Cytochrome P$_{450}$s have various redox partner proteins depending on the sub type

Class I

NAD(P)H $\xrightarrow{2e^-}$ NAD(P)$^+$ — FdR — Fdx — P450

RH + O$_2$ → R-OH + H$_2$O

Class II

NAD(P)H $\xrightarrow{2e^-}$ NAD(P)$^+$ — CPR — P450 — ER membrane

RH + O$_2$ → R-OH + H$_2$O

Class III

NAD(P)H $\xrightarrow{2e^-}$ NAD(P)$^+$ — CPR — P450

RH + O$_2$ → R-OH + H$_2$O

Class IV

NAD(P)H $\xrightarrow{2e^-}$ NAD(P)$^+$ — FMN — FeS — P450

RH + O$_2$ → R-OH + H$_2$O

Class V

NAD(P)H $\xrightarrow{2e^-}$ NAD(P)$^+$ — P450

RH + O$_2$ → R-OH + H$_2$O
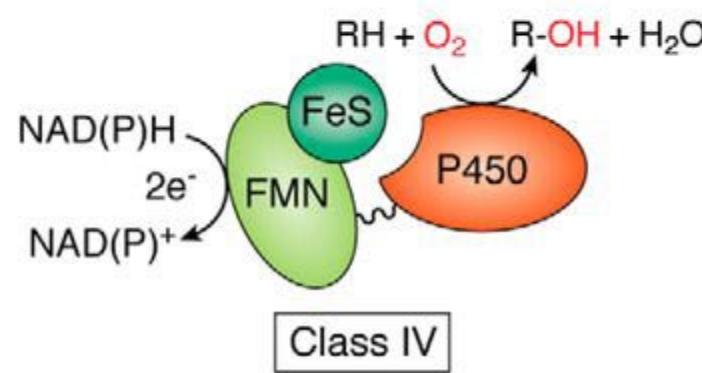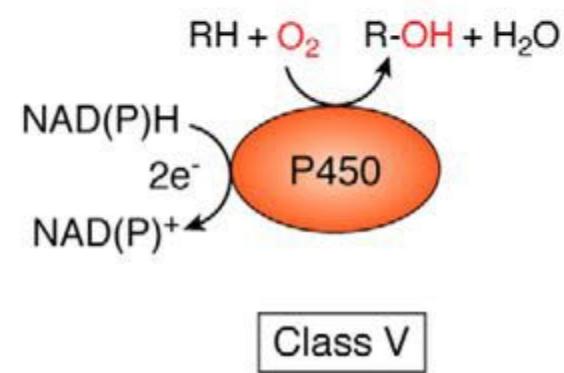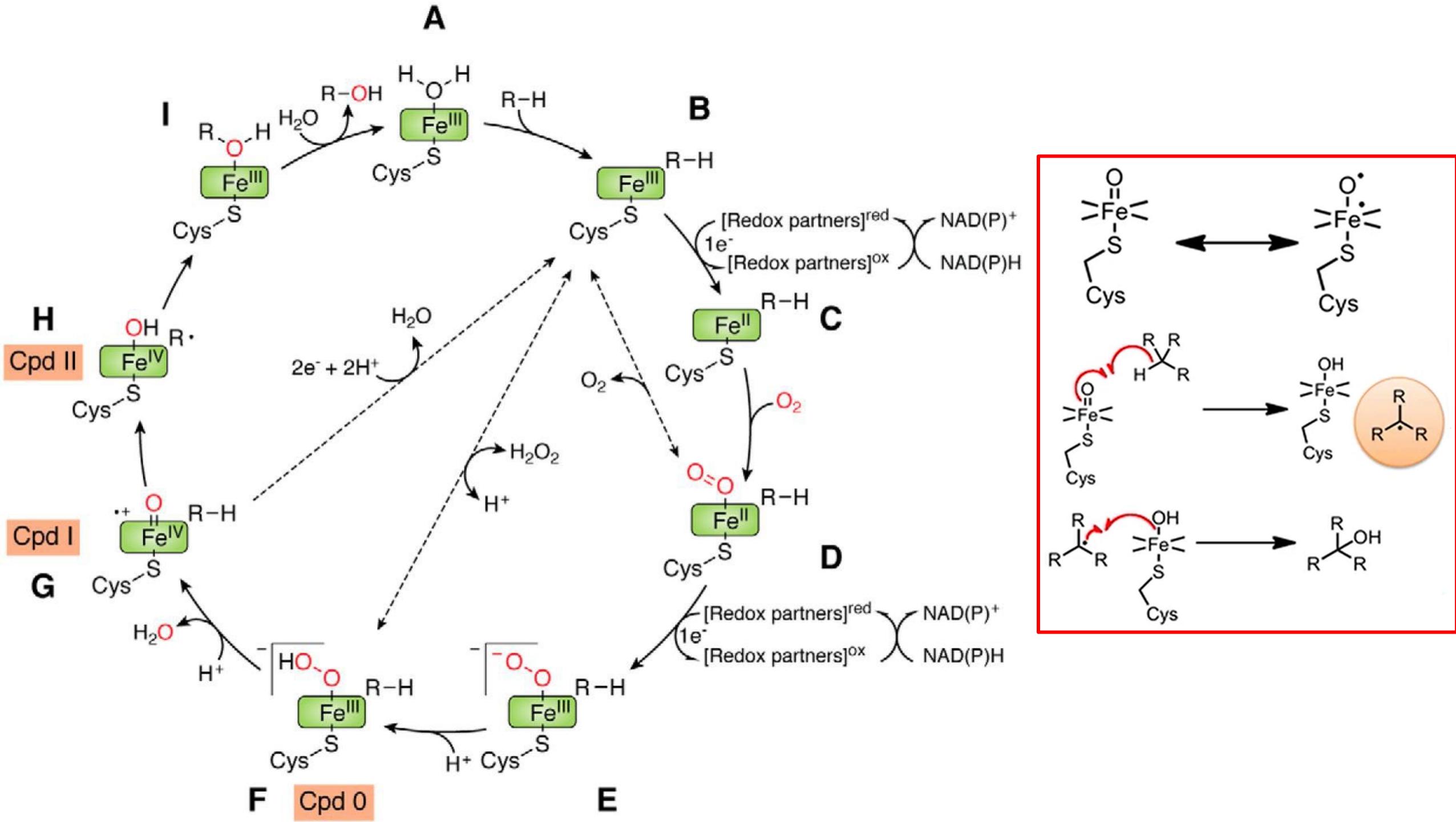
The radical reaction mechanism Activation and hydrogen abstraction:

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
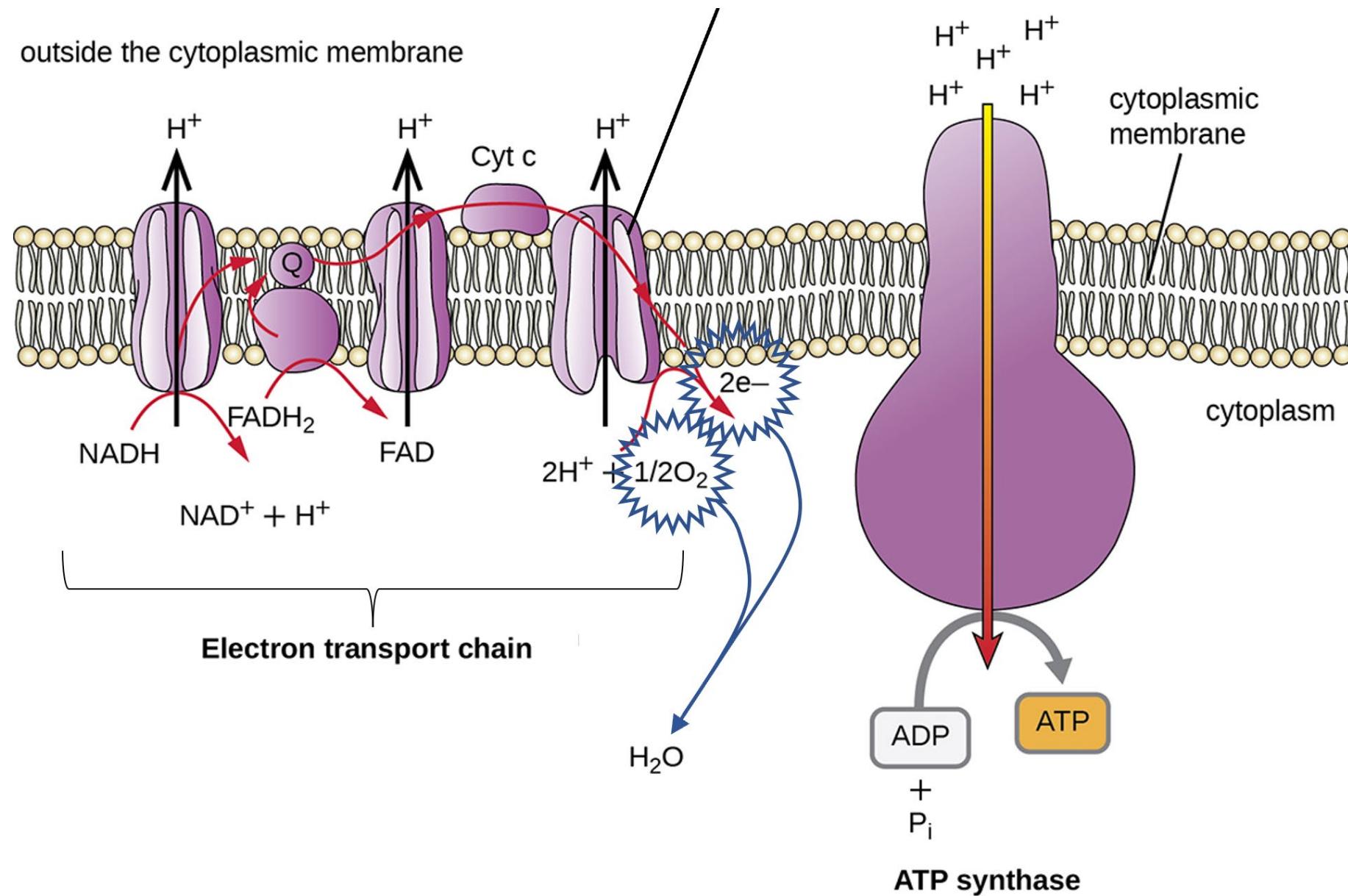
Kinetic isotope effects:

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.
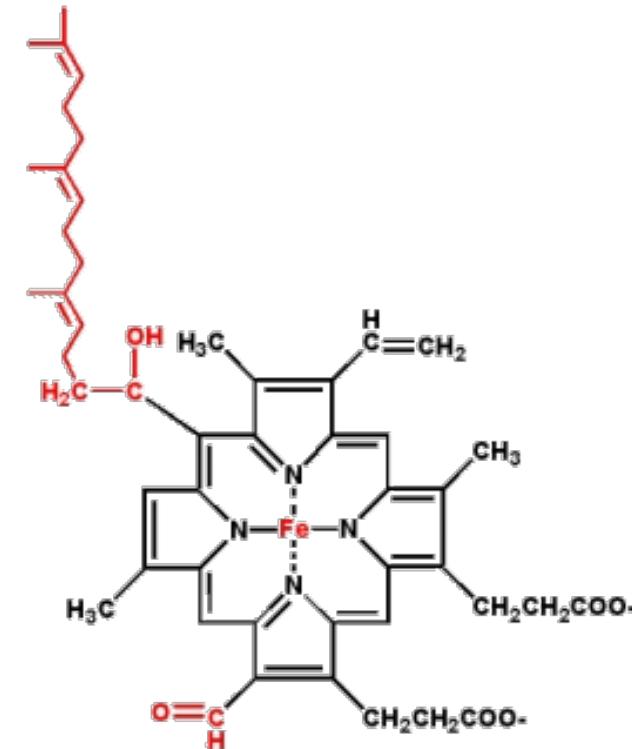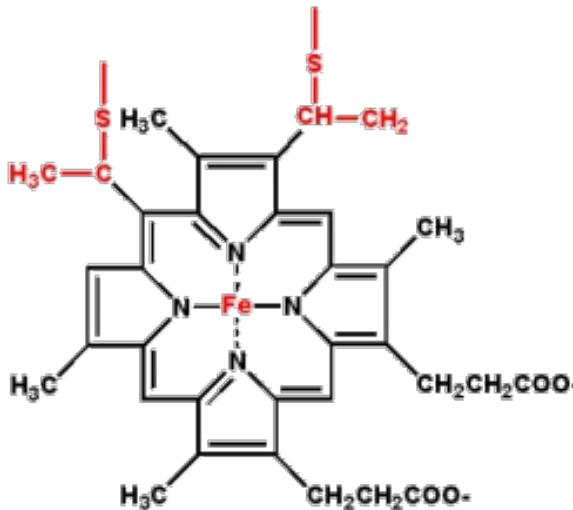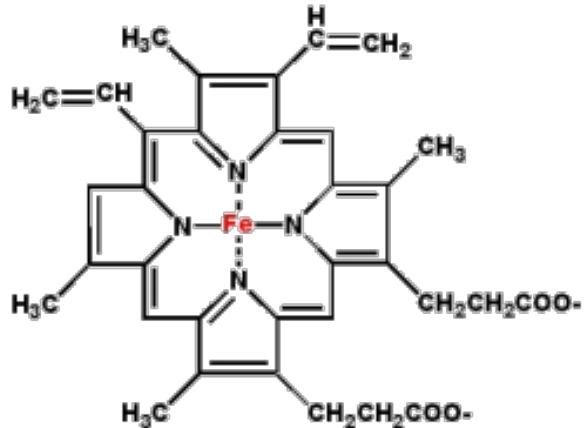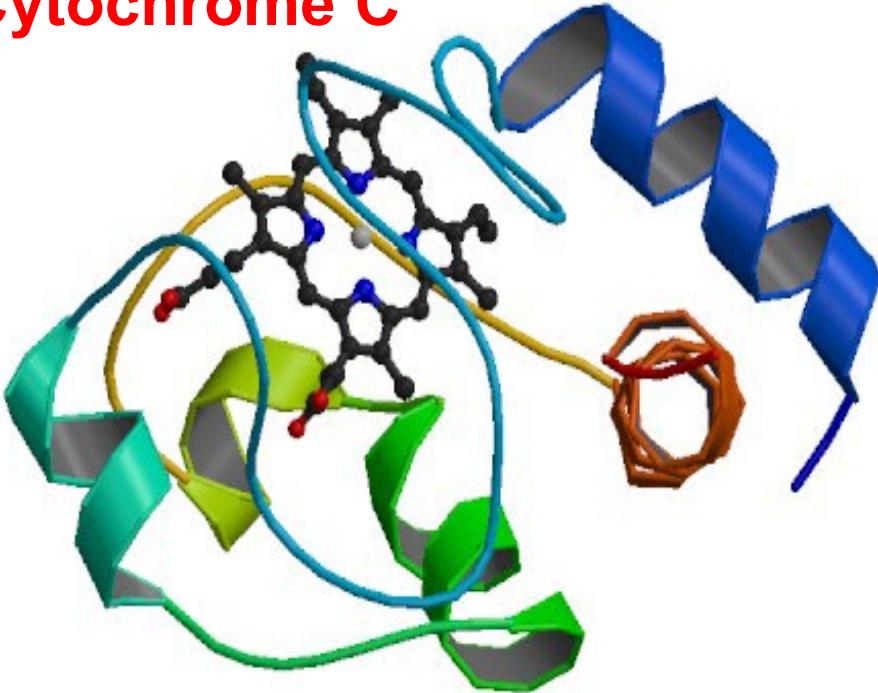
Product stereochemistry:

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

outside the cytoplasmic membrane

H^+

H^+

H^+

Cyt c

H^+ H^+ H^+ H^+ H^+

cytoplasmic membrane

Q

NADH

FADH$_2$

FAD

$2e-$

$2H^+ + 1/2O_2$

$NAD^+ + H^+$

cytoplasm

Electron transport chain

H_2O

ADP

ATP

+

P$_i$

ATP synthase

Cytochrome C

Iron protoporphyrin IX
(cytochrome b, myoglobin, hemoglobin)

Heme C
(cytochrome c)

Heme A
(cytochrome A)
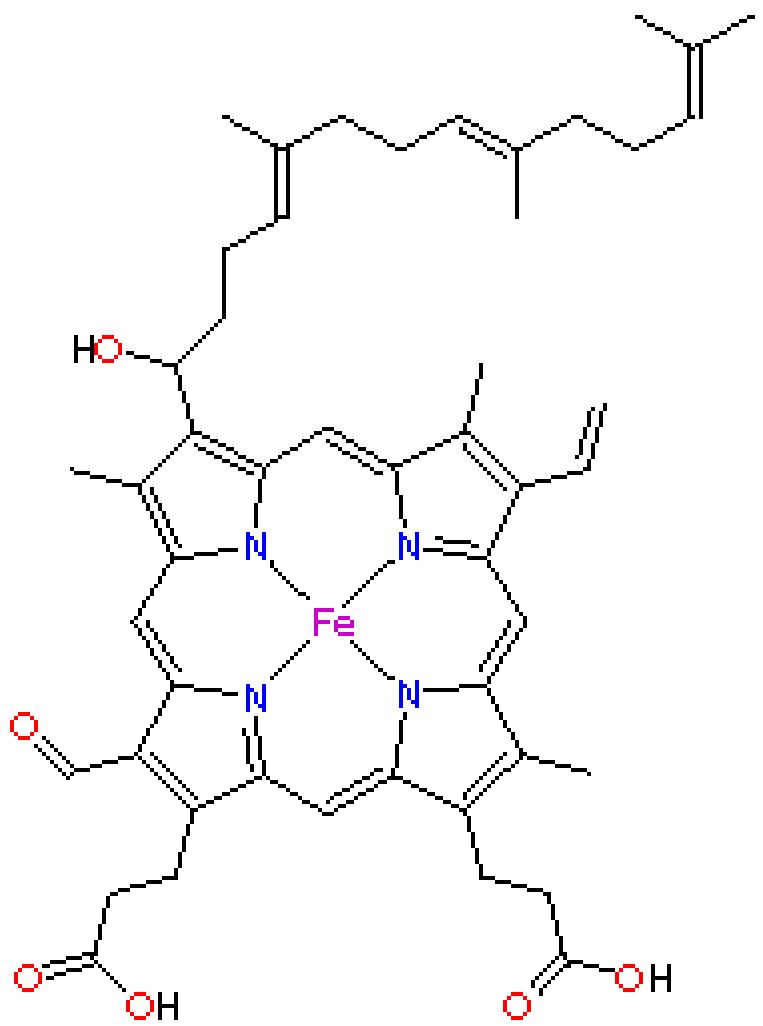
# Cytochrome c oxidase

- 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).
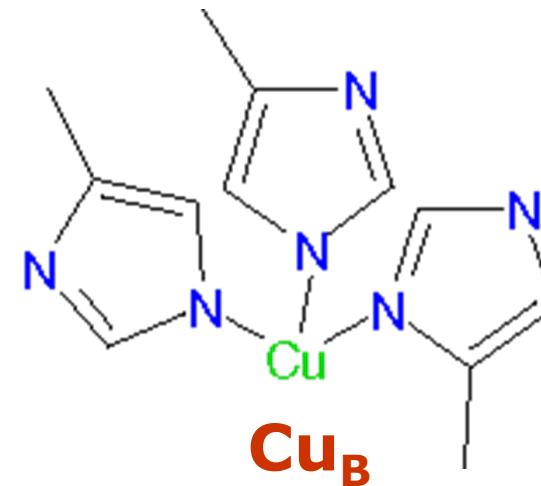
Summary reaction:

- $4 \ Fe^{2+}$-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.
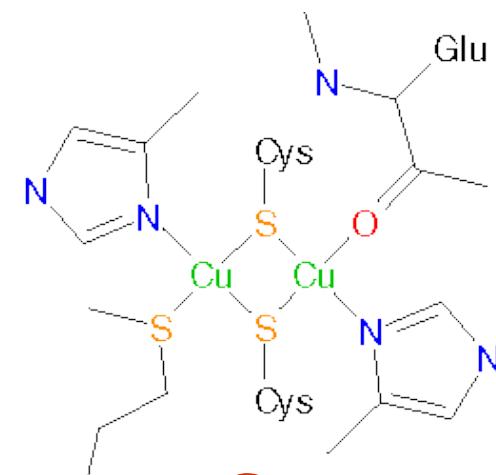
Cytochrome c Oxidase

Heme *a*

Cu$_B$

Cu$_A$

Periodic Table of the Elements

| 1 IA 1A																	18 VIIIA 8A
1 H Hydrogen 1.008	**2 IIA 2A**											**13 IIIA 3A**	**14 IVA 4A**	**15 VA 5A**	**16 VIA 6A**	**17 VIIA 7A**	**2 He** Helium 4.003
3 Li Lithium 6.941	**4 Be** Beryllium 9.012											**5 B** Boron 10.811	**6 C** Carbon 12.011	**7 N** Nitrogen 14.007	**8 O** Oxygen 15.999	**9 F** Fluorine 18.998	**10 Ne** Neon 20.180
11 Na Sodium 22.990	**12 Mg** Magnesium 24.305	**3 IIIB 3B**	**4 IVB 4B**	**5 VB 5B**	**6 VIB 6B**	**7 VIIB 7B**	**8 VIII 8**	**9 VIII 8**	**10 VIII 8**	**11 IB 1B**	**12 IIB 2B**	**13 Al** Aluminum 26.982	**14 Si** Silicon 28.086	**15 P** Phosphorus 30.974	**16 S** Sulfur 32.066	**17 Cl** Chlorine 35.453	**18 Ar** Argon 39.948
19 K Potassium 39.098	**20 Ca** Calcium 40.078	**21 Sc** Scandium 44.956	**22 Ti** Titanium 47.867	**23 V** Vanadium 50.942	**24 Cr** Chromium 51.996	**25 Mn** Manganese 54.938	**26 Fe** Iron 55.845	**27 Co** Cobalt 58.933	**28 Ni** Nickel 58.693	**29 Cu** Copper 63.546	**30 Zn** Zinc 65.38	**31 Ga** Gallium 69.723	**32 Ge** Germanium 72.631	**33 As** Arsenic 74.922	**34 Se** Selenium 78.971	**35 Br** Bromine 79.904	**36 Kr** Krypton 83.798
37 Rb Rubidium 85.468	**38 Sr** Strontium 87.62	**39 Y** Yttrium 88.906	**40 Zr** Zirconium 91.224	**41 Nb** Niobium 92.906	**42 Mo** Molybdenum 95.95	**43 Tc** Technetium 98.907	**44 Ru** Ruthenium 101.07	**45 Rh** Rhodium 102.906	**46 Pd** Palladium 106.42	**47 Ag** Silver 107.868	**48 Cd** Cadmium 112.414	**49 In** Indium 114.818	**50 Sn** Tin 118.711	**51 Sb** Antimony 121.760	**52 Te** Tellurium 127.6	**53 I** Iodine 126.904	**54 Xe** Xenon 131.294
55 Cs Cesium 132.905	**56 Ba** Barium 137.328	**57-71**	**72 Hf** Hafnium 178.49	**73 Ta** Tantalum 180.948	**74 W** Tungsten 183.84	**75 Re** Rhenium 186.207	**76 Os** Osmium 190.23	**77 Ir** Iridium 192.217	**78 Pt** Platinum 195.085	**79 Au** Gold 196.967	**80 Hg** Mercury 200.592	**81 Tl** Thallium 204.383	**82 Pb** Lead 207.2	**83 Bi** Bismuth 208.980	**84 Po** Polonium [208.982]	**85 At** Astatine 209.987	**86 Rn** Radon 222.018
87 Fr Francium 223.020	**88 Ra** Radium 226.025	**89-103**	**104 Rf** Rutherfordium [261]	**105 Db** Dubnium [262]	**106 Sg** Seaborgium [266]	**107 Bh** Bohrium [264]	**108 Hs** Hassium [269]	**109 Mt** Meitnerium [278]	**110 Ds** Darmstadtium [281]	**111 Rg** Roentgenium [280]	**112 Cn** Copernicium [285]	**113 Nh** Nihonium [286]	**114 Fl** Flerovium [289]	**115 Mc** Moscovium [289]	**116 Lv** Livermorium [293]	**117 Ts** Tennessine [294]	**118 Og** Oganesson [294]
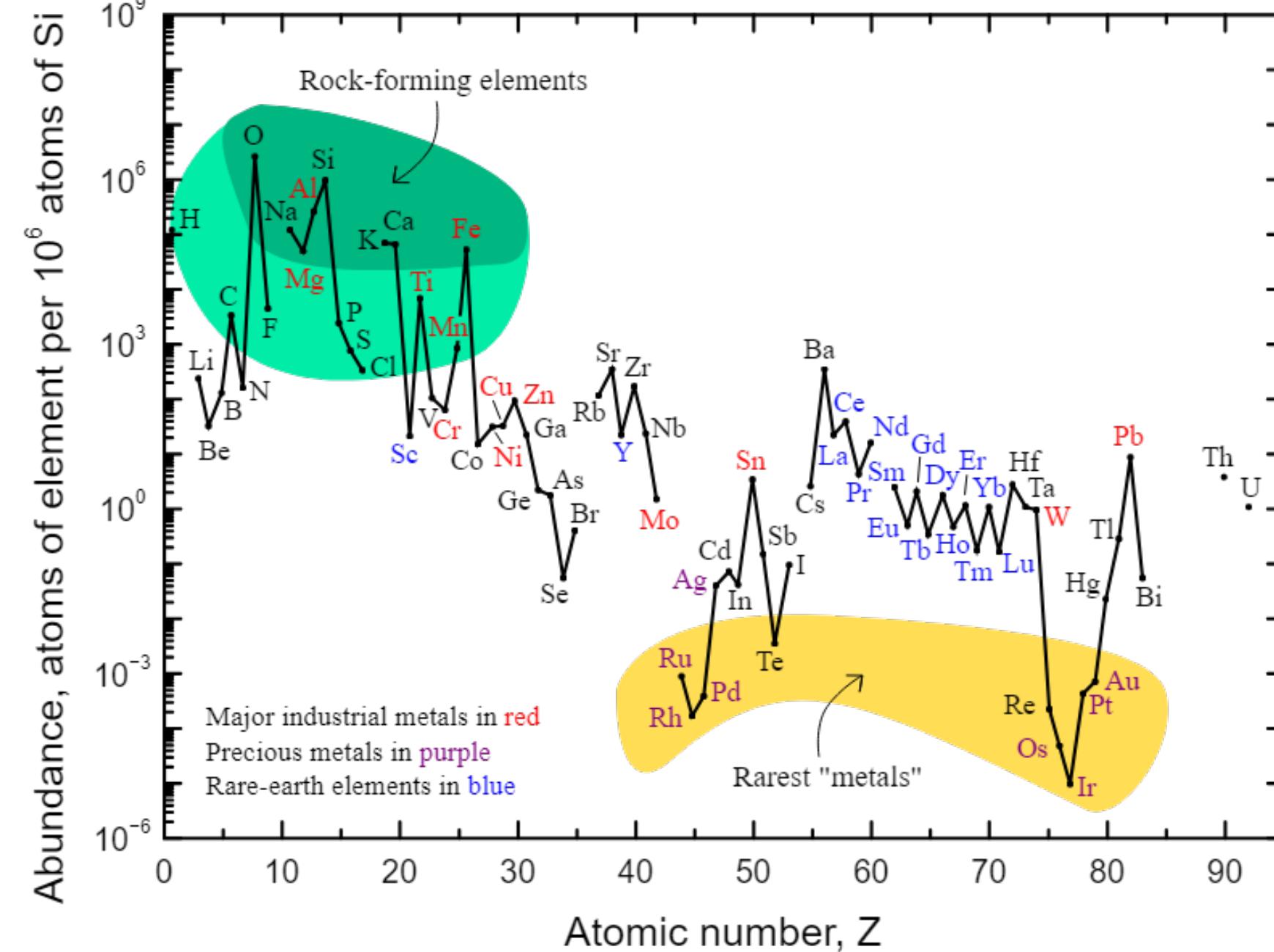
| Lanthanide Series	**57 La** Lanthanum 138.905	**58 Ce** Cerium 140.116	**59 Pr** Praseodymium 140.908	**60 Nd** Neodymium 144.243	**61 Pm** Promethium 144.913	**62 Sm** Samarium 150.36	**63 Eu** Europium 151.964	**64 Gd** Gadolinium 157.25	**65 Tb** Terbium 158.925	**66 Dy** Dysprosium 162.500	**67 Ho** Holmium 164.930	**68 Er** Erbium 167.259	**69 Tm** Thulium 168.934	**70 Yb** Ytterbium 173.055	**71 Lu** Lutetium 174.967
Actinide Series	**89 Ac** Actinium 227.028	**90 Th** Thorium 232.038	**91 Pa** Protactinium 231.036	**92 U** Uranium 238.029	**93 Np** Neptunium 237.048	**94 Pu** Plutonium 244.064	**95 Am** Americium 243.061	**96 Cm** Curium 247.070	**97 Bk** Berkelium 247.070	**98 Cf** Californium 251.080	**99 Es** Einsteinium [254]	**100 Fm** Fermium 257.095	**101 Md** Mendelevium 258.1	**102 No** Nobelium 259.101	**103 Lr** Lawrencium [262]

How did the metal ions get chosen for the enzyme sites?

Let us lay out some basic principles:

1) Rule of abundance

2) Rule of efficiency

3) Rule of basic fitness

4) Evolutionary improvement of efficiency and specificity

Elemental abundance in earth crust

Rule of Abundance

➤ When a function can he accomplished by two or more entities, organisms would utilize the more abundant and readily available one.

➤ The elements essential to organisms are mostly light elements, lighter than atomic number 34 (selenium), with exceptions of iodine and molybdenum.

➤ Four elements mostly found in catalytic site of enzymes – (i) iron (ii) zinc (iii) copper (iv) Molybdenum

❖ Molybdenum, as MoO_4^{2-}, is present in a rather high concentration in sea/oceans --- as high as iron in sea water. The concentration of zinc in sea water is also on the same order of magnitude as that of iron; the copper concentration is about half as high.

➤ The use of the most abundant alkali metals, sodium and potassium, in controlling ion balance and enzyme activities is also in accord with this rule.

➤ Most organisms utilize calcium compounds such as carbonate and phosphate as protective and skeletal material. (e.g. oyesters and we make medicines breaking those shells "ShelCal®")

➤ Undoubtedly this is due to the insolubility of calcium carbonate and phosphate. However, the corresponding strontium compounds are equally insoluble and could substitute calcium compounds. It is obvious that calcium is much more abundant than strontium.

Zinc --- in zinc-enzymes can be in most cases replaced by cobalt in vitro without losing catalytic activity. If organisms are grown in cobalt-rich media, they can produce enzymes in which Zn is replaced by Co.

For example ---

➢E. coli is in the presence of relatively high concentration of Co(II) salts can have the zinc replaced by cobalt in many of their Zn based enzymes. E.g. a Co(II) alkaline phosphatase is obtained by replacing zinc(II) with cobalt(II).

➢ so Zn(II) and Co(II) seem to be interchangeable, but organisms selected zinc because zinc is much more abundant both in sea and earth's upper crust.

Q:- In the above case of Zn(II) not replaced by Co(II) can you think of any other reasons that might prohibit the exchange–think ????
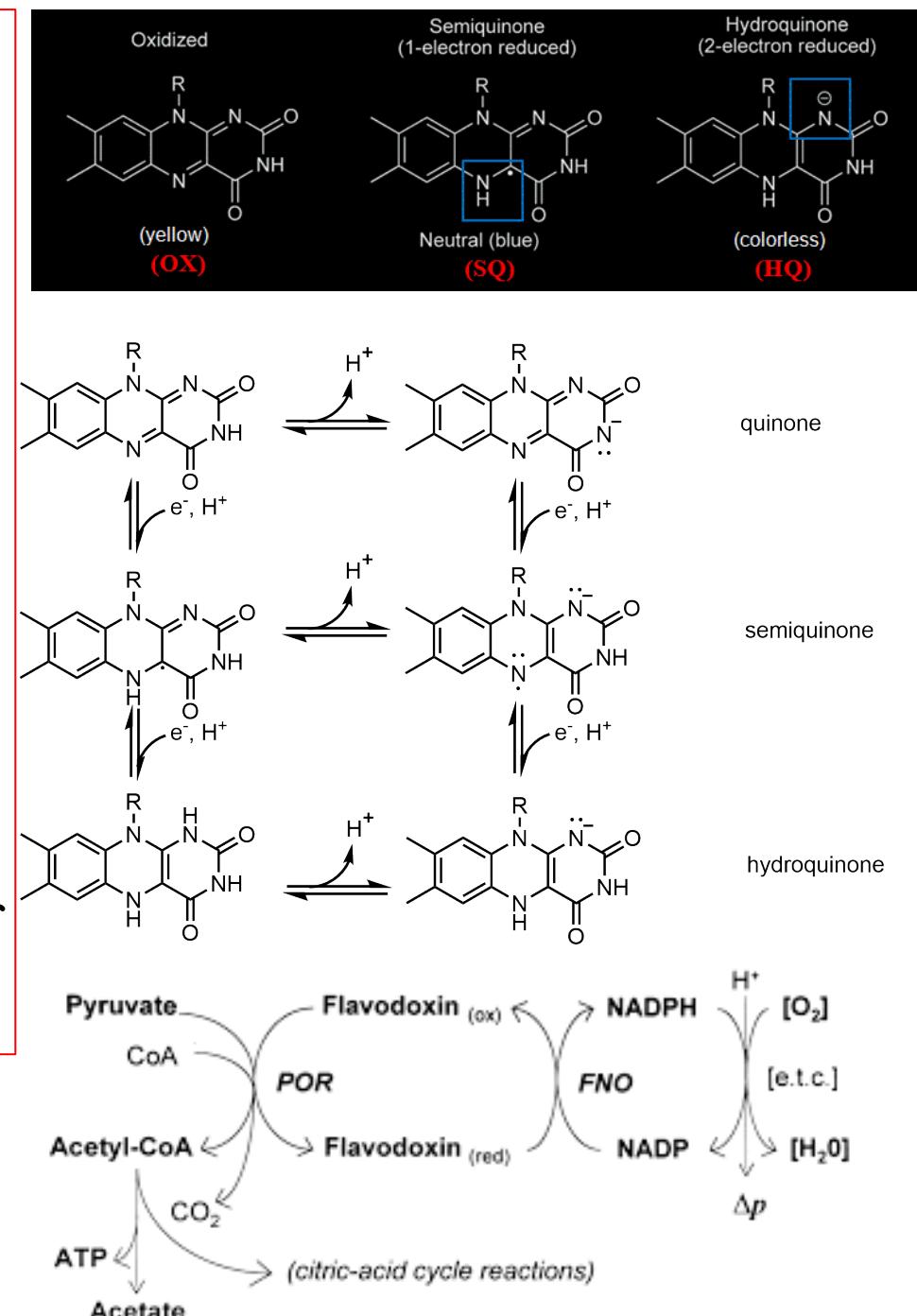
Rule of Efficiency

The rule of efficiency asserts that organisms would choose the more efficient entity as long as it is readily available. Flavodoxins and ferredoxins function as electron-carriers in very similar ways, being interchangeable in most cases.

However, their compositions are entirely different. Flavodoxin contains flavin mononucleotide (FMN) as the prosthetic group, whereas the functional units in ferredoxins are iron-sulfur complexes. Flavodoxin in general is less efficient than ferredoxin.

The synthesis of flavodoxin occurs only during growth in iron poor media in a number of microorganisms.

example, in the case of *Peptostreptococcus elsdenii*, iron-rich cells were found not to contain flavodoxin and iron-deficiencies brought about its *de novo* synthesis (5).

Oxidized / Semiquinone (1-electron reduced) / Hydroquinone (2-electron reduced)
(yellow) (OX) / Neutral (blue) (SQ) / (colorless) (HQ)

quinone

semiquinone

hydroquinone

Importance of Efficiency over abundance

A particular algae known as 'diatom' uses silica to coat their surface instead of Calcium although calcium is much more abundant than silica. Can you tell why???

Well algae needs sunlight and silica coats are more transparent than calcium coats so may be this is the reason we don't know but your knowledge of chemsitry lets you make an educated guess

An element may have ranges of capacities modulated by external factors such as coordinating ligands. However, these ranges are not limitless, and are more or less confined within certain values, particularly when the kinds of external factors are limited.

Some of the important restrictions in the case of ordinary biological systems are:
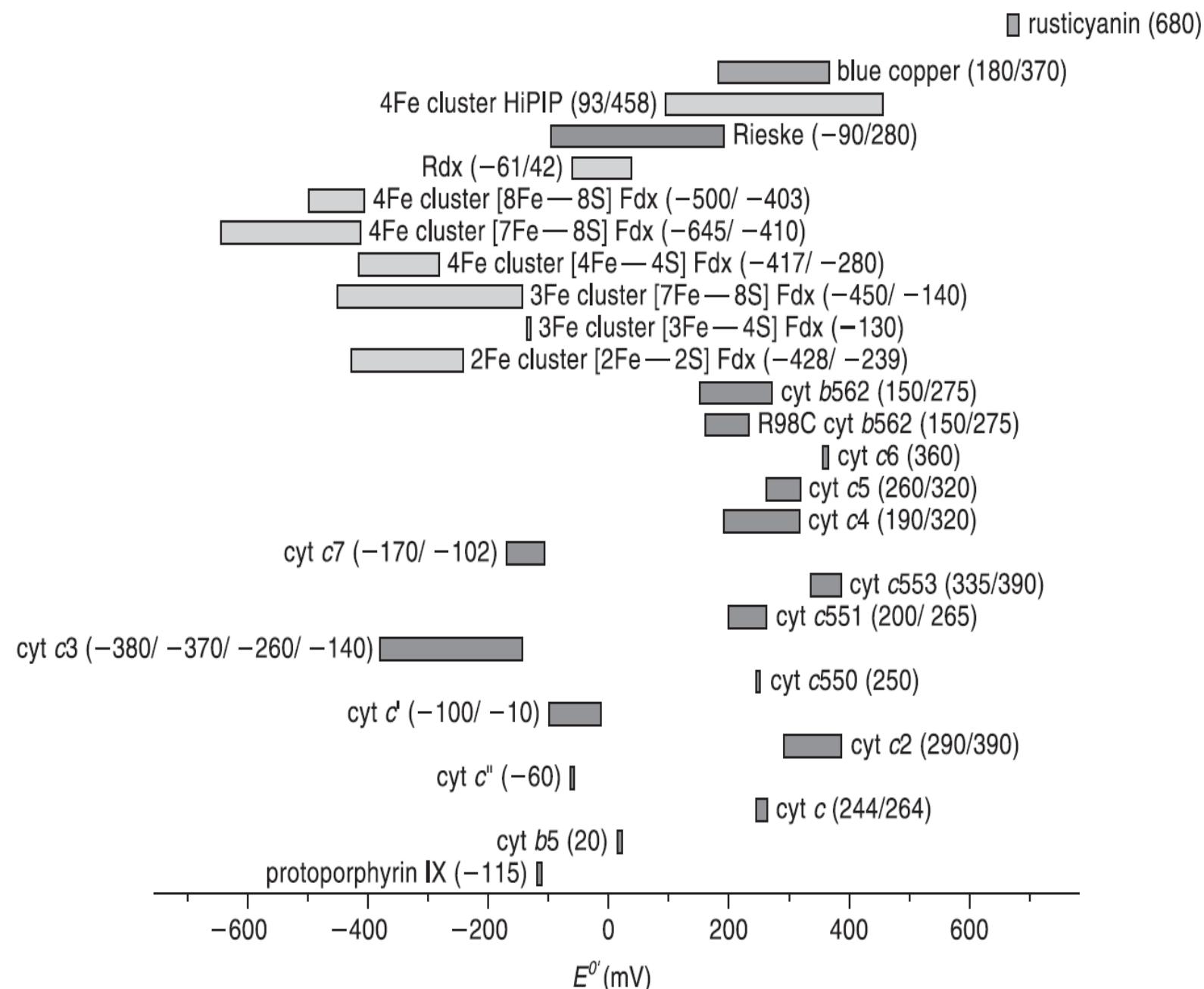
(1) the medium is water

(2) the range of temperature is rather narrow

(3) the possible coordinating ligands are limited; they are proteins, carhohydrates, nucleotides, lipids, and a few other specific ligands such as porphyrins.

The catalytic effects of a metal ion can be characterized by many factors. They are:

(1) continuous parameters such as reduction potential and Lewis acidity

(2) Discontinuous (discrete parameters such as number of valence electrons and favorable coordination number (structure).
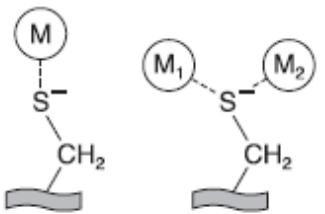
The parameters in category (1) are modulated by ligands and other external factors such as pH of the medium, but those in category (2) are much less influenced by ligands and others.

Reduction potentials of relevant proteins
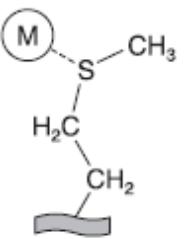
rusticyanin (680)

blue copper (180/370)

4Fe cluster HiPIP (93/458)

Rieske (−90/280)

Rdx (−61/42)

4Fe cluster [8Fe—8S] Fdx (−500/ −403)

4Fe cluster [7Fe—8S] Fdx (−645/ −410)

4Fe cluster [4Fe—4S] Fdx (−417/ −280)

3Fe cluster [7Fe—8S] Fdx (−450/ −140)

3Fe cluster [3Fe—4S] Fdx (−130)

2Fe cluster [2Fe—2S] Fdx (−428/ −239)

cyt b562 (150/275)

R98C cyt b562 (150/275)

cyt c6 (360)

cyt c5 (260/320)

cyt c4 (190/320)

cyt c7 (−170/ −102)

cyt c553 (335/390)

cyt c551 (200/ 265)

cyt c3 (−380/ −370/ −260/ −140)

cyt c550 (250)

cyt c' (−100/ −10)

cyt c2 (290/390)

cyt c'' (−60)

cyt c (244/264)

cyt b5 (20)

protoporphyrin IX (−115)

−600   −400   −200   0   200   400   600

$E^{0'}$ (mV)
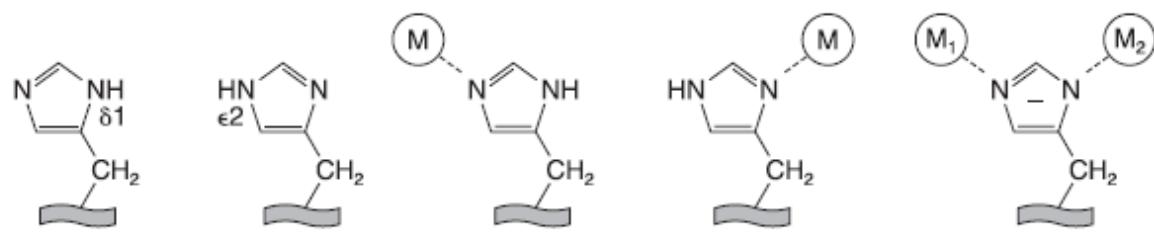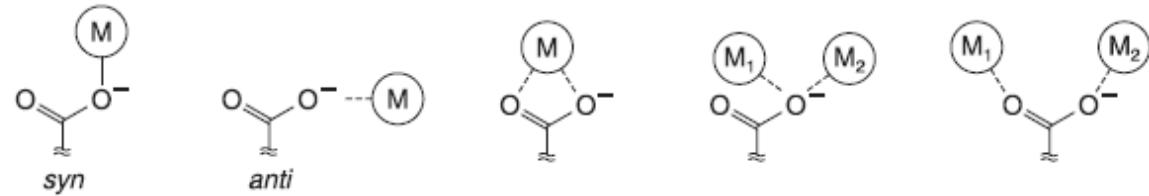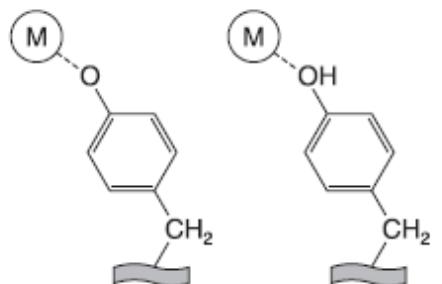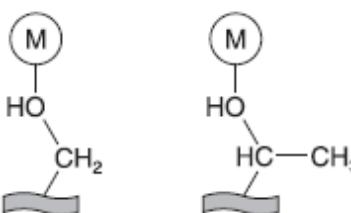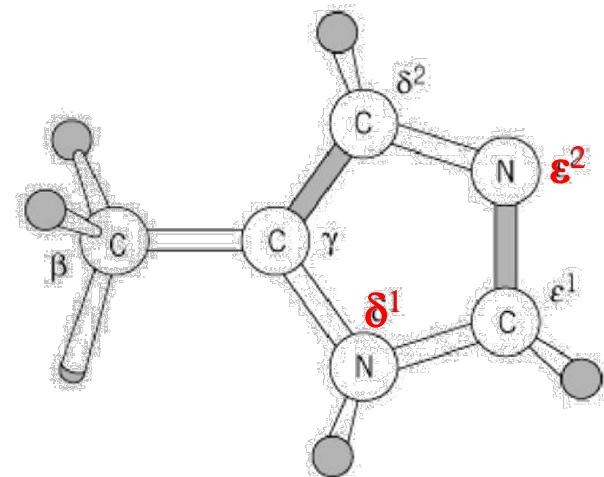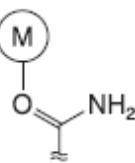
If the reduction potential of $O_2/O_2^{\bullet-}$ and $O_2^{\bullet-}/H_2O_2$ potentials are -0.35 V and +0.95 V, respectively.

Suppose reaction is—
$$O_2^{\bullet-} + M^{n+} \rightarrow M^{(n-1)+}$$
$$M^{(n-1)+} + O_2^{\bullet-} + 2H^+ \rightarrow M^{n+} + H_2O_2$$

Then, a redox system whose reduction potential is between -0.45 and +0.95 V would be an appropriate catalyst for the dismutation of superoxide.

In fact the Cu-Zn superoxide dismutase has
$$E_{Cu(II)/Cu(I)} = +0.43 \text{ V}$$
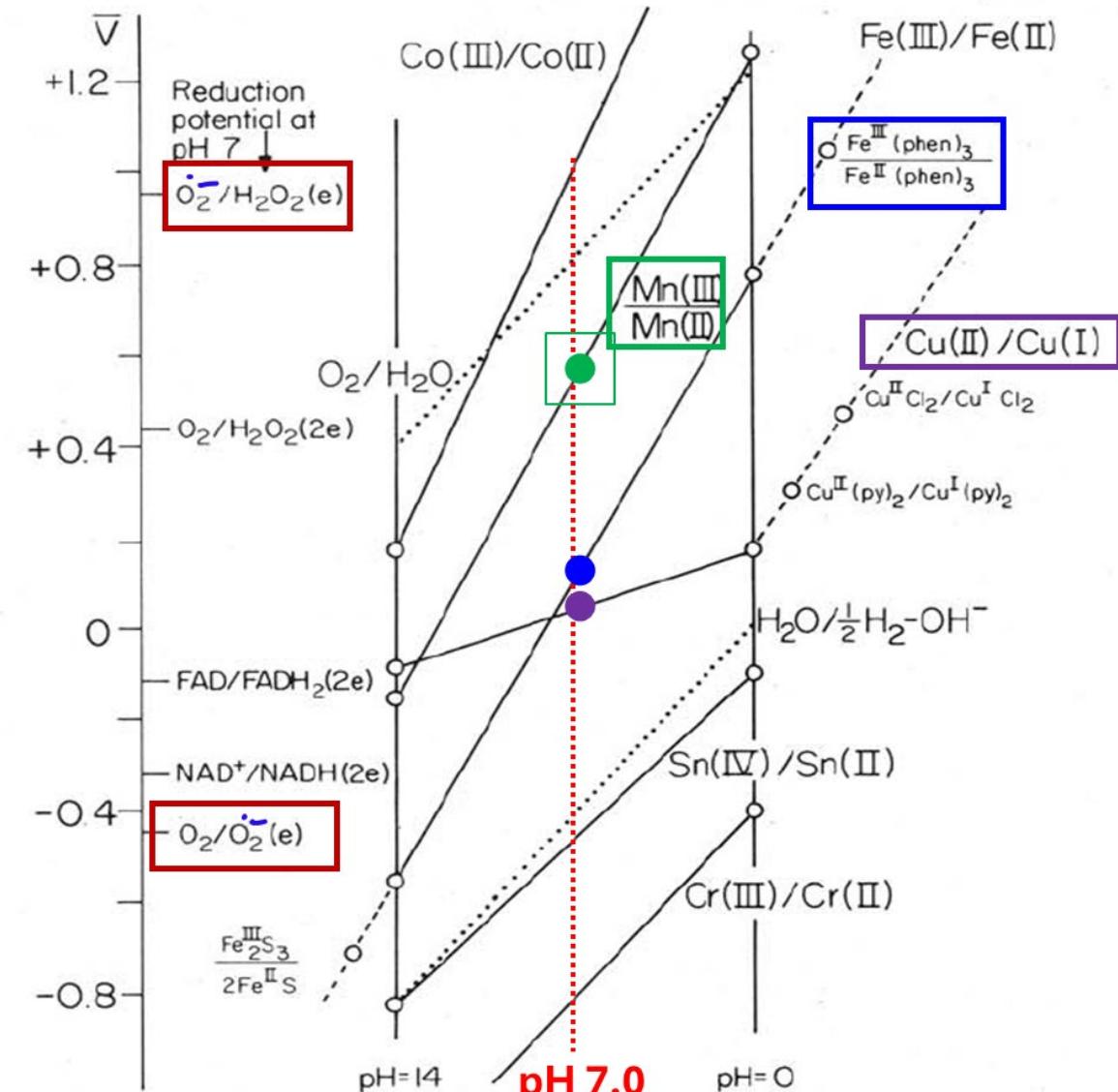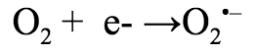
Figure 1. The reduction potentials of some important metal ions and other redox systems. O represents the reduction potential of a metal ion in an aqueous medium either at pH = 0 or at pH = 14, or the reduction potential of a simple metal complex. See the text for a more detailed explanation.

Superoxide Dismutase

Reduction half reactions and their E°' values (at pH 7)
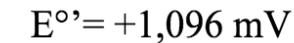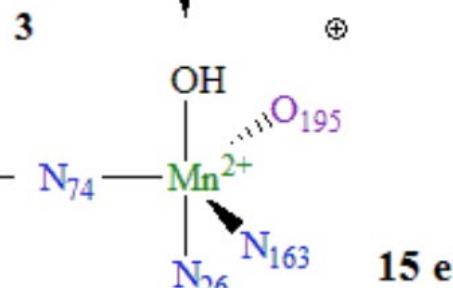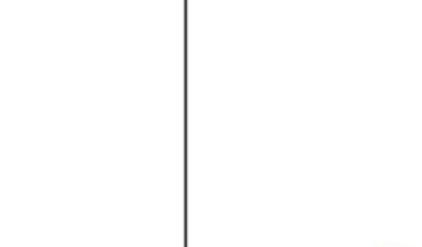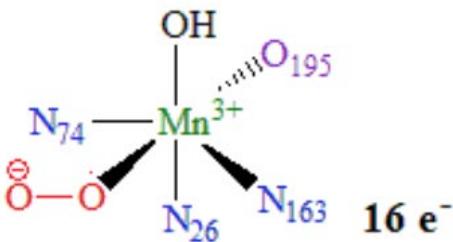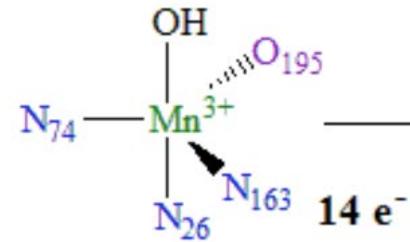
$O_2 + e^- \rightarrow O_2^{\bullet-}$ $\quad\quad$ $E°' = -160$ mV

$\leftarrow +393$ mV for $Mn^{3+}SOD + e^- \leftrightarrow Mn^{2+}SOD$

$O_2^{\bullet-} + e^- + 2H^+ \rightarrow H_2O_2$ $\quad$ $E°' = +850$ mV
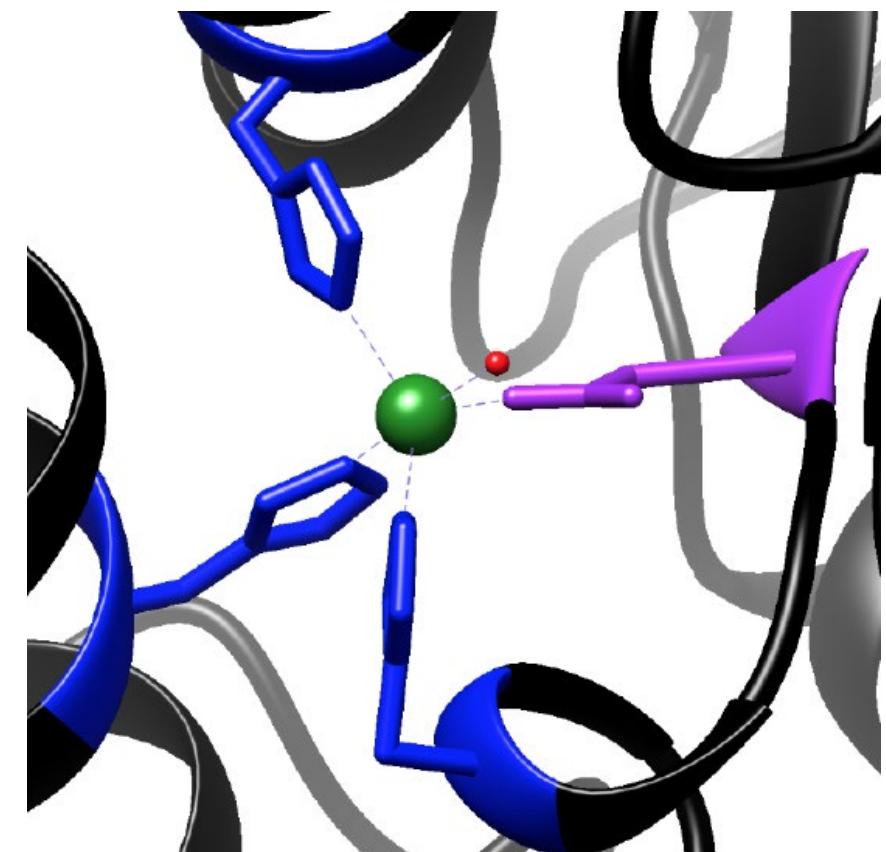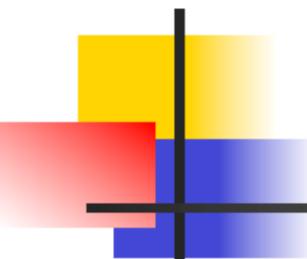
$Mn^{3+} + e^- \leftrightarrow Mn^{2+}$ $\quad\quad$ $E°' = +1,096$ mV $\quad$ ← outside of range

the MnSOD protein is thermodynamically favorable for the catalysis of superoxide to O_2 and H_2O_2

Elimination of Superoxide

❯ Superoxide is eliminated under physiological conditions by a set of superoxide dismutase enzymes that convert it to hydrogen peroxide which is then converted to water and oxygen by catalase.

❯ Mangenese superoxide dismutase is localized in mitochondria while Cu/Zn superoxide dismutases are in the cytoplasm and extracellular.

❯ Bovine Cu/Zn SOD (Orgatein®) was used as a pharmaceutical in Europe for many years for the treatment of osteoarthritis.

❯ In animal models, SOD enzymes have been shown efficacious in models of ischemia-reperfusion injury, inflammation, radiation damage, etc.

Superoxide Diusmutase