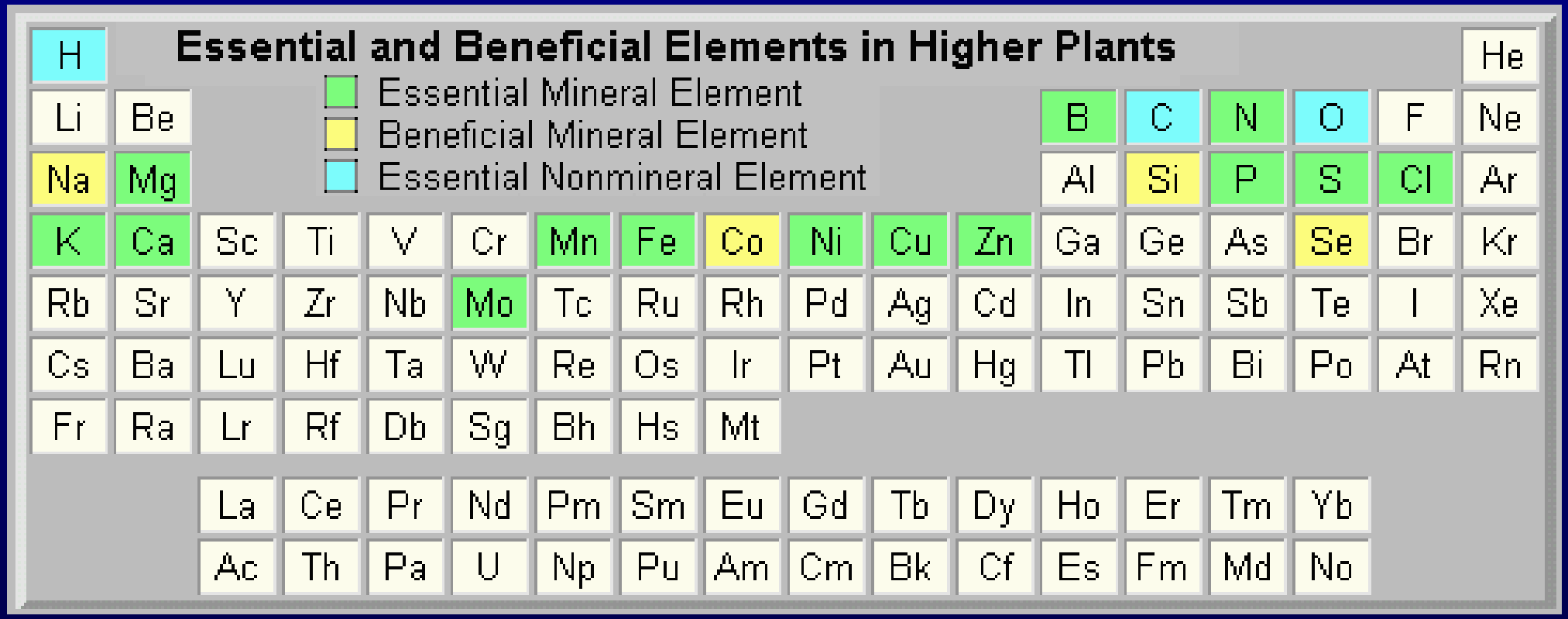
Copper and iron proteins

Hendrik Küpper, Advanced Course on Bioinorganic Chemistry & Biophysics of Plants, summer semester 2025
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

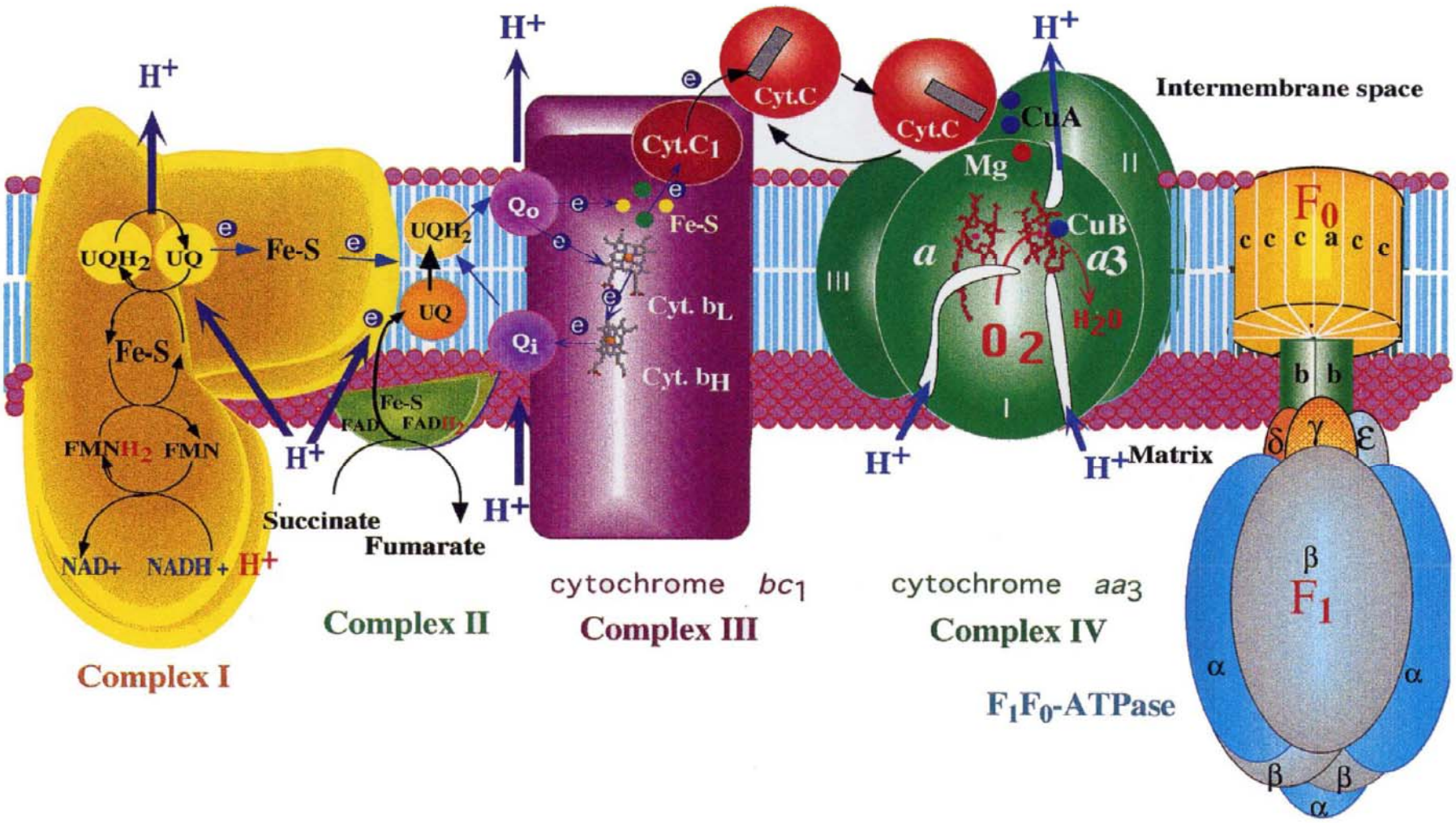
→ Oxygen transport

→ 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

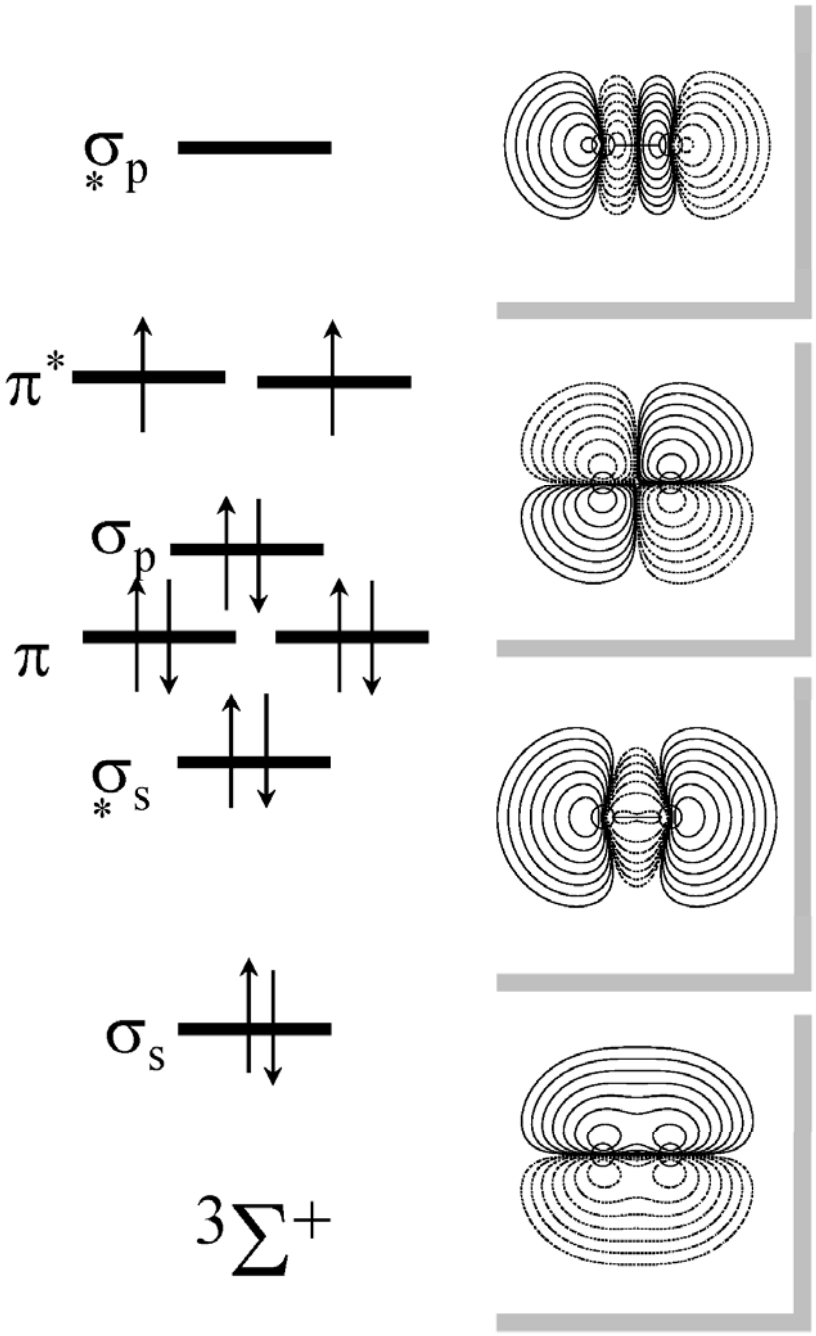
Respiration = reduction of O_2 to H_2O

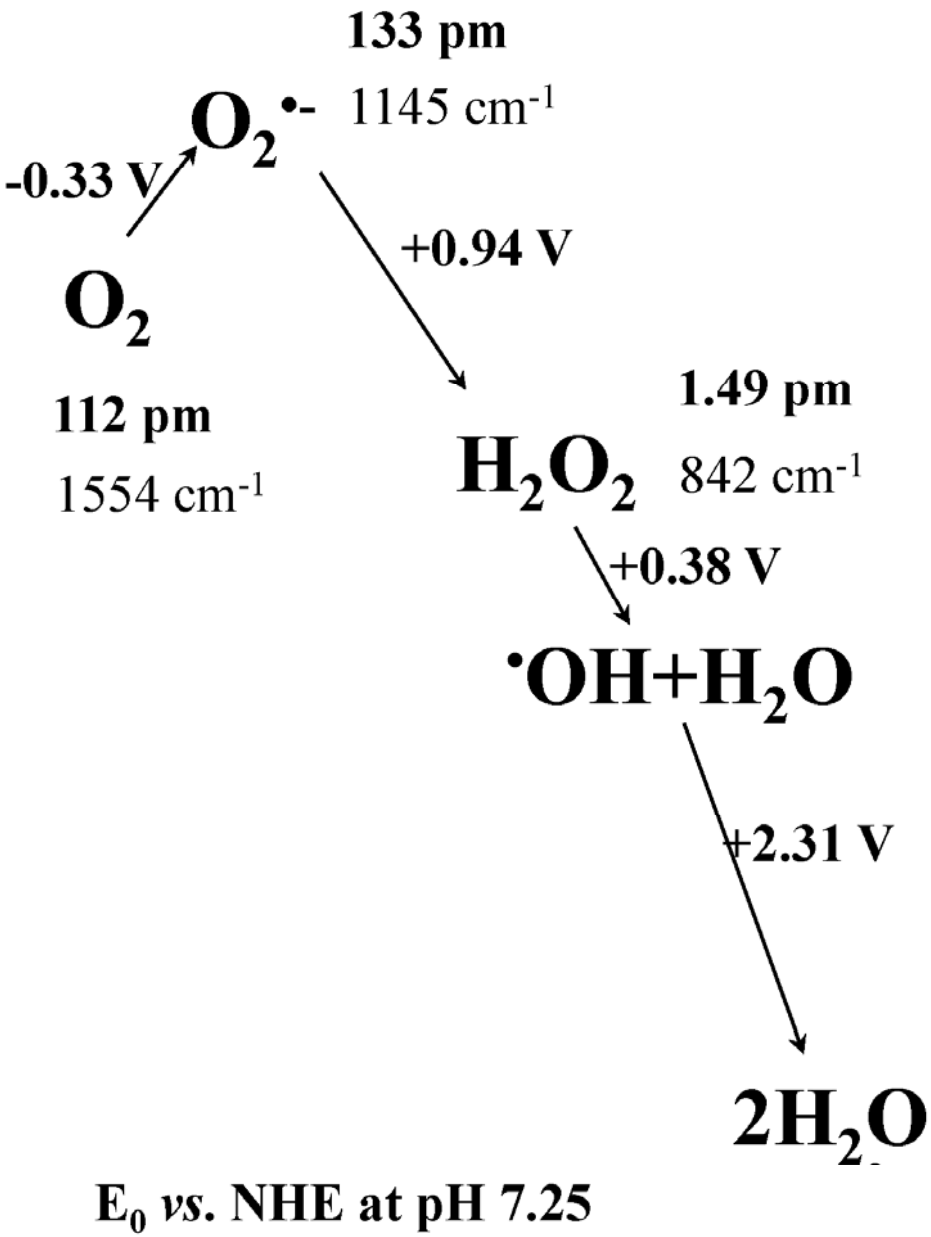
Synthesis of ATP – proton-coupled electron transfer (PCET)



based on a lecture slide of Peter Kroneck

Dioxygen Activation





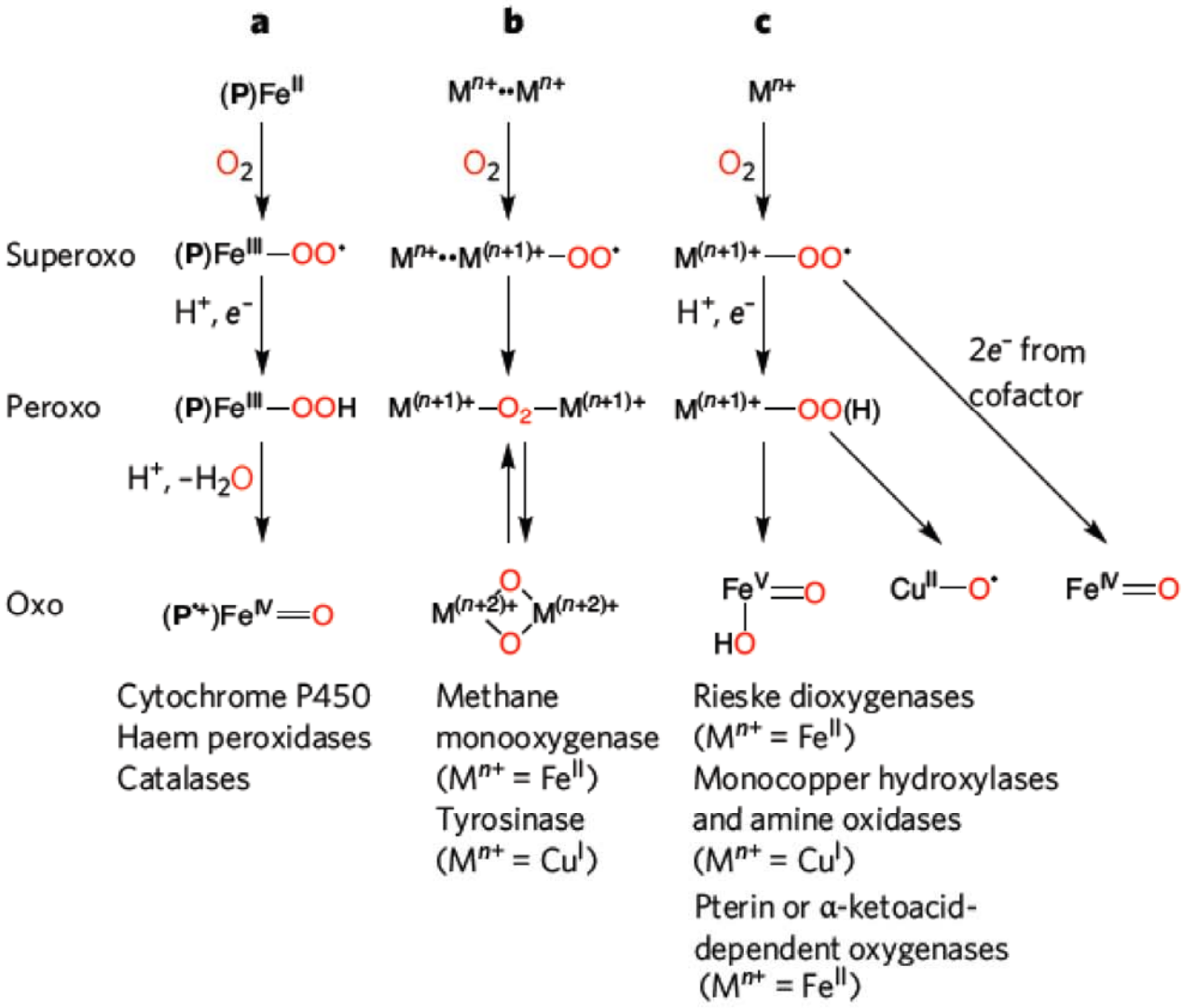
Activation of O_2 – Reaction Types

- **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.

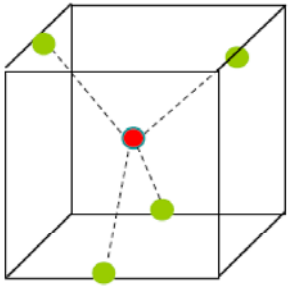
L. Que Jr, W.B. Tolman (2008) NATURE, 455, 333-340



Lecture slide of Peter Kroneck

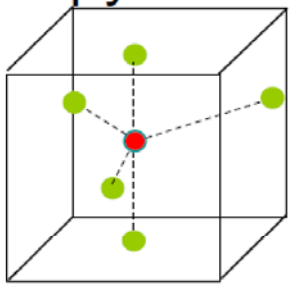
Geometry is important: Iron Proteins

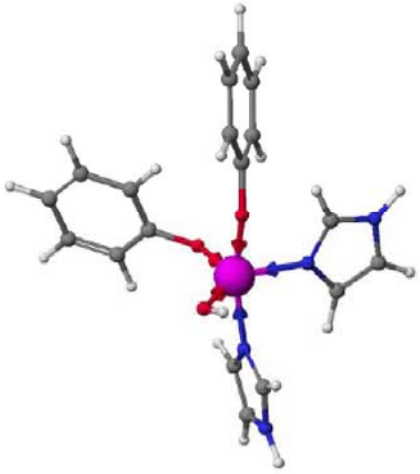
Tetrahedron



Rubredoxin

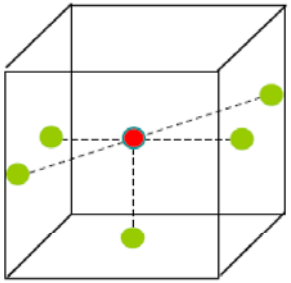
Trigonal Bipyramide





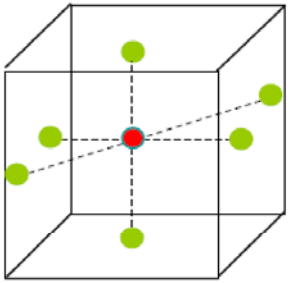
3,4-Protocatechoate Dioxygenase

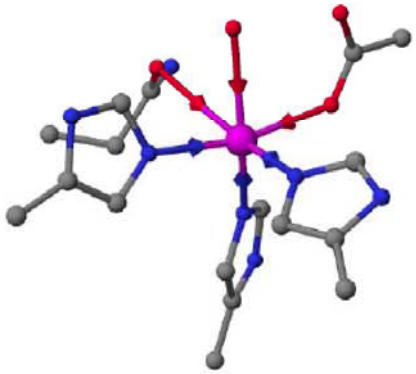
Tetragonal Pyramide



Tyrosine Hydroxylase

Octahedron



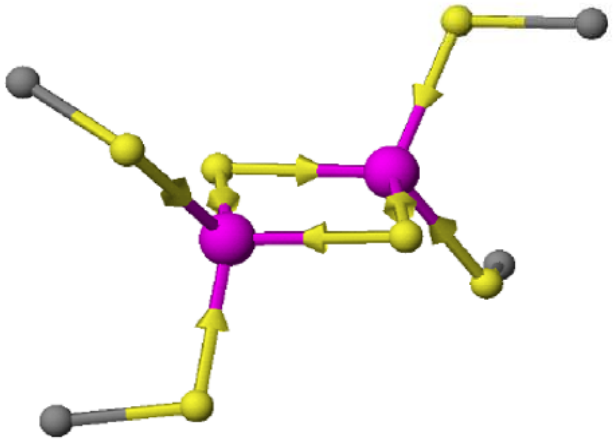


Lipoxygenase

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

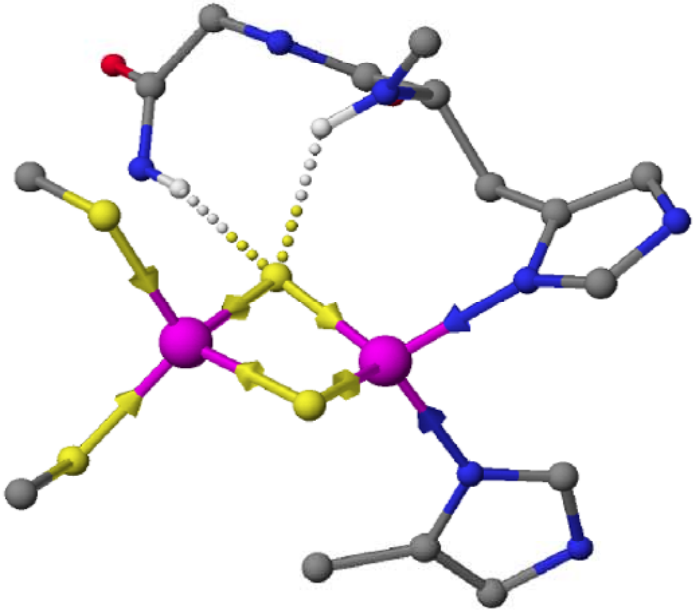
Modulation of Redox potentials (H bridges)

2Fe-2S Ferredoxin



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

2Fe-2S Rieske



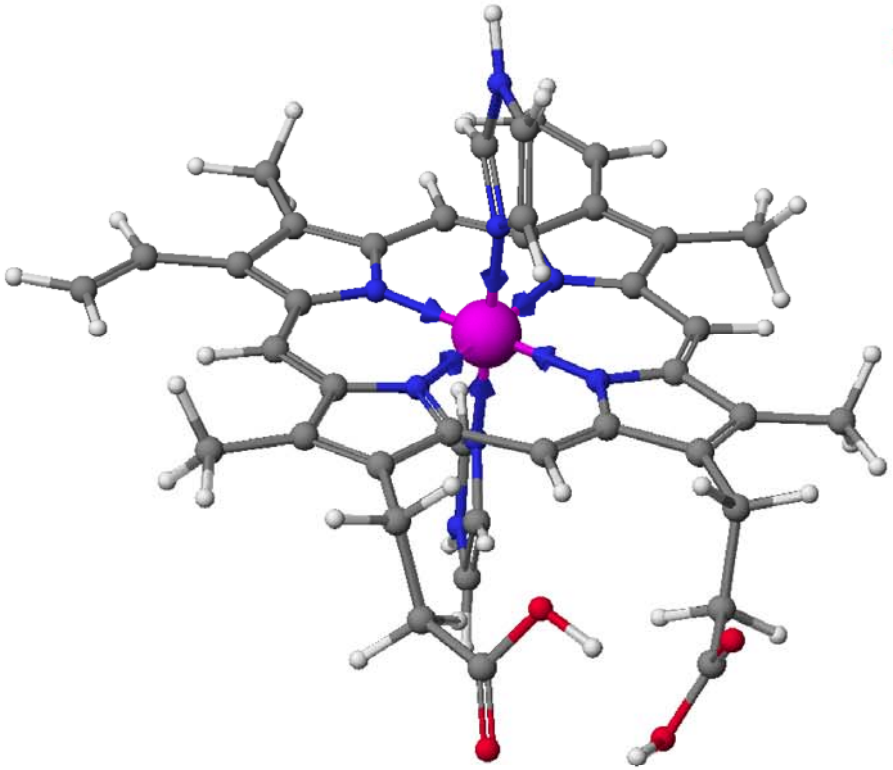
$E^{0`}$ ~ +280 mV

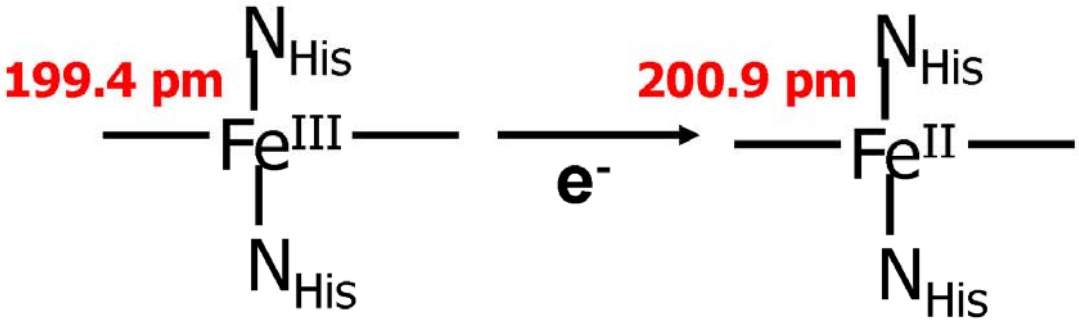
(+150 mV without H bridges)

(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





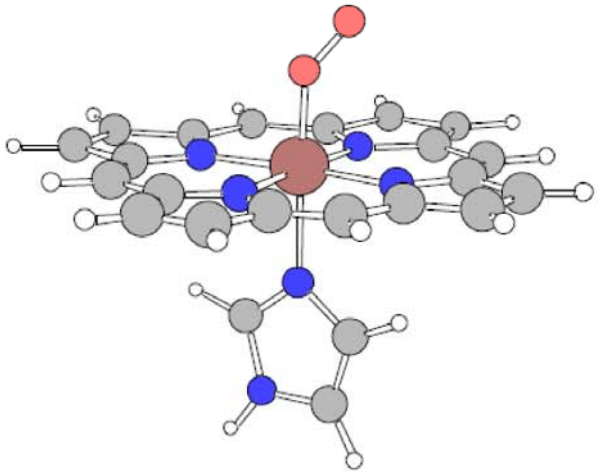
Reorganization Energy

in Cytochromes ≤ 4-5 kcal/mol

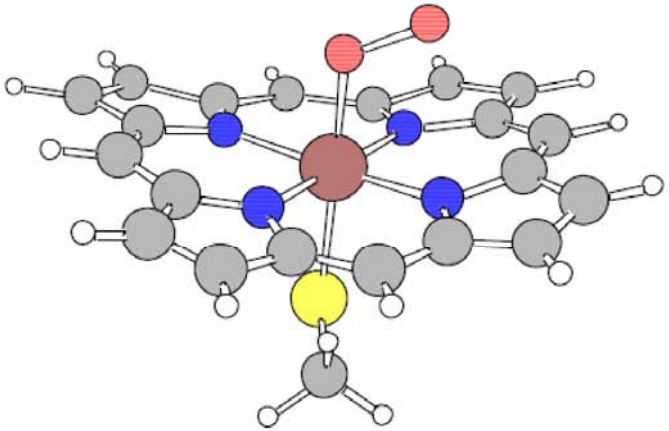
Sigfridsson, E.; Olsson, M.H.M.; Ryde, U. (**2001**) *J. Phys. Chem. B*, 105, 5546

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

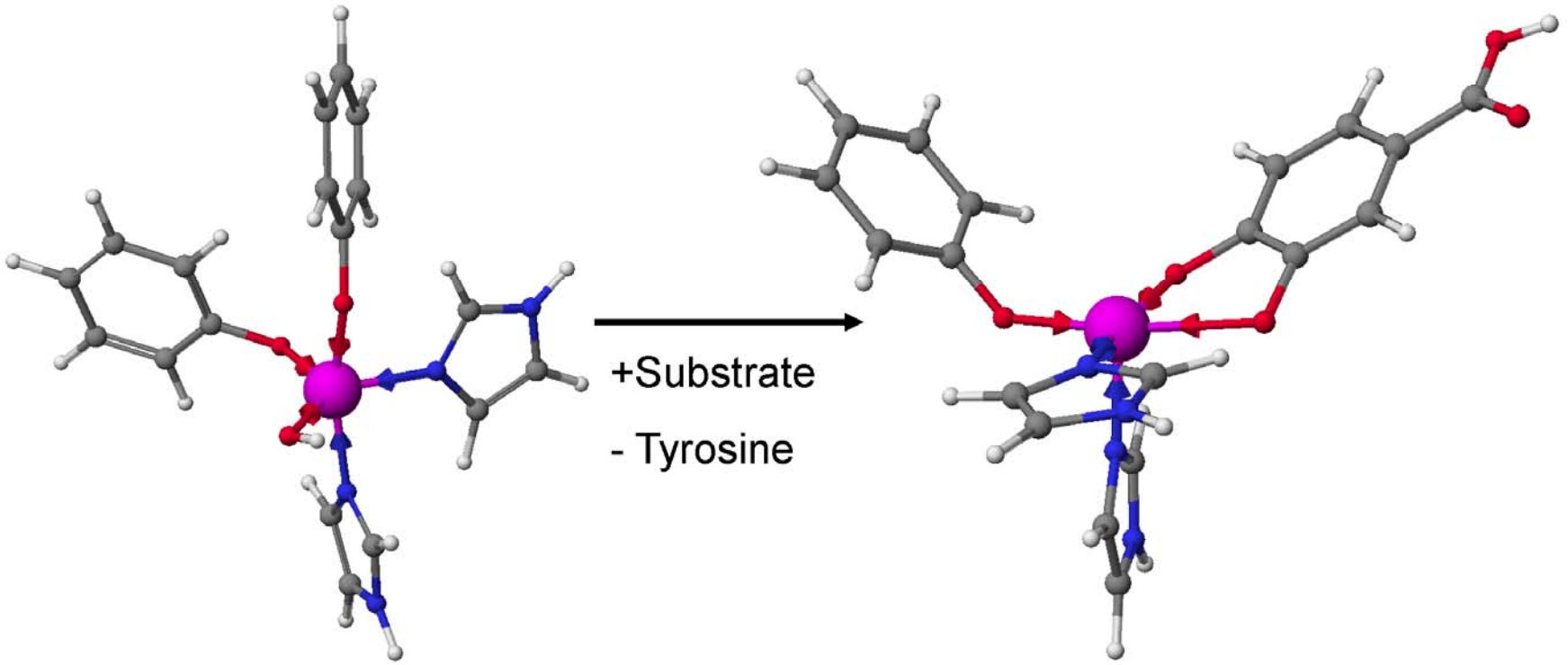


Cytochrome P450
Axial Cysteine
O_2 Activation

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

Opening of Substrate Binding Sites in Enzyme

3,4 Protocatechuate Dioxygenase

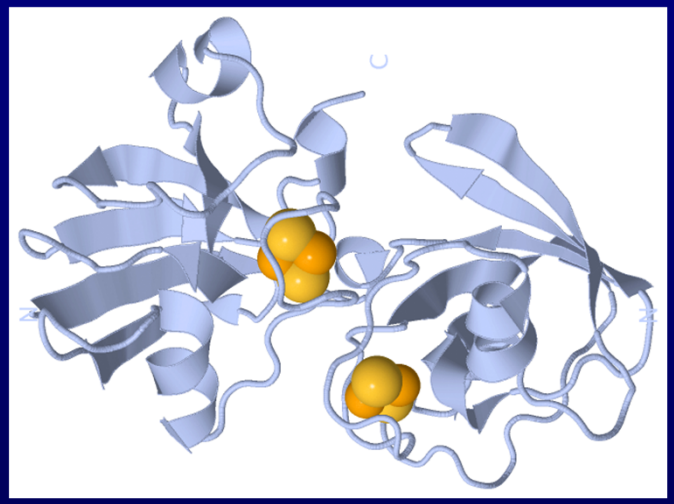


Davis, M.I.; Orville, A.M.; Neese, F.; Zaleski, J.; Lipscomb, J.D.; Solomon, E.I. (**2002**) *J. Am. Chem. Soc. 124, 602*

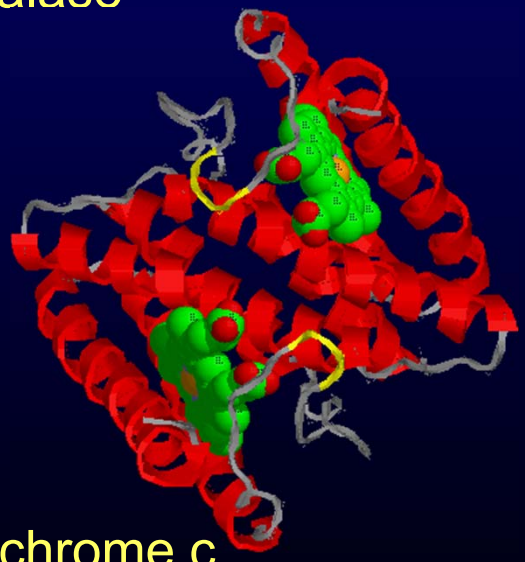
Examples of plant enzymes with iron in the active centre

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

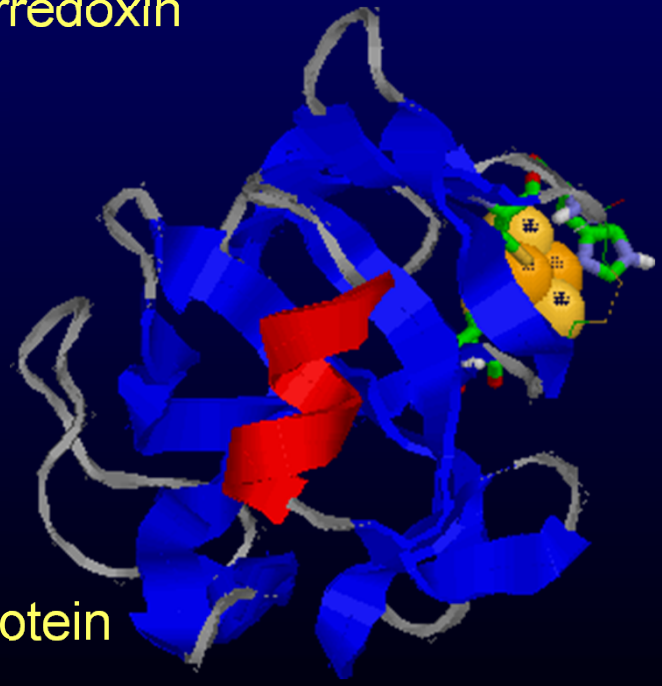
Catalase



Ferredoxin

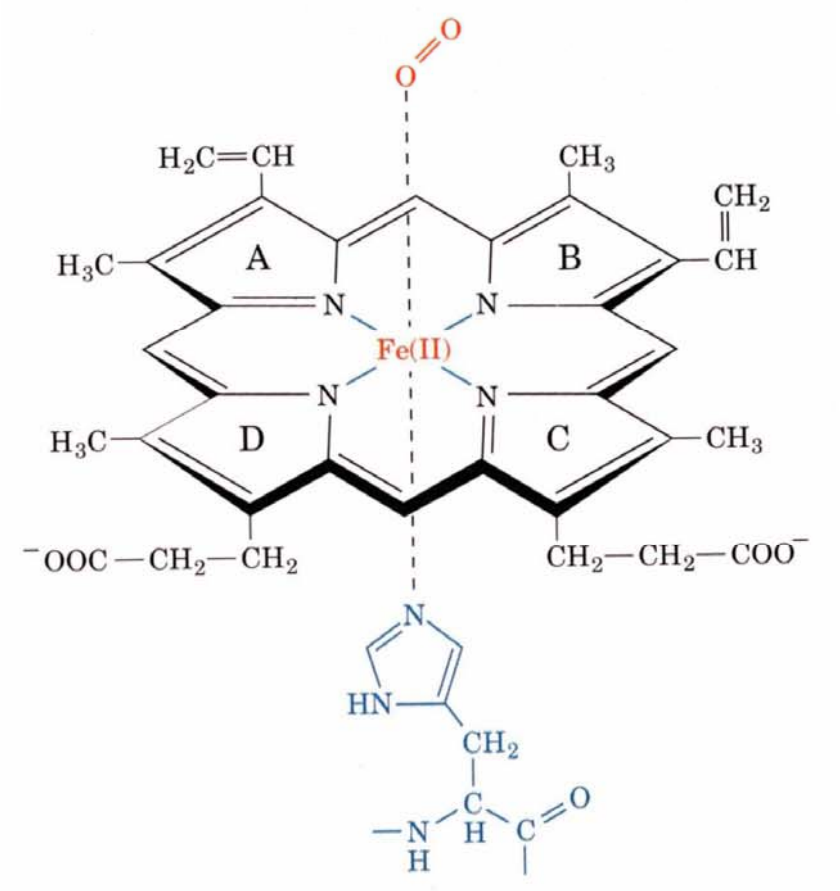


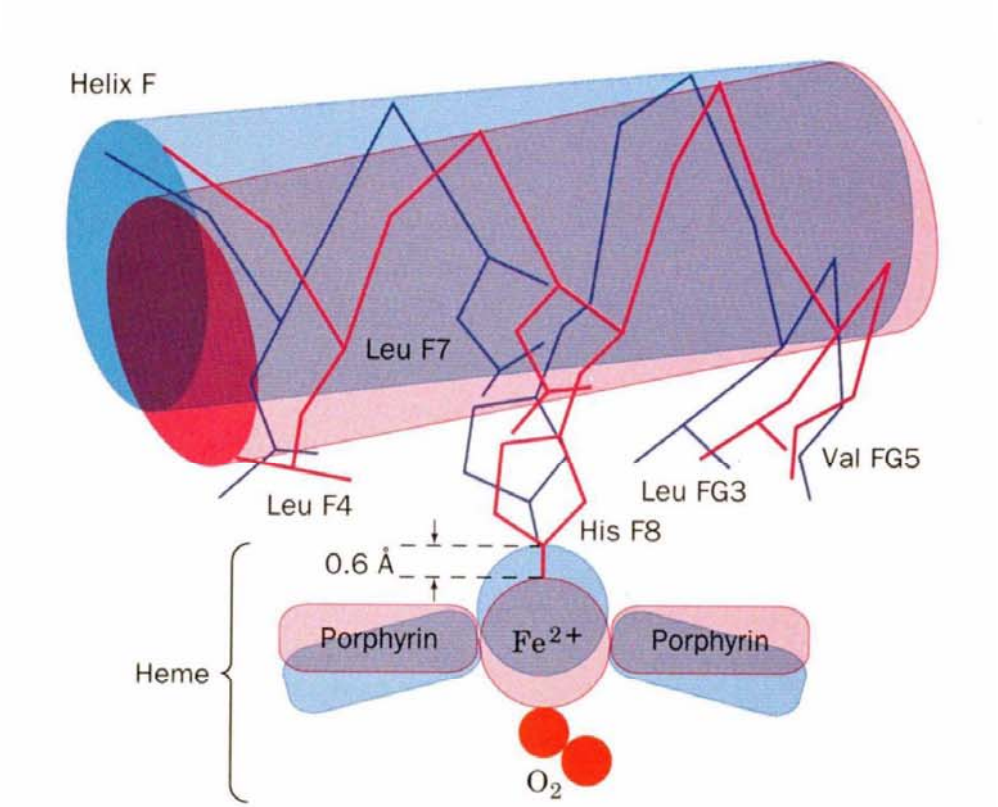
Cytochrome c



Rieske protein

Reversible O_2 Binding Myoglobin and Hemoglobin





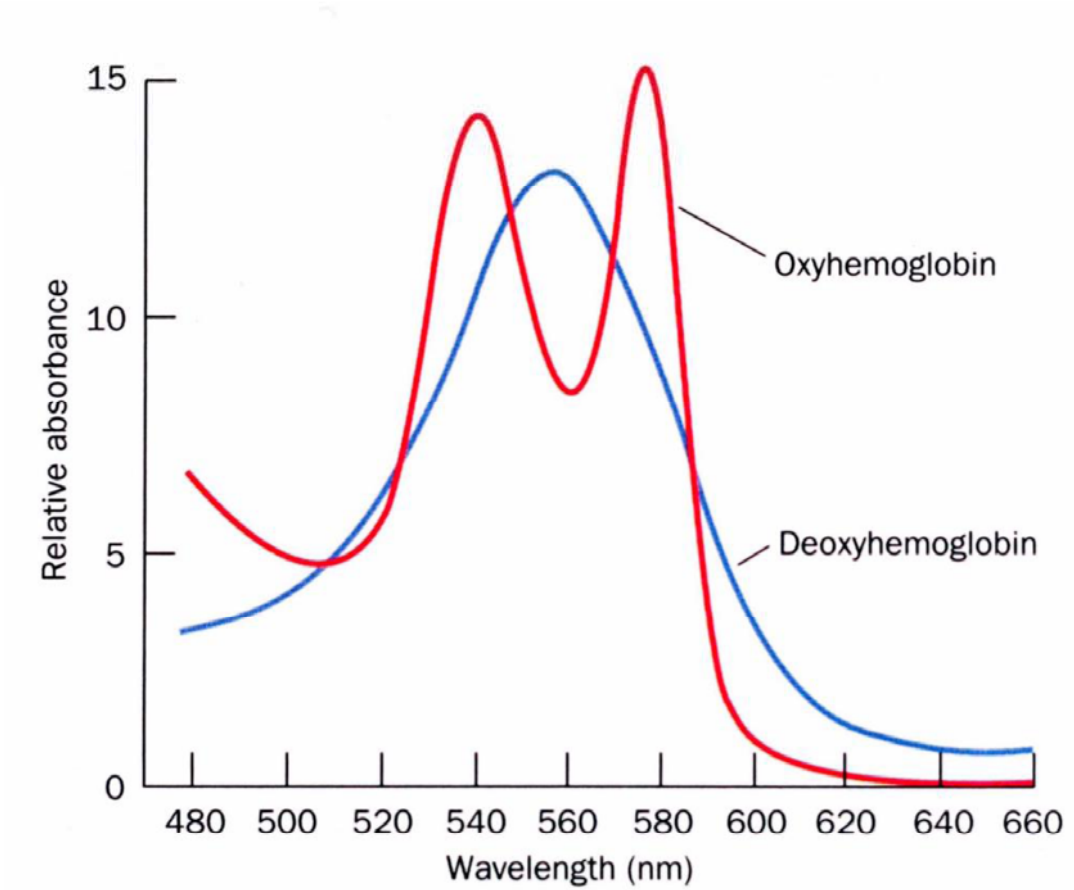
The iron must be in the Fe(II) (ferrous oxidation) state.

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

This causes a difference in the absorption spectra.

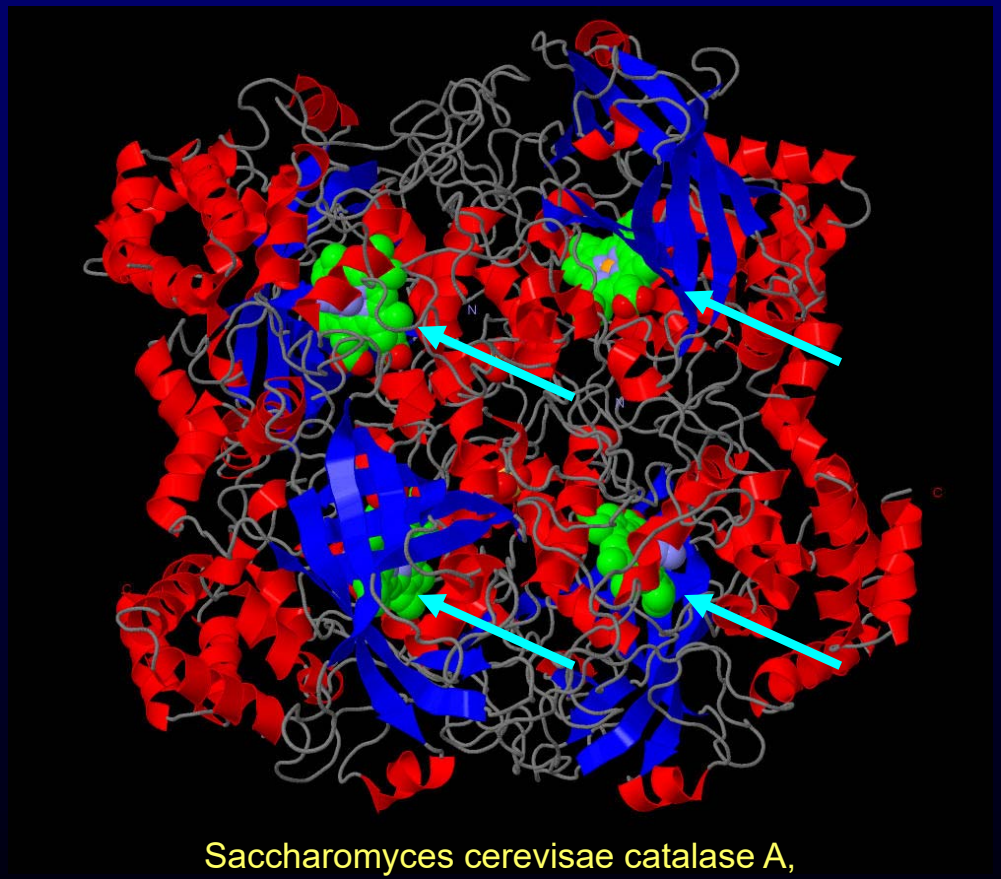
Bluish for deoxy Hb, Redish for Oxy Hb

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

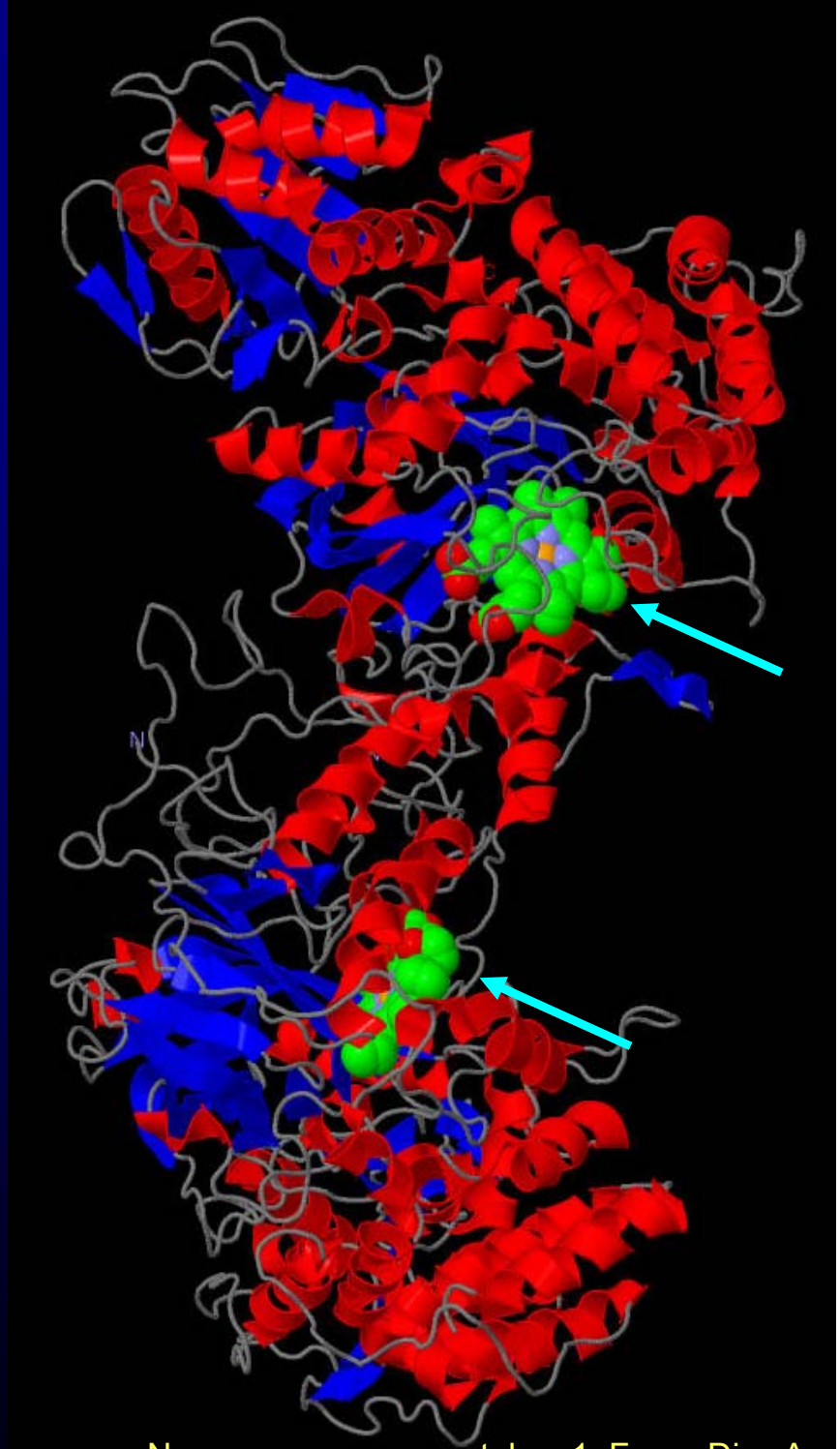


Catalases, some of the most important heme-iron enzymes: (a) function and structure

- 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



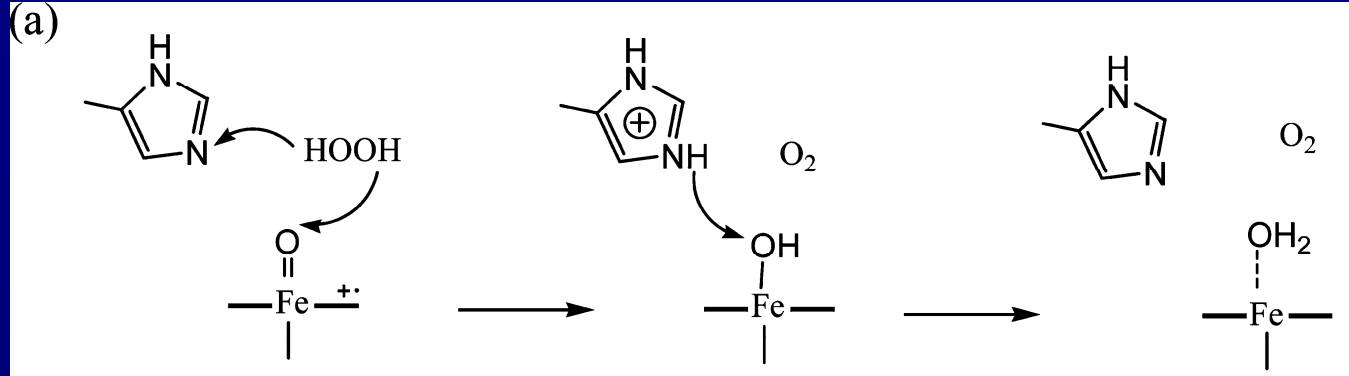
Saccharomyces cerevisae catalase A,
From: Maté JM et al, 1999, J Mol Biol 268, 135-49



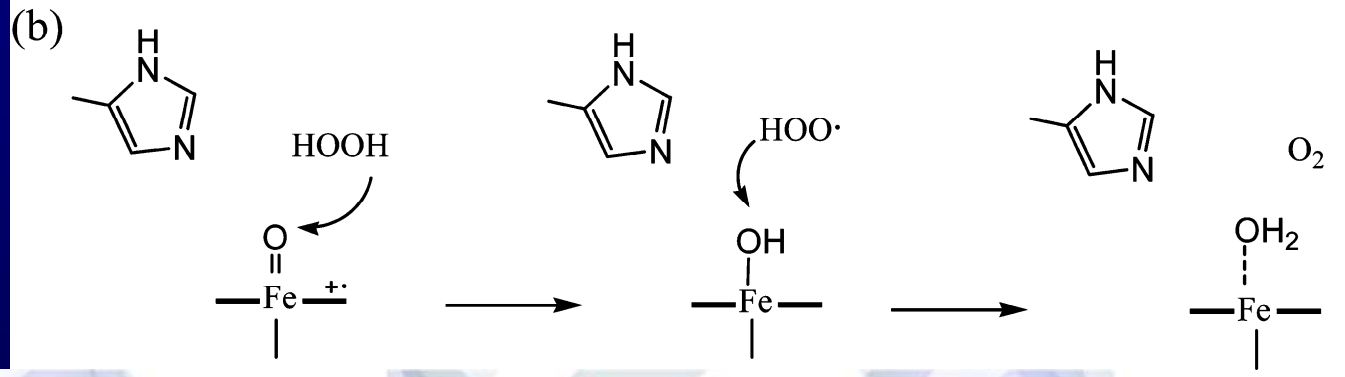
Neurospora crassa catalase1, From: Diaz A et al, 2004, J Mol Biol 342, 971-85

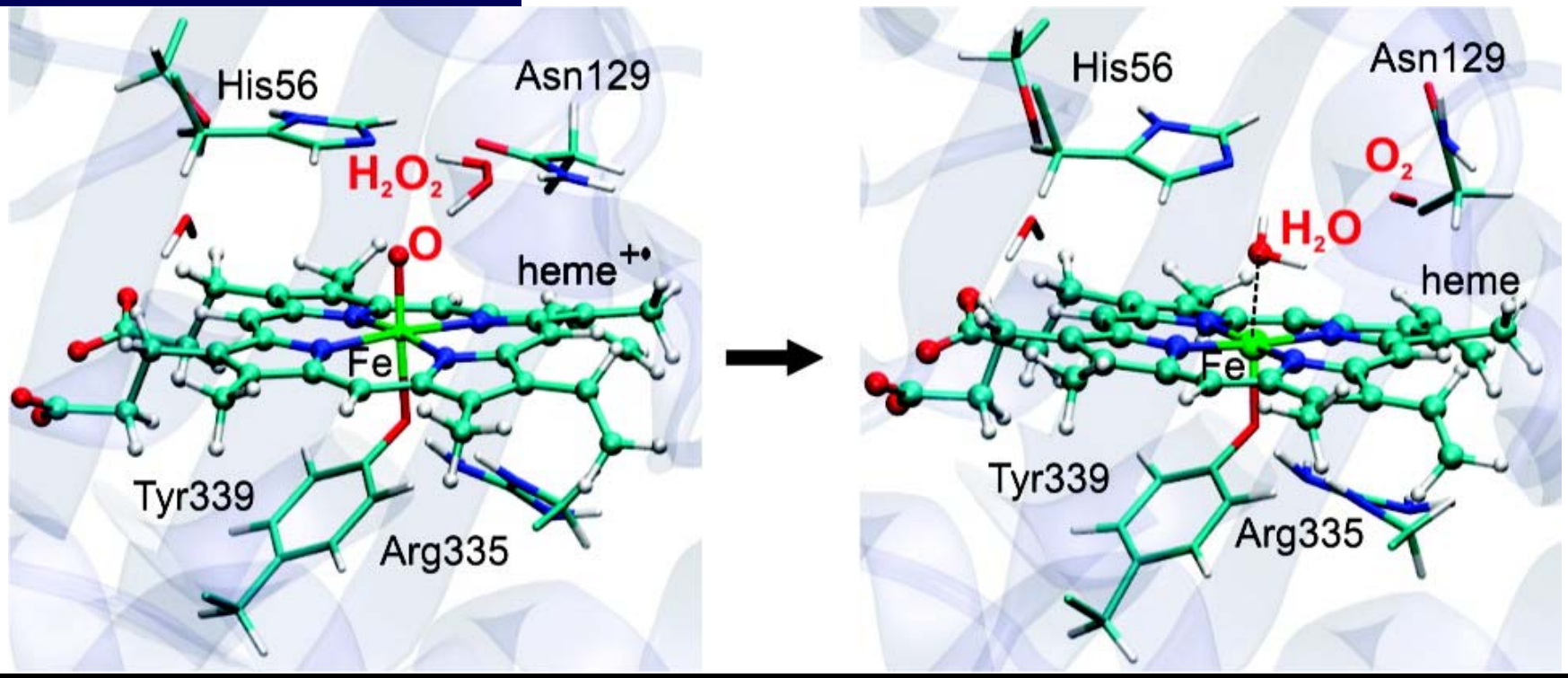
Catalases, heme-iron enzymes (b) catalysis

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

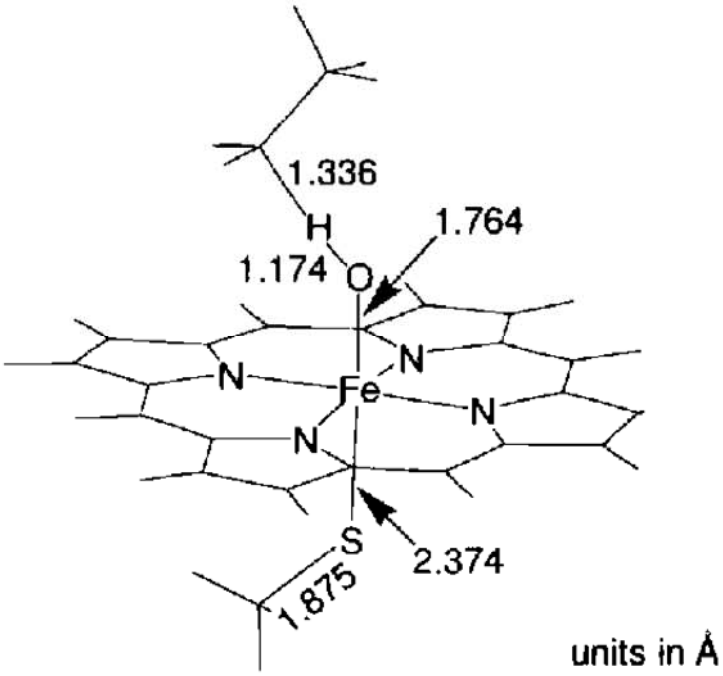


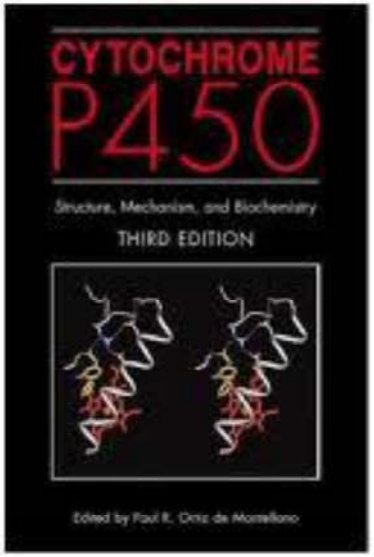
alternative pathways: a) His-mediated mechanism, b) direct mechanism

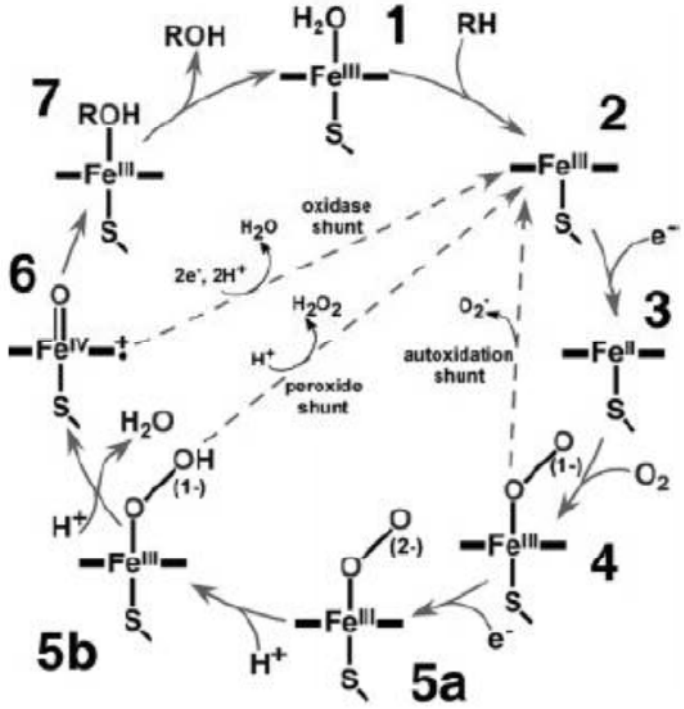




Cytochrome P450







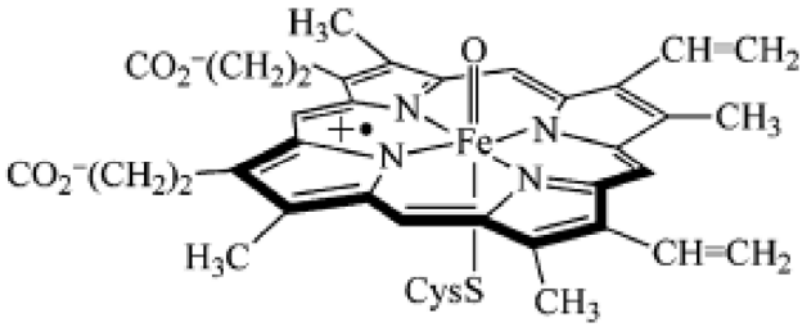
Ortiz de Montellano Chem. Rev. (2010) 110, 932–948; Denisov, Makris, Sligar,Schlichting, Chem. Rev. (2005) 105, 2253-2277

Typical Reactions of Cytochrome P450 (O-Transfer)

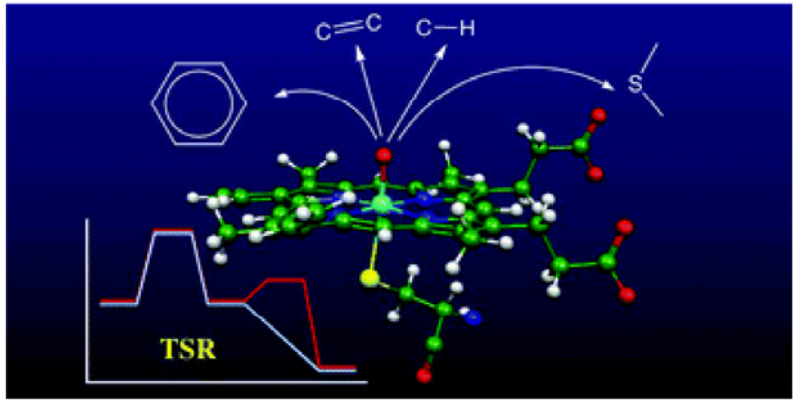
S. SHAIK et al. (2010) Acc. Chem. Res., 43, 1154-1165

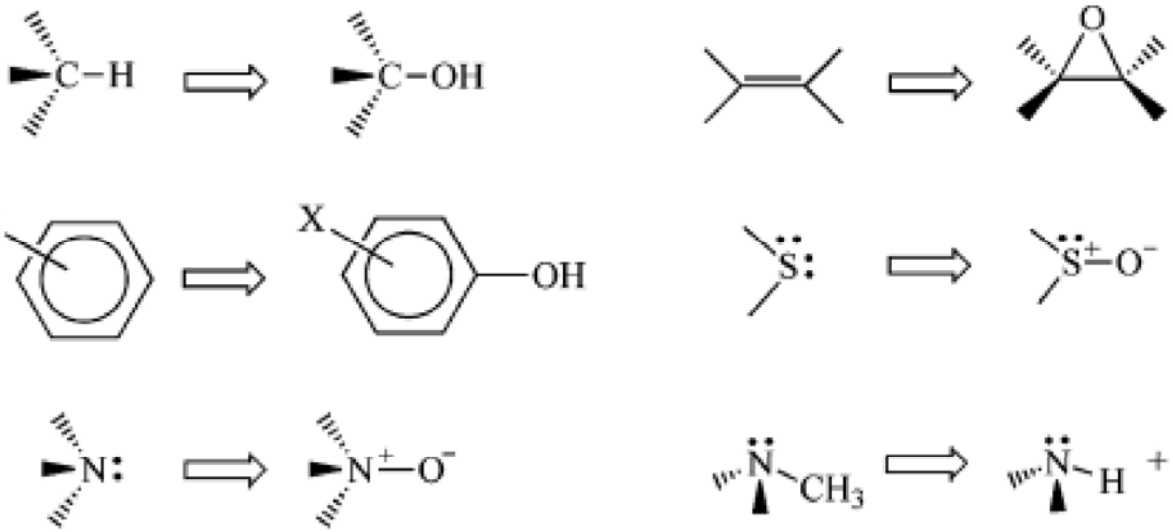
J. Rittle, M. T. Green (2010), SCIENCE 330, 933-936

SCHEME 1



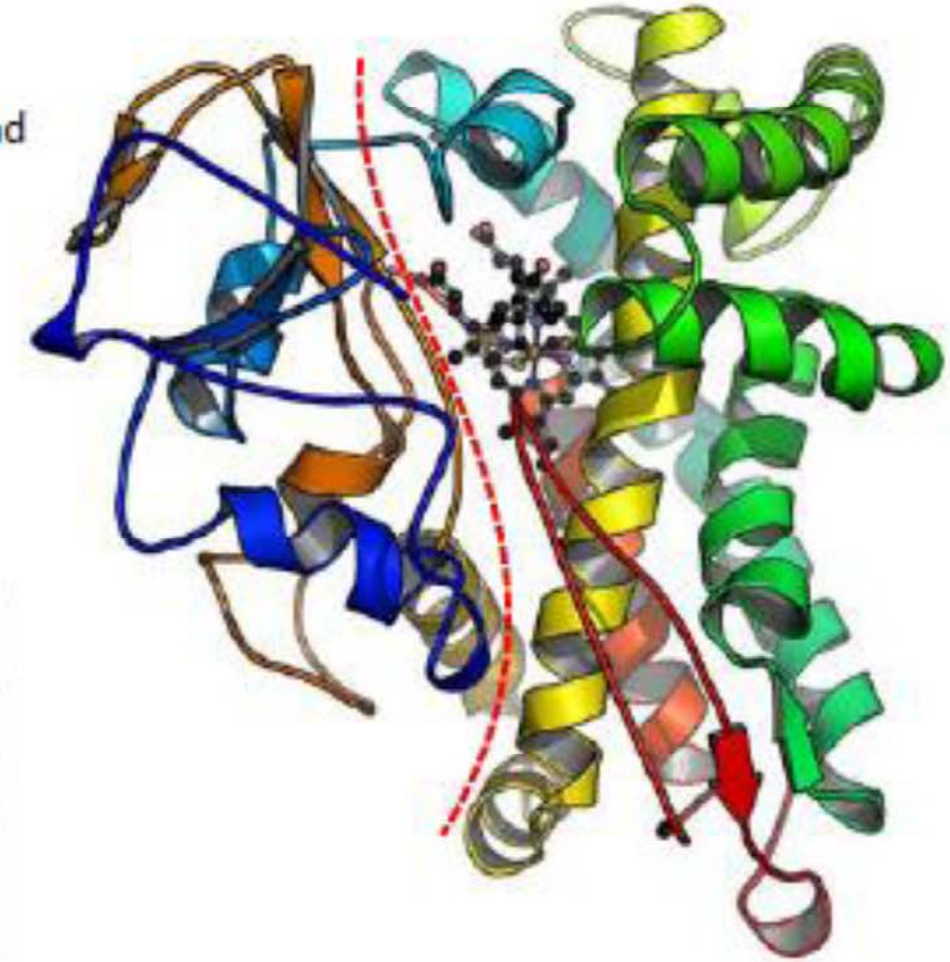
P450 Cpd I





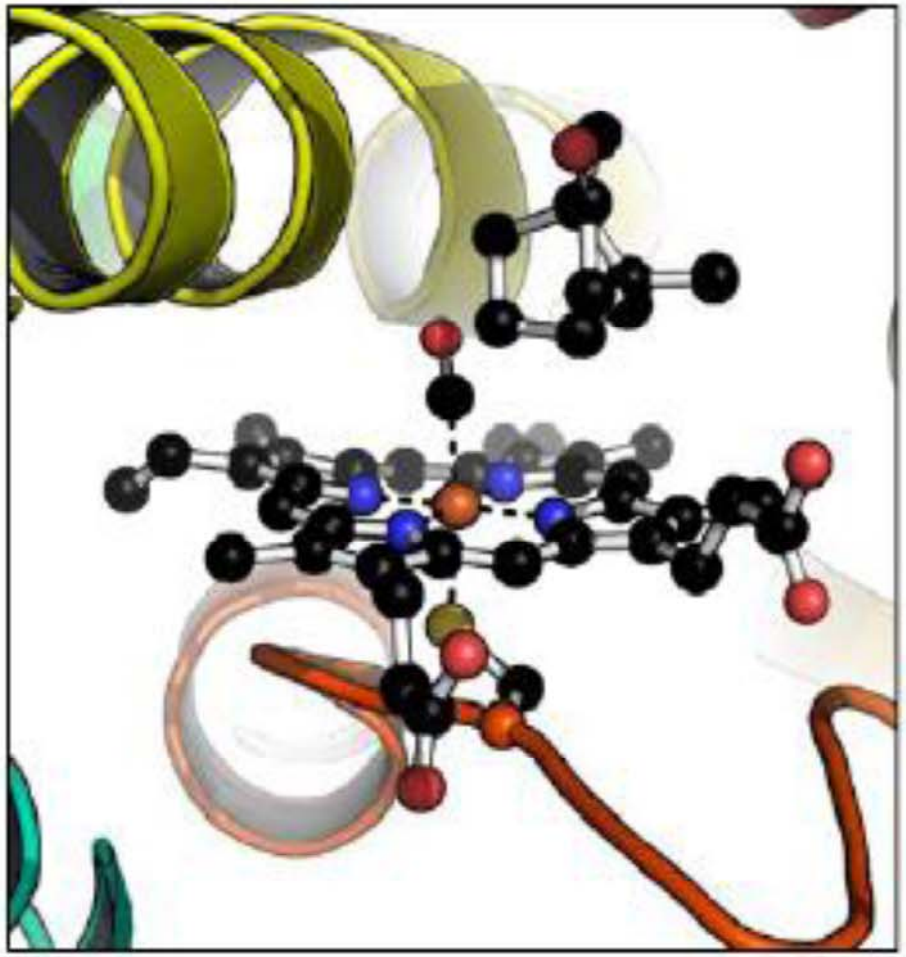
Cytochrome P450 – important enzyme for detoxification of organic compounds

- 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.

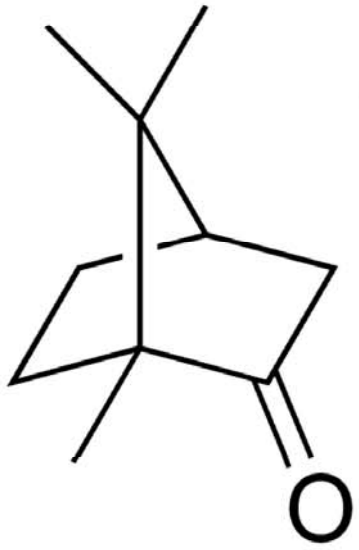
Camphor substrate complex of Cytochrome P450



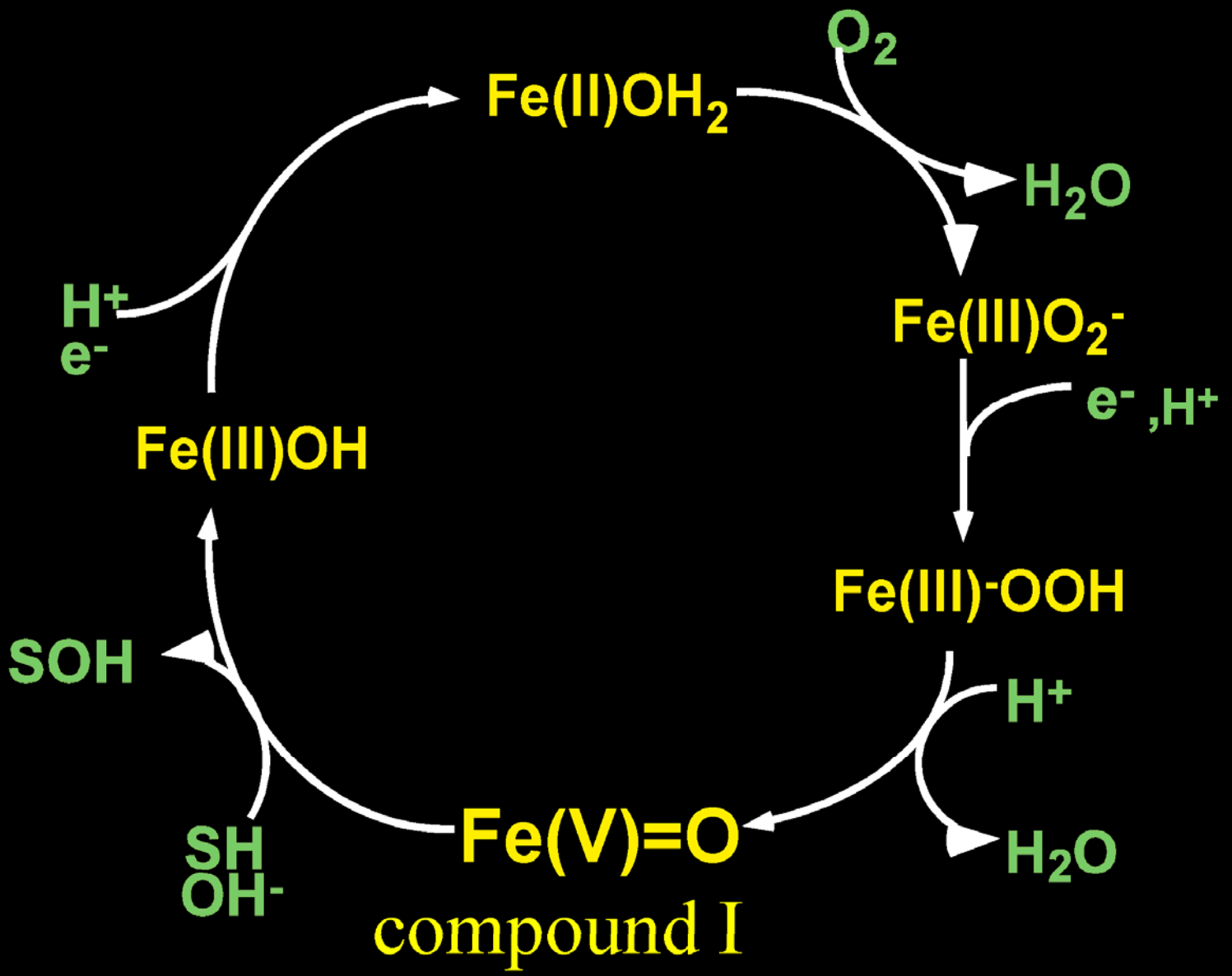
Cytochrome P450cam; PDB-ID 3CPP
Raag & Poulos (1989) *Biochemistry* 28: 7586-7592.

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.



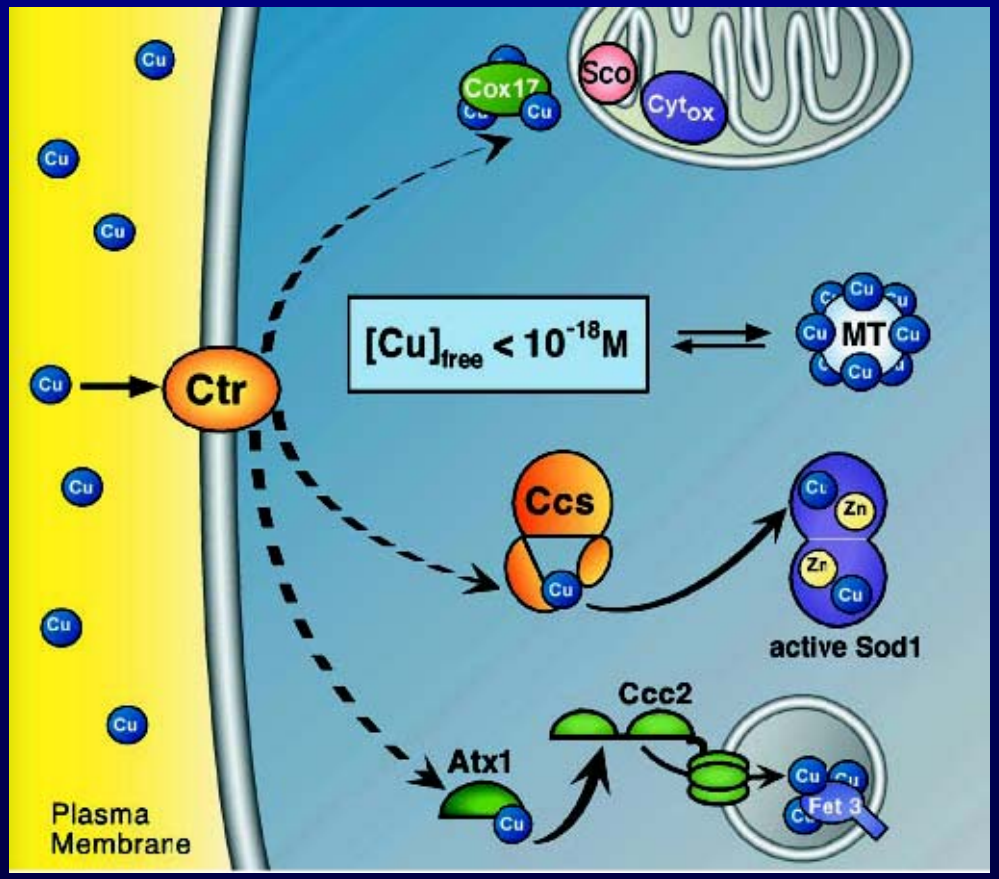
Cytochrome P450 Reaction Cycle



key intermediate

Lecture slide of Peter Kroneck

Copper delivery inside cellular compartments

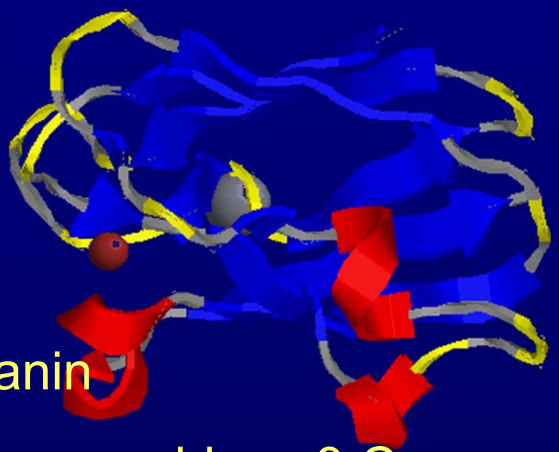


From: OHalloran TV, Culotta VC, 2000, JBC275, 25057-60

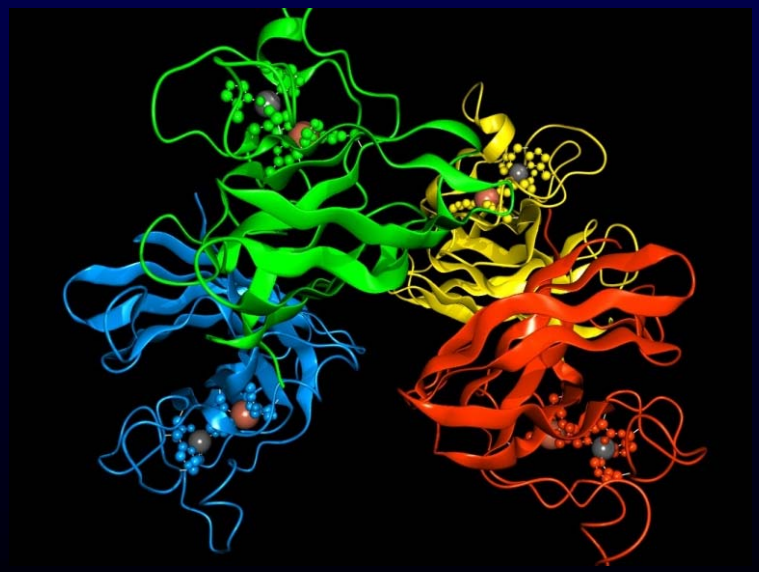
- confusing large number of names for homologous proteins in different organisms
- REALITY: just 3 really different (non-homologous) Cu-chaperones are well known, some more proteins are postulated to be Cu-chaperones

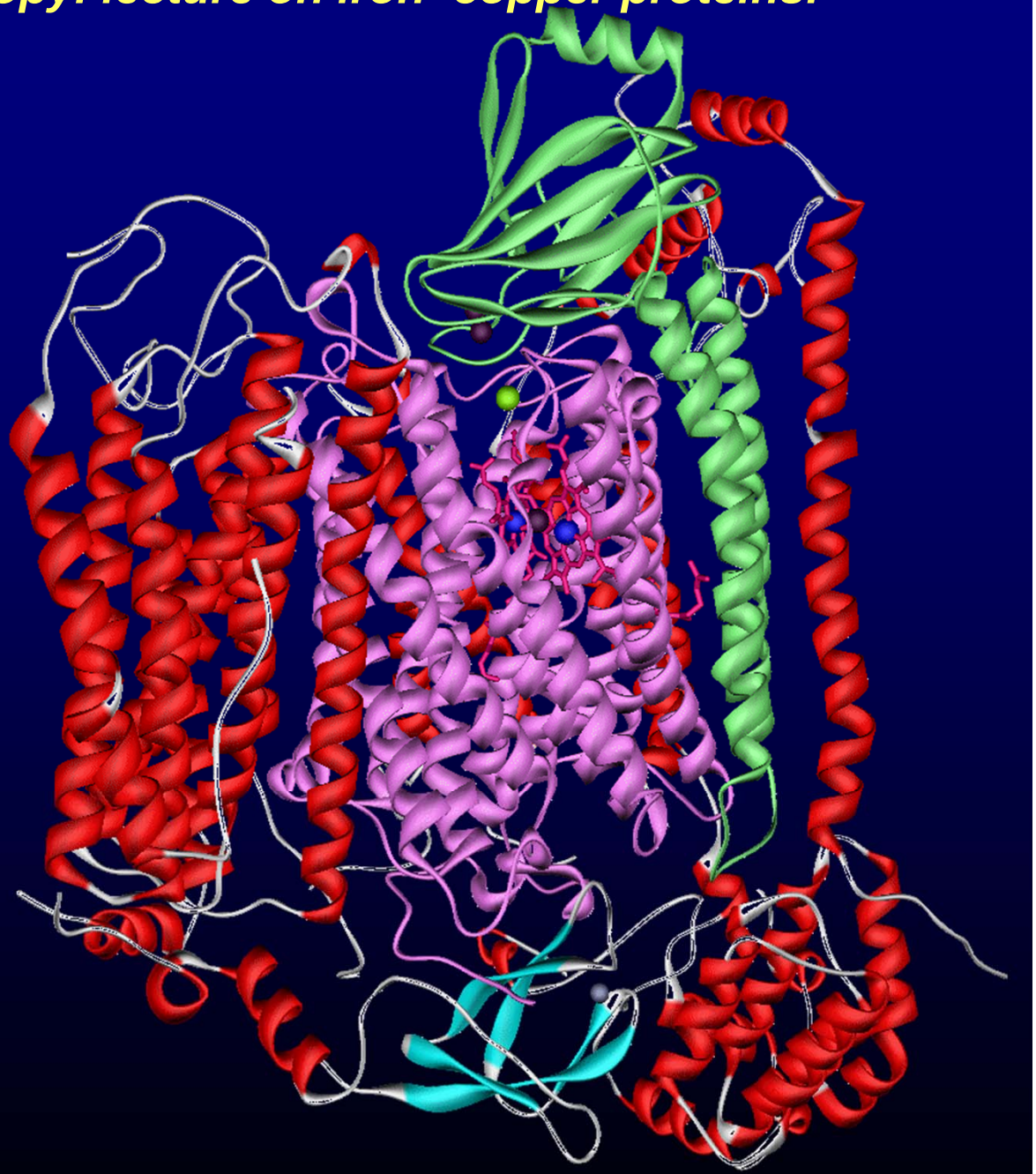
Selected enzymes with copper in the active centre

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

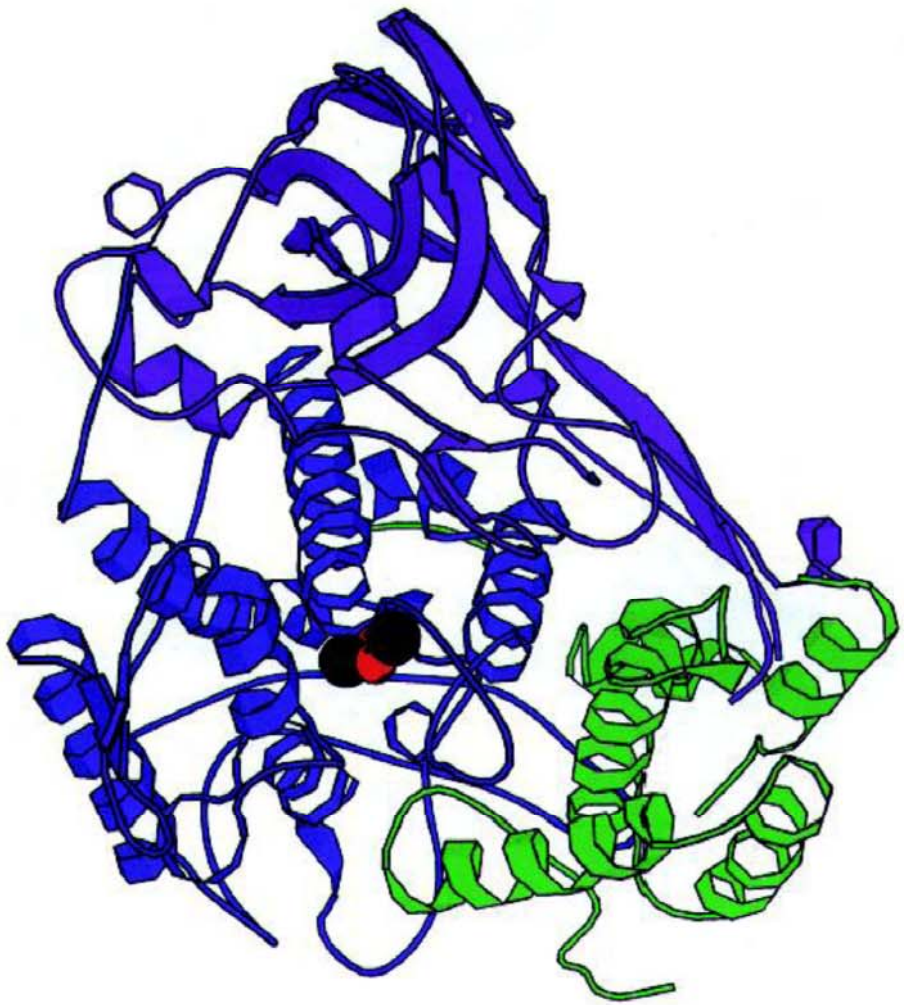
(2) The mononuclear non-blue copper proteins.

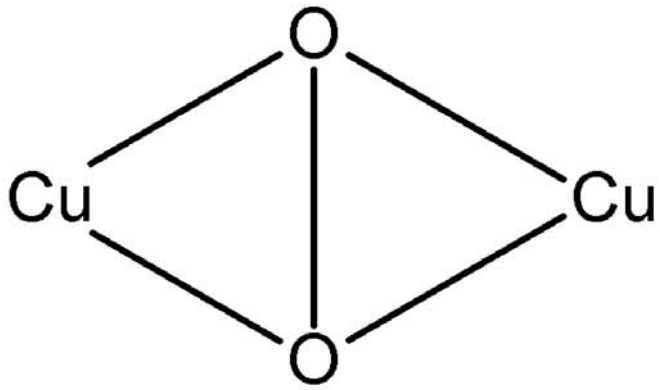
(3) Proteins with a coupled binuclear copper centre.

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)



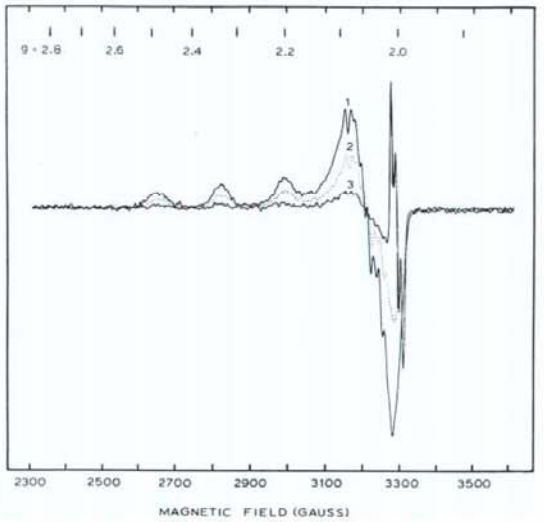


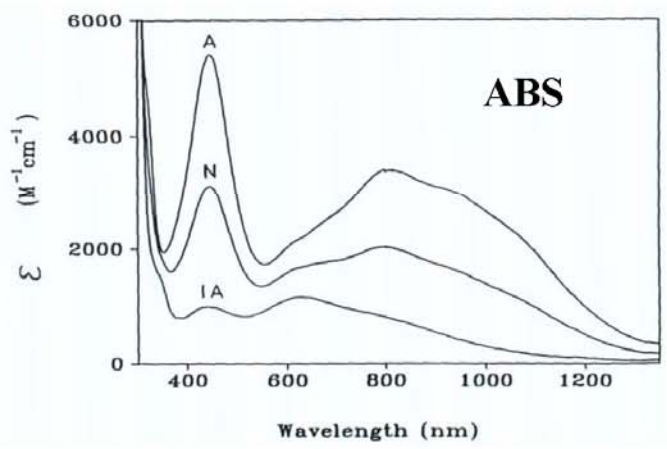
Oxygenated Cu site, see Que, Tolman, NATURE (2008) 455, 333; oxygenated form has a blue colour, **μ-η²:η²-peroxo** binding mode

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

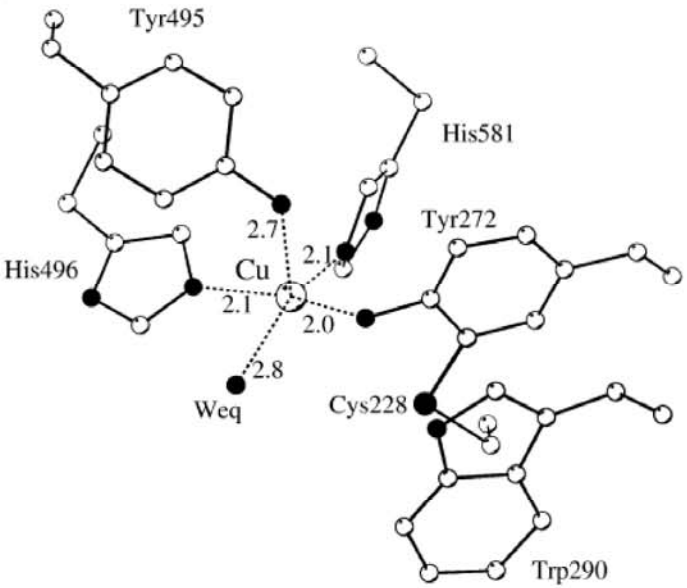
Galactose Oxidase, a Cu-Ligand Radical Enzyme

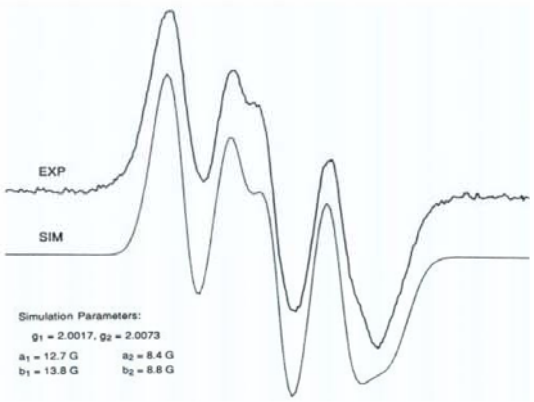
$$RCH_2OH + O_2 \xrightarrow{GalOx} RCHO + H_2O_2$$





(a)

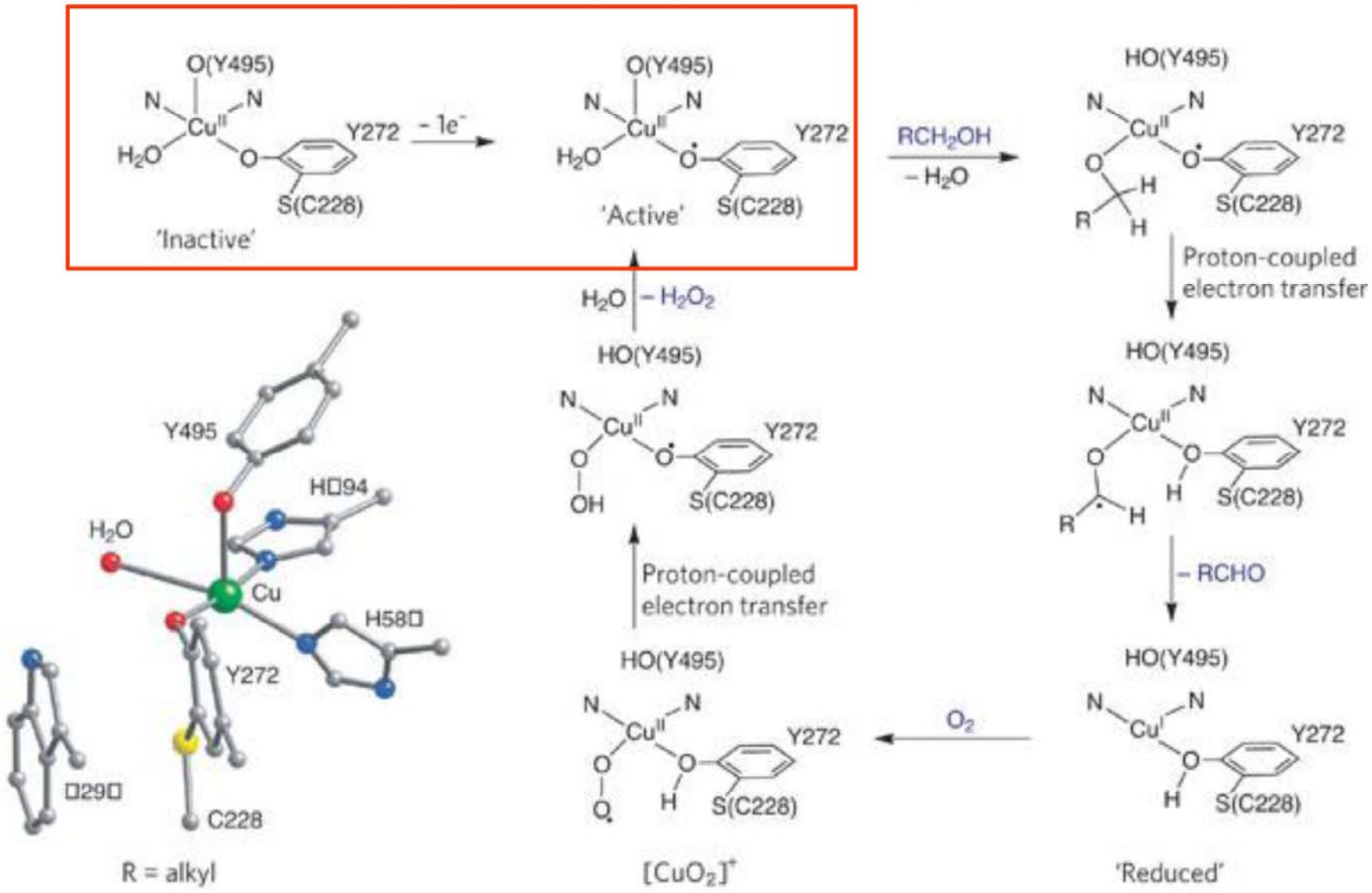




2 different EPR signals (metal-centered and ligand centered) observed during reaction cycle

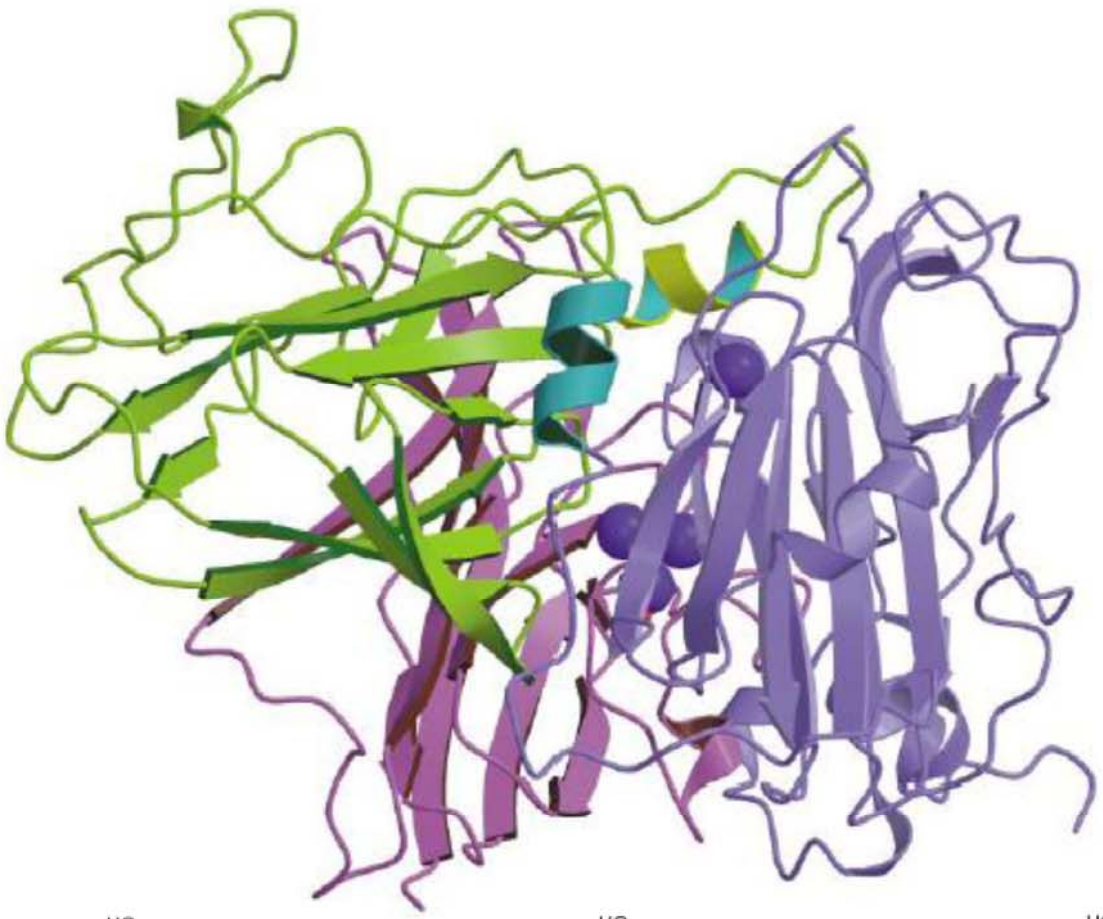
Galactose Oxidase - Mechanism

Que, Tolman (2008) NATURE 455, 333

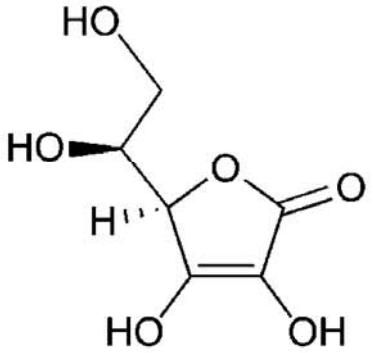


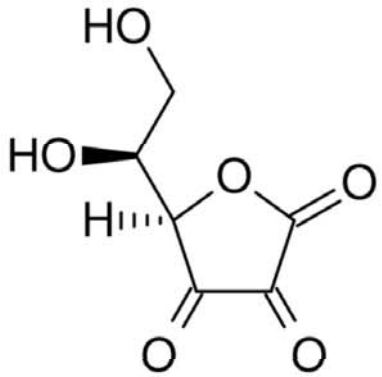
Ascorbic acid oxidase (AOX)

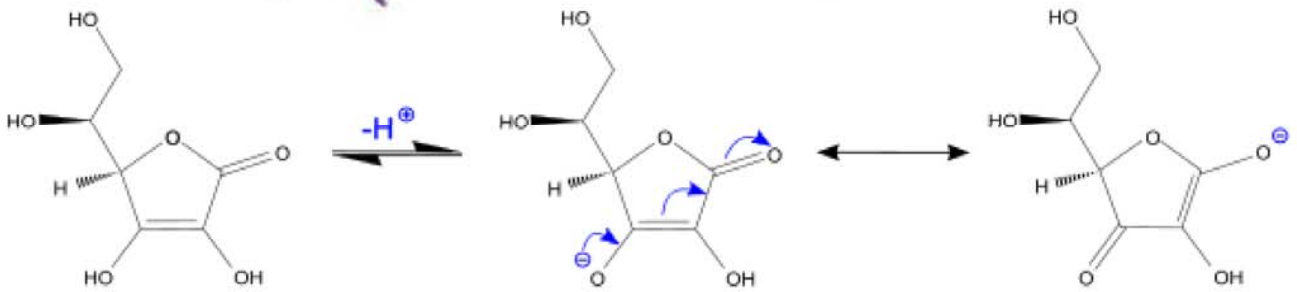
Multi-Copper oxidase (8Cu/homodimer) PDB code 1AOZ



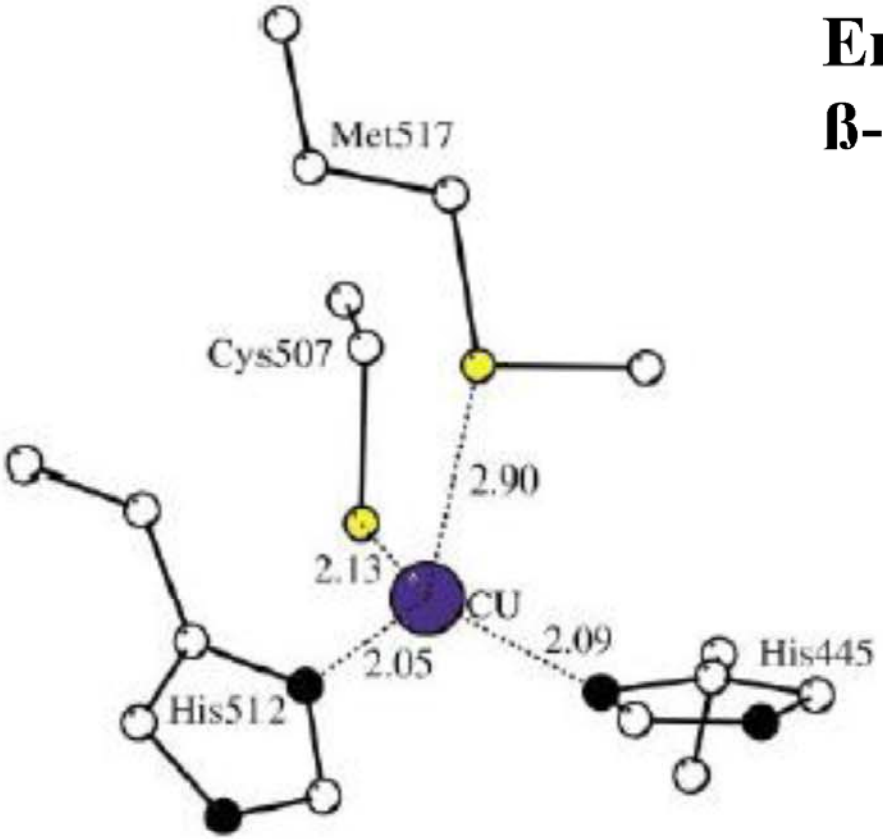
(5*R*)-[(1*S*)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one



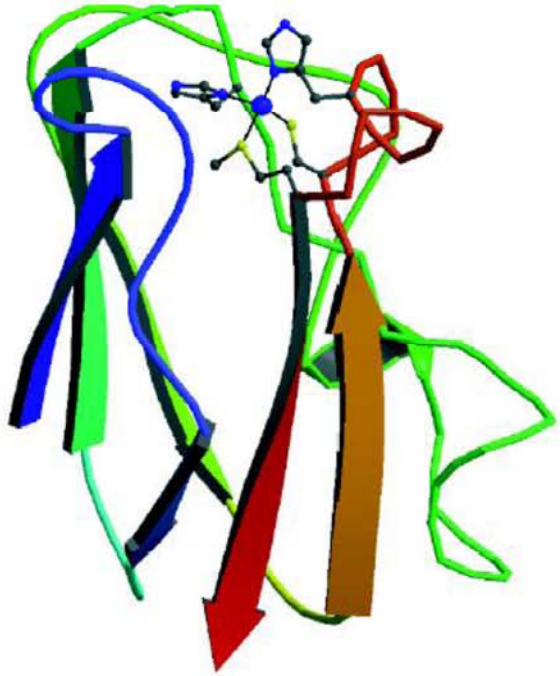




Type 1 electron transfer center of AOX: blue copper protein with high similarity to plastocyanin!

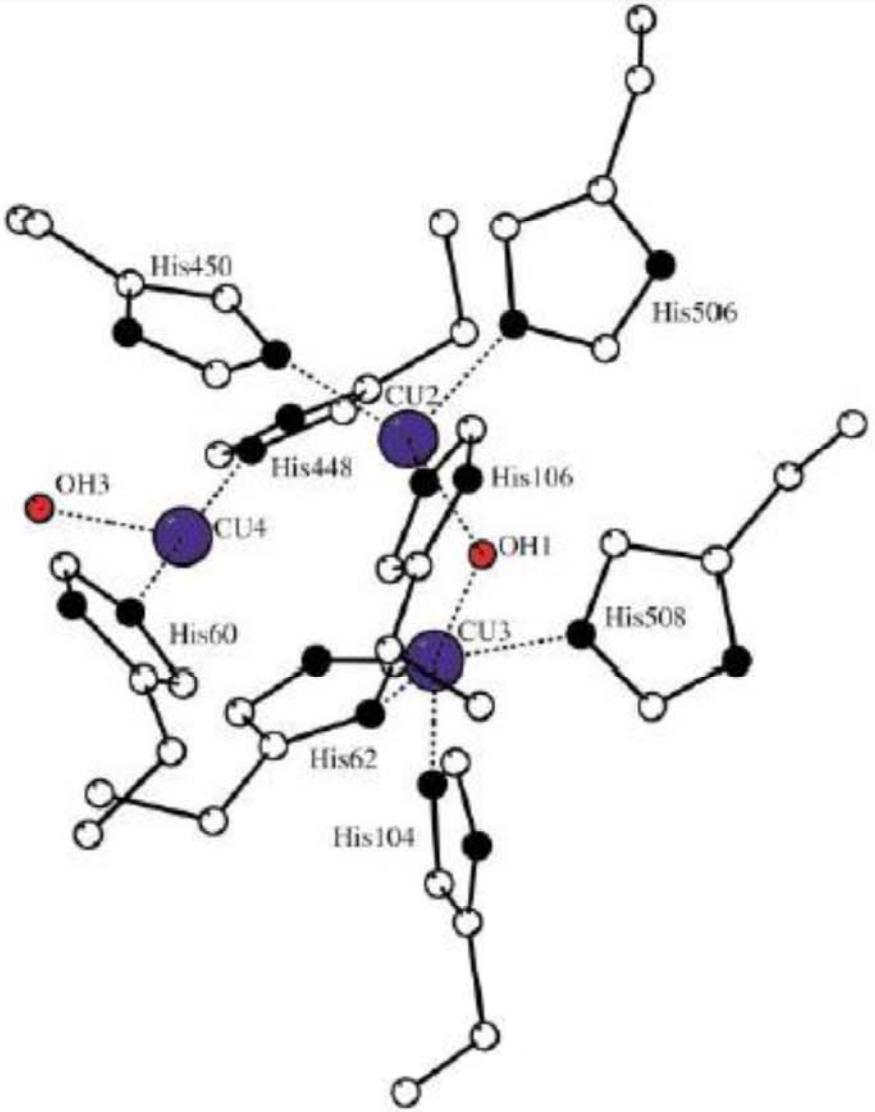


Entrance point for electrons
ß-barrel structural module



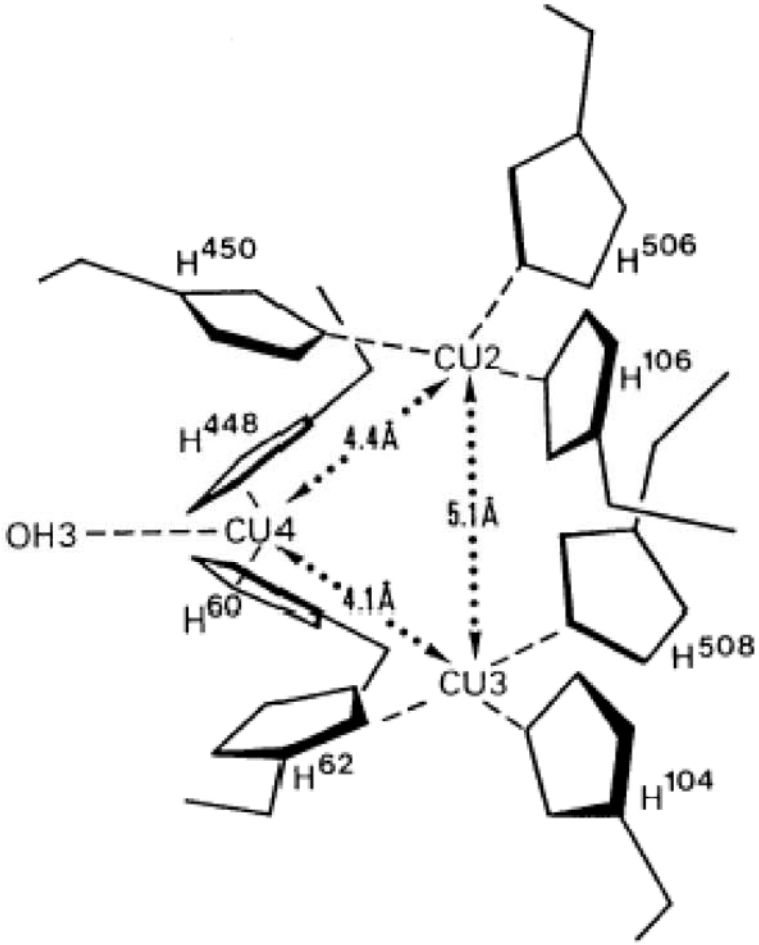
A. Messerschmidt, Handbook of Metalloproteins (2001)

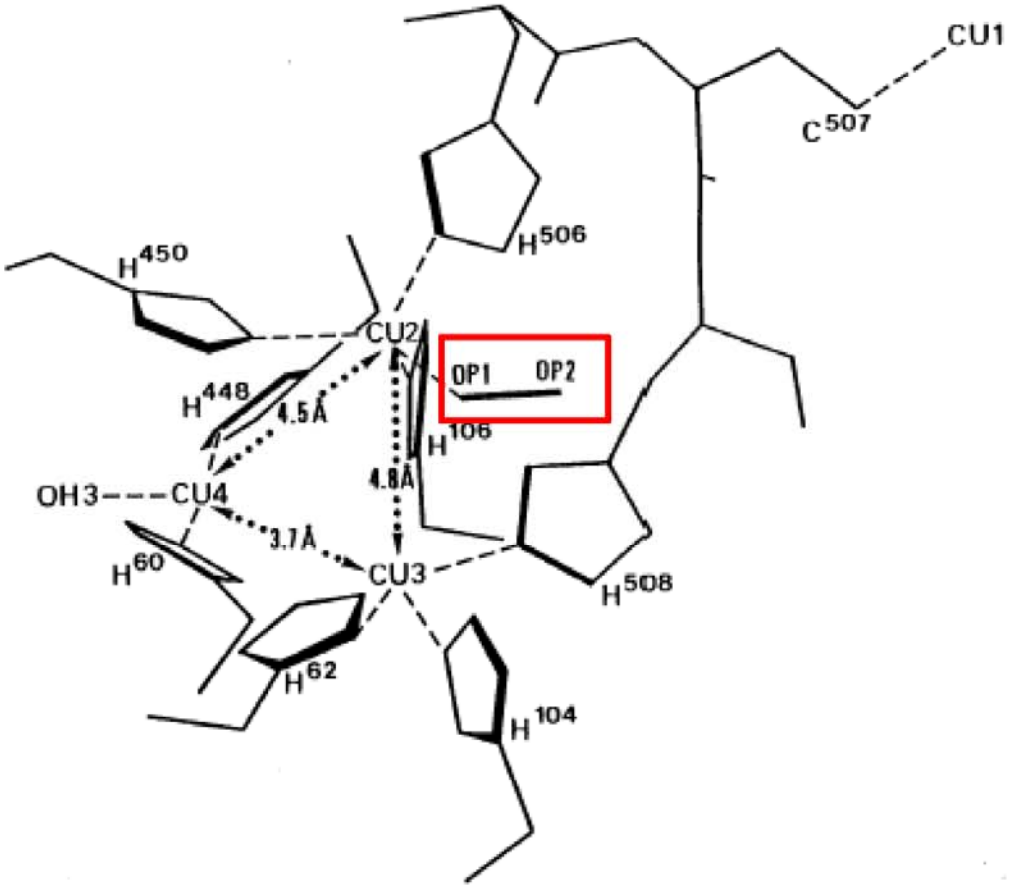
Dioxygen reduction site of AOX: unique trinuclear copper centre



A. Messerschmidt, Handbook of Metalloproteins (2001)

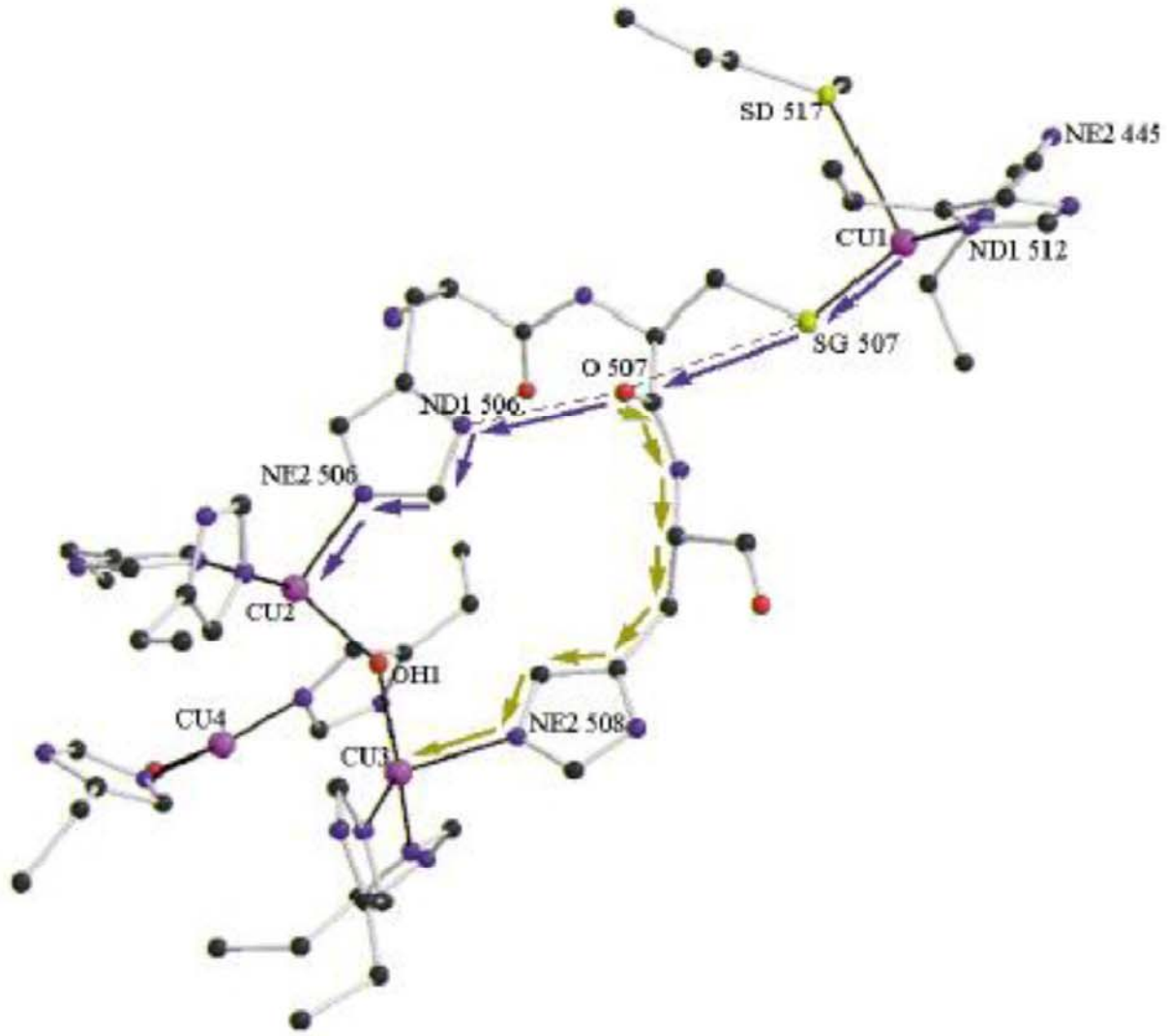
Dioxygen reduction site of AOX: unique trinuclear copper centre - substrate binding





A. Messerschmidt, Handbook of Metalloproteins (2001)

Ascorbic acid oxidase (AOX): electron transport pathways

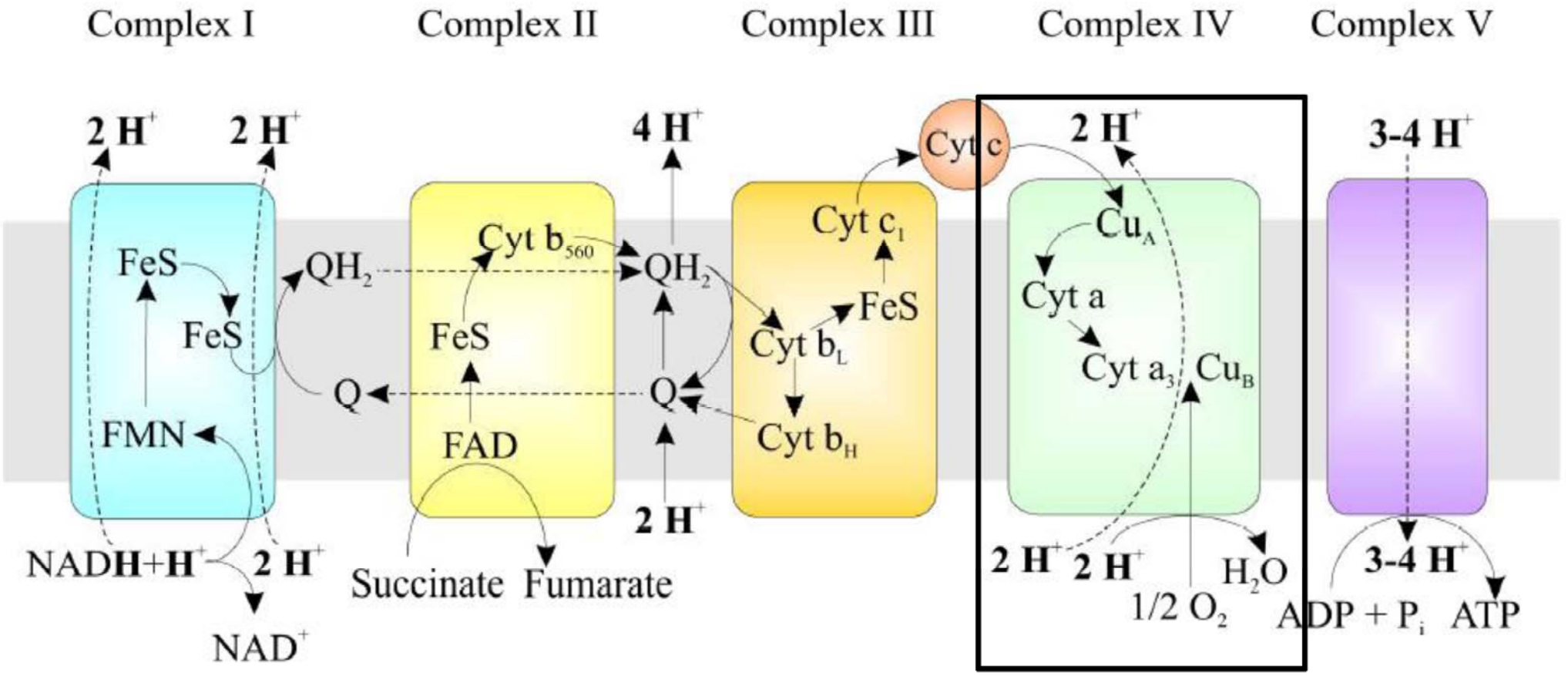


A. Messerschmidt, Handbook of Metalloproteins (2001)

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

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

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

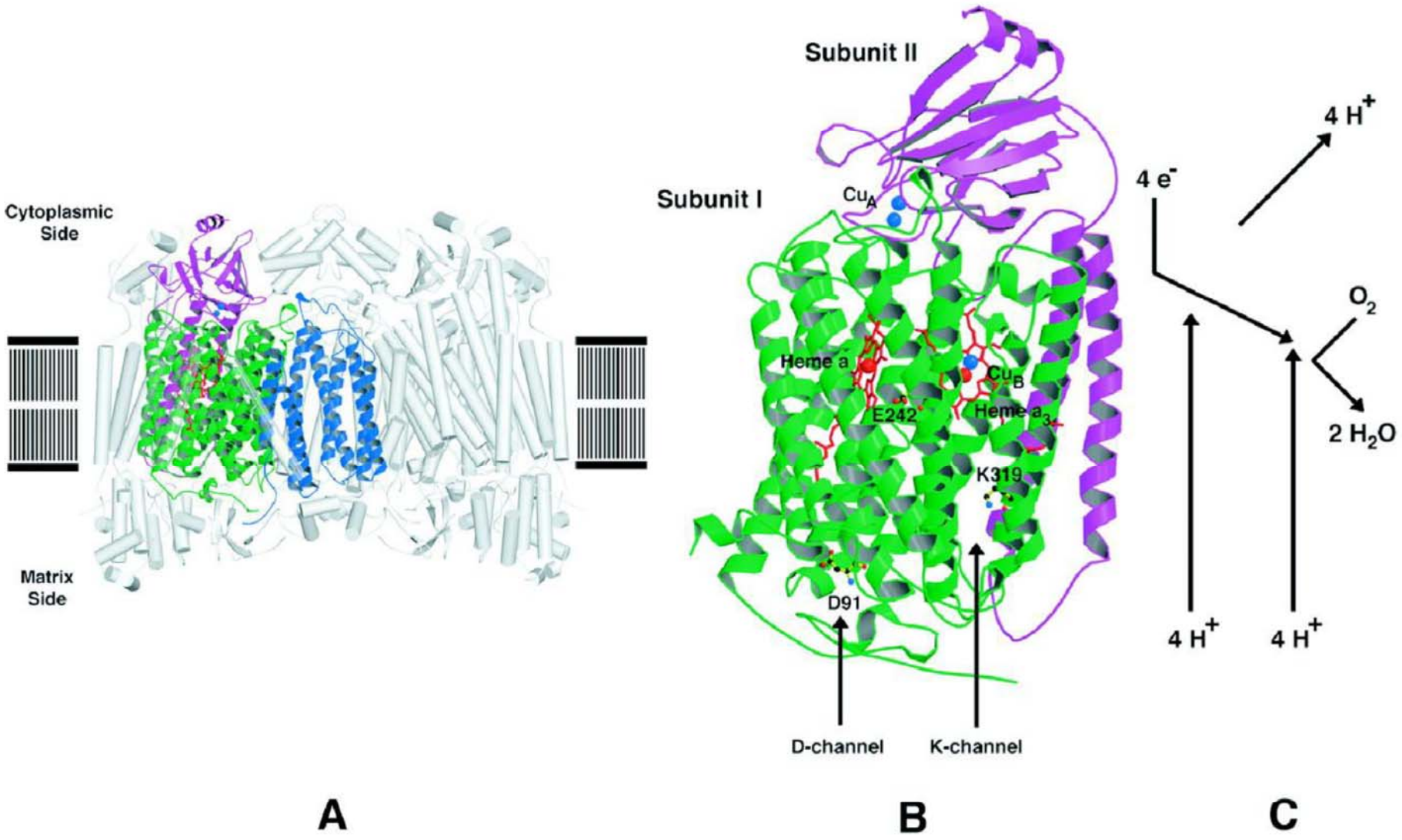
metals (CuA, Fe-heme, Mg, Zn)

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



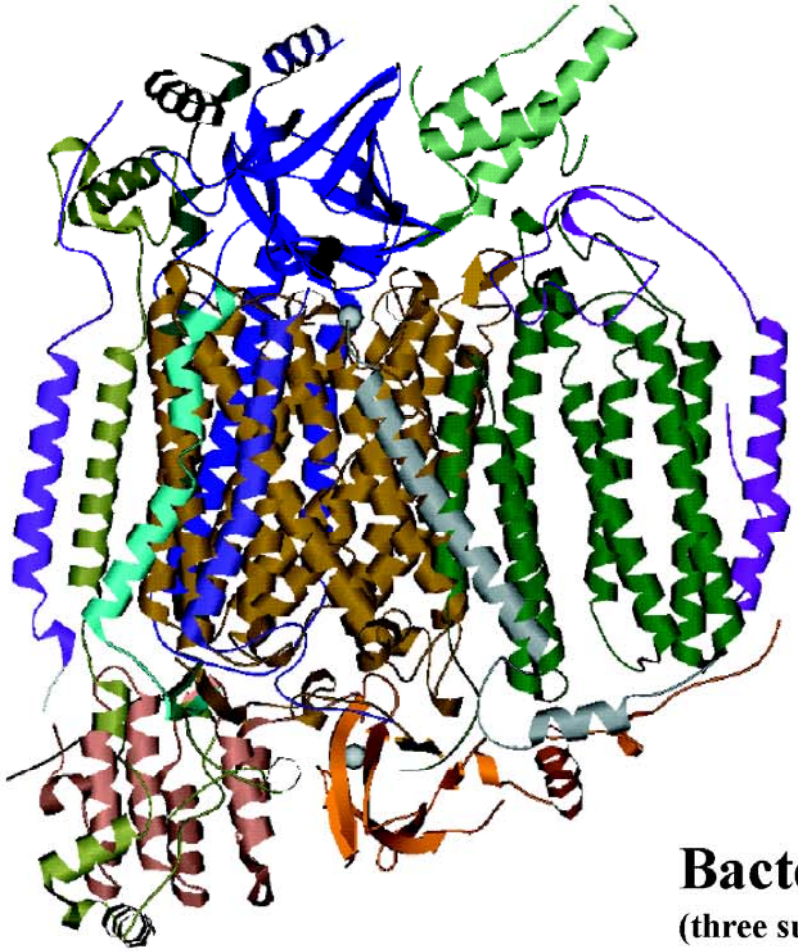
M Saraste Science (1999) 283,1488-1493

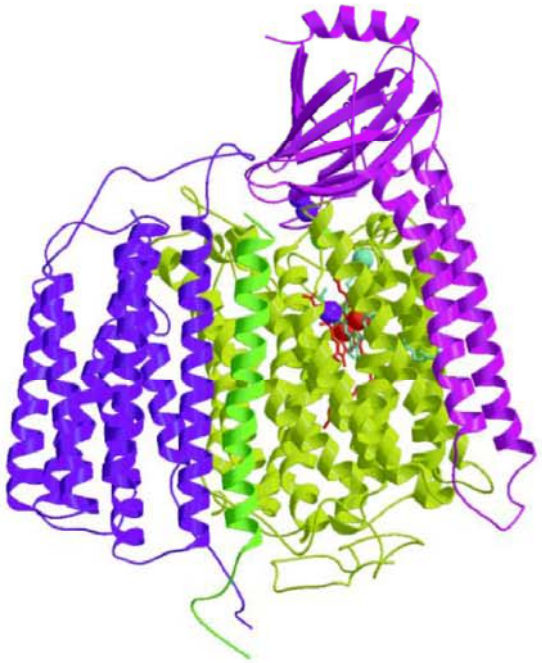
based on a lecture slide of Peter Kroneck

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





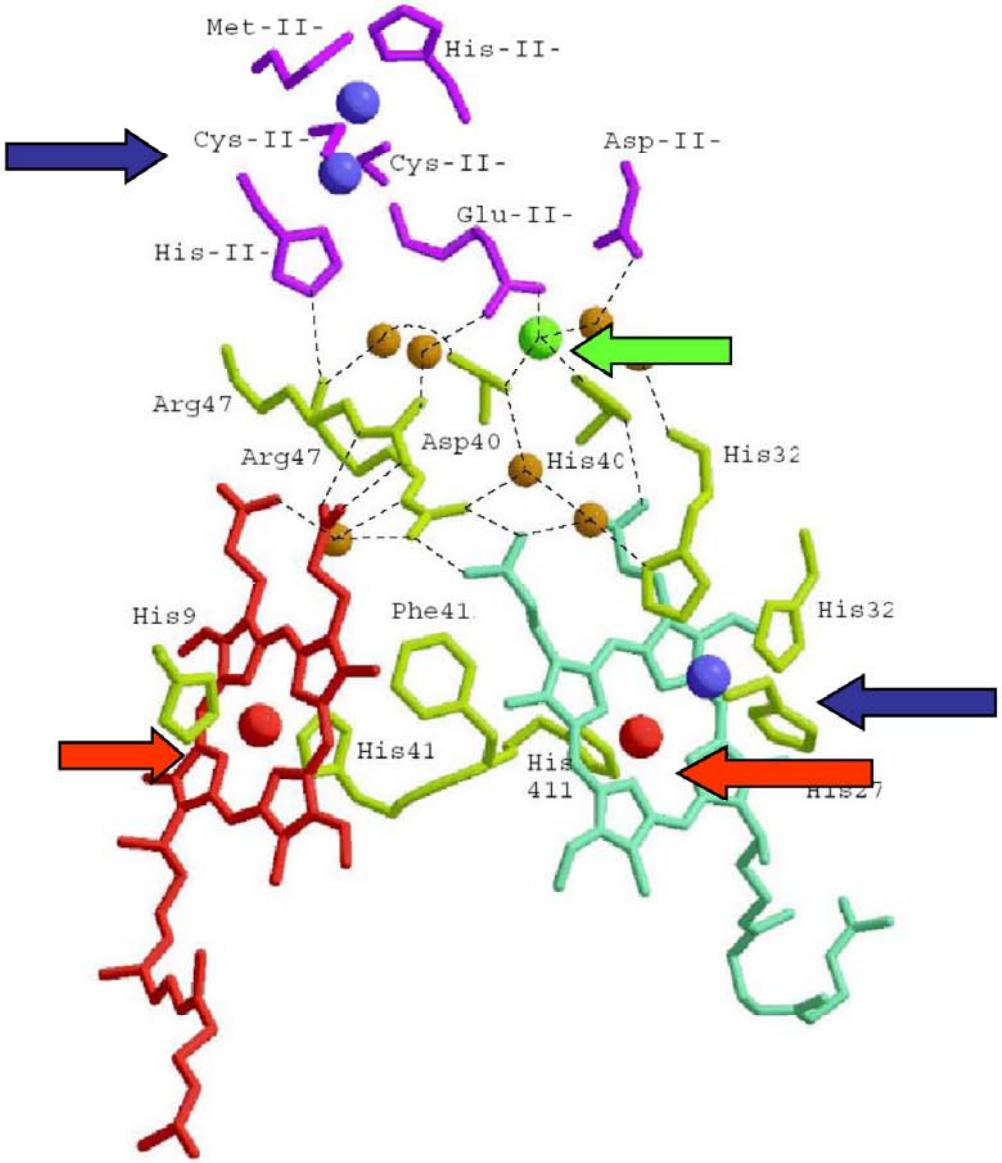
Bacterial COX from *Pseudomonas denitrificans*

(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

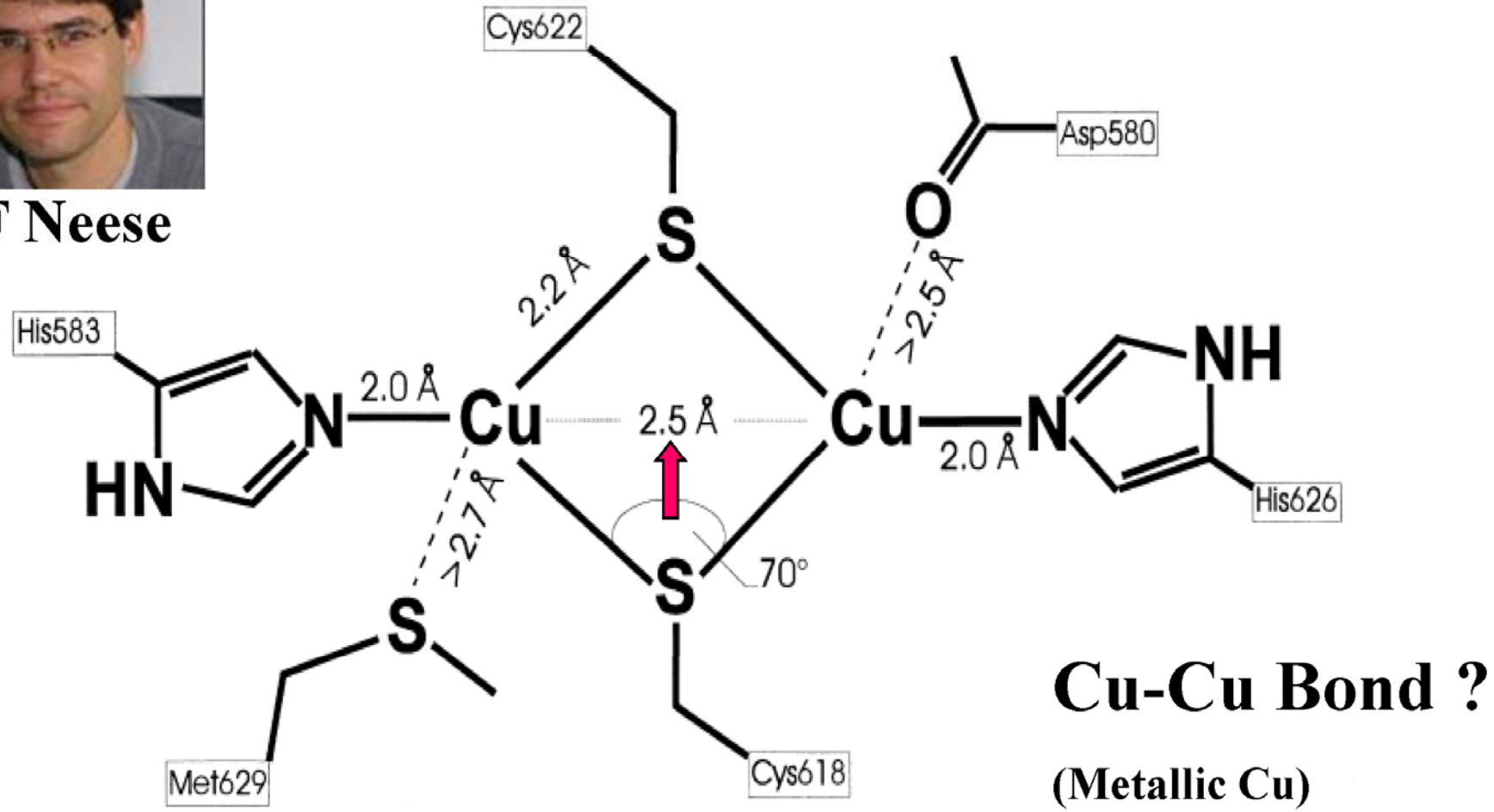


based on a lecture slide of Peter Kroneck

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



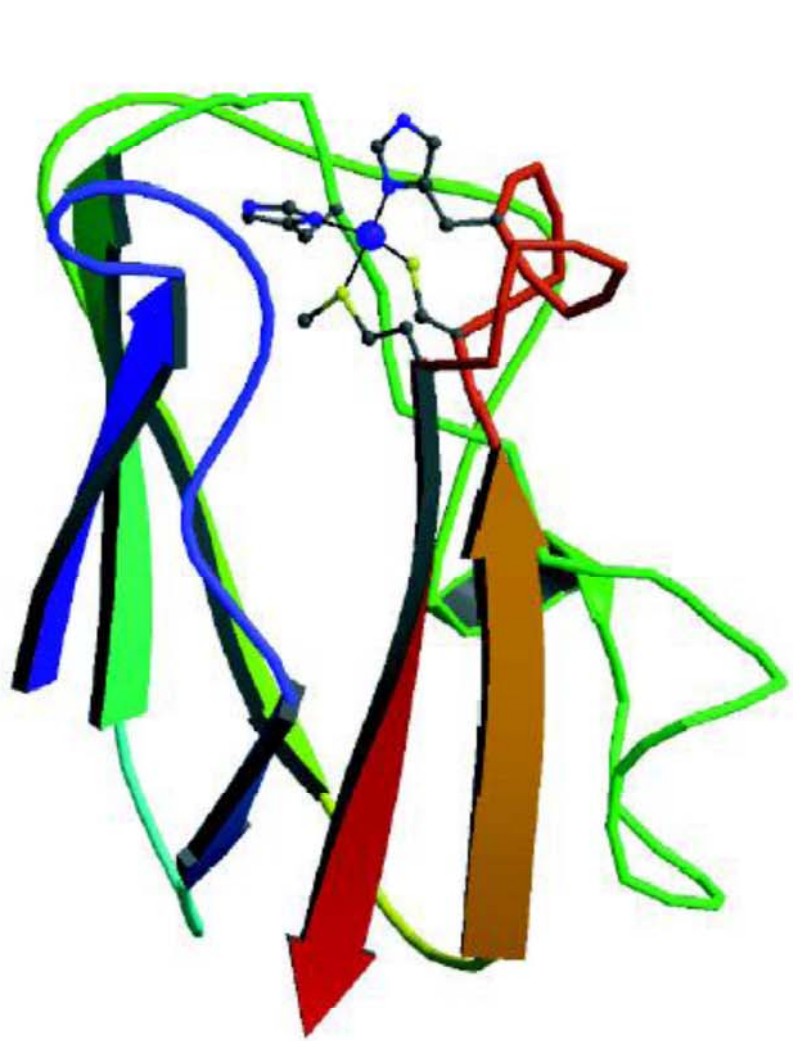
Cu-Cu Bond ?

(Metallic Cu)

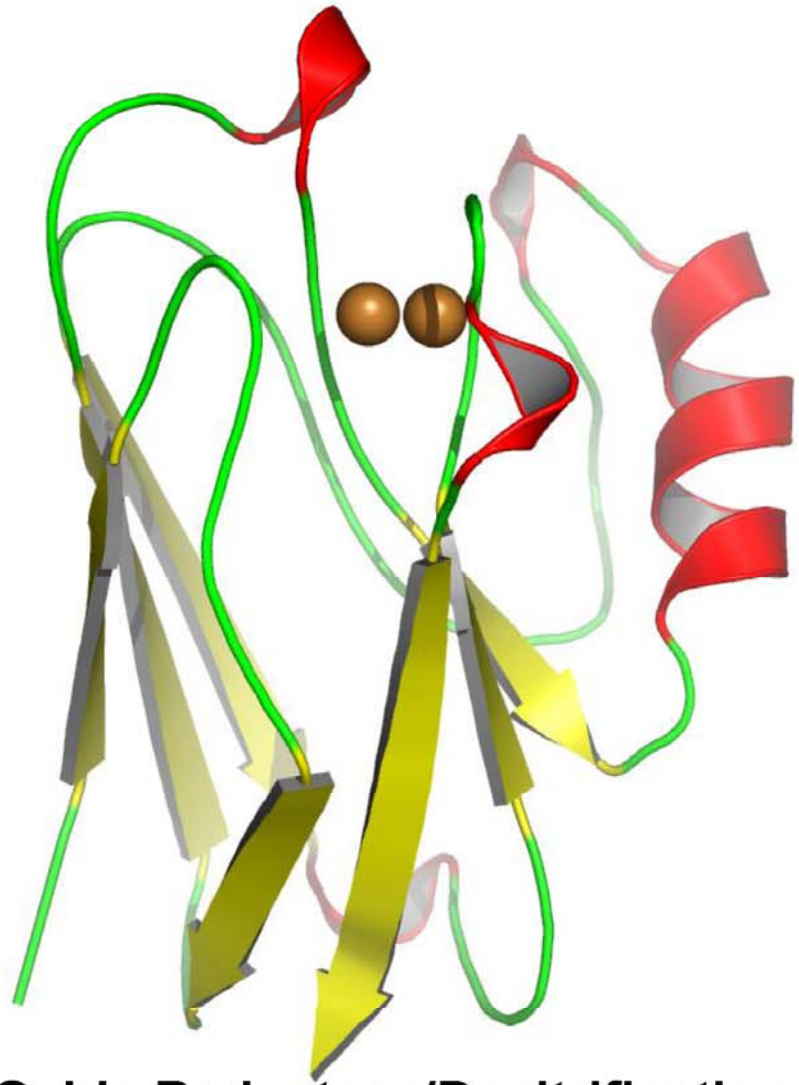
based on a lecture slide of Peter Kroneck

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