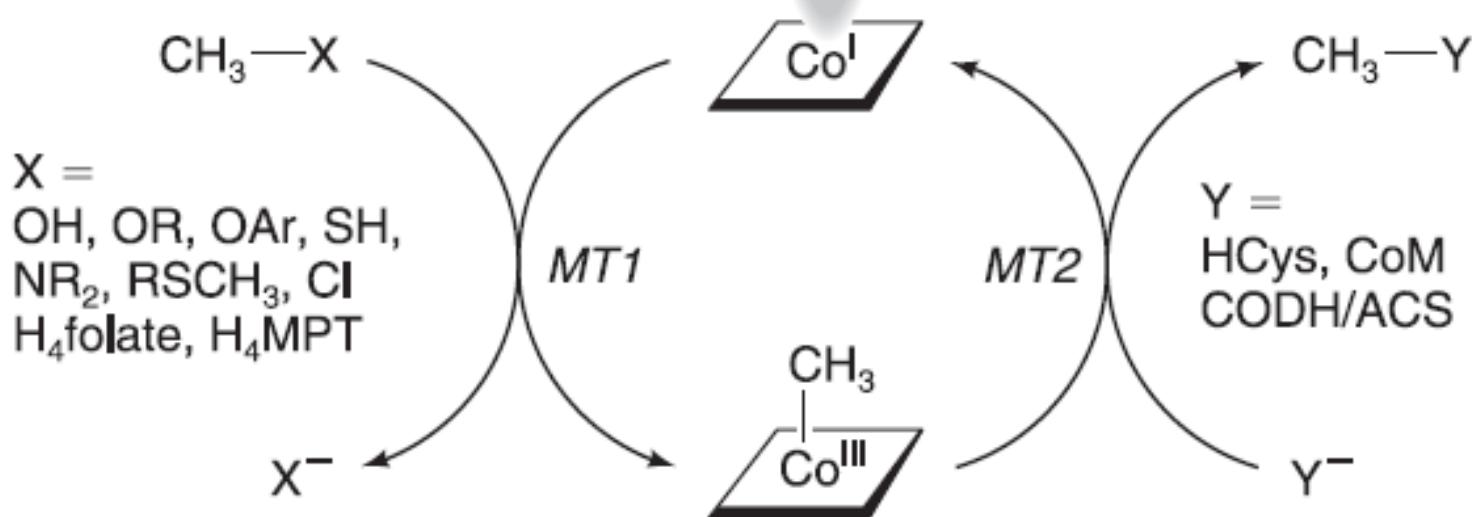


- Three parts (**MT1**, **cobamide (B12)** & **MT2**) are required.
- **MT1** transfers the Me group from a **substrate** (Me-X) to cobamide to form CH₃-B12.
- **MT2** catalyzes the Me transfer from the CH₃-B12 to an **acceptor** (Y).



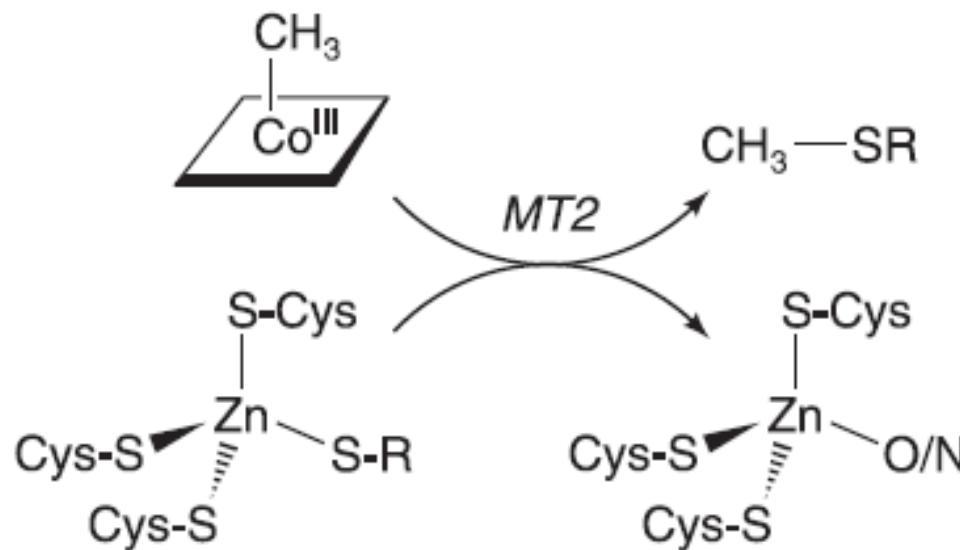
No axial R for methyltransferases (Co(I))

(a)

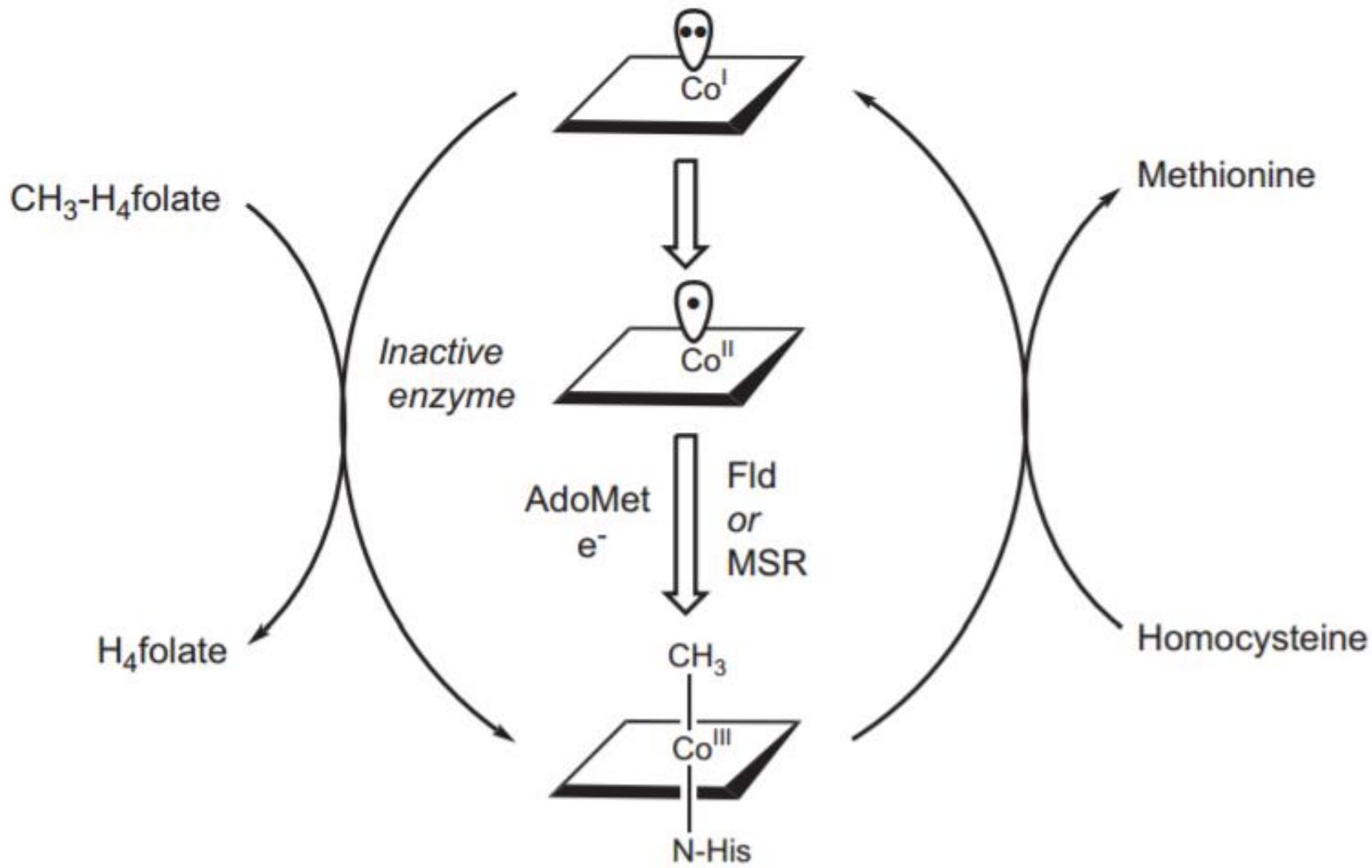


(b)

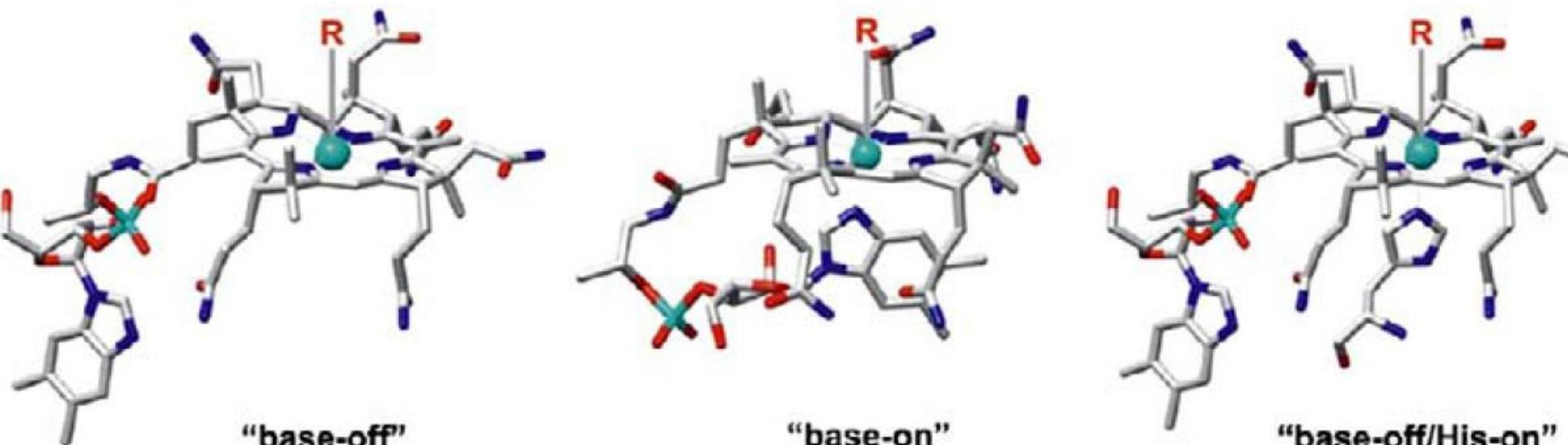
The substrates (Me-X): methanol, amines, thiols, acetyl-CoA, $\text{CH}_3\text{-H}_4$ folate, $\text{CH}_3\text{-H}_4$ MPT, methoxylated aromatics.



- For the **RS**-containing substrate, a **Zn ion** binds & activates the thiol group (e.g., CoMSH) to promote the transfer ($\text{CH}_3\text{-SCoM}$).

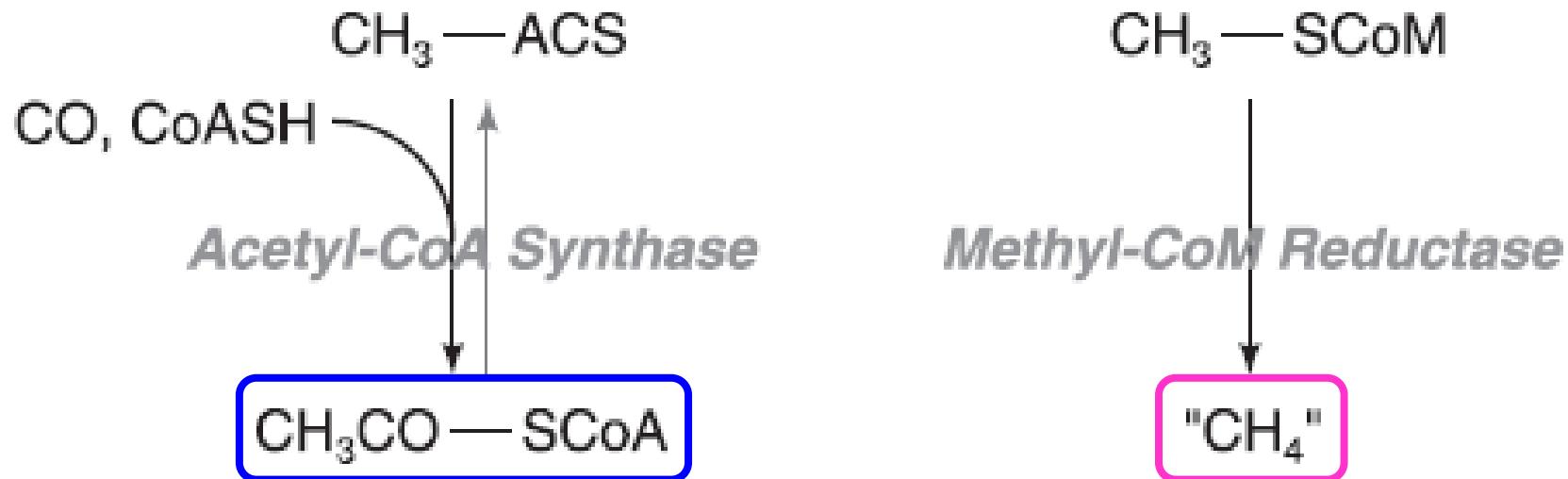


- **Co(I)** state is critical for methyltransferases. When the metal is oxidized to the Co(II) state, **reductive activation** is required by a ET donor (e.g. Fe-S cluster).



- The axial base group of B12 in some proteins dissociates from the Co metal (base-off), while a His of the protein coordinates to the Co metal (His-on) in some enzymes.

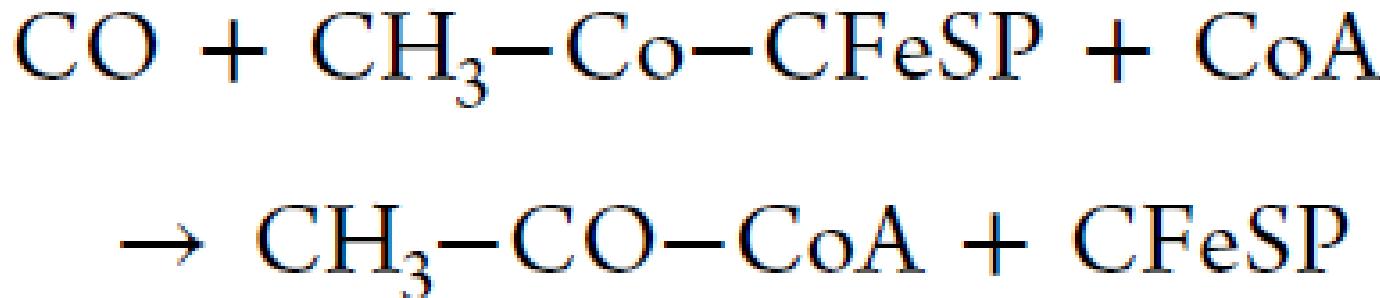
(2C) Carbonylation or Me Group Reduction



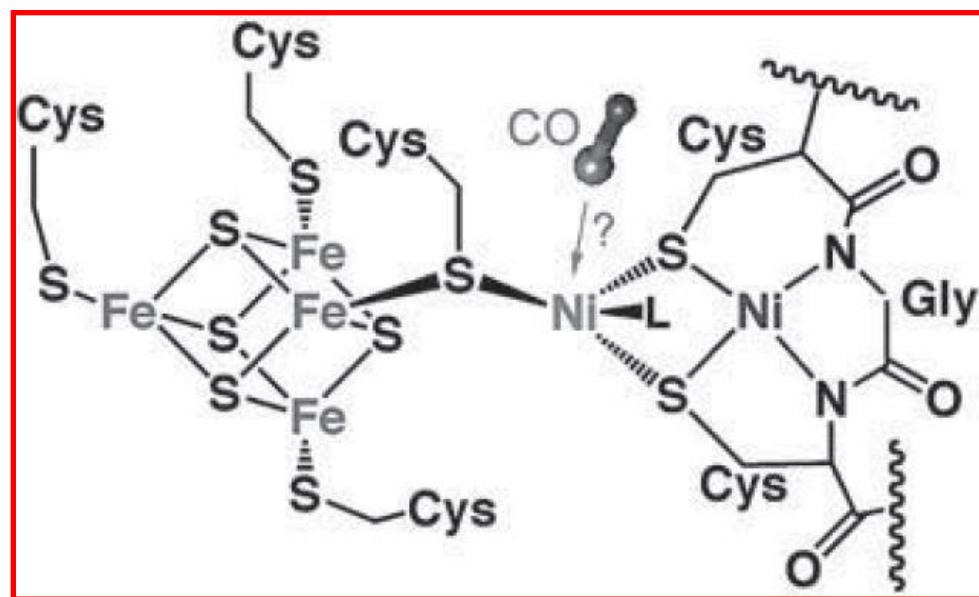
- The pathways of methanogenesis & acetogenesis diverge: Acetogens undergo **carbonylation** reaction with enzyme-bound CH₃-Co(III) to form **acetyl-SCoA**, while methanogens **reduce** CH₃-SCoM to form **CH₄**.

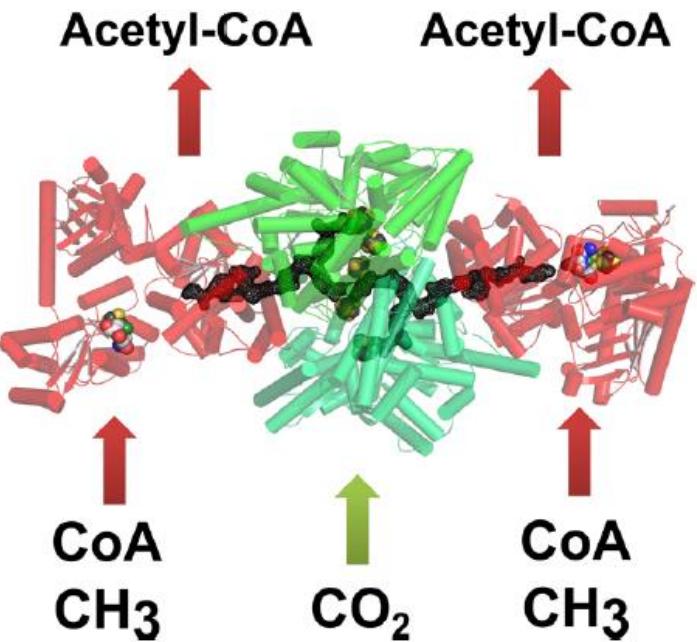
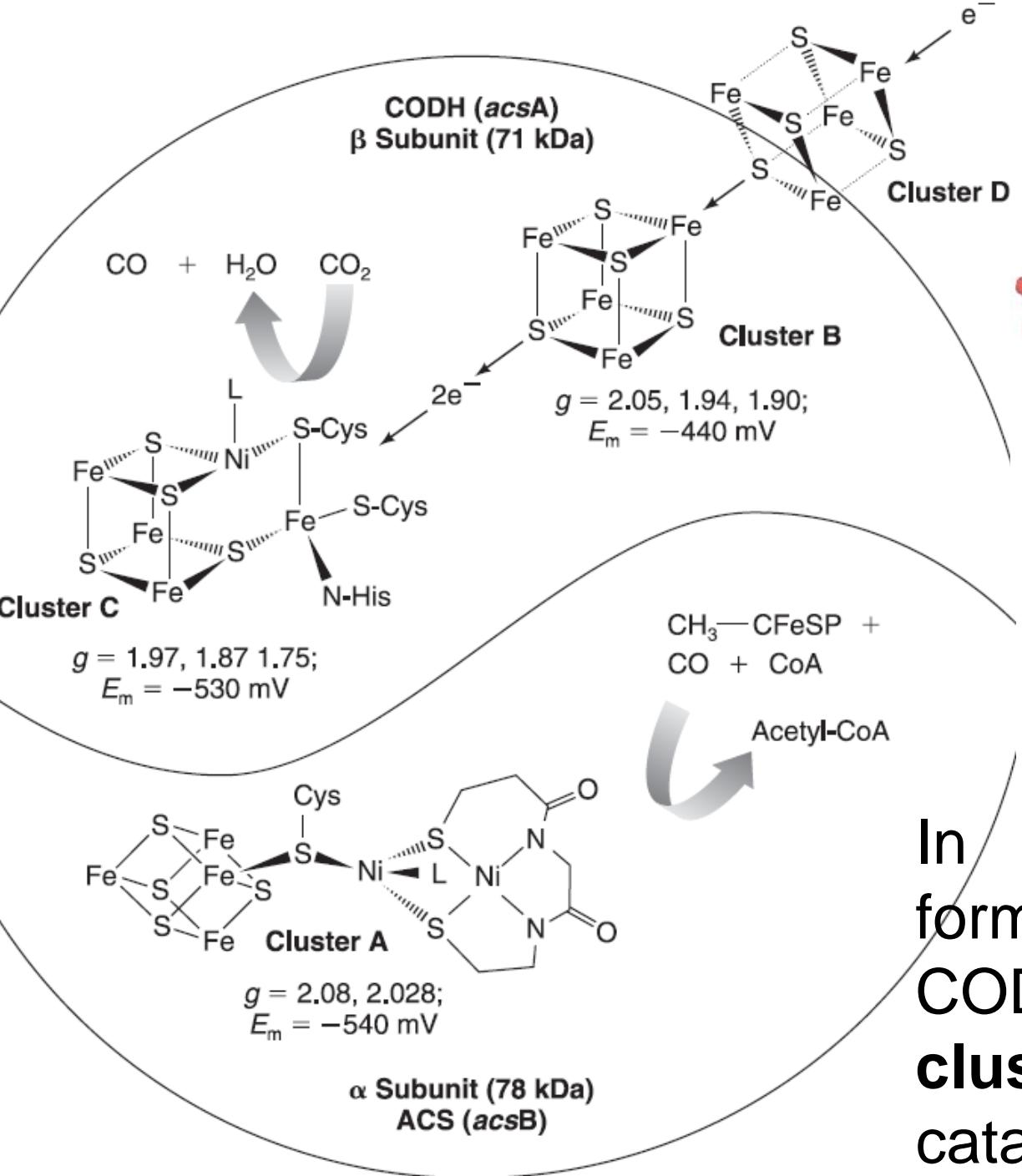
Acetyl-CoA Synthase (ACS)

- Catalyzes condensation of a CH₃ group, CO & CoA to form acetyl-CoA.



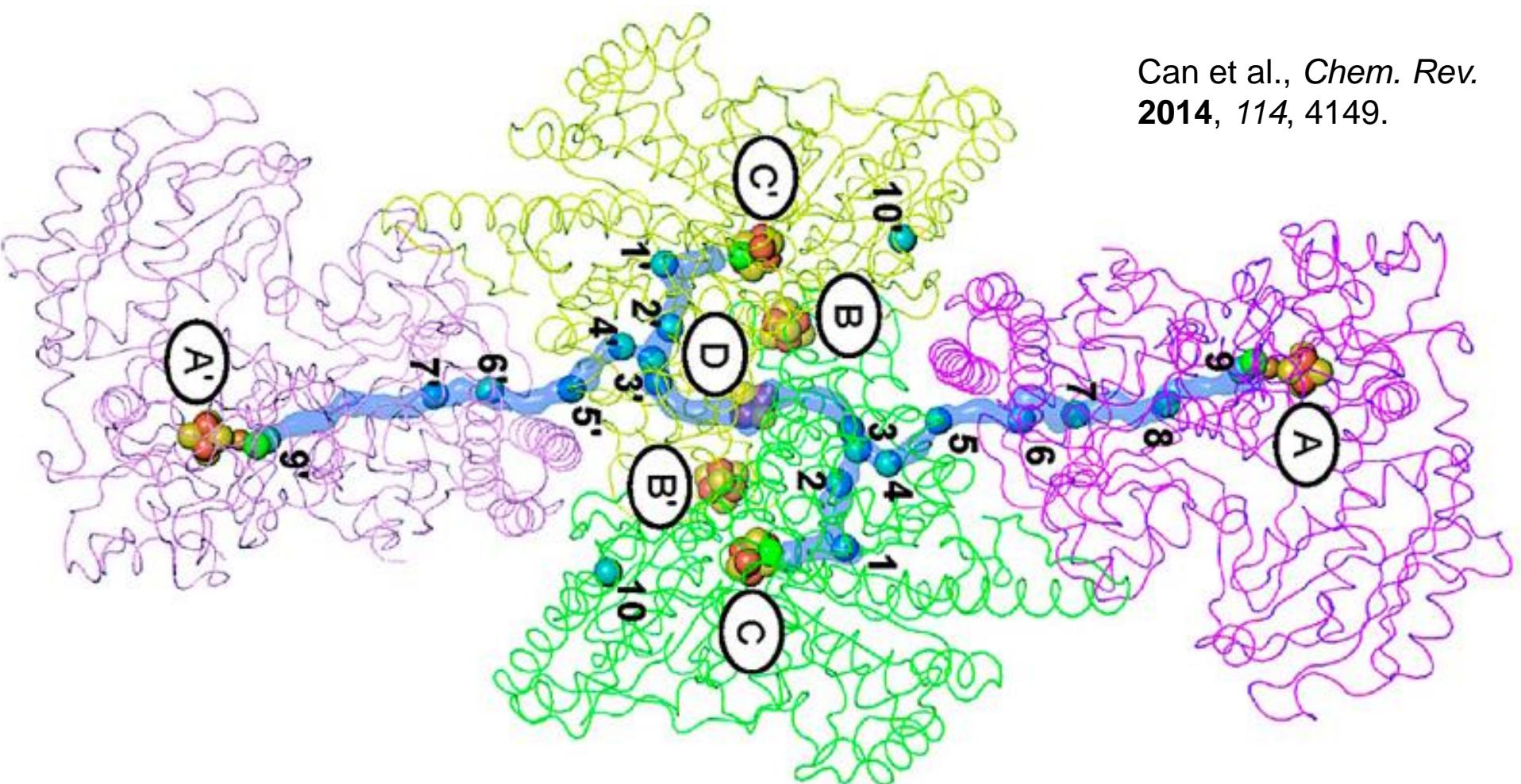
- In a related industrial process (the Monsanto process): Rh-catalyzed formation of MeCO₂H from CH₃OH, CO & HI.



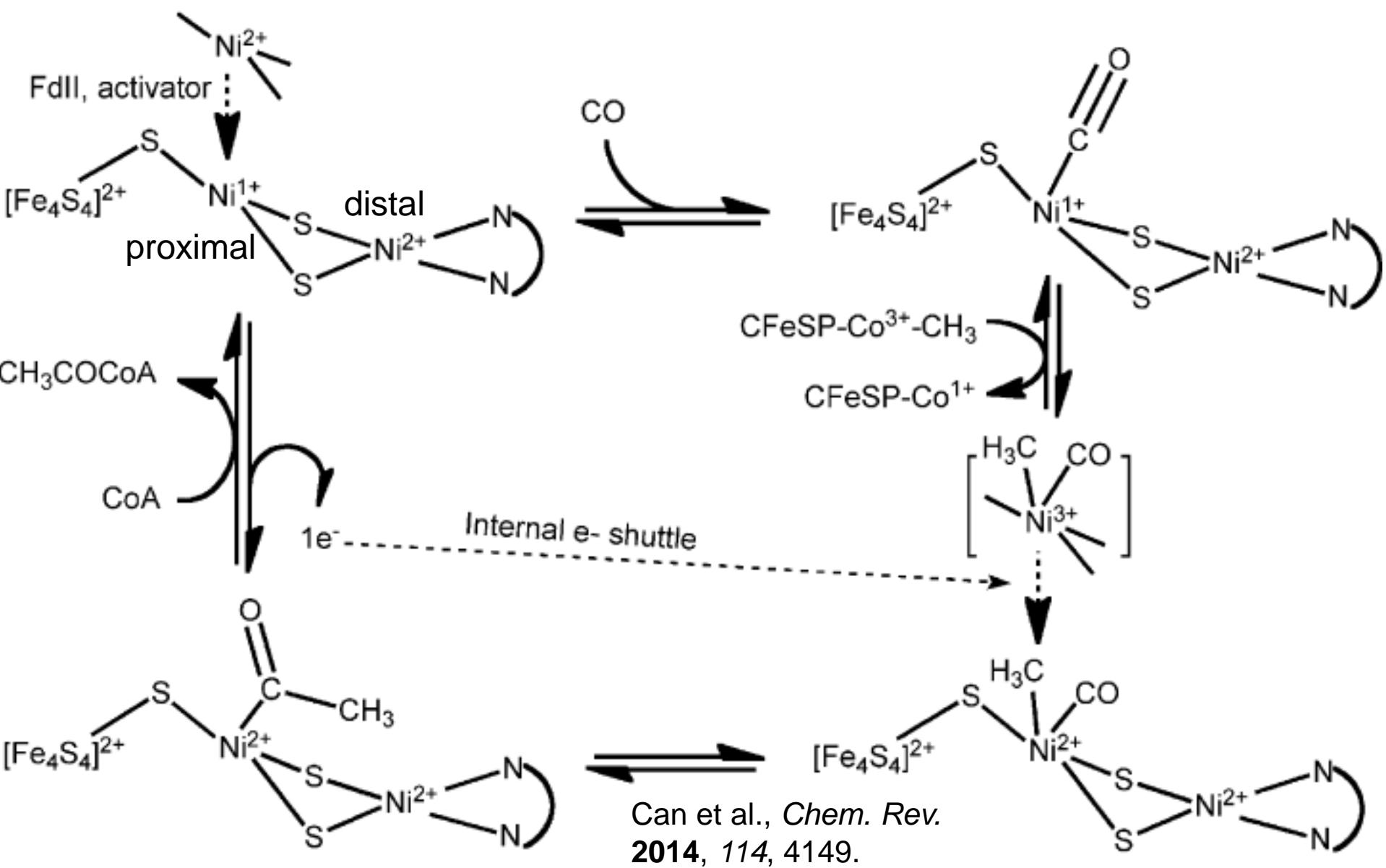


In CODH/ACS, CO formed at cluster C (in CODH) transfers to cluster A (in ACS) to catalyze carbonylation.

- CODH/ACS contains a ~70 Å hydrophobic channel between the clusters C & A to deliver CO to the A cluster. This channel ends just above the proximal Ni in the cluster A, which forms a Ni(I)-CO intermediate.



Proposed Mechanism

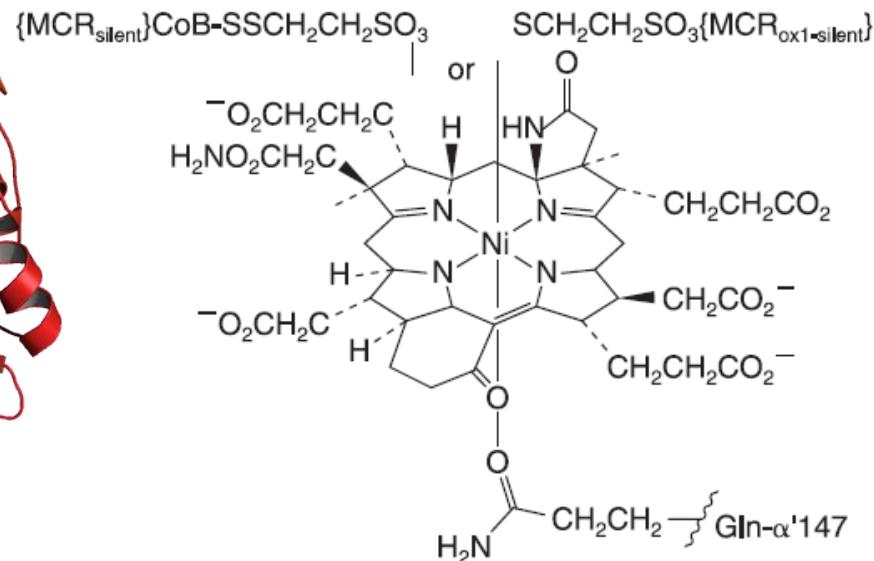
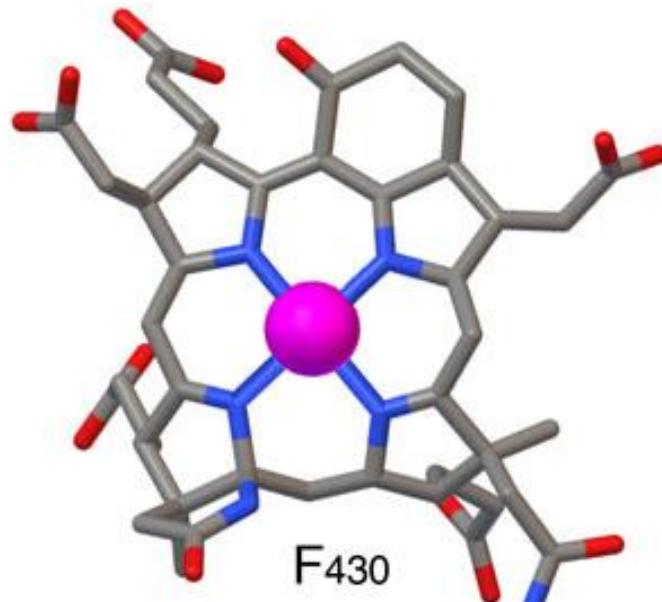
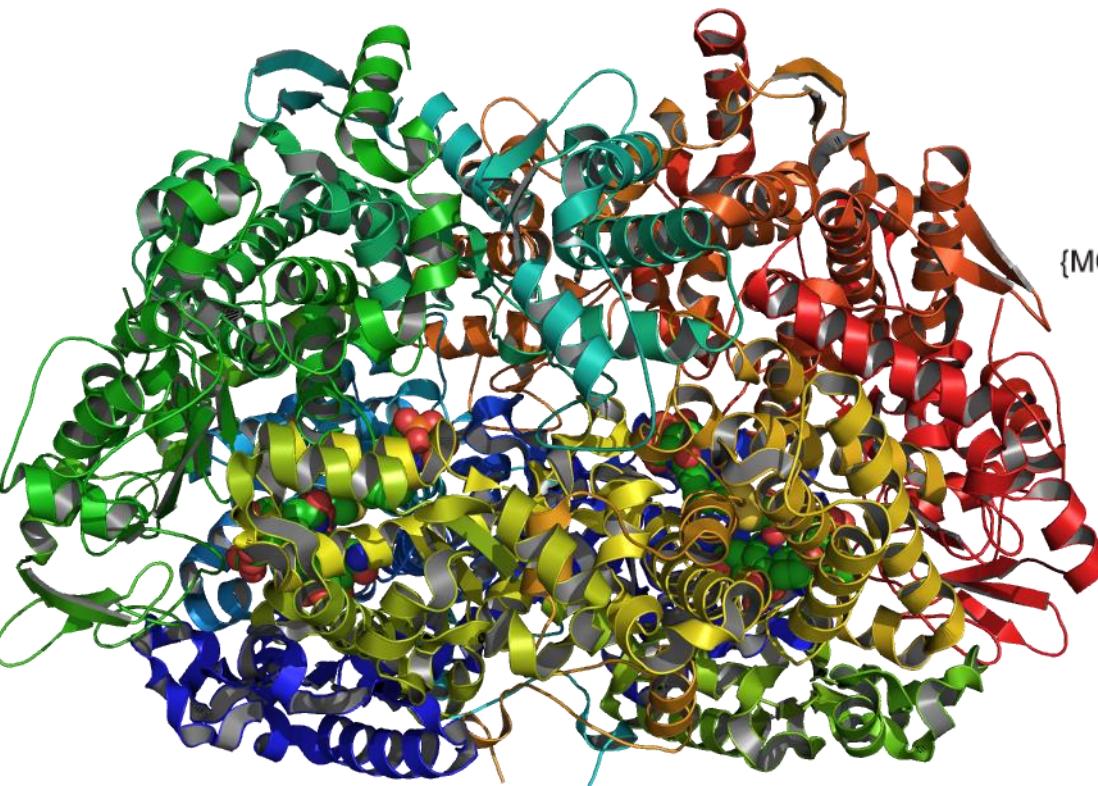


Methyl-CoM Reductase (MCR)

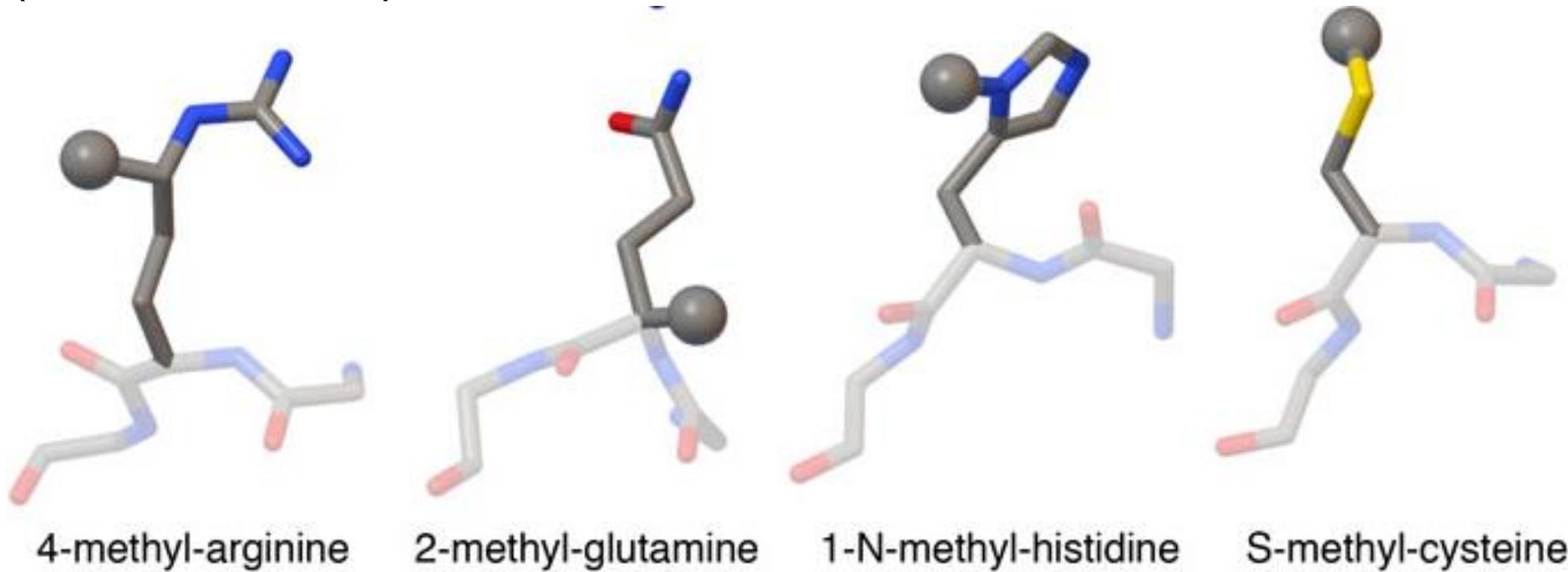


- Reduces $\text{CH}_3\text{-SCoM}$ to give CH_4 (>1 b. tons/year).
- Contains an **unique Ni cofactor**

F₄₃₀

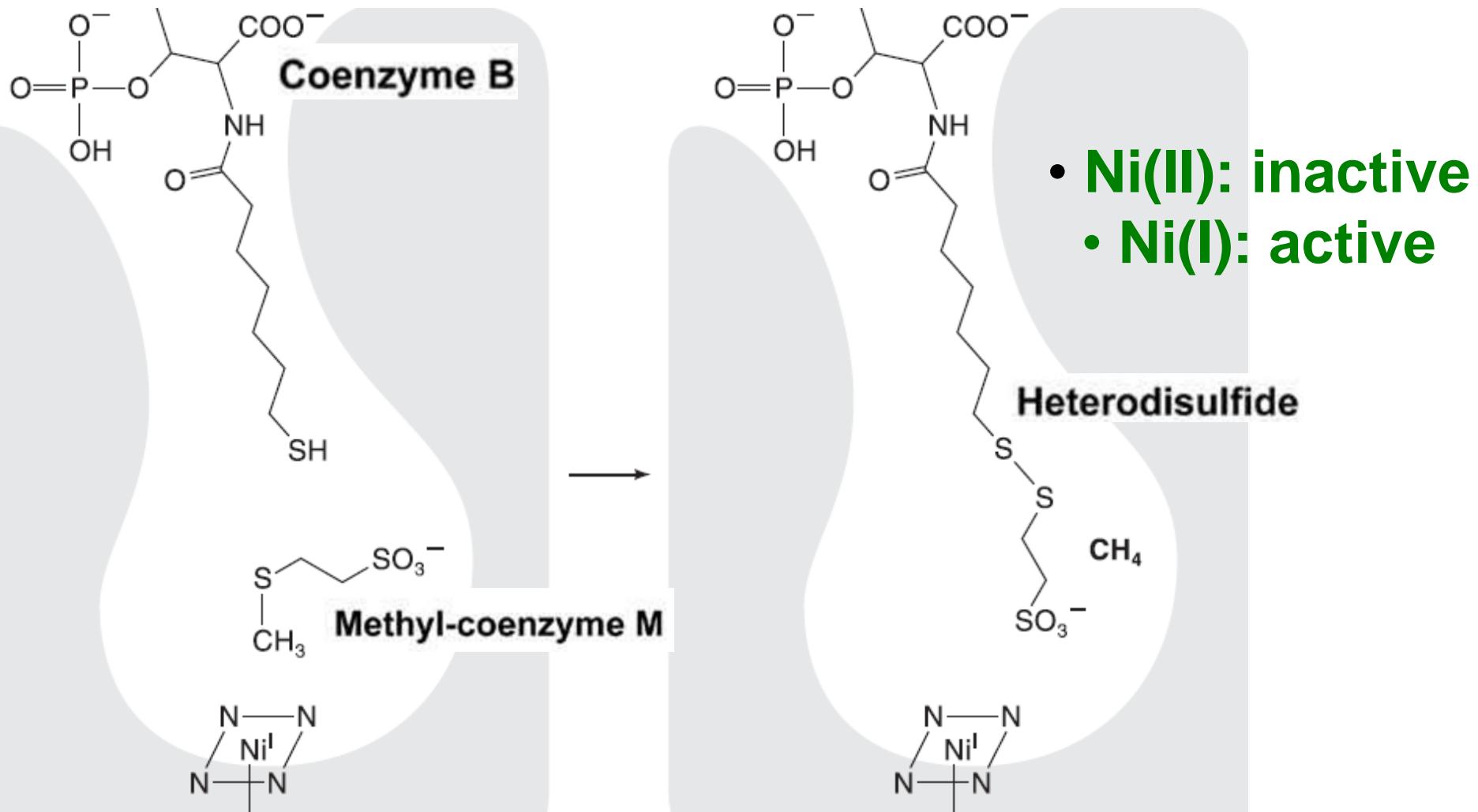


- 5 modified amino acids around the active site in the crystal structure: 4-(S)-CH₃Arg271, 2-CH₃Gln400, 1-N-CH₃His257, S-CH₃Cys452 & unusually, thioglycine445 (C=O → C=S).



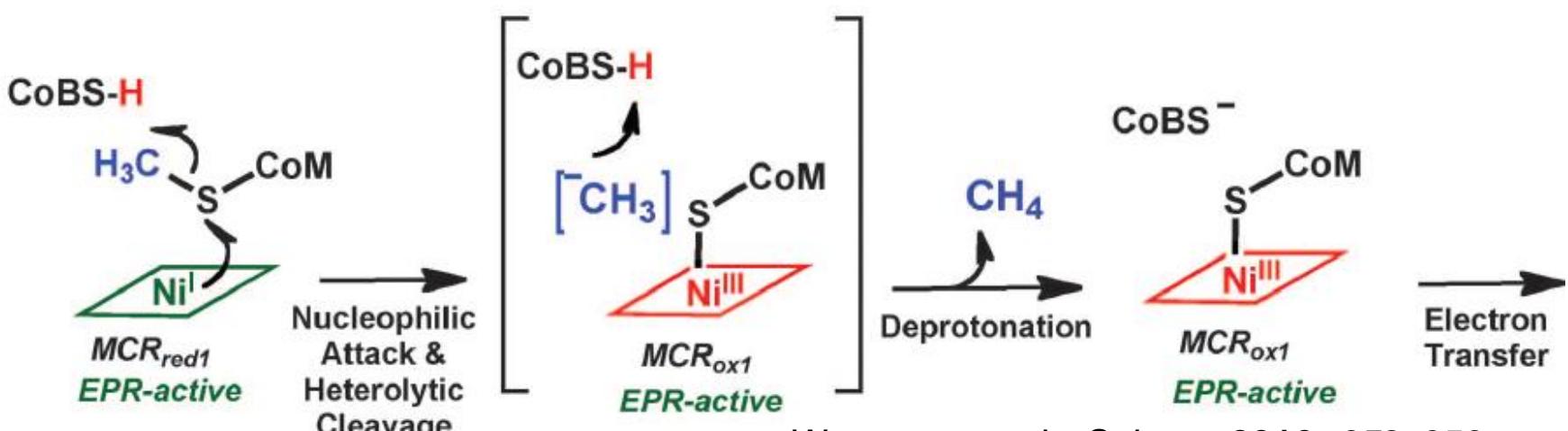
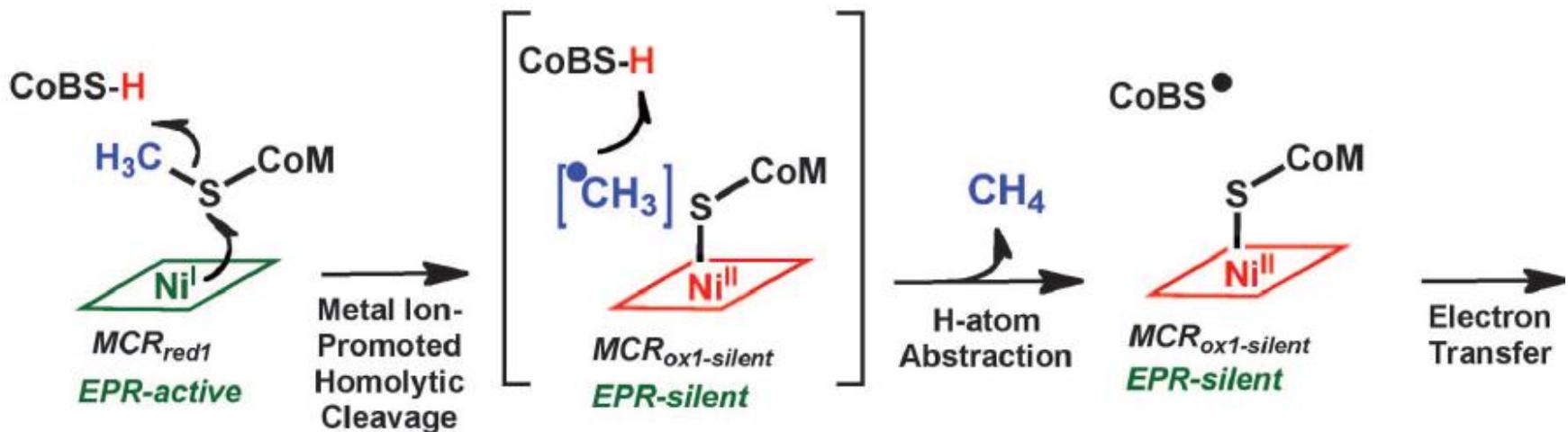
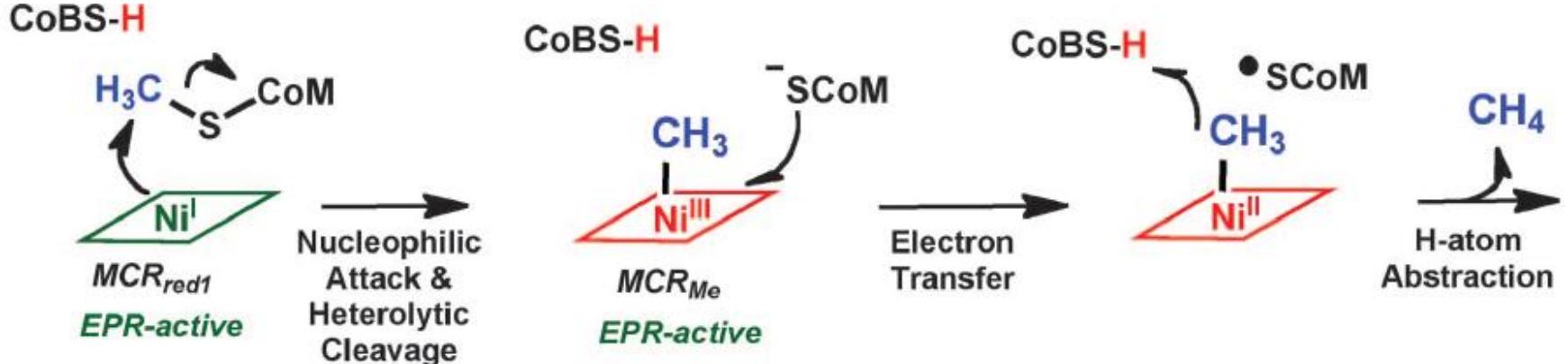
- These CH₃ groups are derived from the Me group of Met through S-adenosyl-methionine (SAM) dependent post-translational or co-translational modifications.

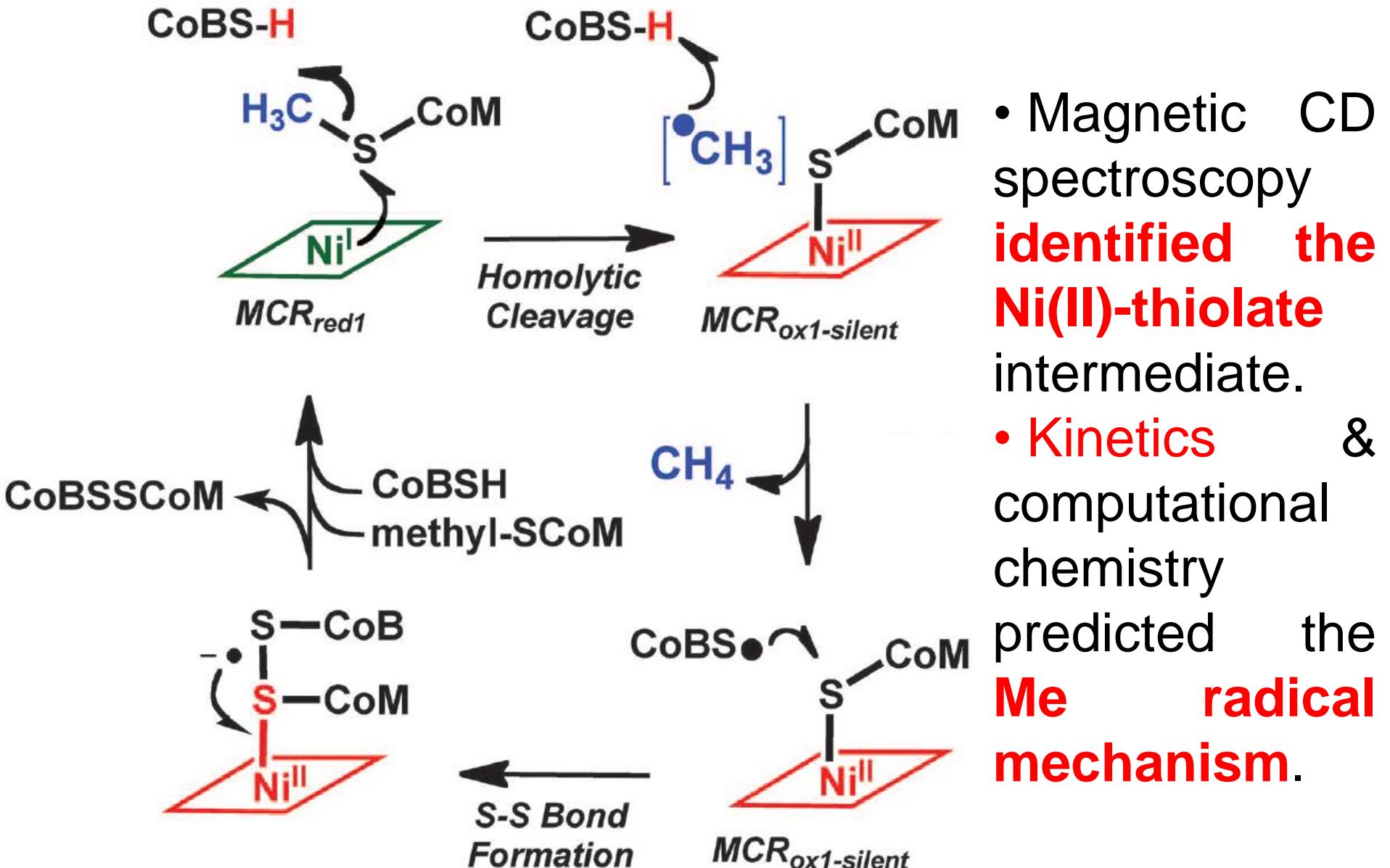
Proposed Mechanisms



- The active site has a narrow long (~30Å) channel.

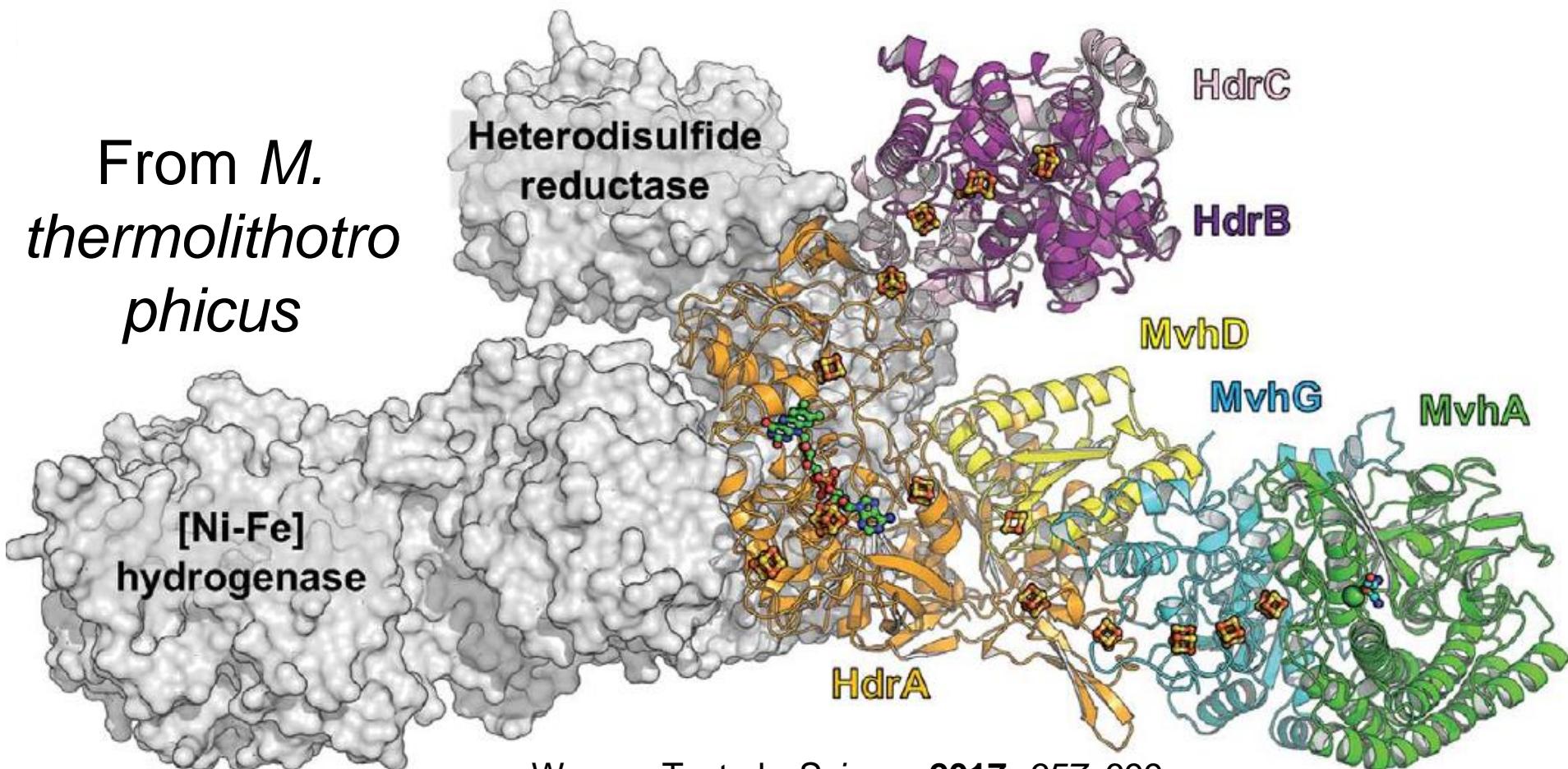
- **Ni(II): inactive**
- **Ni(I): active**

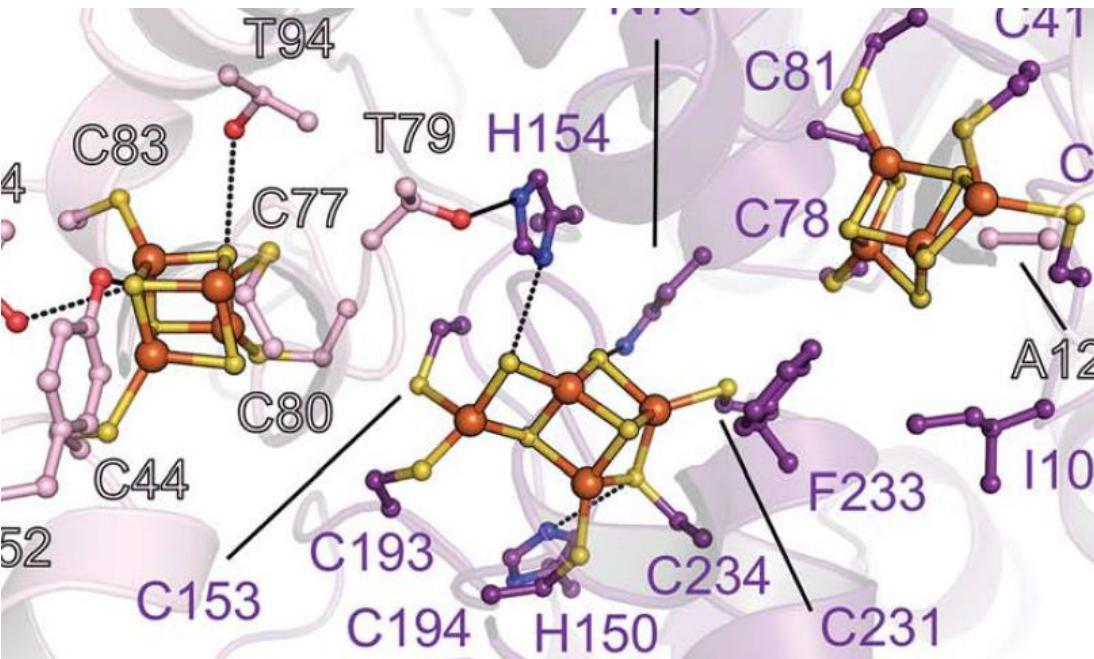




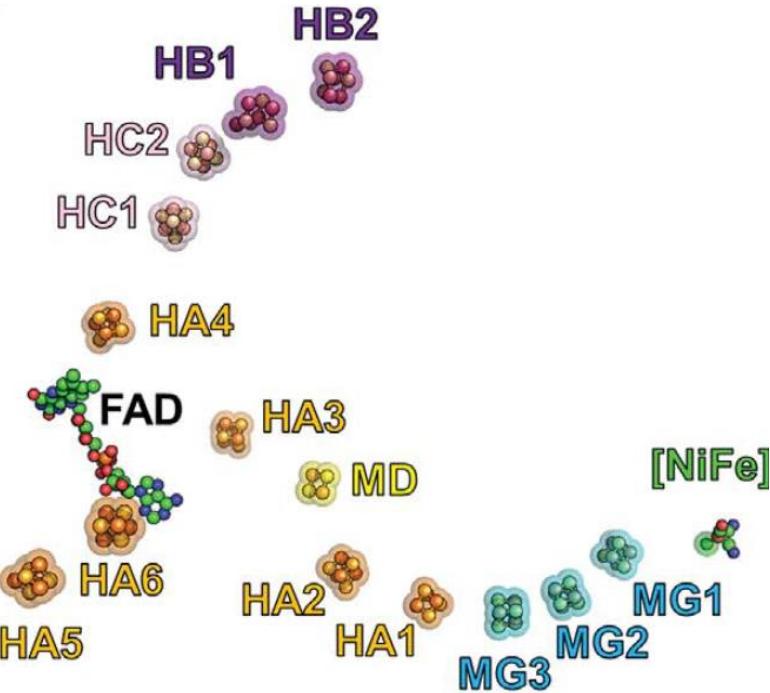
Heterodisulfide Reductase (HDR)

- This Fe-S enzyme catalyzes the reduction of the heterodisulfide (CoM-S-S-CoB) of CoM & CoB:

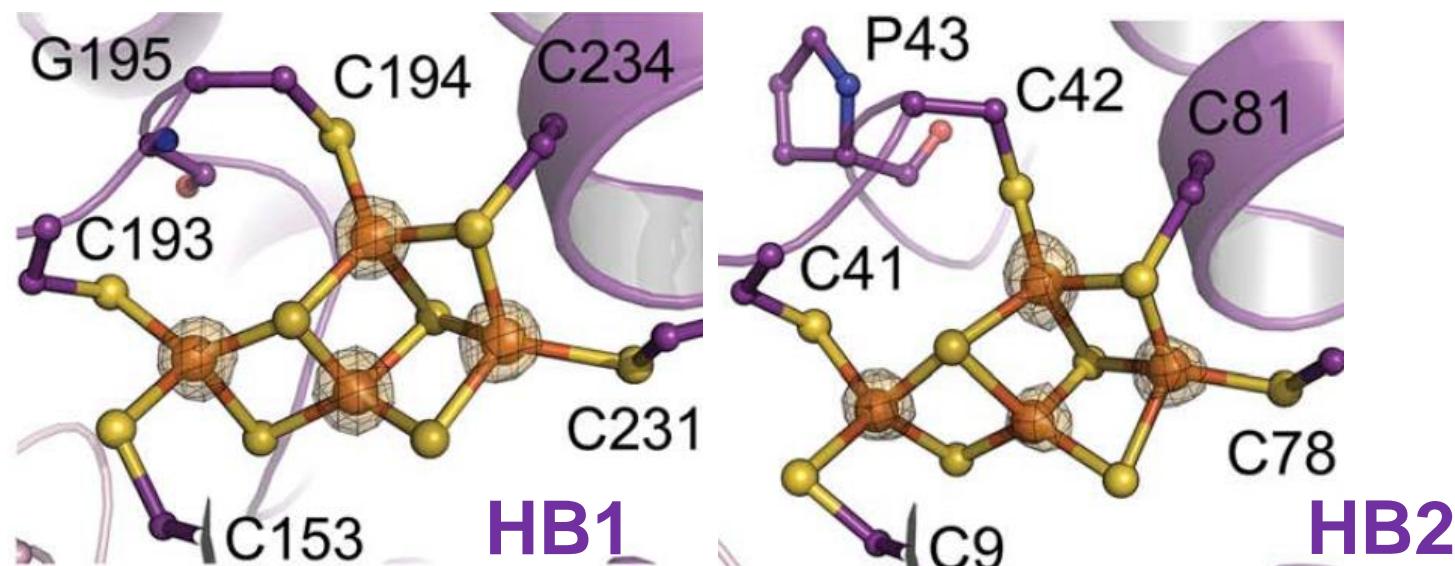




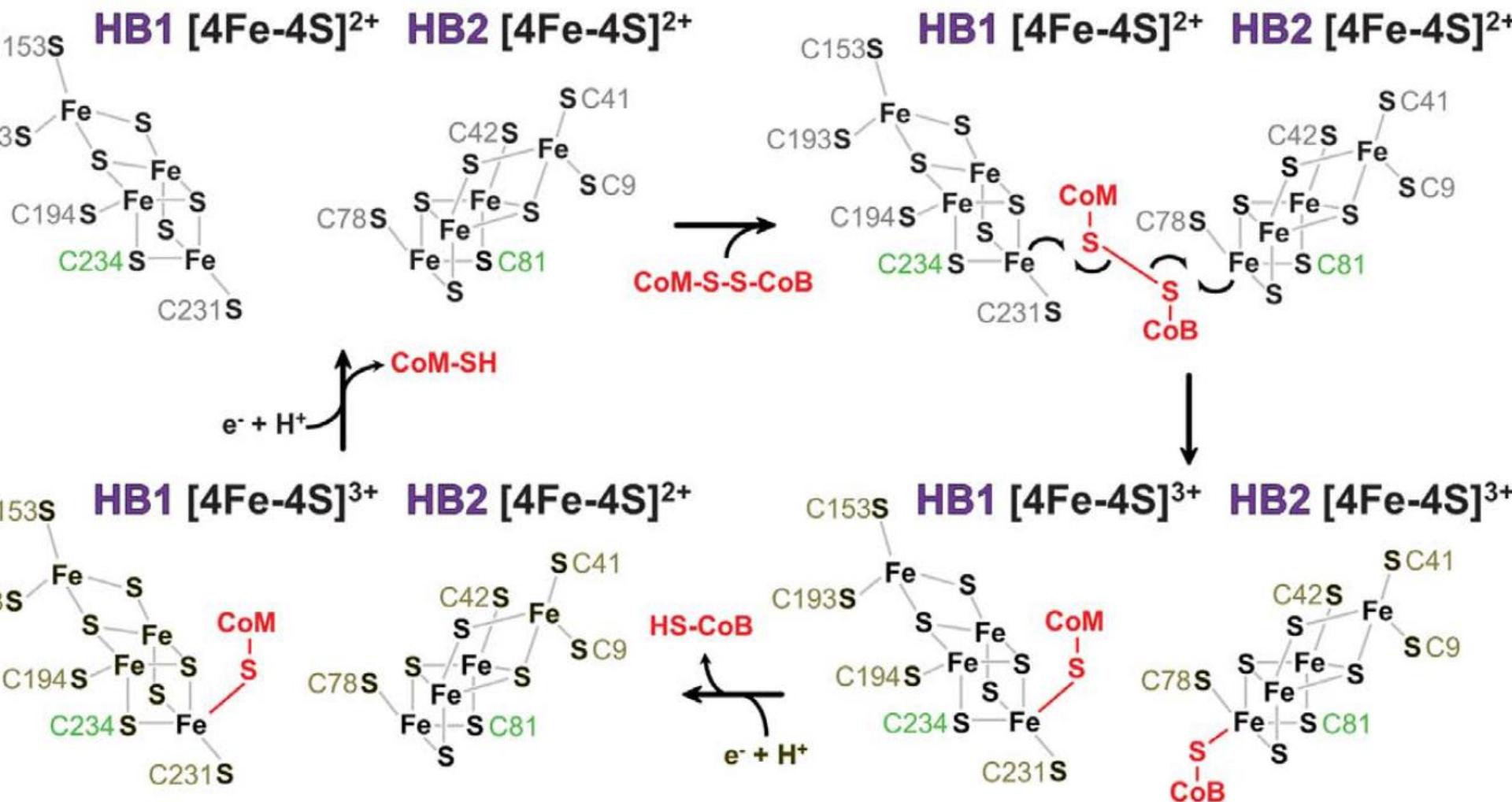
Redox cofactors



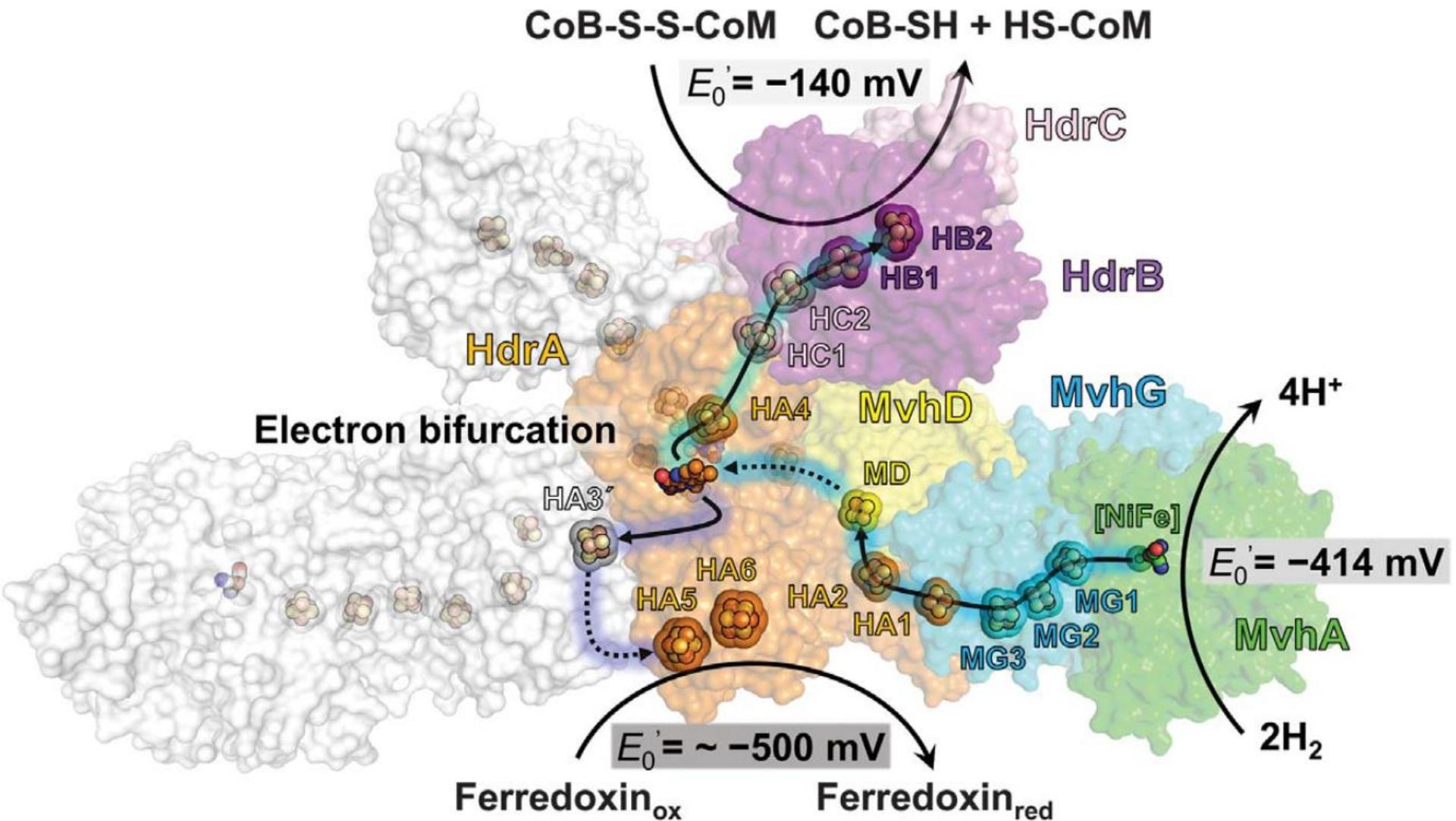
**2 Noncubane [4Fe4S]
clusters in active site (HdrB)**

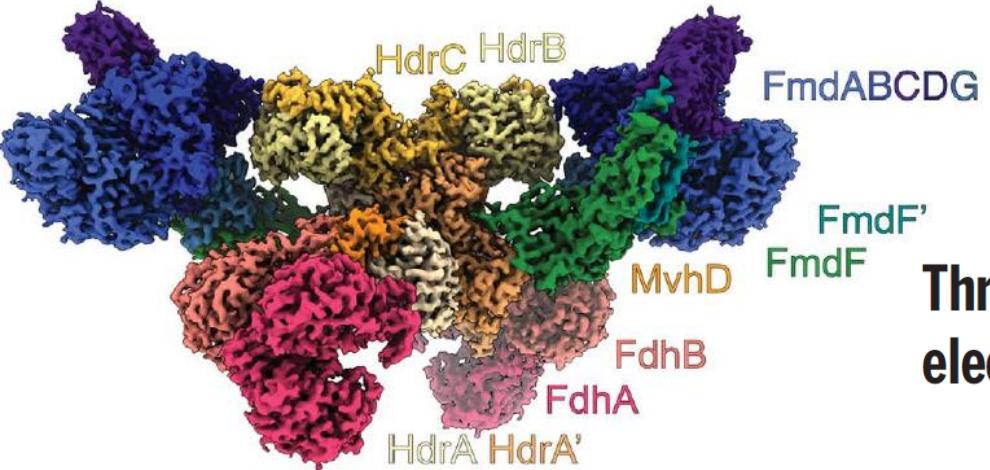


Proposed Mechanism



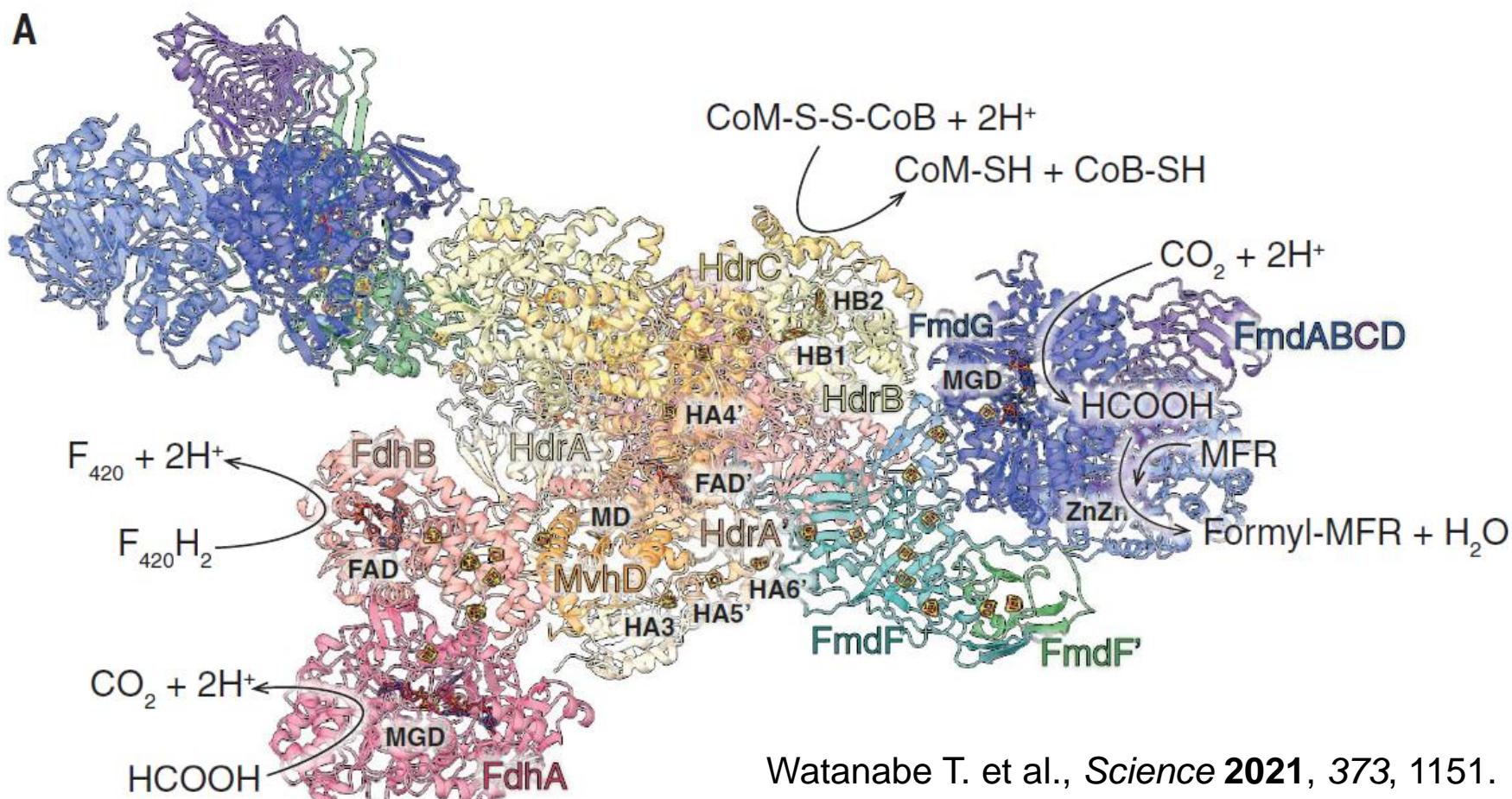
Proposed RT Pathway



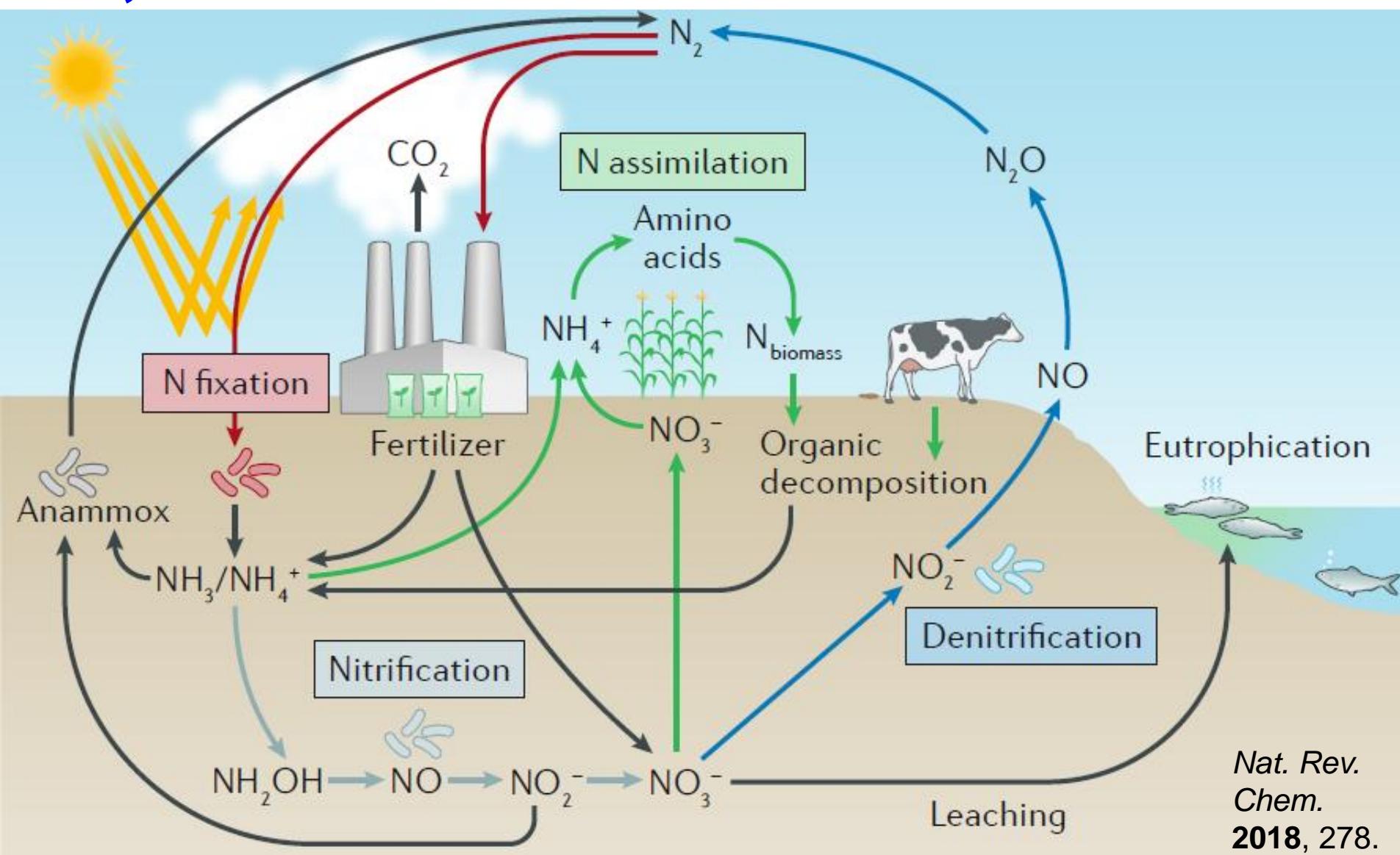


FDH-HDR-FMD Complex (cryo-EM)

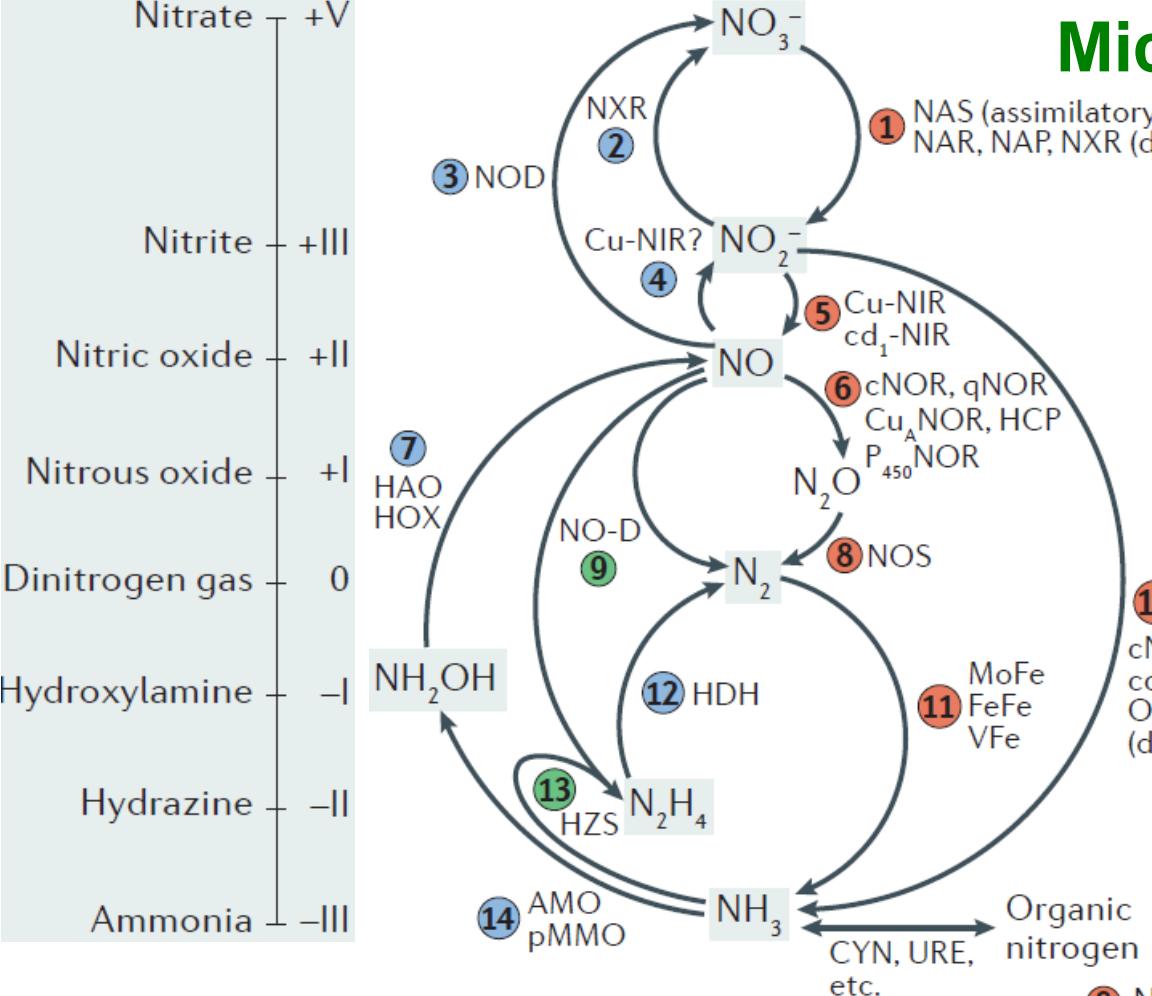
Three-megadalton complex of methanogenic electron-bifurcating and CO₂-fixing enzymes



3. Nitrogen Metabolism: N₂ Fixation, Nitrification & Denitrification



Microbial transformations of N compounds



- ① $\text{NO}_3^- + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$
- ② $\text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + 2\text{e}^- + 2\text{H}^+$
- ③ $\text{NO} + 2\text{H}_2\text{O} \rightarrow \text{NO}_3^- + 3\text{e}^- + 4\text{H}^+$
- ④ $\text{NO} + \text{H}_2\text{O} \rightarrow \text{NO}_2^- + \text{e}^- + 2\text{H}^+$
- ⑤ $\text{NO}_2^- + \text{e}^- + 2\text{H}^+ \rightarrow \text{NO} + \text{H}_2\text{O}$
- ⑥ $2\text{NO} + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O}$
- ⑦ $\text{NH}_2\text{OH} \rightarrow \text{NO} + 3\text{e}^- + 3\text{H}^+$

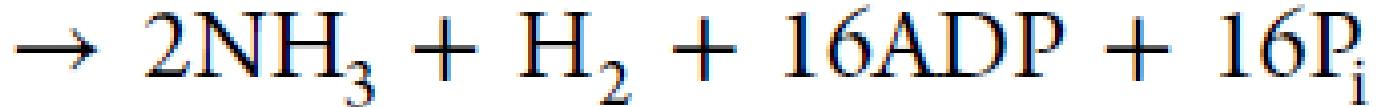
Nat. Rev. Microbio,
2018, 16, 263.

AMO: Ammonia Monooxygenase;
HAO: Hydroxylamine Oxido-reductase; **NaR:** Nitrate Reductase;
NiR: Nitrite Reductase; **NoR:** Nitric Oxide Reductase, **NoS:** Nitrous Oxide Reductase

- ⑧ $\text{N}_2\text{O} + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{N}_2 + \text{H}_2\text{O}$
- ⑨ $2\text{NO} \rightarrow \text{N}_2 + \text{O}_2$
- ⑩ $\text{NO}_2^- + 6\text{e}^- + 8\text{H}^+ \rightarrow \text{NH}_4^+ + 2\text{H}_2\text{O}$
- ⑪ $\text{N}_2 + 8\text{e}^- + 8\text{H}^+ + 16\text{ATP} \rightarrow 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{P}_i$
- ⑫ $\text{N}_2\text{H}_4 \rightarrow \text{N}_2 + 4\text{e}^- + 4\text{H}^+$
- ⑬ $\text{NO} + \text{NH}_4^+ + 3\text{e}^- + 2\text{H}^+ \rightarrow \text{N}_2\text{H}_4 + \text{H}_2\text{O}$
- ⑭ $\text{NH}_4^+ + \text{O}_2 + 2\text{e}^- + \text{H}^+ \rightarrow \text{NH}_2\text{OH} + \text{H}_2\text{O}$

(3A) Nitrogen Fixation: Nitrogenase

- Nitrogen: molecules of life of all organisms, e.g. DNA, RNA & proteins.
- **Biological N₂ fixation**: transform atmospheric **N₂ to NH₃**; whereas **nitrification plus denitrification** convert nitrogen-compounds back to the atmosphere (e.g. N₂).
- Biological N₂: ~60% of total annual fixation.



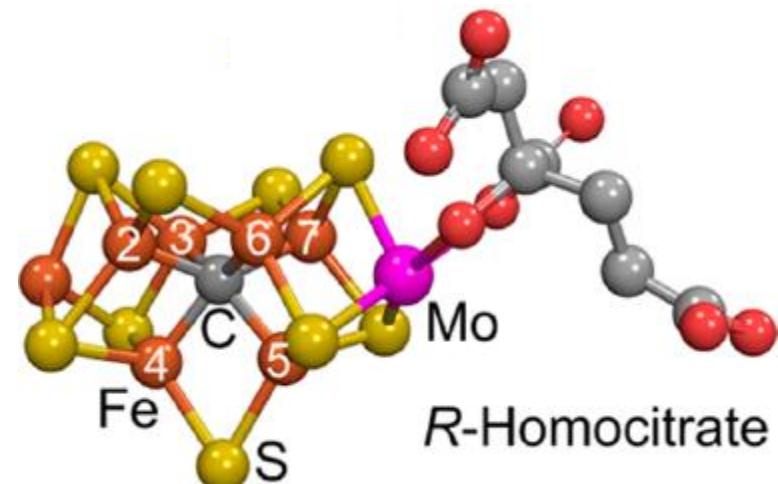
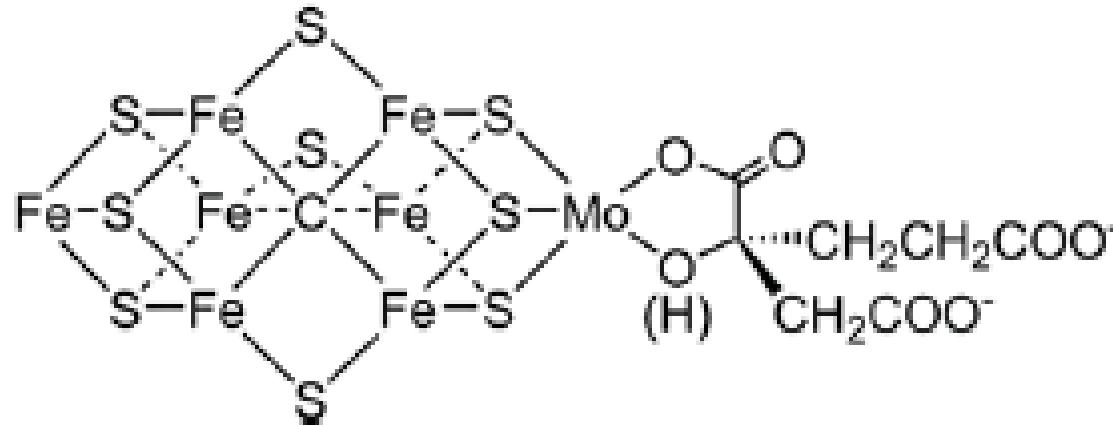
- Industrial N₂ fixation (Haber process):



- Four known types of nitrogenases:

 1. Mo-Nitrogenase (**Mo, Fe, S**)
 2. V-Nitrogenase (**V, Fe, S**)
 3. Fe-only Nitrogenase (**Fe, S**)
 4. *Streptomyces thermoautotrophicus* Nitrogenase

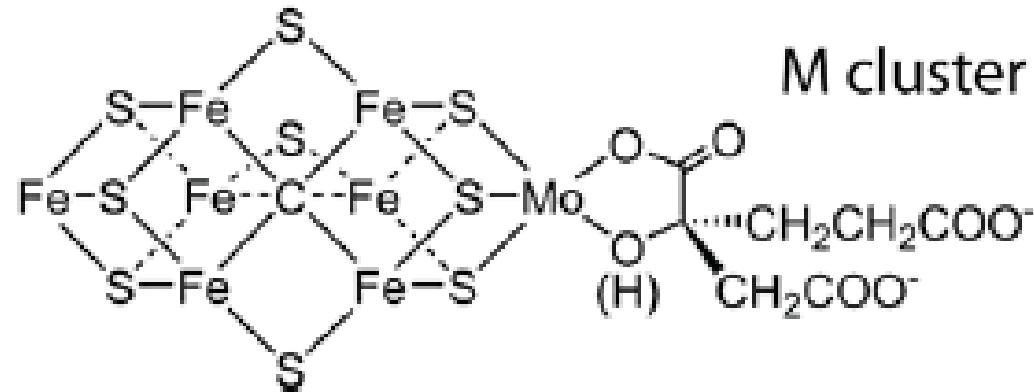
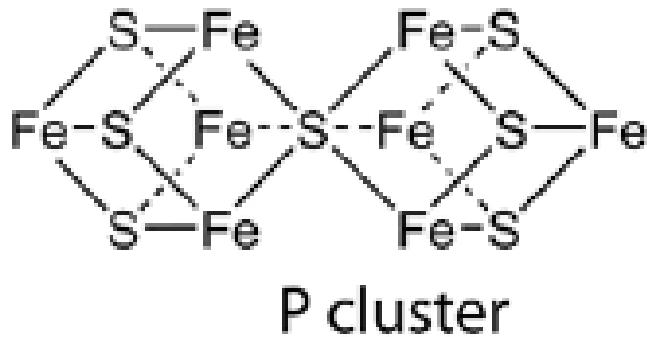
- The **first 3** types are structurally & functionally **similar** (including mechanism), compared to the 4th type.
- Some organisms have only Mo-nitrogenase, whereas others have all three or two types.

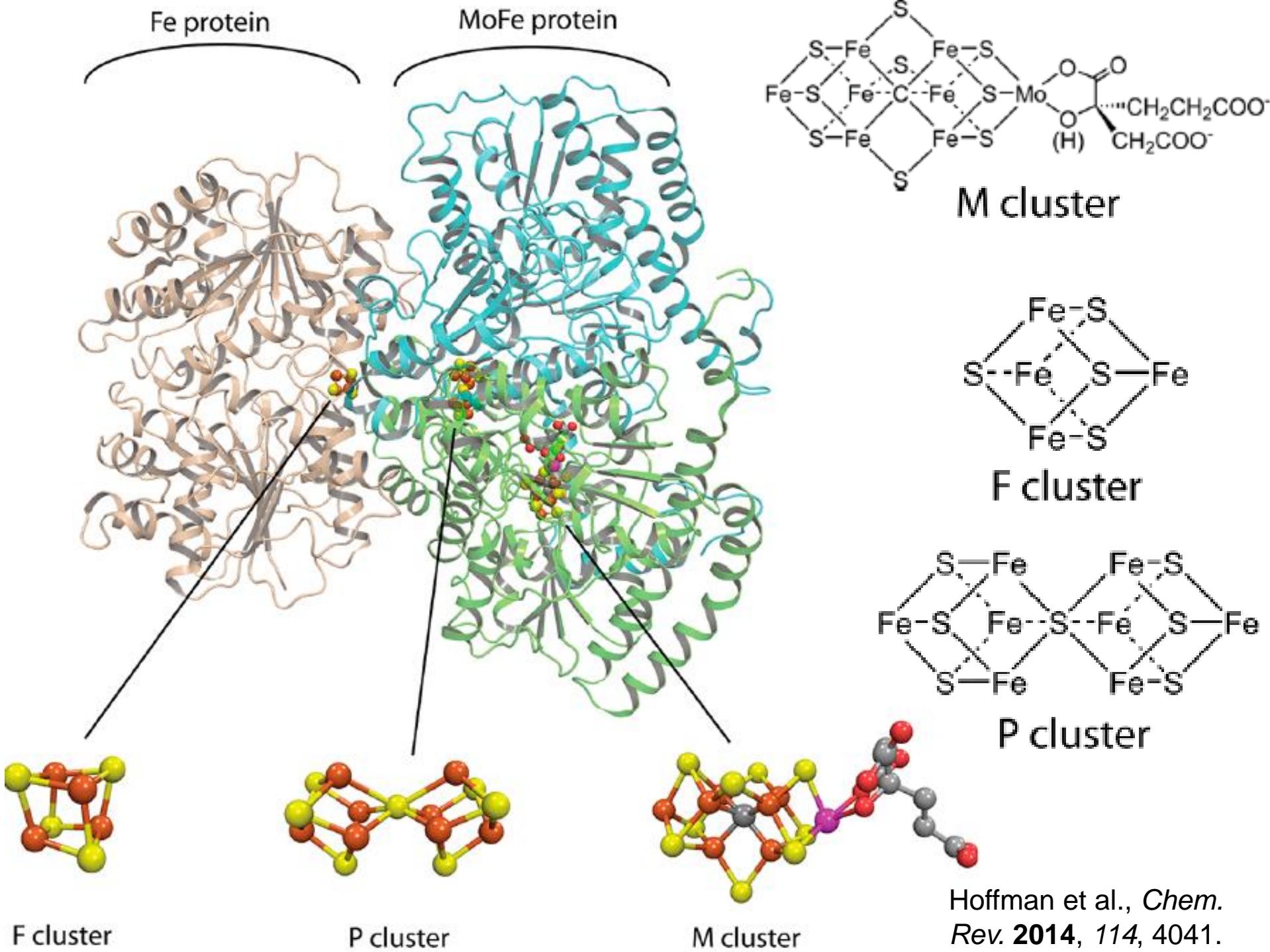


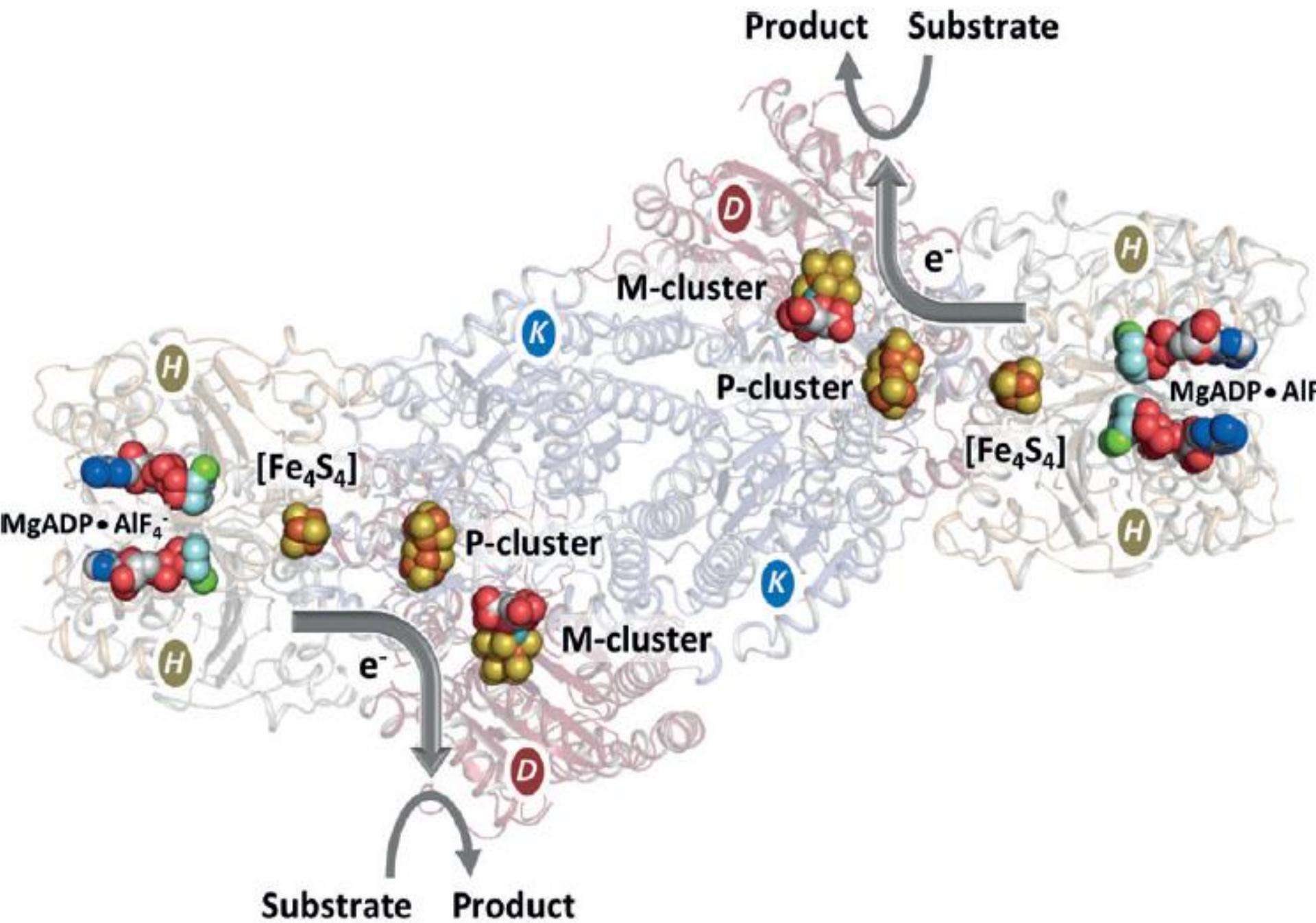
- Availability of the metal ions (either Mo or V) determine expression of the type of nitrogenases:
 - (1) If Mo is available, express the Mo-nitrogenase (& suppress expression of the other two types).
 - (2) else if V is available & Mo is not available, express the V-nitrogenase.
 - (3) else if Mo & V are not available, express the Fe-only nitrogenase.
- Reactivity: *Mo-nitrogenase* > *V-nitrogenase* > *Fe-nitrogenase*. Fe-nitrogenase gives many H₂ gas & less NH₃.

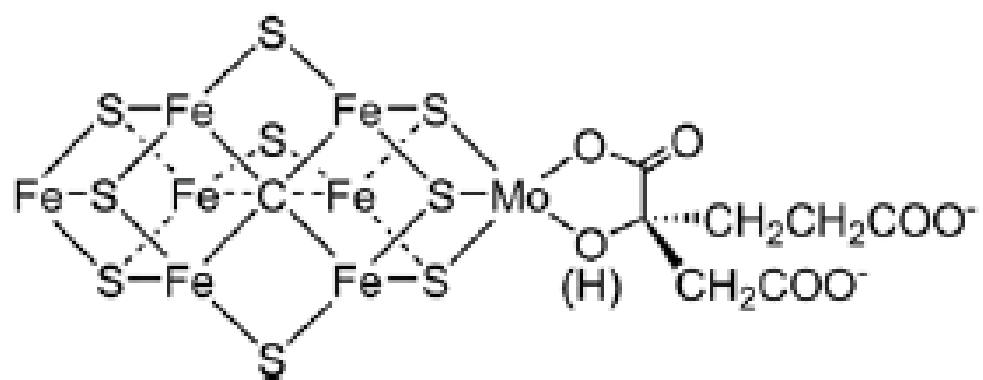
Mo-Nitrogenase

- Consisted of **the Fe protein** (or dinitrogenase reductase) & **the MoFe protein** (or dinitrogenase). The former acts as a reductant for the MoFe protein, which binds & reduces N₂.
- The Fe protein has 2 MgATP binding sites & 1 [4Fe4S] cluster.
- The MoFe protein contains 2 pairs of the **P-cluster** & the **FeMo-cofactor** (or FeMoco or M-cluster), the **most complicated cluster** found in biology.

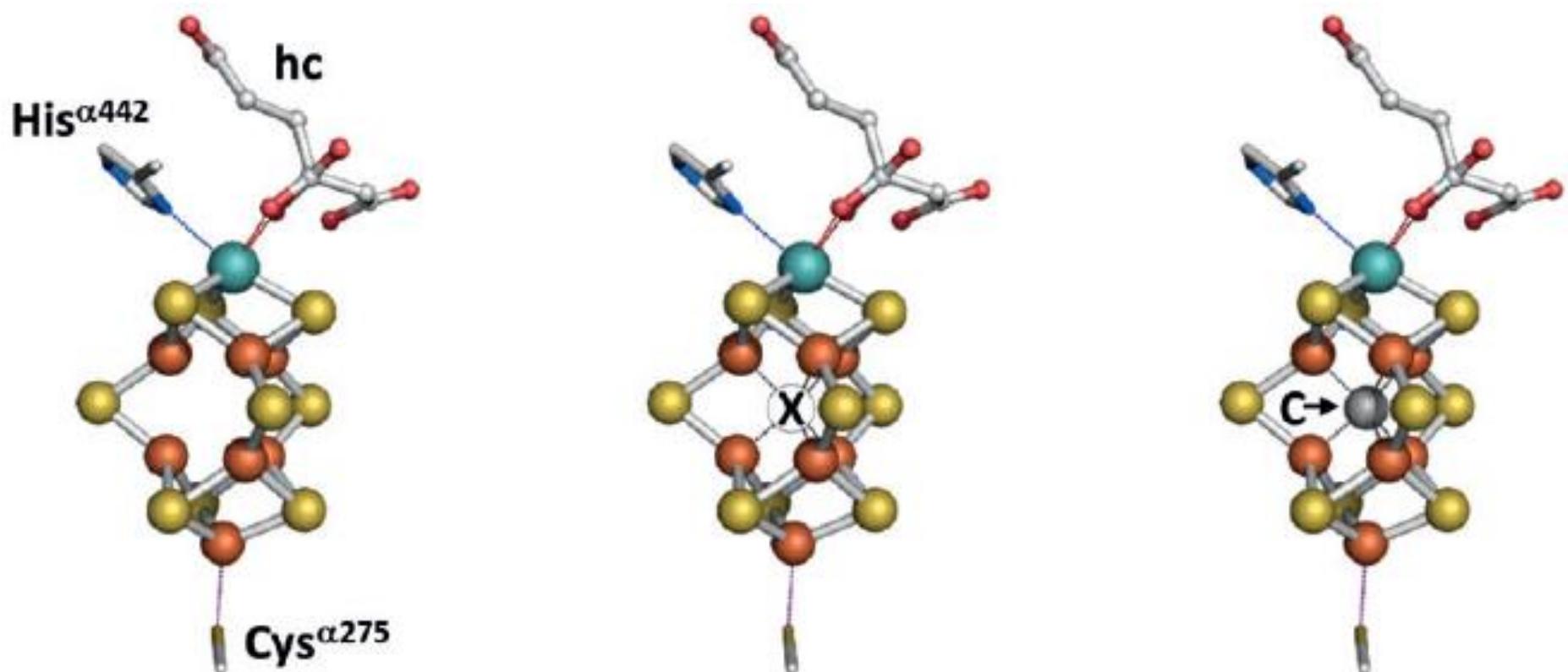








M cluster

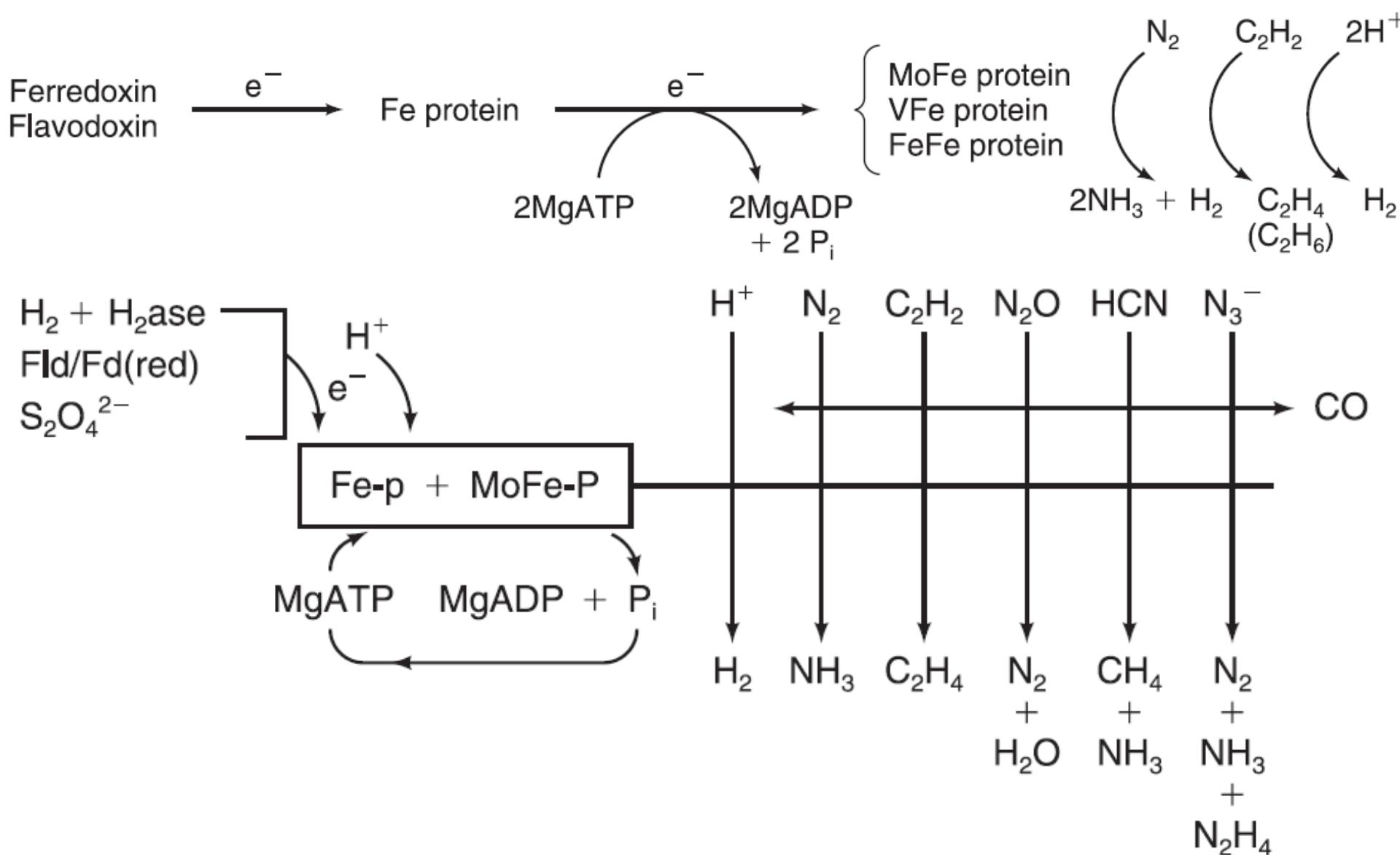


1992

2002

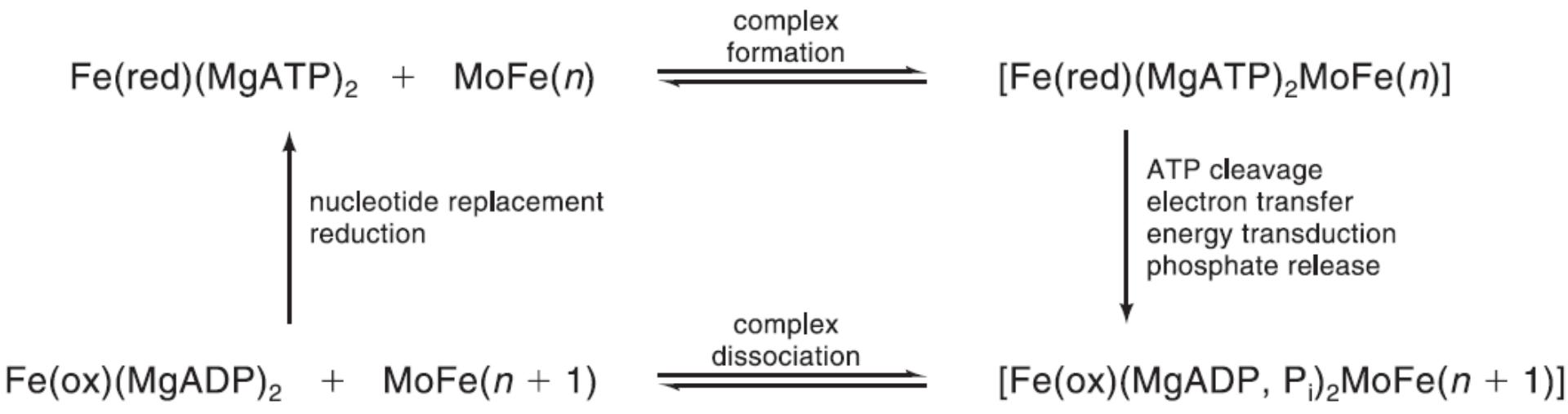
2011

- Also catalyzes reduction of other small molecules with MgATP in an anaerobic environment. CO is a potent inhibitor.



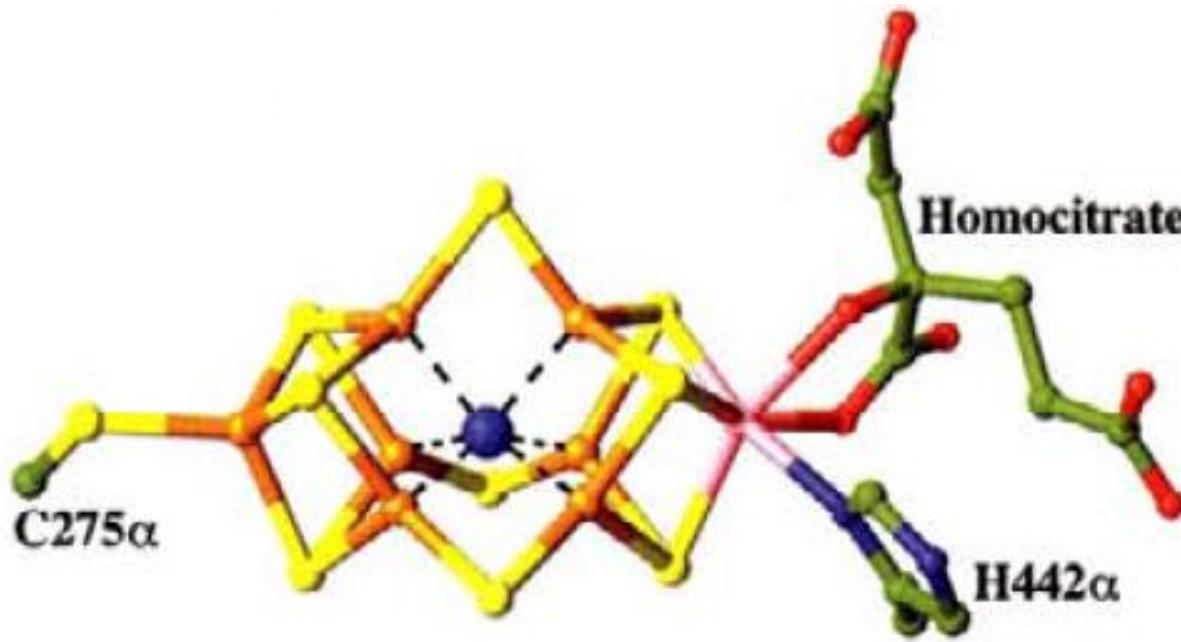
The Fe Protein

- Association of the Fe protein (some conformational change) with the MoFe protein.
- Binding of MgATP induces a significant conformational change.
- Hydrolysis of MgATP induces ET from the [4Fe4S] cluster of the Fe protein to the P cluster & then to the FeMo-cofactor (15 Å away from P-cluster) of the MoFe protein, followed by dissociation of the Fe protein.



The MoFe Protein

- Contains unique **P cluster & FeMo-cofactor**.
- **FeMo-cofactor**: a $[\text{Mo}-\text{Fe}_7-\text{S}_9]$ cluster with an (R) -homocitrate molecule & a **central C atom**. The isolated cofactor is not effective in the N_2 reduction.

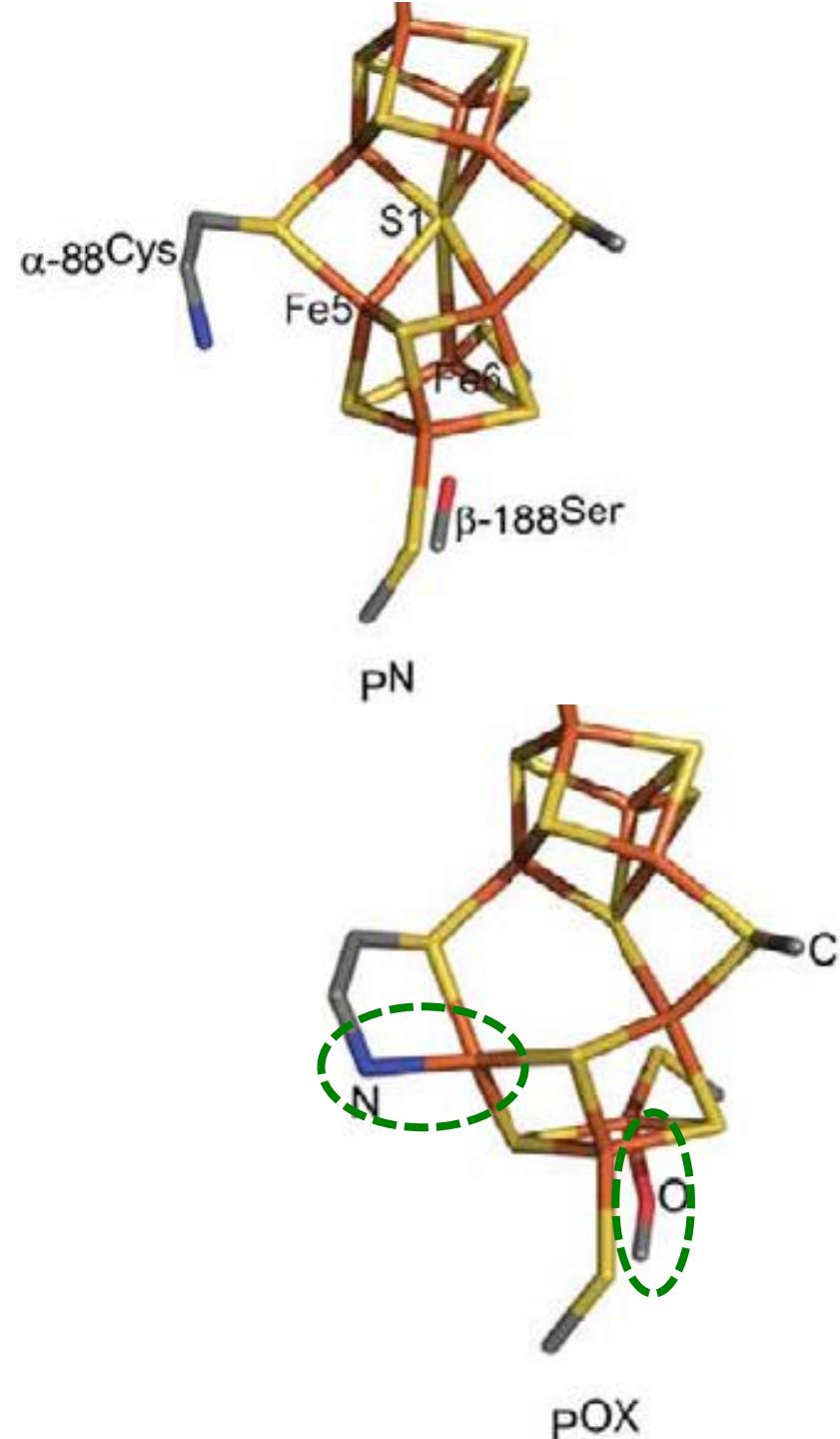


- Unclear how, when, & where $8e^-$ are stored within the MoFe protein.

- P cluster: a $[\text{Fe}_8\text{S}_7]$ cluster.

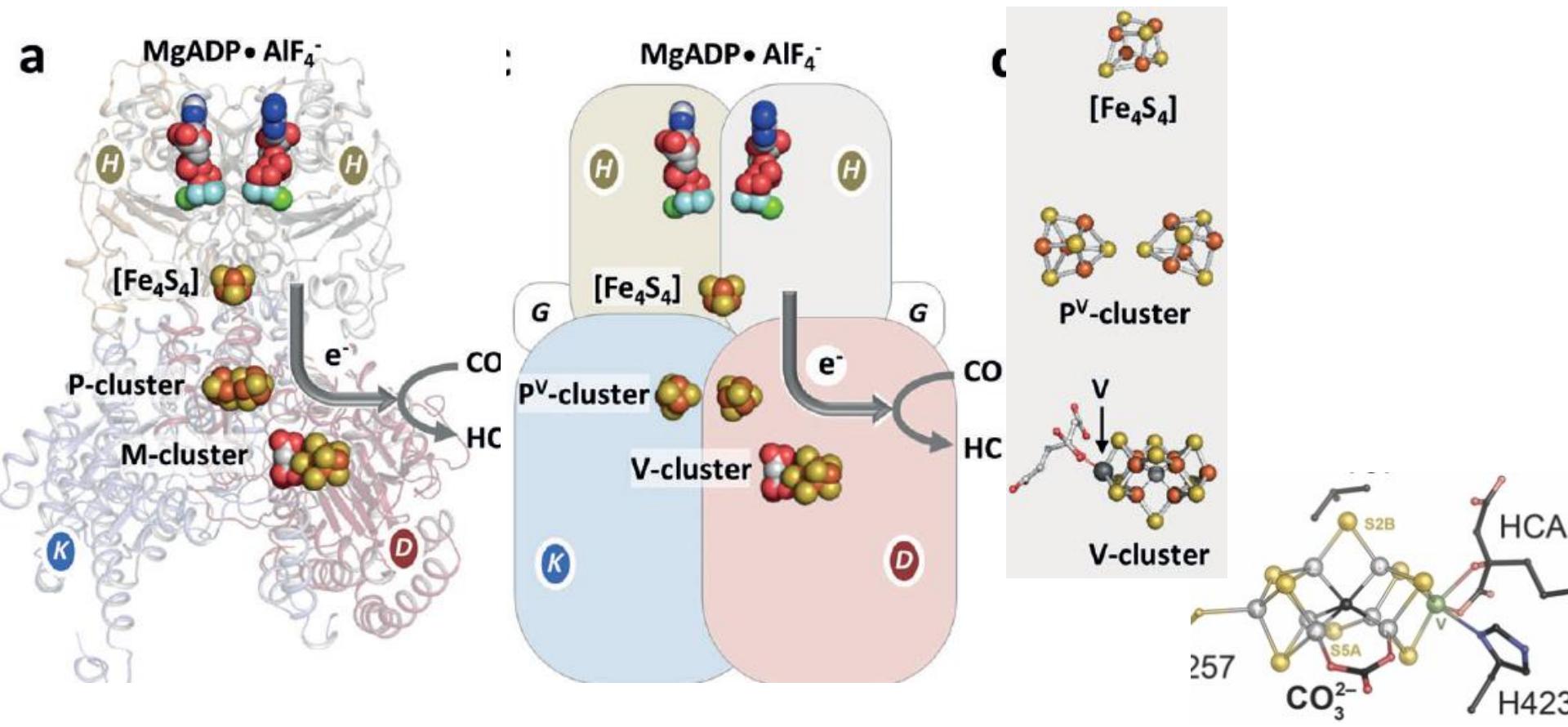
- Upon **oxidation**, 2 of the 4 Fe atoms (Fe5 & Fe6) lose interactions with the **central S** (S1). The Fe6 atom is ligated by **Ser O** & Fe5 atom is ligated by deprotonated backbone **amide-N** of the Cys.

→ **redox-induced ligand changes** of the P-cluster could **release 2 H⁺** from the P cluster.



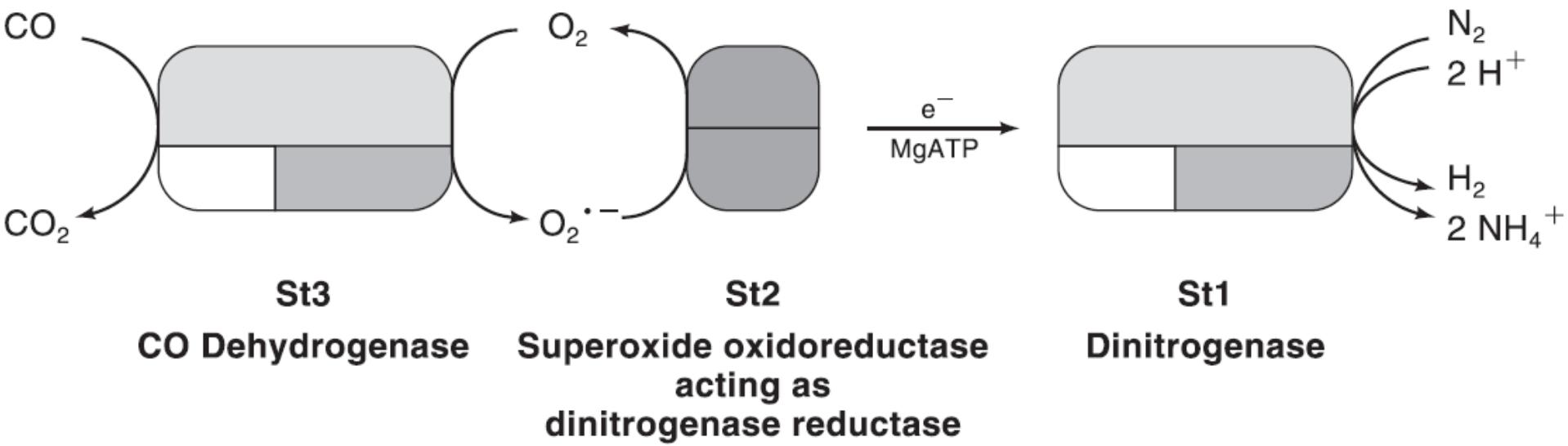
V-Nitrogenase & Fe-only Nitrogenase

- Also consist of the **Fe-protein**. The larger component of the V-nitrogenase has a **VFe-cofactor** (Mo replaced by V).
- The Fe-only nitrogenase has a cofactor in which Mo is replaced by Fe in a **FeFe-cofactor** in a FeFe protein.



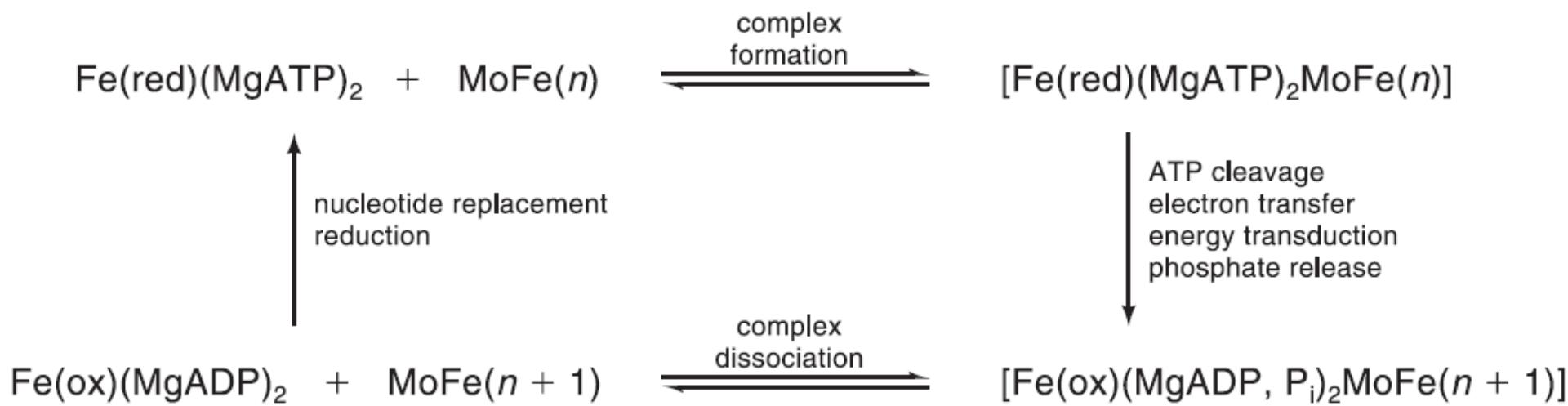
S. thermoautotrophicus Nitrogenase

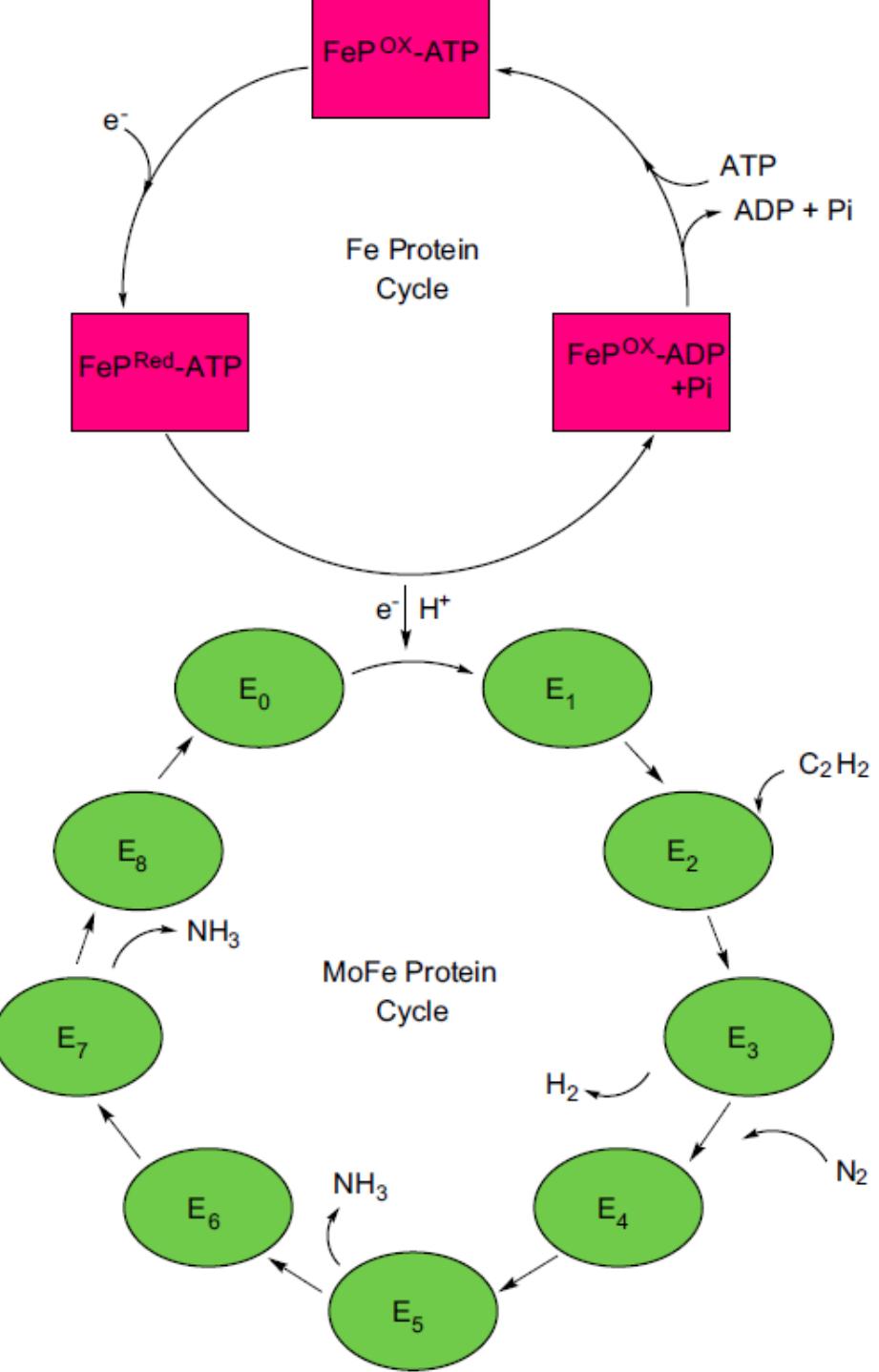
- Unique nitrogenase from *S. thermoautotrophicus*.
- Also contains 2 component proteins (the larger one with Mo, Fe, & S), but **no the Fe-protein**. Instead, a **Mn superoxide oxidoreductase** oxidizes O_2^- to O_2 & transfers e^- to the MoFeS protein.
- Electron donor: a Mo-CODH.



Proposed Mechanisms

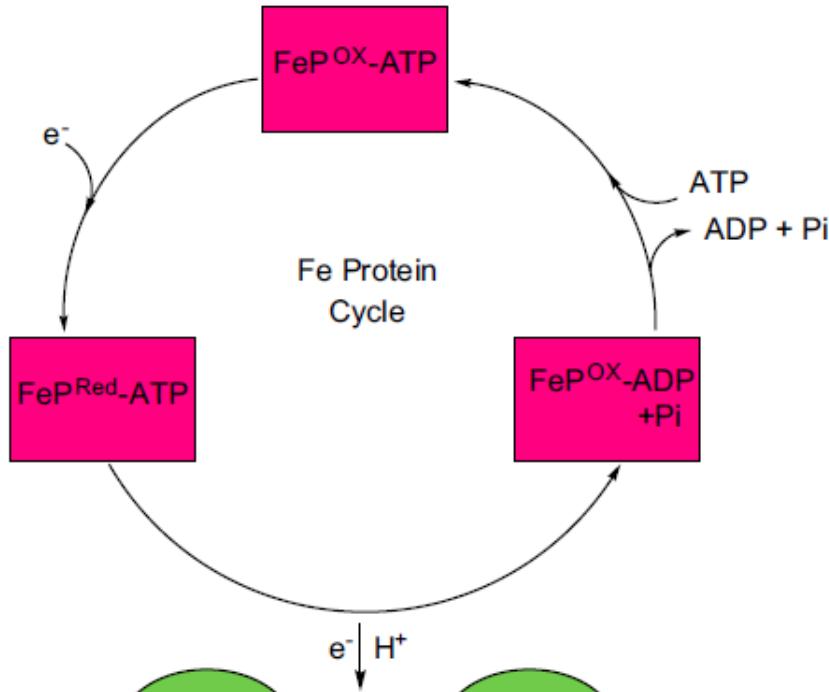
- For catalysis in vitro, the Fe protein **delivers one electron** to the MoFe protein **at one time** coupled with **MgATP binding & hydrolysis**. Both the Fe protein & the MoFe protein are required for the MgATP hydrolysis.
- A computational model (the Lowe-Thorneley (LT) model):



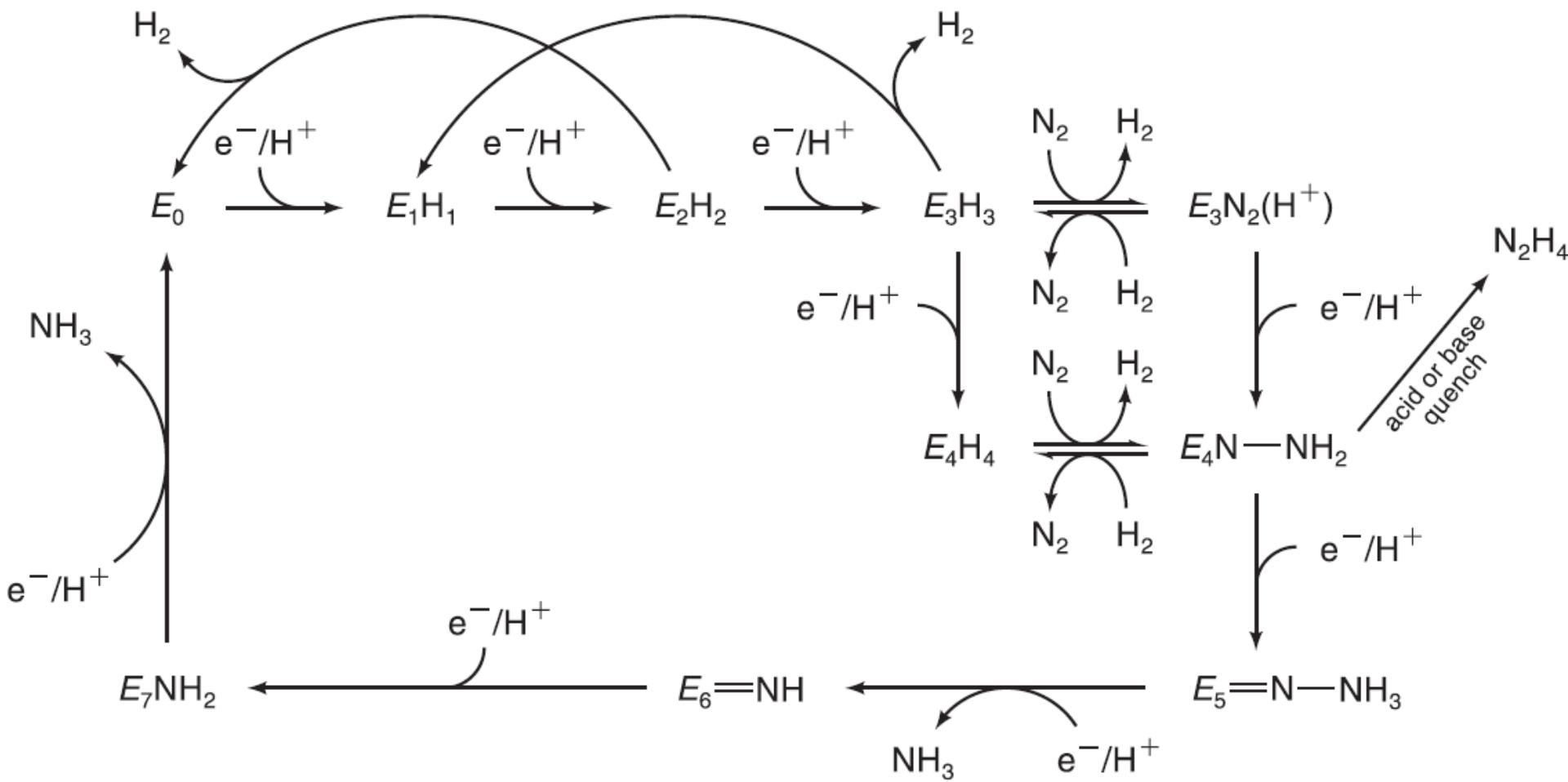


- When **MgATP binds**, the Fe protein undergoes conformational change & **decreases the reduction potential** of its [4Fe4S] cluster (by ~-200 mV to ~-600 mV). The potential of the P cluster is also decreased by ~-100 to ~-400 mV → **larger driving force for ET from the Fe protein to the MoFe protein.**

- The complex MoFe-protein cycle: progressive reduction by 8e⁻ (8 Fe-protein cycle).

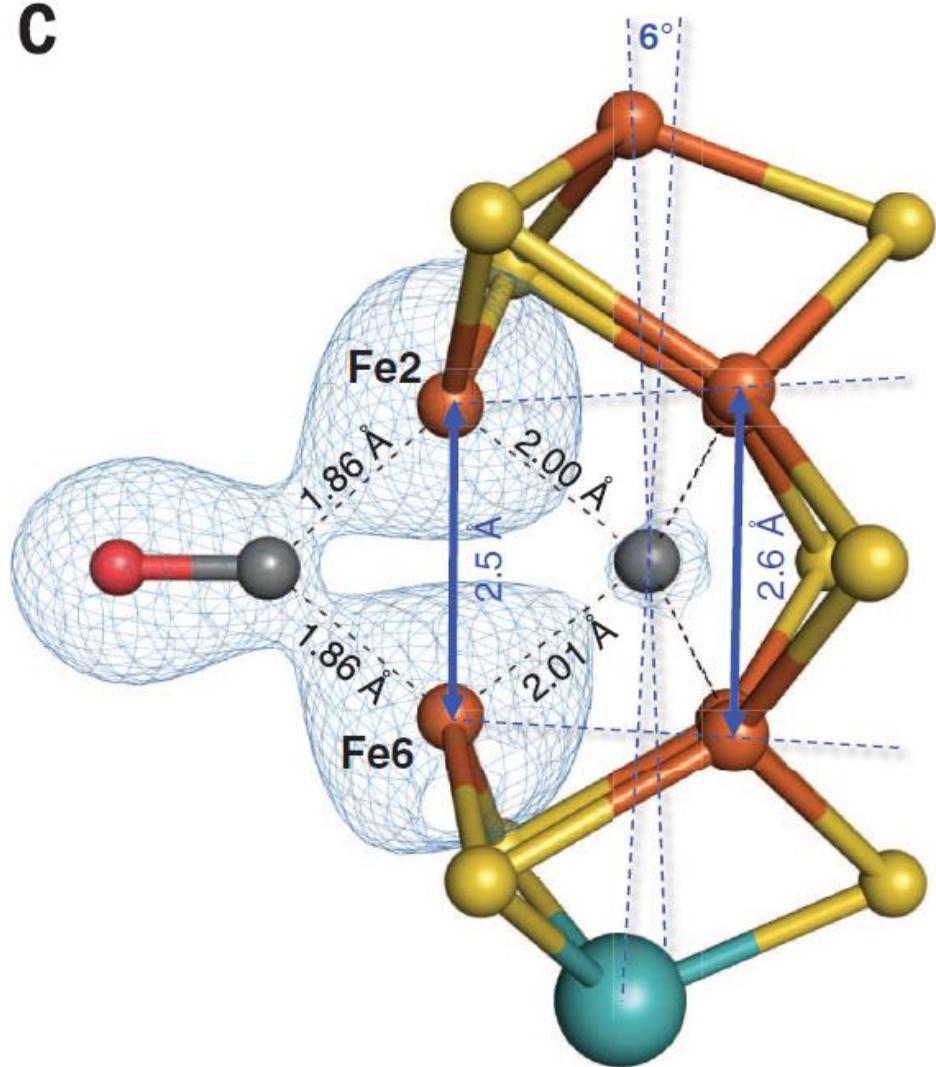


- The MgADP state of the Fe protein induces its conformational change to relax & dissociate.
- So, **MgATP hydrolysis** was proposed to **promote forward ET** to the substrate reduction & to **prevent the backward ET** to the Fe protein.
- The Mo & central Fe atoms: possible substrate binding sites?

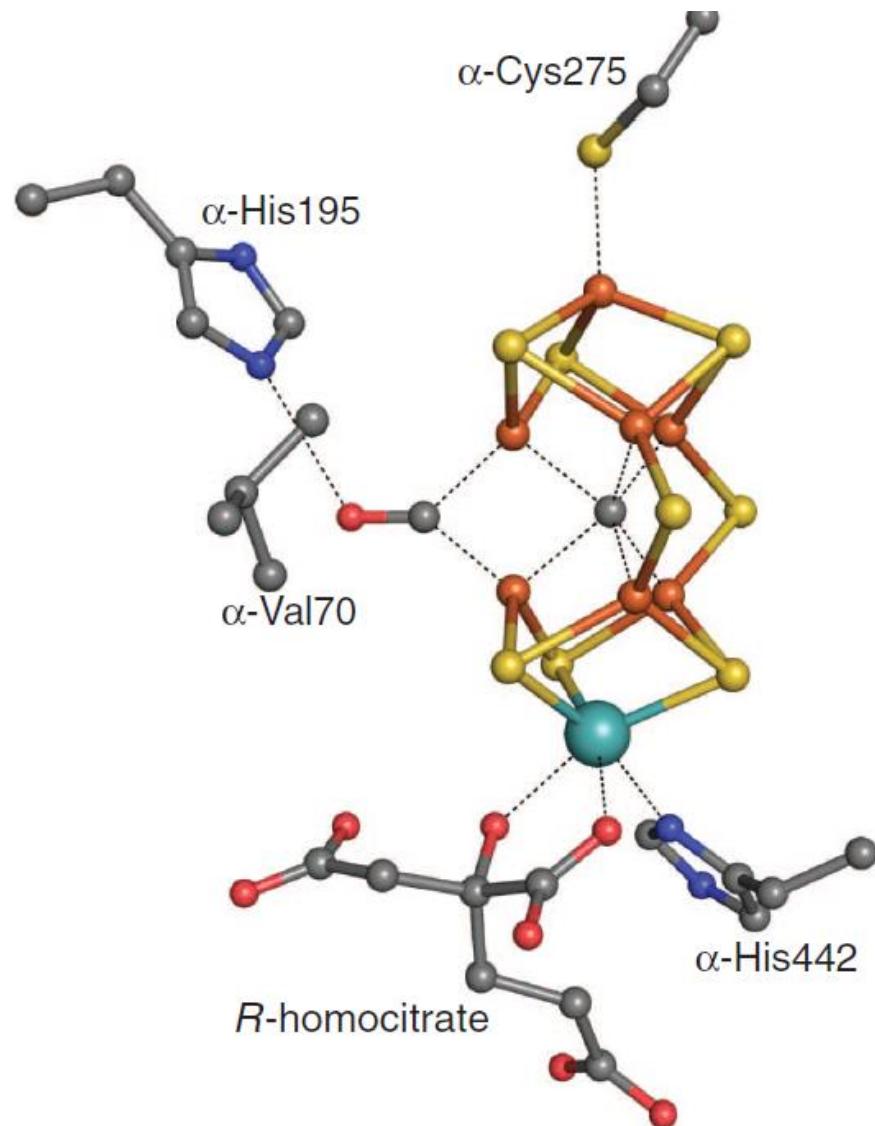


Crystal structure of CO-bound(inhibited) Mo-Nitrogenase

C

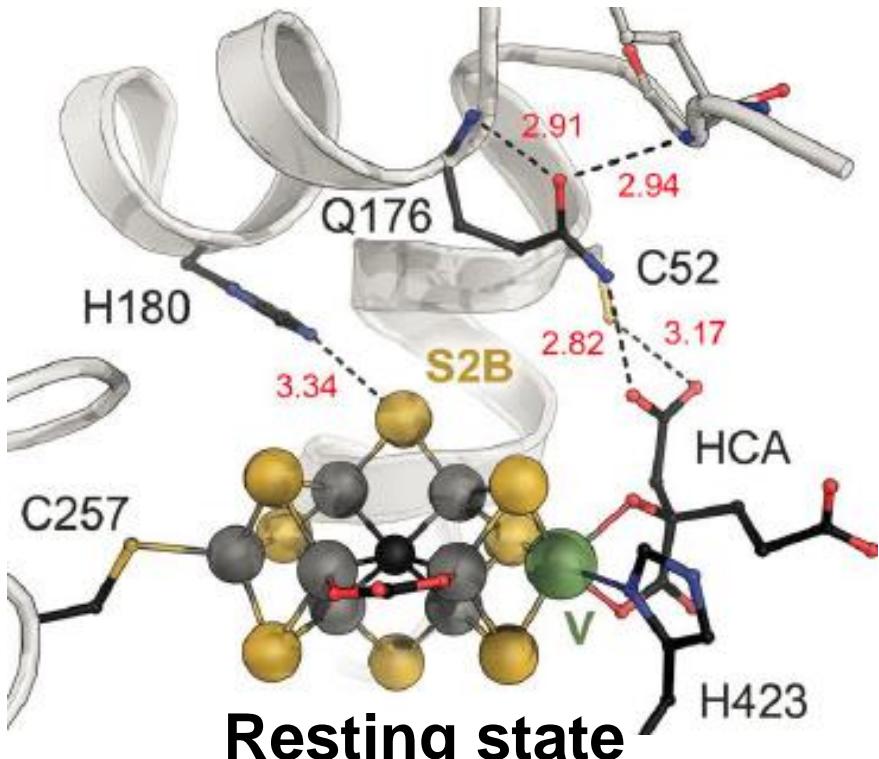


D

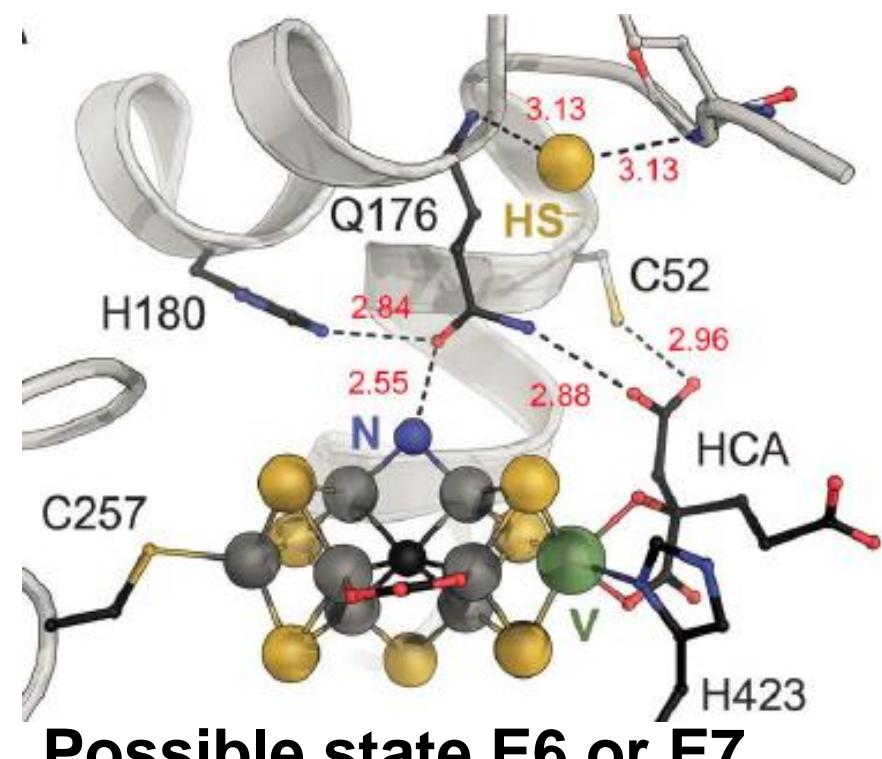


Inactive form; Possible N₂ binding mode

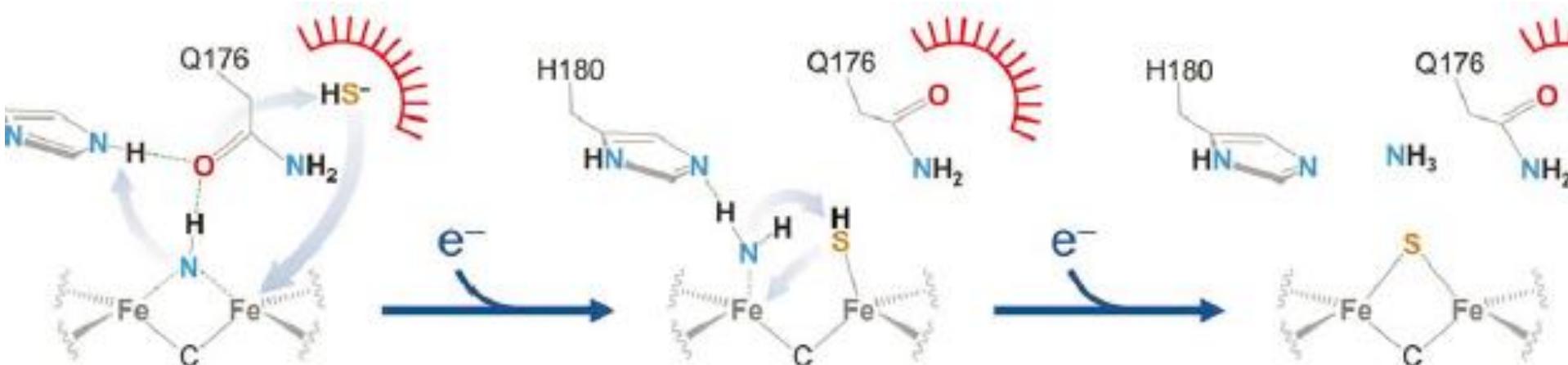
Recent structure of E6 (or E7) in V-Nitrogenase



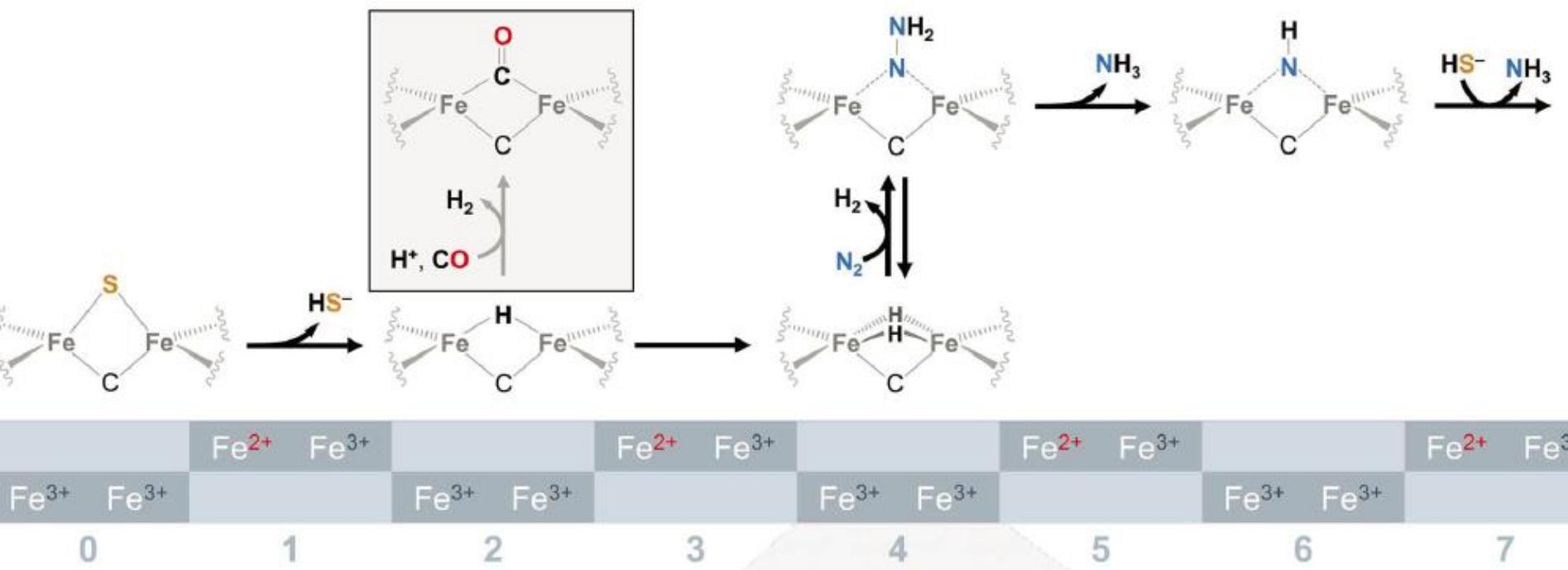
Resting state



Possible state E6 or E7

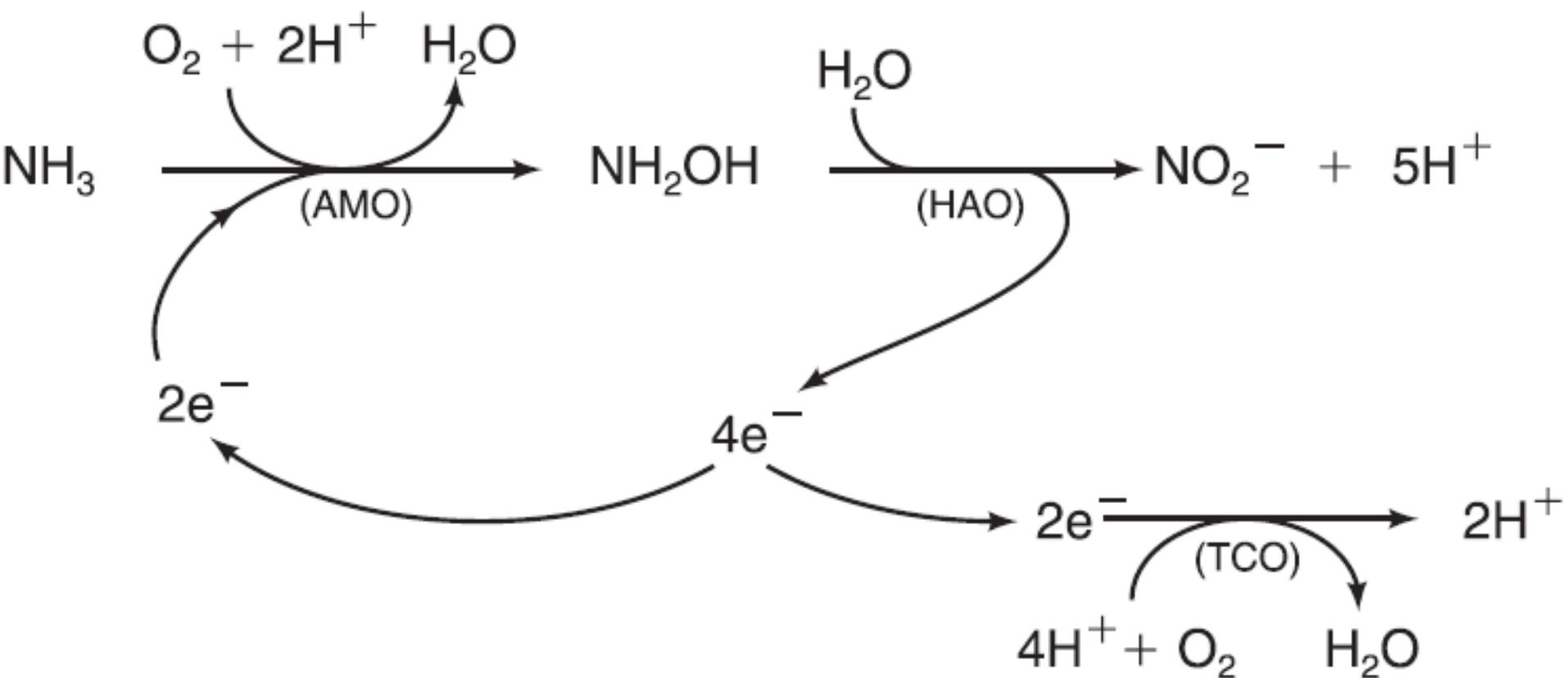


Proposed stepwise reduction of N_2 on nitrogenase cofactor and CO inhibition



(3B) Nitrification

- A series of oxidation reactions to **convert NH₃ into NO₂⁻ or NO₃⁻** (nitrite or nitrate) in microorganisms.
- 3 major steps catalyzed by **3 different enzymes**, ammonia monooxygenase (**AMO**), hydroxylamine oxidoreductase (**HAO**) & nitrite oxidoreductase (**NIO**).



Ammonia Monoxygenase (AMO)

- Catalyzes the first step: **oxidation** of NH_3 to form hydroxylamine, NH_2OH .

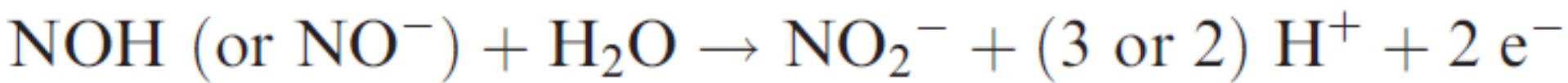


Monoxygenase: oxidation with insertion of one O atom of O_2 into the substrate. Oxidase: oxidation without the oxygen insertion.

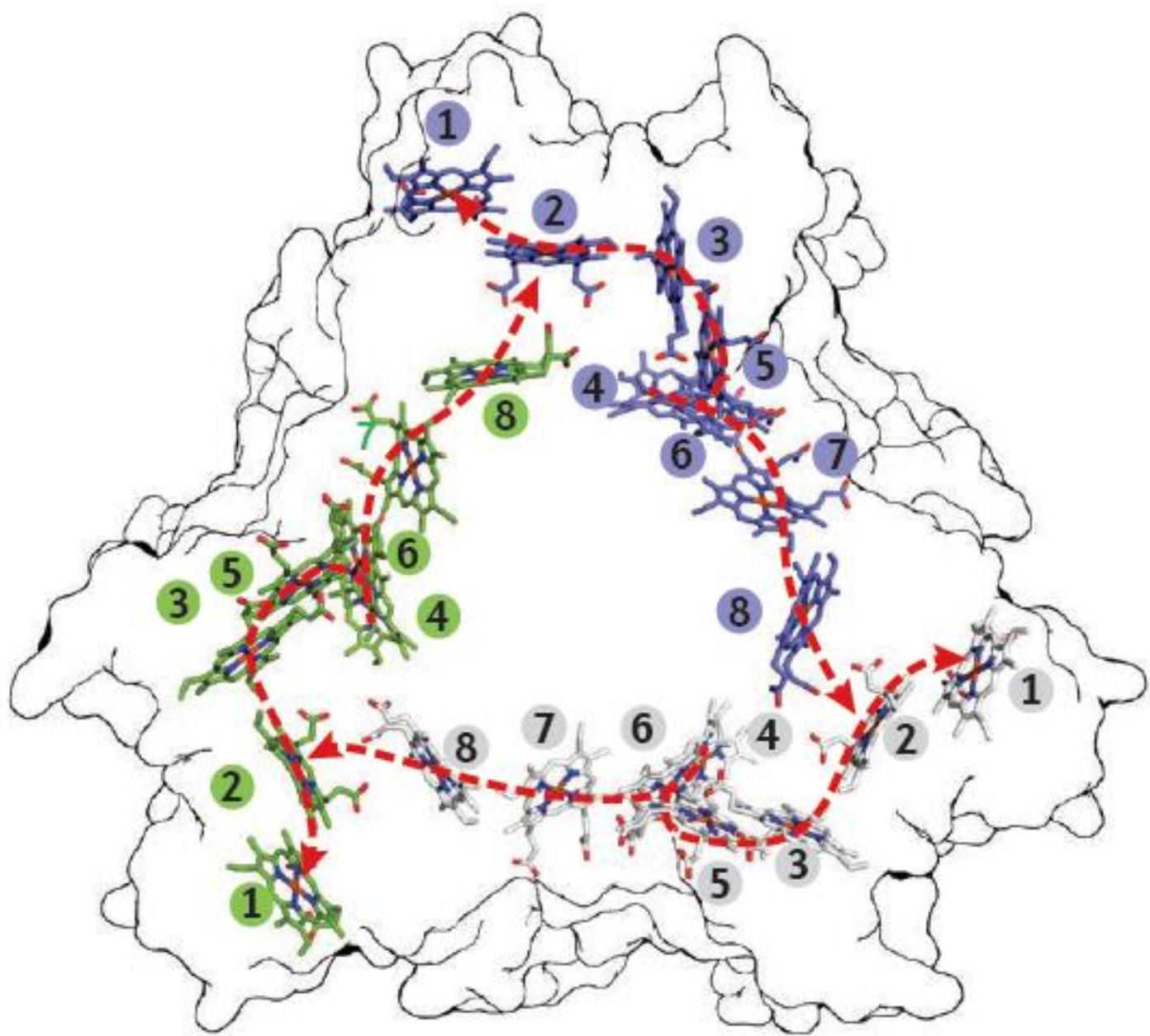
- May form a reactive $\text{M}=\text{O}$ species: hydrogen atom abstraction from the substrate to form hydroxide, followed by recombination to form NH_2OH .
- The electron source: the subsequent oxidation of NH_2OH .

Hydroxylamine Oxidoreductase (HAO)

- Catalyzes the second step: **oxidation of NH_2OH to form NO_2^- .**

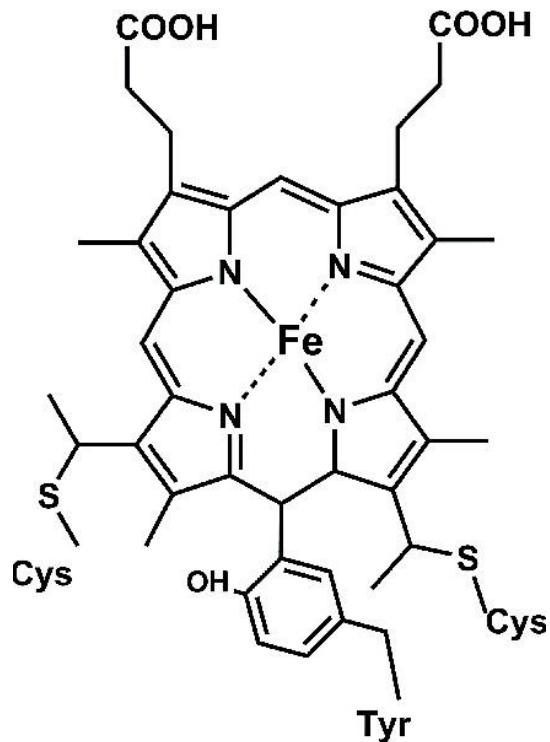
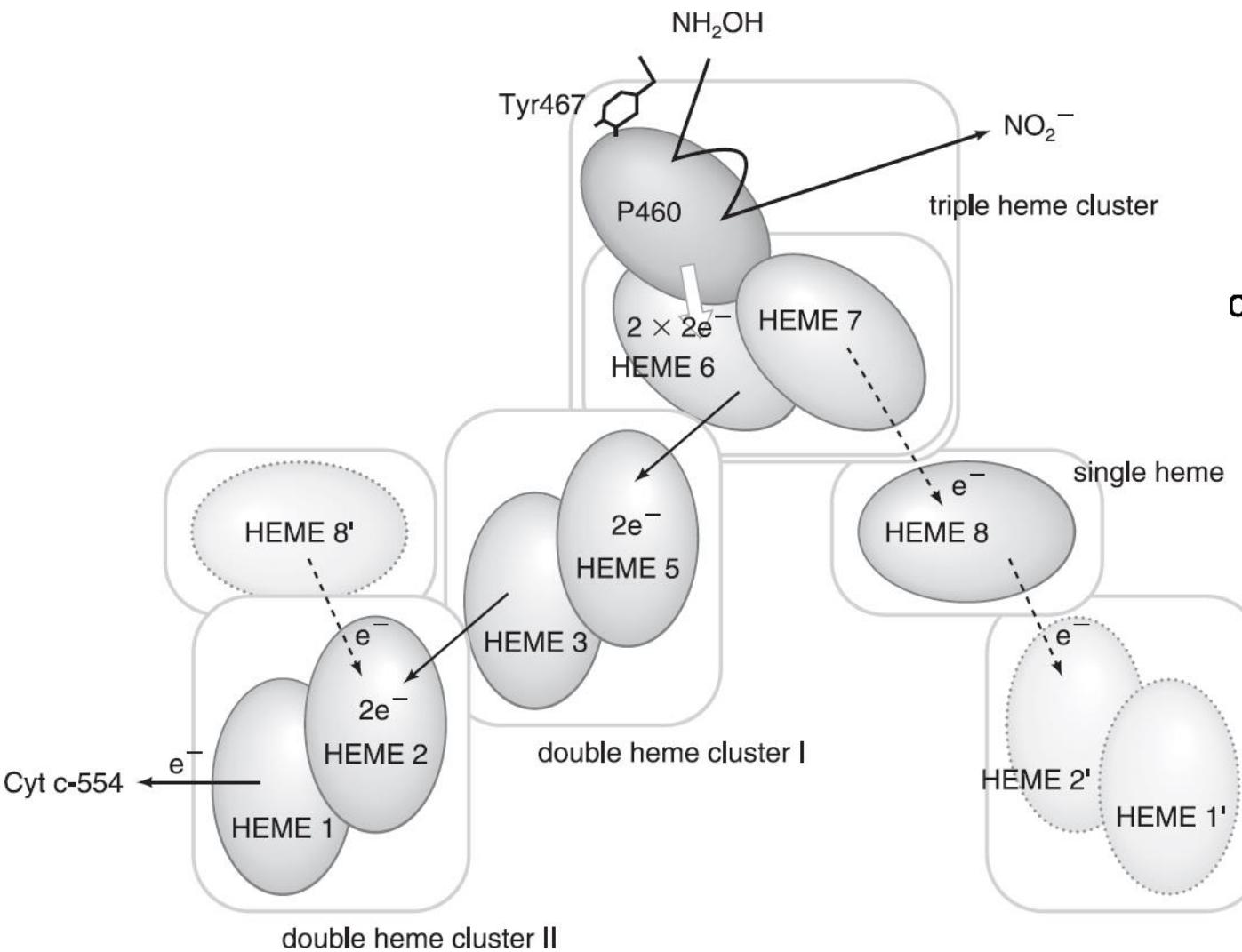


- 2 of 4e⁻ from this NH_2OH oxidation are reused by AMO (the first oxidation step). The other 2e⁻ are transferred to the terminal cytochrome oxidase (TCO, containing Cu₂Cyt-aa₃). TCO reduces O₂ to form H₂O & pumps H⁺ → ATP formation by ATP synthase.



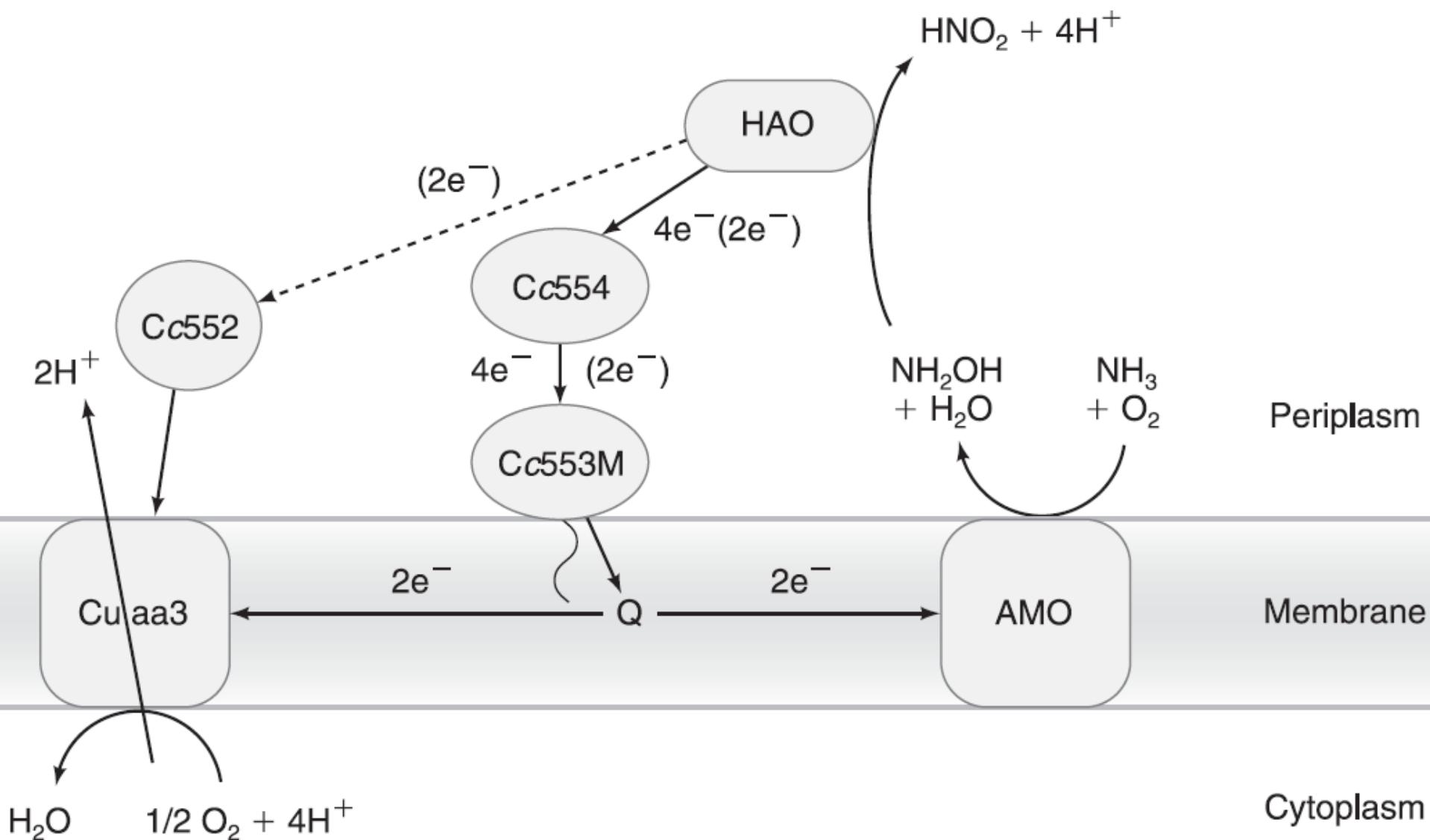
Nat. Rev. Microbio,
2018, 16, 263.

- HAO is trimeric with 8 c-type hemes on each unit, including one (5-coordinated) heme P460 (heme 4).



P460

- P460 heme is linked to a C of Tyr from another subunit.



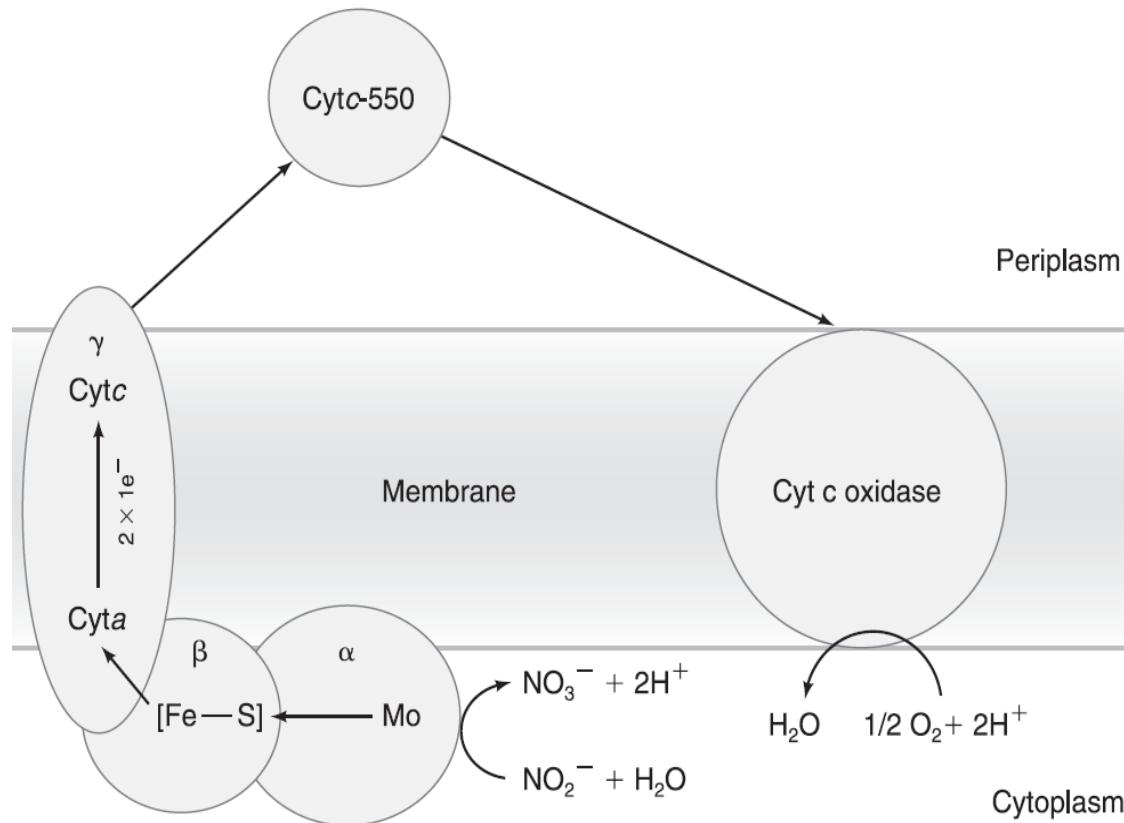
Nitrite Oxidoreductase (NIO)

- Catalyzes the third & final step: **oxidation** of NO_2^- .



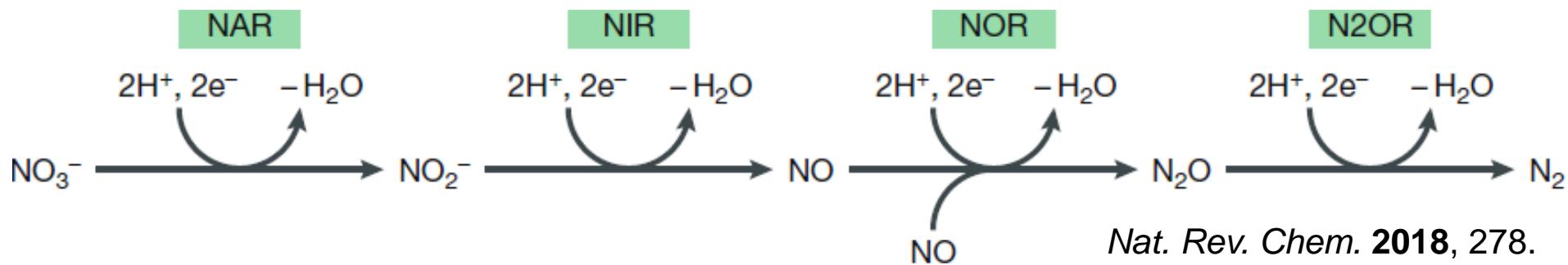
- Contains a **Mo** (catalytic site), [Fe-S] cluster, heme a & heme c.

- 2e^- transferred to Cyt c oxidase.
- Key energy-generating process for *Nitrobacter* & related bacteria.

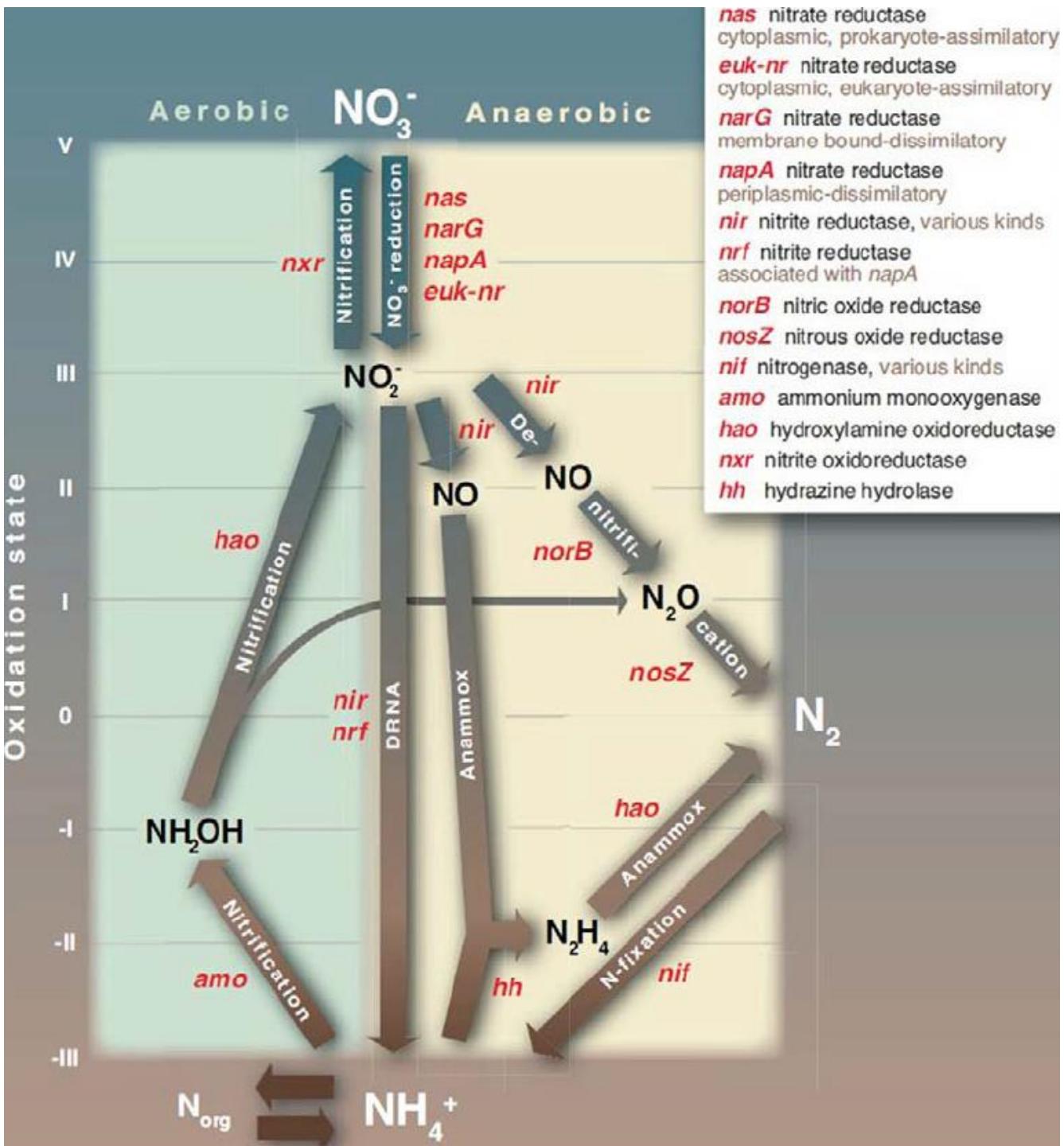


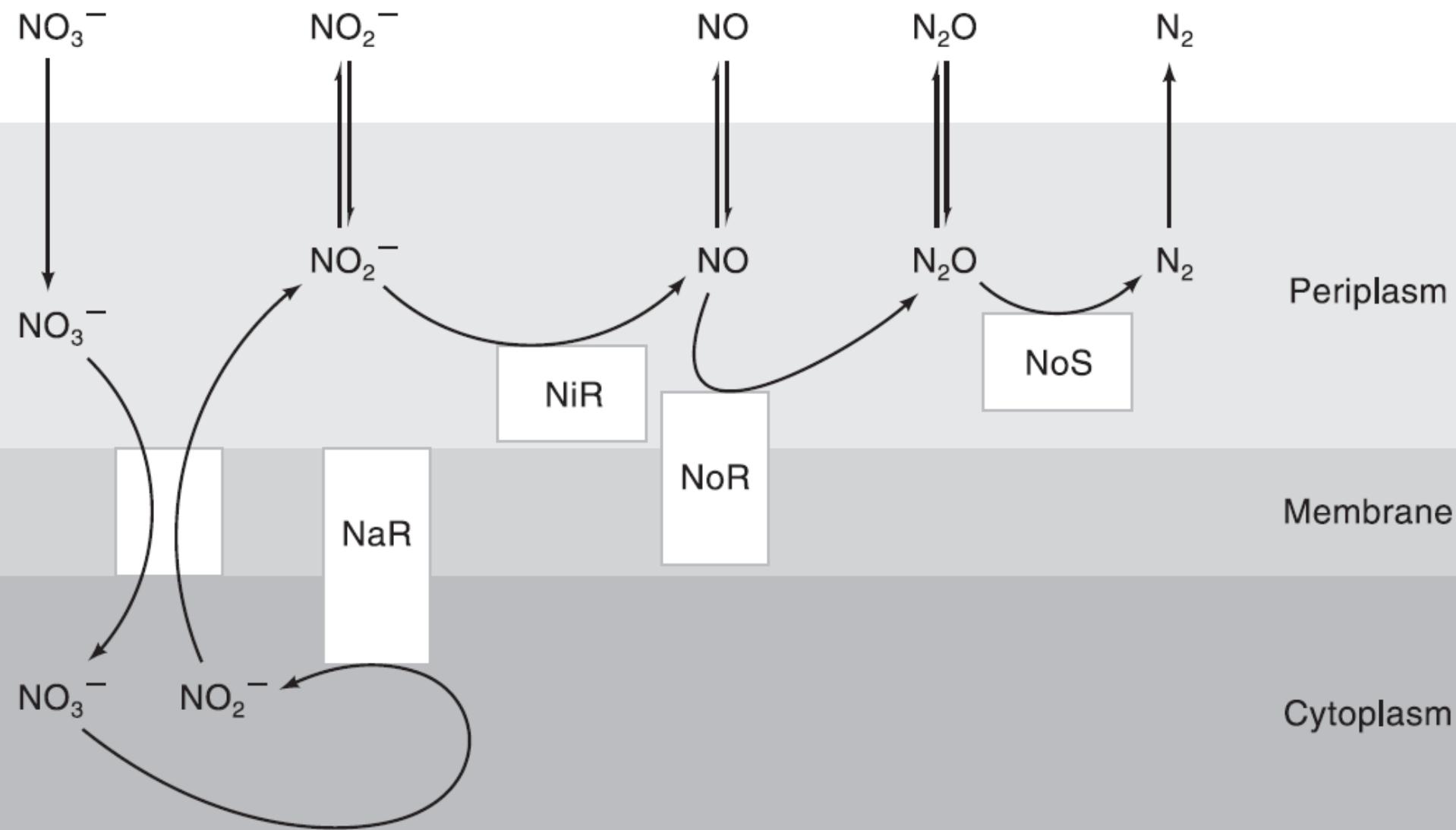
(3C) Denitrification

- An anaerobic process to reduce nitrogen oxide species (NO_3^- , NO_2^- , NO & N_2O) as e^- acceptors to form N_2 in some bacteria: to survive & to gain energy in the absence of O_2 .
- The only way to return fixed nitrogen-compounds to the atmosphere: completes the biological nitrogen cycle.
- 4 enzymatic steps in most organisms, while a few organisms can lack the last enzyme & thus, give N_2O .
- 4 enzymes: Nitrate Reductase (NaR), Nitrite Reductase (NiR), Nitric Oxide Reductase (NoR), & Nitrous Oxide Reductase (NoS or N2OR).



Biological pathways involved in the nitrogen cycle





Dissimilatory Nitrate Reductase (NaR)

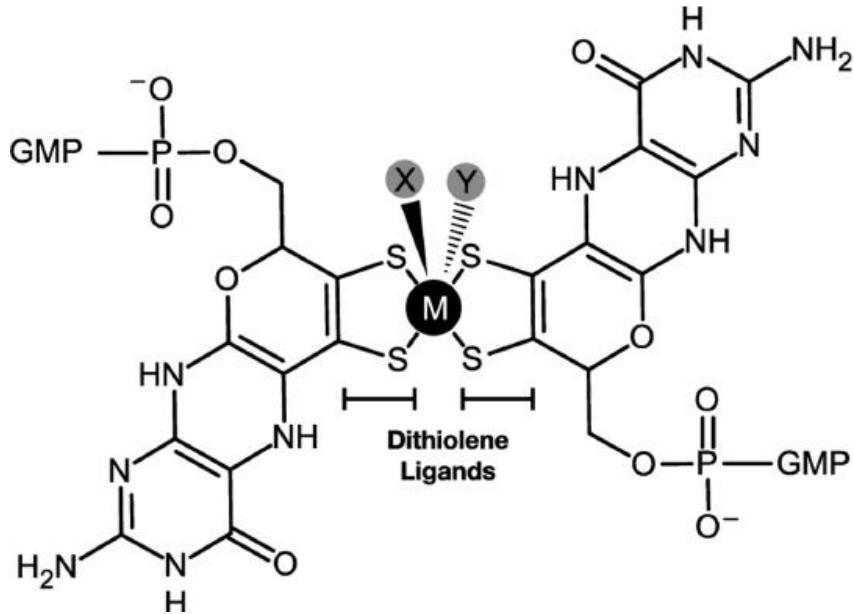
- Catalyzes the first step: **reduction of NO_3^- to give NO_2^- .**

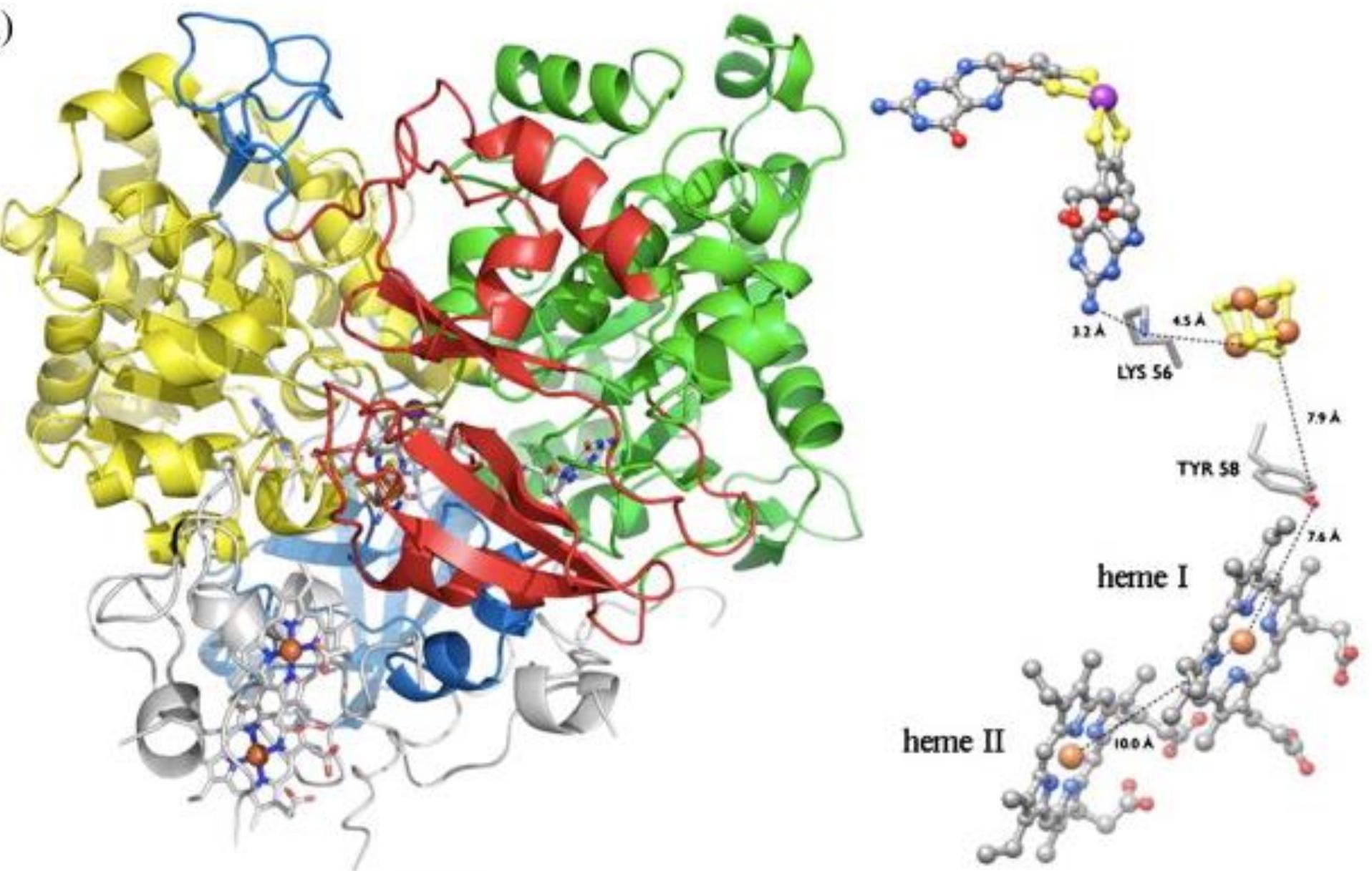


- Contains a **Mo cofactor**, heme & Fe-S cluster; undergoes Mo-catalyzed oxygen-atom transfer reaction.



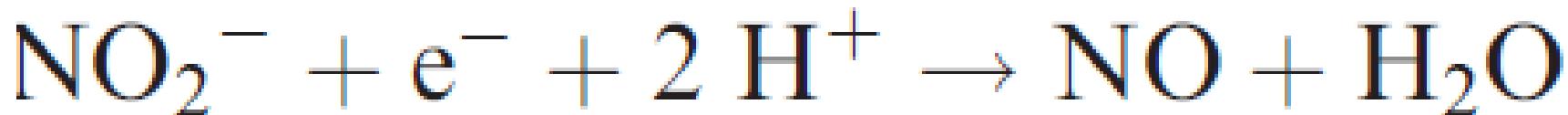
- The Mo(VI) intermediate is reduced to regenerate the active Mo(IV) form.





Dissimilatory Nitrite Reductase (NiR)

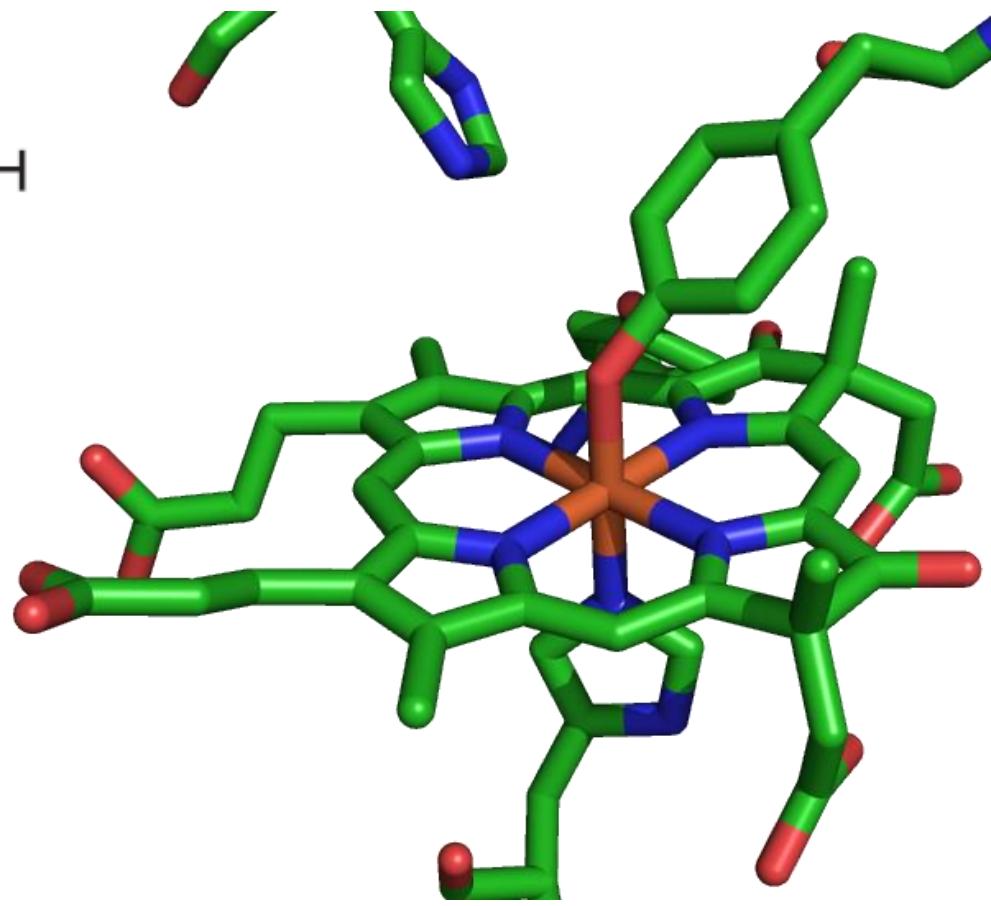
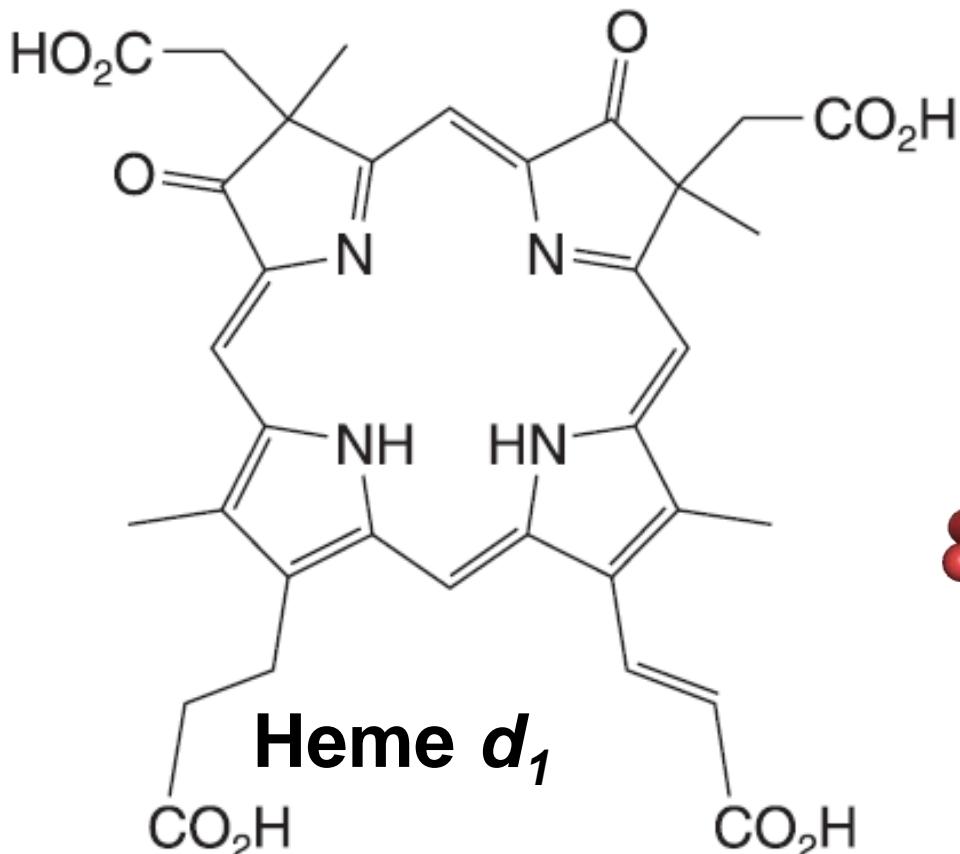
- Catalyzes the second step: **reduction of NO_2^- to give NO.**



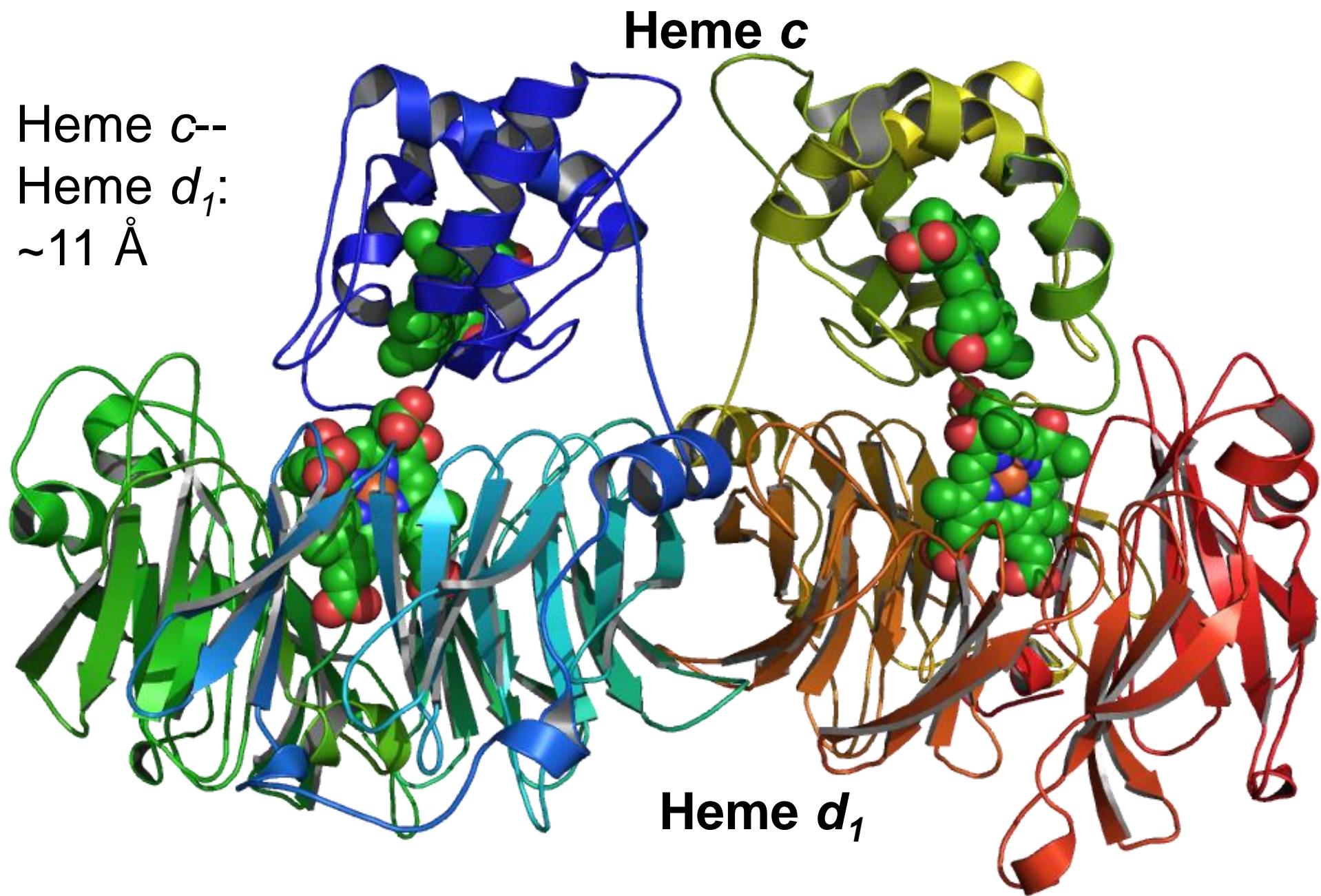
- **2** known different **types** of NiR: Cytochrome cd_1 -containing NiR (hemes c & d_1) & Cu-containing NiR.
- No organism can have both types of NiR.
- The NO product is an inhibitor of NiR.

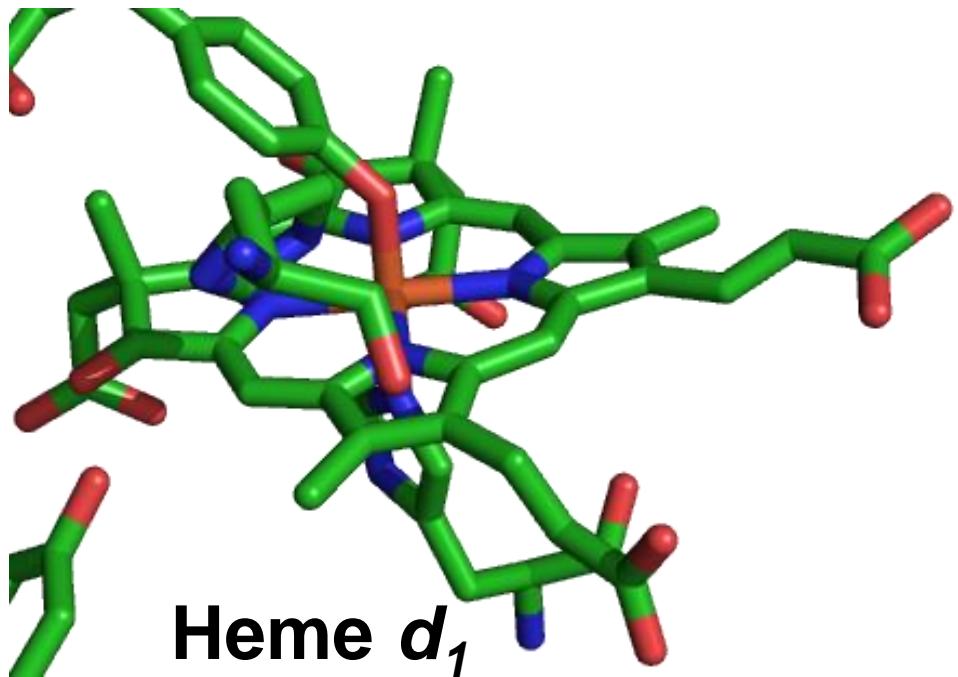
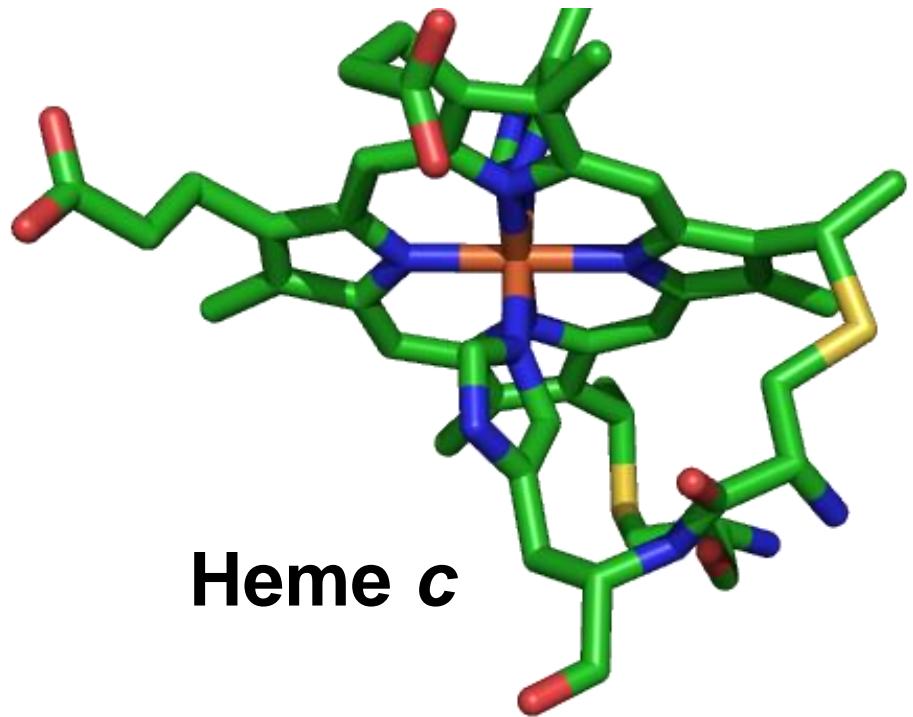
Cytochrome cd_1 -containing NiR

- Contains a **unique** green **heme d_1** , (the **catalytic** site) & **heme c** (the **ET** site).
- A low-spin ferric heme c & a mixture of low- & high-spin ferric heme d_1 , in the oxidized form (resting state).



Heme c--
Heme d_1 :
 $\sim 11 \text{ \AA}$

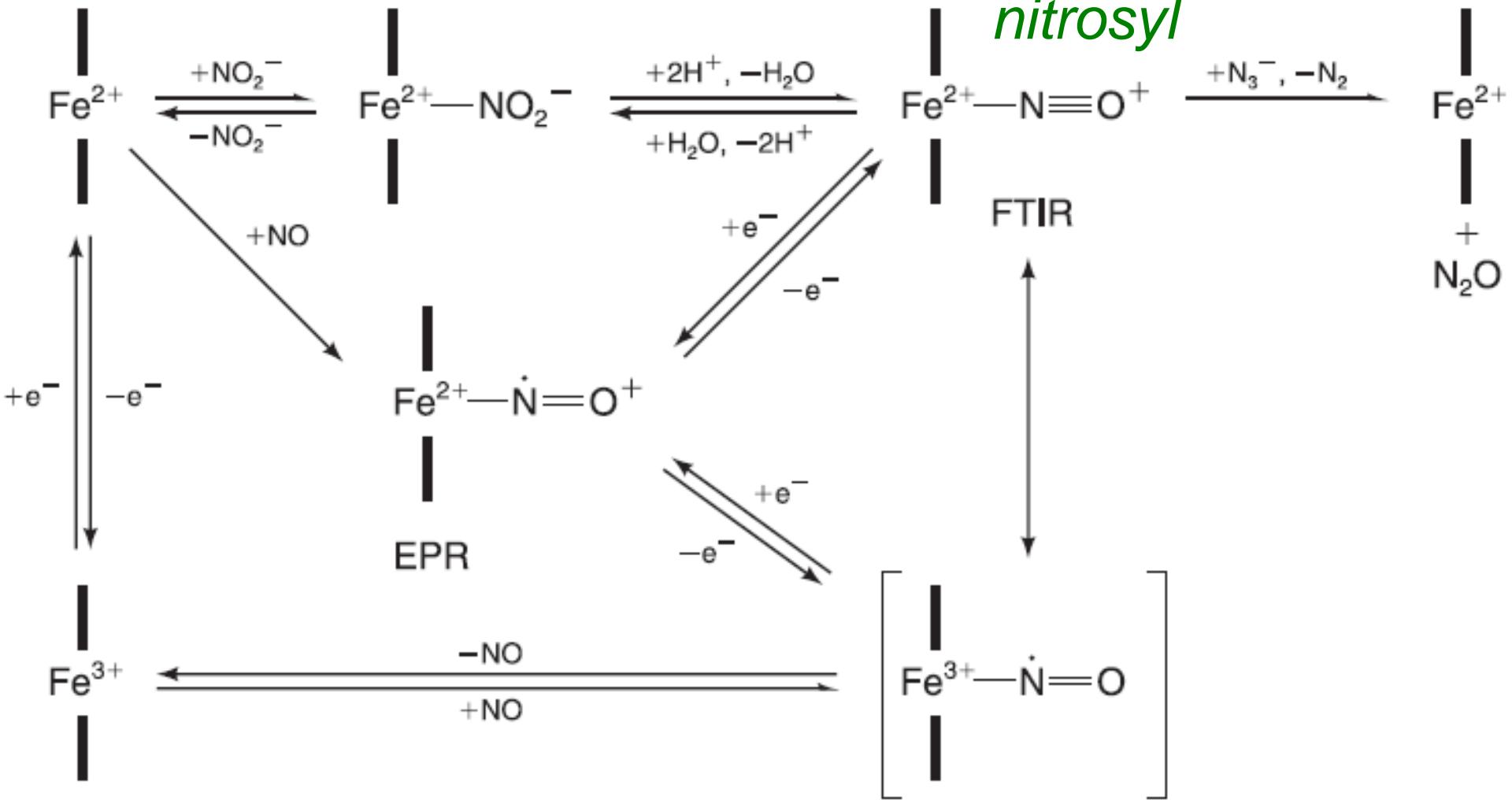


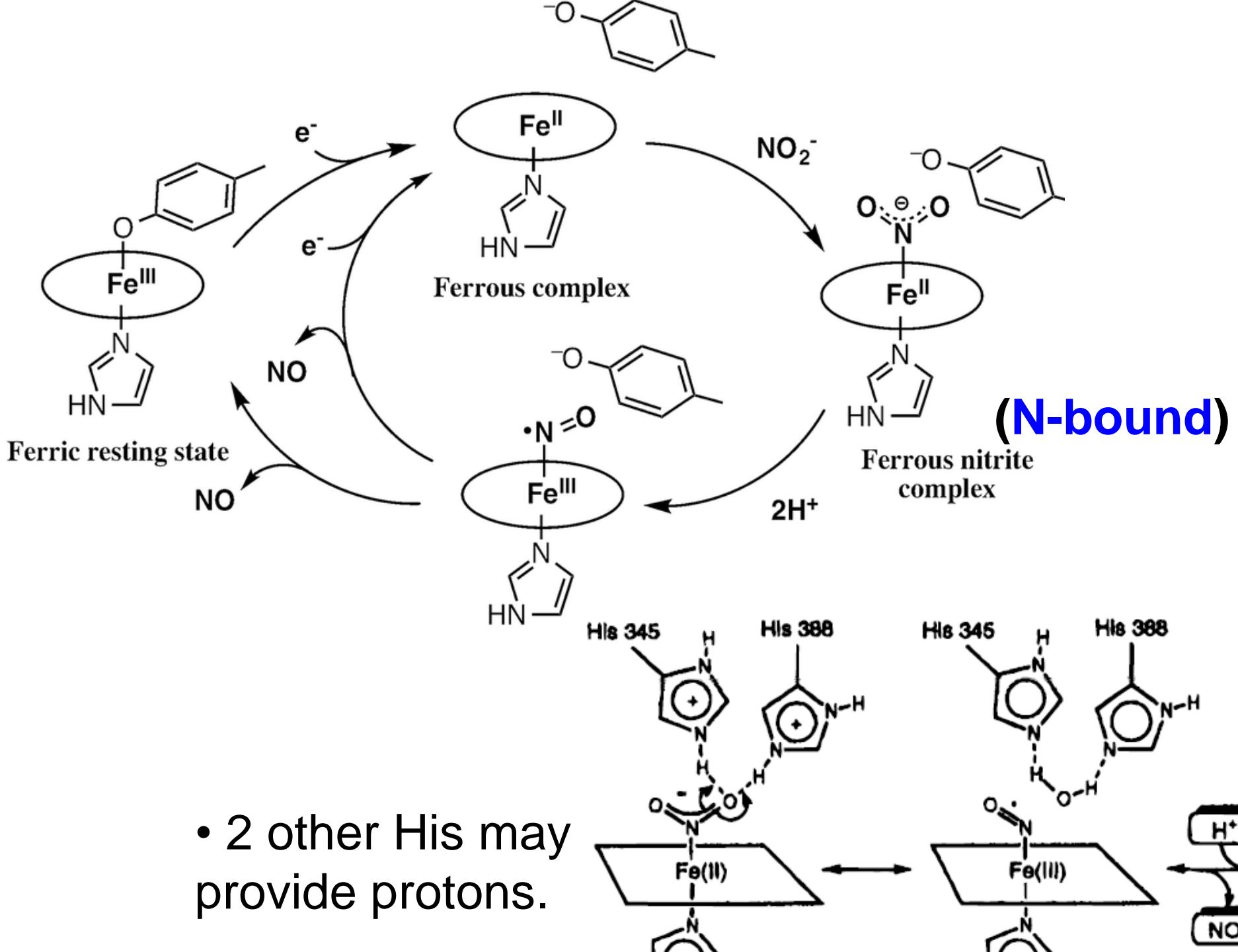


- Difference from common His + Met as the axial ligands, 2 His are the axial ligand in this heme c.
- An unusual axial ligand in heme d_1 : a **phenoxide** of Tyr in the heme c domain. That ligand was proposed to help release NO from the product complex.

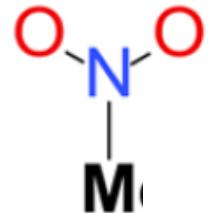
Proposed Mechanism

*electrophilic
nitrosyl*

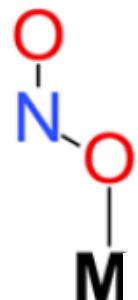




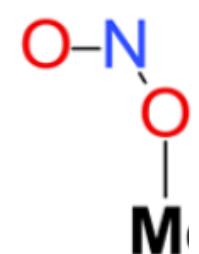
Coordination Modes of M-Nitrite & Electronic Structures of M-NO



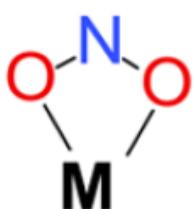
"nitro"
N-bound



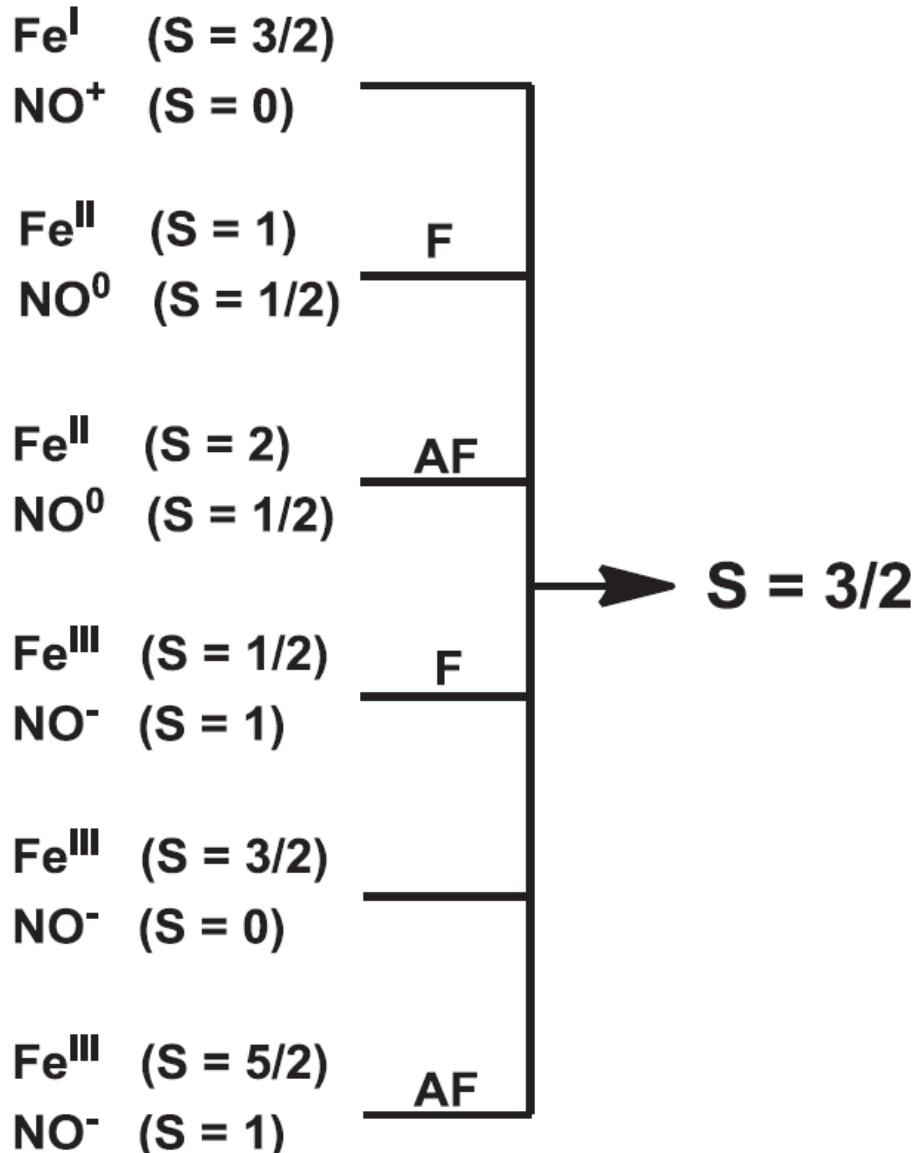
trans "nitrito"
O-bound



cis "nitrito"
O-bound



"bidentate nitrito"
O-bound

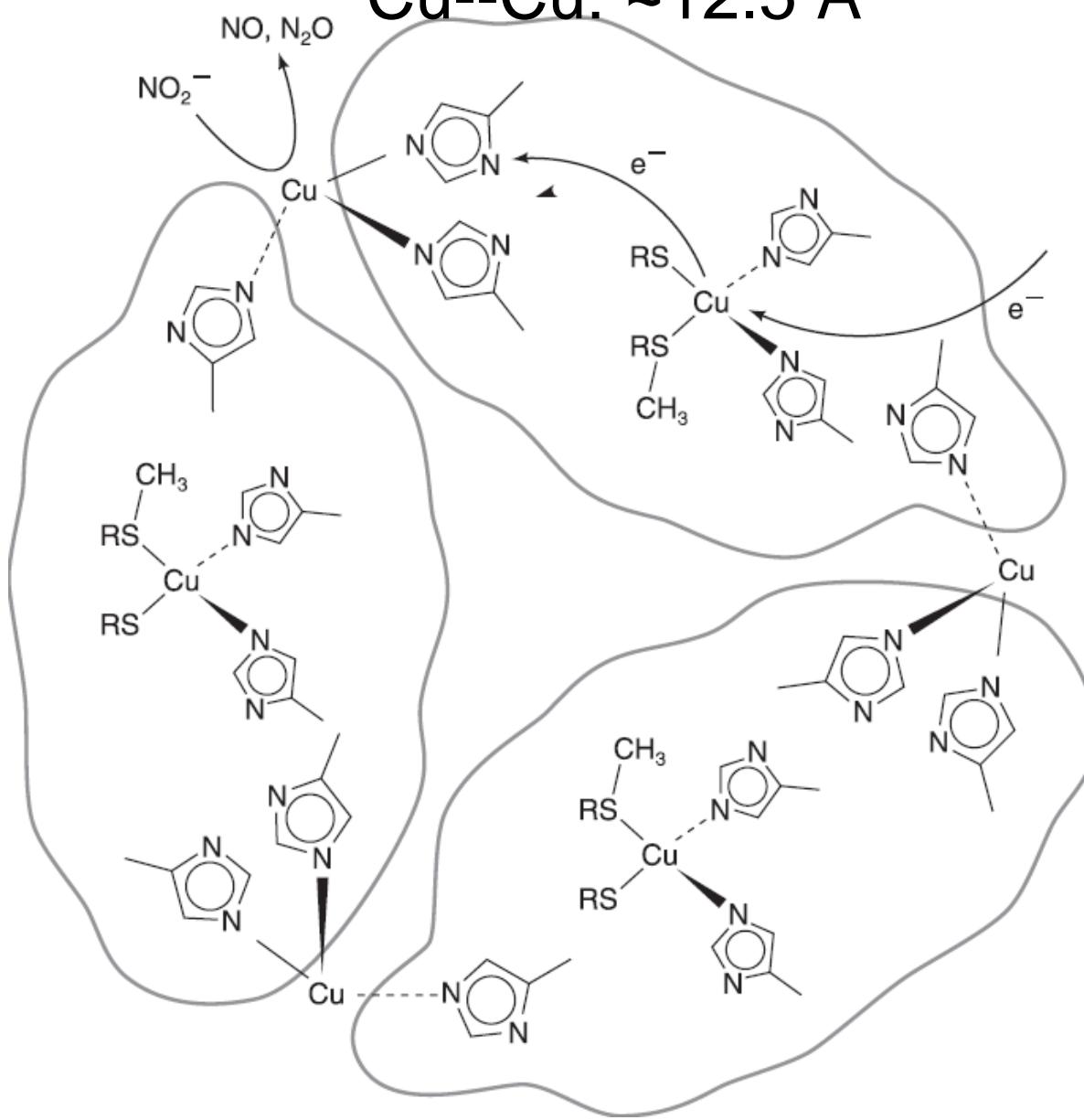


F: ferromagnetic coupling

AF: anti-ferromagnetic coupling

Cu-containing NiR

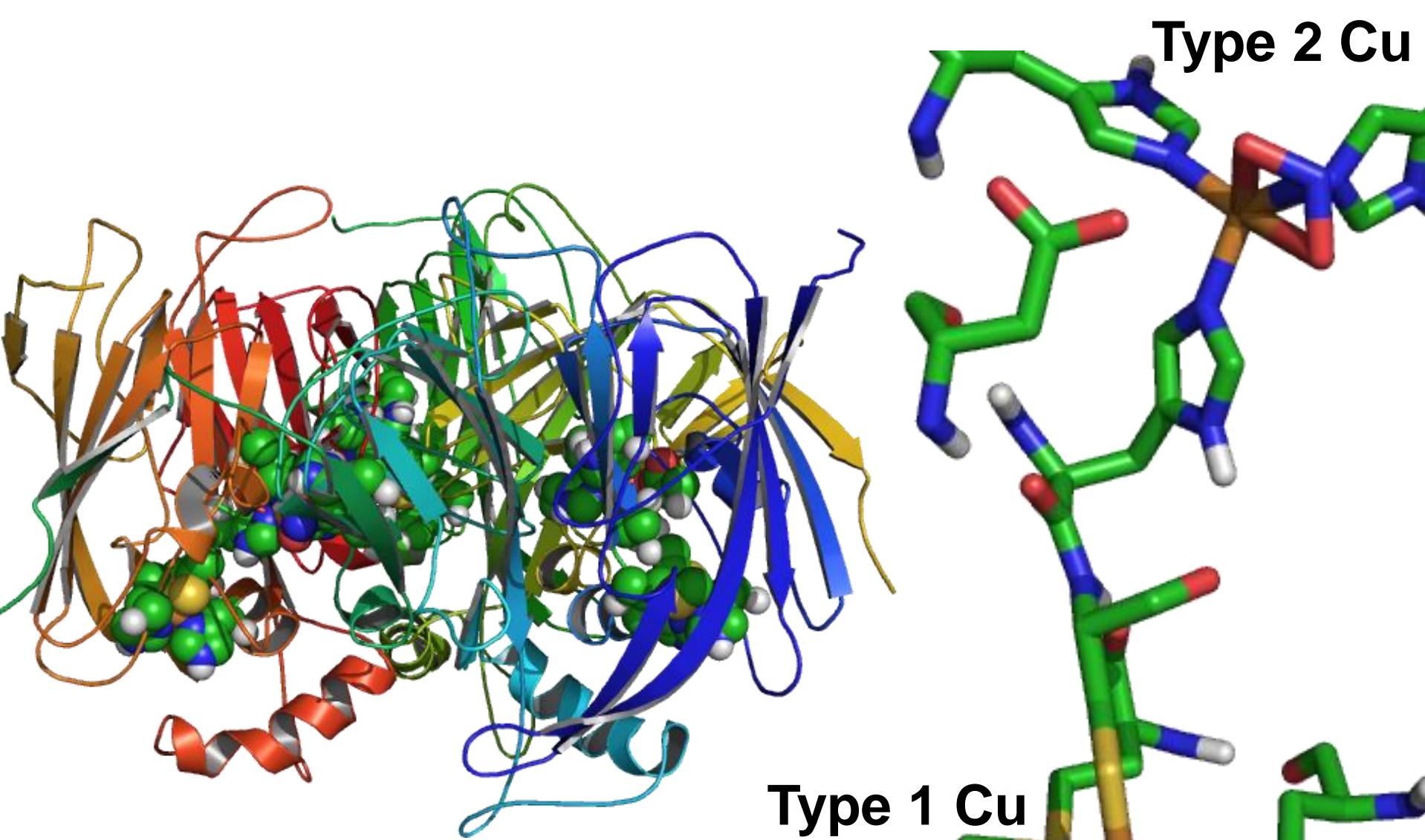
Cu--Cu: ~12.5 Å



- Each subunit contains 2 Cu sites.

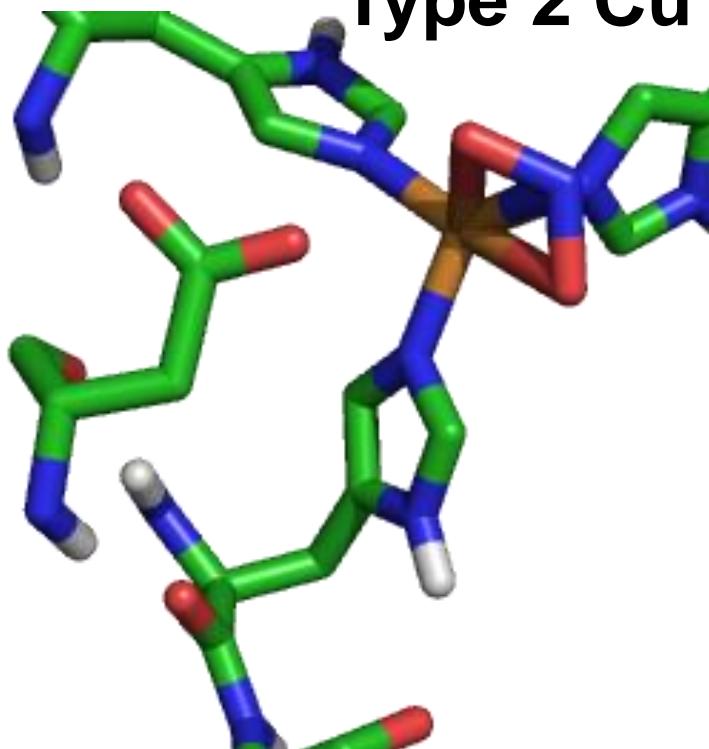
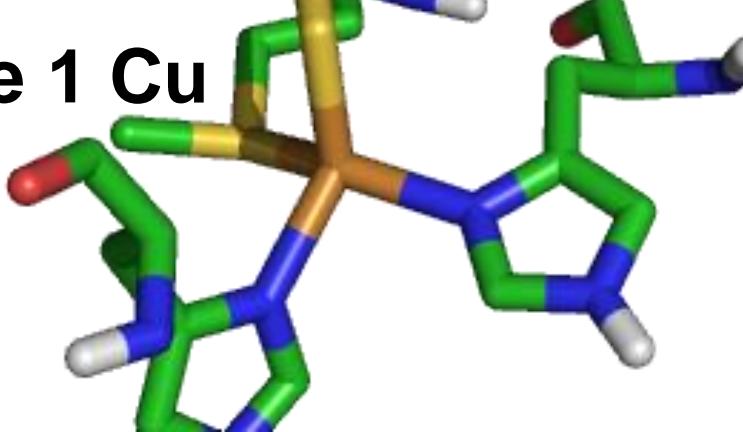
- One unusual green variant of the blue Cu protein (**Type 1 Cu center**, with a Met, 1 Cys & 2 His): the **ET site**.

- One **Type 2 Cu** protein (tetrahedral, with 3 His & 1 H₂O: the **catalytic site**). One His → Lys: inactive.

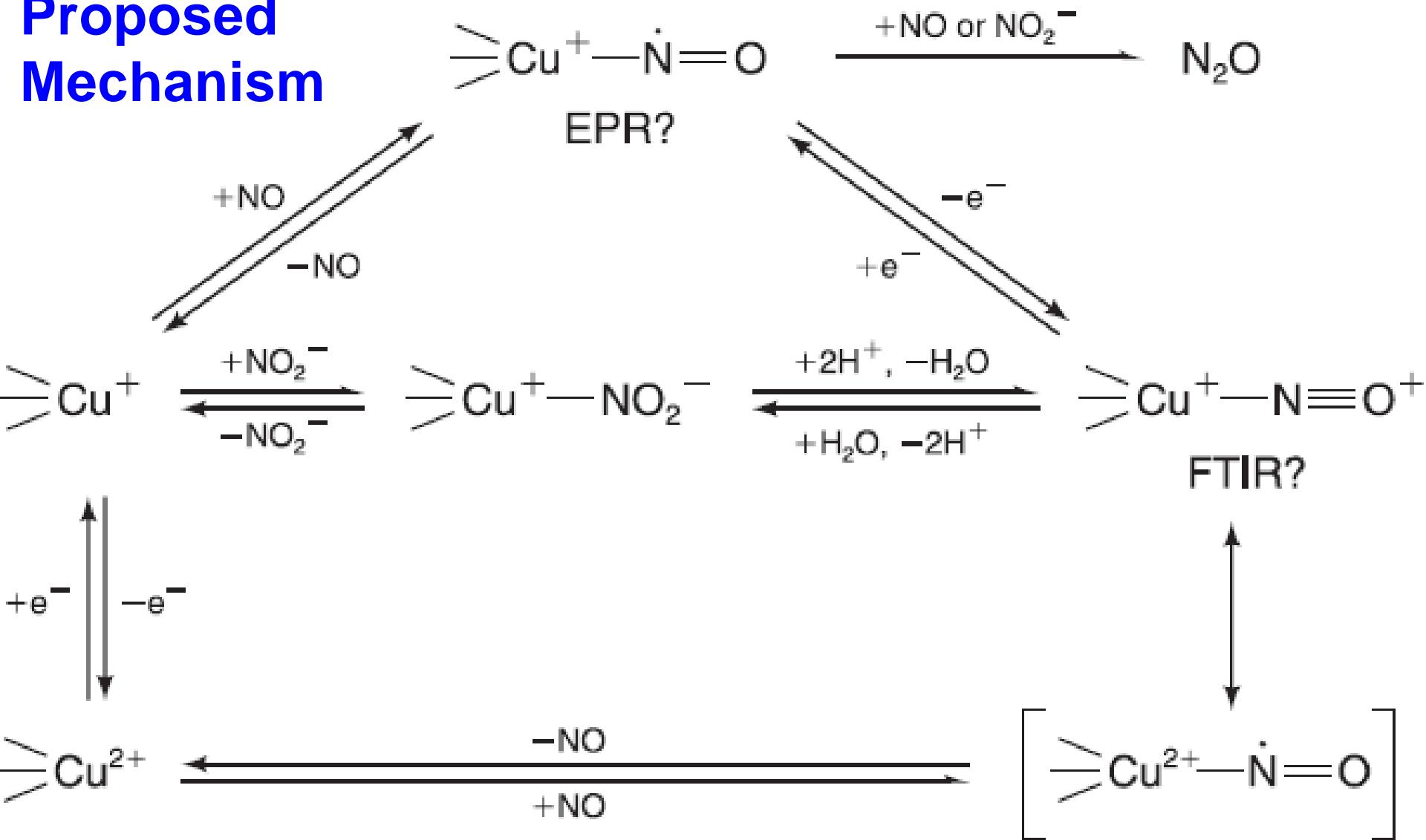


Type 1 Cu

Type 2 Cu

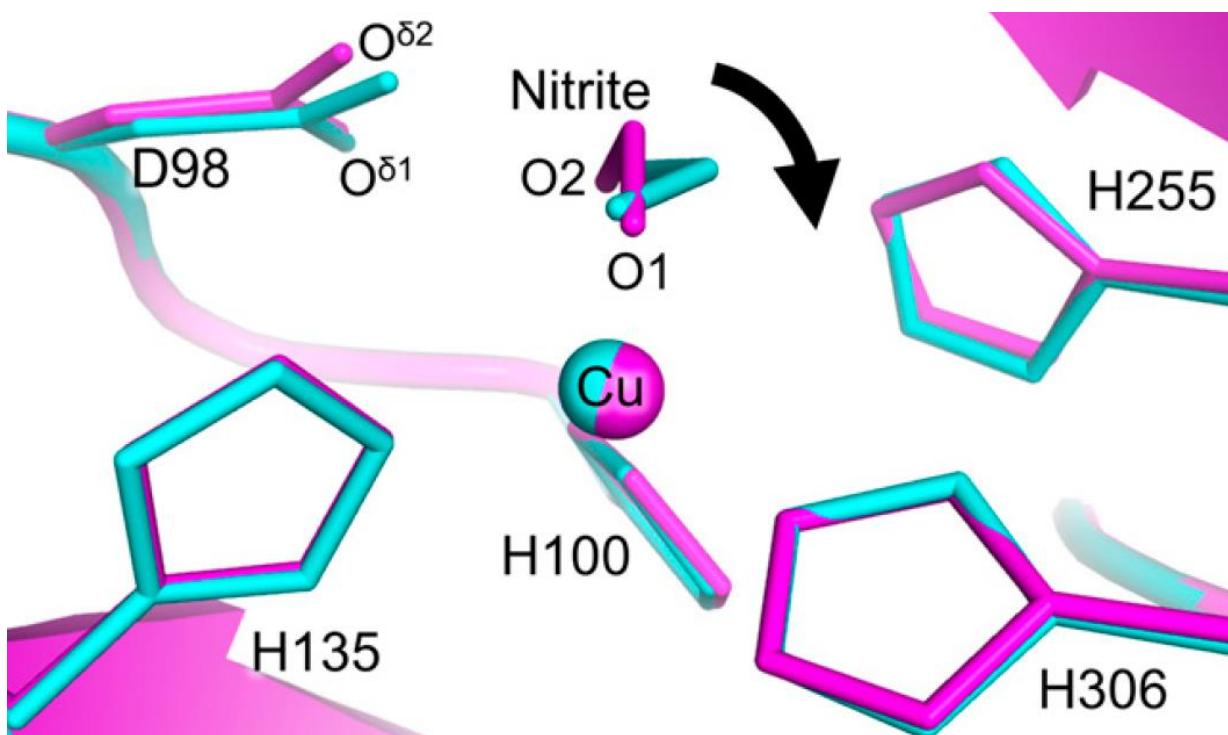


Proposed Mechanism



- Replaced H_2O by NO_2^- in the Type 2 Cu site to form a $\text{Cu}(\text{I})-\text{NO}_2^-$ (N-bound or face-on, vs. O bound is favored for $\text{Cu}(\text{II})$).

Cu NiR (Serial fs X-ray)

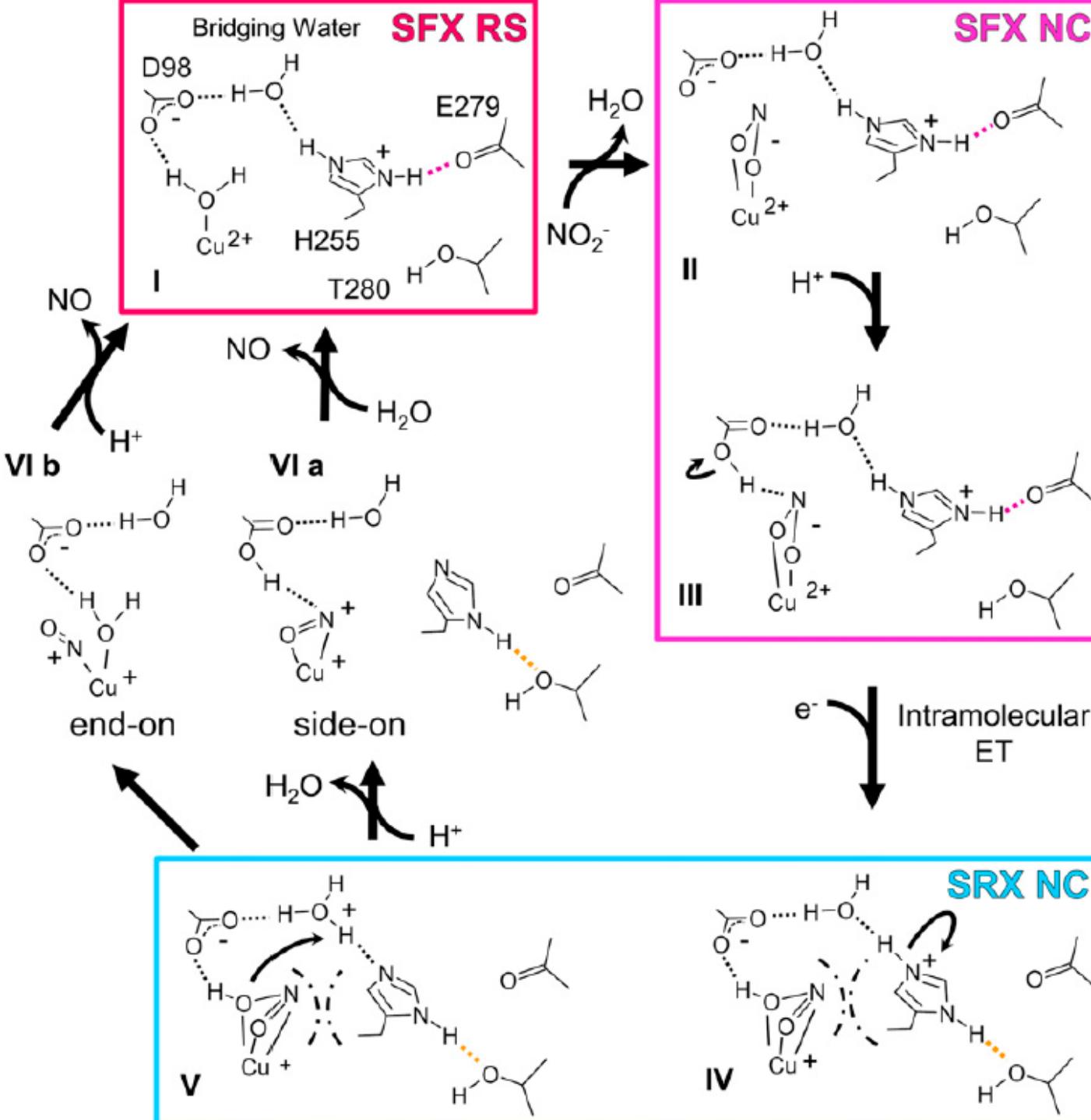


Serial fs
crystallography:
Cu(II)

Synchrotron
radiation
Crystallography:
Cu(I)
Face-on model

Proposed Mechanisms

Fukuda et al., PNAS
2016, 113, 2928.



NC: the nitrite complex

RS: the resting state

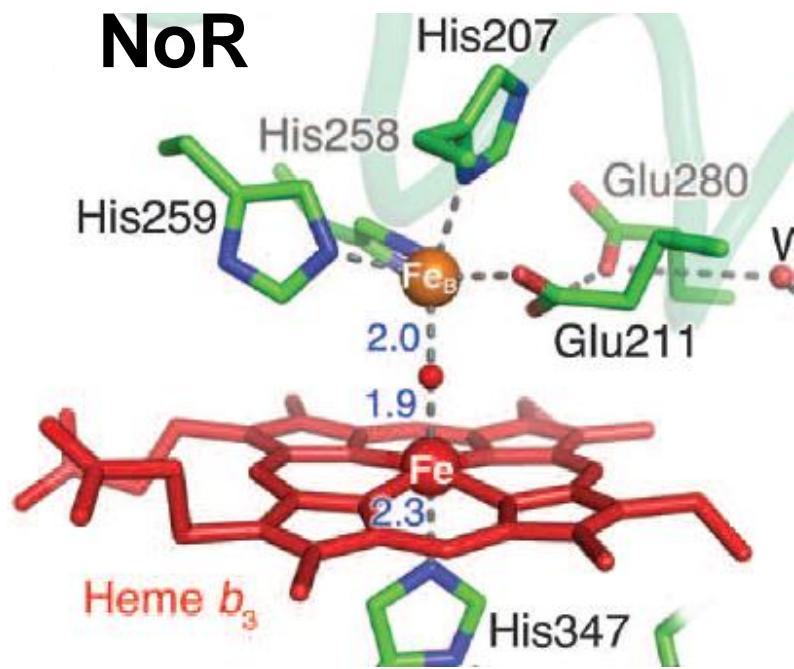
Nitric Oxide Reductase (NoR)

- Catalyzes the third step: **reduction** of NO to give nitrous oxide, N₂O.

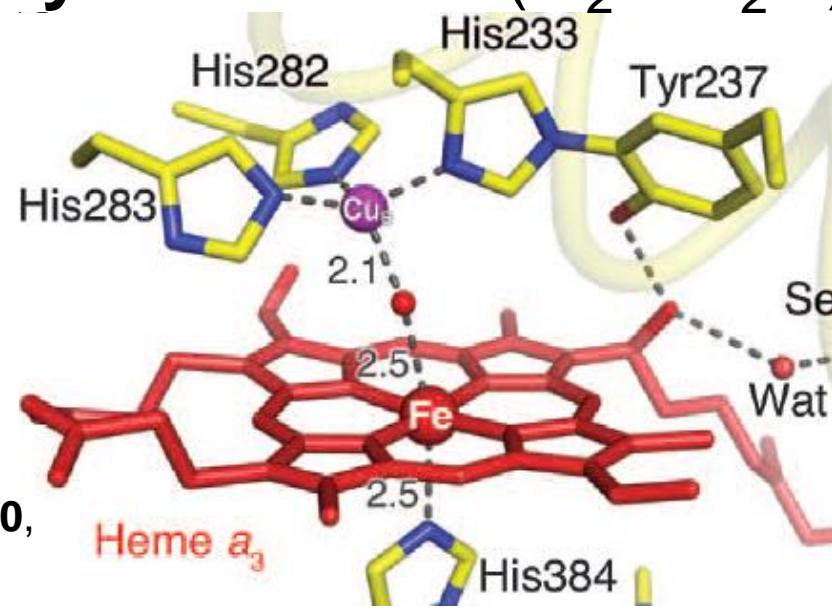


- Contains hemes *b*, *b*₃ & *c* (a mixture of high- & low-spin in the oxidized form), non-heme Fe (**Fe_B**) & Ca²⁺: the **heme-nonheme binuclear center (catalytic)**.

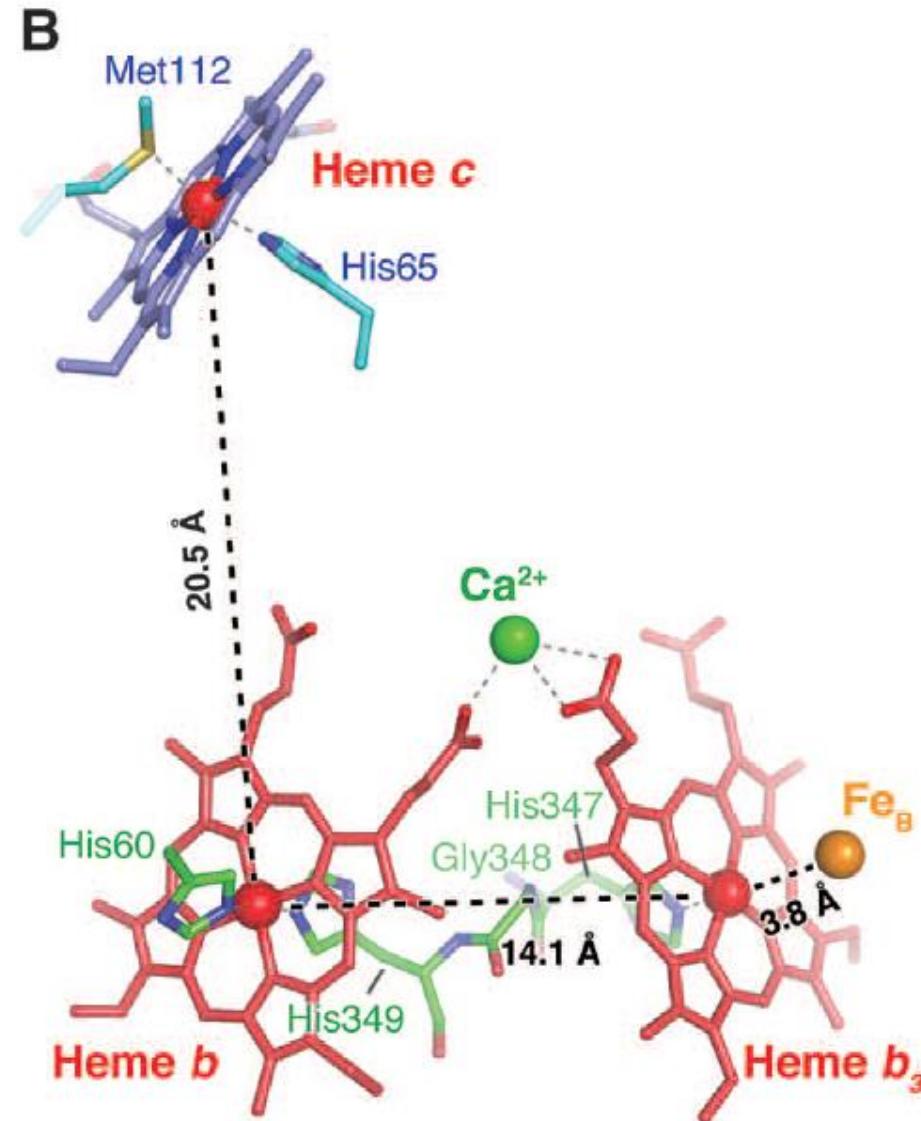
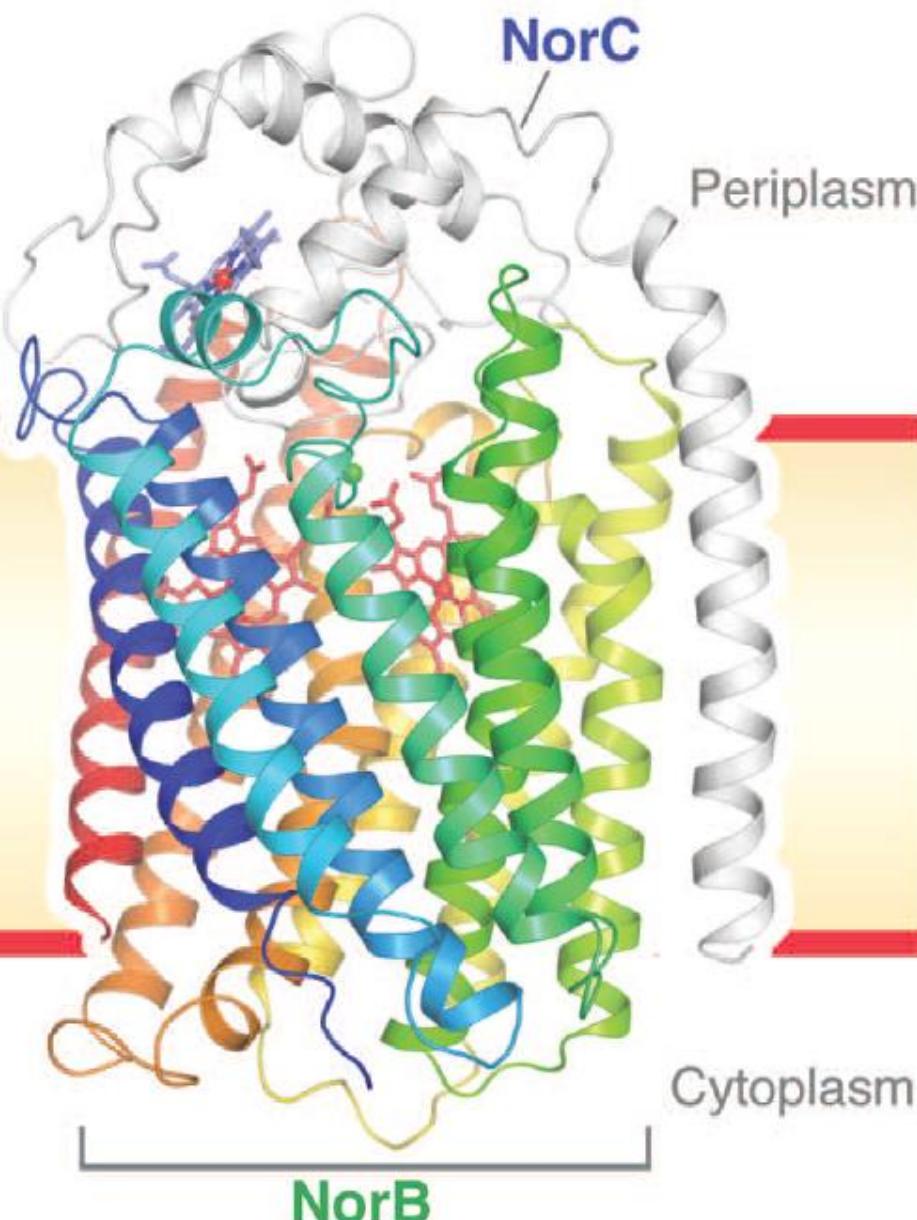
NoR



Cyt c Oxidase (O₂ → H₂O)



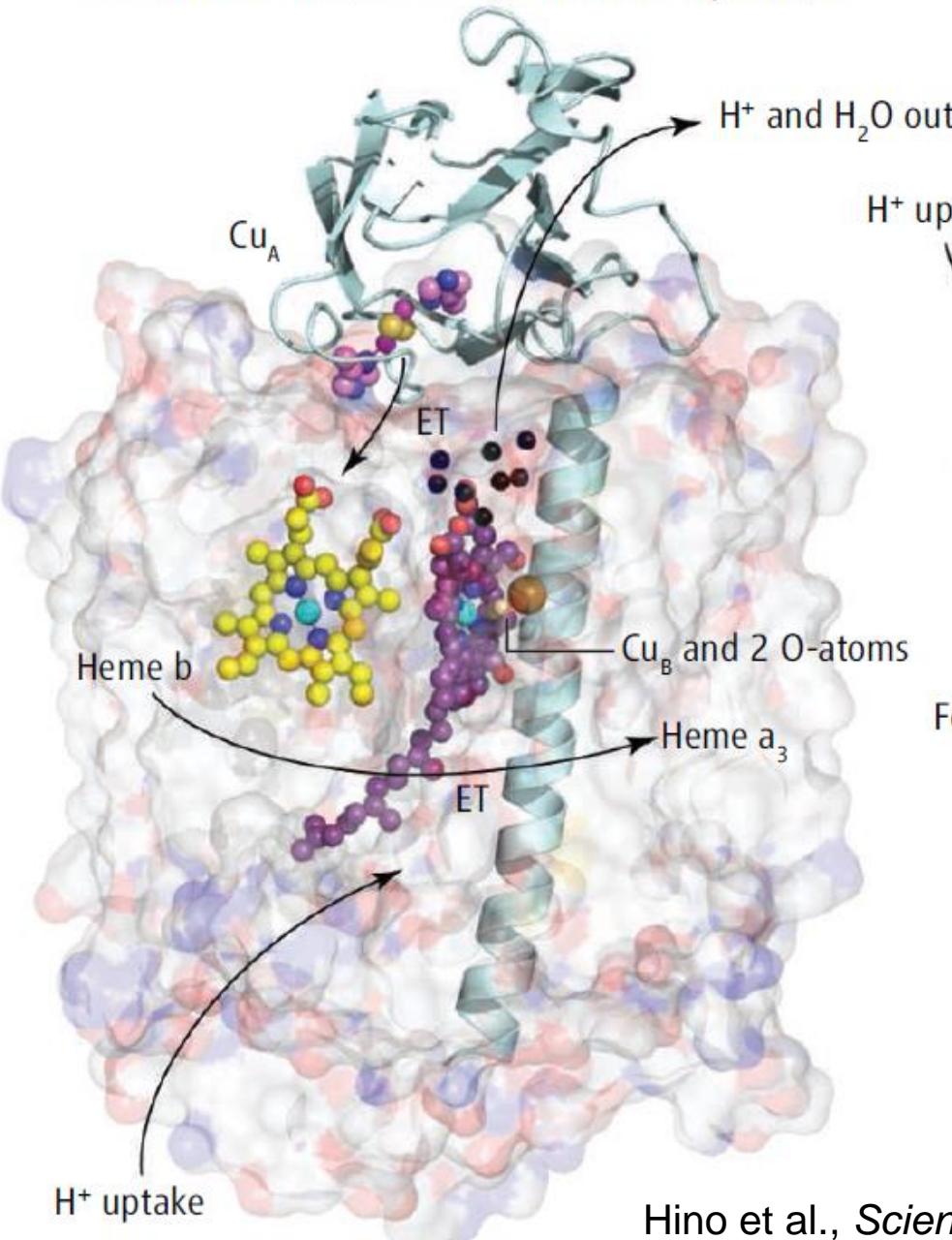
Bacterial NoR



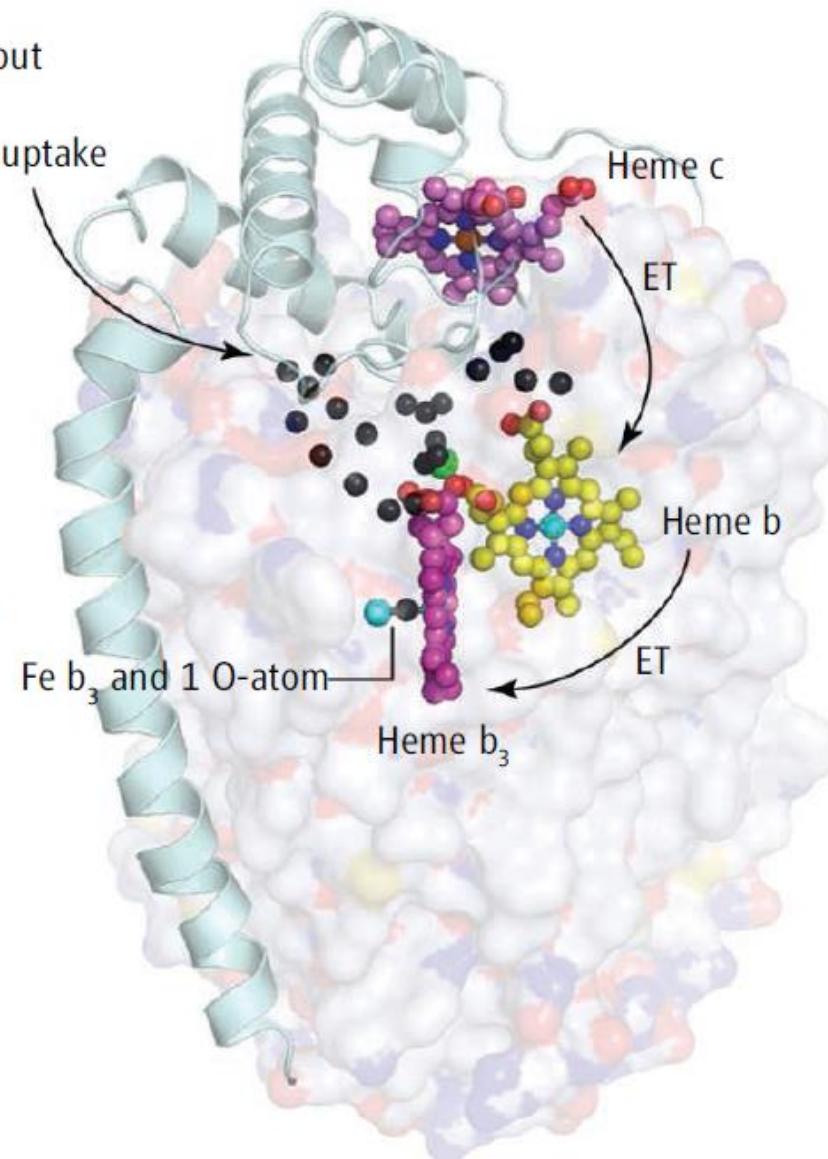
Hino et al., *Science*
2010, 330, 1666.

Proposed Proton & Electron Transfer Pathways

Thermus thermophilus cytochrome ba₃ oxidase

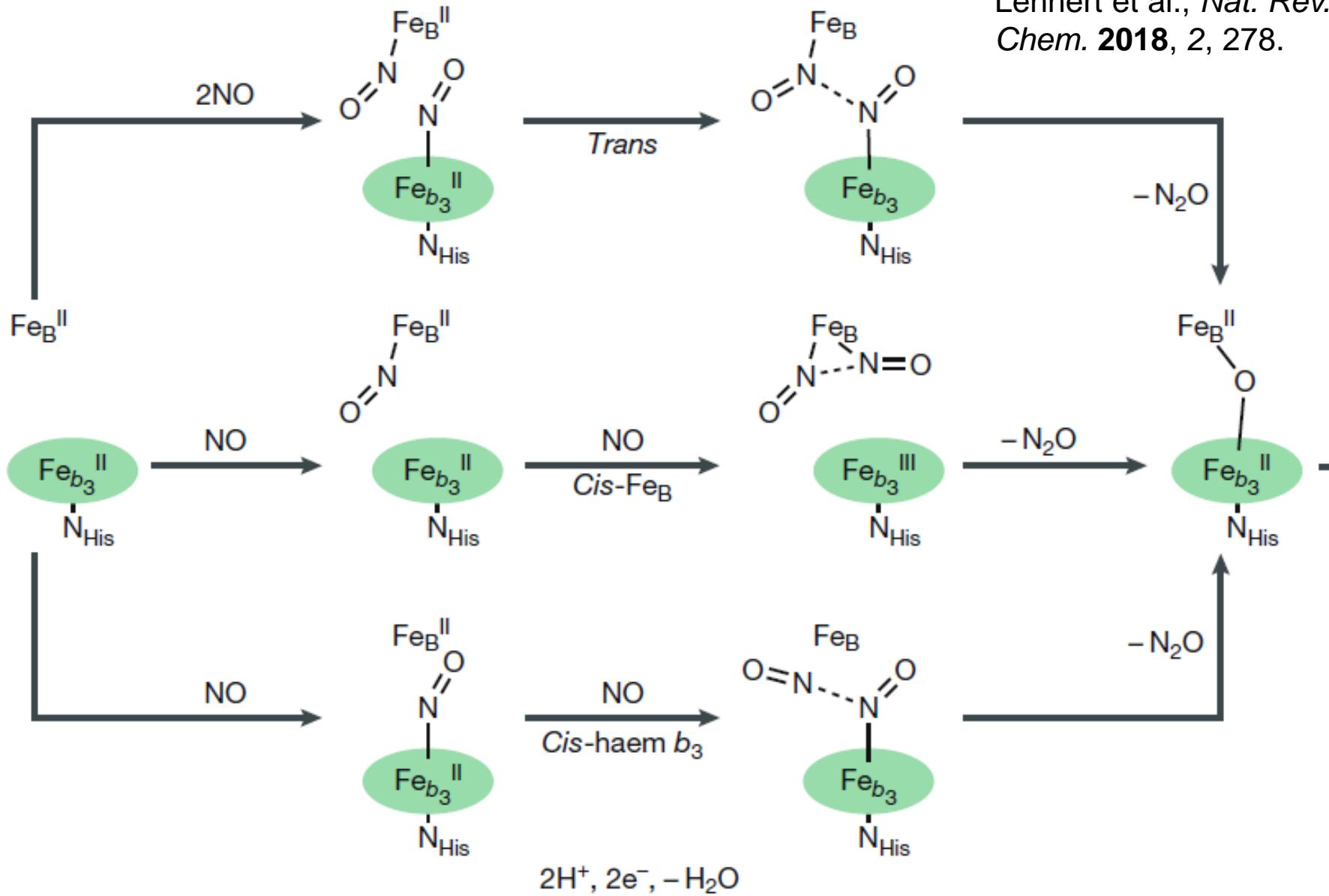


Pseudomonas aeruginosa cNOR



Three Proposed Mechanisms

Lehnert et al., *Nat. Rev. Chem.* 2018, 2, 278.

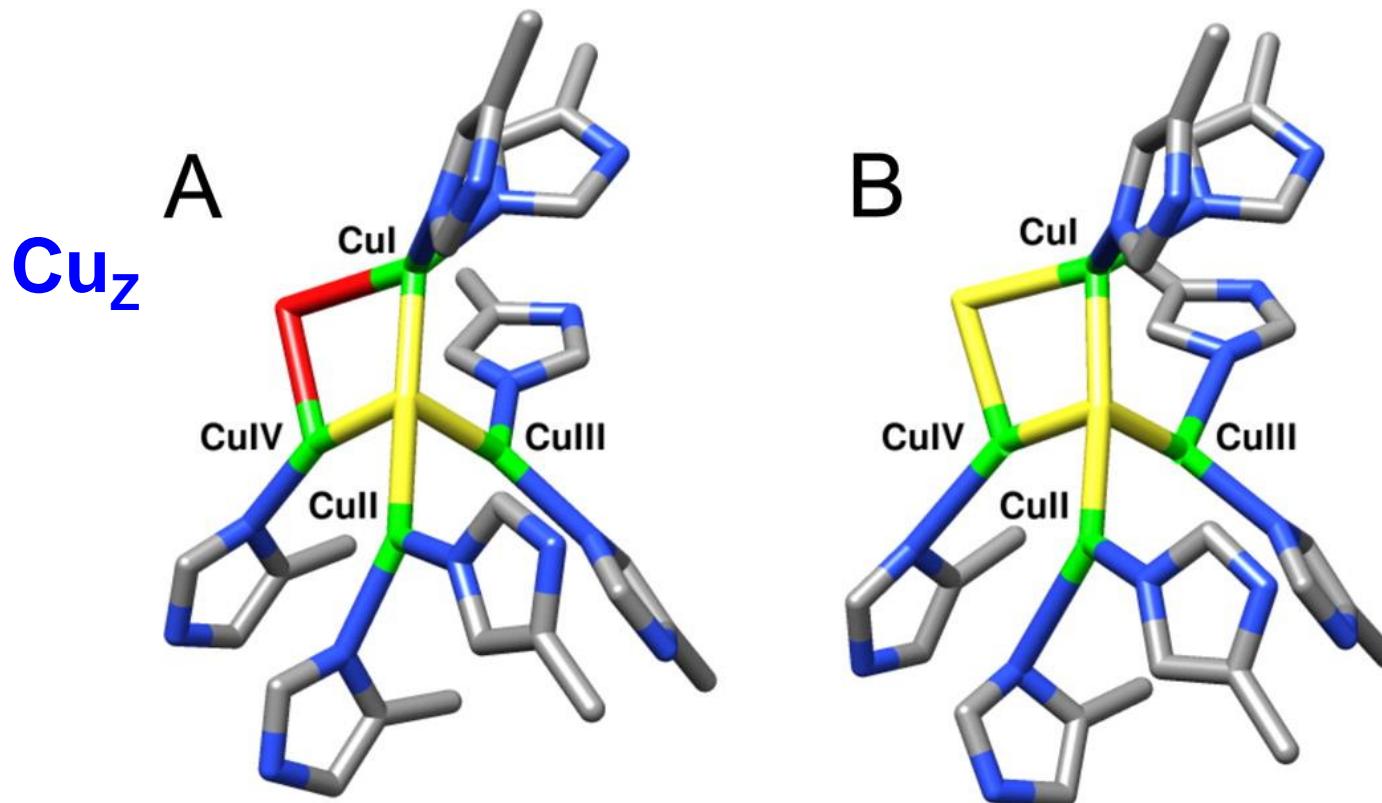


Nitrous Oxide Reductase (NoS)

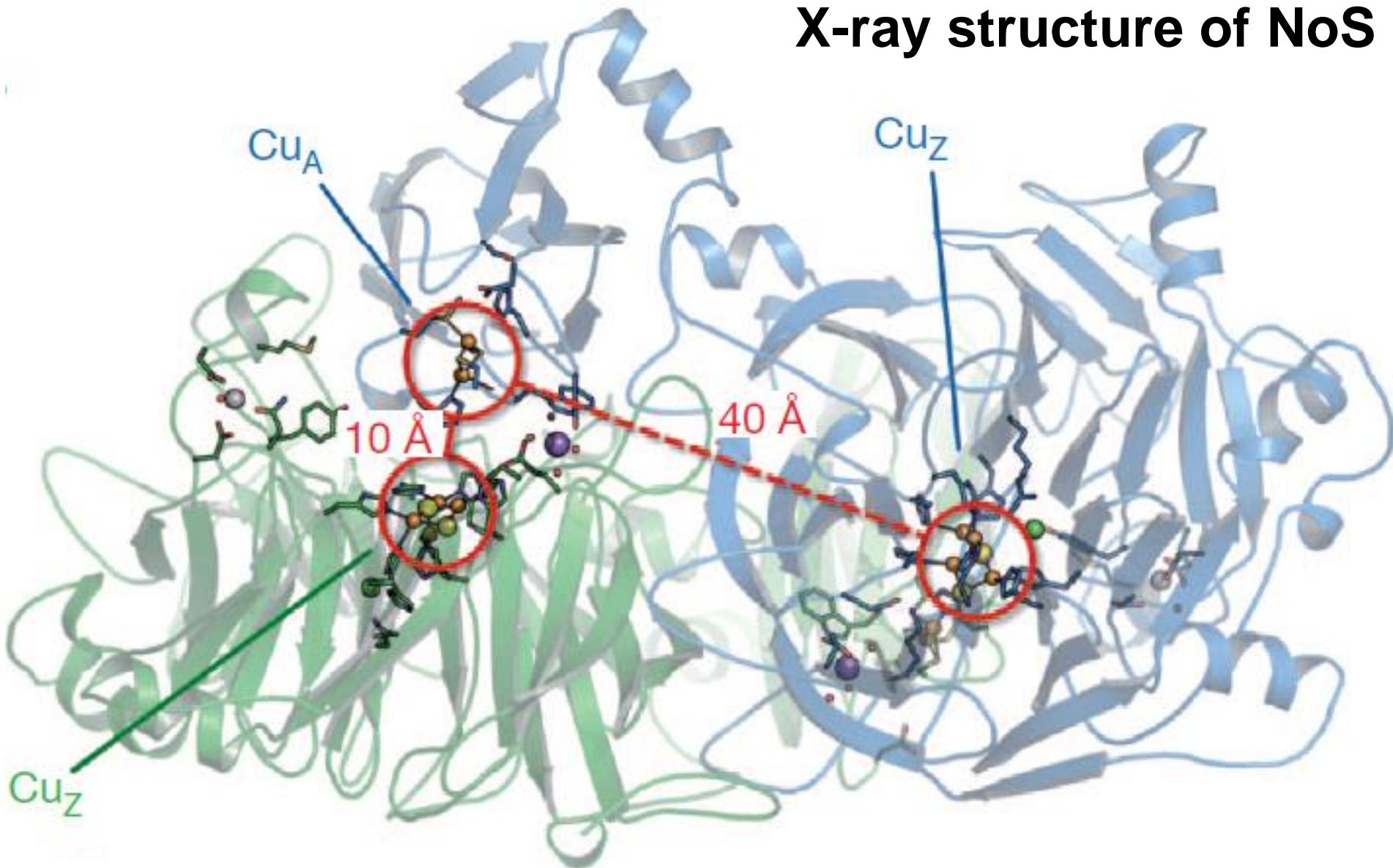
- Catalyzes the final step: **reduction of N₂O to give N₂**.



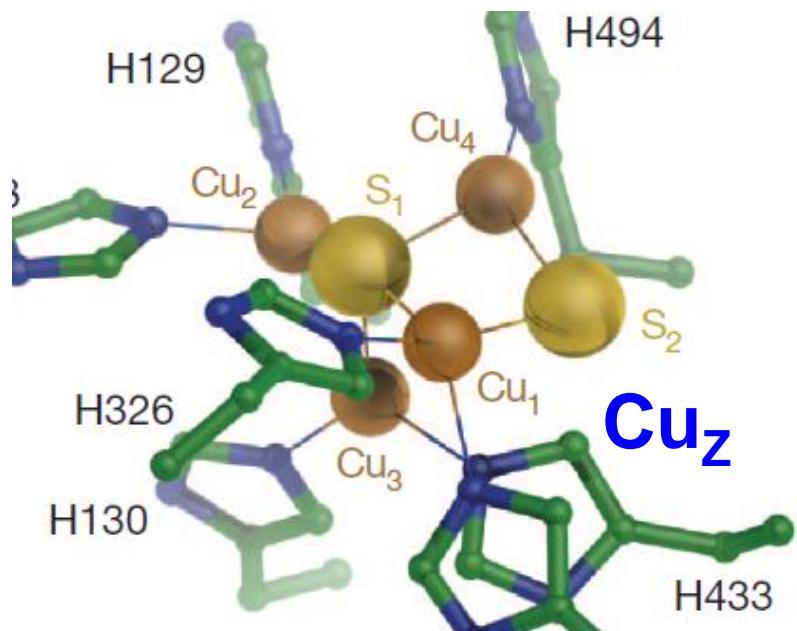
- A novel thiolate-bridged **tetrnuclear Cu_Z** center (catalytic site) & Type 4 Cu protein (**dinuclear Cu_A** center, **ET** site).



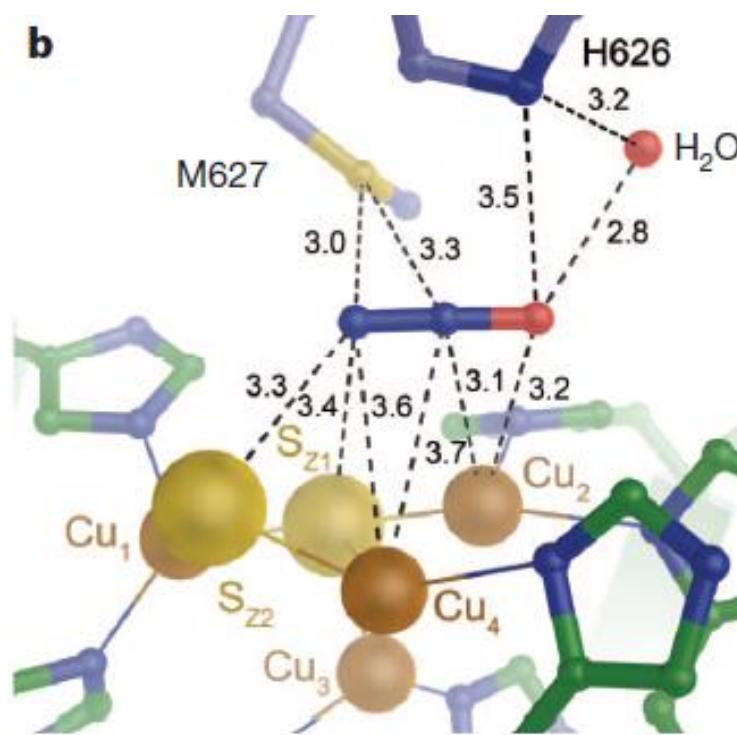
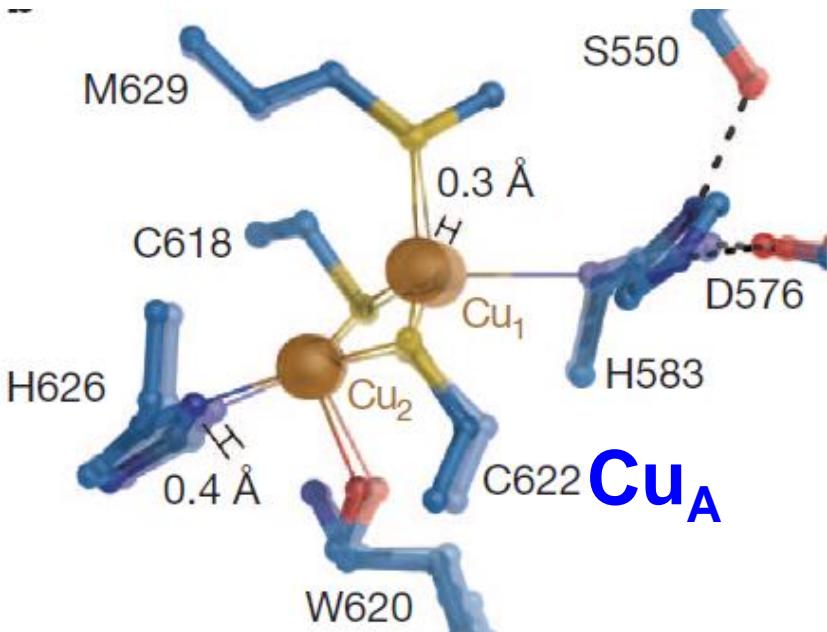
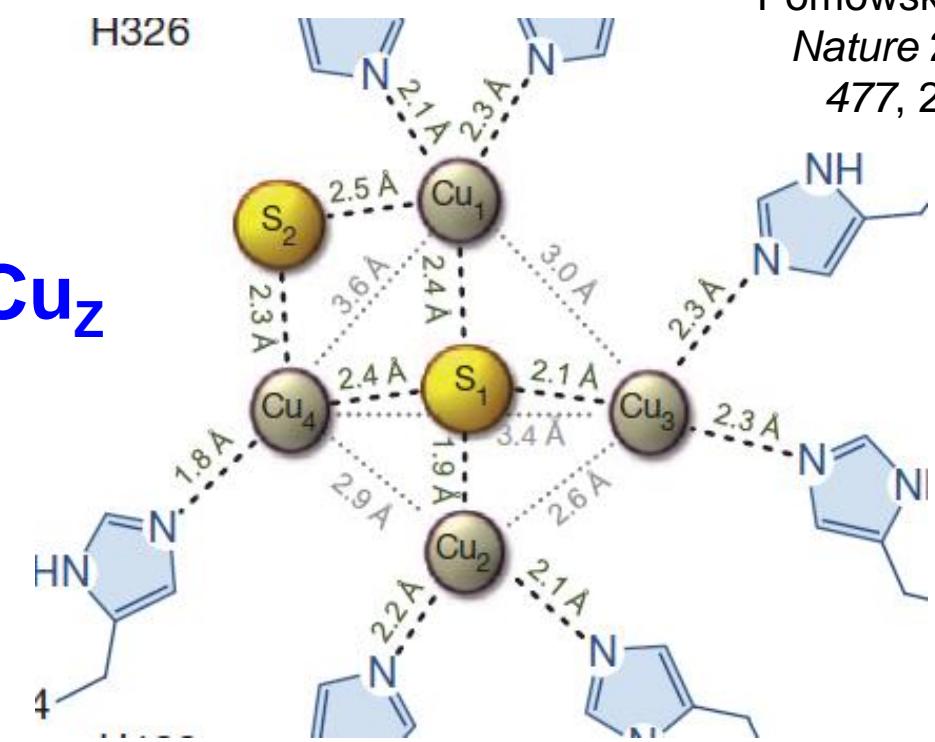
X-ray structure of NoS



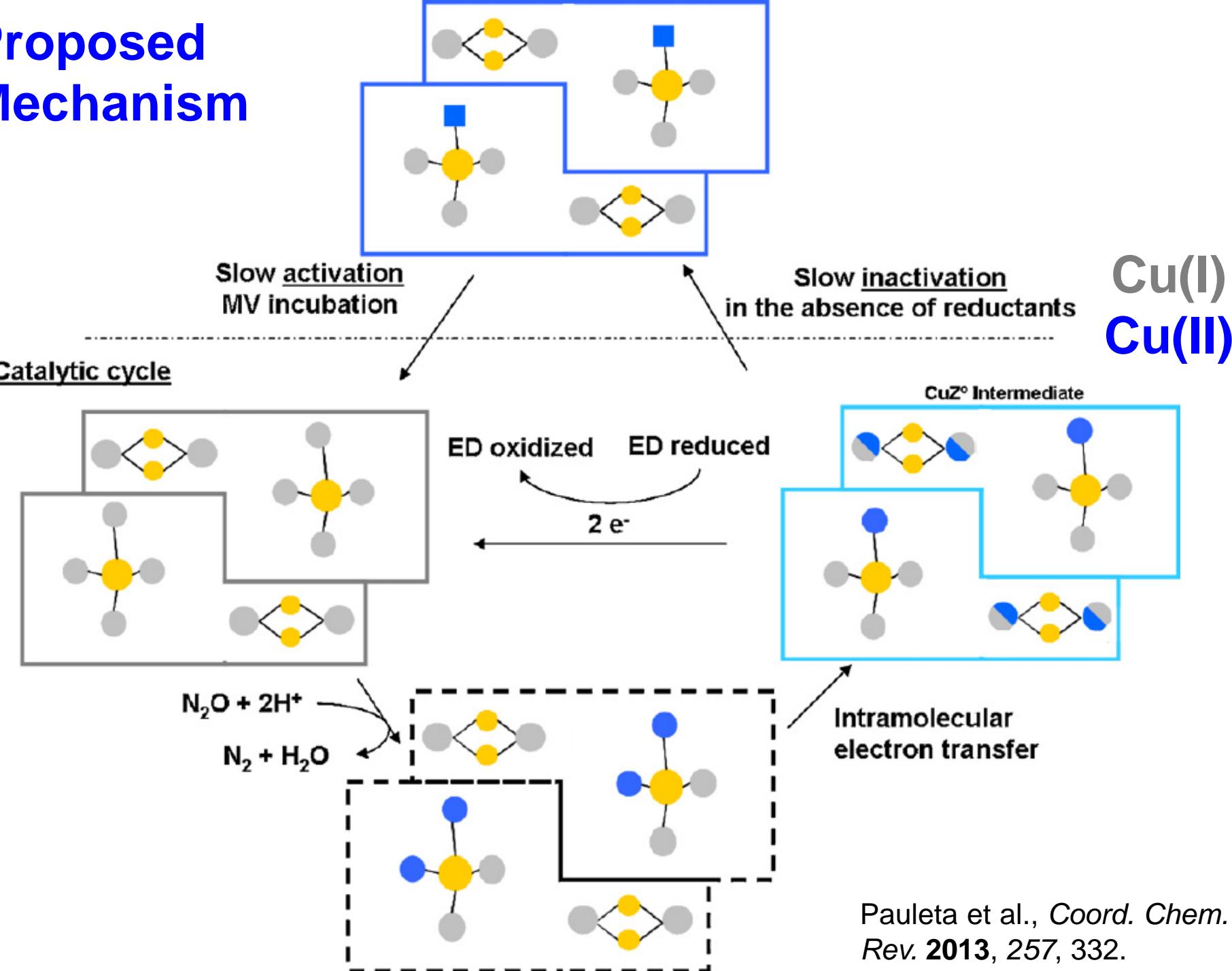
ET: Cu_A of one unit to Cu_Z of the other unit.

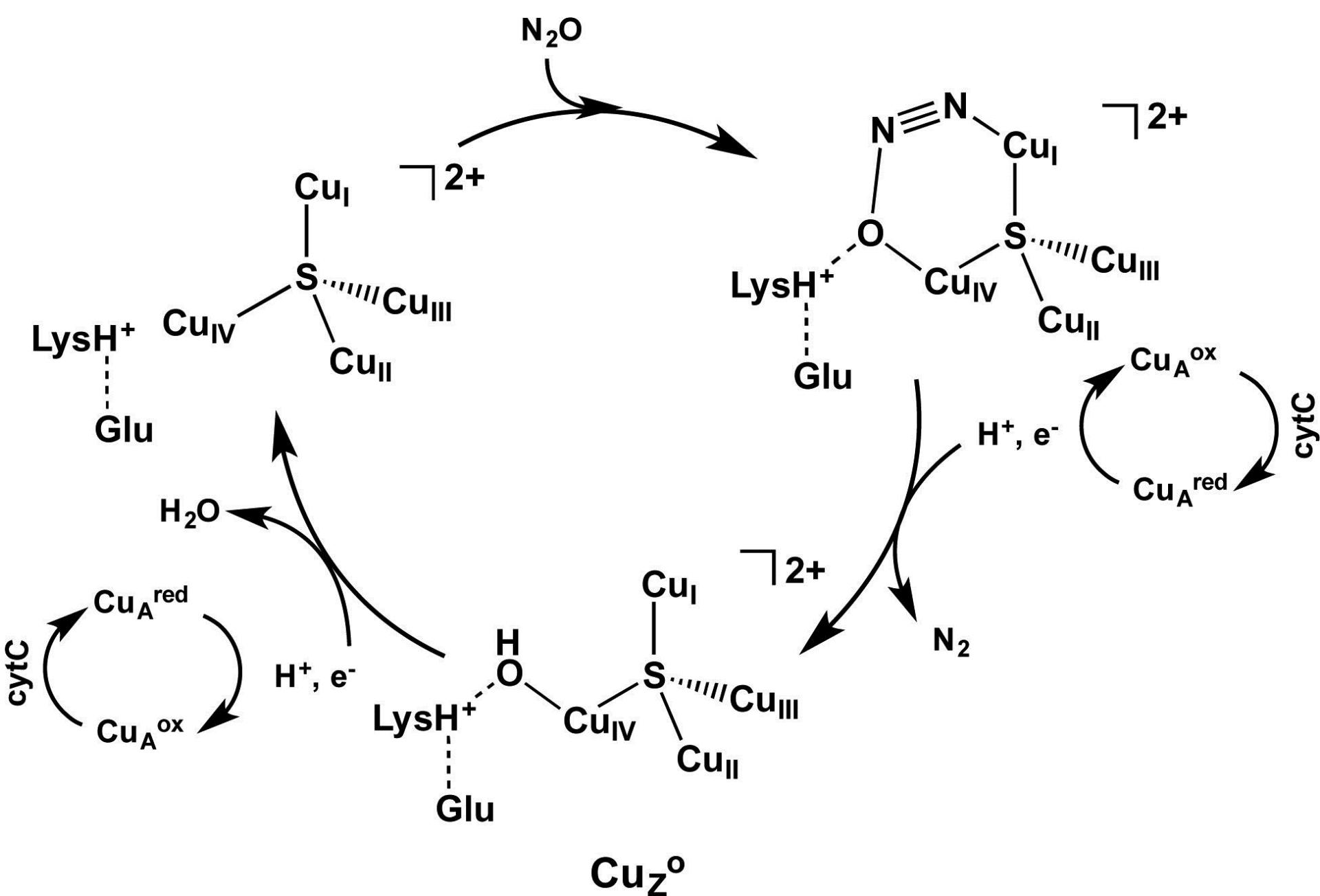


Pomowski et al.,
Nature **2011**,
477, 234.



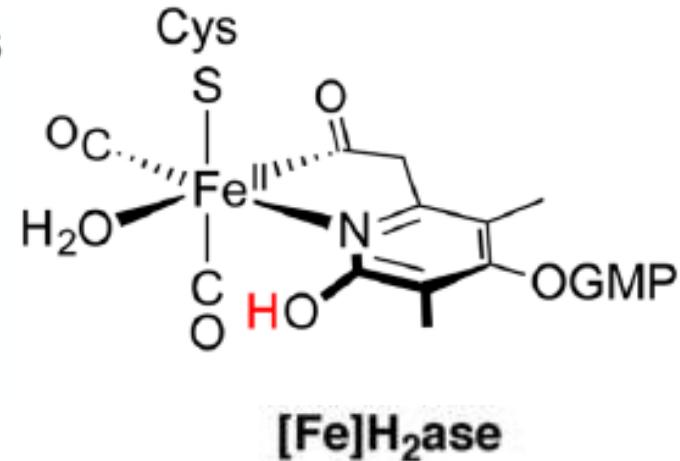
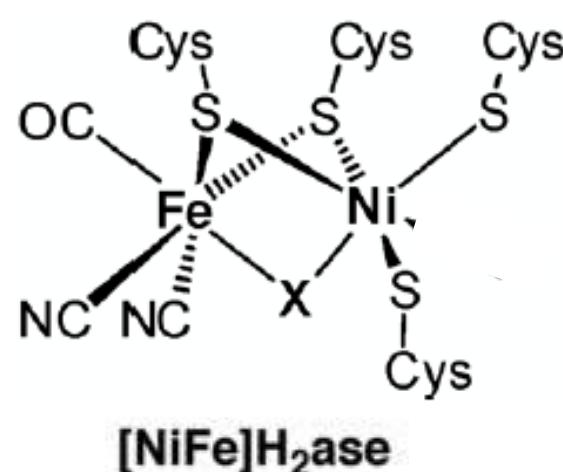
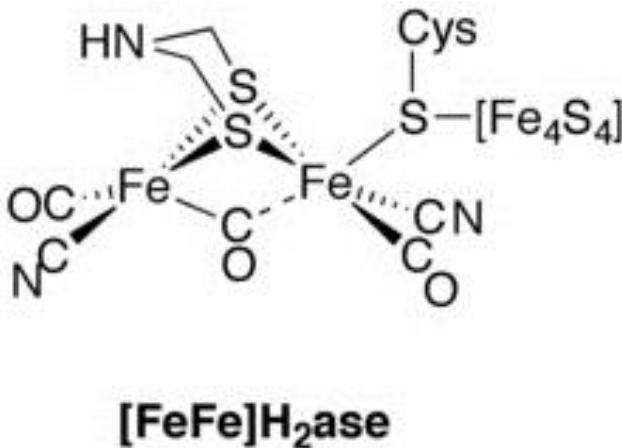
Proposed Mechanism





Key Summary

- **Hydrogenases** (H_2 ases) catalyze oxidation of H_2 or reduction of H^+ : $\text{H}_2 \rightleftharpoons 2 \text{ H}^+ + 2 \text{ e}^-$
- 3 types: **[FeFe] H_2 ase**, **[NiFe] H_2 ase** & **[Fe] H_2 ase**.
- **[FeFe] H_2 ases**: generally involved in the **H_2 formation**; rapidly & irreversibly oxidized/inactivated by O_2 .
- **[NiFe] H_2 ases**: generally involved in the **H_2 oxidation**; more O_2 tolerant.
- **[Fe] H_2 ase (Hmd)**: hydride transfer with the organic cofactor.



- 2 Reductive **CO₂ fixation** pathways: (1) **reduction of CO₂** to give CO catalyzed by carbon monoxide dehydrogenase (**CODH**) or give formate-type (Mo or W enzyme) → (2) add “H” to eventually form **methyl-intermediate** → (3) **methyl transfer** by methyl-transferase → (4) either **carbonylation** catalyzed by **ACS** or **reduction** to form **CH₄** catalyzed by **MCR**.

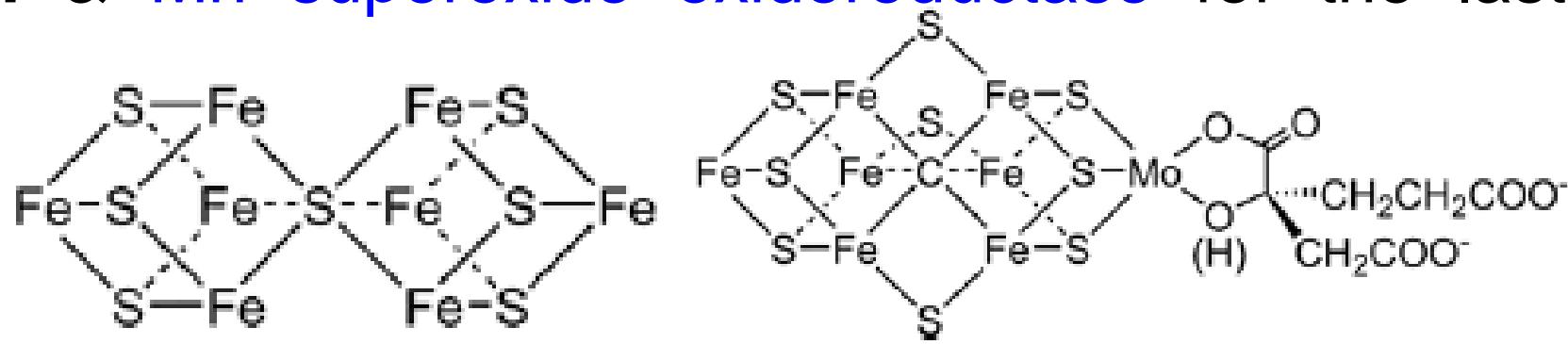
- 3 types of CODH: **Mo-CODH** (with 1 **Cu**), **Ni-CODH** & **Ni-CODH/ACS** (Acetyl-CoA Synthase).
- Methyltransferase: **Co(I)** of **Vitamin B₁₂**.
- **ACS** (**Ni, Fe, S**) catalyzes condensation of a CH₃ group, CO, & CoA to **form acetyl-CoA**.
- **Methyl-CoM Reductase** (MCR, **Ni**) reduces CH₃-SCoM to give **CH₄**.

- Biological N₂ fixation: Nitrogenase



- 4 types of nitrogenase: Mo-nitrogenase (Mo, Fe, S), V-nitrogenase (V, Fe, S), Fe-only nitrogenase (Fe, S) & *S. thermoautotrophicus* nitrogenase (Mo, Fe, S).

- Contains 2 complex cofactors (**P-cluster** & the **FeMo-cofactor**) in the most reactive Mo-nitrogenase.
- **Fe-protein** as the ET protein for the first 3 types, but **CODH** & **Mn superoxide oxidoreductase** for the last type.



- **Nitrification**: oxidation reactions to **convert NH₃ into NO₂⁻ or NO₃⁻**.
- Catalyzed by **3** different enzymes, **ammonia monooxygenase** (AMO), **hydroxylamine oxidoreductase** (HAO, **heme**) & **nitrite oxidoreductase** (NIO, **Mo**).
- **Denitrification**: reduction of **NO₃⁻, NO₂⁻, NO & N₂O** to form N₂.
- Catalyzed by **4** different enzymes, **nitrate reductase** (NaR, **Mo**), **nitrite reductase** (NiR, **heme or Cu**), **nitric oxide reductase** (NoR, **heme-nonheme (Fe_B)**) & **nitrous oxide reductase** (NoS, **Cu_A** & **Cu_Z**).

**Thank You for Your
Attention!
Any Questions?**