PG & RESEARCH DEPARTMENT OF CHEMISTRY
THE NEW COLLEGE (AUTONOMOUS)
(AFFILIATED TO UNIVERSITY OF MADRAS)
CHENNAI – 600 014

BIO-INORGANIC CHEMISTRY

2020-2021

II M.Sc., CHEMISTRY
(SEMESTER – III)

Dr. U. MUHAMMED RAFI, *M.Sc., Ph.D.,*
Assistant Professor of Chemistry

ELEMENTS FOR LIFE

Bulk Elements

C, N, O, H

94% of Body weight

Big C

Major Elements

Na, K, Mg, Ca, P, S, Cl,

3.9% of Body weight

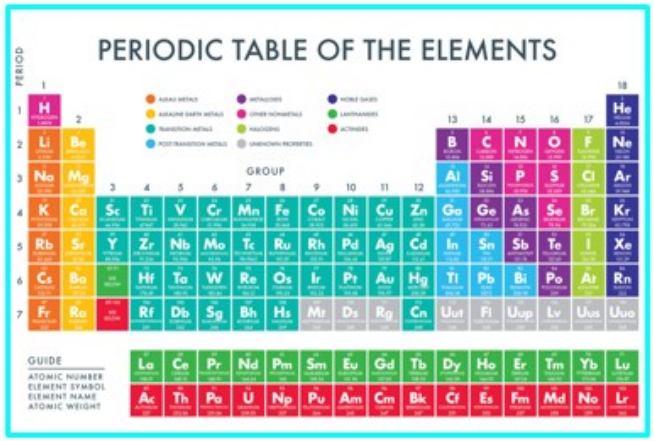
Macro Nutrients

Essential Elements

Fe, Zn, Cu

0.1% of Body weight

Critical Elements



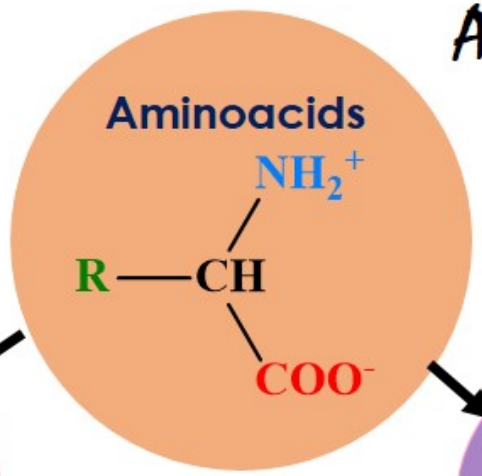
Trace Elements: few grams
Fe, Zn, Cu

Ultra-Trace Elements: few mg
Mn, Mo, Co, V, Ni, Se, Si, As

- These 15 – 20 essential element are important for the proper function of body.
- These are present in many proteins and enzymes and play important role in the biological functions.

1. Fe in hemoglobin for transport of oxygen.
2. Cu in plastocyanin for electron transfer.

AMINOACIDS



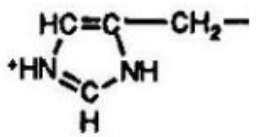
Non polar

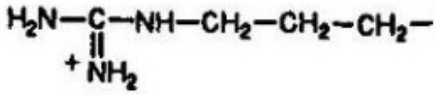
- Alanine R = CH_3
- Glycine R = H
- Leucine R = iso-butyl

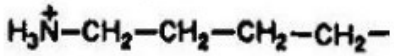
Polar

Basic Polar

- Histidine

  R = 
- Arginine

  R = 
- Lysine

  R = 

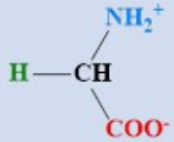
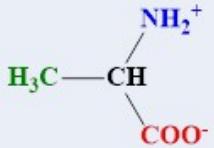
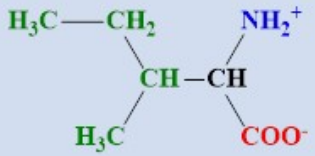
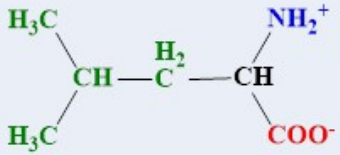
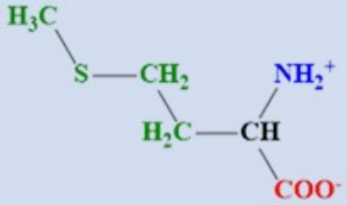
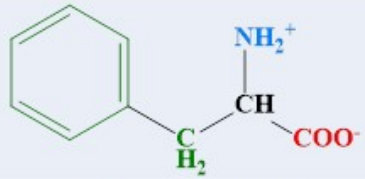
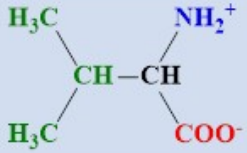
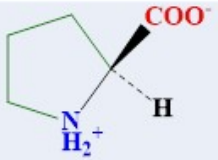
Neutral Polar

- Aspargine R = $H_2N\text{-}CO\text{-}CH_2\text{-}$
- Cysteine R = $HS\text{-}CH_2\text{-}$
- Serine R = $HO\text{-}CH_2\text{-}$

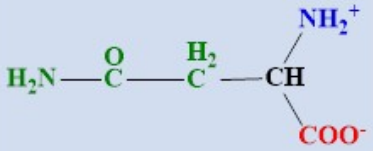
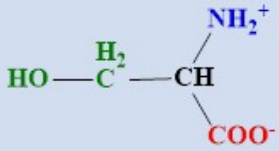
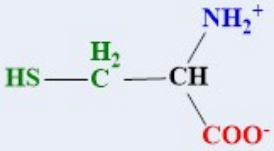
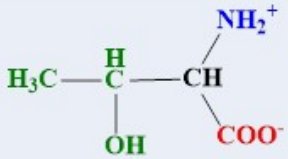
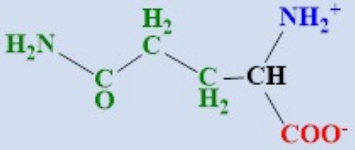
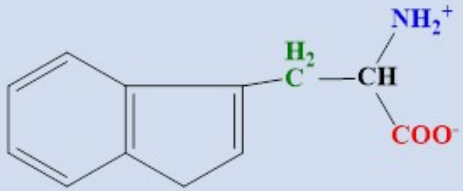
Acidic Polar

- Aspartic acid R = $HOOC\text{-}CH_2\text{-}$
- Glutamic acid R = $HOOC\text{-}CH_2\text{-}CH_2\text{-}$
- Tyrosine R = $HO\text{-}C_6H_5\text{-}CH_2\text{-}$

NON-POLAR AMINOACIDS

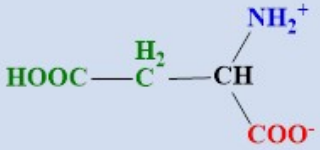
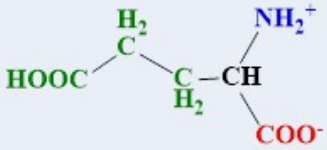
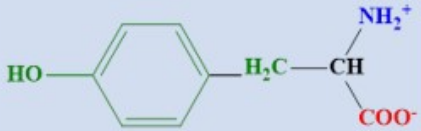
S.No.	Aminoacids	Symbol	Structure
1.	Glycine	Gly, G, 75	
2.	Alanine	Ala, A, 89	
3.	Isoleucine	Ile, I, 131	
4.	Leucine	Leu, L, 131	
5.	Methionine	Met, M, 149 S-donor	
6.	Phenylalanine	Phe, F, 165	
7.	Valine	Val, V, 117	
8.	Proline	Pro, P, 115 N-donor	

NEUTRAL POLAR AMINOACIDS

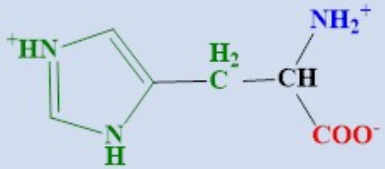
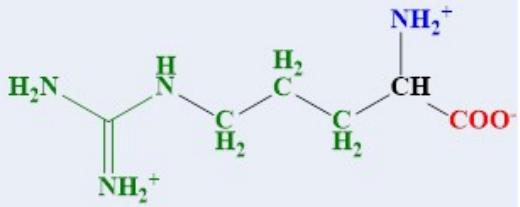
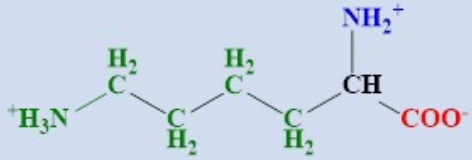
S.No.	Aminoacids	Symbol	Structure	S.No.	Aminoacids	Symbol	Structure
9.	Aspargine	Asn, N, 132 N-donor		12.	Serine	Ser, S, 105 O-donor	
10.	Cysteine	Cys, C, 121 S-donor		13.	Threonine	Thr, T, 119 O-donor	
11.	Glutamine	Gln, Q, 146 N-donor		14.	Tryptophan	Trp, W, 104 N-donor	

Guess the O-donor, S-donor and N-donor?

ACIDIC POLAR AMINOACIDS

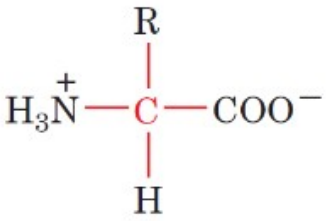
S.No.	Aminoacids	Symbol	Structure
15.	Aspartic acid	Asp, A, 132 O-donor	
16.	Glutamic acid	Glu, E, 147 O-donor	
17.	Tyrosine	Tyr, Y, 181 O-donor	

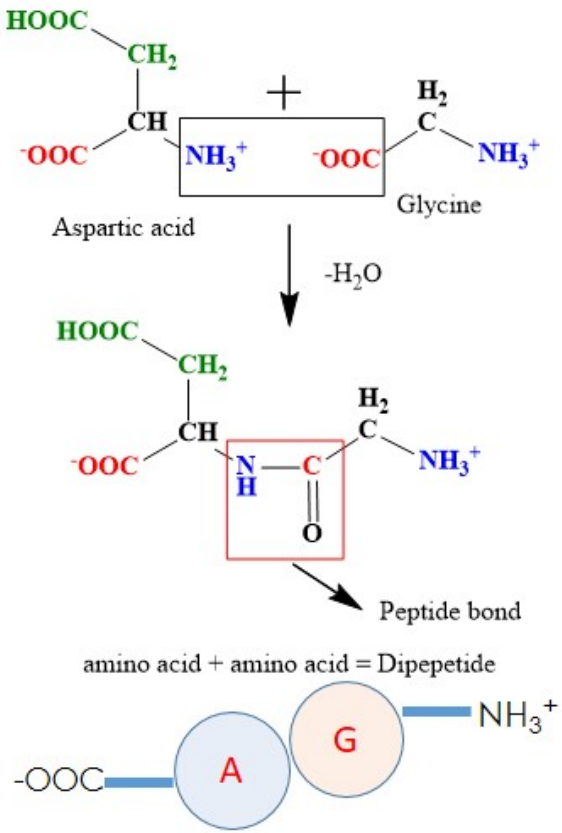
BASIC POLAR AMINOACIDS

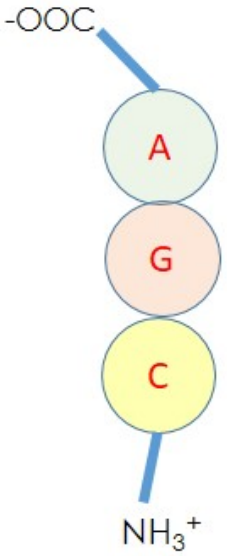
S.No.	Aminoacids	Symbol	Structure
18.	Histidine	His, H, 155 N-donor	
19.	Arginine	Arg, R, 174 N-donor	
20	Lysine	Lys, K, 146 N-donor	

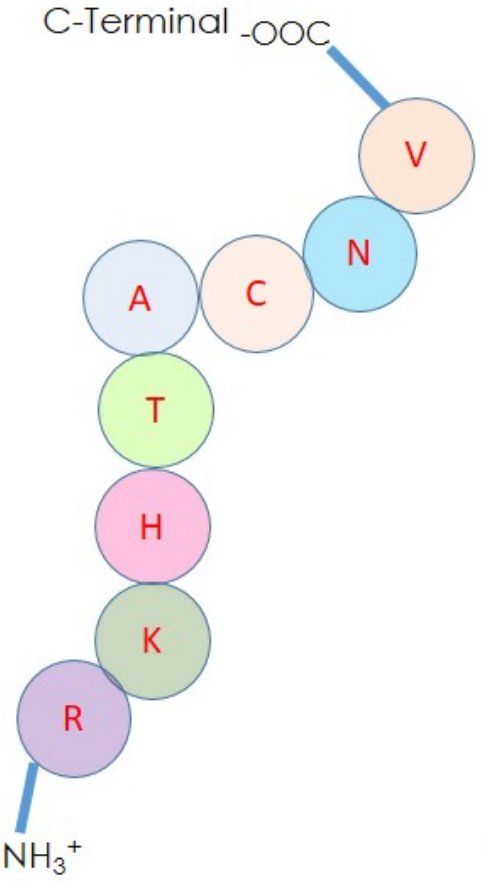
Guess the O-donor and N-donor?

Aminoacids → Dipeptides → Tripeptides → Polypeptides or proteins

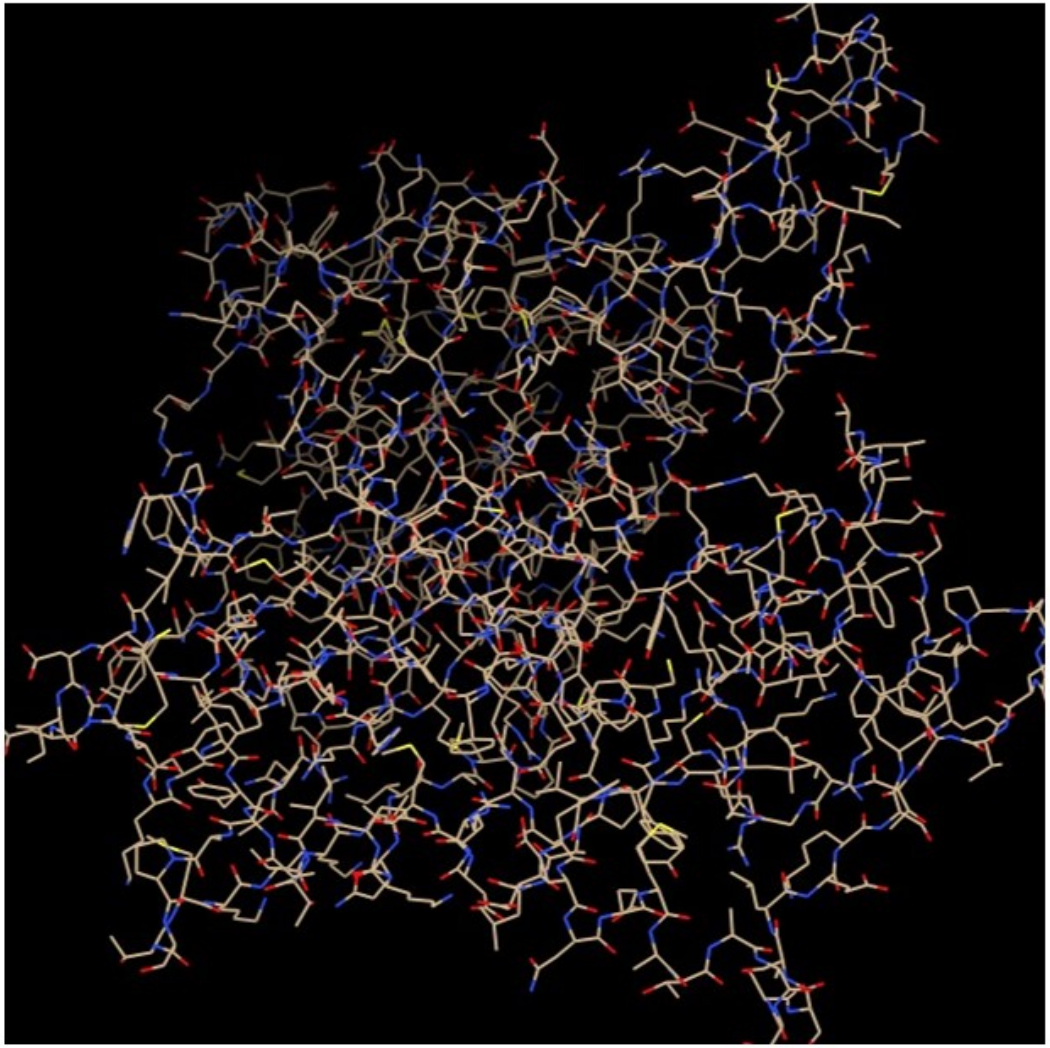




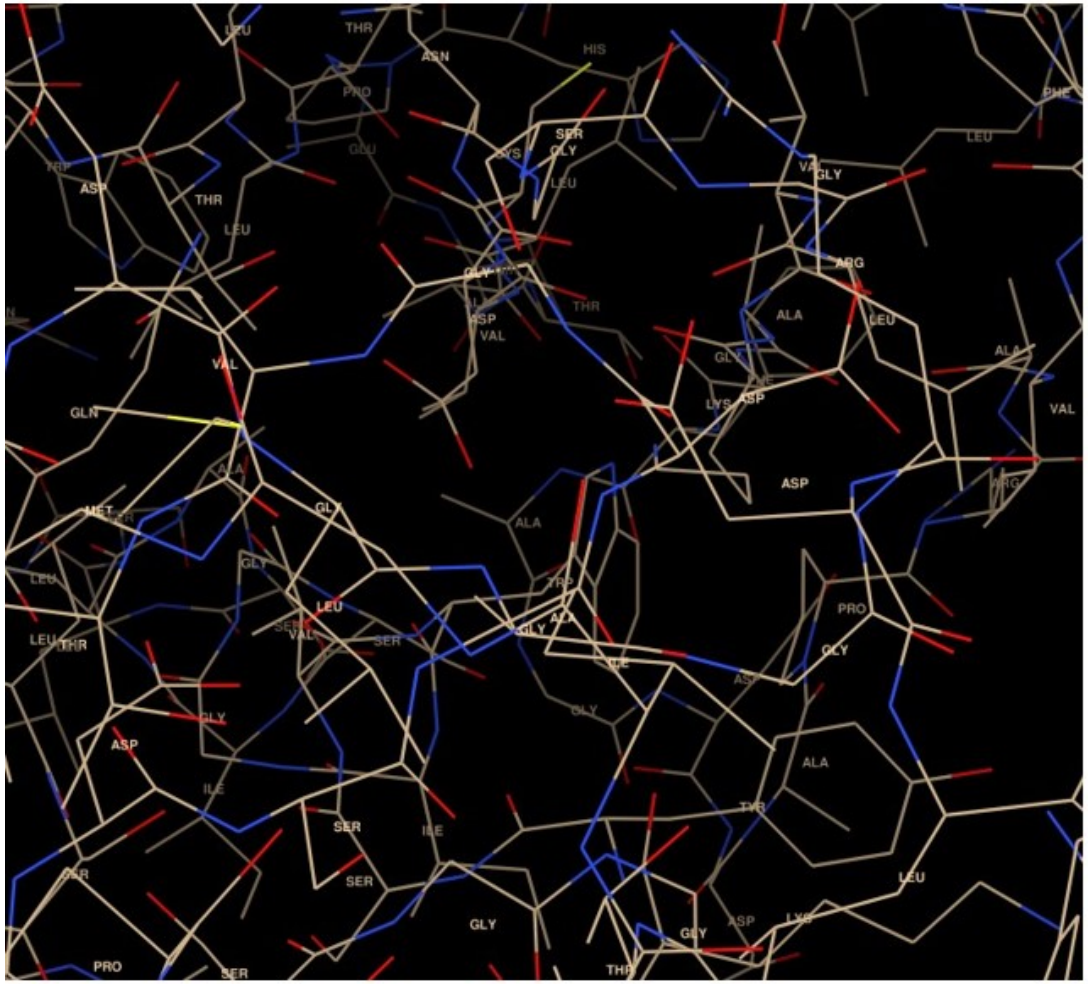


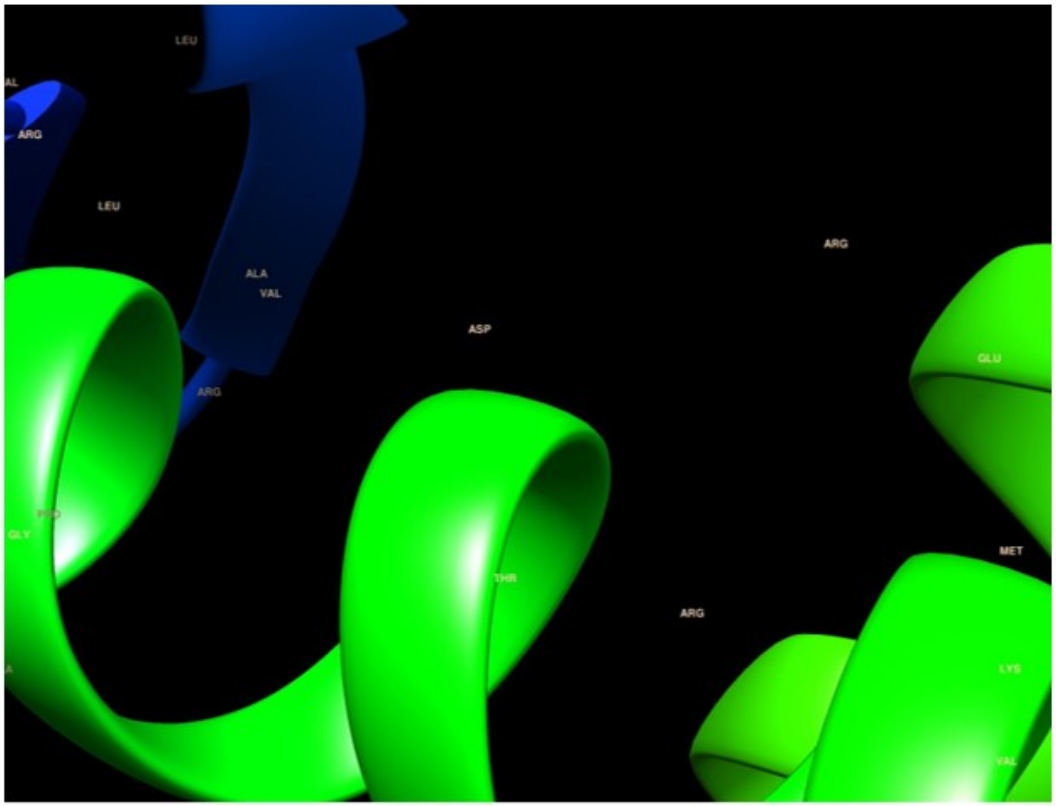


PROTEIN STRUCTURE: ACTIN

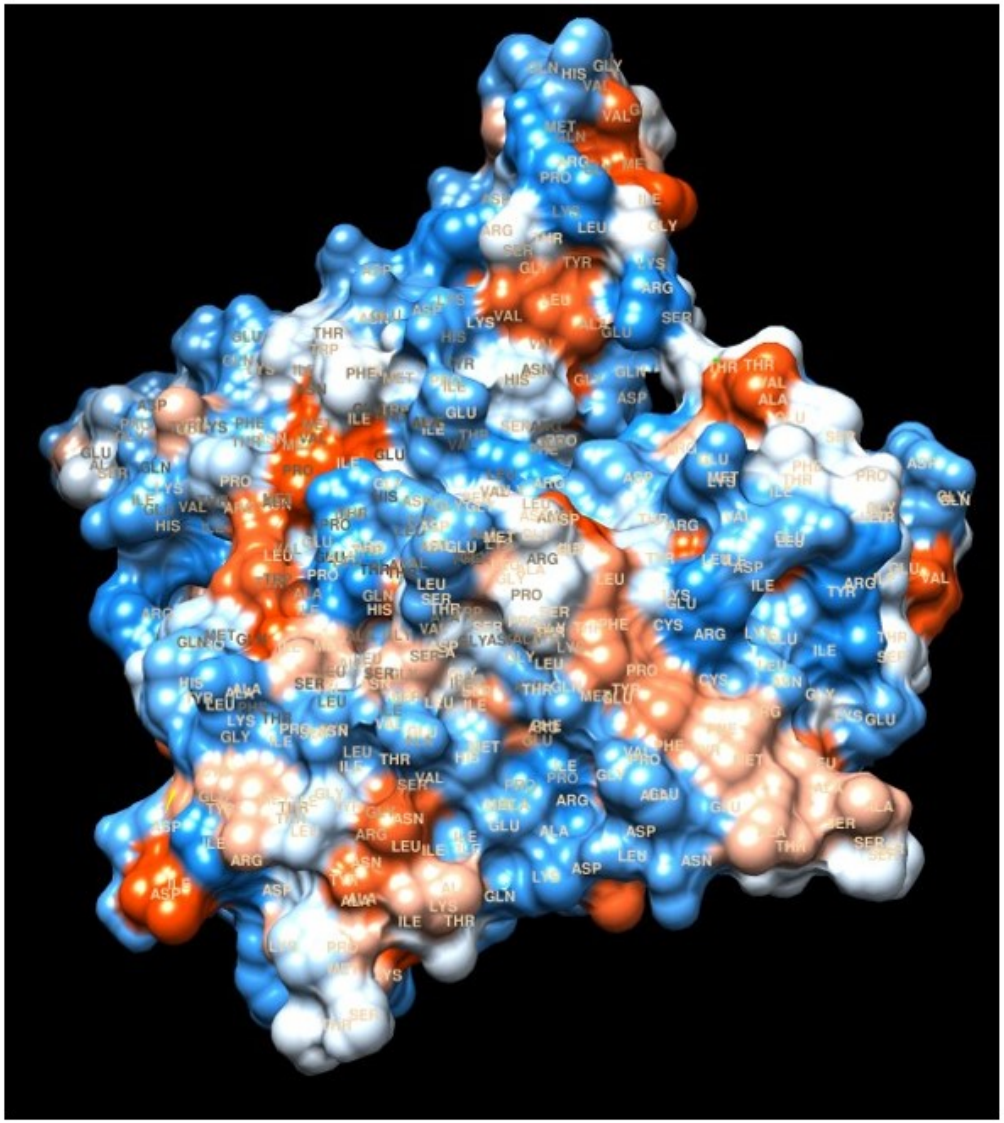


PROTEIN STRUCTURE: ACTIN



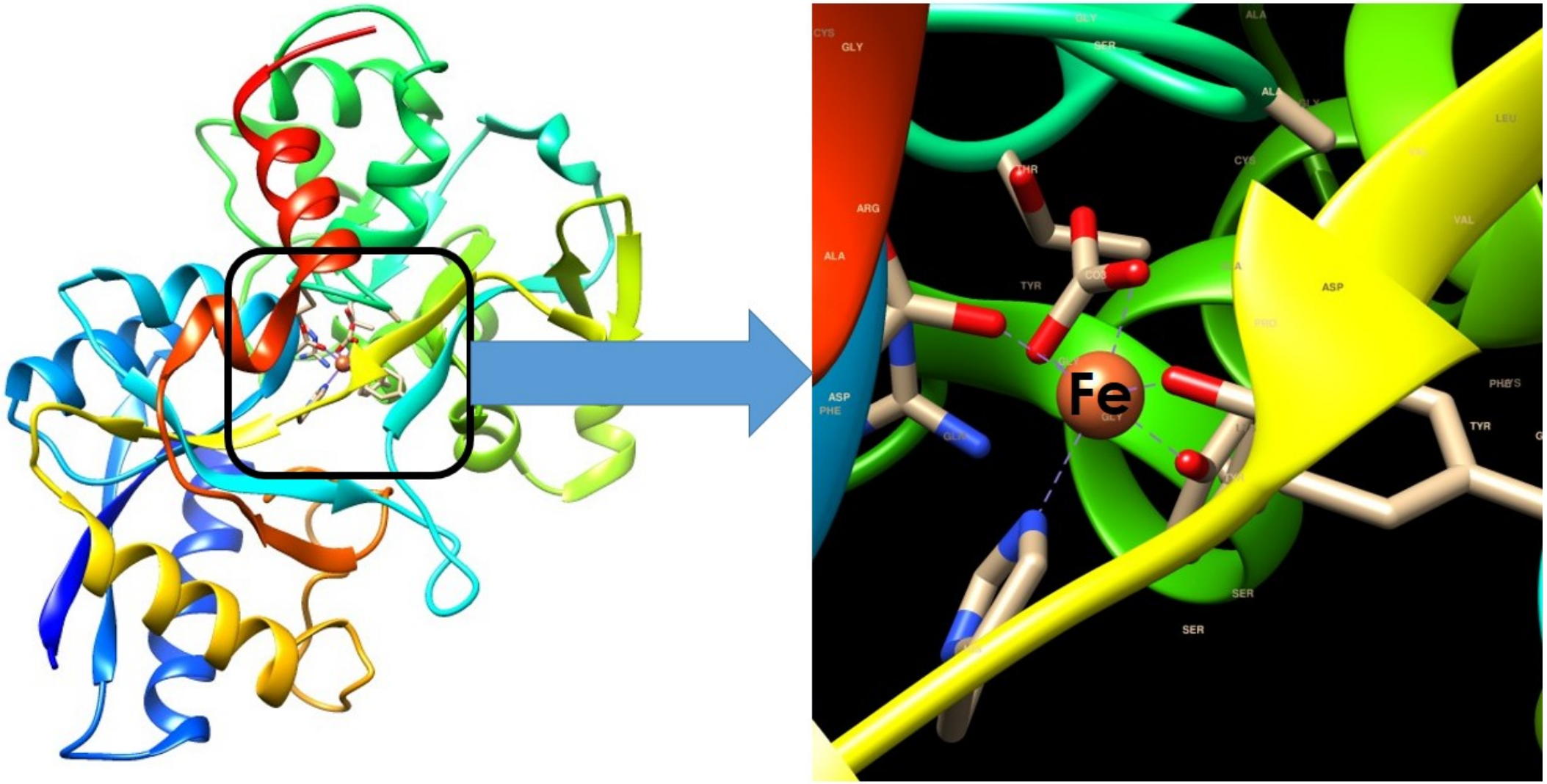


PROTEIN STRUCTURE: ACTIN



Dr. U. Muhammed Rafi, Assistant Professor of chemistry, The New College.

METALLOPROTEIN STRUCTURE: TRANSFERRIN



DEFINITION OF PROTEINS AND ENZYMES

- ✓ **Proteins** are linear **heteropolymers** of a fixed length. A linear chain of amino acids folds into a particular three-dimensional conformation determined by the sequence of the amino acids in the chain. This constitutes the primary level of protein structure.
- ✓ **Protein** is defined as a complex high-molecular-weight organic compound, consisting essentially of combinations of amino acids (AAs) in peptide linkages that contain carbon, hydrogen, oxygen, and nitrogen.
- ✓ **Enzymes** are a specialized class of proteins responsible for catalyzing chemical reactions within the cell.
- ✓ **Enzymes** are the active proteins (except RNAse) that can catalyze biochemical reactions. These are biomolecules required for both syntheses as well as breakdown reactions by living organisms.

DEFINITION OF METALLOPROTEINS/ENZYMES

- Metal ion cofactor coordinated to the protein/enzyme is called **metalloenzyme/metalloprotein**.
- The metal ion is usually **coordinated by nitrogen, oxygen or sulfur** atoms belonging to amino acids in the polypeptide chain and/or a macro cyclic ligand incorporated into the protein.
- The **presence** of the metal ion allows metalloenzymes to perform functions such as redox reactions that cannot easily be performed by the limited set of functional groups found in amino acids.
- The metal ions function like **cofactors**, **imparting activity** to the enzymes

CLASSIFICATION OF ENZYMES

	REACTION	SUBCLASSES	EXAMPLE
1 OXIDOREDUCTASES	redox reactions	Dehydrogenase Oxidases Peroxidases oxygenases	L-lactate:NAD oxidoreductase lactate dehydrogenase. (EC no.: 1.1.1.27.)
2 TRANSFERASES	Group transfer reactions	Transfer of a group amine, carboxyl, carbonyl, methyl, acyl, glycosyl, phosphoryl	L-aspartate:2-oxoglutarate aminotransferase aspartate aminotransferase (EC no.: 2.6.1.1.)
3 HYDROLASES	Bond cleavage by addition of water	Bond cleavage of C–O C–N C–S O–P	Arginase L-arginine amidino hydrolase (EC no.: 3.5.3.1.)

CLASSIFICATION OF ENZYMES

	REACTION	SUBCLASSES	EXAMPLE
4 **LYASES**	cleavage of C—C, C—S, and C—N bondsand form double bonds or cycles	decarboxylases, dehydratases, aldolases Reverse reaction,they are named **synthase** and do not require ATP, GTP	aldolase fructose-bisphosphate aldolase (EC. No.:4.1.2.13.)
5 **ISOMERASES**	interconvert isomers of any type: optical, geometric, or positional	epimerase racemase cis-trans isomerases cycloisomerase tautomerase	d-glyceraldehyde-3-phosphate ketol isomerase triosephosphate isomerase (EC. No.: 5.3.1.1)
6 **LIGASES**	binding of two molecules by hydrolysis of a high-energy bond of a nucleoside triphosphate	Bond formed are C—C, C—S, C—O, or C—N bonds. These enzymes are also called as **synthetases**	glutamine synthetase catalyzes the reaction that uses glutamic acid and ammonia to form glutamine by ATP hydrolysis

NOMENCLATURE OF ENZYMES

International Union of Biochemistry and Molecular Biology (IUBMB)

Nomenclature

Example: Hexokinase (trival or common name)
Catalyzes:
ATP + D-glucose → ADP + D-Glucose 6-phosphate

Systematic name

I name: substrate name
II name: type of reaction ending with *ase*

Systematic name:
ATP-Glucose phosphotransferase
Transfer of phosphoryl group from ATP to glucose

Enzyme Commission number (E.C.)

Has 4 numbers
I digit: Major class
II digit: sub-class
III digit: sub-sub class
IV digit: systematic specific name of the enzyme

E.C. number: 2.7.1.1

I digit, 2 → Transferases (major class)

II digit, 7 → Phosphotransferases (sub-class)

III digit, 1 → Phosphotransferase with a hydroxyl group as acceptor

IV digit, 1 → D-glucose as the phosphoryl group acceptor

Dr. U. Muhammed Rafi, Assistant Professor of chemistry, The New College.

COENZYMES

Many enzymes only perform their catalytic role when associated with another non-protein molecule, of relatively small size, called a ***cofactor***.

Both the protein and nonprotein portions are essential for enzyme activity.

Carbonic anhydrase and alcohol dehydrogenase are enzymes that contain zinc as co-factor.

lactate dehydrogenase, requires a coenzyme called NAD (Nicotinamide adenine dinucleotide).

HALOENZYME =	*APOENZYME* +	*COENZYME*
Whole enzyme	**Protein part** Macromolecule Thermo labile Non-dialyzable	**Non-Protein part** Small in size Thermo stable

SIDEROPHORES

Autotrophs and fungi can obtain Fe from the environment and iron is not available in the form as Fe(II) but mostly as Fe(III).

Siderophores are small **polydentate ligands** that have a very high affinity for Fe(III).

They are secreted from many bacterial cells into the external medium, where they sequester Fe to give a soluble complex that re-enters the organism at a specific receptor. Once inside the cell, the Fe is released.

All **Fe(III) siderophore complexes are octahedral and high spin**.

Because the donor atoms are **hard O or N atoms** and negatively charged, they have a relatively **low affinity for Fe(II)**.

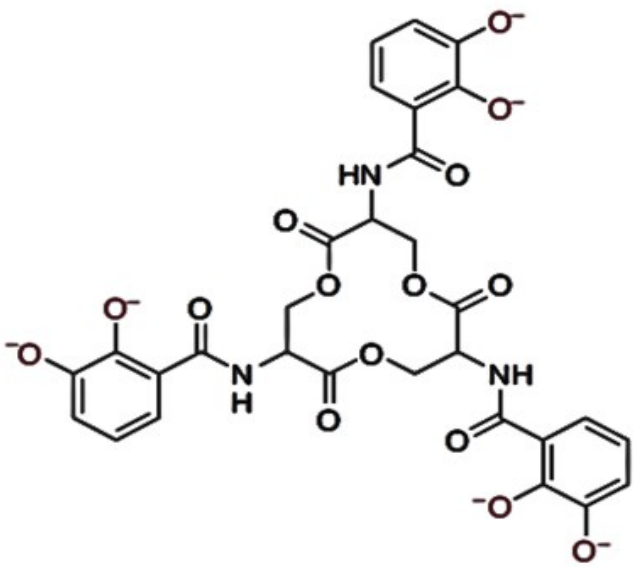
Synthetic siderophores are proving to be very useful agents for the control of '*iron overload*', a serious condition affecting large populations of the world, particularly South-East Asia.

SIDEROPHORES

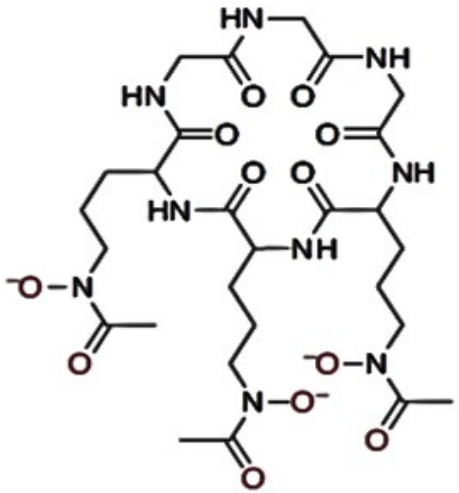
Two main types of siderophore.

1. Based on ***phenolate or catecholate ligands***, for example **enterobactin** (**1**), (association constant for Fe(III) is 1052, enables bacteria to erode steel bridges). (S. typhimurium)

2. Based on ***hydroxamate ligands*** for example **ferrichrome** (**2**), a cyclic hexapeptide consisting of three glycine and three *N*-hydroxyl-l-ornithines. (E-coli)

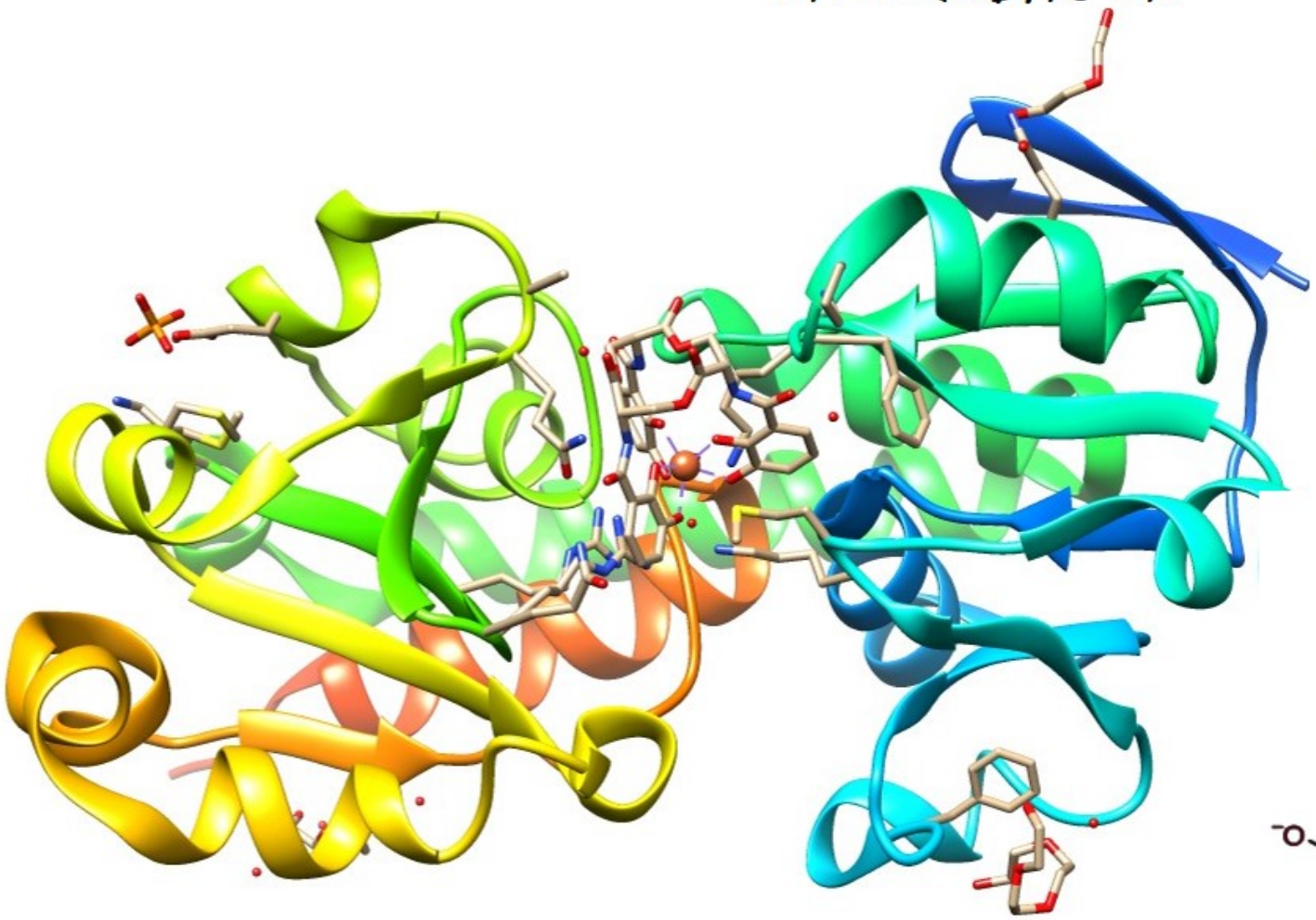


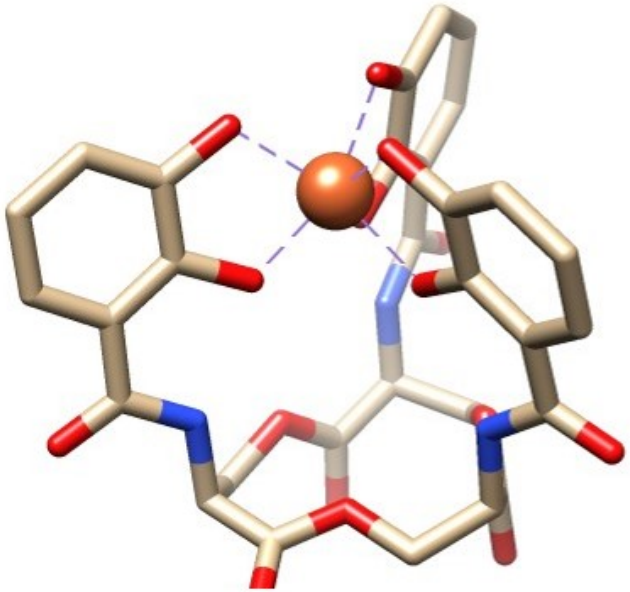
1 Enterobactin

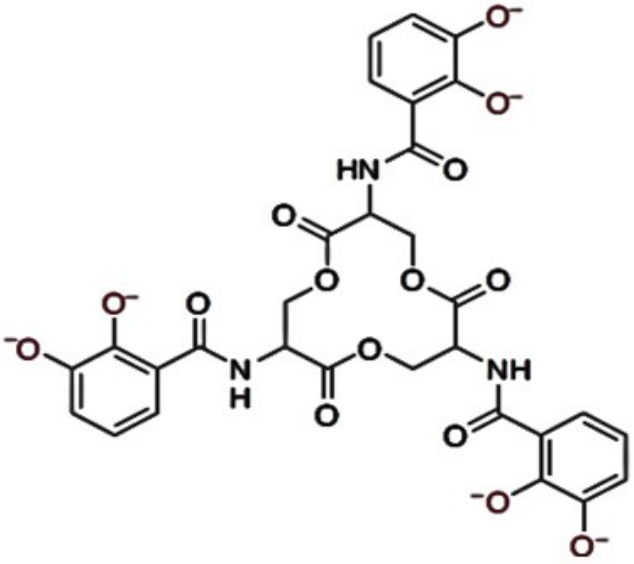


2 Ferrichrome

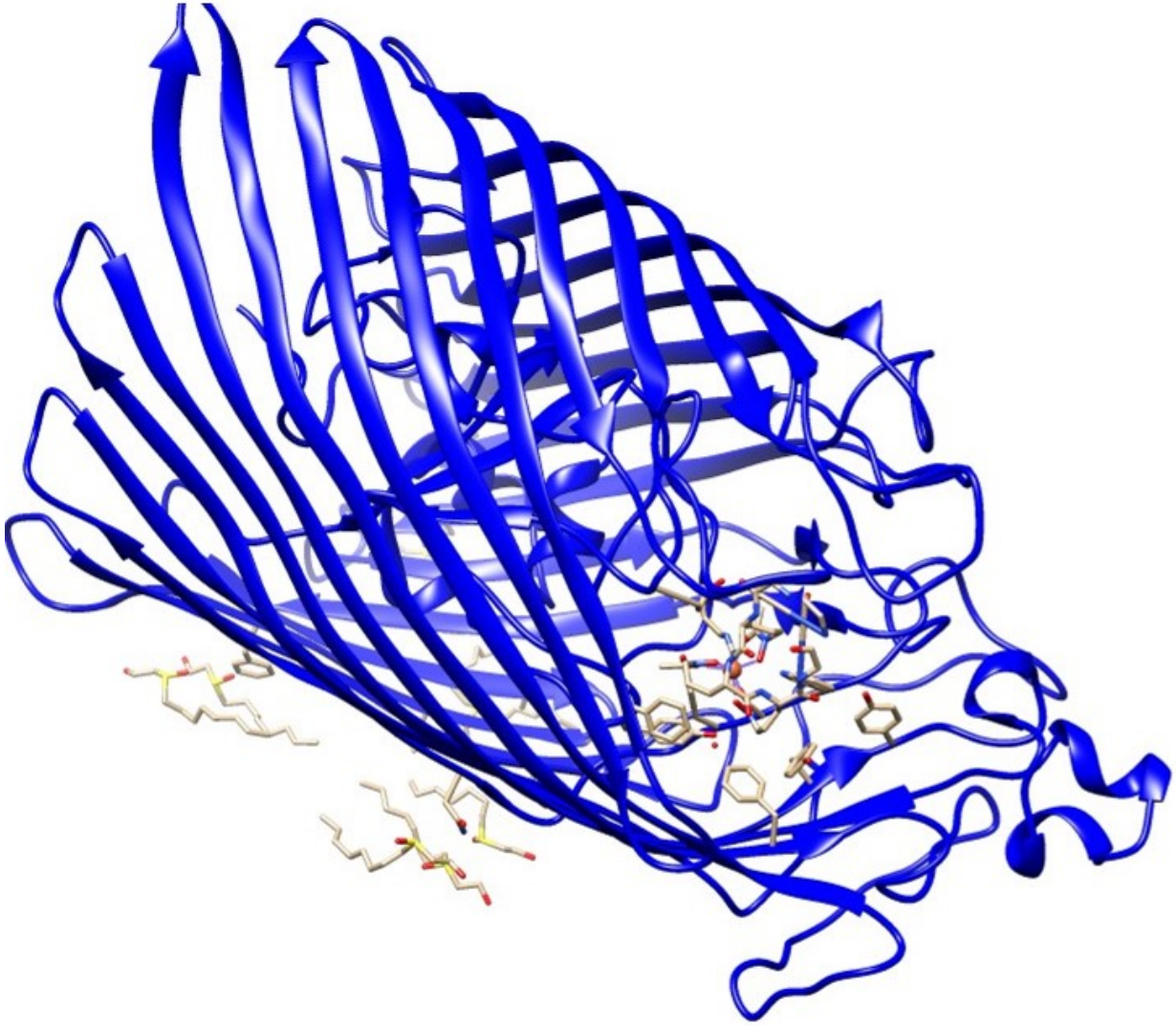
ENTEROBACTIN

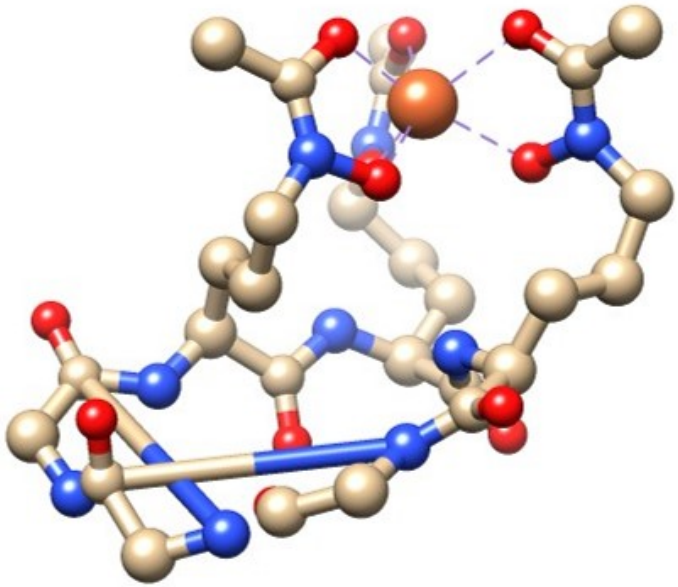


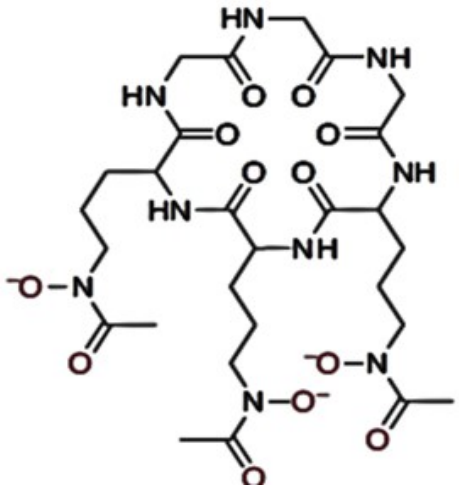




FERRICHROME







TRANSFERRINS

Introduction:

Fe-transport proteins are known collectively as **transferrins**.

Examples:

serum transferrin (in blood plasma), **ovotransferrin** (in egg-white), and **lactoferrin** (in milk).

Uses:

Apotransferrins are potent antibacterial agents as they deprive microbes of their iron.

Transferrins are also present in tears, serving to cleanse eyes after irritation.

STRUCTURE OF TRANSFERRINS

Transferrin

Glycoprotein, 80 kDa, Single polypeptide

N-terminal lobe

C-terminal lobe

N1 sub-domains

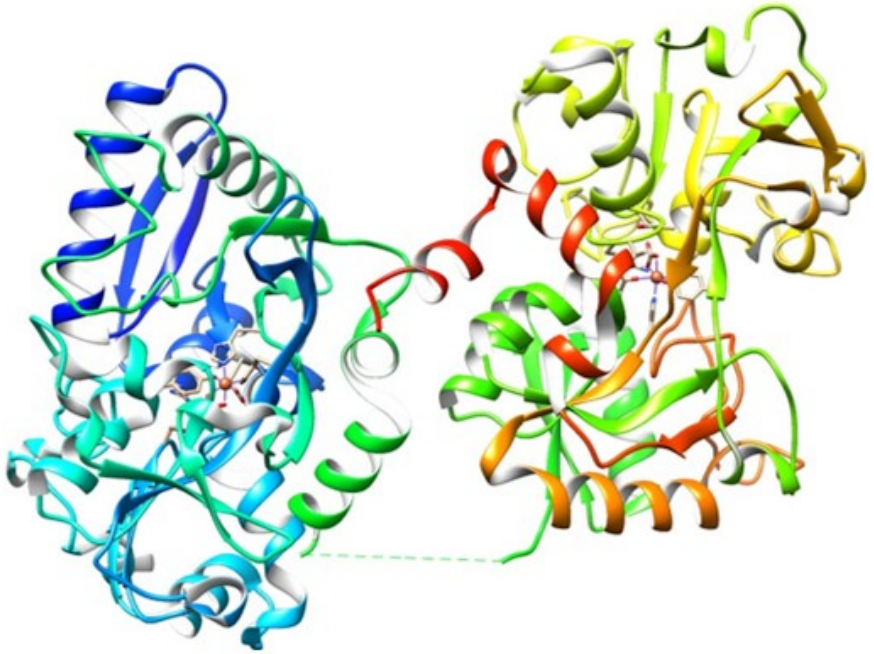
N2 sub-domains

C1 sub-domains

C2 sub-domains

Forms a cleft, to form binding site for Fe(III)

Forms a cleft, to form binding site for Fe(III)



STRUCTURE OF TRANSFERRINS

Coordination of Fe(III) in transferrins

Fe(III) has a

distorted octahedral

geometry

coordinated by

carboxylate-O (Asp),

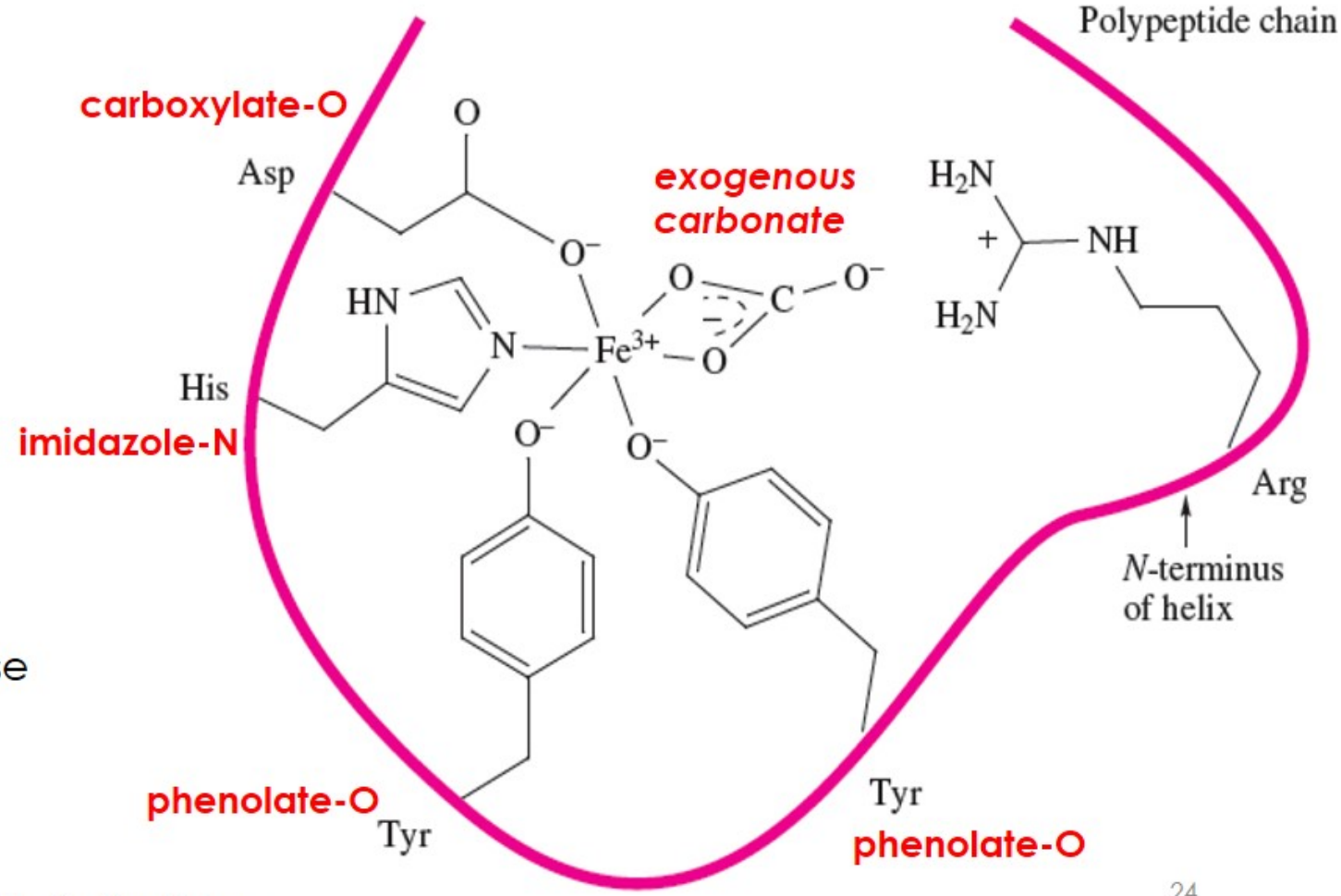
two phenolate-O (Tyr),

and an imidazole-N (His).

exogenous carbonate

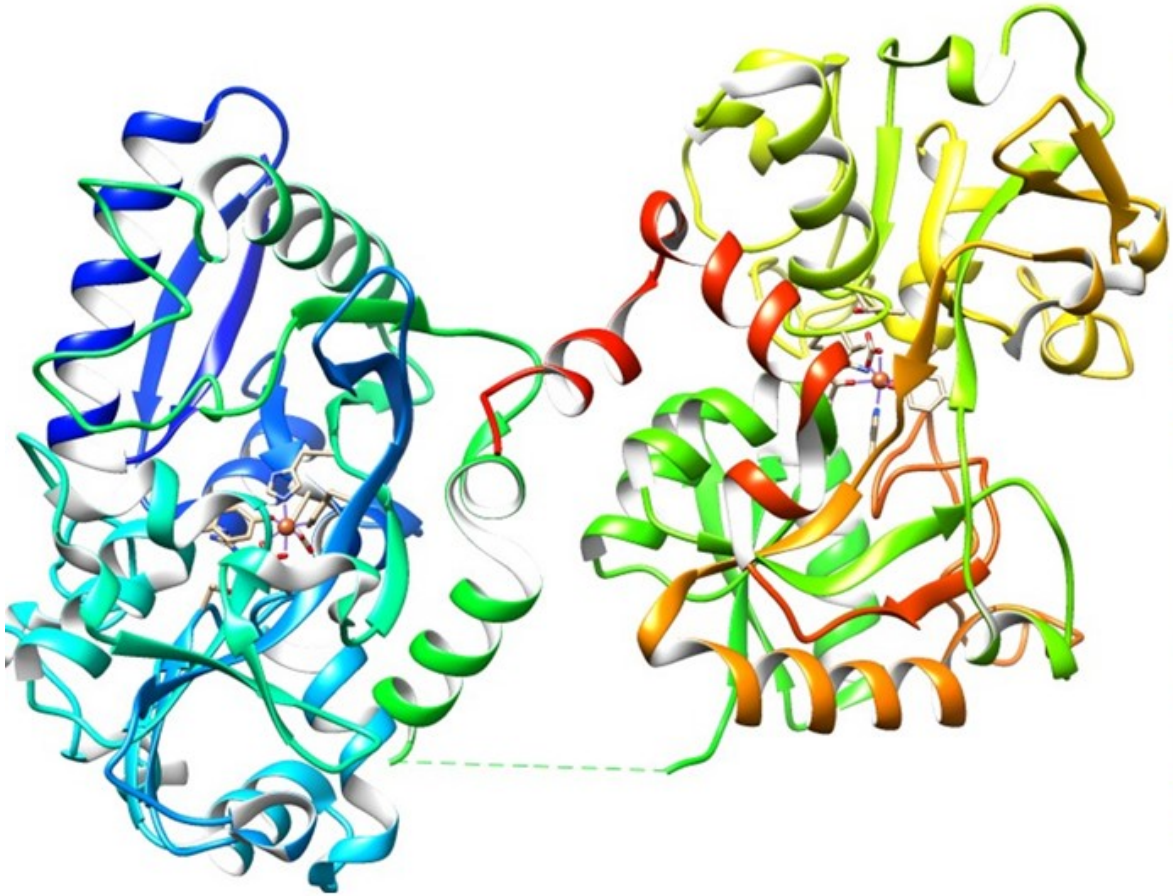
(bidentate)

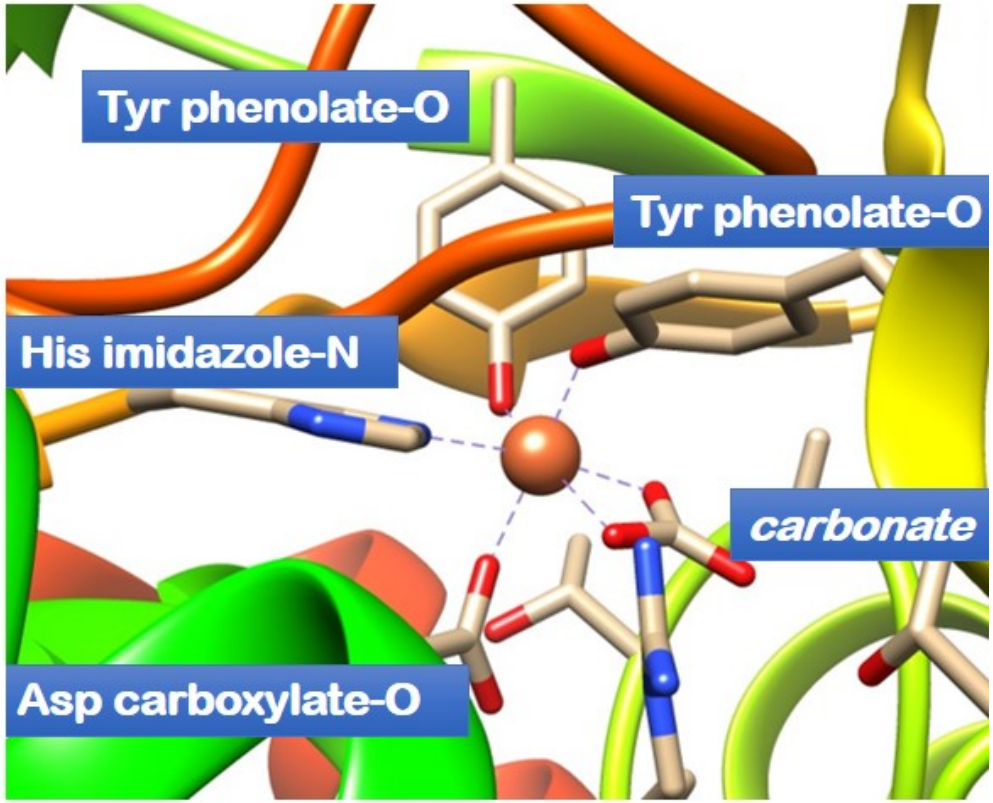
(a **synergistic ligand** because Fe binding depends on its presence).



STRUCTURE OF TRANSFERRINS

Coordination of Fe(III) in transferrins





BINDING OF IRON TO TRANSFERRIN

- Complexation with Fe(III) causes a **conformational change**, causes the **sub-domains** to come together and a single Fe atom is coordinated by amino acid side chains from both domains.

- Complexation of Fe(III) at each site involves simultaneous binding of **HCO_3^- or CO_3^{2-}** and release of H^-:

$$\text{apo-TF} + \text{Fe(III)} + HCO_3^- \rightarrow \text{TF–Fe(III)–}CO_3^{2-} + H^+$$

where TF denotes transferrin.

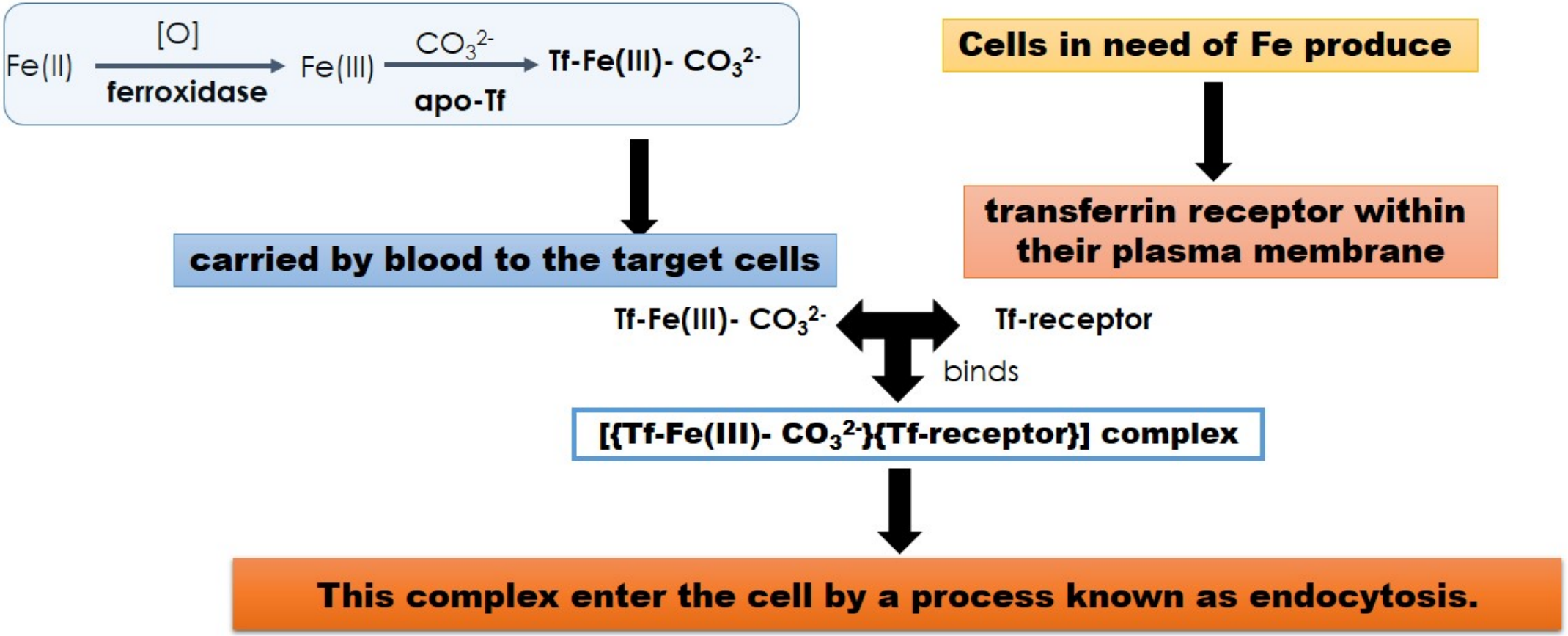
- **Association constant** =1022–1026 (pH = 7).

- The value depends strongly on the **pH**, which control Fe uptake and release.

TRANSFERRINS

- Binding ***synergism*** between CO_3^{2-} and Fe^{3+} is due to the simultaneous (in space) binding to each other as well as to the protein
- In certain cases **oxalate, salicylate, malonate, citrate, glyconate, pyrovate, nitrilotriacetate, phosphate** is bound instead of carbonate.
- As expected from the predominantly anionic ligand set, Fe(III) binds much more tightly than Fe(II).
- However, ions similar to Fe(III), particularly **Ga(III) and Al(III)**, also bind tightly, so that these metals can use the same transport system to gain access to tissues.

RELEASE OF IRON FROM TRANSFERRIN



In endocytosis, a section of the cell membrane is engulfed by the wall, along with its component membrane-bound proteins, to form a vesicle.

RELEASE OF IRON FROM TRANSFERRIN

- The **pH** within this vesicle is then **lowered** by a membrane-bound H^- pumping enzyme that is also swallowed by the cell.
- The binding of Fe is unstable at low pH.
- Indeed, from *in vitro* studies it is known that ***Fe is released by lowering the pH*** to about **5 for serum transferrin** and to **2–3 for lactoferrin**.
- The vesicle then splits and the **TF-receptor complex** is returned to the plasma membrane by **exocytosis**, and Fe(III), probably now complexed by citrate, is released to the cytoplasm.

FERRITIN

- Ferritin is the **principal store of non-haem Fe** in animals (most Fe is occupied in haemoglobin and myoglobin) and, when fully loaded, contains 20 per cent Fe by mass.
- It occurs in all types of organism, from mammals to prokaryotes. In mammals, it is found particularly in the spleen and in blood.
- **Apoferritin** (the protein shell devoid of Fe) can be prepared by treatment of ferritin with reducing agents and an Fe(II) chelating ligand (such as 1,10-phenanthroline or 2,2_-bipyridyl). Dialysis then yields the intact shell.
- Apoferritins have average molar masses in the range **460 to 550 kg mol^{-1}**.

STRUCTURE OF FERRITIN

protein shell.

24 subunits of

H (21 kDa) and **L** (19.9 kDa) **types**

24 subunits forms nearly a **hollow sphere** with ***twofold***, ***threefold***, and ***fourfold*** symmetry axes.

'mineral' core

hydrated Fe(III) oxide with varying amounts of phosphate, which helps anchor it to the internal surface.

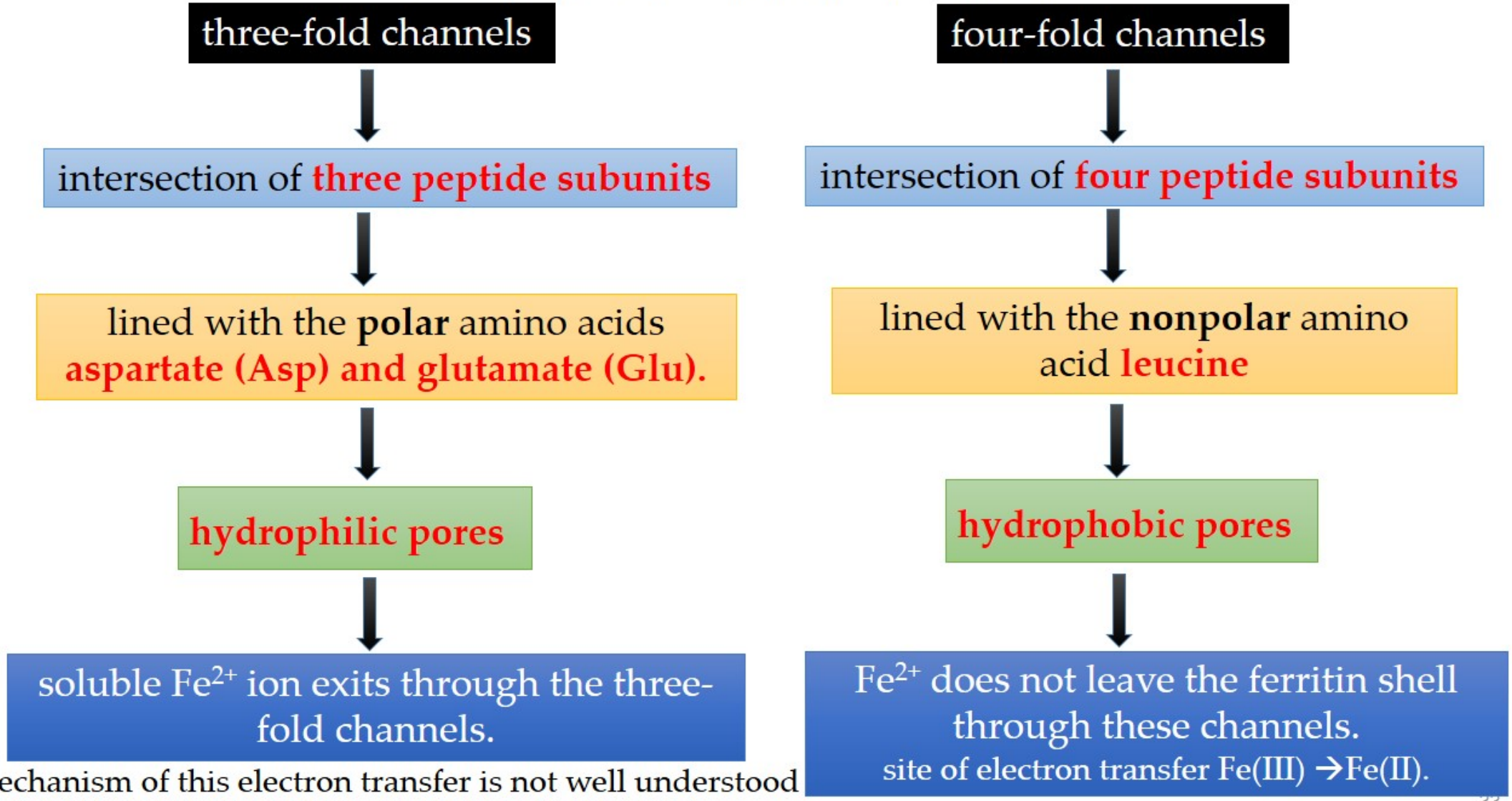
(resembles that of the mineral **ferrihydrite**, **$5Fe_2O_3.9H_2O$**)

4500 Fe atoms (mammalian ferritin)

STORAGE OF IRON IN FERRITIN

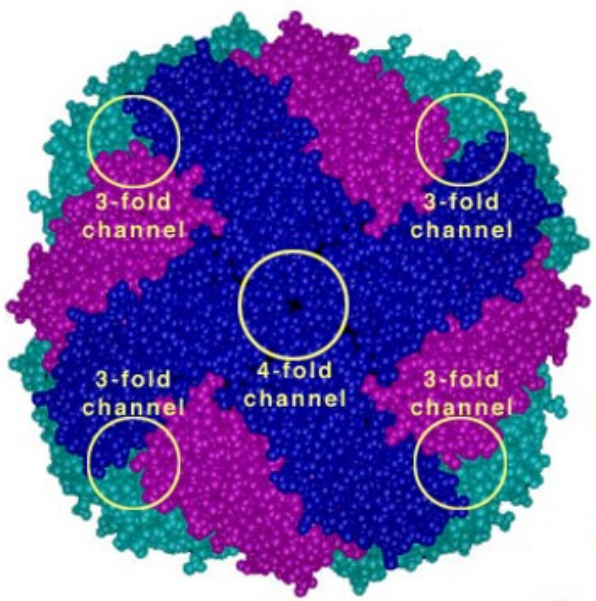
- However, the ferrihydrite core is insoluble but Fe must be mobilized.
- Fe is separated from the ferritin as either Fe(II) or Fe(III), but it is stored as Fe(III)-hydroxide or oxide.
- Oxidation to Fe(III) is thought to occur at specific di-iron binding sites known as **ferroxidase centers**, present in each of the subunits.
- Therefore, oxidation to Fe(II) to Fe(III) involves the ***coordination of O_2 and inner-sphere electron transfer***.
- The mechanism by which Fe is released almost certainly involves its reduction back to the more mobile Fe(II).

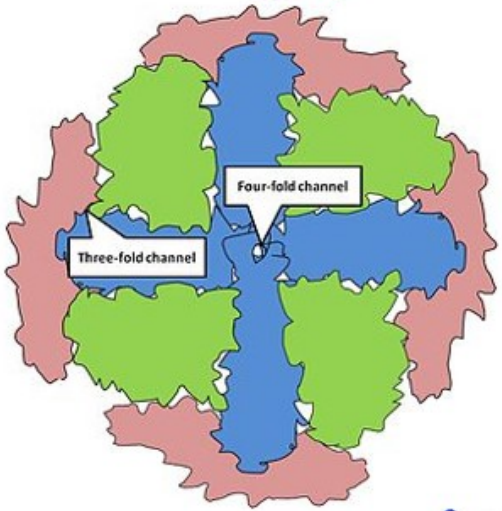
STORAGE OF IRON IN FERRITIN

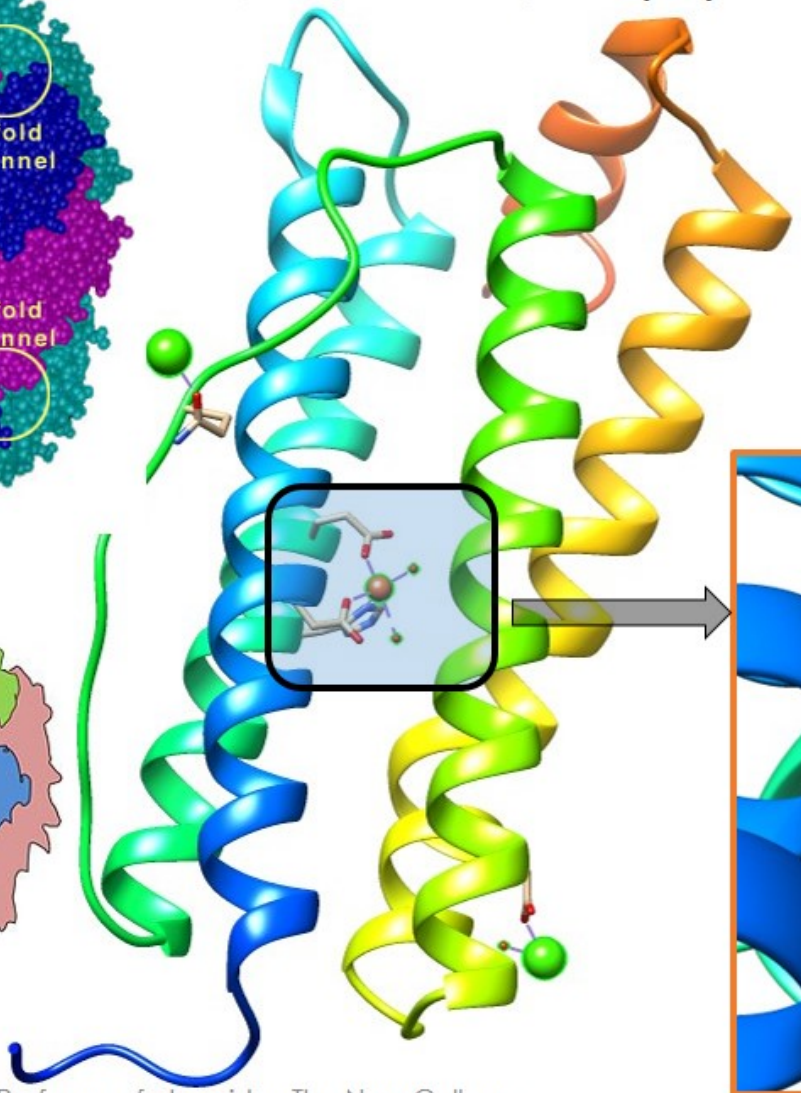


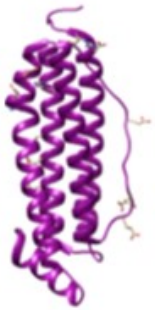
mechanism of this electron transfer is not well understood

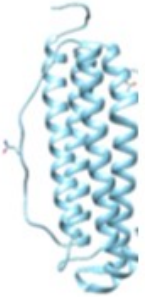
STRUCTURE OF FERRITIN

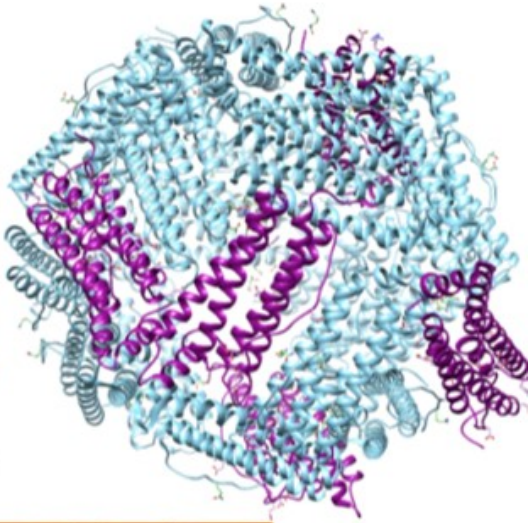


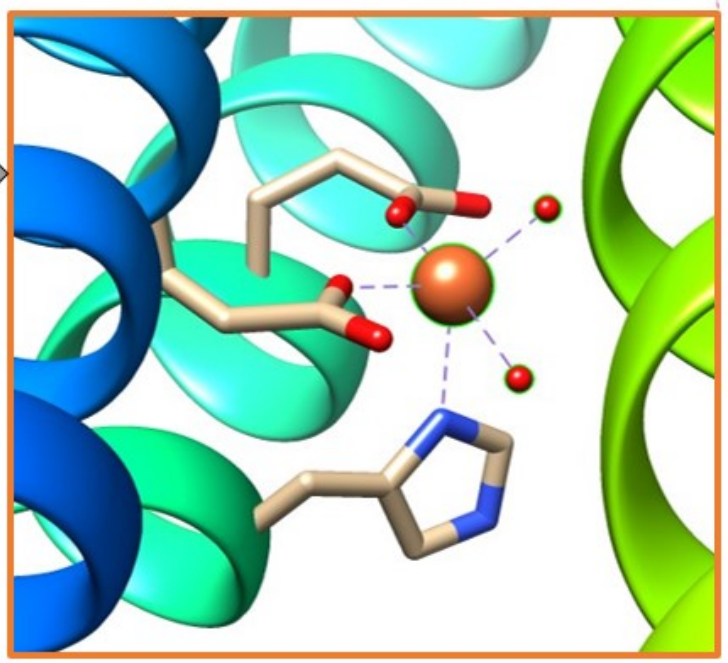












CALCIUM SIGNALLING PROTEINS

- Calcium is particularly ***suited for signaling*** because it has

➢ fast ligand-exchange rates,

➢ intermediate binding constants, and

➢ a large, flexible coordination sphere.

- **Calcium signaling proteins:** They are small proteins that change their conformation depending on the binding of Ca^{2+} at one or more sites; they are thus examples of the metal ion activated proteins.

Troponin C

- Every ***muscle movement*** is stimulated by Ca^{2+} ion binding to a protein known as **troponin C**.

- It has similar structure to calmodulin.

CALMODULIN (CALcium MODULated proteIN)

- The best-studied Ca^{2+}-regulatory protein is **calmodulin** (17 kg mol^{-1}), which

activates calmodulin kinases(II)

↓

catalyze phosphorylation of proteins

↓

involved in cell proliferation, fertilization, learning, memory

activate NO-synthase
Fe-containing enzyme

↓

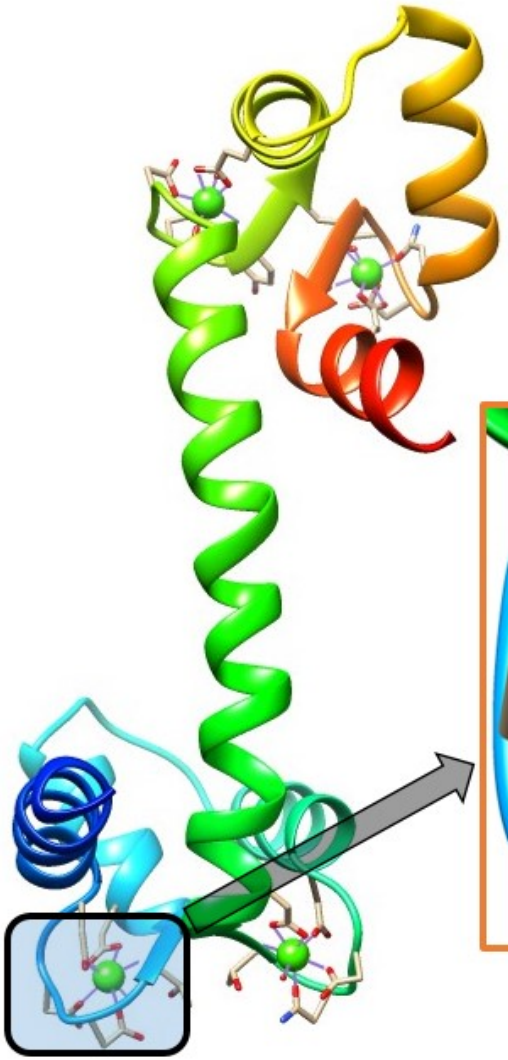
responsible for generating the intercellular signaling molecule nitric oxide

targets **calcineurin**

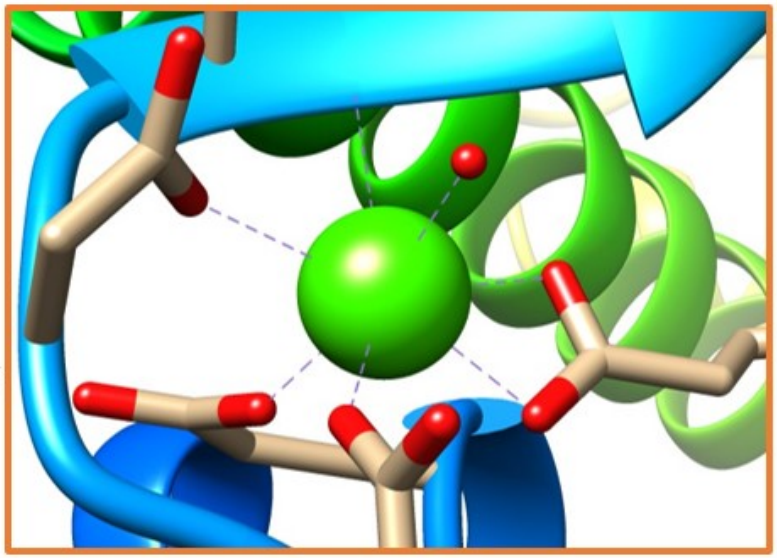
↓

involved in gene expression

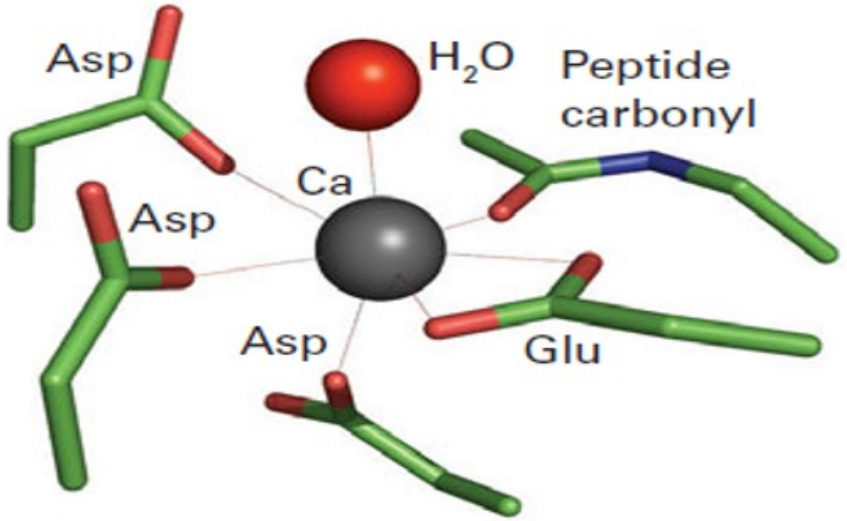
STRUCTURE OF CALMODULIN



The binding of four Ca^{2+} causes a change in the protein conformation.



Calmodulin has four Ca^{2+} -binding sites, which are coordinated by 7 'O' atoms in distorted octahedral shapes, viz., three aspartate, one carbonyl of peptide bond, bidentate glutamate and H_2O.



Calmodulin

ZINC ENZYMES: 1. CARBOXYPEPTIDASE

- **catalyzes** the **hydrolysis of C-terminal amino acids** containing an aromatic or bulky aliphatic side chain.
- CPDs are synthesized as inactive precursors in the **pancreas** for secretion into the digestive tract.
- The inactive form is converted to the active CPA by the enzyme enteropeptidase.

Carboxypeptidase A

- stronger preference for those amino acids containing **aromatic or branched hydrocarbon** chains. (A – aromatic/ aliphatic)

Carboxypeptidase B

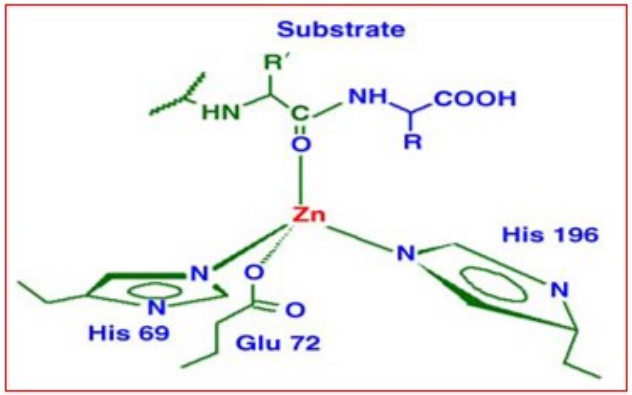
- cleave **positively charged** amino acids like arginine, lysine. (B - basic)

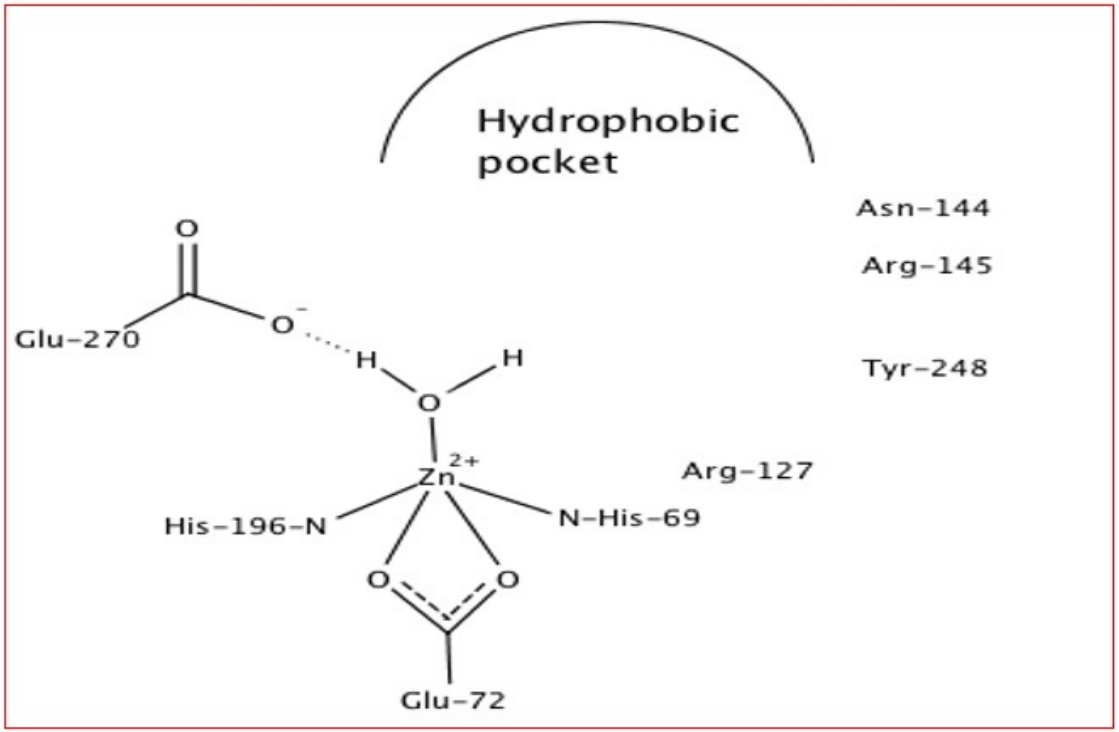
Carboxypeptidase C

cleaves a C-terminal glutamate from the peptide **N-acetyl-L-aspartyl-L-glutamate**

STRUCTURAL FEATURES OF CARBOXYPEPTIDASE

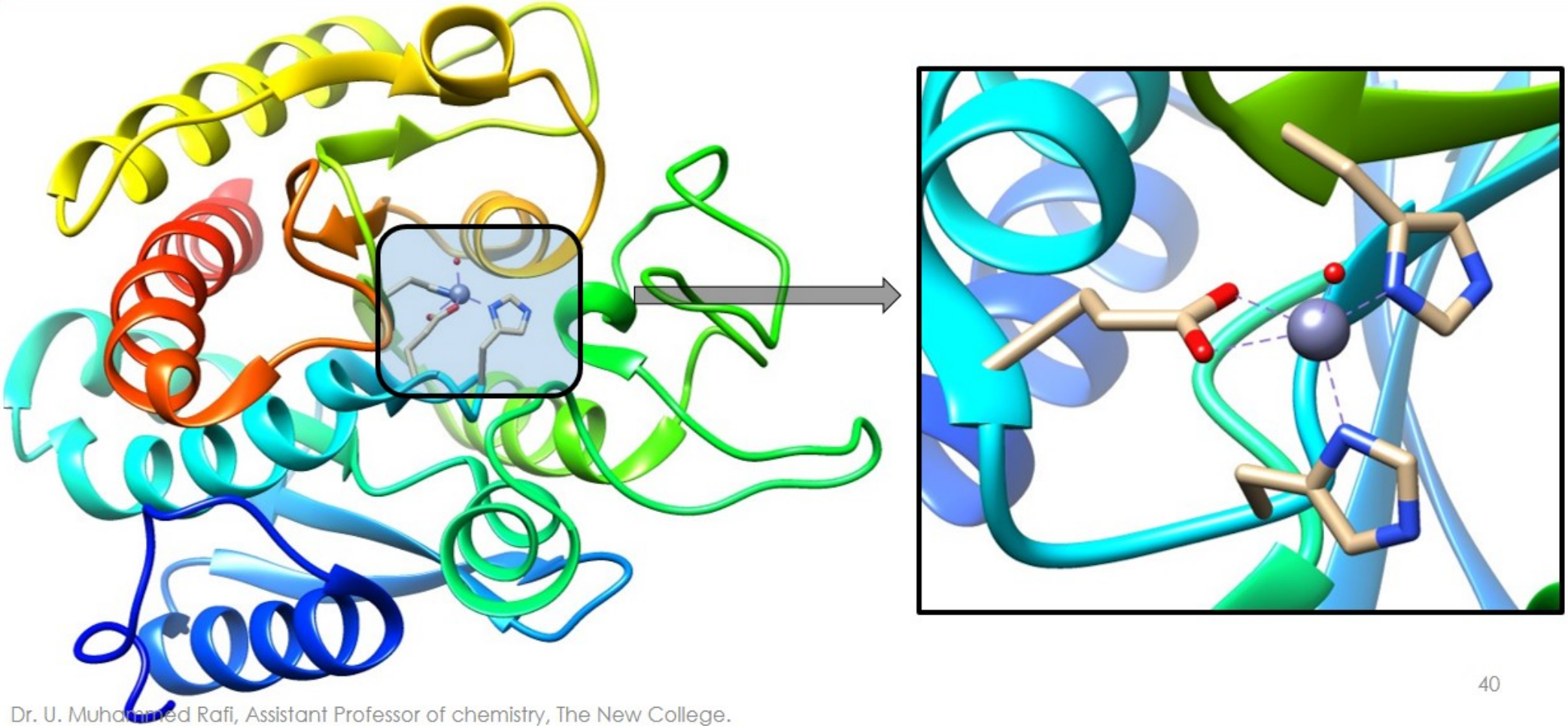
- CPDs consist of *one single peptide chain* and *one atom of zinc*.
- molecular weight is ~34,300.
- CPA has *307 amino acid residues*; CPB has *308* residues.
- Zn^{2+} has a ***distorted penta-coordination*** and is coordinated to ***two histidine*** residues (His69 and His196), a ***glutamate-*** CO_2^-(bidentate) (Glu72) residue, and an H_2O molecule (or OH^-).





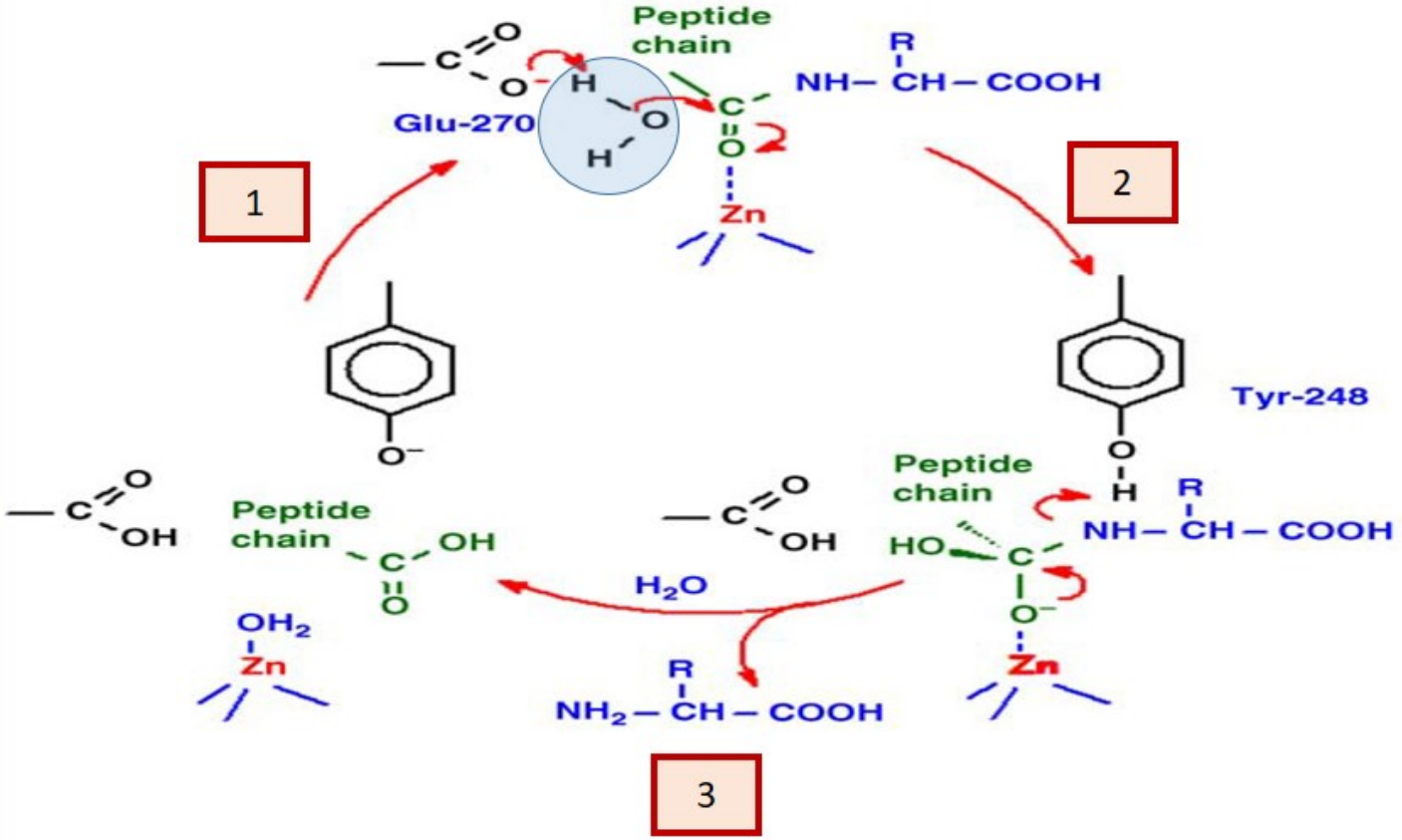
STRUCTURAL FEATURES OF CARBOXYPEPTIDASE

- Zn^{2+} has a ***distorted penta-coordination*** and is coordinated to ***two histidine*** residues (His69 and His196), a ***glutamate-CO_2^-***(bidentate) (Glu72) residue, and an ***H_2O*** molecule (or OH^-).



ROLE OF Zn^{2+} IN CARBOXYPEPTIDASE-A AND CARBOXYPEPTIDASE-B

- The *mechanism* was proposed by **Christianson** and **Lipscomb**.
- Follows **Zn-carbonyl mechanism**
- The active site cavity has a **hydrophobic pocket** that accommodates the **non-polar R′** of the **C- terminal amino acid** of the **substrate** undergoing hydrolysis.
- The crystal structure studies confirm of a neutral water molecule and not a hydroxide ion in the resting state of the enzyme



2. CARBONIC ANHYDRASE

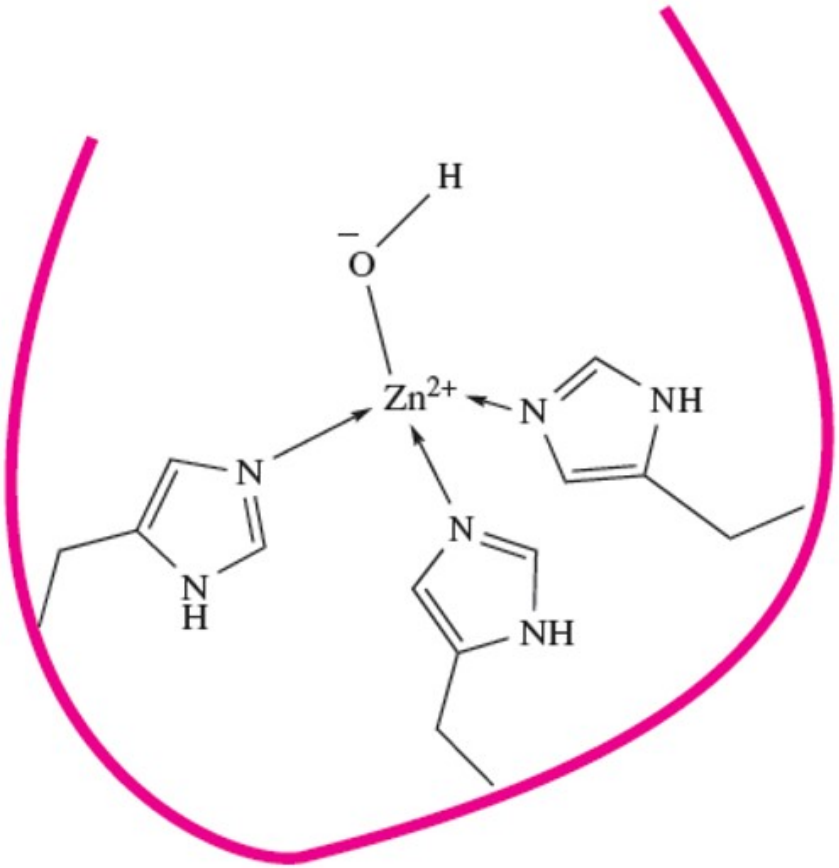
- Carbonic anhydrase (CA, or carbon dioxide dehydratase) was identified in 1940 by Keilin and Mann.
- It is a **lyase** that catalyzes the reversible hydration of carbon dioxide to form the bicarbonate ion

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} HCO_3^- + H^+$$

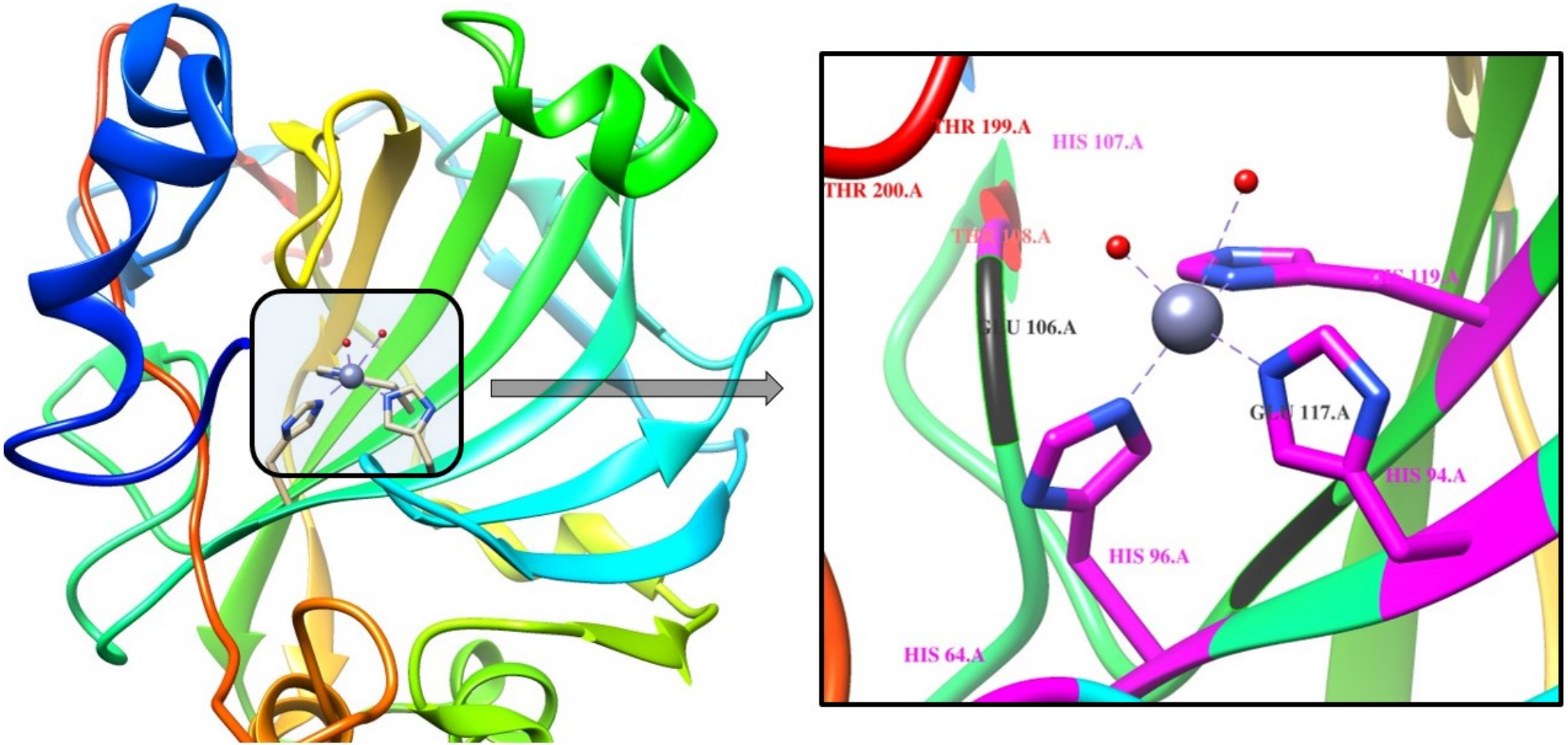
- It catalyzes this reaction with a remarkable rate enhancement.
- The best studied enzyme is **CA II** from red blood cells has a **turnover frequency for CO_2 hydration of about 10^6 s^{-1}**, which is one of the most active of all enzymes.

STRUCTURAL FEATURES OF CARBONIC ANHYDRASE

- There are several forms of CA, all of which are **monomers** with molar mass close to 30 kg mol^{-1} and contain ***one Zn atom per molecule***.
- The protein is composed of **259 amino acids**.
- In CA II, Zn is coordinated by **three His-N ligands** *(His-94, His-96, His-119)* and **one H_2O molecule** in a ***tetrahedral*** **arrangement**.



STRUCTURAL FEATURES OF CARBONIC ANHYDRASE



STRUCTURAL FEATURES OF CARBONIC ANHYDRASE

- The crystal structure of human CA II shows that the Zn atom (active site) is located in a conical cavity about 1.6 nm deep at the **bottom of a cleft** in the enzyme, which is lined with several **histidine** residues.

- The active site pocket also contains ordered network of *water molecules* and other amino acids like **threonine-199, glutamate-106, histidine-64** that are important for hydrogen bonding, ***mediating proton transfer*** (rate determining step in catalytic mechanism) and for ***binding the CO_2 substrate*** (which does not coordinate to Zn).

- Carbonic anhydrases show some variation in their Zn coordination environment, with some CAs from higher plants having **two cysteines** and **one histidine**.

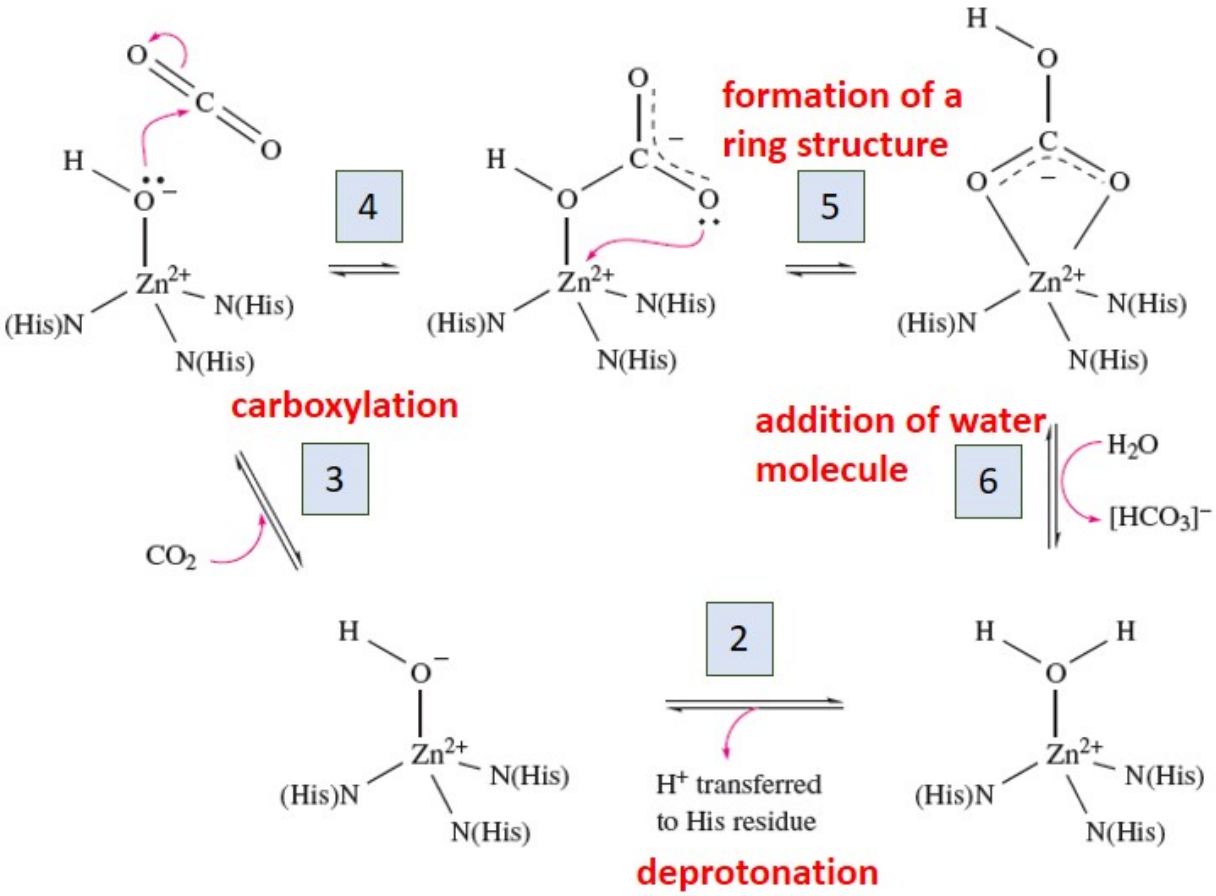
ROLE OF CARBONIC ANHYDRASE

- Follows **Zn-hydroxide mechanism**.

1 Glu106 deprotonates water bound to Zn(II).

3 The formed hydroxide ion bounds to another water via H-bonding, which is H-bonded to Thr199. Now this water is replaced by CO_2. This is how CO_2 is bound by the CA enzyme.

4 The hydroxide ion then attacks the CO_2 by nucleophillic attack.



ZINC ENZYMES
DIFFERENCE BETWEEN CA AND CPD:

Carbonic anhydrase

catalyzes the hydration of carbon dioxide to form **bicarbonate ion and proton**

Zinc(II) coordinated :
(a) **4 Nitrogens**
(1 imidazole, 3 histidine residues) and
(b) **one water molecule**

catalytic mechanism involves four steps **deprotonation, carboxylation, and formation of a ring structure and addition of water molecule** resulting in the formation of bicarbonate

Carboxypeptidase

hydrolyses the peptide bond at carboxyl terminal (C- terminal) end of a **peptide** or protein

Zinc(II) coordinated:
(a) **2 Nitrogens**
(2 histidine residues)
(b) **2 Oxygens** of
(1 **glutamate,** 1 **water molecule**)

catalytic mechanism involves **nucleophilic attack by hydroxide on the carbonyl carbon** of the C – terminal peptide bond (nucleophilic addition)

IRON ENZYMES

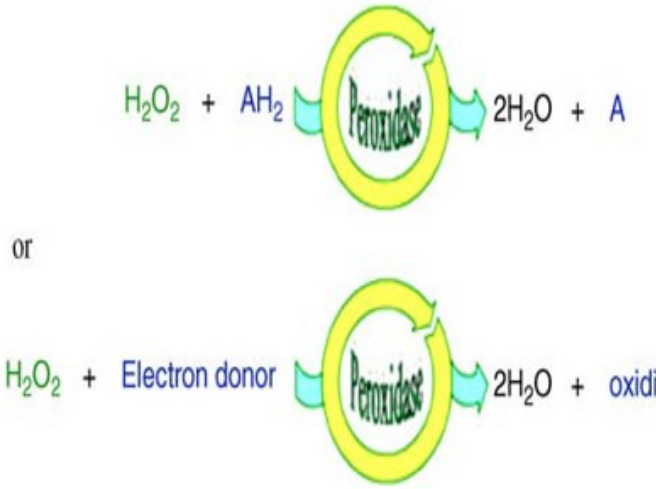
REACTIVE OXYGEN SPECIES OR ROS

- ✓ *O_2 produce various toxic substances such as hydrogen peroxide and superoxide (termed as **reactive oxygen species or ROS**).*
- ✓ *Hydrogen peroxide is a by-product of various reactions in organisms due to oxidative cellular metabolism, which may get converted into highly reactive hydroxyl radicals.*
- ✓ *Hydroxyl radicals cause damage to a variety of cells leading to oxidative stress and ultimately death.*
- ✓ *Hence, rapid and efficient removal of ROS is of utmost significance, which is done by protein complexes called **"catalases"** and **"peroxidases"** that are able to catalyze the decomposition of this reactive molecule (H_2O_2) at reasonable rates.*

2. PEROXIDASES

Peroxidases are ***enzymes*** catalyzing the ***oxidation*** of a variety of organic and inorganic compounds by reducing H_2O_2, also acting as dehydrogenases

Chloroperoxidase:	• halogenates organic substrates
Lactoperoxidase:	• antibacterial, oxidizes NCS
Cytochrome P-450:	• hydroxylates organic substrates
Cytochrome c peroxidase	• : reduces H_2O_2.

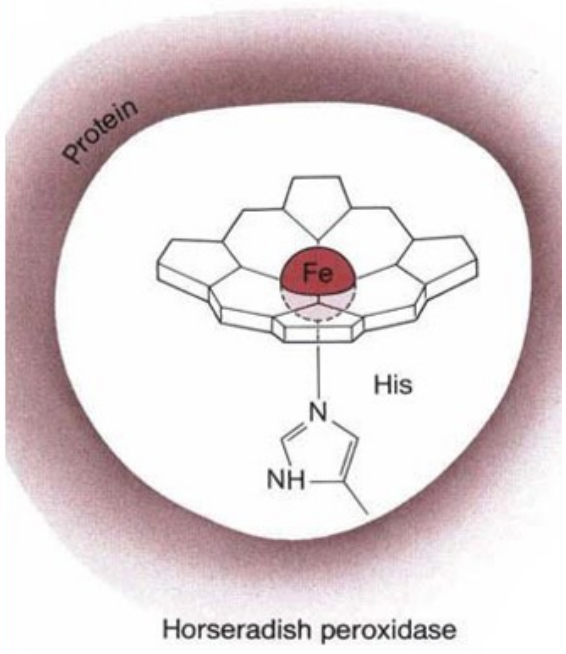


$$H_2O_2 + SH_2 \xrightarrow{\text{peroxidase}} 2H_2O + S \quad \text{where S = substrate}$$

2. PEROXIDASES

Structural features of horseradish peroxidase

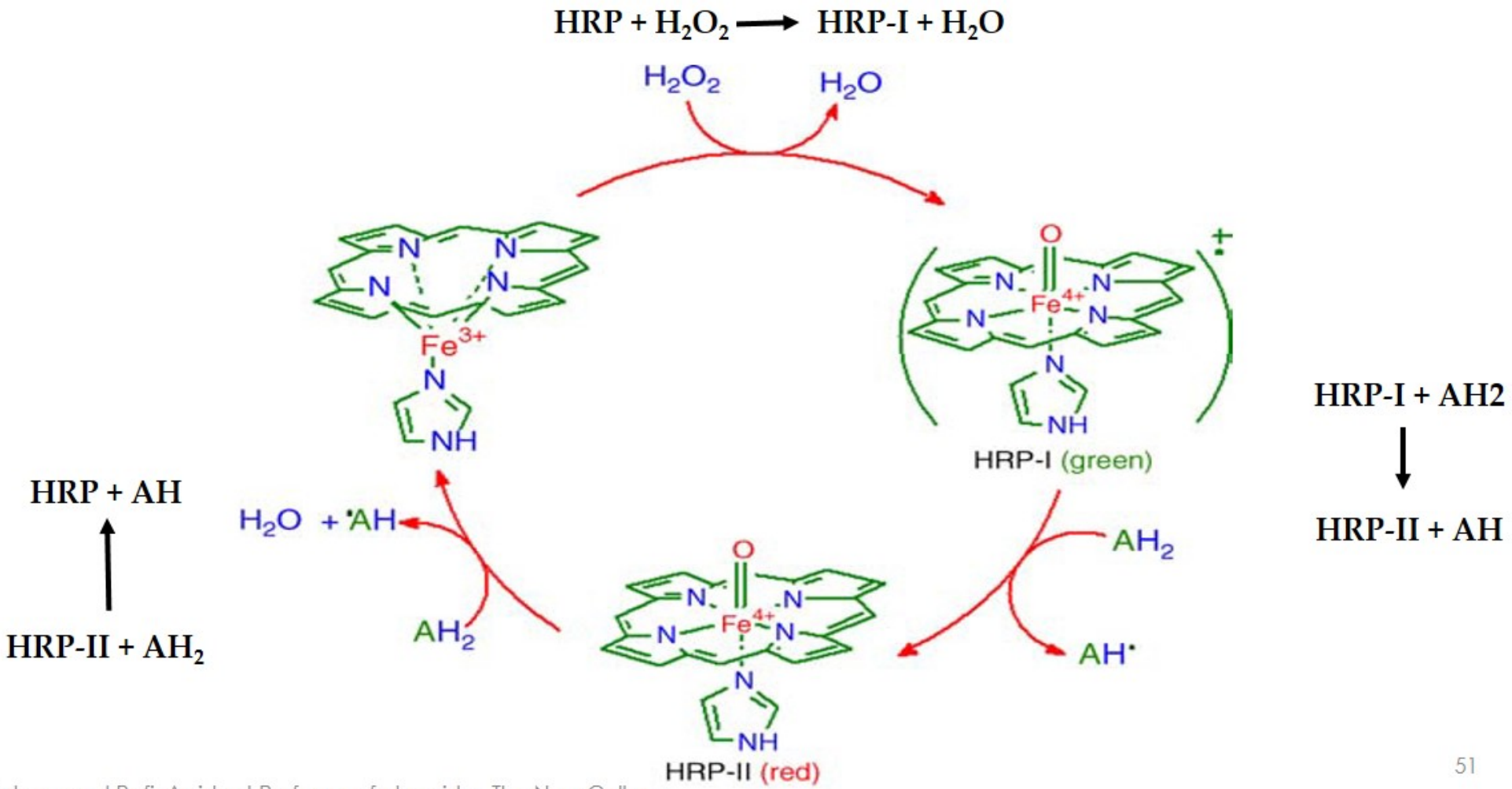
- Most peroxidases are **glycoproteins**. Color brown, molecular weight ~ 40,000
- **HRP C** contains (i) **iron heme group** and (ii) **two calcium atoms**
- The ***heme group*** has a ***planar structure*** with the ***iron atom in the middle*** of a ***porphyrin ring having two open bonding sites***.
- The heme group of HRP is attached to a ***histidine residue (His170)*** and an ***aquo group*** as the fifth and sixth ligands to Fe^{3+}, while a second histidine residue may be hydrogen bonded to the aquo group.
- Each calcium site of the enzyme is ***seven-coordinate*** with oxygen-donor ligands provided by **carboxylates (Asp), hydroxyl groups (Ser, Thr), backbone carbonyls** and a **structural water molecule** (distal site only).
- Ferriperoxidases react reversibly with CN^-, S^{2-}, F^-, N_3^-, and NO.
- In solutions of ***low pH***, HRP is **high spin**, but **low-spin** species are formed at ***high pH*** (pH 11).



Horseradish peroxidase

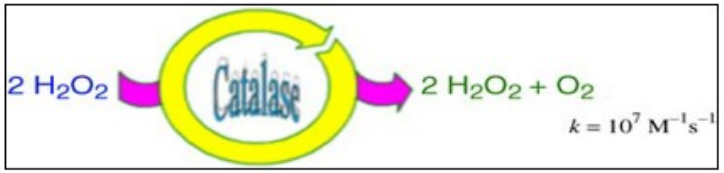
2. PEROXIDASES

Role of peroxidases



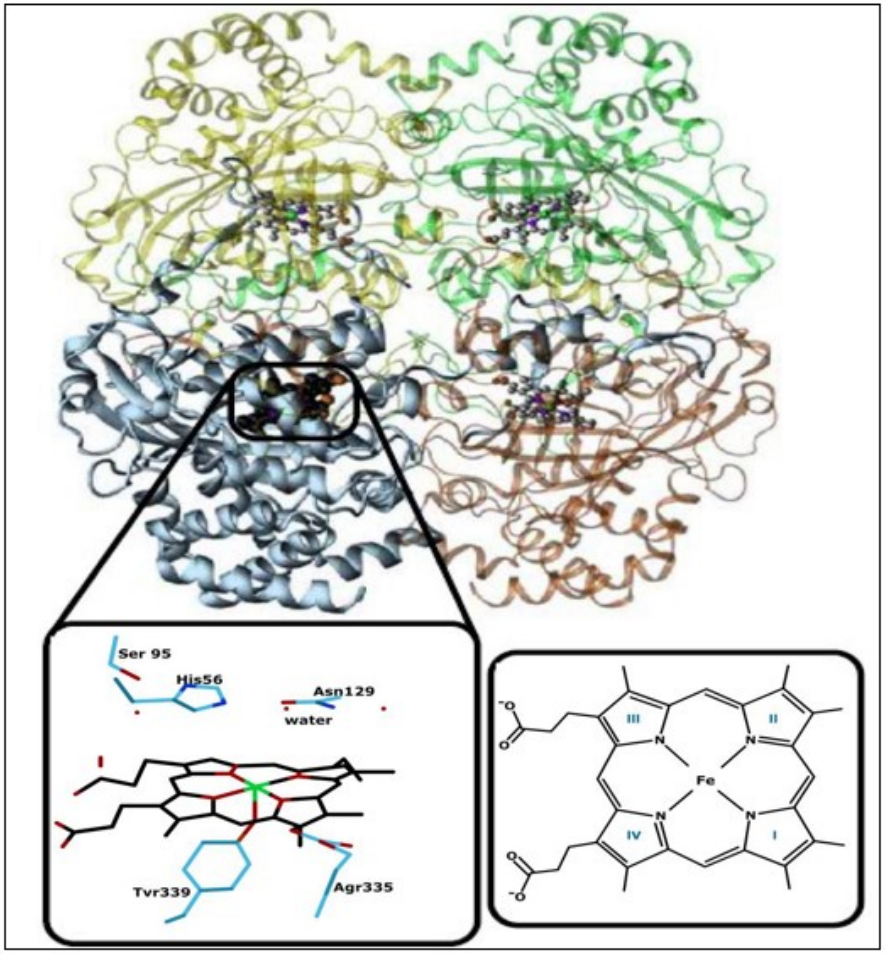
3. CATALASES

Catalases catalyze the ***decomposition of H_2O_2*** into H_2O and O_2



Structure of catalases

- Catalases are composed of ***four identical subunits (tetramers of four polypeptide chains)***, each containing approximately 500 amino acids.
- Every monomer unit has an active heme unit i.e. ***high-spin Fe^{3+}–porphyrin groups*** at the active site.
- It is lined with *hydrophilic residues* at the entrance and with *hydrophobic residues* as the channel descends.
- The Fe(III) of the heme unit exists in high spin state and is coordinated to ***four nitrogen atoms of the porphyrin ring***, while the **fifth and sixth axial positions** are occupied by the ***phenolate O- of the tyrosyl residue*** and ***water*** molecule respectively.



3. CATALASES

Reaction Mechanism

- Chemical modification studies have suggested that ***histidine*** and ***tyrosine*** are involved in the activity of the enzyme.
- During catalytic reactions, the axial water molecule is displaced by H_2O_2.
- The ***axial metal sites*** are occupied by ***water*** and ***an amino acid residue***.

- **Catalase reacts with H_2O_2 to give compound I, which is able to oxidize H_2O_2:**
  - **$H_2O_2 + Fe^{3+} \rightarrow H_2O + Fe$ compound I**
  - **Fe compound I + $H_2O_2 \rightarrow H_2O + O_2 + Fe^{3+}$**

COPPER ENZYMES & THEIR FUNCTIONS

Proteins	Biological Function	Found
"Blue" electron carriers: Azurin, Plastocyanin Stellacyanin, Umecyanin	Electron Transfer (Photosynthesis)	Algae, green leaves and other plants
Blue oxidases: 1. Ceruloplasmin 2. Ascorbate oxidases	Fe and Cu transport Oxidation of L-ascorbic acid	Human and animal serum Plants
Superoxide dismutase	O_2^- detoxification	Red blood cells
Cytochrome c oxidase	Terminal oxidase	Mitochondria
Non-blue oxidases Amine oxidases	Elastin, collagen formation	Animals
Oxygen carrier Hemocyanin	Oxygen transport	Molluscs, arthropods
Copper mono-oxygenases 1. Tyrosinase 2. Dopamine b-hydroxylamine	Melanin synthesis Dopamine to norepinephrine	Animals, plants, insects adrenals
Copper dioxygenases Quercetinase	Quercerti oxidative cleavage	Fungal

TYPES OF COPPER CENTERS

1. **Types 1 centers**

   have a ***single Cu atom***, which has an ***intense blue color***.

2. **Type 2 centers**

   have a ***single Cu atom***, which is almost ***colorless***.

3. **Type 3 centers**

   have a ***di-Cu center*** which is ***EPR-silent***.

4. **Multi-Copper Oxidases:**

   contain Types 1, 2 and 3 centers.

5. **Copper Centers in Cytochrome Oxidase:**

   Two unique copper centers: (i) Cu_A (also known as Cu_d) (ii) a dinuclear centre with cytochrome a_3. It has been referred to as Cu_B

CLASSIFICATION OF BIOLOGICAL COPPER CENTERS

Type	Mononuclear		Dinuclear		Tetranuclear
	Type 1	Type 2	Type 3	CuA	CuZ
UV-Vis Spectrum	Strong absorption~ 600nm & (in some proteins 450nm)	Weak absorption~ 700nm	Weak absorption~ 700nm	Strong absorption~ 480 & 530nm	Strong absorption~ 640nm
EPR Spectrum	4-lines spectrum	4-lines spectrum	Non detectable	7 lines spectrum	2×4-lines spectrum
Common ligands	His, Cys, (Met)	His, Asp, (Tyr)	His, (Tyr)	His, Cys, (Met)	His, S^{2-}
Geometry	Distorted tetrahedral	Square planar or tetragonal	Tetragonal	Trigonal planar	m4-S^{2-} tetracopper cluster
Examples	Azurin, Plastocyanin	Superoxide dismutase	Hemocyanin tyrosinase	Cyt C oxidase NO_2 reductase	NO_2 reductase

PLASTOCYANIN

- Plastocyanin (Pc), a small protein containing **a single Cu atom**, occurs in all higher plants, green algae and some blue-green algae.
- Molecular weight: 10,500, one Cu/molecule, 99 amino acids/molecule.
- Belongs to **Type I "blue" copper proteins** because in the oxidized (Cu^{2+}) state, an extremely strong absorption band near 600 nm gives the proteins an intense blue color.
- These proteins have **low EPR hyperfine splitting** constants in the $g_{||}$ region and relatively high redox potentials

Function:

- It has an essential role in **photosynthesis**, functioning as the last electron carrier from cytochrome-*f* to P700 chlorophyll (between photosystems II and I).

STRUCTURE OF PLASTOCYANIN

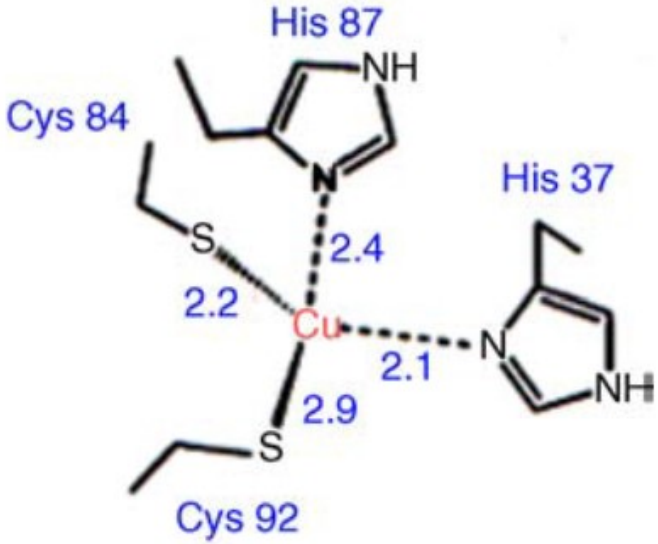
- Copper is **distorted tetrahedral** in geometry.

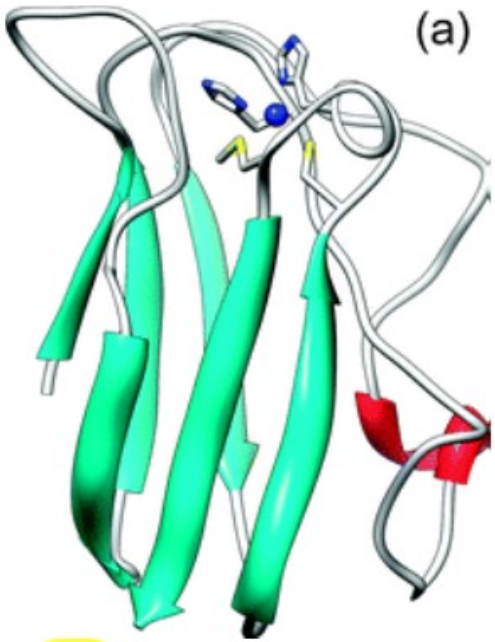
- ***In $Cu^{II}Pc$, the Cu atom is coordinated by***

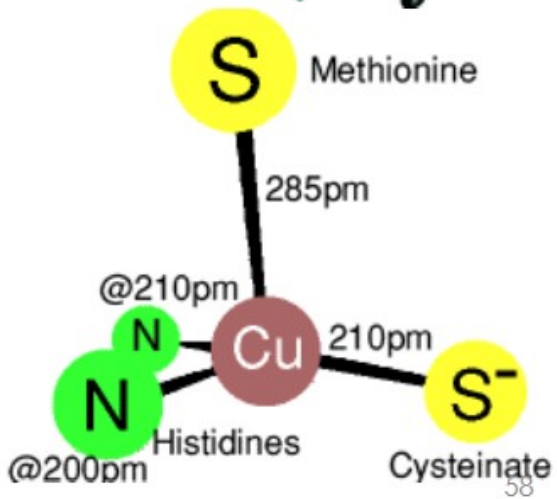
N^{δ} (imidazole) atoms of His37 and His87,

S^{γ} (thiolate) atom of Cys84,

S^{δ} (thioether) atom of Met92







CERULOPLASMIN

- ***Laccase, ascorbate oxidase***, and ***ceruloplasmin*** are the classical members of the multicopper oxidase family also known as ***blue oxidases***.

- The blue oxidases like *ascorbate oxidase, laccase, and ceruloplasmin*, and the terminal oxidases like *cytochrome oxidases and quinol oxidases* are the only enzymes so far known that ***catalyze the direct four-electron reduction of molecular oxygen to water***.

- Ceruloplasmin is exclusively found in the **plasma of vertebrates** and carries about **60% of Cu^{2+} in plasma**.

- Human serum ceruloplasmin is composed of a ***single polypeptide chain of 1046 amino acids***.

- The **six domains** of ceruloplasmin are arranged in a triangular fashion. Each domain consists of a β-barrel composed of eight antiparallel β-strands.

STRUCUTRE OF CERULOPLASMIN

six domains of ceruloplasmin

six to seven copper atoms

domains 2, 4, and 6

mononuclear copper atoms

3 → type I copper atoms

Cu in domains 4 and 6:

2 histidine, 1 cysteine and 1 methionine

Cu in domain 2:

2 histidine, 1 cysteine and 1 leucine

Cysteine in domain 6

Links

interface between **domains 1 and 6**

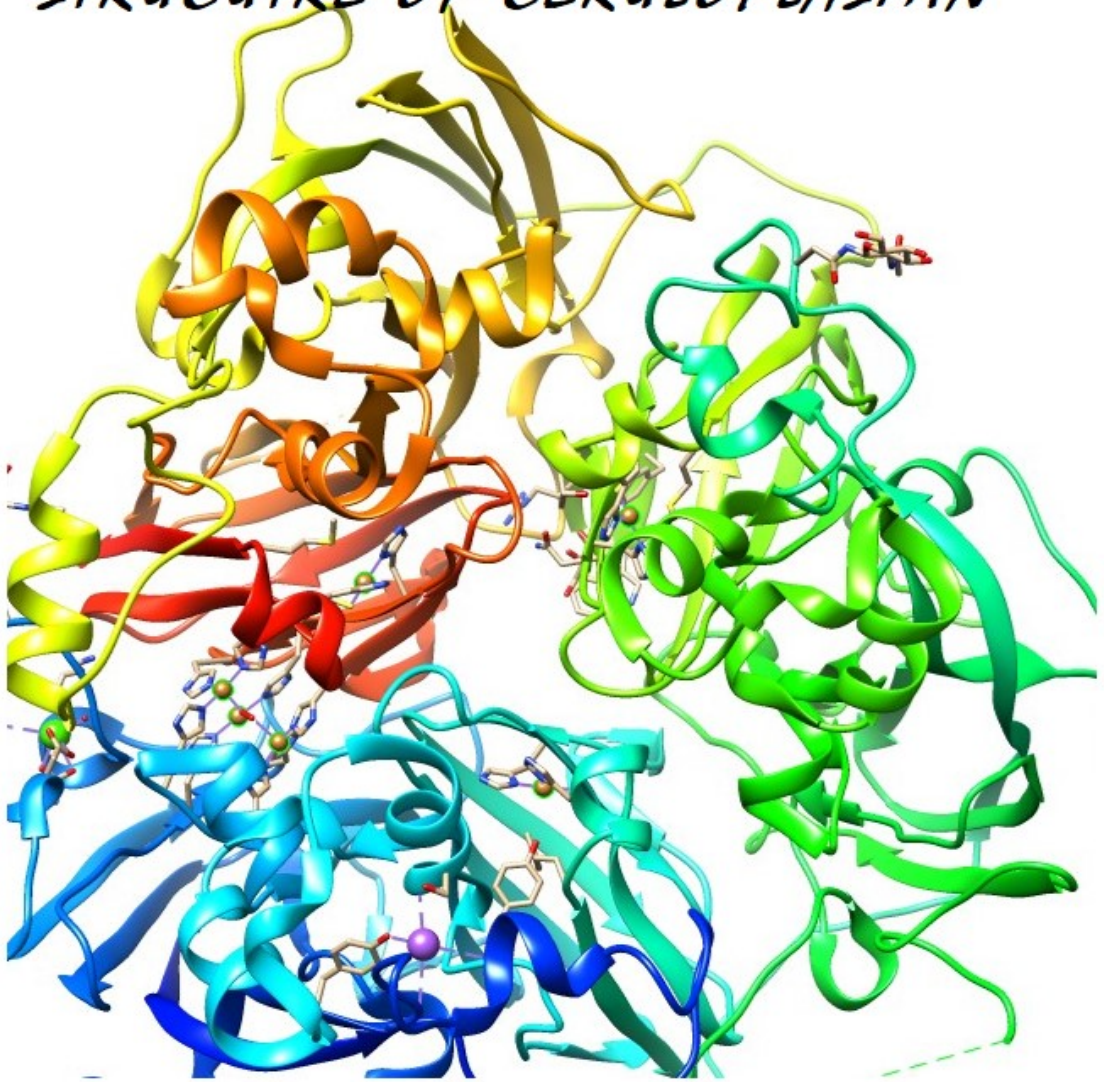
a trinuclear copper cluster

1→ type II copper atoms
2→ type III copper atoms

Cu between domains 1 and 6

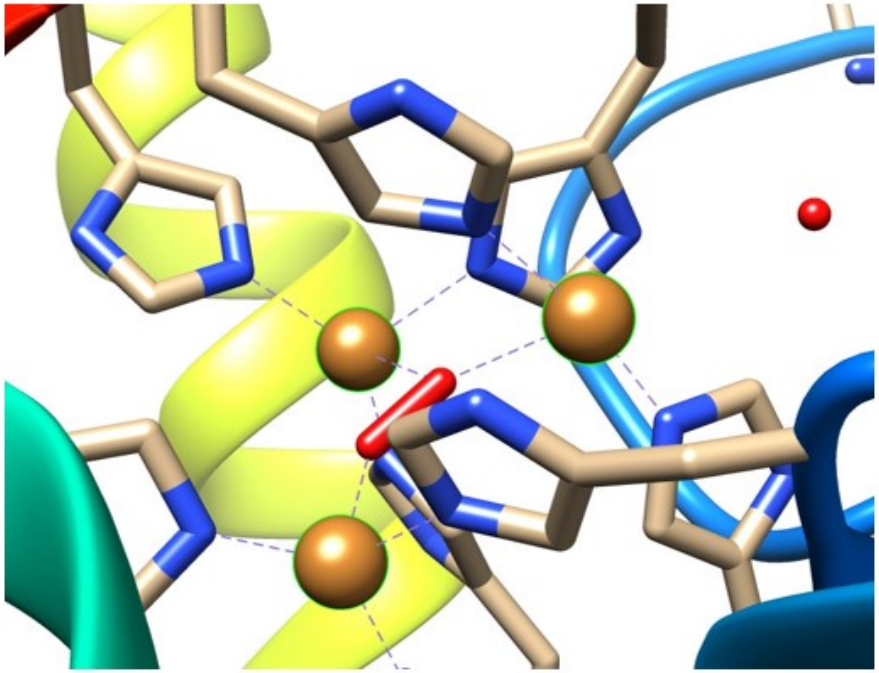
four pairs of histidines

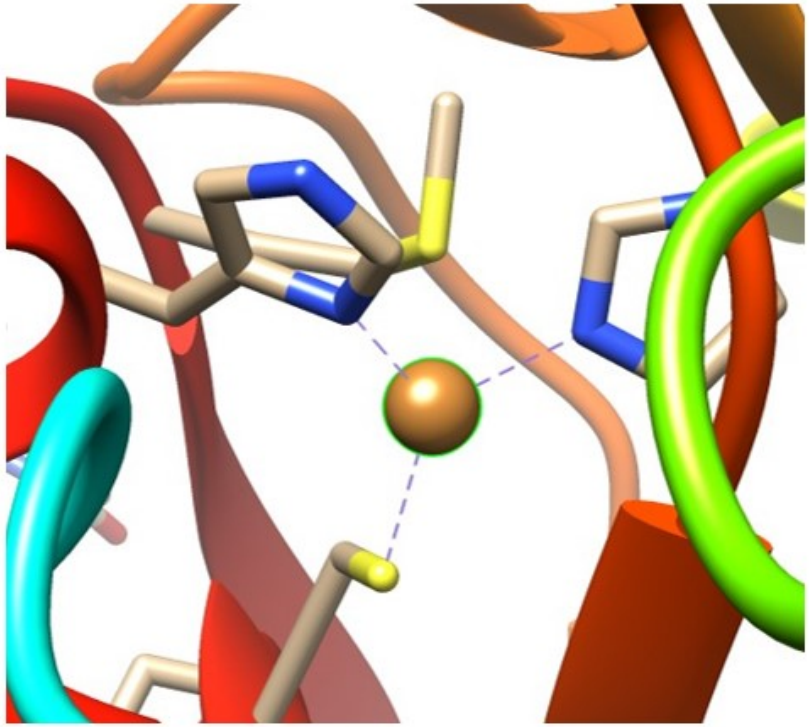
STRUCUTRE OF CERULOPLASMIN



DOMAIN
1 – BLUE
2 – LIGHT BLUE
4 – GREEN
6 – RED

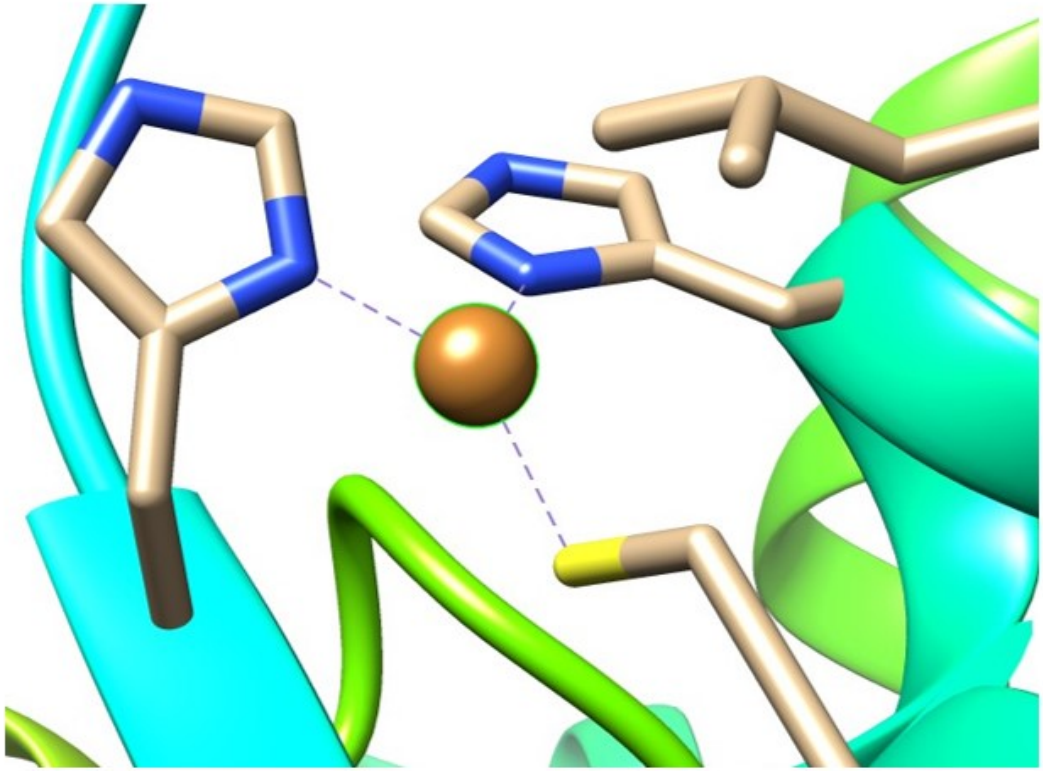
STRUCUTRE OF CERULOPLASMIN

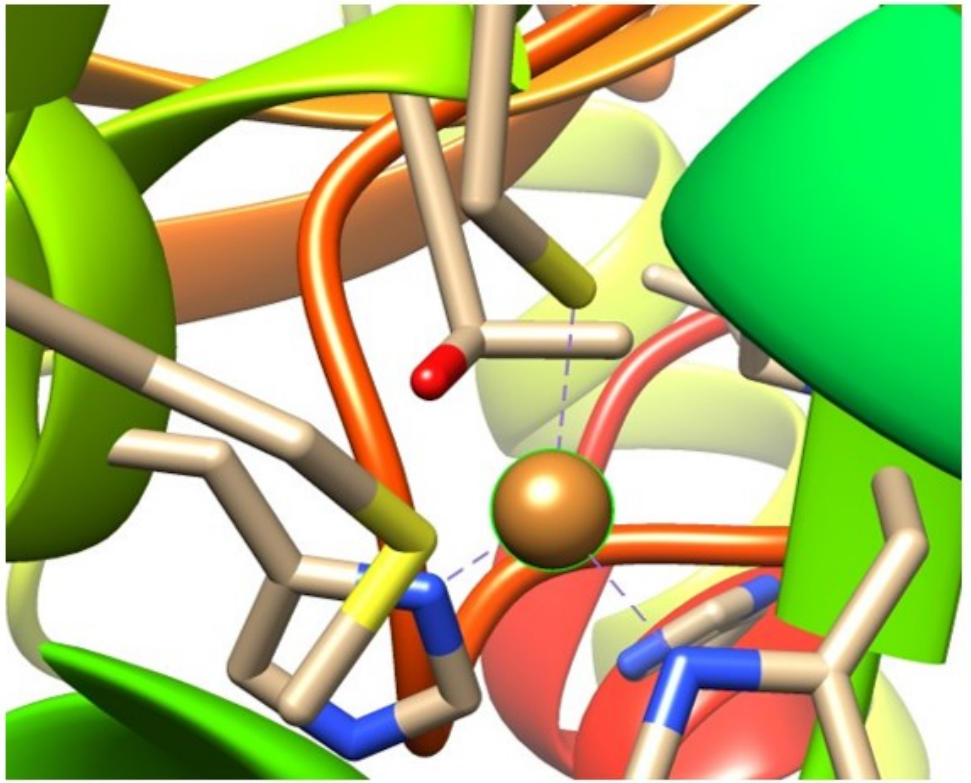




DOMAIN
1 – BLUE
2 – LIGHT BLUE
4 – GREEN
6 – RED

STRUCUTRE OF CERULOPLASMIN





DOMAIN
1 – BLUE
2 – LIGHT BLUE
4 – GREEN
6 – RED

ROLE OF CERULOPLASMIN

1. FERROXIDASE ACTIVITY

transfer of four electrons from Fe(II) iron

mononuclear copper atoms → **a trinuclear copper cluster**

reduction of O_2 to $2H_2O$

Cu's in domain 2 and 4

↓ Electrons transfer

Cu in domain 6

↓ Electrons transfer

a trinuclear copper cluster

- In addition to these integral copper sites, two labile copper binding-sites have been identified in domains 4 and 6 and are 9–10 Å distant from the non-labile sites.
- It has been proposed that the labile binding sites could be used for copper transport by ceruloplasmin as copper would be easily released from these sites.

I type II copper atom → oxygen atom is attached

I type III copper atom
II type III copper atom
→ one oxygen atom bridges

ROLE OF CERULOPLASMIN

1. Ceruloplasmin oxidizes its substrate [Fe(II)] with the concomitant reduction of molecular oxygen. Molecular oxygen binds to the trinuclear cluster and is reduced to two molecules of water by the **transfer of four electrons from the substrate**: Hence it shows ***ferroxidase activity***,

$$4Fe(II) + 4Cu(II) \sim 4Fe(III) + 4Cu(I)$$

$$4H^+ + O_2 + 4Cu(I) \sim 2H_2O + 4Cu(II)$$

2. in the cytoplasm it helps in ***transport and storage of copper***
3. in antioxidant defense as an extracellular ***scavenger of superoxides*** and other oxygen radicals.

➢ Serum Cu^{2+} concentrations and ceruloplasmin are often increased in inflammatory conditions, indicating a positive role for these agents in the ***healing process*** and in ***connective tissue repair***.

ROLE OF CERULOPLASMIN

4. in the cytoplasm in the ***regulation and mobilization of iron***.

- Ceruloplasmin allows the flow of iron from storage sites in the liver to transferrin, for transport to bone marrow and other sites.

- Specifically, **ceruloplasmin** is necessary for **oxidation** of **Fe^{2+}** (which leaves ferritin where it is stored), **to Fe^{3+}** in order to allow attachment to plasma transferrin.

5. Ceruloplasmin is an acute-phase protein in the inflammatory response. The protein is **deficient in Wilson's disease** (defect in copper metabolism).

6. Ceruloplasmin catalyzes the ***oxidation*** of a great variety of both organic and inorganic substances including *amines*, *dopamine*, and *seretonin* as well as *catechol derivatives*, *aminophenols*, and *Fe(II)*.

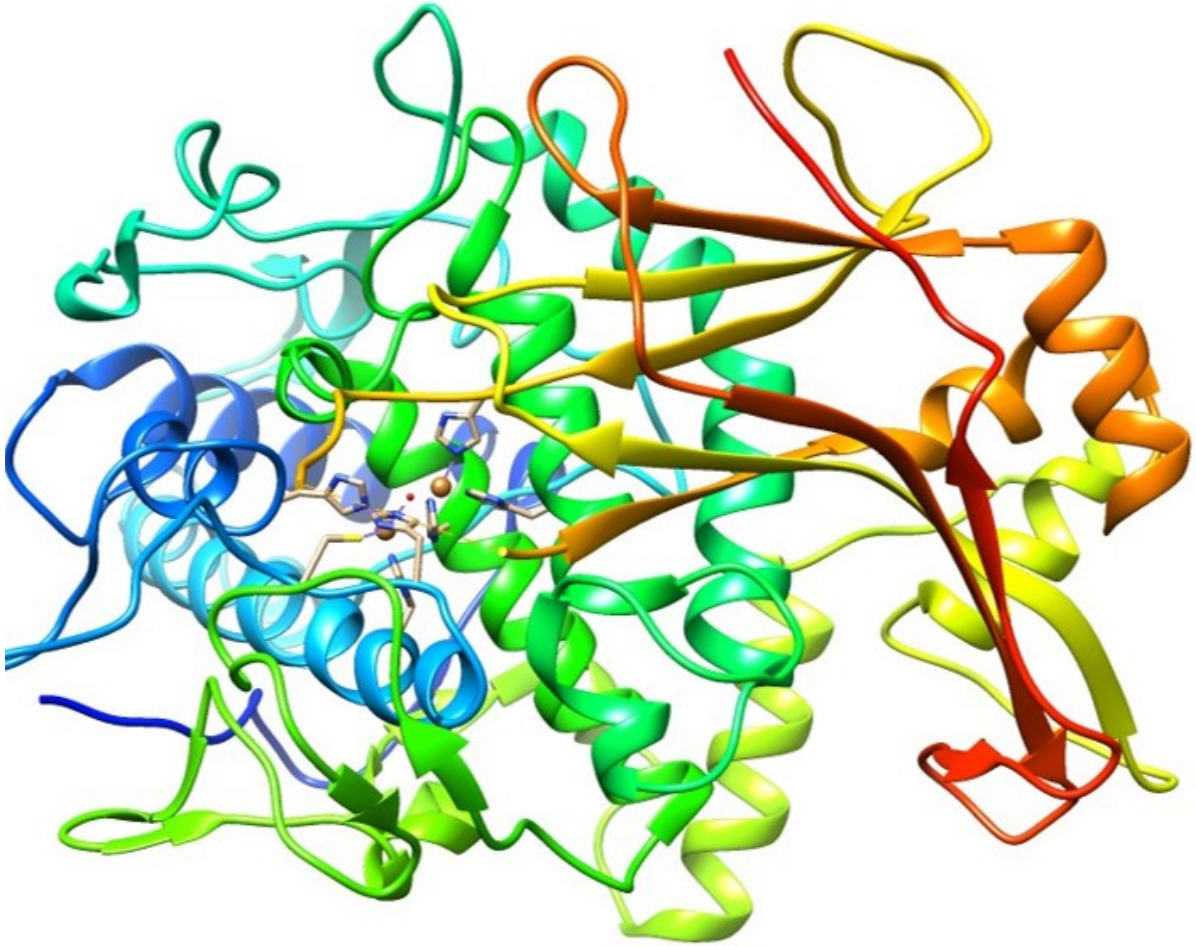
TYROSINASE

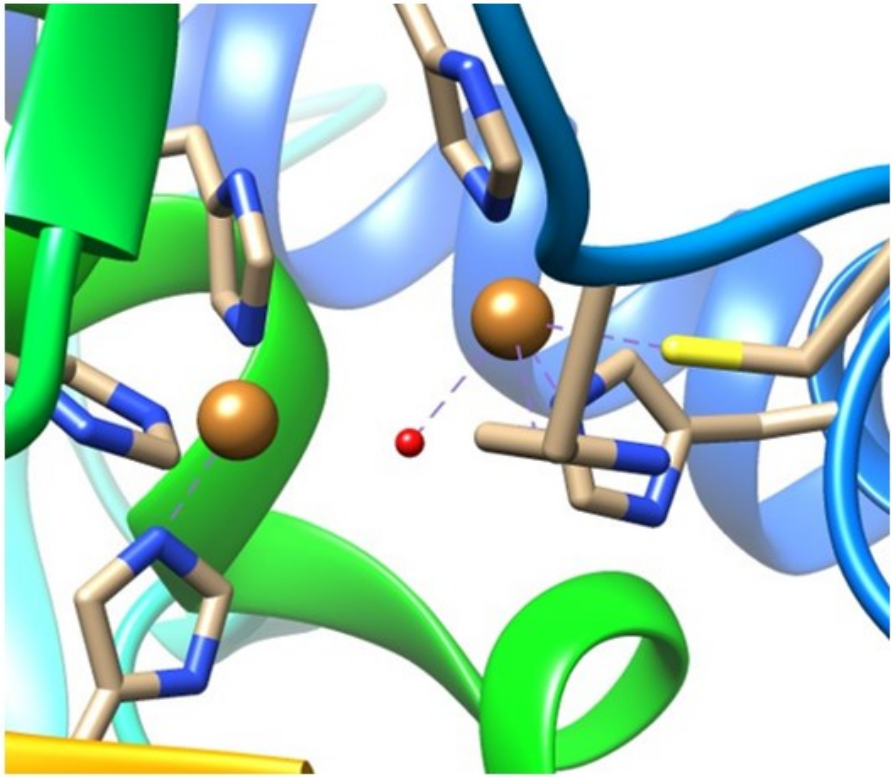
- Tyrosinase is classified into the ***dinuclear type-3 copper protein*** family (as are catechol oxidase and hemocyanin).
- Tyrosinase is found in vegetables, fruits, and mushrooms, is a key enzyme in the **browning** that occurs upon **bruising or long-term storage**.
- In mammals, the enzyme is responsible for **skin pigmentation abnormalities**, such as flecks and defects.
- Tyrosinase is quite significant in the fields of agriculture and industry.

STRUCTURE OF TYROSINASE

- Tyrosinase takes **α-helical structures** with the core of the enzyme, which is formed by a **four-helix bundle**.
- This dicopper center is located at the bottom of the large concavity as a putative substrate-binding pocket, which is formed by the hydrophobic residues.
- One copper ion (designated $\mathbf{Cu_A}$) is coordinated by ***His38, His54,*** and ***His63***.
- The second copper ion ($\mathbf{Cu_B}$) is coordinated by ***His190, His194,*** and ***His216***.

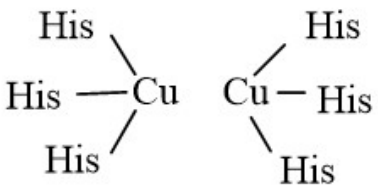
STRUCTURE OF TYROSINASE



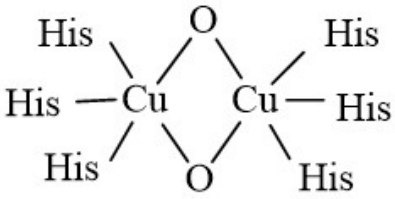


STRUCTURE OF TYROSINASE

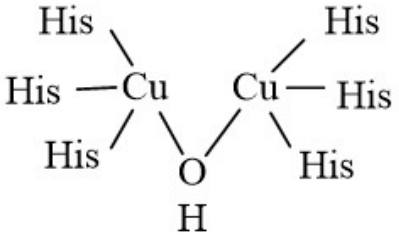
During the catalytic reaction, the type-3 copper center of tyrosinase exists in three redox forms.



Deoxy state



Oxy state



Met state

The **deoxy form** (**Cu(I)–Cu(I)**) is a reduced species, which binds oxygen to give the oxy form

In **oxy form**, molecular oxygen is bound as peroxide in a μ-η^2:η^2 side-on bridging mode (**Cu(II)–O_2^{2-}–Cu(II)**), which destabilizes the O–O bond and activates it.

The **met form** (**Cu(II)–Cu(II)**) is assumed as a resting enzymatic form, where Cu(II) ions are normally bridged to a small ligand, such as a water molecule or hydroxide ion.

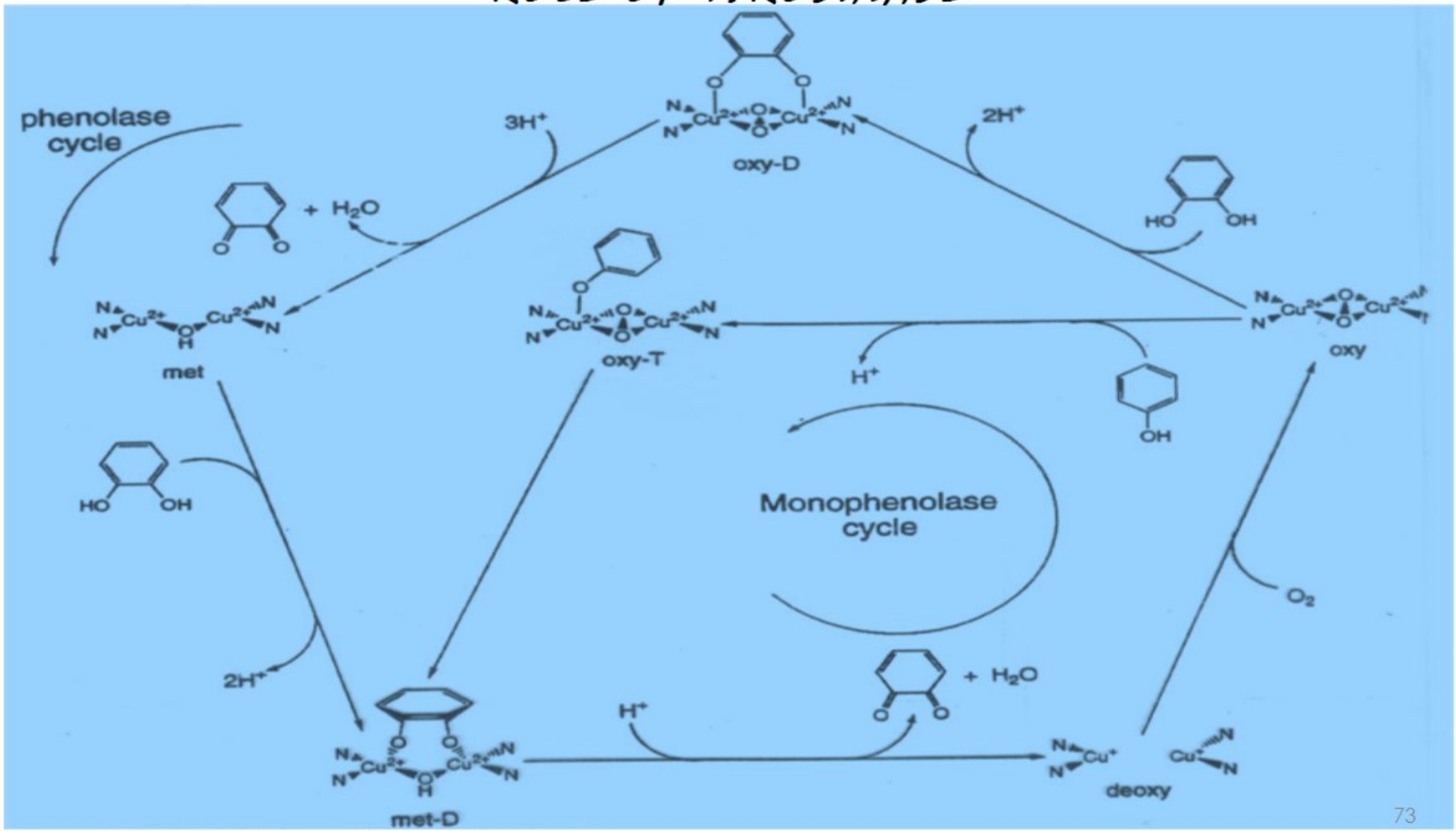
ROLE OF TYROSINASE

- Tyrosinase is one of the key enzymes in ***mammalian melanin synthesis***.
- The pigment is produced in two different cell types: the ***pigmented epithelial cell of the retina***, and the ***melanocyte, a cell of neural crest origin***.
- In pigment cells, melanin is made within specialized organelles, so-called **melanosomes**, by enzymatic reactions starting from tyrosine.
- The first two reactions, the ***hydroxylation of tyrosine to Dopa*** **(3,4-dihydroxyphenylalanine)** and the ***oxidization of Dopa to Dopaquinone***, are ***catalyzed*** by the enzyme ***tyrosinase*** which is therefore regarded as the key enzyme in melanin synthesis.

ROLE OF TYROSINASE

- Without enzymatic action of tyrosinase, the melanin synthetic pathway is blocked, and the result is a true ***albino pheno-type*** characterized by *red eyes* and *unpigmented skin* and *hairs*.
- In humans, absent or reduced activity of tyrosinase is known as the **genetic disorder type 1 oculocutaneous albinism (OCA)**.
- It catalyzes the orthohydroxylation of monophenol and the subsequent oxidation of the diphenolic product to the resulting quinone.

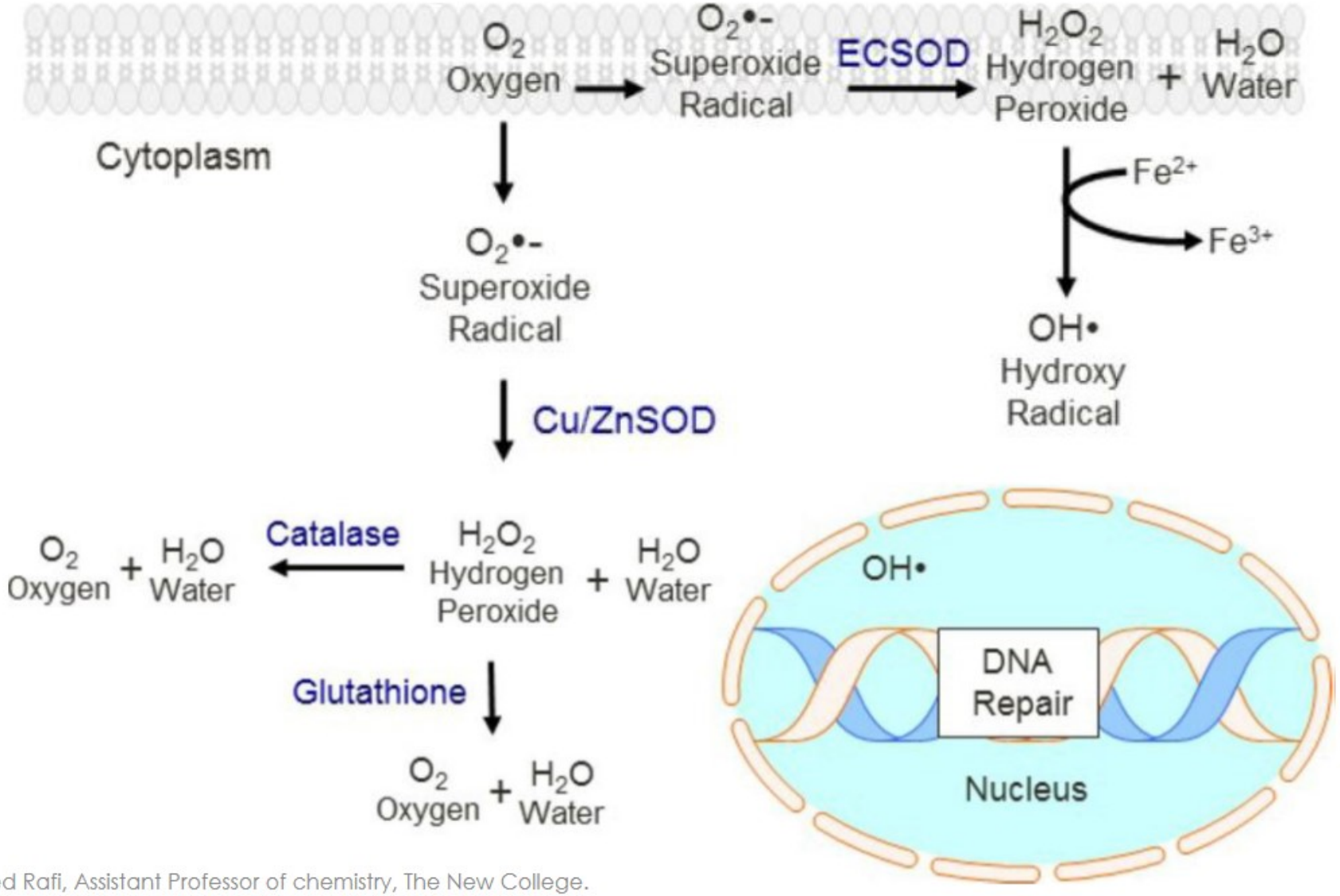
ROLE OF TYROSINASE



SUPEROXIDE DISMUTASE

- Superoxide dismutase (SOD) is present in all aero-tolerant organisms for the purpose of ***minimizing the concentration of superoxide,*** O_2^-, and thus providing protection against oxygen toxicity.
- ***Superoxide dismutases protect cells from reactive oxygen species by catalyzing the disproportionation of superoxide anion radicals into molecular oxygen and hydrogen peroxide.***
- Superoxide is generated by many life processes, which include aerobic metabolism, oxidative phosphorylation and photosynthesis.
- **Superoxide reductase (SOR)** is a class of enzymes that acts only to **reduce** $\mathbf{O_2^{\bullet -}}$ **to** $\mathbf{H_2O_2}$.

SUPEROXIDE DISMUTASE



TYPE OF SUPEROXIDE DISMUTASE

1. iron SOD (FeSOD), 2. manganese SOD (MnSOD),
3. copper-zinc SOD (CuZnSOD), 4. nickel SOD (NiSOD).

- *Cu,ZnSOD also known as **SOD1** and **SOD3** in humans are linked to the fatal neurodegenerative disease **amyotrophic lateral sclerosis** (ALS, also known as **Lou Gehrig's disease**)*

- *FeSOD and MnSOD also referred to as **SOD2** in humans appear to have evolved from a common ancestral gene, with the FeSOD gene observed in primitive eukaryotes, the plastids of plants and in bacteria.*

- The more recently discovered NiSOD has been found only in bacteria.

- Four groups can be found in prokaryotic organisms, while in Eukaryotes ***FeSOD*** can be found in ***chloroplasts***, ***MnSOD*** is the SOD typically found in ***mitochondria*** and can also be found in ***peroxisomes***, and ***CuZnSOD*** is usually the most abundant SOD and can be found in the ***chloroplast***, in the ***cytosol*** and in the ***extracellular space***.

STRUCTURAL FEATURES OF SUPEROXIDE DISMUTASE

CuZnSOD

dimeric enzyme

each monomeric unit

one copper — *one zinc*

bridged by a histidine imidazole.

3 histidines

water molecule.

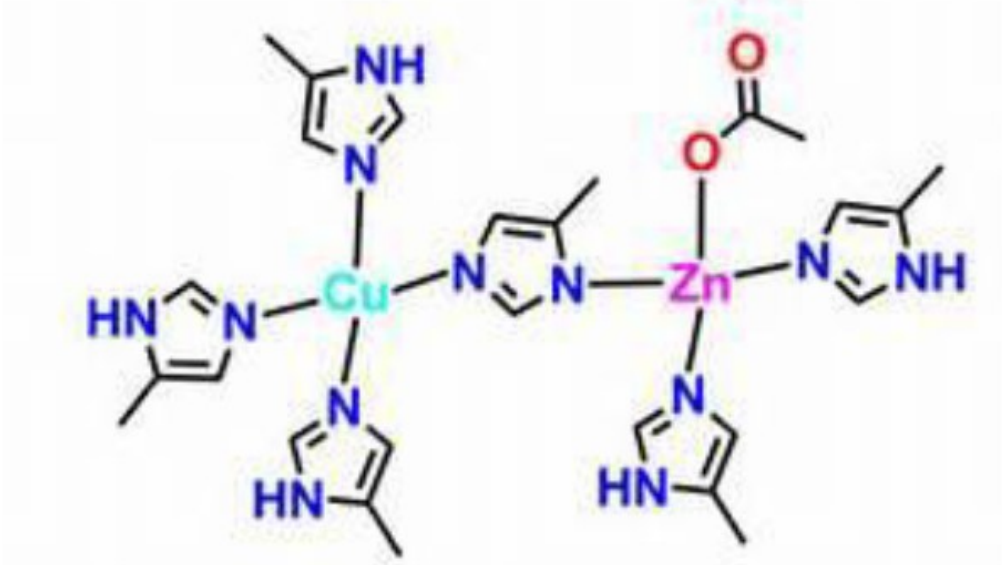
2 histidines

1 aspartate

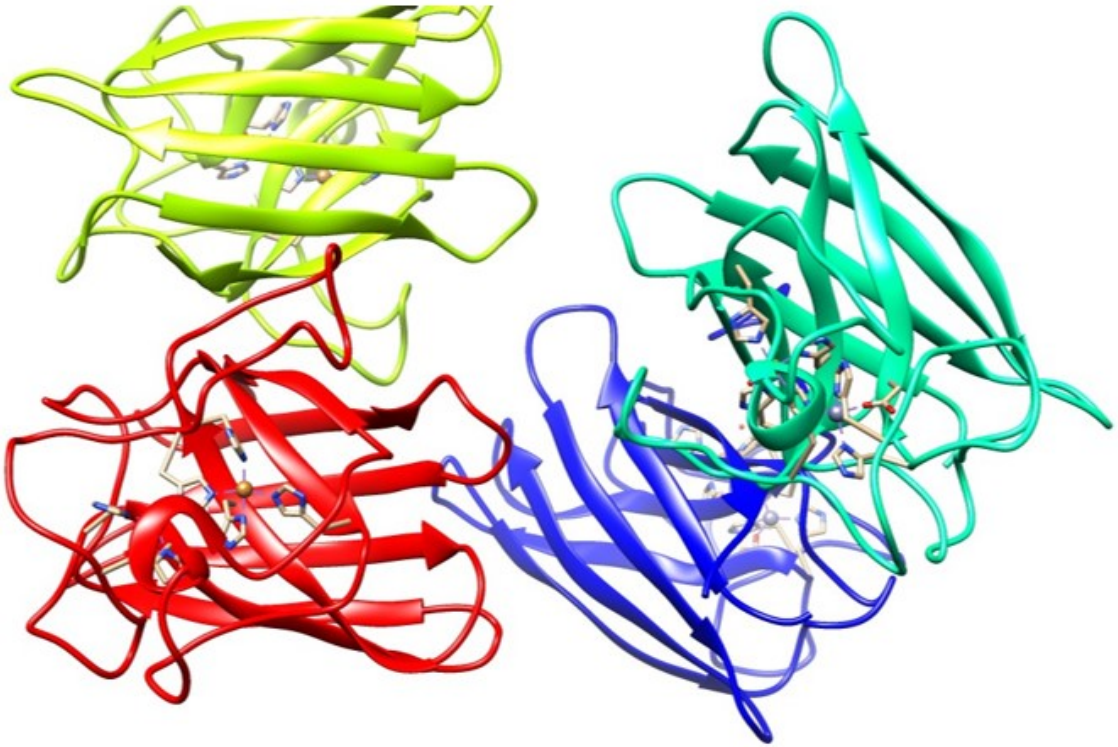
distorted square planar structure

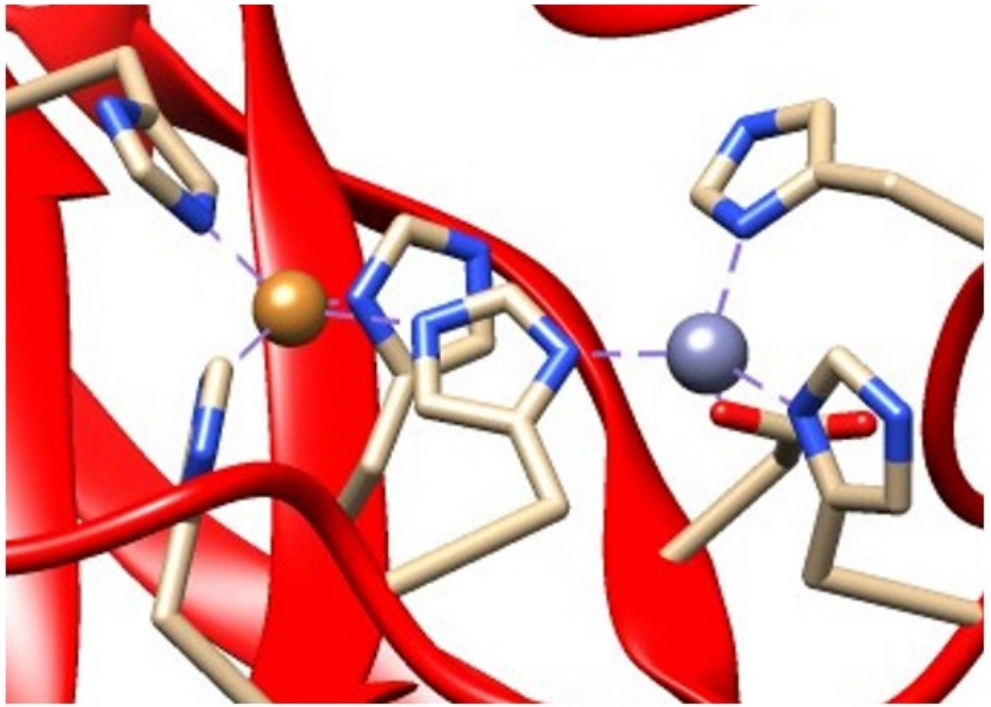
catalytic center.

structural role in the stabilization of the active site.



STRUCTURAL FEATURES OF SUPEROXIDE DISMUTASE

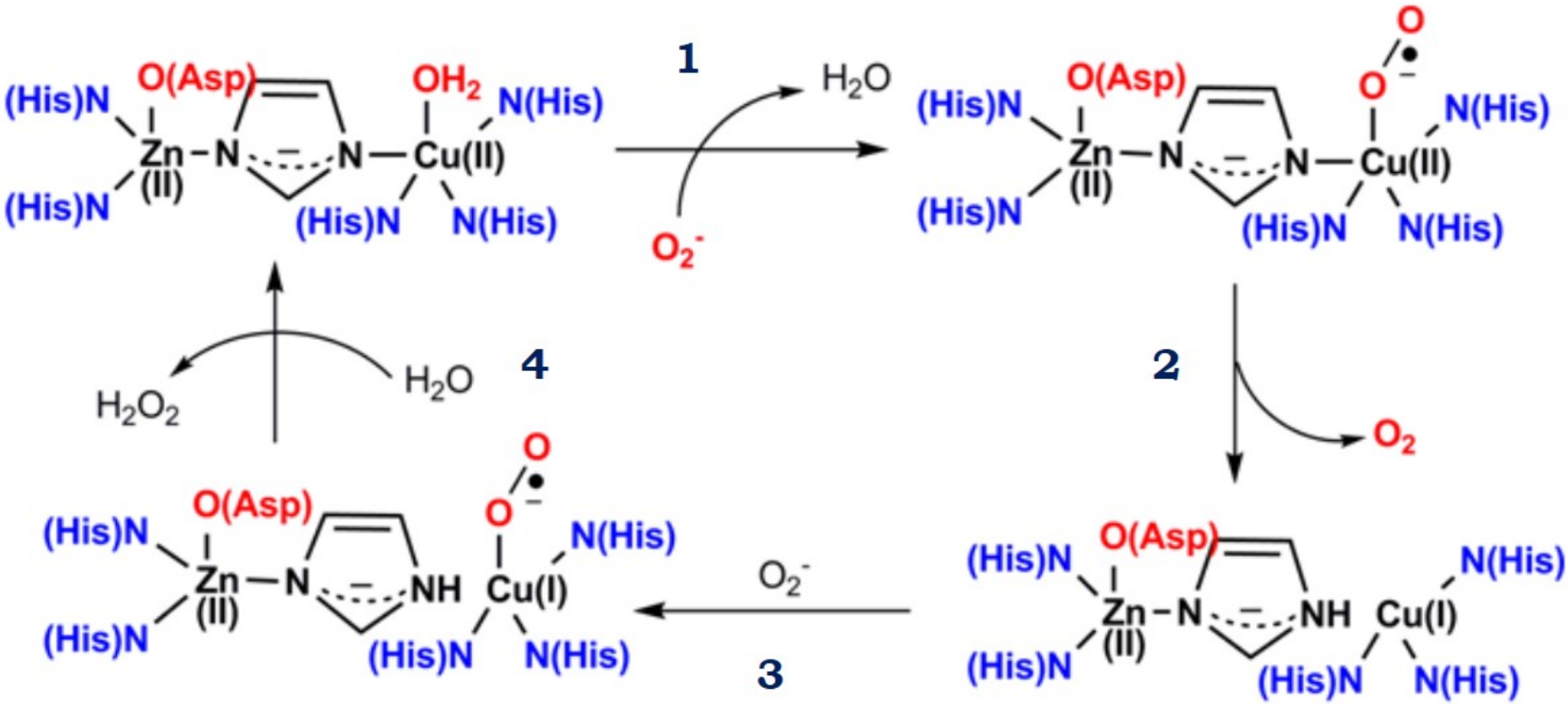




ROLE OF SUPEROXIDE DISMUTASE

- **"ping-pong" mechanism**, at virtually **diffusion controlled rates** with **pH 5–9.5**.
- A general mechanism for Cu,ZnSOD support an **inner sphere mechanism**.
- ***Copper is the redox-active metal***, changing between the +2/+3 oxidation states during catalysis, and zinc appears to play a role in overall enzyme stability and in facilitating a large pH independence in activity.
- ***Superoxide reduces the metal ion in the first step, and then the reduced metal ion is reoxidized by another superoxide, presumably via a metal-peroxo complex intermediate.***
- Upon reduction of the $Cu^{2+}Zn^{2+}SOD$ to $Cu^{+}Zn^{2+}SOD$, the bridging imidazole-copper coordination is lost, as is the bound water and the Cu^{+} shifts position and is three coordinate. Below figure represent the biological role of superoxide dismutase. Cu^{2+} is fully oxidized and Cu^{+} is reduced.

MECHANISM OF SUPEROXIDE DISMUTASE



VITAMIN B12 (CYANO/ADENOSYLCOBALAMIN)

Most corrinoid cofactors are organometallic B_{12}-derivatives and have either methyl or 5′-deoxy-5′-adenosyl groups at the β-coordination site of their Co(III)-ion.

Vitamin B_{12} is an organometallic compound and the only known essential biomolecule with a stable metal-carbon bond.

Structure:

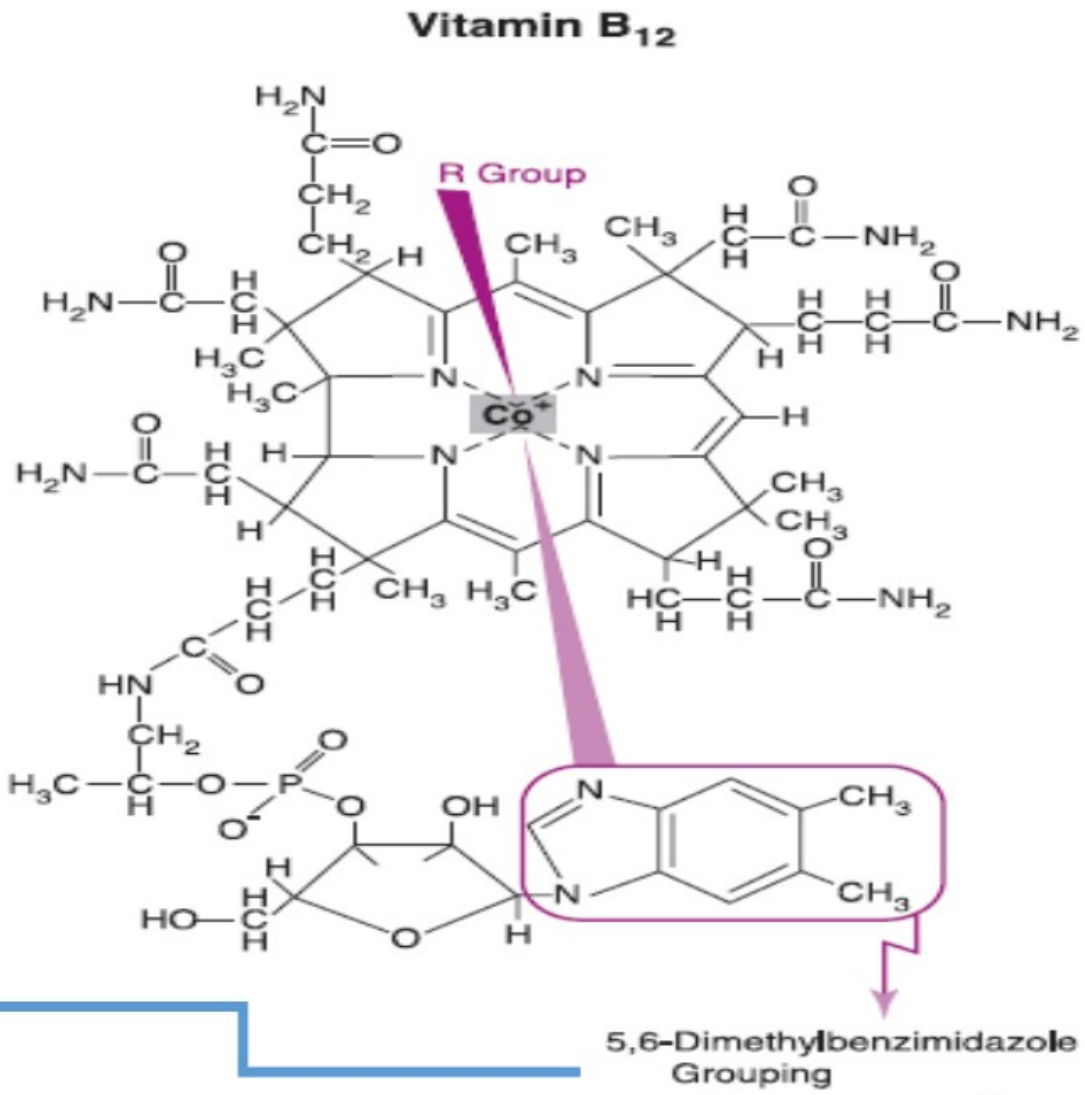
1. Nucleotide
2. Complex of tetrapyrrol ring structure (corrin ring)
3. Cobalt ion at center
4. R-group

R group	Name
Cyanide (CN)	Cyanocobalamin
Hydroxyl (OH)	Hydroxylcobalamin
Adenosyl	Adenosylcobalamin
Methyl (-CH3)	Methylcobalamin

benzimidazole nucleotide is present, it is Cobalamin, and if benzimidazole nucleotide is absent, then it is Cobinamide

B12	X = CN, Co(III)	B12r	Co(II) reduced	B12s	Co(I) Super-reduced
B12a	Aquocobalamin	B12b	Hydroxo		

STRUCTURE OF VITAMIN B12



VITAMIN B12 (CYANO/ADENOSYLCOBALAMIN)

Cobalt-carbon (Co-R) cleavage occur in three ways to produce:

$Co(III) + R^-$

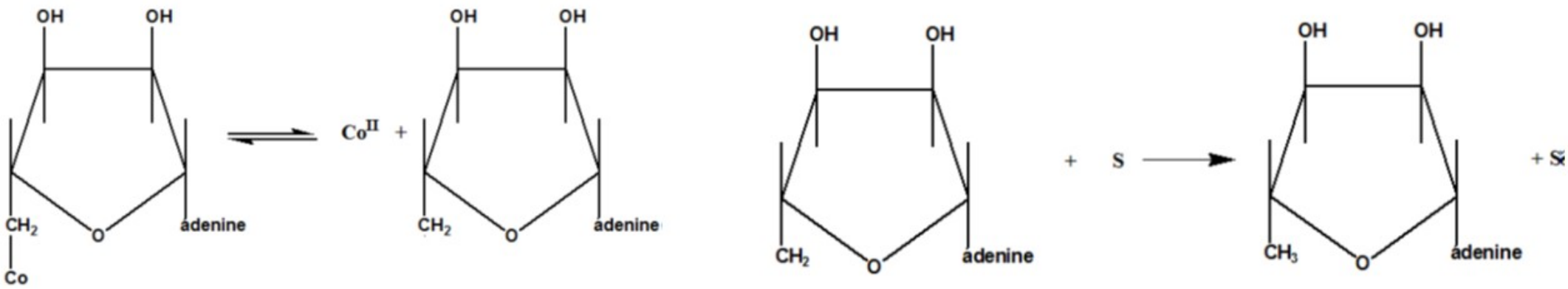
$Co(II) + R^\bullet$

$Co(I) + R^+$

The factors which determine the type of cleavage are as follows:

- ✓ Axial ligand trans to the bond generates the radical. If there is a decrease in the electron donating ability of the axial ligand, then it stabilizes Co(II) and decreases the bond dissociation energy. If there is still large decrease in electron donating ability, then Co(III) is formed. If the electron donating ability increases, then Co(I) is obtained.
- ✓ Steric interactions between the corrin ring and R-group influence the bond dissociation energy of Co–C bond.

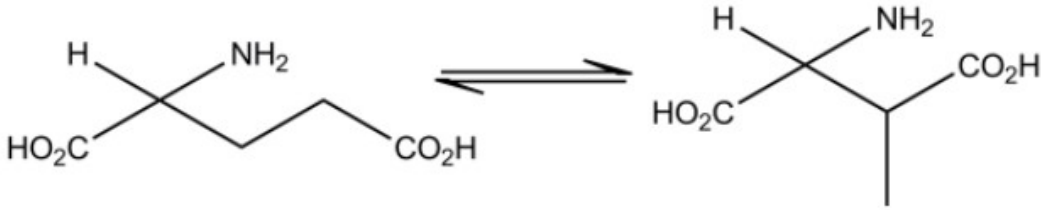
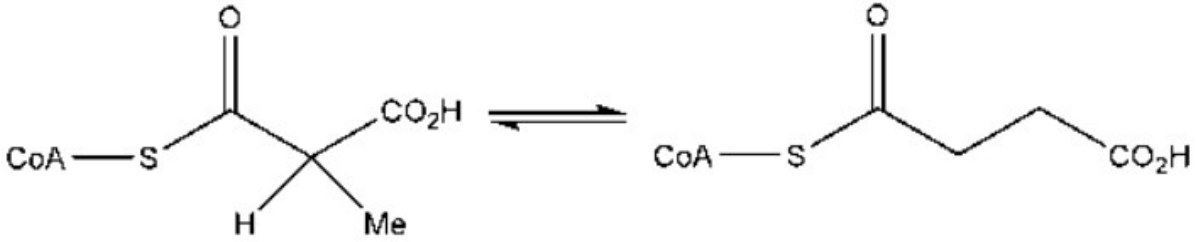
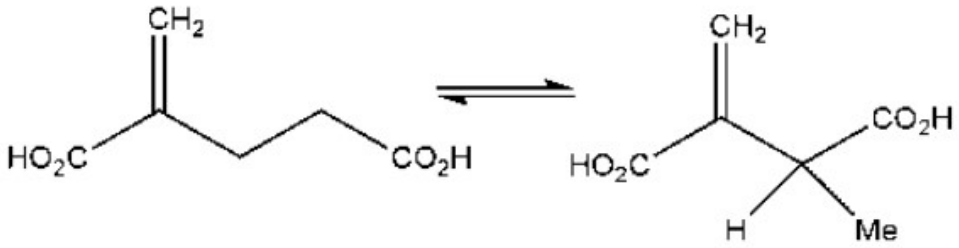
TYPICAL TRANSFORMATION THAT AD-COBALAMIN UNDERGO



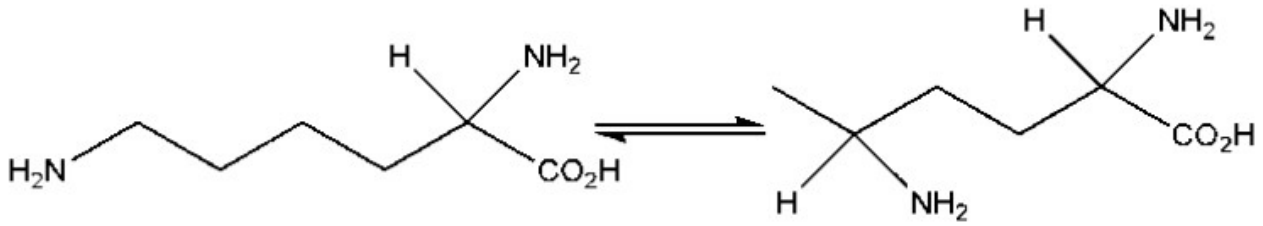
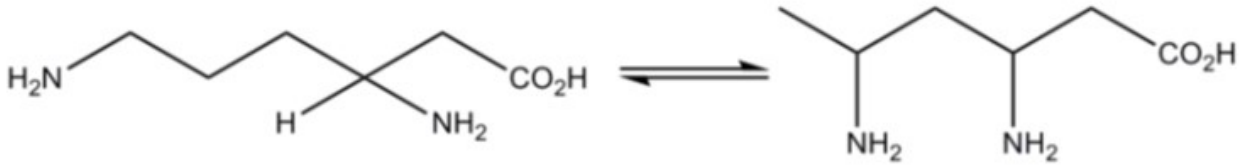
Reactions catalysed by cobalamin based enzymes

- ✓ Cobalamin is a 5-coordinate species.
- ✓ CN is present in cobalamin, it is cyanocobalamin
- ✓ It is involved in 1,2-hydride shift
- ✓ **Class A**: X= alkyl or acyl
- ✓ **Class B**: X= nitrogen containing ligand
- ✓ **Class C**: X = reactions where there is elimination of water or ammonia

REACTIONS CATALYSED BY COBALAMIN BASED ENZYMES

Reactions by cobalamine based enzymes Enzyme	**Class**	
Glutamine mutase	A	
Methyl malonyl-CoA mutase	A	
a-Methylene glutarate mutase	A	

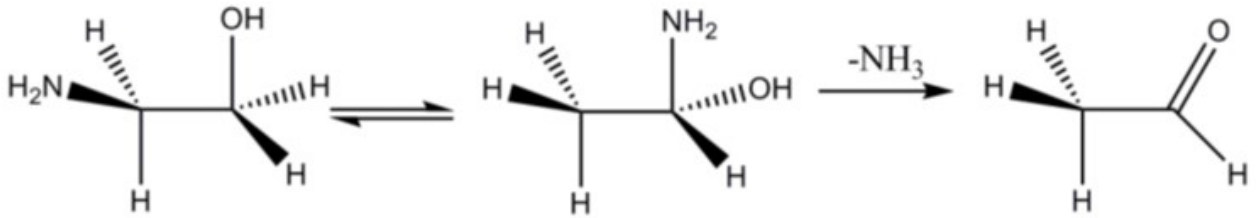
REACTIONS CATALYSED BY COBALAMIN BASED ENZYMES

Reactions by cobalamine based enzymes Enzyme	**Class**	
α-Lysine mutase	B	
β-Lysine mutase	B	

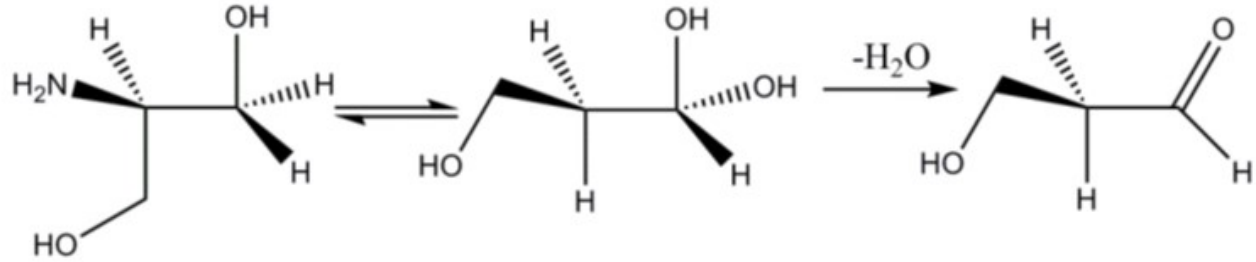
REACTIONS CATALYSED BY COBALAMIN BASED ENZYMES

Reactions (class C) by cobalamine based enzymes

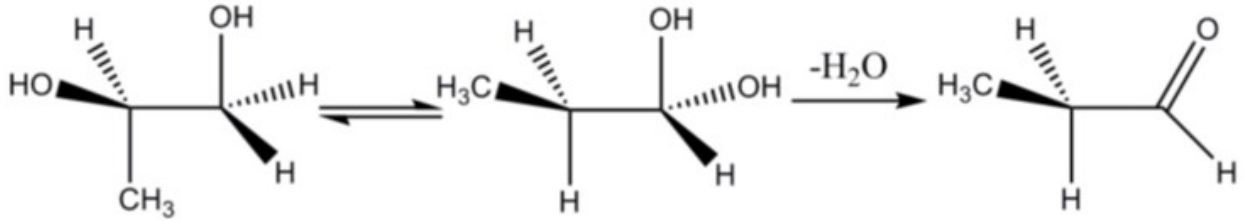
Ethanolamine ammonia lyase →



Glycerol dehydrase →



Propane-1,2-diol dehydratase →

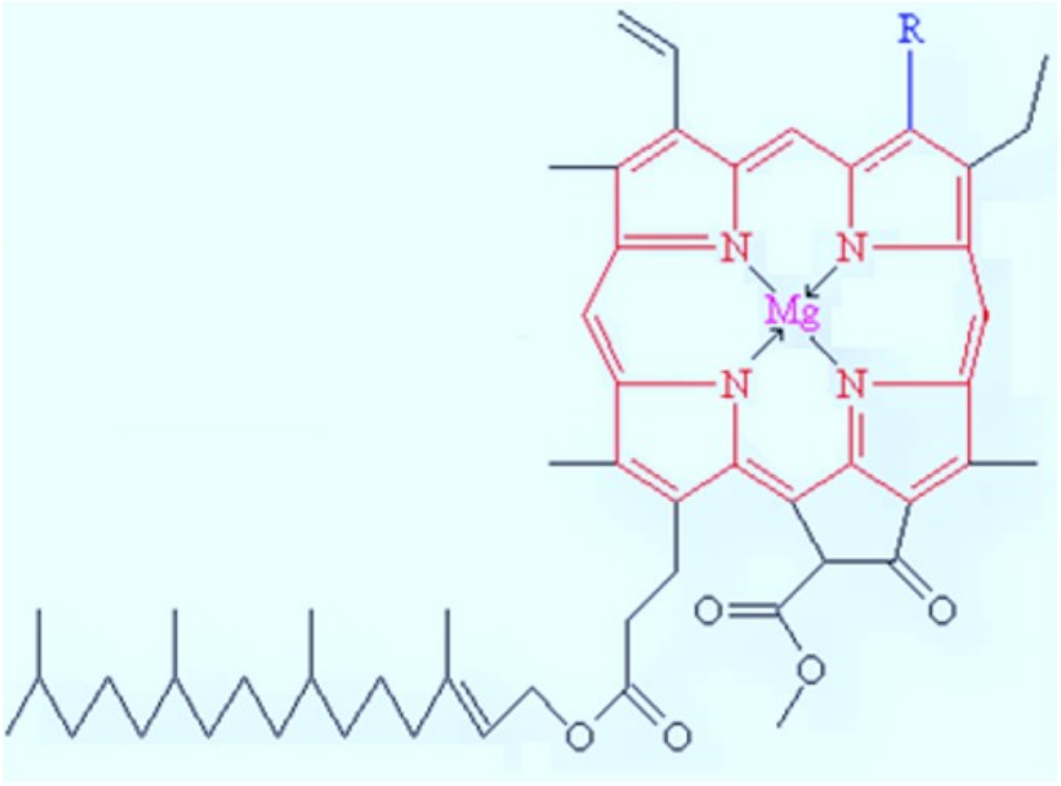


CHLOROPHYLL

- ✓ Chlorophylls are the essential ***components*** for ***photosynthesis*** and occur ***in chloroplasts*** as green pigments in all photosynthetic plant tissues.
- ✓ In ***1864***, ***Stokes*** showed that chlorophyll was a mixture.
- ✓ In ***1912***, ***Willstatter et al***. showed that chlorophyll was a mixture of two compounds, ***chlorophyll-a*** and ***chlorophyll-b***
- ✓ There are at least **five types of chlorophylls** in plants.
- ✓ Chlorophyll 'a' and 'b' occur in higher plants, ferns and mosses. Chlorophyll 'c', 'd' and 'e' are only found in algae and in certain bacteria.

STRUCTURAL FEATURES OF CHLOROPHYLL

- ✓ It is a member of the family of pigments called **tetrapyrroles**.
- ✓ Each chlorophyll molecule contains a **porphyrin (tetrapyrrole)** nucleus with a chelated ***magnesium atom*** at the center and a ***long chain hydrocarbon (phytyl)*** side chain attached ***through a carboxylic acid*** group.
- ✓ chlorophyll-*a* and chlorophyll-*b* are very effective ***photoreceptors*** having a network of ***alternating single and double bonds,*** and the orbitals can delocalize stabilizing the structure.



Chlorophyll *a*, R = CH_3

Chlorophyll *b*, R = CHO

The porphyrin ring is shown in Red

APPLICATIONS OF CHLOROPHYLL

- ✓ Due to the green color of chlorophyll, it has many uses as **dyes and pigments**. It is used in ***coloring soaps, oils, waxes and confectionary***.
- ✓ Chlorophyll is capable of channeling the energy of sunlight into chemical energy through the process of photosynthesis.
- ✓ In this process the energy absorbed by chlorophyll transforms ***carbon dioxide and water into carbohydrates and oxygen***
- ✓ The general process of photosynthesis is described by **Van Niel's equation**:

$$2\,H_2A + CO_2 \rightarrow 2\,A + CH_2O + H_2O$$

- ✓ Van Niel's equation can be applied to oxygenic photosynthesis as:

$$6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2$$

PHOTOSYNTHESIS

In this process the energy absorbed by chlorophyll transforms ***carbon dioxide and water into carbohydrates and oxygen***

$$6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2$$

1. Absorption of light by pigment molecules and transfer of the excitation energy to two reaction centers, Photosystem II (PS II) and Photosystem I (PS I).
2. Light-induced transfer of an electron across the photosynthetic membrane and ***splitting of H_2O into O_2 by PS II***.
3. Light-induced excitation and transfer of an electron across the photosynthetic membrane, generating reducing equivalents in the form of ***nicotinamide adenine dinucleotide phosphate (NADPH) by PS I***.
4. ***Production of ATP*** using the proton gradient generated across the membrane from both H_2O splitting and electron transfer ***through the cytochrome b_6f complex***.
5. Conversion of CO_2 into carbohydrates using ATP and the reducing power of NADPH.

PHOTOSYSTEMS

- ✓ Photosynthesis is a physico-chemical process that utilizes solar energy to convert carbon dioxide and water to glucose.
- ✓ The plant photosynthetic reactions occur in two stages namely "**light reactions**" involving electron-proton transfer processes and **dark reactions** involving the use of CO_2 for the biosynthesis of carbohydrates.
- ✓ During the **light reactions**, the solar energy is converted into ATP and NADPH with the help of multi-pigment protein complexes known as **photosystem I** and **photosystem II**.
- ✓ Photosystem II (P_{680}) performs the light-induced electron transfer reactions in photosynthesis that is responsible for **splitting of water into hydrogen ions and oxygen**.
- ✓ Photosystem I (P_{700}) utilizes light energy to generate high energy electrons which eventually reduce $NADP^+$ to produce NADPH that subsequently enters the Calvin cycle.

PHOTOSYSTEMS I

- ✓ **Photosystem I** is the light-driven ***plastocyanin-ferredoxin oxidoreductase*** present in the ***thylakoid membranes*** of ***cyanobacteria*** and ***chloroplasts***.
- ✓ It is an efficient bio-solar energy converter, capturing the energy from the sun and converting it into electrical energy through a light driven charge separation across the membrane.
- ✓ The reaction center of this photosystem contains ***chlorophyll a*** molecules (P_{700}) that absorb light of **700 nm** wavelength.

STRUCTURE OF PHOTOSYSTEMS I

The structure of photosystem I in a cyanobacterium is a **homotrimer** with each subunit in the trimer containing:

12 different protein molecules bound to:

a) 96 molecules of chlorophyll a

- ✓ *2 molecules of the reaction center chlorophyll P700*
- ✓ *4 accessory molecules closely associated with them*
- ✓ *90 molecules that serve as antenna pigments*

b) 22 carotenoid molecules

c) 4 lipid molecules

d) 3 clusters of Fe_4S_4

e) 2 phylloquinones

PHOTOSYSTEMS II

✓"Photosystem II"- the first link in the photosynthesis chain is a *multi-subunit pigment-protein complex* (**water-plastoquinone oxidoreductase**) embedded in the lipid environment of the thylakoid membranes of plants, algae and cyanobacteria.

✓At the heart of this photosystem is a ***reaction center (RC) core*** containing **chlorophyll a molecules (P_{680})** that absorbs light at λ_{max} *value of 680 nm*.

✓Driven by light, this enzyme catalyzes the chemically and thermodynamically demanding reaction of water splitting.

✓It harnesses solar irradiation **to oxidize two molecules of water to molecular oxygen**, **liberating electrons** which provide the reducing equivalents required for the **conversion of CO_2 into the organic molecules of life**.

STRUCTURE OF PHOTOSYSTEMS II

✓ Photosystem II is also a complex assembly of ***more than 20 different protein molecules bound to***:

a) 50 or more **chlorophyll a** molecules

✓ 2 molecules of the **reaction center chlorophyll P_{680}**

✓ 2 accessory molecules close to them

✓ 2 molecules of pheophytin (chlorophyll without the Mg^{2+})

b) the remaining molecules of **chlorophyll a** serve as **antenna pigments**.

c) Some half dozen **carotenoid** molecules, also serve as antenna pigments.

d) 2 molecules of **plastoquinone**

FUNCTIONS OF PHOTOSYSTEMS

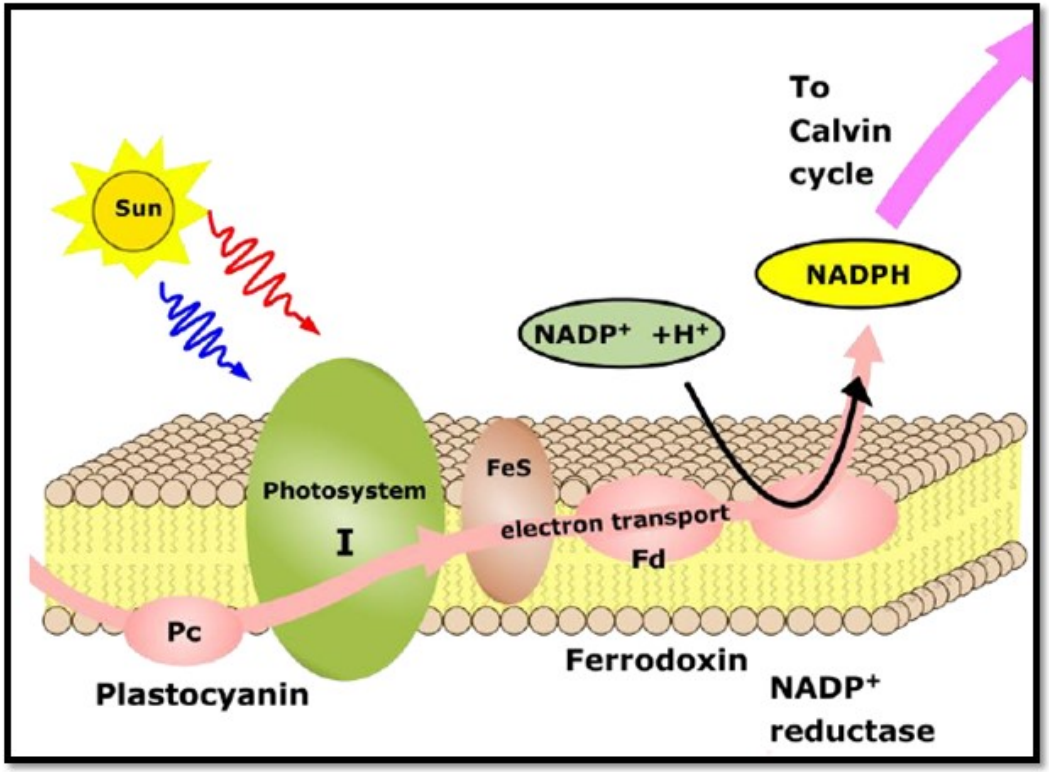
- ✓ The photosystems work through resonance effects.
- ✓ When light is absorbed by photosystem II, the electrons present in the reaction-center get excited which are eventually trapped by the primary electron acceptors.
- ✓ The hole thus created in the reaction center by the departure of photo-excited electron is replenished by the electrons extracted from water through a cluster of four manganese atoms in photosystem II.
- ✓ In this process, oxygen is released when four electrons have been removed from two molecules of water.
- ✓ Further, the light energized electrons travel to photosystem I through the cytochrome $_{b6f}$ complex (an enzyme found in the thylakoid membrane that catalyze the transfer of electrons from plastoquinol to plastocyanin) via an electron transport chain.

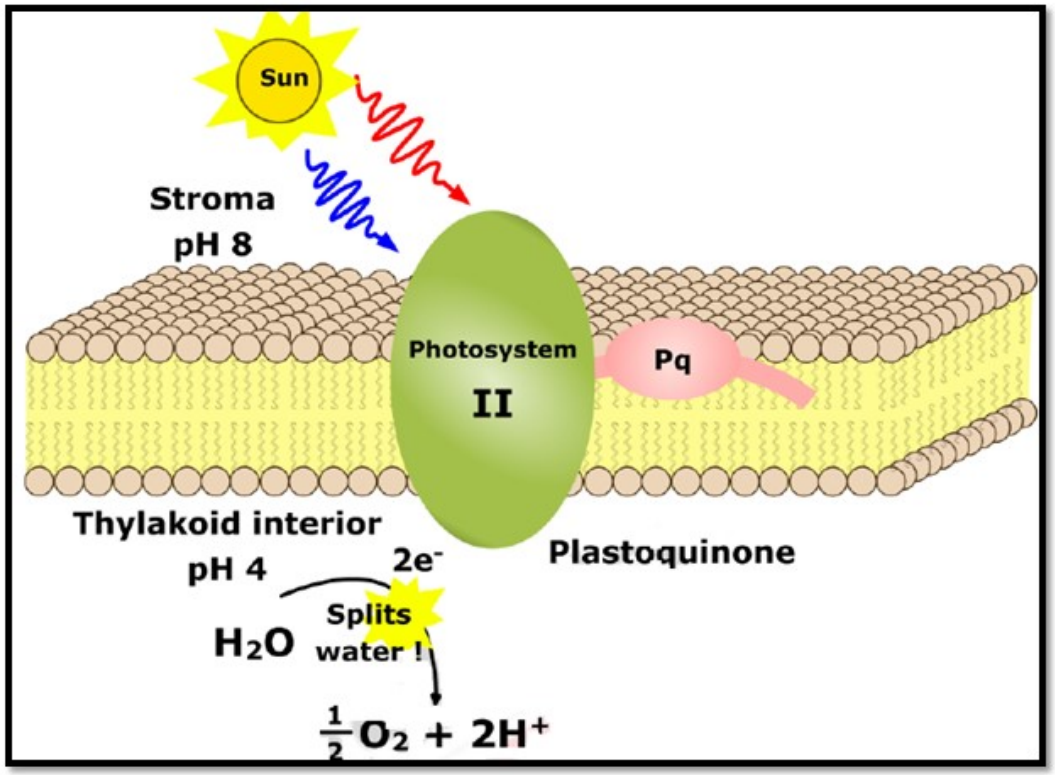
FUNCTIONS OF PHOTOSYSTEMS

- ✓ During this process, (chemiosmosis) transfer of H^+ ions takes place across the thylakoid membrane via plastoquinone which creates a gradient within the chloroplast that is used for the production of ATP molecules.
- ✓ When electron enters photosystem I, it waits until the electron is excited by another photon. The electron thus excited is captured by another electron acceptor which flow down a chain of electron carriers to $NADP^+$ to reduce it to NADPH that enters the Calvin cycle.

$$\mathbf{2\ H_2O + 2\ NADP^+ + 8\ photons \rightarrow O_2 + 2\ NADPH + 2H^+}$$

PHOTOSYSTEM I AND PHOTOSYSTEM II





DIFFERENCE BETWEEN PHOTOSYSTEM I AND PHOTOSYSTEM II

Photosystem I (PS I)	Photosystem II (PS II)
PS I is located at the ***outer surface*** of the grana ***thylakoid membrane***.	PS II is located at the ***inner surface*** of the grana ***thylakoid membrane***.
The photo center is P_{700}.	The photo center is P_{680}.
Pigments absorb ***longer wavelengths*** of light (>680 nm).	Pigments absorb ***shorter wavelengths*** of light (<680 nm).
Participates in cyclic as well as ***non-cyclic photophosphorylation***.	Participates only in ***non-cyclic photophosphorylation***.
It is ***not*** associated with ***photolysis of water***.	It is associated with ***photolysis of water***.
Main function is ***ATP synthesis***.	Main functions are ***ATP synthesis*** and ***hydrolysis of water***.