15BT1001 Unit III

- **Enzymes – Significance, Factors affecting enzyme activity**
- **Mechanism of Enzyme action**
- **Strategies utilized by enzyme to effect catalysis**
    - **Proteases**
    - **Carbonic anhydrase**
    - **Restriction enzymes**
    - **Nucleoside monophosphate kinases**
- **Photosynthesis**
- **Metabolism and Bioenergetics**

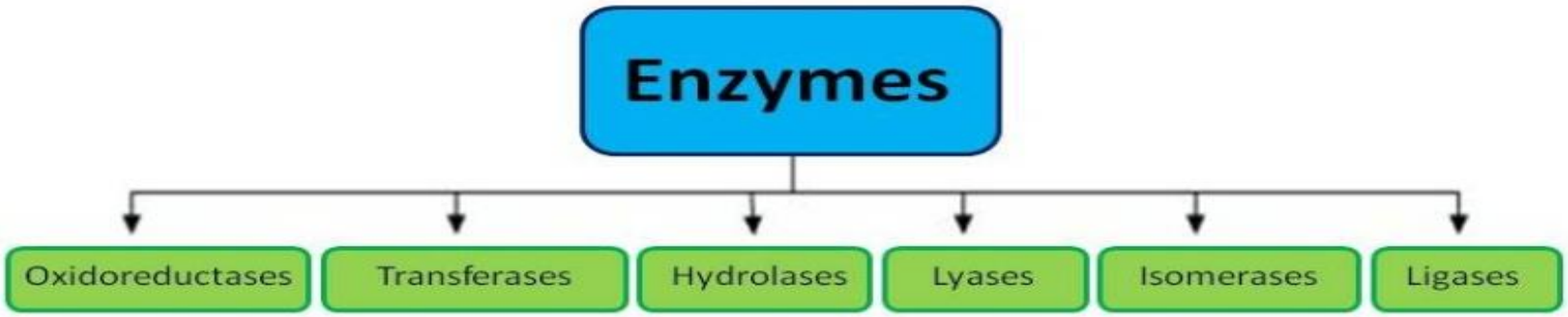
INTRODUCTION

Why Enzymes?

- Natural catalysts
- Speed: 10^6 over un-catalyzed rates!
- Specificity: only the desired reaction occurs
- Permit reactions under mild conditions (pH, temperature etc.)
- All enzyme protein, except – **Ribozyme (non-proteinaceous)**
- Enzyme commission (E.C.) of the Int. Uni. Biochem. & Mol. Biol (IUBMB)

Naming & Classification of Enzyme

- **Names end with "ase" – ex: Urease (Substrate they act on – Hydrolysis of Urea)**
- **Some enzyme named after their source - Protease – Papain (Papaya)**
- **IUBMB – 6 Classes**
- **EC number – Further sub divided into 4**
- **EC number for Papain - 3.4.22.2**
  - **3- Class - Hydrolase**
  - **4 - Sub class - Hydrolyases – that acts on peptide bond - Peptidase**
  - **22 - Peptidase - that acts on Cysteine Endopeptidase**
  - **2 - Cysteine Endopeptidase**

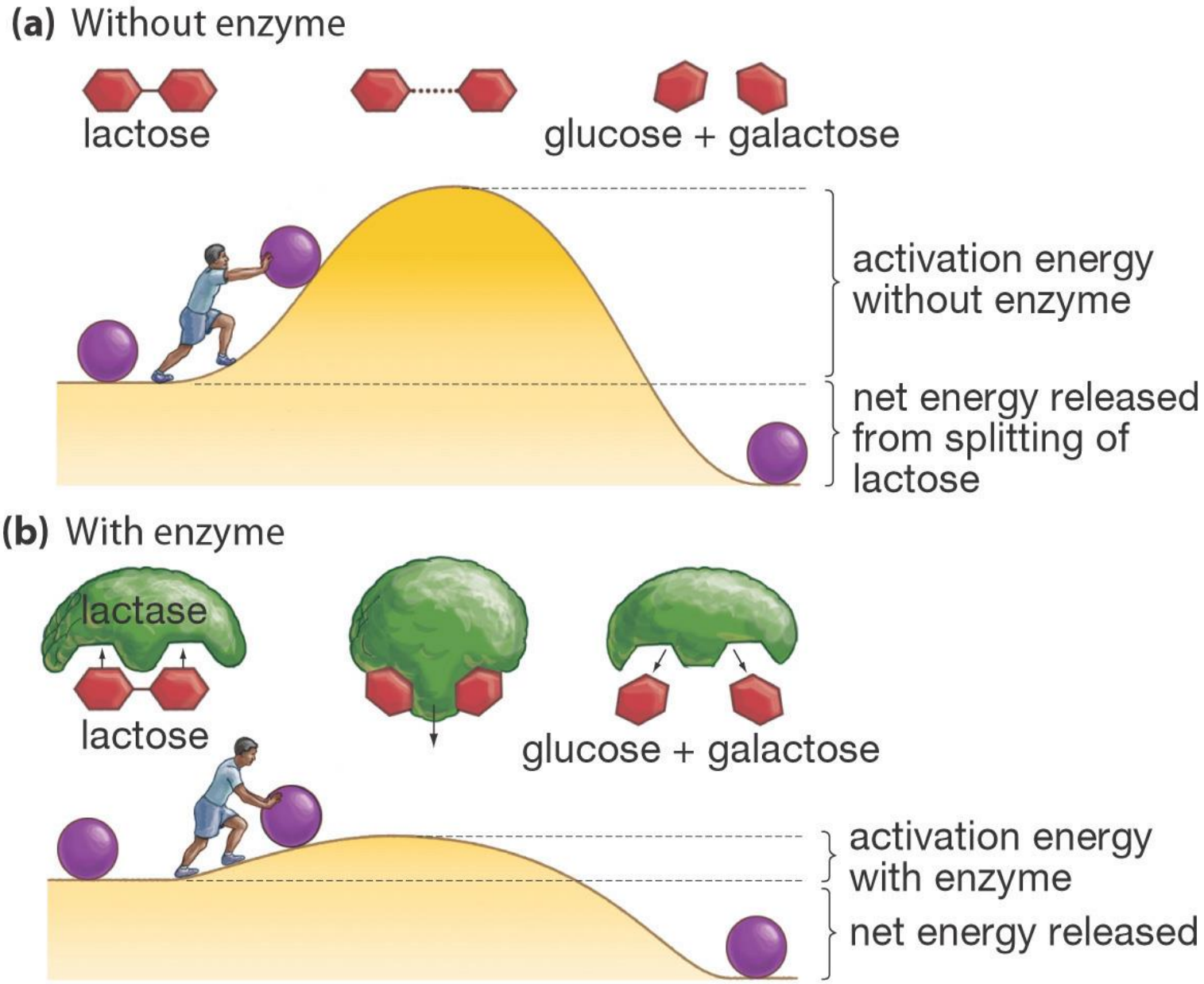


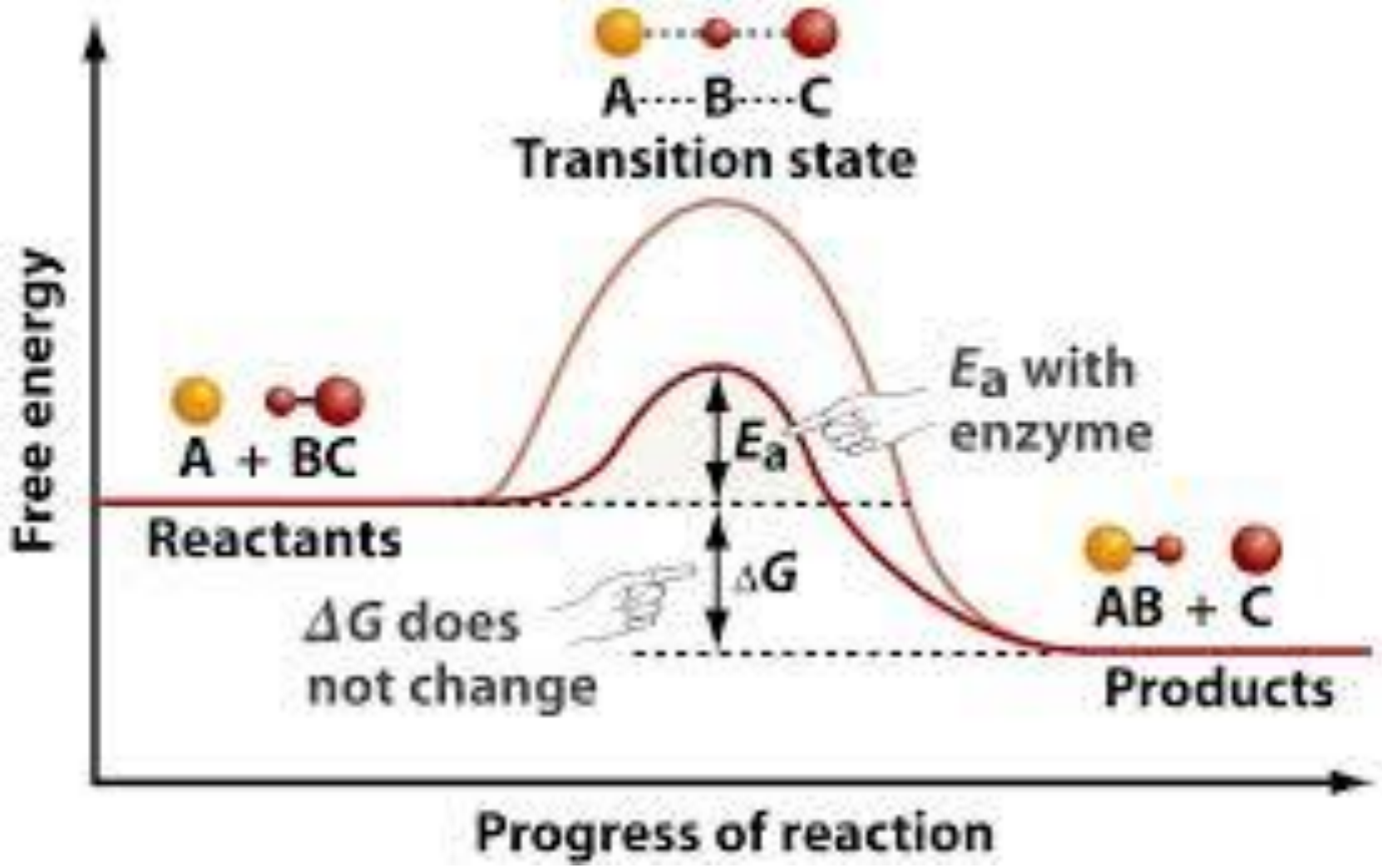
CLASS	DESIGNATION	FUNCTION
EC1	Oxidoreductases	catalyze oxidation/reduction reactions
EC2	Transferases	transfer a functional group (e.g. a methyl or phosphate group)
EC3	Hydrolases	catalyze the hydrolysis of various bonds
EC4	Lyases	cleave various bonds by means other than hydrolysis and oxidation
EC5	Isomerases	catalyze isomerization changes within a single molecule
EC6	Ligases	join two molecules covalent bonds.

Significance of Enzyme

1) Catalytic power (reaction rate)

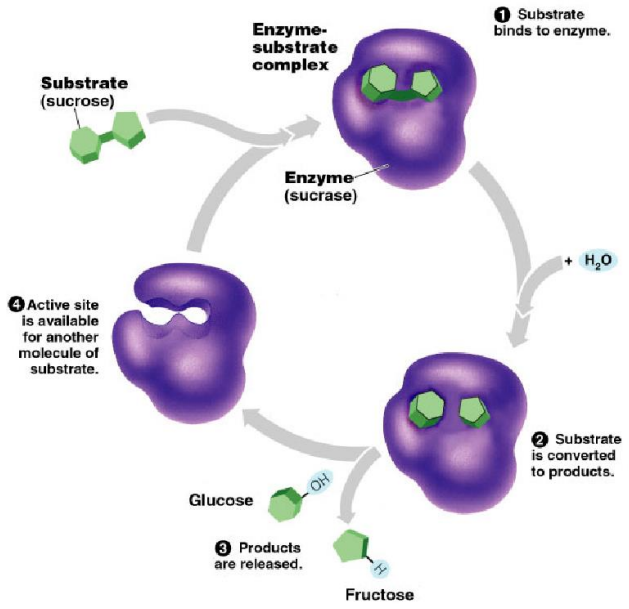
Enzymes work by weakening bonds which lowers activation energy



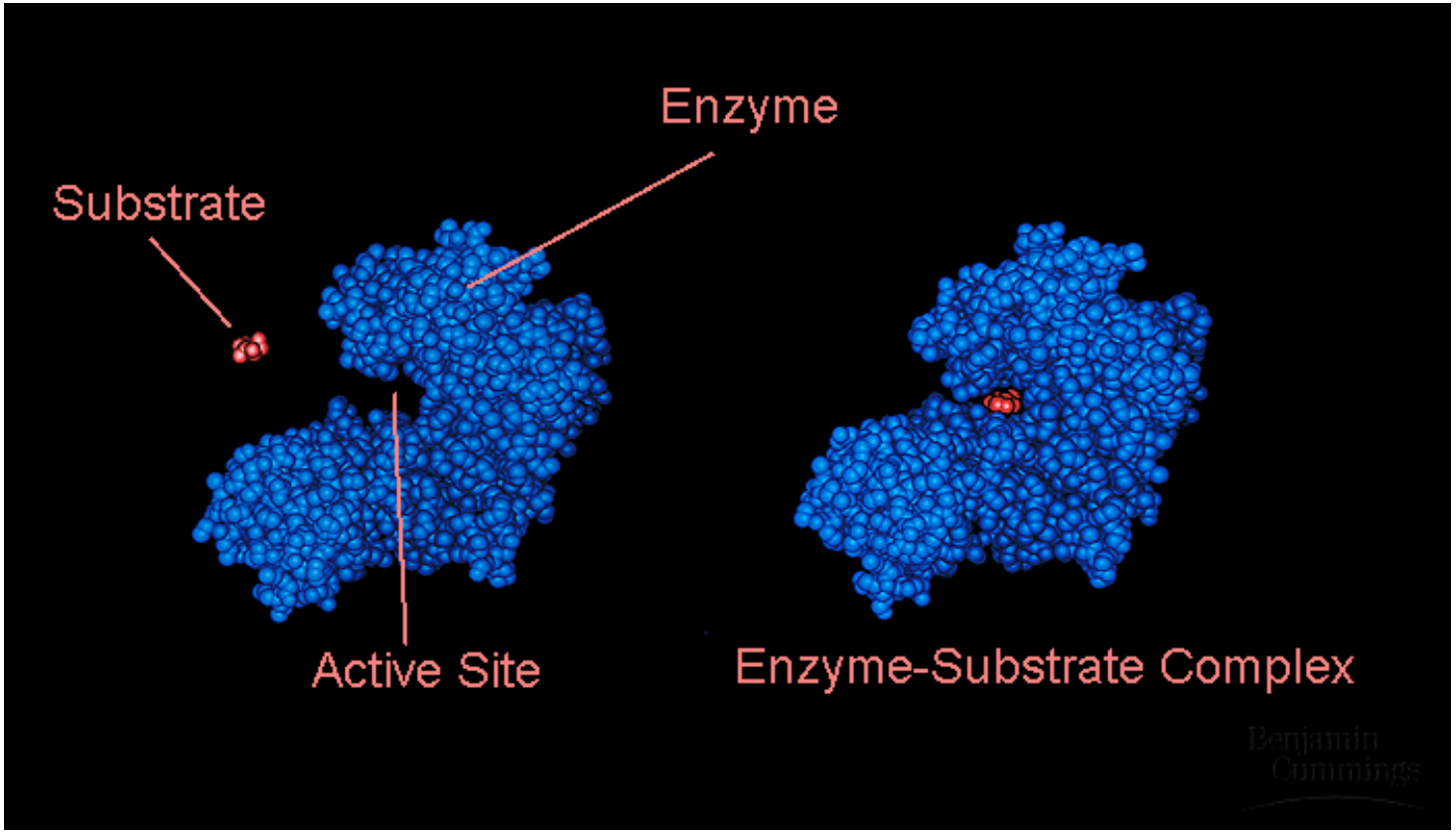

A····B····C
Transition state
Free energy
A + BC
Reactants
E_a
E_a with enzyme
ΔG
ΔG does not change
AB + C
Products
Progress of reaction

2) Specificity

- Enzymes selectively recognize proper substrates over other molecules
- Specificity is controlled by structure – the unique fit of substrate with enzyme controls the selectivity for substrate and the product yield
- 3D, juxtaposition
- Stereo and region specificity

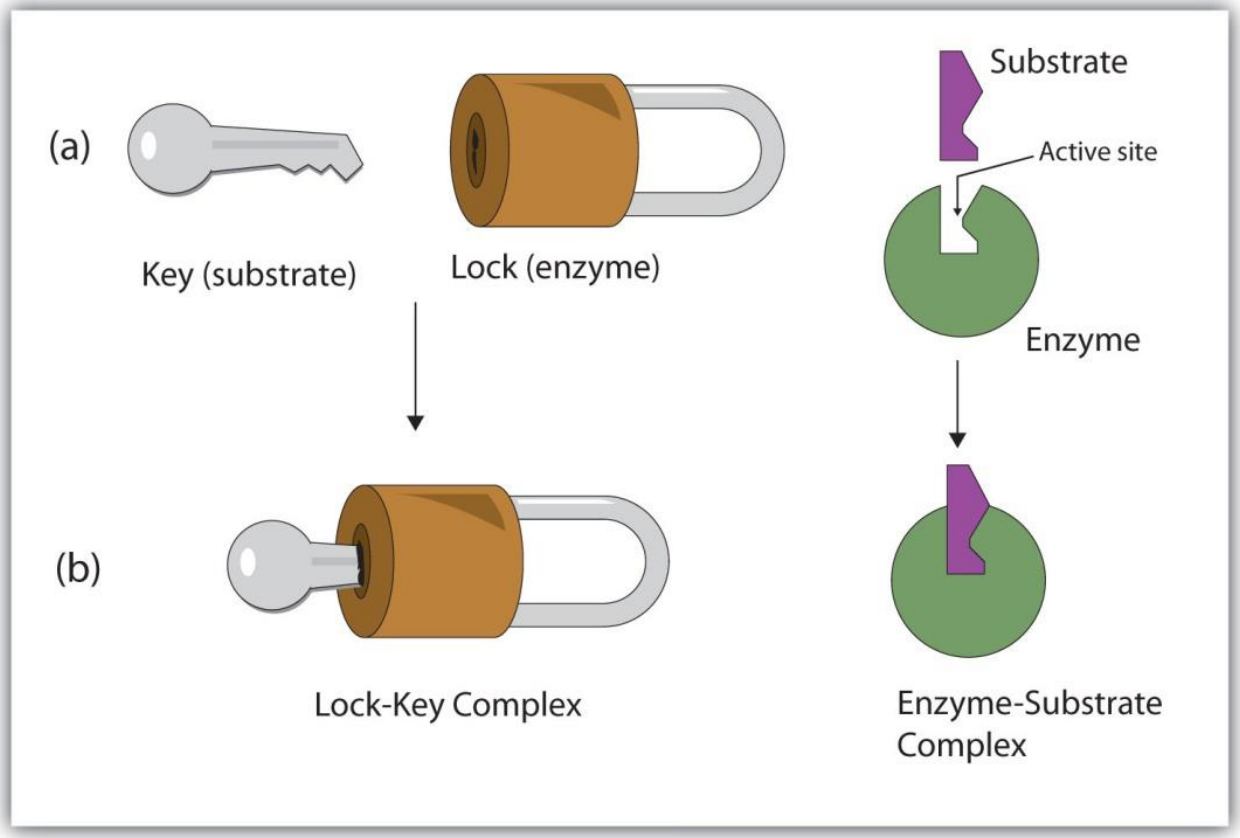


Specificity of Enzyme



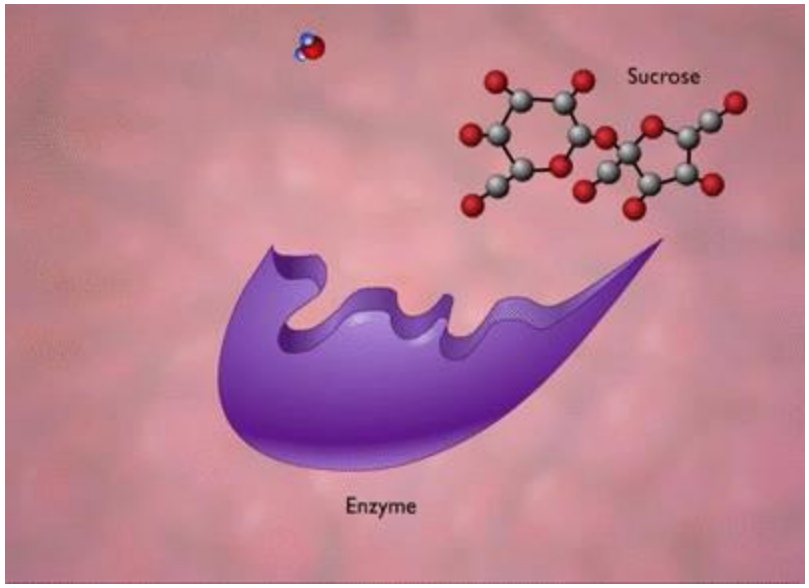
Lock and key model

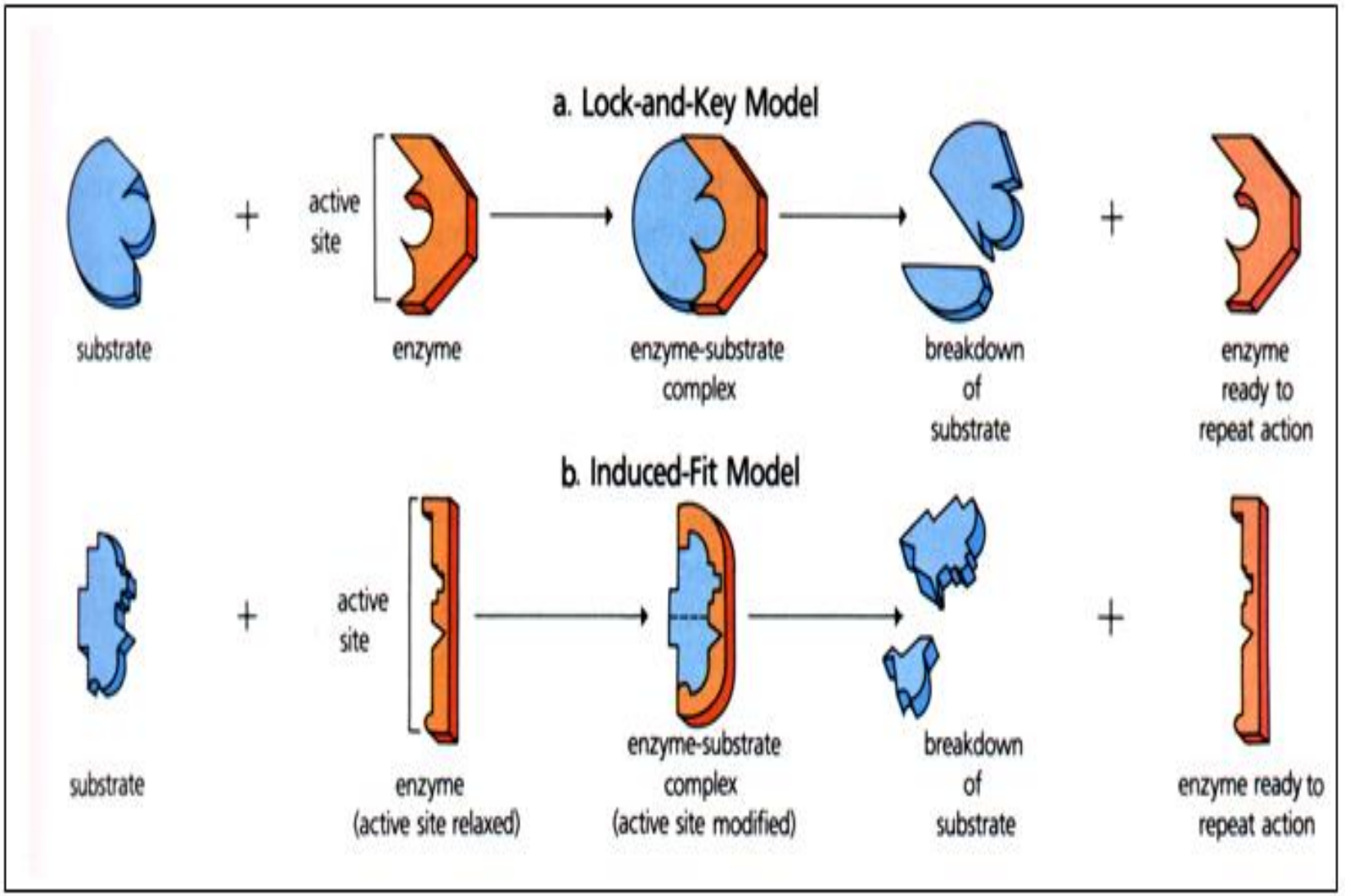
- Emil Fischer, 1894
- Correctly sized key(substrate) fits into the key hole (active site) of the lock(enzyme)



Induced fit model

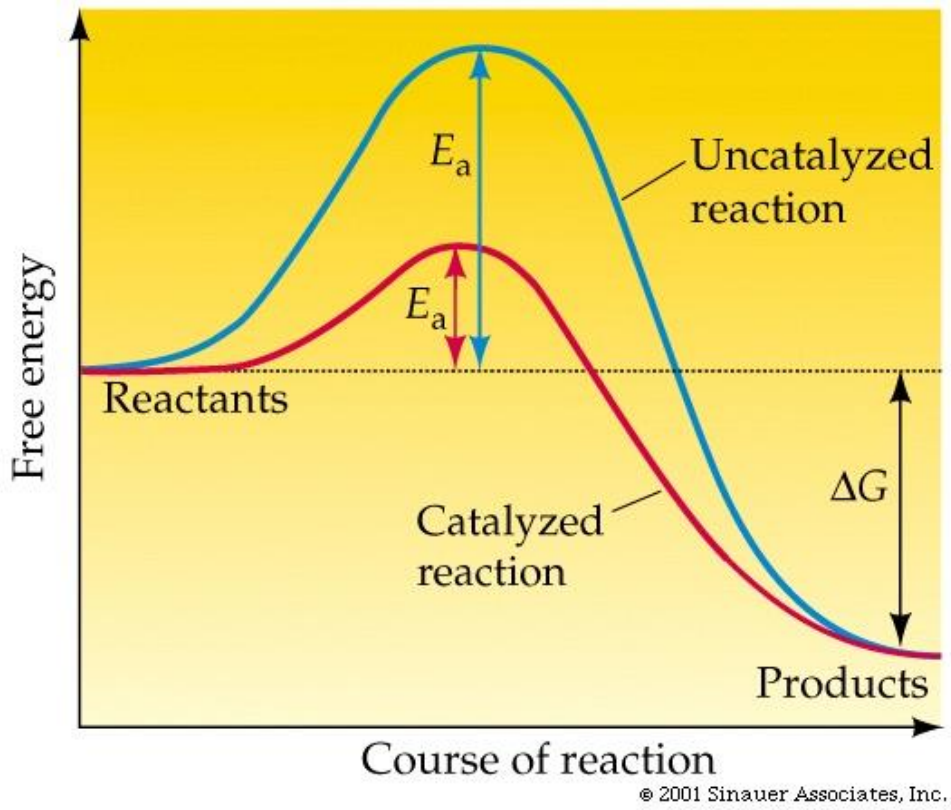
- Daniel Koshland, 1958
- Active site on the enzymes makes contact with the proper substrate, the enzyme molds itself to the shape of the molecule
- **Structural reconfiguration**

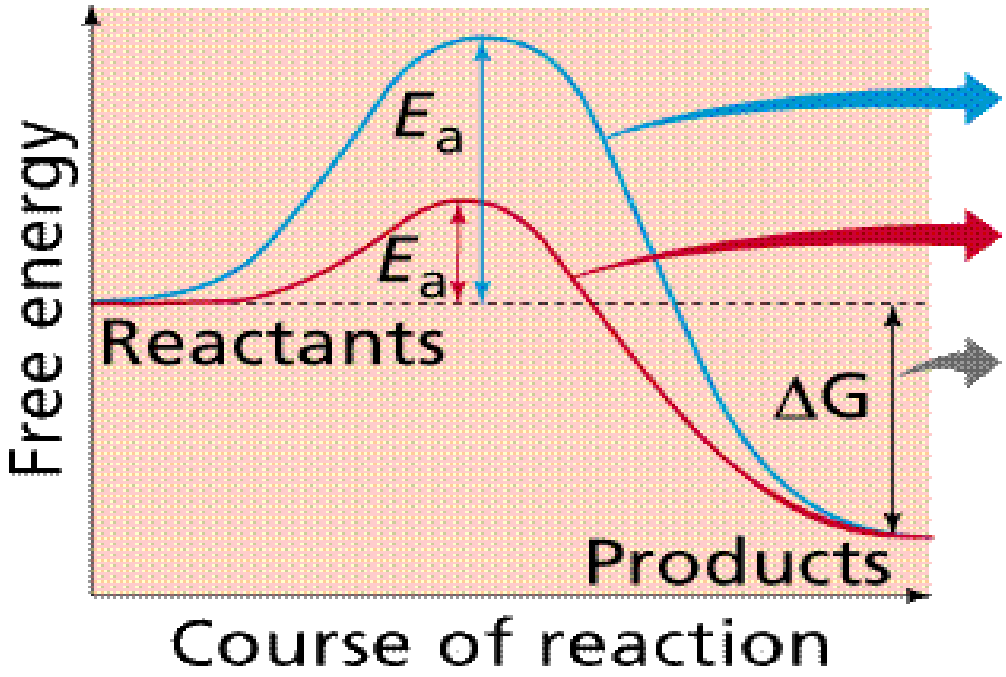



a. Lock-and-Key Model
active site
substrate
enzyme
enzyme-substrate complex
breakdown of substrate
enzyme ready to repeat action
b. Induced-Fit Model
active site
substrate
enzyme (active site relaxed)
enzyme-substrate complex (active site modified)
breakdown of substrate
enzyme ready to repeat action

3) Thermodynamics

- $\Delta G=(G_{product}-G_{reactants})$
- Determines the **feasibility** of the reaction
- Enzymes **lower the activation barrier** and makes the reaction more feasible





An uncatalyzed reaction requires a higher activation energy than does a catalyzed reaction

There is no difference in free energy between catalyzed and uncatalyzed reactions

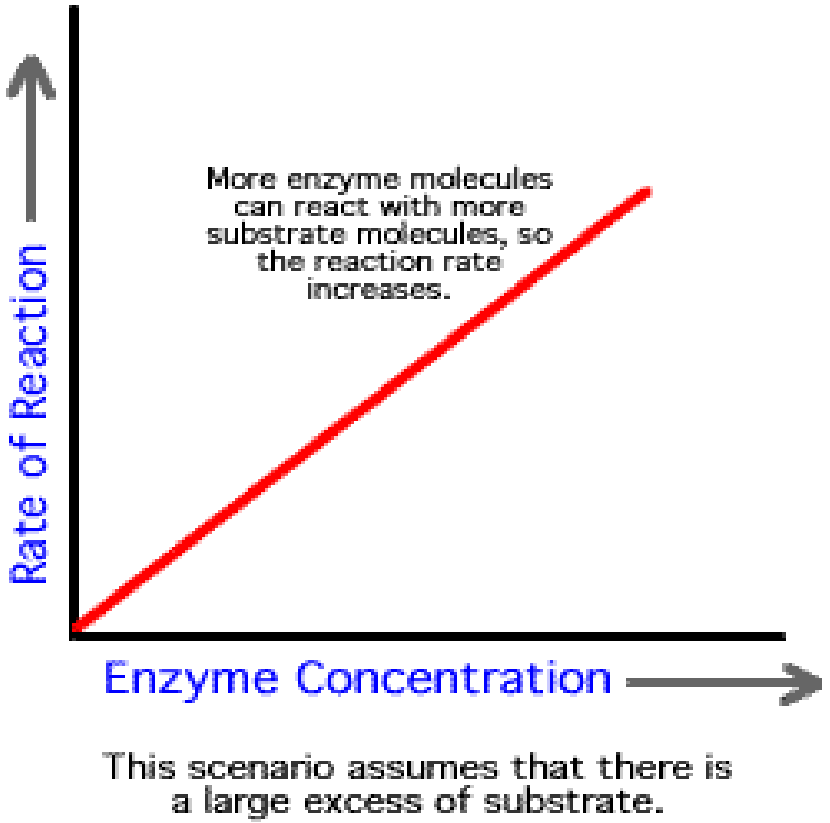
Enzymes cannot alter the final equilibrium but the reaction rate at which the equilibrium attained is altered by surpassing the activation energy

What Affects Enzyme Activity?

1) Enzyme Concentration

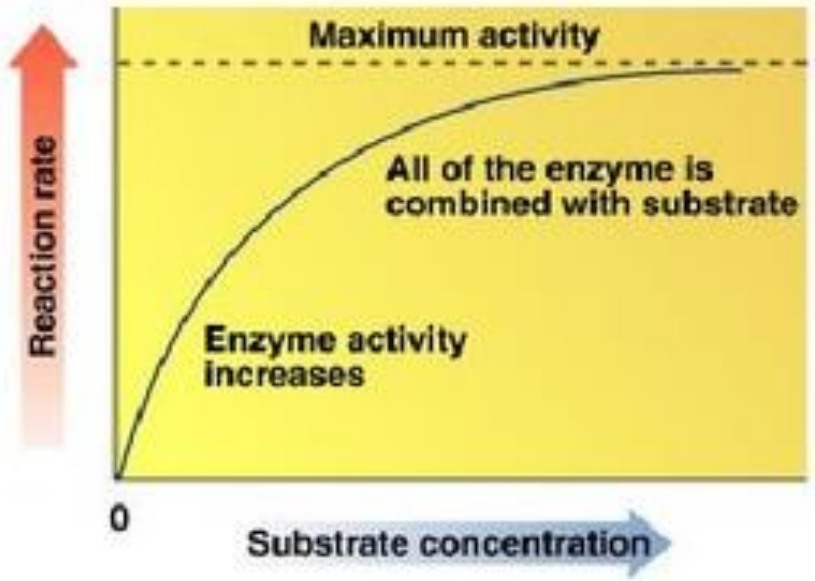
•**Substrate concentration high than enzyme**- substrate concentration considered constant and reaction rate depends on the **enzyme concentration**

• REACTION RATE = k * [E]

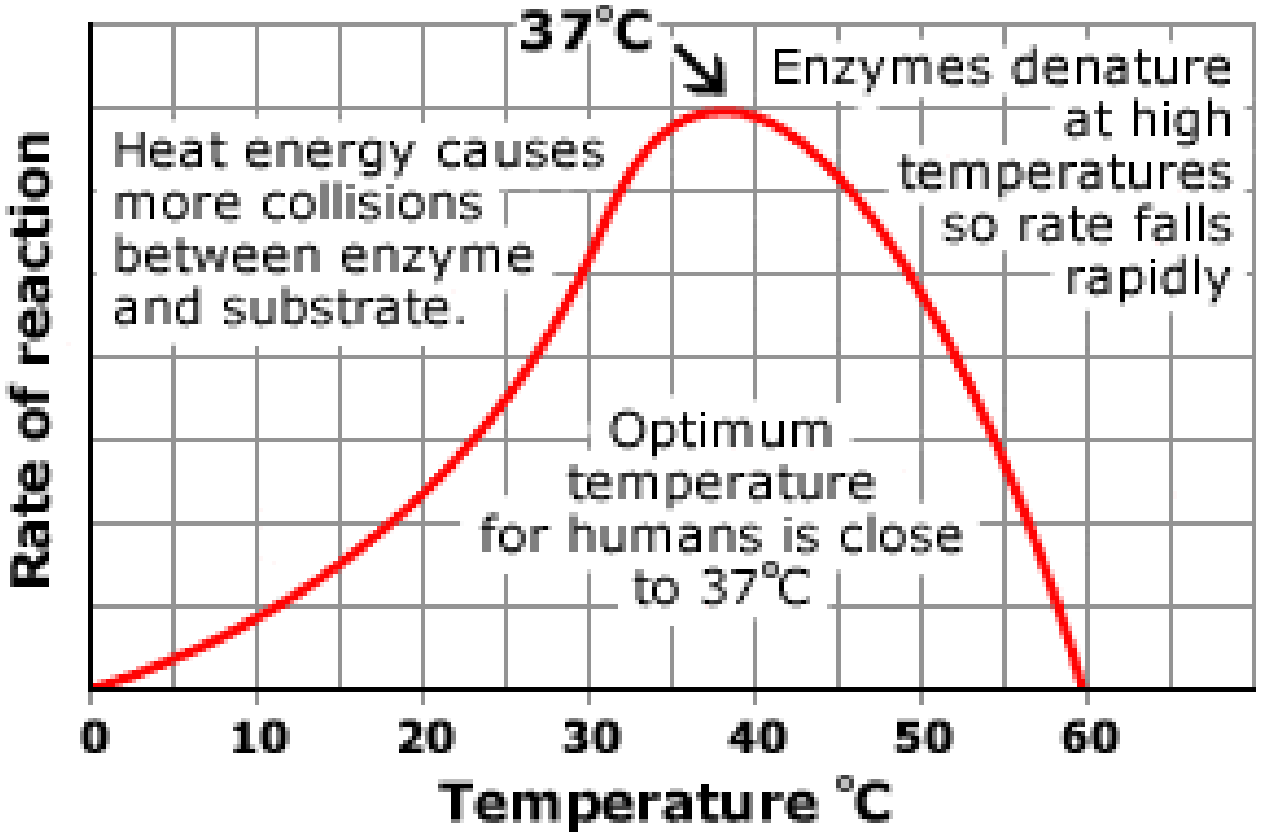


2) Substrate Concentration

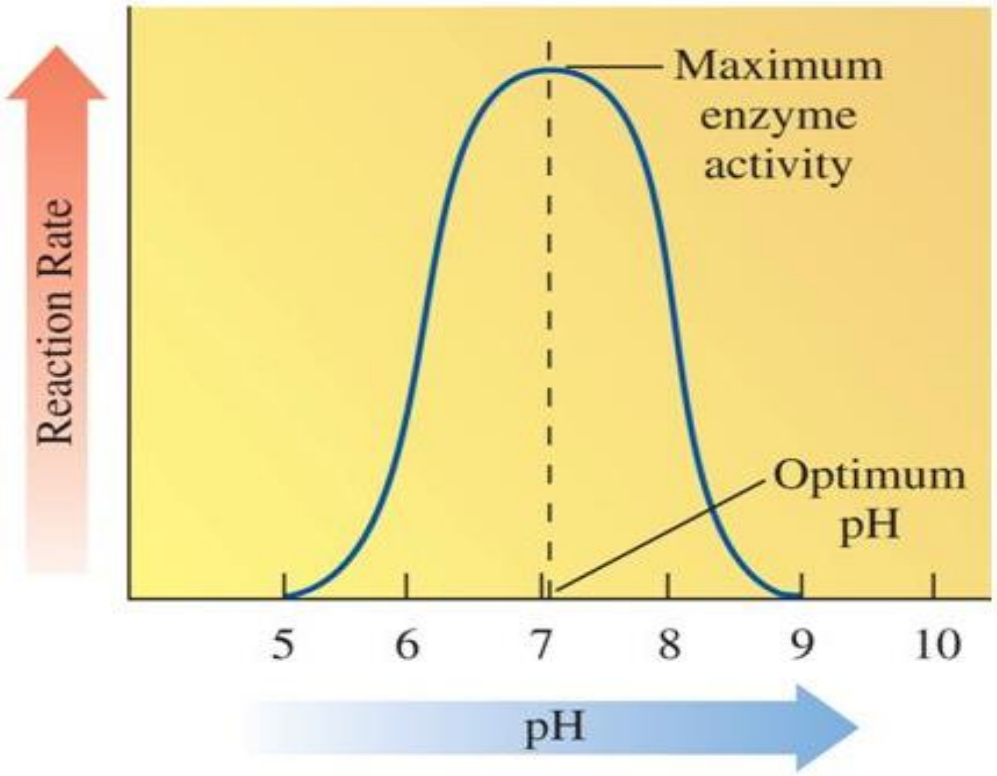
- The rate of reaction increases as substrate concentration increases (at constant enzyme concentration).
- Maximum activity occurs when the enzyme is saturated.



3) Temperature



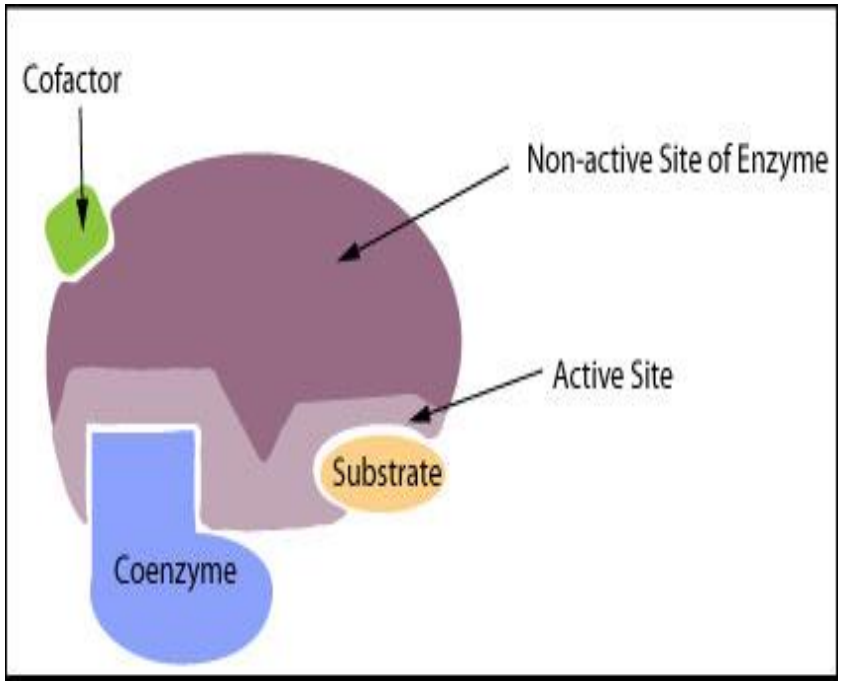
4) pH


Reaction Rate
Maximum enzyme activity
Optimum pH
5
6
7
8
9
10
pH

5) Cofactors (Inorganic) and Coenzymes (Organic)

- Inorganic substances (zinc, iron) and vitamins (respectively) are sometimes need for proper enzymatic activity. Small organic molecules are coenzymes acts at active sites. Eg. TPP, FAD, NAD and CoA.

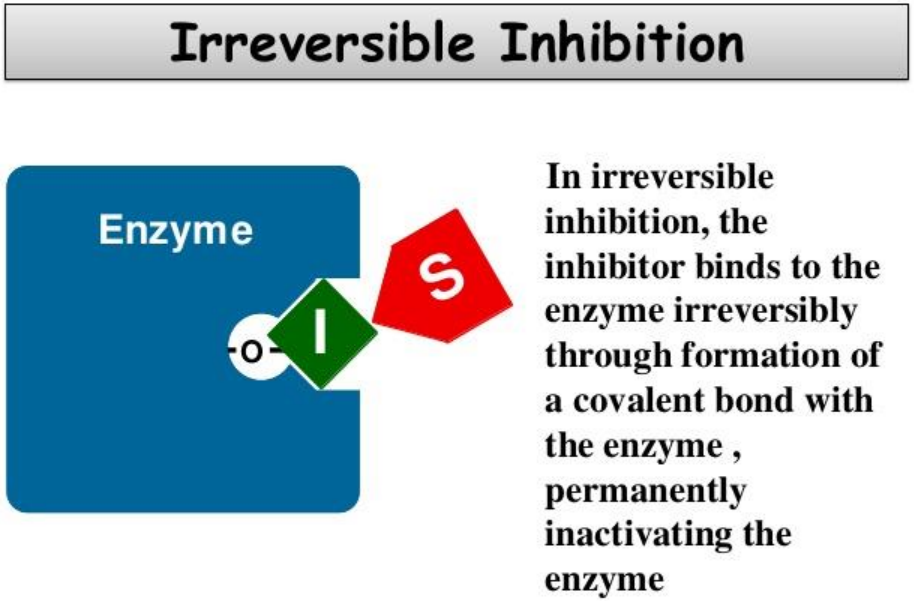
- Example: Iron must be present in the quaternary structure - hemoglobin in order for it to pick up oxygen.



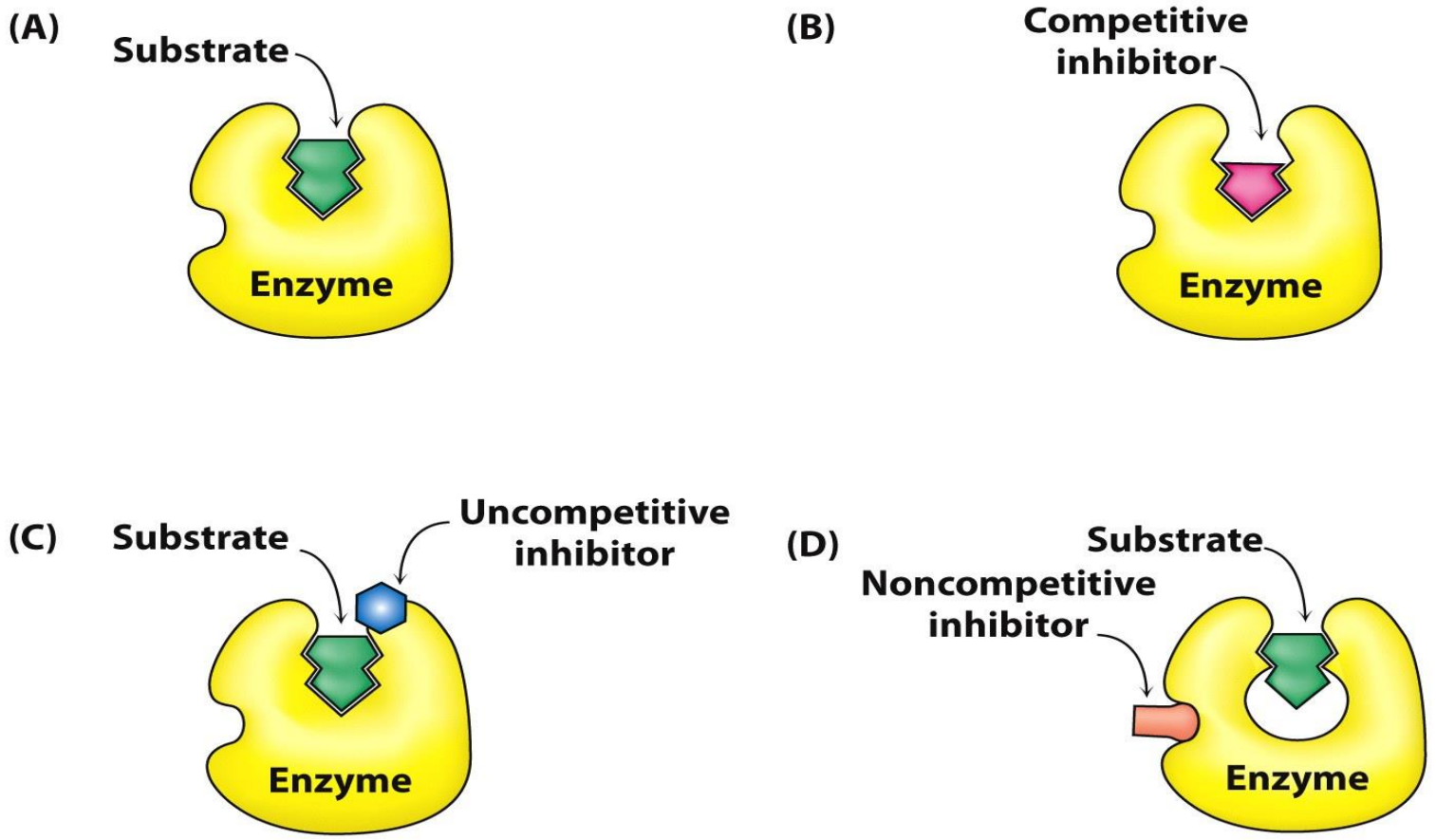
Inhibition

Irreversible Inhibition

- Irreversible (Penicillin inhibitor of transpeptidases of bacterial cell wall)
- Penicillin mimics natural D-alanine-D-alanine dipeptide substrate binding to the transpeptidase enzyme of bacterial cell wall



Reversible Inhibition



Reversible Inhibition

➢Competitive Inhibition

- ❖Similar geometry as substrate
- ❖Compete for enzyme-substrate complex
- ❖Lowers catalytic rate

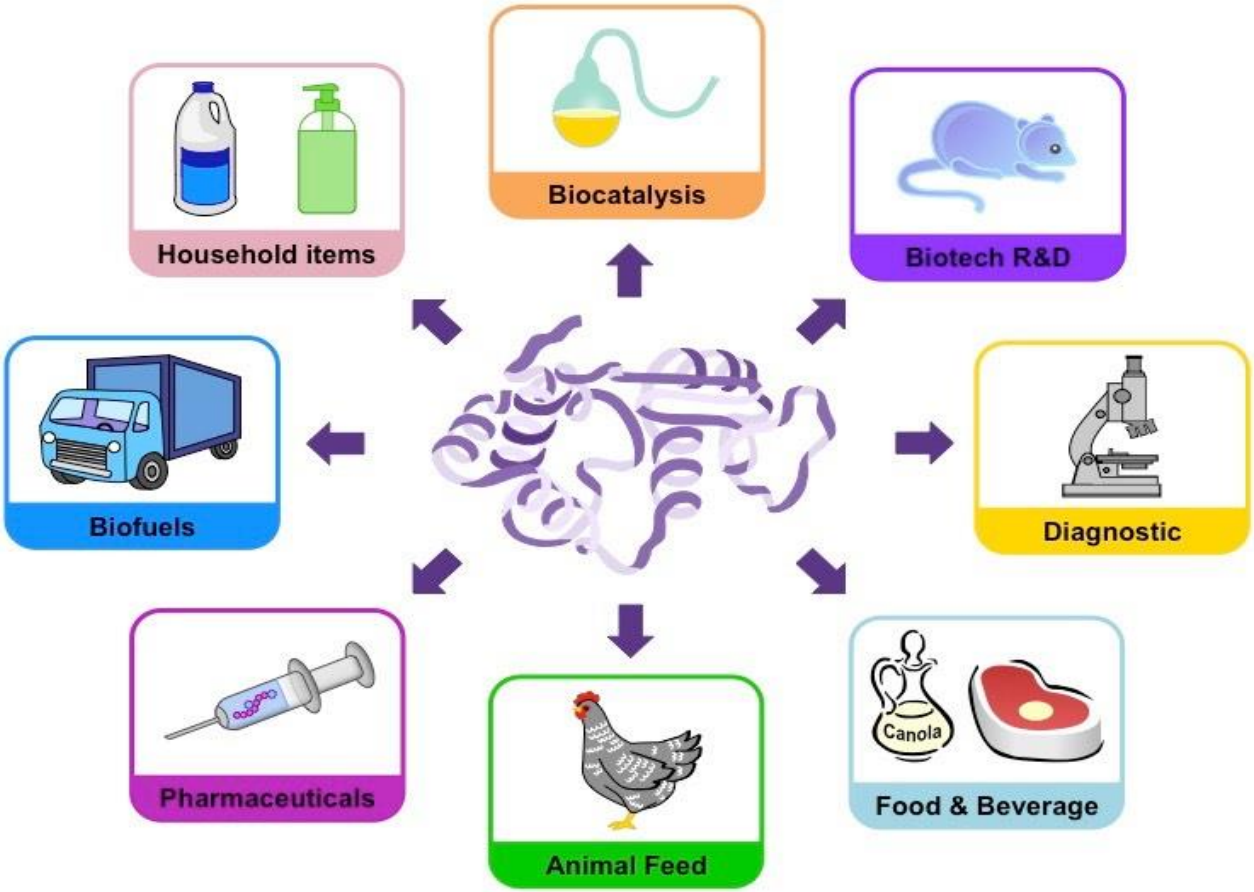
➢Uncompetitive Inhibition

- ❖Unable to bind to free enzyme, but can bind to enzyme-substrate complex
- ❖Reduces reaction rate

➢Noncompetitive Inhibition

- ❖Inhibitor binding site different
- ❖Can bind to free enzyme or enzyme-substrate complex
- ❖Reduces reaction rate

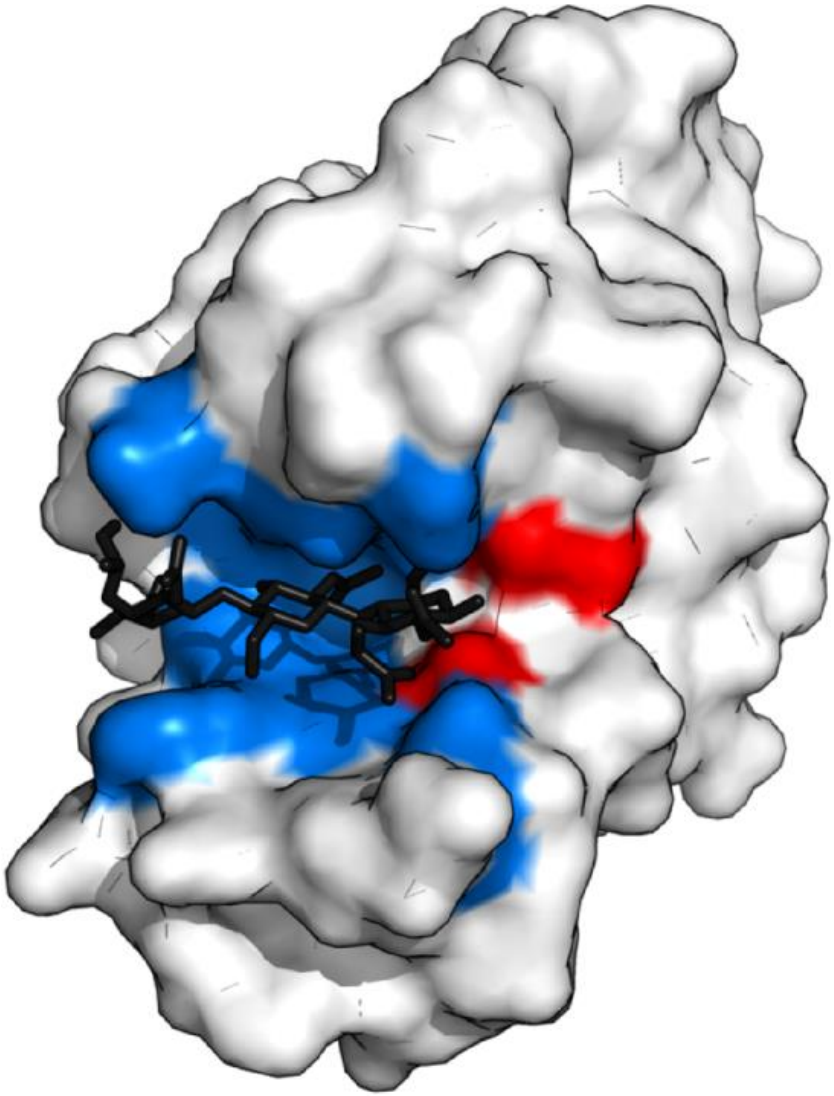
APPLICATION OF ENZYMES



APPLICATION OF ENZYMES

1. **Cellulase** (A polysaccharide found in plant cell wall) – It hydrolyzes cellulose – Used to make paper, cotton & other textile
2. **Hemicellulase** – It hydrolyzes another plant cell wall
3. **Xylanase** (Important industrial enzyme) – It breaks down xylan, a polysaccharide found in plant cell wall – Used in pulp & paper industry
4. **Amylase** – It hydrolyzes starch to sugars – Used in food & brewing industry
5. **Protease** – It hydrolyzes peptide bonds – Used in food, brewing, detergent & leather industry
6. **Lipase** – It breaks down triglycerides to glycerol & fatty acid – Used in food industry

MECHANISM OF ENZYME CATALYSIS

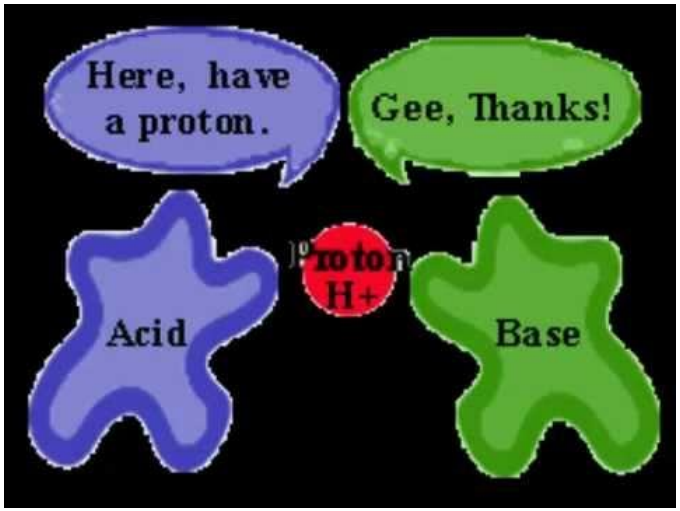


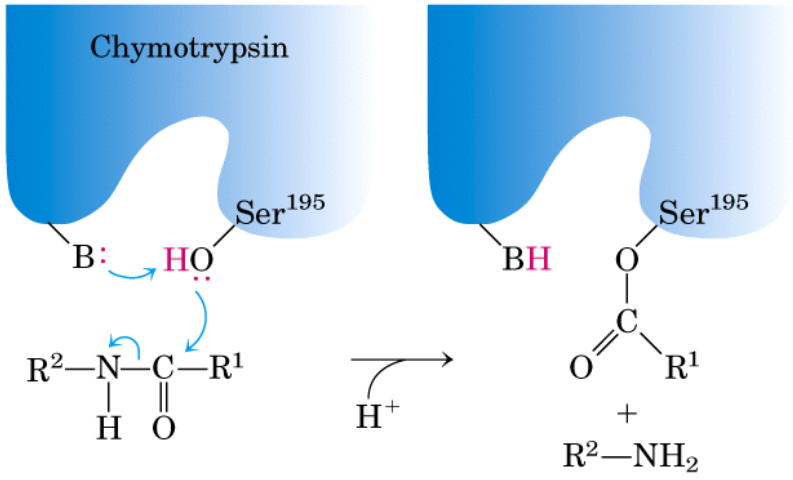
MECHANISM OF ENZYME CATALYSIS

- **GENERAL ACID-BASE CATALYSIS**
- **COVALENT CATALYSIS**
- **METAL ION CATALYSIS**
- **CATALYSIS BY APPROXIMATION (PROXIMITY ORIENTATION)**

1) GENERAL ACID-BASE CATALYSIS

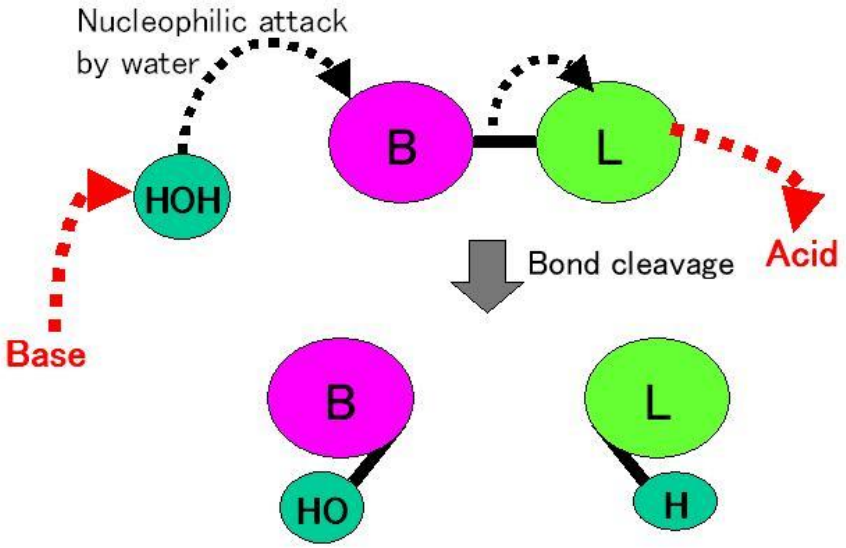
- In an **ENZYME** - active amino acid side chains act as proton donors (acid) and proton acceptor (base)
- By donating or withdrawing protons, the charged intermediates formed in an enzyme catalyzed reaction, will be transformed to product rather than reverting back to form reactant
- Example – Amino acid side chain with neutral pKa values – Glutamic acid, asparatic acid, lysine, arginine, cysteine, serine, histidine, tyrosine.





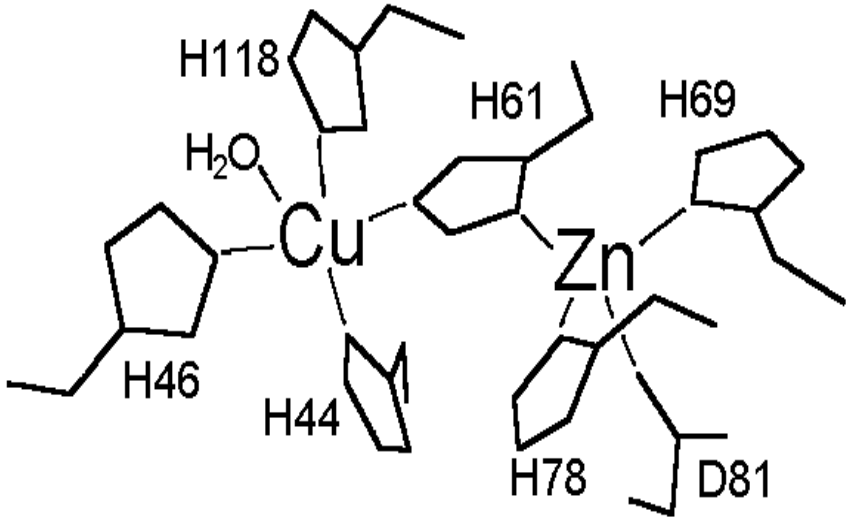
2) COVALENT CATALYSIS

- Amino acid residue which forms nucleophile were used in enzyme catalysis
- Helps in forming temporary covalent bond between enzyme and active site residue, which lowers activation energy to release product
- Example - Papain



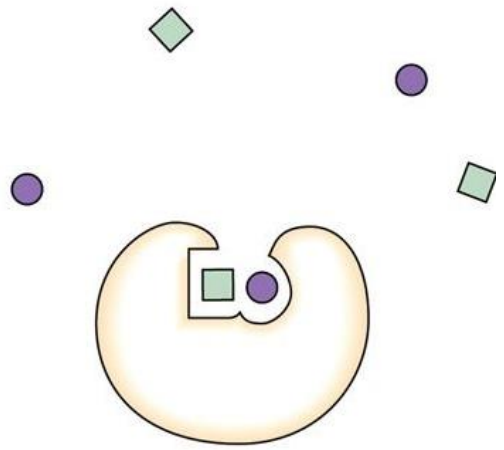
3) METAL ION CATALYST

- Metals attached either in active site of enzyme or to substrate, helps in enzyme catalysis
- Via ionic interactions, metal ions mediate oxidation-reduction reactions and stabilize charged transition states
- Example – Zn+2, Mg+2, Mn+2, Fe+2, Cu+2, K+, Na+

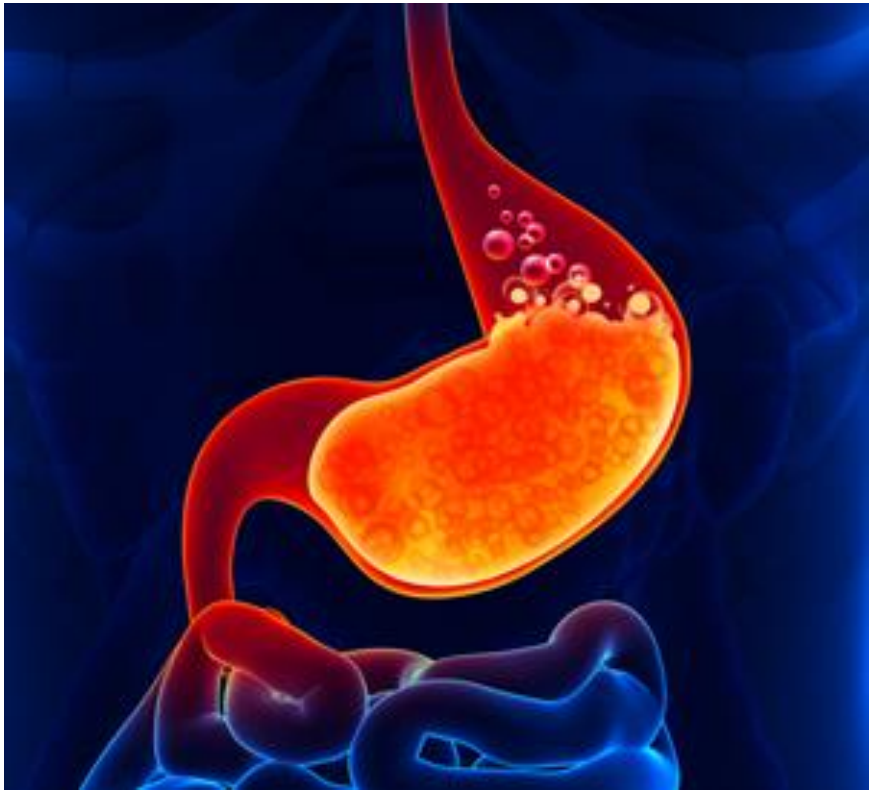


4) CATALYSIS BY APPROXIMATION (PROXIMITY ORIENTATION)

- When two substrates involved, the reaction rate is enhanced by bringing them together on the active binding site of E



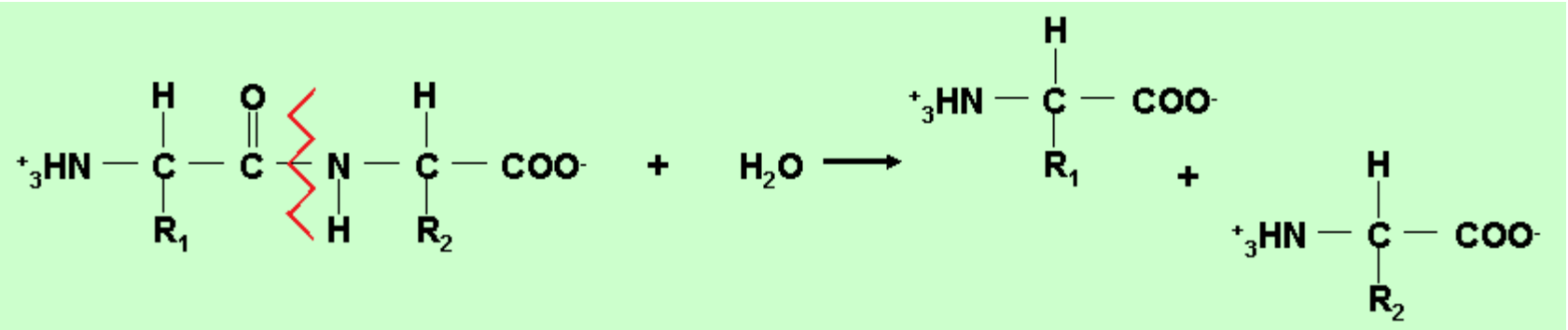
1) PROTEASE



Strategies Utilized by Enzymes To Effect Catalysis

1) Proteases

- Proteases - group of enzymes which break down a variety to proteins into simpler forms
- Proteases break down peptide bonds
- Are also called peptidases or proteinases
- The process of breaking down protein with the help of proteases is called proteolysis.
- Polypeptide chain are broken down into smaller amino acid sequence.



Mechanistic Sets of Proteases

set	feature	inhibitor	examples	function
Serine protease	active site serine	fluorophosphates	trypsin thrombin plasmin coccoonase subtilisin acrosin	digestion blood coagulation lysis of blood clots mechanical digestion sperm penetration
Cysteine protease	active site cysteine	iodoacetate	papain strept. proteinase cathepsin B	digestion digestion intracell. digestion
Acid protease	acidic pH optimum	diazoketones	pepsin chymosin	digestion milk coagulation
Metalloproteases	Zn^{2+}, Zn^{2+}, Ca^{2+}	o-phenanthroline	carboxypeptidase thermolysin	digestion digestion

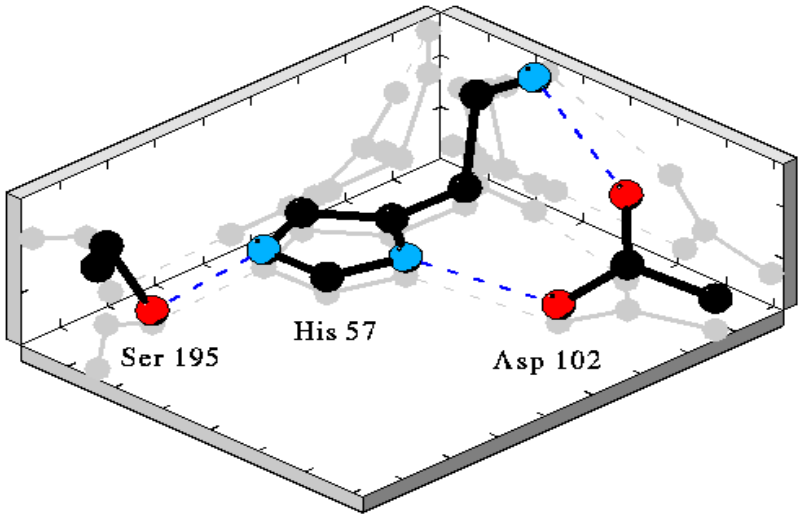
CLASSIFICATION

According to structure and function- 6 basic groups

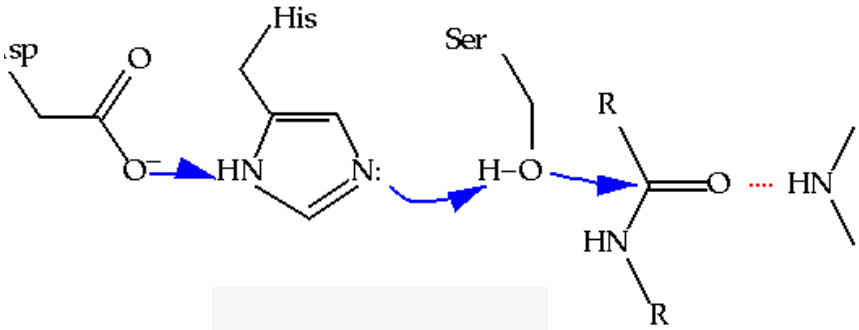
- **Serine proteases**
- **Aspartate proteases**
- **Metalloproteases**
- **Cysteine proteases**
- **Threonine proteases**
- **Glutamic acid proteases**

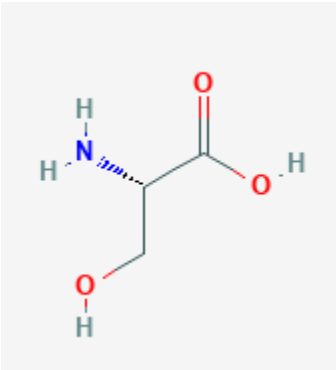
Serine Protease (Catalytic triad)

- The active site in each **serine protease** includes a **serine** residue, a **histidine** residue, & an **aspartate** residue.

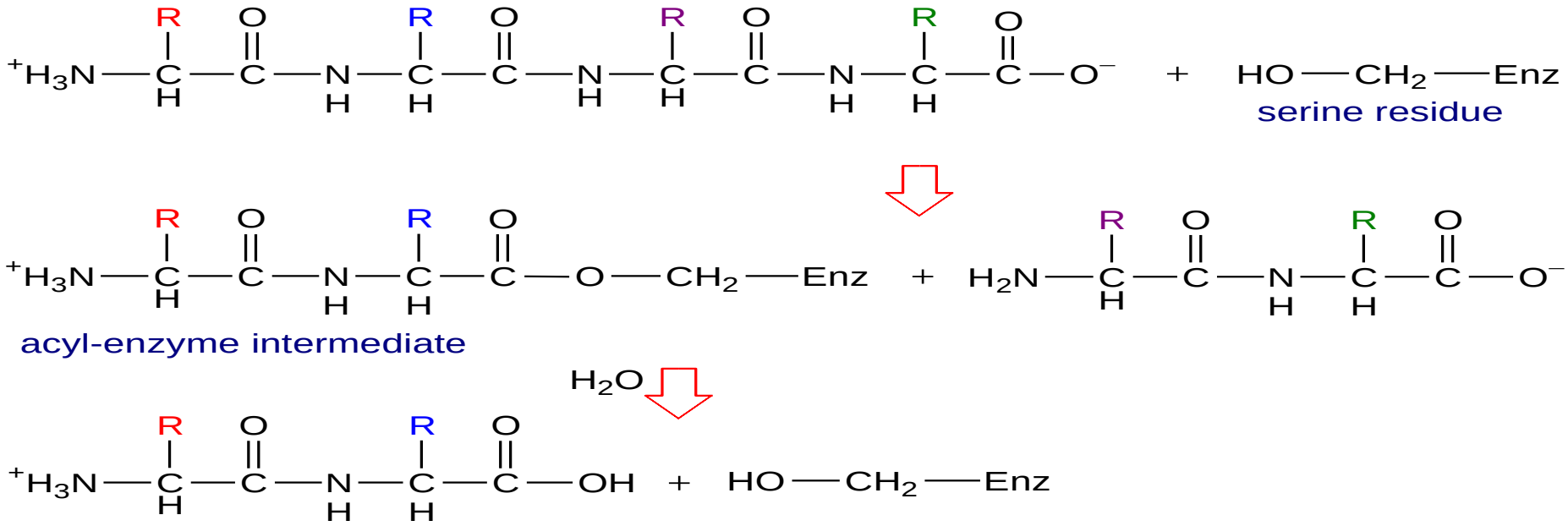


- During attack of the serine hydroxyl oxygen, a proton is transferred from the serine hydroxyl to the imidazole ring of the histidine, as the adjacent aspartate carboxyl is H-bonded to the histidine.





Catalytic Mechanism

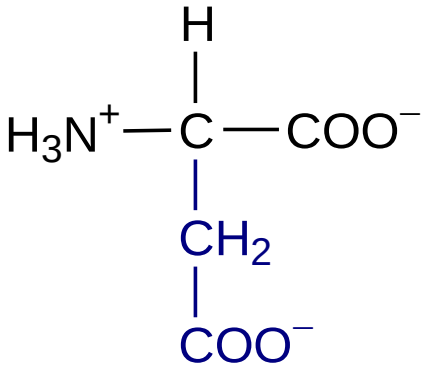


- During catalysis, there is nucleophilic attack of the **hydroxyl O** of a **serine** residue of the protease on the carbonyl **Carbon** of the peptide bond that is to be cleaved.
- An **acyl-enzyme intermediate** is transiently formed.
- In this diagram a small peptide is shown being cleaved, while the usual substrate would be a larger polypeptide.
- Hydrolysis of the ester linkage yields the second peptide product.

Aspartate proteases

- the digestive enzyme **pepsin**
- Some proteases found in **lysosomes**
- the kidney enzyme **renin**
- **HIV-protease**

aspartate (Asp)



➢Two aspartate residues participate in **acid/base catalysis** at the active site.

➢In the initial reaction, one aspartate accepts a proton from an active site **H_2O** which attacks the carbonyl carbon of the peptide linkage.

➢Simultaneously, the other aspartate donates a proton to the oxygen of the peptide carbonyl group.

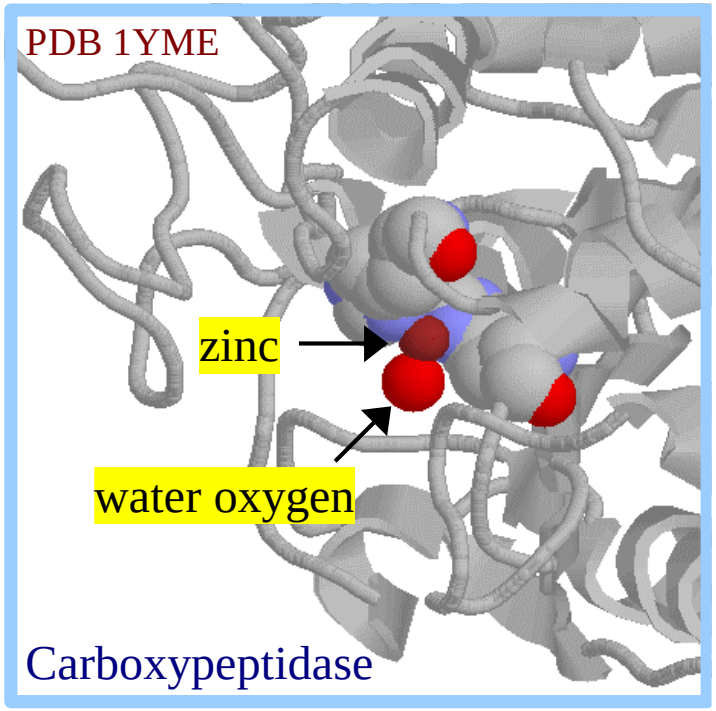
Zinc proteases (metalloproteases)

- Digestive enzymes **carboxypeptidases**
- **Matrix Metalloproteases** (**MMPs**), secreted by cells
- One **lysosomal** protease.

➢Some MMPs (e.g., collagenase) are involved in **degradation of extracellular matrix** during tissue remodeling.

➢Some MMPs have roles in cell **signaling** relating to their ability to release cytokines or growth factors from the cell surface by **cleavage** of membrane-bound **pre-proteins**.

- A zinc-binding motif at the active site of a **metalloprotease includes two His** residues whose imidazole side-chains are ligands to the **Zn^{++}**.
- Colors in Carboxypeptidase image at right: **Zn, N, O**

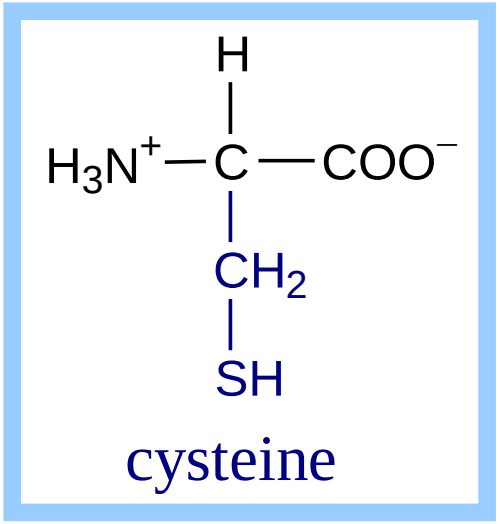


- During catalysis, the **Zn^{++}** promotes nucleophilic attack on the carbonyl carbon by the oxygen atom of a **water** molecule at the active site.
- An active site **base** (Glu in Carboxypeptidase) facilitates this reaction by extracting H^+ from the attacking H_2O.

Cysteine proteases

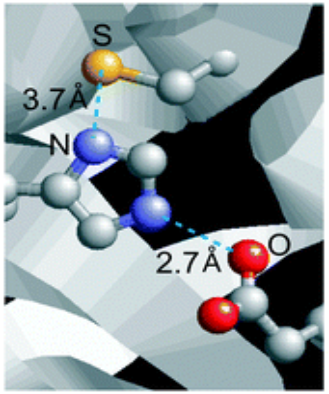
Mechanism of Action

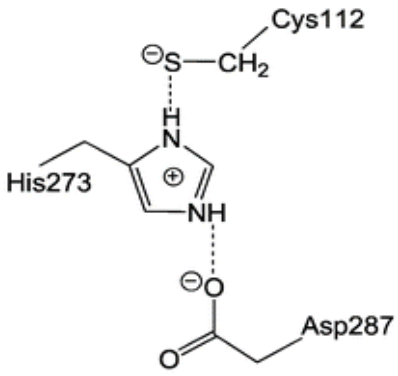
- Acid-base catalysis
- Covalent catalysis



cysteine

- A proton donated from sulfahydrl group of cysteine residue to adjacent imidazole group of histidine residue (Acid-base catalysis)
- A nucleophilic attack by thiolate anion (SH) on - The carbonyl group carbon of Peptide bond - (Covalent catalysis)
- Tetrahedral intermediate formed
- A **thioester** linking the new carboxy-terminus to the cysteine thiol
- This intermediate breaks down followed by hydrolysis – release peptides
- Donate proton back to cysteine and enzyme regenerated





Cysteine proteases:

- **Papain** is a well-studied **plant** cysteine protease.
- **Cathepsins** are a large family of **lysosomal** cysteine proteases, with varied substrate specificities.
- **Caspases** are cysteine proteases involved in activation & implementation of **apoptosis** (programmed cell death).

Caspases get their name from the fact that they cleave on the carboxyl side of an aspartate residue.

- **Calpains** are **Ca^{++}-activated** cysteine proteases that cleave intracellular proteins involved in cell motility & adhesion.

They regulate processes such as cell migration and wound healing.

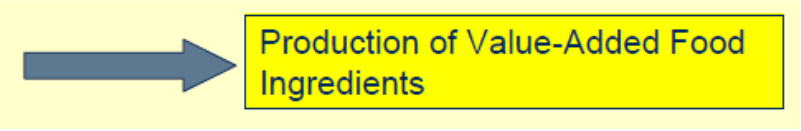
Protease Applications in Food Processing

Basic rationale: Proteases are a powerful tool for modifying the properties of food proteins.

- Improved digestibility
- Improved solubility
- Modified functional properties: emulsification, fat-binding, water-binding, foaming properties, gel strength, whipping properties, etc.
- Improved flavor & palatability
- Improved processing: viscosity reduction, improved drying, etc.

Protease Applications in Food Processing

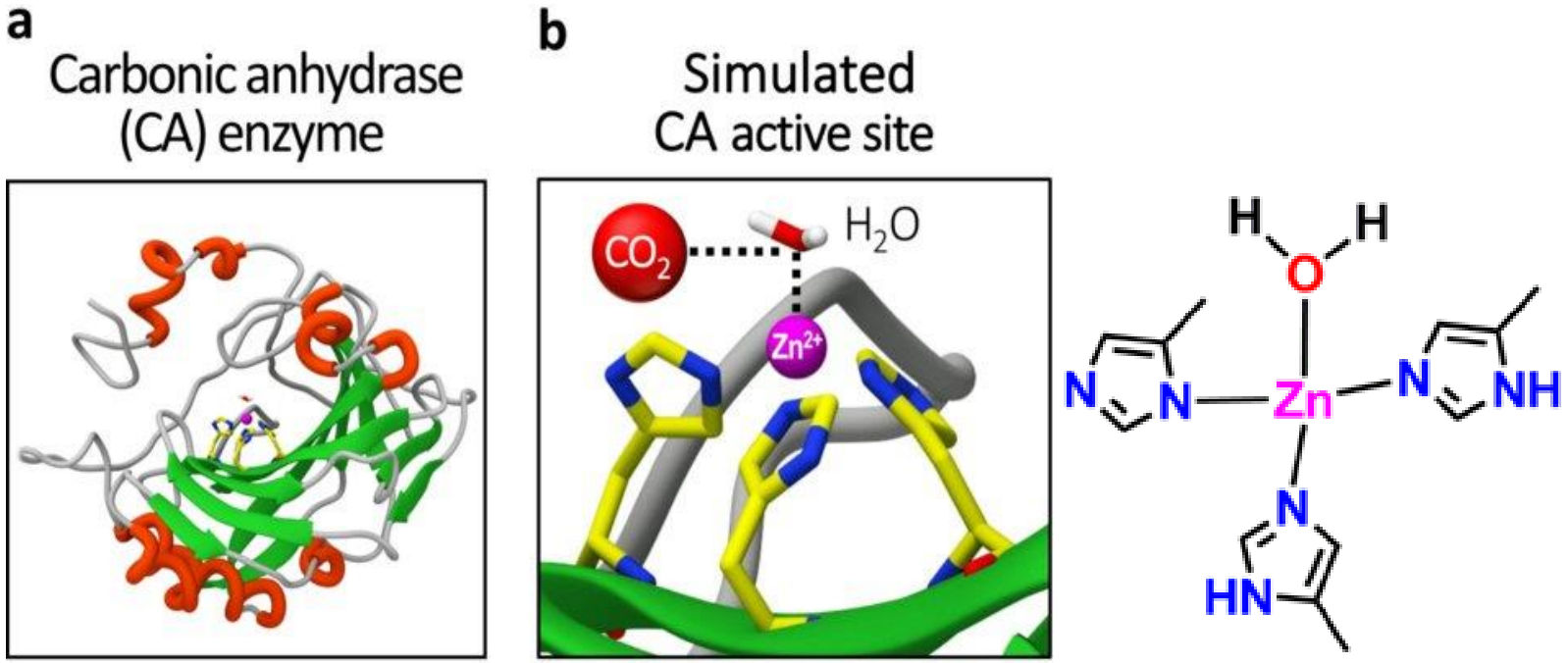
- Proteases are also used in a wide range of foods & food processing applications.
- **Dairy**: milk coagulation, flavor development
- **Baking**: gluten development
- **Fish & seafood processing**: fish meals, enhanced oil recovery, aquaculture
- **Animal protein processing**: improved digestibility, reduced allergenicity, improved flavor, meat tenderization
- **Plant protein processing**: improved functionality & processing, generation of bio-active peptides.
- **Yeast hydrolysis**: flavor compounds.



Nonfood Protease Applications

- Medicine
- Pharmacology & drug manufacture
- Laundry & dishwashing detergents (#1)
- Hard surface cleaning formulations
- Contact lens cleaning formulations
- Waste treatment
- Industrial applications
- Fermentation (fuel EtOH, etc.)
- Chondroitin & heparin production
- Animal feed additives
- Digestive supplements

2) CARBONIC ANHYDRASE

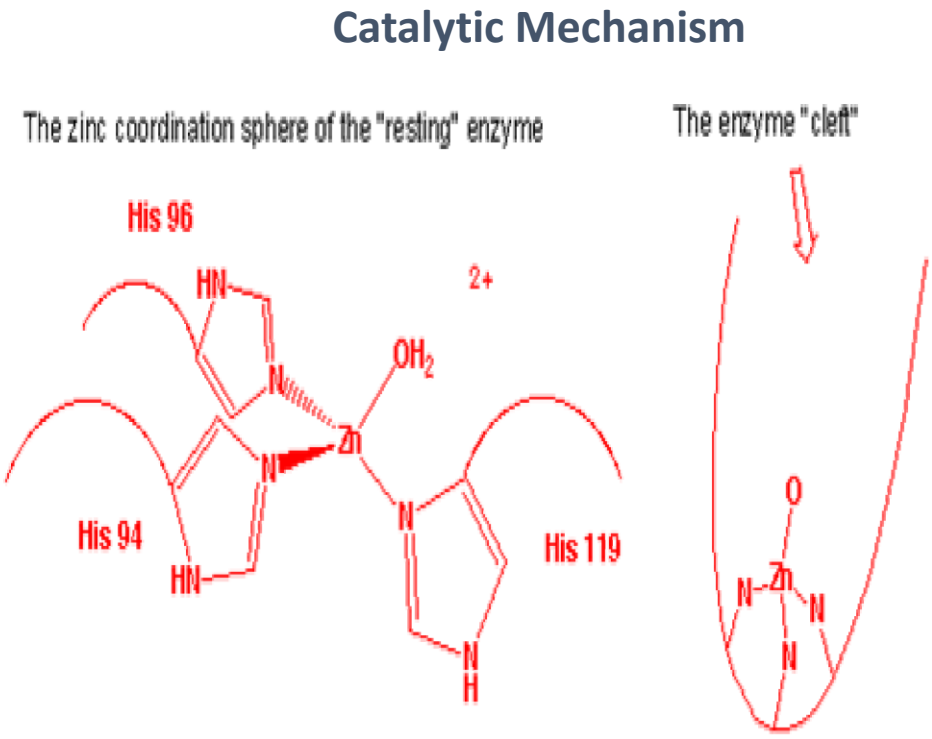


Carbonic anhydrase

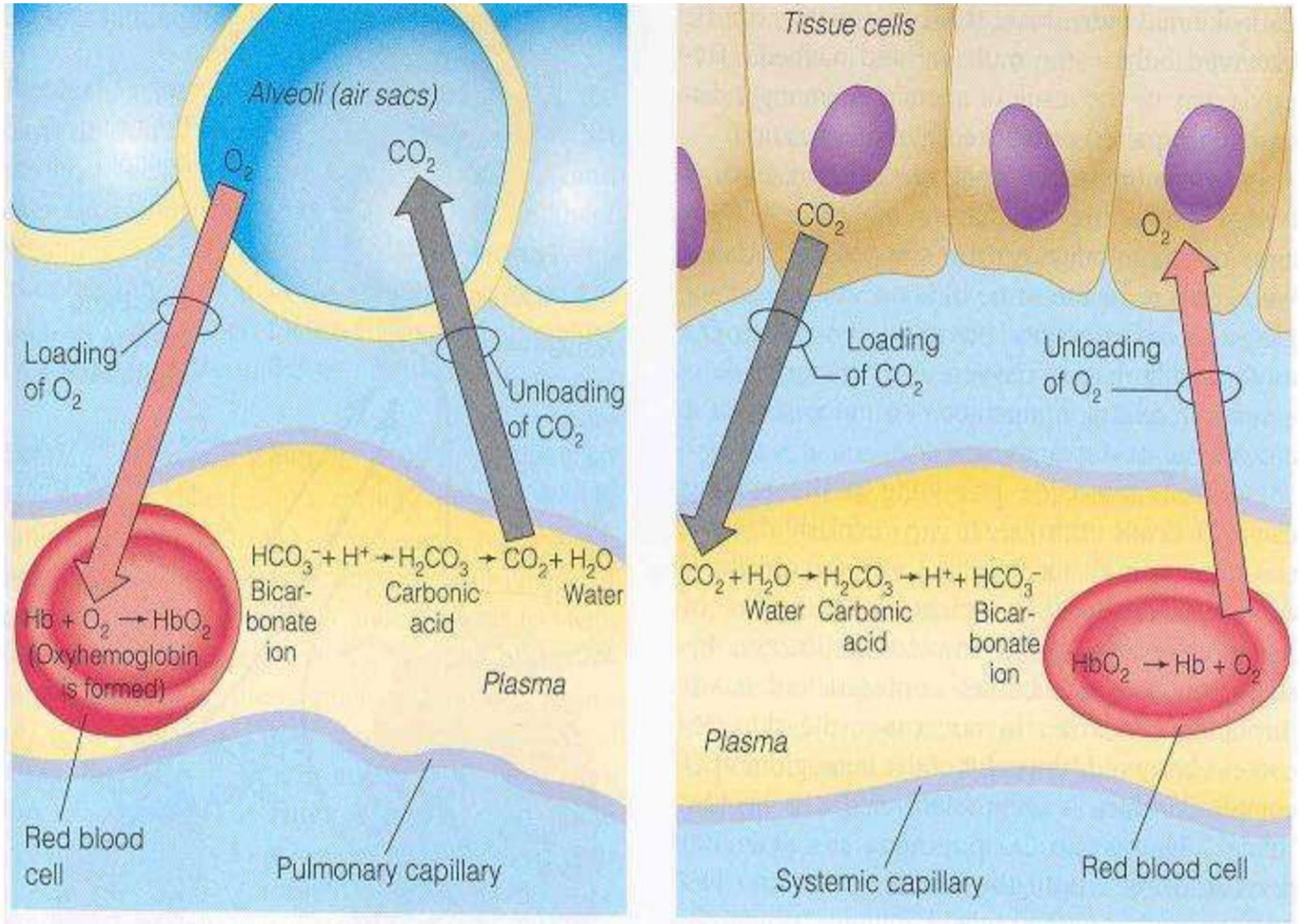
Mechanism of Action

- Acid-base catalysis
- Metal-ion catalysis

- Carbonic anhydrase, a zinc metallo-enzyme (cofactor), is wide spread throughout the bacterial, plant and animal kingdoms.
- It is generally considered one of the most efficient enzymes known as it catalyzes the **reversible interconversion between CO_2 and the bicarbonate ion**.



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



Diagrammatic representation of the major means of oxygen (O_2) and carbon dioxide (CO_2) loading and unloading in the body.

- Carbon dioxide harmful, so converted to soluble form – in blood occurs due to conversion of carbonic acid (formed by reversible hydration)
- Carbonic acid reversibly deprotonated to bicarbonate ion (facilitated to transport via blood)
- Carbonic anhydrase – Active site – Zinc cofactor with imidazole side chain of 3 histidine
- This binds with water – Deprotonation of water -Slightly negative charged hydroxyl ion coordinates with Zinc atom (positively charged)
- Now carbon dioxide in active site react with – nucleophilic hydroxyl ion – form bicarbonate ion

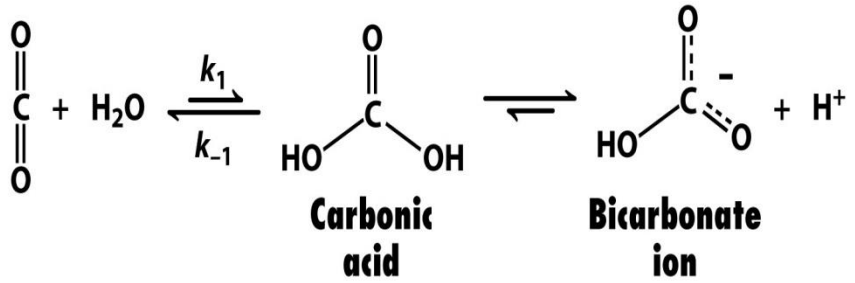
1) **carbon dioxide**

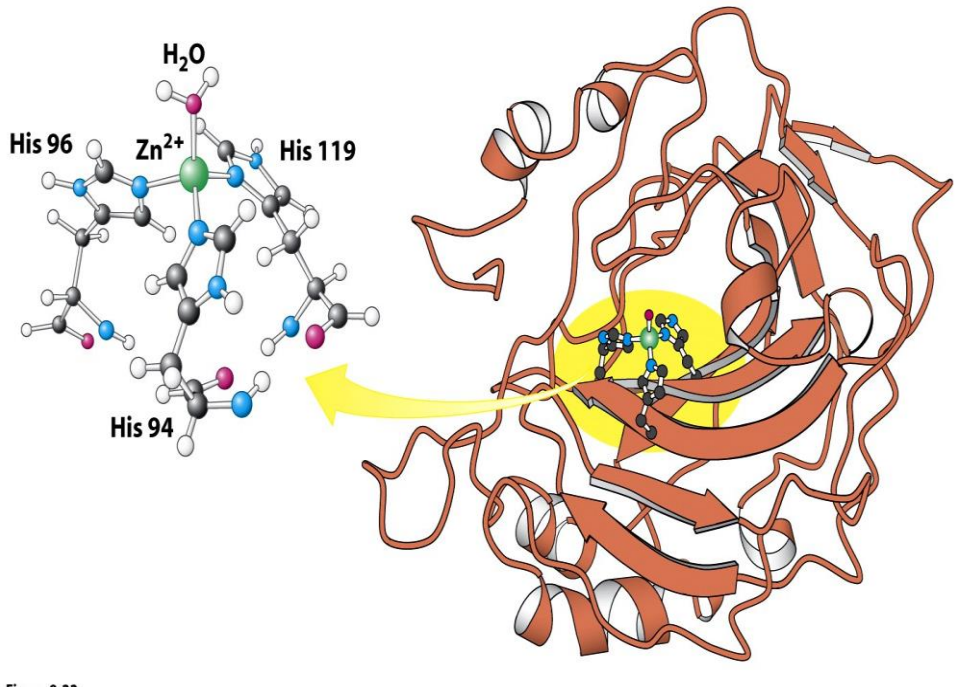
- a major end product of aerobic metabolism

2) **carbonic anhydrase** as Zn^{2+} metalloenzyme

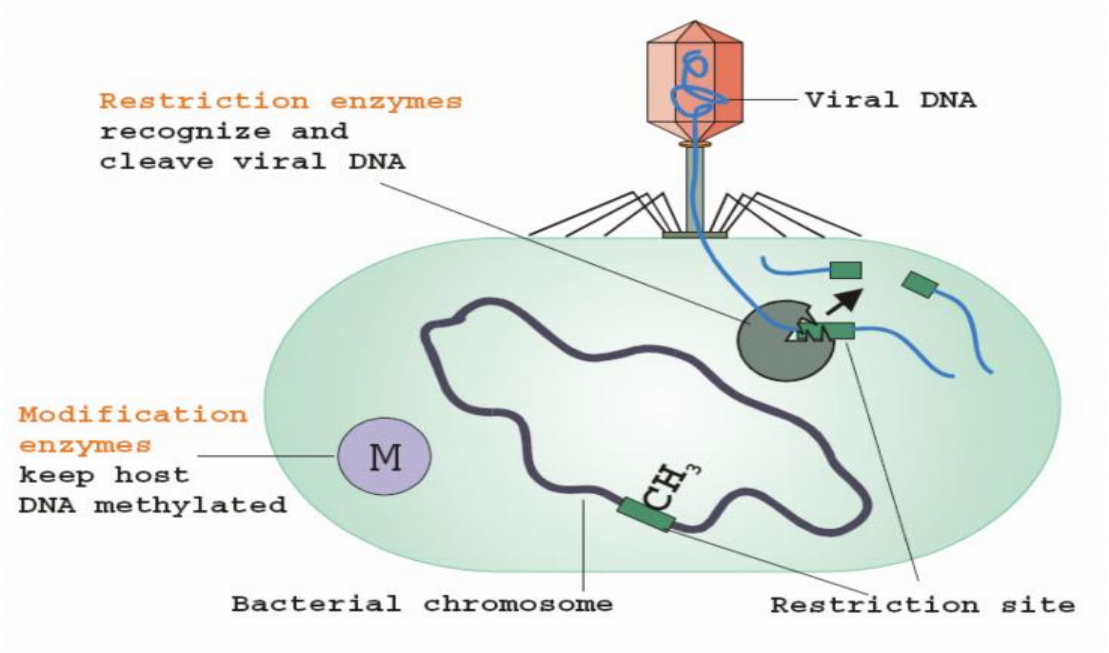
  - positive charge: strong but kinetically labile bonds
  - more than one oxidation state
  - Zn^{+2}: four or more ligands
  - three histidines + water

3) **Zinc activation of water**





3) Restriction Enzymes



Mechanism of Action

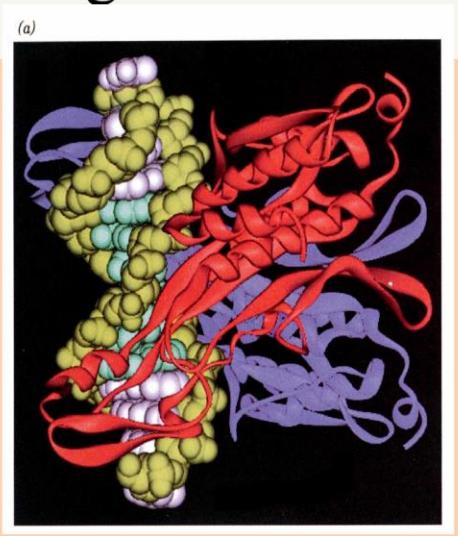
- Metal-ion catalysis
- High substrate specificity
- Modifications to substrate preventing unwanted side reactions

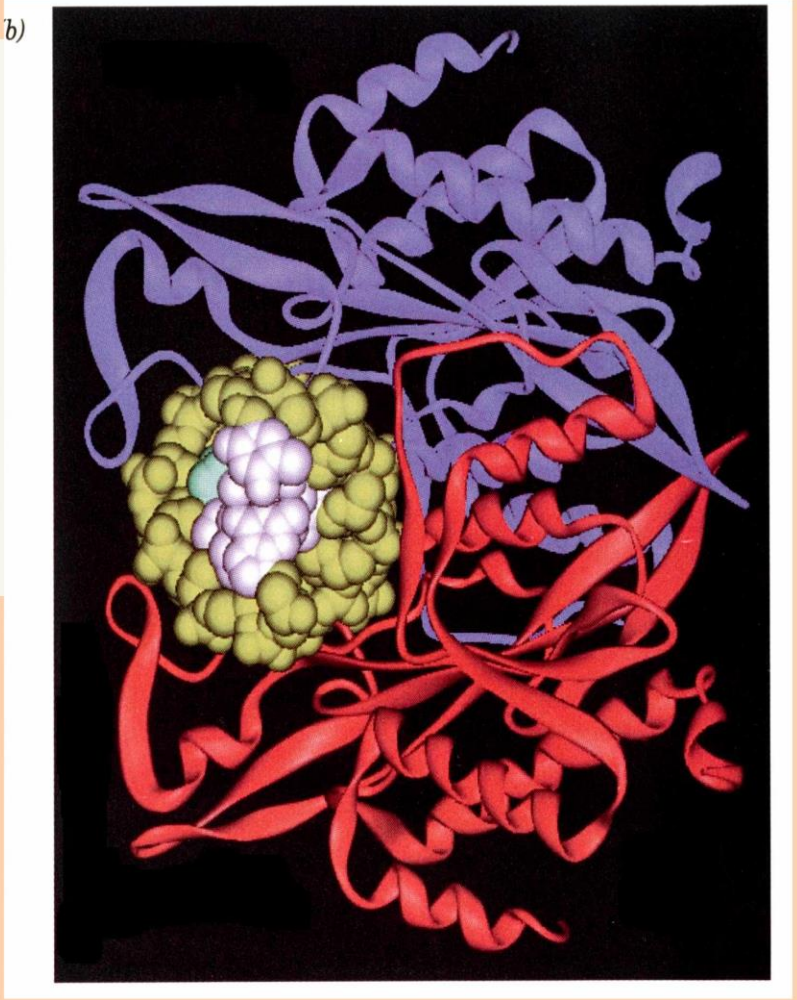
Restriction Enzymes

- Are **DNA-cutting enzymes**
- Often called **restriction endonucleases or molecular scissors**
- A restriction enzyme recognizes and cuts DNA only at a particular sequence (EX: Eco RV) of nucleotides known as recognition sites
- These enzymes catalyses the hydrolysis of phosphodiester bond in the center of recognition sites, thereby producing DNA fragments

Structure of a restriction endonuclease

Restriction enzymes are usually dimers of identical subunits, analogous to the symmetry of their binding sites in DNA.

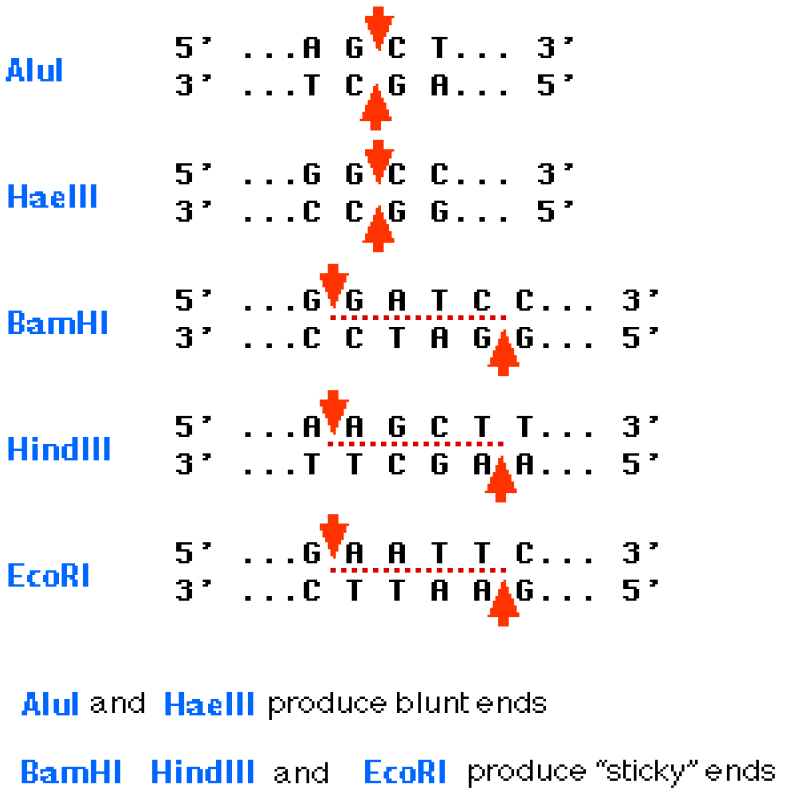




- Most restriction enzymes occur naturally in bacteria.
- Protect bacteria against viruses by cutting up viral DNA.
- Bacteria protects their DNA by modifying possible restriction sites (methylation).
- More than 400 restriction enzymes have been isolated.
- Names typically begin with 3 italicized letters.
    - Enzyme Source
    - *Eco*RI *E. coli* RY13
    - *Hin*dIII *Haemophilus influenzae* Rd
    - *Bam*HI *Bacillus amyloliquefaciens* H
- Many restriction sites are palindromes of 4-, 6-, or 8-base pairs.
- Short restriction site sequences occur more frequently in the genome than longer restriction site sequences, e.g., (1/4)n.

Patterns of DNA cutting by restriction enzymes

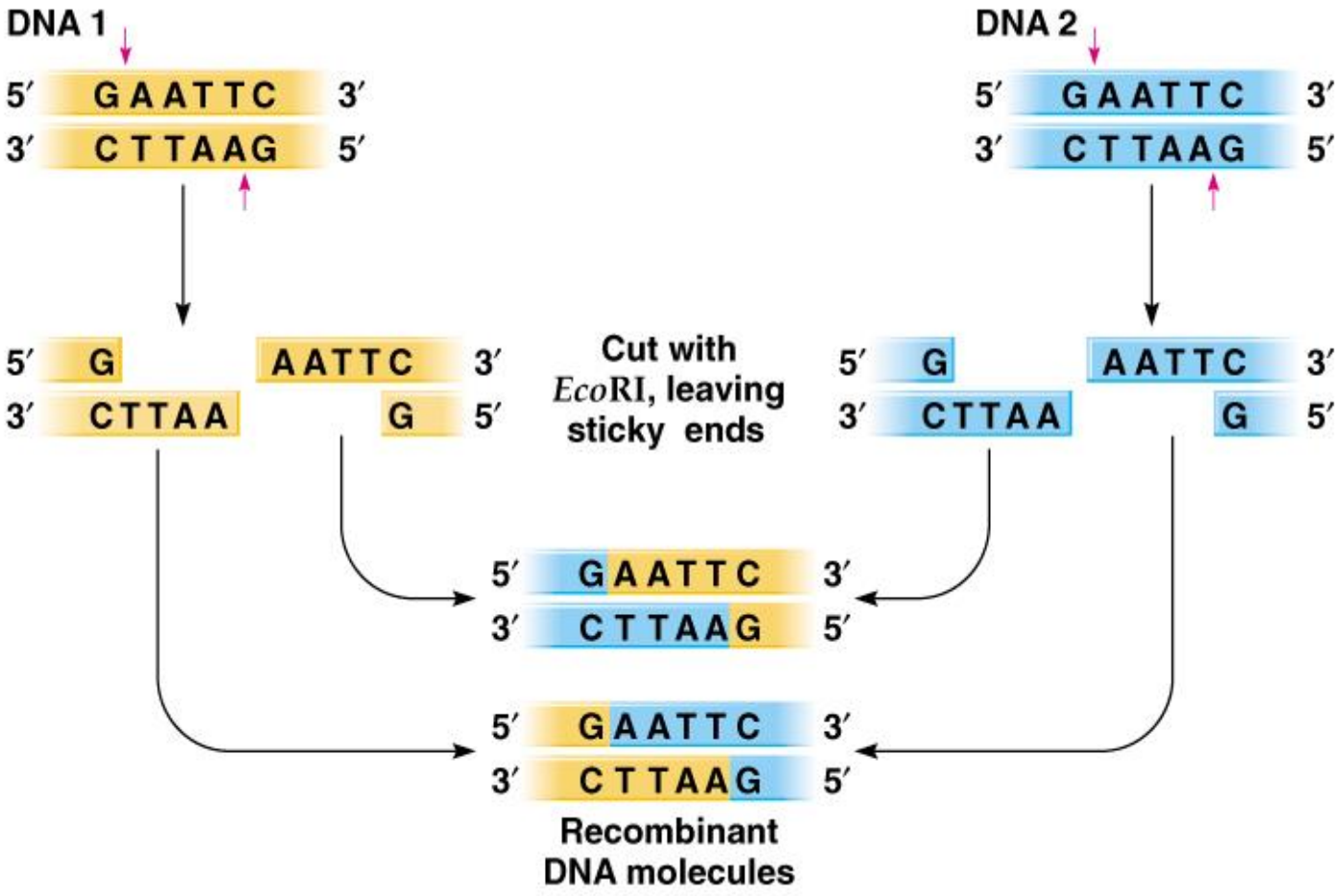
- Base pairing between overhangs with complementary sequences enables two fragments to be joined by another enzyme DNA Ligase.
- A sticky end fragment can be ligated not only to fragment from which it was originally cleaved, but also to any other fragment with a compatible sticky end.
- The result is a molecule of recombinant DNA (rDNA)



Recombinant DNA

DNA molecules constructed *in vitro*, consisting of DNA from 2 or more sources (i.e. cloning vector with foreign DNA inserted)

Cut and ligate 2 DNAs with *Eco*RI ---> recombinant DNA



Applications of Recombinant DNA technologies

- **Pharmaceutical products**
  - insulin – cheaper and safer compared to animal insulin
  - vaccine sub-unit (against hepatitis B) – safer since will not be infected by pathogens
  - DNA of vaccines against malaria, influenza etc.
- **Gene therapy**
  - replacing defective or missing gene with normal gene using adeno~ and retrovirus as vector
- **Gene silencing**
  - known as RNA interference (RNAi) using dsRNA called short interfering RNA (siRNA) that target specific gene (mRNA) and degrade it
- **Human Genome Project (HGP)**
  - 3 billion human genome nucleotides have been sequenced
  - 20,000 – 25,000 genes
  - < 2% encodes function products, 98% intron, transposon (repeating sequence) etc
  - may provide diagnostics and treatments

4) Nucleoside Monophosphate Kinase (NMP Kinase)

MECHANISM USED

- Metal Ion Catalysis
- Proximity Orientation

- NMP kinase are active only in presence of divalent metal ions (Mg^{2+} and Mn^{2+})

1. METAL ION CATALYSIS - Metal ion complexation (formation of a complex) only with substrate instead of enzyme
2. PROXIMITY ORIENTATION - Induced fit mechanism

4) Nucleoside Monophosphate Kinase (NMP Kinase)

- Catalyze the transfer of a phosphate group from NTP to NMP
- If no NMP kinase, then water hydrolyzes NTP, which is an unwanted reaction

$$ATP + H_2O \rightarrow ADP + H_2PO_4 \text{ (Dihydrogen phosphate anion) (Unwanted)}$$

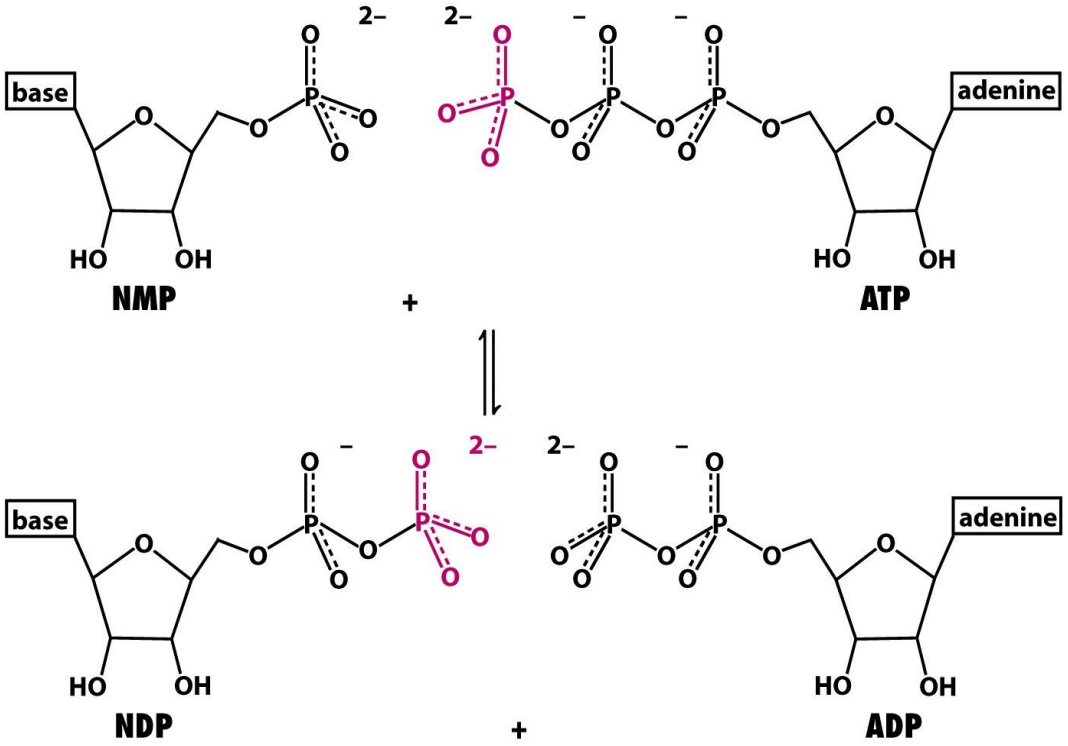
- **NMP Kinase -** Phosphorylates the monophosphate nucleotides

$$NMP + ATP \xrightarrow{\textbf{NMP Kinase}} NDP + ADP$$

- Metal ion coordinates with ATP substrate makes 3D conformation, ATP-Mg^{2+} complex
- This brings NMP in close proximity

AMP + ATP ↔2 ADP by **adenylate kinase**

GMP + ATP ↔GDP + ADP by **guanylate kinase**



Purine synthesis

Purine monophosphates must be converted to the triphosphate forms before they can be utilized in DNA synthesis. This is done in two steps.

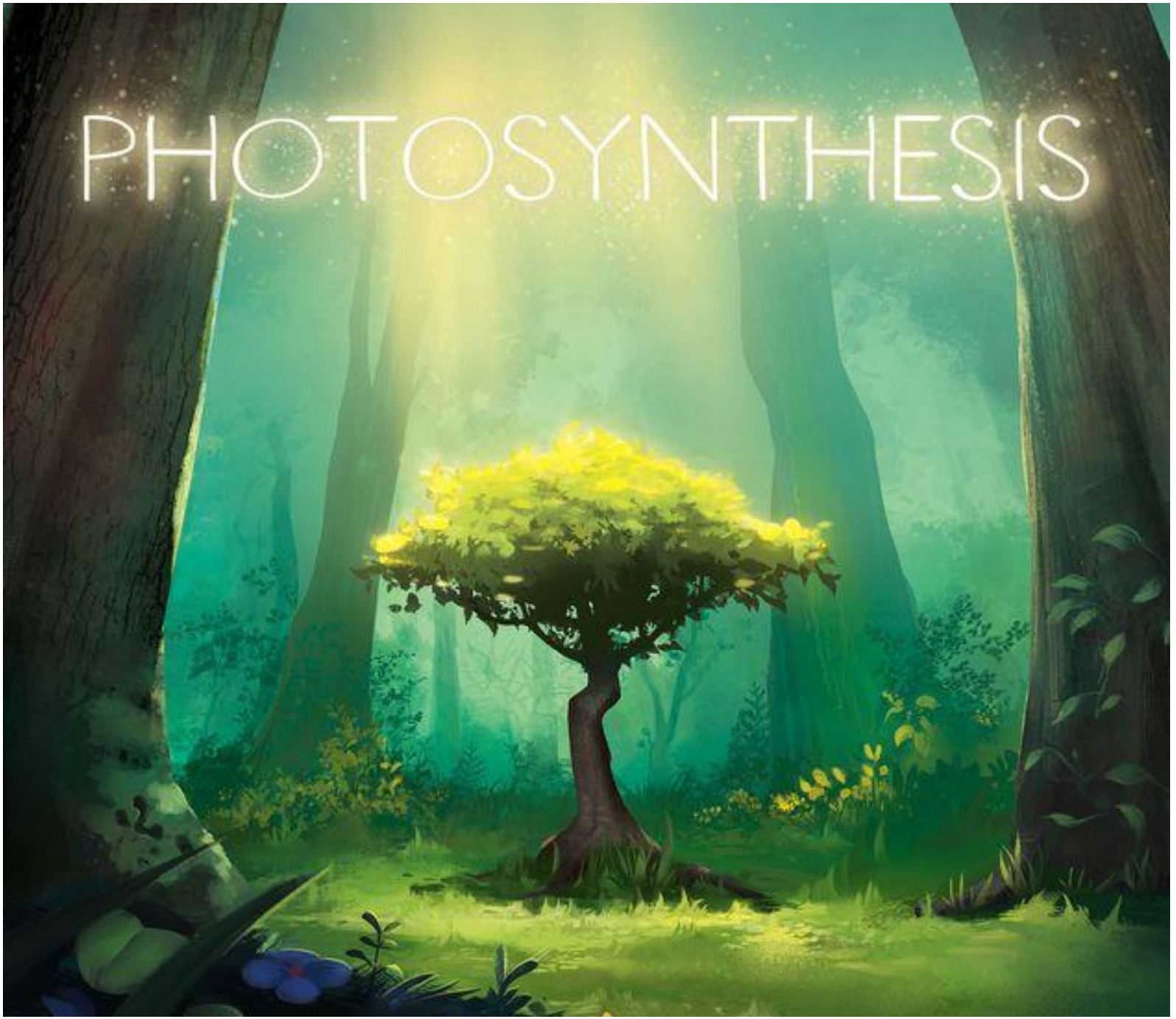
1. The nucleoside monophosphate kinase (NMP kinase) class of enzymes phosphorylates the monophosphate nucleotides.

$$AMP + ATP \leftrightarrow 2\ ADP \text{ by adenylate kinase}$$

$$GMP + ATP \leftrightarrow GDP + ADP \text{ by guanylate kinase}$$

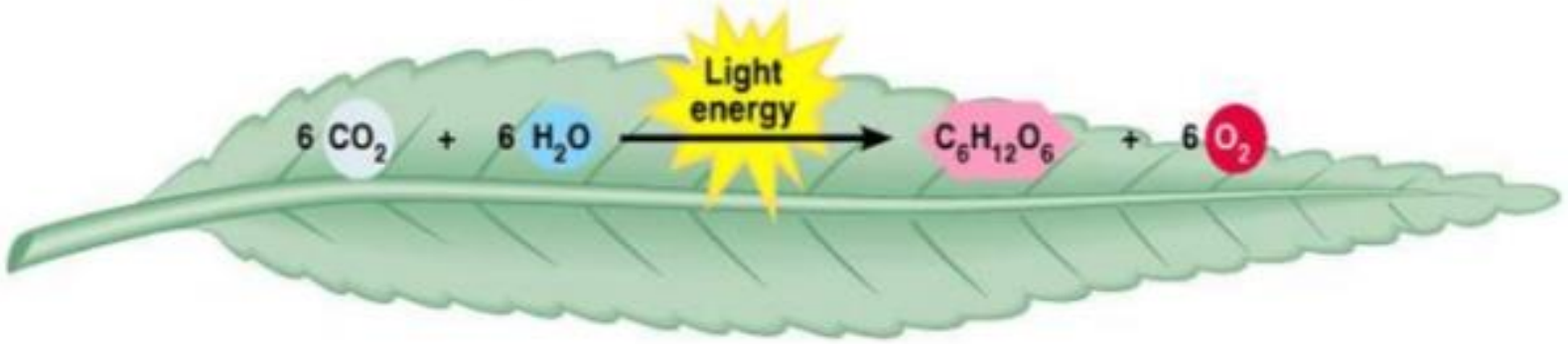
2. The nucleoside diphosphate kinases (NDP kinase) add another phosphate group to the nucleotide diphosphates

$$GDP + ATP \leftrightarrow GTP + ADP$$


PHOTOSYNTHESIS

PHOTOSYNTHESIS

- Is the process by which autotrophic organisms use light energy to make sugar and oxygen gas from carbon dioxide and water.
- Occurs in plants, algae and some prokaryotes
- Anabolic (small molecules combined)
- Endergonic (stores energy)
- Stored as carbohydrate in their bodies.

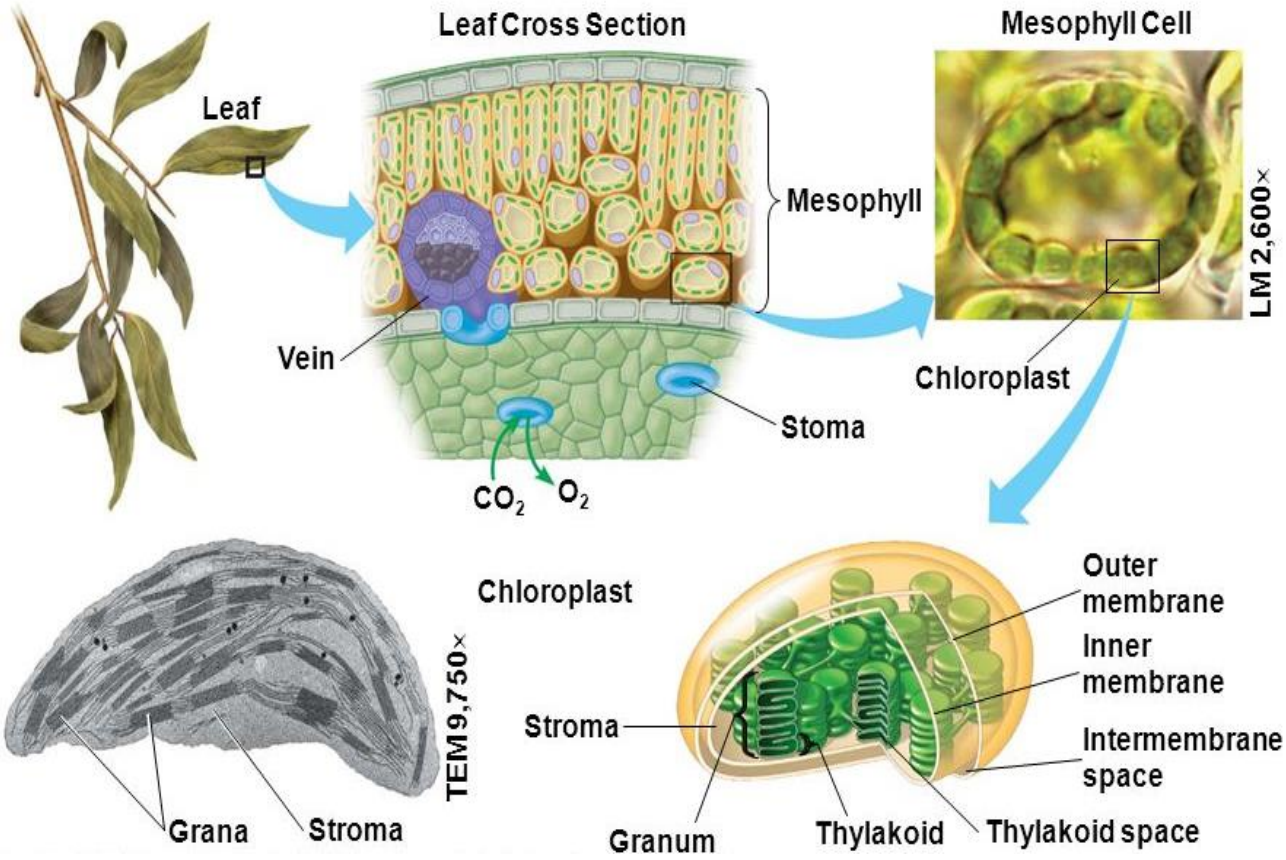


•Plants are Autotrophs – produce their own food (glucose)
•Process - Photosynthesis

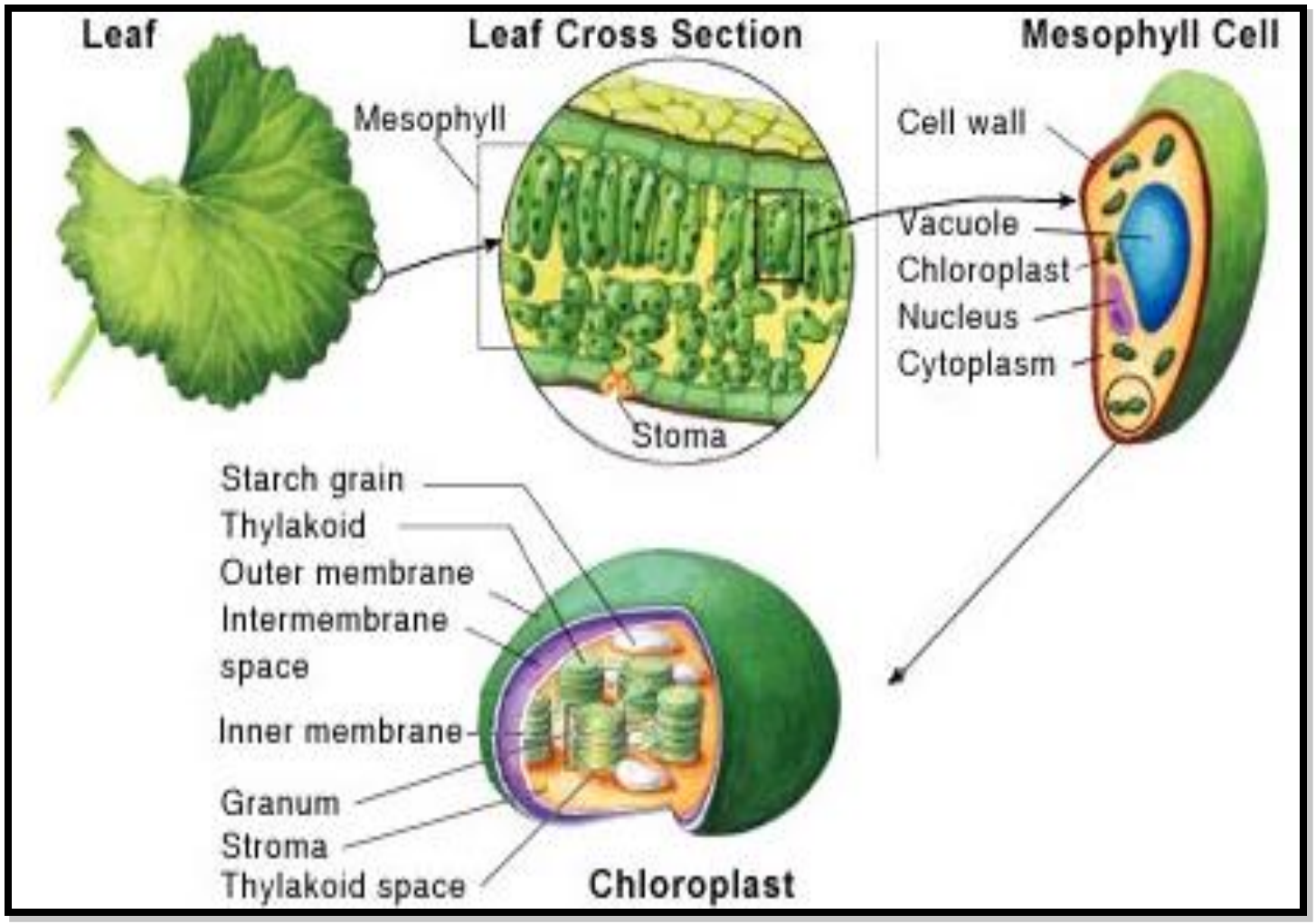
Mainly occurs in the leaves:

a. **Mesophyll cells**

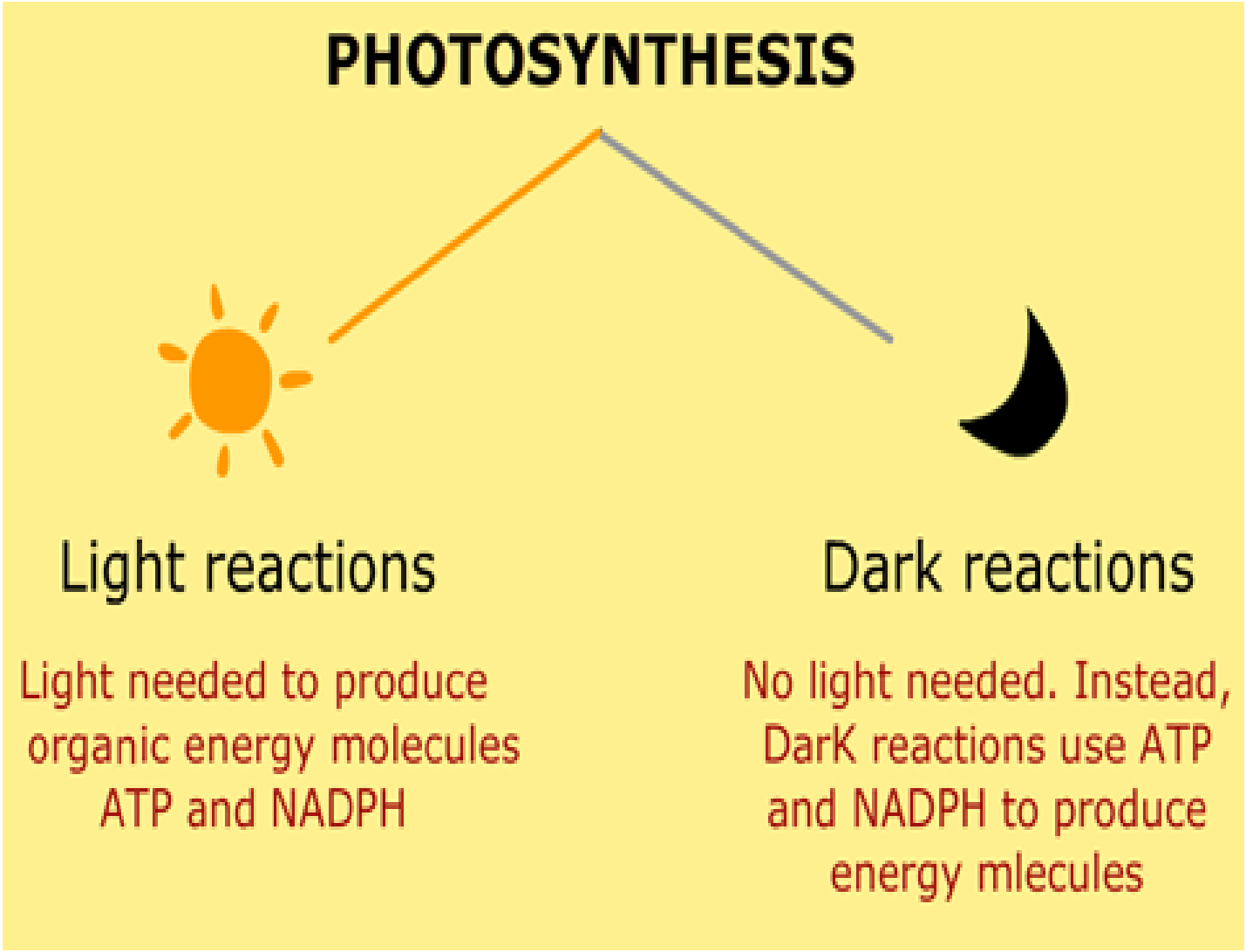
b. **Stoma - pores**



PHOTOSYNTHESIS IN CHLOROPLAST WITH CHLOROPHYLL AS THE KEY ENERGY-TRAPPING MOLECULES



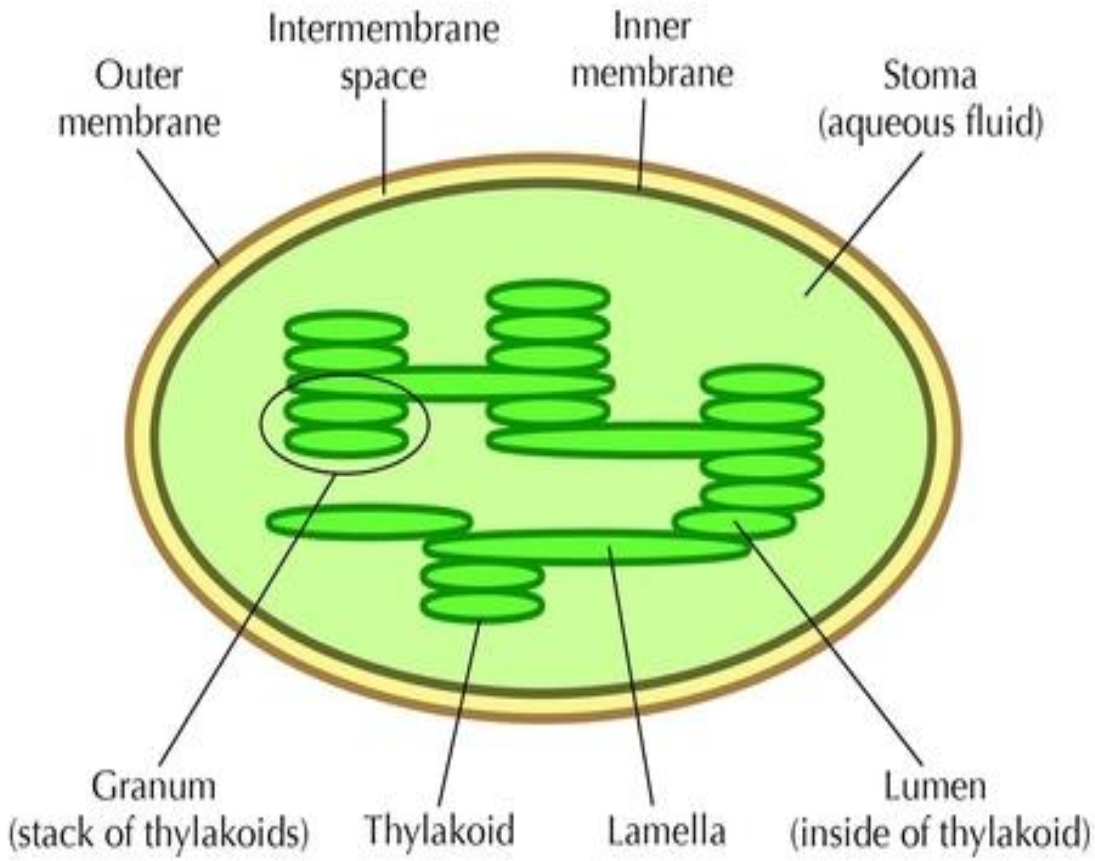
PHOTOSYNTHESIS



PHOTOSYNTHESIS IN CHLOROPLAST

Chlorophyll as energy trapping molecule

STRUCTURE OF CHLOROPLAST



PHOTOSYNTHESIS IN CHLOROPLAST

- Chloroplast – 5µm length with outer, inner and thylakoid membrane
- Chloroplast – Organelle in plant leaf cell – place for photosynthesis

STRUCTURE OF CHLOROPLAST

Stroma

- Space between inner and thylakoid membrane, thylakoids embedded
- Has Enzymatic machinery – Uses ATP & NADPH to reduce CO_2 to Sugars

Thylakoid

- Stacked to form granum
- Has Light harvesting machinery - Light harvesting proteins, Photosystems, Electron Transport Chain & ATP synthase
- Produce NADPH & ATP in stroma

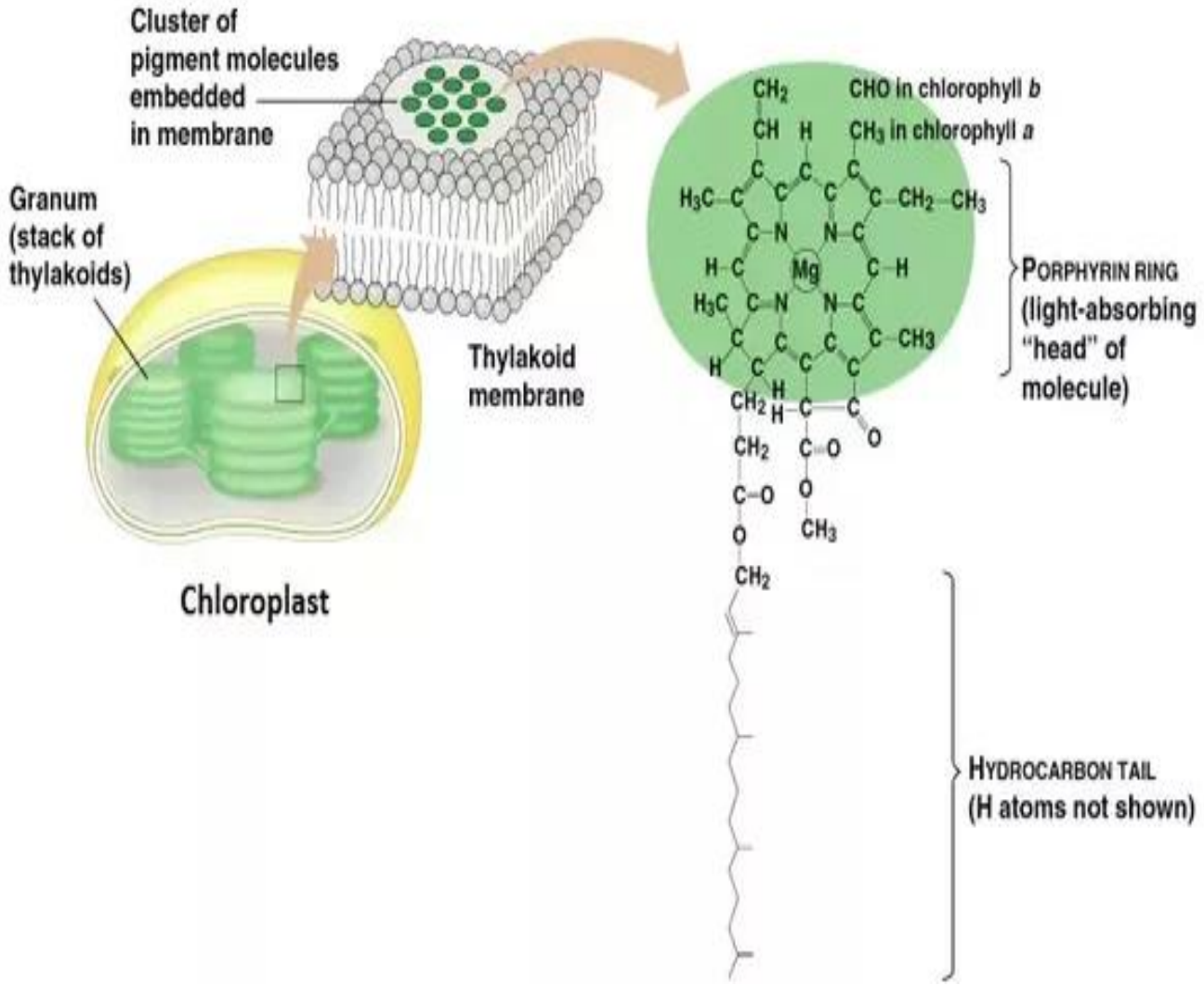
Lamellae

- Grana interconnected with lumen like stroma lamellae

Chlorophyll

- Present in thylakoid
- Green, Photo-receptor, Tetrapyrole with nitrogen coordinated to **magnesium ion center**

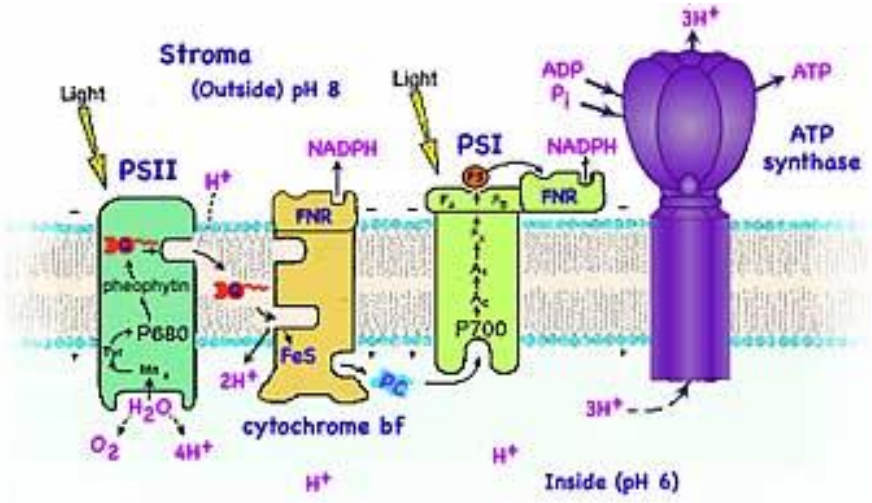
CHLOROPLAST CONTAINING CHLOROPHYLL



PHOTOSYSTEMS IN PHOTOSYNTHESIS

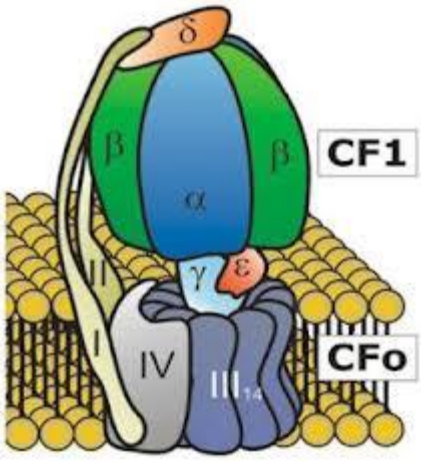
- Photosystem – **2** in number – **Photosystem I (PSI) – P700** & **Photosystem II (PSII) – P680**
- Photosystem (PSI & PSII) – A thylakoid transmembrane protein complex – follow a co-operative interaction to – Harvest light energy from 8 photons
- Light-induced charge separation & electron transport chain – Stores 2 molecule of NADPH
- 2 molecule of water oxidized to produce 1 molecule of oxygen

$2H_2O + NADP^+ + 8h\gamma$ (Photon) $\rightarrow O_2 + 2NADPH$



ATP SYNTHESIS IN CHLOROPLAST

- **Using ATP synthase motors**
- During photosynthesis – proton sourced from stroma & transferred to lumen
- Thylakoid membrane impermeable to proton, lumen more acidic (pH – 4) than stroma – so proton gradient across membrane
- Generates proton-motive force across membrane



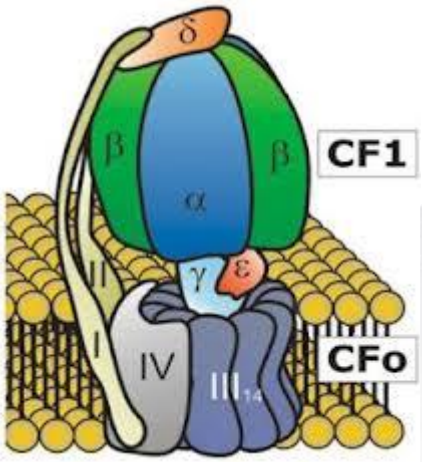
ATP SYNTHESIS IN CHLOROPLAST

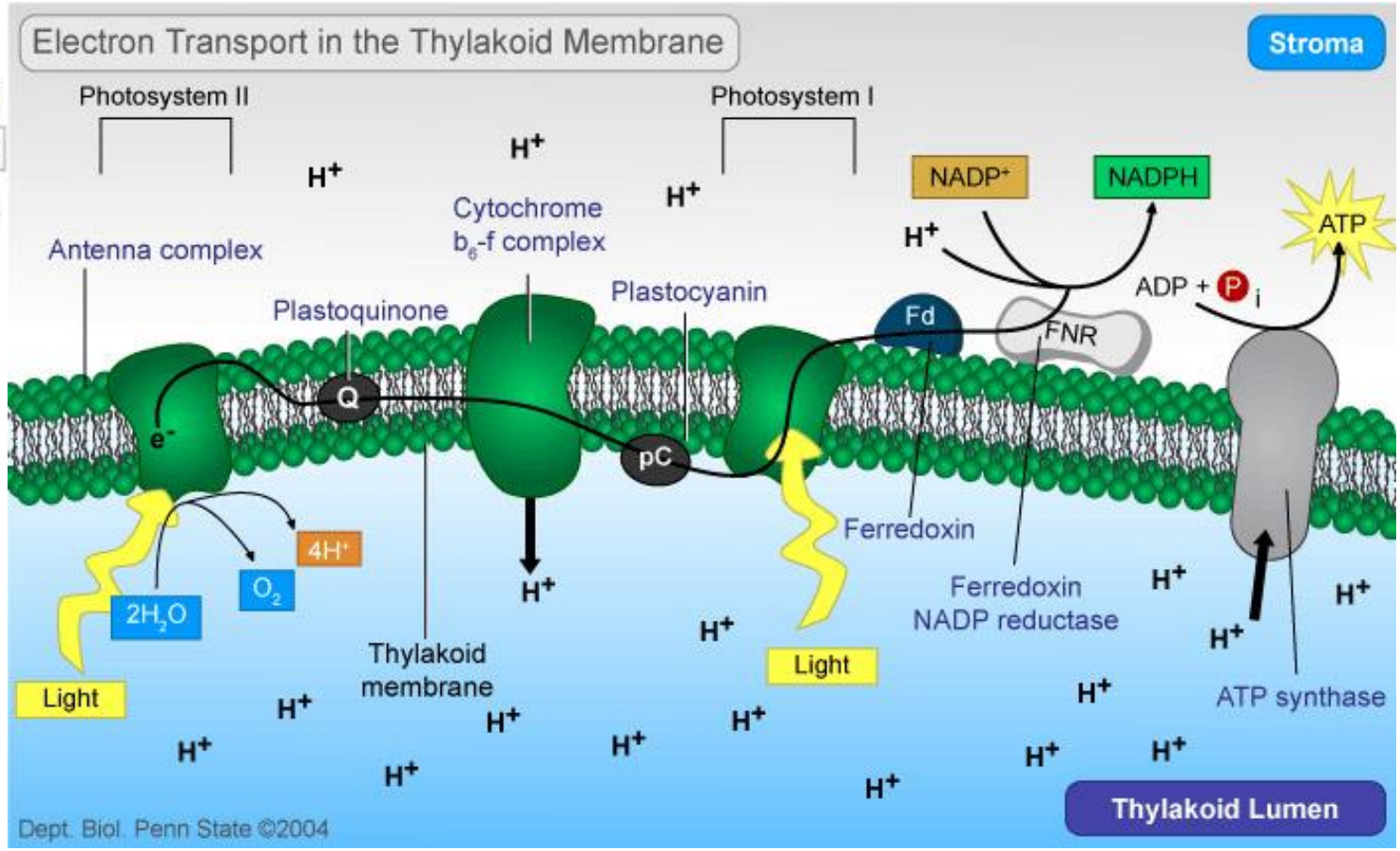
- ATP synthase complex – 2 units (CF_0 and CF_1) - helps to transport protons
- CF_0 - Closer to lumen side of membrane – Routes proton from lumen to stroma
- CF_1 - Present on stromal side – Catalyze the formation of ATP from ADP
- Here $12H^+$ are translocated for catalytic phosphorylation of 3 ADP molecules

PHOTOPHOSPHORYLATION

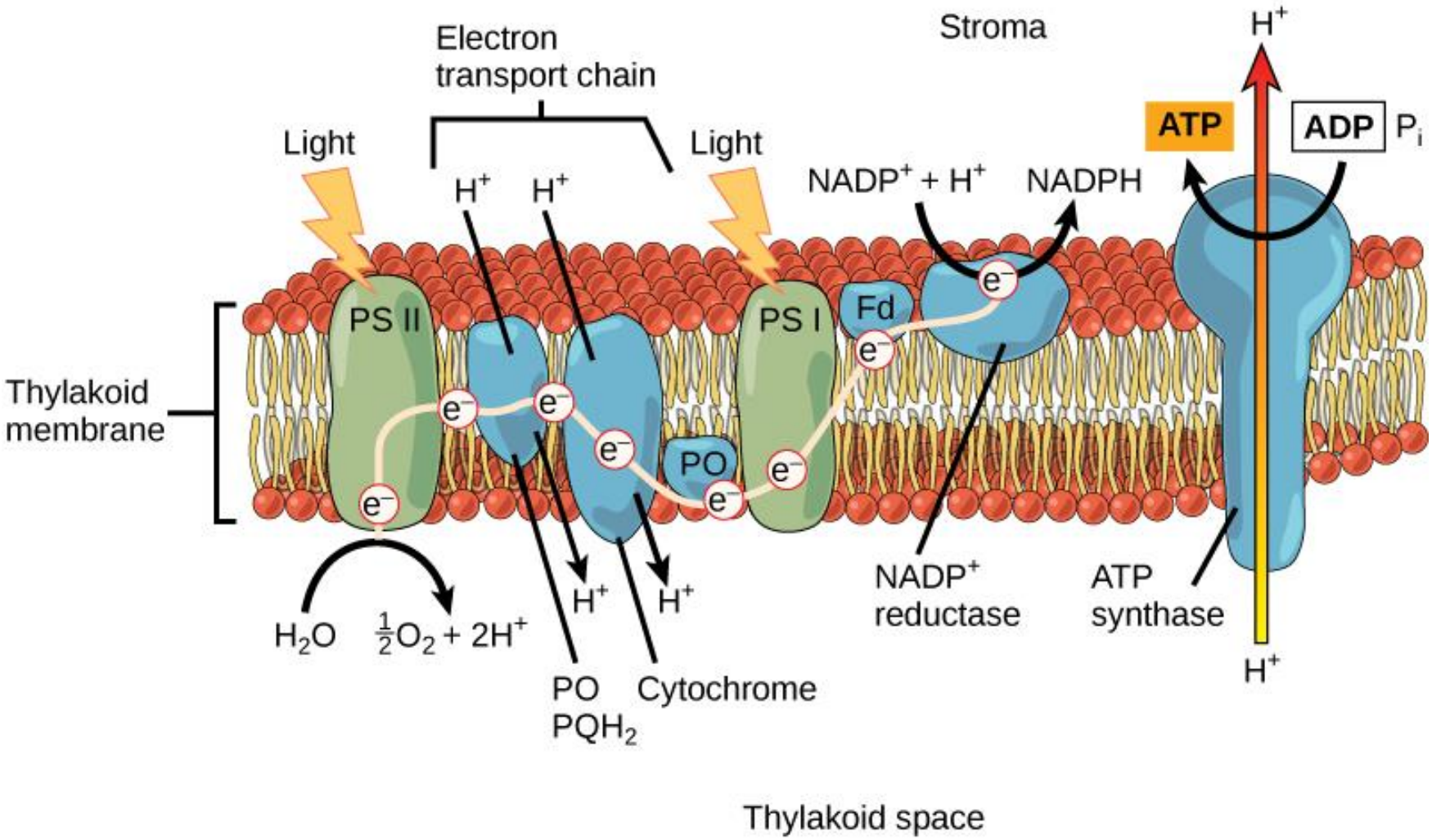
- **3 ADP → 3 ATP →** used for **Dark reaction** to convert CO_2 to carbohydrate (photophosphorylation)

ATP SYNTHESIS IN CHLOROPLAST





LIGHT REACTION



LIGHT REACTION

- Occur in presence of light
- Photon from sunrays (Light) – excite electron in chlorophyll
- Electron transported through electron-transport chain (ETC) – results in proton-motive force
- **NADPH (in reduced form) and ATP produced during light reaction are released into stroma, which is used in dark reaction**

$2\ H_2O + NADP^+ + 3\ ADP + 8\ h\gamma\ (Photon) \rightarrow O_2 + 2\ NADPH + 3\ ATP$

Net Stoichiometry Equation Of Light Reaction

DARK REACTION
(OR)
CALVIN CYCLE
(OR)
LIGHT-INDEPENDENT REACTION

- Also called **Carbon Fixation or C_3 Fixation**
- 3-carbon molecule called Ribulose Biphosphate (RuBP) is used to regenerate the Calvin cycle
- Takes place in stroma of chloroplast
- Utilize ATP & NADPH to reduce atmospheric carbon dioxide into carbohydrates (hexoses)
- Calvin cycle divided into 3 stages

DARK REACTION (OR) CALVIN CYCLE

- Calvin cycle divided into 3 stages

1) Carboxylation/ Carbon Fixation/ Carbon dioxide Fixation

- Fixing of carbon dioxide by ribulose 1,5-bisphosphate (RuBP) (5 Carbon compound)
- This gets hydrolyzed into 2 molecules of 3-Phosphoglycerate (3PGA)

2) Reduction/ Synthesis

- 3-Phosphoglycerate is reduced to Glyceraldehyde 3-phosphate (3 Carbon compound)
- Glyceraldehyde 3-phosphate unite to form glucose (hexose) (6 Carbon Compound)
- Synthesis of hexose sugar from 2 compound of Glyceraldehyde 3-phosphate

DARK REACTION (OR) CALVIN CYCLE

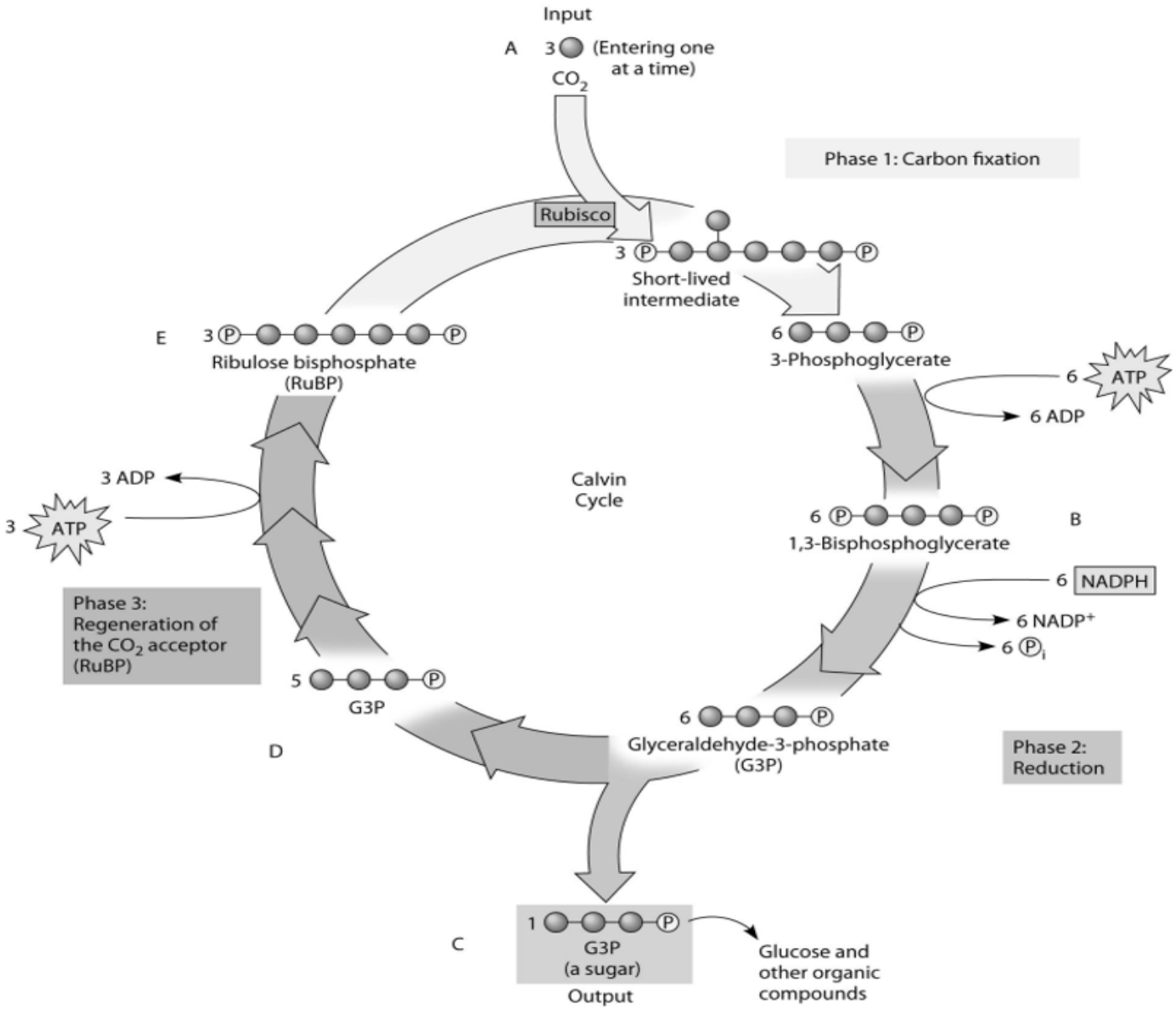
3) Regeneration

- Regeneration of ribulose 1,5-bisphosphate (RuBP)
- RuBP is consumed during carbon fixation. For further carbon fixation cycle to continue, RuBP must be regenerated. This regeneration of RuBP is possible via PCR (photosynthetic carbon reduction) cycle, forming 6 Carbon (hexose), 5 Carbon, 7 Carbon intermediates in carbon shunts
- **Calvin cycle involves Reductive Carboxylation**

$$6\,CO_2 + 6\,H_2O + 18\,ATP + 12\,NADPH \rightarrow C_6H_{12}O_6 + 18ADP + 12\,NADP^+$$

Net Stoichiometry Equation Of Dark Reaction

DARK REACTION (OR) CALVIN CYCLE



PHOTOSYNTHESIS

- Net Stoichiometry for Photosynthesis obtained by multiplying equation of light reaction by 6, and solved to get this;

$$6\ CO_2 + 18\ H_2O + 48\ h\gamma\ (Photon) \rightarrow C_6H_{12}O_6 + 6\ O_2$$

Net Stoichiometry Equation Of Photosynthesis

SIGNIFICANCE OF PHOTOSYNTHESIS

- A fraction of **light energy** is used by plants for photosynthesis to synthesize and **store organic chemical energy reserves in the form of starch, sucrose, micronutrients, etc.,**
- **Herbivores** and man being a **omnivore** (consume both plants and animals) consume the plants and use their carbohydrates as **energy source**.
- Plant produces the **oxygen** is the main source to atmosphere, that we respire.
- **Sun is the ultimate source of all energy** on earth and all organisms are dependent on carbohydrates and oxygen by photosynthesis.

FACTORS AFFECTING THE RATE OF PHOTOSYNTHESIS

- **Amount of available water**
- **Temperature**
- **Amount of available light energy**