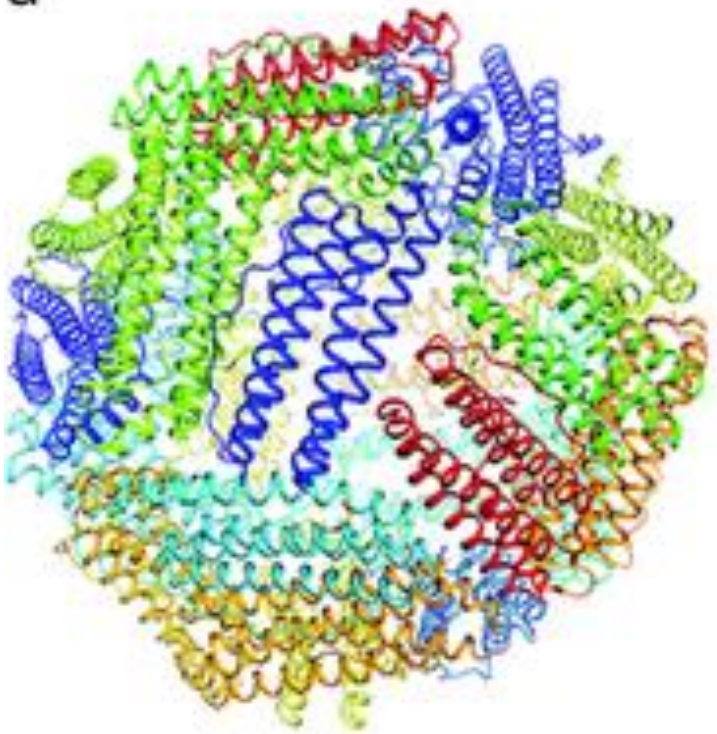
Ferritin : A brief overview

a



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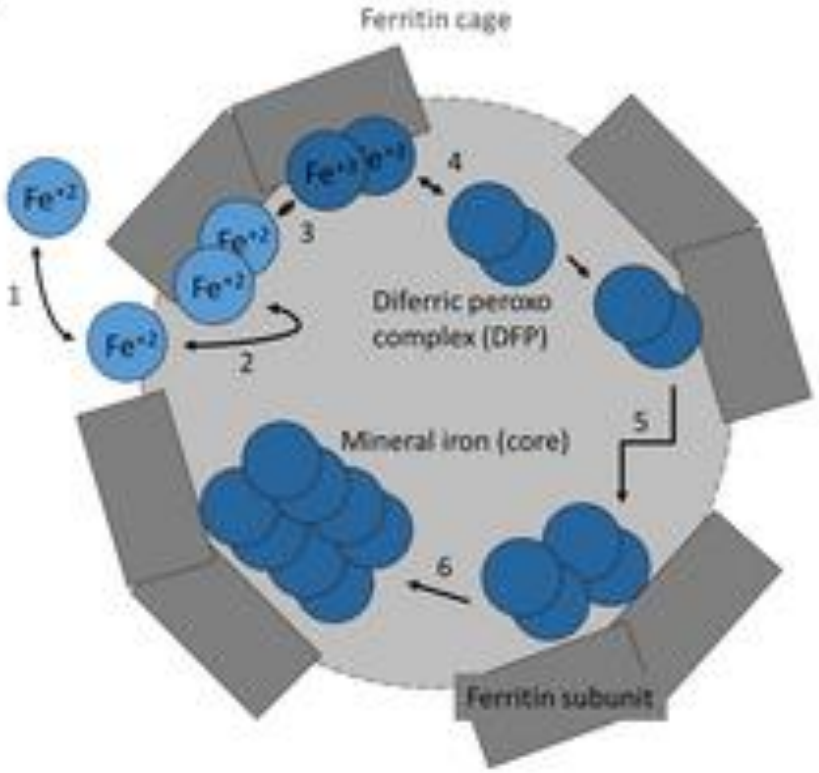
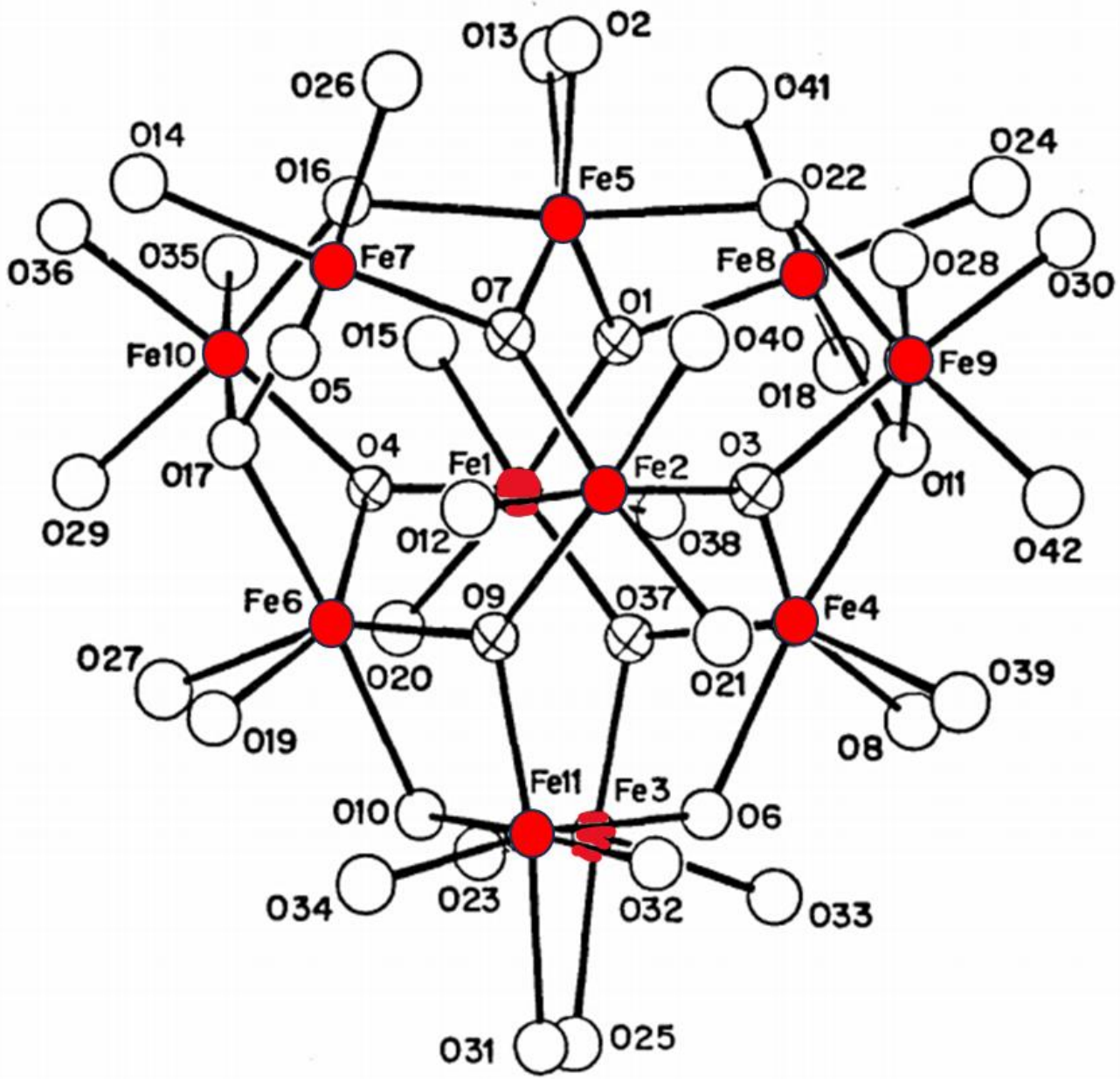


Step	Model Reaction
1. Fe^{+2} transport into ferritin	
2. Fe^{+2} binding to ferroxidase	LIP • — • DFP (Ferritin)
3,4. Fe^{+2} oxidation	
5. Nucleation	DFP • — • core (Ferritin)
6. Mineralization	DFP • — • core (Ferritin, core)

Masison J, Mendes P (2023) Modeling the iron storage protein ferritin reveals how residual ferrihydrite iron determines initial ferritin iron sequestration kinetics. PLOS ONE 18(2): e0281401. https://doi.org/10.1371/journal.pone.0281401
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0281401

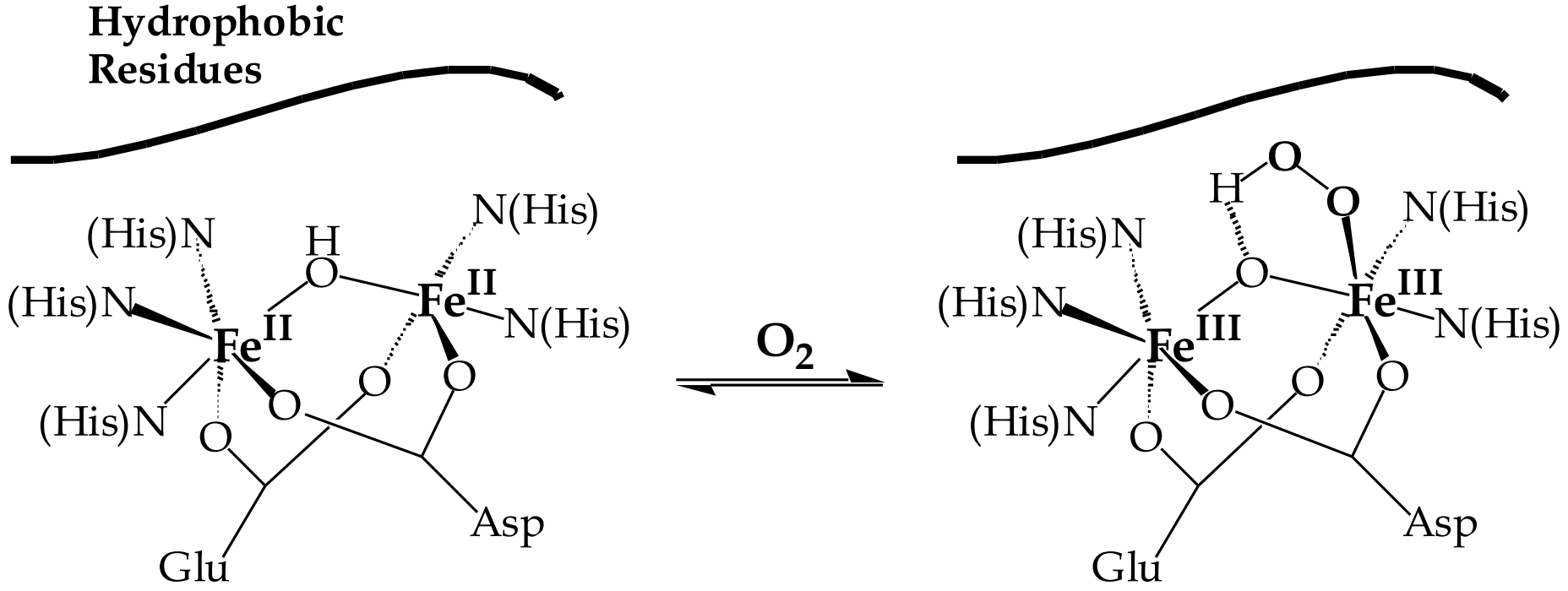
PLOS ONE

FERRITIN



Models for the full iron core of ferritin include ferrihydrite, which matches the ordered regions of ferritin cores that have little phosphate; however, the site vacancies in the lattice structure of ferrihydrite [FeO(OH)] appear to be more regular than in crystalline regions of ferritin cores.

Chemistry at the Active Site of Hemerythrin (Hr)



DeoxyHr
Diferrous

OxyHr
Diferric

Note proton-coupled electron transfer
Evidence for proton transfer comes from resonance Raman work

Hemerythrins - Diiron Dioxygen Carriers

Properties:

Mono- (myo Hr) and multi- (Hr) subunit proteins.
Found in marine invertebrates.

Easily isolated protein; crystallizes after one step!!

Deoxy Hr, colorless, diiron(II)
Oxy Hr, red, diiron(III) peroxo

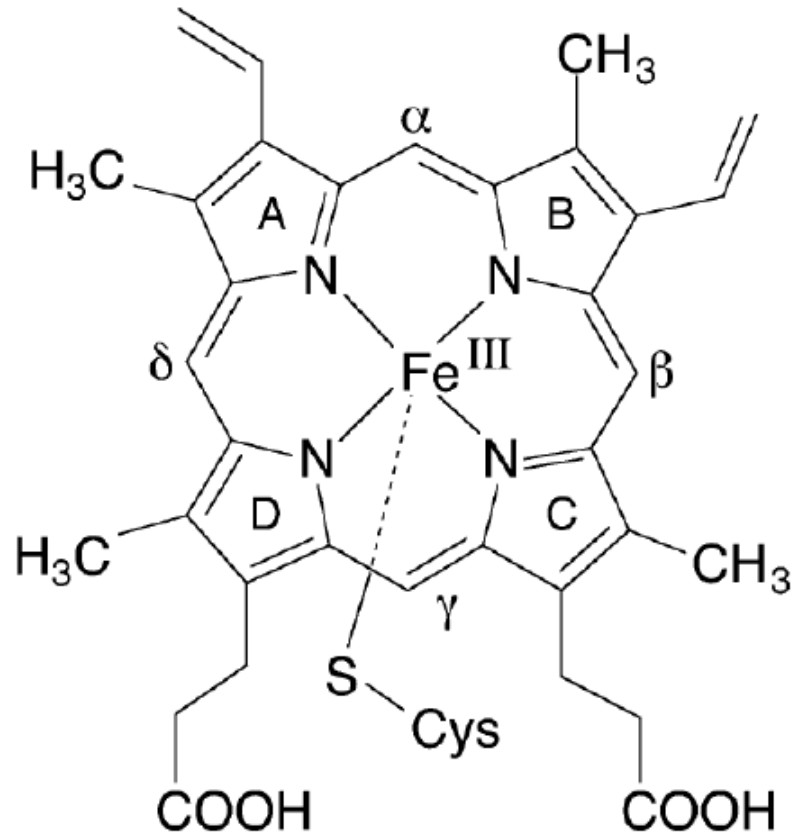
ν_{O-O}, 844 cm^{-1} in the terminally bound peroxide region.

$\nu_{Fe-O-Fe}$, 486 cm^{-1}, resonance enhanced symmetric stretch. The asymmetric stretch occurs at 757 cm^{-1}.

Mixed-valent, semimet Hr, Fe(II)Fe(III): inactive.

Cytochrome P450

- The cytochromes P450 constitute a large family of cysteinato-heme enzymes, are present in all forms of life (plants, bacteria, and mammals), and play a key role in the oxidative transformation of endogeneous and exogeneous molecules
- The total number of members of the cytochrome P450 family is increasing rapidly (over 2000 members.
- In all these cysteinato-heme enzymes, the prosthetic group is constituted of an iron(III) protoporphyrin- IX covalently linked to the protein by the sulfur of a proximal cysteine ligand



Function

These enzymes are potent oxidants that are able

to catalyze the:

(i) hydroxylation of saturated carbon- hydrogen bonds

(ii) epoxidation of double bonds

(iii) oxidation of heteroatoms

(iv) dealkylation reactions

(v) oxidations of aromatics, and so on.

Cytochrome P-450

Enzymes in the cytochrome P-450 family are remarkably versatile 0_2-activating catalysts that can incorporate one of the two oxygen atoms of 0_2 into a broad variety of substrates with concomitant reduction of the other oxygen atom by two electrons to water. In addition to the conversion of unactivated alkanes to alcohols (Eq. 1),

$$\mathbf{R\text{-}H + 0_2 + 2H^+ + 2e^- \rightarrow R\text{-}OH + H_20 \ (1)}$$

P450 enzymes transfer oxygen atoms to a wide range of substrates, transforming alkenes to epoxides, arenes to phenols, and sulfides to sulfoxides and then to sulfones. P450 enzymes also oxidatively cleave C-N and C-O bonds in the metabolism of amines and ethers, respectively, and C-C bonds in the biosynthesis of steroid hormones. Under anaerobic conditions, P-450 will reductively dehalogenate halocarbons to the corresponding alkanes.

P-450 enzymes are generally membrane bound and have been found in plants, animals, yeasts, and bacteria. The best characterized P-450 enzyme is the soluble camphor-metabolizing P450-CAM isolated from *Pseudomna putida*.

The Cytochrome P-450 Reaction Cycle

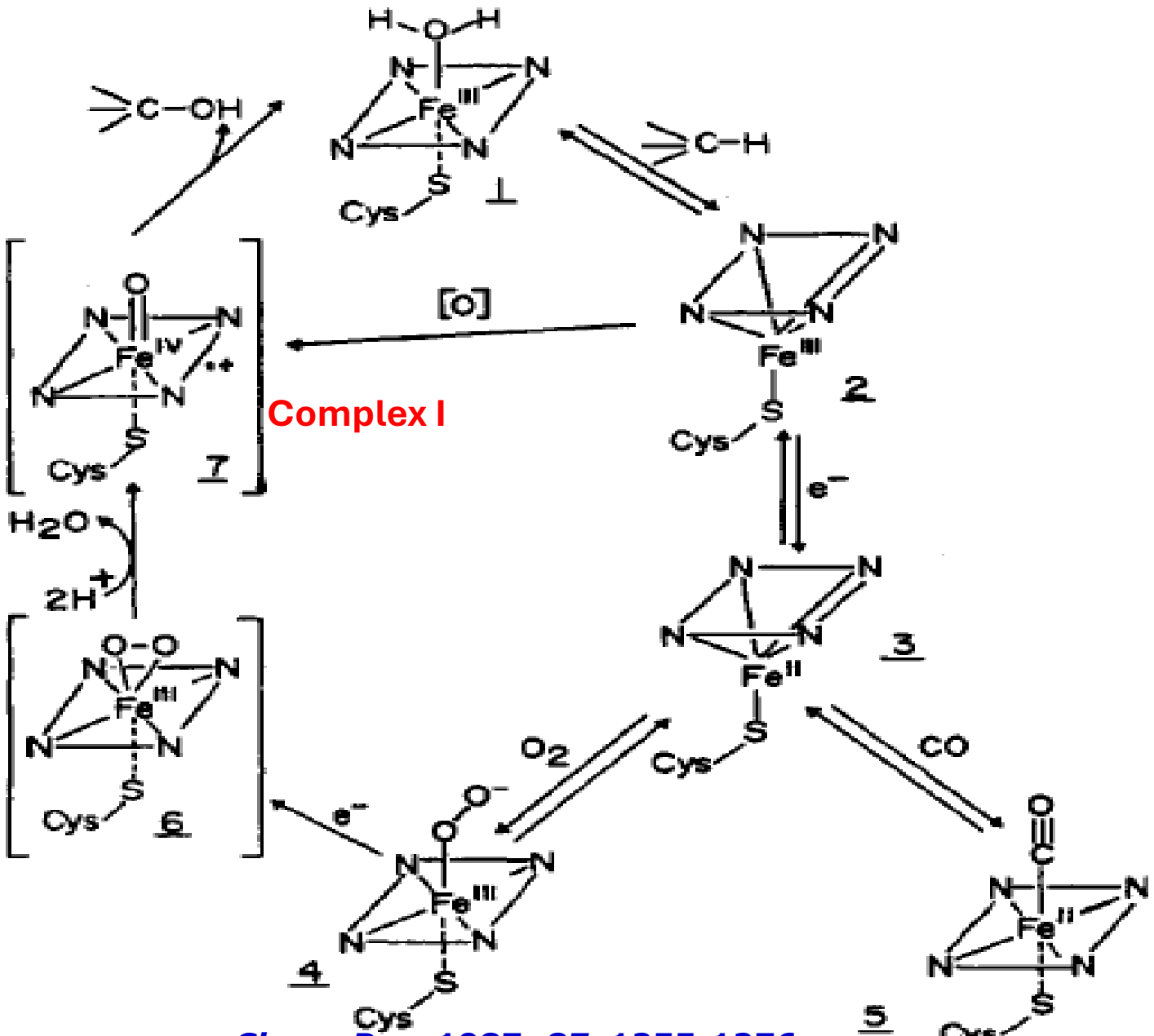
By now we know that -- When an axial site is available on the iron porphyrin, dioxygen can bind and/or be activated there.

In Cyt P-450 with proton-mediated reductive activation of the O_2 molecule, a peroxo intermediate forms that converts to an Fe^{IV}=O species, the ferryl ion.

The ferryl can oxidize hydrocarbons to alcohols, epoxidize olefins, oxidize amines to amine oxides and do related chemistry.

P-450's are mainly based in liver in the endoplasmic reticulum and are enzymes necessary for metabolism and used to convert pro-drugs and pro-carcinogens to their active forms.

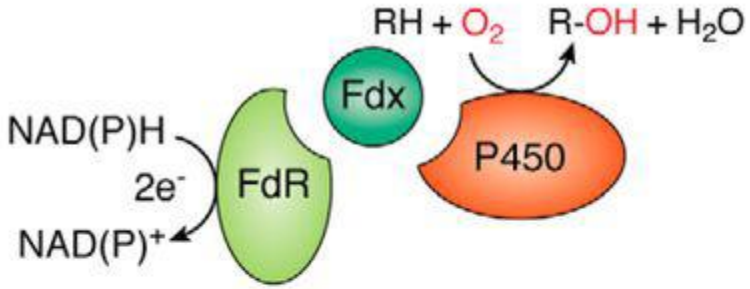
Catalytic Cycle of Cytochrome P450



Chem. Rev. 1987, *87*, 1255-1276

- The binding of carbon monoxide (CO) to cytochrome P450 is reversible.
- The binding is studied extensively using techniques like flash photolysis, which reveals the rapid binding and unbinding of CO.
- This reversible binding is a key feature in the characteristic spectroscopic signature that gives these enzymes their name, as the ferrous (Fe^{2+}) enzyme-CO complex shows a distinct peak at 450 nanometers.
- The name P450 may also arise from the position of the absorption band of the oxoform of the enzyme, *i.e.* oxo form shows absorption band at 450 nm in the UV-Vis spectrum.

Cytochrome P_{450}s have various redox partner proteins depending on the sub type



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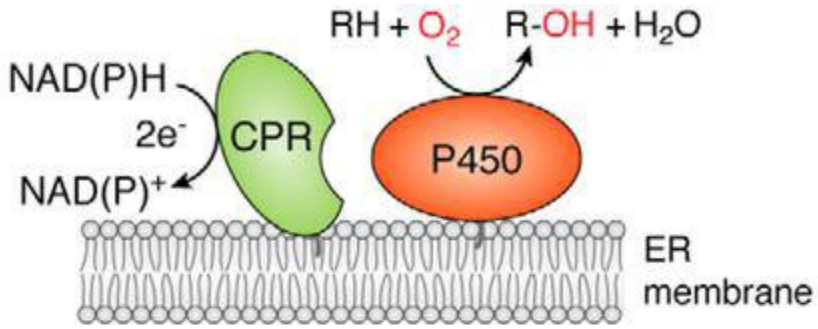

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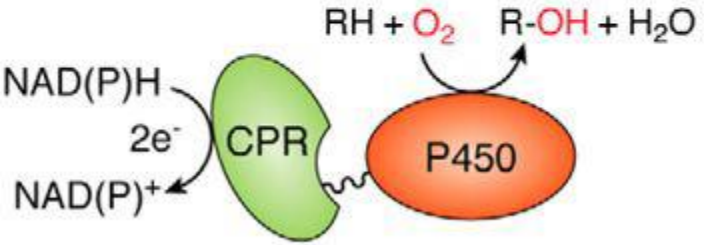

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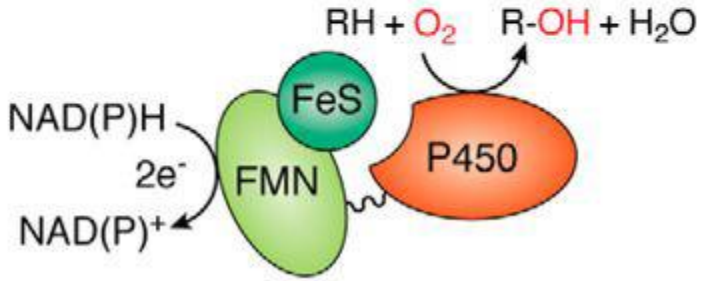

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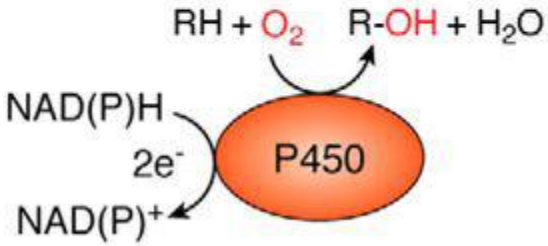
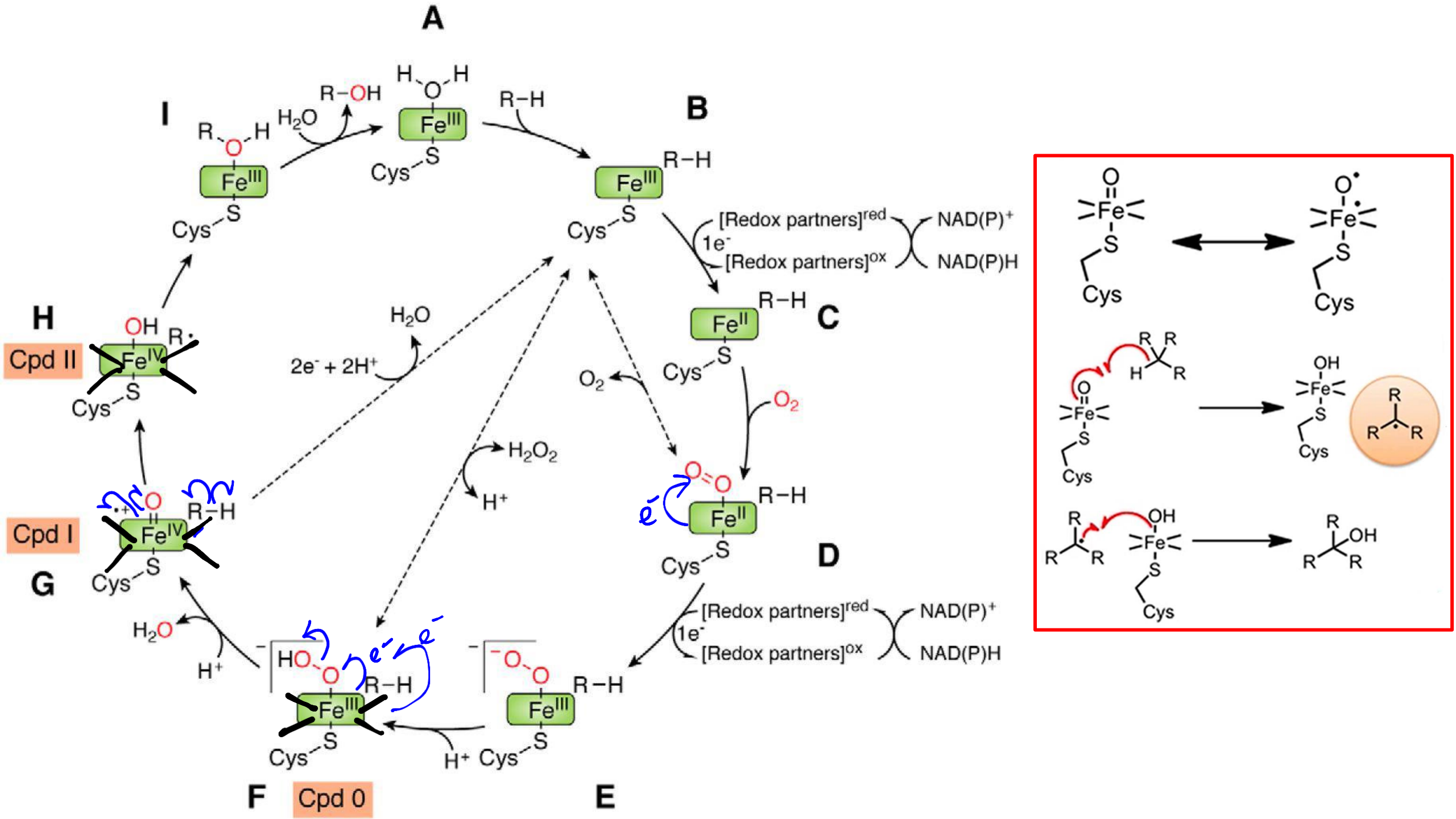


Class V


A
B
C
D
E
F
G
H
I
Cpd I
Cpd II
Cpd 0
R–H
R–OH
H2O
2e- + 2H+
H2O2
O2
[Redox partners]red
[Redox partners]ox
1e-
NAD(P)+
NAD(P)H
Fe^III
Fe^II
Fe^IV
Cys
S

The radical reaction mechanism Activation and hydrogen abstraction:

The process begins with an activated iron species (often called "Compound I") that abstracts a hydrogen atom from the substrate. This generates an organic radical and an iron-bound hydroxyl radical.

Radical recombination:

The organic radical and the iron-bound hydroxyl radical quickly "rebound" and recombine.
Product formation: This recombination results in the insertion of the oxygen atom into the carbon-hydrogen bond, forming an alcohol and returning the enzyme to its resting state.

Evidence for the radical mechanism

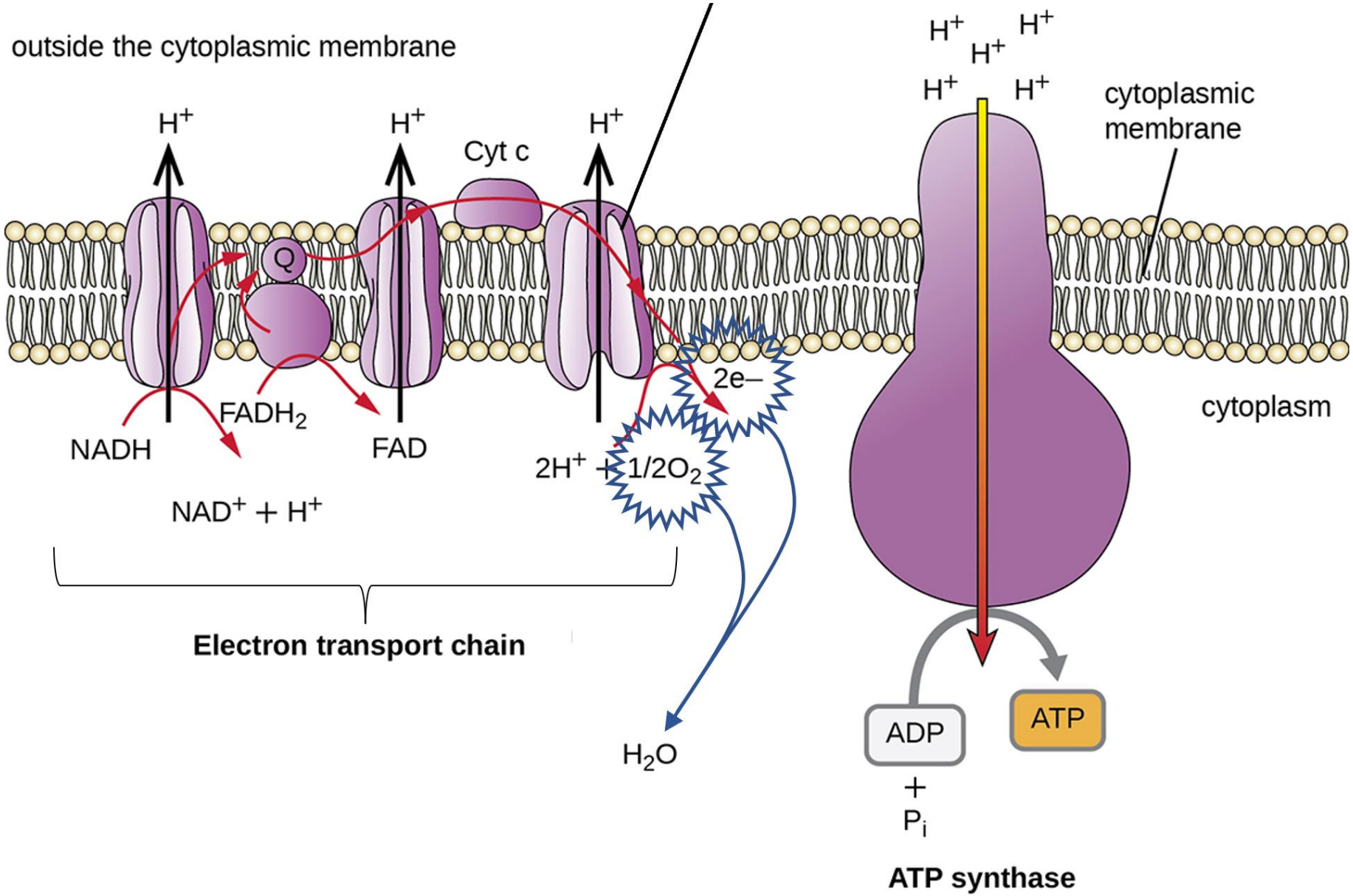
Kinetic isotope effects:

Large kinetic isotope effects k_H/k_D for hydrogen abstraction provide strong evidence for the involvement of a hydrogen atom abstraction step, a key radical process.

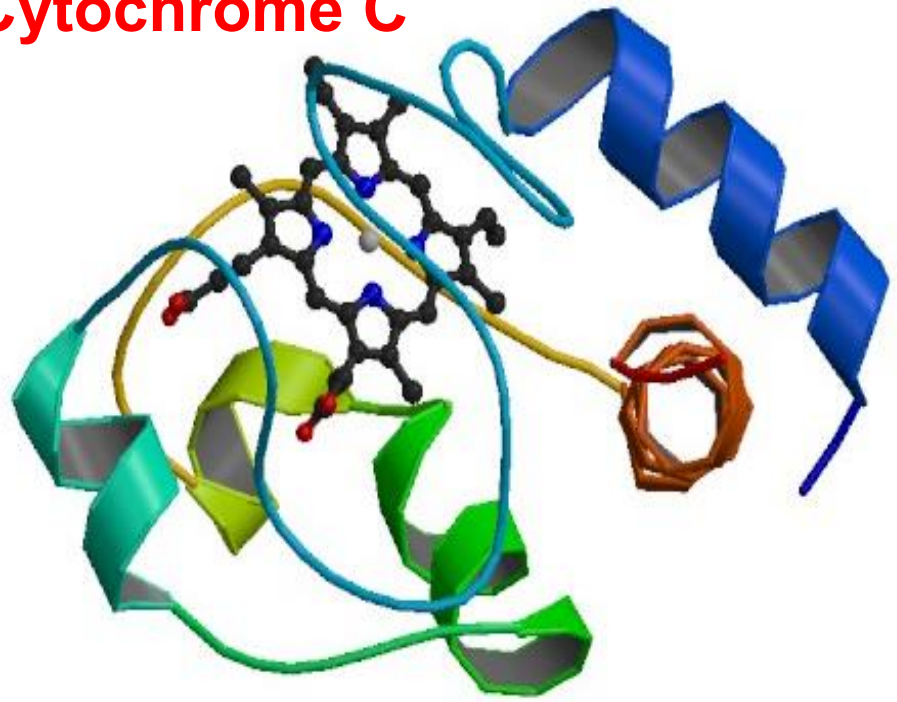
Product stereochemistry:

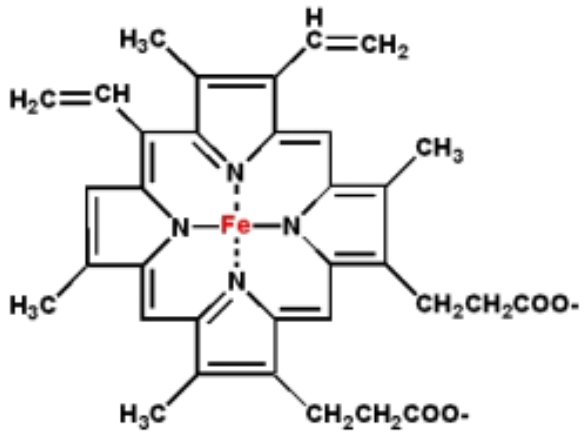
Some reactions show inversion of stereochemistry at the carbon undergoing hydroxylation, which is consistent with a radical intermediate.

Respiration: role of cytochrome C and Cytochrome C oxidase

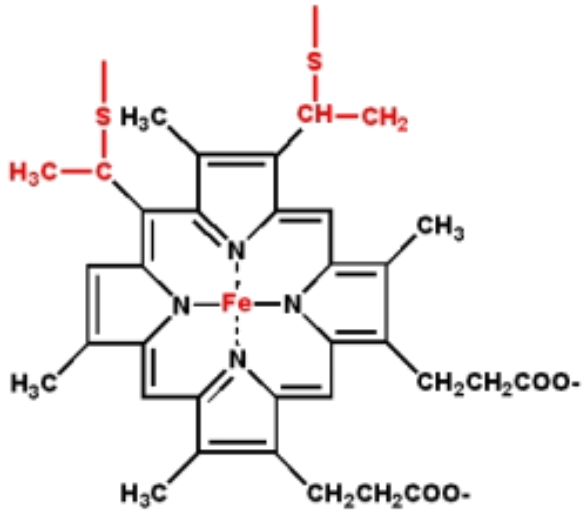


Cytochrome C

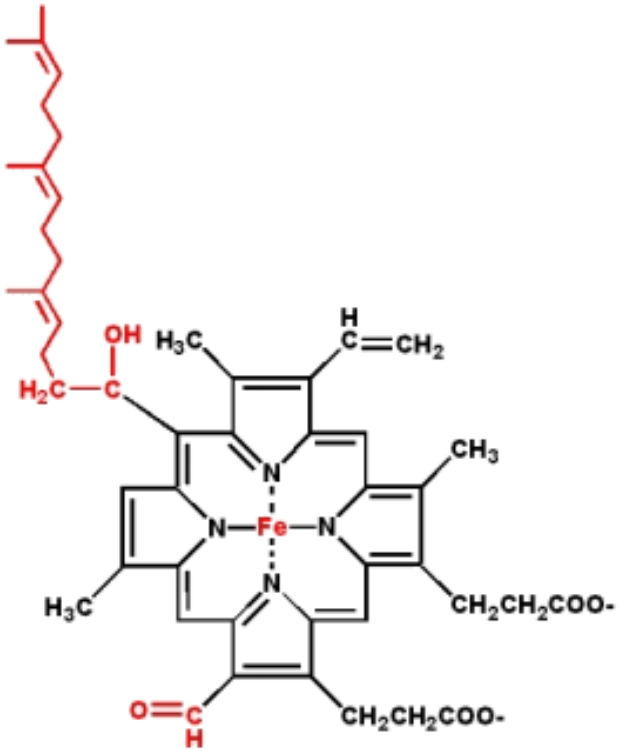




Iron protoporphyrin IX
(cytochrome b, myoglobin, hemoglobin)



Heme C
(cytochrome c)



Heme A
(cytochrome A)

Cytochrome c oxidase

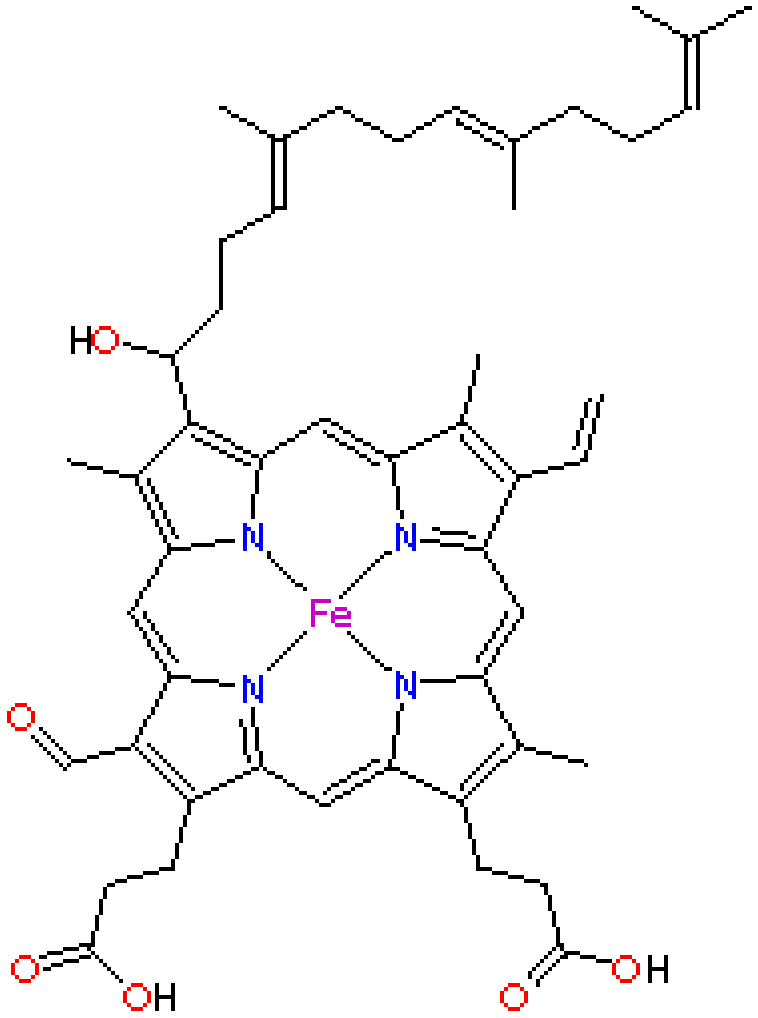
- A large transmembrane protein complex found in bacteria and the mitochondrion.
- It is the last protein in the electron transport chain. It receives an electron from each of four cytochrome *c* molecules, and transfers them to one oxygen molecule, **converting molecular oxygen to two molecules of water.**
- In the process, it translocates four protons, helping to establish a chemiosmotic potential that the ATP synthase then uses to synthesize Adenosine triphosphate (ATP).

Summary reaction:

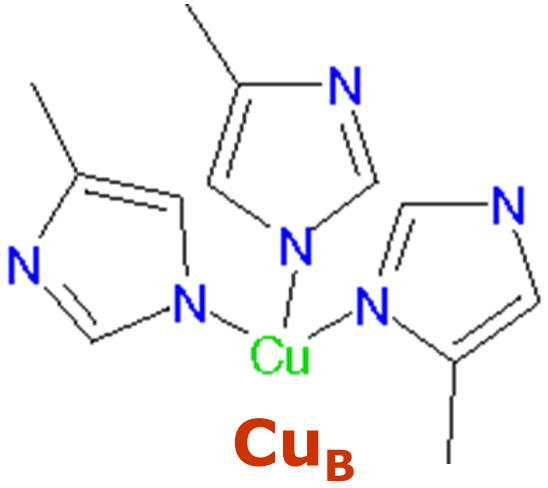
- $4\ Fe^{2+}$-cytochrome *c* + $2\ H^{+}_{in} + O_2 \rightarrow 4\ Fe^{3+}$-cytochrome *c* + $2H_2O + 4\ H^{+}_{out}$

- **The complex contains two hemes, the "a" and "a_3" hemes, and two copper centers, the Cu_A and Cu_B centers.**
- **In fact, the heme "a_3" and Cu_B are a binuclear center that is the site of oxygen reduction.** The mechanism of action of this large complex is still an active research topic.

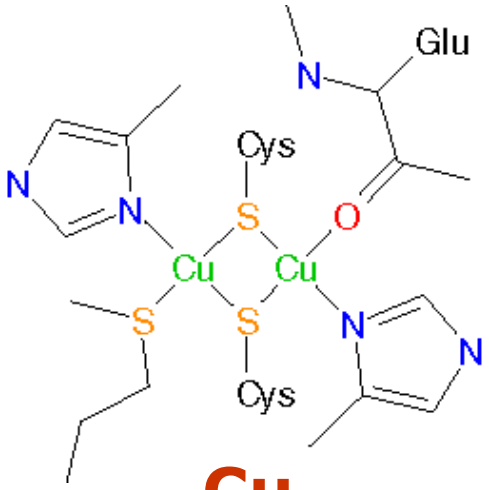
Cytochrome c Oxidase



Heme *a*

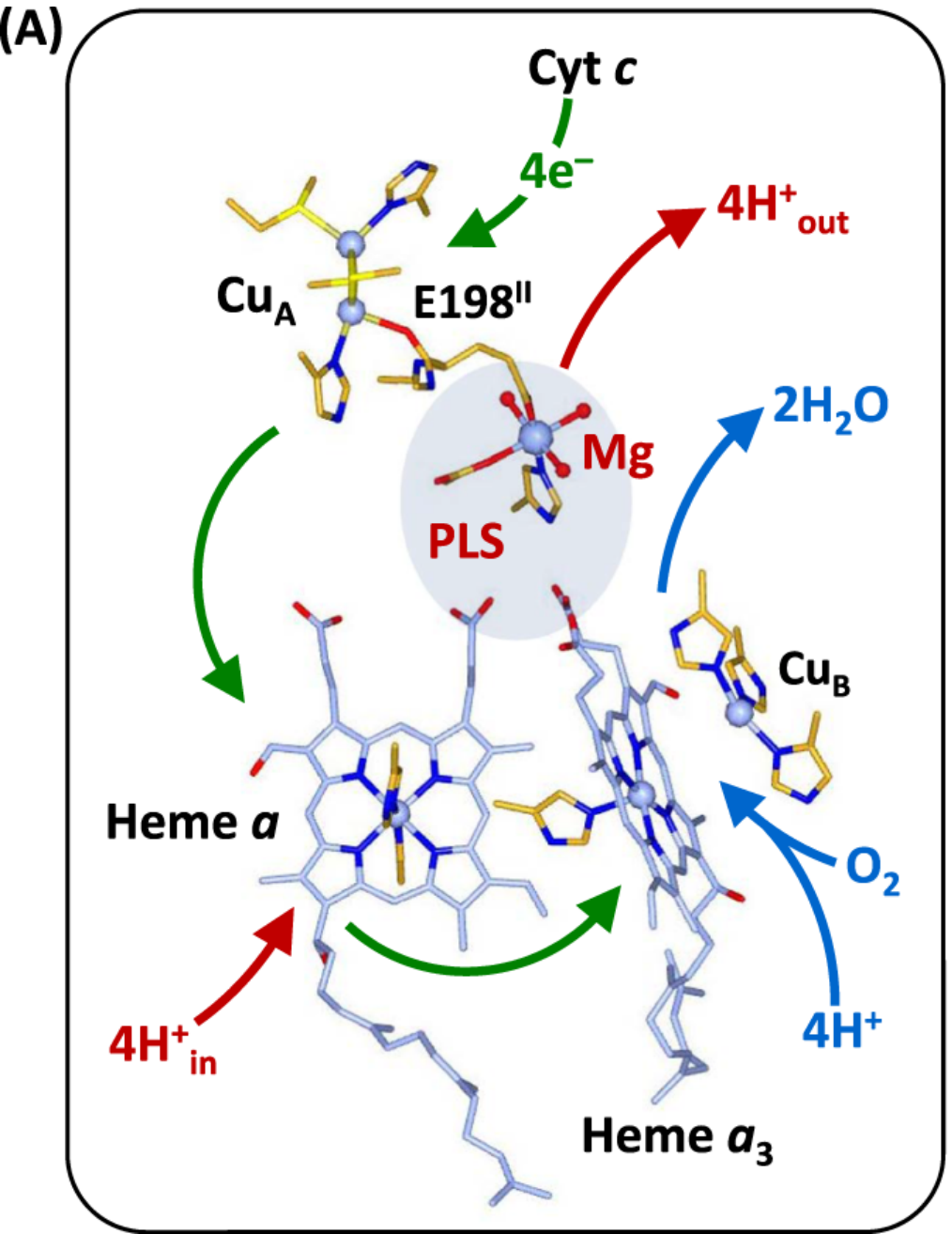


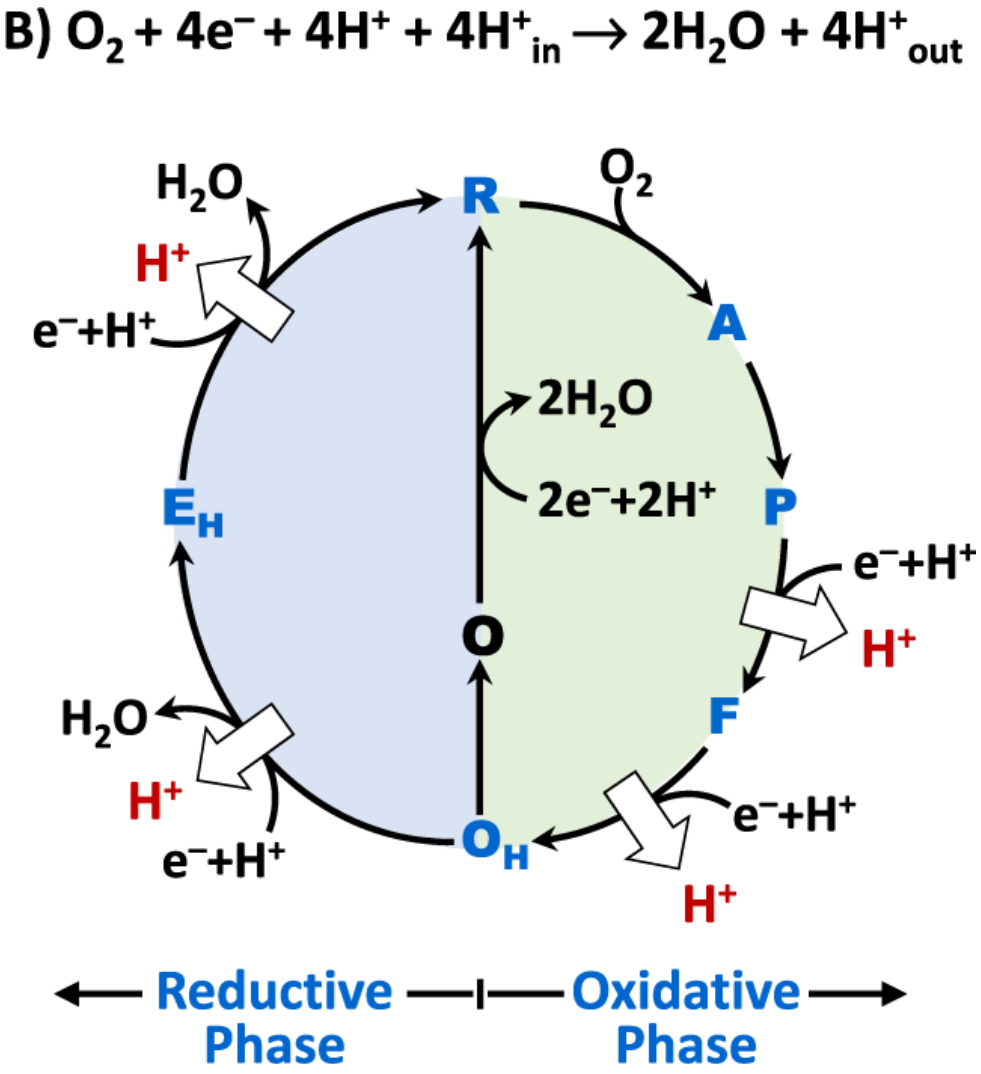
Cu_B



Cu_A

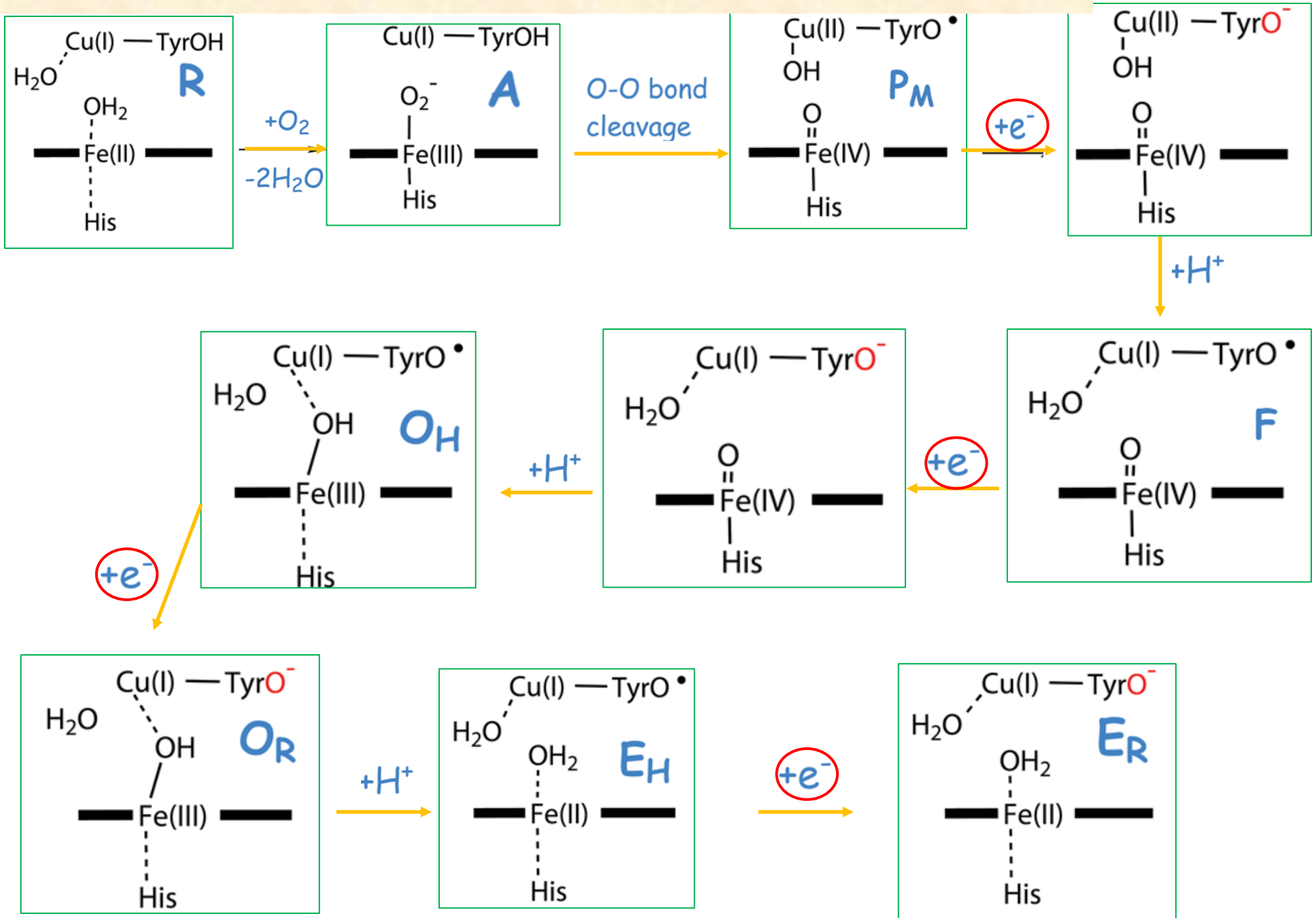
Cytochrome c Oxidase





Ishigami et al. *Nat Commun* **14**, 5752 (2023)

Cytochrome c Oxidase



- The resting oxidized state is suggested to have an extra proton in the active site, as compared to the situation during catalytic turnover, and the uptake of this extra proton occurs slowly when there are no more electrons supplied. This state, labled $\mathbf{O}_P^+$, has a neutral protonated tyrosine, Cu(II) and Fe(III) with a bridging hydroxyl ligand.

- The proton coupled Cu(II) reduction potential for this state is found to be low, in agreement with equilibrium experiments of 0.28–0.35 V. Because of the extra proton there should be no proton pumping coupled to the initial reduction of this state. In contrast, the oxidized state during catalytic turnover, labled $\mathbf{O}_H$, has an unprotonated tyrosine with a tyrosyl radical, Cu(I) and Fe(III), which means that in the two reduction steps from the oxidized to the reduced state, $\mathbf{O}_H \rightarrow \mathbf{R}$, it is the tyrosyl radical and Fe(III) that are reduced.

- A major result from the computational studies is that the intermediate state, which is referred to as the one electron reduced state, and labled $\mathbf{E}_H$, also has an unprotonated tyrosine; i.e., the proton has entered the center of the **binuclear active centre** (BNC). This state is a mixture of two electronic configurations, the main one with a tyrosyl radical and Fe(II) and a secondary one with tyrosinate and Fe(III). In this way the reduction energy of the high potential tyrosyl radical is shared with the low reduction energy of heme a_3 Fe(III), and both reduction steps become exergonic enough to allow proton pumping also with a significant gradient.

- Molecular oxygen binds reversibly to the reduced state with Fe(II) and Cu(I), and after the O–O bond cleavage there is a tyrosyl radical, Cu(II)–OH and Fe(IV)=O ($\mathbf{P}_M$ state).

- According to the computational studies, the first reduction step after the O–O bond cleavage involves reduction of Cu(II) to Cu(I) and formation of a water molecule, and it leaves a tyrosyl radical also in the **F** state. At this stage the proton coupled reduction potential of Cu_B is high, on the order of 1 V.

- In the next reduction step Fe(IV) is reduced to Fe(III), and there is still an unprotonated tyrosyl with radical character in the $\mathbf{O}_H$ state. In these two reduction steps, $\mathbf{P}_M \rightarrow \mathbf{F}$ and $\mathbf{F} \rightarrow \mathbf{O}_H$, it is found to be thermodynamically more favorable to leave the tyrosine unprotonated and let the proton go to the center of the BNC.

- In contrast, in the $\mathbf{O}_H \rightarrow \mathbf{E}_H$ reduction step it has to be assumed that there is a kinetic effect that causes the avoidance of the lower lying **E** state with a protonated tyrosine.

- The K-channel, ending at the active site tyrosine, is only used in the $\mathbf{E}_H \rightarrow \mathbf{R}$ step, in which the tyrosine is reprotonated.

- The presence of the tyrosine in the active site, and the reduction mechanism presented here with an unprotonated tyrosine with radical character in all intermediate steps, are essential for the proton pumping.

- Most likely the uptake of the pump-proton to a temporary loading site, the PLS, is driven by an electrostatic coupling to the electron transfer into the active site. Therefore, it is important that the product of each reduction step has a large enough affinity for uptake of the next electron to the active site already before the chemical proton has arrived.

- The calculations show that the intermediates with a proton in the center of the BNC and the tyrosine unprotonated (radical) secure such a high electron affinity, and essentially the electron goes initially to a tyrosyl radical in all reduction steps.

- The alternative form of the intermediate states where the proton instead is delivered to the tyrosine is found to have a significantly lower electron affinity, most likely requiring uptake of the chemical proton before electron transfer to the active site. Thus, the presence of the tyrosine in the active site enables the uptake of two protons per electron in cytochrome c oxidase.

- It is finally suggested that the tyrosyl radical is not easily observed experimentally because it is erased by the uptake of an extra proton to the active site when the flow of electrons is stopped.