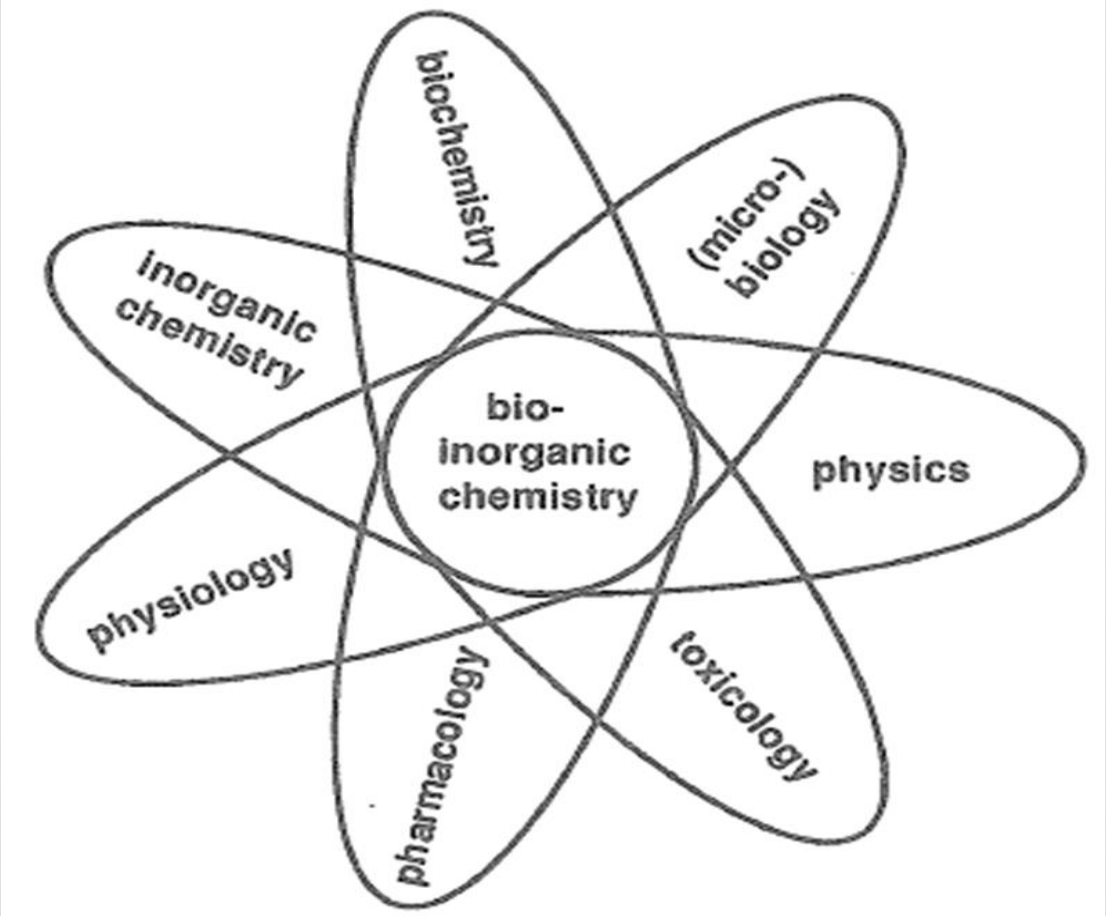
AN INTRODUCTORY LECTURE ON "BIOINORGANIC CHEMISTRY"

DISCIPLINE AT THE INTERFACE BETWEEN INORGANIC CHEMISTRY AND BIOLOGY



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- Classification

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iii. Conditions for Essentiality
iv. Elemental mass abundance in a 70 kg human

- Evolution of biological roles for essential metals
- Factors influencing the acquisition of metal ions by biological systems
- Biological ligands

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iii. Chelate Effect
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v. Metal Ion Complexation by peptides

- Biological functions of metal ions
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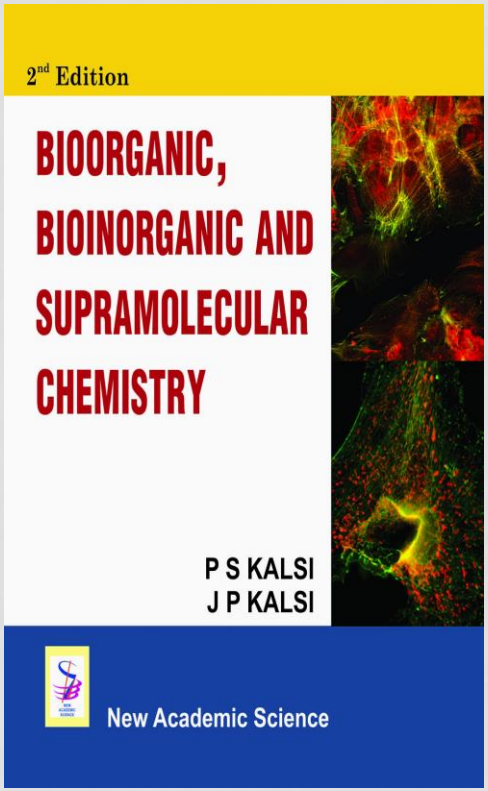
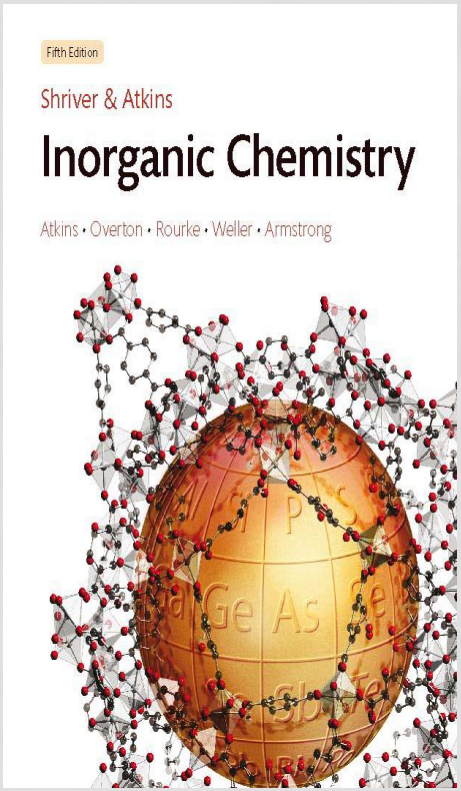
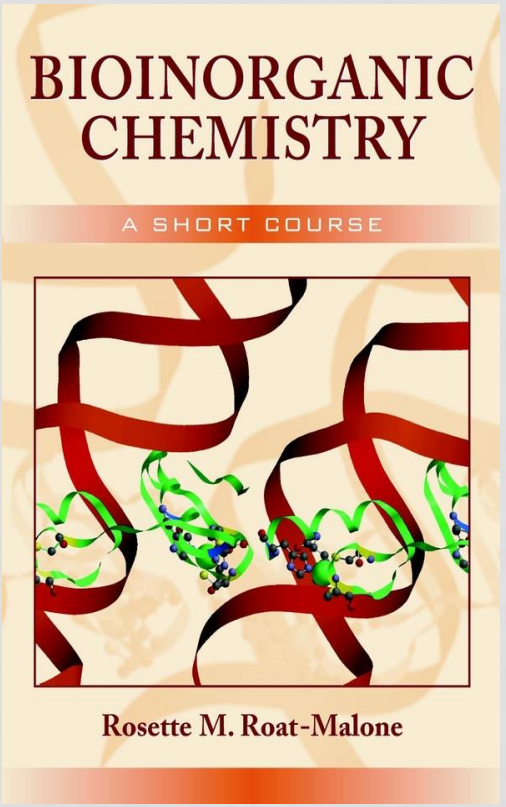
HISTORY AND BACKGROUND







HISTORY AND BACKGROUND

The progress of an inorganic chemistry of biological systems has had a curious history.

- The description of a rapidly developing field of chemistry as "bioinorganic" seems to involve a contradiction in terms, which, however, simply reflects a misconception going back to the beginning of modern science. In the early 19th century, chemistry was still divided into an "organic" chemistry which included only substances isolated from "organisms", and an "inorganic" chemistry of "dead matter".
- This distinction became meaningless after **W¨ohler's** synthesis of *"organic"* urea from *"inorganic"* ammonium cyanide in 1828.

HISTORY AND BACKGROUND

Nowadays, organic chemistry is defined as the chemistry of hydrocarbons and their derivatives, with the possible inclusion of certain nonmetallic heteroelements such as N, O and S, regardless of the origin of the material.

New and more sophisticated trace analytical methods have demonstrated the importance of large number of "inorganic" elements in biochemical processes and have thus revealed their importance.

A corresponding list would include:

- ***Metalloenzymes*** (40% of the known enzymes, especially *oxidoreductases* (**Fe**, **Cu**, **Mn**, **Mo**, **Ni**, **V**) and hydrolases (e.g. peptidases, phosphatases: **Zn**, **Mg**; **Ca**, **Fe**)
- ***Nonenzymatic metalloproteins*** (e.g. hemoglobin: **Fe**)

HISTORY AND BACKGROUND

- ***coenzymes, vitamins*** (e.g. vitamin B_{12}: **Co**);
- ***Nucleic acids:*** (e.g. DNA-M = **Na**, **K**);
- ***Hormones*** (e.g. thyroxine, triiodothyronine: **I**);
- ***Antibiotics*** (e.g. ionophores: valinomycin/**K**);
- ***Biominerals*** (e.g. bones, teeth, shells, coral, pearls: **Ca**, **Si**, . ..).
- ***low-molecular-weight natural products*** (e.g. chlorophyll: **Mg**);

HISTORY AND BACKGROUND

Historically "inorganic" elements were established quite early as essential components of living systems. Examples include following elements:

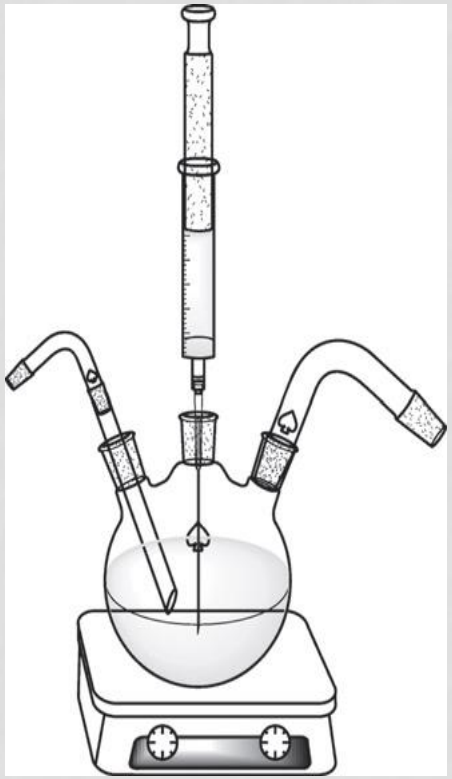
- the extractions of potassium carbonate (K_2CO_3, potash) from plants and of iron-containing complex salts $K_{3,4}[Fe(CN)_6]$ from animal blood in the 18th century.
- The discoveries of elemental phosphorus (as P_4) by dry distillation of urine residues in 1669 and of elemental iodine from the ashes of marine algae in 1811.
- In the middle of the 19th century, Liebig's studies on the metabolism of inorganic nutrients, especially of nitrogen, phosphorus and potassium salts, significantly improved agriculture, so that this particular field of science gained enormous practical importance. However, the theoretical background and the analytical methods of that time were not sufficient to obtain detailed information on the mechanism of action of essential elements because several of these occur only in trace amounts.

HISTORY AND BACKGROUND

- The inorganic elements like iron-containing haemoglobin and magnesium-containing chlorophyll, the **"pigments of life"**, were analysed and characterized later within a special subfield of organic chemistry, the ***chemistry of natural products***.
- It was only after 1960 bioinorganic chemistry became an independent and highly interdisciplinary research area. The following factors have been crucial for this development:
- Biochemical isolation and purification procedures, such as chromatography, physical methods of trace element analysis, such as atomic absorption or emission spectroscopy. All These advanced techniques have made it possible to detect and also characterize trace elements in biological materials.
- An adult human being, for example, contains about 2g of zinc in ionic form (Zn^{2+}). Although zinc cannot be regarded as a true trace element, the unambiguous proof of its existence in enzymes was established only in the 1930s.
- Genuine bio essential trace elements such as nickel and selenium have been known to be present as constitutive components in several important enzymes only since about 1970.

HISTORY AND BACKGROUND

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$$

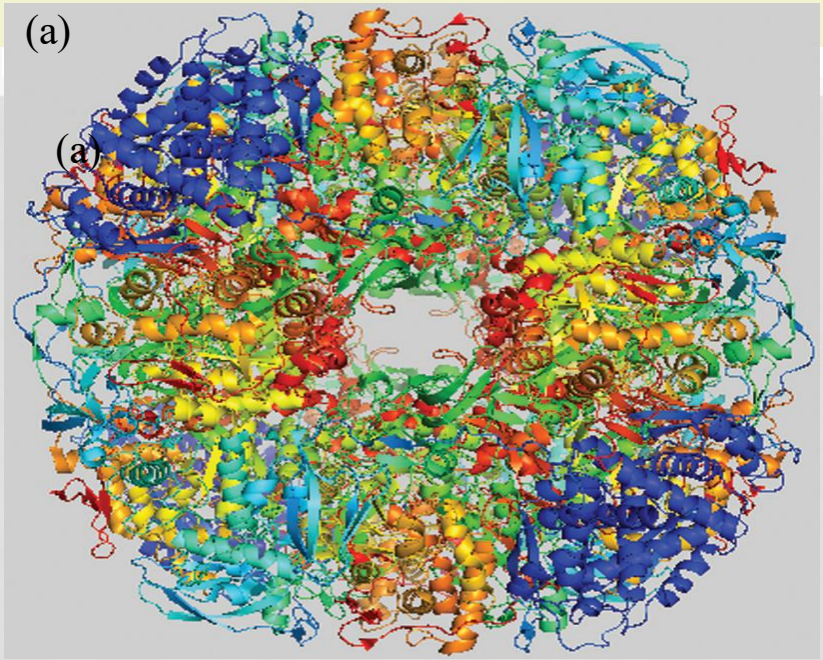


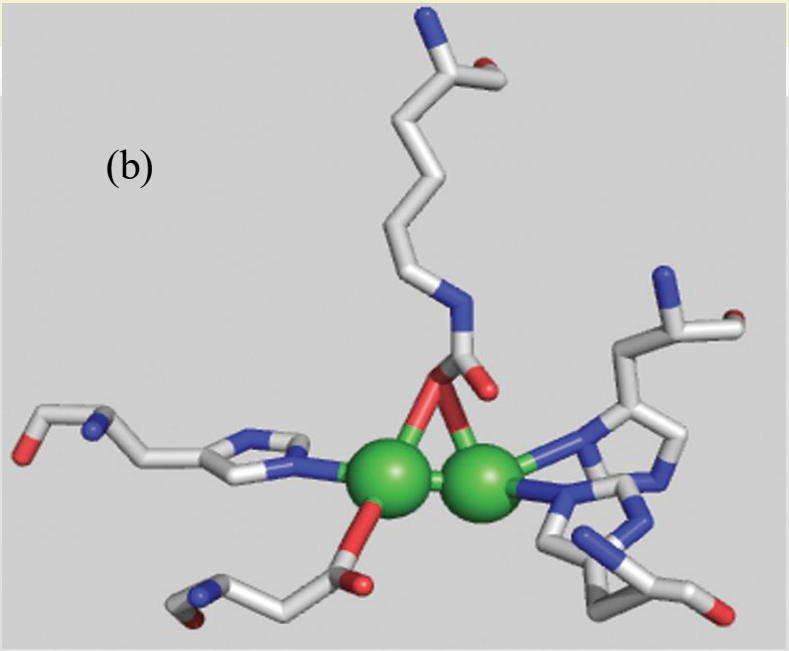
- **In a very different research area, the biological reduction of carbon dioxide by hydrogen to produce methane has been investigated by studying the relevant archaebacteria, which are found in sewage plants. Even though the experiments were carried out under strictly anaerobic conditions and all "conventional" trace elements were supplied (Figure), the results were only partly reproducible.**

- ***Eventually it was discovered that during sampling with a syringe containing a inert stainless steel (Fe/Ni) tip, minute quantities of nickel had dissolved. This accidental generation of Ni^{2+} ions led to a distinctive increase in methane production, and, in fact, several nickel containing proteins and coenzymes have since been isolated.***

Discovery of nickel as an essential trace element in the production of methane by archaea.

HISTORY AND BACKGROUND

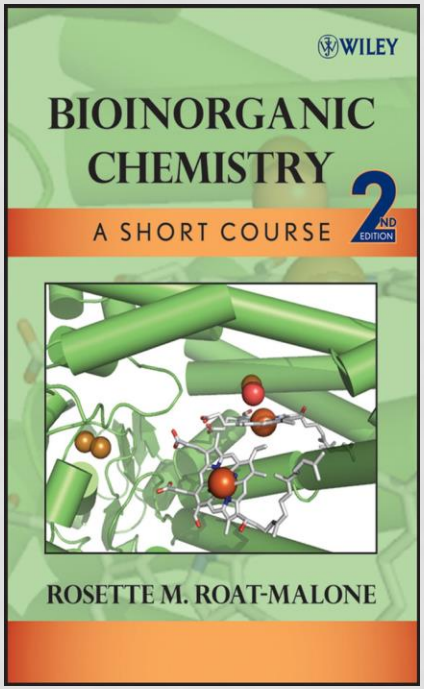


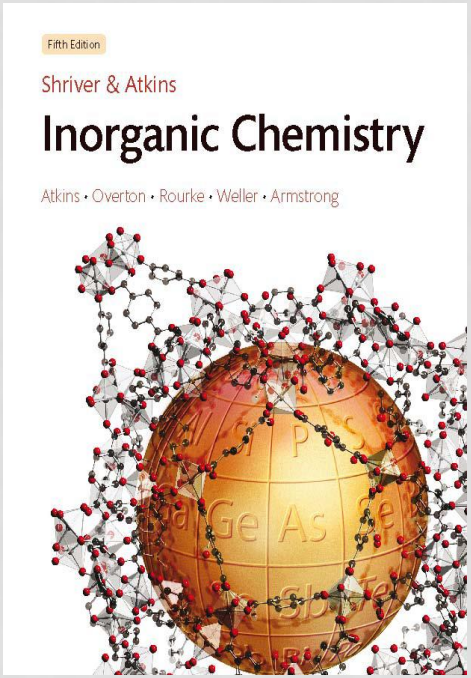


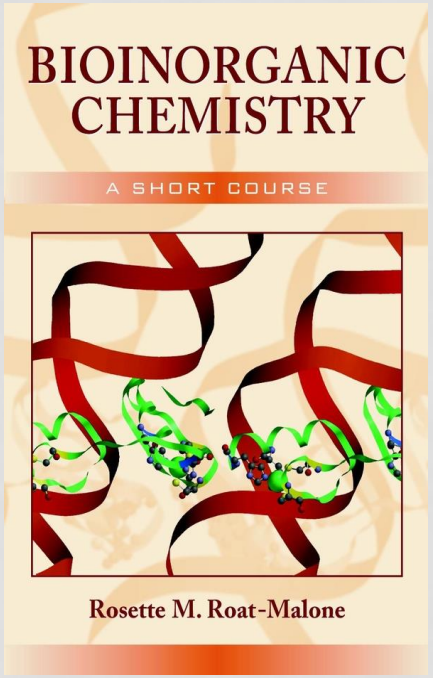
- ***Nickel-containing urease, the first enzyme to be crystallized.***
- **(a)** Crystal structure of the full assembly of *Helicobacter pylori* urease
- **(b)** Active site with two nickel centres (green spheres); histidine, aspartate, and a carbamylated lysine as ligands.

Richard Willstätter (Chemistry Nobel Prize, 1915) ***"Enzymes are not proteins"***
James Sumner*(urease)* (1929) and **John Northrop***(pepsin, trypsin)* (Nobel Prize in 1946) ***"Enzymes are proteins"***

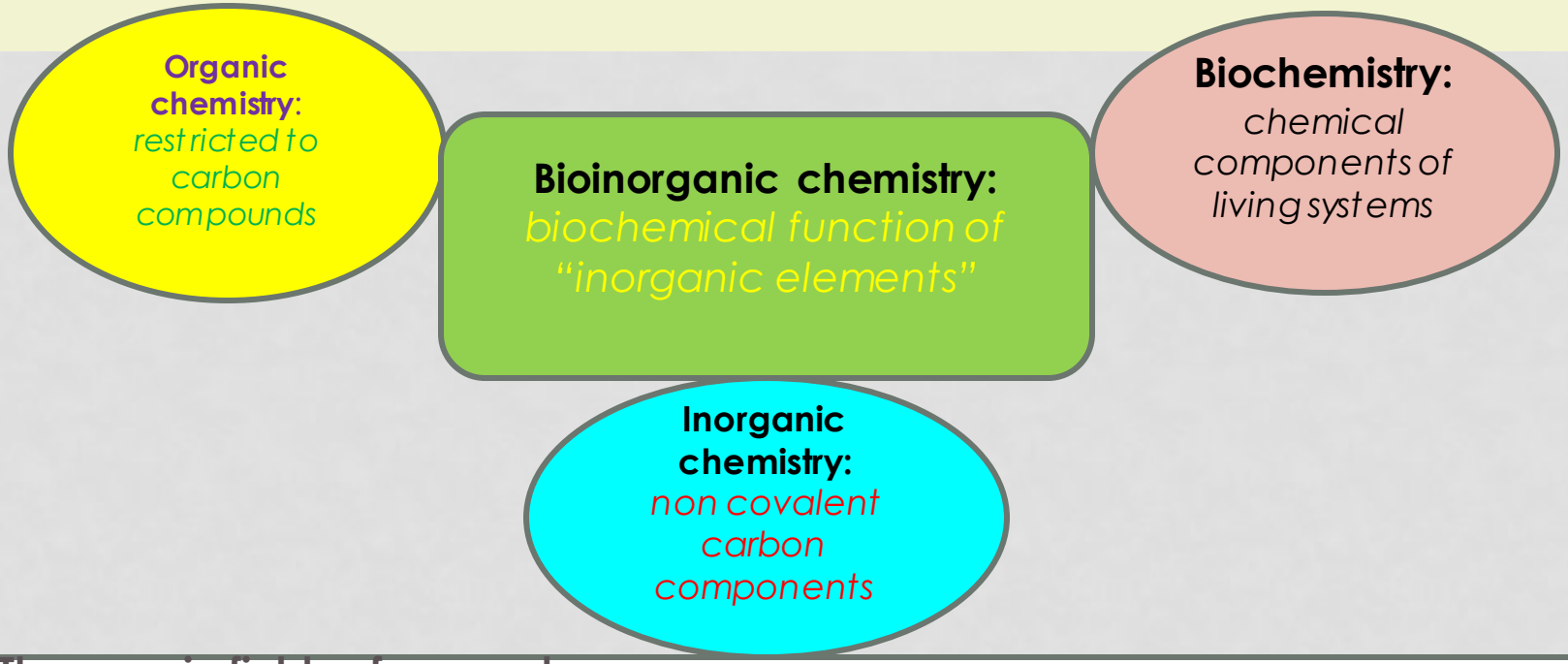
INTRODUCTION







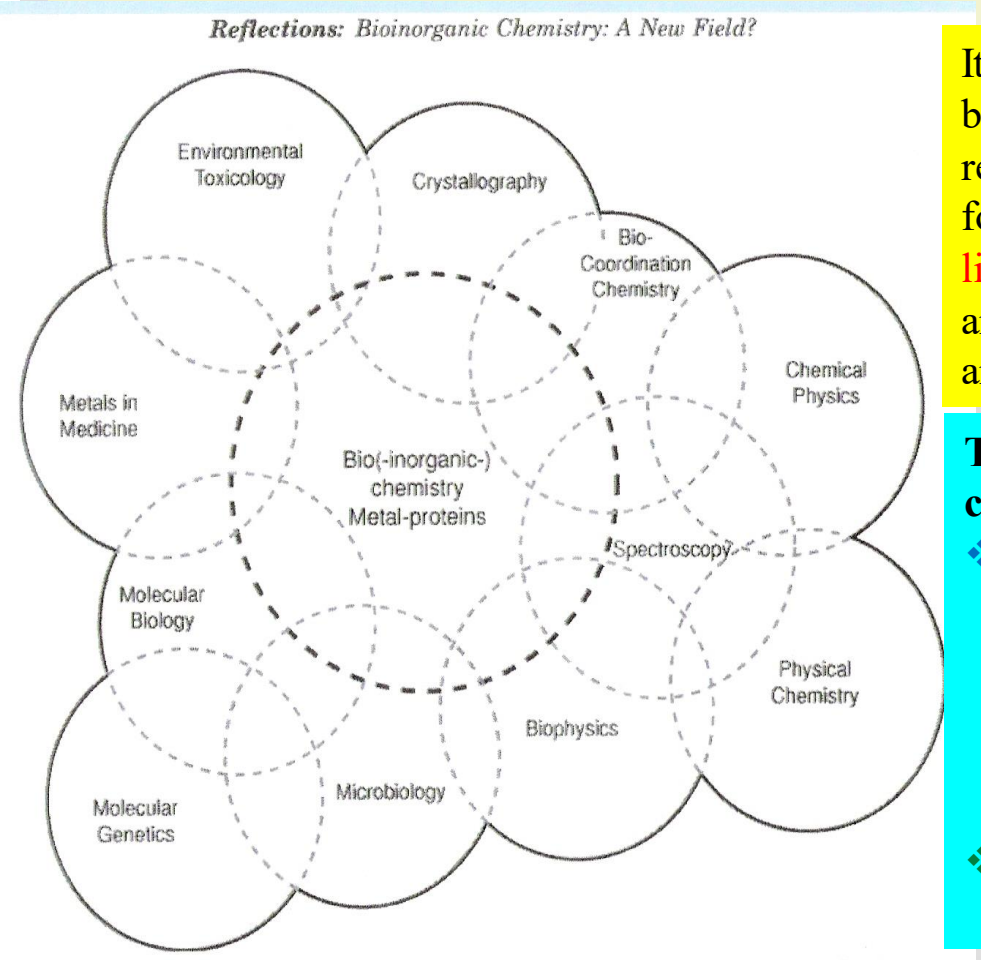
"BIOINORGANIC CHEMISTRY"–A CONTRADICTION?



Three main fields of research

- ***Enzymes, biological relevant complexes: biochemistry and coordination chemistry***
- ***Biomineralization: biochemistry and solid state (materials) chemistry***
- ***Synthesis of biomimiting model systems.***

INTRODUCTION AND DEFINITION

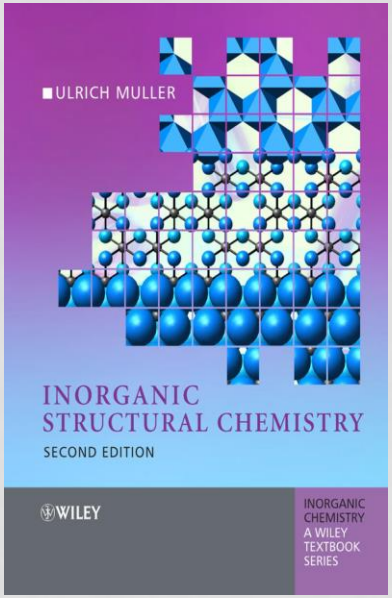


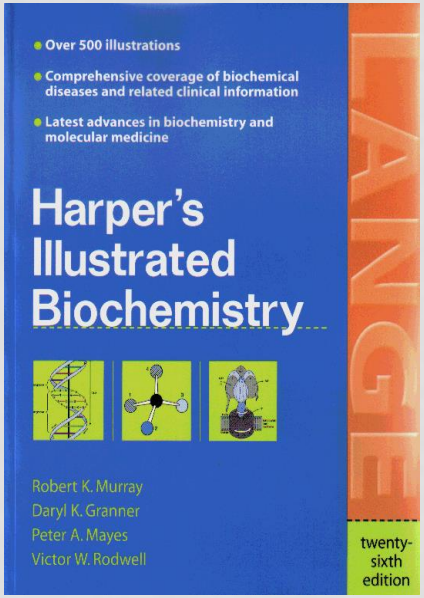
It is at the gateway of inorganic chemistry and biochemistry means it describe the symbiotic relationship between these two sub-disciplines, with focus upon the function of inorganic substances in living systems, including the transport, speciation and eventually, mineralisation of inorganic materials and their use in medicinal therapy and diagnosis.

Two main fields comes under bioinorganic chemistry:

- Investigations of inorganic elements in processes e.g. nutrition, the toxicity of inorganic species, including the ways in which such toxicities are overcome both by natural systems and by human intervention, and of metal-ion transport and storage in biology.
- The introduction of metals (metal complexes) into biological systems as probes and drugs.

CLASSIFICATION



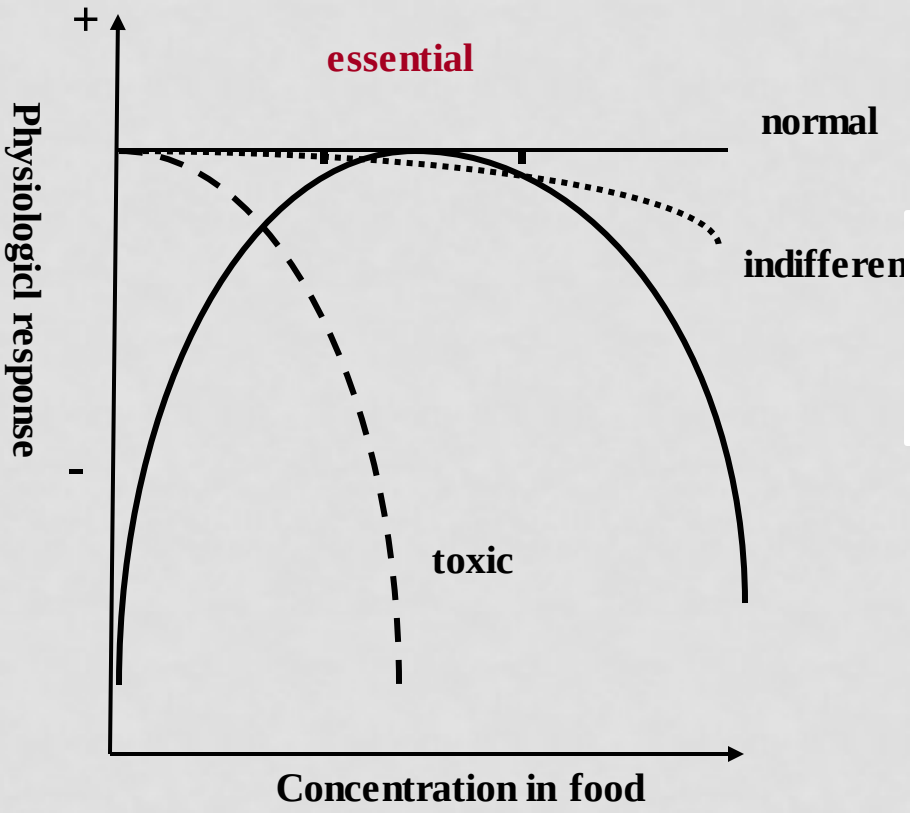


CLASSIFICATION:

ELEMENTAL COMPOSITION OF BIOLOGICAL SYSTEMS

Results of the chemical analysis of biological samples:

practically all elements of the periodic table (min. 50-70 element) can be detected in real biological samples so far by the analytical instruments.



Biological Elements

Essential elements
occur in a given concentration range,
they excert positive biological effects for several different species

Impurity elements
their quantity is a function of environmental effects

Indifferent Elements

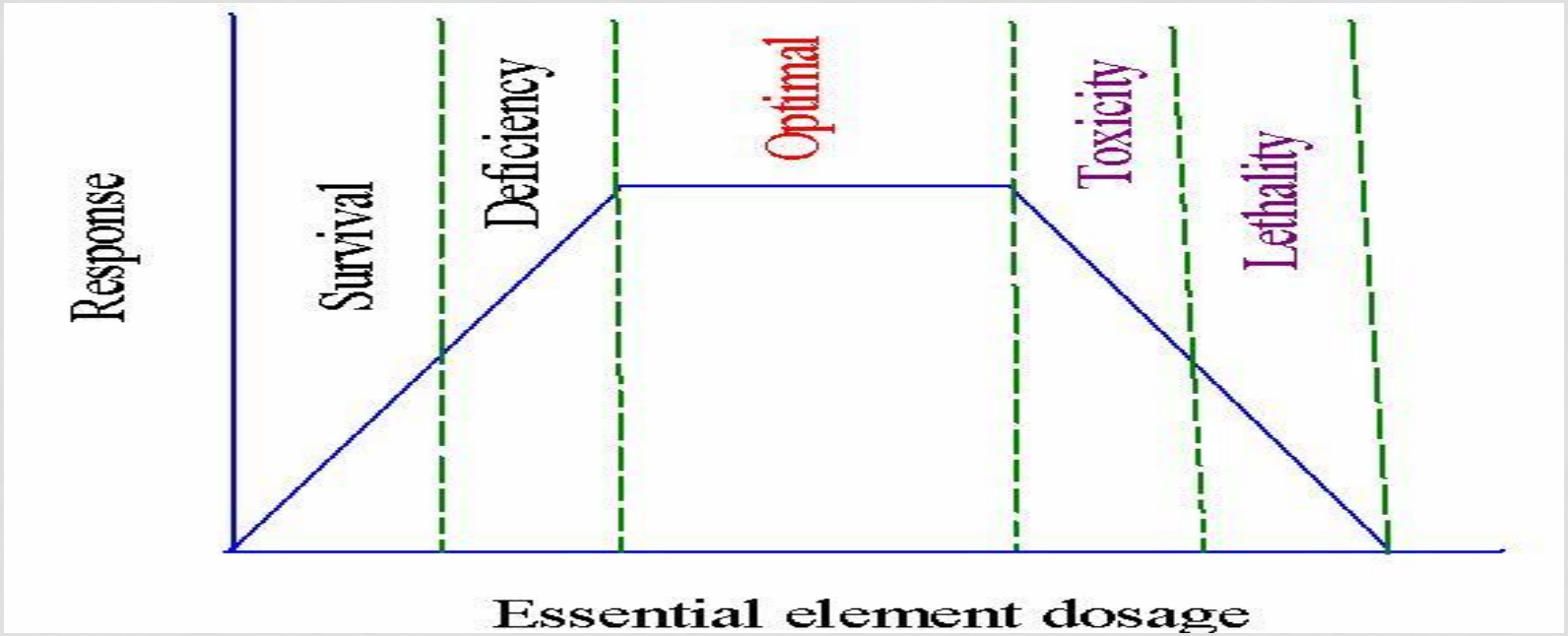
Toxic elements

Beneficial elements

CLASSIFICATION:

ESSENTIAL ELEMENTS (CONDITIONS FOR ESSENTIALITY)

- **Positive physiological response** can be ascribed **to their presence in the case of several species.**
- They occur **in well defined concentration range in each species**
- **Deprival** (from food) will results in reproducible and negative physiological changes. These effects can be reversible or at least reduced by **addition** of the given element.
- **Their deficiency and excess** is connected with well defined **diseseases.**
- The biological presence of the element is connected with **well defined biochemical processes.**



CLASSIFICATION:

ESSENTIAL ELEMENTS (CONDITIONS FOR ESSENTIALITY)

❖ ***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

Consequences:

- **At lowest dosages organism does not survive.**
- **In deficiency regions, the organism exists with less than optimal functions.**
- **After optimal dosage (plateau region), higher dosage cause toxic effects in the organism eventually leading to lethality.**

CLASSIFICATION:

BIOLOGICAL ELEMENTS

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

CLASSIFICATION:

BIOLOGICAL ELEMENTS

Difference between Essential and trace elements?

Essential elements:

- Required for maintenance of life, its absence results in death or malfunctioning
- 30 elements are essential for life processes in plants and animals

Trace Elements:

- Elements required in very small amount (in mg or µg)
- 19 of the 30 elements are trace elements and even out of these ***12 are transition elements***

CLASSIFICATION:

BIOLOGICAL ELEMENTS (CONDITION FOR ESSENTIALITY)

The following makes some elements essential:

- The element must have some unique chemical property that an organism can use to its advantage and without which it cannot survive.
- Adequate amounts of the element must be available in the environment in an easily accessible form.
- Most living matter consists primarily of ***bulk elements*** – oxygen, carbon, hydrogen, nitrogen, and sulfur. They are the building blocks of the compounds that make up our organs and muscles; they also constitute the bulk of our diet.
- Six elements—sodium, magnesium, potassium, calcium, chlorine, and phosphorus—are called ***macrominerals*** and provide essential ions in body fluids and form the major structural components of the body.

Remaining essential elements called ***trace elements*** and are present in small amounts.

CLASSIFICATION:

TRACE ELEMENTS

- It is difficult to detect low levels of some of the essential elements, so the trace elements were relatively slow to be recognized.
- Many compounds of trace elements are toxic.
- How can elements present in small amounts have such large effects on the health of an organism?
- Trace elements participate in an *amplification* mechanism—they are essential components of larger biological molecules that are capable of interacting with or regulating the levels of relatively large amounts of other molecules.

CLASSIFICATION:
BIOLOGICAL ELEMENTS

Bulk Elements(Organic skeleton components)	C, H, O, N, S, P
Macro Minerals (Inorganic skeleton and body-fluid components)	Na, K, Ca, Mg, Cl
Trace elements:	
Main group	Se, Si, Sn, F, I
Transition metal	Fe, Zn, Cu, Mn, Co, Ni, V, Cr, Mo
Non essential (impurity) elements:	
Beneficial	B, Ti, W,... (As, Cd, Pb....)
Toxic	Hg, Cd, Pb, Tl, As, Pt metals, Be, Ba,.

CLASSIFICATION:

ELEMENTAL MASS ABUNDANCE IN A 70 KG HUMAN

Bulk elements

Oxygen	44 kg
Carbon	12.6 kg
Hydrogen	6.6 kg
Nitrogen	1.8 kg
Sulfur	0.1 kg

Macrominerals

Calcium	1700 g
Phosphorus	680 g
Potassium	250 g
Chlorine	115 g
Sodium	70 g
Magnesium	42 g

Trace elements

Iron	5000 mg
Silicon	3000 mg
Zinc	1750 mg
Rubidium	360 mg
Copper	280 mg
Strontium	280 mg
Bromine	140 mg
Tin	140 mg
Manganese	70 mg
Iodine	70 mg
Aluminum	35 mg
Lead	35 mg
Barium	21 mg
Molybdenum	14 mg
Boron	14 mg
Arsenic	~ 3 mg
Cobalt	~ 3 mg
Chromium	~ 3 mg
Nickel	~ 3 mg
Selenium	~ 2 mg
Lithium	~ 2 mg
Vanadium	~ 2 mg

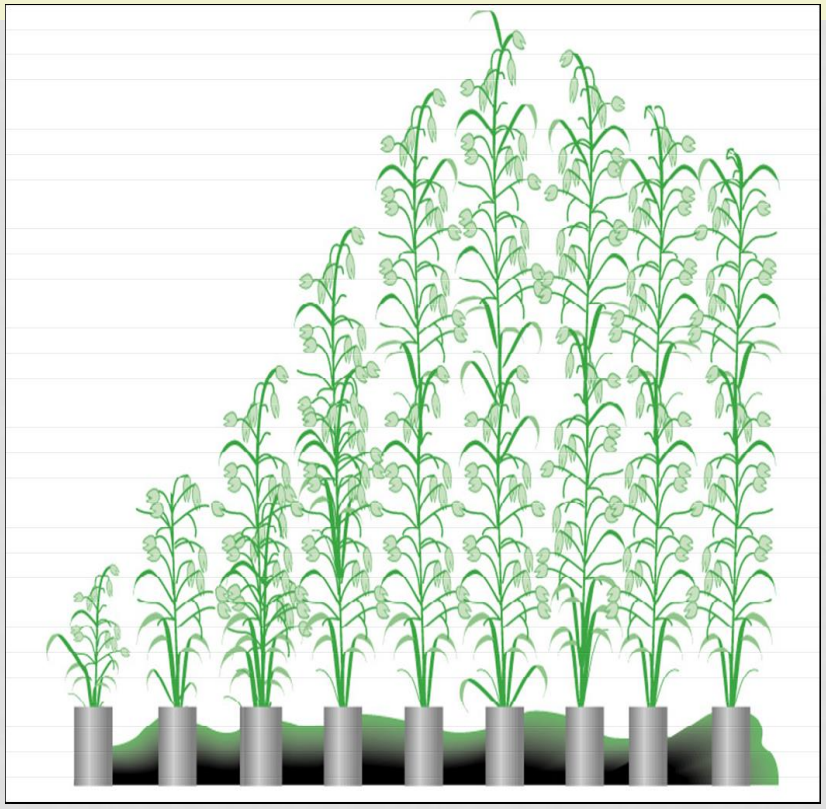
- ✓ *Fe was the first essential transition metal discovered (17th century)*
- ✓ *Zn in 1896*
- ✓ *The rest followed in the 1900s*

CLASSIFICATION:

"ALL THINGS CAN BE POISONS"

Element	Sea Water (M) x 10-8	Human Plasma (M) x 10-8
Fe	0.005-2	2230
Zn	8.0	1720
Cu	1.0	1650
Mo	10	1000
V	4.0	17.7
Mn	0.7	10.9
Cr	0.4	5.5
Ni	0.5	4.4
Co	0.7	0.0025

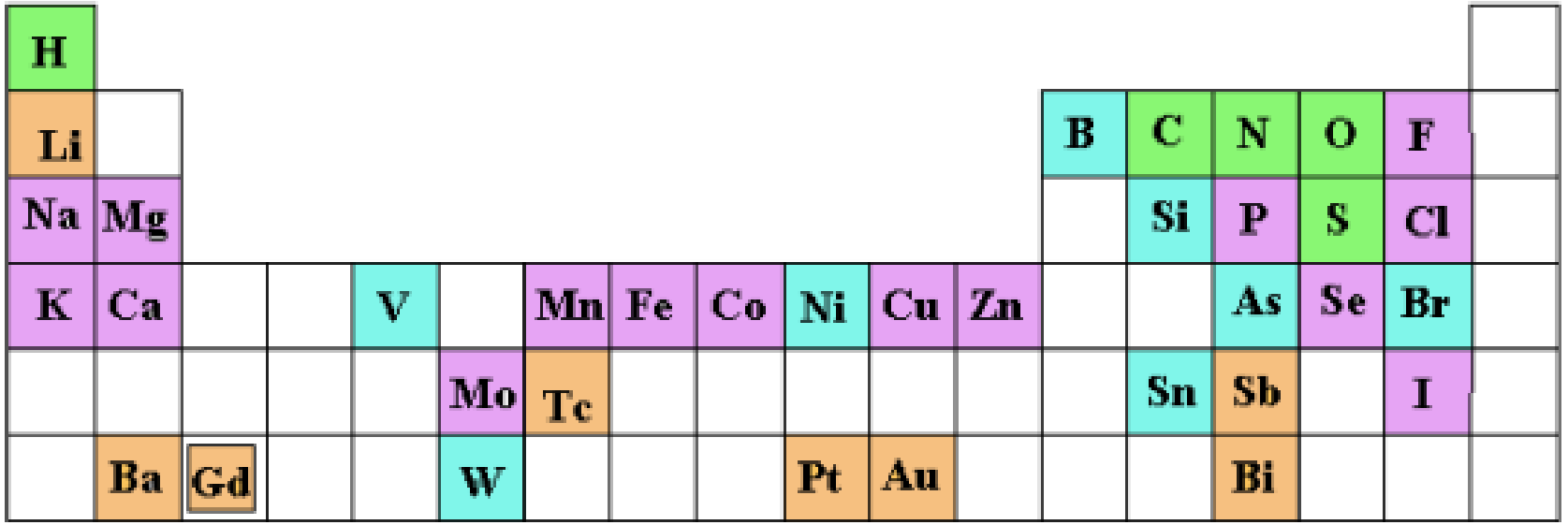
Bertini, I.; Gray, H. B.; Lippard, S. J.; Valentine, J. S. Bioinorganic Chemistry; University Science Books: Sausalito, CA, 1994.



It depends on: dosage, individual health, and way of administration

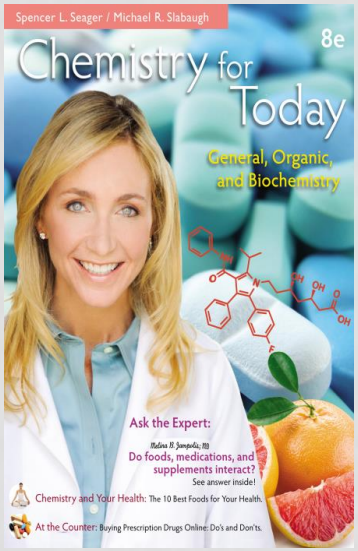
CLASSIFICATION:

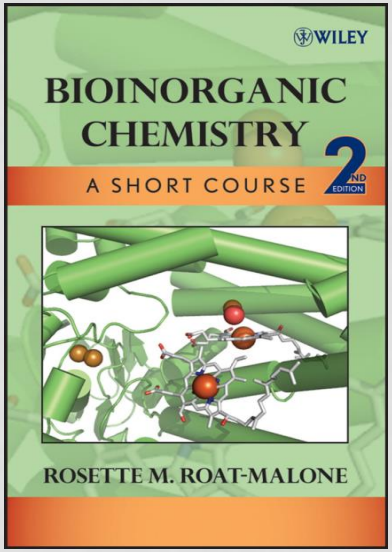
BIOLOGICAL ELEMENTS

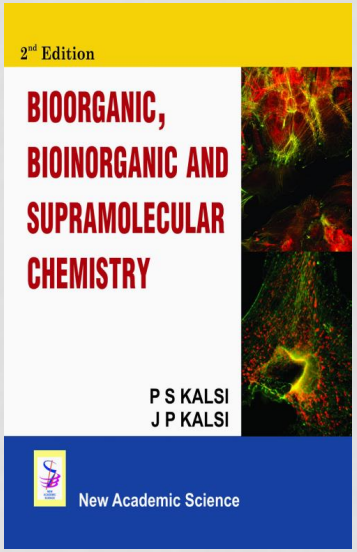


Periodic Table of the bio-elements: *elements building up bio-mass, additional essential elements, essential for some groups of organisms, medicinally important elements.*

EVOLUTION OF BIOLOGICAL ROLES FOR ESSENTIAL METALS







EVOLUTION OF BIOLOGICAL ROLES FOR ESSENTIAL METALS

Why have certain elements been "selected" for use in biological systems?

a. ***Their abundance*** *(availability in the earth's crust or oceans)*
b. ***Their basic fitness*** *(intrinsic chemical suitability)*
c. ***evolutionary adaption*** *to realize critically required specificity.*

Lighter elements are more abundant in general and therefore utilized more. **Therefore 3d metals, rather than 4d, are used as catalytic centers in metalloenzymes**

EVOLUTION OF BIOLOGICAL ROLES FOR ESSENTIAL METALS

Why has Mo (4d) rather than Cr (3d) been utilized more biologically?

Although Mo is rare in the earth's crust, Mo is the most abundant transition metal in sea water as MoO_4^- has fairly high solubility in water. Better correlation exists between the abundance of elements in human body and in sea water than between the human body and the earth's crust.

✓ Taken as evidence for the oceans as the site of evolution of life.

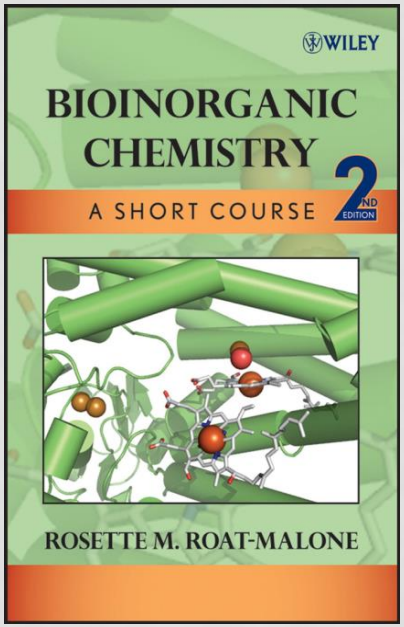
Despite the high abundance of Si, Al and Ti (the 2nd, 3rd and 10th most abundant elements on earth). Why they are not much utilized biologically?

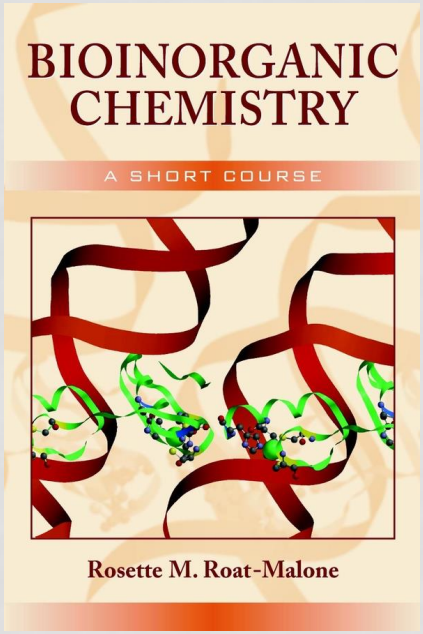
Because of the insolubility of their naturally occurring oxides (SiO_2, Al_2O_3, TiO_2) under physiological conditions. A lower oxidation state is unavailable for Si and Al and unstable for Ti in an aerobic environment and is readily oxidized to Ti(IV) at pH 7.

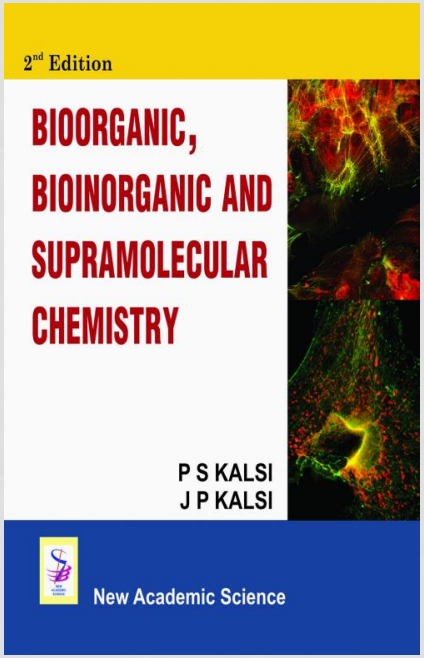
EVOLUTION OF BIOLOGICAL ROLES FOR ESSENTIAL METALS

Why has iron been used so widely in biology although Fe^{3+}, its most stable oxidation state, is highly insoluble at pH 7	Complex biological mechanisms have been developed to accommodate the low solubility of $Fe(OH)_3$ (K_{sp} = 1 x 10–38) ~ pH 7, and take advantage of its high "availability".
Co^{2+} and Zn^{2+} have very similar coordination chemistry and ionic size and can be interchanged in many Zn enzymes without loss of activity. Why is Co not utilized more biologically?	Zn is much more abundant and therefore has been utilized more.
Why has cobalt been given an essential role in cobalamins despite its very low availability?	The unique properties of cobalt (e.g. its oxidation states, redox potentials and coordination chemistry) is needed to achieve essential functions of B_{12} coenzymes.

FACTORS INFLUENCING THE ACQUISITION OF METAL IONS BY BIOLOGICAL SYSTEMS







FACTORS INFLUENCING THE ACQUISITION OF METAL IONS BY BIOLOGICAL SYSTEMS:

The major obstacles preventing the acquisition of metal ions are following:

- **Bioavailability:** This term implies more than just incidence of an element on earth and include its prevalence in environment where life is found. e.g, Zinc sulphide minerals are very common in biosphere but in this form zinc is not very usable.

- The presence of chemical competitors that impedes the acquisition of desired nutrient also reduce the bioavailability e.g, Mo Being most abundant metal ion in ocean present in the form of oxyanion, molybdate, a species that highly soluble and amenable for uptake. It is very similar to sulphate and phosphate oxyanions. Competition from these other oxyanions may seriously hinder the ability to transport molybdate into the cell.

- **Uptake Mechanism:** Cellular membranes are effective permeability barriers that blocks passive diffusion of charged metal ions. Therefore there is a need of ***transporter molecules*** which are embedded in cellular membranes.

FACTORS INFLUENCING THE ACQUISITION OF METAL IONS BY BIOLOGICAL SYSTEMS:

- **Target Delivery:** the critical problem is the delivery of metal ions to their ultimate targets is transport within the cytoplasm or the space between membranes.
  - Cytoplasm is full of natural metal ion chelators such as soluble proteins, peptides ***(glutathione),*** and organic metabolites ***(citrate)*** which may impede the ability of metalloprotiens to acquire their metal cofactor by acting as competitive chelators. E.g, Cu, specific soluble transfer protein called ***copper chaperones*** facilitate safe transfer of Cu from plasma membrane to Cu- Containing proteins. Similar protein for iron transport is ***transferrin.***
- **Storage Mechanism:** The level of a particular metal ion available to an organism in its diet or environment can change drastically over the time.
  - Storage mechanism also provides a mean by which excess metal ions can be detoxified. E.g, excess Fe and Cu can generate reactive oxygen species, that damage DNA, Lipids and proteins.
  - Since metal ions are both essential and toxic, a delicate balance, or ***homeostasis*** must be maintained. This is generally regulated by sensors that governs the activity of transporter, storage molecule and detoxifying enzymes.

FACTORS INFLUENCING THE ACQUISITION OF METAL IONS BY BIOLOGICAL SYSTEMS:

General Transport/Storage Problems:

Capture of Trace Ions from the Environment

- Homeostatic Control of Concentration is essential for life
- Bulk ions present in high concentration
- Trace ions must be actively accumulated
- Trace ions are often insoluble

Selectivity of Ion Uptake is Essential

- Toxic ions must be excluded
- Beneficial ions must be accumulated
- Specialized Molecules have evolved

FACTORS INFLUENCING THE ACQUISITION OF METAL IONS BY BIOLOGICAL SYSTEMS:

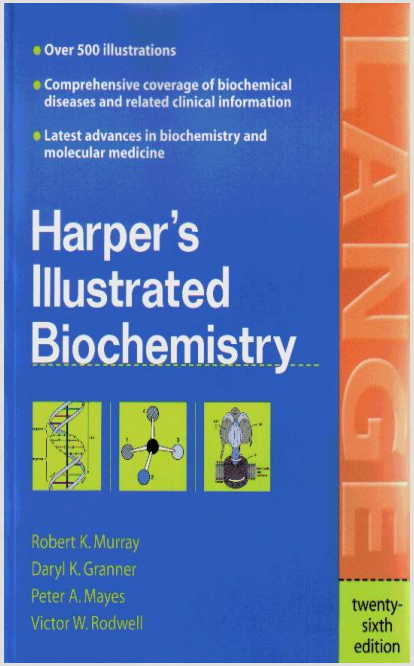
Why does biology utilize transition metals?

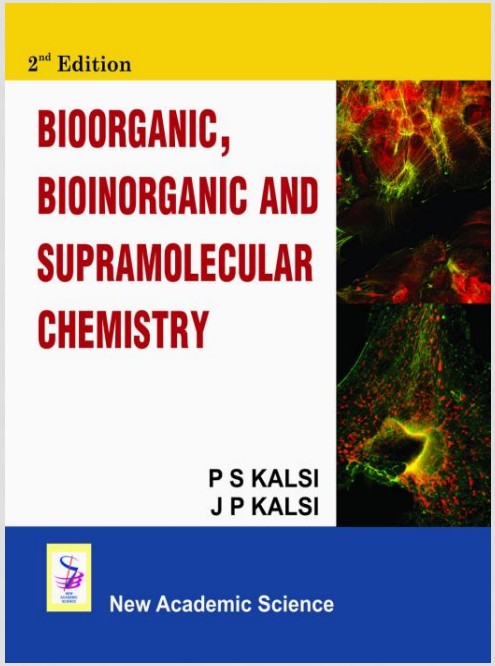
- Transition Metals are extremely good catalytic active sites in enzymes, because they are stable in a variety of geometries and C.N.
- have multiple coordination sites
- Are stable in a variety of oxidation states
- Are able to change the reactivity of ligands
- Have "weak" coordinate bonds (where needed)
- Are capable of stabilizing intermediates.

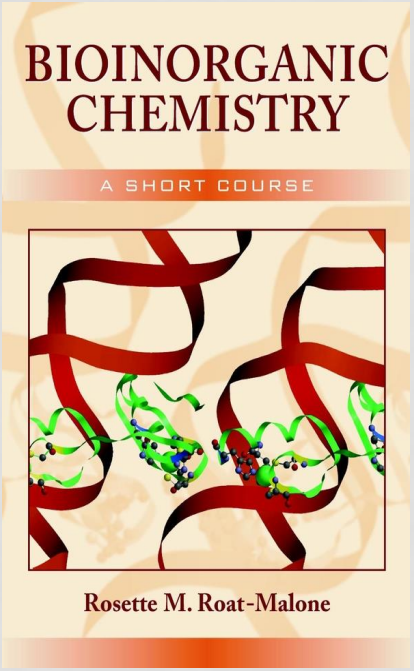
Why does it matter what ligands are attached to the metal ?

- Tune Redox Properties
- Assist in stabilization of multiple oxidation states of transition metals Lability/Stability

BIOLOGICAL LIGANDS







BIOLOGICAL LIGANDS:

TYPES OF LIGANDS IN BIOLOGICAL SYSTEM

- The ligand binding in an typical Coordination M-L Bond (50-150 KJ mol^{-1}) is much weaker than covalent bonding(energy of a single C-C bond is 300-400 KJ mol^{-1}), for hemes and vitamin B_{12} allows much more flexibility in small molecule binding and dissociation (signalling) under biological conditions.

- Other even weaker interactions such as *hydrogen bonding* (**20-60 KJ mol^{-1}**)and *van der Waal's interactions* (<50 KJ mol^{-1}) are crucial for correct structure and functioning of biological system.

- For ***metallodrugs*** these non covalent interactions can play plays an important role in target recognition.

BIOLOGICAL LIGANDS:
HARD AND SOFT LIGAND

- In 1923 the American chemist G.N. Lewis provided a broad definition of acids and bases, which covered acid–base reactions not involving the traditional proton transfer:

an acid is:

an electron-pair acceptor *(Lewis acid)*

a base is:

an electron-pair donor *(Lewis base)*.

- The concept was extended to metal–ligand interactions with the ligand acting as donor, or Lewis base, and the metal ion as acceptor, or Lewis acid.

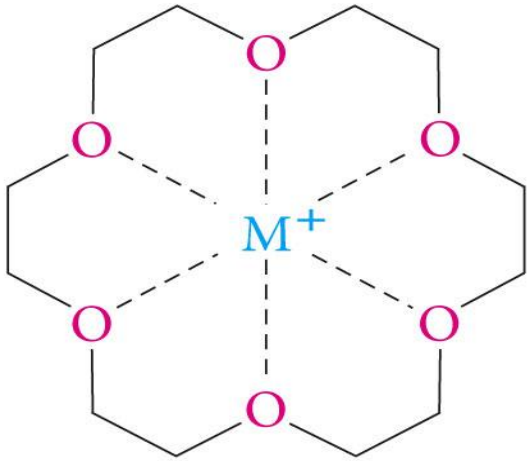
BIOLOGICAL LIGANDS:
HARD AND SOFT LIGAND

*Classification of biologically important metal ions and ligands according to the **'hard–soft acid–base'** concept and their general characteristics*

Acid/acceptor (metal ions)		Base/donor (ligands)
Hard	High charge density, Small ionic radius, No easily excited outer shell electrons **Na+, K+, Mg^{2+}, Ca2+, Cr3+, Fe^{3+}, Co^{3+}**	Low polarizability High electronegativity, Vacant, high-energy orbitals Hard to oxidize H_2O, OH^-, CO_2^-, CO_3^{2-}, NO^{3-}, PO_4^{3-}, $ROPO_3^{2-}$ PO_4^{3-}, $ROPO_3^{2-}$, $(RO)_2PO^{2-}$, ROH, RO^-, R_2O,NH_3, RNH_2, Cl^-
Intermediate	**Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}**	NO^{2-}, SO_3^{2-}, Br^-, N^{3-}, imidazole
Soft	Low-charge density, Large ionic radius, Easily excited outer shell electrons ,**Cu^+, Ag^+, Au^+, Pt^{2+}, Pb^{2+}, Hg^{2+}, Cd^{2+}**	High polarizability, Low-energy vacant orbitals, Easily oxidized RSH, RS^-, CN^-, CO

BIOLOGICAL LIGANDS:
HARD AND SOFT LIGAND

- *In general, hard metal cations form their most stable compounds with hard ligands, whereas soft metal cations form their most stable compounds with soft ligands.* ***(HSAB Principal)***
- ***Crown ethers*** *are hard ligands that have cavities suitable for encapsulating hard metal ions. The [18]-crown-6 ether shown in Figure with its 2.6- to 3.2-A˚ hole provides a good fit for the potassium ion, which has a radius of 2.66A*



M^+ complexed in [18]crown-6

X^-

Cavity diameter		2.6–3.2 Å
Ion diameter	Na^+	1.90 Å
	K^+	2.66 Å
	Cs^+	3.34 Å

Only this ion achieves a snug fit.

BIOLOGICAL LIGANDS:

HARD AND SOFT LIGAND

- It is possible to modify a hard nitrogen ligand toward an intermediate softness by increasing the polarizability of its substituents or the π electron cloud about it. e.g, ***the imidazole nitrogen of the amino acid histidine.***

- Increasing the softness of phosphate ion substituents can transform the hard oxygen ligand of $(RO)_2PO_2^-$ to a soft state in $(RS)_2PO_2^-$. Soft cations and anions are those with highly polarizable, large electron clouds—that is, Hg^{2+}, sulfur ligands as sulfides or thiolates, and iodide ions.

- Hard cations can be thought of as small dense cores of positive charge, whereas hard ligands are usually the small highly electronegative elements or ligand atoms within a hard polyatomic ion—that is, oxygen ligands in $(RO)_2PO_2^-$ or in $CH_3CO_2^-$.

BIOLOGICAL LIGANDS:

CHELATE EFFECT

- ✓Metal ions dissolved in water are effectively complexes to water molecules. Displacing the set of water ligands, partially or entirely by another set, in such aqua metal ions results in forming what is more conventionally known as complexes. Displacement of water molecules by multi-dentate ligands results in more stable complexes than similar systems with none or fewer chelates. Such enhanced stability, referred to as the ***chelate effect.***
- ➢*The chelate effect in proteins is also important, since the three-dimensional (3-D) structure of the protein can impose particular coordination geometry on the metal ion. This determines the ligands available for coordination, their stereochemistry and the local environment, through local hydrophobicity/hydrophilicity, hydrogen bonding by nearby residues with bound and non-bound residues in the metal ion's coordination sphere.*

BIOLOGICAL LIGANDS:
CHELATE EFFECT

- **Chelation is important in medicines.** *Treatment of the hereditary disease* ***thalassaemia*** *requires regular blood transfusion and the excess iron can be removed by the* ***hexadentate chelator desferrioxamine*** ***(Desferal®, DFO)*** *with pFe , depicted in Figure. DFO loses three protons when it binds to* Fe^{3+}.

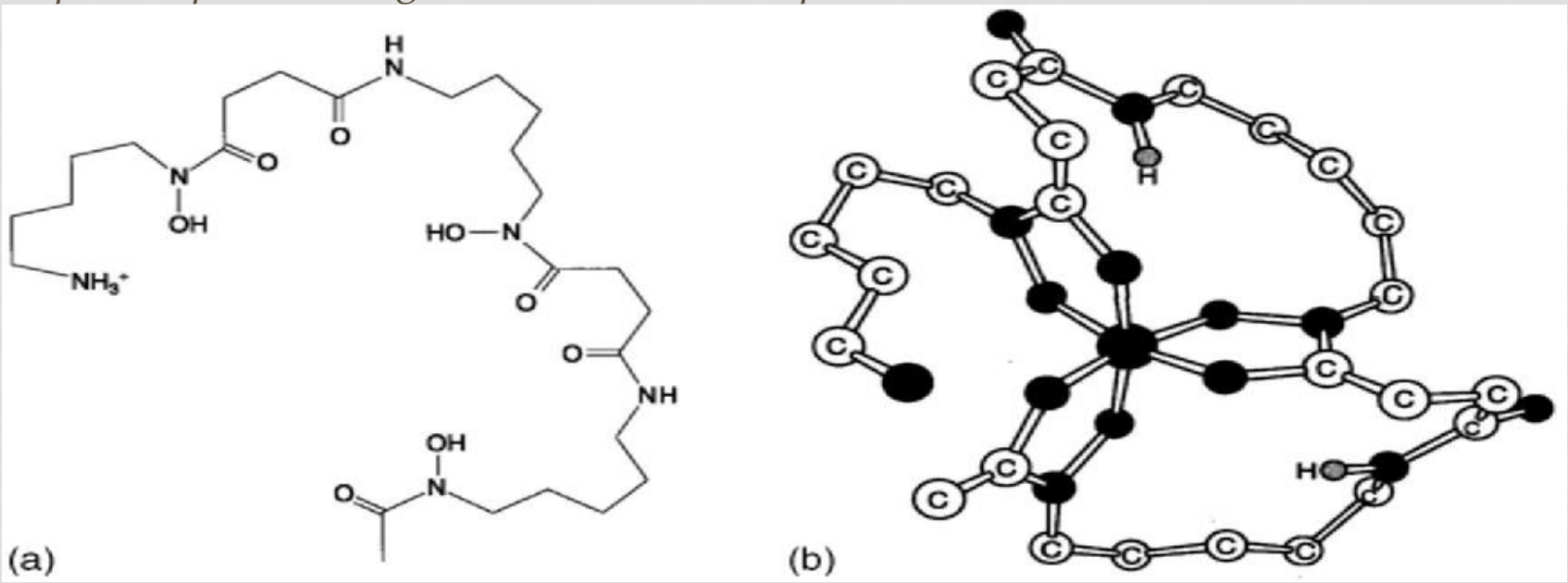
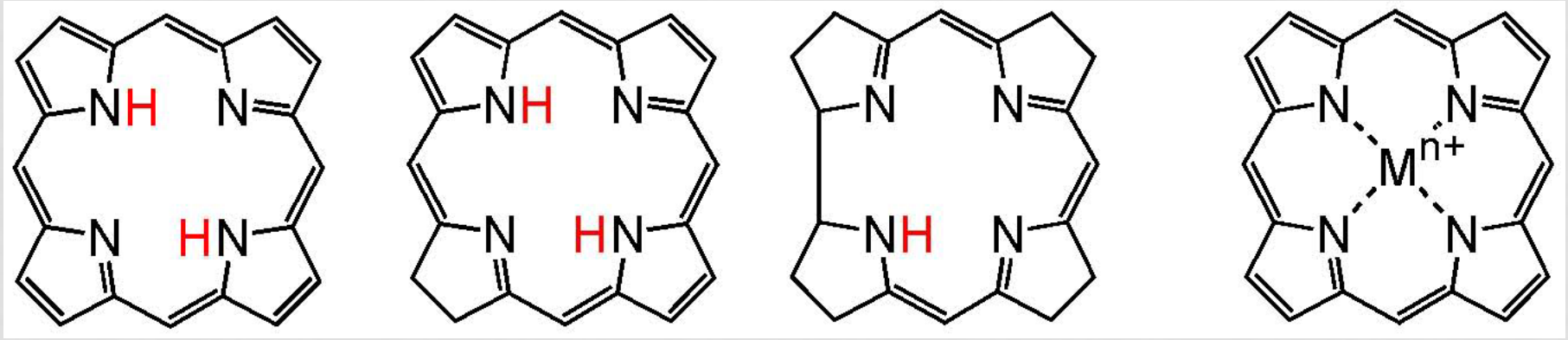


Fig. (a) The metal chelator **desferrioxamine (DFO)** and **(b)** its complex with iron.

BIOLOGICAL LIGANDS:

CHELATE EFFECT

- The corrins and porphyrins are another important class of natural chelator molecules . They are thermodynamically very stable and have four nearly coplanar pyrrole rings, the nitrogen atoms of which can selectively accommodate a number of different metal ions in different oxidation states like **Fe^{2+} in haem**, **Mg^{2+} in chlorophyll** and **Co^{3+} in vitamin B_{12}.**



PORPHYRIN
Hemoglobin
Myoglobin
Peroxidases

CHLORIN
Chlorophyll (Mg2+)
Tunichlorine(Ni2+)

CORRIN
Cobalamin(Co2+)

Metallaporphyrin complex

BIOLOGICAL LIGANDS:

CHELATE EFFECT

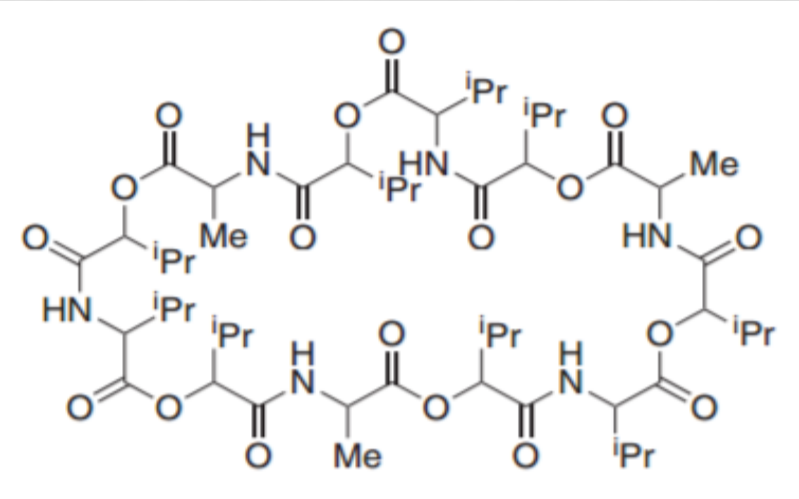
Metal ion	Ionic radius[a] (pm)	Suitability as metal center in complexes with tetrapyrrole macrocycles
Be^{2+}	45	too small
Mg^{2+}	72	proper size; → chlorophyll
Ca^{2+}	100	too big
Al^{3+}	53	rather small
Ga^{3+}	62	gallium(III) porphyrin complexes have been found in crude mineral oil but not in living organisms (very rare element)
In^{3+}	80	rather large, rare element
$O{=}V^{2+}$ (not spherical)	~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+}	~60	proper size; use in synthetic oxidation catalysts
Fe^{2+}(h.s.)	78	too large (*out-of-plane* structure;
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
Co^{2+}(l.s.)	65	proper size; → cobalamins
Ni^{2+}	69	proper size; → F_{430} 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.
[b] h.s., high spin.
[c] l.s., low spin.

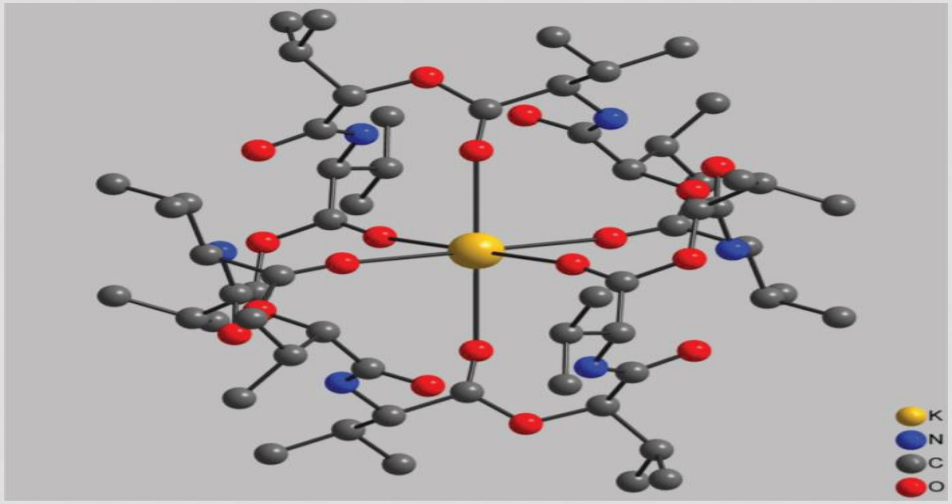
BIOLOGICAL LIGANDS:

CHELATE EFFECT

- Another class of ligands include ***ionophores,*** which are multidentate (≥6) chelate ligands that either exist as ***macrocycles*** (fig-1) or can at least form quasimacrocycles after coordination-induced ring closure via hydrogen bond interactions.
- The alkali metals, which generally form only highly labile complexes, and the rather labile Ca^{2+} ion can be bound in the polar inner cavity of such complex ligands.



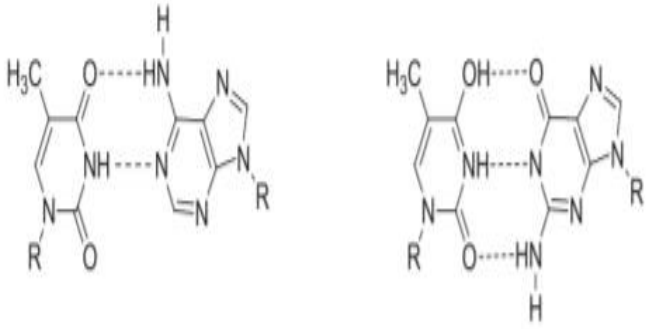
valinomycin

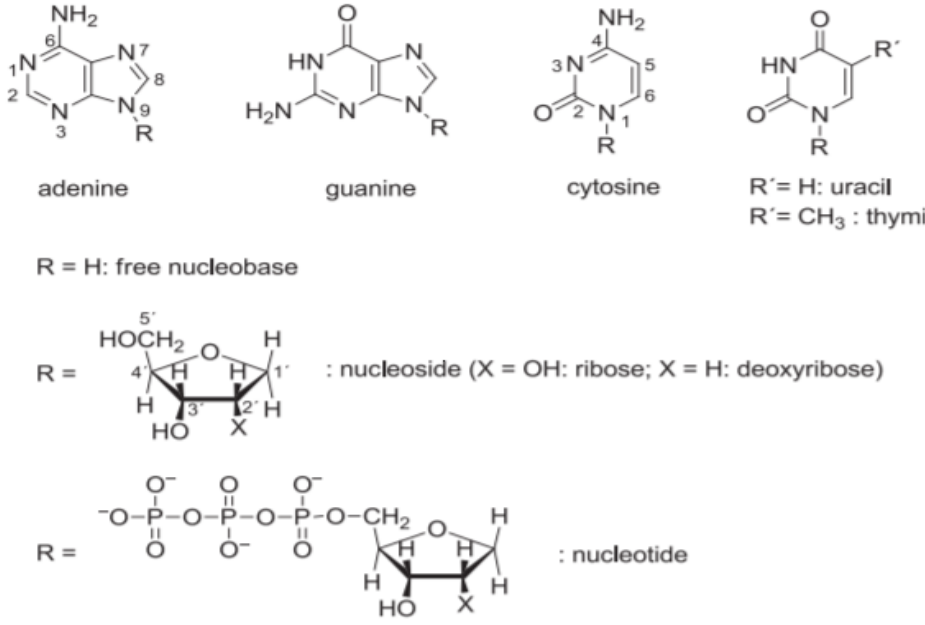


K+/valinomycin complex

BIOLOGICAL LIGANDS:
NUCLEOBASES, NUCLEOTIDES AND NUCLEIC ACIDS (RNA, DNA) AS LIGANDS

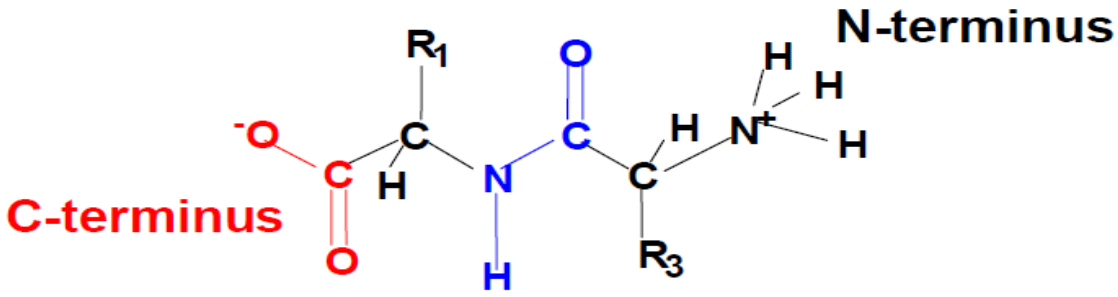
- The negatively charged phosphate/carbohydrate backbone is the obvious first coordination site for monovalent and especially divalent cations.
- The formation, replication and cleavage of nucleic acid polymers (RNA, DNA) as well as their structural integrity (e.g. the double-helical arrangement of conventional DNA) require the presence of metal ions.
- The donor-rich nucleobases and the available regions for "supramolecular" interactions (***→ "secondary bonding"***)



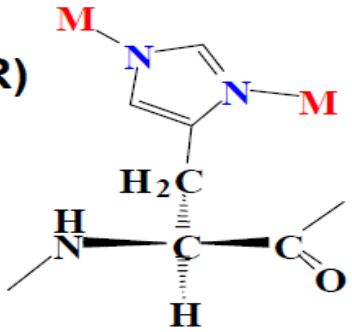


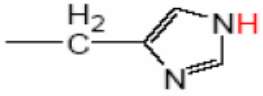
BIOLOGICAL LIGANDS:
METAL ION COMPLEXATION BY PEPTIDES

- Proteins consist of α-amino acids, connected via peptide bonds



- Metal coordination by functional groups in the side chain (R)



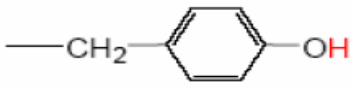


Histidine (both N atoms available, metal-metal briding possible, pKa ~6)

$-CH_2CH_2SCH_3$ Methionine

$-CH_2SH$ Cysteine (metal-metal bridging, pKa ~ 8)

$-CH_2SeH$ Selenocystein ("non-innocent ligand")



Tyrosine ("non-innocent ligand")

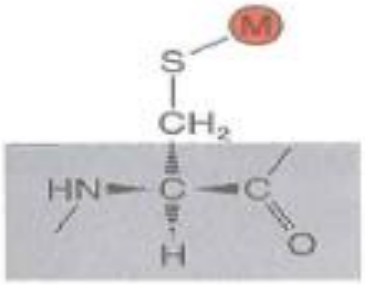
$-CH_2COOH$ Aspartic acid

$-CH_2CH_2COOH$ Glutamic acid

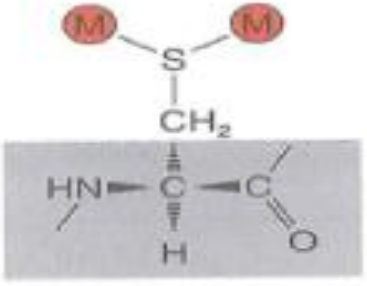
} pKa ~ 4

BIOLOGICAL LIGANDS:

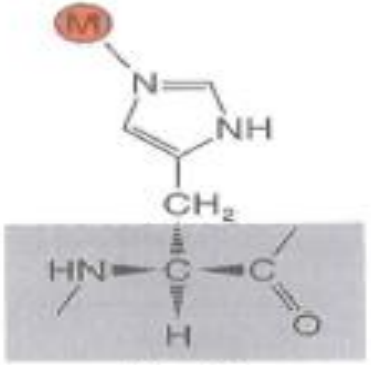
METAL ION COMPLEXATION BY PEPTIDES



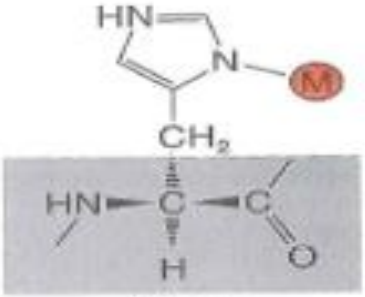
Cys-M



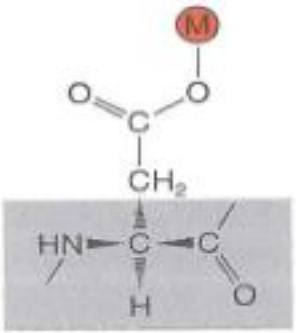
Cys-M_2



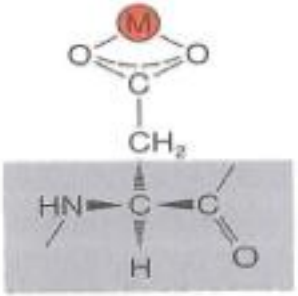
His-M
(ε-nitrogen)



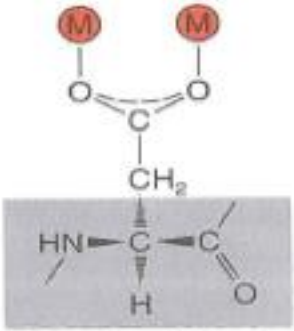
His-M
(δ-nitrogen)



Asp-M
Monodentate

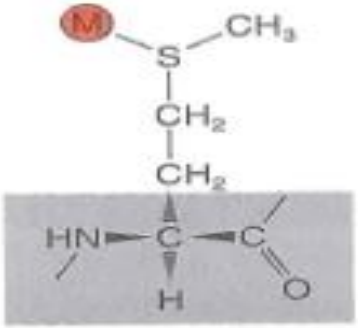


Asp-M
Bidentate, chelating

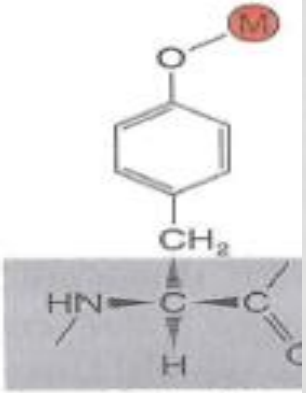


Asp-M_2
Bidentate, bridging

Similar structures are formed by glutamic acid



Met-M

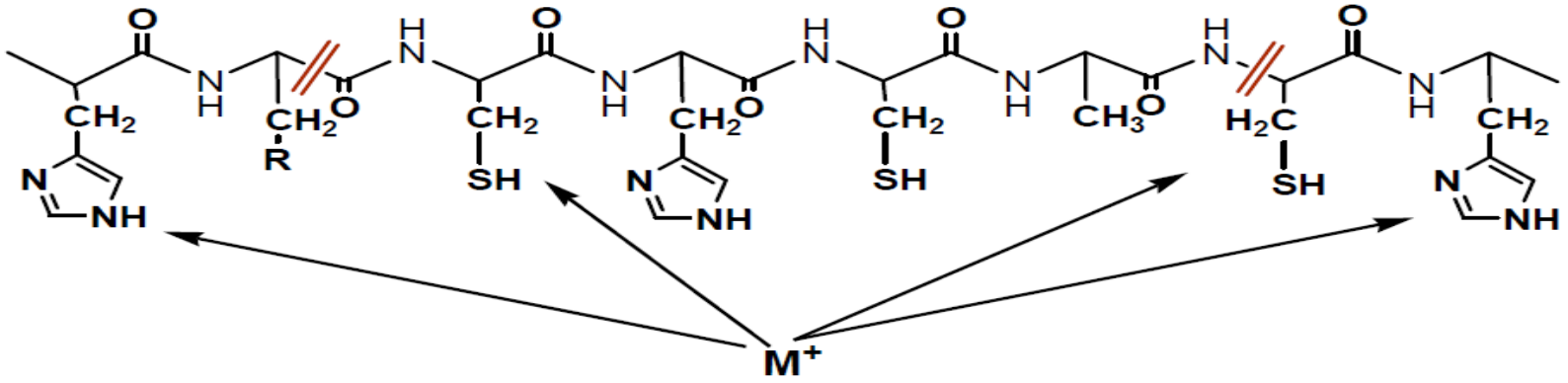


Tyr-M

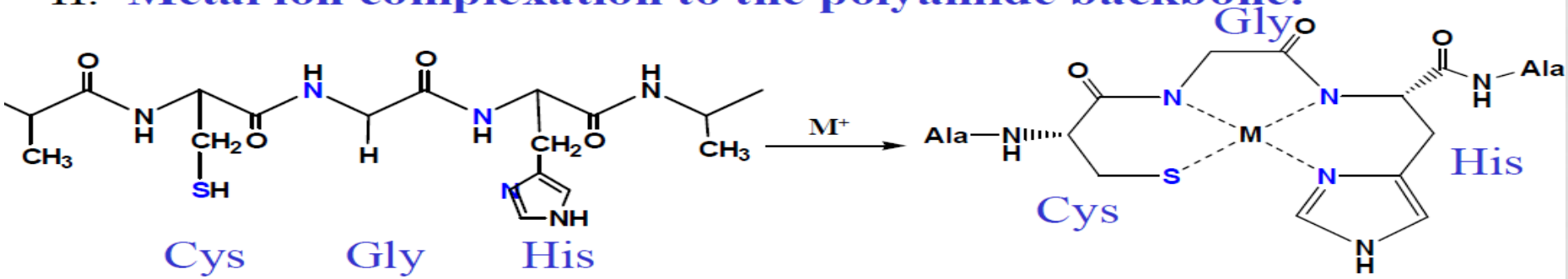
BIOLOGICAL LIGANDS:

METAL ION COMPLEXATION BY PEPTIDES

I. **Metal Ligation by side-chain residues: from two to many amino acid units apart**



II. **Metal ion complexation to the polyamide backbone:**

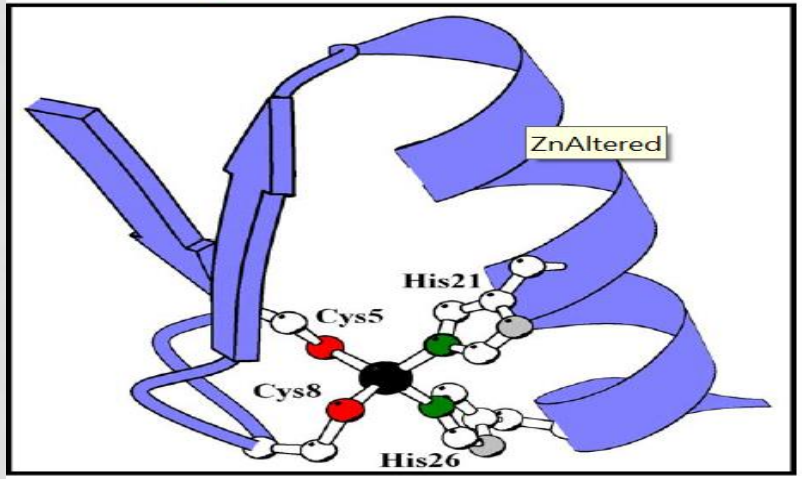


BIOLOGICAL LIGANDS:

METAL ION COMPLEXATION BY PEPTIDES

The Two Classes of Peptide Ligands in Metalloproteins:

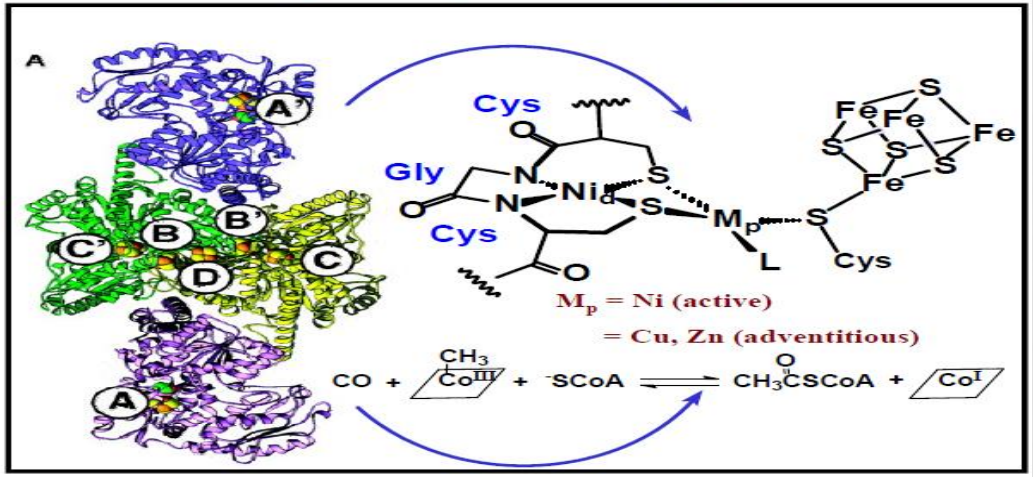
I. Binding to side chain residues



Cys_5XXCys_8~~~~~$His_{21}XXXXHis_{26}$

Zinc Finger

II. Binding to deprotonated peptides



~~~~~Cys_{595}-Gly_{596}-Cys_{597}~~~~~

Acetyl CoA Synthase A-cluster

Metalloprotein vs. Metalloenzyme?

I. Berg, J.M.; Godwin, H.A. *Annu. Rev. Biophys. Biomol. Struct.* **1997**, *26*, 357-371.

II. Drennan, C.L.; et *al. Science*. **2002**, *298*, 567-572.

II. Fontecilla-Camps, J.C.; Lindahl, P.A.; *et al. Nature Structural Biology*. **2003**, 10, 271-279.
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BIOLOGICAL LIGANDS:

METAL ION COMPLEXATION BY PEPTIDES

COMMON TERMS USED IN BIOLOGICAL SYSTEM IN REFRENCE TO PROTIEN:

Apoprotein – amino acids only

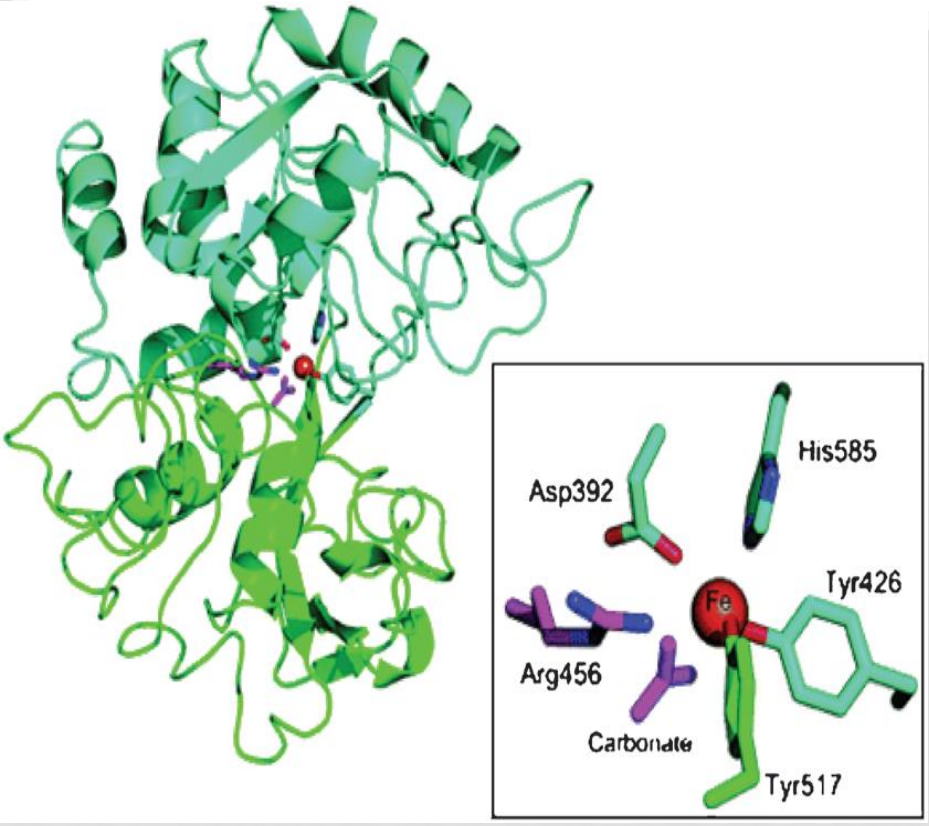
Cofactors – small organic (e.g., vitamins, ATP, NAD, FAD) or inorganic molecules (particularly metal ions) that are required for activity; can be loosely bound ***(coenzymes)*** or tightly bound ***(prosthetic groups)***

Prosthetic group – tightly bound group (e.g., heme) to apoprotein

holoprotein – active protein with cofactors and prosthetic groups attached.

BIOLOGICAL LIGANDS:
NON- PROTEIN LIGAND

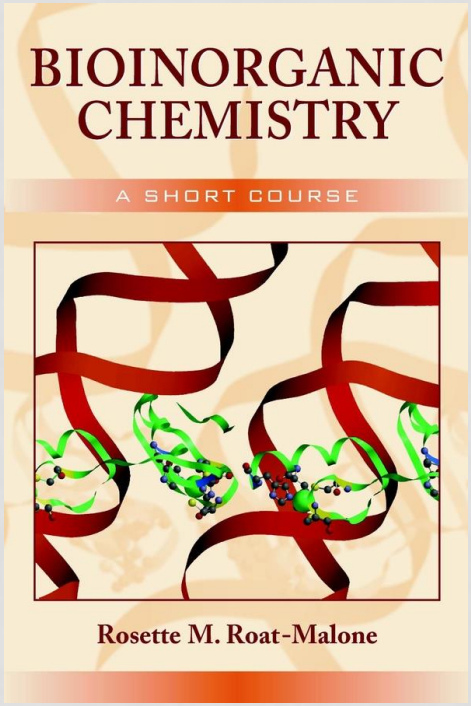
AN EXAMPLE OF A NON-PROTEIN LIGAND: *CARBONATE AND PHOSPHATE*

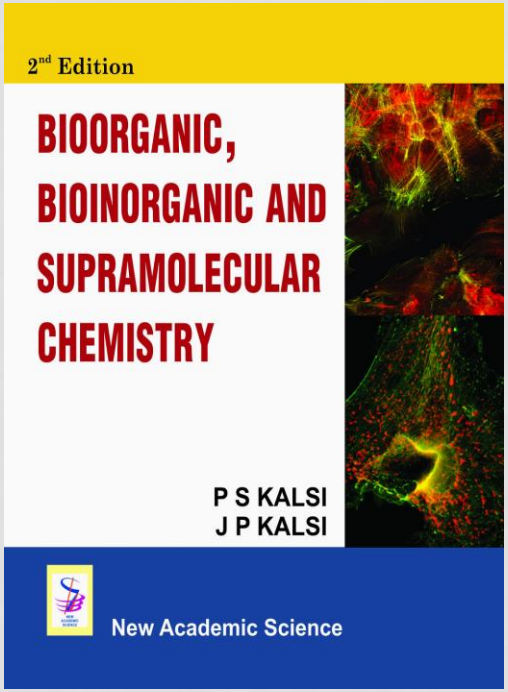


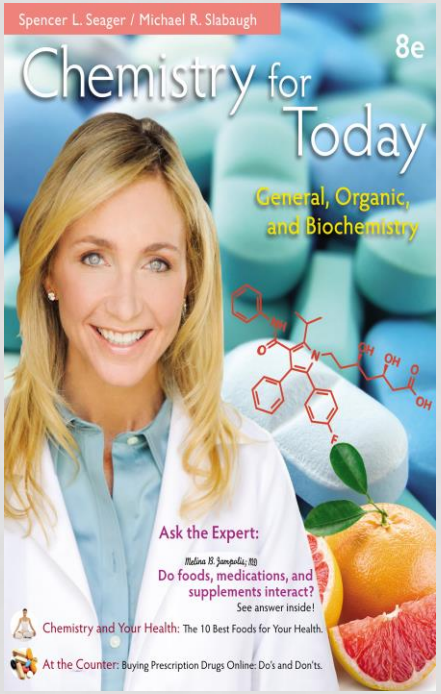
Transferrin includes low molecular weight ligands such as carbonate and phosphate anions
Transferrin are considered as part of *super family of iron-binding proteins,* all of which function by a *'Venus fly trap' mechanism.* They are made up of two homologous lobes, termed N- and C-lobes, each of which binds a single atom of ferric iron together with a 'synergistic' carbonate anion. Each lobe is composed of two domains indicated in Figure which close together upon iron and carbonate binding.

- *Ribbon diagram of the C-lobe of human transferrin with the two domains shown in different colours (cyan for C1 and green for C2).*
- *The inset shows the four protein ligand residues together with the arginine residue which stabilizes binding of the synergistic carbonate ion (both in magenta).*

BIOLOGICAL FUNCTIONS OF METAL IONS

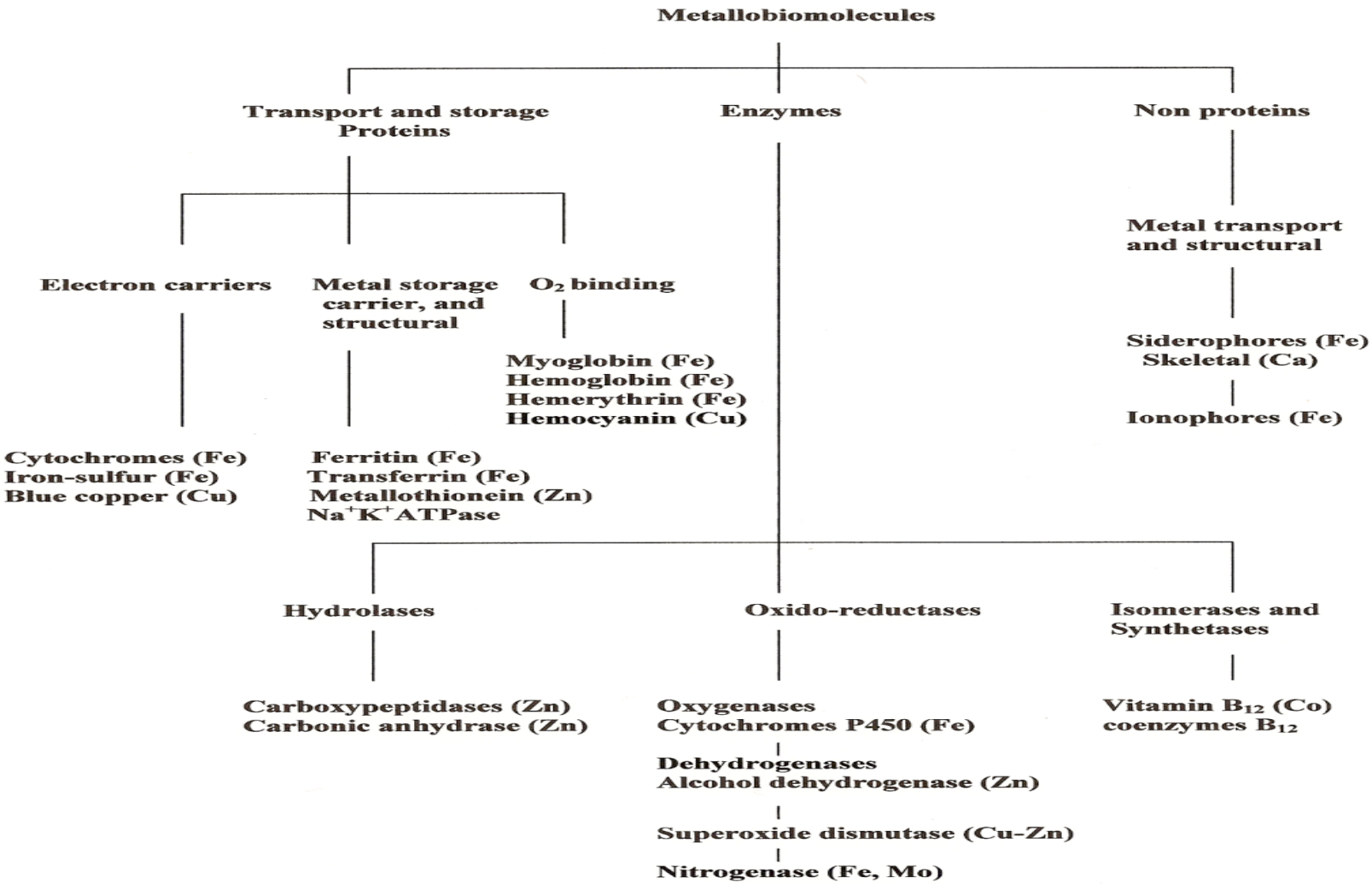






BIOLOGICAL FUNCTIONS OF METAL IONS

Biological functions that can be controlled by different metal ions



BIOLOGICAL FUNCTIONS OF METAL IONS

Metals in Biological Systems: Charge Carriers:

Metal	C. Number, Geometry	Preferred Ligand	Functions and Examples
Sodium, Na^+	6, octahedral	O - Ether, hydroxyl, carboxylate	Charge carrier, osmotic balance, nerve impulses
Potassium, K^+	6 – 8, flexible	O - Ether, hydroxyl, Carboxylate	Charge carrier, osmotic balance, nerve impulses

Metals in Biological Systems: Structural, Triggers:

Metal	C. Number, Geometry	Preferred Ligand	Functions and Examples
Magnesium, Mg^{2+}	6, octahedral	O - Carboxylate, phosphate	hydrolases, isomerases, phosphate transfer, trigger reactions
Calcium, Ca^{2+}	6 – 8, flexible	O - Carboxylate, carbonyl, phosphate	charge carrier, phosphate transfer, trigger reactions
Zinc, Zn^{2+} (d^{10})	4, tetrahedral	O - Carboxylate, carbonyl, S-Thiolate N - imidazole	zinc fingers, gene regulation, anhydrases, dehydrogenases
Zinc, Zn^{2+} (d^{10})	5, square pyramid	O - Carboxylate, carbonyl, N - imidazole	hydrolases, peptidases
Manganese, Mn^{2+} (d^5)	6, octahedral	O - Carboxylate, phosphate, N - imidazole	Structure in oxidases, photosynthesis
Manganese, Mn^{3+} (d^4)	6, tetragonal	O - Carboxylate, phosphate, hydroxide	oxidases, photosynthesis

BIOLOGICAL FUNCTIONS OF METAL IONS

Metals in Biological Systems: Electron Transfer:

Metal	C.Number, Geometry	Preferred Ligand	Functions and Examples
Iron, Fe^{2+} (d^6)	4, tetrahedral	S - Thiolate	Electron transfer, nitrogenases
Iron, Fe^{2+} (d^6)	6, octahedral	O - Carboxylate, alkoxide, oxide, phenolate	Electron transfer in Oxidases
Iron, Fe^{3+} (d^5)	4, tetrahedral	S - Thiolate	Electron transfer, nitrogenases
Iron, Fe^{3+} (d^5)	6, octahedral	O - Carboxylate, alkoxide, oxide, phenolate	Electron transfer in Oxidases
Copper, Cu^+ (d^{10}), Cu^{2+} (d^9)	3, trigonal planar	N - Imidazole	Electron transfer in Type III heme – copper oxidases (Cu_B in cytochrome c oxidase,
Copper, Cu^+ (d^{10}), Cu^{2+} (d^9)	4, tetrahedral	S - Thiolate, thioether, N - imidazole	Electron transfer in Type-I blue copper proteins and Type III heme –copper oxidases (Cu_A in cytochrome c oxidase

Metals in Biological Systems: Dioxygen Transport:

Metal	C. Number, Geometry	Preferred Ligand	Functions and Examples
Copper, Cu^{2+} (d^9)	5, square pyramid 6, tetragonal	O - Carboxylate, N - imidazole	Type II copper oxidases, Type III copper hydroxylases, heamocyanin
Iron, Fe^{2+} (d^6)	6, octahedral	N - Imidazole, porphyrin	Haemoglobin

BIOLOGICAL FUNCTIONS OF METAL IONS

- ***Hydrogen,*** being extremely important in biology used in most of the biological redox reactions along with many non metals such as carbon and nitrogen. It is also used in proton gradients across biological membranes, which are universally used for ATP synthesis.

- ***Lithium,*** Although not required for life, but is used therapeutically in the form of ***lithium carbonate*** for the treatment of manic depression; its mechanism of action still remains a mystery. Effective treatment requires attaining serum lithium concentrations of between 0.8 and 1.2 mmol/L.
- ***Boron*** is an essential trace element for plants, and may well turn out to be essential for mammals as well. The boron-containing polyether–macrolide antibiotic, ***boromycin,*** was isolated as a potent **anti-HIV agent.**

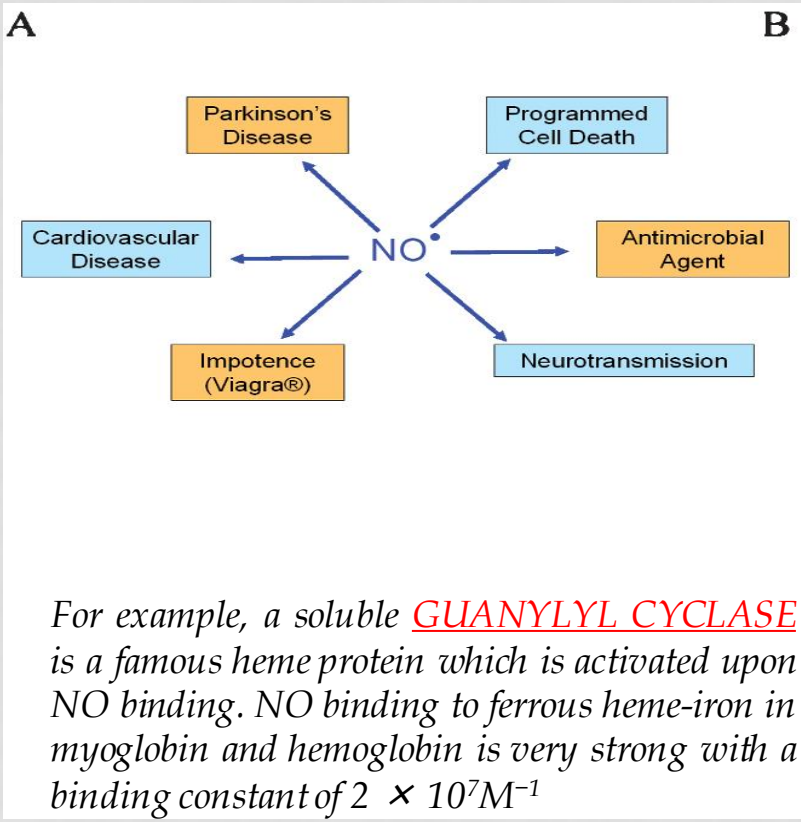
BIOLOGICAL FUNCTIONS OF METAL IONS

- ***Carbon, Nitrogen and Oxygen*** are all essential elements.

- Some compounds of these elements have special role in biological system e.g, Some of the biological effects of the important intracellular messenger, ***nitric oxide, NO,*** which is derived from the amino acid ***arginine***, are illustrated in **figure A**

- ***Fluorine*** in the form of fluoride added to drinking water to retard dental caries is criticized on ground of its potential toxicity although anti-tumour drug ***5-fluorodeoxythymidylate*** **(Figure B)**, a so-called '***suicide substrate***', inhibits the key enzyme of DNA synthesis, named ***"thymidylate synthase".***

BIOLOGICAL FUNCTIONS OF METAL IONS



For example, a soluble GUANYLYL CYCLASE is a famous heme protein which is activated upon NO binding. NO binding to ferrous heme-iron in myoglobin and hemoglobin is very strong with a binding constant of $2 \times 10^7 M^{-1}$

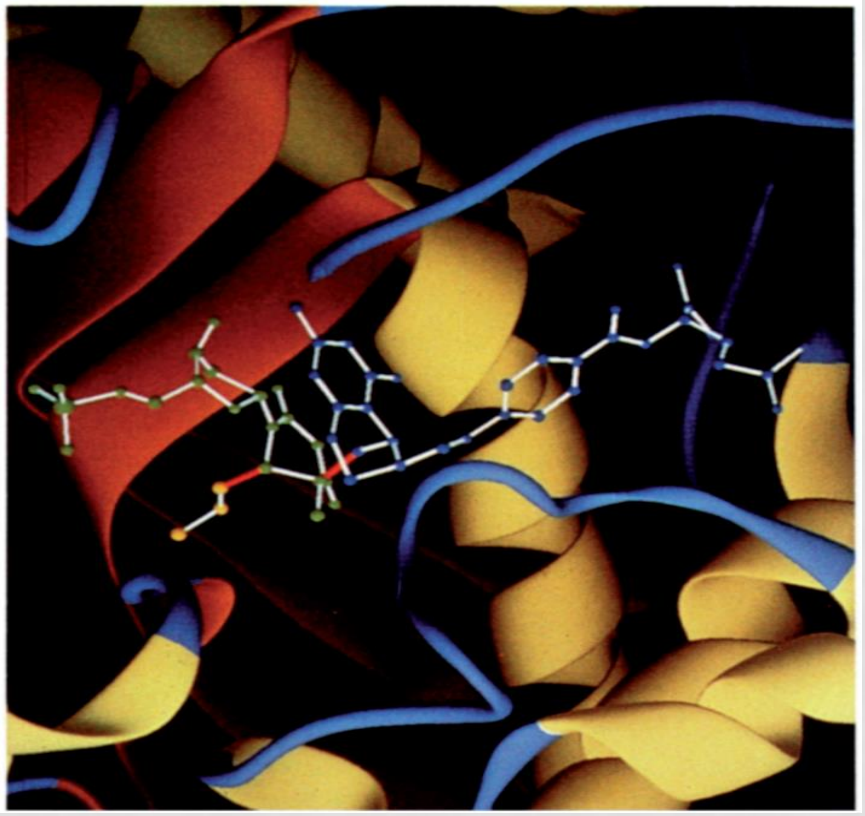
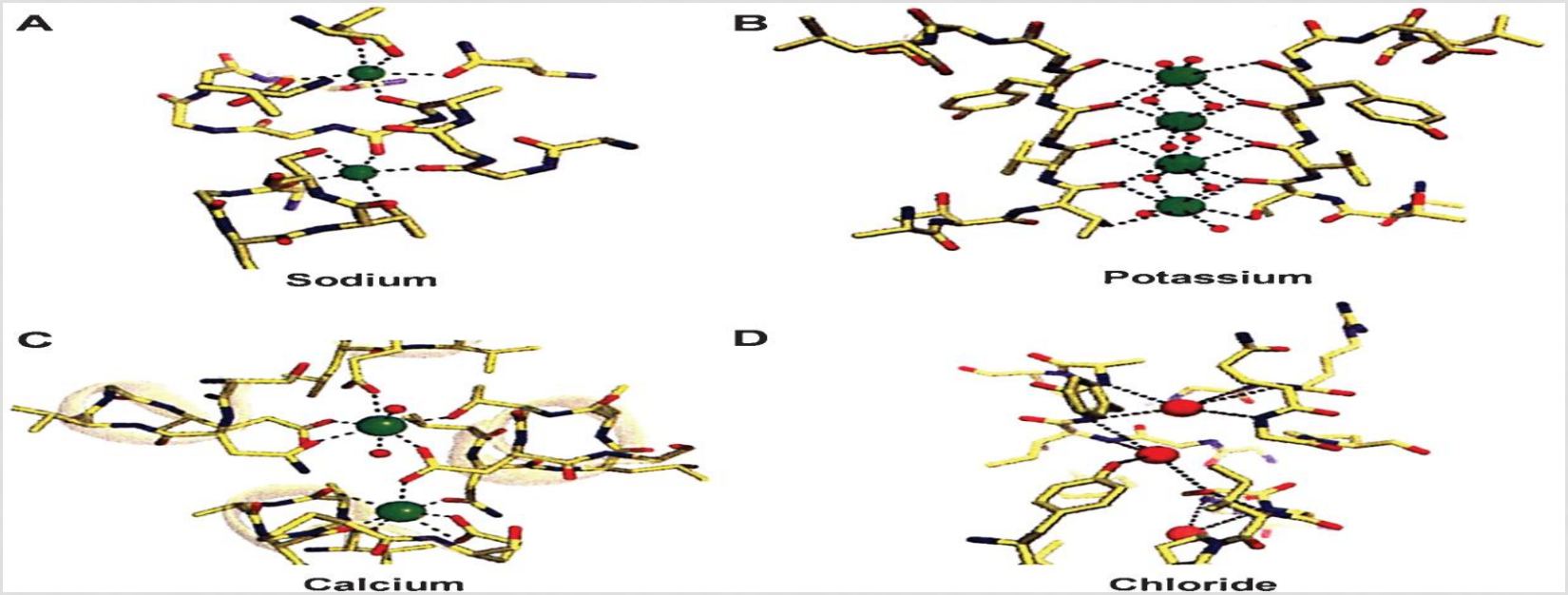


Figure :A) Some biological effects of NO and Figure: B) the structure of thymidylate synthase complexed with the suicide substrate *5-fluorodeoxythymidylate*

BIOLOGICAL FUNCTIONS OF METAL IONS

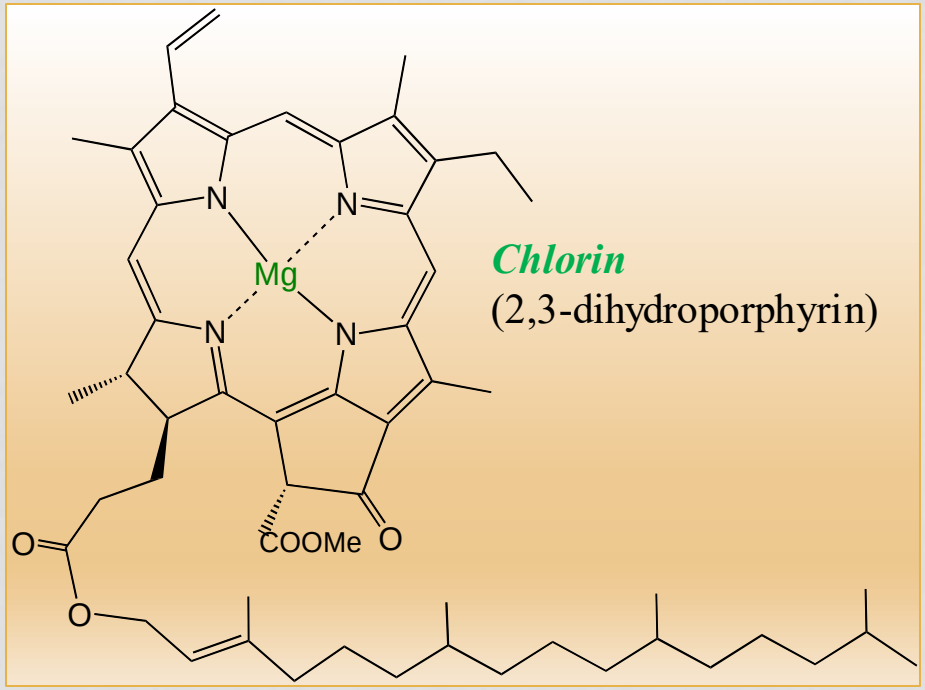
- Sodium is involved in ionic gradients and in osmotic regulation, and, despite its much higher extracellular concentration, has to be kept out of many cells by the action of an energy consuming Na/K ATPase pump. The following figures explain the selective-binding sites for Na^+, K^+, Ca^{2+} and Cl^- in transport proteins.



(a) Two Na^+ binding sites in the LeuT Na-dependent pump. **(b)** Four K binding sites in the KcsA K channel. **(c)** Two Ca^{2+} binding sites in the Ca^{2+} ATPase pump. **(d)** Two central Cl^- binding sites in a mutant ClC Cl^-/H^+ exchanger.

BIOLOGICAL FUNCTIONS OF METAL IONS

Magnesium has its role intimately intertwined with phosphate: in many ***phosphoryl transfer reactions***, and also as ***Mg-ATP*** in *muscle contraction*. It also help in the stabilization of nucleic acid structures as well as in the *catalytic activity of ribozymes* (catalytic RNA molecules). It also serves as a structural component of enzymes, and is found as the metal centre in chlorophylls.



Chlorin
(2,3-dihydroporphyrin)

Structure of chlorophyll a

BIOLOGICAL FUNCTIONS OF METAL IONS

Aluminium, while extremely abundant in the earth's crust, is not used by living organisms. It is a notorious neurotoxin,

❖ Acid rain can change the usual association of aluminium in the soil with silicate (predominant above pH 6.5) for phosphate, rendering aluminium more toxic. This may be the reason why silicon is essential, because it keeps aluminium in a non-toxic form as *aluminium silicate*. The importance of phosphorus and sulphur is obvious, the latter often associated with iron in an important family of proteins that contains iron–sulfur clusters.

Silicon, it is among trace elements and part of *Glutathione peroxidase enzyme* which catalyses the removal of hydrogen peroxide from biological system.

$$GSH + H_2O_2 \longrightarrow GSSG + H_2O_2$$

GSH= Reduced Glutathione

GSSG= Oxidised Glutathione

BIOLOGICAL FUNCTIONS OF METAL IONS

Chromium, Nickel

These metal ions are required in very small quantities, and they have uncertain biological roles.

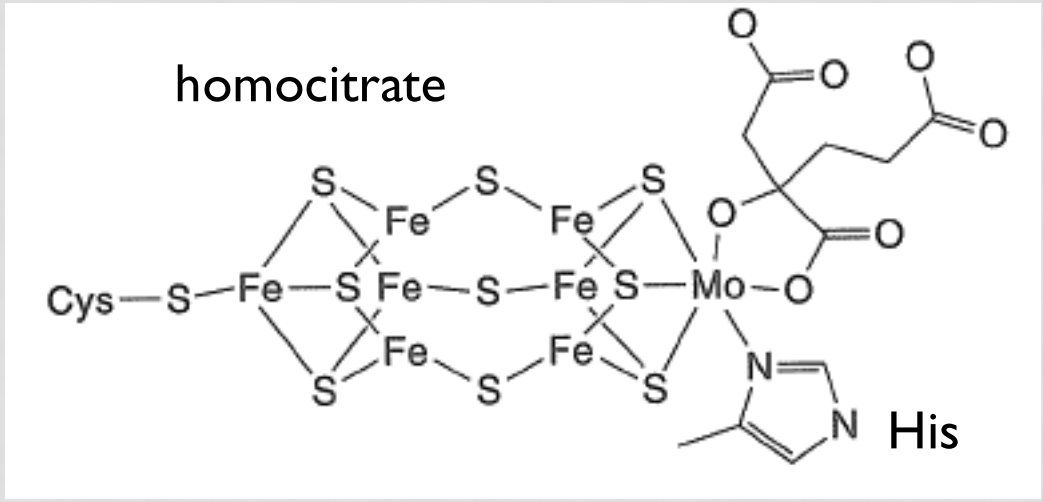
- Cr being used in glucose metabolism, potentiate insulin and serve as a component of glucose tolerance factor.
- Ni have role only in plants and bacteria (role in CH_4 production) and *SOD enzymes*.

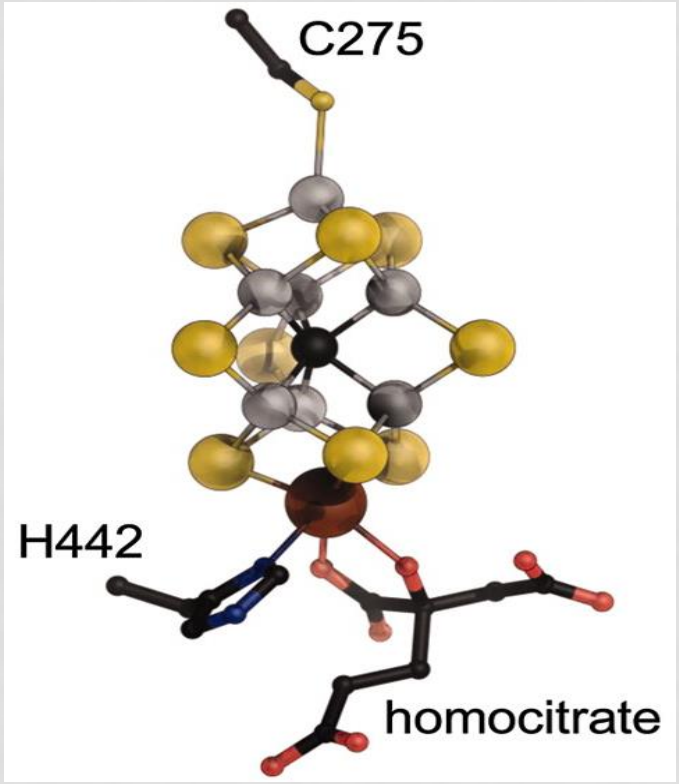
Manganese, Molybdenum

- Critical role in photosynthetic reaction centers, and SOD enzymes.
- Support brain function and reproduction. It is also required for blood sugar formation and part of bone structure
- Central role in nitrogenase enzymes catalyzing $N_2 \rightarrow NH_3$, $NO_3^- \rightarrow NH_3$

BIOLOGICAL FUNCTIONS OF METAL IONS

SPECIAL COFACTOR LIGANDS FOR Mo – NITROGENASE



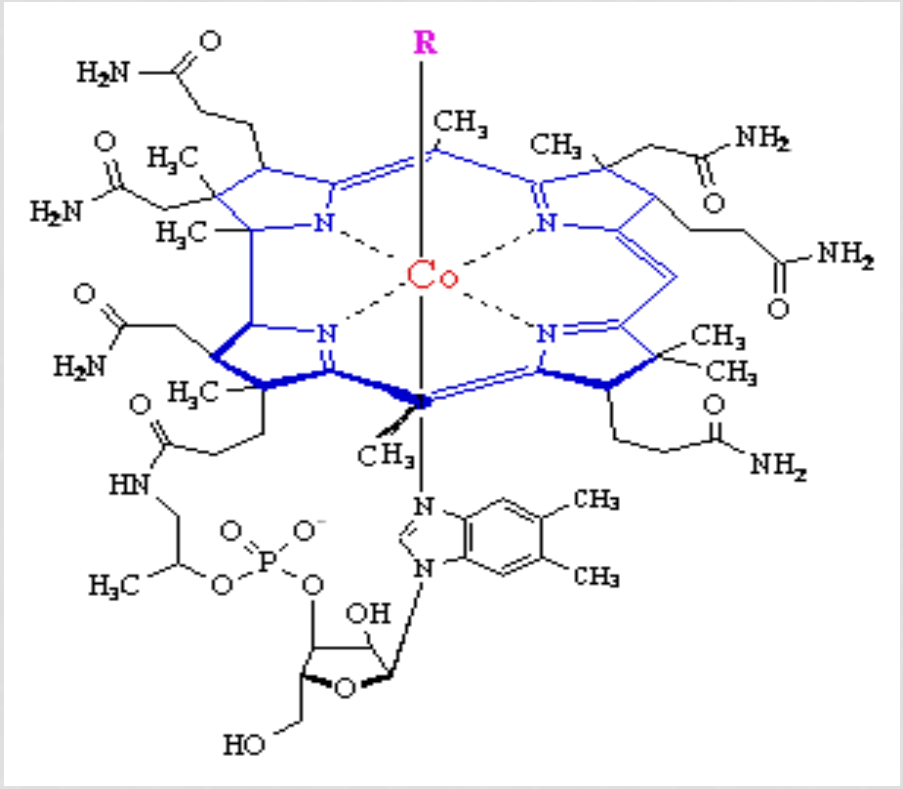


In fact, recent evidence indicates that there is a carbon in the middle of the Fe-Mo cofactor of nitrogenase:
Science ***2011****, 334, 940*
Science ***2011****, 334, 974*

BIOLOGICAL FUNCTIONS OF METAL IONS

Cobalt.

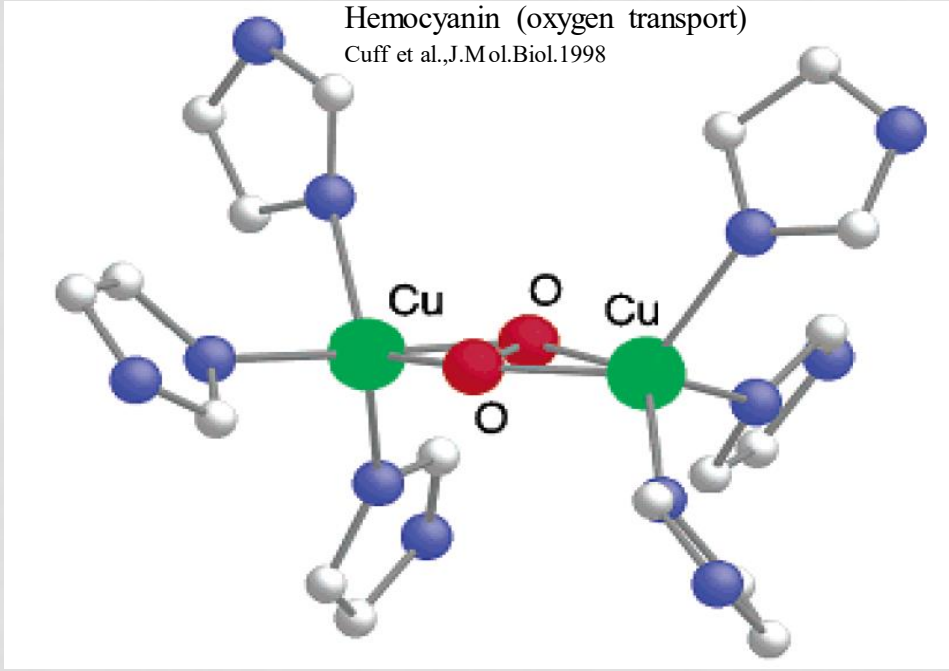
- Unique biological role in cobalamin (B_{12}-coenzymes) isomerization reactions.
- Naturally occurring organometallic compound



BIOLOGICAL FUNCTIONS OF METAL IONS

Copper

- Often participate together with Fe in proteins or has equivalent redox roles in same biological reactions. *Reversible* O_2 *binding*, O_2 *activation*, *electron transfer*, O_2^- *dismutation (SOD).*



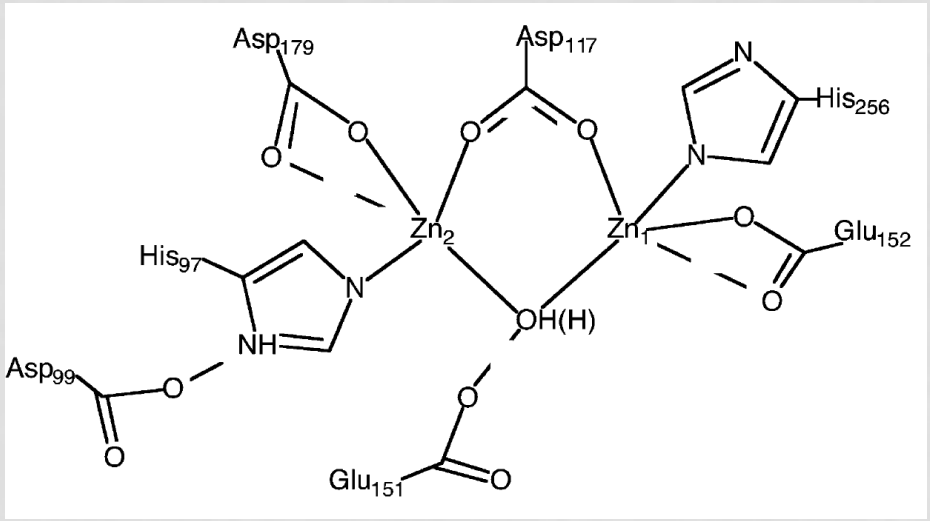
BIOLOGICAL FUNCTIONS OF METAL IONS

Zinc.

- Relatively abundant metal. Major concentration in *metallothionein* (which also serves as a reservoir for other metals, e.g. Cd, Cu, Hg). Many well characterized Zn proteins, including redox proteins, hydrolases and nucleic acid binding proteins.

Aminopeptidase from Aeromanas proteolytica

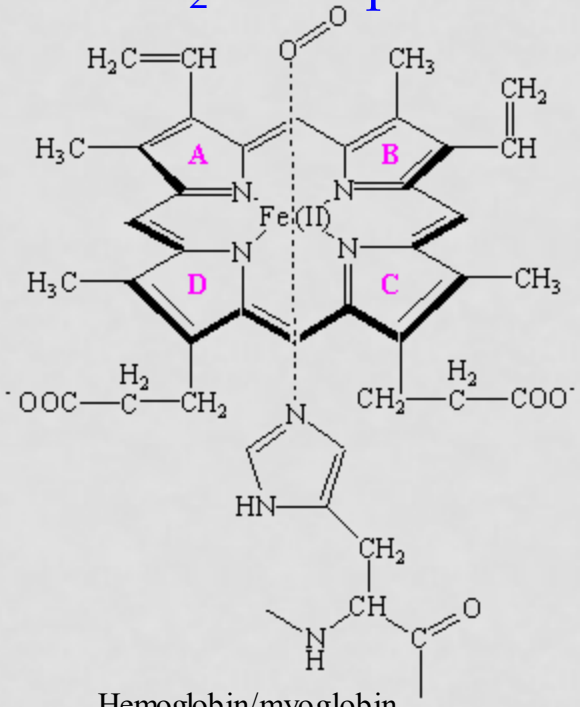
(Stamper et al., Biochemistry 2004, 43, 9620-9628)



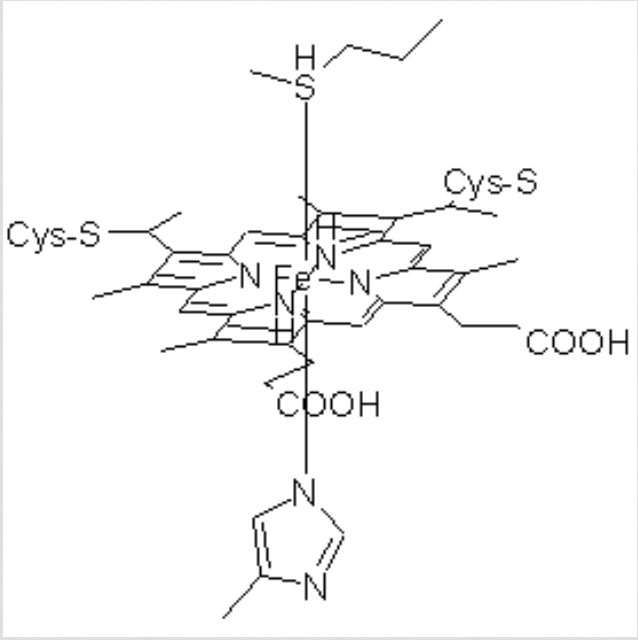
BIOLOGICAL FUNCTIONS OF METAL IONS

Iron.

- Most abundant metal in biology, used by all plants and animals including bacteria. Some roles duplicated by other metals, while others are unique to Fe. Iron use has survived the evolution of the O_2 atmosphere on earth.



Hemoglobin/myoglobin



Cytochrome c (involved in respiratory chain)

CONCLUSION

Bioinorganic chemistry with aim to

GENERAL TRANSPORT/STORAGE PROBLEMS

- Charged Ions must pass through a Hydrophobic Membrane
  - Neutral gases (O_2, CO_2) and low charge density ions (anions) can move directly through the membrane
  - High charge density cations require help
- Once inside the cell, metal ions must be transported to the location of their use, then released or stored for later
  - Release from ligand is often not trivial
  - Storage requires additional molecules

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MECHANISMS FOR MEMBRANE TRANSPORT

- ***Ionophores***: special carrier molecules that wrap around metal ions so they can pass through the membrane by diffusion

- ***Ion Channels***: large, membrane-spanning molecule that form a hydrophilic path for diffusion

- ***Ion Pumps***: molecules using energy to transport ions in one direction through a membrane

MECHANISMS FOR MEMBRANE TRANSPORT

- ***Passive Transport***: moves ions down the concentration gradient, requiring no energy source
  - Ionophores and Ion Channels are Passive
- ***Active Transport***: moves ions against the concentration gradient, requiring energy from ATP hydrolysis
  - Ion Pumps are Active
- Choice of Transport Mechanism
  - Charge
  - Size
  - Ligand Preference