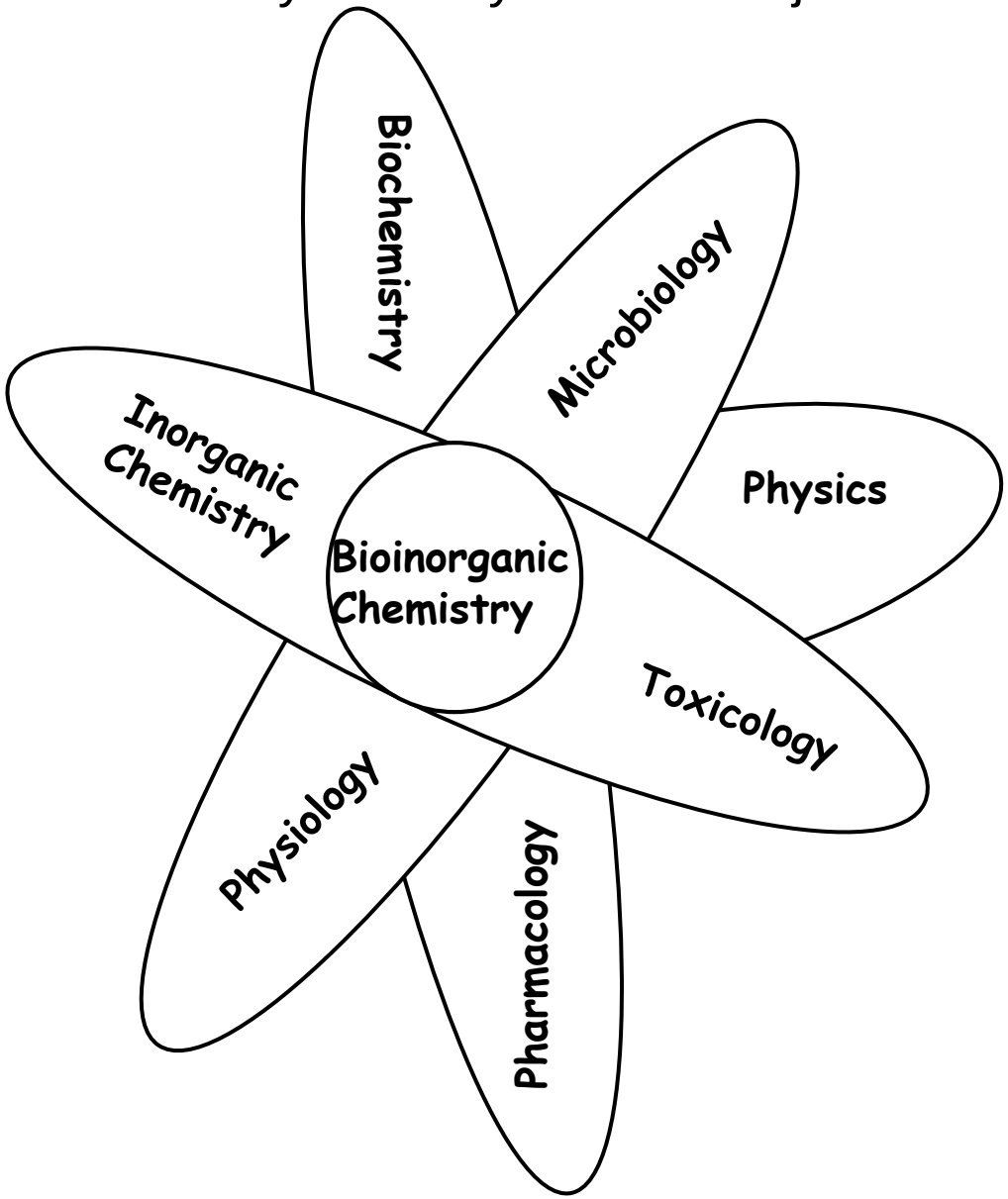
Metal ions in biological system and Potential Medicine

Avinash Kumbhar
University of Pune,Pune

Bioinorganic Chemistry is a very difficult subject



Bioinorganic Chemistry is a highly interdisciplinary research field

What is Bioinorganic Chemistry?

It's a study of the role of naturally occurring metal ions in biological systems as well as the role of externally introduced metal ions.

To search the answers for the following questions-

- Which metal ions are used in biological systems?
- How nature chose these elements?
- How do these elements get into the cells?
- How the concentration of these elements are regulated?
- How these metals bind to biopolymers?
- How these metals help in folding the biopolymers?

- How these metals help in folding the biopolymers?
- How are these metals inserted into the active site?
- What are the major roles of metal ions in biological systems?
- Which metal ions play a role in medicinal chemistry?
- Which metal ions are toxic to biological systems?

The study of metal ions with the aim of understanding the life processes.

The Bioorganic/Bioinorganic Periodic Table

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	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Metals — Essential elements for humans (daily requirement: 25 mg)

Non metals — Presumably essential elements

Conclusions derived from the above periodic table:

1. **“Chemistry of life “ is the chemistry of lighter elements”**
2. **Biological elements have been selected from practically all groups and**
   sub groups except IIIA and IVA and inert gases.

Chemical elements essential to life forms can be divided into the following

(i) Bulk elements: C, H, N, O, P, S

(ii) Macro minerals and ions: Na, K, Mg, Ca, Cl, PO_4^{3-}, SO_4^{2-}

(iii) Trace elements: Fe, Zn, Cu

(iv) Ultratrace elements comprises of

(a) non-metals: F, I, Se, Si, As, B

(b) metals: Mn, Mo, Co, Cr, V, Ni, Cd, Sn, Pb, Li

Essentiality of elements is defined by,

(1) A physiological deficiency appears when the element is removed from the diet

(2) The deficiency is relieved by the addition of that element to the diet

(3) A specific biological function is associated with the element

Why are metal ions important in biology ?

- **Catalysing reactions via:**

– Hydrolytic e.g. carbonic anhydrase, carboxypeptidase

– Substrate transfer e.g. haemoglobin, myoglobin

– Electron transfer e.g. cytochrome C oxidase

– Thermodynamic and kinetic considerations

- **Stabilising structure:**

– Protein

– DNA

– Skelet**al**

- **Charge balancing**

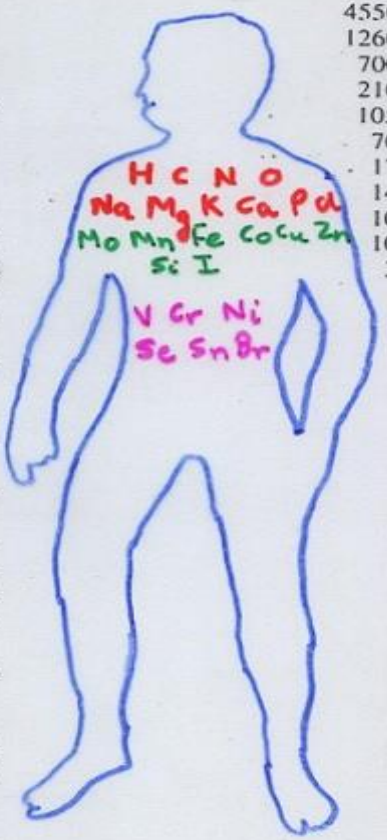
– Osmotic balance

– Nerve function

- **Replication and information encoding**

Table 2.1 Average elemental composition of a human body (adult, 70 kg [1])

element and symbol		mass (g)	year of discovery as an essential element
oxygen	O	45500	
carbon	C	12600	
hydrogen	H	7000	
nitrogen	N	2100	
calcium	Ca	1050	
phosphorus	P	700	
sulfur	S	175	
potassium	K	140	
chlorine	Cl	105	
sodium	Na	105	
magnesium	Mg	35	
iron	Fe	4.2	17th century
zinc	Zn	2.3	1896
silicon	Si	1.4	1972
rubidium[a]	Rb	1.1	
fluorine	F	0.8	1931
zirconium[a]	Zr	0.3	
bromine[b]	Br	0.2	
strontium[a]	Sr	0.14	
copper	Cu	0.11	1925
aluminum[a]	Al	0.10	
lead[a]	Pb	0.08	
antimony[a]	Sb	0.07	
cadmium[b]	Cd	0.03	(1977)
tin[b]	Sn	0.03	(1970)
iodine	I	0.03	1820
manganese	Mn	0.02	1931
vanadium[b]	V	0.02	(1971)
selenium	Se	0.02	1957
barium[a]	Ba	0.02	
arsenic[b]	As	0.01	1975
boron[b]	B	0.01	
nickel[b]	Ni	0.01	(1971)
chromium	Cr	0.005	1959
cobalt	Co	0.003	1935
molybdenum	Mo	<0.005	1953
lithium[b]	Li	0.002	

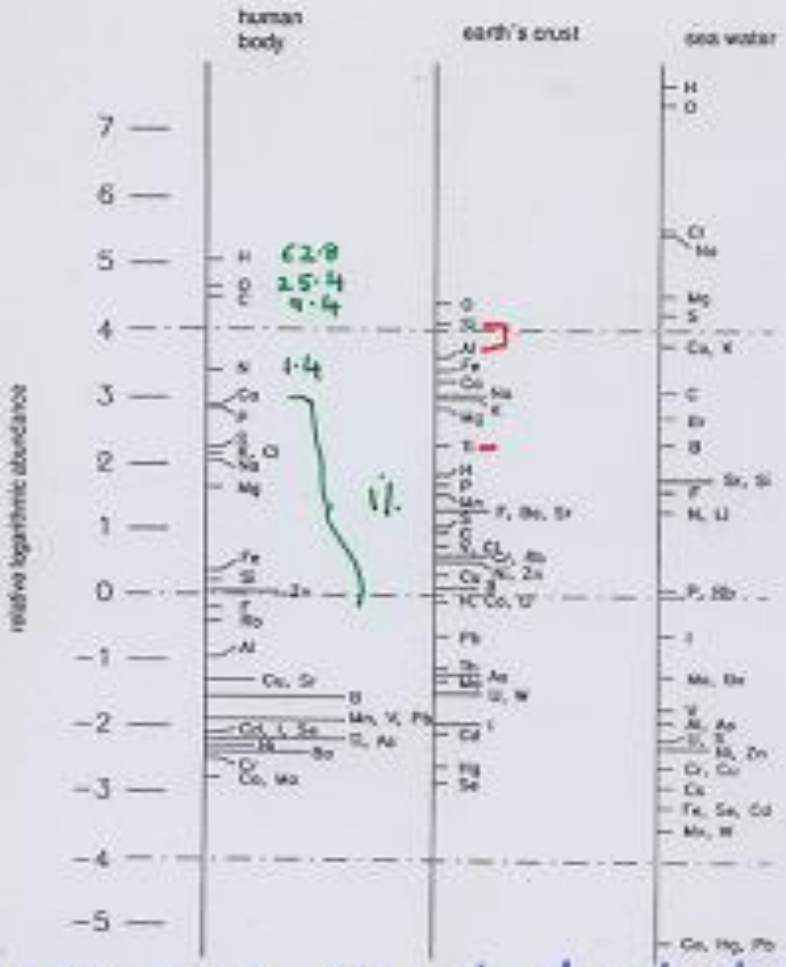


[a] Not essential. [b] Essentiality uncertain.

Pt Ba
Tc Ru
Au
B
Se

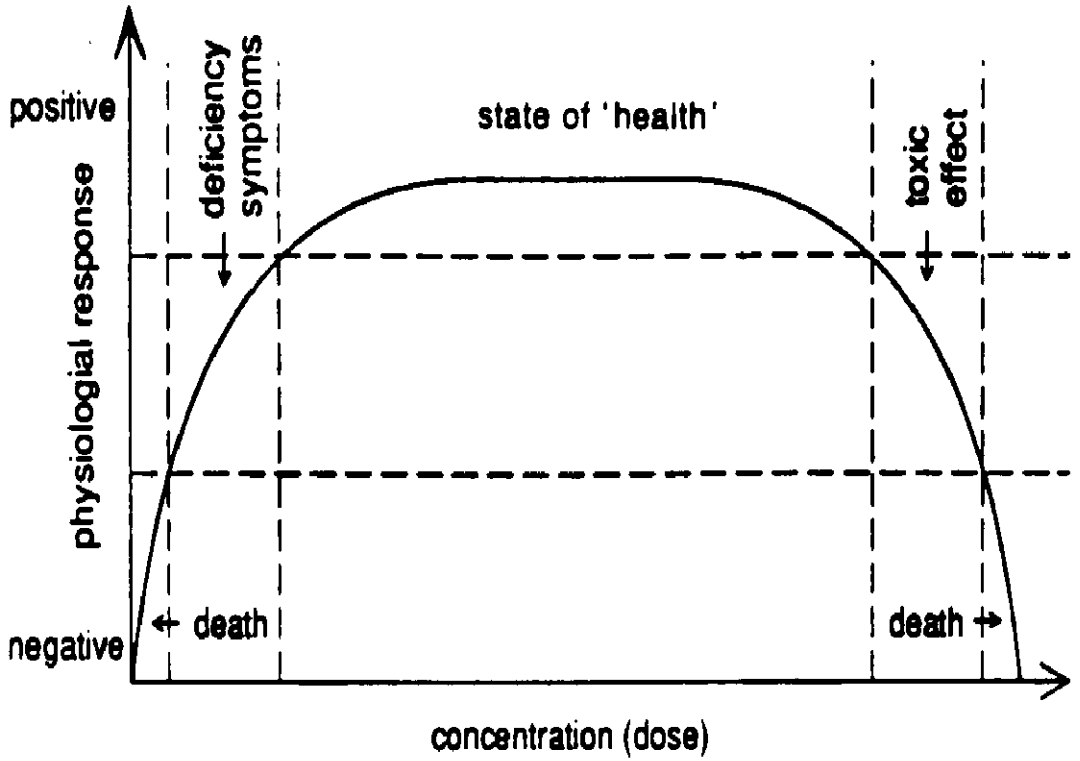
} therapeutics
Diagnostics

Relative abundance of elements



why Si, Ti, Al though abundant have no fn. in biology.

Ans: May be related to the difficulty in solubilizing and mobilizing the common ions Si^{4+}, Al^{3+}, Ti^{4+} in biological fluids at pH 6–8.


positive
physiologial response
negative
deficiency symptoms
state of 'health'
toxic effect
death
death
concentration (dose)

The economical use of resources- abundance and availability

❖The element to be used in the biological systems must be abundant and must be available in a extractable form in water

❖ Nature responded to abundance and availability following a principle of ‘the economical utilization of resources’ i.e. choosing those elements less costly in terms of energy required for uptake, given the function for which they are required

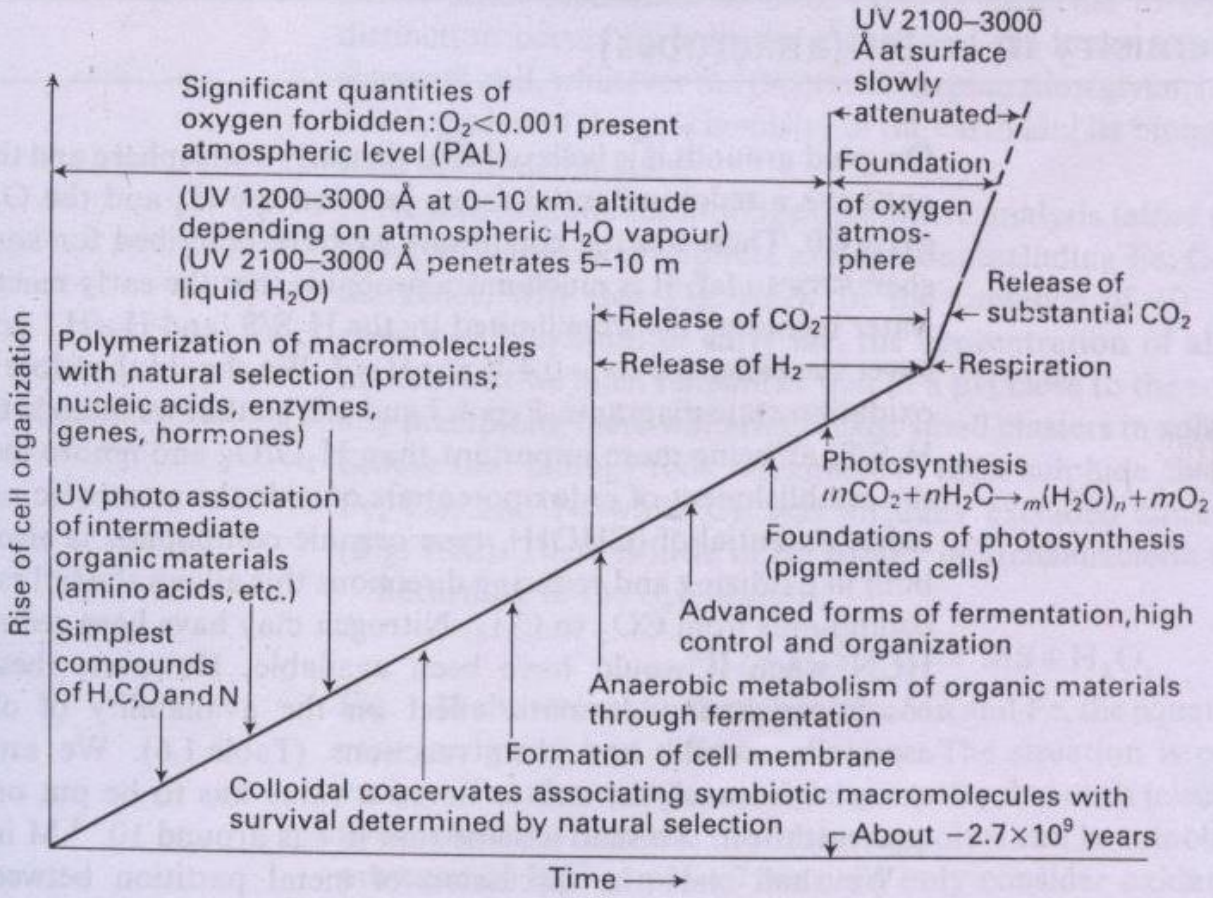


Fig. 1.11 The involvement of the elements in life has to be seen against the progression of the development of organization over historical time. We suppose earth has existed for over 4×10^9 years. We guess that organic molecules were formed haphazardly for a long period both through the action of light and on catalytic surfaces. Organization of chemistry in space and time evolved and is called living once it could reproduce. For a long period only simple cells, prokaryotes, developed. They had no internal boundaries. At some stage from about 2.7×10^9 years an evolution of dioxygen in the atmosphere developed due to prokaryote metabolism. A vast change took place by some 570×10^6 years ago bringing internal cellular structures of great diversity and multi-cellular organisms. (This figure is after Bernal (1967) and will be used in a somewhat different form in Chapter 21.)

What is special in Metals?

1. Geometry preferences
   (Oxidation-state dependent; size dependent)
2. Binding strengths (weak, strong)
3. Binding kinetics (fast, slow)
4. Binding preferences for ligands (HSAB)
5. Reactivity patterns
   a) Redox properties
   b) Photoreactivity and charge transfer
   c) Coordination changes (catalysis)
   d) Powerful template possibilities
6. Cluster formation possibilities

BIOLOGICAL FUNCTIONS OF INORGANIC ELEMENTS

1) **STRUCTURAL FUNCTION -**

endo and exo-skeletons—membrane " filling material" DNA helical structure maintained in presence of cations.
Solid-state/structural functions are represented mainly by elements Ca, Mg (as cations) and P,O,C,S,Si,F (as anions).
Zn (Zinc fingers)

2) **CHARGE CARRIERS/ INFORMATION TRANSFER-**

Transmembrane concentration gradient, ion pumps, electrical impulses in nerves, trigger mechanisms: muscle contraction. Represented by Na^{+}, Ca^{2+}, K^{+}.

3) **FORMATION, METABOLISM AND DEGRADATION OF ORGANIC COMPOUNDS-**

Acid-base catalysis, hydrolysis Zn^{2+} and Mg^{2+}

4) **ENERGY CONVERSION –**

Transfer of electrons , redox-active metal centers $Fe^{II}/Fe^{III}/Fe^{IV}$, Cu^{I}/Cu^{II}, $Mn^{II}/Mn^{III}/Mn^{IV}$, $Mo^{IV}/Mo^{V}/Mo^{VI}$, $Co^{I}/Co^{II}/Co^{III}$, $Ni^{I}/Ni^{II}/Ni^{III}$.

5) **ACTIVATION OF SMALL SYMMETRICAL MOLECULES-**

(a) Reversible uptake, transport, storage and conversion of oxygen (Fe,Cu)
(b) Generation of oxygen (Mn)
(c) Fixation of nitrogen and its conversion to ammonia (Fe,Mo,V)
(d) Reduction of carbon dioxide to methane (Ni, Fe)

6) **FACILE GENERATION OF RADICALS –**

Typical organometallic like reactivity (Co-alkyl)

This diversity is shrouded in evolutionary history
bioavailability of a given element at the biosphere/geosp. interface
pressure to evolve multiple pathways for survival..

Functions of Metal ions in Biology

Metal	Function	Typical Deficiency Symptoms
Na, K	charge carrier, osmotic balance	death
Mg	Structure, hydrolase, isomerase	Muscle cramps
Ca	Structure, trigger, charge carrier	Retarted skeletal growth
V	Nitrogen fixation, oxidase	N/A
Cr	Glucose intolerance	Diabetes symptoms
Mo	N_2 fixation, oxidase, oxo transfer	Retardation of cell growth
Mn	Photosynthesis, oxidase, structure	Infertility, impaired growth
Co	Oxidase, carbon group transfer	Pernicious anemia
Fe	O_2 transport and storage, oxidase, electron transfer, N_2 fixation	Ananemia, disorders of the immune system
Ni	Hydrogenase, hydrolase	Growth depression dermatitis
Cu	E-transfer, O_2 Transport, oxidase	Artery weakness, liver disorders
Zn	Structure, hydrolase, male fertility	Skin damage, stunted growth, retarted sexual maturation, impaired development
Se, As	Puberty (?) and growth	Impaired development

Subdivisions of Bioinorganic Chemistry

- **Natural selection of elements-related to evolution**
  Economical use of resources.
  Biological environment and elemental ability.
  Homeostasis.

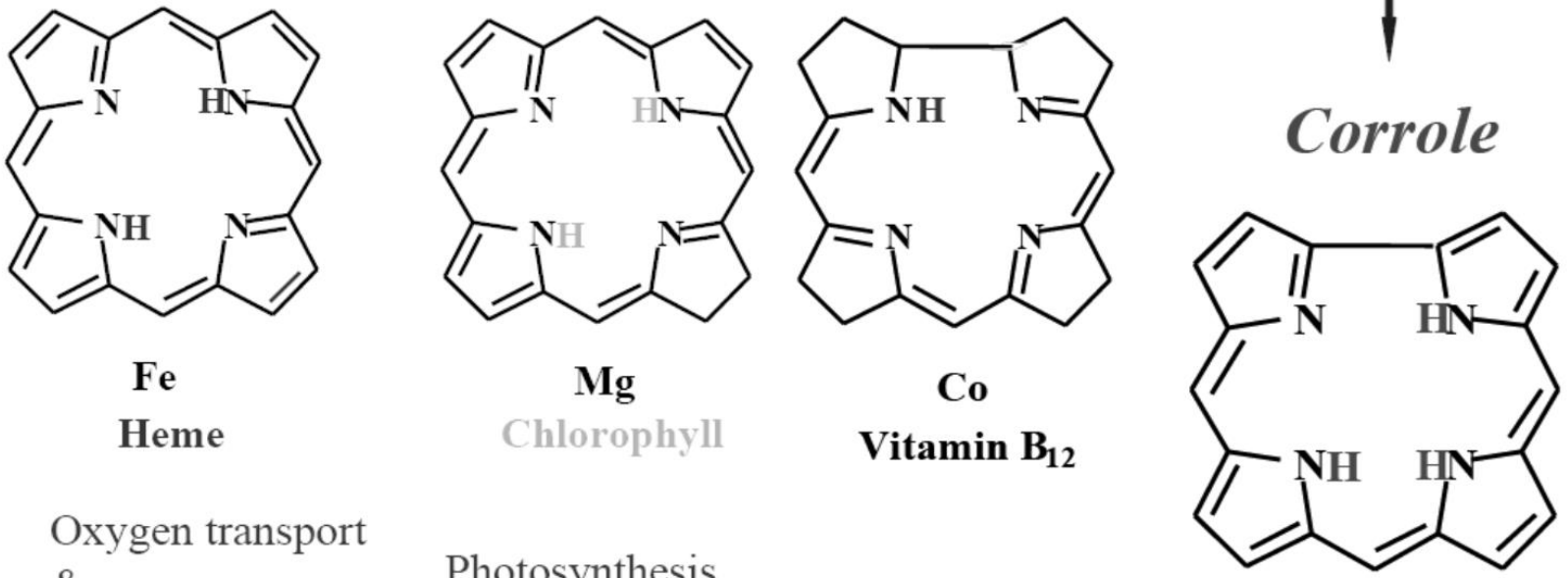
- **Biomimetic Chemistry**
  Structural and functional modeling of enzymes and proteins**.**

- **Medicinal Inorganic Chemistry**
  Synthesis and application of inorganic compounds as drugs and diagnostic agents.
  Toxicology of metals and metalloids.
  Chemical Nucleases

- **Biomineralization**
  Controlled assembly of advanced materials in biology

Macrocyclic ligands in Bioinorganic Chemistry

Porphyrin ***Chlorin*** ***Corrin*** ***Corrole***



Fe
Heme

Oxygen transport
&
Oxygen activation

Mg
Chlorophyll

Photosynthesis

Co
Vitamin B_{12}

Nobel prizes related to tetrapyrroles

1. R.Wilstatter (1915) –constituton of chlorophyll
2. H.Fischer (1930) – constitution of the heme system
3. J.C.Kendrew and M.F.Perutz (1962)- X-ray structure of myoglobin and hemoglobin
4. D.Crawfoot-Hodgkin (1964)-X-ray structure of vitamin B12
5. R.B.Woodward (1965)-natural product synthesis of chlorophyll and vitamin B12
6. J.Disenhofer,H.Michel,R.Huber (1988)-X-ray structure of heme and chlorophyll containing photosynthetic reaction centre in bacteria.

Characteristic features of tetrapyrole bioligands

a) **Planar or nearly planar ring system** are very stable (no geometric stress) . All bond lengths (134-145pm) and angles (107-126°) as well as torsional angles (< 10°) are normal for the neighboring sp^2-hybridized carbon and nitrogen centers.

b) Tetradentate chelate ligands after **deprotonation carry single or double negative charge** can bind coordinatively labile metal ions. Kinetic stability (dissociation only if all metal-to-ligand bonds are broken simultaneously (which is unlikely)

c) Macrocyclic ligands are **selective with regard to the size of the coordinated ion** as they are rigid because of conjugated double bonds. Spherical ions with radii 60-70pm are suited to fit the cavity (in-plane coordination).

d) **Extensively congugated π systems.** The Huckel rule for aromatic cyclic systems (4n + 2) π=18 πelectrons.

(i) Thermally stable.

(ii) Ligands and metal complexes show intense absorption bands in the visible region " pigments of life"

(iii) One electron reduction and oxidation is facilitated because of the narrowing of the π frontier orbital gap and the resulting anion and cation radicals are stable.

These are useful in electron buffering and storage in biological energy transformations – photosynthesis and respiration.

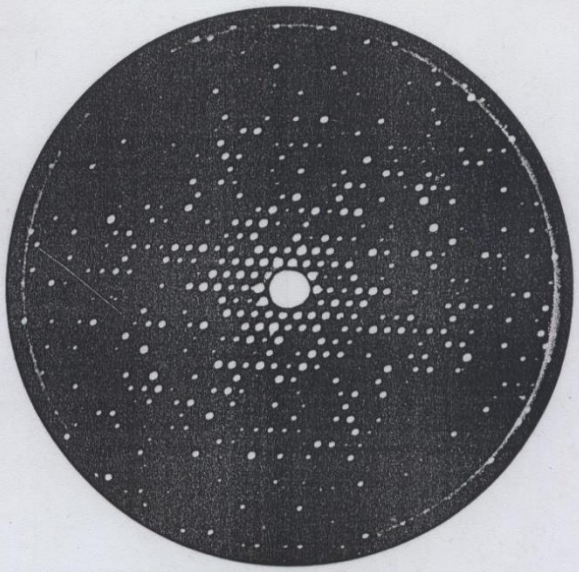
e) **Coordinatively unsaturated.** Two axial sites vacant (X and Y) for controlled stoichiometric or catalytic activation of substrates using trans-effect (i) hemoglobin - X = O_2 and Y = proximal histidine (ii) cobalmin - X = CH_2R and Y = benzimadazole

f) **Tetragonal distortion** of the octahedral symmetry causes characteristic splitting of the d orbitals affecting chemical reactivity. Deoxy-hemoglobin and deoxy-myoglobin and coenzyme F430 (Ni) feature a very critical high spin metal (II) center with out-of-plane complexation.

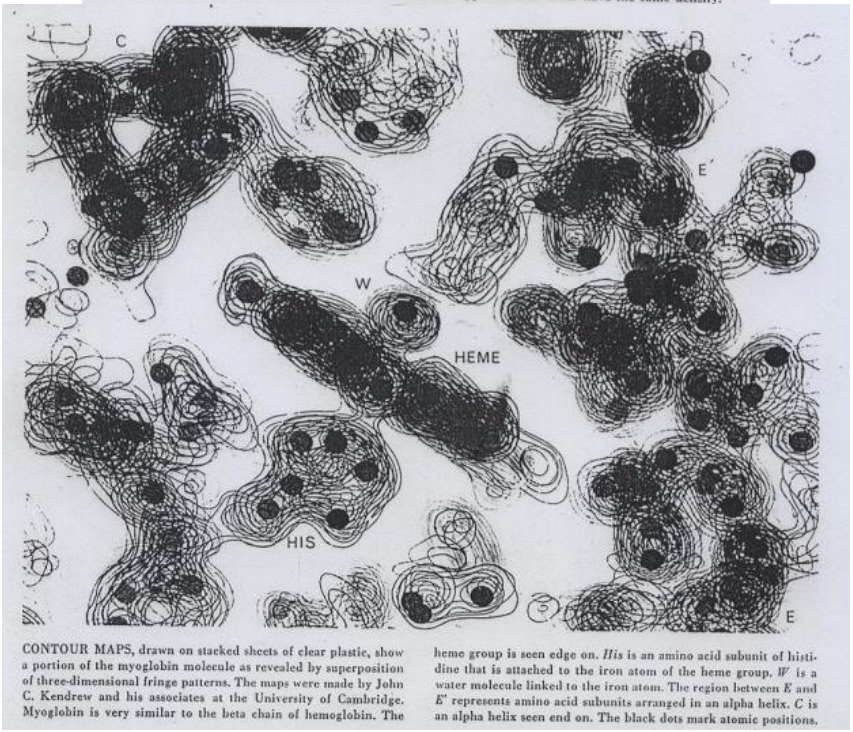
Table 2.7 Ionic radii and (biological) complexation of metal ions by tetrapyrrole ligands

metal ion	ionic radius[a] (pm)	suitability as metal center in complexes with tetrapyrrole macrocycles
Be^{2+}	45	too small
Mg^{2+}	72	proper size, → chlorophyll (Chap. 4.2)
Ca^{2+}	100	too big
Al^{3+}	53	rather small
Ga^{3+}	62	gallium(III) porphyrin complexes have been found in crude mineral oil but not in living organisms (very rare element)
In^{3+}	80	rather large, rare element
$O{=}V^{2+}$ (not spherical)	ca. 60	vanadyl porphyrins are relatively abundant in certain crude oil fractions where they interfere with the catalytic removal of N and S in refineries; they have not been observed in living organisms
Mn^{2+}(h.s.)[b]	83	too large (?)
Mn^{3+}	ca. 60	proper size, use in synthetic oxidation catalysts
Fe^{2+}(h.s.)	78	too large (*out-of-plane* structure, compare Fig. 5.4)
Fe^{2+}(l.s.)[c]	61	proper size
Fe^{3+}(h.s.)	65	proper size
Fe^{3+}(l.s.)	55	rather small
average value for $Fe^{2+/3+}$	65	→ heme system with Fe^{n+} in various oxidation and spin states (Chapters 5 und 6)
Co^{2+}(l.s.)	65	proper size, → cobalamins (Chap. 3)
Ni^{2+}	69	proper size, –: F430 (Chap. 9.5), tunichlorin
Cu^{2+}	73	relatively large; Cu porphyrins have not been found in organisms, strong bonds are formed mainly with histidine in proteins
Zn^{2+}	74	relatively large; Zn porphyrins have not been found in organisms, strong bonds are formed e.g. with histidine or cysteinate in proteins

[a] For coordination number 6, from [43] [b] h.s.: high-spin. [c] l.s.: low-spin.



X-RAY DIFFRACTION PATTERN was made from a single crystal of hemoglobin that was rotated during the photographic exposure. Electrons grouped around the centers of the atoms in the crystal scatter the incident X rays, producing a symmetrical array of spots. Spots that are equidistant from the center and opposite each other have the same density.



CONTOUR MAPS, drawn on stacked sheets of clear plastic, show a portion of the myoglobin molecule as revealed by superposition of three-dimensional fringe patterns. The maps were made by John C. Kendrew and his associates at the University of Cambridge. Myoglobin is very similar to the beta chain of hemoglobin. The heme group is seen edge on. *His* is an amino acid subunit of histidine that is attached to the iron atom of the heme group. *W* is a water molecule linked to the iron atom. The region between *E* and *E'* represents amino acid subunits arranged in an alpha helix. *C* is an alpha helix seen end on. The black dots mark atomic positions.

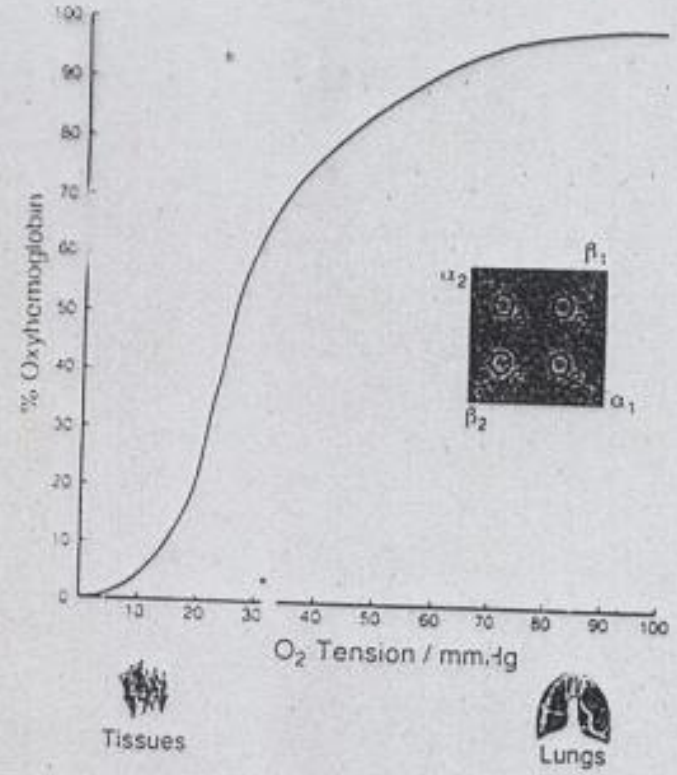


Figure 1. The classical oxygen dissociation curve of Hb. Hemoglobin's oxygen dissociation curve is sigmoidal, whereas other oxygen-carrying molecules (such as myoglobin) have hyperbolic dissociation curves. Only the sigmoidal curve is characteristic of the cooperative process by which the release of one oxygen molecule alters the affinity for the remaining oxygens bound to the other proteic subunits. The 4-subunit arrangement in Hb ($\alpha_1,\alpha_2,\beta_1,\beta_2$) accomplishes a specific function in the vertebrates as Hb moves from an extreme gradient of oxygen partial pressure (or oxygen tension) from lungs to hypoxic tissues. The dashed diagonal lines in the inset indicate that oxygen molecules are bound to α/β subunits (to the 6th coordination positions of Fe^{2+} ions on the heme planes).

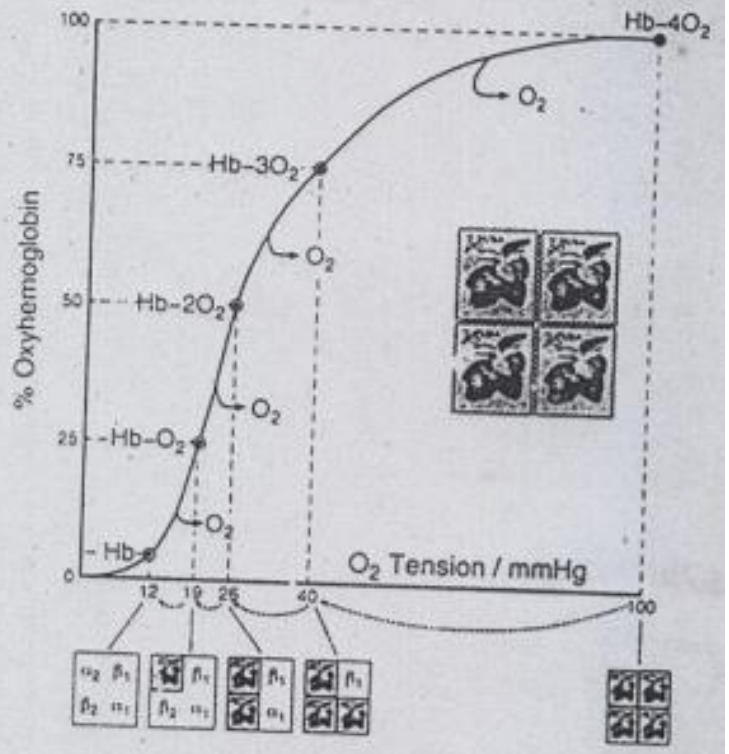
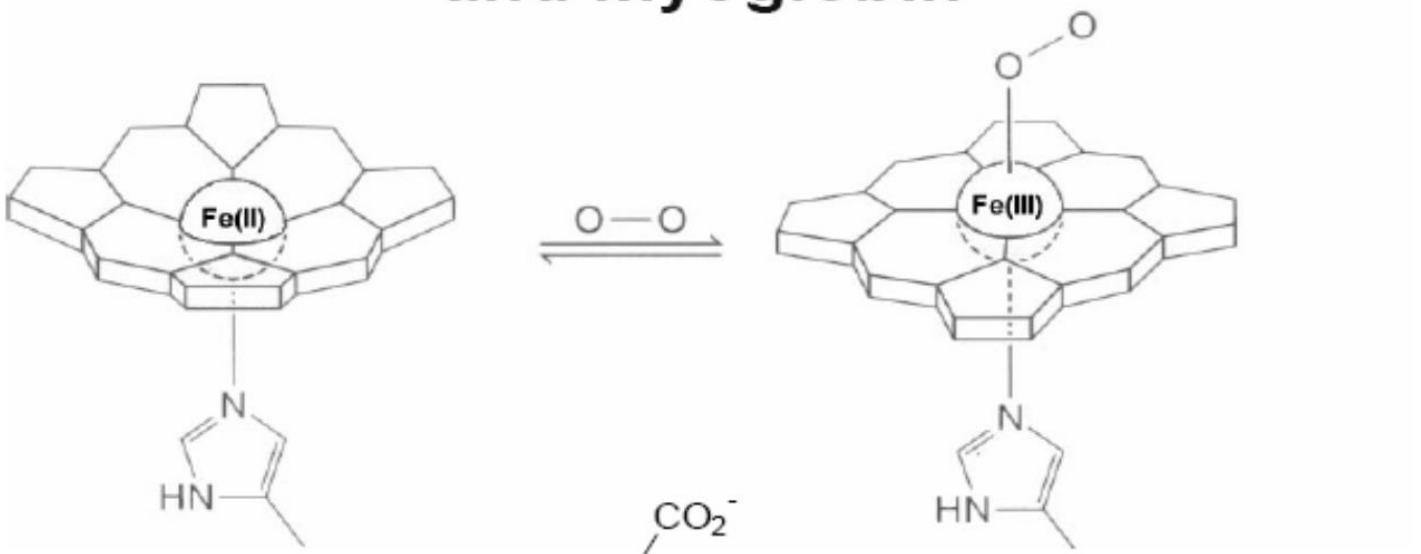
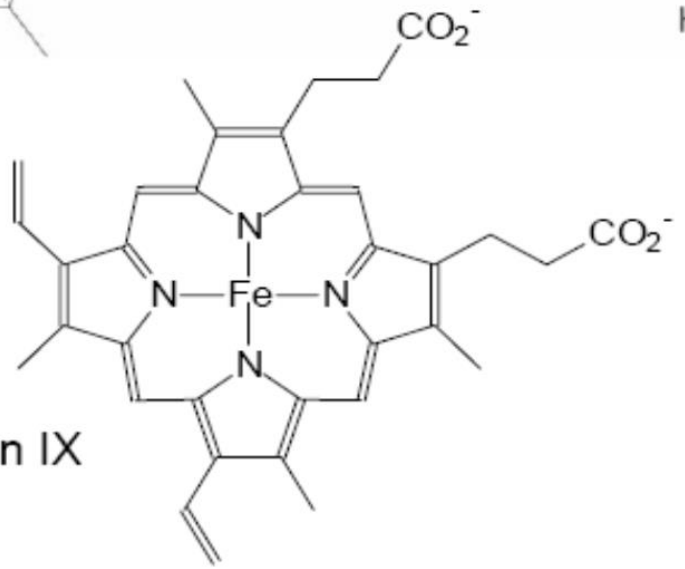


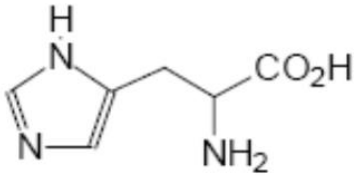
Figure 2. The postage-stamp analogy. To release single stamps from a block of four, we have to make two cuts to release the first stamp and only one cut to release the second; with a final cut we release the last two stamps, thus each time needing less "energy" to do the job. Similarly, oxygen remains tightly bound to Hb in the lungs but will be progressively released as partial oxygen pressure drops in the tissues of the body. The release of the second, and even more so the third, oxygen molecule requires a smaller drop in pressure as the Hb-carrying erythrocyte moves farther from the lungs. In the analogy, $Hb\text{-}4O_2$ exists as "four stamps bound to the 4 Hb subunits"; $Hb\text{-}3O_2$ exists as "three stamps bound + 1 Hb subunit free"; and so on.

Heme-based oxygen carriers: hemoglobin and myoglobin





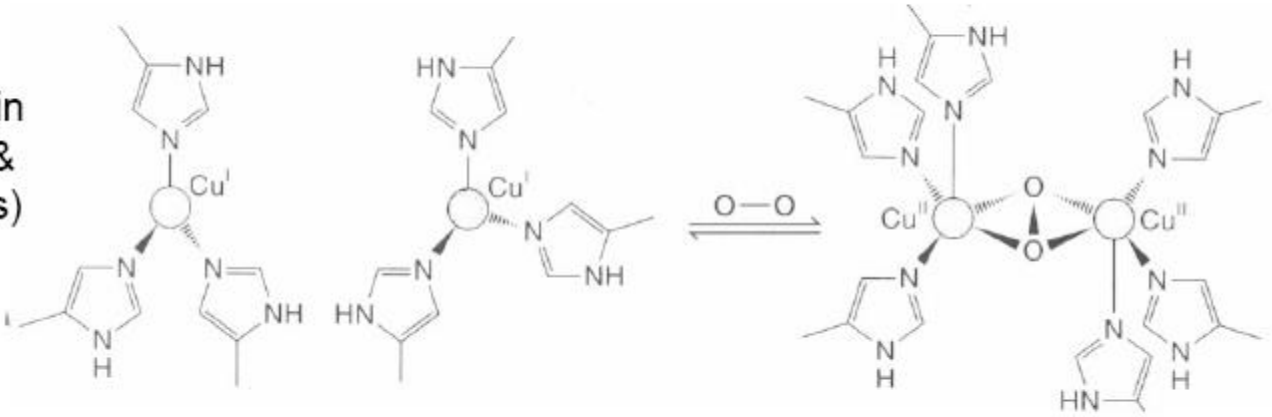
iron protoporphyrin IX



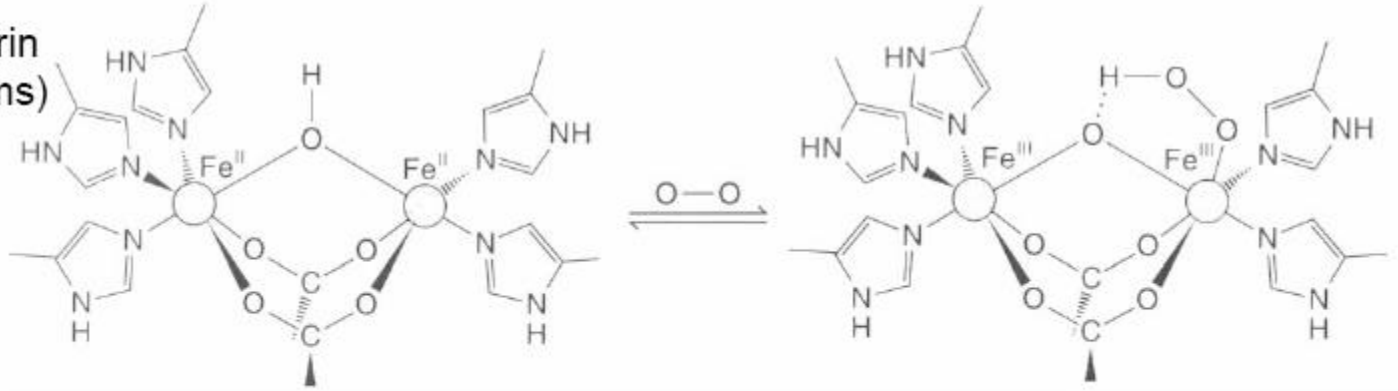
histidine

Non-heme oxygen carriers

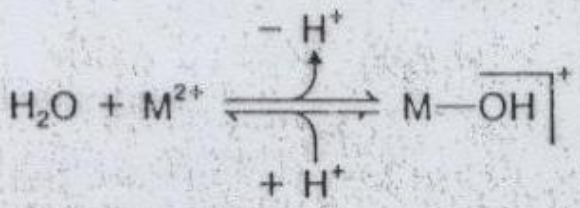
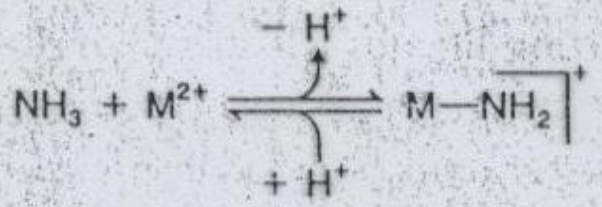
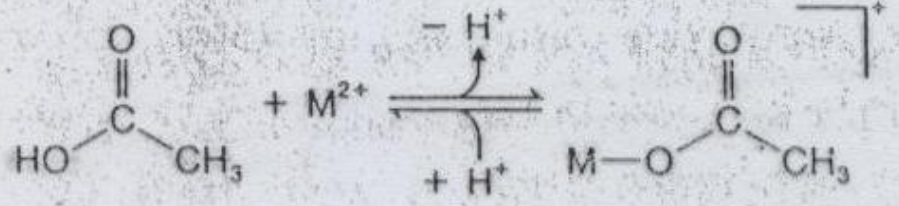
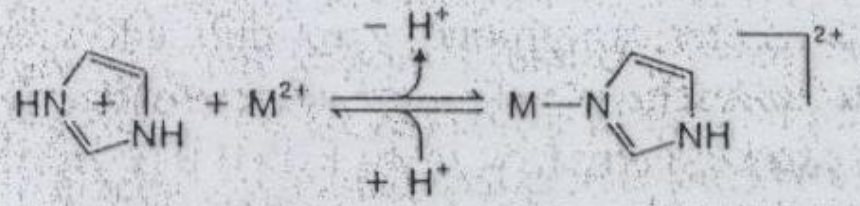
hemocyanin
(mollusks &
anthropods)



hemerythrin
(sea worms)



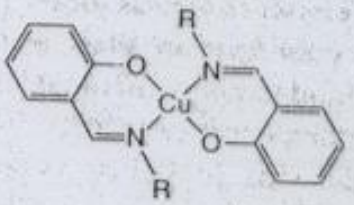
pK$_a$ values for selected ligands with and without metal ions

Ligand and reaction	Metal ion	Log K (25°C, 0.1 M)
	None	14.0
	Ca^{2+}	13.4
	Mn^{2+}	11.1
	Cu^{2+}	10.7
	Zn^{2+}	10.0
	None	35.0
	Co^{2+}	32.9
	Cu^{2+}	30.7
	Ni^{2+}	32.2
	None	4.7
	Mg^{2+}	4.2
	Ca^{2+}	4.2
	Ni^{2+}	4.0
	Cu^{2+}	3.0
	None	7.0
	Co^{2+}	4.6
	Ni^{2+}	4.0
	Cu^{2+}	3.8

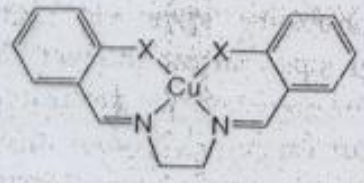
Alterations in the ligand donor atom and stereochemistry at metal center Produces differences in the potential at which e⁻ transfer reactions will occur:

Effect of ligands on Cu(I)/Cu(II) reduction potential in DMF solution

Compound name	$E_{1/2}$, V[a]
Cu(*O*-sal)$_2$en	−1.21
Cu(Me-sal)$_2$	−0.90
Cu(Et-sal)$_2$	−0.86
Cu(*S*-sal)$_2$en	−0.83
Cu(*i*-Pr-sal)$_2$	−0.74
Cu(*t*-Bu-sal)$_2$	−0.66



Cu(*R*-sal)$_2$



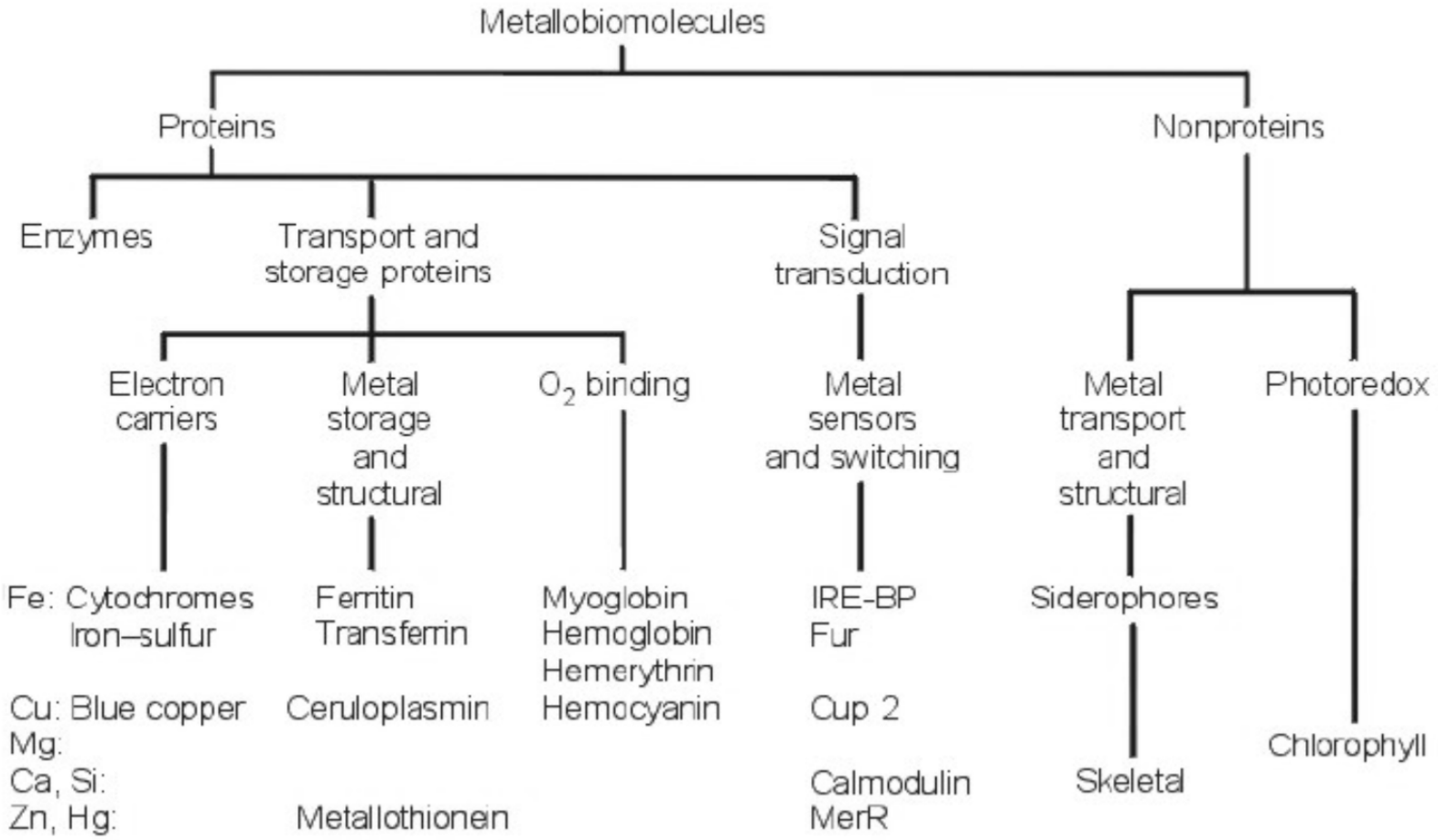
Cu(*X*-sal)$_2$en
X = *O* or *S*

[a] Potential at which the complex is half-oxidized and half-reduced.

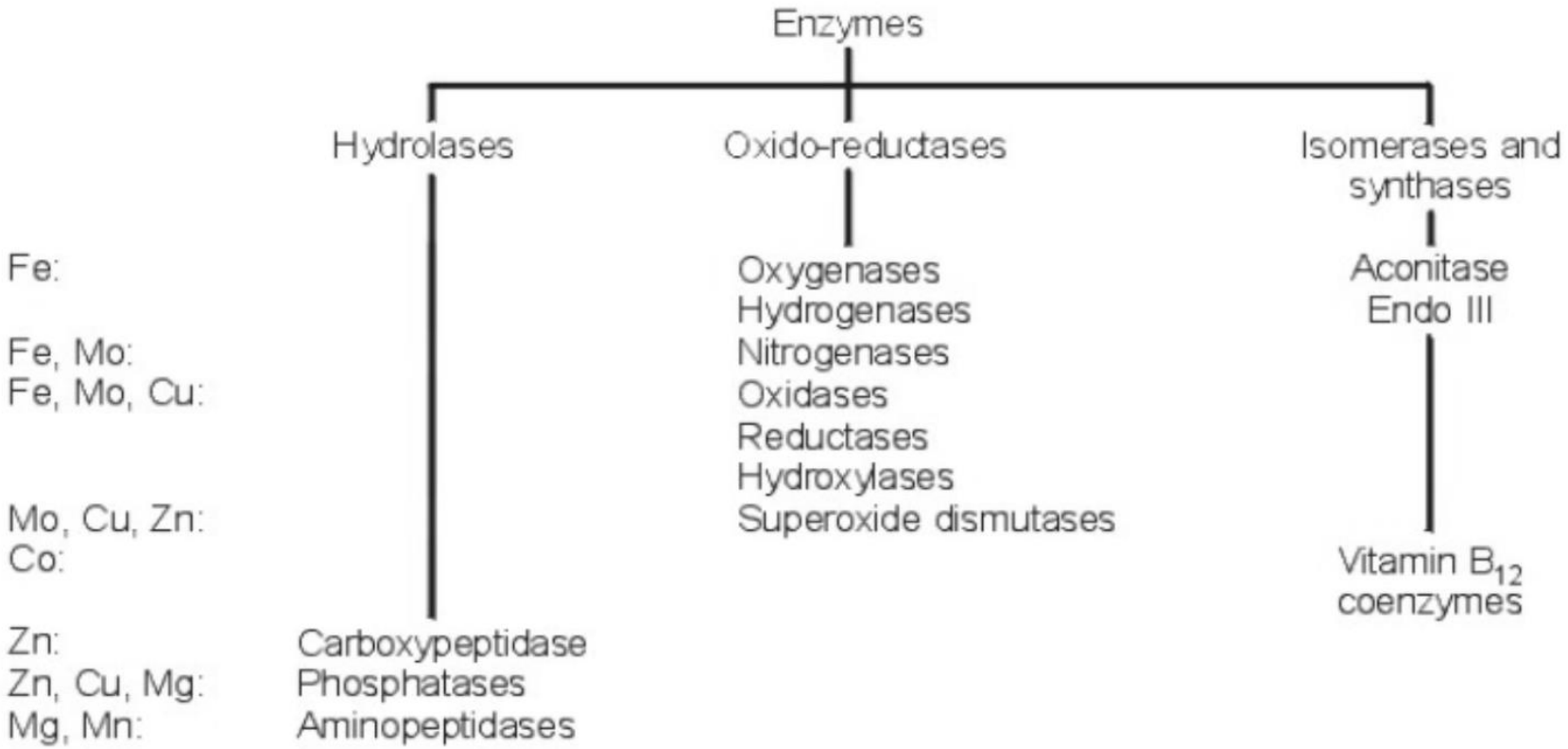
Cu(I)
d^{10}
Prefers Td

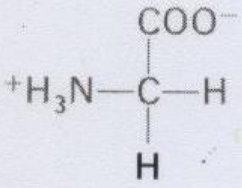
Cu(II)
d^9
Prefers Sqplanar

Categories of metallobiomolecules

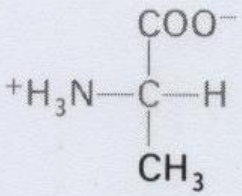


Metalloenzymes

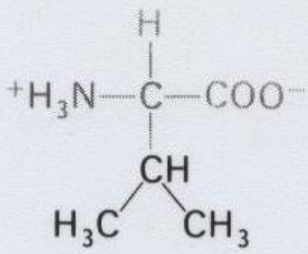




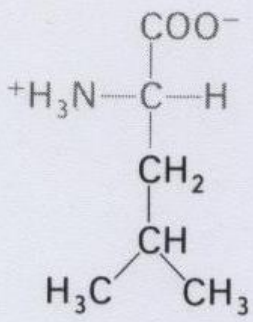
Glycine
(Gly, G)



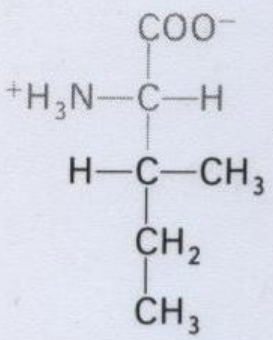
Alanine
(Ala, A)



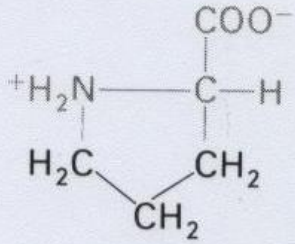
Valine
(Val, V)



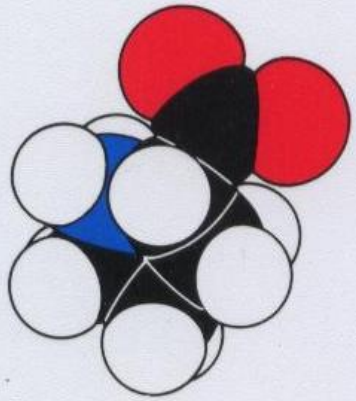
Leucine
(Leu, L)

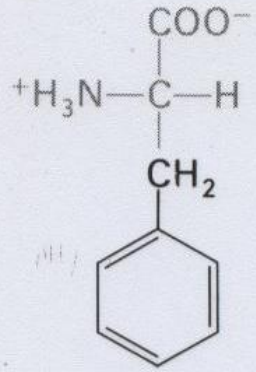


Isoleucine
(Ile, I)

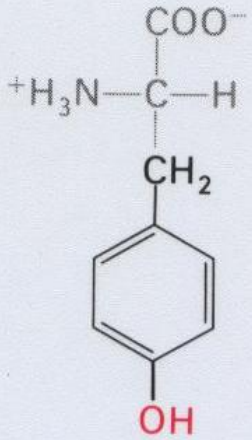


Proline
(Pro, P)

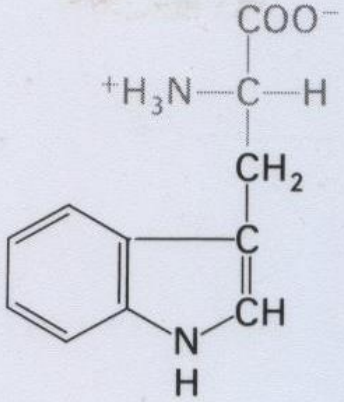




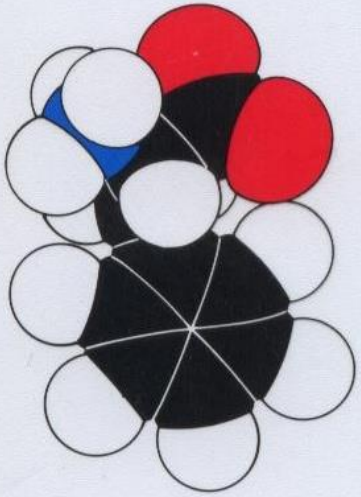
Phenylalanine
(Phe, F)



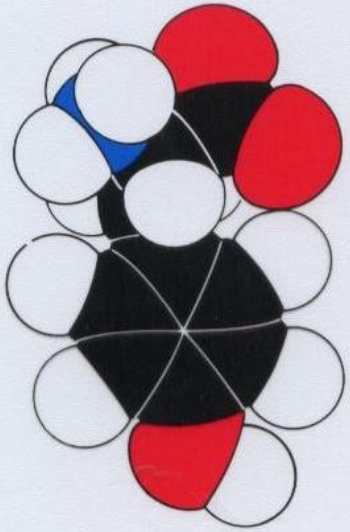
Tyrosine
(Tyr, Y)



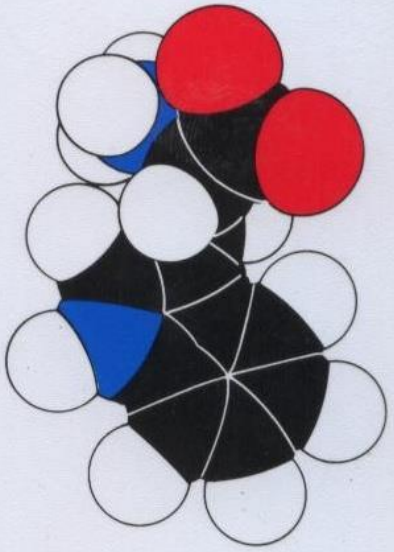
Tryptophan
(Trp, W)



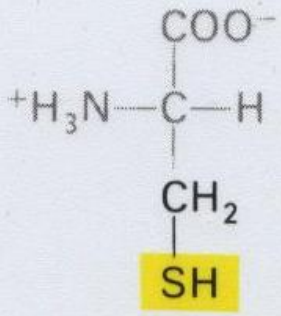
Phe



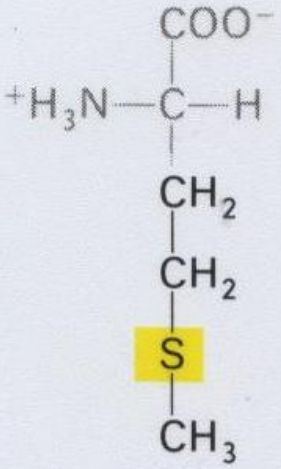
Tyr



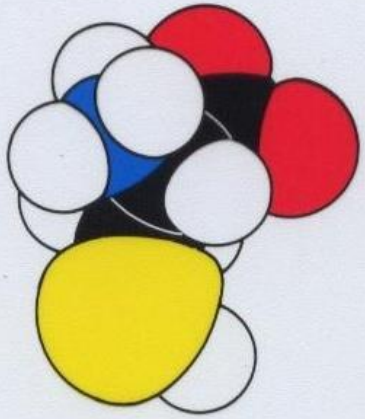
Trp



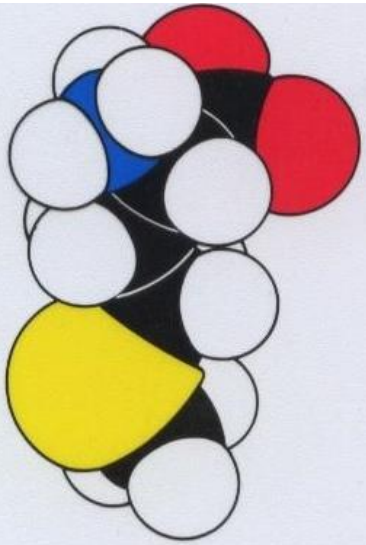
Cysteine
(Cys, C)



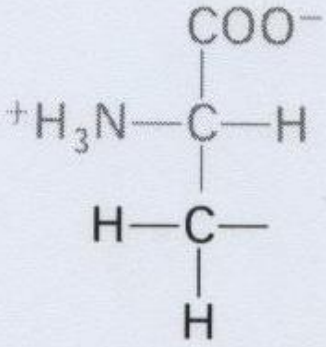
Methionine
(Met, M)



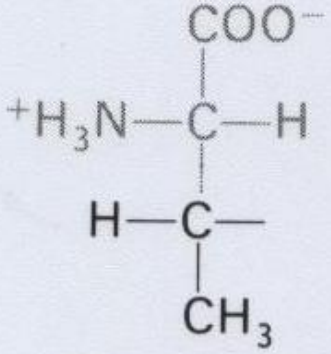
Cys



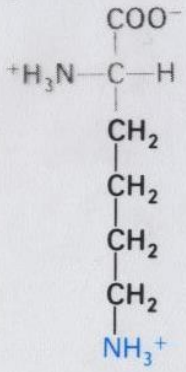
Met



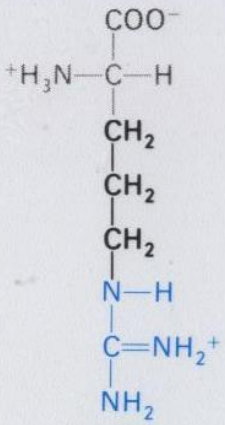
Serine
(Ser, S)



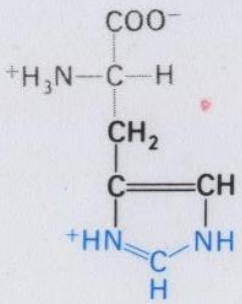
Threonine
(Thr, T)



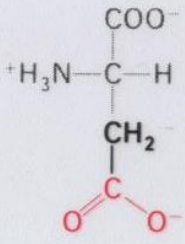
Lysine
(Lys, K)



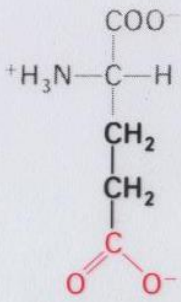
Arginine
(Arg, R)



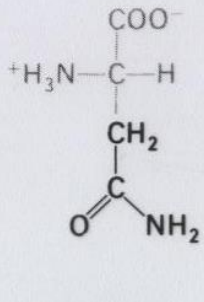
Histidine
(His, H)



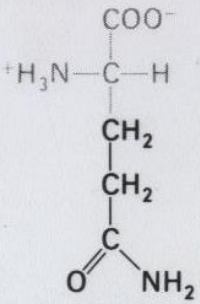
Aspartate
(Asp, D)



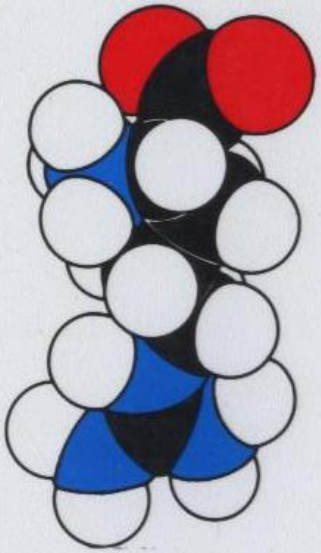
Glutamate
(Glu, E)



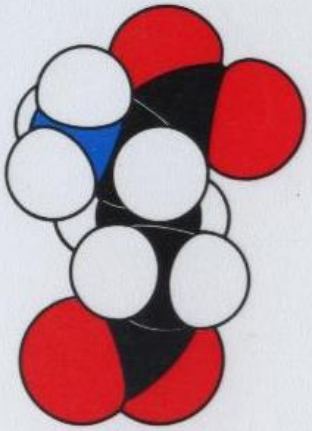
Asparagine
(Asn, N)



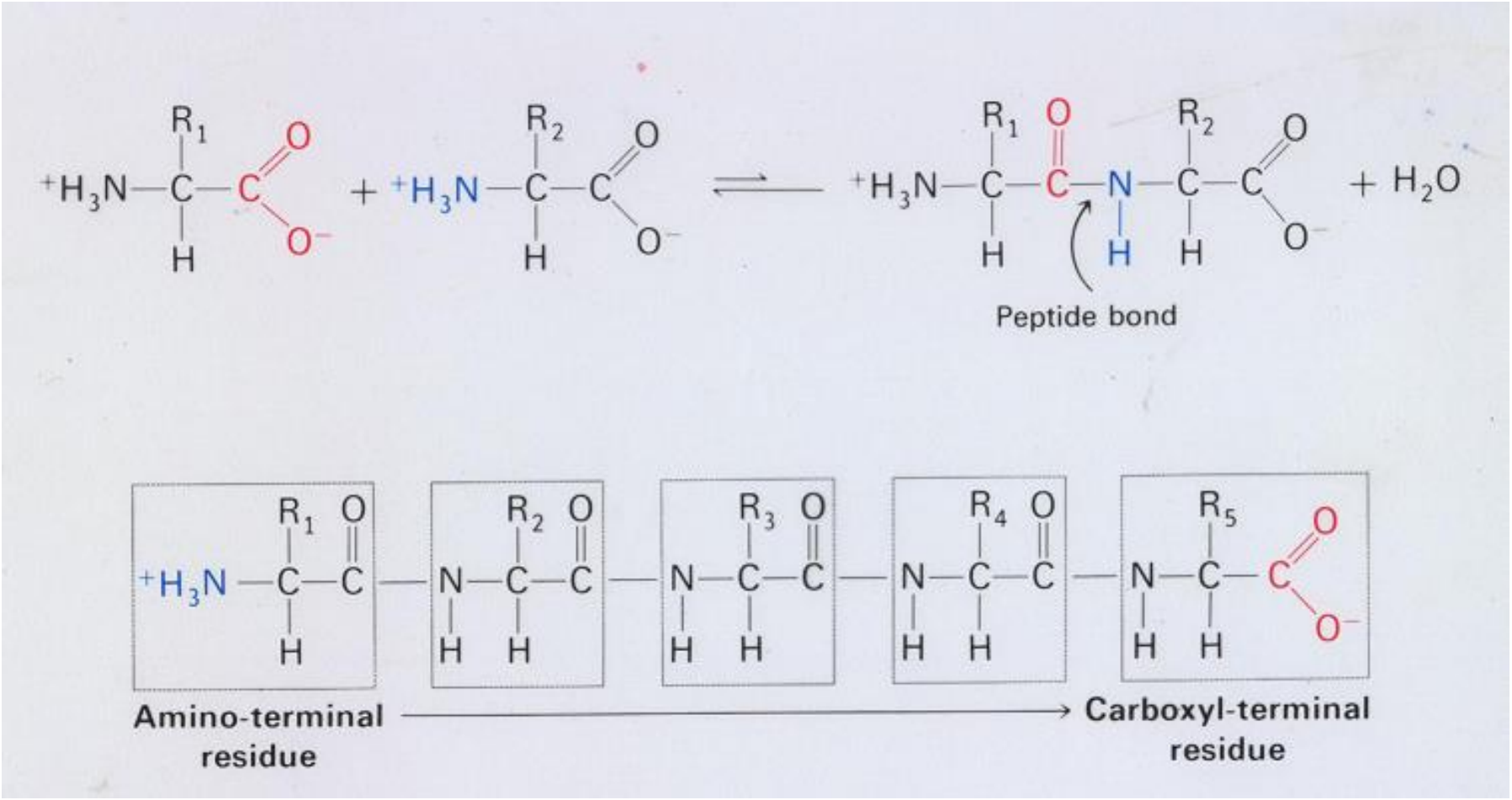
Glutamine
(Gln, Q)



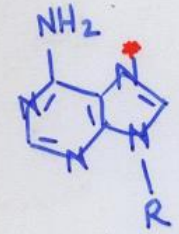
Arg



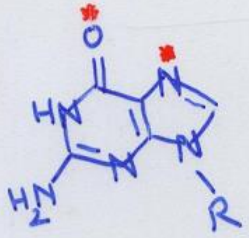
Glu


Peptide bond
Amino-terminal residue
Carboxyl-terminal residue

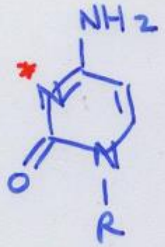
Nucleobases, Nucleotides and Nucleic acids



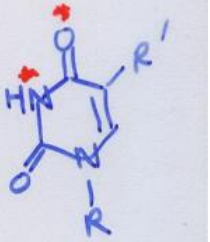
adenine



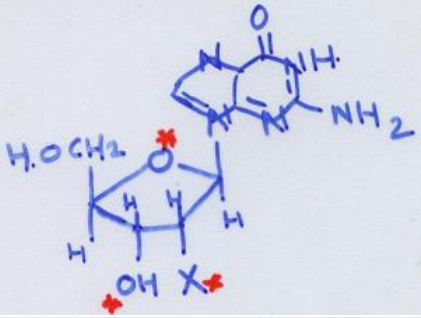
guanine



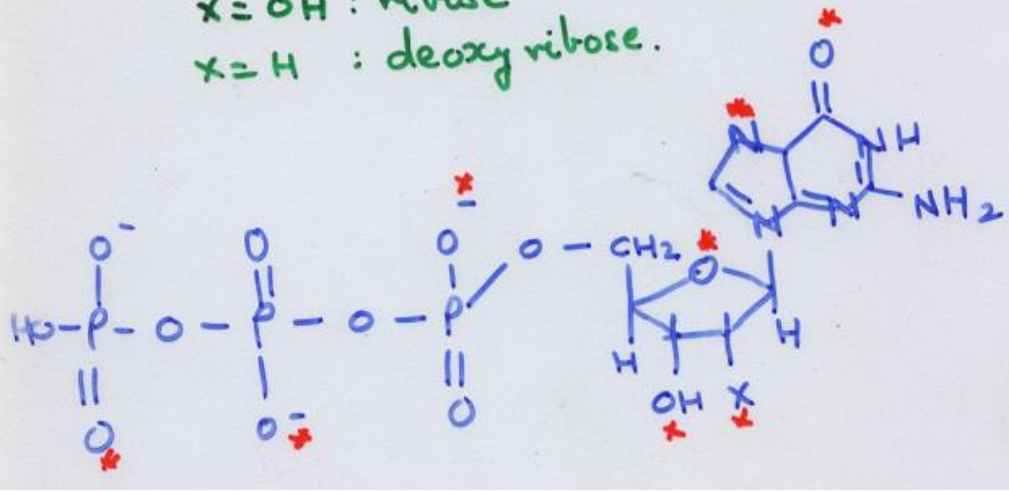
cytosine



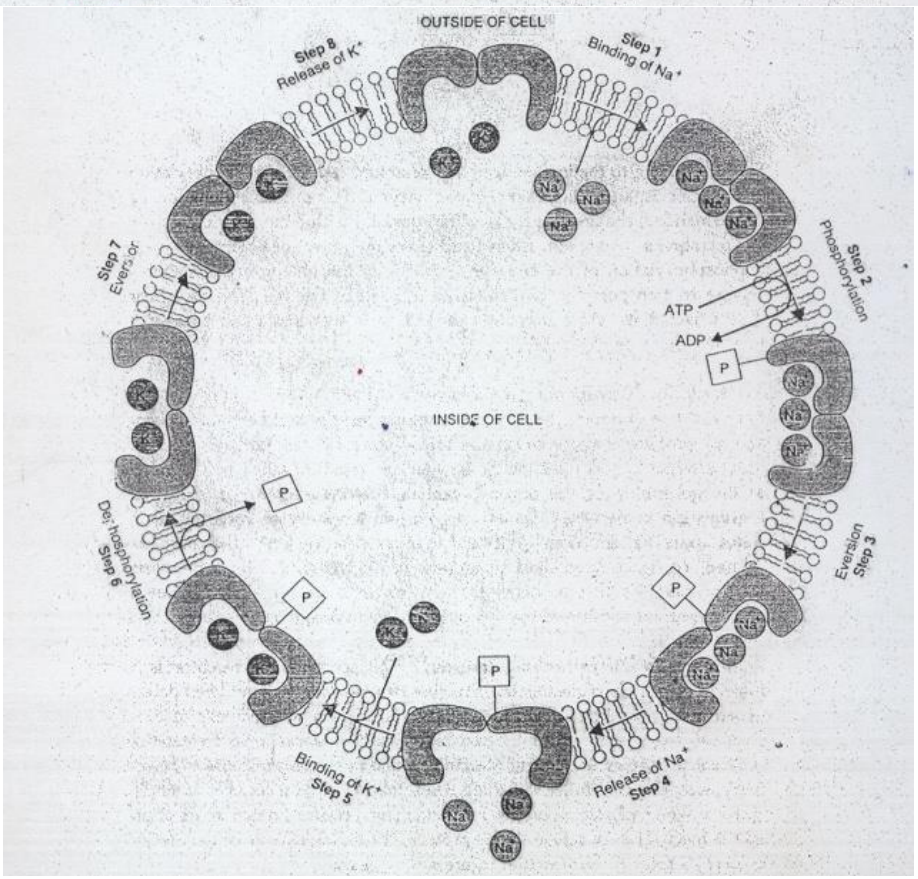
R′ = H uracil
R′ = CH_3 thym



x = OH : ribose
x = H : deoxyribose.



Ion	Extracellular conc.	Intracellular Conc.	Ratio $[ion]_o/[ion]_i$	E (mV)
Na^+	145	12	12	+68
K^+	4	155	0·026	-99
Ca^{2+}	1·5	$<10^{-7}$ M	715,000	7+128
Cl^-	123	4·2	30	-90



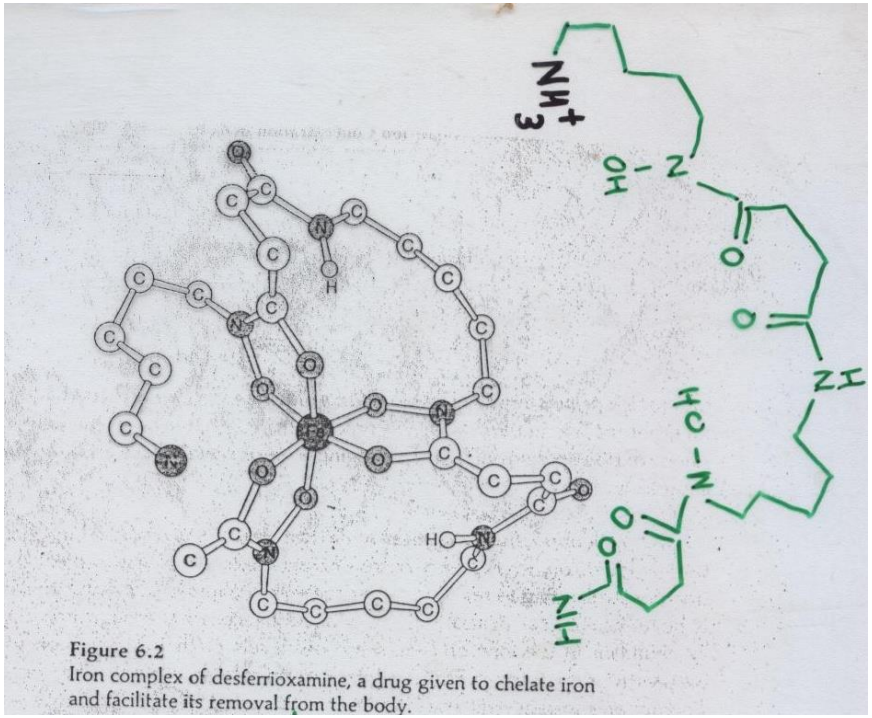


Figure 6.2
Iron complex of desferrioxamine, a drug given to chelate iron and facilitate its removal from the body.

Transport of iron. transferrin.
Synergistic binding of CO_3^{2-}
Oxygen rich environment favorable for Fe (III) hard.

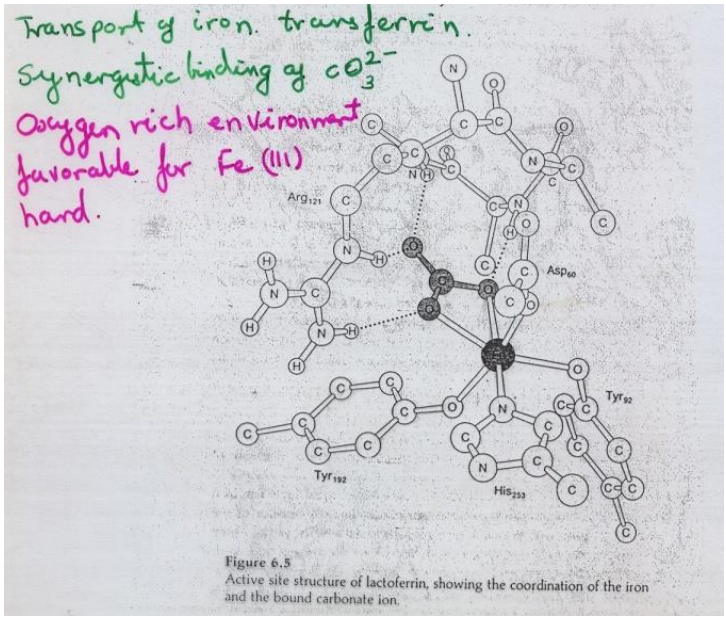
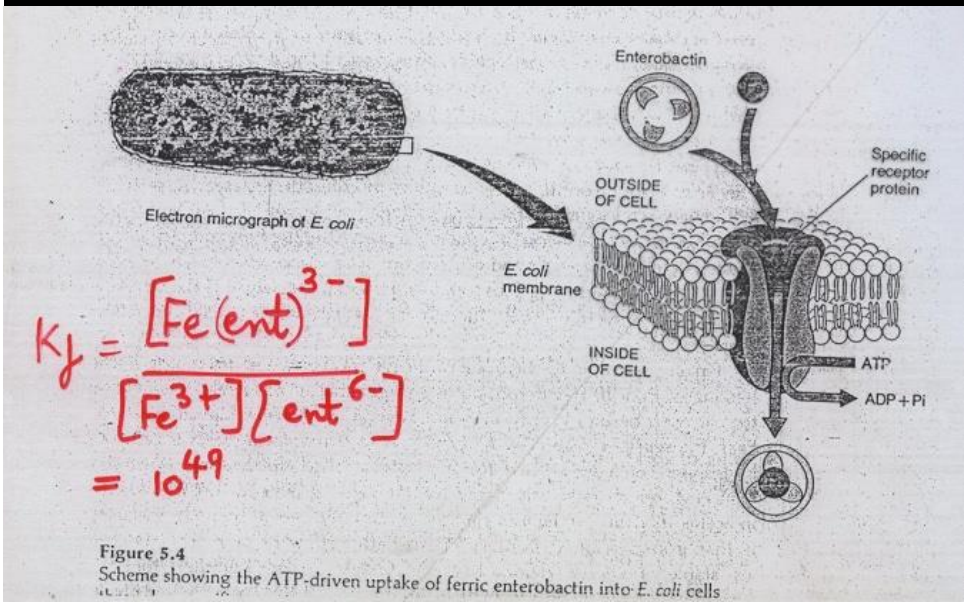


Figure 6.5
Active site structure of lactoferrin, showing the coordination of the iron and the bound carbonate ion.

Bioavailability of Metal Ions



$$K_f = \frac{[Fe(ent)^{3-}]}{[Fe^{3+}][ent^{6-}]} = 10^{49}$$

Figure 5.4
Scheme showing the ATP-driven uptake of ferric enterobactin into *E. coli* cells

Model Compounds- Biomimetic Chemistry

- Large size of metallobiomolecules and high resolution structure of metal coordination difficult

❖ If X-ray crystal structure is known it is possible to design a replica of the coordination environment.----------replicative models

❖ If X-ray crystal structure is not known we test postulated structure by spectroscopy by synthesizing models----- speculative models

✓ If models are only structurally similar--------- structural models

✓ If models are functionally similar ----------functional models

- **Biomimetic approach has helped in the study of**

1. Assignment or verification of the metal oxidation states
2. effects of distance and medium on electron transfer rates
3. role of steric and electronic factors
4. Identity of likely intermediates of enzyme catalyzed reactions

- **Strategy for models complexes- spontaneous self assembly**

- **Nature adopted the a similar strategy based on available chemistry in the geosphere during evolution .**

Biomimetic chemistry

Copper proteins by function

[1] Catalysis

Oxidoreductases
Amine oxidase
Ammonia monooxygenase
Ascorbate oxidase
Ceruloplasmin
Cu,Znsuperoxide dismutase
Cytochrome c oxidase
Diamine oxidase
Dopamine ßhydroxylase
Galactose oxidase
Laccase
Lysyl oxidase
Methane monooxygenase
N_2O reductase
Nitrite reductase
Peptidylglycinehydroxylating monooxygenase
Phenylalanine hydroxylase
Tyrosinase
Ubiquinone oxidase

[2] Electron transfer

Auracyanin
Azurin
Phytocyanin family
Plastocyanin family
Rusticyanin

Copper –An alternative to biological iron

Function	Fe protein	Cu protein
O_2 transport	Hemoglobin (h) Hemerythrin (nh)	Hemocyanin
oxygenation	Cytochrome P-450(h) Methane monooxygenase(nh) Catechol dioxygenase nh)	tyrosinase
oxidase	Peroxidases(h)	Amine oxidases laccase

Electron transfer	Cytochromes (h)	Blue copper proteins
Antioxidative	Peroxidases (h) Bacterial SOD (nh)	SOD
NO2- reduction	Nitrite reductase(h)	Nitrite reductase

Importance of Copper in Biological Systems

Copper in life systems

- Third most abundant trace metal in Human
- 80-120 mg in Normal Human Body

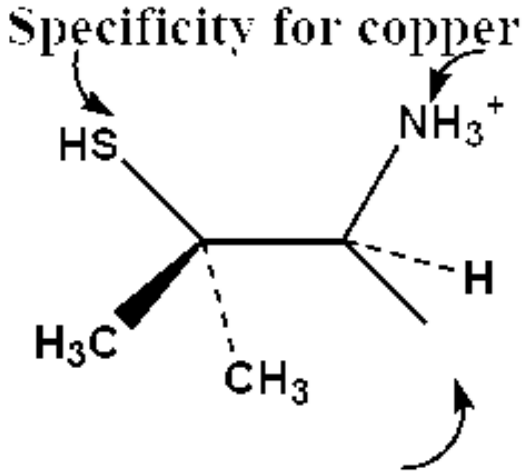
Copper deficiency

- Menkes Disease (Brain), kinky hair, connective tissue formation
- Life expectancy less than 3 years, new borns, insufficient oxygen utilization in brain
- Low activity of Cu enzymes
- Disfunction of intracellular Cu-transport/storage cerulosplasmin and metallothionein

Copper Excess

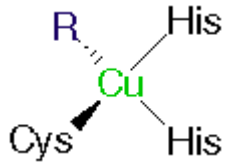
Wilsons disease (Brain, Liver, Eyes)

- Hereditary disfunction of cerulosplasmin
- D-penicillamine
  Cu-specific chelating ligand



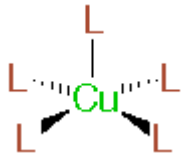
Superfluous copper

Copper Proteins/Enzymes



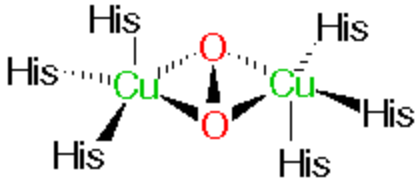
$Cu(N^{\delta}_{His})_2S^{\gamma}_{Cys}R$

R = S Met (azurin, plastocyanin, accase)
R = O Glu (phytocyanins)
R = H2O (ceruloplasmin)



$Cu(N^{\varepsilon}_{His})_mR_n$

L = N, O or S ligands; R = O or S ligands
m = 1 to 4; *n* = 0 to 3; *m*+*n* = 4 or 5



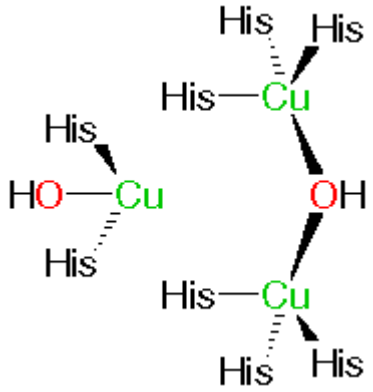
$\mu O_2[Cu(N^{\varepsilon}_{His})_3]_2$

Type I (blue copper proteins)

- Small blue proteins
  - Auracyanin
  - Azurin
  - Phytocyanin family
  - Plastocyanin family
  - Rusticyanin
- Blue oxidases
  - Ascorbate oxidase
  - Ceruloplasmin
  - Laccase
- Nitrite reductase

Type II

- Cu,Znsuperoxide dismutase
- Dioxygenases
- Monooxygenases
  - Dopamine ßhydroxylase
  - Methane monooxygenase
  - Peptidylglycine hydroxylating monooxygenase
  - Phenylalanine hydroxylase
- Nitrite reductase
- Nonblue oxidases
  - Amine oxidase
  - Diamine oxidase
  - Galactose oxidase
  - Lysyl oxidase



$Cu(N^{\varepsilon}_{His})_2OH{\cdot}\mu OH[Cu(N^{\varepsilon}_{His})_3]_2$

Copper Proteins: Important Functions

- Electron transfer
- Dioxygen binding and transport
- Metal ion storage and transport

Electron Transfer Proteins

$$Cu^{II} + e^{-} \rightleftharpoons Cu^{I}$$

Blue Copper Proteins

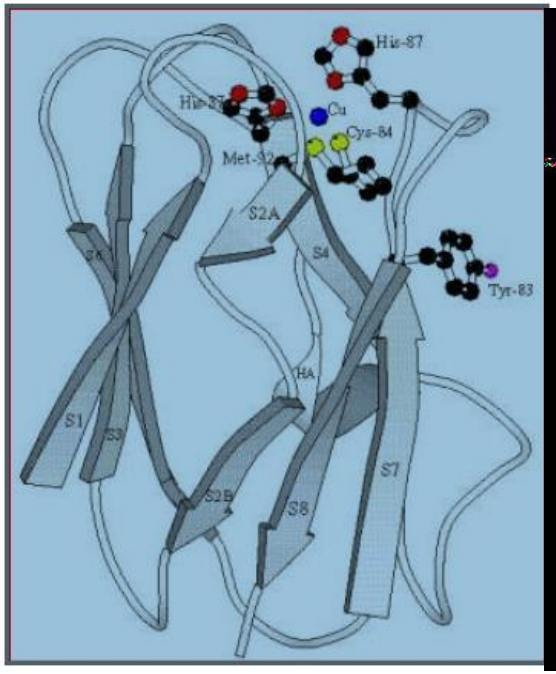
- Function: Electron-Transfer in Photosynthesis
- Novel Coordination Geometry
- Cu^{II}-SR Bond Stable
- Abnormal Spectral Properties:

  Intense Visible Absorption 600 nm (ε, 3000 M^{-1})

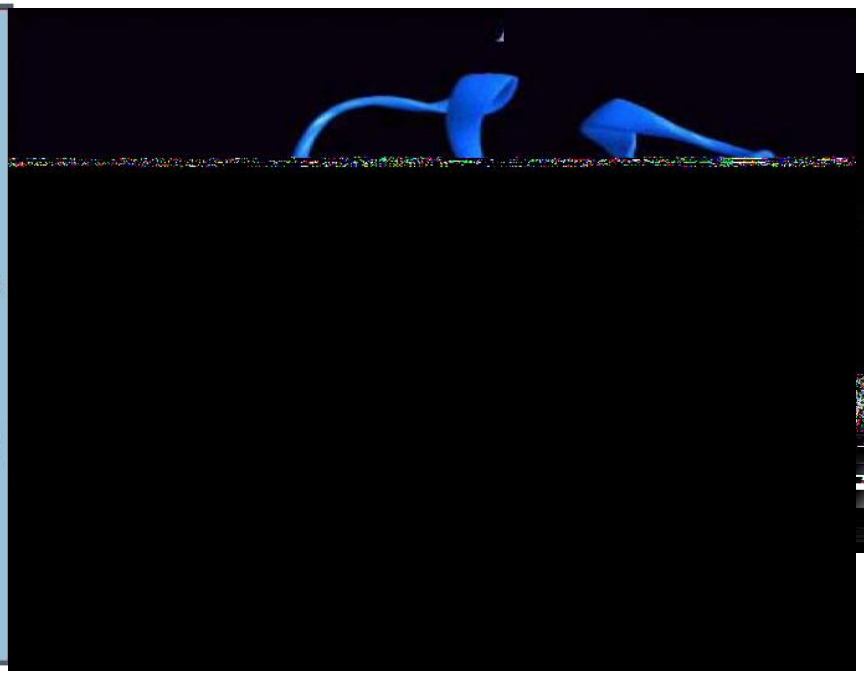
  $A_{||}$ (30 - 70 G)
- Rather high Cu^{II}/Cu^{I} Redox Potential

  $\Delta G = -nFE = -ve$

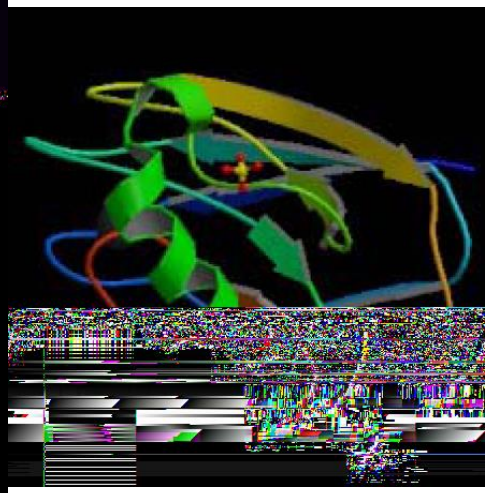
Plastocyanin



Azurin



Auracyanin



Hemocyanins

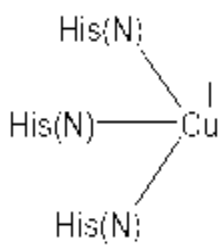
- Oxygen carriers in the hemolymph of molluscs and anthropods, **snails, quids, cuttle fish,octopus**

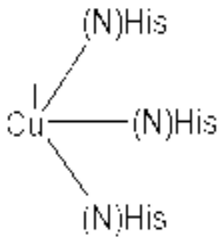
- 2 Cu(I) ions
  Each Cu(I) bound to 2/3 Imidazoles (histidine)

- 345 nm (20,000)
  570 nm (1000) O_2^{2-} —— Cu(II)

- Peroxide —— 750 cm^{-1} band

Deoxy-Hemocyanin diamagnetic/colourless





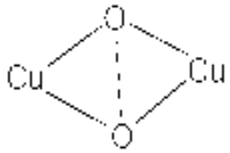
Cu—Cu

3.45 A^0

Most stable in trigonal geometry;
Enormous strain at O_2 binding site

Oxy-Hemocyanin : Cu(II) 4-coordinated (normal)
transformation is facile

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



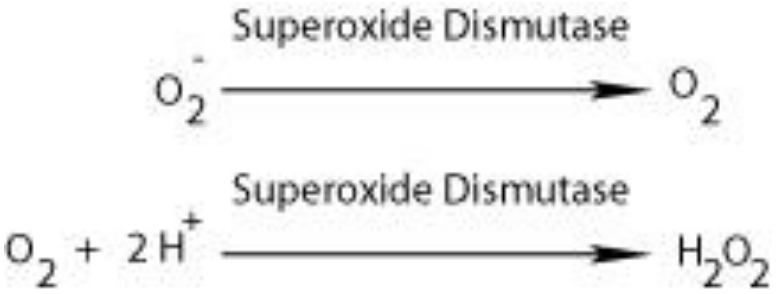
$\mu\text{—}\eta^2 : \eta^2\text{—}O_2^{-}$

Cu—Cu

3.6 A^0

Antiferromagnetic interaction

The enzyme **superoxide dismutase** (**SOD**, EC 1.15.1.1), catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. As such, it is an important antioxidant defense in nearly all cells exposed to oxygen. One of the exceedingly rare exceptions is *Lactobacillus plantarum* and related lactobacilli, which use a different mechanism.



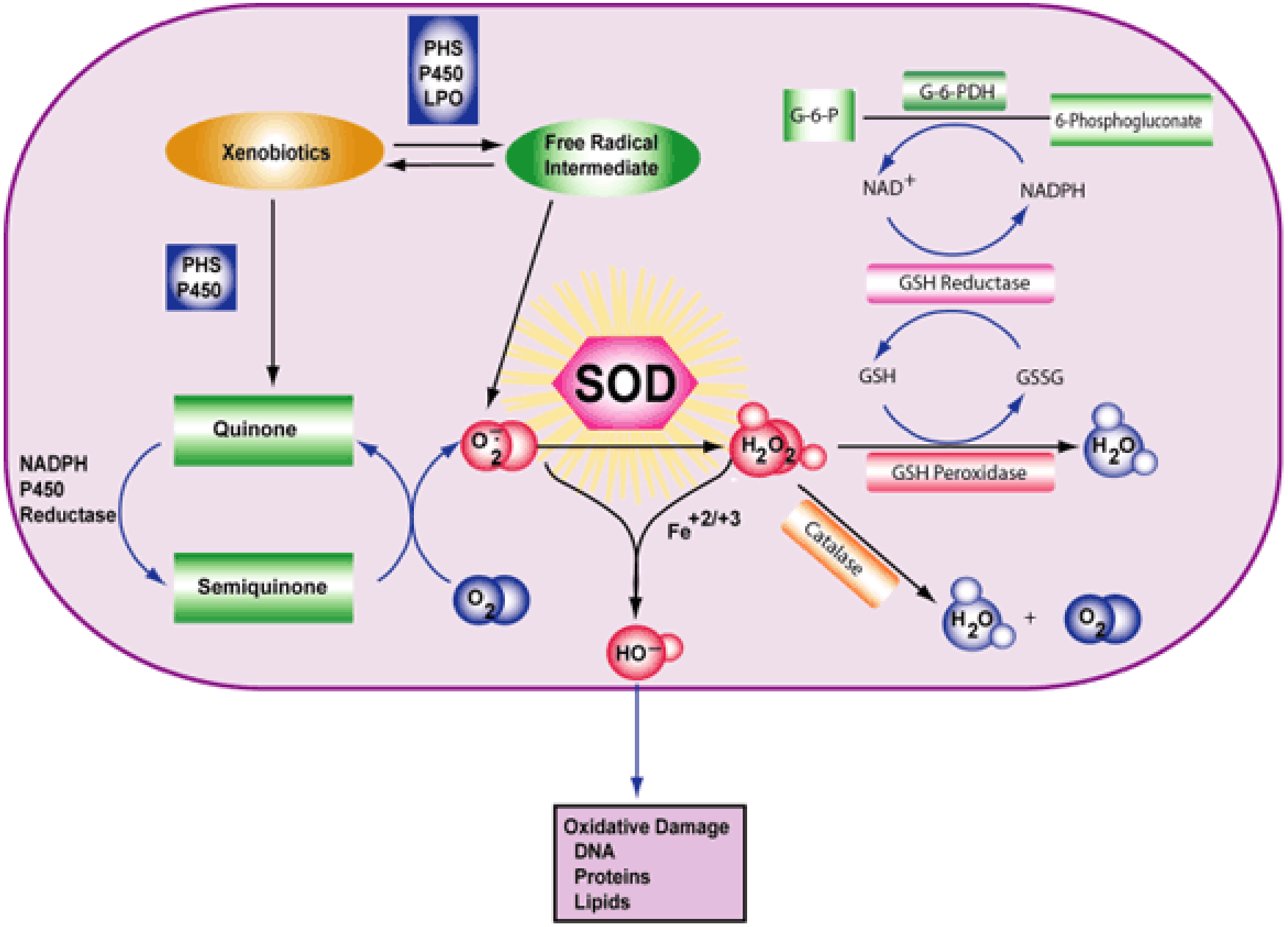
Reaction

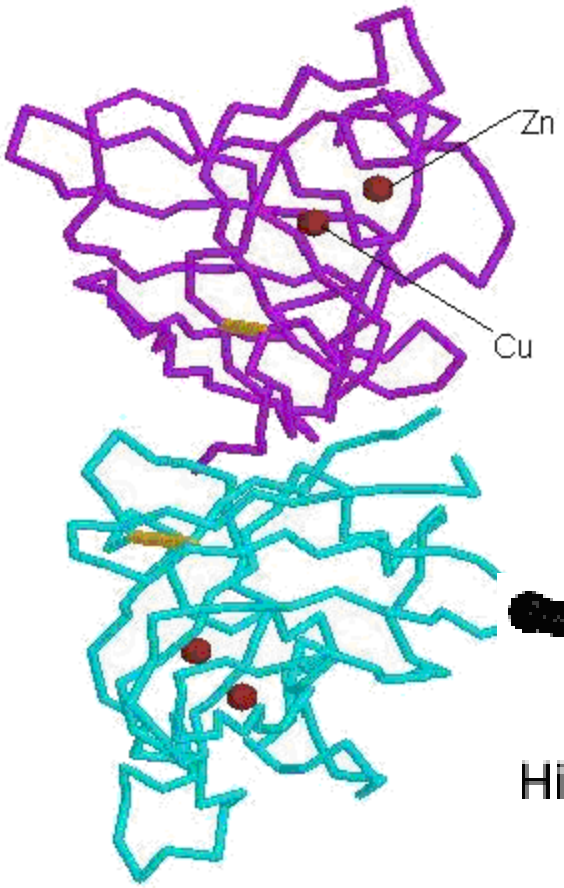
The SOD-catalysed dismutation of superoxide may be written with the following half-reactions :

$$M^{(n+1)+} - SOD + O_2^- \rightarrow Mn^+ - SOD + O_2$$
$$M^{n+} - SOD + O_2^- + 2H^+ \rightarrow M^{(n+1)+} - SOD + H_2O_2.$$

where M = Cu (n=1) ; Mn (n=2) ; Fe (n=2) ; Ni (n=2).

In this reaction the oxidation state of the metal cation oscillates between n and n+1.


PHS
P450
LPO
Xenobiotics
Free Radical
Intermediate
PHS
P450
G-6-P
G-6-PDH
6-Phosphogluconate
NAD+
NADPH
GSH Reductase
GSH
GSSG
SOD
Quinone
NADPH
P450
Reductase
Semiquinone
O2
H2O2
H2O
GSH Peroxidase
Fe+2/+3
Catalase
HO−
H2O
+
O2
Oxidative Damage
DNA
Proteins
Lipids

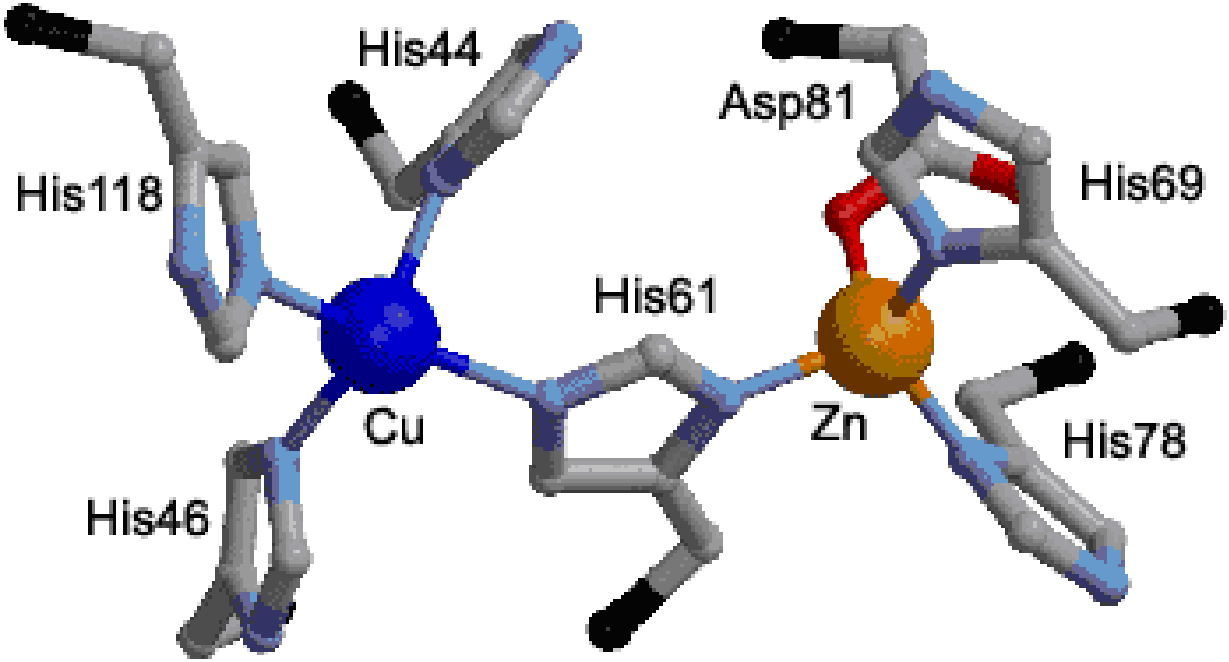


$Cu^{2+} + O_2^{-\cdot} \rightarrow Cu^{+} + O_2$

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

Net Reaction: $2\ O_2^{-\cdot} + 2H^{+} \rightarrow H_2O_2 + O_2$

K=(~$10^5\ M^{-1}\ s^{-1}$ at pH 7)



Manganese in Biology

Manganese(II) ions function as cofactors for a number of enzymes and the element is thus a required trace mineral for all known living organisms.

Biological role:

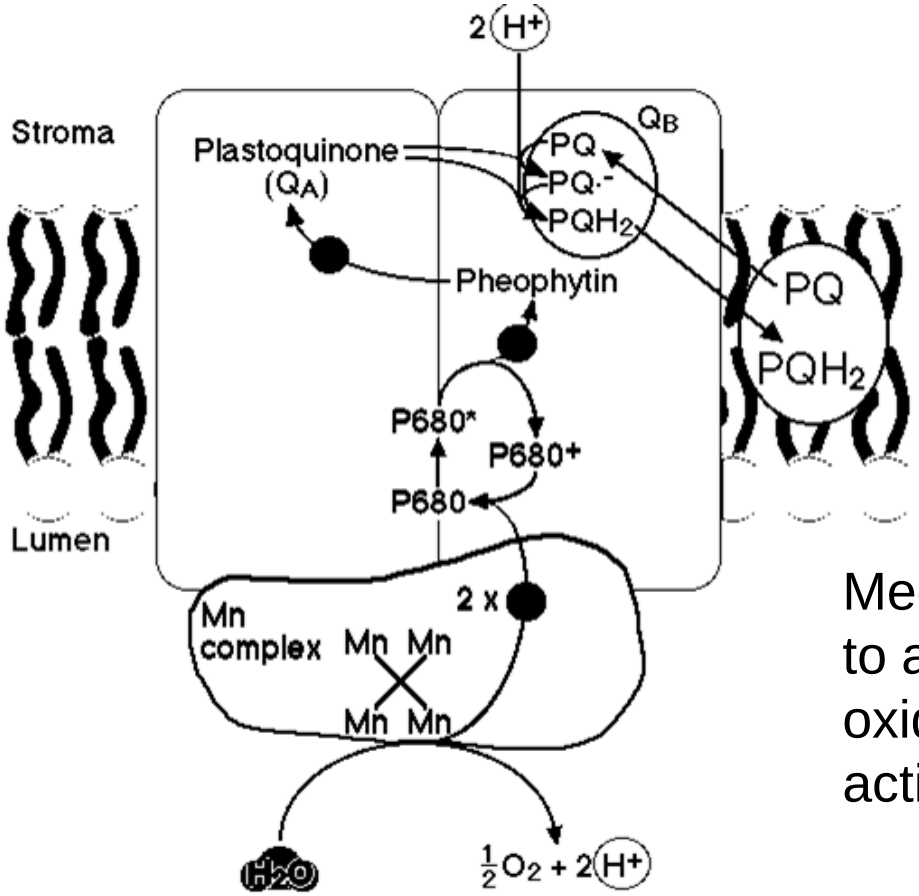
- The classes of enzymes that have manganese cofactors are very broad and include such classes as oxidoreductases, transferase, hydrolases, lyases, isomerases, ligases, lectins and integrins.

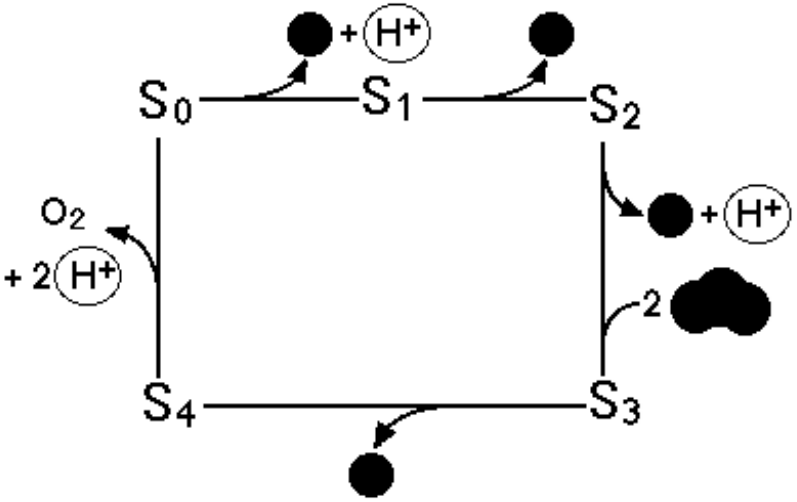
- Mn-SOD is the type of SOD present in eukaryotic mitochondria, and also in most bacteria. The Mn-SOD enzyme is probably one of the most ancient, for nearly all organisms living in the presence of oxygen use it to deal with the toxic effects of superoxide, formed from the 1-electron reduction of dioxygen.

- Manganese is also important in photosynthetic oxygen evolution in Chloroplasts in plants, which are also evolutionarily of bacterial origin.

- The Oxygen evolving complex (OEC), a water-oxidizing enzyme contained in chloroplast membrane, and which is involved in the terminal photo oxidation of water during the light reactions of photosynthesis, has a metalloenzyme core containing four atoms of manganese**.**

Photosystem II

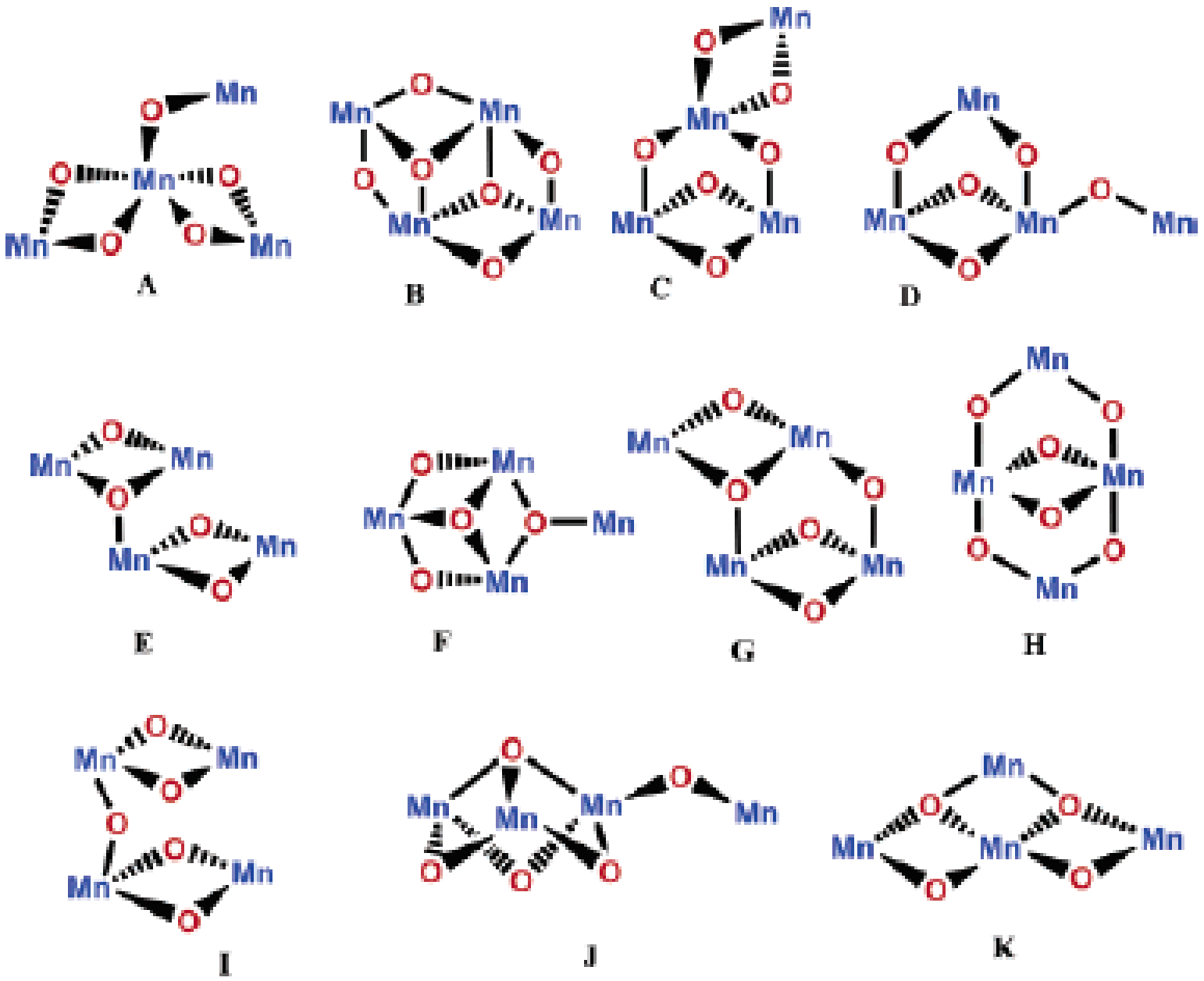




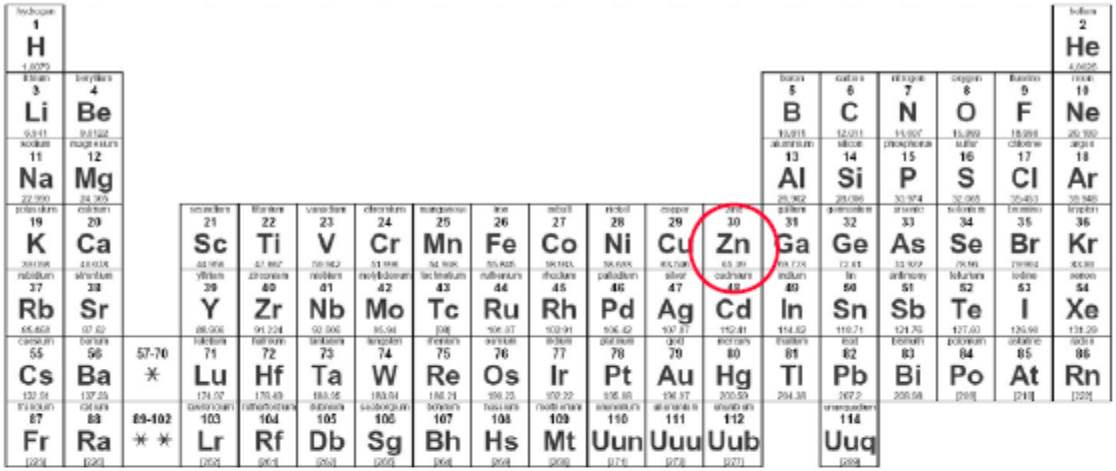
Mechanistic cycle (commonly referred to as the Kok catalytic cycle) for water oxidation in the photosystem II (PSII) active site.

$$H_2O + CO_2 \rightleftharpoons 1/n(CH_2O)_n + {}^3O_2 \qquad \Delta H = +470\ KJ/mole$$

Proposed PSII Mn_4 Structures



Zinc in Biology



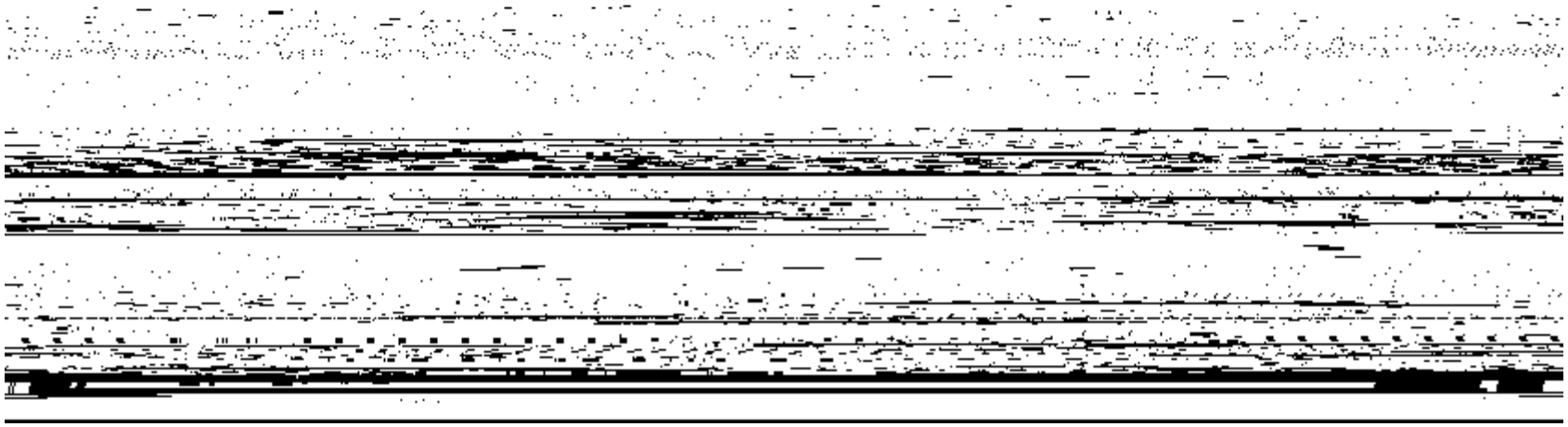
Human body: 2-3 g zinc

- **Zinc** - essential trace element; the 2nd most abundant transition metal in biology.
- Zinc exists always in its +2 oxidation state, and colorless
- More than 300 enzymes with diverse biological roles
- Cd(II) and Hg(II) can replace Zn(II) → toxic
- **Zinc enzymes** are redox inactive and the zinc ion in enzymes has several accessible geometries
- The oxygen and sulfur ligands are labile, which allows substrate binding.
- Zinc in enzymes is a good Lewis acid – **Zinc Hydrolases**

Zinc Enzymes

Zinc is the only metal to be found in all six classes of enzymes.

Main Class	Type of Reaction Catalyzed
Oxidoreductases	**Oxidation-reduction reactions** **Alcohol Dehydrogenase**
Transferases	**Transfer of an amino group between substrates** **Aspartate carbamoyltransferase**
Hydrolases	**Hydrolysis of ester, amide, phosphate groups** **Carboxypeptidase, ACE, β-Lactamases**



CLASSES OF ENZYMES

Hydrolytic enzymes

- Addition or removal of elements of water in a substrate molecule.
  - Carbonic Anhydrase

    $CO_2 + H_2O \rightarrow HCO_3^- + H^+$

  - Carboxypeptidases

    $-CO-NH- + H_2O \rightarrow -COOH + H_2N-$

  - Esterases
  - Alkaline Phosphatases
- Metal ions: Zn^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}

  Avoid undesired electron transfer

Zinc and filamentous structures

1. Collagens	Zinc collagenase
2. Proteoglycans	Zinc stromelysin
3. Denatured collagens	Zinc gelatinase
4. Keratins	Zinc cross links

Zinc enzymes in synthesis

1. RNA	RNA polymerase
2. DNA synthesis	Reverse transcriptase
3. Viral synthesis	terminal dNT transferase
4. Transfer RNA	tRNA synthetase
5. Essential amino acids	dehydroquinate synthase
6. Essential amino acids	aspartate transcarbamylase

Zinc proteins related to peptide hormonal action

1. Insulin	Zinc associated with hormone storage
2. Angiotensin	Zinc in angiotensin conversion enzyme
3. Enkephalin	Zinc in enzyme enkephalinase
4. Neurotensin	degradation by zinc enzyme

Zinc in Degradation

1. Pancreatic juice	Carboxypeptidase
2. extracellular digestion	Thermolysin
3. Breakdown of DNA	Nucleotidase and RNA

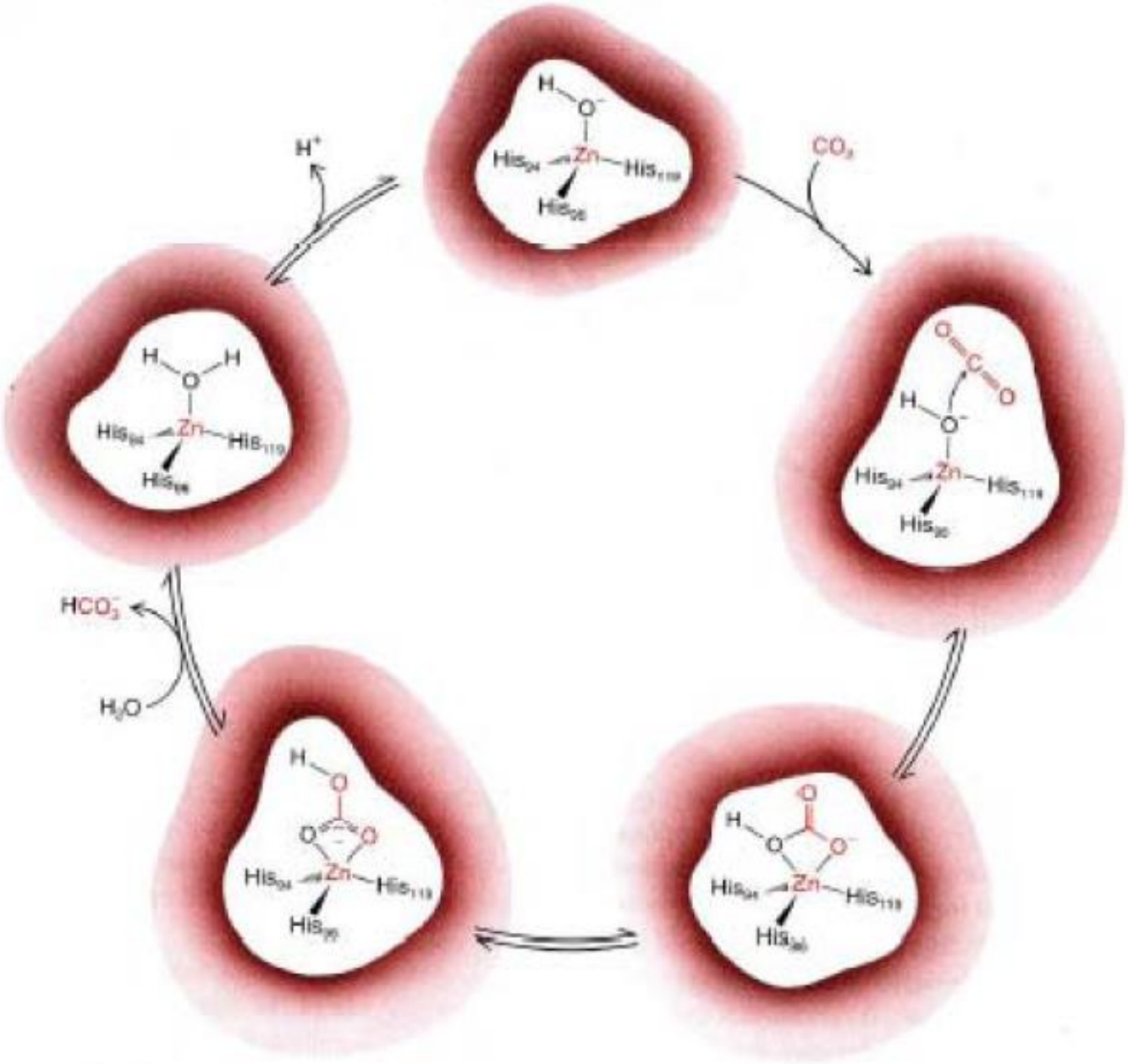
Carbonic Anhydrase

$$H_2O + CO_2 \rightleftharpoons HCO_3^- + H^+$$

- TOF = 10^6 s^{-1}. 10^8 times faster than without enzyme!
- For humans, 7 different CAs have been indentified. A high concentration is found in the

- The Zn^{2+} is coordinated to three His side chains in a 15 Å deep cavity. The water molecule is acidic with a pK of ca. 7.

Carbonic Anhydrase – The Reaction Mechanism

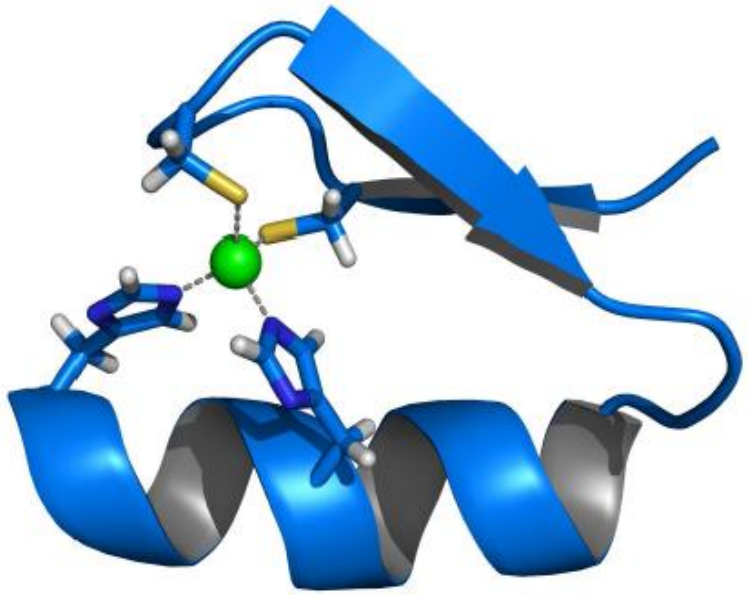


Zinc fingers

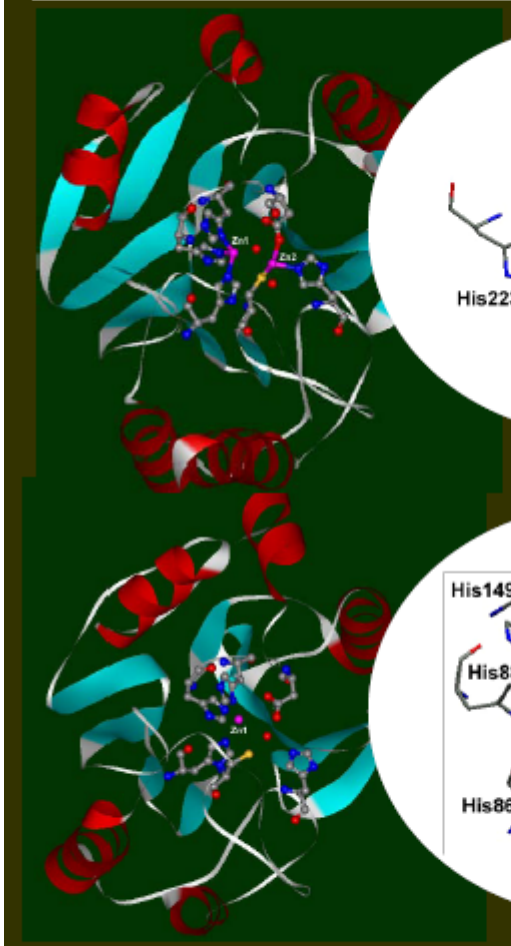
- Typical feature of transcription factor proteins
- Ligand environment: tetrahedral, 2 His and 2 Cys
- Bind to specific regions of DNA
- Binding specificity determined by geometry: large scale interaction

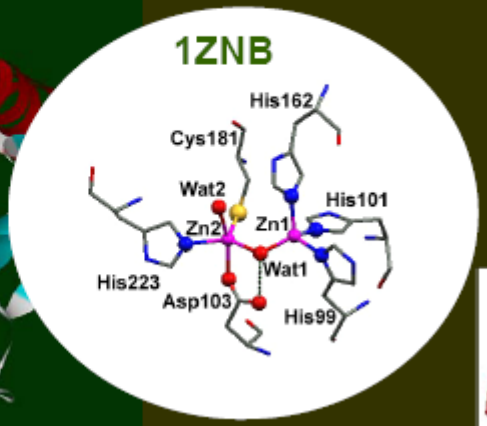
→"Zincless" finger can be made

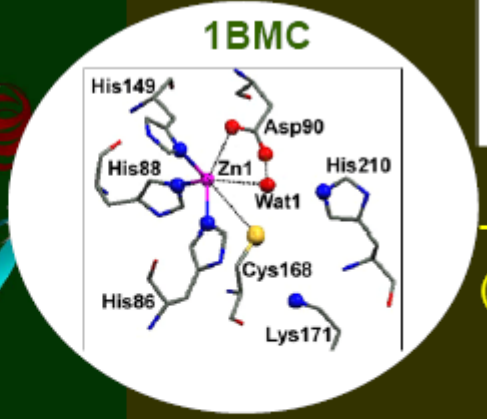
- Target of molecular genetic engineering



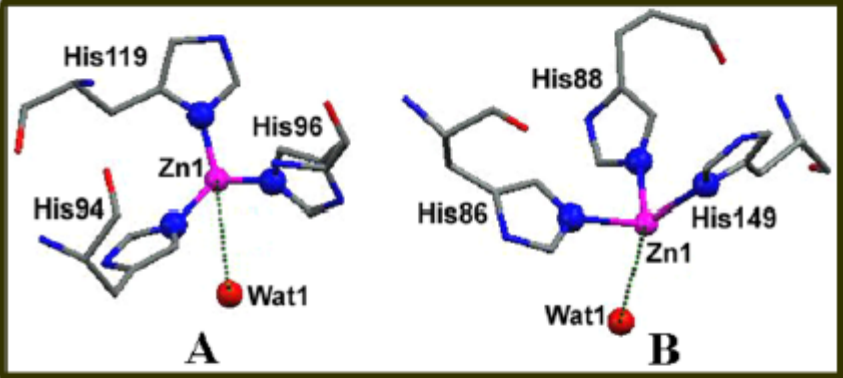
Metallo-β-Lactamases – Zinc Hydrolases







Hydrolyze a wide range of β-lactams including **cephamycins** and **imipenem** – resistant to the serine lactamases.



The active site of (A) carbonic anhydrase (1G3Z); (B) metallo-β-lactamase (1BMC).

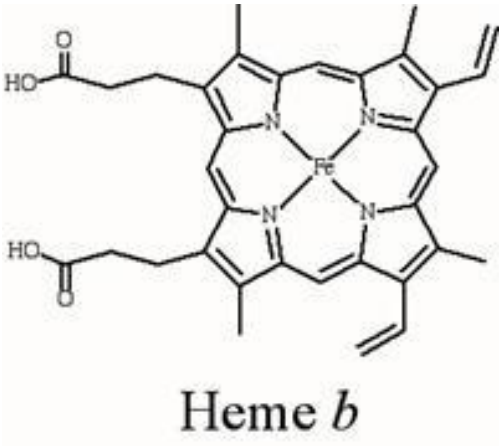
The active site of (a) binuclear zinc enzyme from *B. fragilis* (PDB code: 1ZNB) and (b) mononuclear zinc enzyme from *B. cereus* (PDB code: 1BMC).

Iron in Biology

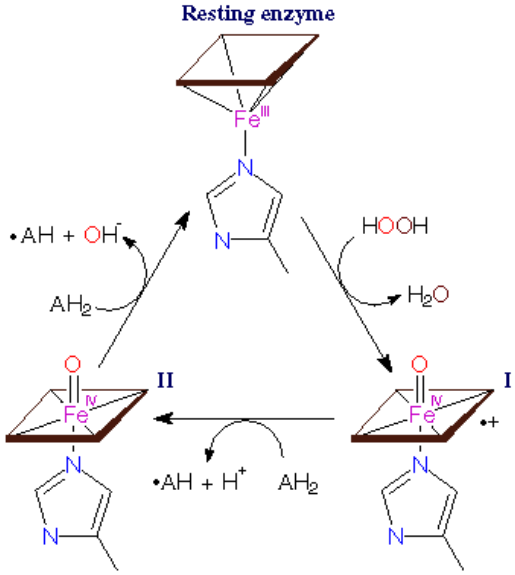
Peroxidases are haemcontaining enzymes that use hydrogen peroxide (H_2O_2) as the electron acceptor to catalyse a number of oxidative reactions.
Most haem peroxidases follow the reaction scheme-

Haem proteins by function

- Catalysis
- Electron transfer
- Oxygen transport and storage
- Nitric oxide transport



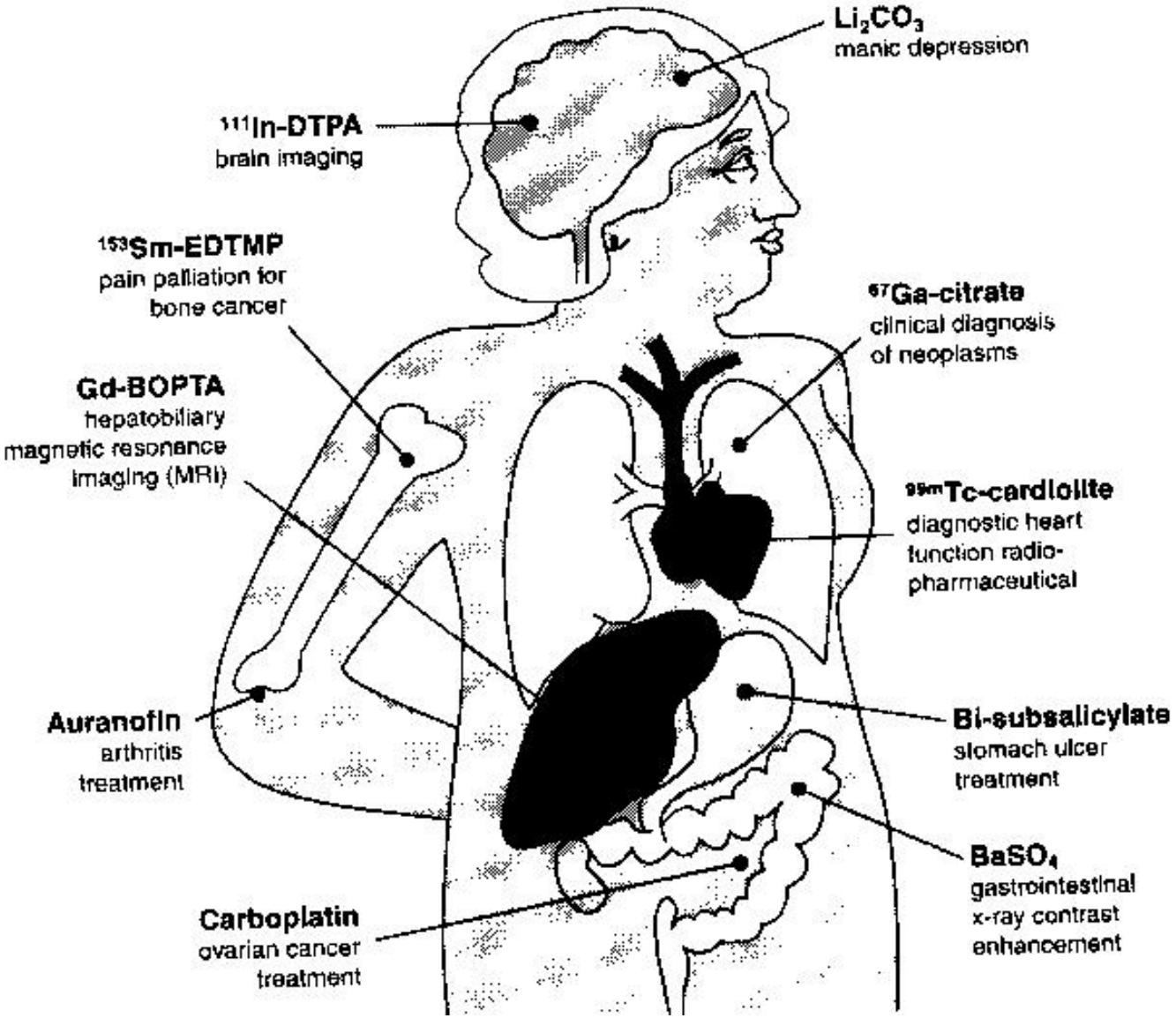
Heme *b*



- Peroxidases typically catalyze a reaction of the form:

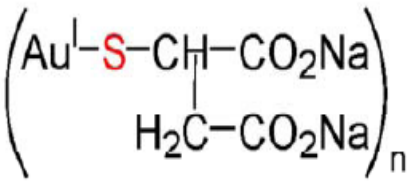
ROOR' + electron donor (2 e-) + $2H^+ \rightarrow$ ROH + R'OH

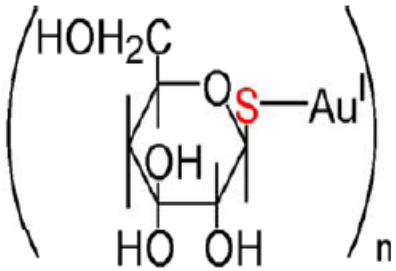
Medicinal Inorganic Chemistry



Application of Metals in Medicine

- Li^+: Treatment of depression (Li_2CO_3, low doses)
- Gd^{3+}: Contrast agent (NMR)
- $BaSO_4$: Contrast agent (radiography)
- ^{99}mTc: radio diagnostics (thyroid)
- Au(I): Rheumatism





- Sb(III): Eczema
- Bi(III): Gastric ulcer
- Cd: Carboanhydrase(Thalassiosira weissflogii)

Metal based Anti-Cancer Drugs

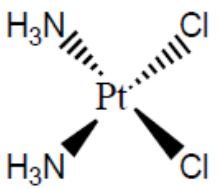
- In 1965 Rosenberg discovered antiproliferative effect of a cisplatin whilst conducting studies on bacteria under in an electric field produced by platinum complexes

- He was able to show that the compound cisplatin was responsible for the effect and this was found to be effective against treating some cancers.

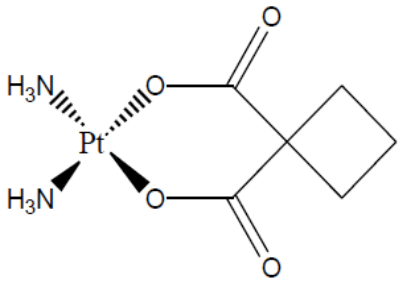
- Cisplatin is now THE MOST used anti-cancer drug
  BUT CONTAINS NO CARBON ATOMS !!

- HOW DOES *CIS-PLATIN* TARGET CANCER ?

- By reaction with DNA?

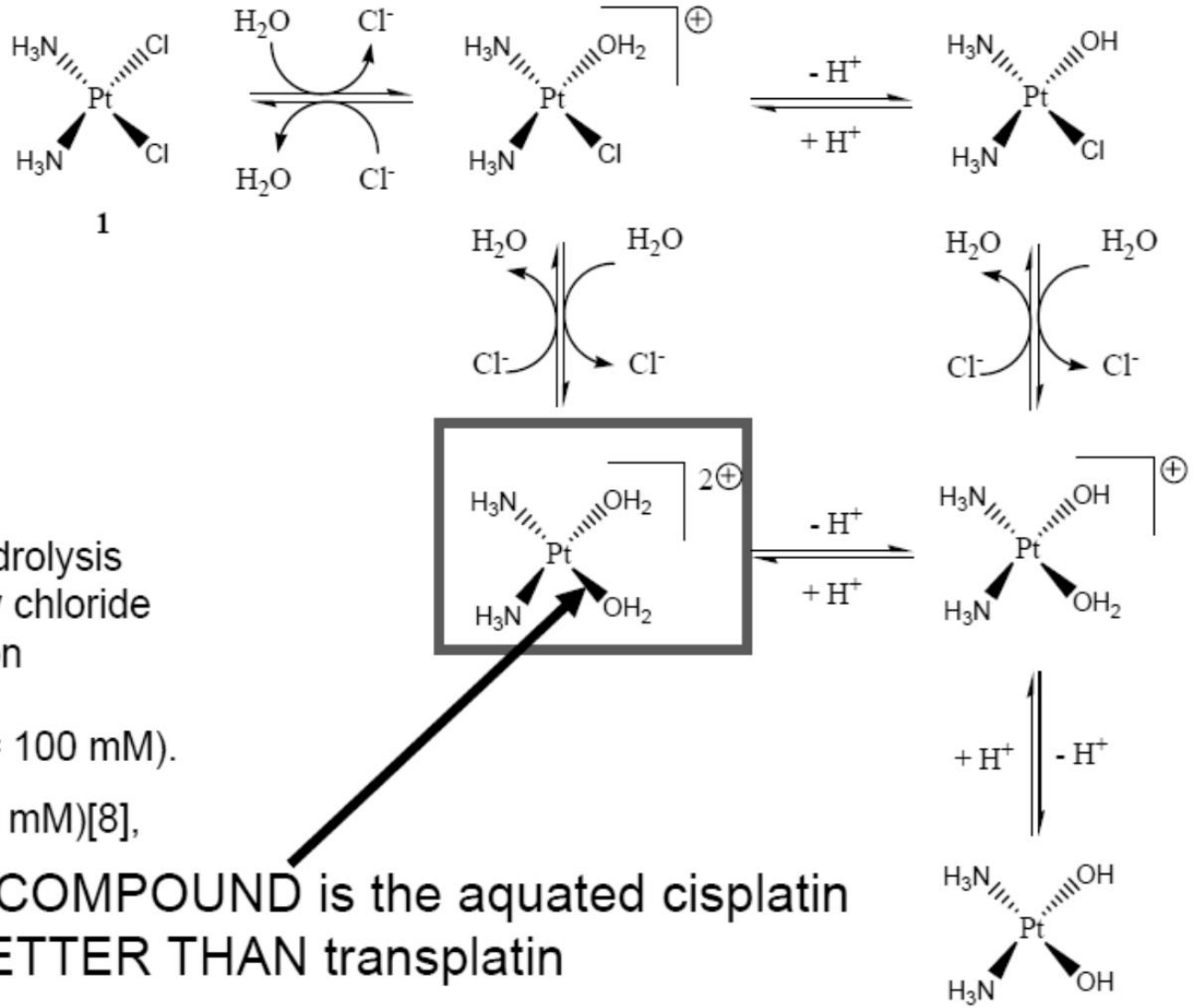


Cisplatin
(**1**)



Carboplatin
(**2**)

Reactions of *cis-platin* under physiological conditions

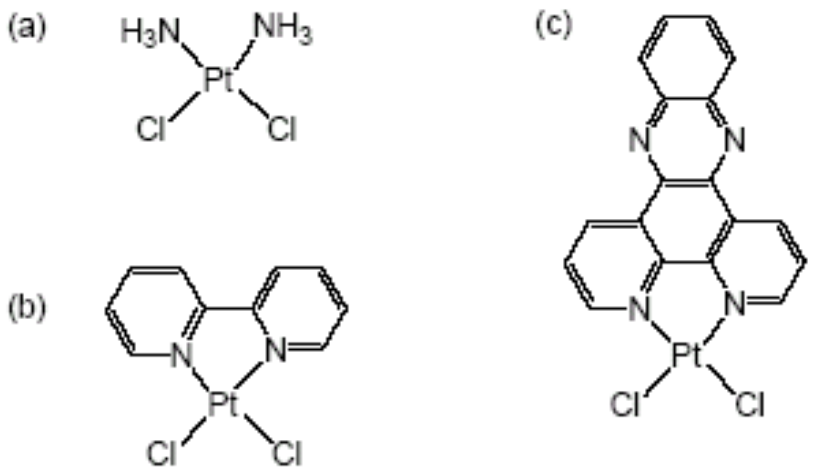


Cisplatin hydrolysis regulated by chloride concentration

([Cl-blood] = 100 mM).

([Cl-cell] = 3 mM)[8],

Reactive COMPOUND is the aquated cisplatin
MUCH BETTER THAN transplatin



. Molecular structures of (a) cisplatin, (b) $Pt(bpy)Cl_2$, and (c) $Pt(dppz)Cl_2$.

Disadvantages of *cis*-platin

- Applicable in relatively narrow range of tumors.
- Limited solubility in Aqueous solution.
- Intravenous administration, inconvenience to outpatient treatment.
- Nephrotoxicity,neurotoxicity and nausia.
- Higher toxicity leading to lower doses of 100 mg/day.

cis-platin and new drugs

- From a clinical point–of-view the current challenges in drug development include:

  (i) addressing the poor solubility of cisplatin and analogues in water

  (ii) cellular resistance of cancer cells to cisplatin

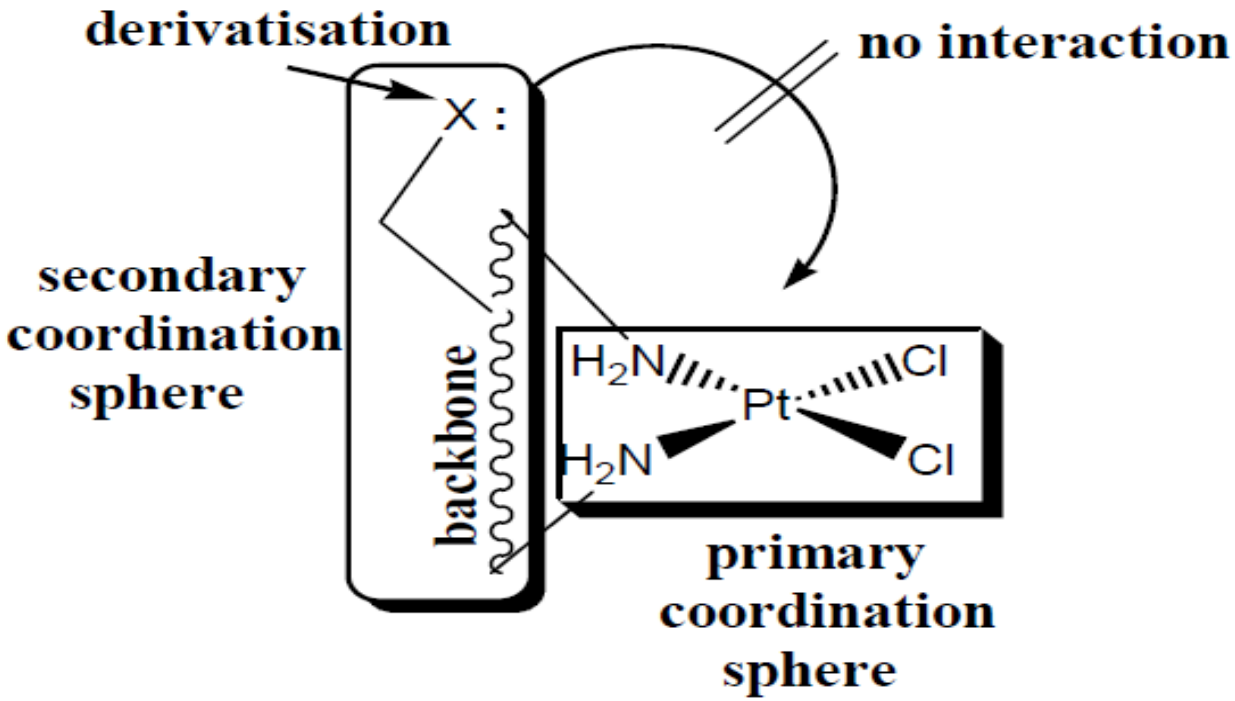
  (iii) toxic side effects of cisplatin

  (e.g. nausea, neurotoxicity, kidney damage)

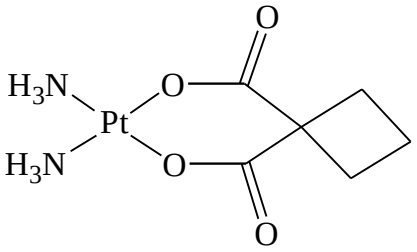
  (iv) use of platinum-based therapeutics to treat cisplatin resistant cell lines

The need for new *cis-platin* drugs

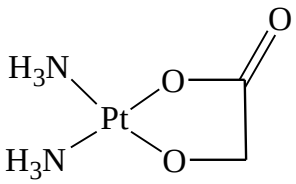
- For understanding
- To address toxicity and cellular resistance



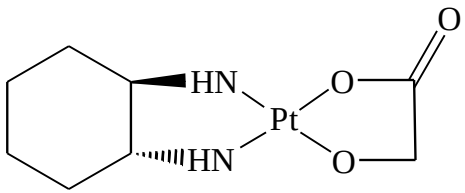
Second line Anticancer Drugs



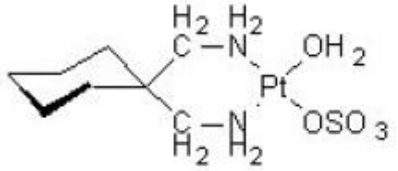
Carboplatin



Nedaplatin



Oxaliplatin



spiroplatin
aqua-1,1-bis(aminomethyl)-cyclohexanesulfatoplatinum(II)

Carboplatin is less toxic than cisplatin and can be given in higher doses (2000 mg/dose). Routinely used in clinics.

Oxaplatin is approved for secondary treatment of metastatic colorectal cancer in France and other European countries and

Nedaplatin has received approval for use in Japan.

The search continues for an improved Pt-anticancer agents which are less toxic, orally active and non-cross-resistant with *cis*-platin and *trans*-platin.

Vanadium-based drugs for diabetes

- Insulin is the mainstay of treatment of Type-I (insulin dependent-10%) and Type-II (insulin-independent –90%) diabetic patients.

- Daily subcutaneous injections of insulin to insulin-defecient patients lowered the blood glucose to normal values and interrupts a fatal metabolic disorder.

- Oral administration is ineffective in mammals.

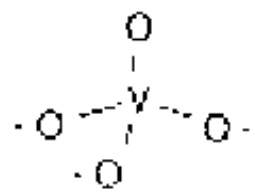
- Insulin stimulates the uptake of glucose (glycogen in liver and muscle) fatty acids (triglycerides in adipose tissue) and amino acids (proteins in muscle) from blood circulation for further storage and utilization.

- Insulin also inhibits the action of other hormones that trigger the breakdown of glycogen,fatty acids and proteins.

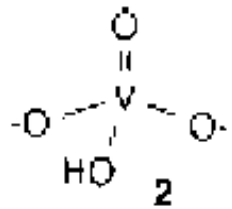
Need and search for insulin substitutes

- Development of Insulin resistance.
- Development of methods for preparation on insulin responsive cells in 1970 facilitated investigation of mechanism of insulin action as well as identification of several agents that mimic the insulin action.
- Proteins (trypsin, lectins, and antibodies) H_2O_2,Zn, Mn ions effective in rat adipocytes but FAIL in animal model.

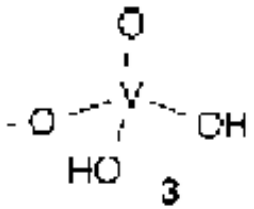
Vanadate : a structural and electronic analogue of phosphate



1



2

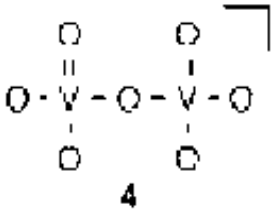


3

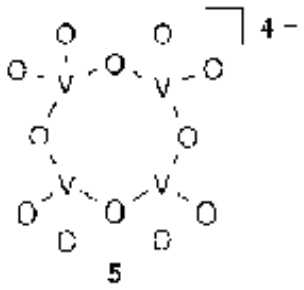
$H_3VO_4 + H_2O \leftrightarrow H_2VO_4^- + H_3O^+$ pK_a 3.5 (1)

$H_2VO_4^- + H_2O \leftrightarrow HVO_4^{2-} + H_3O^+$ pK_a 7.8 (2)

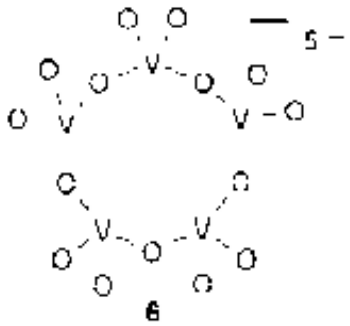
$HVO_4^{2-} + OH^- \leftrightarrow VO_4^{3-} + H_2O$ pK_a 12.7 (3)



4



5



6

Analogues of pyrophosphate, oligomeric and polymeric phosphate
Contain anhydride bonds

Schematic representation of the activation of glucose intake by insulin

(a & b) activation of glucose intake by insulin

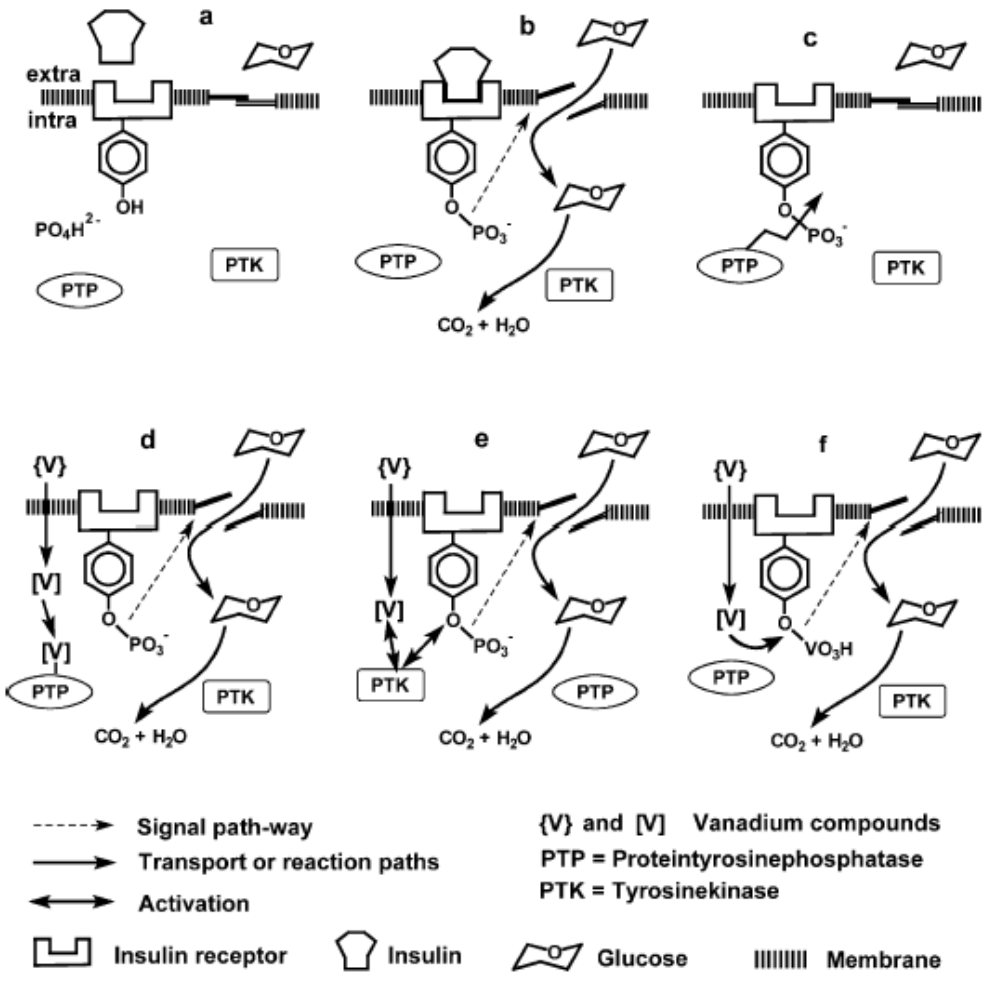
(c) Blockage of glucose intake in absence of insulin

Counteractions by vanadium compounds

(d) Activation of a phosphatase

(e) Activation of a non-membrane kinase

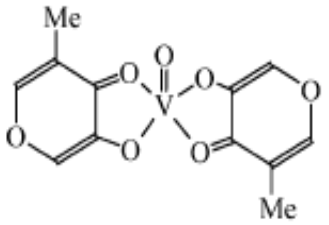
(f) Vanadylation of the insulin receptor tyrosine



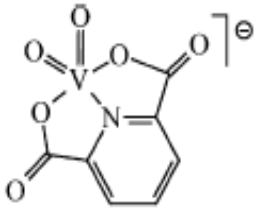
D. Rehder et al. Coordination Chemistry Review, 237 (2003) 53/63

Insulin-mimetic Vanadium compounds in various stages of clinical tests

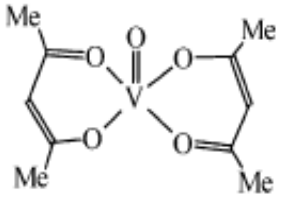
- Vanadium(IV) maltalato complex (28) has been introduced in clinical tests in humans.



28

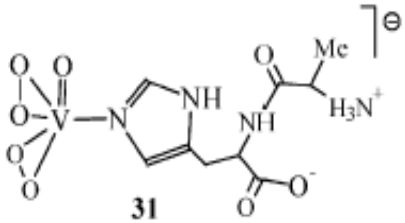


29

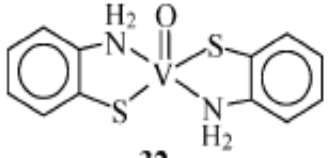


30

- V(V)-bispicolinato complex (29) has been successful in curing diabetic cats



31

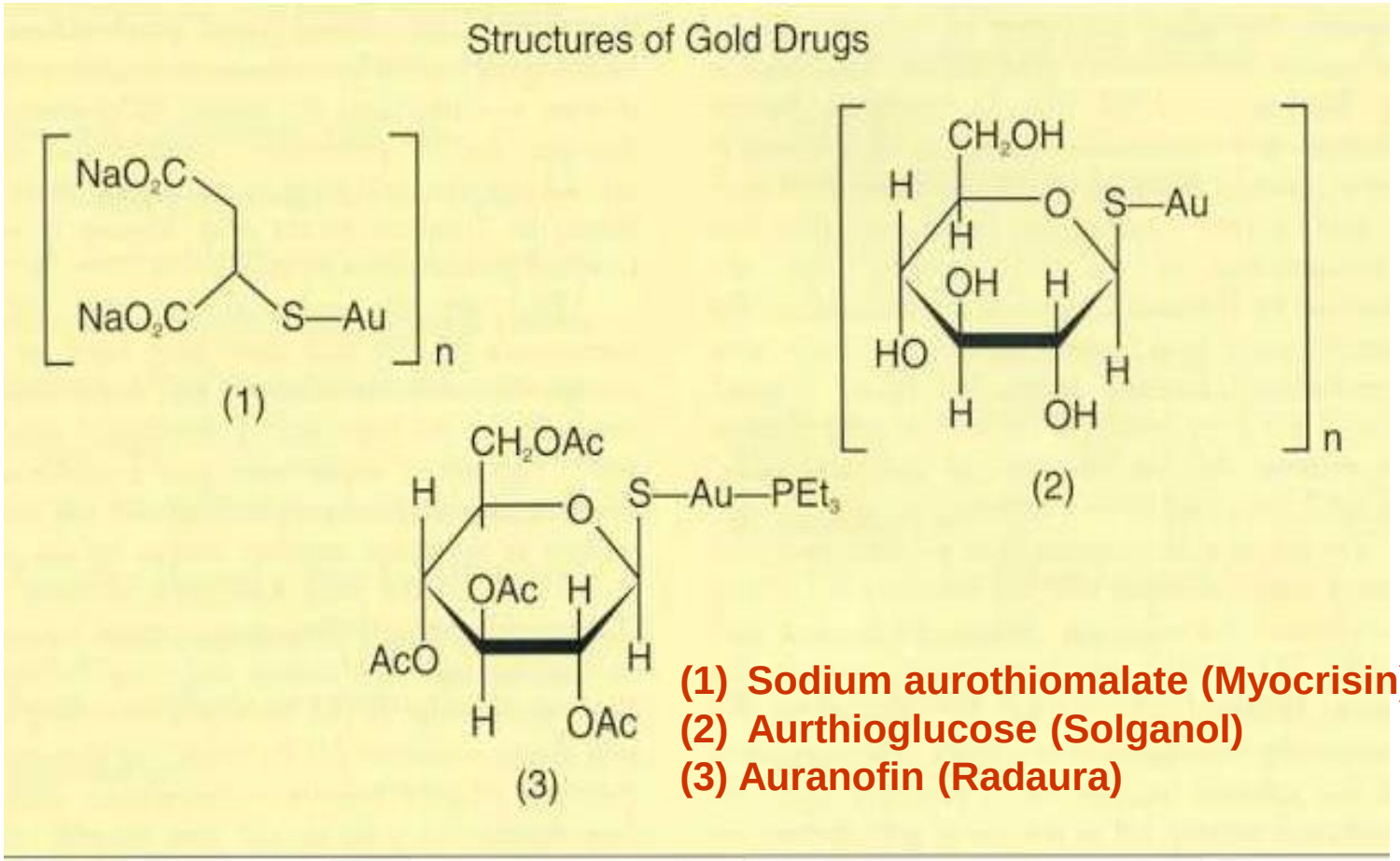


32

Slow development of Vanadium compounds in pharmaceutical industry

1. Toxicity of vanadate
2. Heavy metals not accepted by the market
3. Vanadium is retained in the bone, Half-life of VO^{2+} is one month
4. Market logistics and competing interference

Gold in treatment of rheumatoid arthritis



(1) Sodium aurothiomalate (Myocrisin)
(2) Aurthioglucose (Solganol)
(3) Auranofin (Radaura)

Nucleases (Restriction Enzymes)

Enzymes which carry out hydrolysis of internucleotide linkages in nucleic acids at relatively specific points.

A] Endonucleases:-Hydrolysis at internal position in DNA at nucleotide or RNA strand**.**

B] Exonucleases:-Hydrolysis only at terminal linkage,some at 5` and others at 3`end**.**

Required for controlled fragmentation of DNA and RNA into smaller pieces at specific points.This property has opened a new area in the Biochemistry of genes systematic dissection and mapping of chromosomes.Therefore possible to splice or recombine genes from one organism into the genome of another.

Werner Arber(Switzerland),Daniel Nathans(USA) and Hamilton Smith (USA) – Nobel Prize in Medicine in 1978 for discovery of Restriction Endonucleases.

Transition metal complexes as chemical Nucleases

Basics of Nucleic acid interactions:-

A] Nucleic acid structures

B] Nucleases

C] Fundamental interactions of metal complexes with nucleic acids

i] Coordination

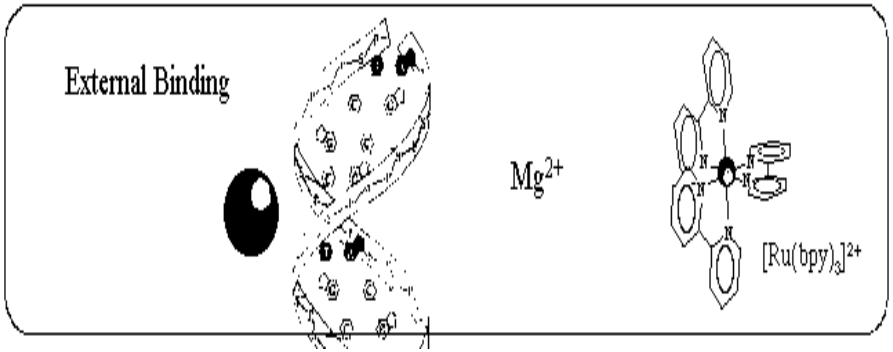
ii] Intercalation

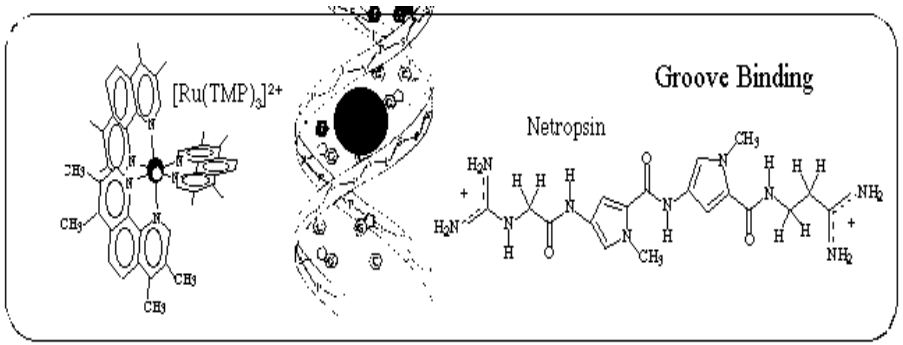
D] Fundamental reactions of metal complexes with nucleic acids

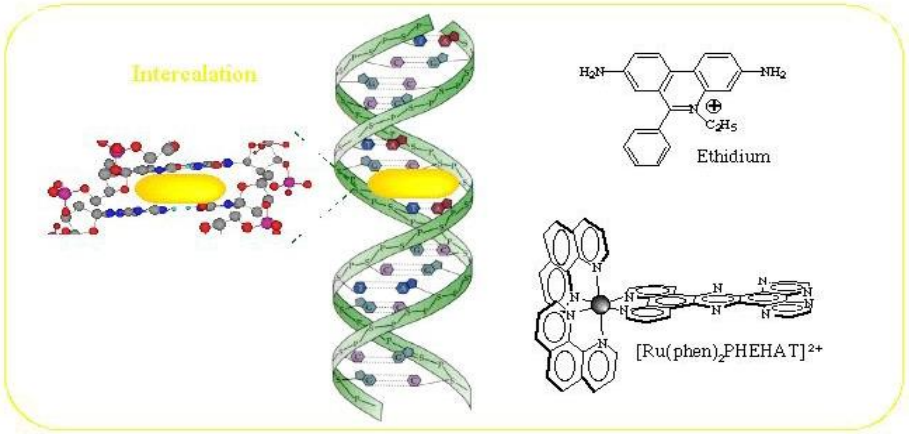
i] Redox

ii] Hydrolysis

Metal DNA interactions



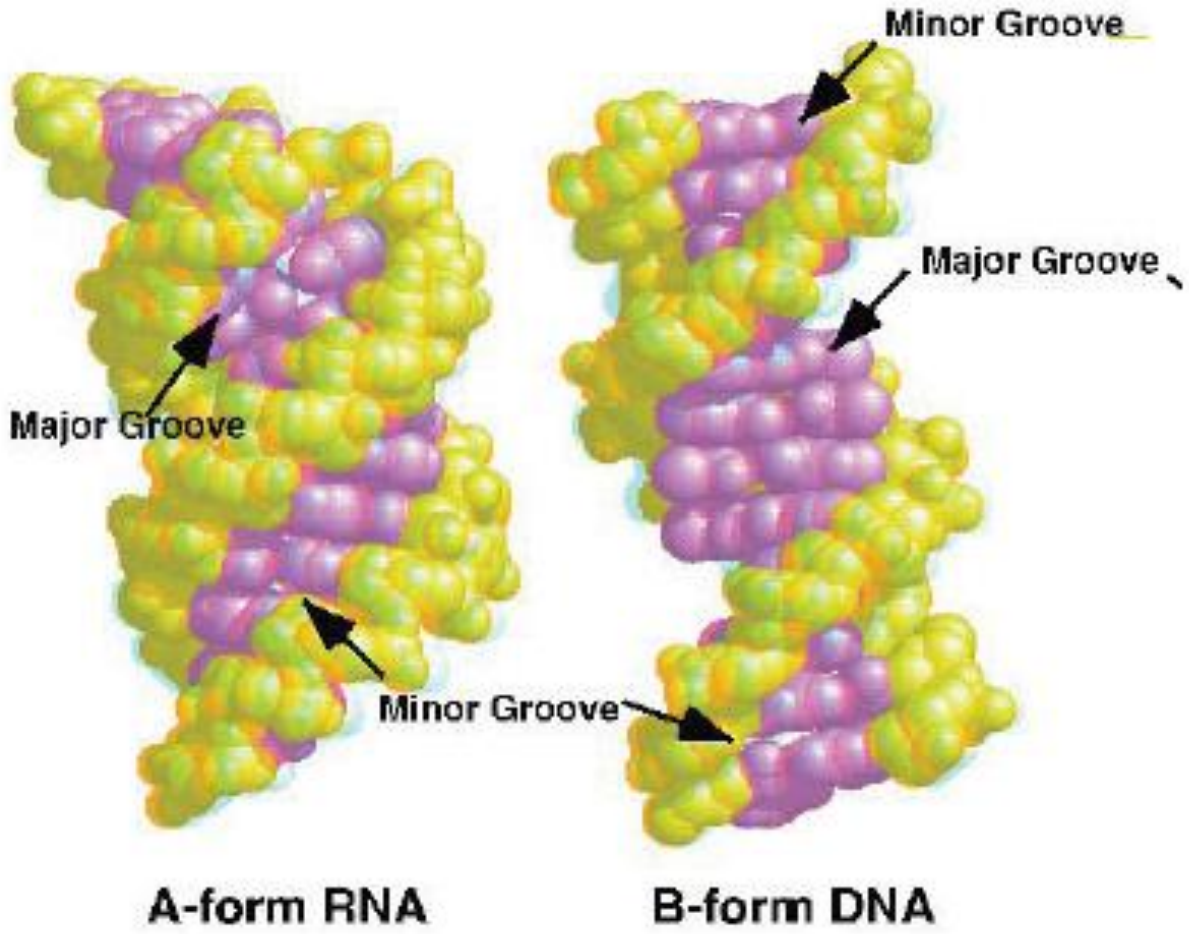




- Ionic or electrostatic interaction
  eg.-$[Ru(bpy)_3]Cl_2$
- Hydrogen bonding
  eg.-$[Co(NH_3)_6]^{3+}$
- Groove binding
  eg.-$[Cu(phen)_2]^{+}$
- Covalent binding

a) heterocyclic bases (Pt;N_7guanine)
b) phosphate oxygens (Mg^{2+})
c) sugar oxygens (Cu^{2+},Os^{3+})

- Intercalation
  Noncovalent stacking interaction between planar aromatic moiety of metal complex and the DNA base pairs


Minor Groove
Major Groove
Major Groove
Minor Groove
A-form RNA
B-form DNA

	A-DNA	B-DNA	Z-DNA
Screw sense	Right handed	Right handed	Left handed
Shape	Broadest	Intermediate	Most elongated
Rise per base pair	2.3 Å	3.4 Å	3.8 Å
Glycosidic bond	Anti	Anti	Anti for C,T and Syn for G
Base pair per turn of helix	11	10.4	12
Pitch per turn of helix	25.3 Å	35.4 Å	45.6 Å
Major groove	Narrow and very deep	Wide and quite deep	Flat
Minor groove	Broad and shallow	Narrow and quite deep	Narrow and deep

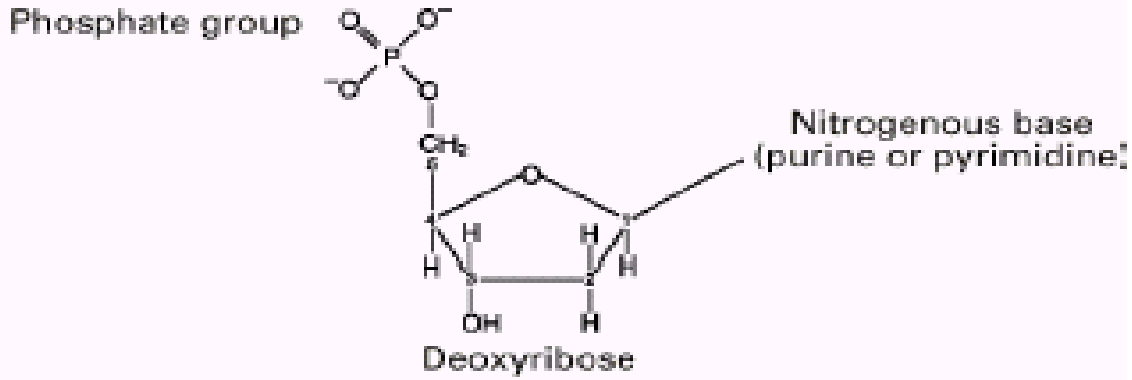


Figure 2. Structure of a nucleotide.

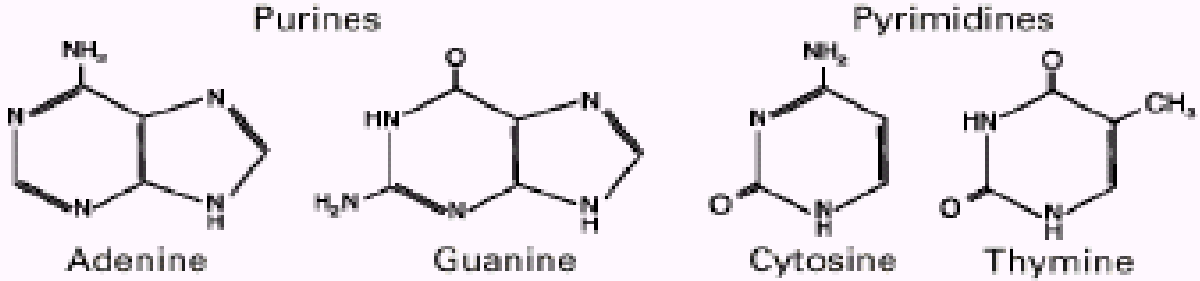
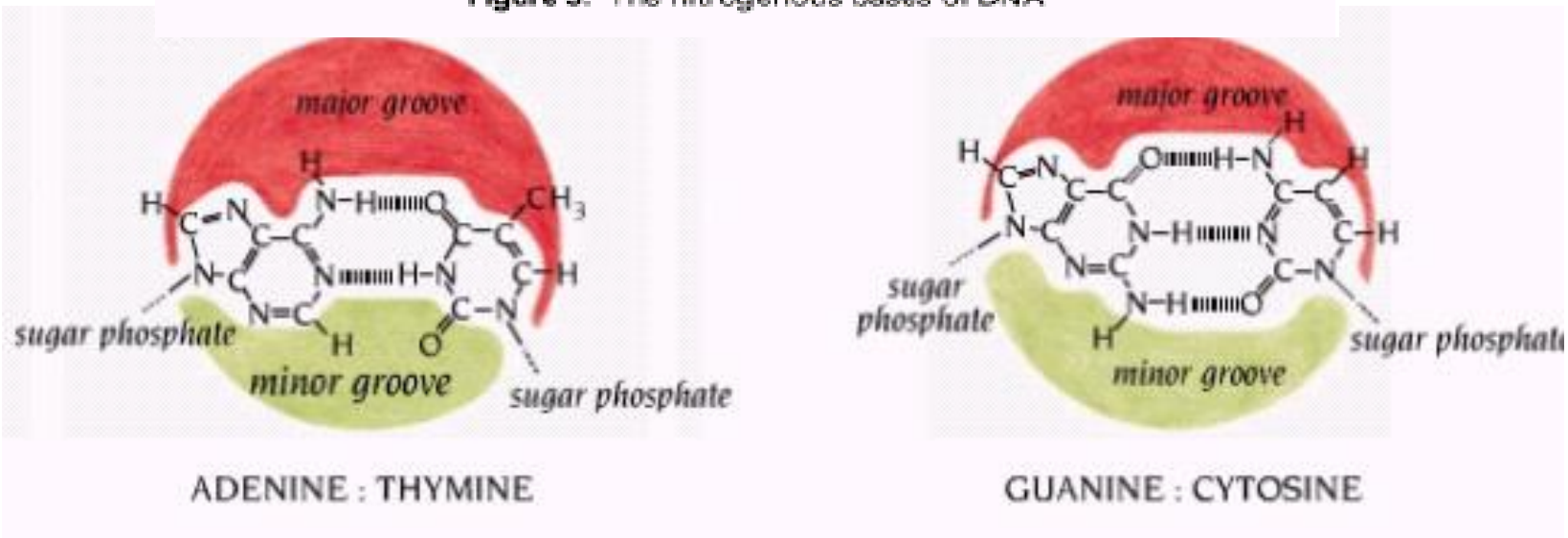


Figure 3. The nitrogenous bases of DNA



ADENINE : THYMINE

GUANINE : CYTOSINE

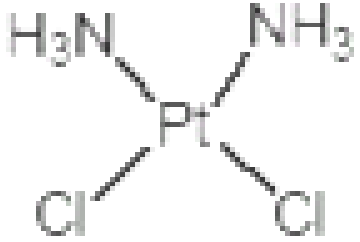
Families of well studied Coordination complexes

A] Cis-platin as anticancer agent

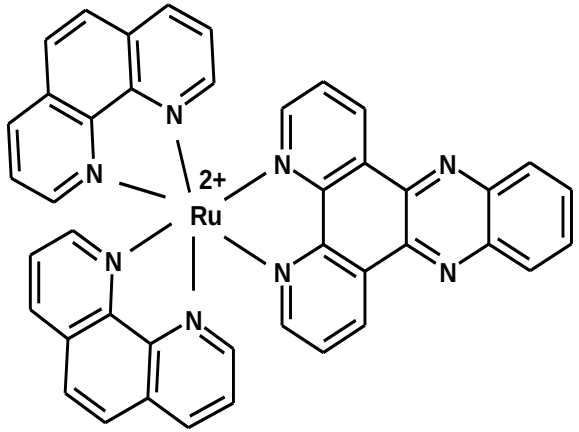
B] $Cu(Phen)_2^+$ as chemical nuclease

C] $Fe(EDTA)^{2-}$ as footprinting agent

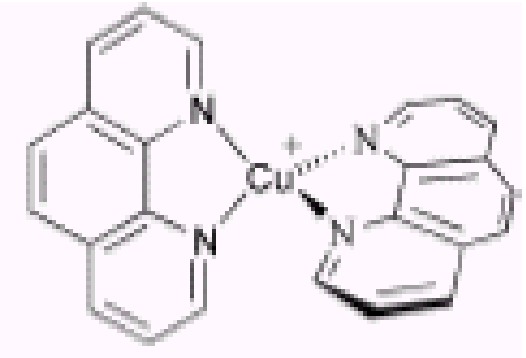
D] $M(phen)_3$ (M=Ru,Rh,Os) as spectroscopic probes



cis-platin



$[Ru(phen)_2dppz]^{2+}$



$[Cu(phen)_2^+$

Why study Metal-DNA interactions?

- For evolving molecular biological tools (Synthetic restriction enzymes)
- The genetic basis of several major diseases has been recognized and therefore necessary to target aberrant DNA by direct binding or chemical excision
- To develop novel DNA-sequence reading and cleavage systems that are amenable to synthetic manipulation and have suitable biocompatibility (stability,cellular penetration and recycling)
- To understand DNA reactivity and detect DNA structures

Techniques used to study metal nucleic acid interactions

A] Viscosity measurements

B] ^{1}H NMR Studies

C] UV-Visible studies

D] Emission spectroscopy

E] Cyclic voltammetric studies

F] Circular Dichroism

G] Gel electrophoresis

H] Resonance Raman studies

I] Equilibrium dialysis

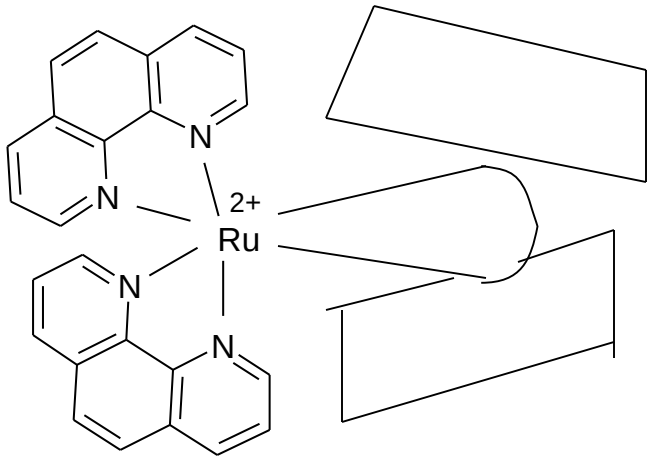
J] Covalent Binding assay

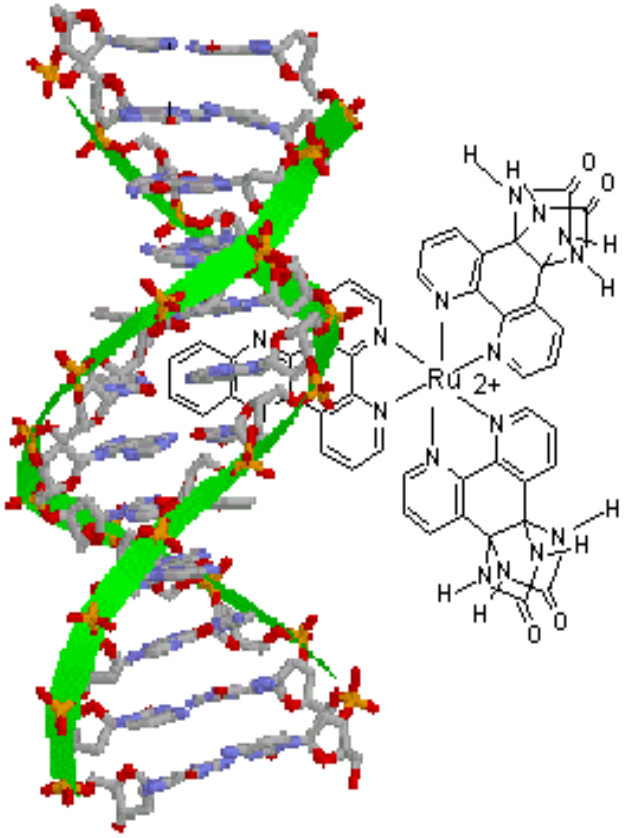
Possible Metal-DNA interactions

- Ionic or electrostatic interaction
  eg.-$[Ru(bpy)_3]Cl_2$
- Hydrogen bonding
  eg.-$[Co(NH_3)_6]^{3+}$
- Groove binding (major and minor)
  eg.-$[Cu(phen)_2]^{+}$
- Covalent binding
  a) heterocyclic bases (Pt ; N_7 guanine)
  b) phosphate oxygens (Mg^{2+})
  c) sugar oxygens (Cu^{2+},Os^{3+})
- Oxidation
  eg.-$[Ru(bpy)_3]^{2+}$

Intercalation

Non covalent stacking interaction between planar aromatic moiety of metal complex and the DNA base pairs





Modification of the ancillary ligand influences the optical and DNA binding properties of the complexes.

Following changes occur upon intercalation

A] Unwinding and lengthening of the DNA helix
B] Electronic interaction of the intercalator within the helix
C] DNA Rigidity and orientation of the intercalator within the helix

Experimental criteria that establish intercalation can be classified as follows:

A] Experiments that evaluate structural changes in the DNA helix
1.Changes in solution viscosity of bulk DNA.
2.Changes in sedimentation coefficient.
3.Downfield shifts in the ^{31}P NMR spectrum

B] Experiments that indicate an electronic interaction between the
intercalator and DNA bases.

1.Hypochromism
2.Bathochromic shift
3.Emission enhancement
4.^{1}H NMR up field shifts in the aromatic protons of the intercalated
molecule

C] Experiments that demonstrate molecular orientation or rigidity

1.Dichroic technique
2.Changes in luminescence polarization

[A] Viscosity Measurements

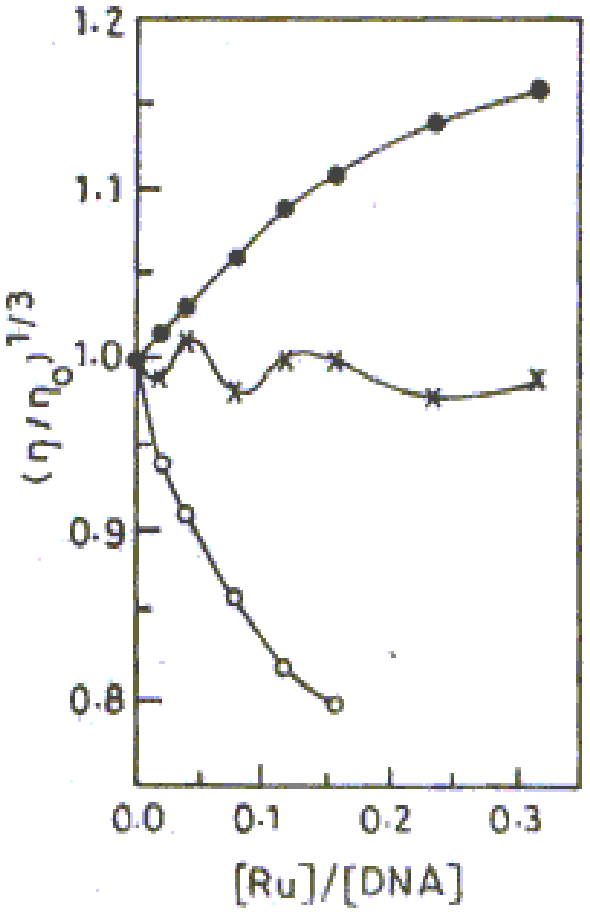


Fig. —Effect of increasing amounts of $[Ru(bpy)_3]^{2+}$ (×), $[Ru(bpy)_2(pztp)]^{2+}$ (•) and $[(bpy)_2Ru(pztp)Ru(bpy)_2]^{4+}$ (o) on the relative viscosities of calf-thymus DNA (ref. 32).

[B] *1H* NMR Studies

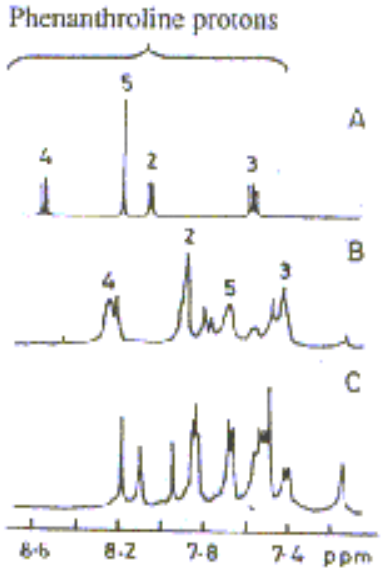


Fig. —^{1}H NMR spectra of (A) Δ-[Ru-(phen)$_3$]$^{2+}$, (B) the dodecanucleotide with Δ-[Ru(phen)$_3$]$^{2+}$ in the aromatic proton region at a metal complex to dodecanucleotide ration of 1, in 10 mM phosphate buffer (*p*H 7) and (C) the free dodecanucleotide d(TCGGGATCCCGA)$_2$ (ref. 19).

- Shifts in ^{1}H NMR resonance's of both DNA binding complex and the oligonucleotide are evidence of increased association
- These shifts can be used empirically to gain structural insights into binding modes of complexes such as M(phen)$_3^{2+}$ where (M=Ru,Rh)

[C] UV-Visible Studies

- Hypochromism and red shift are observed on binding with DNA
- These spectroscopic perturbations can be used to define equilibrium binding affinities and chiral preferences as well as extent of intercalation

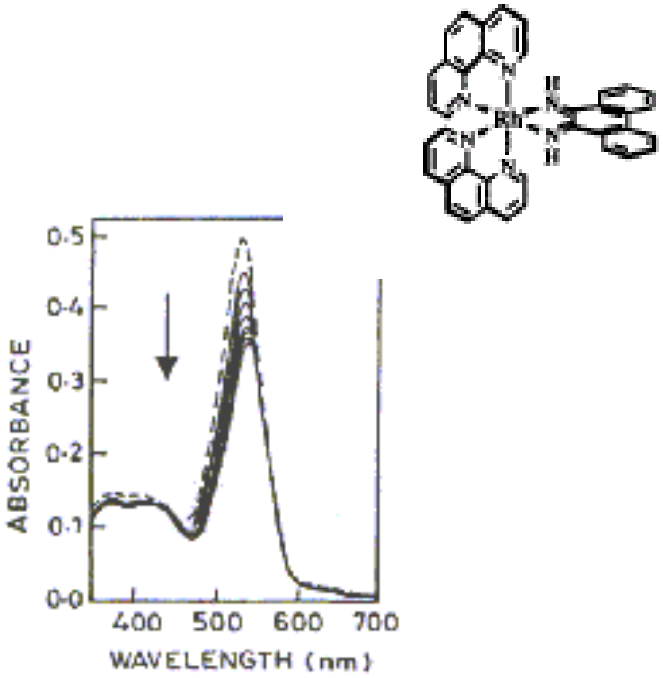


Fig. —Visible absorption spectrum of $[Ru(phen)_2(phi)]^{2+}$ (10 μM) in the absence (----) and presence (——) of increasing amounts of DNA (ref. 27).

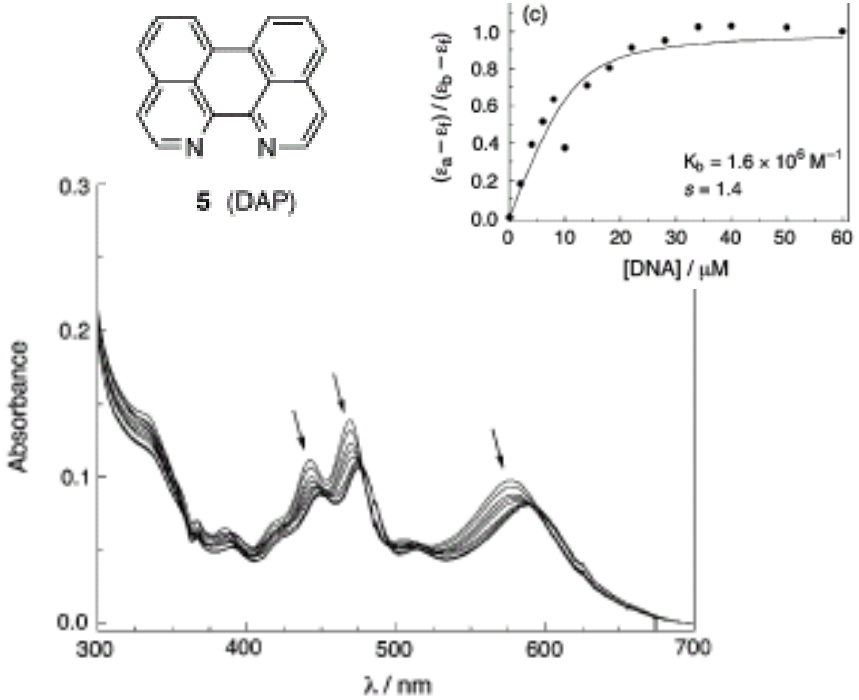
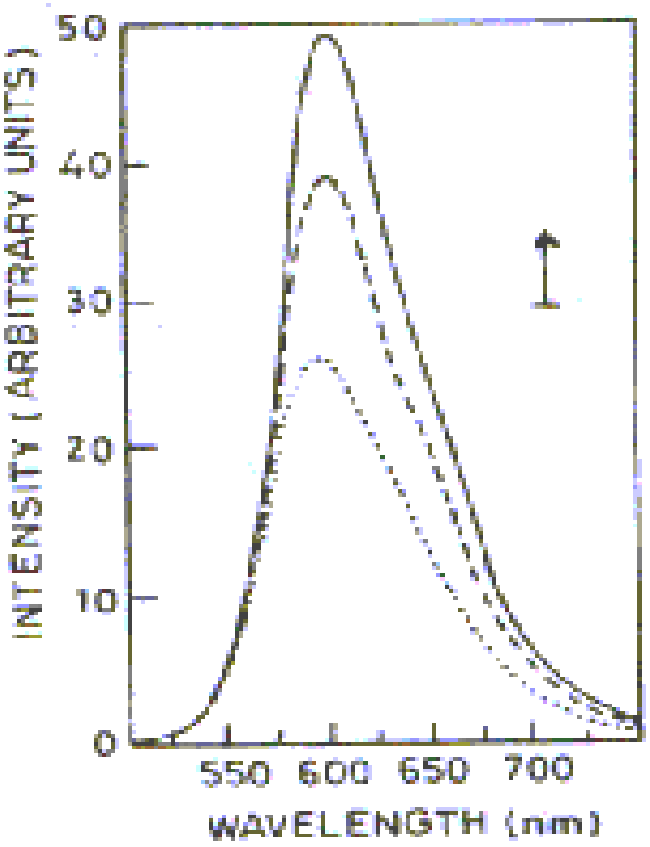


Figure 3. Changes in the electronic absorption spectrum of 4.0 μM $[Ru(5)_2(bpy)]^{2+}$ in 5 mM Tris buffer, pH = 7.5, 50 mM NaCl upon addition of 0, 2, 4, 6, 8, 14, 18, 22, 28, 34, 40, 50, and 60 μM calf-thymus DNA.

$$DNA / \varepsilon_a - \varepsilon_f = DNA / \varepsilon_b - \varepsilon_f + 1 / K_b (\varepsilon_a - \varepsilon_f)$$

[D] Emission spectroscopy



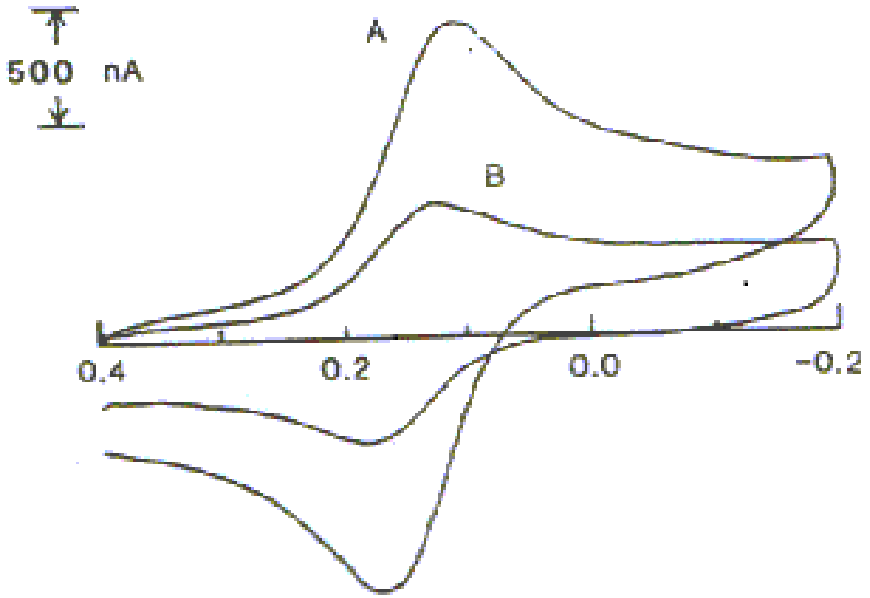
Increase in Fluorescence intensity and lifetime of excited state due to intercalation

Figure-Emission spectrum of free $[Ru(phen)_3]^{2+}$(……) $[\Lambda\text{-}Ru(phen)_3]^{2+}$ in the presence of DNA (--------), and Δ-$[Ru(phen)_3]^{2+}$(-) in the presence of DNA showing the enantioselective binding of the complexes to the helix.

[E] Voltammetric studies of the interaction of tris(phen) complexes with DNA

- Coordination complexes of 1,10-phenanthroline and bipyridine with Co^{3+},Ru^{3+},Fe^{3+} are known to intercalate between base pairs of DNA
- Interaction of M-DNA interaction with reduced and oxidised metal
- Differentiates betwwen intercalation and electrostatic binding
- Estimates binding parameters (binding site sizes and binding constant)

- Figure- Cyclic voltammograms of 1×10^{-4} M $[Co(phen)_3]^{3+}$ In the (A) absence and (B) presence of 5mM nucleotide phosphate (NP).Sweep rate,100mV/s.



[F] Circular dichroism

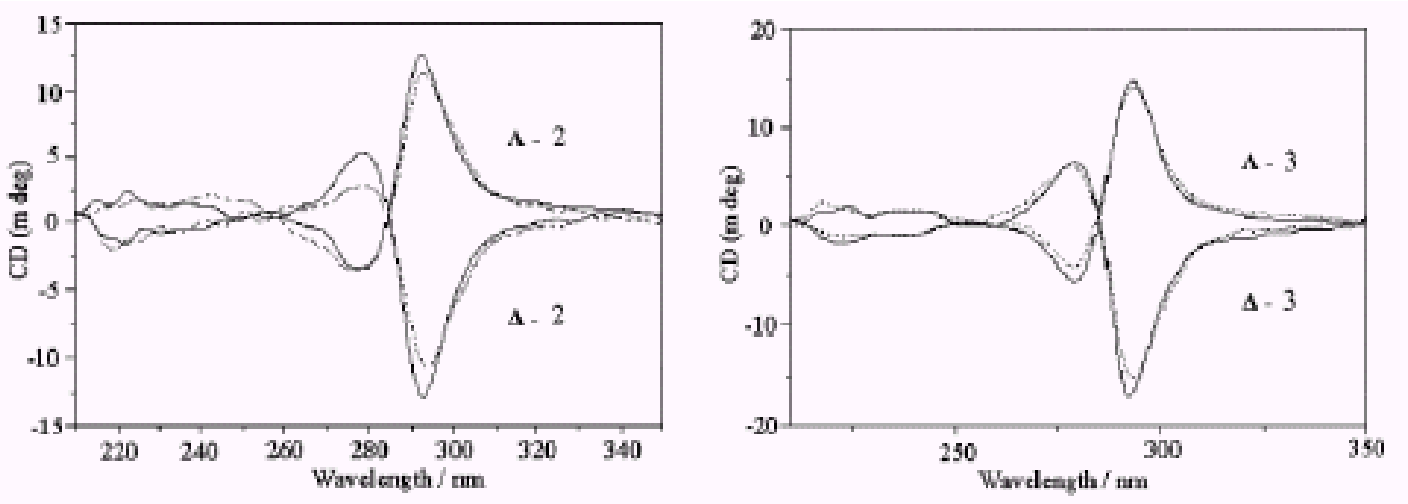
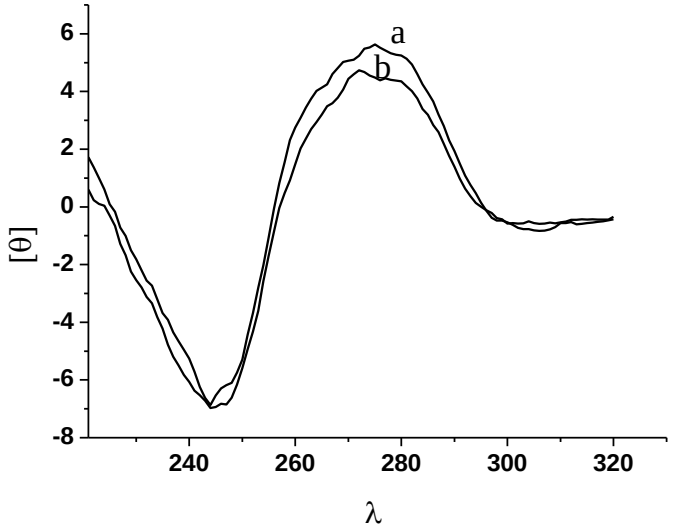


Fig. 3 CD spectra of Δ-2, Λ-2, Δ-3 and Λ-3 in 5 mM Tris-HCl buffer (pH 7.2), 50 mM NaCl in the absence (—) and in the presence (---) of CT-DNA. [Ru] = 1.0×10^{-5} M; [DNA] = 1.5×10^{-4} M; path length 1.0 cm. The CD spectrum of CT-DNA was subtracted from those of the mixtures.

Figure - Circular Dichroism spectra of calf thymus DNA (20 μM) in the absence (a) and presence (b) of $[Co(dppz)_2Cl_2]Cl$ (10 μM) complex.



G] Covalent binding assay for bis-phenantroline dichloro Ruthenium (II) complexes to B-DNA

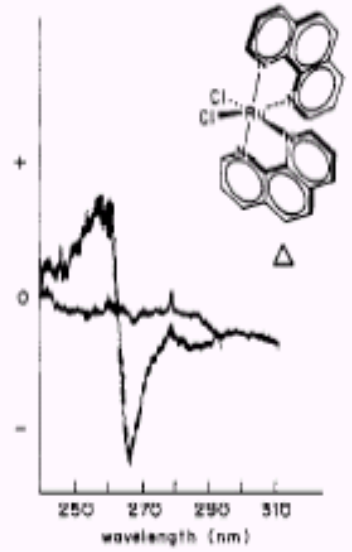


Figure 2. Circular dichromism of the supernatant after ethanol precipitation of the ruthenium complex bound to B-DNA. Binding to B-DNA is stereoselective and leads to enrichment of the supernatant in the unbound Δ isomer (inset).

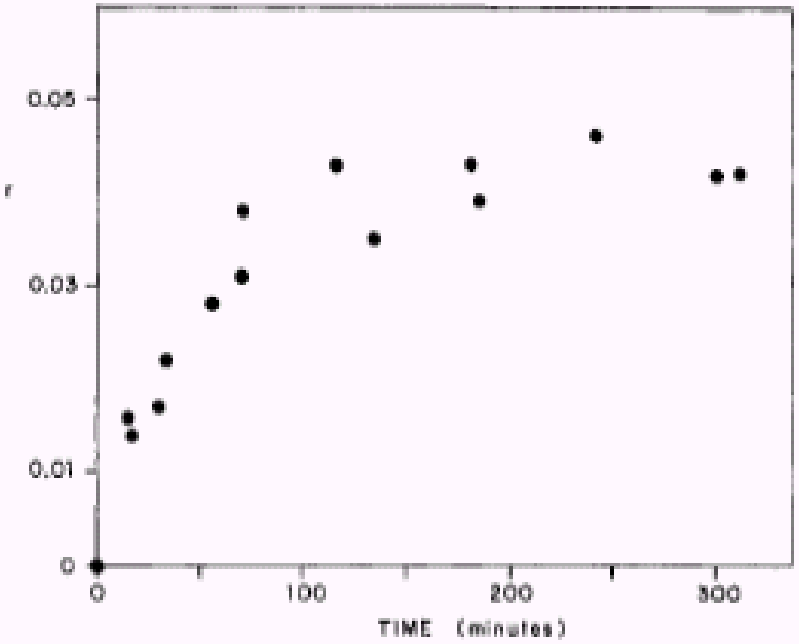


Figure 1. Plot of $(phen)_2RuCl_2$ binding to calf thymus DNA as a function of time; r is the ratio of bound ruthenium to nucleotide concentrations.

Applications of different metal complexes that bind nucleic acids are

[A] *Spectroscopic probes*

- Tris (phenanthroline) Ru (II) complexes offer a novel spectroscopic probe of nucleic acids
- Derivatives of the tris(phenanthroline) metal complexes that may become exceedingly useful as spectroscopic probes

e.g.-$[Ru(bpy)_2dppz]^{2+}$ and $[Ru(phen)_2dppz]^{2+}$ (dppz=dipyridophenazine).

- Quite novel luminescent phenanthroline and diphenylphenanthroiline complexes of copper (I) are extremely valuable as cleavage *probes.*

[B] Metallofootprinting agents

- Derivative of a tris (phenanthroline) metal complexes e.g. $[Rh(phi)_2bpy]^{3+}$ currently being applied in footprinting experiments
- $[Cu(phen)_2]^{+}$ and manganese porphyrins have been used to footprint DNA-binding proteins

[C] Conformational probes

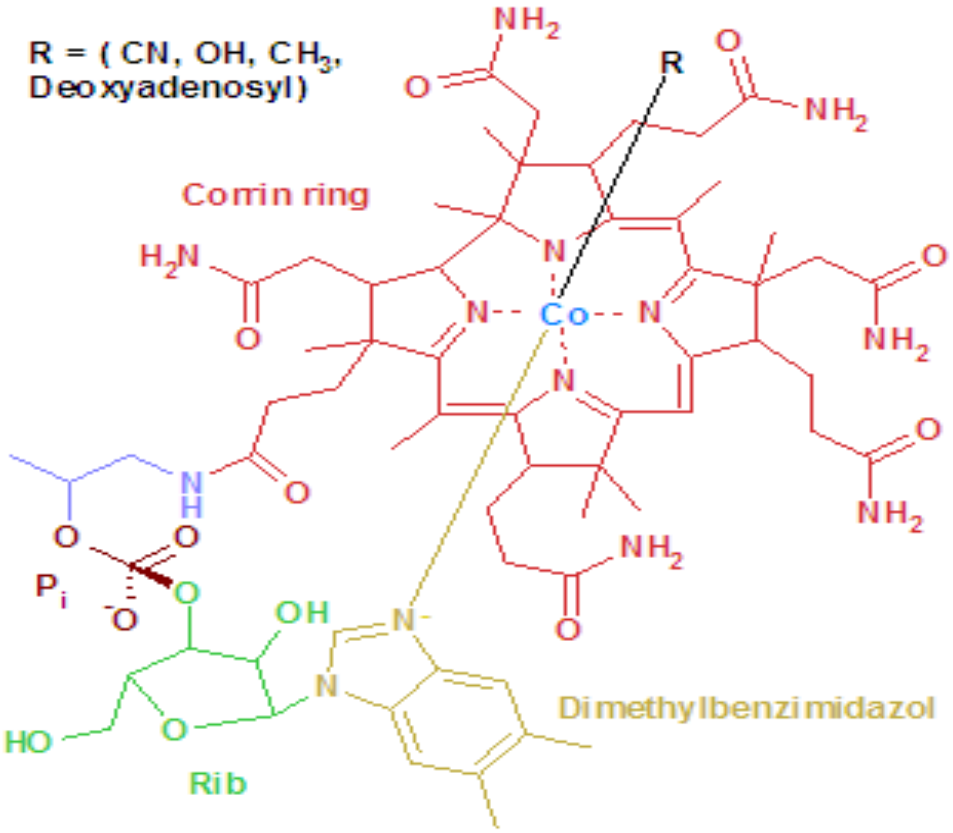
- Wide application in probing the local variations in conformation that arise along nucleic acid polymers
- Used in probing the structural variations in nucleic acids

Conclusion

- Study, understand and teach Bioinorganic Chemistry – it is closest to life

Cobalt in Biology

Vitamin B12



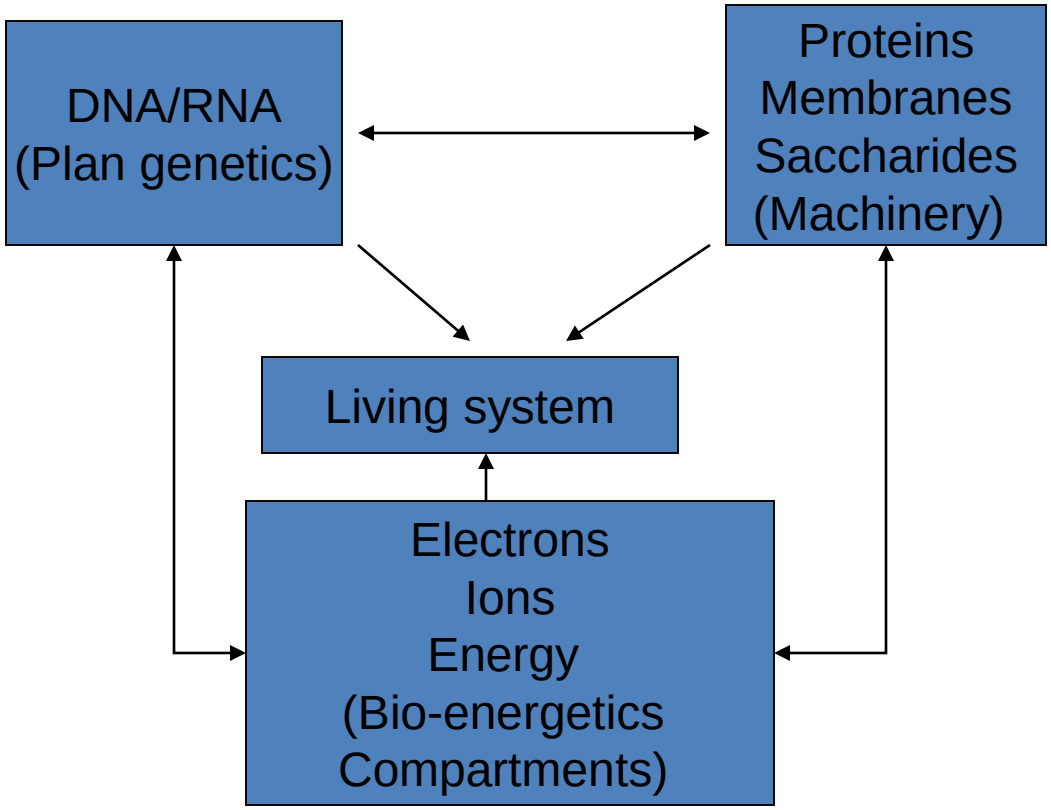
Vitamin B12 is the only known essential biomolecule with a stable metal-carbon bond, that is, it is an organometallic compound.

- ✓ a methyl group - as in methylcobalamin
- ✓ a 5'-deoxyadenosine at the the 5' positon - as in adenosylcobalamin (coenzyme B12)
- ✓ a cyanide group - as in Vitamin B12 - as supplied from drug companies

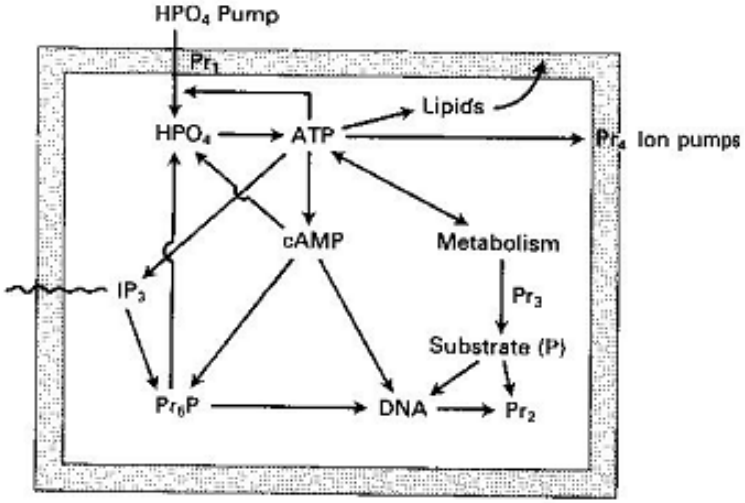
Homeostasis

- All living species have two different ‘environments ‘ one the external surroundings the ‘habitat’ and the other ‘milieu’ made up of the biological fluids e.g. the blood plasma.

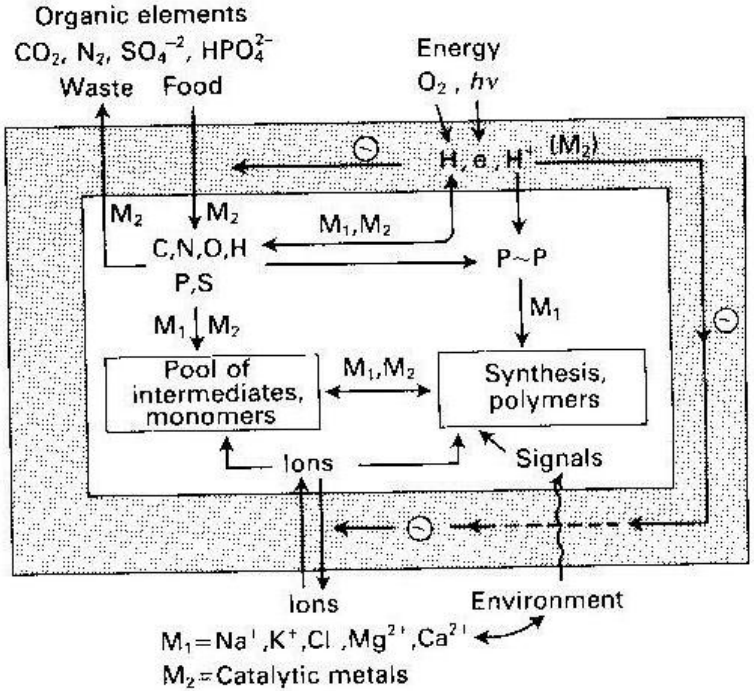
- This conservation of conditions in a living system, homeostasis, does not correspond to a state of equilibrium in thermodynamic terms (which would be equivalent to death) but to a series of related and controlled states in a dynamic process of continuous material, charge, and energy flows through the cells, with forced and fixed directions.



Scheme showing involvement of phosphorus



Scheme showing the close connections in a prokaryote cell between a variety of elements



DNA binding

- Major adducts of platinum drugs with DNA are the 1,2-GpG and 1,2-ApG intrastrand crosslinks – 90%

- Structural studies show that the Pt cross links induce bending and unwinding of DNA and cause destacking of the purine bases.

- *cis*-$\{Pt(NH_3)_2\}^{2+}$- d(CCTG*G*TCC)·d(GGACCAGG) indicates that the B DNA backbone conformation is significantly altered to accommodate the platinated lesion

- Spectroscopic and calorimetric studies on the major adduct of the *cis-platin* with a 20-mer DNA duplex containing a GG intrastrand-crosslink have suggested that platination induces a conformational shift from an B-like to an A-like form – may be important in HMG recognition.

- Spectroscopic and calorimetric studies on the major adduct of the *cis-platin* with a 20-mer DNA duplex containing a GG intrastrand-crosslink have suggested that platination induces a conformational shift from an B-like to an A-like form – may be important in HMG recognition.

- It is known that platinum forms bifunctional DNA adducts with the following order of sequence preference: -GG- > -AG- >> -GA and platination is kinetically controlled.

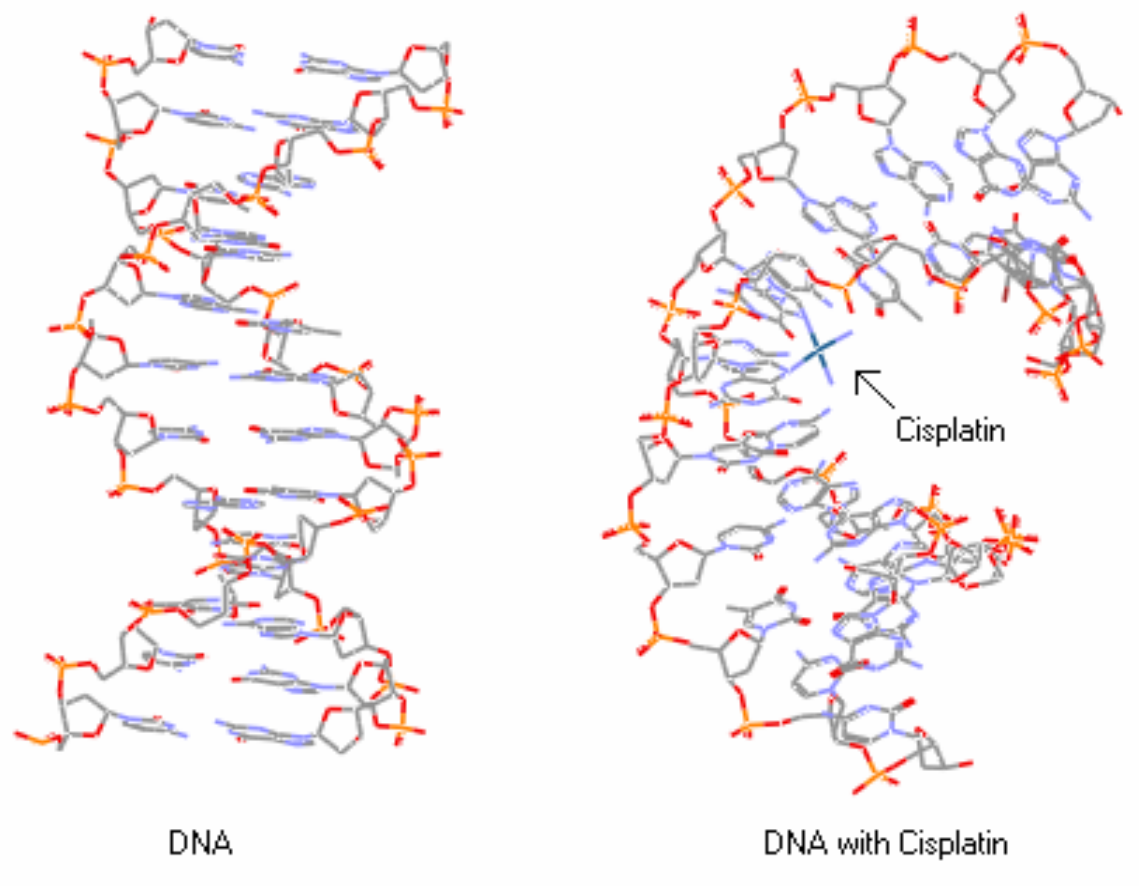
- Inter strand cross links can also be generated between DNA and *cis-platin* between to G's on opposite sides of the duplex

- Monofunctional adducts can also form and can be long lived (t(1/2) = 80hrs)

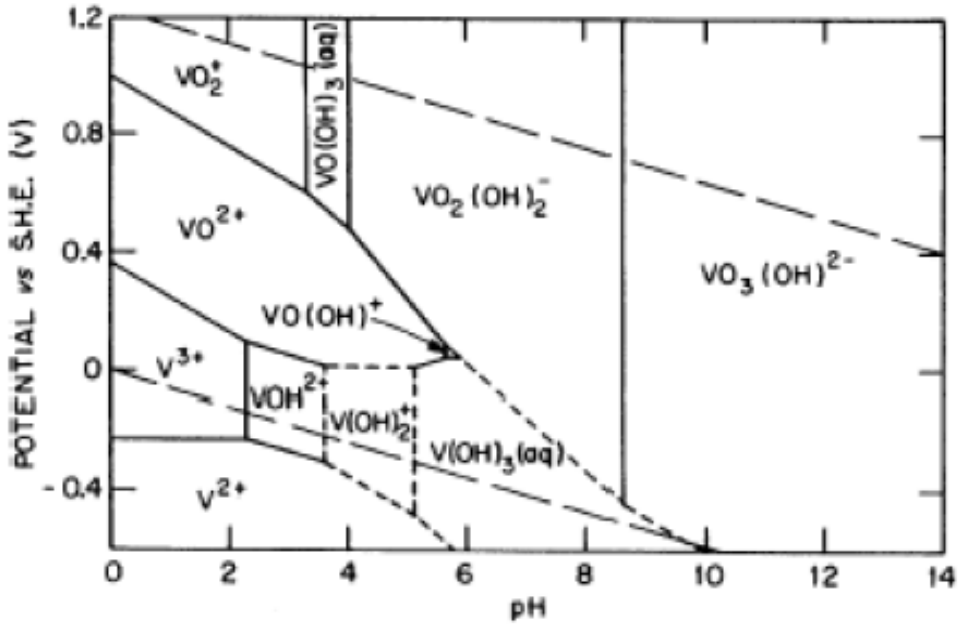
DNA binding – GpG INTRA STRAND

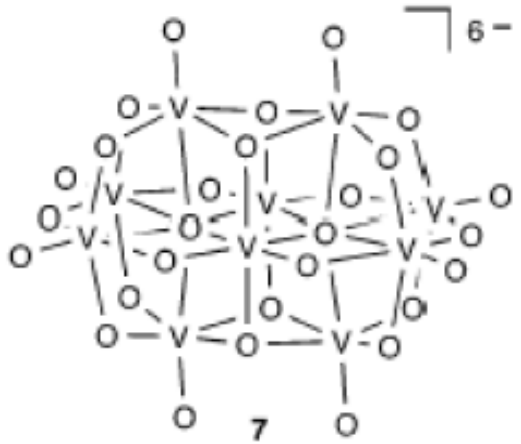
- *cis-platin* binds to DNA and causes a critical structural change in the DNA – a bend of 45 degrees

cis-platin binds to two Adjacent G's at N7 on the DNA in an INTRA strand cross-link



Oxidation state of vanadium species as a function of pH and reduction potential





7

High pH V(V) is the most stable form of vanadium

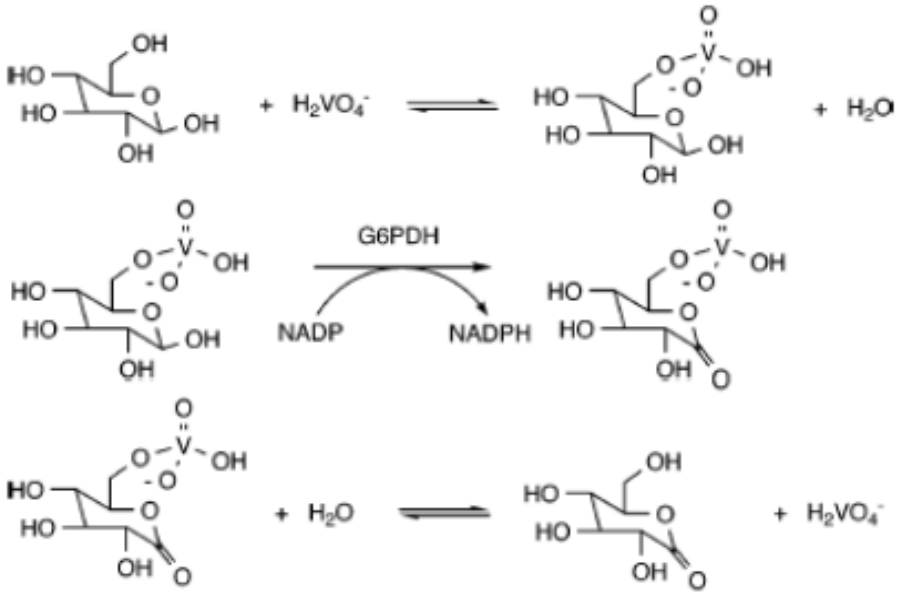
Low pH V(IV) is most favoured

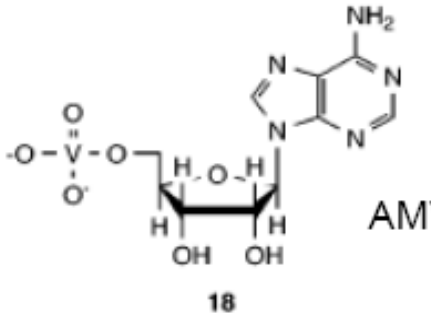
pH 2 to 6 the major V(V) species is the decamer

$[V_{10}O_{28}]^{6-}$ oxoanion contains 3 different vanadium atoms, the most unusual being the non-oxo VO_6 type at the centre

Vanadate esters : functional analogues of phosphate esters

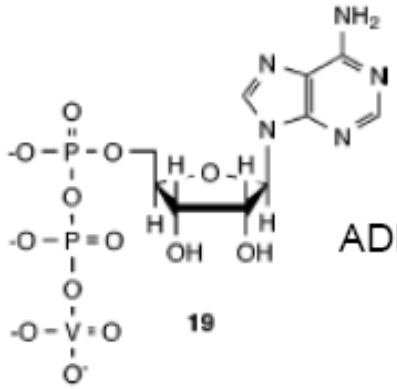
Reaction of D-glucose with vanadate to form D-glucose-6-vanadate





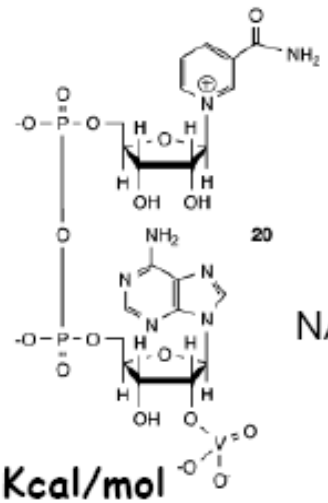
AMV

18



ADPV

19

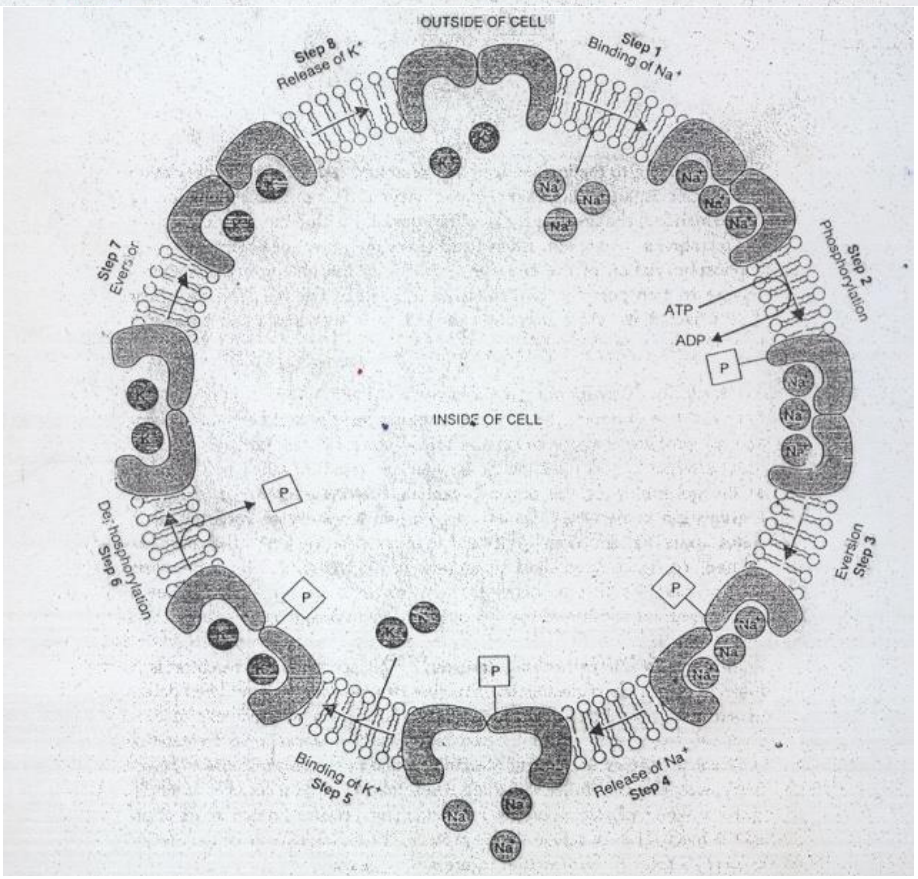


20

NADV

Pyrophosphate bond 7 Kcal/mol while hetero-vanadate bond 2-3 Kcal/mol

Ion	Extracellular conc.	Intracellular Conc.	Ratio $[ion]_o/[ion]_i$	E (mV)
Na^+	145	12	12	+68
K^+	4	155	0·026	-99
Ca^{2+}	1·5	$<10^{-7}$ M	715,000	7+128
Cl^-	123	4·2	30	-90



Model Compounds- Biomimetic Chemistry

✓ Large size of metallobiomolecules and high resolution structure of metal coordination difficult

✓ If X-ray crystal structure is known it is possible to design a replica of the coordination environment.----------replicative models

✓ If X-ray crystal structure is not known we test postulated structure by spectroscopy by synthesizing models-----speculative models

✓ If models are only structurally similar--------- structural models

✓ If models are functionally similar ----------functional models

Biomimetic approach has helped in the study of

1. Assignment or verification of the metal oxidation states

2. effects of distance and medium on electron transfer rates

3. role of steric and electronic factors

4. Identity of likely intermediates of enzyme catalyzed reactions

- Strategy for models complexes- spontaneous self assembly

- Nature adopted the a similar strategy based on available chemistry in the geosphere during evolution .

Manganese in Biology

Manganese(II) ions function as cofactors for a number of enzymes and the element is thus a required trace mineral for all known living organisms.

Biological role

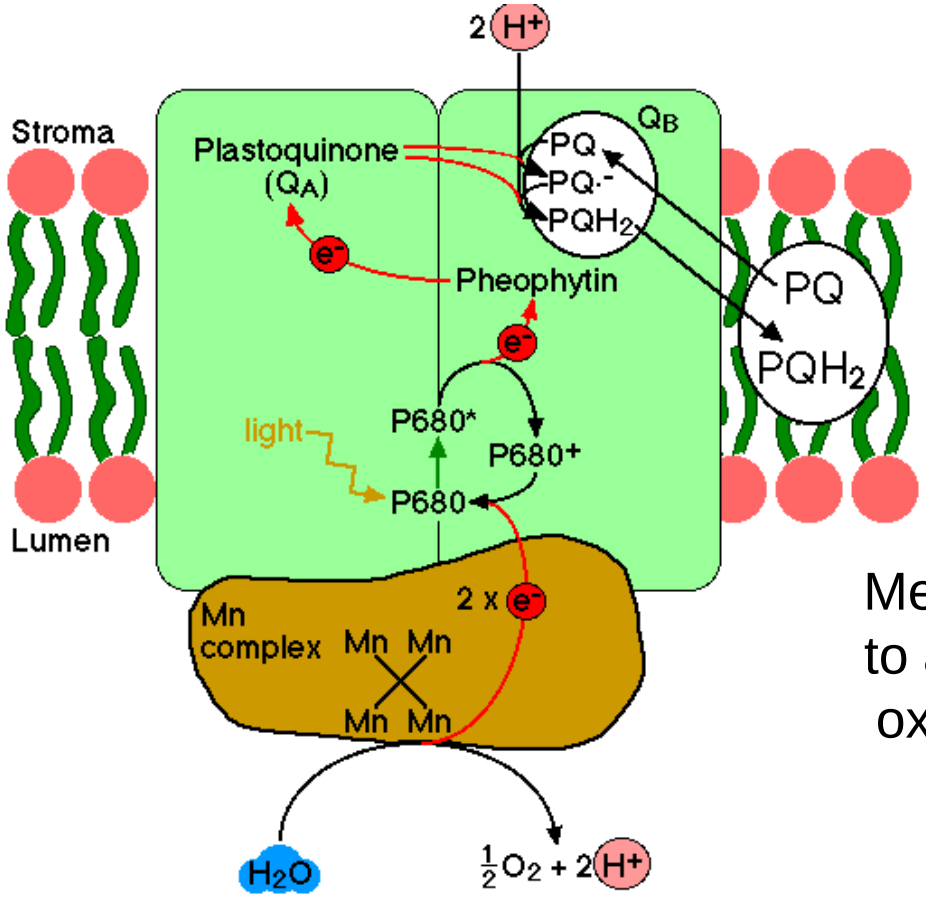
- The classes of enzymes that have manganese cofactors are very broad and include such classes as oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, lectins, and integrins.

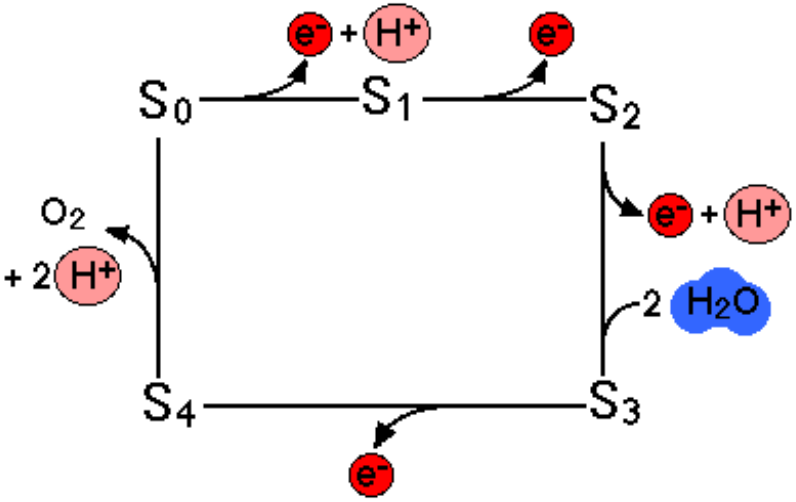
- Mn-SOD is the type of SOD present in eukaryotic mitochondria, and also in most bacteria. The Mn-SOD enzyme is probably one of the most ancient, for nearly all organisms living in the presence of oxygen use it to deal with the toxic effects of superoxide, formed from the 1-electron reduction of dioxygen.

- Manganese is also important in photosynthetic oxygen evolution in chloroplasts in plants, which are also evolutionarily of bacterial origin.

- The oxygen evolving complex (OEC), a water-oxidizing enzyme contained in chloroplast membrane, and which is involved in the terminal photooxidation of water during the light reactions of photosynthesis, has a metalloenzyme core containing four atoms of manganese.

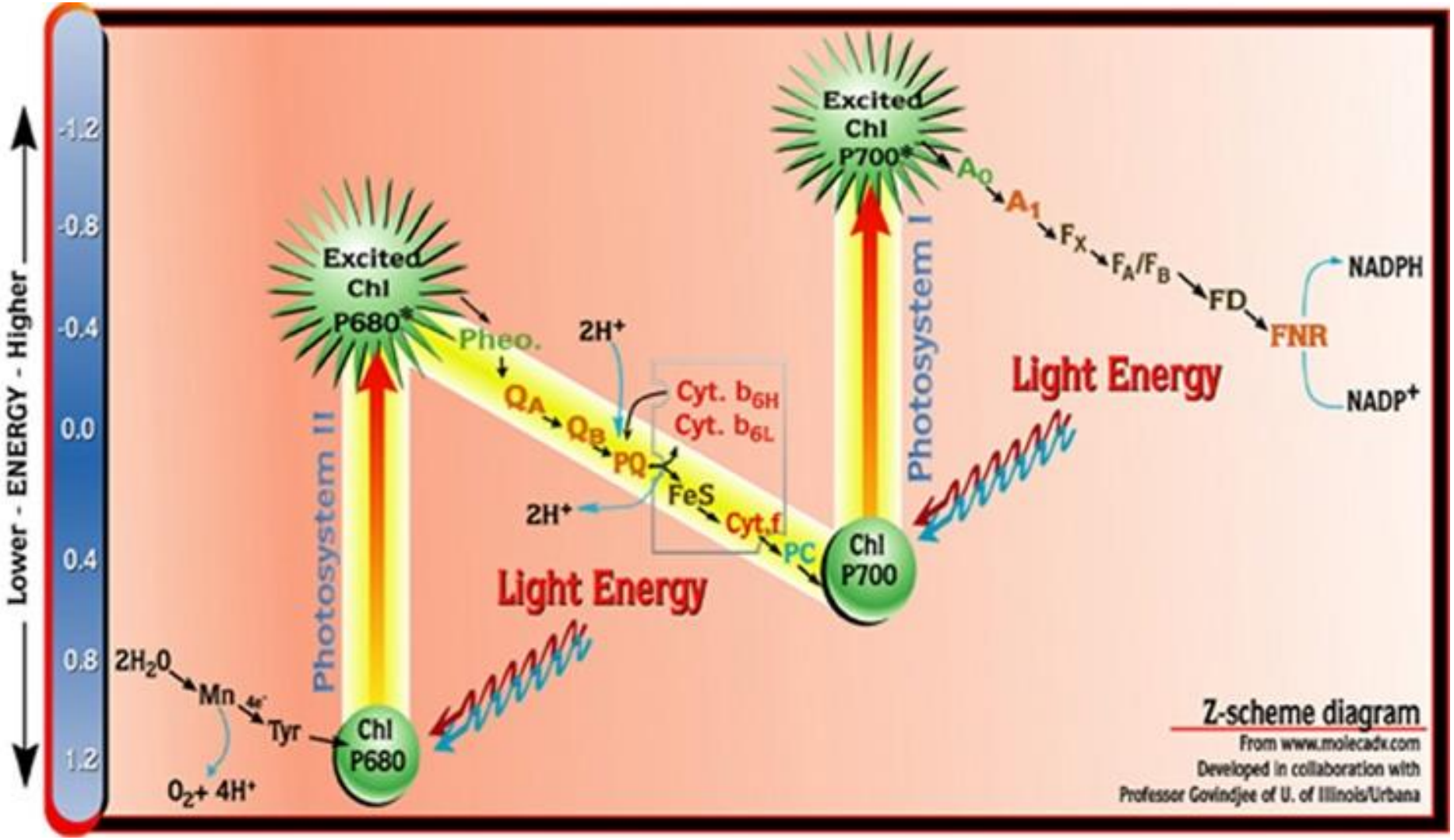
Photosystem II



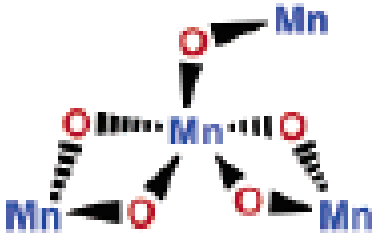


Mechanistic cycle (commonly referred to as the Kok catalytic cycle) for water oxidation in the photosystem II (PSII) active site.

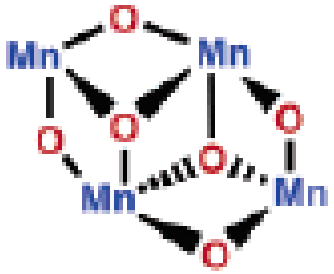
$$H_2O + CO_2 \rightleftharpoons 1/n(CH_2O)_n + {}^3O_2 \qquad \Delta H = +470 \text{ KJ/mole}$$


Lower - ENERGY - Higher
-1.2
-0.8
-0.4
0.0
0.4
0.8
1.2
Excited Chl P680*
Pheo.
Q_A
Q_B
PQ
$2H^+$
Cyt. b_{6H}
Cyt. b_{6L}
FeS
Cyt.f
PC
Chl P700
Excited Chl P700*
A_0
A_1
F_X
F_A/F_B
FD
FNR
NADPH
$NADP^+$
Light Energy
Photosystem II
Photosystem I
$2H_2O$
Mn
Tyr
Chl P680
$O_2 + 4H^+$
Z-scheme diagram
From www.molecadx.com
Developed in collaboration with
Professor Govindjee of U. of Illinois/Urbana

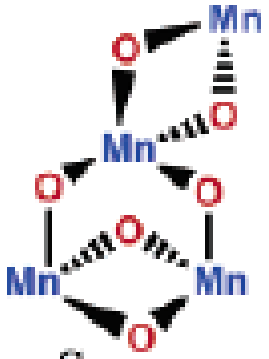
Proposed PSII Mn_4 Structures



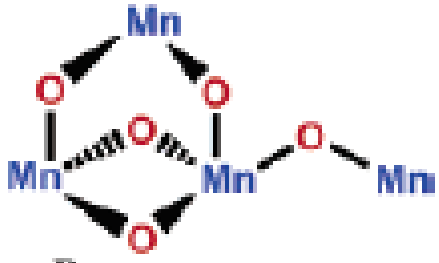
A



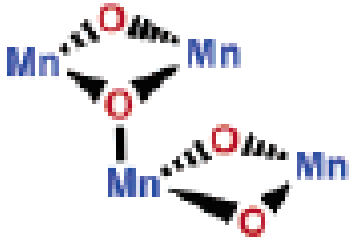
B



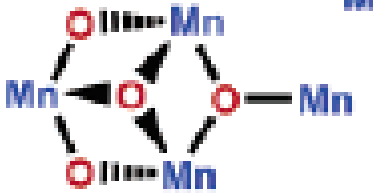
C



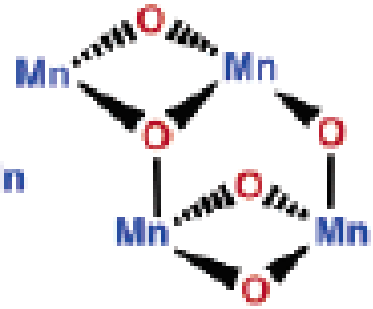
D



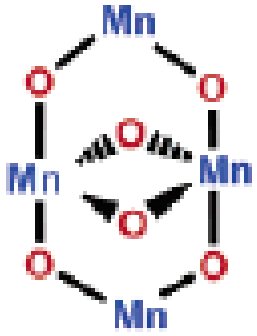
E



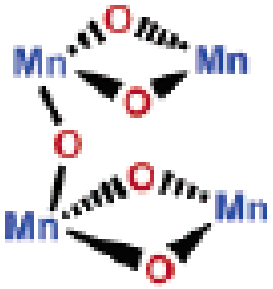
F



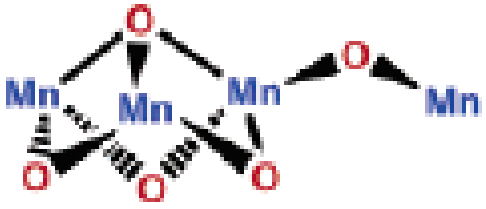
G



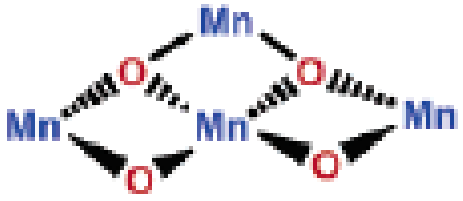
H



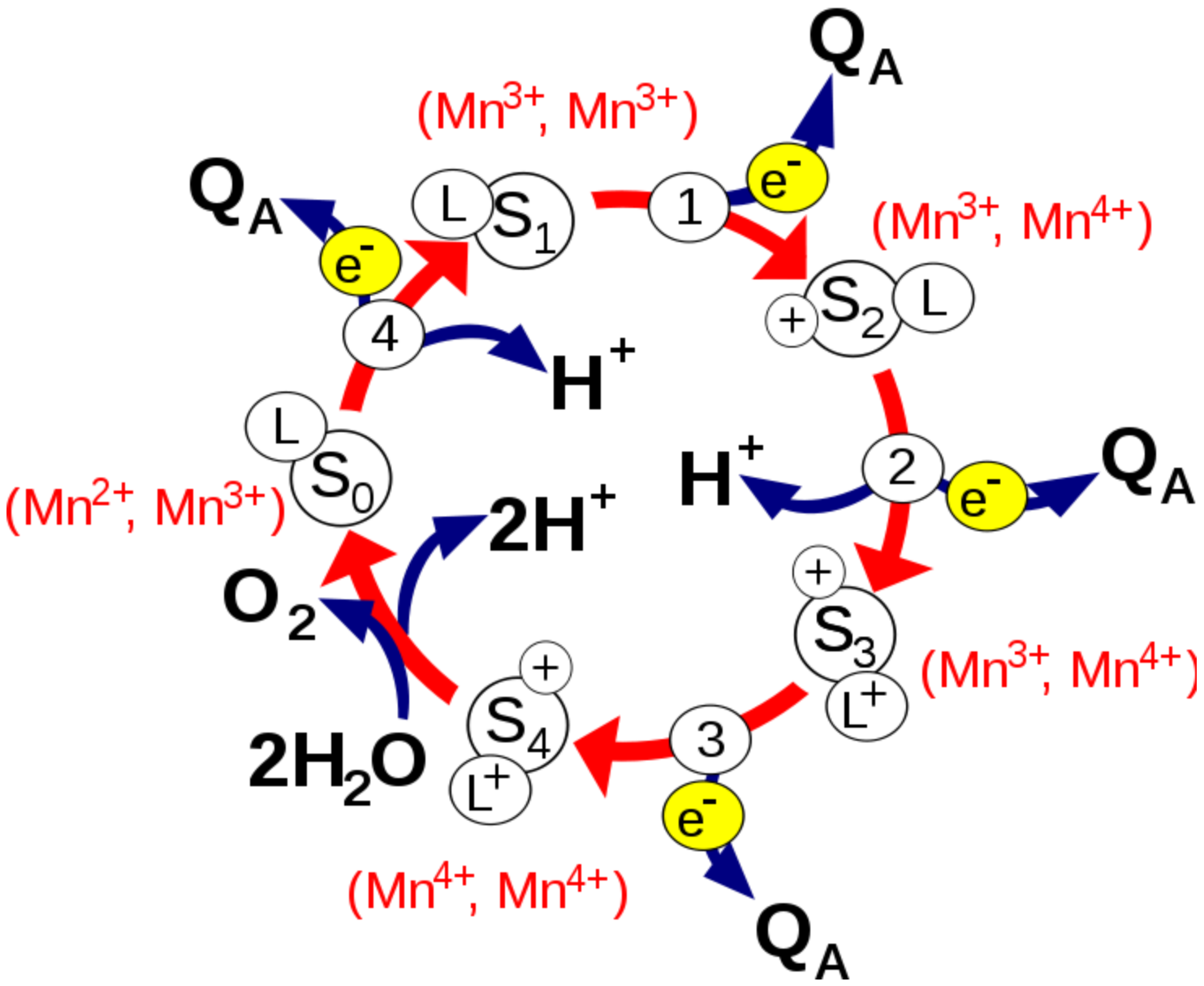
I



J



K


Q_A
(Mn^{3+}, Mn^{3+})
Q_A
e-
L
S_1
1
e-
(Mn^{3+}, Mn^{4+})
+
S_2
L
4
H^+
L
S_0
H^+
2
e-
Q_A
(Mn^{2+}, Mn^{3+})
$2H^+$
+
S_3
O_2
(Mn^{3+}, Mn^{4+})
L^+
$2H_2O$
+
S_4
3
L^+
e-
(Mn^{4+}, Mn^{4+})
Q_A