BHARATHIDASAN UNIVERSITY

Tiruchirappalli 620024

Tamil Nadu India

Programme: M.Sc., Chemistry

Course Title: Bio-Inorganic and Organometallic Chemistry

Course Code: CHE621CC

UNIT – I

Introduction to Bioinorganic Chemistry; Bioenergetics and ATP cycle

UNIT-II

Electron Transfer in Biology; Heme and non-Heme proteins

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UNITS I and II

CHE621CC – Bioinorganic Chemistry

By

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UGC – Assistant Professor

School of Chemistry

Unit - I

Introduction to bioinorganic chemistry

Metallobimolecules and its classification – Essential and trace elements in biology, Biological Ligands (Porphyrins, corrin and chlorin). Organic electron carrier couples- NADH, NADPH, $FMNH_2$, FADH, Quinones and ubiquinone.

Transport across the membrane-Active and passive transport, Ionophores, sodium/potassium pump (Na/K ATPase enzyme mechanism), Vitamin B12 – Structural features, Chemistry of cobalamins, Co-C bond cleavage, isomerase reactions and bioalkylation (synthesis of methionine).

Bioenergetics and ATP Cycle

Features of ATP, phosphate group transfer potential of various phosphate compounds, glycolysis (glucose to pyruvate conversion) and glucose storage as glycogen. Photosynthesis: structural features of chlorophylls, role of Mg(II), Pigments involved in Photosystems I and II (PS I and II), cleavage of water using PSI and PS II in photosynthesis (Z-scheme), Oxygen evolving complex (OEC) and its involvement in oxidation of water to O_2

UNIT II

Electron Transfer in Biology

Iron-sulphur proteins-Ferredoxins, Rubredoxins, Rieske's protein

Cytochromes-classifications, structural features, O_2 activation using CytC oxidase, oxidative phosphorylation, and respiratory chain (ATP synthesis and blocking of respiratory chain), CN poisoning.

Blue copper proteins: classification (type 1, 2 and 3) with examples (azurin and plastocyanin), Function of Cu, Zn-Superoxide dismutase

Heme and Non-heme Proteins

Oxygen transport and storage: heme-dioxygen bonding, Structural features of heme group in hemoglobin (Hb) and myoglobin (Mb), O_2 transport mechanism, Allosteric effect, cooperative effect, Bohr effect, role of 2,3-bisphosphoglycerate (BPG), irreversible oxidation of heme, -Hematin formation, CO poisoning, Hb in acid-base balance and CO_2 transport, Structure and functions of Hemerythrin and hemocyanin.

UNIT III

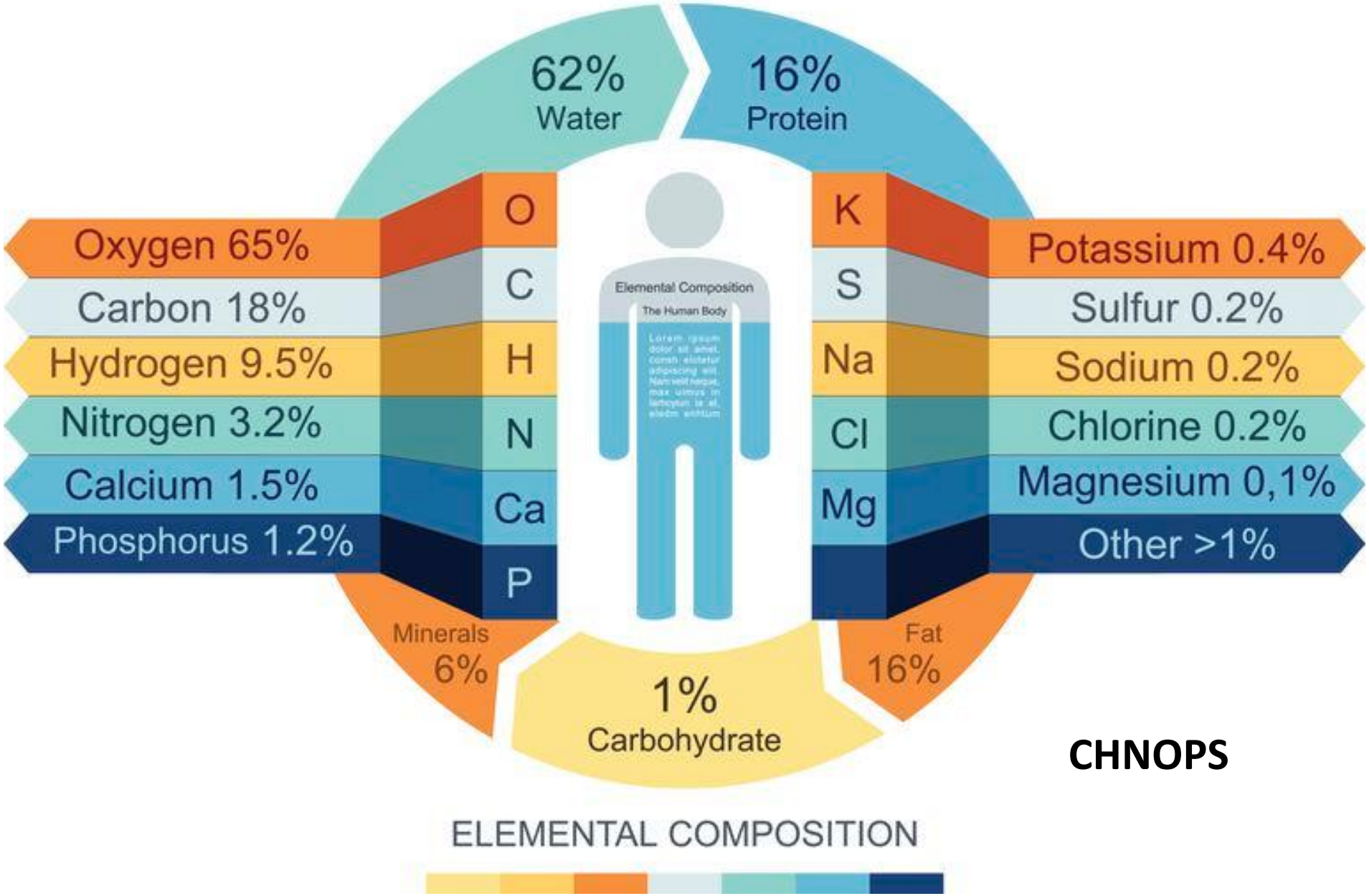
Nickel and Molybdenum containing Enzymes

Structure and functions of Urease, Hydrogenases, biological nitrogen fixation using molybdenum nitrogenase - spectroscopic and other evidences, other nitrogenase model systems.

Metals in Medicine: cis-platin and its mode of action, side effects, Gold containing drugs as anti-rheumatic agents and their mode of action - Lithium in pschycopharmocological drugs. Copper, Zinc deficiency and its treatment, Wilson disease, Menke's disease, Arsenic, Mercury poisoning and chelating drugs used for detoxification.

BASICS – Chapter 1 of Unit I

ESSENTIAL ELEMENTS OF THE HUMAN BODY



CHNOPS

Trace Elements: Cr, Mn, Fe, Cu, Zn, Se, Mo, I

TE	*Physiological function(s)*	*Symptoms of dietary deficiency*	*Symptoms in patients on long-term PN without added TE or inadequate provision of TE*
Selenium (Se)	Component of >25 selenoproteins representing the functional form of selenium and involved in redox signalling, the antioxidant defence system (glutathione peroxidase), thyroid hormone metabolism and immune response	Cardiomyopathy, chronic osteoarthritis, poor immune function, cognitive decline, increased risk of autoimmune thyroid disease, impaired reproductive capacity In the acute-phase of critical illness: oxidative stress, infectious complications, worsening organ failure, higher mortality rates	Nail and hair changes in children, skeletal muscle myopathy, cardiomyopathy
Zinc (Zn)	As a component of >300 zinc enzymes: essential for health and well-being, has an important role in wound healing, required for the structural integrity of proteins regulating gene expression and nuclear binding proteins acting as transcription factors	Growth retardation, delay in sexual maturation, diarrhoea, increased susceptibility to infections, dermatitis, the appearance of behavioural change, alopecia, delayed wound healing, impaired resistance to infection, reduced growth rate	Eczematous rash, nail changes, alopecia, mental apathy and depression, visual dysfunction, impaired immune function
Copper (Cu)	Component of copper metalloenzymes (cuproenzymes) required for normal function of the haematologic, vascular, skeletal, antioxidant and neurologic systems	Anaemia, leukopenia, bone abnormalities, decreased pigmentation of skin and hair, neurological derangements	Anaemia, pancytopenia, neutropenia
Manganese (Mn)	Cofactor for the activity of many metalloenzymes involved in antioxidant protection and amino acid, lipid, protein, and carbohydrate metabolism	A specific deficiency syndrome has not been described in humans.	No cases in adults, one isolated case of a paediatric patient with manganese deficiency (short stature, low serum levels, depletion of bone manganese levels)
Chromium (Cr)	Required for normal glucose tolerance and lipid metabolism (promotion of insulin action in peripheral tissues)	Postulated as a contributing factor to the development of type II diabetes.	Syndrome of glucose intolerance similar to diabetes
Iron (Fe)	Major component of several important classes of functional proteins (haeme-proteins, enzymes, storage and transport proteins) involved in O_2 and electron transport	Anaemia, reduced resistance to infection; in clinical setting: adverse effects on outcome parameters	Iron deficiency anaemia
Molybdenum (Mo)	Part of the molybdenum co-factor (molybdopterin) of several flavo- and haeme enzymes involved in oxidation–reduction reactions, amino acid and purine metabolism	A dietary deficiency of molybdenum has never been reported in humans	One isolated case reported (progressive tachycardia, tachypnoea, neurological and visual changes, and coma), associated with increased excretion of sulphite, hypoxanthine and xanthine, and reduced excretion of uric acid and sulphate.
Iodine (I)	Major component of the thyroid hormones,	Iodine deficiency disorders: goitre,	Not yet reported in patients on PN due

- **Carbon** is found in proteins, carbohydrates, lipids, and nucleic acids. It's also found in carbon dioxide
- **Hydrogen** much of the hydrogen exists in water, functions to transport nutrients, remove wastes, lubricate organs and joints, and regulate body temperature. Hydrogen is also important in energy production and use. The H^+ ion can be used as a hydrogen ion or proton pump to produce ATP and regulate numerous chemical reactions
- **Nitrogen** is found in amino acid that make up the proteins and nucleic acids that make up the DNA.
- **Calcium** is used to build bones and teeth, plus it's important for muscle contraction
- **Phosphorus** found in nucleic acids; most of them are in the bones and teeth; primary energy molecule ATP in the body
- **Potassium** (charge carrier) electrolyte and is used in nerve conduction. It helps to regulate the heart beat and vital for electrical signalling in nerves.
- **Sulfur** is found in some amino acids and proteins; present in keratin which form skin, hair and nails

- **Water**: Water is the most abundant chemical compound in living human cells, accounting for **65 percent to 90 percent** of each cell. It's also present between cells. For example, blood and cerebrospinal fluid are mostly water.

- **Fat**: The percentage of fat varies from person to person, but even an obese person has more water than fat.

- **Protein**: In a lean male, the percentages of protein and water are comparable. It's about **16 percent by mass**. Muscles, including the heart, contain a lot of muscle. Hair and fingernails are protein. Skin contains a large amount of protein

- **Minerals**: Minerals account for about 6 percent of the body. They include salts and metals. Common minerals include sodium, chlorine, calcium, potassium, and iron.

- **Carbohydrates**: Although humans use the sugar glucose as an energy source, there isn't that much of it free in the bloodstream at any given time. Sugar and other carbohydrates only account for about 1% of body mass

Chang, Raymond (2007). *Chemistry*, Ninth Edition. McGraw-Hill. pp. 52

- **Sodium** helps regulate fluid volume, temperature and blood pressure, the electrolyte balance in the body and maintain homeostasis with respect to the volume of water in the blood and cells
- **Magnesium** helps to regulate the heart beat, blood pressure, and blood glucose levels. It is used in protein synthesis and metabolism. It is needed to support proper immune system, muscle, and nerve function and essential for more than 300 metabolic reactions
- **Iron** (0.006%) is a key element in the metabolism of almost all living organisms. It is also found in hemoglobin, which is the oxygen carrier in red blood cells.
- **Fluorine** (0.0037%) is found in teeth and bones. Outside of preventing tooth decay, it does not appear to have any importance to bodily health.
- **Zinc** (0.0032%) is an essential trace element for all forms of life. Several proteins contain structures called "zinc fingers" help to regulate genes. Zinc deficiency has been known to lead to dwarfism in developing countries.
- **Copper** (0.0001%) is important as an electron donor in various biological reactions. Without enough copper, iron won't work properly in the body

- **Iodine** (0.000016%) is required for making of thyroid hormones, which regulate metabolic rate and other cellular functions. Iodine deficiency, which can lead to goiter and brain damage, is an important health problem throughout much of the world
- **Selenium** (0.000019%) is essential for certain enzymes, including several anti-oxidants. Unlike animals, plants do not appear to require selenium for survival, but they do absorb it, so there are several cases of selenium poisoning from eating plants grown in selenium-rich soils
- **Chromium** (0.0000024%) helps regulate sugar levels by interacting with insulin, but the exact mechanism is still not completely understood
- **Manganese** (0.000017%) is essential for certain enzymes, in particular those that protect mitochondria — the place where usable energy is generated inside cells — from dangerous oxidants
- **Molybdenum** (0.000013%) is essential to virtually all life forms. In humans, it is important for transforming sulfur into a usable form. In nitrogen-fixing bacteria, it is important for transforming nitrogen into a usable form
- **Cobalt** (0.0000021%) is contained in vitamin B_{12}, which is important in protein formation and DNA regulation

Occurrence and Availability of Inorganic Elements in Organism

Some biological essential elements with their functions:

Charge balance and electrolytic conductivity: **Na, K, Cl**

Structure and templating: **Ca, Zn, Si, S, Mo, Ni**

Signaling: **Ca, B, N, O**

Brønstead Acid-Base Buffering: **P, Si, C**

Lewis Acid-Base Catalysis: **Zn, Fe, Ni, Mn**

Electron Transfer: **Fe, Cu,**

Group Transfer (e.g. CH_3 , O, S): **V, Fe, Co, Ni, Cu, Mo, W**

Redox Catalysis: **V, Mn, Fe, Co, Ni, Cu, W, S, Se**

Energy Storage: **H, P, S, Na, K, Fe**

Biomineralization: **Ca, Mg, Fe, Si, Sr, Cu, P**

Energy generation: **Ca, Mg**

Elemental composition of a human body of 70 kg

Oxygen	43 kg (61%, 2700 mol)
Carbon	16 kg (23%, 1300 mol)
Hydrogen	7 kg (10%, 6900 mol)
Nitrogen	1.8 kg (2.5%, 129 mol)
Calcium	1.0 kg (1.4%, 25 mol)
Phosphorus	780 g (1.1%, 25 mol)
Potassium	140 g (0.20%, 3.6 mol)
Sulfur	140 g (0.20%, 4.4 mol)
Sodium	100 g (0.14%, 4.3 mol)
Chlorine	95 g (0.14%, 2.7 mol)
Magnesium	19 g (0.03%, 0.78 mol)
Iron	4.2 g
Fluorine	2.6 g
Zinc	2.3 g
Silicon	1.0 g
Rubidium	0.68 g
Strontium	0.32 g

Bromine	0.26 g
Lead	0.12 g
Copper	72 mg
Aluminum	60 mg
Cadmium	50 mg
Cerium	40 mg
Barium	22 mg
Iodine	20 mg
Tin	20 mg
Boron	18 mg
Nickel	15 mg
Selenium	15 mg
Chromium	14 mg
Manganese	12 mg
Arsenic	7 mg
Lithium	7 mg
Molybdenum	6 mg
Cobalt	6 mg

Emsley, John, The Elements, 3rd ed., Clarendon Press, Oxford, 1998

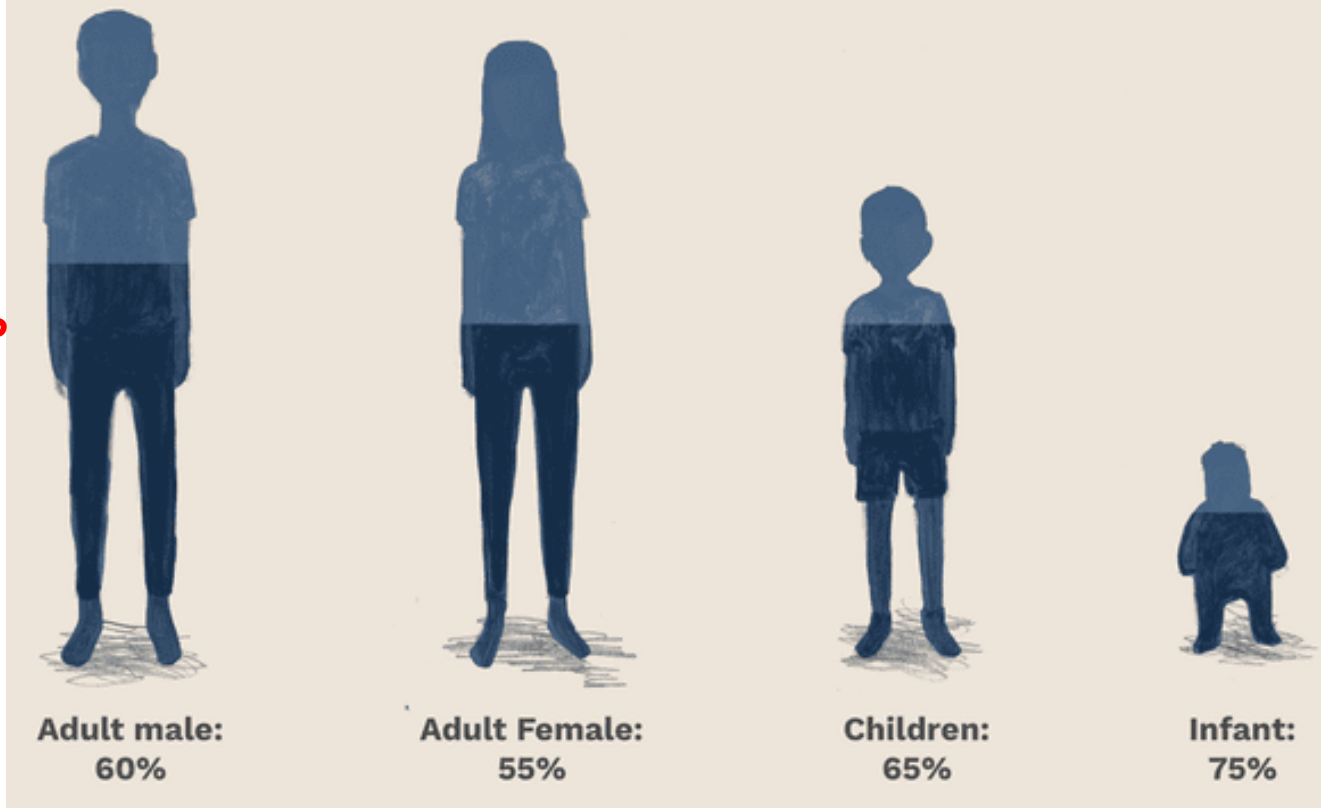
What Is the Function of Water in the Body?

- Water acts as an insulator, regulating internal body temperature. This is partly because water has a high specific heat, plus the body uses perspiration and respiration to regulate temperature
- Water is needed to metabolize proteins and carbohydrates used as food. It is the primary component of saliva, used to digest carbohydrates and aid in swallowing food
- The compound lubricates joints
- Water insulates the brain, spinal cord, organs, and fetus. It acts as a shock absorber
- Water is used to flush waste and toxins from the body via urine
- Water is the principal solvent in the body. It dissolves minerals, soluble vitamins, and certain nutrients

How Much Water is in Your Body

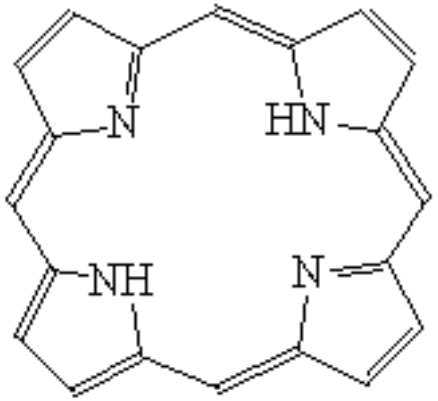
- Infants typically has 75-78% dropping to 65% by one year of age
- Babies and children > Adult men > Adult women > Obese men and women

- **Heart and brain - 73%**
- **Lungs 83%**
- **Muscles and kidneys 79%**
- **Skin 64%**
- **Bones 31%**



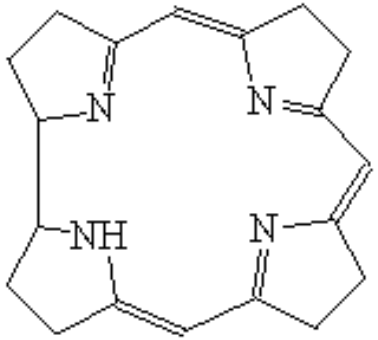
How Much Water is in Your Body

Porphyrin, Corrin and Chloring Ring System



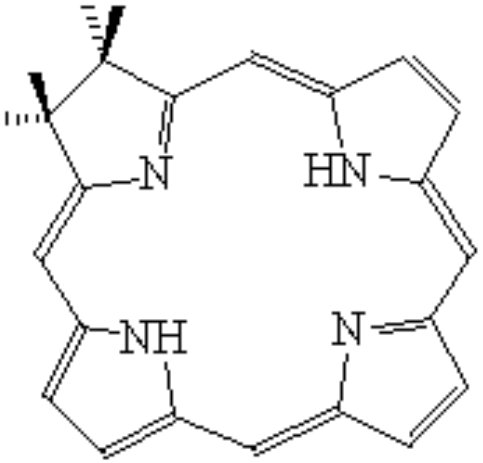
Porphyrin

All Heme proteins contains porphyrin ring system – e.g. Hemoglobin, myoglobin, Cytochromes (a, b and c)



Corrin

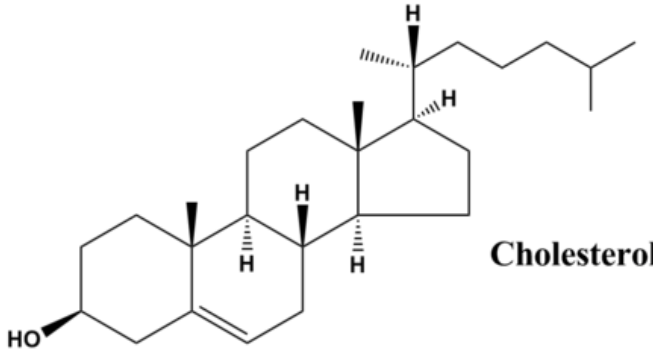
Vitamin B_{12} contains corrin ring



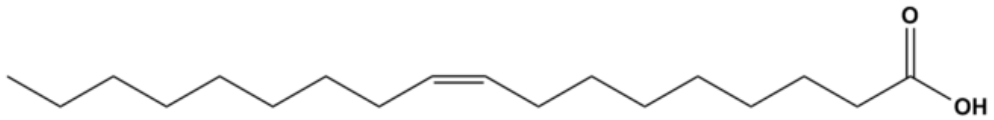
Chlorin

Chlorophyll contains chlorin ring

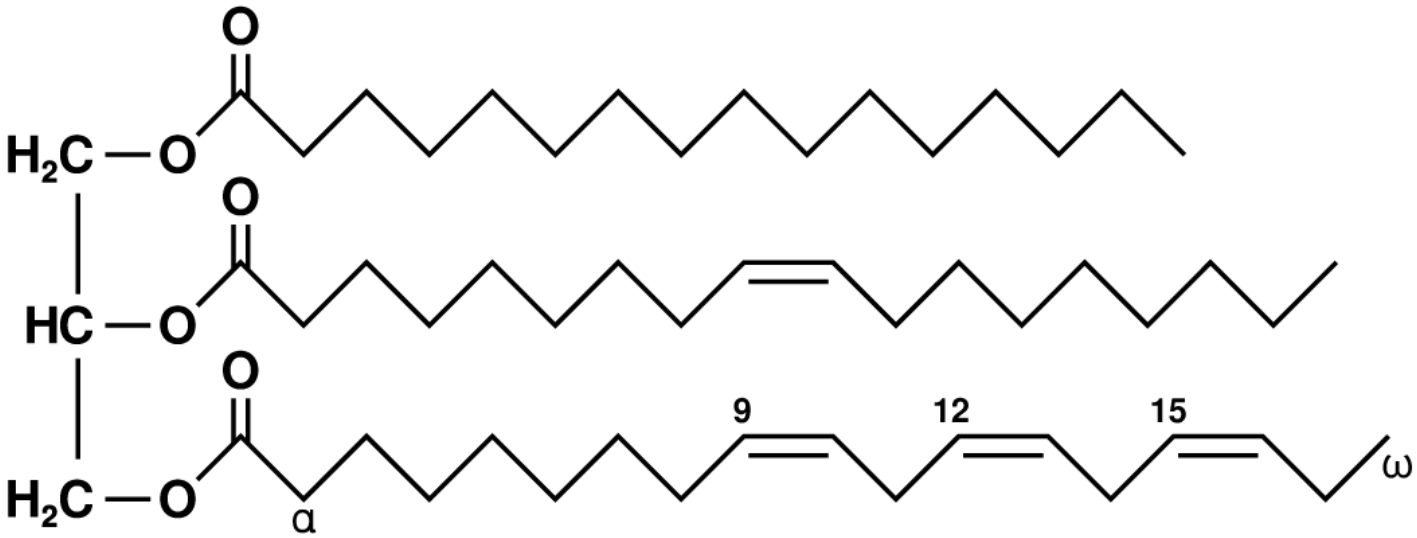
Biomolecules: Lipids Greek *lipos* (fat)



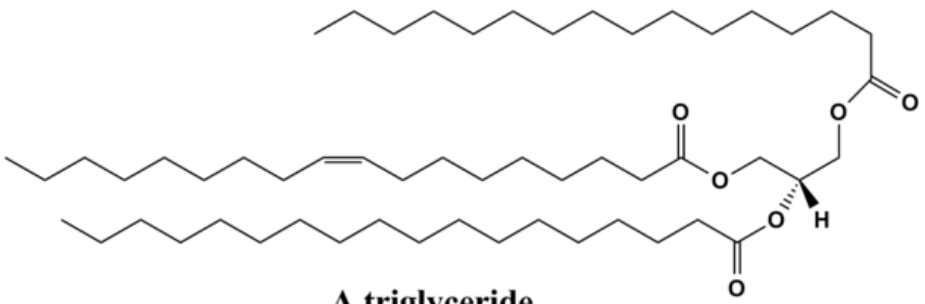
Cholesterol



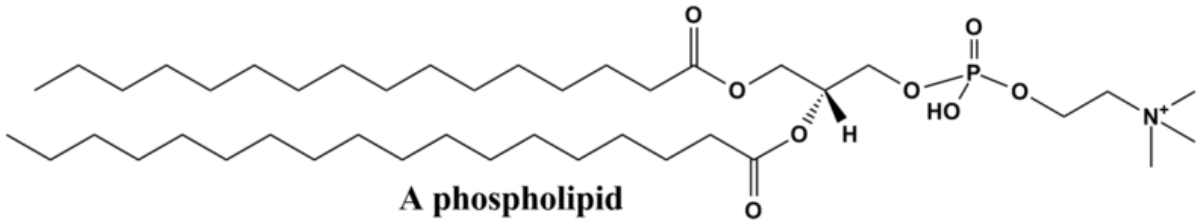
A free fatty acid



palmitic acid, oleic acid, alpha-linolenic acid.



A triglyceride

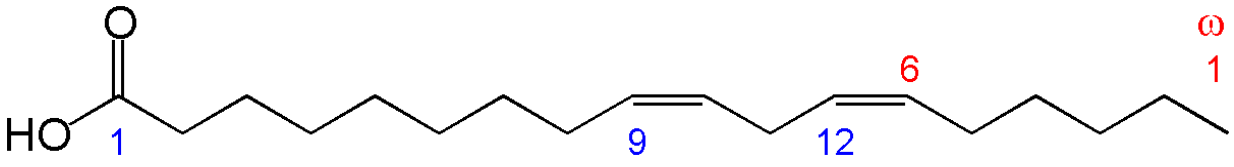


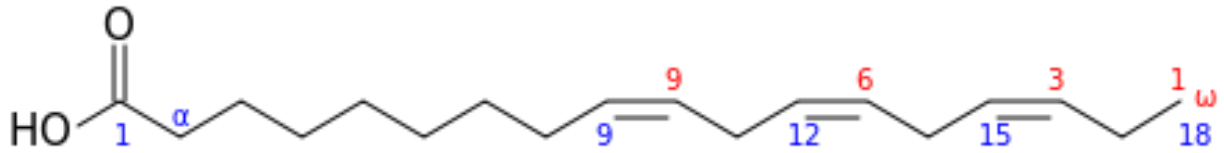
A phospholipid

Biomolecules: Lipids Greek *lipos* (fat)

The major dietary lipids for humans and other animals are animal and plant triglycerides, sterols, and membrane phospholipids

Humans and other mammals have a dietary requirement for certain essential fatty acids, such as linoleic acid (an omega-6 fatty acid) and alpha-linolenic acid (an omega-3 fatty acid) because they cannot be synthesized from simple precursors in the diet

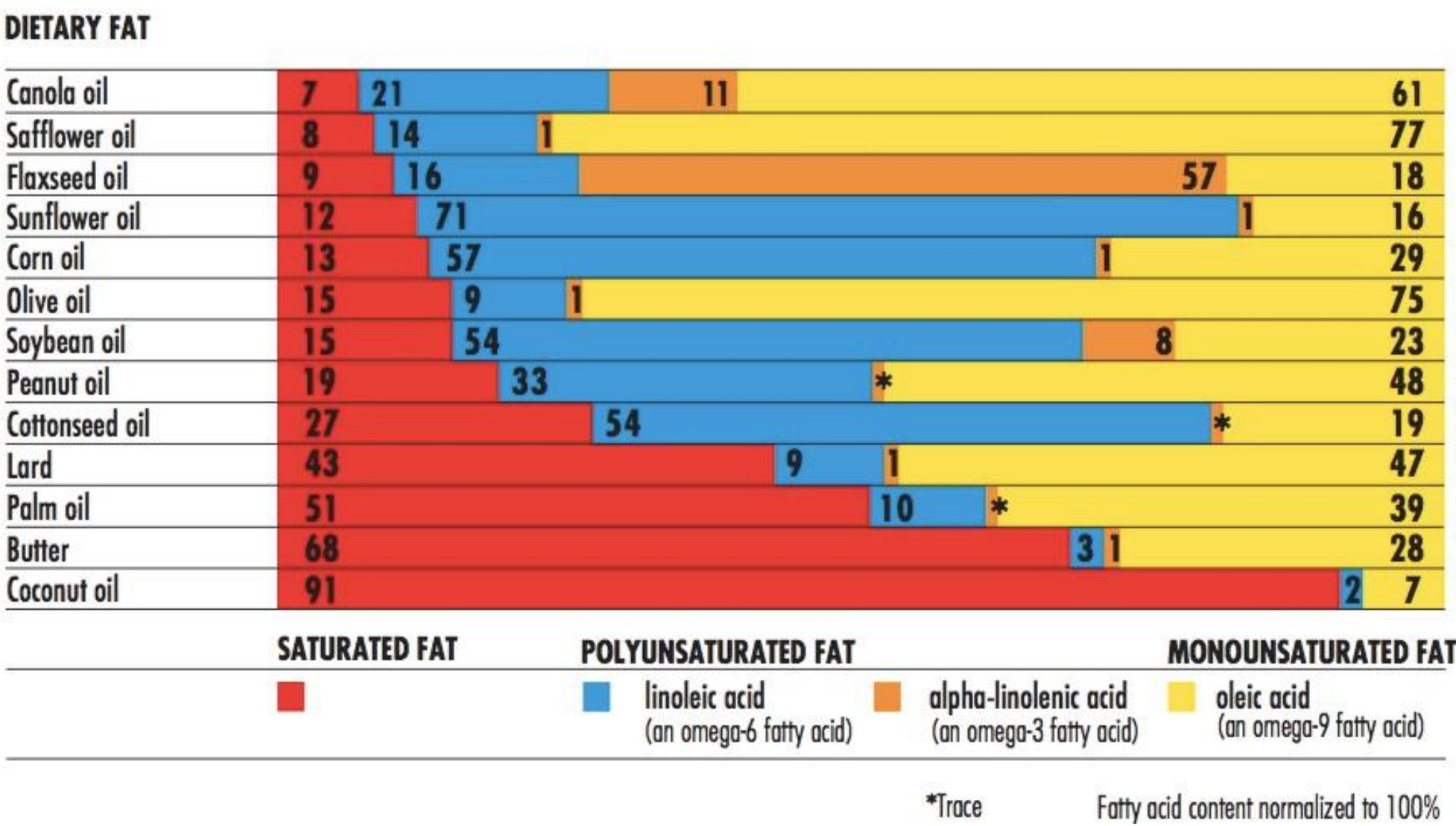




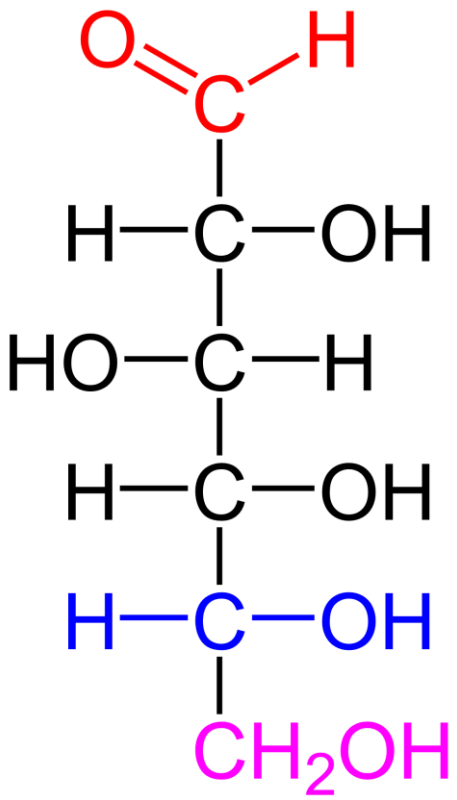
Triglycerides, stored in adipose tissue, are a major form of energy storage both in animals and plants. In comparison to glycogen which would contribute only half of the energy per its pure mass, carbohydrate carbons are all bounded to hydrogens unlike in carbohydrates.

The complete oxidation of fatty acids provides high caloric content, about 38 kJ/g (9 kcal/g), compared with 17 kJ/g (4 kcal/g) for the breakdown of carbohydrates and proteins. Migratory birds that must fly long distances without eating use stored energy of triglycerides to fuel their flights.

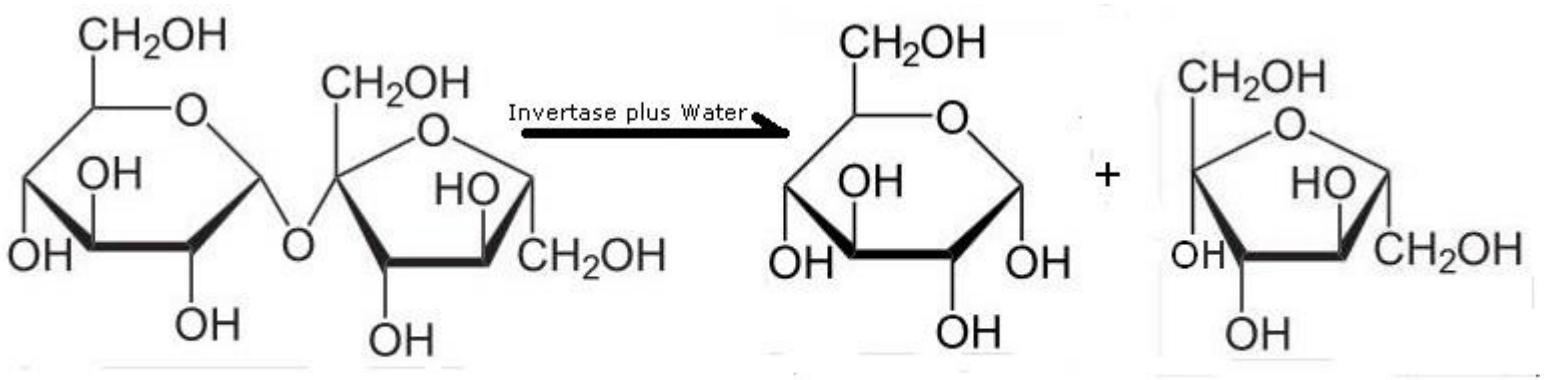
Comparison of Dietary Fats



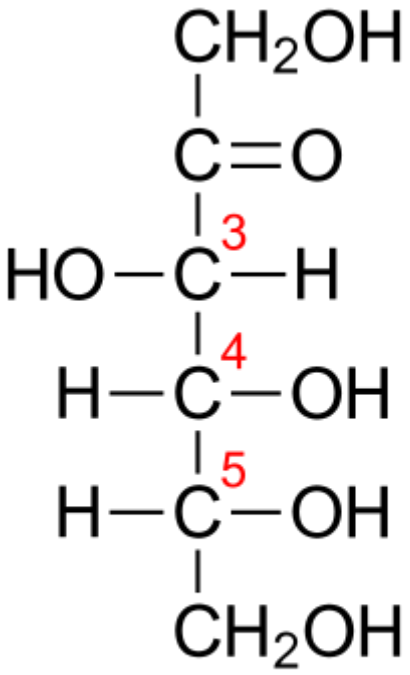
Biomolecules: Carbohydrates



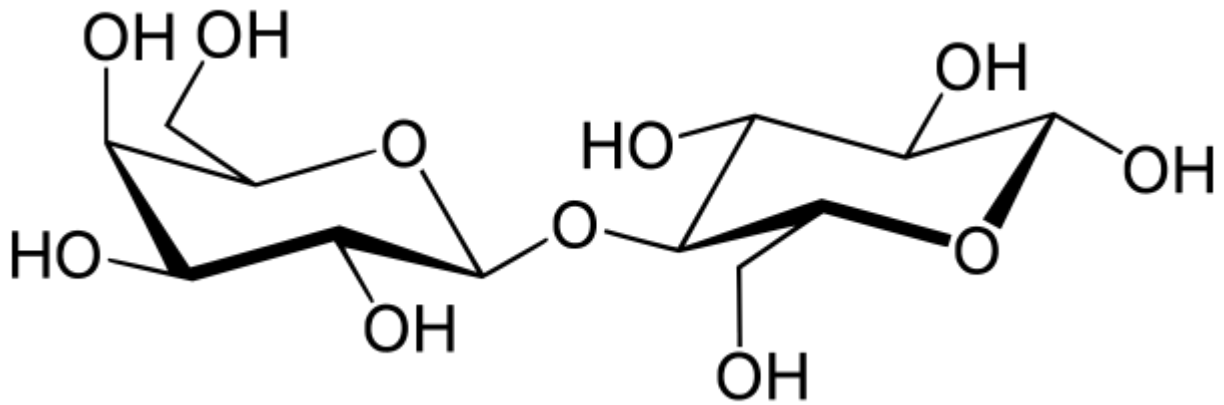
D-glucose



Sucrose (Table Sugar) Glucose Fructose



D-Fructose



Lactose

LivingHealthyMom.com	Sucanat	White Sugar
-Notice how almost all the nutrients have been removed from white sugar during processing, leaving it as “empty calories.”		
Calories	570	770
Carbs	135g	199g
Fat	0	0
Sodium	.5mg	0
Potassium	1125mg	4mg
Vitamin A	1600IU	0
thiamin (B1)	.21mg	0
riboflavin (B2)	.21mg	0.038
niacin	.20mg	0
calcium	165mg	2mg
iron	6.5mg	0.10mg
vitamin B6	.60mg	0
magnesium	127mg	0.008mg
zinc	2.3mg	0.02mg
copper	.3mg	0.014
pantothenic acid	1.8mg	0
chromium	40mcg	unknown
phosphorus	48mg	0
According to the USDA National Database		

Jaggery = sucrose (70%) + Glucose & Fructose (15%) + Fats (0.1%) +Proteins (0.4%) +Minerals (2%)+Water (10%) + Ash (1%)

Sugar =99% Sucrose + 1% water

https://www.quora.com/Why-is-jaggery-considered-more-healthy-than-white-sugar

https://ehp.niehs.nih.gov/doi/10.1289/ehp.94102s5211

According to one source, 100 grams (half a cup) of jaggery may contain (4):

- **Calories:** 383.
- **Sucrose:** 65–85 grams.
- **Fructose and glucose:** 10–15 grams.
- **Protein:** 0.4 grams.
- **Fat:** 0.1 grams.
- **Iron:** 11 mg, or 61% of the RDI.
- **Magnesium:** 70-90 mg, or about 20% of the RDI.
- **Potassium:** 1050 mg, or 30% of the RDI.
- **Manganese:** 0.2–0.5 mg, or 10–20% of the RDI.

https://www.walshmedicalmedia.com/open-access/review-on-recent-advances-in-value-addition-of-jaggery-based-products-2157-7110-1000440.pdf

https://www.healthline.com/nutrition/jaggery#TOC_TITLE_HDR_4

Glycemic Index

Low GI (<55), Medium GI (56-69) and High GI (70>)

Grains / Starchs		Vegetables		Fruits		Dairy		Proteins	
Rice Bran	27	Asparagus	15	Grapefruit	25	Low-Fat Yogurt	14	Peanuts	21
Bran Cereal	42	Broccoli	15	Apple	38	Plain Yogurt	14	Beans, Dried	40
Spaghetti	42	Celery	15	Peach	42	Whole Milk	27	Lentils	41
Corn, sweet	54	Cucumber	15	Orange	44	Soy Milk	30	Kidney Beans	41
Wild Rice	57	Lettuce	15	Grape	46	Fat-Free Milk	32	Split Peas	45
Sweet Potatoes	61	Peppers	15	Banana	54	Skim Milk	32	Lima Beans	46
White Rice	64	Spinach	15	Mango	56	Chocolate Milk	35	Chickpeas	47
Cous Cous	65	Tomatoes	15	Pineapple	66	Fruit Yogurt	36	Pinto Beans	55
Whole Wheat Bread	71	Chickpeas	33	Watermelon	72	Ice Cream	61	Black-Eyed Beans	59
Muesli	80	Cooked Carrots	39						
Baked Potatoes	85								
Oatmeal	87								
Taco Shells	97								
White Bread	100								
Bagel, White	103								

GLYCEMIC INDEX CHART

Low Glycemic (55 or Below) High Glycemic (70 or Higher)

SNACKS	G.I.	STARCH	G.I.	VEGETABLES	G.I.	FRUITS	G.I.	DAIRY	G.I.
Pizza	33	Bagel, Plain	33	Broccoli	10	Cherries	22	Yogurt, Plain	14
Chocolate Bar	49	White Rice	38	Pepper	10	Apple	38	Yogurt, Low Fat	14
Pound Cake	54	White Spaghetti	38	Lettuce	10	Orange	43	Whole Milk	30
Popcorn	55	Sweet Potato	44	Mushrooms	10	Grapes	46	Soy Milk	31
Energy Bar	58	White Bread	49	Onions	10	Kiwi	52	Skim Milk	32
Soda	72	Brown Rice	55	Green Peas	48	Banana	56	Chocolate Milk	35
Doughnut	76	Pancakes	67	Carrots	49	Pineapple	66	Yogurt, Fruit	36
Jelly Beans	80	Wheat Bread	80	Beets	64	Watermelon	72	Custard	43
Pretzels	83	Baked Potato	85	Onions	75	Dates	103	Ice Cream	60

GLYCEMIC INDEX CHART

0 10 20 30 40 50 60 70 80 90 100

Food Item Low GI(0-55)

- Apple 39
- WheatCereal 31
- Soybean 18
- Cashews 21
- Grapes 46
- Honey 55
- Brown Rice 55

Food Item Medium GI(56-69)

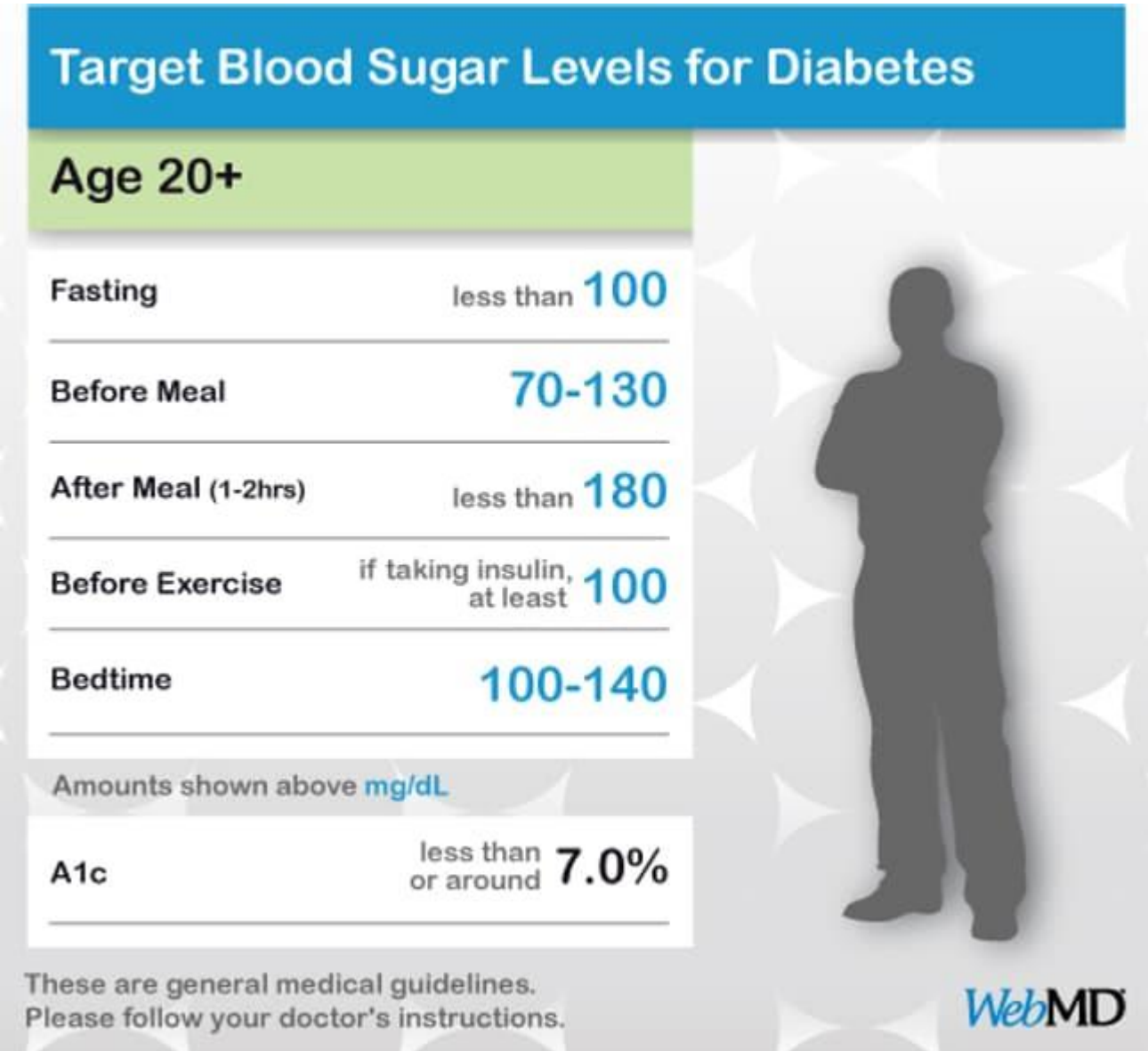
- Sugar 65
- Raisins 64
- Cheese Pizza 60
- Pineapple 66
- Wheat Thins 67

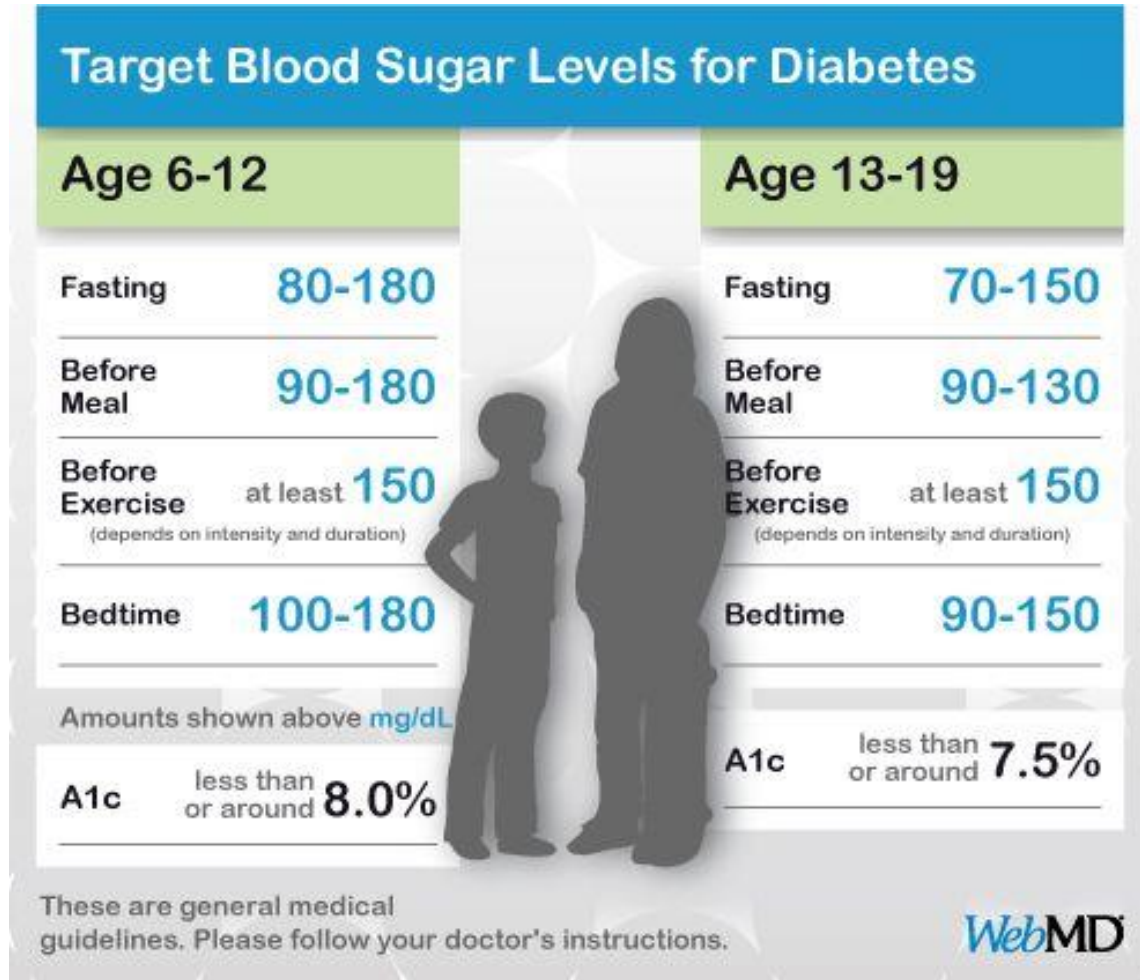
Food Item High GI(70-100)

- Corn Chips 72
- Gatorade 78
- Pumpkin 75
- Pretzels 83
- White Rice 89

https://www.publichealth.com.ng/low-glycemic-index-foods-list/

https://www.mayoclinic.org/diseases-conditions/diabetes/symptoms-causes/syc-20371444





https://www.webmd.com/diabetes/guide/normal-blood-sugar-levels-chart-adults

The Benefits of *Raw Honey*

Raw, unprocessed honey is much better as a sweetener than white sugar

Because it is not pasteurized, it still has the natural enzymes in it

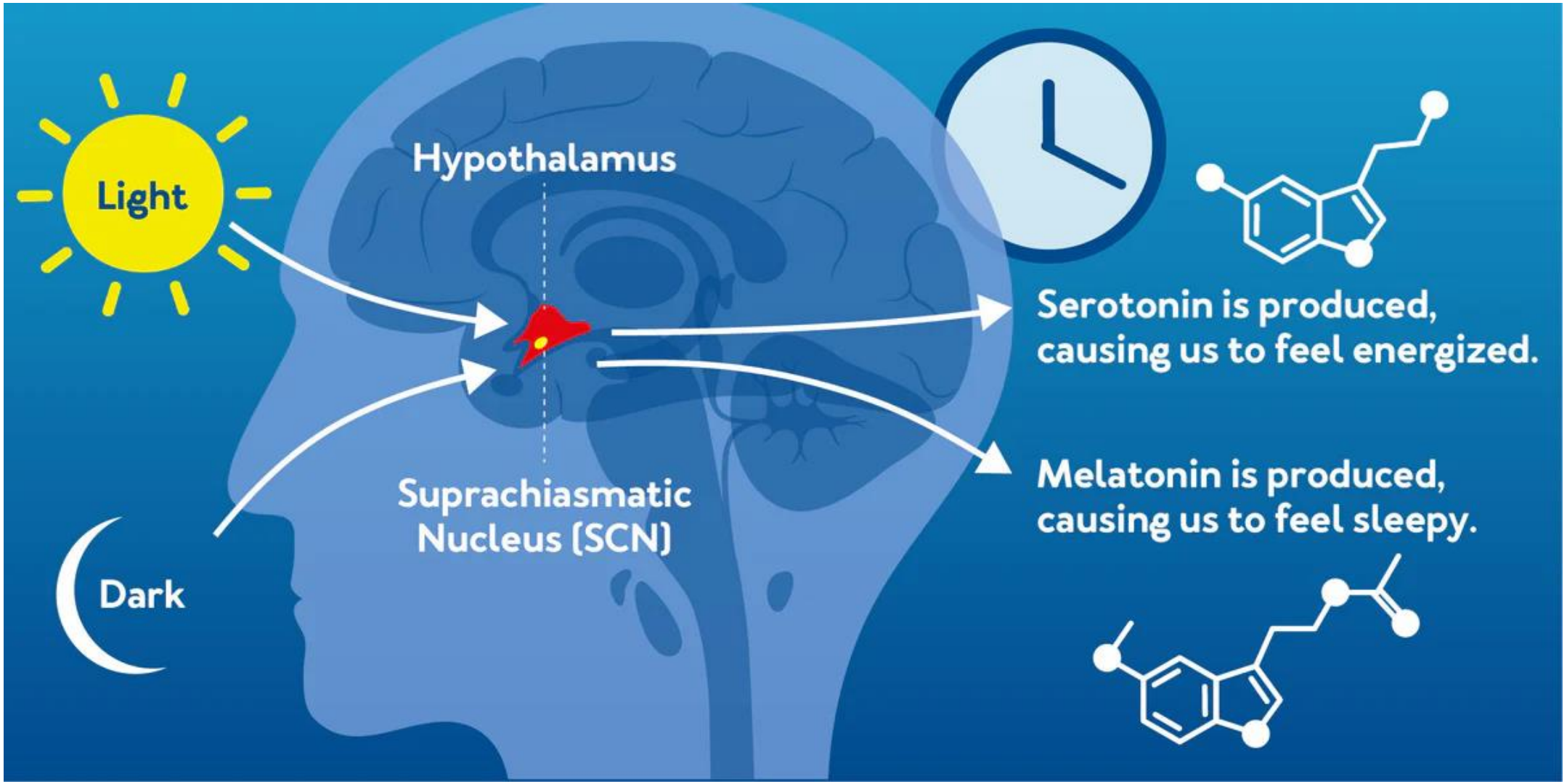
Honey naturally contains vitamins B2, B3, B5, B6 & C

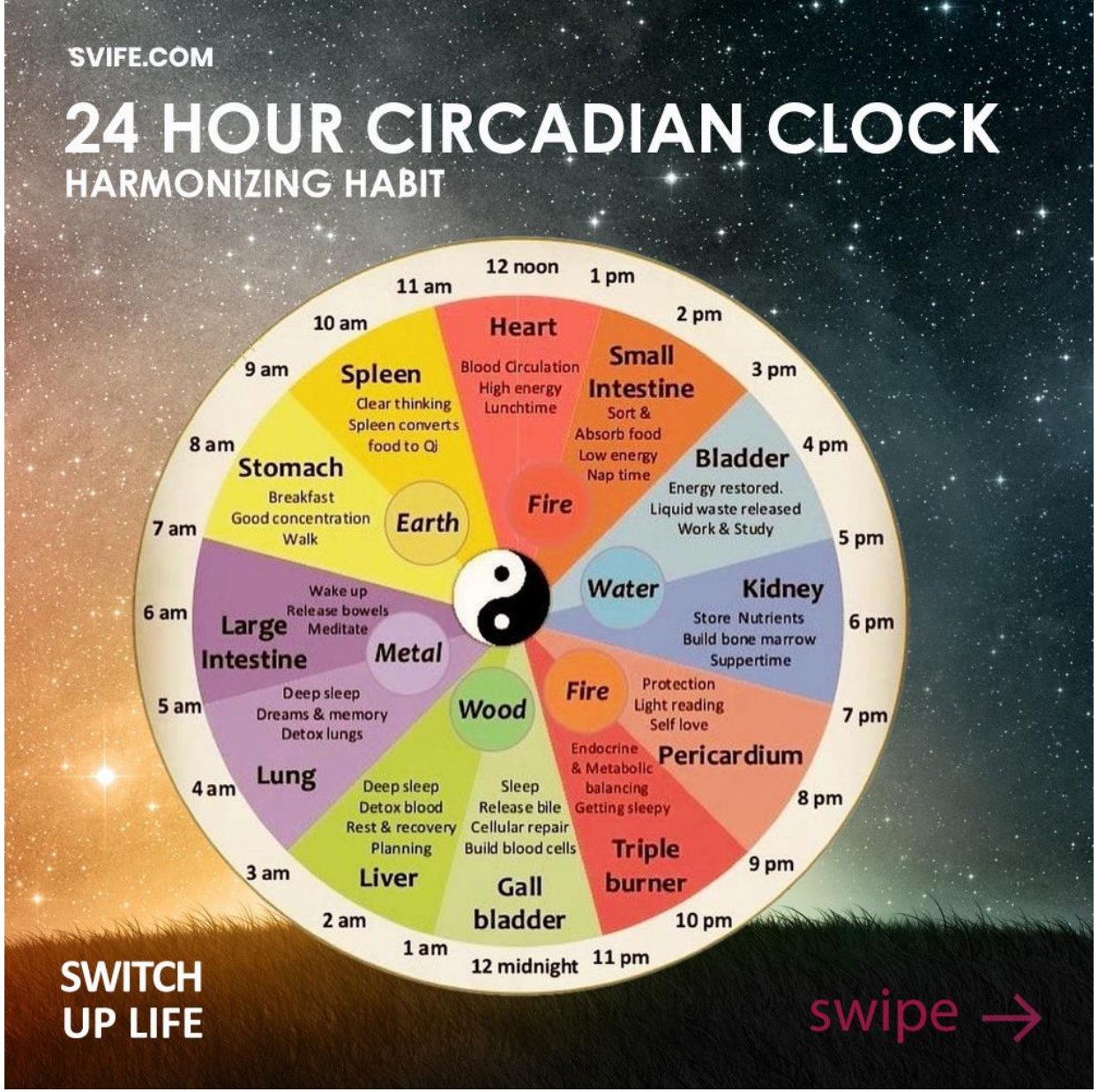
Minerals that can be found in honey, include: potassium, magnesium, zinc and iron

Honey helps fight indigestion and can soothe a sore throat

Honey has antimixrobial & antifungal benefits because it contains: propolis, something the bees use to protect their hives from unwanted organisms

#APL


Light
Hypothalamus
Suprachiasmatic Nucleus (SCN)
Dark
Serotonin is produced, causing us to feel energized.
Melatonin is produced, causing us to feel sleepy.



https://carex.com/blogs/resources/circadian-rhythm https://www.svife.com/what-are-circadian-rhythms/

https://www.news-medical.net/health/Circadian-Rhythm.aspx

Top 10 Sources of Veggie Protein

design / layout by:
Q-Mars Imandel
www.facebook.com/viberider

Where do you get your protein?

(brought to you by The GIVE Project)

The GIVE project
thegiveproject.org
www.facebook.com/giveproject

Spinach
49% protein

Kale
45% protein

Broccoli
45% protein

Cauliflower
40% protein

Mushrooms
38% protein

Parsley
34% protein

Cucumbers
24% protein

Green Pepper
22% protein

Cabbage
22% protein

Tomatoes
18% protein

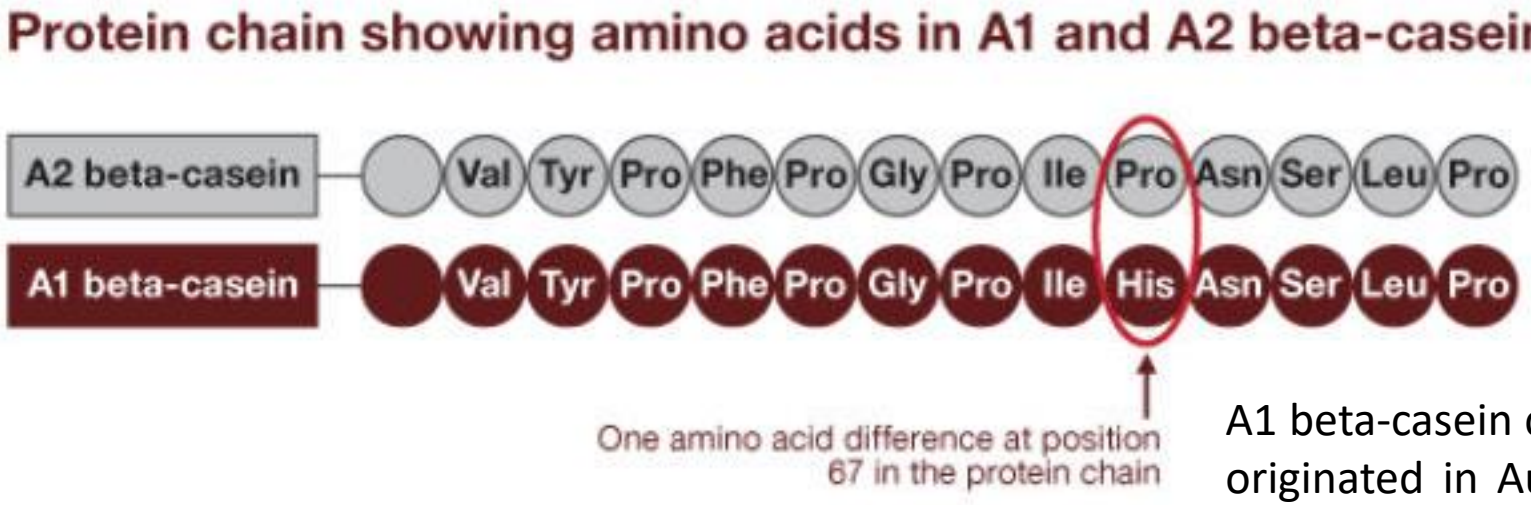
Protein in Meat:

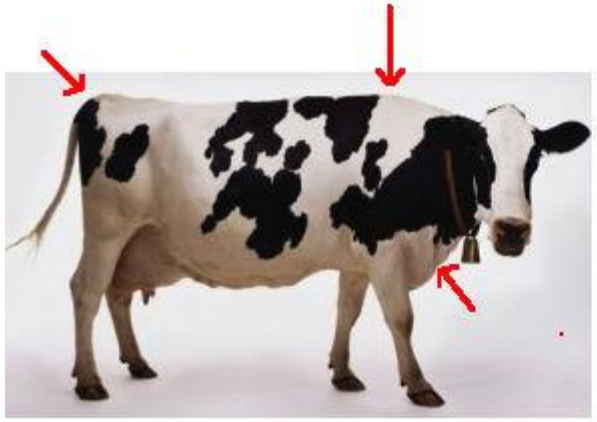
Beef
25.8% protein

Chicken
23% protein

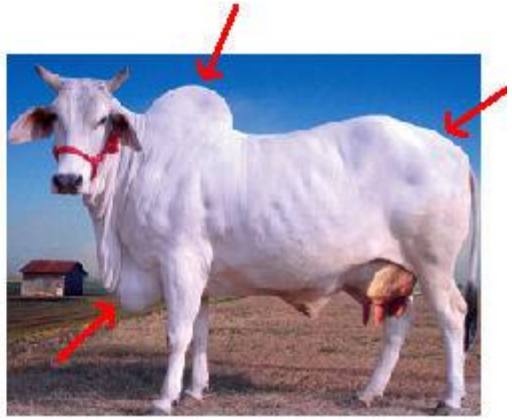
Eggs
12% protein

Importance of amino acid





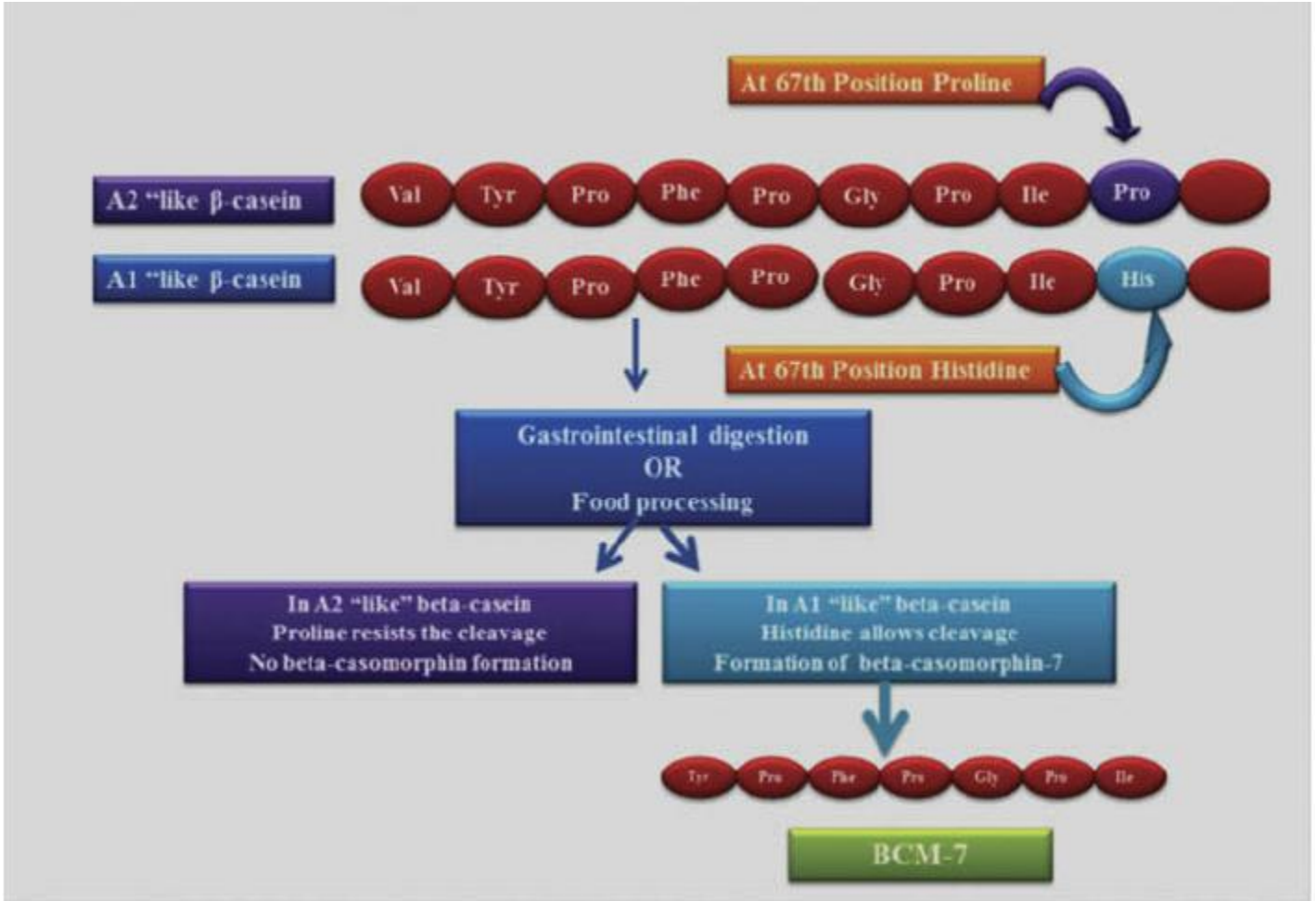
ஹைப்ரிட் பசு



இந்திய பசு!

A1 beta-casein comes from the most common cow breed that originated in Australia, United States, and Northern Europe. Holstein, Friesian, Ayrshire, and British Shorthorn features A1 beta-casein genetic material. A1 beta-casein can be found on all commercially-prepared milk

BCM7 Beta-casomorphin-7 an opioid peptide in A1 beta-casein is produced as a result of the breaking off of histidine in the number 67 amino acid chain during digestion found in A1 milk is known to have opioid or narcotic side effects and is identified to be the culprit of lactose intolerance in 1 out 4 Americans. The absorption of BCM7 into the bloodstream leads to the high incidence of autism, schizophrenia, and other neurological disorders



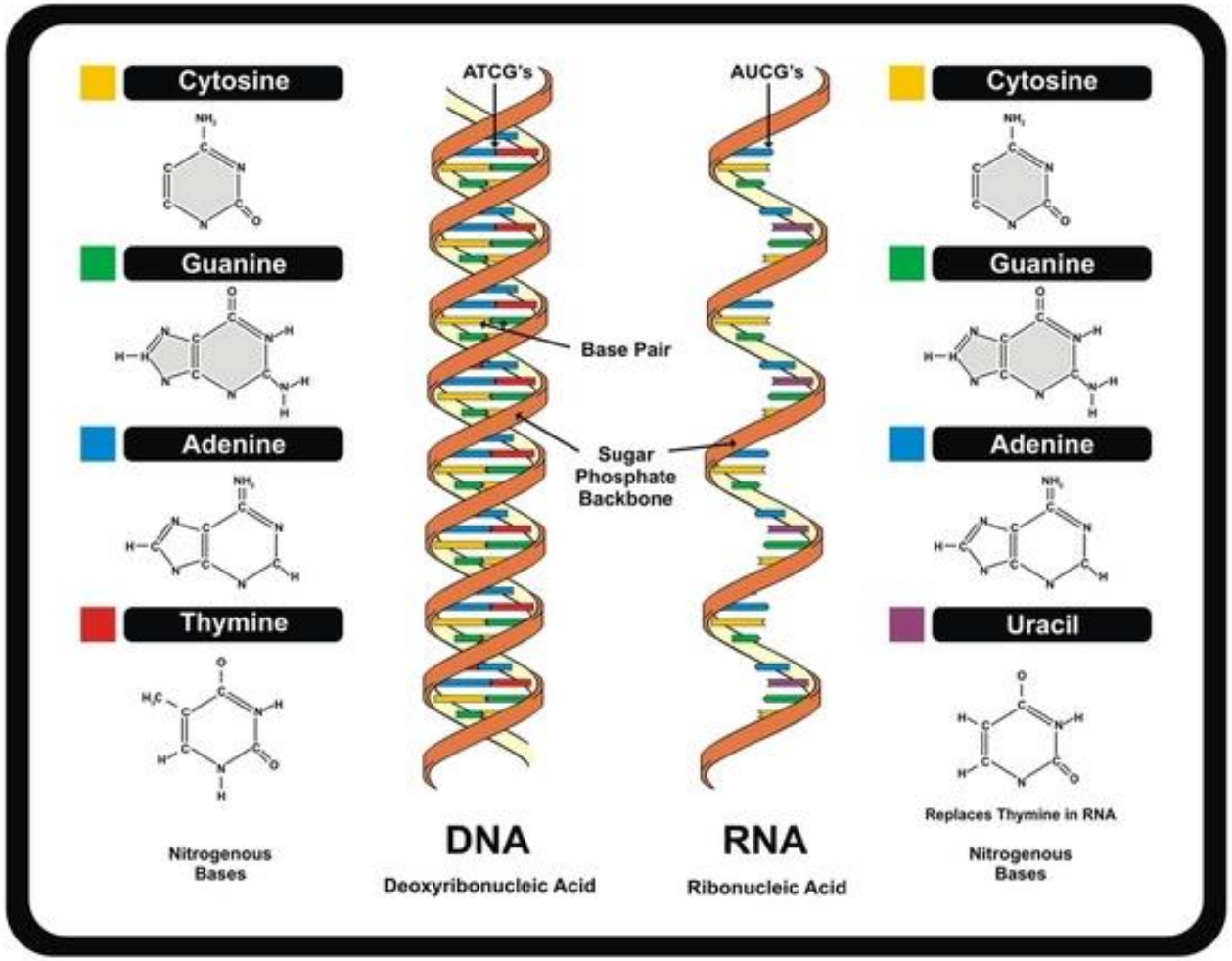
http://www.thehealthedgepodcast.com/wp-content/uploads/2015/03/a1-vs-a2.jpg

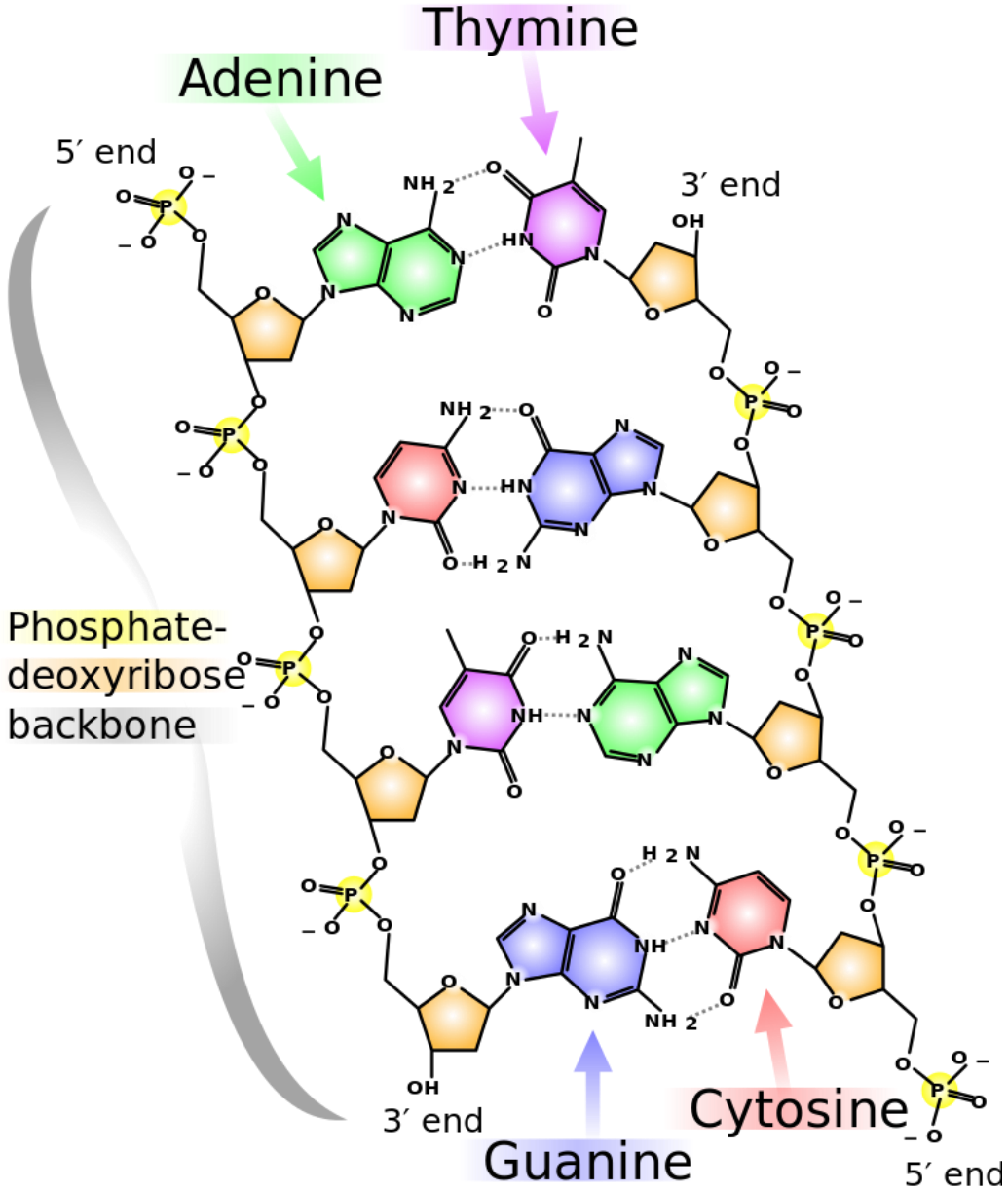
https://a2milk.co.uk/wp-content/uploads/hcp-explain3.png

Desi Cow milk	Ordinary milk
Indian Desi cows produce A2 milk which contains A2 Beta casein.	Jersey cow produce A1 milk which contains A1 Beta casein.
Desi cow milk only contains the A2 protein and no A1.	All ordinary milk has a mixture of A1 and A2 proteins.
High level of Omega 3 that cleans the cholesterol deposits of blood vessels	Harmful to human body.
Cerebrosides present in A2 milk increases brain power.	Autism, Schizophrenia, Stomach Ulcer, Type 1 diabetes and cardiac disease
Strontium of A2 milk enhances the body immunity and protects from harmful radiation.	Holsteins and Friesians are not native breeds of India.

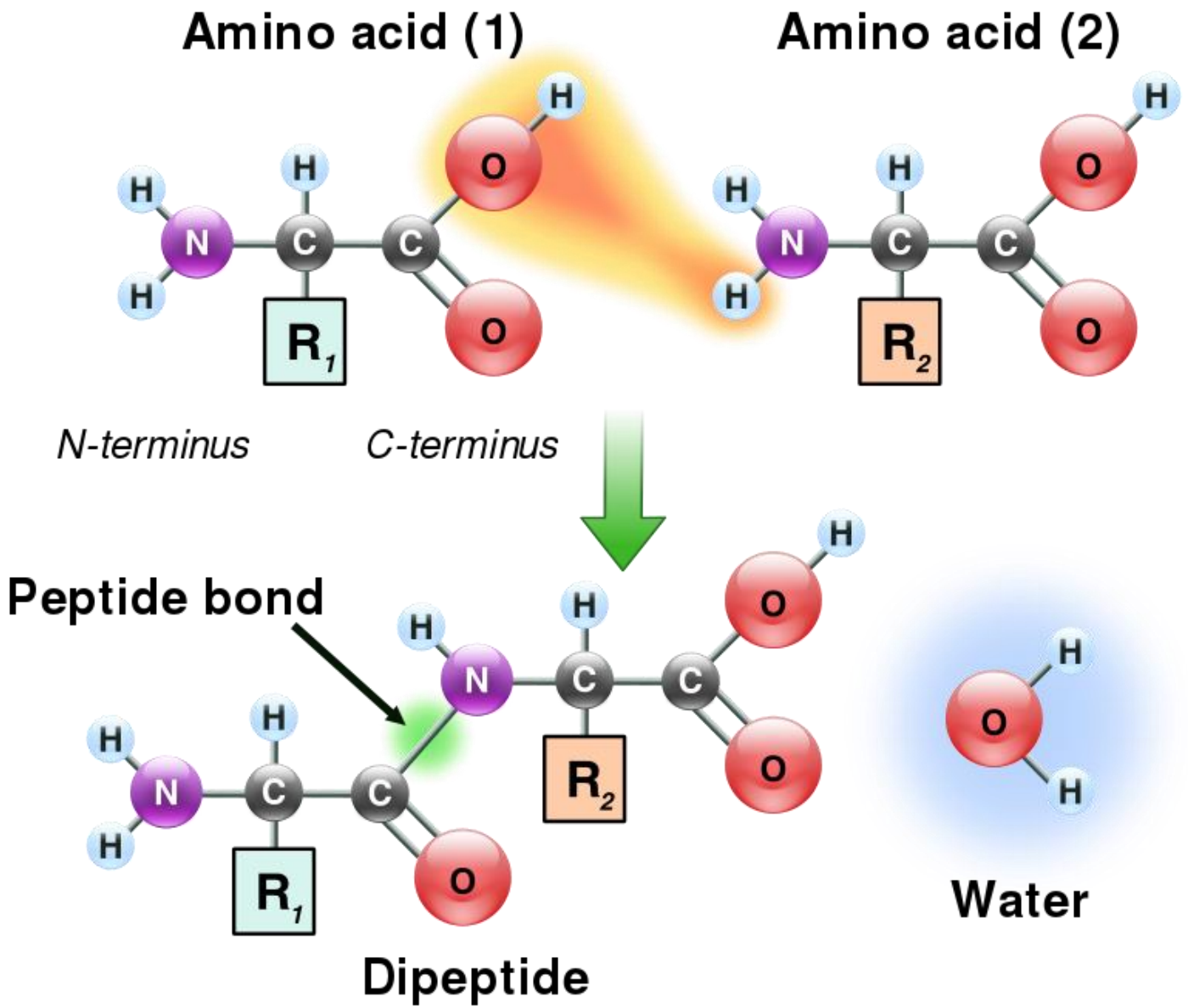
Biomolecules: Nucleic Acid

ATGCGCTTAG
TACGCGAAC



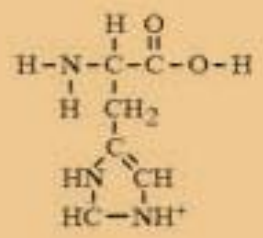


Biomolecules: Proteins

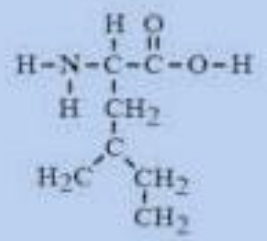


THE ESSENTIAL AMINO ACIDS

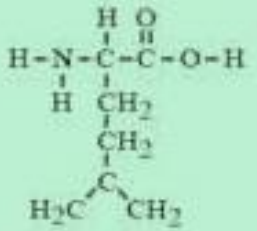
(WHICH OUR BODIES CANNOT MAKE) :



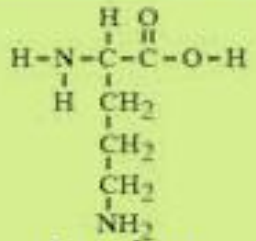
Histidine



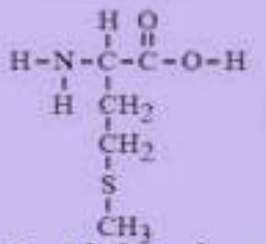
Isoleucine



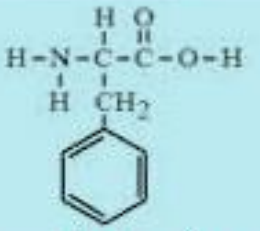
Leucine



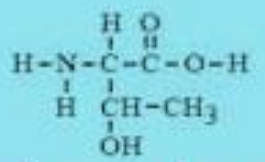
Lysine



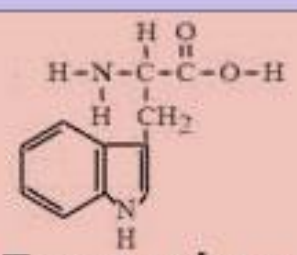
Methionine



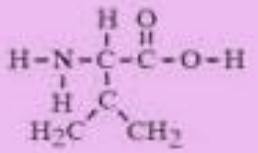
Phenylalanine



Threonine

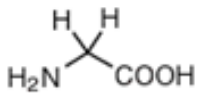


Tryptophan

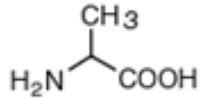


Valine

Small

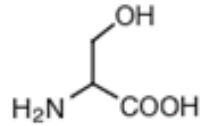


Glycine (Gly, G)
MW: 57.05

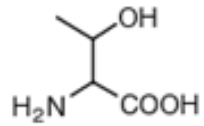


Alanine (Ala, A)
MW: 71.09

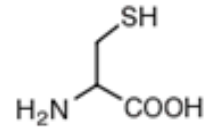
Nucleophilic



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

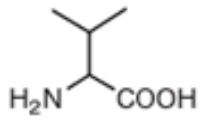


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

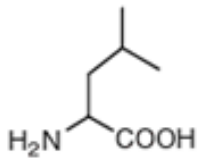


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

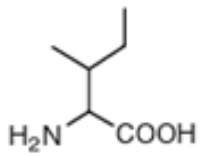
Hydrophobic



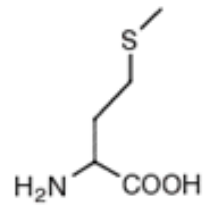
Valine (Val, V)
MW: 99.14



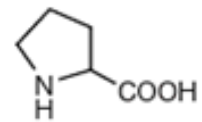
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

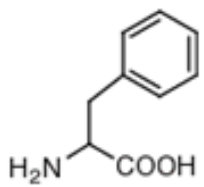


Methionine (Met, M)
MW: 131.19

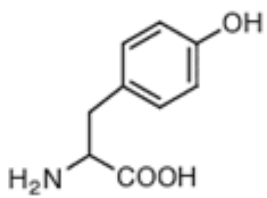


Proline (Pro, P)
MW: 97.12

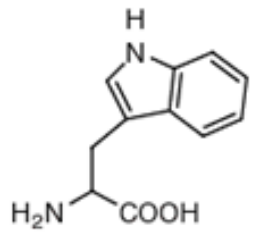
Aromatic



Phenylalanine (Phe, F)
MW: 147.18

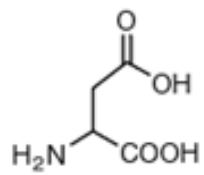


Tyrosine (Tyr, Y)
MW: 163.18

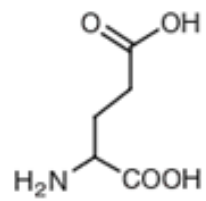


Tryptophan (Trp, W)
MW: 186.21

Acidic

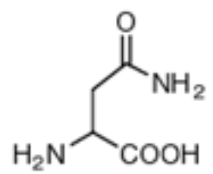


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

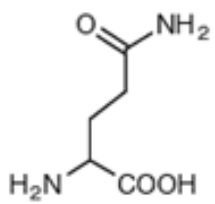


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

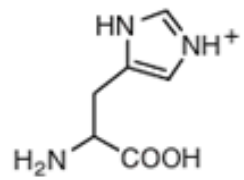


Asparagine (Asn, N)
MW: 114.11

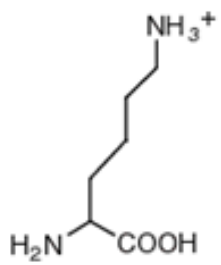


Glutamine (Gln, Q)
MW: 128.14

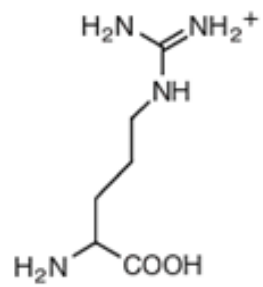
Basic



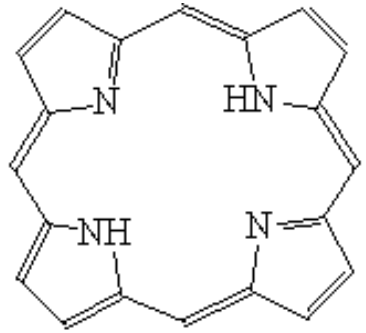
Histidine (His, H)
MW: 137.14, pK_a = 6.04



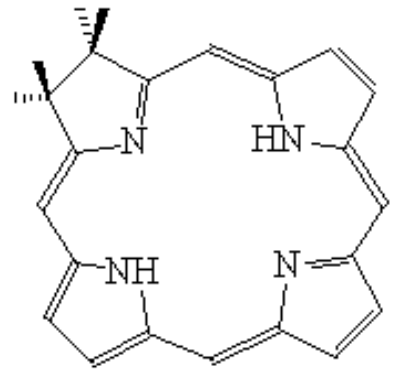
Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



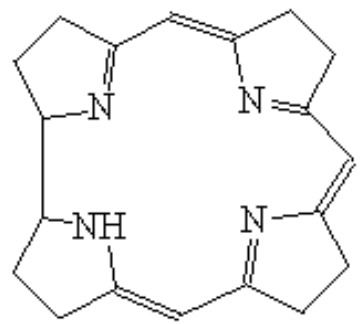
Arginine (Arg, R)
MW: 156.19, pK_a = 12.48



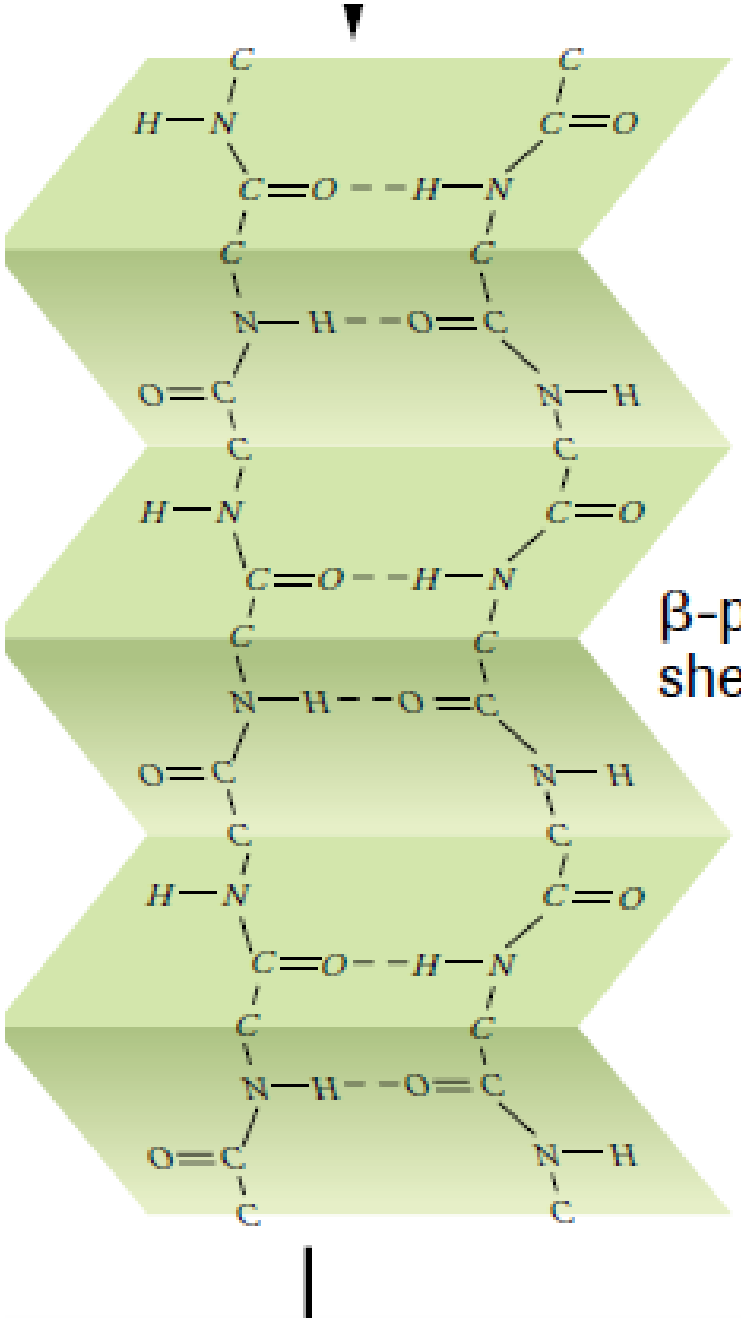
Porphyrin



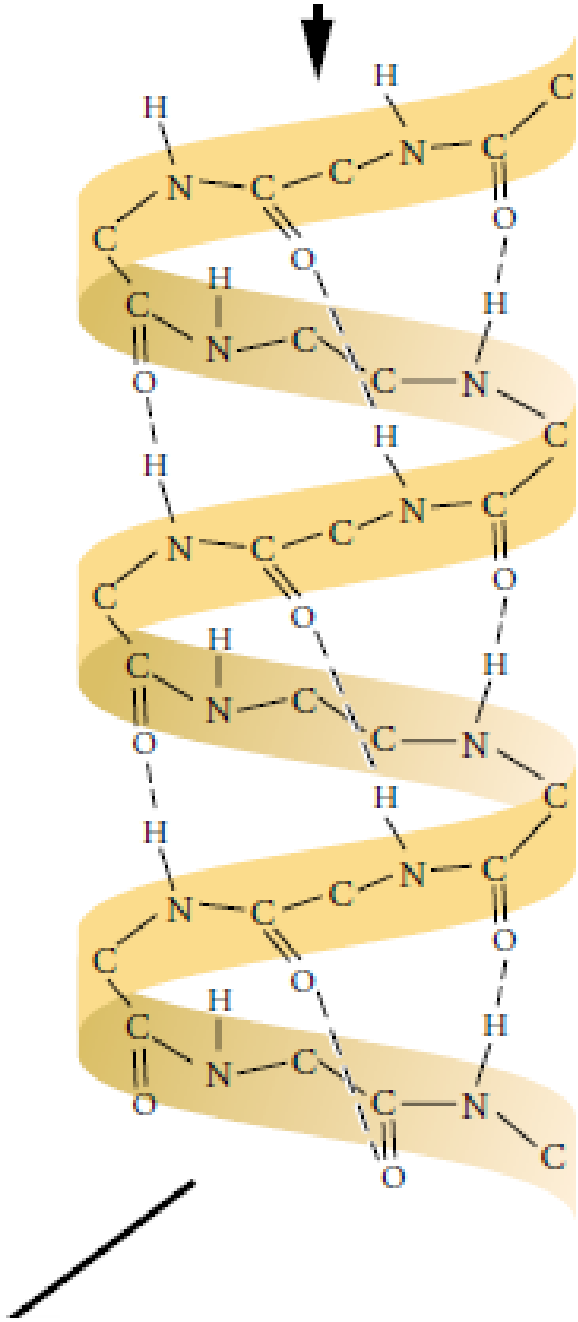
Chlorin



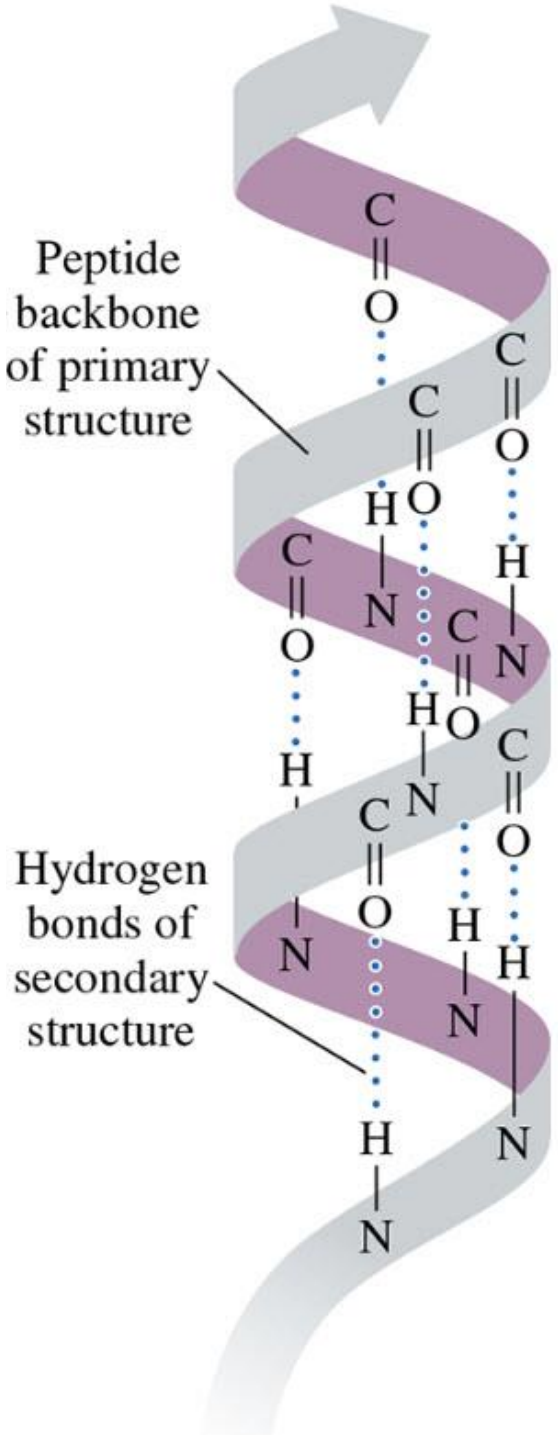
Corrin



β-pleated sheet



α helix



Metallobiomolecules

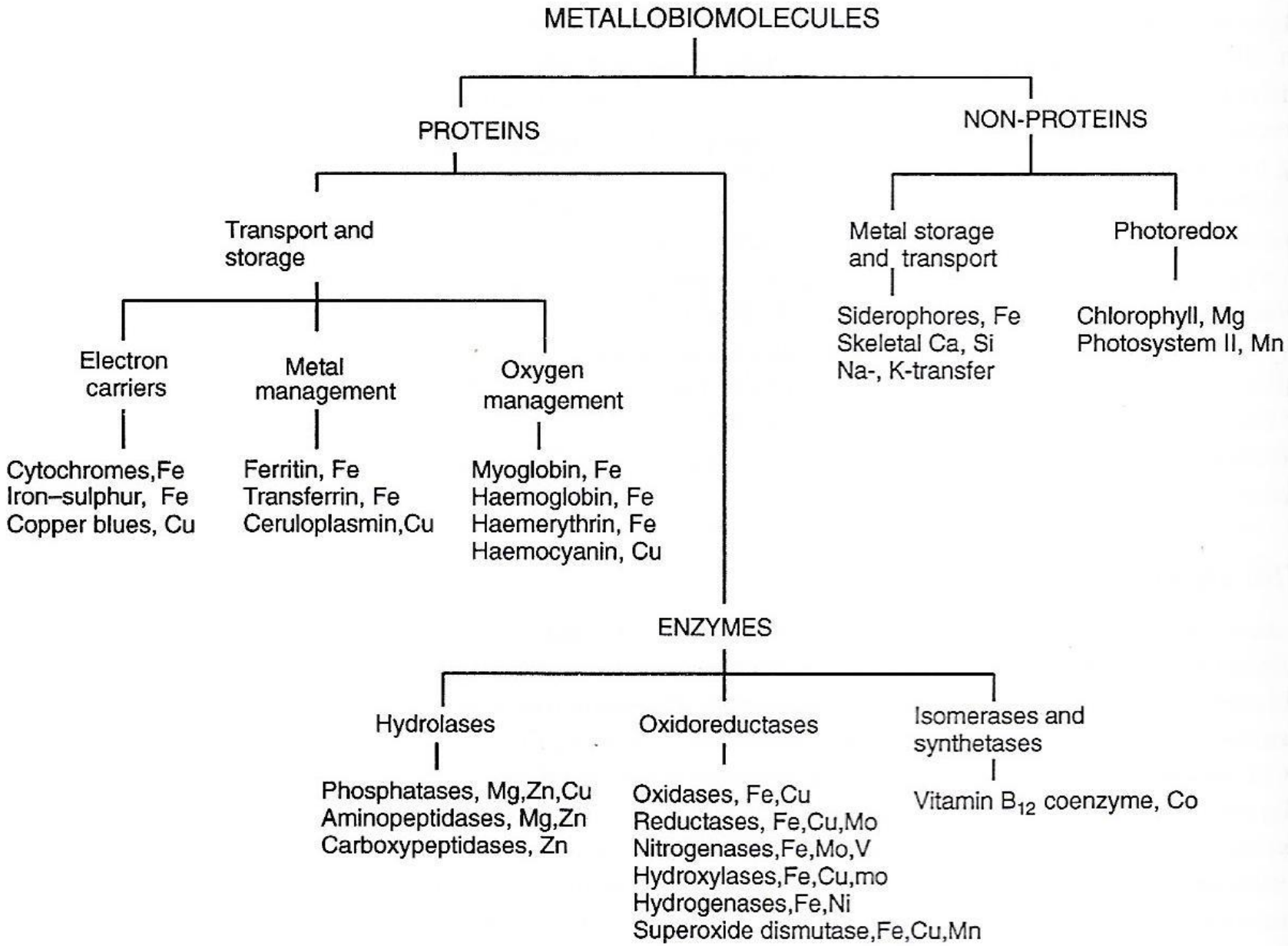


Fig 1.2. A classification of metallobiomolecules.

Enzyme: A biologically active compound containing one or more polypeptide units that are folded in a globular or fibrous form and catalyzes chemical reactions is called enzyme.

Apoenzyme: Many enzymes required an additional molecule to catalyze the particular chemical reaction. The small molecule is known as cofactor. It could be metal ion(s) or non-protein organic molecules. An enzyme without a cofactor is called apoenzyme.

Holoenzyme: An enzyme with a complete complement of cofactors is known as a holoenzyme.
So it can be written; Holoenzyme = Apoenzyme + coenzyme

Metalloenzyme: Enzyme that contains metal ion(s) in its active site and metal ion(s) participate(s) in the biological transformation.

Enzymes are biological catalysts that increase the rate of reaction without affecting the equilibrium of the reaction. All chemical reactions within a biological cells are catalyzed by enzymes.

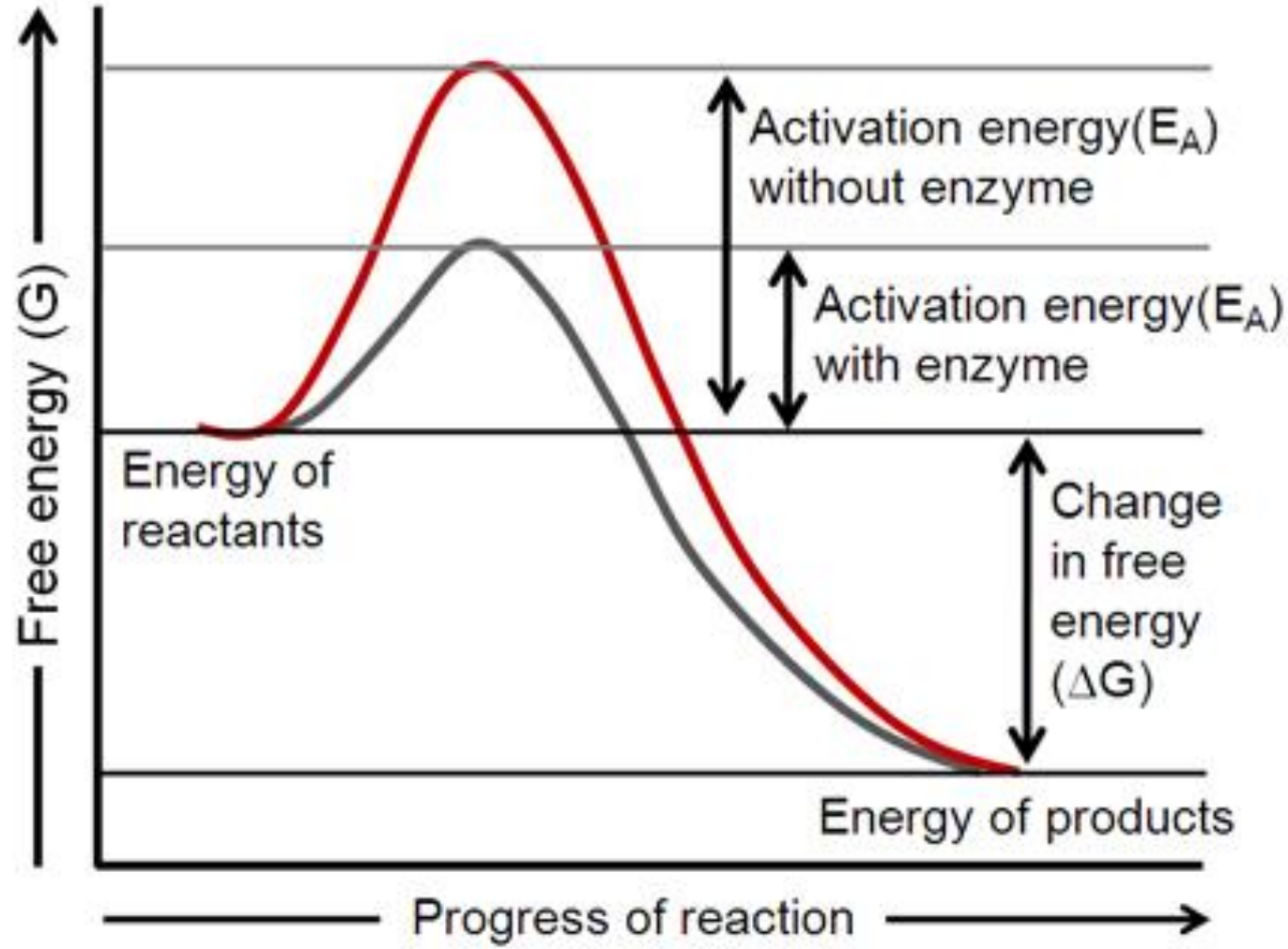
They work by lowering the activation energy (E_a) for a reaction, thereby increasing the reaction rate leading to faster formation of products and rapid achievement of the equilibrium state

Cofactors :
Many enzymes require an additional small molecule, known as a cofactor to aid the catalytic activity. A cofactor is usually a non-protein molecule; it can be either inorganic molecules (metals) or small organic molecules (coenzymes). For example Zn^{2+} serves as cofactor for carbonic anhydrase and alcoholdehydrogenase, Fe^{+}/Fe^{3+} act as cofactor for ferredoxin, hemoglobin and cytochrome

Coenzymes :
Coenzymes are organic nonprotein molecules that are mostly derivatives of water soluble vitamins soluble; they bind an apoenzyme protein molecule to produce an active holoenzyme

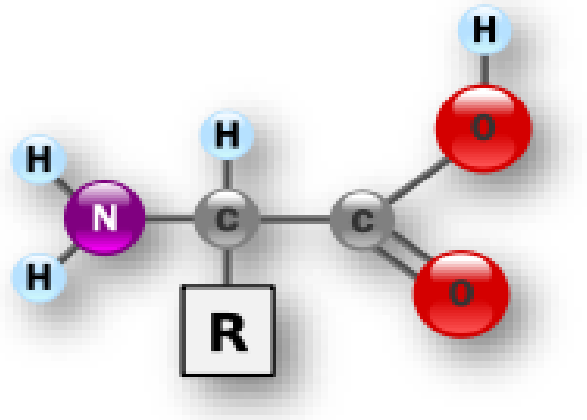
Apoenzyme :
An apoenzyme is an inactive enzyme, activation of which occurs upon the binding of an organic or inorganic cofactor
Apoenzyme + cofactor = holoenzyme

Holoenzyme :
An apoenzyme together with its cofactor leads to formation of a catalytically active holoenzyme. Most cofactors are not covalently bound but instead are tightly bound. However, organic prosthetic groups such as an iron ion or a vitamin may also be covalently bound


Free energy (G)
Activation energy(E_A) without enzyme
Activation energy(E_A) with enzyme
Energy of reactants
Change in free energy (ΔG)
Energy of products
Progress of reaction

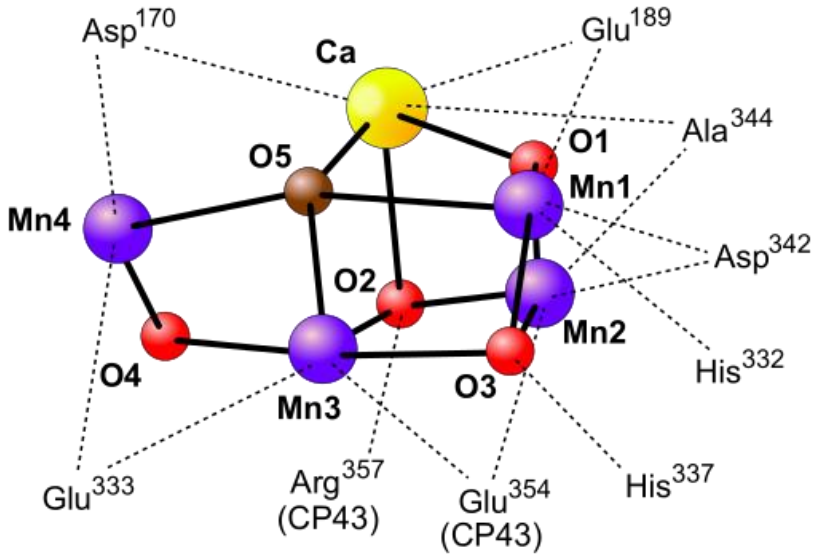
Metalloproteins:

- About 20% of the human body is made up of proteins. Aminoacids are the building blocks of proteins and plays a vital role in biological processes

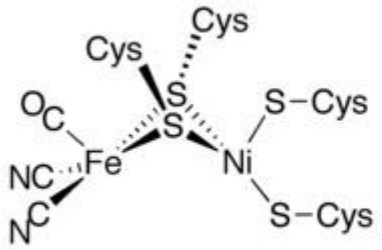


- Proteins that contains metal ion as co factor is called metalloproteins. It is co-ordinated through N, O and S atom of the amino acid side chain and forms a stable five membered chelate ring

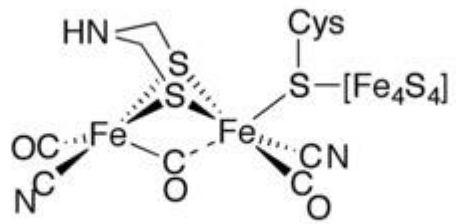
Importance of metal – amino acid complexes



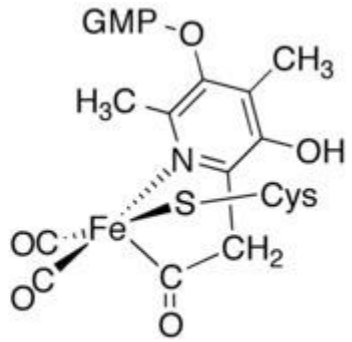
Oxygen Evolving Complex



[NiFe]H_2ase



[FeFe]H_2ase

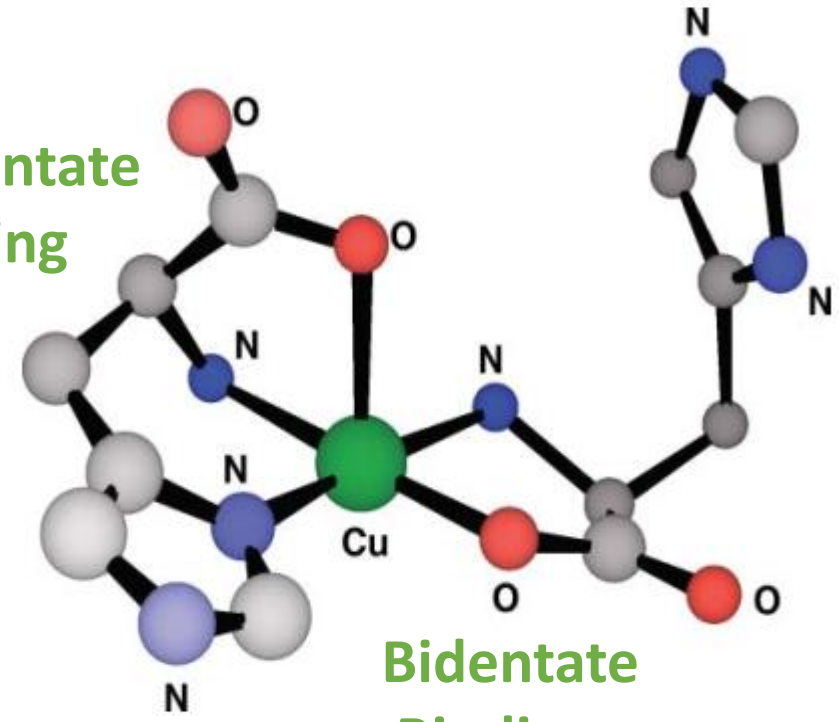


[Fe]H_2ase

$H_2 \rightleftharpoons 2\ H^+ + 2\ e^-$ (1)

$H_2 \rightleftharpoons H^+ + H^-$ (2) (2)

Medicinal Use of Metal Amino acids complexes

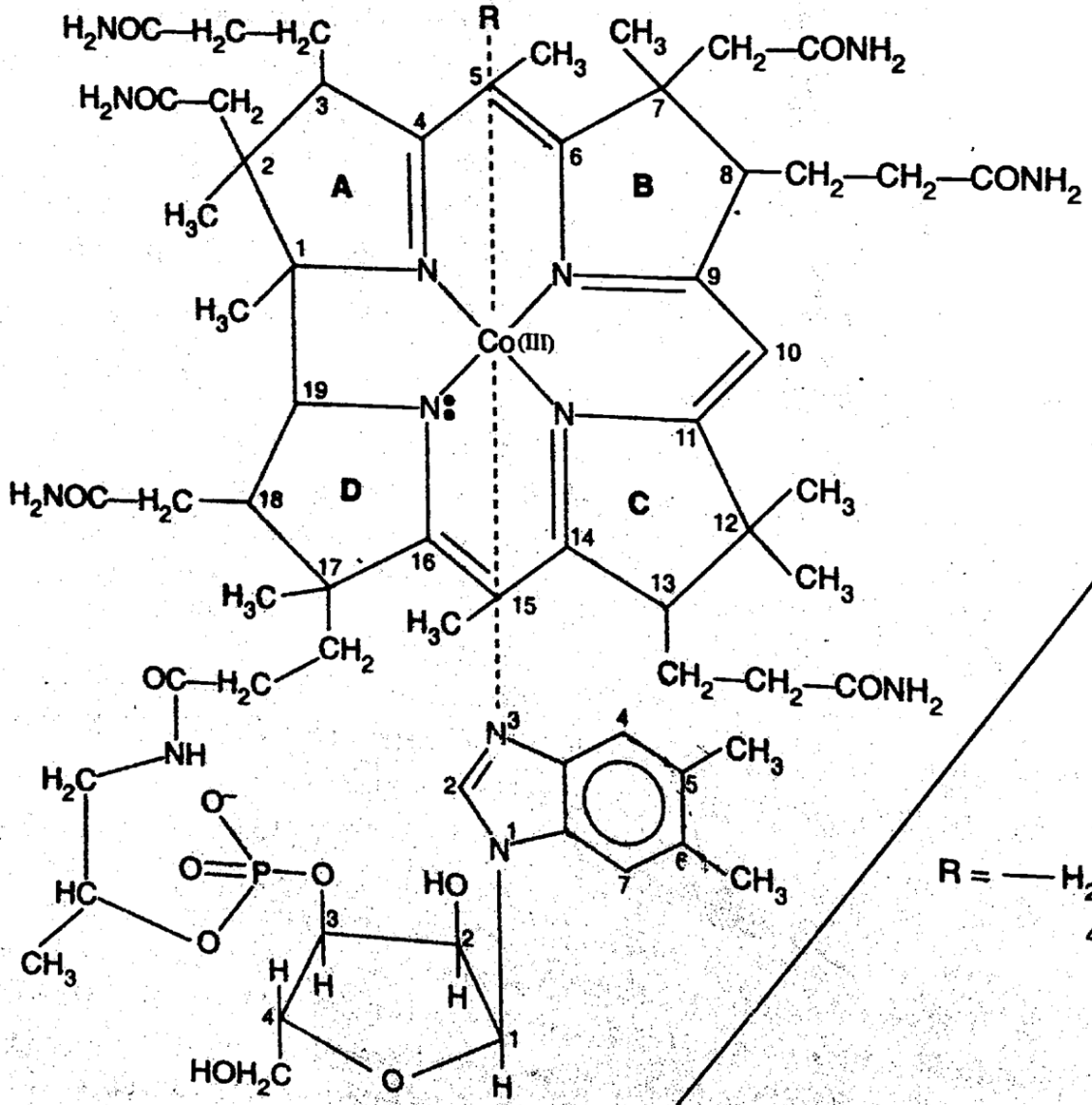


➢ One of the imidazole ring remains uncoordinated and both the imidazole rings coordinated. Cu[(L-His)(D-His)(H_2O)].

➢ The unique structure of [$Cu(His)_2$] Copper Bis-Histidine which has been used for the treatment of "Menkes disease" was determined by Sarkar.

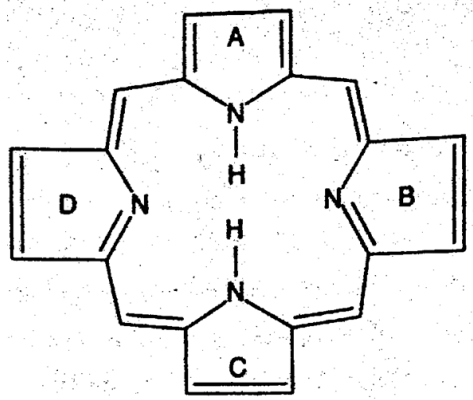
Vitamin B_{12} – Cobalt containing protein

Vitamin B_{12} Anti-pernicious anaemia factor

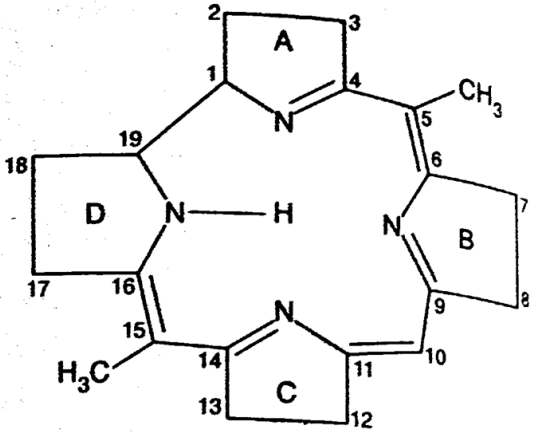


(5′–deoxyadenosyl)

Figure 10.1.1.2: Structural representation of **5′- deoxyadenosylcobalamin.**



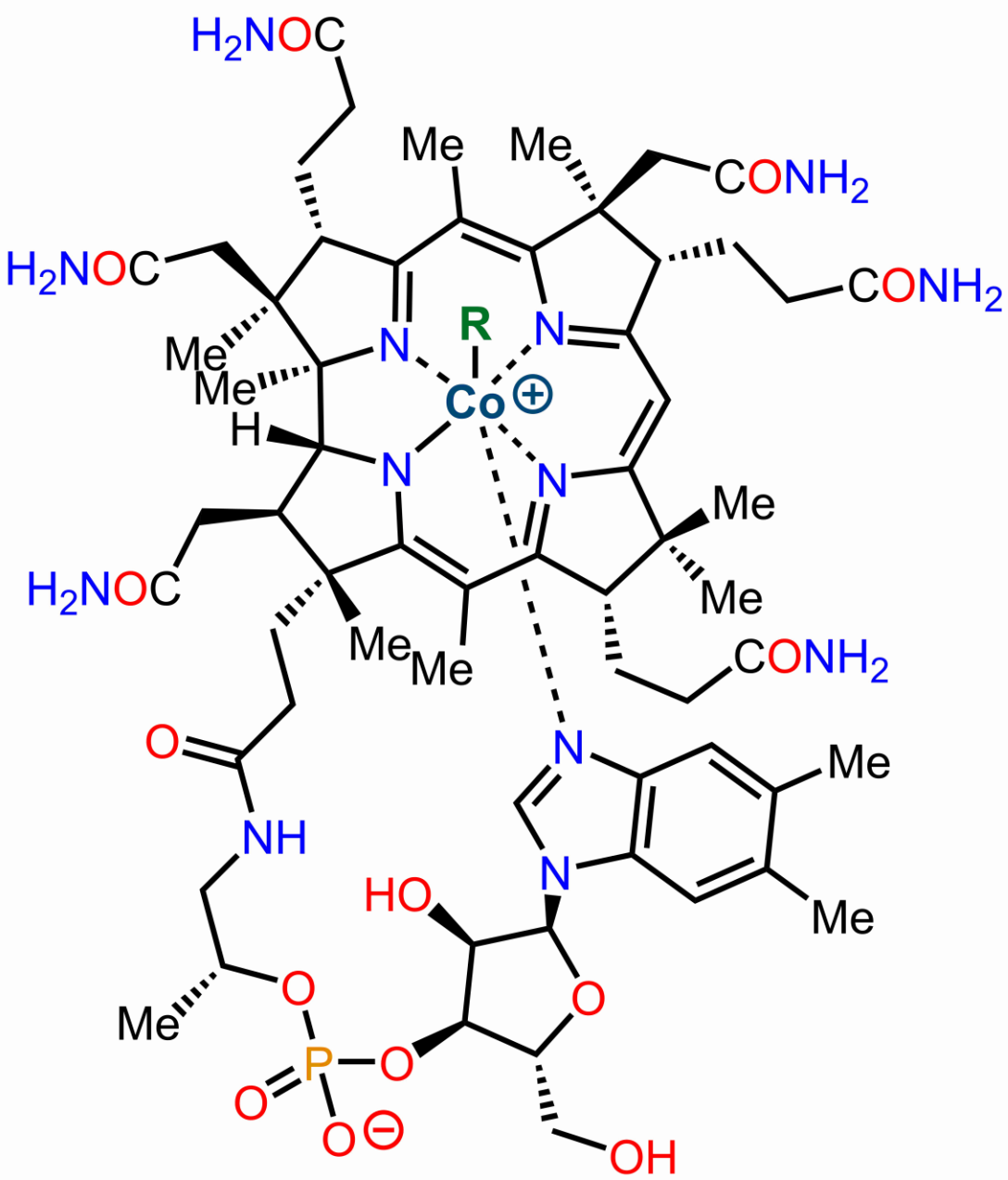
(Porphyrin ring)



(Corrin ring)

Figure 10.1.1.1: *Structural representation of porphyrin and corrin ring.*

Cobalt containing protein: Vitamin B_{12}, B_{12r} and B_{12s}



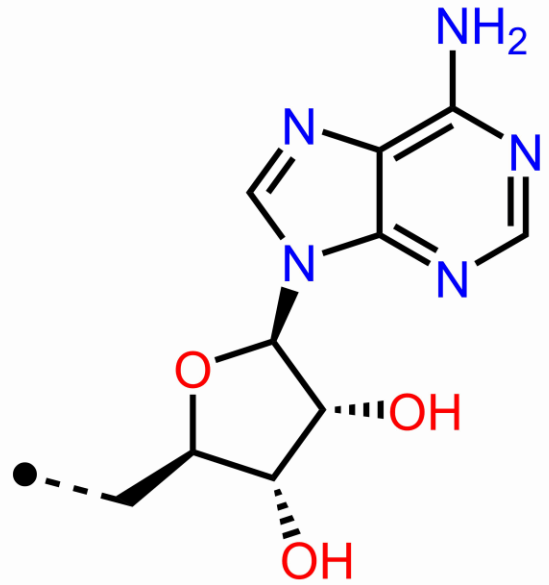
R group:

CN: *cyanocobalamin*

OH: *hydroxocobalamin (vitamin B_{12a})*

Me: *methylcobalamin (MeCbl)*

Ado: *adenosylcobalamin (coenzyme B_{12}, AdoCbl)*



Ado = 5'-deoxyadenosyl

Cobalt containing protein: Vitamin B_{12}, B_{12r} and B_{12s}

Isolation – 1958 by Barker; Structural Elucidation by Crowfoot – Hodgkin in 1961; B12 is cyanocobalamin and adenosyl cobalamin is coenzyme B12.
pernicious anemia (PA) is a disease in which not enough red blood cells are produced due to a deficiency of vitamin B_{12} and the The term "pernicious" means "deadly"

Vit B12 is required for the synthesis of Succinyl coA which in turn needed for the synthesis of porphyrin

Deficiency of vitamin B-12 lead to anemia, fatigue, mania, and depression, while a long term deficiency can cause permanent damage to the brain and central nervous system

cobalt normally exists as Co(III); under reducing conditions, it reduced to Co(II) or even Co(I), usually **denoted as B_{12r} and B_{12s}**, for reduced and super reduced, respectively

B_{12r} (Co^{2+}) and B_{12s} (Co^{+}) can be prepared from cyanocobalamin by controlled potential reduction, or chemical reduction using **sodium borohydride in alkaline solution**, **zinc in acetic acid**, or by the action of thiols. In biological system done by FAD.

Both B_{12r} and B_{12s} are stable indefinitely under oxygen-free conditions. B_{12r} appears orange-brown in solution, while B_{12s} appears bluish-green under natural daylight, and purple under artificial light

10.1.6 Special Characteristics of B_{12} Coenzyme

The special properties of cobalamins have been already discussed in Sec. 10.1.3. Here the characteristic properties of B_{12} coenzyme are summarised below to understand *why nature has selected cobalt for vitamin* B_{12}.

(i) Labilisation of 'Co—C' bond is greatly influenced by the protein chain. The *trans*-axial ligand also influences the Co—C bond strength.

(ii) The cobalt centre can have the oxidation states, +1 (d^8) or +2 (d^7) or +3 (d^6). Cob(III)alamin can easily dissociate (*effect of the strain in corrin ring, cf.* Sec. 10.1.3) the weakly bound sixth ligand to generate the 5-coordinate intermediate which can act as a **good electrophile**. In the +1 state (*i.e.* B_{12s}), it acts as a *strong nucleophile*. The 6-coordinate Co(III) (d^6) can experience **reductive elimination** while the 4-coordinate B_{12s} can experience **oxidative addition**. In fact, d^8 (16*e*) system is the ideal centre for *oxidative addition* and d^6 (18*e*) is the ideal centre for *reductive elimination*.

(iii) The flexibility of the corrin ring allows it to attain different conformations. This change in conformation can change the lability of the Co—C bond.

(iv) It may be noted that cobalt-porphyrin analogues of B_{12} cannot be reduced to Co(I) in aqueous solution. But the presence of corrin ring in cobalamin makes the process possible.

(v) From methylcobalamine, the methyl group can be released as $^{+}CH_3$ or $^{\bullet}CH_3$ or $^{-}CH_3$ depending upon the situation.

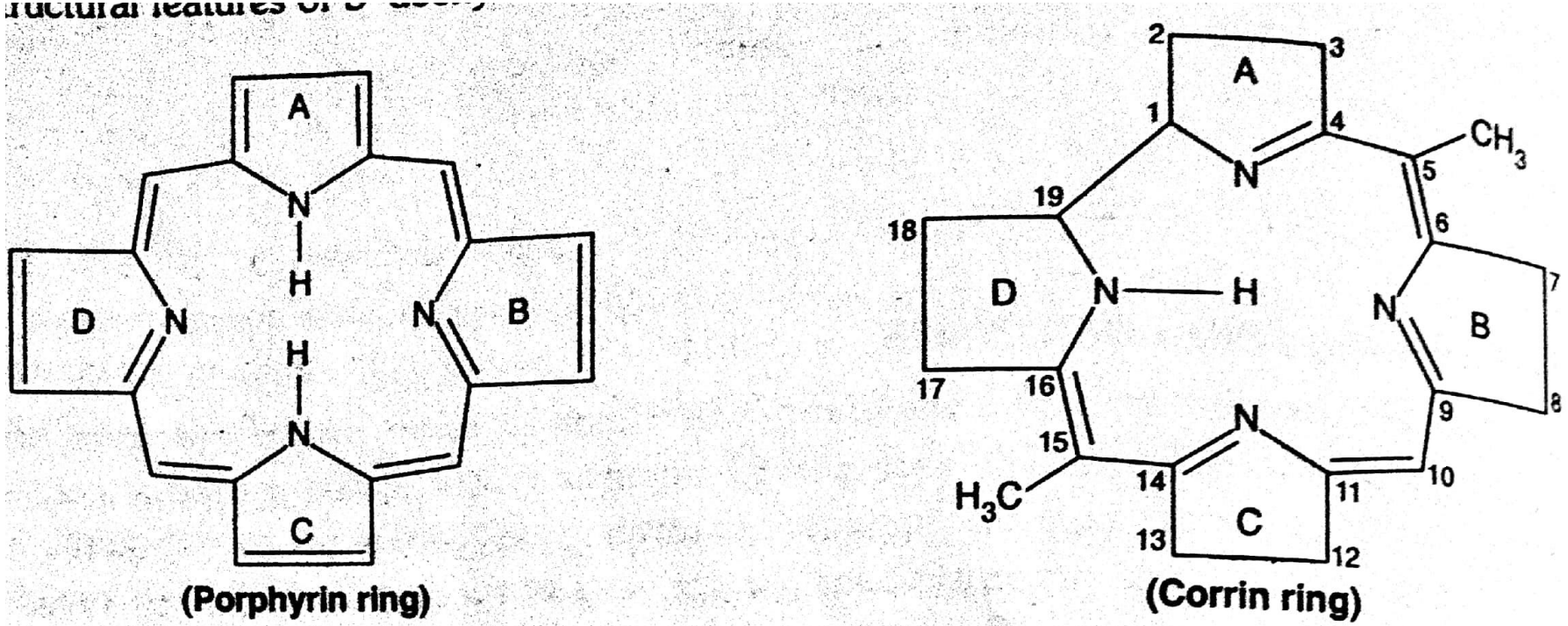


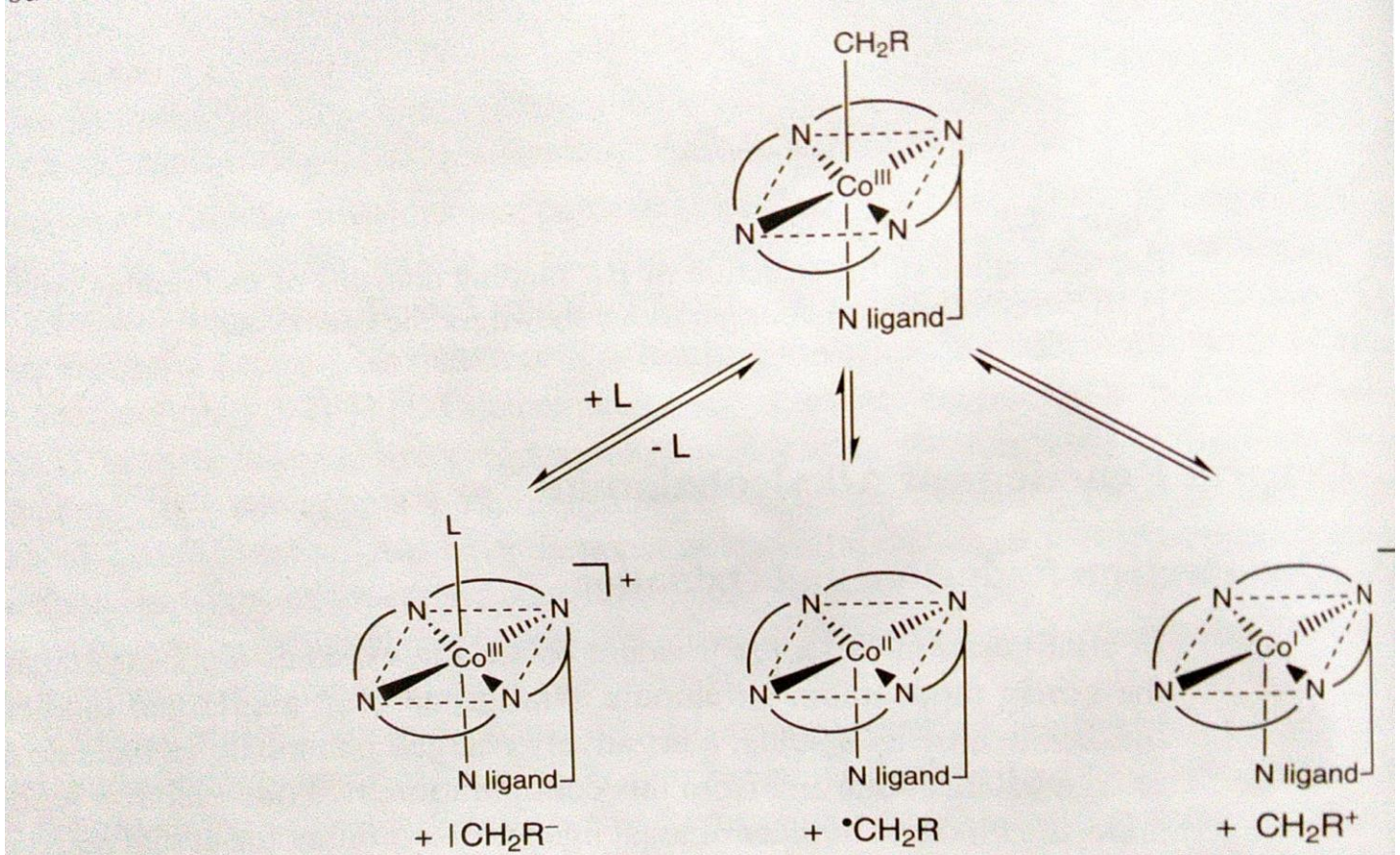
***Figure 10.1.1.1**: Structural representation of porphyrin and corrin ring.*

$Co\text{-}CR_3 \longrightarrow Co(III) + {}^{-}CR_3$ heterlytic cleavage, carbanion

$Co\text{-}CR_3 \longrightarrow Co(II)^{\bullet} + {}^{\bullet}CR_3$ homolytic cleavage, free radical

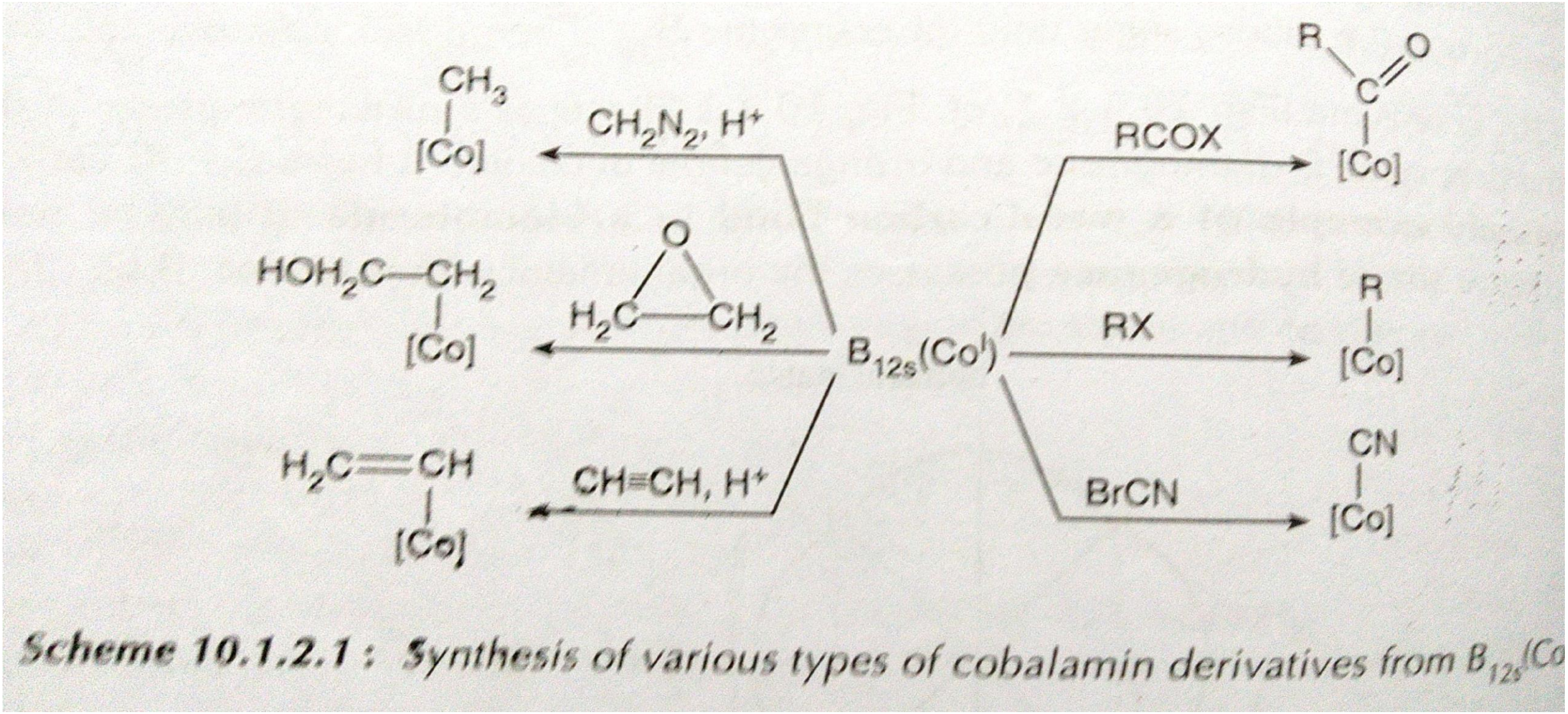
$Co\text{-}CR_3 \longrightarrow Co(I) + {}^{-}CR_3$ heterolytic cleavage, carbocation

Co-C bond Cleavage



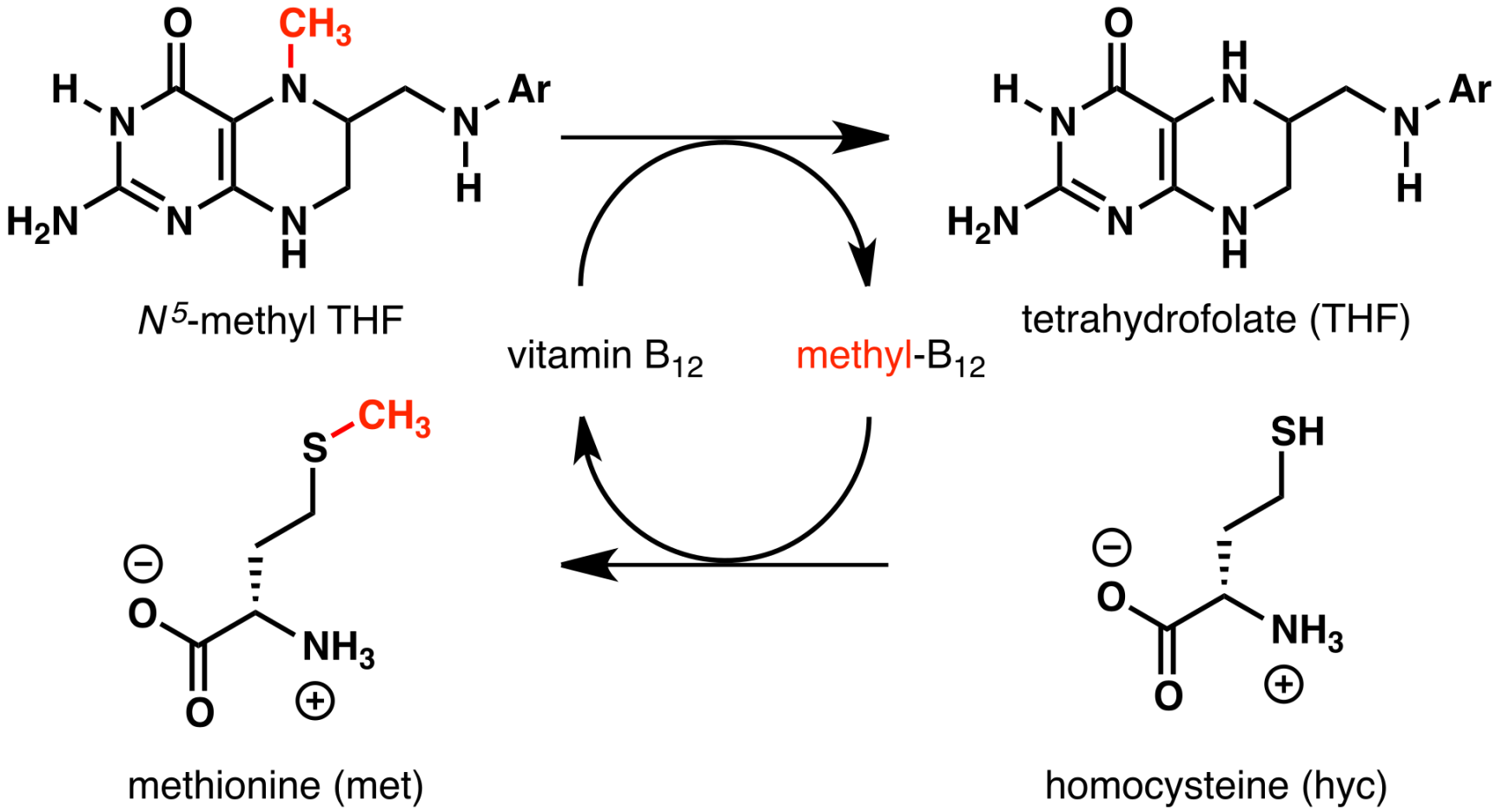
type of reaction:	heterolysis	homolysis	heterolysis
metal configuration in the product	d^6 low-spin, stable, inert	d^7 low-spin, 1 unpaired electron $(d_{z^2})^1$	d^8, "super-nucleophilic" $(d_{z^2})^2$
alkyl ligand, eliminated as:	"carbanion", nucleophilic	primary alkyl radical, very reactive	"carbocation", electrophilic
app. electrochemical potential equivalent[a]:	> 0 V	0 to -0.4 V	< -0.9 V

Reactions of Co(I) $B12_s$



Scheme 10.1.2.1 : Synthesis of various types of cobalamin derivatives from B_{12s}(Co

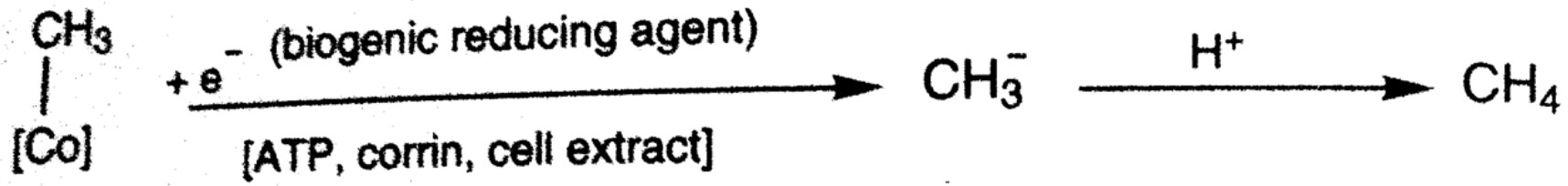
Alkylation Reactions of vit-B12



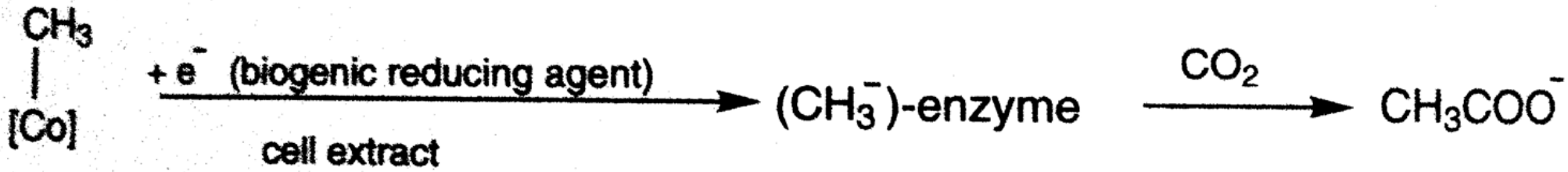
This reaction is catalyzed by the **enzyme methionine synthase with B_{12} as an essential cofactor**
During B_{12} deficiency, this reaction cannot proceed, **which leads to the accumulation of 5-methyltetrahydrofolate**.

Alkylation Reactions of vit-B12

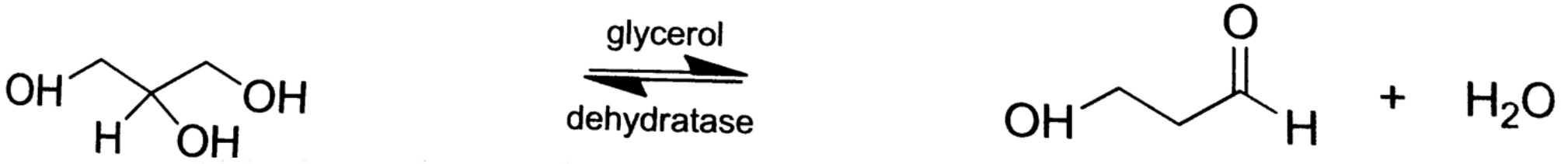
(b) Synthesis of methane



(c) Synthesis of acetate

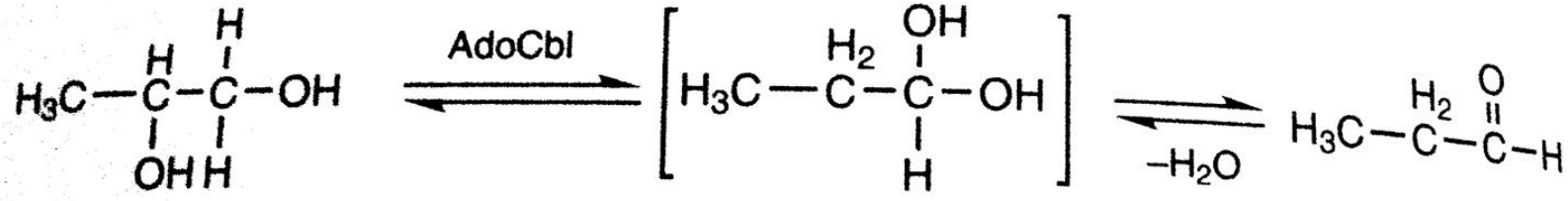


Isomerisation Reactions



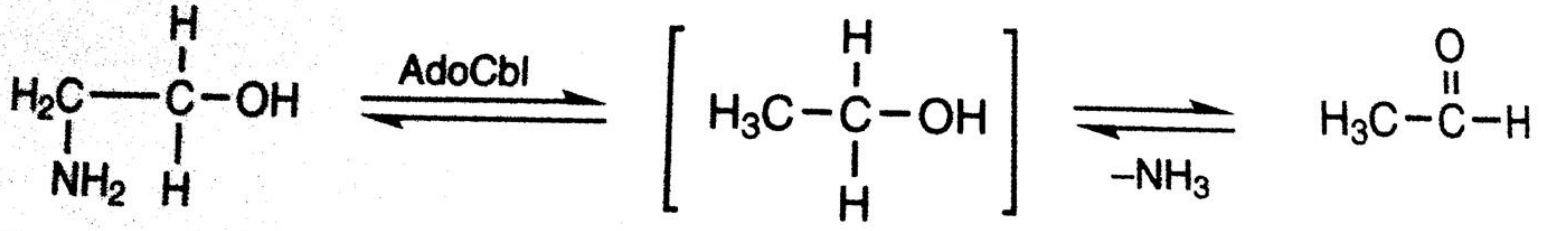
(ii) *Reactions which require AdoCbl*

(d) *Diol dehydrase*



diol dehydrase

(e) Ethanolamine ammonia lyase

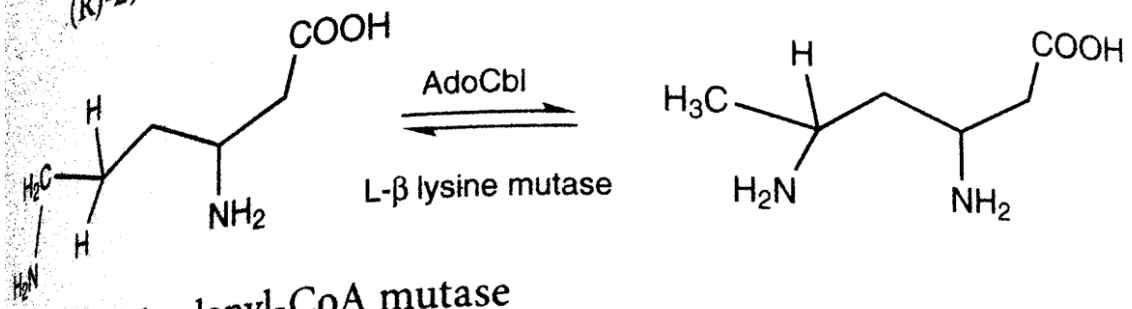


ethanolamine ammonia lyase

Isomerisation Reactions

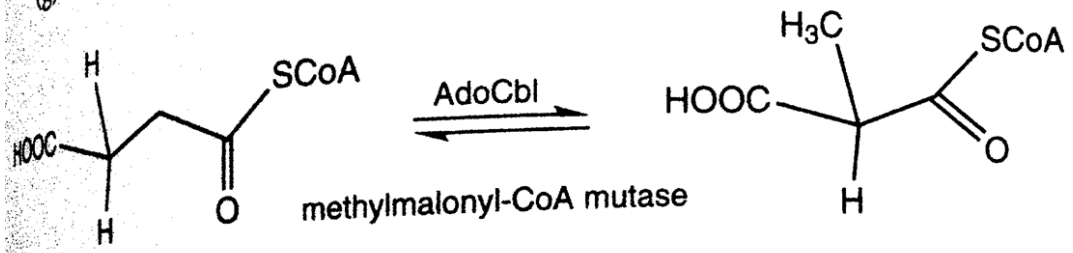
Several mutases in which hydrogen and another group on an adjacent carbon exchange places are shown below.

(f) Aminomutase utilising (S)-3,6-diaminohexonoate, (R)-2,6-diaminohexonoate, (R)-2,5-diaminopentanoate or α and β-leucine.



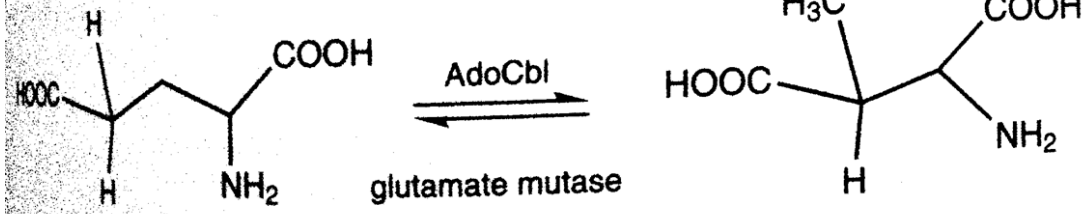
(20.6)

(g) Methylmalonyl-CoA mutase

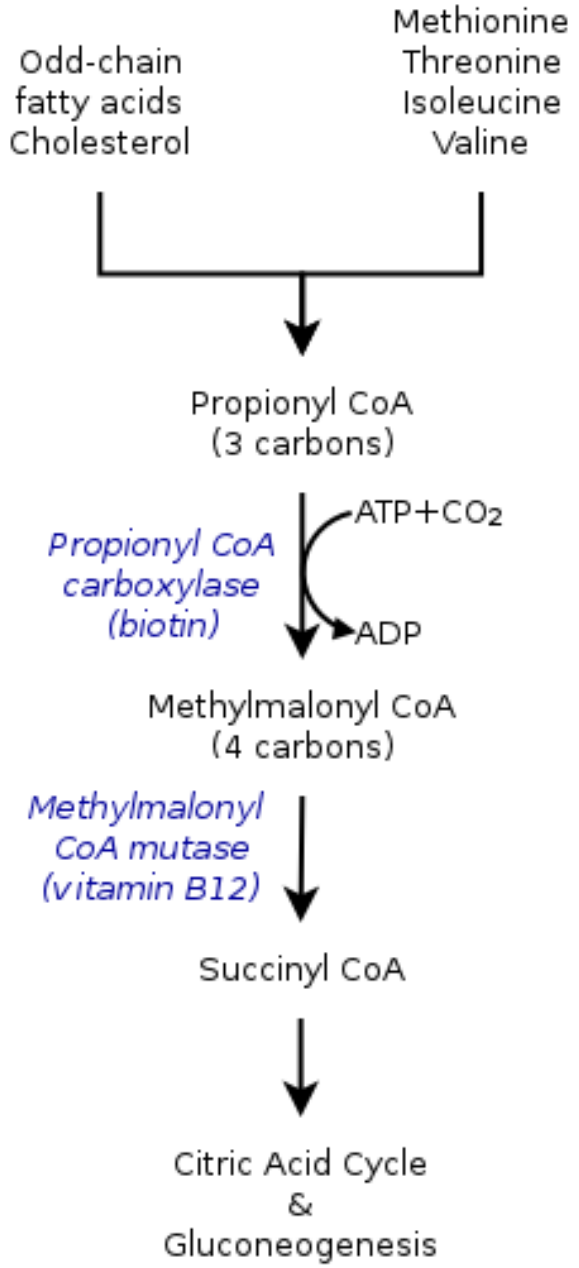


(20.7)

(h) Glutamate mutase

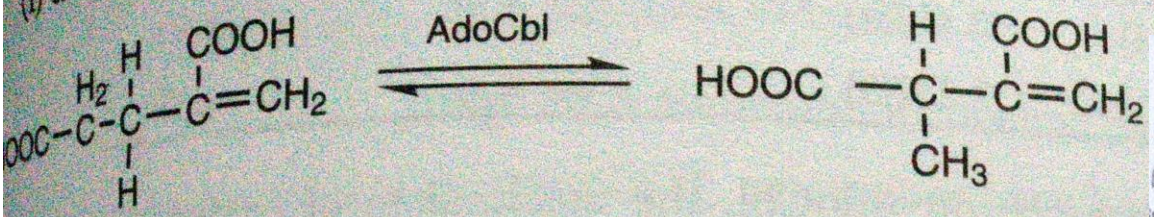


(20.8)



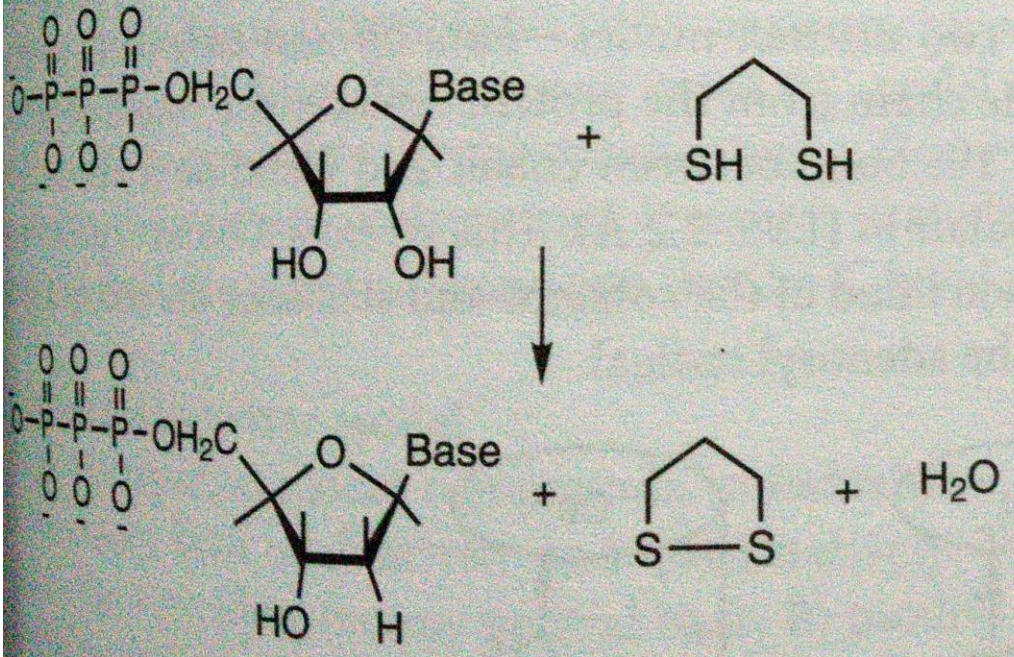
Mechanism of Isomerisation Reactions

(i) α-Methyleneglutarate mutase



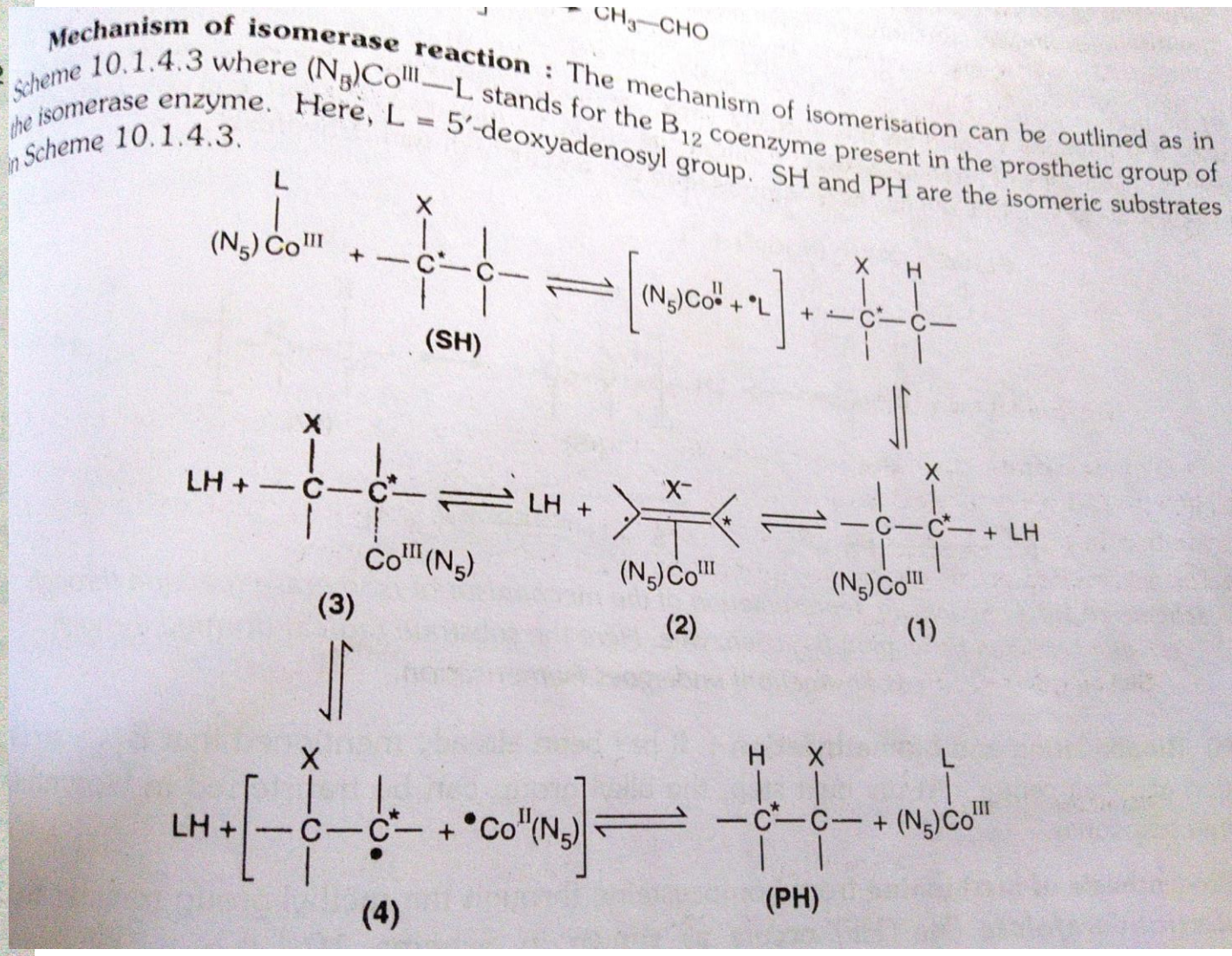
(j) Ribonucleotide reductase

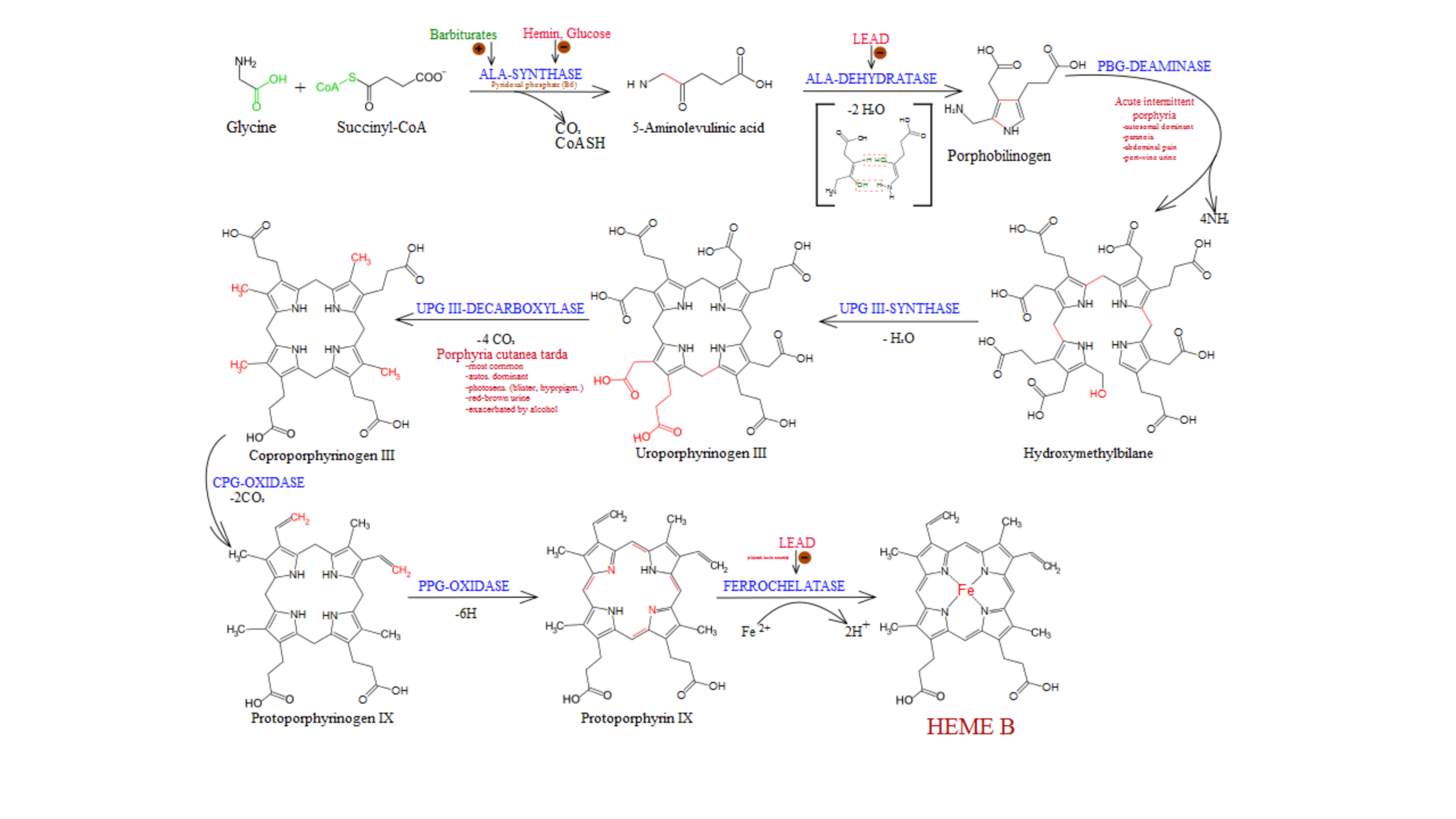
Here ribose is reduced to deoxyribose.



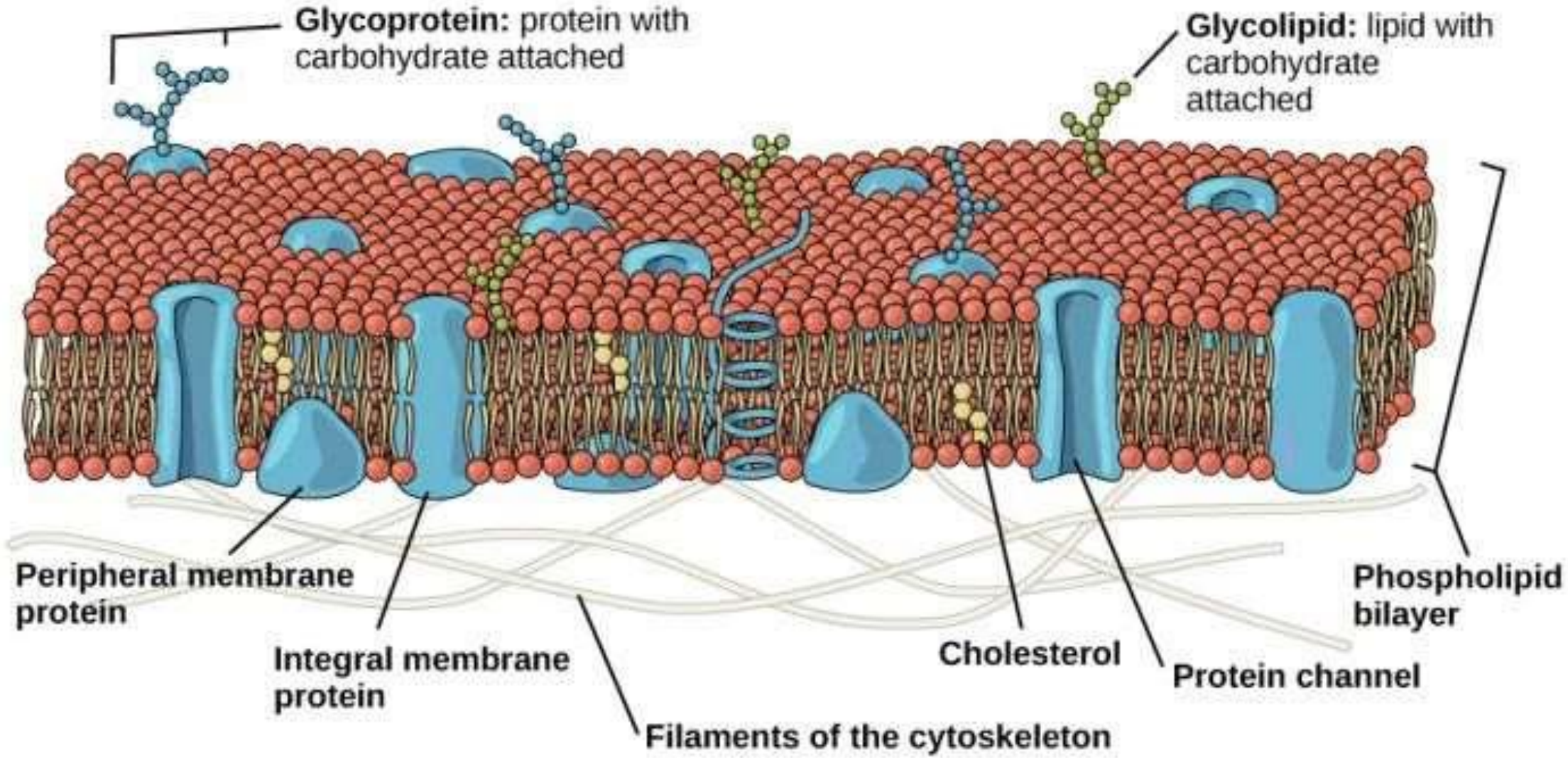
ribonucleotide reductase

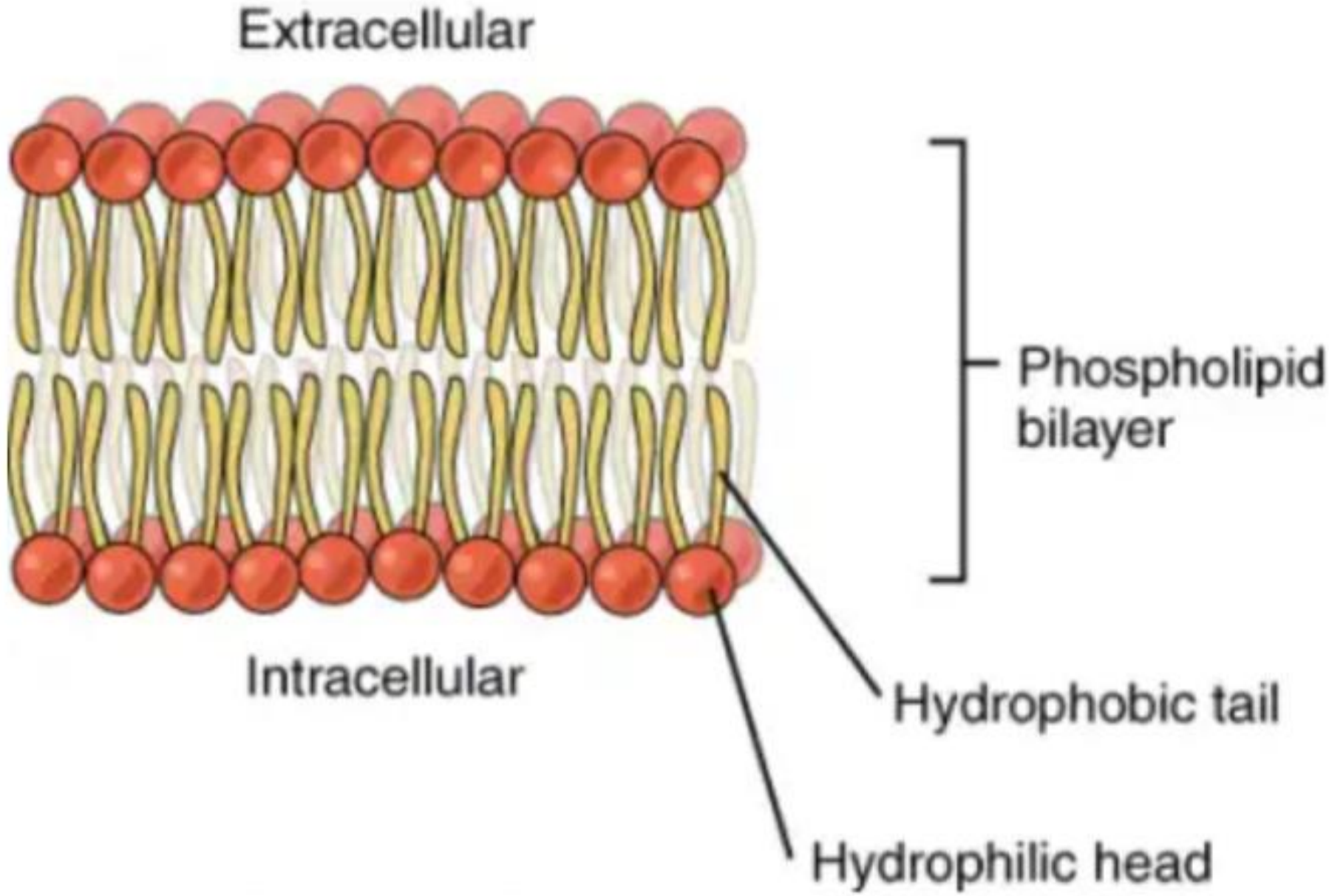
Mechanism of isomerase reaction : The mechanism of isomerisation can be outlined as in Scheme 10.1.4.3 where $(N_5)Co^{III}$—L stands for the B_{12} coenzyme present in the prosthetic group of the isomerase enzyme. Here, L = 5′-deoxyadenosyl group. SH and PH are the isomeric substrates in Scheme 10.1.4.3.




Barbiturates
Hemin, Glucose
ALA-SYNTHASE
Pyridoxal phosphate (B6)
Glycine
Succinyl-CoA
CO₂
CoASH
5-Aminolevulinic acid
LEAD
ALA-DEHYDRATASE
-2 H₂O
Porphobilinogen
PBG-DEAMINASE
Acute intermittent porphyria
-autosomal dominant
-psychosis
-abdominal pain
-port-wine urine
4NH₃
Hydroxymethylbilane
UPG III-SYNTHASE
- H₂O
Uroporphyrinogen III
UPG III-DECARBOXYLASE
-4 CO₂
Porphyria cutanea tarda
-most common
-autos. dominant
-photosens. (blister, hyperpigm.)
-red-brown urine
-exacerbated by alcohol
Coproporphyrinogen III
CPG-OXIDASE
-2CO₂
Protoporphyrinogen IX
PPG-OXIDASE
-6H
Protoporphyrin IX
LEAD
FERROCHELATASE
Fe 2+
2H+
HEME B

Ionophores


Glycoprotein: protein with carbohydrate attached
Glycolipid: lipid with carbohydrate attached
Peripheral membrane protein
Integral membrane protein
Filaments of the cytoskeleton
Cholesterol
Protein channel
Phospholipid bilayer


Extracellular
Intracellular
Phospholipid bilayer
Hydrophobic tail
Hydrophilic head

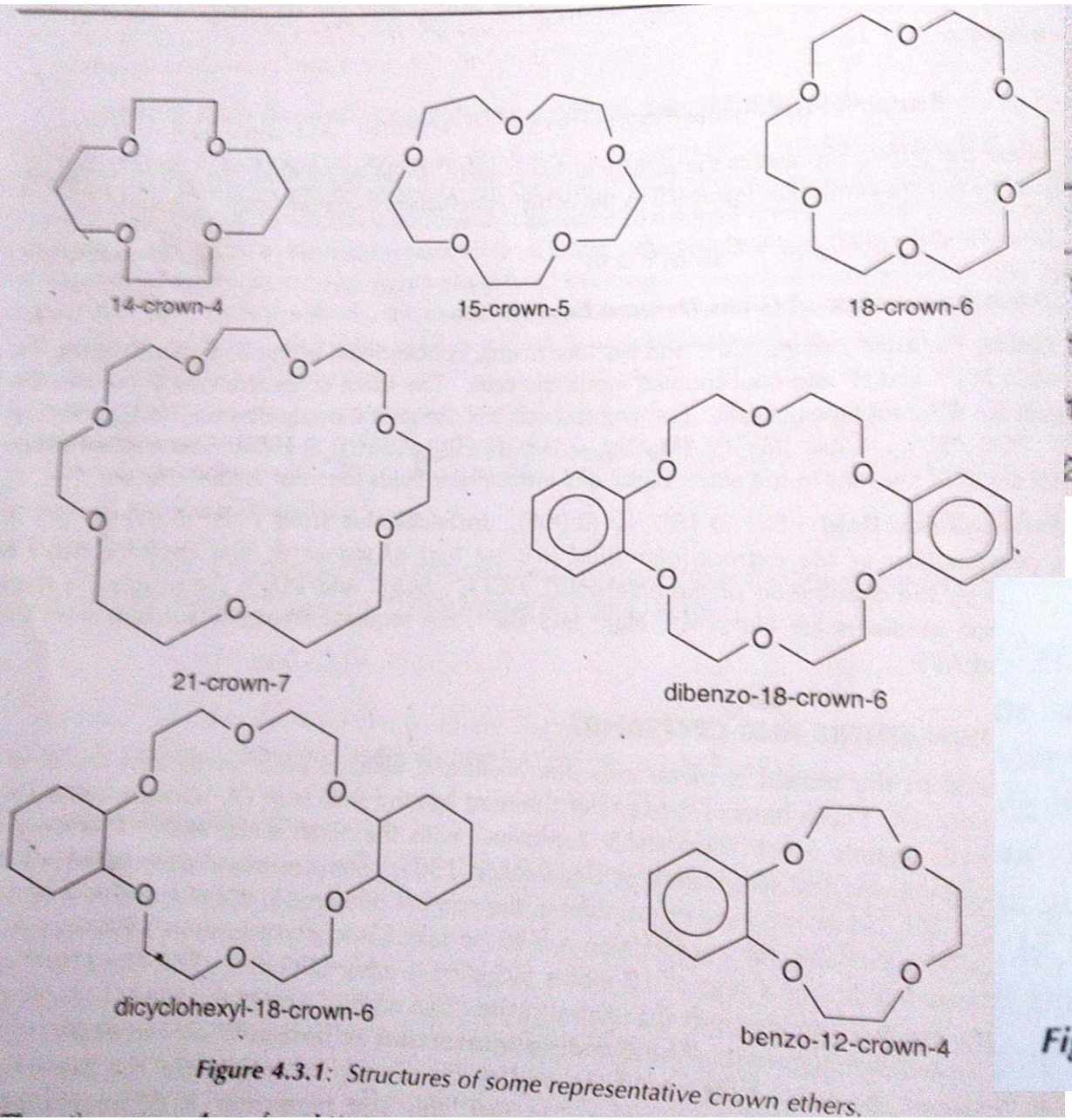


Figure 4.3.1: Structures of some representative crown ethers.

Table 4.3.1

Diameters (in pm) of crown ether cavity and metal ions.

Crown ether	Metal ions
14-crown-4 (120-150)	Li^+ (140), Na^+ (190), Ca^{2+} (200)
15-crown-5 (180-220)	K^+ (270), Ba^{2+} (270), Rb^+ (300)
18-crown-6 (260-320)	Cs^+ (330)
21-crown-7 (340-430)	

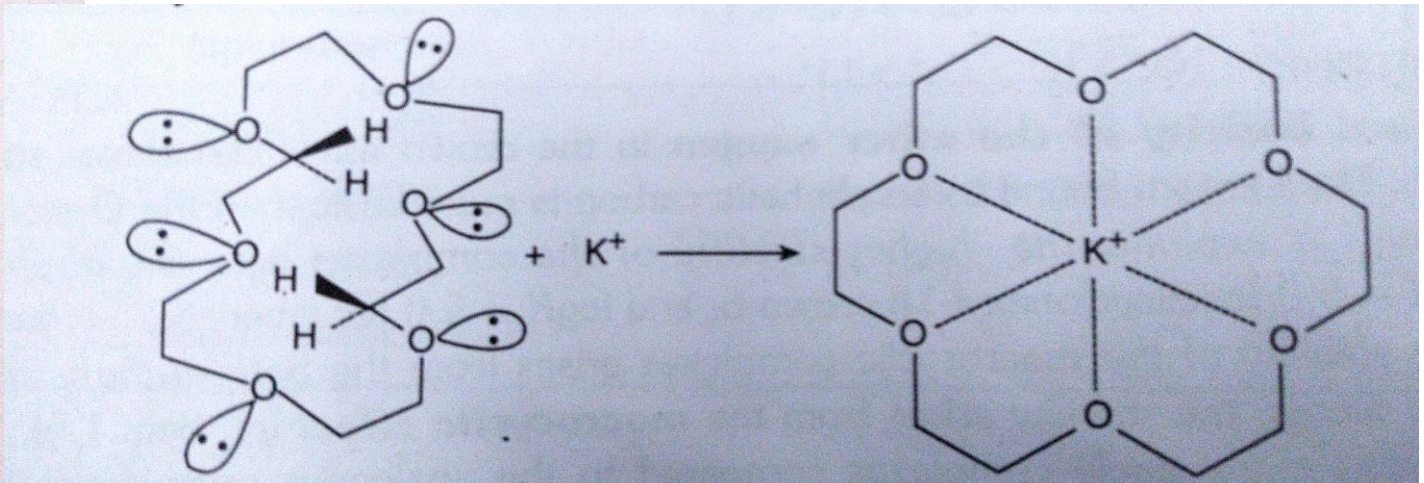


Figure 4.3.2 : Change of conformation of 18-crown-6 during complexation

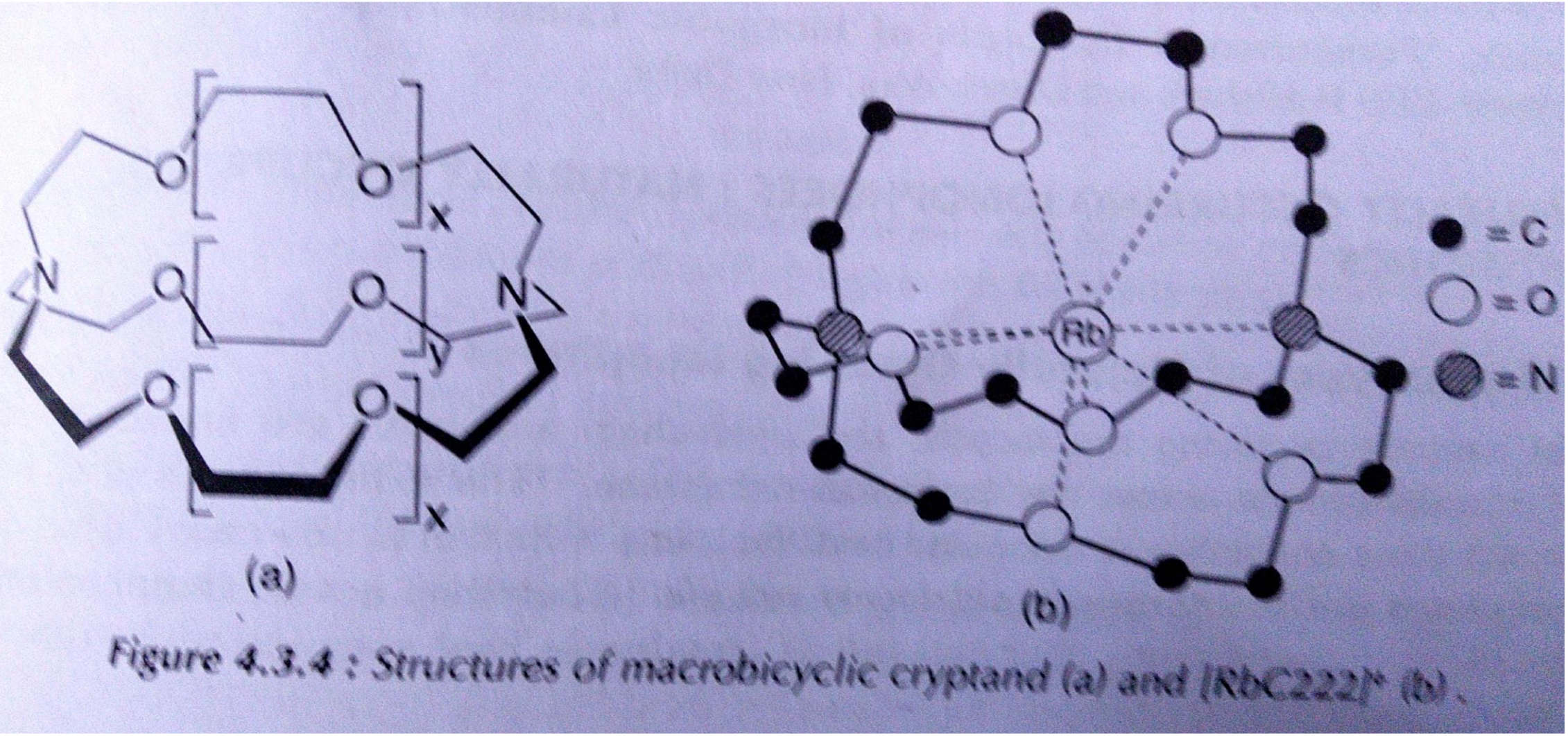
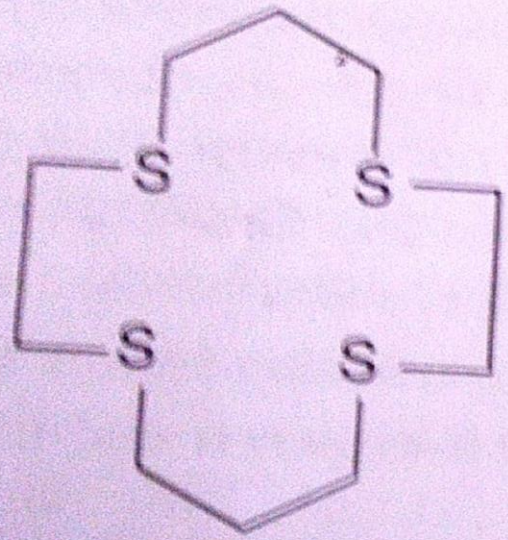
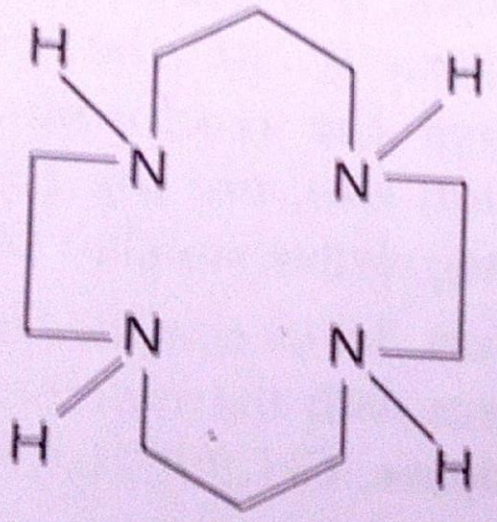


Figure 4.3.4 : Structures of macrobicyclic cryptand (a) and $[RbC222]^+$ (b) .



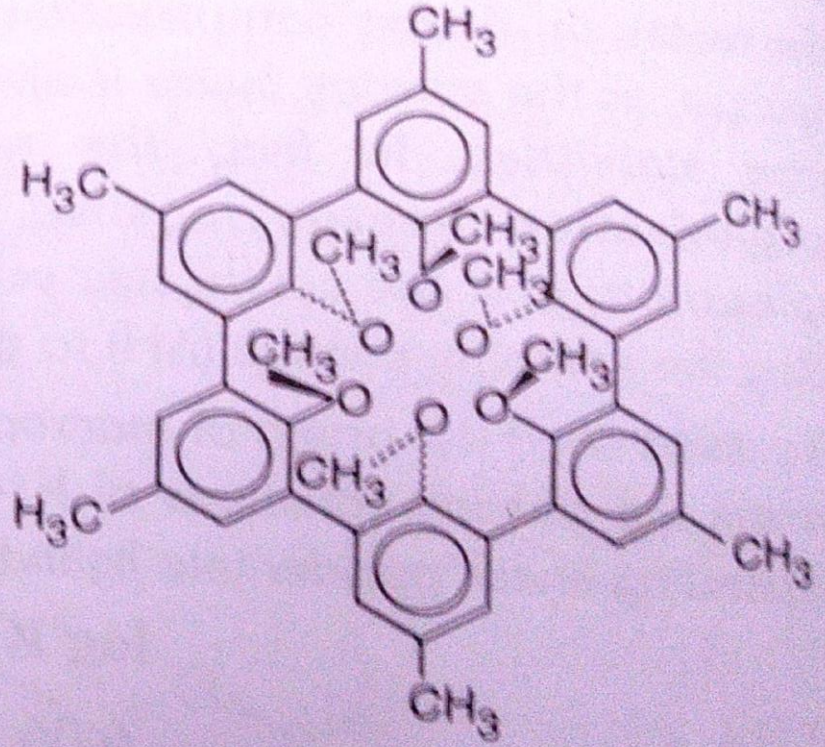
Thiocrown

(a)



Cyclam

(b)



Spherand (methoxytolune based)

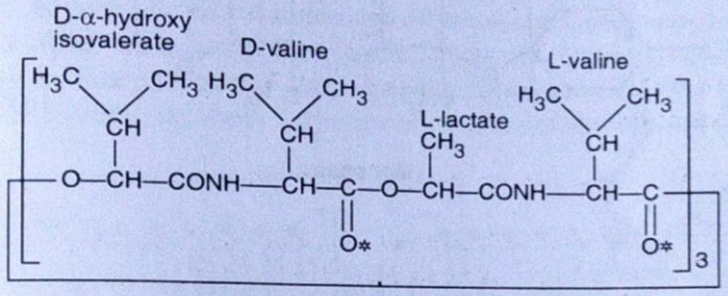
(c)

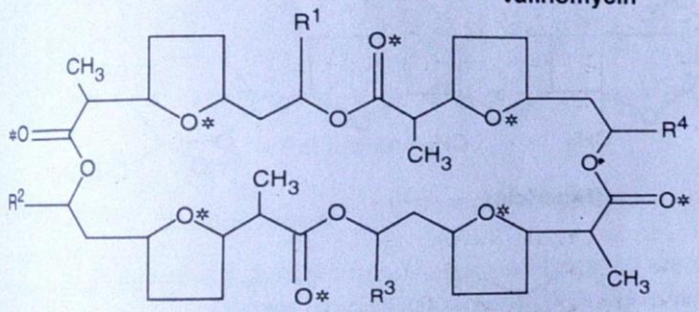
Figure 4.3.3 : *Structures of (a) thiocrown; (b) cyclam and (c) spherand, a typical example. (a) 1,4,8,11-tetrathiacyclotetradecane, (b) 1,4,8,11-tetraazacyclotetradecane*

Based on their structural properties, they are classified into two classes.

(i) **Class I :** These are *cyclic ionophores* (Fig. 4.4.1.1) and are neutral at physiological *pH*. Thus their metal complexes are cationic in nature. This group is subdivided into **macrotetrolides** (*e.g.* **actins** like nonactin, monactin, dinactin, trinactin, tetranactin) and **cyclodepsipeptides** (condensation products of amino acids and carboxylic acids, *e.g.* valinomycin, enniatin A, enniatin B)

(ii) **Class II :** These are *open-chain* carboxylic acid ionophores (Fig. 4.4.1.2). These can cyclize through hydrogen bonding. They form neutral complexes (*e.g.* $RCO_2^-Na^+$) after deprotonation of the $-CO_2H$ group. The examples are : monensin, nigericin, dianemycin.





$R^1 = R^2 = R^3 = R^4 = CH_3$, Nonactin,
$R^1 = R^2 = R^3 = CH_3$ and $R^4 = C_2H_5$, Monactin
$R^1 = R^3 = CH_3$ and $R^2 = R^4 = C_2H_5$, Dinactin
$R^1 = CH_3$ and $R^2 = R^3 = R^4 = C_2H_5$, Trinactin
$R^1 = R^2 = R^3 = R^4 = C_2H_5$, Tetranactin

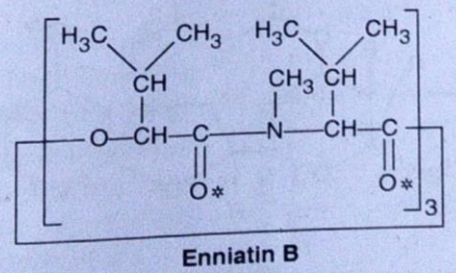
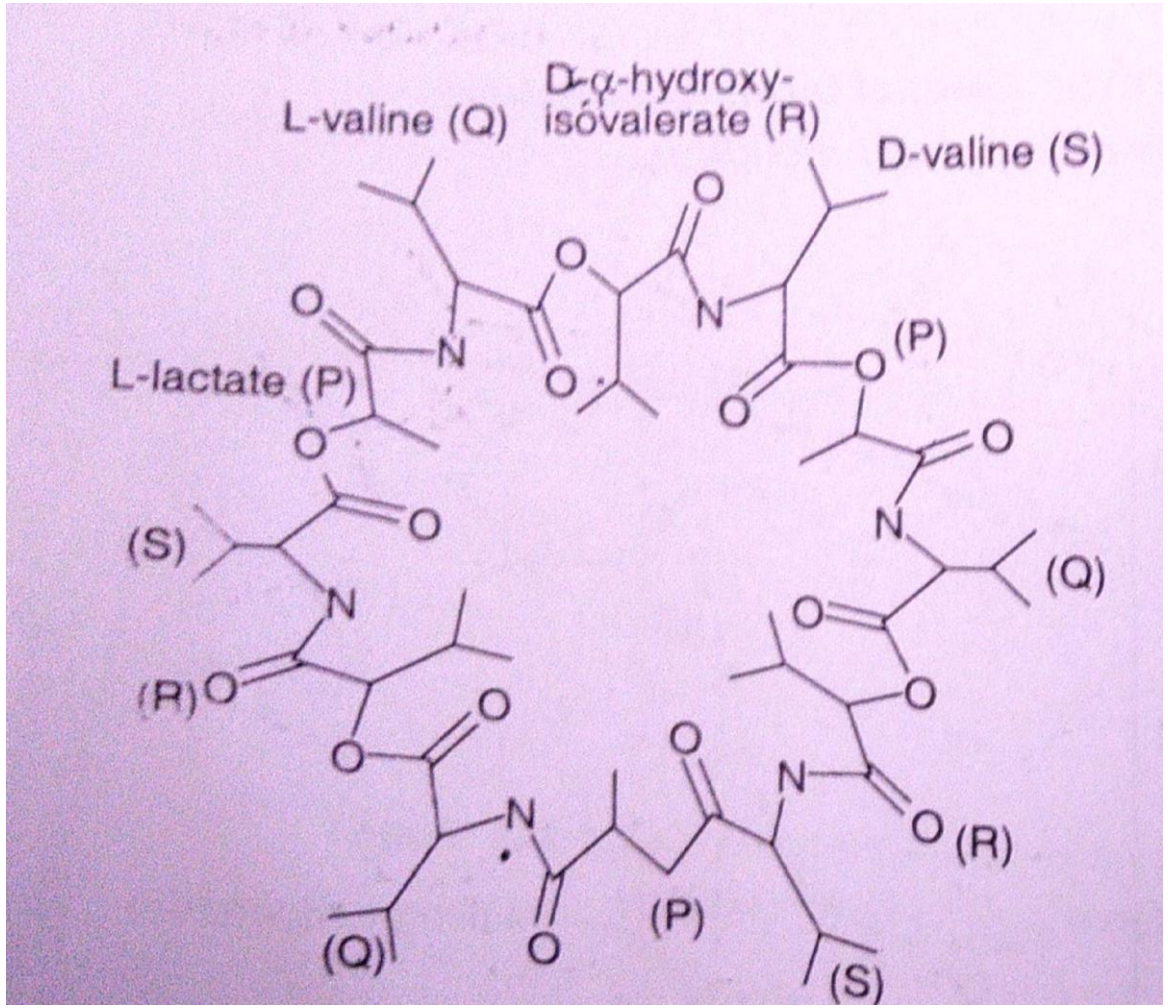


Figure 4.4.1.1: Structures of some representative naturally occurring cyclic ionophores, i.e. antibiotics of Class I. (Represents the donor sites).*



Valinomycin – binds K^+ more tightly than Na^+

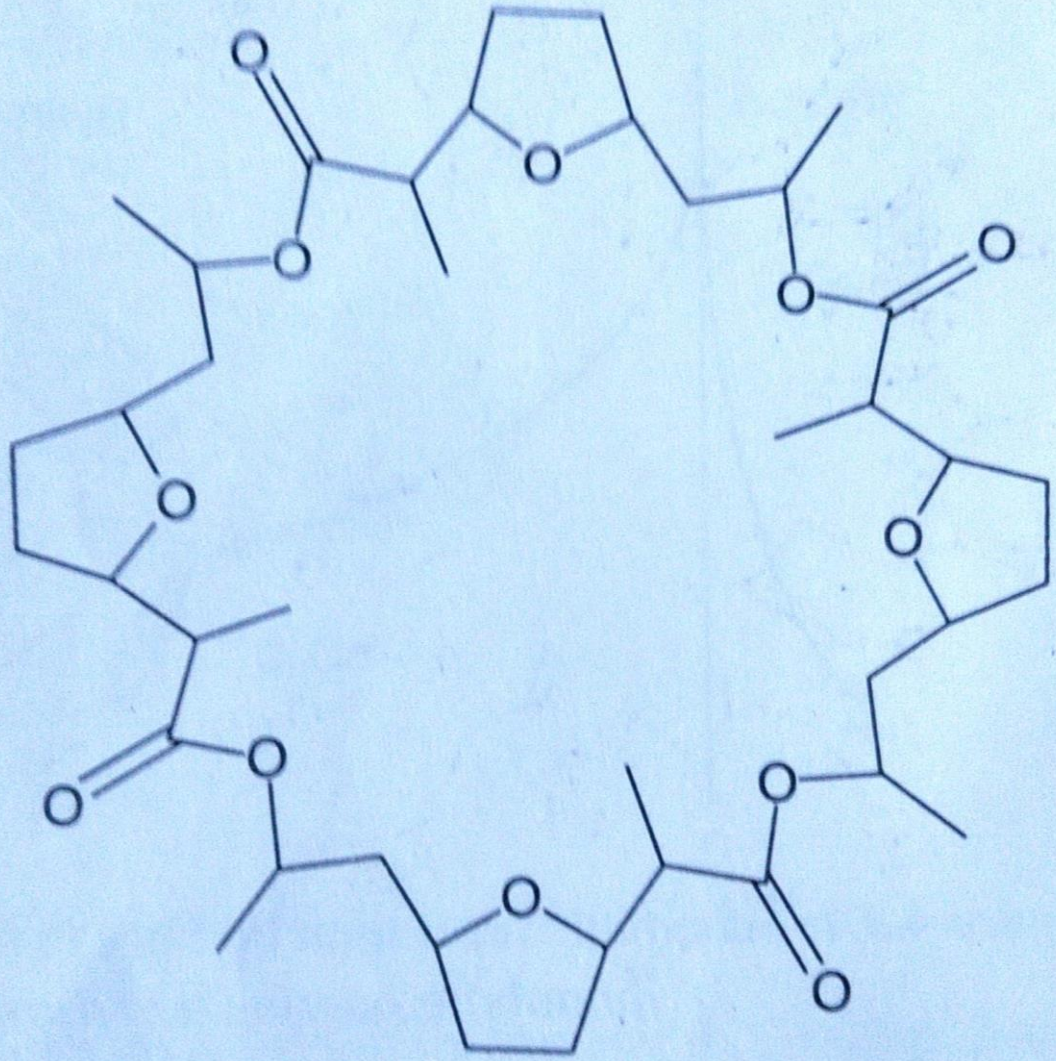
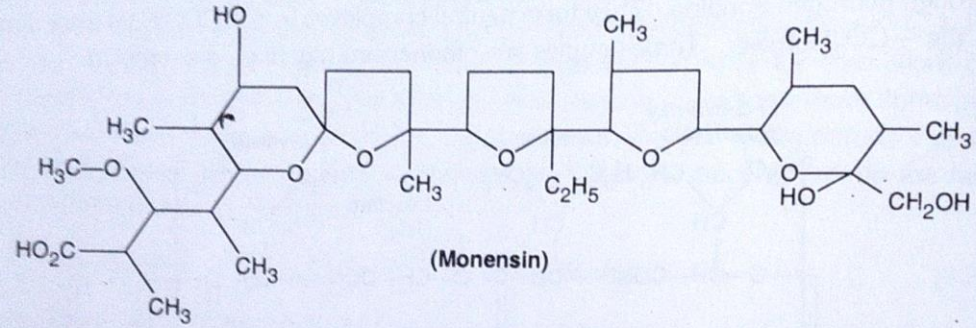


Figure 4.4.4.1: *Structural representation of the macrocyclic ionophore, nonactin (cf. Fig. 4.4.1.1).*

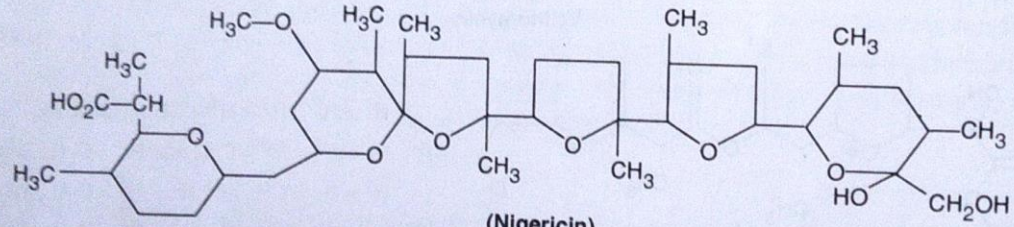
Selectivity sequence of the naturally occurring antibiotics is shown below.

Valinomycin: $Rb^+ > K^+ > Cs^+ > Na^+$; *Actins:* $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$; *Nigericin:* $K^+ > Rb^+ > Na^+ > Li^+$; *Monensin* : $Na^+ > K^+ > Rb^+$; *Dianemycin:* Na^+, K^+, $Rb^+ > Li^+$.

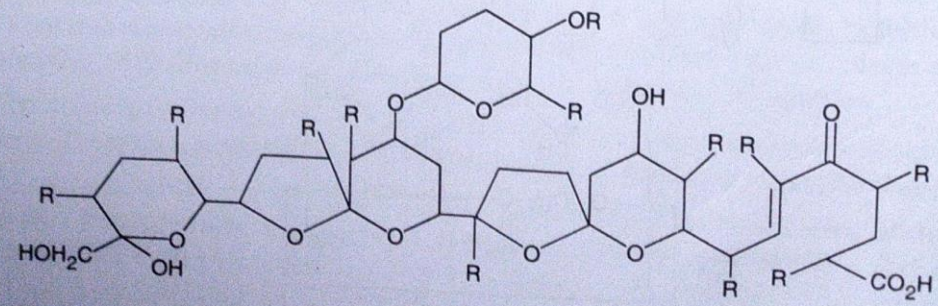
Valinomycin and *nonactin* are very much selective towards K^+ compard to Na^+. In fact, based on this property, these antibiotics have been used in the development of *metal selective membrane electrodes* which may be used to estimate K^+ in the presence of Na^+. On the other hand, the antibiotic *actinomycin* binds Na^+ preferably compared to K^+.



(Monensin)



(Nigericin)



(Dianemycin) (R = CH_3)

Figure 4.4.1.2: *Structures of some representative open-chain naturally occurring carboxylic acid ionophores, i.e. antibiotics of Class II.*

Mechanism of Channel forming (class II) and Carrier (Class I) Ionophore

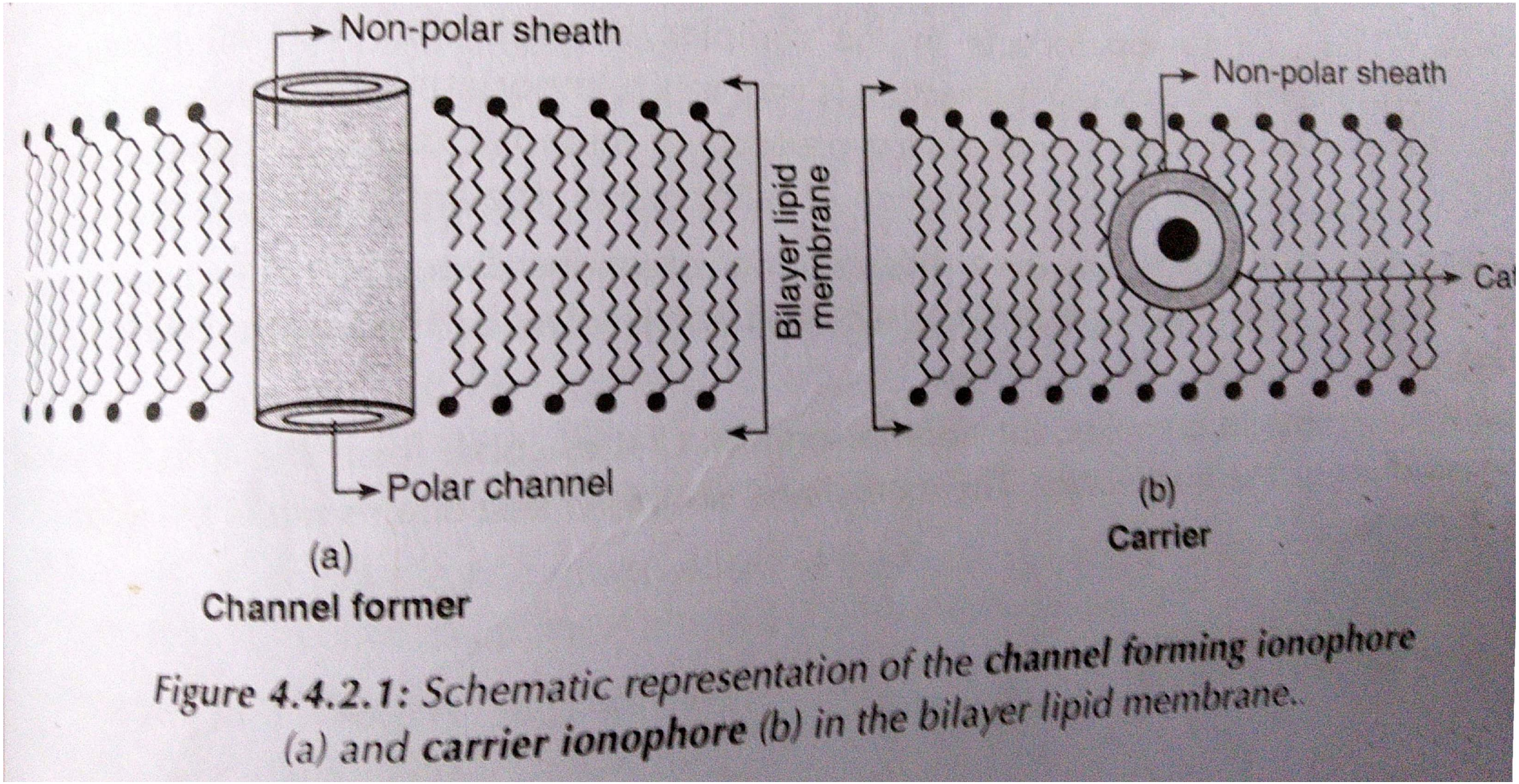


*Figure 4.4.2.1: Schematic representation of the **channel forming ionophore** (a) and **carrier ionophore** (b) in the bilayer lipid membrane.*

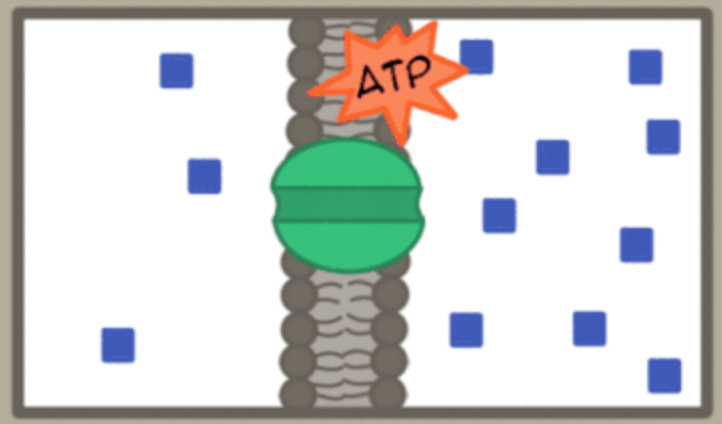
Na/K Transfer across the membarane – Na/K ATPase

- Active – against concentration gradient; and passive transport – Higher end to lower end – spontaneous process –ve deltaG
- 0.6 mmol of electrons/sec = 60 ampere of current in resting human
- Extracellular fluid: Na (0.15) and K (0.005) - Ca and Na in mol/dm^3
- Intracellular fluid: Na (0.01) and K (0.15) – Mg and K mol/dm^3
- $Na_{out}/Na_{in} = 15$; $Ca_{out}/Ca_{in} = 1000$; $K_{in}/K_{out} = 25$ and $Mg_{in}/Mg_{out} = 100$

CELL TRANSPORT @AmoebaSisters

Requires Energy

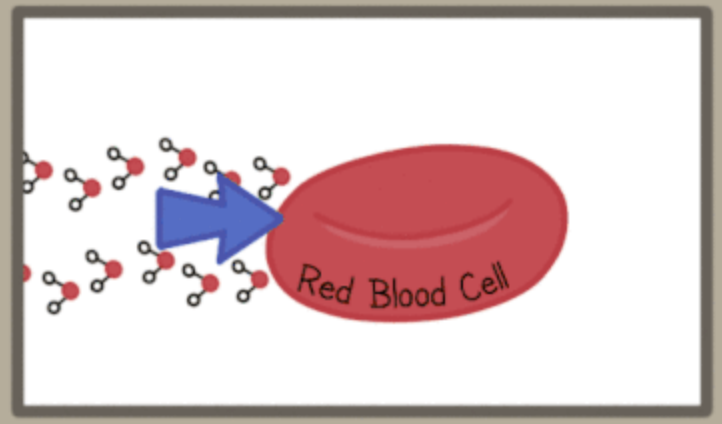
Active Transport



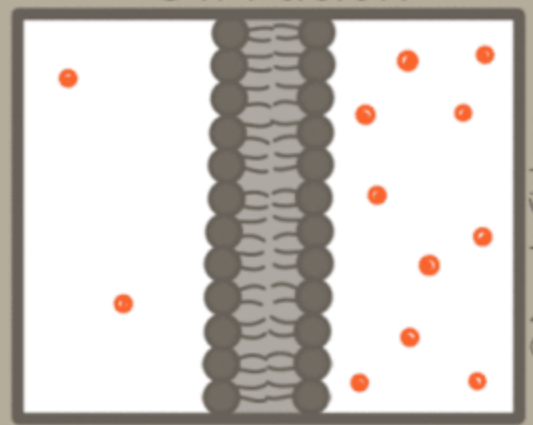
Bulk Transport (ex: Endocytosis)

Does Not Require Energy
(Passive Transport)

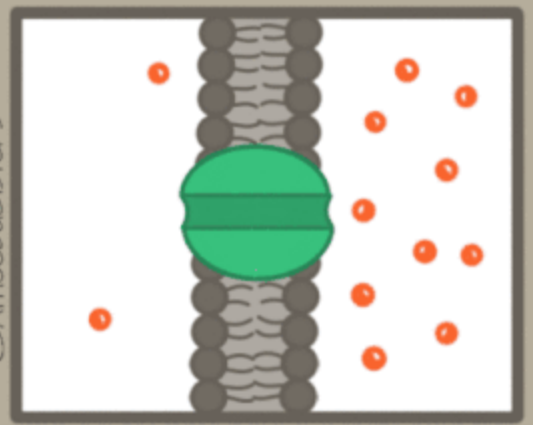
Osmosis



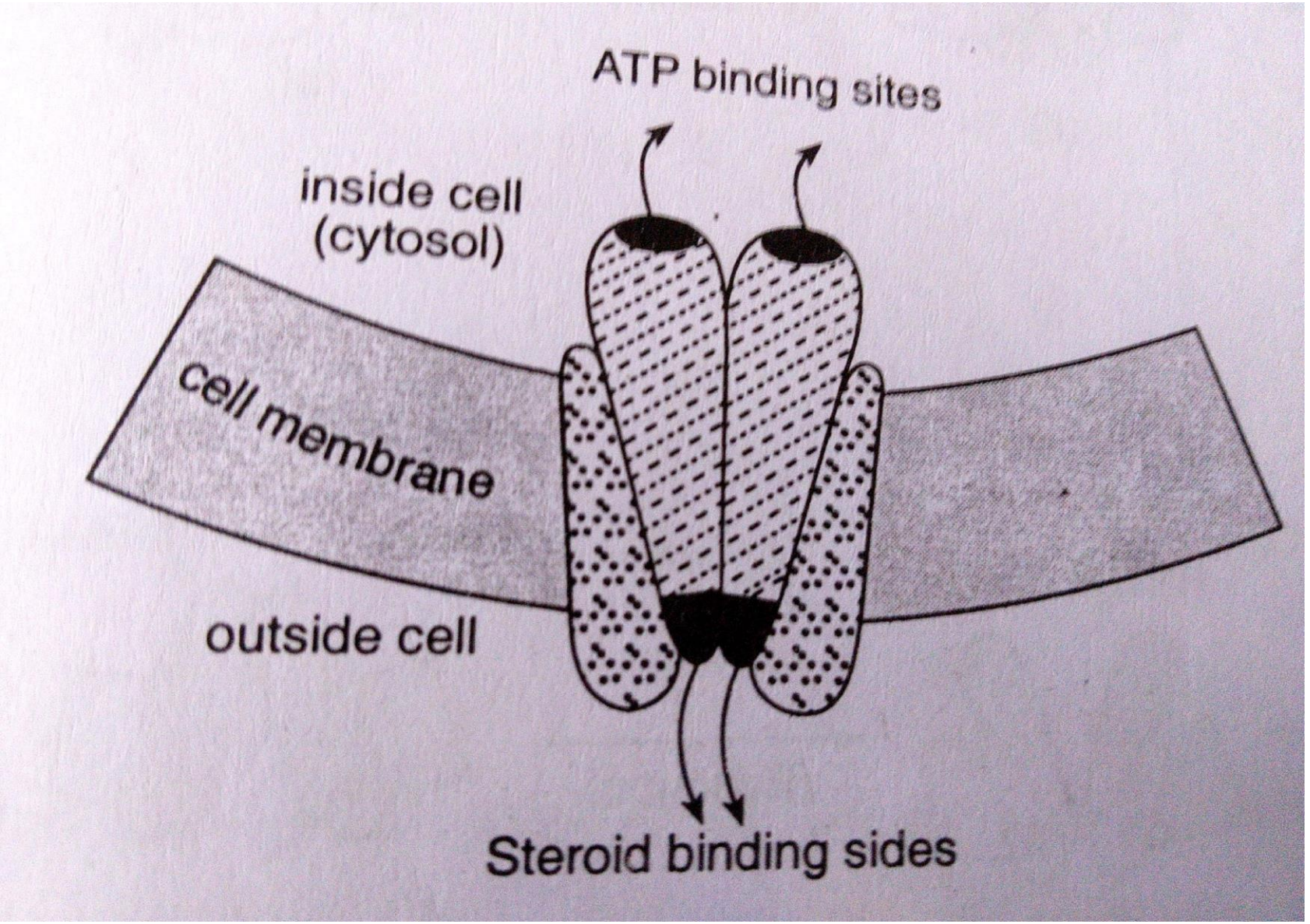
Diffusion



Facilitated Diffusion



Na/K ATPase



Mol wt 280 kDa
$\alpha_2\beta_2$ tetramer
α unit – 100 kDa
β unit – 80 kDa

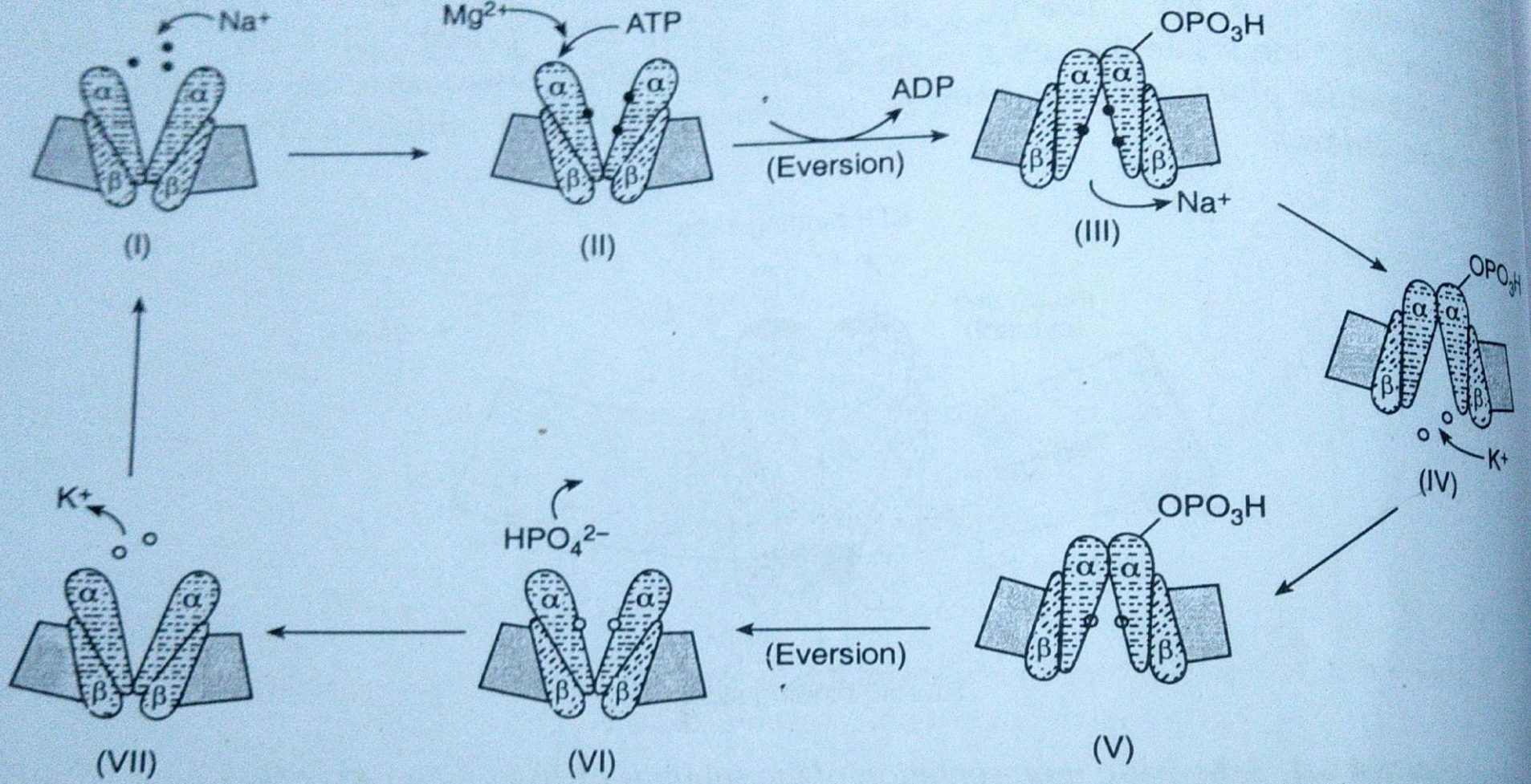


Figure 4.6.2 : *Schematic representation of the functioning of Na^+-K^+-pump.*

The overall process is :

$$3(Na^+)_{in} + 2(K^+)_{out} + MgATP^{2-} + H_2O \longrightarrow 3(Na^+)_{out} + 2(K^+)_{in} + MgADP^- + HPO_4^{2-} + H^+ \quad (4.6.1)$$

The Na+/K+ - ATPase enzyme is a tetrameric protein with two *α*- subunits and two *β*- subunits.
The *α*- sub units are close and contact to each other, while *β*- subunits are situated apart from each other (Figure). The ions exchange proceeds via following steps;

Step 1. Inside the cell **three Na^+ ions bind to the *α*- subunits** of the enzyme which is consisting of six oxide units as binding site.

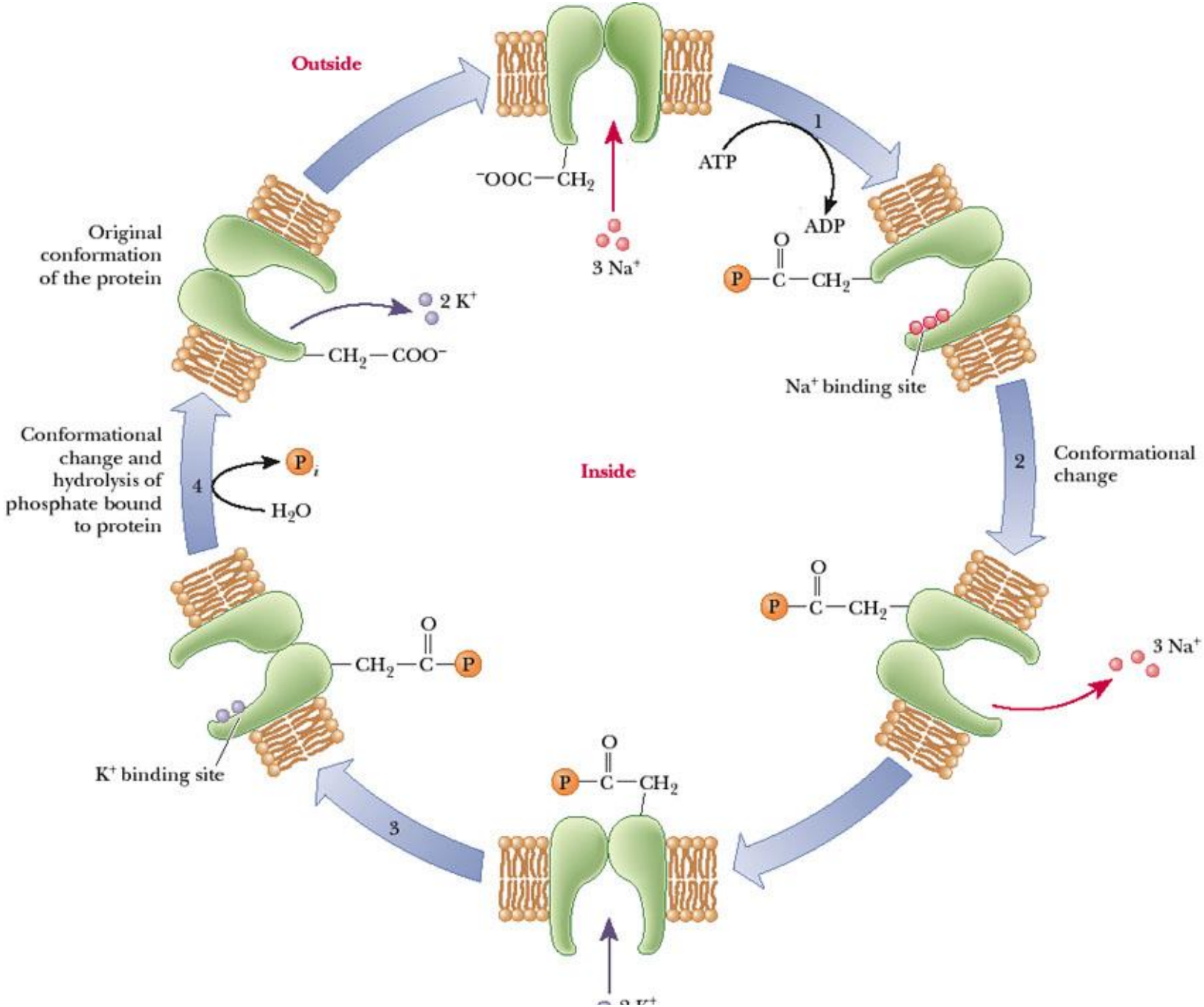
Step 2. The attachment of Na^+ ions to the *α*- subunits **changes the local polarity that helps an ATP to bind with a *α*- subunit**. The bound ATP is hydrolyzed to ADP and covalently bound with a phosphate ester (P). Due to this phosphorylation a conformation change (the inside cavity is closed and outside cavity is opened) took place in the *α*- subunit.

Step 3. The weakly bound three Na^+ ions are released outside the cell.

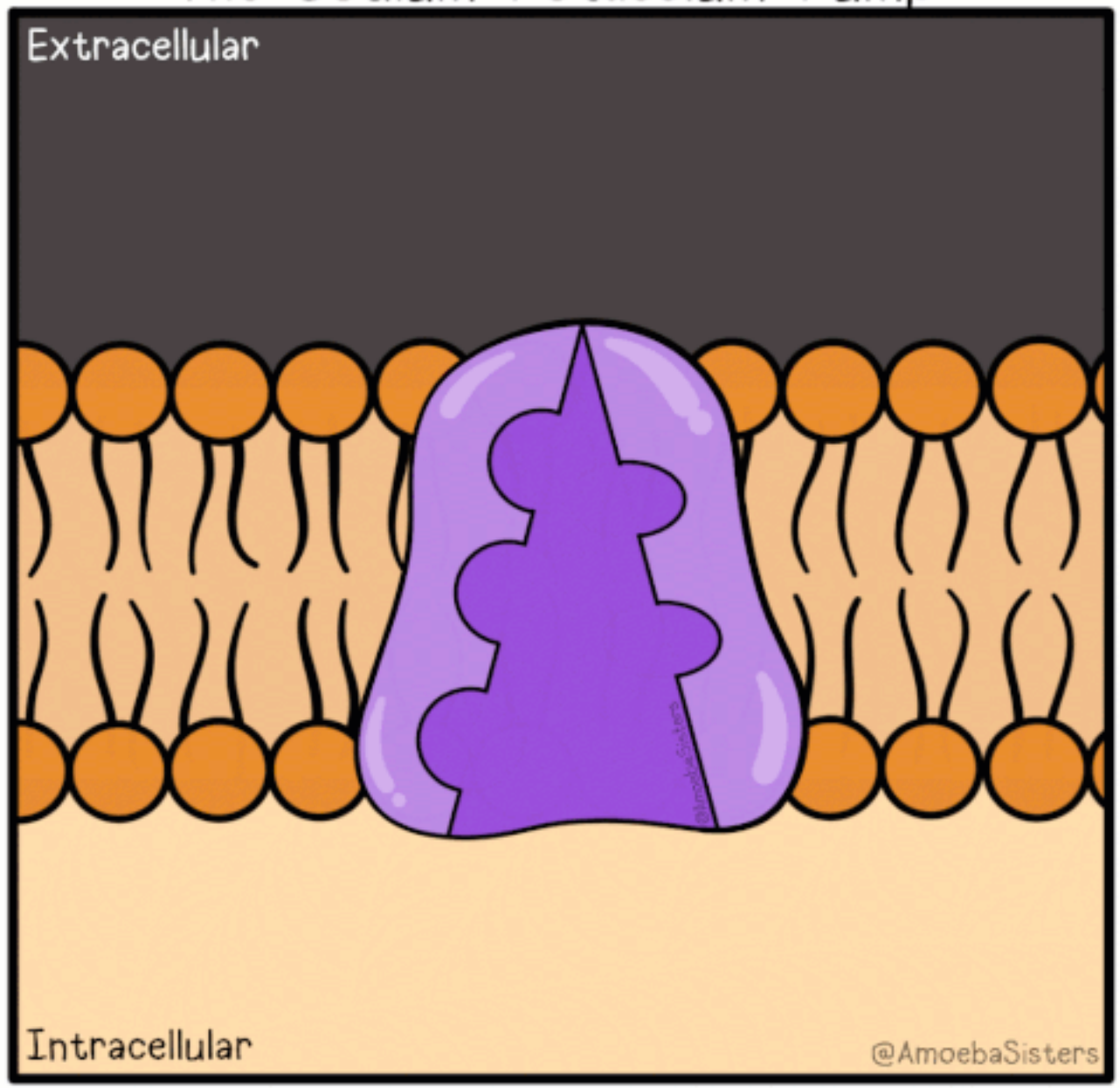
Step 4 and Step 5. Two K^+ ions outside the cell bind to the open cell cavity and simultaneously dephosphorylation takes place. Because of this a configurational change happens and two K+ ions move inside the cell with the help of *α* - subunits.

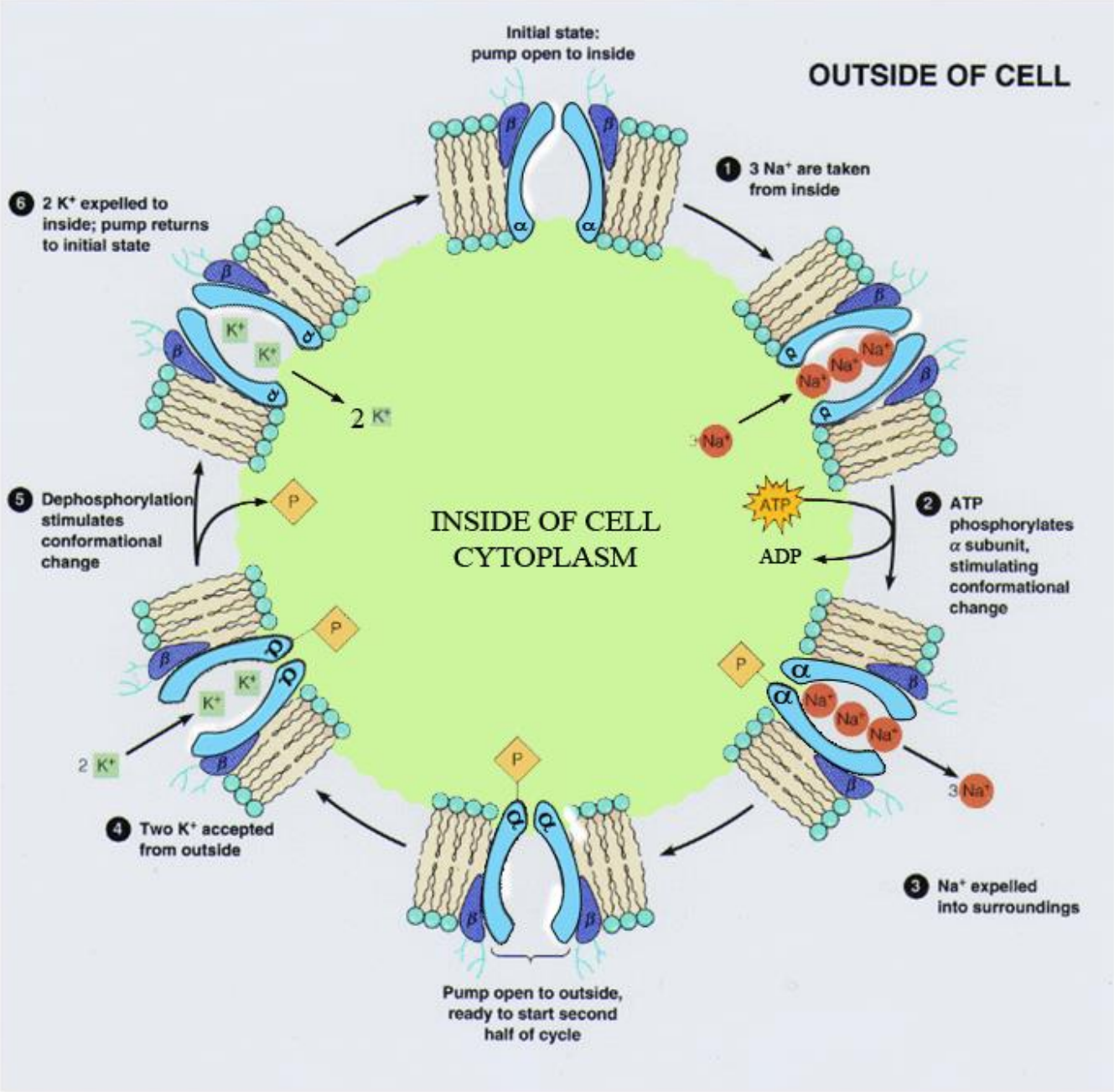
Step 6. The bound two K^+ ions are released inside the cell and initial configuration is achieved by the enzyme.

Use: In the pumping process three positively charged Na^+ ions are released from the cell and two positively charged K^+ ions are accepted inside the cell. Hence, a charge difference is produced by the process. This charge difference creates potential gradient across the cell. For good example, signal transmission across neuron cells.


Outside
$^{-}OOC-CH_2$
3 Na^+
1
ATP
ADP
P
O
C
CH_2
Na^+ binding site
2
Conformational change
3 Na^+
3
K^+ binding site
CH_2-C-P
2 K^+
CH_2-COO^-
Original conformation of the protein
4
P_i
H_2O
Conformational change and hydrolysis of phosphate bound to protein
Inside

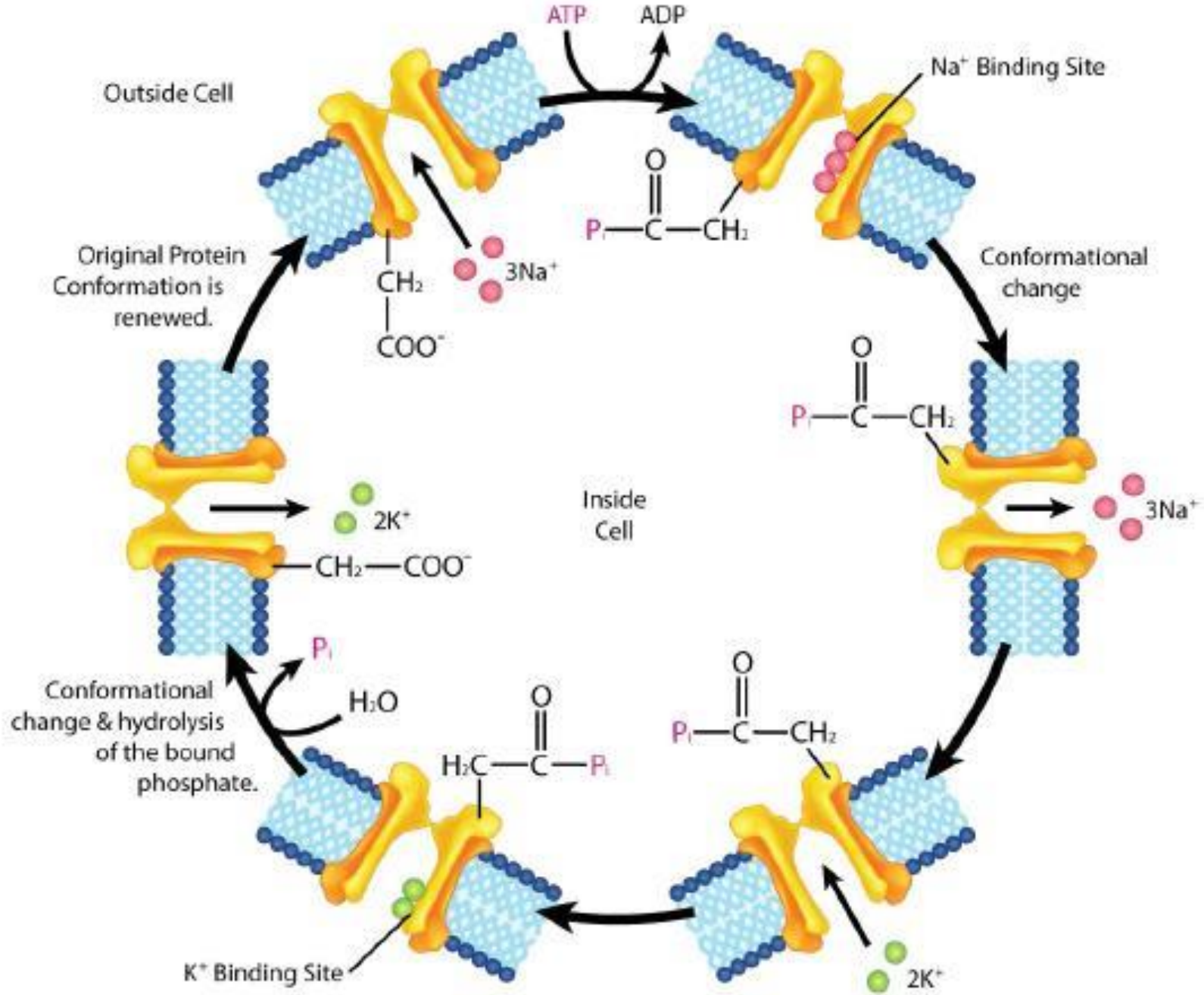
The Sodium Potassium Pump

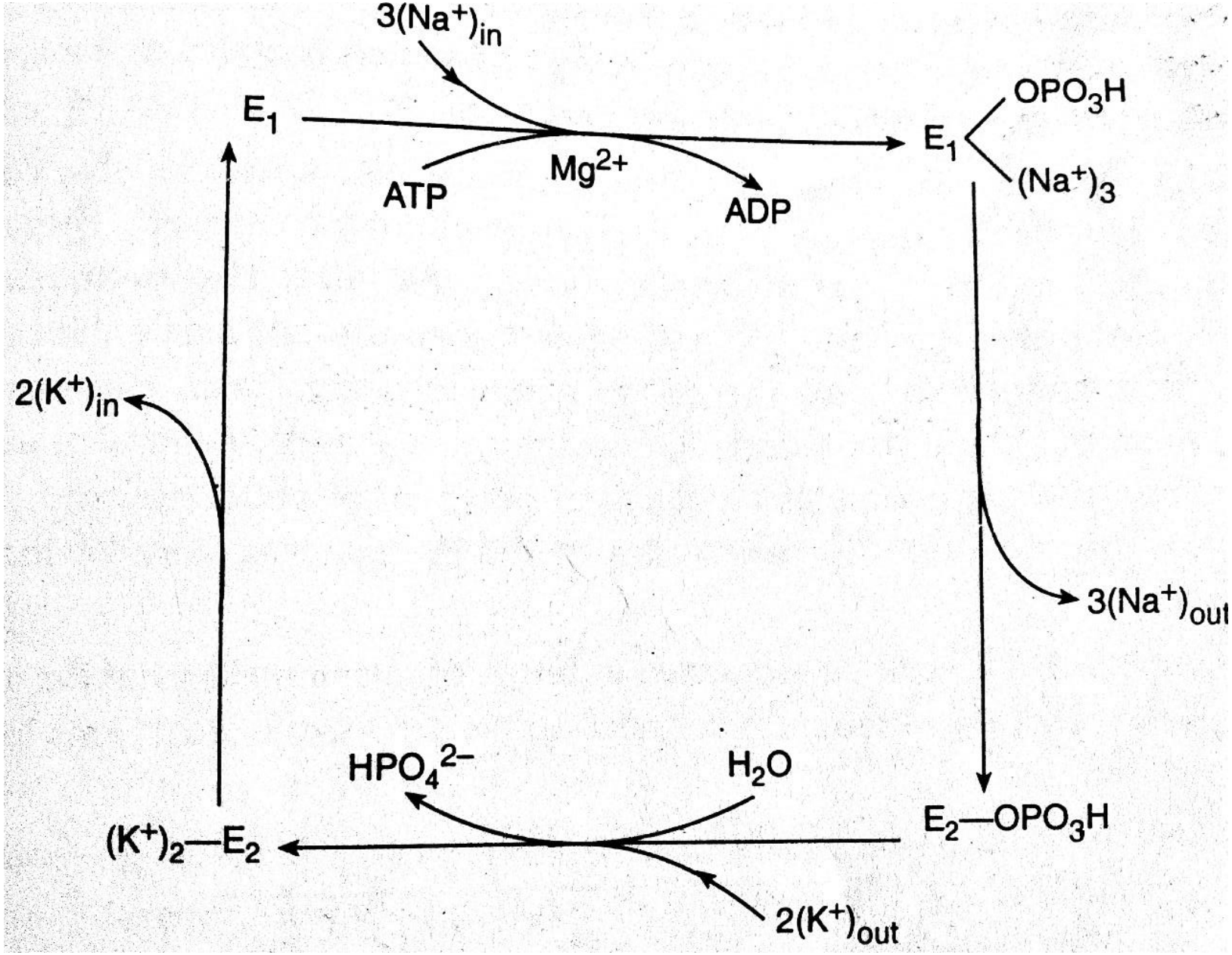




The Na^+/K^+ pump:

The Na^+/K^+ pumping between inside and outside a cell is assisted by a membrane-bound **Na+/K+ - ATPase** enzyme that catalyzes the movement of ions in the both direction across a cell. Through this pumping process the concentration difference of the ions inside and outside a cell is maintained and hence, a constant cell potential achieved.


ATP
ADP
Outside Cell
Na⁺ Binding Site
Original Protein Conformation is renewed.
3Na⁺
Conformational change
2K⁺
Inside Cell
3Na⁺
Pi
H₂O
Conformational change & hydrolysis of the bound phosphate.
K⁺ Binding Site
2K⁺


3(Na+)in
E1
ATP
Mg2+
ADP
E1
OPO3H
(Na+)3
2(K+)in
3(Na+)out
HPO4 2–
H2O
(K+)2—E2
E2—OPO3H
2(K+)out

4.7 ACTIVE TRANSPORT OF Na^+ AND K^+ IONS AND NERVE IMPULSE : Na^+ - AND K^+- CHANNELS

A nerve cell, *neuron*, is star-shaped with a number of finger-like extensions (called *dendrites*). The cell body is associated with a long tube called *axon*. The axon is ended at the *synapse* through which it is connected with the dendrites of the next nerve cell.

At the resting condition, like other cells, the neuron has also a much higher concentration of K^+ ions inside the cell, while Na^+ ions are more concentrated outside the cell. These concentration gradients are maintained at the cost of metabolic energy. **At rest, the membrane is rather, permeable to K^+ compared to Na^+.** Cl^- and other anions present inside the cell maintain the electroneutrality. K^+ ions tend to leave the cell to attain the equilibrium concentration, but this leaves behind an excess of anions. It prevents the tendency towards concentration equilibration. At rest, the concentration gradient generates an electrical potential of about –60 mV. The potentials can be measured by inserting the microelectrodes in the giant nerve fibre of the *squid* having diameter about 1 mm.

The electrical potential (E) can be expressed in terms of permeabilities of the involved ions by **Goldman – Hodgkin – Katz equation**. By considering the K^+, Na^+ and Cl^- ions to be involved in the process, E is given by :

$$E = \frac{RT}{F} \ln \frac{P_K[K^+]_{out} + P_{Na}[Na^+]_{out} + P_{Cl}[Cl^-]_{in}}{P_K[K^+]_{in} + P_{Na}[Na^+]_{in} + P_{Cl}[Cl^-]_{out}}$$

where P gives the permeability. The permeability changes on excitation as follows :

$P_K : P_{Na} : P_{Cl} \approx 1 : 0.04 : 0.45$, (at resting state)

and, $P_K : P_{Na} : P_{Cl} \approx 1 : 20 : 0.45$, (at excited state)

Thus at rest the potential arises mainly due to the permeability of K^+ while at the excited state it is due to the permeability of Na^+. This is why, ***resting potential*** (– 60 mV) is called ***K^+-potential*** while the potential at the excited state is called the ***Na^+-potential*** (+ 30 mV). Since the concentration gradient of Na^+ is opposite to that of K^+, the K^+-potential and Na^+-potential are opposite in nature. *Thus, on excitation there is a swing from K^+-*

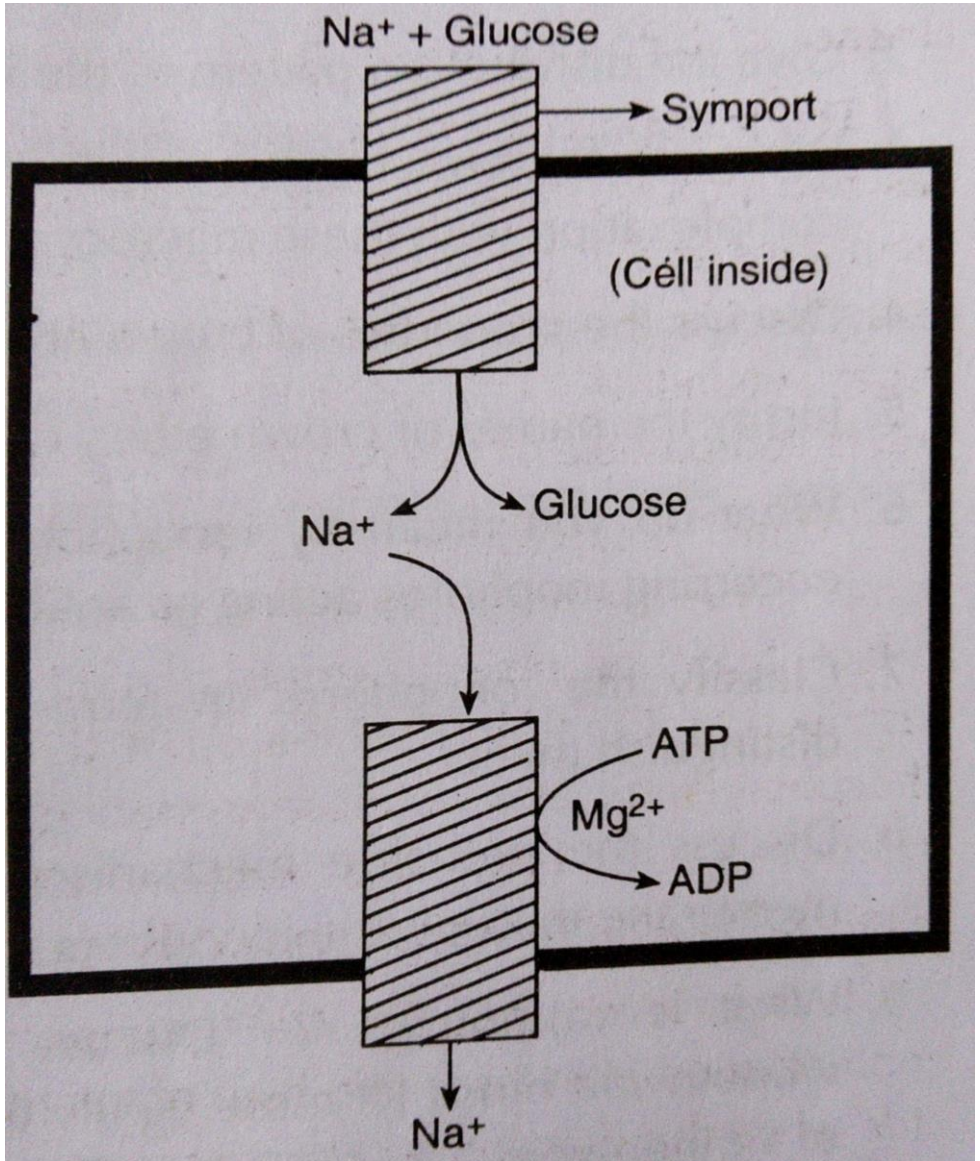
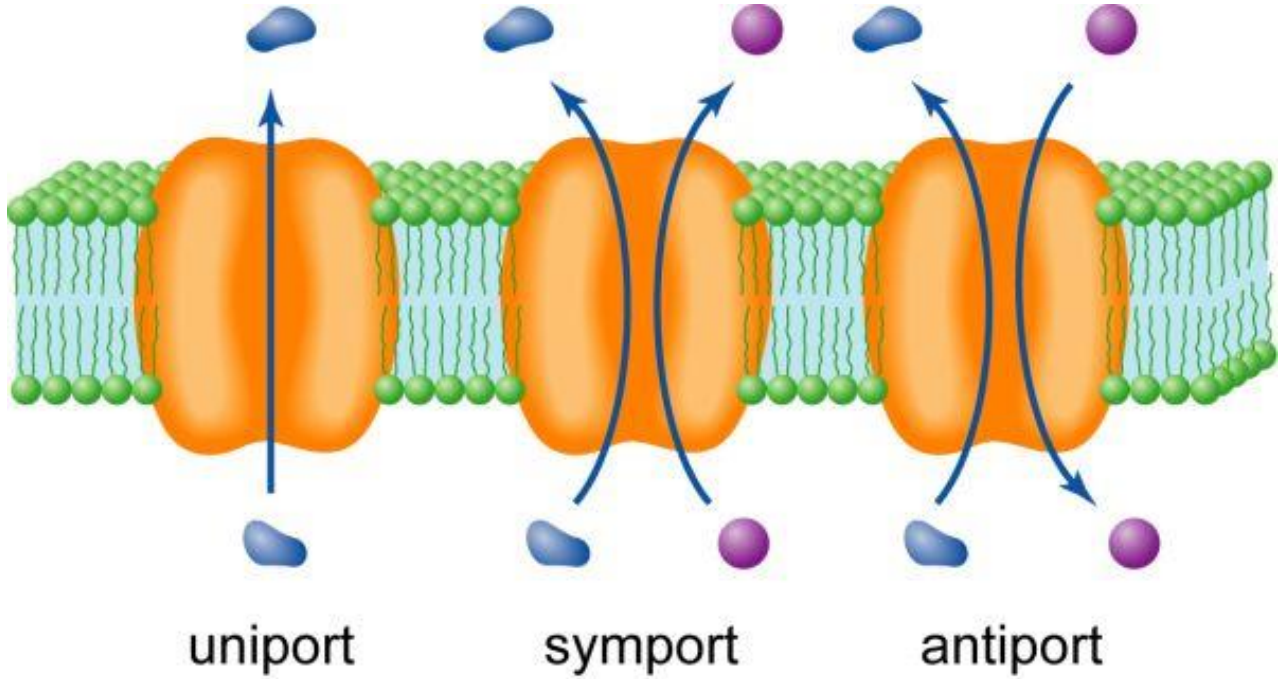


Figure 4.9.1: *Schematic representation of cotransport of glucose driven by Na^+-inflow.*

UNIPORT VS SYMPORT VS ANTIPORT

UNIPORT	SYMPORT	ANTIPORT
An integral membrane protein, which transports a single type of substrate species across the cell membrane	Another integral membrane protein involved in the transport of two different molecules in the same direction through the cell membrane	The third type of integral membrane protein involved in the secondary active transport of two different molecules in opposite directions
Types of molecules: One	Types of Molecules: Two	Types of Molecules: Two
Direction of Transport: Single	Direction of Transport: Single	Direction of Transport: Both
Transporter Proteins: Carrier proteins	Transporter Proteins: Cotransporters	Transporter Proteins: Cotransporters
Uses primary active transport	Uses secondary active transport	Uses secondary active transport
Driving Force: ATP	Driving Force: Electrochemical gradient	Driving Force: Electrochemical gradient
Examples: Channel proteins	Examples: Na/glucose symporter	Examples: Na/H antiporter

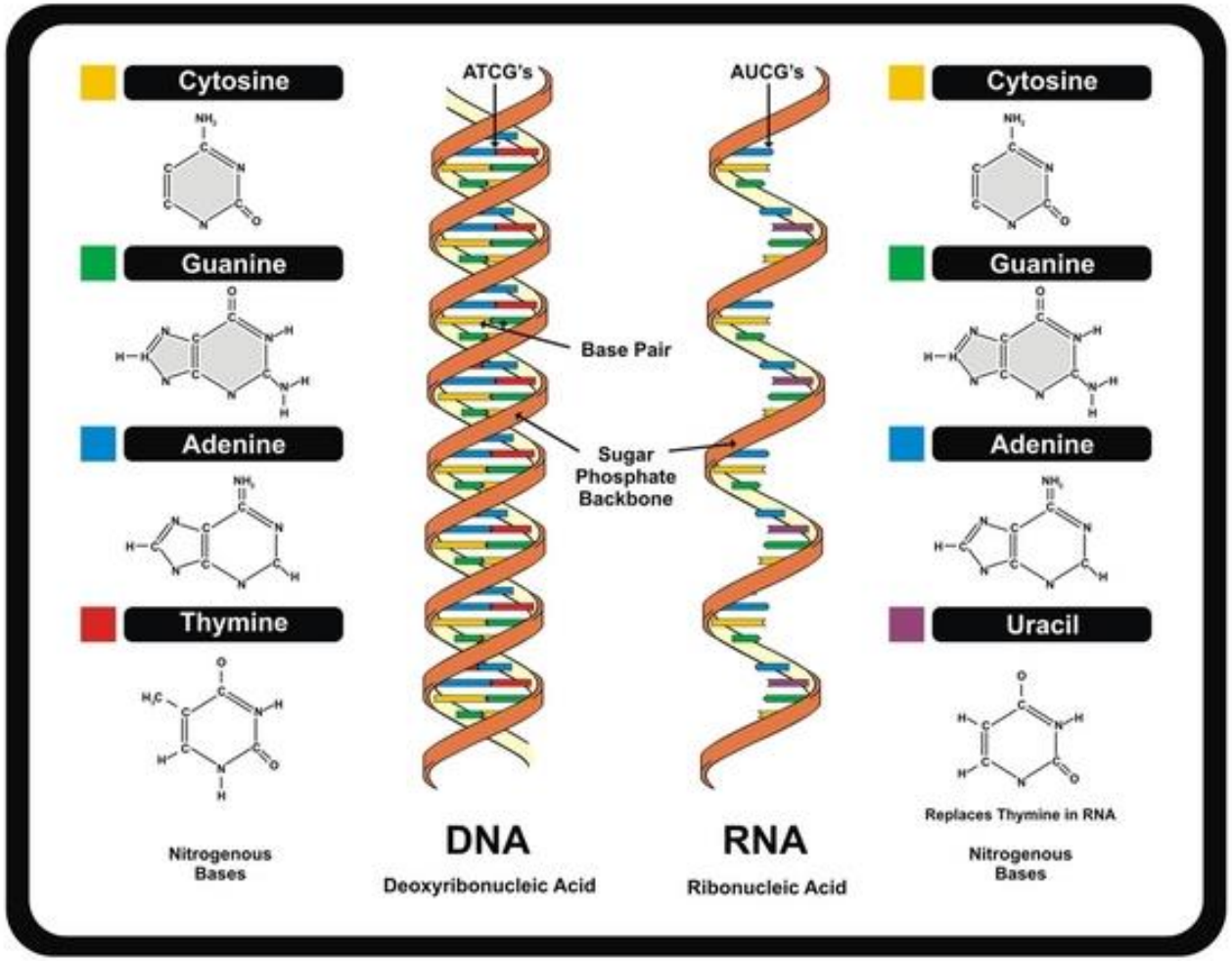


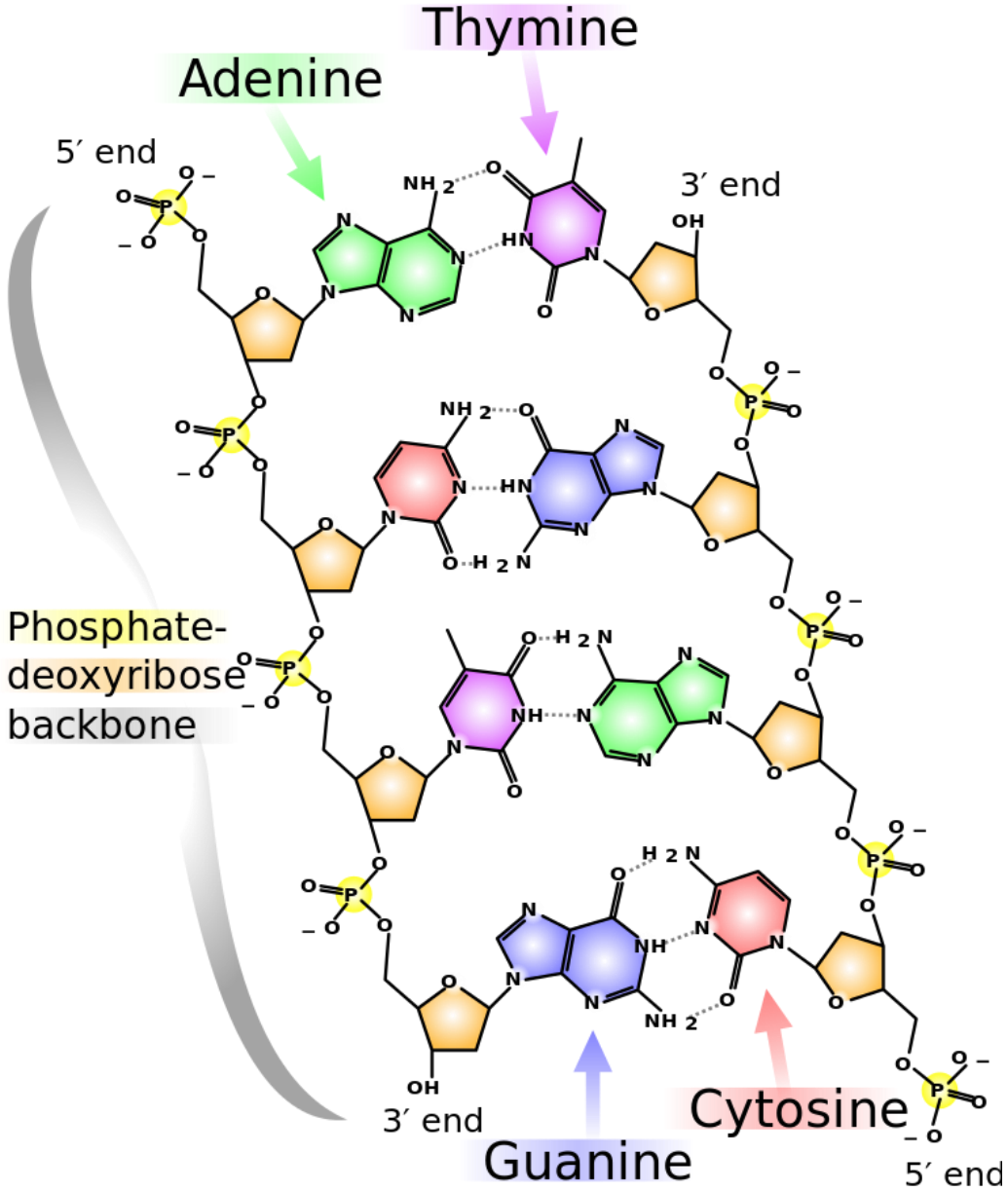
2. Bioenergetics and ATP Cycle

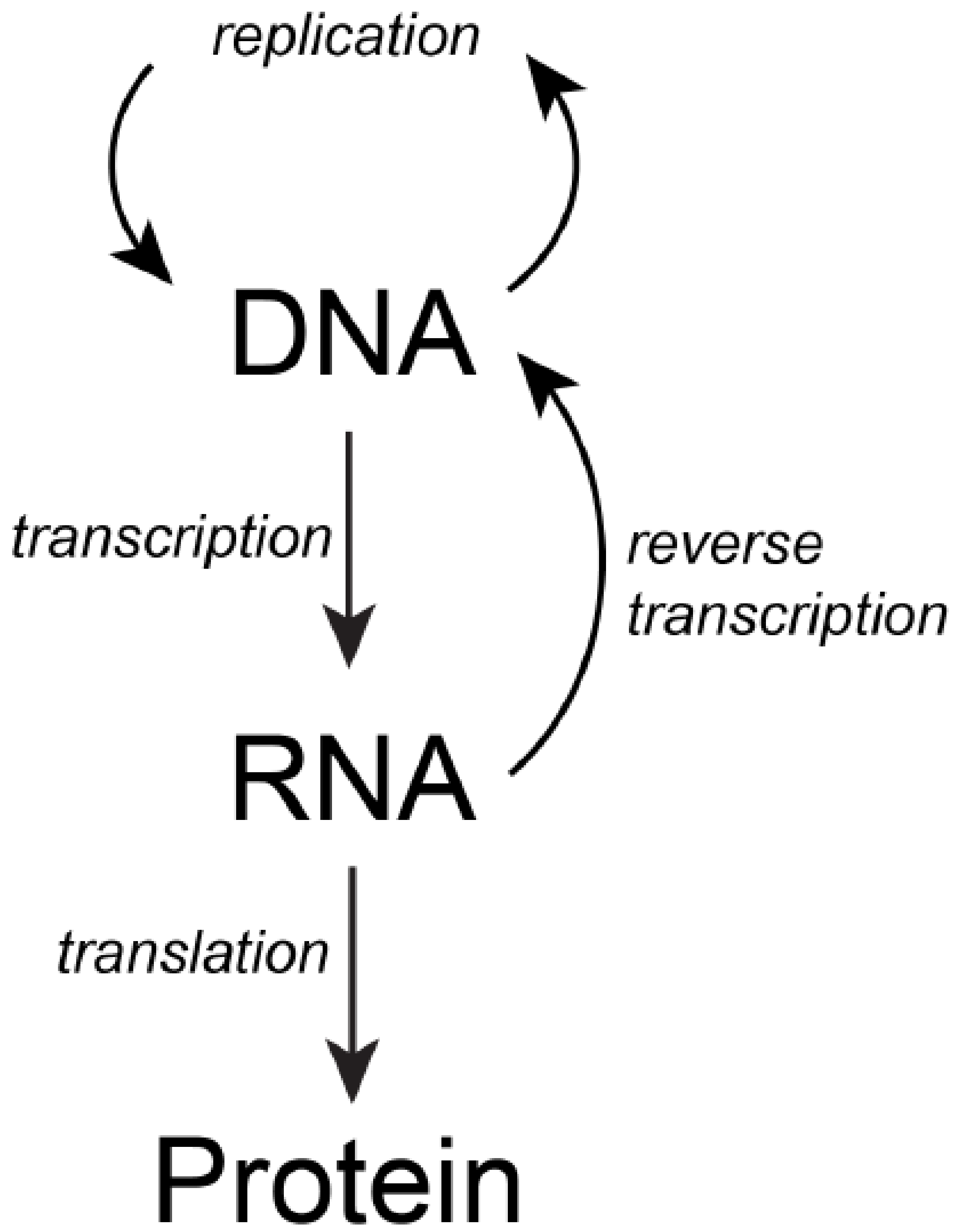
DNA Polymerase

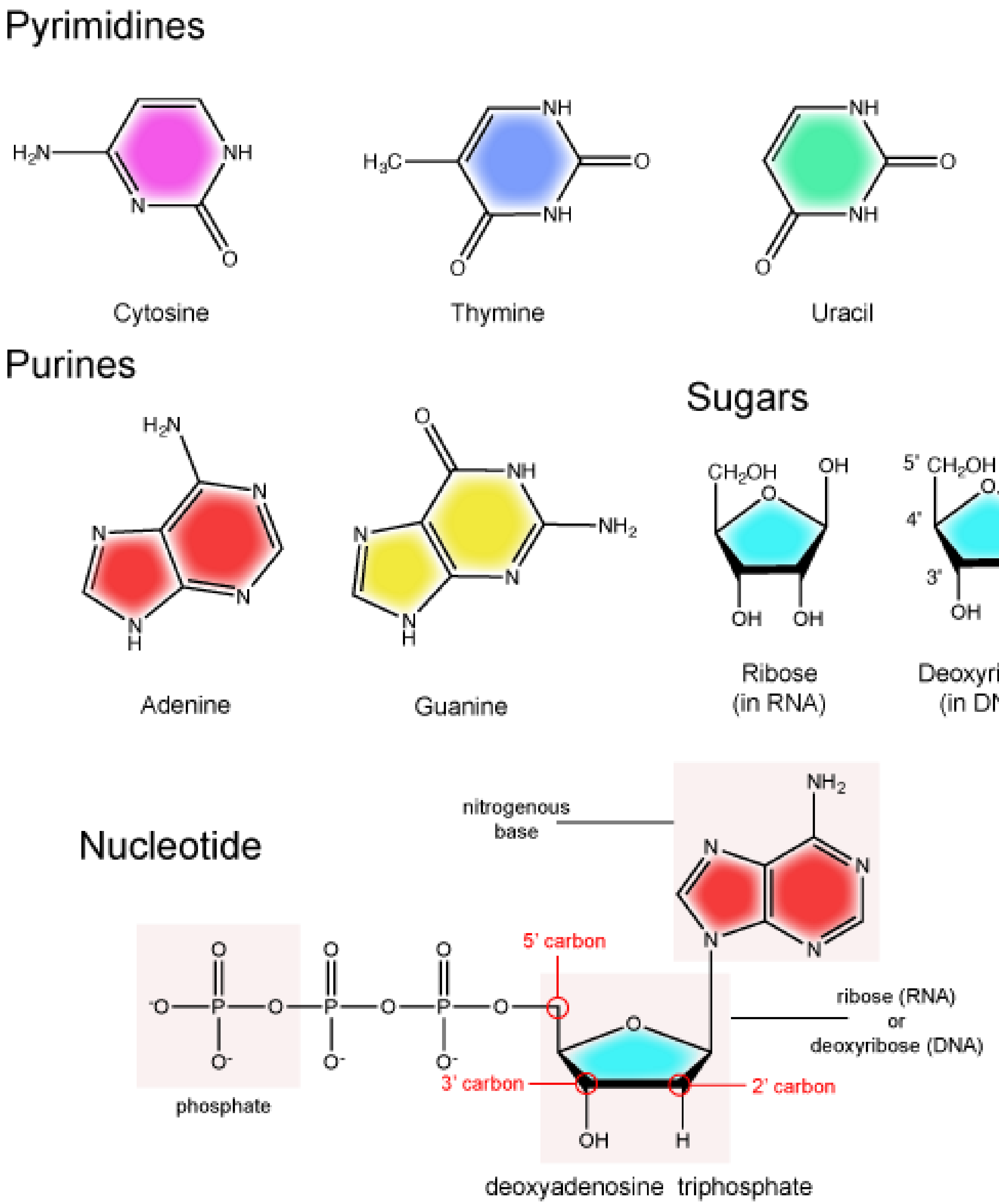
Biomolecules: Nucleic Acid

ATGCGCTTAG
TACGCGAAC



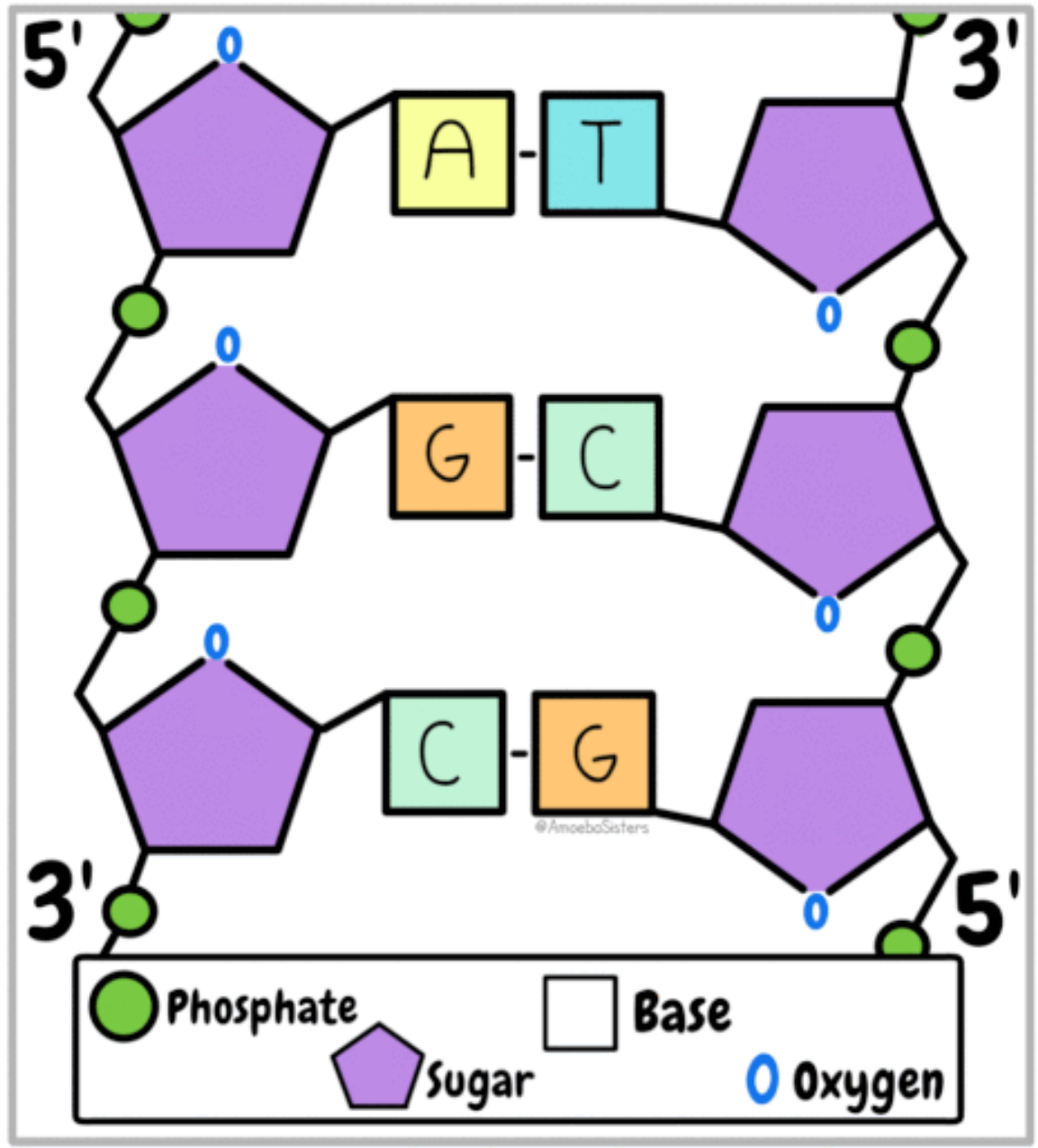



replication
DNA
transcription
reverse
transcription
RNA
translation
Protein


Pyrimidines
H_2N
NH
N
O
Cytosine
H_3C
NH
O
NH
O
Thymine
NH
O
NH
O
Uracil
Purines
H_2N
N
N
N
N
H
Adenine
O
NH
NH_2
N
N
N
H
Guanine
Sugars
CH_2OH
OH
O
OH
OH
Ribose
(in RNA)
5' CH_2OH
OH
O
4'
1'
3'
2'
OH
H
Deoxyribose
(in DNA)
Nucleotide
nitrogenous
base
NH_2
N
N
N
N
5' carbon
$^-$O
P
O
O
O
O^-
phosphate
ribose (RNA)
or
deoxyribose (DNA)
3' carbon
2' carbon
OH
H
deoxyadenosine triphosphate

5' to 3' and 3' to 5' in DNA

@AmoebaSisters

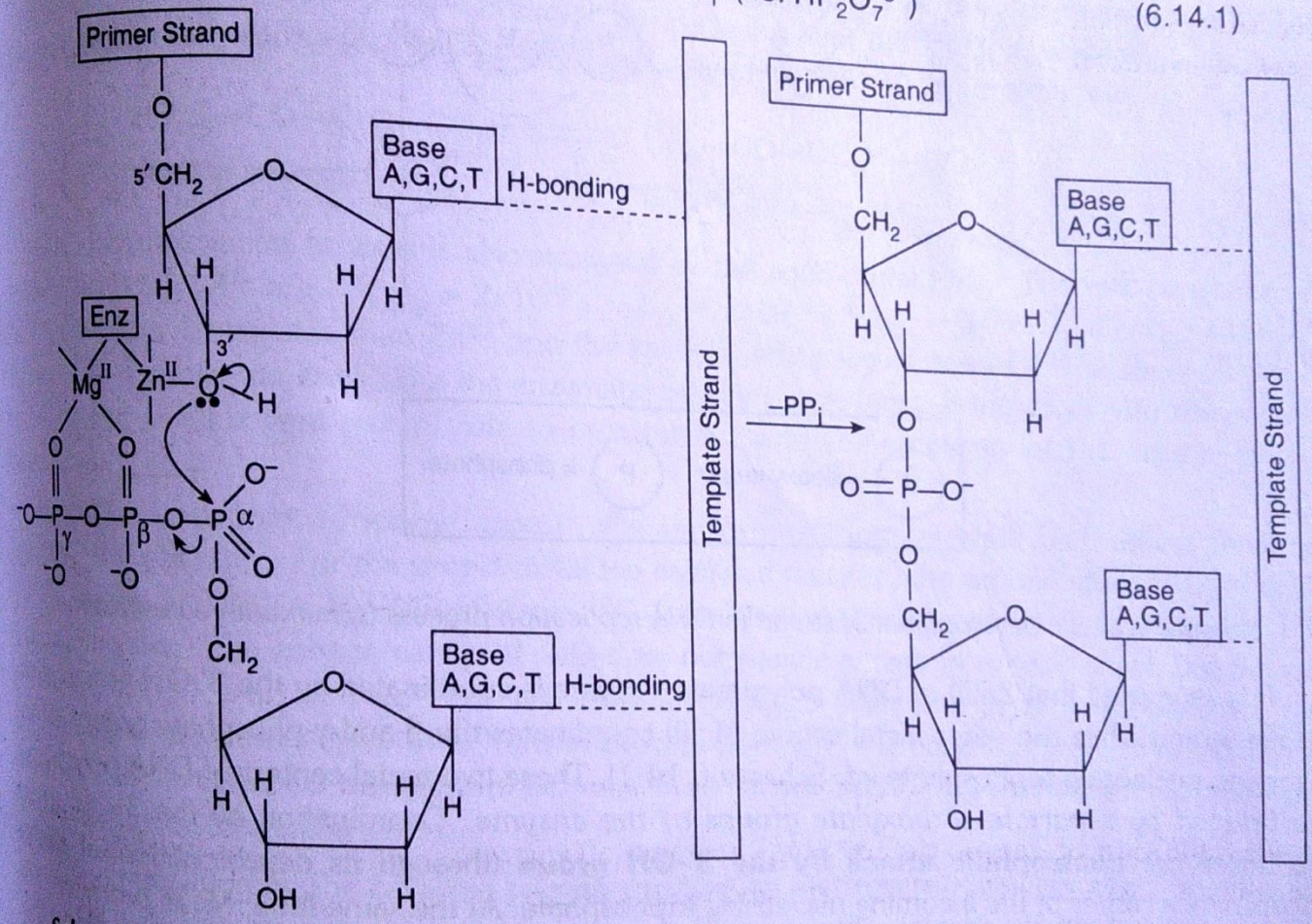


sphere. coordinate intermediate

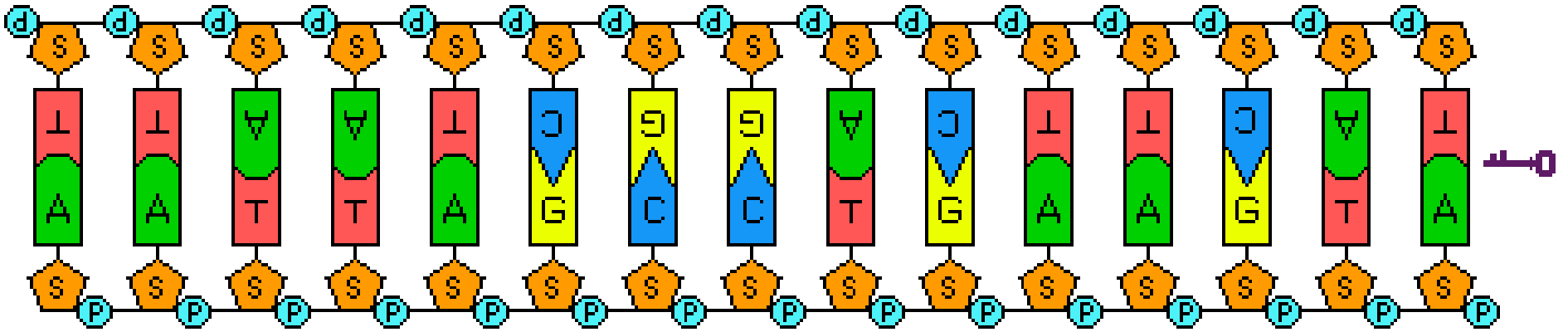
6.14 DNA POLYMERASE : STRUCTURE AND REACTIVITY

DNA polymerase enzyme is responsible for the synthesis of new DNA molecules having the same nucleotide sequence of the parent DNA. Thus the enzyme is important in the DNA synthesis process denoted by the following reaction.

$$(DNA)_n + d\,NTP \rightleftharpoons (DNA)_{n+1} + PP_i \ (i.e.\ HP_2O_7^{3-}) \qquad (6.14.1)$$



Scheme 6.14.1 : *Schematic representation of DNA polymerase activity in DNA chain elongation process.*



https://in.pinterest.com/pin/575968239841102600/

Leading and Lagging Strand in DNA Replication

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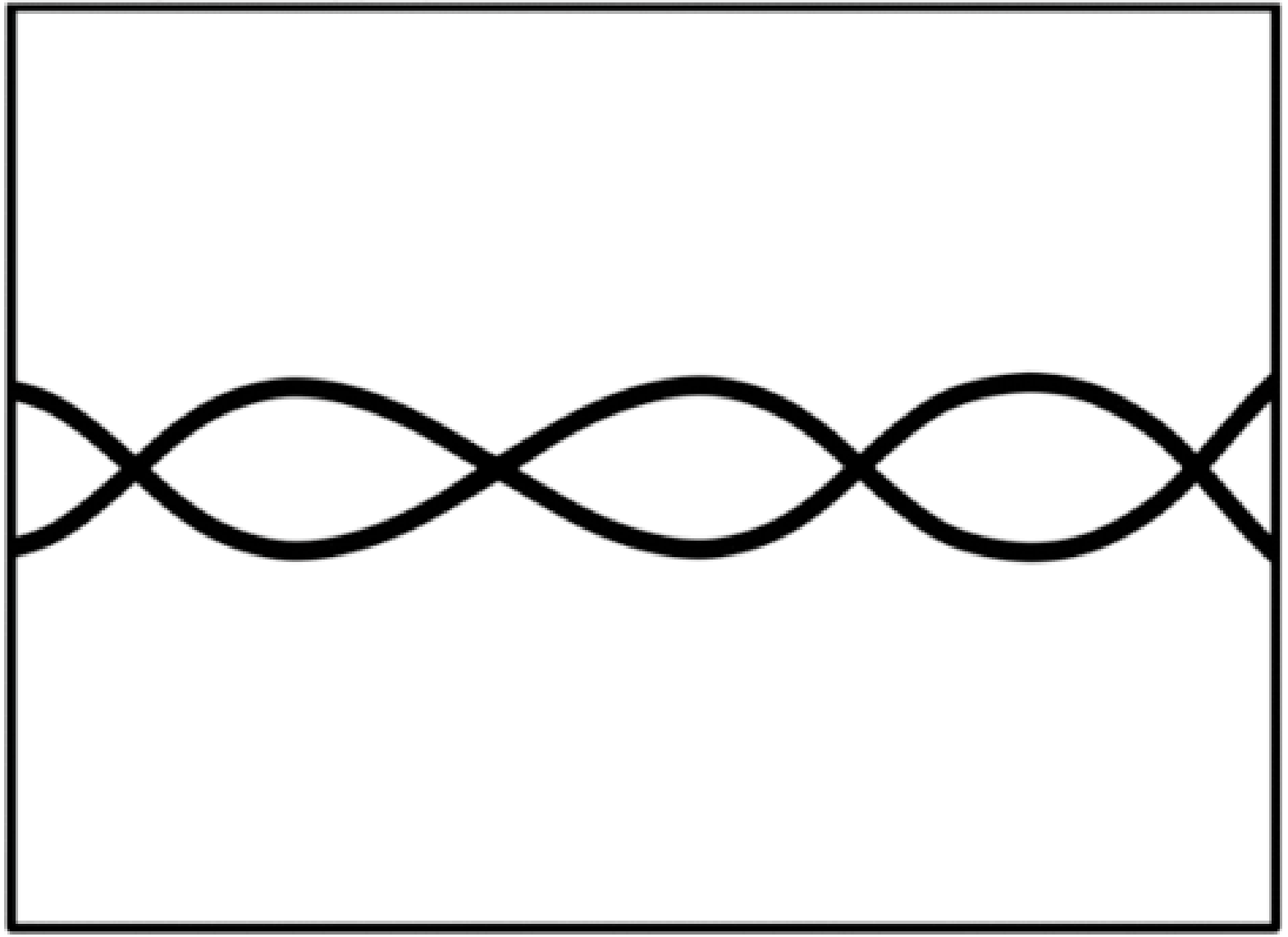


- In DNA replication
  - The parent molecule unwinds, and two new daughter strands are built based on base-pairing rules

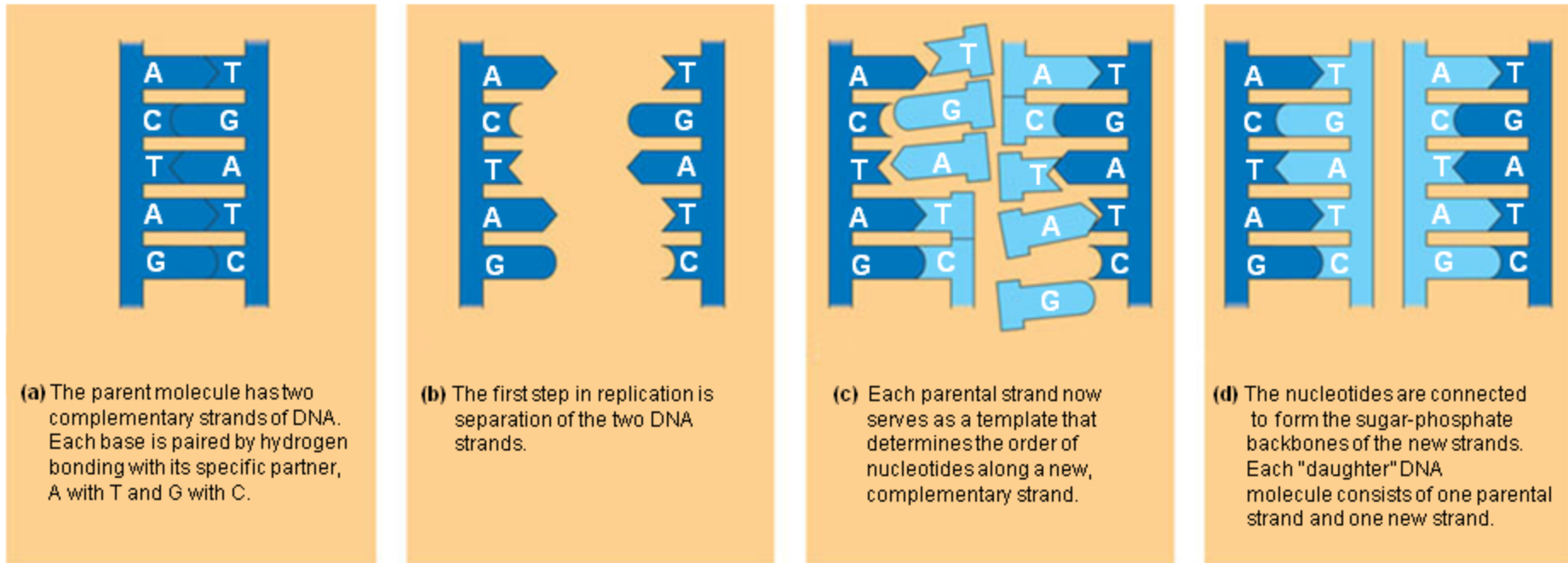


Figure 16.9 a–d

Elongation of DNA

– Is catalyzed by enzymes called DNA polymerases, which add nucleotides to the 3′ end of a growing strand

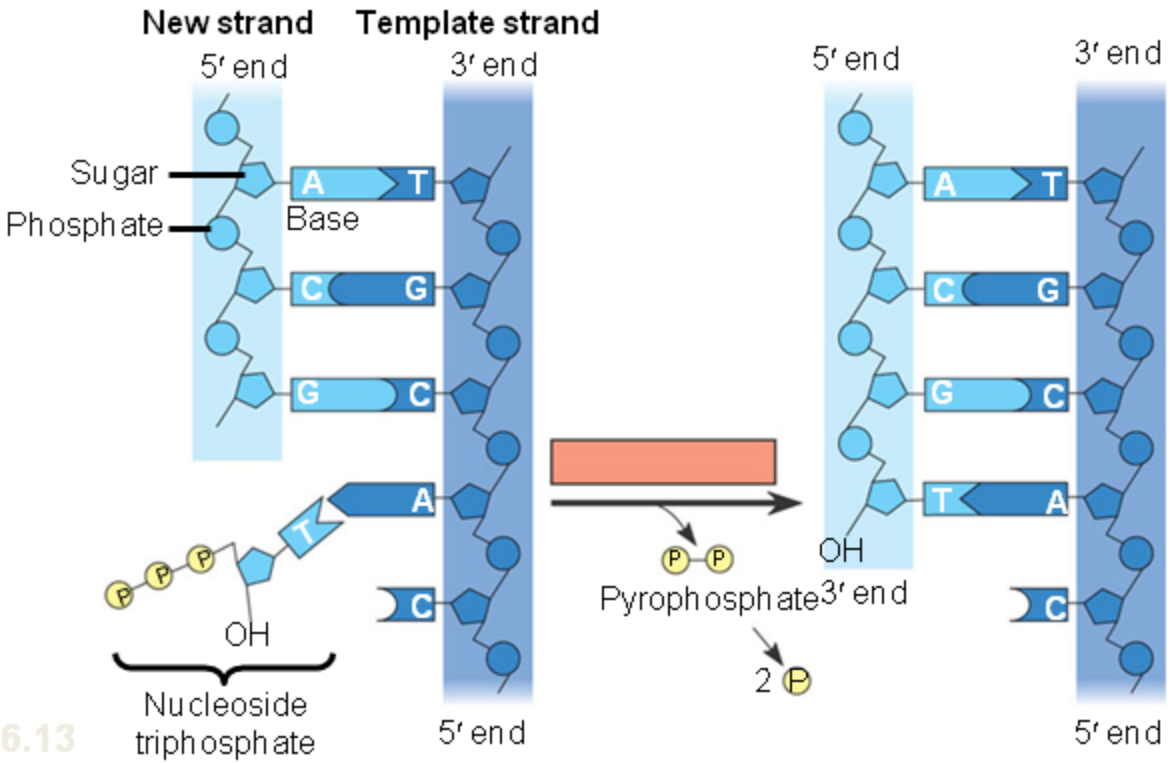


Figure 16.13

www.sliderbase.com

http://www.sliderbase.com/spitem-1465-3.html#1

- A summary of DNA replication

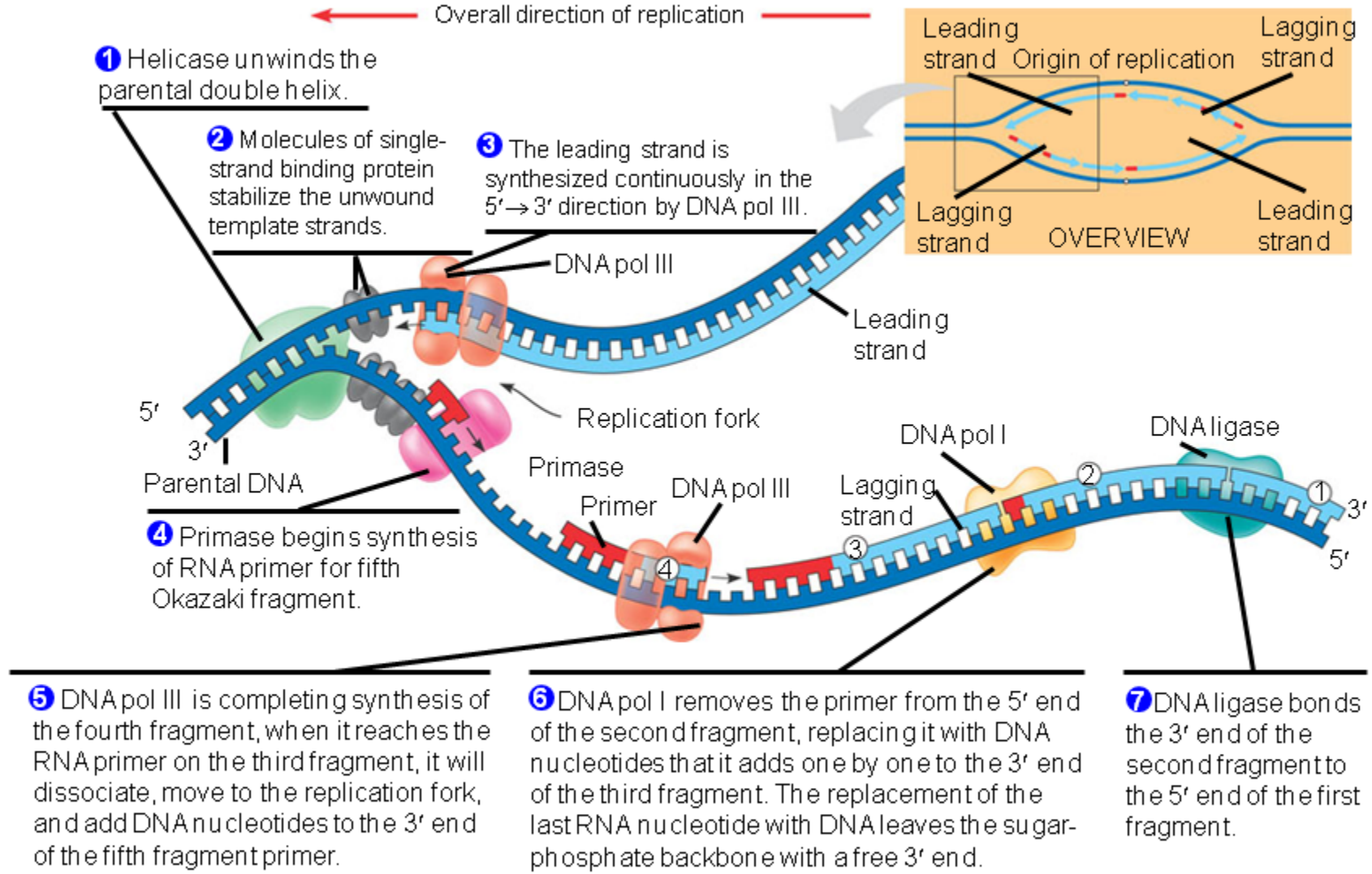


Figure 16.16

www.sliderbase.com

http://www.sliderbase.com/spitem-1465-4.html

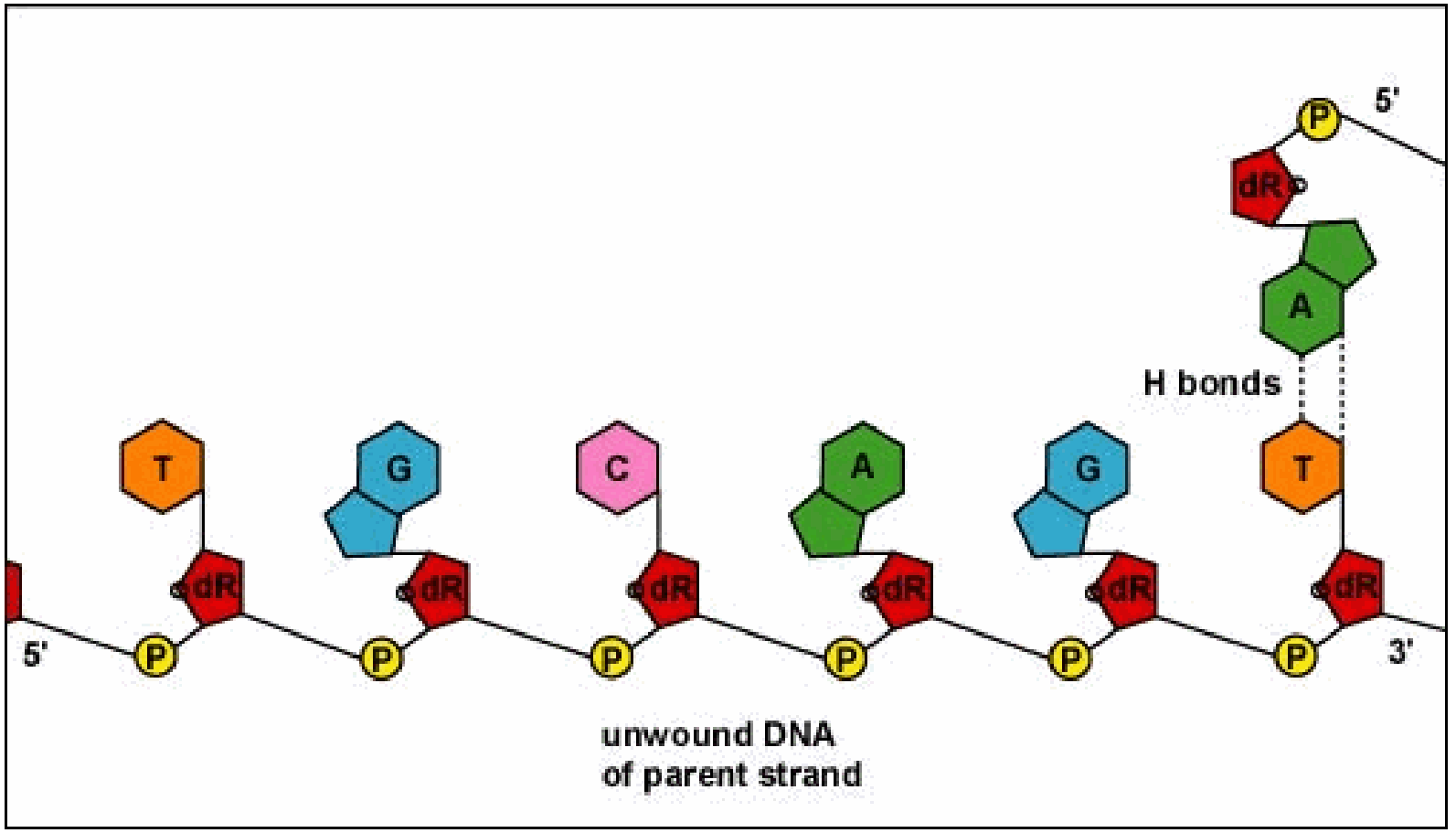
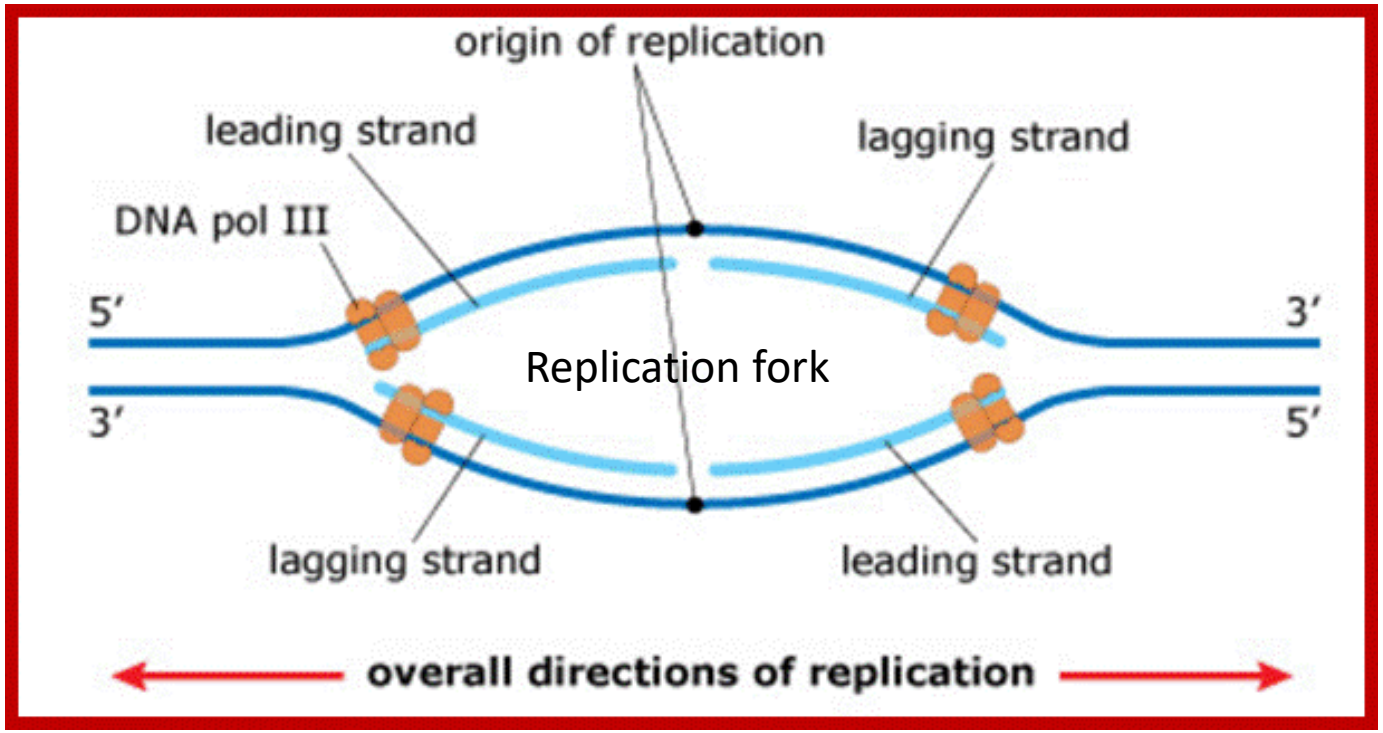

5'
P
dR
A
H bonds
T
G
C
A
G
T
dR
P
5'
3'
unwound DNA
of parent strand

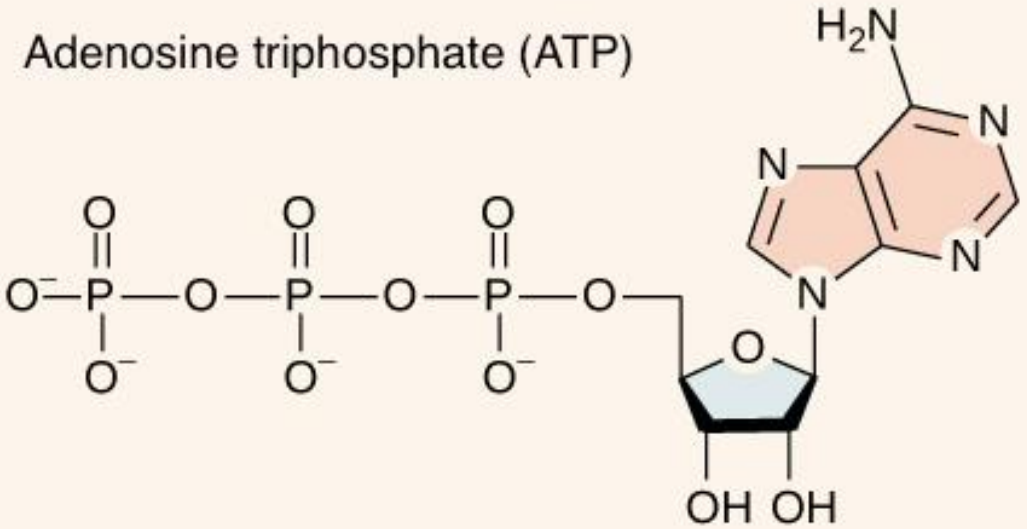
Table 1. The Molecular Machinery Involved in Bacterial DNA Replication

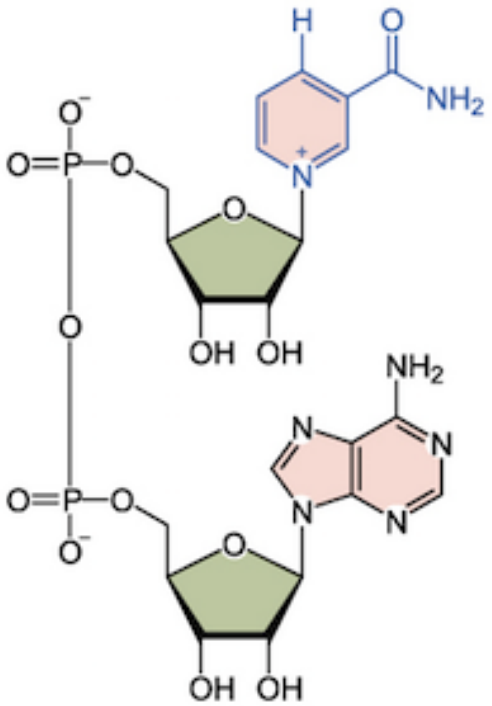
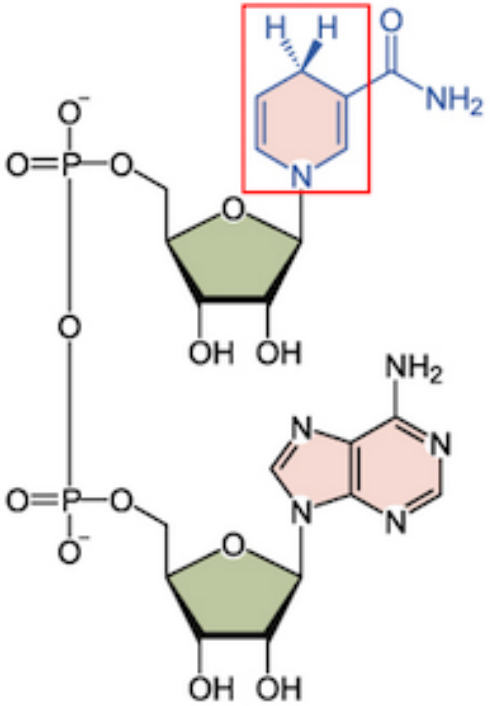
Enzyme or Factor	Function
DNA pol I	Exonuclease activity removes RNA primer and replaces it with newly synthesized DNA
DNA pol III	Main enzyme that adds nucleotides in the 5′ to 3′ direction
Helicase	Opens the DNA helix by breaking hydrogen bonds between the nitrogenous bases
Ligase	Seals the gaps between the Okazaki fragments on the lagging strand to create one continuous DNA strand
Primase	Synthesizes RNA primers needed to start replication
Single-stranded binding proteins	Bind to single-stranded DNA to prevent hydrogen bonding between DNA strands, reforming double-stranded DNA
Sliding clamp	Helps hold DNA pol III in place when nucleotides are being added
Topoisomerase II (DNA gyrase)	Relaxes supercoiled chromosome to make DNA more accessible for the initiation of replication; helps relieve the stress on DNA when unwinding, by causing breaks and then resealing the DNA
Topoisomerase IV	Introduces single-stranded break into concatenated chromosomes to release them from each other, and then reseals the DNA


origin of replication
leading strand
lagging strand
DNA pol III
5′
3′
Replication fork
3′
5′
lagging strand
leading strand
overall directions of replication

Glycolysis

Adenosine triphosphate (ATP)



NAD^+	NADH
	

Energy Rich Phosphoanhydride Bond

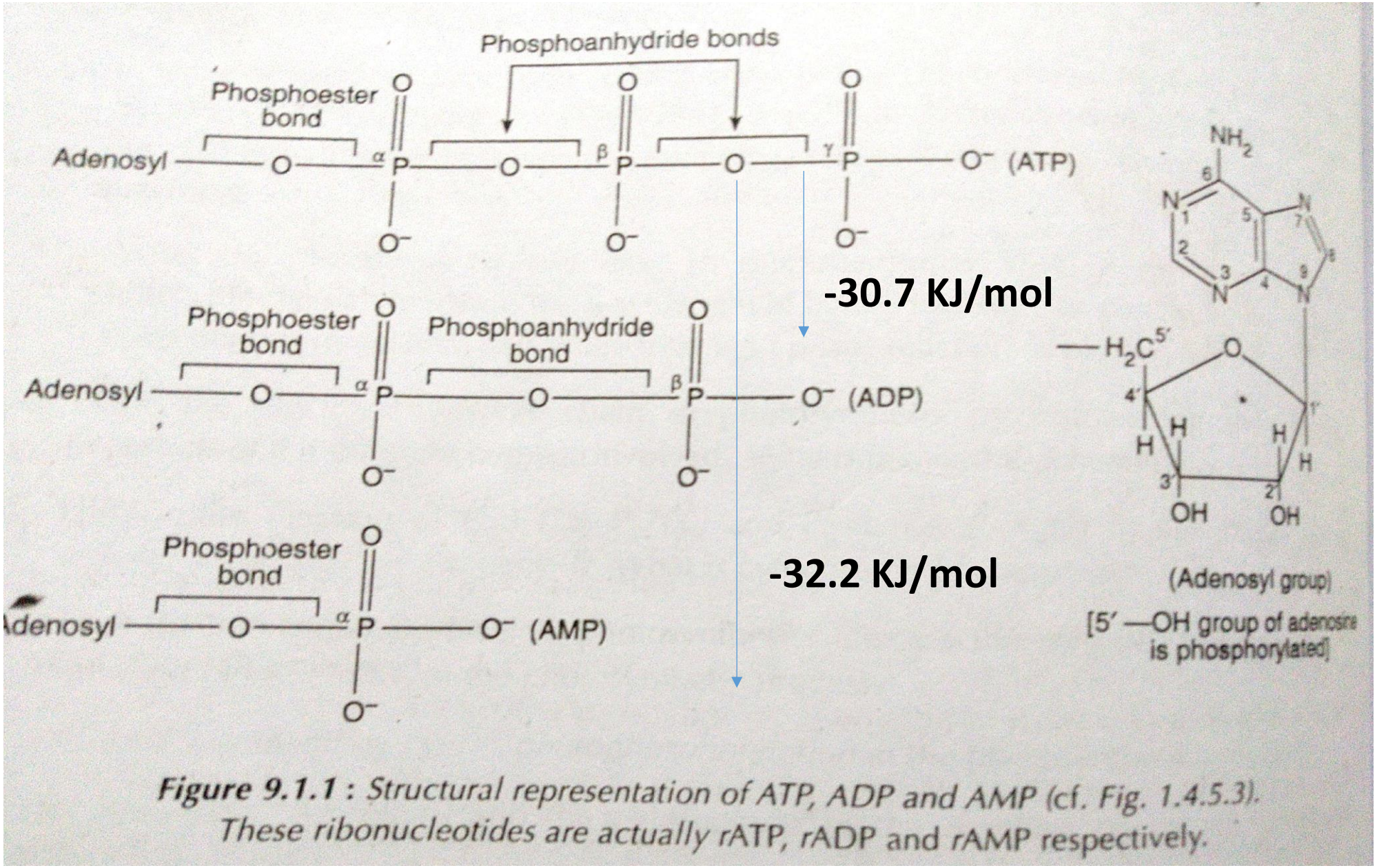


Figure 9.1.1 *: Structural representation of ATP, ADP and AMP (cf. Fig. 1.4.5.3). These ribonucleotides are actually rATP, rADP and rAMP respectively.*

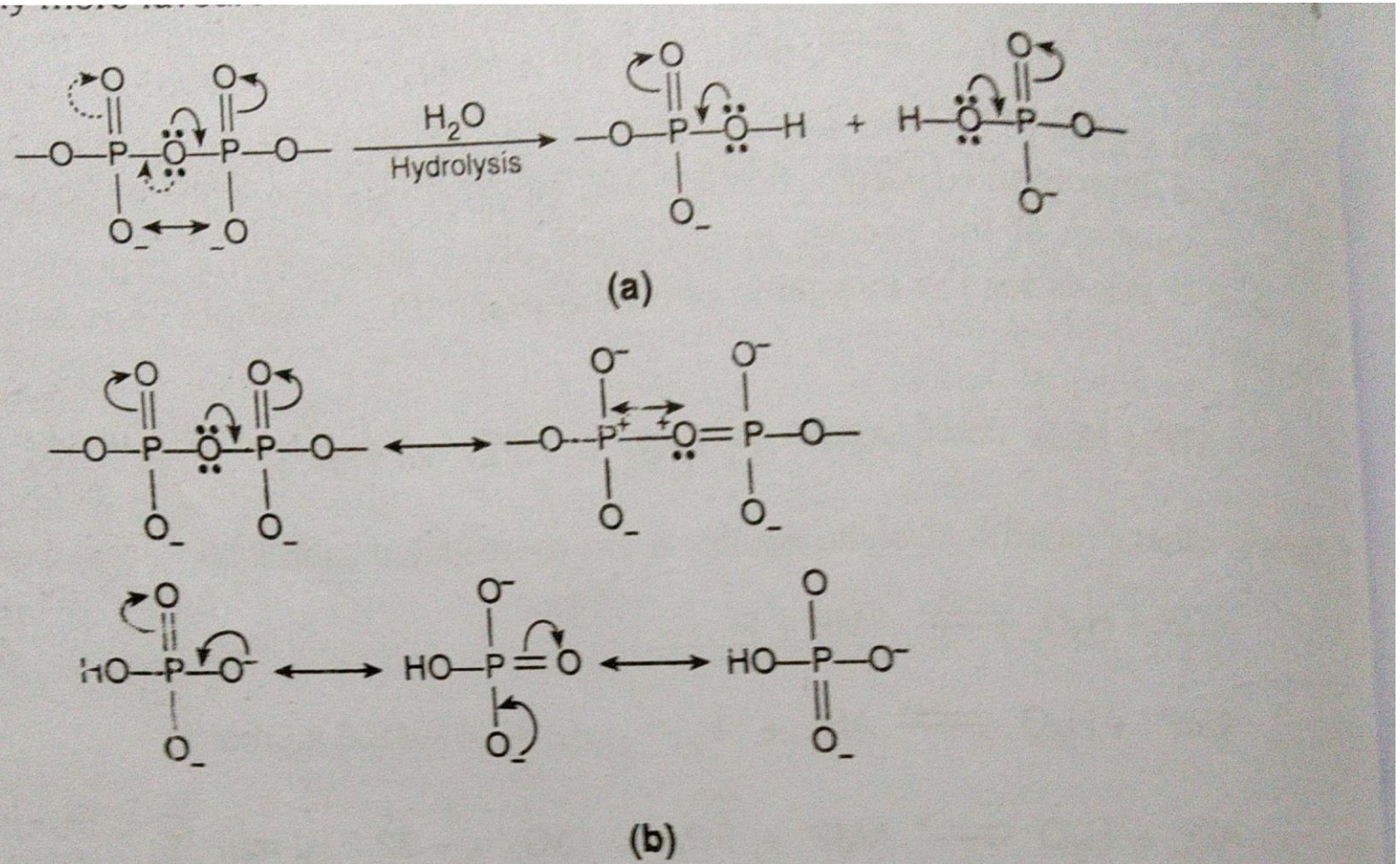


Figure 9.1.2 : *(a) Electrostatic repulsion (denoted by↔) between the adjacent phosphoryl groups and competing resonance between the phosphoryl groups of a phosphoanhydride segment contribute to destabilise it with respect to its hydrolysed products. (b) An important resonating structure of a phosphoanhydride segment destabilises the system due to an electrostatic repulsion (denoted by ↔) between the positive charges developed on the adjacent atoms; no such destabilising resonating structure exists for the hydrolysed product orthophosphate.*

Phosphate Group Transfer Potential

Table 9.1.1

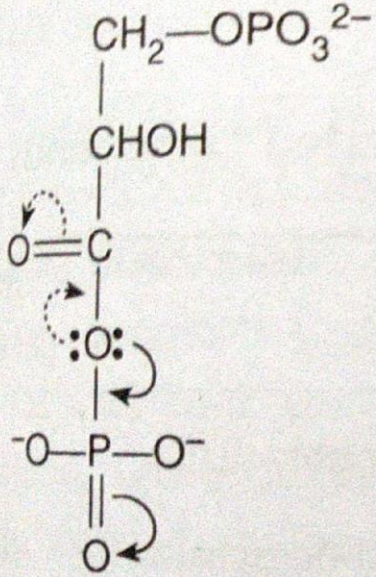
Free energies ($\Delta G_0'$ at pH ≈ 7.0, 25°C) of hydrolysis of some phosphorylated compounds.

Compounds	$\Delta G_0'$ (kJ mol^{-1})	Compounds	$\Delta G_0'$ (kJ mol^{-1})	Compounds	$\Delta G_0'$ (kJ mol^{-1})
(High transfer potential)		**(Medium transfer potential)**		**(Low transfer potential)**	
Phosphoenolpyruvate	– 62.0	Pyrophosphate (PP_i)	– 33.6	Glucose-1-phosphate	– 21.0
1,3-Diphosphoglycerate	– 49.6	ATP to PP_i	– 32.2	Fructose-6-phosphate	– 13.8
Acetyl phosphate	– 43.3	ATP (to ADP)	– 30.7	Glucose-6-phosphate	– 13.9
Creatine phosphate	– 43.3			Glycerol-3-phosphate	– 9.2

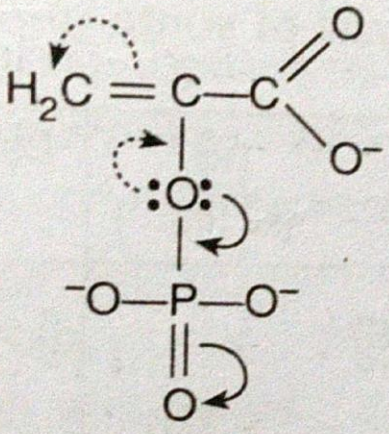
High Phosphate Group Transfer Potential

Glucose + ATP $\rightleftharpoons$ Glucose-6-phosphate + ADP + H^+, (9.1.7)

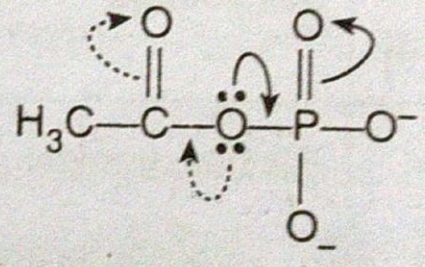
The $\Delta G_0'$ for this process is given by : $-(30.7 - 13.9) = -16.8$ kJ mol^{-1}.



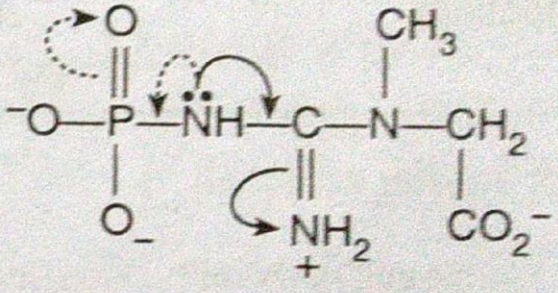
(1, 3- diphosphoglycerate)



(Phosphoenolpyruvate)

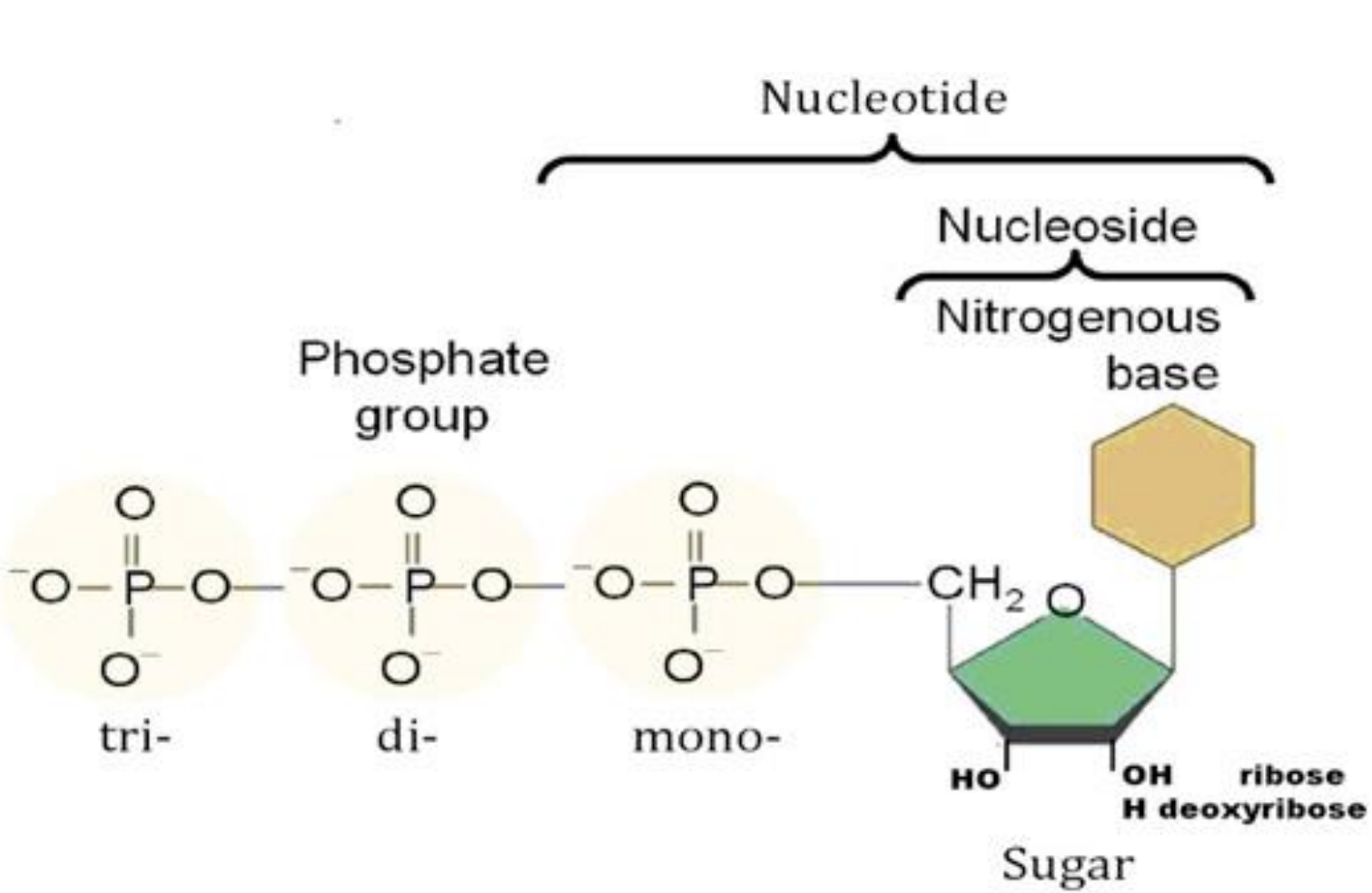


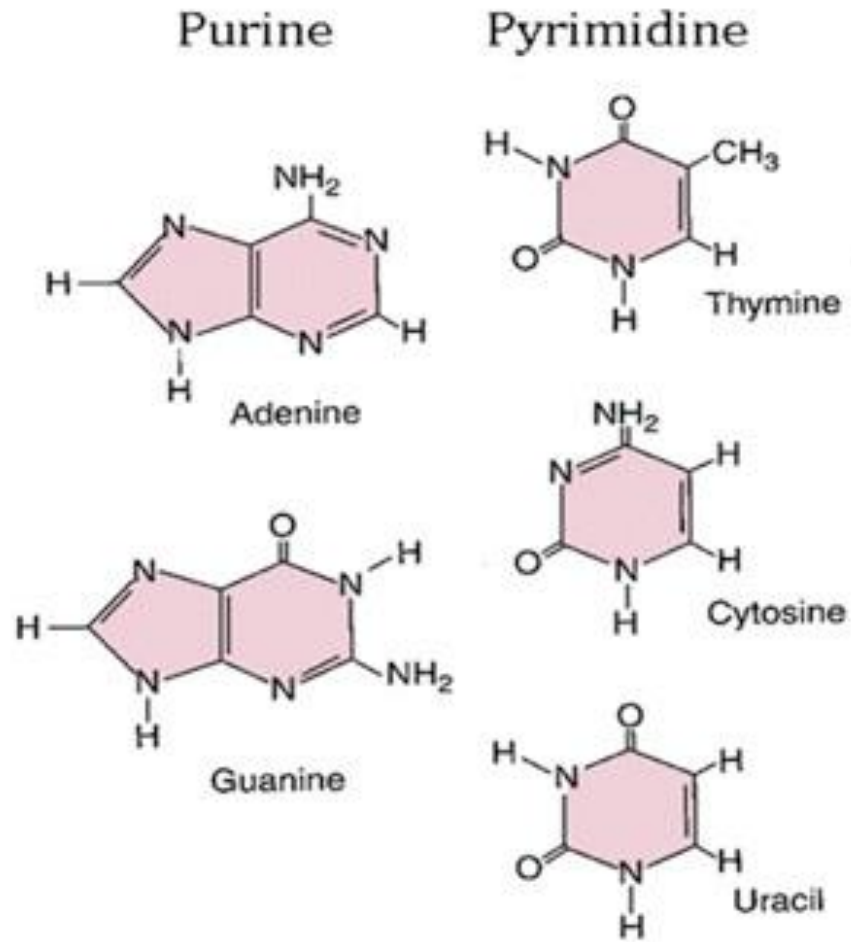
(Acetyl phosphate)

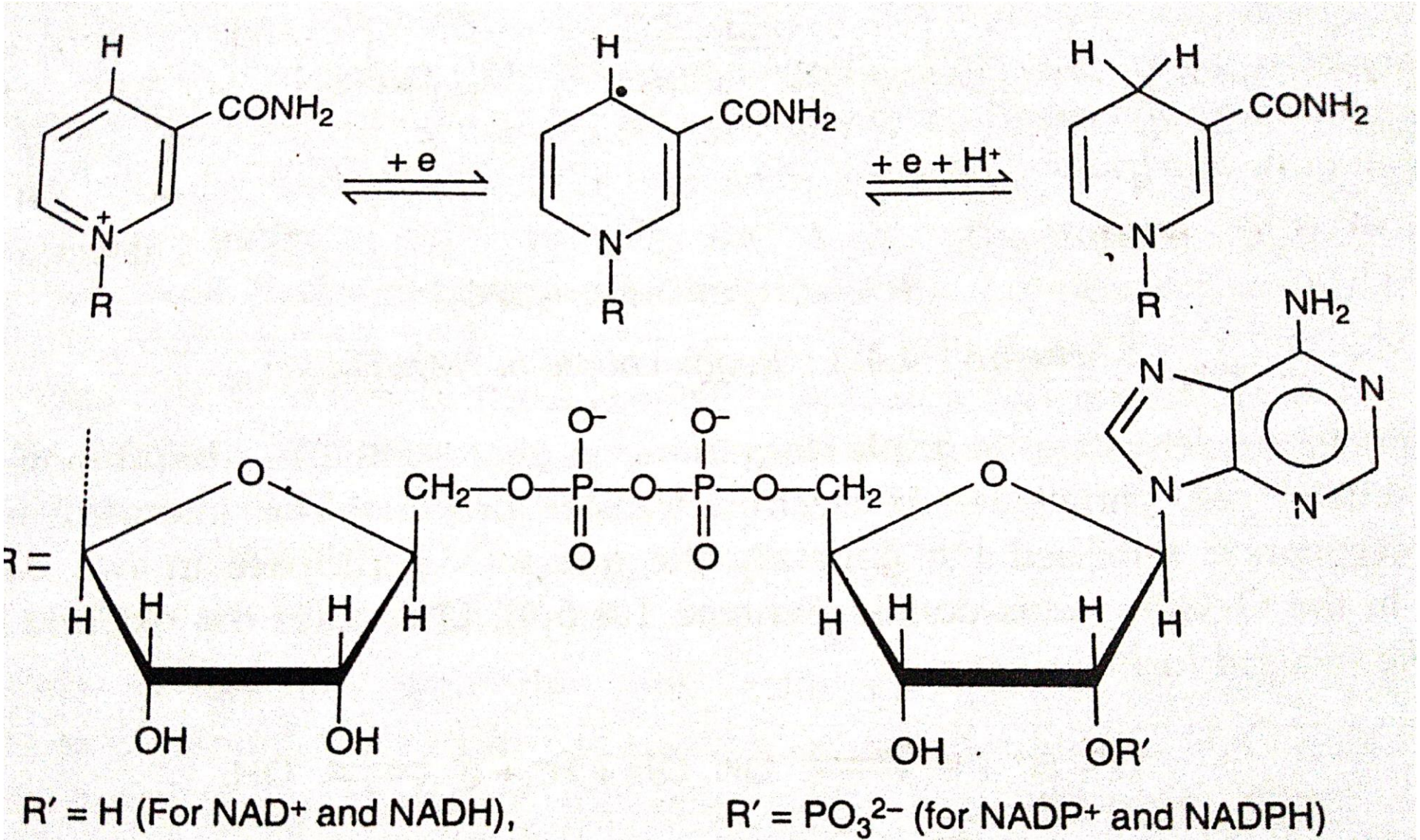


(Creatine phosphate)

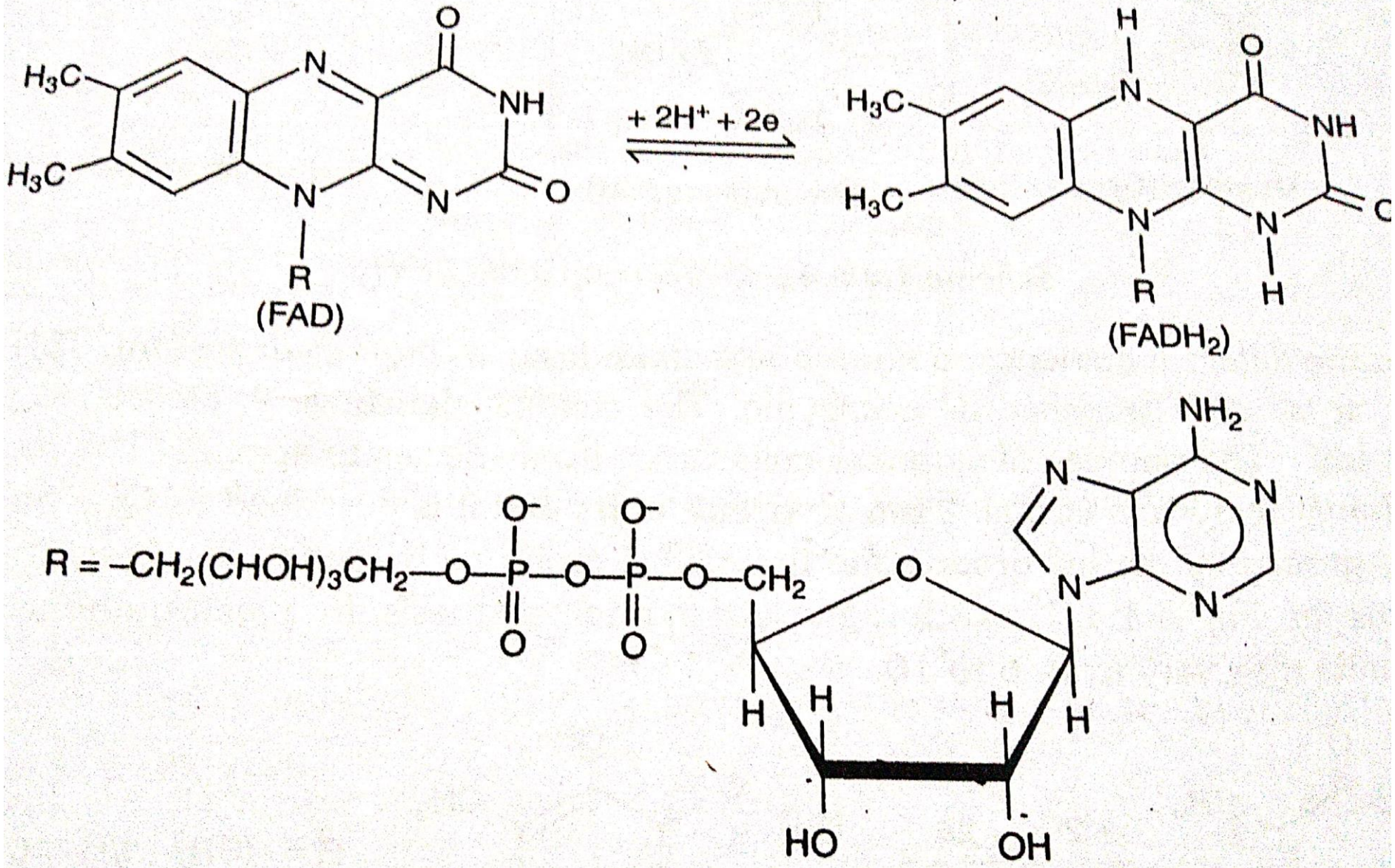
*Figure 9.1.3 : Structural representation of some compounds having **high phosphate group transfer potentials.** Competing resonance to destabilise (with respect to the hydrolysed products) the compounds is schematically shown.*


Nucleotide
Nucleoside
Nitrogenous base
Phosphate group
tri-
di-
mono-
HO
OH ribose
H deoxyribose
Sugar

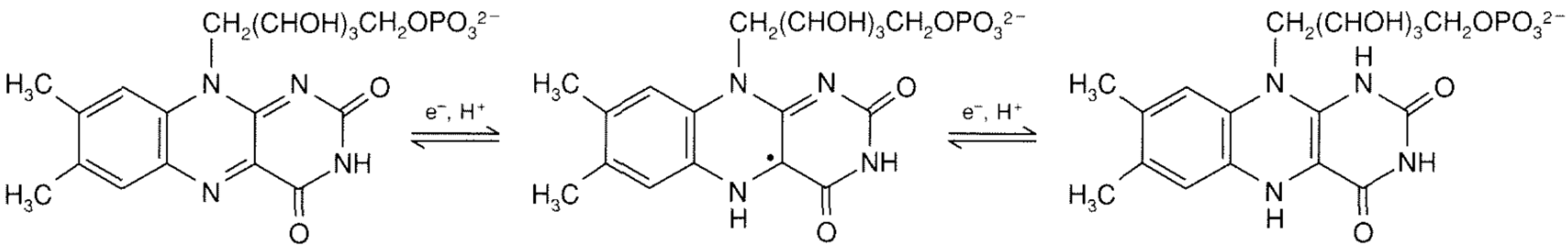

Purine
Pyrimidine
Adenine
Thymine
Guanine
Cytosine
Uracil



Scheme 1.4.6.1 : *Redox couples of NAD⁺/NADH and NADP⁺/NADPH.*



Scheme 1.4.6.2: *Redox couple of FAD/FADH$_2$.*

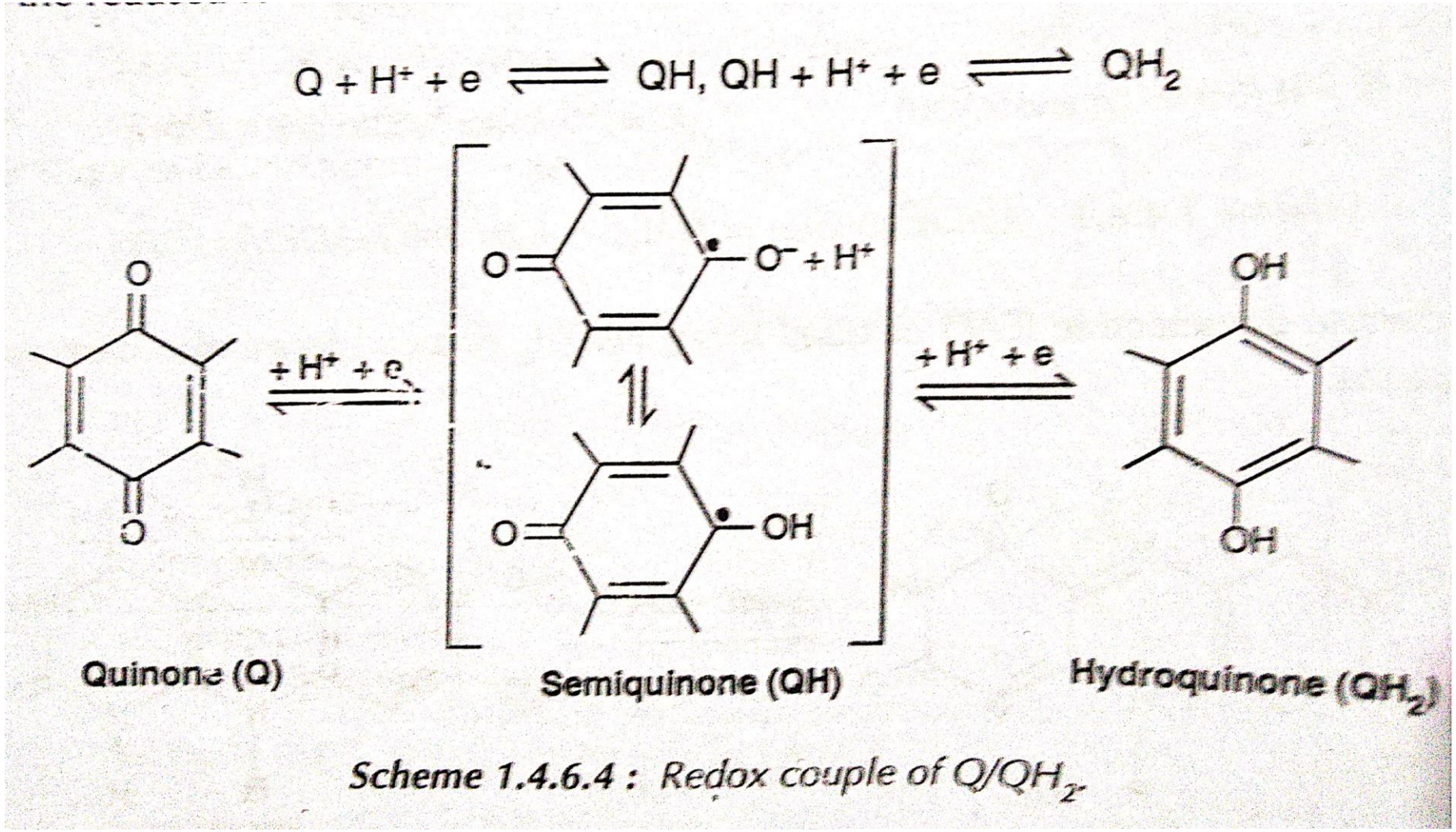


λ_{max} ~337,445 nm

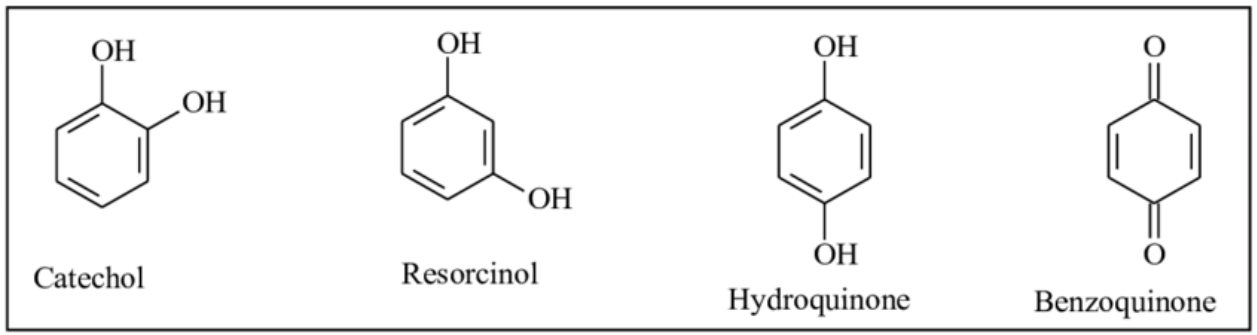
λ_{max} ~565 nm

colorless

Figure 6.3
Reduction of FMN.



Scheme 1.4.6.4 : Redox couple of Q/QH_2



of isoprene units may vary from

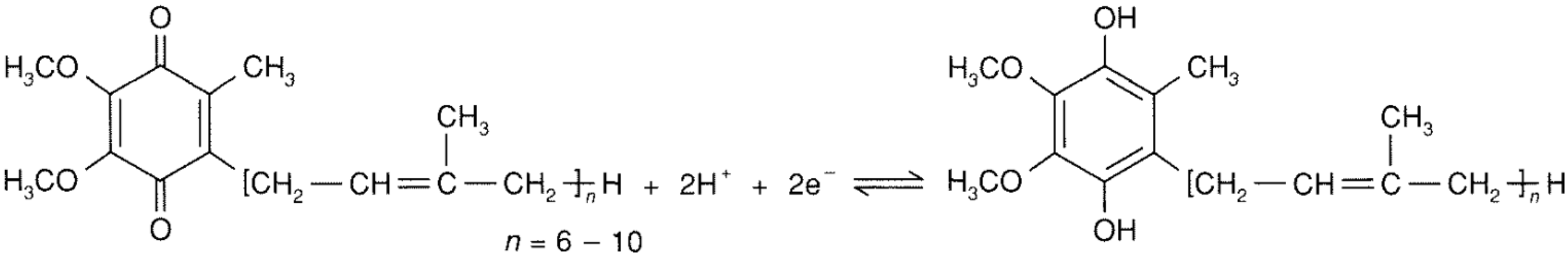


Figure 6.2
Reduction of coenzyme Q (ubiquinone) to ubiquinol.

9.4 ROLE OF PHOSPHATE IN GLUCOSE OXIDATION : GLYCOLYSIS AND CITRIC ACID CYCLE

9.4.1 Glycolysis

Metabolic oxidation of glucose is an important phenomenon. It occurs in two stages. The first stage involves the oxidative breakdown of glucose (6-C skeleton) into two molecules of pyruvic acid (3-C skeleton) through a series of reactions known as ***glycolysis***.

$$\text{Glucose} + 2NAD^+ + 2ADP^{3-} + 2HPO_4^{2-} \longrightarrow 2\ \text{pyruvate}^- + 2ATP^{4-} + 2NADH + 2H^+ + 2H_2O \quad (9.4.1.1)$$

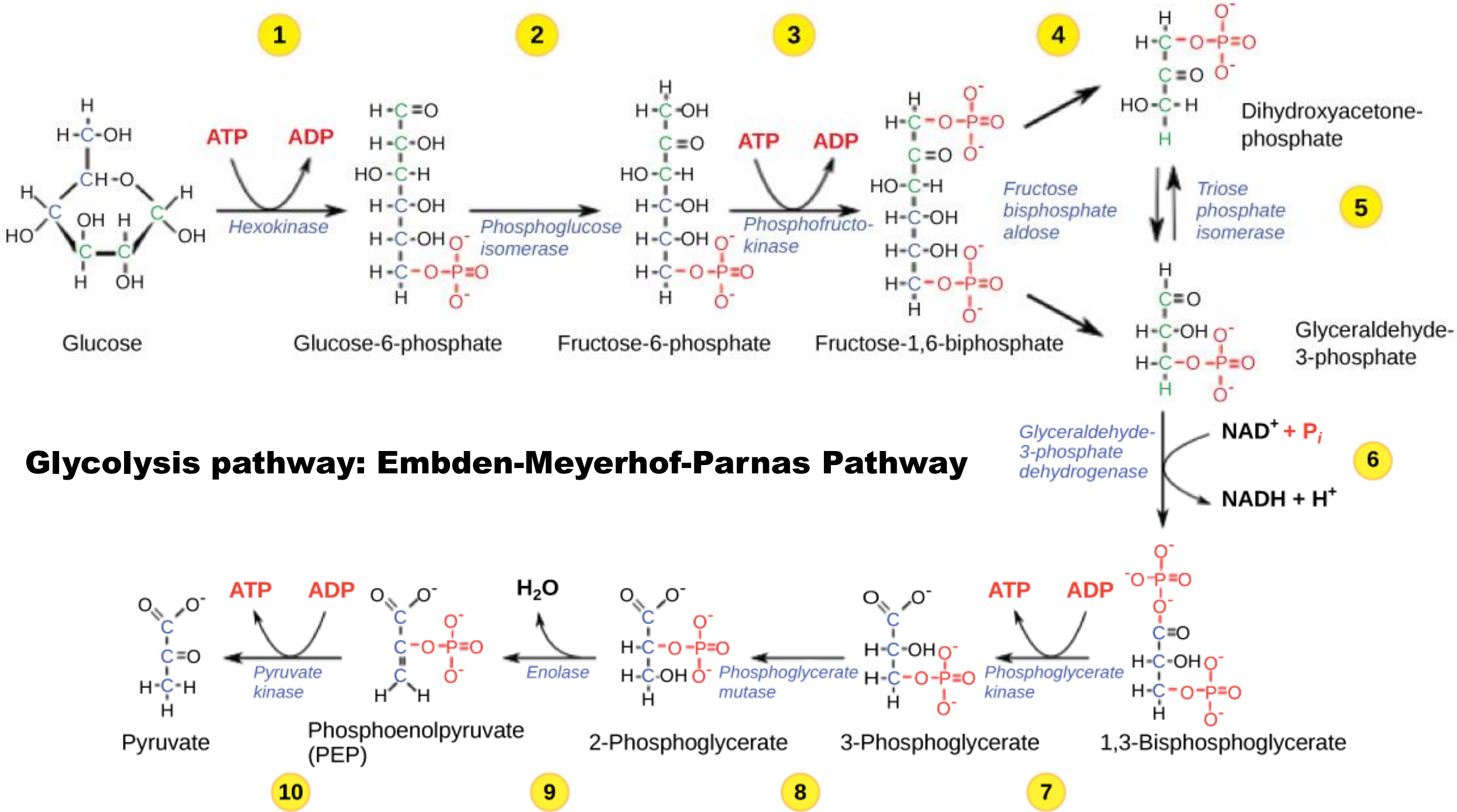
In the first stage, glucose is activated to glucose-6-phosphate through phosphorylation by ATP. Hexokinase enzyme catalyses the process (*cf.* Scheme 9.3.4). Insertion of a phosphoryl group (PO_3^-) into the skeleton of glucose makes it *chemically reactive due to the unfavourable electrostatic strain* originated from the negative charge of the phosphate group. At different steps of glycolytic pathway, phosphorylation and dephosphorylation reactions occur. These are catalysed by different enzymes. In phosphorylation process, phosphoryl group is transferred from a compound having higher phosphate group transfer potential

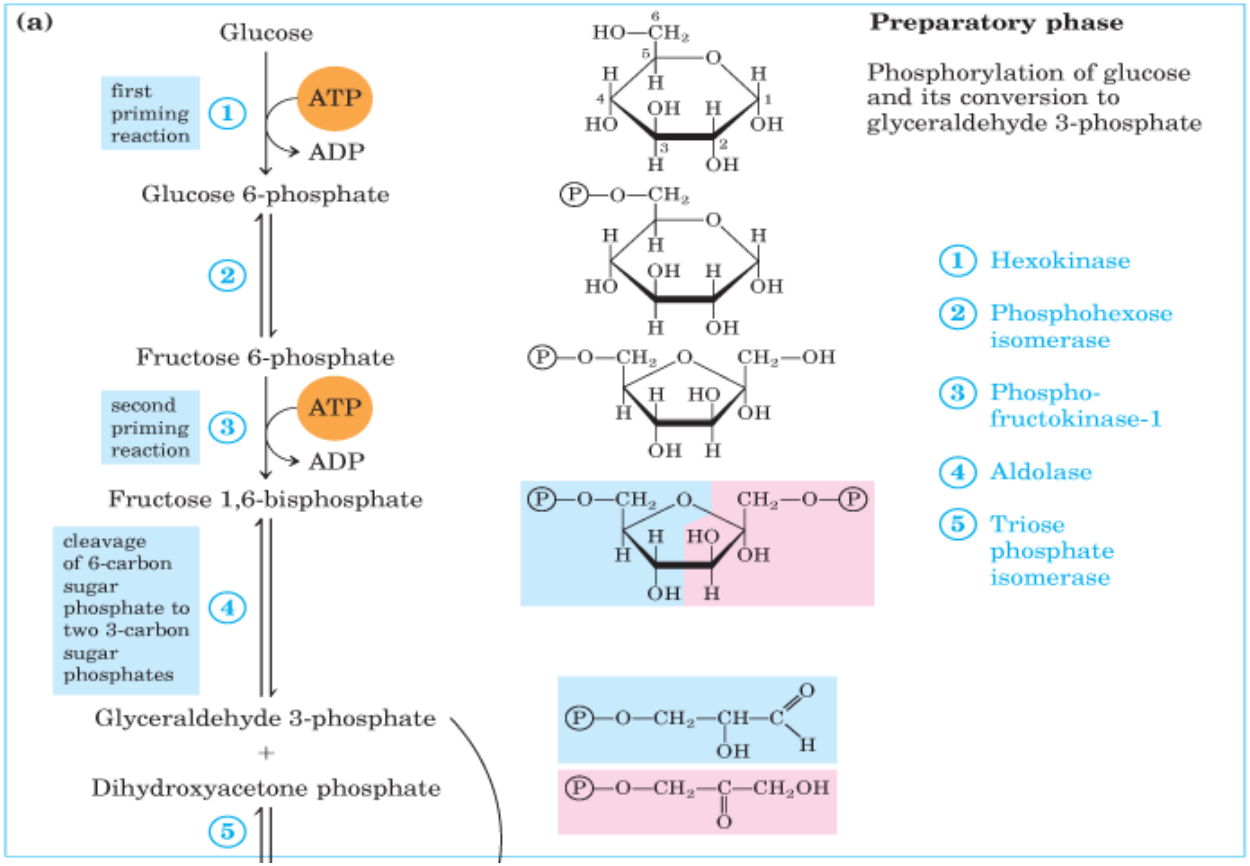
In the glycolytic pathway (called ***Embden-Meyerhof-Parnas pathway***), there are three irreversible steps catalysed by *hexokinase* (conversion of glucose to glucose-6-phosphate, *cf.* Scheme 9.3.4), *phosphofructokinase* (conversion of fructose-6-phosphate to fructose-1,6-diphosphate) and *pyruvate kinase* (conversion of phosphoenolpyruvate to pyruvate, *cf.* Scheme 9.3.5).

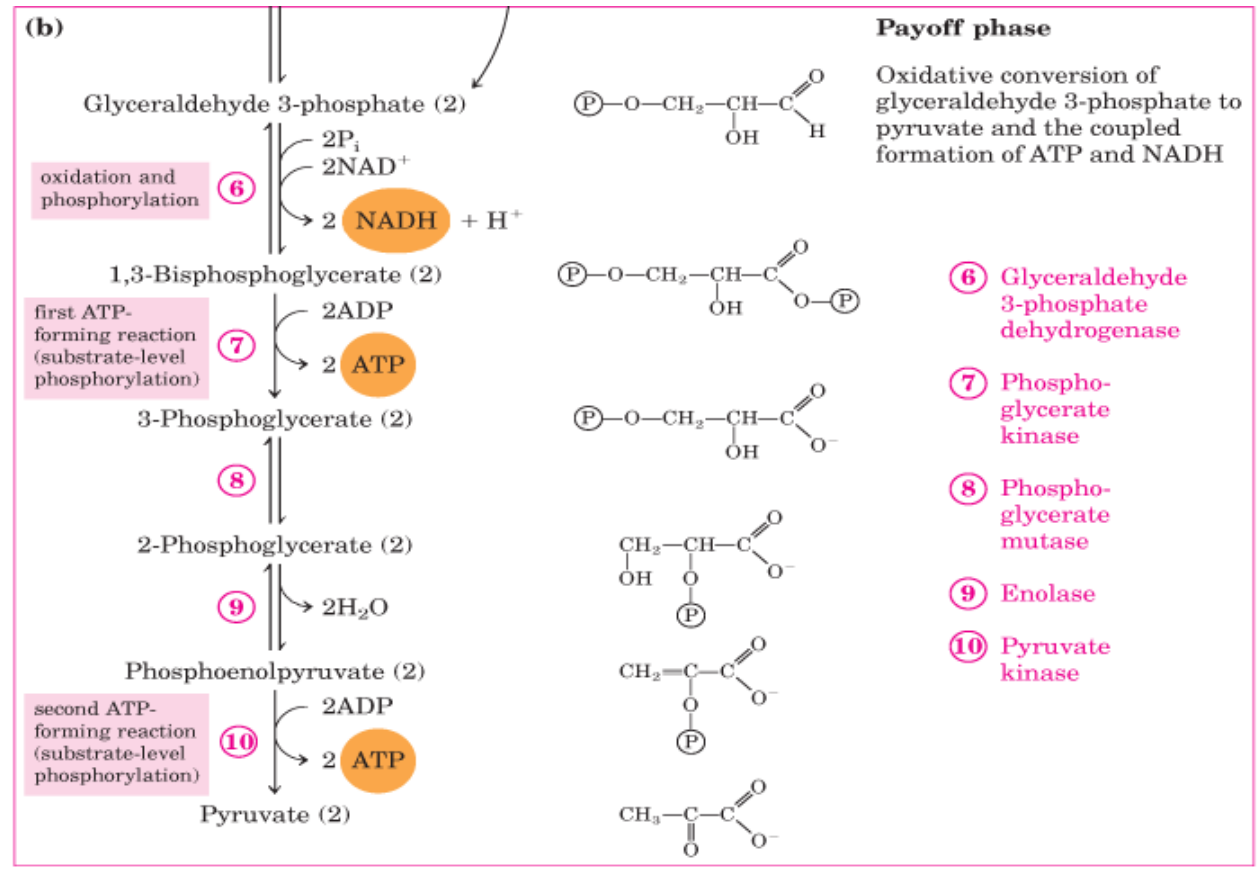
Reactions in glycolysis : (i) The first stage converts glucose to fructose-1,6-diphosphate by three successive reactions: phosphorylation, isomerisation and a second phosphorylation. This stage consumes 2 molecules of ATP per molecule of glucose. (ii) The second stage involves the cleavage of fructose-1,6-diphosphate (6-C skeleton) by aldolase into glyceraldehyde-3-phosphate (3-C skeleton) and dihydroxyacetone phosphate (3-C skeleton) which are mutually interconvertible. The active compound, glyceraldehyde-3-phosphate is converted to 1,3-diphosphoglycerate (1,3-DPG) through oxidation and phosphorylation. 1,3-diphosphoglycerate having a high *phosphate group transfer potential* can generate ATP from ADP and it is itself converted into 3-phosphoglycerate which isomerises to 2-phosphoglycerate. (iii) At the last stage, phosphoenolpyruvate is formed from 3-phosphoglycerate through a phosphoryl shift followed by dehydration. Phosphoenolpyruvate finally converts ADP to ATP and it is itself converted to pyruvate which can act as a ***building block for the biosynthesis of different cellular components***.

Consumption and generation of ATP in glycolysis : The phosphorylation and dephosphorylation reactions are controlled by the ***phosphate group transfer potential***. A compound having a higher phosphate group transfer potential can phosphorylate a compound having a lower phosphate group transfer potential. The following reactions involve ATP. ATP can

Glycolysis pathway: Embden-Meyerhof-Parnas Pathway




(a)
Glucose
first priming reaction
1
ATP
ADP
Glucose 6-phosphate
2
Fructose 6-phosphate
second priming reaction
3
ATP
ADP
Fructose 1,6-bisphosphate
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
4
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
1 Hexokinase
2 Phosphohexose isomerase
3 Phospho-fructokinase-1
4 Aldolase
5 Triose phosphate isomerase


(b)
Glyceraldehyde 3-phosphate (2)
oxidation and phosphorylation
6
2Pi
2NAD+
2 NADH + H+
1,3-Bisphosphoglycerate (2)
first ATP-forming reaction (substrate-level phosphorylation)
7
2ADP
2 ATP
3-Phosphoglycerate (2)
8
2-Phosphoglycerate (2)
9
2H2O
Phosphoenolpyruvate (2)
second ATP-forming reaction (substrate-level phosphorylation)
10
2ADP
2 ATP
Pyruvate (2)
Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
6 Glyceraldehyde 3-phosphate dehydrogenase
7 Phospho-glycerate kinase
8 Phospho-glycerate mutase
9 Enolase
10 Pyruvate kinase

1.The first step in glycolysis is the conversion of D-glucose into glucose-6-phosphate. The enzyme that catalyzes this reaction is hexokinase.

2.The second reaction of glycolysis is the rearrangement of glucose 6-phosphate (G6P) into fructose 6-phosphate (F6P) by glucose phosphate isomerase (Phosphoglucose Isomerase).

3.Phosphofructokinase, with magnesium as a cofactor, changes fructose 6-phosphate into fructose 1,6-bisphosphate.

4.The enzyme Aldolase splits fructose 1, 6-bisphosphate into two sugars that are isomers of each other. These two sugars are dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP).

5.The enzyme triophosphate isomerase rapidly inter- converts the molecules dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GAP). Glyceraldehyde phosphate is removed / used in next step of Glycolysis.

6.Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) dehydrogenates and adds an inorganic phosphate to glyceraldehyde 3-phosphate, producing 1,3-bisphosphoglycerate.

7.Phosphoglycerate kinase transfers a phosphate group from 1,3-bisphosphoglycerate to ADP to form ATP and 3-phosphoglycerate.

8.The enzyme phosphoglycero mutase relocates the P from 3- phosphoglycerate from the 3rd carbon to the 2nd carbon to form 2-phosphoglycerate.

9.The enzyme enolase removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvic acid (PEP).

10.The enzyme pyruvate kinase transfers a P from phosphoenolpyruvate (PEP) to ADP to form pyruvic acid and ATP Result in step 10.

11.Although 2 ATP molecules are used in steps 1-3, 2 ATP molecules are generated in step 7 and 2 more in step 10. This gives a total of 4 ATP molecules produced. If you subtract the 2 ATP molecules used in steps 1-3 from the 4 generated at the end of step 10, you end up with a net total of 2 ATP molecules produced.

Glycolysis in the Cytoplasm

glucose C_6

ATP → ADP

glucose 6-phosphate C_6-P

fructose 6-phosphate C_6-P

ATP → ADP

2x glyceraldehyde 3-phosphate P-C_3

2P

2 NAD+ → 2 NADH

2x 1,3-biphosphoglycerate P-C_3-P

2 ADP → 2 ATP

2x 3-phosphoglycerate P-C_3

H_2O

2x phosphoenolpyruvate P-C_3

2 ADP → 2 ATP

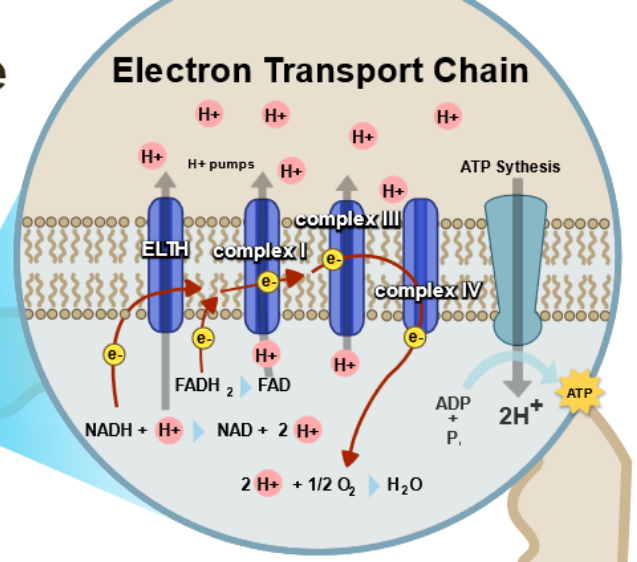
2x pyruvate C_3

-2 ATP
Energy Investment Stage

+4 ATP
Energy Harvesting Stage

Net Gain:
2 ATP

Citric Acid Cycle in the Mitochondria



NADH

CO_2 (waste)

NAD+

Ketoglutarate C_5

NAD+

Citrate C_6

Krebs Cycle

NADH

CO_2

ATP

Oxaloacetate C_4

Succinate C_4

FAD

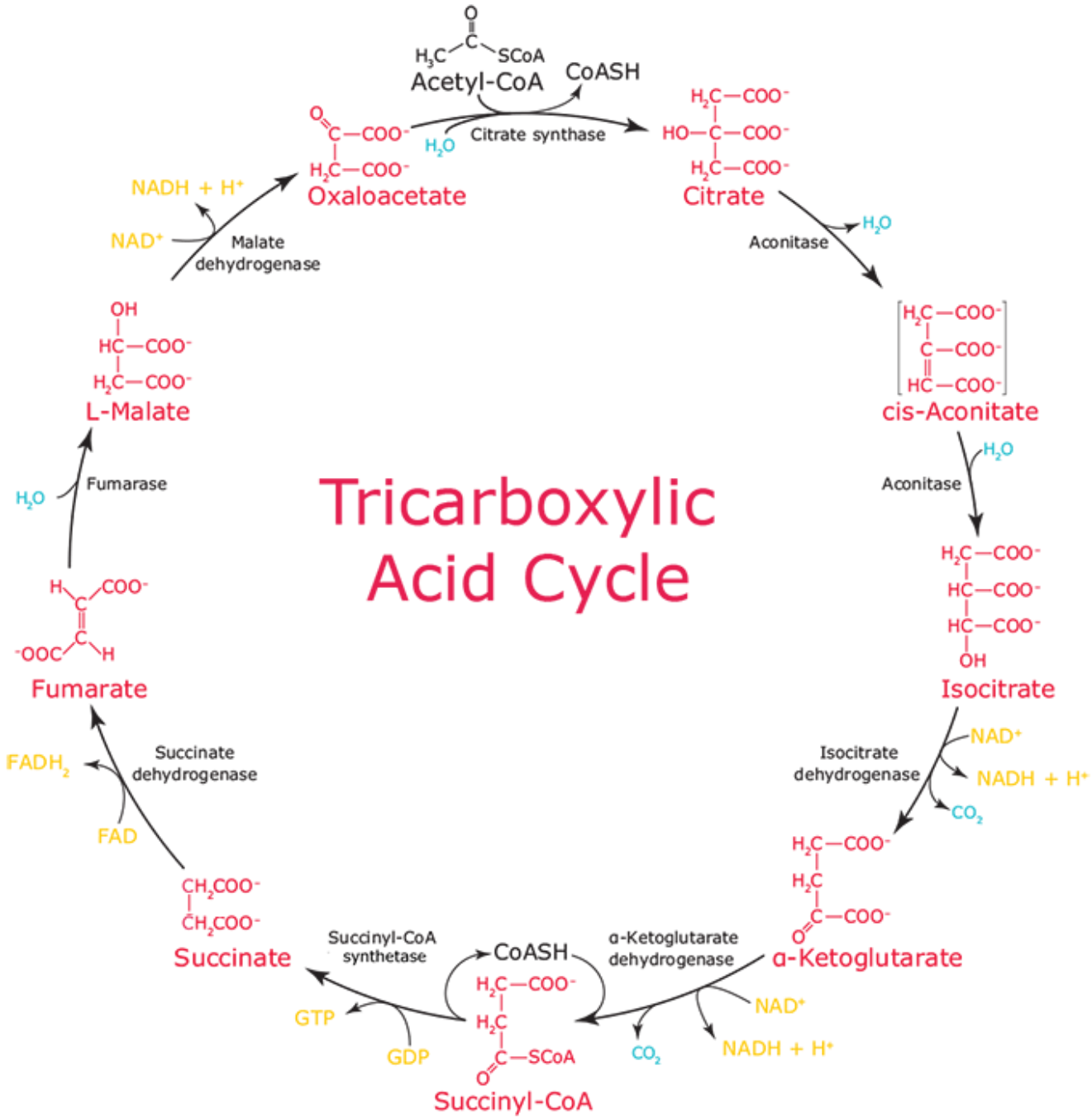
NADH

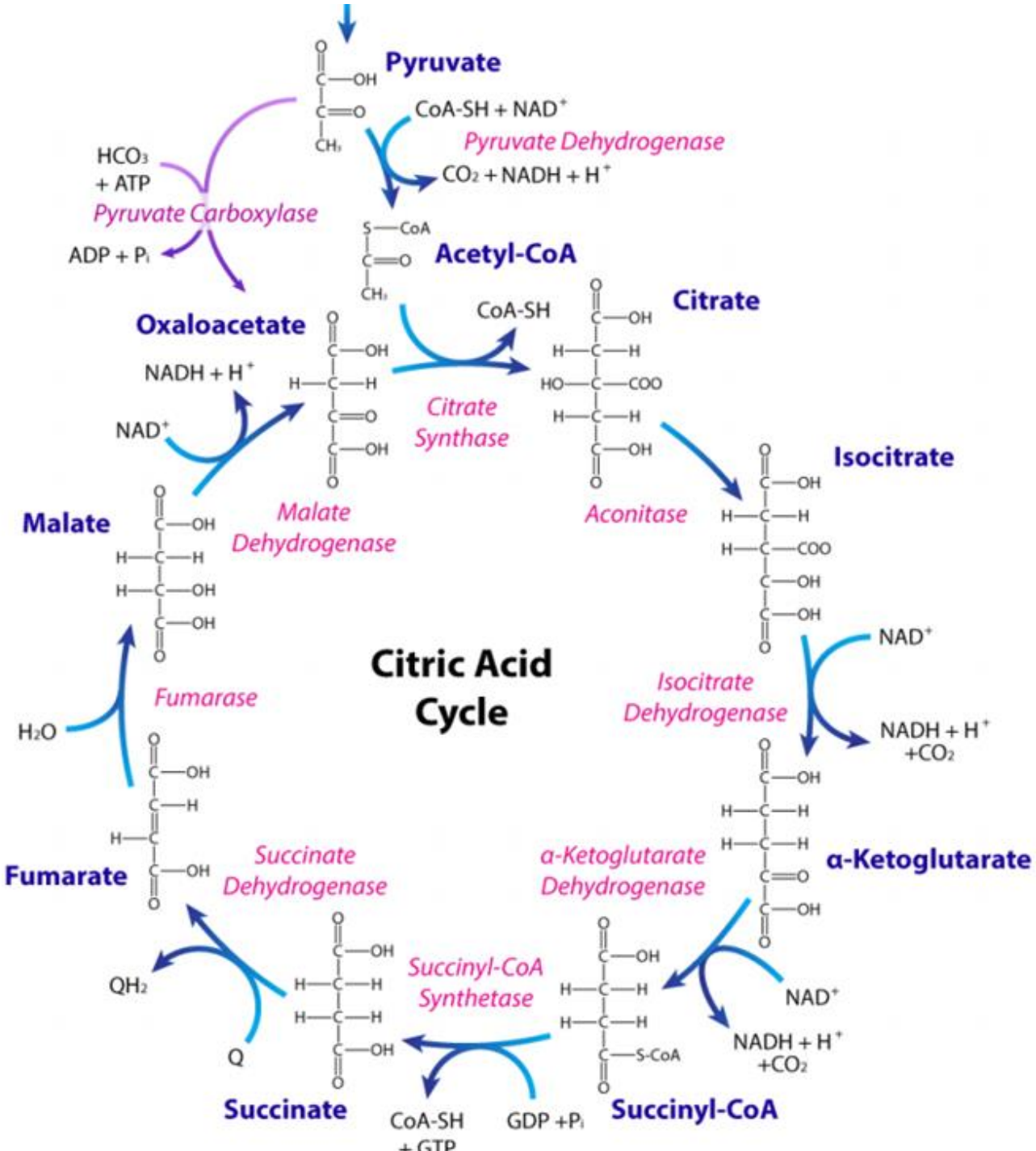
Fumarate C_4

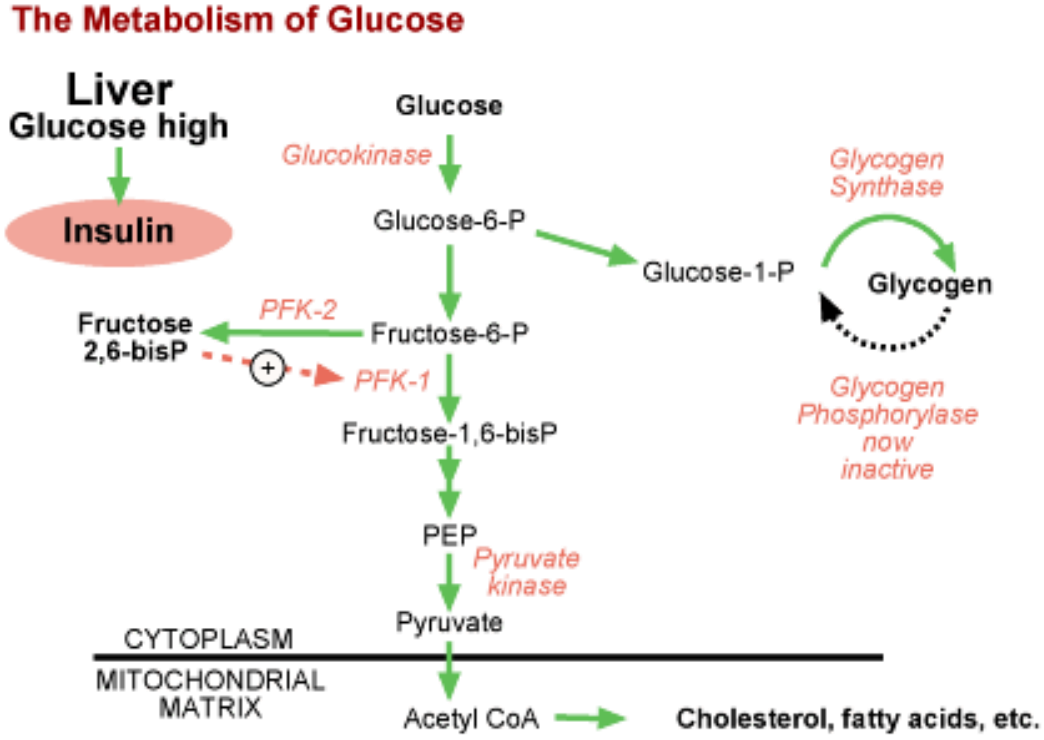
$FADH_2$

NAD+

CoA

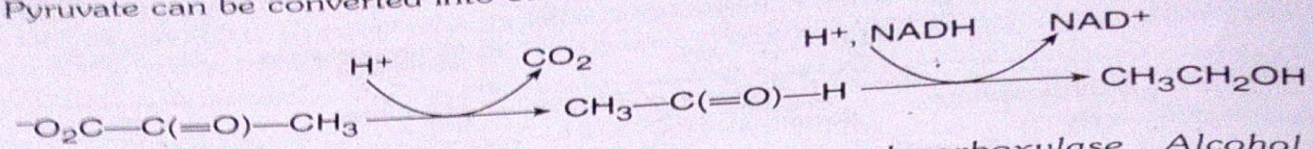

Tricarboxylic Acid Cycle
Acetyl-CoA
CoASH
Citrate synthase
Oxaloacetate
Citrate
Aconitase
cis-Aconitate
Aconitase
Isocitrate
Isocitrate dehydrogenase
α-Ketoglutarate
α-Ketoglutarate dehydrogenase
Succinyl-CoA
Succinyl-CoA synthetase
Succinate
Succinate dehydrogenase
Fumarate
Fumarase
L-Malate
Malate dehydrogenase
NADH + H+
NAD+
FADH2
FAD
GTP
GDP
CO2


Pyruvate
CoA-SH + NAD+
Pyruvate Dehydrogenase
CO2 + NADH + H+
HCO3 + ATP
Pyruvate Carboxylase
ADP + Pi
Acetyl-CoA
Oxaloacetate
CoA-SH
Citrate
NADH + H+
NAD+
Citrate Synthase
Malate
Malate Dehydrogenase
Aconitase
Isocitrate
Citric Acid Cycle
NAD+
Isocitrate Dehydrogenase
NADH + H+ +CO2
H2O
Fumarase
Fumarate
Succinate Dehydrogenase
α-Ketoglutarate Dehydrogenase
α-Ketoglutarate
QH2
Q
Succinyl-CoA Synthetase
NAD+
NADH + H+ +CO2
Succinate
CoA-SH + GTP
GDP +Pi
Succinyl-CoA


The Metabolism of Glucose
Liver
Glucose high
Insulin
Glucose
Glucokinase
Glucose-6-P
Glucose-1-P
Glycogen Synthase
Glycogen
PFK-2
Fructose 2,6-bisP
Fructose-6-P
PFK-1
Glycogen Phosphorylase now inactive
Fructose-1,6-bisP
PEP
Pyruvate kinase
Pyruvate
CYTOPLASM
MITOCHONDRIAL MATRIX
Acetyl CoA
Cholesterol, fatty acids, etc.

9.4.2 Fate of Pyruvate

Pyruvate can be converted into ethanol, lactate or acetyl coenzyme as follows :



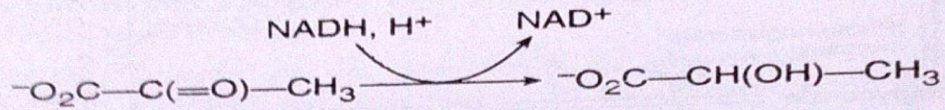
(9.4.2.1)

Decarboxylation of pyruvate is catalysed by *pyruvate decarboxylase*. *Alcohol dehydrogenase* (Sec. 7.12) catalyses the reduction of acetaldehyde to ethanol by NADH through a hydride transfer mechanism.

The overall reaction known as *alcoholic fermentation* is :

$$\text{Glucose} + 2H^+ + 2ADP + 2P_i \longrightarrow 2\text{Ethanol} + 2CO_2 + 2ATP + 2H_2O \qquad (9.4.2.2)$$

Pyruvate can also be reduced by NADH to lactate by *lactate dehydrogenase*.



(9.4.2.4)

The net reaction is :

$$\text{Glucose} + 2P_i + 2ADP \longrightarrow 2\text{Lactate} + 2ATP + 2H_2O \qquad (9.4.2.4)$$

In the conversion of pyruvate to lactate or ethanol, NAD^+ is regenerated and glycolysis process can continue *in anaerobic condition*. If NAD^+ were not regenerated, glycolysis could not proceed beyond the formation of glyceraldehyde-3-phosphate. *Anaerobic oxidative breakdown of glucose to ethanol or lactate produces a small amount of energy.* But aerobic oxidation of glucose through ***citric acid cycle*** (*i.e.* **tricarboxylic acid cycle or Krebs cycle**) releases much more energy. Formation of **acetyl-CoA (acetyl coenzyme)** is the starting point of *aerobic oxidation of glucose*.

$$^-O_2C—C(=O)—CH_3 + CoA—SH + NAD^+ \longrightarrow CoA—S—C(=O)CH_3 + CO_2 + NADH \qquad (9.4.2.5)$$

This reaction is catalysed by ***pyruvate dehydrogenase complex*** (*cf.* Scheme 9.4.3.7). The cofactor called coenzyme A (*i.e.* CoA, A stands for acetylation) has an active terminal –SH group which can participate in acetylation and deacetylation reaction. ***Acetyl CoA is biologically very important*** (see Fig. 8.7.2 for its structure). The free energy change for its hydrolysis is negative ($\Delta G_0' = -31.5$ kJ mol^{-1}).

$$CH_3CO–S–CoA + H_2O \rightleftharpoons CH_3CO_2^- + CoA–SH + H^+, \qquad (9.4.2.6)$$

Thus acetyl CoA has a **high *acetyl group transfer potential*** and it acts as a carrier of activated acetyl group (*cf.* ATP has a high phosphate group transfer potential and it is a carrier of activated *phosphoryl group*). In aerobic oxidation, in the citric acid cycle NAD^+ is regenerated from NADH to sustain the glycolysis.

• ***Phosphoglycerate kinase*** catalyses (Scheme 9.3.4b) the conversion of 1,3-phosphoglycerate into 3-phosphoglycerate. In the activity of phosphoglycerate kinase, Mg^{2+} probably plays the similar role as in the case of hexokinase. Here, a β-phosphoryl oxygen of Mg-ADP complex makes a nucleophilic attack on the P-atom of 1,3-diphosphoglycerate to displace the phosphoryl group giving rise to Mg-ATP complex and 3-phosphoglycerate.

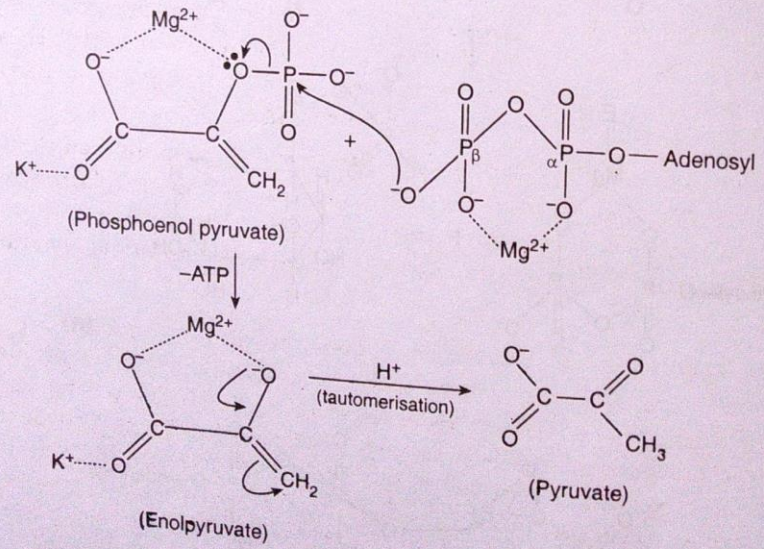
• ***Pyruvate kinase*** catalyses (Scheme 9.3.5) the conversion of phosphoenolpyruvate to pyruvate. It requires Mg^{2+}/Mn^{2+} for activity. It is also suggested that in addition to the divalent metal ion, the monovalent cation K^+ also participates in controlling the enzymatic activity of pyruvate kinase. Here, the transfer of a phosphoryl group from phosphoenolpyruvate to Mg-ADP complex occurs in the same way as in the case of phosphoglycerate kinase catalysed conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate. At the next step, enolpyruvate tautomerises to pyruvate and this step is thermodynamically highly favoured ($\Delta G_0' = -31.4$ kJ mol^{-1}).

The $\Delta G_0'$ (= − 31.3 kJ mol^{-1}) of the overall process is obtained as follows :

$$\text{PHosphoenolpyruvate} + H_2O \rightleftharpoons \text{Pyruvate} + P_i, \qquad \Delta G_0' = -62 \text{ kJ mol}^{-1}$$

$$ADP + P_i \rightleftharpoons ATP + H_2O, \qquad \Delta G_0' = +30.7 \text{ kJ mol}^{-1}$$

i.e. $$\text{Phosphoenolpyruvate} + ADP \rightleftharpoons \text{Pyruvate} + ATP, \qquad \Delta G_0' = (-62 + 30.7) = -31.3 \text{ kJ mol}^{-1}$$



Scheme 9.3.5 : *Mechanistic steps of* ***pyruvate kinase*** *(Mg^{2+}-dependant) catalysed conversion of phosphoenolpyruvate to enolpyruvate and then thermodynamically favoured tautomerisation to pyruvate.*

• ***Adenylate kin***

9.3 MECHANISM OF HYDROLYSIS OF ATP AND PHOSPHATE GROUP TRANSFER FROM ATP : ATP-ASE AND KINASE ENZYMES

(a) Kinase Enzyme Activity

In biological system, the *terminal phosphoryl group* of ATP is very often transferred to the receptor substrate having a lower phosphate group transfer potential compared to that of ATP.

$$ATP^{4-} + HX \rightleftharpoons ADP^{3-} + XPO_3^{2-} + H^+ \qquad (9.3.1)$$

As for example :

$$\text{Glucose} + \text{ATP} \xrightleftharpoons{\text{Hexokinase}} \text{Glucose-6-phosphate} + \text{ADP} + H^+, (\Delta G_0' = -16.8 \text{ kJ mol}^{-1}) \qquad (9.3.2)$$

This type of reaction is catalysed by the enzymes called *kinase* which requires the bivalent metal ions (generally Mg^{2+} or Mn^{2+}) for its activity. Several kinase enzymes are involved in glycolysis (Sec 9.4). The hydrolysis of ATP to ADP is catalysed by ATP-ase.

Kinases are actually *transferase* enzymes. In some kinases (*e.g.* nucleoside diphosphate kinase) phosphoryl group transfer occurs to a group belonging to the enzyme itself. It also happens so in alkalaline phosphatase (Sec. 6.9). In other kinases, the phosphoryl group transfer occurs directly from the donor to the acceptor through the formation of a *ternary complex*.

be generated if the released free energy (ΔG°) is at least 30.7 kJ mol^{-1}. There are two such generating reactions in the total glycolytic path. Glycolysis also produces 2 molecules of NA which can be oxidized (in respiratory electron transport chain) back to NAD^+ to produce 6 mole of ATP. Thus in glycolysis, 1 molecule glucose ≡ 2NADH + 2ATP (*cf.* citric acid cycle).

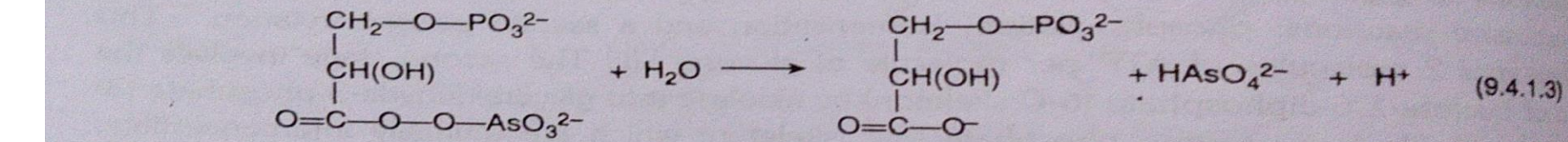
Reaction	ATP changes per molecule of glucose
Glucose → Glucose-6-phosphate	- 1 (*i.e.* consumption)
Fructose-6-phosphate → Fructose-1,6-diphosphate	- 1 (*i.e.* consumption)
2 (1,3-Diphosphoglycerate → 3-Phosphoglycerate)	+ 2 (*i.e.* generation)
2 (Phosphoenolpyruvate → Pyruvate)	+ 2 (*i.e.* generation)
Total	+ 2 (*i.e.* generation)

The glycolysis performs three roles : generation of ATP, NADH (2 per glucose molecule yruvate. **Pyruvate is used as a building block in different biosynthesis.** In aerobic oxid yruvate enters into **Krebs cycle** and electron transport chain. In anaerobic oxidation, pyruv onverted to ethanol and/or lactate.

Arsenate (AsO_4^{3-}) acts as an uncoupler in glycolysis : Arsenate (AsO_4^{3-}) resembles phosphate (PO_4^{3-}) in structure and reactivity. In the presence of AsO_4^{3-}, it can enter into the glycolytic path by replacing PO_4^{3-}. During glycolysis, glyceraldehyde-3-phosphate is converted into 1,3-diphosphoglycerate (1,3-DPG) or 1, 3-bisphosphoglycerate (1, 3-BPG) through oxidation coupled by phosphorylation. 1,3-DPG is a *high-energy phosphate compound which can produce ATP.*

$$^{-2}O_3P{-}OCH_2{-}CH(OH){-}CHO + NAD^+ + P_i \rightleftharpoons$$
$$^{-2}O_3P{-}OCH_2{-}CH(OH){-}C(=O){-}OPO_3^{2-}\ (1,3\text{–DPG}) + NADH \qquad (9.4.1.2)$$

In the presence of AsO_4^{3-}, the above reaction produces 1-arseno-3-phosphoglycerate, $^{-2}O_3P{-}OCH_2{-}CH(OH){-}C(=O){-}OAsO_3^{2-}$, instead of 1,3-DPG. This arseno-compund is ***kinetically unstable*** (*i.e.* kinetically labile) and it is rapidly hydrolysed to 3-phosphoglycerate.



(9.4.1.3)

(1-arseno-3-phosphoglycerate) **(3-phosphoglycerate)**

Thus in the presence of AsO_4^{3-}, the net reaction is :

$$\text{Glyceraldehyde-3-phosphate} + NAD^+ + H_2O \longrightarrow$$
$$\text{3-Phosphoglycerate} + NADH + 2H^+ \qquad (9.4.1.4)$$

In normal glycolytic path, during the conversion of glyceraldehyde-3-phosphate to 3 phosphoglycerate *via* 1,3-DPG, *ATP is generated.* **But, *in the presence of arsenate, this ATP generation does not occur.*** In the presence of phosphate, oxidation being coupled by phosphorylation produces the high-energy phosphate compound, 1,3-DPG, but *arsenate uncouples the reaction* and only the oxidation product, 3-phosphoglycerate is produced.

Glycolysis is the metabolic process that serves as the foundation for both aerobic and anaerobic cellular respiration. In glycolysis, glucose is converted into pyruvate. Glucose is a six- memebered ring molecule found in the blood and is usually a result of the breakdown of carbohydrates into sugars. It enters cells through specific transporter proteins that move it from outside the cell into the cell's cytosol. All of the glycolytic enzymes are found in the cytosol.

$$C_6H_{12}O_6 + 2\ NAD^+ + 2\ ADP + 2\ P \longrightarrow 2\ \text{pyruvic acid, } (CH_3(C{=}O)COOH + 2\ ATP + 2\ NADH + 2\ H^+$$

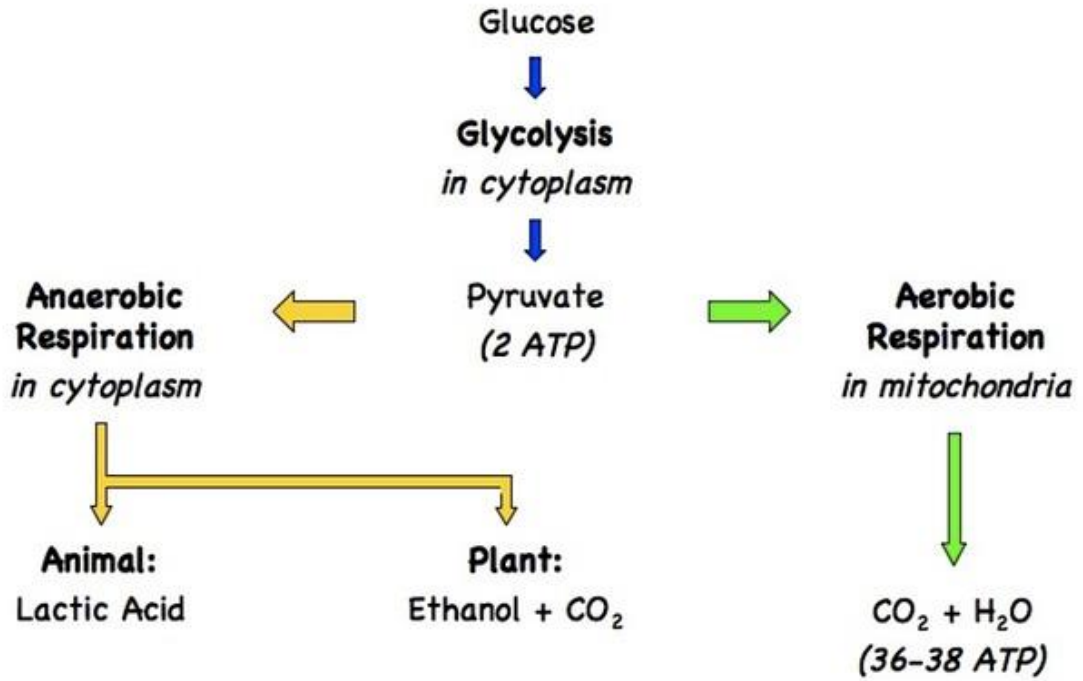
The simplified reaction is as follows:

$$C_6H_{12}O_6\ (s) + 6\ O_2\ (g) \rightarrow 6\ CO_2\ (g) + 6\ H_2O\ (l) + \text{heat}$$

ΔG = -2880 kJ per mole of $C_6H_{12}O_6$

A negative ΔG indicates that the reaction can occur spontaneously.

- The steps of glycolysis remain the same till the formation of pyruvate in both aerobic and anaerobic modes of respiration. **After pyruvate formation, depending on the availability of oxygen the pyruvate will have different fates in different organisms.**
- In animals, where there is sufficient oxygen supply **pyruvate enters the mitochondria and is completely oxidized to carbon dioxide. In the absence of oxygen, it enters the anaerobic respiration where it is converted to lactate**.
- So, Under aerobic conditions, the net formation of ATP until the formation of pyruvate is **2 ATP + (1 NADH = 3 ATP) = 5 ATP**. However, under anaerobic conditions, **the NADH is not converted to ATP**; therefore, the net production of ATP is only 2 ATP.

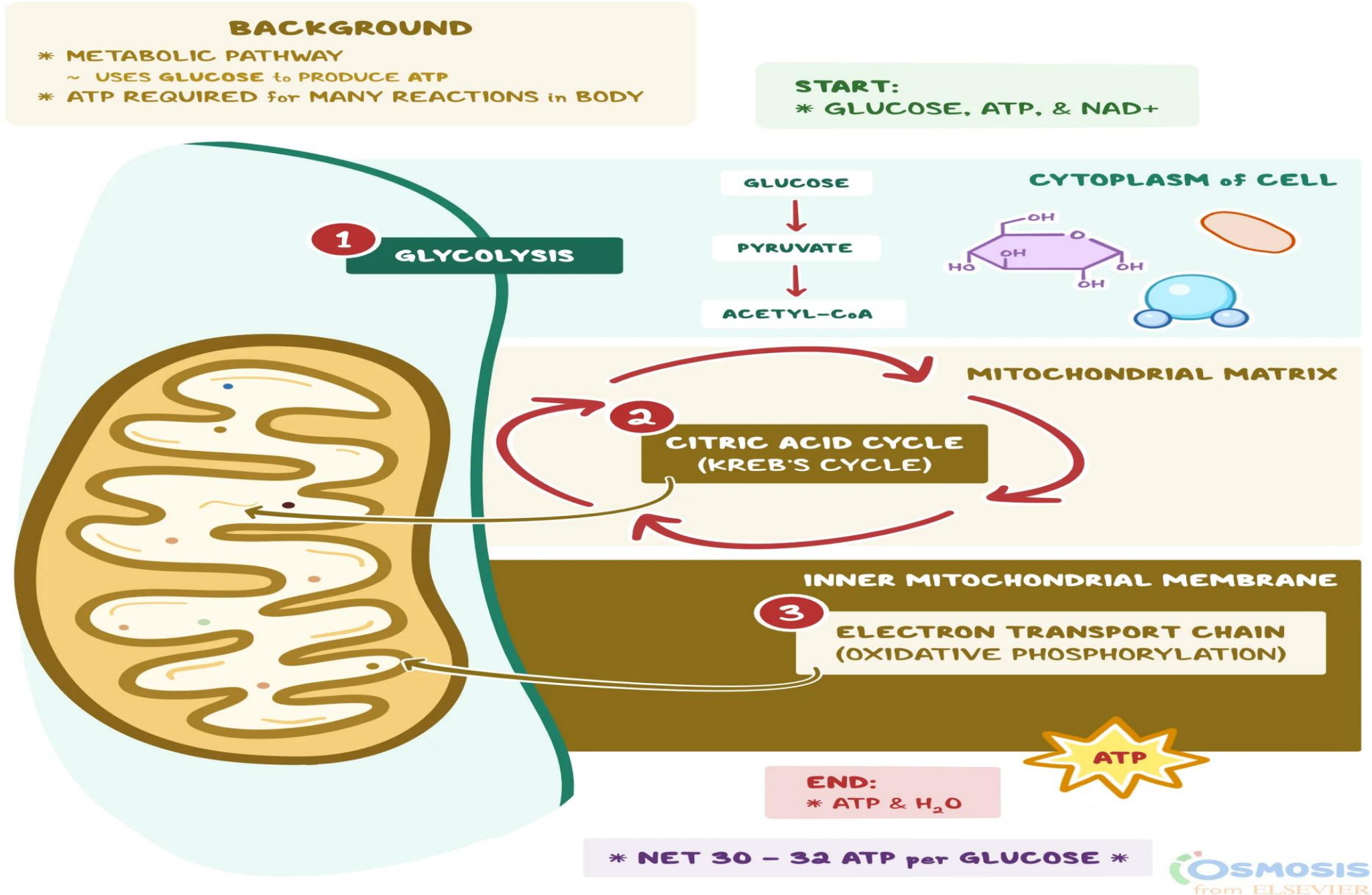


one molecule of glucose oxidized by aerobic respiration in prokaryotes yields the following:
Glycolysis
2 net ATP from substrate-level phosphorylation
2 NADH yields 6 ATP (assuming 3 ATP per NADH) by oxidative phosphorylation
Transition Reaction
2 NADH yields 6 ATP (assuming 3 ATP per NADH) by oxidative phosphorylation
Citric Acid Cycle
2 ATP from substrate-level phosphorylation
6 NADH yields 18 ATP (assuming 3 ATP per NADH) by oxidative phosphorylation
2 $FADH_2$ yields 4 ATP (assuming 2 ATP per $FADH_2$) by oxidative phosphorylation

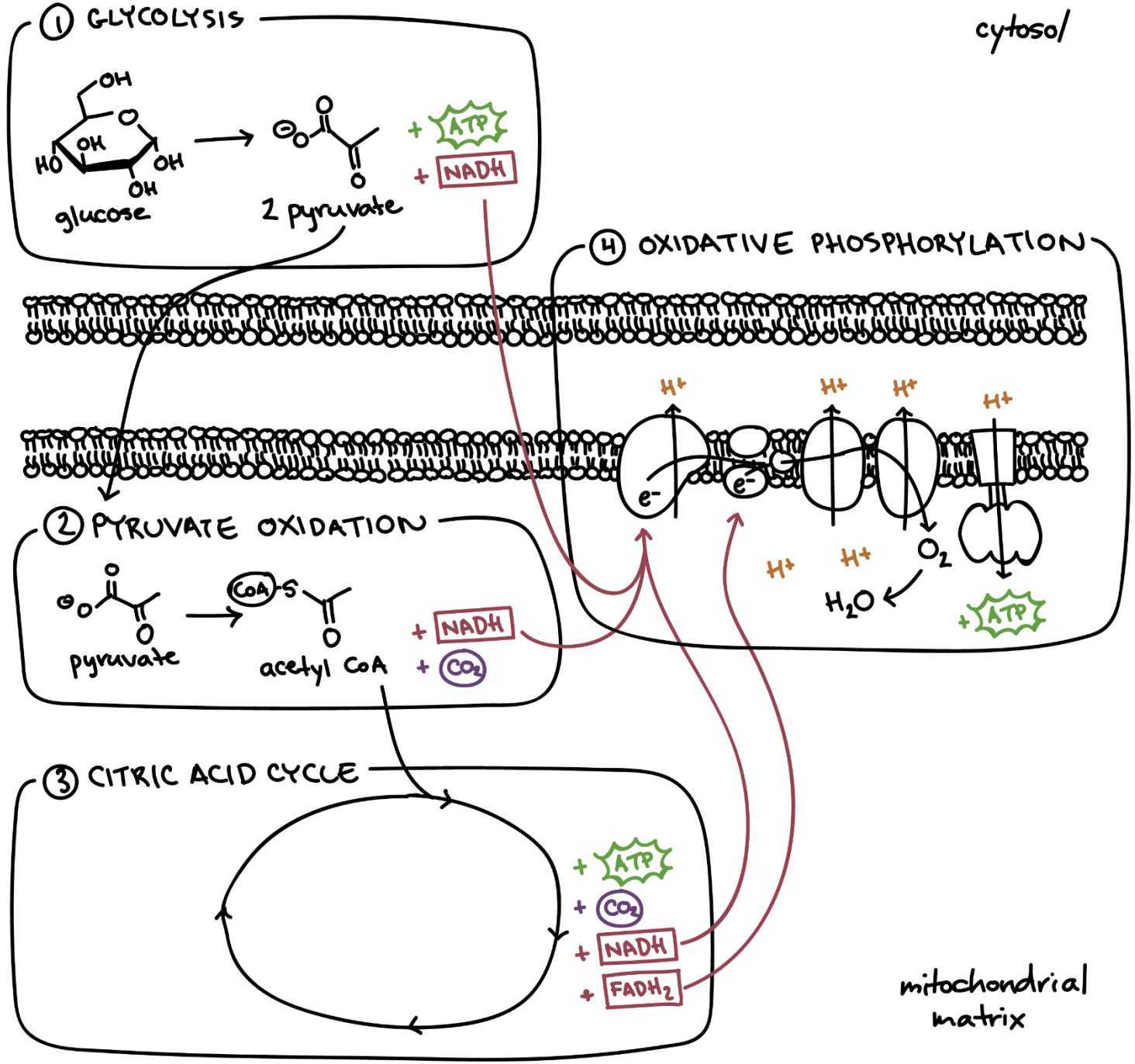
Total Theoretical Maximum Number of ATP Generated per Glucose in Prokaryotes

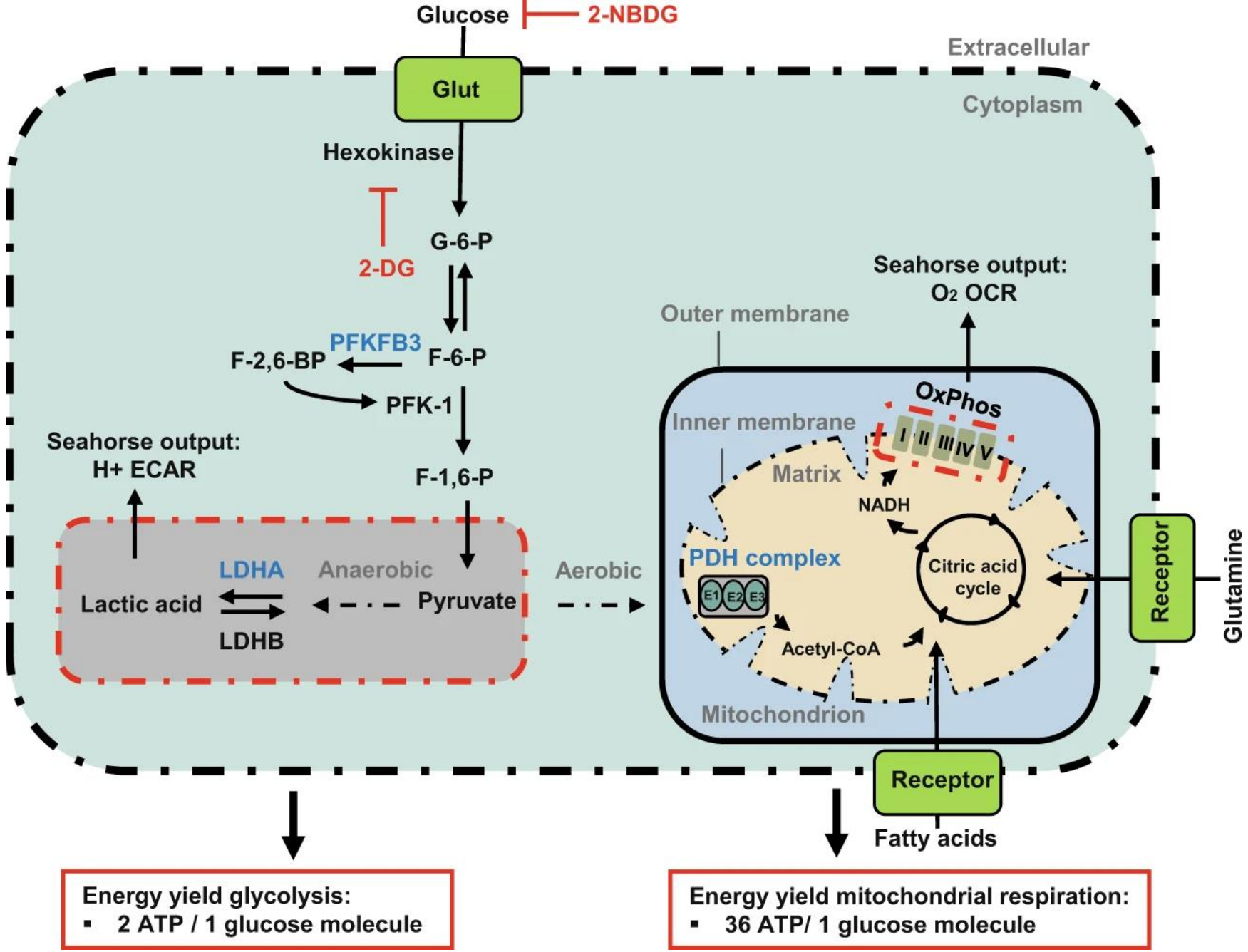
38 ATP: 4 from substrate-level phosphorylation; 34 from oxidative phosphorylation.

In eukaryotic cells, the theoretical maximum yield of ATP generated per glucose is 36 to 38, depending on how the 2 NADH generated in the cytoplasm during glycolysis enter the mitochondria and whether the resulting yield is 2 or 3 ATP per NADH.



https://www.osmosis.org/answers/cellular-respiration


cytosol
1 GLYCOLYSIS
glucose
2 pyruvate
+ ATP
+ NADH
4 OXIDATIVE PHOSPHORYLATION
H+
e-
O2
H2O
+ ATP
2 PYRUVATE OXIDATION
pyruvate
acetyl CoA
CoA
+ NADH
+ CO2
3 CITRIC ACID CYCLE
+ ATP
+ CO2
+ NADH
+ FADH2
mitochondrial matrix

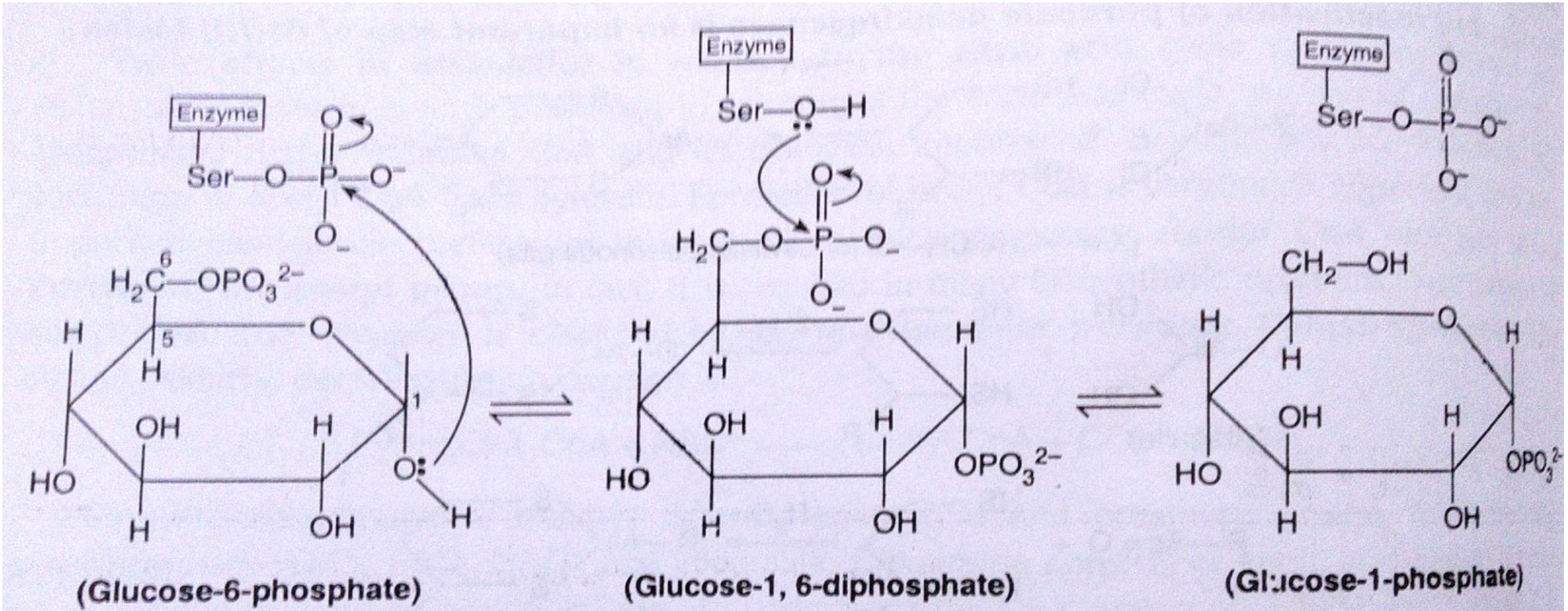


https://doi.org/10.1038/s41598-019-48676-2 Sci.Rep. 2019, 9, 12608

Glucose storage as Glycogen

- Although glucose is the primary fuel for cells, **it is not an efficient molecule for long-term storage in complex** (i.e. greater than single-celled) organisms. Therefore, in both plants and animals, the glucose molecules are linked together to form **polysaccharides known as glucans**
- **In animals, the glucan formed is glycogen**, which consists of glucose molecules linked by α(1->4) glycosidic bonds, and branching α(1->6) bonds approximately between 8 to 14 residues apart. The average size of a glycogen unit is a cytoplasmic granule containing over 100000 glucose molecules
- **Glycogen synthesis begins with UDP-glucose phosphorylase**, which combines the nucleotide uridine triphosphate (UTP) with glucose-1-phosphate to release pyrophosphate (PP_i) and form UDP-glucose
- **In the next step, glycogen synthase attaches the UDP-glucose to the pre-existing glycogen chain with an α(1->4) linkage**. It cannot join two individual glucoses together, only add to a pre-existing chain
- **Similarly, in plants, the major disaccharide is sucrose**, formed by the linkage of UDP-glucose and fructose-6-phosphate. This results in sucrose-6-phosphate, which is then readily dephosphorylated to sucrose

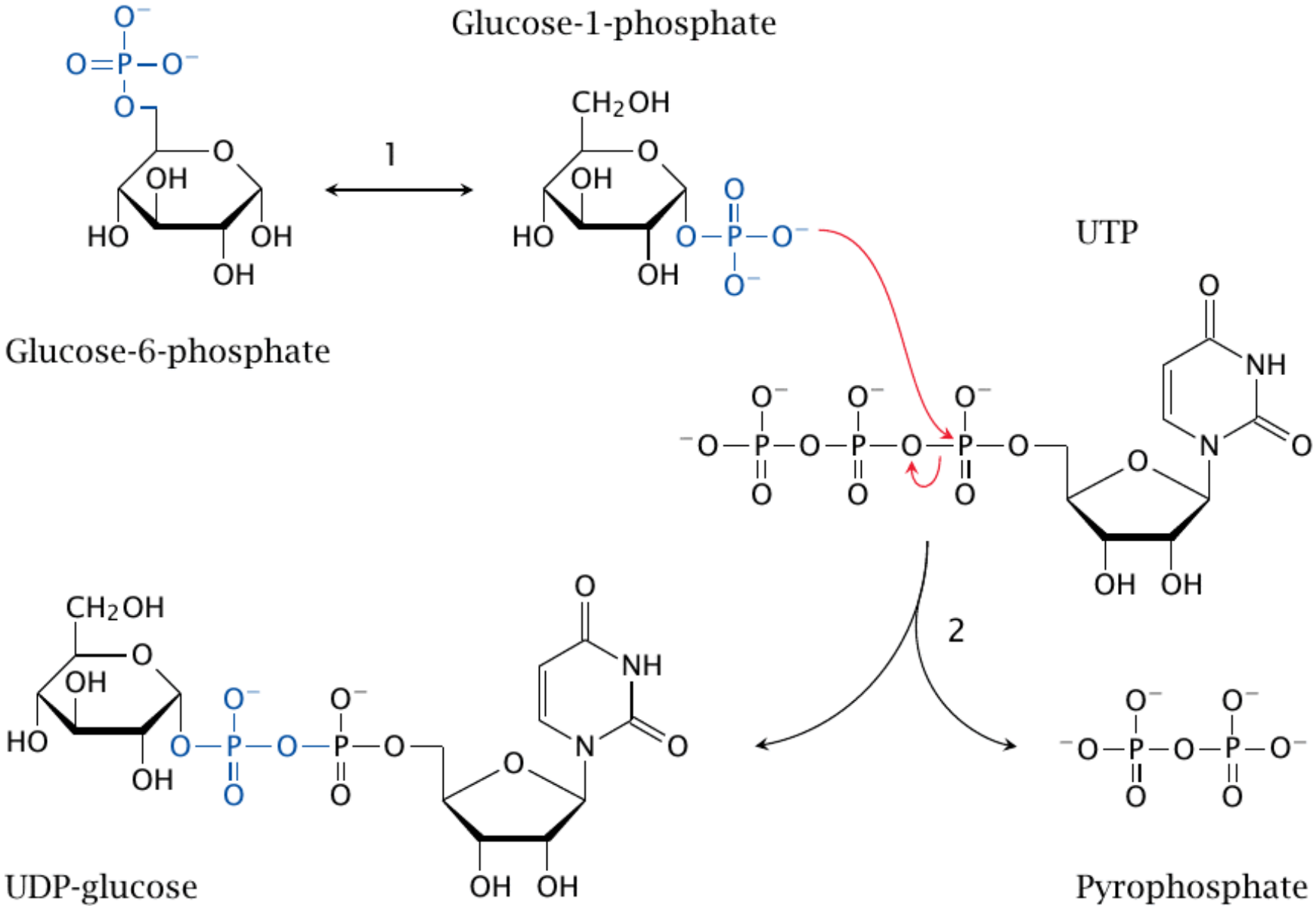
Glycogenesis - Glucose storage as Glycogen



Scheme 9.4.4.2 : *Mechanistic steps of phosphoglucomutase catalysed inetrconversion of glucose-6-phosphate to glucose-1-phosphate. Binding of the substrate by the C_3-OH group with the Mg(II) center of the enzyme is not shown here.*

Thus the seryl-phosphate group transits between the 1- and 6-positions of glucose. Rotation of glucose-1,6-diphosphate around the Mg(II)—OH bond (using the C_3-OH group) leads to the

Glucose storage as Glycogen



Glucose storage as Glycogen

required protein conformation change and then the C_6-phosphate is transferred to the serine —OH group. Thus the *first step* of glycogen synthesis is:

Glucose-6-phosphate $\xrightarrow{\text{(phosphoglucomutase)}}$ glucose-1- phosphate

The *second step* of glycogen synthesis is the formation of UDPG (*uridine diphosphate glucose*) from UTP (*uridine triphosphate*).

UTP + Glucose-1- phosphate $\longrightarrow$ UDPG + PP_i (pyrophosphate); $PP_i \xrightarrow{Mg^{2+}} 2P_i$

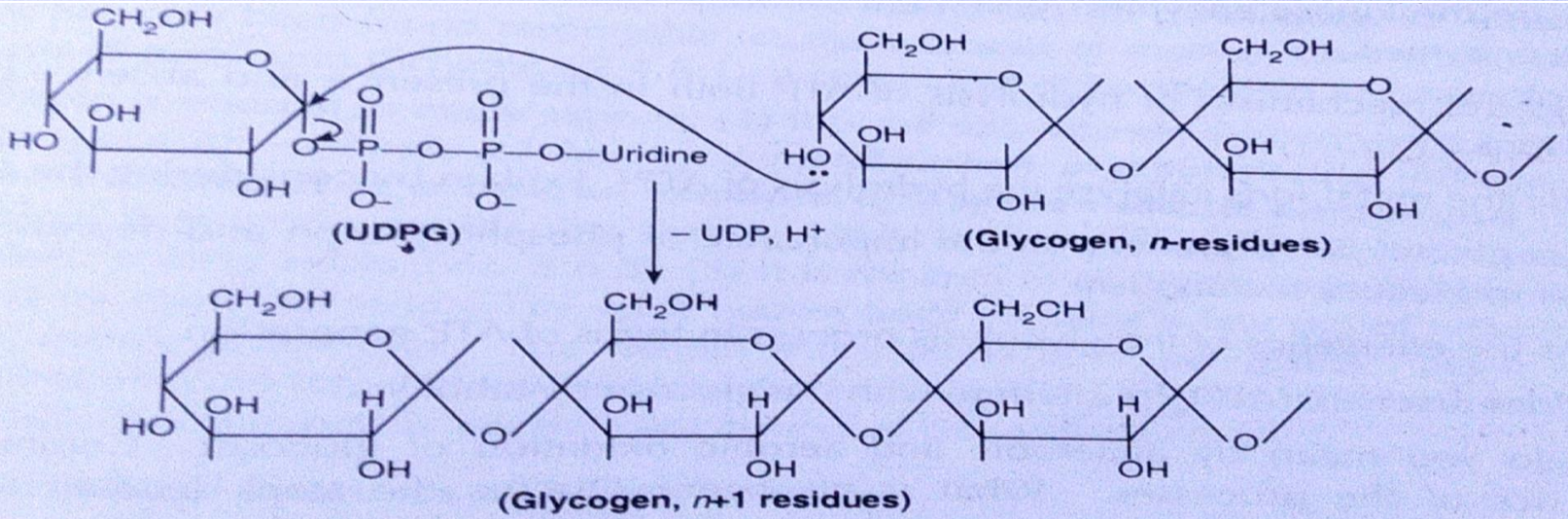
UDPG is *an activated form of glucose* and it participates in glycogen synthesis.

UDPG + glycogen (*n*-residues) $\xrightarrow{Mg^{2+}}$ glycogen (*n* + 1 residues) + UDP

ATP + UDP $\longrightarrow$ ADP + UTP

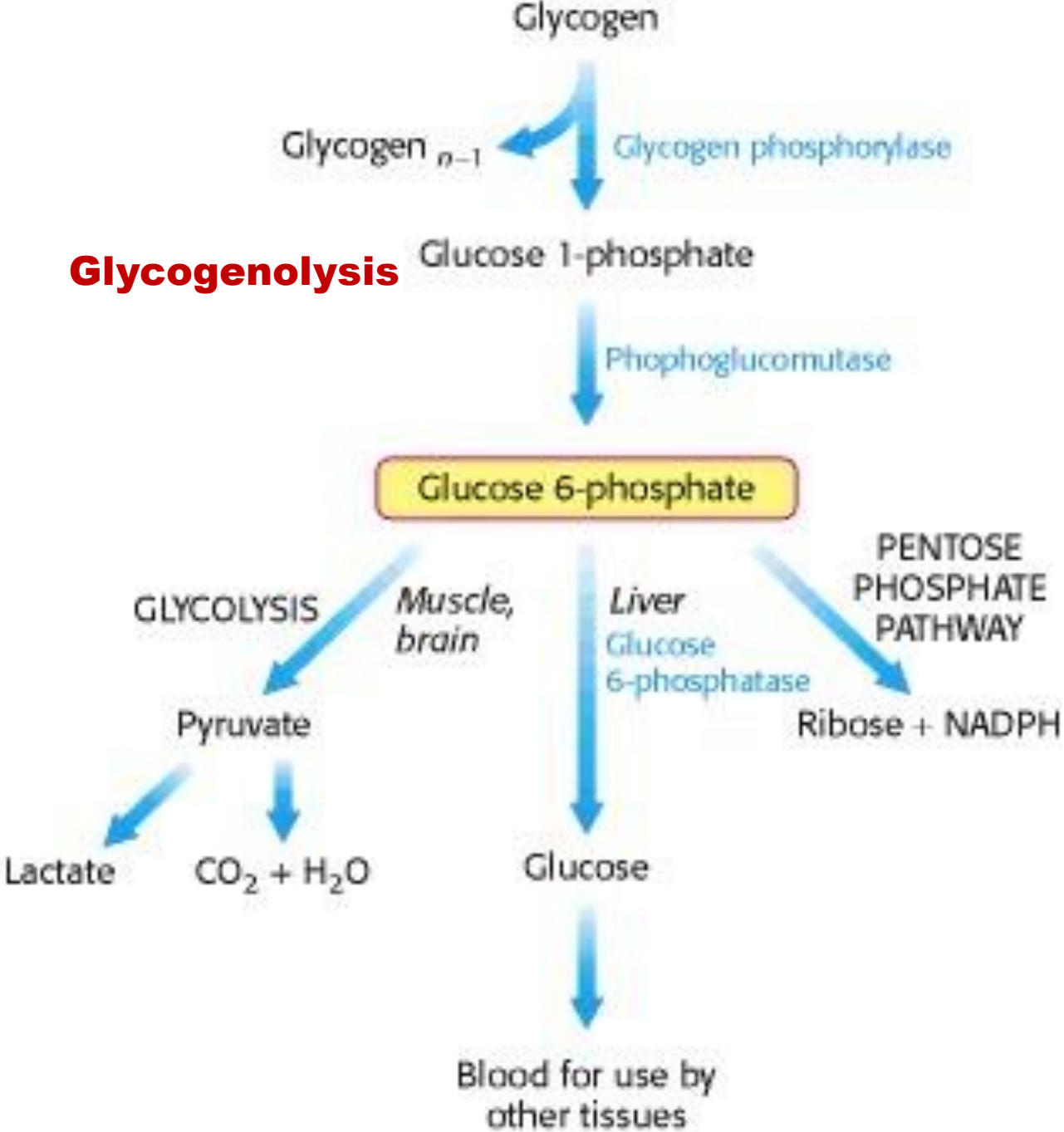
Thus the net reaction leading to glycogen synthesis is given by :

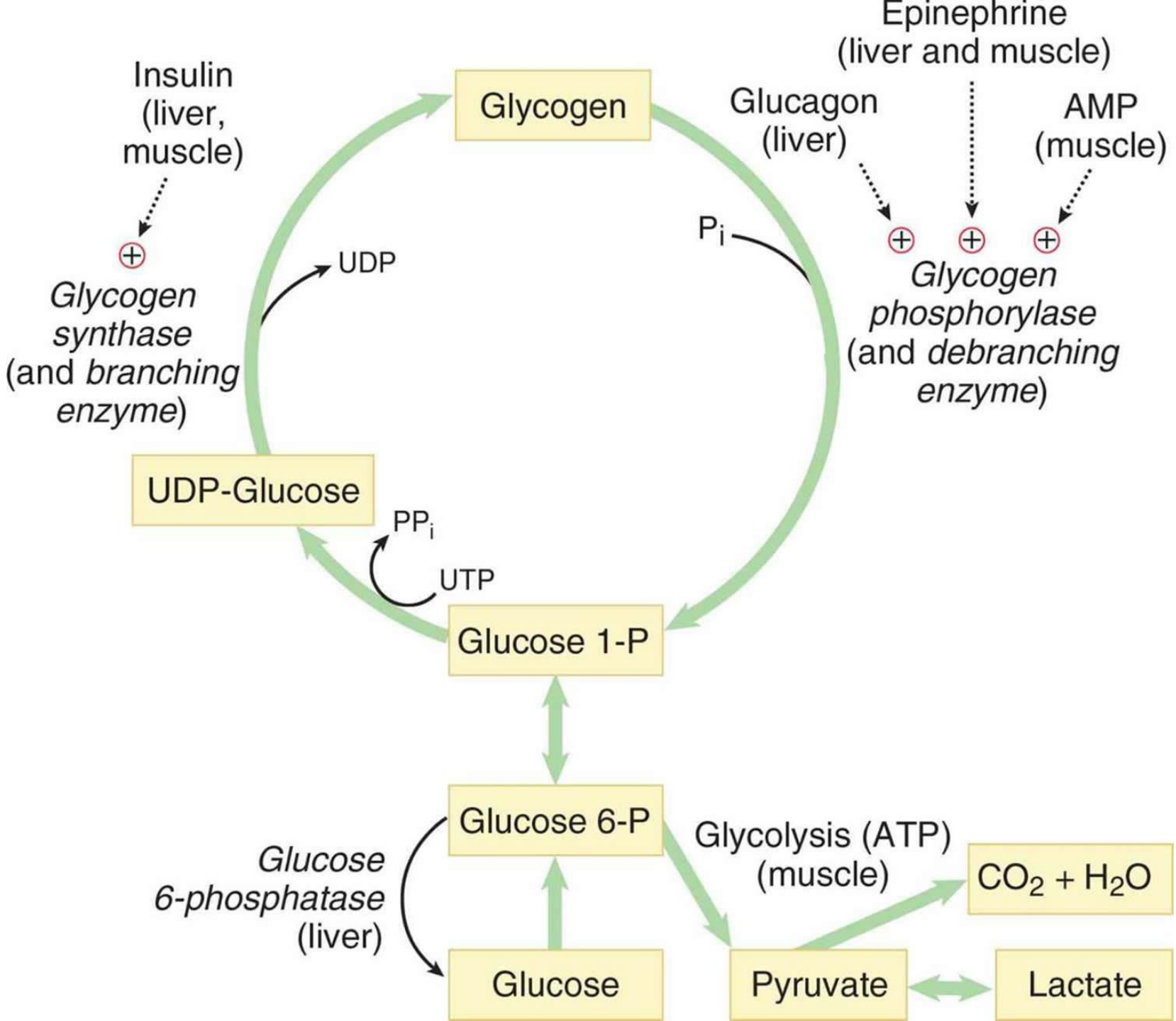
Glucose-6-phosphate + ATP + $glycogen_n$ + H_2O $\longrightarrow$ $glycogen_{n+1}$ + ADP + $2P_i$

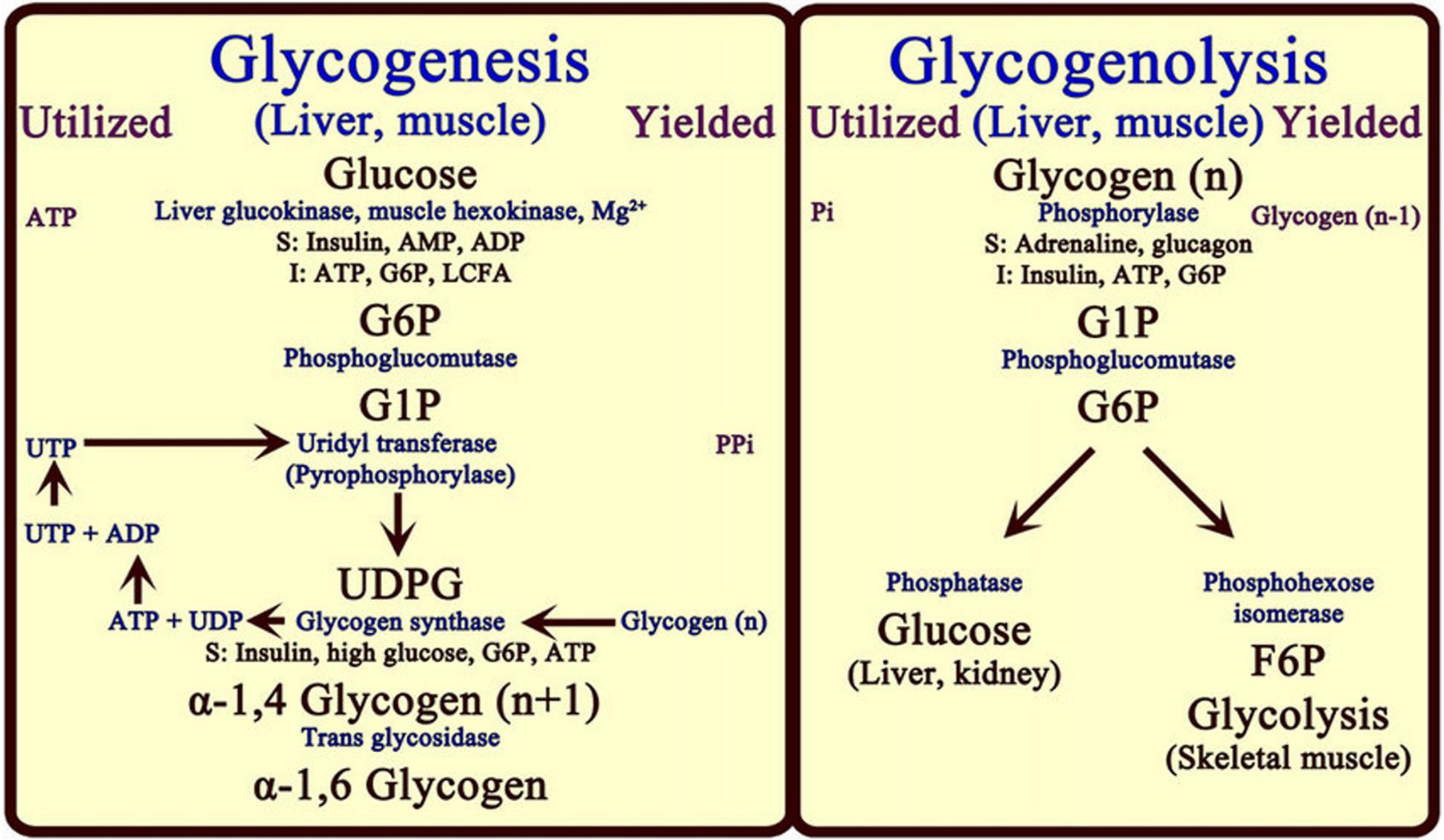


Scheme 9.4.4.3 : *Glycogen synthase catalysed glycogen synthesis. The intermediate carbonium ion is stabilised by the lone pair of cyclic oxygen giving rise to glucosyl oxonium ion.*

Glycogenolysis




Epinephrine
(liver and muscle)
Insulin
(liver,
muscle)
Glycogen
Glucagon
(liver)
AMP
(muscle)
Pi
UDP
Glycogen
synthase
(and branching
enzyme)
Glycogen
phosphorylase
(and debranching
enzyme)
UDP-Glucose
PPi
UTP
Glucose 1-P
Glucose 6-P
Glycolysis (ATP)
(muscle)
Glucose
6-phosphatase
(liver)
CO2 + H2O
Glucose
Pyruvate
Lactate


Glycogenesis
Utilized
(Liver, muscle)
Yielded
Glucose
ATP
Liver glucokinase, muscle hexokinase, Mg2+
S: Insulin, AMP, ADP
I: ATP, G6P, LCFA
G6P
Phosphoglucomutase
G1P
UTP
Uridyl transferase
(Pyrophosphorylase)
PPi
UTP + ADP
UDPG
ATP + UDP
Glycogen synthase
Glycogen (n)
S: Insulin, high glucose, G6P, ATP
α-1,4 Glycogen (n+1)
Trans glycosidase
α-1,6 Glycogen
Glycogenolysis
Utilized (Liver, muscle) Yielded
Glycogen (n)
Pi
Phosphorylase
Glycogen (n-1)
S: Adrenaline, glucagon
I: Insulin, ATP, G6P
G1P
Phosphoglucomutase
G6P
Phosphatase
Glucose
(Liver, kidney)
Phosphohexose
isomerase
F6P
Glycolysis
(Skeletal muscle)

Reactions Of Glycogenesis

1. Glucose is phosphorylated to glucose-6-phosphate catalyzed by glucokinase (in liver) and hexokinase (in muscle).

2. Glucose-6-phosphate is converted to glucose-1-phosphate by phospho-gluco-mutase.

3. Glucose-1-phosphate reacts with UTP to form UDP-glucose and releases pyrophosphate. This reaction is catalyzed by UDP-glucose pyrophosphorylase enzyme.

4. Now the glycogen synthase enzyme transfers the glucose monomer from UDP-glucose to the 4th position of glycogen primer to form alpha-1,4 glycosidic linkage.

5. The step 4 continues up to the chain elongated to minimum of 2nd monomer. after that the second enzyme known as branching enzyme transfers a portion of 1,4 chain to a near by branch to form 1,6 glycosidic linkage.

5. The branch again grow with 1,4 linkage using UDP-glucose and then further branching by 1,6 glycosidic linkage.

https://gpatindia.com/glycogen-metabolism-glycogenesis-and-mcqs-for-gpat-neet-csir-net-ssc/

Regulation of Glycogenesis

The principal enzyme for controlling the glycogenesis is the glycogen synthase enzyme. This enzyme is regulated by several allosteric effectors like hormone and cyclic AMP.

Enzymes like epinephrine and glucagon inhibits the glycogenesis. Insulin inhibits the cAMP which further inhibits the glycogen synthase.

High concentration of Glucose-6-phosphate stimulates the synthesis of new glycogen.

Significance of Glycogenesis

1.It removes the excess glucose from the circulation and store it in form of glucose.

2.Keep blood glucose level normal

3.Remove lactate from skeletal muscles and RBCs.

4.Supply glucose at active skeletal muscle

5.Replenish the liver glycogen

6.Regulates acid base balance

Photosynthesis

J. Deisenhofer and R. Huber, H. Michel, Nobel Prize – 1988 – Structural elucidation of a bacterial photosynthetic reaction center
R. A. Marcus, Nobel Prize – 1992 – theoretical description of the underlying electron – transfer process

In plants the primary photosynthesis events take place in the highly folded disk-shaped thylakoid memberane vesicles inside of chloroplasts and even in simple bacteria the process is membrane spanning.

Since immobilization and a defined orientation of pigments and of reaction centers are crucial for the success of photosynthesis, all chlorophyll molecules feature a long aliphatic phytyl side chain by which they anchor these pigments in the hydrophobic phospholipid membrane, which has a thickness of about 5 nm.

Photosynthetic output of green plants in normal sunlight is usually assumed to be about 1g of glucose per hour per $1m^2$ of leaf surface area.
The photosynthetically active algae phytoplankton also play an important role on a global scale, since the water coverage of the earth is about 71%.

About 200 billion tonnes of carbohydrate equivalents are produced from CO_2 each year.

$$2Fe^{2+} \xrightarrow{[O]} Fe_2O_3 + \text{energy}$$

$$8H_2S \xrightarrow{[O]} S_8 + 8H_2O + \text{energy}$$

$$S_8 + 8H_2O \xrightarrow{[O]} 8SO_4^{2-} + 16H^+ + \text{energy}$$

Nitrifying bacteria utilise the following reactions for energy.

$$2NH_3 \xrightarrow{[O]} 2NO_2^- + 3H_2O + \text{energy}$$

$$NO_2^- \xrightarrow{[O]} NO_3^- + \text{energy}$$

Chemolithotropic bacteria

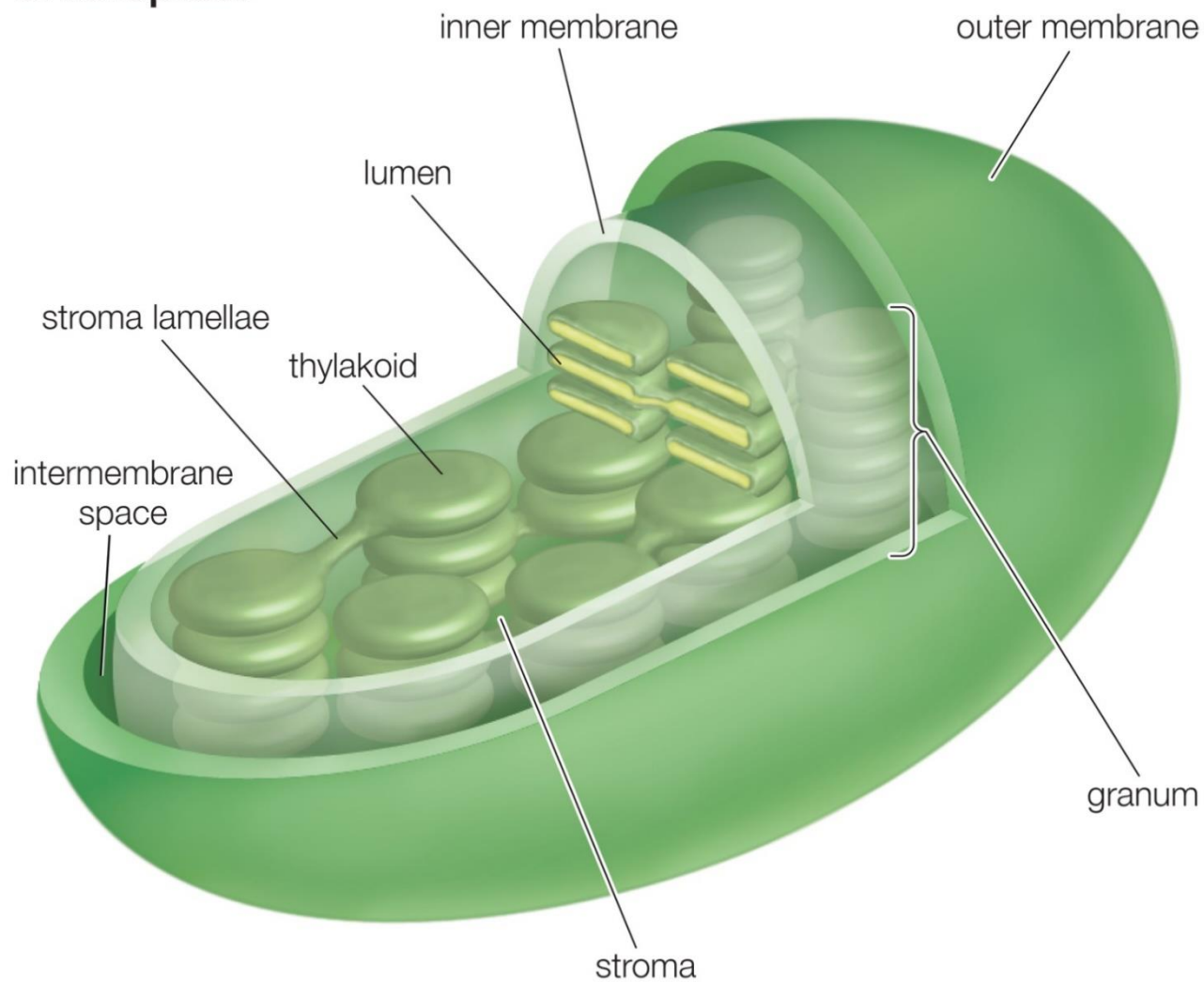
Some bacteria called sulfate reducers can transfer electrons to sulfate (SO_4^{2-}) reducing it to H_2S. Other bacteria, called nitrate reducers, can transfer electrons to nitrate (NO_3^-) reducing it to nitrite (NO_2^-). Other nitrate reducers can reduce nitrate even further to nitrous oxide (NO) or nitrogen gas (N_2).

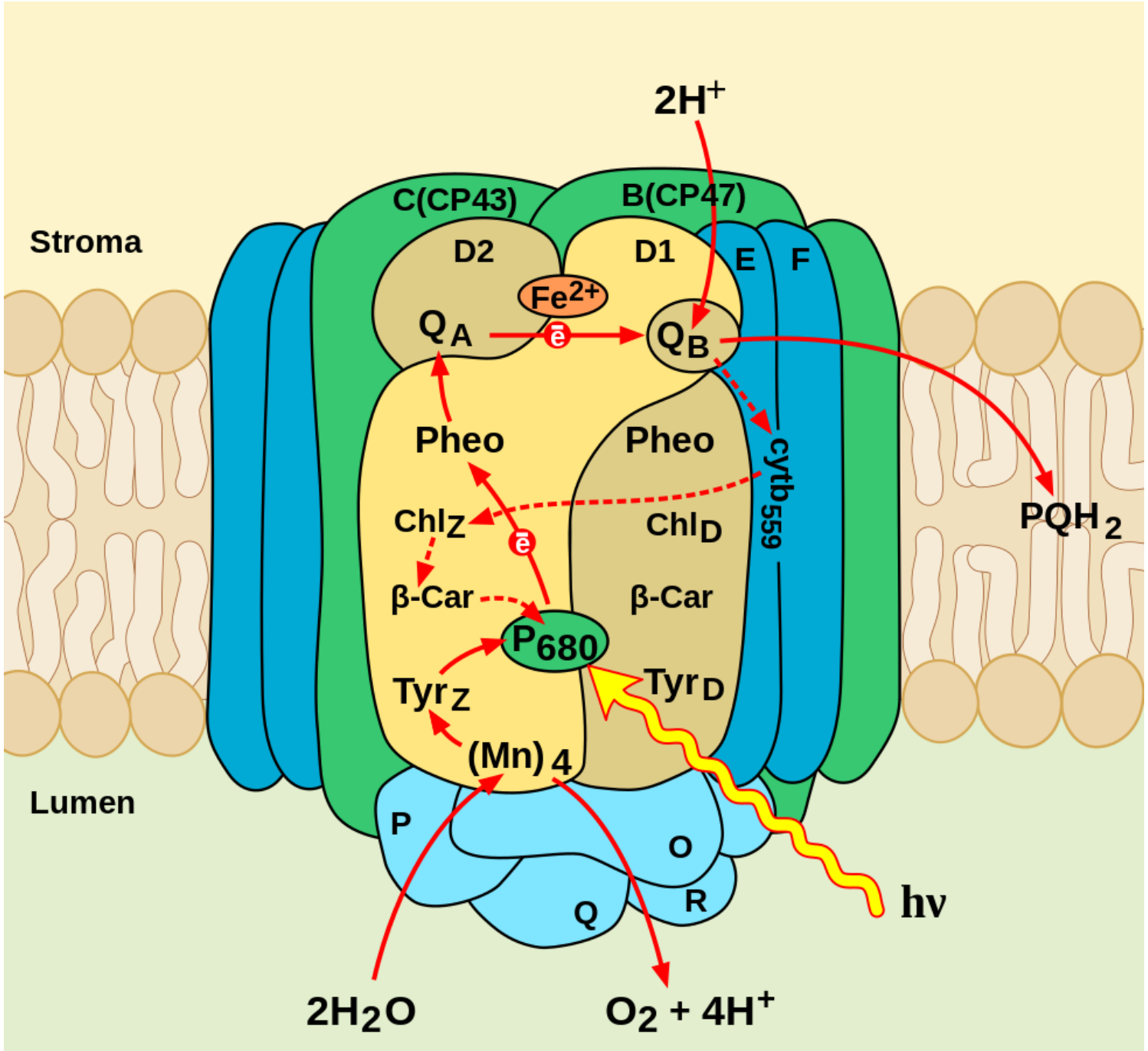
In fact, in the photosynthetic redox reaction, different electron donors (*e.g.* H_2O, H_2S, etc.) and different electron acceptors (*e.g.* CO_2, NO_3^-, etc.) may be used. But, *commonly, the photosynthesis reaction means the involvement of* H_2O *as an electron donor and* CO_2 *as an electron acceptor.* Robert Hill showed that the isolated chloroplasts on being illuminated can evolve O_2 and reduce an artificial electron acceptor like $Fe(CN)_6^{3-}$.

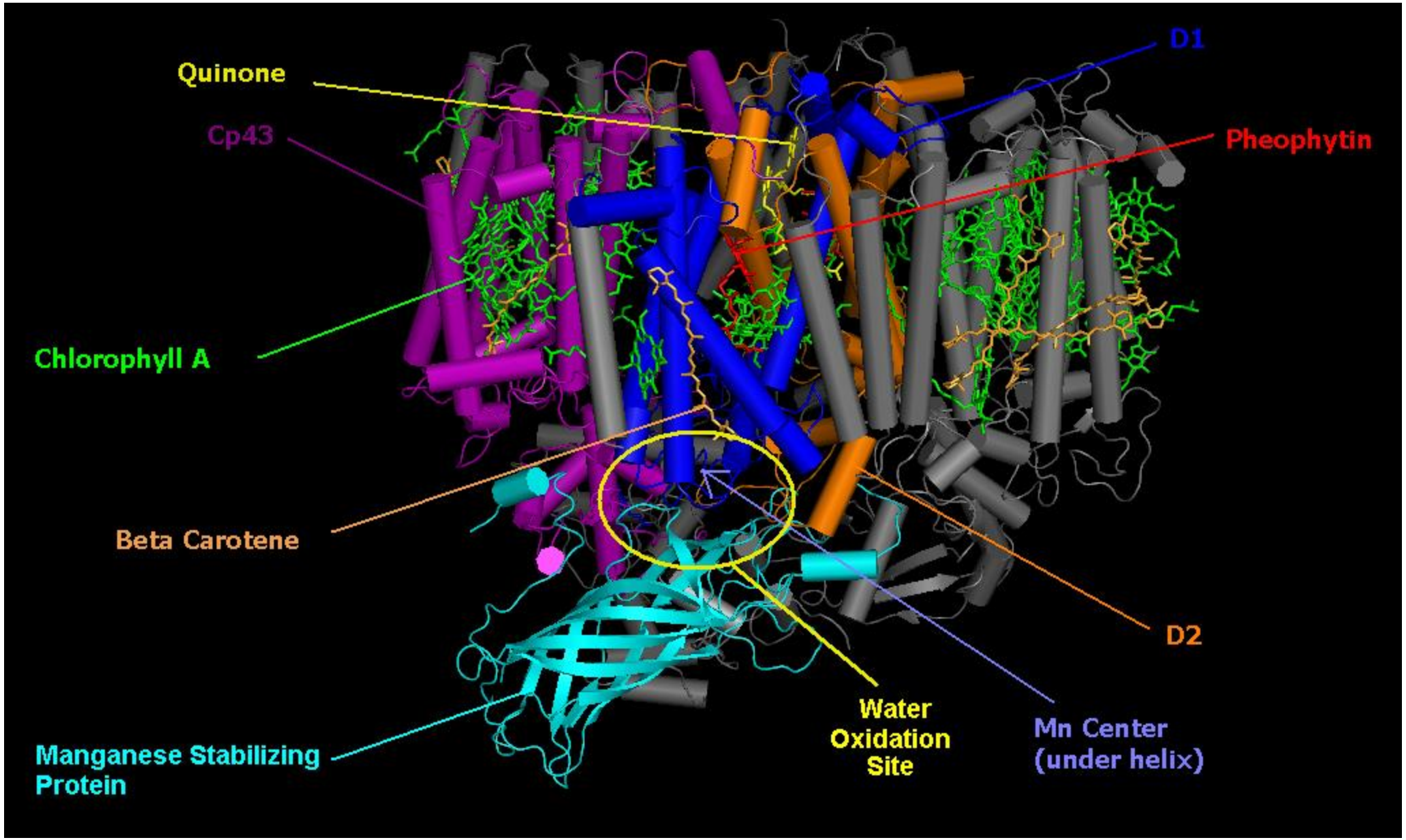
$$2H_2O + 4Fe(CN)_6^{3-} \xrightarrow[\text{chloroplast}]{\text{illuminated}} O_2 + 4H^+ + 4Fe(CN)_6^{4-} \qquad (8.5.1.4)$$

This ***Hill reaction*** proves that O_2 evolution can occur even in the absence of CO_2 and an artificial electron acceptor can substitute CO_2.

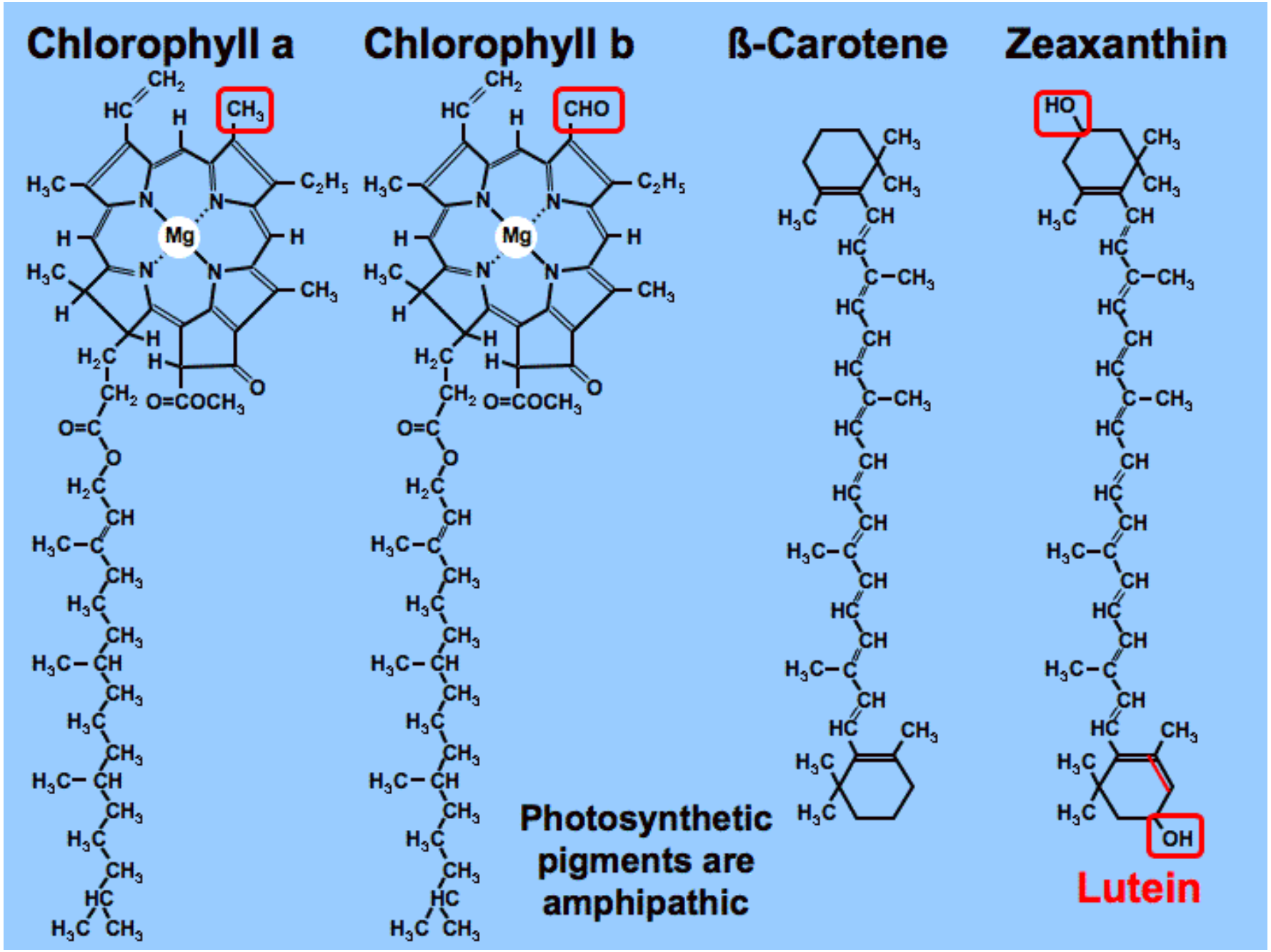
Chloroplast

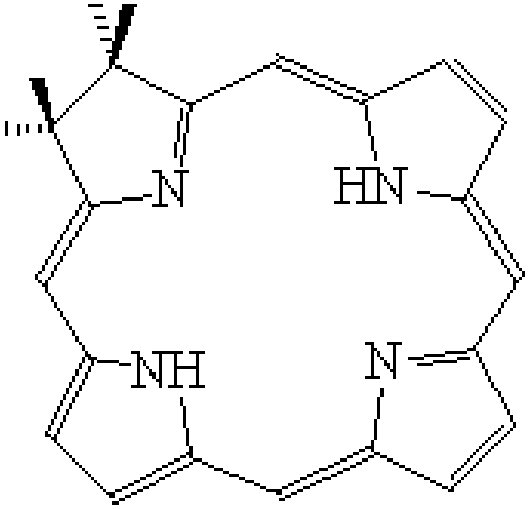



2H+
C(CP43)
B(CP47)
Stroma
D2
D1
E
F
Fe2+
QA
ē
QB
Pheo
Pheo
cytb559
PQH2
ChlZ
ChlD
ē
β-Car
β-Car
P680
TyrZ
TyrD
(Mn)4
Lumen
P
O
Q
R
hν
2H2O
O2 + 4H+



Cyanobacterial photosystem II


Chlorophyll a
Chlorophyll b
ß-Carotene
Zeaxanthin
Photosynthetic pigments are amphipathic
Lutein



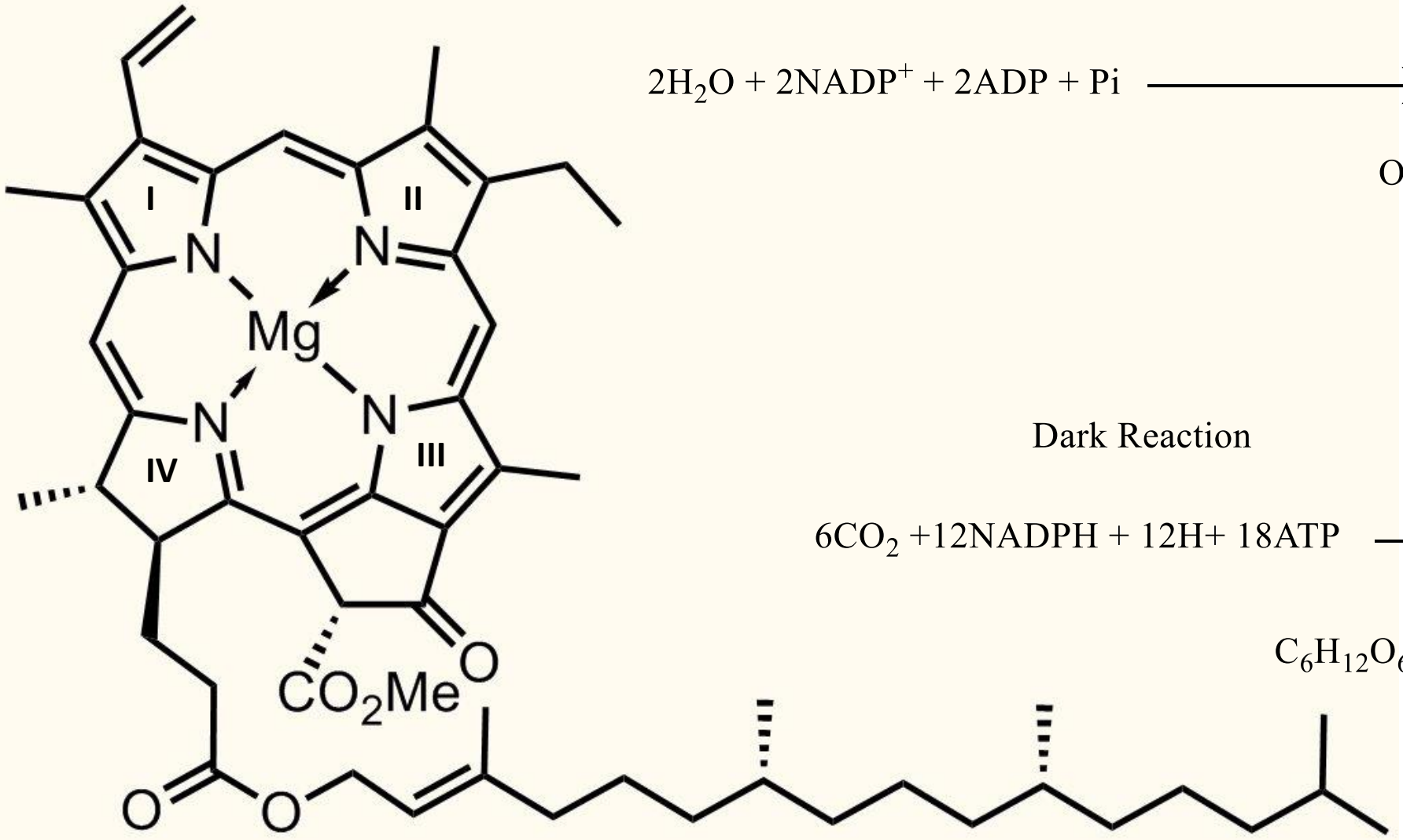
Chlorin

Light Reaction

$2H_2O + 2NADP^+ + 2ADP + Pi \longrightarrow O_2 + 2NADPH + 2ATP + 2H^+$

Dark Reaction

$6CO_2 + 12NADPH + 12H+ \ 18ATP \longrightarrow C_6H_{12}O_6 + 12ADP^+ + 6H_2O + 18(ADP + Pi)$



Chlorophyll a

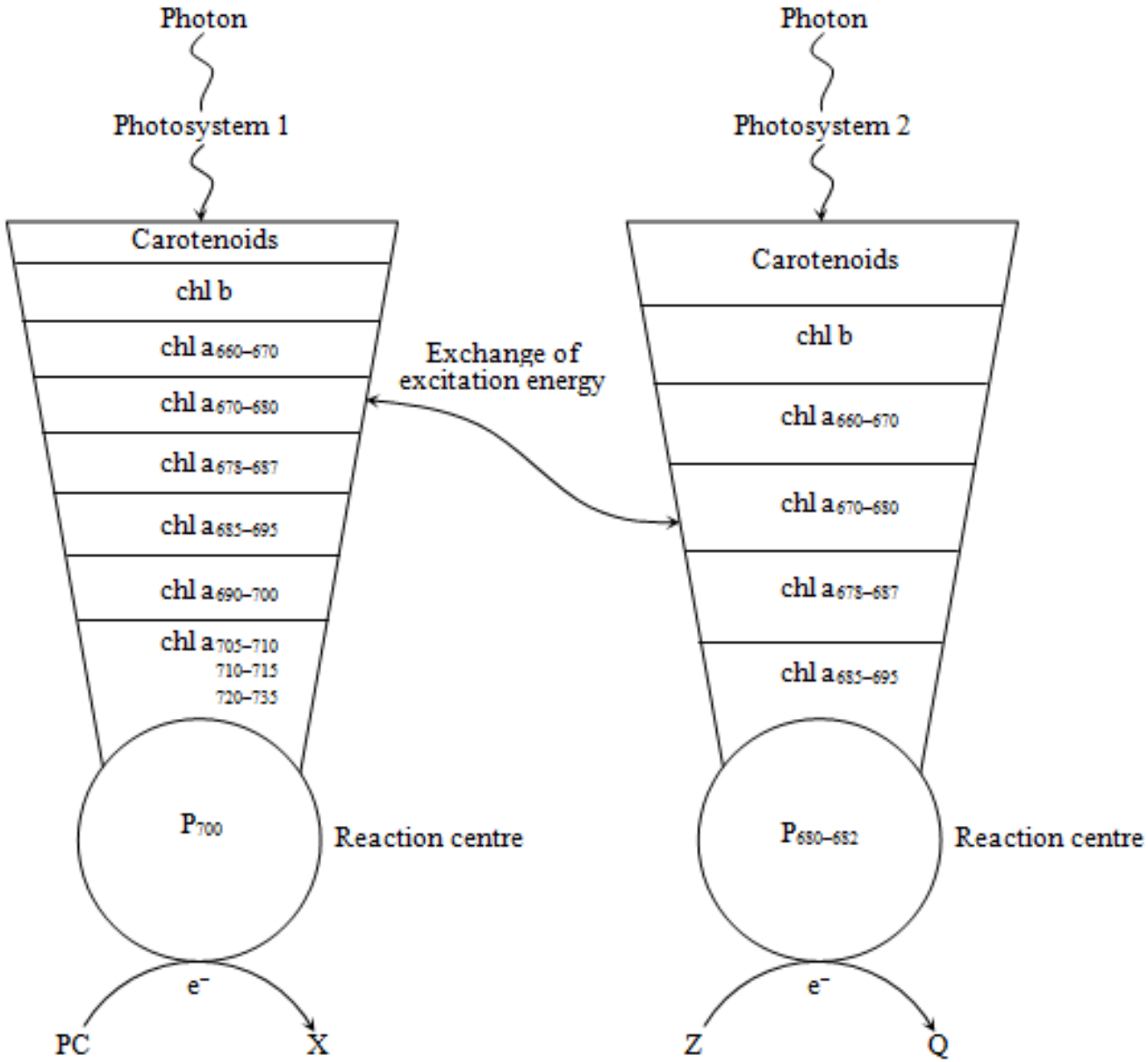


Fig : Distribution of pigments in the two photosystems or pigment systems

Number	Component
photosystem I (including cytochrome *b/f* complex)	
~200	antenna chlorophylls
~50	carotenoids
1	reaction center P_{700}
1	chlorophyll *a* (primary acceptor A_o)
1	vitamin K_1 (secondary acceptor A_1)
3	Fe/S-clusters (FeS)
1	bound ferredoxin (Fd)
1	soluble ferredoxin (Fp)
1	plastocyanin (PC, primary donor)
1	Rieske Fe/S center
1	cytochrome *f* (cyt *f*)
2	cytochromes b_6 (cyt b_6)
photosystem II (including oxygen-evolving complex, OEC)	
~200	antenna chlorophylls
~50	carotenoids
1	reaction center P_{680}
2	chlorophylls
2	pheophytins (primary acceptor)[a]
2	plastoquinones (PQ)
2	tyrosine residues (primary donor)[b]
4	manganese centers
1	calcium ion Ca^{2+}
several	chloride ions Cl^-
1	cytochrome b_{559}

https://qforquestions.com/photosynthesis-photochemical-reaction/

In plants, light-dependent reactions occur in the thylakoid membranes of the chloroplasts where they drive the synthesis of ATP and NADPH.

How many pigments are involved in Photosynthesis?

"Pigments are the substances that possess the ability to absorb light at specific wavelength." Leaves of plants have four types of pigments, i.e. **Chlorophyll a** (**bright or blue green** in chromatogram), **Chlorophyll b** (**yellow green**), **Carotenoids** (**yellow to yellow – orange**) and **Xanthophylls (yellow**). **Photosynthesis takes place in red and blue regions of spectrum** and some photosynthesis also takes place at other wavelengths. **Chlorophyll is the major pigment that traps the light energy and other pigments are referred as accessory pigment which traps light and transfer the energy to chlorophyll a.**

Types of Photosynthetic Reactions

Photosynthetic Reactions are of two types, i.e.

Light Dependent Reaction – *In these reactions, the energy from sunlight is absorbed by chlorophyll and transformed into chemical energy in the form of ATP and NADPH (electron carrier molecule).*

Light Independent Reaction or Dark Reaction – This reaction is also referred as **Calvin Cycle**. In this reaction, the **energized electron from light dependent reactions provides energy to form carbohydrates from CO_2 molecules**

Quantum Yield

(i) Rate or yield of photosynthesis is measured in terms of quantum yield or O2 evolution, which may be defined as, "Number of O2 molecules evolved per quantum of light absorbed in photosynthesis."

(ii) **Quantum requirement in photosynthesis = 8, i.e., 8 quanta of light are required to evolve one mol. of O2**.

(iii) **Hence quantum yield = 1 /8 = 0.125 (i.e., a fraction of 1) as 12%.**

Emerson effect and Red drop: R. Emerson and C.M. Lewis (1943) observed that the quantum yield of photosynthesis decreases towards the far red end of the spectrum (680nm or longer). Quantum yield is the number of oxygen molecules evolved per light quantum absorbed. **Since this decrease in quantum yield is observed at the far region or beyond red region of spectrum is called red drop.**

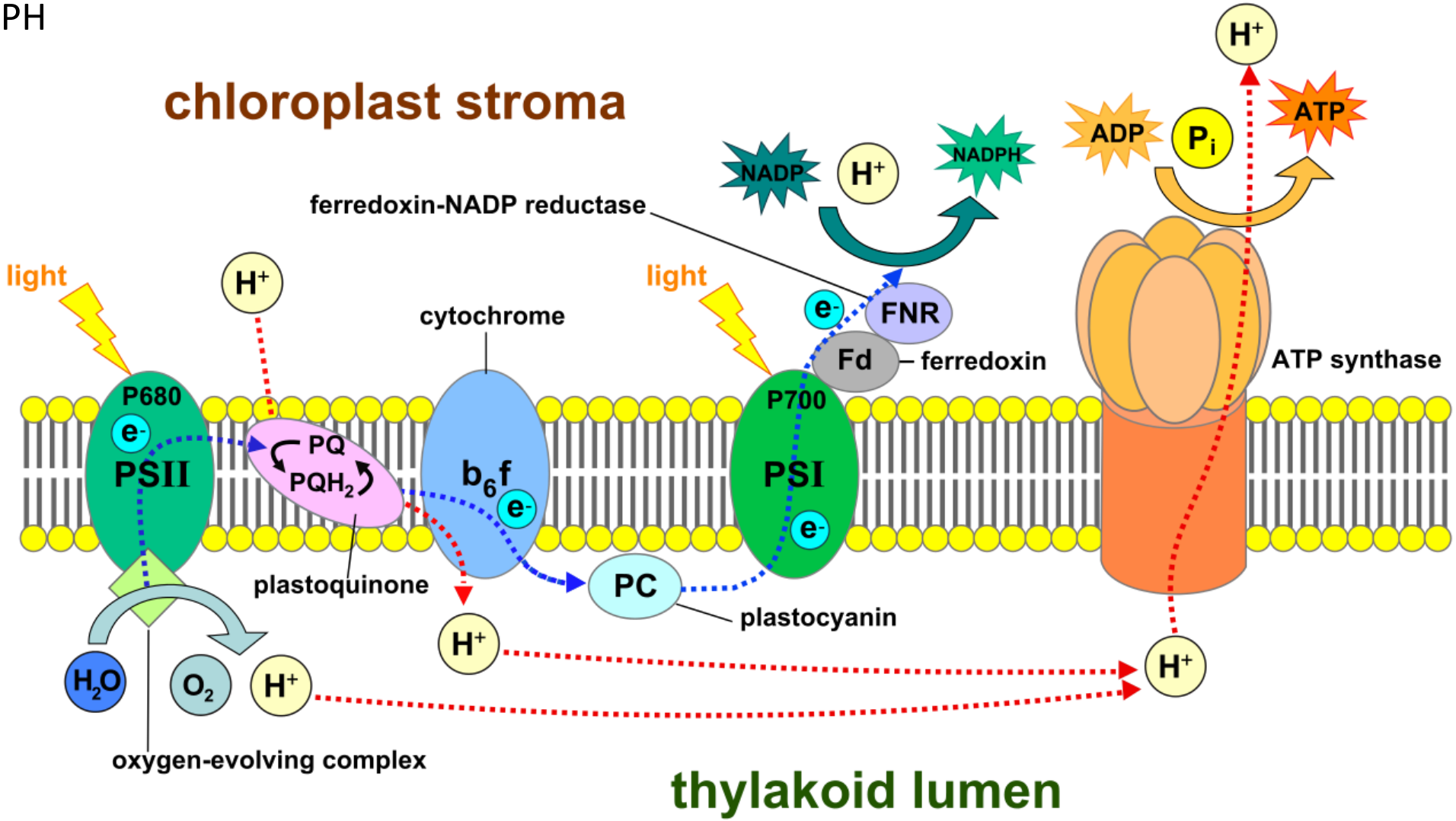
Emerson et al. (1957) further observed that **photosynthetic efficiency of light of 680nm or longer is increased if light of shorter wavelengths (Less than 680nm) is supplied simultaneously**. **When both short and long wavelengths were given together the quantum-yield of photosynthesis was greater than the total effect when both the wavelengths were given separately**. This increase in photosynthetic efficiency (or quantum yield) **is known as Emerson effect or Emerson enhancement effect**.

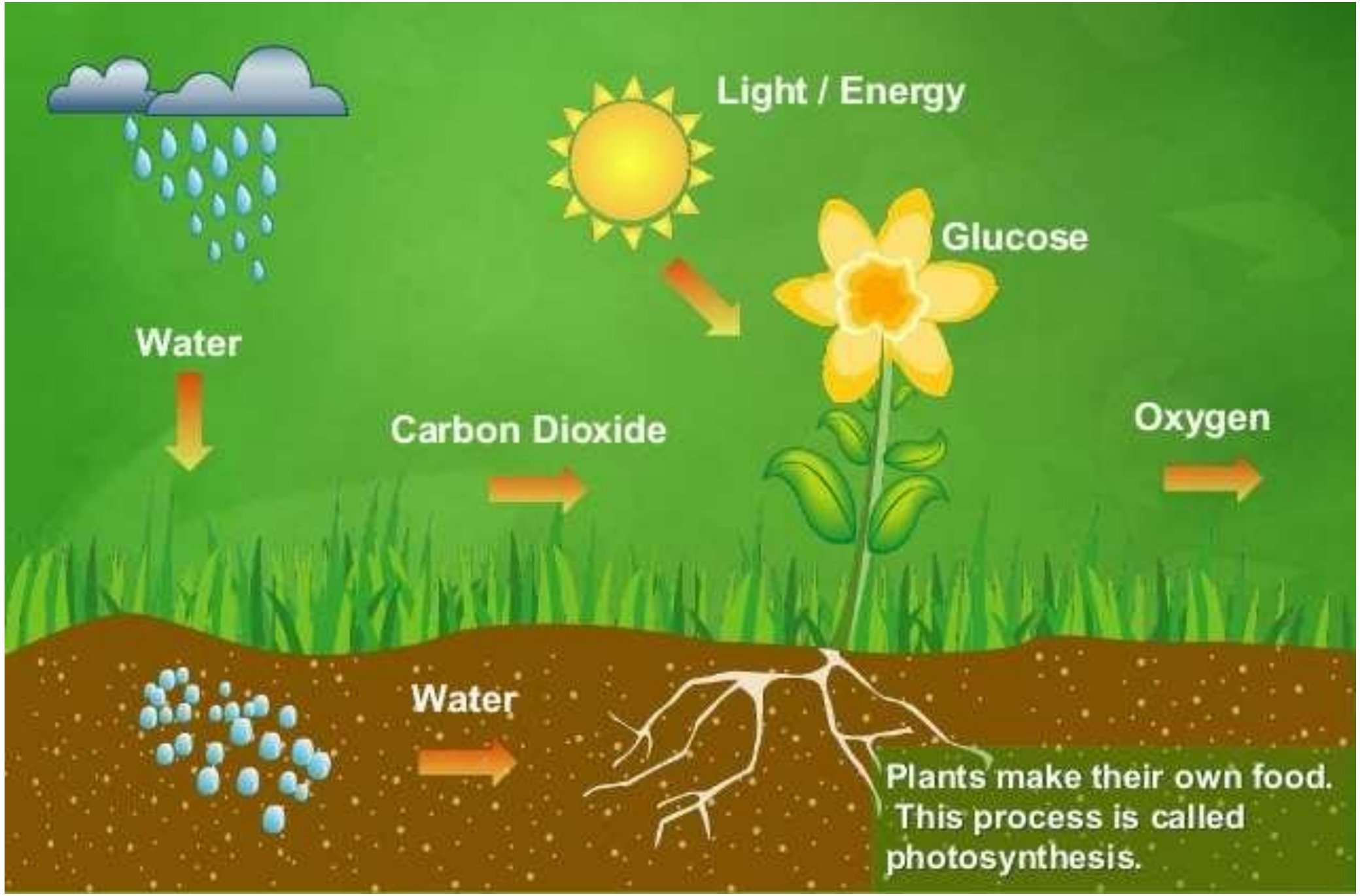
(i) Pigment system I or Photosystem I: The important pigments of this system are **chlorophyll a 670, chlorophyll a 683, chiorophyll a 695, P700' Some physiologists also include carotenes and chlorophyll b in pigment system I. P700 acts as the reaction centre. Thus, this system absorbs both wavelengths shorter and longer than 680nm**.

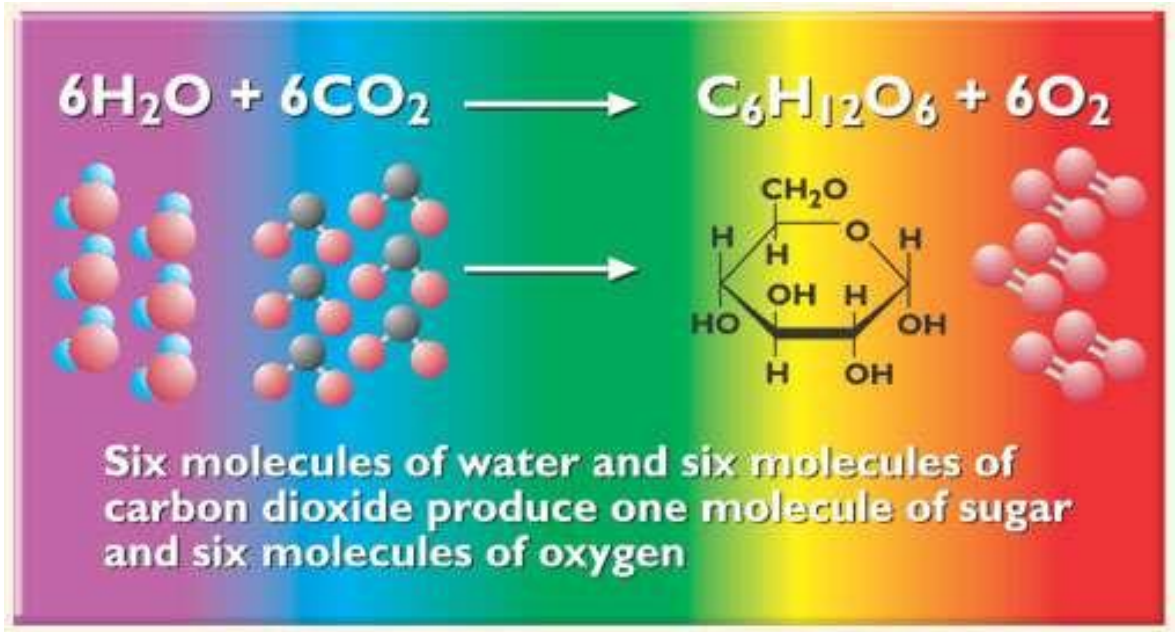
(ii) Pigment system IT or photosystem II: **The main pigments of this system are chlorophyll a 673, P680, chlorophyll band phycobilins**. **This pigment system absorbs wavelengths shorter than 680nm only. P680 acts as the reaction centre**

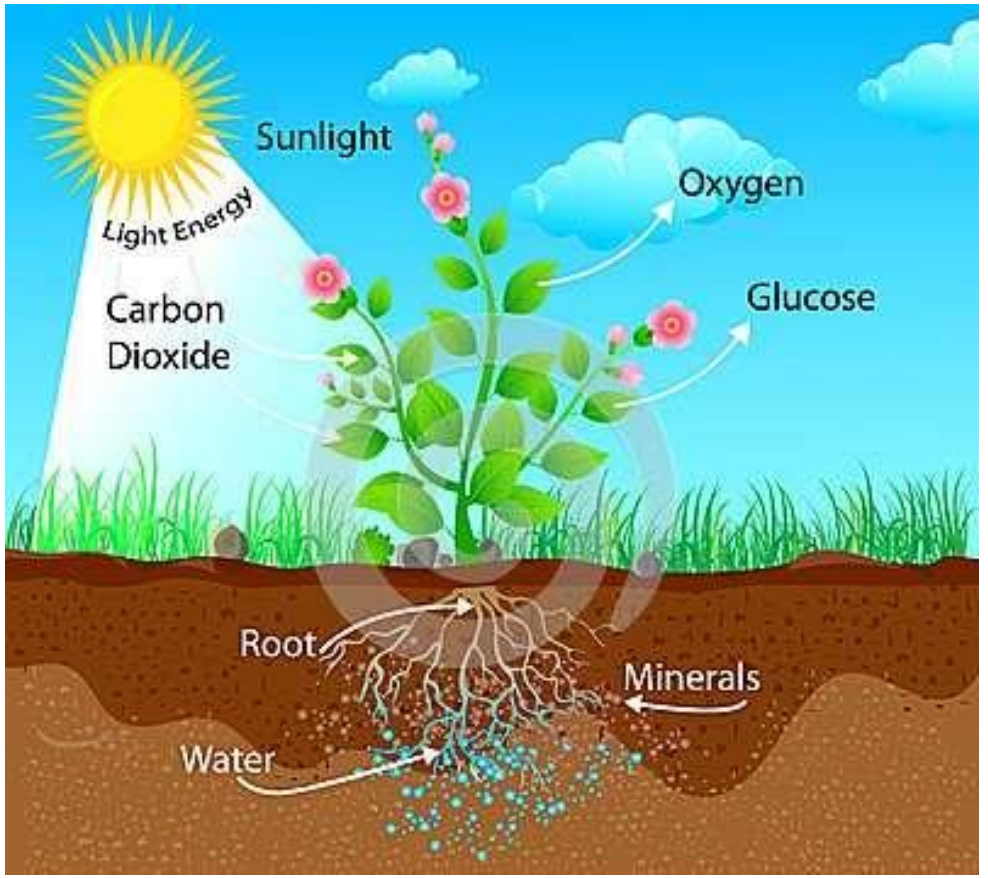
Photosystem II light-harvesting proteins are the intrinsic transmembrane proteins CP43 (PsbC) and CP47 (PsbB) occurring in the reaction centre of photosystem II. These polypeptides bind to chlorophyll a and beta-carotene and pass the excitation energy on to the reaction centre.[1] This family also includes the iron-stress induced chlorophyll-binding protein CP43' (IsiA,CP43'), which evolved in cyanobacteria from a PSII protein to cope with light limitations and stress conditions.
Under iron-deficient growth conditions, CP43' associates with photosystem I to form a complex that consists of a ring of 18 or more CP43' molecules around a PSI trimer, which significantly increases the light-harvesting system of PSI. IsiA can also provide photoprotection for PSII.[2]
Plants, algae and some bacteria use two photosystems, PSI with P700 and PSII with P680. Using light energy, PSII acts first to channel an electron through a series of acceptors that drive a proton pump to generate ATP, before passing the electron on to PSI. Once the electron reaches PSI it has used most of its energy in producing ATP, but a second photon of light captured by P700 provides the required energy to channel the electron to ferredoxin, generating reducing power in the form of NADPH. The ATP and NADPH produced by PSII and PSI, respectively, are used in the light-independent reactions for the formation of organic compounds. This process is non-cyclic, because the electron from PSII is lost and is only replenished through the oxidation of water. Hence, there is a constant flow of electrons and associated hydrogens from water for the formation of organic compounds. It is this stripping of hydrogens from water that produces the oxygen we breathe.
IsiA has an inverse relationship with the Iron stress repressed RNA (IsrR). IsrR is an antisense RNA that acts as a reversible switch that responds to changes in environmental conditions to modulate the expression of IsiA.

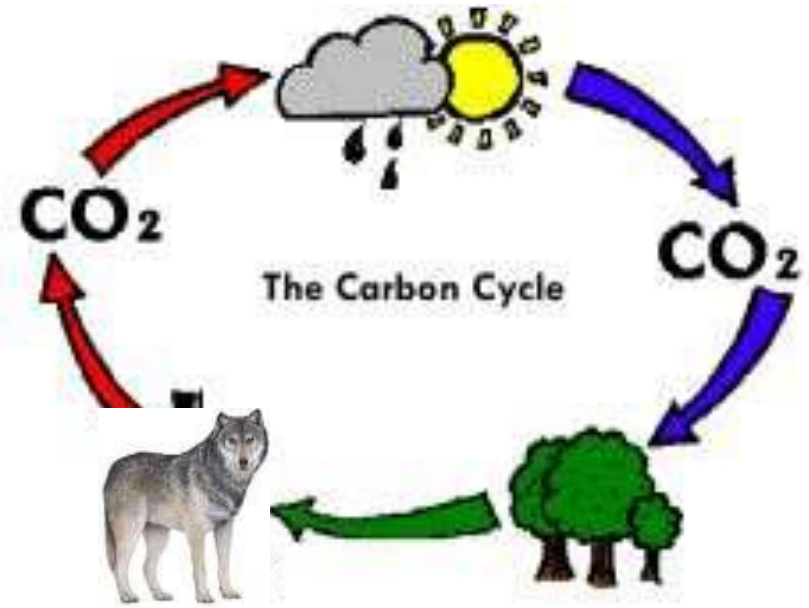
- In photosynthesis, the **light-dependent** reactions take place on the thylakoid membranes. The inside of the thylakoid membrane is called the lumen, and outside the thylakoid membrane is the stroma, where the light-independent reactions take place
- There are four major protein complexes in the thylakoid membrane: Photosystem II (PSII), Cytochrome b6f complex, Photosystem I (PSI), and ATP synthase. These four complexes work together to ultimately create the products ATP and NADPH




Light / Energy
Glucose
Water
Carbon Dioxide
Oxygen
Water
Plants make their own food.
This process is called
photosynthesis.







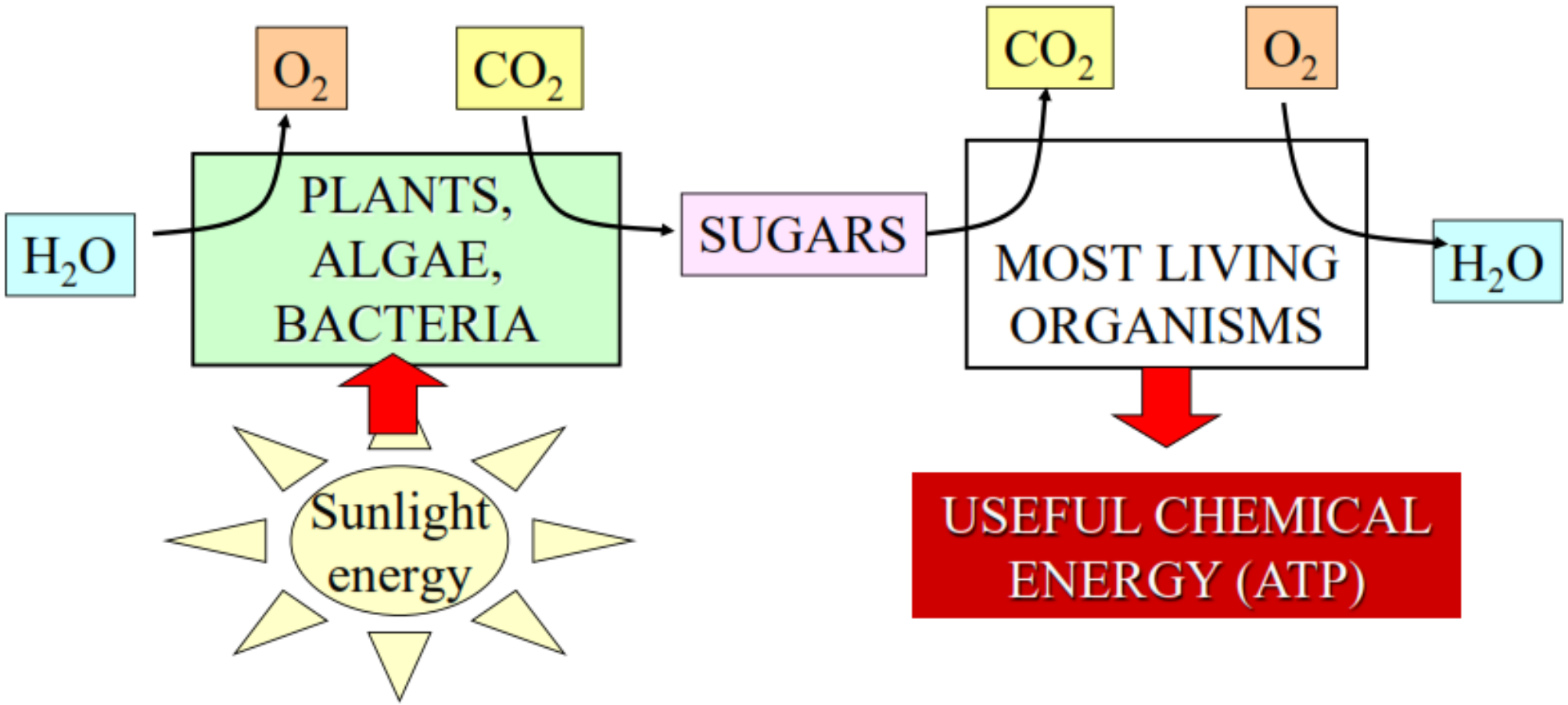
Carbon moves from atmosphere to plants to animals and back to atmosphere

PHOTOSYNTHESIS

$CO_2 + H_2O \rightarrow O_2 + SUGARS$

RESPIRATION

$SUGARS + O_2 \rightarrow H_2O + CO_2$



Summary of Photosynthesis:

1. Light energy absorbed by *chlorophyll a* drives the reactions of photosynthesis.

2. Converts light energy into chemical energy to make organic compounds.

3. CO_2 and H_2O used to produce $C_6H_{12}O_6$ (glucose) and O_2 (gas).

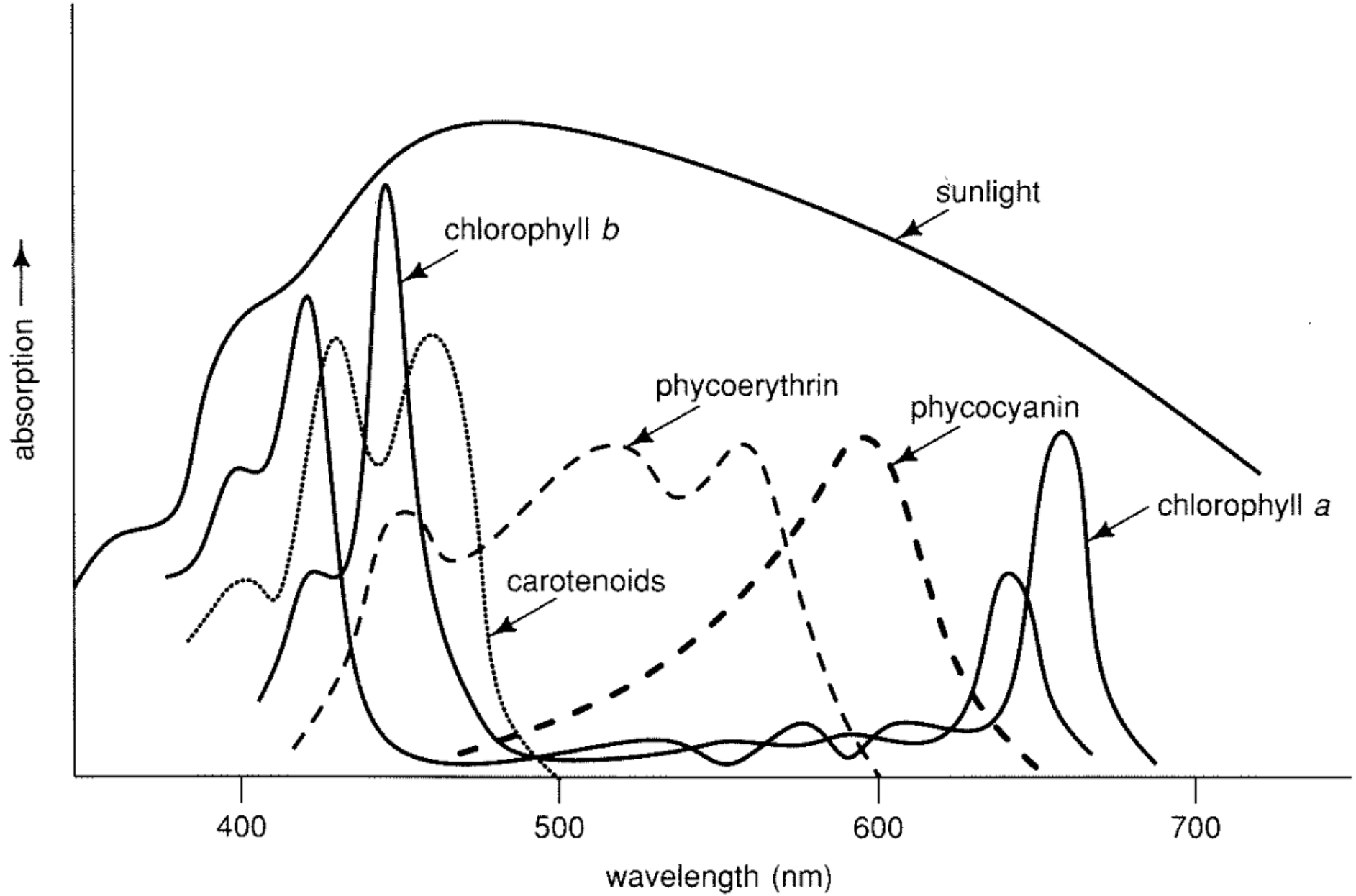
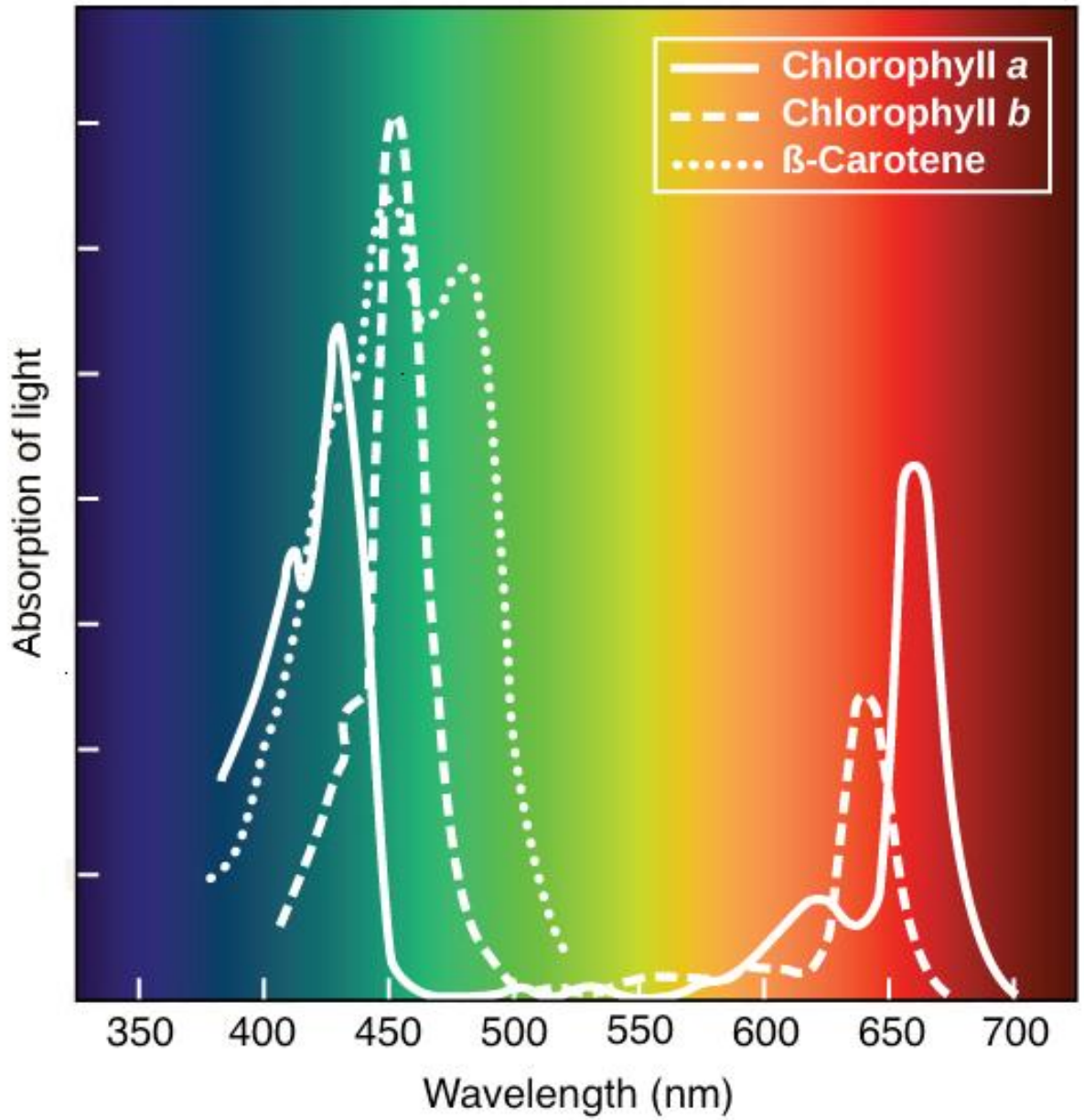
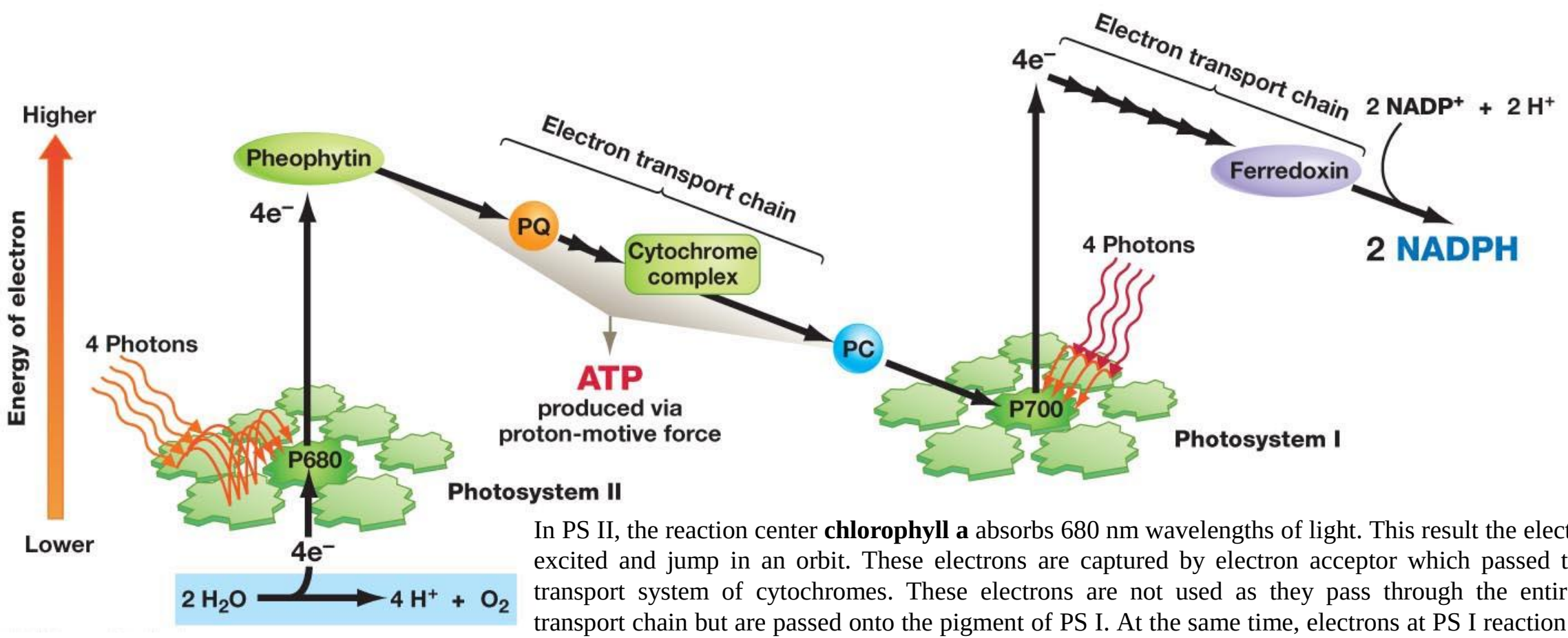


Figure 6.13
Absorption spectra of the photosystem pigments.

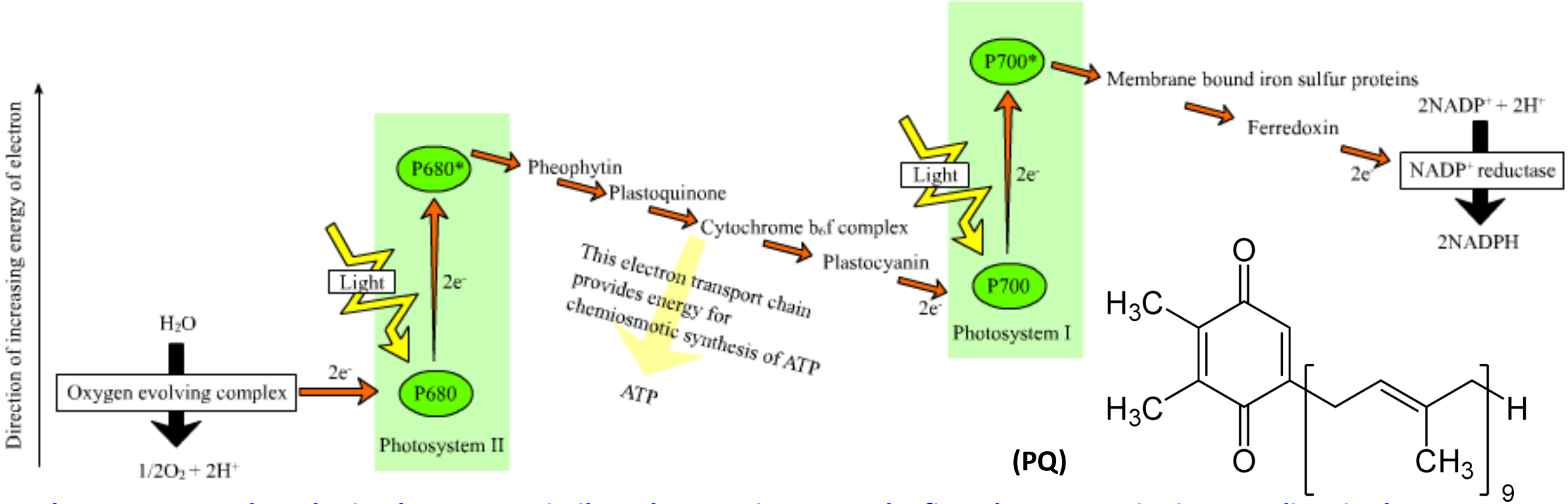
Absorption Spectra of Pigments





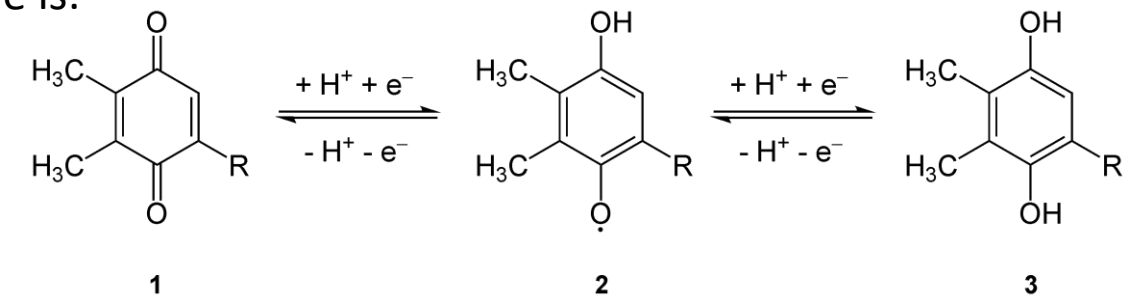
In PS II, the reaction center **chlorophyll a** absorbs 680 nm wavelengths of light. This result the electrons to be excited and jump in an orbit. These electrons are captured by electron acceptor which passed to electron transport system of cytochromes. These electrons are not used as they pass through the entire electron transport chain but are passed onto the pigment of PS I. At the same time, electrons at PS I reaction center are also excited when they receive red light of wavelength 700 nm. These electrons are transferred to another acceptor molecule with greater redox potential. In this electron transport chain, electrons are not used rather they are passed to the pigments of b. At the same time, electrons present in reaction center of PS I also gets excited after receiving red light of wavelength 700 nm. Then, these electrons are transferred to another acceptor molecule with greater redox potential. These electrons, then move downhill and this time to energy rich molecule i.e. NADP+, whose addition reduces NADP+ to NADPH + H+.

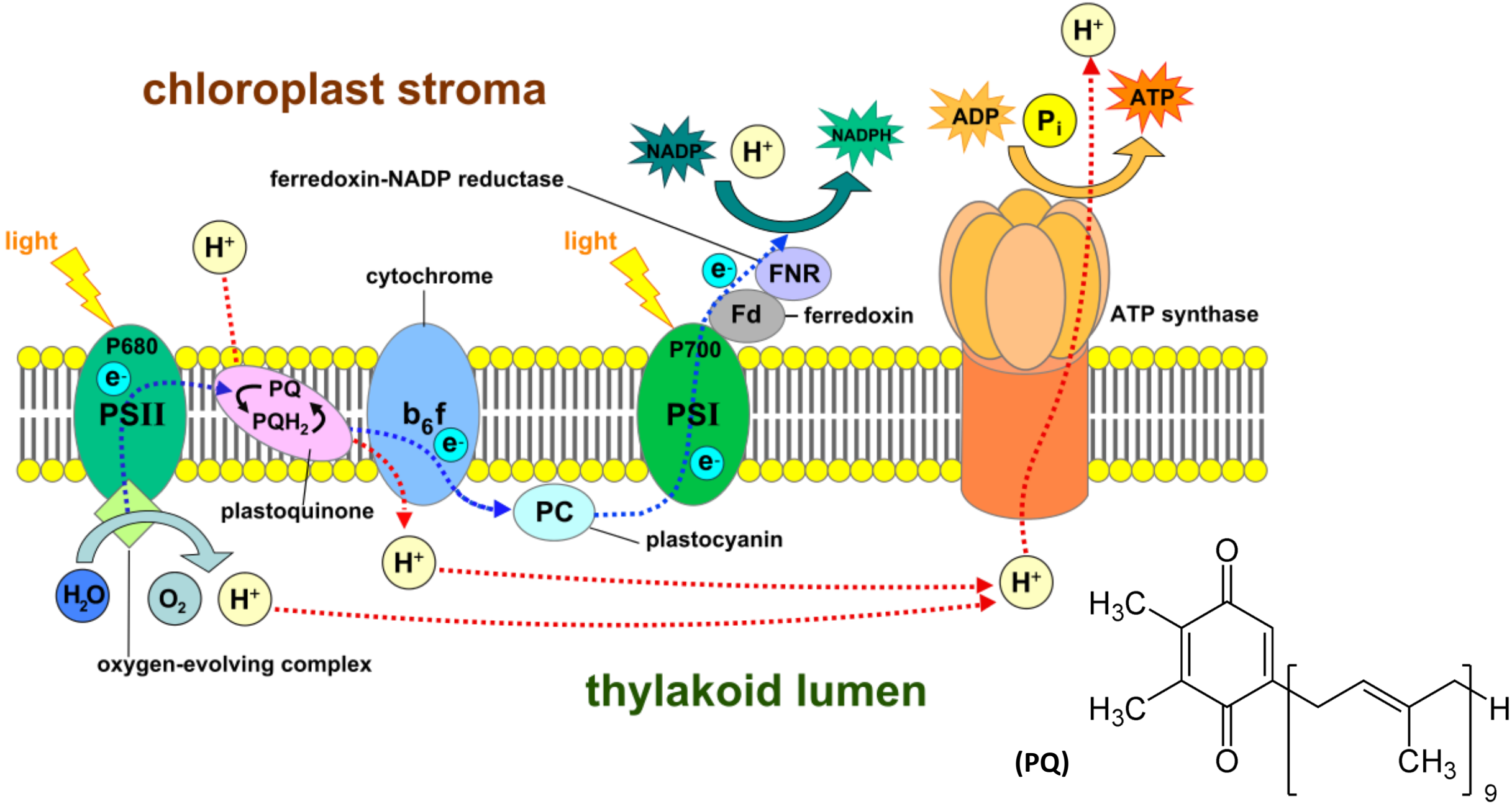
This entire scheme of transfer of electrons starting from PS II to uphill then down the electron chain to PS I, excitation of electrons, transferring to another acceptor and ultimately downhill to NADP+ resulting in formation of NADPH + H+ is referred as Z Scheme.



In photosystem II, pheophytin plays a very similar role. It again acts as the first electron carrier intermediate in the photosystem. After P680 becomes excited to P680*, it transfers an electron to pheophytin, which converts the molecule into a negatively charged radical. The negatively charged pheophytin radical quickly passes its extra electron to two consecutive plastoquinone molecules. Eventually, the electrons pass through the cytochrome b_6f molecule and leaves photosystem II. The reactions outlined above in the section concerning purple bacteria give a general illustration of the actual movement of the electrons through pheophytin and the photosystem. The overall scheme is:

1.Excitation
2.Charge separation
3.Plastoquinone reduction
4.Regeneration of substrates




chloroplast stroma
H+
ADP
Pi
ATP
NADP
H+
NADPH
ferredoxin-NADP reductase
light
H+
cytochrome
light
e-
FNR
Fd
ferredoxin
ATP synthase
P680
e-
PSII
PQ
PQH2
b6f
e-
P700
PSI
e-
plastoquinone
PC
plastocyanin
H+
H+
H2O
O2
H+
oxygen-evolving complex
thylakoid lumen
H3C
H3C
O
O
CH3
H
9
(PQ)

Reaction in Photosystem II

Pheophytin is the first electron carrier intermediate in the photoreaction center (RC P870) of purple bacteria. Its involvement in this system can be broken down into 5 basic steps. The first step is excitation of the bacteriochlorophylls $(Chl)_2$ or the special pair of chlorophylls. This can be seen in the following reaction.

$(Chl)_2 + 1\ photon \rightarrow (Chl)_2^*$ (excitation)

The second step involves the (Chl)2 passing an electron to pheophytin, producing a negatively charged radical (the pheophytin) and a positively charged radical (the special pair of chlorophylls), which results in a charge separation.

$(Chl)_2^* + Pheo \rightarrow \cdot(Chl)_2^+ + Pheo^-$ (charge separation)

The third step is the rapid electron movement to the tightly bound menaquinone, QA, which immediately donates the electrons to a second, loosely bound quinone (QB). Two electron transfers convert QB to its reduced form (QBH2).

$2\cdot Pheo^- + 2H^+ + QB \rightarrow 2Pheo + QBH_2$ (quinone reduction)

The fifth and final step involves the filling of the “hole” in the special pair by an electron from a heme in cytochrome c. This regenerates the substrates and completes the cycle, allowing for subsequent reactions to take place.

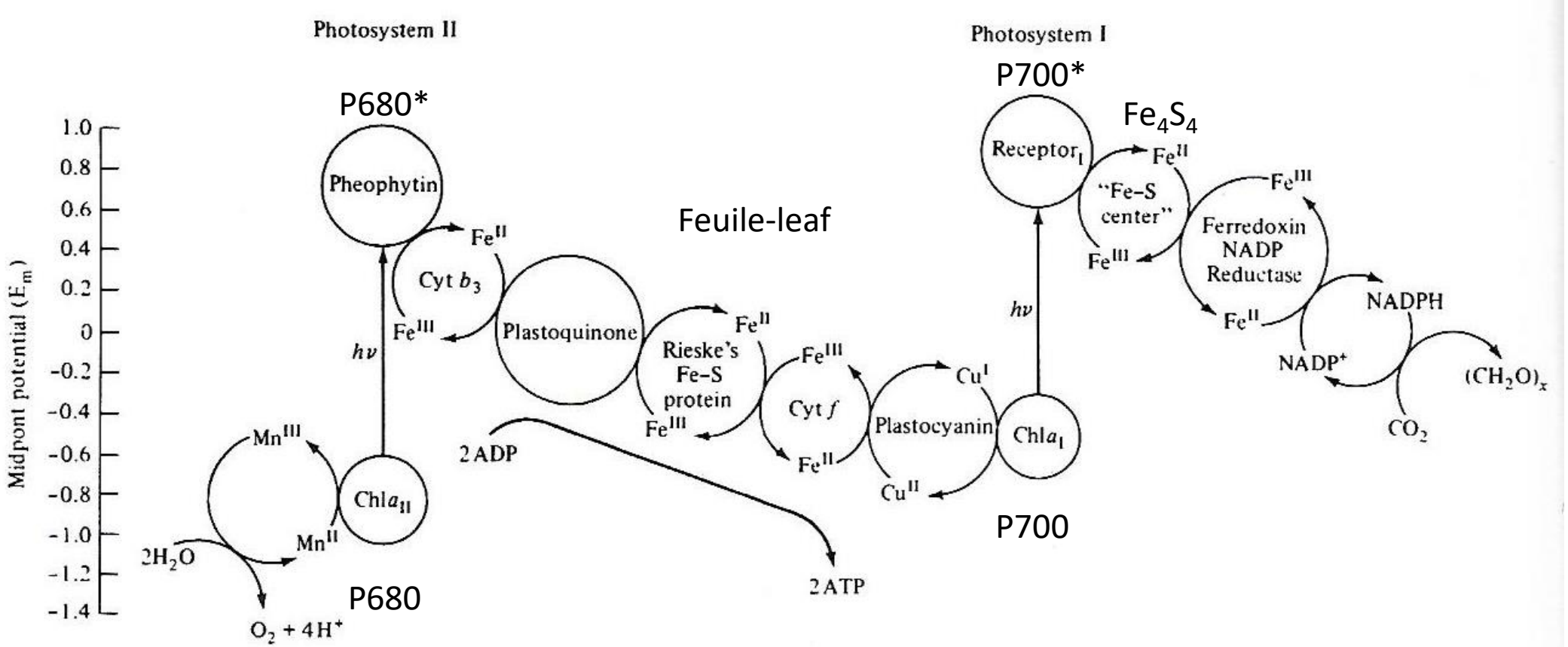


Fig. 4.13. Electron flow in photosystems I and II ('Z'-scheme). The vertical axis gives mid-point redox potential with reducing species (top) and oxidizing species (bottom). Reproduced with permission from Harper Collins.

PS-II (P-680) consists of Chl-a-660, Chl-a-673, Chl-a-680, Chla-690, Chl-b, or Chl-c or Chl-d, carotenoids & phycobilins. Phycobilins present only in PS II

PS-I (P-700) consists of Chl-'a' 670, Chl-a-683, Chl-'a'-695, carotenoids, some molecules of chl- 'b' & reaction centre Chl-'a'-700/P-700

ferredoxin-NADP$^+$ reductase

2 reduced ferredoxin + $NADP^+ + H^+ \longrightarrow$ 2 oxidized ferredoxin + NADPH

Journal of chemical education – **2005, 82(5), 791-94**

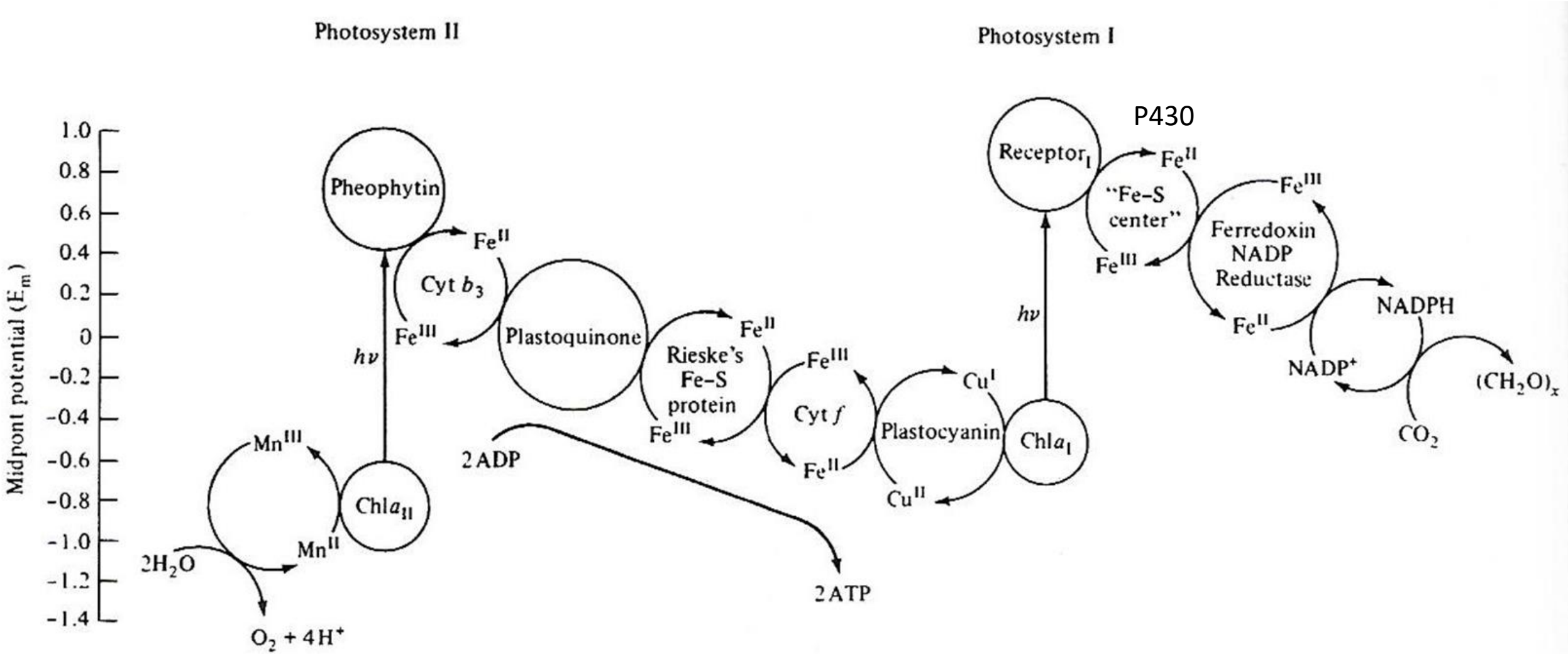
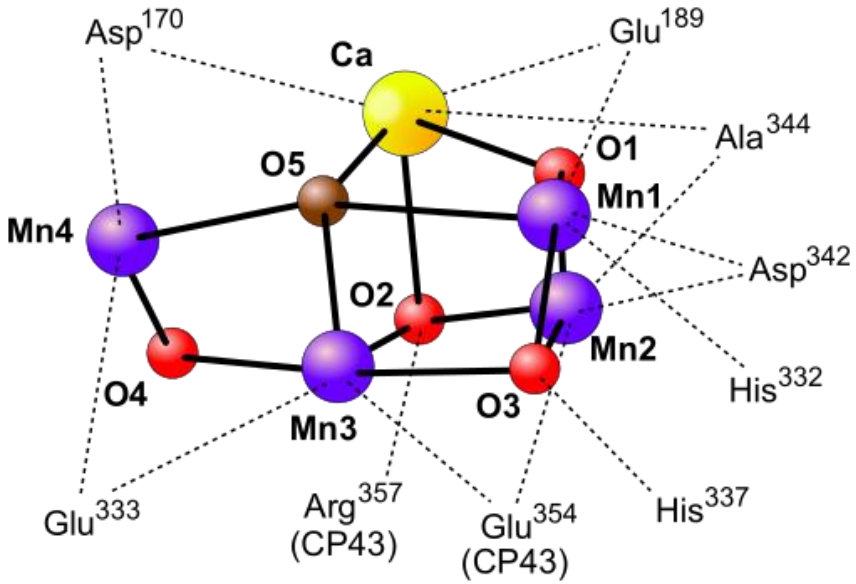


Fig. 4.13. Electron flow in photosystems I and II ('Z'-scheme). The vertical axis gives mid-point redox potential with reducing species (top) and oxidizing species (bottom). Reproduced with permission from Harper Collins.

Oxygen-evolving complex (OEC)



KOK Cycle

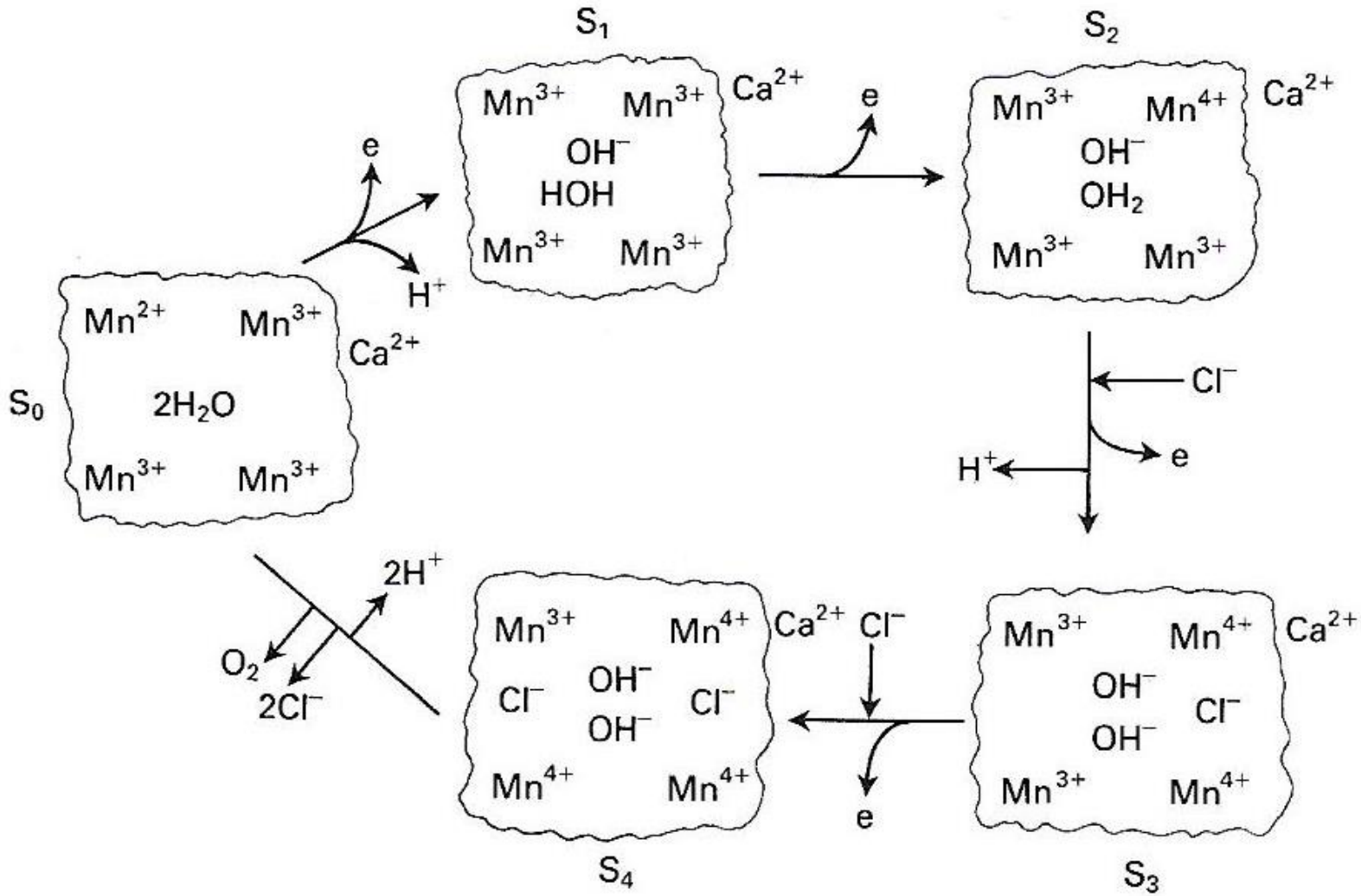
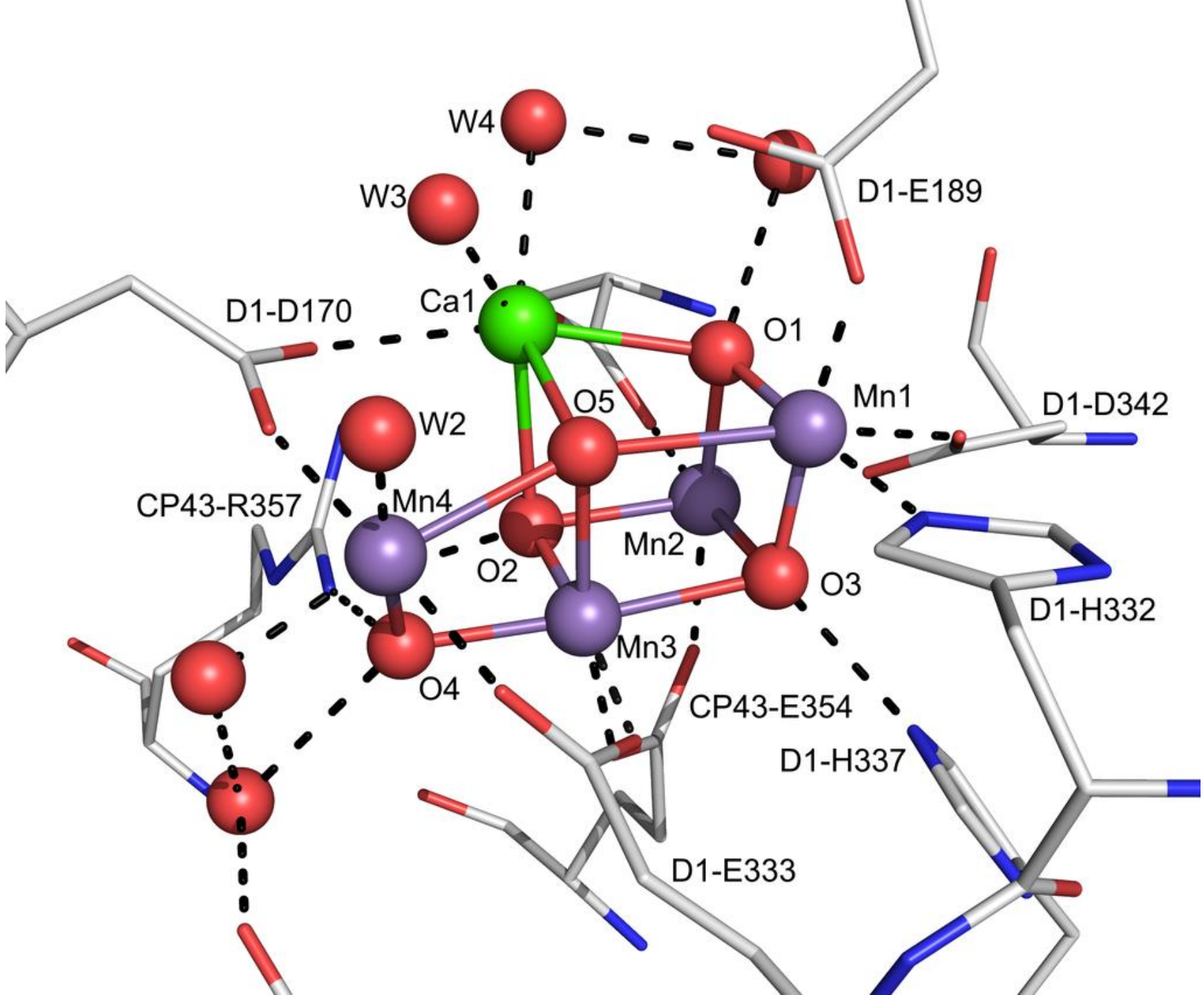
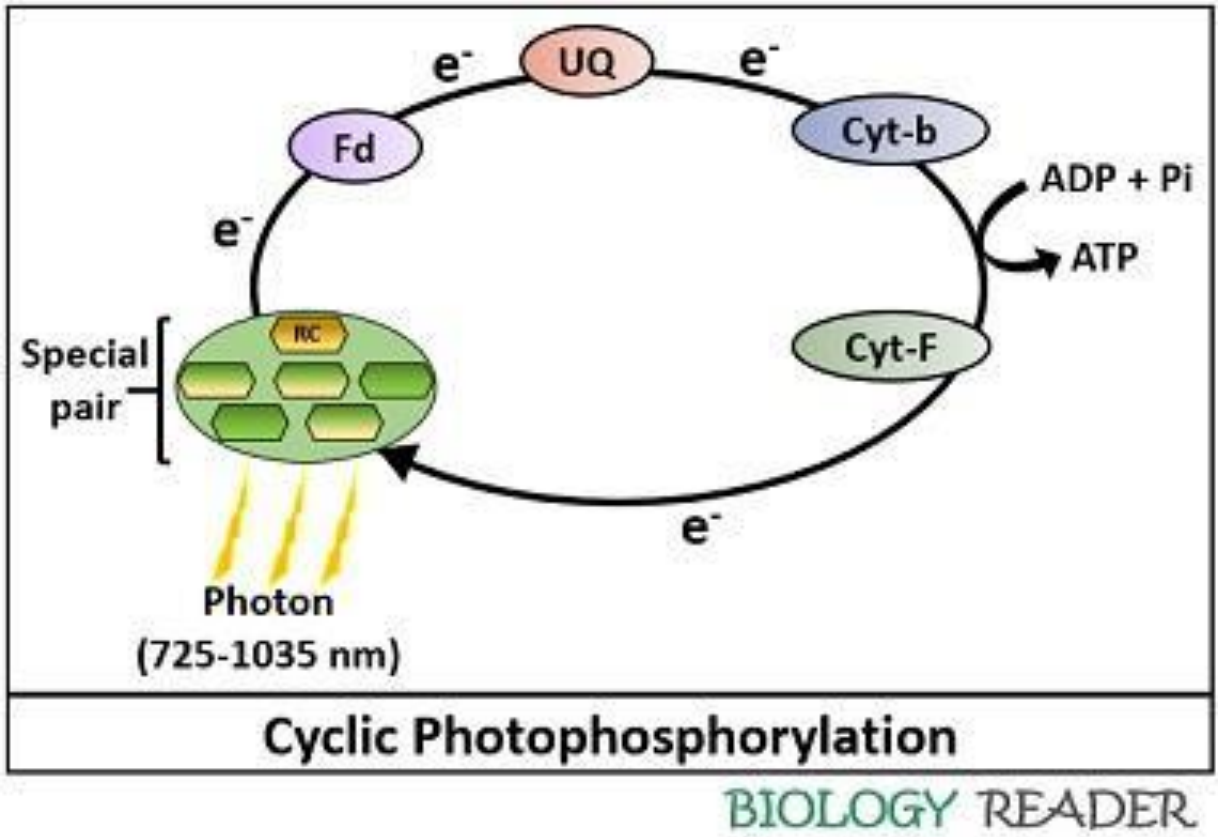
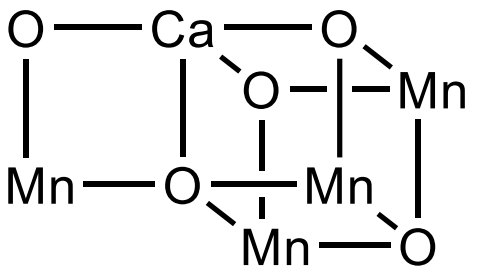


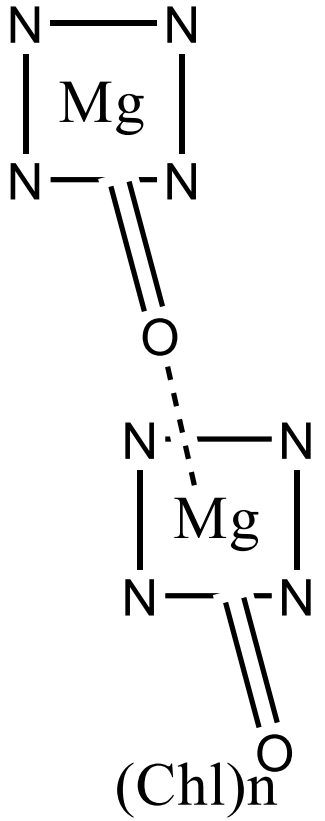
Fig. 4.15. A chemical scheme for O_2 production at a four-manganese cluster involving also Ca^{2+} and Cl^- (reproduced with permission from Oxford University Press).

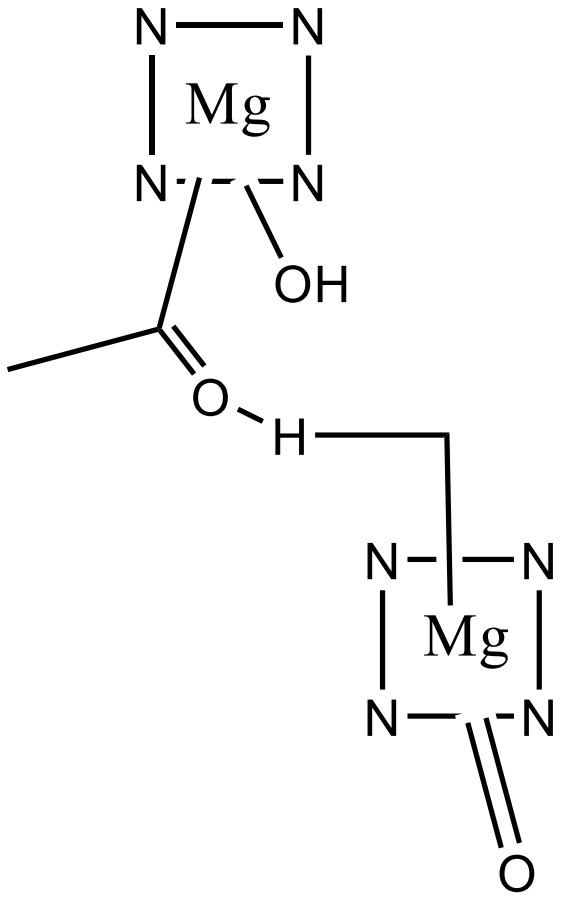

W4
W3
D1-E189
D1-D170
Ca1
O1
Mn1
D1-D342
O5
W2
CP43-R357
Mn4
Mn2
O2
O3
D1-H332
Mn3
O4
CP43-E354
D1-H337
D1-E333

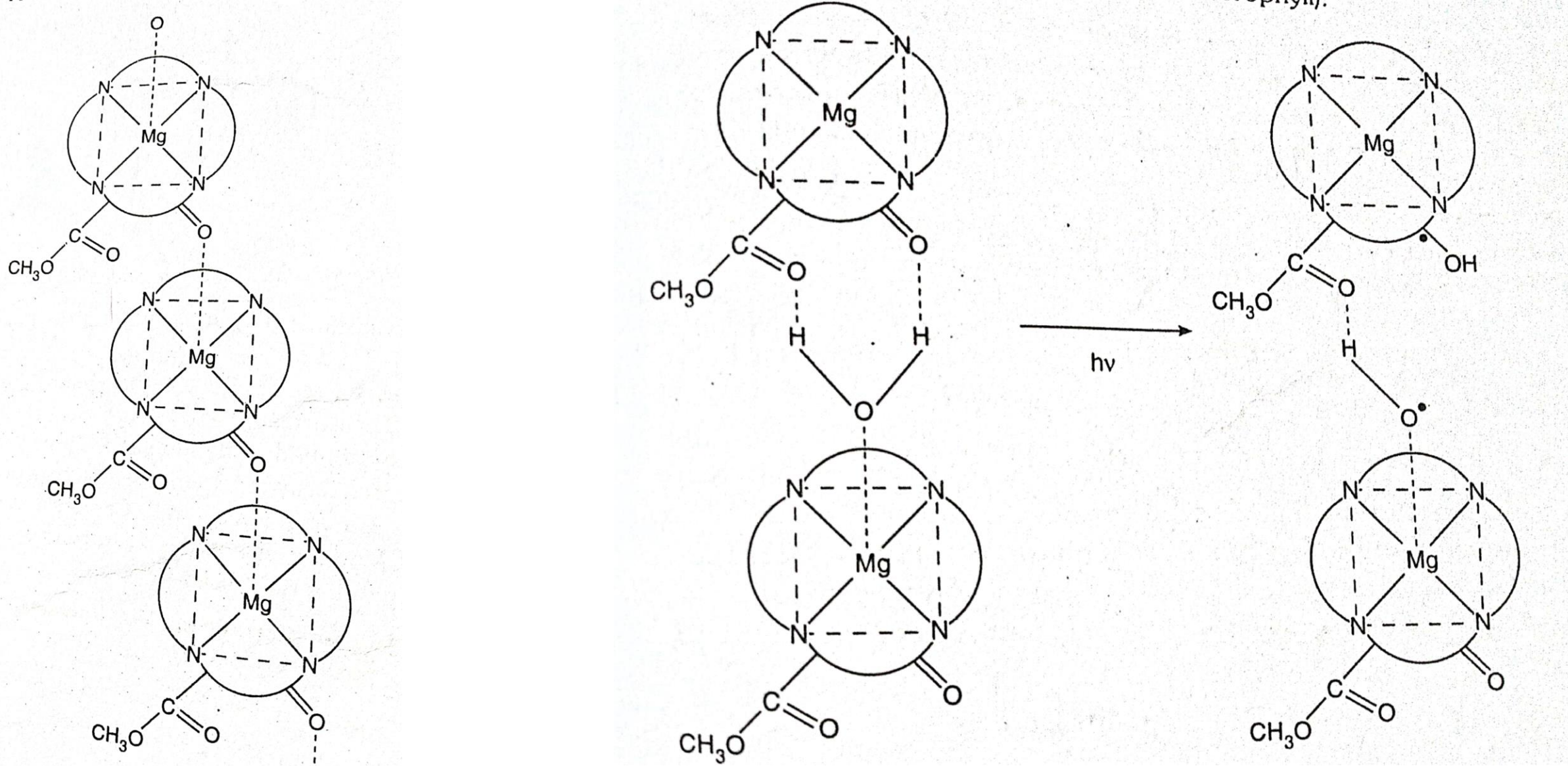
Antenna Chlorophyll



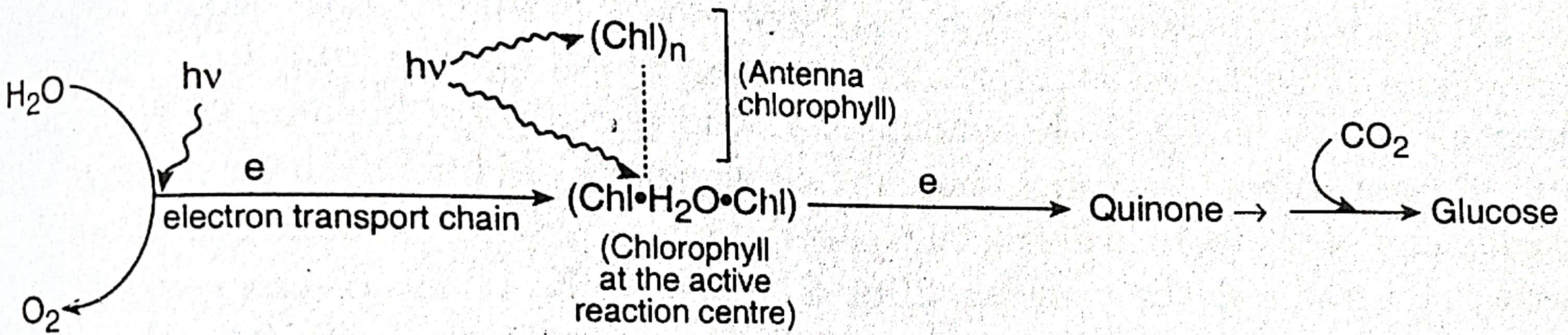







Mg
N
O
C
CH_3O
H
OH
hν

The total electron transport system leading to reduction of CO_2 in photosynthes schematically represented in Scheme 8.5.6.1.



Scheme ***8.5.6.1*** *: Schematic representation of photoelectron transfer process in photosynthesi*

We now turn to the molecular mechanism of DNA replication. The full replication machinery in cells comprises more than 20 proteins engaged in intricate and coordinated interplay. In 1958, Arthur Kornberg and his colleagues isolated the first known of the enzymes, called DNA polymerases, that promote the formation of the bonds joining units of the DNA backbone.

https://www.youtube.com/watch?v=R4-c3hUhhyc

https://www.news-medical.net/life-sciences/DNA-Polymerase-Families.aspx

https://www.wehi.edu.au/wehi-tv/molecular-visualisations-dna

https://www.ncbi.nlm.nih.gov/books/NBK22513/

https://www.khanacademy.org/science/ap-biology/ap-cellular-respiration-and-fermentation

Iron Containing Proteins

Chapter 3. Heme and Non-heme Proteins

- Brief introduction to proteins
  - Difference between A1 and A2 milk; sickle cell anemia due to valine
- Structure of porphyrin (drawing and numbering scheme)
  - Substituents (PIX), tetranionic nature
  - How Fe(II) binds with PIX, coordination sphere, geometry of T and R state
  - Proximal and distal histidine
- Fe-O bonding – Fe^{2+} HS to Fe^{3+} LS
  - Nature: whether Fe(III)-O-O^- or Fe(II)-O=O, the evidence from FT-IR
  - Inertness of triplet O_2 and mode of binding of O_2
  - Importance of globular protein surrounding the heme
  - Eight possible salt bridge interactions
  - The presence of bisphosphoglycerate (BPG) and its uses
  - P(O2) vs % oxygenation-effect of pH, CO2, allosteric interactions, Hill equation and coefficient
- Importance of heme group

- How DPG helps to feed O_2 to fetus from mother
- Bohr and Haldane effect
- Allosteric interaction
- Hill equation and coefficient
- Other storage and transport proteins

Iron Proteins

Iron containing proteins are mainly belonging to four categories.

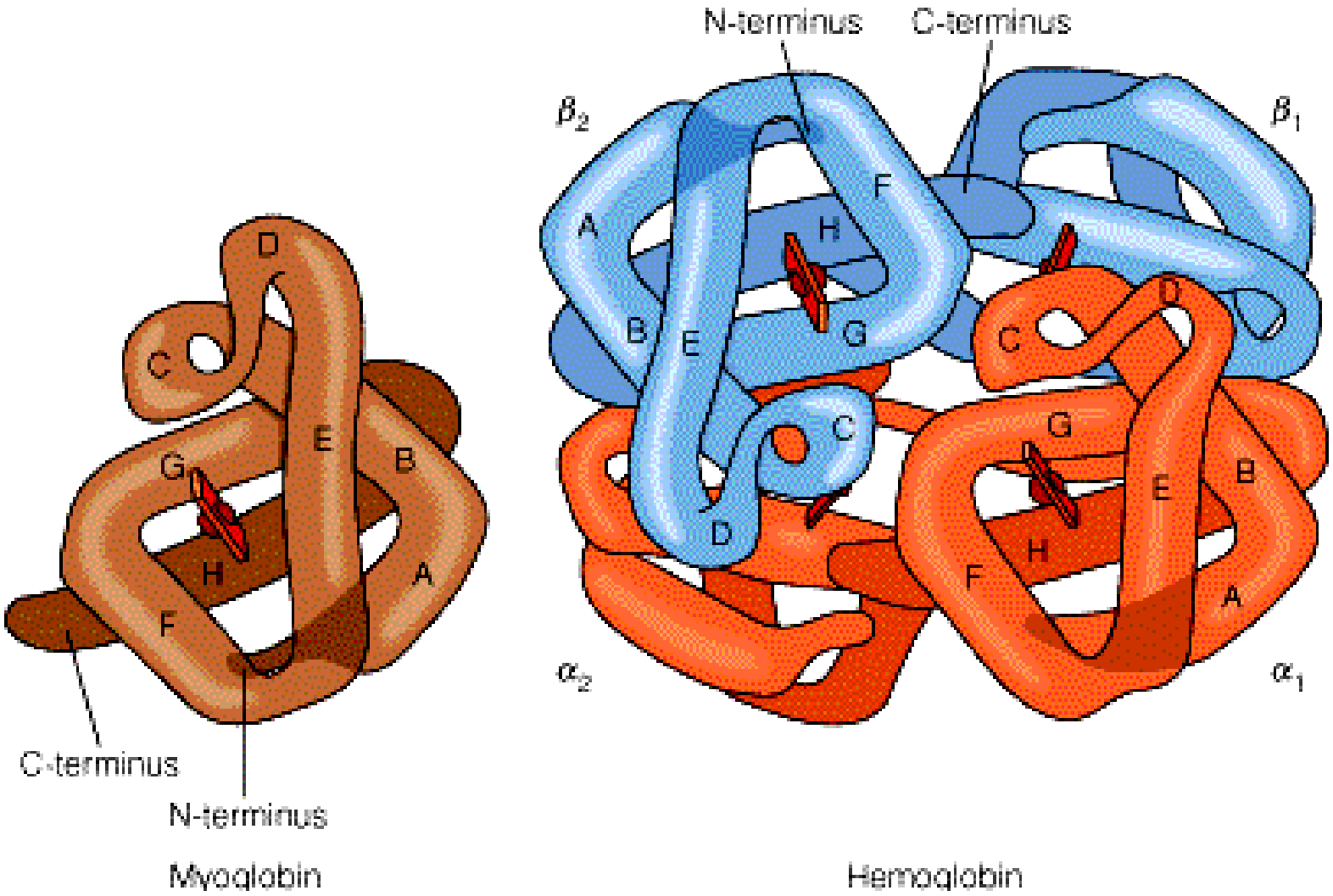
A.Iron-porphyrin proteins: hemoglobin, myoglobin, cytochrome P450, etc; all of them contains one of more iron-porphyrin units. They are mainly involved in oxygen transfer, oxygen storage, and electron transfer.

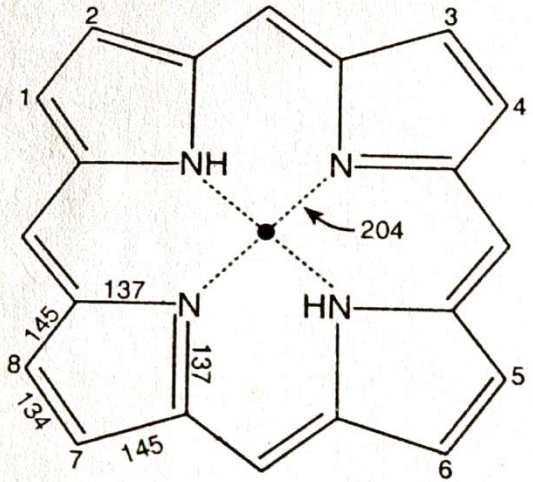
B.Non-heme iron proteins: ferritin, transferrin, hemesiderin, etc; they are mostly involved in iron storage and transport

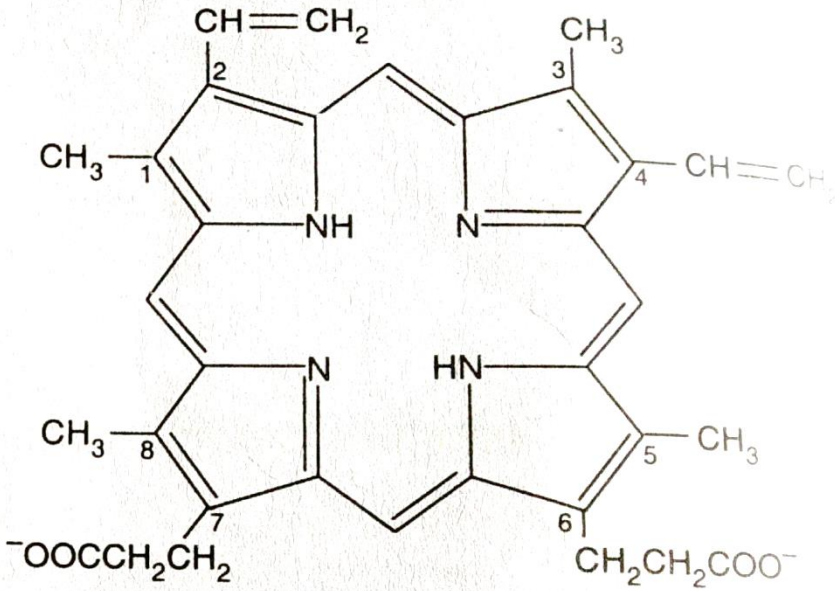
C.Non-heme diiron oxo-bridged species: ribonucleotide reductase, hemerythrin, methane monooxygenase, etc.

D.Non-heme iron-sulfur cluster proteins: nitrogenase, ferredoxins, rubredoxins. They are mainly involved in biological electron transfer reactions.

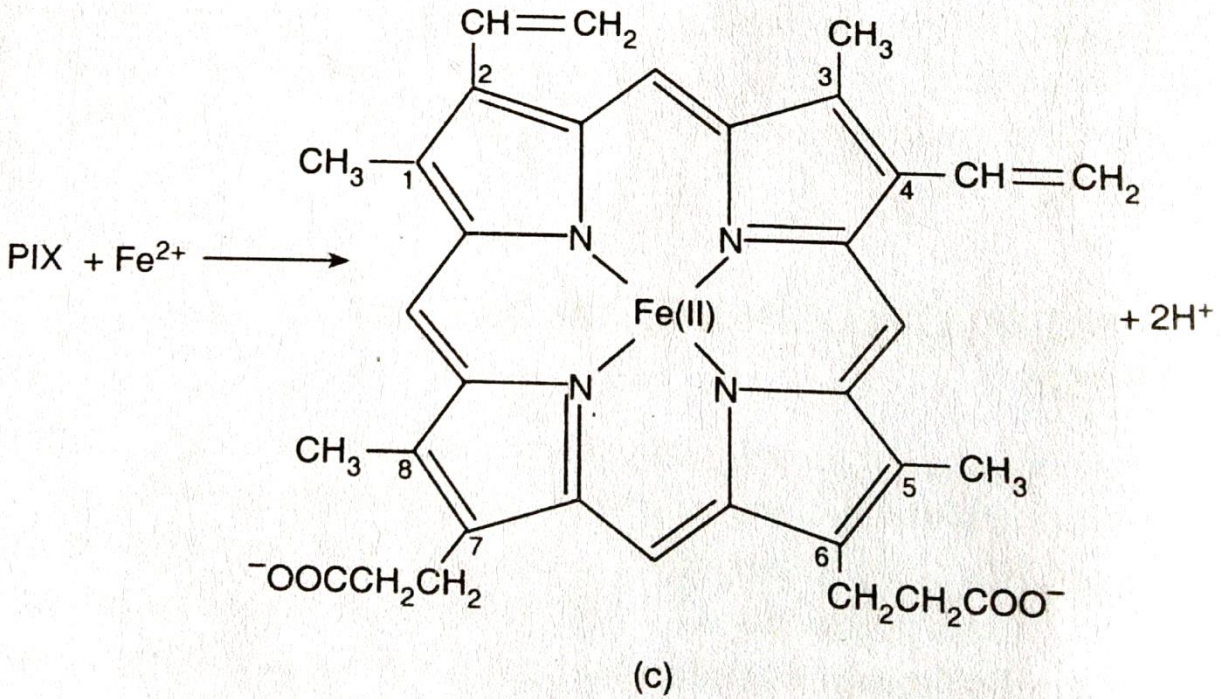
Heme Proteins – Hemoglobin and Myoglobin

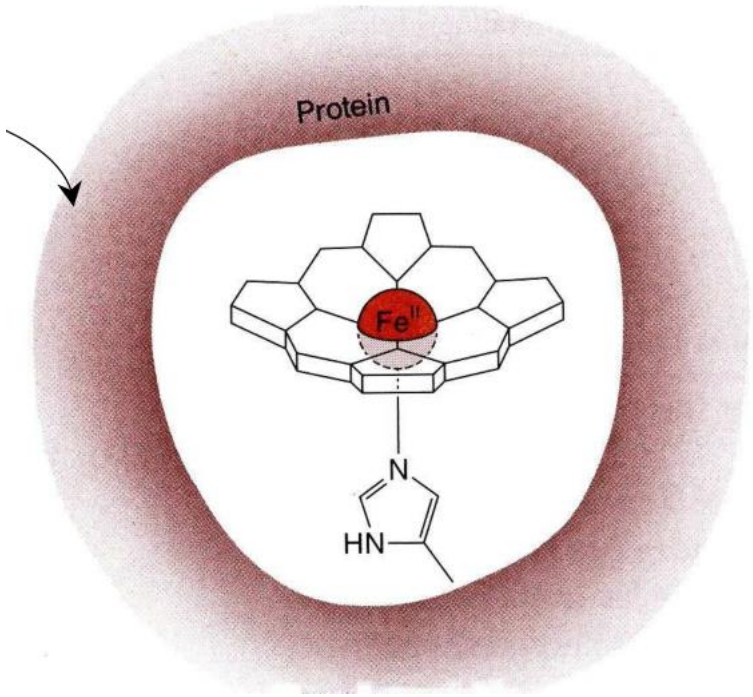


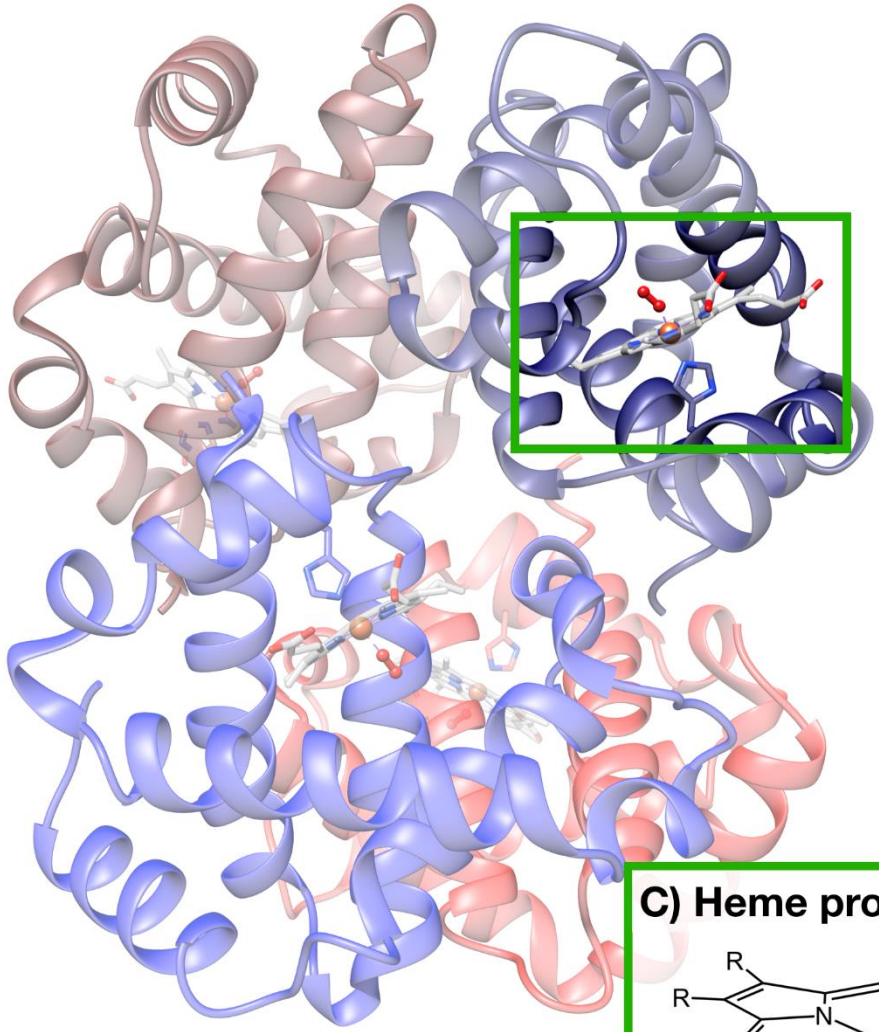


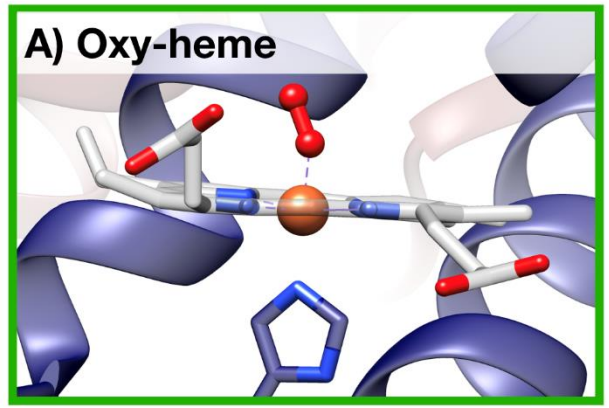


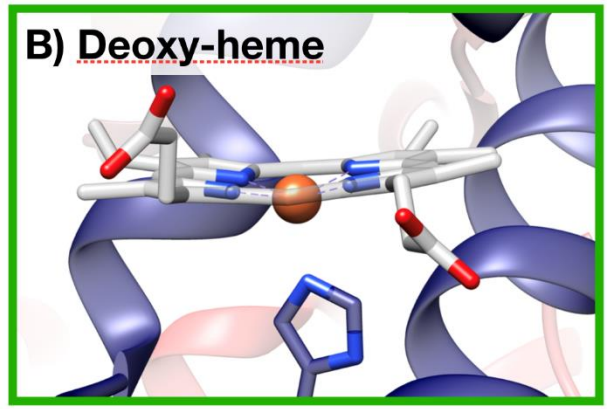
Protoporphyrin – IX (PIX)



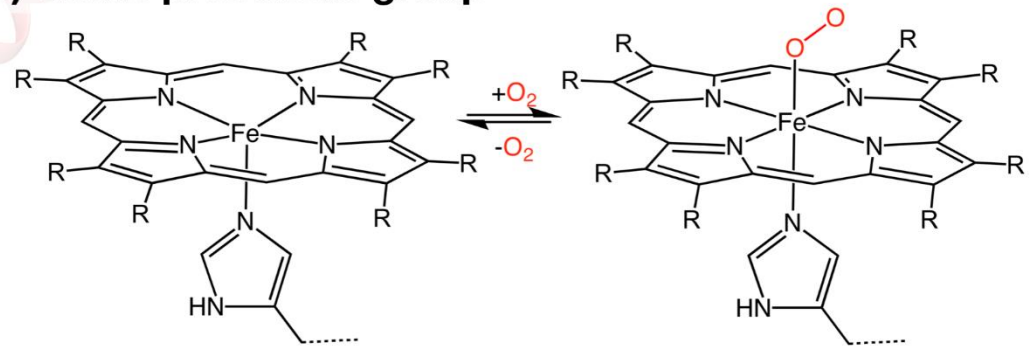






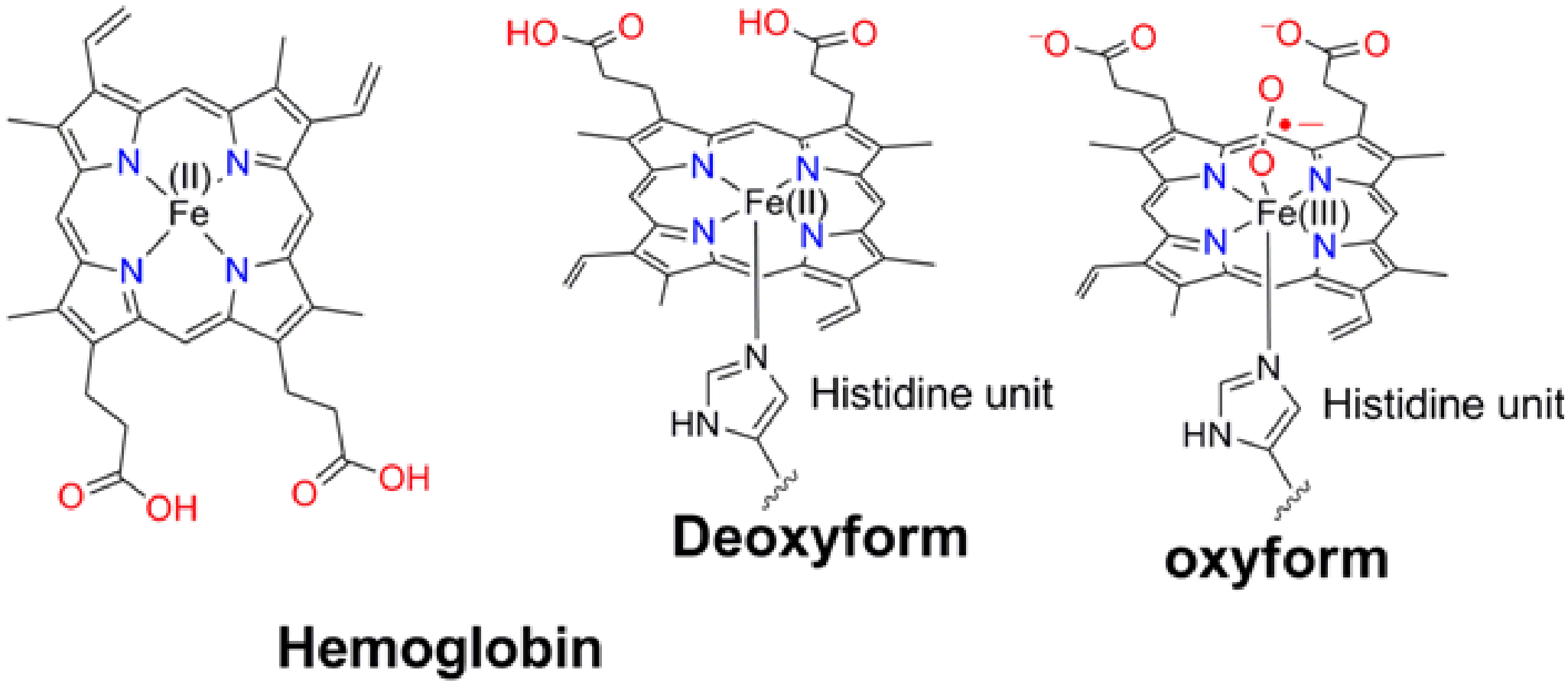


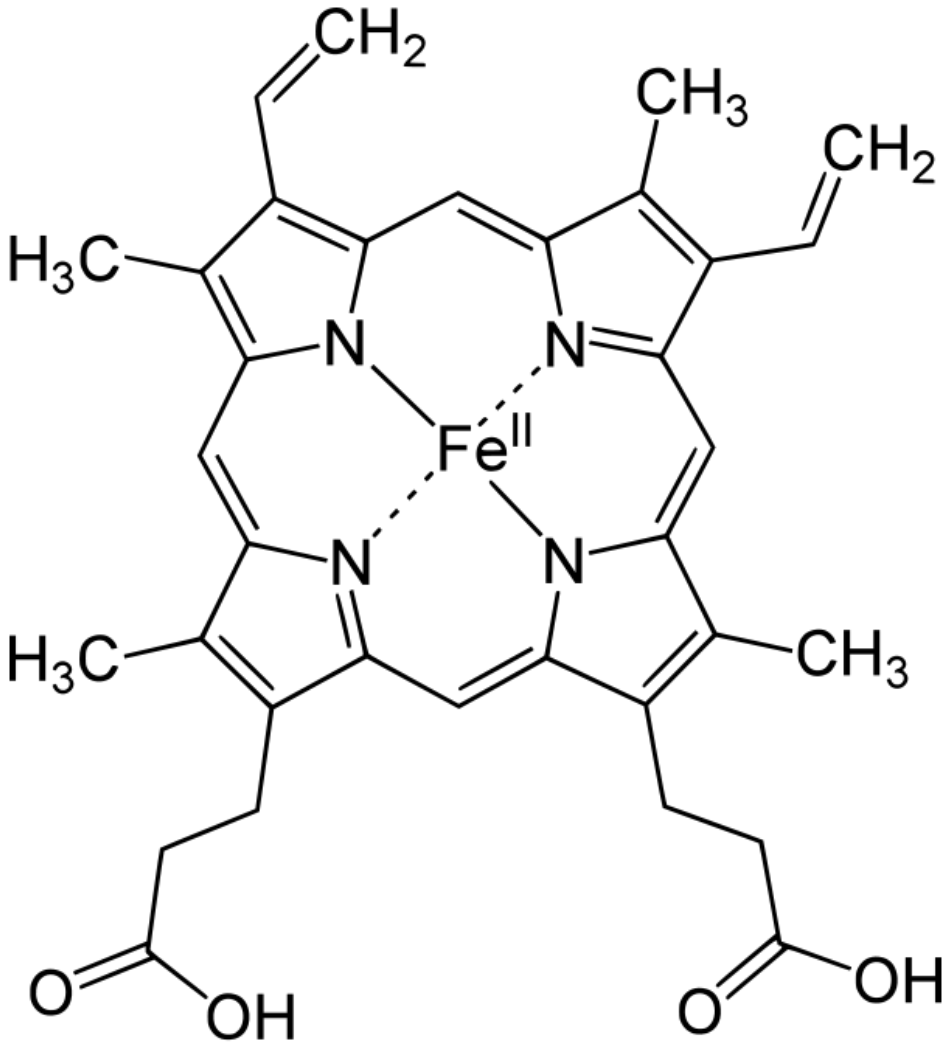
C) Heme prosthetic group



Hemoglobin and Myoglobin:

Hemoglobin is an essential iron protein for molecular oxygen (dioxygen) transport and found in red blood cells. Both are globular proteins; myoglobin is engaged in storage of molecular oxygen in muscle tissues and controlled transport of molecular oxygen for the oxidative reactions.





- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids))
- Removal of waste such as carbon dioxide, urea, and lactic acid
- Immunological functions, including circulation of white blood cells and detection of foreign material by antibodies
- Coagulation the response to a broken blood vessel, the conversion of blood from a liquid to a semi-solid gel to stop bleeding.
- Messenger functions, including the transport of hormones and the signaling of tissue damage
- Regulation of body pH
- Regulation of core body temperature
- Hydraulic functions

A hemoglobin unit is composed of has **four protein** chains each of which contains one porphyrin ring coordinated to iron (known as heme) packed in a roughly tetrahedral α2β2 cluster. The α unit contains 141 amino acids residue and the β unit contains 146 amino acids residue.

In hemoglobin, a high-spin Fe(II) is coordinated to four N atoms of porphyrin ring. The fifth coordination site is occupied by a histidine group. In this condition the protein containing four heme compartments is in stain condition **and called tense (T)** state. After binding to molecular oxygen **high-spin Fe(II) changes to low-spin Fe(III)** and molecular oxygen is transferred to superoxide. Covalent radii of Fe(II) is too large to fit into the cavity created by four N atoms of the porphyrin ring. Formation of low-spin Fe(III), the radii of iron decreases. Moreover, transformation of square pyramidal deoxy form to octahedral oxy form leads iron center closer to heme cavity. The protein is now in comparatively less stain relaxed form (R). In this was all the four iron centers in hemoglobin are transferred to oxo-hemoglobin forms.

Binding of molecular oxygen to the iron center of the hemoglobin tetramer causes **release of protons from the acid units which minimize the pH**. This lowering of pH favours molecular oxygen release to tissues and conversion of Fe(III) to Fe(II). This deoxyhemoglobin picks up 2 protons and 2 molecules of CO_2 from tissues and carried to the lungs, where the CO_2 is released. After that, deoxyhemoglobin which further binds to molecular oxygen and the O_2 carrying and CO_2 returning processes from tissues continues.

Myoglobin is a single chain heme protein containing 154 amino acids and it contains several region of α–helix. The structure of the active site (where the reaction occurs) and the oxygen carrying mechanism is same as hemoglobin. The oxygen uptake capacity of myoglobin is thermodynamically more when compared to hemoglobin and hence, oxygen uptake in myglobin is more than that of hemoglobin.

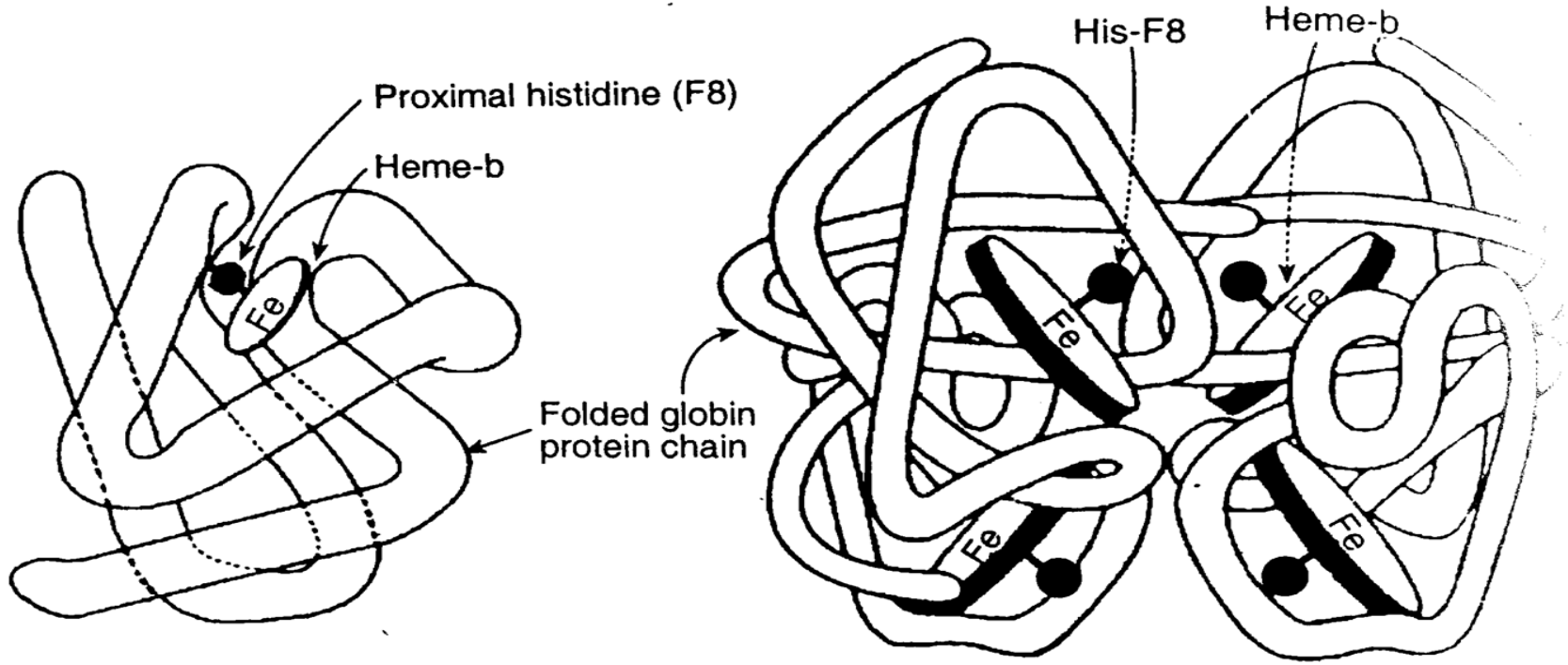


Figure 5.5.1.3 *: Structure of myoglobin (Mb) and Hemoglobin (Hb) with the globin protein cha*

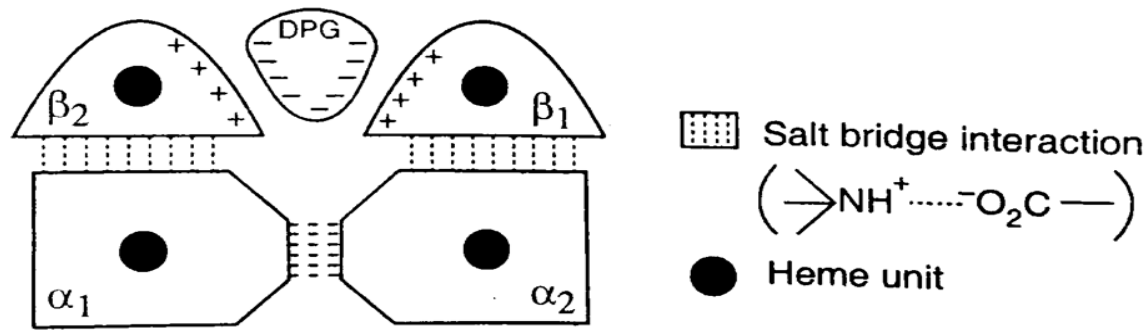
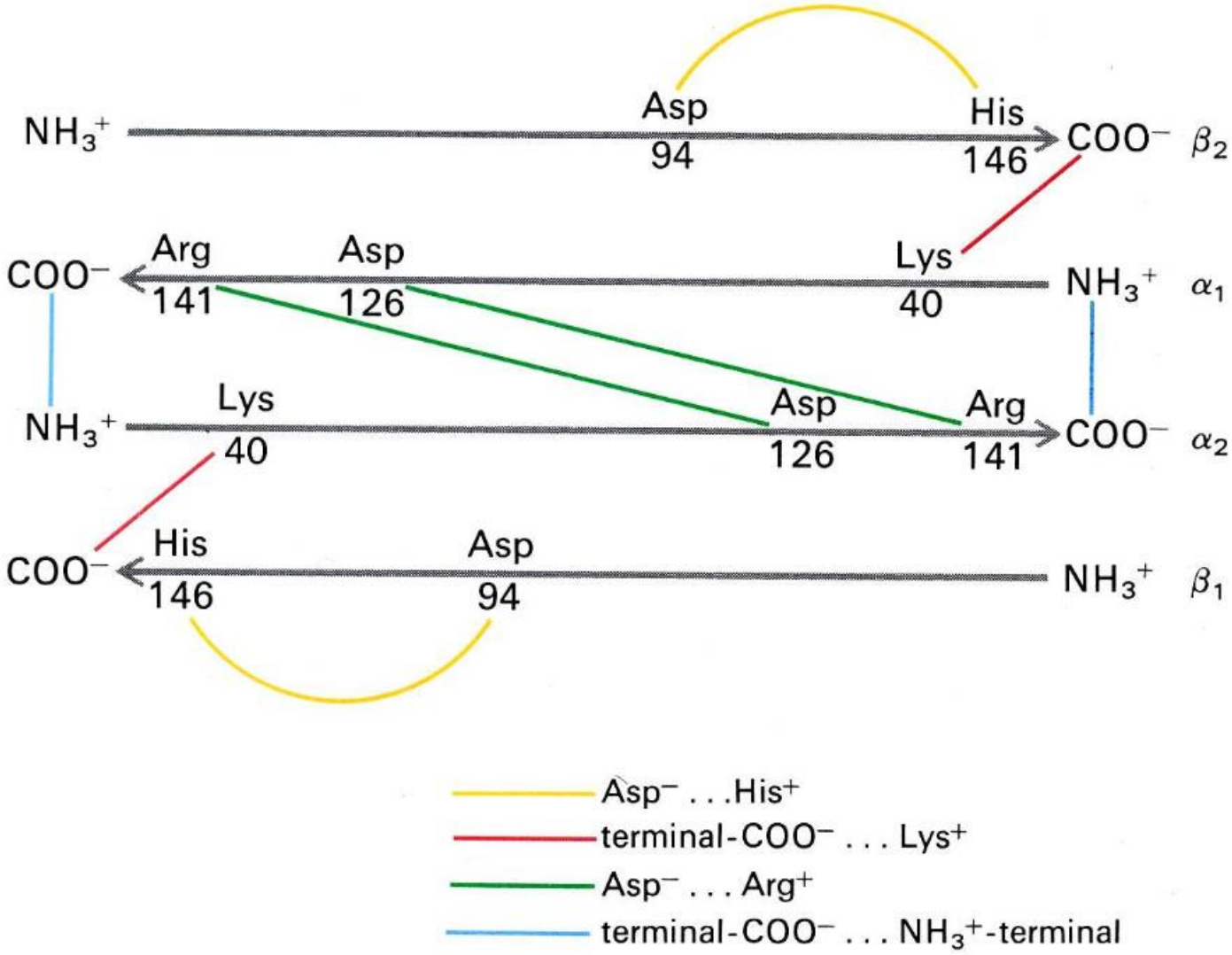


Figure 5.5.1.4 *: Schemetic representation of tetrameric hemoglobin (Hb-A)*



Salt links between different subunits in deoxyhemoglobin. These noncovalent, electrostatic cross-links are disrupted on oxygenation.

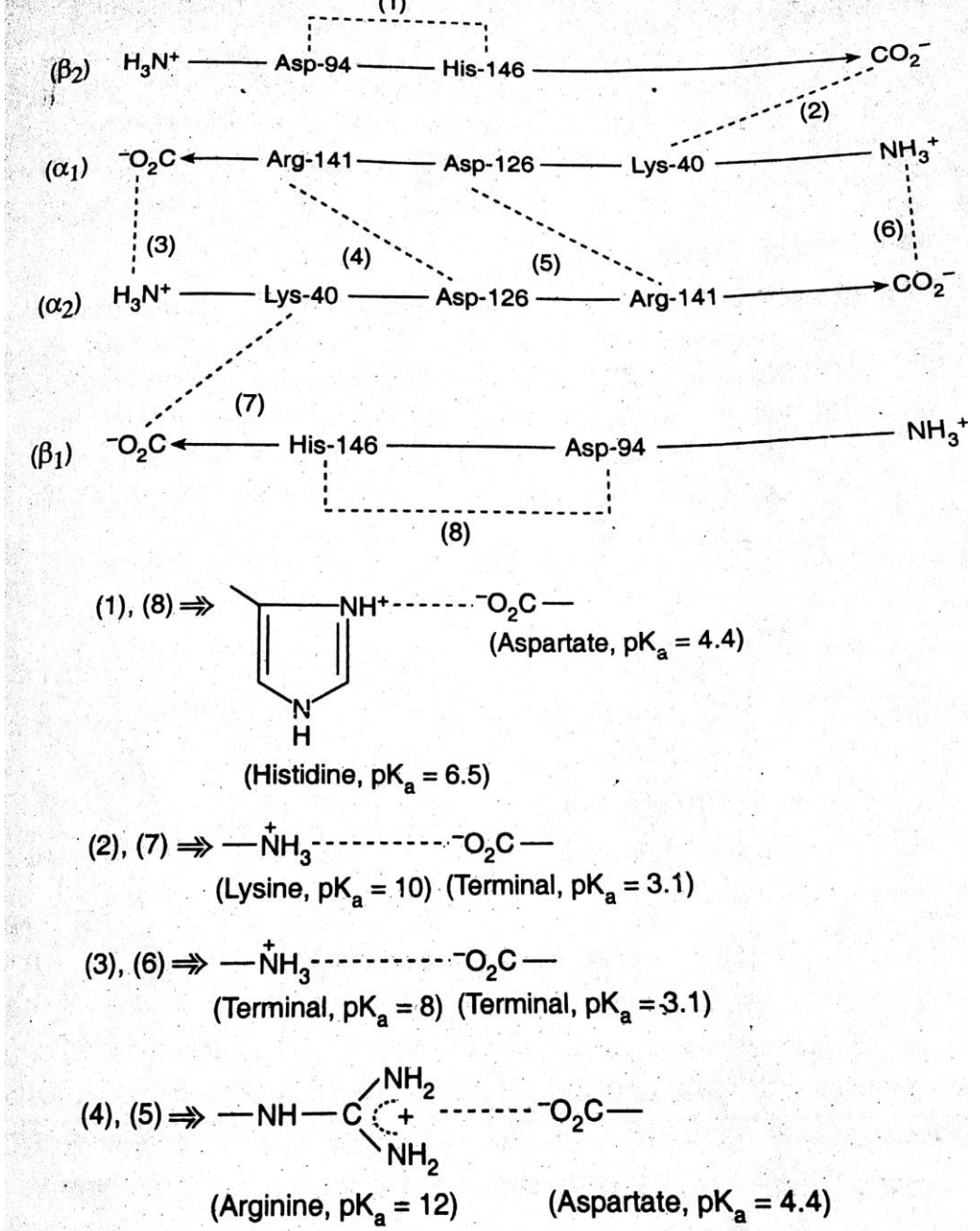


Figure 5.5.1.5 *: Salt-bridge interactions in Hb-A ($\alpha_2\beta_2$). Given pK_A values correspond to the respective conjugate acids.*

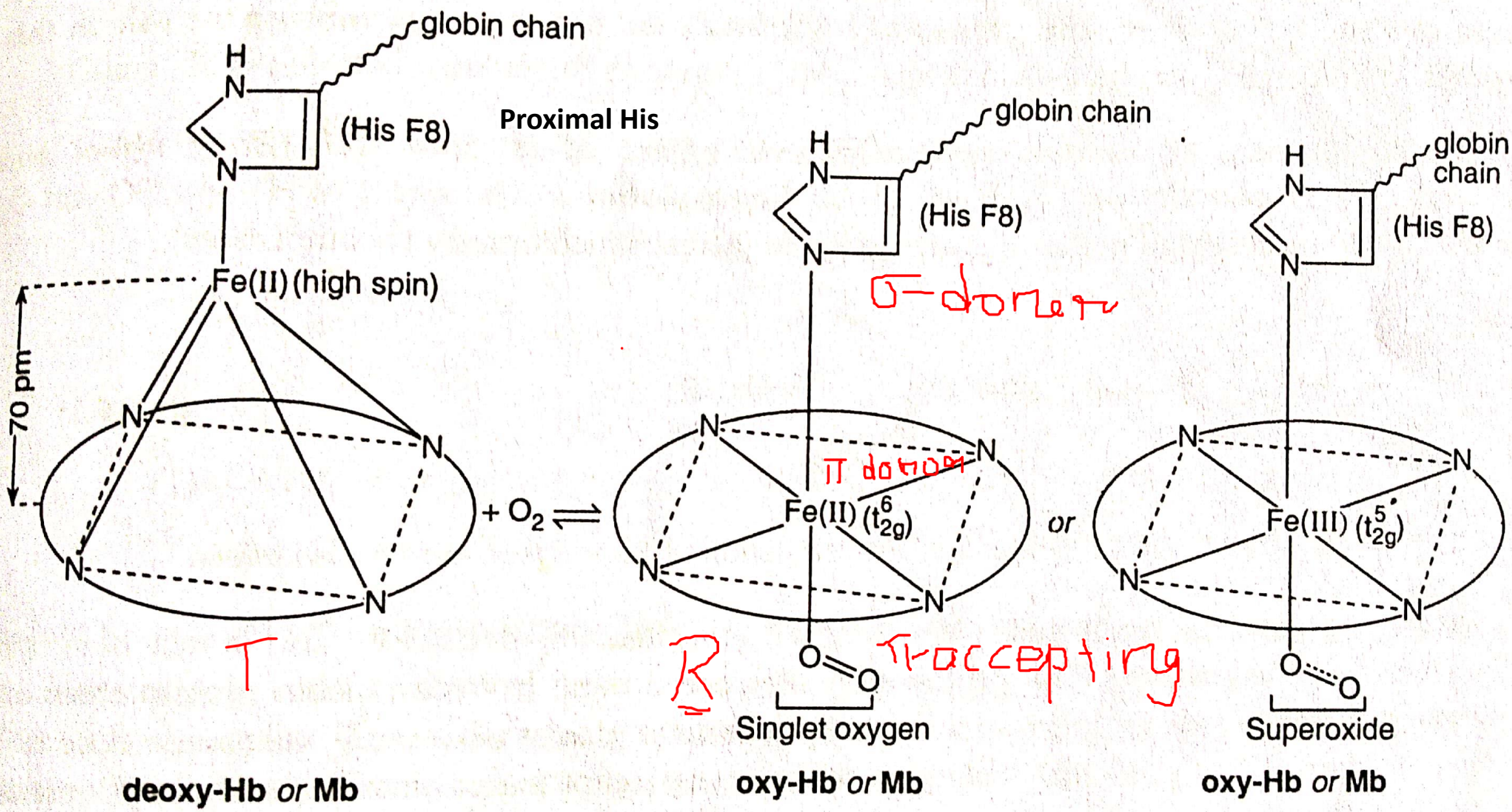
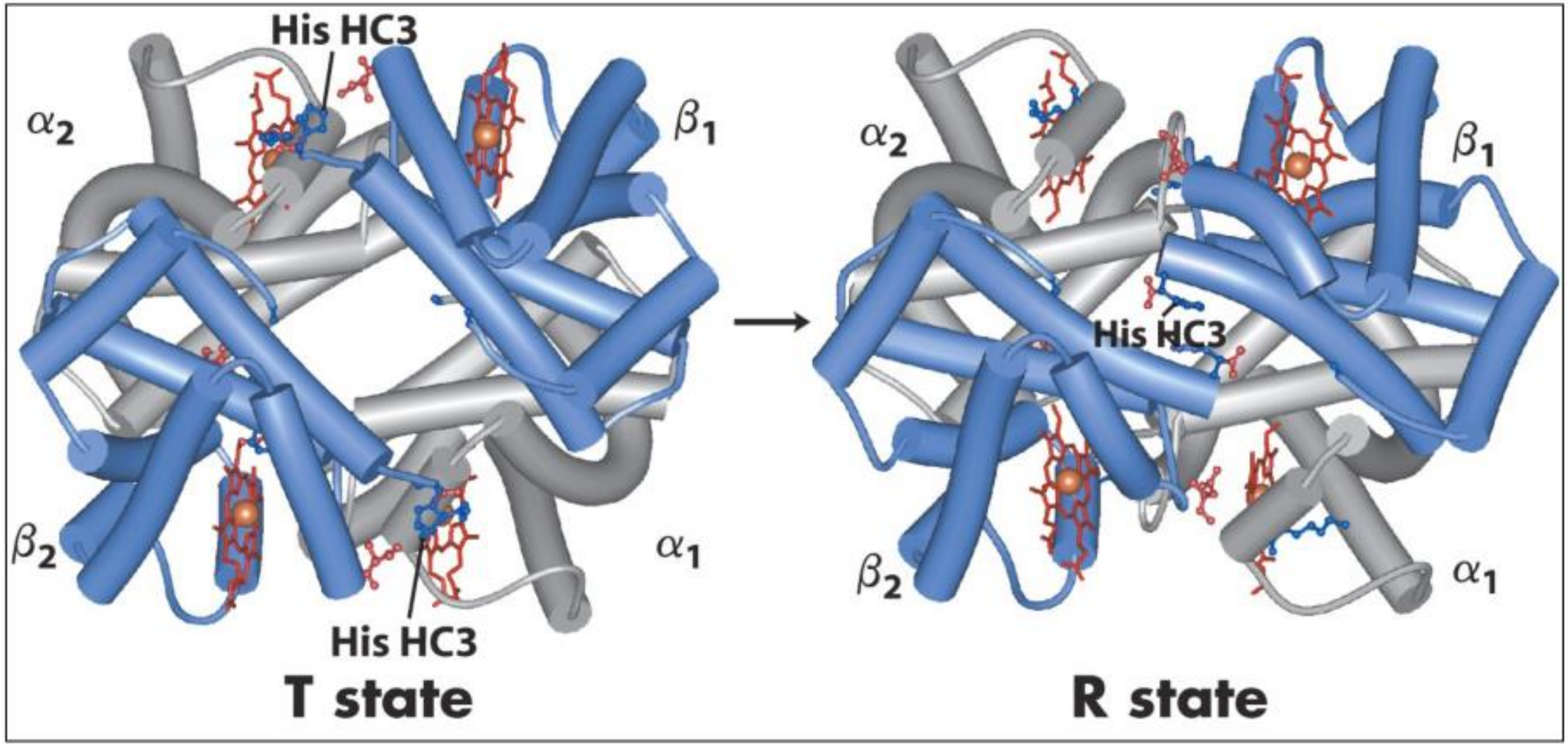
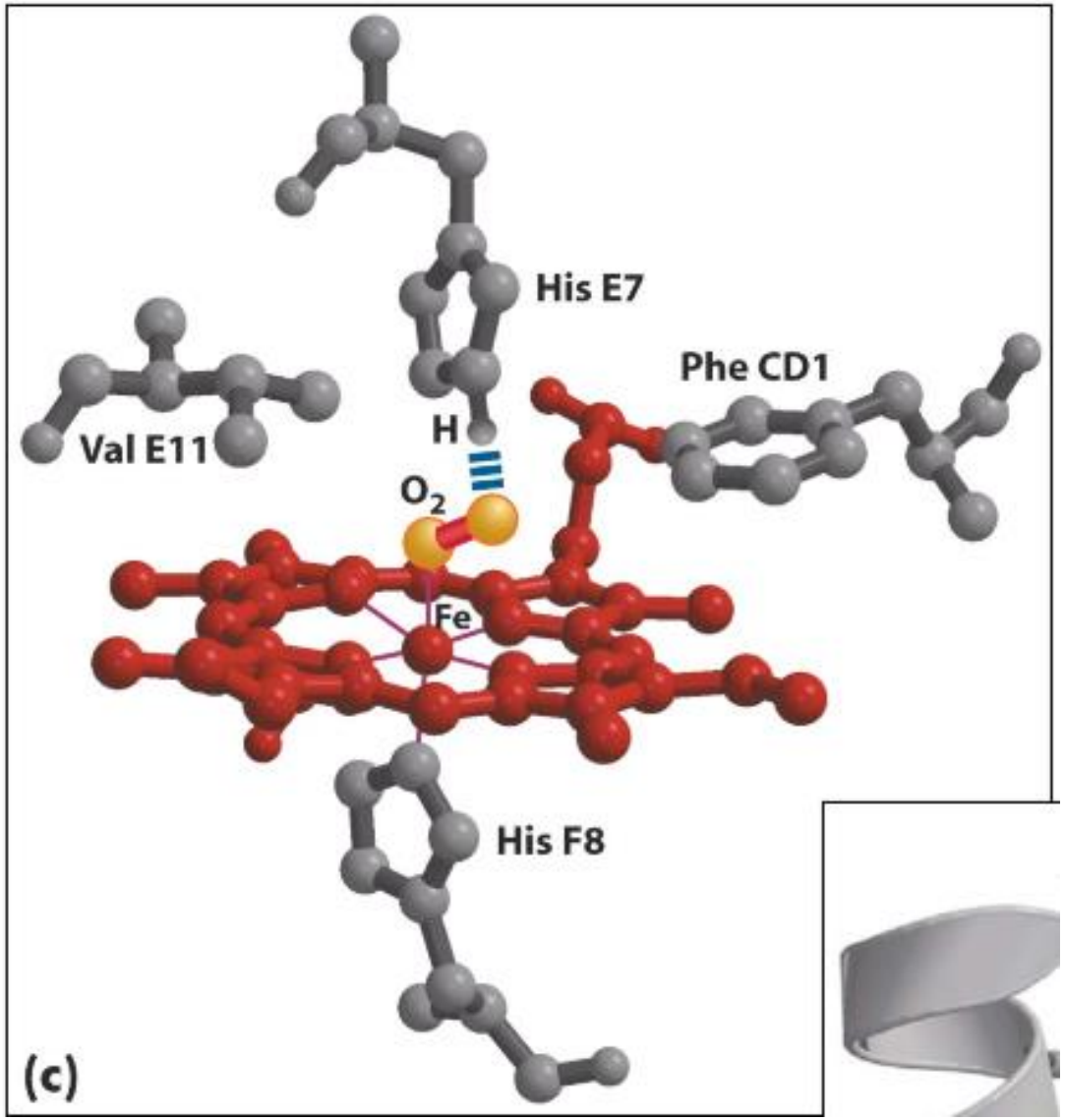
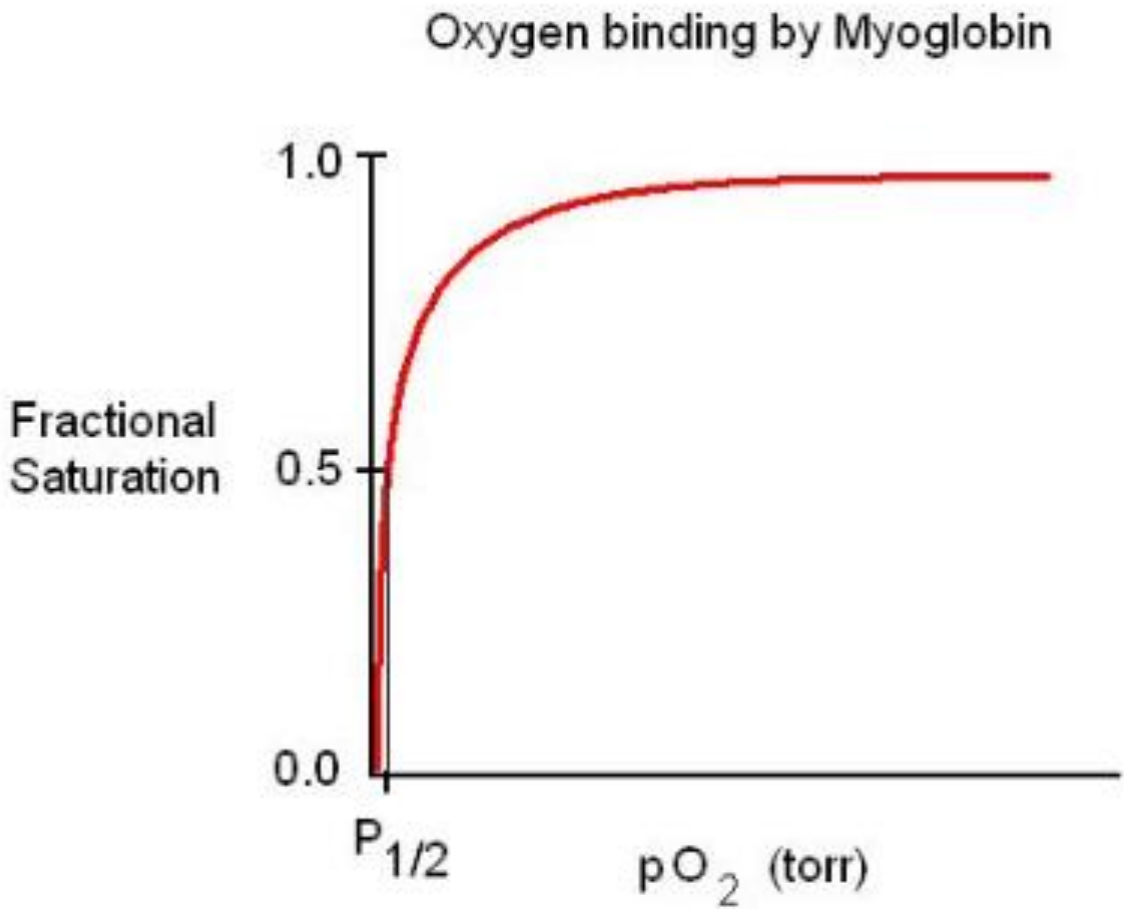


***Figure 5.5.4.1**: Change of coordination sphere of Fe(II) in Hb or Mb during oxygenation.*


His HC3
α2
β1
β2
α1
His HC3
T state
α2
β1
His HC3
β2
α1
R state

Distal histidine





Vibrational and geometrical properties of dioxygen species

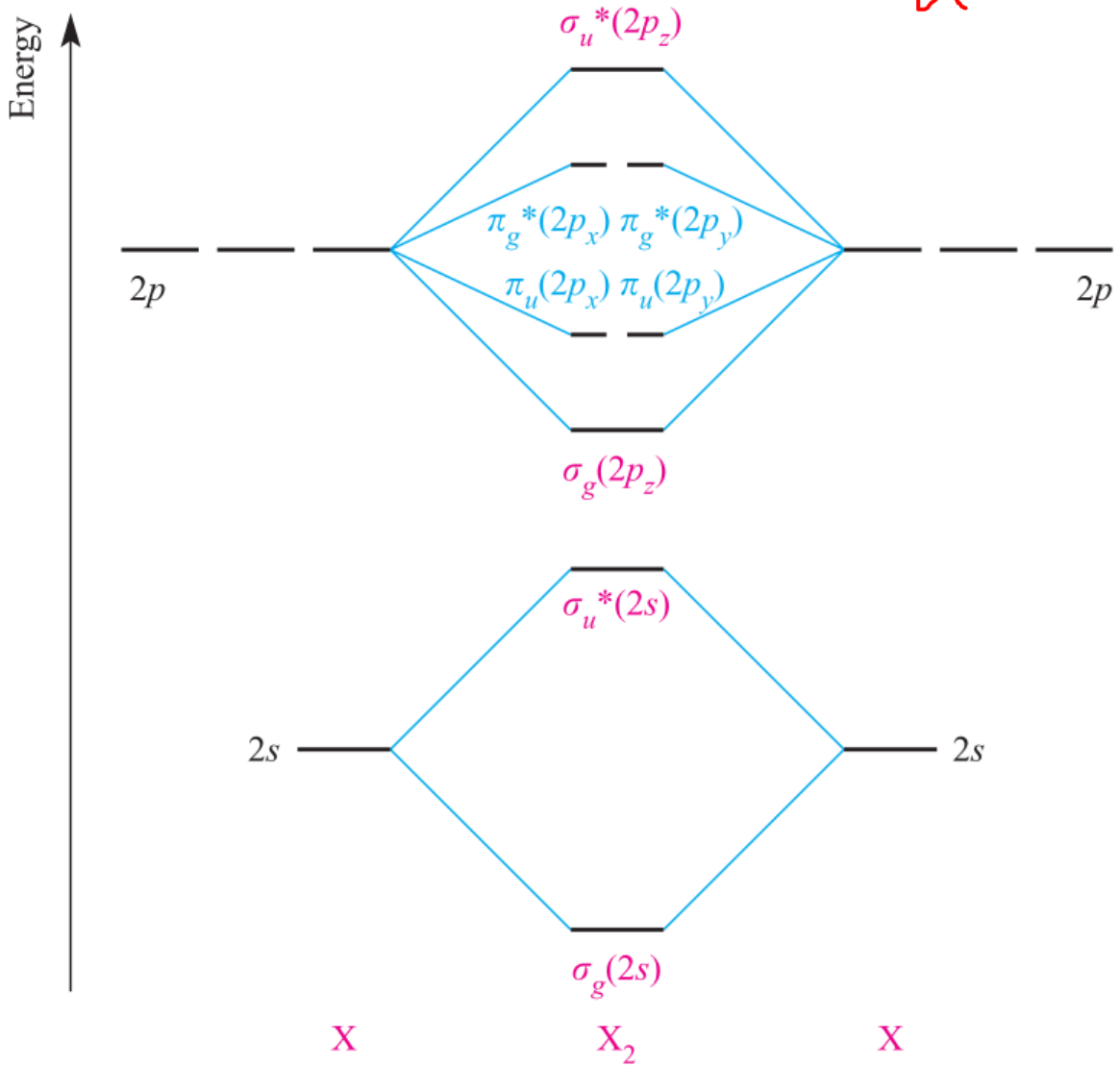
Species	ν_{O-O} (cm^{-1})	d_{O-O} (Å)
O_2^+	1,905	1.12
O_2	1,580	1.21
O_2^-	1,097	1.33
O_2^{2-}	802	1.49

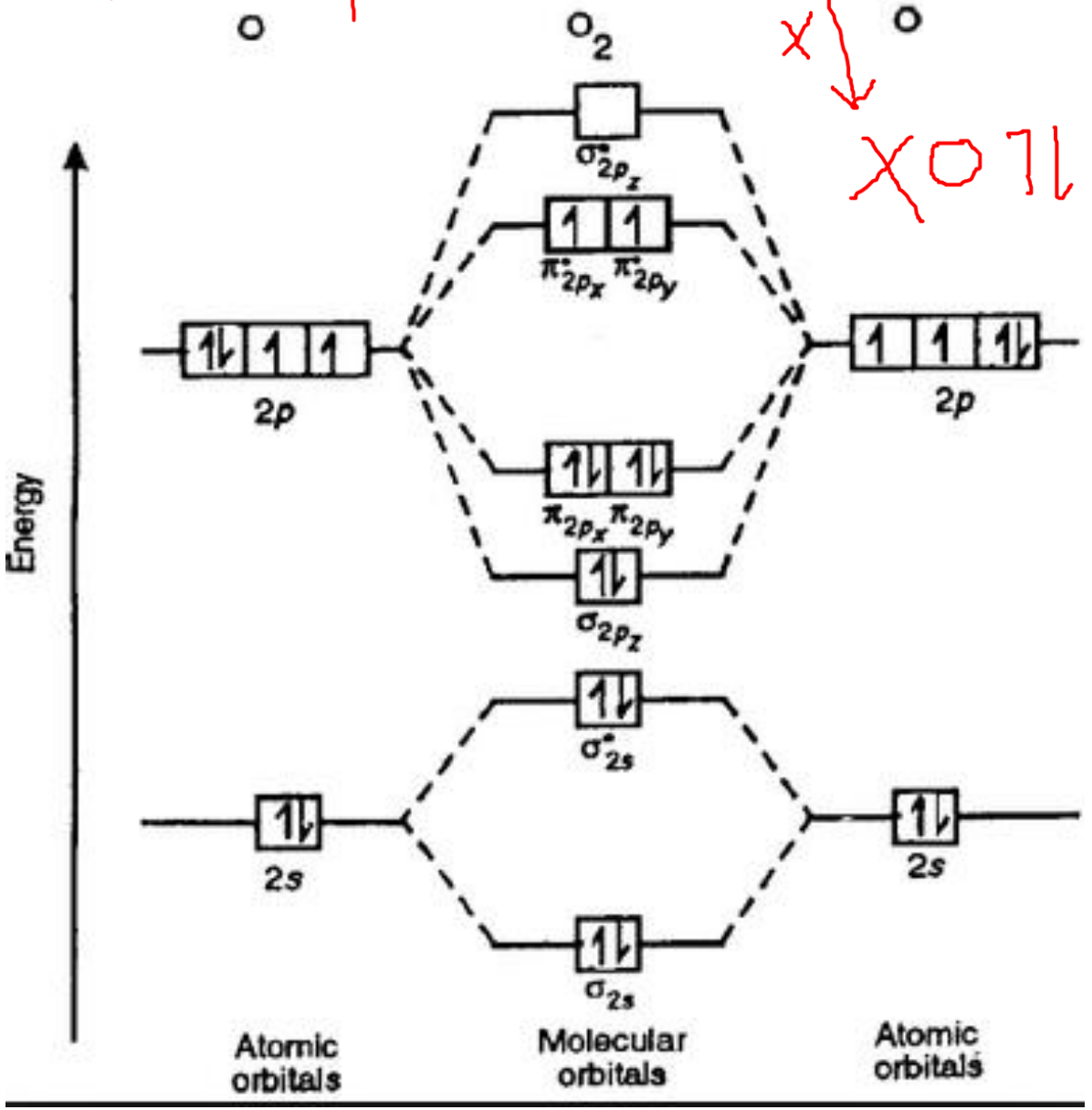
Oxy-Myoglobin	Oxy-porphyrin $Fe^{(II)}O_2$	Oxy-hemerythrin (peroxide)
ν_{O-O}: 1105 cm^{-1}	$\nu_{^{16}O-^{16}O}$: 1139 cm^{-1} $\nu_{^{18}O-^{18}O}$: 1076 cm^{-1}	ν_{O-O}: 844 cm^{-1}
ν_{Fe-O}: 572 cm^{-1}	ν_{Fe-O}: 568 cm^{-1}	ν_{Fe-O}: 503 cm^{-1}

O2 117 + 97 kJ/mol → O2 가스

x ↓ O

X O 가스





$O_2 + M \longrightarrow$

17

$sML_n + O_2 \underset{k}{\rightleftharpoons} sL_nMO_2 \xrightarrow{Rate} L_nM + S_{ox} + O_{2\,red}$

K o

Hb — $k >> k$

$k >> K$ → Oxidase P450

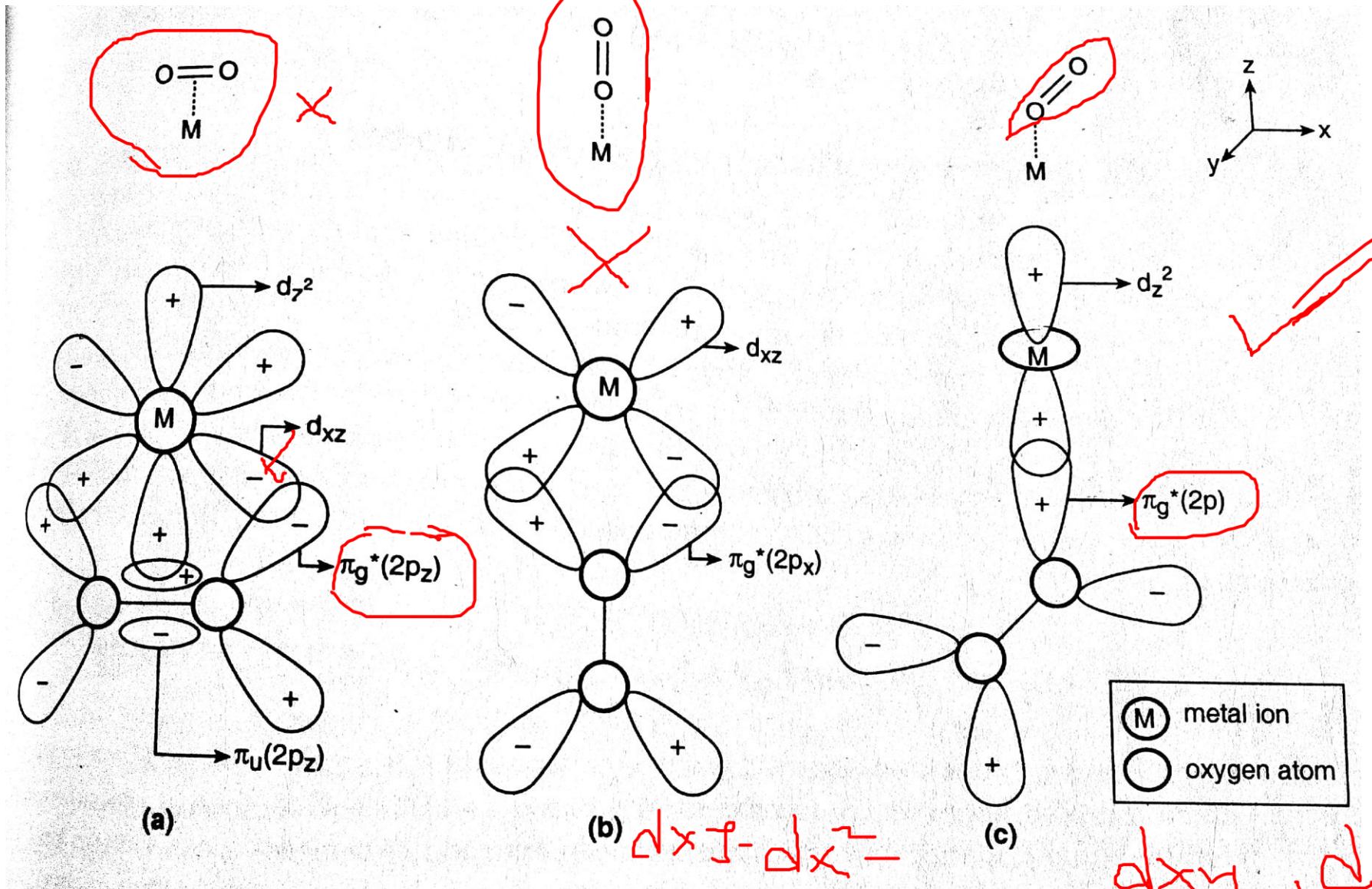
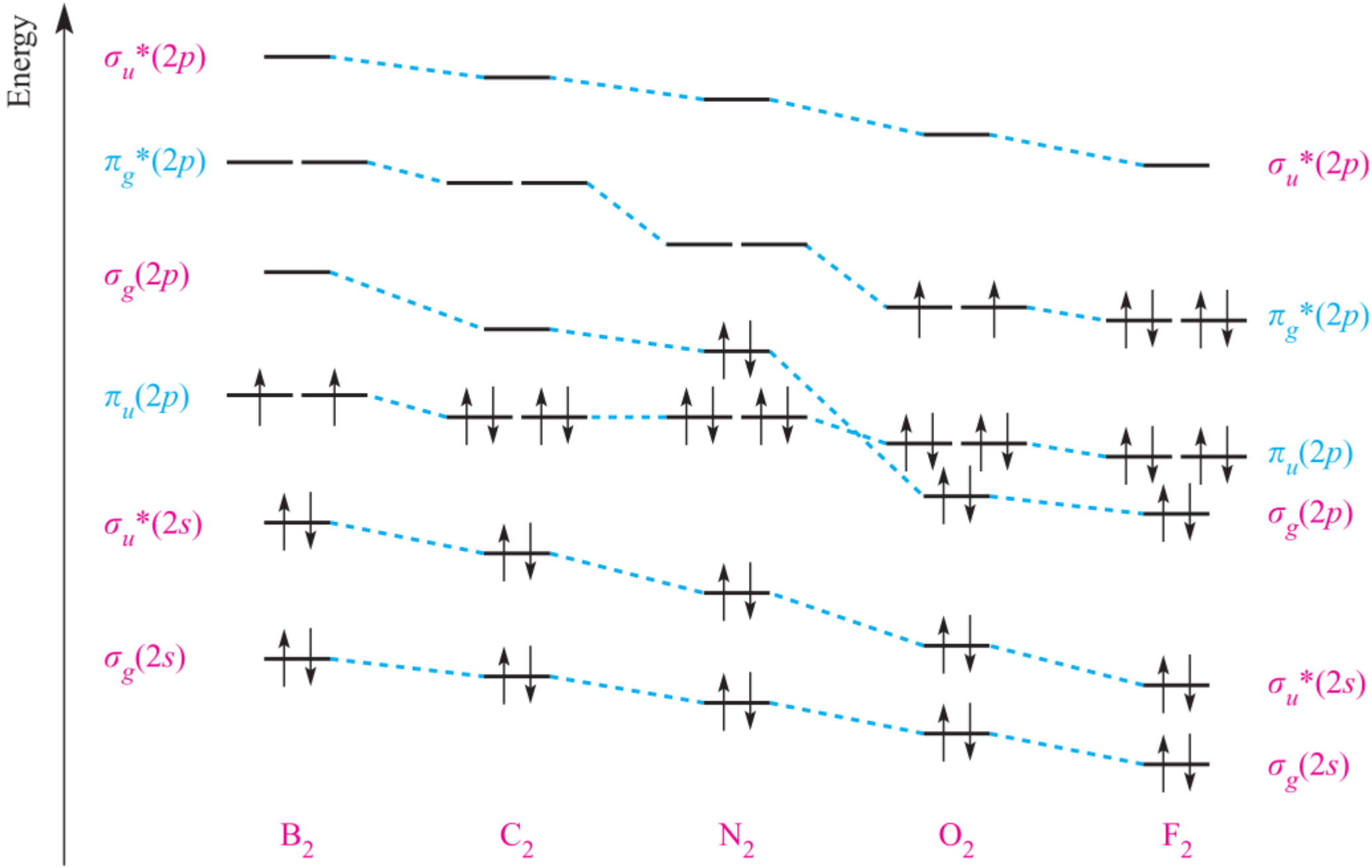


***Figure 5.1.2.1**: Different modes of bonding in M — O_2 complexes. (a) Perpendicular mode (edge-on overlap); (b) Linear mode (end-on overlap); (c) Bent end-on overlap.*


Energy
$\sigma_u^*(2p)$
$\pi_g^*(2p)$
$\sigma_g(2p)$
$\pi_u(2p)$
$\sigma_u^*(2s)$
$\sigma_g(2s)$
$\sigma_u^*(2p)$
$\pi_g^*(2p)$
$\pi_u(2p)$
$\sigma_g(2p)$
$\sigma_u^*(2s)$
$\sigma_g(2s)$
B_2
C_2
N_2
O_2
F_2

5.5.3 Characteristics of O_2-Binding Interaction with Hb and Mb

The relative O_2 affinity of Hb can be explained by different types of ***allosteric interactions*** by O_2, H^+, CO_2, Cl^- and 2,3-diphosphoglycerate (DPG) towards O_2-binding with Hb. *These allosteric effects are absent in Mb*. These effects will be discussed in the following sections.

(1) Cooperativity, Hill-plot, and allosteric effect : The O_2-binding curves (Fig. 5.5.2.1) can be explained by considering the *cooperative interaction* among the four heme units of Hb and ***noncooperative*** O_2-binding with the heme unit of Mb. For Mb, the curve is **hyperbolic** while for Hb it is **sigmoidal (S shaped)** in nature. The O_2 affinity can be measured by p_{50} which gives the partial pressure [$p(O_2)$] of O_2, at which 50% of oxygenation is attained. For Mb, p_{50} is ~1.0 Torr while for Hb, p_{50} is ~26 Torr. Mb has only one O_2-binding site and the following equilibrium is relevant.

$$Mb + O_2 \rightleftharpoons MbO_2,\ K_M = [MbO_2]/[Mb][p(O_2)] \qquad (5.5.3.1)$$

The parameter f_M (fraction of total Mb bearing O_2) is :

$$f_M = [MbO_2]/\{[Mb] + [MbO_2]\} \qquad (5.5.3.2)$$

K_M can be expressed in terms of f_M.

$$K_M = f_M/\{(1 - f_M)\}\{p(O_2)\}/ = \frac{1}{p_{50}} \qquad (5.5.3.3)$$

or, $f_M = K_M p(O_2)/\{1 + K_M p(O_2)\}$ (5.5.3.4)

At the value of $f_M = 0.5$ (*i.e.* 50% of total Mb is oxygenated), the corresponding $p(O_2)$ is denoted by p_{50} and it leads to $1/K_M = p_{50}$. The corresponding ***Hill equation*** is :

$$\log\left(\frac{f_M}{1 - f_M}\right) = \log\{p(O_2)\} + \log K_M = \log\{p(O_2)\} - \log(p_{50}) \quad (5.5.3.5)$$

In the case of Hb, the corresponding expressions are complicated and the results are empirically formulated as follows for the process : $Hb + nO_2 \rightleftharpoons Hb(O_2)_n$

$$K_H = \frac{[Hb(O_2)_n]}{[Hb]\{p(O_2)\}^n} = \frac{f_H}{(1 - f_H)\{p(O_2)\}^n} = \frac{1}{(p_{50})^n} \; ; \; f_H = K_H\{p(O_2)\}^n / [1 + K_H\{p(O_2)\}^n] \quad (5.5.3.6)$$

$$\log\left(\frac{f_H}{1 - f_H}\right) = n \log\{p(O_2)\} + \log K_H = n \log\{p(O_2)\} - n \log(p_{50}) \quad (5.5.3.7)$$

For Hb, the exponent n (= 2.8) referred to as ***Hill coefficient*** is obtained from Hill equation (Eqn. 5.5.3.7). In fact, for Mb, $n = 1$. For Hb, $n > 1$, and it indicates that O_2-binding in the subunits of Hb is *interdependent* and it suggests **positive *cooperativity*** among the heme units due to *heme-heme interaction*.

Hills Plot

❖**Hill co-efficient value n:**

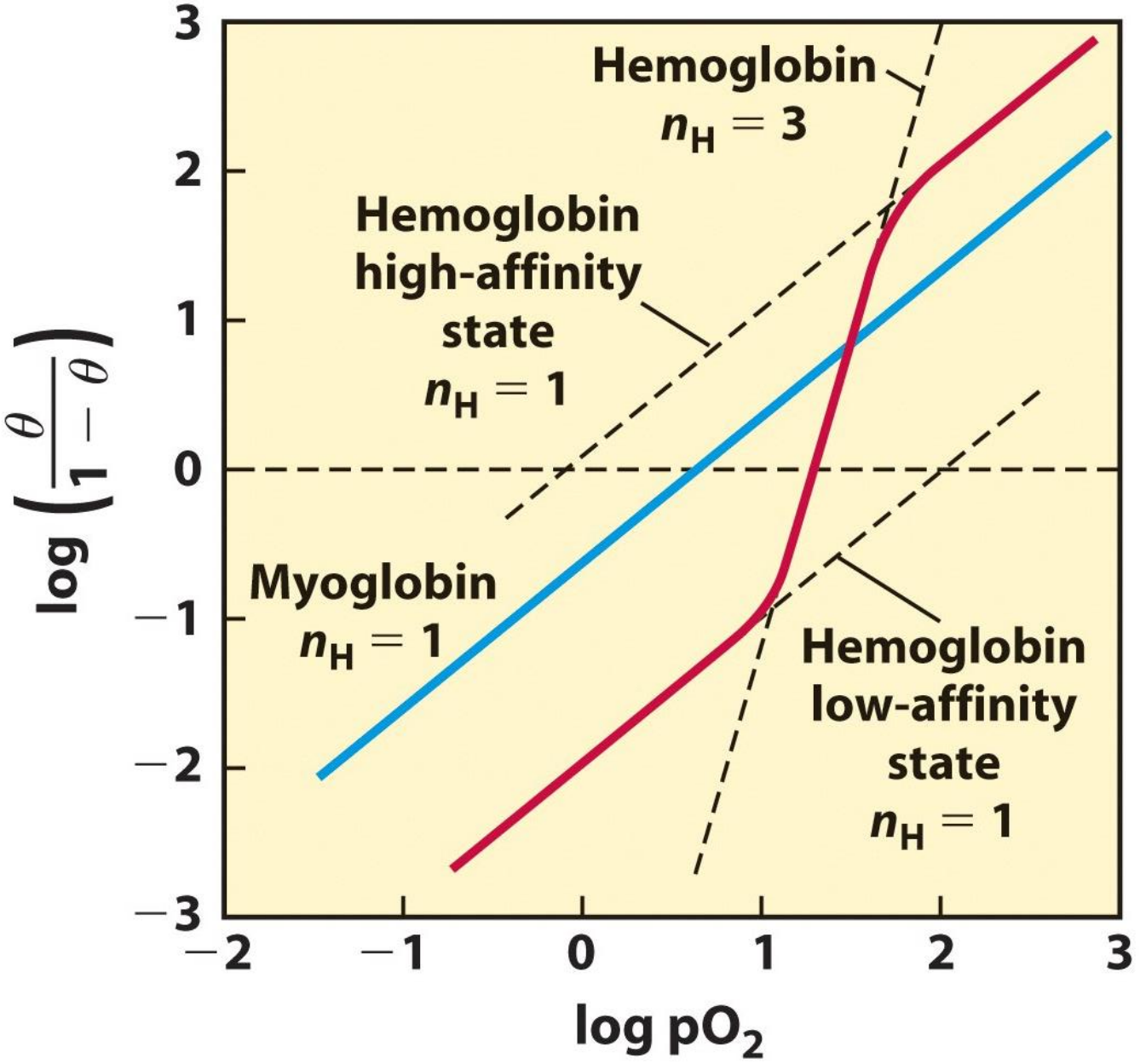
For O_2 Storage protein n = 1

e.g. Myoglobin, Hemerythrin

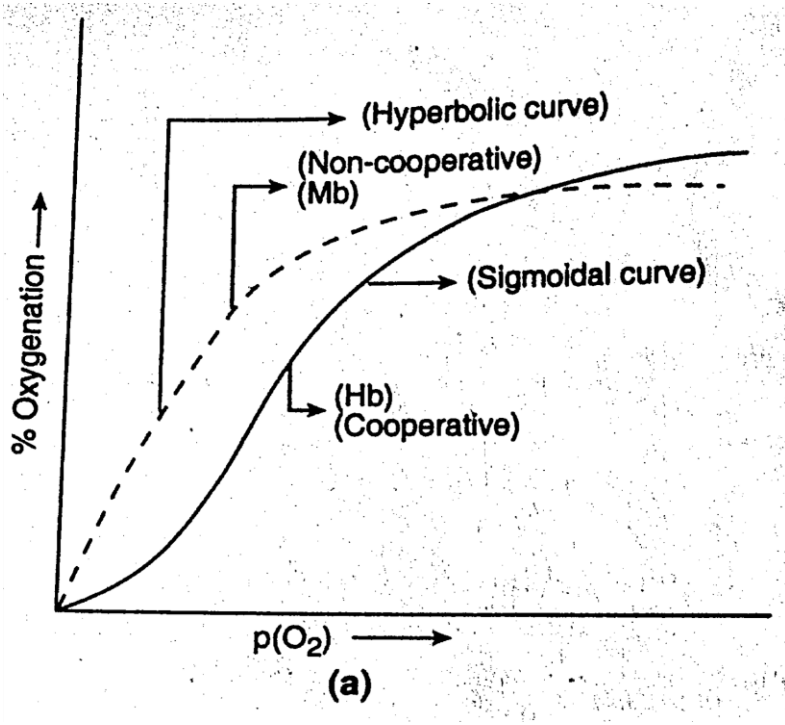
❖**For O_2 Transport proteins**

✓ Hemoglobin n = 3

✓ Hemocyanin n =9



Allosteric Interaction – Effect of Cooperative effect on Hemoglobin



(a)

The phenomena of cooperativity and noncooperativity can be explained by the Hill plot, $\log\{f/(1-f)\}$ *versus* $\log\{p(O_2)\}$ (Fig. 5.5.3.1). The linear Hill plot with a *slope of unity indicates noncooperativity* (as for Mb), *while the slope greater than unity suggests positive cooperativity.* To characterise the cooperativity for Hb, the O_2-binding data at intermediate saturation (*i.e.* $0.2 < f < 0.8$) yield a straight line in Hill plot with a slope close to 3. It is worth noting that at low values of f (< 0.2) and high values of f (> 0.8) for Hb, **no cooperativity (i.e. n = 1)** is noticed from the corresponding Hill plot. For *hemocyanine (Hc)*, the cooperativity is very high and the Hill coefficient is as high as 9. For hemerythrin (Hr), the Hill coefficient is close to unity. *Generally,* **cooperativity (n > 1)** *is noted for the oxygen transport proteins (e.g. Hb, Hc) while noncooperativity* ($n = 1$) *is noted for the oxygen storage proteins (e.g. Mb, Hr).*

The phenomenon of cooperativity in Hb can also be explained by considering the corresponding step-wise O_2-binding constants.

$$Hb + O_2 \rightleftharpoons Hb(O_2),\ K_{1(H)} \qquad (5.5.3.8)$$

$$Hb(O_2) + O_2 \rightleftharpoons Hb(O_2)_2,\ K_{2(H)} \qquad (5.5.3.9)$$

$$Hb(O_2)_2 + O_2 \rightleftharpoons Hb(O_2)_3,\ K_{3(H)} \qquad (5.5.3.10)$$

$$Hb(O_2)_3 + O_2 \rightleftharpoons Hb(O_2)_4,\ K_{4(H)} \qquad (5.5.3.11)$$

Due to the positive cooperativity among the subunits of Hb, the successive O_2-binding constants increase, (*i.e.* $K_{1(H)} < K_{2(H)} < K_{3(H)} < K_{4(H)}$) against the **normal statistical order** $K_{1(H)} > K_{2(H)} > K_{3(H)} > K_{4(H)}$.

The cooperative interaction where the binding of one molecule of a substance (*e.g.* binding of O_2 in Hb) influences the binding of next molecules of *the same kind* is described to as a ***homotropic allosteric interaction.*** Similarly, ***a heterotropic allosteric interaction*** involves

the cooperative interaction among the different types of substances binding with the target protein. All these allosteric interactions are absent in Mb.

(2) *Effect of H^+, CO_2 and Cl^- on O_2-binding interaction with Hb* : Both H^+ and CO_2 show a *heterotropic allosteric interaction* due to which increasing concentration of these species reduces the affinity of deoxy-Hb towards O_2. In the working tissues, CO_2 and lactic acid are produced. Lactic acid is produced from the incomplete oxidation of glucose due to insufficient supply of O_2. Thus in the working tissues, the lower pH *stimulates* the release of O_2 from oxy-Hb. The effect of pH on O_2 affinity of Hb is described as ***Bohr effect*** (Fig. 5.5.2.1b) (named after the discoverer, Christan Bohr, father of physicist Niels Bohr). A *very large Bohr effect* leading to a sharp decrease of O_2 affinity with decreasing pH is known as ***Root effect*** which is important for some kinds of fish, probably in maintaining proper buoyancy.

In the alveolar capillaries of the lungs, the high concentration of O_2 unloads H^+ and CO_2 from hemoglobin through oxygenation. This effect is described as *Haldane effect*, a reciprocal Bohr effect (due to which high concentration of H^+ and CO_2 in the active tissues removes off O_2 from oxy-Hb). Cl^- binds more strongly with the deoxy-Hb and thus O_2-affinity of Hb decreases with the increase of Cl^- concentration.

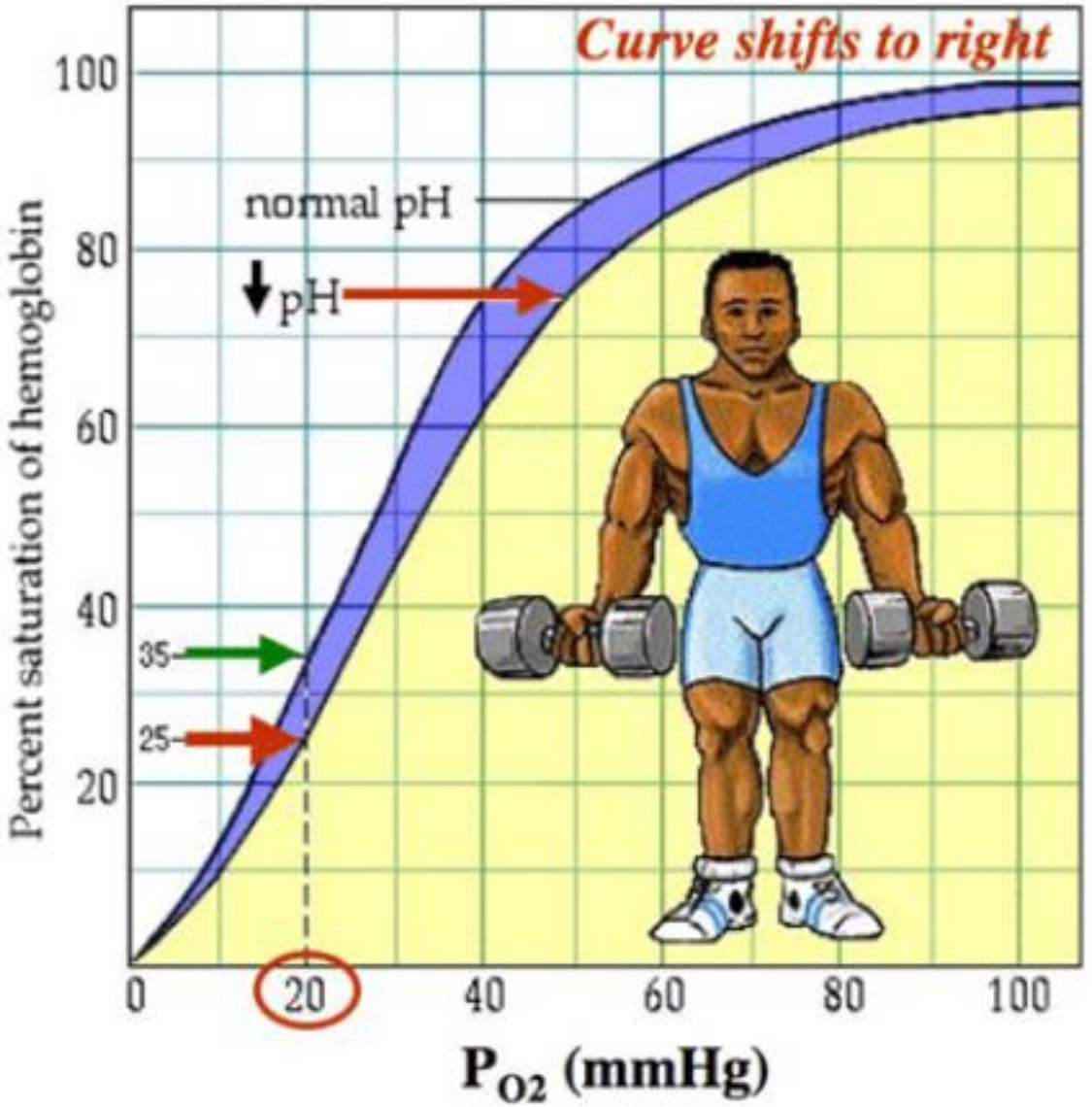
Bohr Effect

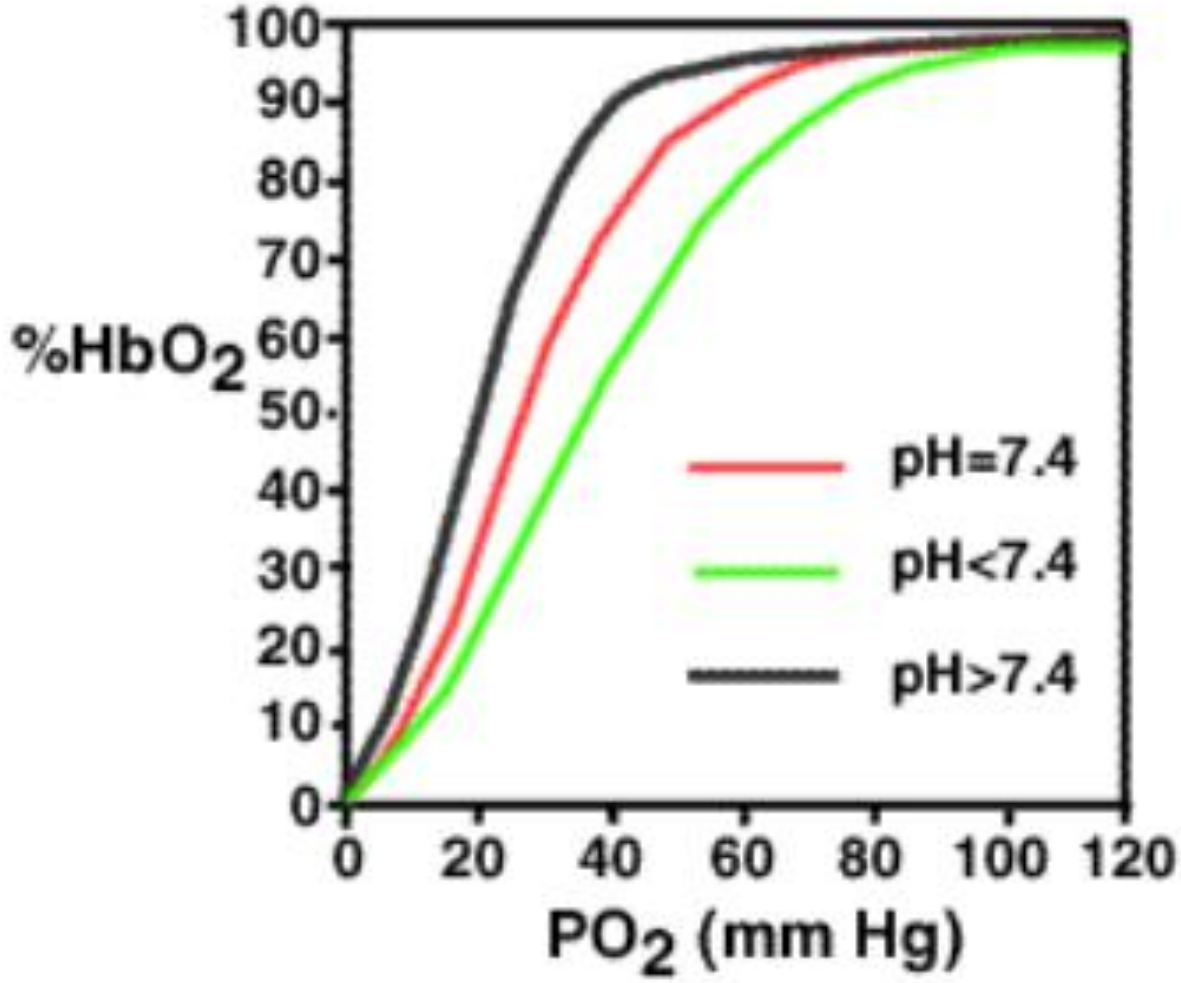
Oxygen-hemoglobin Dissociation: Exercise

Factors shifting curve to right

- ↓ **pH** (more acidic)
- ↑ **Temperature**
- ↑ P_{CO2}
- ↑ **2,3-BPG**

Called Bohr effect





The red line on the graph represents the dissociation curve at a normal pH (pH = 7.4).

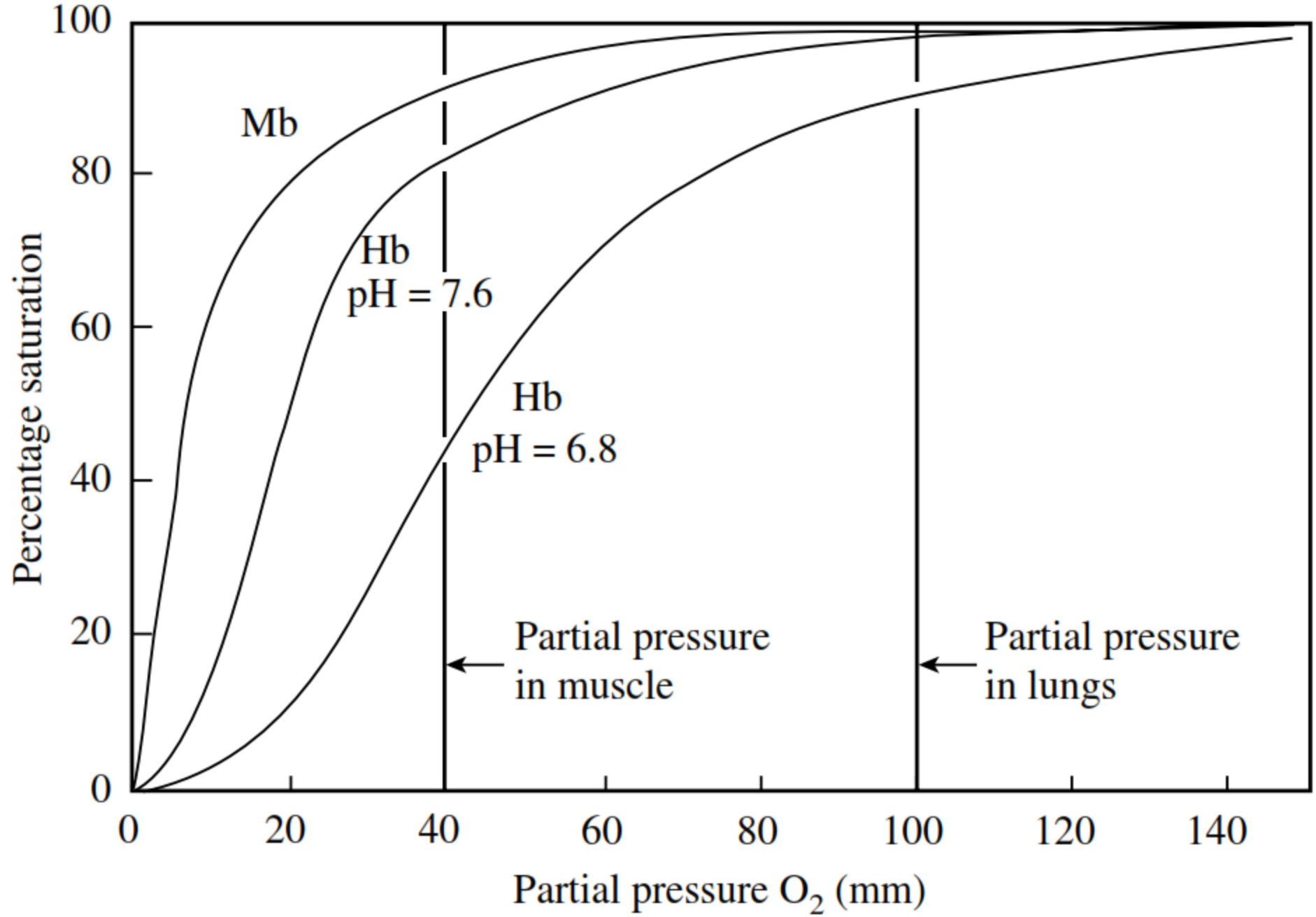
The green line represents the curve in the blood of exercising tissues, shifted to the right of the normal curve.

The black line represents the curve in the blood at the lungs, shifted to the left of the normal curve.

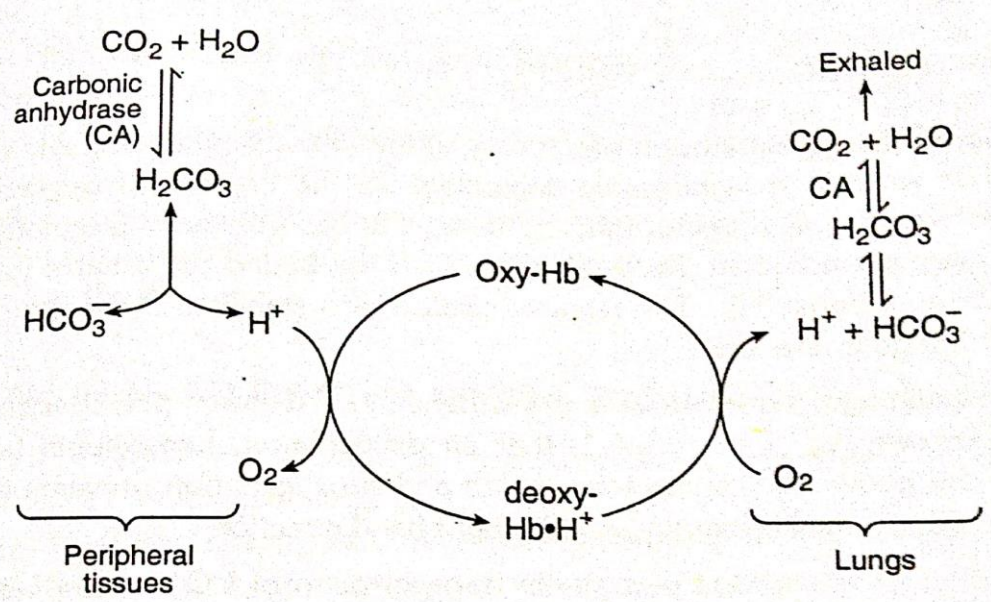
Higher PCO_2 = Higher [H+] = Lower pH = Shift to the RIGHT

Lower PCO_2 = Lower [H+] = Higher pH = Shift to the LEFT

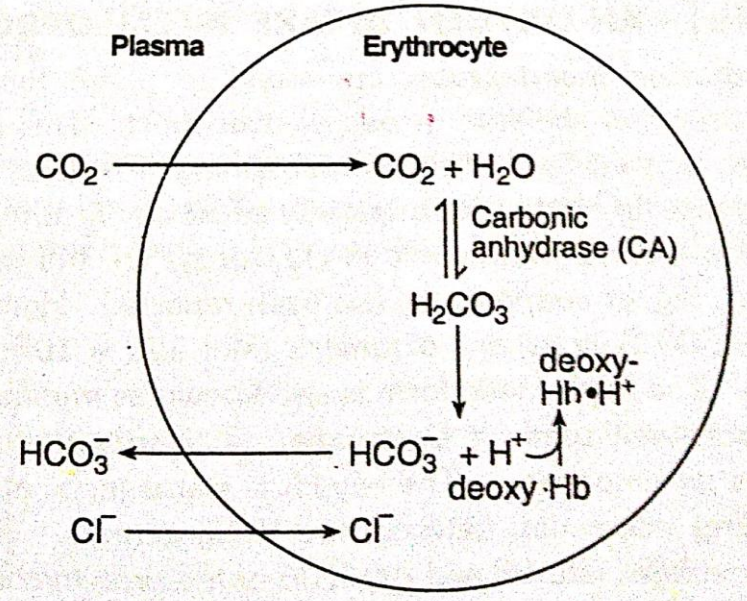
- Increased temperature, such as in exercising tissues, shifts the curve to the right releasing oxygen.
- Decreased temperature shifts the curve to the left, enhancing oxygen uptake.


100
80
60
40
20
0
Percentage saturation
Mb
Hb
pH = 7.6
Hb
pH = 6.8
Partial pressure
in muscle
Partial pressure
in lungs
0
20
40
60
80
100
120
140
Partial pressure O_2 (mm)

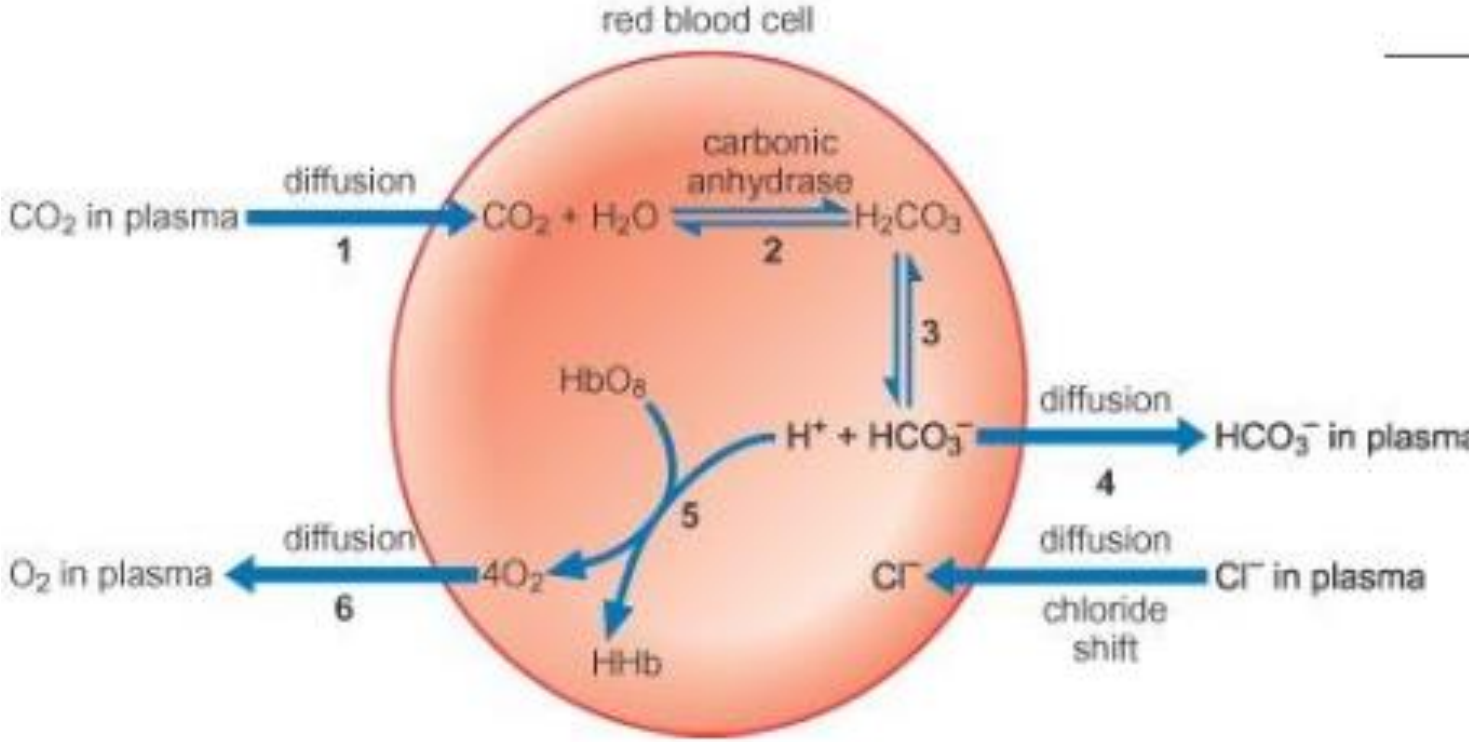
Transport of CO_2 through Hemoglobin

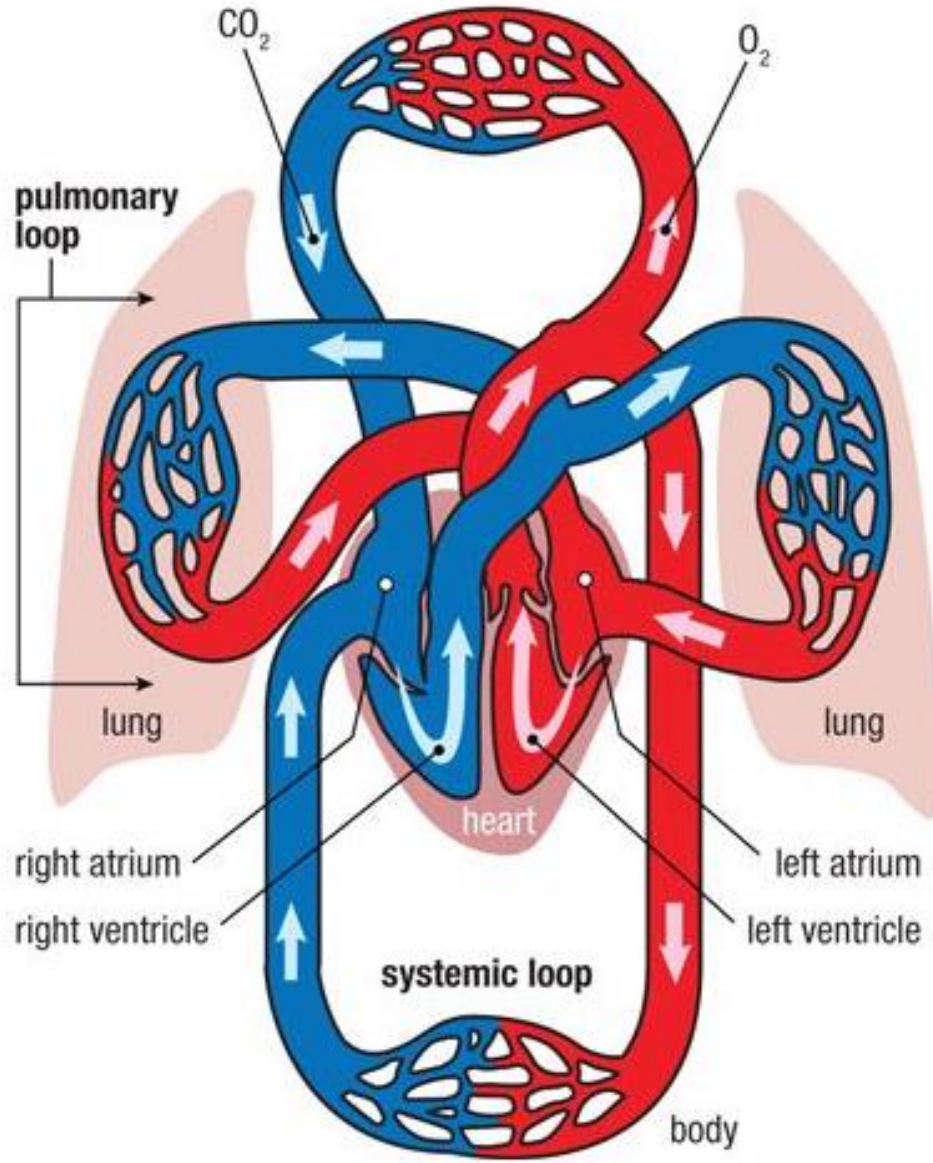


Scheme 5.6.1: *Transport of CO_2 through the mediation of hemoglobin.*



Scheme 5.6.2 : *Generation of bicarbonate by the erythrocyte and the chloride shift phenomenon*


red blood cell
diffusion
CO_2 in plasma
1
$CO_2 + H_2O$
carbonic anhydrase
2
H_2CO_3
3
HbO_8
$H^+ + HCO_3^-$
diffusion
HCO_3^- in plasma
4
5
diffusion
O_2 in plasma
6
$4O_2$
HHb
Cl^-
diffusion
Cl^- in plasma
chloride shift


CO_2
O_2
pulmonary loop
lung
lung
heart
right atrium
right ventricle
left atrium
left ventricle
systemic loop
body

Importance of 2,3-diphosphoglycerate

(3) Effect of 2,3-diphosphoglycerate (DPG) (presenty described as 2,3-bisphosphoglycerate, i.e. 2,3-BPG) on O_2 affinity of Hb : DPG shows a heterotropic allosteric effect due to which O_2 affinity of Hb decreases with the increase of DPG concentration. In the active tissues, the higher concentration of DPG stimulates the release of O_2 from oxy-Hb. In human red cells, DPG is present at about the same molar concentration as Hb. In the absence of DPG, p_{50} is ~1 Torr like that of Mb while in the presence of DPG, normal blood cells show $p_{50} \approx 26$ Torr for Hb. In fact, DPG binds with Hb in 1 : 1 molar complex with the binding constants ($K_1 >> K_2$) in the following equilibria.

$$\text{deoxy-Hb} + \text{DPG} \rightleftharpoons \text{deoxy-Hb•DPG}, \quad K_1 \qquad (5.5.3.12)$$

$$\text{oxy-Hb} + \text{DPG} \rightleftharpoons \text{oxy-Hb•DPG}, \quad K_2 \qquad (5.5.3.13)$$

It leads to favour the following equilibrium,

$$\text{oxy-Hb} + \text{DPG} \rightleftharpoons \text{deoxy-Hb•DPG} + O_2 \qquad (5.5.3.14)$$

In fact, this function of DPG as a sensitive control to release O_2 from oxy-Hb has been exploited in adaptation to **hypoxia** (when there is a disorder in O_2 delivery in active tissues). The DPG concentration also *increases at higher altitudes and helps to release more oxygen from oxy-Hb at a given pressure of oxygen*. In *anemic condition*, the DPG concentration also increases to cope with the oxygen demand of the body. The effect of DPG on O_2 affinity of Hb is shown in Fig. 5.5.3.2.

Another interesting example involving the allosteric effect of DPG explains the mechanism of transfer of O_2 from mother to developing fetus across the placenta. It has been found that the DPG binds more strongly to adult **Hb-A ($\alpha_2\beta_2$)** than to **fetal Hb-F ($\alpha_2\gamma_2$)**. Consequently, fetal Hb-F has a high O_2 affinity compared to that of mother's adult Hb-A and this enables the transfer of O_2 from mother to fetus.

Fe(II)/Fe(III) Irreversible Oxidation – Hematin Formation

5.5.5 Chemical and Steric Protection of Heme from its Irreversible Oxidation

In hemoglobin and myoglobin, iron is present as Fe^{2+} in deoxy-forms. At biological pH (~7.0), free heme-group (*without the globin protein*) gets *irreversibly oxidised by air* (*i.e.* O_2) in aqueous media to give ***hemin* or *hematin*** consisting of Fe(III).

$$\text{Heme-[Fe(II)]} \xrightarrow{O_2,\ \text{water}} \text{Hemin-[Fe(III)]} \qquad (5.5.5.1)$$

The oxidised forms containing Fe(III) of hemoglobin and myoglobin are described as ***methemoglobin*** (Met-Hb) and ***metmyoglobin*** (Met-Mb) respectively. These *irreversibly oxidised forms* are of no use from the standpoint of oxygen transport. *It is the basic requirement of an oxygen carrier to deliver the bound O_2 unchanged.* There are some evidences to support the fact that in oxy-Hb, iron remains as Fe(III) and O_2 remains as O_2^- (superoxide), *i.e.* a redox reaction has occurred upon oxygenation (all these facts will be discussed in dealing with the bonding mechanism *cf.* Sec. 5.5.9 and Fig. 5.5.9.2). Obviously, *to act as an oxygen carrier, oxy-Hb must be able to reverse the reaction rapidly at the cell where the O_2 is to be delivered.* In fact, *this condition of reversibility* is attained only when the heme unit is folded by the globin protein. ***Without this globin protein, Fe(II) will be irreversibly oxidised.*** Before to understand how the globin protein prevents this irreversible oxidation, it is important to discuss the mechanism of irreversible oxidation of Fe(II)—L. These are shown in the following reactions.

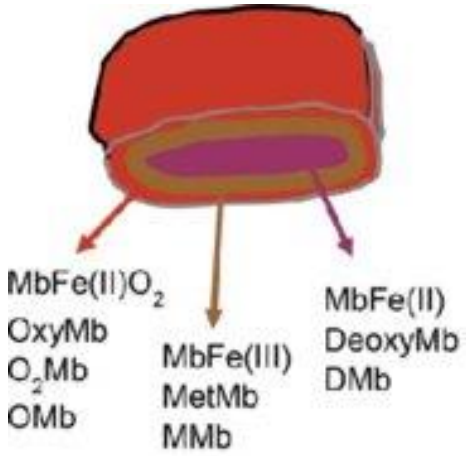
$$\text{L—Fe(II)} + O_2 \rightleftharpoons \text{L—Fe(III)—}O_2^- \qquad (5.5.5.2)$$

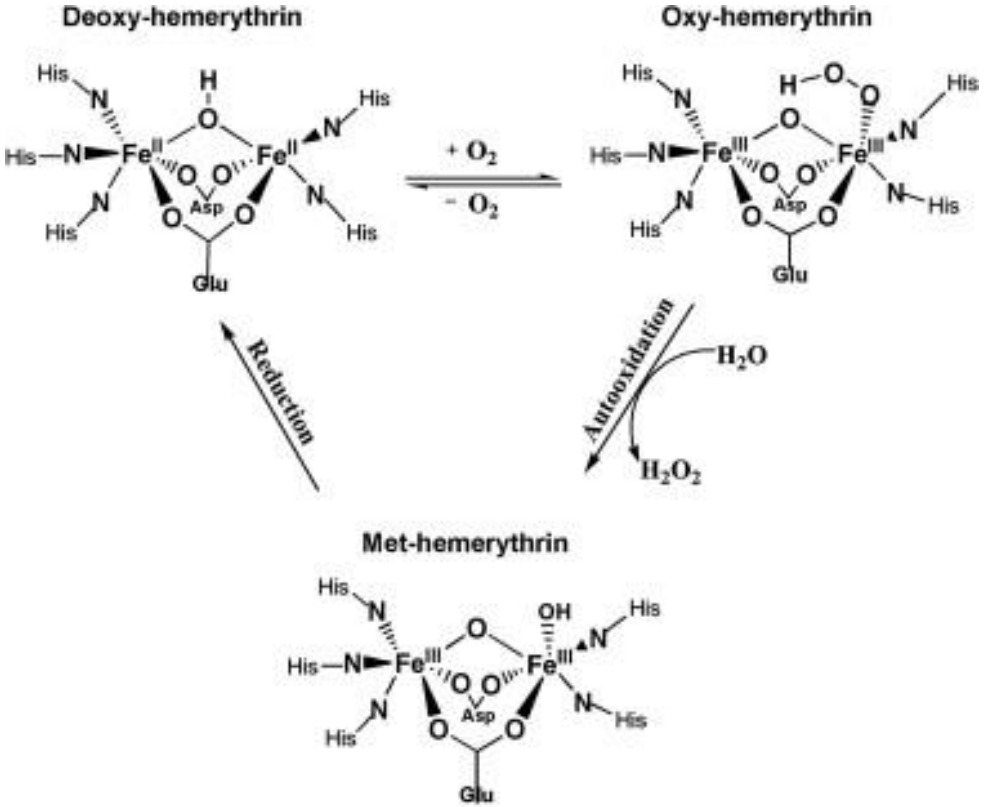
$$\text{L—Fe(III)—}O_2^- + \text{Fe(II)—L} \rightleftharpoons \text{L—Fe(III)—}O_2^{2-}\text{—Fe(III)—L} \qquad (5.5.5.3)$$

$$\text{L—Fe(III)—}O_2^{2-}\text{—Fe(III)—L} \xrightarrow{\text{fast}} 2\ \text{L—Fe(IV)}{=}O^{2-} \qquad (5.5.5.4)$$

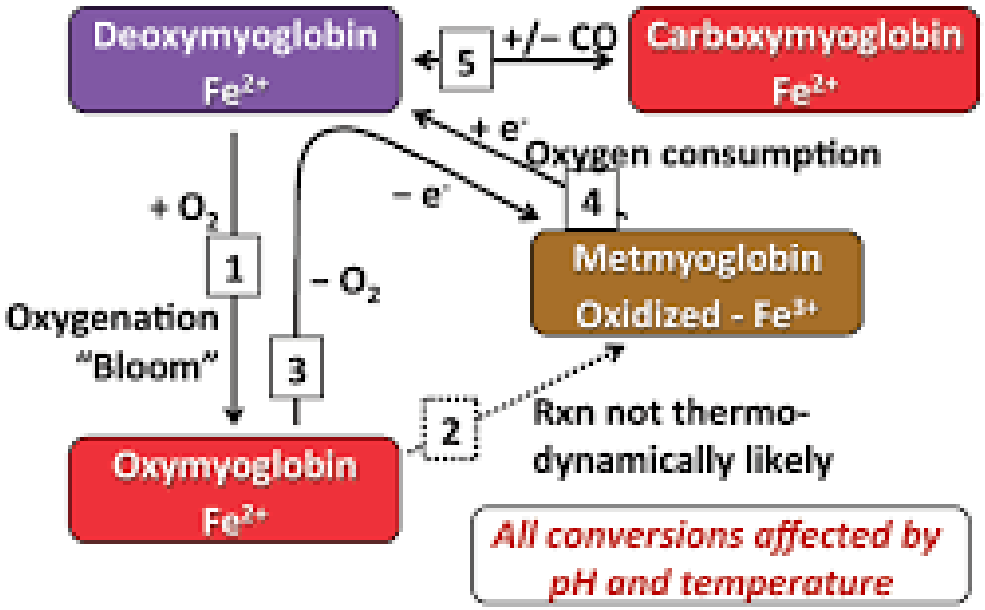
$$\text{L—Fe(IV)}{=}O^{2-} + \text{Fe(II)—L} \xrightarrow{\text{fast}} \text{L—Fe(III)—O—Fe(III)—L} \qquad (5.5.5.5)$$

Nature protects heme-[Fe(II)] as follows : (i) Thus the irreversible oxidation passes through the formation of *peroxo- and oxo-bridged binuclear complexes.* Formation of this type of binuclear



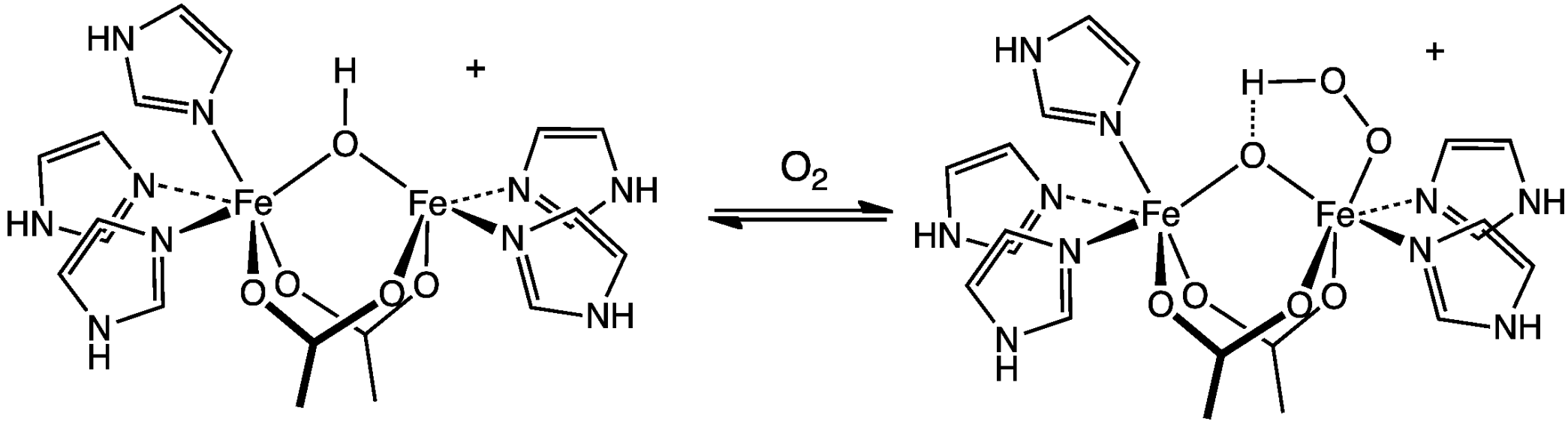


Chemistry of the Fresh Meat Color Triangle



Hemerythrin

Deoxy form (Colourless) **oxy form (Violet)- 360nm absorption**



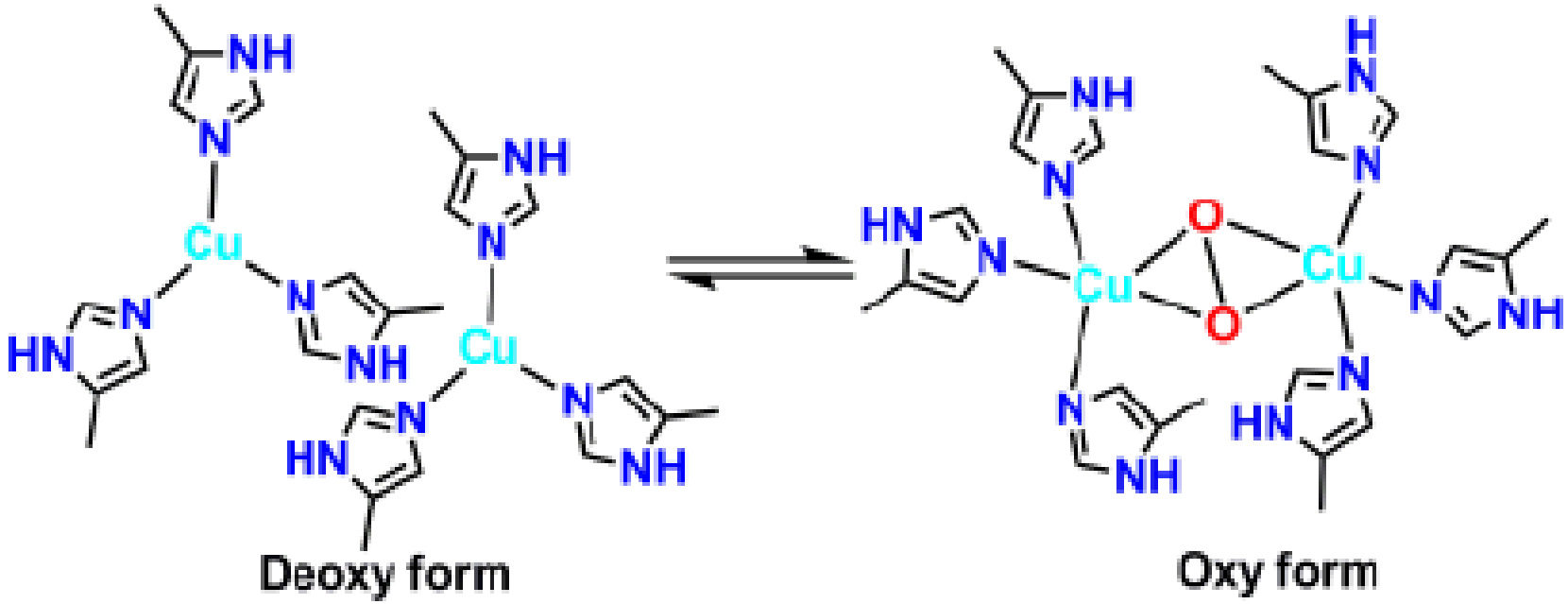
Marine invertebrates; **monomeric – 13500 Da and octameric – 108,000 Da Hill coefficient of 1.2 to 1.4**; The uptake of O_2 by hemerythrin is accompanied by two-electron oxidation of the diferrous centre (Glu58 and Asp106) to produce a diferric hydroperoxide (OOH^-) complex FT-IR ν_{o-o} = 844 cm^{-1}

Deoxyhemerythrin contains two high-spin ferrous ions bridged by hydroxyl group. One iron is hexacoordinate and another is pentacoordinate. A hydroxyl group serves as a bridging ligand but also functions as a proton donor to the O_2 substrate. This proton-transfer result in the formation of a single oxygen atom (μ-oxo) bridge in oxy-hemerythrin (diamagnetic, Fe(III)-Fe(III))

O_2 binds to the pentacoordinate Fe^{2+} centre at the vacant coordination site. Then electrons are transferred from the ferrous ions to generate the binuclear ferric (Fe^{3+},Fe^{3+}) centre with bound peroxide.

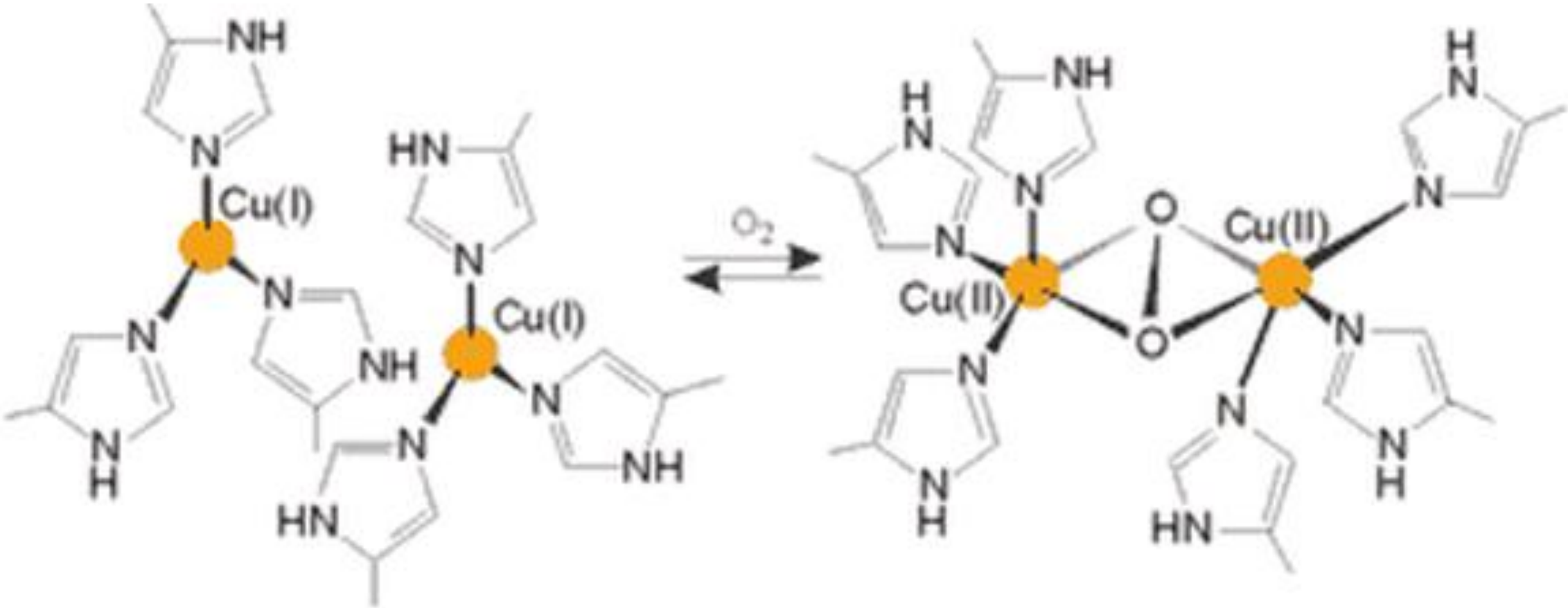
Hemocyanine:

In many organisms, like arthropods (lobster, scorpion 77,000 D) and molluscs (snail, octopi etc 53000 D), oxygen is transported by Cu - containing haemocyanin protein. HC are polymeric of 6, 12, 24, 48 subunits and no monomer is found. These metalloproteins contain two copper atoms that reversibly bind a single oxygen molecule (O_2) Hill co eff - 9



The deoxy form of the protein contains Cu(I) colorless and diamagnetic that undergoes oxidation to Cu(II) in its oxy form, peroxo-bridged dicopper(II) species, intense blue (λ = 580 nm $\varepsilon = 10^4\ M^{-1}\ cm^{-1}$. In the deoxy form each copper center is tri-coordinated by three histidine units. The distance between the two copper centers is 4.60 A. This shows no direct interactions between the two atoms. The deoxy form is colorless. Upon coordination with oxygen copper centers undergo to Cu(II) from Cu(I) and hence, a blue color appears due to peroxo-to-Cu(II) charge transfer FT-IR $\nu_{o-o} = 744\ cm^{-1}$.

Hemocyanine



Summary of O2 storage and Transport Proteins

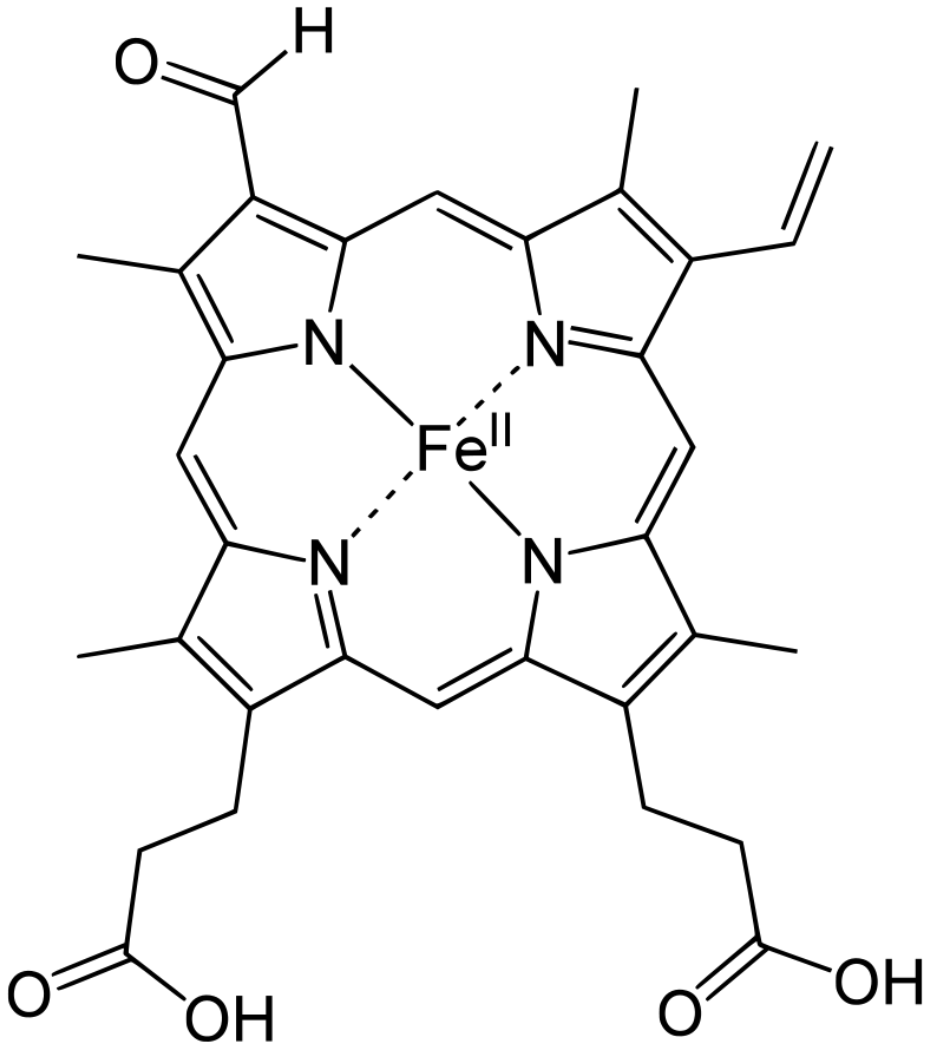
Table 6.1. Comparison of some properties of respiratory pigments

S. No.	Property	Myoglobin	Hemoglobin	Hemerythrin	Hemocyanin
1	Metal	Iron	Iron	Iron	Copper
2	Metal : O_2	Fe : O_2	$[Fe:O_2]_4$	$2Fe:O_2$	$2Cu:O_2$
3	Oxidation state of metal	Fe(II)	Fe(II)	Fe(II)	Cu(I)
4	Ligand unit	Porphyrin	Porphyrin	Protein side chains	Protein side chains
5	Number of subunits present	1	4	8	Variable
6	Molecular weight	17,000	65,000	1,08,000	4 to 90 lakhs
7	Colour				
	Oxy form:	Red	Red	Violet pink	Blue
	Deoxy form:	Red-blue	Red-blue	colourless	colourless

Table 4.1 Some Properties of Oxygen Transport Proteins

O_2 carrier:	Myoglobin	Hemoglobin	Hemerythrin	Hemocyanin
Source:	Higher animals, some invertebrates	Higher animals, some invertebrates	invertebrates	Arthropods, mollusks
Metal:	Fe	Fe	Fe	Cu
Metal:bound O_2 stoichiometry (ligands):	Fe:O_2 (heme, histidine)	Fe:O_2 (heme, histidine)	2 Fe:O_2 (nonheme, protein side chains)	2 Cu:O_2 (nonheme, protein side chains)
Metal ox state in deoxy form/*d* electrons (color):	II/d^6 (red-purple, violet)	II/d^6 (red-purple, violet)	II/d^6 (colorless)	I/d^{10} (colorless)
Metal ox state in oxy form/*d* electrons (color):	II/d^6–O_2 or III/d^5–O_2^- (red)	II/d^6–O_2 or III/d^5–O_2^- (red)	III/d^5 (burgundy)	II/d^9 (blue)
Approximate molecular weight (kDa):	17	65	108	400 to 2×10^4
Number of subunits:	1	4 (some species have up to 10)	8	Many

Pigment	Colour	Metal element	Animal
hemoglobin	red	iron	Mammals Birds Reptiles Amphibians Fishes
hemocyanin	blue	Copper	Molluscs
Chlorocruorin	Green	Iron	Some annelids
hemoerythrin	red	Iron	Some annelids



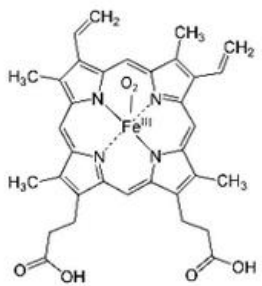
Chlorocruorin

RED

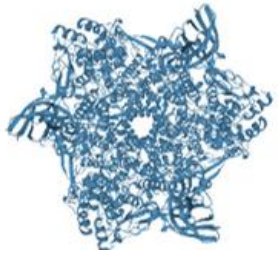
Humans and the majority of other vertebrates

Iron

HEMOGLOBIN



Heme B
(oxygenated form)

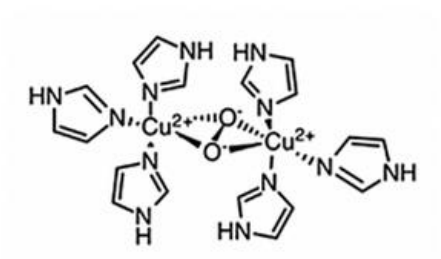


BLUE

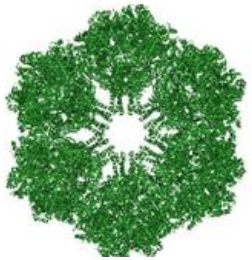
Spiders, crustaceans, some molluscs, octopuses and squids

Copper

HEMOCYANIN



(oxygenated form)

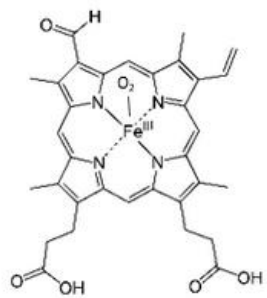


GREEN

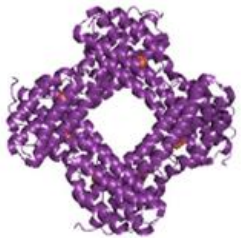
Some segmented worms, some leeches, and some marine worms

Iron

CHLOROCRUORIN



(oxygenated form)

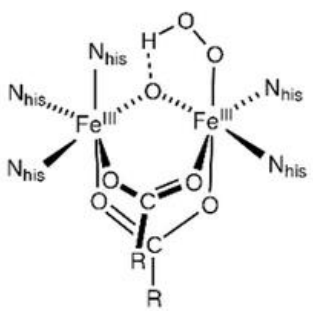


VIOLET

Marine worms including peanut worms, penis worms and brachiopods

Iron

HEMERYTHRIN



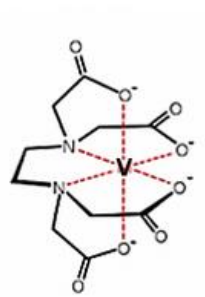
(oxygenated form)

YELLOW

Beetles, sea squirts, sea cucumber

Vanadium

VANABIN



(oxygenated form)

https://www.ebi.ac.uk/pdbe/news/what-do-snails-spiders-octopods-and-queen-england-have-common

Table 3.1.1.2

Average Fe distribution in a normal adult

Protein	Mol. Wt. (kDa)*	Function	Oxidation state	Amount (g) (% total Fe)	Coordination sphere (No. of Fe-sites per molecule)
Hemoglobin (Hb)	64.5	O_2 transport in plasma	II	2.6 (65)	Heme (4 Fe)
Myoglobin (Mb)	17	O_2 storage in muscle	II	0.13 (6)	Heme (1 Fe)
Transferrin	76	Fe transport in plasma	III	0.007 (0.2)	Non-heme (2 Fe)
Ferritin	444	Fe storage in cells	III	0.52 (13)	Non-heme (approx. 4500 Fe)
Hemosiderin	–	Fe storage in cells	III	0.48 (12)	Non-heme (approx. 5000 Fe)
Catalase	280	H_2O_2 metabolism	III/IV/V	0.004 (0.1)	Heme (1 Fe)
Cytochrome *c*	12.5	Electron transport	II/III	0.004 (0.1)	Heme (1 Fe)
Peroxidase	44	H_2O_2 metabolism	III/IV/V	–	Heme (1 Fe)
Cytochromes and oxidase**	–	Oxidation	II/III	0.02 (< 0.5)	Heme

* 1 Dalton (Da) = 1amu = 1u = 1.66×10^{-27} g, *e.g* H_2 is a two Dalton molecule.

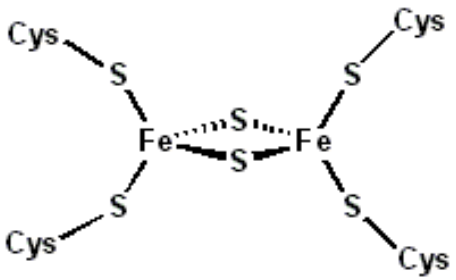
** Cytochrome P-450 involves the oxidation states III/IV/V.

Table 1.3
Average human Fe distribution.

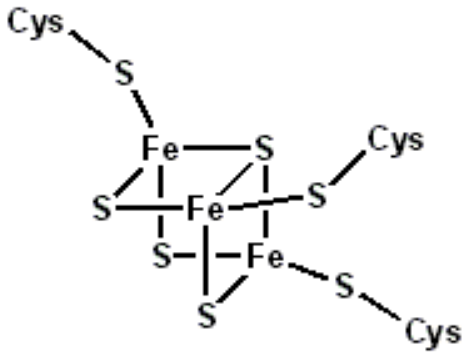
Protein	Function	Oxidation state of Fe	Amount of Fe (g)	Percent of total
Hemoglobin	Plasma O_2 transport	2	2.6	65
Myoglobin	Muscle O_2 storage	2	0.13	6
Transferrin	Plasma Fe transport	3	0.007	0.2
Ferritin	Cell Fe storage	3	0.52	13
Hemosiderin	Cell Fe storage	3	0.48	12
Catalase	H_2O_2 metabolism	2	0.004	0.1
Cytochrome *c*	Electron transport	$\frac{2}{3}$	0.004	0.1
Other	Oxidases, other enzymes, etc.		0.14	3.6

Chapter 4. Electron Transfer in Biology

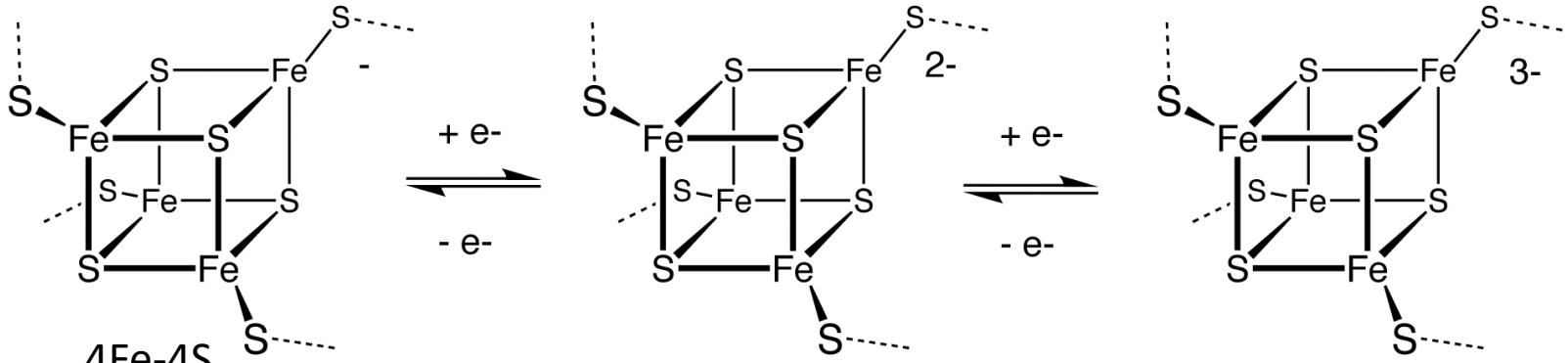
Fe-S proteins: Rubredoxin and Ferredoxin



2Fe-2S



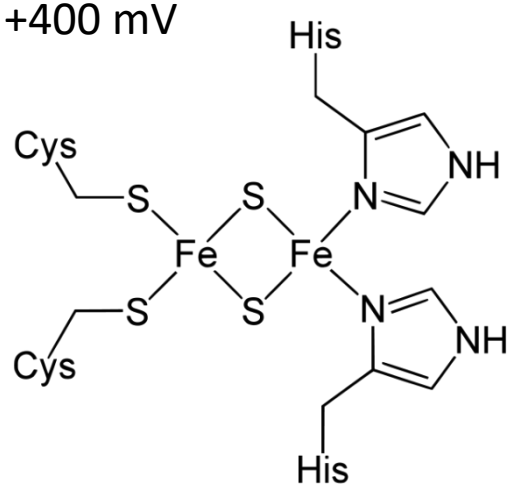
3Fe-4S

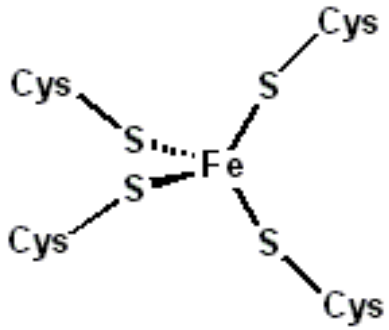


4Fe-4S

The formal oxidation numbers of the iron ions can be [$2Fe^{3+}$, $2Fe^{2+}$] or [$1Fe^{3+}$, $3Fe^{2+}$] in low-potential ferredoxins. The oxidation numbers of the iron ions in high-potential ferredoxins can be [$3Fe^{3+}$, $1Fe^{2+}$] or [$2Fe^{3+}$, $2Fe^{2+}$]

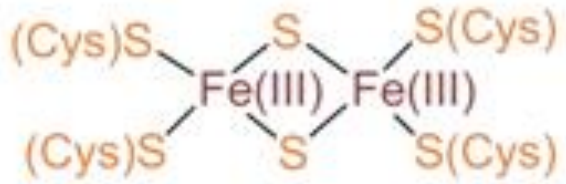
Rieske proteins: It is a unique [2Fe-2S] cluster in that one of the two Fe atoms is coordinated by two histidine residues rather than two cysteine residues. They have since been found in plants, animals, and bacteria with widely ranging electron reduction potentials from -150 to +400 mV





Rubredoxin: 1Fe-0S proteins

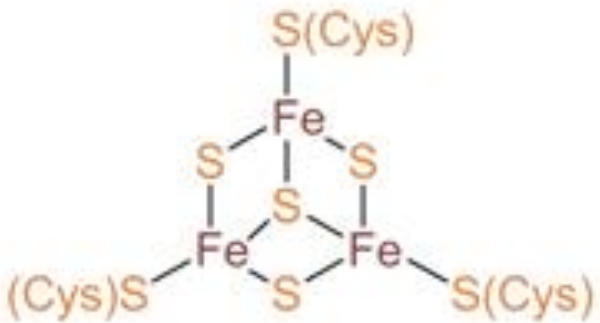
This iron-sulphur protein is an electron carrier, and it is easy to distinguish its metallic centre changes: the oxidized state is reddish (due to a ligand metal charge transfer), while the reduced state is colourless (because the electron transition has an energy of the infrared level, which is imperceptible for the human eye).



[2Fe-2S]$^{2+}$

[2Fe-2S]$^{2+}$ $\xrightarrow[E° = 0 \text{ to } -0.4 \text{ V}]{+e^-}$ **[2Fe-2S]$^{1+}$**

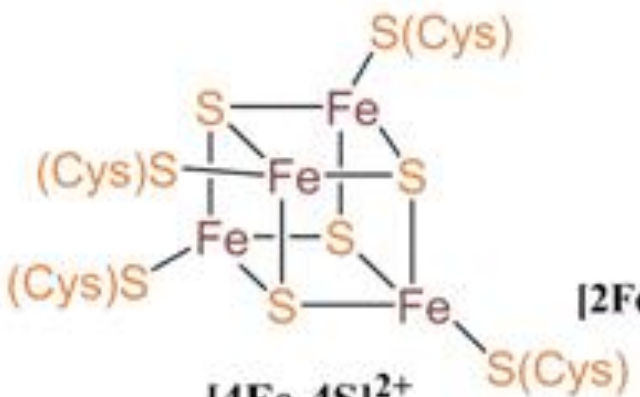
[2Fe(III)] **[Fe(II)Fe(III)]**



[3Fe-4S]$^{1+}$

[3Fe-4S]$^{2+}$ $\xrightarrow[E° = +0.1 \text{ to } -0.4 \text{ V}]{+e^-}$ **[3Fe-4S]^{0}**

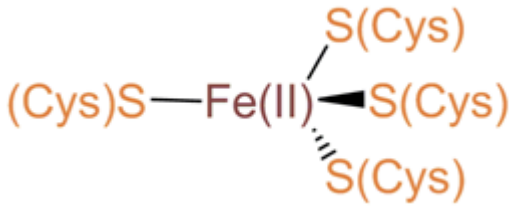
[3Fe(III)] **[Fe(II)2Fe(III)]**



[4Fe-4S]$^{2+}$

[4Fe-4S]$^{2+}$ $\xrightarrow[E° = +0.1 \text{ to } -0.4 \text{ V}]{+e^-}$ **[4Fe-4S]^{0}**

[2Fe(II)2Fe(III)] **[3Fe(II)Fe(III)]**



Rubredoxin: 1Fe-0S proteins

Iron-sulfur proteins:
The iron-sulfur proteins occurs extensively in all living organisms and take part in a wide range of electron-transfer processes, either as redox centers (*e* . *g* . ferredoxins, rubredoxins) or as catalysts (*e* . *g* . hydrogenase, nitrogenase, etc).
[1Fe-0S] proteins:

Iron-sulfur proteins with no bridging sulfur (0S) or sulfide atom is known as rubredoxins. It is mainly found in bacteria and acts as one electron donor-acceptor. The arrangement around the iron center is tetrahedral and the Fe(II) center is surrounded by four sulfur atoms from four cystine (Cys) moieties .

Ferredoxins are most important family of iron-sulfur proteins. Three major categories of ferredoxins are, [2Fe-2S], [3Fe-4S], and [4Fe-4S]. The [4Fe-4S] is most important.

[2Fe-2S]: Isolated from mammals, plants, and bacteria. Both the iron centers are in tetrahedral coordination environment and linked by two inorganic sulfide bridges. Both Fe(III) centers are antiferromagnetically coupled to each other. Hence, a diamagnetic ground state results. After accepting a electron one center becomes Fe(II) and the other is Fe(III). After antiferromagnetic coupling between the S = ½ ground state appeared.

[3Fe-4S]: Found in *Azobacter vinlandii* , and *Desulfovibrio gigas* and also in pig heart. All the iron centers are in tetrahedral coordination environment and linked with each other by two inorganic sulfide bridges.

[3Fe-4S]: It is found is several iron containing metalloenzymes like nitrogenase, hydrogenase, etc. The structure is a distorted cubic core. The alternative corners of the cube are occupied by iron and inorganic sulfide. Irons are connected to each other via two sulfide bridges. All the iron centers are tetrahedral.

Cytochromes

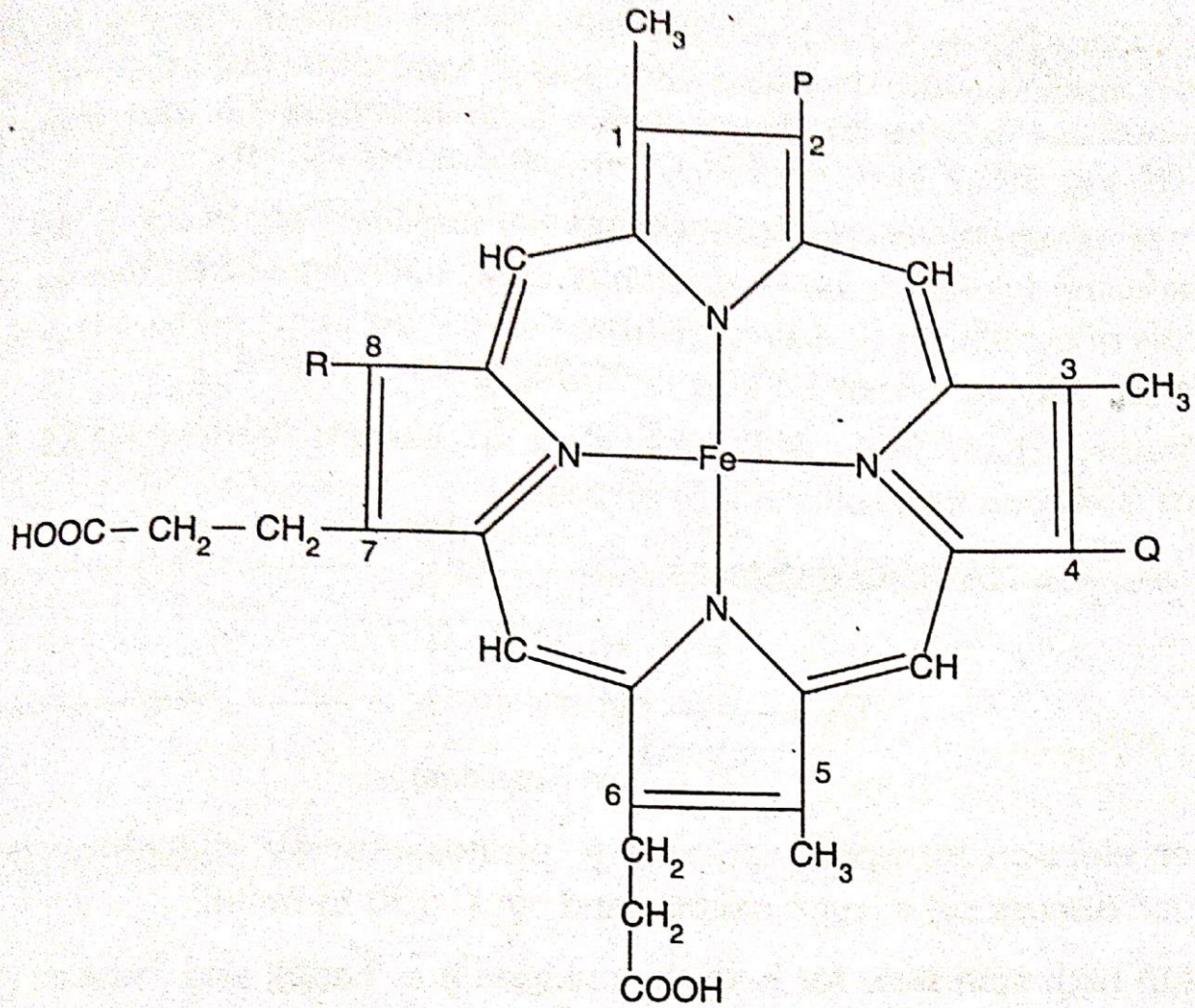
- **Cytochromes** are iron containing hemeproteins central to which are heme groups that are primarily responsible for the generation of ATP via electron transport
- On the basis of the position of their lowest energy absorption band in the reduced state, named as **cytochromes *a* (605 nm), *b* (565 nm), and *c* (550 nm)**
- Cytochromes are, capable of performing oxidation and reduction. Because the cytochromes (as well as other complexes) are held within membranes in an organized way, the redox reactions are carried out in the proper sequence for maximum efficiency

Cytochrome C

- The polypeptide chain contains amino acids ranging from 103 (in some fishes) to 112 (in terrestrial vertebrates). A N atom from histidine and S atom from methionine are present in 5^{th} and 6^{th} position

- So it reacts indirectly by electron transfer mechanism – reduce dioxygen and transmit its oxidizing power towards burning of food and release of energy in respiration

- Responsible for unusually severe and rapid toxicity of CN- poisoning which binds the 6^{th} position and stabilizes the Fe(III) to such an extent that it can no longer be reduced and take part in the electron shuttle.



Heme a

P = —CHOHCH$_2$—(CH$_2$CH=CCH$_2$)$_3$—H (*i.e.* $C_{17}H_{29}O$),
with a CH$_3$ branch on the CCH$_2$ unit

Q = —CH=CH$_2$, R = —C(=O)H

Heme b (i.e. protoheme or protoporphyrin IX)

P = Q = —CH=CH$_2$, R = —CH$_3$

Heme c

P = Q = —CH(CH$_3$)SCH$_2$CH(NH$_2$)CO$_2$H, R = —CH$_3$

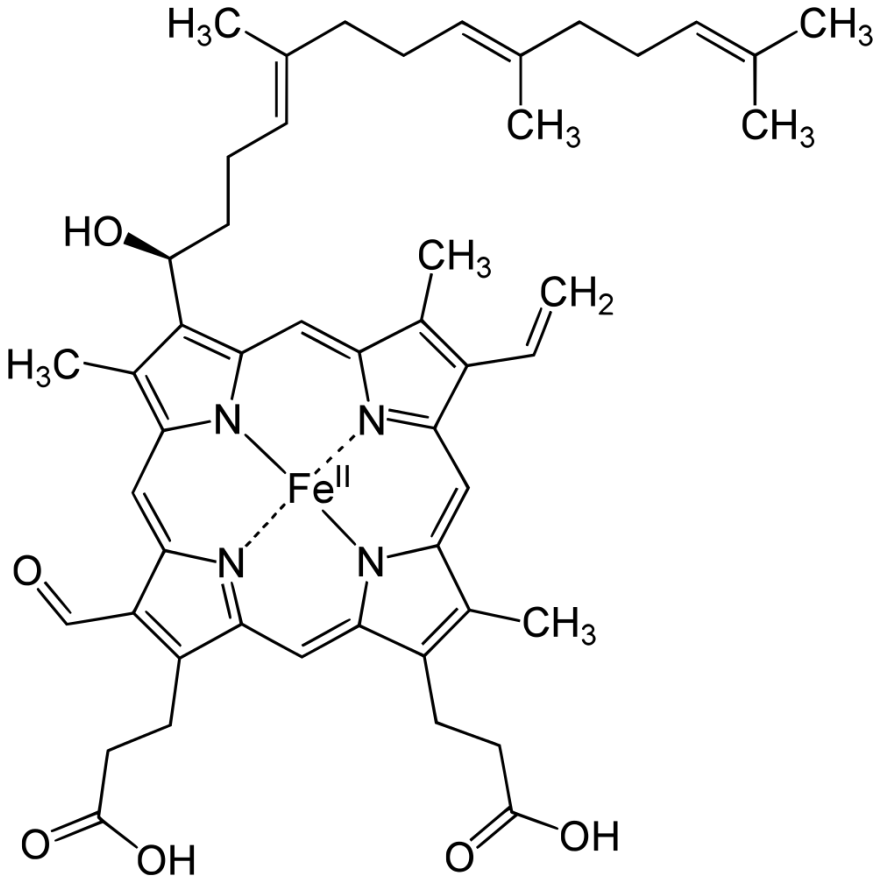
Note : The P and Q moietes are linked covalently to the protein chain through cystein residues.

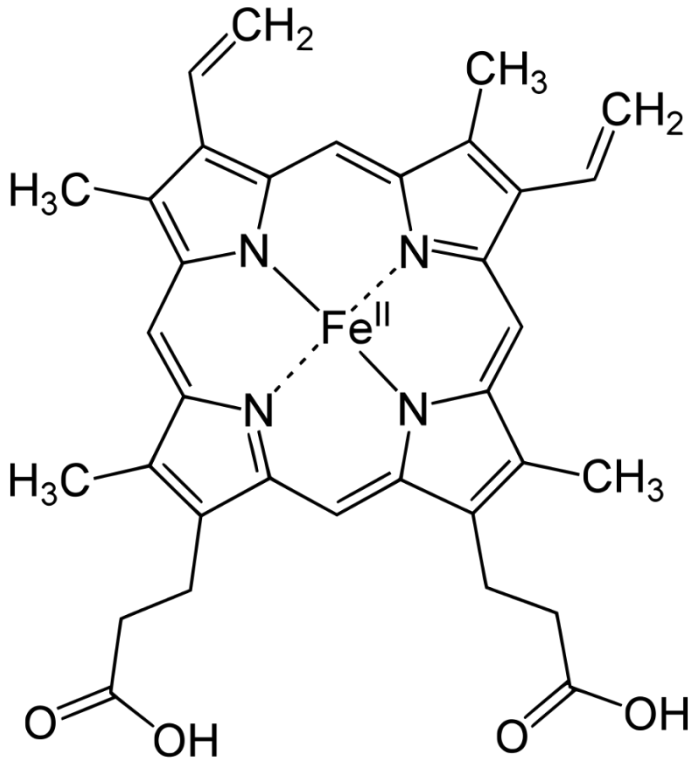
Chloroheme

P = —CH=CH$_2$, Q = —C(=O)H, R = —CH$_3$

Type A: cytochromes a

cytochromes *a* (605 nm), *b* (≈565 nm), and *c* (550 nm)





Soret Band

Phytyl chain

Type B: Hemoglobin, myoglobin, peroxidase and cytochromes b

Type C: R1 = R2 = CH(CH3)S-protein; cytochrome C

R1 = C(H)=O R2 = CH=CH2 called chloroheme found in chlorocruorin

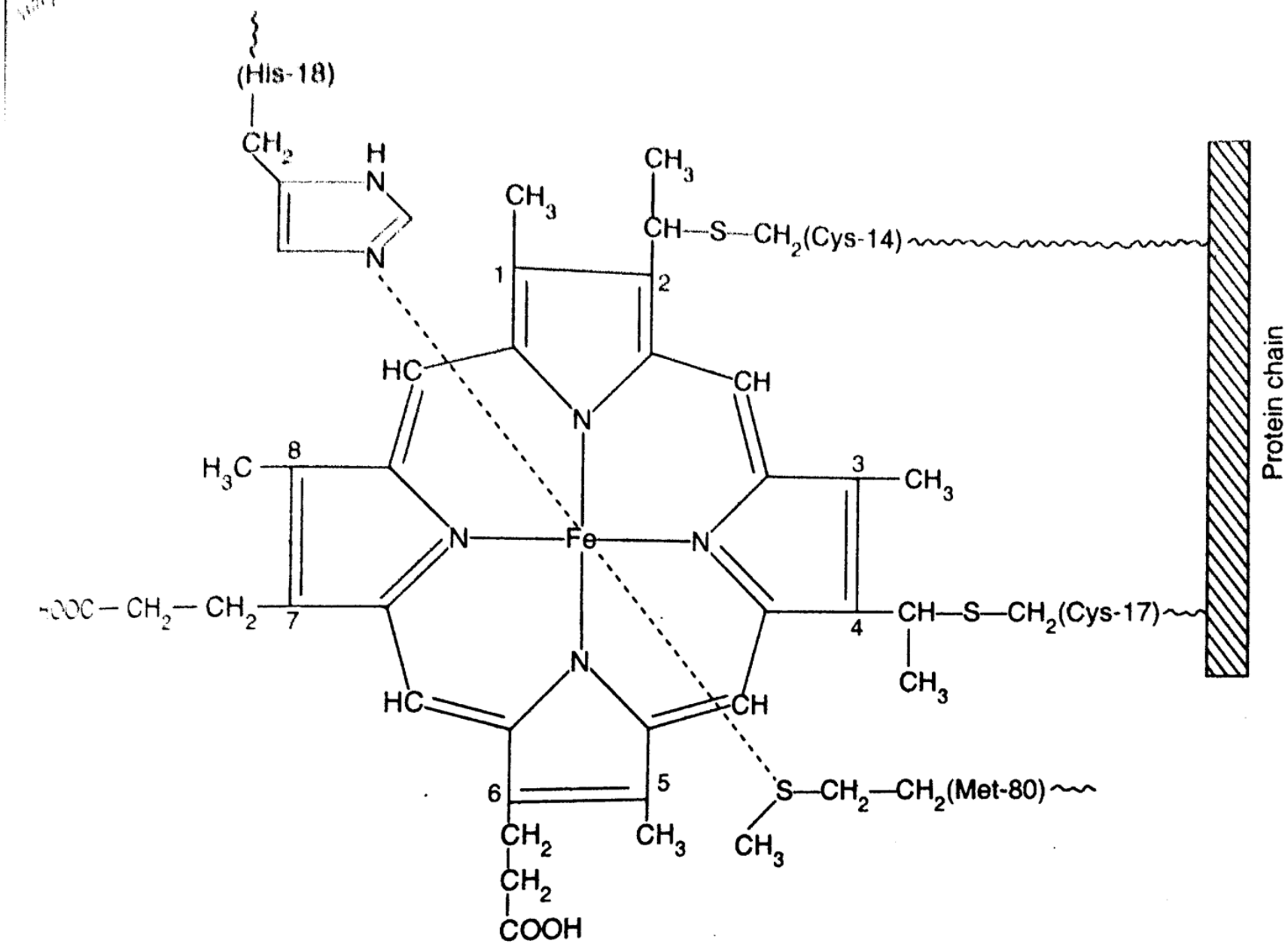
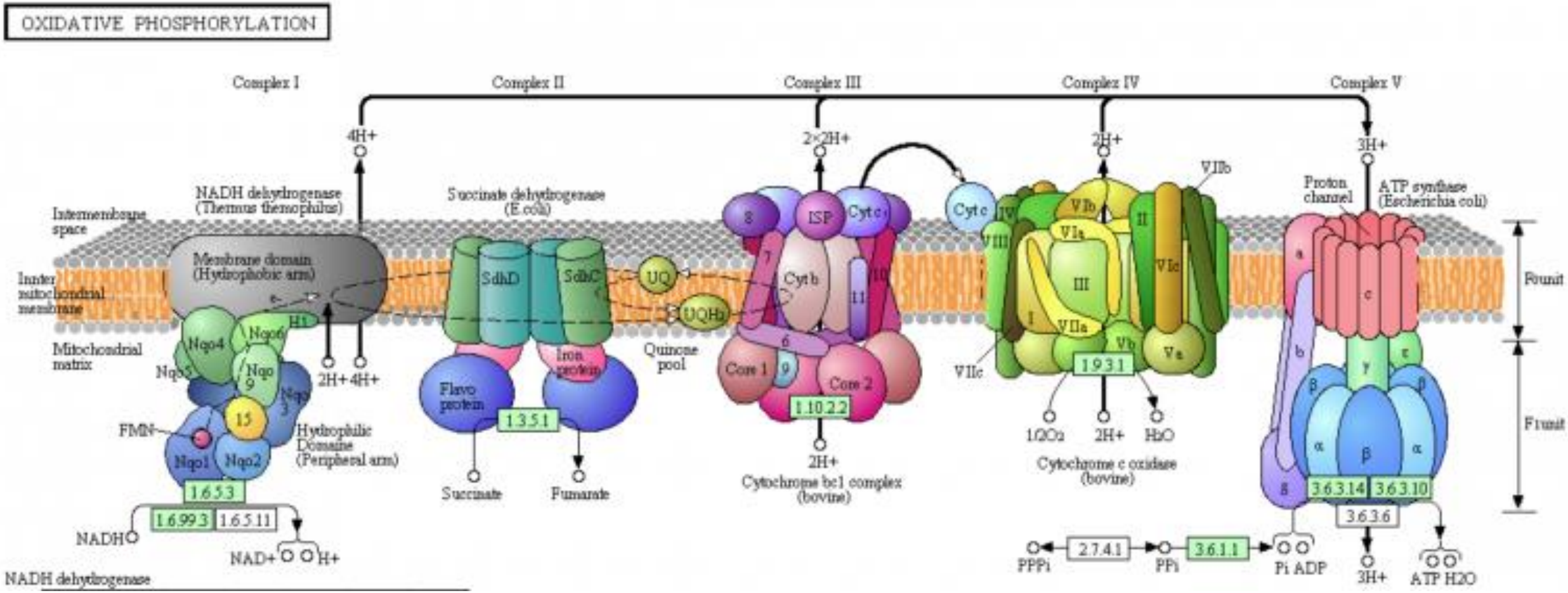


Figure 7.5.2.1: *Structural representation of the active site of cyt c where the heme group is covalently bonded with the protein chain through two cysteine side chains at the 2 and 4 positions.*


OXIDATIVE PHOSPHORYLATION
Complex I
Complex II
Complex III
Complex IV
Complex V
4H+
2×2H+
2H+
3H+
NADH dehydrogenase (Thermus thermophilus)
Succinate dehydrogenase (E.coli)
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
Membrane domain (Hydrophobic arm)
Hydrophilic Domaine (Peripheral arm)
Nqo1
Nqo2
Nqo3
Nqo4
Nqo5
Nqo6
Nqo9
Nqo15
H1
FMN
1.6.5.3
1.6.99.3
1.6.5.11
NADH
NAD+
H+
2H+ 4H+
SdhD
SdhC
Flavo protein
Iron protein
1.3.5.1
Succinate
Fumarate
UQ
UQH2
Quinone pool
Cyt c1
ISP
Cyt b
Core 1
Core 2
1.10.2.2
2H+
Cytochrome bc1 complex (bovine)
Cyt c
VIIb
VIIa
VIIc
VIa
VIb
VIc
Va
Vb
1.9.3.1
1/2O2
2H+
H2O
Cytochrome c oxidase (bovine)
Proton channel
ATP synthase (Escherichia coli)
Fo unit
F1 unit
3.6.3.14
3.6.3.10
3.6.3.6
2.7.4.1
3.6.1.1
PPPi
PPi
Pi ADP
3H+
ATP H2O
NADH dehydrogenase

Respiratory Chain (O_2 reduction)

- **The electron flow is from cyt b – cyt c – cyt a – O_2 at least some of the cyt a binds O_2 and to reduce them.**
- It means that **cyt a** is the last link of the food processing chain – it must be five coordinated in absence of O_2.

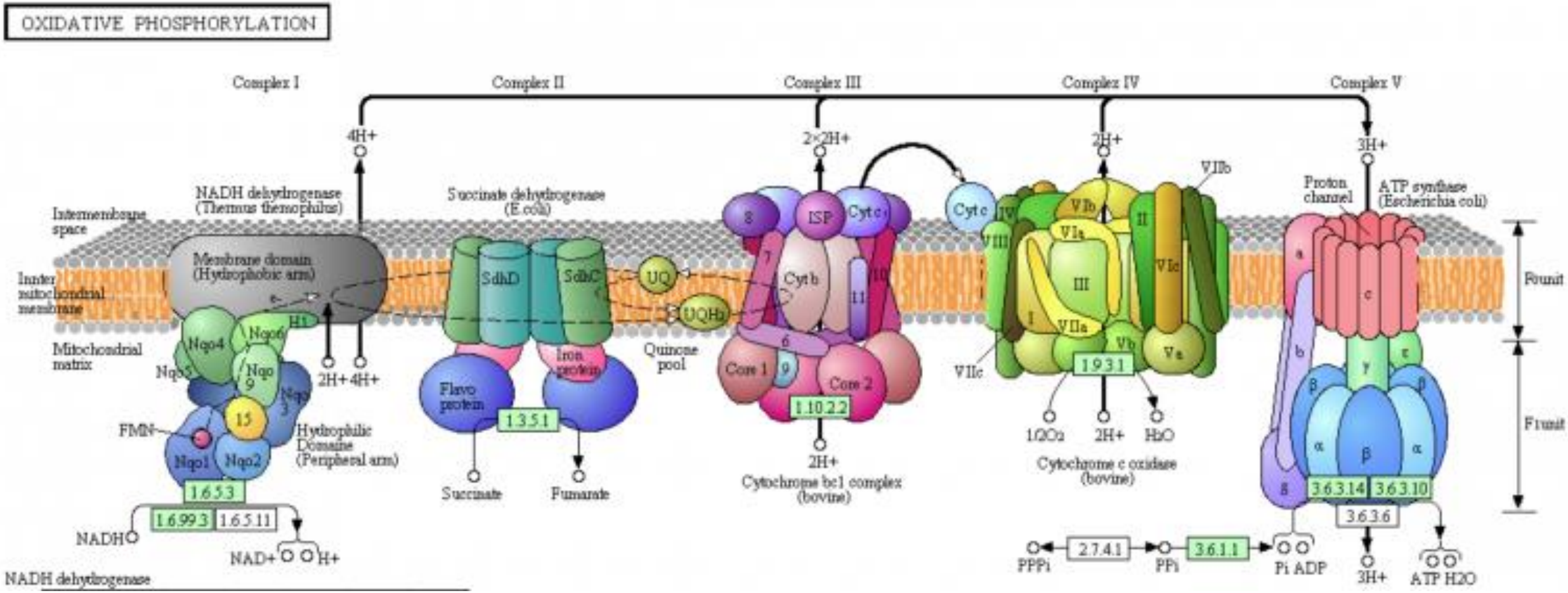
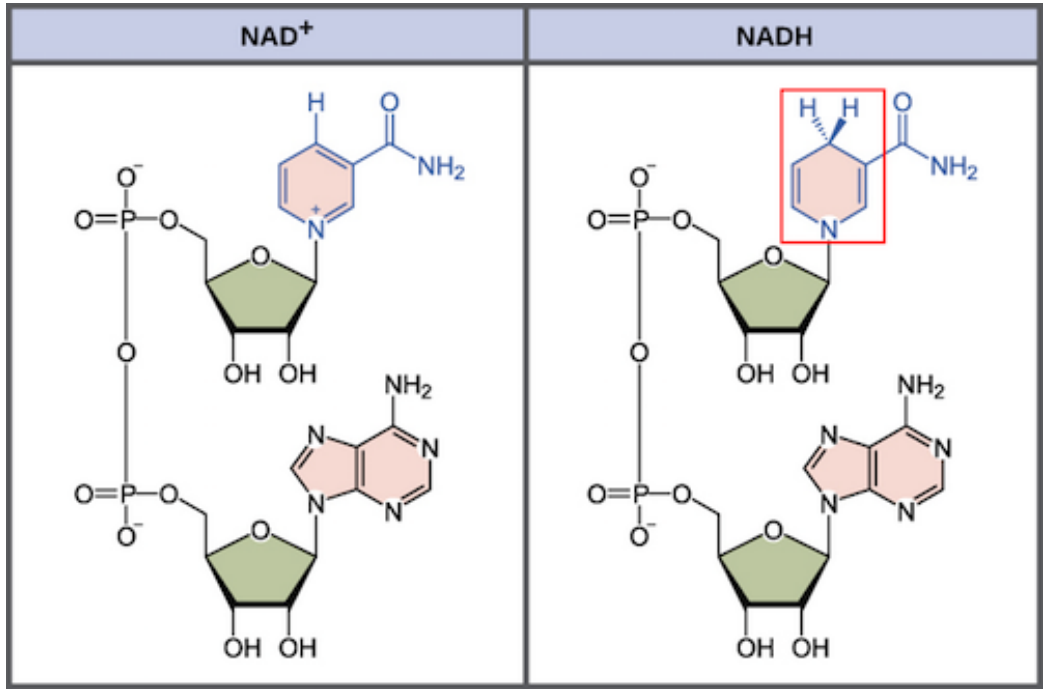
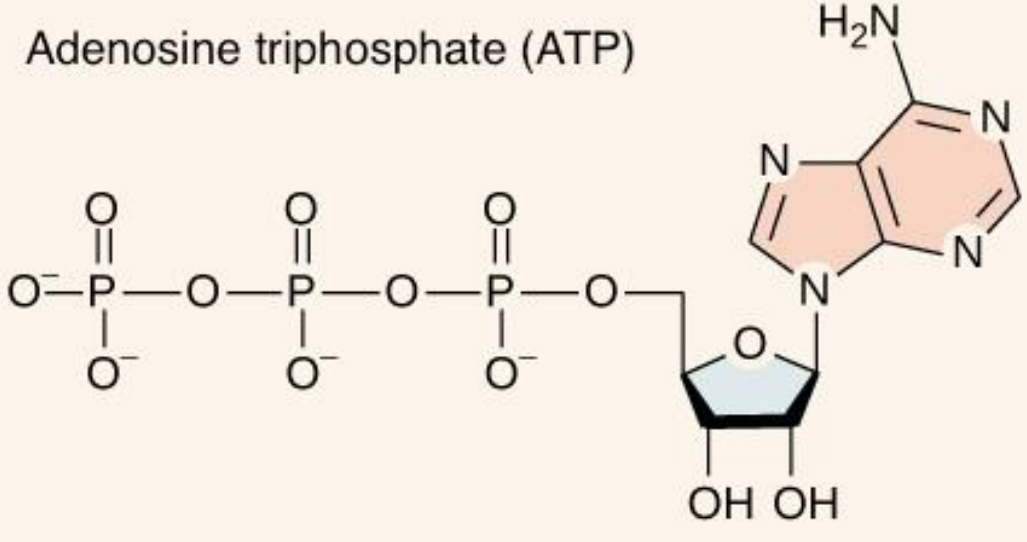

OXIDATIVE PHOSPHORYLATION
Complex I
Complex II
Complex III
Complex IV
Complex V
4H+
2×2H+
2H+
3H+
NADH dehydrogenase (Thermus thermophilus)
Succinate dehydrogenase (E.coli)
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
Membrane domain (Hydrophobic arm)
Hydrophilic Domaine (Peripheral arm)
Nqo1
Nqo2
Nqo3
Nqo4
Nqo5
Nqo6
Nqo9
Nqo15
H1
FMN
1.6.5.3
1.6.99.3
1.6.5.11
NADH
NAD+
H+
2H+ 4H+
SdhD
SdhC
Flavo protein
Iron protein
1.3.5.1
Succinate
Fumarate
UQ
UQH2
Quinone pool
Cyt c1
ISP
Cyt b
Core 1
Core 2
1.10.2.2
2H+
Cytochrome bc1 complex (bovine)
Cyt c
VIIb
VIIa
VIIc
VIa
VIb
VIc
Va
Vb
1.9.3.1
1/2O2
2H+
H2O
Cytochrome c oxidase (bovine)
Proton channel
ATP synthase (Escherichia coli)
Fo unit
F1 unit
3.6.3.14
3.6.3.10
3.6.3.6
2.7.4.1
3.6.1.1
PPPi
PPi
Pi ADP
3H+
ATP H2O
NADH dehydrogenase

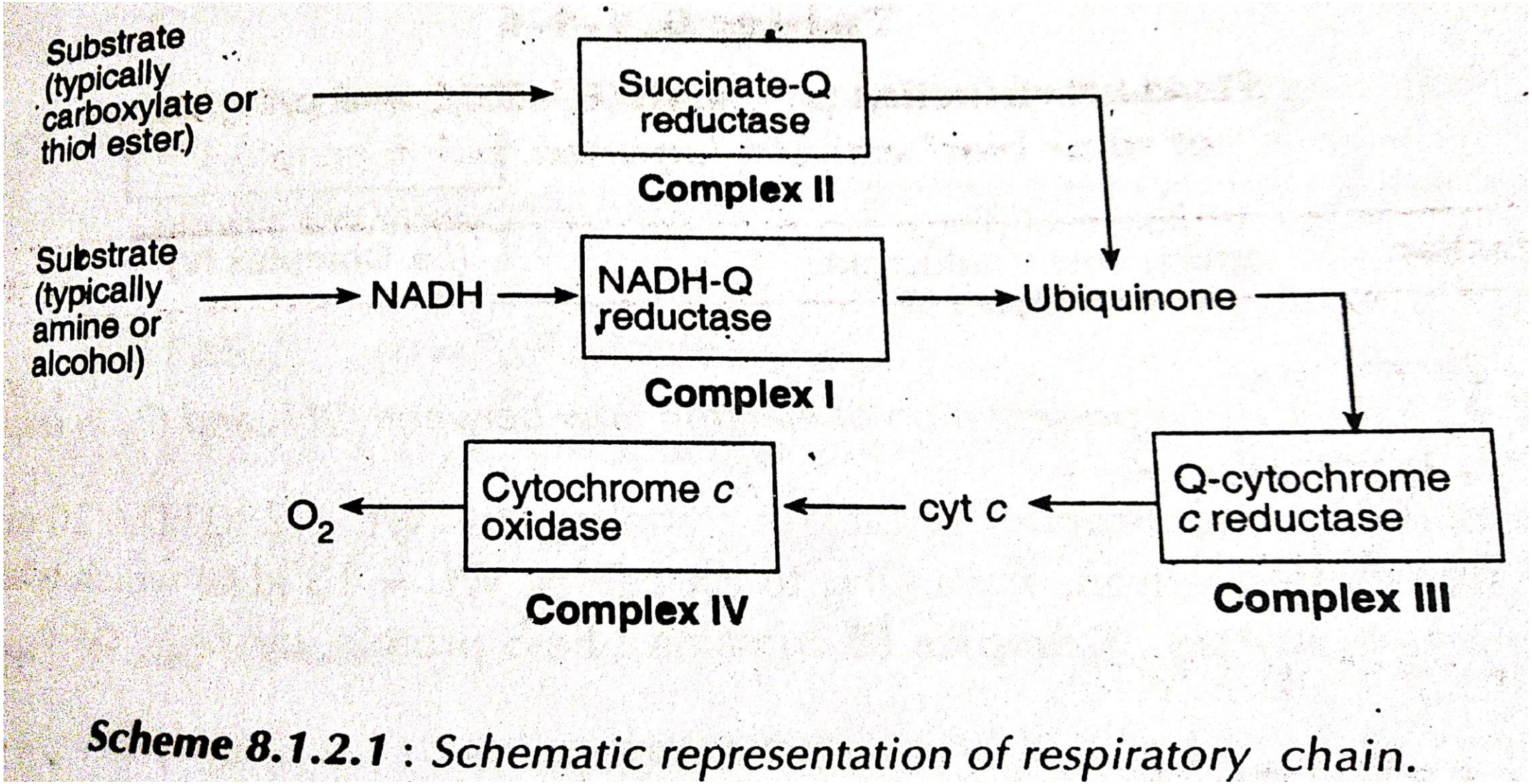
Table: 8.1.1.1

Standard reduction potentials (E_0', 25°C and pH 7.0) of some biochemically important redox couples)

Half Reaction	E_0' (V)
$O_2 + 2H^+ + 2e \rightleftharpoons H_2O$	+ 0.82
cyt a_3 (Fe^{3+}) + $e \rightleftharpoons$ cyt a_3 (Fe^{2+})	+ 0.39
$O_2 + 2H^+ + 2e \rightleftharpoons H_2O_2$	+ 0.30
cyt a (Fe^{3+}) + $e \rightleftharpoons$ cyt a (Fe^{2+})	+ 0.29
cyt c (Fe^{3+}) + $e \rightleftharpoons$ cyt c (Fe^{2+})	+ 0.24
cyt b (Fe^{3+}) + $e \rightleftharpoons$ cyt b (Fe^{2+})	+ 0.06
Ubiquinone + $2H^+ + 2e \rightleftharpoons$ Ubiquinol	+ 0.05
Fumarate + $2H^+ + 2e \rightleftharpoons$ Succinate	+ 0.03
FAD + $2H^+ + 2e \rightleftharpoons FADH_2$	0.0 (in flavoproteins) . – 0.22 (in free coenzyme)
Pyruvate + $2H^+ + 2e \rightleftharpoons$ Lactate	– 0.19
Acetaldehyde + $2H^+ + 2e \rightleftharpoons$ Ethanol	– 0.20
$NAD^+ + H^+ + 2e \rightleftharpoons$ NADH	– 0.32
$NADP^+ + H^+ + 2e \rightleftharpoons$ NADPH	– 0.32
Cystine + $2H^+ + 2e \rightleftharpoons$ 2 Cysteine	– 0.34
$H^+ + e \rightleftharpoons \frac{1}{2} H_2$	– 0.42
Fd_{ox} (Fe^{3+}) + $e \rightleftharpoons$ Fd_{red} (Fe^{2+})	– 0.45
Acetate + $3H^+ + 2e \rightleftharpoons$ Acetaldehyde + H_2O	– 0.58

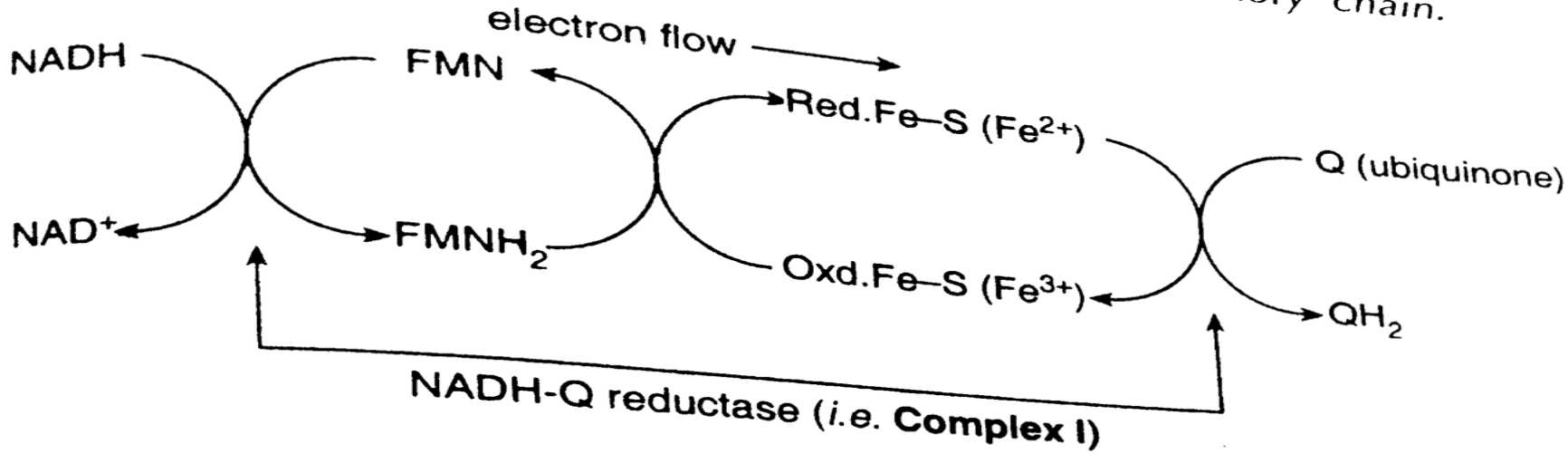




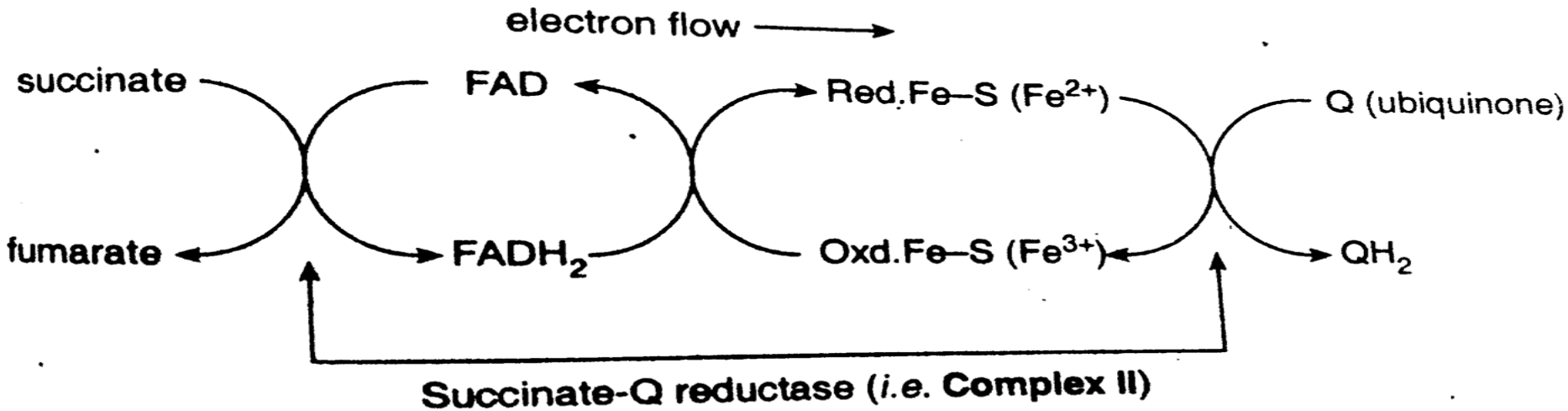


Scheme 8.1.2.1 : *Schematic representation of respiratory chain.*

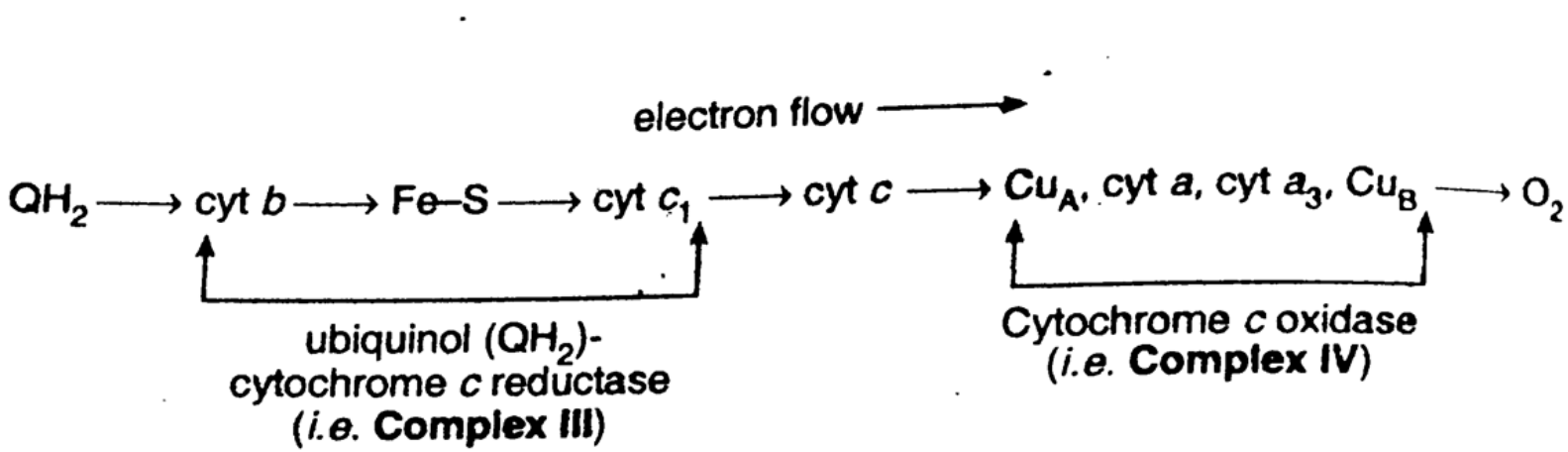
Coenzyme Q is the only electron carrier which Is not covalently bound with the protein chain in the respiratory system. Thus is function as a mobile carrier of electron.



Scheme 8.1.2.2 : *Schematic representation of electron flow in NADH-Q reductase (i.e. Complex I) of respiratory chain.*

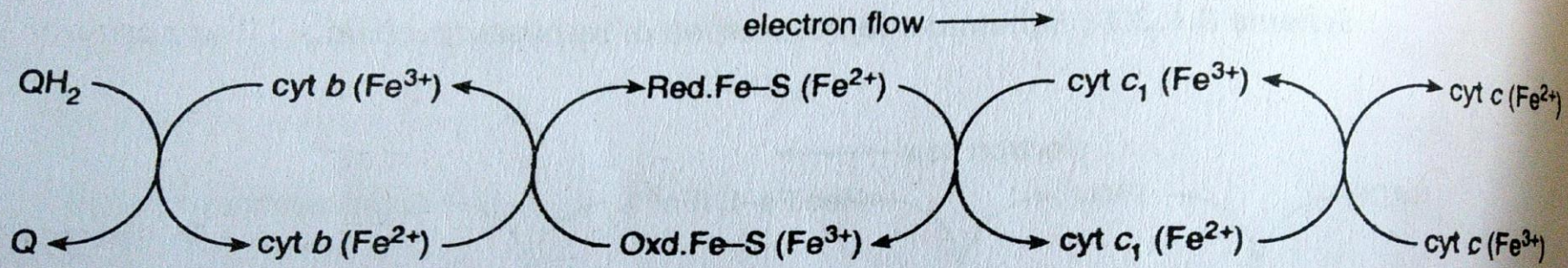


Scheme 8.1.2.3 : *Schematic representation of electron flow in succinate-Q reductase (i.e. Complex II) of respiratory chain.*



Scheme 8.1.2.4 : *Schematic representation of electron flow between QH_2 and O_2 in respiratory ch*

The ubiquinol *(QH_2)-cytochrome c reductase* complex (Mol. Wt. ~ 280 kDa, 10 subunits), known as **complex III** transports electrons from QH_2 to cyt *c* (Mol. Wt. = 13 kDa) which is a *water soluble peripheral membrane protein.* **Complex III** contains a Fe-S protein, cyt b_{562}, cyt b_{566} and cyt c_1.



Scheme 8.1.2.5 : *Schematic representation of electron flow in QH_2-cytochrome c reductase (i.e. complex III) of respiratory chain.*

Then the overall reaction is :

$$\frac{1}{2} O_2 + NADH + H^+ \rightleftharpoons H_2O + NAD^+, \qquad \Delta E_0' = +1.14\ V$$

The net reaction, *i.e.* oxidation of one mole of NADH, involves the Gibbs free change ($\Delta G_0'$) as follows:

$$\Delta G_0' = -n F \Delta E_0', \quad (n = 2,\ F = \text{faraday})$$

$$= -2 \times 96500 \times 1.14 \text{ volt} \times \text{coulomb} \times \text{mol}^{-1}, \quad (1\ F = 96{,}500\ \text{C mol}^{-1})$$

$$= -2 \times 96500 \times 1.14 \text{ J mol}^{-1}, \quad (1 \text{ volt} \times 1 \text{ coulomb} = 1 \text{ Joule})$$

$$\approx -220 \text{ kJ mol}^{-1}$$

The free energy change in the synthesis of ATP from ADP by mitochondrial ATP-ase enzyme is given below.

$$HADP^{2-} + HPO_4^{2-} \rightleftharpoons ATP^{4-} + H_2O, \qquad \Delta G_0' = +30.7 \text{ kJ mol}^{-1}$$

The above process is very often represented simply as: $ADP + P_i \rightleftharpoons ATP$ where P_i denotes the inorganic *ortho*-phosphate moiety. The free energy change in the above mentioned process depends on many factors (*cf.* Sec. 9.1). The above value is generally considered for calculation in biological conditions. Thus, if the total energy (220 kJ mol^{-1}) released for oxidation of 1 mole of NADH (*i.e.* transfer of 2 mole of electrons) by O_2 is available for ATP synthesis, then it will yield about 7 moles of ATP. But, in reality, it cannot produce 7 moles of ATP. This is because, the difference of reduction potential ($\Delta E_0'$) between the adjacent couples must be sufficiently high to release 30.7 kJ mol^{-1}. The minimum value of required $\Delta E_0'$ for the synthesis of one mole of ATP is given by :

$$\Delta E_0' = -\Delta G_0'/n\,F = (30.7 \times 1000/2 \times 96{,}500)\ V = 0.16\ V.$$

In the respiratory chain, there are three sites (*cf.* Fig. 8.1.3.1) where $\Delta E_0'$ value between the adjacent redox couples exceeds 0.16 V. The ATP generating sites are :

Site I : Complex I (*i.e.* NADH-Q reductase) catalysed oxidation of NADH by Q.

$$Q + NADH + H^+ \xrightarrow{\text{Complex I}} QH_2 + NAD^+$$

E_0' (Q) = + 0.045 V, E_0' (NAD^+) = − 0.32 V, $\Delta E_0'$ = 0.365 V, $\Delta G_0'$ = $-nF\Delta E_0'$ = −70.4 kJ mol^{-1} (n = 2, F = 96500 C mol^{-1})

Site II : Complex III (*i.e.* QH_2-cytochrome *c* reductase) catalysed oxidation of QH_2 by cyt *c* (oxidised form).

$$QH_2 + \text{cyt } c \text{ (oxidised)} \xrightarrow{\text{Complex III}} Q + \text{cyt } c \text{ (reduced)}$$

E_0' (cyt c) = + 0.235 V, E_0' (Q) = + 0.045 V, $\Delta E_0'$ = 0.19 V, $\Delta G_0'$ = −36.7 kJ mol^{-1}

Site III : Complex IV (*i.e.* cytochrome *c* oxidase) catalysed oxidation of cyt *c* (reduced form)

Cyt C Oxidase: Catalytic Cycle and Mechanism of Proton Pumping

- Biochemistry, 1999, 15129 https://doi.org/10.1021/bi9910934

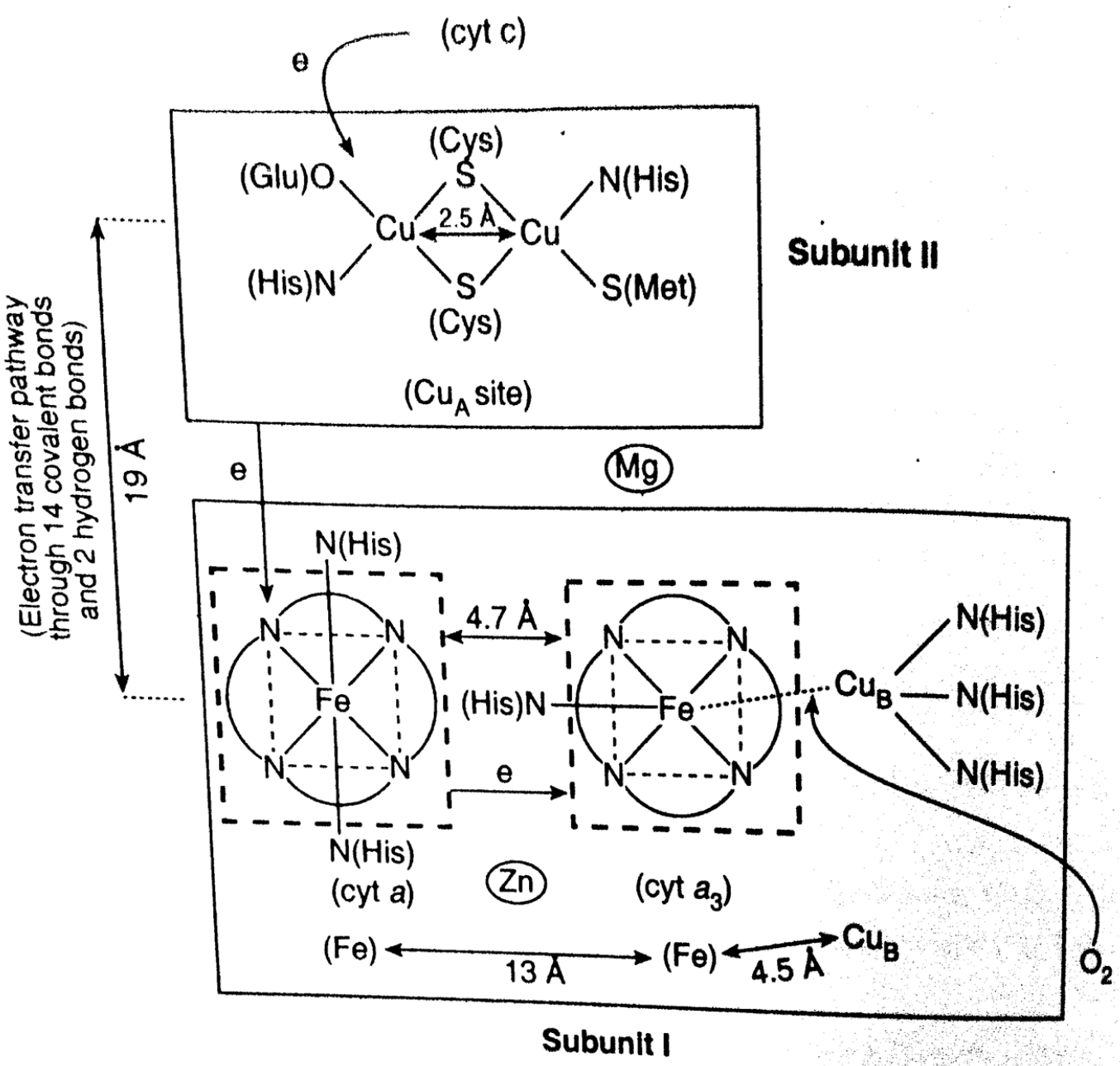


Figure 7.6.1.1: *Schematic representation of the position of different components in cyt c oxidase and activity of cyt c oxidase*

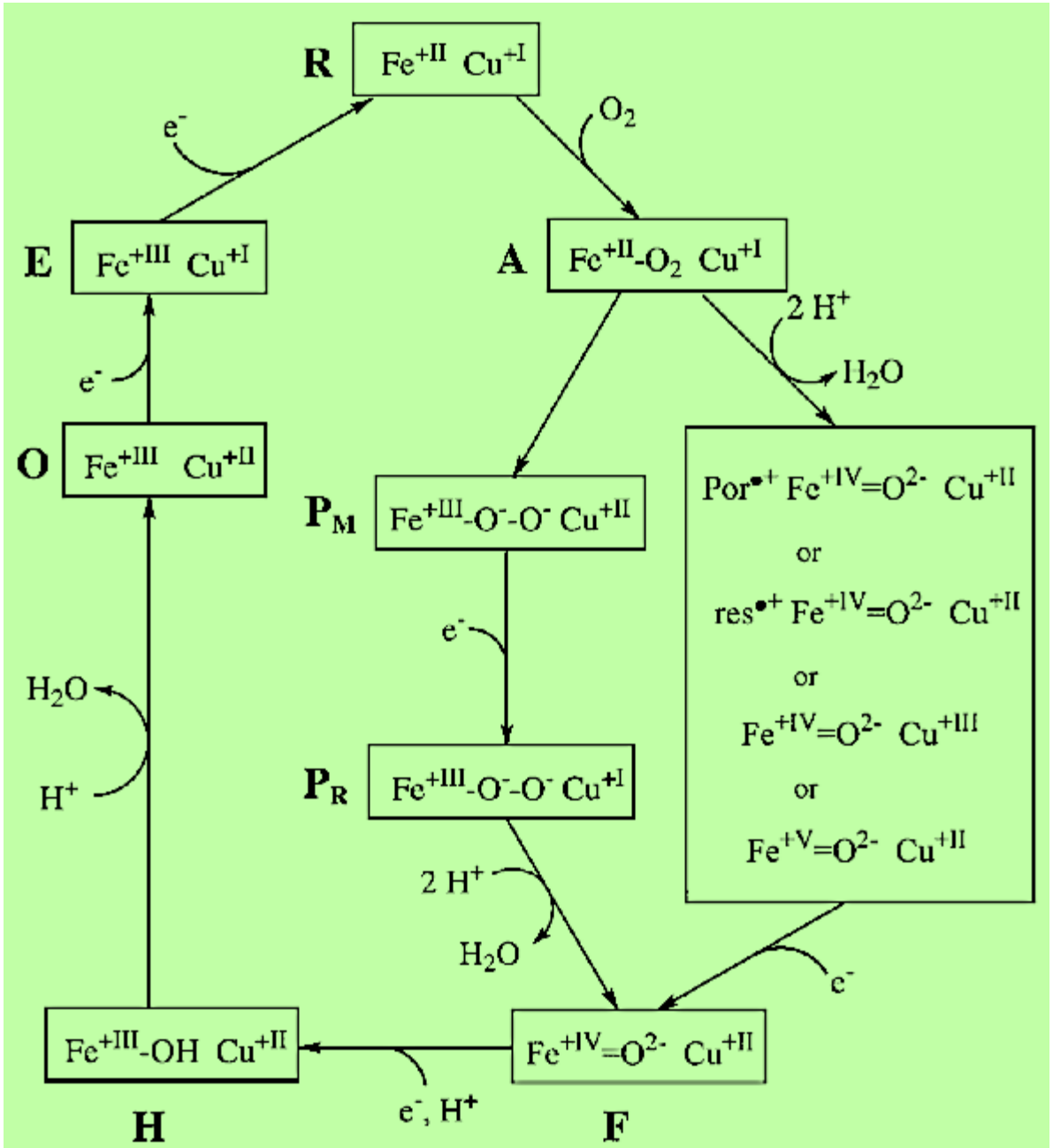
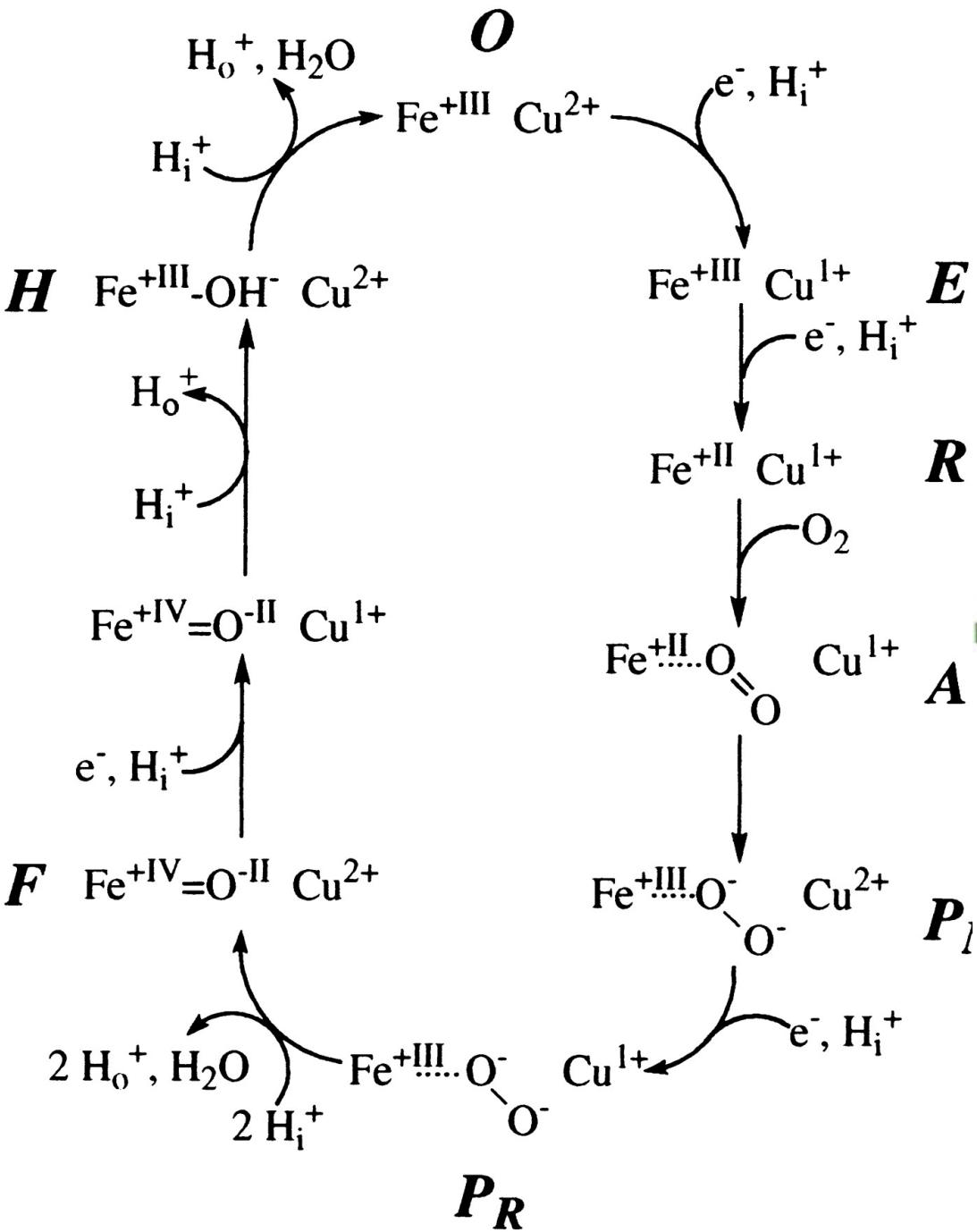
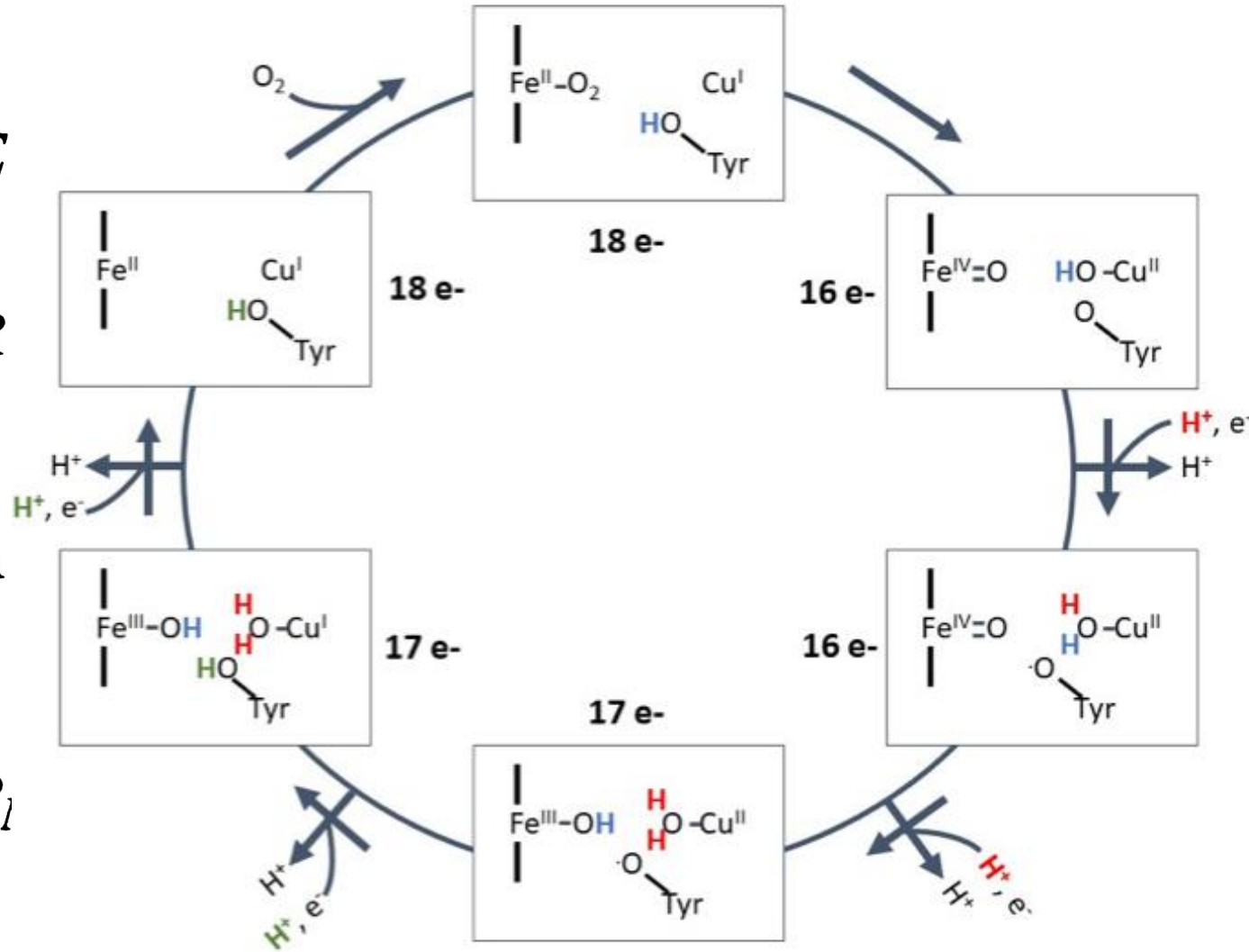
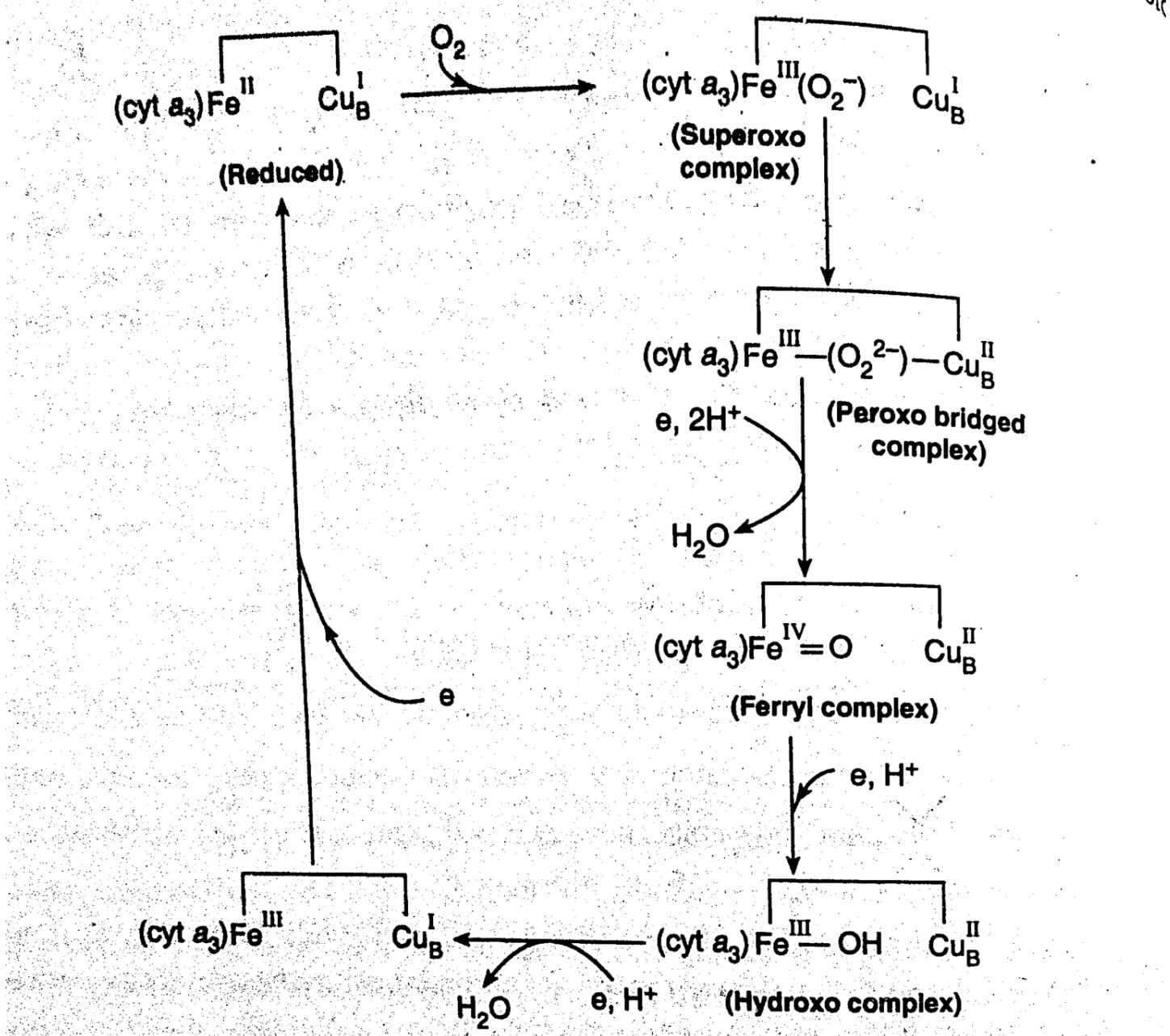


Figure 4 The catalytic cycle of cytochrome *c* oxidase as derived from optical and resonance Raman spectroscopy (23, 49). Starting from the oxidized form (*O*), the one-electron reduced form (*E*) and then the doubly reduced form (*R*) are generated. Upon oxygen binding compound A is observed. Next the peroxy-intermediates P_M, P_R are formed (see also text). Alternative structures are presented on the *right*, based on the proposal that the O-O bond is already split in these states. However, one electron is missing, which could be provided by a porphyin-ring system (por), an amino acid residue (res), Cu_B (leading to a Cu^{3+}-state), or the heme a_3-Fe. There is general agreement about the structure of the oxoferryl-state (*F*) and a hydroxy-state (*H*) formed after protonation of the iron-bound oxygen atom. After water formation and release, the O-state is regenerated.

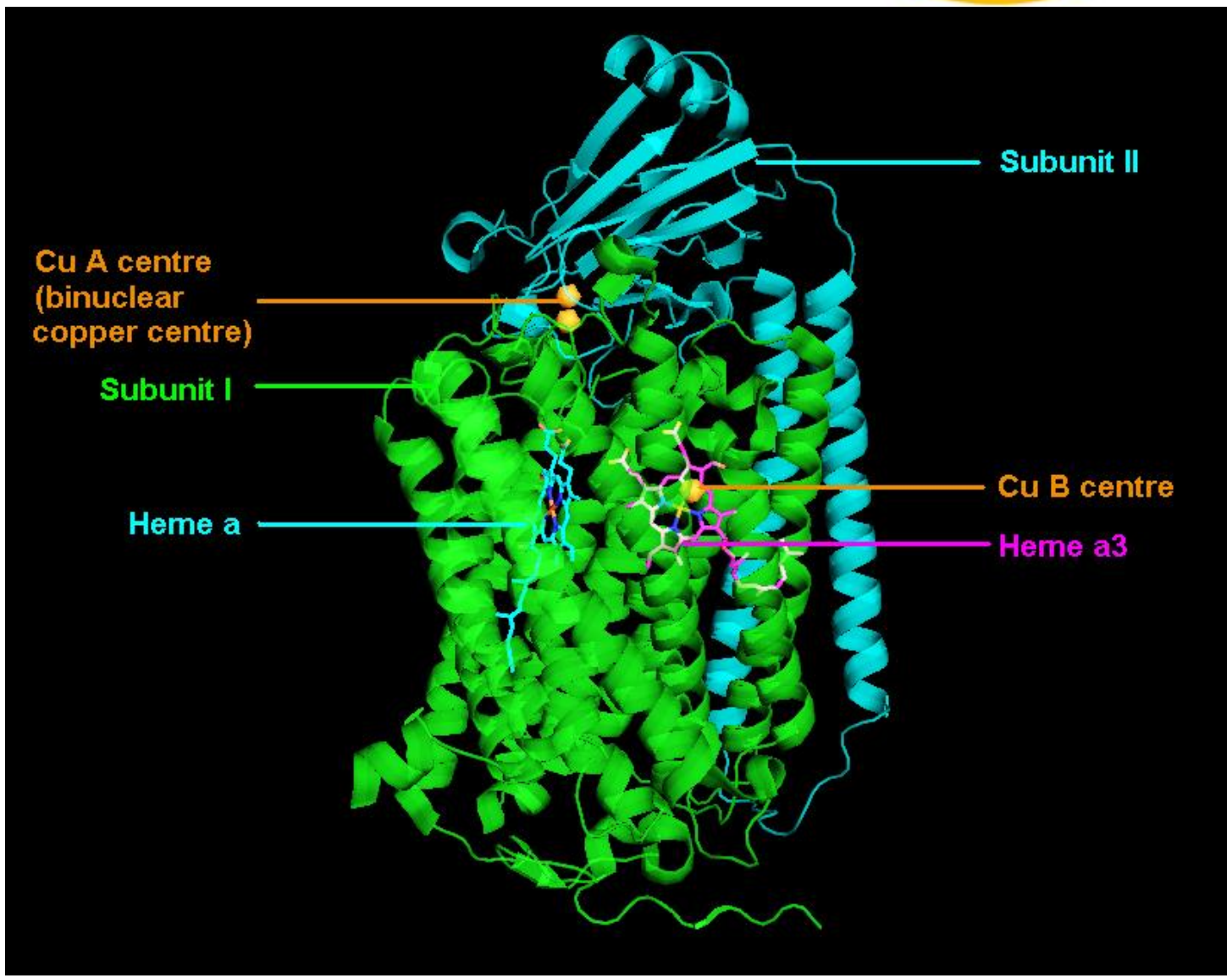
4 Fe^{2+}-cytochrome *c* + 4 H^+_{in} + O_2 → 4 Fe^{3+}-cytochrome *c* + 2 H_2O + 4 H^+_{out}

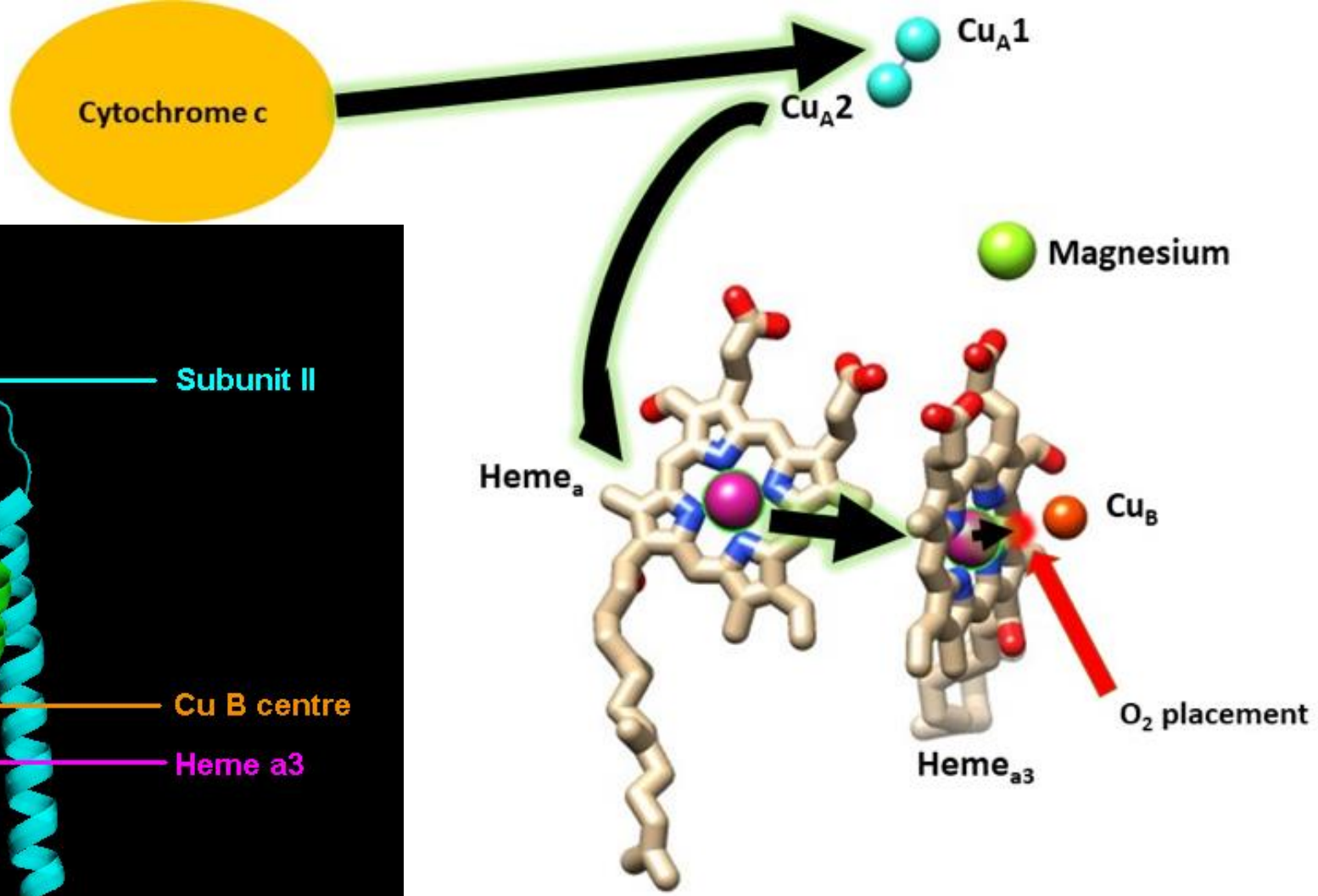

O
H_o^+, H_2O
Fe^{+III} Cu^{2+}
e^-, H_i^+
H_i^+
H Fe^{+III}-OH^- Cu^{2+}
Fe^{+III} Cu^{1+} E
e^-, H_i^+
H_o^+
Fe^{+II} Cu^{1+} R
H_i^+
O_2
Fe^{+IV}=O^{-II} Cu^{1+}
Fe^{+II} O_2 Cu^{1+} A
e^-, H_i^+
F Fe^{+IV}=O^{-II} Cu^{2+}
Fe^{+III} O_2^- Cu^{2+} P_I
e^-, H_i^+
2 H_o^+, H_2O
Fe^{+III} O_2^-
Cu^{1+}
2 H_i^+
P_R

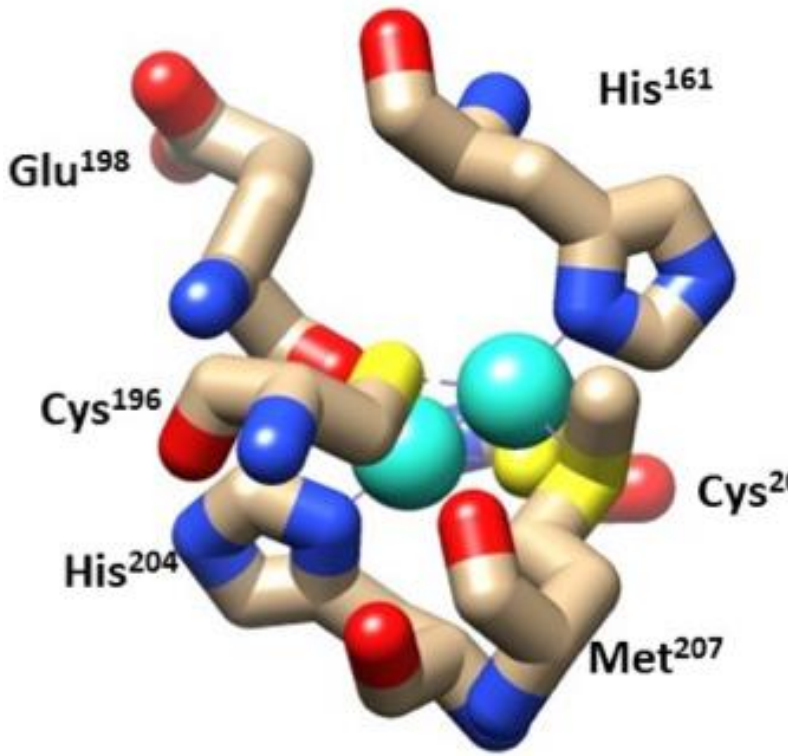

O_2
Fe^{II}-O_2 Cu^I
HO Tyr
18 e-
Fe^{II} Cu^I
HO Tyr
18 e-
16 e-
Fe^{IV}=O HO-Cu^{II}
O Tyr
H^+, e^-
H^+
H^+
H^+, e^-
Fe^{III}-OH H O-Cu^I
H HO Tyr
17 e-
16 e-
Fe^{IV}=O H O-Cu^{II}
H O Tyr
17 e-
Fe^{III}-OH H O-Cu^{II}
H O Tyr
H^+
H^+, e^-
H^+
H^+, e^-

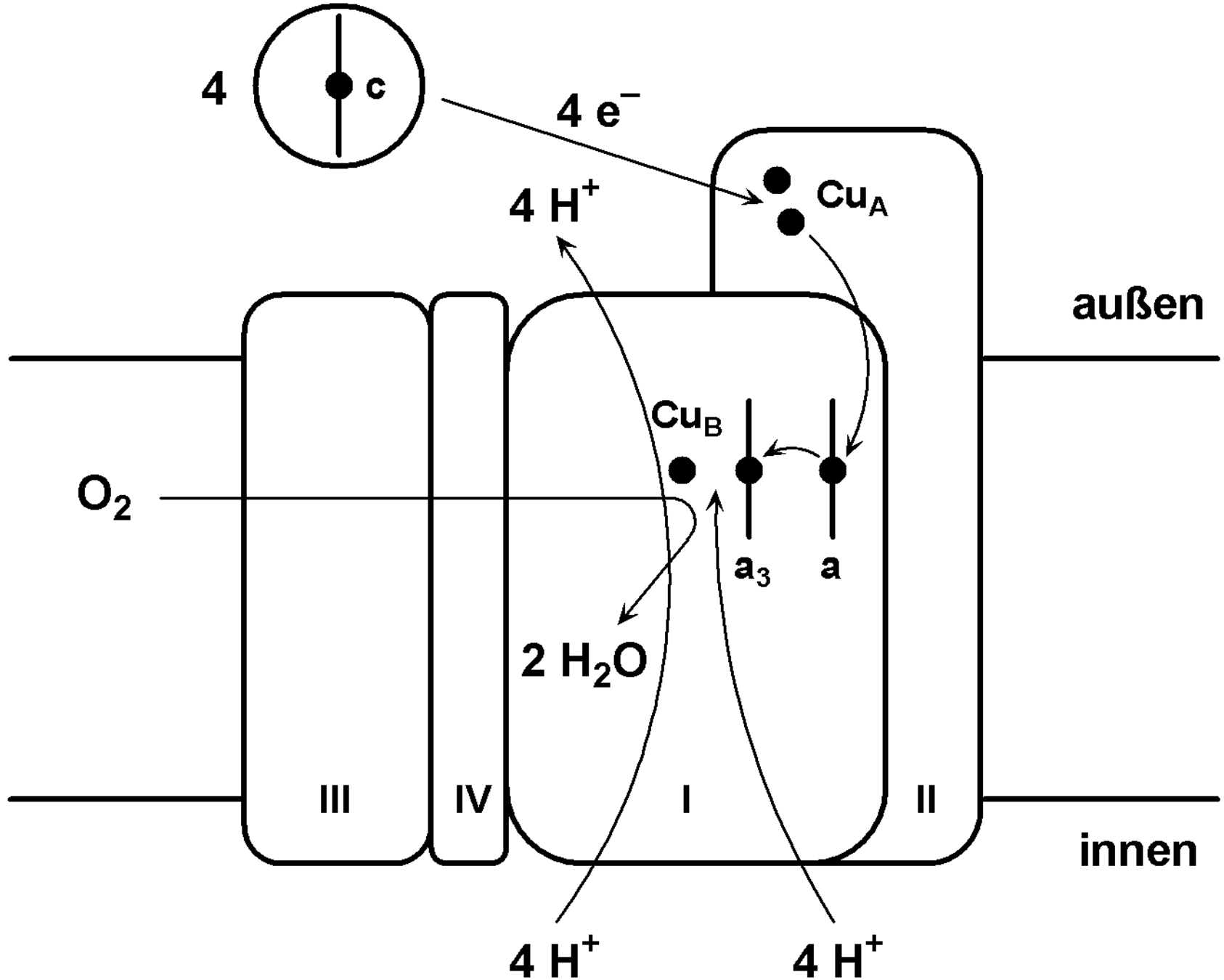


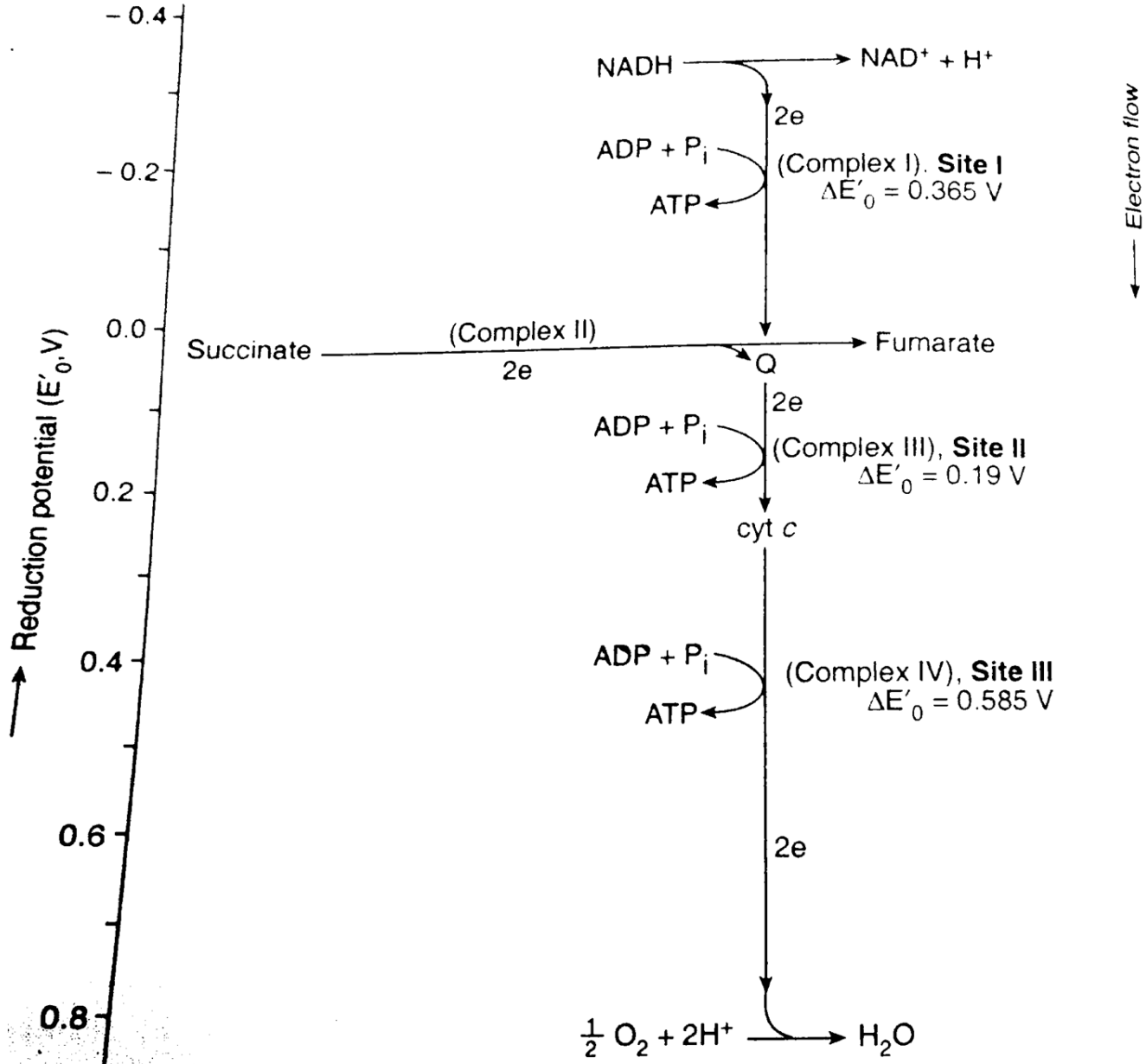
Scheme 7.6.2.1 : *Schematic representation of the mechanism of reduction of O_2 catalysed by cytochrome c oxidase.*


Subunit II
Cu A centre
(binuclear
copper centre)
Subunit I
Cu B centre
Heme a
Heme a3


Cytochrome c
Cu_A1
Cu_A2
Magnesium
$Heme_a$
Cu_B
O_2 placement
$Heme_{a3}$


His161
Glu198
Cys196
Cys2
His204
Met207


4
c
4 e⁻
4 H+
CuA
außen
CuB
O2
a3
a
2 H2O
III
IV
I
II
innen
4 H+
4 H+



3.1 : Mitochondrial respiratory chain representing the ATP generating sites. Redu
potentials (E_0' at pH ≈ 7, 25 °C) of the redox couples are shown.

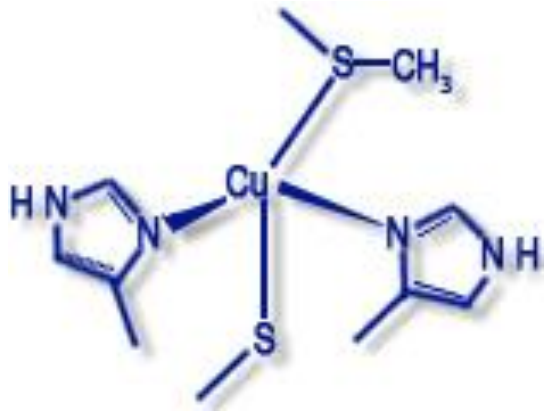
CN Poisoning and blocking of Respiratory Chain

8.1.4 Blocking of the Respiratory Chain and Inhibition of the Electron Flow

If the ATP generating sites (*i.e.* site I, II and III) are blocked then ATP synthesis will be prevented and it will inhibit the electron flow to end the life process. *Rotenone* (a plant toxin) and *amytal* (a barbutarate) specifically inhibit the activity of NADH-Q reductase to block the **site I**. It is important to note that this blocking cannot stop the electron flow from oxidation of succinate (*cf.* succinate enters into the respiratory chain via **complex II**), as in this route electrons enter in the respiratory chain beyond the **site I** at ubiquinone (*cf.* Scheme 8.1.2.1). *Antimycin A* (an antibiotic) can block the **site II**. This blocking can be bypassed by the addition of ascorbate which directly can reduce cyt *c* to keep alive the **site III**. The **site III** is blocked by the ligands like CO, CN^-, N_3^-, etc. In cyt *c* oxidase (Sec. 7.6), cyt a_3 (5 coordinate Fe) and Cu_B are coordinatively unsaturated and the ligands may occupy the vacant site of either Fe or Cu or both. CO is believed to complex with the Fe(II) state of cyt a_3 and it stabilises the Fe(II) state and prevents its reoxidation to the Fe(III) state. N_3^- is expected to stabilise the Fe(III) state of cyt a_3. The deadly toxic effect of CN^- is due to its complexation with iron [cyt a_3] or copper [Cu_B] at the vacant coordination site. CN^- preferably stabilises Fe(III) in Fe(III)/Fe(II) couple while it stabilises preferably Cu(I) in Cu(II)/Cu(I) couple. Consequently, regeneration of Cu(II) and Fe(II) by the adjacent redox couples is not possible. To remove this blocking at site III, CN^- is to be removed from cytochrome *c* oxidase. Fe(III) in met-Hb and met-Mb can thermodynamically and kinetically snatch the bound CN^- from the **site III**. Probably, due to the positive charge on $(Fe^{III}$–heme$)^+$ present in **met-Hb or met-Mb**, it binds CN^- more strongly than either cyt *c* oxidase or hemoglobin/myoglobin. This charge neutralization gives also an **entropic favour** due to relaxation of electrorestriction over the surroundings. This is why, in the treatment of CN^- poisoning, met-Hb is to be produced by the injection of $NaNO_2$ solution or inhalation of the amylnitrite vapour, which can oxidise some of the hemoglobins [Fe(II)-heme] to met-Hb. Then the met-Hb bound CN^- is destroyed in spleen. Presently, Co(II)-edta complex is administered to detoxify CN^- toxicity as this complex binds CN^- very strongly and it snatches away CN^- from cytochrome *c* oxidase.

Classification of Biological Copper Centers

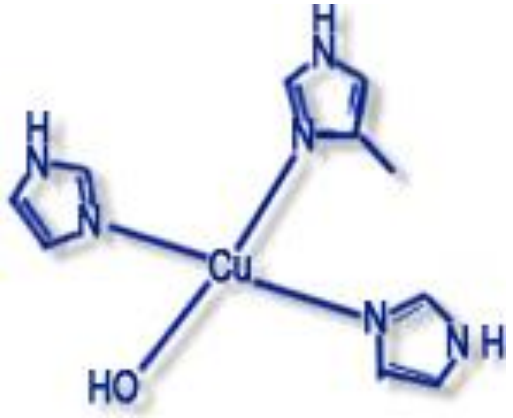
	Mononuclear		Dinuclear		Tetranuclear
Type	**Type 1**	**Type 2**	**Type 3**	**Cu_A**	**Cu_Z**
UV-vis Spectrum	Strong absorption ~ 600 nm and (in some proteins) 450 nm	Weak absorption ~ 700 nm	Weak absorption ~ 700 nm	Strong absorption ~ 480 and 530 nm	Strong absorption ~ 640 nm
EPR spectrum	4-line ($A_{\|}$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



Type I ("blue" copper proteins)

- Strongly distorted coordination sphere (3 + 1) comprised of 2x histidine, 1x methionine, 1x cysteine
- Absorption at ca. 600 nm (blue)
- EPR spectrum with small Cu coupling and g-value anisotropy:

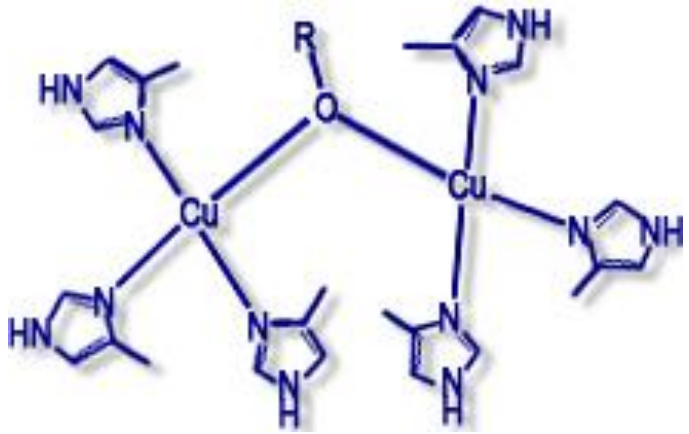
Examples: plastocyanin, azurin, nitrite reductase



Type II ("non-blue" copper proteins)

- Essentially planar coordination sphere with 3x histidine und 1x H_2O or substrate molecule
- Weak absorption in the visible region
- EPR spectrum with axial symmetry ("normal" EPR spectrum):

Examples: galactose oxidase, amine oxidase, dopamine monooxidase



Type III (dimers)

- Oxygen bridged dimer with a Cu-Cu distance of ca. 360 pm
- After oxygen uptake shows intense absorptions at 350 nm and 600 nm
- EPR silent; antiferromagnetically coupled d^9 centers:

Examples: haemocyanin, tyrosinase

http://www.e-learning.chemie.fu-berlin.de/en/bioanorganik/kupfer/molekuele/cu_proteine/index.html

There are three classes of copper centres in blue copper proteins:

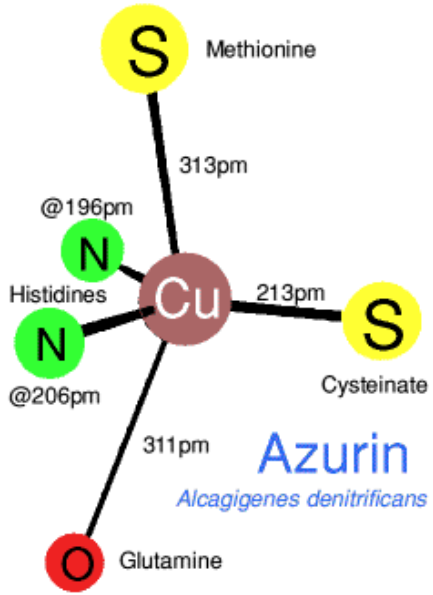
- A Type 1 centre is characterized by an intense absorption in the electronic spectrum with $\lambda_{max} \approx 600\,nm$, and $\varepsilon_{max} \approx 100$ times greater than that of aqueous Cu^{2+}. The absorption is assigned to charge transfer from a cysteine ligand to Cu^{2+}. In the EPR spectrum (Cu^{2+} has one unpaired electron), narrow hyperfine splitting is observed (see Section 4.9).
- A Type 2 centre exhibits electronic spectroscopic characteristics typical of Cu^{2+}, and the EPR spectrum is typical of a Cu^{2+} centre in a simple coordination complex.
- A Type 3 centre exhibits an absorption with $\lambda_{max} \approx 330\,nm$ and exists as a pair of Cu(II) centres which are antiferromagnetically coupled to give a diamagnetic system. Hence, there is no EPR spectroscopic signature. The Cu_2-unit can function as a 2-electron transfer centre and is involved in the reduction of O_2.

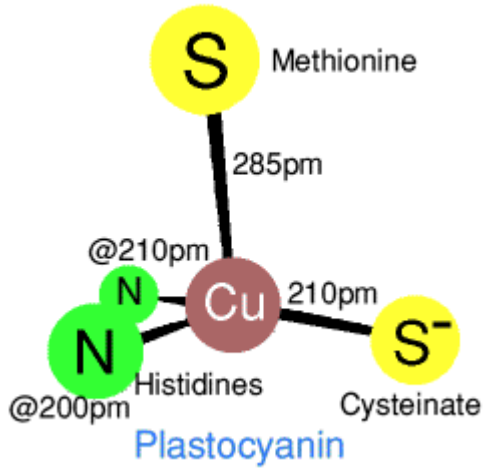
"Classical" Copper Centers in Proteins

Copper participates in many biological processes involving electron transfer reactions. Its roles are as widely varied as simple electron transfer, oxygen activation, and oxygen transport.

In this sense, the copper proteins often have functions which can be carried out by iron centers. This is an indication that natural evolution was "success-oriented" and not "structure-oriented." A good example of this is the enzyme nitrite reductase. Its active site can be either an iron haem center or a type I copper complex.

Copper proteins are often classified as type I, type II, or type III centers, depending on the environment of the metal ion and spectroscopic characteristics (EPR spectrum, color, etc).

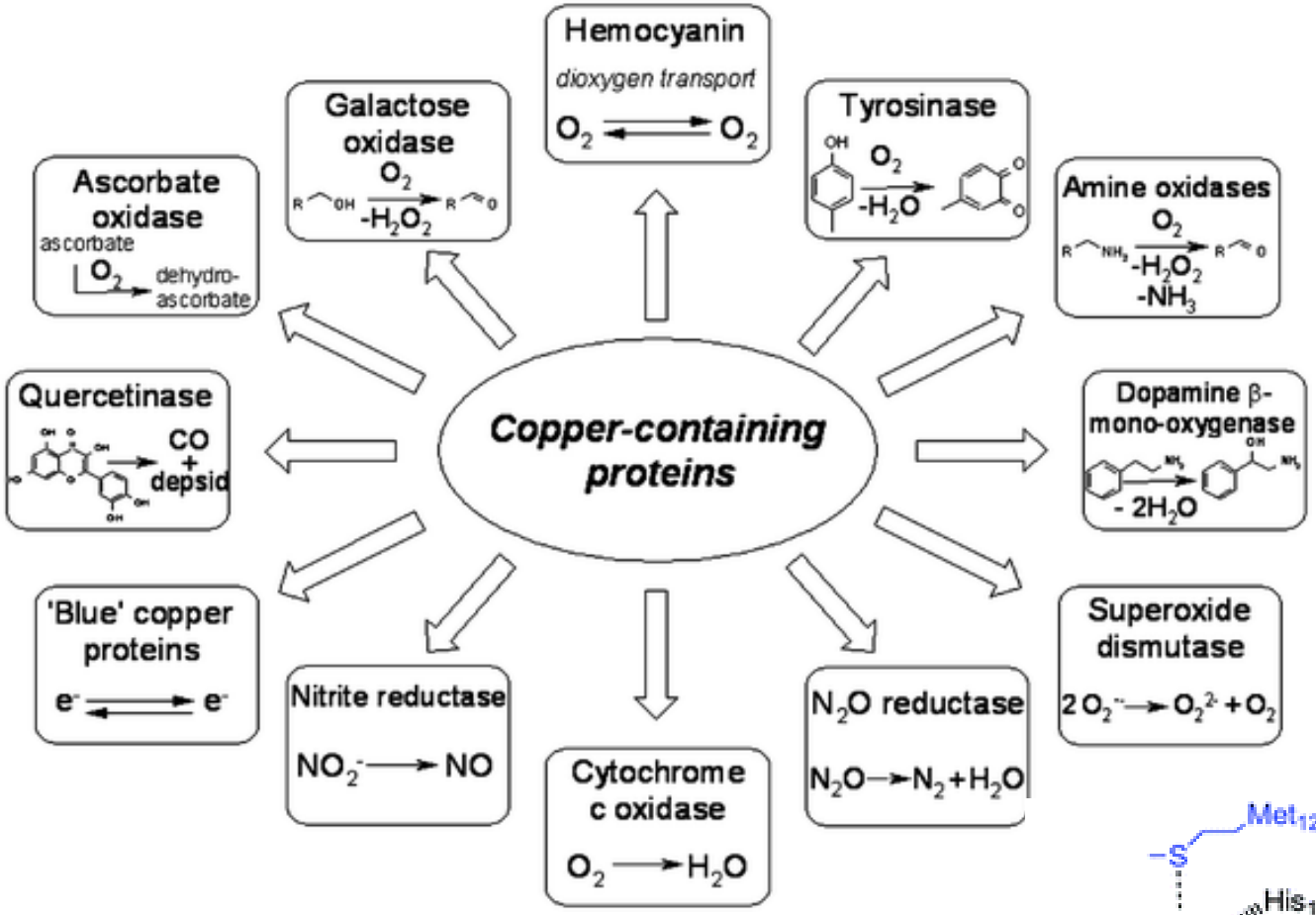


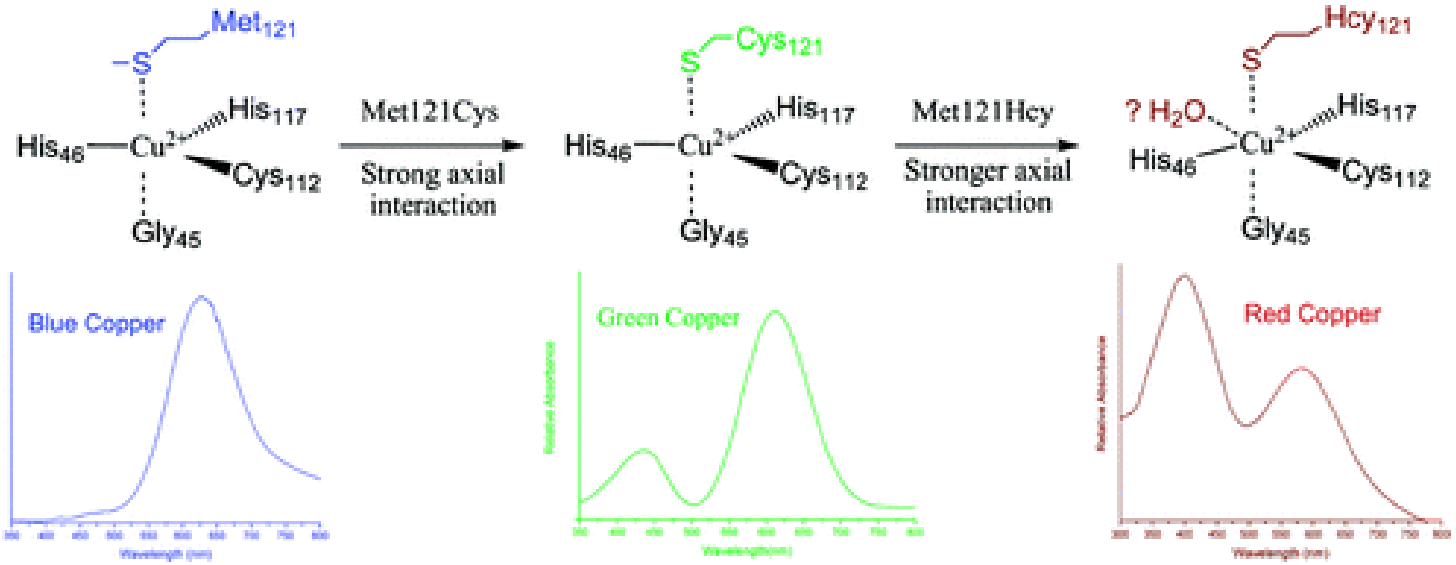


Type I or blue – intense UV-Vis absorption at 600 nm ε>3000 cm^{-1} arising from cys to Cu(II)

Plastocyanin: found in higher plants – involved in the electron transfer between PSI and PSII

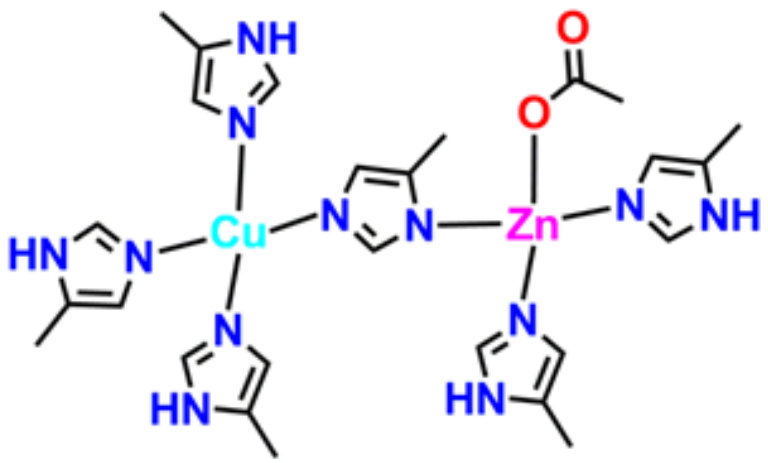
Azurin: found in denitrifying bacteria – involved in the respiratory chain where the role is to transfer electron between cyt c551 to cyct oxidase


Copper-containing proteins
Hemocyanin
dioxygen transport
$O_2 \rightleftharpoons O_2$
Galactose oxidase
O_2
$-H_2O_2$
Tyrosinase
O_2
$-H_2O$
Ascorbate oxidase
ascorbate
O_2
dehydro-ascorbate
Amine oxidases
O_2
$-H_2O_2$
$-NH_3$
Quercetinase
CO
+
depsid
Dopamine β-mono-oxygenase
$-2H_2O$
'Blue' copper proteins
$e^- \rightleftharpoons e^-$
Superoxide dismutase
$2\,O_2^{\cdot-} \rightarrow O_2^{2-} + O_2$
Nitrite reductase
$NO_2^- \rightarrow NO$
N_2O reductase
$N_2O \rightarrow N_2 + H_2O$
Cytochrome c oxidase
$O_2 \rightarrow H_2O$


Met_{121}
His_{46}
Cu^{2+}
His_{117}
Cys_{112}
Gly_{45}
Met121Cys
Strong axial interaction
Cys_{121}
Met121Hcy
Stronger axial interaction
Hcy_{121}
? H_2O
Blue Copper
Green Copper
Red Copper

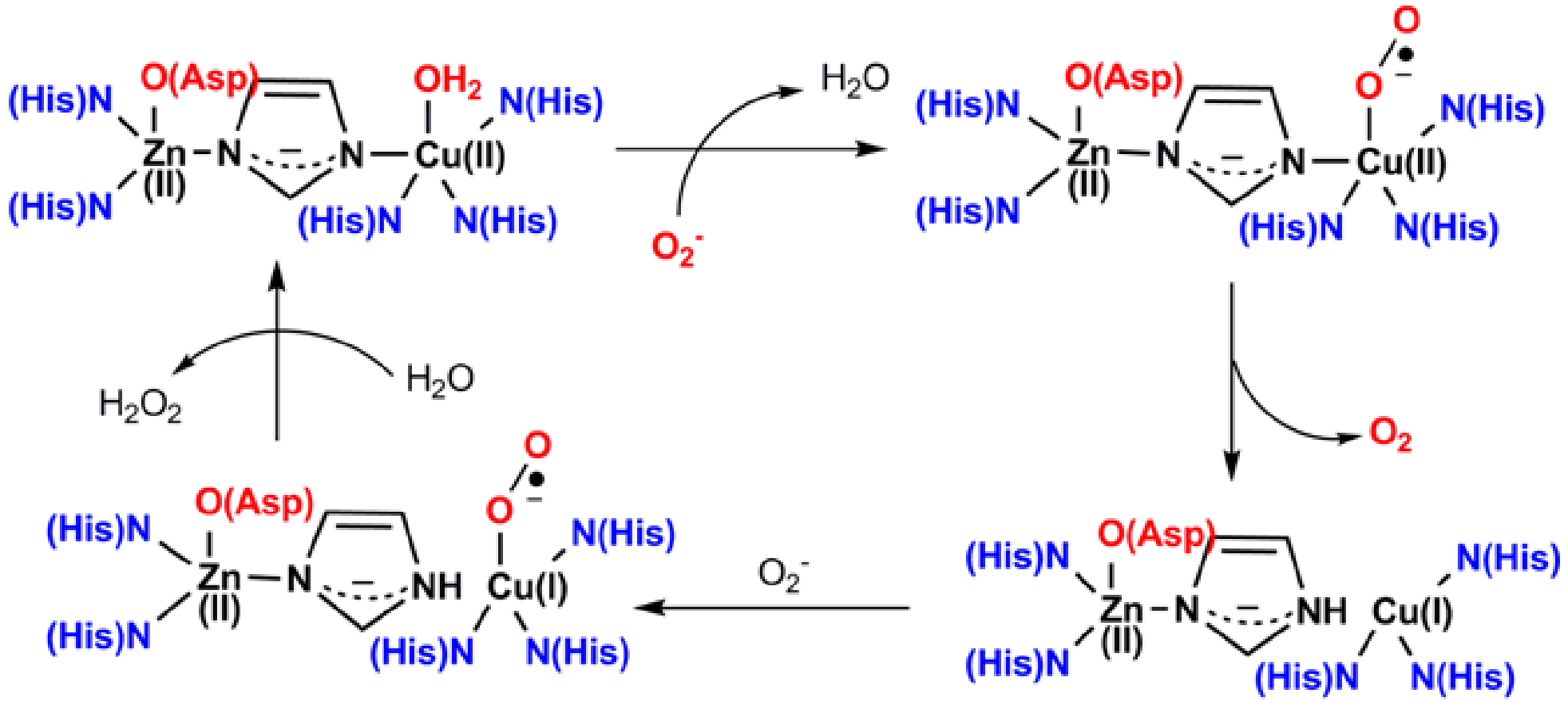
Cu-Zn Superoxide dismutase: Type 2 protein – non-blue type
Superoxide dismutase catalyses the following dismutation reaction

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$



The active site of this enzyme is dinuclear and contains copper and zinc atom. The copper center is surrounded by three histidine units, one bridging hisitine residue and one axial weakly bound solvent (water) molecule. The zinc center is tetradentate and surrounded by two histidine residues, one bridging hinsitine, and one Asp acid units. Copper and zinc centers are in +II oxidation state and about 6.0 A apart from each other.

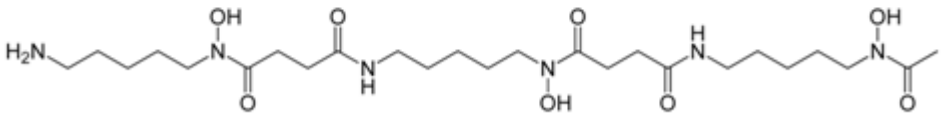
The active form of Cu/Zn-SOD enzyme contains one Zn and one Cu atoms and both are in +II oxidation state. Zn(II) center does not participates in the catalysis process, rather, its presence in the active site controls the reactivity shown by the Cu(II) center. One superoxide molecule, initially, binds to the Cu(II) centers and then hemolytic cleavage of the Cu-O bond reduces Cu(II) to Cu(I) and superoxide oxidizes to molecular oxygen. A vital structural change occurs upon transformation of Cu(II) to Cu(I). The Cu-N bond from the bridging histidine unit breaks. The second molecule of superoxide binds to Cu(I) and consequently, reduced to peroxide by Cu(I) that reoxidized to Cu(II). The labile peroxide is replaced by solvent water molecule (**Ping-pong** mechanism)



Siderophores: It means **iron carriers in greek** are small, high-affinity iron-chelating compounds secreted by microorganisms such as bacteria, fungi and grasses. Siderophores are amongst the strongest soluble Fe^{3+} binding agents known

Hydroxamate siderophores:

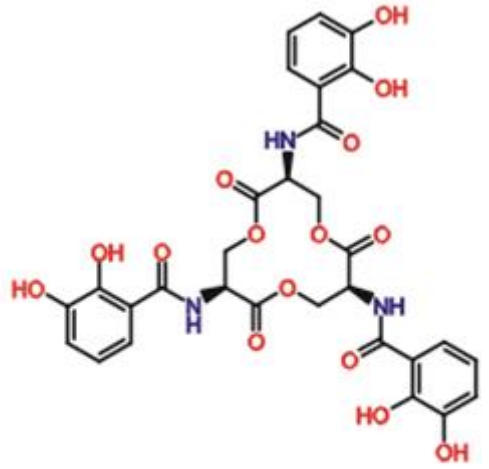
Siderophore	Organism
Ferrochrome	*Ustilago sphaerogena*
Desferrioxamine B	*Streptomyces pilosus*
(Deferoxamine)	*Streptomyces coelicolor*
Desferrioxamine E	*Streptomyces coelicolor*
Fusarinine C	*Fusarium roseum*
Ornibactin	*Burkholderia cepacia*



Desferrioxamine B

Catecholate siderophores:

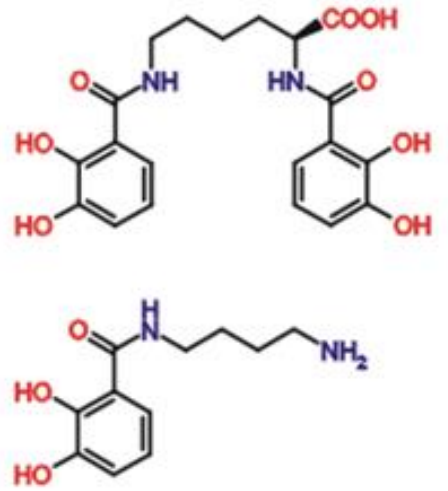
Siderophore	Organism
Enterobactin	*Escherichia coli* enteric bacteria
Bacillibactin	*Bacillus subtilis* *Bacillus anthracis*
Vibriobactin	*Vibrio cholerae*



enterobactin of *E. coli*

Mixed ligands:

Siderophore	Organism
azotobactin	*Azotobacter vinelandii*
pyoverdine	*Pseudomonas aeruginosa*
yersiniabactin	*Yersinia pestis*



azotochelin (top) and aminochelin (bottom) of *A. vinelandii*

Ionophores are lipid soluble molecules. They transport **ions across the lipid bilayers** of a cell membrane. Example: Synthetic ionophores are **crown ethers**, **cryptands**, **calixarenes**, etc.

Nickel containing Protein: Urease

- Urease are found in numerous bacteria, fungi, algae, plants and some invertebrates, as well as in soils, as a soil enzyme
- In 1926, James B. Sumner showed that urease is a protein by examining its crystallized form and awarded Nobel prize in chemistry in 1946
- They are nickel-containing metalloenzymes of high molecular weight
- It is a bis-μ-hydroxo dimeric nickel center, with an interatomic distance of ~3.5 Å octahedrally coordinated Ni(II) ions are high spin and weakly antiferromagnetically coupled
- Jack bean meal, watermelon seeds, and pea seeds have all proven useful sources of urease

Urease

Urease (urea amidohydrolase: EC 3.5.1.5) catalyzes the hydrolysis of urea to yield ammonia and carbamate. The latter compound spontaneously decomposes to yield another molecule of ammonia and carbonic acid

$$H_2N-CO-NH_2 + H_2O \xrightarrow{\text{Urease}} NH_3 + H_2N-C(O)OH$$

$$H_2N-C(O)OH + 2H_2O \rightarrow NH_3 + H_2CO_3$$

In aqueous solutions, the released carbonic acid and the two molecules of ammonia are in equilibrium with their deprotonated and protonated forms, respectively. The net effect of these reactions is an increase in pH.

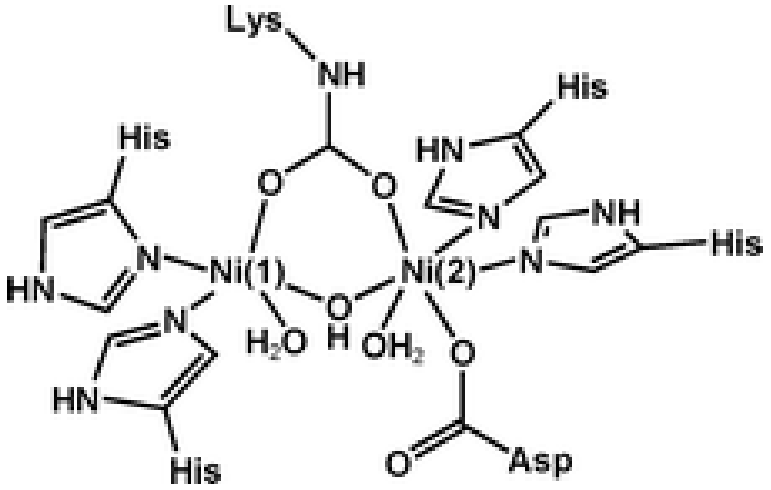
$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$

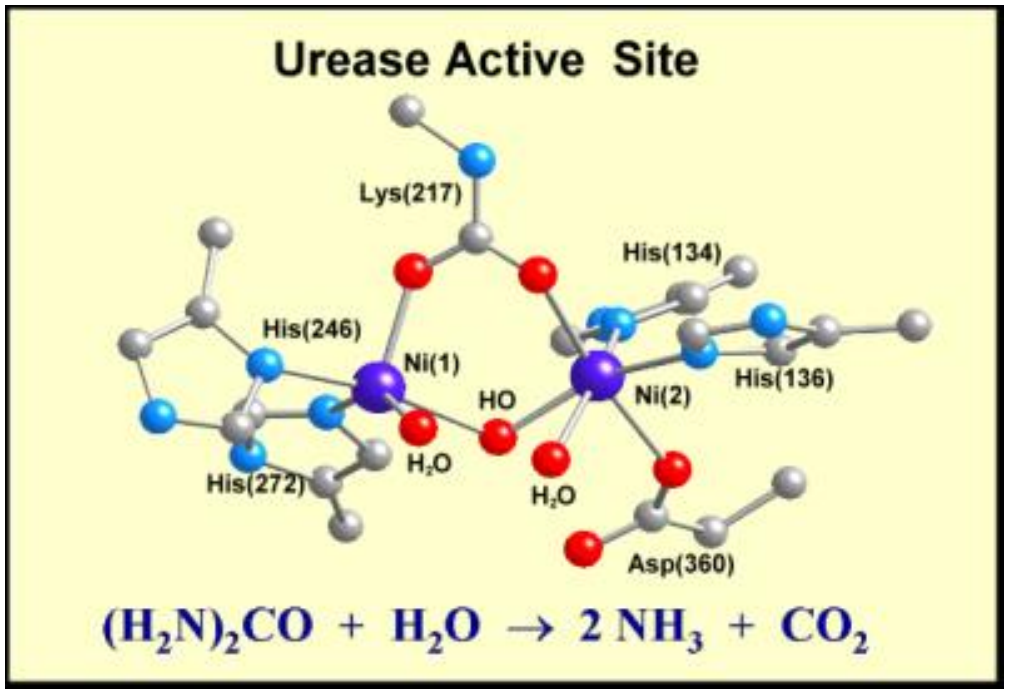
$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-$$

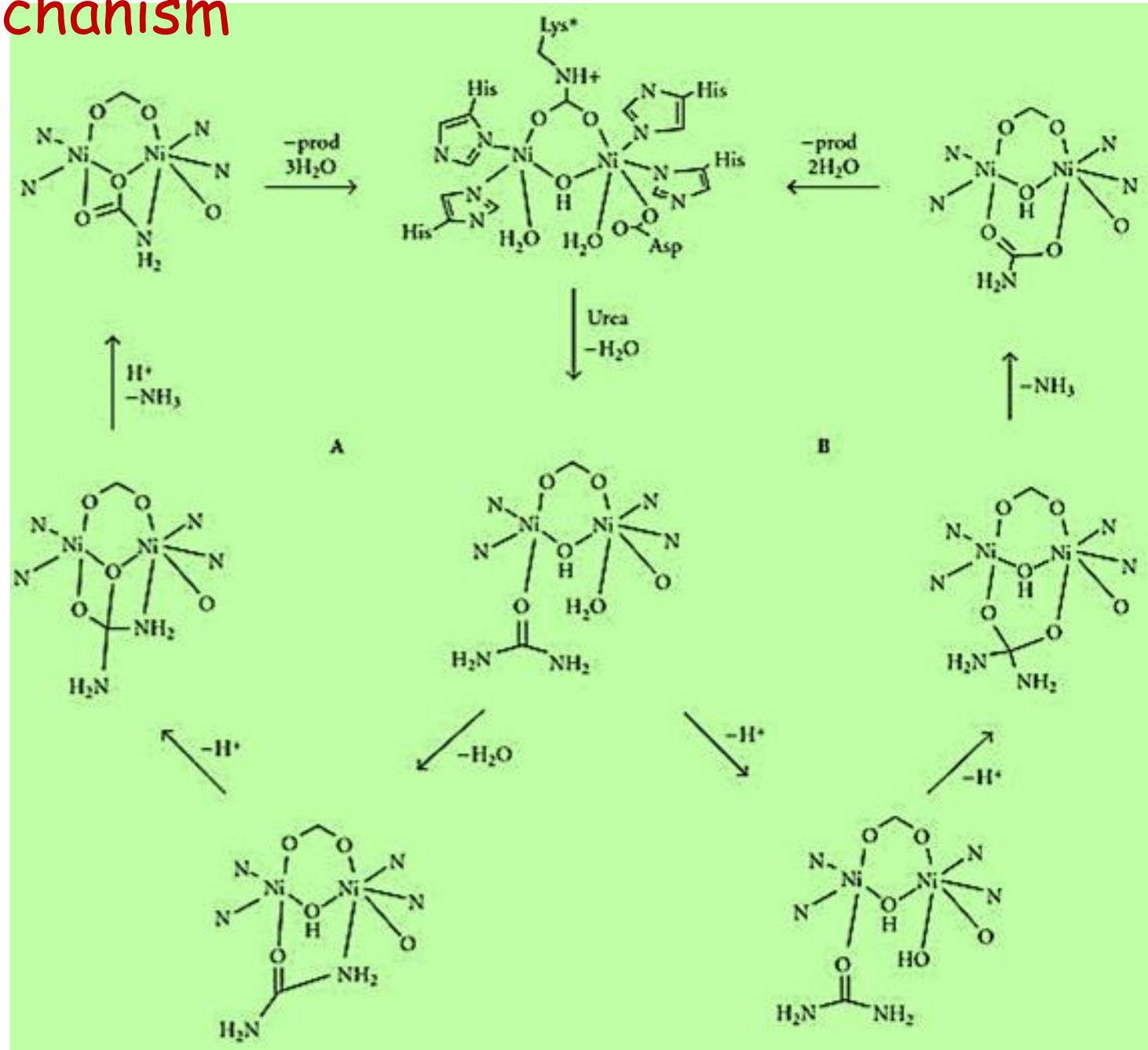
Ammonia, a preferred nitrogen source for bacteria and the product of urea hydrolysis, is assimilated into protein and other nitrogenous compounds in bacteria by a single pathway. Glutamine synthetase (EC 6.3.1.2) catalyzes the reaction:

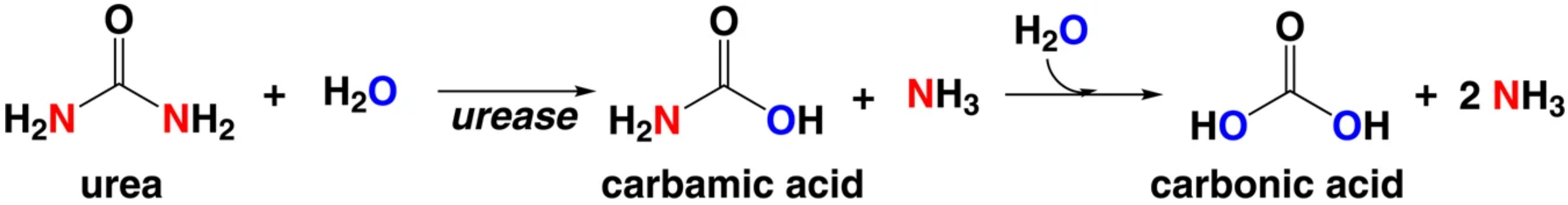
$$NH_3 + \text{glutamate} + ATP \rightarrow \text{glutamine} + ADP + P_i$$

Urease - mechanism

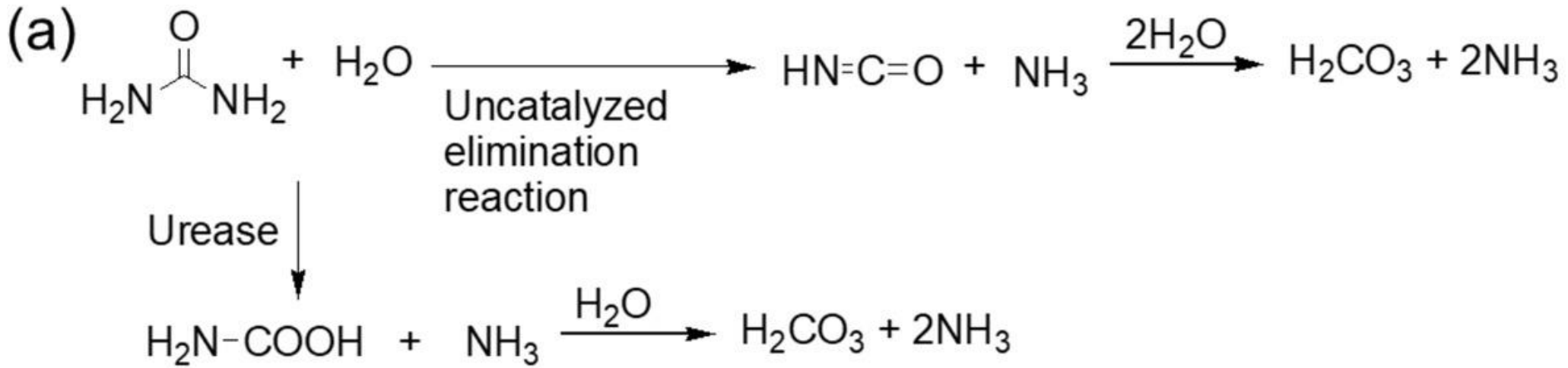


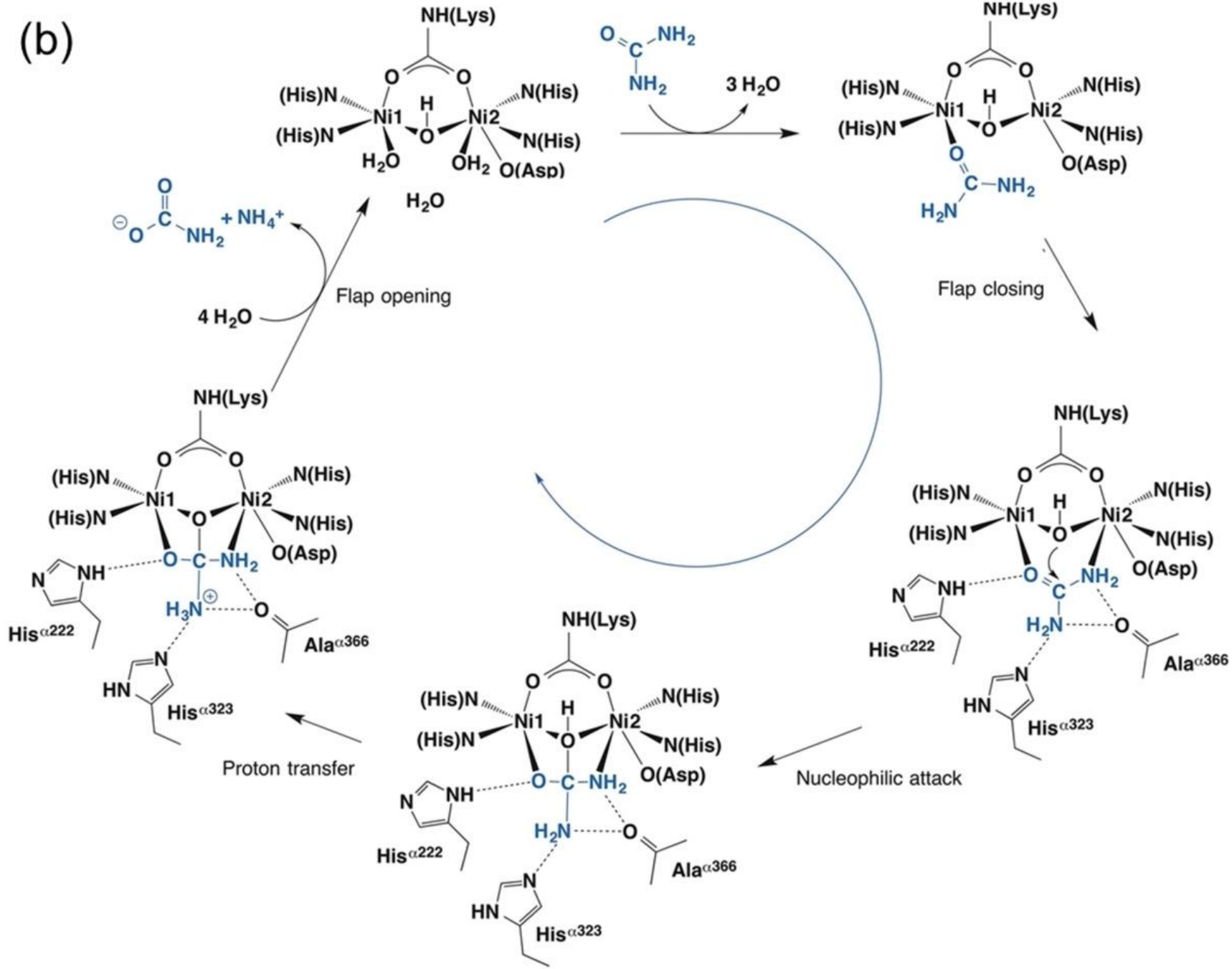


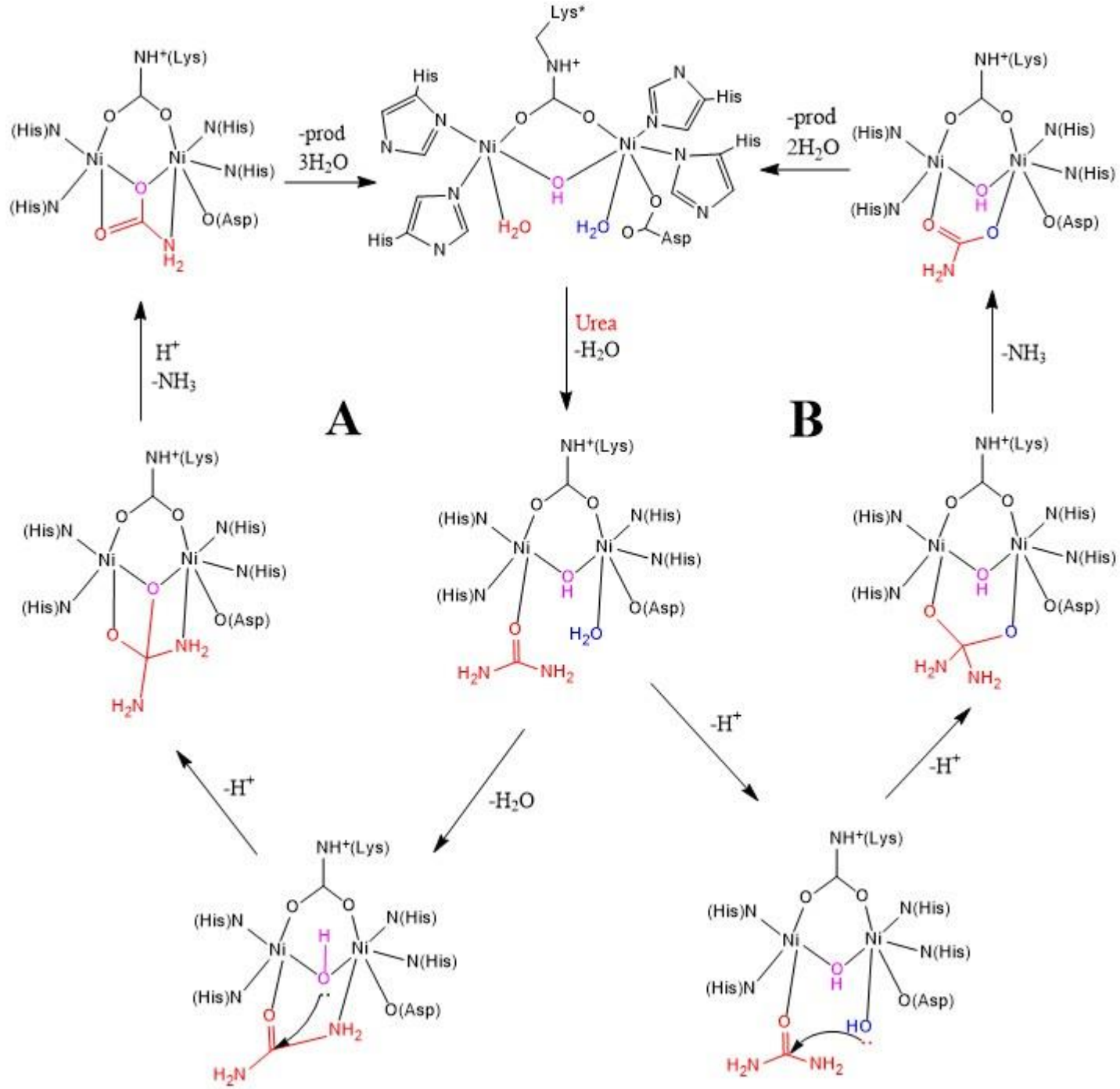




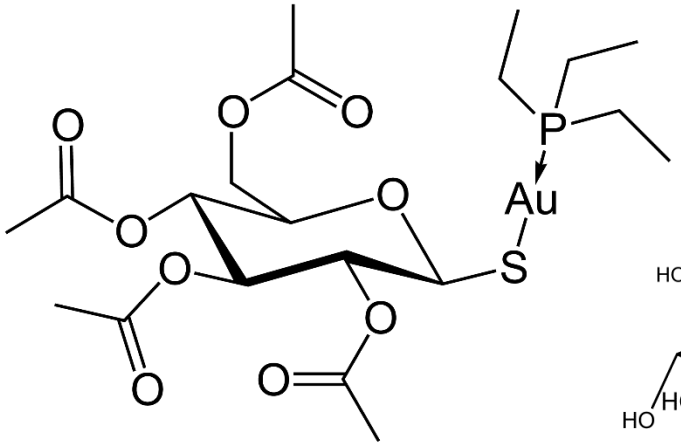
M.Wt 600 kDa, six subunits – each subunits contain two nickel (II) centres and are 3.5 A apart.



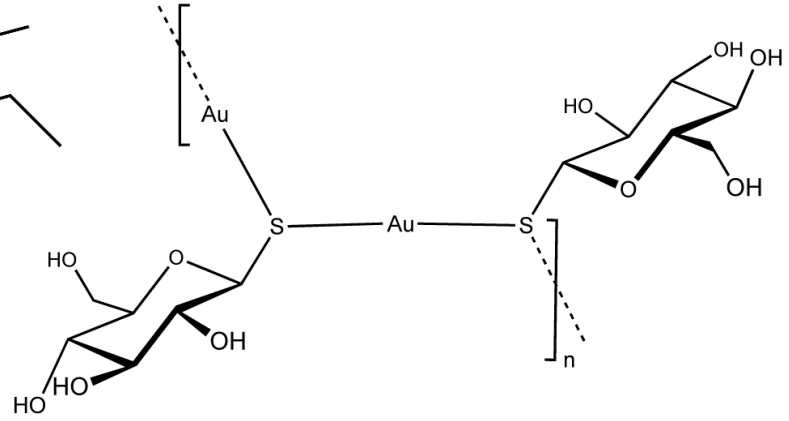

(b)
NH(Lys)
(His)N
N(His)
Ni1
Ni2
H
O
H2O
O(Asp)
3 H2O
NH2
Flap closing
Nucleophilic attack
Proton transfer
Flap opening
4 H2O
+ NH4+
His α222
His α323
Ala α366


-prod
3H2O
-prod
2H2O
Urea
-H2O
H+
-NH3
-NH3
A
B
-H+
-H2O
-H+
-H+

Gold drugs in rheumatoid arthritis

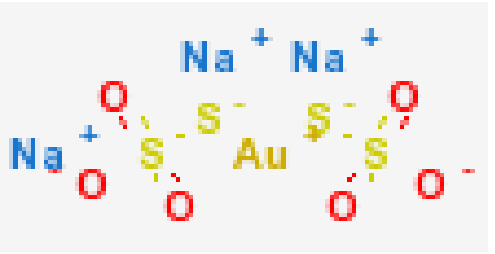


Auranofin



Solganol (gold thioglucose)

Myochrysine (gold disodium thiomalate)



sanocrysine (gold trisodium thiosulphate)

The use of gold salts in the treatment of rheumatoid arthritis is known as **chrysotherapy**. Except Auranofin other three of the above mentioned drugs administered intramuscularly while Auranofin can be administered orally.

Lithium drugs: lithium carbonate – are primarily used as a psychiatric medication for psychiatric mind disorder and schizopheranic symptoms. This includes the treatment of major depressive disorder that does not improve following the use of other antidepressants, and bipolar disorder. In these disorders, it reduces the risk of suicide. Lithium is taken by mouth.

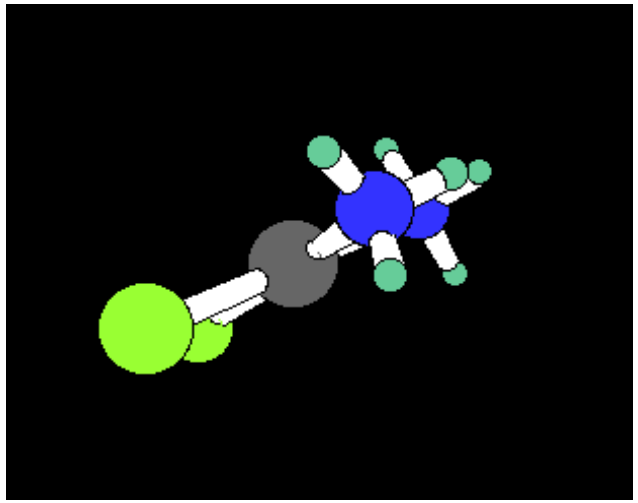
Common side effects include increased urination, shakiness of the hands, and increased thirst. Serious side effects include hypothyroidism, diabetes, and lithium toxicity. Blood level monitoring is recommended to decrease the risk of potential toxicity. If levels become too high, diarrhea, vomiting, poor coordination, sleepiness, and ringing in the ears may occur. If used during pregnancy, lithium can cause problems in the baby. It appears to be safe to use while breastfeeding. Lithium salts are classified as mood stabilizers. How lithium works is not specifically known.

Cis- Platin

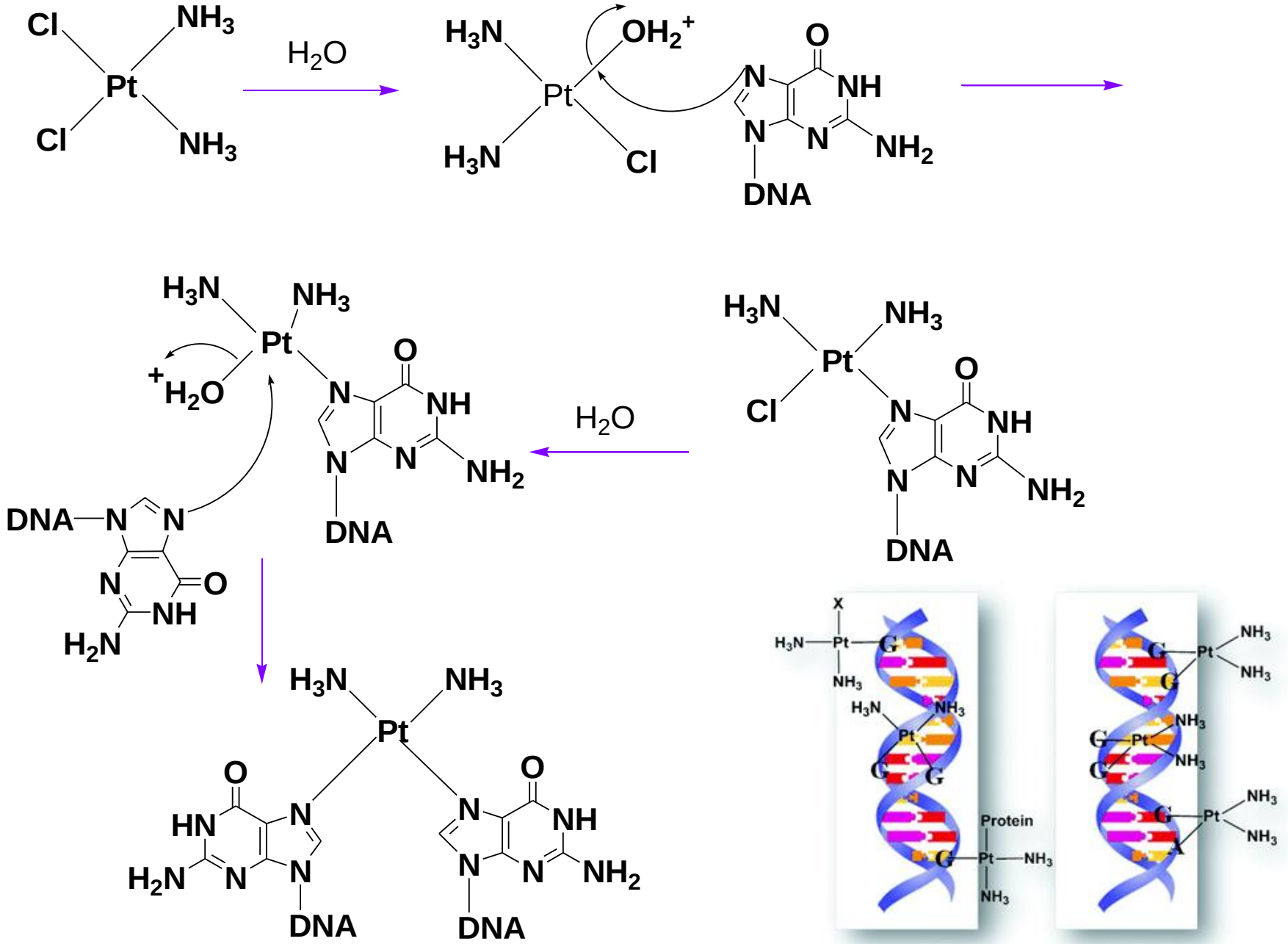
- Barnett Rosenberg in 1965 accidentally discover cis platin. It is a **chemotherapy** agent. In addition it is also used for **Auger therapy** (low energy radiation therapy).
- It is used to treat various types of **cancers** but particularly effective against **testicular cancer**.

Side effects:

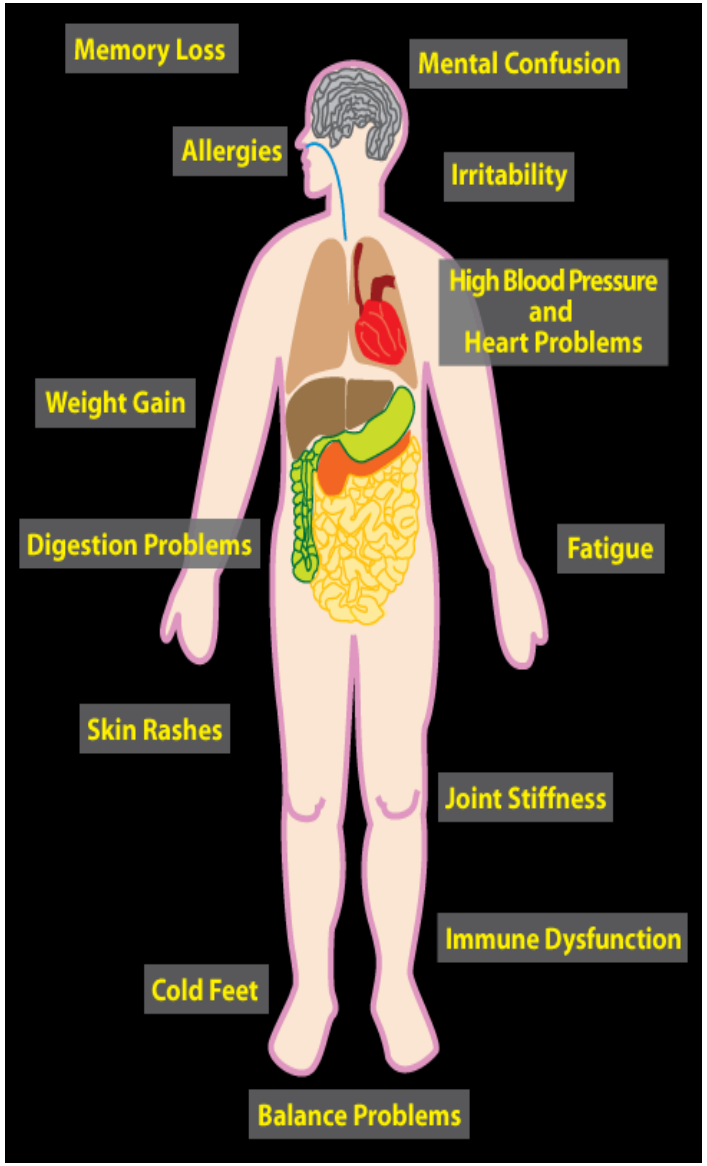
- ✓ **Nephrotoxicity** (kidney damage)
- ✓ **Neurotoxicity** (nerve damage)
- ✓ **Ototoxicity** (hearing loss)
- ✓ **Nausea** and **vomitting**



Mechanism of cisplatin :



Mercury Poisoning



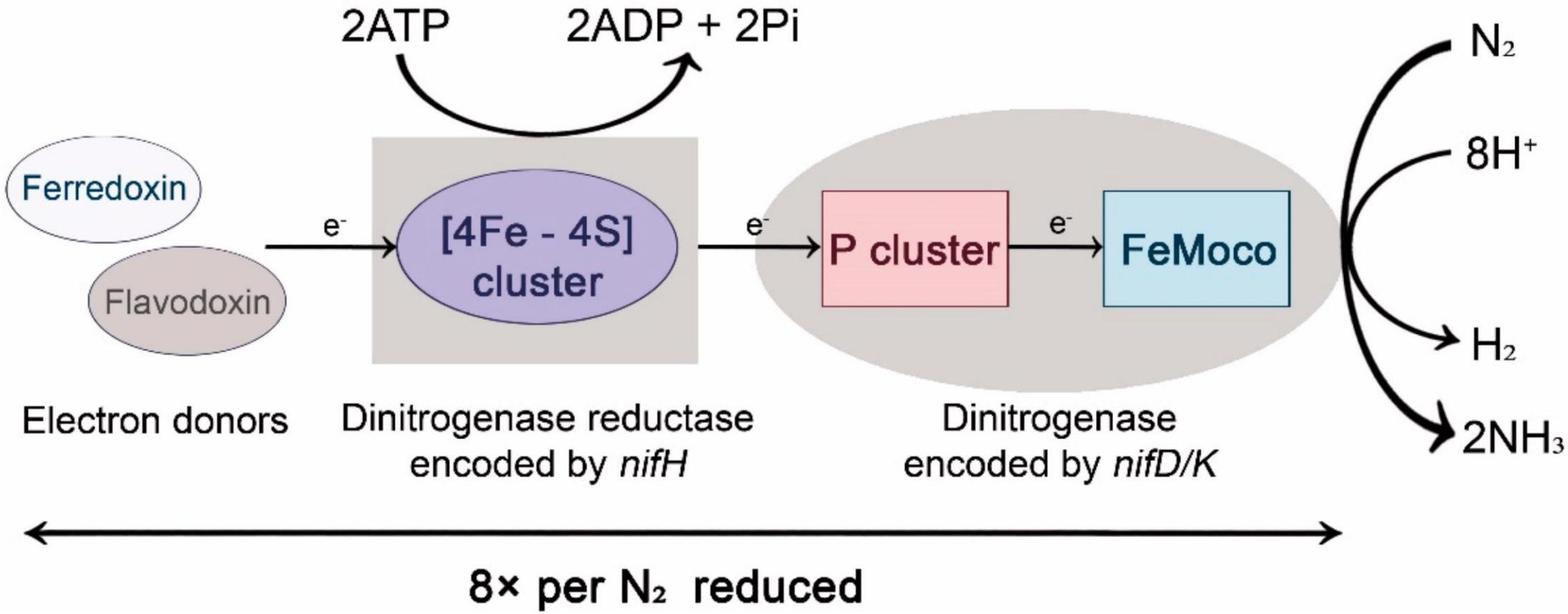
Its toxic effects can cause damage to the brain, kidneys, and lungs with symptoms including sensory impairment (vision, hearing, speech); lack of coordination; loss of hair, teeth, and nails; memory impairment; and insomnia

Nitrogenase and Hydrogenase

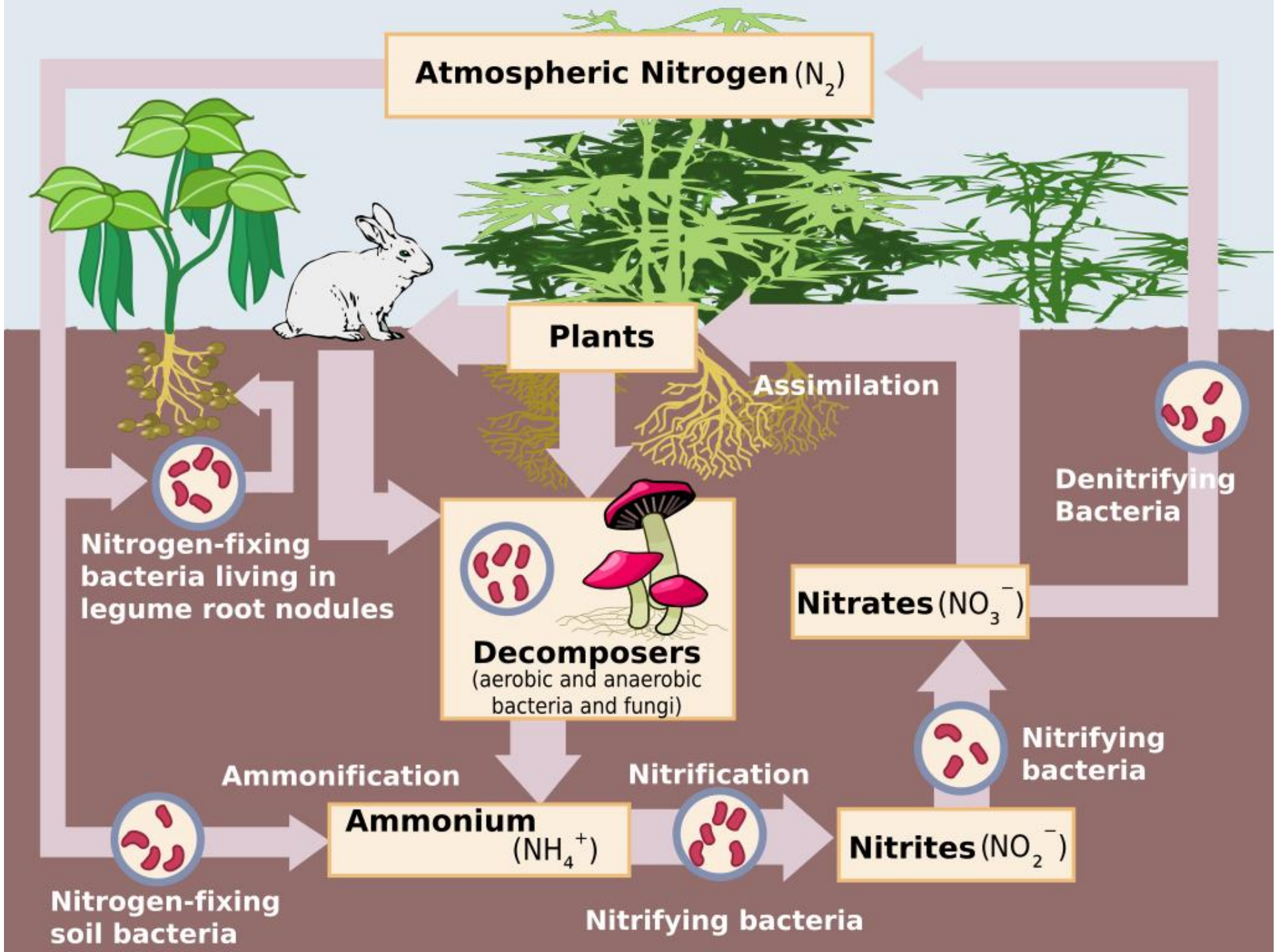
A

$$N_2 + 8H^+ + 8e^- + 16ATP \longrightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

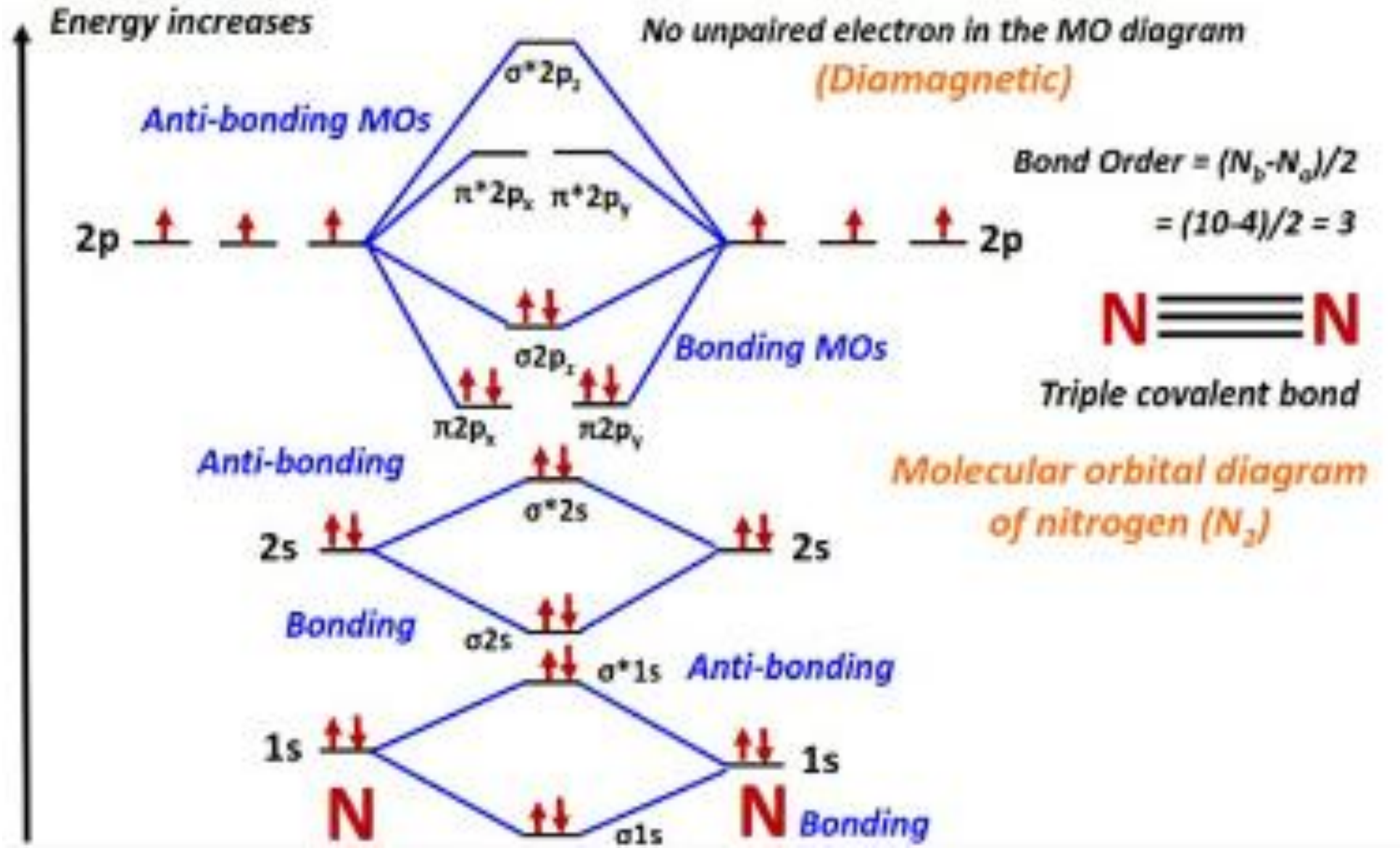
B



https://www.mdpi.com/2075-4450/13/1/84


Atmospheric Nitrogen (N_2)
Plants
Assimilation
Denitrifying Bacteria
Nitrogen-fixing bacteria living in legume root nodules
Decomposers
(aerobic and anaerobic bacteria and fungi)
Nitrates (NO_3^-)
Nitrifying bacteria
Ammonification
Nitrification
Ammonium (NH_4^+)
Nitrites (NO_2^-)
Nitrogen-fixing soil bacteria
Nitrifying bacteria

HOMO = -15.6 eV
LUMO = -7.0 eV



For Nitrogenase Reading:

1. https://pdb101.rcsb.org/motm/26; 2. https://www.rcsb.org/structure/1n2c;

3. https://www.nature.com/articles/387370a0. Structure of ADP·AlF_4^--stabilized nitrogenase complex and its implications for signal transduction

4. https://www.nature.com/articles/s42004-023-01046-6 Fe protein docking transduces conformational changes to MoFe nitrogenase active site in a nucleotide-dependent manner and references therein

5. https://doi.org/10.1021/acs.accounts.8b00112 Energy Transduction in Nitrogenase

6. Nitrogenase assembly

There are two types of bacteria that synthesize nitrogenase and are required for nitrogen fixation. These are:

•Free-living bacteria (non-symbiotic), examples include:

- Cyanobacteria (blue-green algae)
- Green sulfur bacteria
- *Azotobacter*

•Mutualistic bacteria (symbiotic), examples include:

- *Rhizobium*, associated with leguminous plants
- *Spirillum*, associated with cereal grasses
- *Frankia*

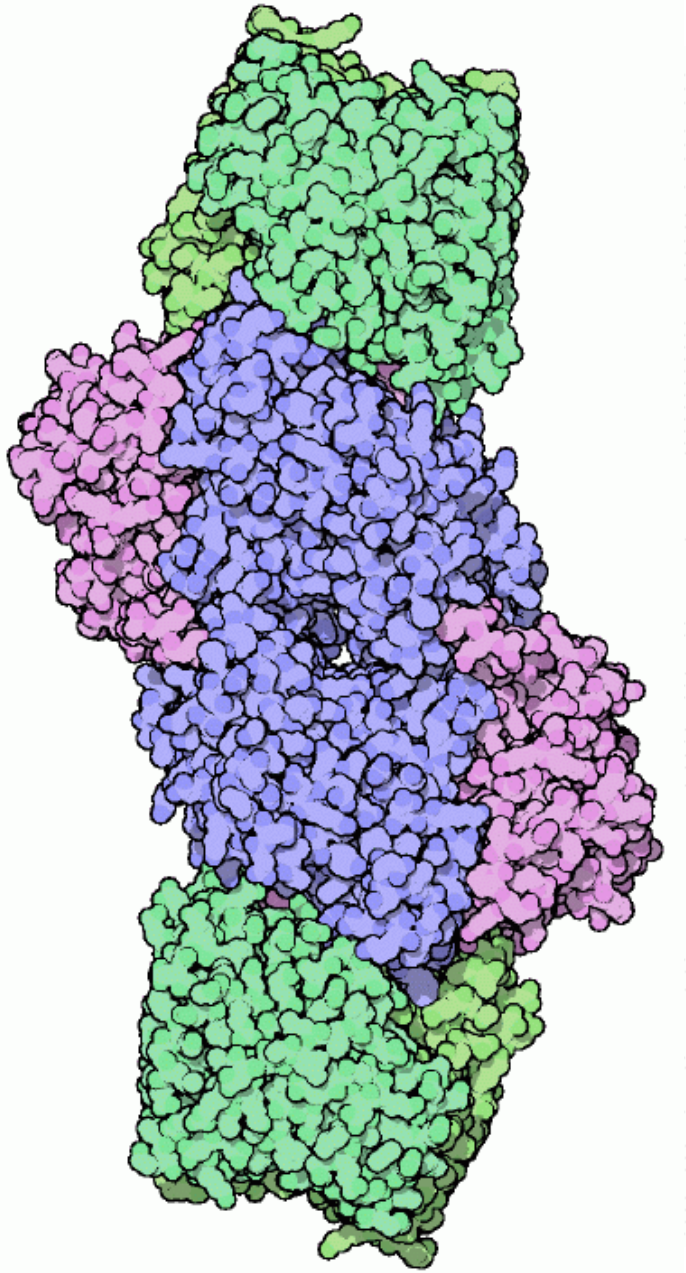
Table 9.2. Properties of nitrogenase proteins

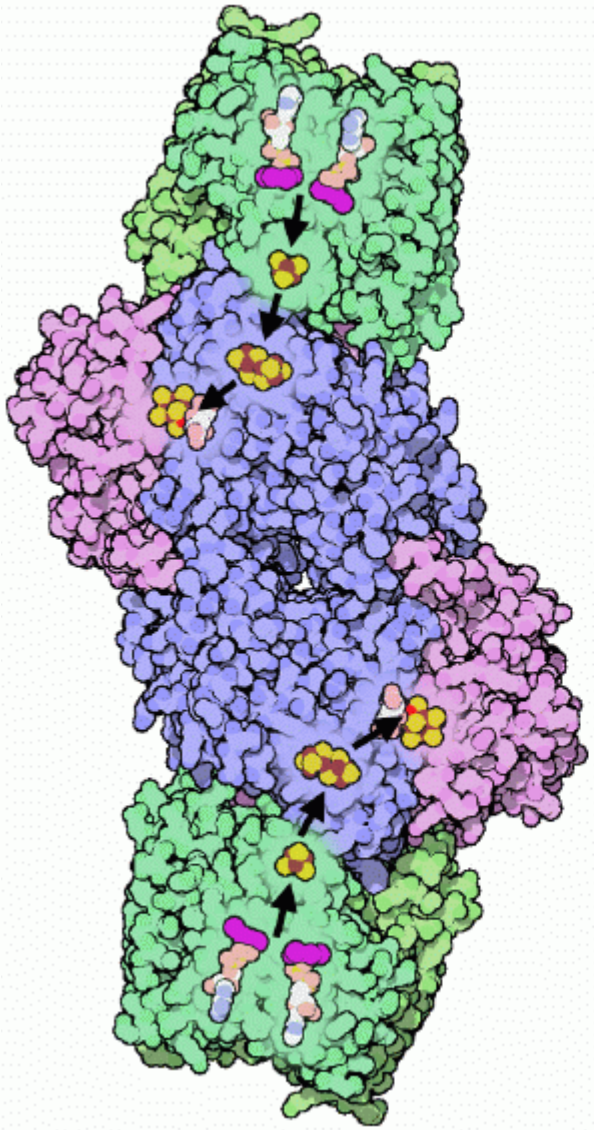
Sl. No.	Property	Fe-protein	Mo-Fe protein
1	Colour	Yellow	Brown
2	Molecular weight	65,000	2,00,000-2,22,000
3	Number of metal atoms per molecule	4 Fe atoms	2 Mo atoms* 24 Fe atoms**
4	Number of sulphur atoms	4	24
5	Structure	Monomer	Tetramer
6	Sensitivity to oxygen	Irreversibly sensitive to even by the brief exposure to air.	Reversibly sensitive to oxyge It can withstand for brief exposure to oxygen.
7	Specific activity† (N_2 reduced/min. mg/protein)	460-530 n moles	350 n moles

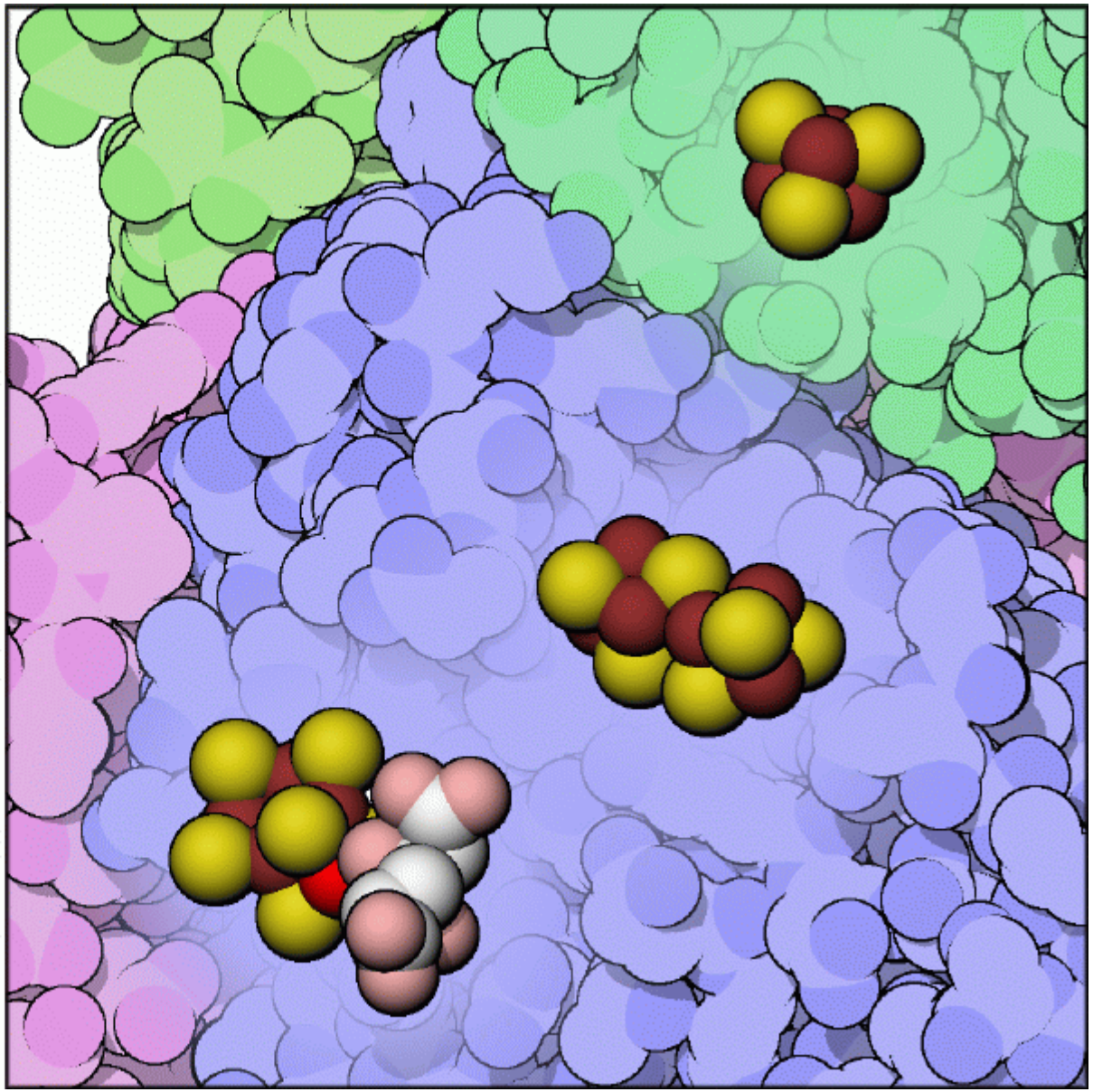
* 1.3-1.8 Mo atoms reported.

** 18-35 Fe atoms reported.

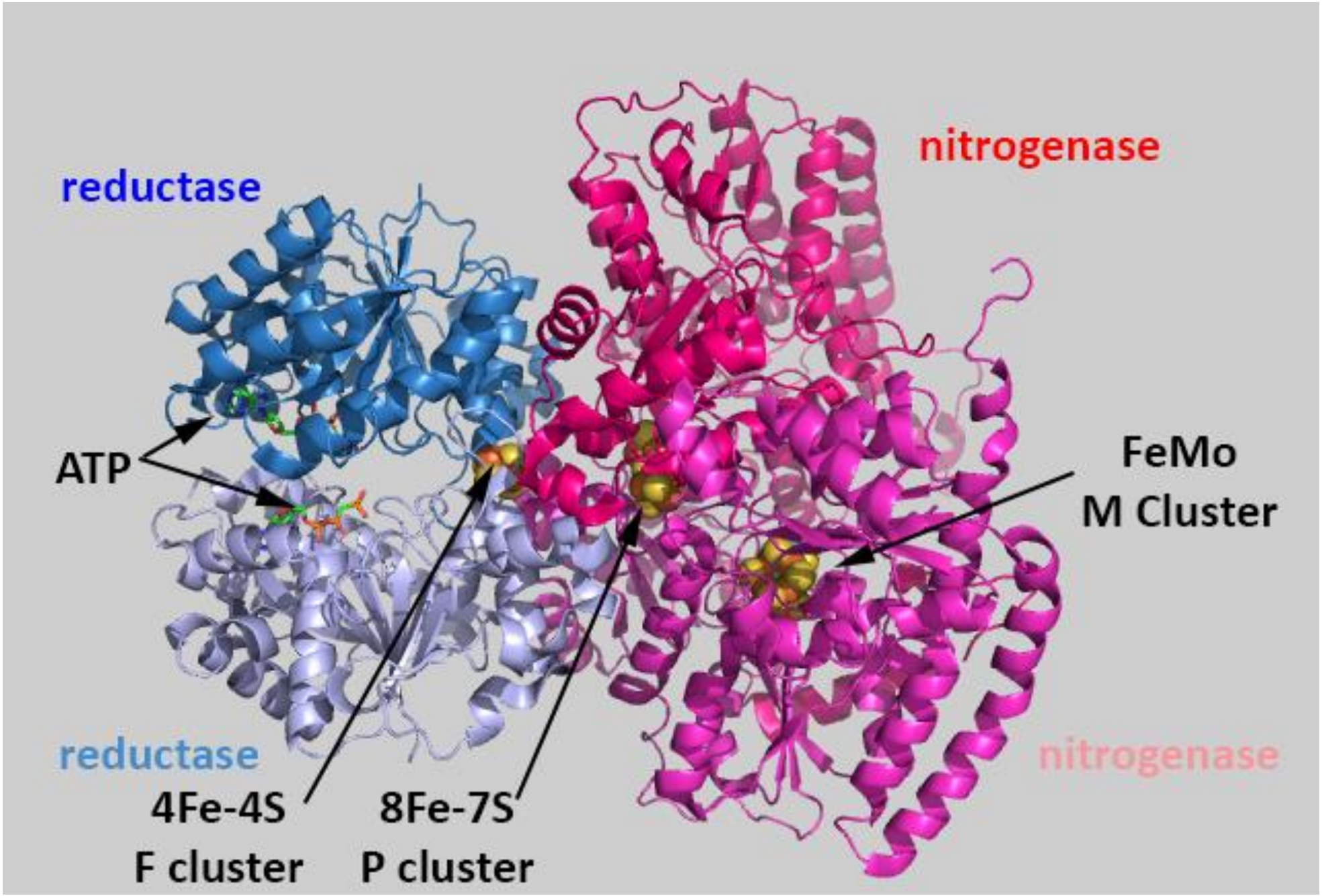
† The activity of N_2ase is mainly due to the combined effect contributed by both proteins.

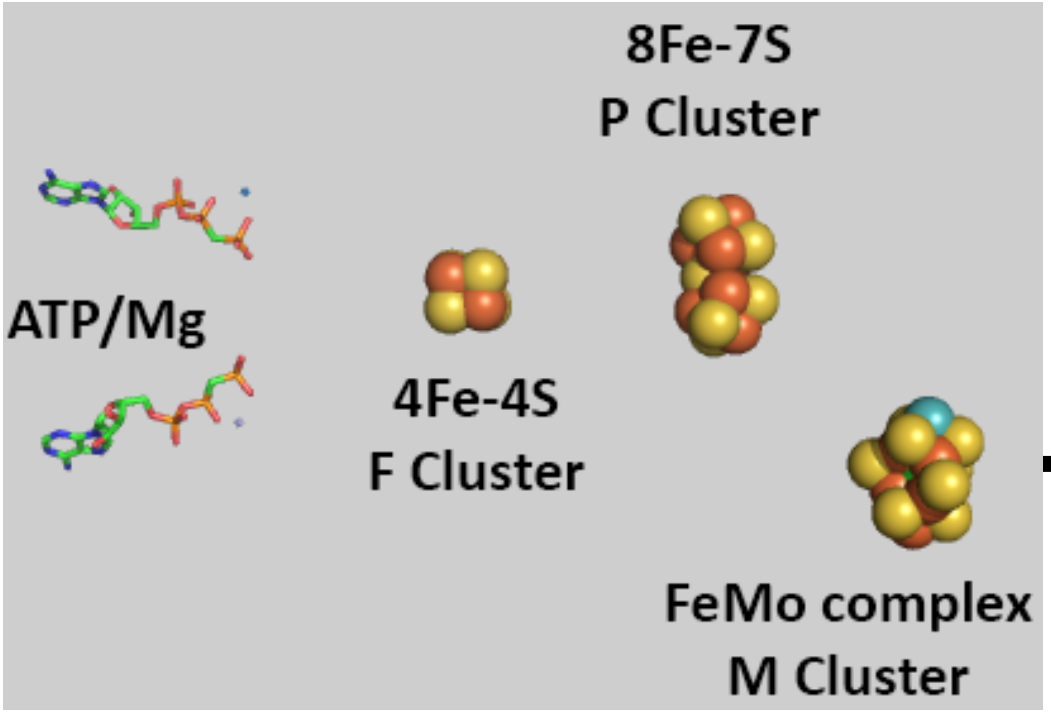




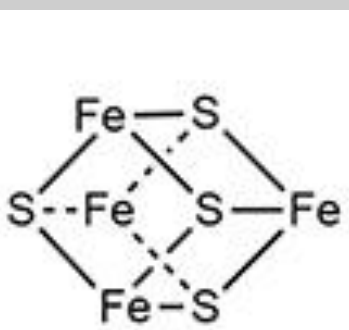


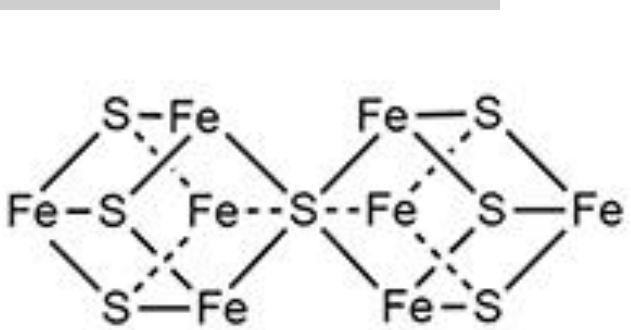
https://pdb101.rcsb.org/motm/26

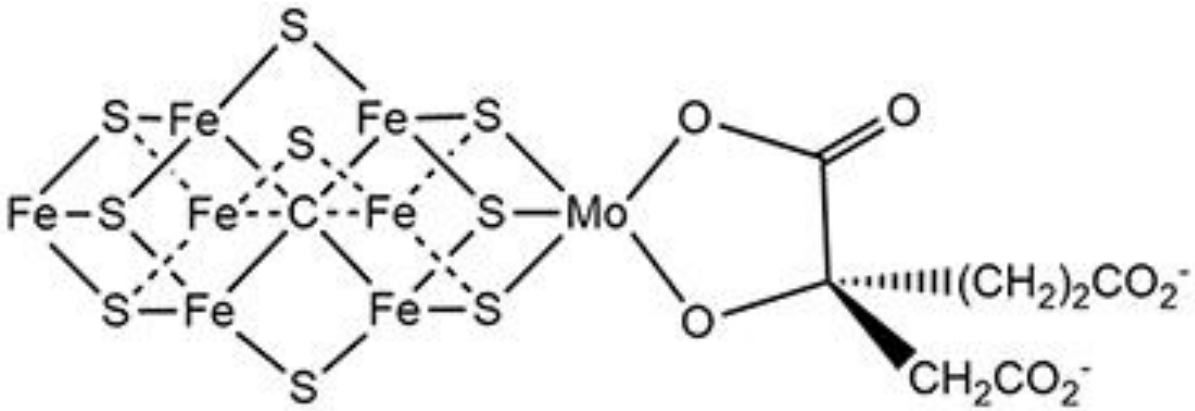

reductase
nitrogenase
ATP
FeMo
M Cluster
reductase
nitrogenase
4Fe-4S
F cluster
8Fe-7S
P cluster

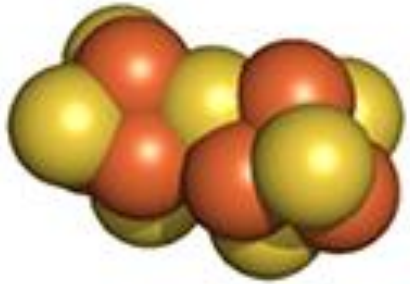


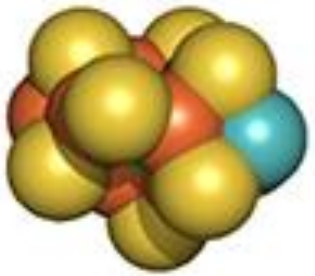
→ 2 Mo, 30 Fe and Sulphide, 40 cys units

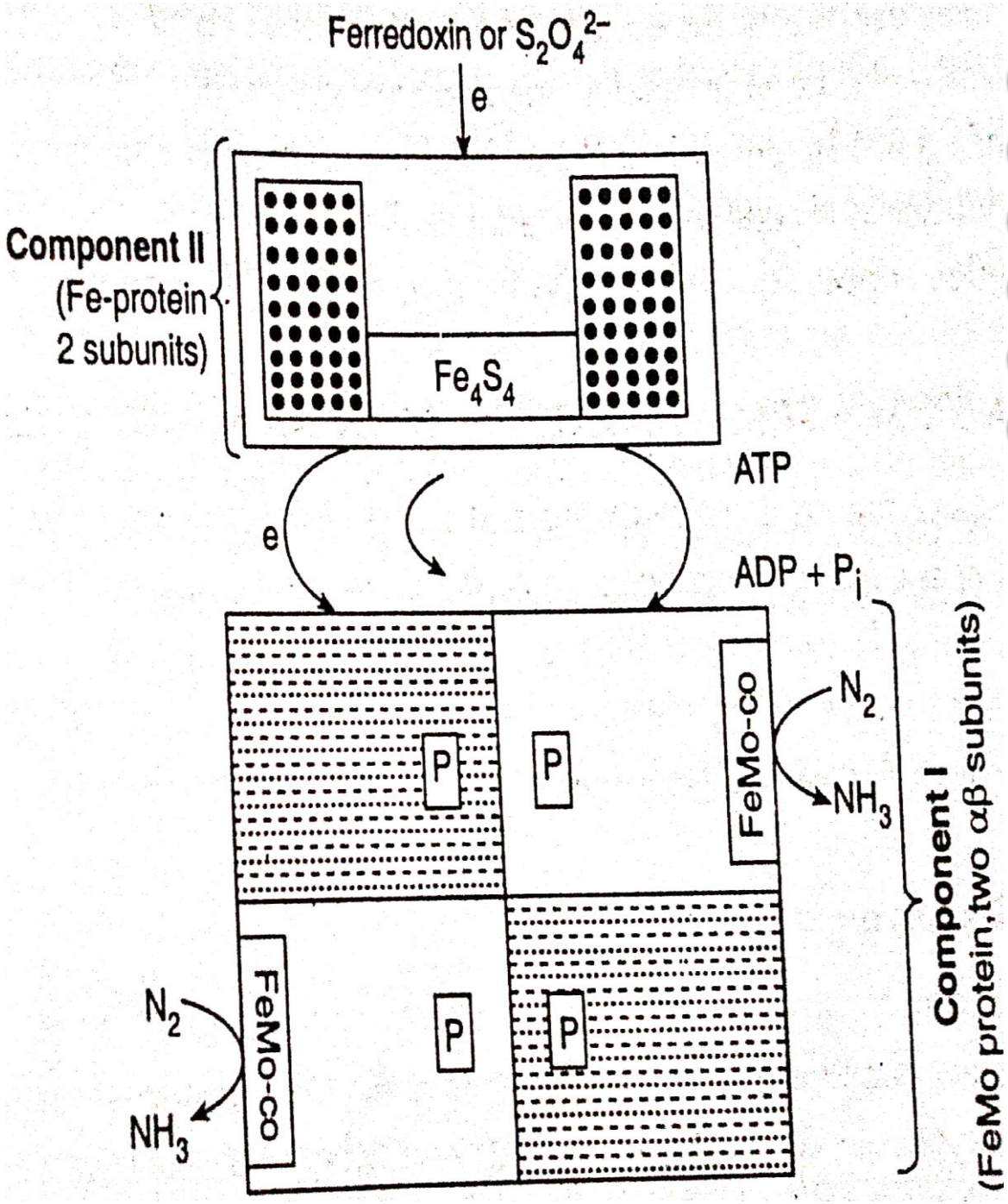


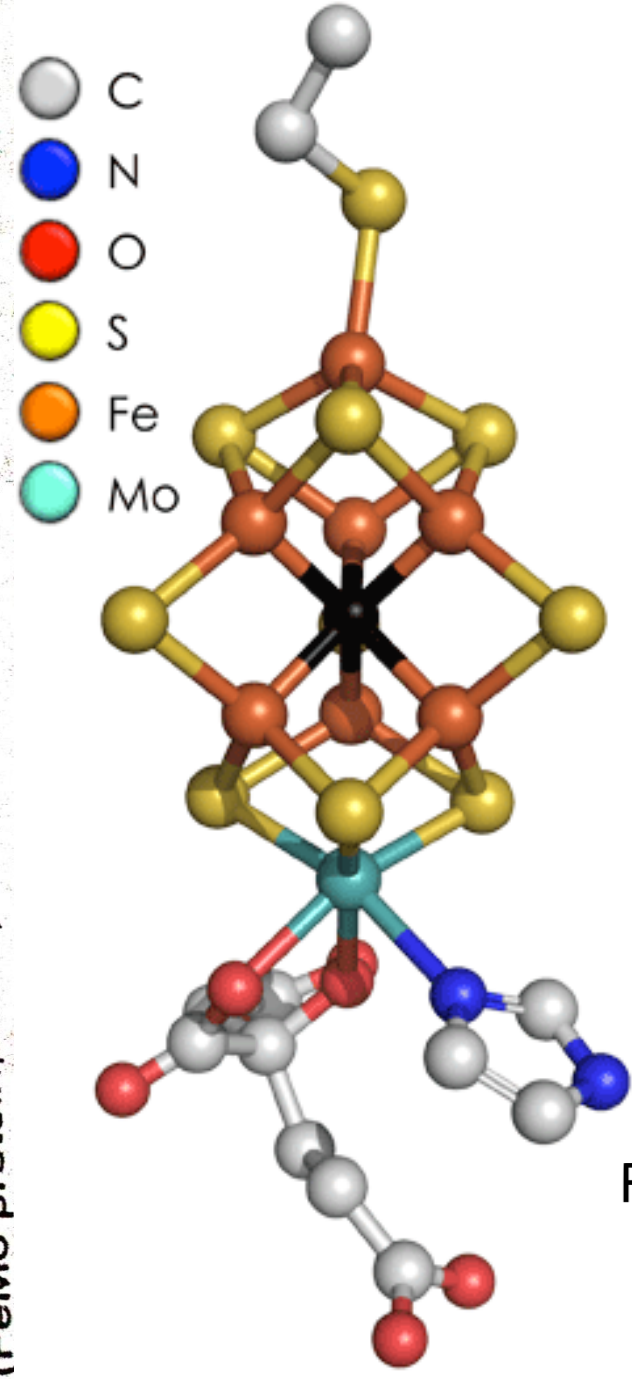


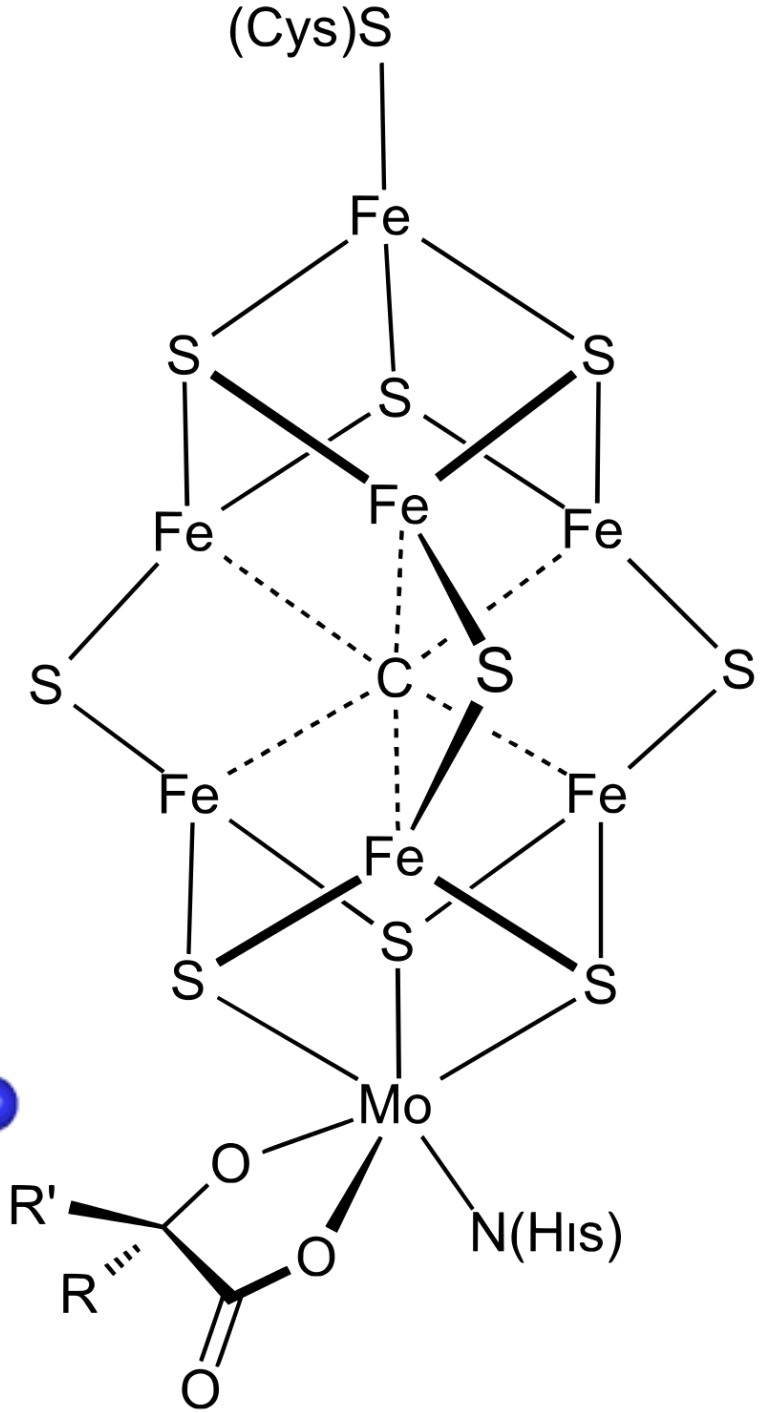




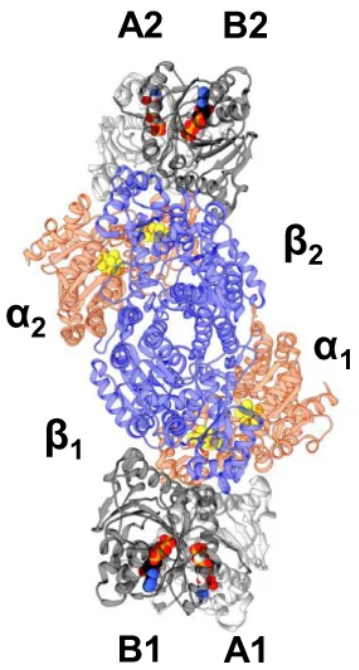


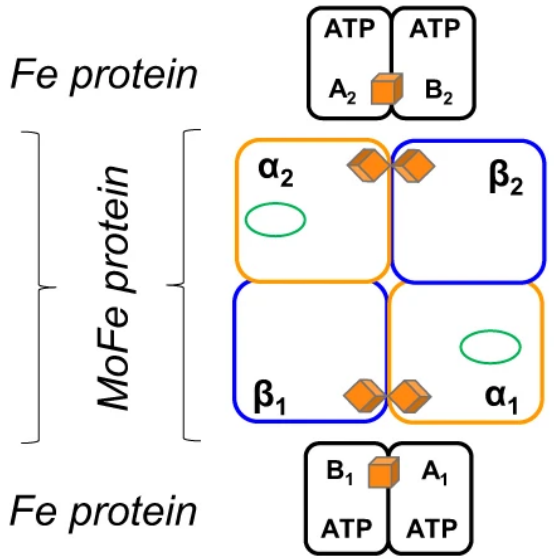

Ferredoxin or $S_2O_4^{2-}$
e
Component II
(Fe-protein
2 subunits)
Fe_4S_4
ATP
e
ADP + P_i
P
P
FeMo-co
N_2
NH_3
P
P
FeMo-co
N_2
NH_3
Component I
(FeMo protein, two αβ subunits)

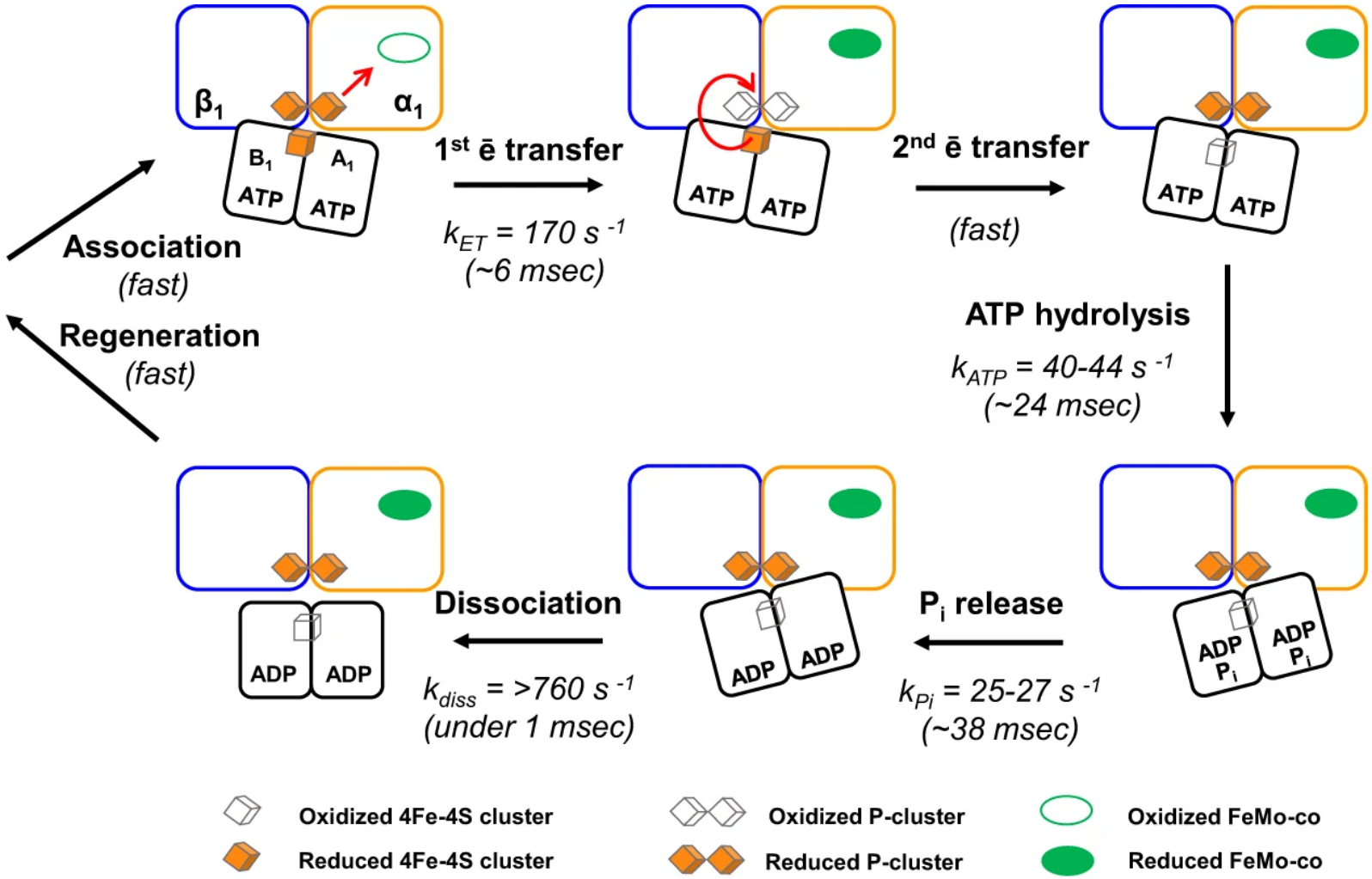

C
N
O
S
Fe
Mo


(Cys)S
Fe
S
S
S
Fe
Fe
Fe
S
C
S
S
Fe
Fe
Fe
S
S
S
Mo
O
R'
R
O
O
N(His)

$$N_2 + 8e^- + 8H^+ + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i$$







https://www.nature.com/articles/s42004-023-01046-6

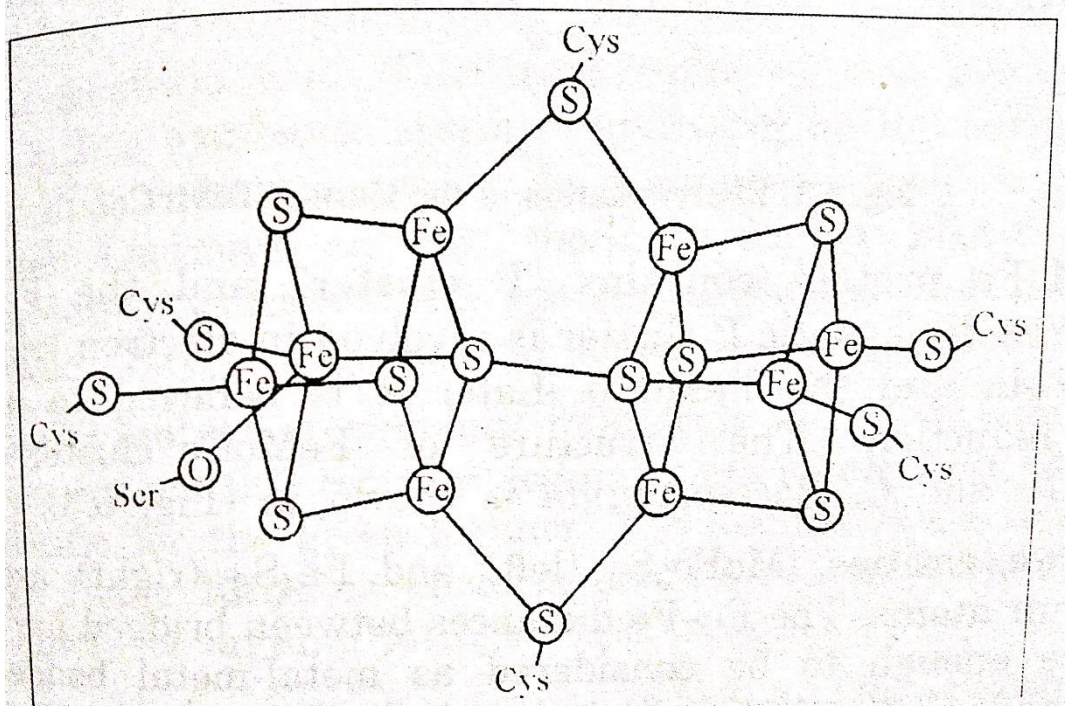
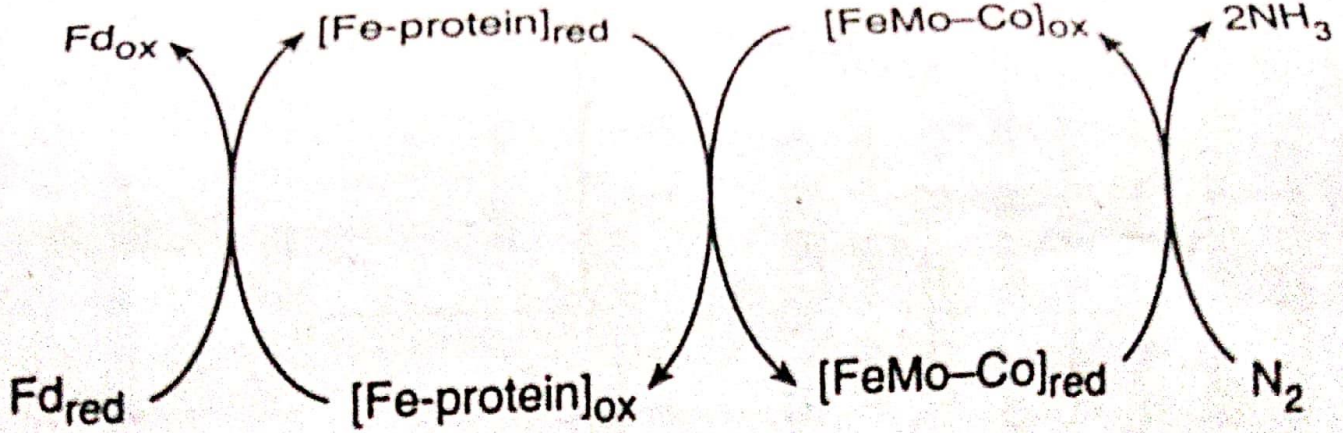


Fig. 6.2. Model for the structure of the P cluster in nitrogenase.



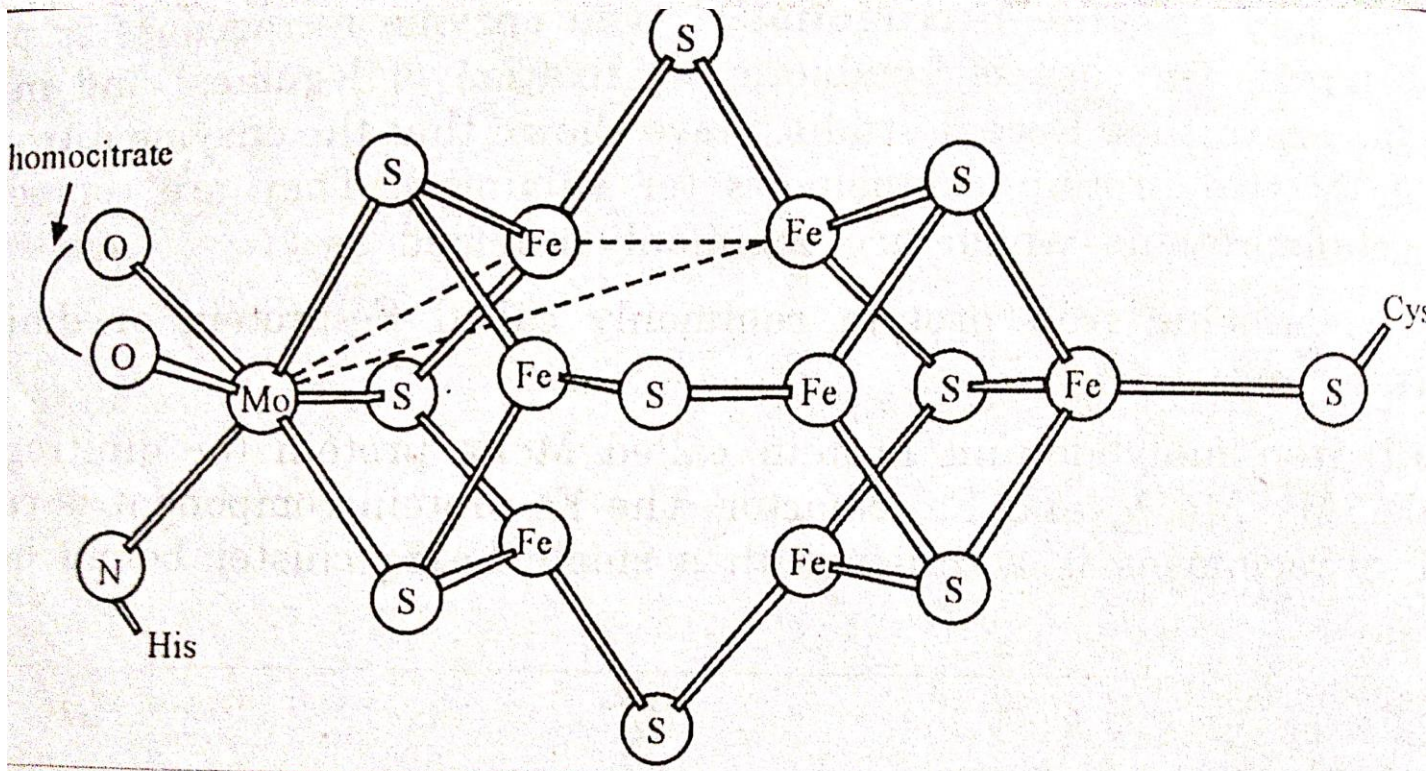


Fig. 6.3. Representation of the Core of FeMoCo.

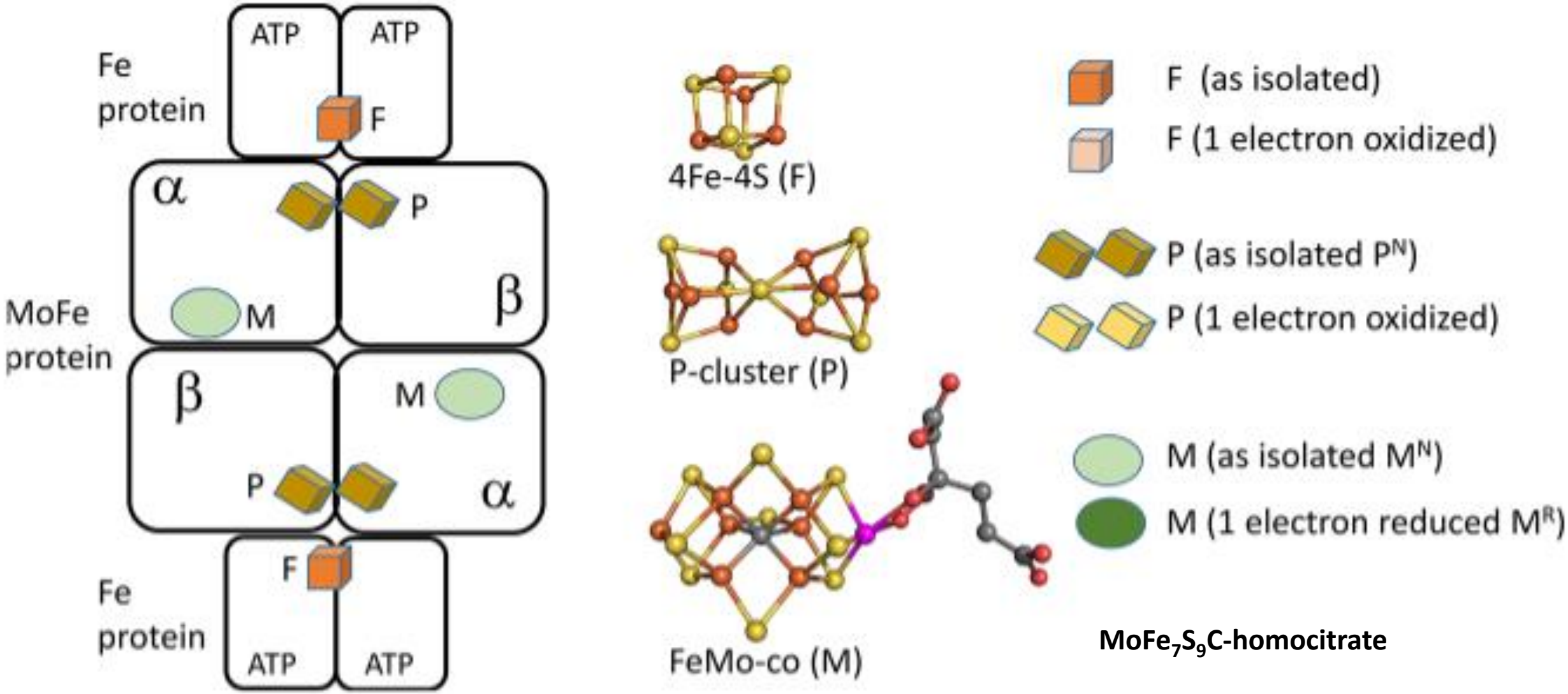
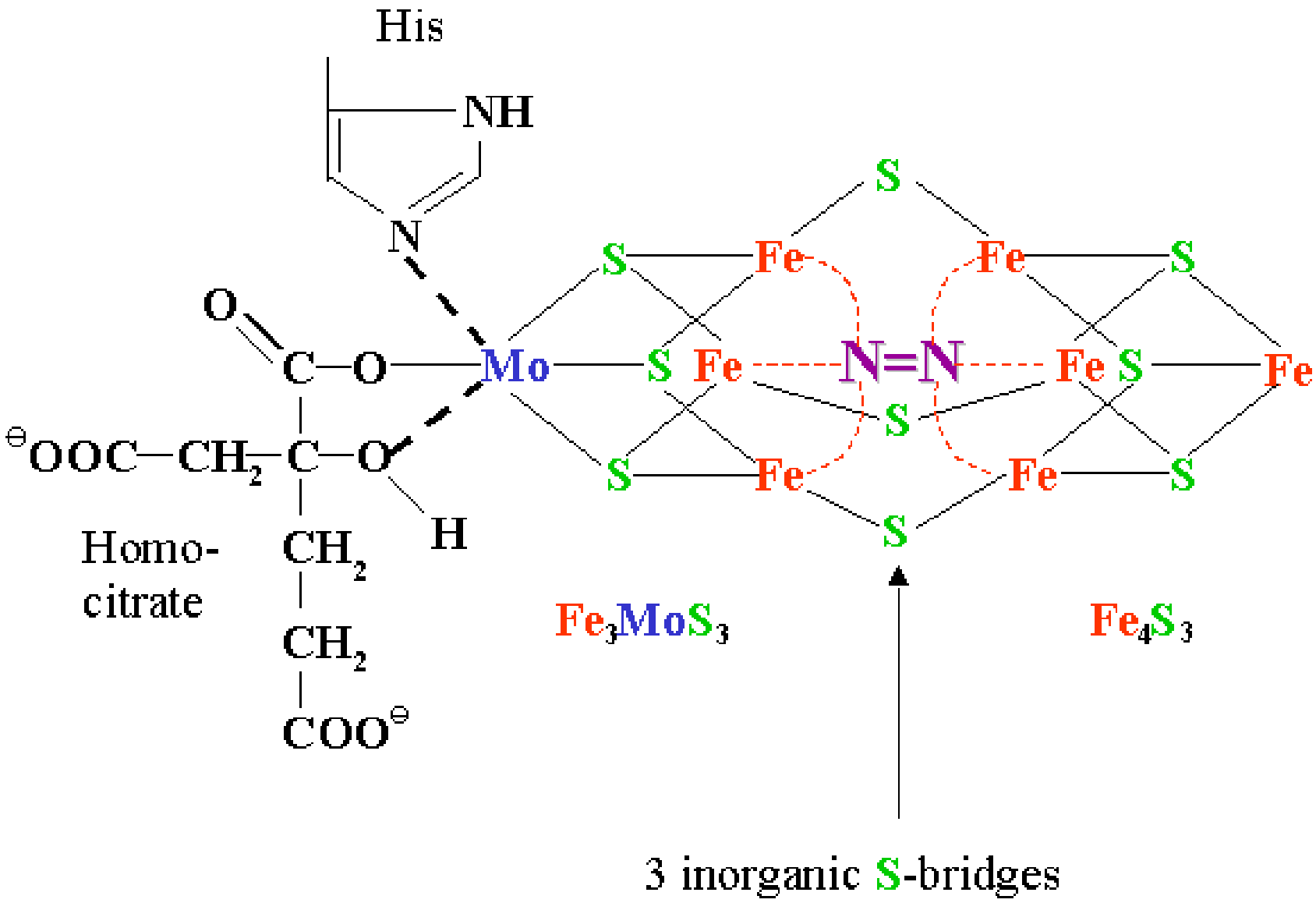


Figure 1. Diagram of the nitrogenase proteins and the metal-containing cofactors. (left) Schematic representation of the Fe protein component and the MoFe protein component with metal cofactors. (center) Structures of the 4Fe–4S cluster (F), the P-cluster (P), and the FeMo cofactor (M). Structures are from PDB entry 4WZA. (right) Legend showing representations of the metal clusters and oxidation states.

https://www.nature.com/articles/s42004-023-01046-6



https://www.uky.edu/~dhild/22/lect22.html

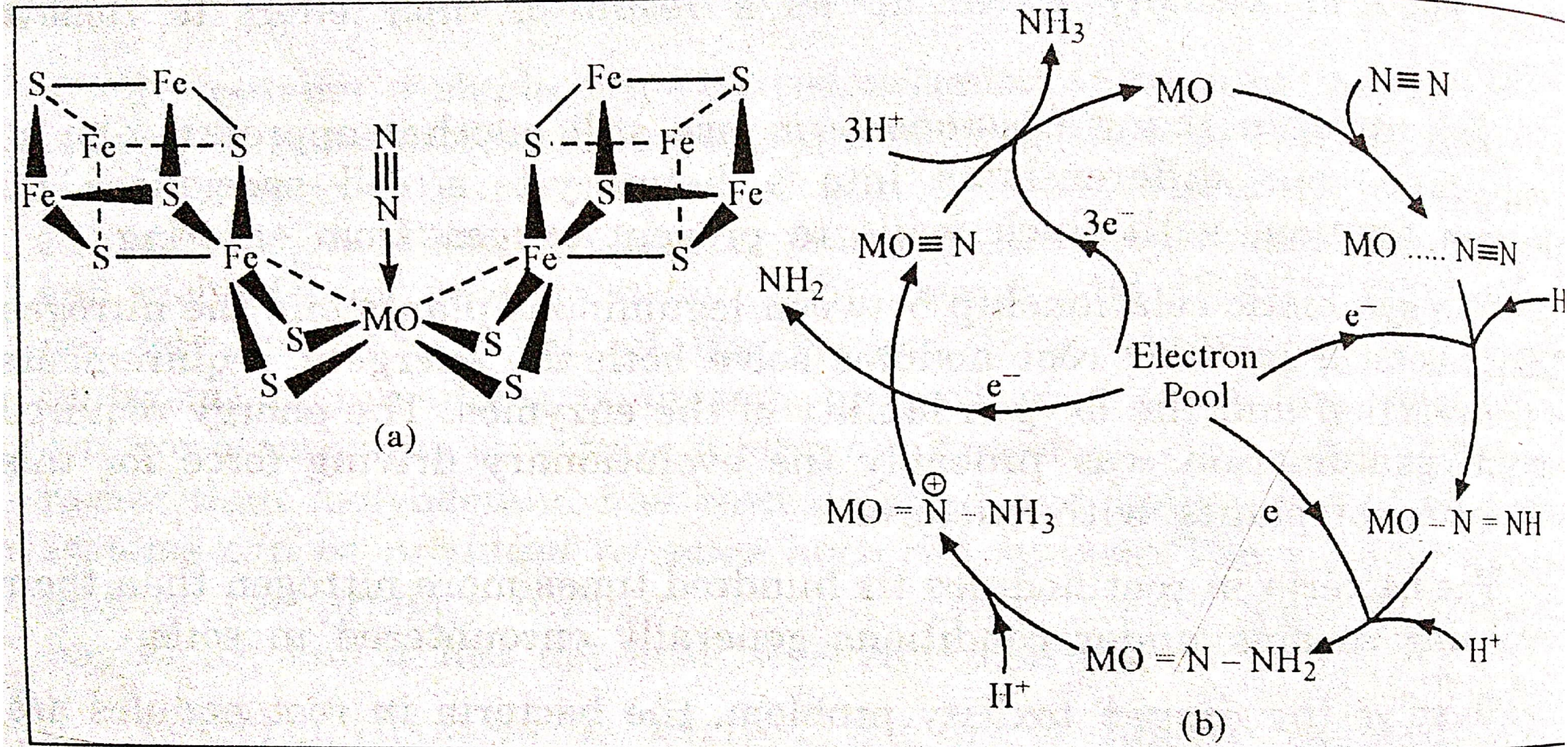
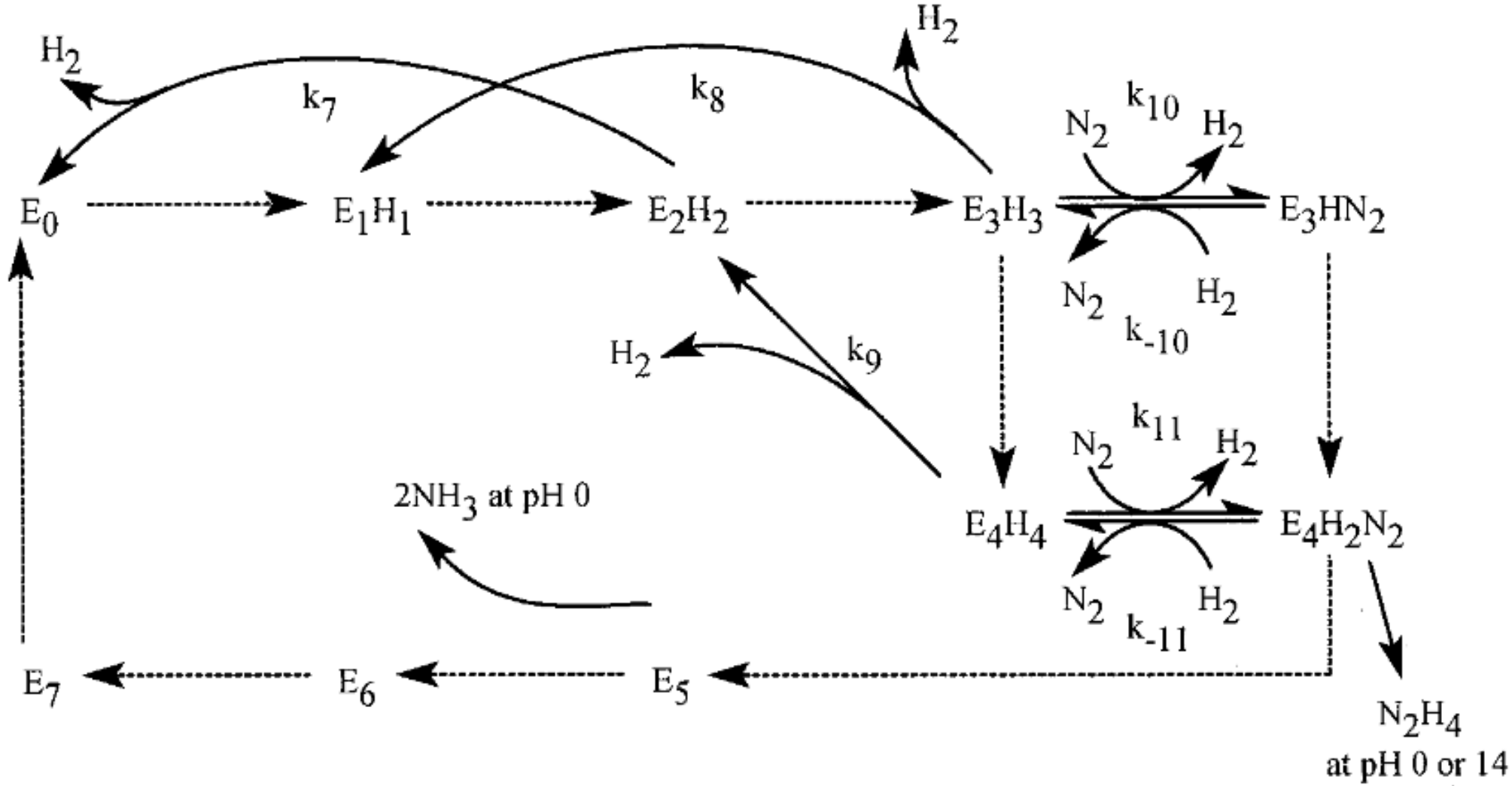
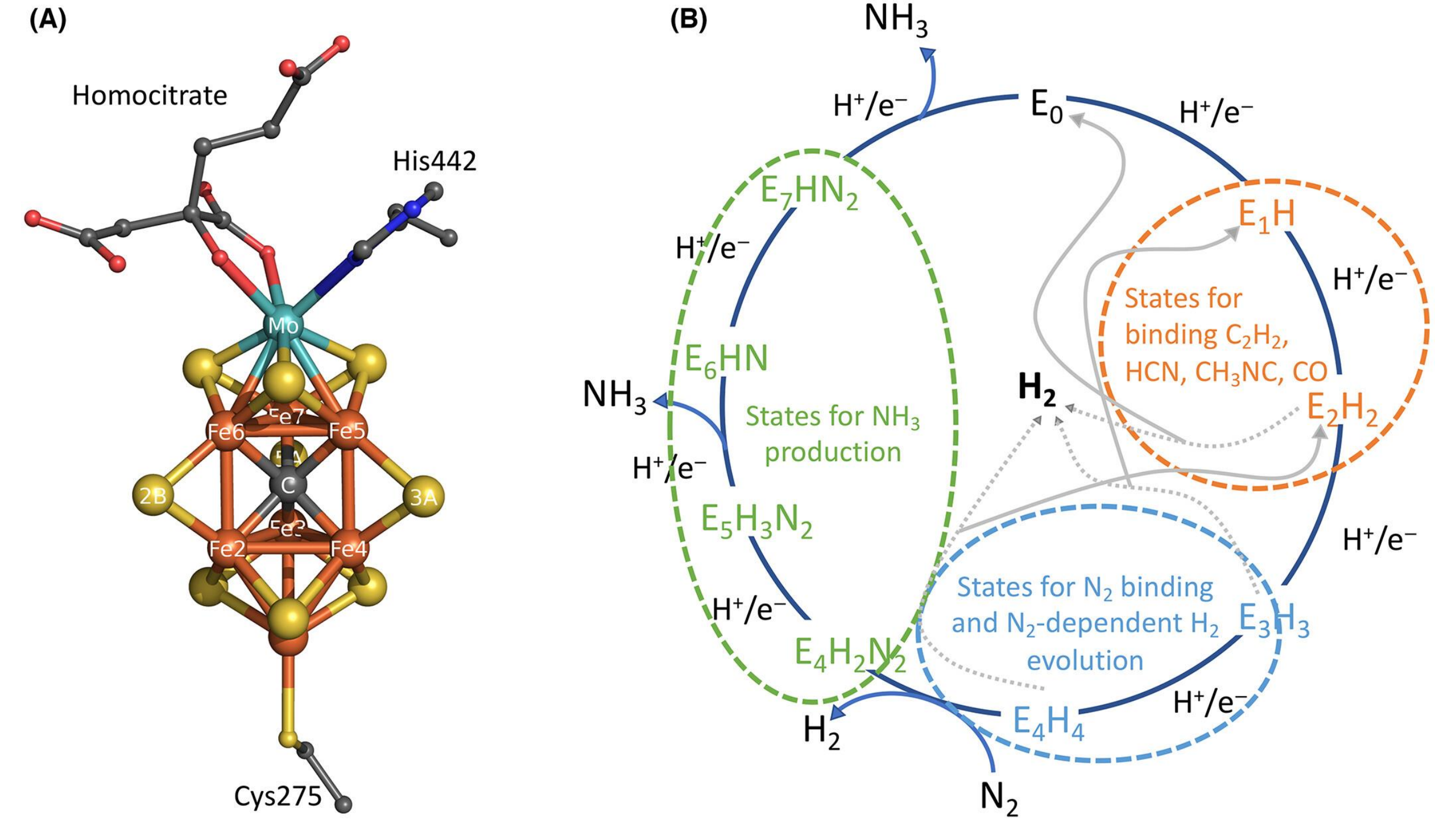


Fig. 6.6. (a) Proposed arrangement of Mo, S and Fe in the active site of nitrogenase. (b) Proposed catalytic cycle of nitrogen fixation and conversion.

Scheme 2. The MoFe Protein Cycle of Nitrogenase[a]




(A)
Homocitrate
His442
Mo
Fe6
Fe5
2B
C
3A
Fe2
Fe4
Cys275
(B)
NH3
H+/e−
E0
H+/e−
E7HN2
H+/e−
E6HN
NH3
H+/e−
E5H3N2
H+/e−
E4H2N2
H2
N2
E4H4
H+/e−
E3H3
H+/e−
E2H2
H+/e−
E1H
H+/e−
H2
States for NH3 production
States for binding C2H2, HCN, CH3NC, CO
States for N2 binding and N2-dependent H2 evolution

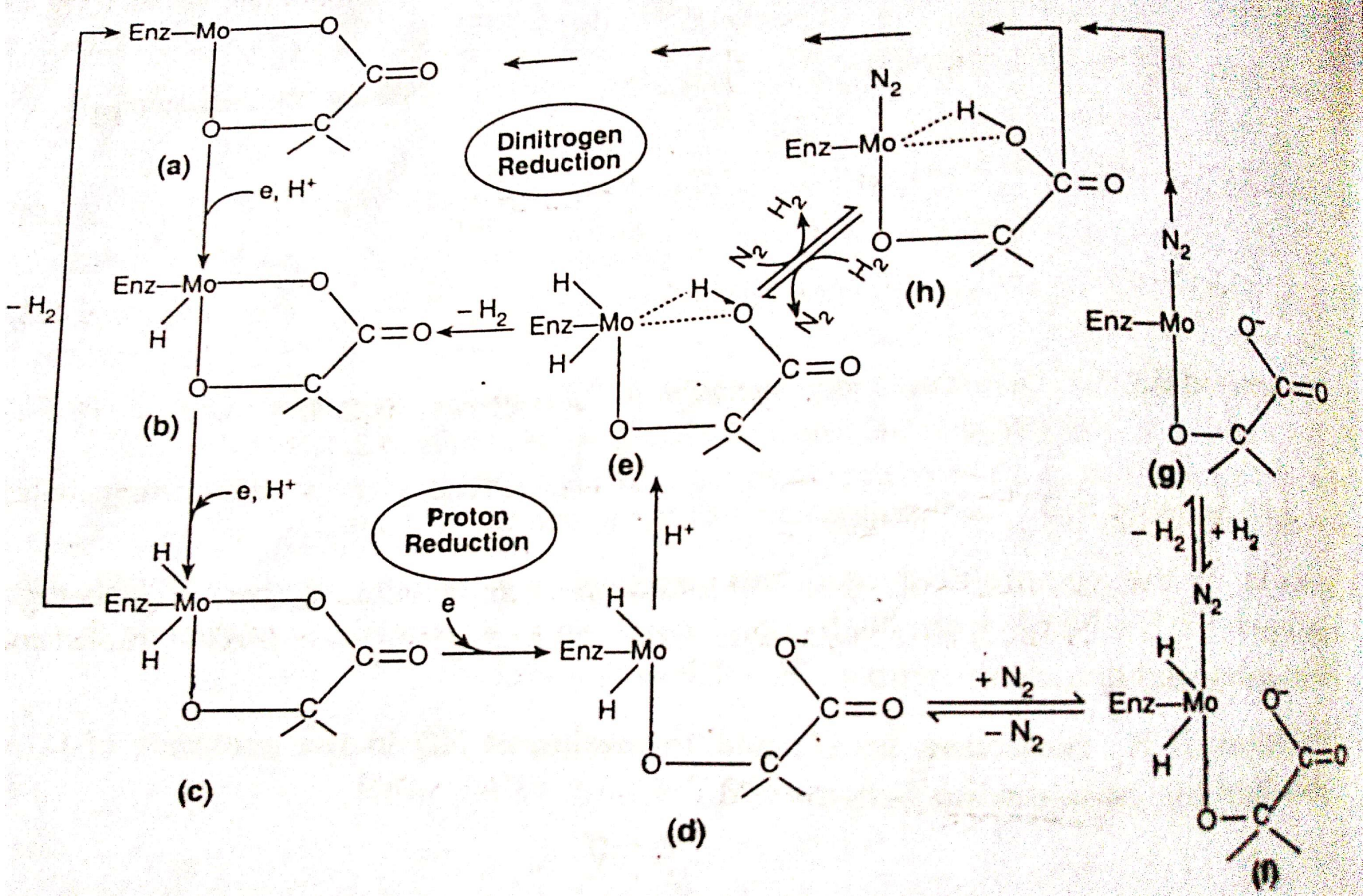
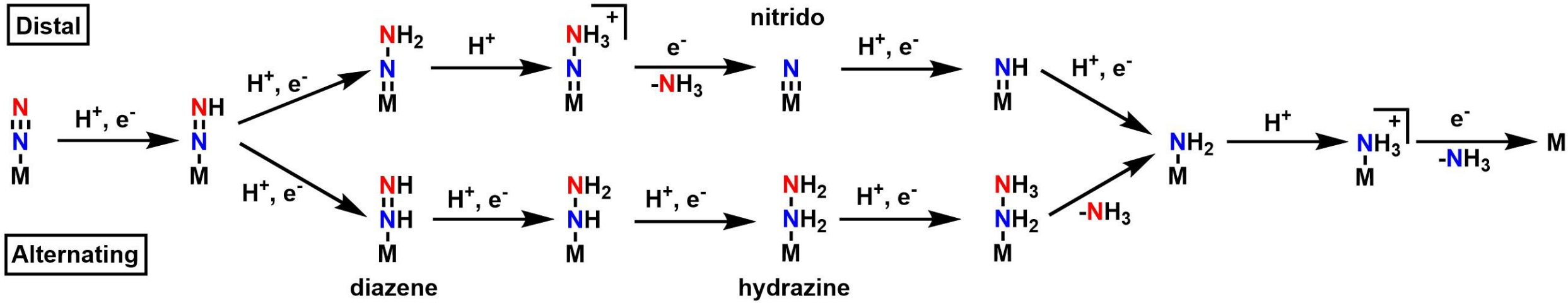
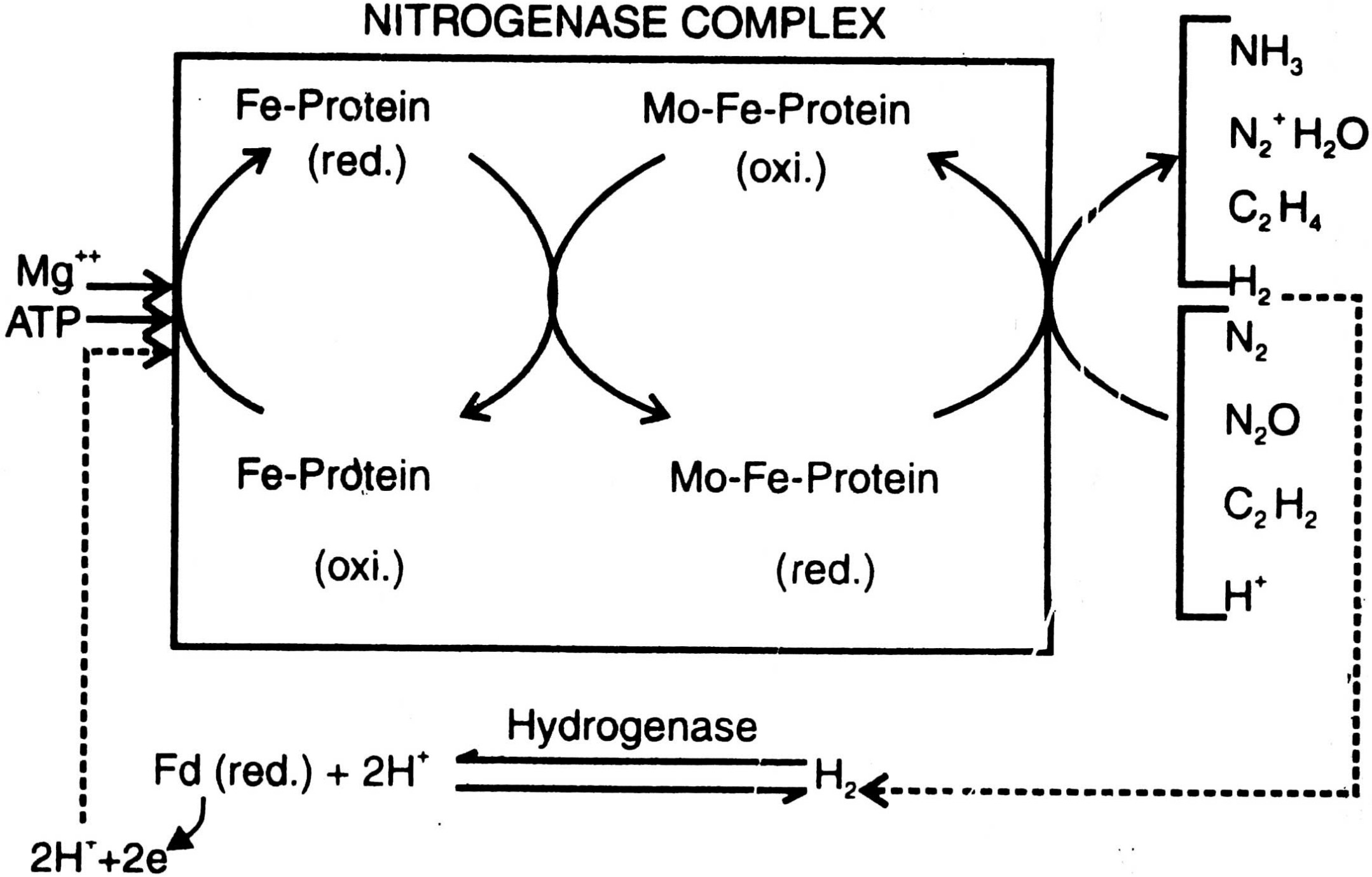

Enz—Mo
(a)
e, H+
Dinitrogen Reduction
(b)
– H2
(c)
Proton Reduction
e
(d)
H+
(e)
– H2
(h)
N2
H2
(g)
– H2
+ H2
(f)
+ N2
– N2

Table 8. Rate Constants of the Reactions in Schemes 1 and 2[a]

rate constant	value	comment
k_1	$5 \times 10^7\ M^{-1}\ s^{-1}$	responsible for lower activity at low protein concentrations
k_{-1}	$15\ s^{-1}$	
k_2	$200\ s^{-1}$	electron transfer from FeP to MoFeP
k_3	$4.4 \times 10^6\ M^{-1}\ s^{-1}$	responsible for lower activity at high protein concentrations
k_{-3}	$6.4\ s^{-1}$	rate-limiting step when substrates and FeP are saturating
k_4	$3 \times 10^6\ M^{-1}\ s^{-1}$	rate of reduction of $FeP(MgADP)_2$ complex
k_6	$1.2 \times 10^9\ M^{-1}\ s^{-1}$	rate of dissociation of $S_2O_4^{2-}$ into $2SO_2^{\bullet-}$
k_{-6}	$1.75\ s^{-1}$	rate of association of $2SO_2^{\bullet-}$ to $S_2O_4^{2-}$
k_7	$250\ s^{-1}$	gives increased H_2 evolution at low electron flux
k_8	$8\ s^{-1}$	slow to maximize E_3 concentration and hence N_2 binding
k_9	$400\ s^{-1}$	rapid H_2 evolution from most reduced hydridic species
k_{10}	$4 \times 10^5\ M^{-1}\ s^{-1}$	determine K_m^{N2} and K_I^{H2} at low electron flux
k_{-10}	$8 \times 10^4\ M^{-1}\ s^{-1}$	
k_{11}	$2.2 \times 10^6\ M^{-1}\ s^{-1}$	determine K_m^{N2} and K_I^{H2} at high electron flux
k_{-11}	$3 \times 10^6\ M^{-1}\ s^{-1}$	

[a] The values are for *K. pneumoniae* nitrogenase at 23 °C, pH 7.4.[13]


Distal
Alternating
nitrido
diazene
hydrazine
H+, e-
H+
e-
-NH3
-NH3
M


NITROGENASE COMPLEX
Fe-Protein
(red.)
Mo-Fe-Protein
(oxi.)
Fe-Protein
(oxi.)
Mo-Fe-Protein
(red.)
Mg++
ATP
NH3
N2 + H2O
C2H4
H2
N2
N2O
C2H2
H+
Hydrogenase
Fd (red.) + 2H+
H2
2H+ + 2e

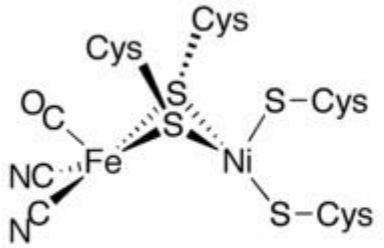
The MoFe protein can reduce many substances

- The MoFe protein can reduce many substrates
- Although under natural conditions the MoFe only reacts with N_2 and H^+.

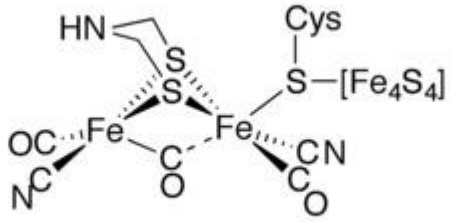
TABLE 12.4
Reactions catalyzed by nitrogenase

$N_2 \rightarrow NH_3$	Molecular nitrogen fixation
$N_2O \rightarrow N_2 + H_2O$	Nitrous oxide reduction
$N_3^- \rightarrow N_2 + NH_3$	Azide reduction
$C_2H_2 \rightarrow C_2H_4$	Acetylene reduction
$2\ H^+ \rightarrow H_2$	H_2 production
$ATP \rightarrow ADP + P_i$	ATP hydrolytic activity

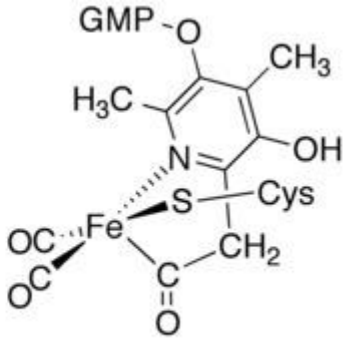
Hydrogenase



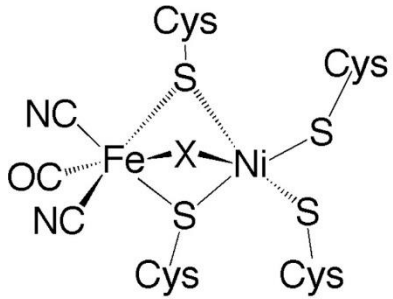
[NiFe]H_2ase



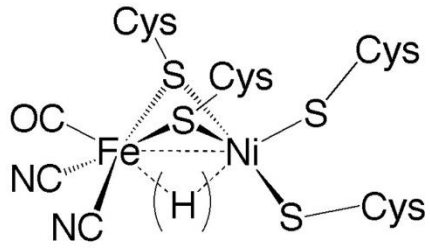
[FeFe]H_2ase



[Fe]H_2ase



oxidized form



reduced form

the [FeFe] and [NiFe] hydrogenases are true redox catalysts, driving H_2 oxidation and proton (H^+) reduction (equation 1) while the [Fe] hydrogenases catalyze the reversible heterolytic cleavage of H_2 shown by reaction (2)

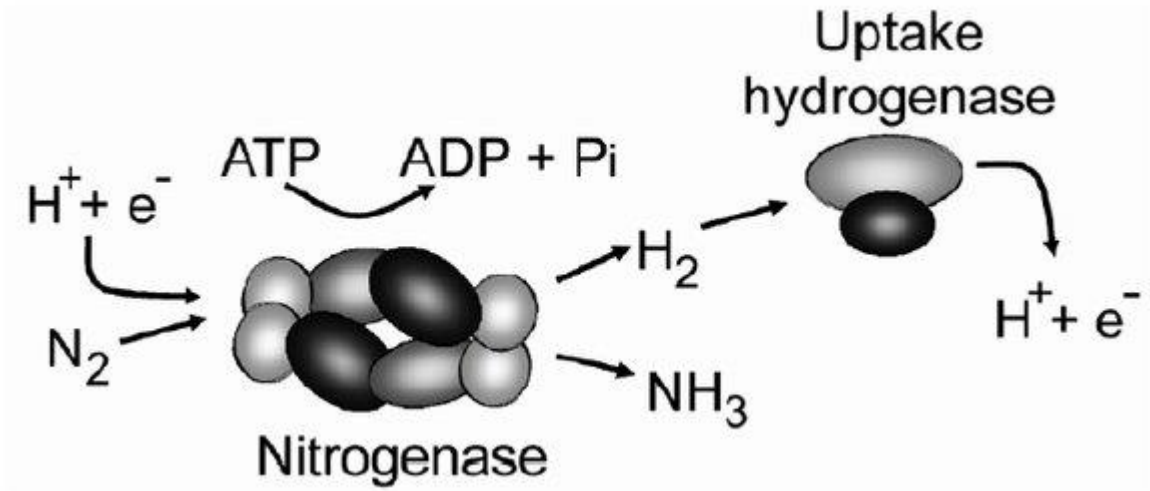
$H_2 \rightleftharpoons 2\ H^+ + 2\ e^-$ (1)

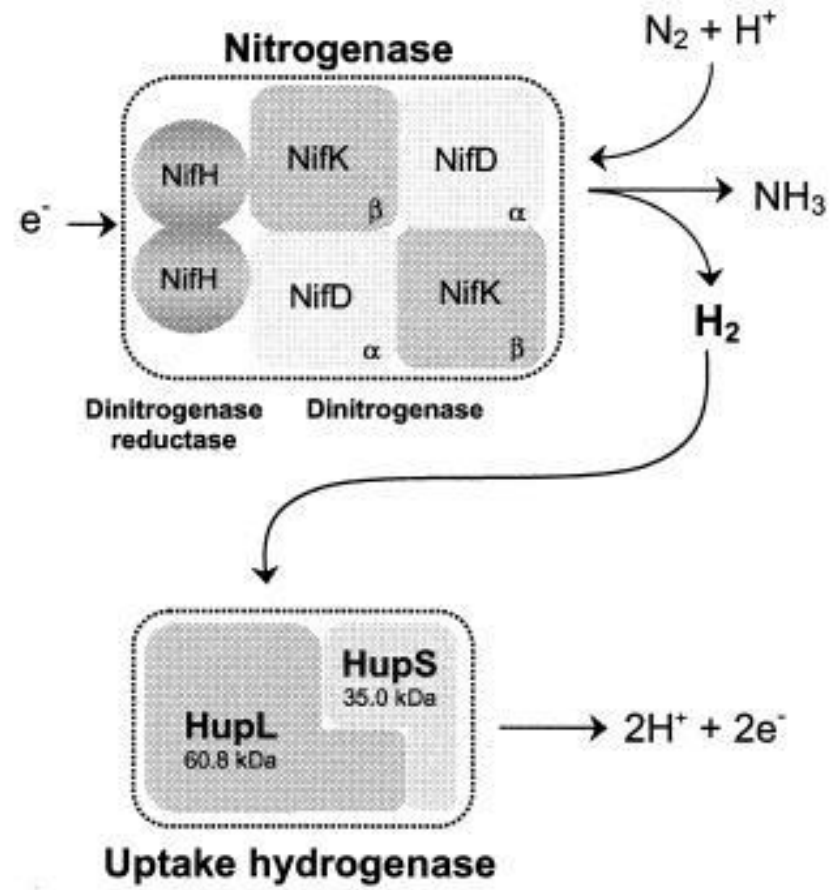
$H_2 \rightleftharpoons H^+ + H^-$ (2) (2)

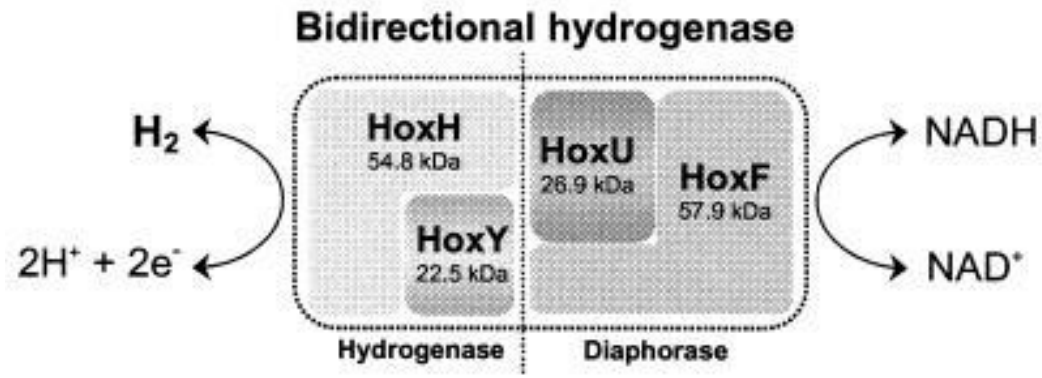
A **hydrogenase** is an enzyme that catalyzes the reversible oxidation of molecular hydrogen (H_2), as shown below:

(1) $H_2 + A_{ox} \rightarrow 2H^+ + A_{red}$

(2) $2H^+ + D_{red} \rightarrow H_2 + D_{ox}$

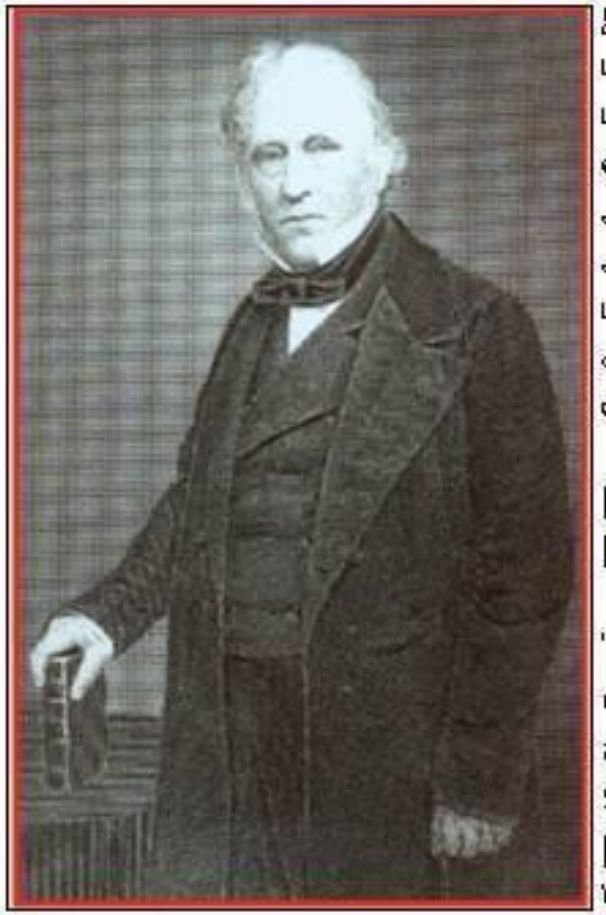

Uptake
hydrogenase
ATP
ADP + Pi
H+ + e-
H2
H+ + e-
N2
NH3
Nitrogenase


Nitrogenase
N2 + H+
NifH
NifK
NifD
β
α
e-
NH3
NifH
NifD
NifK
α
β
H2
Dinitrogenase
reductase
Dinitrogenase
HupS
35.0 kDa
HupL
60.8 kDa
2H+ + 2e-
Uptake hydrogenase


Bidirectional hydrogenase
H2
HoxH
54.8 kDa
HoxU
26.9 kDa
HoxF
57.9 kDa
NADH
HoxY
22.5 kDa
2H+ + 2e-
NAD+
Hydrogenase
Diaphorase

- 1835 Dominance of English language as a medium of employment and education
- Establishment of teacher-training schools for all levels of instruction.
- led towards the nationalization of many universities
- Many advantages but one biggest loss :

Education lost its own esteem, and Indian touch



இத்தருணத்தில், சுமார் 170 ஆண்டுகளுக்கு முன்பு பிரிட்டிஷ் நாடாளுமன்றத்தின், பிரபல உறுப்பினரும், பிரிட்டிஷ் அரசாங்கத்தில் பல முக்கியப் பதவிகளை வகித்தவரும், 1834-ம் ஆண்டு பிரிட்டிஷ் அரசு அமைத்த 'சுப்ரீம் கவுன்ஸில் ஆஃப் இந்தியா' என்ற அமைப்பின் முக்கிய உறுப்பினருமான மெக்காலே பிரபு நான்காண்டுகள் நமது நாட்டைச் சுற்றிப்பார்த்துவிட்டு ஆங்கிலேய அரசுக்கு எழுதியதைக் கீழே தந்துள்ளோம்.

LORD MACAULAY'S ADDRESS TO THE BRITISH PARLIAMENT 2 FEBRUARY, 1835

"I have travelled across the length and breadth of India and I have not seen one person who is a beggar, who is a thief. such wealth I have seen in this country, such high moral values, people of such caliber, that I do not think we would ever conquer this country, unless we break the very backbone of this nation, which is her spiritual and cultural heritage, and, therefore, I propose that we replace her old and ancient education system, her culture, for if the Indians think that all that is foreign and English is good and greater than their own, they will lose their selfesteem, their native culture and they will become what we want them, a truly dominated nation."