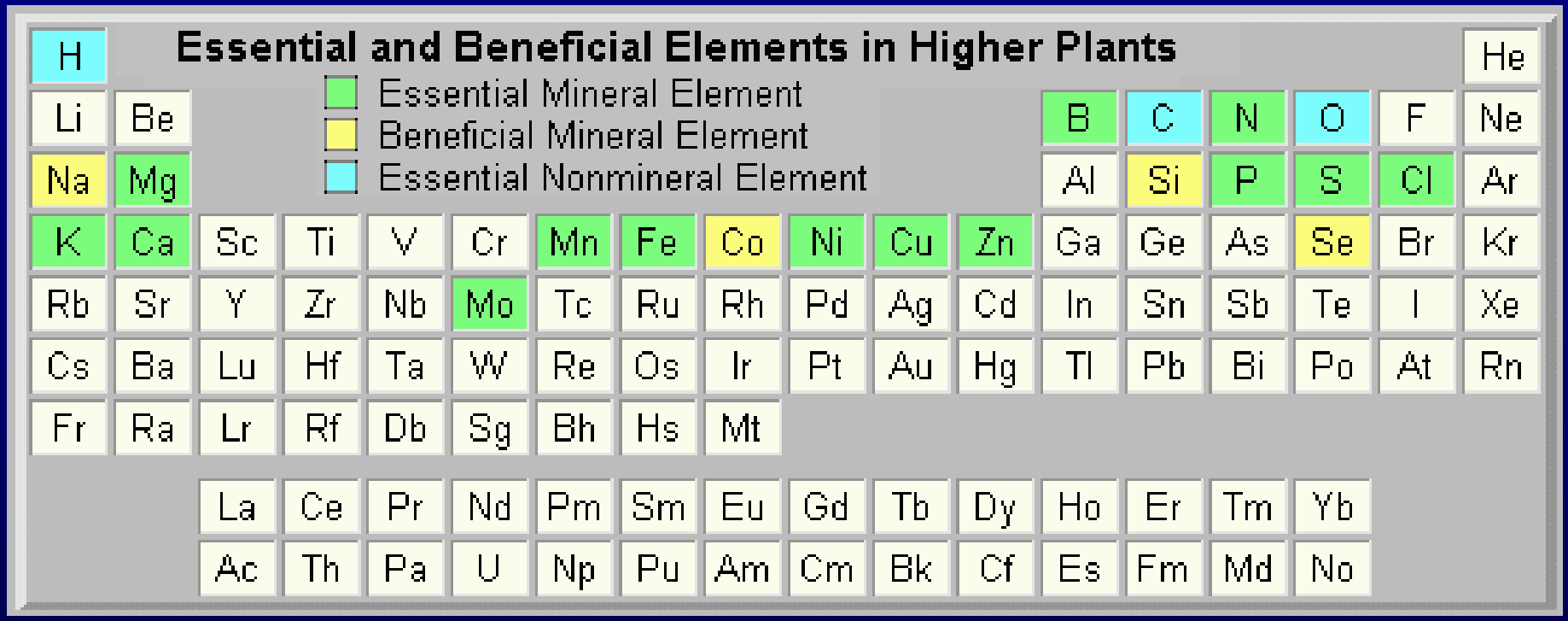
Copper and iron proteins

Hendrik Küpper, Advanced Course on Bioinorganic Chemistry & Biophysics of Plants, summer semester 2021
based on a lecture of Peter Kroneck, Universität Konstanz

Elements that are known to be essential for plants



Typical roles of copper and iron proteins

→ Reductions and oxidations of substrates

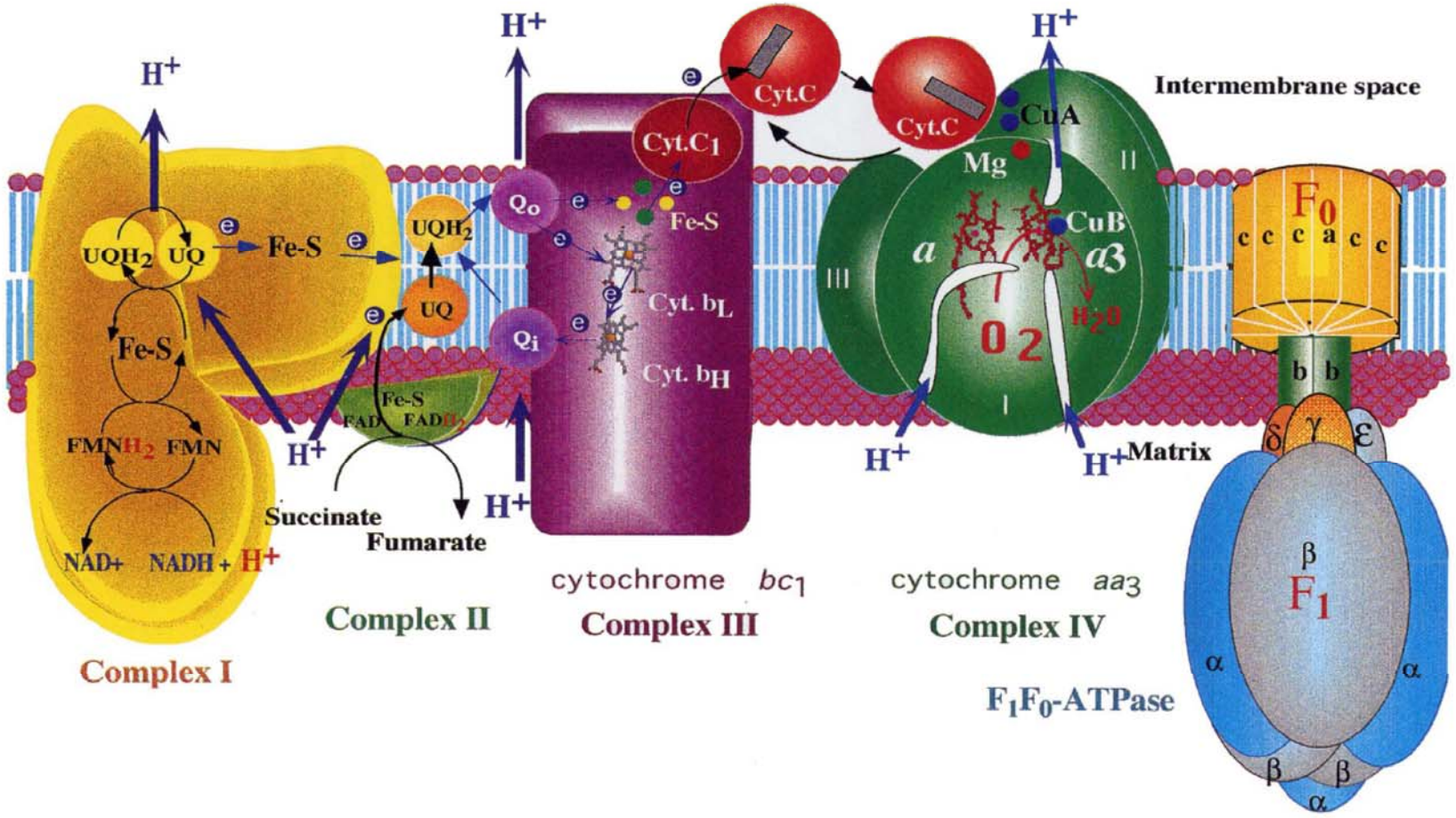
→ Oxygen transport

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

periodic table from http://soils.wisc.edu/facstaff/barak/soilscience326/periodic.gif

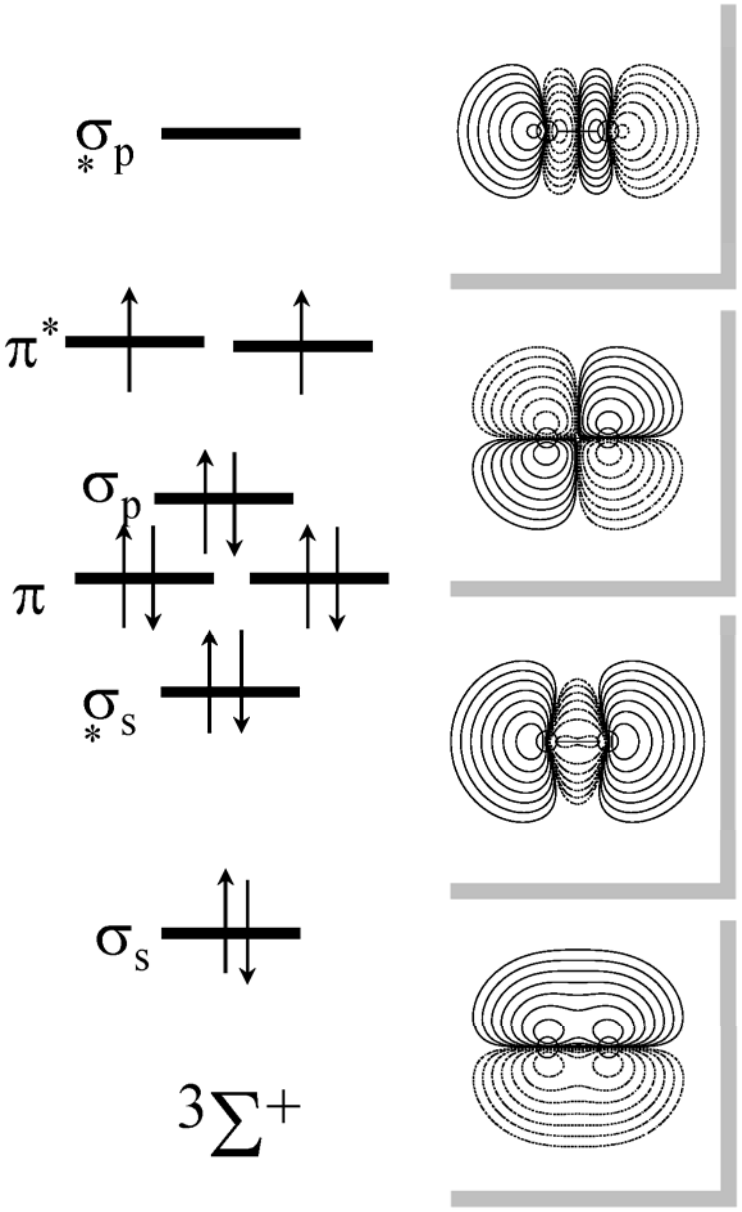
Respiration = reduction of O_2 to H_2O

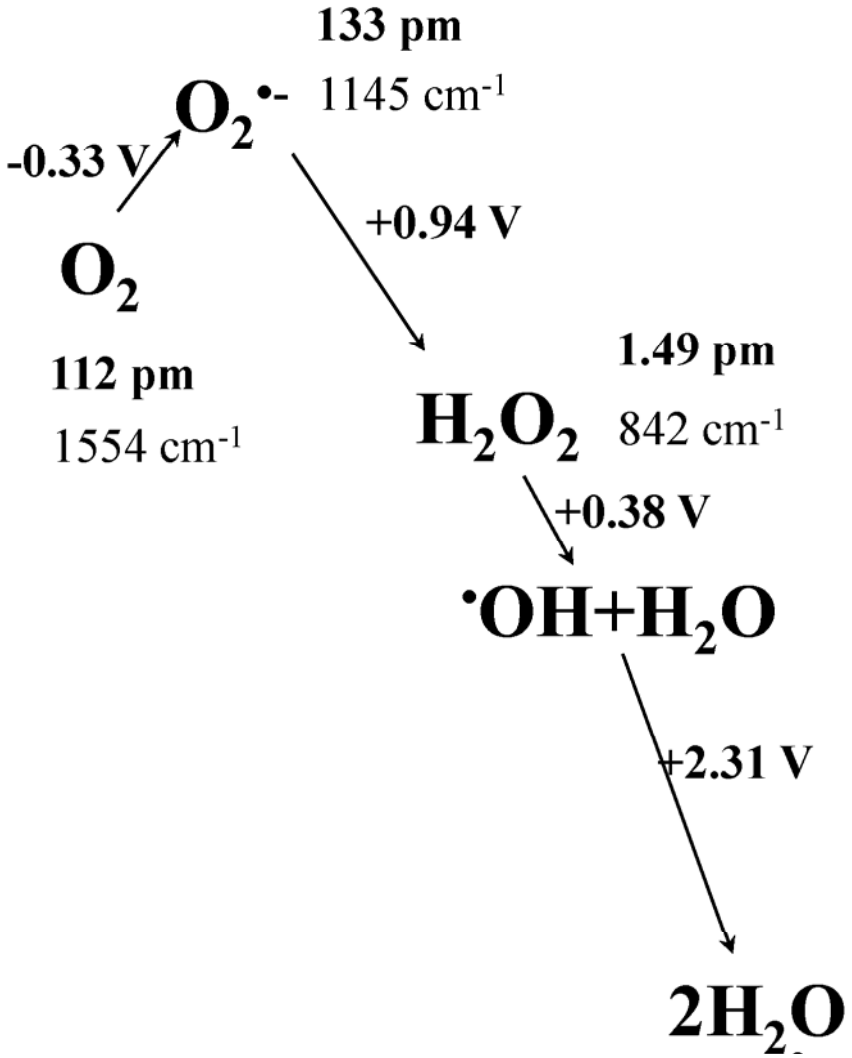
Synthesis of ATP – proton-coupled electron transfer (PCET)



based on a lecture slide of Peter Kroneck

Dioxygen Activation





E_0 *vs.* NHE at pH 7.25

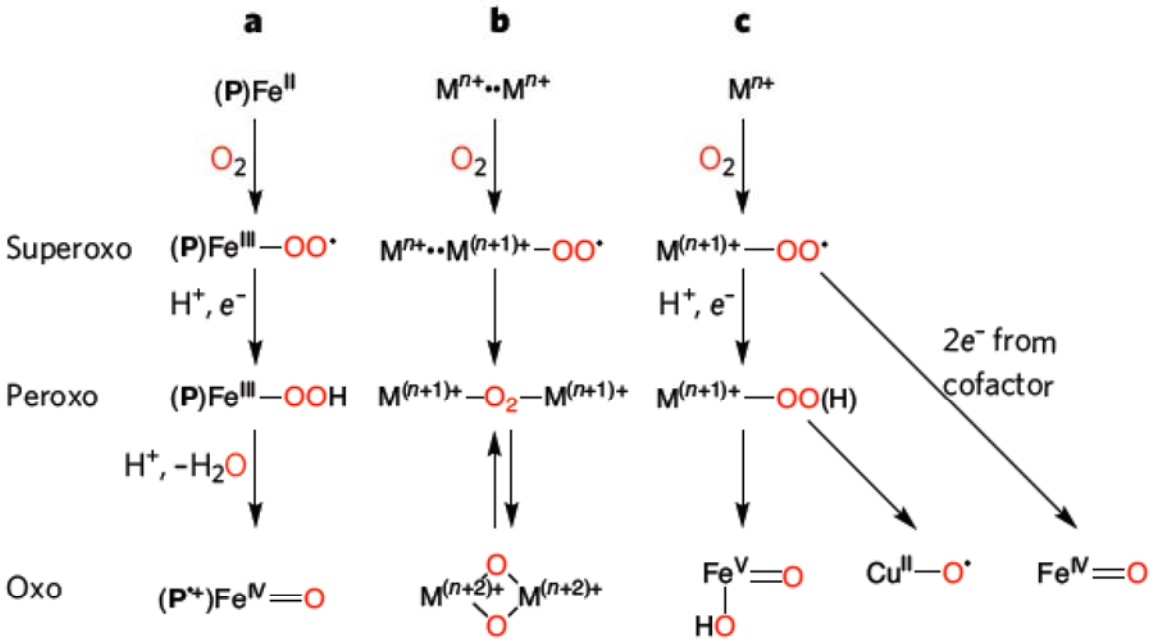
Activation of O_2 – Reaction Types

- **Reversible binding of O_2 – Myoglobin, Hemoglobin (Fe), Hemocyanin (Cu-Cu)**
- **$O_2^{\cdot -}$ dismutation – Superoxide Dismutase (Mn, Fe, Ni, Cu, Zn)**
  $O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow O_2 + H_2O_2$
- **H_2O_2 decomposition – Catalase (Mn, heme-Fe)**
  $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$
- **Oxygenases (focus on Monooxgenase Cytochrome P450)**
  $R\text{-}H + O_2 + NADPH + H^+ \rightarrow R\text{-}OH + H_2O + NADP^+$
- **Oxidases (2-electron reduction to H_2O_2; Fe, Cu)**
  $O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$ (focus on Cu enzyme Galactose Oxidase)
- **Oxidases (4-electron reduction to H_2O; heme-Fe, Cu)**
  $O_2 + 4e^- + 4H^+ \rightarrow 2\ H_2O$ (focus on Cu enzyme Ascorbic Acid Oxidase and Fe,Cu enzyme Cytochrome *c* Oxidase)

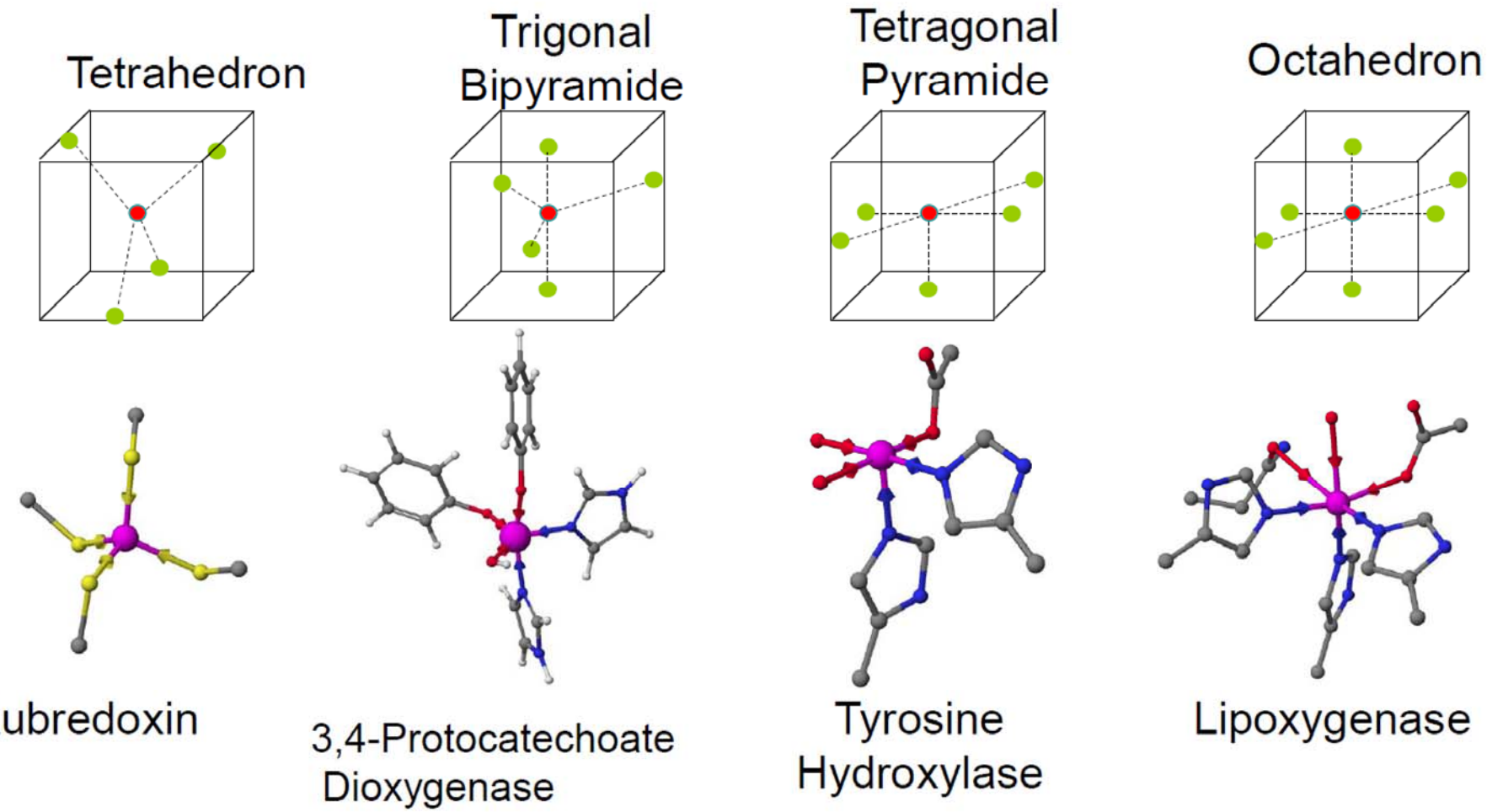
O_2 activation by Metallo-Oxygenases

Mechanisms involve the formation of an initial O_2 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



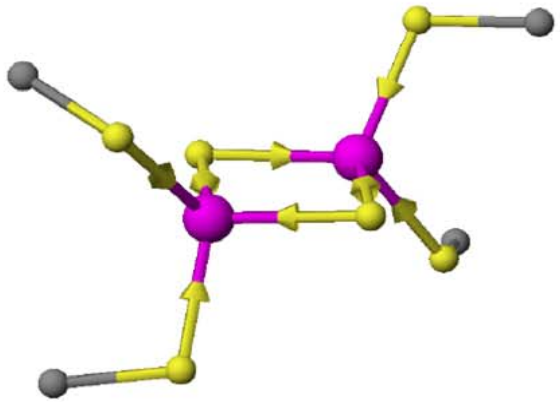
Geometry is important: Iron Proteins



El Solomon, *et al.* (**2000**), Chem. Rev., *100*, 235–350

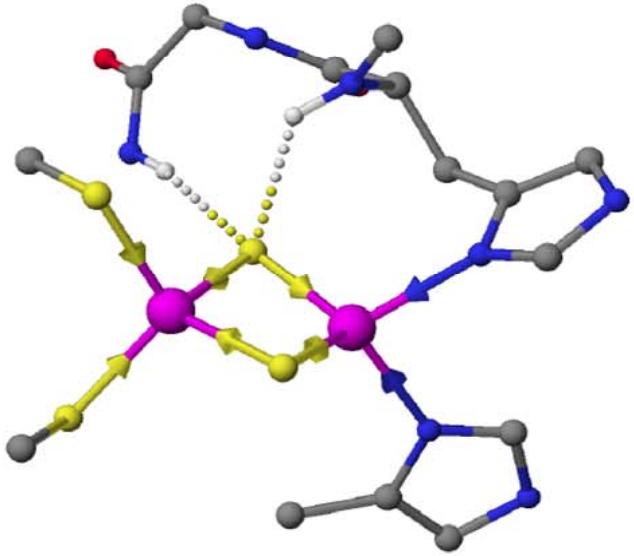
Modulation of Redox potentials (H bridges)

2Fe-2S Ferredoxin



$E^{0`}$ ~ -400 mV

2Fe-2S Rieske



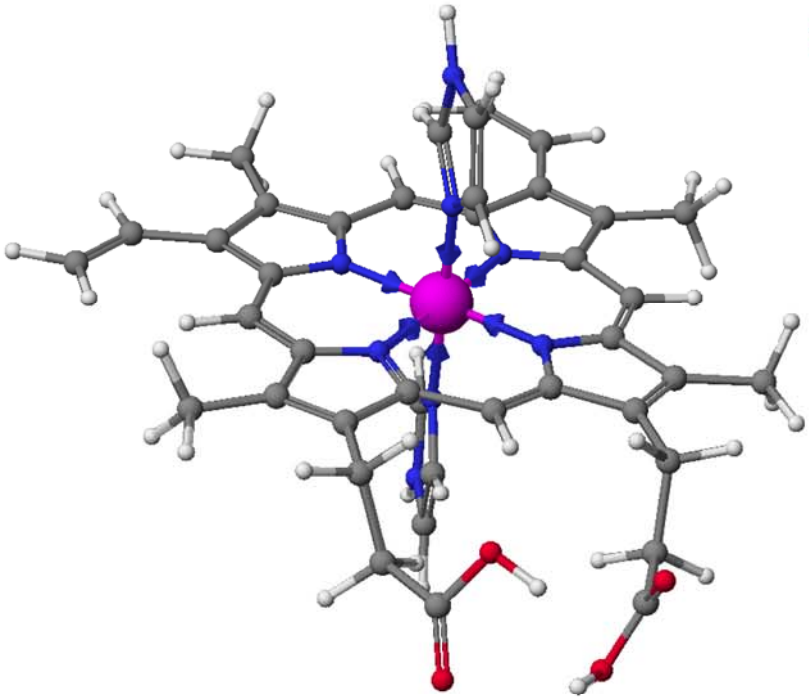
$E^{0`}$ ~ +280 mV

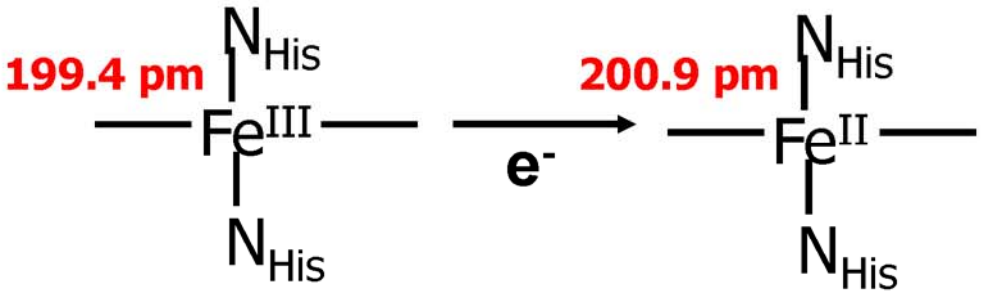
(+150 mV without H bridges)

(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





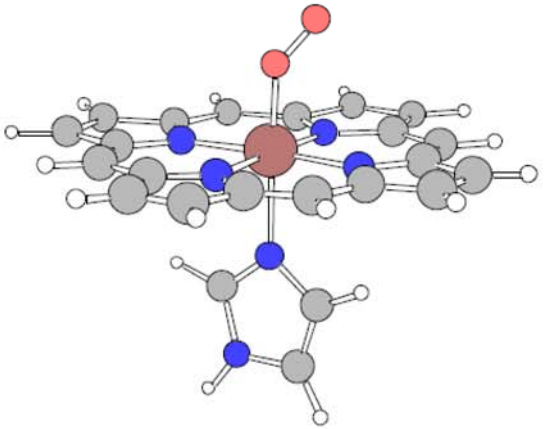
Reorganization Energy

in Cytochromes ≤ 4-5 kcal/mol

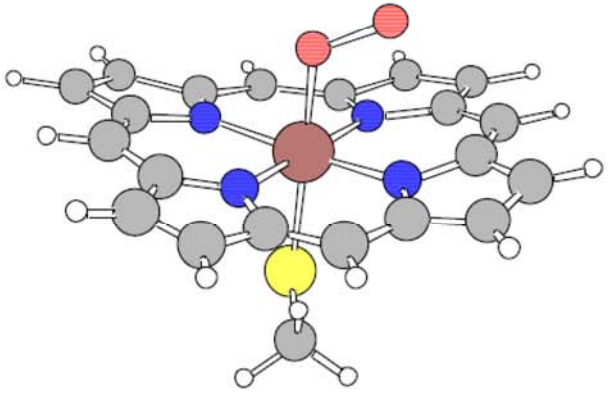
Sigfridsson, E.; Olsson, M.H.M.; Ryde, U. (**2001**) *J. Phys. Chem. B*, 105, 5546

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
O_2 Transport

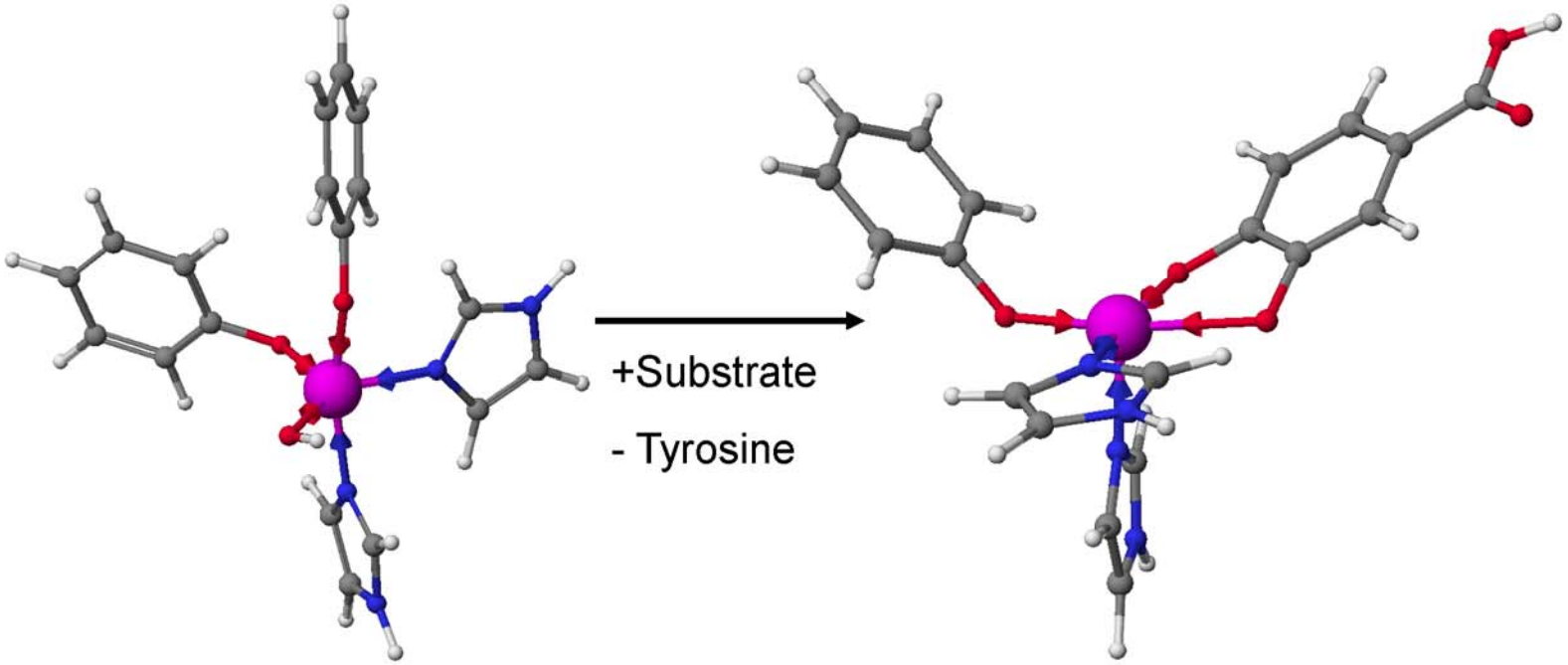


Cytochrome P450
Axial Cysteine
O_2 Activation

$$RH + O_2 + 2H^+ + 2e^- \rightarrow R\text{-}OH + H_2O$$

Opening of Substrate Binding Sites in Enzyme

3,4 Protocatechuate Dioxygenase

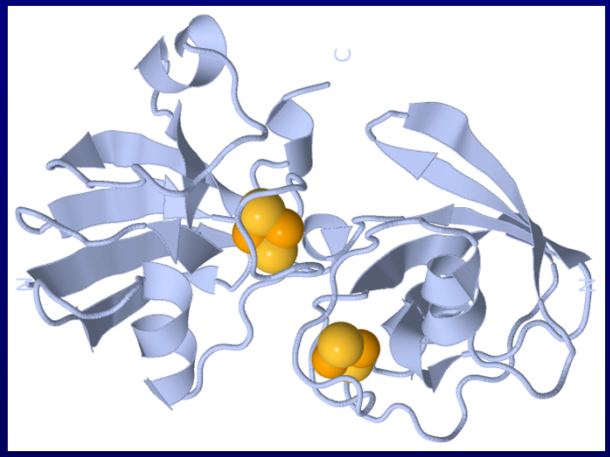


Davis, M.I.; Orville, A.M.; Neese, F.; Zaleski, J.; Lipscomb, J.D.; Solomon, E.I. (**2002**) *J. Am. Chem. Soc. 124, 602*

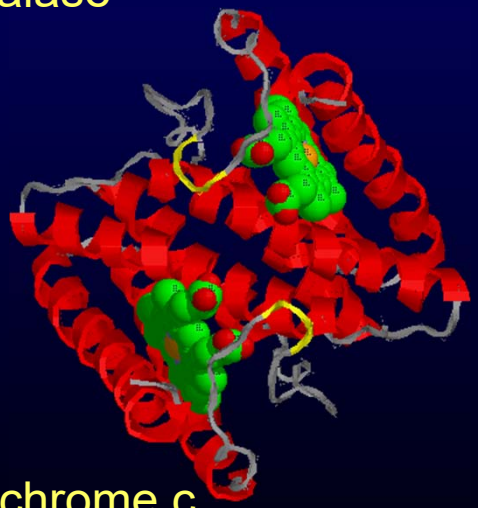
Examples of plant enzymes with iron in the active centre

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

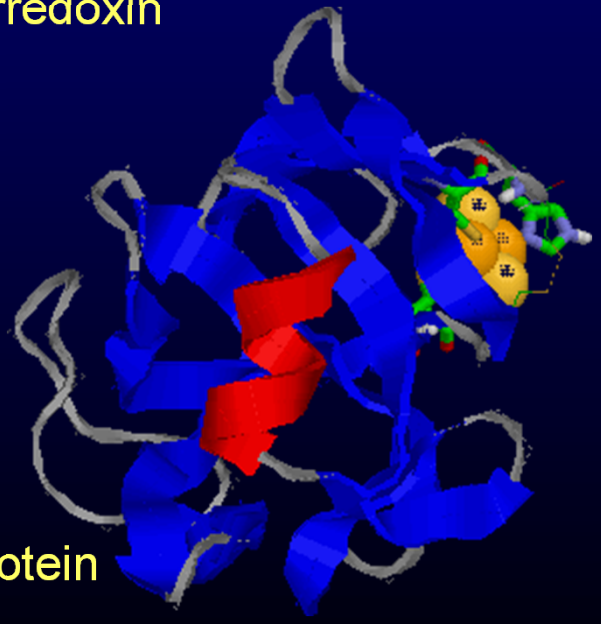
Catalase



Ferredoxin

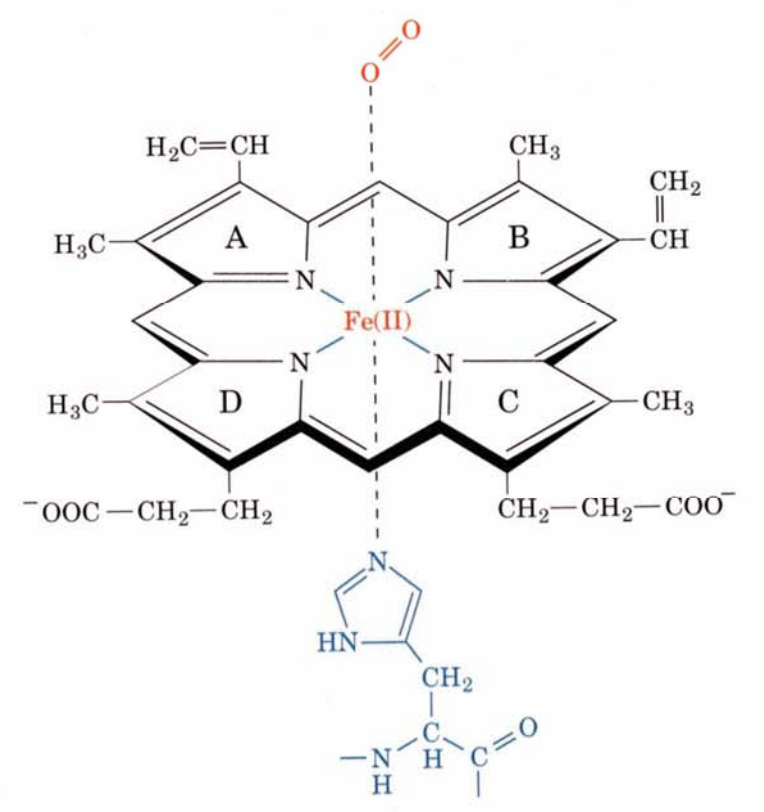


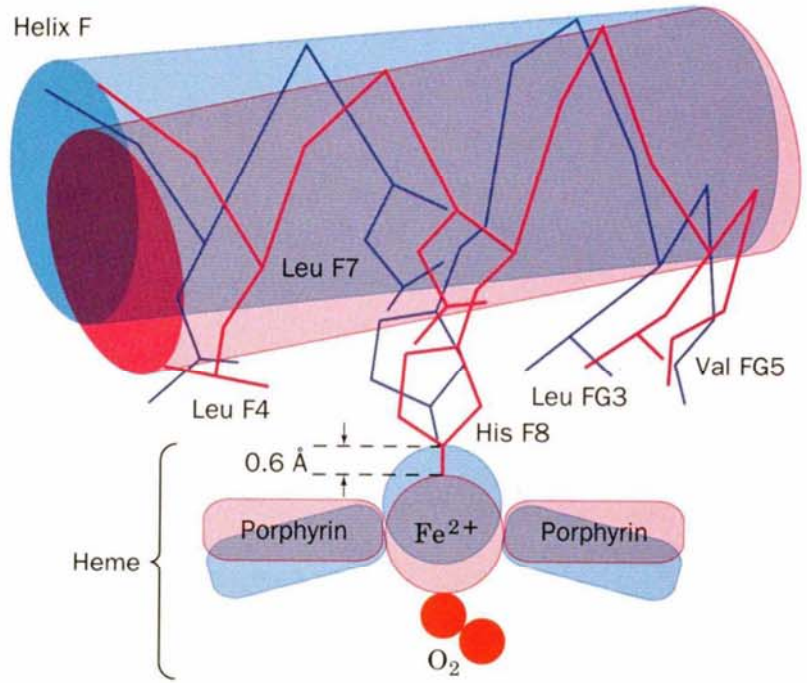
Cytochrome c



Rieske protein

Reversible O_2 Binding Myoglobin and Hemoglobin





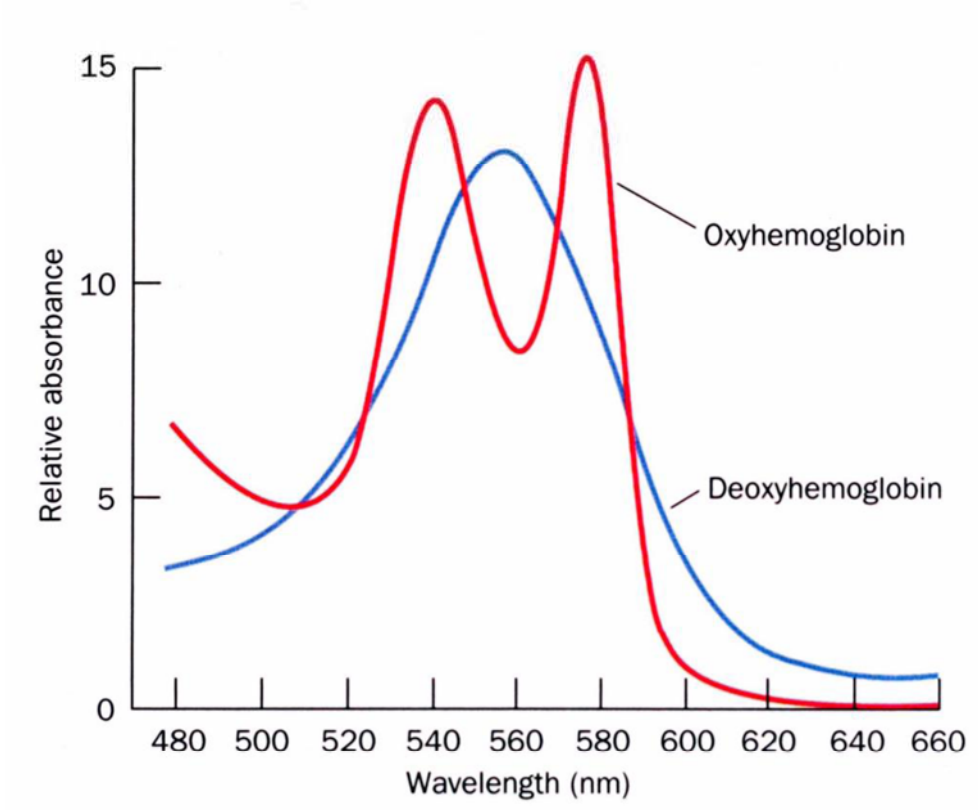
The iron must be in the Fe(II) (ferrous oxidation) state.

Binding of O_2 rearranges the electronic distribution and alters the d orbital energy.

This causes a difference in the absorption spectra.

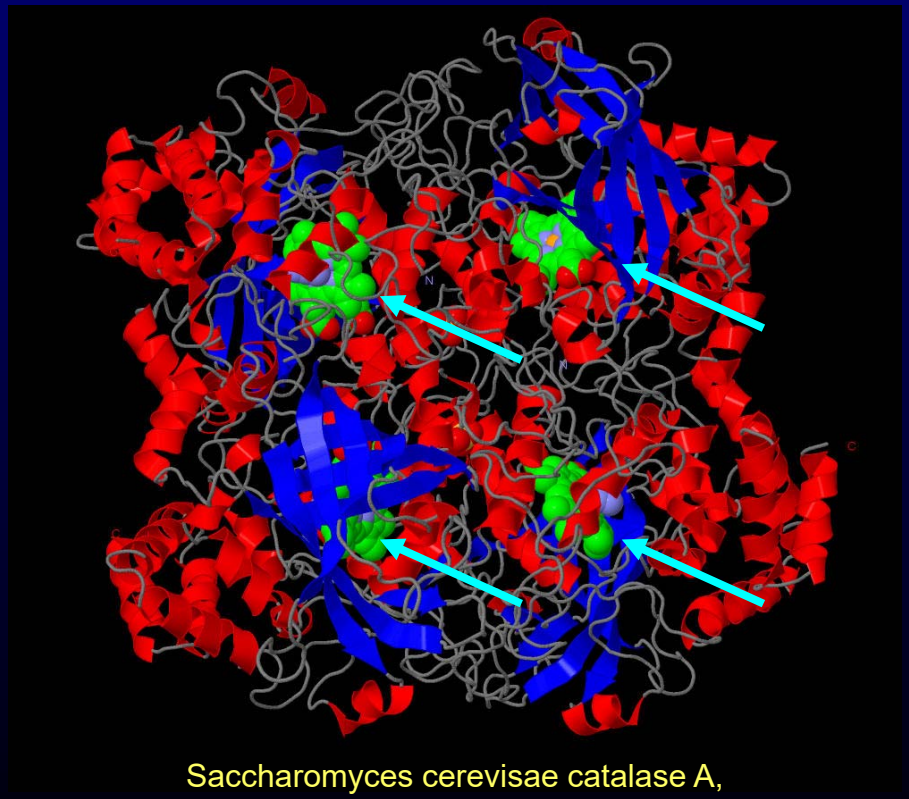
Bluish for deoxy Hb, Redish for Oxy Hb

Measuring the absorption at 578 nm allows an easy method to determine the percent of O_2 bound to hemoglobin.

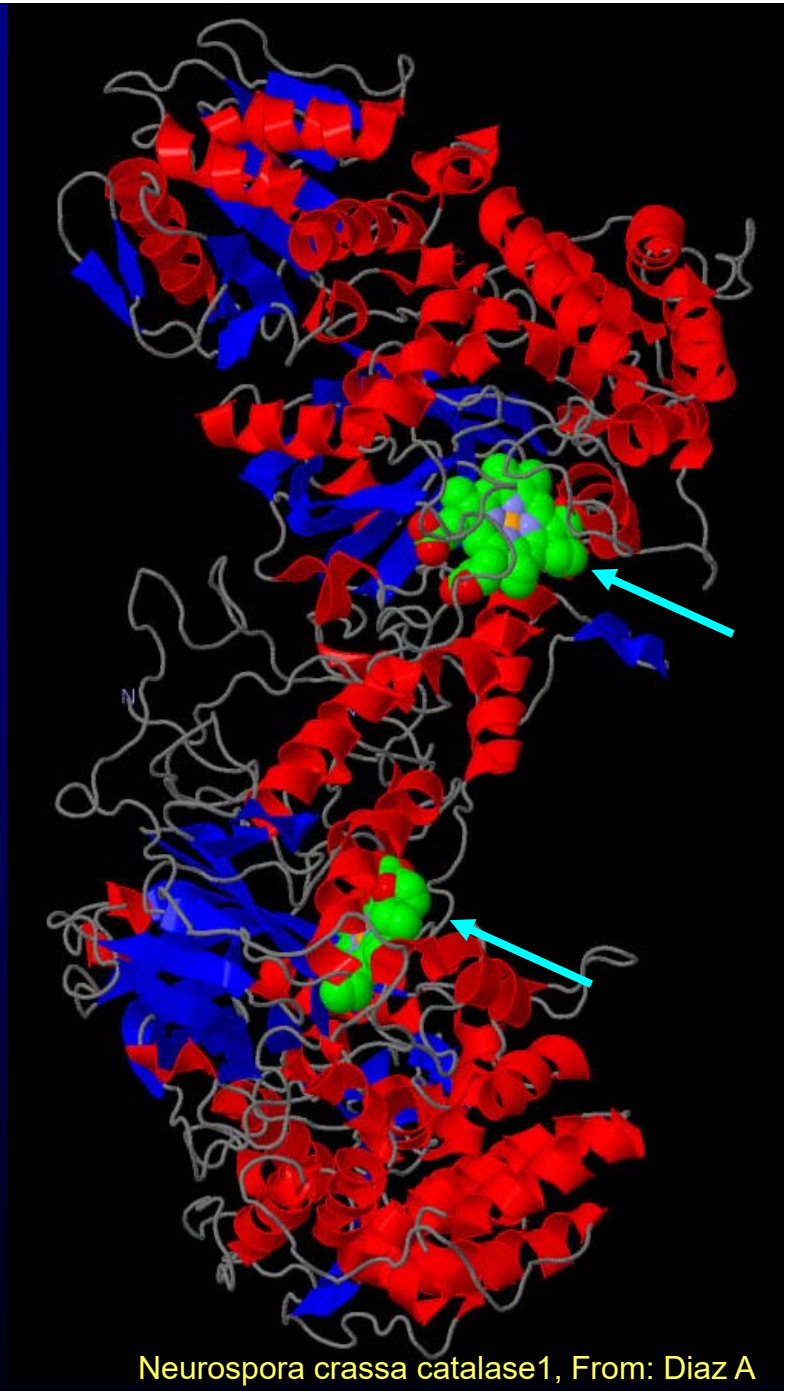


Catalases, some of the most important heme-iron enzymes: (a) function and structure

- 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



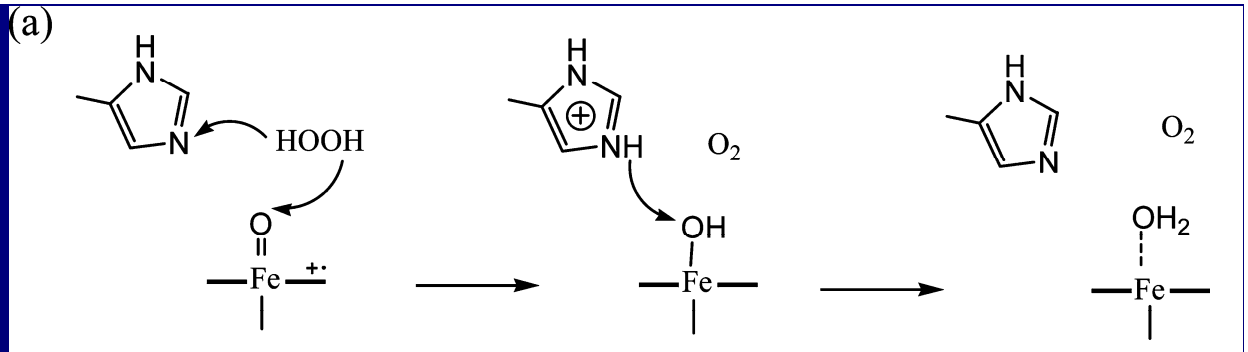
Saccharomyces cerevisae catalase A,
From: Maté JM et al, 1999, J Mol Biol 268, 135-49



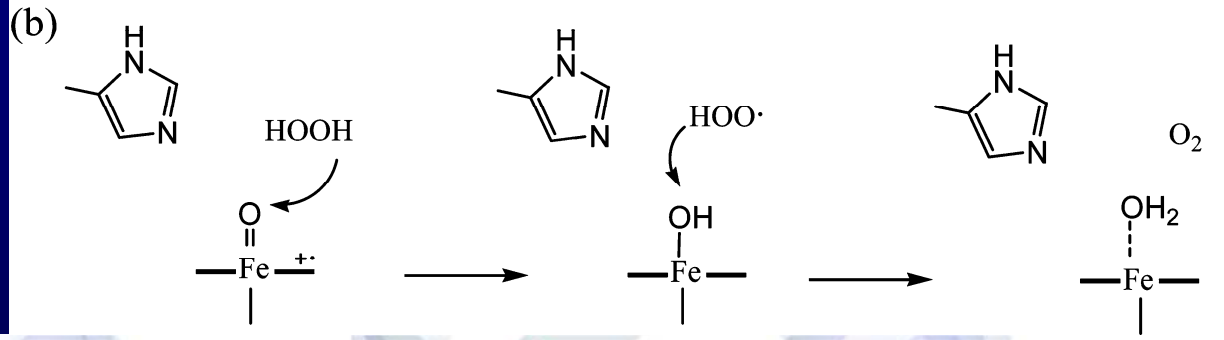
Neurospora crassa catalase1, From: Diaz A et al, 2004, J Mol Biol 342, 971-85

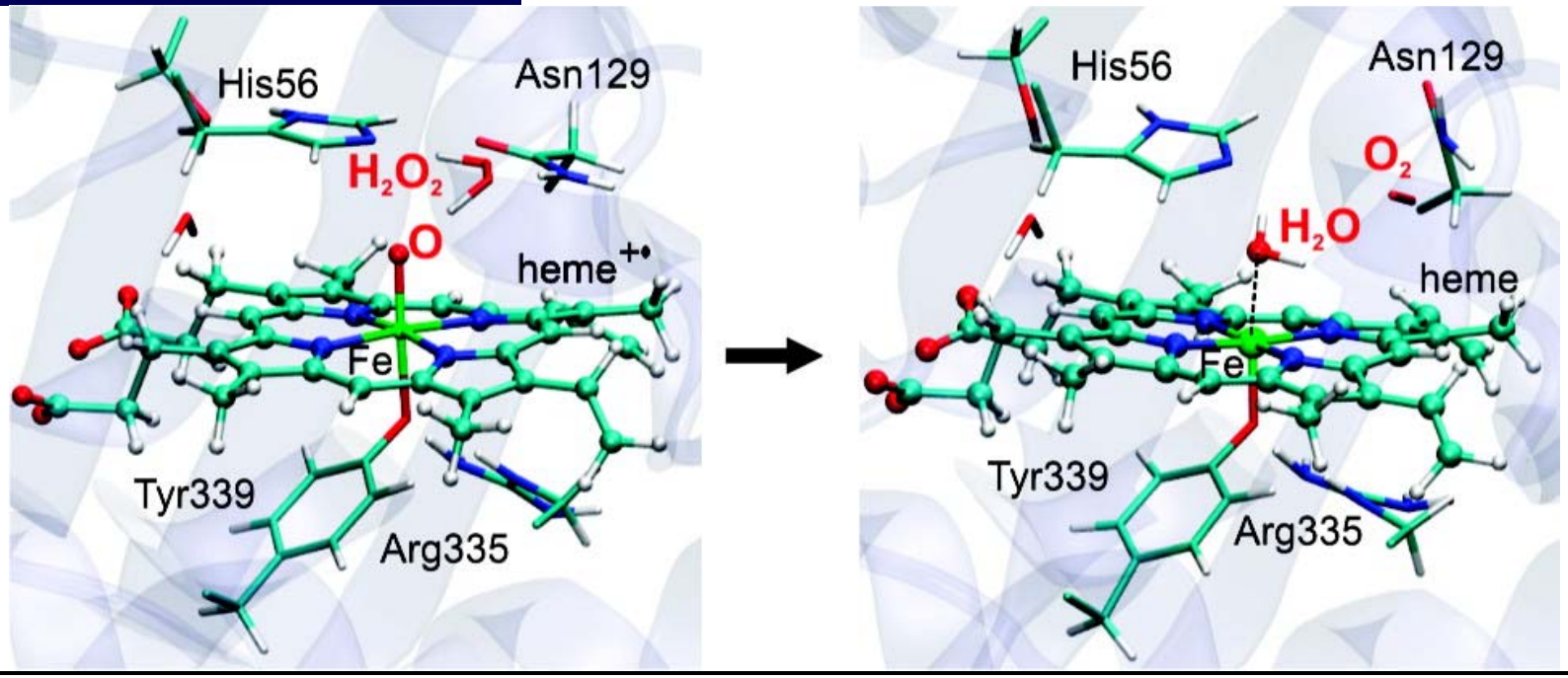
Catalases, heme-iron enzymes (b) catalysis

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

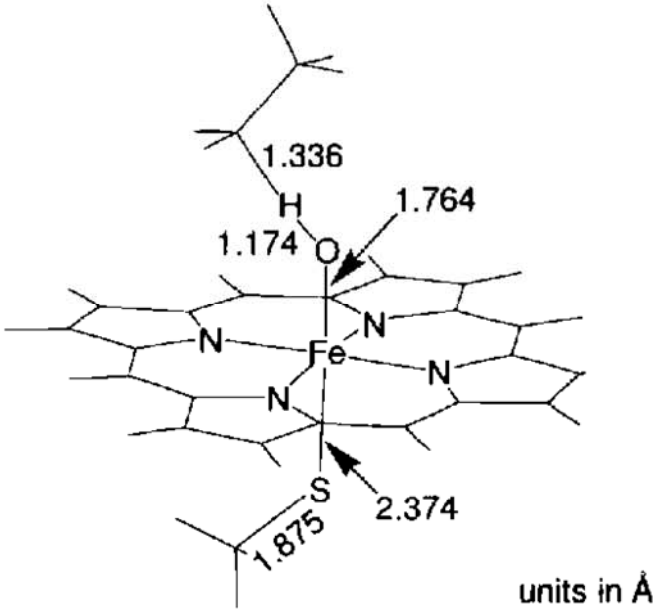


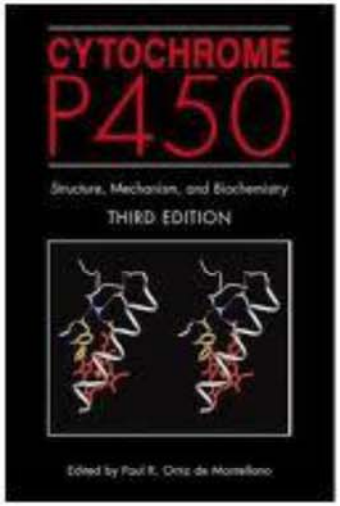
alternative pathways: a) His-mediated mechanism, b) direct mechanism

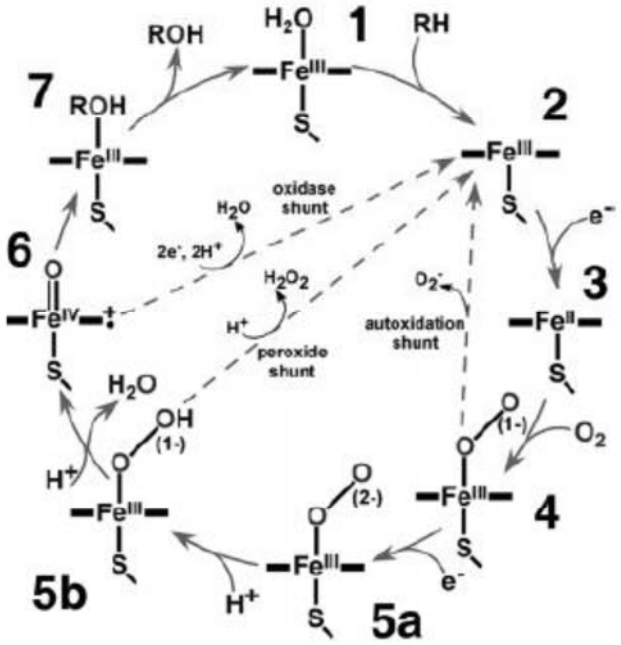




Cytochrome P450







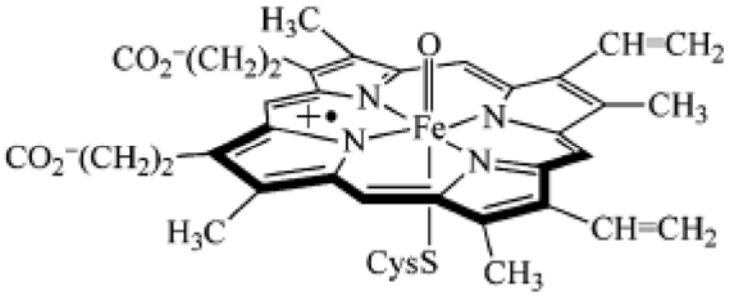
Ortiz de Montellano Chem. Rev. (2010) 110, 932–948; Denisov, Makris, Sligar,Schlichting, Chem. Rev. (2005) 105, 2253-2277

Typical Reactions of Cytochrome P450 (O-Transfer)

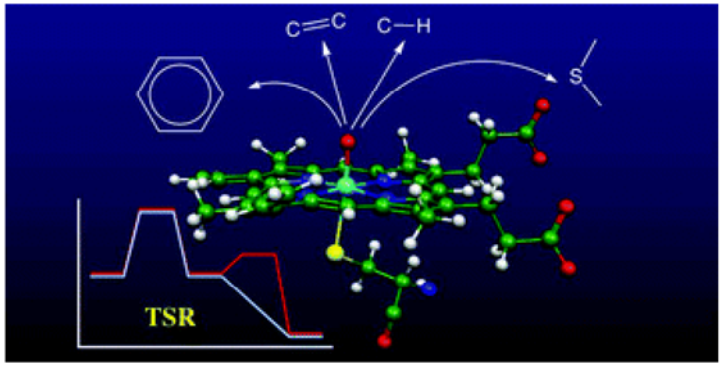
S. SHAIK et al. (2010) Acc. Chem. Res., 43, 1154-1165

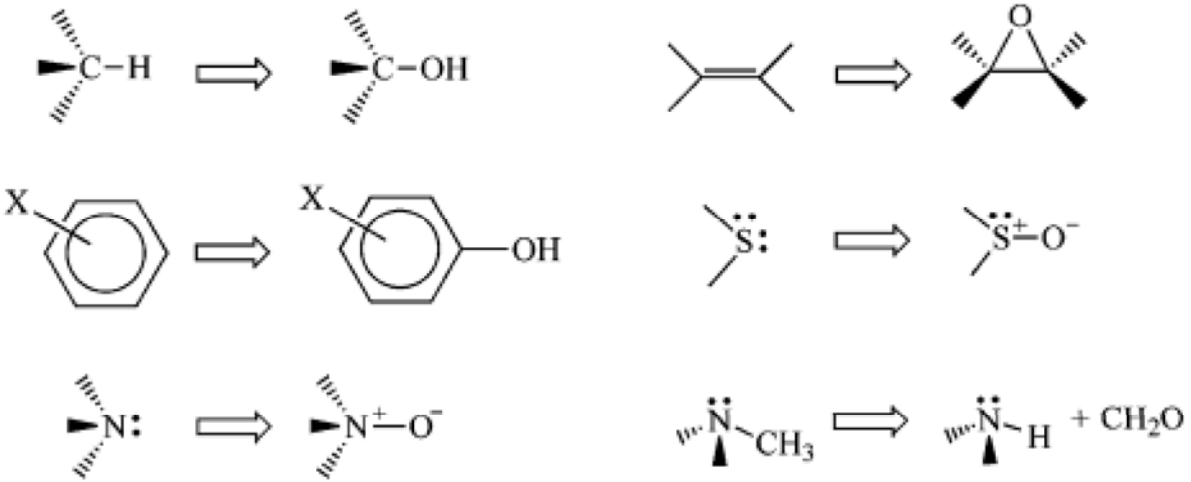
J. Rittle, M. T. Green (2010), SCIENCE 330, 933-936

SCHEME 1



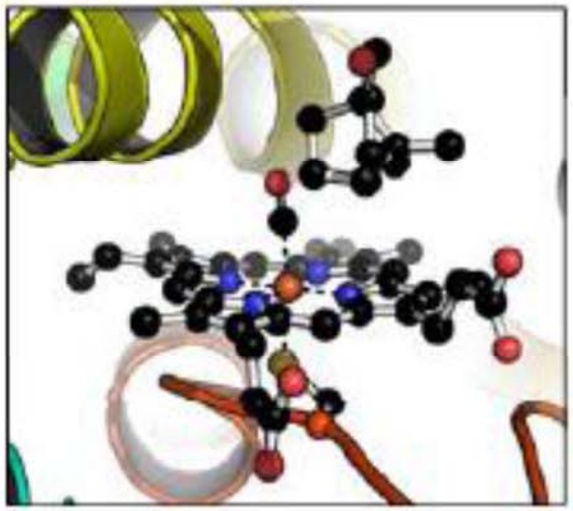
P450 Cpd I

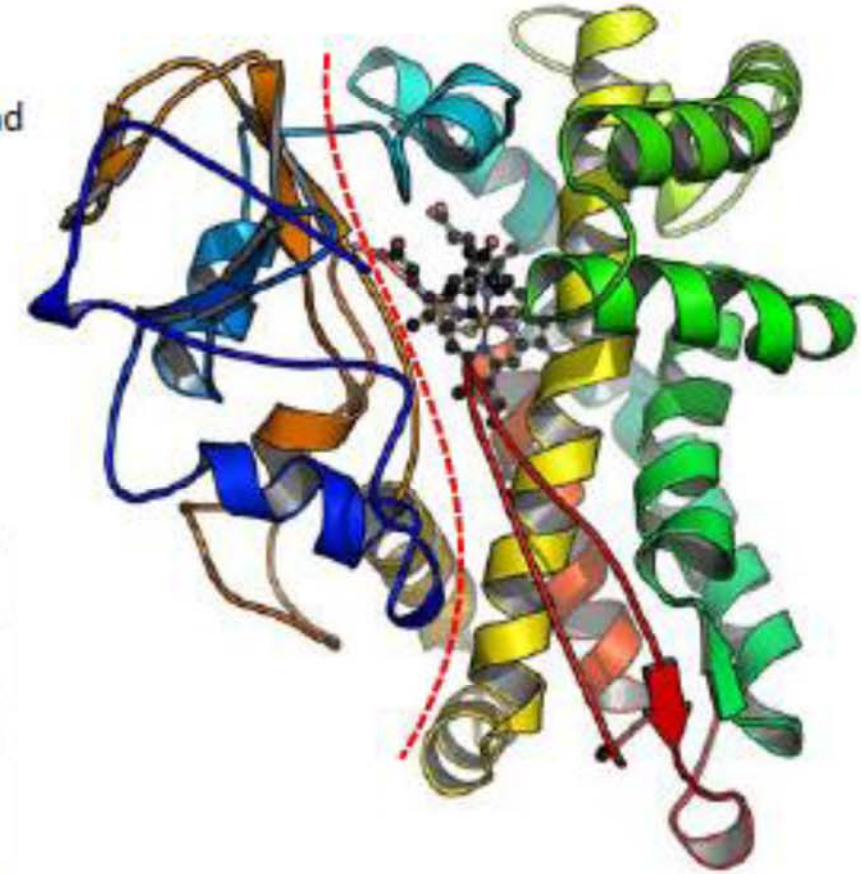




Cytochrome P450 – important enzyme for detoxification of organic compounds

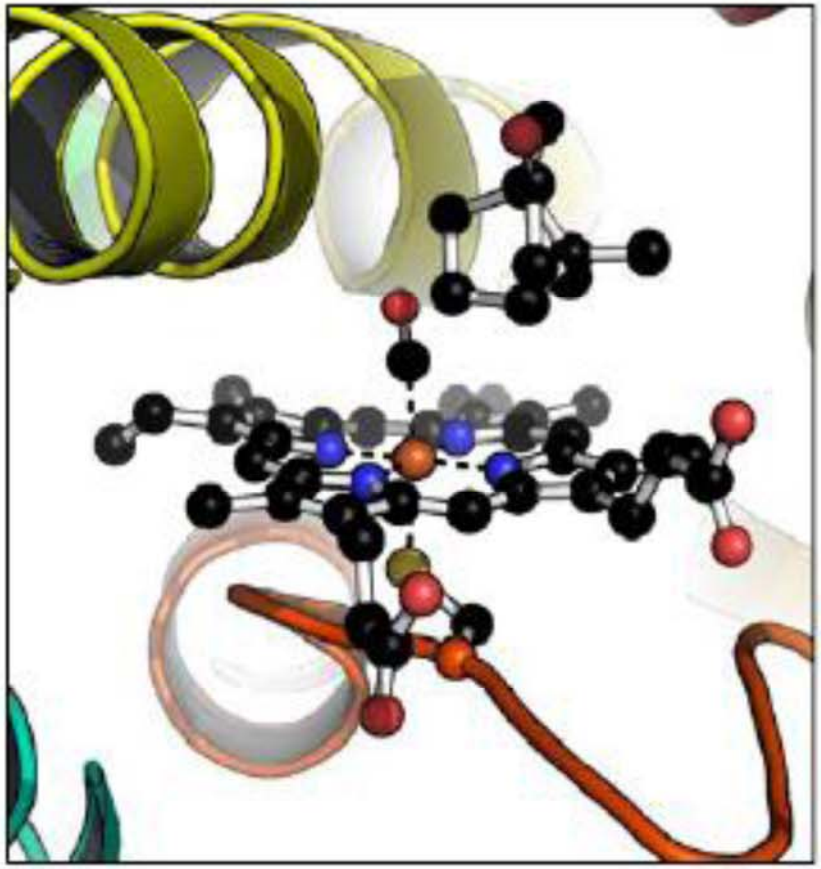
- Single *b*-type heme group
- Cys thiolate as proximal axial ligand
- Triangular prism
- No domain structure, but a more α-helical half and a half containing β-strands.
- High degree of structural conservation within the family.





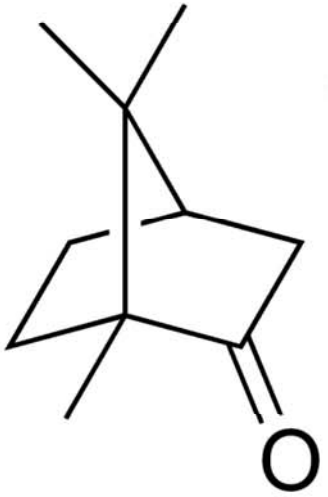
Cytochrome P450cam; PDB-ID 1CPP
Poulos *et al.* (1987) *J.Mol.Biol.* 195: 687-700.

Camphor substrate complex of Cytochrome P450



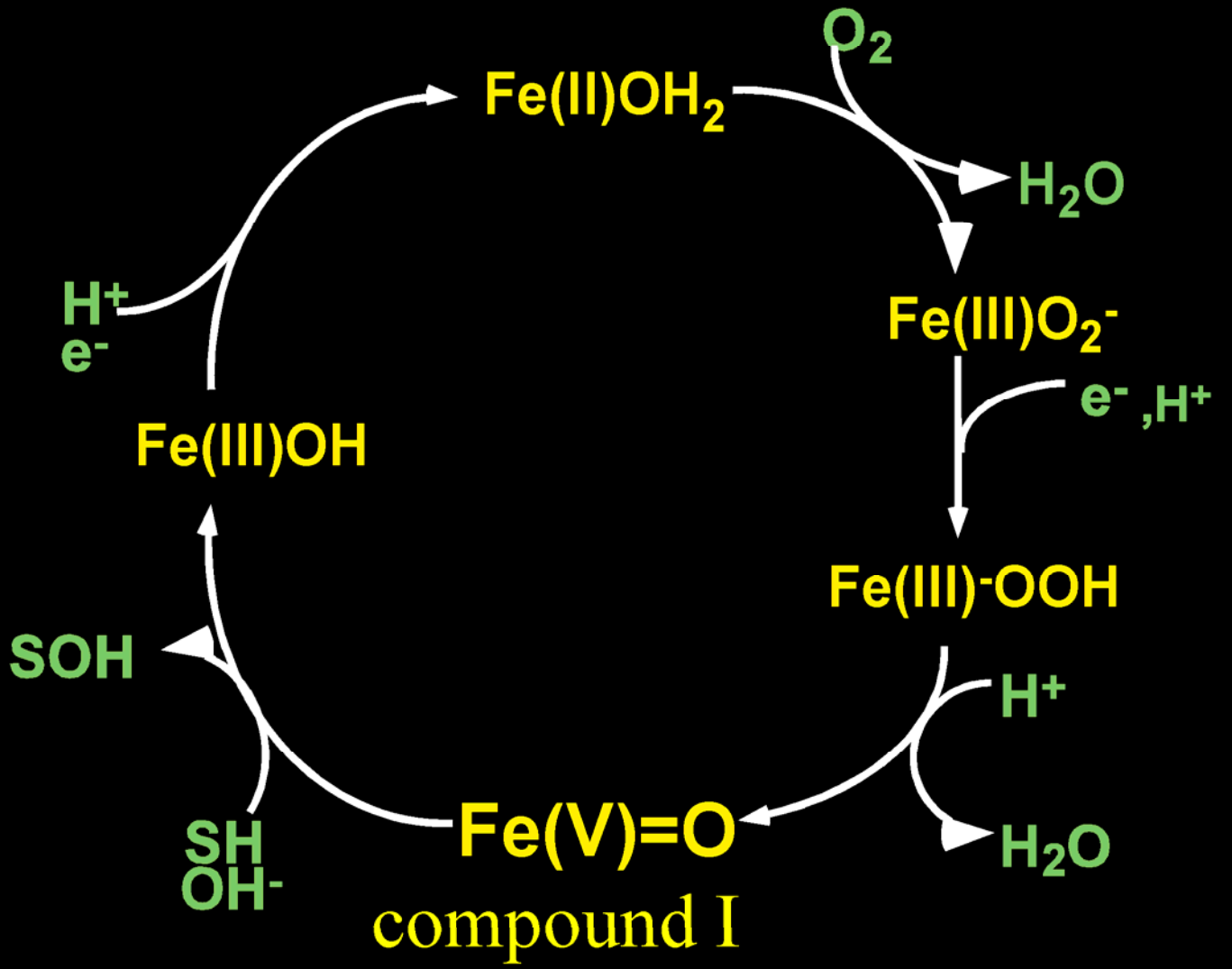
Multiple substrate complexes of P450s were readily obtained, but no intermediates of the reaction cycle.

In the P450cam substrate complex, CO binds to heme, presumably analogous to O_2.



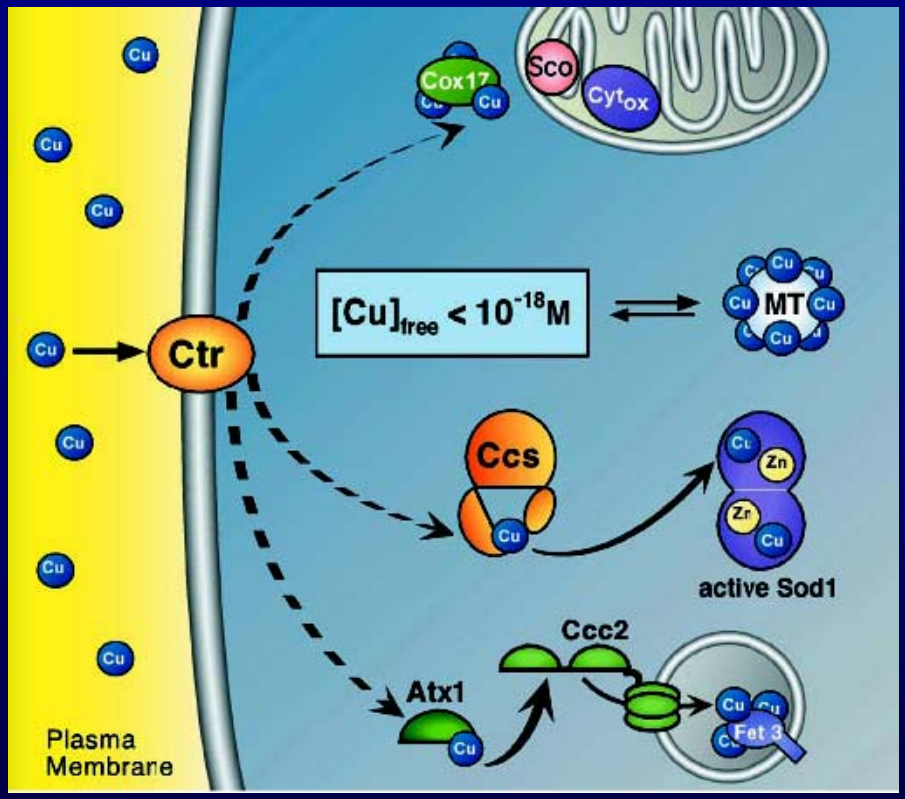
Cytochrome P450cam; PDB-ID 3CPP
Raag & Poulos (1989) *Biochemistry* 28: 7586-7592.

Cytochrome P450 Reaction Cycle



key intermediate

Copper delivery inside cellular compartments

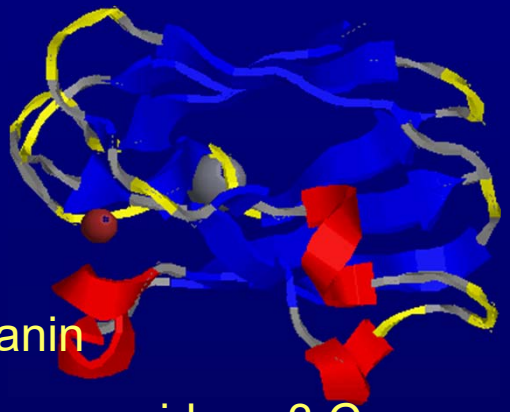


From: OHalloran TV, Culotta VC, 2000, JBC275, 25057-60

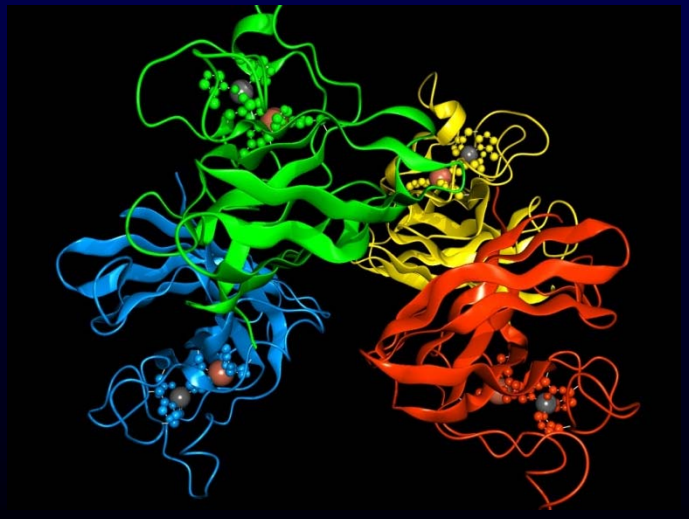
- confusing large number of names for homologous proteins in different organisms
- REALITY: just 3 really different (non-homologous) Cu-chaperones are well known, some more proteins are postulated to be Cu-chaperones

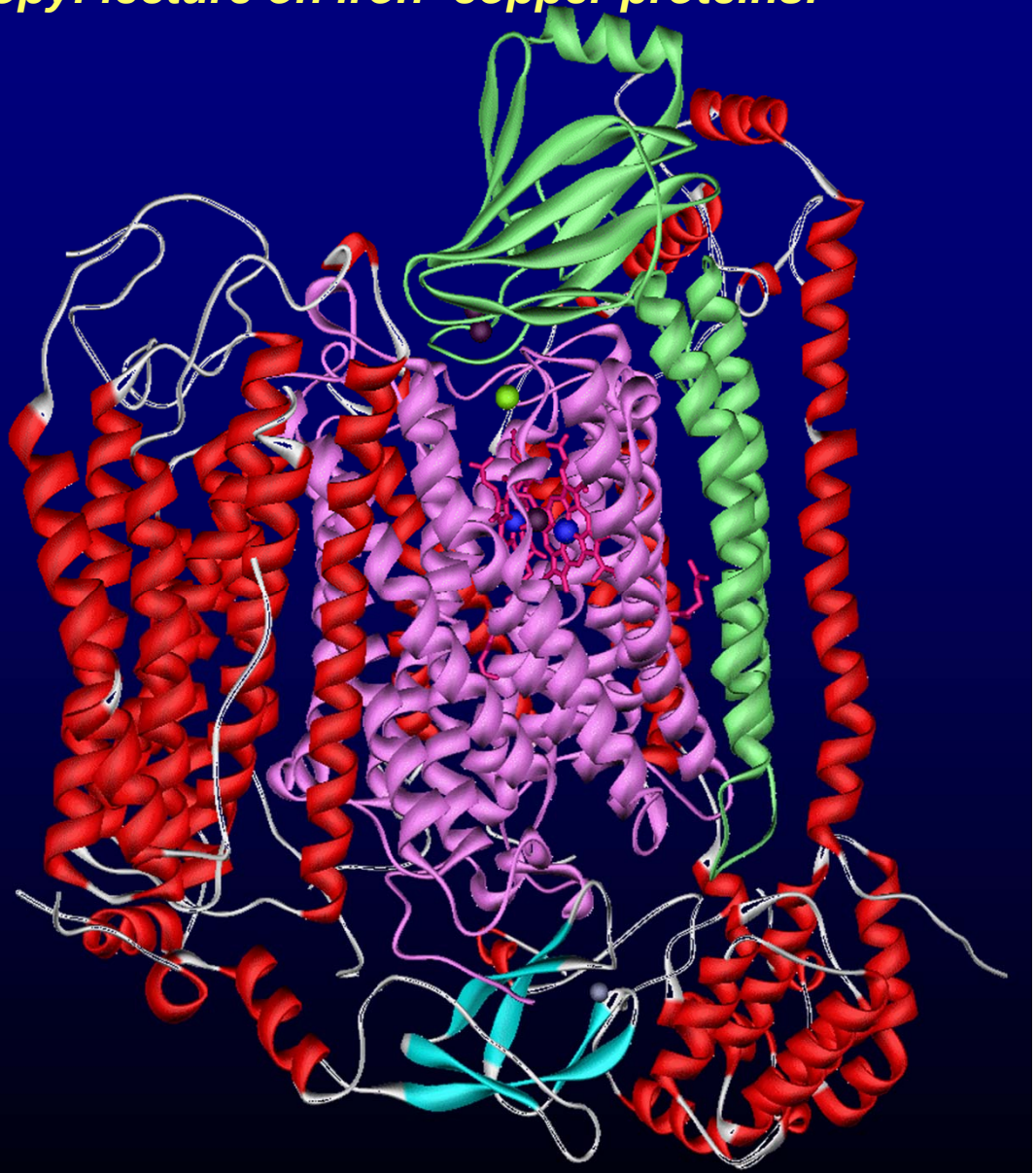
Selected enzymes with copper in the active centre

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

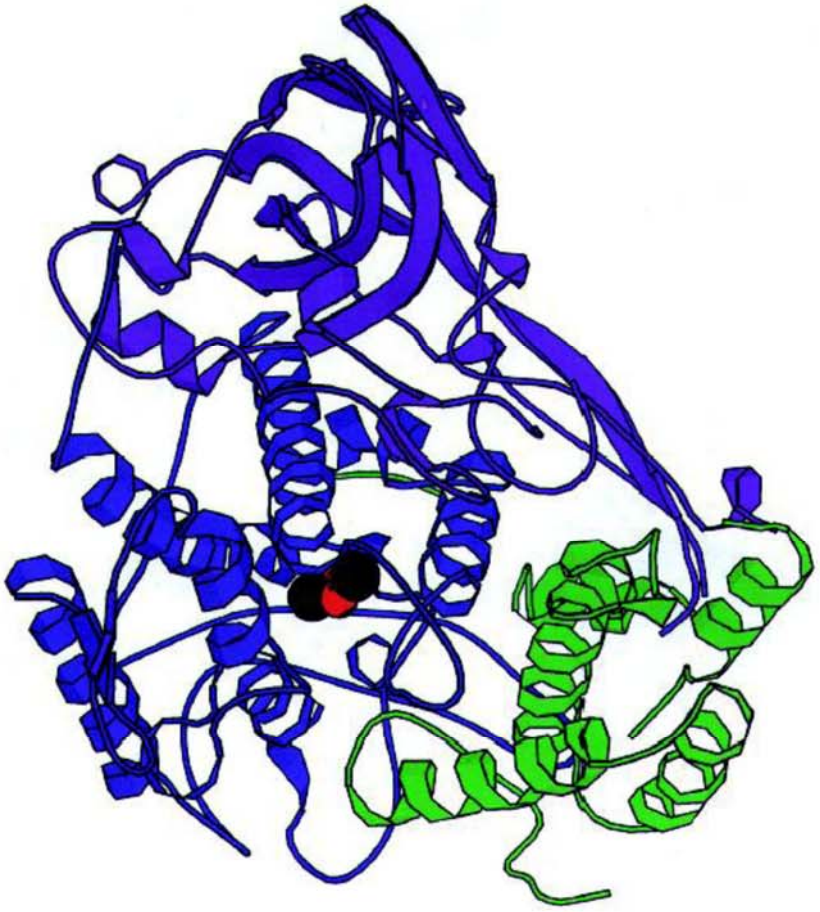
(2) The mononuclear non-blue copper proteins.

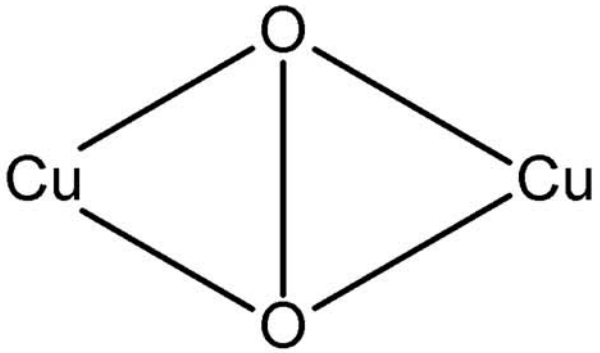
(3) Proteins with a coupled binuclear copper centre.

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

Solomon EI, Heppner DE, Johnston EM, Ginsbach JW, Cirera J, Qayyum M, Kieber-Emmons MT, Kjaergaard CH, Hadt RG, Tian L. 2014. Copper active sites in biology. Chemical Reviews 114, 3659–3853.

Hemocyanin (reversible O_2 binding)



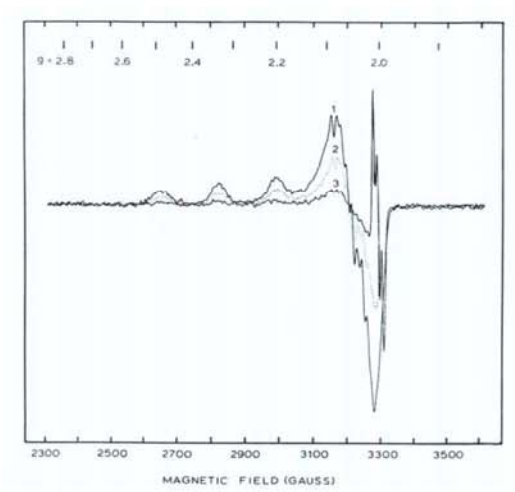


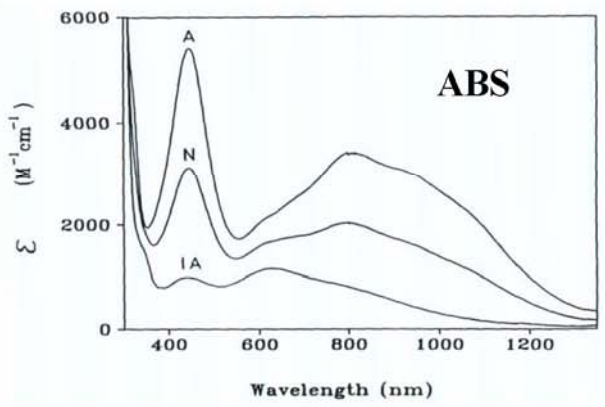
Oxygenated Cu site, see Que, Tolman, NATURE (2008) 455, 333; oxygenated form has a blue colour, **μ-η²:η²-peroxo** binding mode

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

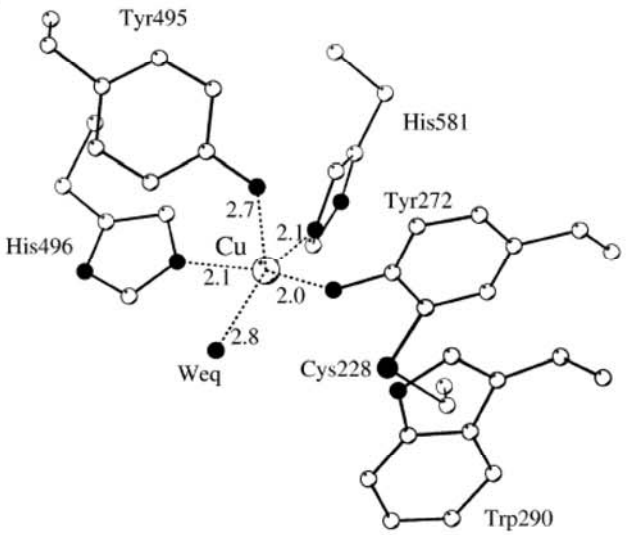
Galactose Oxidase, a Cu-Ligand Radical Enzyme

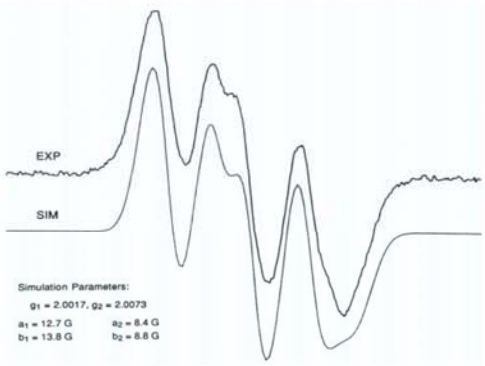
$$RCH_2OH + O_2 \xrightarrow{GalOx} RCHO + H_2O_2$$





(a)

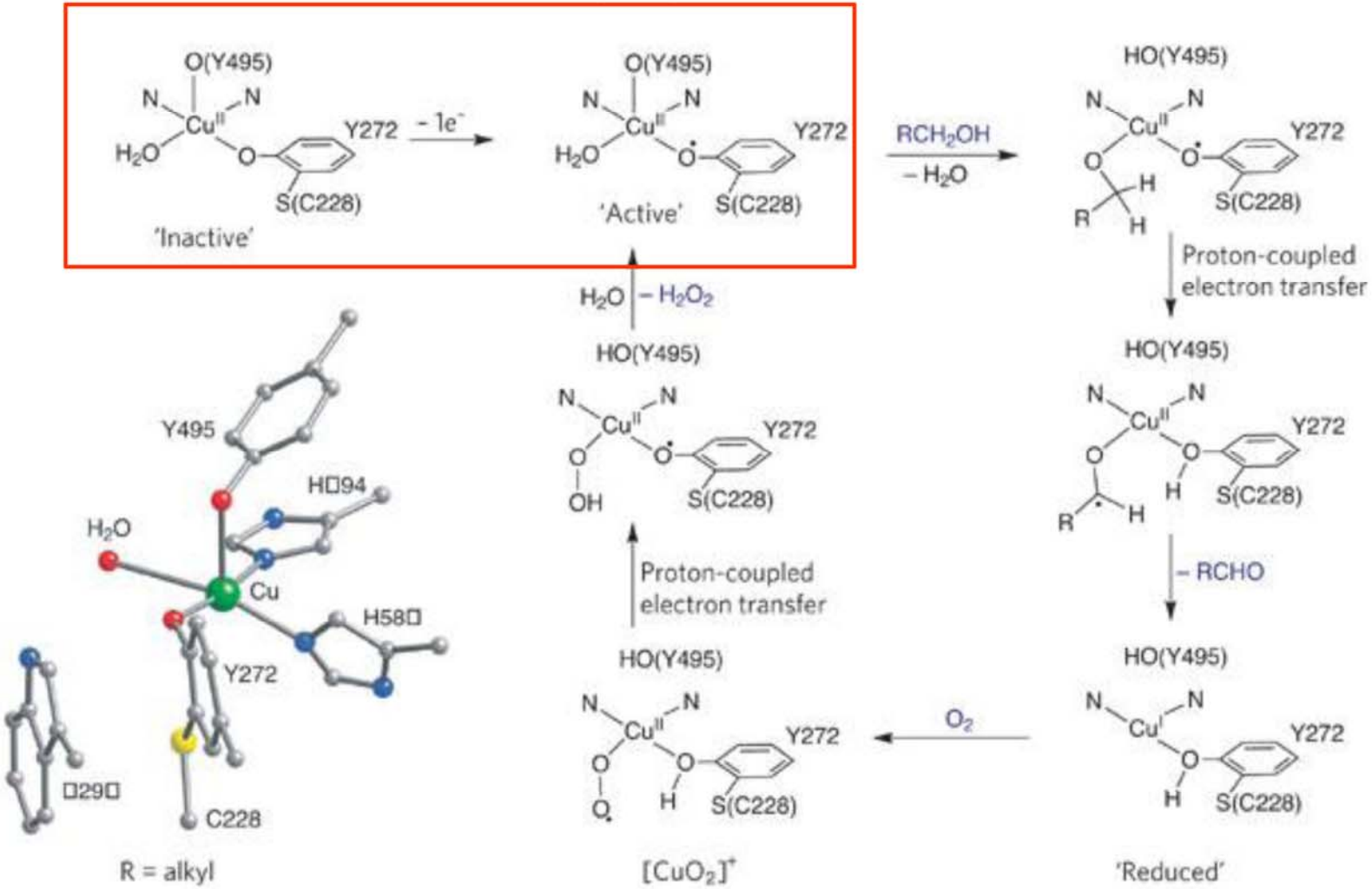




2 different EPR signals (metal-centered and ligand centered) observed during reaction cycle

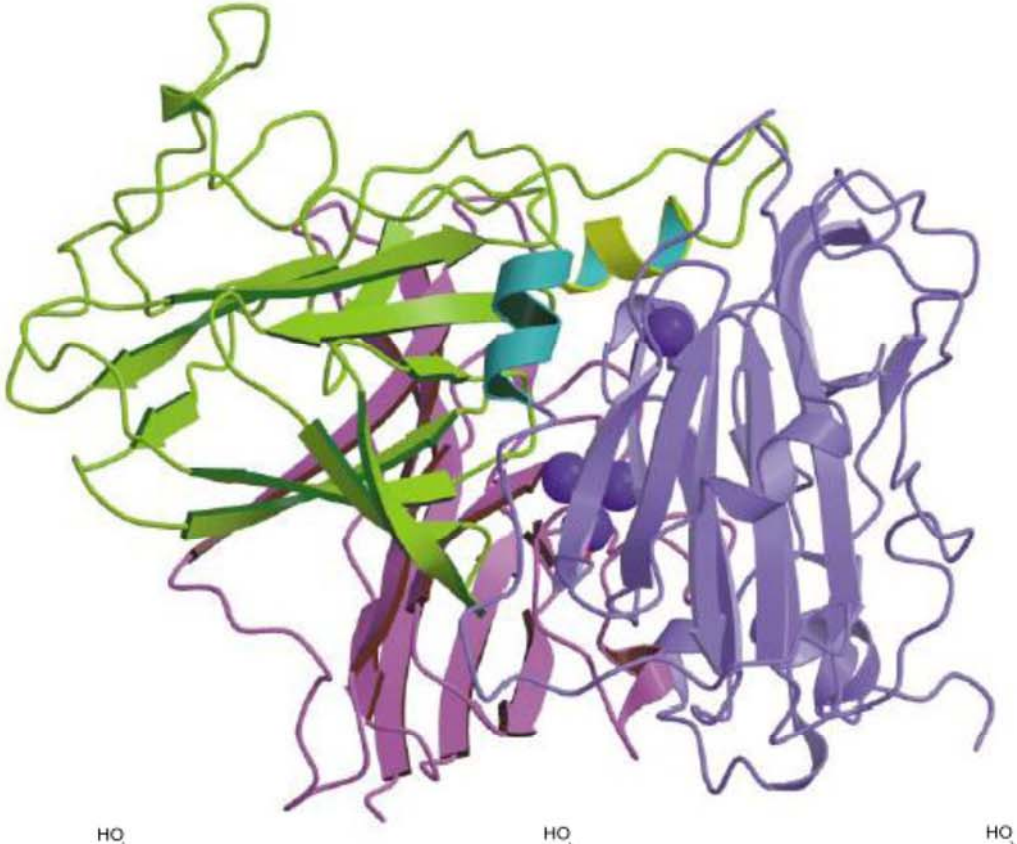
Galactose Oxidase - Mechanism

Que, Tolman (2008) NATURE 455, 333

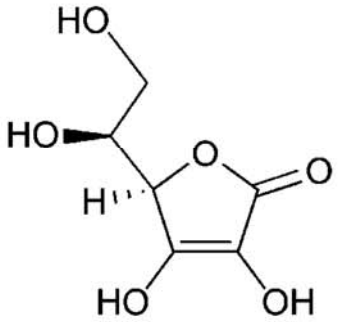


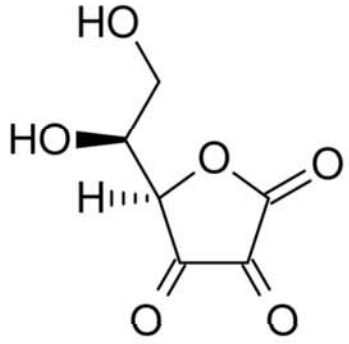
Ascorbic acid oxidase (AOX)

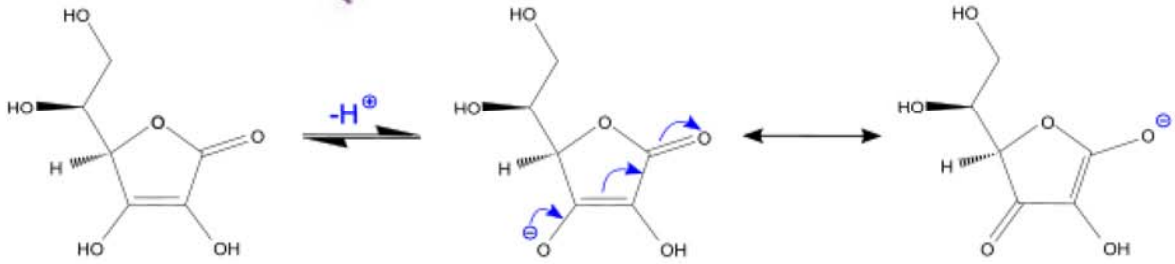
Multi-Copper oxidase (8Cu/homodimer) PDB code 1AOZ



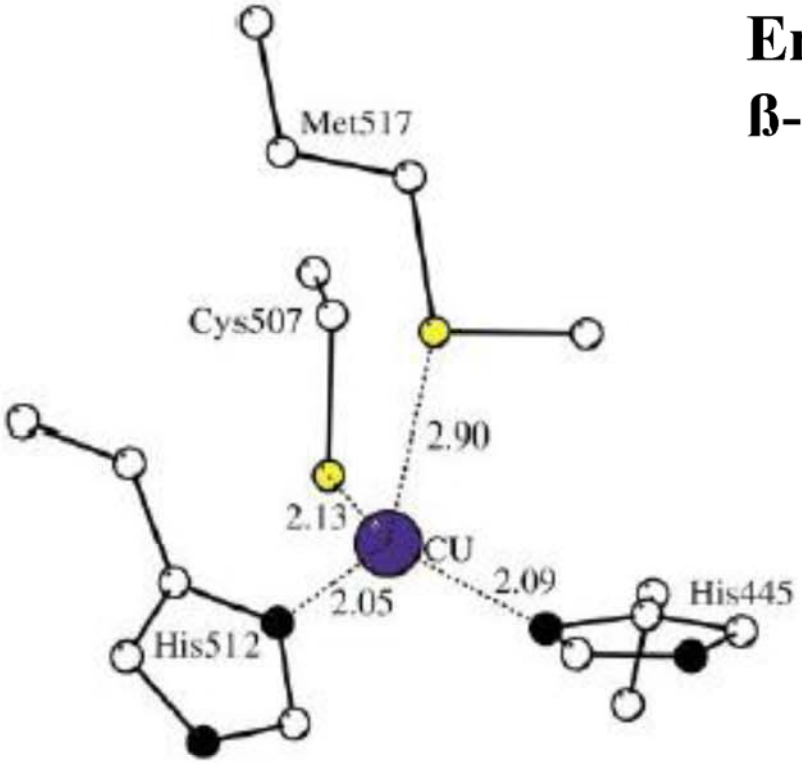
(5*R*)-[(1*S*)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one



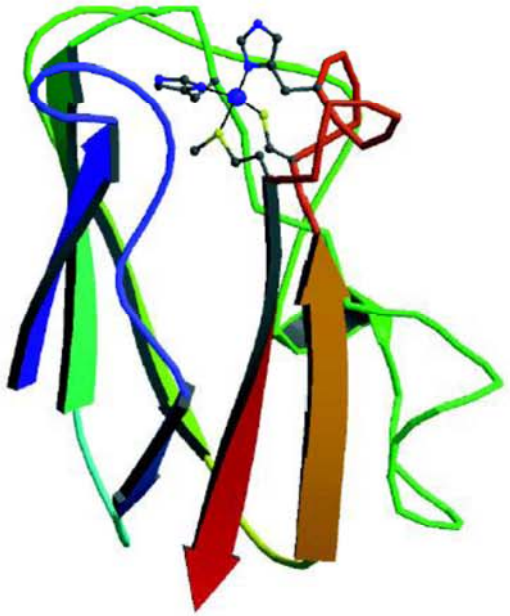




Type 1 electron transfer center of AOX: blue copper protein with high similarity to plastocyanin!

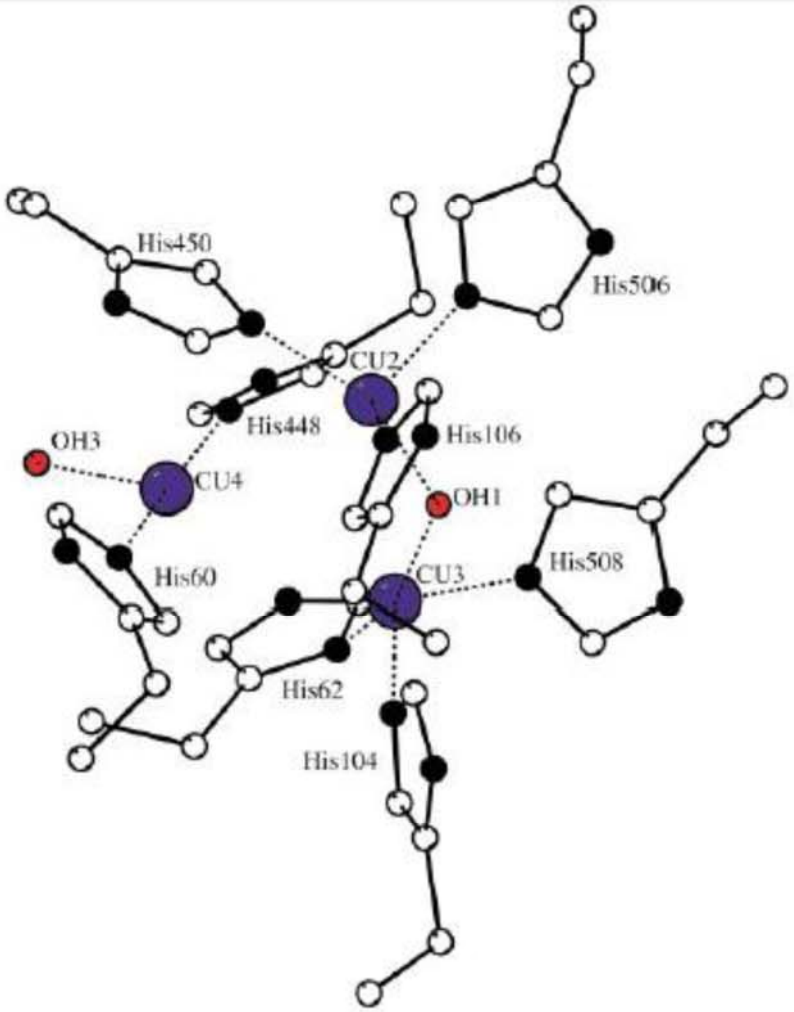


Entrance point for electrons
ß-barrel structural module



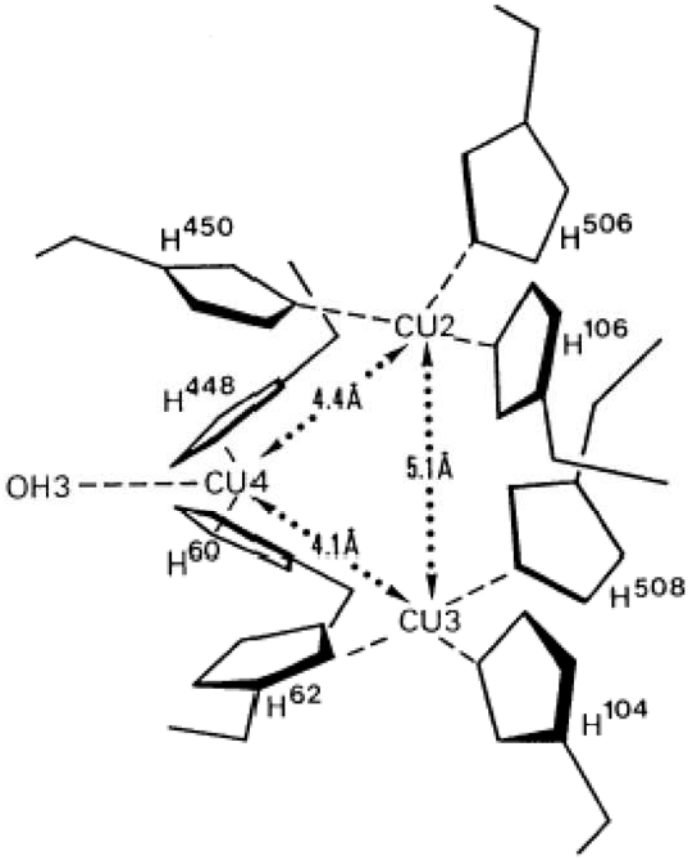
A. Messerschmidt, Handbook of Metalloproteins (2001)

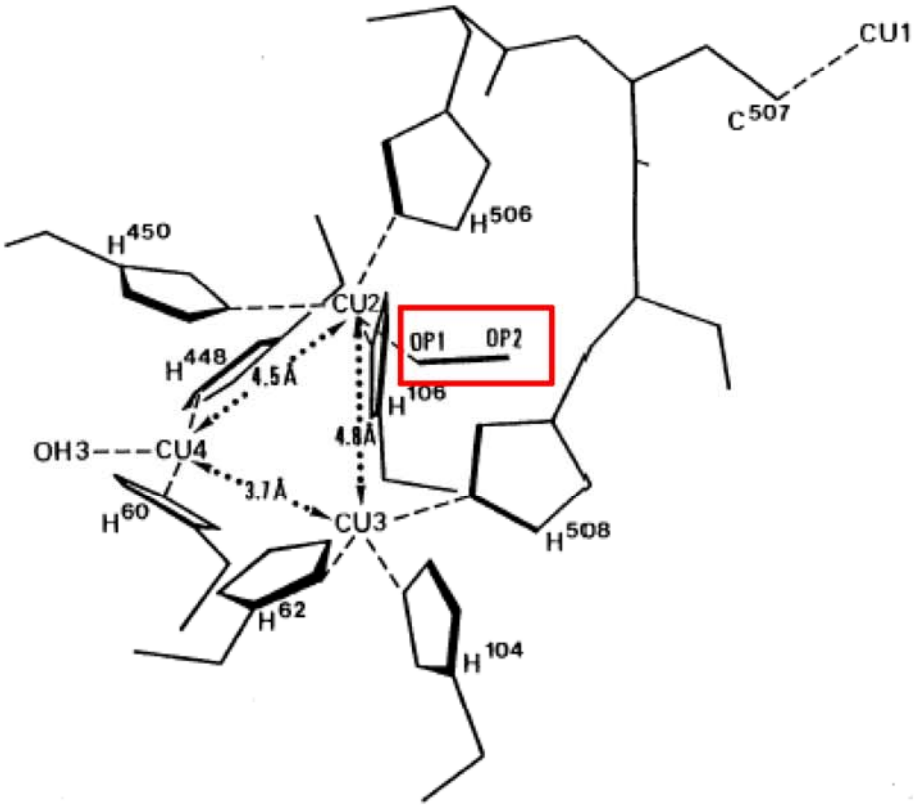
Dioxygen reduction site of AOX: unique trinuclear copper centre



A. Messerschmidt, Handbook of Metalloproteins (2001)

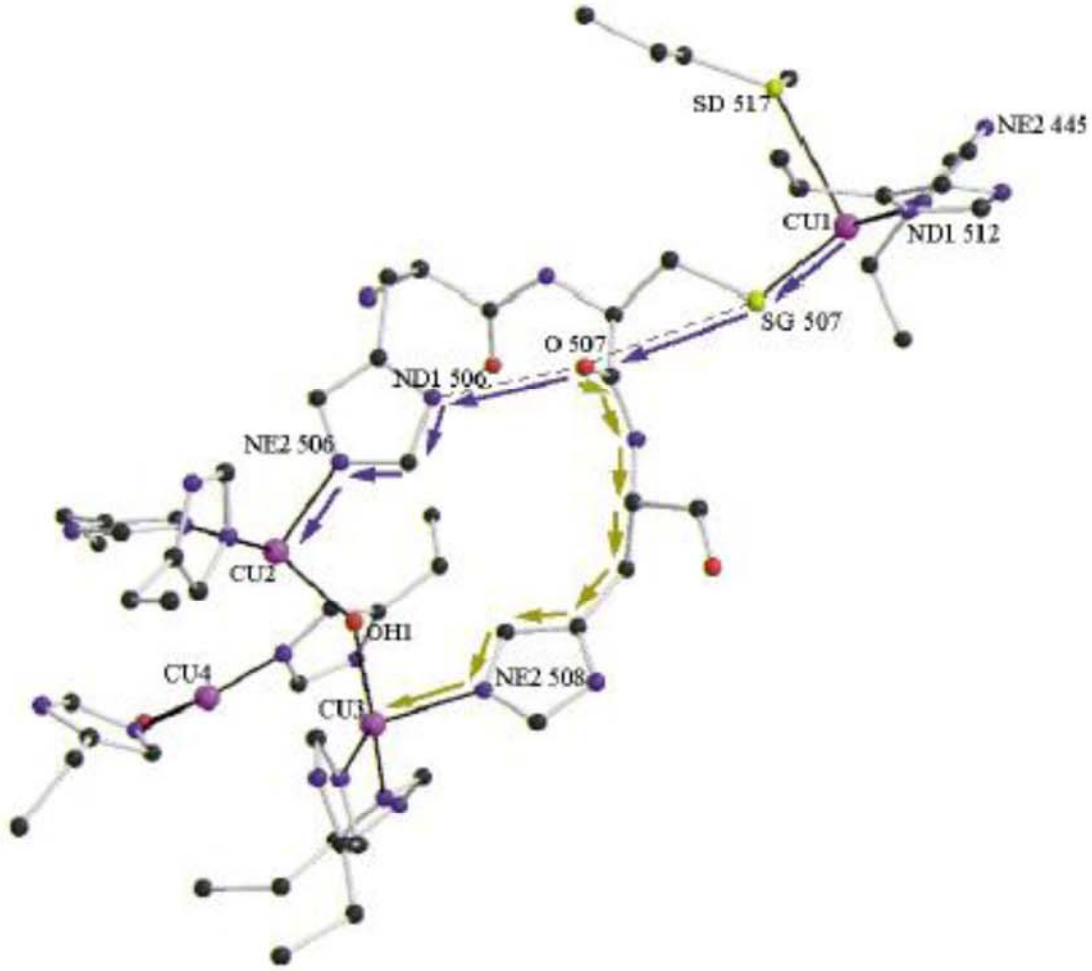
Dioxygen reduction site of AOX: unique trinuclear copper centre - substrate binding





A. Messerschmidt, Handbook of Metalloproteins (2001)

Ascorbic acid oxidase (AOX): electron transport pathways

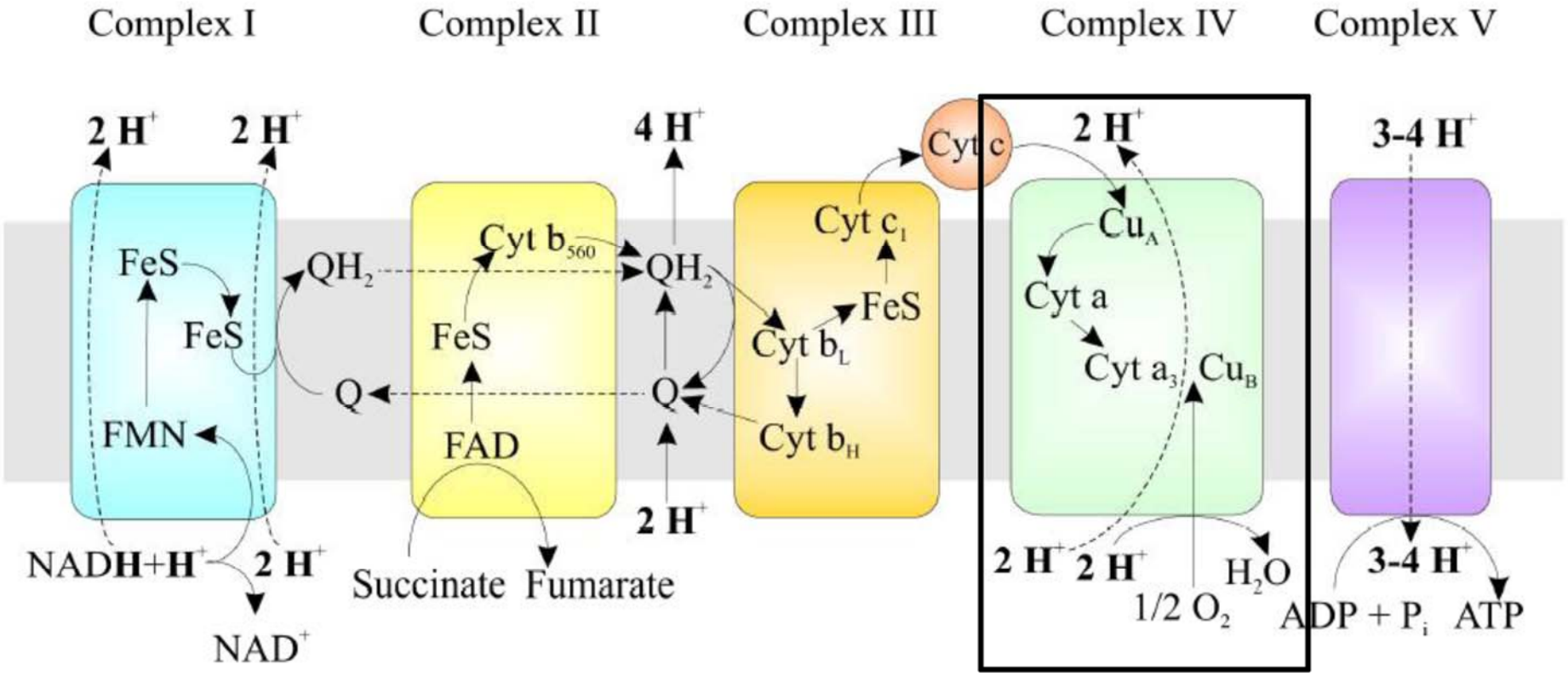


A. Messerschmidt, Handbook of Metalloproteins (2001)

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

$$4\,\text{cyt}\ c^{2+} + 8\,H_i^+ + O_2 \rightarrow 4\,\text{cyt}\ c^{3+} + 4\,H_o^+ + 2\,H_2O$$



based on a lecture slide of Peter Kroneck

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

$$O_2 + 4H^+ + 4H_i^+ + 4e^- \rightarrow H_2O + H_2O + 4H_o^+ \ (+818\ mV)$$

$$N_2O + 2H^+ \ 2e^- \rightarrow N_2 + H_2O \quad (+1355\ mV)$$
$$2NO + 2H^+ + 2e^- \rightarrow N_2O + H_2O \quad (+1175\ mV)$$

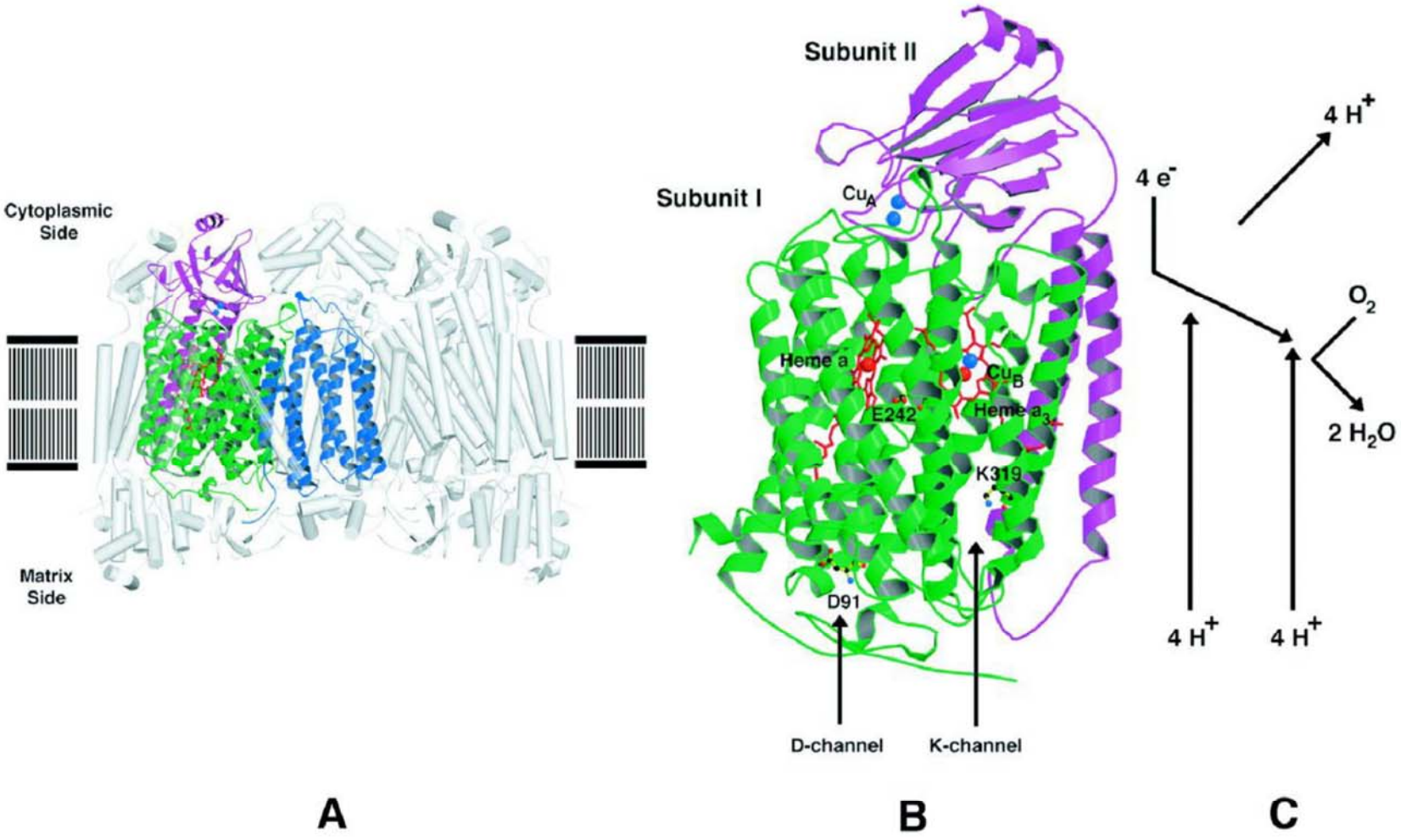
metals (CuA, Fe-heme, Mg, Zn)

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

metal centers: CuA → ET; Fe-CuB → O_2 reduction

based on a lecture slide of Peter Kroneck

COX: structure and reaction scheme



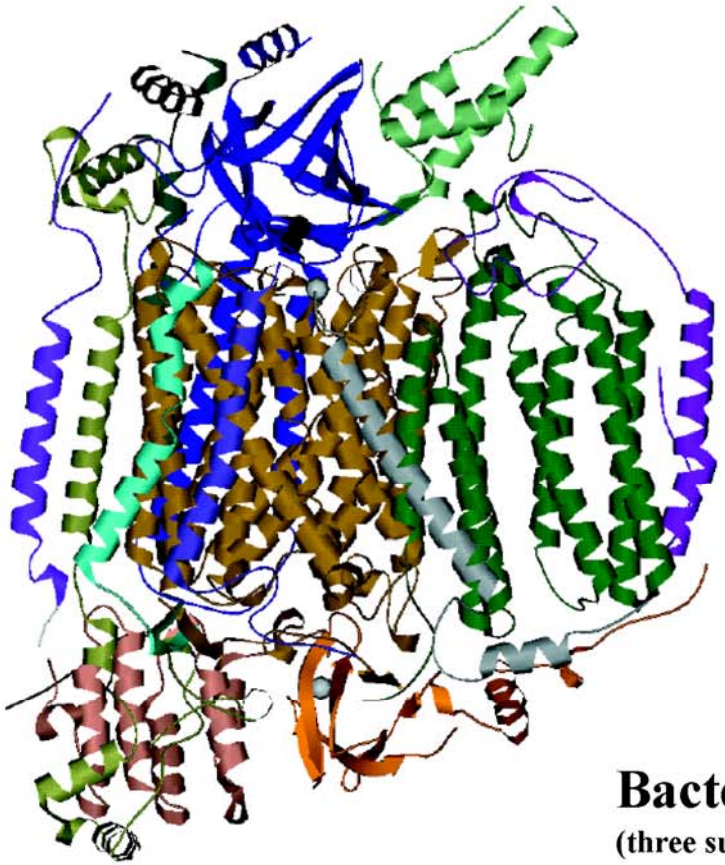
M Saraste Science (1999) 283,1488-1493

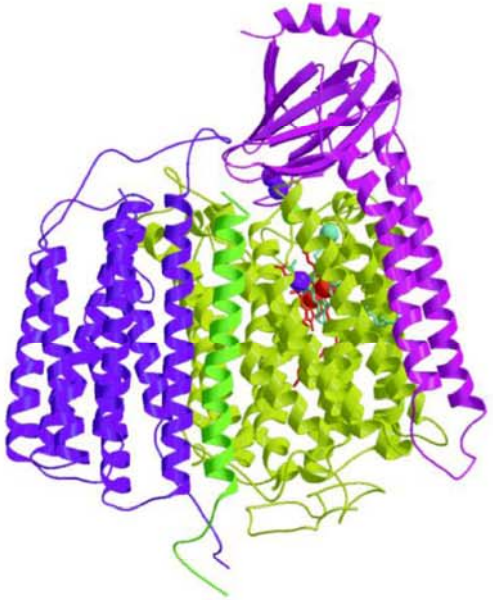
based on a lecture slide of Peter Kroneck

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





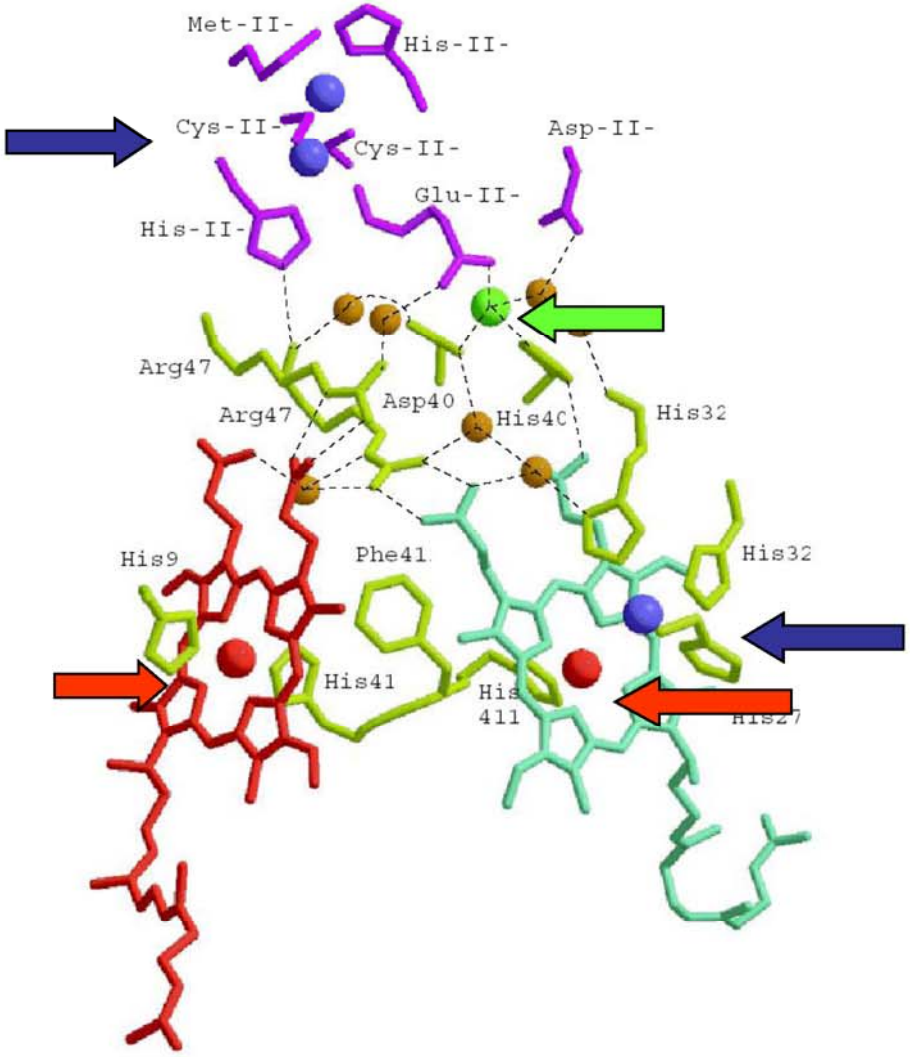
Bacterial COX from *Pseudomonas denitrificans*

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

based on a lecture slide of Peter Kroneck

Metal Centers in bacterial COX

Cu Fe Mg

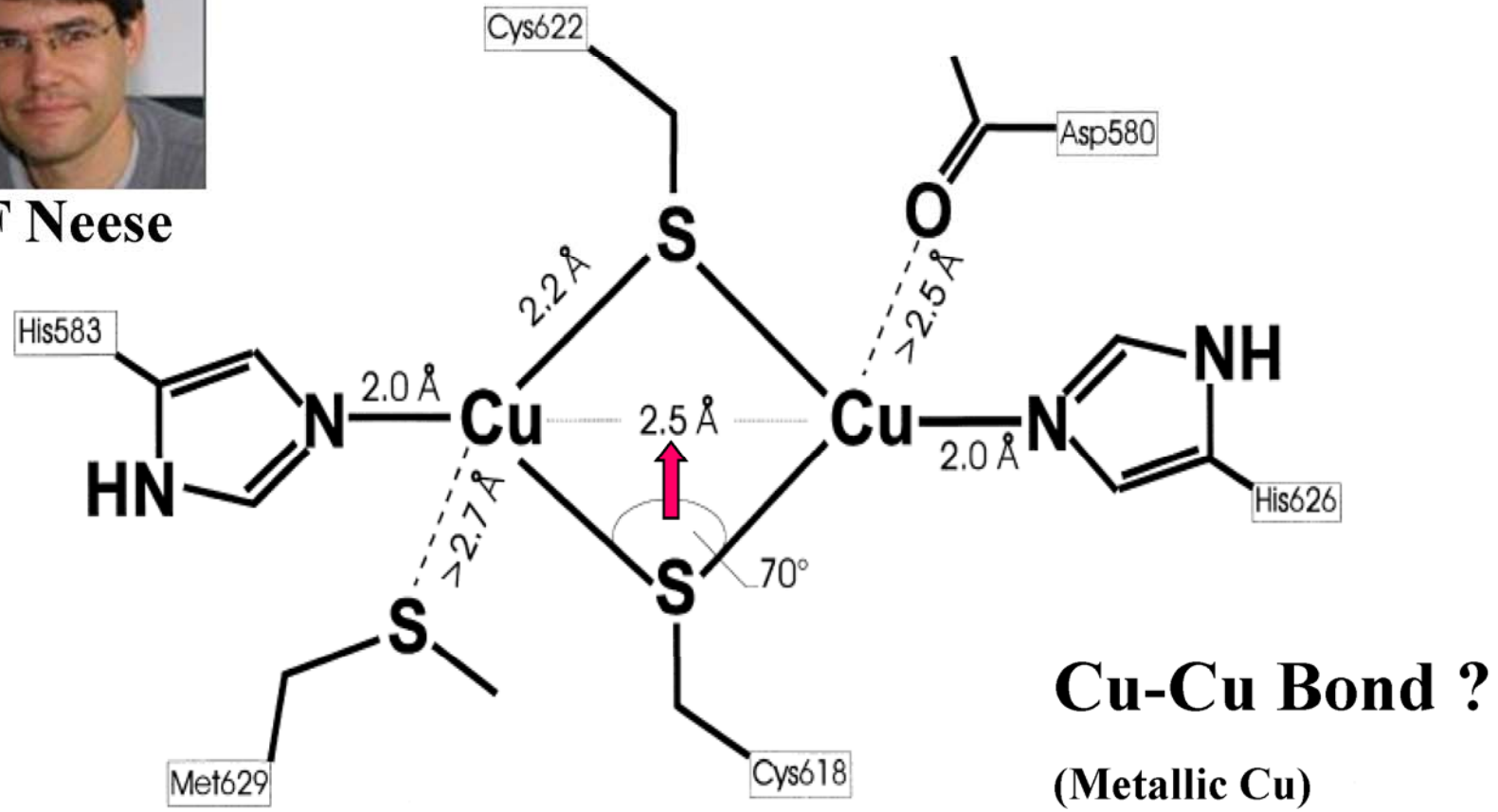


based on a lecture slide of Peter Kroneck

The CuA site of COX: a mixed-valence Cu_2S_2 rhomb

formal oxidation state: $Cu^{1.5+}$, in reality 1 unpaired delocalised e^- per 2 Cu

F Neese



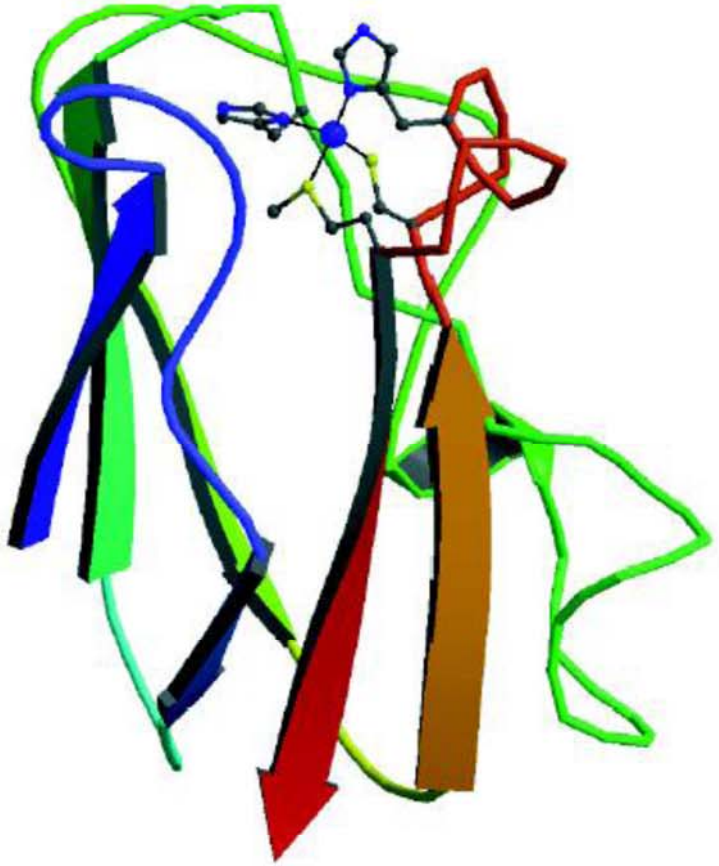
Cu-Cu Bond ?

(Metallic Cu)

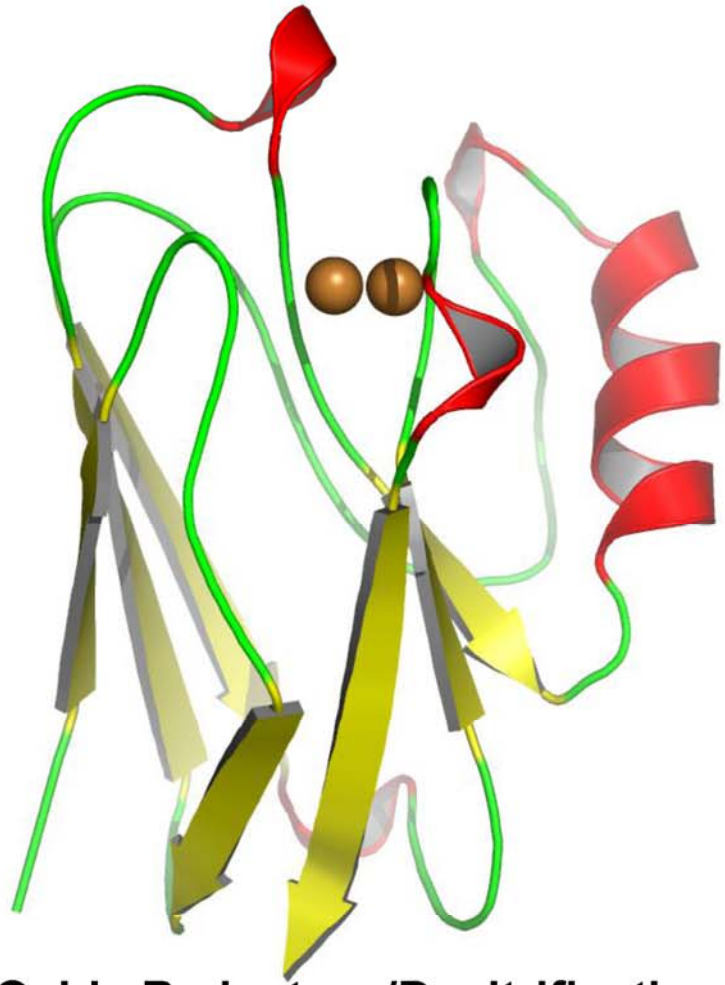
based on a lecture slide of Peter Kroneck

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