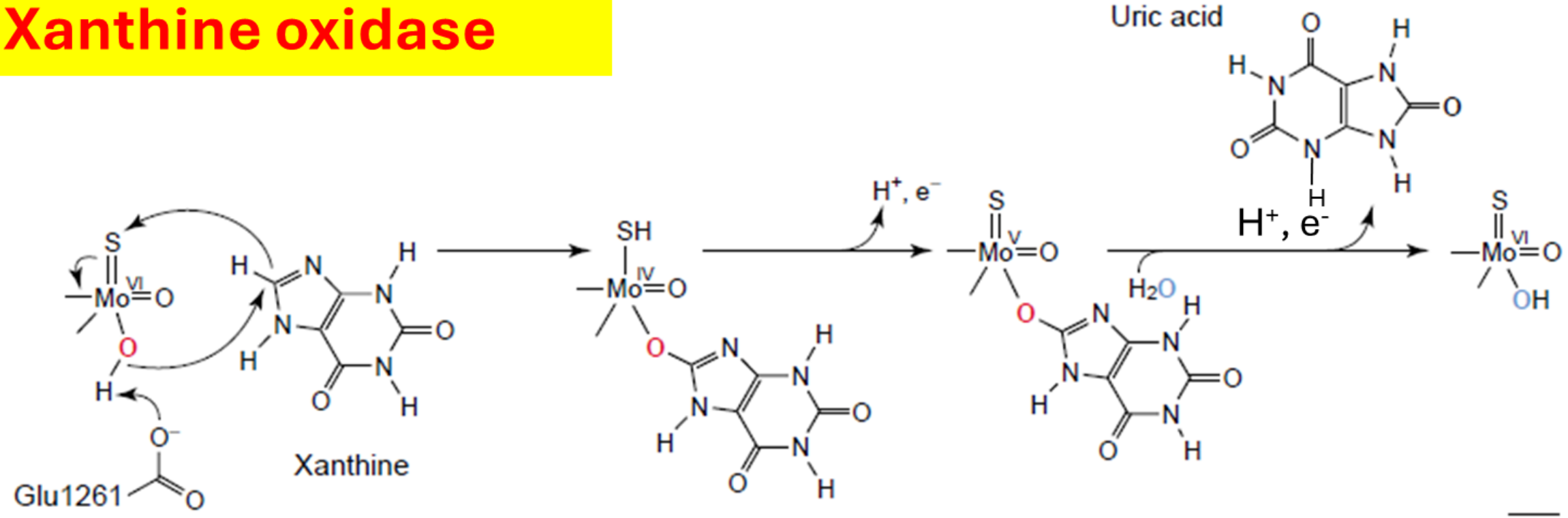
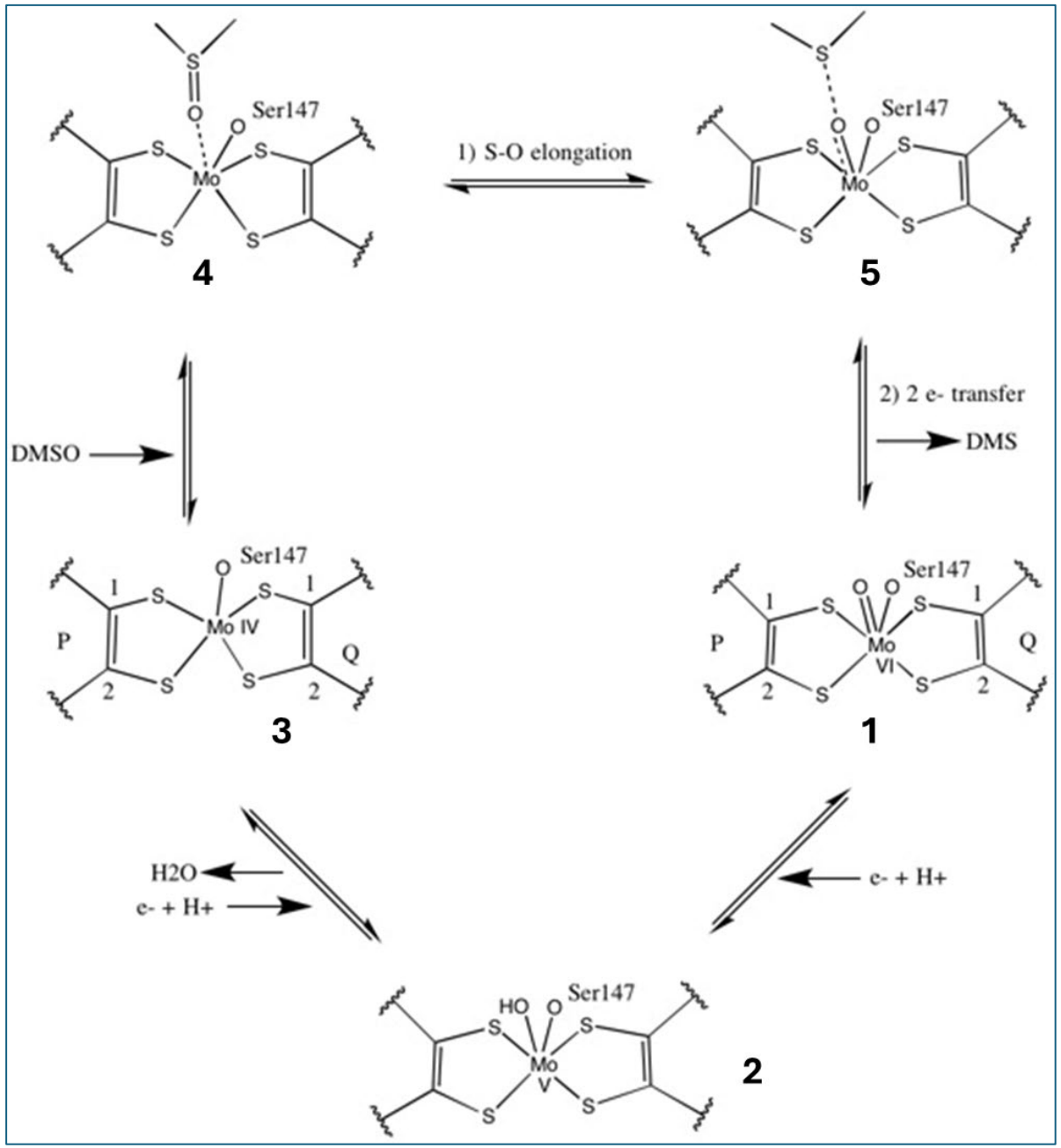
Xanthine oxidase



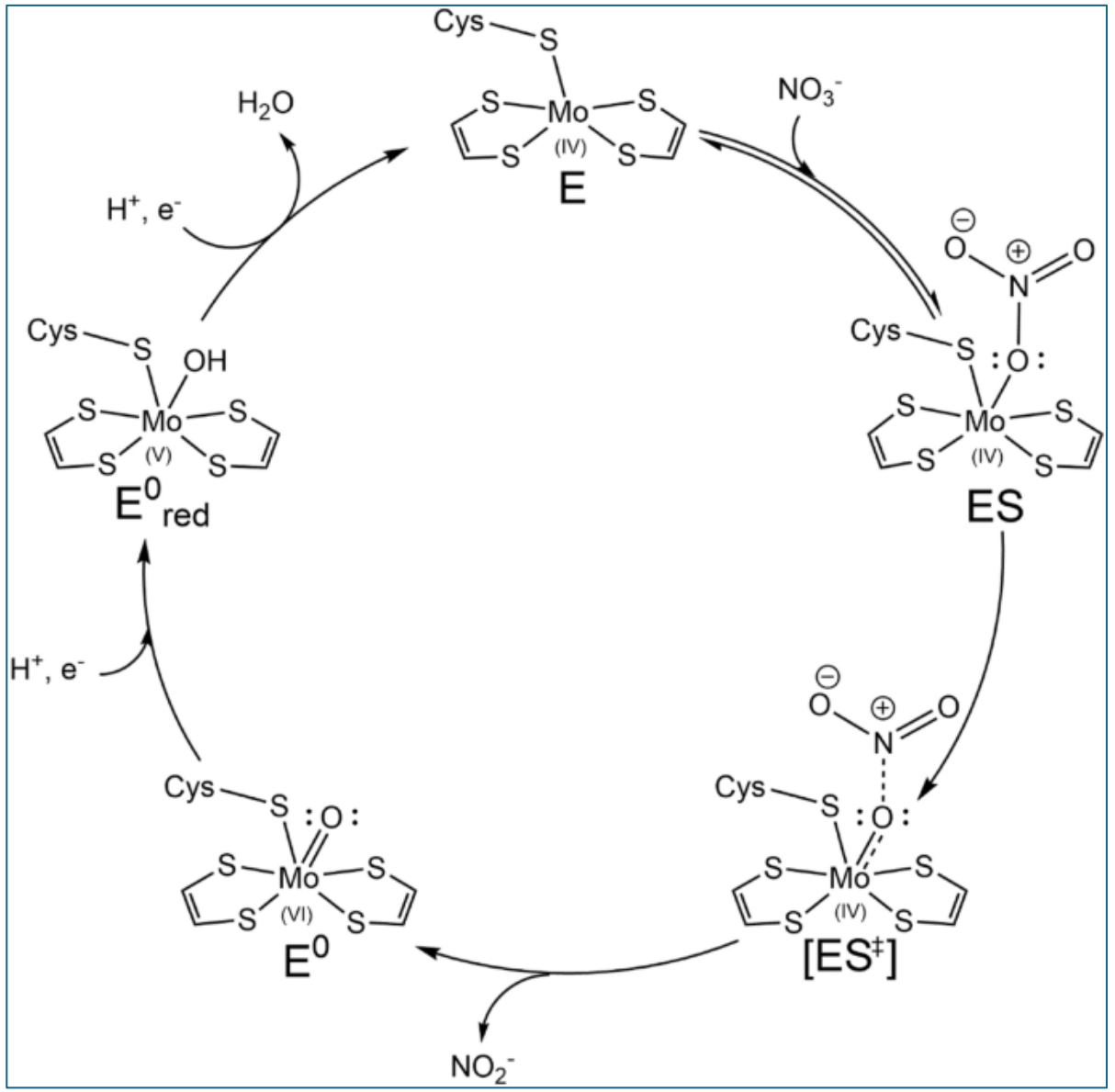
- The reaction mechanism of xanthine oxidase. Evidence indicates that catalysis is initiated by base-assisted nucleophilic attack of the Mo^{VI}–OH group on the C-8 position of the substrate.
- Hydride transfer to the Mo=S group leads to a reduced $LMo^{IV}O(SH)(OR)$ species, where OR represents the product coordinated to the molybdenum.
- Successive one-electron oxidation steps take the enzyme through an electron-paramagnetic-resonance-active Mo^{V} state before reaching the fully re-oxidized enzyme.
- Transfer of **oxygen** from the molybdenum center of the active site to uric acid is indicated in red and the Mo–OH group in blue.

Xanthine oxidase (inhibition)

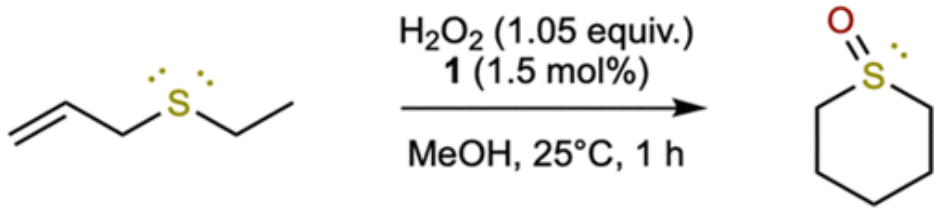
DMSO reductase

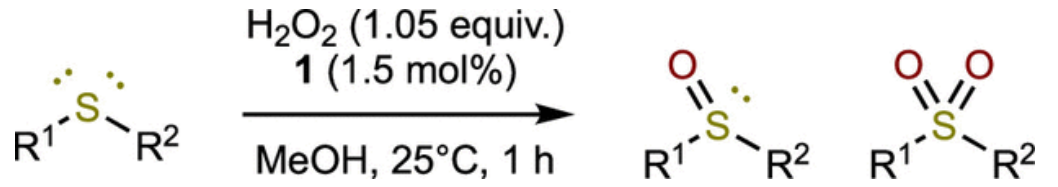


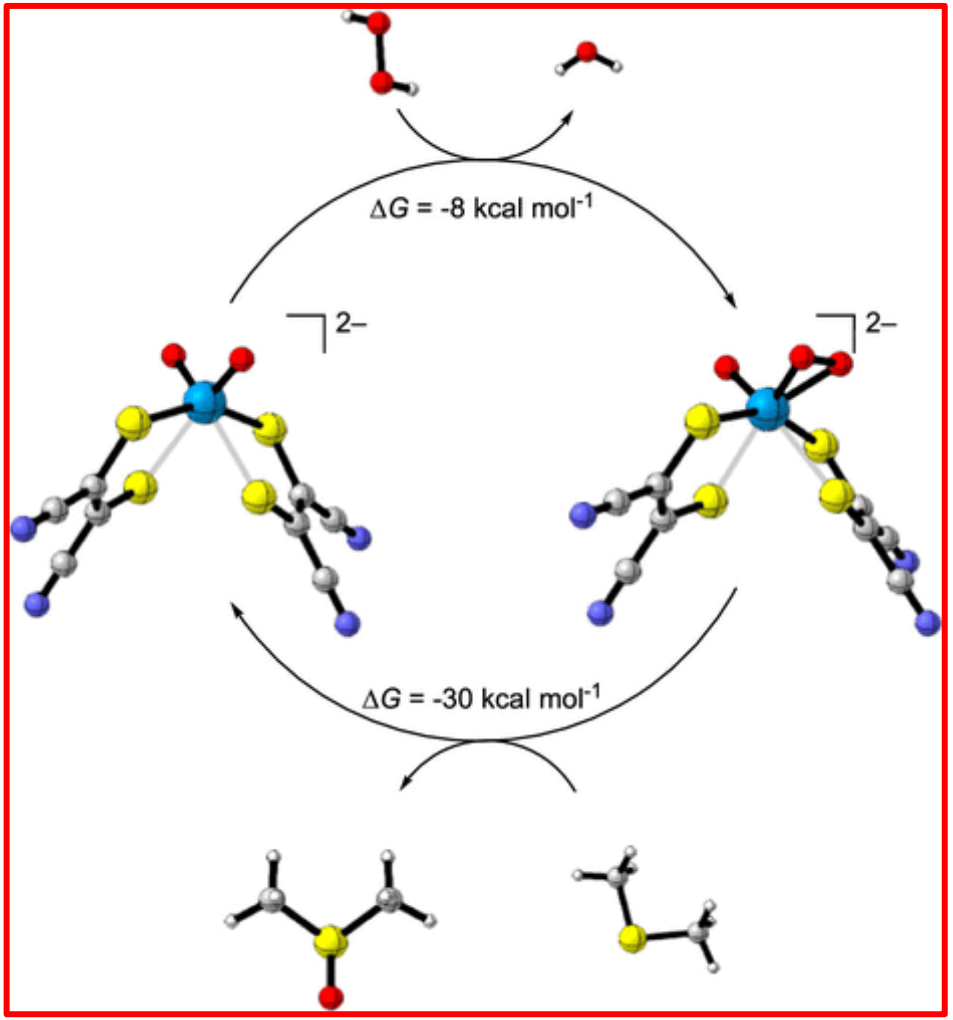
Nitrate reductase

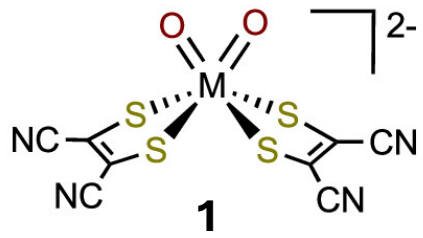


Detoxifying sulfur mustard



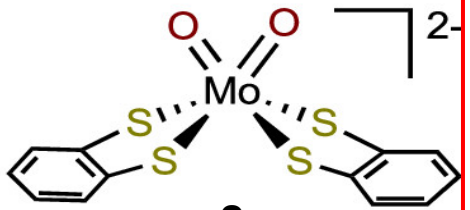






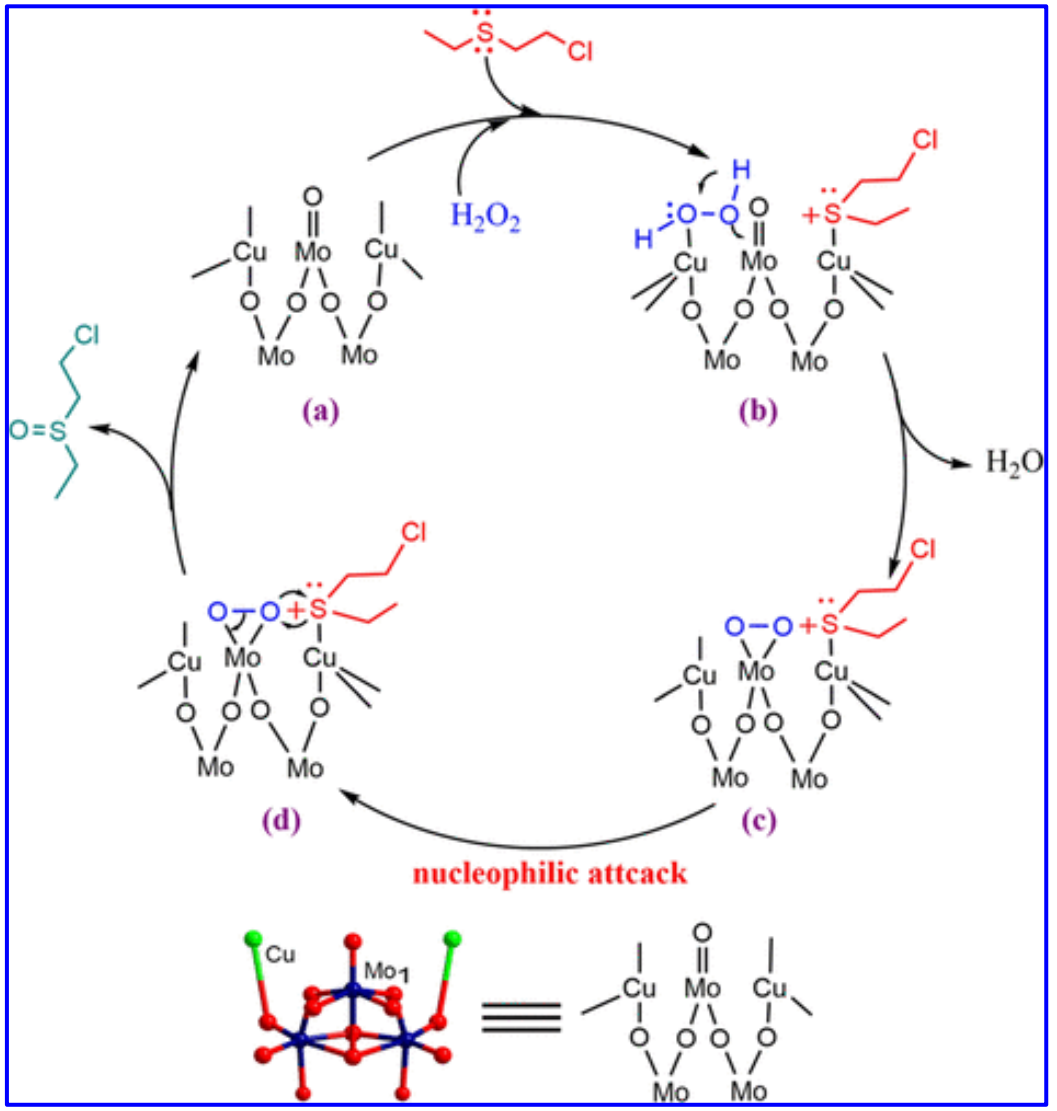
1

$[MO_2(mnt)_2]^{2-}$
M = Mo or W



2

$[MoO_2(bdt)_2]^{2-}$



2024: we are still learning from nature!
(Mankad et al. ACS catalysis 2024, 14, 9323-9327

Liu et al. *Inorg. Chem.* 2024, 63, 1, 346−352

Nitrogen reduction by nitrogenase

0 -3

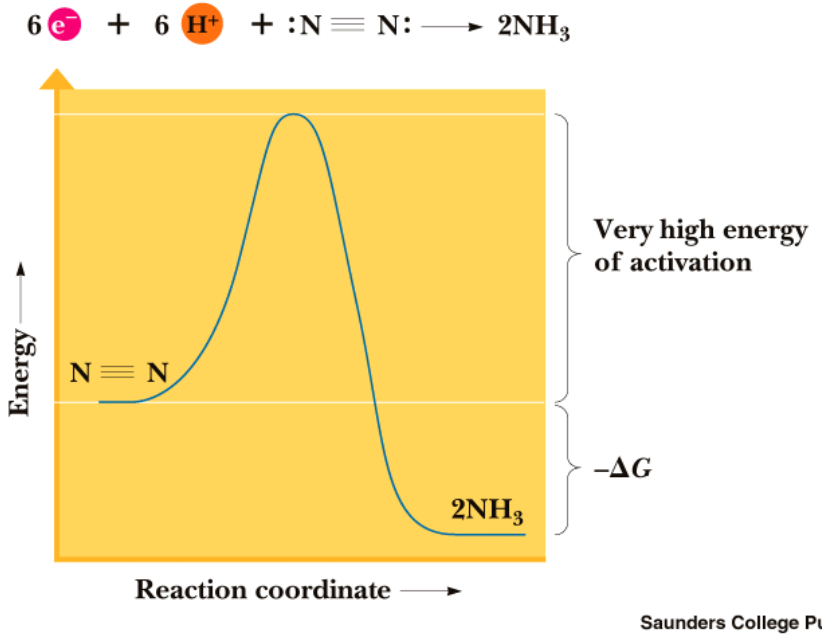
$N_2 + 3H_2 \rightarrow 2NH_3$ $\Delta G'^{o} = -33.5$ kJ/mol

However, N-N triple bond is a significant kinetic barrier **Bond energy = 930 kJ/mol**

Nitrogen is fixed by anaerobic bacteria

$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP + 16H_2O \rightarrow 2\,NH_3 + 16\,ADP + 16\,P_i + H_2$

Garrett & Grisham: Biochemistry, 2/e
Figure 26.4



Saunders College Publishing

Why should nitrogenase need ATP???

- N_2 reduction to ammonia is thermodynamically favorable
- However, the activatior barrier for breaking the N-N triple bond is enormous
- 16 ATP provide the needed activation energy

The FeMoCo

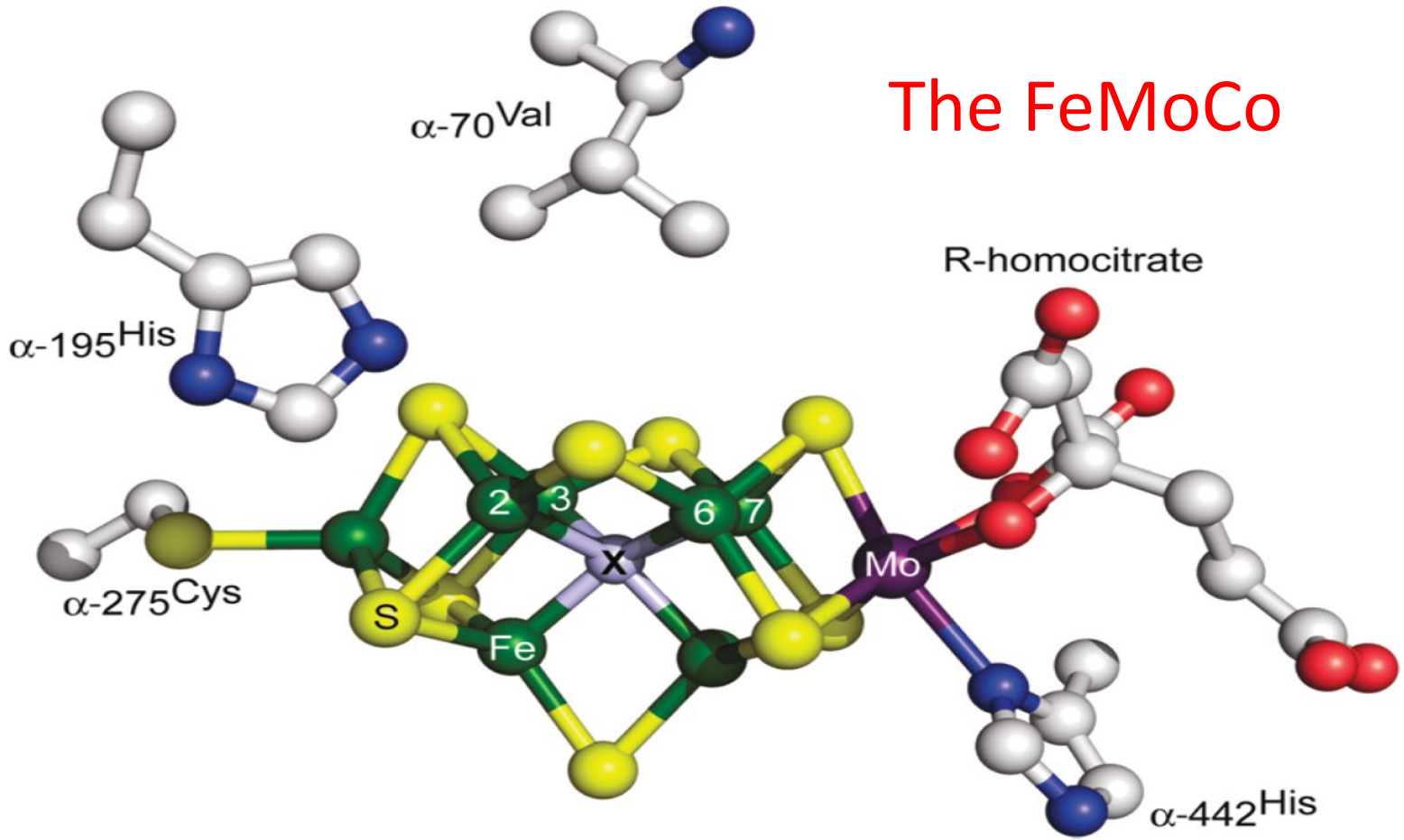
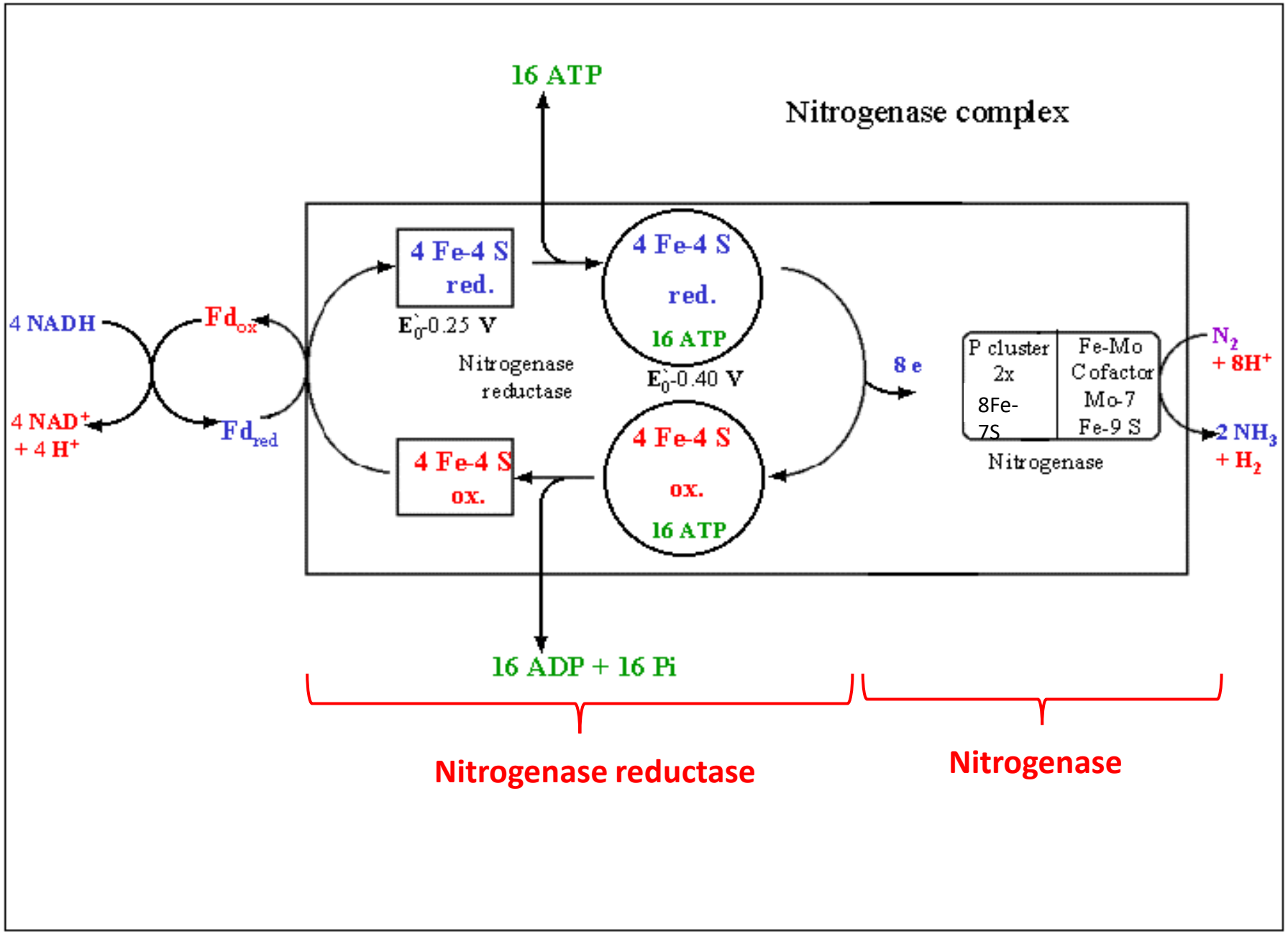
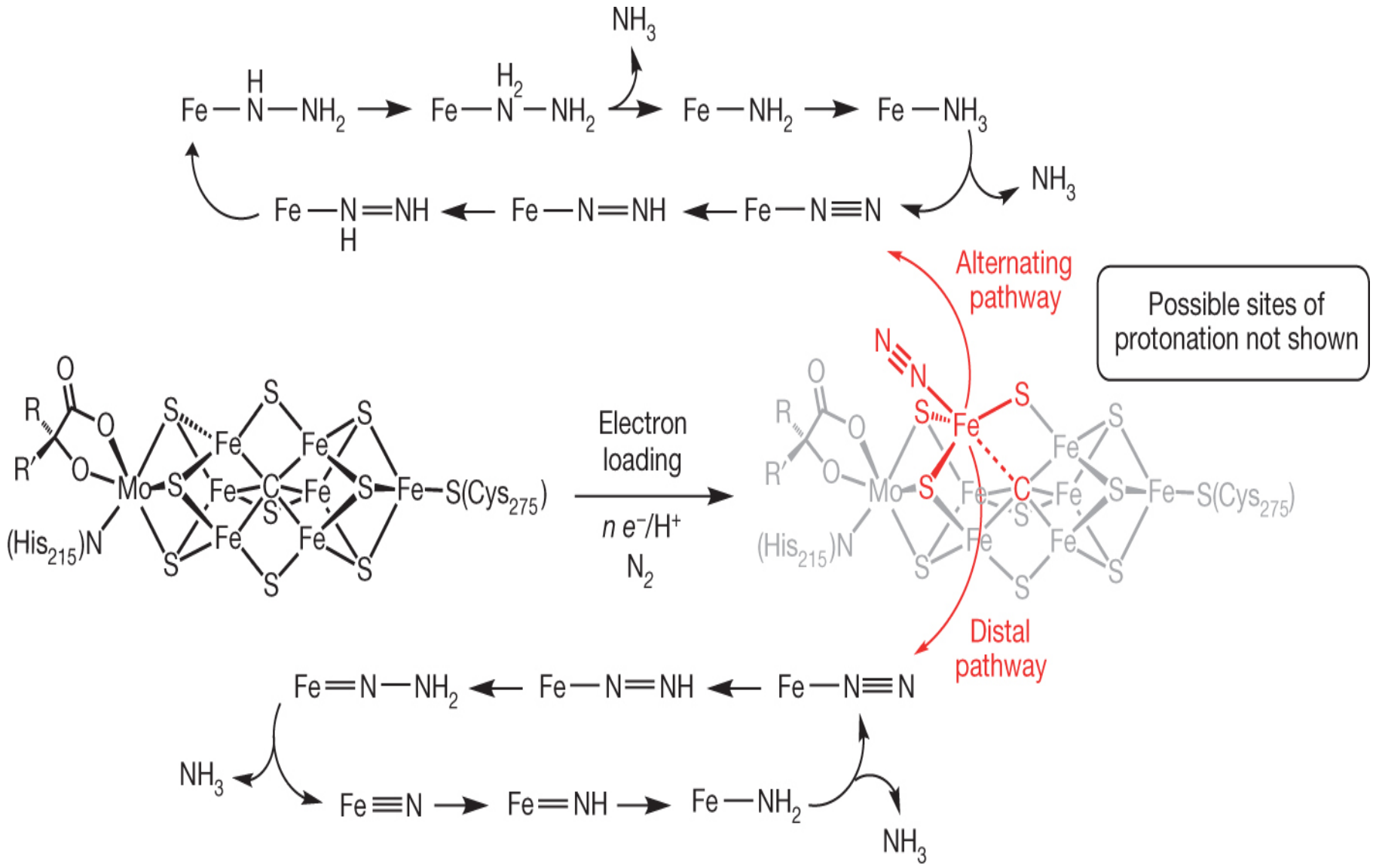


FIGURE 2. Structure of FeMo-co, including the two residues that covalently link it to the apoprotein and the two implicated in function, α-V70 as substrate "gatekeeper" and α-H195 as agent for proton delivery.

Nitrogen reduction by nitrogenase: role of nitrogenase reductase



Steps of nitrogen reduction by the FeMo-cofactor of nitrogenase.



JS Anderson *et al. Nature* **501**, 84-87 (2013) doi:10.1038/nature12435

nature

What do we know?

- the cycle is controlled by the electron donation of the Fe-protein cycle,
- protons and electrons are added one-by-one to the MoFe-protein,
- N_2 binds after the protein has been reduced by 3-4 electrons.

The conversion of N_2 to ammonia requires six electrons, thus consumes 12 units of MgATP. Each sacrificially produced mole of hydrogen additionally consumes 4 moles of MgATP. Thus the general reaction equation of nitrogenase turnover may be written as

$$N_2 + 8H^+ + 8e^- + 16MgATP + 16H_2O \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i \quad (3.2)$$

with MgATP being hydrolyzed to MgADP and phosphate (P_i). In pH-neutral conditions most of the ammonia will be protonated to NH_4^+.

1. H_2 is always produced during N_2 turnover but the ratio between N_2 and H_2 does not seem to be fixed in neither direction. This is called OHE (ordinary hydrogen evolution). H_2 is released when N_2 binds to the cluster [79] and can on the other hand displace bound N_2. H_2 is a competitive inhibitor[4] of N_2 turnover, but of no other substrate

2. H_2 is produced by nitrogenase if no other substrate is available

What do we know?

Charge state of the resting state. One of the most significant measurable quantities of the cofactor is its $S = 3/2$ spin signal. This shows that a well-defined, odd number of electrons always resides on the cluster. This defines its charge state.

The chemistry of the cofactor changes upon reduction and protonation. Therefore reliable information about the charge state of the resting state is essential for qualitatively correct simulations of the mechanism.

Atom type of the central ligand. Crystallographic analysis has restricted the central ligand to be C, N, or O. While there are some indications that it is nitrogen, its identity has not been clearly determined up to now. The different sizes of these atom types change the reactivity of the central cage.

Binding modes of N_2. At the heart of the open questions is that of the binding modes of dinitrogen to FeMoco. They are the starting points for the reduction mechanism. The protonation states have to be investigated before answering the question of N_2 binding modes.

There is little evidence for binding of any substrate to the resting state of the MoFe protein, and N_2 does not appear to bind until the MoFe protein has been activated by the accumulation of three or four electrons and protons.