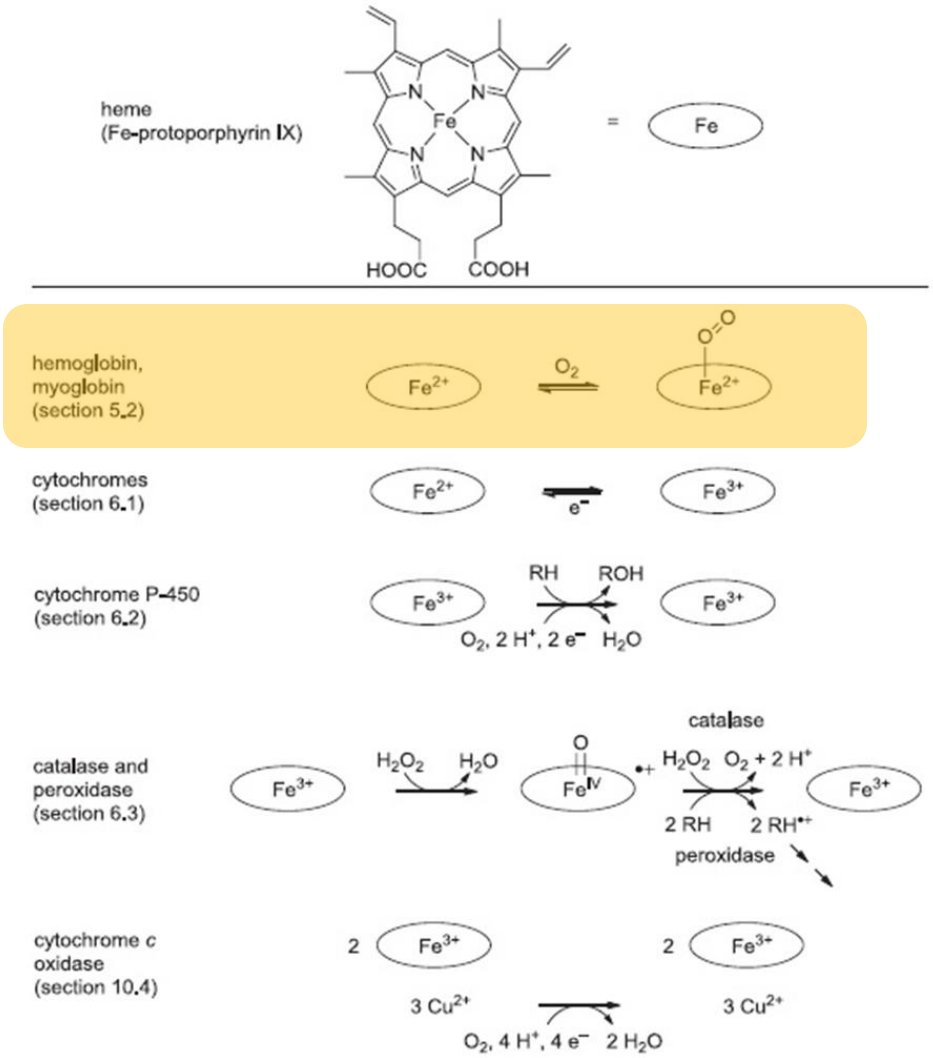
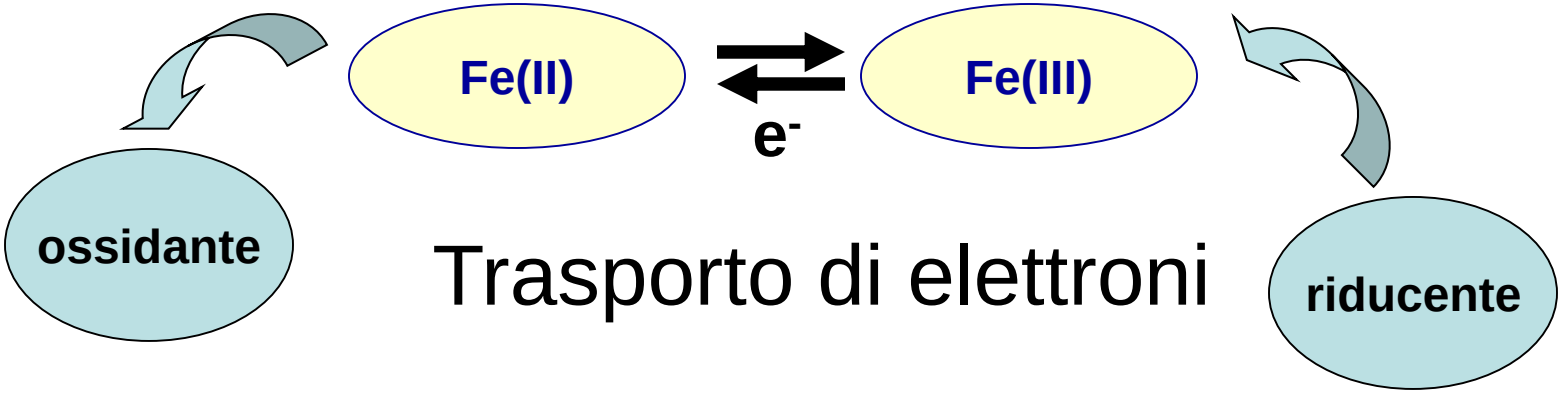
# Type Heme Iron Proteins



# Citocromi

## Contenuti sia nelle piante che negli animali




**Mediatore capacità redox di alcuni elementi e composti ad es. $O_2$**

# Cytochromes

**Absorbance**

$$A = \log \frac{I_{out}}{I_{in}}$$

**Transmittance**

$$\%T = \frac{I_{out}}{I_{in}} \times 100\,\%$$

- **Cytochromes are proteins with heme prosthetic groups. They absorb light at characteristic wavelengths.**

- **Absorbance changes upon oxidation/reduction of the heme iron provide a basis for monitoring the redox state of the heme.**

- **Some cytochromes are part of large integral membrane complexes, each consisting of several polypeptides and including multiple electron carriers.**

- **Cytochrome c is instead a small, water-soluble protein with a single heme group.**

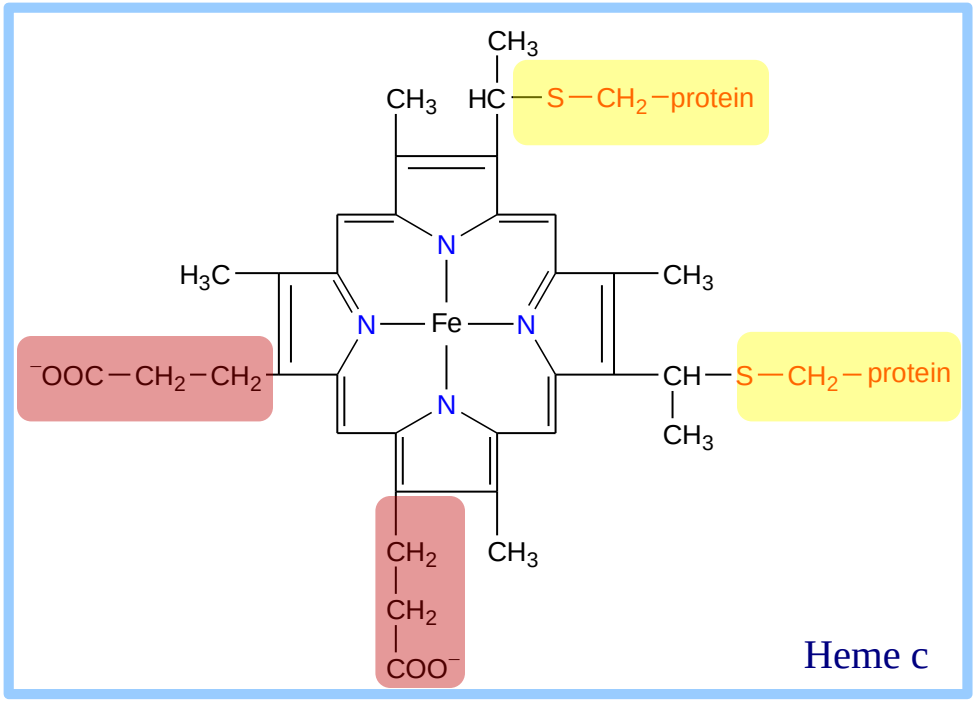
# Cytochromes

## Different structures and properties

Hemes in the 3 classes of cytochrome (**a**, **b**, **c**) differ slightly in substituents on the porphyrin ring system

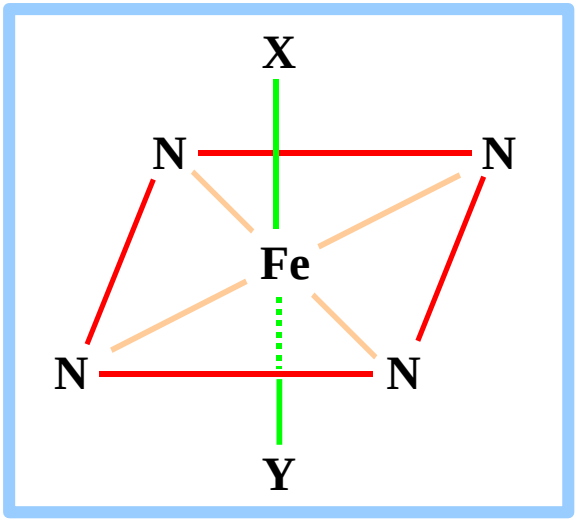
A common feature is 2 propionate side-chains.

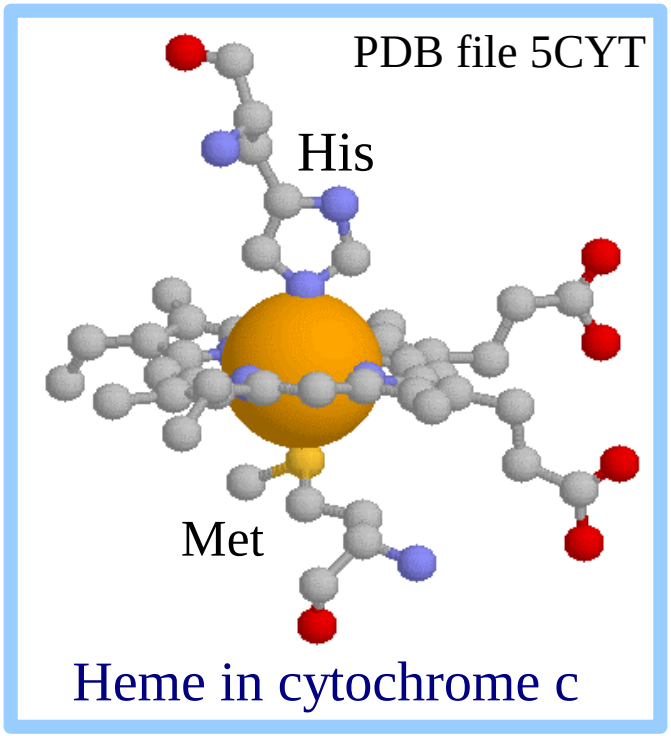
Only **heme c** is covalently linked to the protein via thioether bonds to **cysteine** residues.



The porphyrin ring is planar.

The heme **Fe** is usually bonded to **2 axial ligands**, above & below the heme plane (X,Y) in addition to **4 N** of porphyrin**.**



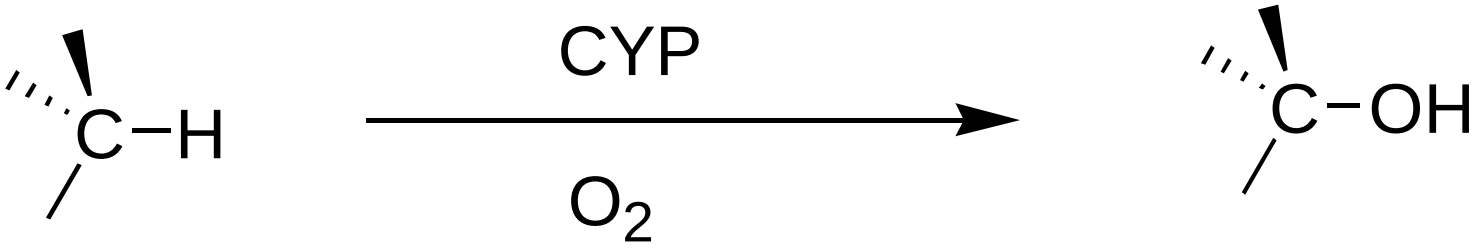

**Axial ligands may be S or N atoms of amino acid side-chains.**

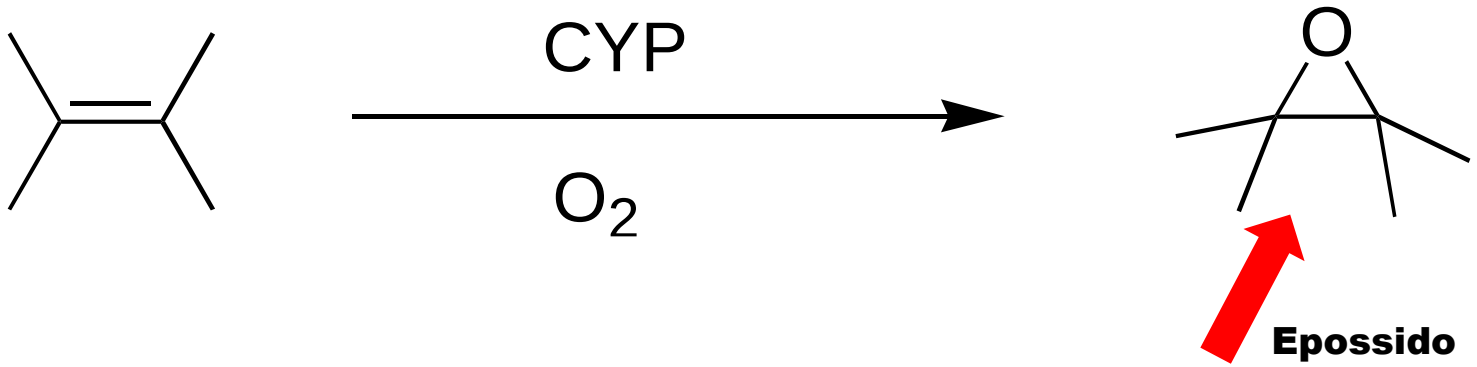
**Axial ligands in cyt c are Met S (yellow) and His N (blue).**

# Cytochrome P-450

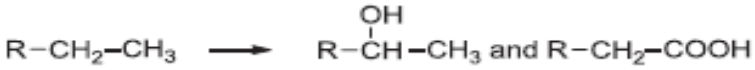
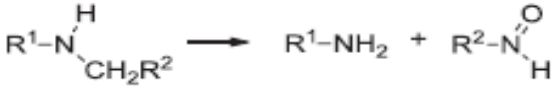
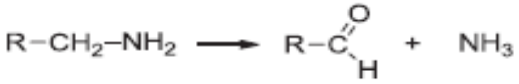
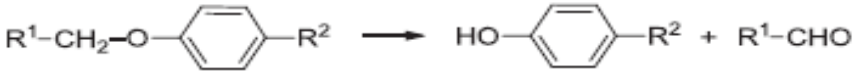
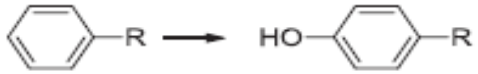
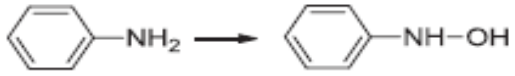
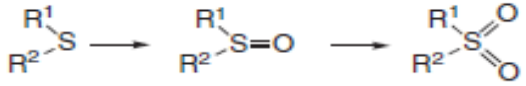
**Picco di Soret**
assorbimento massimo a una lunghezza d'onda di 450 nm: il ferro del gruppo eme ($Fe^{2+}$) è legato al monossido di carbonio

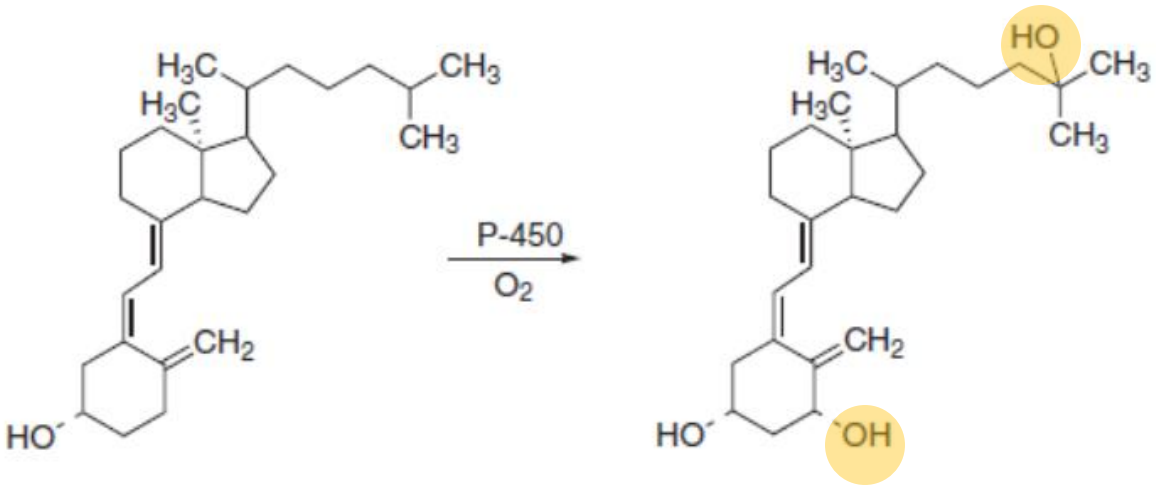
## Introduzione dell'ossigeno in substrati inerti all'ossidazione



# Metabolismo ossidativo di prodotti farmaceutici

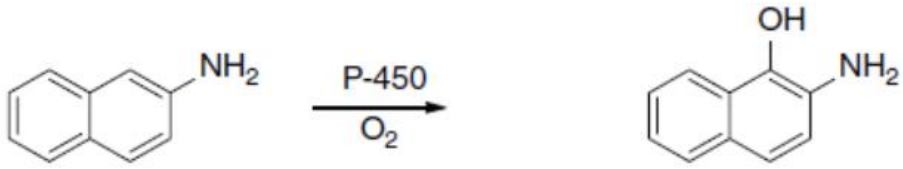
| Reaction type | Equation | Examples |
|---|---|---|
| oxidation of aliphatic chains |  | barbiturates |
| oxidative N-dealkylation |  | ephedrine |
| oxidative deamination |  | histamine<br>norepinephrine<br>mescaline |
| oxidative O-de-alkylation |  | phenacetin<br>codein<br>mescaline |
| para-hydroxylation of aromatic compounds |  | phenobarbital<br>chlorpromazine |
| oxidation of aromatic amines |  | aniline derivatives |
| S-oxidation |  | phenothiazine |




colecalciferol (vitamin $D_3$)

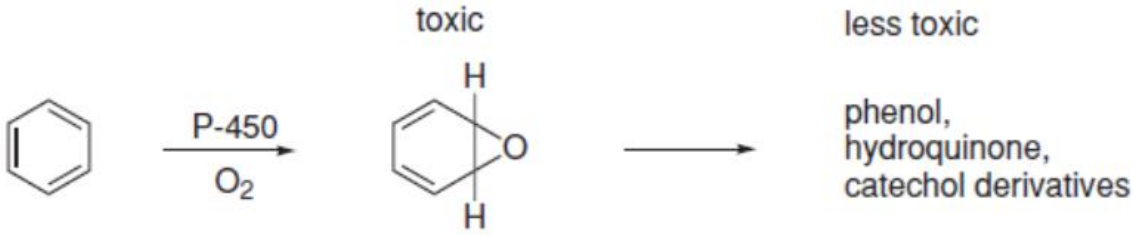
1,25-dihydroxy-colecalciferol

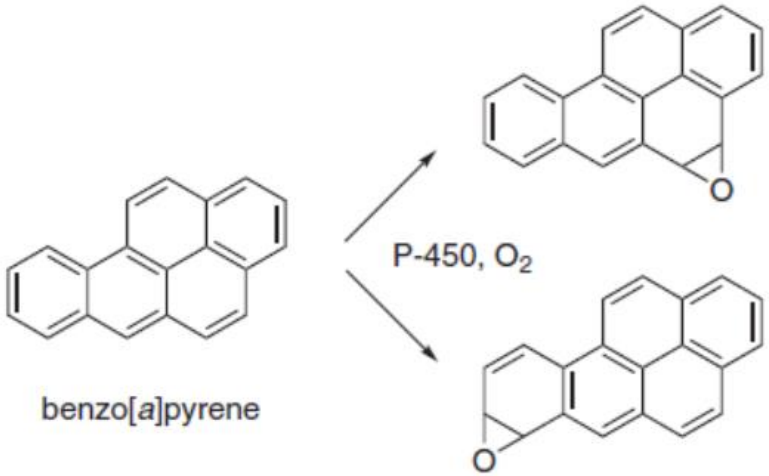
Forma attiva




β-naphthylamine

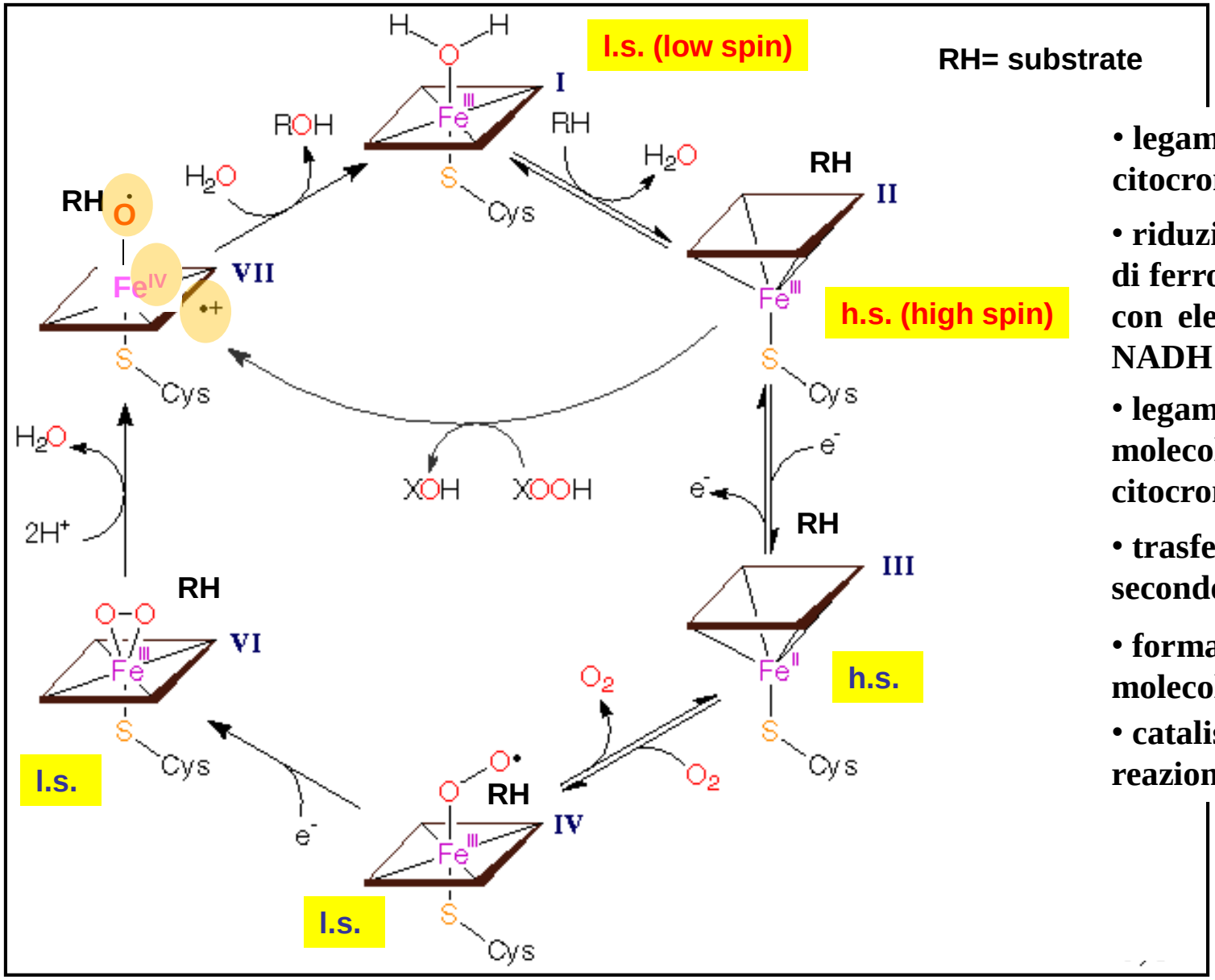
α-hydroxy-β-aminonaphthalene
(carcinogenic)



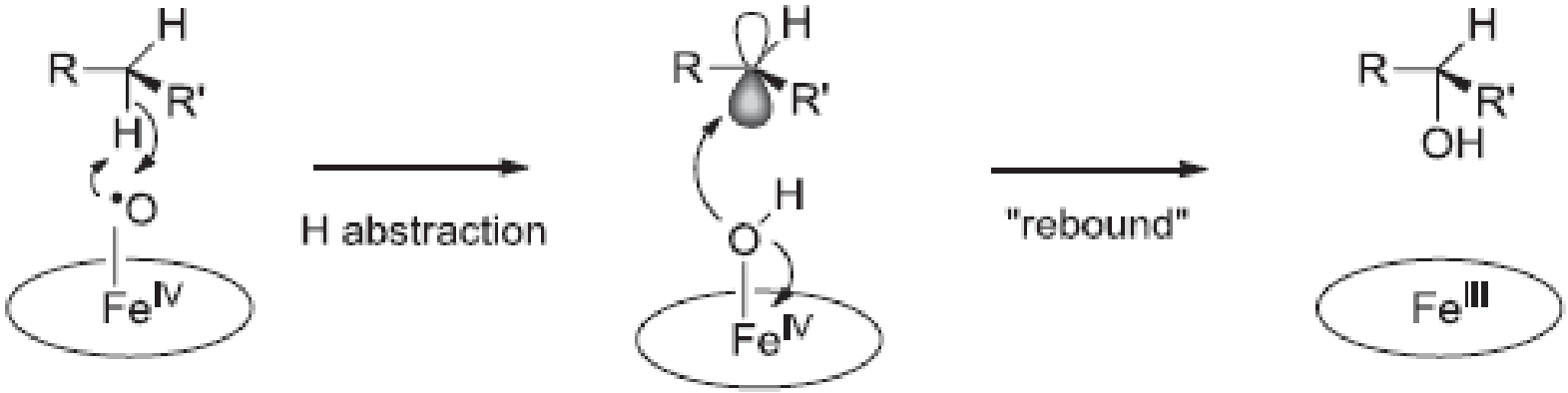




benzo[*a*]pyrene

# Cytochrome P450 reaction cycle

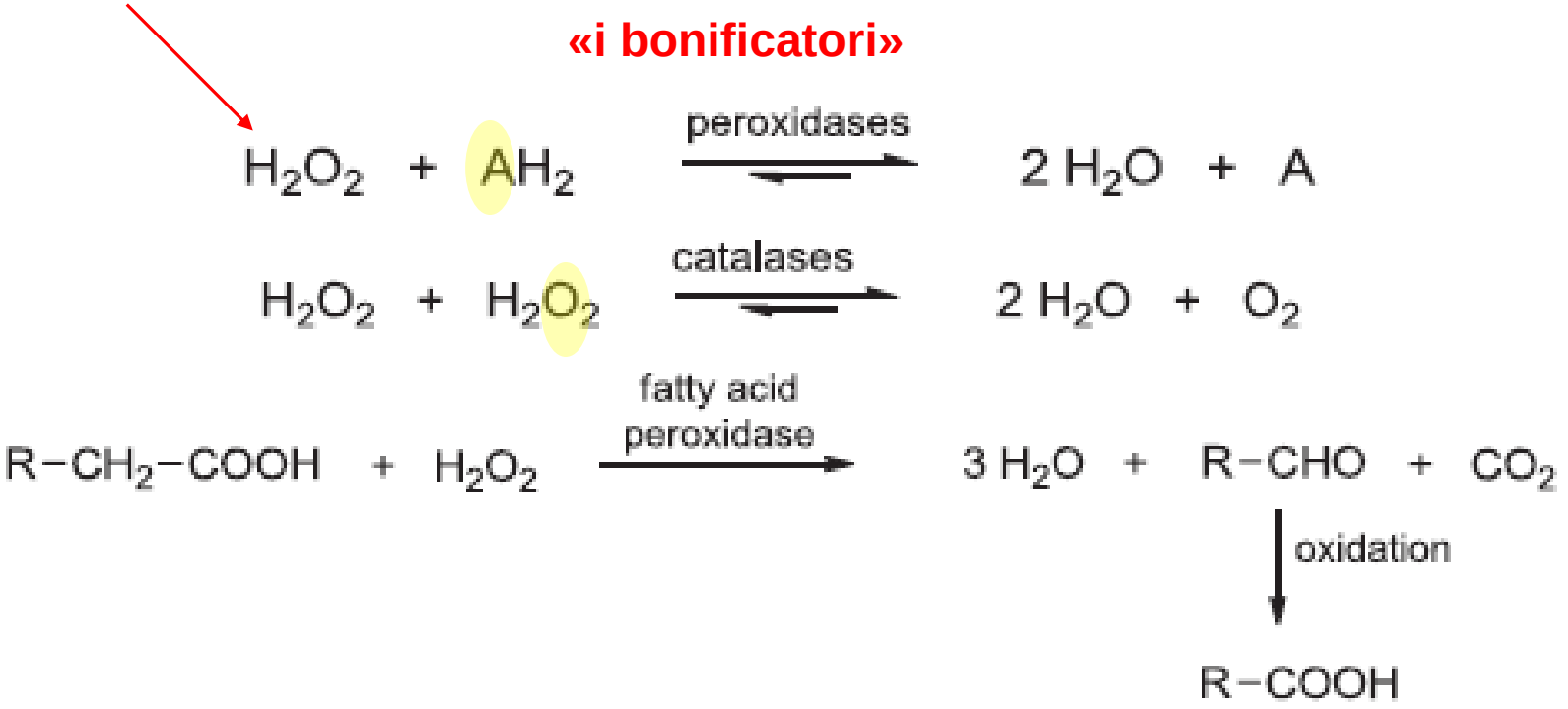



- **legame del substrato al citocromo P450**
- **riduzione dell'atomo di ferro del gruppo eme con elettroni forniti da NADH o NADP(H)**
- **legame dell'ossigeno molecolare al citocromo**
- **trasferimento di un secondo elettrone**
- **formazione di una molecola d'acqua**
- **catalisi della specifica reazione**


R
H
R'
H
O
Fe^IV
H abstraction
R
H
R'
H
O
Fe^IV
"rebound"
R
H
R'
OH
Fe^III

# Perossidasi e Catalasi

## «i bonificatori»




$$H_2O_2 + AH_2 \rightleftharpoons 2\,H_2O + A$$

$$H_2O_2 + H_2O_2 \rightleftharpoons 2\,H_2O + O_2$$

$$R{-}CH_2{-}COOH + H_2O_2 \xrightarrow{\text{fatty acid peroxidase}} 3\,H_2O + R{-}CHO + CO_2$$

$$R{-}CHO \xrightarrow{\text{oxidation}} R{-}COOH$$

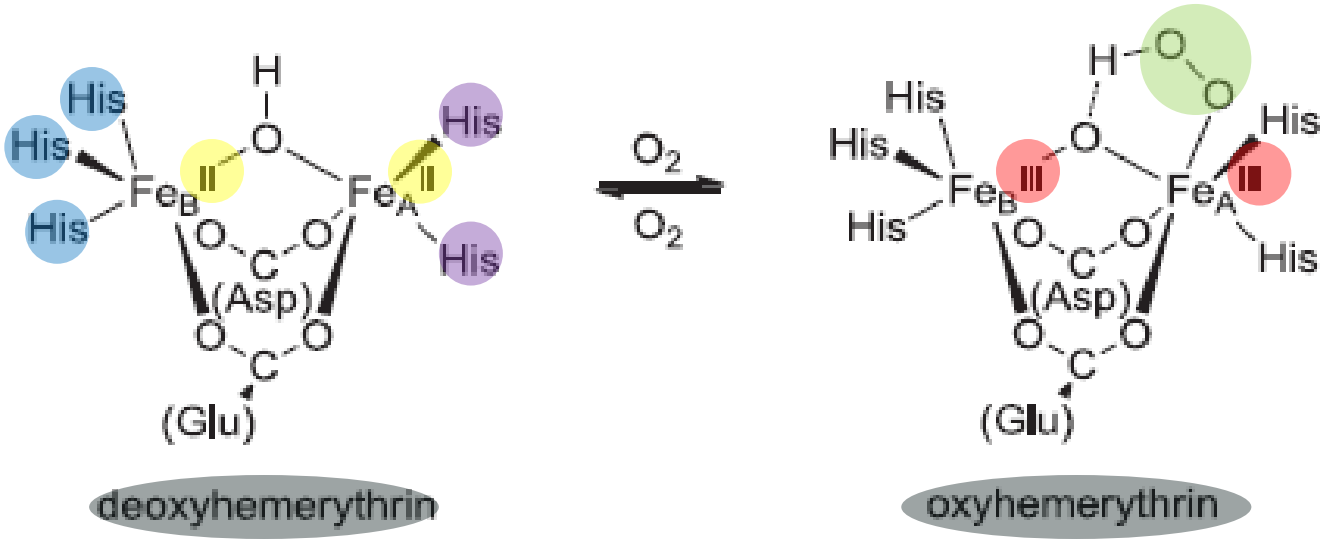
## coordination

## Fe-proteine di tipo non-eme

- **Trasferimento di ossigeno: emeritrina**
- **Proteine Fe-S**
- **Proteine di immagazzinamento e di trasporto:**
  **transferina, ferritina ed emosiderina**

# Il trasferimento di ossigeno in invertebrati (crostacei, molluschi, vermi marini):

## L’ emeritrina

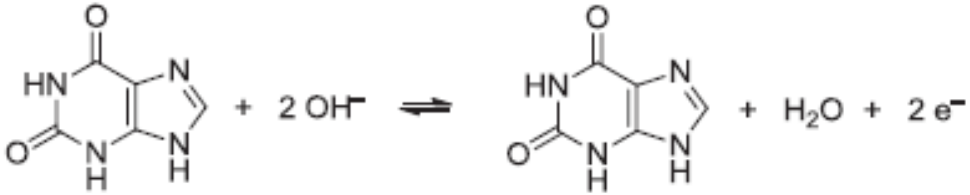


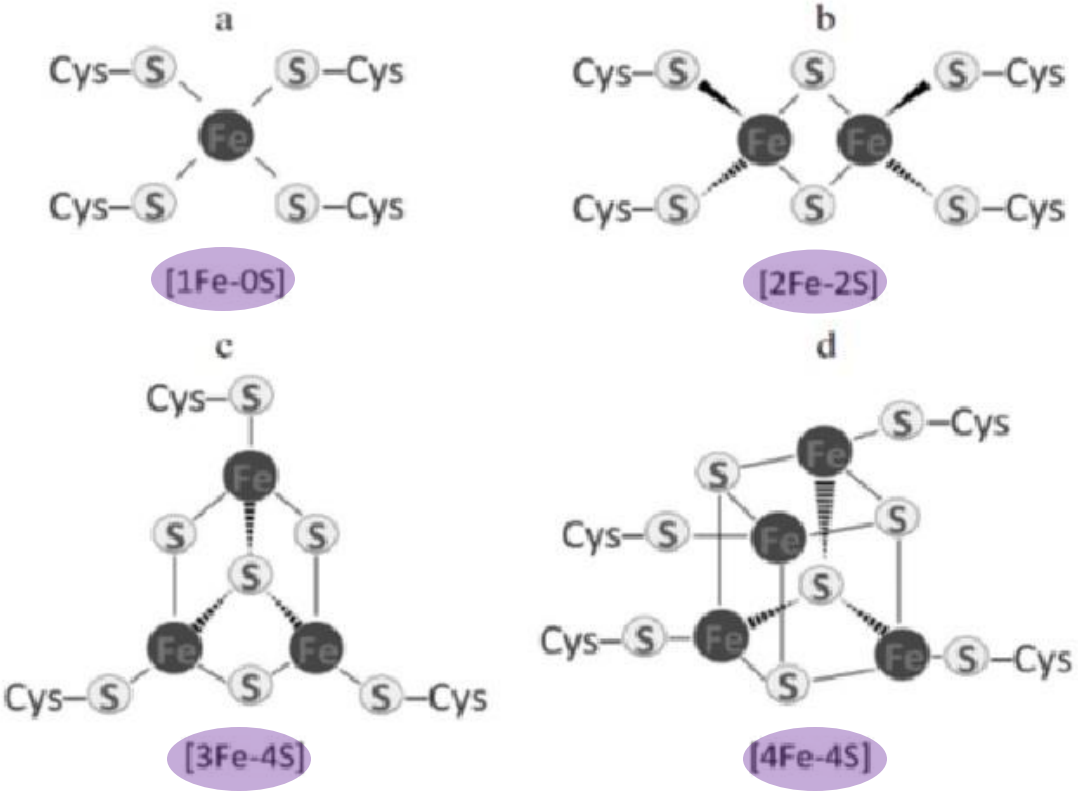

**Atomi di ferro non equivalenti**

**Ossigeno legato sotto forma perossidica**

# Proteine Fe-S

La maggior parte sono coinvolte in reazioni di trasferimento elettronico a potenziali tipicamente negativi

| Enzymes | Catalyzed reaction |
|---|---|
| hydrogenases | $2\,H^+ + 2\,e^- \rightleftharpoons H_2$ |
| nitrogenases | $N_2 + 10\,H^+ + 8\,e^- \rightleftharpoons NH_4^+ + H_2$ |
| sulfite reductase | $SO_3^{2-} + 7\,H^+ + 6\,e^- \rightleftharpoons HS^- + 3\,H_2O$ |
| aldehyde oxidase | $R\text{-}CHO + 2\,OH^- \rightleftharpoons R\text{-}COOH + H_2O + 2e^-$ |
| xanthine oxidase |  |
| NADP oxidoreductase | $NADP^+ + H^+ + 2\,e^- \rightleftharpoons NADPH$ |

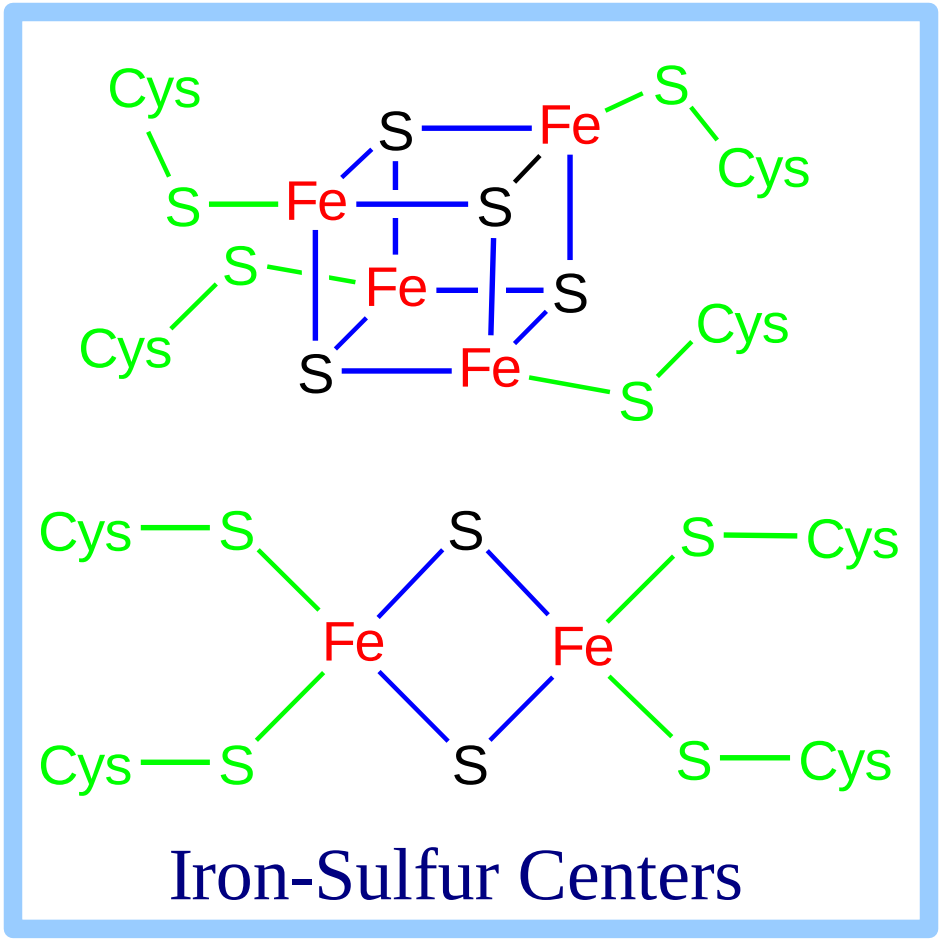



**Iron-sulfur centers** (**Fe-S**) are prosthetic groups containing **1-4 iron atoms** complexed to elemental & cysteine **S** atoms.

Electron transfer proteins may contain multiple Fe-S centers.

**4-Fe** centers have a tetrahedral structure, with **Fe** & **S** atoms alternating as vertices of a cube.

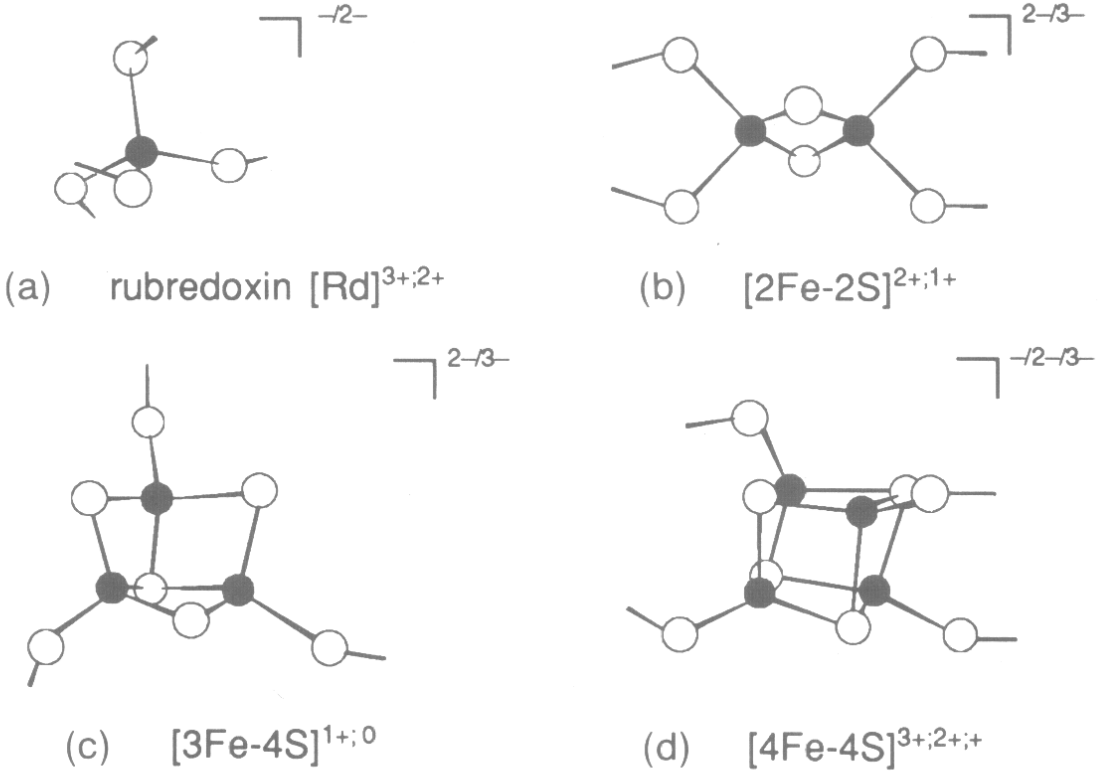
Iron-sulfur centers transfer only **one electron**, even if they contain two or more iron atoms




This is due to the close proximity of the iron atoms

# Potenziali redox di alcune Fe/S proteine

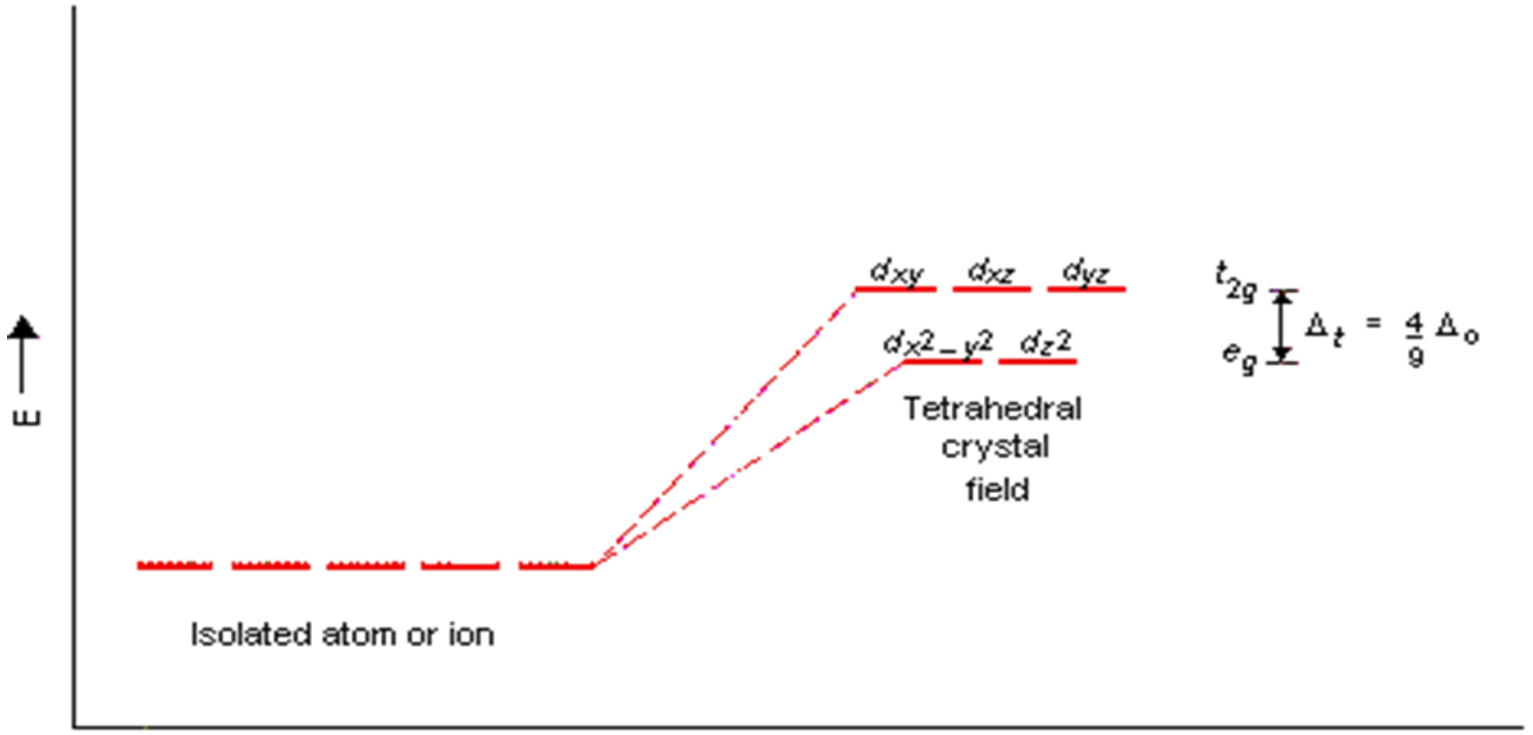
| Protein | Typical origin | Type of Fe/S center | Molecular mass (kDa) | E (mV) |
|---|---|---|---|---|
| rubredoxin | *Clostridium pasteurianum* | $[Rd]^{2+;3+}$ | 6 | −60 |
| 2Fe ferredoxin | spinach | $[2Fe\text{-}2S]^{1+;2+}$ | 10.5 | −420 |
| adrenodoxin | adrenal mitochondria | $[2Fe\text{-}2S]^{1+;2+}$ | 12 | −270 |
| Rieske center | adrenal mitochondria | $[2Fe\text{-}2S]^{1+;2+}$ | 250 ($bc_1$ complex) | +280 |
| 4Fe ferredoxin | *Bacillus stearothermophilus* | $[4Fe\text{-}4S]^{1+;2+}$ | 9.1 | −280 |
| 8Fe ferredoxin | *Cl. pasteurianum* | $2[4Fe\text{-}4S]^{1+;2+}$ | 6 | −400 |
| High-potential iron–sulfur protein (HiPIP) | *Chromatium vinosum* | $[4Fe\text{-}4S]^{2+;3+}$ | 9.5 | +350 |
| ferredoxin II | *Desulfovibrio gigas* | $[3Fe\text{-}4S]^{n+}$ | 24 | −130 |
| ferredoxin I | *Azotobacter vinelandii* | $[3Fe\text{-}4S]^{n+}$ $[4Fe\text{-}4S]^{n+}$ | 14 | −460 |

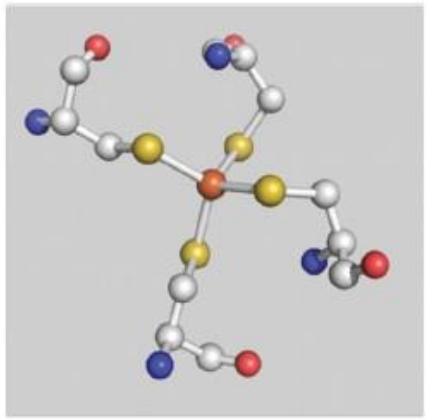



- Four S atoms in a tetrahedral setup
- S steric hindrance «forbids» the usual hexacoordination

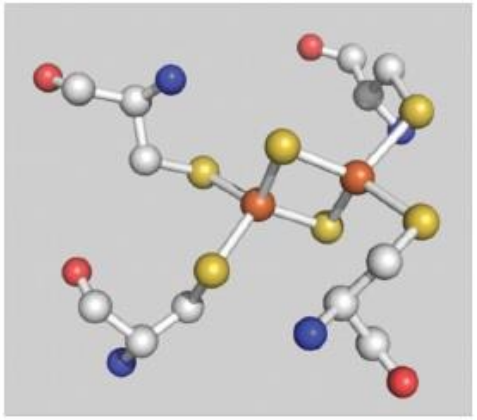
- High spin complexes only!

# Tetrahedral orbital splitting

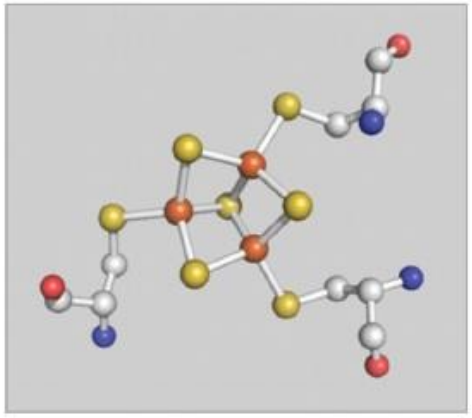




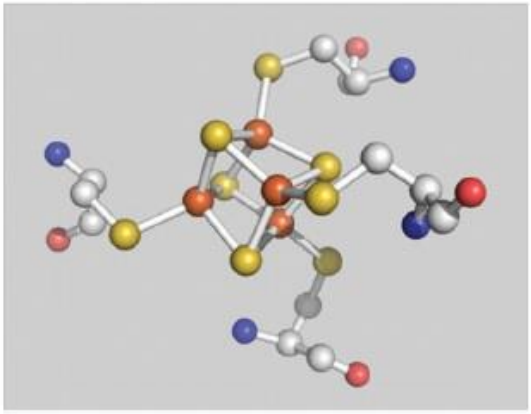
(a) $[Rd]^{3+;2+}$



(b) $[2Fe\text{-}2S]^{2+;+}$



(c) $[3Fe\text{-}4S]^{+;0}$

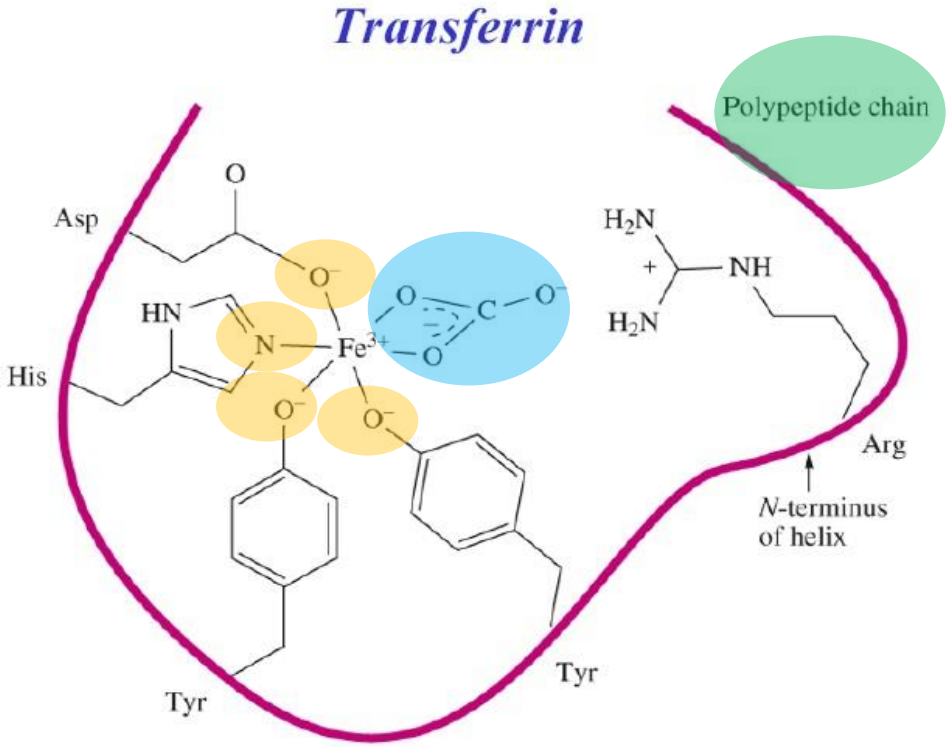


(d) $[4Fe\text{-}4S]^{3+;2+;+}$

## *Serum Transferrin*

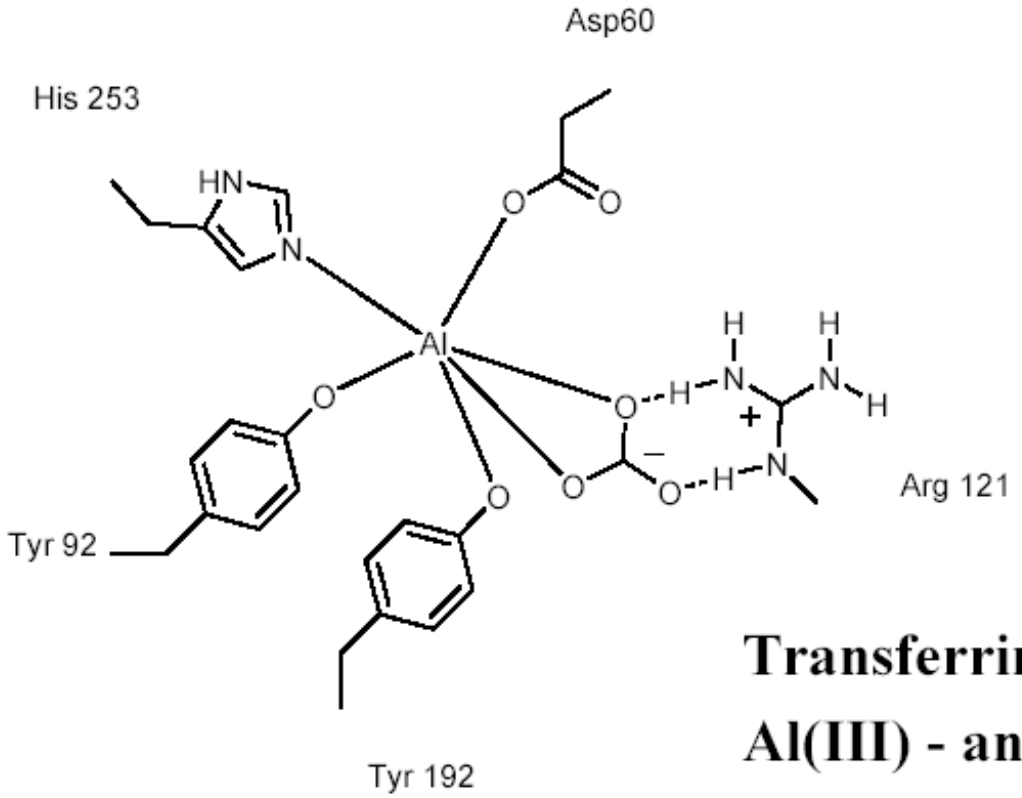
- ***Transferrin*** **transports ~ 40 mg of iron/day to the bone marrow. Structure is a single polypeptide coiled in such a way as to contain two pockets suitable for binding Fe(III).**

1. **Each pocket presents N- and O donors to the metal centre but the presence of a carbonate $CO_3^{2-}$ or bicarbonate $HCO_3^-$ ion is also essential.**
2. **The exact mechanism for Fe uptake and release is unknown.**
3. **The actual X-ray structure of human *lactoferrin* has been elucidated by X-ray crystallography.**




**A Fe(III) binding site in *transferrin***

# *Transferrin*




**Transferrin can also bind Al(III) - and allow it to cross the blood-brain barrier**

**This is believed to be one of the causes of Alzheimers' disease**

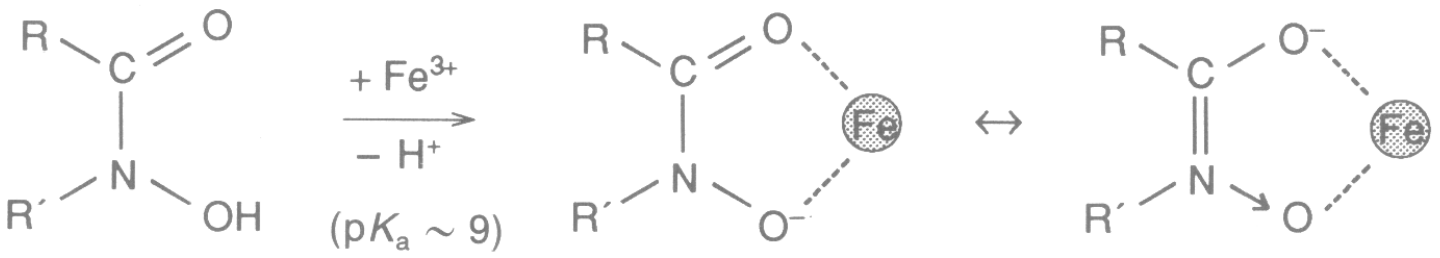
# *Siderophores*

- **Aerobic micro-organisms cannot absorb iron from their aqueous environment since for $Fe(OH)_3$ $K_{sp} \sim 2.65 \times 10^{-39}$. They use polydentate ligands called *siderophores* to scavenge iron**

1. **Each ligand supplies six O-donors for chelation in a potentially octahedral geometry.**
2. **Examples are *enterobactin*, *desferrichrome* and *desferrioxamine*.**
3. **As the complexes of Fe(III) have very high stability. It is likely that an Fe(II) intermediate is involved in release as stability constant $10^2$-$10^3$ lower.**

The majority of effective siderophores can be divided into two groups: hydroxamates and catecholates.

(a) hydroxamate coordination

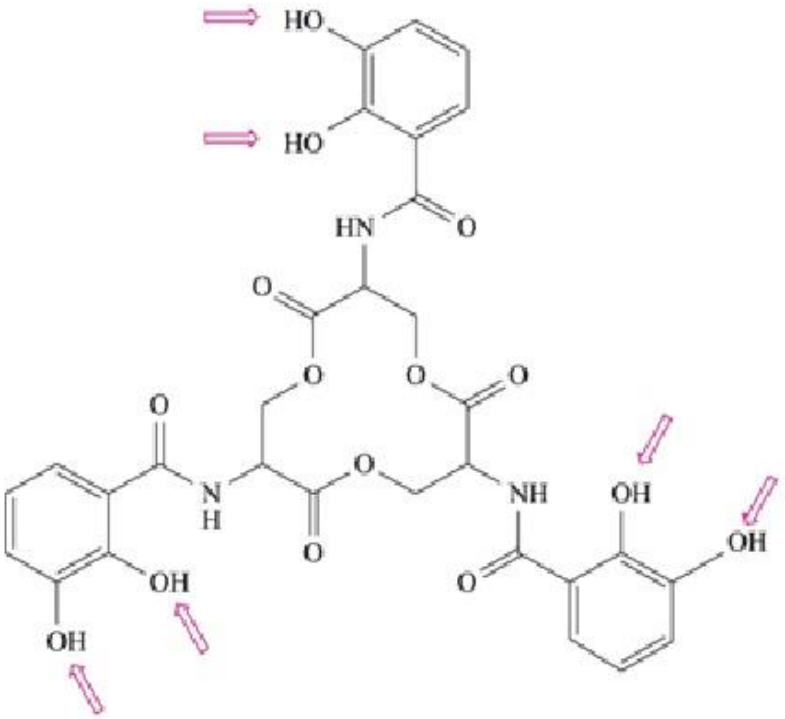



(b) catecholate complex


(8.6)

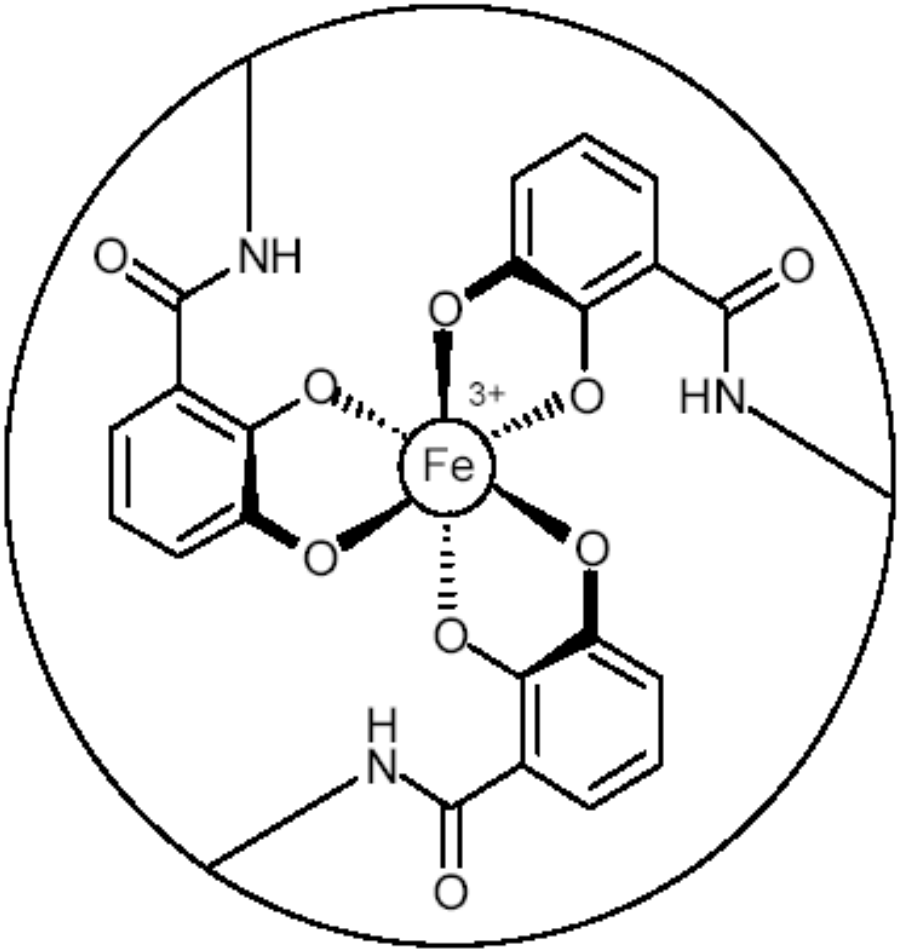
# *Enterobactin – A Bio-Ligand for Fe(III)*

Catecholate

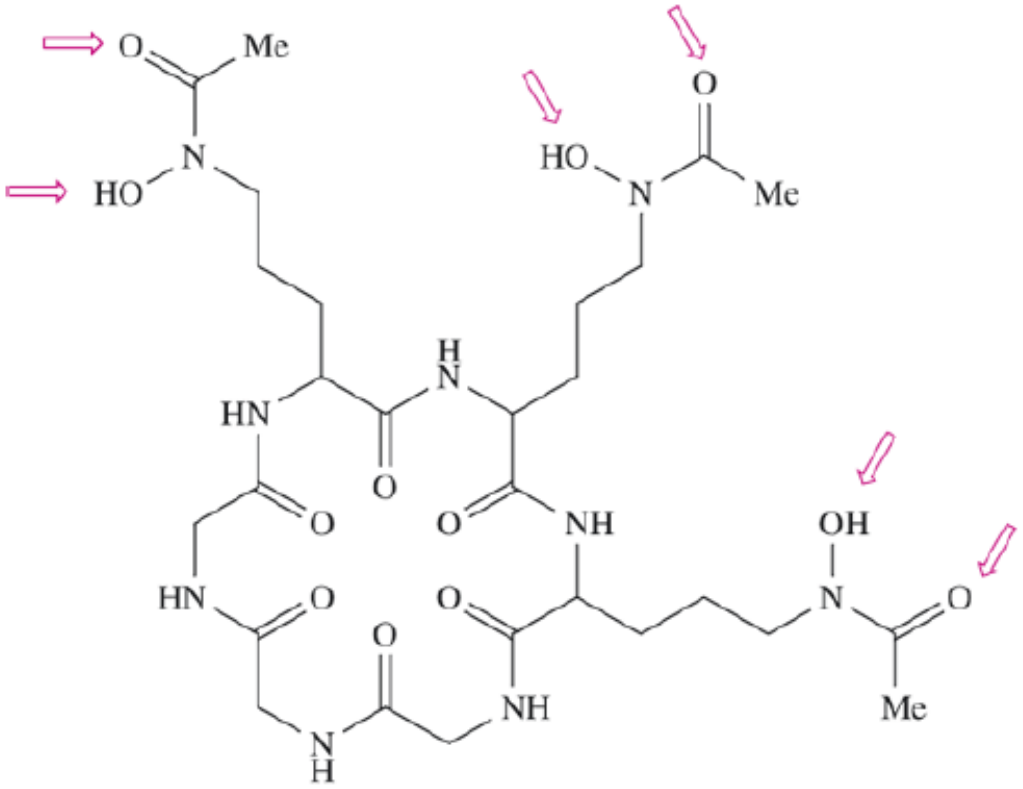



- **Enterobactin, contains 3 catechol groups and can deprotonate to bind Fe(III) as an ~octahedral complex $[FeL]^{3-}$.**

# *Siderophores - Enterobactin*



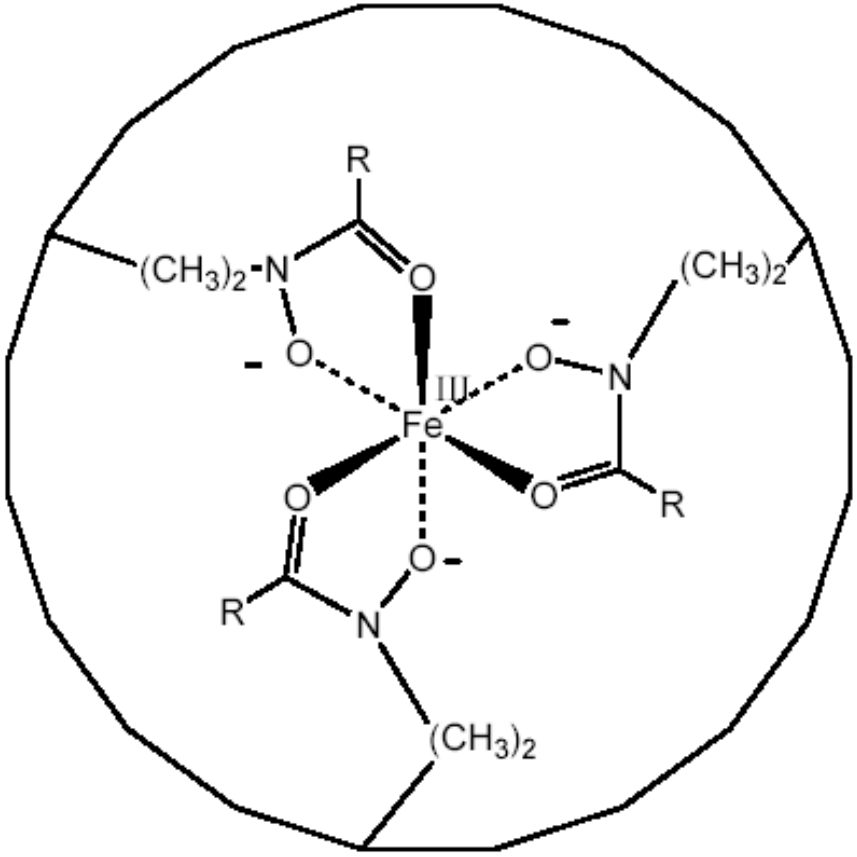
# *Siderophores - Ferrichromes*




Hydroxamate

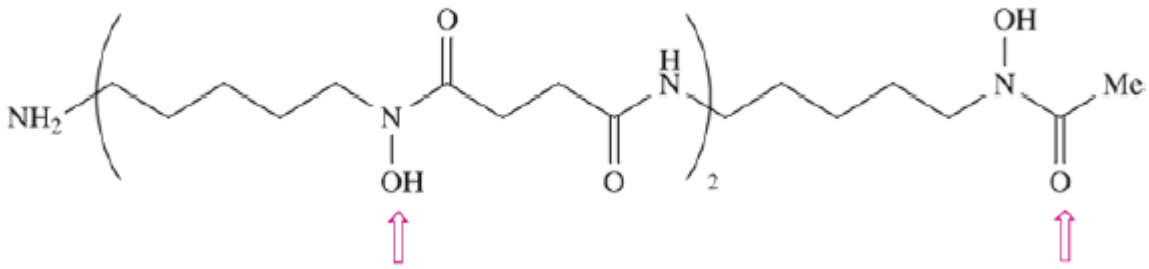
**Cyclic peptide siderophores produced by Fungi**

# *Siderophores - Ferrichromes*



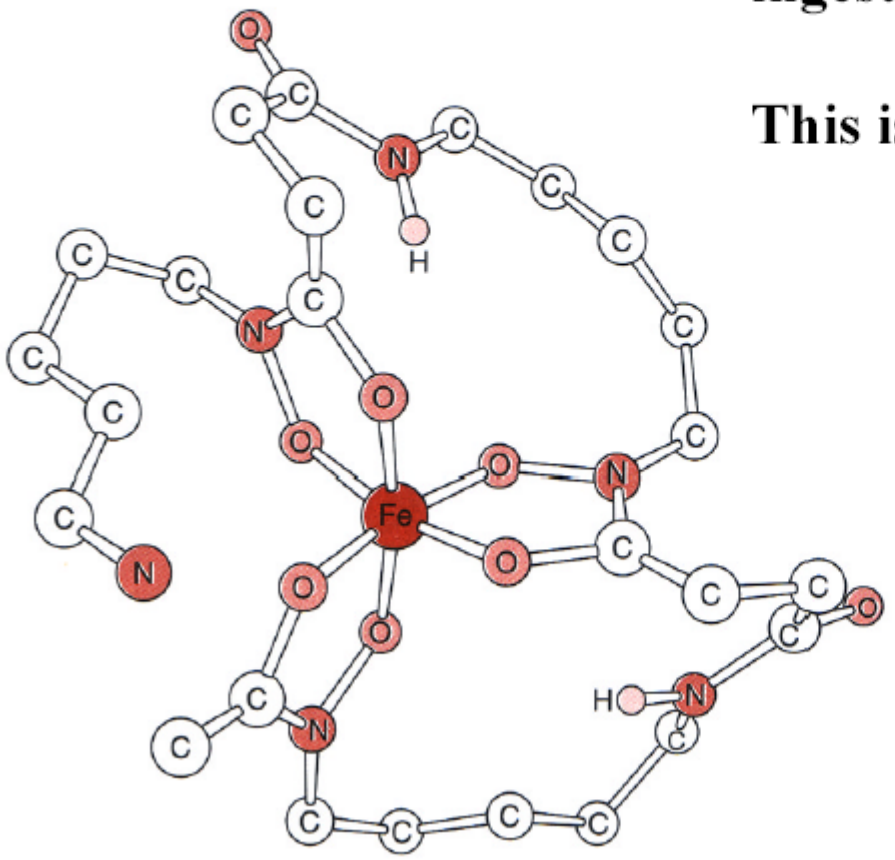
# *Other Siderophores*

**Desferrioxamine**



**Desferal**

# *Desferrioxamine*




**Drug given to patients who have too much iron from accidental ingestion.**

**This is called *chelation therapy***