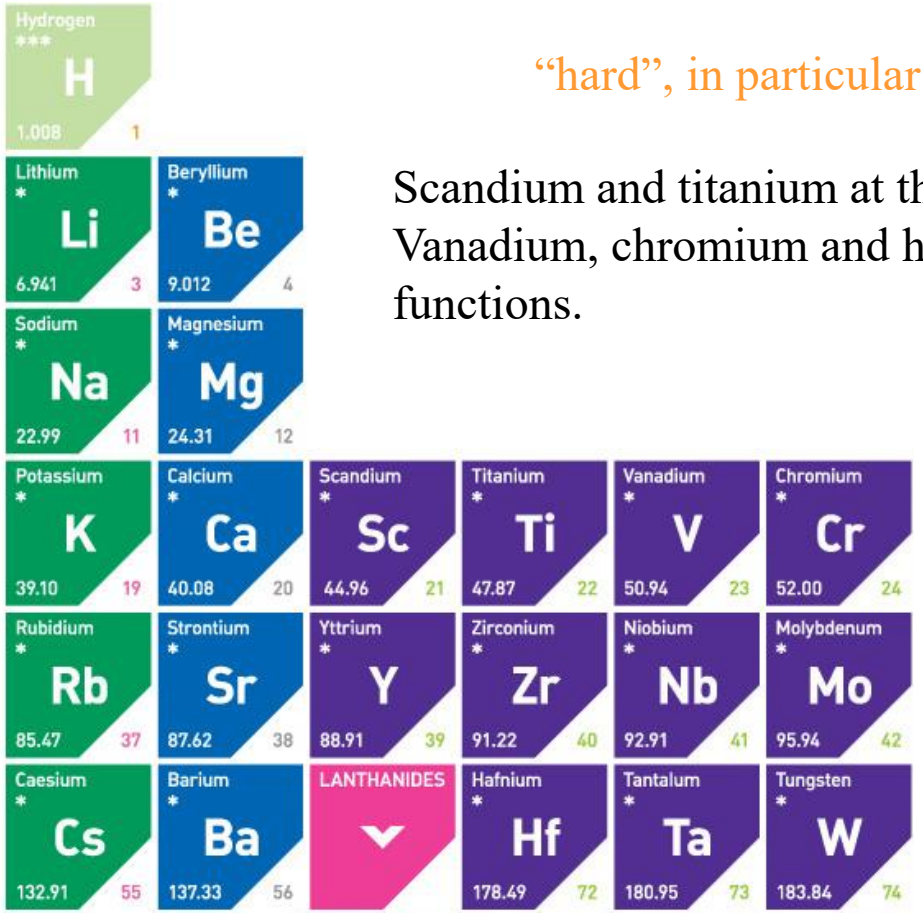
Molybdenum Oxotransferase Enzymes

In contrast to “late” transition metals such as cobalt, nickel and copper, the metals from the left half of the transition-element periods favor

high oxidation states

high coordination numbers

“hard”, in particular negatively charged, oxygen donor coordination centers



Scandium and titanium at the beginning of the first (3d) row do not have any biochemical relevance
Vanadium, chromium and homologues molybdenum and tungsten exhibit several physiological functions.

Molybdenum is the biologically most important element in this series:

oxygen transfer

nitrogen fixation

Molybdenum is the **only element from the second (4d) period** of the transition metals **with a biological function**.

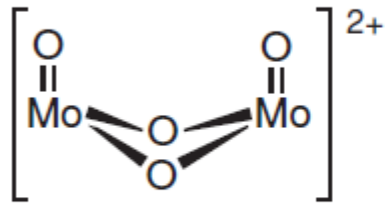
One explanation: its bioavailability

Although molybdenum is quite rare in the earth's crust, like all other heavy metals from this part of the periodic table, it is quite soluble in (sea)water at pH 7 (approximately 100 mM) in its most stable, hexavalent form as molybdate(VI), MoO_4^{2-}.

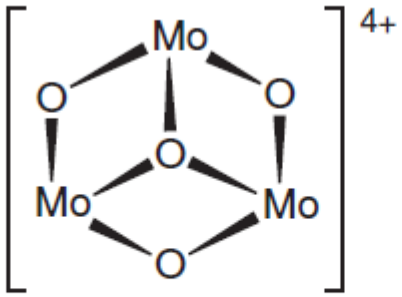
The **chromate(VI) ion, CrO_4^{2-}**, which is also quite soluble at pH 7, behaves as a strong oxidant and is thus **unstable under physiological conditions**

For **oxidation states lower than +VI**, the chemistry of molybdenum in aqueous solution is characterized by **aggregation phenomena**

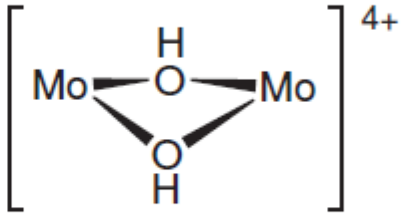
-formation of "clusters"-



Mo^{V}



Mo^{IV}



Mo^{III}

Mo^{II}

Mo ions connected by hydroxo or oxo bridges and coordinatively saturated by water ligands

Such aggregation can be suppressed **in the presence of a protein** acting as a multidentate and protecting chelate system or **under utilization of special cofactor ligands**

The physiologically relevant oxidation states of **molybdenum** lie **between +IV and +VI**, and the corresponding **redox potentials of about −0.3 V** are physiologically acceptable

At these oxidation states, molybdenum shows affinity towards:

negatively charged O *and* S ligands such as oxide, sulfide, thiolates and hydroxide and nitrogen ligands

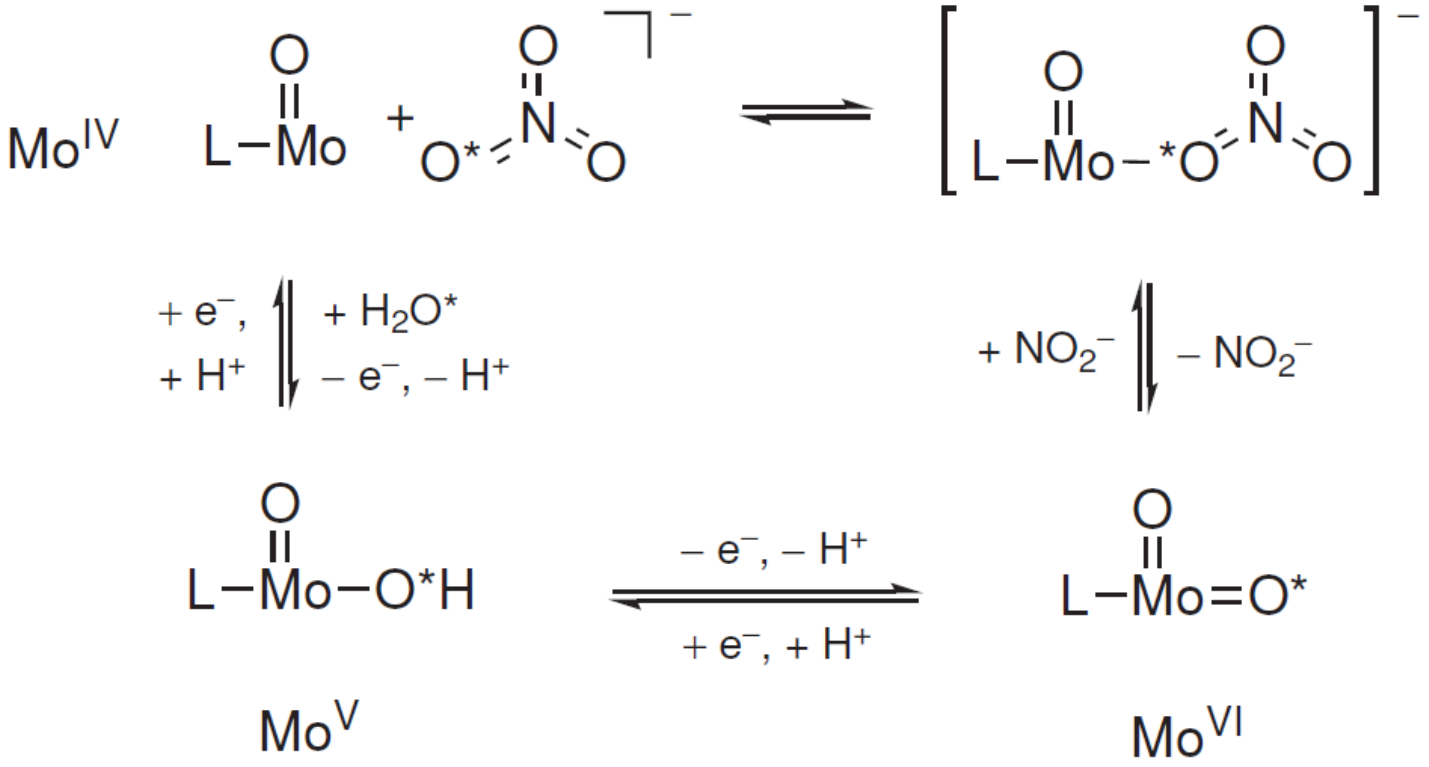
Molybdenum in enzymes is the **catalysis of controlled oxygen transfer to or from a two-electron substrate**, which involves spatially separated one-electron-transferring components such as cytochromes, Fe/S centers or flavins

Coupling of electron transfer and oxide exchange leads to the formal transfer of an oxygen

$$LMo(VI)O_2 + X \Rightarrow LMo(IV)O + XO$$

The transferred oxygen **does *not* originate from O_2**!!

Temporal and spatial separation of the electron transfer and the actual oxygen translocation



Regeneration of the reduced enzyme may proceed **via O_2 as the eventual oxidant** yield peroxides or superoxide

L: ligands in the coordination sphere of molybdenum

O*: ^{18}O labelling

Some molybdenum-containing hydroxylases and correspondingly catalyzed reactions

the best characterized are **xanthine oxidase**, **sulfite oxidase** and **nitrate reductase**

Enzyme	Molecular mass (kDa)	Prosthetic groups	Typical function
xanthine oxidase	275 (dimer)	2 Mo, 4 Fe_2S_2, 2 FAD	oxidation of xanthine to uric acid in liver and kidney
nitrate reductase	228 (dimer)	2 Mo, 2 cyt *b*, 2 FAD	nitrate/nitrite transformation in plants and microorganisms $NO_3^- + 2\,H^+ + 2\,e^- \rightleftharpoons NO^- + H_2O$
aldehyde oxidase	280 (dimer)	2 Mo, 4 Fe_2S_2, 2 FAD	oxidation of aldehydes, heterocycles, amines and sulfides in liver
sulfite oxidase	110 (dimer)	2 Mo, 2 cyt *b*	sulfite/sulfate transformation in liver (sulfite detoxification) $SO_3^{2-} + H_2O \rightleftharpoons SO_4^{2-} + 2\,e^- + 2\,H^+$
arsenite oxidase	85	Mo, Fe_nS_n	transformation of thiolate-blocking AsO_2^- by microorganisms: $AsO_2^- + 2\,H_2O \rightleftharpoons AsO_4^{3-} + 2\,e^- + 4\,H^+$
formate dehydrogenase (Mo)	>100	Mo, Fe_nS_n, Se	CO, reduction by microorganisms: $HCOO^- \rightleftharpoons CO_2 + 2\,e^- + H^+$
formate dehydrogenase (W)	340	W, Fe_nS_n, Se	CO, reduction by microorganisms: $HCOO^- \rightleftharpoons CO_2 + 2\,e^- + H^+$

Examples of malfunctions of molybdoenzymes in higher organisms

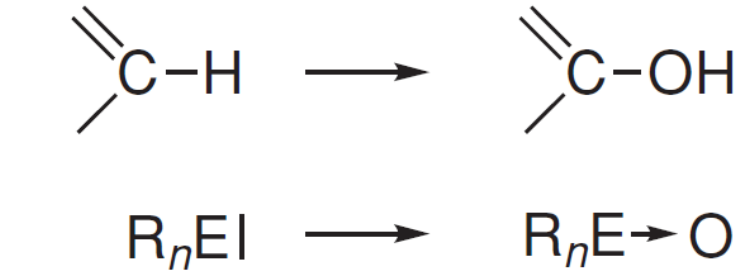
- dysfunction of xanthine oxidase impacts the purine metabolism (→ gout)
- sulfite oxidase dysfunction generates neurological disorders

The Cu/MoS antagonism known from cattle breeding

Tetrathiomolybdates(VI), which are formed in the complex stomach of ruminants from molybdate and electron-rich sulfide, act as efficient chelating ligands for positively charged, albeit electron-rich ("soft"), metal ions such as Cu^{+}.

Secondary deficiency of copper (necessary for collagenases) which is responsible for a weakening of connective tissue in animals that graze on molybdenum-rich soil.

In general terms, molybdoenzymes catalyze the reactions



E = N, S, As

In biological oxidations that are not directly dependent on O_2, the oxidation equivalents are made available in one-electron steps via electron-transfer proteins.

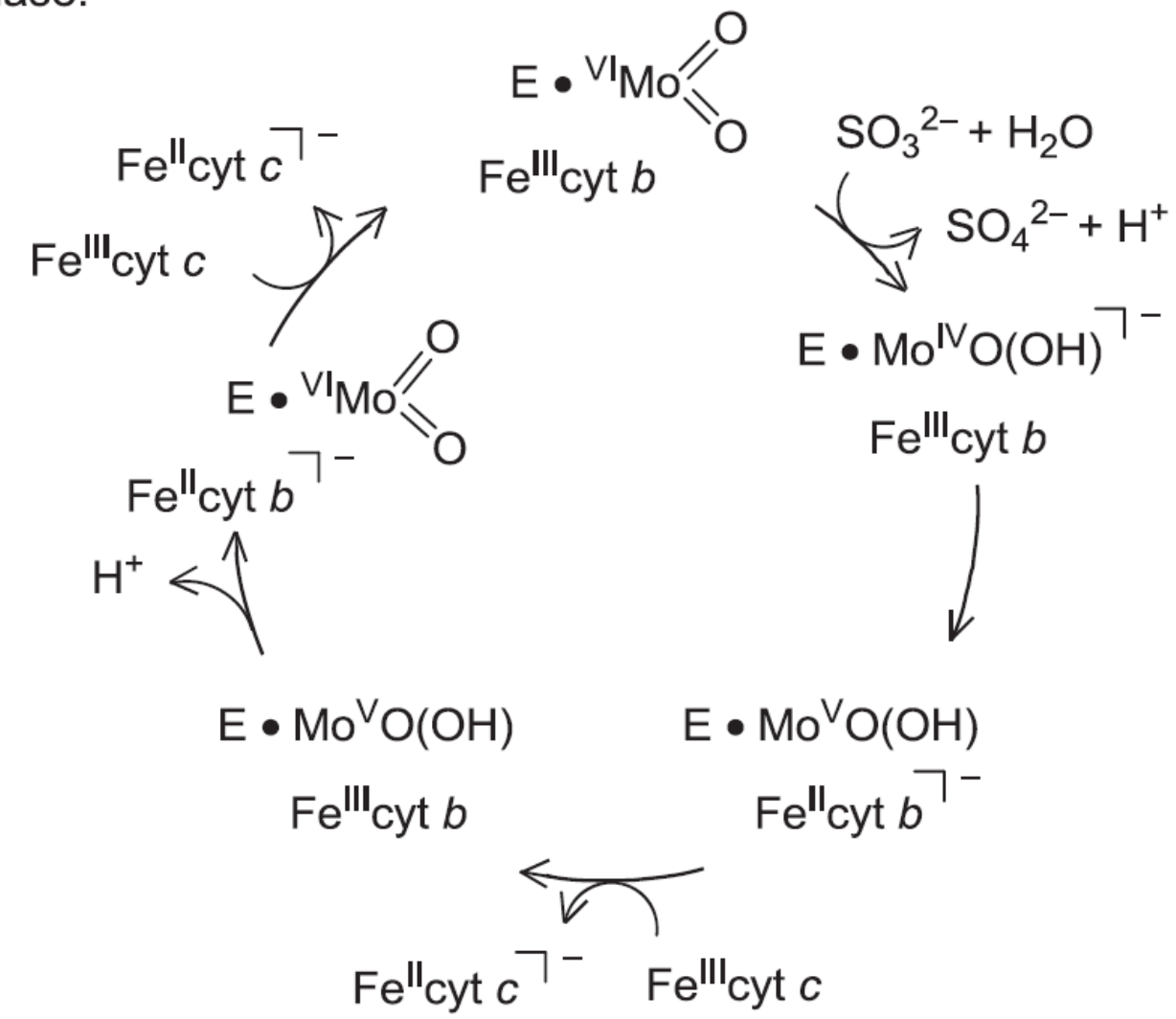
Molybdoenzymes **contain electron-transfer components** such as cytochromes, Fe/S centers or flavins

Catalytic cycle for the oxidation of sulfite to sulfate

d_1-configurated Mo(V) is observed by EPR

Oxide ligands either get protonated after reduction due to their strongly increased basicity **or** will be **replaced by hydroxide from the surrounding water** after complete oxygen atom transfer.

sulfite oxidase:



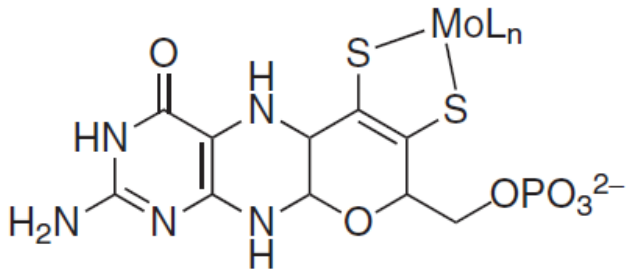
E: (apo-)enzyme; Fe cyt: cytochrome with iron oxidation state

overall reaction: $SO_3^{2-} + H_2O + 2\ Fe^{III}cyt\ c \longrightarrow SO_4^{2-} + 2\ H^+ + 2\ Fe^{II}cyt\ c$

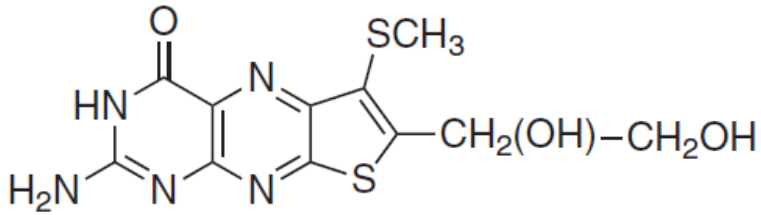
All molybdenum- or tungsten-containing hydroxylases contain cofactor, consisting of molybdenum (or tungsten) and a **special molybdopterin/tungstopterin** ligand.

It is a tetrahydropterin derivative, “pyranopterin”, which has a very characteristic metal-coordinating ene-1,2-dithiolate/“dithiolene” (non-innocent) chelate function.

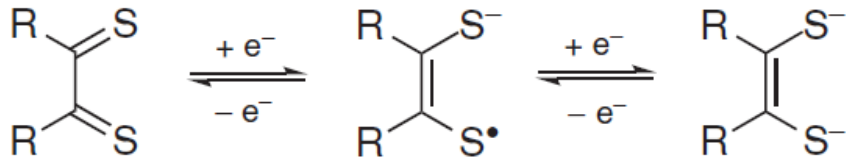
It is metabolized at urothione.



Mo-cofactor ("Moco"); L_n: ligands



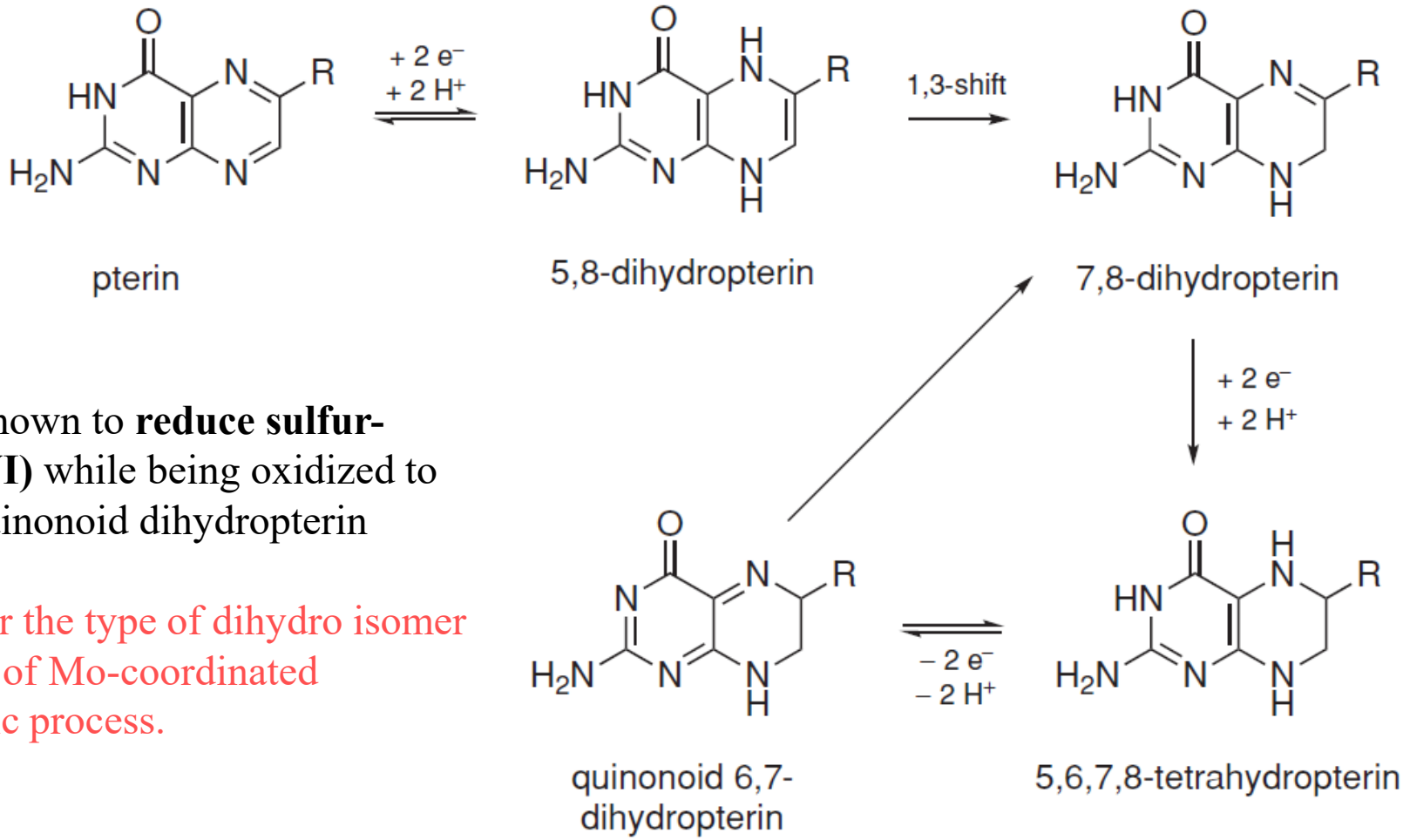
urothione



1,2-dithio-1,2-diketone

enedithiolate(2–)

Dithiolenes and the pterins are metal-coordinating potentially non-innocent ligands

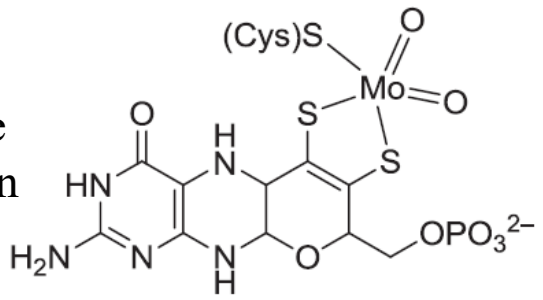


Tetrahydropterins have been shown to **reduce sulfur-coordinating molybdenum(VI)** while being oxidized to the enzymatically reducible quinonoid dihydropterin

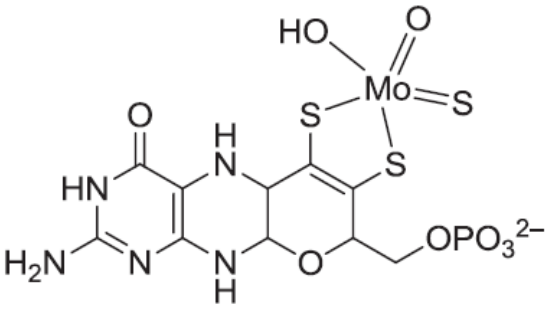
No unambiguous evidence for the type of dihydro isomer *in vivo* or of a redox reactivity of Mo-coordinated molybdopterin in the enzymatic process.

Three families of molybdoenzyme are distinguished:

The **sulfite oxidase family** is characterized by one S,S-coordinated pyranopterin and one cysteinate ligand and by oxo functions at the metal, which may be converted on reduction



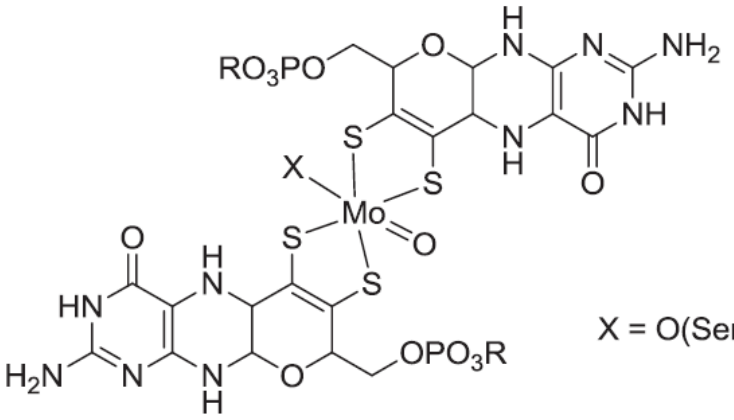
sulfite oxidase type



xanthine oxidase type

Mo(VI) state of enzymes of the **xanthine oxidase family**, one **sulfide ligand is bound at** an **Mo**–S distance of 215 pm

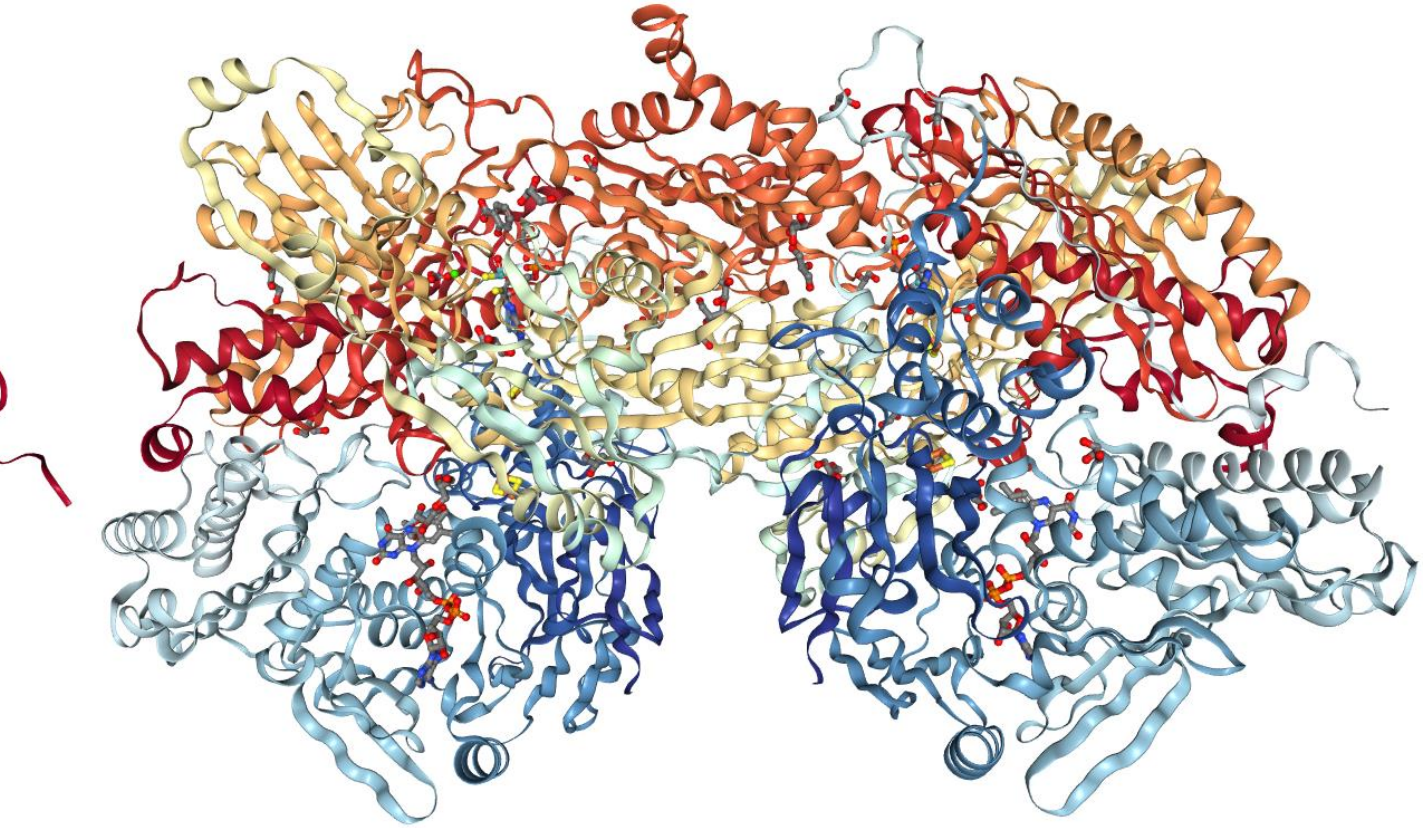
DMSO reductase family involves two pyranopterin ligands, an oxo function and a further monodentate ligand such as cysteinate, selenocysteinate, serin or aspartate

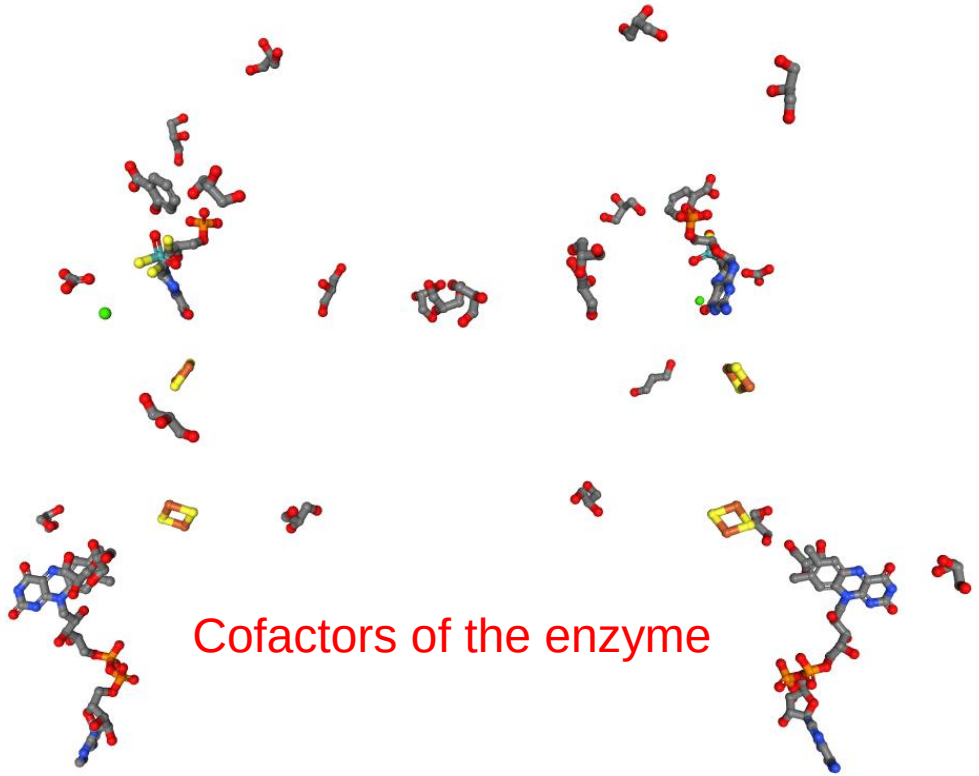


X = O(Ser) or Se(Cys)

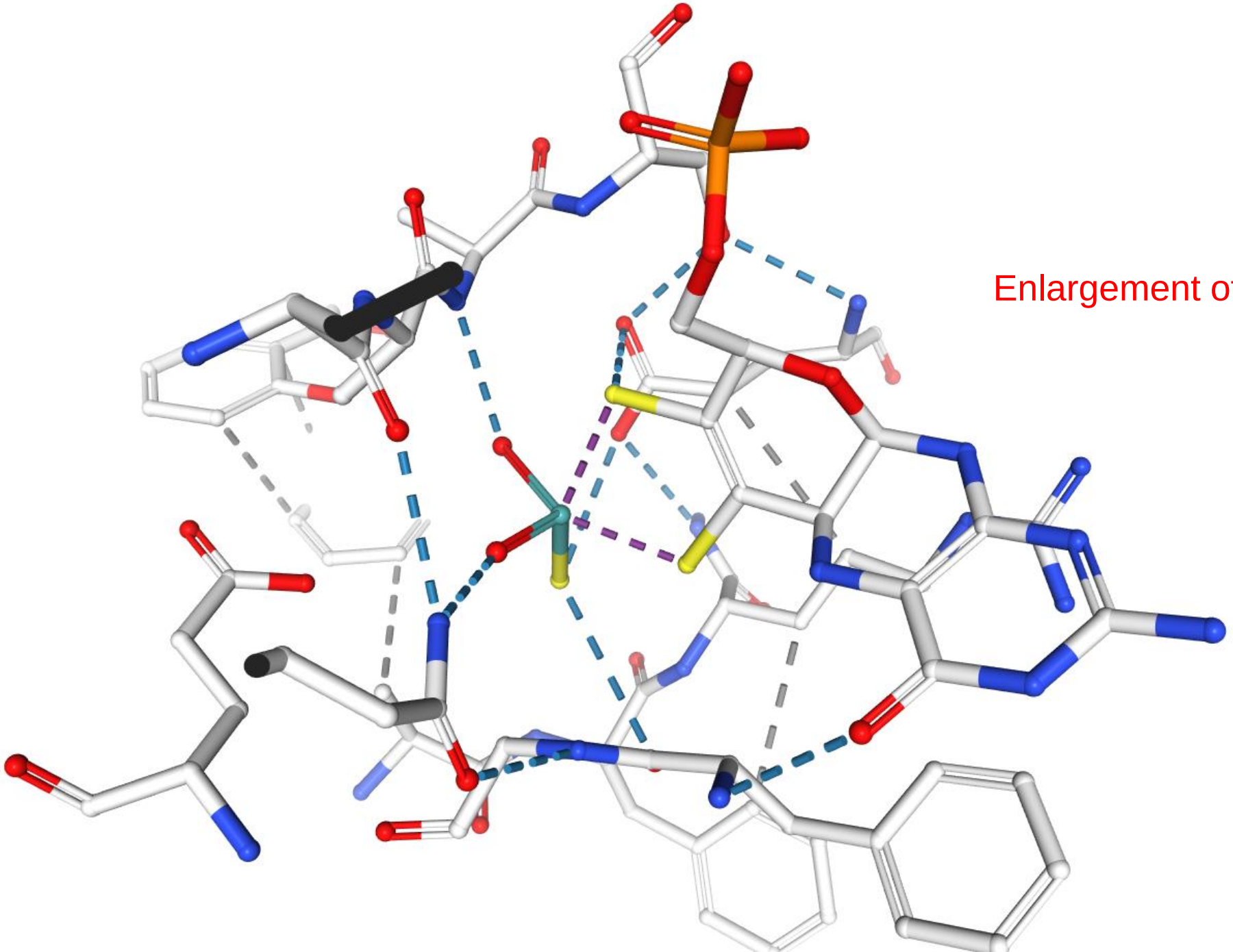
DMSO reductase type

3UNC
Crystal Structure of Bovine Milk Xanthine Dehydrogenase





Cofactors of the enzyme

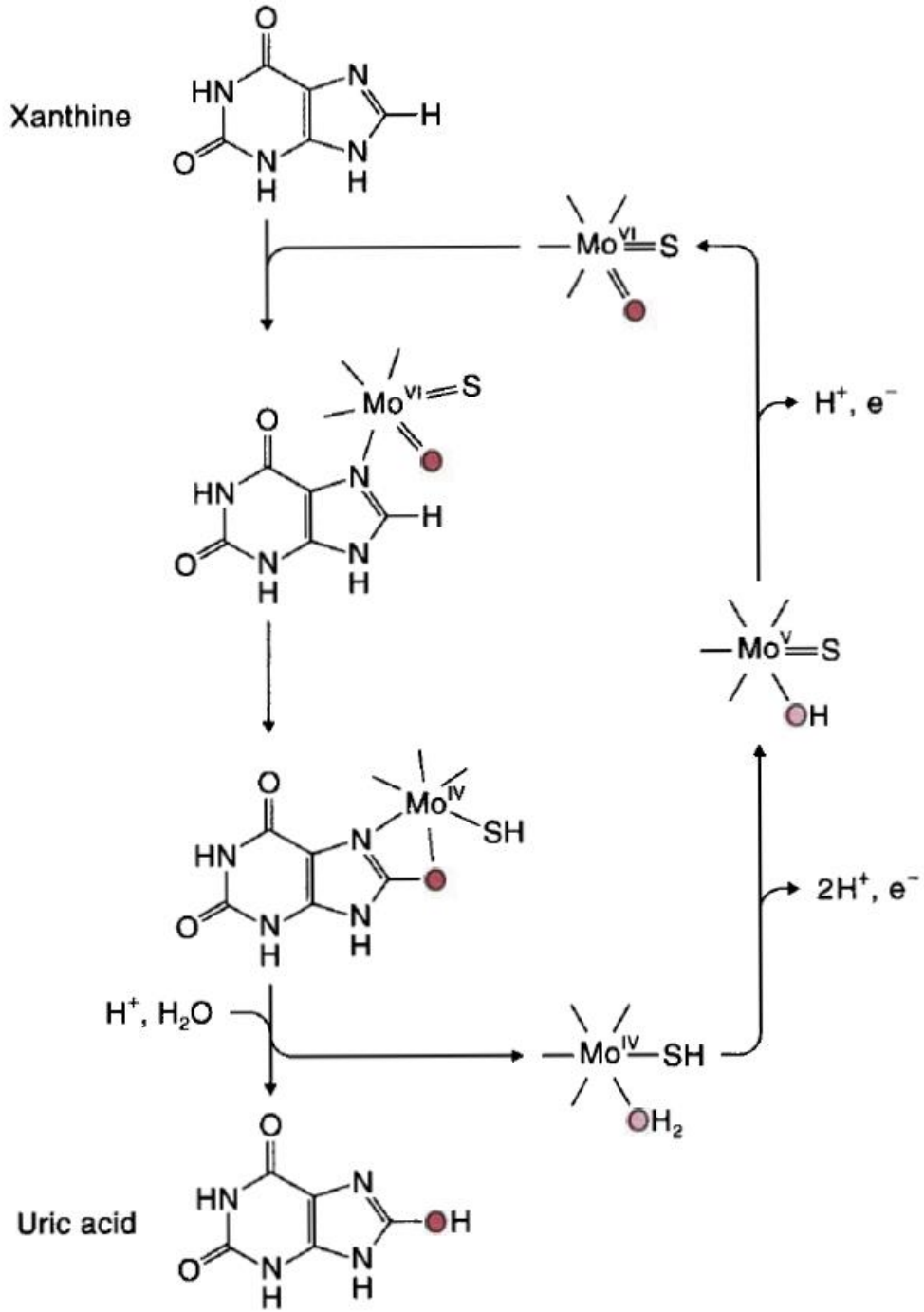


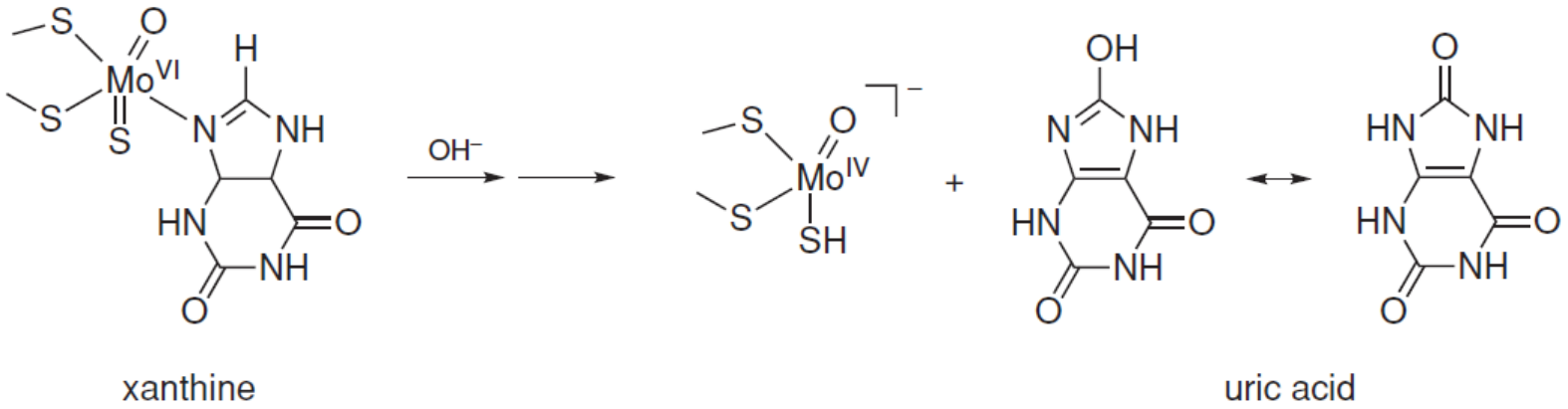
Enlargement of the Mo-pyranopterin

Scheme for xanthine oxidase reaction

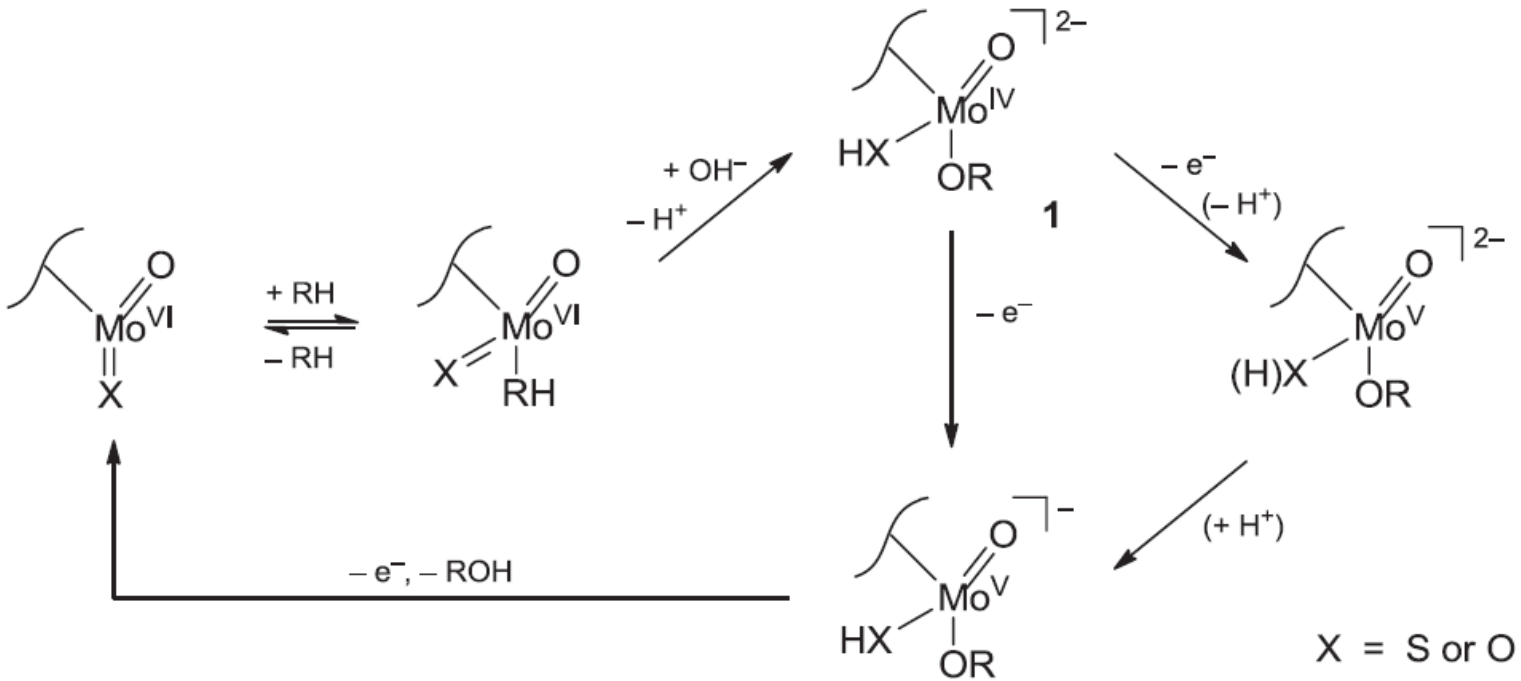
An oxygen atom is transferred from the molybdenum center to xanthine, with the terminally bound sulfido group acting as a base for hydrogen that is removed from the substrate.

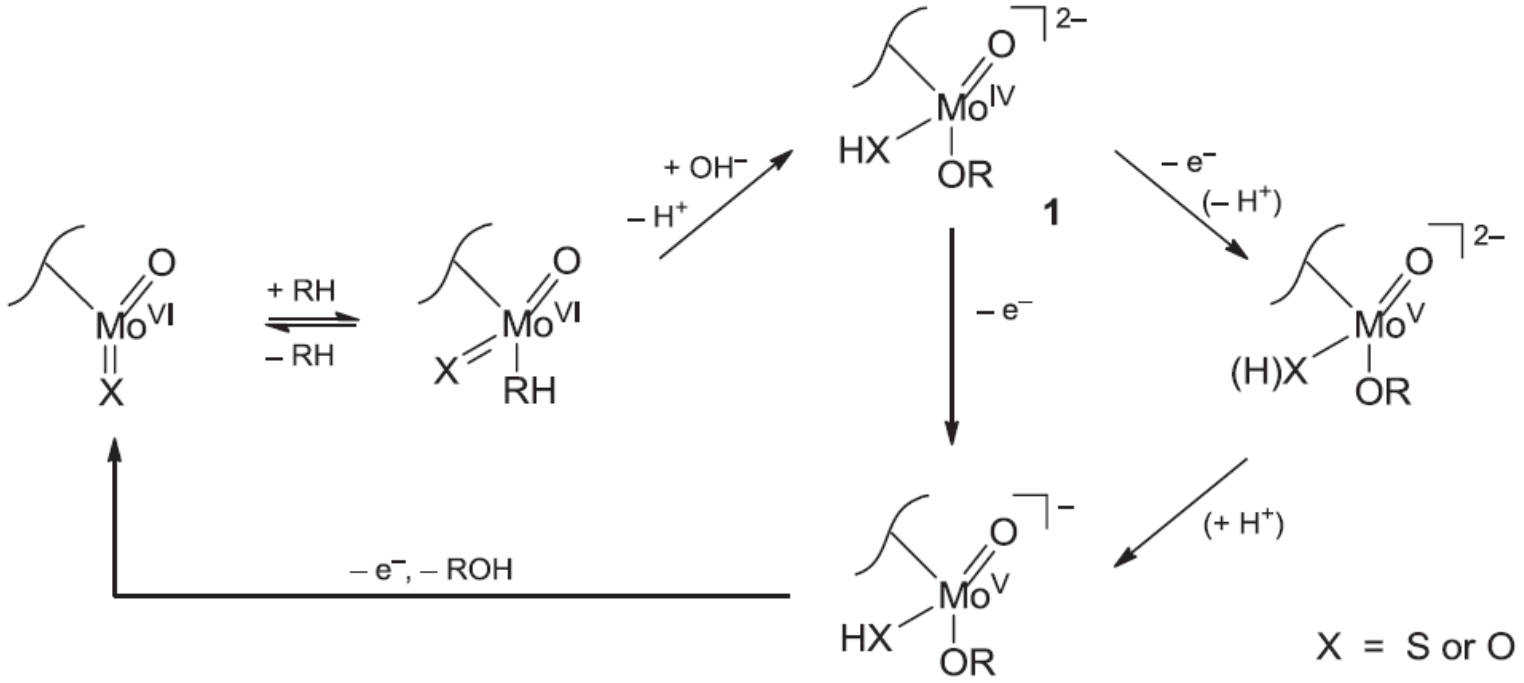
The oxo group is then restored at the Mo center from a water molecule via a series of coupled deprotonation and electron transfer steps.





Mo(V) species are frequently observed by EPR spectroscopy during the stepwise reoxidation of Mo(IV) after completed oxygen transfer to the substrate





The **activated complex 1** of tetravalent molybdenum with one oxo, one hydroxyl or sulfhydryl and one indirectly coordinated substrate ligand, R, **can be one-electron oxidized under optional deprotonation**.

Loss of the second (and last) d electron causes **release of the oxidized substrate** and the **resting state of the enzyme** is obtained, which can again react with substrate and base to **1** in an intramolecular electron-transfer process.

Molybdenum-dependent Nitrogen Fixation

The inorganic-biological nitrogen cycle is of huge importance!

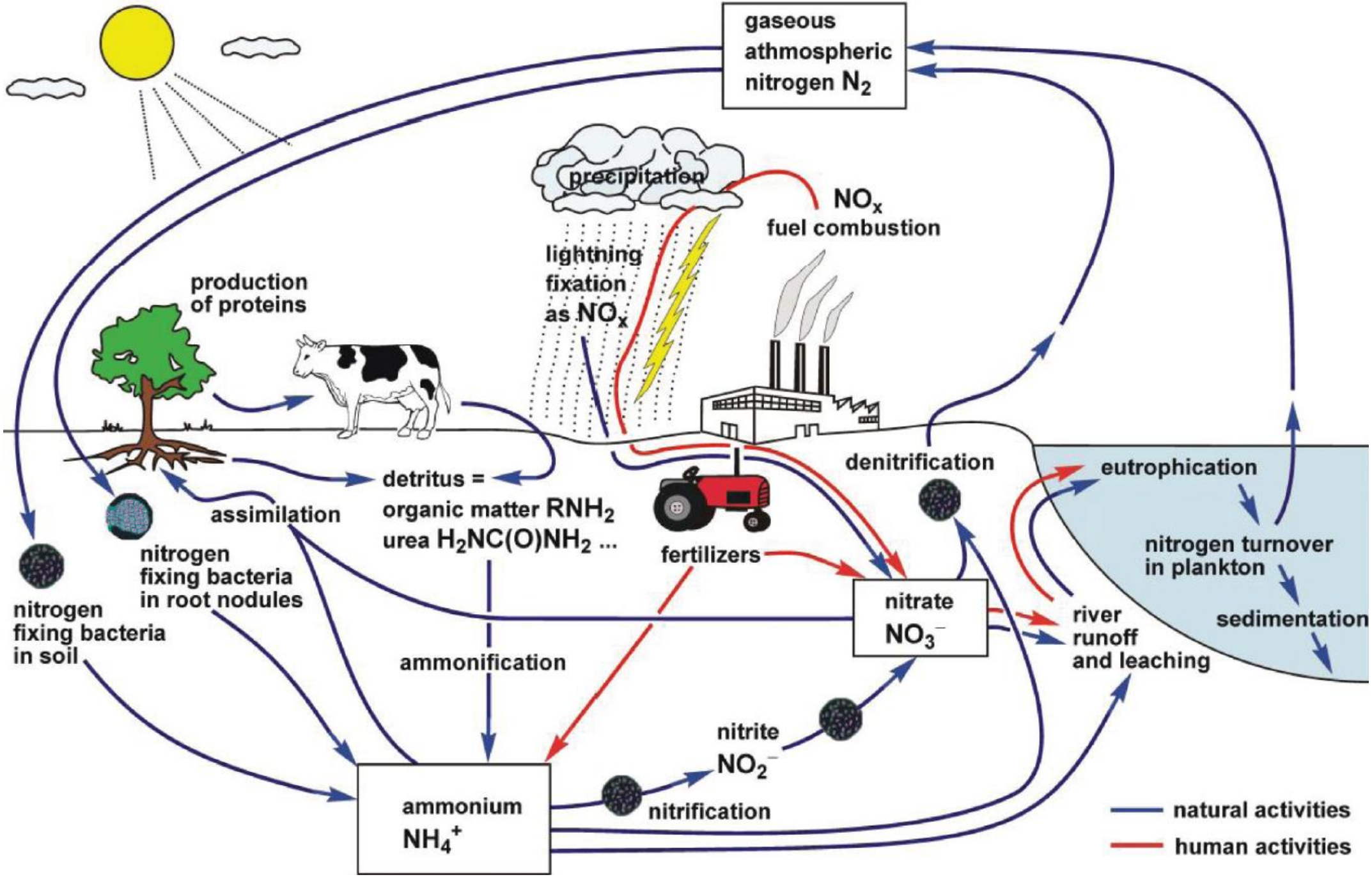
Only the technical nitrogen fixation realized in ammonia synthesis (according Haber and Bosch) guarantees to supplement the often **growth-limiting nitrogen content of the soil** and thus guarantee a food production adequate for the growing human population.

Ammonia continues to be one of the leading products of the chemical industry. **The overall turnover of products from synthetic nitrogen fixation tends to exceed that of the biological process.**

There are physical-atmospherical reactions, which transform N_2 during thunderstorm discharges, of limited importance.

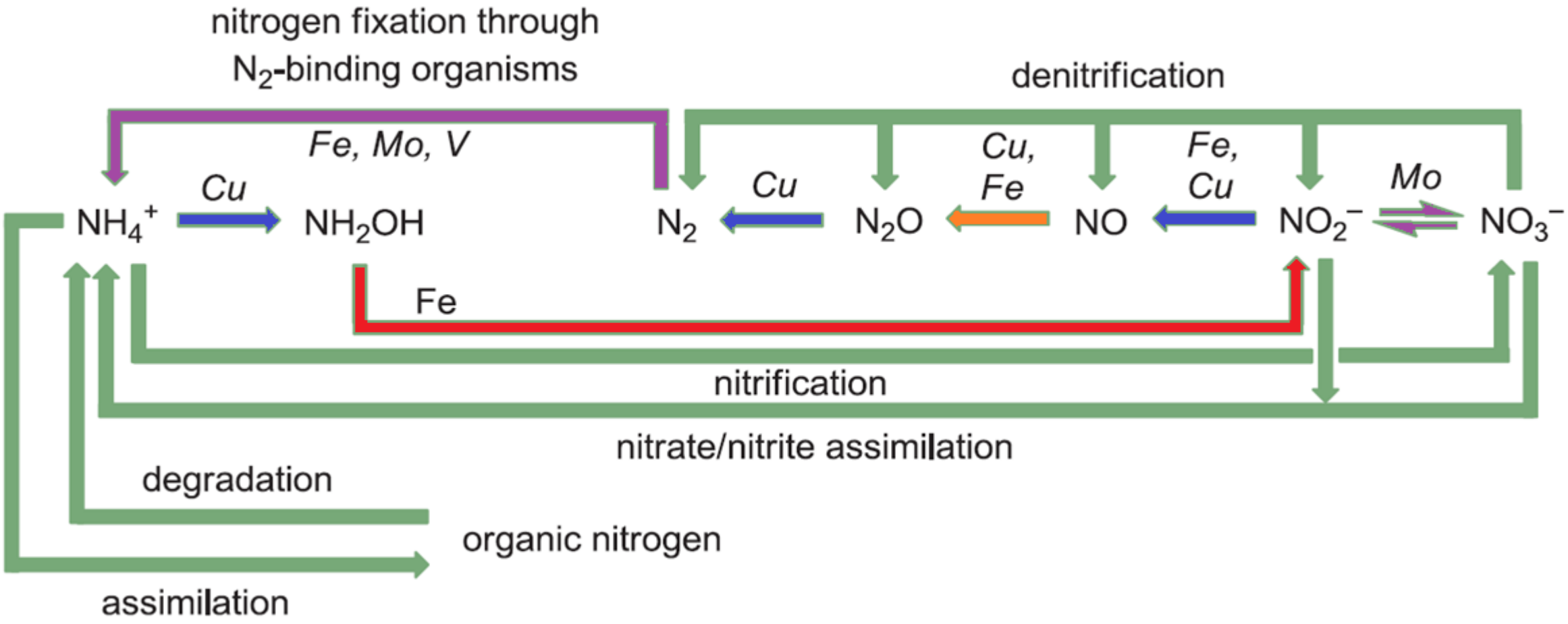
The drawbacks of excessive fertilizer use lie in the **pollution of soil or ground and surface water with ammonium and nitrate.**

The toxic gases NO and NO_2 ("NO_x"), as well as the greenhouse gas N_2O are formed in increasing concentrations.


gaseous
athmospheric
nitrogen N_2
precipitation
NO_x
fuel combustion
lightning
fixation
as NO_x
production
of proteins
detritus =
organic matter RNH_2
urea $H_2NC(O)NH_2$ ...
assimilation
denitrification
eutrophication
nitrogen turnover
in plankton
sedimentation
river
runoff
and leaching
fertilizers
nitrogen
fixing bacteria
in root nodules
nitrogen
fixing bacteria
in soil
nitrate
NO_3^-
ammonification
nitrite
NO_2^-
nitrification
ammonium
NH_4^+
natural activities
human activities

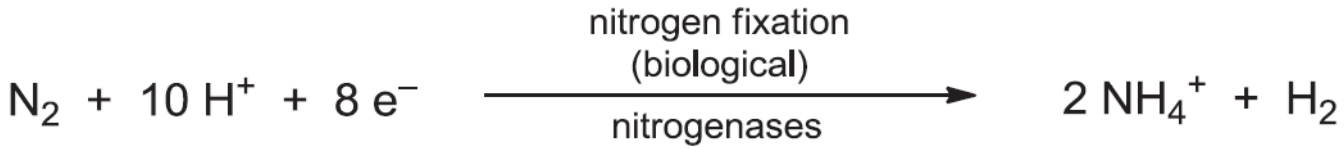
Chemical representation of the nitrogen cycle

Most of the biological systems which participate in the global nitrogen cycle contain metal-requiring enzymes

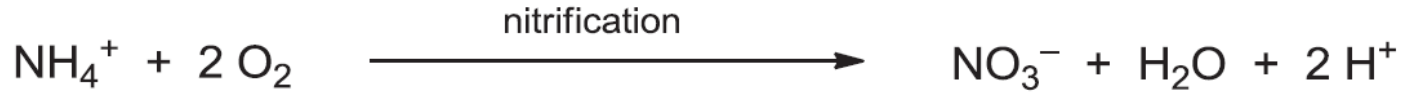


Three main processes can be distinguished:
Nitrogen fixation, nitrification and denitrification

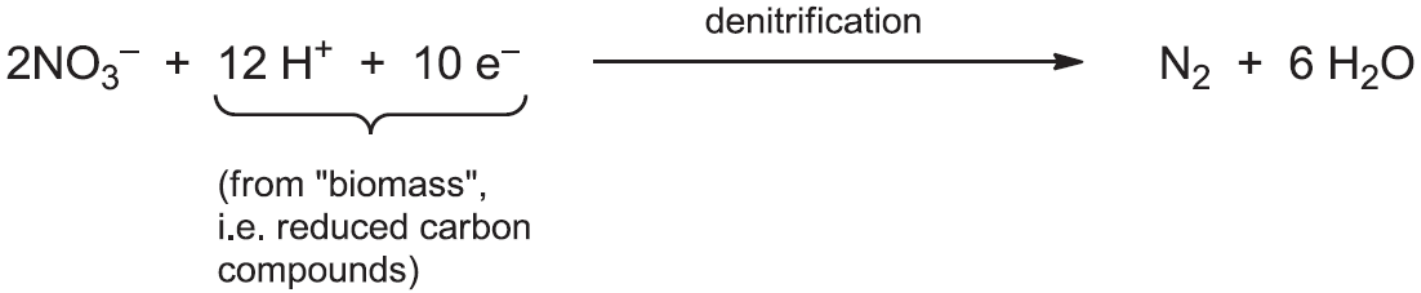
$$N_2 + 3\,H_2 \longrightarrow 2\,NH_3 \quad \text{(gas phase)}$$



$$N_2 + 10\,H^+ + 8\,e^- \longrightarrow 2\,NH_4^+ + H_2$$



$$NH_4^+ + 2\,O_2 \longrightarrow NO_3^- + H_2O + 2\,H^+$$



$$2NO_3^- + \underbrace{12\,H^+ + 10\,e^-}_{\text{(from "biomass", i.e. reduced carbon compounds)}} \longrightarrow N_2 + 6\,H_2O$$

The oxidative process of nitrification yields **nitrate**: the oxidation state in which **nitrogen can be assimilated by most higher plants**.

Challenge in nitrification (*Nitrosomas* bacteria):

Avoid the formation of the stable dinitrogen molecule, N_2, the zerovalent state.

Aerobic conditions and sufficient buffer capacity for the resulting protons in soil or mineral material (atmospheric weathering) are indispensable

Metalloenzymes for certain stages of *de*nitrification:

molybdenum-containing nitrate(N^{+V}) reductase (→ nitrite)

the copper or heme-iron containing nitrite(N^{+III}) reductases

the copper-containing dinitrogen monoxide(N^{+I}) reductase

Denitrification requires rather **anaerobic conditions** and **organic substances as reductants**.

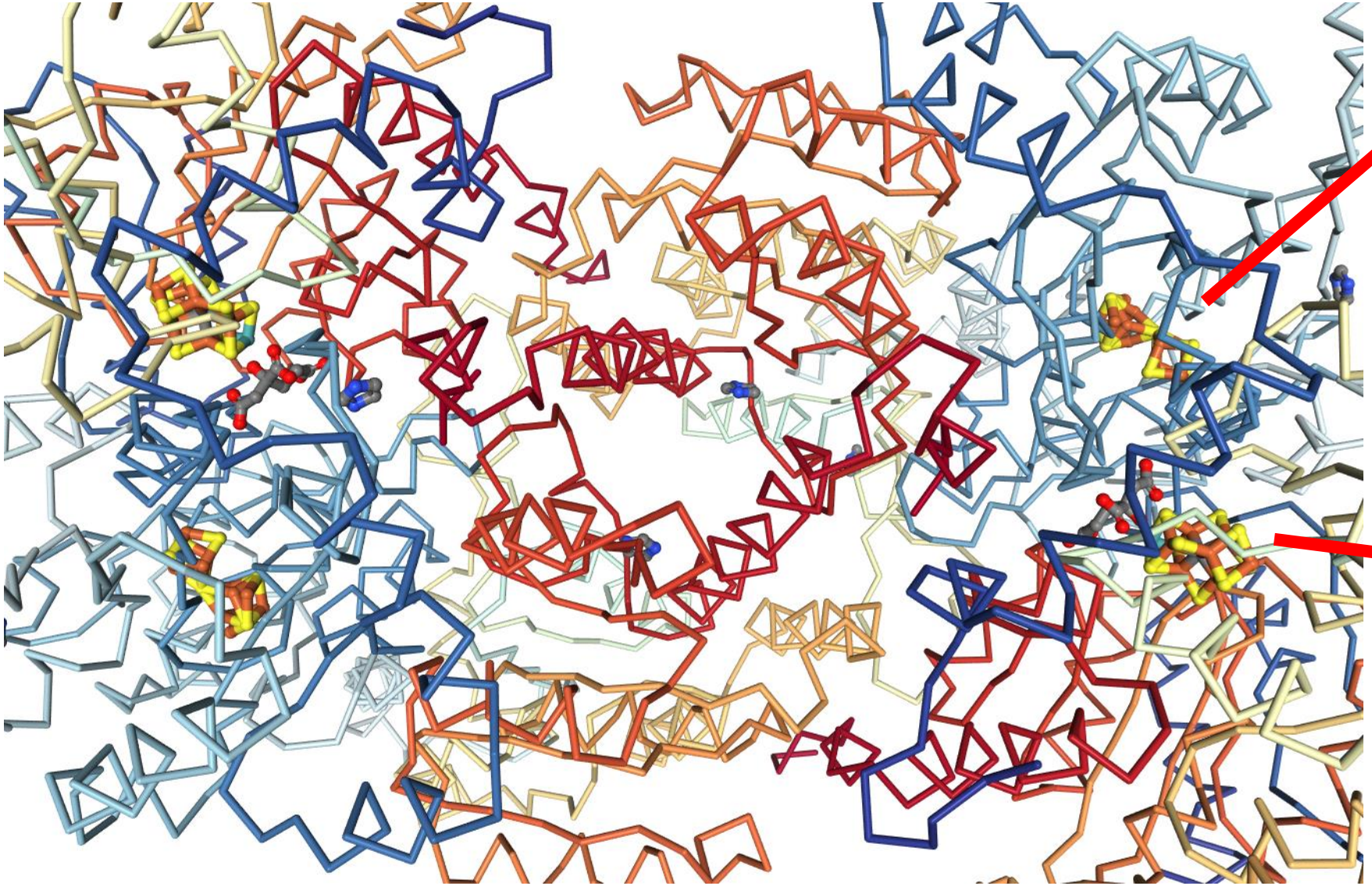
The most extensively studied molybdenum-dependent form of nitrogenase has an $(\alpha_2\beta_2)(\gamma_2)$ two-protein composition

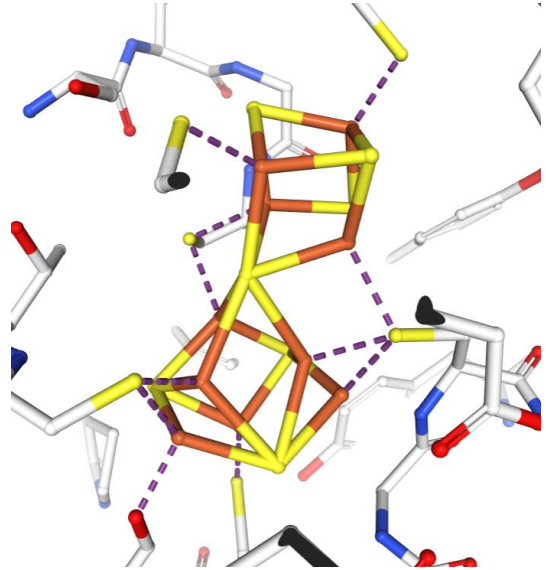
The special dimeric iron protein (γ_2) is the "dinitrogenase reductase" (about 60 kDa), being essential for the function of the enzyme.

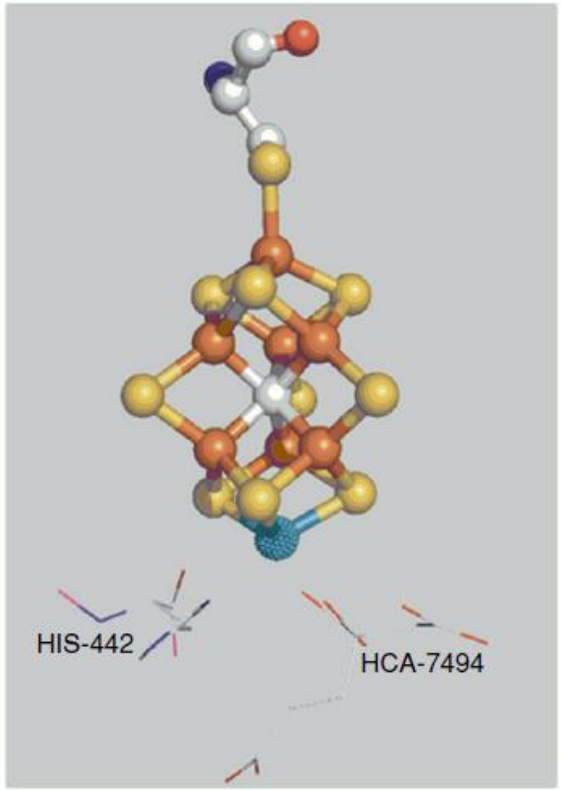
Bound *between* the two γ subunits of this protein is a single [4Fe–4S] cluster, which can be reduced to a paramagnetic form at −0.35V.
This "Fe protein" of nitrogenase contains two Mg^{2+}/ATP receptors, because two ATP molecules have to be hydrolyzed for each transferred electron.
Binding of ATP or ADP further lowers the potential by about 0.1V.

The second component, **the actual "dinitrogenase" or "FeMo protein",** is an **$\alpha_2\beta_2$ tetramer (220 kDa),** which contains two very special Fe/S systems, the **[8Fe-7S] P** clusters between and subunits and two "**FeMo cofactors**" in the subunits, each with the inorganic composition of **$MoFe_7S_9$**.

PDB = 3U7Q
A. vinelandii nitrogenase MoFe protein

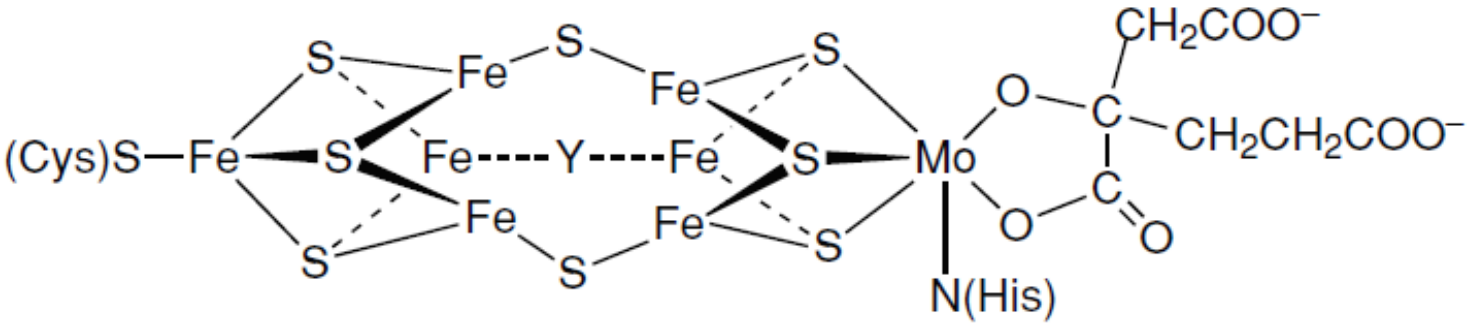






The actual structure of the $MoFe_7S_9$ cluster, consists of **two subclusters**, [MoFe3S3] and [Fe4S3], **bridged by three sulfide ions**.

Known from Fe and Mo extended x-ray absorption fine structure (EXAFS) measurements

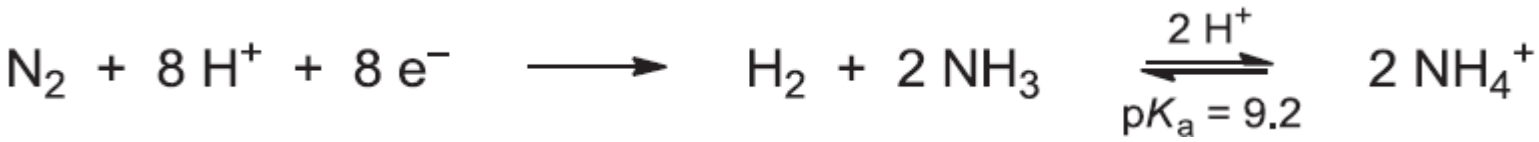


Y: presumably C

A hexacoordinate (μ6) atom surrounded by iron atoms at the center of the M cluster has been identified as a carbido (C^{4-}) ligand, **originating from the methyl group of S-adenosylmethionine**

In **the reduced state**, the **cofactor contains tetravalent molybdenum**; however, the total electron spin of ***S* = 3/2 is mostly located at the various different sulfur-coordinated iron centers**, which, according to Mossbauer data, show some degree of electron delocalization.

The possibly multiple function of the homocitrate ligand (structural, catalysis support, assembly factor) is still being investigated.



Due to the **thermodynamic stability of the dinitrogen molecule**, its reduction requires a large amount of energy in the form of **several ATP equivalents** (with Mg^{2+} as the hydrolysis catalyst) and (at least) **six electrons per N_2 at a physiologically quite negative potential** of less than −0.3V

Role of dinitrogenase reductase

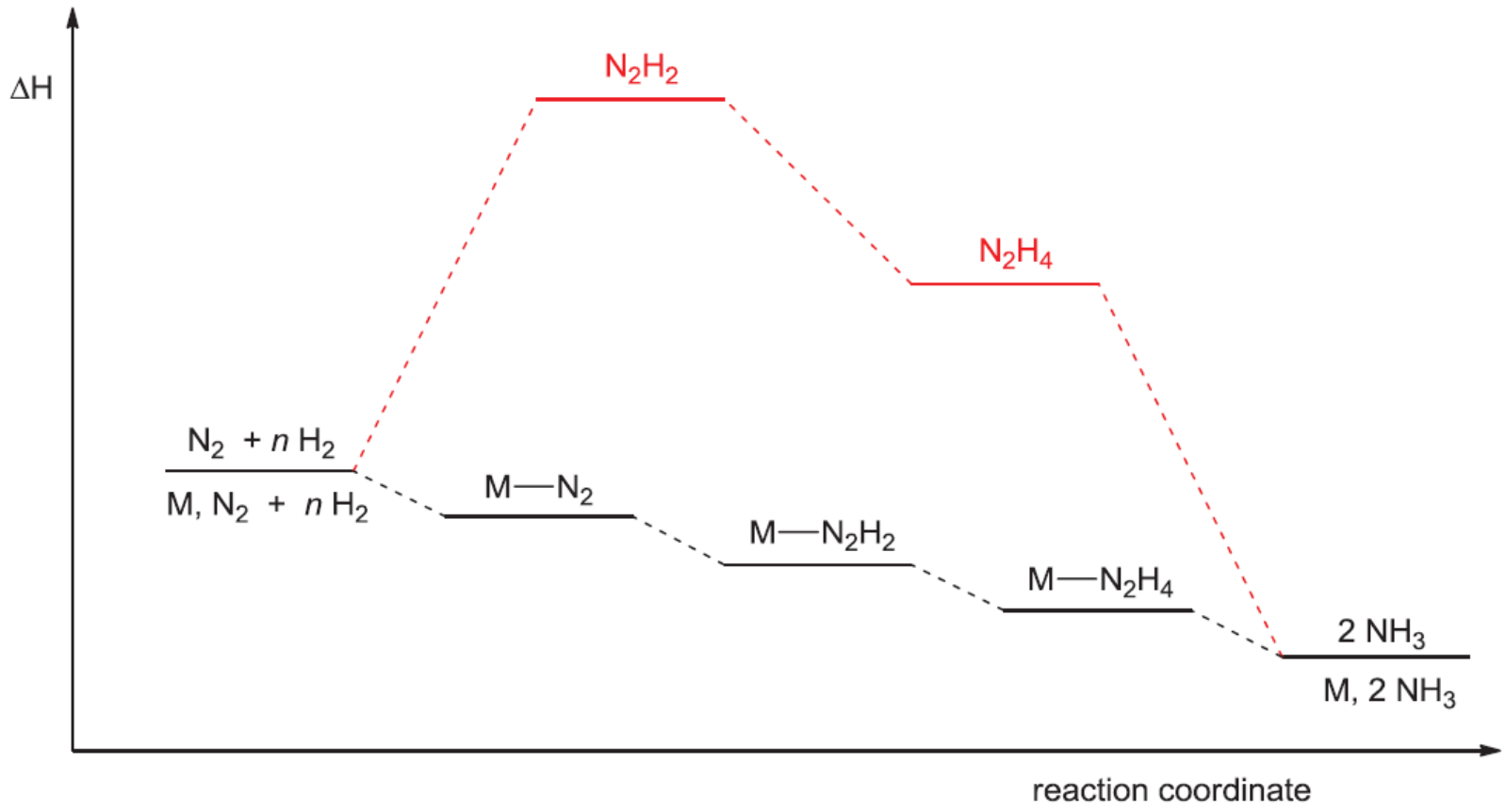
[4Fe–4S] cluster provides an electron flow at low potential using simultaneous ATP hydrolysis as the driving force (two ATP per electron).

Inside the FeMo protein, **the polynuclear P clusters** can be **reduced to the all-Fe(II)** form, and presumably **regulate the low potential transfer of electrons to the FeMo cofactors**.

Mechanism has not been clarified yet

A **multistep reaction** sequence is necessary for this energetically and mechanistically demanding process. According to the heterogeneously catalyzed technical synthesis of ammonia, **a catalyst is required to prevent the formation of "free", high-energy intermediates**.

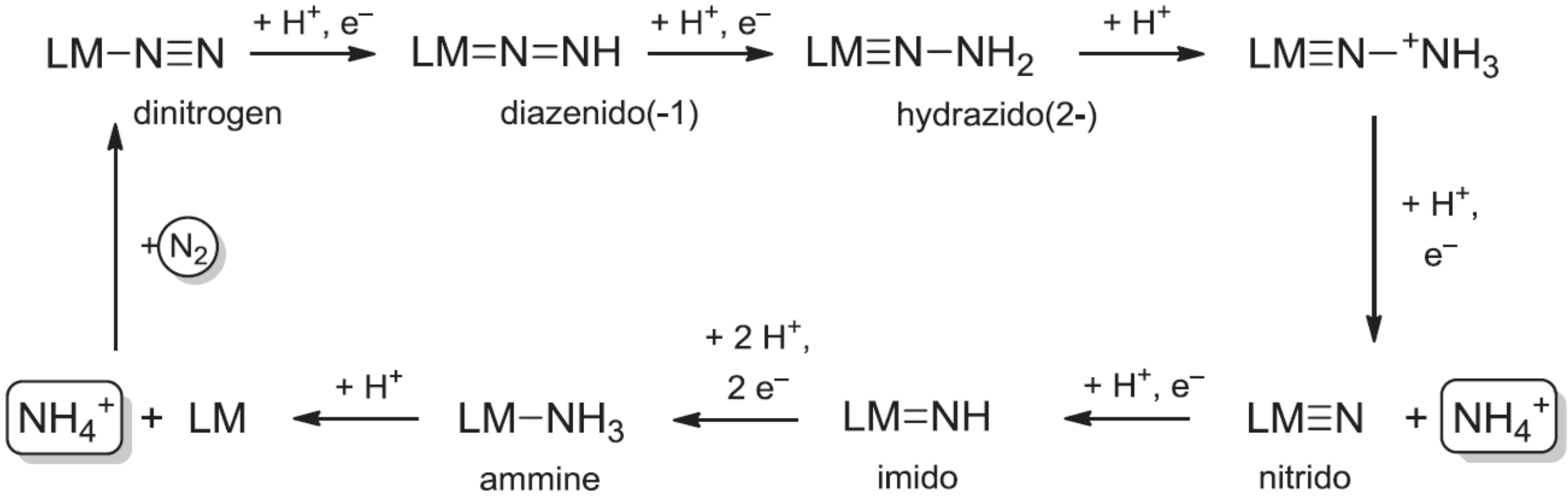
Stabilization of high-energy intermediates in the conversion of $N_2 \longrightarrow NH_3$ through binding to a metal center, M:



The coordinative saturation of the Mo center and the existence of heteroatomfree nitrogenases suggests an **iron rather than a molybdenum coordination of N_2**

A catalysis mechanism based on model reactions of complexes with nonbridging, end-on coordinated N_2 can be formulated

LM = ligand metal with one free coordination site



The low redox potentials and the necessarily high reactivity of nitrogenase enzymes further require the absence of competing molecules such as dioxygen, O_2.

N_2-assimilating microorganisms are either obligatory anaerobes or posses complex protection mechanisms for the exclusion of O_2 (iron-containing proteins, which serve as O_2 sensors).

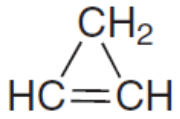
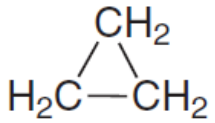
Nitrogenase activity is also inhibited by the isoelectronic substrates carbon monoxide, −C≡O+, and N≡O+; characteristic reaction products are obtained with several other small molecules containing multiple bonds

In the case of molybdenum-containing nitrogenase, both the exclusive *Z*(*cis*)-hydrogenation of acetylene to the ethylene state and the cleavage of the isocyanide triple bond are remarkable.

Nitrogenases also possess an intrinsic hydrogenase activity, which leads to the obligatory production of dihydrogen, H_2, during the biological N_2 fixation reaction

$$N_2 + 8\,H^+ + 8\,e^- \longrightarrow H_2 + 2\,NH_3 \underset{pK_a = 9.2}{\overset{2\,H^+}{\rightleftharpoons}} 2\,NH_4^+$$

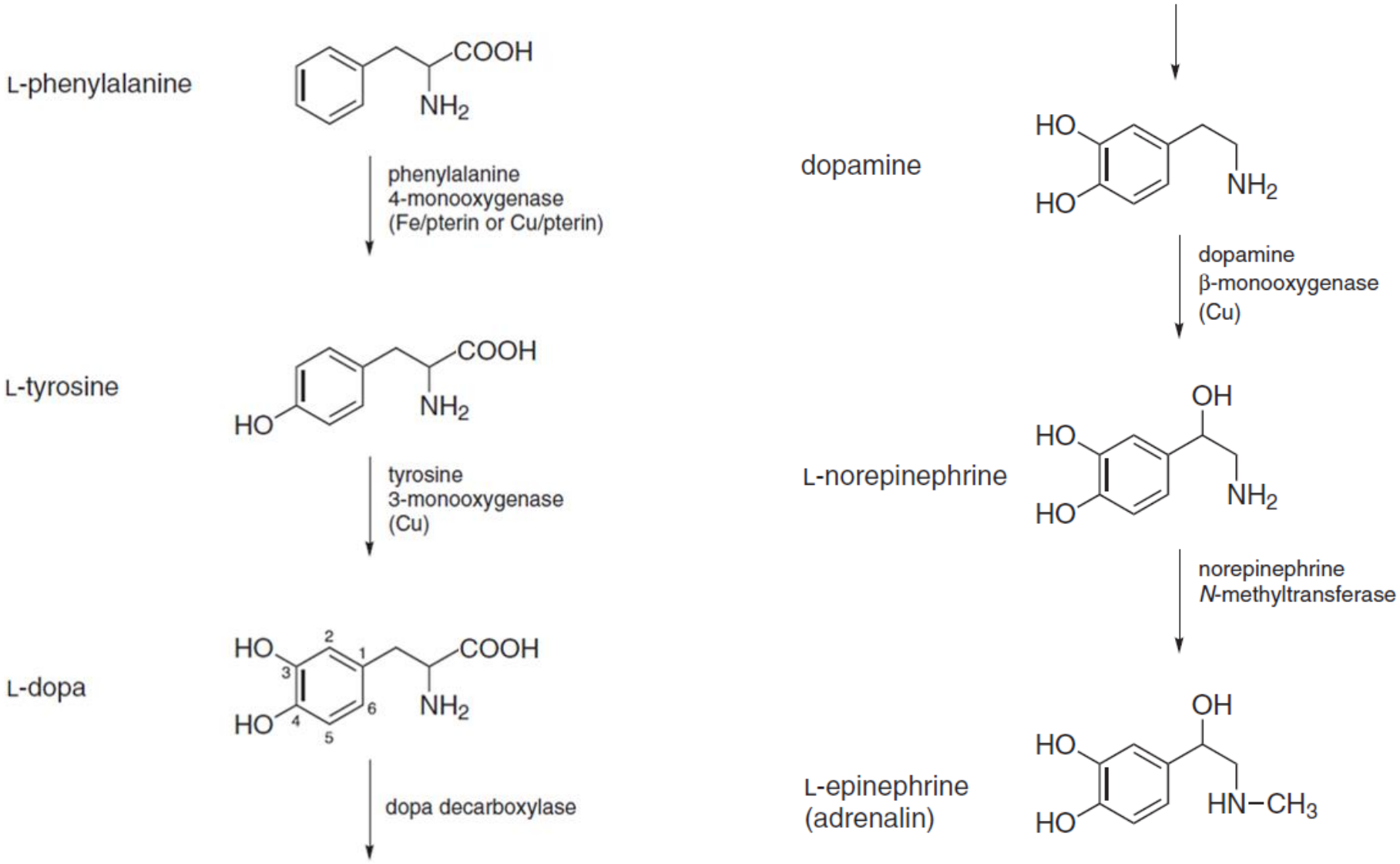
Reduction (hydrogenation) reactions catalyzed by nitrogenases.

Substrate	Products	Number of electrons required
$IN{\equiv}NI$	$2\ NH_3\ +\ H_2$	$8\ e^-$
$H{-}C{\equiv}C{-}H$	C_2H_4 or Z-$C_2H_2D_2$ (from C_2D_2)[a]	$2\ e^-$
$H{-}C{\equiv}NI$	$CH_4\ +\ NH_3\ (CH_3NH_2)$	$6\ e^-\ (4\ e^-)$
$H_3C{-}N^+{\equiv}Cl^-$	$CH_3NH_2\ +\ CH_4$	$6\ e^-$
$^-\langle N{=}N^+{=}N\rangle^-$	$N_2H_4\ +\ NH_3\ (N_2\ +\ NH_3)$	$6\ e^-\ (2\ e^-)$
$^-\langle N{=}N^+{=}O\rangle$	$N_2\ +\ H_2O$	$2\ e^-$
	$1/3$  $+\ 2/3\ H_3C{-}CH{=}CH_2$	$2\ e^-$
$2\ H^+$	H_2	$2\ e^-$

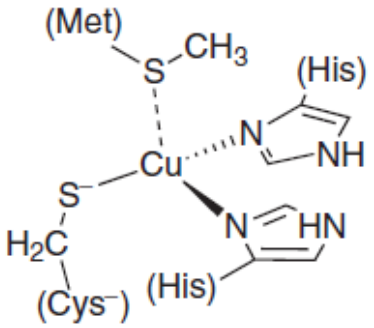
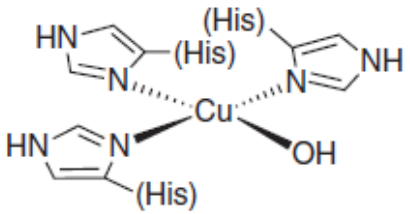
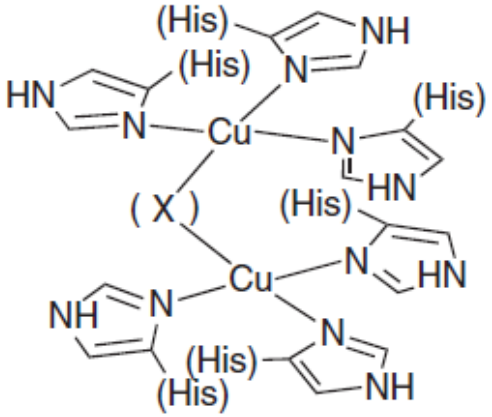
[a] Partially four-electron reduction to C_2H_6 in vanadium-dependent nitrogenase.

Cu-based monooxygenase enzymes

They include copper-containing enzymes such as **tyrosinase and dopamine -monooxygenase**.

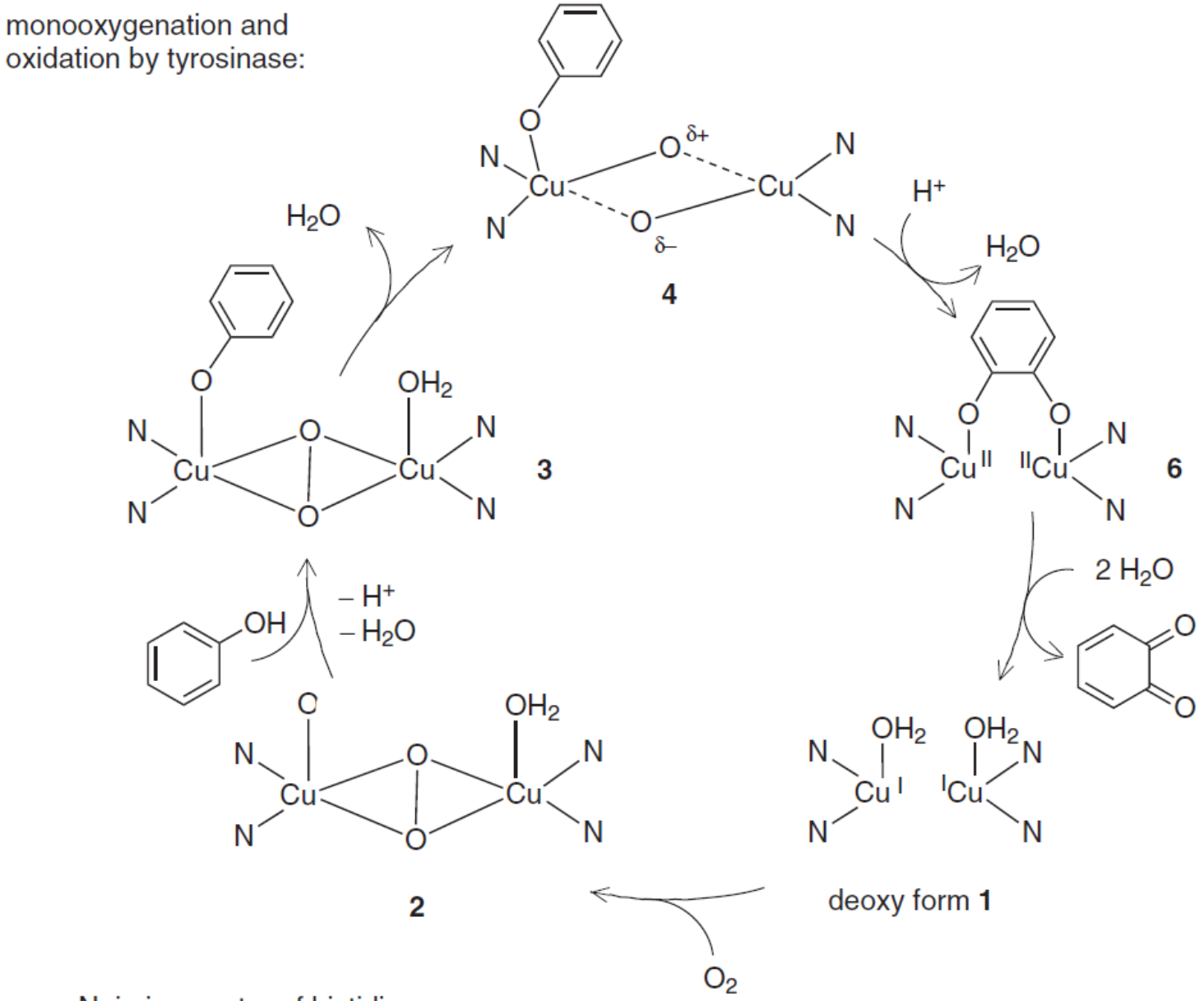


Classification of copper centers based on structural and spectroscopic point of view

Generalized coordination geometry	Function, structure, characteristics
type 1 	**type 1:** "blue" copper centers function: reversible electron transfer $Cu^{II} + e^- \rightleftarrows Cu^{I}$ structure: strongly distorted, (3 + 1) coordination absorption of the copper(II) form at about 600 nm, molar extinction coefficient $\varepsilon > 2000\ M^{-1}\ cm^{-1}$, LMCT transition S(Cys) → Cu^{II} EPR/ENDOR of the oxidized form: small $^{63,65}Cu$ hyperfine coupling and *g* anisotropy, interaction of the electron spin with -**S-CH**$_2$-; Cu^{II} → S(Cys) spin delocalization
type 2 	**type 2:** normal, "non-blue" copper function: O_2 activation from the Cu^{I} state in cooperation with organic coenzymes structure: essentially planar with weak additional coordination (Jahn–Teller effect for Cu^{II}), typically weak absorptions of Cu^{II}, $\varepsilon < 1000\ M^{-1}\ cm^{-1}$, ligand–field transitions (d → d) normal Cu^{II} EPR
type 3 	**type 3:** copper dimers function: O_2 uptake from the Cu^{I}–Cu^{I} state structure: (bridged) dimer, Cu–Cu distance about 360 pm after O_2 uptake, intense absorptions around 350 and 600 nm, $\varepsilon = 20\,000$ and $1000\ M^{-1}\ cm^{-1}$, LMCT transitions O_2^{2-} → Cu^{II} EPR-inactive Cu^{II} form (antiferromagnetically coupled d^9 centers)

Mechanism for tyrosine monoxygenase

The oxygen atom incorporated in the substrates has been found to **come from O_2**



overall reaction: $\text{Aryl(H)OH} + O_2 \longrightarrow \text{Aryl(O)}_2 + H_2O$

Cu,Zn- Superoxide Dismutases

SODs catalyze the disproportionation ("dismutation") of toxic $O_2^{\bullet -}$ to O_2 and H_2O_2.

This reaction is also catalyzed by many non-enzymatic transition metal species, albeit in a less controlled fashion

!"free" transition metals ions are physiologically undesirable!

In addition to the Cu,Zn-containing SOD from the cytoplasm of eucaryotes, there are other forms which contain nickel, iron or manganese (SODs from mitochondria and bacteria)

Metals present in the early phases of life evolution

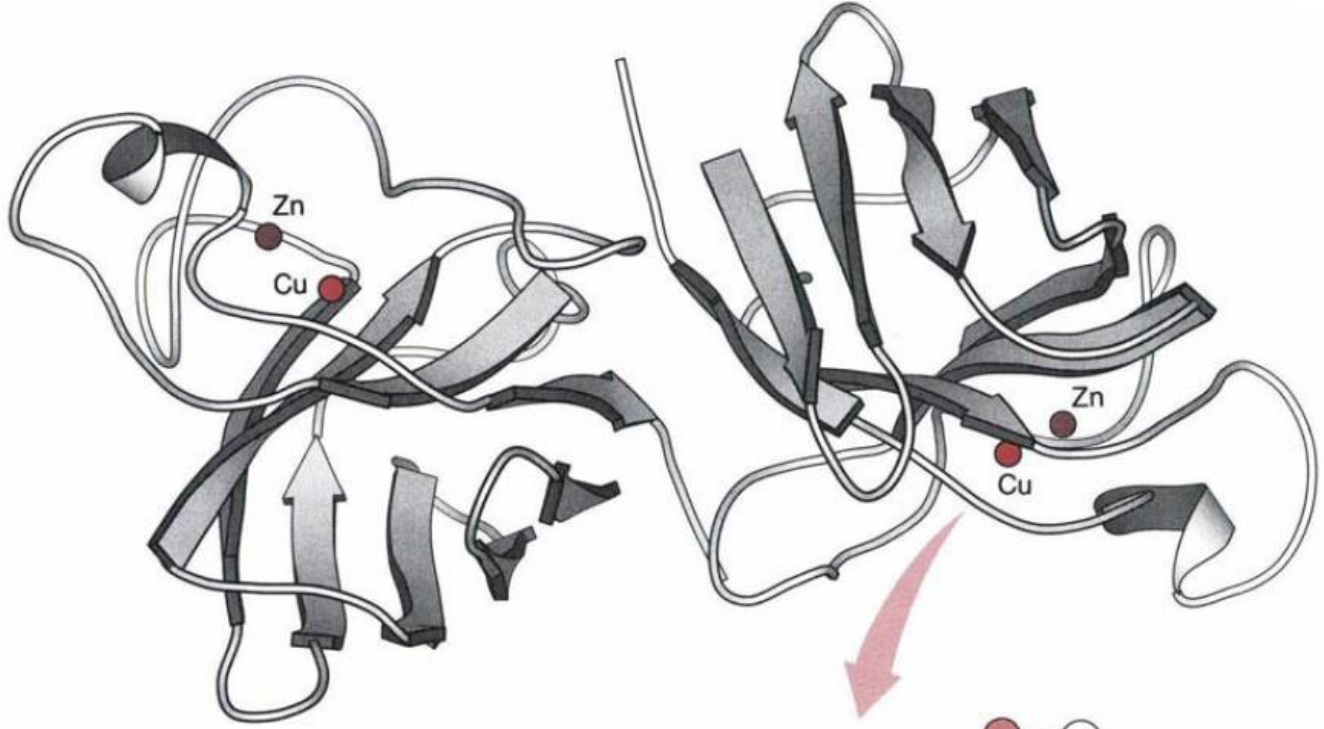
The hydrogen peroxide formed in this process can further disproportionate to yield O_2 and H_2O in reactions catalyzed by catalases, or it can be utilized via peroxidase enzymes

Considering the metastability and reactivity of the radical anion $O_2^{\bullet-}$, **there seems to be no need for a special catalyst specificity of SODs**

However under physiological circumstances, the **dismutation of $O_2^{\bullet-}$ must proceed very rapidly (i.e. at the diffusion limit)** in order to prevent uncontrolled oxidations by this radical anion or its follow-up products in reactions with transition metal ions.

A major requirement for SODs is therefore **their resistance to the aggressive substrate, $O_2^{\bullet-}$, *and* to the products O_2^{2-} and O_2.**

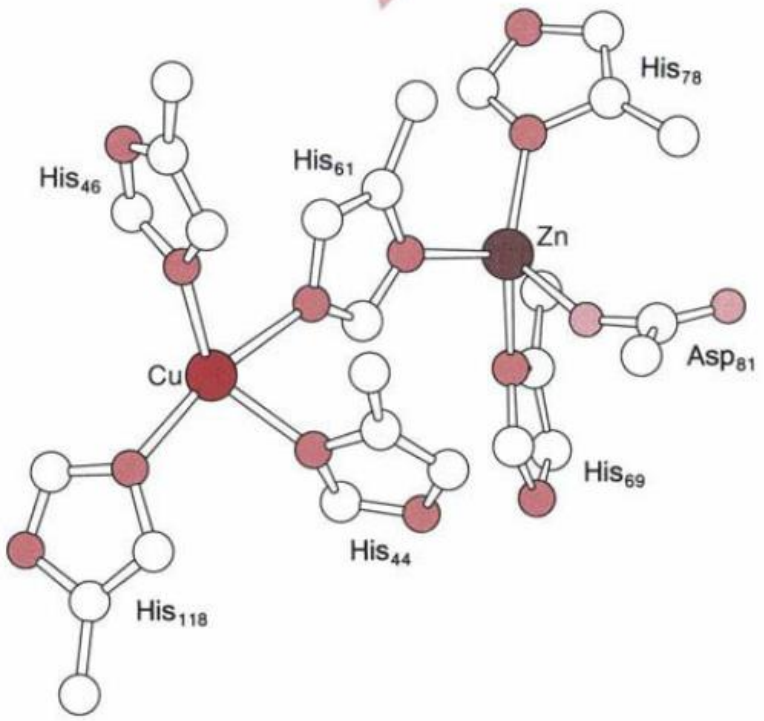
The relatively small (2×16 kDa) Cu,Zn-SOD from erythrocytes

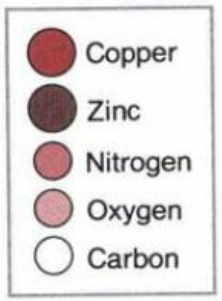


Each subunit contains **one copper and one zinc ion**, bridged by a deprotonated, **resonance-stabilized imidazolate** ring of a histidine side chain.

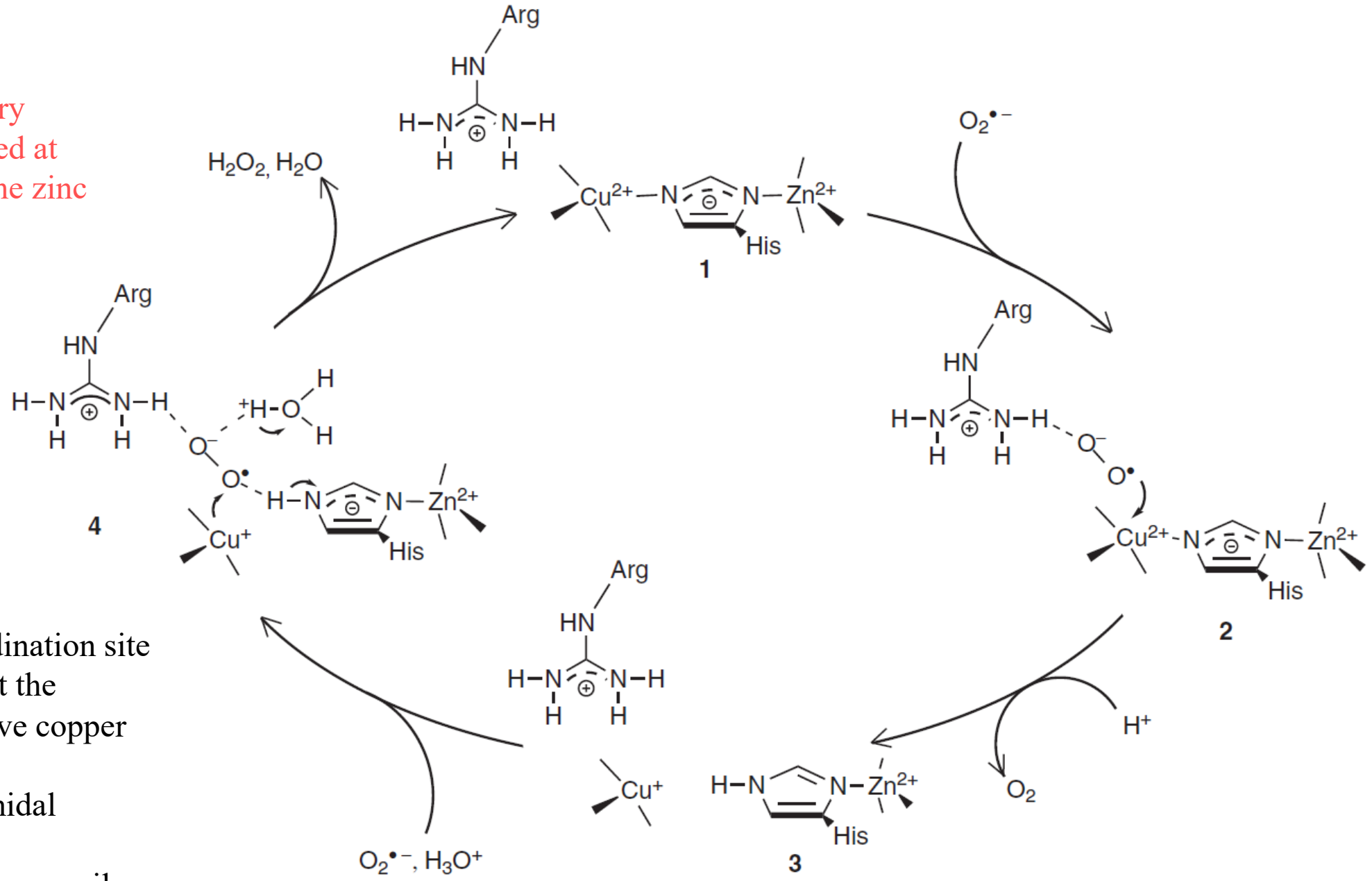
The other amino acid ligands are three His (Cu) and two His and one Asp^- (Zn), respectively.

PDB code 2SOD





Compared to a regular tetrahedron, the geometry is more severely distorted at the copper site than at the zinc center



Additional coordination site for $O_2^{\bullet-}$ is thus at the catalytically active copper center
(→ square pyramidal arrangement)
which may be temporarily occupied by labile H_2O.

It is assumed that $O_2^{\bullet -}$ is oxidized to O_2 by the Cu(II) species **1** (SODs must *not* be oxidatively attacked by O_2!)

the reduced copper(I) center can then be replaced at the imidazole ring by a proton, resulting in a normal, geometrically relaxed zinc complex **3** with histidine.

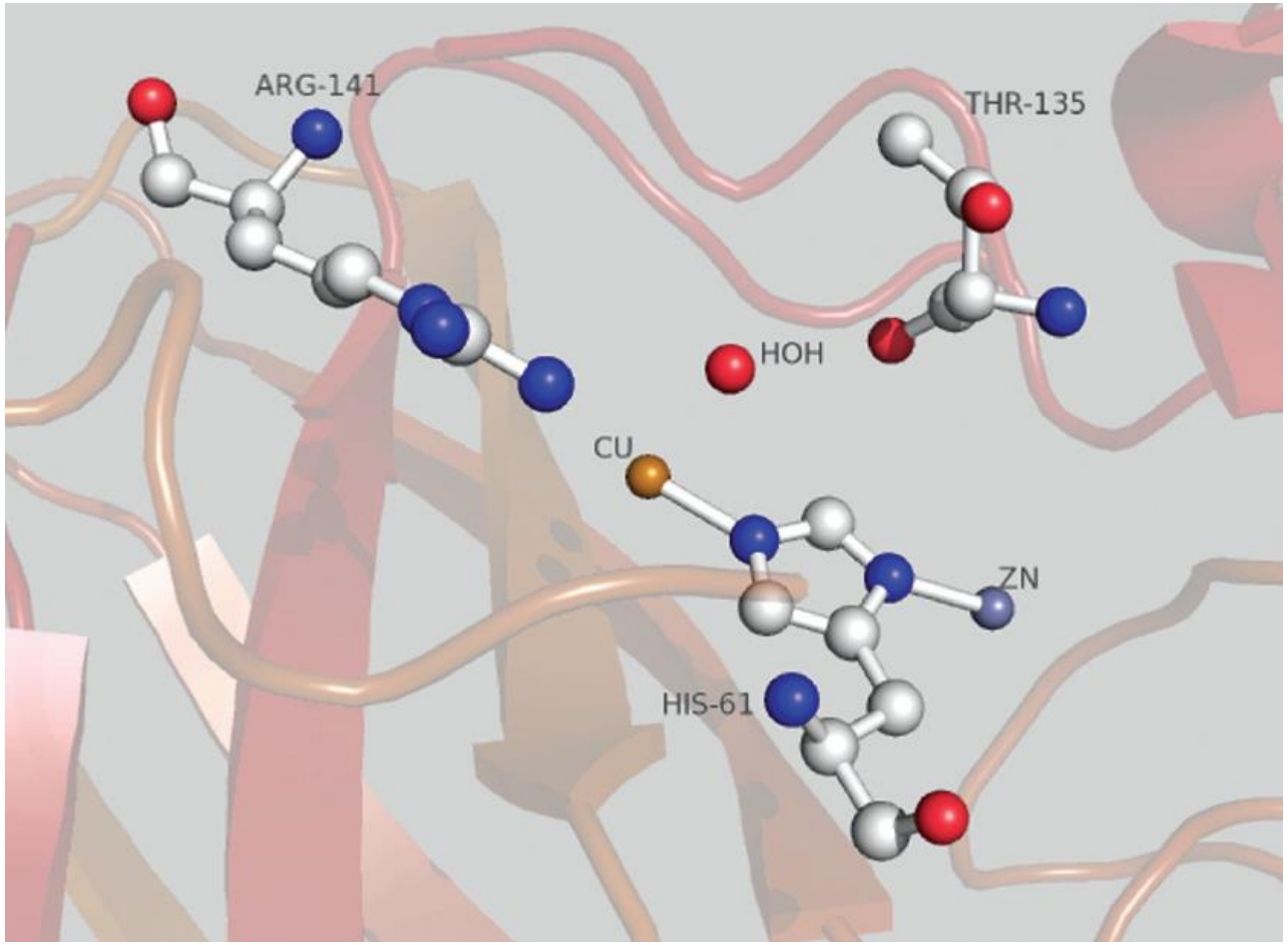
Next, the coordinatively unsaturated copper(I), which is still anchored in the protein, can be oxidized by hydrogen-bonded superoxide anion (**4**). The thus formed basic (hydro)peroxide is then protonated (e.g. by the imidazole ring of still Zn-coordinated histidine) and transformed into H_2O_2.

The driving force for this reaction lies in the affinity of copper(II) for the imidazole nitrogen of histidine. Removal of the Zn^{2+} ion seems to cause only a small decrease in the enzymatic activity; the role of this metal ion appears to be the introduction of structural stabilization.

$$\overset{(-0.5)}{2\,O_2^{\bullet -}} + 2\,H^+ \xrightarrow{\text{SOD}} \overset{(-I)}{H_2O_2} + \overset{(0)}{O_2}$$

(oxygen oxidation states)

The very rapid, almost diffusion-controlled reaction of the enzyme with $O_2^{\bullet-}$ is strongly assisted by electrostatic interactions, which lead the small monoanion $O_2^{\bullet-}$ into the protein through a funnel-shaped, approximately 1–2 nm deep channel.



Schematic representation of the cavity of $O_2^{\bullet-}$ conversion in Cu,Zn-SOD, with one water molecule extending into the cavity

PDB code 2SOD

The apparently toxic natural product $O_2^{\bullet -}$ can be utilized and even deliberately produced by organisms for certain special purposes.

E.g. **Phagocytes (neutrophils)**, which are essential for the immune system of higher organisms, **produce large amounts of superoxide and follow-up products such as H_2O_2 and ClO^-** with the help of a "**respiratory burst oxidase**" in order to kill invading microorganisms

A SOD therapy is also indicated after exposure to ionizing radiation, which mainly produces oxygen-containing radicals

Connections between SOD activity and the rate of aging and the role of defect mutants for neurodegenerative diseases such as amylotrophic lateral sclerosis are under investigation.

Overview of the most important activities of biological copper (apart from regulatory, transport and storage functions)

- Close association with dioxygen, its reaction products such as N/O compounds and its metabolites, and with inorganic and organic radicals

- Only copper(I) and, as part of the Cu(I/II) redox system, copper(II) have been established as biological copper oxidation states: a clear contrast with the wider oxidation state range of iron from Fe(I) to at least Fe(IV).