Subject: Advanced Inorganic Chemistry: Bioinorganics

Professor: Isabel Castro Bleda

Teaching guide: In Bioinorganic Chemistry (Topic 2 of the Advanced Inorganic Q. subject) the presence of metallic elements in living beings will be studied, their interaction with biological ligands, the properties they are capable of developing (catalysis, electron transfer) , structural ...), and the most recent advances in medicinal inorganic chemistry.

1.Introduction. Essential elements. Terminology and biological ligands.

2.Techniques in Bioinorganics. Spectroscopic techniques for the characterization of metallic centers. Techniques for studying the interaction / cutting of DNA.

3.Storage and transport of Fe. Storage and transport of O_2. Siderophores, transferrin and ferritin. Myoglobin, hemoglobin and other O_2 transport systems.

4.Electronic transfer processes. Cytochromes, Fe-S clusters, blue Cu proteins.

5.Catalytic processes. Acid-base catalysis (enzymes of Zn, Fe and Mg); reduction of H_2O_2 and O_2 (peroxidases, oxidases and oxygenases); Catalytic processes of enzymes containing Co.

6.Genetic transcription. Fingers of Zn.

7.Biological cycles. N_2 cycle and H_2 cycle.

8.Chemistry of metallic elements in medicine. Cancer treatment, antiarthritics, chelation therapy, diagnostic imaging.

5.- Catalytic processes

Classic role of enzymes, highly selective catalysts in the very diverse reactions of living beings. We will see only the most important…

- Acid-base catalysis
  - Zinc enzymes
    - Carbonic anhydrase: 1939
    - Carboxypeptidase: 1964
    - Alkaline phosphatase
    - Alcohol dehydrogenase
    - ß-lactamase
    - Termolysin
    - Magnesium enzymes
  - Manganese enzymes
  - Iron enzymes

- O_2 and H_2O_2 reduction
  - Peroxidases
  - Oxidases
  - Oxygenases

- Catalytic processes of cobalt enzymes

5.- Catalytic processes: Acid-base catalysis: Zinc enzymes

- Properties of the Zn^{2+} ion responsible for its **bioselection for acid-base catalysis**
  - Abundance: among the elements of the 1st transition series, Zn is the 2nd most abundant, only overcome by Fe: in the human body of an average adult there are ~ 3 g of Zn and ~ 4 g of Fe
  - Of all the transition metal ions, Zn^{2+} is the most effective Lewis acid together with Cu^{2+} and Ni^{2+}
  - But unlike Cu^{2+} and Ni^{2+}, it is inactive from the redox point of view, since it only presents an Oxidation State (O.S.), eliminating the risk that free radicals are generated
  - Flexibility in the coordination geometry since with a d^{10} configuration there is no stabilization of the ligand field: no preference for a given coordination number (C.N.). This flexibility is very important due to the structural restrictions imposed by the protein itself.
    - Most common C.N.s in metalloenzymes: 4 or 5
    - C.N. and geometry determined by the load and size of the ligands
  - It forms kinetically strong bonds with amino donor groups or other protein ligand donors, particularly with the histidines of the side chains.
  - And yet, it forms kinetically labile bonds with non-protein ligands, including water. In fact, not only the ligand exchange reactions are rapid, but also the intramolecular ligand redistributions.
- These characteristics favor a **high catalytic activity** since they allow rapid binding of the substrate, its activation, changes in the binding of intermediate species and rapid dissociation of the products formed.

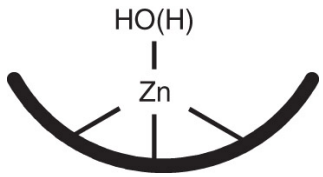
- **Ligands present in zinc catalytic sites**

Enzima	Ligando 1	Ligando 2	Ligando 3	Ligando 4
Clase I				
Alcohol deshidrogenasa	Cisteína	Histidina	Cisteína	H_2O
Clase III				
Carboxipeptidasa A	Histidina	Glutamato	Histidina	H_2O
Carboxipeptidasa B	Histidina	Glutamato	Histidina	H_2O
Termolisina	Histidina	Histidina	Glutamato	H_2O
β-lactamasa	Histidina	Histidina	Histidina	H_2O
Fosfatasa alcalina	Aspartato	Histidina	Histidina	H_2O
Clase IV				
Anhidrasa carbónica I	Histidina	Histidina	Histidina	H_2O
Anhidrasa carbónica II	Histidina	Histidina	Histidina	H_2O

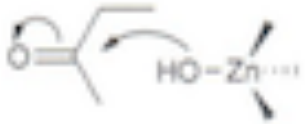
- **Cadmium as a zinc substituent:** element considered highly toxic, it is an essential nutrient for certain marine organisms; a carbonic anhydrase has been isolated from marine phytoplankton containing Cd^{2+}, a fact explained by the low concentration of Zn in the oceans.

5.- Catalytic processes: Acid-base catalysis: Zinc enzymes

- Zinc coordination sphere



- Mechanism of zinc enzymes, between two borderline cases:
  - Zn-hydroxide mechanism, the Zn^{2+} ion promotes the deprotonation of a coordinated water molecule, creating a nucleophile OH-, optimally positioned to attack the carbonyl C atom



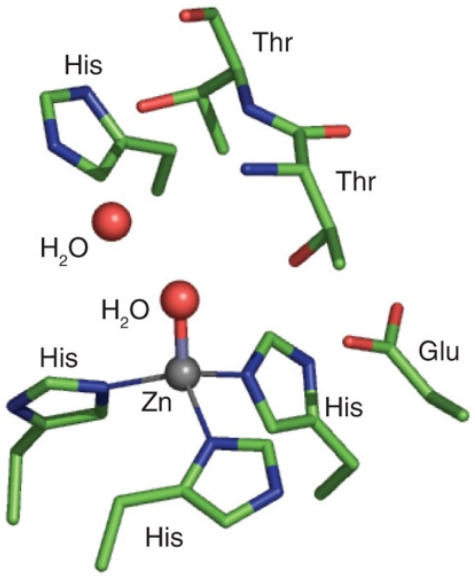
  - Zn-carbonyl mechanism, the Zn^{2+} ion acts like a Lewis acid and accepts the e- pair of the carbonyl O atom

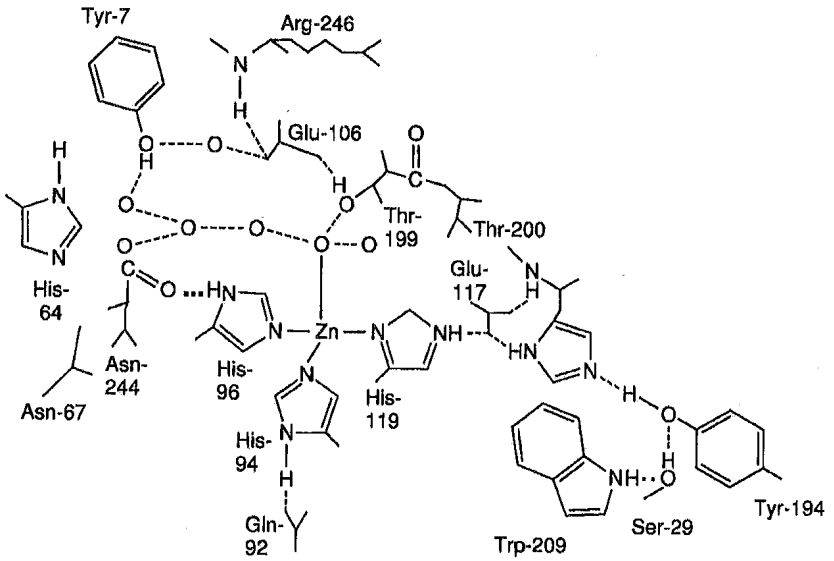
  - These mechanisms also occur with other X = O groups, in particular, the P = O groups of the phosphate esters

5.- Catalytic processes: Acid-base catalysis: Zinc enzymes

Carbonic anhydrase catalyzes the reversible conversion of carbon dioxide and water to hydrogen carbonate anions and proton cations according to the equilibrium:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$





In nature several forms of carbonic anhydrase are found, but in all of them the active center is a Zn monomer.

-In the α-type carbonic anhydrase present in animals, the zinc ion is coordinated to the N atoms of the imidazole rings of three histidine residues. The primary function of the enzyme in animals is to interconvert carbon dioxide and bicarbonate to maintain the acid-base balance in blood and other tissues and help transport carbon dioxide out of tissues.

- The plants contain β-type carbonic anhydrase, which, although it is a different enzyme, participates in the same reaction and also has a zinc ion in the active site. In plants, carbonic anhydrase helps raise the concentration of CO_2 within the chloroplast. This is the reaction that integrates CO_2 into carbohydrates during photosynthesis.

5.- Catalytic processes: Acid-base catalysis: Zinc enzymes

Carbonic anhydrase: de Neumol Cir Torax, 2010, 69, 200-209

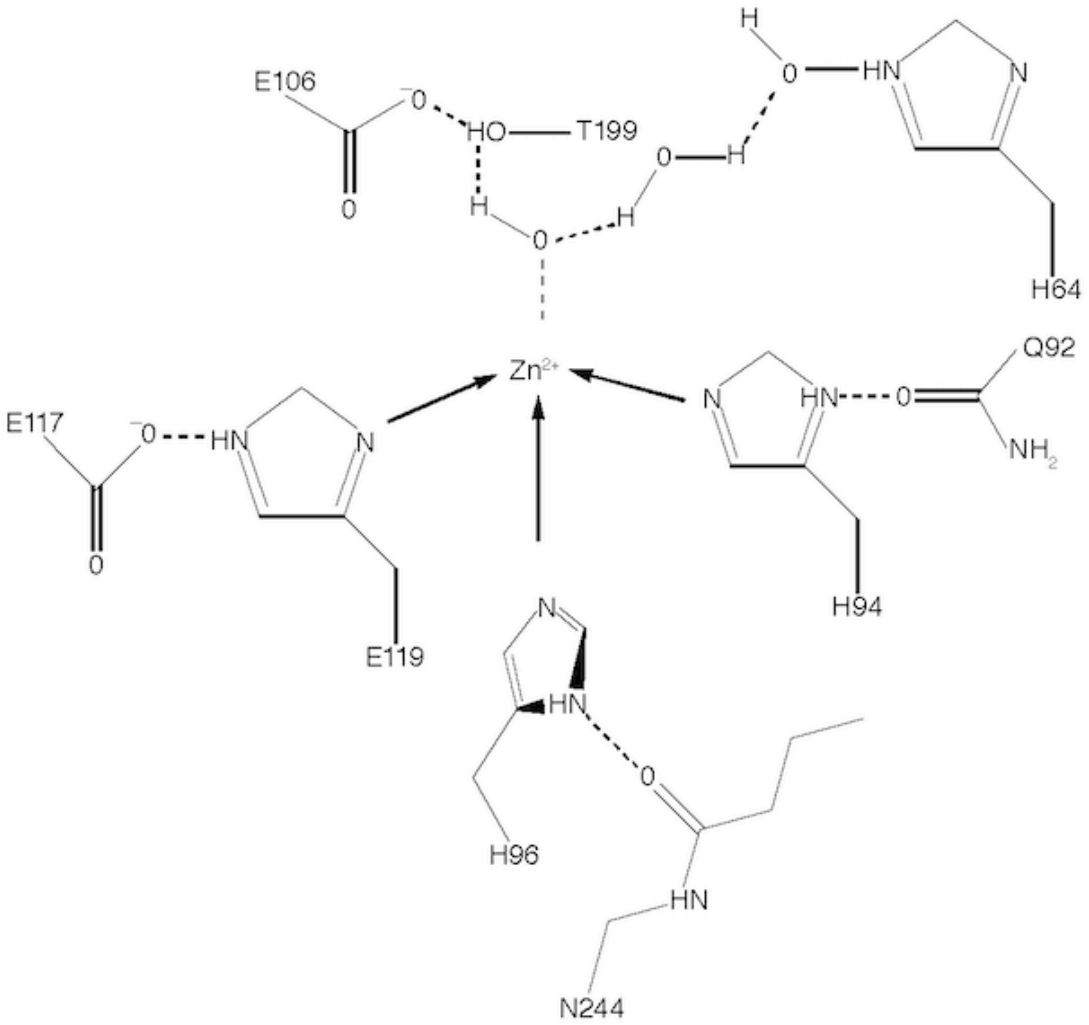
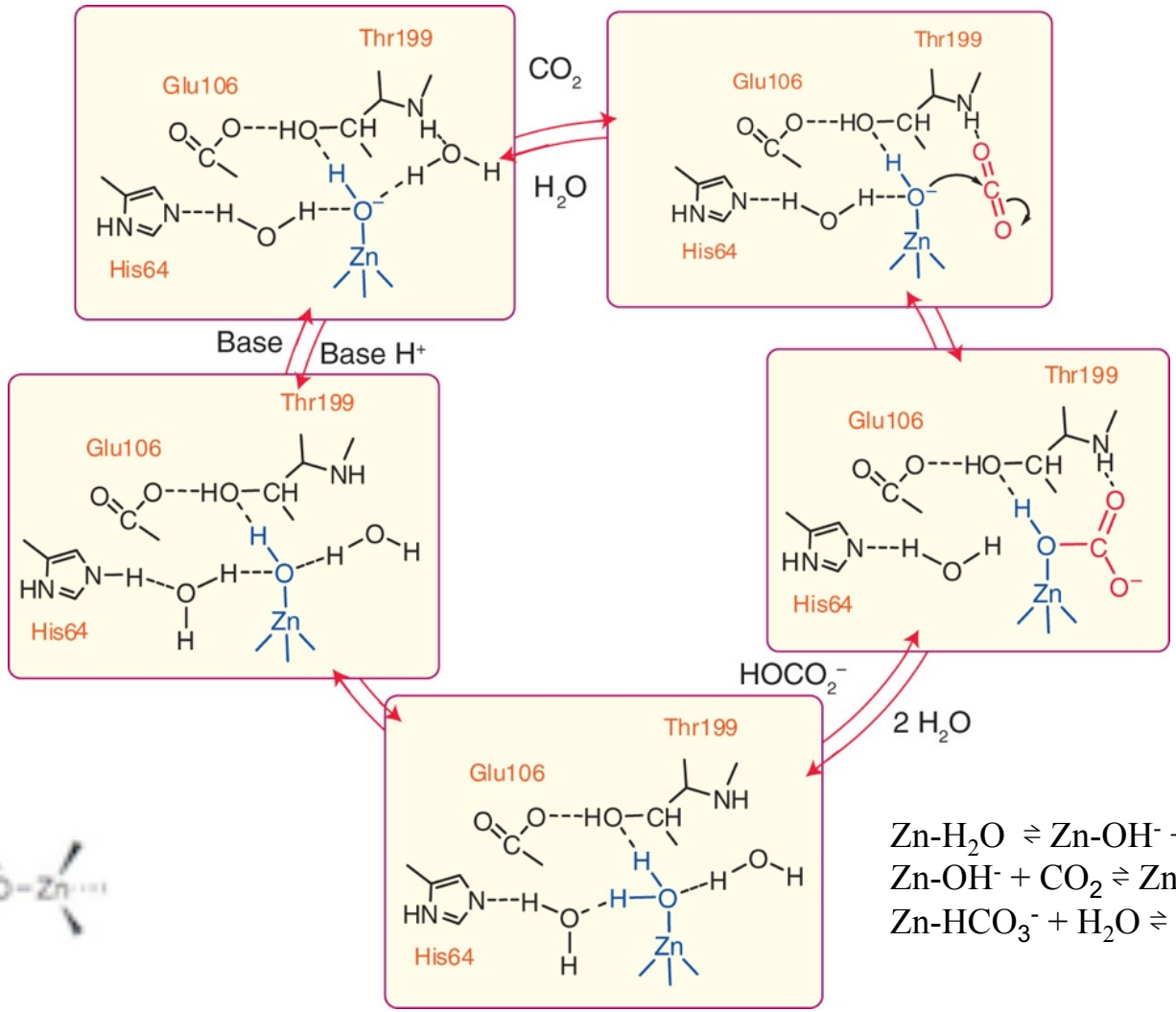
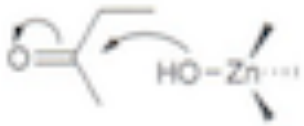


Figura 1. Estructura de la anhidrasa carbónica. En la figura se observa la molécula de zinc en el sitio activo, ésta coordina con los anillos imidazol de histidina 94, 96 y 119.

Carbonic anhydrase: mechanism of action





$Zn\text{-}H_2O \rightleftharpoons Zn\text{-}OH^- + H^+$

$Zn\text{-}OH^- + CO_2 \rightleftharpoons Zn\text{-}HCO_3^-$

$Zn\text{-}HCO_3^- + H_2O \rightleftharpoons Zn\text{-}H_2O + HCO_3^-$

5.- Catalytic processes: Acid-base catalysis: Zinc enzymes

Carbonic anhydrase: de Neumol Cir Torax, 2010, 69, 200-209

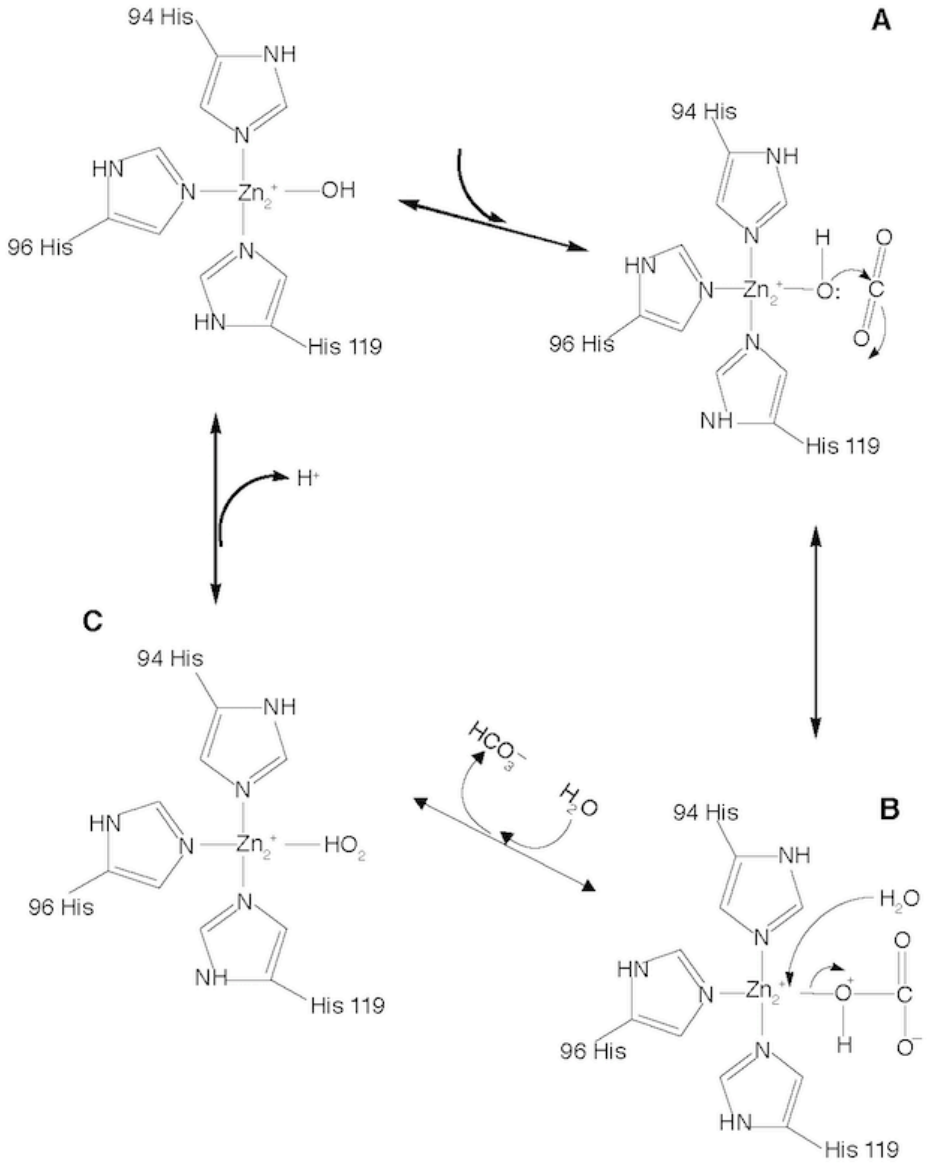
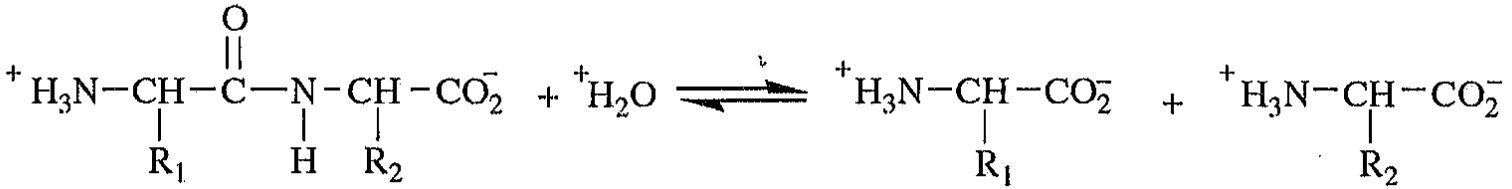
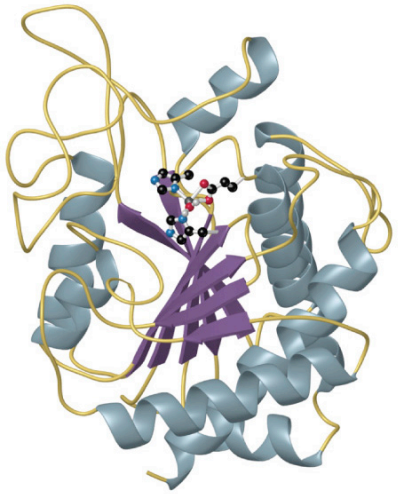


Figura 2. Mecanismo de reacción de la anhidrasa carbónica. La hidratación del CO_2 se lleva a cabo de la siguiente manera: el carbonilo del CO_2 es atacado por el hidroxilo de la AC y forma una molécula de HCO_3^- que queda unida al zinc; posteriormente, la hidrólisis produce la liberación del HCO_3^-. Enseguida el H^+ se transfiere al amortiguador externo mediante un transportador (H64 en la AC-II) para regenerar la especie catalítica activa, y el zinc se une nuevamente al hidroxilo.

5.- Catalytic processes: Acid-base catalysis: Zinc enzymes

Carboxypeptidase: is any enzyme from the group of peptidases or proteases capable of catalyzing the hydrolysis of a C-terminal peptide bond of polypeptide substrates, thus releasing the amino acid located at the end of the chain.





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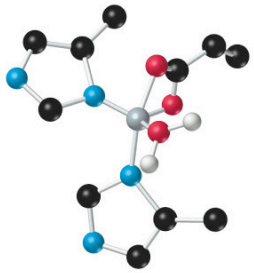

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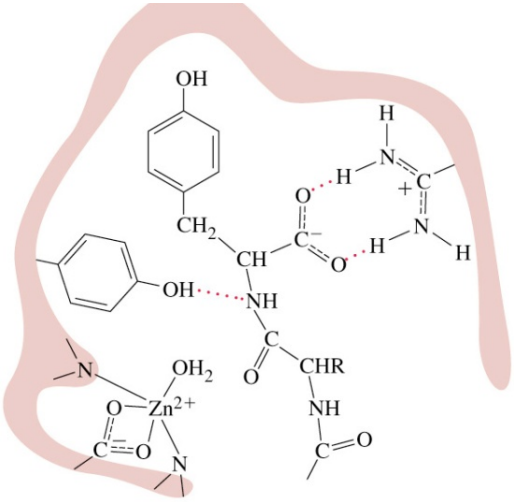
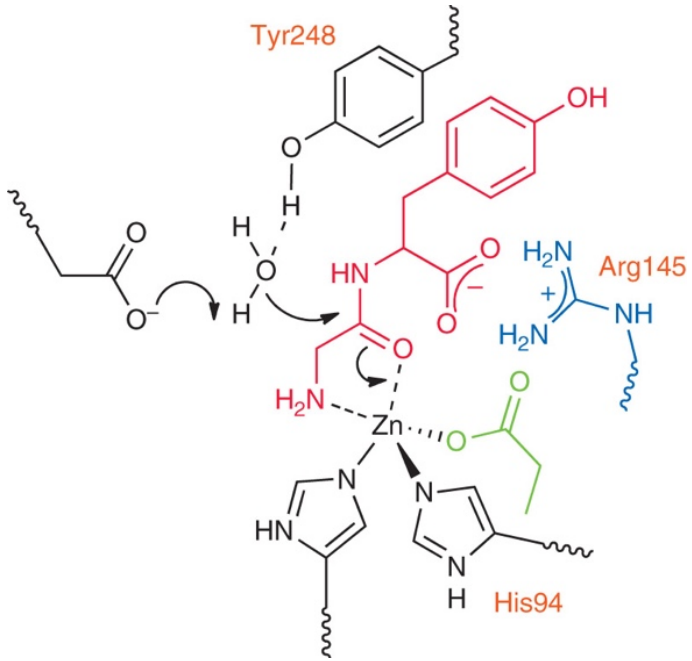
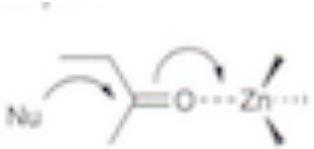


- Zn is pentacoordinated to two ligands histidine-N, a glutamate-CO_2^- (that acts as a bidentate) and a weakly bound water molecule.
- Additionally, in the active cavity there are several amino acid residues that are functionally important.

Carboxypeptidase: mechanism of action





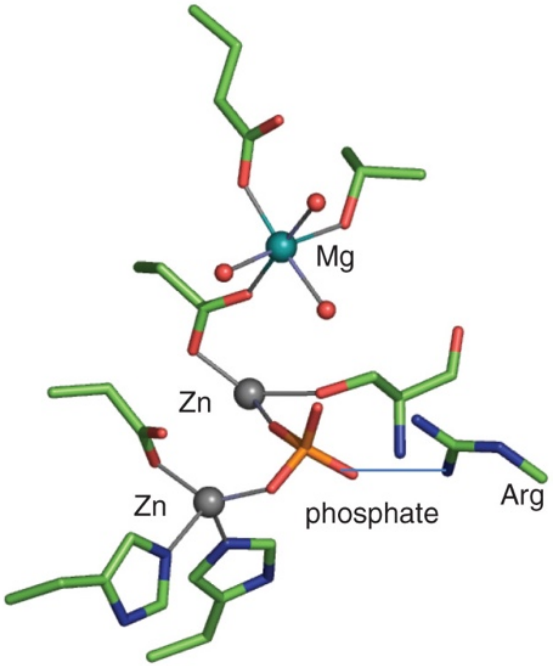
The figure shows the structure of the enzyme obtained in the presence of glycine inhibitors (in red), where Zn is coordinated to the glycine O-carbonyl instead of the H_2O molecule.

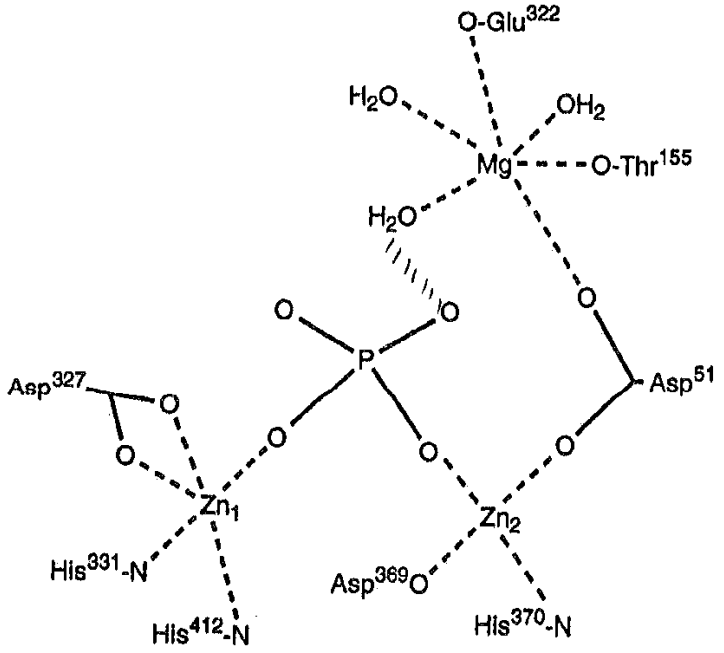
This suggests a Zn-carbonyl mechanism, where the Zn^{2+} ion acts as a Lewis acid and accepts the e- pair of the carbonyl O atom.

5.- Catalytic processes: Acid-base catalysis: Zinc enzymes

Alkaline phosphatase catalyzes the hydrolysis of the phosphate monoesters of various types of molecules such as nucleotides, proteins and alkaloids, including ATP.
This enzyme is very versatile because it can also act as phototransferase in the presence of a phosphate acceptor.
Alkaline phosphatases are more effective in a basic medium, the optimum pH being of the order of 8.

$$R{-}OPO_3^{2-} + H_2O \rightarrow ROH + HOPO_3^{2-}$$



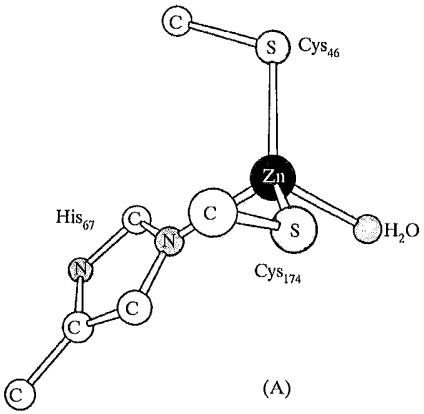


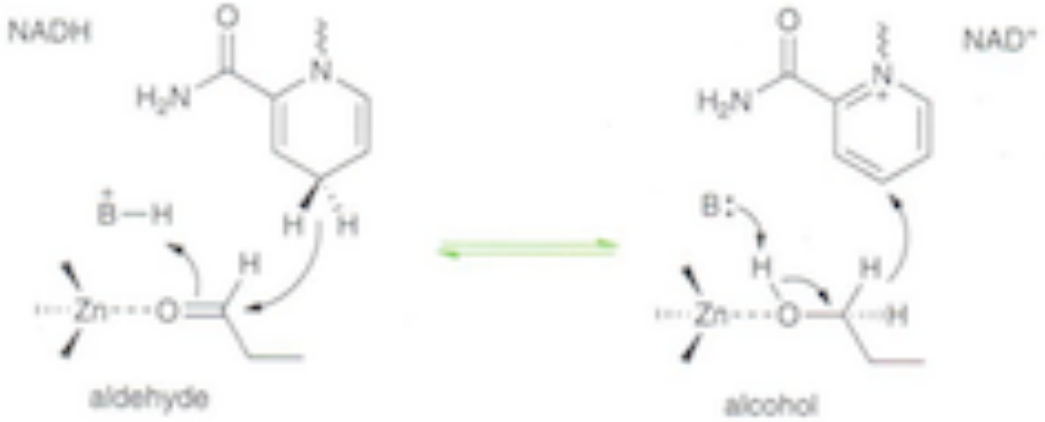
5.- Catalytic processes: Acid-base catalysis: Zinc enzymes

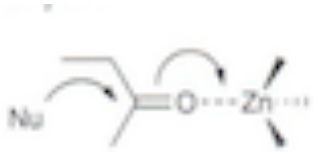
Alcohol dehydrogenase: the alcohol dehydrogenases are a group of seven enzymes that are present in many organisms and facilitate the interconversion between alcohols and aldehydes (or between alcohols and ketones). The catalyzed reaction is the reduction of NAD^+ by alcohol:

$$\text{Alcohol} + \text{NAD} + \rightleftharpoons \text{Aldehyde or Ketone} + \text{NADH}$$

As cofactors in the reaction it is possible to use Zn or Fe depending on the type of alcohol dehydrogenase.







5.- Catalytic processes: Acid-base catalysis: magnesium enzymes

- Properties of the Mg^{2+} ion for acid-base catalysis
  The Mg^{2+} ion is less acidic than Zn^{2+} (it polarizes less to coordinate ligands), but the exchange rate of ligands is higher

- The most important enzyme of Mg is RuBisCo (ribulose 1,5-bisphosphate carboxylase-oxygenase)

  - It is responsible for removing CO_2 from the atmosphere and fixing it in plants
  - It is found in the chloroplast of autotrophic organisms
  - This enzyme has a double behavior that justifies its name, catalyzing two opposite processes:

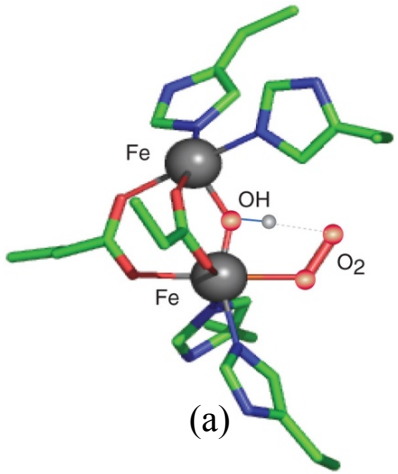
  - Carbon fixation. The RuBisCO catalyzes the first step of the Calvin cycle (stages of photosynthesis that take place in the dark), that is, the fixation of CO_2 to an organic form (which justifies its classification as carboxylase). In the reaction, a CO_2 molecule binds to the hydrocarbon chain of the RuBisCO. Through a transition state of six carbons, two molecules of 3-phosphoglyceric acid are formed:

$$\text{Ribulose-1,5-bisphosphate} + CO_2 + H_2O \rightleftharpoons 2\ \text{3-phosphoglycerate} + 2\ H^+$$

  - The photorespiration. The RuBisCO catalyzes the fixation of O2 on the same substrate (which justifies its classification as oxygenase), ends up releasing CO2 and dissipating energy.

5.- Catalytic processes: Acid-base catalysis: iron enzymes

- **Acid phosphatases** are a subgroup of phosphatases, a type of enzyme used to release phosphate groups attached to other molecules. The acid phosphatases are those that present optimal activity at acidic pH. They are widely distributed in the body (erythrocytes, serum, platelets, leukocytes, spleen, liver, osteoclasts and in glandular epithelia of prostate, breast, stomach and colon).
  - They contain a dinuclear metal center, Fe(III) with another metallic ion, Fe, Mn or Zn
    - In animals the active center is Fe(III)-Fe(II)
    - In plants, the active center is Fe(III)-Mn(II) or Fe(III)-Zn (II)
  - Catalyze the hydrolysis of phosphate esters in mild acidic conditions
- The acid phosphatases of Fe(III)-Fe(II) (a):
  - They are known as purple acid phosphatases (PAPs) due to their intense purple color due to a charge transfer transition of Fe(III)-ligand at 510-550 nm
  - The two Fe are of high spin and remain in that state of spin during the reactions
- Acid phosphatases of Fe(III)-M(II) (b): Fe-Mn active site of an acid potato phosphatase



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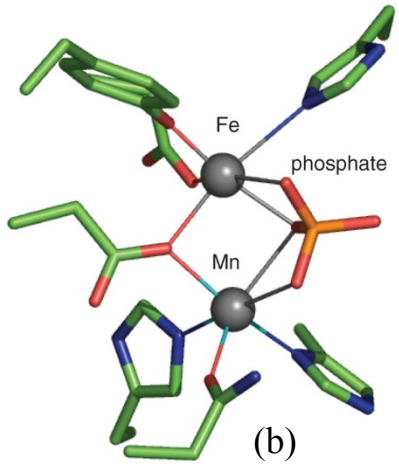


(b)

5.- Catalytic processes: Acid-base catalysis: Zinc enzymes

- Acid phosphatases: mechanism of action

5.- Catalytic processes

Classic role of enzymes, highly selective catalysts in the very diverse reactions of living beings. We will see only the most important…

- Acid-base catalysis
  - Zinc enzymes
    - Carbonic anhydrase: 1939
    - Carboxypeptidase: 1964
    - Alkaline phosphatase
    - Alcohol dehydrogenase
    - ß-lactamase
    - Termolysin
    - Magnesium enzymes
  - Manganese enzymes
  - Iron enzymes

- O_2 and H_2O_2 reduction
  - Peroxidases
  - Oxidases
  - Oxygenases

- Catalytic processes of cobalt enzymes

5.- Catalytic processes

Many **the most important biological functions of iron proteins** of the metalloenzymes in this chapter contain iron, so we will remenber here :

- Transport and storage of O_2
- Transportation of e-
- Catalysis of redox reactions
- Redox enzymes of Fe are generally associated with the biological use of O_2 and related species such as H_2O_2 and can be: oxygenases, oxidases, peroxidases, catalases and superoxide dismutases. In this chapter we will see the most important ones.
- But they can also catalyze other simple inorganic species as happens in the enzymes nitrite reductase, sulfite reductase, hydrogenases etc.
- Catalysis of non-redox reactions such as hydration / dehydration of many organic molecules
- Regulation of gene expression

According to the active center, Fe proteins can be classified into 4 groups:

- Heme proteins, which have a Fe porphyrin as a prosthetic group: in Hb and Mb, iron is Fe(II), while in peroxidases (and also catalases), iron is Fe(III), then these last proteins can not interact with O_2 but they can interact with H_2O_2.
- Fe-S proteins (non-heme), which have a Fe-S cluster as a prosthetic group.
- Non-heme proteins with a dinuclear active center with 2 iron ions bound together by a bridge carboxylate ligand.
- Non-heme proteins with a mononuclear core that have a single iron ion attached to the polypeptide chain.

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

Peroxidases

- They belong to the category of oxidoreductases.
- They catalyze redox bisubstrate reactions; the **first substrate is hydrogen peroxide that acts as an oxidant** (to which they owe their name) and the **second substrate is a substance with reducing characteristics that is oxidized by peroxide.**

$$\text{Donor} + H_2O_2 \rightleftharpoons \text{Oxidized donor} + H_2O$$

- Virtually all peroxidases are haemoproteins (active center an heme group Fe(II)-porphyrin) but some may contain as active center cysteine or redox-selenocysteine residues such as glutathione peroxidase, which is a selenoprotein..
- The high affinity of Fe for H_2O_2 causes that the Fe of the heme group can be coordinated by the two planes of the active center, the superior and the inferior, giving rise to an inhibition by excess of substrate since when both positions are occupied by the hydrogen peroxide the binding of the other substrate is not possible.
- The nature and chemical structure of the second substrate can be very different, whether we refer to the "in vivo" oxidized substrates or the substrates that are used "in vitro" in order to detect this enzymatic (clinical) activity.
  - Phenols, aromatic amines, complex organic molecules or halide ions.
  - This poor specificity for the reducing substrate makes the affinity also small.

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

Peroxidases (cont.)

Actually, there **are two major groups:**

- **When there is an oxidizable substrate** (previous slide). In these cases, in addition to destroying the $_{H2O2}$, avoiding its toxic action, a substance of interest is synthesized or degrades one that interests to eliminate. The nature of the oxidized substrate determines the biological function of the enzyme. Some important examples:

  ✓ Organic molecules (phenols, aromatic amines, complex organic molecules ...) as substrates:

  $$\text{ROH} + \text{R}'\text{OH} \xrightarrow{\text{Peroxidase}} \text{ROOR}' + 2e^- + 2H^+$$

  $$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O$$

  $$\text{ROH} + \text{R}'\text{OH} + H_2O_2 \rightarrow \text{ROOR}' + 2H_2O$$

  ✓ Halide ions as substrate:

  myeloperoxidase, one of the active species in phagocytosis, where the substrate is a chloride ion that oxidizes to hypochlorous acid:

  $$H_2O_2 + Cl^- + H^+ \rightarrow H_2O + HOCl$$

  haloperoxidases, biohalogenation reactions:

  $$H_2O_2 + I^- + RH + H^+ \rightarrow 2H_2O + RI$$

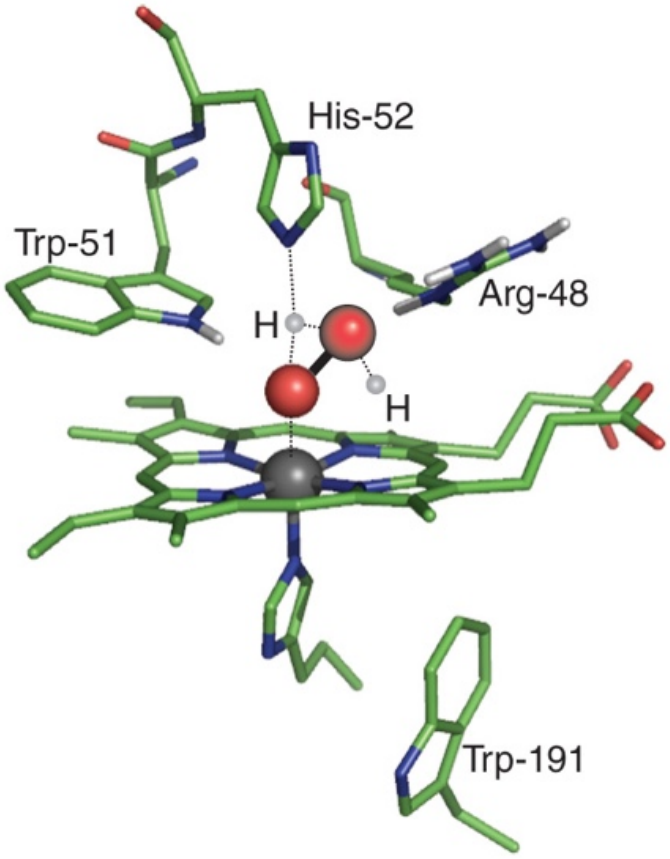
- **When there is no oxidizable substrate;** H_2O_2 is reduced to water by the contribution of 2 e- which come from the peroxidase itself.

  $$H_2O_2 + 2e^- + 2H^+ \rightarrow 2H_2O$$

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

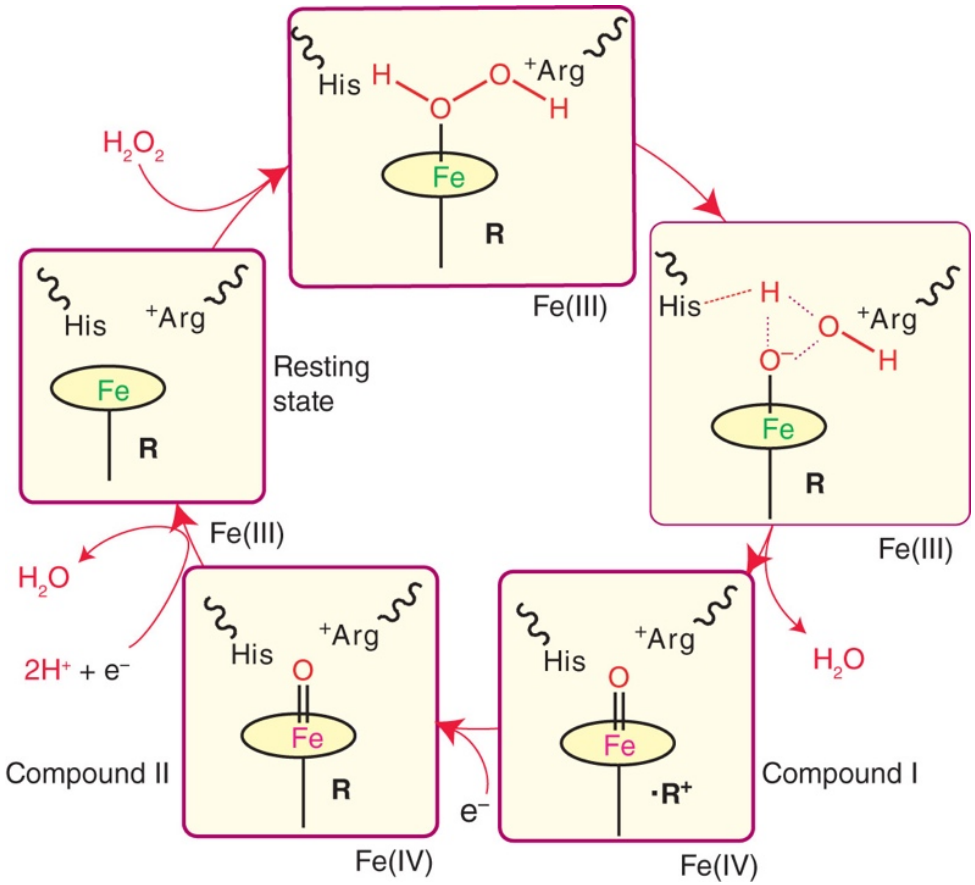
Peroxidases (cont.)

The peroxidases contain a pentacoordinated heme group in which the nearest axial ligand is an imidazole ligand of a histidine while the distal position is occupied by another imidazole ligand of another histidine. They are exemplified by **cytochrome c peroxidase**, which is shown in the figure.



5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

Peroxidases (cont.): catalytic cycle of peroxidases containing heme groups



$PFe(III) + H_2O_2 \rightarrow PFe(III)\text{-}OOH + H^+ \rightarrow {}^{\cdot}PFe(IV)\text{-}O + H_2O$

a) In cytochrome c peroxidase and in peroxidases whose substrates are organic molecules, compound I undergoes two monoelectronic reductions through a new intermediate species, an oxoporphyrin of Fe (IV):

$${}^{\cdot}PFe(IV)\text{-}O + 1e^- \rightarrow PFe(IV)\text{-}O$$
$$PFe(IV)\text{-}O + 1e^- + 2H^+ \rightarrow PFe(III) + H_2O$$

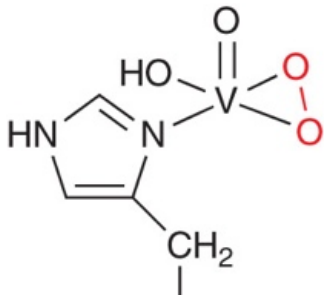
b) In thyroid peroxidase (catalyzes the iodination of phenolic groups of the thyroglobulin protein), compound I undergoes a one-step reduction by 2 e-, where the iodide ion is oxidized to hypoiodite, the active halogenating agent:

$${}^{\cdot}PFe(IV)\text{-}O + I^- \rightarrow PFe(III)\text{-}OI^-$$

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

Peroxidases (cont.) for the catalysis of other halogenated compounds

- H_2O_2 is used by biological systems for the synthesis of halogenated compounds. This process, particularly important in algae, is catalyzed by a class of peroxidases that contain V instead of Fe
- They exploit the acidity of the V(V)
- The active site of **bromoperoxidase** consists of an oxo-V(V) unit coordinated to a donor atom N of histidine
- The catalytic mechanism involves the activation of the coordinated peroxide and the transfer of an O atom to a Br^- ion, forming a BrO^- species that attacks the organic substrate



Catalases, a subgroup of peroxidases that also contain a pentacoordinated heme group with the axial ligand being a phenolate tyrosine group, catalyze the **disproportion of H_2O_2**, protecting organisms from their toxic effects. Almost all aerobic organisms possess this enzyme.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

The first part of the cycle is analogous to that of the previous peroxidases, but in this case the compuete I oxidizes a second molecule of H_2O_2 to molecular oxygen and water, returning to its initial state:

$$PFe(III) + H_2O_2 \rightarrow PFe(III)\text{-}OOH + H^+ \rightarrow {}^{\cdot}PFe(IV)\text{-}O + H_2O$$

$$^{\cdot}PFe(IV)\text{-}O + H_2O_2 \rightarrow PFe(III) + O_2 + H_2O$$

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

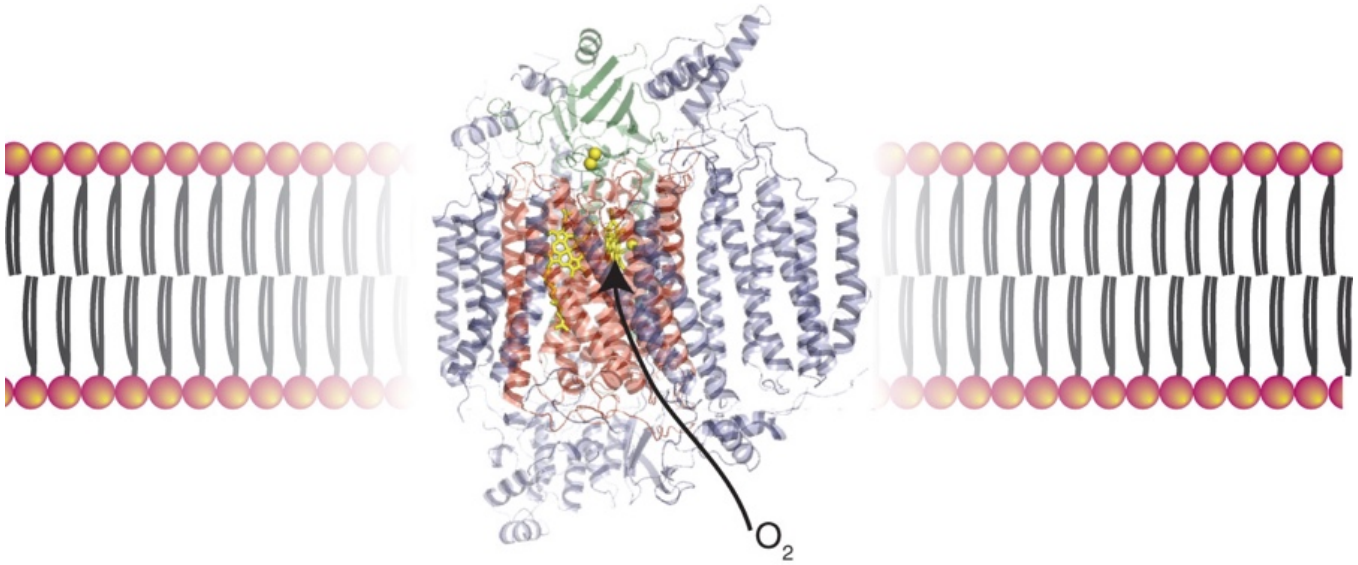
Oxidases: catalyze the reduction of O_2 to H_2O without incorporation of O atoms in the oxidizable substrate. We will see the cytochrome *c* oxidase, the blue copper oxidases, the polyphenol oxidases and the amino oxidases.

Cytochrome c oxidase (1st type of oxidases)

It is the most important oxidase and forms the basis of all higher life forms. It is found in the inner membrane of the mitochondria.

It catalyzes the reduction by 4 e- of O_2 to H_2O using cytochrome c as electronic donor. The reaction includes $4H^+$ that are not chemically consumed, but are 'pumped' through the membrane against a potential gradient. These enzymes are called 'proton pump'.

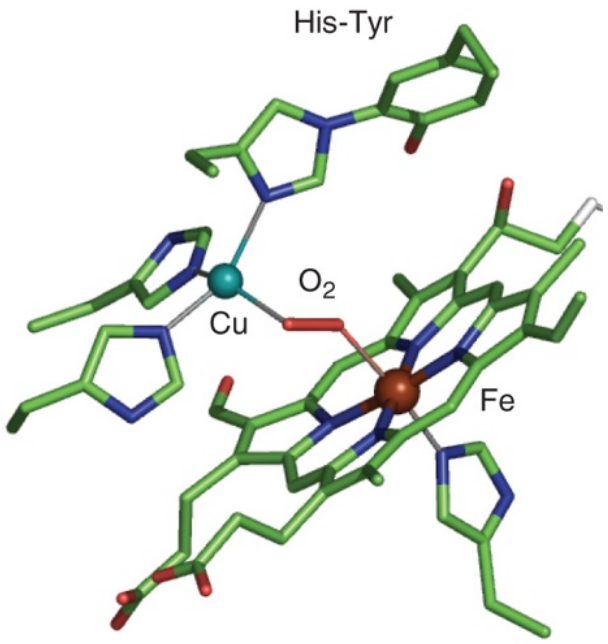
$$O_2(g) + 4e^- + 8H^+(inside) \rightarrow 2H_2O(l) + 4H^+(outside)$$

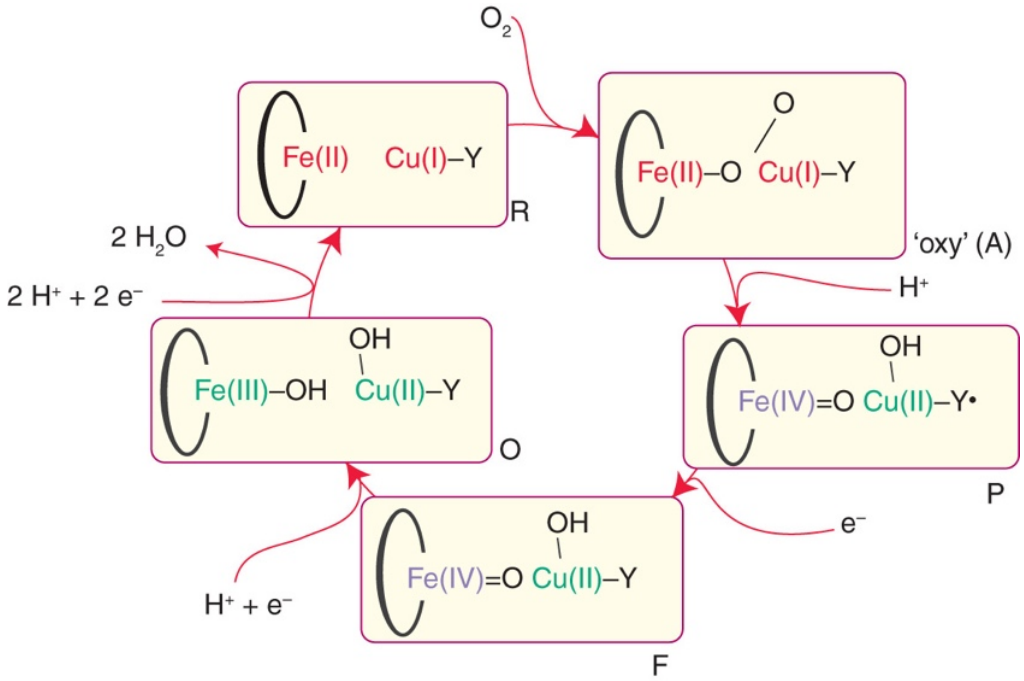


Cytochrome *c* oxidase contains 3 Cu atoms, 2 Fe-heme atoms, as well as an atom of Mg and a atom of Zn, these last two of structural relevance.

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

Cytochrome *c* oxidase (cont.): the active site for the reduction of O_2 consists of a Fe(II)-heme center, which has an adjacent Cu(I) semi-heme center coordinated to 3 N of histidine ligands (one of the his is coordinated to a tyrosine). The e- are supplied to the dinuclear entity by a second Fe-porphyrin.





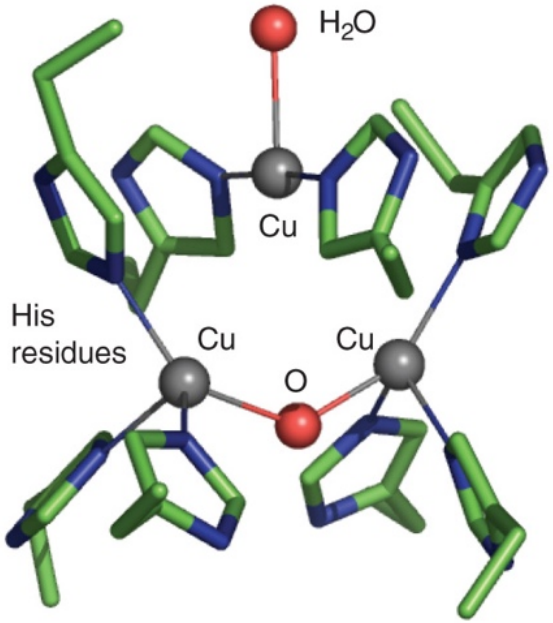
$$O_2(g) + 4e^- + 4H^+ \rightarrow 2H_2O$$

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

Blue copper oxidases (2nd type of oxidases)

The native form contains a dinuclear center of Cu(II), which is reduced by the substrate, passing to the active form (entity with two atoms of Cu (I) linked by an O-bridge atom that joins a third atom of Cu next forming a trinuclear entity) and that catalyzes the reduction of O_2 to H_2O.
Two important and well characterized enzymes of this type:

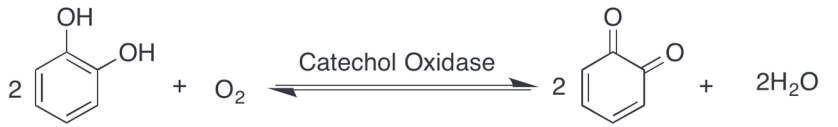
- ascorbate oxidase (skin of vegetables such as cucumbers and pumpkins, with two functions, to protect the interior of the fruit of O2 and oxidize phenolic substrates to intermediates that will form the skin)
- the family of laccases (in plants and fungi to catalyze the oxidation of phenolic substrates)

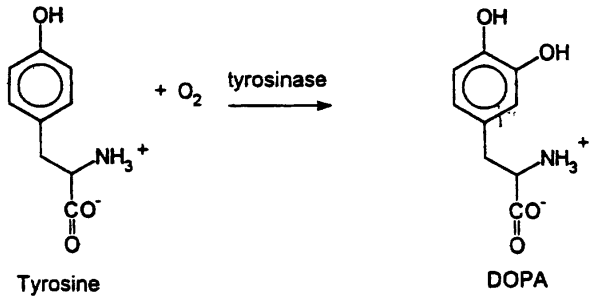


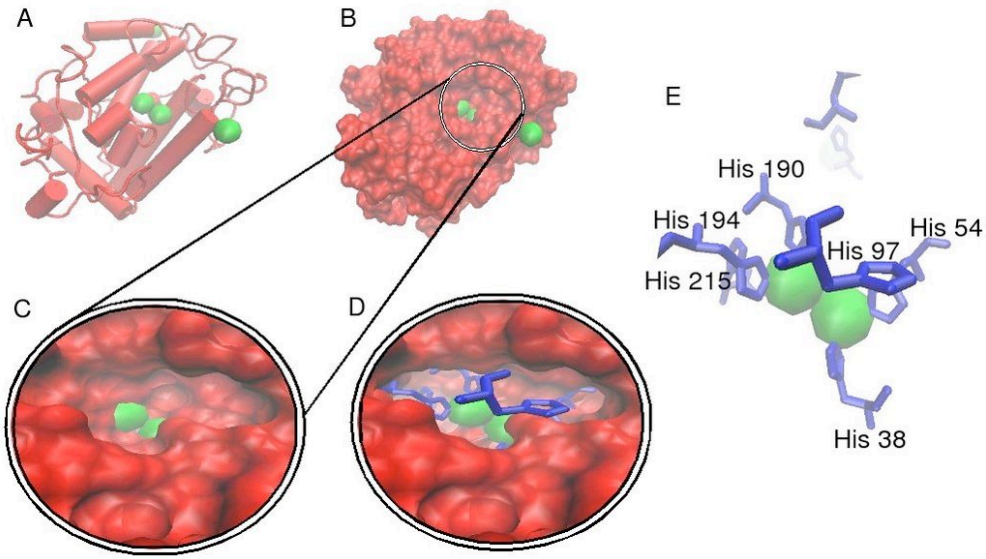
Polyphenol oxidases (3rd type of oxidases)

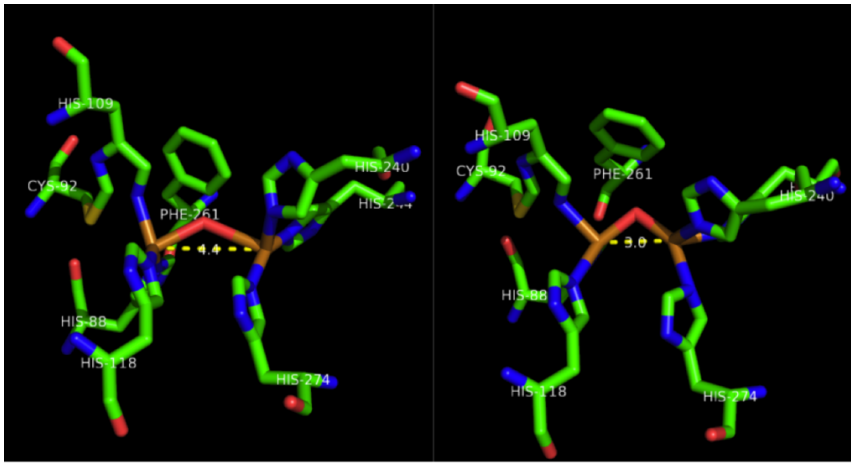
It is a family of metalloenzymes that contain a dinuclear center of Cu type III in its active centers, that is, each Cu atom is coordinated to three histidine residues. Among them, it is worth mentioning tyrosinase and catechol oxidase, responsible for the production of melanin-type pigments.

Both enzymes catalyze the oxidation of o-diphenols substrates to their corresponding o-quinones. Tyrosinases can also catalyze the hydroxylation of monophenols to diphenols.







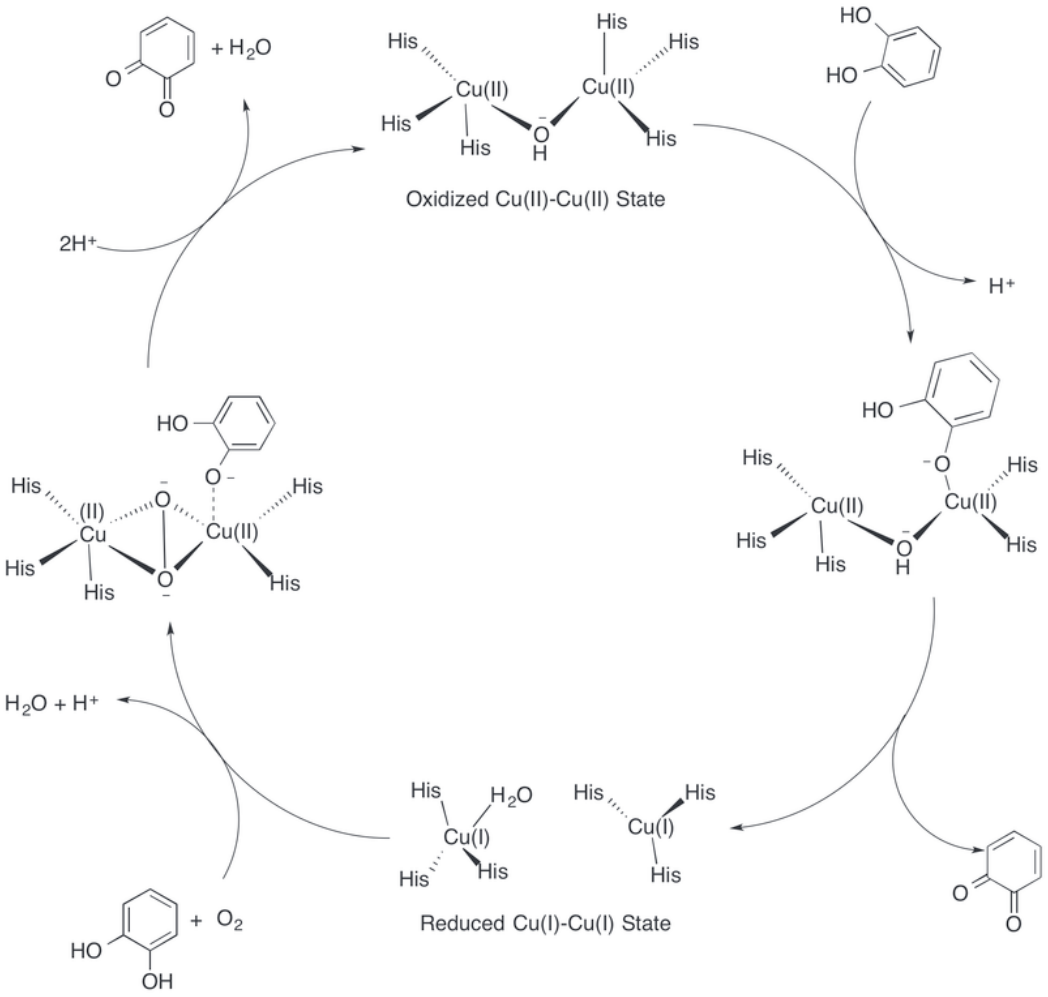


Reduced Cu(I)—Cu(I) state

Native Cu(II)—Cu(II) state

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

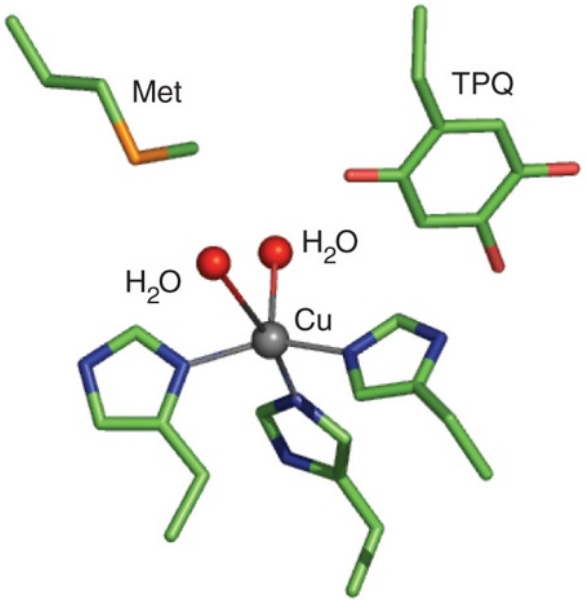
•Catalytic cycle proposed for catechol oxidase (only for illustrative purposes)



5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

Amino oxidases (4th type of oxidases)

- Catalyze the oxidation of amines to aldehydes
- They carry out a reduction of O_2 to H_2O_2 (reduction by 2e-) with an active center of a single Cu atom that ranges from Cu(II) to Cu(I)
- Like cytochrome *c* peroxidase and cytochrome c oxidase, amino oxidases have an additional oxidant source located near the metal, in this case, a cofactor called topaquinone (TPQ) that is formed by oxidation of tyrosine.



5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

Oxygenases

•They catalyze the insertion of one or both O atoms of O_2 in an organic substrate (the difference with oxidases is that in the latter both O atoms end up as H_2O or H_2O_2)

- Monooxygenases catalyze the insertion of one O atom while the other O atom is reduced to H_2O
- Dioxigenases catalyze the insertion of both O atoms
- Oxygenases are called hydroxylases when the O atom is inserted into a C–H bond

•Many oxygenases contain Fe, other Cu or flavin (an organic cofactor)

•Monooxygenases catalyze reactions of the type

$$R–H + O_2 + 2H^+ + 2e^- \rightarrow R–O–H + H_2O$$

where the e- are provided by an electronic donor such as a FeS protein

•Dioxigenases catalyze reactions of the type

$$H–R–R' + O_2 \rightarrow H–O–R–R'–O–H$$

where no additional electronic donor is required
and where two C–H bonds of the same molecule can be oxygenated

•The oxygenases of Fe are grouped into two large groups: heme and non-heme, which in general are monoxigenases and dioxygenases respectively.

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

Hemoxygenases

•They are widely distributed and are usually monoxigenases

•Cytochrome P450 (or only 'P450') encompasses an important group of monooxygenases that contain the heme group

•In eukaryotic cells they are located in the mitochondria and in the animals they are concentrated in the tissue of the liver

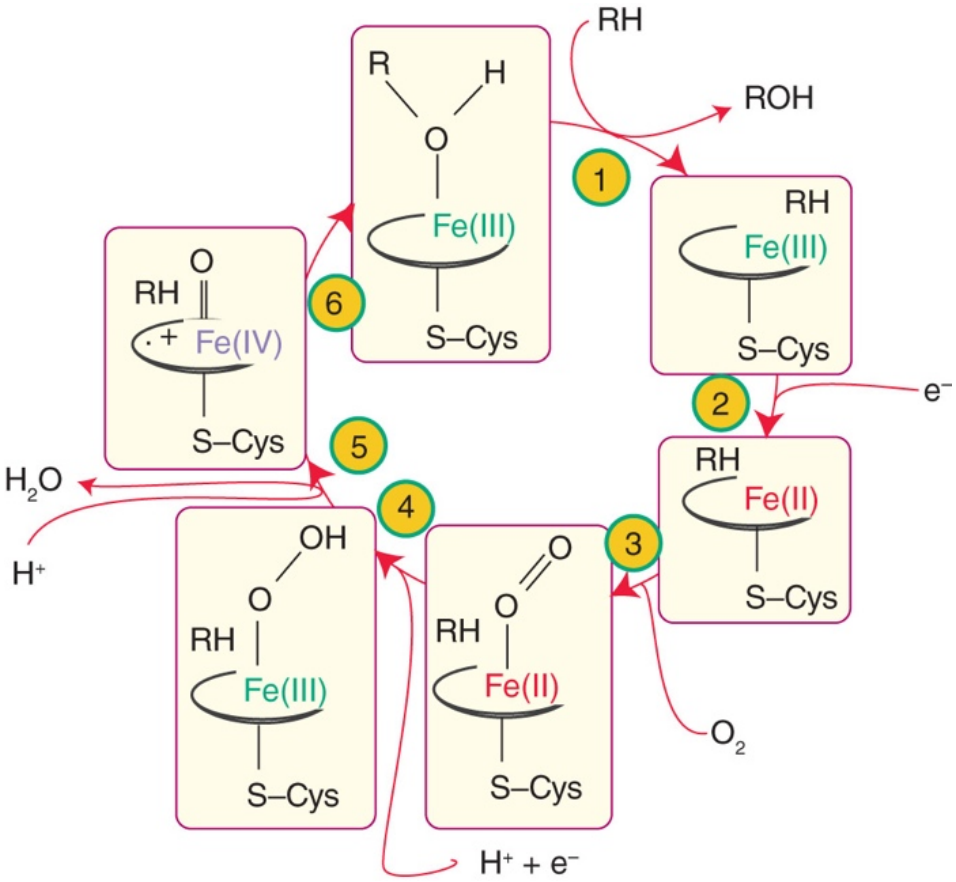
•They play an essential role in biosynthesis, for example in steroid transformations such as progesterone production

•The designation 'P450' comes from the appearance of an intense absorption band at 450 nm

•Most P450s are complex enzymes bound to membranes, so it is difficult to isolate and characterize them. Most of what is known about P450s comes from an enzyme, P450cam, which is isolated from the bacterium Psuedomonas putida. This organism uses camphor as its sole carbon source and the first stage is the oxygenation of position 5

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

•Hemoxygenases: cytochrome P450 catalytic cycle. It is thought that the mechanism of other P450s is similar, although they differ in the architecture of the active center.

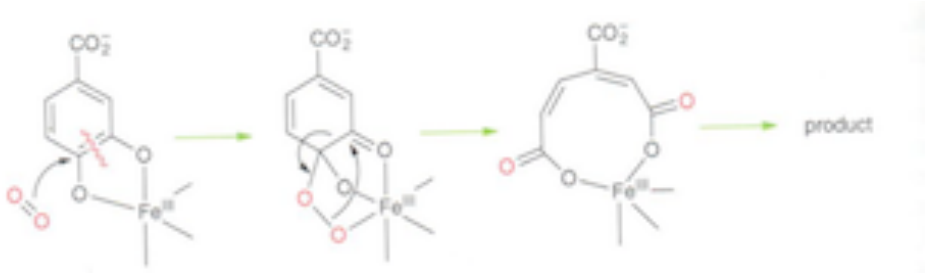


•As an exercise

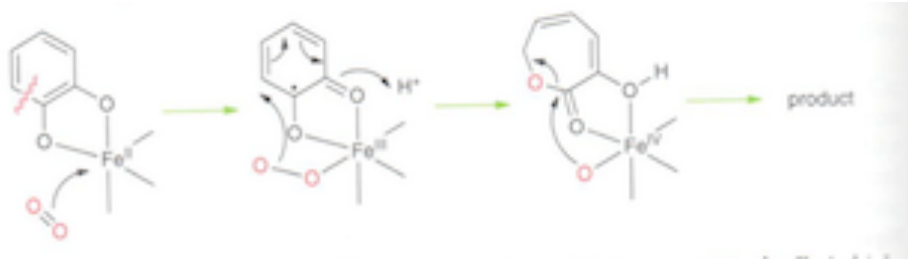
5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

No-heme oxygenases

- They are widely distributed and are usually dioxygenases
- Most contain a single Fe atom in the active center and are classified according to whether the active species in the protein is Fe(III) or Fe(II)
- Non-heme oxygenases of Fe(III) (or intradiol oxygenases), the Fe atom functions as a Lewis acid and activates the organic substrate without coordinating O_2



- Non-heme oxygenases of Fe(II) (or extradiol oxygenases), the Fe atom coordinates directly with O_2 and activates it to attack organic substrates



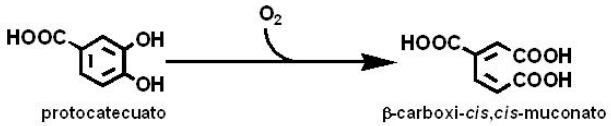
5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

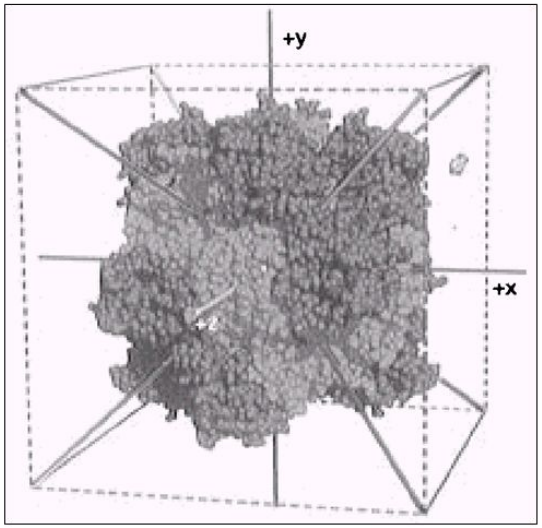
•Non-heme oxygenases

The non-heme oxygenases of Fe(III) are exemplified by the protocatequate 3,4-dioxygenase

- The Fe is closely coordinated by a set of ligands of the protein that include two His-N and two Tyr-O, these last two donor atoms stabilize Fe(III) against Fe(II)
- They are dark red due to a transfer transition of tyrosinate-to-Fe (III) charge

Protocatecuato 3,4-dioxigenasa $(\alpha\beta Fe^{3+})_{12}$
P. putida





Ohlendorf *et al.*, 1988

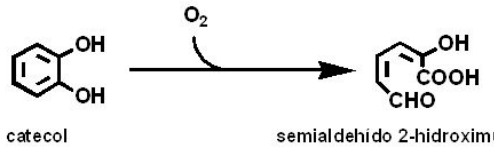
5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

•Non-heme oxygenases

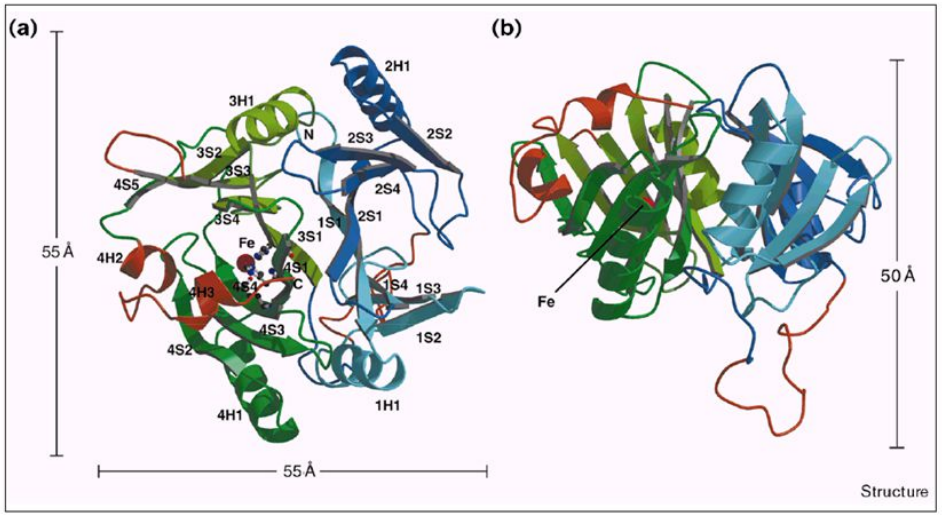
The non-heme oxygenases of Fe(II) are exemplified by catechol 2,3-dioxygenase

- The Fe is coordinated by a set of ligands of the protein that include two His-N and a carboxylic group. The lability of Fe(II) together with the difficulty of observing spectroscopic signals (such as EPR spectra) make these enzymes difficult to study.

Catecol 2,3-dioxigenasa $(\alpha Fe^{++})_4$
***P. putida* mt-2**



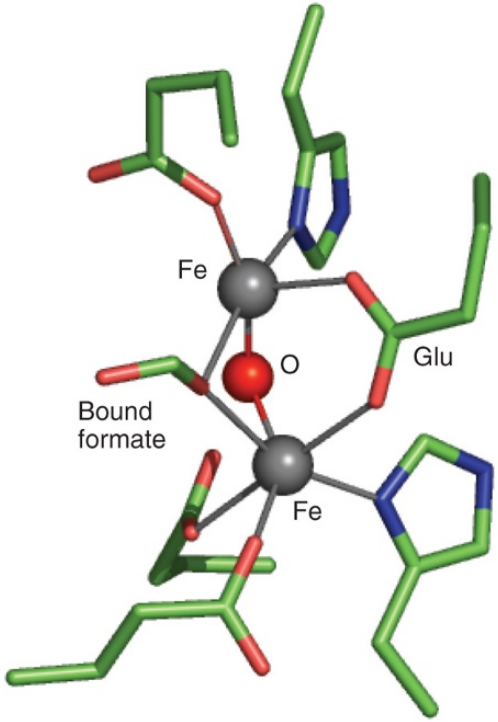
catecol — semialdehído 2-hidroximucónico

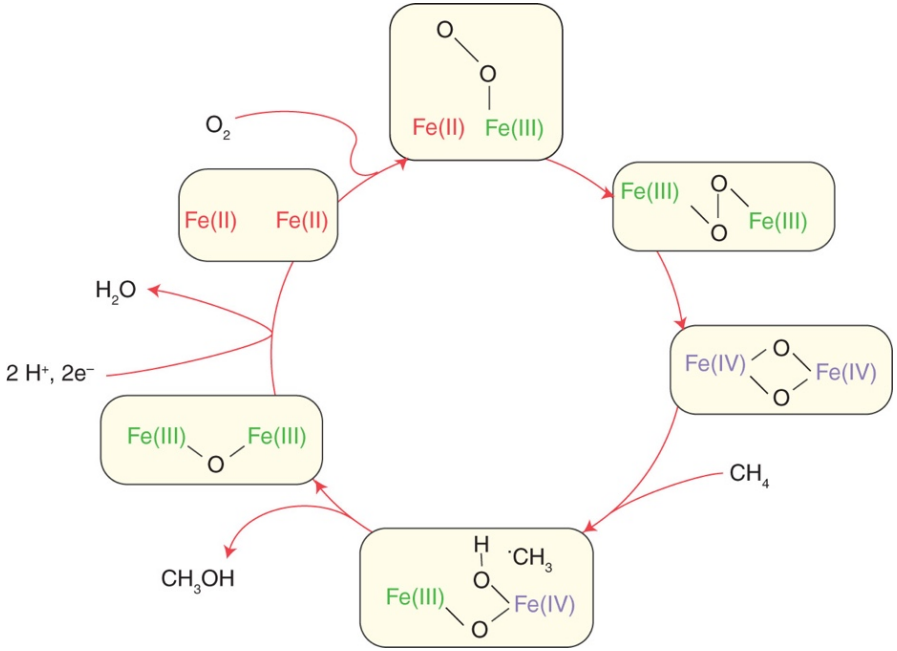


Kita *et al.*, 1999

5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

•Oxygenases and their crucial role in the metabolism of methane, a greenhouse gas. Active center (Fe dinuclear) and plausible catalytic cycle of methane monooxygenase.

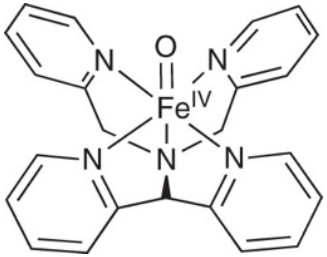




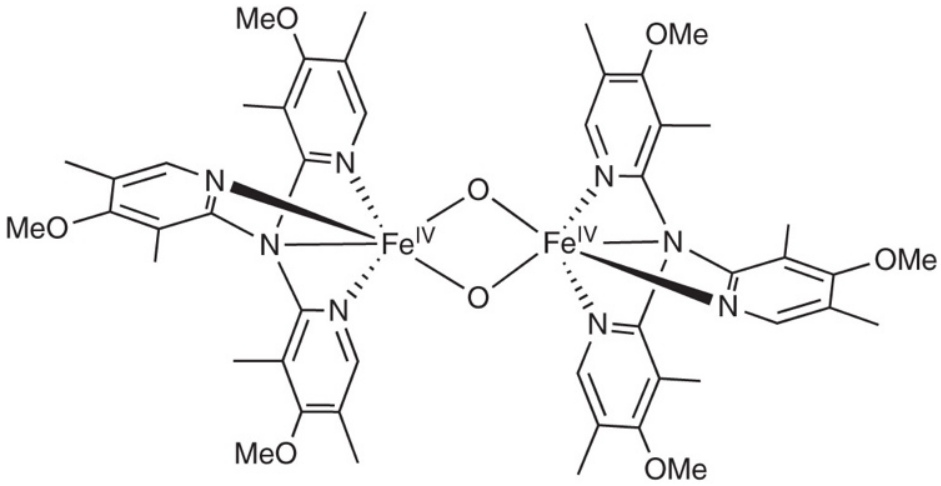
5.- Catalytic processes: Reduction of O_2 and H_2O_2: peroxidases, oxidases, oxygenases

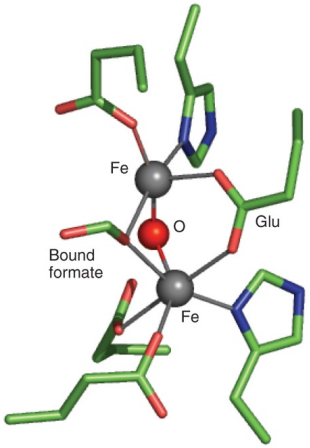
- Models for the study and characterization of enzymatic intermediates of Fe(IV)

  - Oxo mononuclear complex of Fe(IV) containing a pentadentate pentaaza ligand that oxygenates C-H bonds in a variety of hydrocarbons including cyclohexane



  - Bis(μ-oxo) dinuclear complex of Fe(IV) has been proposed as a structural analog of active intermediate formed in methane monooxygenase





5.- Catalytic processes

Classic role of enzymes, highly selective catalysts in the very diverse reactions of living beings. We will see only the most important…

- Acid-base catalysis
  - Zinc enzymes
    - Carbonic anhydrase: 1939
    - Carboxypeptidase: 1964
    - Alkaline phosphatase
    - Alcohol dehydrogenase
    - ß-lactamase
    - Termolysin
    - Magnesium enzymes
  - Manganese enzymes
  - Iron enzymes

- O_2 and H_2O_2 reduction
  - Peroxidases
  - Oxidases
  - Oxygenases

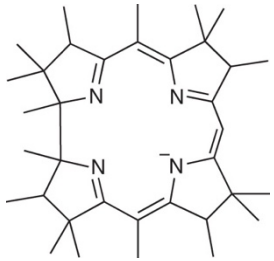
- Catalytic processes of cobalt enzymes

5.- Catalytic processes: Catalytic processes of cobalt enzymes

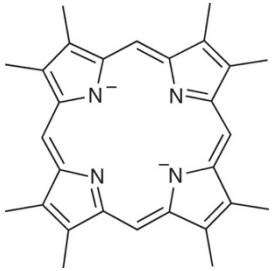
The macrocyclic complexes of Co are cofactors in enzymes that catalyze:

- Reactions of methyl transfer or transmethylation
- Dehalogenation reactions
- Isomerization reactions

The macrocycle is a corrin ring, similar to profirin, except that there is less conjugation and it is smaller (15-member ring instead of 16)



Corrine ring



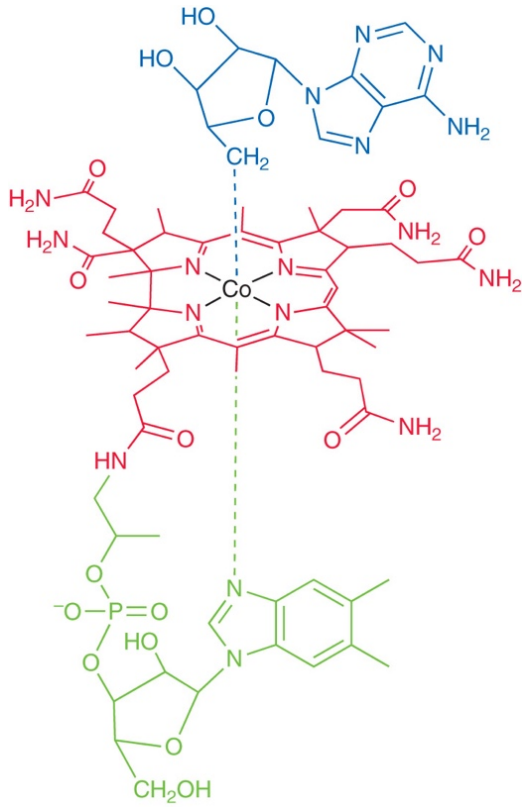
Porphyrin ring

5.- Catalytic processes: Catalytic processes of cobalt enzymes

- Structure of coenzyme B_{12}

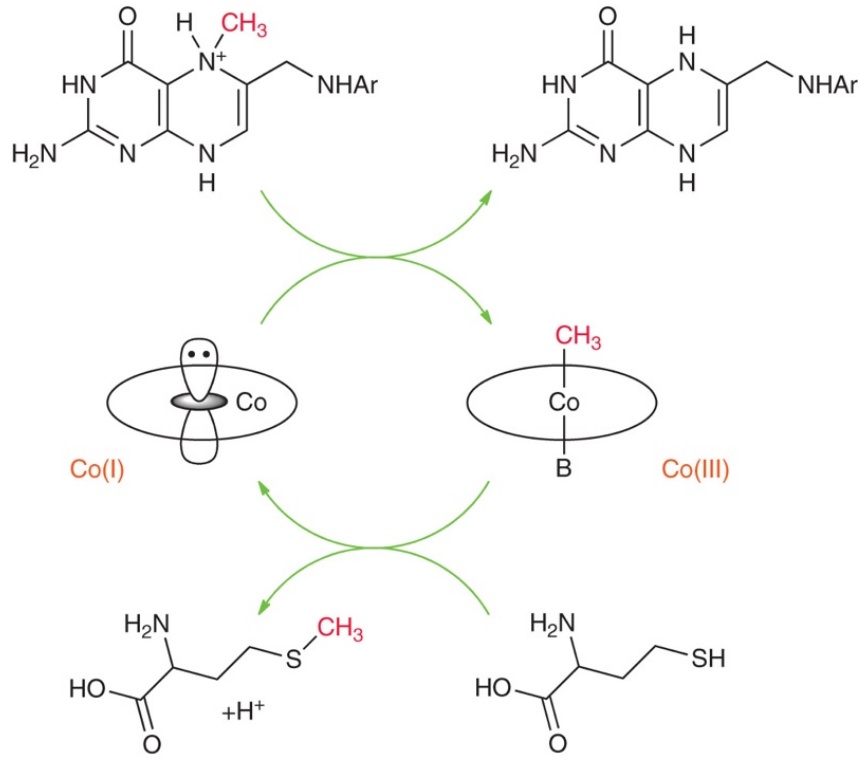
The pentacoordinated complex known as cobalamin has a fifth donor atom N in one of the axial positions: normally this ligand is a dimethylbenzimidazole which is covalently linked to the corrin ring through a nucleotide, although it is also common to find a histidine residue.

Coenzyme B_{12} is formed when the sixth ligand, R, is 5'-deoxyadenosine, which is coordinated to the Co atom through a $-CH_2-$ group. This sixth ligand is interchangeable. This enzyme is not synthesized by animals, only by microorganisms and is ingested in the form of aquacobalamin, hydroxocobalamin or cyanocobalamin, which are known collectively as vitamin B_{12}.



5.- Catalytic processes: Catalytic processes of cobalt enzymes

- Cobalamines that catalyze methyl transfer reactions or transmethylation.
- One of the most important is methionine synthase, which catalyzes the last step in the regeneration of methionine from homocysteine.
- Reaction mechanism: Co(I) is a strong nucleophile and attacks the electrophilic CH_3 group of 5-methyltetrahydrofolate (which transforms into tetrahydrofolate) to form Co(III) methylcobalamin that transfers a CH_3^+ to homocysteine (to form methionine).

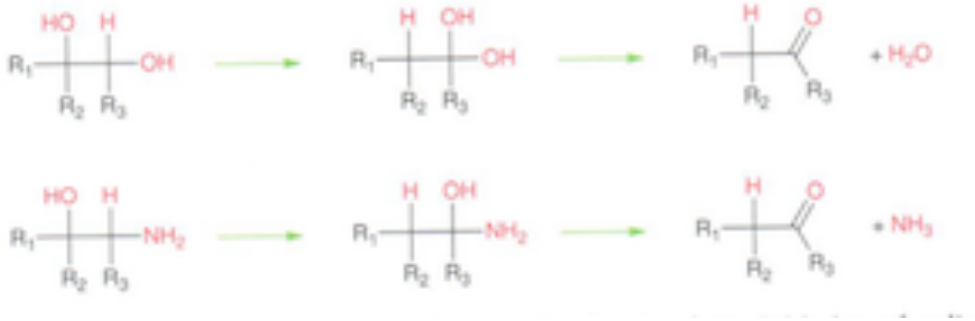


5.- Catalytic processes: Catalytic processes of cobalt enzymes

- Cobalamins that catalyze reordering reactions based on radicals, two types:

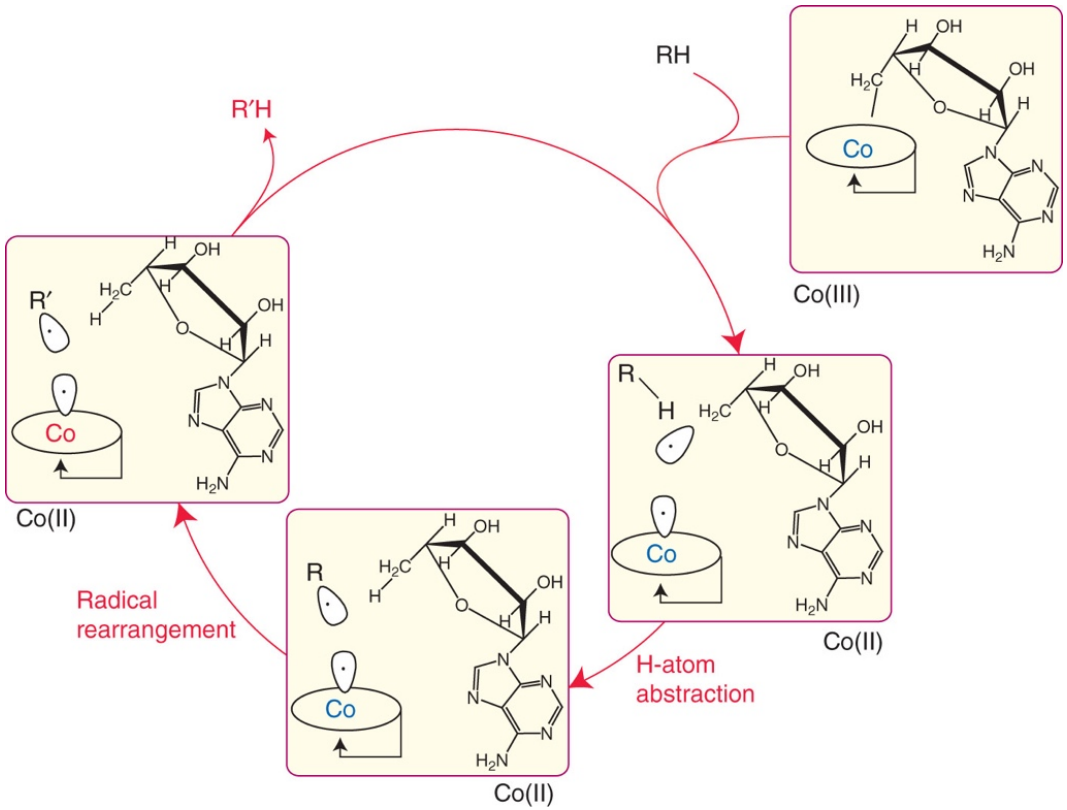
  - Isomerizations (mutases): catalyze the displacement of a functional group from one position to another within the same molecule.

  - Dehydration or deamination (liases): catalyze the breakdown of several chemical bonds by means other than hydrolysis and oxidation.



5.- Catalytic processes: Catalytic processes of cobalt enzymes

Reaction mechanism of cobalamins that catalyze reordering reactions based on radicals. The homolytic cleavage of the Co-C bonds results in a low spin Co(II) complex and a carbon radical that extracts an H atom from the RH substrate. The substrate radical is retained in the active site and undergoes rearrangement (from R to R ') before the hydrogen atom is transferred.

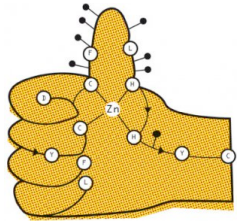


6.- Genetic transcription

- **Transcription** is the first step of gene expression, in which a particular segment of DNA is copied to RNA by the enzyme RNA polymerase. Both DNA and RNA are nucleic acids, which use nucleotide base pairs as a complementary language. During transcription, a strand of complementary RNA is produced.

- **Transcription factors** are proteins that bind to DNA to control genes. These regulatory proteins stimulate or repress the transcriptional rate of their target genes by binding to specific regions of these. This activates or deactivates gene signaling cascades. Transcription factors have fundamental functions in almost all biological processes (development, growth and responses to environmental factors) and it is assumed that they have a preponderant role in the evolution of species.

- The '**zinc fingers**' are present in an important family of transcription factors. They are structural domains of zinc metalloproteins that are able to "anchor" in the grooves of the DNA double helix as if they were fingers. The coordination to the Zn(II) ions stabilizes the folds of the protein chain.



- **Electronic and structural characterization:**

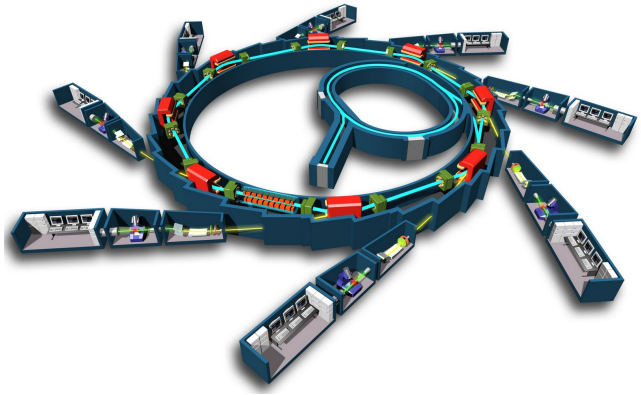
✓ Zn^{2+} ion has a d^{10} configuration (diamagnetic and colorless) so magnetic and electronic techniques are not useful. For its electronic characterization, it is substituted by the Co^{2+} ion (paramagnetic and colored and that also easily forms T_d complexes), or by the Cd^{2+} ion (similar since it is just below in the SP).

✓ In the absence of monocrystals suitable for XR diffraction, we use structural characterization techniques that do not require them, **X-ray absorption spectroscopy** (XAS).

1 cont.- Techniques in Bioinorganics

Synchrotron radiation source in Spain: ALBA

Synchrotron radiation is electromagnetic radiation generated by charged particles (such as e-) that move along a curved path at high speed (close to that of light) in a magnetic field.



ALBA consists of a linear particle accelerator or LINAC, a synchrotron preacelerator or booster and a main synchrotron or storage ring of 270 m perimeter. In the first two devices, the e- are accelerated to speeds close to that of light, reaching an energy of up to 3 GeV. Said e- are injected into the storage ring, from which the light lines emerge.

The obtained electromagnetic radiation (continuum of wavelengths, from the IR to the RX, very intense, polarized and with a temporal scheme) is fundamental in all the fields of science and technology in which it is necessary to analyze samples of small dimensions such as crystalline structures, new materials, biological samples, pollutants or archaeological remains. It can also have applications in the design of new drugs and in imaging and medical therapies.

1 cont.- Techniques in Bioinorganics

- **Beamlines in ALBA**
- **BL01: Infrared Microscopy (MIRAS).** Devoted to Fourier Transform Infrared (FTIR) spectroscopy and microscopy, a very potential tool to identify the vibrational signatures and therefore the chemical composition of materials.
- **BL04: Materials science and powder diffraction (MSPD).** High-resolution powder diffraction and high pressure powder diffraction using diamond anvil cells.
- **BL09: Soft X-Ray Microscopy (MISTRAL).** Cryo nano-tomography for biological applications. Spectroscopic imaging with various X-ray absorption edges.
- **BL11: Non-crystalline Diffraction (NCD).** Small Angle X-ray Scattering (SAXS) experiments provide structural and dynamic information of large molecular assemblies such as polymers, colloids, proteins and fibres, covering a wide range of research areas.
- **BL13: Macromolecular Crystallography (XALOC).** It aims to provide Structural Biology groups with a flexible and reliable tool to help in solving structures of macromolecules and complexes.
- **BL22: Core Level Absorption & Emission Spectroscopies (CLÆSS)** provides a simultaneous and unified access to two complementary techniques: absorption and emission spectroscopies.
- **BL24: Photoemission Spectroscopy And Microscopy (**CIRCE) is a variable polarization soft X-ray beamline dedicated to advanced photoemission experiments.
- **BL29: Resonant Absorption And Scattering (BOREAS).** The variable polarization soft X-ray beamline is dedicated to fundamental, as well as applied, polarization-dependent spectroscopic investigation of advanced materials.

The study of metalloproteins is approached from two complementary techniques:

- The diffraction of RX on monocrystal (XALOC): determination of the three-dimensional structure of the protein.
- XAS (X-ray absorption spectroscopy) (CLAESS): determination of the oxidation state and the coordination environment of the metal ion.

Given the importance of knowledge of the structure of proteins to establish their operating model, one of the three new beamlines of ALBA (Phase III - in design) is XAIRA: MICROFOCUS BEAMLINE FOR MACROMOLECULAR CRYSTALOGRAPHY.

1 cont.- Techniques in Bioinorganics

XAS: Application to Biological Systems

- **EXAFS (Extended X-ray Absorption Fine Structure)** is a powerful technique that furnishes *atomic* resolution local structures of metalloprotein active site.
- **XANES (X-ray Absorption Near-Edge Structure)** is a powerful technique to obtain valuable information of the electronic structures of metalloproteins.

- Sample Requirement
  - 100 uL in volume, 20-30% glycerol/glassing agent
  - 0.1-1 mM for heavy metals Z > Cu, ~2mM for Z < Fe

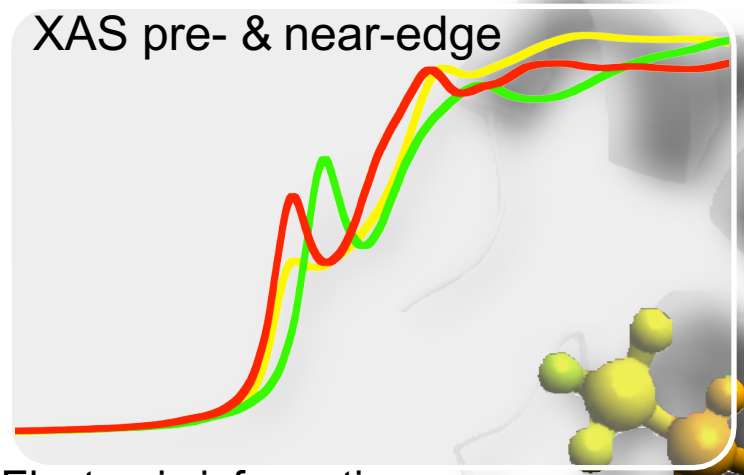
- Measurement Time
  - Time : 5-15 hours (per-sample, excluding duplicates)
  - Reproducibility : at least once

- Direct Comparison to Crystallography

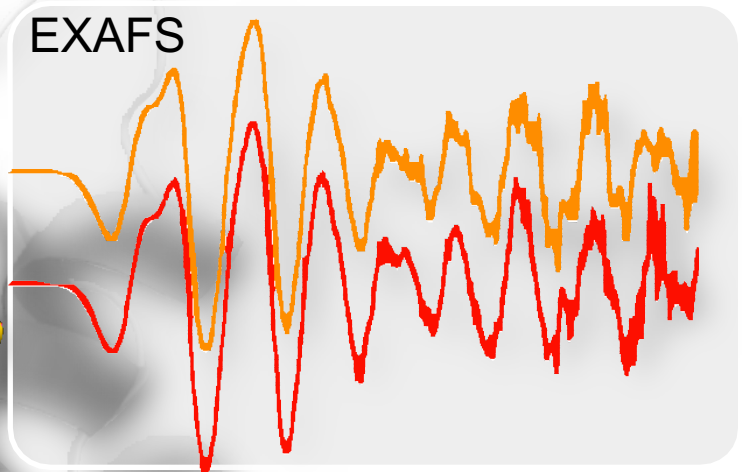
  - Solution EXAFS may vary from crystallography due to changes in H-bonding or due to crystal packing effects, i.e., solution and crystalline structures of metalloproteins may vary *intrinsically*
  - Researchers should feel encouraged to combine XAS and Crystallography, *routinely*

1 cont.- Techniques in Bioinorganics

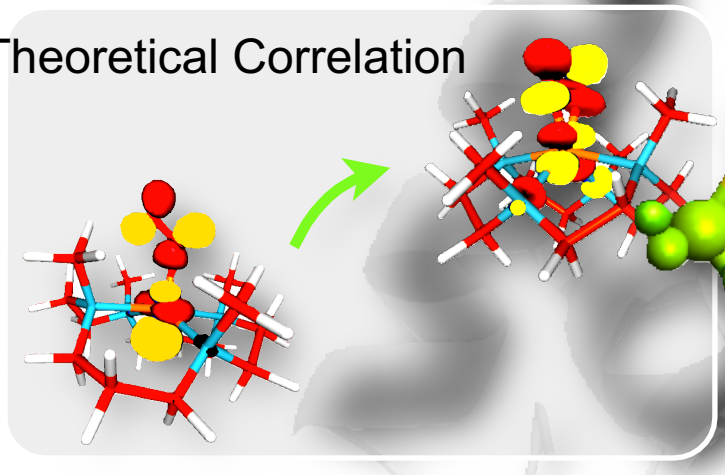
Combining Experiment and Theory



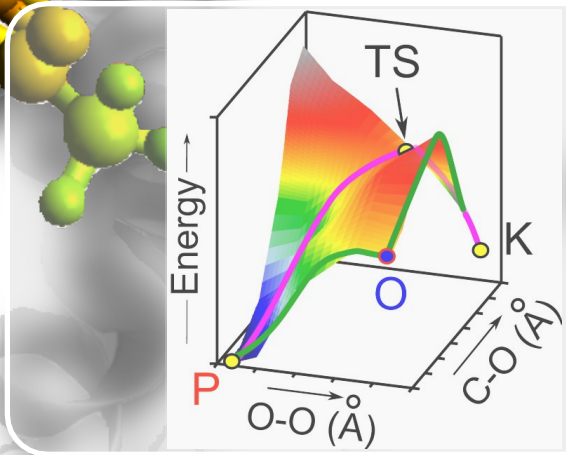
Electronic information:
covalency, bond strength, type of ligands



Structure: Information on Ligands
How many, *What* type, *How* far.



Detailed Electronic Information

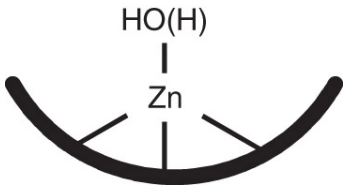


Structure Function Correlation

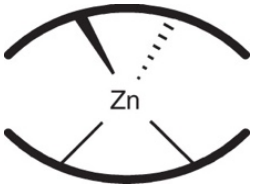
6.- Genetic transcription

- Typical Zn^{2+} ion environments depending on the role played
  - In the catalytic sites: three permanent protein ligands and an interchangeable ligand (H_2O)
  - In structural sites: four permanent protein ligands.

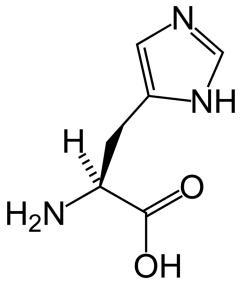
Structural

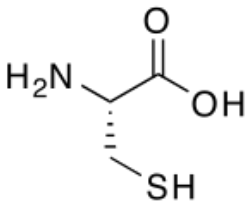


Catalyst



- The Zn^{2+} ion is particularly suitable for binding to proteins and keeping them in a particular conformation since they form stable complexes, in particular with S and N donors. Therefore, proteins are frequently coordinated to histidine and cysteine residues.





6.- Genetic transcription

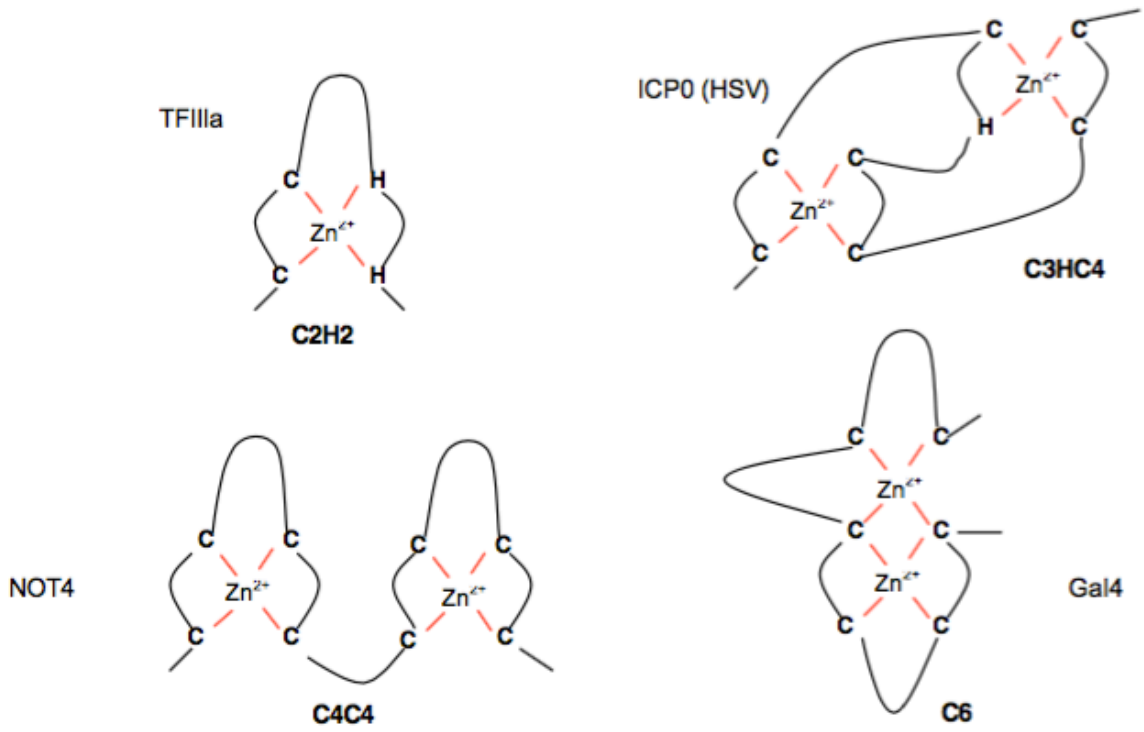
Definition of Zn finger: a protein motif that binds to DNA and whose amino acids are folded into a single structural unit around a Zn atom of T_d geometry.

Zn ions are coordinated to a combination of cysteine and histidine residues.

On the classical finger Zn, a Zn atom is bound to two cysteines and two histidines. Between Cys and His there are 12 residues that form a fingertip that binds to DNA.

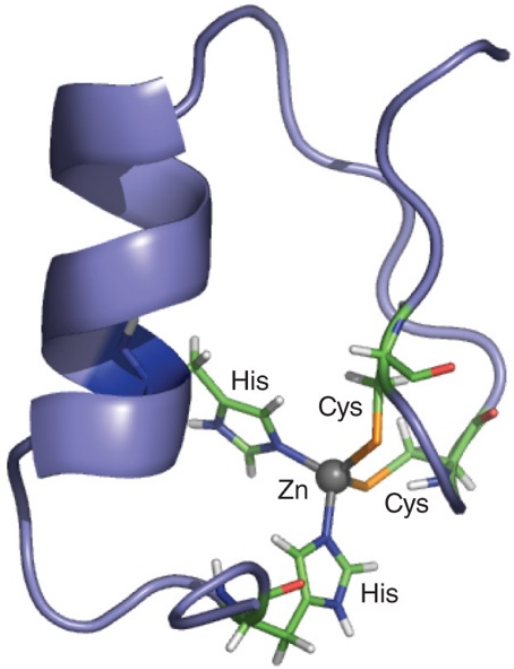
By variations in the composition of the fingertip sequences and spacing of the tandem repeats of the motif, the Zn fingers can form a large number of specific binding sites with different sequences.

Classification of Zn fingers: originally the name and the number of the residues were used to classify the different types of zinc fingers (for example, Cys2His2, Cys4 and Cys6). There are at least 15 kinds of zinc fingers. Some of the most important are:



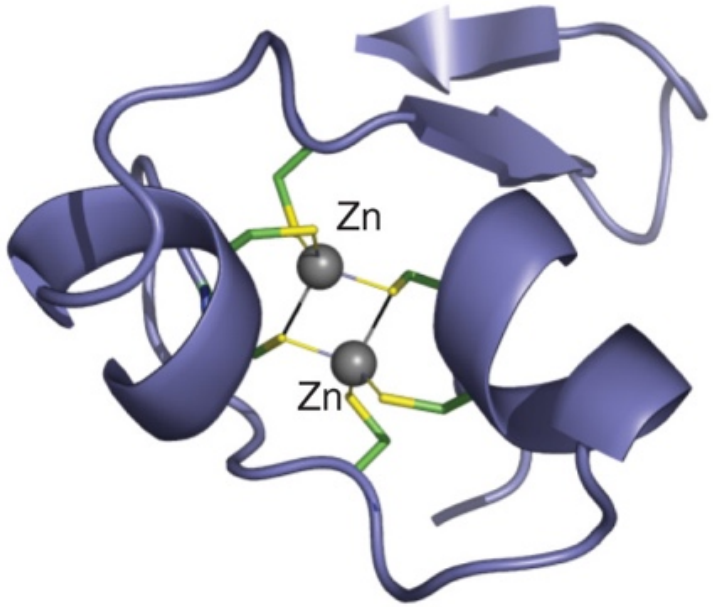
6.- Genetic transcription

- **Zn fingers C2H2 or Cys2His2:** contain mononuclear Zn(II) complexes in which the Zn ion is coordinated to two amino acid pairs of the lateral chains of the finger; one side of the finger tip provides two S-cysteine donors and the other side provides two N-histidine donors..



Example of C2H2 in the TFIIIA protein.

- **Zn fingers C6 or Cys6:** contain dinuclear Zn (II) complexes where two Zn ions are coordinated to 6 cysteine residues.

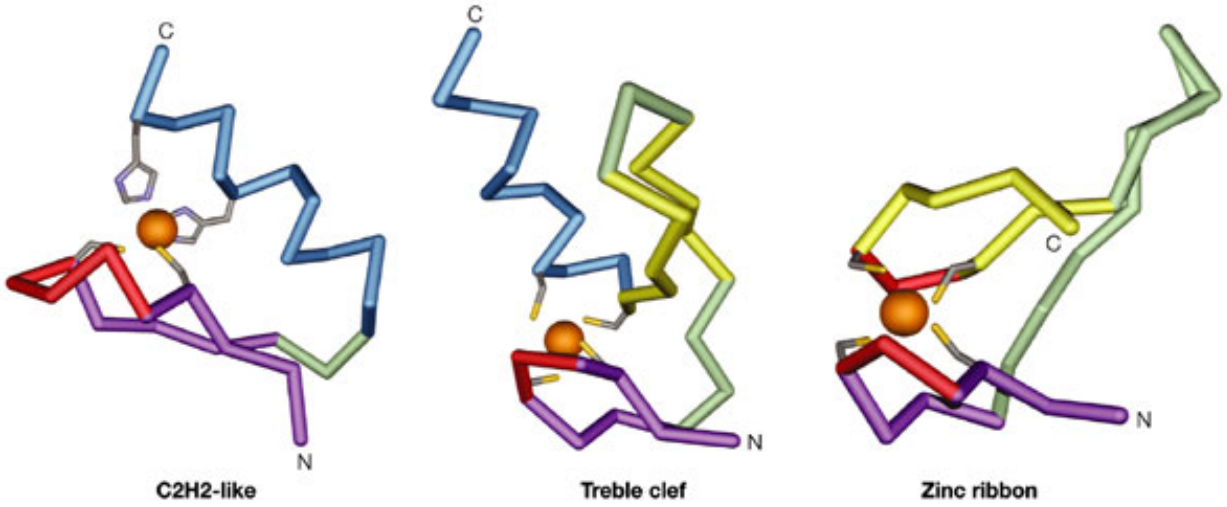


Example of C6 in the Ga14 protein of the yeast.

6.- Genetic transcription

- At present, proteins are classified according to the general form of the main chain in the folding domain.

- The most common "folding groups" are:
  - Cys2His2-like (classical Zn fingers)
  - reble clef Zn fingers
  - ribbon Zn fingers



6.- Genetic transcription

Types and structure of "zinc finger proteins". The structures depend on the type of zinc finger and generally contain a series of zinc fingers.

1. Proteins with Zn fingers Cys2His2-like. The amino acid sequence of a zinc finger of this type is:

$$\mathbf{Cys\text{-}X_{2\text{-}4}\text{-}Cys\text{-}X_{12}\text{-}His\text{-}X_{3\text{-}5}\text{-}His}$$

where X are different amino acids. The finger itself contains about 23 amino acids and the connector between the fingers is almost always formed by 7-8 amino acids.
Generally, the fingers are organized as simple series of tandem repetitions. Occasionally there is more than one group of fingers. The stretch of fingers goes from 9 repetitions that occupy almost the complete protein (as in the case of TFIIIA) to a single small domain with 2 fingers (as in the ADR1 regulator of Drosophila). The general transcription factor SP1 has a DNA binding domain that has thre zinc fingers (Fig. A).

2. Proteins with Zn fingers type Cys2Cys2 (**steroid receptors** and some other proteins). Sequence:

$$\mathbf{Cys\text{-}X_{2}\text{-}Cys\text{-}X_{13}\text{-}Cys\text{-}X_{2}\text{-}Cys}$$

The proteins with this type of fingers often have non-repeated fingers, unlike the tandem repetition of the previous fingers. The receptors for glucocorticoids and estrogen each have 2 fingers (Fig. B).

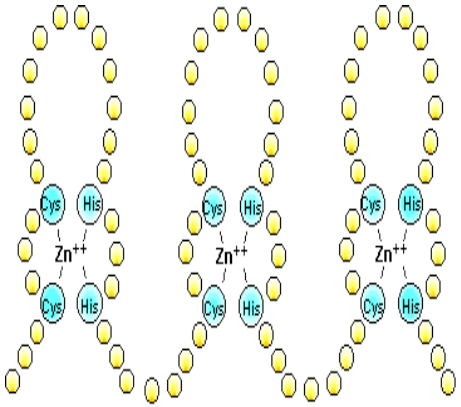


Fig. a) Series of three fingers of zinc (example of the SP1 factor)

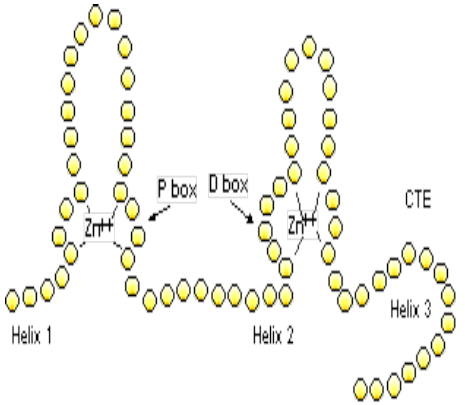
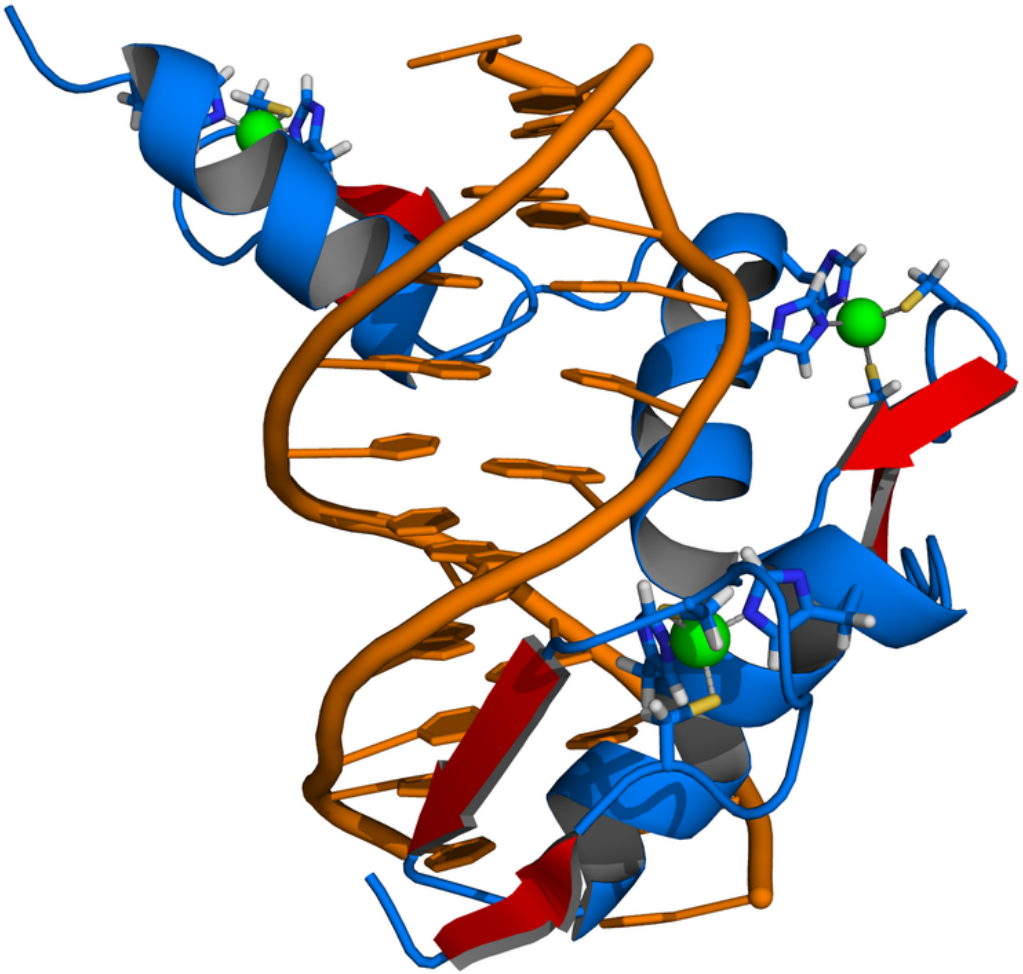


Fig. b) DNA binding domain of a steroid receptor

6.- Genetic transcription

Representation of Zif268 protein (blue) containing three zinc fingers in complex with DNA (orange). Coordinating amino acids and zinc ions are highlighted (green).

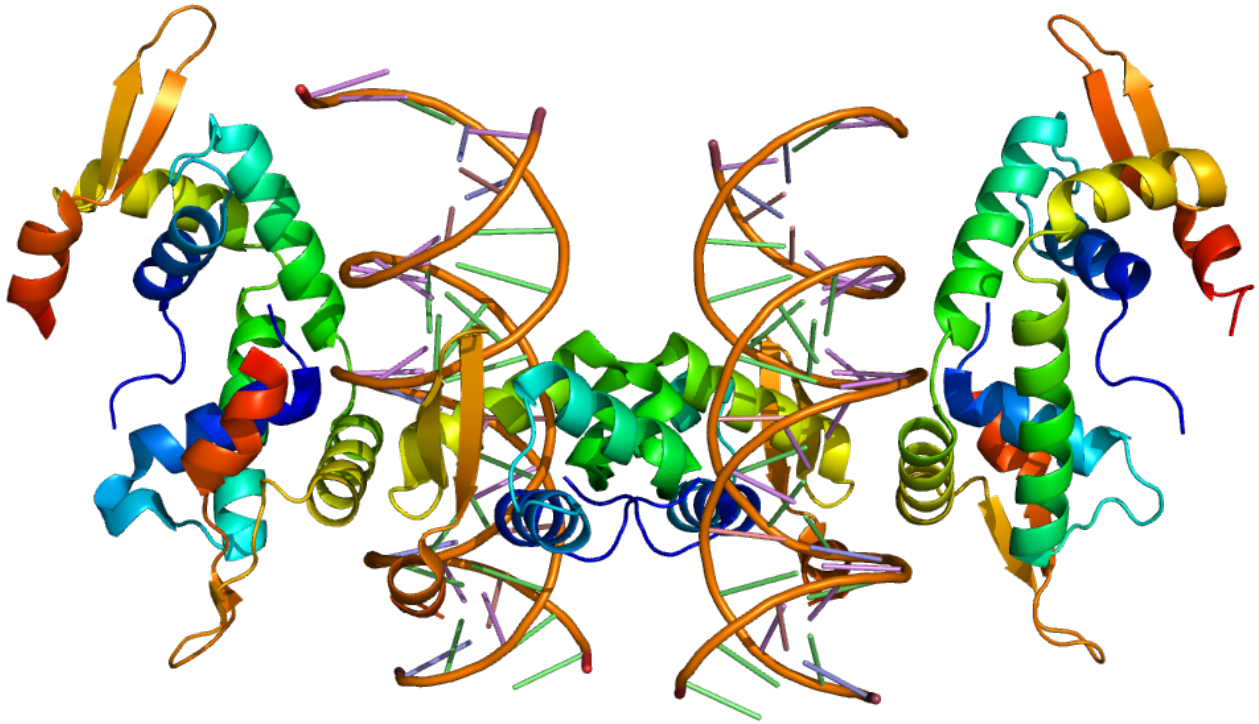


6.- Genetic transcription

- The discovery at the end of the 90s by S. Fisher et al. of the FoxP2 gene and the study at the beginning of the 21st century of the language disorders suffered by 50% of the members of an English family known as KE that carried a mutated version of this gene, constituted the indisputable proof of the genetic basis of our language. It has also been seen that there is a significant association between this gene and auditory hallucinations in schizophrenia.

- It is not a gene unique to humans (in fact it is believed to be present in all vertebrates), but a mutation that took place about 200.000 years ago, which modified the sequence of a few nucleotides, could be responsible for learning of language in homo sapiens.

- The protein contains an area of polyglutamine, a zinc finger and a leucine zipper.



7.- Biological cycles: N_2 cycle and H_2 cycle

- Nature is extraordinarily economical and maximizes the use of elements that have been taken from the non-biological world, often with great difficulty. We have already seen how iron is assimilated through the use of special ligands and how this valuable resource is stored in ferritin. That is, the useful species are recycled instead of returned to the environment.

- Two important examples are nitrogen, so difficult to fix from its gaseous source by chemical processes but easily fixed by many microorganisms and hydrogen, which is rapidly achieved by microbes in processes analogous to electrolytic cells.

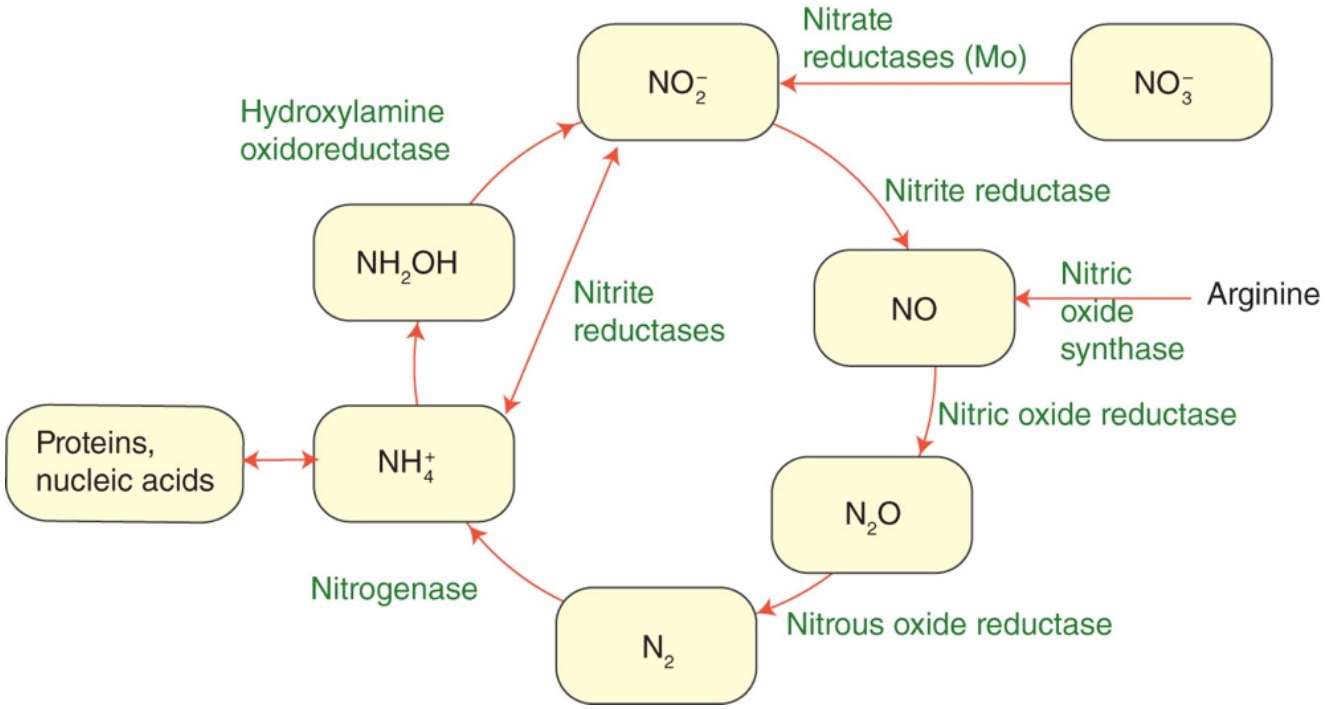
7.- Biological cycles: N_2 cycle and H_2 cycle

N_2 cycle

•It involves many different organisms and a wide variety of metalloenzymes (Fe, Cu and Mo).

•It can be divided into two parts, nitrogen uptake usable from NO_3^- or N_2 (assimilation) and denitrification or desasimilation.

•Many of the compounds are toxic or environmentally challenging: ammonia is essential for the synthesis of amino acids, NO is produced in small quantities that serve as cellular signaling agents, N_2O is a greenhouse gas, its release to the atmosphere depends of the balance between the activity of NO reductase and N_2O reductase in the biological world.



7.- Biological cycles: N_2 cycle and H_2 cycle

Nitrogenase

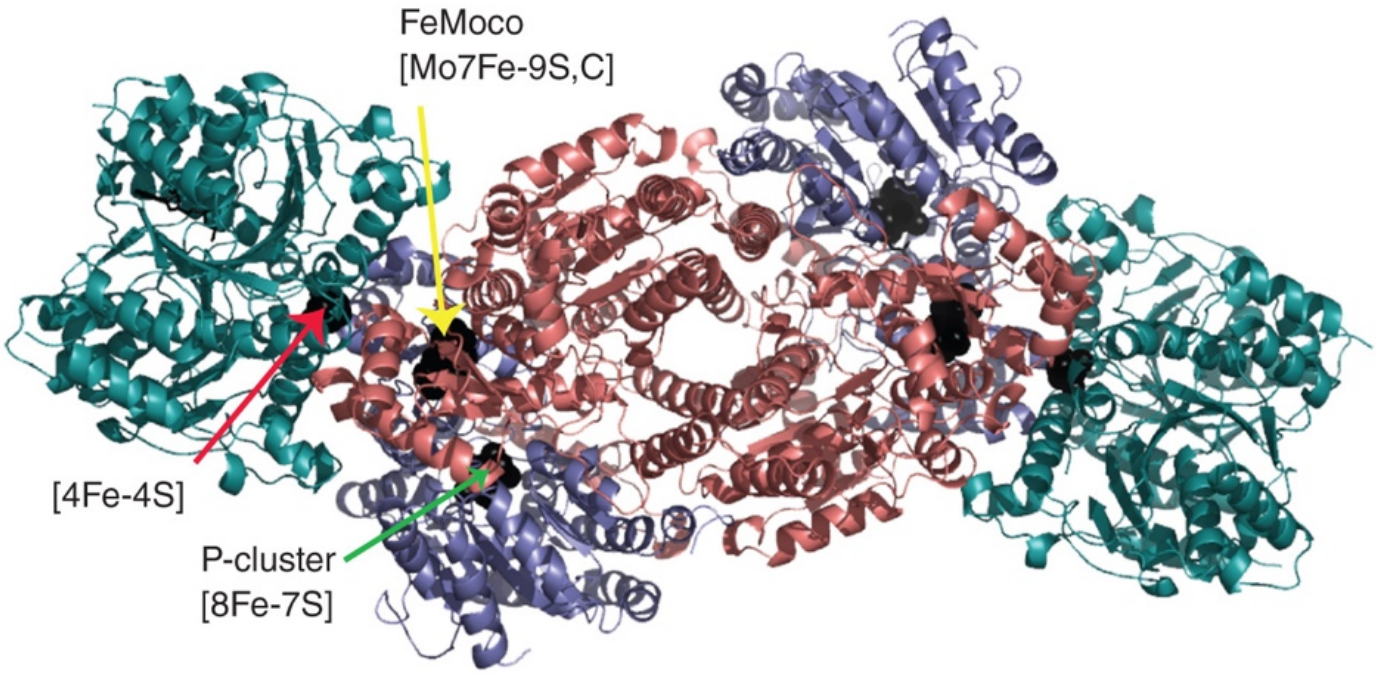
•It is an enzyme present in the so-called nitrogen-fixing bacteria (in the soil and in the nodules of some roots)

•It catalyzes the reduction of N_2 to NH_3 in a reaction that is coupled to the hydrolysis of 16 molecules of ATP:

$$N_2 + 8H^+ + 8e^- + 16\,ATP \rightarrow 2NH_3 + H_2 + 16\,ADP + 16\,Pi\ (inorganic\ phosphate)$$

•Nitrogenase is an enzyme formed by two types of proteins:

- ✓ The largest is the so-called MoFe protein
- ✓ The little one is the so-called Fe protein

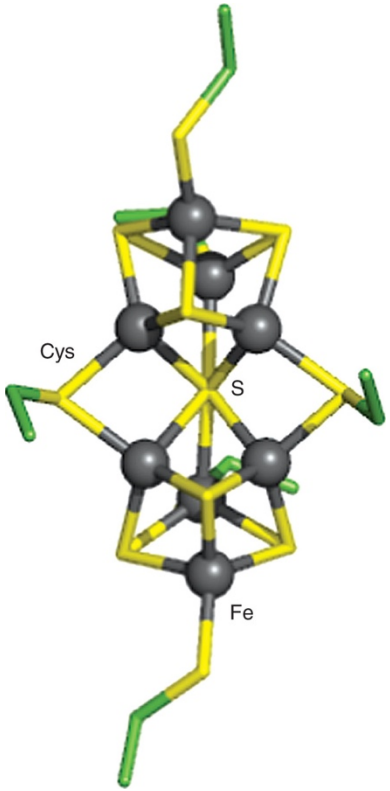


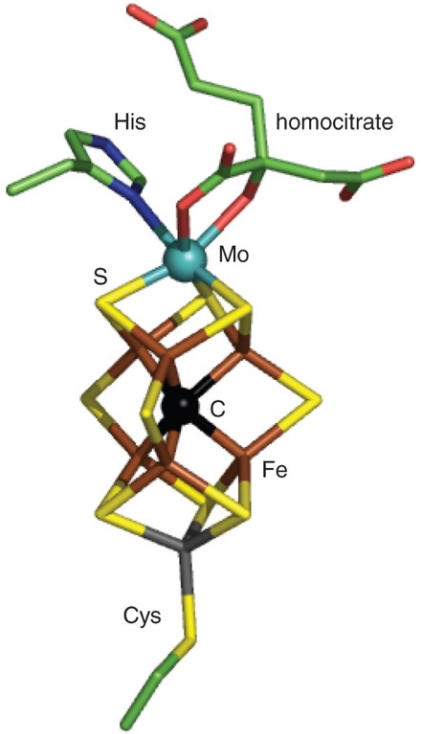
7.- Biological cycles: N_2 cycle and H_2 cycle

- **The Fe protein**
  - ✓ It contains a single cluster [4Fe-4S] that is coordinated by 2 cysteine residues.
  - ✓ Its function is the transfer of electrons from a reducing agent, such as ferredoxin or flavodoxin, to the MoFe protein.
  - ✓ Each e-transfer requires the hydrolysis of 2 molecules of ATP, bound to the Fe protein.
  - ✓ The hydrolysis of ATP also causes a conformational change in nitrogenase, causing the Fe protein and MoFe protein to be closer together to facilitate the transfer of e-.

7.- Biological cycles: N_2 cycle and H_2 cycle

- The **MoFe protein** is constituted by two alpha subunits and two beta subunits, each alpha-beta unit contains two types of superclusters:
  - [8Fe-7S] known as 'P-cluster' and which is believed to be an electronic transfer center.
  - [Mo7Fe-9S, C] known as 'FeMoco' (cofactor FeMo) and which is believed to be the center where the N_2 is reduced to NH_3. The Mo is also coordinated to a N-imidazole of histidine and to 2 O atoms of an exogenous molecule, R-homocitrate. In the center of the cage formed by the 6 atoms of Fe in trigonal coordination, there is a C atom that stabilizes the cage, which otherwise would collapse. The reduction mechanism is unknown, the question is whether the N_2 is bound and reduced in the Mo or in one or more of the Fe.





7.- Biological cycles: N_2 cycle and H_2 cycle

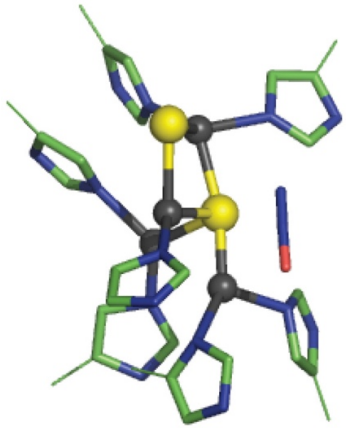
Nitrate reductase: is a Mo enzyme involved in the transfer of an O atom and catalyzes the reduction of NO_3^- to NO_2^-.

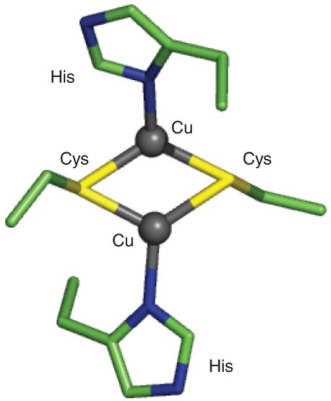
The remaining enzymes of the N_2 cycle contain heme or Cu groups as active centers.

Nitrite reductase: two classes
•Multihemic enzymes that can reduce NO2- to NH3
•Enzymes that contain Cu and carry out a transfer of 1e-, producing NO

NO (nitric oxide) reductases: enzymes that manipulate NO are
•Nitric oxide synthase, a heme enzyme responsible for producing NO by oxidation of L-arginine
•Nitric oxide reductase, which is what properly reduces NO to N_2O

N_2O (nitrous oxide) reductases:
•There is a cofactor that is a cluster [4Cu-2S] where the 4 Cu atoms are coordinated to the protein by imidazole ligands and held together by two inorganic sulfur ions. The coordination and activation of the N_2O molecule is difficult since it is a very weak ligand; the structure reveals that the N_2O molecule occupies a non-coordinating position at about 3 Å of one of the Cu.
•The long-distance transfer is carried out by a dinuclear Cu center, the same one found in the ciochrome c oxidase.





7.- Biological cycles: N_2 cycle and H_2 cycle

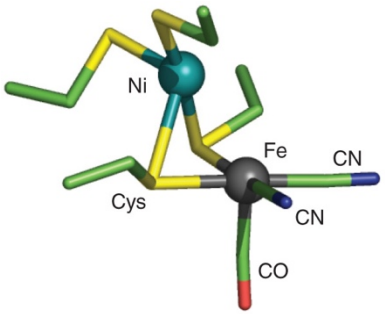
H_2 cycle

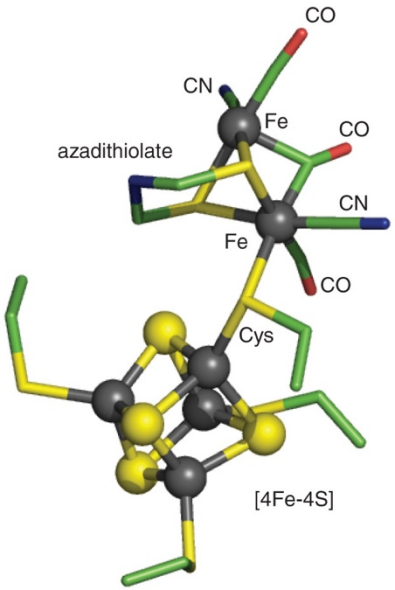
•99% of all organisms use H_2 and possess extremely active metalloenzymes (**the hydrogenases**) that catalyze the interconversion of H_2 into H^+ (in the form of H_2O).

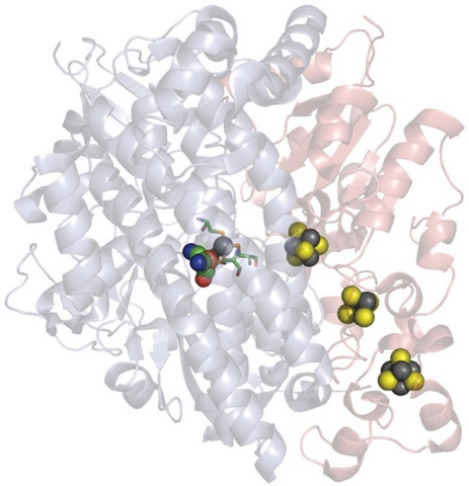
•H_2 is produced by some organisms (as a waste product) and is used by other organisms as fuel, which explains the small amount of H_2 in the atmosphere.

•All hydrogenases contain Fe and some also contain Ni. The best characterized are [NiFe] and [FeFe]: both classes contain at least one CO ligand, at least one CN- ligand and one cysteine (or sometimes selenocysteine). In particular, the hydrogenases [FeFe], contain a bidentate ligand, the azadithiolate, $(SCH_2)_2NH$, which forms a bridge between the Fe atoms and also a cluster [4Fe-4S] that is coordinated to one of the Fe by a ligand bridge cysteine.

•These fragile active sites are buried deep in the enzyme, requiring special pores and pathways for the transmission of H_2 and H^+ as well as a series of FeS clusters that provide a way for the transfer from e- to long distance to the active site of the hydrogenase.

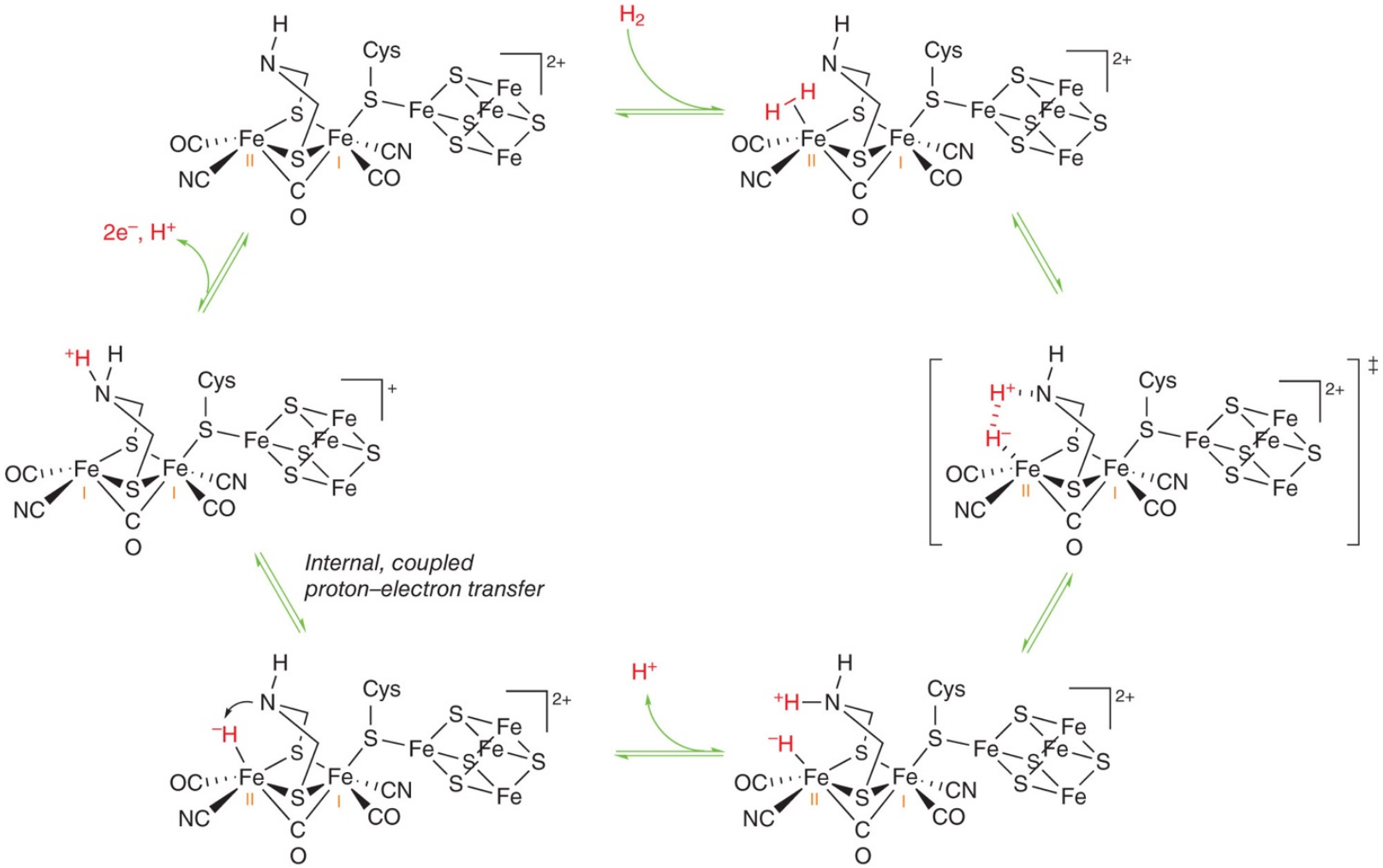






7.- Biological cycles: N_2 cycle and H_2 cycle

- **Catalytic cycle proposed for hydrogenases [FeFe]**



Este material docente ha sido elaborado en el marco de una convocatoria de ayudas para el desarrollo de proyectos de innovación educativa y mejora de la calidad docente (convocado por el Vicerectorat de Polítiques de Formació i Qualitat Educativa de la Universitat de València, en el curso 2017-2018). Código: UV-SFPIE_RMD17-725369

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