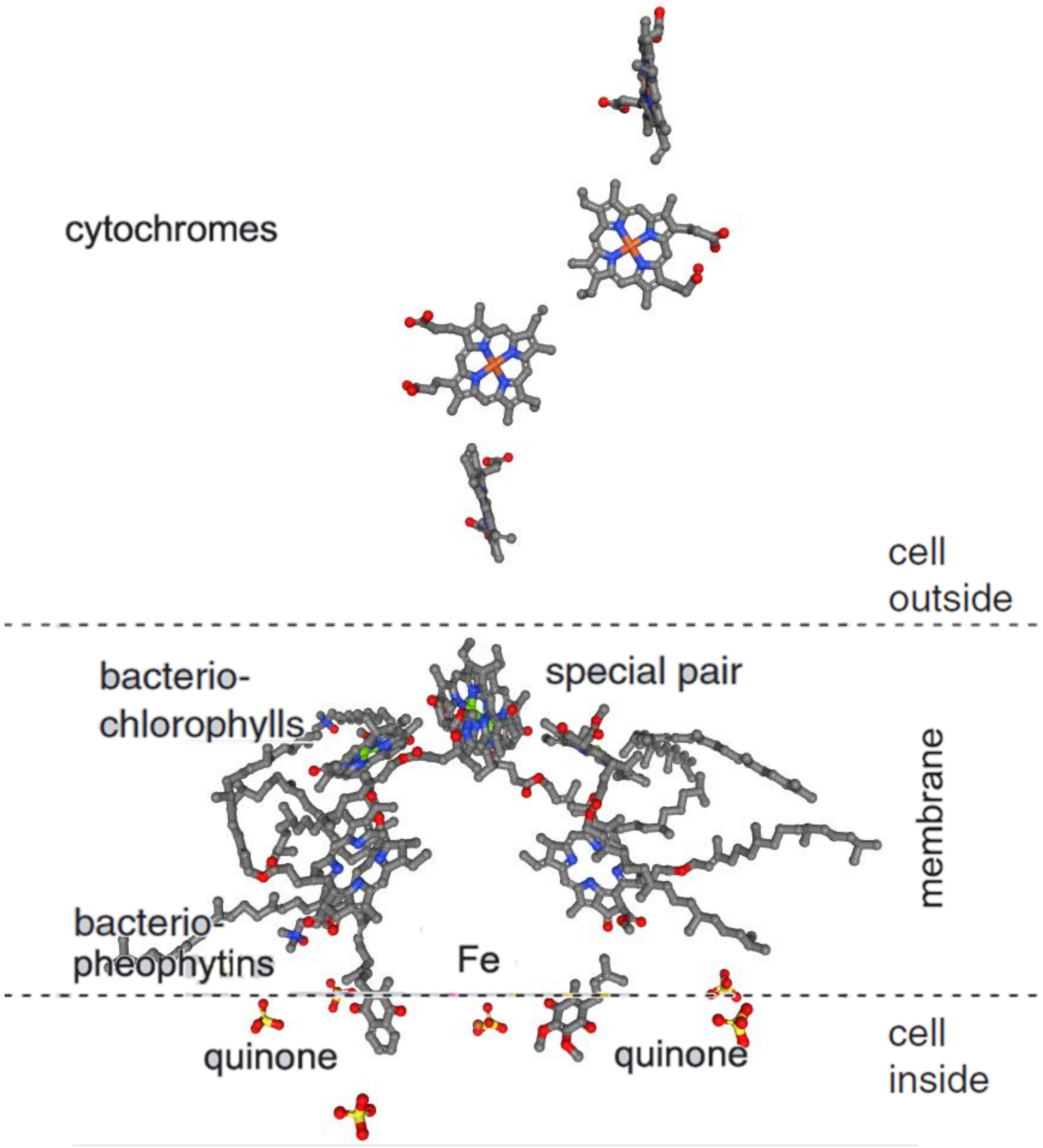
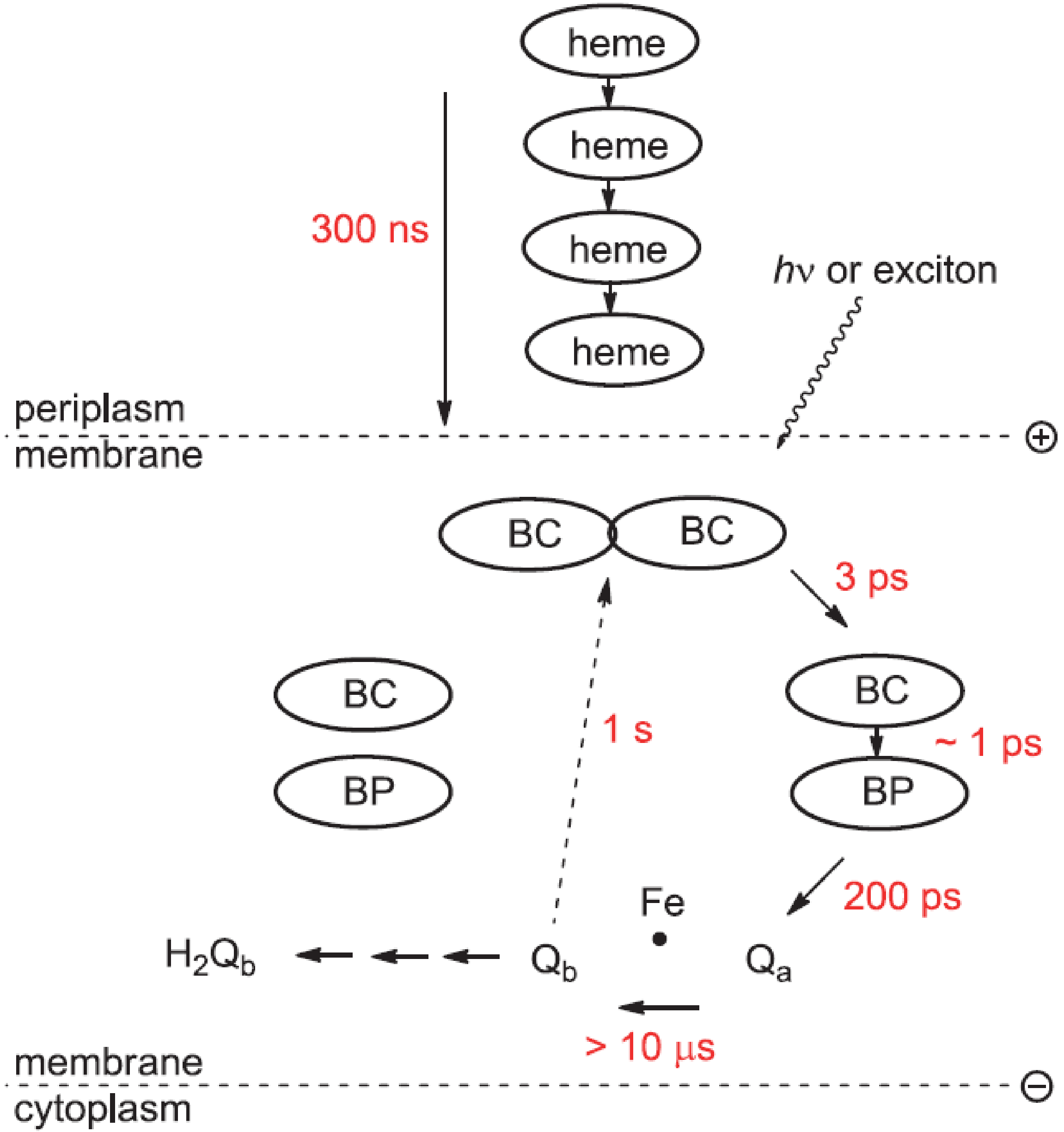
ET: Oxidation of Water to O_2





Quinone Qb or hydroquinone H2Qb, is not tightly bound to the protein but exchanges with quinones in the “**quinone pool**” of the membrane, so that electrons can now be transported further outside the protein.

Simple bacteria => the electron gradient gives rise to a coupled H^+ gradient and photosynthetic phosphorylation takes place (ATP synthesis).

Higher organisms => further steps, the “dark reactions”, which eventually lead to the production of the electron-rich coenzyme NADPH and to Mg^{2+}-requiring CO_2 reduction (Calvin cycle).

The **radical cation** of the “special pair” that remained after the initial charge separation is reduced after a relatively long time (hundreds of nanoseconds) through regulated electron flow via the heme centers of cytochrome proteins.

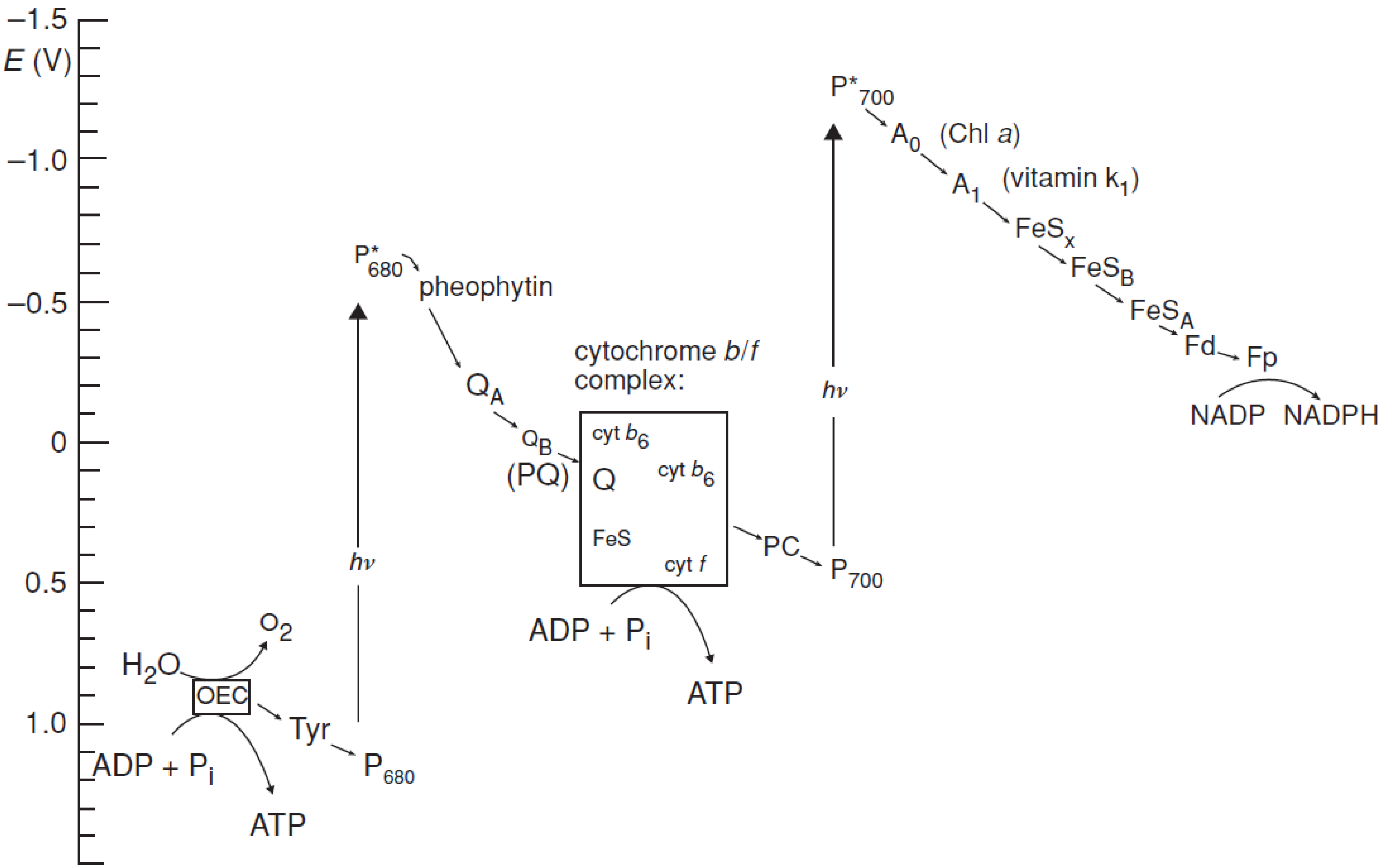
The electron deficiency or “hole” is thus further translocated and:
Simple bacteria => finally filled up through back electron transfer at a different site in the membrane

Higher organisms => the hole created at the special pair may be the starting point for substrate oxidation which requires an additional photosynthetic system consisting of another light-harvesting complex, a reaction center and an oxidase enzyme.

The photosynthesis in plants and cyanobacteria features *two* separately excitable photosystems. These two systems can be connected in a redox potential diagram, **the "Z scheme".**

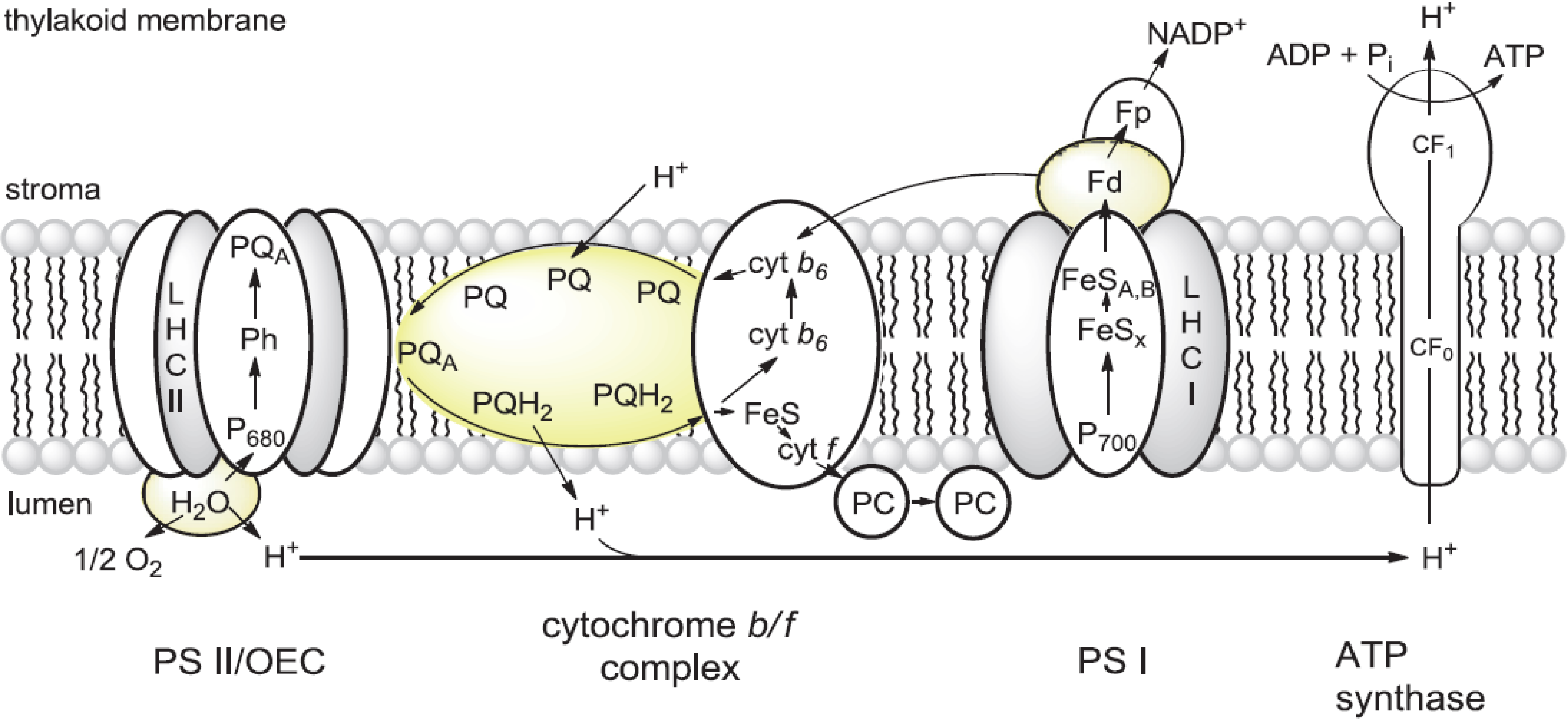
In addition to **photosystem I** (PS I, absorption maximum 700 nm), a **photosystem II** (PS II, absorption maximum 680 nm) exists, which provides electrons for phosphorylation and for PS I.

The electron holes remaining in PS II represent a **very positive potential**, which is used to oxidize two water molecules to dioxygen in an overall four-electron process



photosystem II **photosystem I**

Structural organization of the lamellar thylakoid membrane of higher plants, with the following components: **two photosystems** (PS) and **two light-harvesting complexes** (LHC), an **oxygen-evolving complex** (OEC) at PS II, a **cytochrome *b*/*f* complex**, **plastoquinones and plastocyanin**, several **iron–sulfur centers** , soluble **ferredoxin** (Fd) and the **flavoprotein Fp** (Ferredoxin/NADP reductase) and **ATP synthase** as the center of photosynthetic phosphorylation.



Photosynthetic fixation of CO2 (carboxylation) requiring the RuBisCO enzyme, polarizing Mg^{2+} ions and the universal “hydride” carrier NADPH.

$$\boxed{4\,e^- + 4\,H^+ + CO_2 \rightarrow 1/n\ (CH_2O)_n\ +\ H_2O}$$

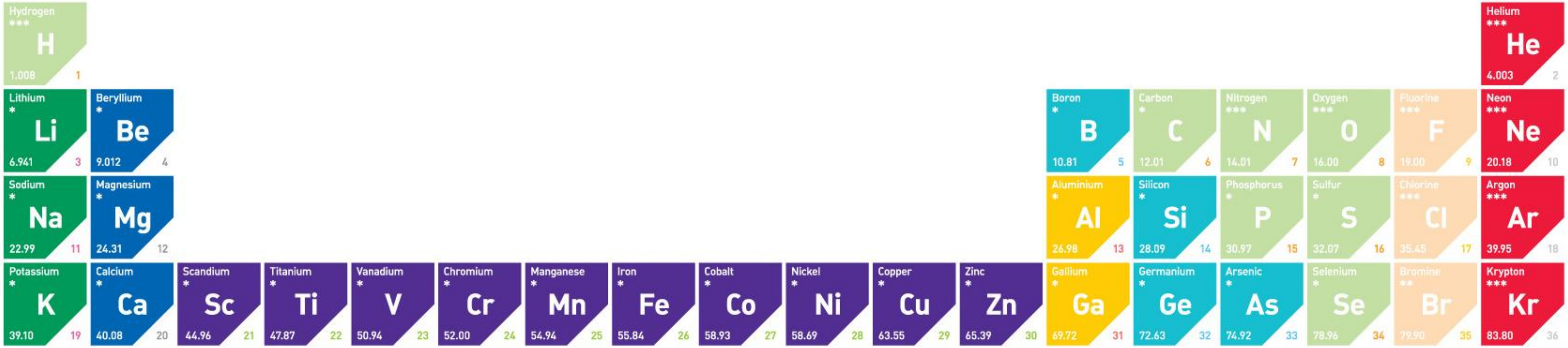
Manganese-catalyzed Oxidation of Water to O2

$$\boxed{2\,H_2O \rightarrow O_2 + 4\,H^+ + 4\,e^-}$$

Crucial catalytic function of a polynuclear manganese complex

What are the **properties** that make **manganese centers** particularly well suited for the catalysis of water oxidation and for the rapid evolution of dioxygen?

- a **large variety** of stable or at least metastable **oxidation states** (+II, +III, +IV, +VI, +VII);
- the often very **labile** binding of **ligands**;
- a pronounced preference for **high-spin states**, due to inherently small d orbital splitting, resulting in a complex magnetic behavior.



Hydrogen *** H 1.008 1																	Helium *** He 4.003 2
Lithium * Li 6.941 3	Beryllium * Be 9.012 4											Boron * B 10.81 5	Carbon * C 12.01 6	Nitrogen *** N 14.01 7	Oxygen *** O 16.00 8	Fluorine *** F 19.00 9	Neon *** Ne 20.18 10
Sodium * Na 22.99 11	Magnesium * Mg 24.31 12											Aluminium * Al 26.98 13	Silicon * Si 28.09 14	Phosphorus * P 30.97 15	Sulfur * S 32.07 16	Chlorine *** Cl 35.45 17	Argon *** Ar 39.95 18
Potassium * K 39.10 19	Calcium * Ca 40.08 20	Scandium * Sc 44.96 21	Titanium * Ti 47.87 22	Vanadium * V 50.94 23	Chromium * Cr 52.00 24	Manganese * Mn 54.94 25	Iron * Fe 55.84 26	Cobalt * Co 58.93 27	Nickel * Ni 58.69 28	Copper * Cu 63.55 29	Zinc * Zn 65.39 30	Gallium * Ga 69.72 31	Germanium * Ge 72.63 32	Arsenic * As 74.92 33	Selenium * Se 78.96 34	Bromine ** Br 79.90 35	Krypton *** Kr 83.80 36

Also, freshly precipitated manganese dioxide forms a typically nonstoichiometric mixed-valent (+IV,+III) system of the composition $MnO_2^{-x} \times n\, H_2O$,

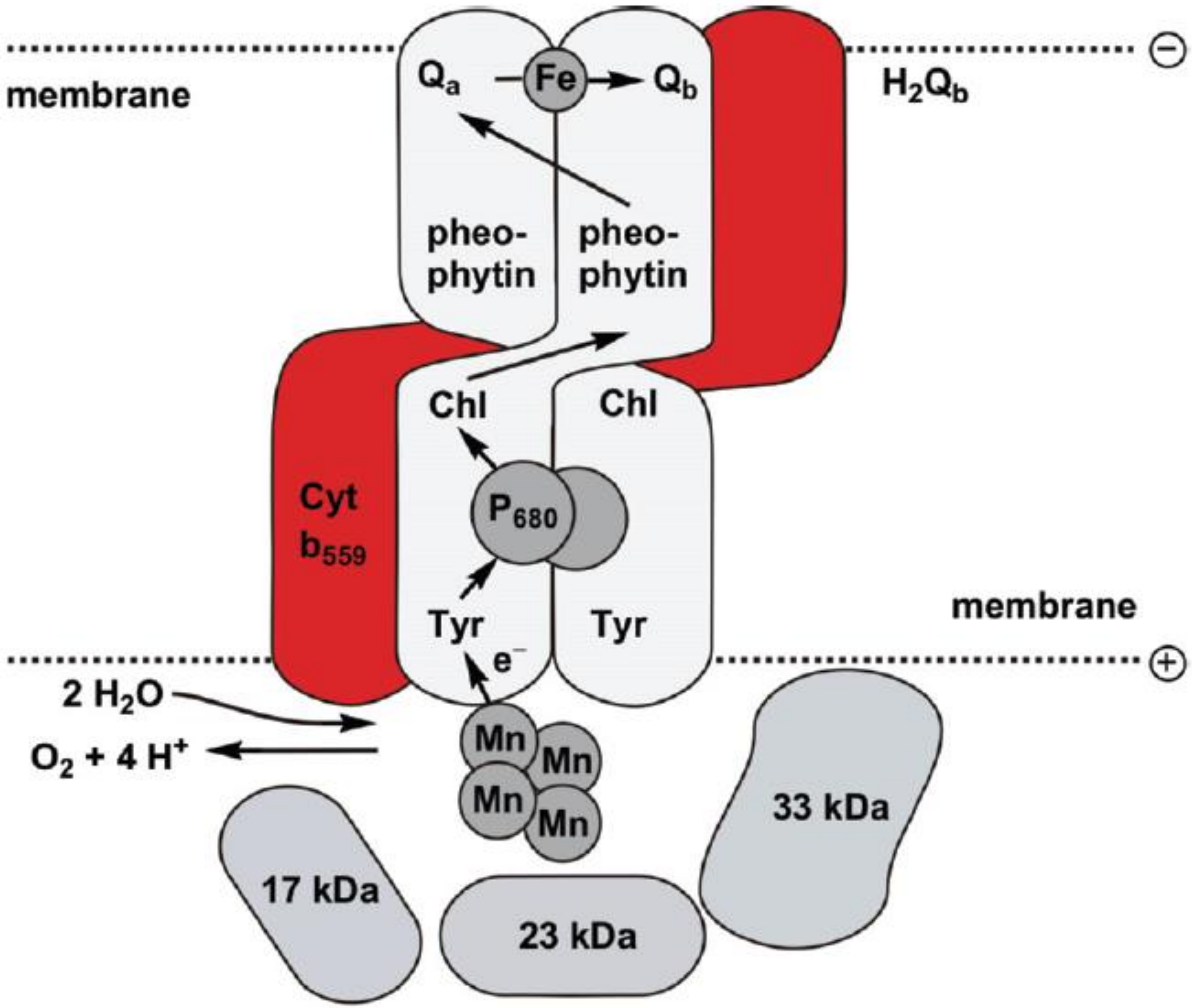
↓

good heterogeneous catalyst for the decomposition of hydrogen peroxide to dioxygen and water.

Mn(III,IV) oxides or hydroxides were certainly available in sea water under the conditions of developing photosynthesis about 3×10^9 years ago; oxidic manganese nodules with about 20% Mn content are quite abundant on the sea floor.

Selection during evolution of life

The importance of manganese for the O_2 metabolism is not restricted to photosynthesis; further established examples include:
manganese-containing superoxide dismutase, an **azide-insensitive catalase** and other **peroxidases.**



OEC = oxygen-evolving complex

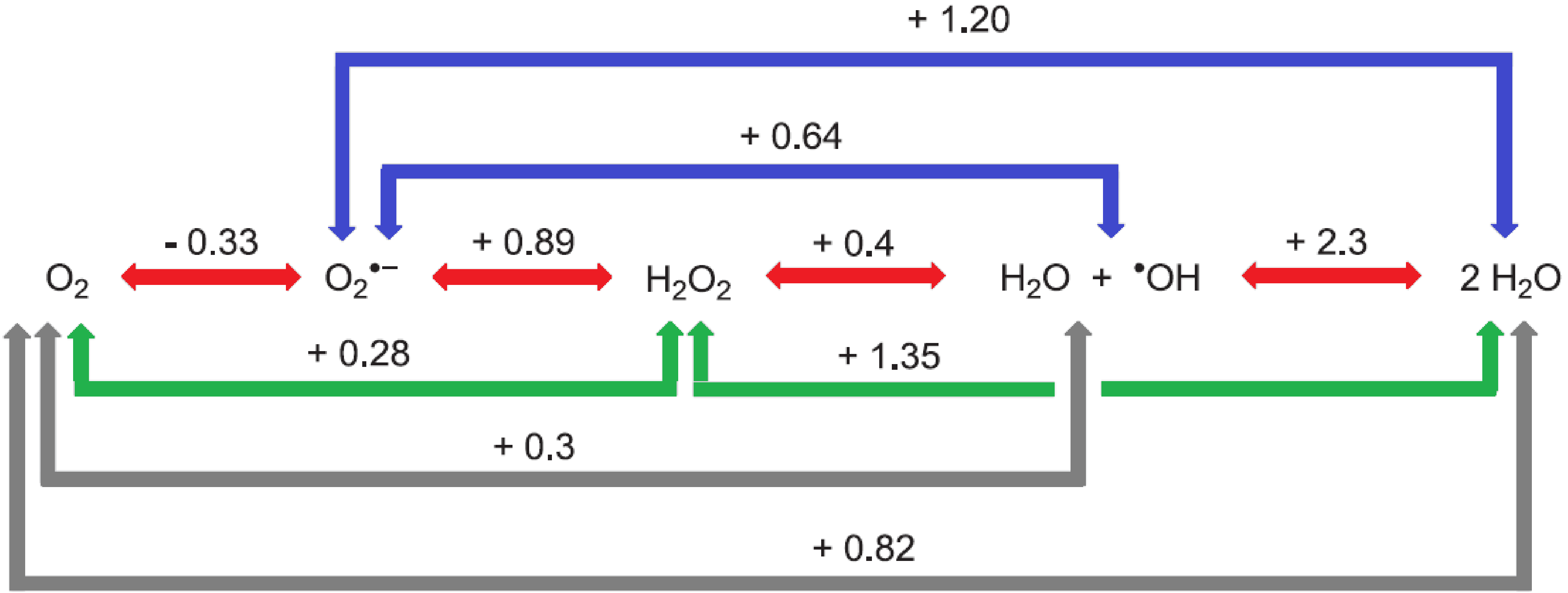
WOC = water-oxidizing complex

Schematic structure of PS II, including the OEC: protein subunits, electron-transferring components and electron-flow.

Because of the higher excitation energy in comparison with bacterial pigments, the PS II of plants can utilize the **tyrosine/tyrosine radical cation redox pair** (*E*0 = +0.95 V)
to transfer electrons to the OEC (also called WOC);

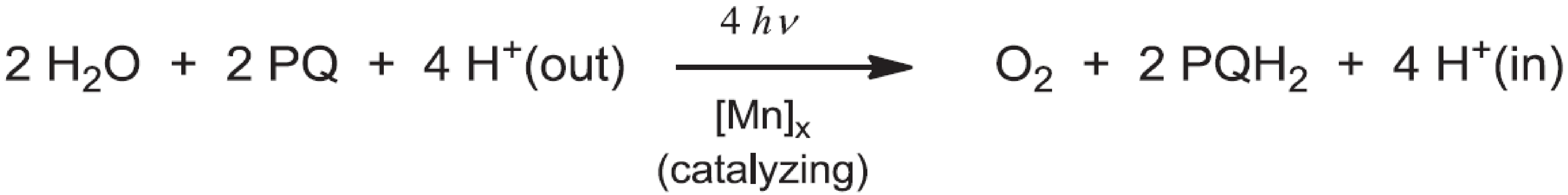
a secondary electron donor for P_{700} is the copper-containing protein plastocyanin.

Redox potential scheme of water

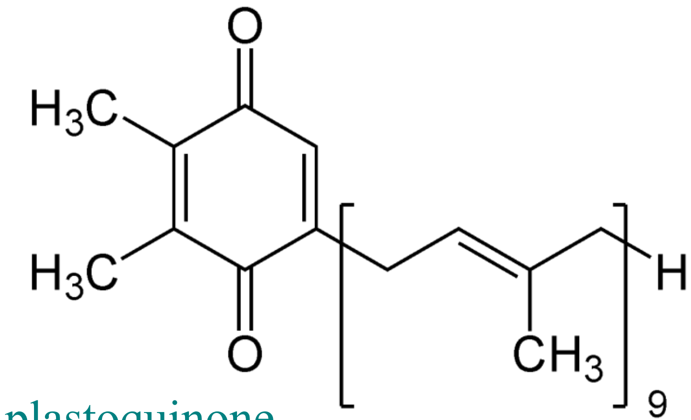


The removal of one, two or three instead of four electrons per two H_2O molecules requires **rather high potentials** and leads to such reactive high-energy products as hydroxyl radical (•OH), hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$). All of these are potentially harmful substances for biological membranes, particularly in the presence of transition metal ions. Important functions of water oxidation catalysts are therefore to **lower the oxidation potential**, to **prevent the formation of free reactive intermediates**, and to **guarantee substrate specificity**, since many molecules are more easily oxidized than H_2O.

The overall reaction in PS II can be summed up as in the equation, employing **quinonoid proton** and **electron carriers** (PQ: plastoquinone, PQH_2: plastohydroquinone).



$$2\,H_2O + 2\,PQ + 4\,H^+(\text{out}) \xrightarrow[\substack{[\text{Mn}]_x \\ \text{(catalyzing)}}]{4\,h\nu} O_2 + 2\,PQH_2 + 4\,H^+(\text{in})$$



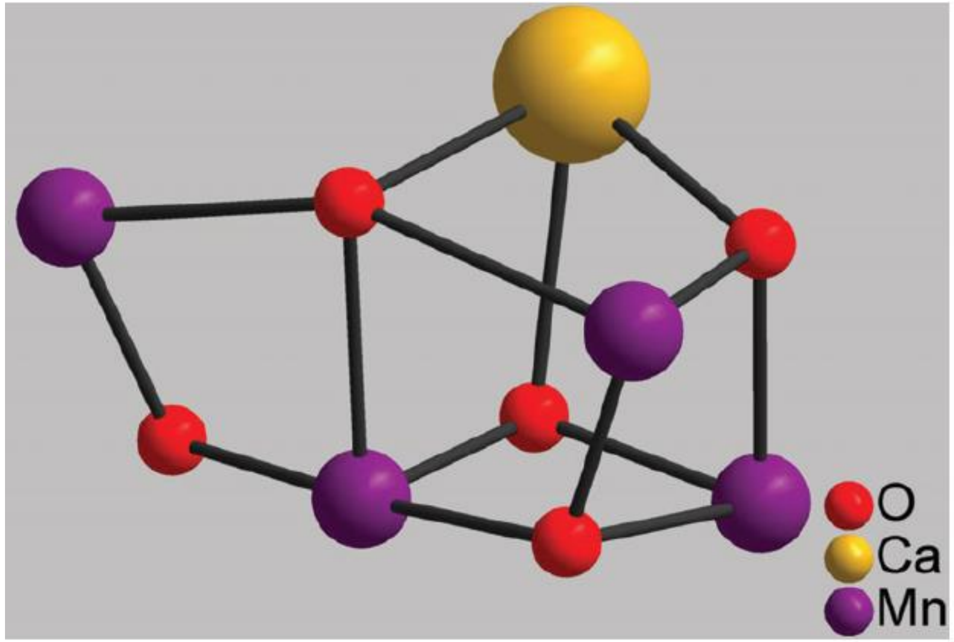
plastoquinone

The required four oxidation equivalents for O_2 production are available **only after excitation** with at least **four photons in PS II**; measurements of the actual quantum yield for photosynthesis have shown that about eight photons are needed for every molecule of converted CO_2.

In addition to inorganic Ca^{2+} and several chloride ions, effective dioxygen generation requires manganese ions as redox-active components.

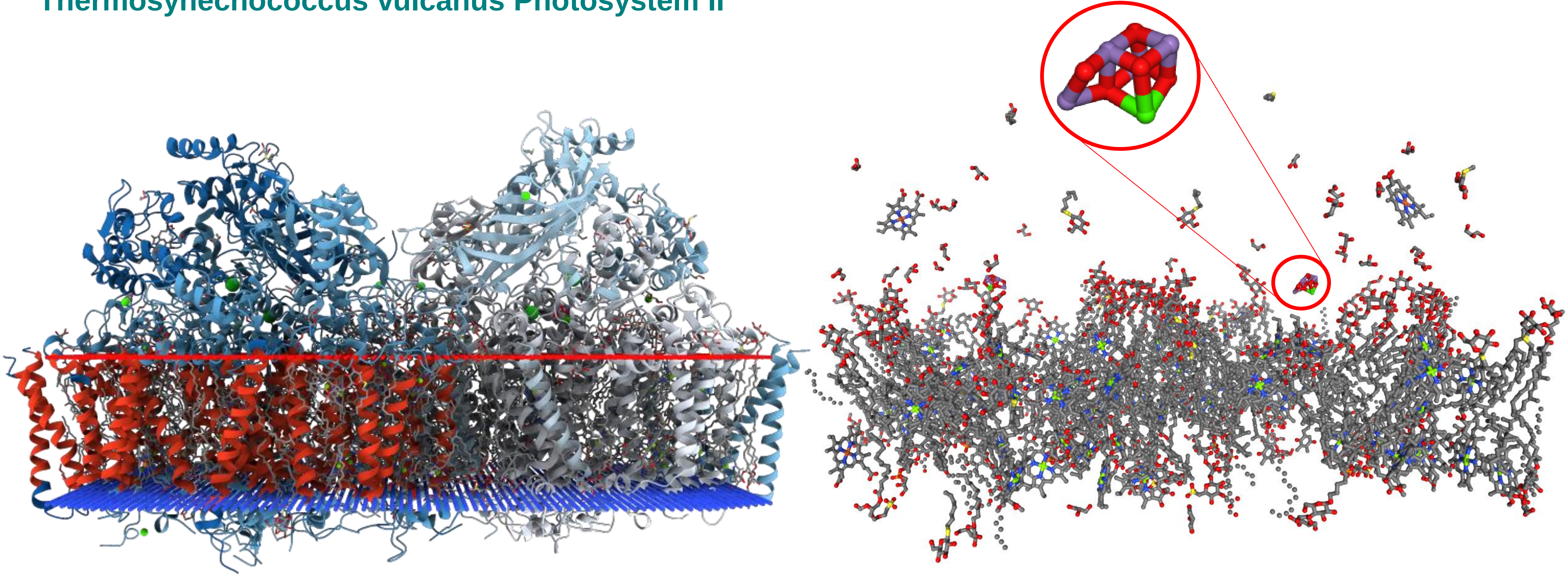
EPR spectroscopically detectable **manganese deficiency** thus **impairs** the **growth** of plants.

The catalyzing oligoprotein complex OEC contains a total of **four manganese centers** in one of its subunits (33 kDa).



PDB = 3WU2

Thermosynechococcus vulcanus Photosystem II



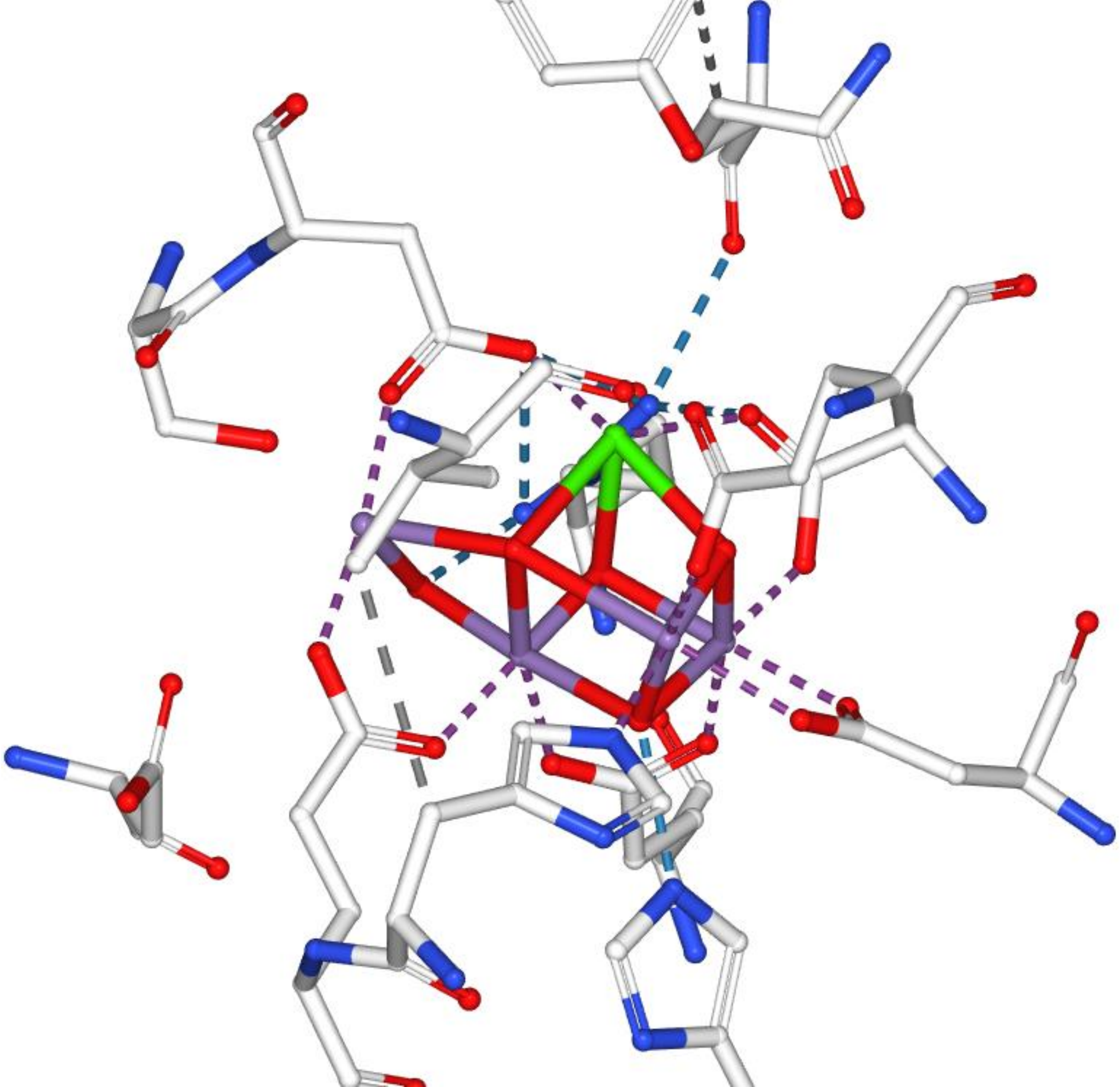
In view of the **intense x-ray irradiation** needed to analyze the crystals of very large protein complexes, the assignment of structurally suggested oxidation states requires great caution, as **radiation** can cause **redox reactions**.

The structure of the metal cluster contains a distorted Mn_3CaO_4 heterocubane with one added (“dangling”) Mn centre, connected to one of the cubane oxygen atoms and to one of the manganese sites via a bridging oxide.

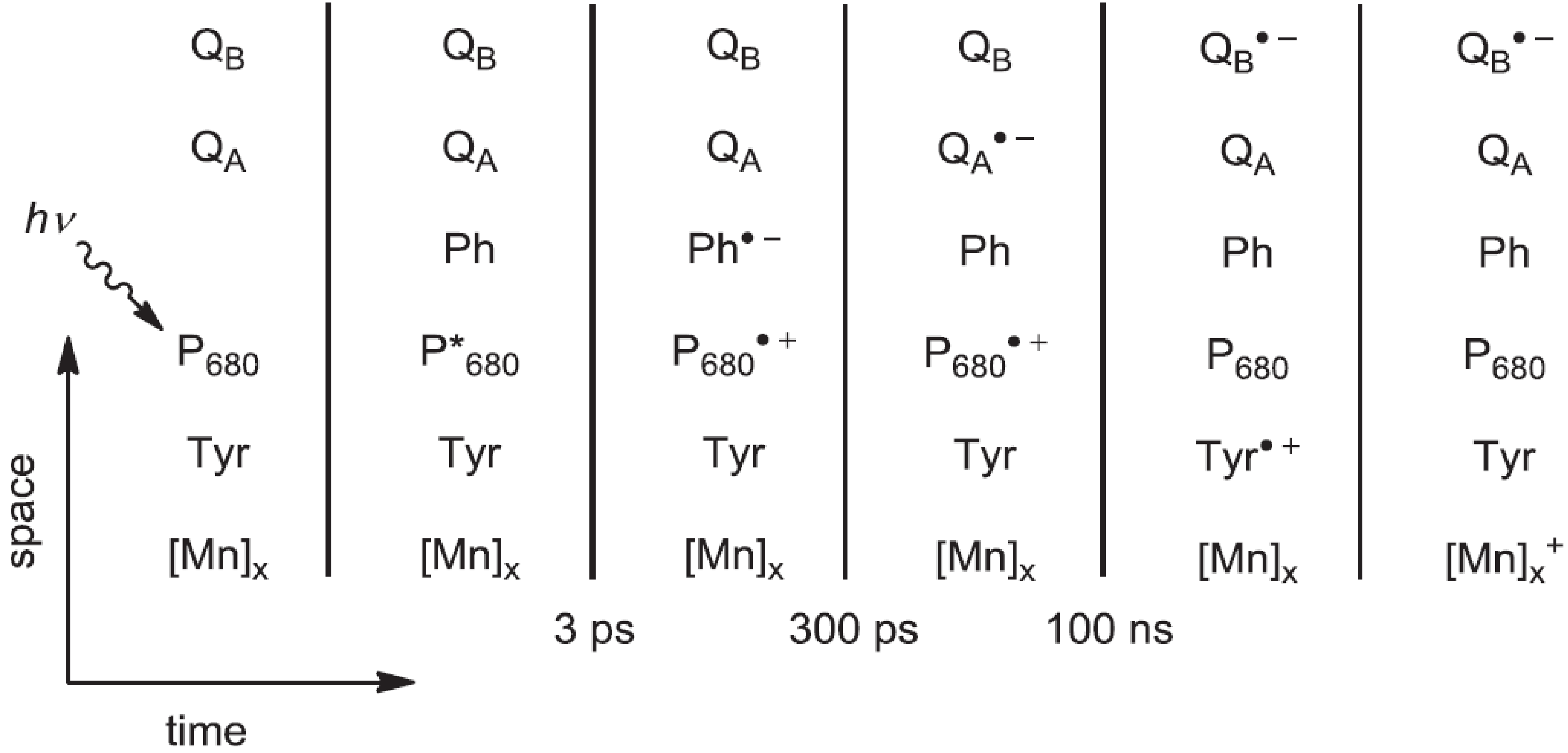
The first-shell coordination is complemented by carboxylates (glutamate, aspartate) and a histidine.

Two non-metal-binding chloride ions can be located near the cluster and participate in structure stabilization and hydrogen bonding.

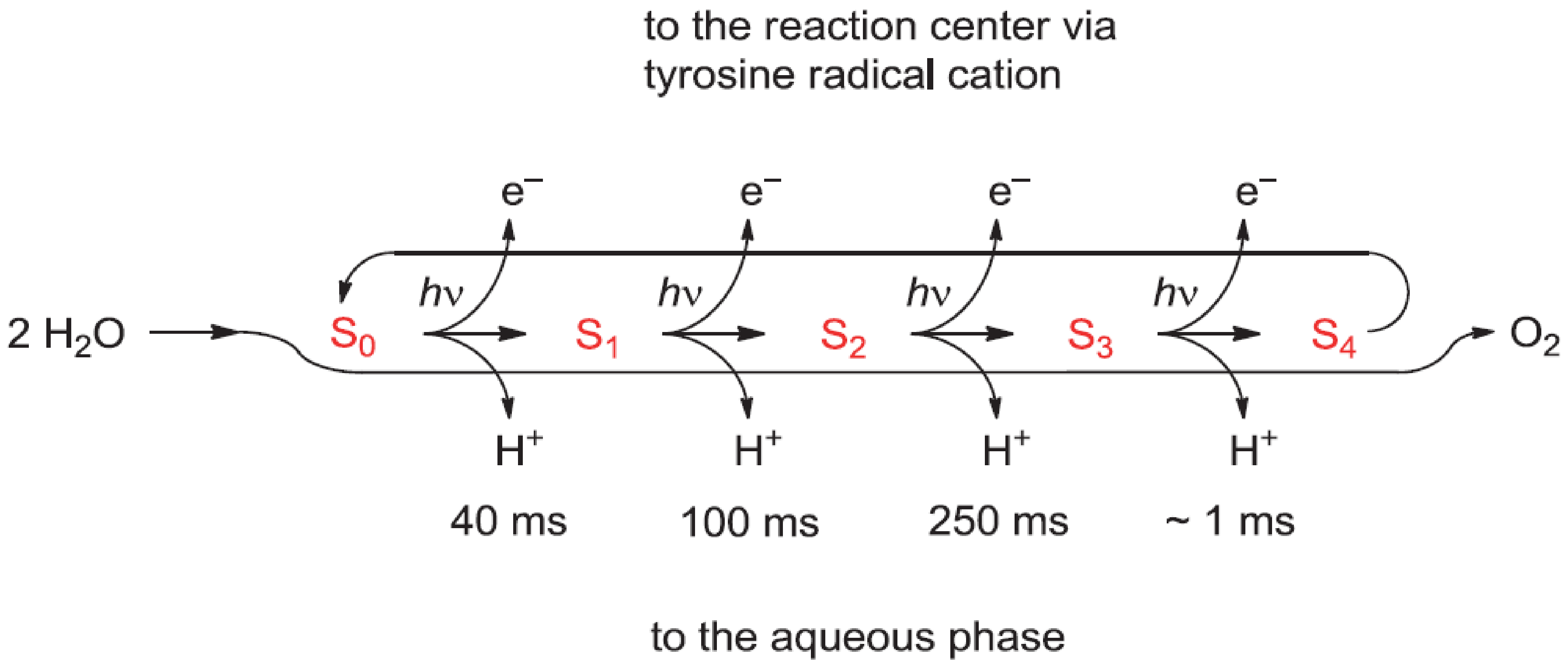
The location of the substrate water (converted to O_2) and the details of the manganese oxidation states involved are being studied both experimentally and by computational methods.



The **charge-separation cascade** illustrated in the scheme shows how single oxidation equivalents become available for the polynuclear manganese cluster via a **tyrosine radical cation** (“$Tyr^{\bullet +}$”) as a primary acceptor.



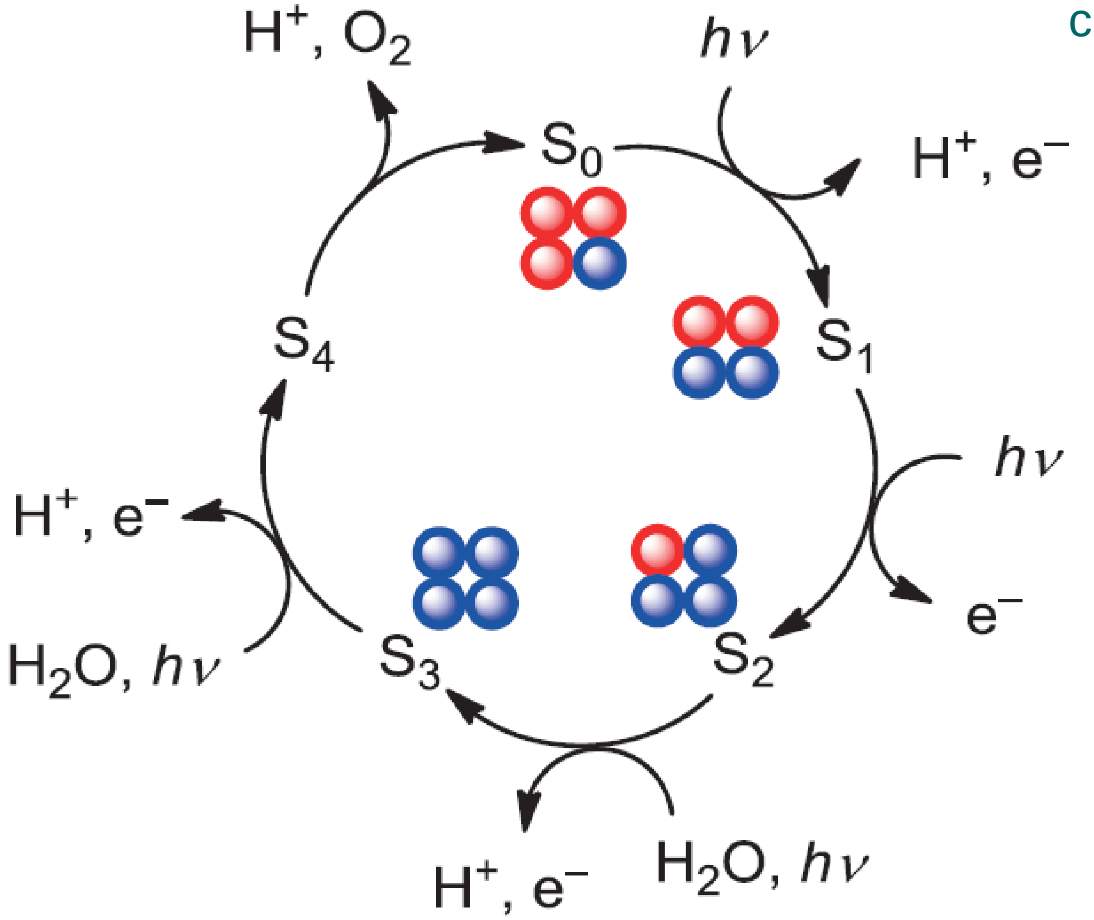
Until two molecules of water have been oxidized *stepwise* to dioxygen through successive light excitation/charge separation events, **five exactly tuned (oxidation) states of the PS II**, referred to as S_0 to S_4 (**Kok cycle**), are observed in the millisecond range by flash-photolysis techniques.



According to manganese XAS near edge studies, the metal oxidation state changes from S_0 via S_1 to S_2.

S_2 => S_3 probably a not-innocent ligand involved

The relatively long-lived intermediate state S_2 was characterized using EPR finding an **Mn(III)Mn(IV)$_3$** configuration



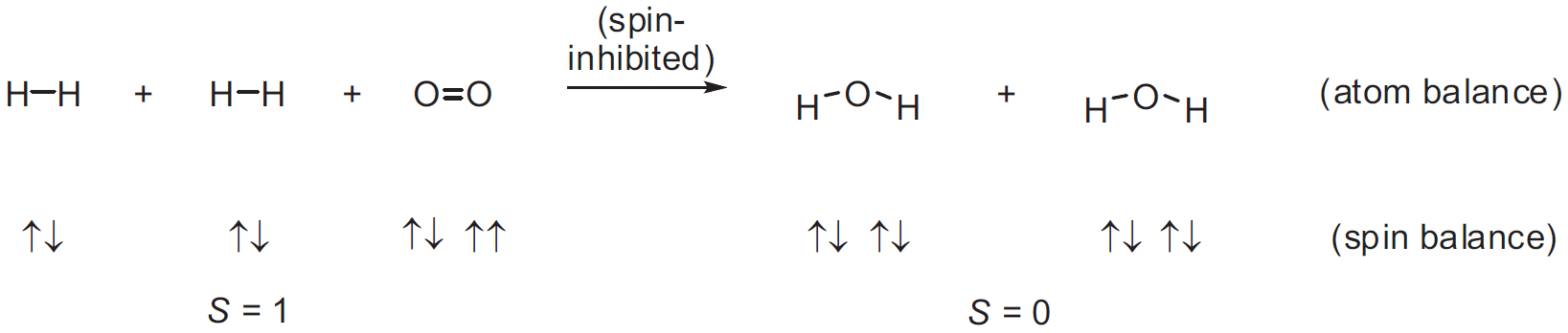
🔴 Mn^{3+}

🔵 Mn^{4+}

The spin issue

It would require a “flipping” of electron spins to make an odd-electron catalyst and an even electron systems such as O_2 compatible; however, spin-flipping processes during chemical reactions often implicate a **high activation energy** because of their **low probability** (→ statistical aspect of the reaction rate).

Example: the H_2/O_2 mixture, which reacts to give water only after activation by a bond-breaking catalyst or after ignition (= local bond breaking) in a radical chain reaction.



Due to at least partial antiferromagnetic coupling between the individual high-spin metal centers, the **total spin** of the **manganese cluster** in the OEC is **distinctly small**

In fact smaller than that of some synthetic polymanganese compounds with $S > 10$ ground states.

Considering this fact, as well as the already mentioned availability of **fairly high oxidation** states and the **high lability** regarding coordinated ligands

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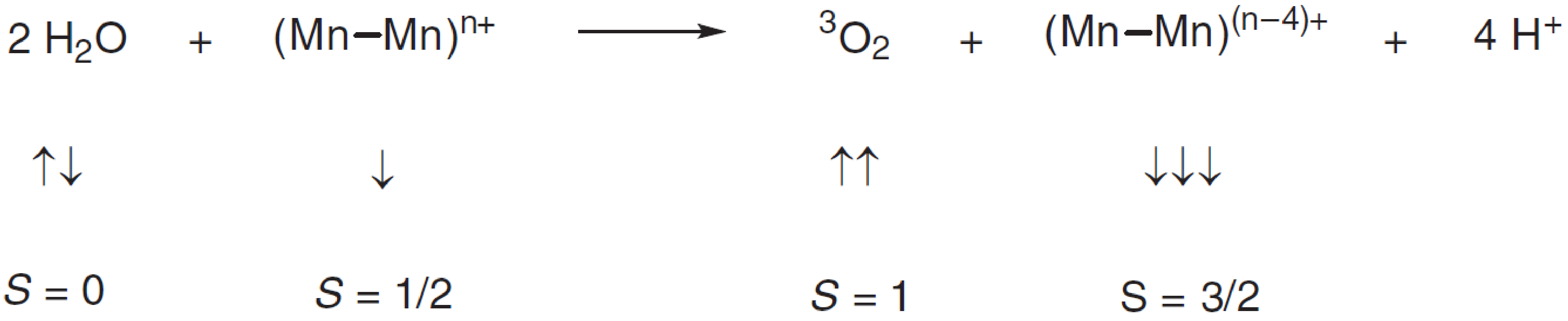
manganese centers uniquely suited to catalyzing the generation and *release* of the molecule O_2 in its triplet ground state; that is, with an *even* number of unpaired electrons.

To appreciate this behavior, one must remember that the reaction of transition metals with dioxygen normally involves an irreversible binding.

The hypothetical spin balance in the scheme shows the possible function of catalytic metal centers with variable spin quantum numbers *S* = n/2.



$$\begin{array}{ccccccccc} 2\,H_2O & + & (Mn{-}Mn)^{n+} & \longrightarrow & {}^3O_2 & + & (Mn{-}Mn)^{(n-4)+} & + & 4\,H^+ \\ \uparrow\downarrow & & \downarrow & & \uparrow\uparrow & & \downarrow\downarrow\downarrow & & \\ S = 0 & & S = 1/2 & & S = 1 & & S = 3/2 & & \end{array}$$

In the four-electron oxidation ofwater to O_2, the polymanganese system thus acts

- as an electron reservoir, accumulating charge in an exactly controlled fashion at physiologically high redox potential
- as a *non*-${}^3O_2$-retaining catalyst.

Atom- and Group-Transfer Chemistry

Both substrate binding and redox changes occur during atom- and group-transfer chemistry , even when the overall reaction does not involve a net redox change.

Reactions involving **dioxygen** and its metabolic products are the largest subset of such functions.

Key features

Two electron-process can employ:

- metals, such as molybdenum, which undergo facile multielectron redox level changes, for example, between $M^{(n+2)+}$ and M^{n+}.
- a dimetallic center involving redox metals such as iron or copper
- coupling of one of these metals with a redox-active ligand such as a porphyrin

Geometric changes resulting from ligand-mediated redox reactions at a metal center can trigger coordinated movements of nearby protein chains, resulting in cooperative behavior.

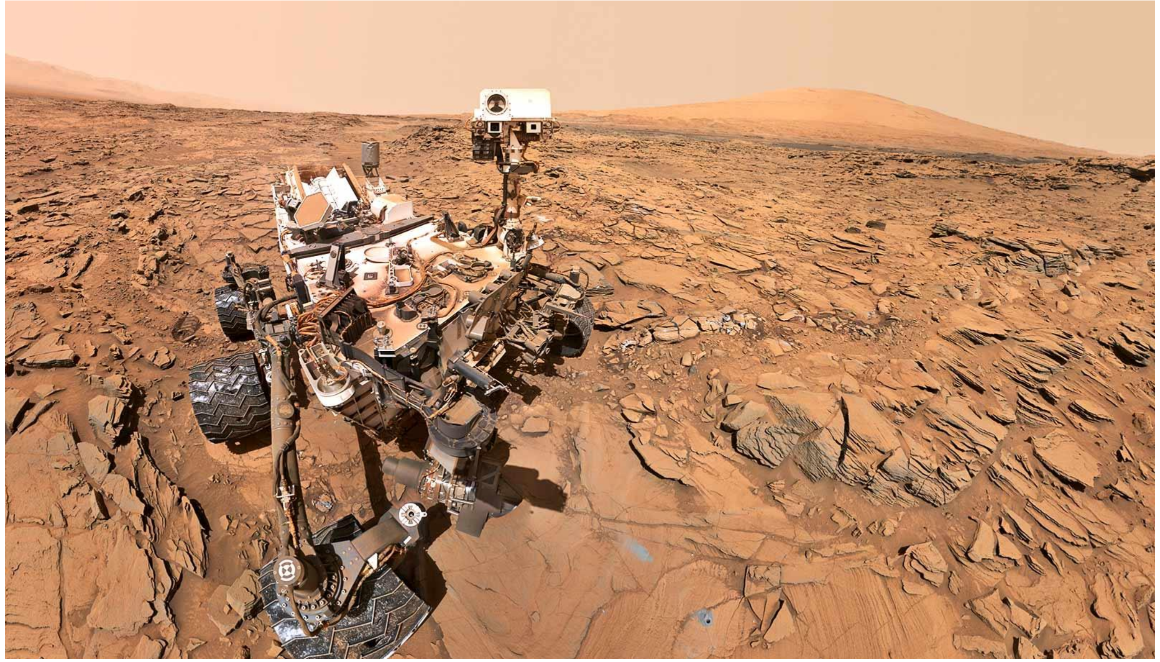
Group-transfer reactions can also occur through the generation of radicals at a metal center

Bioinorganic systems can facilitate this chemistry
-binding and transferring of electrons through metal-mediated redox transformations-

The Dioxygen Molecule, O_2: Uptake, Transport and Storage of an Inorganic Natural Product

A bit of history…

Studies of the atmospheres of planets and moons have generally shown **O_2 contents of far less than 1 vol. %**, and the primordial atmosphere of the earth until about 2.5 billion years ago is assumed to have been very similar.

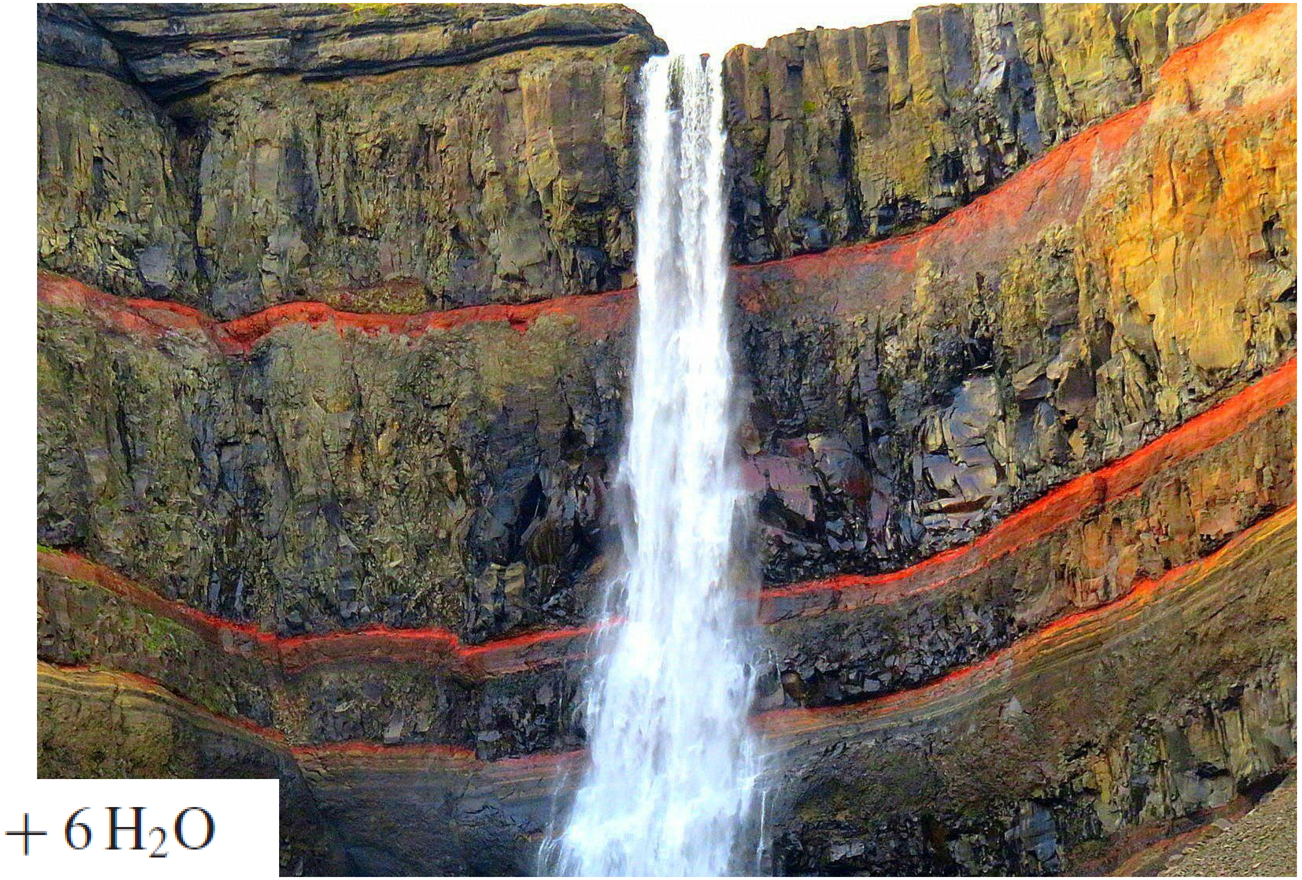


O_2 is a **natural product,** that is, a secondary metabolic product, just like alkaloids or terpenes. Initially, it even represented an exclusively toxic waste product.

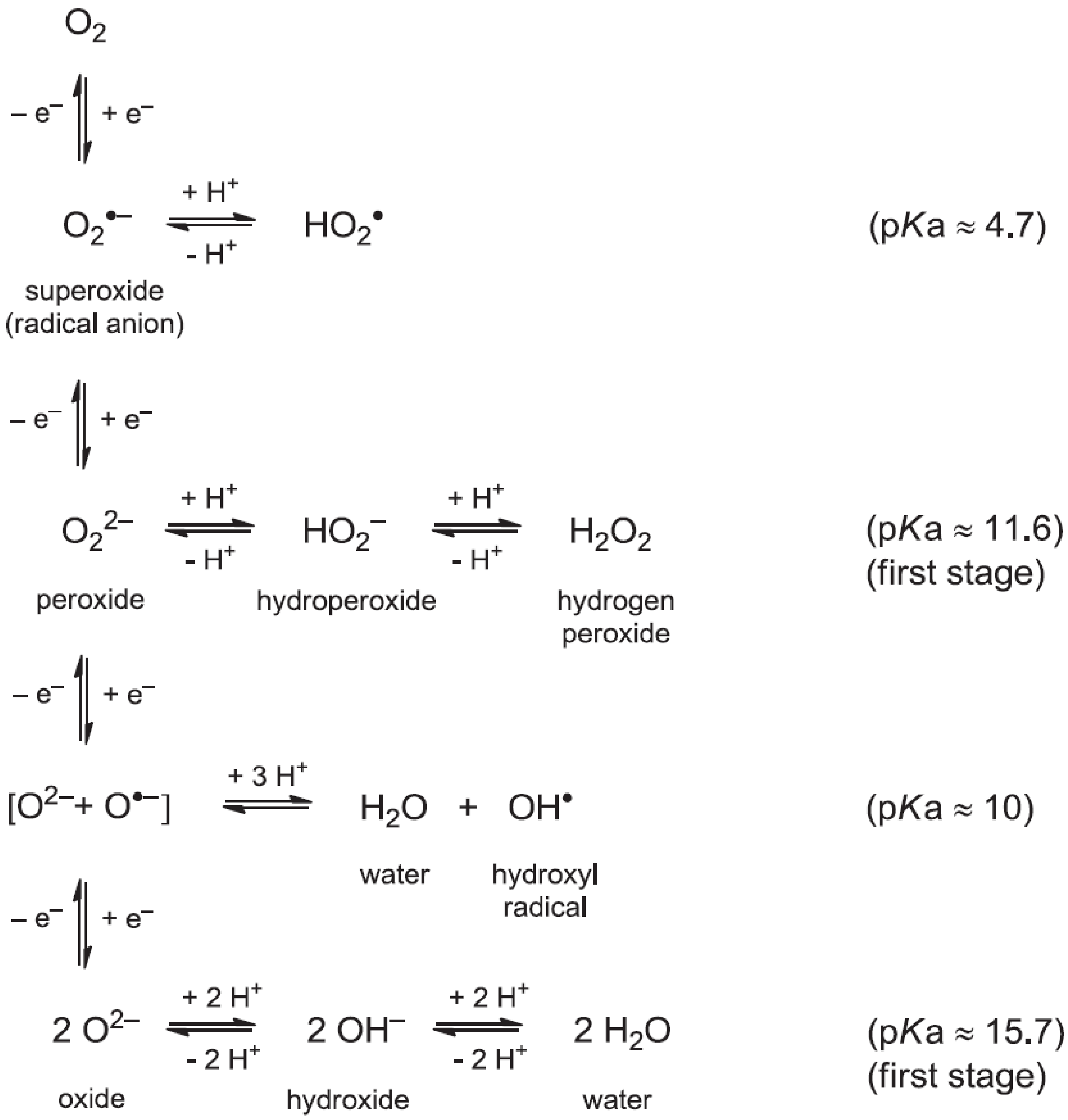
The amount of photosynthesis increased to such an extent that the simultaneously produced oxidation equivalents could no longer be scavenged by auxiliary substrates such as sulfur(−II) and iron(II) compounds;

Eventually, a (Mn-)catalyzed oxidation of the surrounding water to dioxygen developed

For a while, the "environmentally" extremely harmful O_2 could be **deactivated by reaction with reduced compounds**, particularly soluble ions such as Fe^{2+} and Mn^{2+}, to form massive **oxidic sediments**, such as "**banded iron formations**"



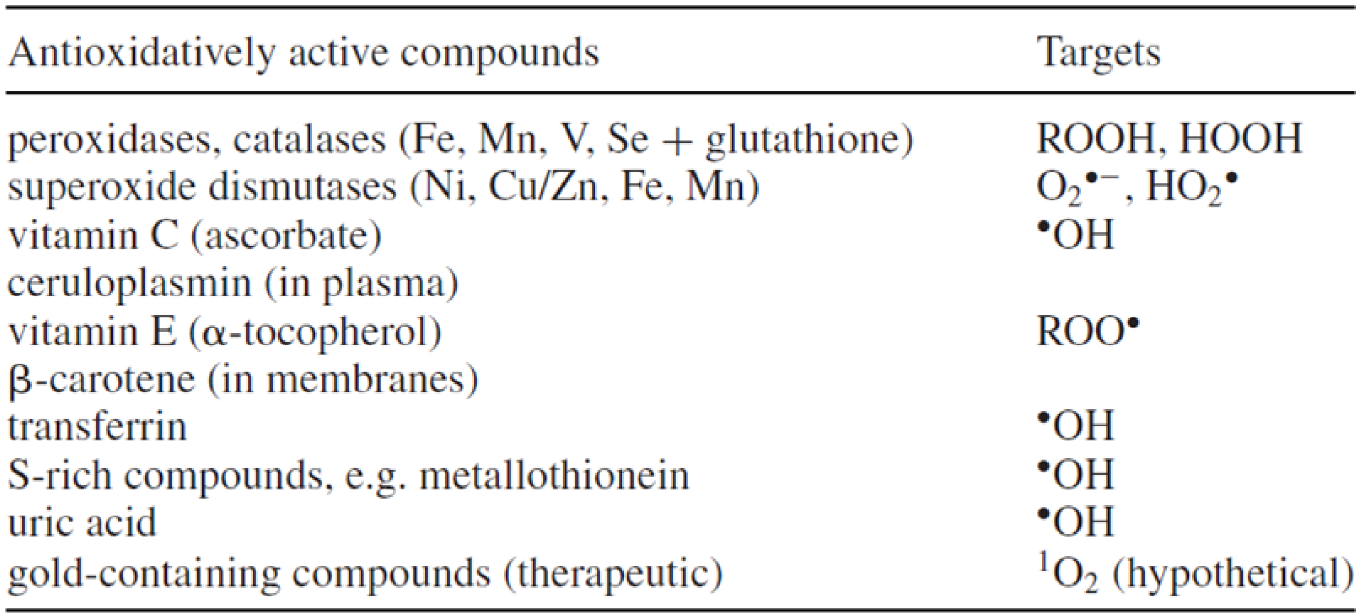
$$4\,Fe^{2+} + O_2 + 2\,H_2O + 8\,OH^- \rightarrow 4\,Fe(OH)_3 + 6\,H_2O$$



About 2.5 billion years ago the concentration of O_2 in the atmosphere started to increase, and from about **700 million years ago** another rise in O_2 concentration led to the **current equilibrium between biogenic production and a general, biogenic and non biogenic consumption** of O_2.

In addition to the oxidizing character of O_2, partially reduced and highly reactive species are easily formed through catalysis by transition metals such as Fe^{II}.

Detoxification with regard to these species has made it necessary to develop a multitude of **biological antioxidants**



Antioxidatively active compounds	Targets
peroxidases, catalases (Fe, Mn, V, Se + glutathione)	ROOH, HOOH
superoxide dismutases (Ni, Cu/Zn, Fe, Mn)	$O_2^{\bullet -}$, $HO_2^{\bullet}$
vitamin C (ascorbate)	$^{\bullet}OH$
ceruloplasmin (in plasma)	
vitamin E (α-tocopherol)	$ROO^{\bullet}$
β-carotene (in membranes)	
transferrin	$^{\bullet}OH$
S-rich compounds, e.g. metallothionein	$^{\bullet}OH$
uric acid	$^{\bullet}OH$
gold-containing compounds (therapeutic)	$^{1}O_2$ (hypothetical)

Therefore, only those ***aerobic* organisms** that were able to **develop protective mechanisms** against O_2 *and* the very toxic intermediates (often radicals) resulting from its partial reduction **have survived** in contact with the atmosphere

Exploiting oxygen: a new way to reach prosperity?

Organisms that were able to use the **reverse process of photosynthesis** in an oxygen-containing atmosphere for a **much more efficient and only indirectly light-dependent metabolic energy conversion** and thus for a more dynamic form of life.

This so-called “**respiration**” may be viewed as a “cold” combustion of reduced substrates (“food”).

Other requirements/possibilities for new living being were:

- to obtain a useful degradation of partially reduced oxygen intermediates such as H_2O_2, $O_2^{\bullet -}$ and $\bullet OH$
- the ability to cope with a drastically changed bioavailability of some elements and their compounds

Incidentally, the biogenic O_2 production **increased the ozone (O_3) layer** in the stratosphere

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a more controlled development of organisms by **protecting** them from the **high-energy components of solar radiation**

O_2 properties

According to its position in the periodic table and the resulting second highest electronegativity of all elements, **O_2 is strongly oxidizing**.

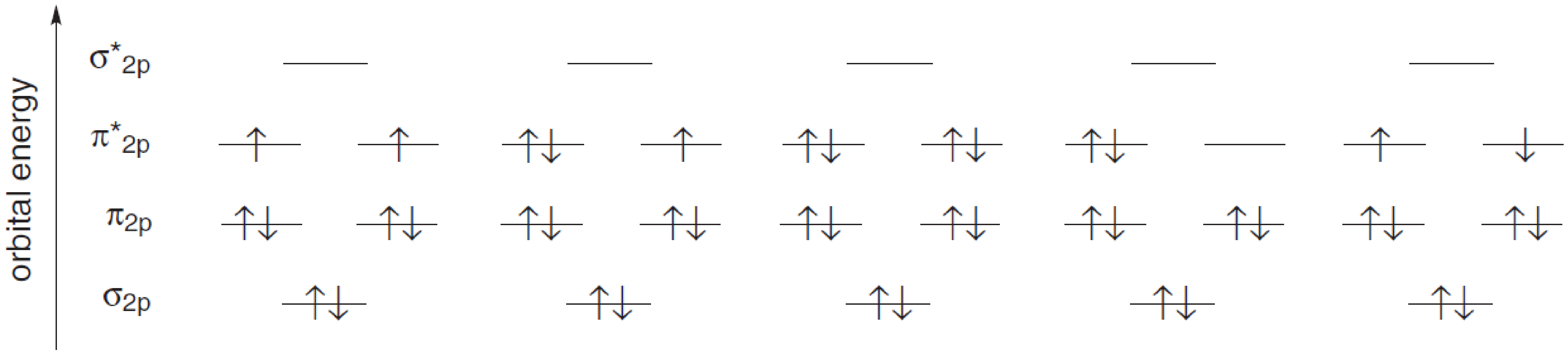
Many substances react very exothermically with dioxygen, although activation is often required, due to **inhibited reactivity**.

Why?

triplet ground state of the O_2 molecule with two unpaired electrons

$^3O_2$ with two unpaired electrons in the ground state is favored over both singlet states $^1O_2$ ($^1\Delta$) and $^1O_2$ ($^1\Sigma$), by more than 90 and 150 kJ/mole, respectively.

Consequence of the molecular orbital scheme and Hund's rule



	$^3O_2$	$^2O_2^{\bullet-}$	$^1O_2^{2-}$	$^1O_2(^1\Delta)$	$^1O_2(^1\Sigma)$
bond order	2	1.5	1		
bond length (pm)	121	≈ 128	≈ 149		
vibrational frequency (cm^{-1})	1560	1150-1100	850-740		

This phenomenon is responsible for the present **metastable situation** at the earth's surface, with the simultaneous **presence of combustibles** (wood, fossil fuels, carbohydrates etc.) and an **oxygen-rich atmosphere without** the instant **formation** (via fire) of the **lowest-energy products, water and CO_2**.

Therefore, chemically or photogenerated singlet dioxygen $^1O_2$ represents a toxic form of oxygen
the diamagnetic elemental modification ozone, O_3, also shows uninhibited and therefore uncontrolled oxidation behavior towards biomolecules.

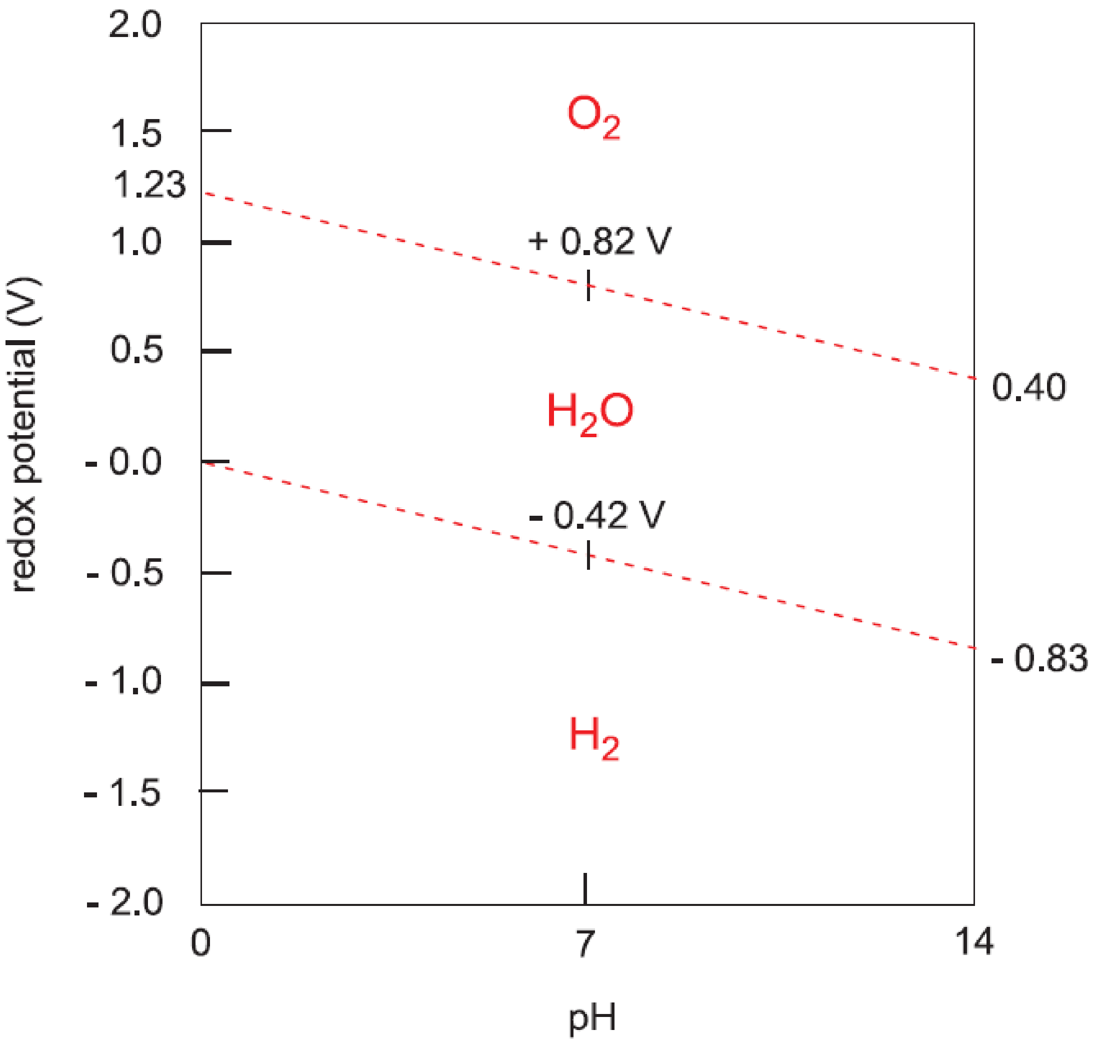
The "spin-verbot" (the impediment to a spin-forbidden process) is not valid for those reaction partners of $^3O_2$ that can easily undergo single electron-transfer reactions or which already contain unpaired electrons. Including:

- radicals ($S = 1/2$), either as stable free species or as intermediates produced by, for example, ignition;
- compounds with photochemically produced excited triplet states ($S = 1$);
- paramagnetic transition metal centers ($S \geq 1/2$).

What about metal?

Almost all reactions between O_2 and **metal complexes proceed irreversibly**, as illustrated and in most of these reactions, **oxygen is eventually reduced** to the (−II) state so that oxide (O^{2-}), hydroxide (OH^-) or water (H_2O) ligands result

The **redox potentials** for the reduction of O_2 **decrease** in the **presence of electrophiles** such as metal ions and H^+.



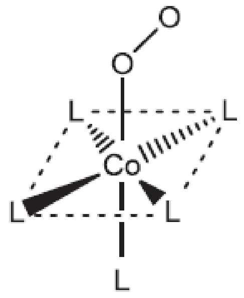
Stability diagram of water, illustrating the thermodynamic equilibrium between H_2O and H_2 or O_2 as a function of potential and of pH, respectively all other states are metastable.

About O_2 coordination

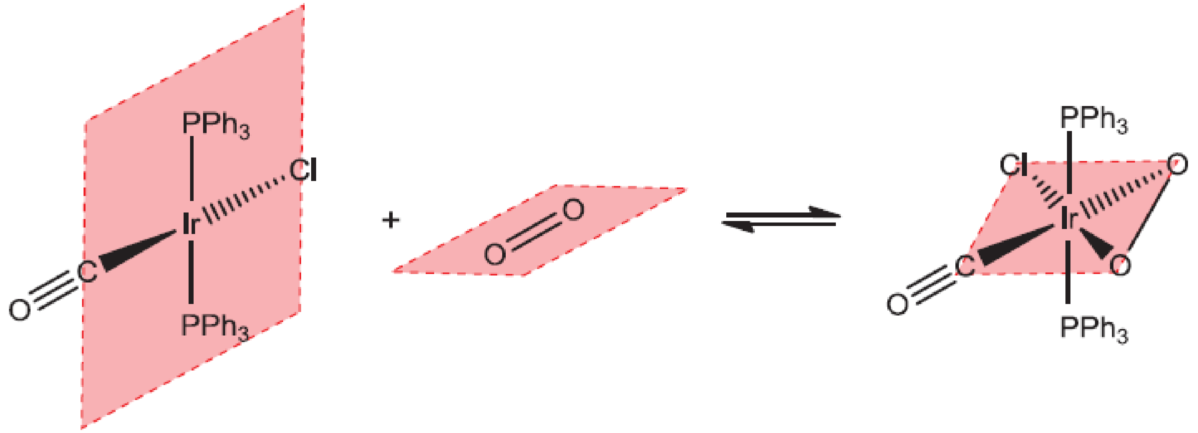
Inorganic and organometallic compounds of the metals **cobalt, rhodium and iridium** from group 9 of the periodic table form *reversibly* dioxygen-coordinating complexes (simple models)

Vaska's iridium complex shows reversible uptake and release of **"side-on" (η^2) coordinated O_2**, while pentacyano–, salen– and pentaammine–cobalt complex fragments can coordinate **"end-on" η^1-O_2**

end-on (η^1)

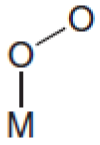
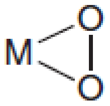
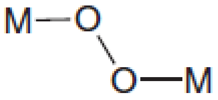


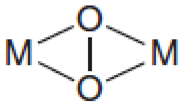
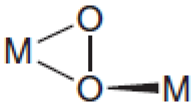
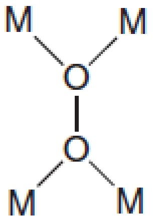
L = NH_3, CN^-



side-on (η^2)

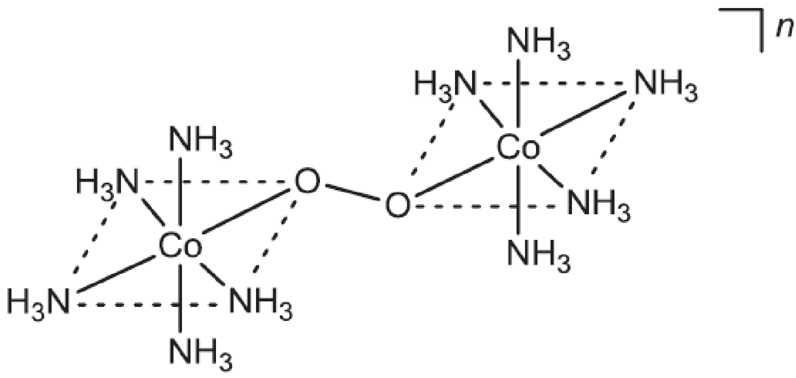
Further possibilities for O_2^{n-} coordination geometries

type of structure:	mode of O_2^{n-} coordination:	
	η^1	end-on
	η^2	side-on
	$\mu-\eta^1:\eta^1$	end-on bridging

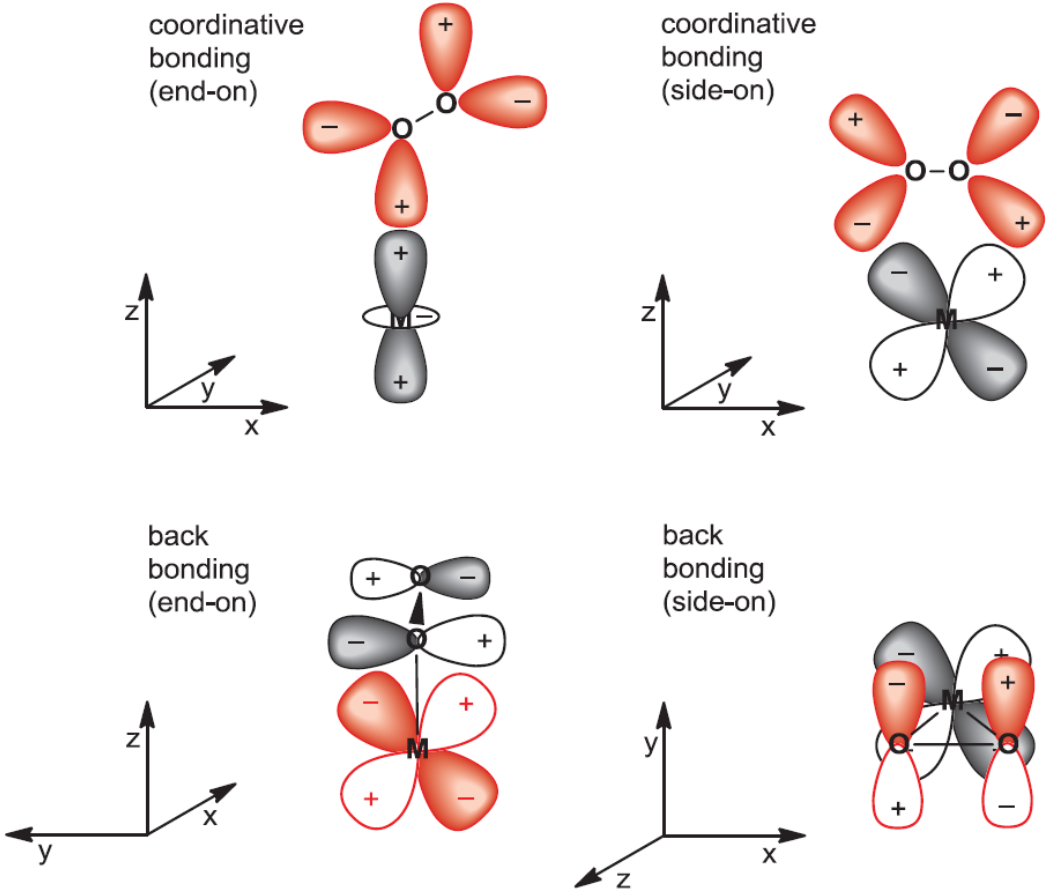
type of structure:	mode of O_2^{n-} coordination:	
	$\mu-\eta^2:\eta^2$	side-on bridging
	$\mu-\eta^1:\eta^2$	end-on/side-on bridging
	$\mu_4-(\eta^1)_4$	end-on, fourfold bridging

Structural and spectroscopic studies (electron paramagnetic resonance, EPR) suggest that the **redox-active "non-innocent" dioxygen ligand** is mainly bound in **singly or doubly reduced** form.

-can act as a bridging ligand between metals-



$n = 5$, d(O–O) = 131 pm, $O_2^{\bullet -}$ ligand
$n = 4$, d(O–O) = 147 pm, O_2^{2-} ligand



The unsaturated molecule O_2 is a **σ or π donor/π acceptor** ligand.

Electron density is transferred to the electropositive metal center via the free electron pairs or the double bond

high-energy, partly filled d orbitals of electron-rich metal centers can contribute to back-bonding via π back-donation into one of the partly filled π* orbitals of O_2

Oxygen Transport and Storage

What functions are required by organisms for a controlled utilization of O_2?

Before dioxygen can be metabolized, it has to be **taken up reversibly** from the atmosphere and **transported to oxygen-depleted tissue**, where it must be stored until actual use.

Strategies for O_2 coordination

Groups of mollusks, crustaceans, spiders and worms

dinuclear metal arrangements with amino acid coordination, namely the **copper protein hemocyanin** (*Hc*) or the **iron protein hemerythrin** (*Hr*)

Majority of other organisms, particularly vertebrates

heme system: monoiron complexes of a certain porphyrin macrocycle, protoporphyrin IX. The corresponding proteins are the **tetramer hemoglobin** (*Hb*, O2 uptake in the lungs and transport in the blood stream) and the **monomer myoglobin** (muscles)

Dioxygen transport is **not a catalytic** but a “**stoichiometric**” function

Huge number of devoted proteins!

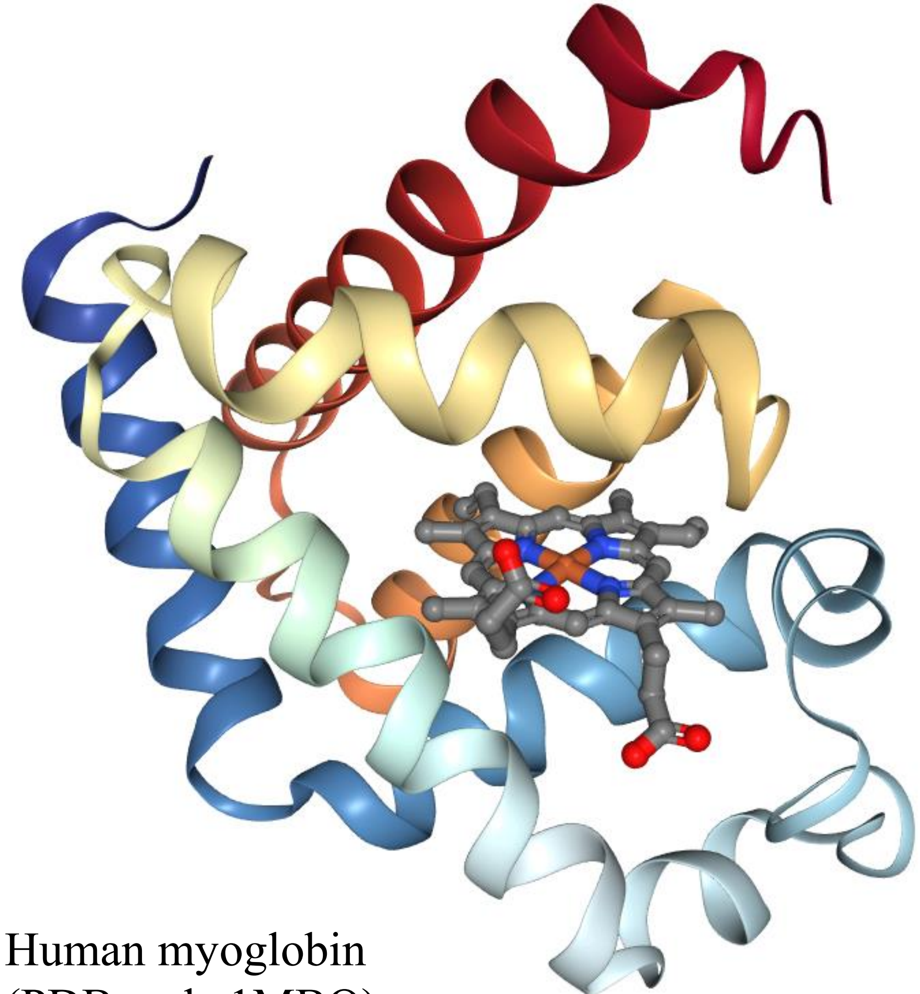
About 65% of the iron present in the human body is confined to the transport protein hemoglobin (*Hb*) alone; the content of the oxygen-storage protein myoglobin is roughly 6%.

The share of O_2 in air is only about 21 vol.%and a sufficient level has to be maintained in the tissue even under unfavorable circumstances (e.g. at higher altitude)

Human blood has an approximately 30 times higher “solubility” for O_2 than water.

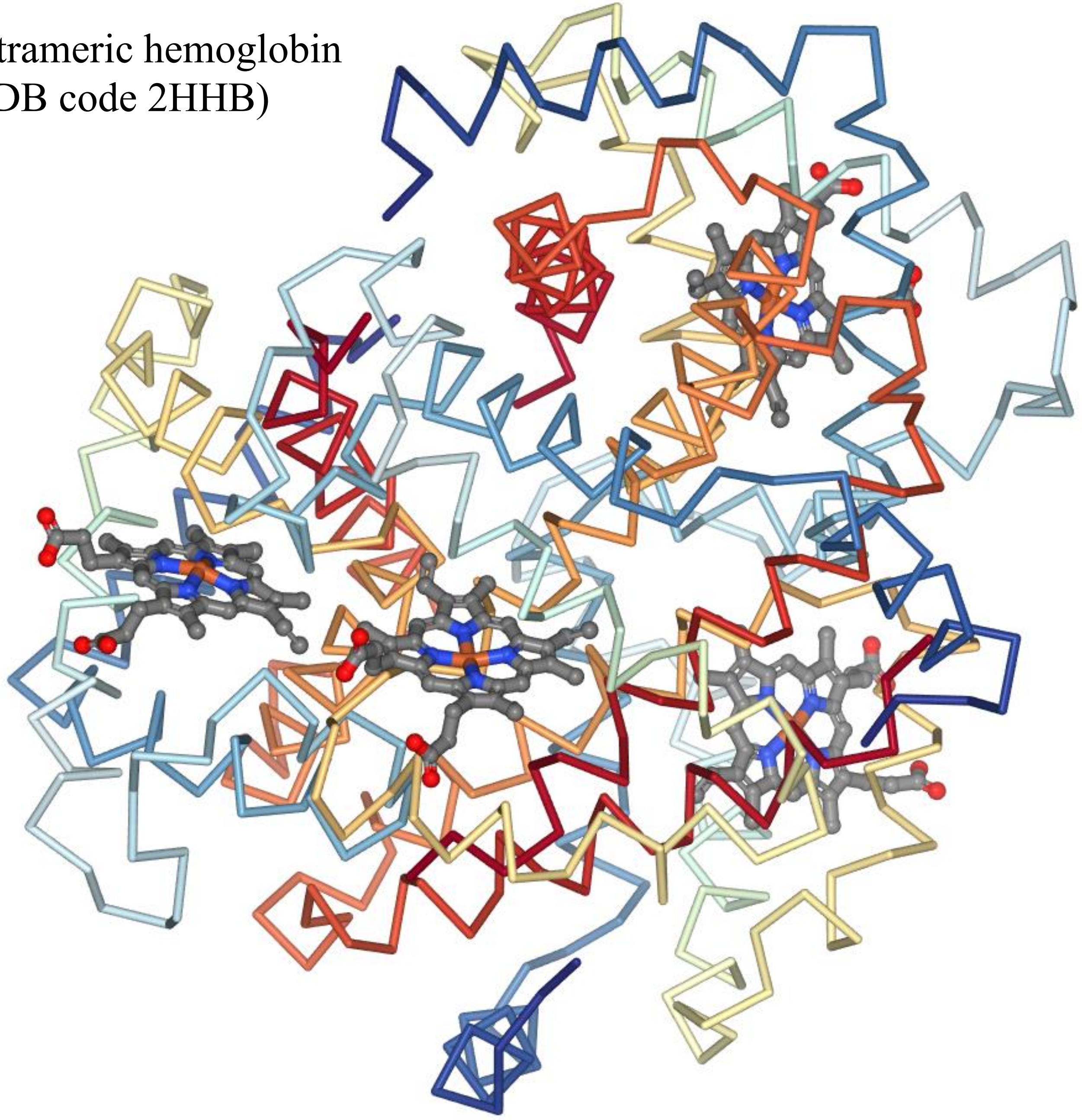
Metal-storage proteins like ferritin constitute most of the rest of the iron in the human body.

The catalytically active enzymes are present in only minute amounts.



Human myoglobin
(PDB code 1MBO)

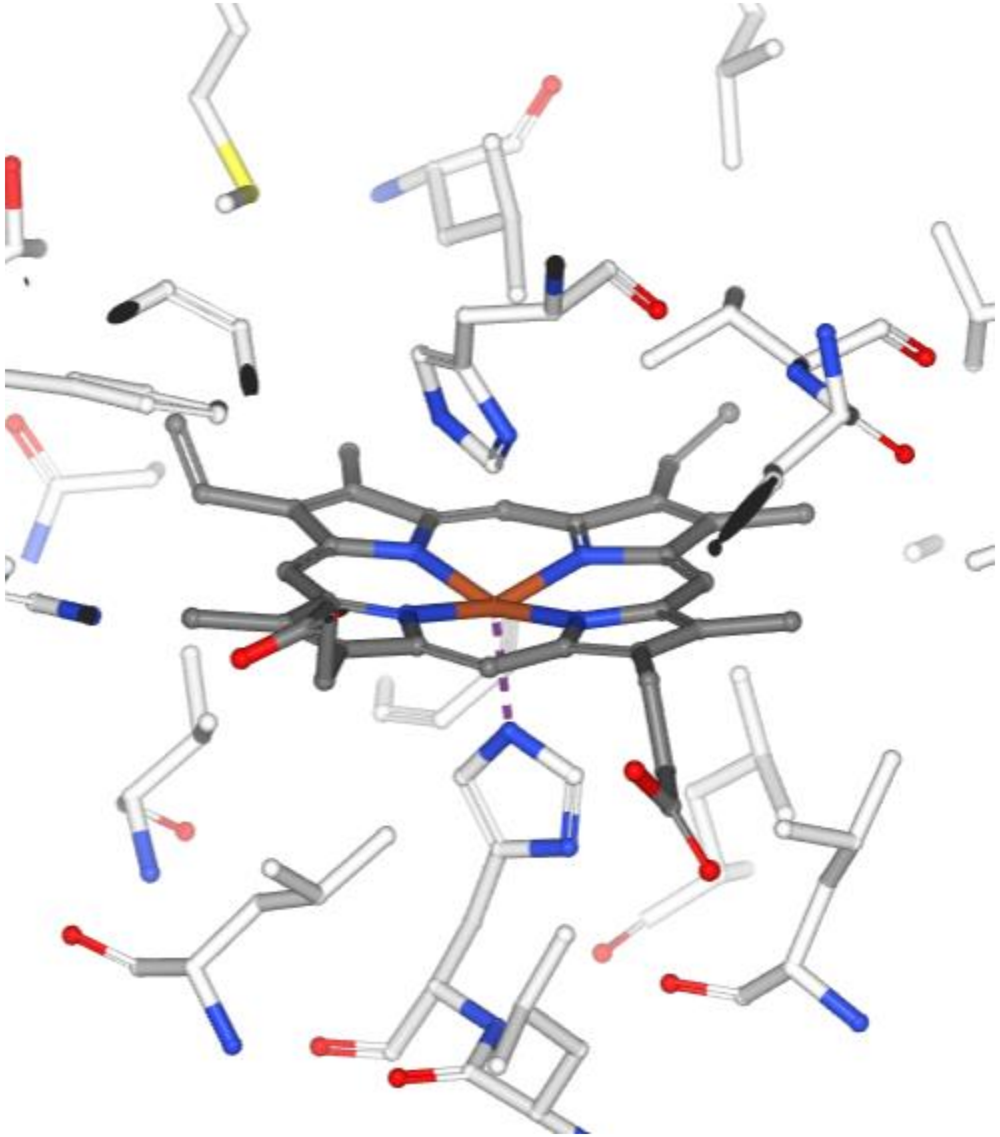
Tetrameric hemoglobin
(PDB code 2HHB)



Myoglobin (Mb) is the O_2-binding protein found principally in muscle tissues of vertebrates.

It consists of a single polypeptide chain of 153 amino acids called globin, made up of seven α-helical and six nonhelical segments.

Attached to the chain by coordination to the imidazole ring of a histidine residue is the dioxygen-binding **prosthetic group, iron(II) protoporphyrin IX.**



Hemoglobin (Hb) transports O_2 in blood, taking dioxygen from air in the lungs and delivering it to Mb in tissues.

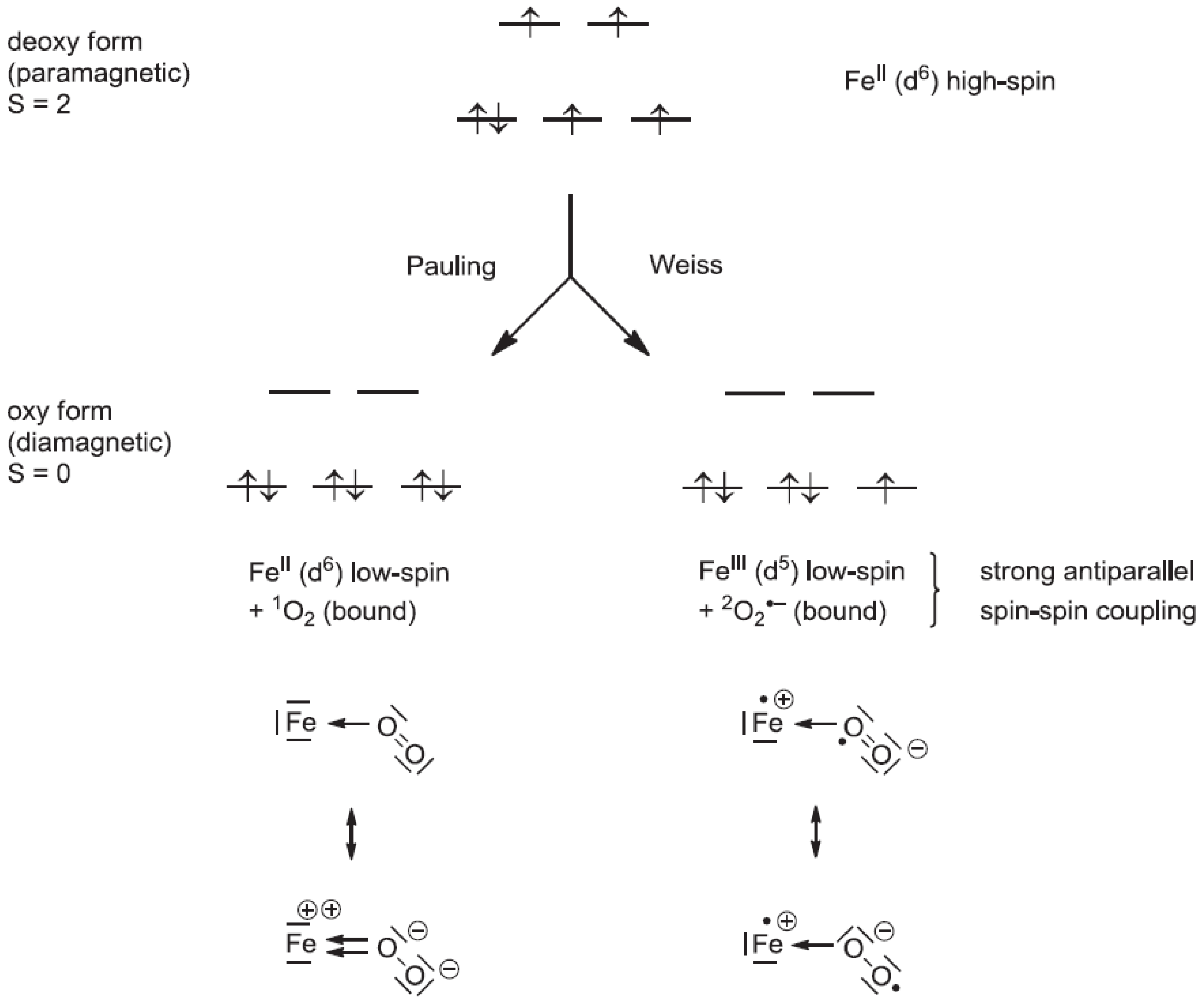
Hemoglobin is a multisubunit protein, with two *α* and two *β* polypeptide chains. In **each chain** there is an **iron protoporphyrin IX group held by a proximal histidine** imidazole residue, as in Mb.

The presence of **high-spin Fe(II)** in the deoxy forms of *Hb* and *Mb* is established and an ***S* = 2 ground** state with **four unpaired electrons** is observed.

The presence of an **even number of unpaired electrons is favorable** for a rapid, **non-spin-inhibited binding** of $^3O_2$, with its S = 1 ground state.

For the **diamagnetic oxy forms (S = 0)** with **end-on coordinated O_2** in a nonlinear arrangement and with an Fe-O-O angle of about 120°, the oxidation state assignment is less unambiguous because of the non-innocent nature of coordinated dioxygen

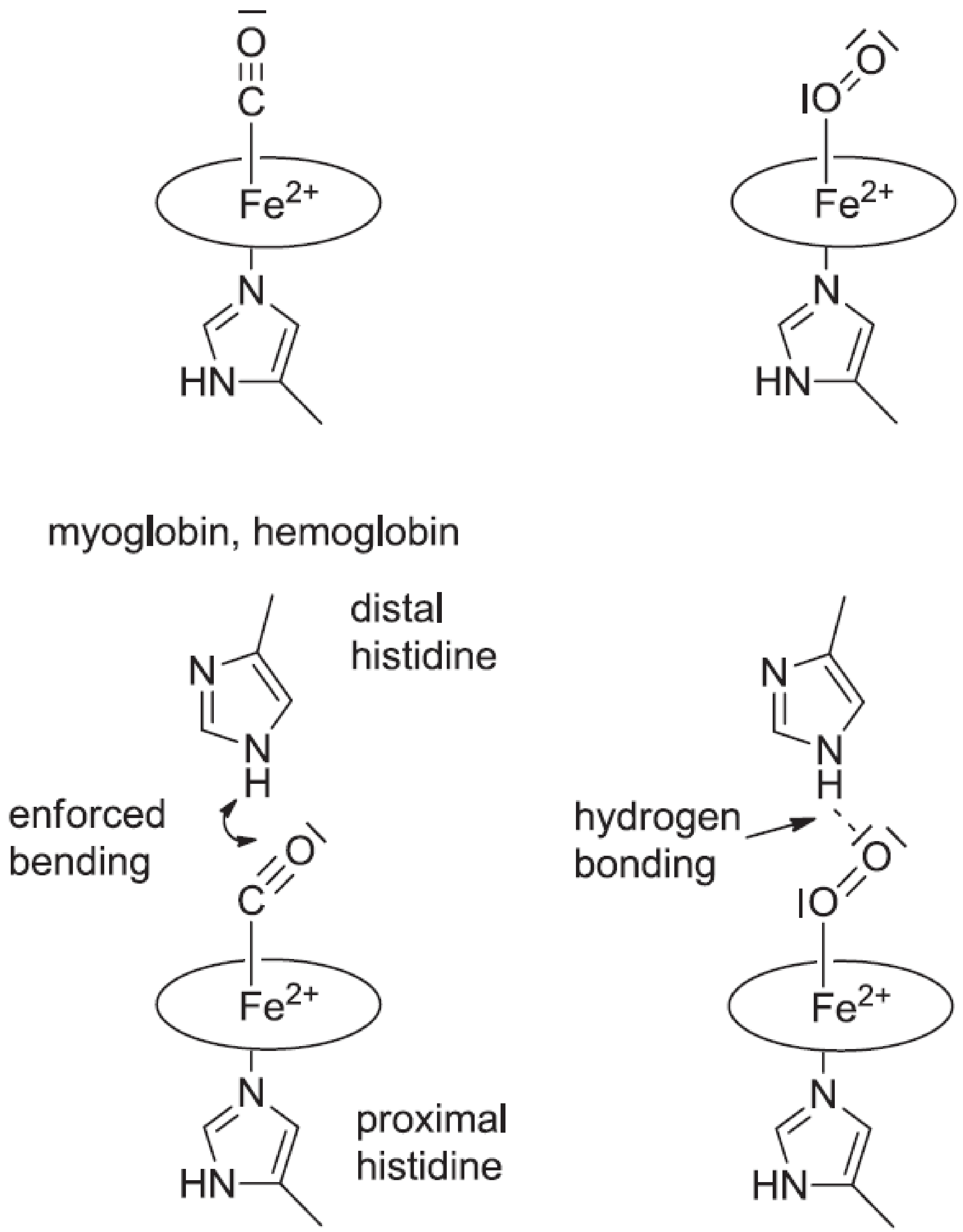
-Pauling and Weiss models-



A system to protect against CO toxicity

η^1(C)-coordinating **CO** in protein-free model systems **prefers a linear arrangement**, whereas end-on coordinated **O_2** prefers a **bent situation**, with an Fe-O-O angle of about 120◦, due to the remaining free electron pair at the coordinating oxygen atom.

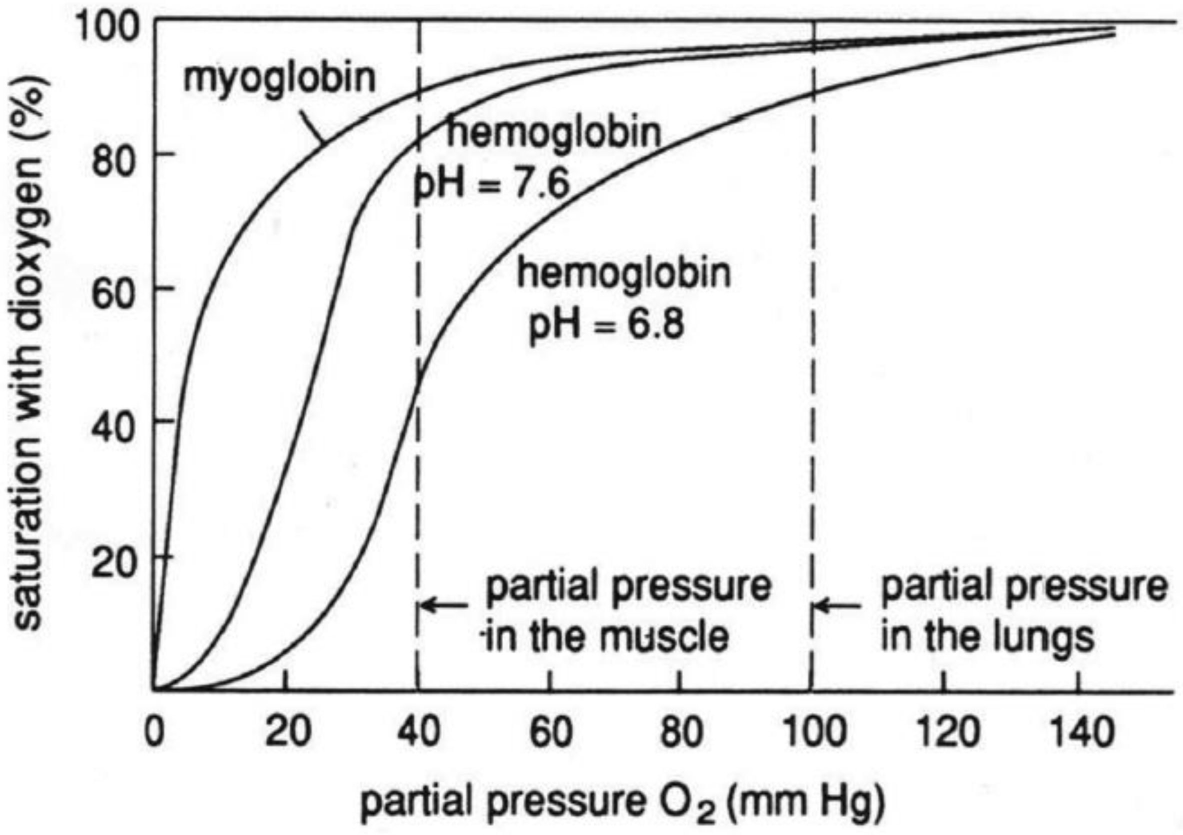
In myoglobin, spatial restrictions through the protein environment and the possibility of hydrogen bond formation with the distal histidine cause a more favorable equilibrium situation for binding of the definitely weaker -acceptor O_2 (KCO/KO2 ≈ 200 vs 25 000 in protein-free heme model systems); nevertheless, it is well known that **only small concentrations of CO in air can be tolerated.**



Cooperative effect in Hb

The transport system for O_2 has to take up this molecule as effectively as possible in its ground-state form, $^3O_2$, from the gas phase, in order to transport it in specialized blood cells, the erythrocytes, via the circulatory system to an intermediate storage site and to release it there completely.

The storage system always has to have a higher affinity for O_2 than the nonetheless efficient transport system. In the case of hemoglobin, with its total of four heme sites, this efficiency is guaranteed by the cooperative effect. In the course of loading with four molecules O_2, corresponding to 1 ml O_2 per 1 g *Hb*, the oxygen affinity increases, as graphically illustrated by a sigmoidal, nonhyperbolic saturation curve.



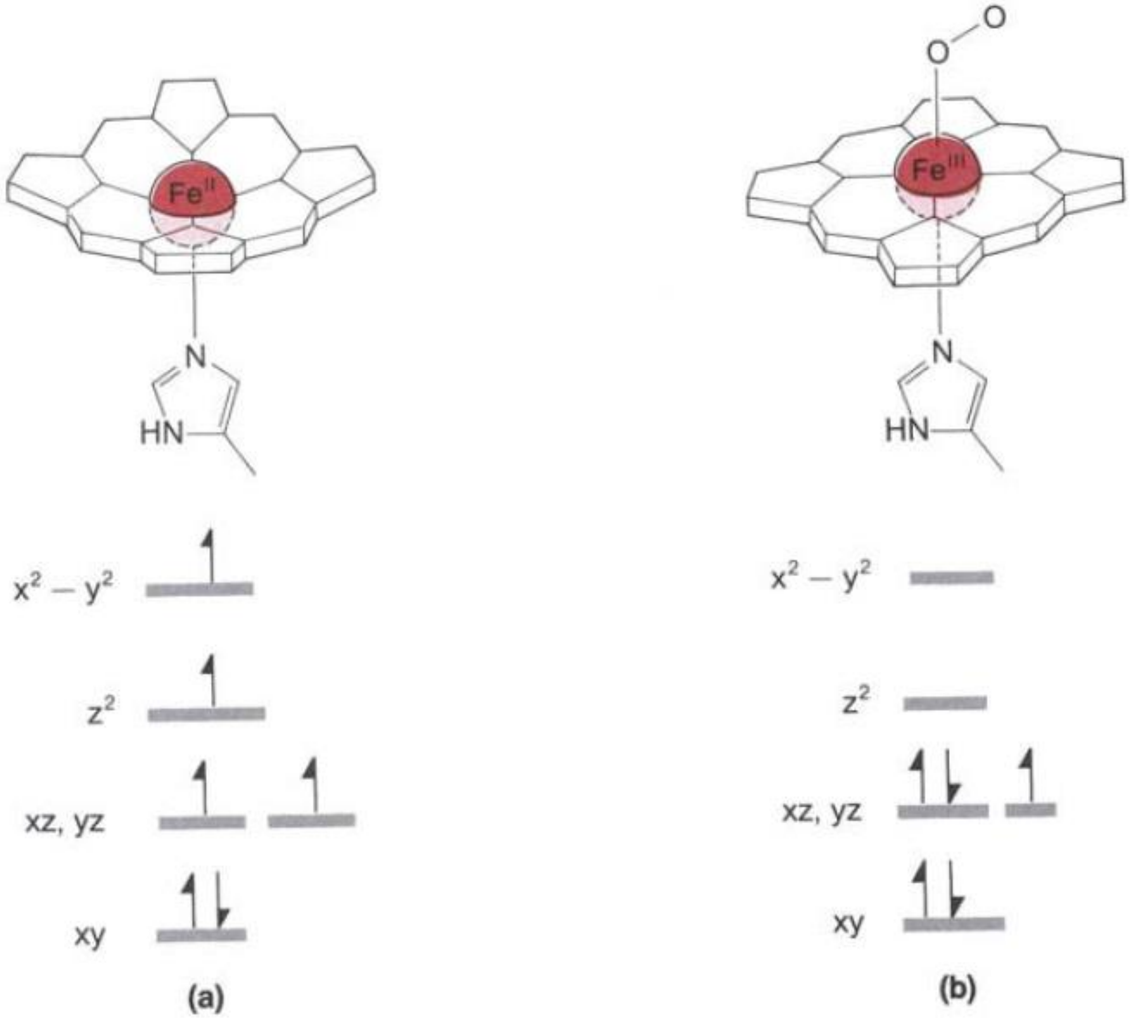
The cooperative effect guarantees an efficient transfer of O_2 to the storage site: the less O_2 is present in the transport system, the more completely it is released

In deoxy Hb, the iron lies 0.36 to 0.40 Å out of the porphyrin ring plane but moves within 0.12 Å of the plane upon binding of dioxygen.
A similar phenomen is observed in Mb.

For this apparent entatic state situation, the relatively light “pull” from coordinating O_2 or CO is sufficient to effect at least a **partial charge transfer from the metal to the incoming ligand** and a spin crossover of the metal center.

The thus effected **contraction of the metal** and the relative **movement of about 20 pm** towards the now more strongly coordinating macrocycle are **probably essential factors for the cooperative effect**.

Another significant structural change during O_2 coordination concerns the straightening of the Fe–N bond towards the proximal histidine with respect to the porphyrin plane.

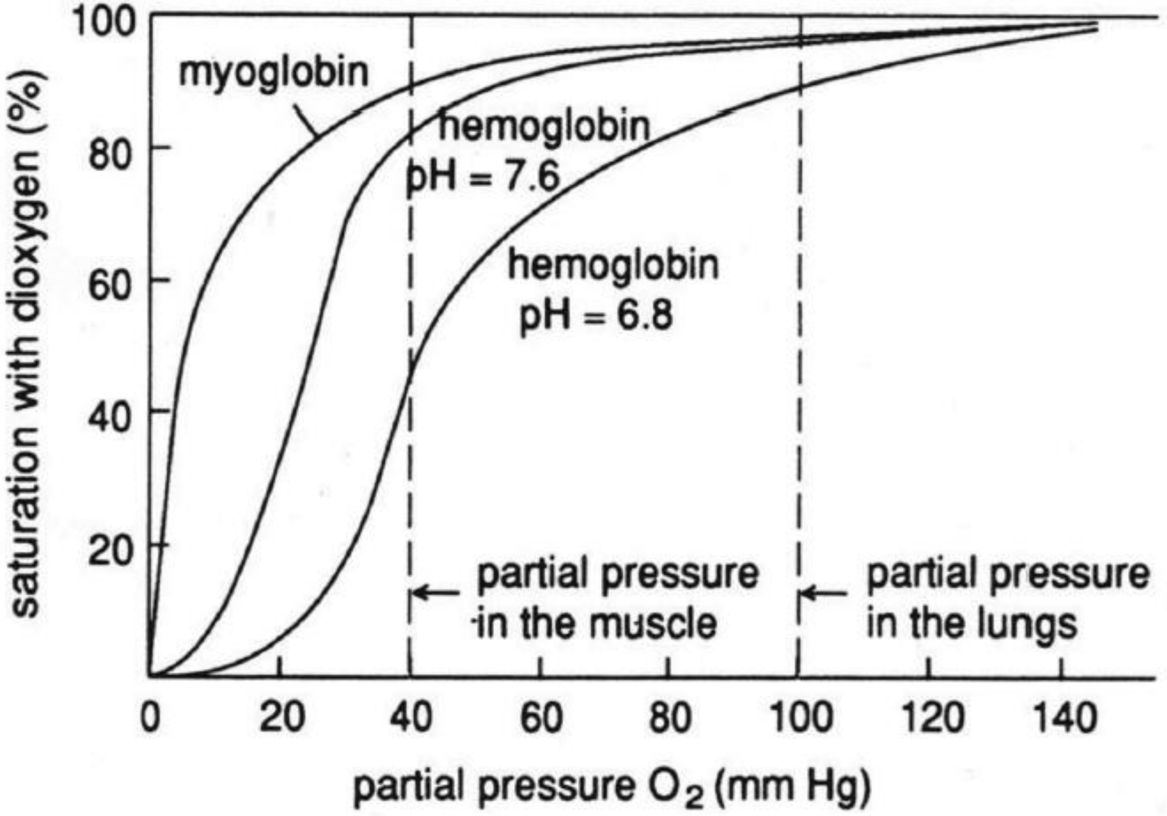


The **(positive) cooperative effect** of the prototypical “allosteric” protein hemoglobin is thus due to the variable interaction between the four heme-containing units of tetrameric *Hb*, the changes being **triggered by O_2 coordination to individual heme sites**.

Each of the four protein chains, which are linked to each other by electrostatic interactions (“salt bridges”), shows a geometrical change upon dioxygen coordination. This transformation is “transduced” to the other units by a process which, in the most simple concept, can be visualized by a “spring-tension” model involving a conformational change of the quarternary structure.

A **two-state model** consisting of a tense (**T, low O_2 affinity**) and a relaxed state (**R, high O_2 affinity**) for each heme describes the allosteric behavior of the tetrameric protein.

According to this scheme, there are **four different states for the individual heme system** (oxy and deoxy form, T and R state). The T form in particular features a steric interaction of the proximal histidine with the porphyrin ring, thus favoring a porphyrin bending, as it is found in the nonplanar deoxy form.



What about pH dependence (Bohr effect)?

It originates from the fact that the binding of CO_2 (which is formed as a respiratory end product) to a terminal amino group of hemoglobin reduces its capability to bind oxygen; that is, oxy-*Hb* is a stronger acid than deoxy-*Hb*. *Hb* thus acts as an O_2 *and* CO_2 transport protein.

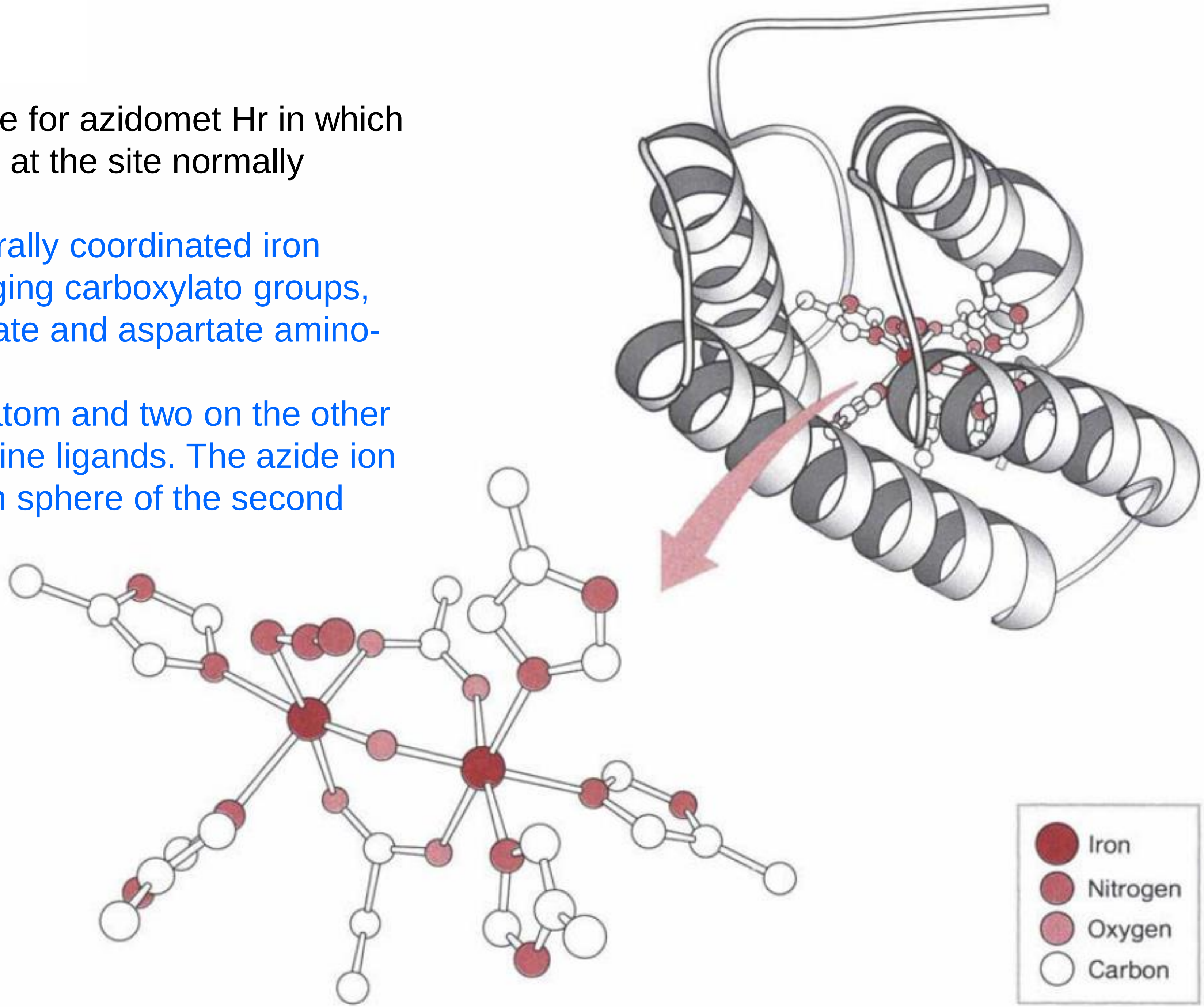
With the release of O_2, *Hb* takes up protons and helps to convert carbonic acid, H_2CO_3, to bicarbonate, HCO_3^-.
The sigmoidal character of O_2 binding is more pronounced in (carbonic) acidic solution and the biological relevance for this is immediately obvious: during an increased CO_2 production, the CO_2/O_2 gas exchange is promoted in that the transport protein becomes completely loaded with O_2.

Hemerythrin

X-ray structural information is available for azidomet Hr in which the azide anion (N_3^-) is bonded to iron at the site normally occupied by O_2 in oxy Hr.

The structure consists of two octahedrally coordinated iron atoms joined by a μ-oxo and two bridging carboxylato groups, the latter being contributed by glutamate and aspartate amino-acid residues from the protein chain.

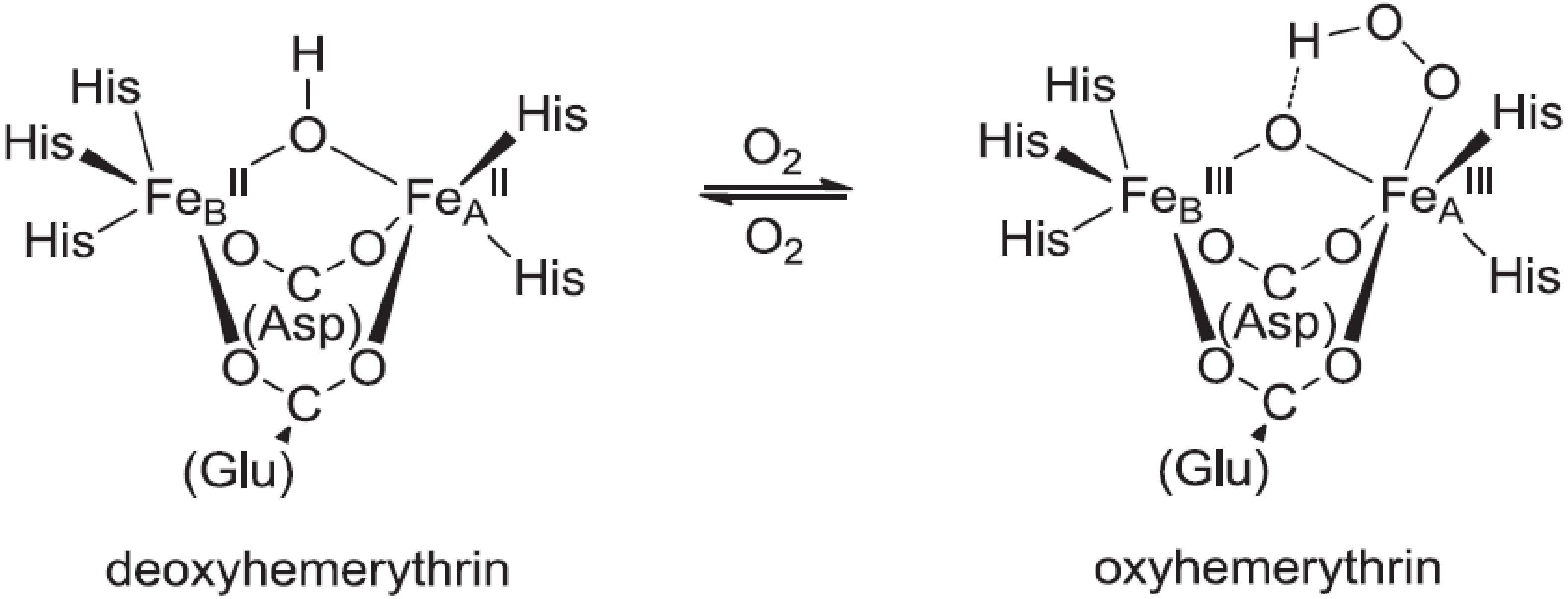
Three terminal positions on one iron atom and two on the other are filled by imidazole groups of histidine ligands. The azide ion completes the octahedral coordination sphere of the second iron atom.



When dioxygen is bound there, *both* centers are oxidized to Fe(III) under simultaneous reduction of the substrate to the peroxide state and the metal centers are more tightly linked by formation of an oxo bridge ("superexchange"→antiferromagnetic coupling).

The O_2 taken up presumably exists as hydroperoxo ligand, HOO^-, allowing a hydrogen bond to be formed with the bridging oxide ion.

This kind of reactivity between O_2 and Fe(II) is not surprising, but it is astonishing that the O_2 binding in the protein is ***reversible***.



deoxyhemerythrin

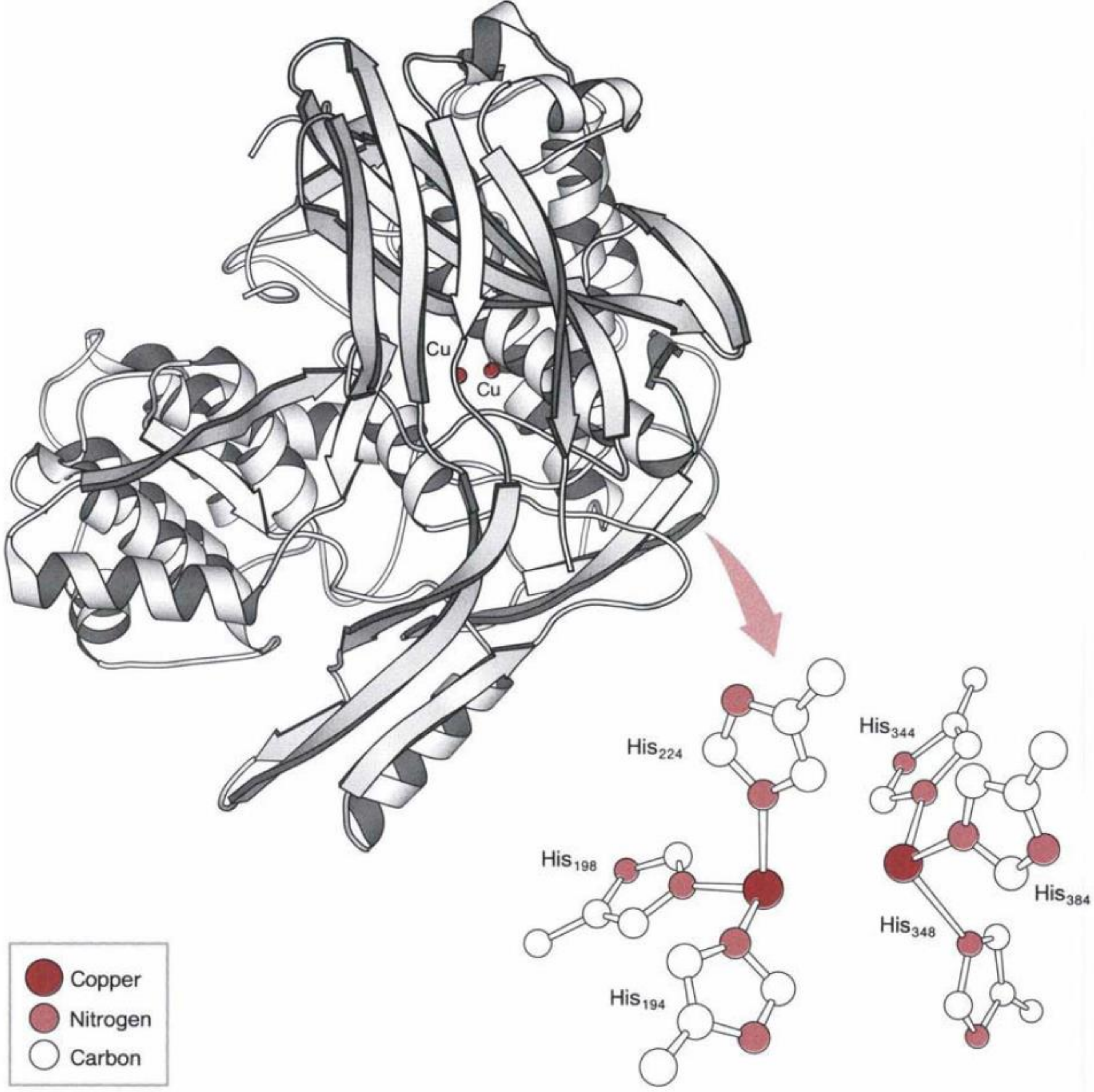
oxyhemerythrin

Hemocyanin

The structure of the **colorless, deoxy form of the protein** is known, and the chemical contents of its **dinuclear copper core**.

In deoxy Hc, two **cuprous ions are each coordinated by three histidine** residues at an internuclear distance of 3.7 ±0.3 Å.

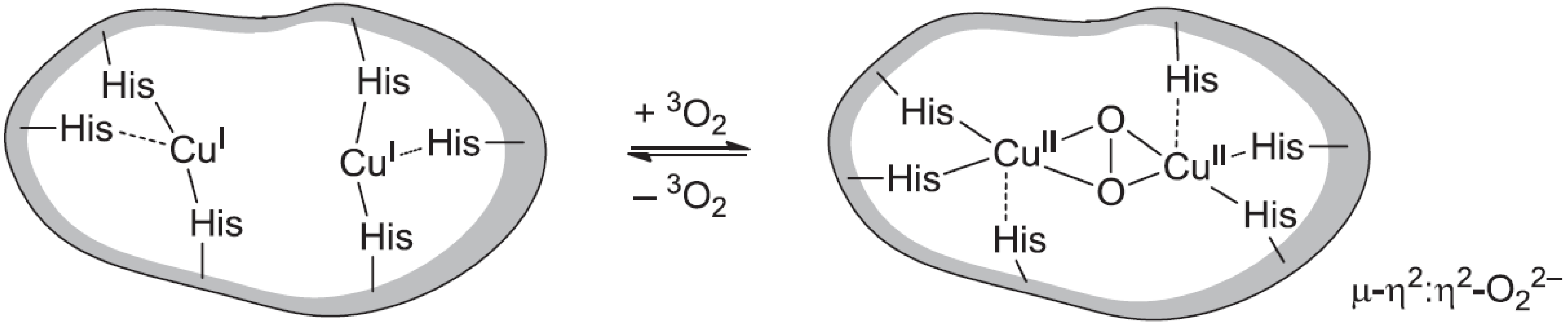
An **empty cavity** is available between the metals to accommodate the dioxygen molecule.



The core contains either *cis*-μ-η^1:η^1 or μ-η^2:η^2 coordinated "dioxygen" in the peroxide oxidation state.

The **latter alternative** was only seriously considered after corresponding model compounds had been prepared this alternative **does not require an additional ligand** and is in **better agreement with the strongly weakened O–O bond** and the **little changed Cu–Cu** distance after O_2 binding.

While the reaction of O_2 with Cu(I) is not unexpected, the reversibility of the dioxygen binding by the protein remains incredible given the **distinct changes in oxidation** states and the **multiple coordination**.



deoxyhemocyanin (deoxy-*Hc*)

oxyhemocyanin (oxy-*Hc*)

$d_{Cu\text{-}Cu} \approx 360$ pm