Copper Proteins and Enzymes

By David Crochet

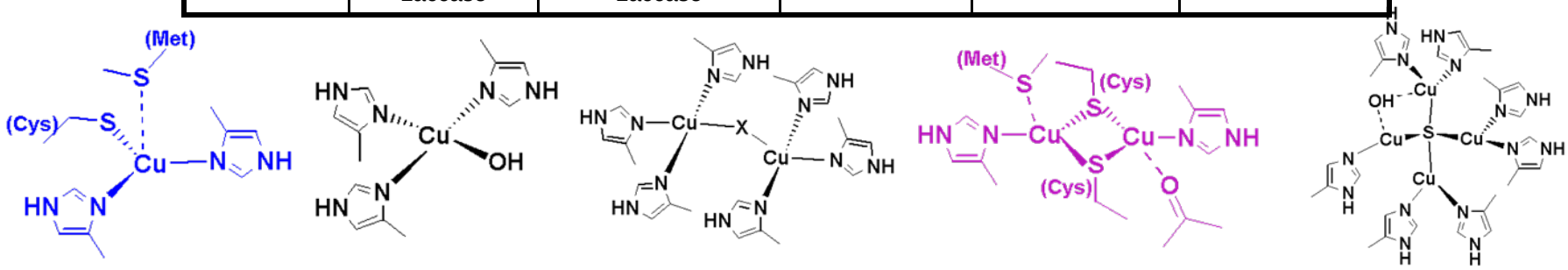
Properties of Copper

- Two isotopes Cu-63 and Cu-65
- Both have a nuclear spin of 3/2
- Is usually ligated by Histidine, Cystiene, Aspartic acid, Tyrosine, or sulfide
- Cu^0 and Cu(I) are d^{10} Cu(II) is d^9
- Cu(II) exhibits the Jahn-Teller effect when in an octahedral complex
- Usually forms tetrahedral-like complexes

- The human body contains about 100-150 mg of copper.
- The recommended daily dose is about 1-3 mg/day
- Copper exists as Cu^{+} and Cu^{2+} ions
- Copper deficiency
  - Anemia
  - steatohepatitis –inflammation of the liver
- Copper poisoning
  - Germicidal effect
  - Wilson's disease

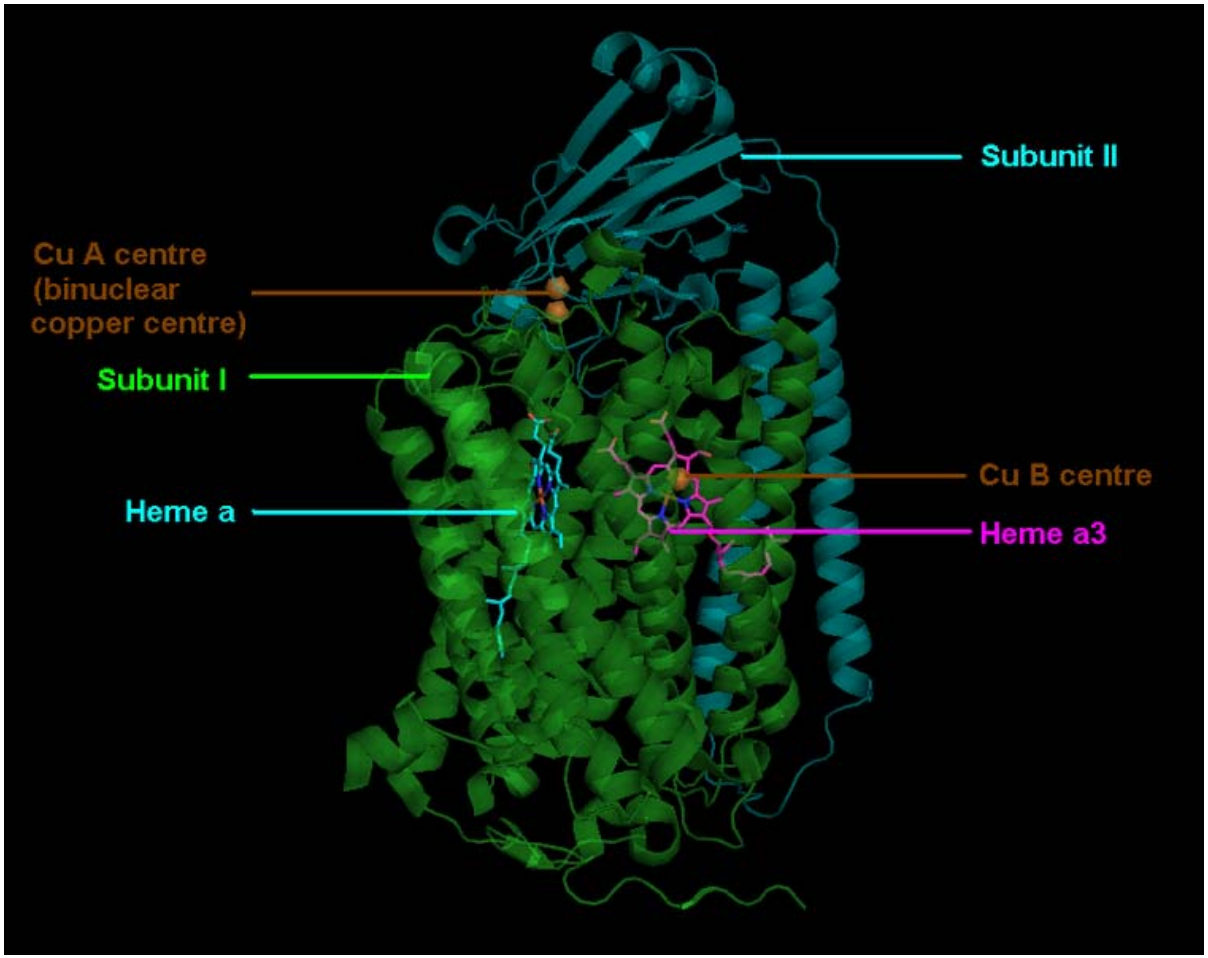
Classification of Biological Copper Centers

	Mononuclear		Dinuclear		Tetranuclear
Type	**Type 1**	**Type 2**	**Type 3**	**Cu_A**	**Cu_Z**
UV-vis Spectrum	Strong absorption ~ 600 nm and (in some proteins) 450 nm	Weak absorption ~ 700 nm	Weak absorption ~ 700 nm	Strong absorption ~ 480 and 530 nm	Strong absorption ~ 640 nm
EPR spectrum	4-line ($A_{\|\|}$ < 80 x 10^{-4} cm^{-1})	4-line ($A_{\|\|}$ ~ (130-180) x 10^{-4} cm^{-1})	non-detectable	7-line ($A_{\|\|}$ ~ 30-40 x 10^{-4} cm^{-1})	2x4-line ($A_{\|\|}$ ~ 61x 10^{-4} cm^{-1} & $A_{\|\|}$ ~ 24 x 10^{-4} cm^{-1})
Common ligands	His, Cys, (Met)	His, Asp, (Tyr)	His, (Tyr)	His, Cys, (Met)	His, S^{2-}
Active site geometry	Distorted tetrahedral	Distorted tetragonal	Tetragonal	Trigonal planar	m_4-S^{2-} tetracopper cluster
Examples	Azurin Plastocyanin Stellacyanin Nitrite reductase Laccase	Superoxide dismutase Galactose oxidase Amine oxidase Nitrite reductase Laccase	Hemocyanin Tyrosinase Catechol oxidase Laccase	Cyt c oxidase N_2O reductase Menaquinol NO-reductase	N_2O reductase



Yi Lu, "Cupredoxins", in "*Comprehensive Coordination Chemistry II: From Biology to Nanotechnology*", Jon McCleverty and Tom J. Meyer, Eds., Vol 8 (Biocoordination Chemistry, Lawrence Que, Jr. and William B. Tolman, Eds.) pp 91-122 (2003).

Cytochrome-C oxidase

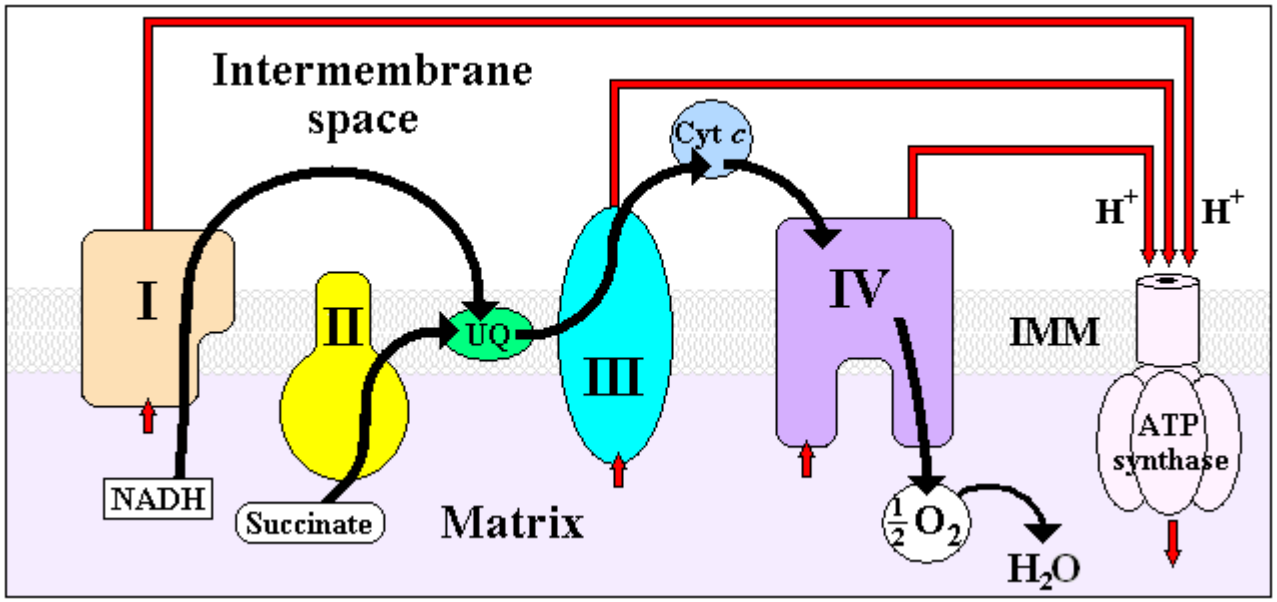


Nevue, Curtis 2008

This Lecture will focus on

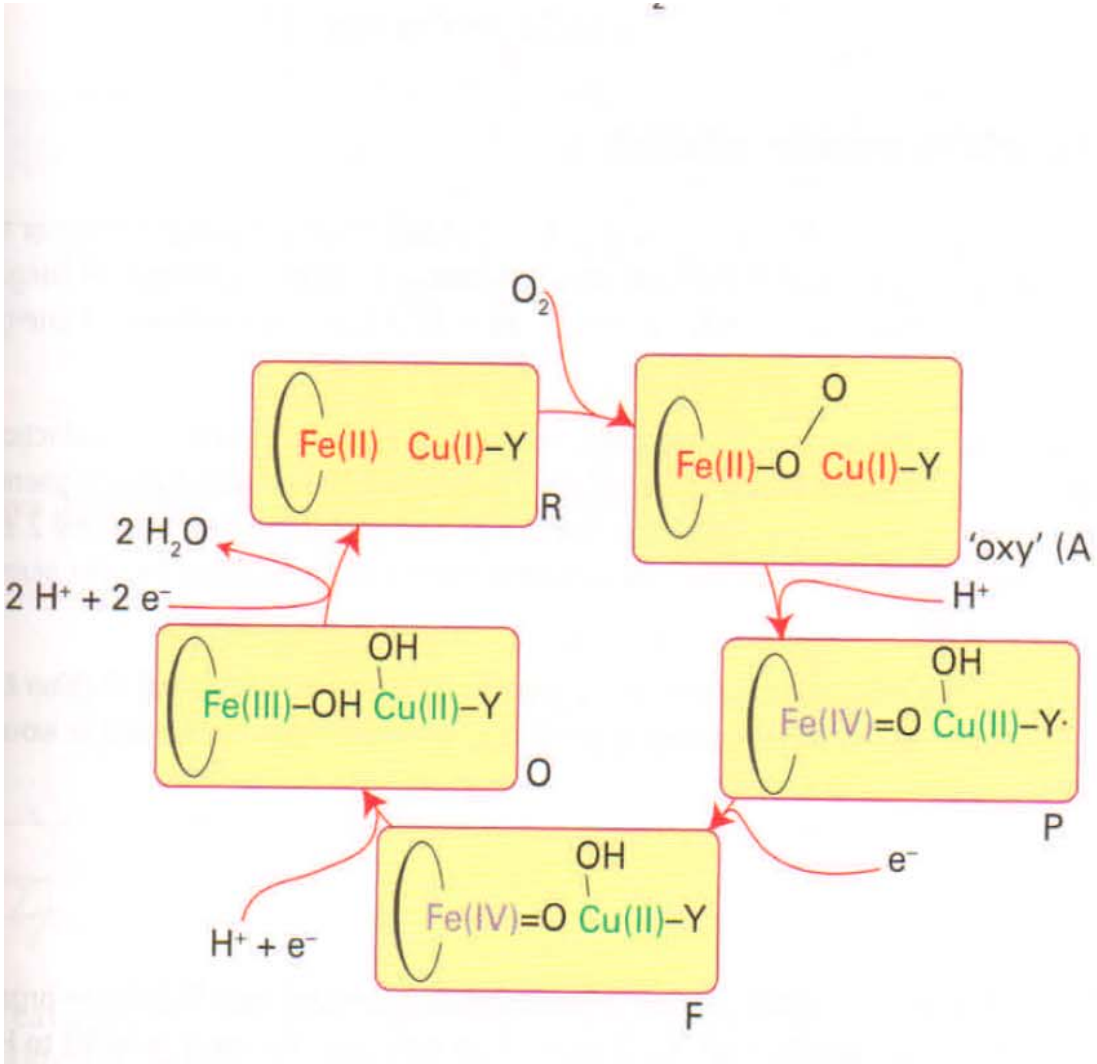
- Cytochrome c Oxidase
- Hemocyanin
- Copper-Zinc Superoxide Dismutase
- Plastocyanin

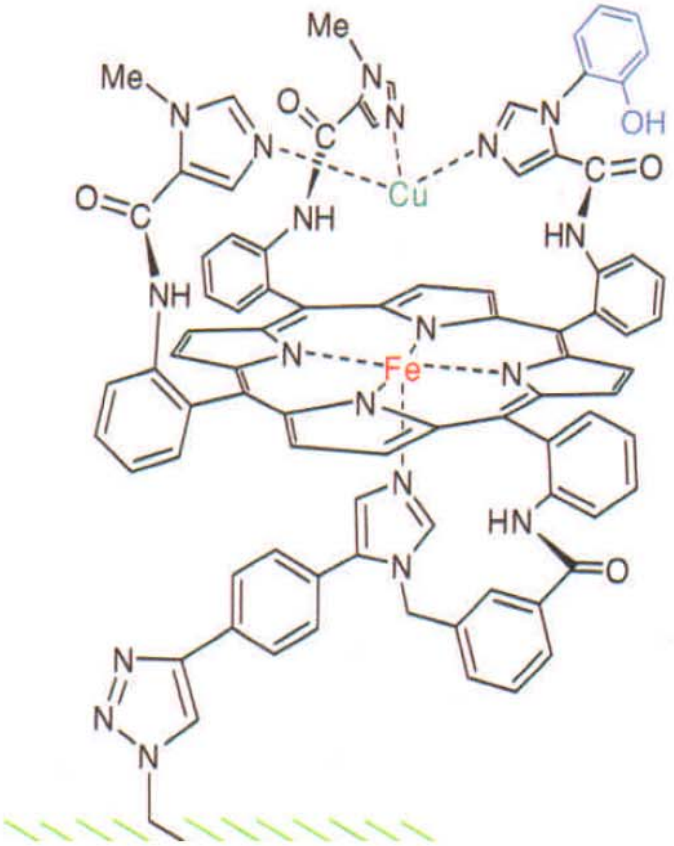
Cytochrome c oxidase: What does it do?



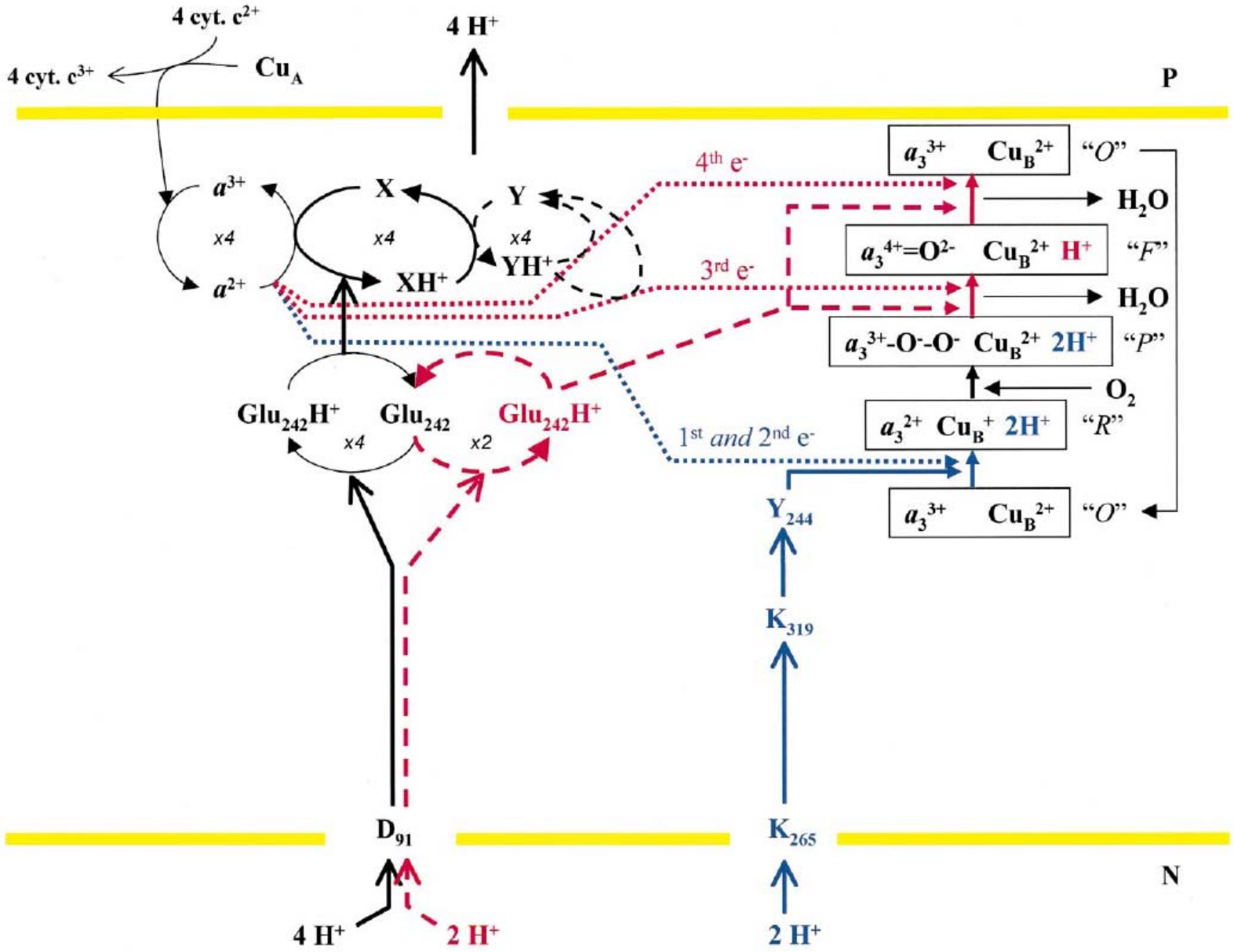
- Found in the mitochondrial membrane
- Serves to convert O_2 into $2H_2O$
- $4\ Fe^{2+} + 8\ H^+_{in} + O_2 \rightarrow 4\ Fe^{3+} + 2\ H_2O + 4\ H^+_{out}$

Copper and Iron act in concert to split O_2



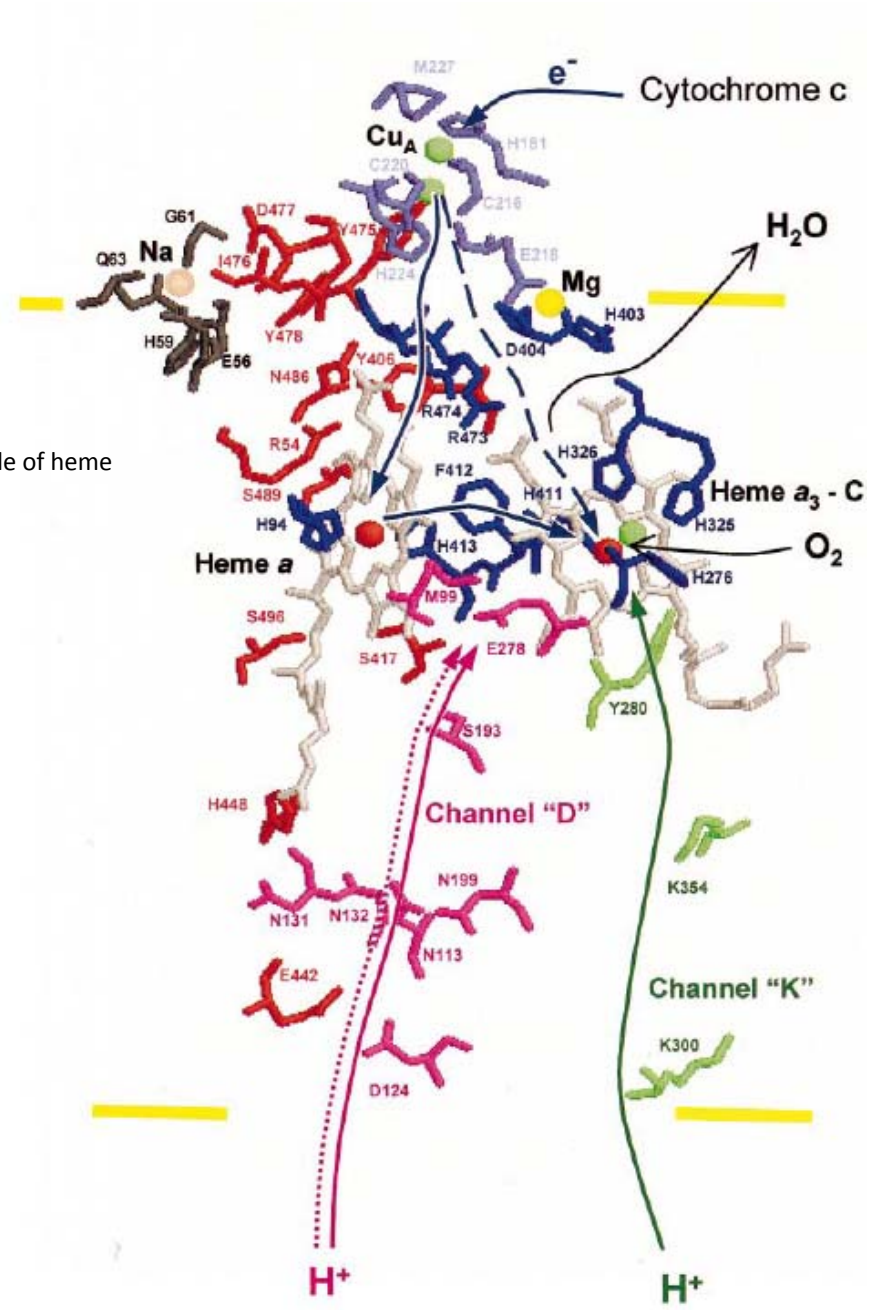


Organic Chemistry. Peter Atkins, Tina Overton, et al. p. 753


4 cyt. c2+
4 cyt. c3+
CuA
4 H+
P
a3+
a2+
x4
X
XH+
Y
YH+
4th e-
3rd e-
a33+ CuB2+ "O"
H2O
a34+=O2- CuB2+ H+ "F"
H2O
a33+-O--O- CuB2+ 2H+ "P"
O2
a32+ CuB+ 2H+ "R"
1st and 2nd e-
a33+ CuB2+ "O"
Glu242H+
Glu242
Glu242H+
x4
x2
Y244
K319
D91
K265
N
4 H+
2 H+
2 H+

Another view of cytochrome c oxidase

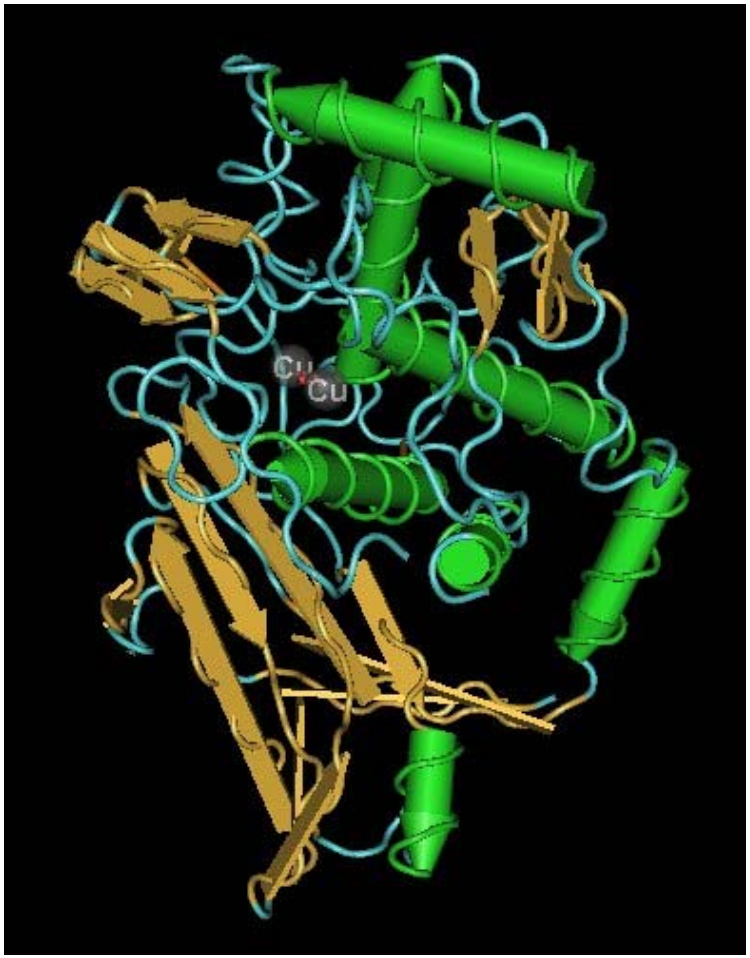
A cooperative model for protonmotive heme-copper oxidases. The role of heme *a* in the proton pump of cytochrome *c* oxidase
FEBS Letters, *Volume 439, Issues 1-2*, *13 November 1998*, *Pages 1-8*
Sergio Papa, Nazzareno Capitanio, Gaetano Villani



Additional features of Cytochrome c oxidase

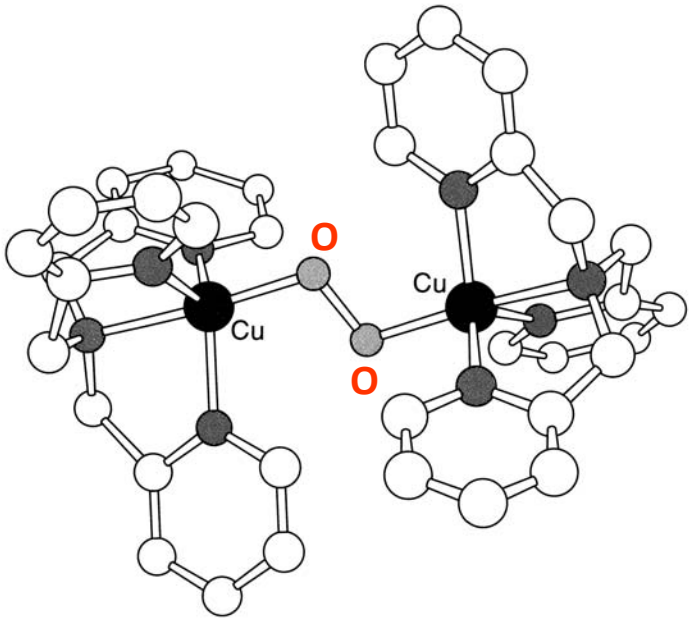
- cyanide, sulfide, azide, and carbon monoxide can bind to the active site causing asphyxiation of the cell
- mutations of the complex are almost always fatal and are the cause of Leigh's syndrome, some cardiomyopathies, and some anemias

Hemocyanin: An O_2 carrier

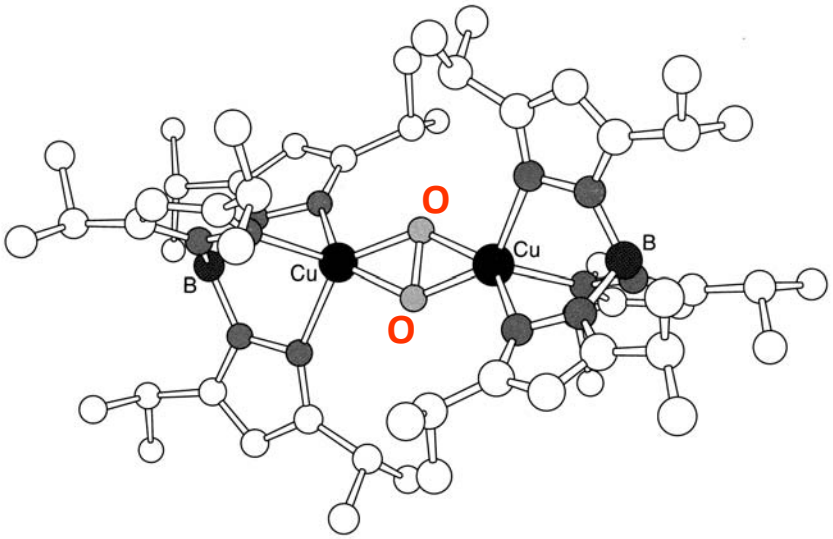


Model Compounds of Oxy-Hc

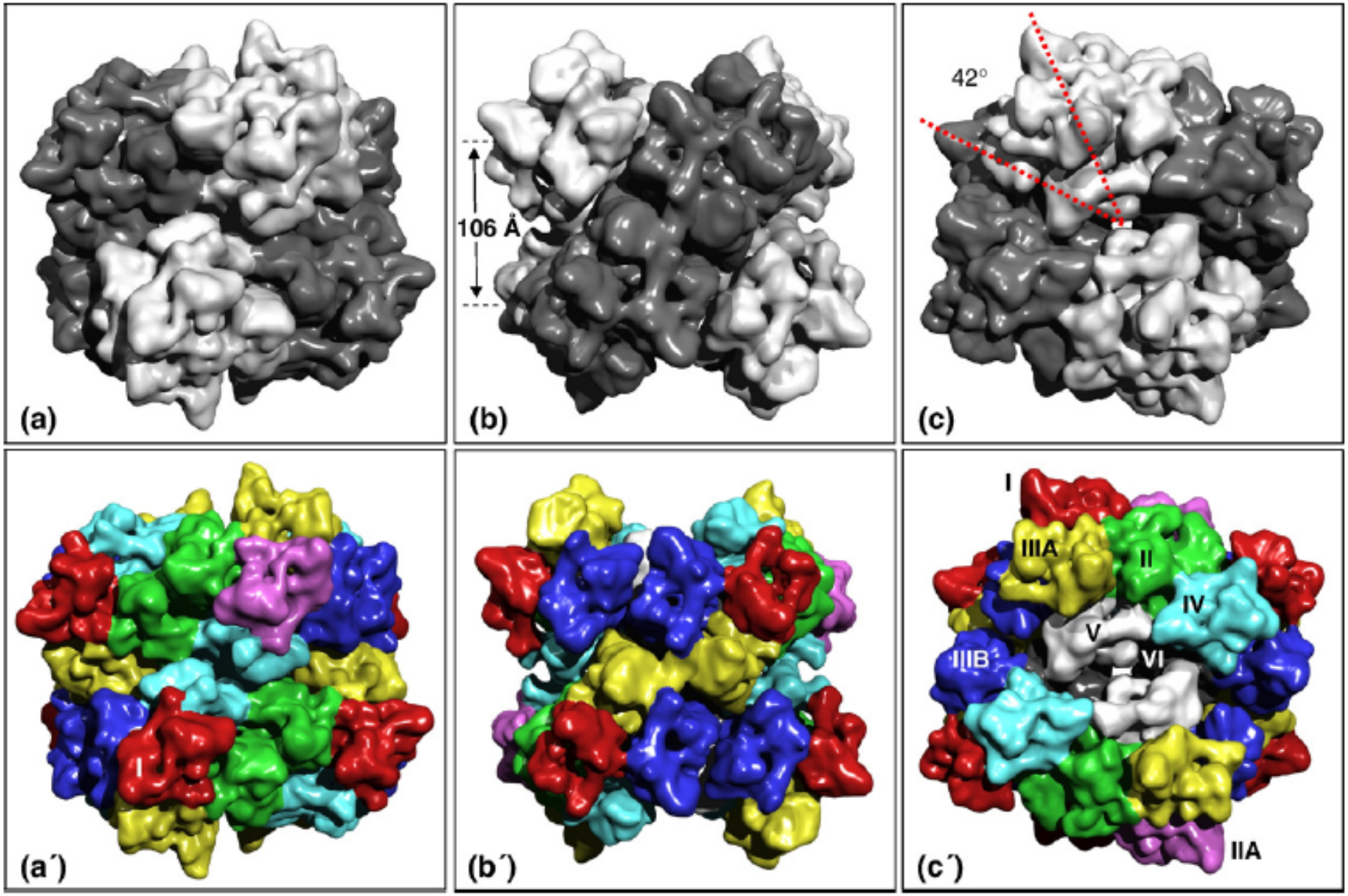
η^1, η^1-bridged:



η^2, η^2-bridged:



- Oxygen transport in most mollusks and arthropods
- Unlike hemoglobin, hemocyanin is found freely floating in the hemolymph, as opposed to being bound to red blood cell-like cells
- Hemocyanin is usually arranged in clusters of bimers of hexamers which can weigh over 1500 kDa
- Binding within dimers and especially hexamers is very co-operative



***Limulus polyphemus* Hemocyanin: 10 Å Cryo-EM Structure, Sequence Analysis, Molecular Modelling and Rigid-body Fitting Reveal the Interfaces Between the Eight Hexamers**

Journal of Molecular Biology, *Volume 366, Issue 4*, *2 March 2007*, *Pages 1332-1350*

Andreas G. Martin, Frank Depoix, Michael Stohr, Ulrich Meissner, Silke Hagner-Holler, Kada Hammouti, Thorsten Burmester, Jochen Heyd, Willy Wriggers, Jürgen Markl

Superoxide Dismutase

- Copper and zinc form the active site

Prevents radical reactions such as

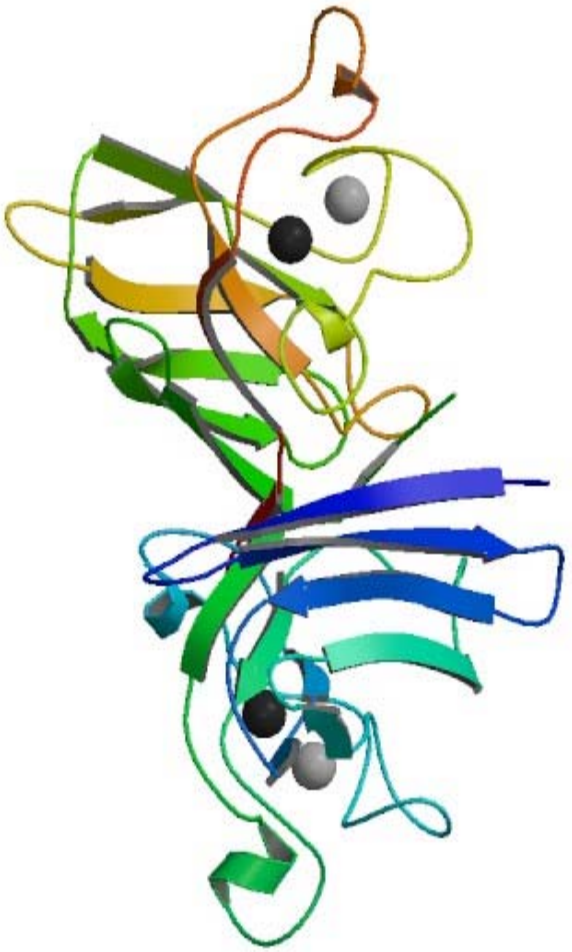
$NO + O_2^- \rightarrow ONOO^-$

Which form extremely toxic compounds

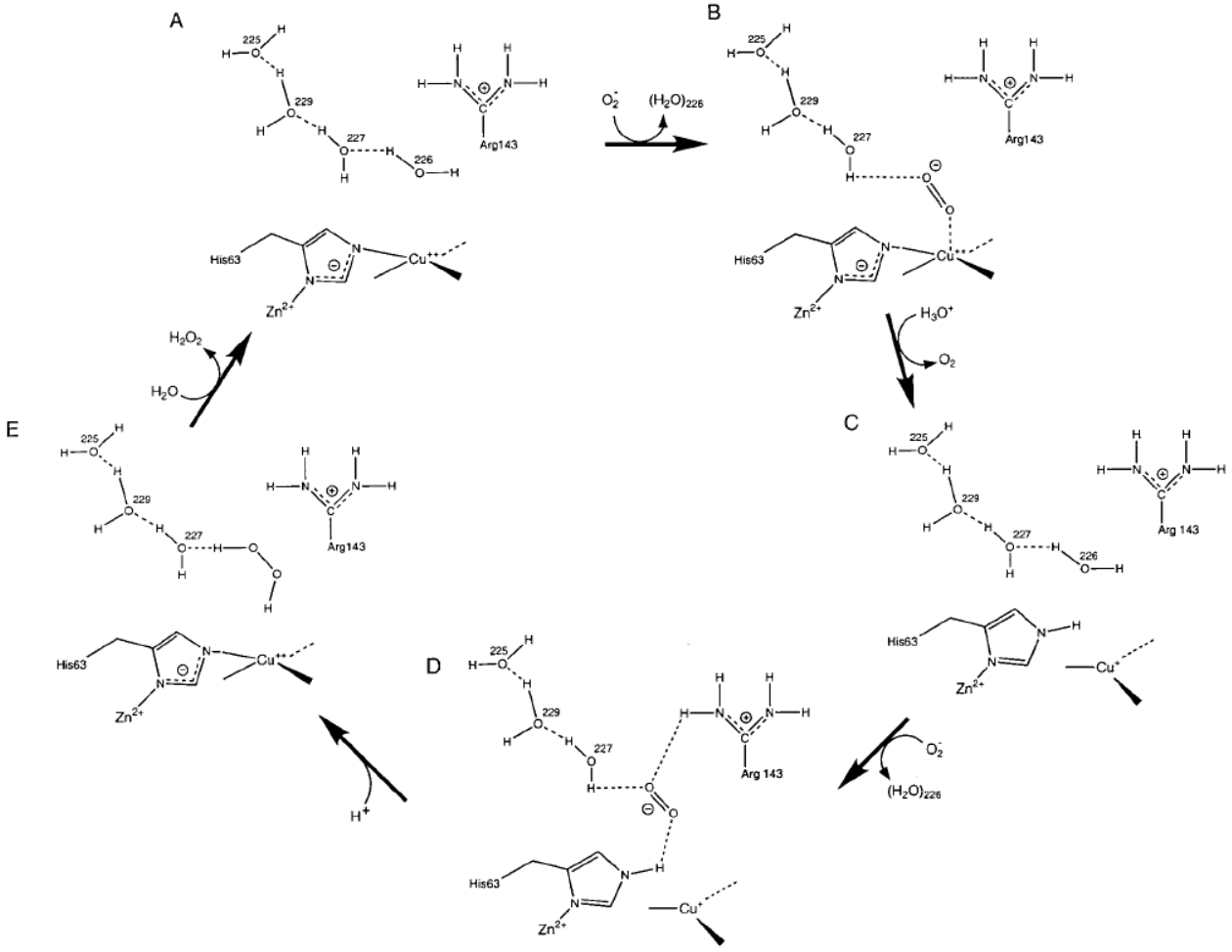
Superoxide is removed by the reactions

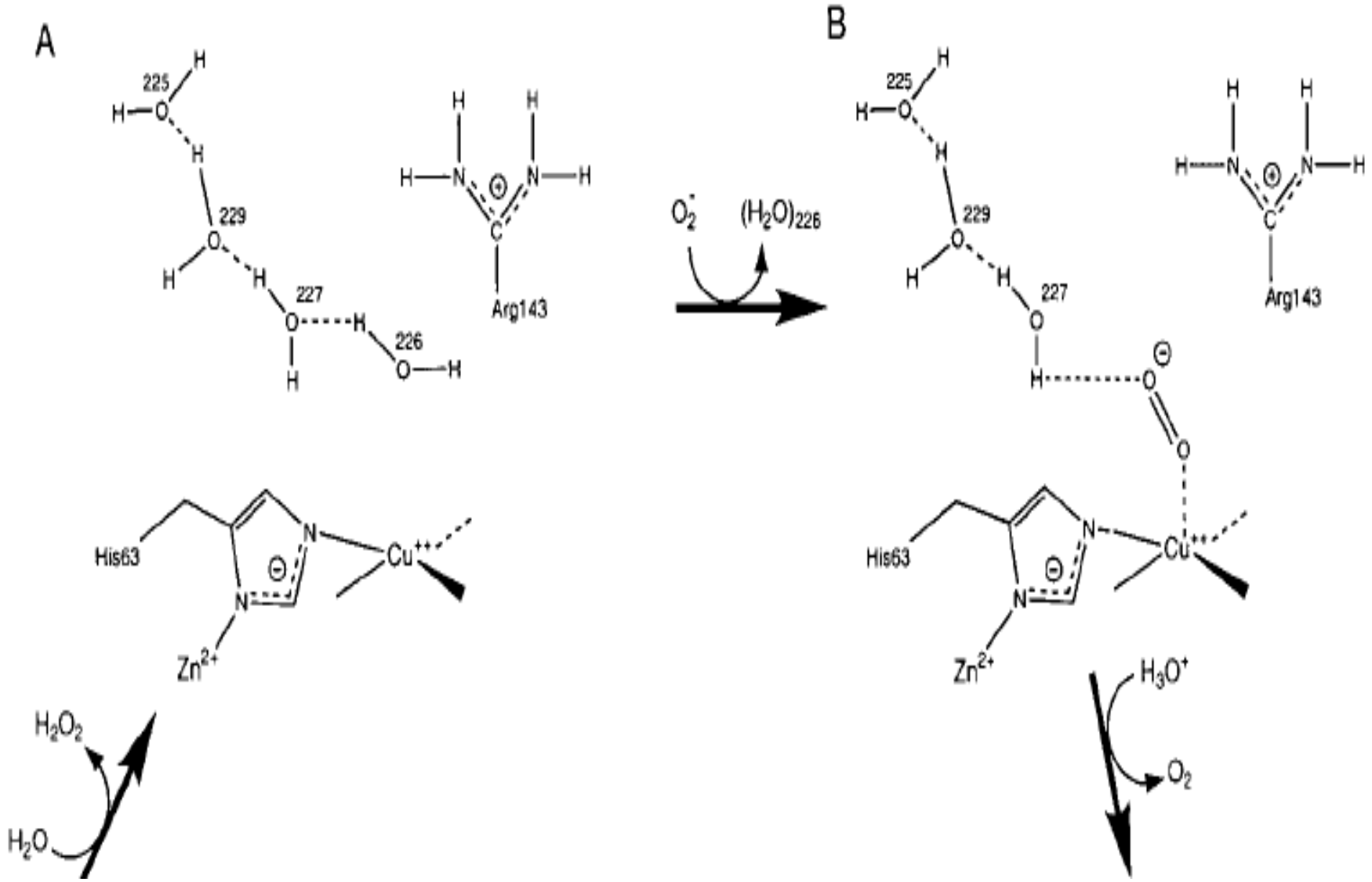
$Cu^{2+} + O_2^- \rightarrow Cu^+ + O_2$

$Cu^+ + O_2^- + 2H^+ \rightarrow Cu^{2+} + H_2O_2$

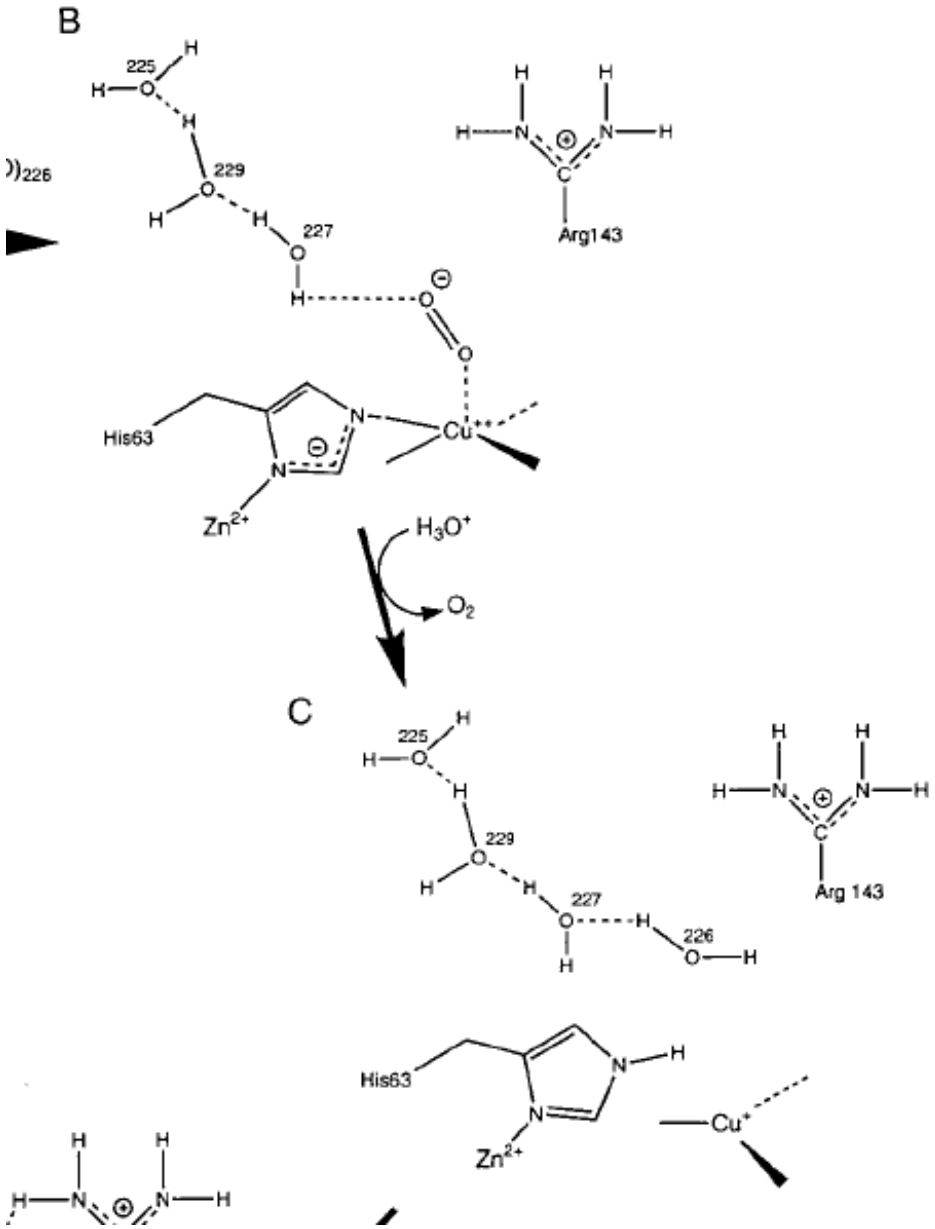


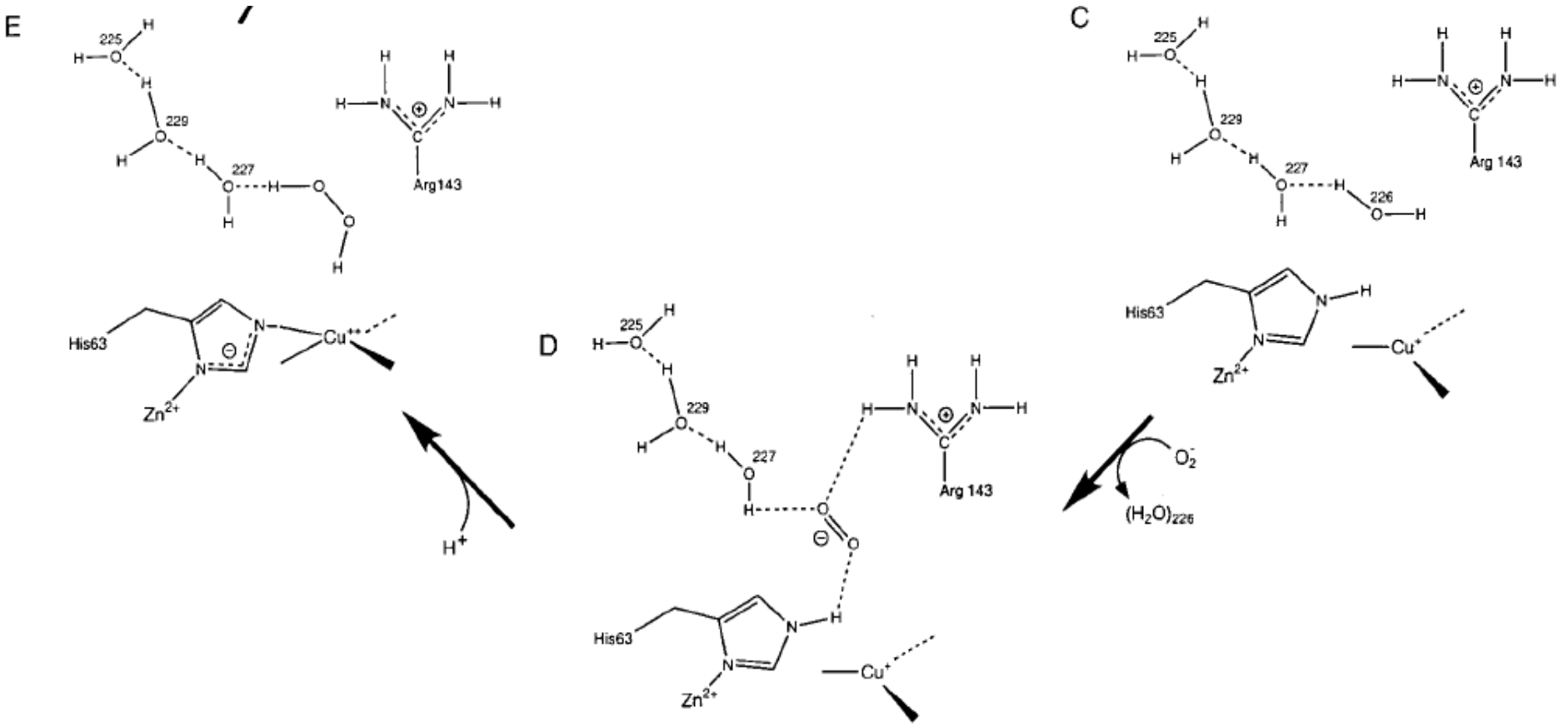
Fe,Zn SOD mechanism

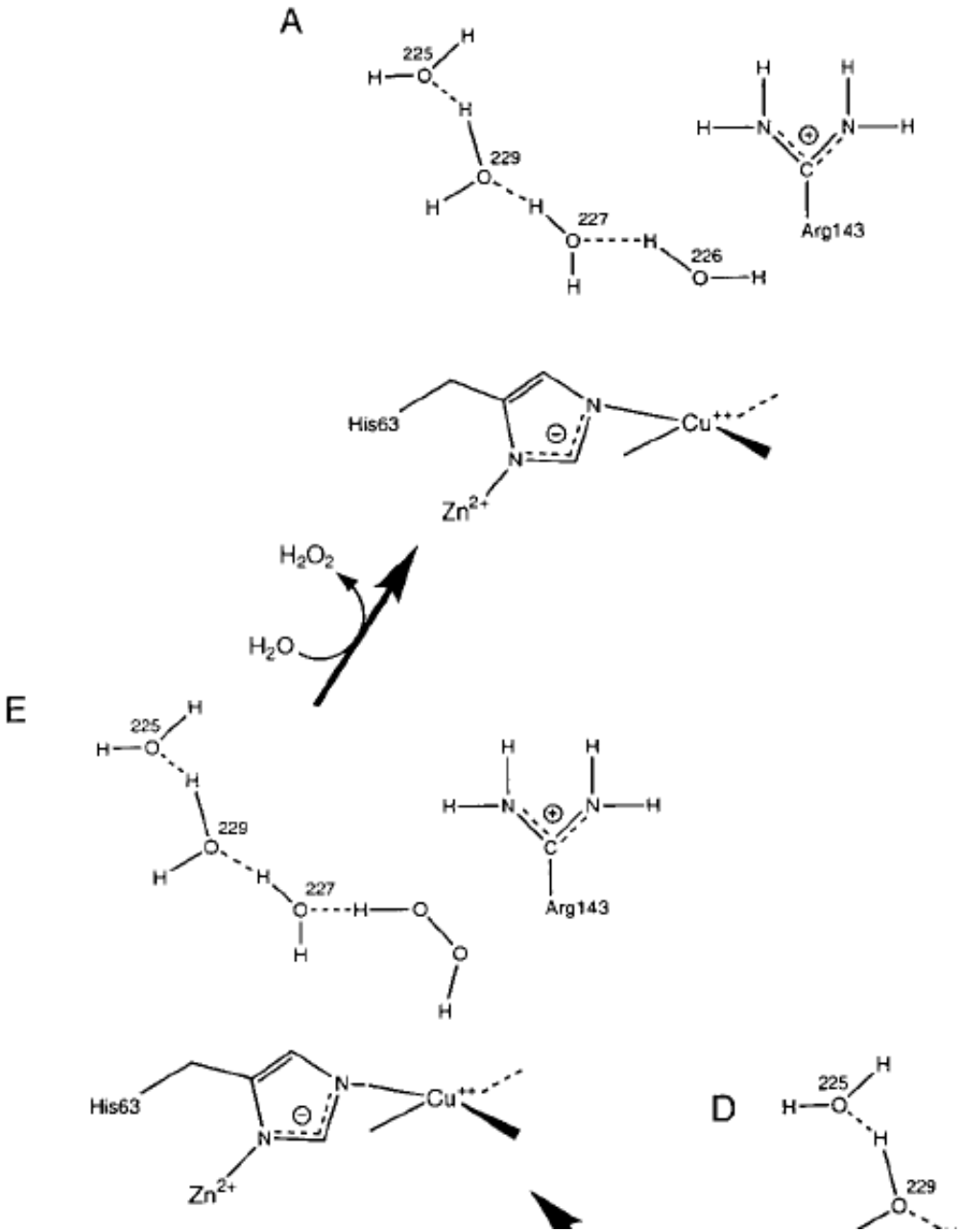




A structure-based mechanism for copper-zinc superoxide dismutase Hart, P. John, Balbirnie, Melinda M. , Ogihara, Nancy L. Biochemistry (American Chemical Society) v. 38 no. 7 (February 16 1999) p. 2167-78

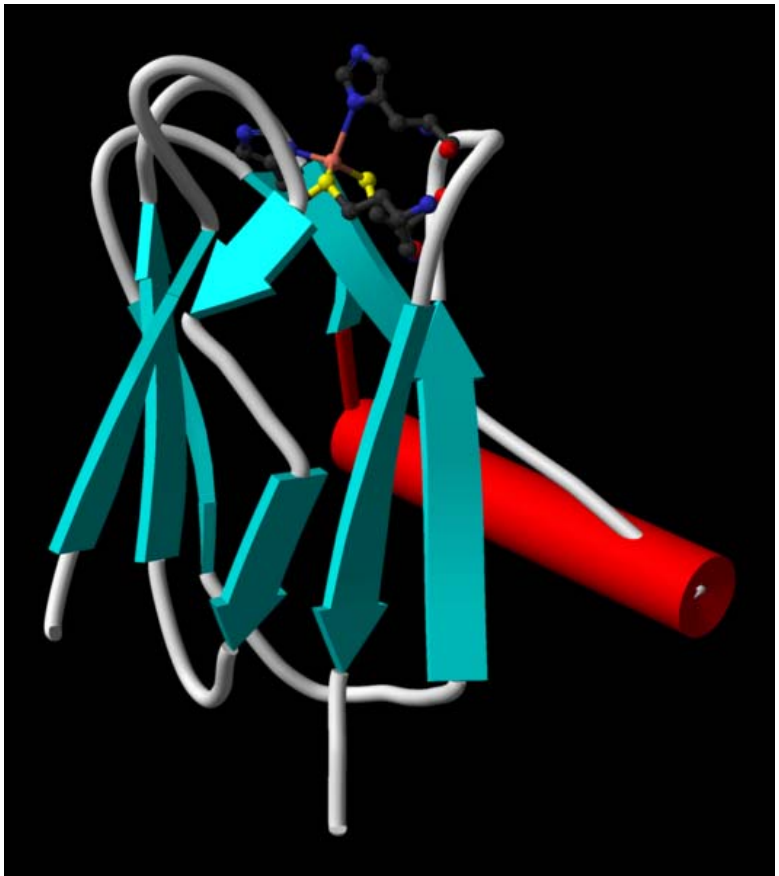

B
225
229
227
Arg143
His63
Zn2+
H3O+
O2
C
225
229
227
226
Arg 143
His63
Zn2+
Cu+


E
225
229
227
Arg143
His63
Cu+
Zn2+
H+
D
225
229
227
Arg 143
His63
Cu+
Zn2+
C
225
229
227
226
Arg 143
His63
Cu+
Zn2+
O2-
(H2O)226

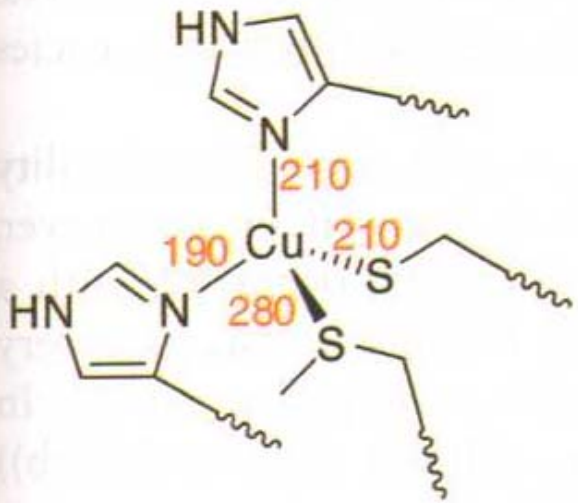

A
225
229
227
226
Arg143
His63
Cu++
Zn2+
H2O2
H2O
E
225
229
227
Arg143
His63
Cu++
Zn2+
D
225
229

Plastocyanin

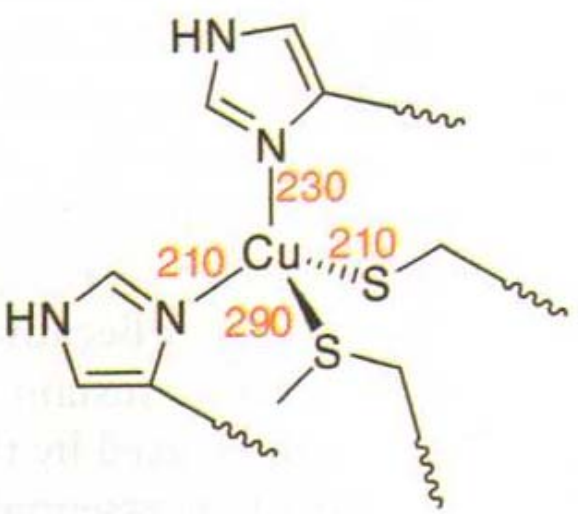
- Plastocyanin is a monomeric protein which assists in electron transport during photosynthesis.



Copper Center

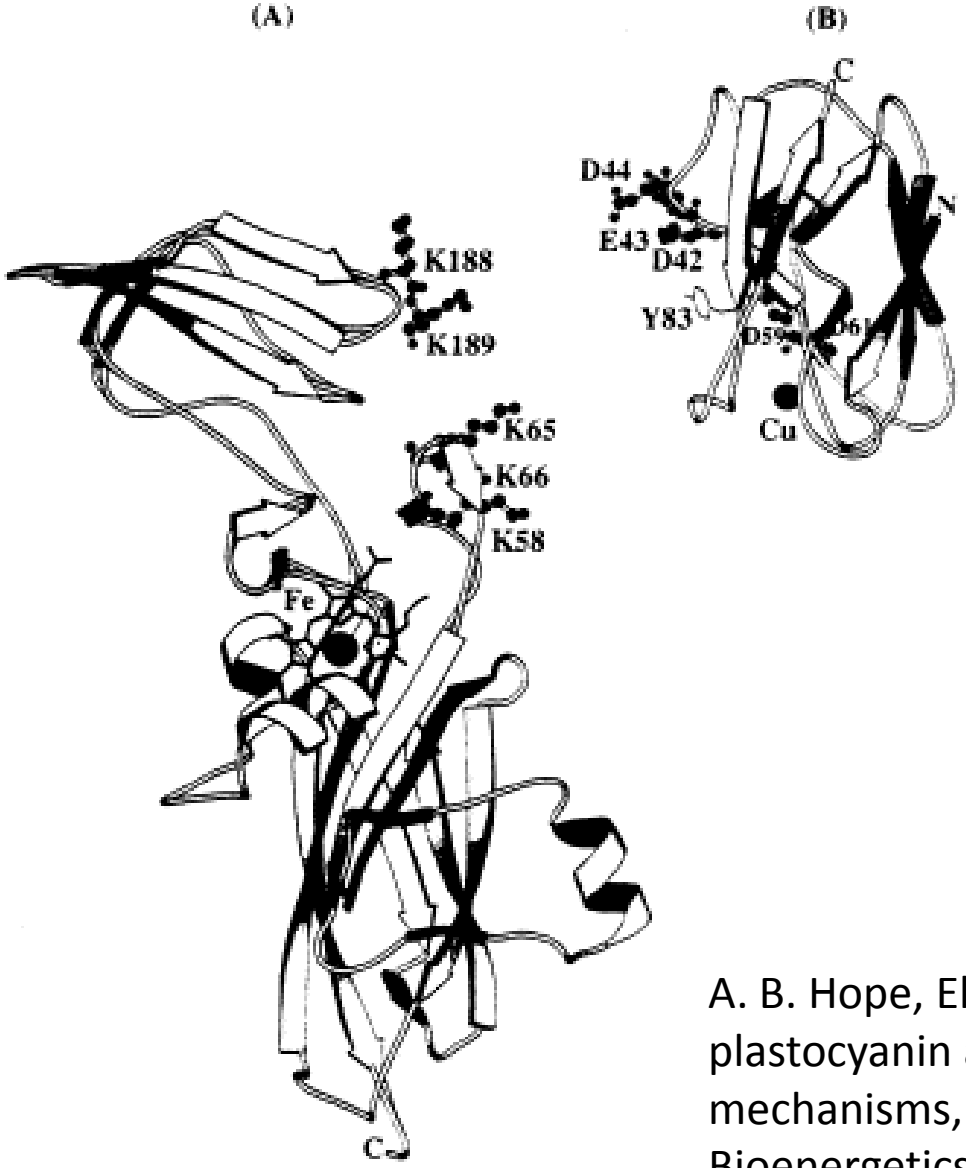


29a Oxidized plastocyanin



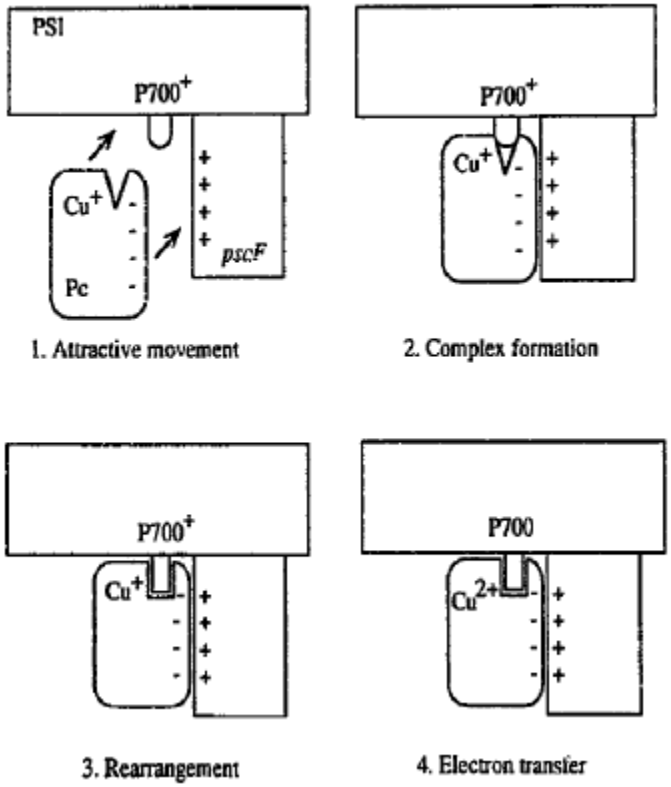
29b Reduced plastocyanin

Cytochrome f and Plastocyanin



A. B. Hope, Electron transfers amongst cytochrome f, plastocyanin and photosystem I: kinetics and mechanisms, Biochimica et Biophysica Acta (BBA) - Bioenergetics, Volume 1456, Issue 1, 3 January 2000, Pages 5-26, ISSN 0005-2728

Plastocyanin and Photosystem I



Reduction of photosystem I by cytochrome c_6 and plastocyanin: molecular recognition and reaction mechanism
Bioelectrochemistry and Bioenergetics, *Volume 42, Issue 2*, *May 1997*, *Pages 249-254*
Manuel Hervás, JoséA. Navarro, Berta De la Cerda, Antonio Díaz, Miguel A.De la Rosa