

Copper and iron proteins

Elements that are known to be essential for plants

Essential and Beneficial Elements in Higher Plants																	
H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Lr	Rf	Db	Sg	Bh	Hs	Mt									
		La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb		
		Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No		

Typical roles of copper and iron proteins

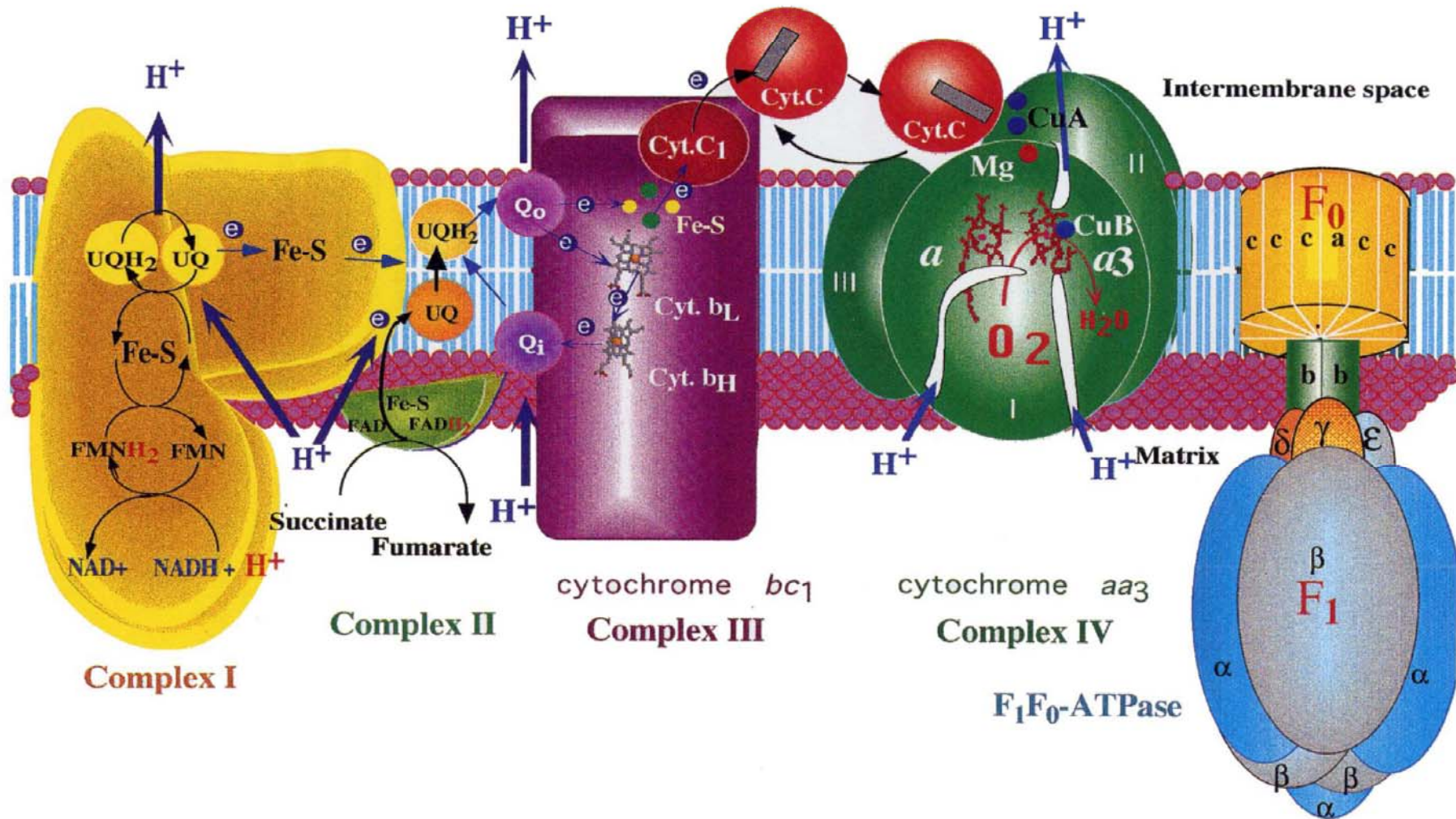
→ Reductions and oxidations of substrates

→ Oxygen transport

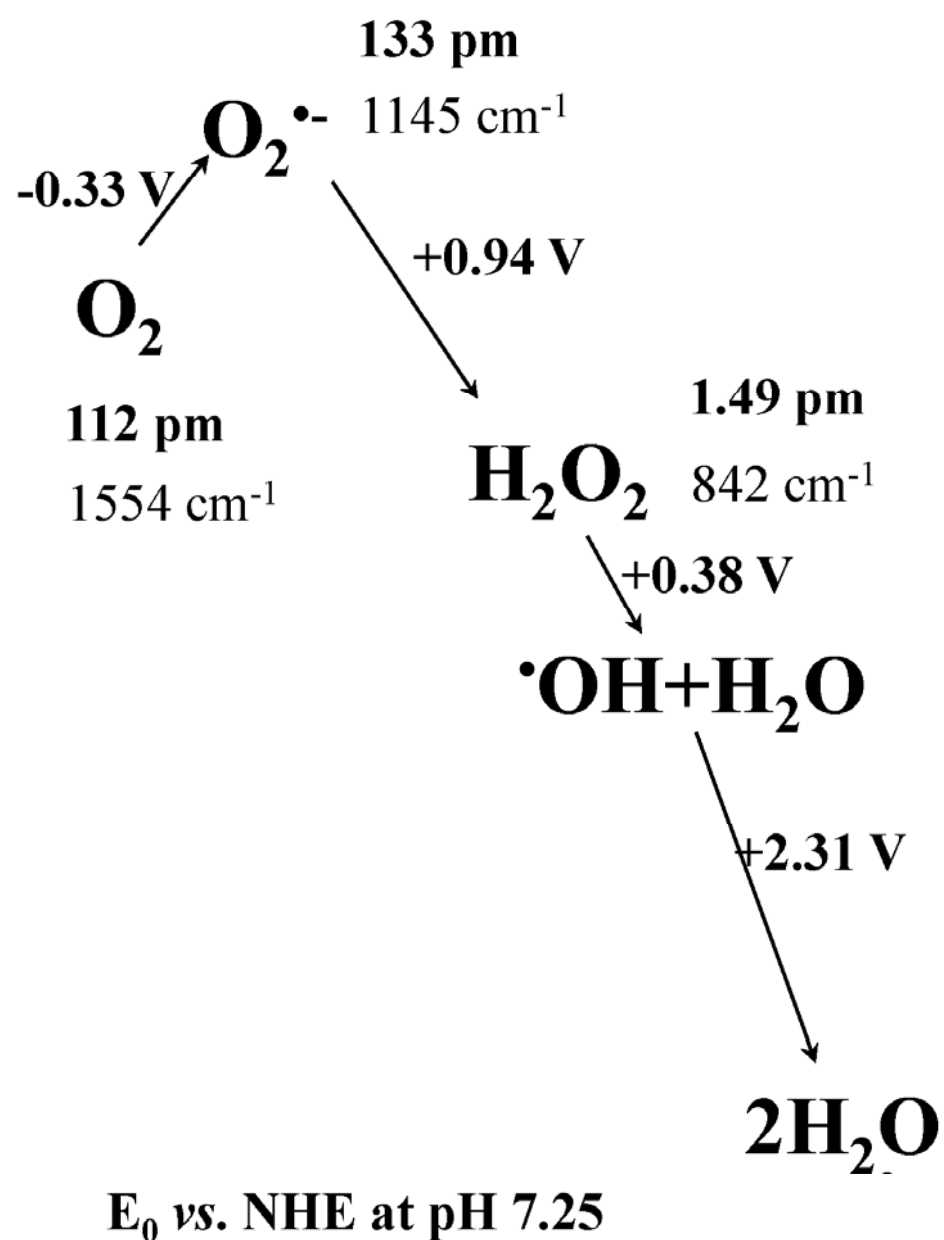
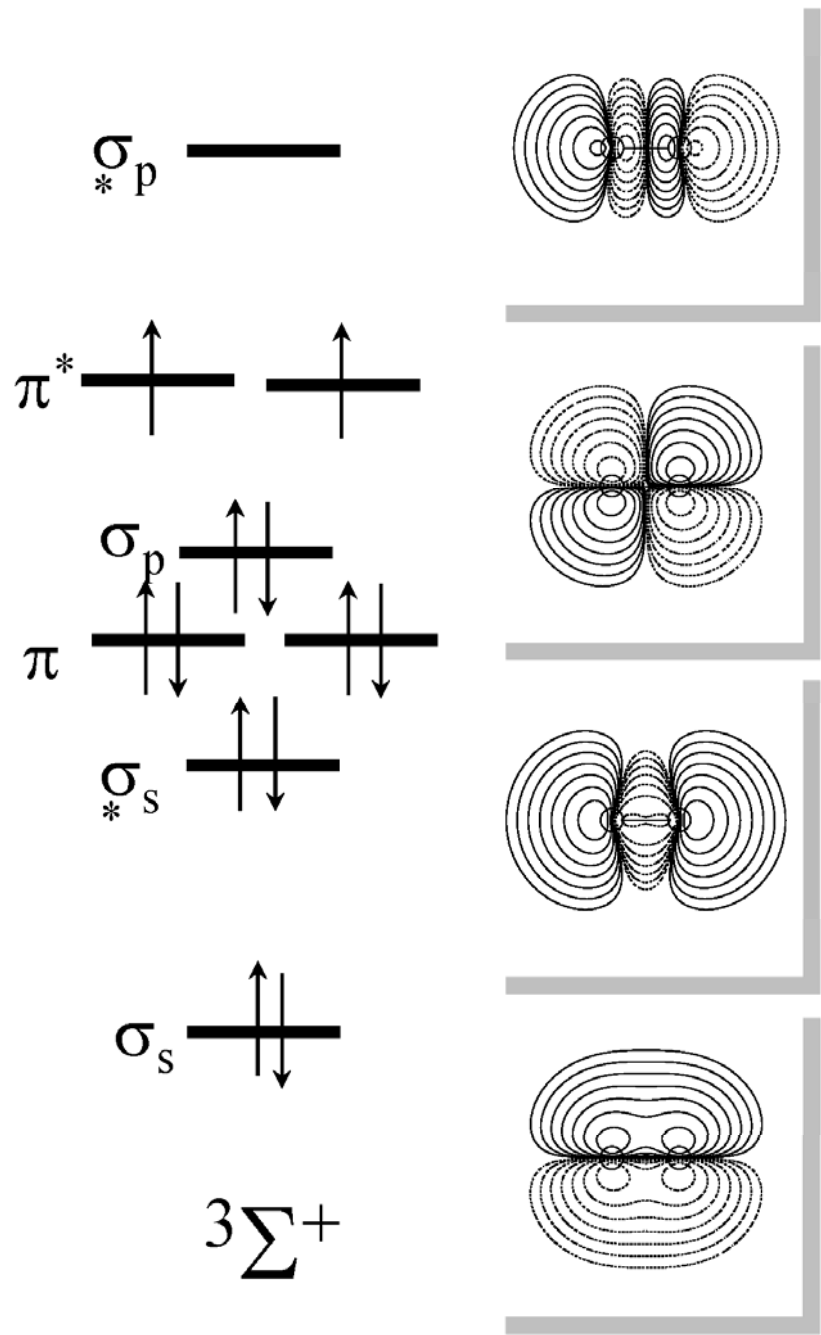
→ In several metabolic functions, enzyme variants exist that can replace each other if an organism suffers from deficiency of one of these two metals

Respiration = reduction of O_2 to H_2O

Synthesis of ATP – proton-coupled electron transfer (PCET)



Dioxygen Activation



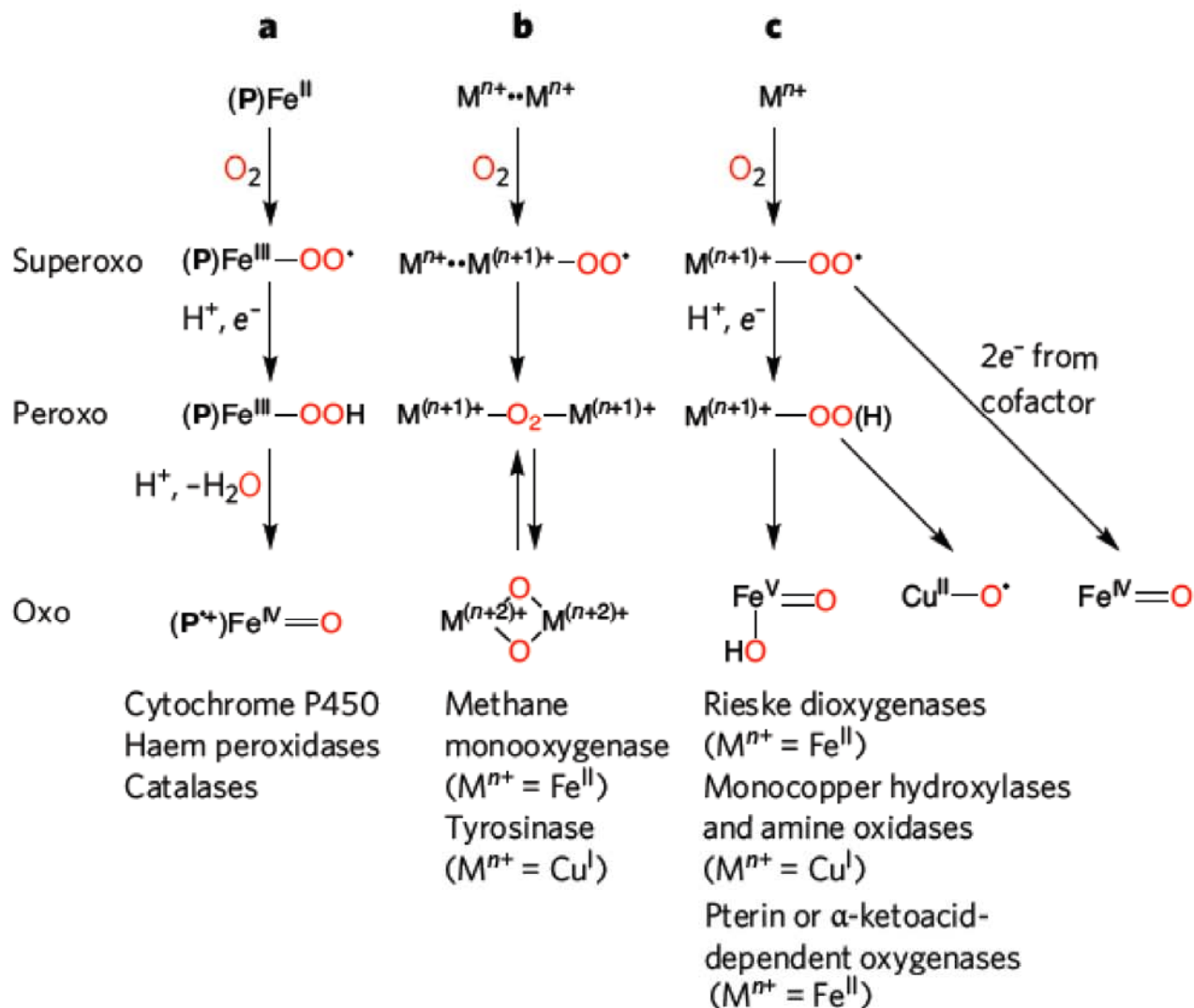
Activation of O₂ – Reaction Types

- **Reversible binding of O₂ – Myoglobin, Hemoglobin (Fe), Hemocyanin (Cu-Cu)**
- **O₂^{•-} dismutation – Superoxide Dismutase (Mn, Fe, Ni, Cu, Zn)**
$$\text{O}_2^{\cdot-} + \text{O}_2^{\cdot-} + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$$
- **H₂O₂ decomposition – Catalase (Mn, heme-Fe)**
$$2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$$
- **Oxygenases (focus on Monooxygenase Cytochrome P450)**
$$\text{R-H} + \text{O}_2 + \text{NADPH} + \text{H}^+ \rightarrow \text{R-OH} + \text{H}_2\text{O} + \text{NADP}^+$$
- **Oxidases (2-electron reduction to H₂O₂; Fe, Cu)**
$$\text{O}_2 + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2$$
 (focus on Cu enzyme Galactose Oxidase)
- **Oxidases (4-electron reduction to H₂O; heme-Fe, Cu)**
$$\text{O}_2 + 4\text{e}^- + 4\text{H}^+ \rightarrow 2 \text{H}_2\text{O}$$
 (focus on Cu enzyme Ascorbic Acid Oxidase and Fe,Cu enzyme Cytochrome *c* Oxidase)

O₂ activation by Metallo-Oxygenases

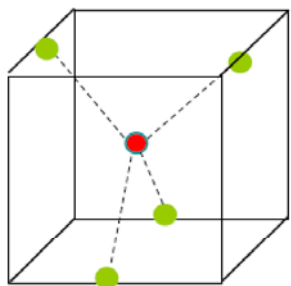
Mechanisms involve the formation of an initial O₂ adduct (**superoxo**), conversion to a metal–peroxide (**peroxo**), and subsequent O–O bond cleavage to yield a high-valent oxidant (**oxo**). Oxygen atoms involved are shown in red. M, metal; P, porphyrin.

L. Que Jr, W.B. Tolman (2008) NATURE, 455, 333-340

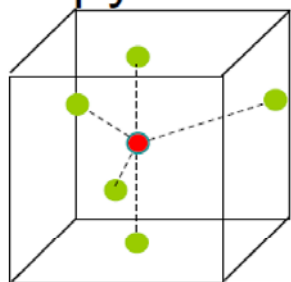


Geometry is important: Iron Proteins

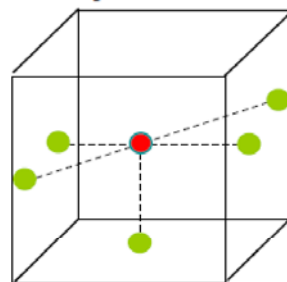
Tetrahedron



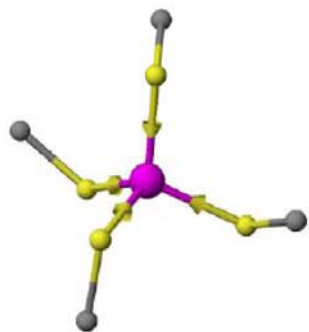
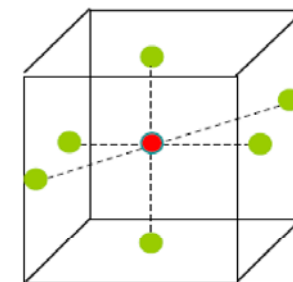
Trigonal Bipyramide



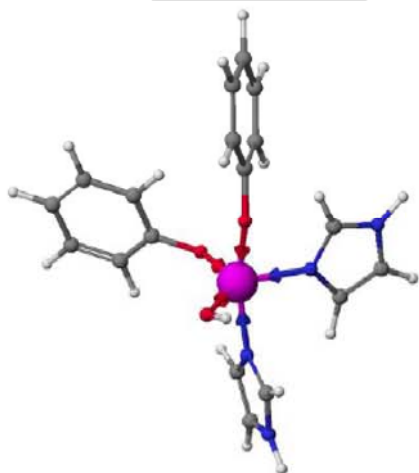
Tetragonal Pyramide



Octahedron



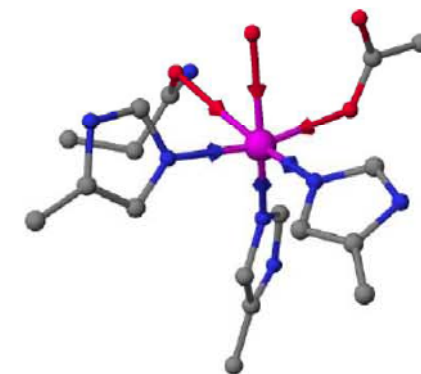
Rubredoxin



3,4-Protocatechoate
Dioxygenase



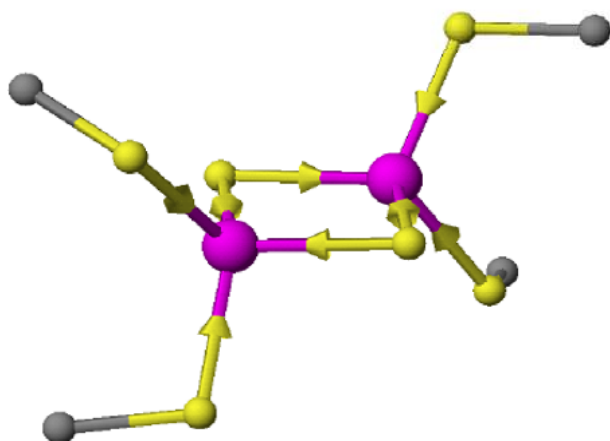
Tyrosine
Hydroxylase



Lipoxygenase

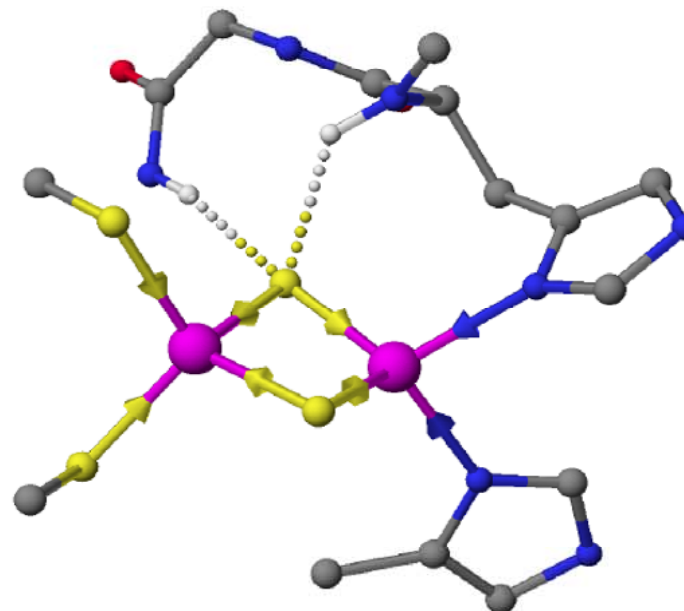
Modulation of Redox potentials (H bridges)

2Fe-2S Ferredoxin



$E^0 \sim -400 \text{ mV}$

2Fe-2S Rieske



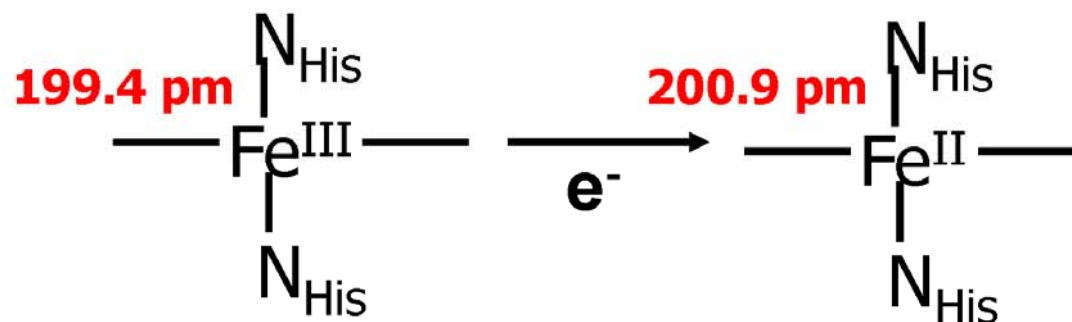
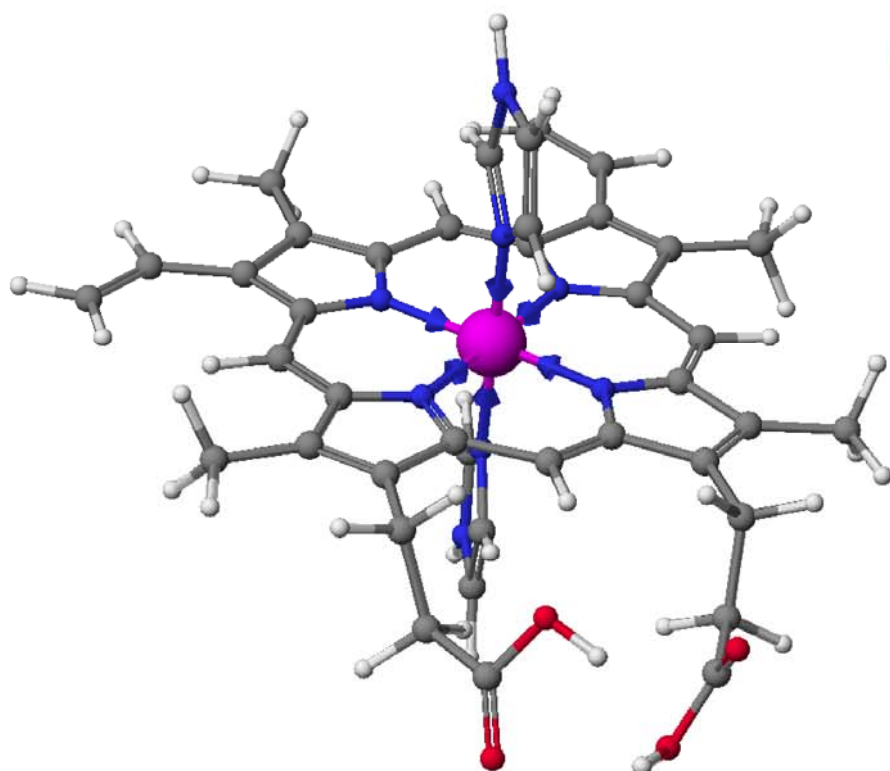
$E^0 \sim +280 \text{ mV}$

(+150 mV without H bridges)

- (a) Stephens, P.J.; Jollie, D.R.; Warshel, A. (1996) *Chem. Rev.*, 96, 2491
(b) Link, T.A. (1999) *Adv. Inorg. Chem.*, 47, 83

Low (zero) Reorganization Energy

Low-Spin Heme center

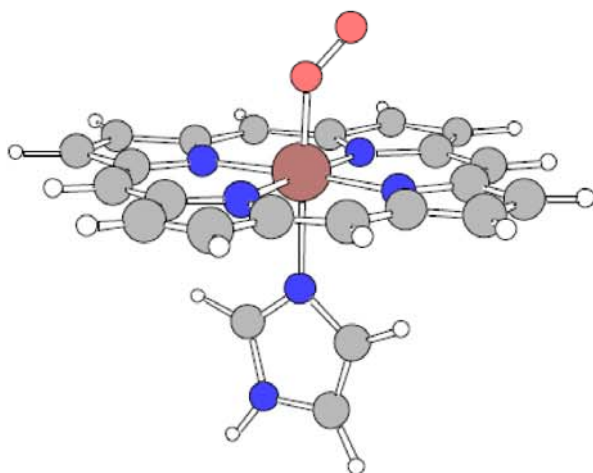


Reorganization Energy

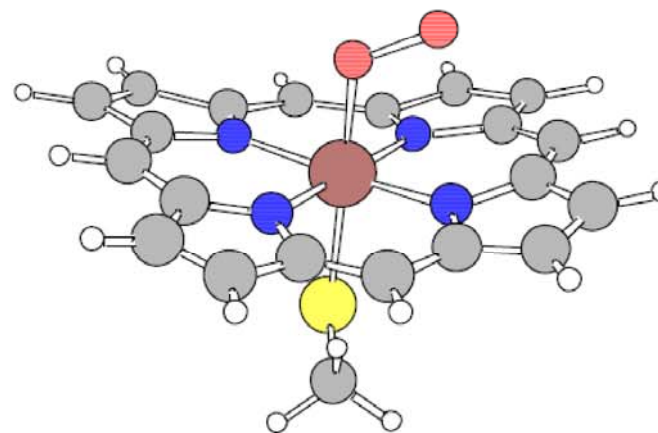
in Cytochromes $\leq 4\text{-}5$ kcal/mol

Trans-Effect - Tuning reactivity

A ligand *X trans* to a second ligand *Y* can influence the stability of the M-Y bond. With *X* being a strong Lewis base, the M-Y bond will be weakened



Myoglobin
Axial Histidine
O₂ Transport

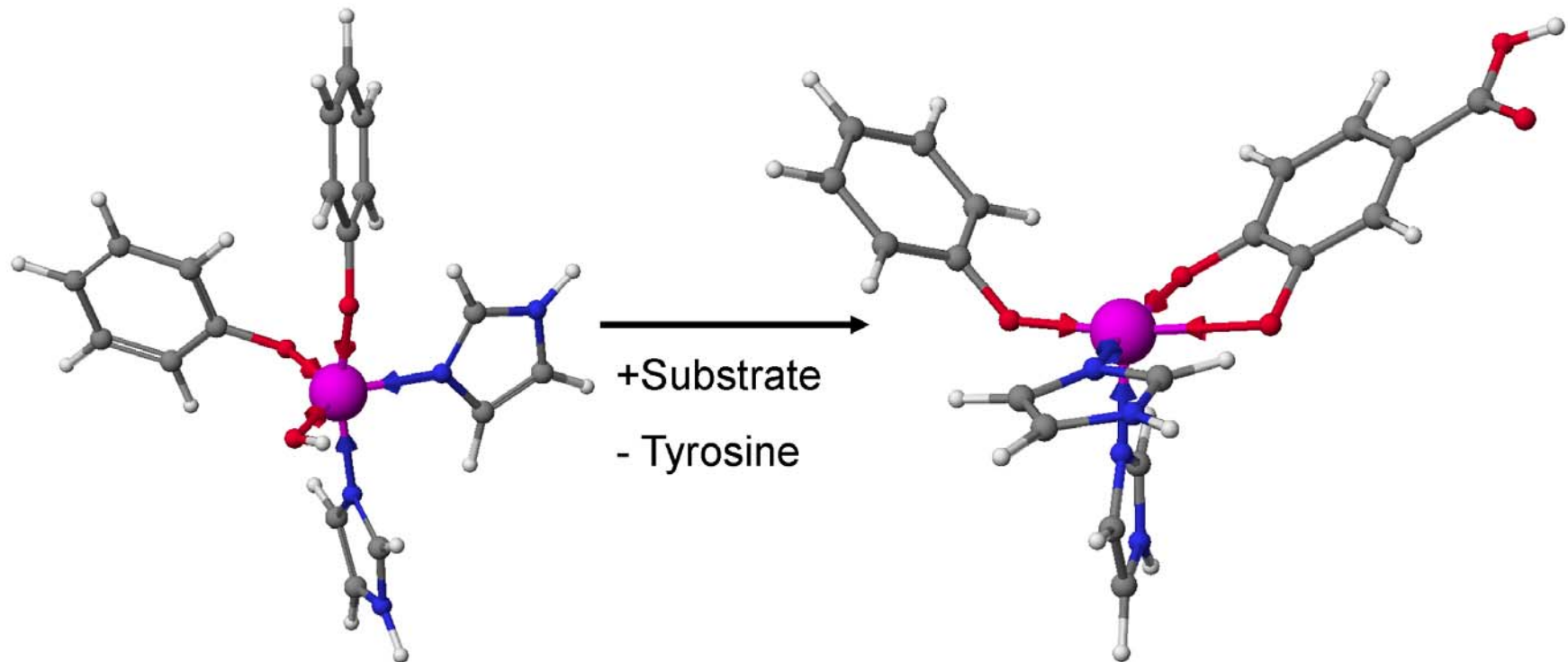


Cytochrome P450
Axial Cysteine
O₂ Activation



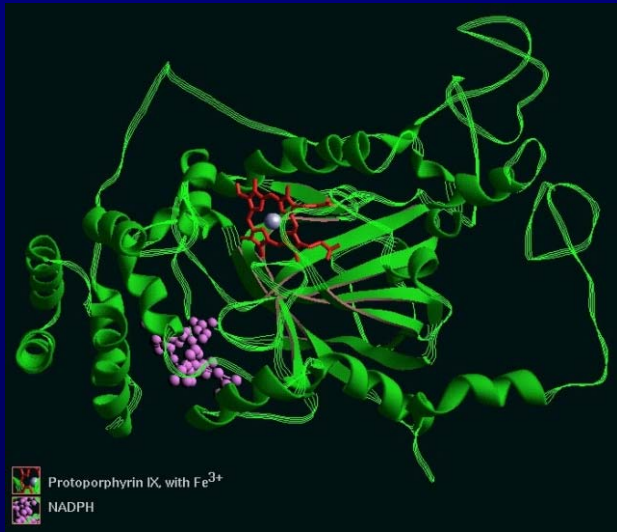
Opening of Substrate Binding Sites in Enzyme

3,4 Protocatechuate Dioxygenase

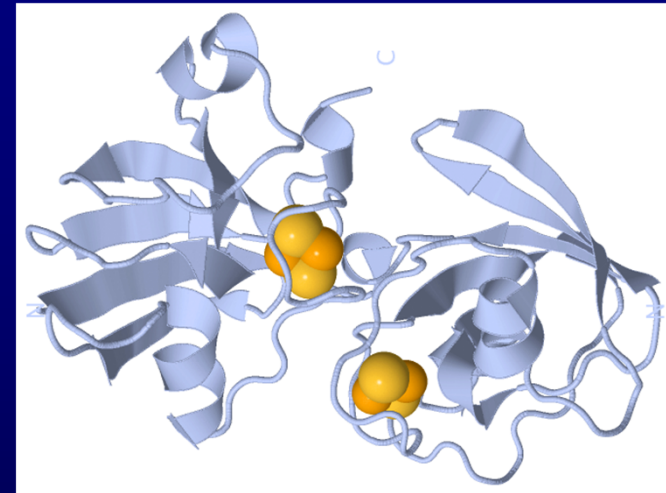


Examples of plant enzymes with iron in the active centre

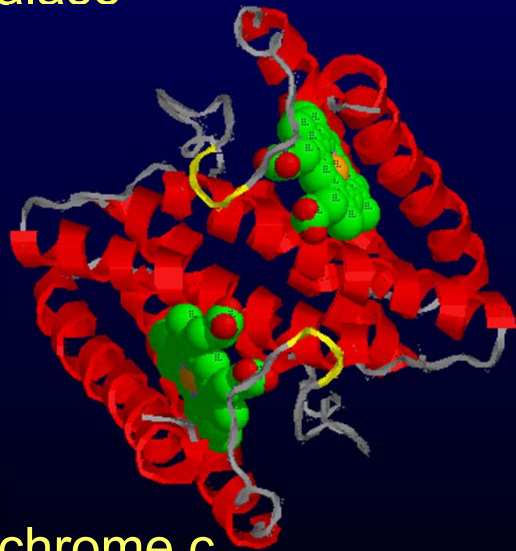
Details on biochemistry & spectroscopy: lecture on iron+copper proteins!



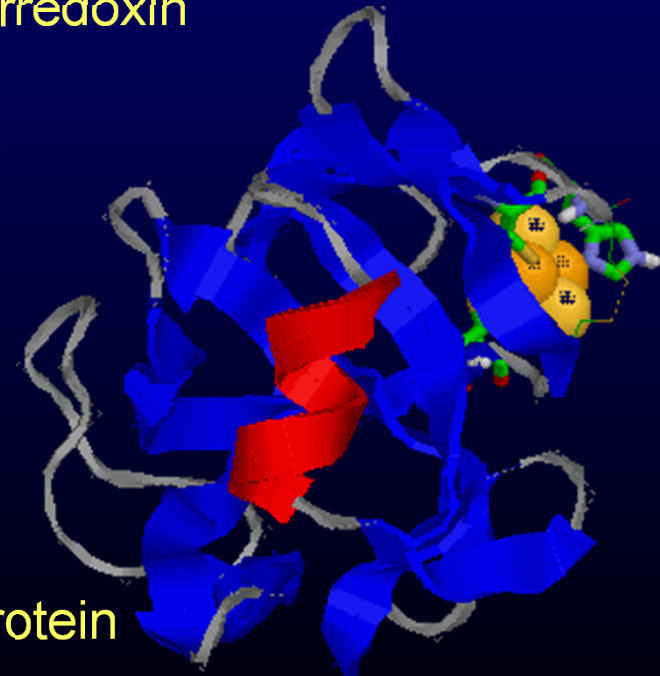
Catalase



Ferredoxin

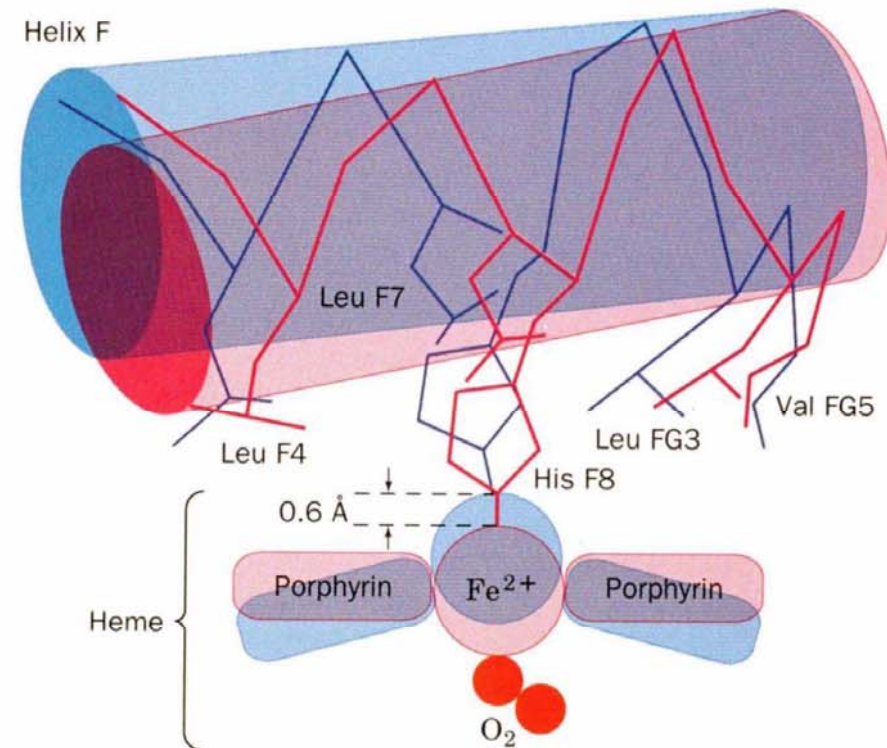
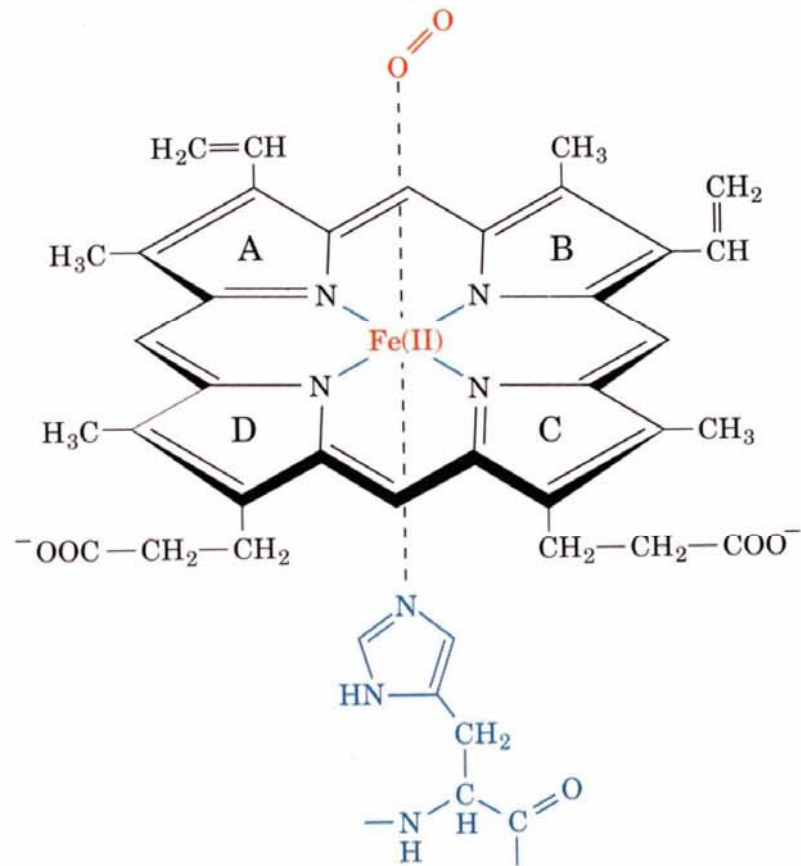


Cytochrome c



Rieske protein

Reversible O₂ Binding Myoglobin and Hemoglobin



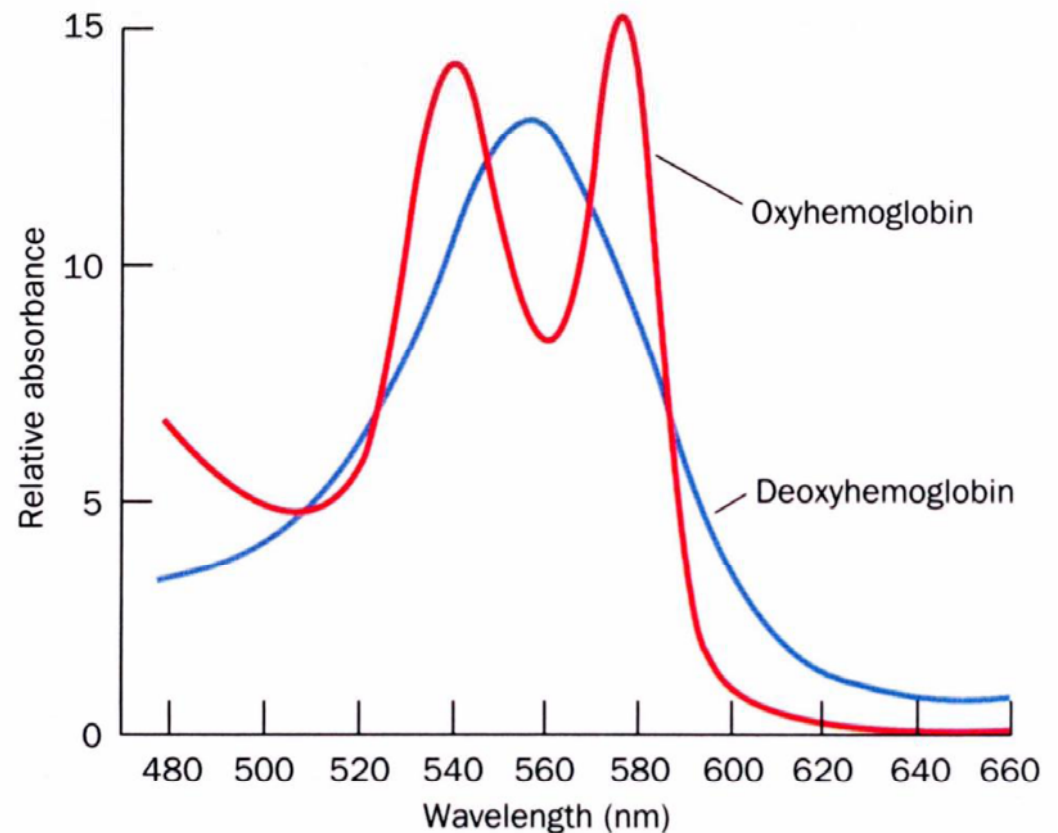
The iron must be in the Fe(II) (ferrous oxidation) state.

Binding of O₂ rearranges the electronic distribution and alters the d orbital energy.

This causes a difference in the absorption spectra.

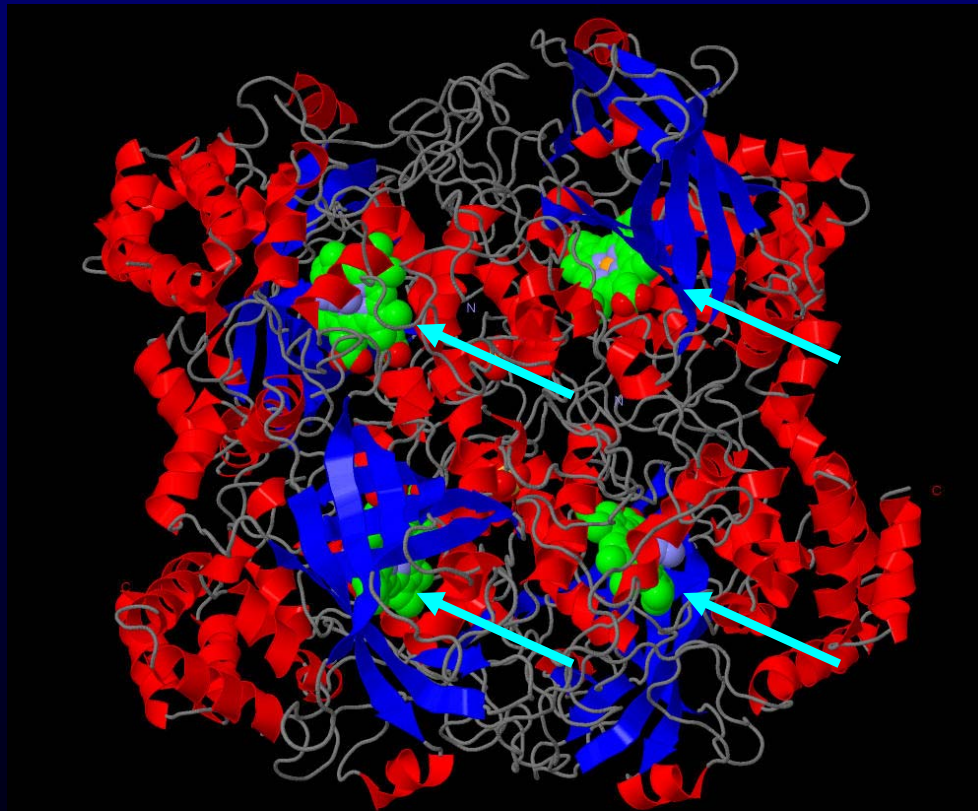
Bluish for deoxy Hb, Redish for Oxy Hb

Measuring the absorption at 578 nm allows an easy method to determine the percent of O₂ bound to hemoglobin.

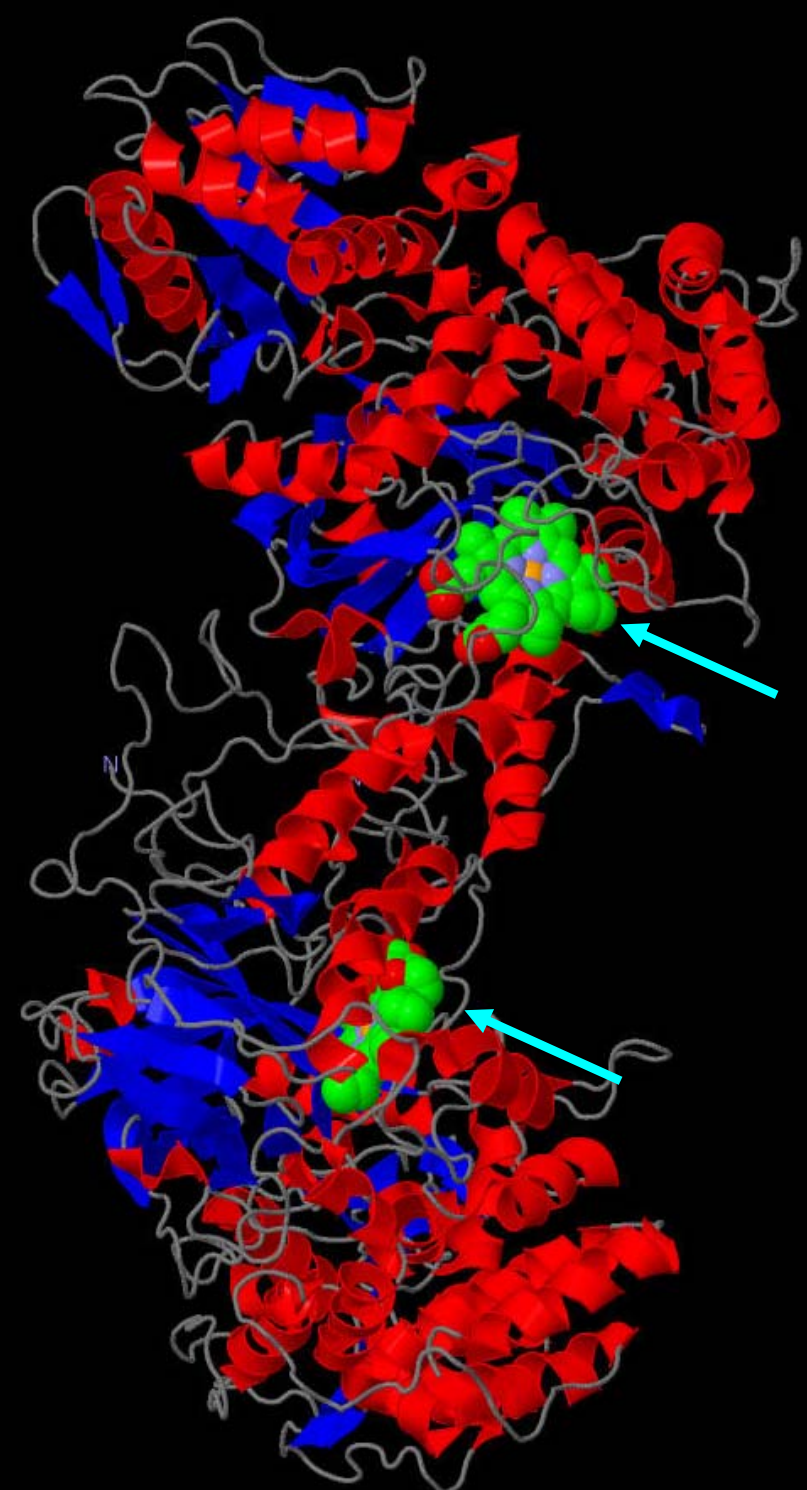


Catalases, some of the most important heme-iron enzymes: (a) function and structure

- Detoxify hydrogen peroxide (H_2O_2)
- Various forms found in all kinds of aerobic organisms from bacteria to plants and animals
- Iron always present as heme-iron



Saccharomyces cerevisiae catalase A,
From: Maté JM et al, 1999, J Mol Biol 268, 135-49

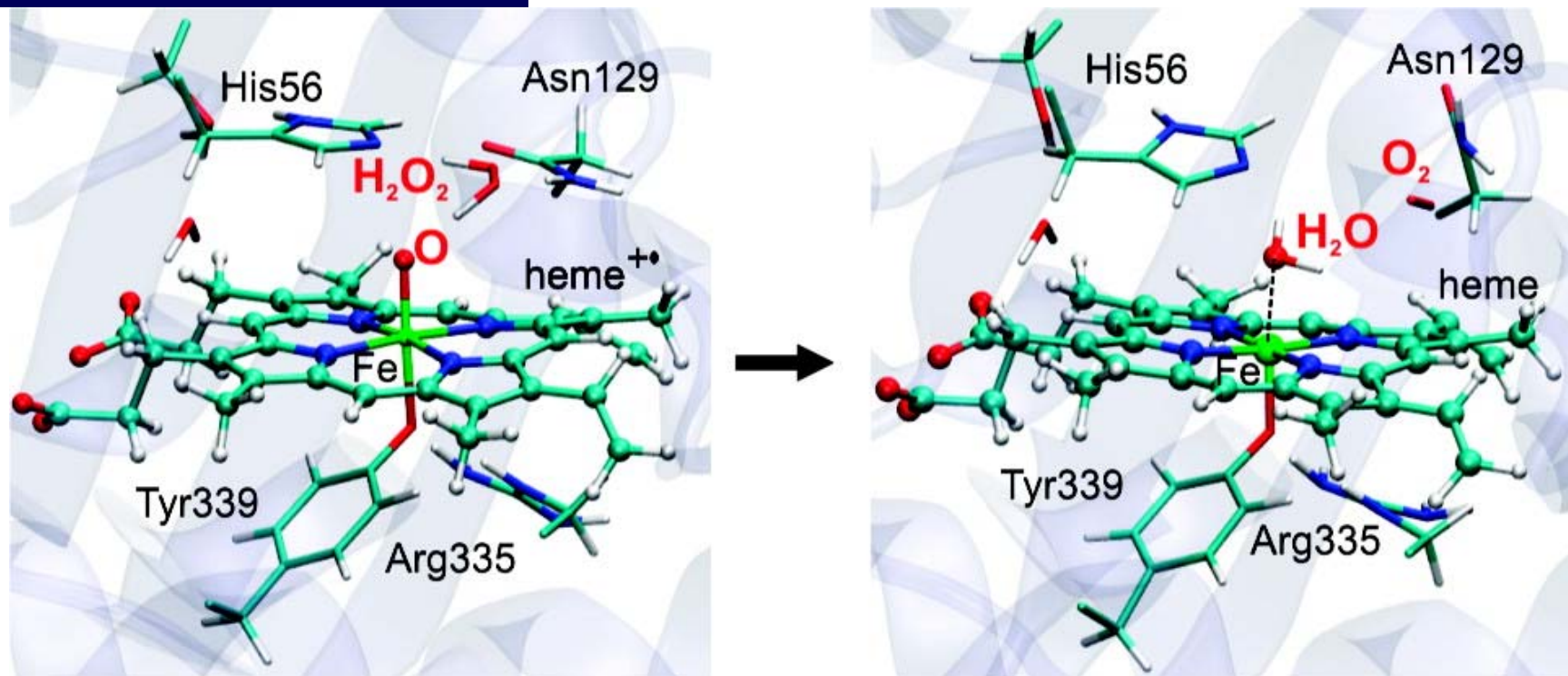
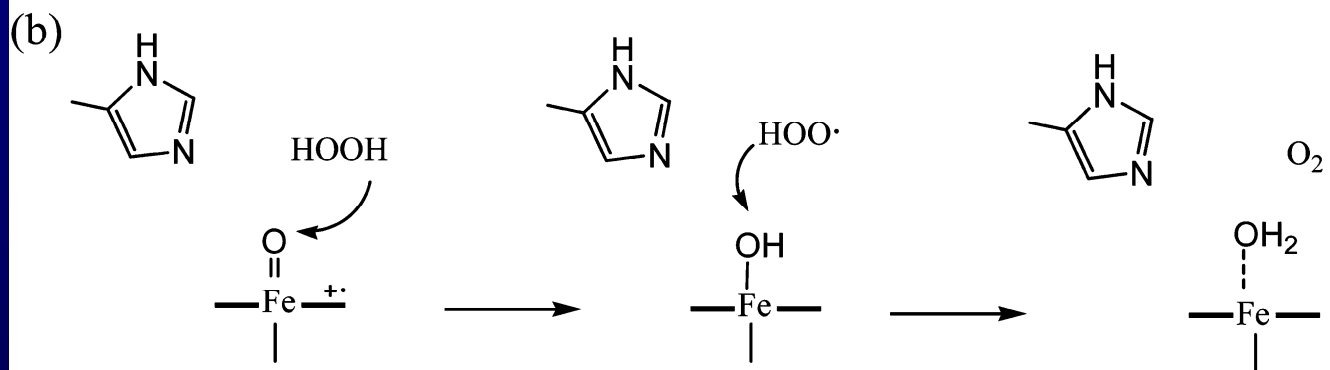
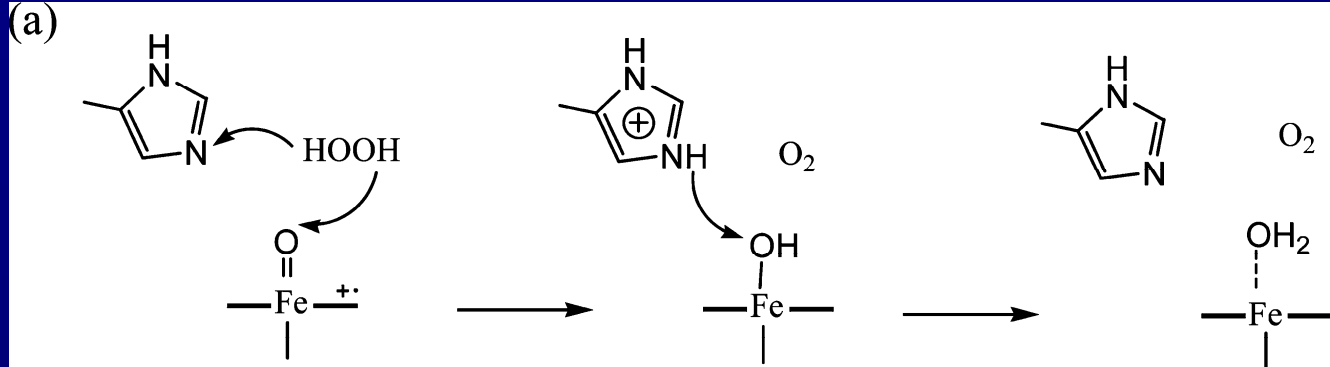


Neurospora crassa catalase1, From: Diaz A
et al, 2004, J Mol Biol 342, 971-85

Catalases, heme-iron enzymes

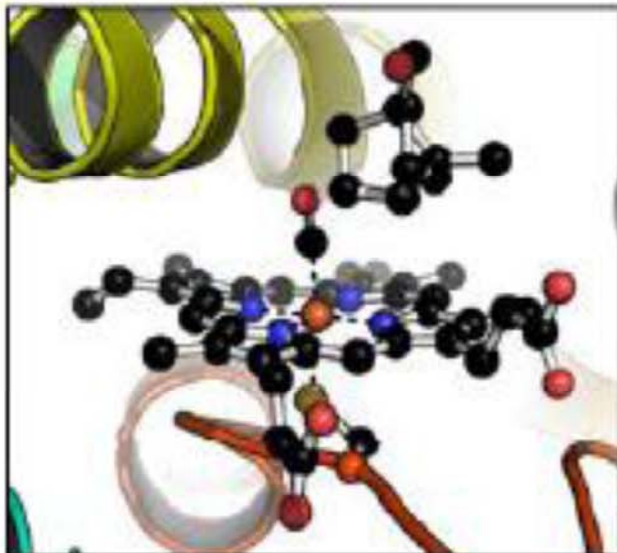
(b) catalysis

From: Alfonso-Prieto M et al., 2009, J. Am. Chem. Soc. 131, 11751-61.



Cytochrome P450 – important enzyme for detoxification of organic compounds

- Single *b*-type heme group
- Cys thiolate as proximal axial ligand
- Triangular prism
- No domain structure, but a more α -helical half and a half containing β -strands.
- High degree of structural conservation within the family.



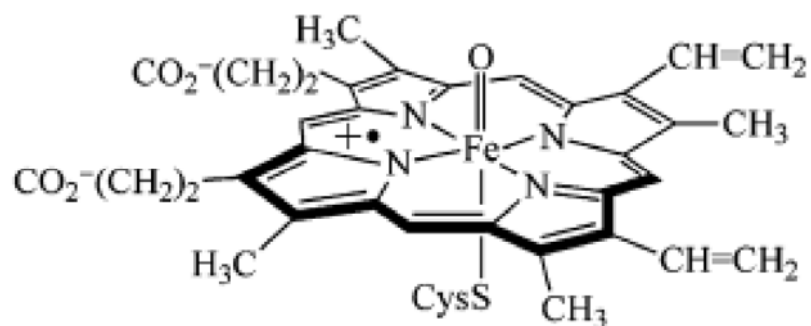
Cytochrome P450cam; PDB-ID 1CPP
Poulos et al. (1987) *J.Mol.Biol.* 195: 687-700.

Typical Reactions of Cytochrome P450 (O-Transfer)

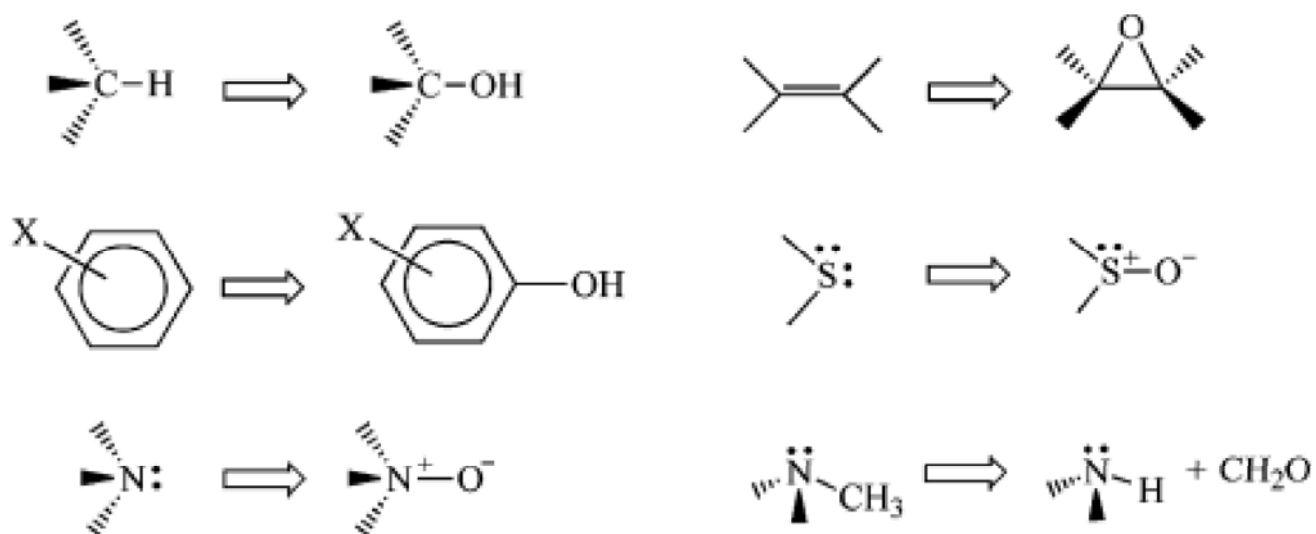
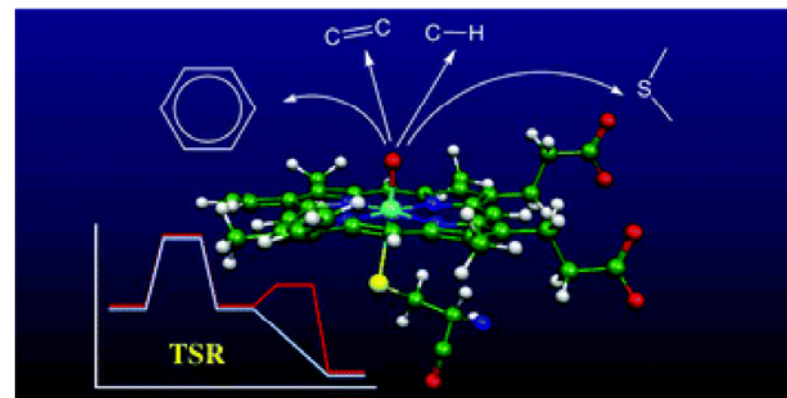
S. SHAIK et al. (2010) *Acc. Chem. Res.*, **43**, 1154-1165

J. Rittle, M. T. Green (2010), *SCIENCE* **330**, 933-936

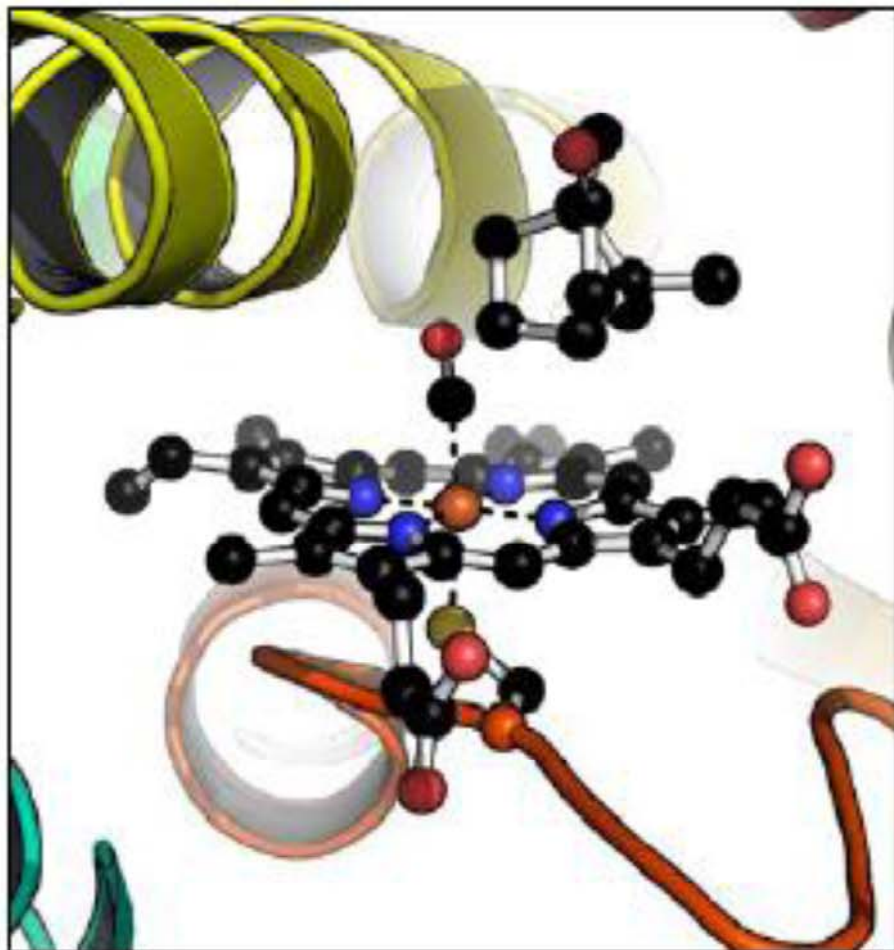
SCHEME 1



P450 Cpd I

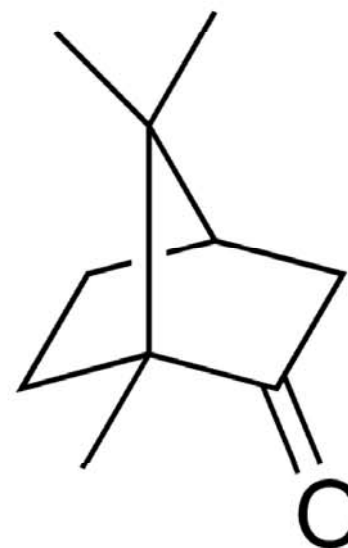


Camphor substrate complex of Cytochrome P450



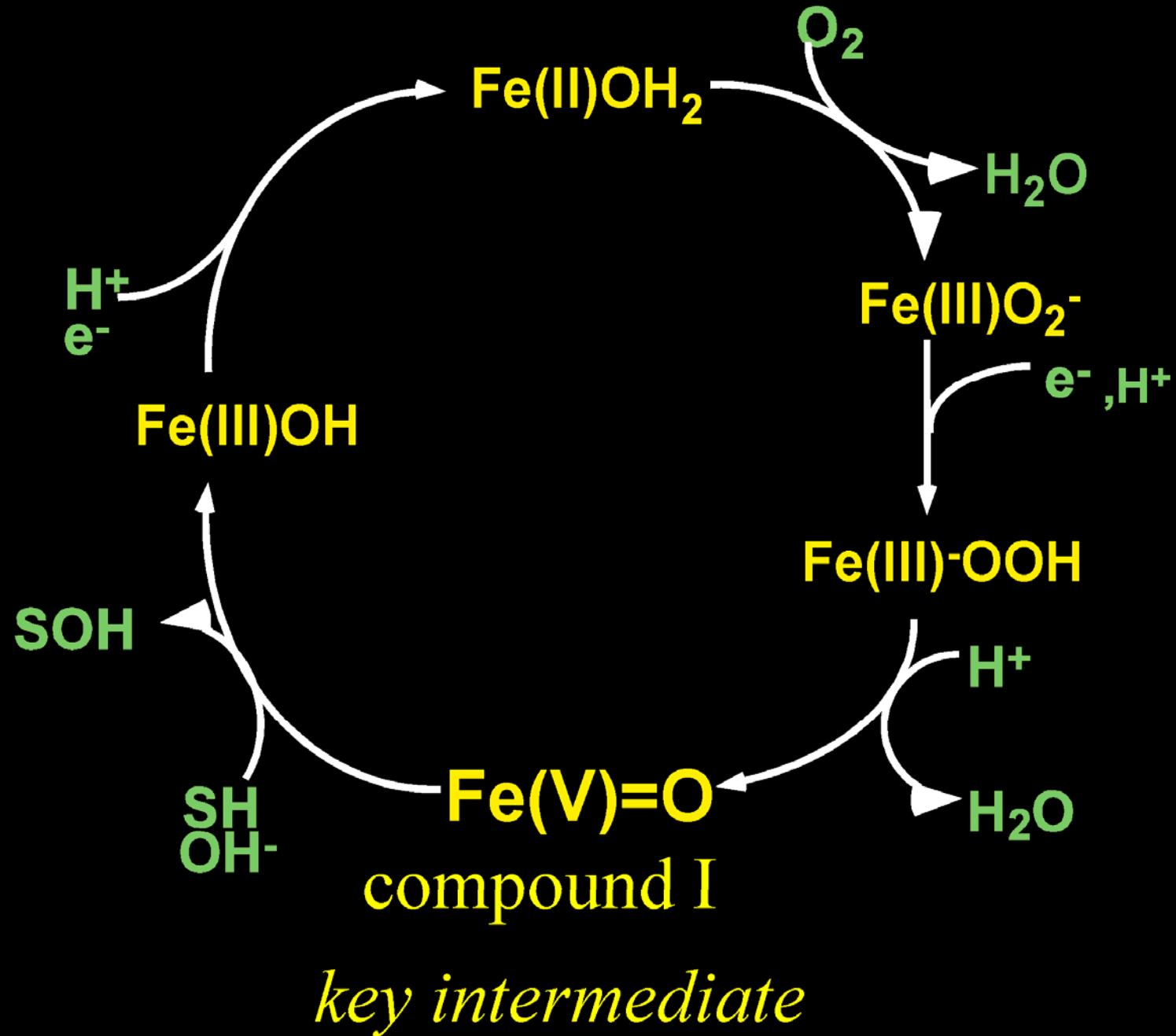
Multiple substrate complexes of P450s were readily obtained, but no intermediates of the reaction cycle.

In the P450cam substrate complex, CO binds to heme, presumably analogous to O₂.

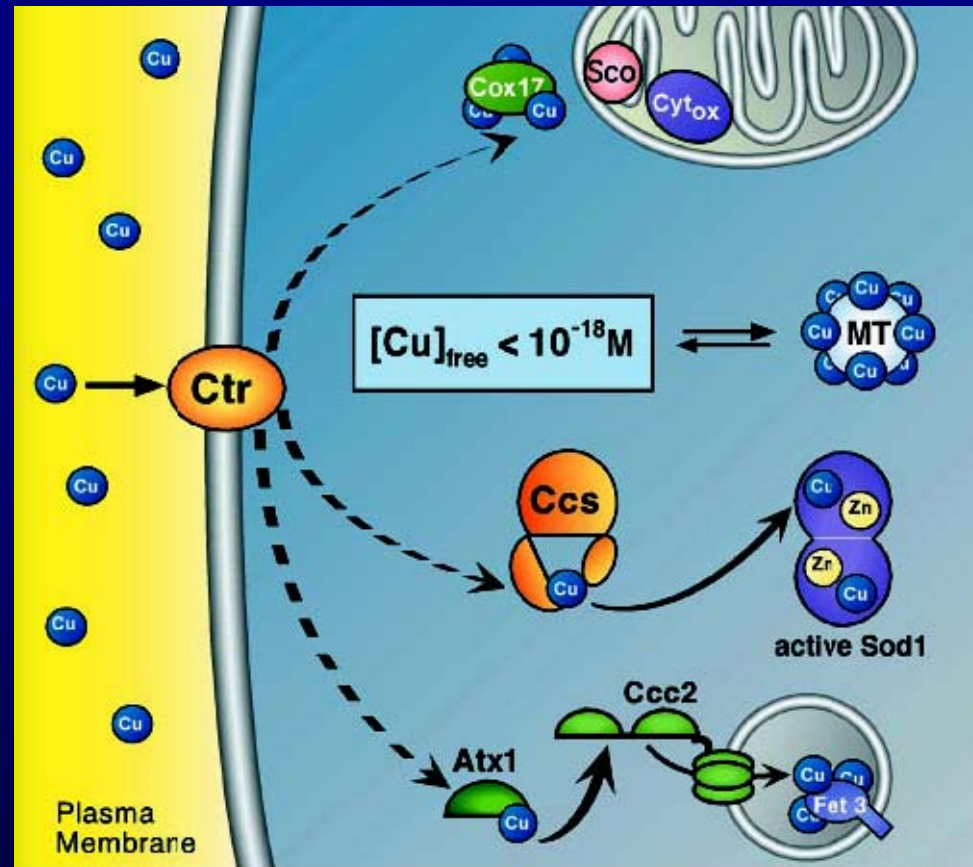


Cytochrome P450cam; PDB-ID 3CPP
Raag & Poulos (1989) *Biochemistry* 28: 7586-7592.

Cytochrome P450 Reaction Cycle



Copper delivery inside cellular compartments

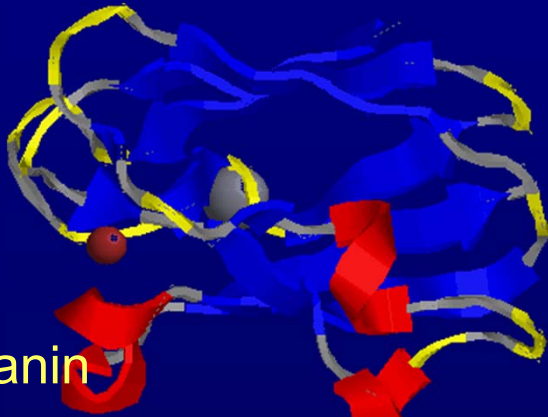


From: O'Halloran TV, Culotta VC, 2000, JBC275, 25057-60

- confusing large number of names for homologous proteins in different organisms
- REALITY: just 3 really different (non-homologous) Cu-chaperones are well known, some more proteins are postulated to be Cu-chaperones

Selected enzymes with copper in the active centre

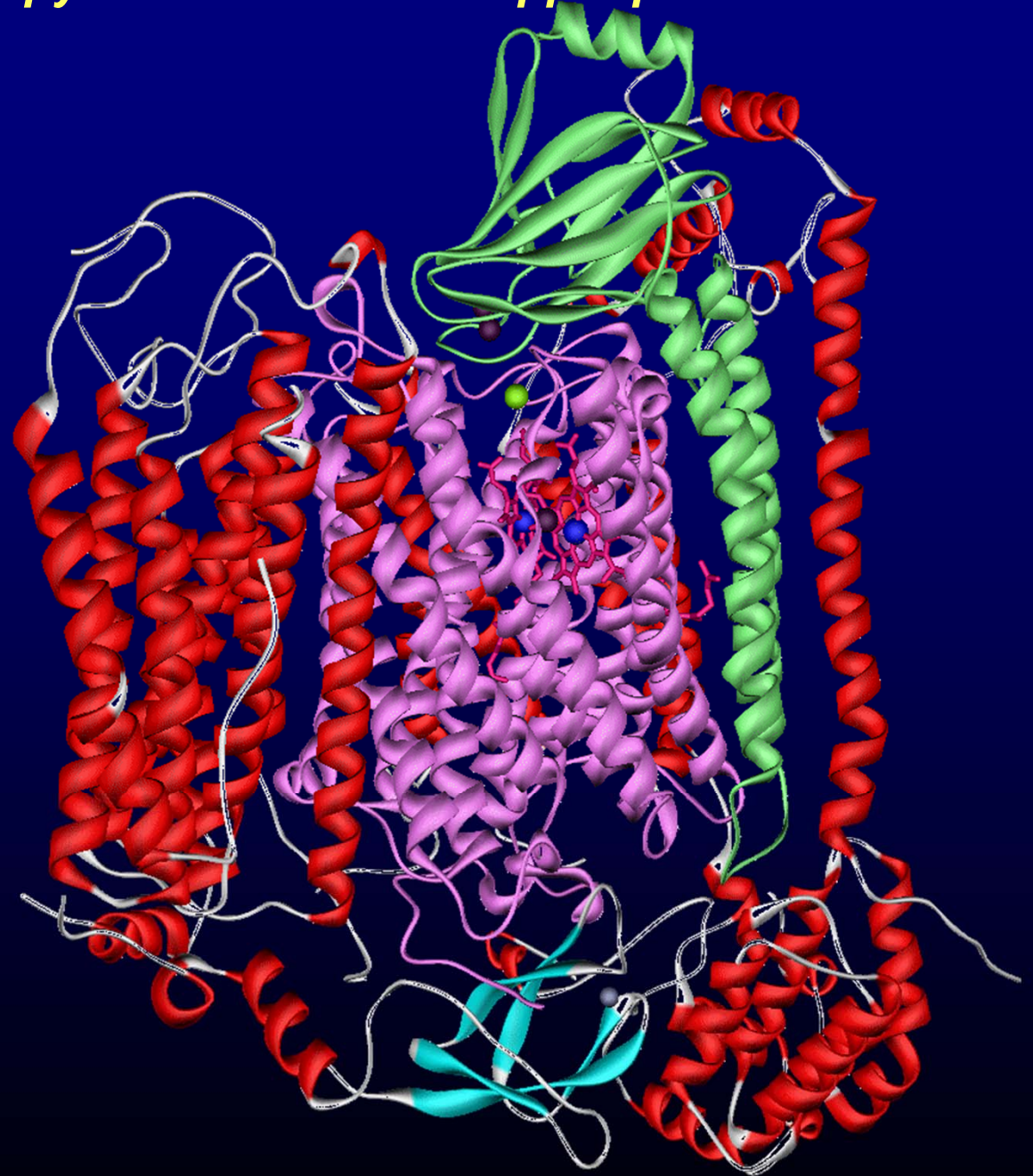
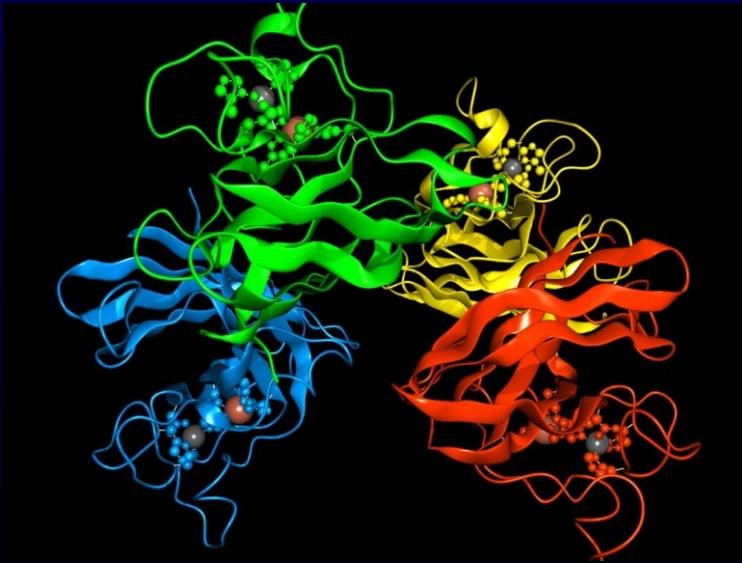
Details on biochemistry & spectroscopy: lecture on iron+copper proteins!



- Plastocyanin

- Cytochrome c oxidase 3 Cu (binuclear site and mononuclear site)

- SOD = Superoxide dismutase

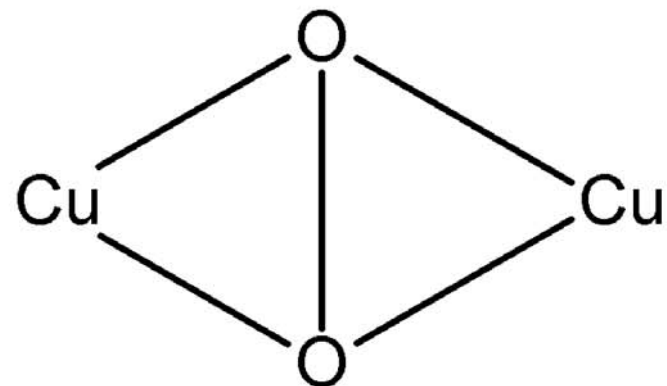
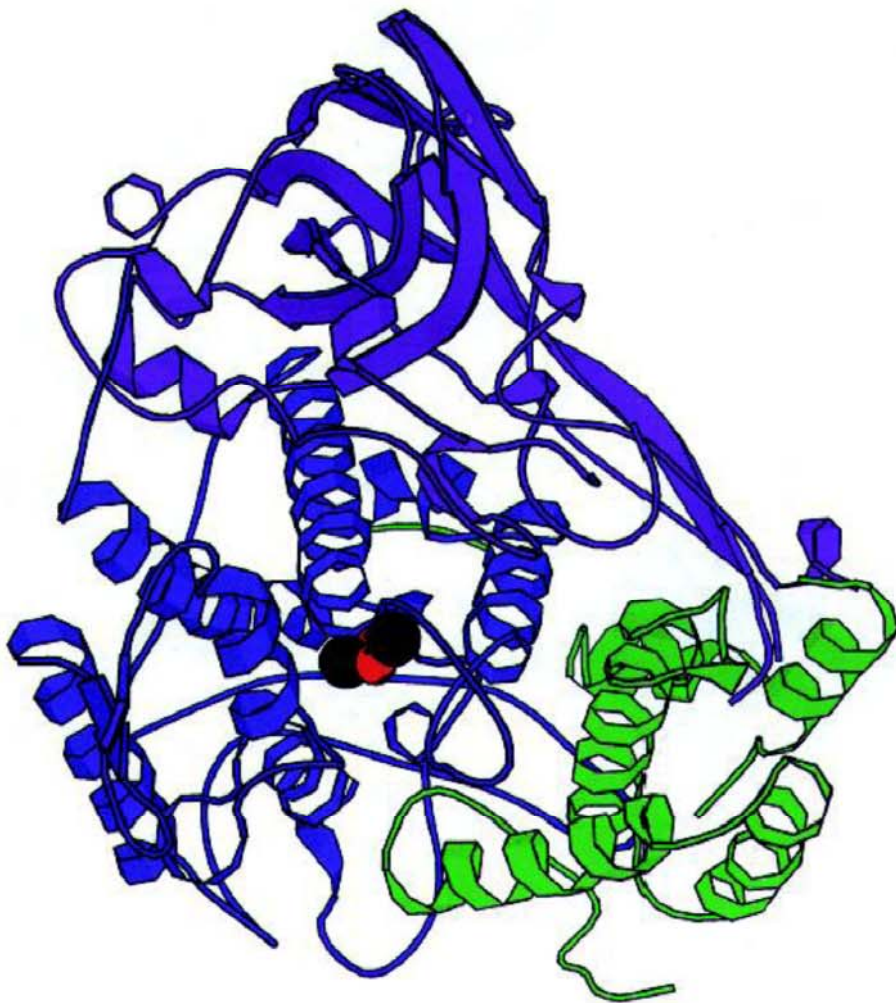


Classically, copper proteins are divided into three classes

- (1) The mononuclear blue copper proteins characterized by an extremely strong absorption band at ~600 nm, caused by a ligand–metal charge transfer between the sulphur of a cysteine residue and the copper. Most famous example: plastocyanin
- (2) The mononuclear non-blue copper proteins.
- (3) Proteins with a coupled binuclear copper centre.

More recently, further specialised types of copper centres have been found

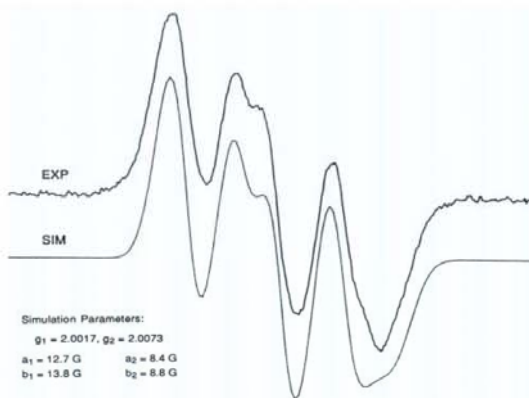
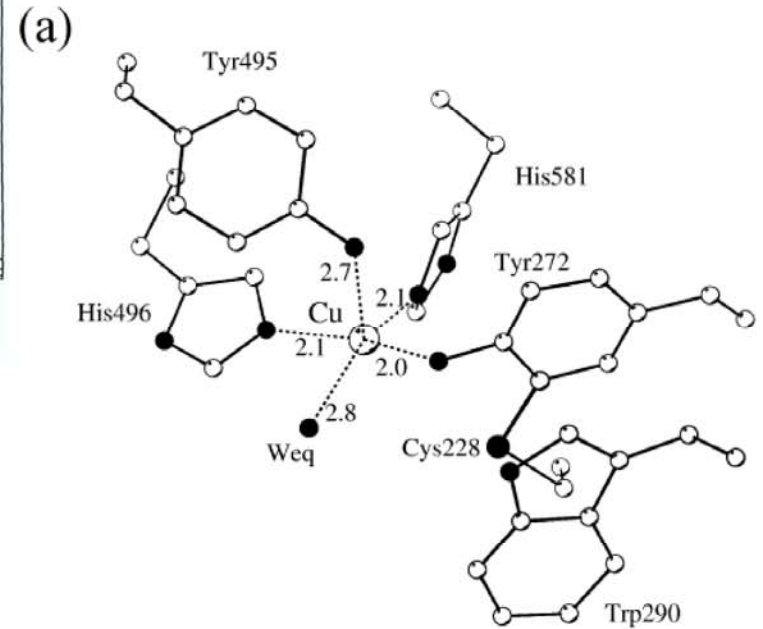
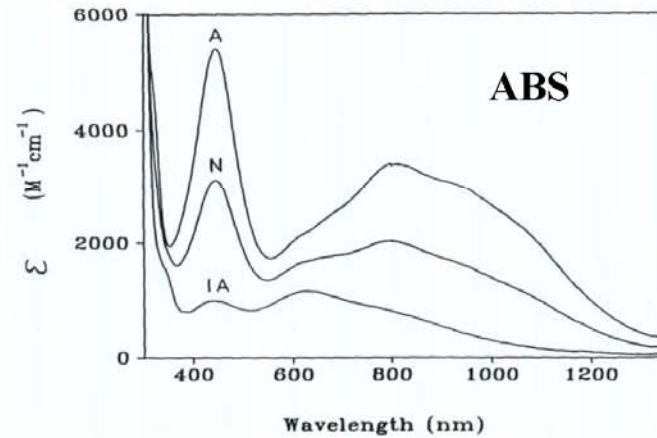
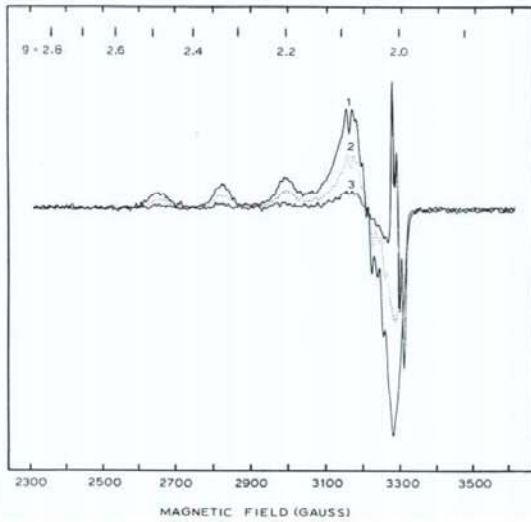
Hemocyanin (reversible O₂ binding)



Oxygenated Cu site, see Que, Tolman, NATURE (2008) 455, 333; oxygenated form has a blue colour, $\mu\text{-}\eta^2\text{:}\eta^2\text{-peroxo}$ binding mode

K Magnus, *Limulus polyphemus* (atlantic horseshoe crab) Hemocyanin, PDB 1OXY, Handbook of Metalloproteins (2001)

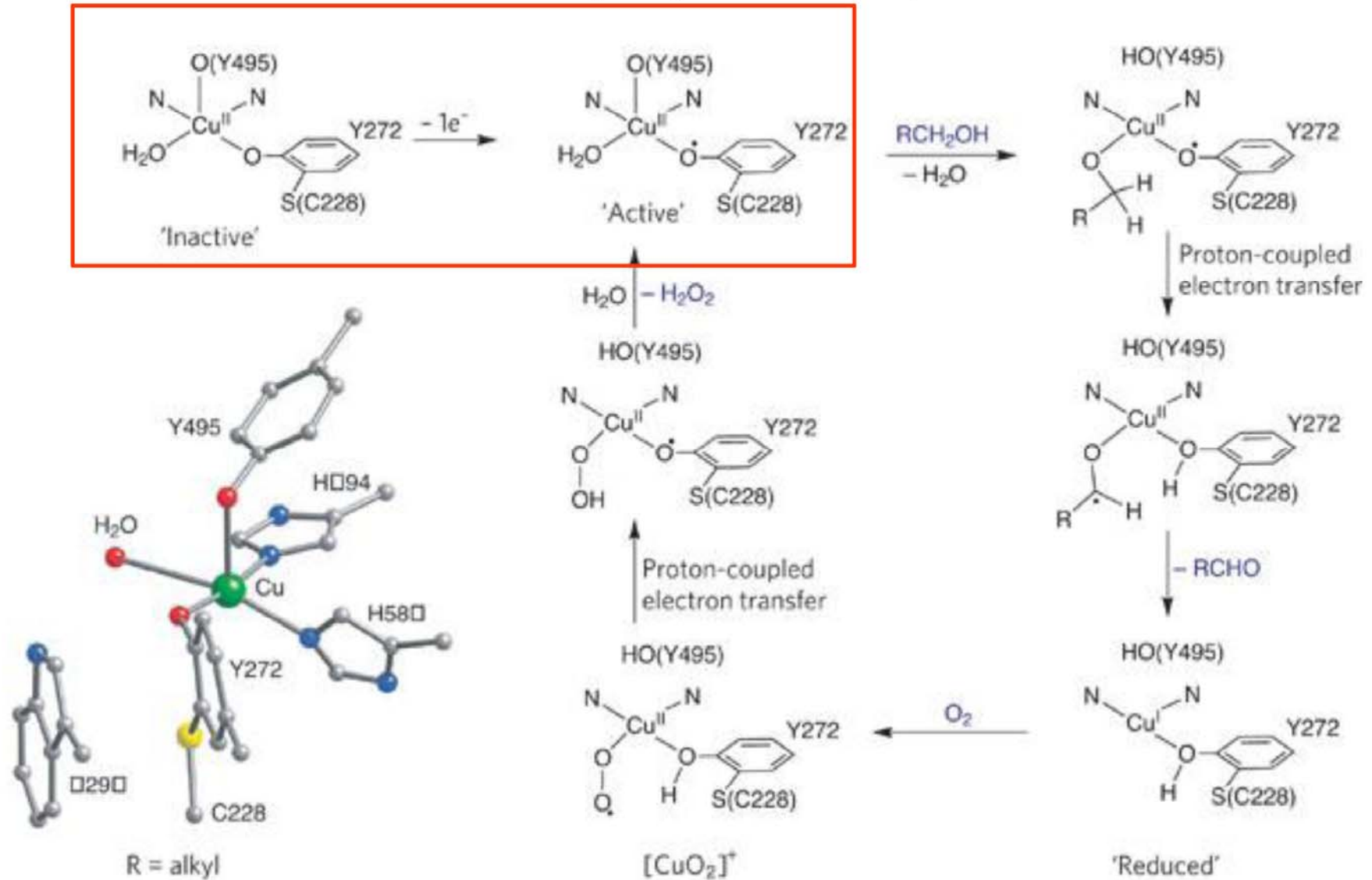
Galactose Oxidase, a Cu-Ligand Radical Enzyme



2 different EPR signals (metal-centered and ligand centered) observed during reaction cycle

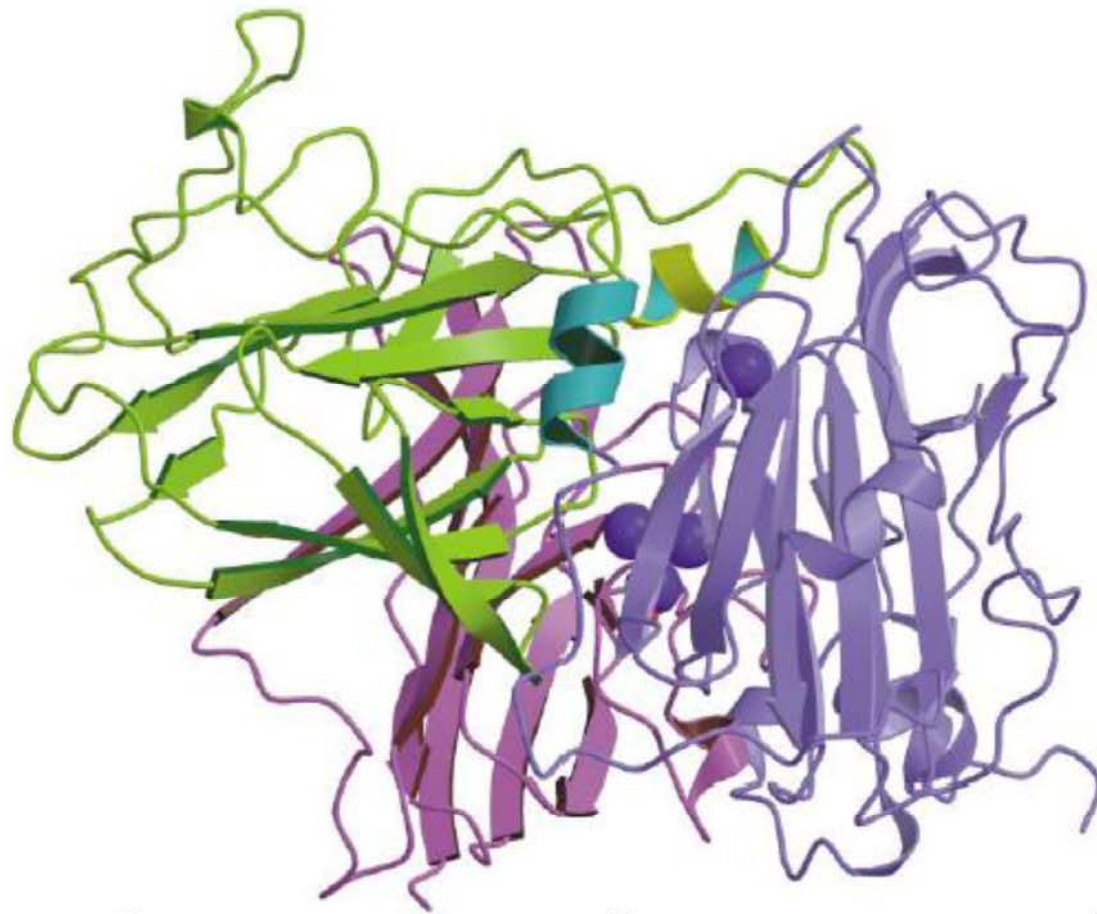
Galactose Oxidase - Mechanism

Que, Tolman (2008) NATURE 455, 333

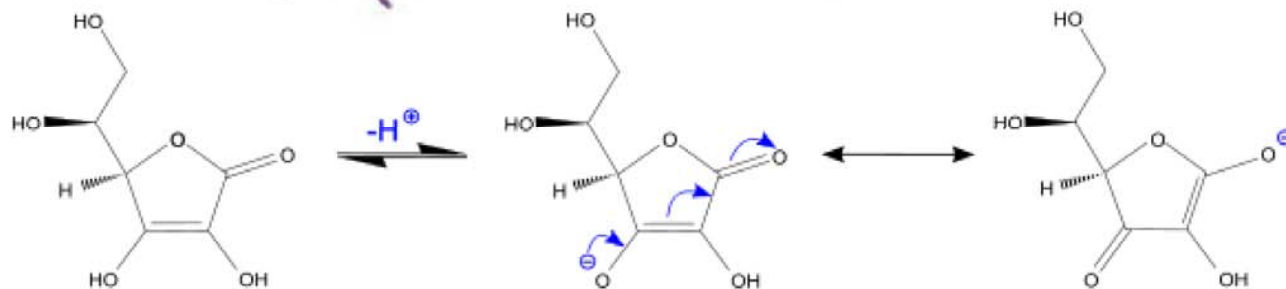
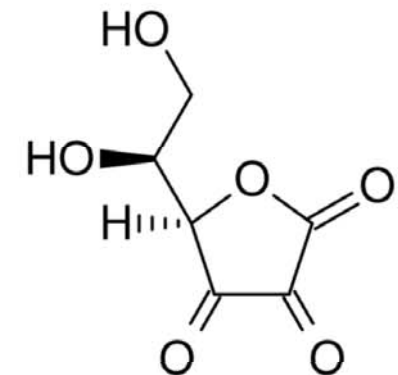
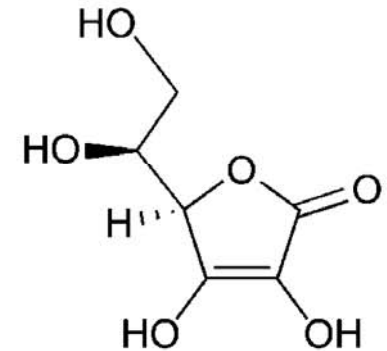


Ascorbic acid oxidase (AOX)

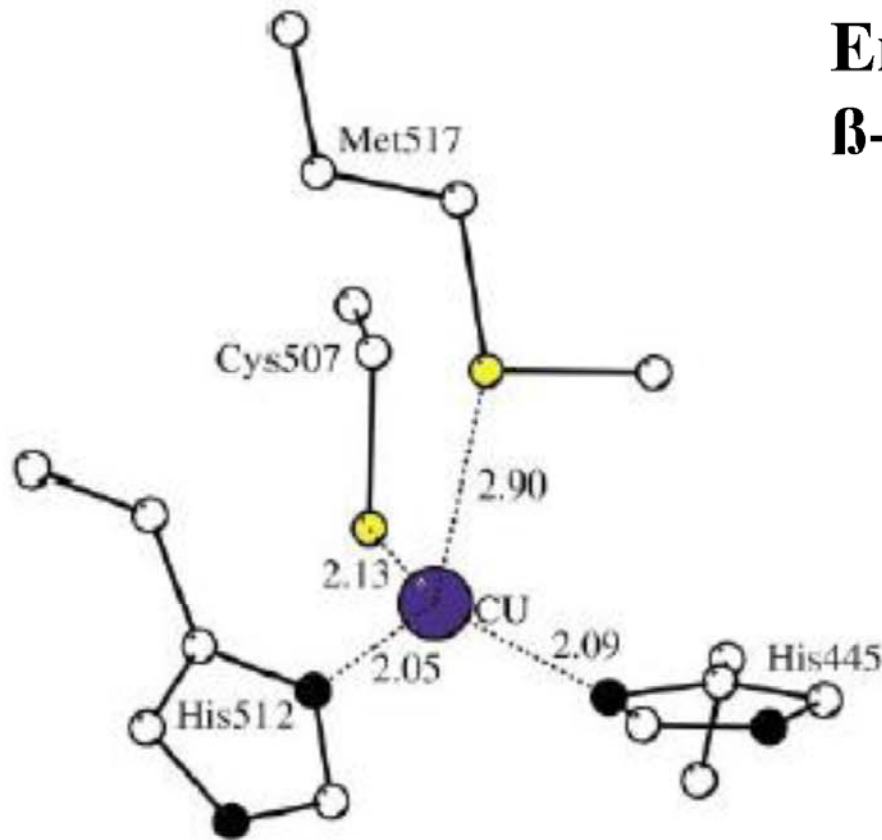
Multi-Copper oxidase (8Cu/homodimer) PDB code 1AOZ



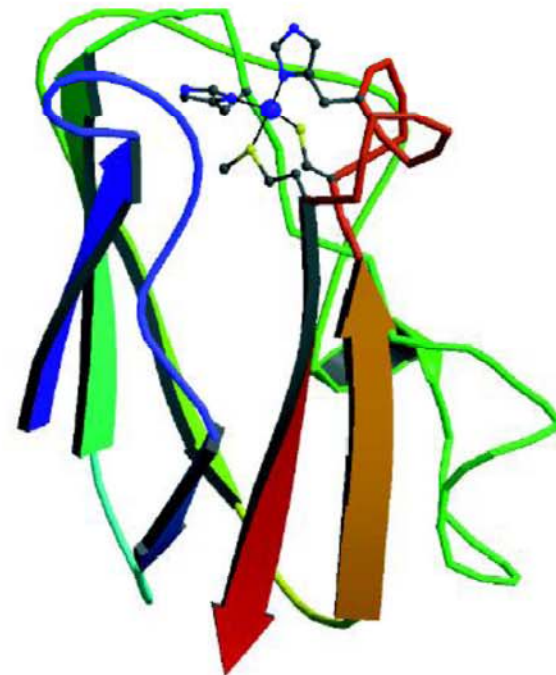
(5*R*)-[(1*S*)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one



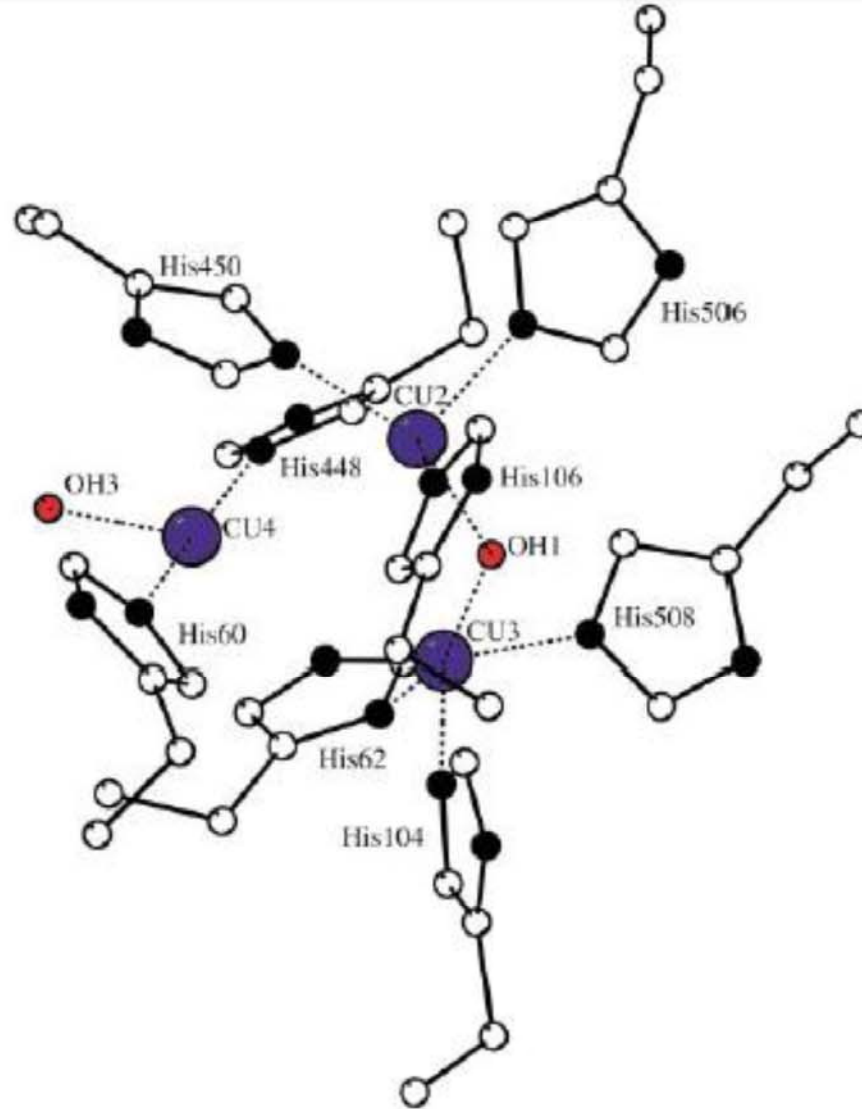
Type 1 electron transfer center of AOX: blue copper protein with high similarity to plastocyanin!



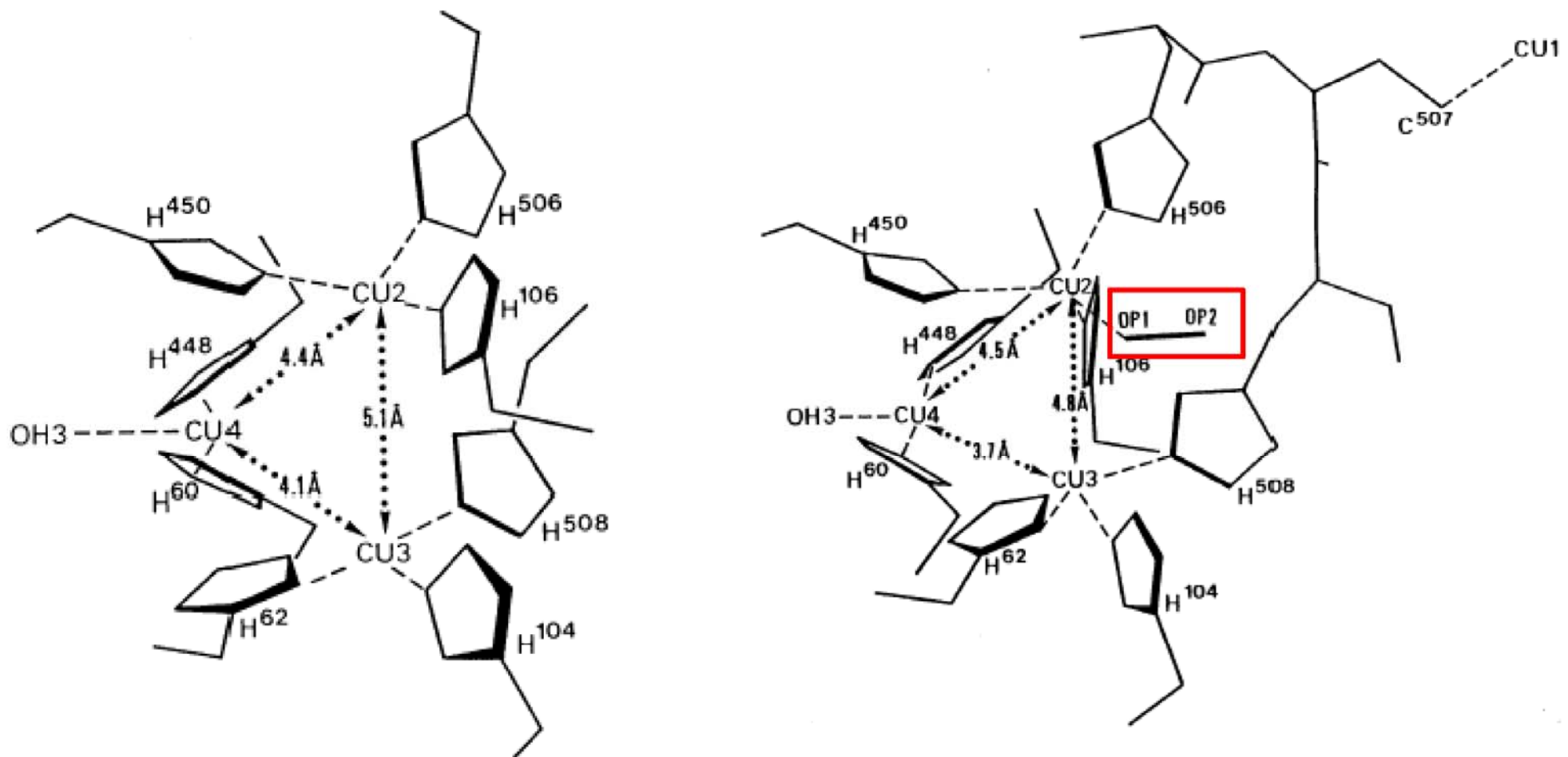
**Entrance point for electrons
 β -barrel structural module**



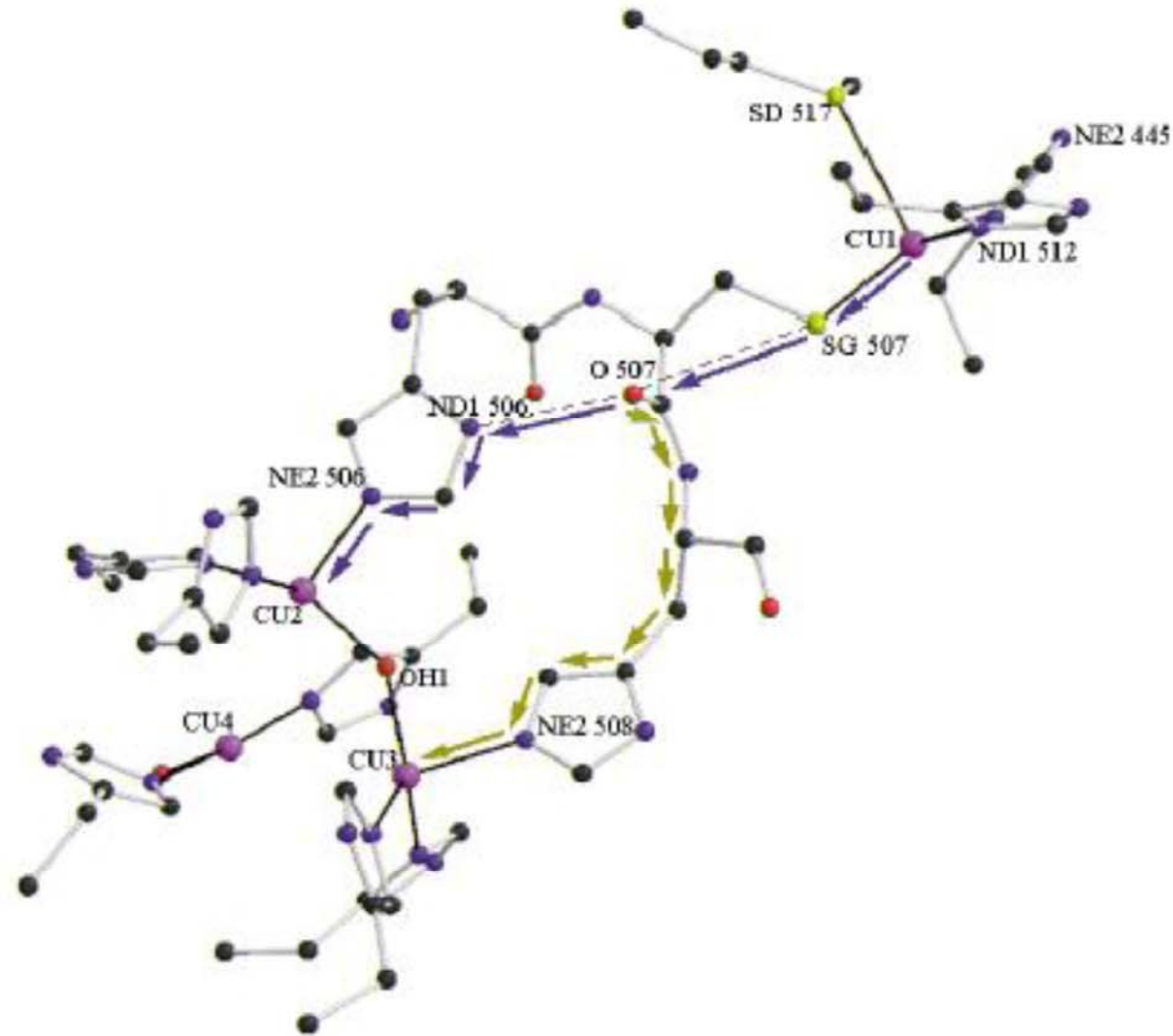
Dioxygen reduction site of AOX: unique trinuclear copper centre



Dioxygen reduction site of AOX: unique trinuclear copper centre - substrate binding

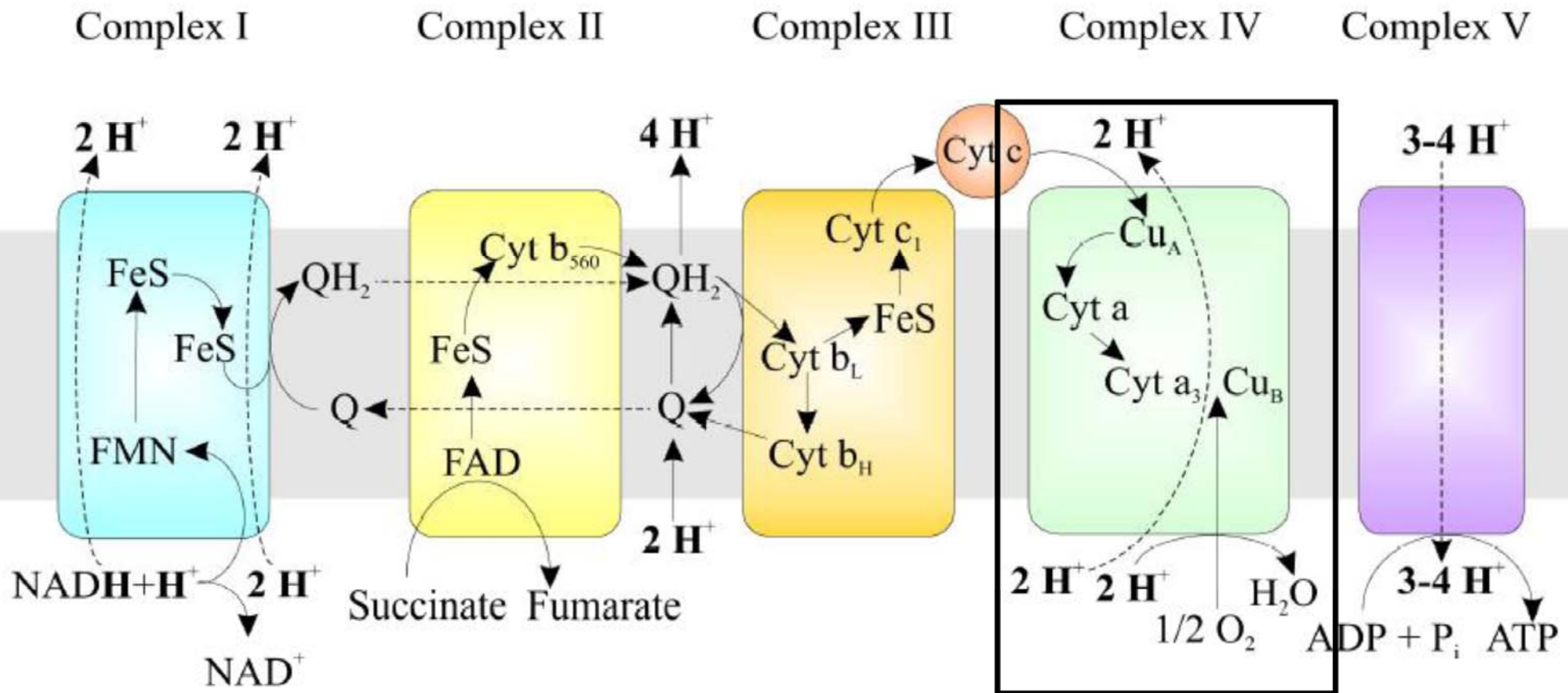
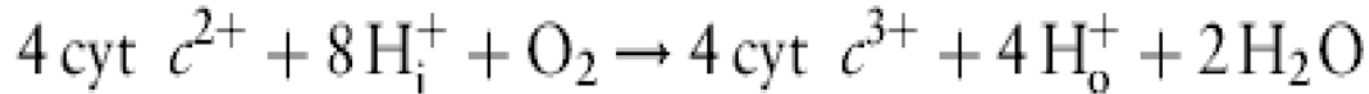


Ascorbic acid oxidase (AOX): electron transport pathways



Combining the best of Fe and Cu: Cytochrome c oxidase (COX), a redox-driven proton pump in the respiratory chain

Kaila, Verkhovsky, Wikstroem, Chemical Reviews (2010) 110, 7062–7081



COX: reaction scheme, alternative substrates, active centres

S. Yoshikawa, K. Muramoto, K. Shinzawa-Itoh *Annu. Rev. Biophys.* (2011) 40, 205–23

Tomoya Hino, et al. *SCIENCE* (2010) 330, 1666-1670

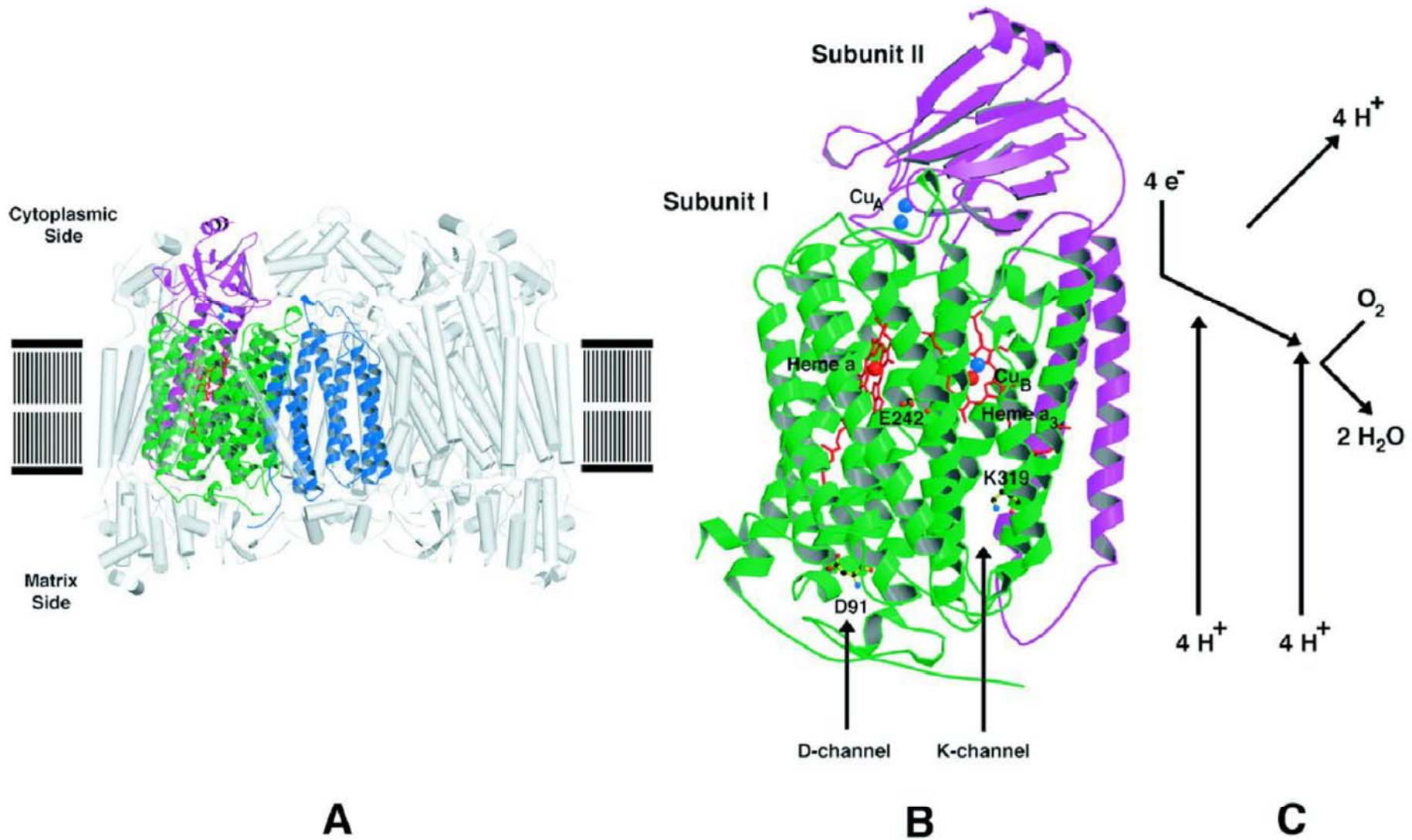


metals (CuA, Fe-heme, Mg, Zn)

e⁻ transfer (redox; tyrosyl radical ?), H⁺ transfer (pump)

metal centers: CuA → ET; Fe-CuB → O₂ reduction

COX: structure and reaction scheme

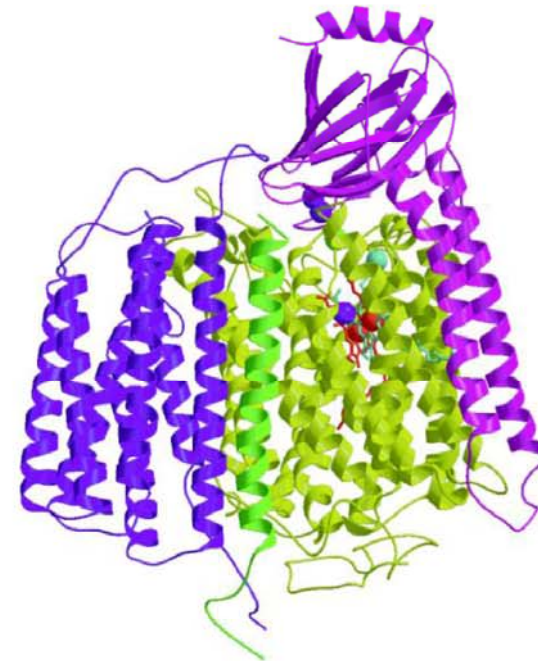
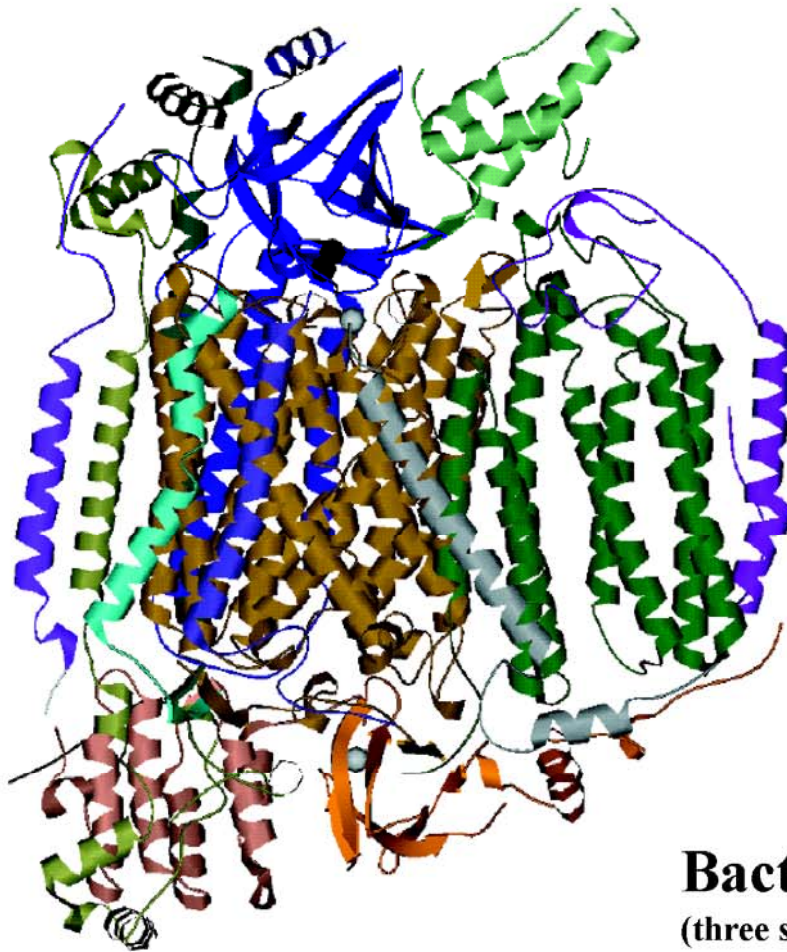


M Saraste Science (1999) 283,1488-1493

COX: structure with organism-specific variations

(representation of the monomer from bovine heart/13 subunits)

Tsukihara et al., SCIENCE 1995, 269, 1069; Yoshikawa et al., SCIENCE 1998, 280, 1723

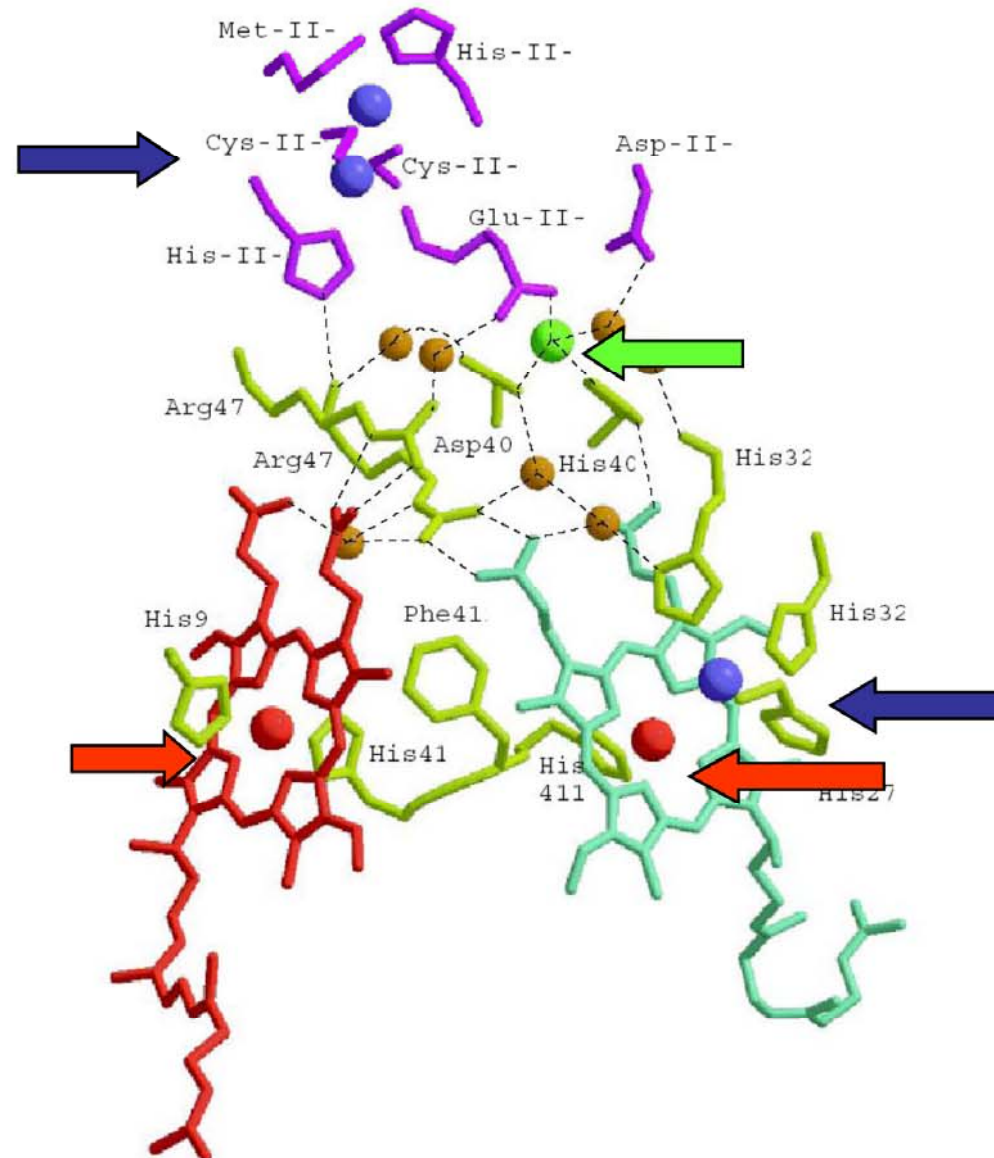


Bacterial COX from *Pseudomonas denitrificans*

(three subunits; Iwata et al., NATURE, 1995, 376, 660)

Metal Centers in bacterial COX

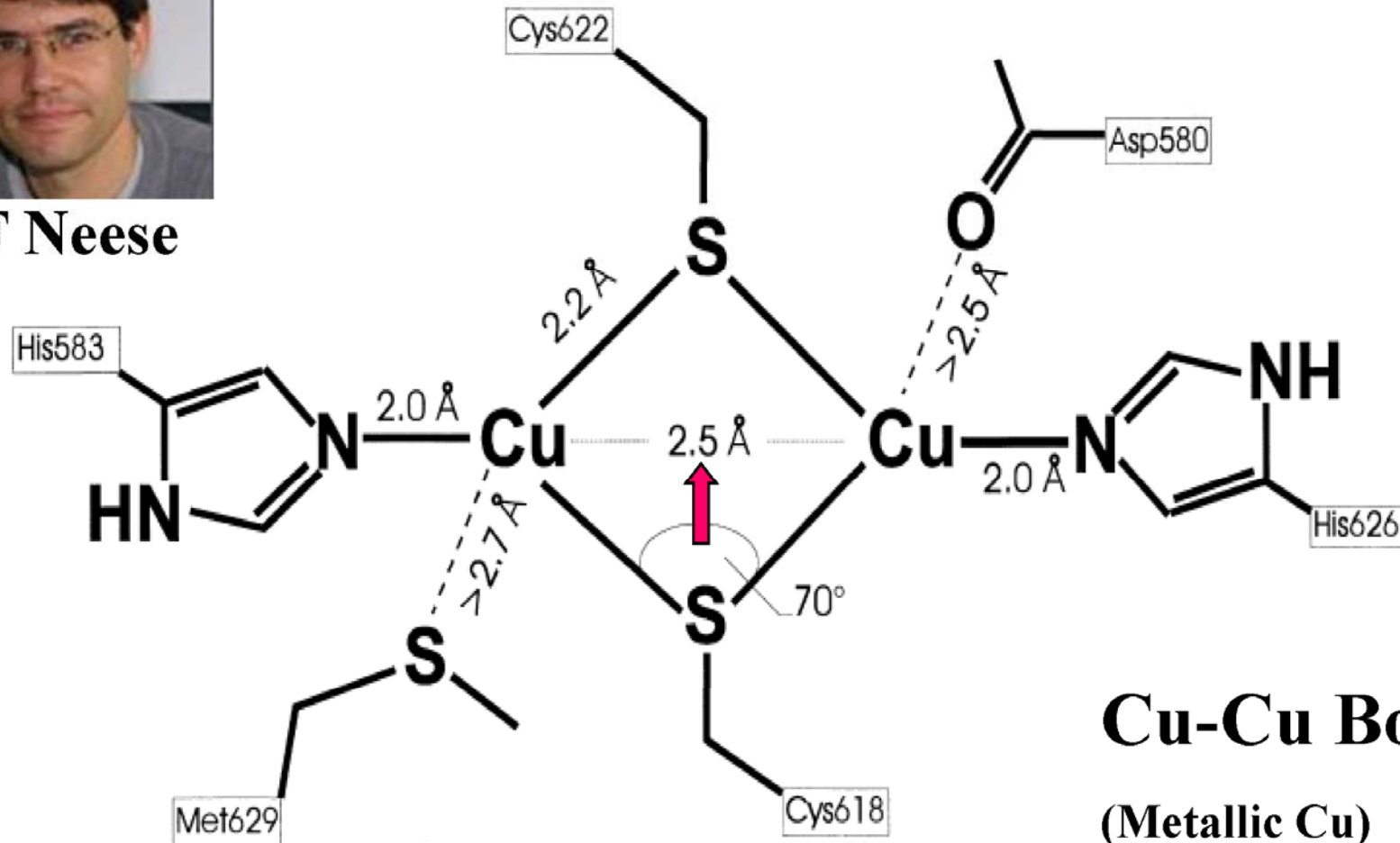
Cu Fe Mg



The CuA site of COX: a mixed-valence Cu_2S_2 rhomb formal oxidation state: $\text{Cu}^{1.5+}$, in reality 1 unpaired delocalised e^- per 2 Cu



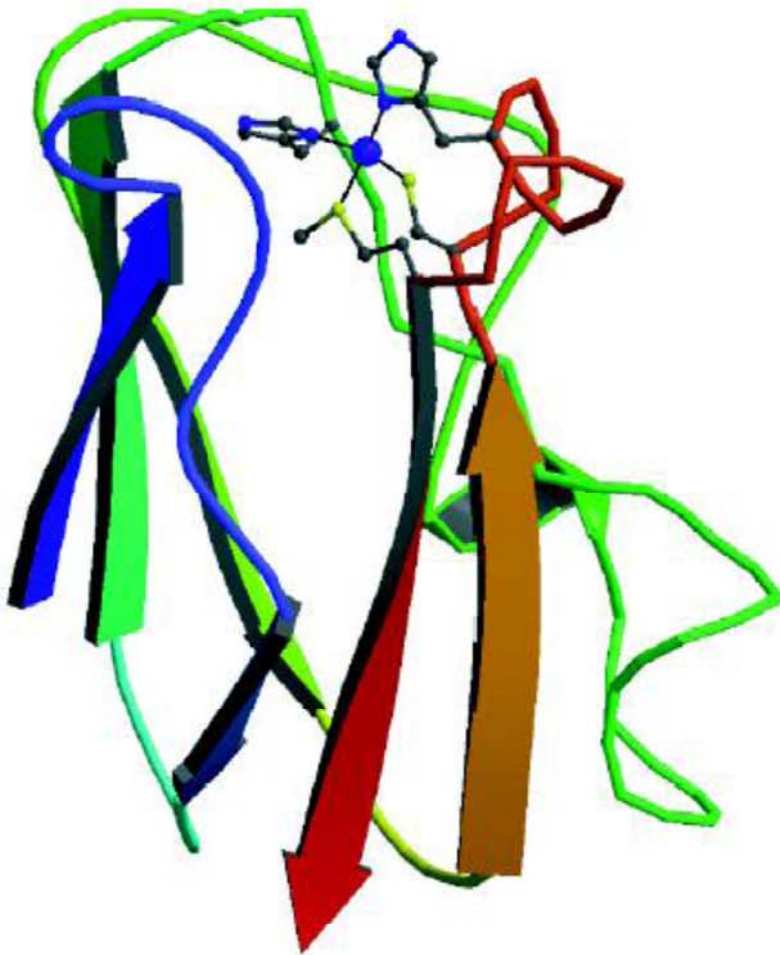
F Neese



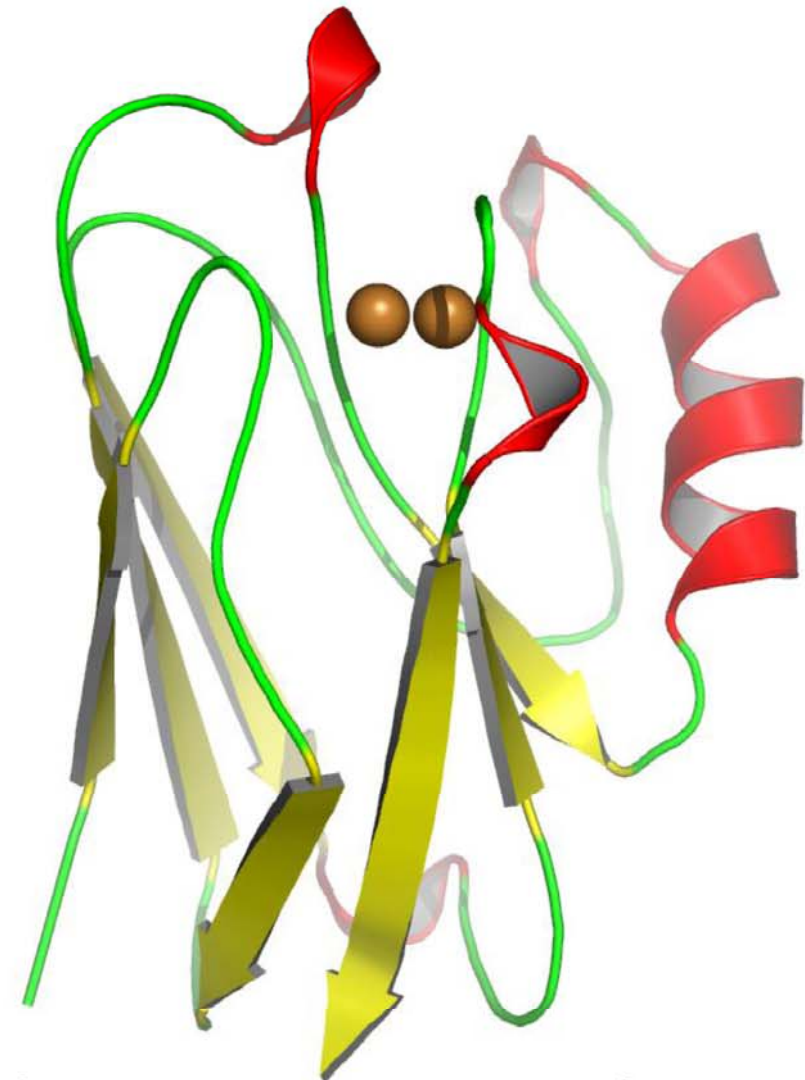
Evolution and Bioengineering through Loop directed Mutagenesis

From a Blue Mononuclear Cu to a Purple Dinuclear CuA

MG Savelieff, Y Lu, J Biol Inorg Chem, 15, 967-976 (2010)



Plastocyanin/Photosynthesis



Nitrous Oxide Reductase/Denitrification
Cytochrome c Oxidase/Respiration

**All slides of my lectures can be downloaded
from my workgroup homepage**

Biology Centre CAS → Institute of Plant Molecular Biology → Departments
→ Department of Plant Biophysics and Biochemistry,
or directly

http://webserver.umbr.cas.cz/~kupper/AG_Kuepper_Homepage.html