Amino Acids & Peptides

By: Inaam Ahmed Amin

Amino Acids

- More than 300 naturally occurring amino acids, only 20 form the monomer units of proteins. called proteinogenic or natural amino acids(standard amino acids)
- These are normal or primary amino acids that are incorporated in proteins and that are coded in the standard genetic code (available codons to the 20 L - α-amino acids).
- Both one- and three-letter abbreviations for each amino acid used to represent the amino acids in peptides and proteins, example Glycine Gly [G], Alanine Ala [A], Serine Ser [S] and so on.
- Non-Standard amino acids that are found in proteins are formed by post translational modification of an amino acid already present in a peptide during protein synthesis.
- These modifications increase the biologic type of proteins by altering their solubility, stability, and interaction with other proteins.
- Examples, the carboxylation of glutamate(a calcium-binding a.a residue found in the blood clotting protein and the hydroxylation of proline for maintaining connective tissues.

Selenocysteine, the 21st L - α -Amino Acid

- Selenocysteine is an L - α -amino acid found in many types of proteins, including certain peroxidases and reductases.
- where it participates in the catalysis of electron transfer reactions.
- As its name implies, a selenium atom replaces the sulfur of its structural analog, cysteine.
- The p*K 3* of selenocysteine, 5.2, is 3 units lower than that of cysteine.
- Selenocysteine is inserted into polypeptides during translation, it is commonly referred to as the "21st amino acid." However, unlike the other 20 genetically encoded amino acids, selenocysteine is not specified by a simple three-letter codon.

Stereoisomerism of A.As

- **Enantiomers**, also known as **optical isomers**, are two stereoisomers that are related to each other by mirror images of each other that are non-superimposable.
- Every stereogenic center in one has the opposite configuration in the other. have the same physical properties, except for the direction in which they rotate polarized light.

- COOH

  |

H - C – NH2

  |

  R

D (+) amino acid

COOH

|

H2N– C – H

|

R

L (-) amino acid

Only L - α -Amino Acids Occur in Proteins

- The -carbon of amino acids is chiral(except glycine, all share the absolute configuration of L -glyceraldehyde and thus are L- $\boldsymbol{\alpha}$ -amino acids.
- Several free L - $\boldsymbol{\alpha}$ -amino acids fulfill important roles in metabolic processes. Examples include ornithine, citrulline, and argininosuccinate that participate in urea synthesis; tyrosine in formation of thyroid hormones; and glutamate in neurotransmitter biosynthesis.
- D -Amino acids that occur naturally include free D -serine and D -aspartate in brain tissue, D -alanine and D -glutamate in the cell walls of gram-positive bacteria, and D -amino acids in certain peptides and antibiotics produced by bacteria, fungi, reptiles, and other non mammalian species.

Amino Acids May Have Positive, Negative, or Zero Net Charge

- Charged and uncharged forms of the ionizable —COOH and —$NH3^+$ weak acid groups exist in solution in protonic equilibrium:
- R-COOH ↔ R-COO^{--}+ H^+
- R-NH_3^+ ↔ R-NH_2 + H^+
- While both R—COOH and R—$NH3^+$ are weak acids, R—COOH is a far stronger acid than R—$NH3^+$. At physiologic pH (pH 7.4), carboxyl groups exist almost entirely as R—COO^- and amino groups predominantly as R—$NH3^+$.

At Its Isoelectric pH (pI), an Amino Acid Bears No Net Charge

- Molecules that contain an equal number of ionizable groups of opposite charge and that therefore bear no net charge are termed **zwitterions,** Zwitterions are one example of an **isoelectric** species— a molecule that has an equal number of positive and negative charges and thus is electrically neutral.
- The isoelectric pH, also called the pI, is the pH midway between p*K* a values on either side of the isoelectric species. For an amino acid such as alanine that has only two dissociating groups. The first p*K* a (R—COOH) is 2.35 and the second p*K* a (R—NH3+) is 9.69. The isoelectric pH (pI) of alanine thus is

$$\text{pI} = \frac{pK_1 + pK_2}{2} = \frac{2.35 + 9.69}{2} = 6.02$$

Amino Acid Sequence Determines Primary Structure

- The number and order of all of the amino acid residues in a polypeptide establish its primary structure.

- Amino acids present in peptides are called aminoacyl residues and are named by replacing the -*ate* or -*ine* suffixes of free amino acids with -*yl* (eg, alan*yl,* aspart*yl,* tyros*yl*).

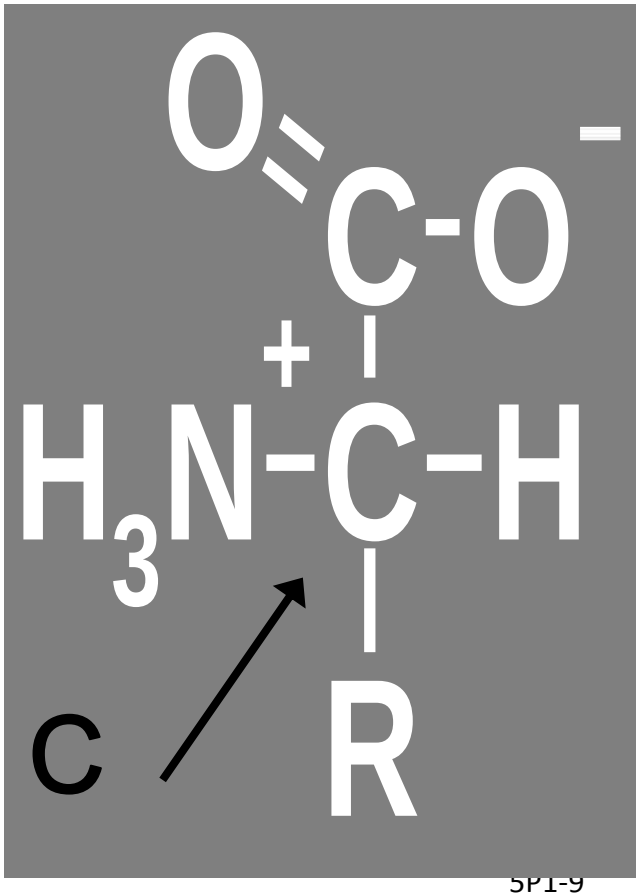
- Peptides are then named as derivatives of the carboxyl terminal aminoacyl residue. For example, Lys-Leu-Tyr-Gln is called lys*yl* -leuc*yl* -tyros*yl* -glutam*ine.*

- The –*ine* ending on glutamine indicates that its -carboxyl group is *not* involved in peptide bond formation.

Amino Acids and Peptides

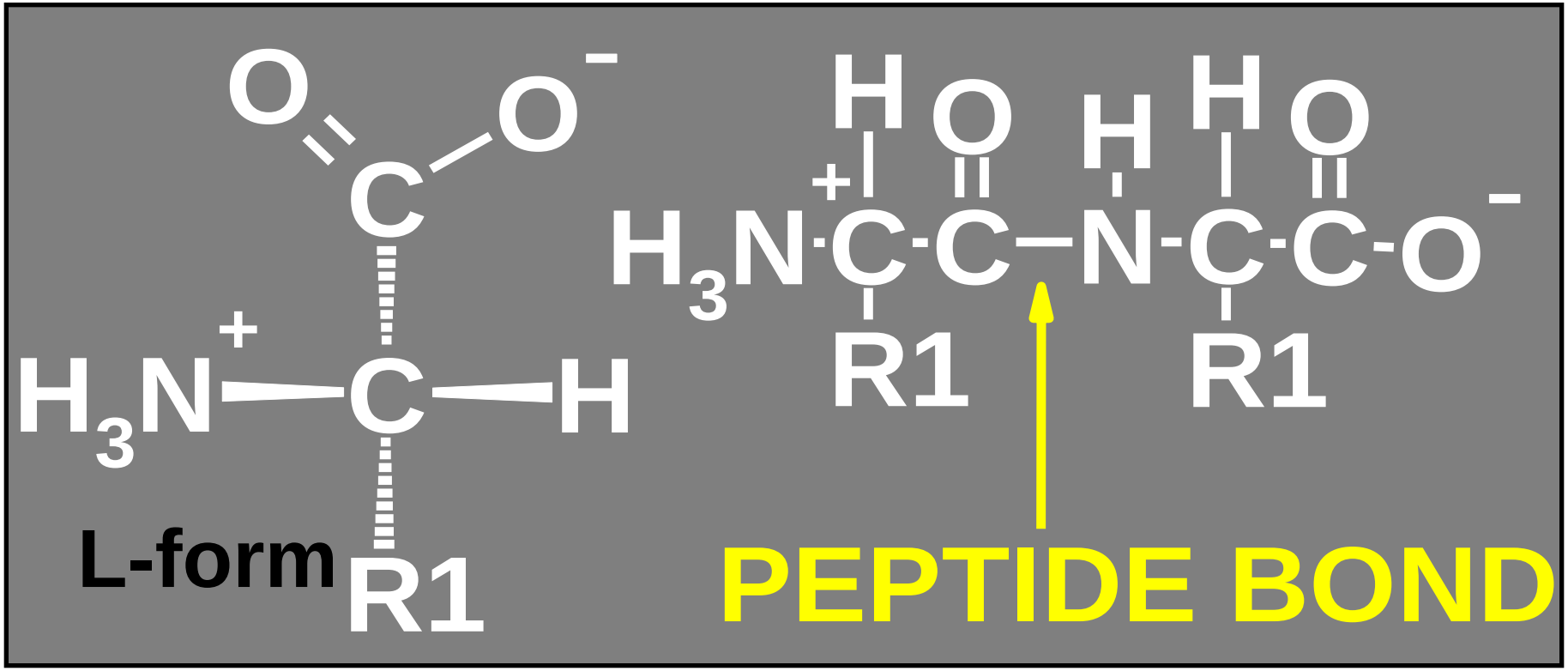
- An α- amino acid is a carboxylic acid with an amino group on the carbon alpha to the carboxylic acid .
- The α- carbon also has an R group side chain except for glycine which has two Hs.

Generic amino acid at physiological pH: zwitterion form



Amino Acids

- General form:
- 1. an amino acid (AA);
- 2. two AA linked to form the peptide bond.



THE -R GROUPS DETERMINE THE PROPERTIES OF AMINO ACIDS(1)

- Since glycine, the smallest amino acid, can be adapted in places difficult to other amino acids, it often occurs where peptides bend sharply.
- The hydrophobic R groups of alanine, valine, leucine, and isoleucine and the aromatic R groups of phenylalanine, tyrosine, and tryptophan typically occur primarily in the core (central part) of cytosolic proteins.
- The charged R groups of basic and acidic amino acids stabilize specific protein conformations via ionic interactions, or salt bridges.
- A salt bridge is a non-covalent interaction between two ionized sites. It has two components: a hydrogen bond and an electrostatic interaction.
- In a salt bridge, a proton migrates from a carboxylic acid group to a primary amine or to the guanidine group in Arg.
- Typical salt bridges involve Lys or Arg as the bases and Asp or Glu as the acids. Of all the non-covalent interactions, salt bridges are among the strongest.

THE -R GROUPS DETERMINE THE PROPERTIES OF AMINO ACIDS(2)

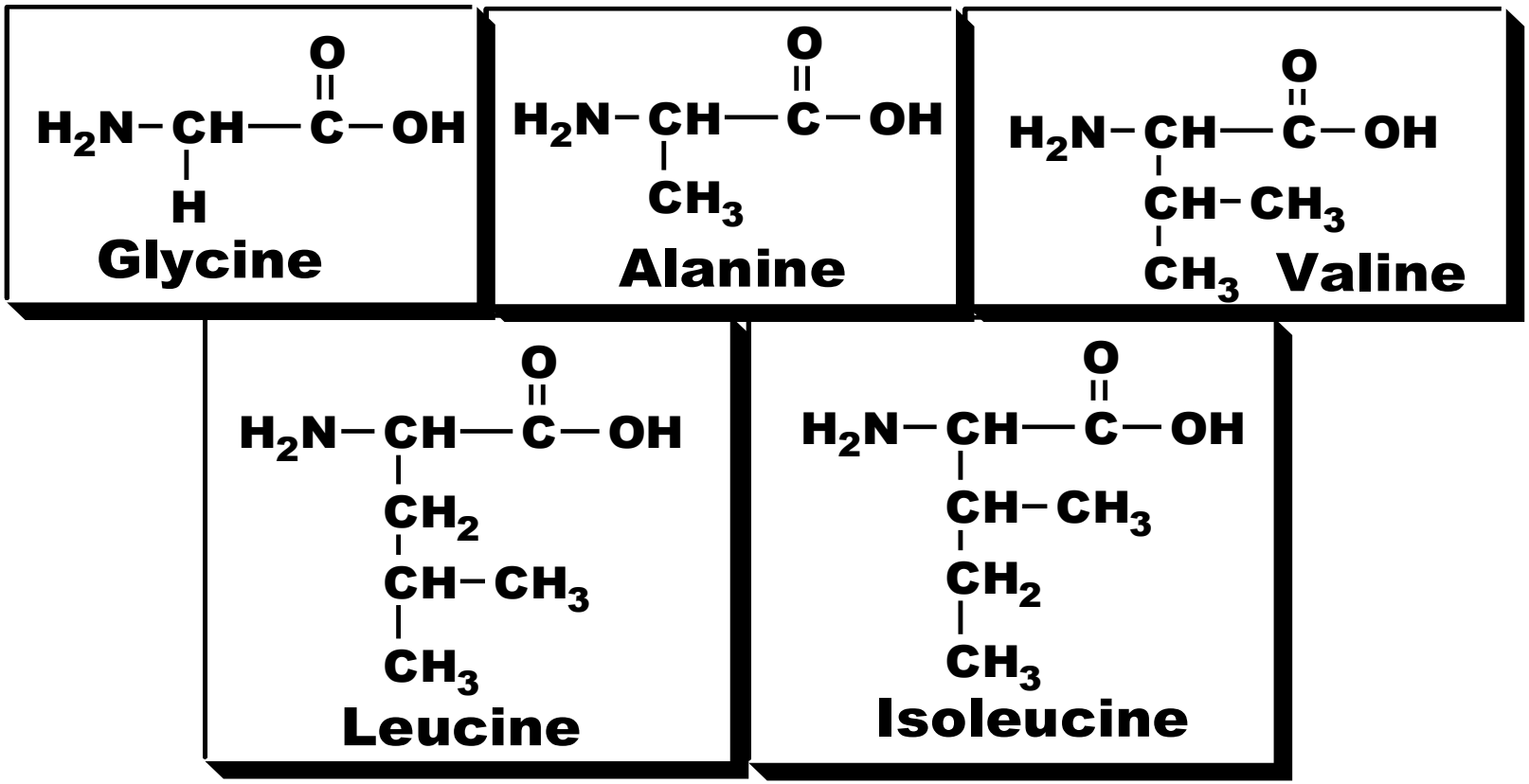
- Histidine plays exclusive roles in enzymatic catalysis. The p*K* a of its imidazole proton permits it to function at neutral pH as either a base or an acid catalyst.
- The —OH groups of serine, tyrosine, and threonine also participate in regulation of the activity of enzymes whose catalytic activity depends on the phosphorylation state of these residues.
- The primary alcohol group of serine and the primary thio alcohol (—SH) group of cysteine are excellent nucleophiles and can function during enzymatic catalysis.

• Classification of Amino Acids

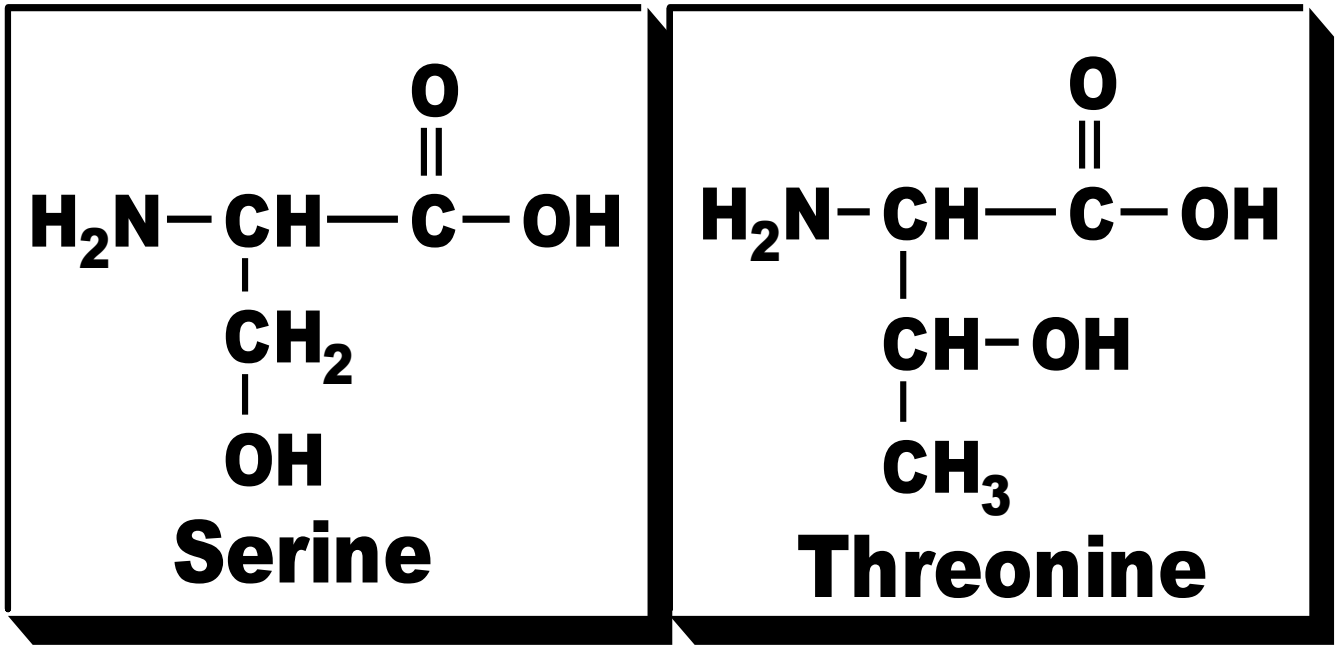
- Amino acids can be classified by one of three methods:
- **I-Chemical classification**: Based upon the number of amino groups or carboxyl groups in the amino acid:

1. Neutral amino acids (mono-amino, mono-carboxylic).
2. - Acidic amino acids (mono-amino, dicarboxylic).
3. - Basic amino acids (diamino, mono-carboxylic).

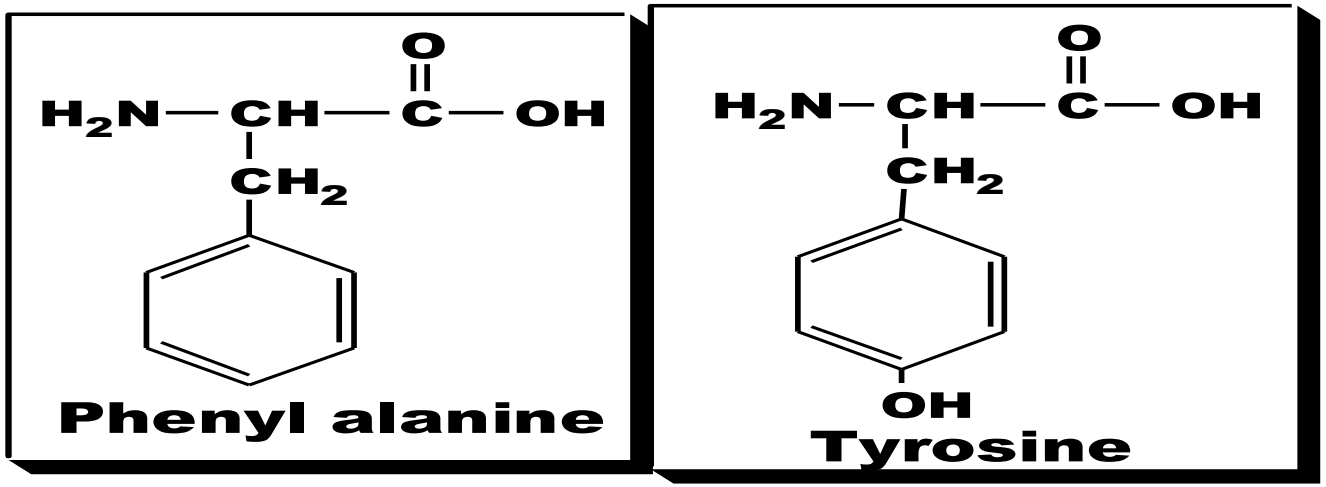
- **A) Neutral amino acids**
- They contain one amino group and one carboxyl group. They have 5 types:
- **1-Aliphatic amino acids: e.g.,**



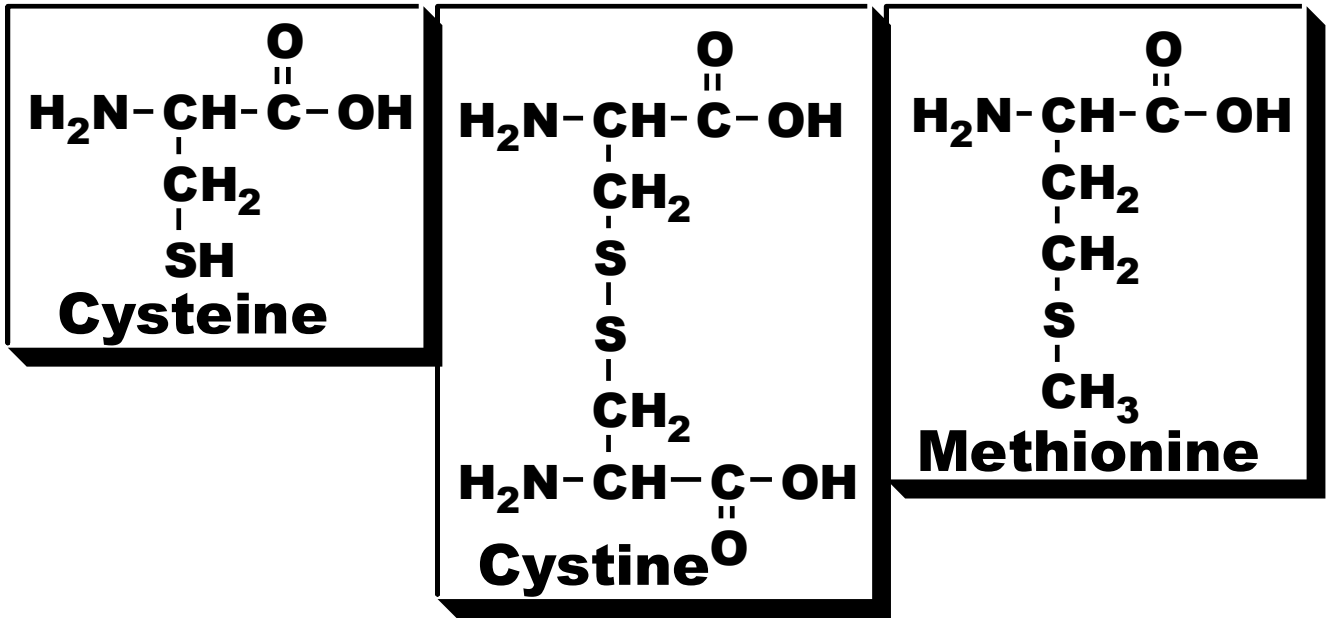
- 2. Hydroxy amino acids: e.g.,
- serine, threonine,.



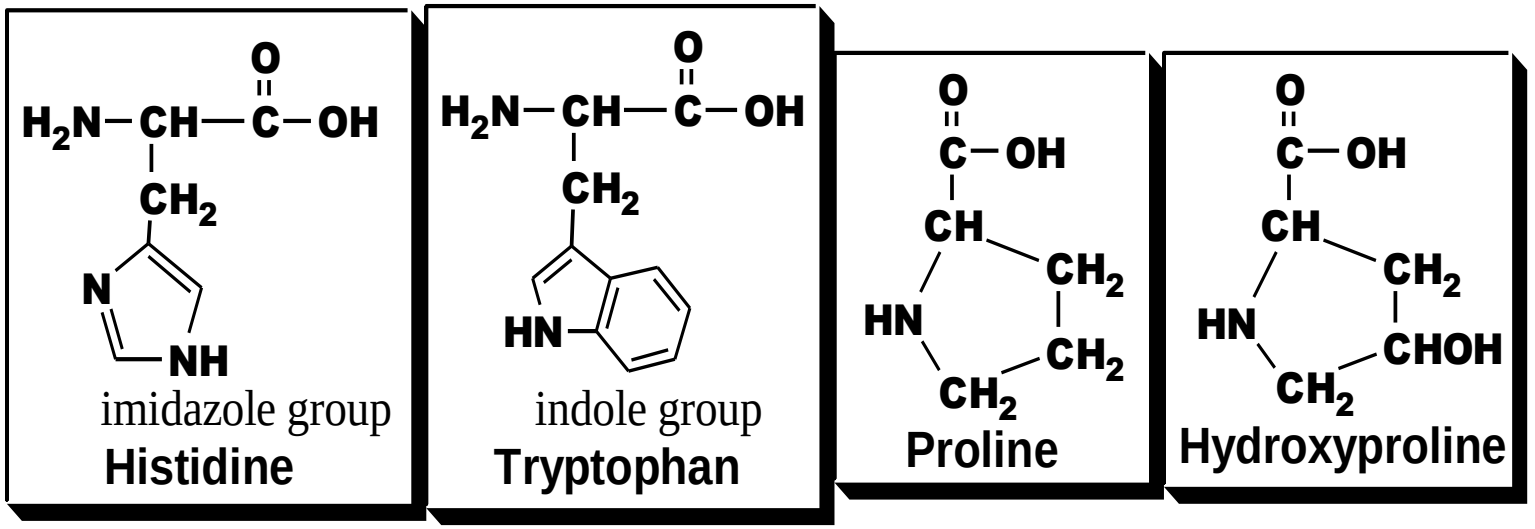
- 3. Aromatic amino acids: e.g.,
- phenylalanine and tyrosine .
- Tyrosine is synthesized from phenyl alanine and both give triiodothyronine and thyroxin, adrenaline and noradrenaline.
- Melanin pigment and cresol ,phenol in the body, e.g.,



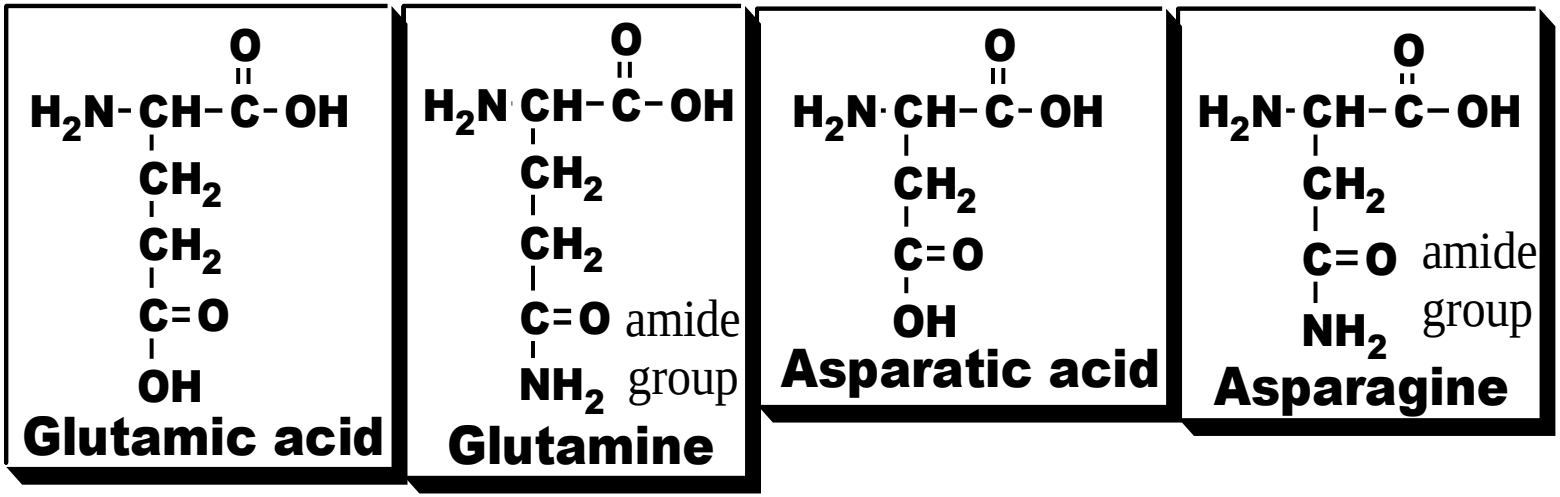
- **4-Sulfur-containing amino acids:** e.g.,
- Cysteine gives cystine and its SH group is very essential in activity of many proteins particularly the active sites of enzymes.



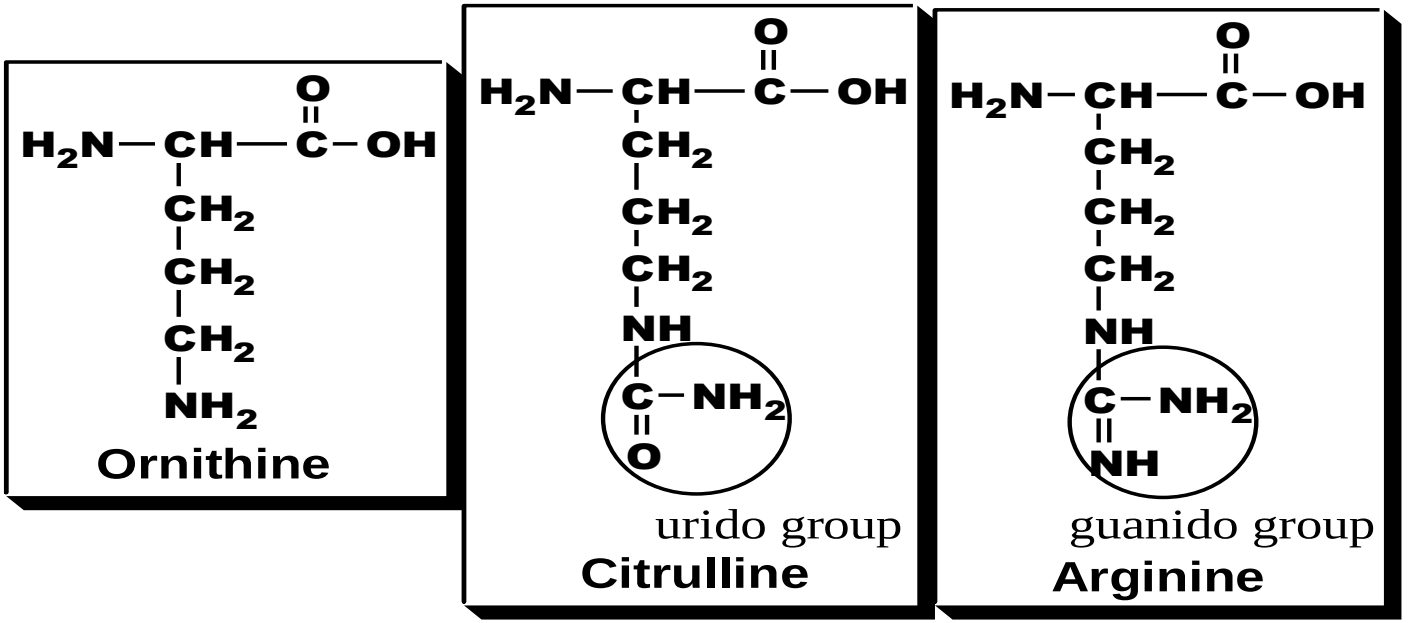
- **5-Heterocyclic amino acids**: e.g.,
- Histidine gives histamine a very important inflammatory mediator.
- Proline gives hydroxyproline that is essential for collagen cross-linking.
- Tryptophan gives nicotinic acid, melatonin, serotonin and indican in the body.



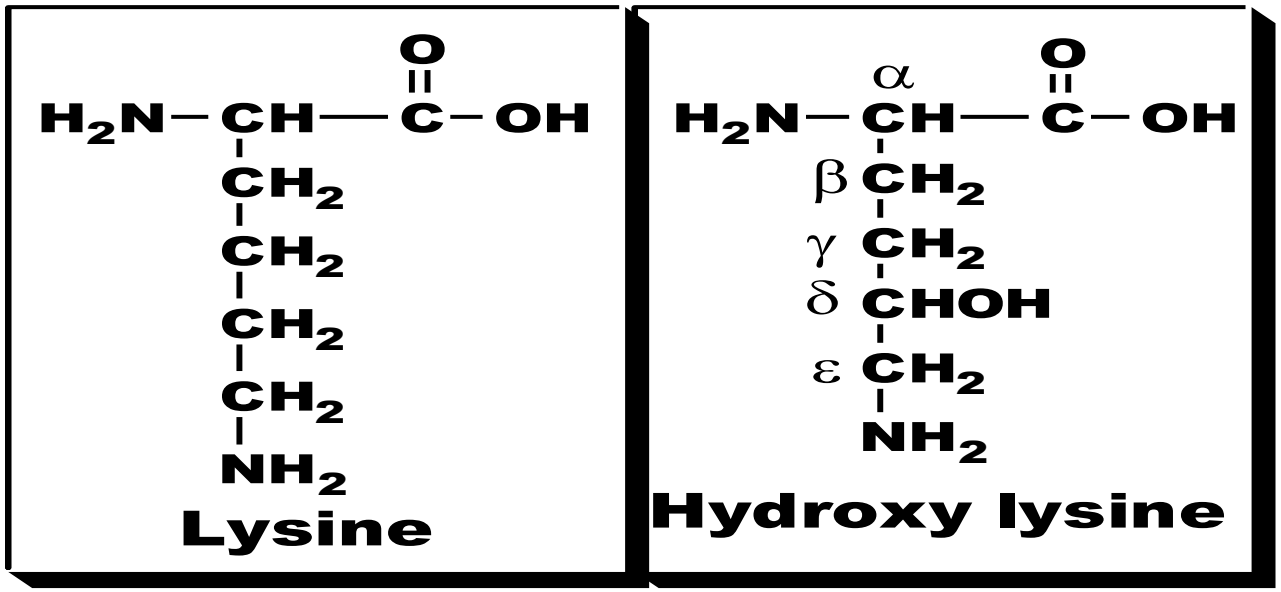
- **B) Acidic amino acids**
- They contain 2 carboxyl groups and one amino group, e.g., glutamic acid and asparatic acid.
- These acidic amino acids can occur in the tissue in the form of amides, e.g., glutamic acid ⇒ glutamine and asparatic acid ⇒ asparagine.



- ## C) Basic amino acids
- They contain 2 amino groups and one carboxyl group, e.g., Ornithine and Arginine. Ornithine does not enter in the synthesis of proteins and is usually present in the free form. It is synthesized from arginine. Citrulline is formed from ornithine during urea synthesis

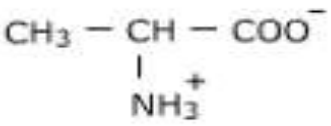


- Lysine and Hydroxy lysine: They participate in protein cross-linking.

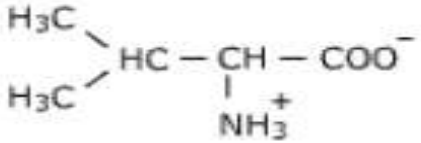


Nonpolar & polar amino acids

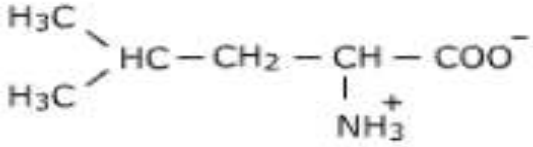
- Non-polar A.As
- Non Polar Amino Acids have equal number of amino and carboxyl groups and are neutral. These amino acids are hydrophobic and have no charge on the 'R' group like **alanine, valine, leucine, isoleucine, phenyl alanine, glycine, tryptophan, methionine and proline.**



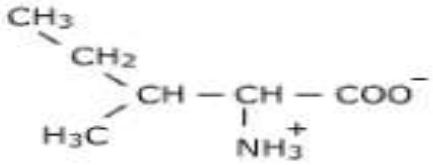
Alanine



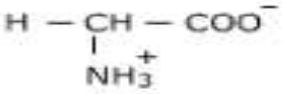
Valine



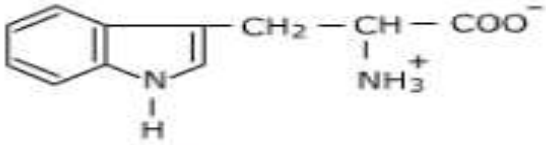
Leucine



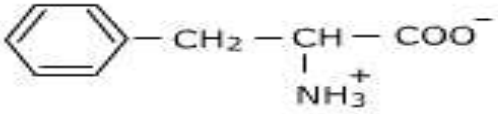
Isoleucine



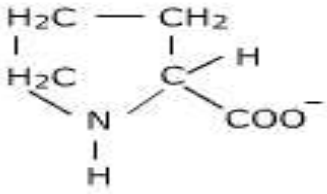
Glycine



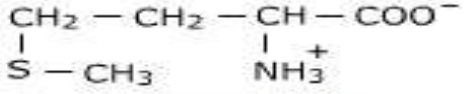
Tryptophan



Phenyl alanine



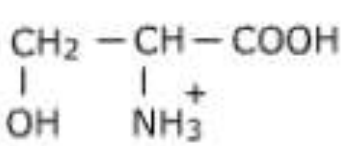
Proline



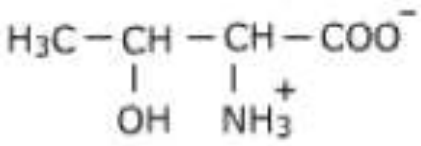
Methionine

Polar amino acids(1)

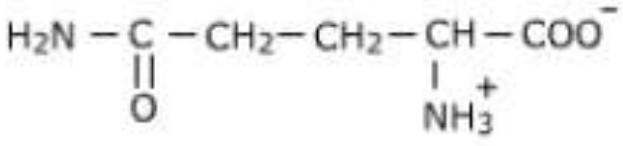
- **Polar Amino Acids with no Charge**
- These amino acids do not have any charge on the 'R' group. These amino acids participate in hydrogen bonding of protein structure. These amino acids includes **serine, threonine, tyrosine, cysteine, glutamine and asparagine.**



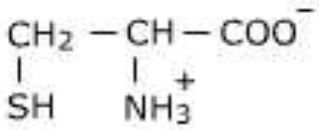
Serine



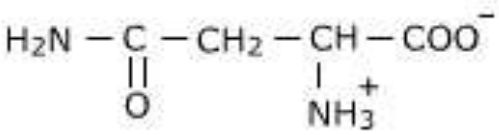
Threonine



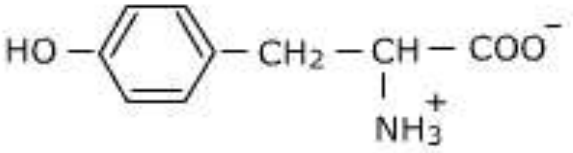
Gultamine



Cysteine



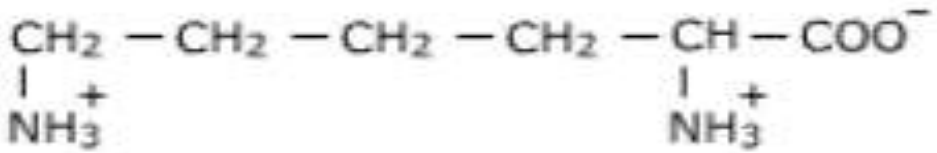
Asparqine



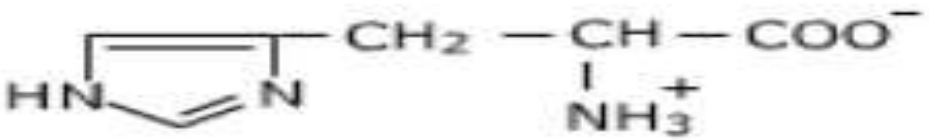
Tyrosine

Polar Amino Acids(2)

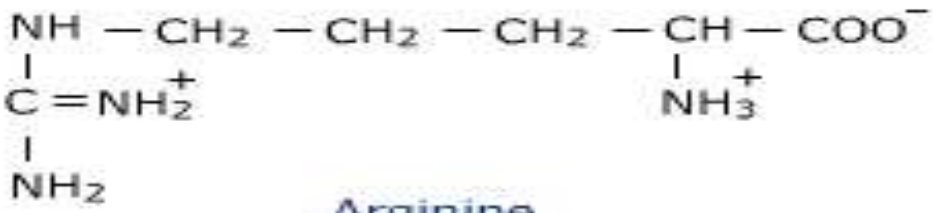
- **Polar Amino Acids with Positive Charge**
- Polar amino acids with positive charge have more amino groups as compared to carboxyl groups making it basic. The amino acids, which have positive charge on the 'R' group. They are **lysine, arginine and histidine**.



Lysine



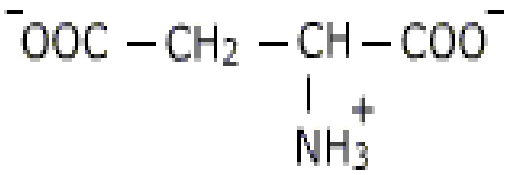
Histidine



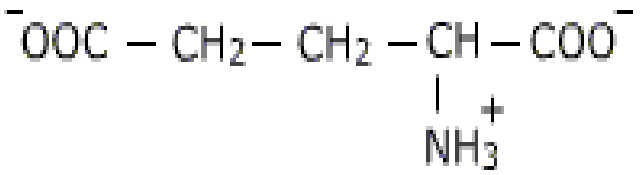
Arginine

Polar Amino Acids(3)

- **Polar Amino Acids with Negative Charge**
- Polar amino acids with negative charge have more carboxyl groups than amino groups making them acidic. The amino acids, which have negative charge on the 'R' group called as **dicarboxylic mono-amino acids**. They are **aspartic acid and glutamic acid**.



Aspartic acid



Glutamic acid

- **II-Biological or nutritional classification: Based upon whether the amino acids can be synthesized in human body or not:**

1. **Essential amino acids**: Not synthesized in the body and must be supplied in the diet, and these include: Valine, Isoleucine, Threonine, Tryptophan, Leucine, Lysine, Methionine and Phenylalanine.(deficiency affect the grow and health)
2. **Semi-essential amino acids:** Two amino acids are grouped under semi-essential amino acids since they can be synthesized within the organism but their synthesis is not in sufficient amounts. In that they should also be provided in the diet, these amino acids are arginine and histidine.
3. **Non-essential amino acids:** The rest of amino acids that can be synthesized in the body and not essential to be present in diet. .(deficiency not affect the grow and health).

- **III-Metabolic Classification**: **Based upon the fate of amino acid inside the body**:
- 1-- **Glucogenic amino acids**, (these amino acids that can be converted to glucose through gluconeogenesis) . The production of glucose from glucogenic amino acids involves these amino acids being converted to alpha keto acids and then to glucose, with both processes occurring in the liver. This mechanism predominates during catabolysis, rising as fasting and starvation increase in severity. Glucogenic amino acids includes: Alanine, cysteine, glycine, Arginine, glutamine, Isoleucine, tyrosine.

- 2-Ketogenic amino acids, (that can be converted to ketone bodies). Those amino acids in which their carbon skeleton is degraded to Acetoacetyl CoA, or acetyl CoA. then converted to acetone and β-hydroxy butyrate which are the main ketone bodies are called ketogenic amino acids. Phenylalanine, tyrosine, tryptophan, isoleucine, leucine, and lysine. These AAs have ability to form ketone bodies which is particularly evident in untreated DM in which large amounts of ketone bodies are produced by the liver (i.e. not only from fatty acids but also from ketogenic amino acids). Degradation of Leucine which is an exclusively ketogenic AA makes a large supply of ketone bodies during starvation

- **3-Mixed function amino acids**,
- can be converted to both glucose and ketone bodies, The division between ketogenic and glucogenic amino acids is not sharp for amino acids. (Tryptophan, phenylalanine, tyrosine and Isoleucine are both ketogenic and glucogenic). Some of the AAs that can be converted in to pyruvate, particularly (Alanine, Cysteine and serine, can also potentially form acetoacetate via acetyl CoA especially in severe starvation and untreated diabetes mellitus.

Ketogenic	*Ketogenic & glucogenic*	*Glucogenic*
Leucine	**Lysine**	**Rest of amino acids**
	Isoleucine	
	Tyrosine	
	Tryptophan	
	Phenyl alanine	

Non protein functions

- In addition to providing the monomer units from which the long polypeptide chains of proteins are synthesized.
- the L - -amino acids and their derivatives participate in cellular functions as diverse as: nerve transmission and the biosynthesis of porphyrins, purines, pyrimidines, and urea.
- Short polymers of amino acids called *peptides* perform prominent roles in the neuroendocrine system as hormones, hormone-releasing factors , neuromodulators, or neurotransmitters.
- While proteins contain only L - -amino acids, microorganisms involved peptides that contain both D - and L - -amino acids. Several of these peptides are of therapeutic value, including the antibiotics bacitracin and gramicidin A and the antitumor agent bleomycin.

Non protein functions(2)

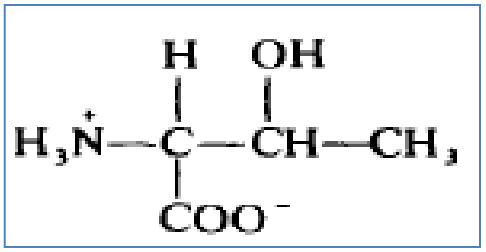
- . Many free amino acids are used to synthesize other molecules for example
- Tryptophan is a precursor of the neurotransmitter serotonin
- Tyrosine is a precursor of the neurotransmitter dopamine
- Glycine is a precursor of porphyrins such as heme
- Aspartate, glycine and glutamine are precursors of nucleotides

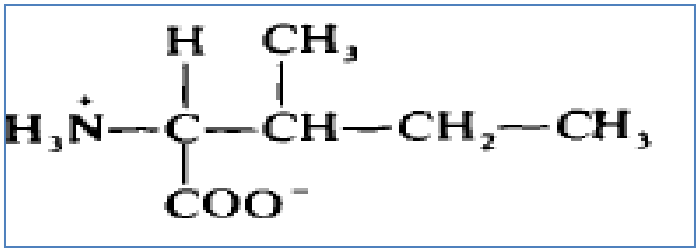
Physical & Chemical properties of amino acids

By: Inaam Ahmed Ameen

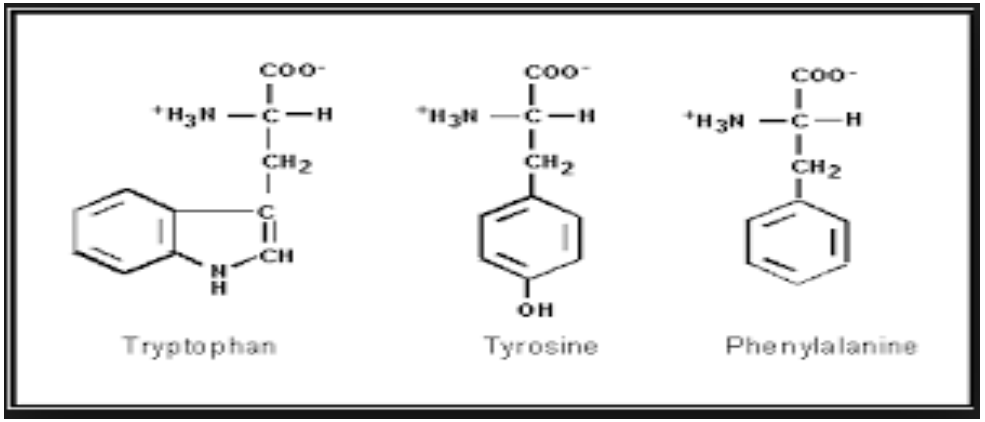
- **I. Physical properties of amino acids:**
- **1. Solubility:**
- All amino acids are soluble in water, diluted acids and alkalis. The charged functional groups of amino acids ensure that they are readily solvated by—and thus soluble in—polar solvents such as water and ethanol but insoluble in nonpolar solvents such as benzene, hexane, or ether.
- Their melting points are extremely high (usually exceeding 200°C)

- ## 2. Optical activity:
- All amino acids, except glycine, are optically active, i.e., they contain asymmetric carbon atom (α-carbon), thus they can turn the plane polarized light either to the right(Dextro-D) or to the left (Levo-L).
- Optical activities depend on the pH & side chain.
- Few amino acids like Thr & Ile have an additional asymmetric carbon in their structure.



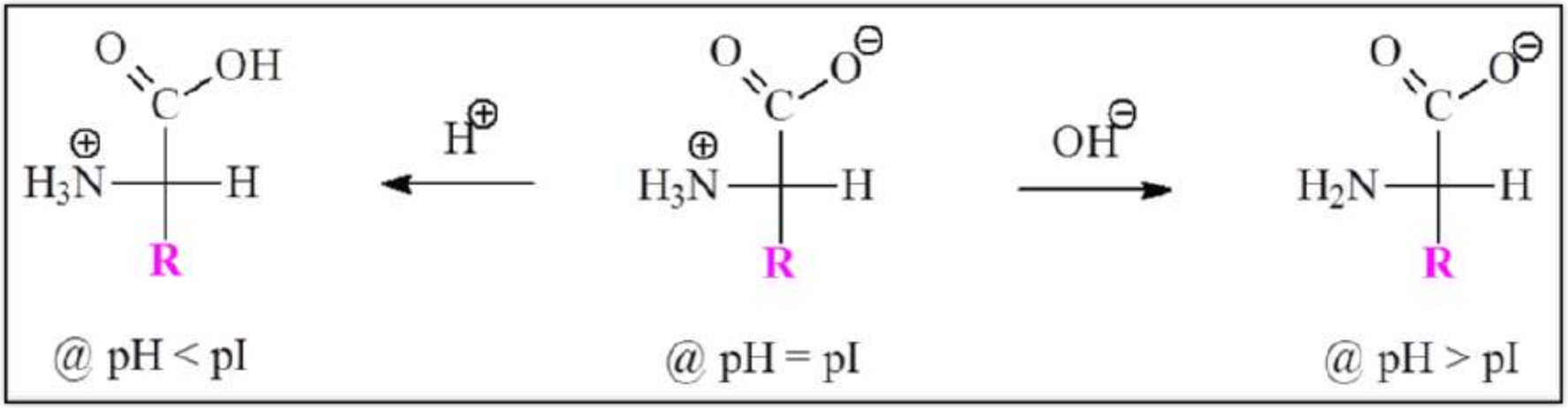


- **3. Absorption of ultraviolet light:** Amino acids do not absorb visible light and thus are colorless. However, aromatic amino acids(tyrosine, phenylalanine, and tryptophan) especially tryptophan absorb high-wavelength (250–290 nm) ultraviolet light. Because it absorbs ultraviolet light about ten times more efficiently than either phenylalanine or tyrosine, tryptophan makes the major contribution to the ability of most proteins(for analysis) to absorb light in the region of 280 nm.



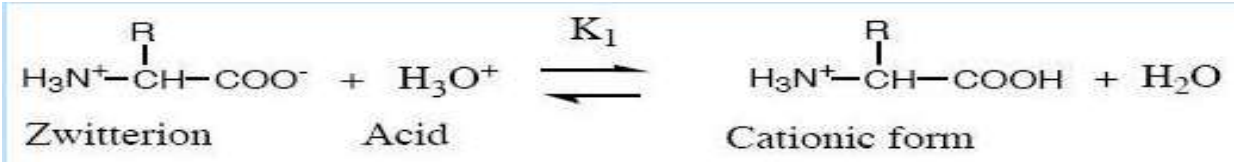
4-Amphoteric nature & isoelectric pH:

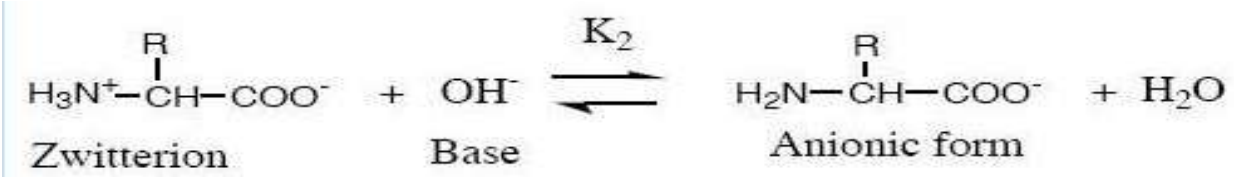
- Molecules that contain an equal number of ionizable groups of opposite charge and that therefore bear no net charge are termed **zwitterions,** Zwitterions are one example of an **isoelectric** species— a molecule that has an equal number of positive and negative charges (electrically neutral).
- The isoelectric pH, also called the pI, is the pH midway between p*K* a values on either side of the isoelectric species.
- The ionization state of amino acids:
- At a pH less than the value of the isoelectric point, the amino acid is protonated and has a +ve charge; at a pH greater than the pI the amino acid is deprotonated and has a –ve charge



The calculation isoelectric pH (pI) of amino acids

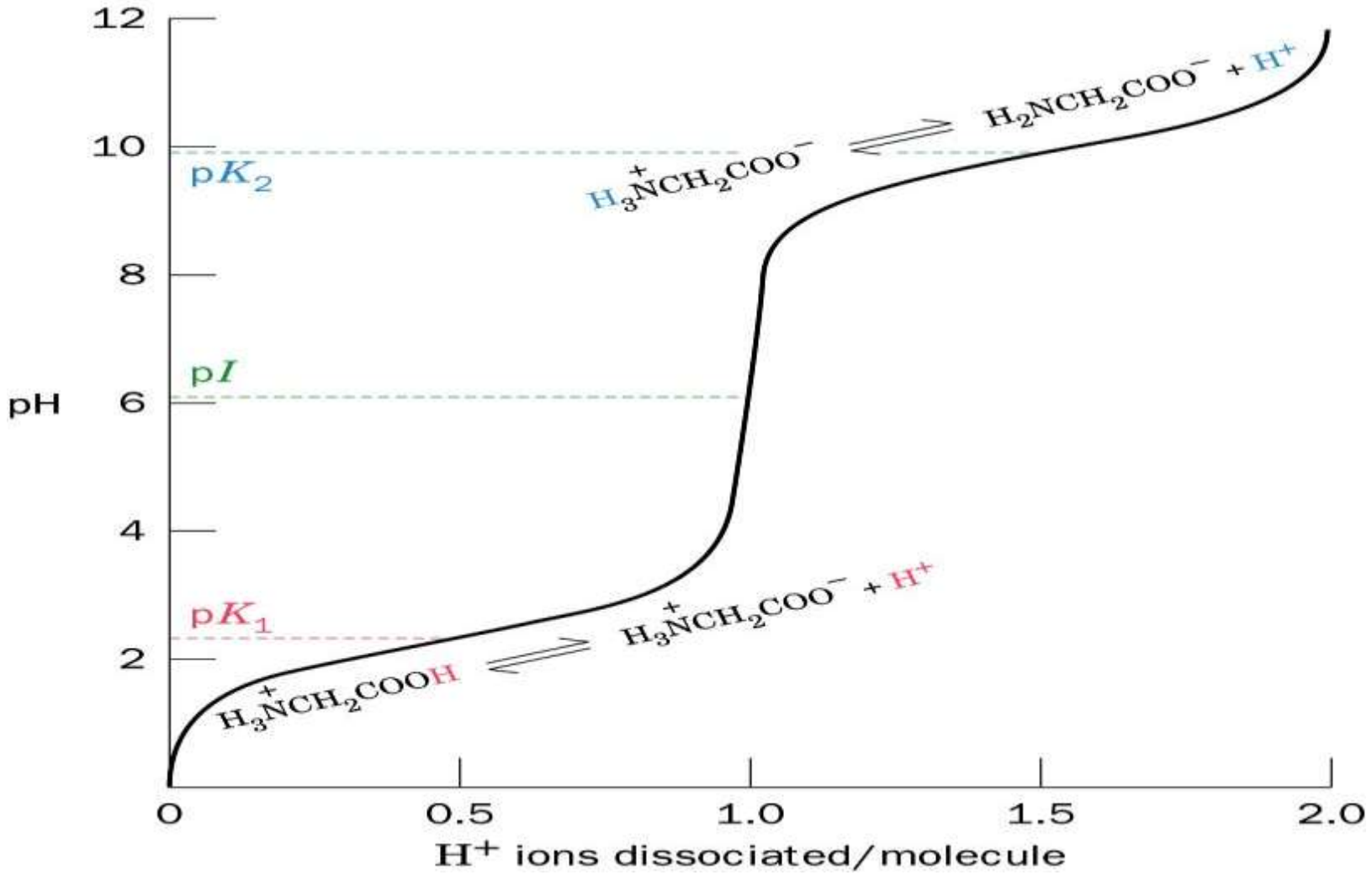
- Each amino acid has its own pI value. The Ionizable groups of amino acids act as weak acids or bases, giving off or taking on protons when the pH is altered. Simply, common amino acids are weak polyprotic acids.
- Titration curves are produced by checking the pH of given volume of a sample solution after successive addition of acid or alkali. Titration curves are usually plots of pH against the volume of titrant added or more correctly against the number of equivalents added per mole of the sample.
- Upon titration of amino acid with acid, it acts as a base, and upon titration with base, it acts as an acid.



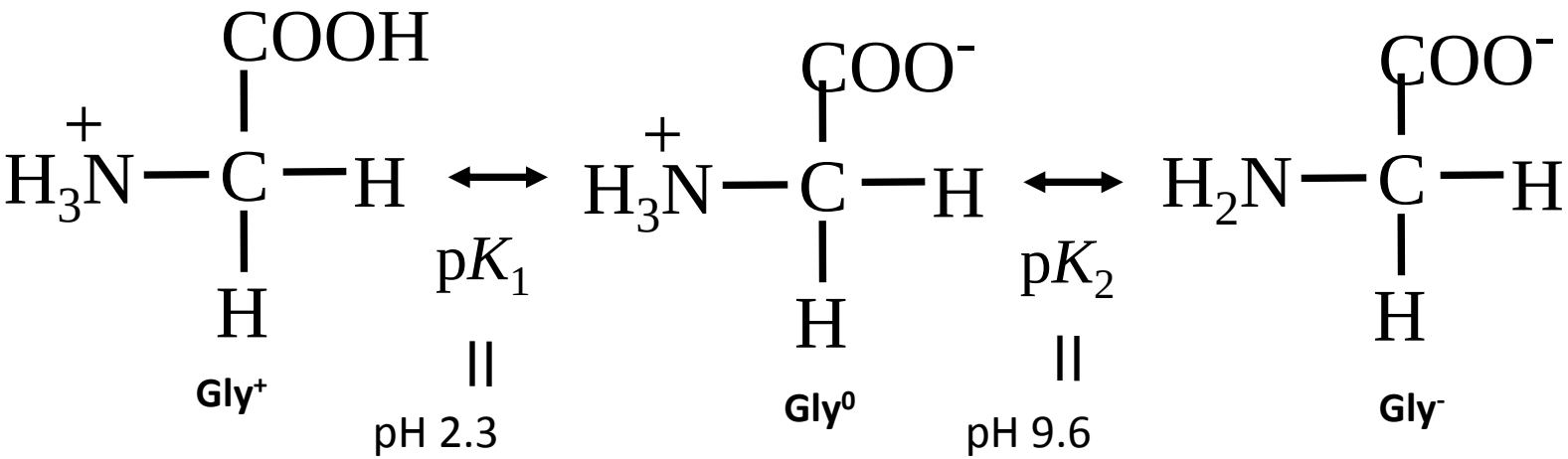


Titration curves of amino acids

- Amino acids can be titrated and give titration curve. For example glycine titration curve:



Titration of Gly



From the p*K* values we can calculate the pI (isoelectric point) where the amino acid is neutral.

pI ≈ average of (p*K* *below* neutral+ p*K* *above* neutral), the points at which the glycine have it s maximum buffering capacity
So, for Gly,

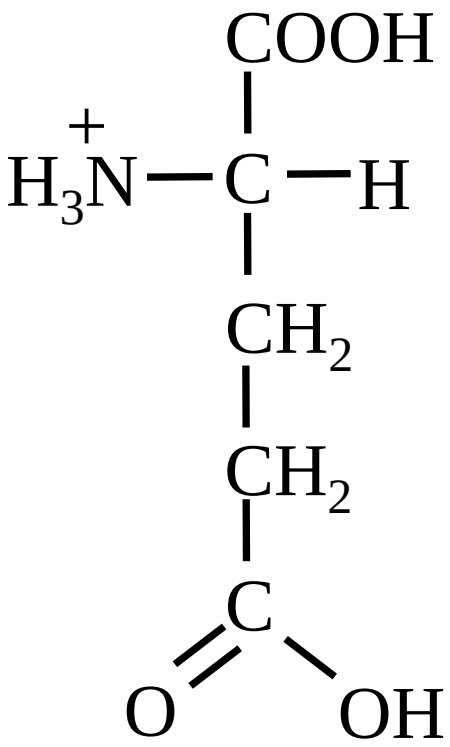
$$pI = (pK_1 + pK_2)/2$$
$$= (2.3 + 9.6)/2 \approx 6$$

General rules for amino acid ionization

- Alpha carboxylic acids ionize at acidic pH and have pKs less than 6; So in titrating a fully protonated amino acid, alpha carboxylic acids lose the proton first.
- Alpha amino groups ionize at basic pH and have pKs greater than 8; So after acids lose their protons, amino groups lose their proton.
- Most of the 20 amino acids are similar to Gly in their ionization properties because their side chains do not ionize at biological pHs.
- However, there are 5 exceptions worth noting (the amino acids with polar charged side chains)
- Glu, Asp, Lys, Arg, His
- Each has 3 ionizible groups and thus, 3 pKs.

Charged polar (acidic) side chains

9.5 2.1



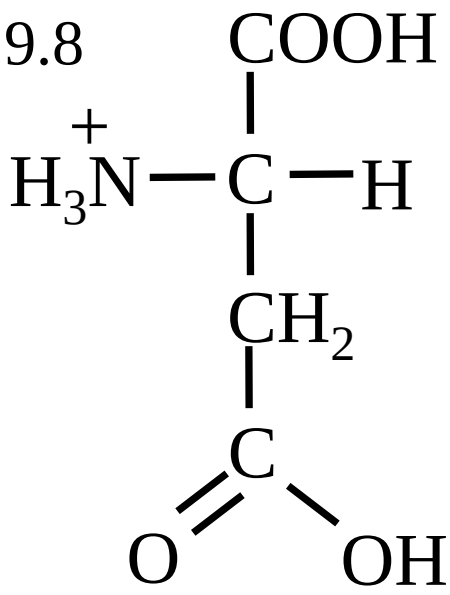
4.1

Glutamic acid

Glu

E

9.8 2.0



3.9

Aspartic acid

Asp

D

Titration Curve for Glutamic Acid

pK_1 carboxylic acid = 2.2

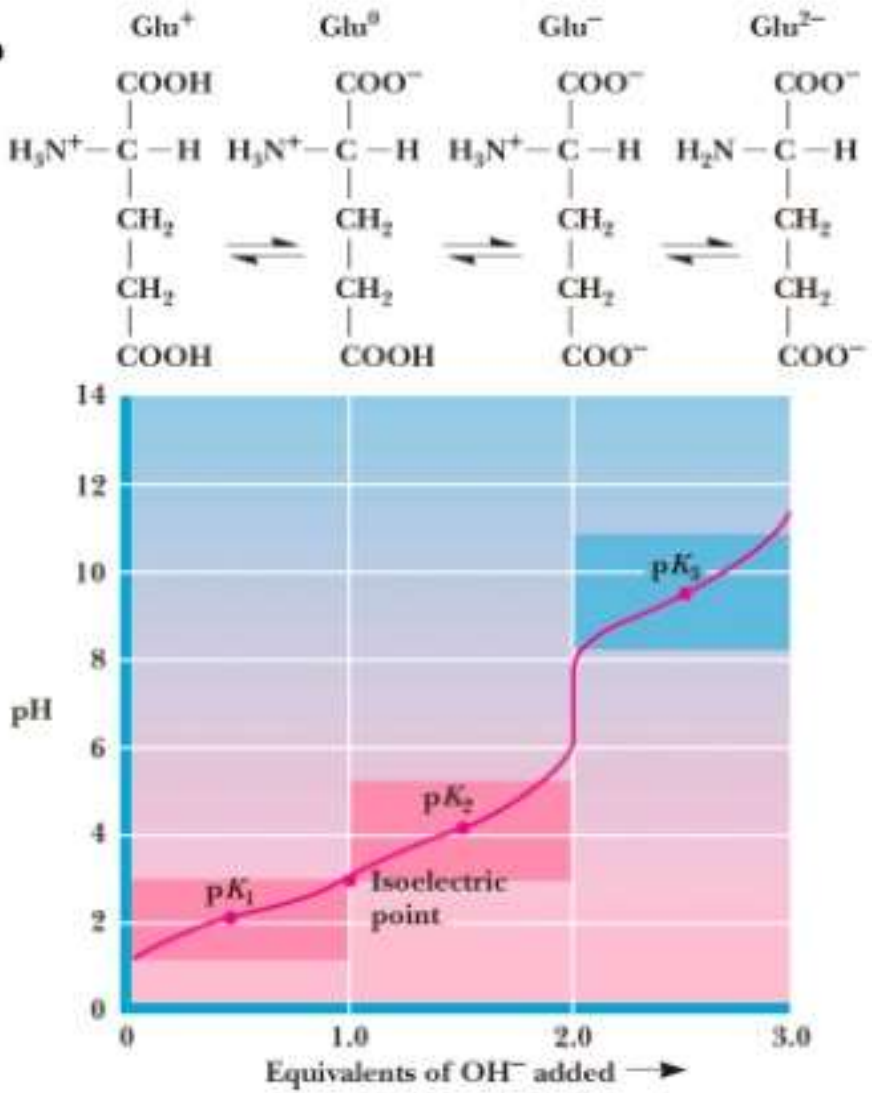
pK_2 R group = 4.3

pK_3 amino group = 9.7

$pI = (pK_1 + pK_2)/2$

$pI = (2.2+4.3)/2$

$pI = 3.25$



Titration Curve for Lysine

pK_1 carboxylic acid = 2.2

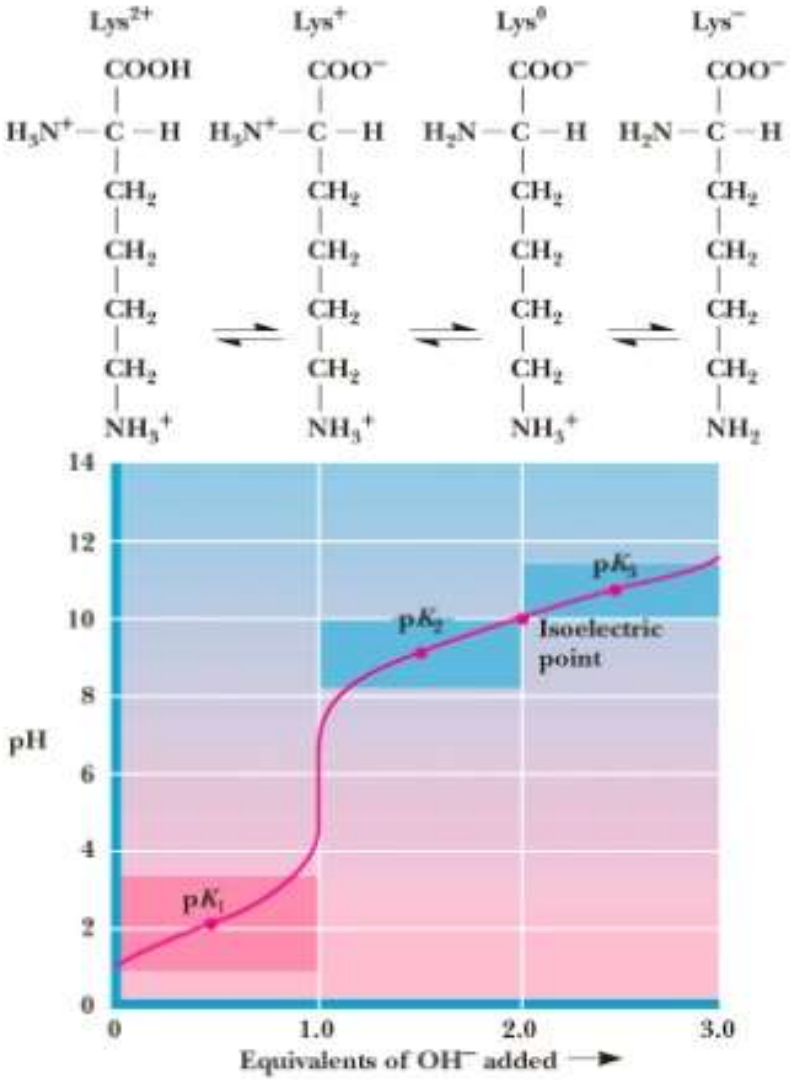
pK_2 amino group = 9.0

pK_3 R group = 10.5

$pI = (pK_2 + pK_3)/2$

pI = (9+10.5)/2

pI = 9.75



How to calculate the pI of a compound with more than 2 p*K*s

- Find the amino acid form with no net charge (total charge = 0).
- Take the p*K* of the amino acid form going towards +1 form as the lower p*K*.
- Next find the amino acid form going towards the -1 form.
- Finally, average these two p*K*s to get the p*I*.

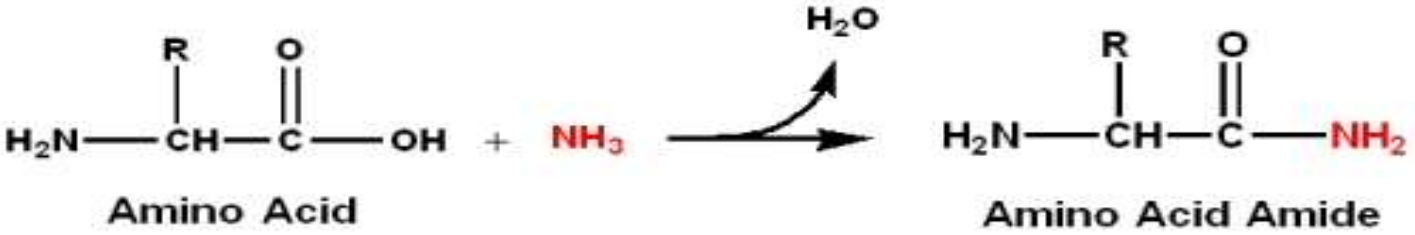
Chemical reactions of amino acids

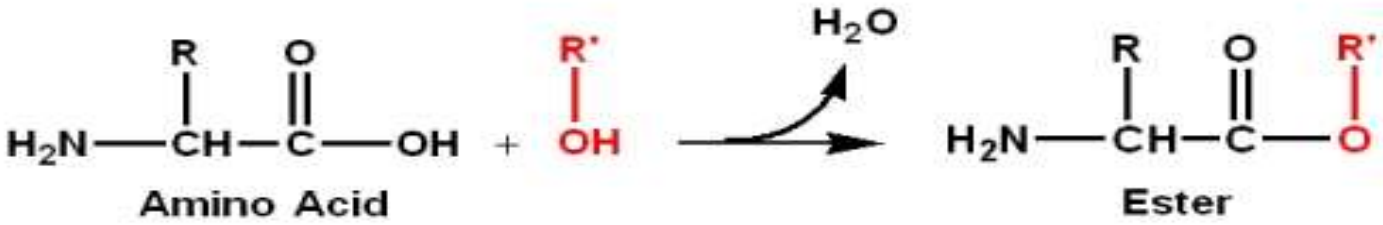
- **Acid-base Chemistry of Amino Acids**
- Amino acids by themselves have amino (pKa ~9.0-10.5) and carboxyl groups (pKa ~2.0-2.4) that can be titrated
- At neutral pH the amino group is protonated, and the carboxyl group is deprotonated.
- The side chains of acid and basic amino acids, and some polar amino acids can also be titrated:

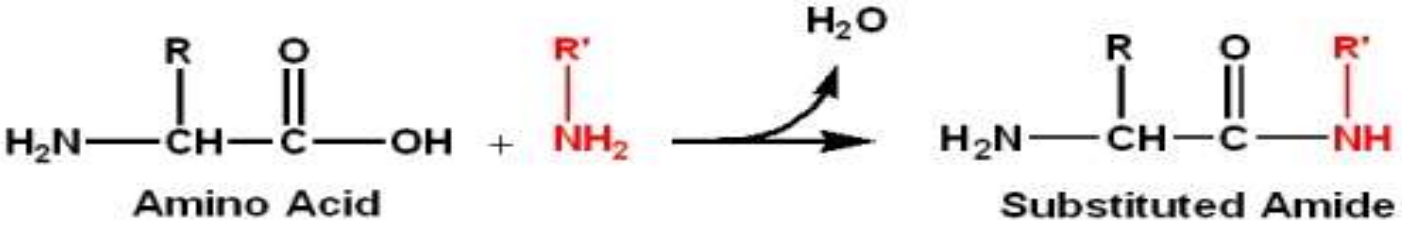
Amino acid	Functional Group	Side chain pKa
Cysteine	-SH	8.3
Serine	-OH	13
Threonine	-OH	13
Tyrosine	-OH	10.1
Aspartic acid	-COOH	3.9
Glutamic acid	-COOH	4.3
Histidine	Imidazole ring	6.0
Arginine	Guanidino	12.5
Lysine	$-NH_2$	10.5

Reactions of amino acids

- Free amino acids (excluding proline) share similar chemical reactivities due to the common amino and carboxyl groups.
- Different amino acid side chains have different chemical reactivities.
- Some common carboxyl-group reactivities(these can leads to the polymerization of amino acids):



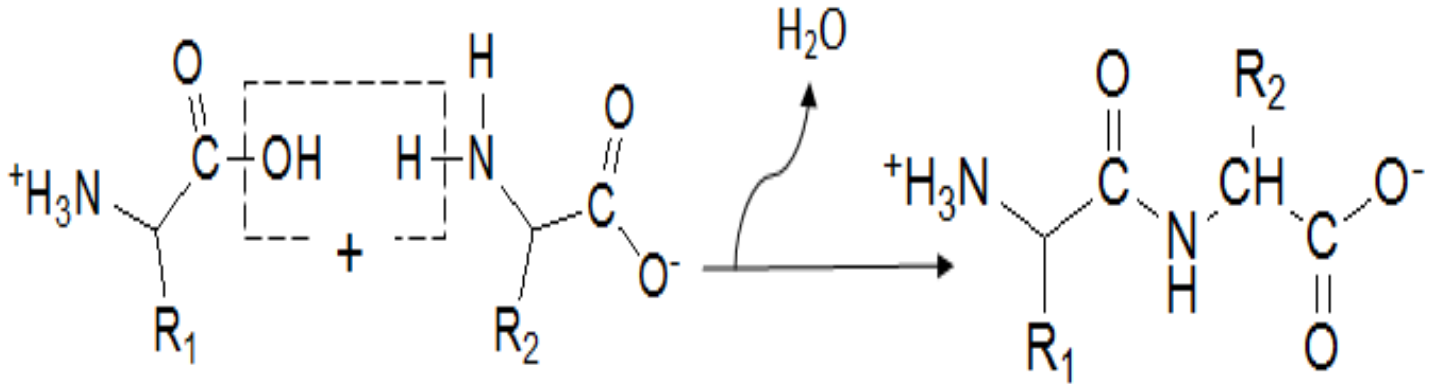




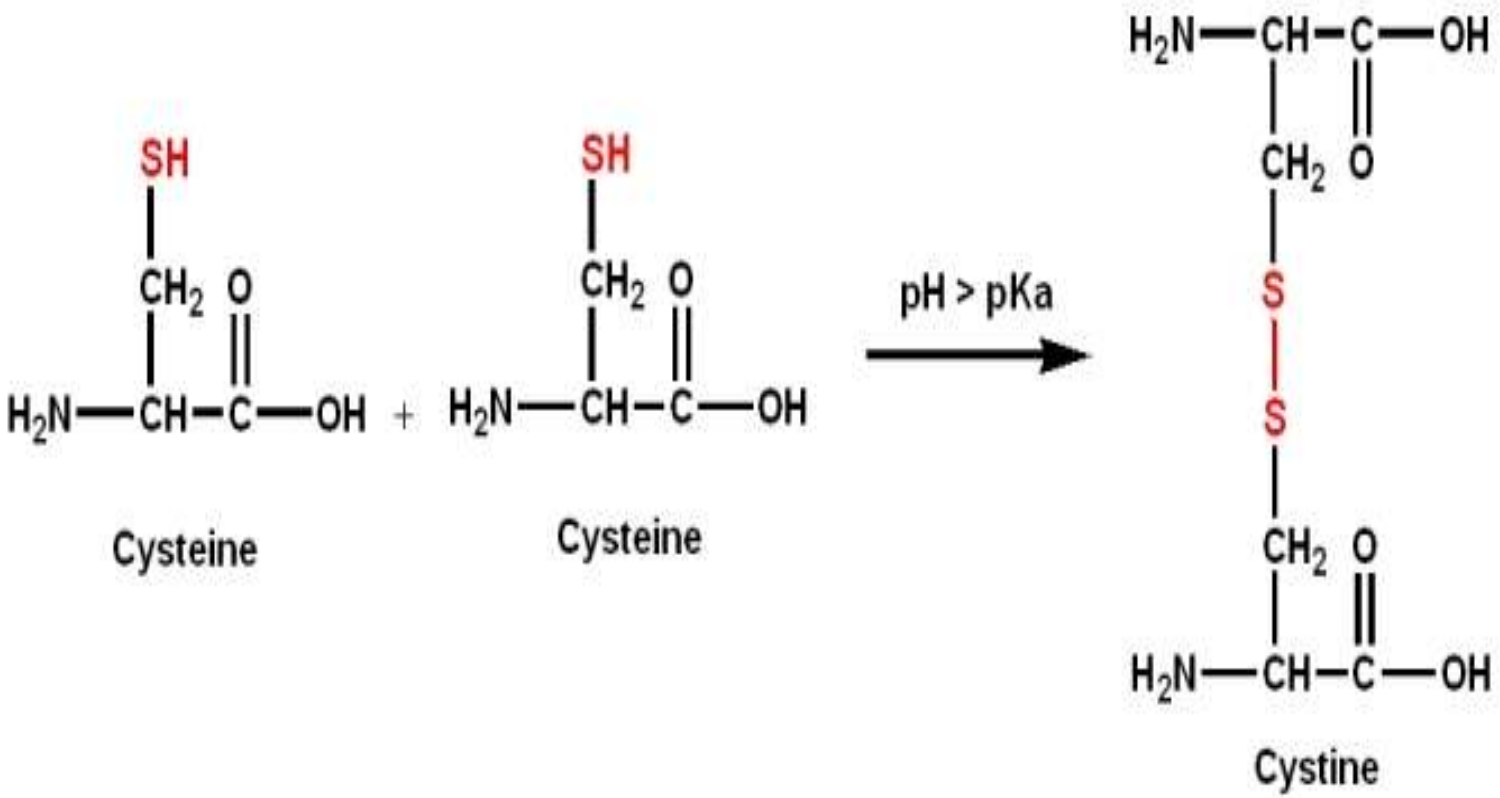
Common side chain reactions

- **– COOH group**
- Carboxyl group in Asp, Glu side chain in addition to α – COOH can be participating in formation of ester, peptide bond (amides bond) also (acid anhydrides, oxidation and reduction, decarboxylation). These reactions used for detection for amino acids.
- **– NH_2 group**
- NH_2 group in side chain of Lys and the α – NH_2 group can ionization, acylation and esterfication. These processes are important in the **[detoxification]**.
- **– SH**
- SH group can be oxidation, alkylation. This important to stabilizes proteins.
- **– OH**
- OH group can be esterfication.
- In general, ionized group (or charged group) in amino acid stabilize protein configuration by formation of [salt bonds].
- **Example:**
- Rupture and formation salts bonds accompany oxygenation and deoxygenation of Hb.

- . The most important reaction of amino acids is peptide bond formation.
- H_2O
- The reactions involve removal of water molecule between α – NH_3^+ group of amino acid and COO^- group of second amino acid.



- A common side chain reaction involving cysteine:



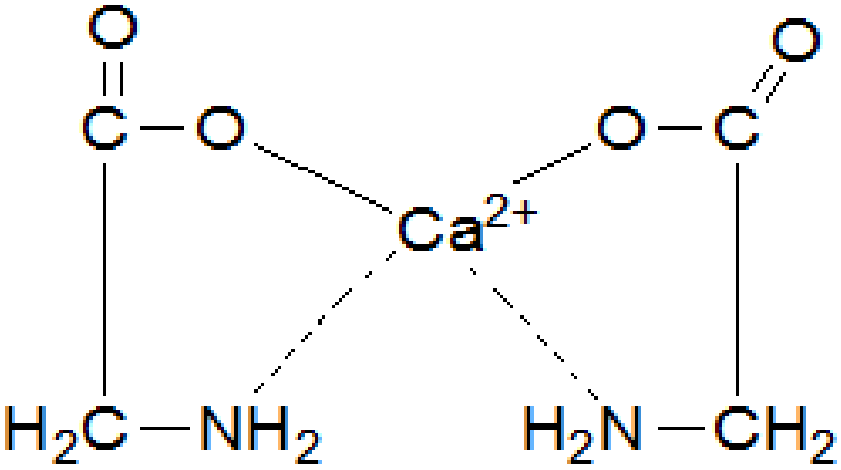
- **This can covalently link two polypeptide chains in a "disulfide bond" crosslink**

Chemical test for amino acids

- There are different reactions for detections amino acids in general like ninhydrin test or fluorescamine, or for detection specific amino acids like: -
- Millon test → for Tyr
- Salkguchi test → for Arg
- Nitroprusside test → for Cys
- Hopkin test → for Trp

Other amino acids reactions

- There are reactions for free α amino group like: {Sanger reaction and Edman reaction}, these used to detect the first amino acid in primary structure of protein.
- Some peptides of amino acids are due to both – NH_2 and – COOH group together like chelating of amino acid with certain heavy metals and other ions like Cu^{2+}, Co^{2+},Mn^{2+} and Ca^{2+}.



- Calcium diglycinate (soluble calcium complex)
- This chelating may be used to remove Ca from bones and teeth and could development dental caries.

General reaction for amino acids

- **Ninhydrin test**
- In the pH range of 4-8, all α- amino acids react with ninhydrin (triketohydrindene hydrate), a powerful oxidizing agent to give a purple colored product (diketohydrin) termed Rhuemann's purple. All primary amines and ammonia react similarly but without the liberation of carbon dioxide. The imino acids proline and hydroxyproline also react with ninhydrin, but they give a yellow colored complex instead of a purple one. Besides amino acids, other complex structures such as peptides, peptones and proteins also react positively when subjected to the ninhydrin reaction.
- **Xanthoproteic acid test**
- Aromatic amino acids, such as Phenyl alanine, tyrosine and tryptophan, respond to this test. In the presence of concentrated nitric acid, the aromatic phenyl ring is nitrated to give yellow colored nitro-derivatives

Millon's test

- Phenolic amino acids such as Tyrosine and its derivatives respond to this test. Compounds with a hydroxybenzene radical react with Millon's reagent to form a red colored complex. Millon's reagent is a solution of mercuric sulphate in sulphuric acid.

.

- **Histidine test**
- This test was discovered by Knoop. This reaction involves bromination of histidine in acid solution, followed by neutralization of the acid with excess of ammonia. Heating of alkaline solution develops a blue or violet coloration.

- **Hopkins cole test**
- This test is specific test for detecting tryptophan. The indole moiety of tryptophan reacts with glyoxilic acid in the presence of concentrated sulphuric acid to give a purple colored product.
-
- **Sakaguchi test**
- Under alkaline condition, α- naphthol (1-hydroxy naphthalene) reacts with a mono-substituted guanidine compound like arginine, which upon treatment with hypobromite or hypochlorite, produces a characteristic red color.
-
- **Lead sulphide test**
- Sulphur containing amino acids, such as cysteine and cystine. upon boiling with sodium hydroxide (hot alkali), yield sodium sulphide. This reaction is due to partial conversion of the organic sulphur to inorganic sulphide, which can detected by precipitating it to lead sulphide, using lead acetate solution.

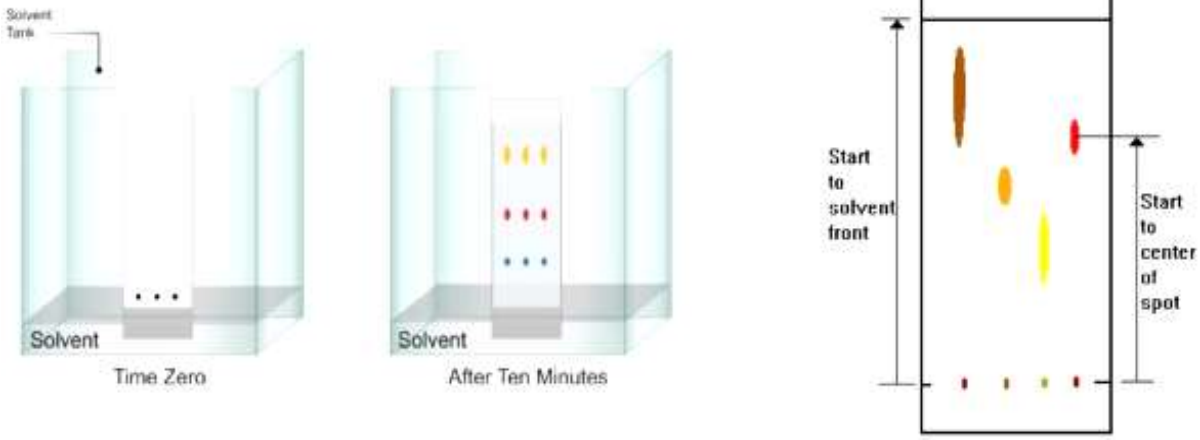
Separation of Amino acids

- Several methods are used for separation of amino acids depend on
- 1-Molecular Wt of a.a
- 2-Solubility(polarity) of a.a
- 3-Electrical charge
- Methods for separation includes
- Electrophoresis
- Chromatography

Separation of amino acids

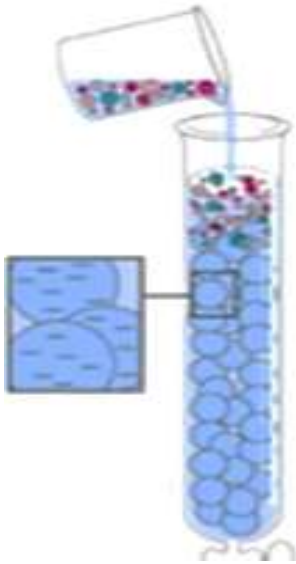
- Properties of amino acids that can be used to separate a mixture of them:
- Charge differences: By increasing the PH the amino acids were deprotonated and have more --ve charge, while by decreasing the PH tends to be protonated and have more + ve charge
- R- group polarity differences: According to the chemical concept(like dissolves in like),the polar amino acids dissolve in polar solvents, while non-polar amino acids dissolves in non-polar solvents.
- Chromatography: is the techniqueused to separate amino acids, two types:
- 1-Thin Layer Chromatography(TLC)
- 2-Ion-exchange chromatography(IEC)

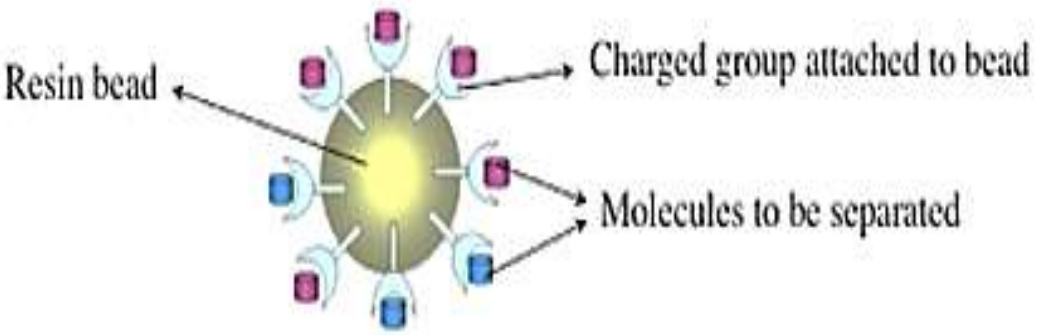
TLC-Thin Layer Chromatography



- Steps:
- 1-Samples are spotted onto the paper(stationary phase)
- 2-Paper is put into a container holding a solvent(mobile phase)
- 3-Solvent runs up paper, separating samples.
- 4-Paper is sprayed with ninhydrin, which dyes the colorless amino acids purple
- 5-Rf values are calculated(= distance traveled by sample／distance traveled by solvent)

IEC-Ion Exchange Chromatography

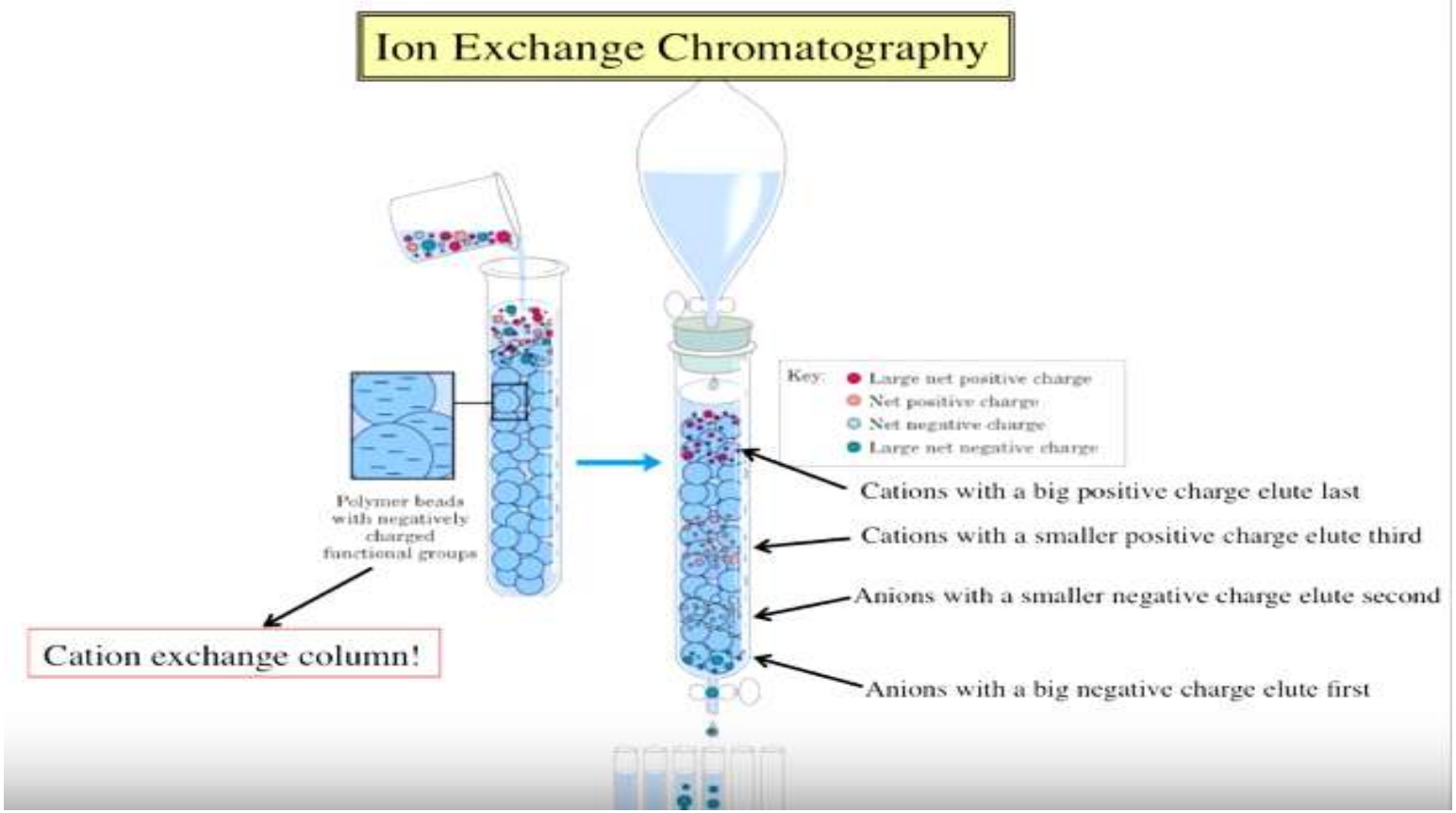




- Steps:
- 1-A column containing charged resin beads is prepared
- 2-Mixture of amino acids is run through the column
- 3- Fractions of given volumes are collected from the column

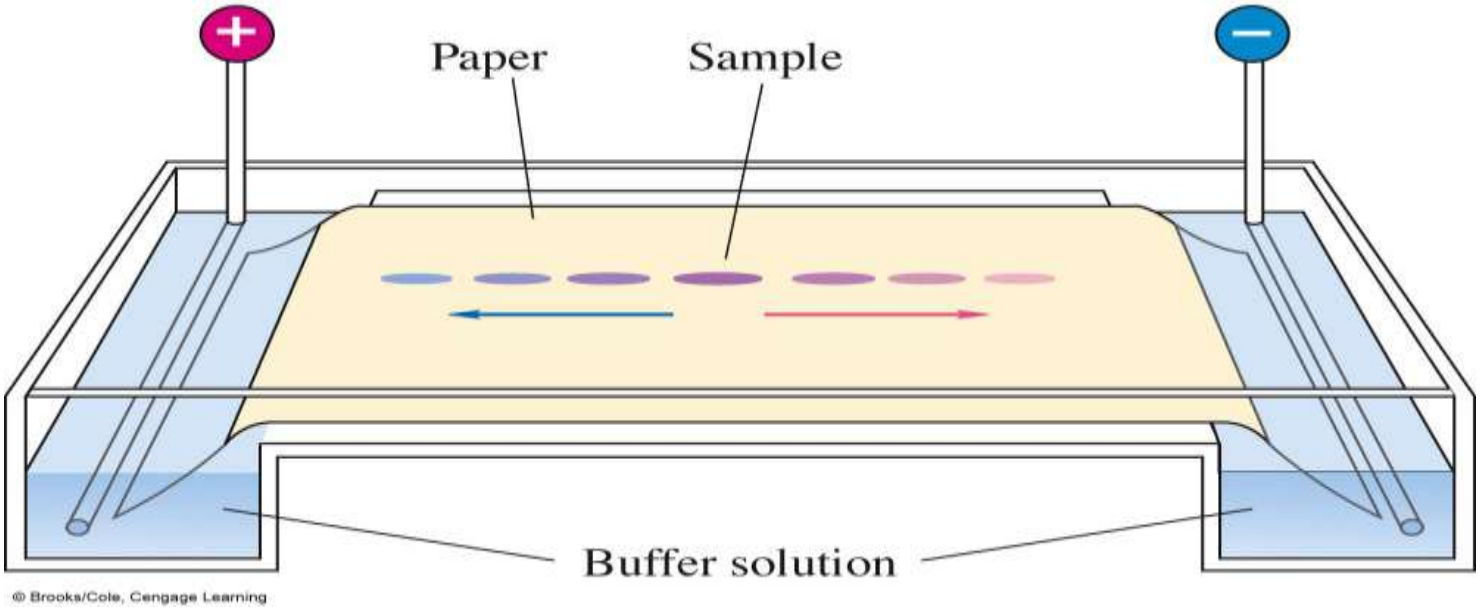
Ion-exchange chromatography

- Two types of ion-exchange column:
- Cation exchange column
- Anion exchange column



Electrophoresis

- **Electrophoresis:** The process of separating compounds on the basis of their electric charge.
  - electrophoresis of amino acids can be carried out using paper, starch, polyacrylamide and agarose gels, and cellulose acetate as solid supports.

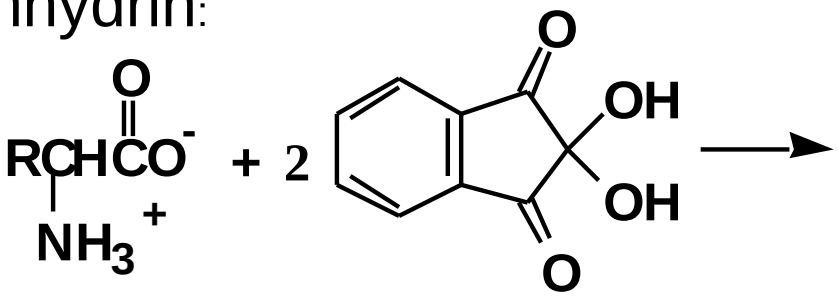


Electrophoresis

- A sample of amino acids is applied as a spot on the paper strip.
- An electric potential is applied to the electrode vessels and amino acids migrate toward the electrode with charge opposite their own.
- Molecules with a high charge density move faster than those with low charge density.
- Molecules at isoelectric point remain at the origin.
- After separation is complete, the strip is dried and developed to make the separated amino acids visible.
- After detection with ninhydrin , 19 of the 20 amino acids give the same purple-colored anion; proline gives an orange-colored compound.

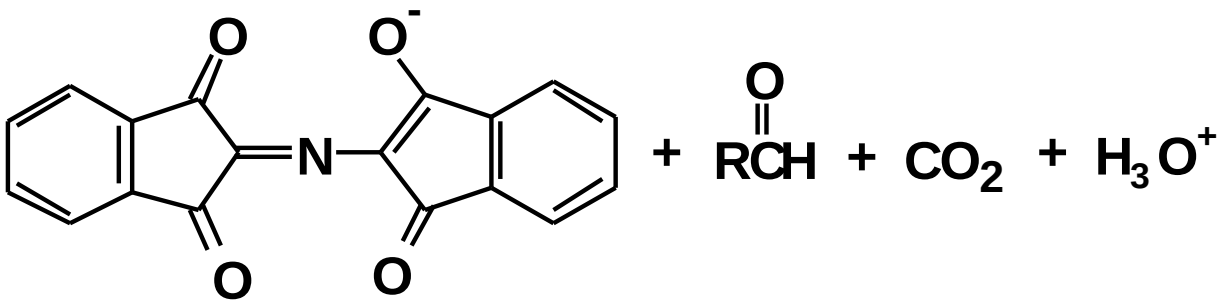
Electrophoresis

The reagent commonly used to detect amino acid is ninhydrin:



An α-amino acid

Ninhydrin



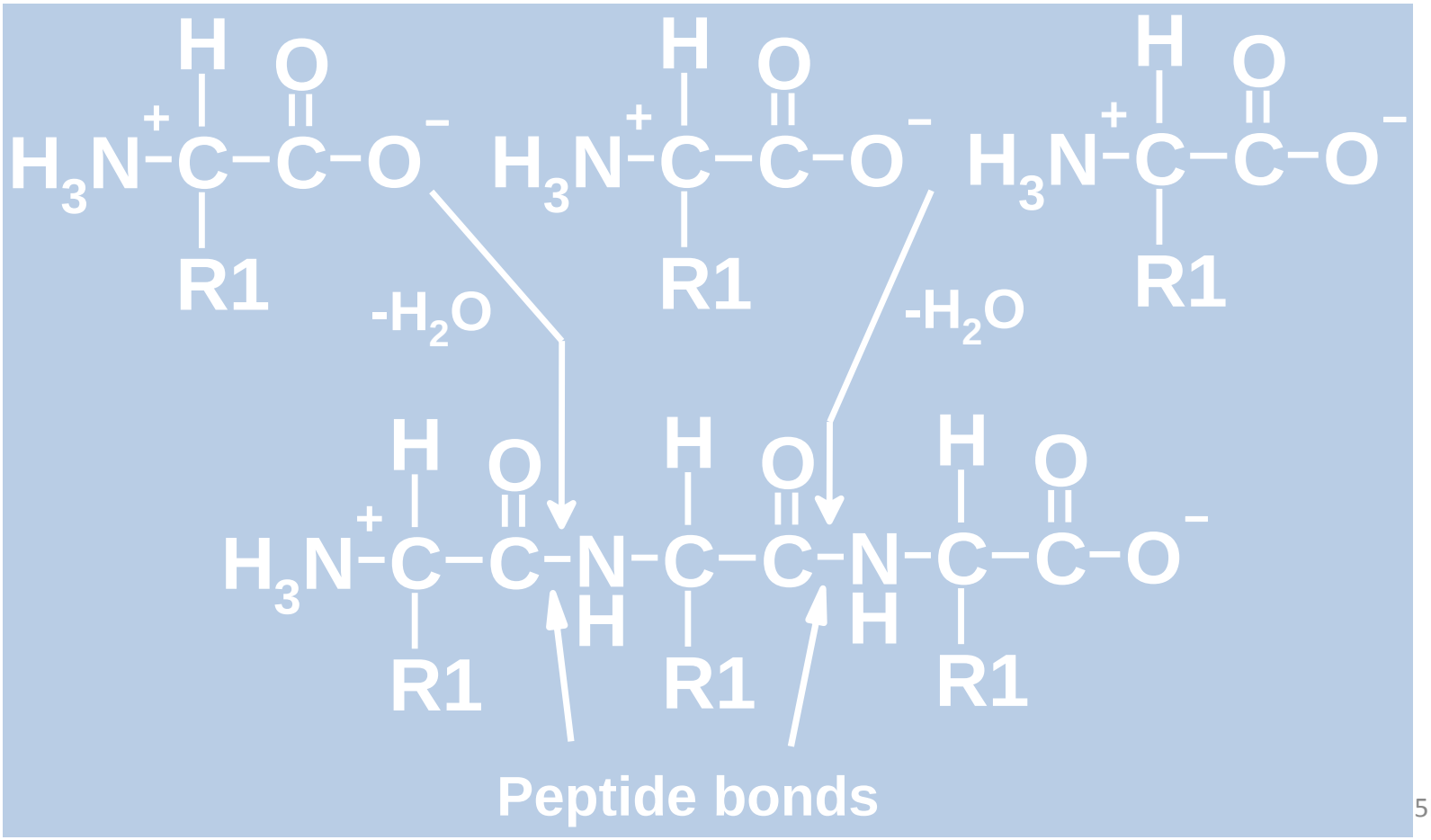
Purple-colored anion

Peptides

- Lecture (3) by : Inaam Ahmed Ameen

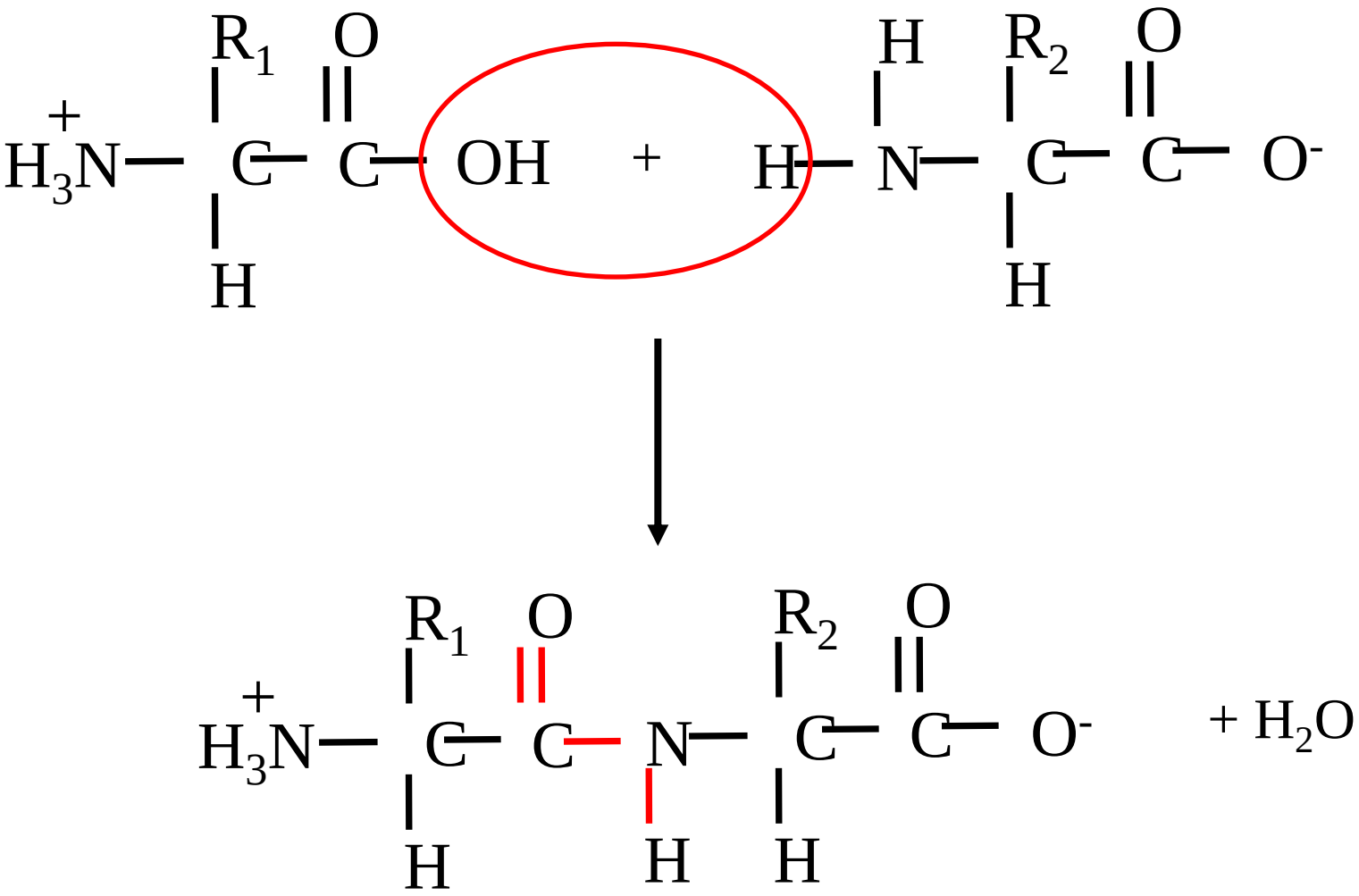
Peptides

- Peptide: a polymer of about 2-100 AAs linked by the peptide(amide) bond. The reaction between α – amino group and α – carboxyl group is a simple elimination of H_2O molecules, when peptides are drawn; their amino terminal written on ***left*** and carboxyl group to ***right***.



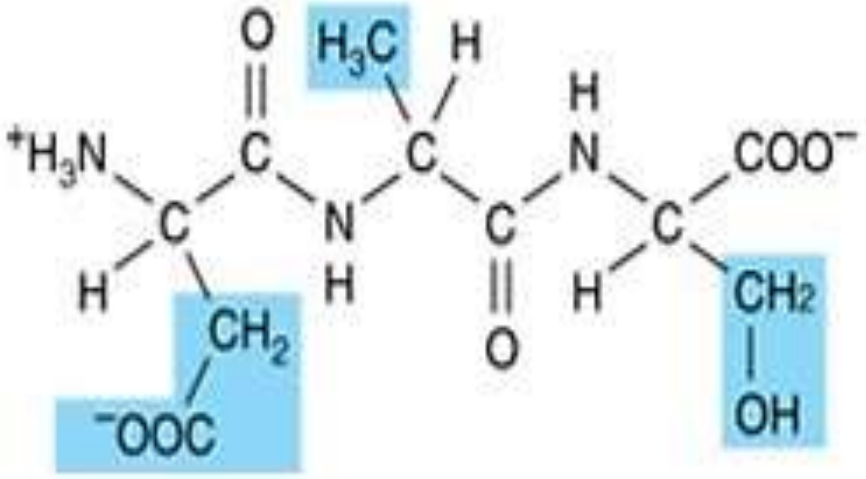
Peptide bonds

- Amide linkage



- The peptide usually written in a *Zigzag* form with repeating sequence of backbone atoms: -
- α – nitrogen, α – carbon, carbonyl carbon.

- Then add a hydrogen atom to each -carbon and to each peptide nitrogen, and an oxygen atom to the carbonyl carbon. Finally, add the appropriate R groups (shaded) to each -carbon atom.



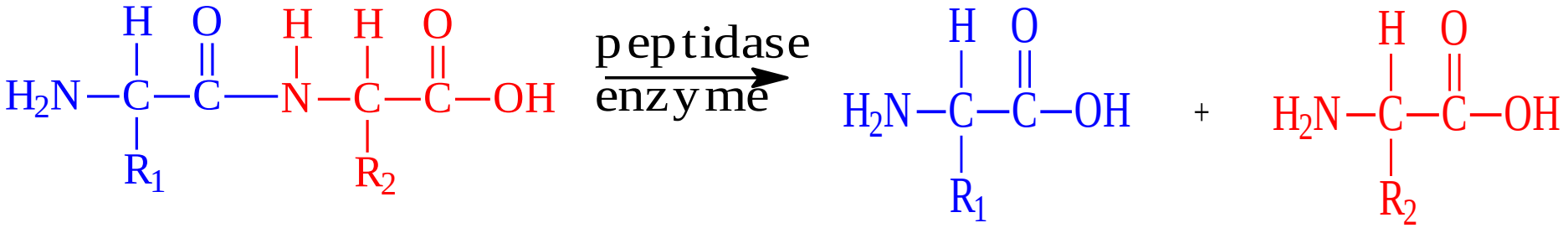
- Amino acid unit in a peptide is called a ***residue*** (the part after losing a hydrogen atom from its amino group and a hydroxyl moiety from its carboxyl group).
- Peptides are named as derivatives of the carboxyl terminal aminoacyl residue. For example: -
- Lys – Leu – Tyr – Glu
- Lysyl – leucyl – tyrosyl – glutamine
- The sequence of amino acids in peptides or protein determined the primary structure.

Peptides Are Polyelectrolytes

- The peptide bond is uncharged at any pH of physiologic concern.
- Formation of peptides from amino acids is therefore accompanied by a net loss of one positive and one negative charge per peptide bond formed.
- Peptides however are charged at physiologic pH owing to their terminal carboxyl and amino groups and, where present, their acidic or basic R groups.
- As for amino acids, the net charge on a peptide depends on the pH of its environment and on the pKa values of its dissociating groups.

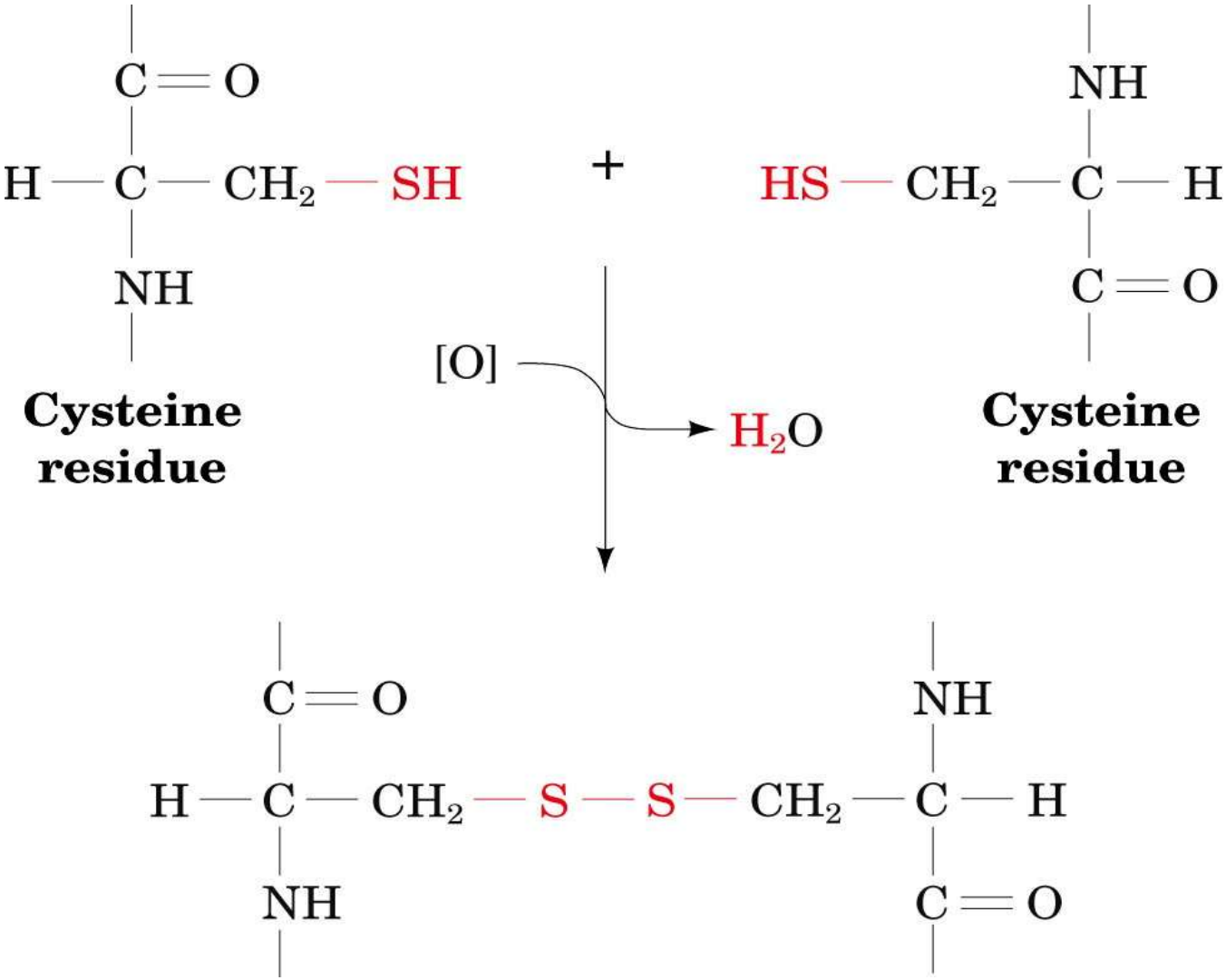
Peptide bonds

- Amino acids are covalently bonded to one another by amide linkages (bonds) between the carboxylic acid group of one amino acid and the amino group of the next amino acid.
- Amide bonds are strong and are resistant to hydrolysis, but there are enzymes that catalyze their hydrolysis (to the amino acids).



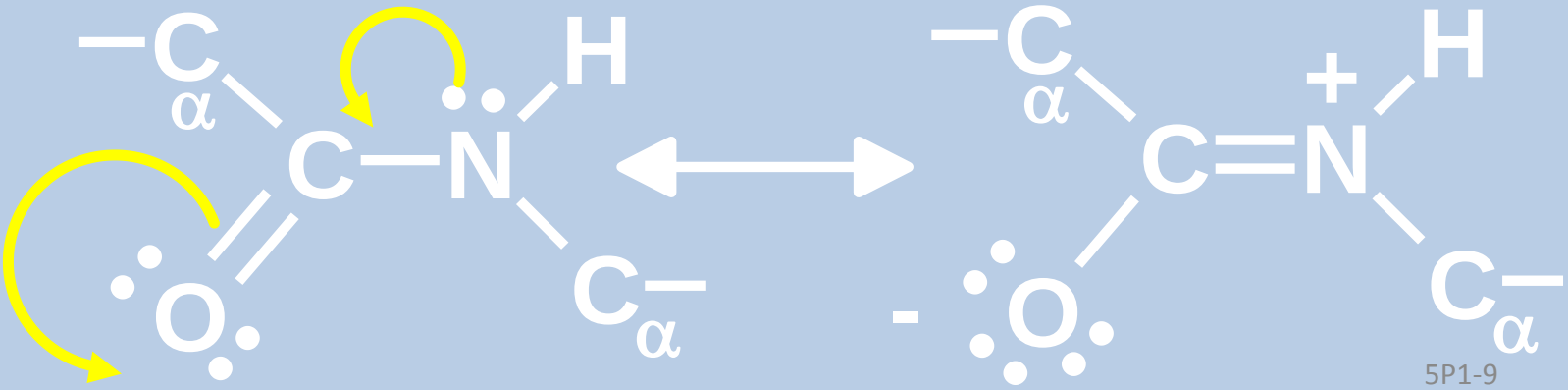
- In addition to amide bonds, a second kind of covalent bond exists in some peptides in which two cysteine residues (amino acid units) are connected through a disulfide bond formed by oxidation (dehydrogenation) of the sulfhydryl (SH, thiol) groups (next slide).

Formation of cystine



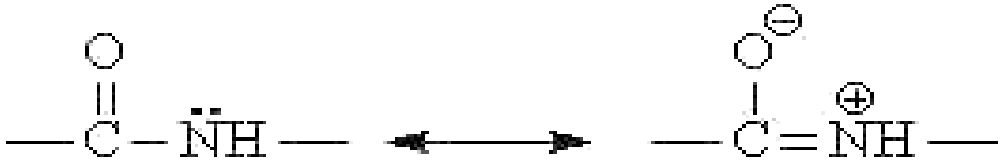
Peptides

- A peptide is written with the N-terminal end to the left and the C-terminal end to the right.
- H_2N-Tyr-Ala-Cys-Gly-COOH
- Name = Tyrosyl alanyl cysteinyl glycine
- The peptide bond is rigid and planar due to the resonance contribution.

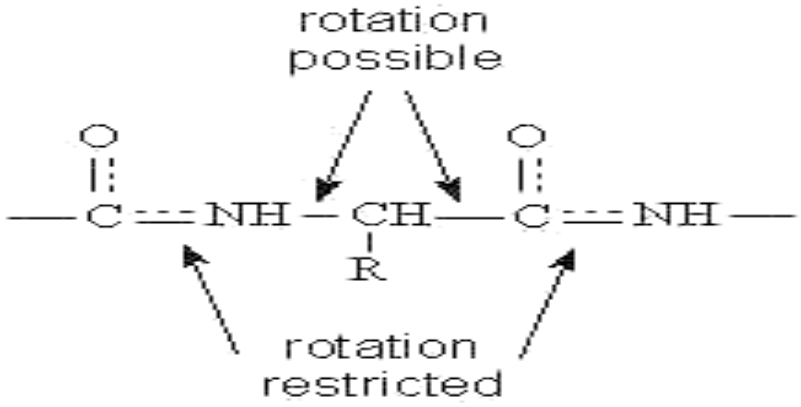


Resonance of the Peptide bond

- The amide structure has two resonance contributors:



- Because the bond between the carbonyl carbon and the nitrogen has a partial double bond character, rotation around this bond is restricted. Thus, the peptide unit is a planar, rigid structure and rotation in the peptide backbone is restricted to the bonds involving the α carbon



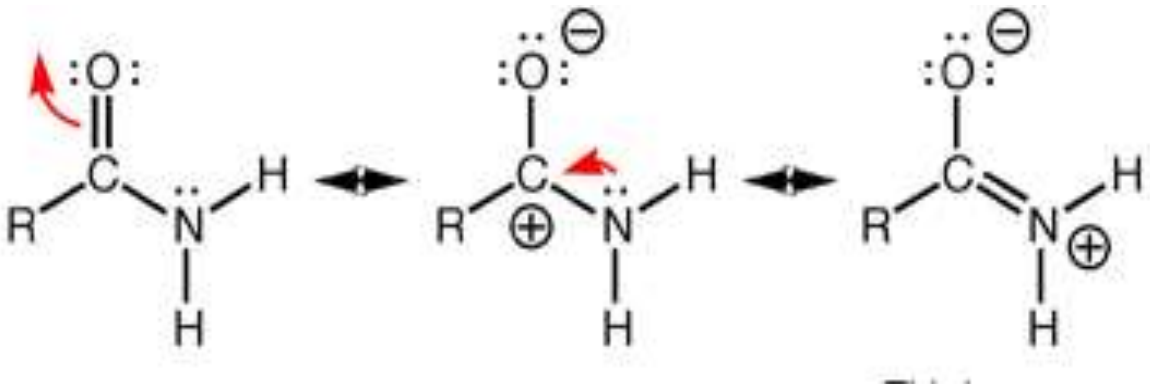
- The rigid, planar nature of the peptide unit has effects for the detailed three-dimensional structure of peptides,

Characteristics of Peptide Bonds

- Peptide bonds are strong with partial double bond character:
  - They are not broken by usual denaturing agents like heating or high salt concentration.
  - They can be broken by:
    - Prolonged exposure to strong acid or base at elevated temperatures.
    - Specific enzymes such as digestive enzymes.
- Peptide bonds are rigid and planner resisting free rotation, therefore they stabilize protein structure

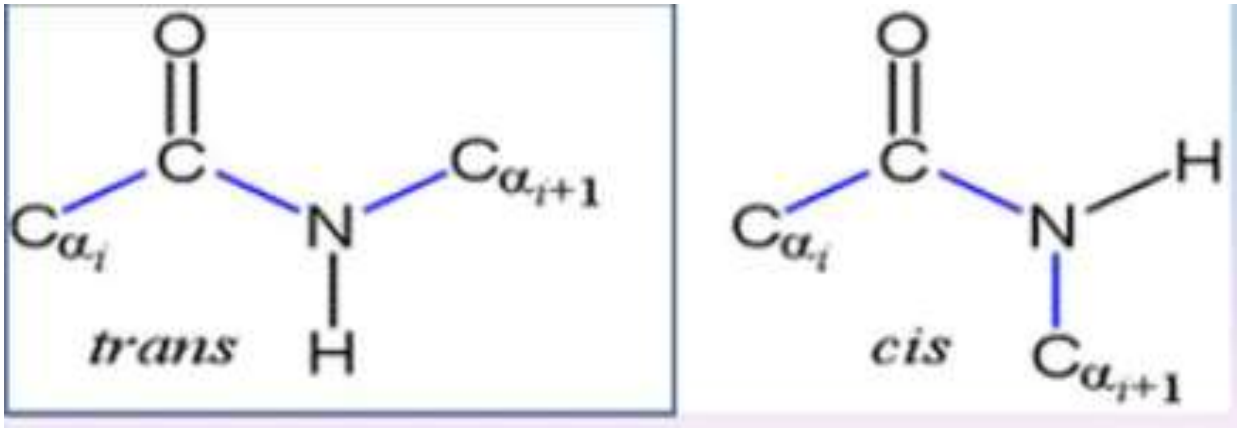
The stability of the peptide bond

- Is due to the resonance of amides. With resonance, the nitrogen is able to donate its lone pair of electrons to the carbonyl carbon and push electrons from the carbonyl double bond towards the oxygen, forming the oxygen anion.



- This resonance effect is very stabilizing because the electrons can be delocalized over multiple atoms, with one especially stable resonance structure containing the highly electronegative oxygen as an anion. .

- The partial double bond renders the amide group planar, occuring either in cis or trans isomers. The most commonly observed is the trans isomer



- the <u>trans</u> form is usually favored because in cis conformation the R bulky group on adjacent α – carbon usually interfere "steric effect" the exception is the Pro.

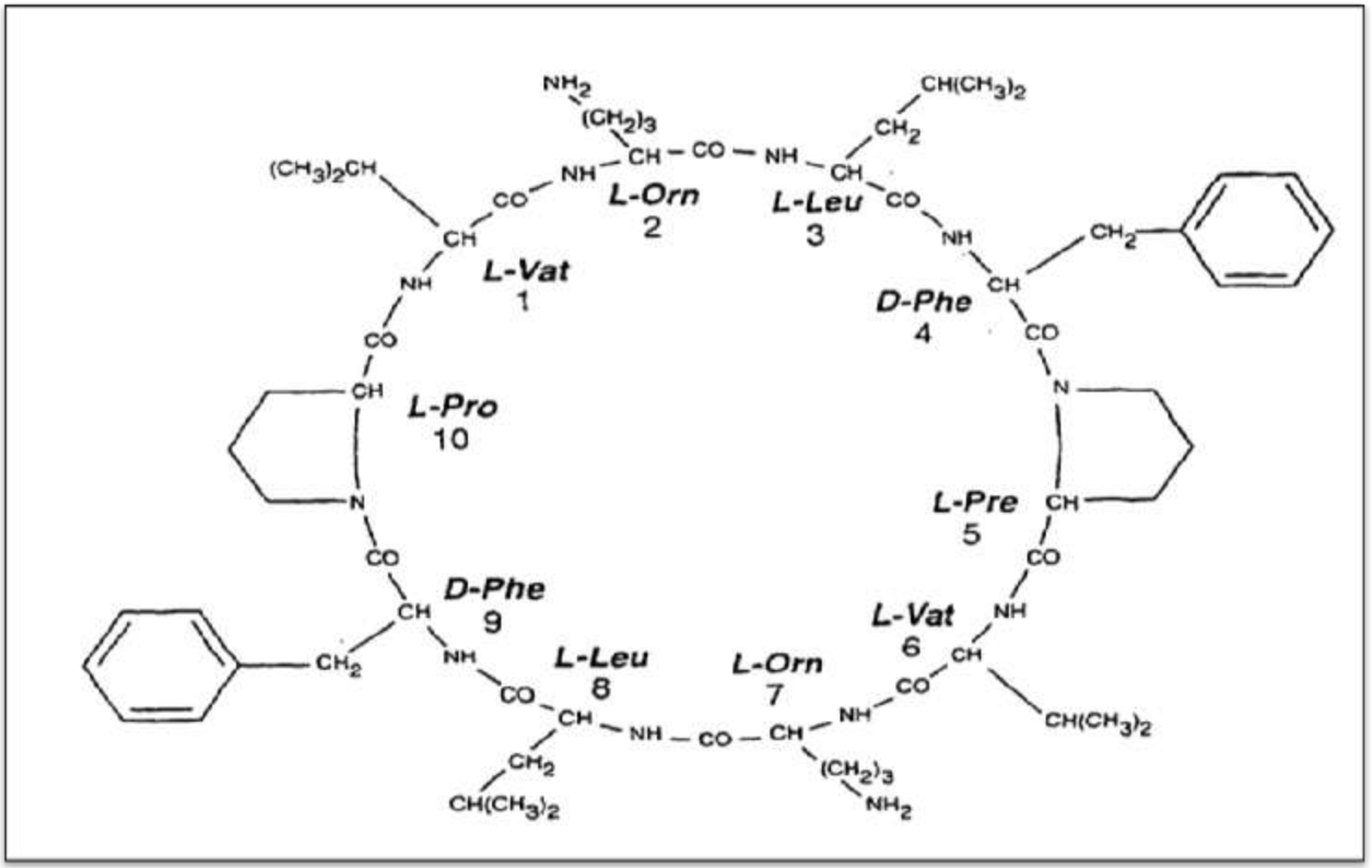
Some Peptides Contain Unusual Amino Acids

- In mammals, peptide hormones typically contain only the -amino acids of proteins linked by standard peptide bonds.
- Other peptides may, however, contain non-protein amino acids, derivatives of the protein amino acids, or amino acids linked by an atypical peptide bond.
- For example, the amino terminal glutamate of glutathione, which participates in protein folding and in the metabolism of xenobiotics (substances that are foreign to the body), is linked to cysteine by a non-peptide Bond.
- The amino terminal glutamate of thyrotropin-releasing hormone (TRH) is cyclized to pyroglutamic acid, and the carboxyl group of the carboxyl terminal prolyl residue is amidated.
- The nonprotein amino acids D -phenylalanine and ornithine are present in the cyclic peptide antibiotics tyrocidin and gramicidin S.

- Peptides can be simple *dipeptide.*
- amino acid residues fewer than 10 these called *oligopeptides.*
- long peptides more than 10 amino acids called *polypeptides.*
- And longer than 100 amino acids called *protein*.

Cyclic peptide

- Some cyclic oligopeptides in which N & C terminal have been linked like “antibiotic polypeptide from Bacillus” this peptide form complexes with metal ions and disruption transport across the cell membrane and killing certain bacteria.



Biological importance of peptides

- Peptides are found throughout every cell and tissue in the body and are an integral part of most biologic processes.
- Maintenance of appropriate concentration and activity levels of peptides is necessary to achieve homeostasis and maintain health.
- The function of a peptide is determined by its size and amino acid sequence.

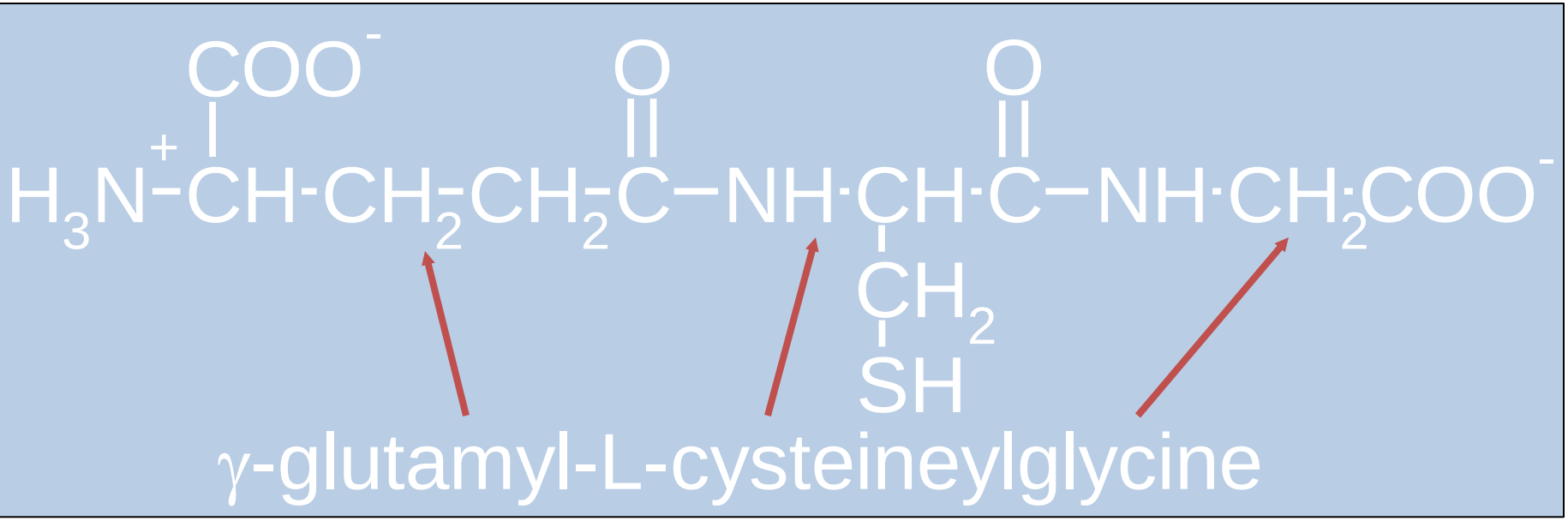
Number of amino acids:

- A *dipeptide* has two amino acids. (Carnosine)
- A *tripeptide* has three amino acids(Glutathione)
  A *tetrapeptide* has four amino acids.
- A *pentapeptide* has five amino acids.(Enkephalin)
- A *hexapeptide* has six amino acids.
- A *heptapeptide* has seven amino acids.
- An *octapeptide* has eight amino acids (angiotensin II).
- A *nonapeptide* has nine amino acids (oxytocin).
- A *decapeptide* has ten amino acids (gonadotropin-releasing hormone & angiotensin I).

Glutathione

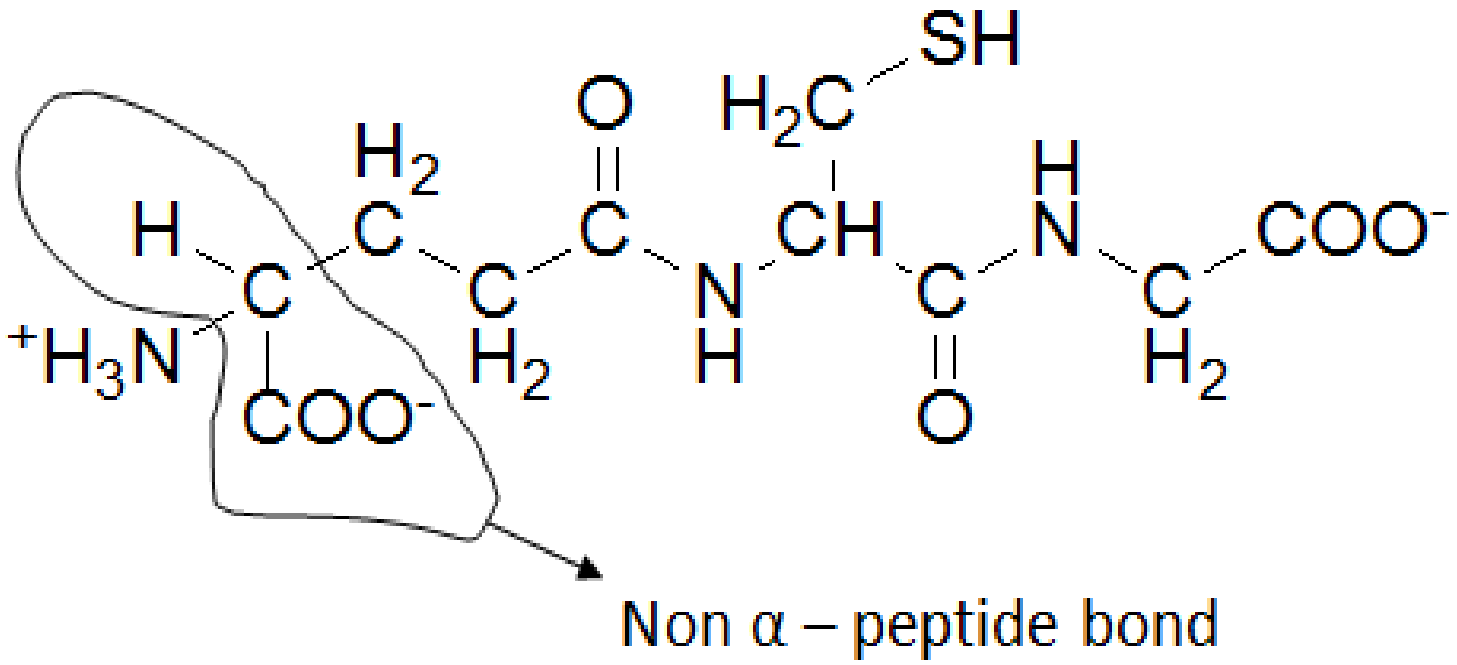
- **Glutathione (GSH)** is an important antioxidant in plants, animals, fungi, and some bacteria.
- Glutathione is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals.
- It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side chain and the amine group of cysteine, and the carboxyl group of cysteine is attached by normal peptide linkage to a glycine.
- Glutathione exists in both reduced (GSH) and oxidized (GSSG) states. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ($H^+ + e^-$) to other molecules, such as reactive oxygen species to neutralize them.

Glutathione



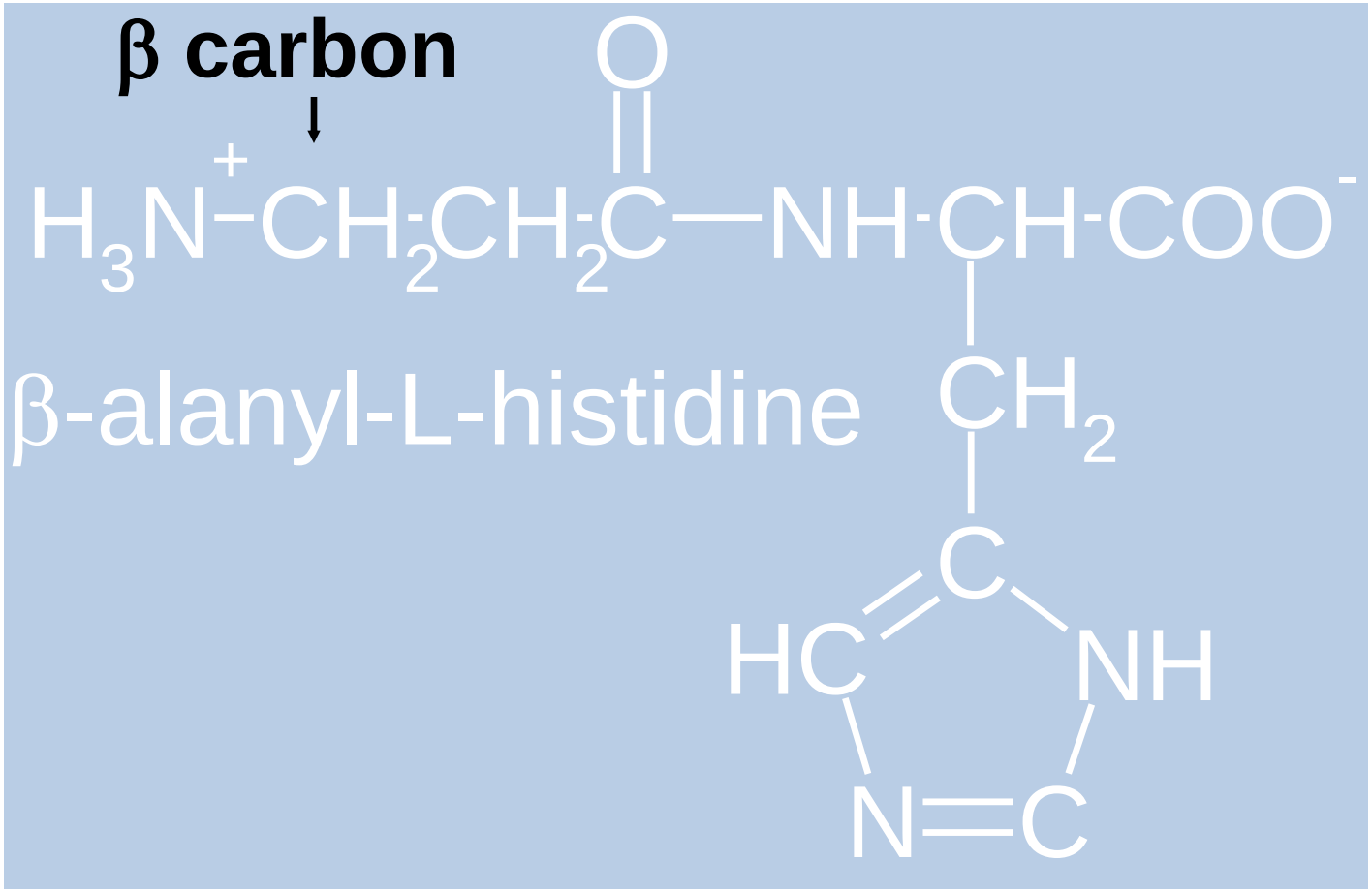
Glutathione: the reduced form reduces oxidizing agents by dimerizing to form the disulfide bond with release of 2 H.

Note: the non- peptide bond that links Glu to Cys



- Glutathione (-glutamyl-cysteinyl-glycine).

Carnosine



which is water soluble, dipeptide of β – Alanine and Histidine found in muscle tissue

Enkephalin

Tyr-Gly-Gly-Phe-Leu

N-terminal AA **C-terminal AA**

Pentapeptide Endogenous naturally occurring peptide that has potent painkilling effects and is released by neurons in the central nervous system and by cells in the adrenal medulla.

Oxytocin&Vassopressin

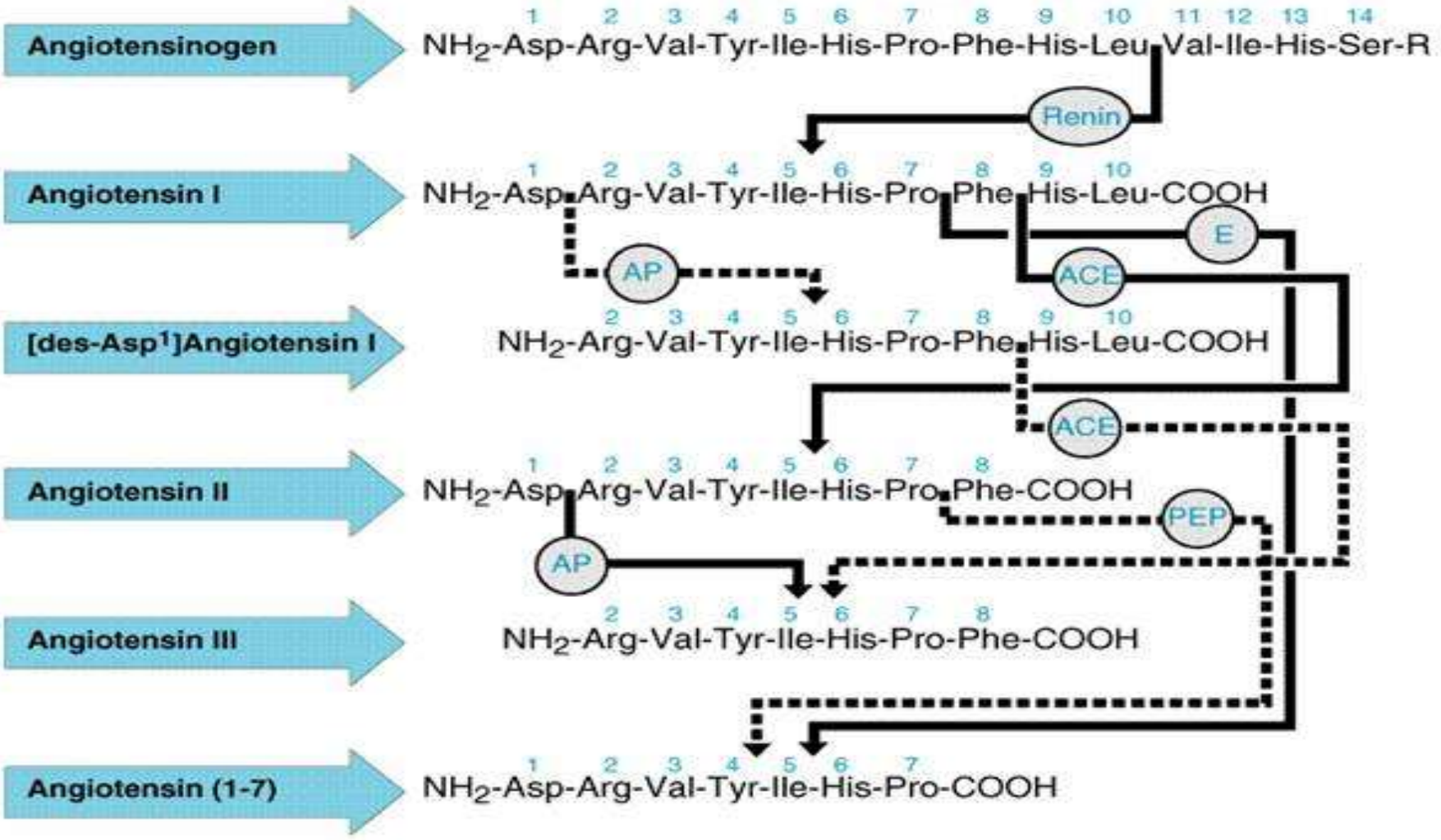
- these two cyclic peptide hormones which secreted from pituitary gland.
- **Oxytocin:** is a peptide with 9 amino acids secreted by posterior pituitary and act on uterine muscles during labor.
- **Vasopressin:** this hormone called also (ADH) anti diuretic hormone, which effect the reabsorption of H_2O from the distal and collection tubule of kidneys.

Oxytocin&Vassopressin

Oxytocin Induces labor and aids in forcing milk from the mammary glands.

Vassopressin has a Phe at position 3 instead of Ile and an Arg at position 8 instead of a Leu. Its role is in regulating blood pressure.

Angiotensin



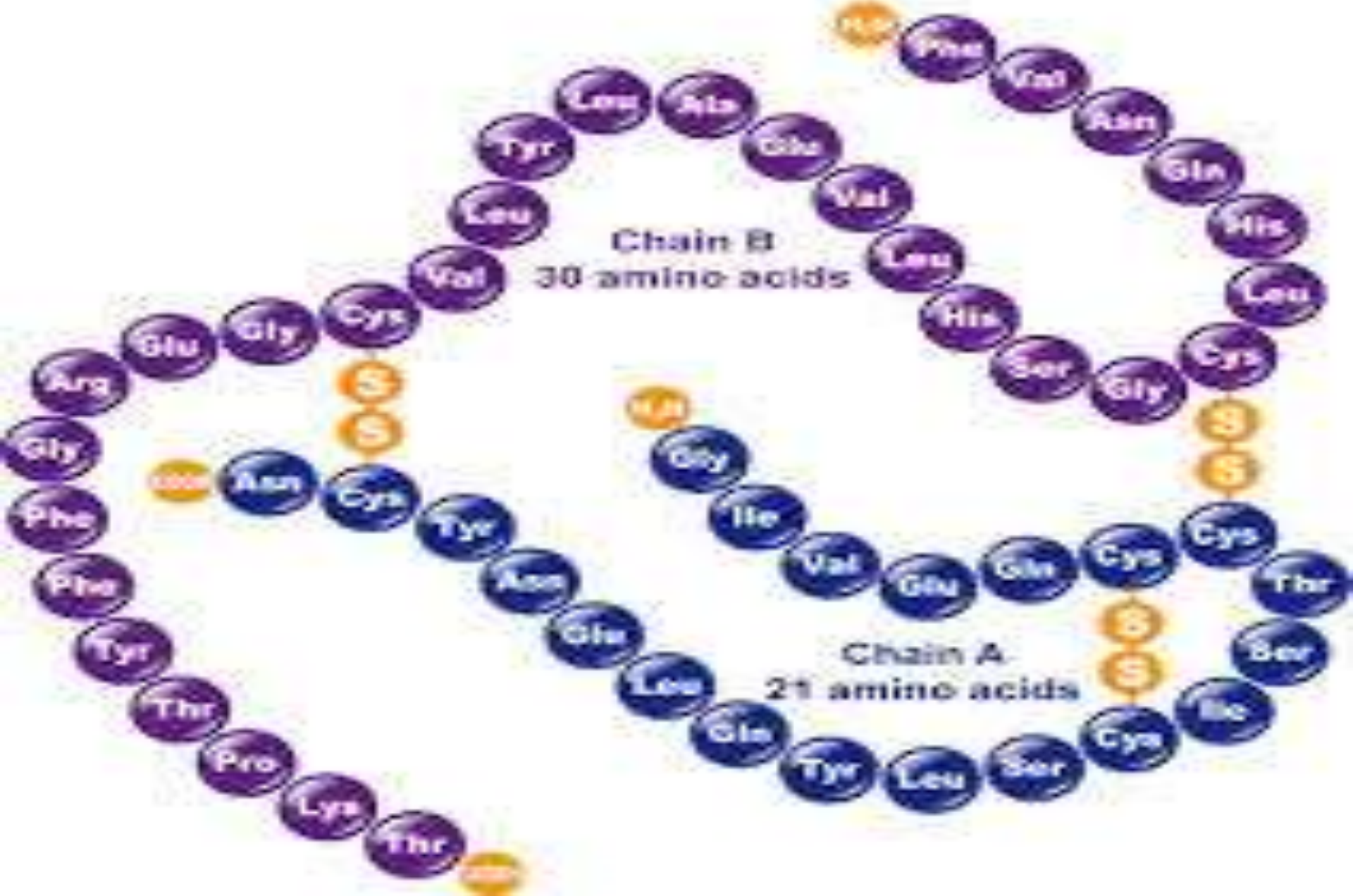
Angiotensin

- A peptide hormone derived from angiotensinogen (485aa). There are three forms of angiotensin. Angiotensin I (decapeptide) is produced by the action of renin (an enzyme produced by the kidneys) on a angiotensinogen, which is formed by the liver.
- Angiotensin I is transformed into angiotensin II in the blood by the action of angiotensin-converting enzyme (ACE).
- Angiotensin II acts directly on blood vessels, causing their constriction and thereby raising blood pressure.
- This substance also can cause vessel constriction through indirect mechanisms, such as by stimulating the release of the steroid hormone aldosterone and catecholamines from the adrenal glands and by blocking the reuptake of the hormone norepinephrine into neurons.
- Angiotensin III is a metabolite of angiotensin II and shares similar, though less potent, actions.

Insulin

- **Insulin** is a peptide hormone produced by beta cells of the pancreatic islets, and it is considered to be the main anabolic hormone of the body.
- It regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of, especially, glucose from the blood into fat, liver and skeletal muscle cells.
- In these tissues the absorbed glucose is converted into either glycogen via glycogenesis or fats (triglycerides) via lipogenesis , or, in the case of the liver, into both.
- Insulin consists of two polypeptide chains, the A- and B- chains, linked together by disulfide bonds.

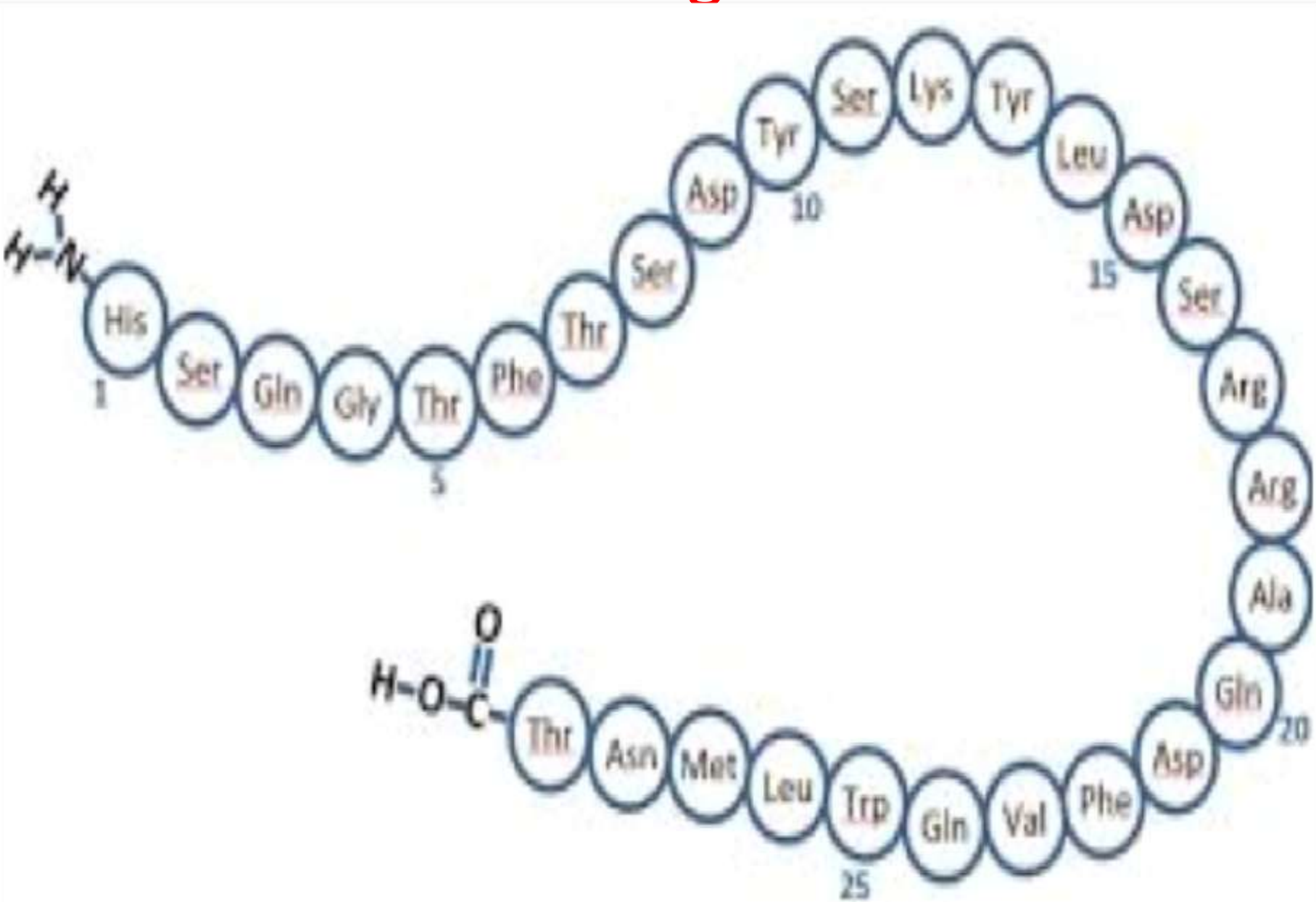
Human Insulin



Glucagon

- **Glucagon** is a peptide hormone , produced by alpha cells of the pancreas.
- It raises the concentration of glucose and fat in the bloodstream, and is considered to be the main catabolic hormone of the body.
- Its effect is opposite to that of insulin, which lowers the extracellular glucose.
- Glucagon is a 29-amino acid polypeptide.

Glucagon


His
Ser
Gln
Gly
Thr
Phe
Thr
Ser
Asp
Tyr
Ser
Lys
Tyr
Leu
Asp
Ser
Arg
Arg
Ala
Gln
Asp
Phe
Val
Gln
Trp
Leu
Met
Asn
Thr
1
5
10
15
20
25
H-O-C
O

Proteins

Lec (4) by : Inaam Ahmed Ameen

Proteins

- Proteins are essential parts of all living organisms and participate in every process within cells.
- Form a major part of body, next to water.
- Essential component of Protoplasm.
- Essential component of the biological membranes (cell membrane, Nuclear membrane and membranes of the organelles).
- Make the basic structure of muscles, skin, hair, nail, bones and all the tissues of the body.
- Essential for the repair of damaged tissues.

BIO-MEDICAL IMPORTANCE of proteins

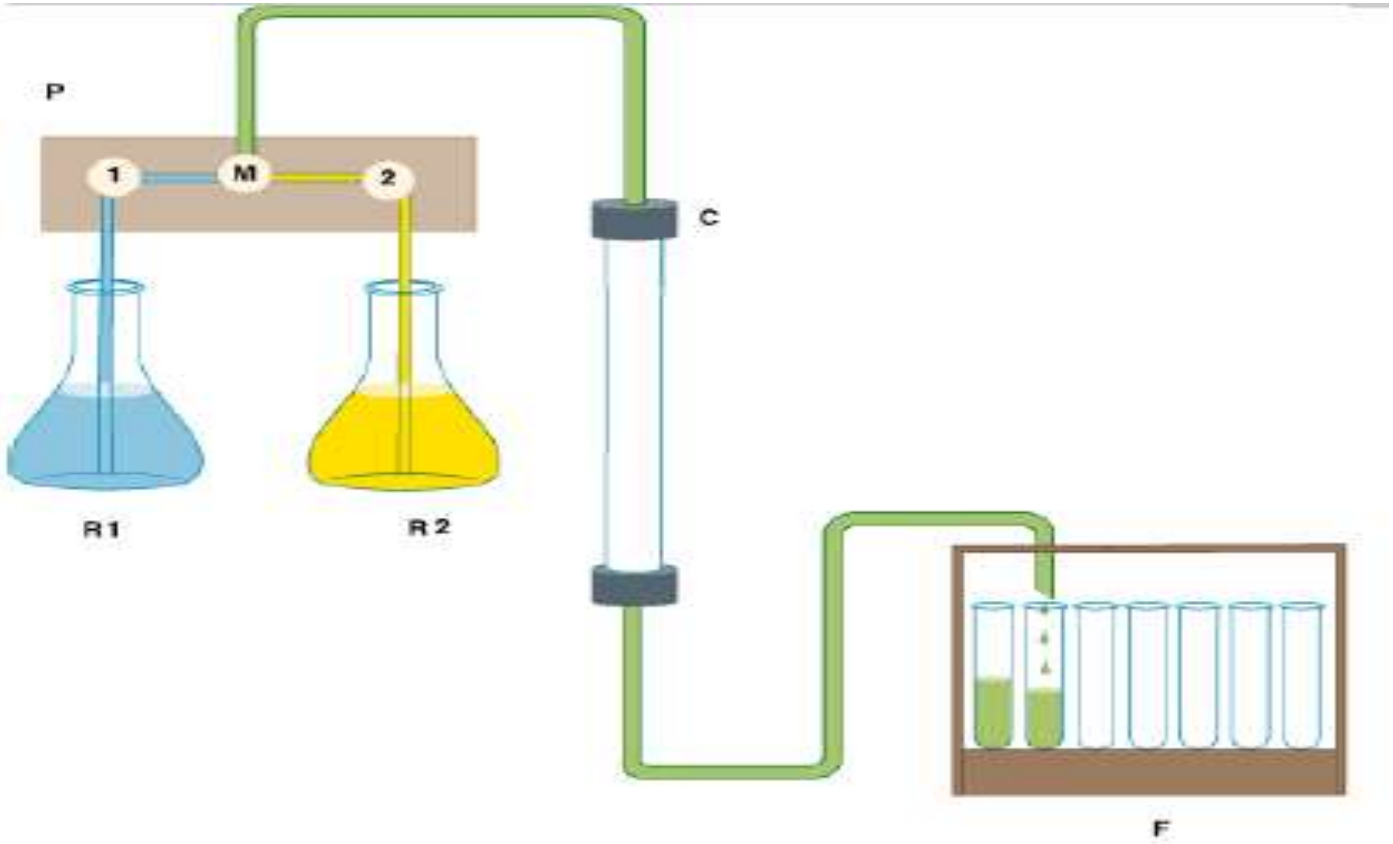
- Proteins are physically and functionally complex macromolecules that perform multiple critically important roles. An internal protein network, the cytoskeleton, maintains cellular shape and physical integrity.
- Actin and myosin filaments form the contractile machinery of muscle. Hemoglobin transports oxygen , while circulating antibodies search out foreign invaders.
- Enzymes catalyze reactions that generate energy, synthesize and degrade biomolecules, replicate and transcribe genes, process mRNAs, etc .
- Receptors enable cells to sense and respond to hormones and other environmental signals.
- An important goal of molecular medicine is the identification of proteins and those events in their life cycle whose presence, absence, or deficiency is associated with specific physiologic states or diseases .

PROTEINS purification&separation

- Highly purified protein is essential for the detailed examination of its physical and functional properties.
- Cells contain thousands of different proteins, each in widely varying amounts.
- The isolation of a specific protein in quantities sufficient for analysis may require multiple successive purification techniques.
- Classic approaches use differences in relative solubility of individual proteins as a function of pH (isoelectric precipitation).
- polarity (precipitation with ethanol or acetone), or salt concentration (salting out with ammonium sulfate).
- Chromatographic separations partition molecules between two phases, one mobile and the other stationary.

Column Chromatography

- Column chromatography of proteins employs as the stationary phase small spherical beads of modified cellulose, acrylamide, or silica whose surface coated with chemical functional groups.
- The beads are packed in a cylindrical container, or column.
- These stationary phase media interact with proteins based on their charge, hydrophobicity, and ligand-binding properties.
- A protein mixture is applied to the column and the liquid mobile phase is penetrated through it.
- Small portions of the mobile phase or eluant are collected as they leak out.



Components of a typical liquid chromatography apparatus:

R1 and **R2:** Reservoirs of mobile phase liquid. **P:** Programable pumping system containing two pumps, 1 and 2, and a mixing chamber, **M.**

The system can be set to pump liquid from only one reservoir, to switch reservoirs at some predetermined point to generate a step gradient, or to mix liquids from the to reservoirs in proportions that vary over time to create a continuous gradient.

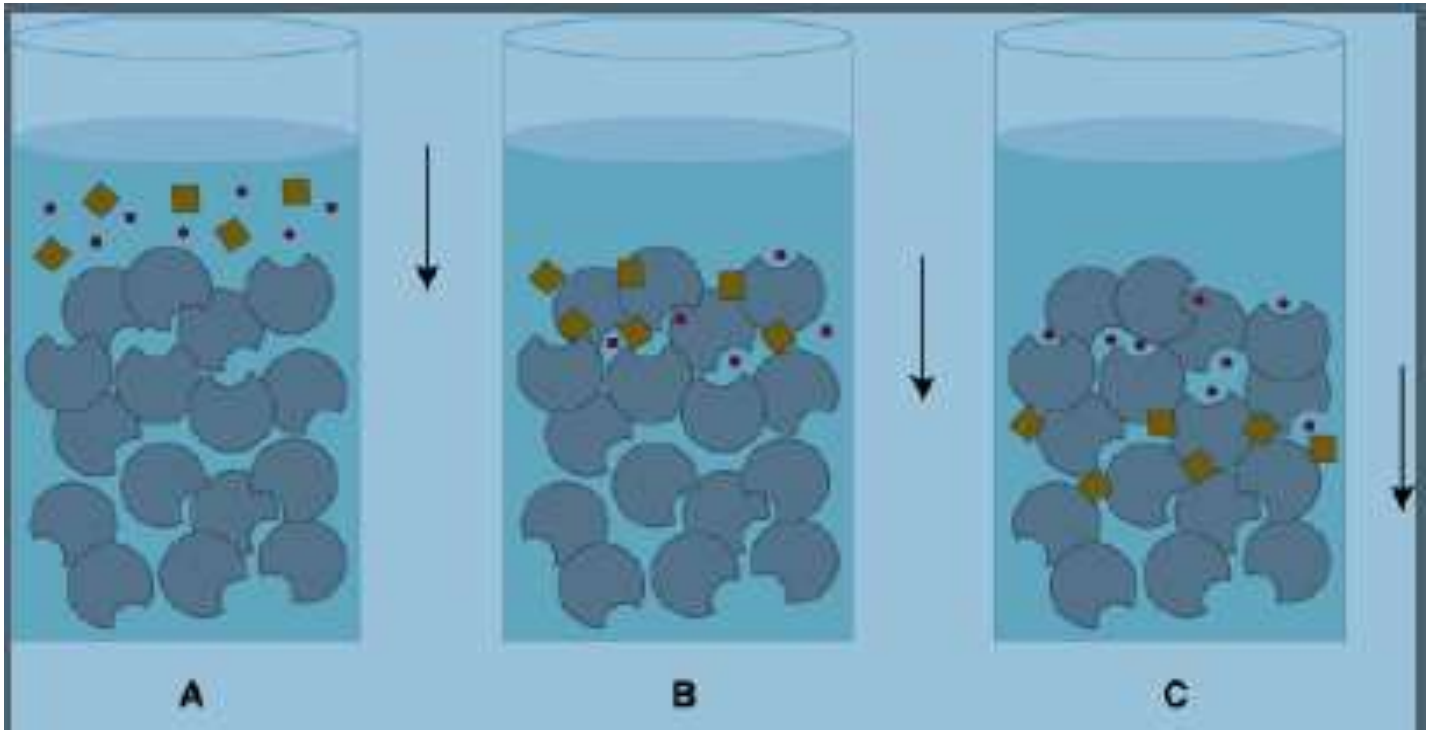
C: Glass, metal, or plastic column containing stationary phase.

F: Fraction collector for collecting portions, (*fractions),* of the eluant liquid in separate test tubes.

Partition Chromatography

- Column chromatographic separations depend on the relative affinity of different proteins for a given stationary phase and for the mobile phase.
- In partition chromatography, association between each protein and the matrix is weak and transient.
- Proteins that interact more strongly with the stationary phase are retained longer.
- Best separation of the protein of interest from other proteins can be achieved by careful control of the composition of the two phases.

Size Exclusion Chromatography (gel filtration):



- **A:** A mixture of large molecules (brown) and small molecules (red) are applied to the top of a gel filtration column.
- **B:** Upon entering the column, the small molecules enter pores in the stationary phase matrix (gray) from which the large molecules are excluded.
- **C:** As the mobile phase (blue) flows down the column, the large, excluded molecules flow with it, while the small molecules, which are protected from the flow when inside the pores, lag farther and farther behind.

Other techniques used for separation of proteins:

- **Ion Exchange Chromatography**
- **Hydrophobic Interaction Chromatography**
- **Affinity Chromatography**
- **Absorption Chromatography**

Purification of peptides

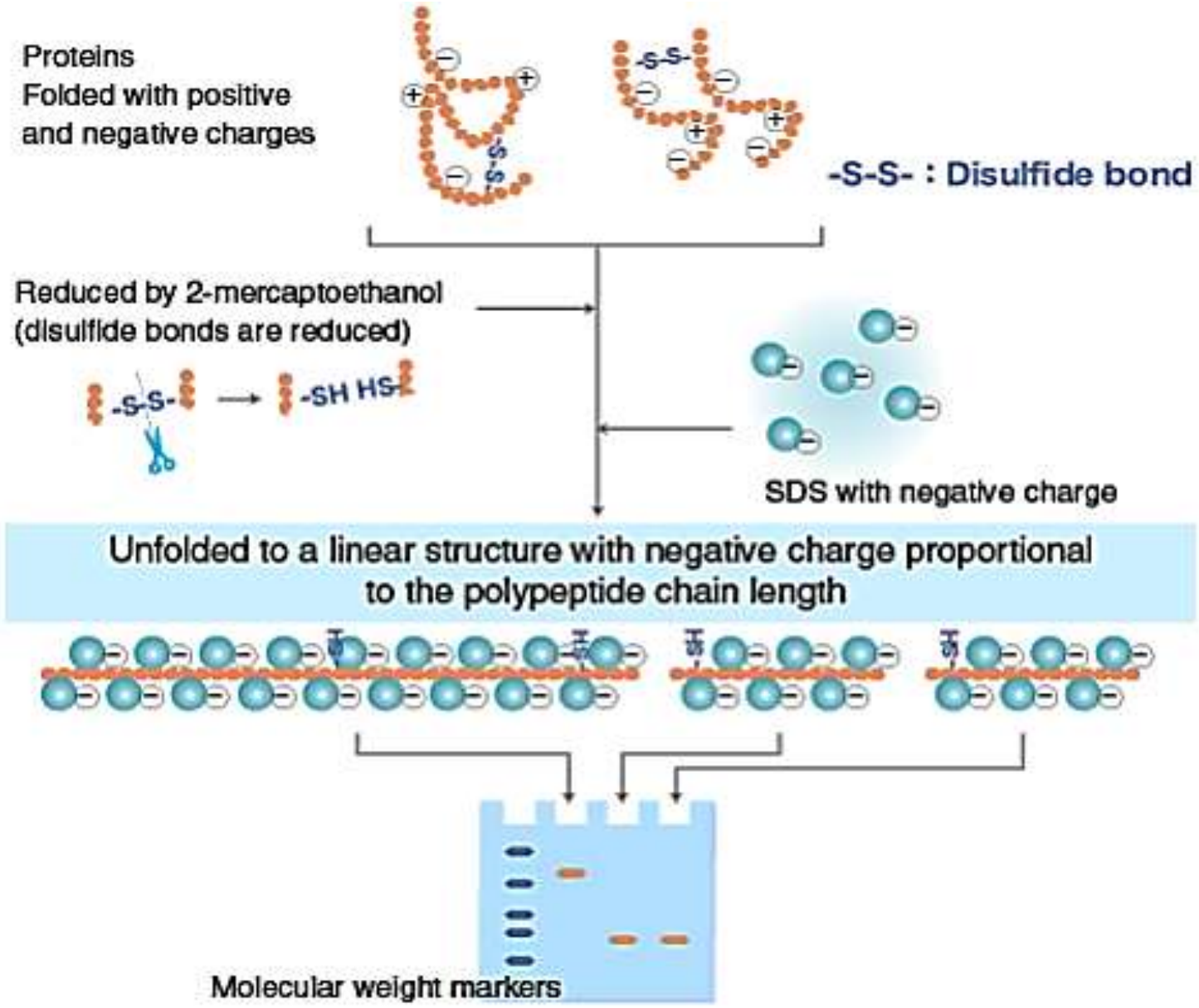
- Peptides Are Purified by Reversed-Phase High-Pressure Chromatography:
- High-pressure liquid chromatography (HPLC) employs incompressible silica or alumina microbeads as the stationary phase and pressures of up to a few thousand psi.
- Incompressible media permit both high flow rates and enhanced resolution.
- HPLC can resolve complex mixtures of lipids or peptides whose properties differ only slightly.
- Peptide mixtures are eluted using a gradient of a water-miscible organic solvent such as acetonitrile or methanol.

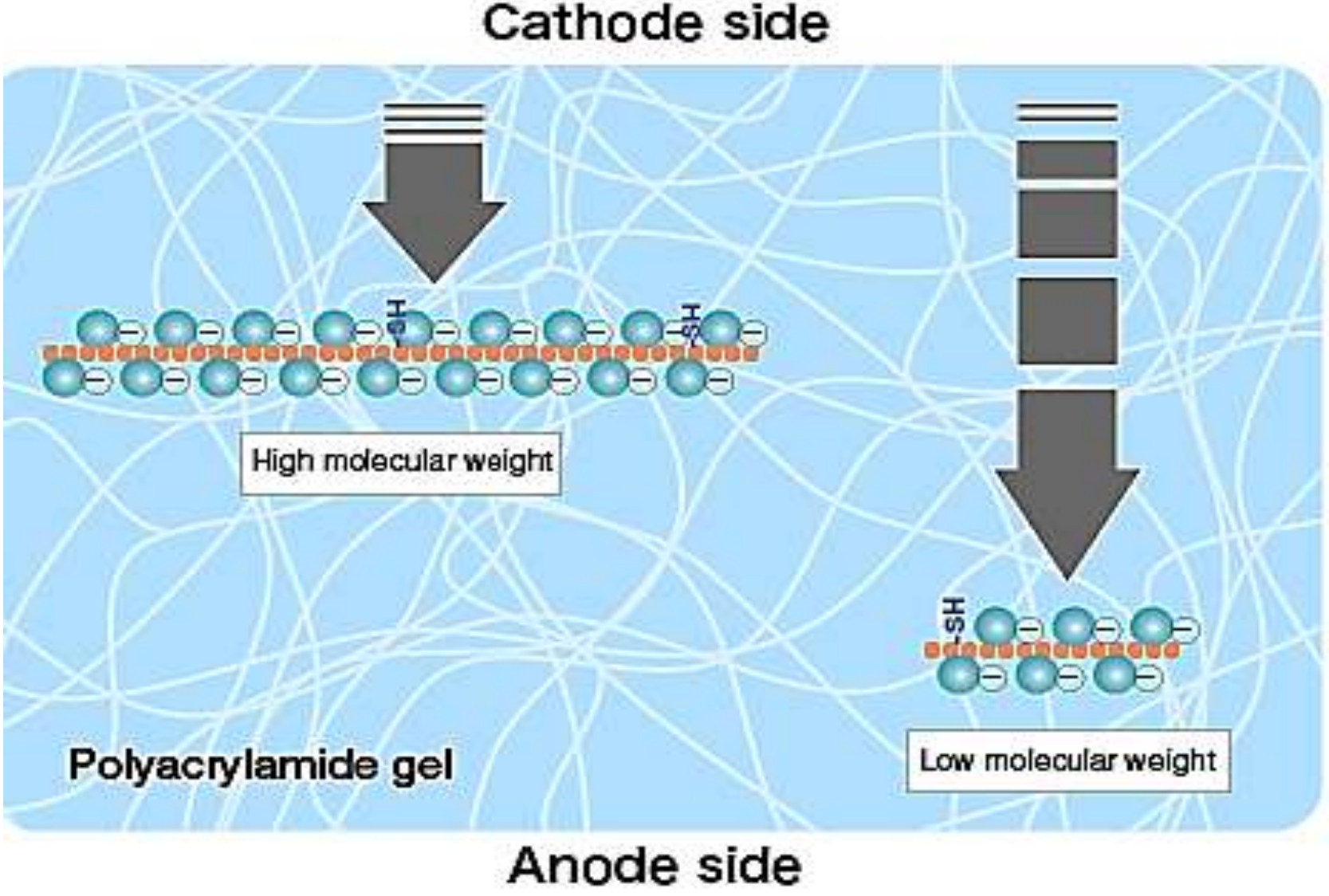
Purification of proteins

- Protein Purity Is Assessed by Polyacrylamide Gel Electrophoresis (PAGE):
- The most widely used method for determining the purity of a protein is SDS-PAGE—polyacrylamide gel electrophoresis (PAGE) in the presence of the anionic detergent sodium dodecyl sulfate (SDS).
- The principle:
- When proteins are separated by electrophoresis through a gel matrix, smaller proteins migrate faster due to less resistance from the gel matrix. Other affect on the rate of migration through the gel matrix include the structure and charge of the proteins.

- In SDS-PAGE, the use of sodium dodecyl sulfate (SDS, also known as sodium lauryl sulfate) and polyacrylamide gel largely eliminates the influence of the structure and charge, and proteins are separated solely based on polypeptide chain length.

- SDS is a detergent with a strong protein-denaturing effect and binds to the protein backbone at a constant molar ratio. In the presence of SDS and a reducing agent that cleaves disulfide bonds critical for proper folding, proteins unfold into linear chains with negative charge proportional to the polypeptide chain length.


Proteins
Folded with positive
and negative charges
-S-S-
-S-S- : Disulfide bond
Reduced by 2-mercaptoethanol
(disulfide bonds are reduced)
-S-S-
-SH HS-
SDS with negative charge
Unfolded to a linear structure with negative charge proportional
to the polypeptide chain length
Molecular weight markers

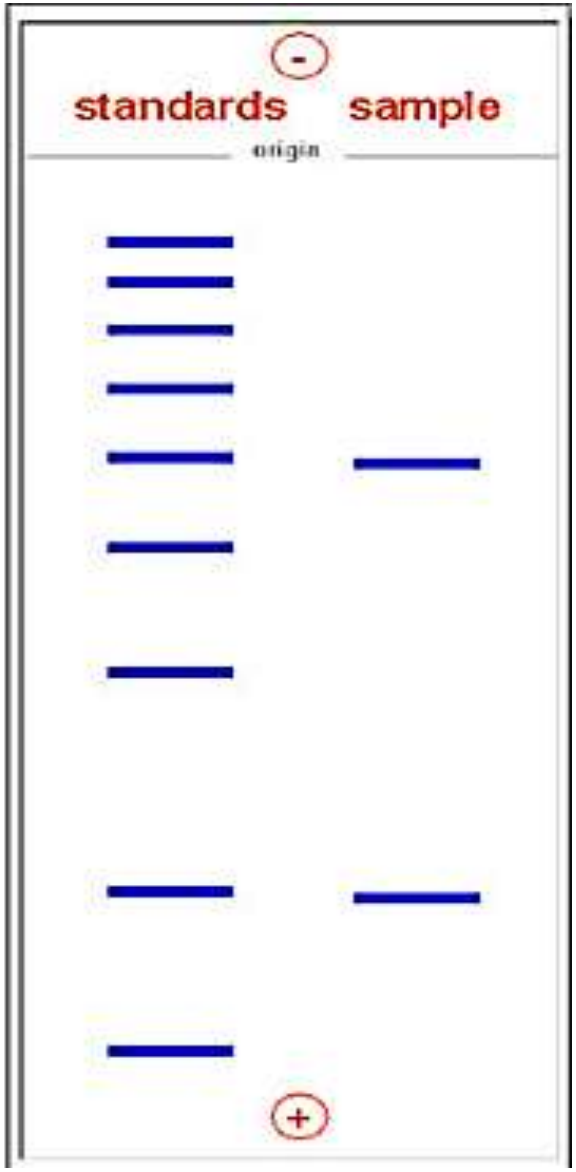

Cathode side
High molecular weight
Low molecular weight
Polyacrylamide gel
Anode side

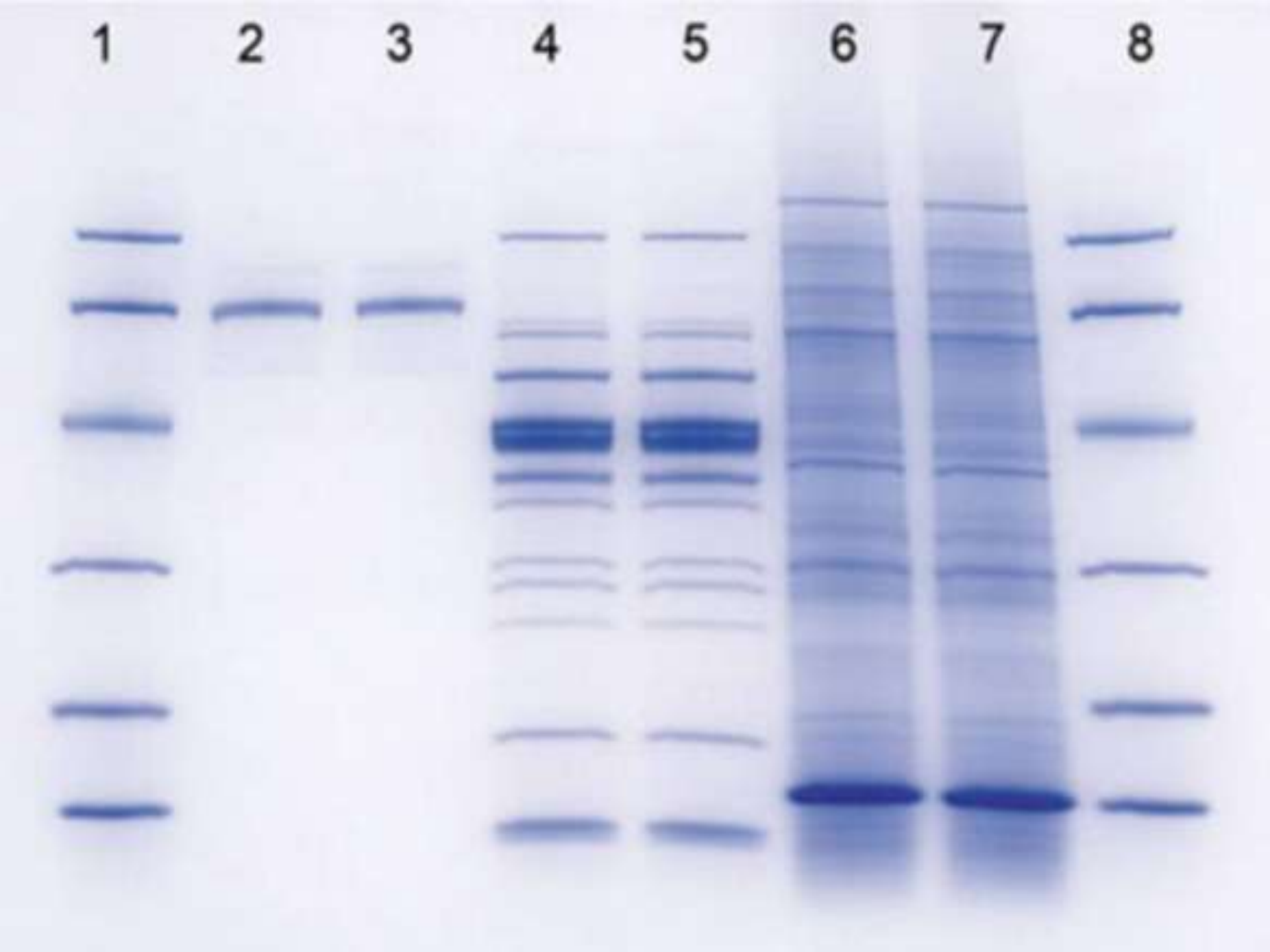
native protein

CH_3-$(CH_2)_{10}$-CH_2-O-SO_3^- Na^+

CH_3-CH_2-SH

denatured by SDS + mercaptoethanol




1
2
3
4
5
6
7
8

The first Determination of the Sequence of a polypeptide by sanger:

- Mature insulin consists of the 21-residue A chain and the 30-residue B chain linked by disulfide bonds.
- Frederick Sanger reduced the disulfide bonds, separated the A and B chains, and cleaved each chain into smaller peptides using trypsin, chymotrypsin, and pepsin (partial hydrolysis by enzymes).
- The resulting peptides were then isolated and treated with acid to hydrolyze peptide bonds and generate peptides with as few as two or three amino acids. Each peptide was reacted with 1-fluoro-2,4-dinitrobenzene (Sanger's reagent), which exclude the exposed α-amino groups of the amino-terminal residues.
- The amino acid content of each peptide was then determined and the amino-terminal amino acid identified.

Steps for determination of the Sequence of a polypeptide

- Cleave the peptide into smaller fragments, and determine the amino acid composition of these smaller fragments.
- Identify the N-terminus and C-terminus in the parent peptide and in each fragment.
- The C-terminal amino acid can be determined by carboxypeptidase-catalyzed hydrolysis.
- Identifying the N-terminus, depending on the fact that the amino N at the terminus is more nucleophilic than any of the amide nitrogens.
- So the key reagent in Sanger's method for identifying the N-terminus is 1-fluoro-2,4-dinitrobenzene which is very reactive toward nucleophilic aromatic substitution.
- Endopeptidases cleave proteins at specific sites within the chain.
- Organize the information so that the sequences of small fragments can be overlapped to show the full sequence.

Overlapping approach

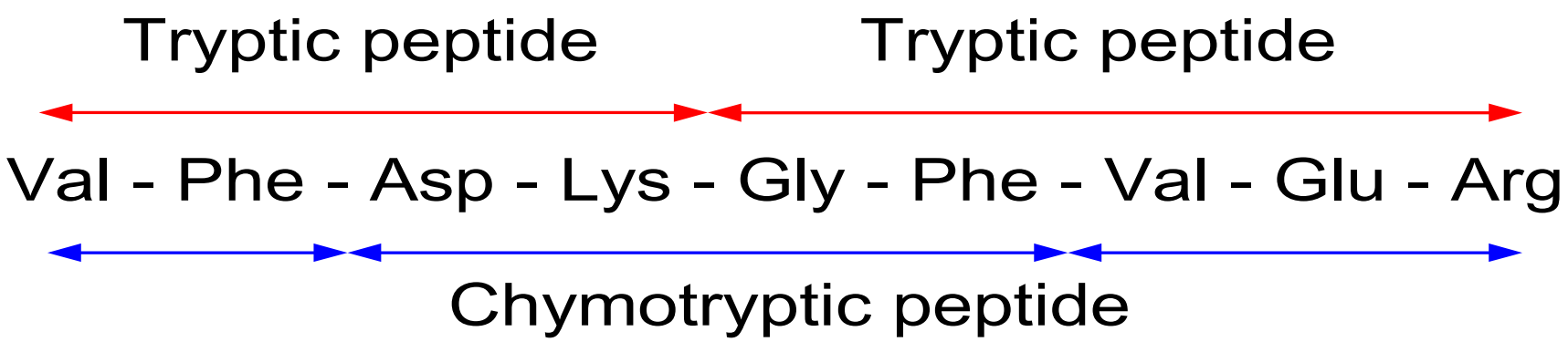
Tryptic cleavage generates two peptides

Gly-Phe-Val-Glu-Arg, Val-Phe-Asp-Lys

Chymotryptic cleavage generates three peptides

Val-Phe, Val-Glu-Arg, Asp-Lys-Gly-Phe

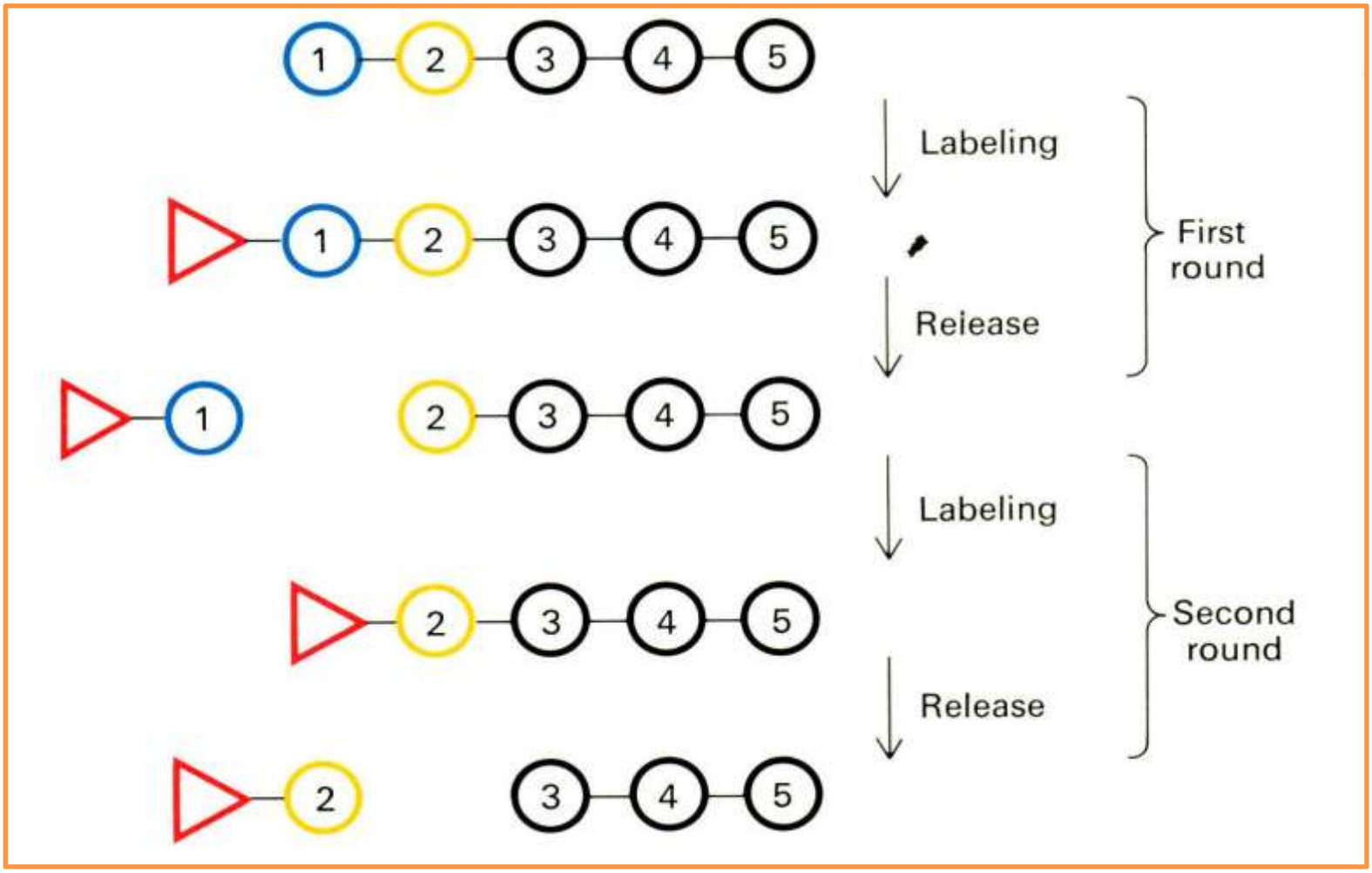
Overlapping the sequenced peptides

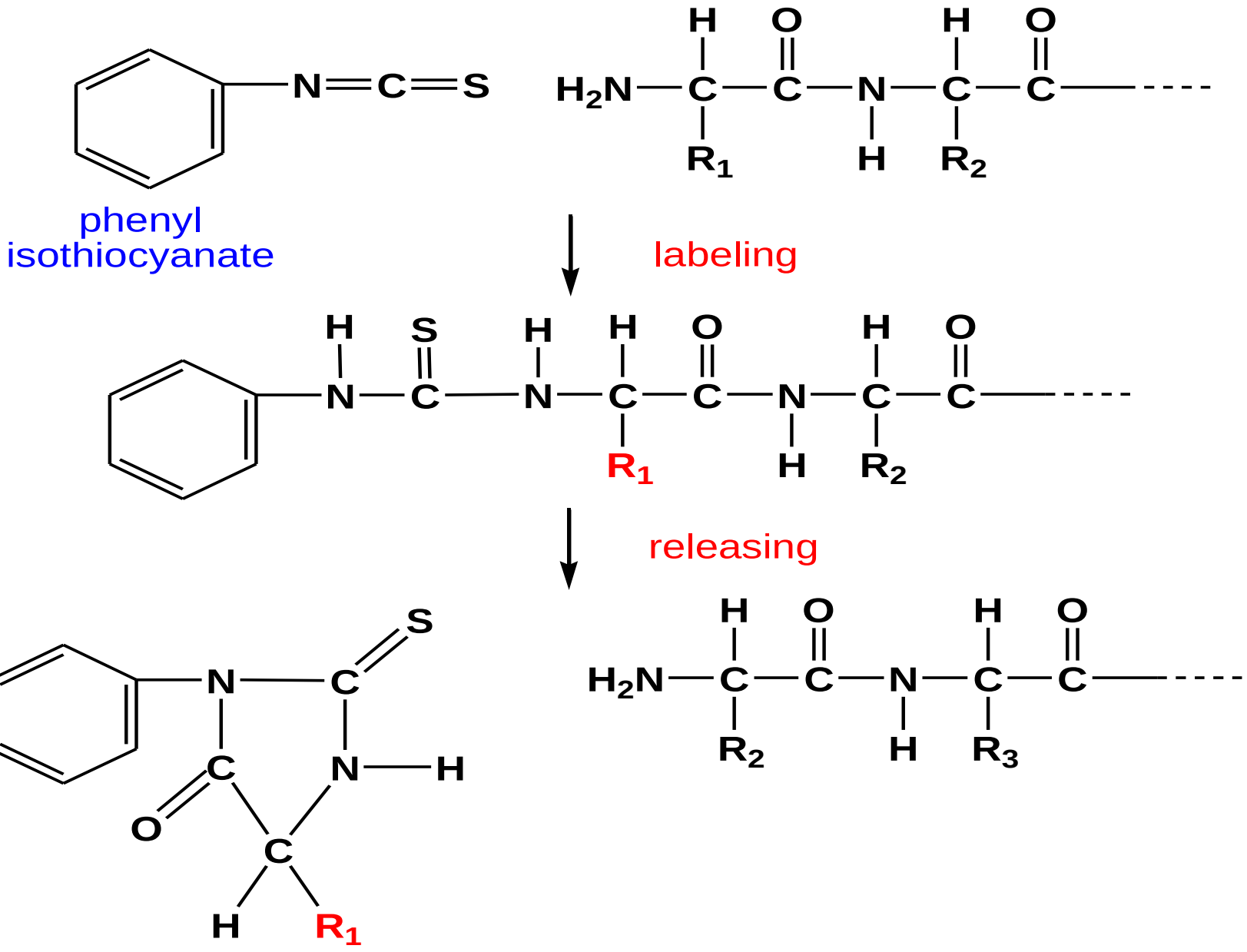


Edman's reagent

- Pehr Edman introduced phenylisothiocyanate (Edman's reagent) to selectively label the amino-terminal residue of a peptide.
- Successive rounds of derivative formation with Edman's reagent can be used to sequence many residues of a single sample of peptide.
- The first 20–30 residues of a peptide can readily be determined by the Edman method, but most polypeptides contain several hundred amino acids.
- Consequently, most polypeptides must first be cleaved into smaller peptides prior to Edman sequencing.

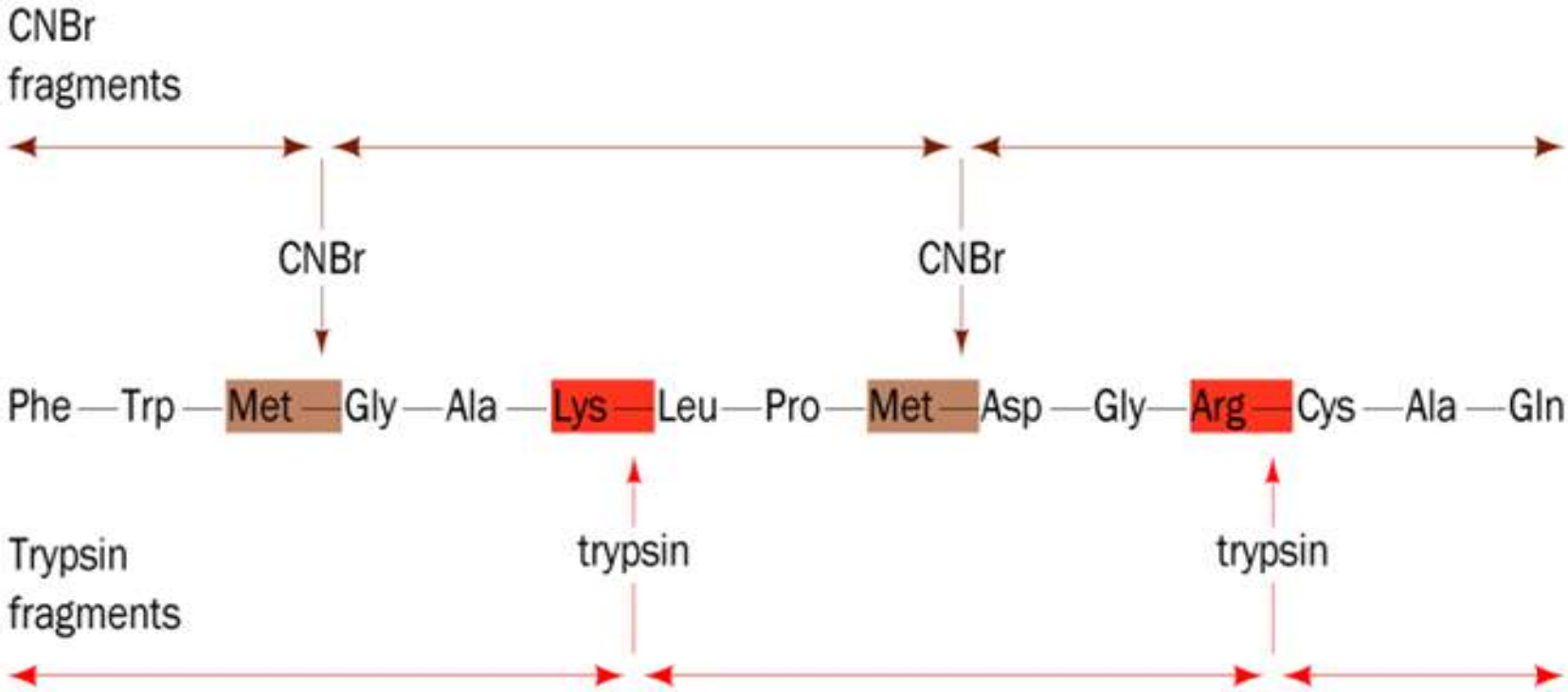
Edman degradation




phenyl
isothiocyanate
labeling
releasing

Specific chemical cleavage reagents

- Cyanogen bromide (CNBr) cleaves at methionine (Met) residues; formic acid cleaves at aspartic acid-proline (Asp-Pro) peptide bonds; hydroxylamine cleaves at asparagine-glycine (Asn-Gly) peptide bonds, and 2-nitro-5-thiocyanobenzoic acid (NTCB) cleaves at cysteine (Cys) residues.



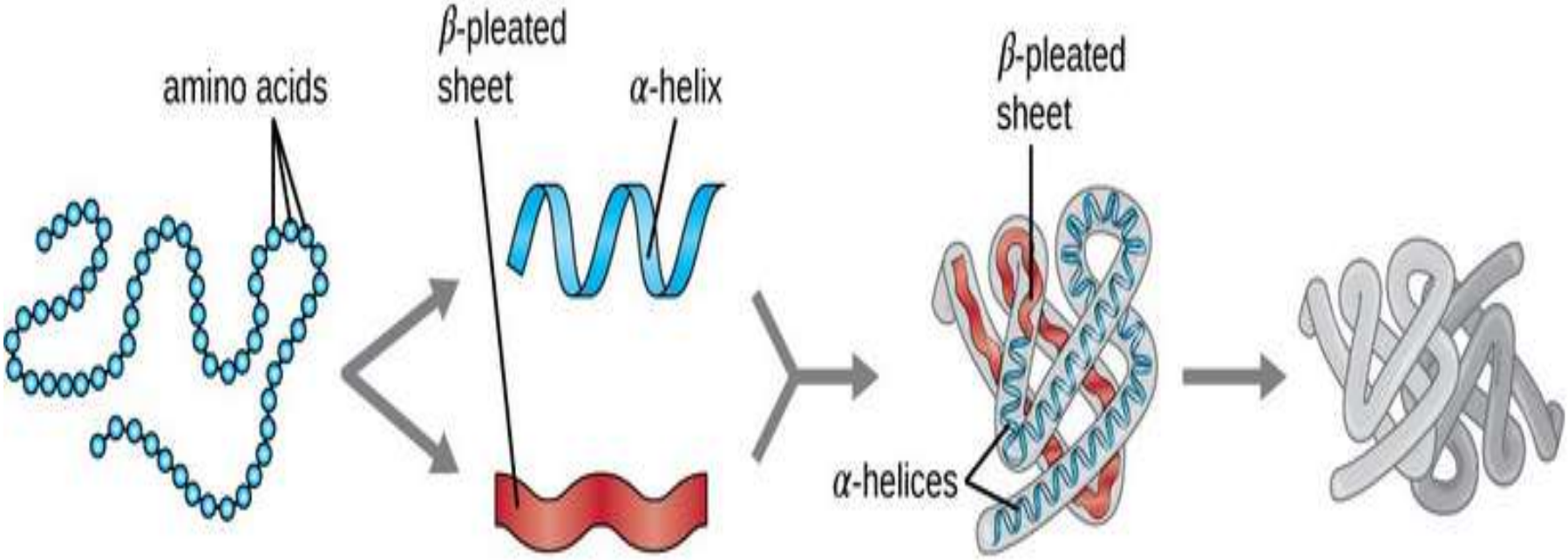
Selective Peptide Cleavage

Name	Type	Specificity
Cyanogen Bromide	Chemical	Carboxyl Side of Methionine
Trypsin	Enzymatic	Carboxyl Side of Basic Amino Acids e.g. Lys & Arg
Chymotrypsin	Enzymatic	Carboxyl Side of Aryl Amino Acids e.g. Phe, Tyr & Trp

Protein Structure

Lec (5) by: Inaam Ahmed Ameen

Protein structure

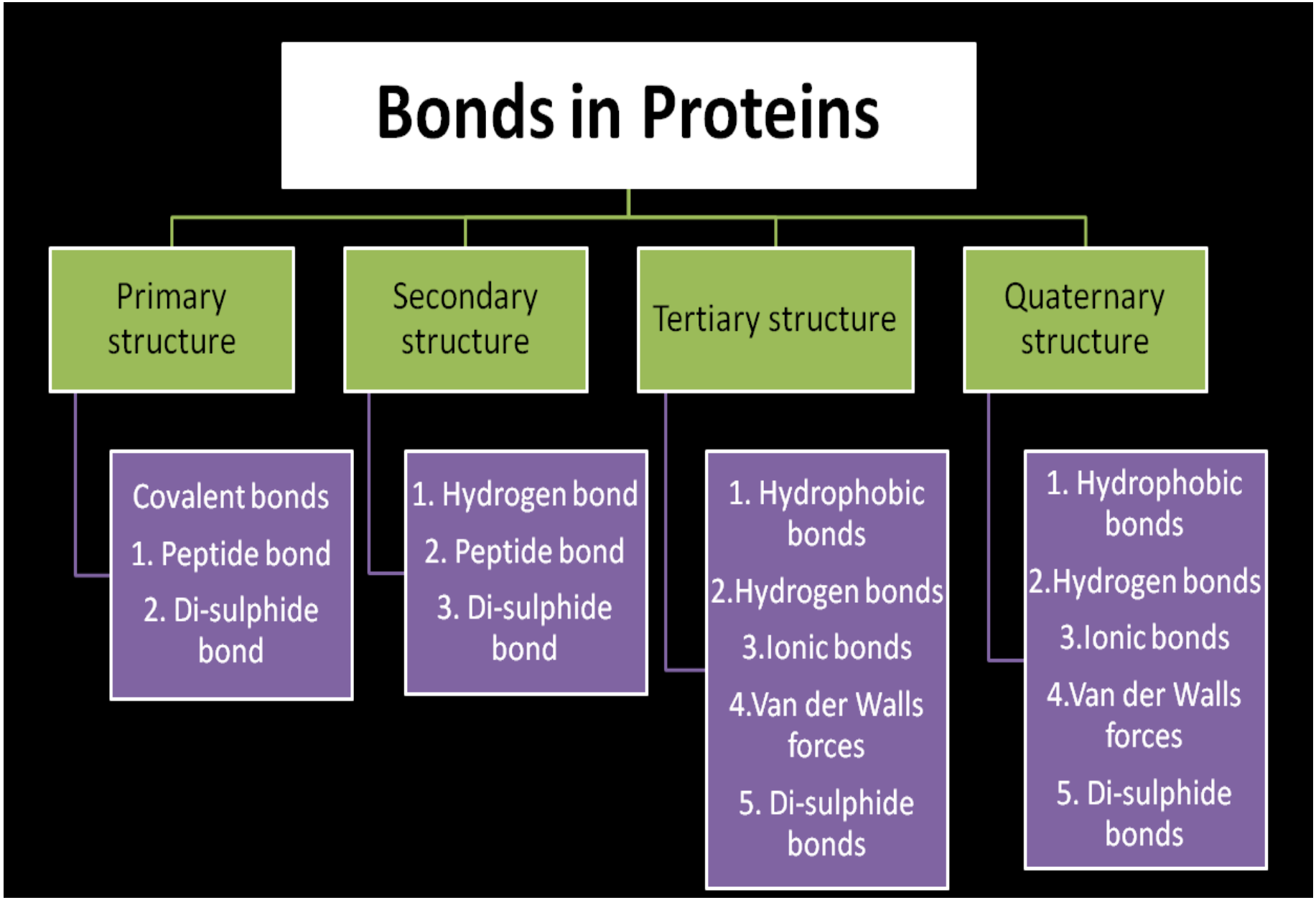


Primary Protein Structure
Sequence of a chain of amino acids

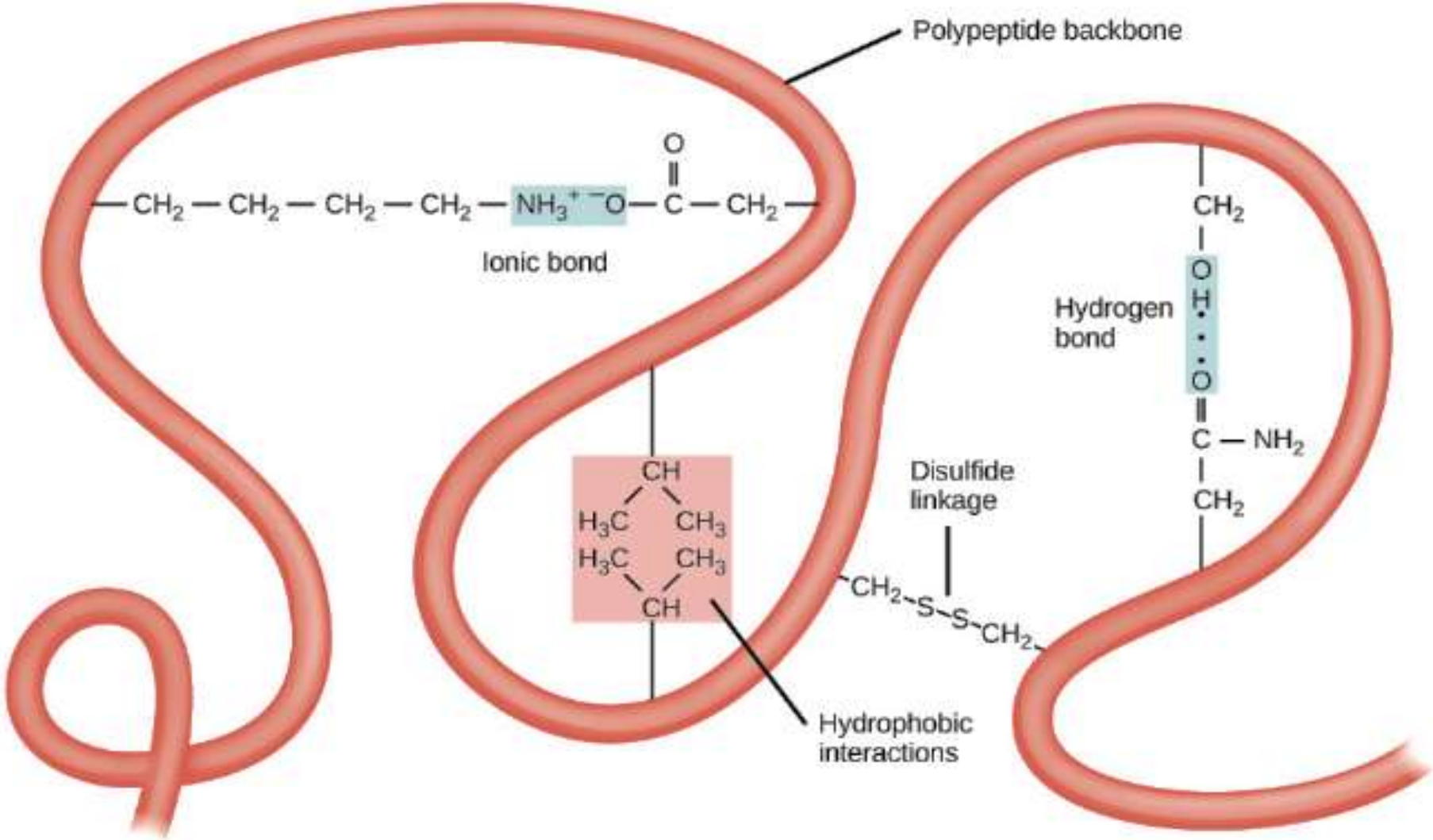
Secondary Protein Structure
Local folding of the polypeptide chain into helices or sheets

Tertiary Protein Structure
three-dimensional folding pattern of a protein due to side chain interactions

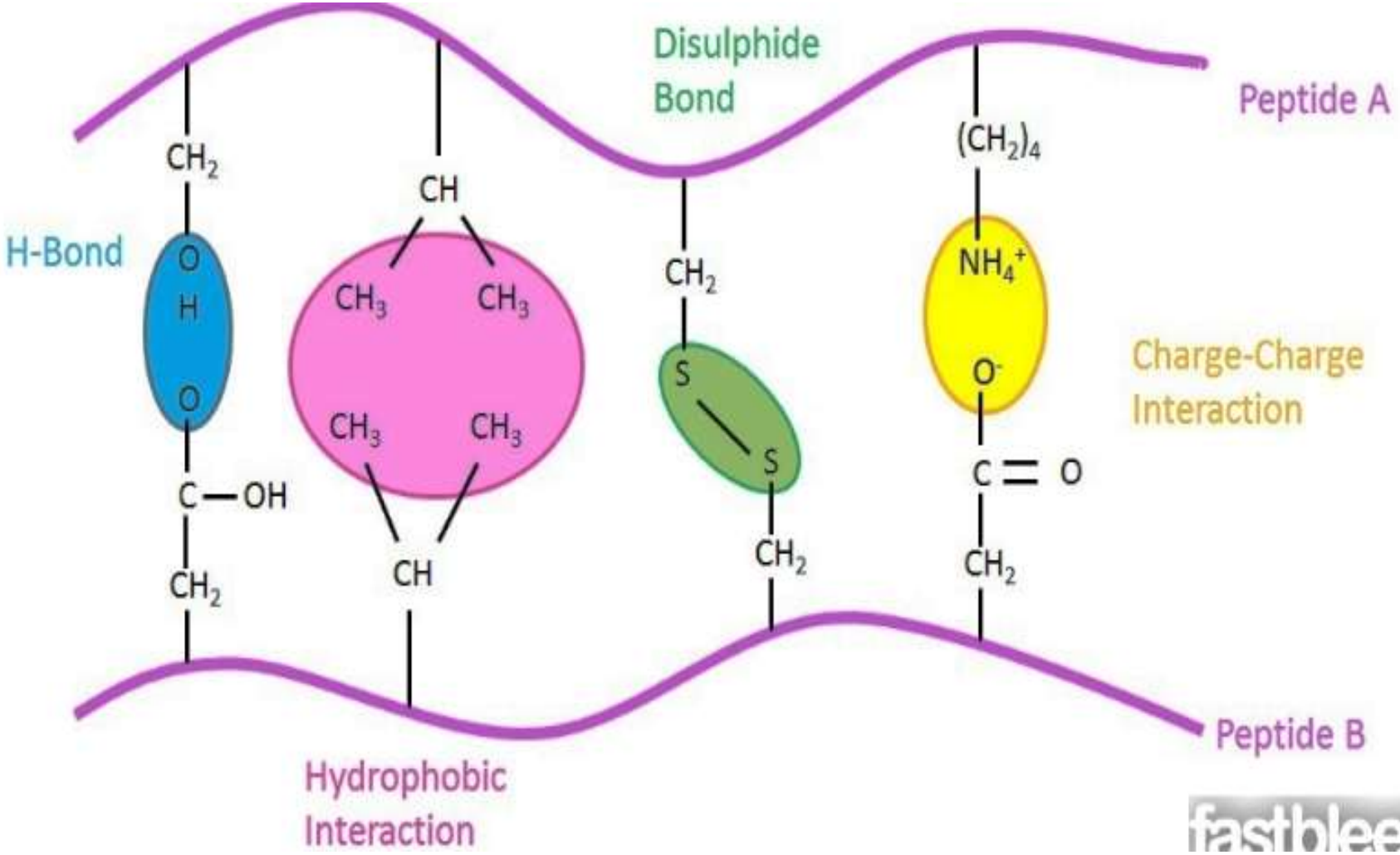
Quaternary Protein Structure
protein consisting of more than one amino acid chain


Bonds in Proteins
Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Covalent bonds
1. Peptide bond
2. Di-sulphide bond
1. Hydrogen bond
2. Peptide bond
3. Di-sulphide bond
1. Hydrophobic bonds
2.Hydrogen bonds
3.Ionic bonds
4.Van der Walls forces
5. Di-sulphide bonds
1. Hydrophobic bonds
2.Hydrogen bonds
3.Ionic bonds
4.Van der Walls forces
5. Di-sulphide bonds

Bonds in proteins within the same polypeptide

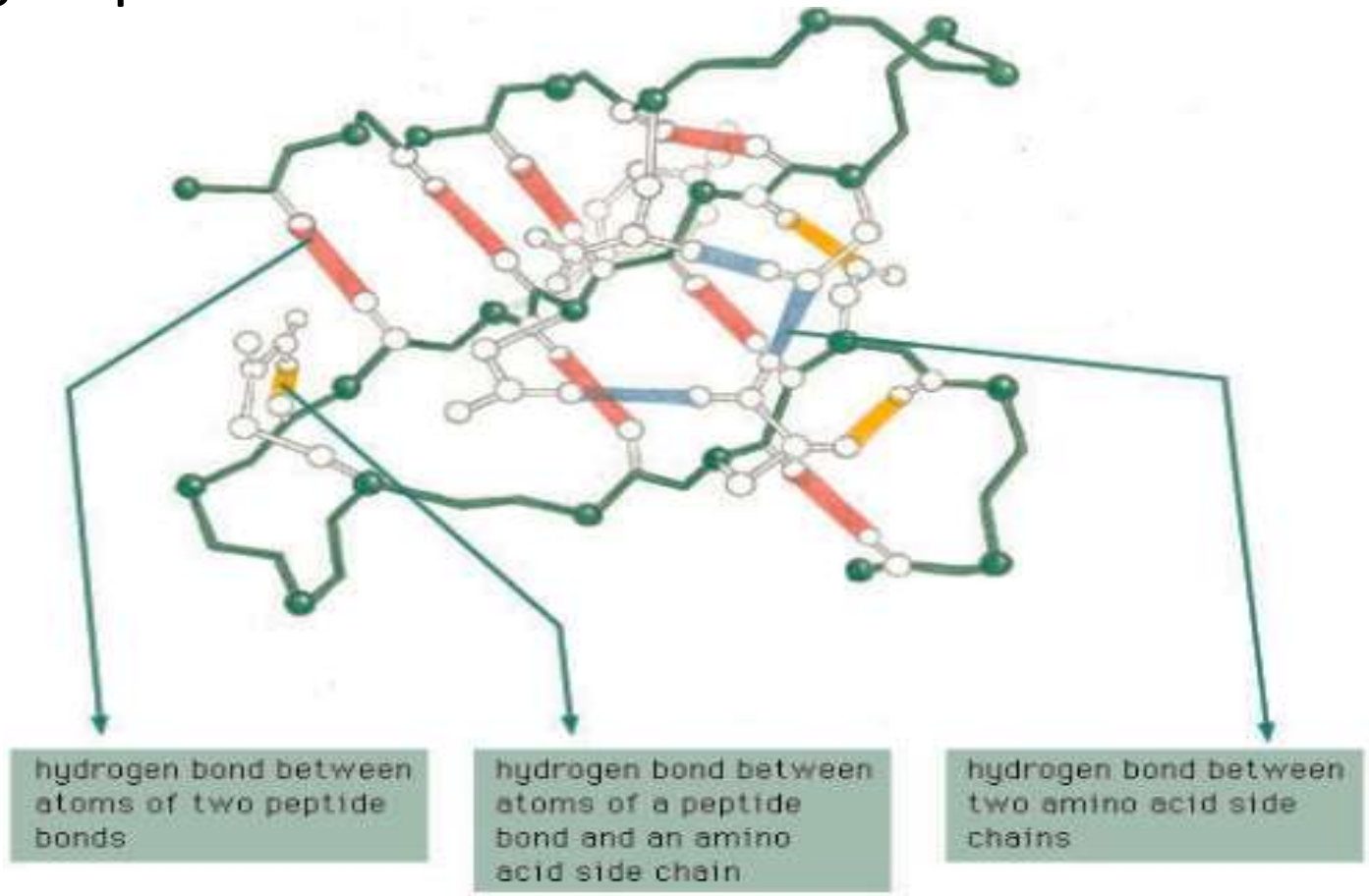


Bonds between different polypeptids



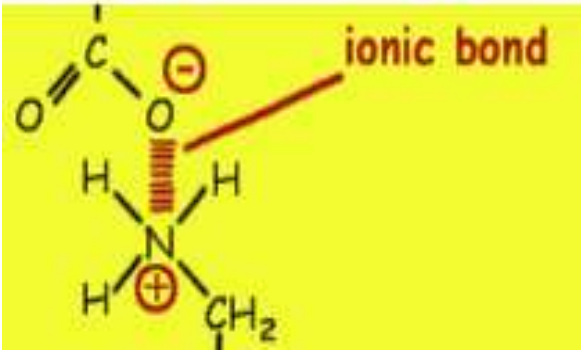
Hydrogen bonds in proteins

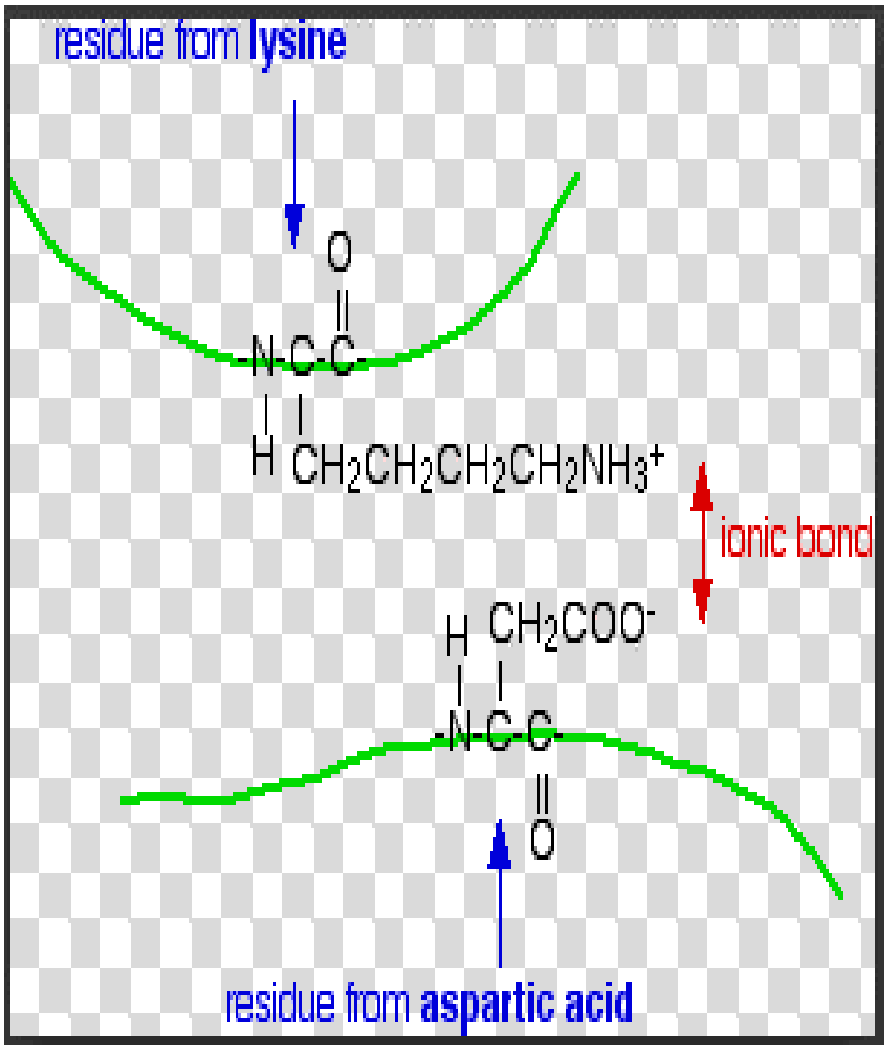
- H-bonds form between 1-atoms involved in the peptide bond. 2-peptide bond atoms and R groups. 3-R groups.



Ionic or electrostatic bond

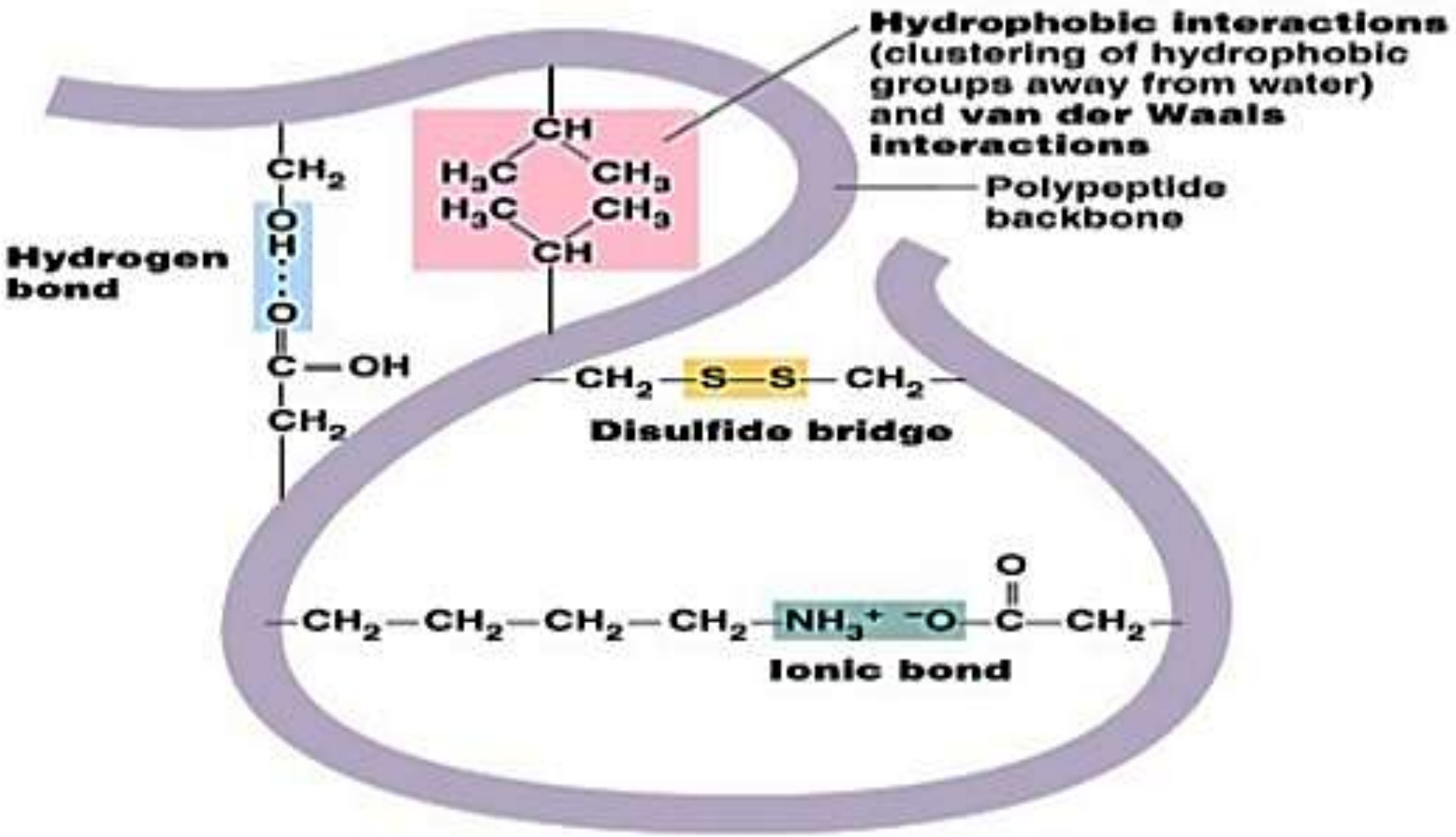
- An ionic bond or salt bridge can be formed between the carboxylate ion of an acidic residue such as aspartic acid or glutamic acid and an ammonium ion of a basic residue such as lysine, arginine or histidine.
- This is the strongest of the intramolecular bonds.





Hydrophobic bonds

- Between nonpolar side chains



Levels of protein structure

- Primary (1°)
- Secondary (2°)
- Tertiary (3°)

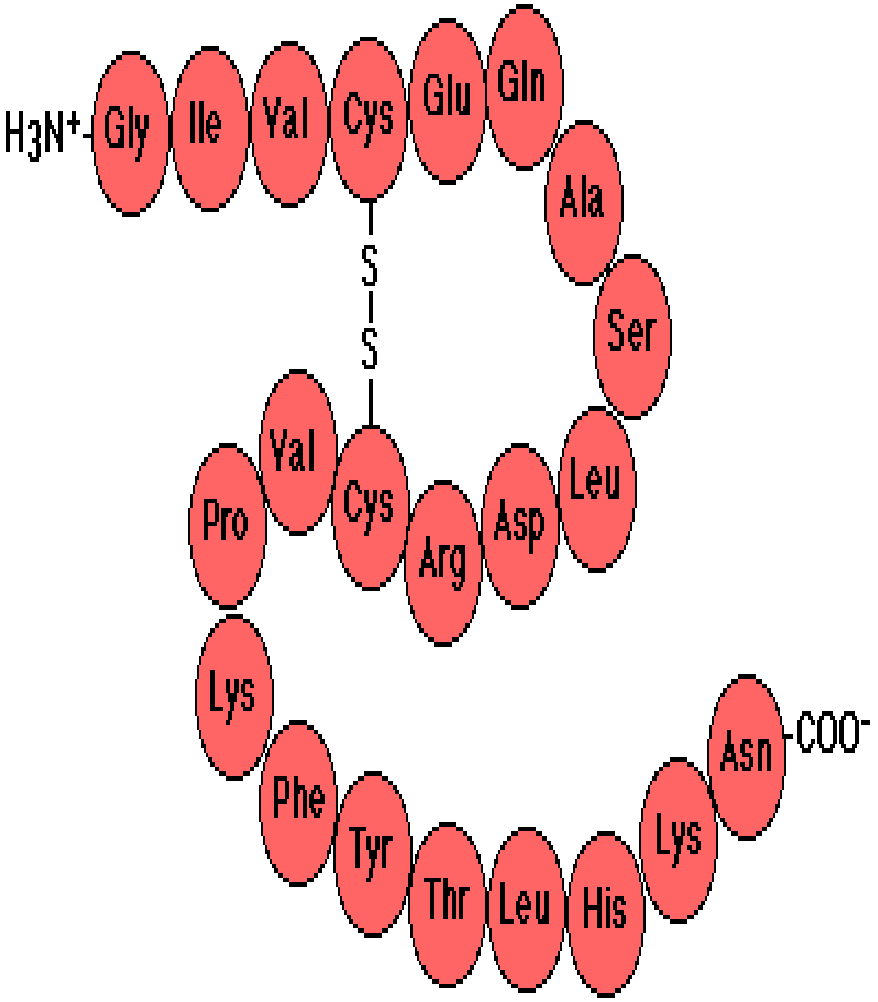
} organizes folding within a single polypeptide

- Quaternary (4°)

} interactions between two or more polypeptides that make a protein

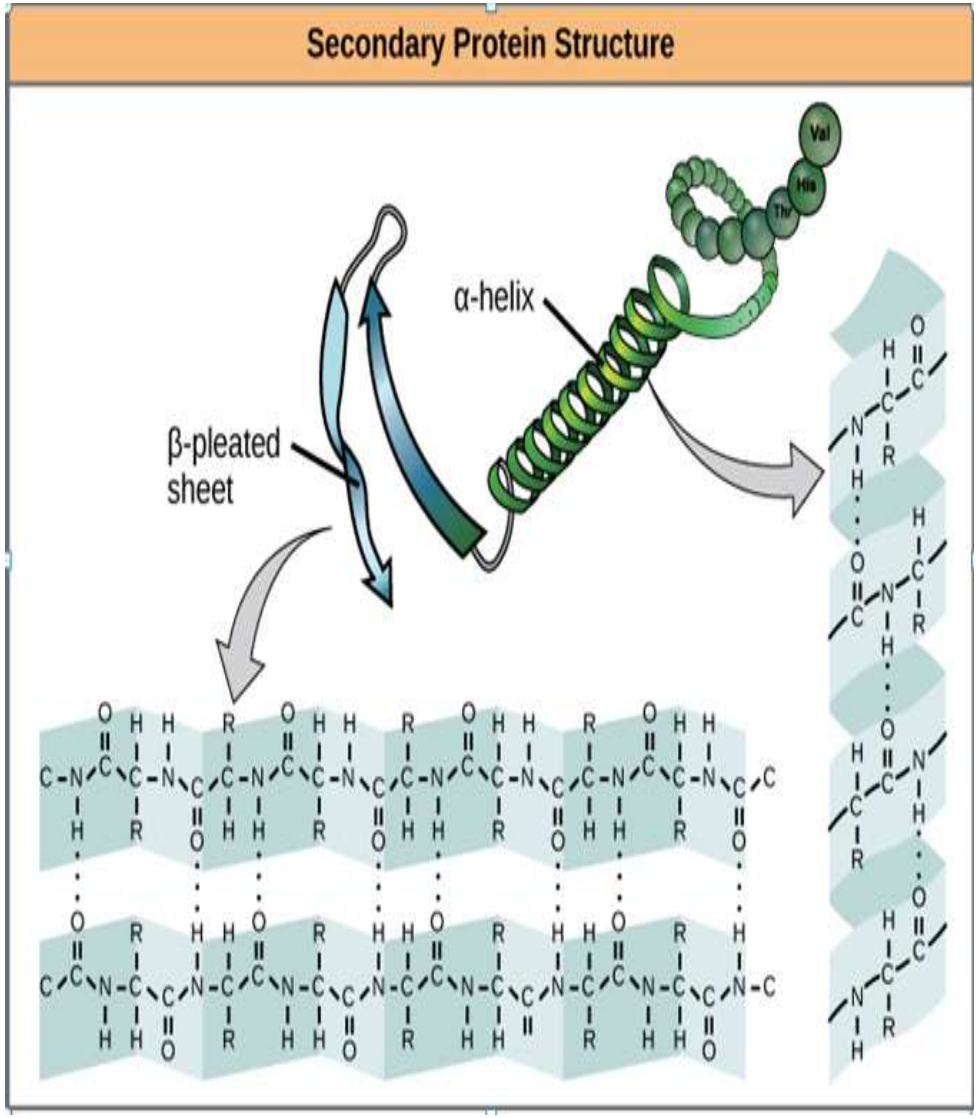
Primary Structure of Proteins

- Primary structure is simply the sequence of residues making up the protein, involves only the covalent bonds linking residues together.
- linear
- ordered
- 1 dimensional
- sequence of amino acid polymer written from amino end to carboxyl end
- a perfectly linear amino acid polymer

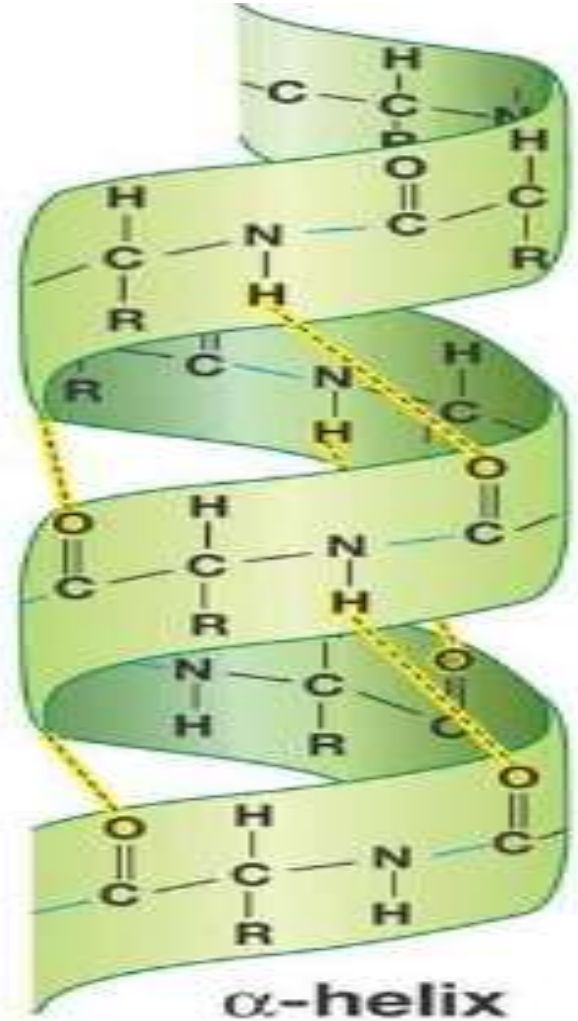


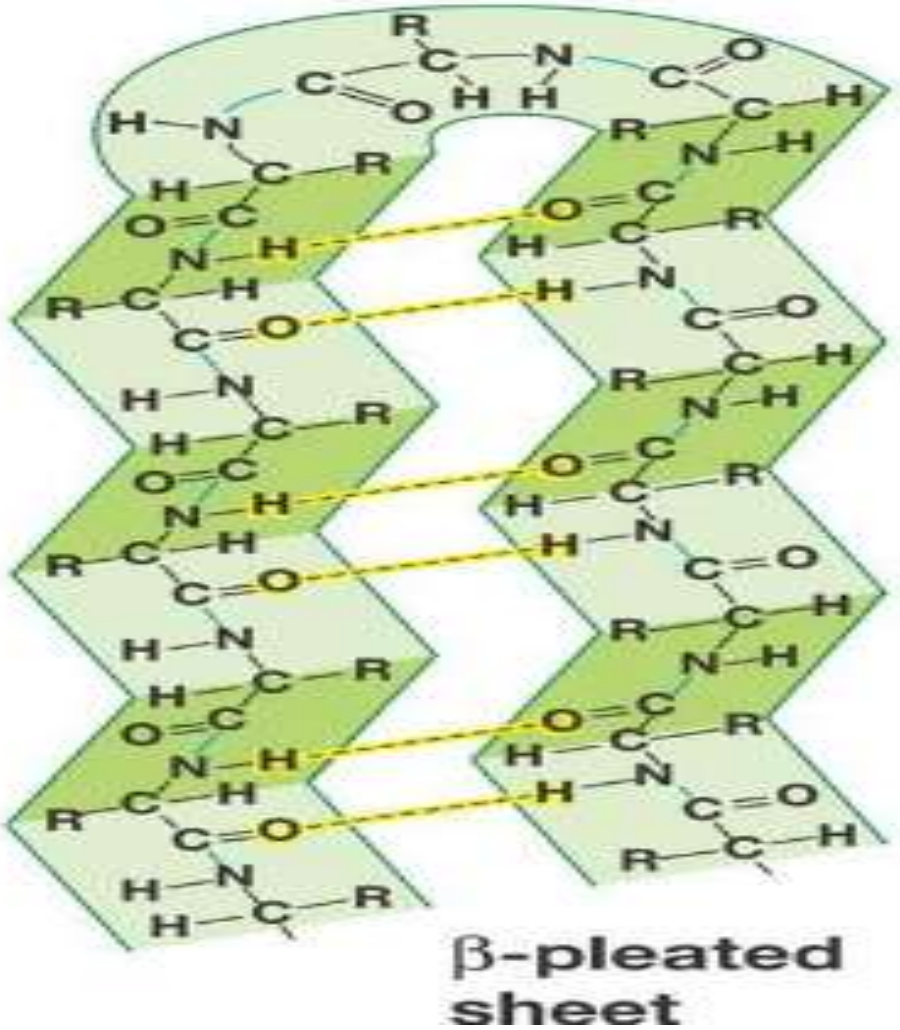
Secondary Structure

- non-linear
- 3 dimensional
- localized to areas of an amino acid chain
- formed and stabilized by hydrogen bonding.



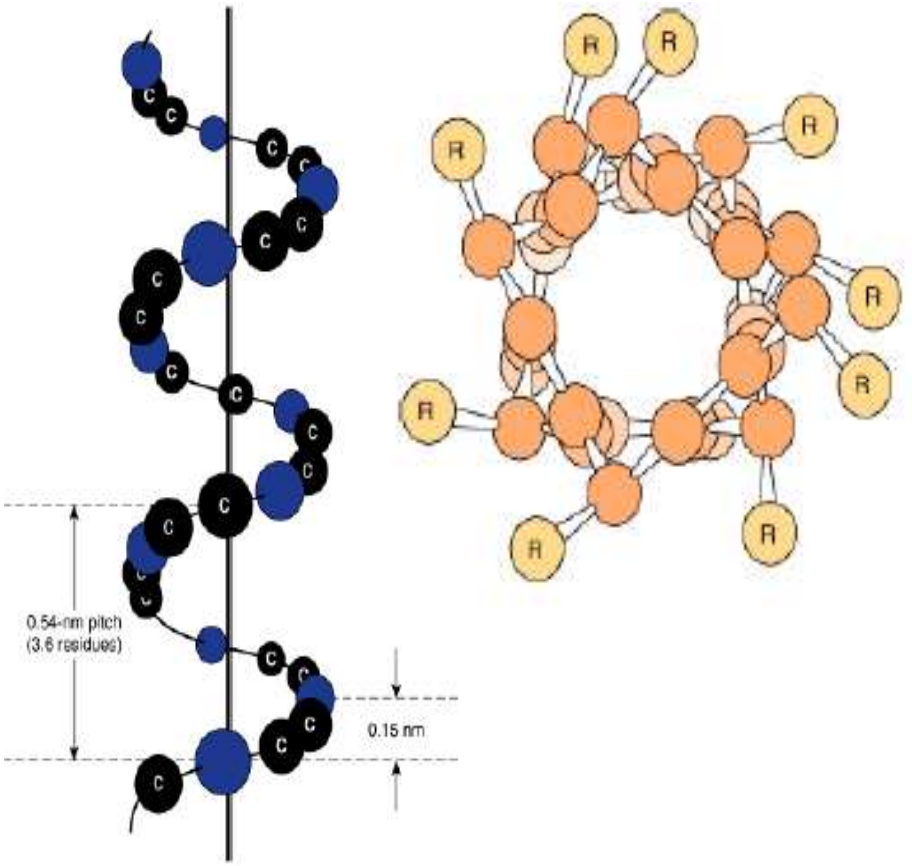
Hydrogen bonds stabilize secondary structure of proteins





Helix α

- The polypeptide backbone of an α helix is twisted by an equal amount about each –carbon.
- A complete turn of the helix contains an average of 3.6 amino residues (amino acid residues spaced three or four residues apart in the primary sequence are spatially close together when folded in the α-helix), and the distance it rises per turn is 0.54 nm.
- The R groups of each aminoacyl residue in an α helix face outward.
- Proteins contain only L α -a mino acids, for which a right-handed α helix is by far the more stable, and only right-handed helices are present in proteins.
- Schematic diagrams of proteins represent helices as coils or cylinders



Stability of α helix

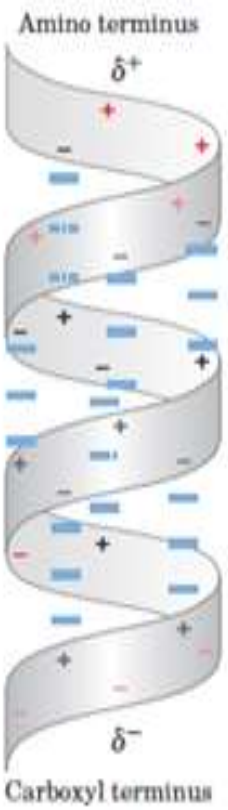
- The stability of an α helix arises primarily from hydrogen bonds formed between the oxygen of the peptide bond carbonyl and the hydrogen atom of the peptide bond nitrogen of the fourth residue down the polypeptide chain.
- Since the peptide bond nitrogen of proline lacks a hydrogen atom to contribute to a hydrogen bond, proline can only be stably adapted within the first turn of an α helix. When present elsewhere, proline disrupts the conformation of the α helix, producing a bend.
- Because of its small size, glycine also often induces bends in helices.
- Large numbers of charged amino acids (glutamate, aspartate, histidine, lysine, or arginine) also disrupt the helix by forming ionic bonds, or by electro statically repelling each other.
- Amino acids with bulky side chains (tryptophan , valine or isoleucine), branch at the β-carbon (the first carbon in the R-group, next to the α-carbon) can interfere with formation of the α-helix if they are present in large numbers.

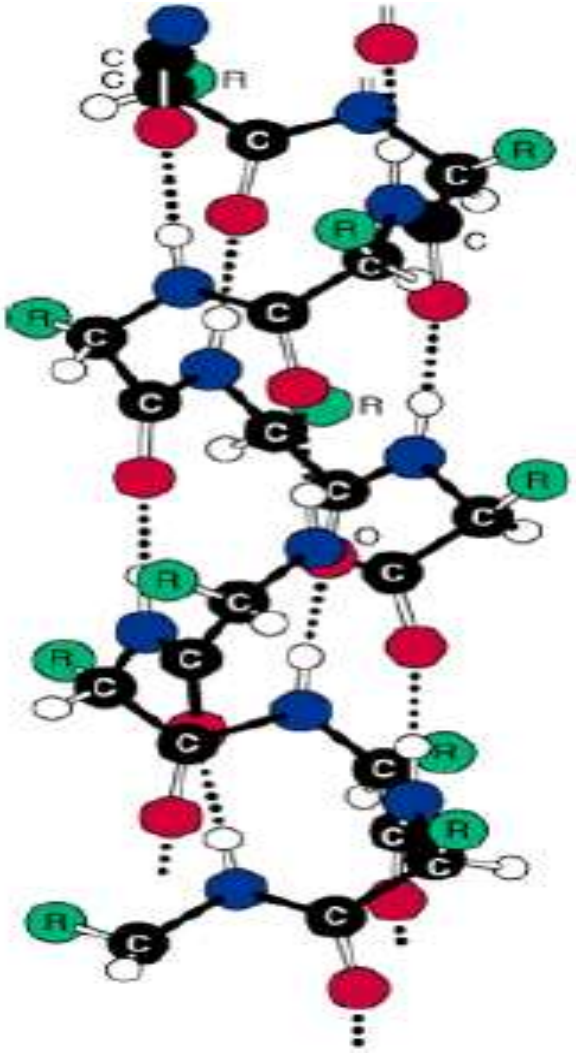
α helix

Many α helices have predominantly hydrophobic R groups on one side of the axis of the helix and predominantly hydrophilic ones on the other.

These **amphipathic helices** are well adapted to the formation of interfaces between polar and nonpolar regions such as the hydrophobic interior of a protein and its aqueous environment.

Clusters of amphipathic helices can create a channel, or pore, that permits specific polar molecules to pass through hydrophobic cell membranes.





- Hydrogen bonds (dotted lines) formed between H and O atoms stabilize a polypeptide in an α -helical conformation

β-Sheet

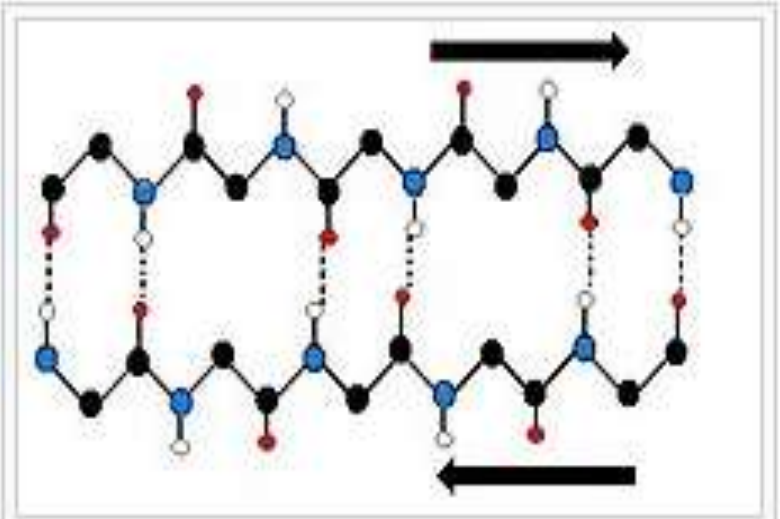
Beta Sheets are multiple strands of polypeptides connected to each other through hydrogen bonding in a sheet.

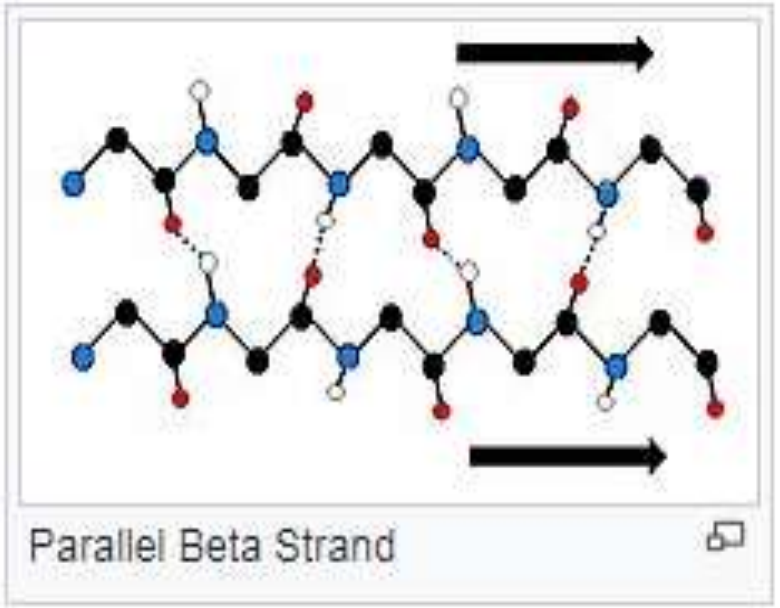
Hydrogen bonding occurs between the NH and CO groups(all of the peptide bond components are involved) between two different strands & **not within one strand .**

The beta strands can be arranged in a parallel, anti-parallel, or mixed (parallel and anti-parallel) manner.

The anti-parallel configuration is the simplest. The N and C terminals of adjacent polypeptide strands are opposite to one another, meaning the N terminal of one peptide chain is associated with the C terminal of an adjacent chain.

The parallel arrangement occurs when neighboring polypeptide chains run in the same direction. As a result, an amino acid cannot bond directly to the complementary amino acid in an adjacent chain as in the anti-parallel configuration. Instead, the amino group from one chain is bonded to a carbonyl group on the adjacent chain.





Differences between alpha helix & beta sheet

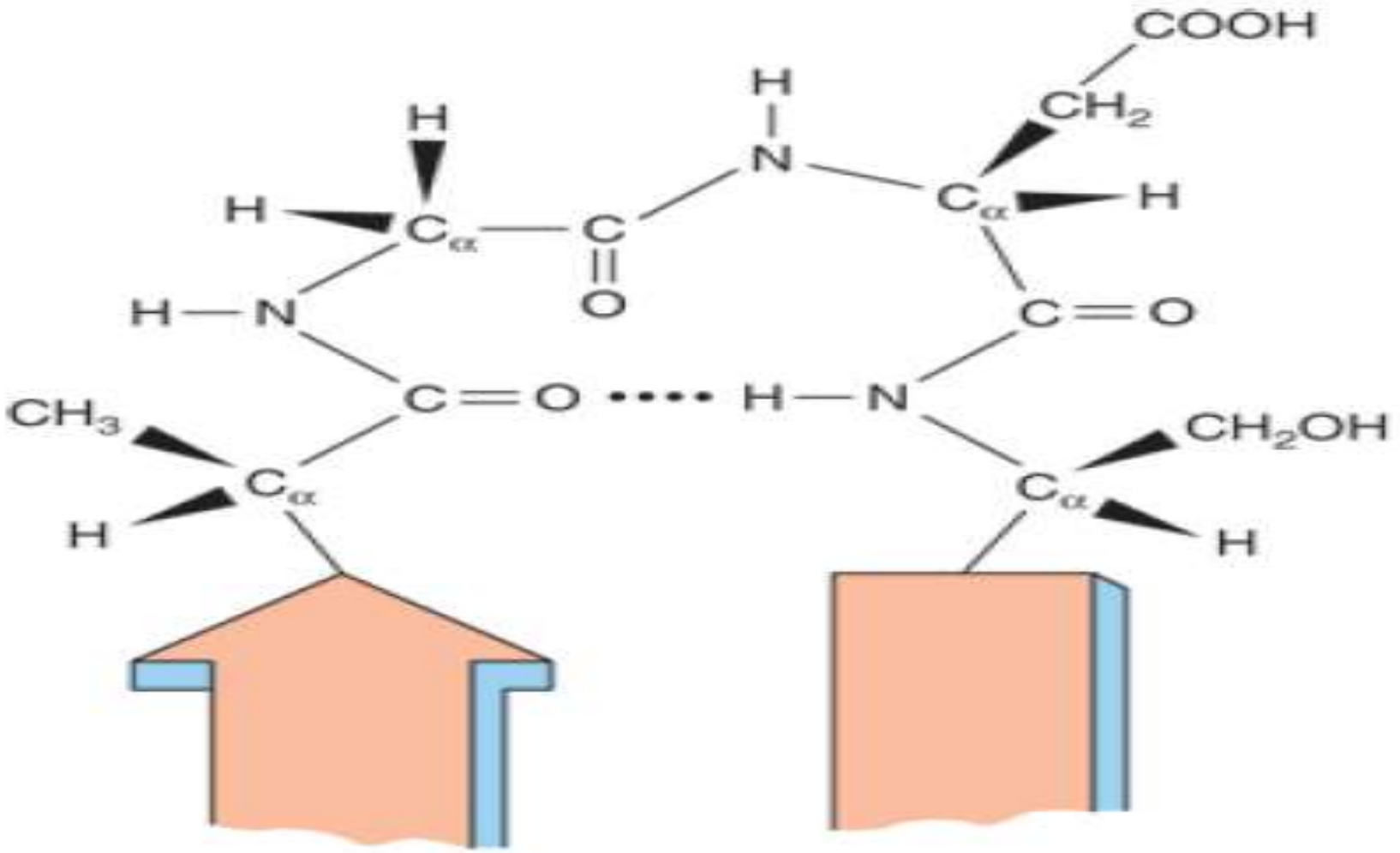
They have two different shapes to do a specific job

- In alpha helix structure, there are 3.6 amino acids per turn of the α helix.
- All the peptide bonds are trans and planar, and the N-H groups in the peptide bonds point in the same direction, which is approximately parallel to the axis of the helix.
- The C=O groups of all peptide bonds point in the opposite direction, and they are parallel to the axis of the helix.
- The C=O group of each peptide bond is bonded to the N-H group of the peptide bond forming a hydrogen bond.
- All R- groups are pointed outward from the α helix.

- Each peptide bond in the beta pleated sheet is planar and has the trans-conformation.
- The C=O and N-H groups of peptide bonds from adjacent chains are in the same plane and point toward each other forming hydrogen bonding between them.
- form a zigzag or pleated pattern in which the R groups of adjacent residues point in opposite directions(above and below the plane of the β sheet)
- the peptide backbone of the β sheet is highly extended
- Clusters of twisted strands of β sheet form the core of many globular proteins

Loops & Bends

- Roughly half of the residues in a "typical" globular protein reside in αhelices and β sheets and half in loops, turns, bends, and other extended conformational features.
- Turns and bends refer to short segments of amino acids that join two units of secondary structure, such as two adjacent strands of an antiparallel βsheet.
- A turn involves four aminoacyl residues, in which the first residue is hydrogen-bonded to the fourth, resulting in a tight 180-degree turn.
- Proline and glycine often are present in turns.
- Loops are regions that contain residues within the minimum number necessary to connect adjacent regions of secondary structure.
- Irregular in conformation, loops on the other hand serve key biologic roles.
- For many enzymes, the loops that link areas responsible for binding substrates often contain aminoacyl residues that participate in catalysis.



A turn that links two segments of anti-parallel β sheet. The dotted line indicates the hydrogen bond between the first and fourth amino acids of the four-residue segment Ala-Gly-Asp-Ser.

β bends

- Reverse the direction of a polypeptide chain, helping in formation of a compact, globular shape.
- They are usually found on the surface of protein molecules, and often include charged residues.
- are generally composed **of 4 amino acids**, one of which may be proline—the amino acid that causes a “bend” in the polypeptide chain, also glycine **,** with the smallest R-group, is frequently found in β-bends.
- Are stabilized by the **formation of H- & ionic bonds**

Supersecondary structures (motifs)

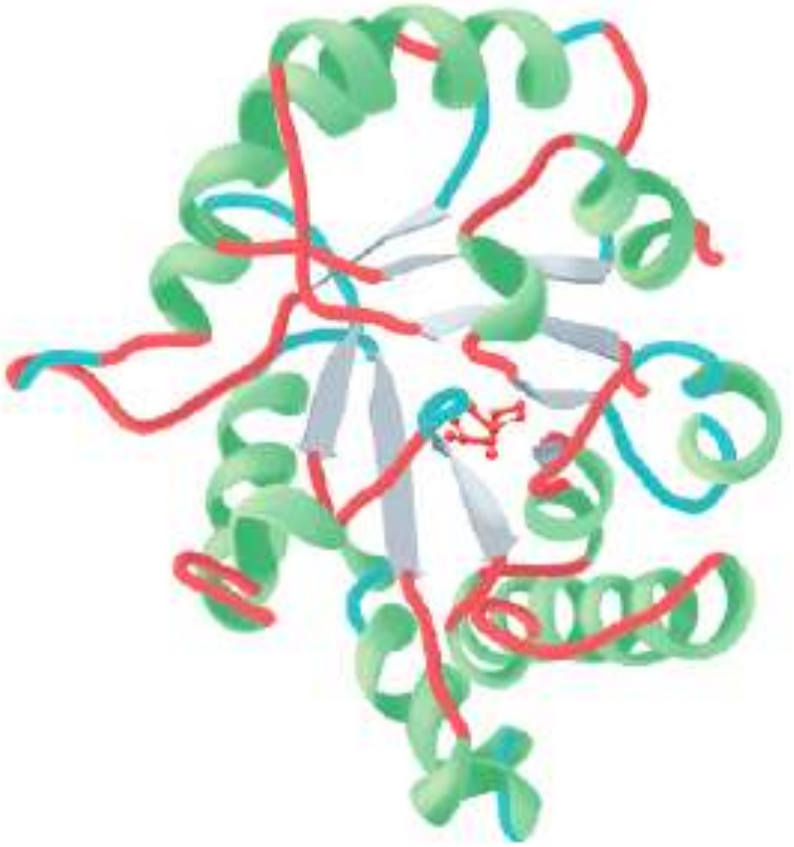
- Structural motifs such as the helix-loop-helix
- motif that are intermediate between secondary and tertiary structures are often termed supersecondary structures.
- Since many loops and bends reside on the surface of proteins and are thus exposed to solvent, they constitute readily accessible sites, or epitopes**,** for recognition and binding of antibodies.
- While loops lack apparent structural regularity, they exist in a specific conformation stabilized through hydrogen bonding, salt bridges, and hydrophobic interactions with other portions of the protein.
- Proteins may contain "disordered" regions, often at the extreme amino or carboxyl terminal, characterized by high conformational flexibility.
- This structural flexibility enables such regions to control &switches the protein structure and function.

Tertiary structure

- The "tertiary structure" refers to the entire three-dimensional conformation of a polypeptide.
- It indicates, in three-dimensional space, how secondary structural features—helices, sheets, bends, turns, and loops—connect to form domains and how these domains relate spatially to one another.
- A **domain** is a section of protein structure sufficient to perform a particular chemical or physical task such as binding of a substrate or other ligand.
- Proteins containing multiple domains also can be made through the association of multiple polypeptides

- Domains are distinct functional and or structural units in a protein.
- Usually they are responsible for a particular function or interaction, contributing to the overall role of a protein.
- Domains may exist in a variety of biological situations, where similar domains can be found in proteins with different functions.

- Simple proteins, particularly those that interact with a single substrate, such as lysozyme or triose phosphate isomerase and the oxygen storage protein myoglobin, often consist of a single domain.



- The enzyme triose phosphate isomerase complexed with the substrate analog 2-phosphoglycerate (red). Note the elegant and symmetrical arrangement of alternating βsheets (blue) and helices (green), with the β sheets forming a β -barrel core surrounded by the α helices.

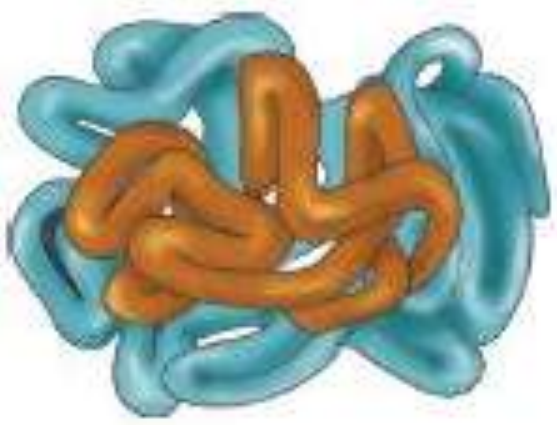
- By contrast,lactate dehydrogenase is comprised of two domains, an N-terminal NAD+-binding domain and a C-terminal-binding domain for the second substrate, pyruvate.
- Lactate dehydrogenase is one a family of oxidoreductases which share a common N-terminal NAD(P)+-binding domain known as the **Rossmann fold.** By fusing the Rossmann fold module to a variety of C-terminal domains, a large family of oxidoreductases have evolved that utilize NAD(P)+/NAD(P)H for the oxidation and reduction of a wide range of metabolites.
- Examples include alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, malate dehydrogenase, quinone oxidoreductase, 6-phosphogluconate dehydrogenase, D-glycerate dehydrogenase and others.

- Polypeptides containing two domains. Shown is the three dimensional structure of lactate dehydrogenase with the substrates NADH (red) and pyruvate (blue) bound.

- **Monomeric** proteins consist of a single polypeptide chain.
- **Dimeric** proteins contain two polypeptide chains. **Homodimers:**contain two copies of the same polypeptide chain, while in a **heterodimer:** the polypeptides differ.
- Greek letters (α, β, γ , etc.) are used to distinguish different subunits of a heterooligomeric protein, and subscripts indicate the number of each subunit type. For example, $\alpha 4$ designates a homotetrameric protein, and $\alpha 2\ \beta 2\ \gamma$ a protein with five subunits of three different types.

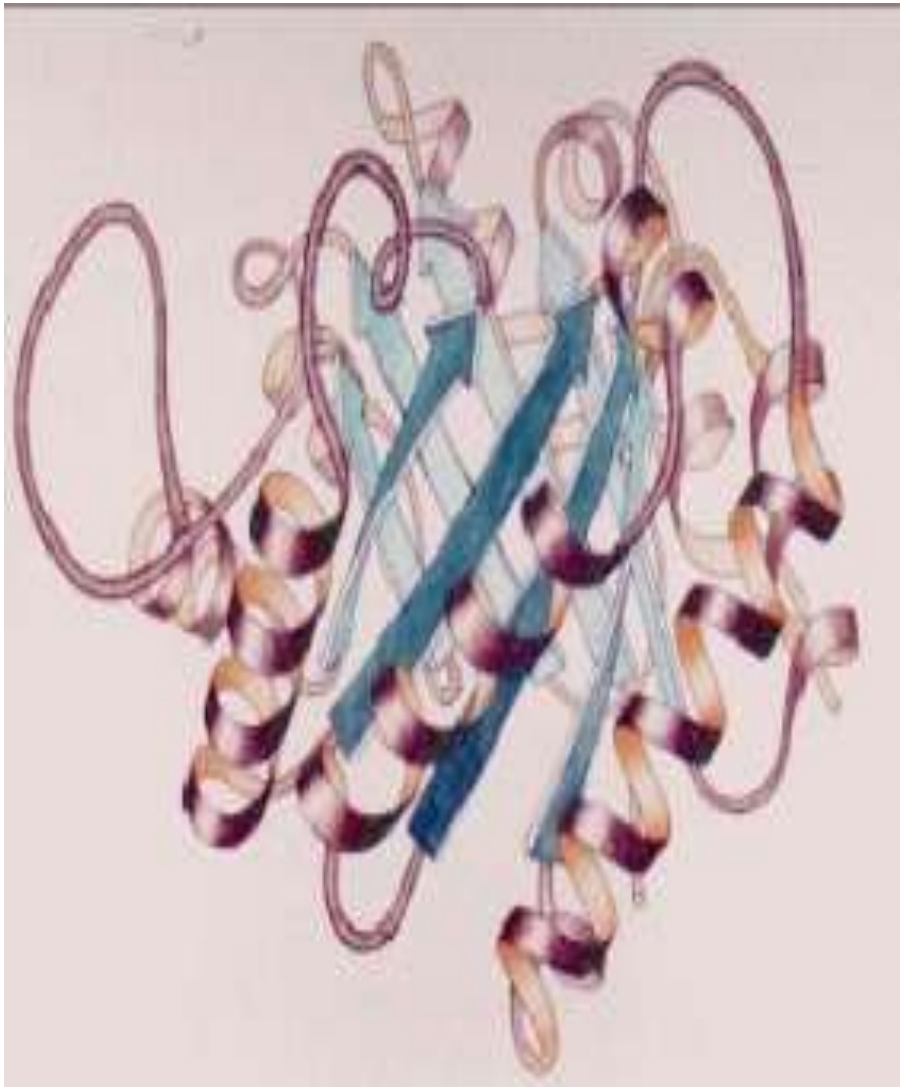
Quaternary structure

- A structural level in which several proteins (or polypeptide subunits) interact through non-covalent bonds to form one functional protein complex.
- Many proteins are made up of multiple polypeptide chains(referred as *protein subunits)*. These subunits may be the same (as in a homodimer) or different (as in a heterodimer).
- The quaternary structure refers to how these protein subunits interact with each other and arrange themselves to form a larger aggregate protein complex.

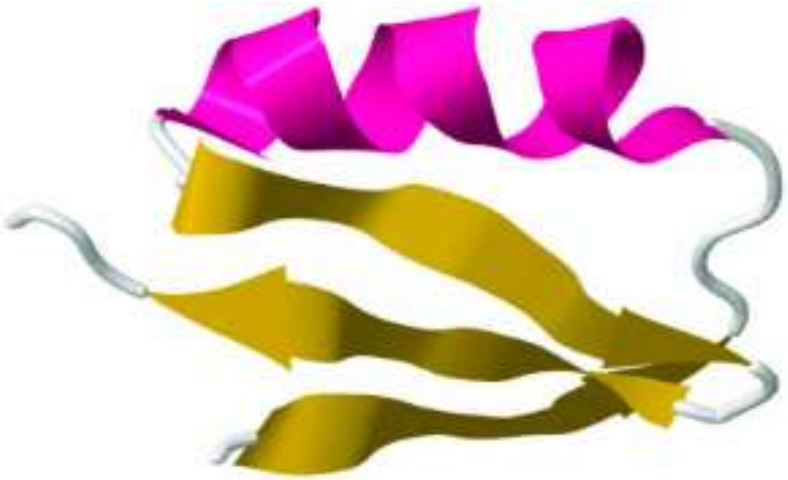


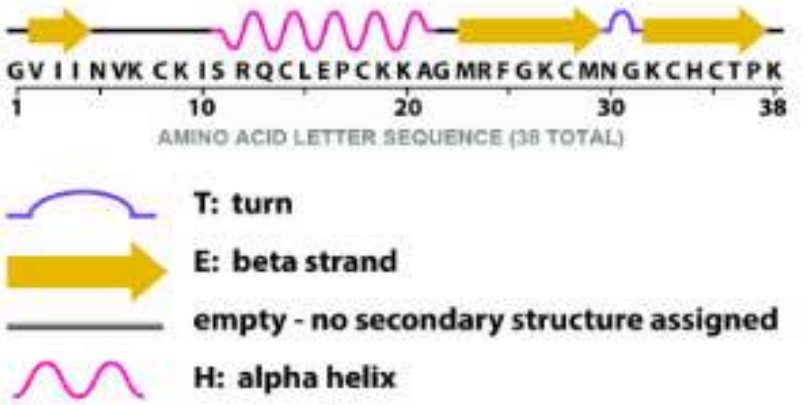
Ribbon Diagrams

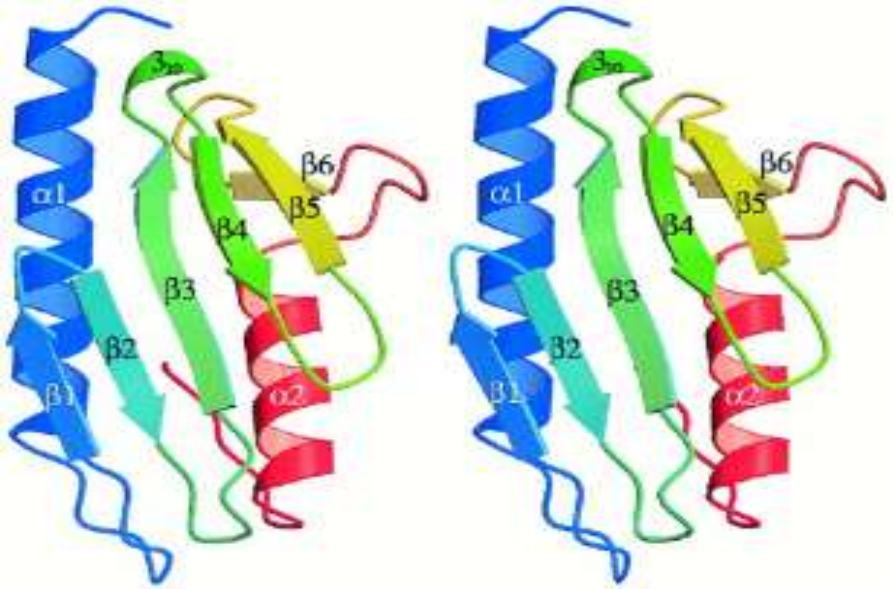
- This was one of Jane Richardson s first hand drawn and colored diagrams of a protein
- Ribbon diagrams draw the conformation of the polypeptide backbone, with cylinders and arrows indicating regions of αhelix and βsheet, respectively.
- line segments that link the carbons indicate the path of the polypeptide backbone.



Examples for ribbon diagrams







Overall fold of *E. coli*

MULTIPLE FACTORS STABILIZE TERTIARY &QUATERNARY STRUCTURE:

- Higher orders of protein structure are stabilized primarily (often exclusively) by noncovalent interactions.
- Hydrophobic interactions that drive most hydrophobic amino acid side chains into the interior of the protein, protecting them from water.
- hydrogen bonds and salt bridges between the carboxylates of aspartic and glutamic acid and the oppositely charged side chains of protonated lysyl, argininyl, and histidyl residues.
- Some proteins contain covalent disulfide (S—S) bonds that link the sulfhydryl groups of cysteinyl residues.
- Intrapolypeptide disulfide bonds further enhance the stability of the folded conformation of a peptide.
- interpolypeptide disulfide bonds stabilize the quaternary structure of certain oligomeric proteins.

Protein classification

Lec (6) by: Inaam Ahmed Ameen

Protein classification

- **Methods of protein classification**
- Protein classification based on chemical composition
- Protein classification based on shape
- Protein classification based on biological functions
- Protein classification based on solubility

Protein classification based on chemical composition:

- Simple proteins
- Conjugated proteins
- Derived proteins

- Simple proteins
- Also known as homoproteins, they are made up of only amino acids. Examples are plasma albumin, collagen, and keratin.

Conjugated proteins

- Conjugated proteins (holoprotein)consist of polypeptide (apoprotein)part and nonprotein (prosthetic group, cofactor, coenzyme) components needed for proper function.
- Examples:
- glycoprotein ,lipoprotein, nucleoprotein,phosphoprotein, metalloprotein , hemoprotein , and flavoprotein.

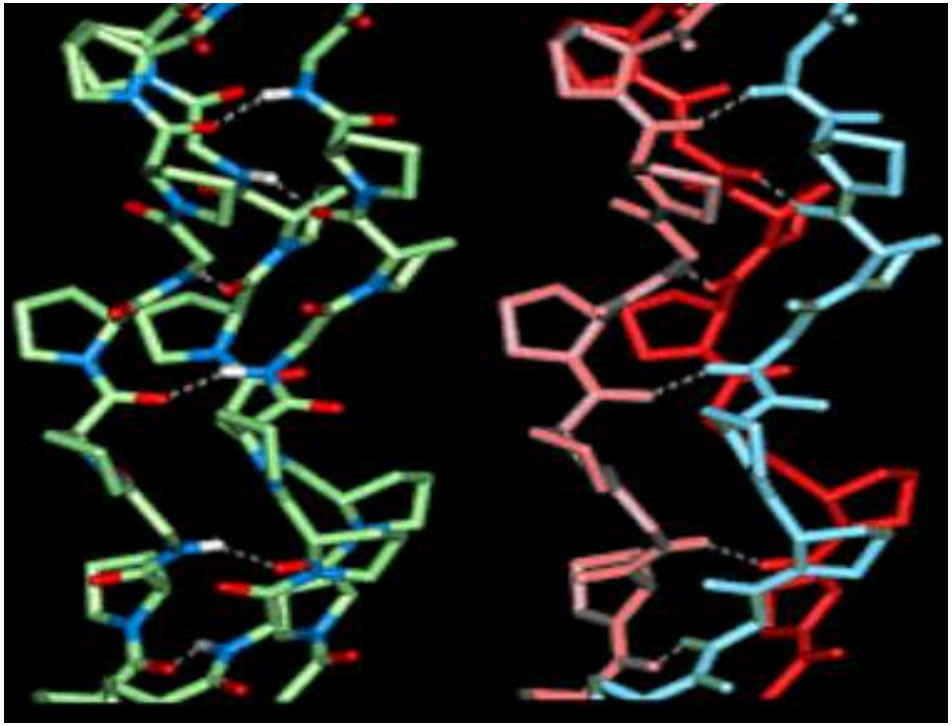
Derived proteins

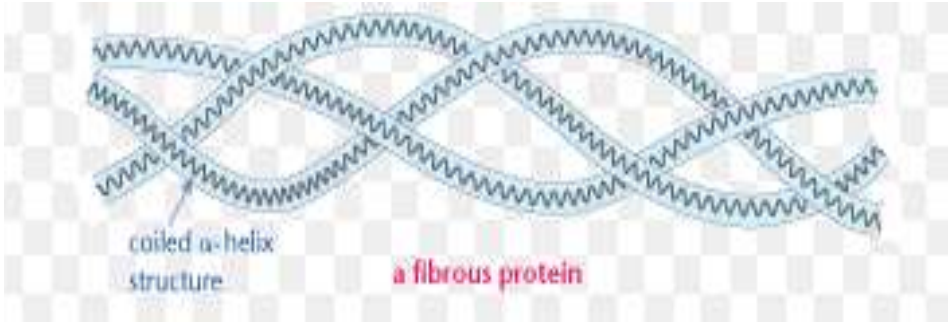
- **Derived proteins:** this class of proteins formed from the native protein (simple and conjugated proteins) by the action of heat, physical forces or chemical factors like: coagulated proteins and denatured protein by heat or X – ray or UV – ray or vigorous shaking or acid or alkali or progressive hydrolysis of protein.

Protein classification based on shape

- Divided into two classes:
- fibrous and globular.

- Fibrous proteins are spiral and helical and are cross linked by disulfide and hydrogen bonds.
- Examples are collagen, keratin, elastin, myosin





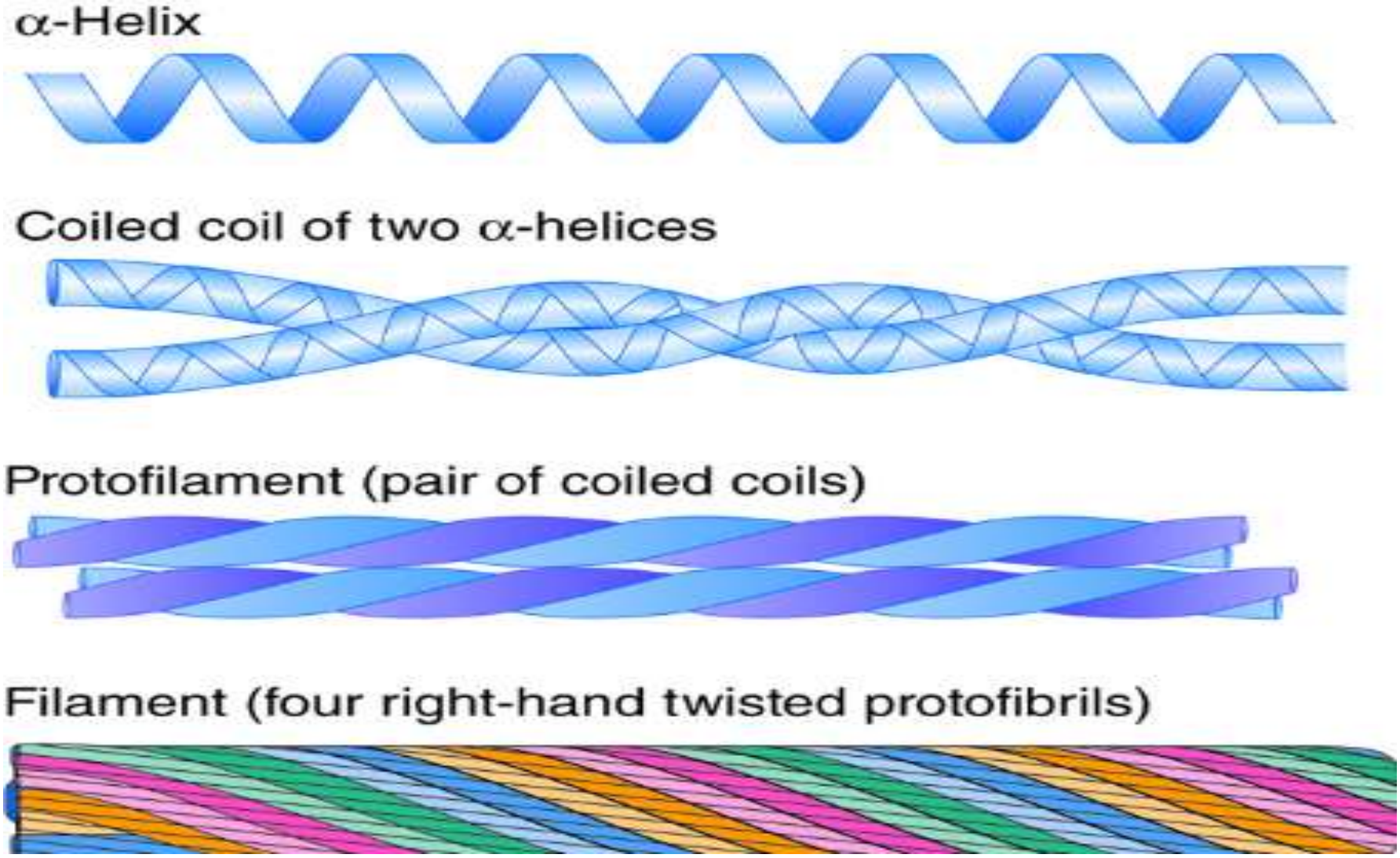
Collagen

- As the main component of connective tissue, it is the most abundant protein in mammals, making up from 25% to 35% of the whole-body protein content. Collagen consists of amino acids spiral together to form triple-helices to form of elongated fibrils. It is mostly found in fibrous tissues such as tendons, ligaments and skin.
- Depending upon the degree of mineralization, collagen tissues may be rigid (bone), compliant (tendon), or have a gradient from rigid to compliant (cartilage). It is also abundant in corneas, cartilage, bones, blood vessels, the gut, intervertebral discs, and the dentin in teeth.
- It consists of a triple helix made of the repetitious amino acid sequence glycine, proline or hydroxyproline . collagen triple helix has 3.3 residues per turn. Each of the three chains is stabilized by the steric repulsion due to the pyrrolidine rings of proline and hydroxyproline residues.

α-Keratins

- α-Keratins is a type of keratin found in mammals. This protein is the primary component of nails, claws, hair, wool, and a large part of the outer layer of the skin.
- The different stiffness and flexibility of these structures is a consequence of the number of disulfide bonds that participate, together with other binding forces, to stabilize the protein structure.
- And this is the reason why wool keratins, which have a low number of disulfide bonds, are flexible, soft and extensible, unlike nails and claw keratins that are rich in disulfide bonds.
- Two keratin proteins come together and the helices wind around themselves to form a quaternary structure of a coiled-coil dimer. These dimers then connect into protofilaments and then filaments.

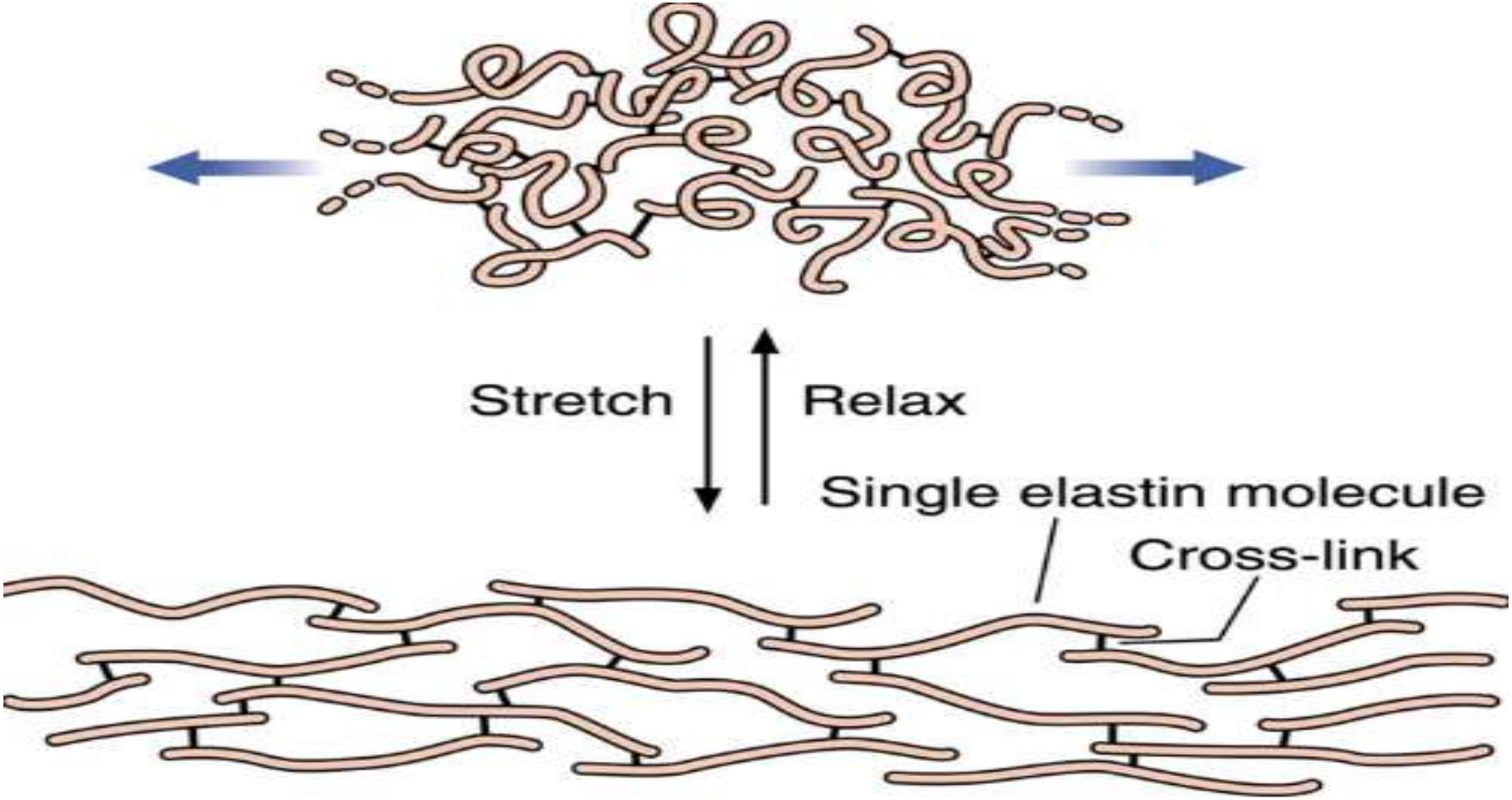
α *Keratin* structure

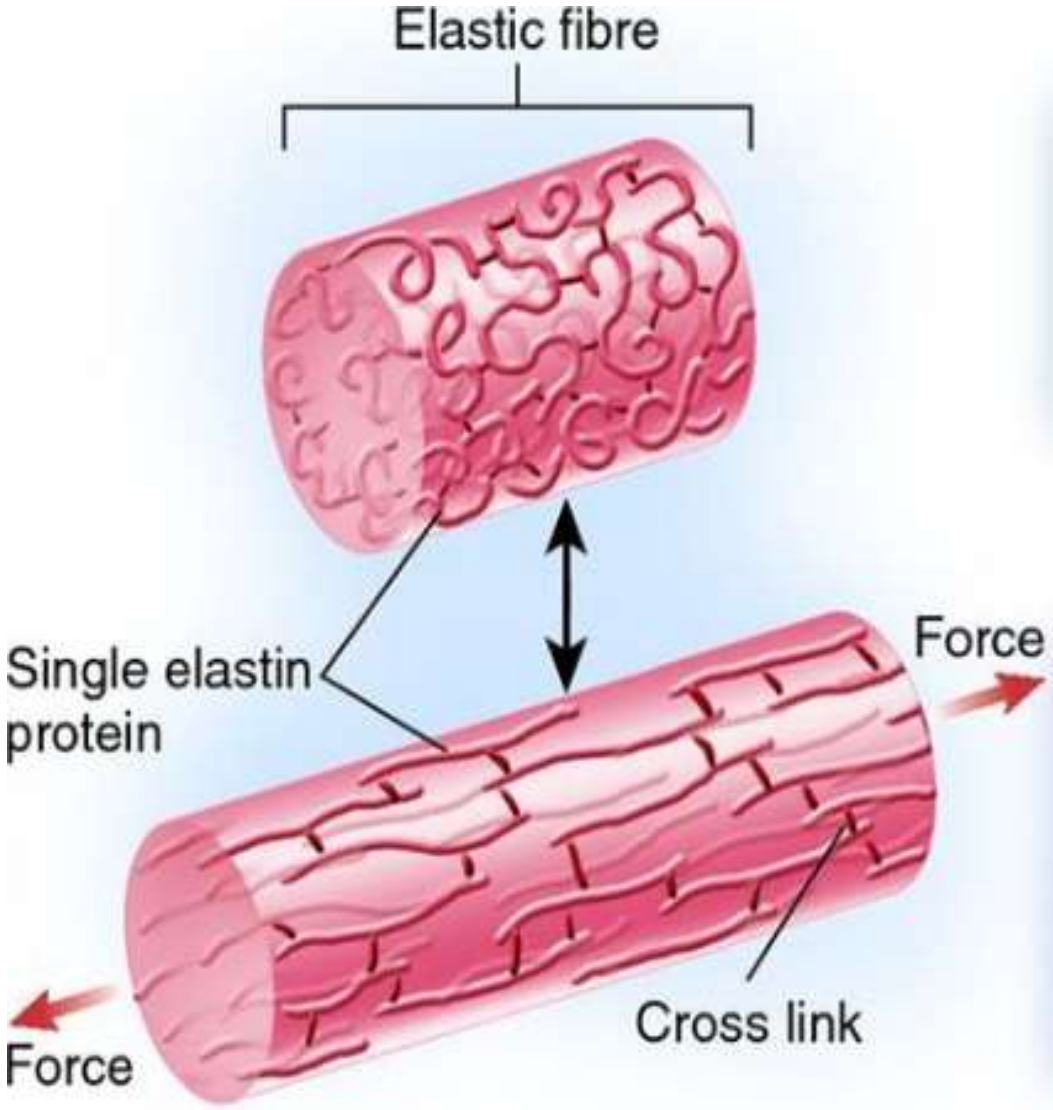


Elastin

- Elastin: is a highly elastic protein in connective tissue and allows many tissues in the body to return their shape after stretching or contracting.
- this protein provides elasticity to the skin and blood vessels, a consequence of its random coiled structure, that differs from the structures of the α-keratins and collagens.

Stretched and relax elastin





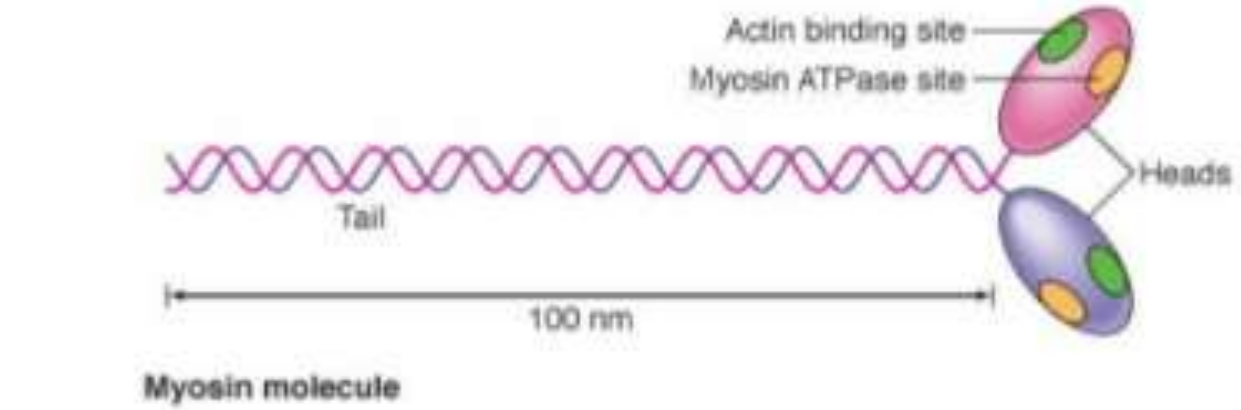
In the absence of a stretching force, the elastin proteins are in a compact conformation.

When subjected to a stretching force, the elastin proteins elongate but remain attached to each other via cross links.

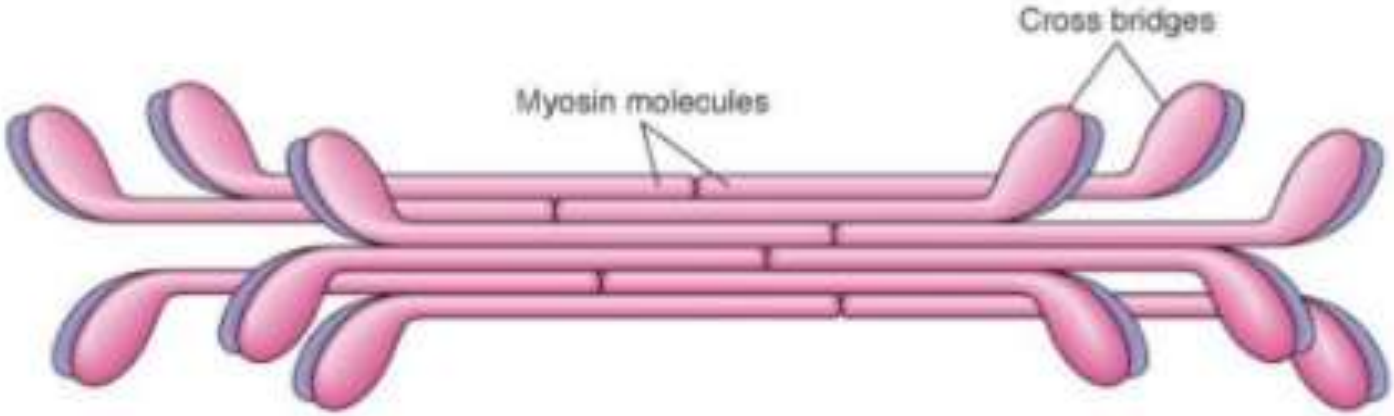
Myosin

- Component of thick filament composed of six polypeptide chains.
- The two heavy chains wrap spirally around each other form a double helix; however one end of each of these chains is folded into a globular protein mass called the head; the elongated portion is called the tail.
- Tails oriented toward center of filament and globular heads protrude outward at regular intervals
- Heads form cross bridges between thick and thin filaments.
- Cross bridge has two important sites critical to contractile process
- An actin binding site
- A myosin ATPase(ATP splitting) site

Structure and Arrangement of Myosin Molecules Within Thick Filament



Myosin molecule

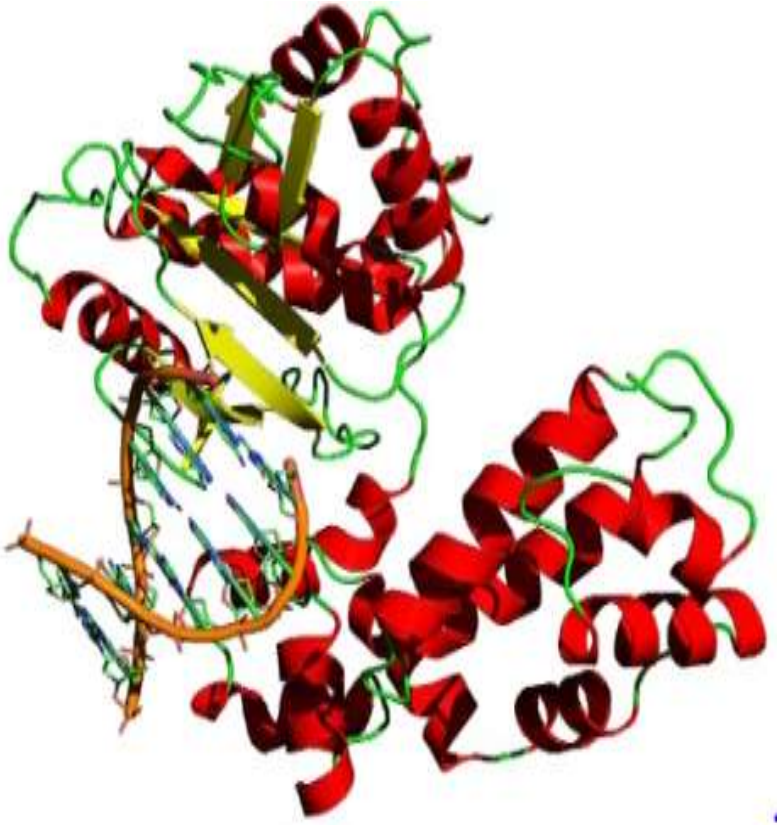


Thick filament

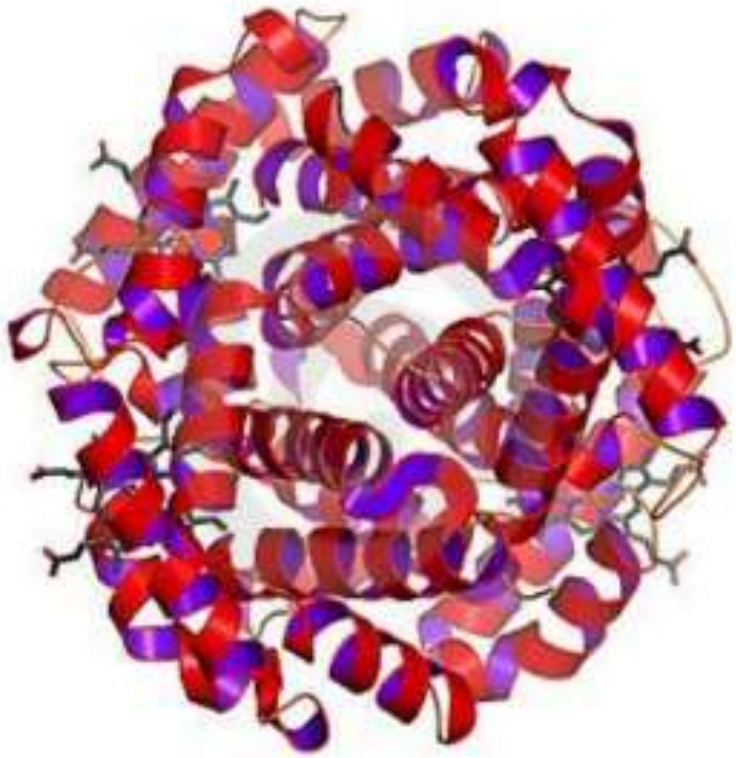
Globular proteins

- Most of the proteins belong to this class . They have a compact and more or less spherical structure, more complex than fibrous proteins. In this regard, motifs, domains, tertiary and quaternary structures are found, in addition to the secondary structures.
- The amino acid chain twists and folds in a manner that enhances the protein's solubility in water by placing polar groups of atoms at the protein's surface (where they can participate in attractive interactions with water molecules).
- This twisting and folding that determine the overall shape of a protein molecule (its tertiary structure) are due largely to the very complex interaction of intramolecular forces that exists among different groups of atoms within the molecule, and to intermolecular forces acting between groups of atoms on the protein and molecules in the protein's immediate surroundings.
- The aqueous solubility of globular proteins allows them to exist in biological fluids as individual molecules or in small clusters

Quaternary Structure: Globular



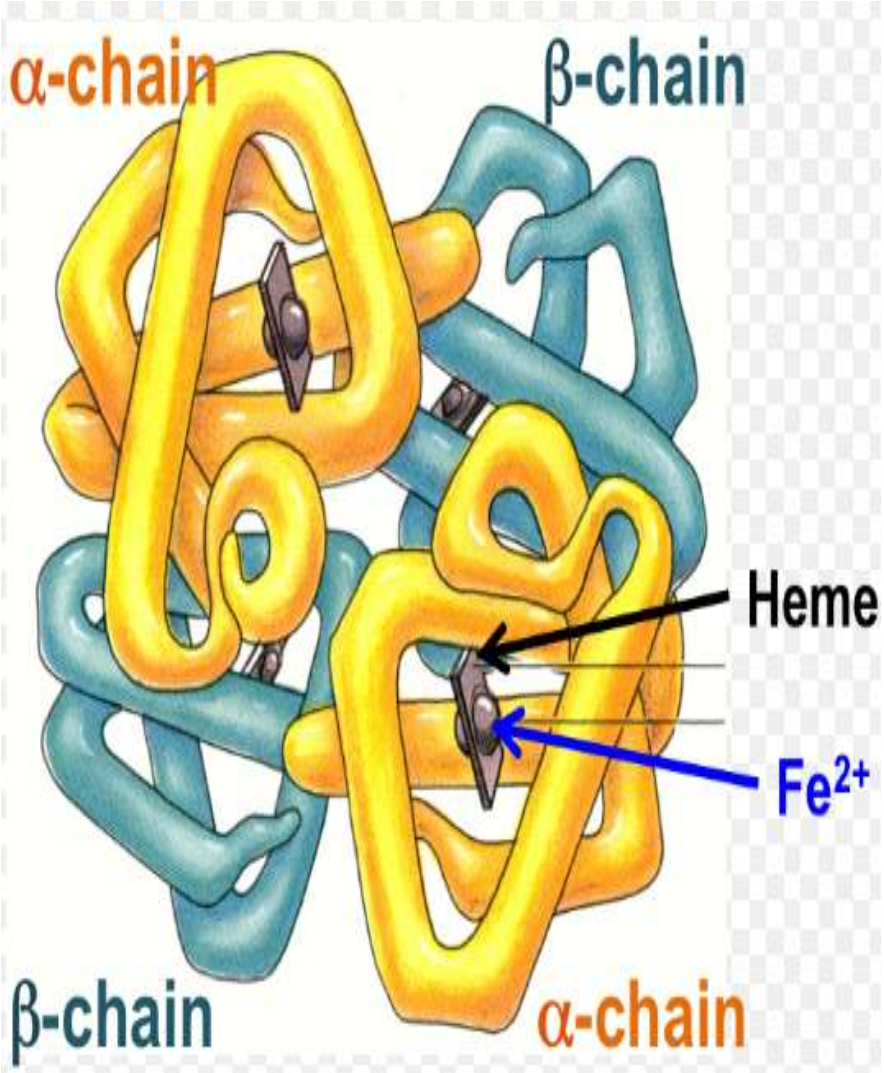
- DNA polymerases



Hemoglobin

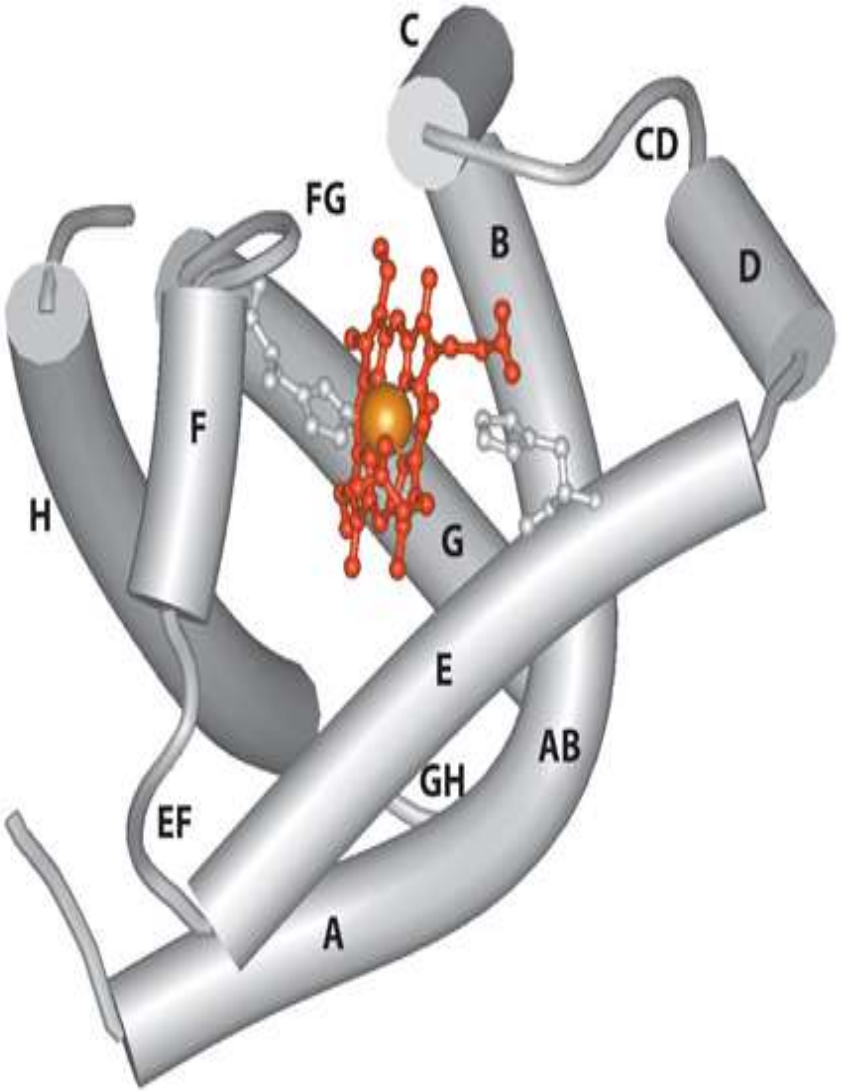
Hemoglobin

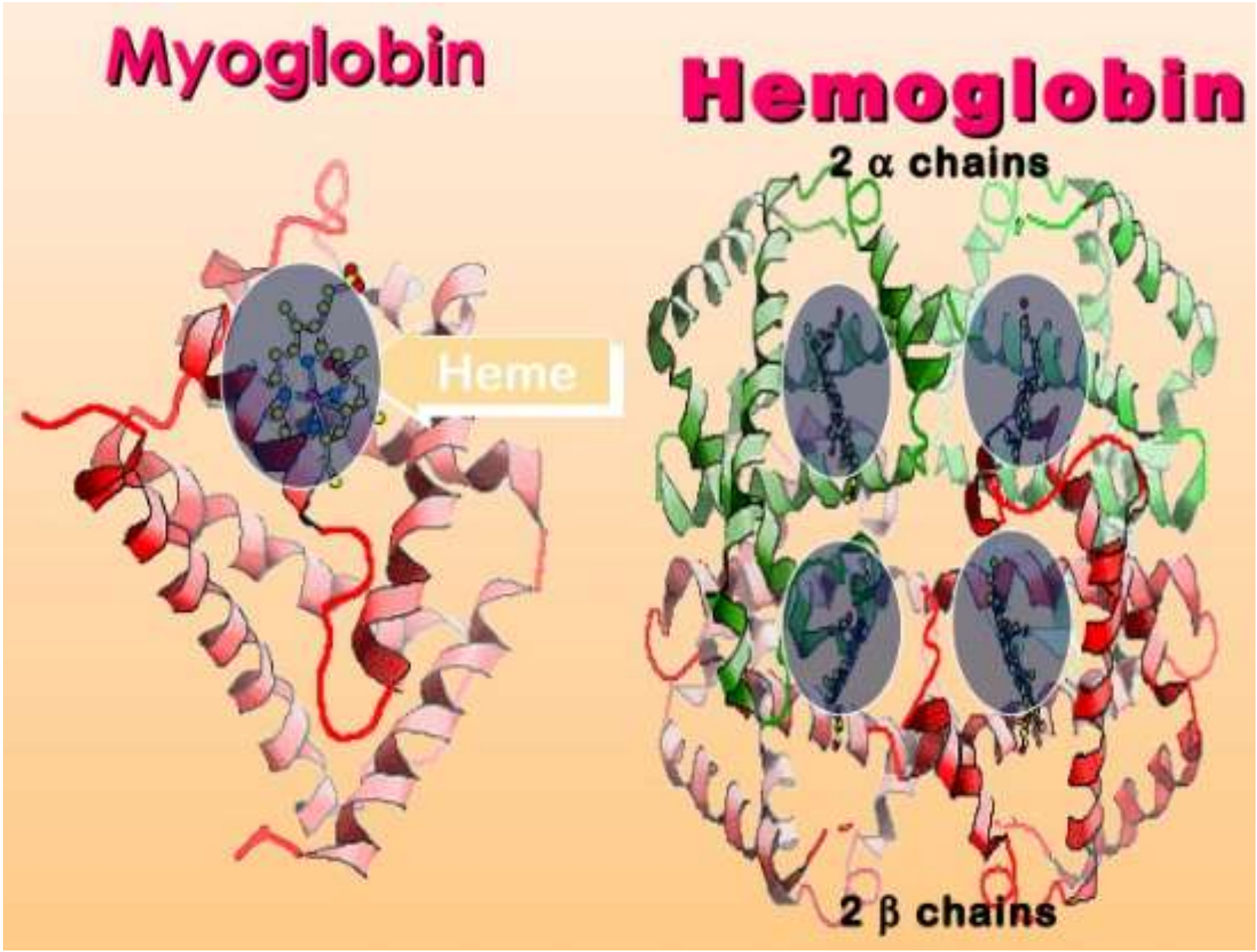
- Hemoglobin is a protein found in red blood cells and is the primary vehicle for transporting oxygen in the blood.
- It is composed of the protein globin(a polypeptide), and the pigment heme.
- Globin: four chains ($2\alpha\ and\ 2\beta$)
- Heme: porphyrin ring with central iron. Iron is the site of attachment with O2.
- There are 4 heme groups each attached to one globin chain. So one Hb molecule can carry up to 4 O2 Molecules.
- The major (96%) type of hemoglobin present in adults called HbA and it has
- 2 alpha globin chains and
- 2 beta globin chains($\alpha 2\beta 2$)



Myoglobin

- It is present in heart and skeletal muscles.
- Function as reservoir of oxygen and as an oxygen carrier.
- It increases the rate of transport of oxygen within the muscle cell by enhancing the solubility of oxygen.
- Monomer(one globin chain), tertiary structure, consists of eight α –helices (A to H).
- Contains one heme group in its internal hydrophobic cleft and therefore can bind one oxygen molecule forming oxymyoglobin.
- Binds oxygen more tightly




Myoglobin
Heme
Hemoglobin
2 α chains
2 β chains

	Fibrous	Globular
Shape	Long and narrow	Round/spherical
Purpose	Structural	Functional
Acid sequence	Repetitive amino acid sequence	Irregular amino acid sequence
Stability	Less sensitive to changes in pH, temperature, etc.	More sensitive to changes in pH, temperature, etc.
Examples	Collagen, myosin, fibrin, actin, keratin, elastin	Enzymes, haemoglobin , insulin, immunoglobulin
Solubility	(Generally) insoluble in water	(Generally) soluble in water
Structure	Extended protein	Compact protein
Secondary structure	Simple based on one type only	Complex : mixture of $\boldsymbol{\alpha\_helix}$, $\boldsymbol{\beta\_sheet\ and\ loop\ structu}$
Quaternary structure	Usually held together by covalent bridges	Held together by non covalent forces

Protein classification based on biological functions:

- From the functional point, they may be divided into several groups:
- Structural proteins:
- Form the component of the connective tissue, bone ,tendons, cartilage, ligaments, skin, nail, hairs and horn.
- Most of them are fibrous proteins and are insoluble in water.
- Example: collagen, keratin and elastin.
- Note:
- Tendons connect muscle to bone. Whereas ligaments attach one bone to an other. Tendons are non elastic but ligaments can be stretched, because they contain, in addition to collagen fibres, the protein elastin

- Enzymes (biochemical catalysts).
- Reduce the activation energy of reactants and speed-up the metabolic reactions in the cells.
- Most of them are globular conjugated proteins.
- Examples: DNA polymerase,Nitrogenase, Lipase.

Hormones:

- Like insulin and glucagon

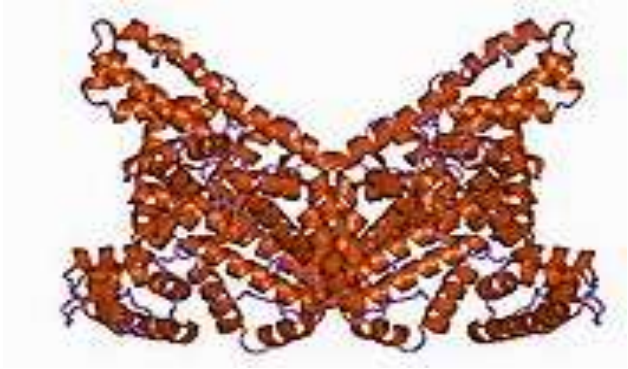
- Respiratory pigments:
- They are colored proteins
- All of them conjugated proteins and they contain pigments(chrome) as their prosthetic group

- Transport proteins:
- They transport the materials in the cells
- They form channels in the plasma membrane
- They also form one of the components of blood and lymph
- Example: Serum albumin
- Contractile proteins
- They are the force generators of muscles
- They can contract with the expense of energy from ATP molecule
- Example: Actin, myosin
- Storage proteins
- They act as the store of metal ions and amino acids in the cells
- Found in seeds, egg and milk
- Example: Ferritin which stores iron, cassein, ovalbumin, gluten of wheat

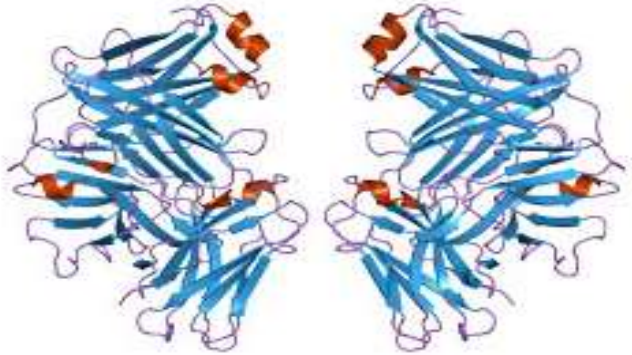
Protein classification based on solubility

- On the basis of solubility, the proteins are classified as follows:
- Albumins, these are simple proteins soluble in water, dilute salt solutions, dilute acids and bases. Albumins may coagulate on heating. Examples: Egg albumin, serum albumin and milk albumin.
- Globulins: these are simple proteins insoluble in water, dissolve in dilute salt solutions. Examples: Globulins of white egg and blood serum.
- Globins : Globins are highly soluble proteins. Also dissolve in ammonium hydroxide. Example : Haemoglobins.
- Protamines: These are also a highly soluble proteins which can be dissolved in ammonium hydroxide, Example: proteins of fish sperm.

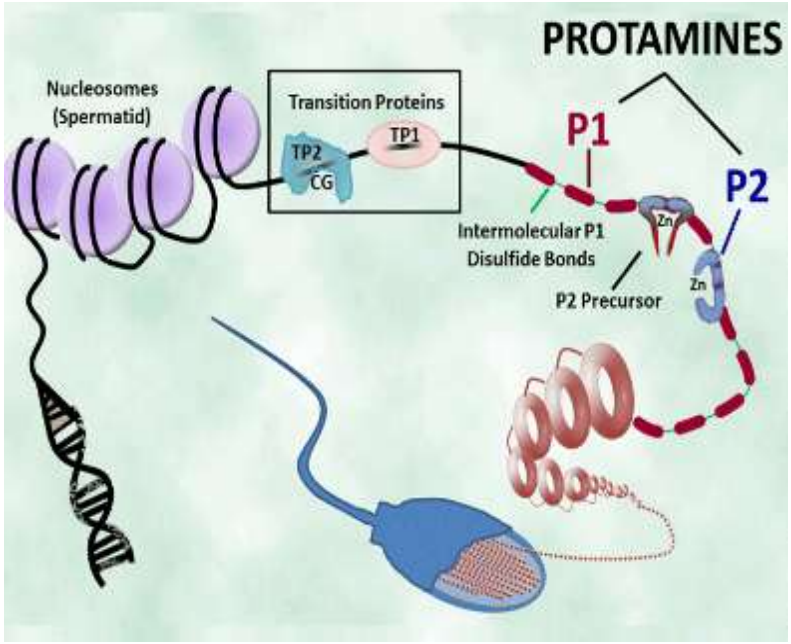
- Histones: Are soluble in water but insoluble in ammoniun hydroxide.These do not coagulate on heating. Example: Nucleoproteins.

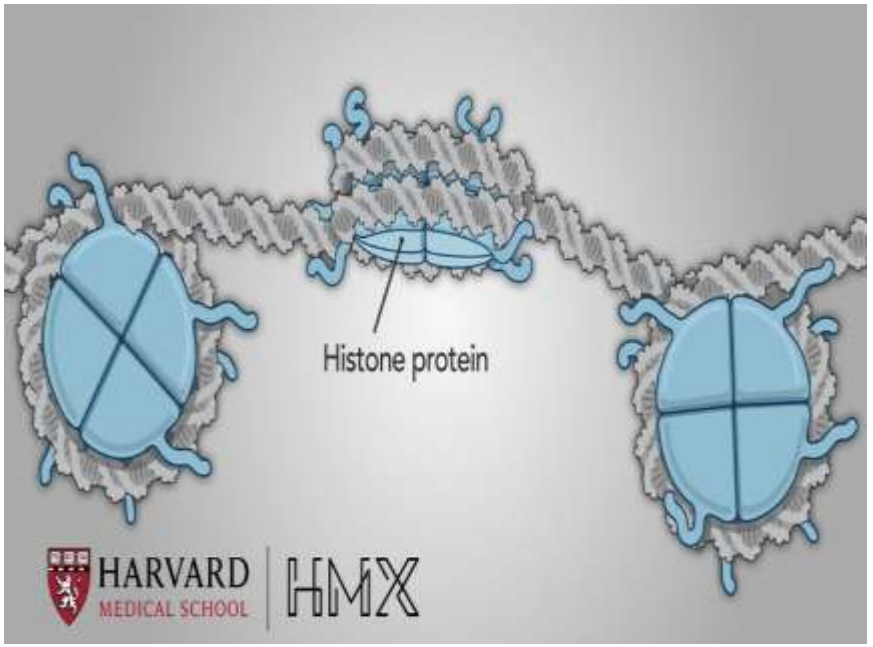


- Albumin



Globulin





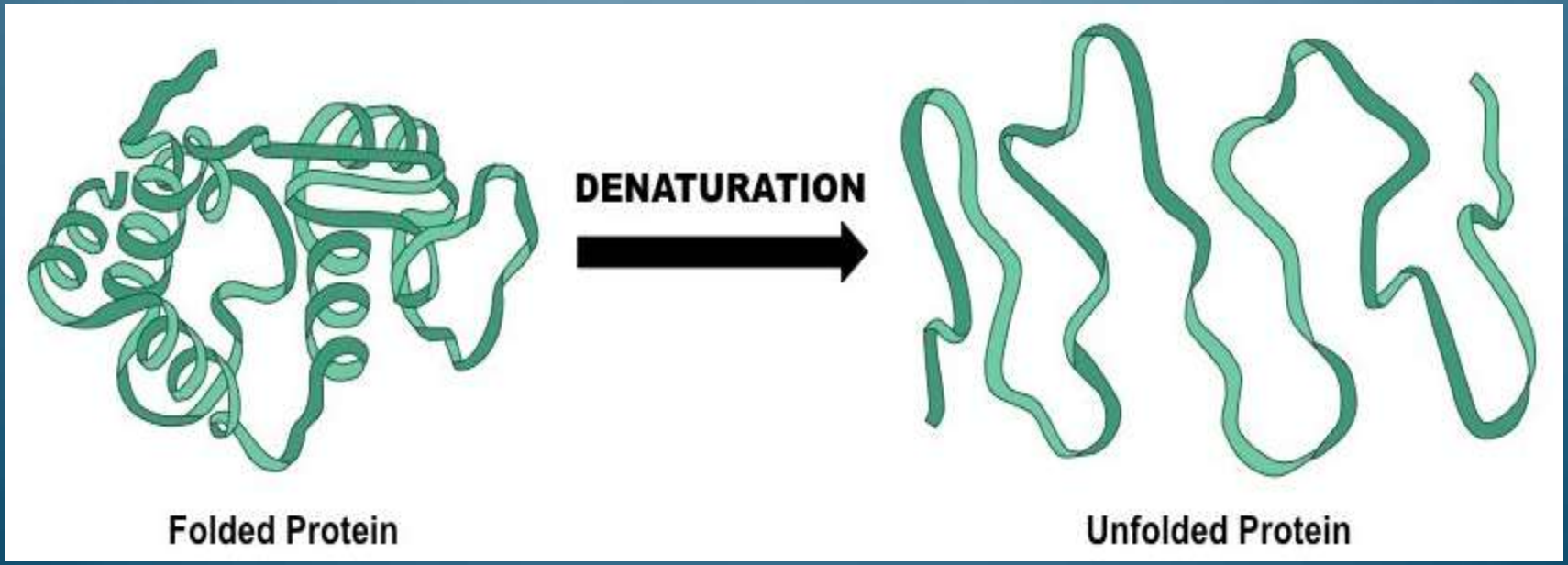
Denaturation of proteins

Lec (7) by: Inaam Ahmed Ameen

Denaturation of proteins

Denaturation is a structural change in a protein that results in the loss of its biological properties

Because the way a protein folds determines its function, any change of the tertiary structure will alter its activity



Denaturation

- The conversion of a biologically functional molecule into a non-functional form
- There are many denatured states but one native state
- Proteins can regenerate to their native state but slowly
- Denatured proteins have a greater tendency to aggregate.

- Denaturation is a process in which a protein loses its native shape due to the disruption of weak chemical bonds and interactions, thereby becoming biologically inactive.
- When protein denature, the cells go throught a series of changes, first loosening then tightening.
- A loss of three- dimentional structure, sufficient to cause loss of function.
- Loss of secondary , tertiary and quaternary structure of proteins. Change in physical, chemical and biological properties of protein molecules.

For example

- Changing pH denatures proteins because it changes the charges on many of the side chains. This disrupts electrostatic attractions and hydrogen bonds.
- Certain reagents such as urea and guanidine hydrochloride denature proteins by forming hydrogen bonds to the protein groups that are stronger than the hydrogen bonds formed between the groups.
- Detergents such as sodium dodecyl sulphate denature proteins by associating with the non-polar groups of proteins, thus interfering with the normal hydrophobic interactions.
- Organic solvents such as acetone alcohols denature proteins by disrupting hydrophobic interactions.
- Proteins can also be denatured by heat. Heat increase molecular motion which can disrupt the attractive forces.

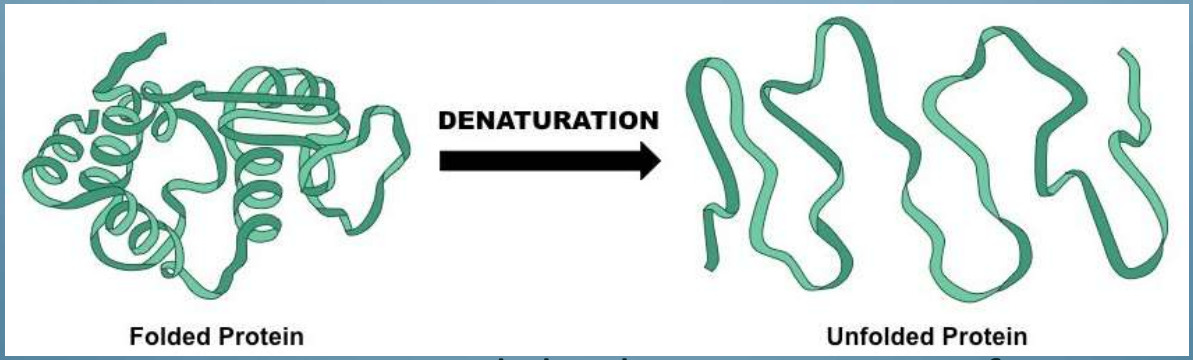
- None of these agents breaks peptide bonds, so the primary structure of a protein remains intact when it is denatured.
- When a protein is denatured, it loses its function.
- Example:
- A denatured enzyme stops function
- A denatured antibody no longer can bind its antigen.
- The denatured state dose not necessarily associate with complete unfolding of the protein and randomization of conformation.
- Under most conditions, denatured proteins exist in a set of partially folded states that are poorly understood.

Agents of denaturation

- **Physical agents**:
- 1- Heat
- 2-Violent shaking
- 3-X-rays
- 4-Hydrostatic pressure(5000-10000 atm)
- 5-UV radiation

Heat

- High levels of thermal energy may disrupt the hydrogen bonds that hold the protein together
- As these bonds are broken, the protein will begin to unfold and lose its capacity to function as intended



- If the temperature is increased slowly, a proteins conformation generally remains intact until an sudden loss of structure(and function) occurs over a narrow temperature range.
- Temperatures at which proteins denature may vary, but most human proteins function optimally at body temperature (~37ºC)
- Optimum temp:
- the temp at which the enzyme has its maximum activity

Thermal denaturation

- Trypsinogen 55 C
- Pepsinogen 60 C
- Lysozyme 72 C
- Myoglobin 79 C
- Soy Glycinin 92 C
- Oat globulin 108 C
- Affected by pH, water, solutes

Violent shaking

- Agitation also denatures protein. We see this clearly in the stirring of egg whites.

- Hydrostatic pressure(5,000—10,000 atm)
- Pressure destabilization of hydrophobic aggregates.
- Pressure denatured proteins, unlike heat denatured proteins, retain a compact structure.

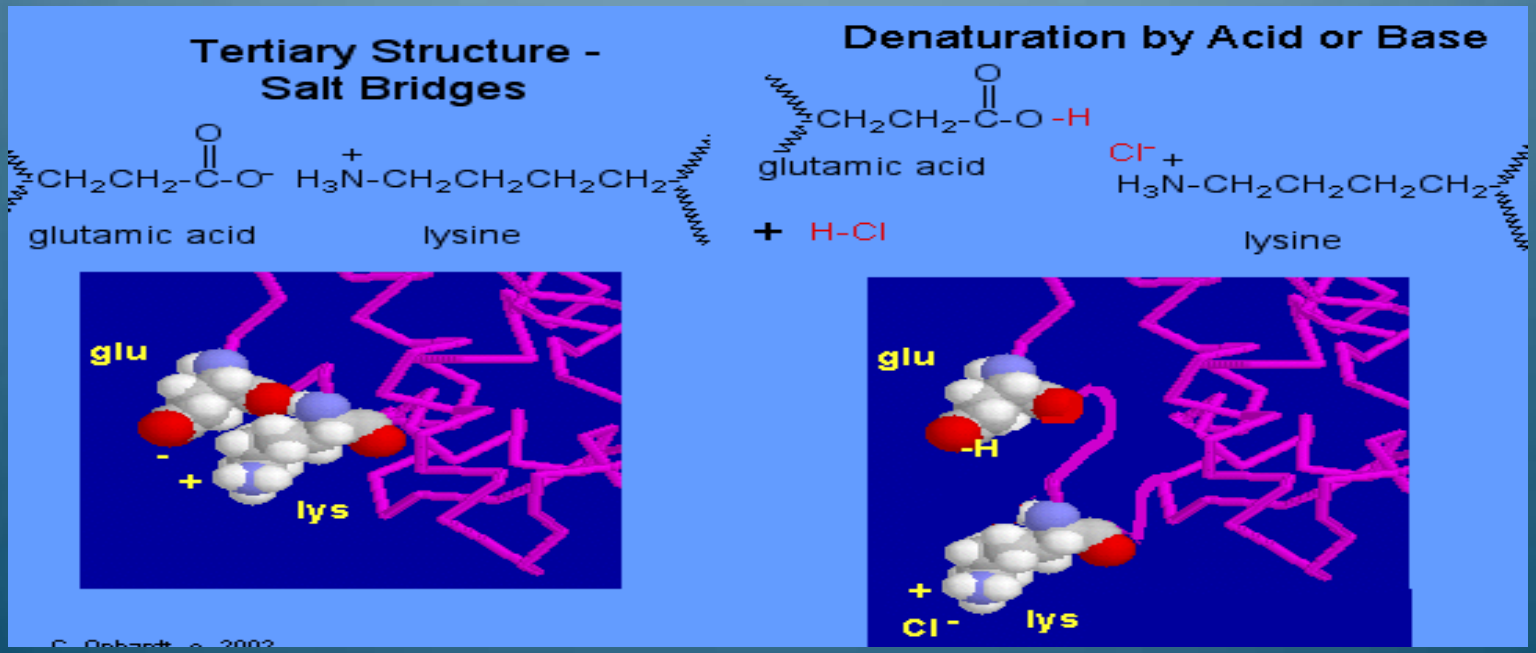
- **UV Radiation**
- UV radiation supplies kinetic energy to protein molecules, causing their atoms to vibrate more rapidly and disrupting relatively weak hydrogen bonding and dispersion forces.

Chemical agents:

- Acids and alkalies
- Organic solvents(ether, alcohol).
- Salts of heavy metals(Pb, Hg).
- Detergents
- Altered pH

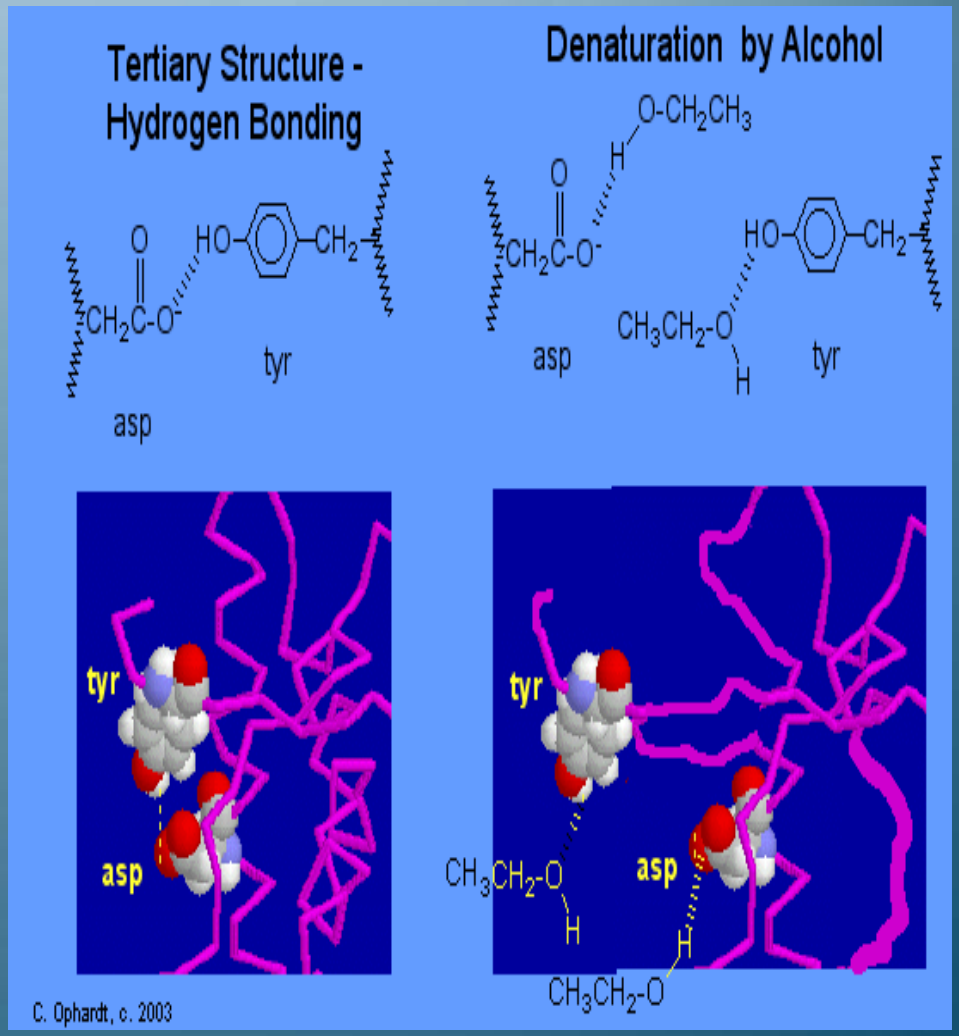
Acids and bases

- Acids and bases disrupt salt bridges held together by ionic charges.
- Double replacement reaction occurs where the positive and negative ions in the salt change partners with the positive and negative ions in the new acid or base added.
- This reaction occurs in the digestive system, when the acidic gastric juices cause the coagulating of milk.



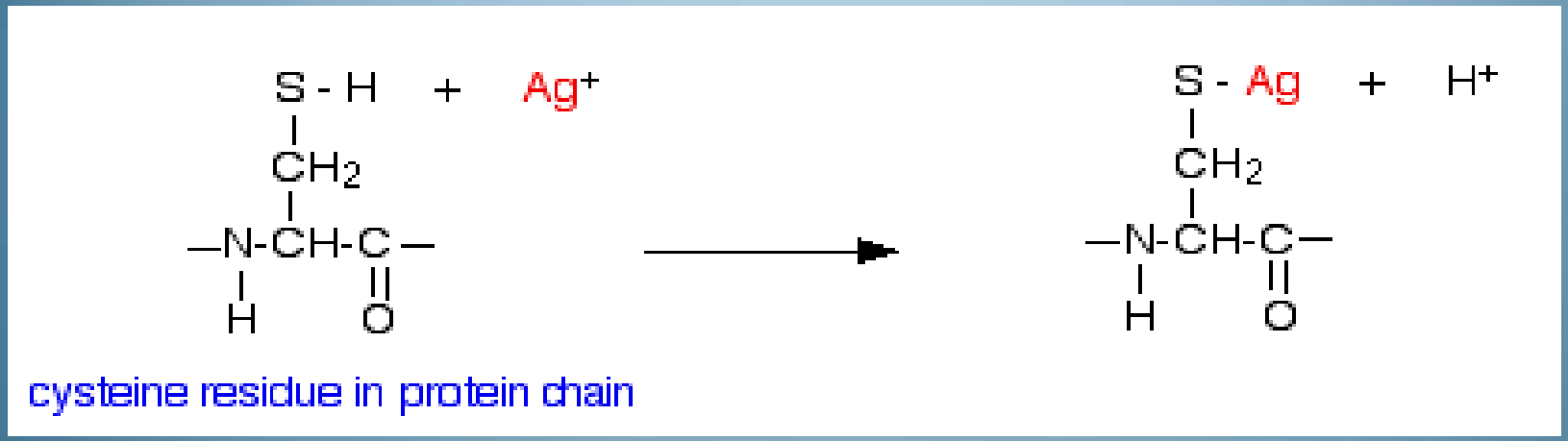
Organic solvents(Ether, Alcohol)

- Alcohol disrupts hydrogen bonding:
- Hydrogen bonding between "side chains" occurs in tertiary protein structure in a variety of amino acid combinations. All of these are disrupted by the addition of another alcohol.
- New hydrogen bonds are formed instead between the new alcohol molecule and the protein side chains.
- For example:
- In the prion protein, tyr 128 is hydrogen bonded to asp 178, which cause one part of the chain to be bonding with a part some distance away. After denaturation, the graphic show significant structural changes.

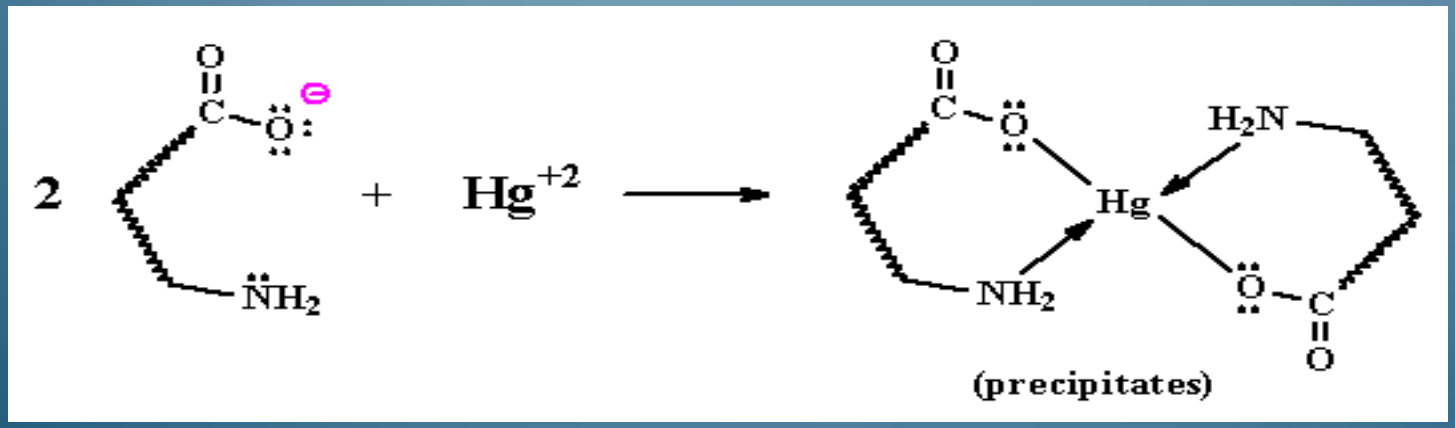


Salts of heavy metals (Pb, Hg)

- Heavy metals salts usually contain Hg++, Pb++ , Ag+, and other metals with high molecular weight. Since salts are ionic they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.
- **Heavy metals denature proteins by:**
- Disruption of the salt bridges that stabilize the structure of the protein
- Disruption of disulfide bonds that stabilize the structure due to the high affinity of heavy metals to sulfur (displacement reaction)
- Formation of water-insoluble metal protein salts



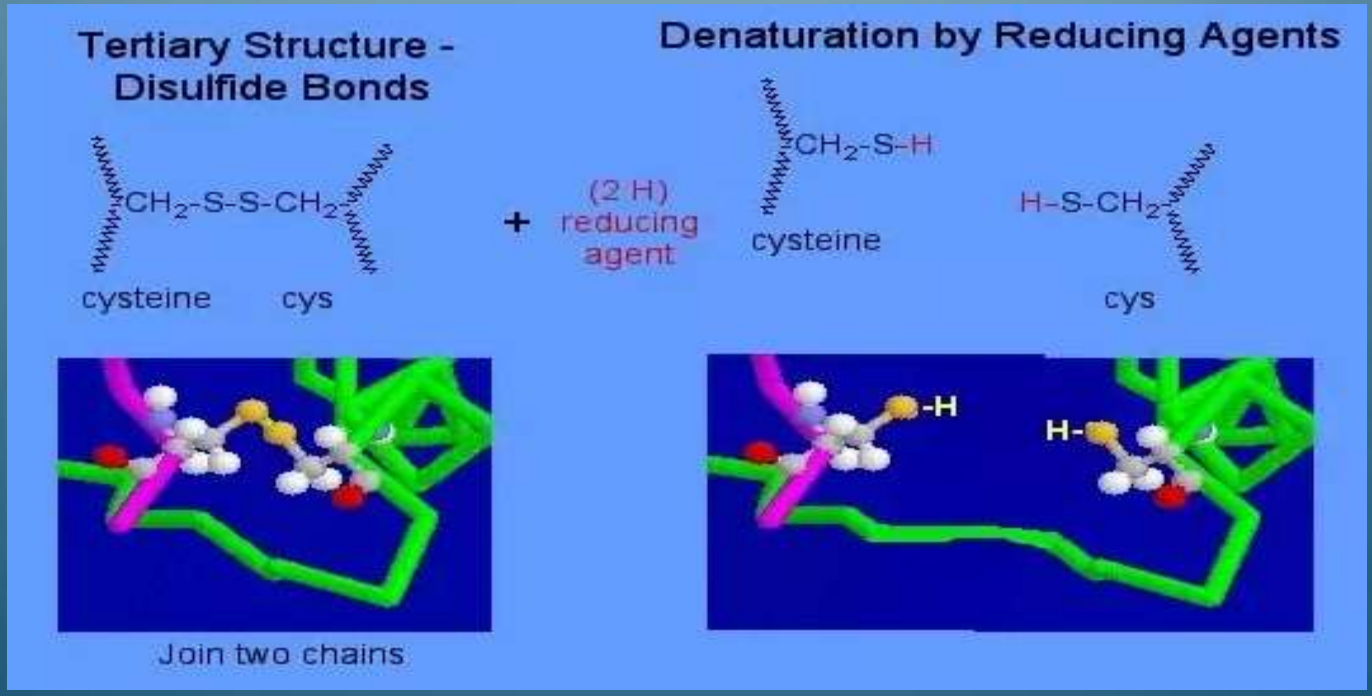
- Displacement reaction causing disruption of disulfide bonds.



- Reaction of mercury with protein to form a water-insoluble salt.

reducing agents denature proteins: disrupt disulfide bonds

- Reducing agents disrupt the disulfide bonds. Disulfide bonds are formed by oxidation of sulfhydryl groups on cysteine. Different protein chains or loops within a single chain are held together by the strong covalent disulfide bonds. If oxidizing agents cause the formation of a disulfide bond, then reducing agents, of course, act on any disulfide bonds to split it apart.

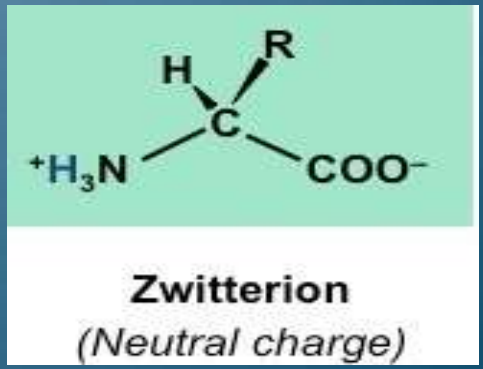


Denaturation of proteins by detergents

- Detergents are amphiphilic molecules(both hydrophobic and hydrophilic parts).
- Example:
- Hydrophobic part of detergent associated With hydrophobic parts of the protein (coating with detergent molecules)
- Hydrophilic ends of the detergent molecules interact with water(nonpolar parts of the protein become coated with polar groups that allow their association with water)
- Hydrophobic parts of the protein no longer need to associate with each other
- Dissociation of the non-polar R groups can lead to unfolding of the protein chain.
- Detergents such as sodium dodecyl sulphate denature proteins by associating with the non-polar groups of protein, thus interfering with the normal hydrophobic interactions.

Effect of pH on Protein Structure

- Amino acids are zwitterions, neutral molecules possessing both negatively (COO^-) and positively (NH_3^+) charged regions
- Changing the pH will alter the charge of the protein, which in turn will alter protein solubility and overall shape
- All proteins have an optimal pH which is dependent on the environment in which it functions (e.g. stomach proteins require an acidic environment to operate, whereas blood proteins function best at a neutral pH)
- Optimum pH:
- The pH at which the enzyme has its maximum activity



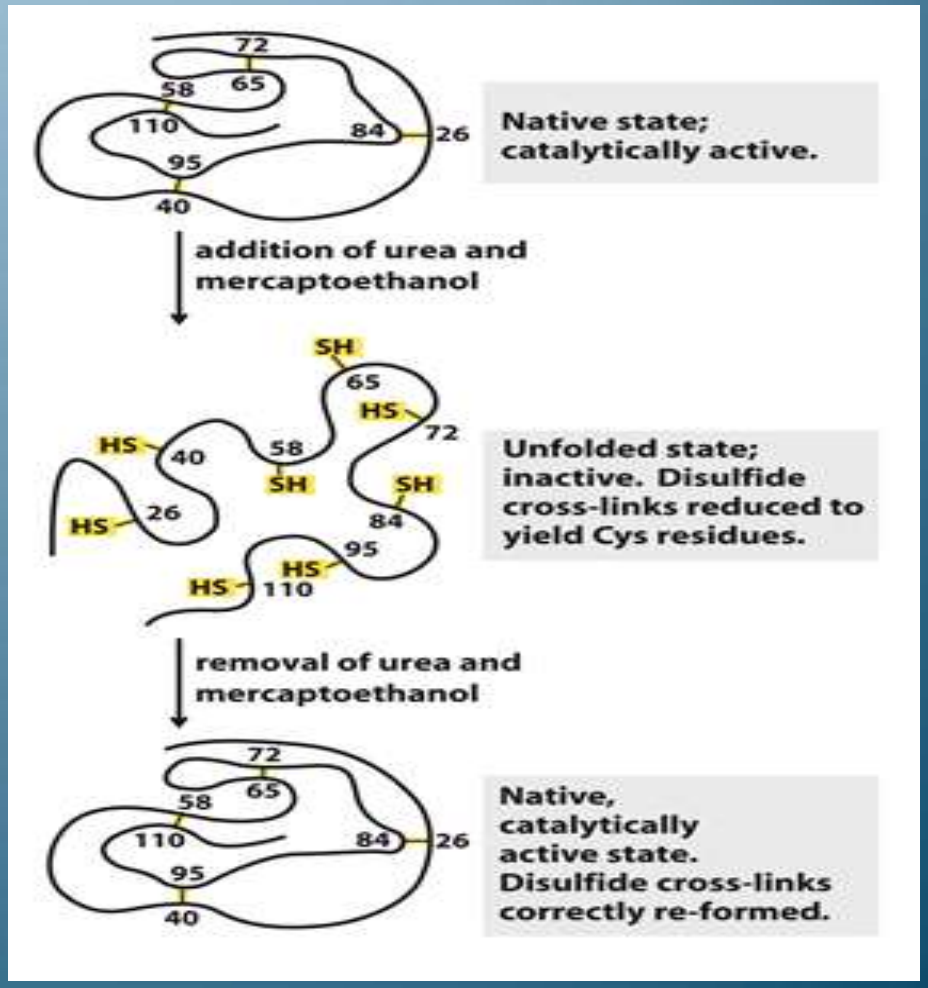
Characteristics of denaturation

- The native helical structure of protein is lost
- The primary structure of a protein with peptide linkages remains intact(peptide bonds are not hydrolyzed).
- The protein loses its biological activity.
- Denatured protein becomes insoluble in the solvent in which it was originally soluble.
- The viscosity of denatured protein(solution) increases while its surface tension decreases.
- Denaturation is associated with increase in ionizable and sulfhydryl groups of protein. This is due to loss of hydrogen and disulfide bonds.
- Decrease in size and shape of the protein molecule

- Denatured protein is more easily digested. This is due to increased exposure of peptide bonds to enzymes. Cooking causes protein denaturation and, therefore, cooked food(protein) is more easily digested.
- Denaturation is usually irreversible, example prepared omelet from an egg(protein-albumin) but the reversal is not possible.
- Careful denaturation is some times reversible(known as renaturation). For example: Hemoglobin undergoes denaturation in the presence of salicylate. By removal of salicylate, hemoglobin is renatured. Other example the renaturation of ribonuclease after denaturation with urea and mercaptoethanol as shown below:

Renaturation of proteins

- purified ribonuclease was denatured to its unfolded inactive state by exposure to a concentrated solution of urea and the reducing agent, mercaptoethanol.
- This unfolded the protein and reduced all disulfide cross-links to cysteine residues. When urea and mercaptoethanol were removed, the randomly-coiled, denatured ribonuclease spontaneously refolded to its catalytically active state



Additional information:

- Denaturation may not require complete unfolding of proteins. It might be still a folded structure but in random conformation.
- Denaturation is cooperative (changes in one part of protein accelerate the unfolding of the other part.
- Some proteins are resistant to denaturation by heat(proteins of hot spring bacteria stable at 100 c).
- Heat destabilizes H-bonding
- Detergents, urea, organic solvents: destabilize hydrophobic interactions
- Extreme pH conditions: cause ionization of side chains resulting in electrostatic repulsion and breakdown of structure.

Plasma proteins

Lec (8) By: Inaam Ahmed Ameen

Origins of plasma proteins

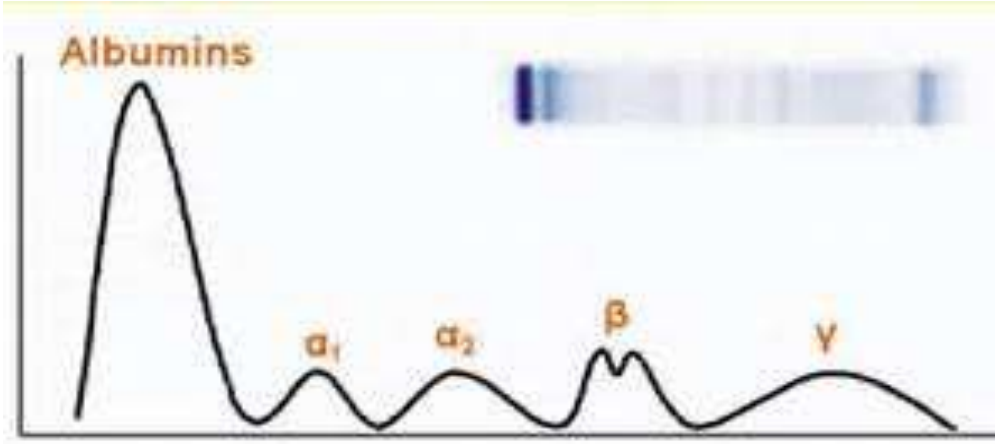
- 90% made in liver
- Antibodies made by plasma cells
- Peptide hormones made by endocrine organs
- The total concentration of proteins in plasma is 6-8 g/dL.

Plasma proteins and their role

- Plasma proteins include :
- Albumin (65-85 g/l)
  - Transportation
  - Regulation of oncotic pressure
  - Regulation of pH
- Globulin (28 g/l)
  - α
  - β
  - Transportation
  - γ - Defense
- Fibrinogen (3 g/l) - Blood clotting (haemostasis)

Plasma Proteins

- The proteins present in the plasma of human blood are a mixture of simple proteins, glycoproteins, lipoproteins and other conjugated proteins are called “Plasma Proteins“.
- These may be separated by salt precipitation, immunological technique and electrophoresis.
- The three major fractions of plasma proteins are known as Albumin, globulin and Fibrinogen.



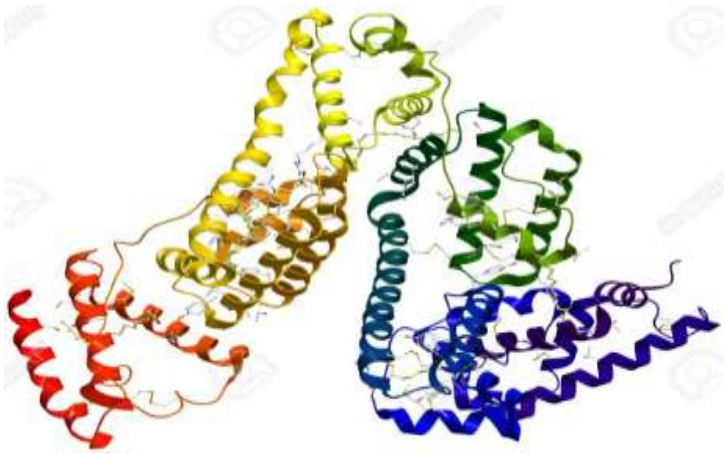
- Normal electrophoretic graph and blood proteins

Fractions of plasma protein

Fraction
Albumins: Albumin, pre-albumin (transthyretin)
α_1-globulins: Thyroxin-binding globulin, transcortin, α_1-acid glycoprotein, α_1-antitrypsin, α_1-lipoprotein (HDL), α_1-fetoprotein
α_2-globulins: Haptoglobin, macroglobulin, ceruloplasmin
β-globulins: Transferrin, hemopexin, lipoprotein (LDL), fibrinogen, C-reactive protein, C_3 and C_4 components of the complement system
γ-globulins: IgG, IgM, IgA, IgD, IgE

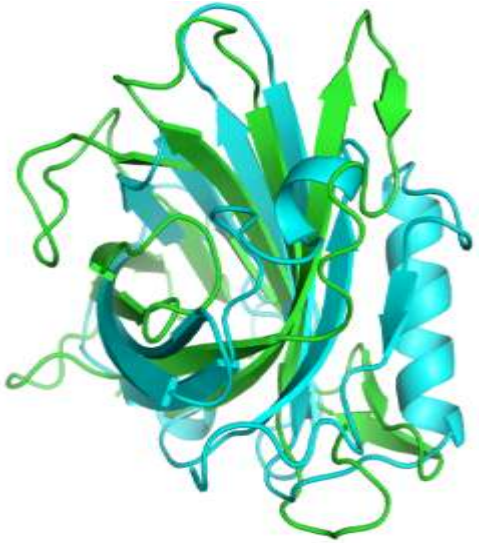
Albumin

- This is the most abundant class of plasma proteins (2.8 to 4.5 gm/100ml) with highest electrophoretic mobility.
- It is soluble in water and it synthesized in liver and consists of a single polypeptide chain of 610 amino acids (with 17 disulfide bonds).
- It is rich in some essential amino acids, contain both acidic and basic residues.
- The presence of these residues makes the molecule highly charged with positive and negative charge.
- Besides having a nutritive role, albumin acts as a transport carrier for various biomolecules such s fatty acids, trace elements and drugs, also maintenance of osmotic pressure and fluid distribution between blood and tissues.



Globulins

- Although globulins make up a smaller proportion of blood plasma protein, they perform the very important function of providing antibodies.
- Globulin protein is actually subdivided into four major categories: gamma globulin, alpha-1 globulin, alpha-2 globulin, and beta globulin.
- Gamma globulins are also classified as immunoglobulin and are the specific group of plasma protein that functions as antibodies providing protection against disease on a cellular level.
- The alpha and beta globulins primarily act as transporters for fat soluble vitamins, hormones and lipids.
- Alpha and beta globulins are synthesized in the liver.
- gamma globulins are created by the lymphoid tissue

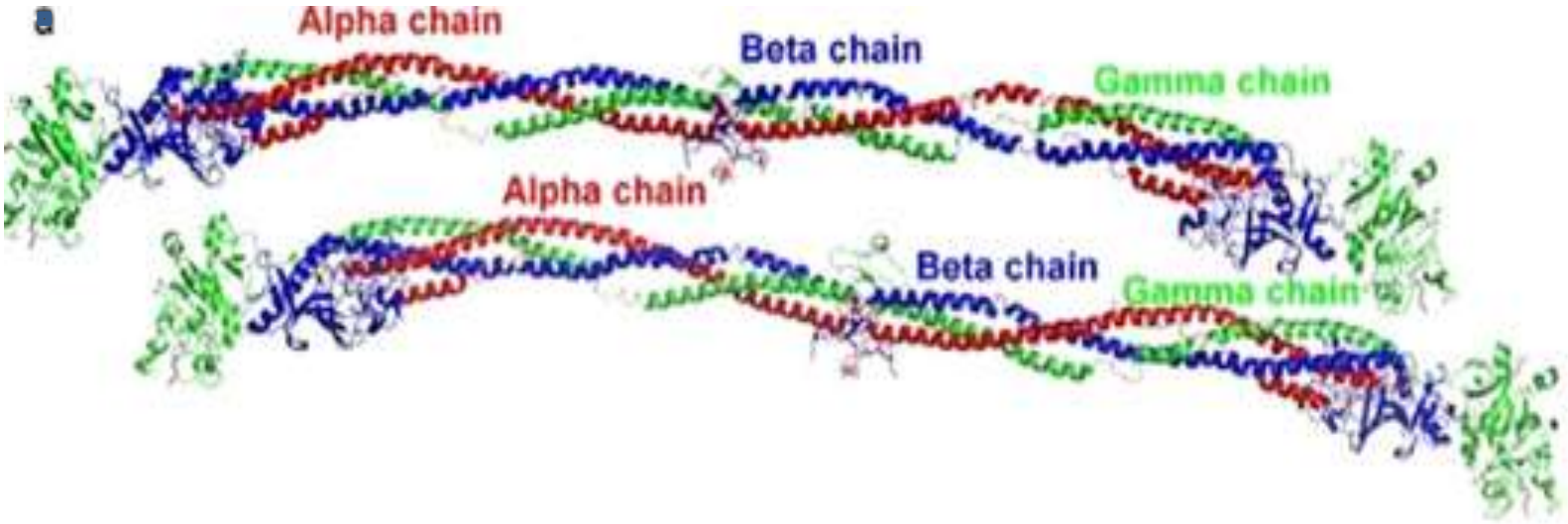


Fibrinogen

- Fibrinogen is also created by the liver. Its primary function is to work with blood platelets to form blood clots.
- Abnormally low levels of fibrinogen can lead to excessive bleeding and hemorrhaging.
- Elevated levels of fibrinogen, however, can be a strong predictor of stroke.
- Fibrinogen circulates in the plasma at a concentration of approximately 200 to 400 mg/dL, with a half-life of four days .
- Fibrinogen has numerous functional interactions and plays a pivotal role in the hemostatic balance:
- It is the substrate for fibrin clot formation.
- It binds to platelets to support platelet aggregation.
- It has a role in wound healing.

Fibrinogen structure

- Human fibrinogen is a complex dimeric glycoprotein composed of two identical chains centrally connected by three disulfide bonds. Each half consists of three polypeptide chains (alpha, beta, and gamma) synthesized by hepatocytes. The synthesis of fibrinogen takes place in the liver, carbohydrate side chains are added to the beta and gamma chains before the molecule is secreted into the plasma.



The concentration of Plasma Proteins is determined by 3main factors

- **Rate of synthesis.** [Most plasma proteins are synthesized in the liver, some example like immunoglobulin are produced at other sites in **lymphocytes**, apo lipoproteins produced by **enterocytes**].

- **Rate of catabolism:** most proteins are degraded after being taken up by cells within the body.

- **The volume of fluid in which proteins are distributed:** the proteins distribution in fluid compartments and loss into third space (ascites, pleural exudates) or to the outside (protein urea).

Functions of plasma proteins

1-Transport substance*:* many substances are transported in blood bound to proteins such as: thyroxin, cortisol, salicylates, bilirubin and vitamin A.

2- Maintaining plasma oncotic pressure (the osmotic pressure due to protein): the pps (albumin responsible for 80% of plasma oncotic pressure) are the major determinant of the distribution of fluid between the intra vascular and extra vascular components and thus plasma volume.

3- Buffering pH changes: pps “particularly albumin”has the maximum buffering capacity due to the high concentration and the presence of large number of histidine residues, which contribute maximally towards maintenance of acid base balance.

4- Enzyme activity.

5- Clotting and the acute inflammatory response.

6- Immunity.

METHODS OF PLASMA PROTEIN SEPARATION

COMMON METHODS of protein separation into albumin, fibrinogen, alpha, beta and gamma globulins

- Paper electrophoresis
- Ultracentrifugation
- Affinity chromatography
- Fractional precipitation method
- Immune electrophoresis

For example Electrophoresis separation pattern depends on:

- Size
- Shape
- Composition
- Electrical charge

Measurement of Plasma Proteins

A) Quantitative measurement of a specific protein:

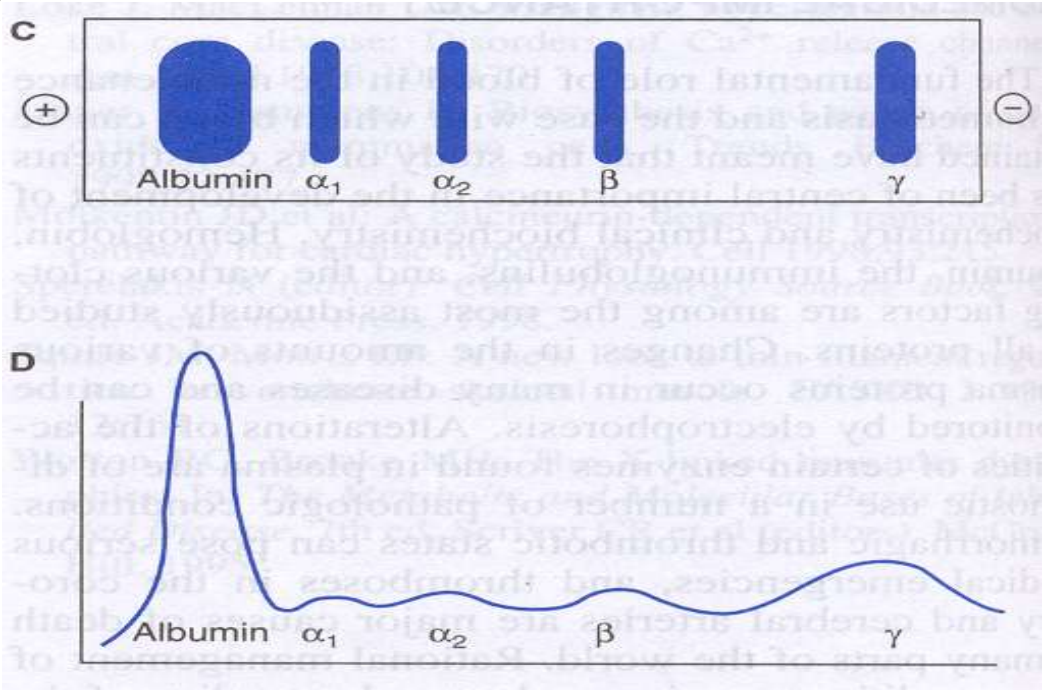
Chemical or immunological reactions

B) Semiquantitative measurement by electrophoresis:

- Proteins are separated by their electrical charge in electrophoresis
- Five separate bands of proteins are observed

- The amounts of these five bands can be easily quantified by use of densitometric scanning machines.
  - These bands change in disease

- **Albumin**
- **α_1-Globulins:**
  - **α_1-Antitrypsin, α-fetoprotein**
- **α_2-Globulins:**
  - **Ceruloplasmin, haptoglobin**
- **β-Globulins:**
  - **CRP, transferrin, β2-microglobulin**
- **γ- Globulins**



Characteristics of plasma proteins

Plasma proteins are large molecules:

with molecular weights ranging mostly from 50,000 to 300,000 Dalton.

Most plasma proteins are glycoproteins:

- They generally contain either N- or O-linked oligosaccharide chains, or both. Albumin is the major exception; it does not contain sugar residues. The oligosaccharide chains have various functions, they are responsible for certain properties of plasma proteins like solubility, viscosity, charge, denaturation etc.

Many plasma proteins exhibit polymorphism**:**

Polymorphism is a Mendelian trait that exists in the population in at least two phenotypes, neither of which is rare. Plasma proteins showing polymorphism are haptoglobin, transferring, ceruloplasmin, and immunoglobulin.

- Large size of the protein molecules:

they can be separated from the plasma by ultracentrifugation (unlike electrolytes or other smaller molecules).

Their size and shape:

Owing to their size and particularly their shape, they greatly contribute to blood viscosity. The plasma protein fibrinogen is a significant contributor to blood viscosity. Due to the presence of polar residues on their surfaces.

- EACH PLASMA PROTEIN HAS A CHARACTERISTIC HALF-LIFE IN THE CIRCULATION:
- the half-life of the protein (the time for the activity to decline from its peak value to one-half of its peak value)
- Half life**:** Transferrin 8 days,albumin 20 days,haptoglobin 5days, IgE2 days
- In certain diseases, the half-life of a protein may be markedly altered.

For instance, in some gastrointestinal diseases such as regional ileitis (Crohn disease), considerable amounts of plasma proteins, including albumin, may be lost into the bowel through the inflamed intestinal mucosa. Patients

with this condition have a protein-losing gastroenteropathy**,** and the half-life of albumin in these subjects may be reduced to as little as 1 day.

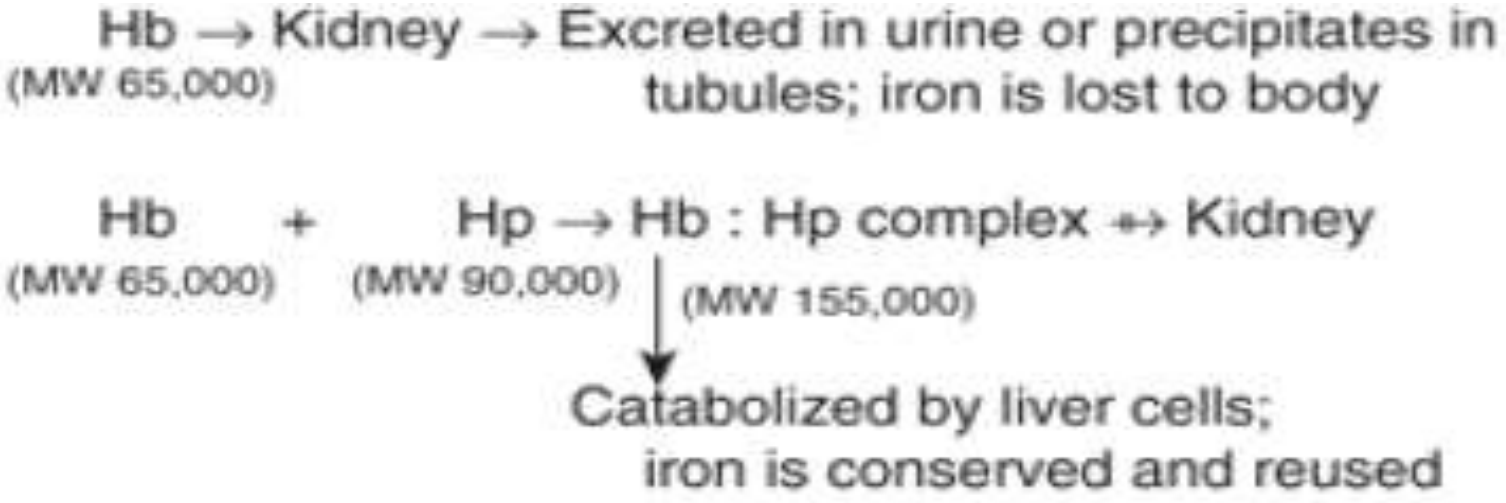
The level of certain proteins in plasma increase during acute inflammatory states or secondary to certain types of tissue damage

- These proteins are called "acute-phase proteins" and include C-reactive protein α1 -antitrypsin, haptoglobin, α1 -acid glycoprotein,and fibrinogen.
- The elevations of the levels of these proteins vary from as little as 50% to as much as 1000-fold in the case of CRP.
- Their levels are also usually elevated during chronic inflammatory states and in patients with cancer.
- These proteins are believed to play a role in the body's response to inflammation. For example, C-reactive protein can stimulate the classic complement pathway, and α1 -antitrypsin can neutralize certain proteases released during the acute inflammatory state.
- CRP is used as a marker of tissue injury, infection, and inflammation,

and there is considerable interest in its use as a predictor of certain types of cardiovascular conditions secondary to atherosclerosis.

Haptoglobin Binds Extracorpuscular Hemoglobin, Preventing Free Hemoglobin from Entering the Kidney:

- Haptoglobin (Hp) is a plasma glycoprotein that binds extracorpuscular hemoglobin (Hb) in a tight noncovalent complex (Hb-Hp).
- Approximately 10% of the hemoglobin that is degraded each day is released into the circulation and is thus extracorpuscular.
- Free hemoglobin passes through the glomerulus of the kidney, enters the tubules, and tends to precipitate within.
- The Hb-Hp complex is too large to pass through the glomerulus.
- The function of Hp thus to prevent loss of free hemoglobin into the kidney. This preserves the valuable iron present in hemoglobin.



- The levels of haptoglobin in human plasma vary and are of some diagnostic use. Low levels of haptoglobin are found in patients with hemolytic anemias. This is explained by the fact that whereas the half-life of haptoglobin is pproximately 5 days, the half-life of the Hb-Hp complex is about 90 min, the complex being rapidly removed from plasma by hepatocytes.
- Thus, when haptoglobin is bound to hemoglobin, it is cleared from the plasma about 80 times faster than normally. Accordingly, the level of haptoglobin falls rapidly in situations where hemoglobin is constantly being released from red blood cells, such as occurs in hemolytic anemias.

Transferrin

- Transferrin (Tf) is a plasma protein that plays a central role in transporting iron around the body to sites where it is needed.
- Iron is important in the human body because of its occurrence in many hemoproteins such as hemoglobin, myoglobin, and the cytochromes.
- Transferrin is a $\beta 1$ –globulin, It is a glycoprotein and is synthesized in the liver. About 20 polymorphic forms of transferrin have been found. It plays a central role in the body's metabolism of iron because it transports iron (2 mol of Fe3+ per mole of Tf) in the circulation to sites where iron is required, eg, from the gut to the bone marrow and other organs. Approximately 200 billion red blood cells (about 20 mL) are catabolized per day, releasing about 25 mg of iron into the body—most of which will be transported by transferrin.

Ferritin Stores Iron in Cells

- Ferritin is another protein that is important in the metabolism of iron. Under normal conditions, it stores iron that can be called for use as conditions require. In conditions of excess iron (eg, hemochromatosis), body stores of iron are greatly increased and much more ferritin is present in the tissues, such as the liver and spleen.

Page 1125

Plasma proteins electrophoresis for diagnosis of different disorders:

- Plasma protein electrophoresis is used to identify patients with multiple myeloma and other plasma protein disorders.
- Electrophoresis separates proteins based on their physical properties, and the subgroups of these proteins are used in understanding the results.
- Plasma protein levels show practically certain changes in response to acute inflammation, malignancy, trauma, necrosis, infarction, burns, and chemical injury.

Plasma Protein Electrophoresis and Associated Conditions or Disorders

Increased albumin

Dehydration

Increased $\alpha 1$ globulins

Pregnancy

Increased $\alpha 2$ globulins

Adrenal insufficiency

Adrenocorticosteroid therapy

Nephrotic syndrome

Increased $\beta 1 or \beta 2$ globulins

Biliary cirrhosis

Cushings disease

Hypothyroidism

Iron deficiency anemia

Obstructive jaundice

Increased γ globulins

Cirrhosis

Malignant lymphoma

Multiple myeloma

Decreased albumin

Chronic infections

Hemorrhage, burns, or protein-losing

Entero pathies

Impaired liver function resulting from decreased synthesis of albumin

Malnutrition

Nephrotic syndrome

Pregnancy

Decreased $\alpha 1$ globulins

Alpha 1-antitrypsin deficiency

Decreased $\alpha 2$ globulins

Malnutrition

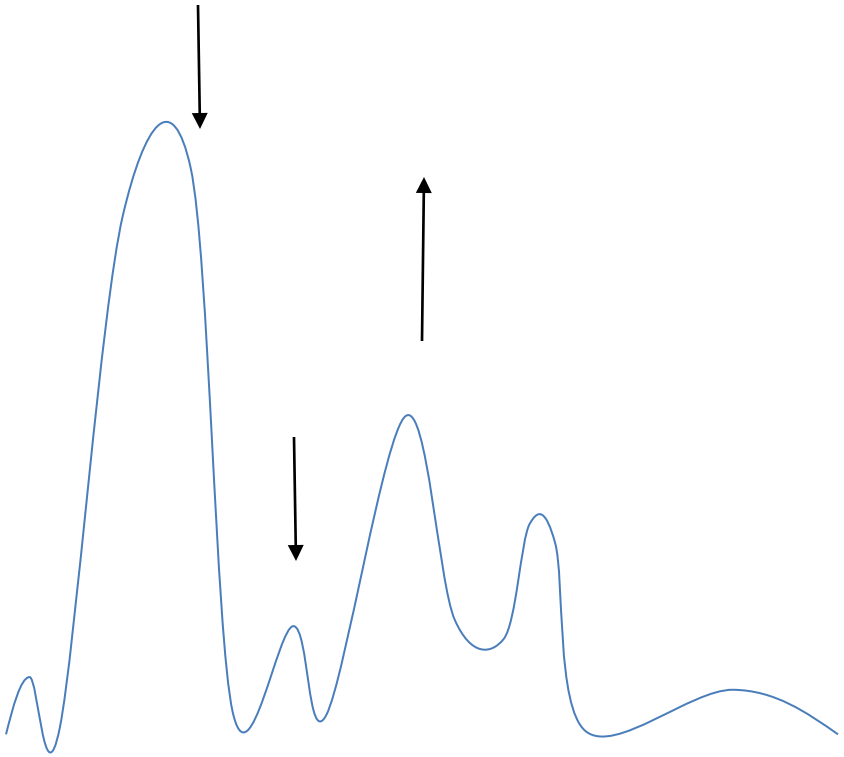
Megaloblastic anemia

Decreased $\beta 1 or \beta 2$ globulins

Protein malnutrition

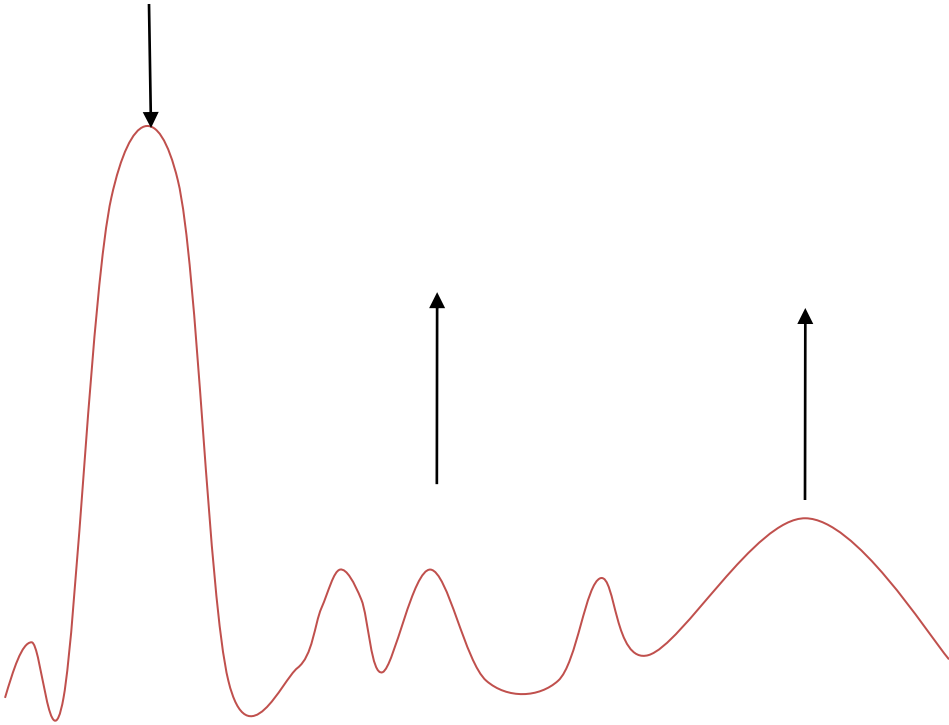
Acute inflammation

↓ Albumin
↓ α_1 – globulin
↑ α_2 – globulin
n γ – globulin



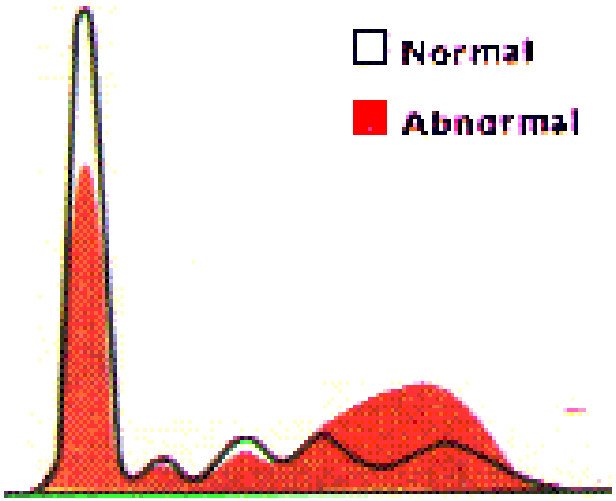
Chronic inflammation

↓ Albumin
n α_1 - globulin
↑ α_2 – globulin
n β – globulin
↑ γ – globulin



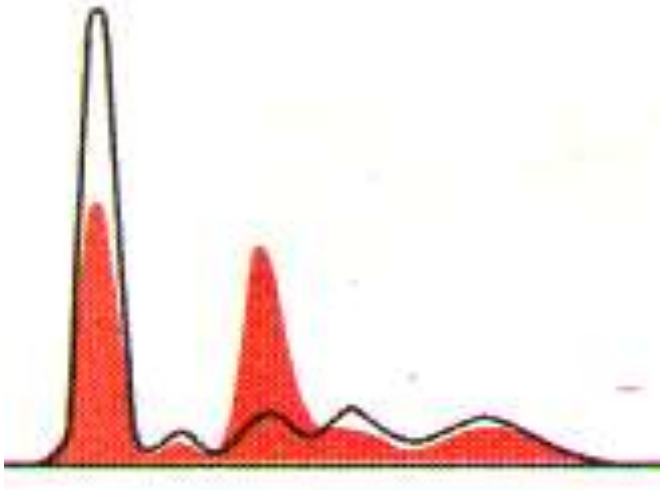
Liver cirrhosis

↓↓ Albumin
n α_1 - globulin
n α_2 – globulin
n β – globulin
↑↑ γ – globulin



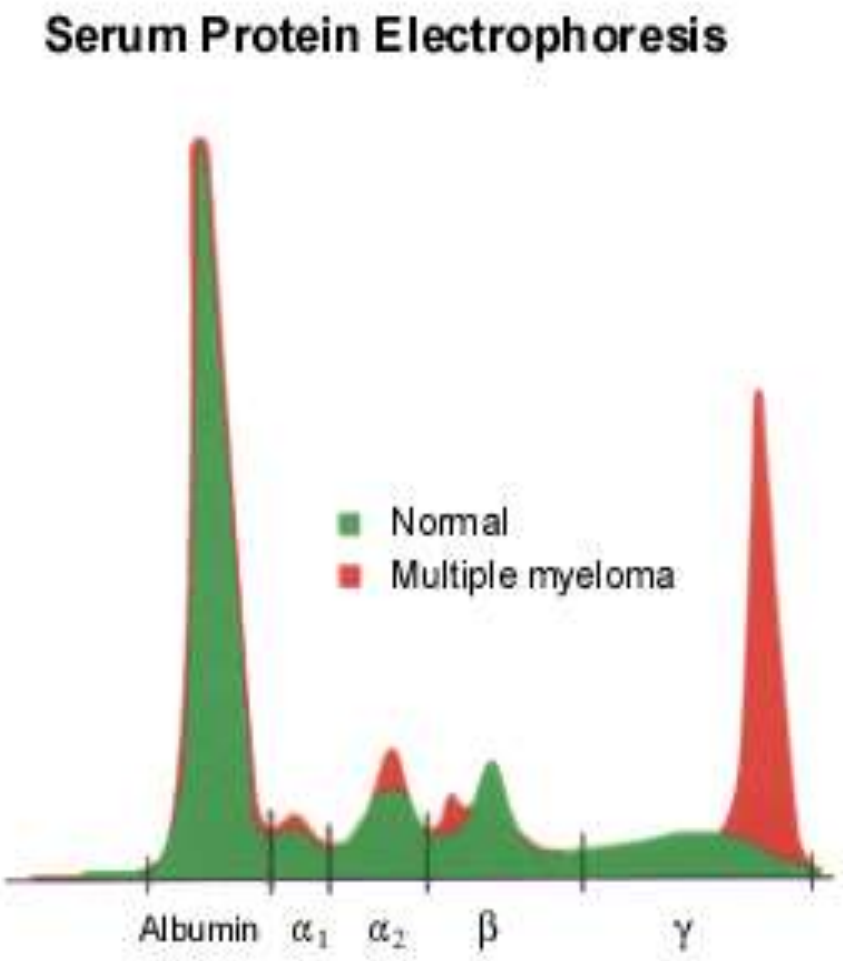
Nephrotic syndrome

↓↓ Albumin
n α_1 - globulin
↑↑ α_2 – globulin
↑↑ β – globulin
↓ γ – globulin



Multiple Myeloma

- B-cell malignancy characterised by abnormal proliferation of plasma cells able to produce a monoclonal immunoglobulin (M protein) characterized by the presence of a sharp, well defined band.

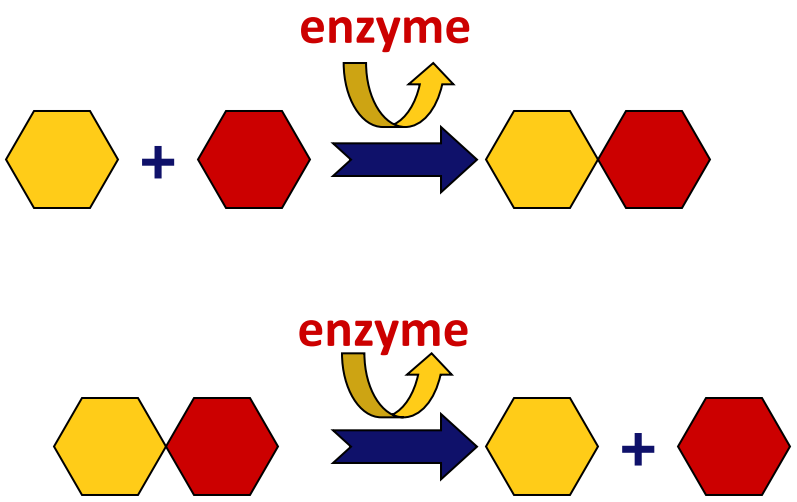


Enzymes

Inaam Ahmed Ameen

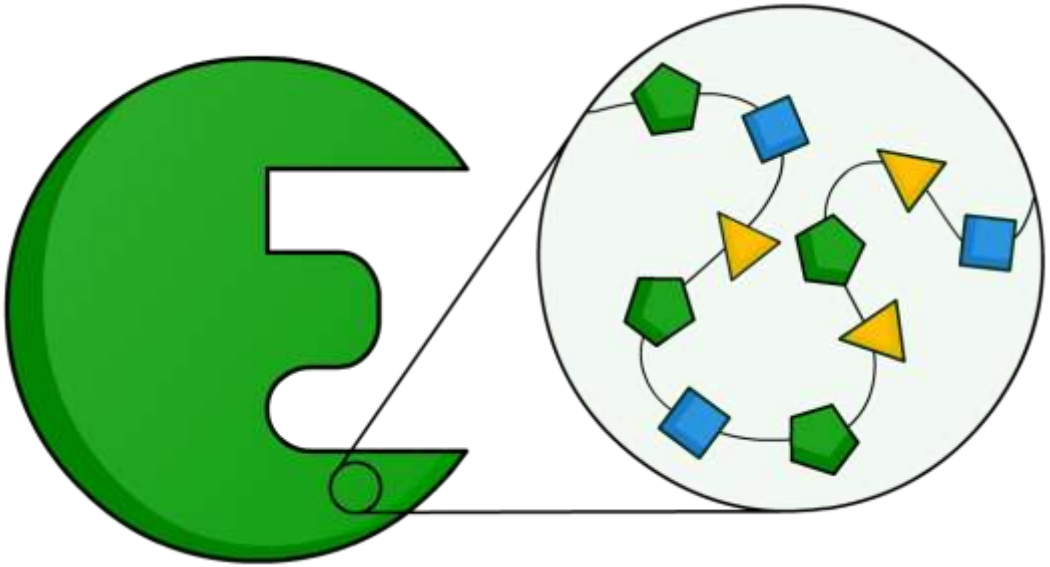
Enzymes are important for Life

- all chemical reactions in living organisms require enzymes to work
  - building molecules
    - synthesis enzymes
  - breaking down molecules
    - digestive enzymes
- enzymes speed up reactions
  - "catalysts"



Enzymes are protein molecules, and so are made up of amino acids. Most enzymes contain between 100 and 1,000 amino acids

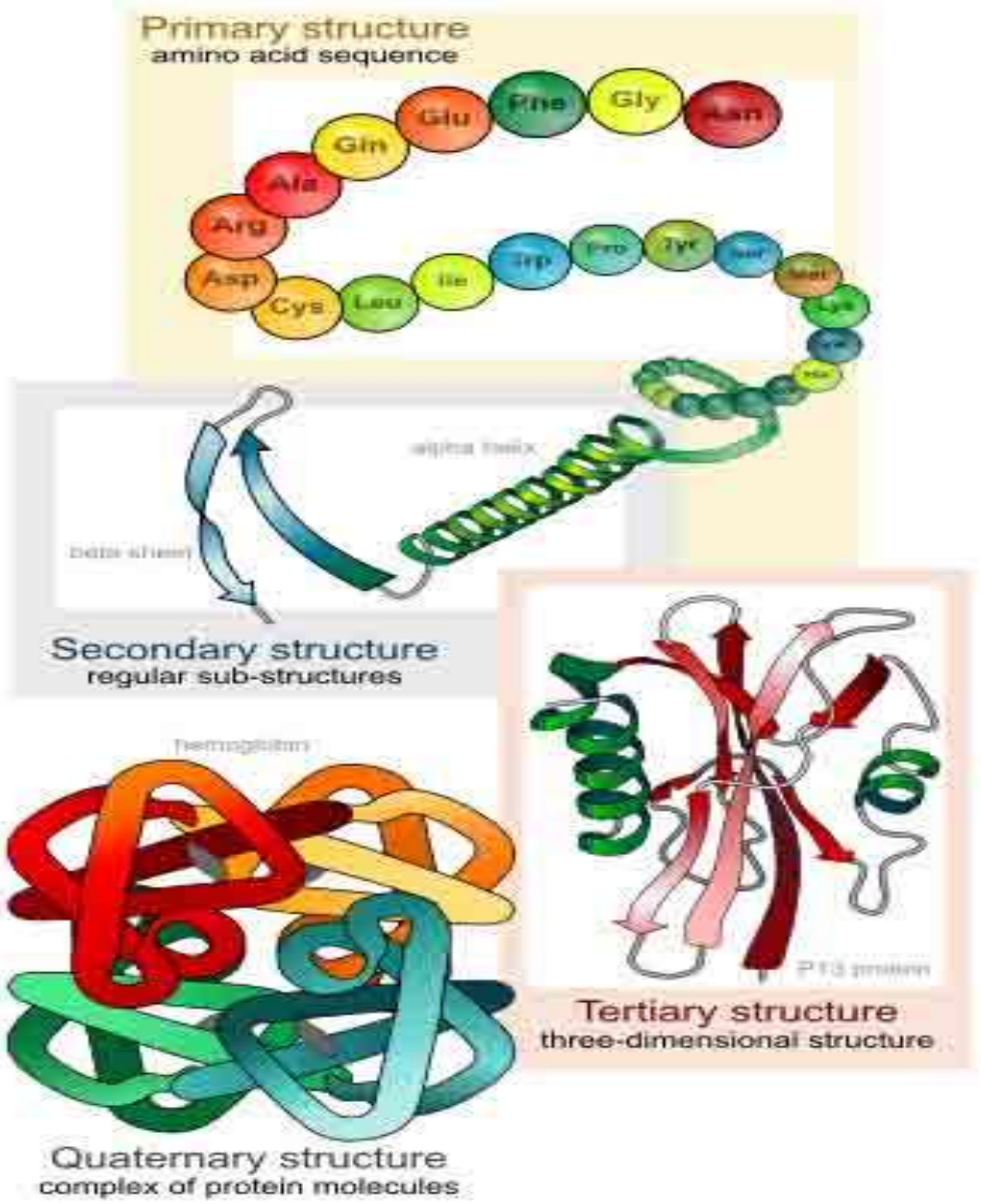
These amino acids are joined together in a long chain, which is folded to produce a unique 3D structure.



Different types of enzymes have different shapes and functions because the sequence and type of amino acids in their structure is different.

Enzymes are tertiary and quaternary structures

- Enzymes catalytic activity depends on the integrity of their native protein conformation.
- If the enzyme is denaturated, dissociated or broken down in to its component amino acids its catalytic activity is destroyed



Enzymes

- Enzymes act as biological catalysts. Catalyze the convertion of one or more compounds(substrates) in to one or more different compounds(products).

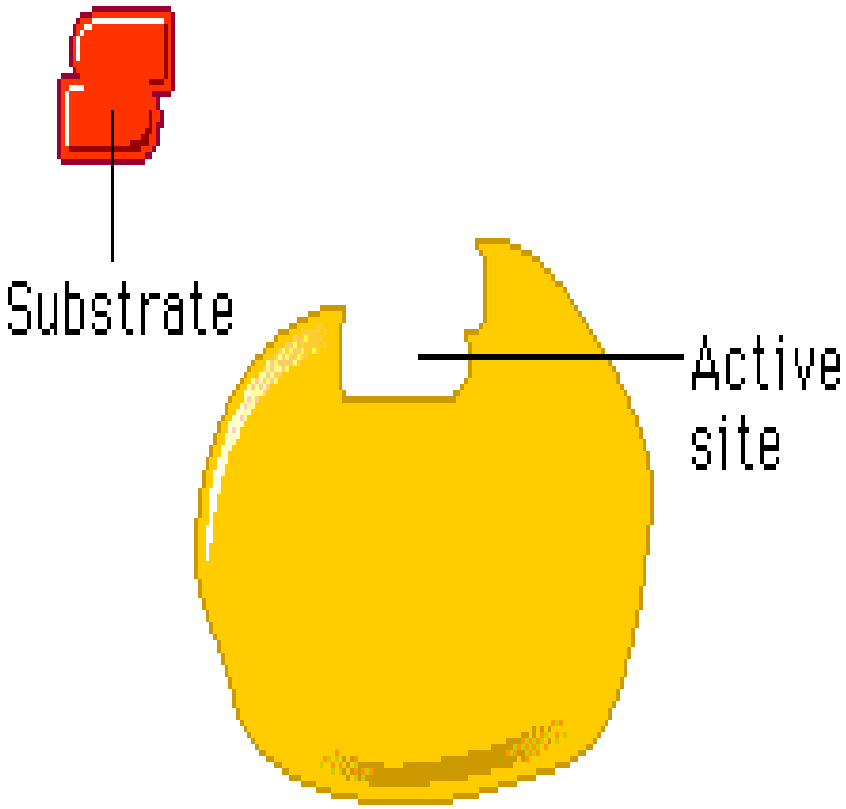
- cells use enzymes to speed up chemical reactions that take place in cells.

- Enzyme speed up reactions by lowering the activation energies.

- Because a particular enzyme catalyzes only one reaction, there are thousands of different enzymes in a cell catalyzing thousands of different chemical reactions

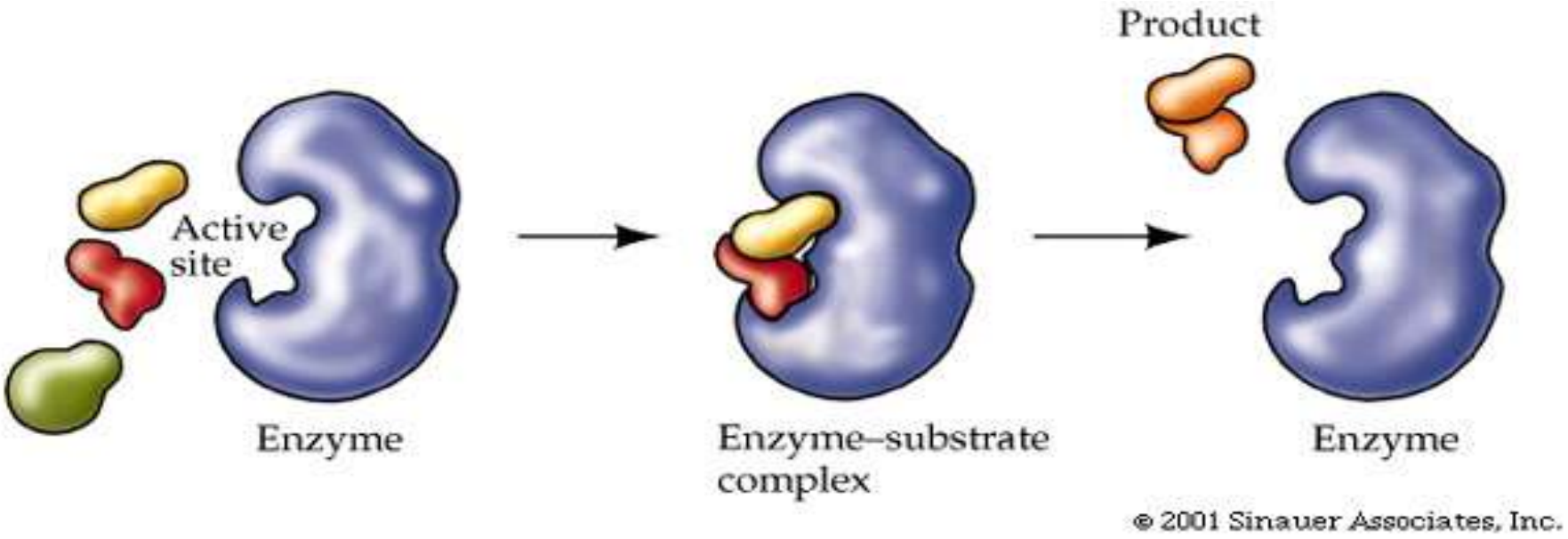
The Enzyme Substrate complex

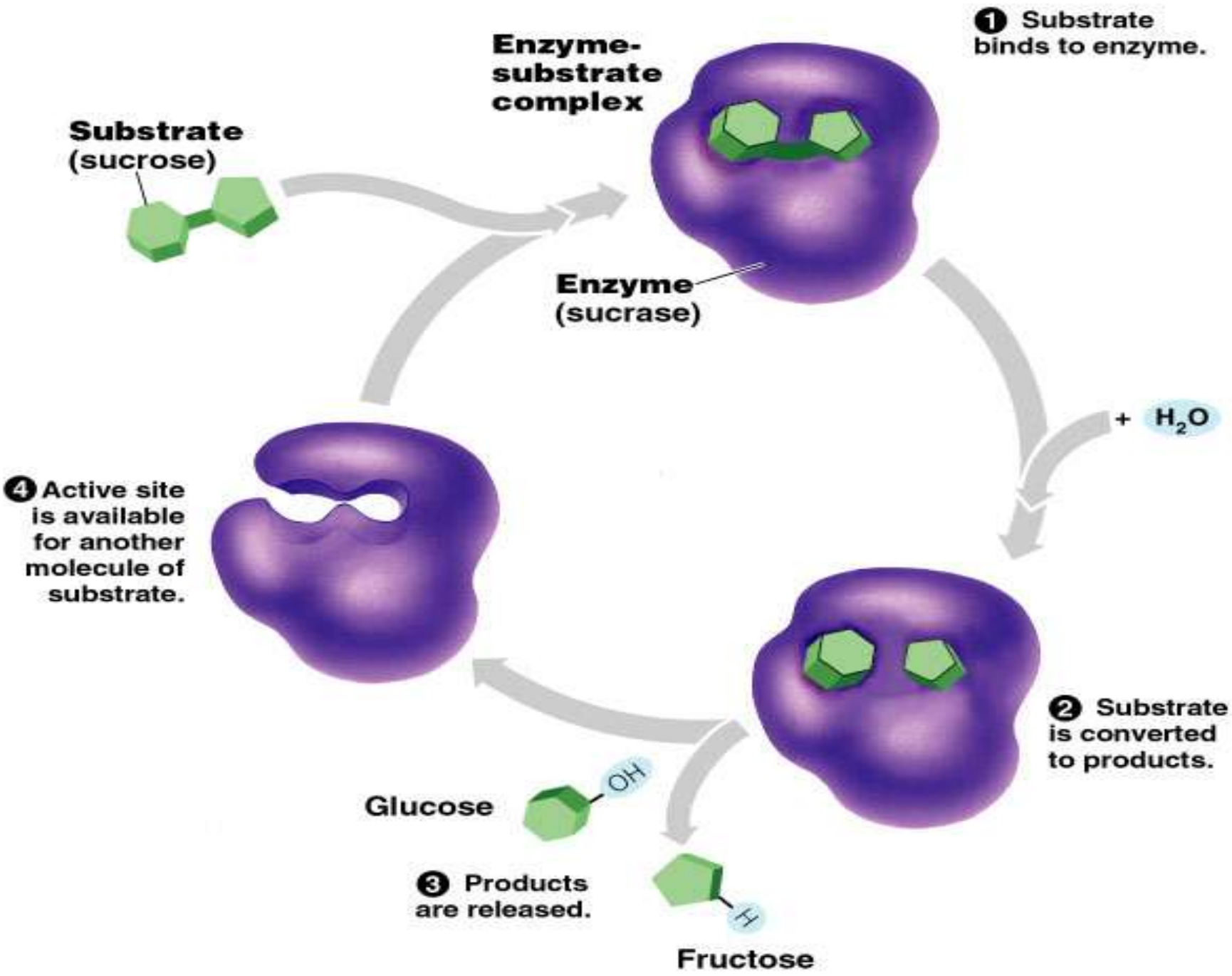
- When enzymes function the active site interacts with the substrate.
- The active site shape matches the substrates shape.
- Once the substrate and active site meet a change in shape of the active site causes a stress that changes the substrate and produces an end product.



All enzymes have an active site, where substrates are attracted to.

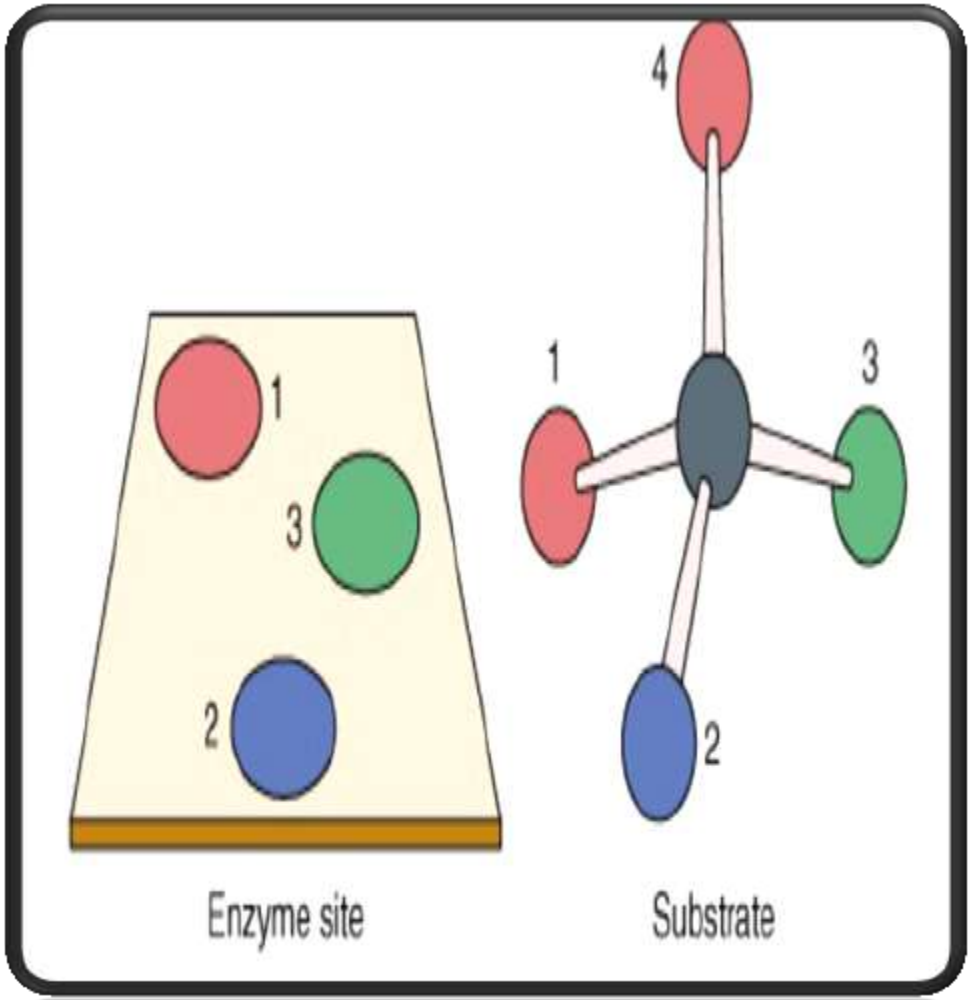
- Enzymes are used over and over again.





General properties of enzymes

- Unlike most catalysts used in synthetic chemistry, enzymes are specific both for
  1. Type of reaction
  2. a single substrate or a small set of closely related substrates
- Enzymes are also stereospecific catalysts and typically catalyze reactions of only one stereoisomer of a given compound—for example, D- but not L-sugars, L- but not D-amino acids. Since they bind substrates through at least "three points of attachment.



Enzyme Nomenclature

Each enzyme is given two names:

1. The first is its short, **Recommended Name**, convenient for everyday use.
2. The second is the more complicated **Systematic Name**, which is used when an enzyme must be identified without doubtness.

Recommended Name

Recommended Name :

- The commonly used names for most enzymes describe the type of reaction catalyzed, followed by the suffix *-ase.* For example:
  - *dehydrogen**ase**s remove hydrogen atoms*
  - *prote**ase**s* hydrolyze proteins
  - isomer**ase**s catalyze rearrangements in configuration.
- Modifiers may precede the name to indicate:
  - the substrate (*xanthine oxidase)*
  - *the source of the enzyme* (*pancreatic ribonuclease)*
  - *its regulation (hormone-sensitive lipase)*
- Alphanumeric designators are added to identify multiple forms of an enzyme (eg, RNA polymerase *III; protein kinase C).*

Systematic Name

- The International Union of Biochemists (IUB) developed a clear system of enzyme nomenclature in which each enzyme has a unique name and code number that identify the type of reaction catalyzed and the substrates involved.
- Despite the clarity of the IUB system, the names are relatively cumbersome, so we generally continue to refer to enzymes by their traditional names.
- In this system Enzymes are grouped into the following six classes.

Enzyme classes

1. **Oxidoreductases:**

   Enzymes that catalyze oxidations and reductions.

2. **Transferase:**

   Enzymes that catalyze transfer of moieties such as glycosyl, methyl, or phosphoryl groups.

3. **Hydrolases:**

   Enzymes that catalyze *hydrolytic cleavage of C—C, C—O, C—N and other covalent bonds.*

4. **Lyases:**

   Enzymes that catalyze cleavage of C—C, C—O, C—N and other covalent bonds by *atom elimination, generating double bonds.*

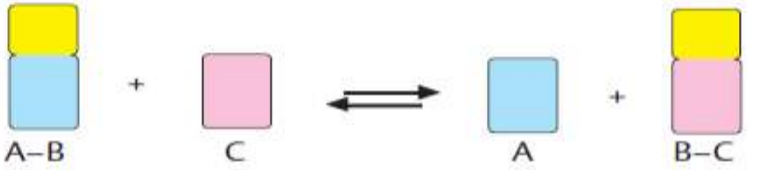
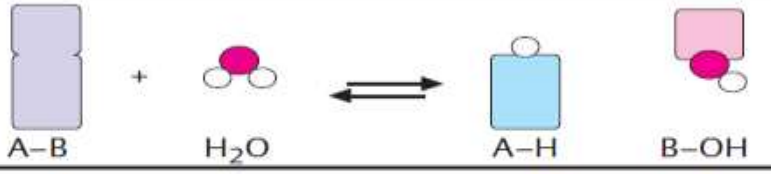
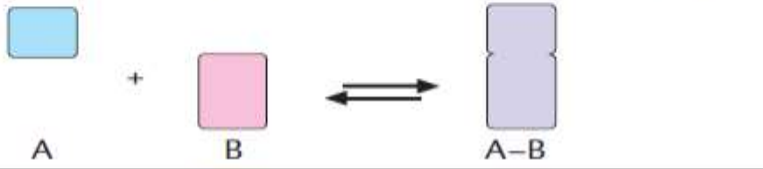
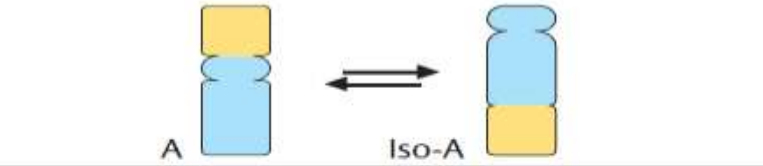
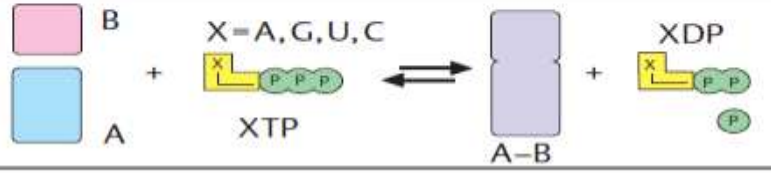
5. **Isomerases:**

   Enzymes that catalyze geometric or structural changes *within a molecule.*

6. **Ligases:**

   Enzymes that catalyze the joining together (ligation) of two molecules in reactions coupled to the hydrolysis of ATP.

C. The enzyme classes

Class	Reaction type	Important subclasses
1 Oxidoreductases	○ = Reduction equivalent  	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	 	C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	 	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	 	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	 	Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")	 	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

Example:

- The IUB name for hexokinase illustrates both the clarity of the IUB system and its complexities.
- The IUB name of hexokinase is :

ATP:D-hexose 6-phosphotransferase E.C.2.7.1.1.

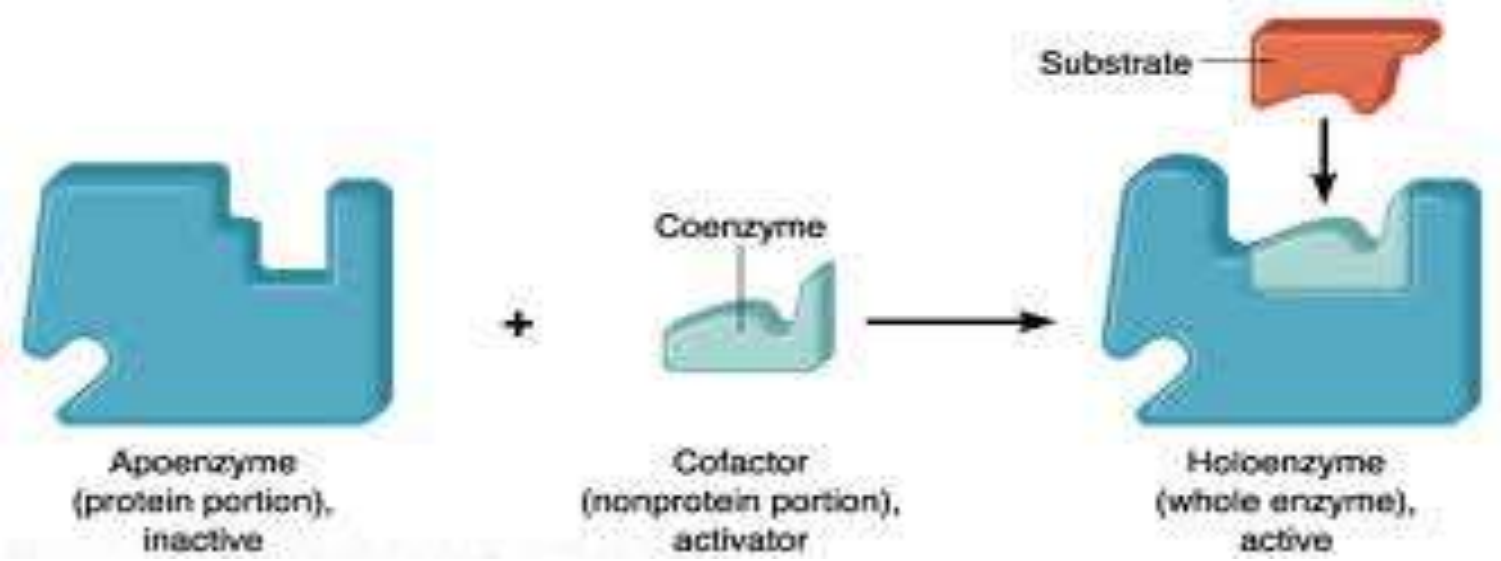
  - This name identifies hexokinase as a member of class 2 (transferases)
  - subclass 7 (transfer of a phosphoryl group)
  - subsubclass 1 (alcohol is the phosphoryl acceptor)
  - "hexose-6" indicates that the alcohol phosphorylated is on carbon six of a hexose

Practical importance of enzymes

- In some inherited diseases there may be a deficiency or even a total absence of one or more enzymes
- The measurements of the activities of enzyme in blood, plasma, erythrocytes or tissue samples are important in diagnosting certain diseases
- Many drugs exert their biological effects through interactions with enzymes
- Enzymes are important practical tools in chemical industry, food processing and agriculture

APOENZYME and HOLOENZYME

- The enzyme without its non protein moiety is termed as apoenzyme (proenzyme or zymogen)and it is inactive.
- Holoenzyme is an active enzyme with its non protein component.



Important Terms to Understand Biochemical Nature And Activity of Enzymes

- **Cofactor:**
  - A cofactor is a non-protein chemical compound that is bound (either tightly or loosely) to an enzyme and is required for catalysis.
  - Types of Cofactors:
    - Coenzymes.
    - Prosthetic groups.

Types of Cofactors

- **Coenzyme:**
  The non-protein component, loosely bound to apoenzyme by non-covalent bond.
- **Examples : vitamins or compound derived from vitamins.**
- **Prosthetic group**
  The non-protein component, tightly bound to the apoenzyme by covalent bonds is called a Prosthetic group.

Enzyme Specificity

- Enzymes have different types of specificity
- Enzymes specificity subdivided to

  - Bond specificity

  -Group specificity

  -Substrate specificity

  -Optical or stereo specificity

  -Geometrical specificity

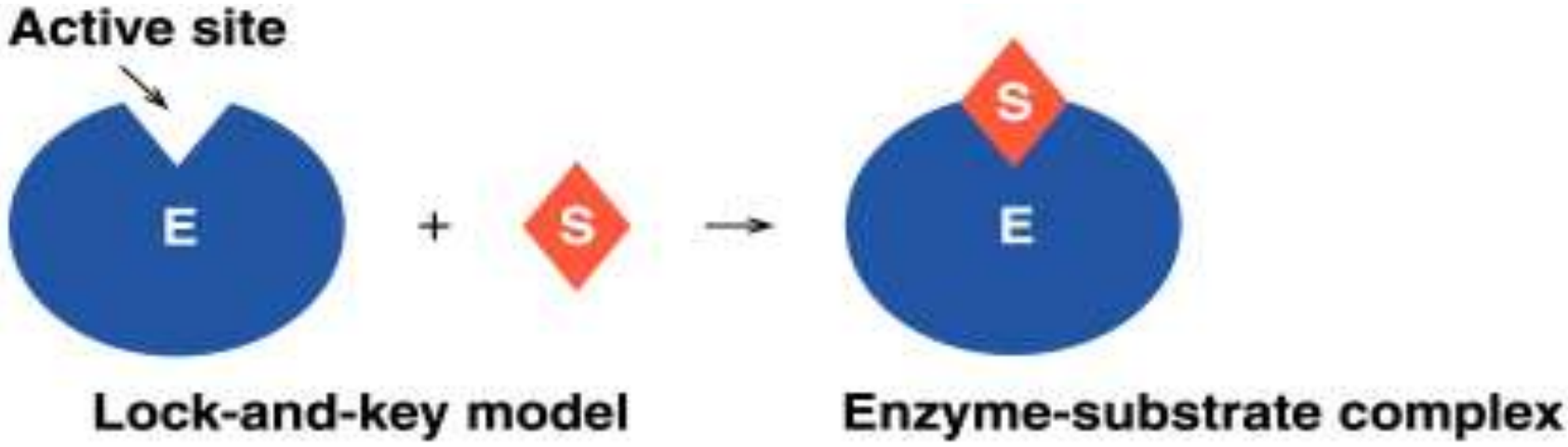
  -Co-factor specificity

Mechanism of Action of Enzymes

- Enzymes increase reaction rates by decreasing the Activation energy:
- Enzyme-Substrate Interactions:
  - Formation of Enzyme substrate complex by:
  - Lock-and-Key Model
  - Induced Fit Model

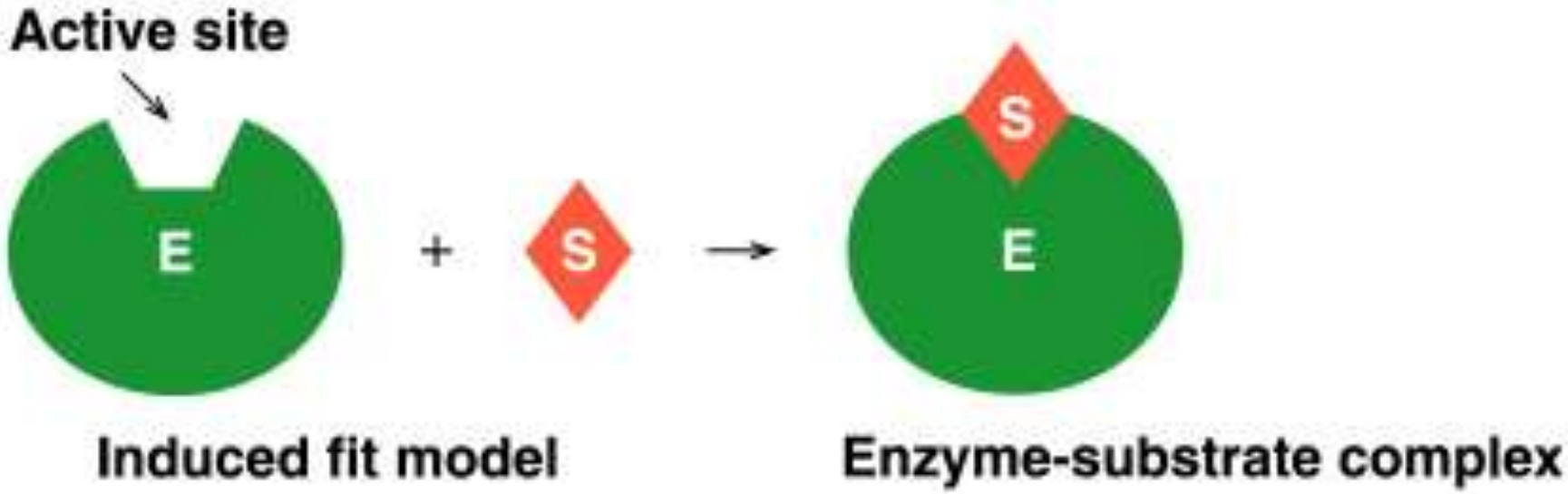
Lock-and-Key Model

- In the lock-and-key model of enzyme action:
  - the active site has a rigid shape
  - only substrates with the matching shape can fit
  - the substrate is a key that fits the lock of the active site
- This is an older model, however, and does not work for all enzymes



Induced Fit Model

- In the induced-fit model of enzyme action:
  - the active site is flexible, not rigid
  - the shapes of the enzyme, active site, and substrate adjust to maximumize the fit, which improves catalysis
  - there is a greater range of substrate specificity
- This model is more consistent with a wider range of enzymes



Enzyme catalytic mechanisms

Mechanisms employed by enzymes to facilitate catalysis

Catalysis by proximity

Acid –Base catalysis

Covalent catalysis

Metal ion catalysis

- Additional free energy is obtained through the "Binding Energy" (binding of the substrate to the enzyme.)
- Binding energy often helps stabilize the transition state, lowering $\Delta G^{\ddagger}$.

1-Catalysis by proximity

- The higher the concentration of reacted molecules the more frequently they will encounter one another, and the greater will be the rate of their reaction.
- When an enzyme binds substrate molecules at its active site, it creates a region of high local substrate concentration.
- This environment also orients the substrate molecules spatially in a position ideal for them to interact, resulting in rate enhancements of at least thousand fold.
- Catalysis by proximity

2- Acid Base catalysis

- Many biochemical reactions involve the formation of unstable charged intermediates that tend to break down rapidly to their reactant species
- The charged intermediates can often be stabilized by the transfer of protons to or from the substrate or intermediate to form a species that breaks down more readily to products than reactants
- The ionizable functional groups of aminoacyl side chains and (where present) of prosthetic groups contribute to catalysis by acting as acids or bases(act as proton donors and acceptors)
- These groups can be positioned in an enzyme active site to allow proton transfers , providing rate enhancements of about 10^2– 10^3
- This type of catalysis is the most common one

3-Covalent Catalysis

- A transient covalent bond is formed between the enzyme and the substrate
- The functional groups of some enzymes cofactors can serve as nucleophiles in the formation of covalent bonds with substrates
- On completing the reaction the enzyme returns to it, s original unmodified state
- Covalent catalysis common with enzymes that catalyze group transfer reactions
- Residues on the enzyme that participate in covalent catalysis generally are cysteine or serine and occasionally histidine

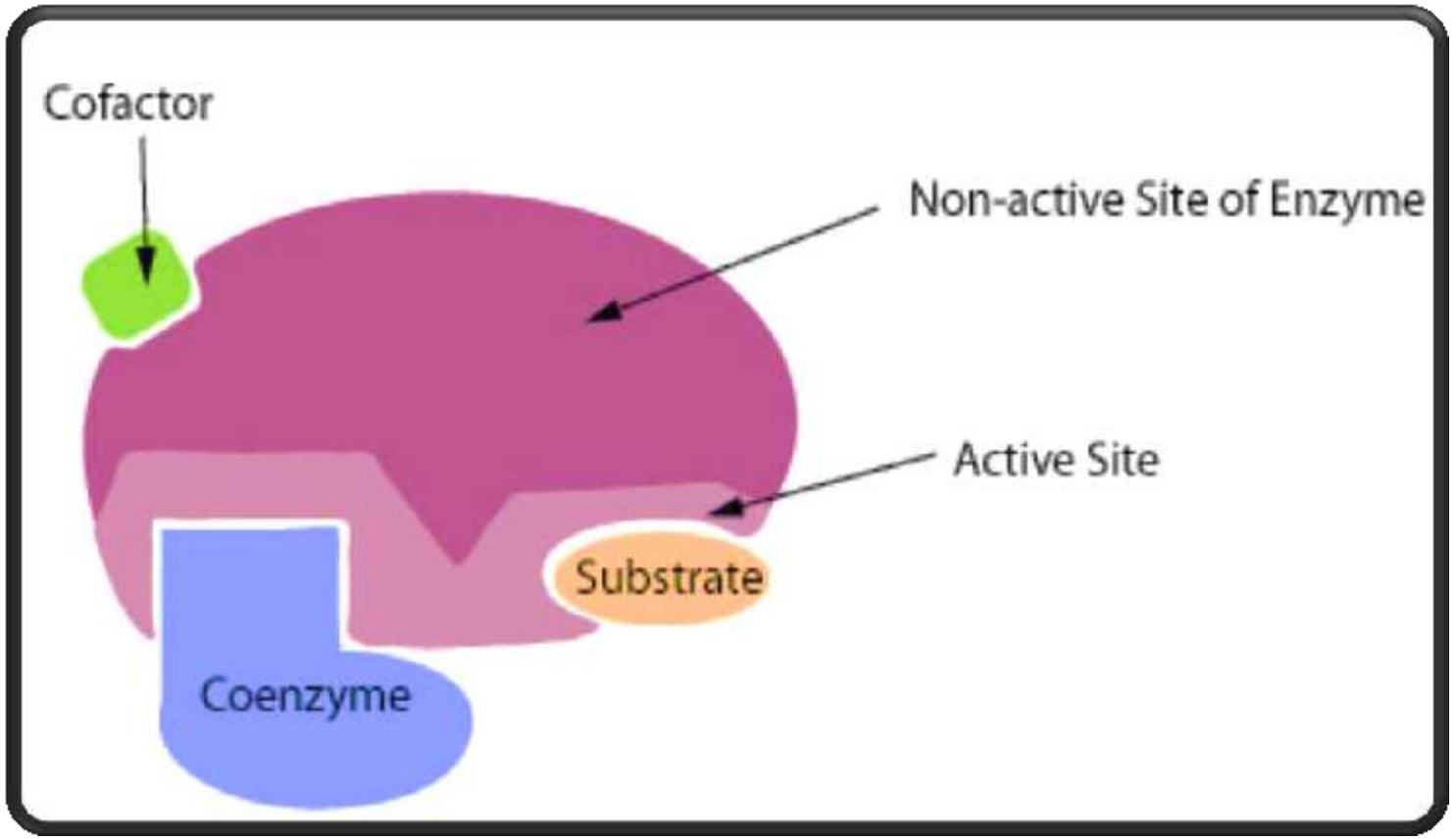
4-Metal ion catalysis

- Metals, whether tightly bound to the enzyme or taken up from solution a long with the substrate, can participate in catalysis in several ways
- Ionic interactions between an enzyme– bound metal and a substrate can help orient the substrate for reaction or stabilize charged reaction transition states
- Metals can also mediate oxidation - reduction reactions by reversible changes in the metal ions oxidation state
- Nearly a third of all known enzymes required one or more metal ions for catalytic activity
- Most of enzymes employ a combination of several catalytic mechanisms to bring about a rate enhancement , example: chymotrypsin enzyme in which both covalent & acid-base catalysis are employed

Enzyme Lec (10)

By: Inaam Ahmed Ameen

Enzyme structure components

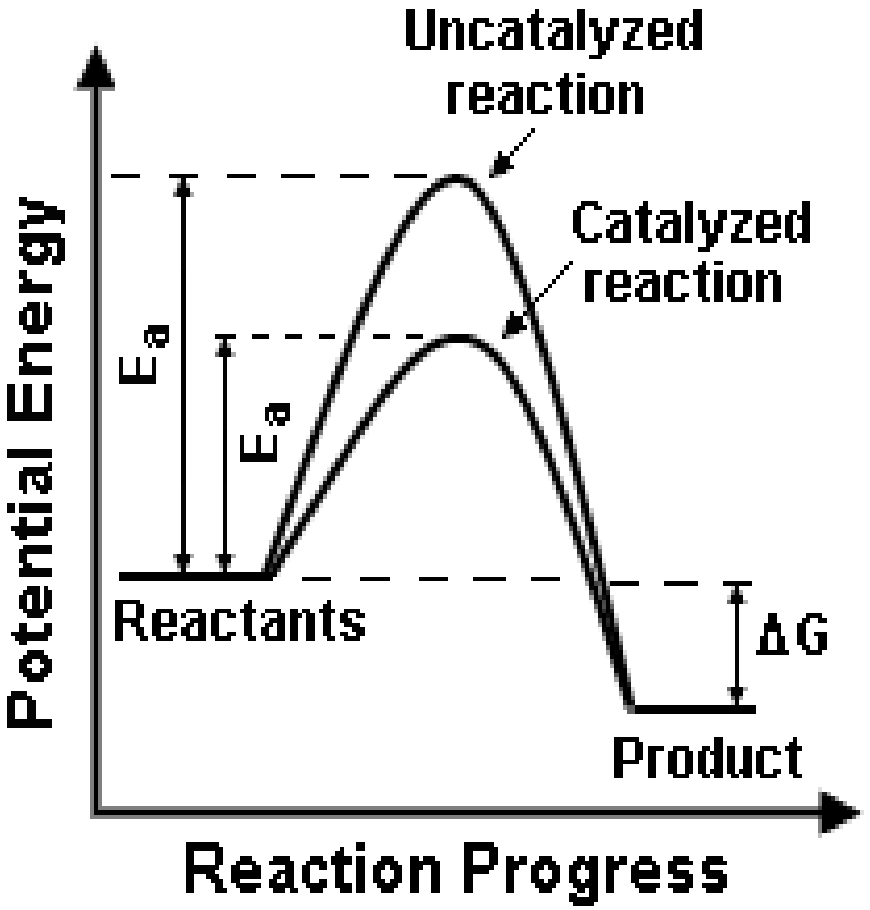


CHANGES IN FREE ENERGY DETERMINE THE DIRECTION &EQUILIBRIUM STATE OF CHEMICAL REACTIONS

- The Gibbs free energy change G (also called either the free energy or Gibbs energy) describes both the *direction* in which a chemical reaction will tend to proceed and the concentrations of reactants and products that will be present at equilibrium.
- G for a chemical reaction equals the sum of the free energies of formation of the reaction products Gp minus the sum of the free energies of formation of the substrates Gs.

Activation energy

- ***activation energy*** is the energy that must be overcome in order for a chemical reaction to occur.
- Activation energy may also be defined as the minimum energy required to start a designated chemical reaction. It is denoted by E_a in units of kilojoules per mole (kJ/mol).
- It may be considered as the "energy barrier" that must be overcome to start a chemical reaction.
- The speed of reaction depends very steeply (sharply) on the height of the energy (also known as transition) barrier.
- A small lowering of the transition barrier can yield a drastic increase in speed.

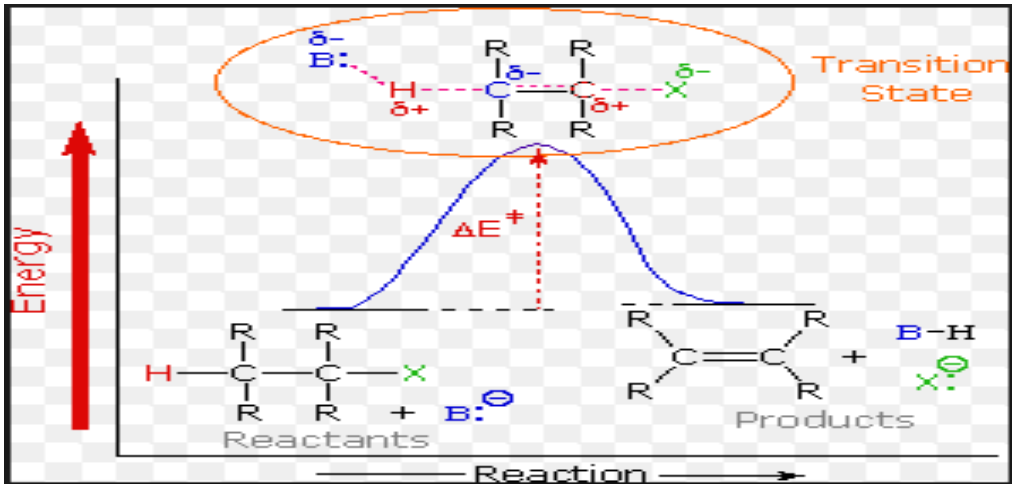


ΔG is the change in Gibbs free energy between the reactants and the product

E_a is the activation energy for each of of the two reaction paths

Transition state

- In a chemical reaction, the **transition state** is the point where there is a maximum value of energy.
- This energy is called the activation energy. When two or more molecules are mixed, they will hit each other. If they hit with enough energy to go through the transition state, they will react and form new molecules.
- At the transition state, new bonds are formed while the old ones are broken. In a graph or a drawing, the transition state is often marked with the double dagger ‡ symbol.
- Bond breaking and formation require energy, which is the reason that reactions have an **activation energy** to overcome.
- Activation energy is the minimum amount of energy needed for a reaction to occur.

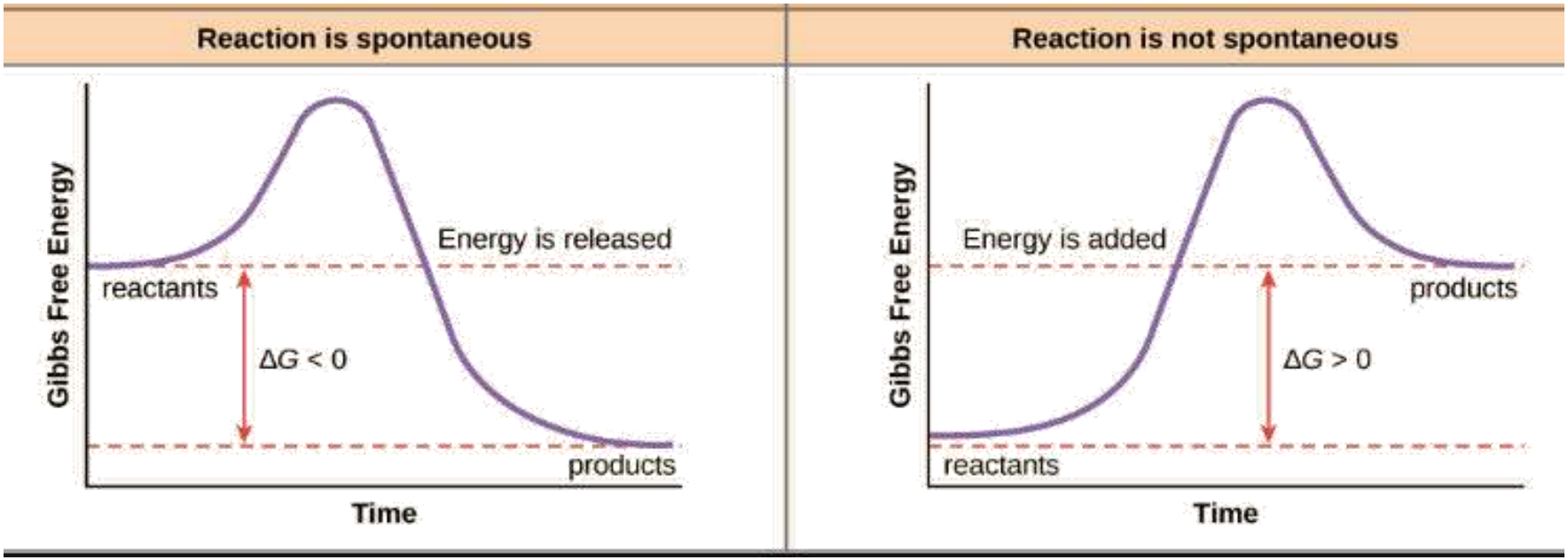


The presence of the catalyst provides an alternative reaction path, which is definitely more complex because it involves the catalyst, but energetically much more favorable.

The activation energy of the catalyzed reaction is significantly smaller than that of the uncatalyzed reaction. Given its exponential dependence on activation energy, the rate of the catalyzed reaction is much higher.

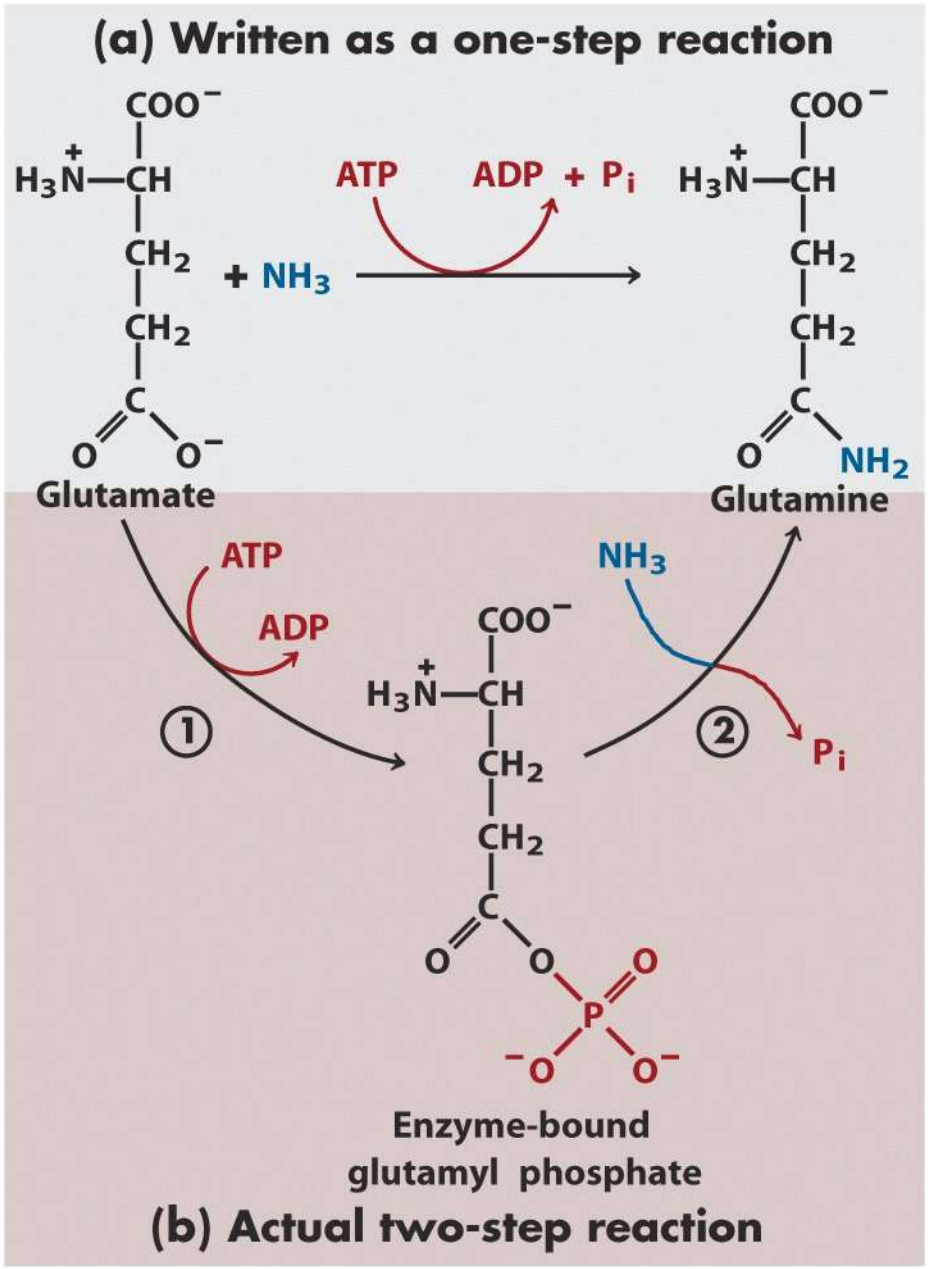
Since the overall change in Gibbs free energy is the same for the catalyzed reaction as for the uncatalyzed reaction, the reaction equilibrium constant (which is a function only of the Gibbs free energies of reactants and products) is not affected by the catalyst. As noted above, the catalyst does not change the chemical thermodynamics of the reaction.
turn over frequency (TOF):
Moles of product formed per second per mole of catalyst
Moles of reactants converted per second per active site
In both of the above definitions, the unit of time is sometimes designated as an hour rather than a second.

Spontaneous and nonspontaneous reactions



- This type of graph is called a reaction coordinate diagram. In the case of Spontaneous reaction, the figure indicates two key things: (1) the difference between the free energy of the reactants and products is negative and (2) the progress of the reaction requires some input of free energy (shown as an energy hill). Reactions that have a negative ΔG are termed exergonic reactions. These reactions are said to occur spontaneously

- A chemical reaction with a positive ΔG means that the products of the reaction have a higher free energy than the reactants.
- These chemical reactions are called endergonic reactions, and they are NOT spontaneous. An endergonic reaction will not take place on its own without the transfer of energy into the reaction.
- The building of complex molecules, such as sugars, from simpler ones is an anabolic process and is endergonic. On the other hand, the catabolic process, such as the breaking down of sugar into simpler molecules, is generally exergonic.
- the equilibrium constant *K* is a value defining the ratio of the concentrations of the products to the concentrations of the reactants in a reversible chemical reaction, at the point when the reactants and products reach steady -state values, a point otherwise known as equilibrium.
- Equilibrium in a chemical reaction is the state in which both reactants and products are present in concentrations that have no further tendency to change with time. Usually, this state results when the forward reaction proceeds at the same rate as the reverse reaction.
- But it does not mean that there is no interconversion between substrates and products—it means that when the reactant(s) are converted to product(s) that product(s) are converted to reactant(s) at an equal rate



Provide Free Energy

Enzyme Catalyzed Reactions

- When a substrate (S) fits properly in an active site, an **enzyme-substrate (ES) complex** is formed:

$$E + S \leftrightarrows ES$$

- Within the active site of the ES complex, the reaction occurs to convert substrate to product (P):

$$ES \rightarrow E + P$$

- The products are then released, allowing another substrate molecule to bind the enzyme

  - this cycle can be repeated millions (or even more) times per minute

- The overall reaction for the conversion of substrate to product can be written as follows:

$$E + S \leftrightarrows ES \rightarrow E + P$$

Factors affecting the rates of enzyme—catalyzed reactions:

- substrate concentration
- Enzyme concentration
- Temperature
- PH
- Physical agents
- Activators
- inhibitors

Substrate concentration

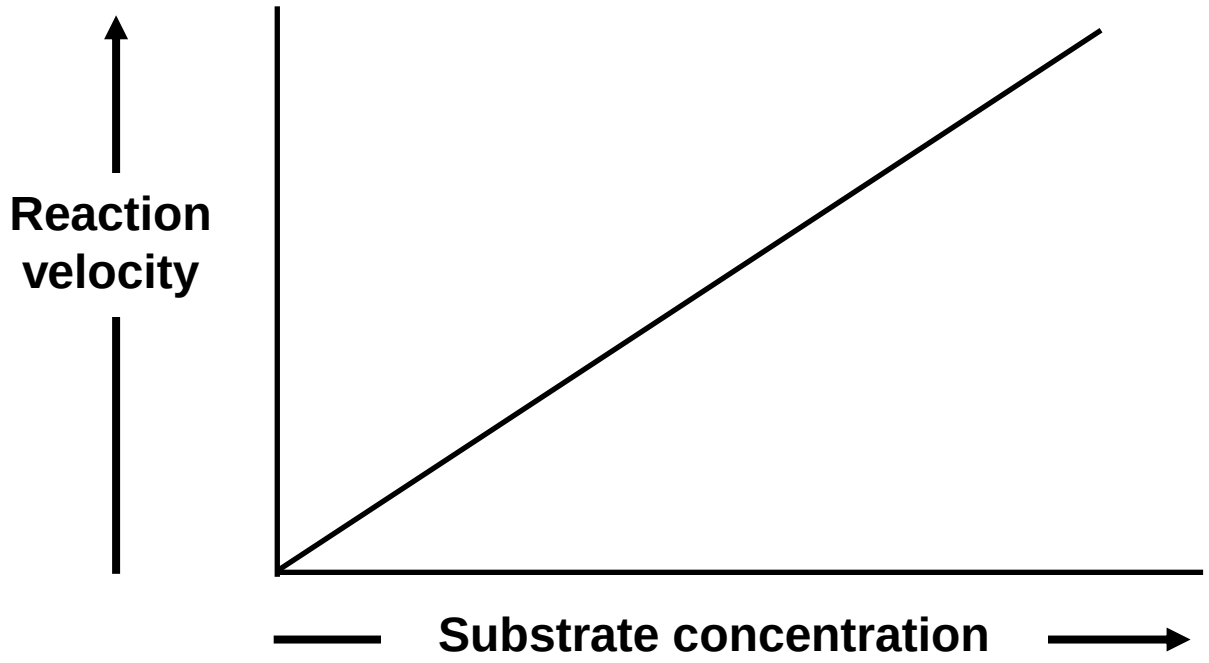
The relationship between substrate concentration and rate of an enzymatic reaction is represented by a **hyperbolic curve**.

a) At low substrate concentrations the rate of the enzymatic reaction is **directly proportional** to the substrate concentration (since many active sites are available for the substrate to bind to).

b) At medium substrate concentrations the relationship deviates from linearity, since less active sites are available .

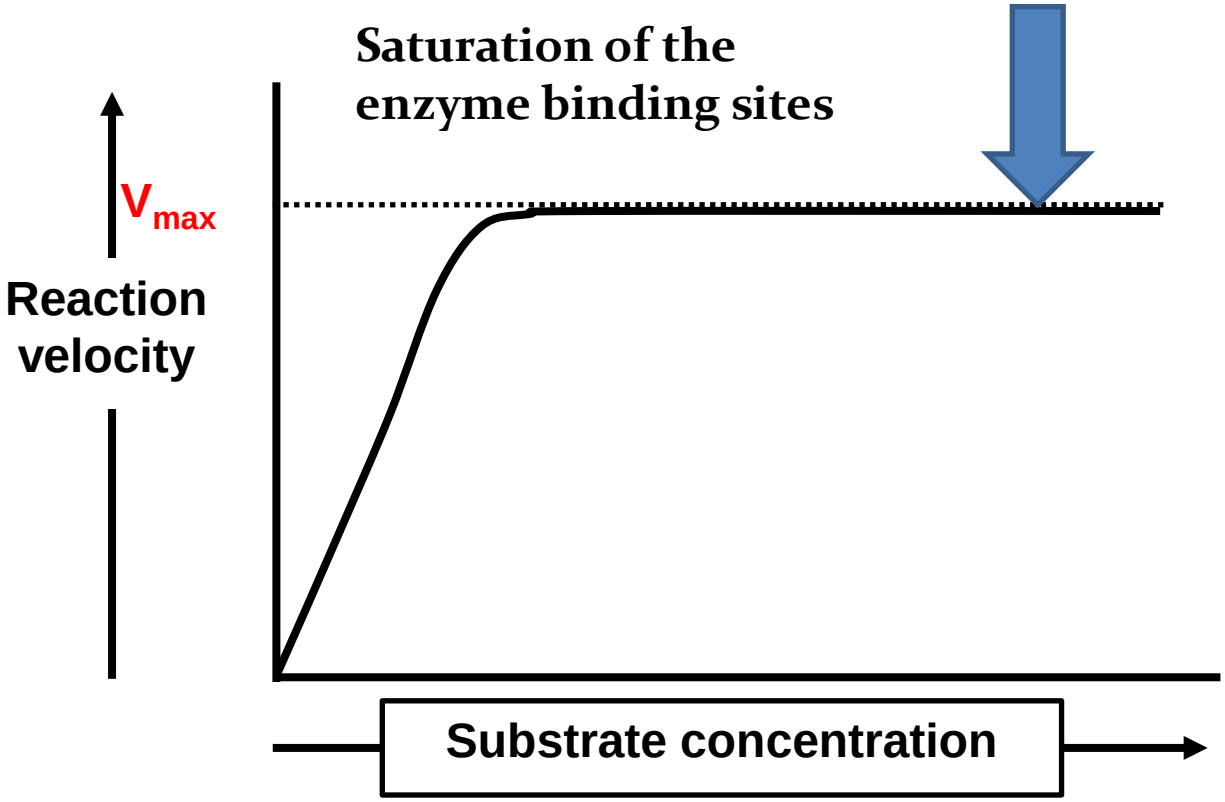
c)At high substrate concentrations the rate of the enzymatic reaction reaches maximum values and remains constant even with increased substrate concentrations ,since all the active sites of the enzyme are saturated by the substrate (saturation effect).

Substrate concentration: Non-enzymic reactions



- The increase in velocity is proportional to the substrate concentration

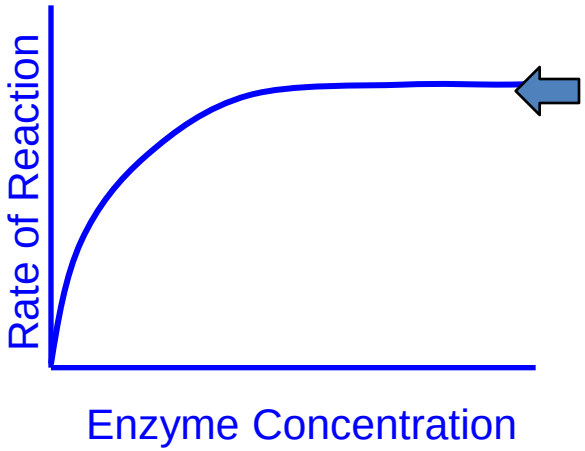
Substrate concentration: Enzymatic reactions hyperbolic curve:



- Faster reaction but it reaches a saturation point when all the enzyme molecules are occupied.
- If you alter the concentration of the enzyme then $\mathbf{V_{max}}$ will change too.

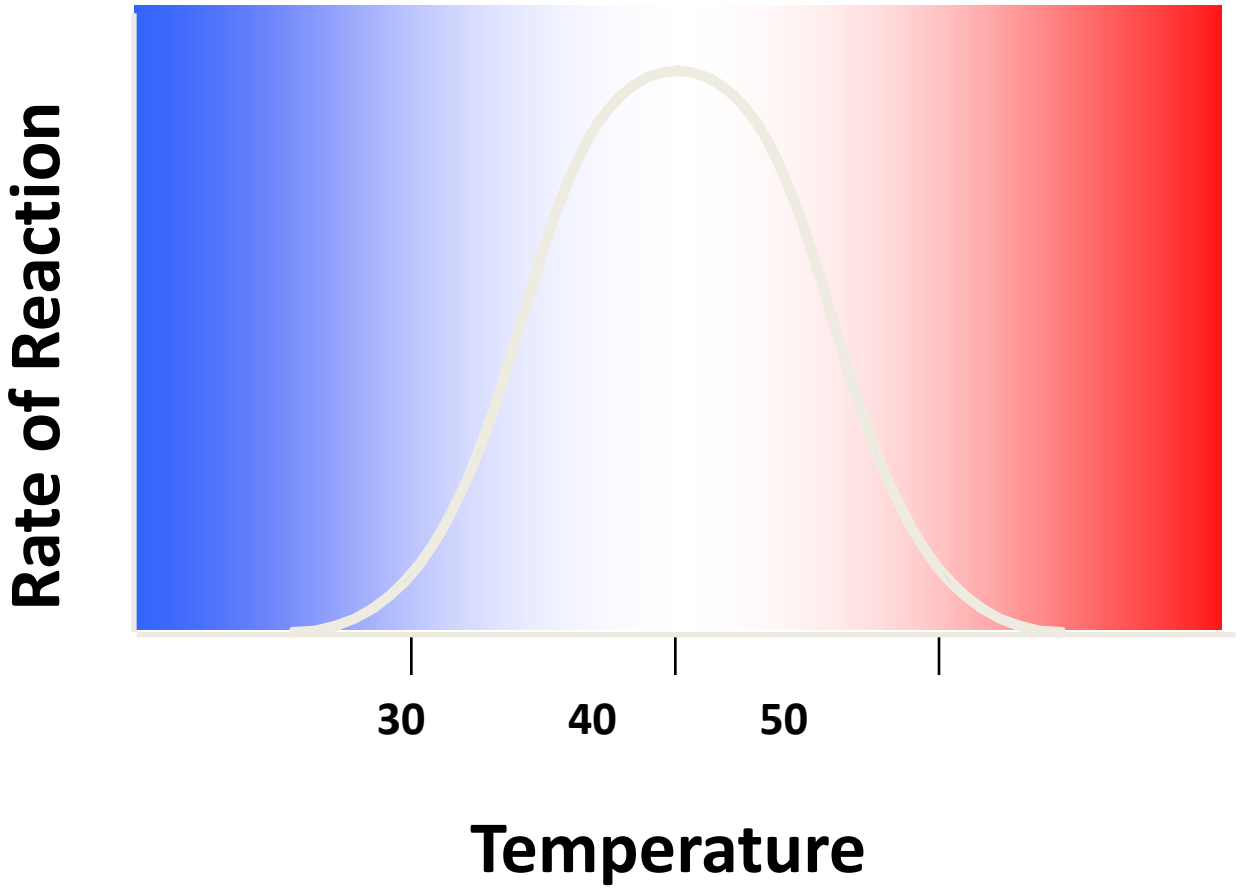
Enzyme concentration

- If there is insufficient enzyme present, the reaction will not proceed as fast as it, because there is not enough enzyme for all of the reactant molecules.
- As the amount of enzyme is increased, the rate of reaction increases. If there are more enzyme molecules than are needed, adding additional enzyme will not increase the rate. Reaction rate therefore increases as enzyme concentration increases but then it levels off.

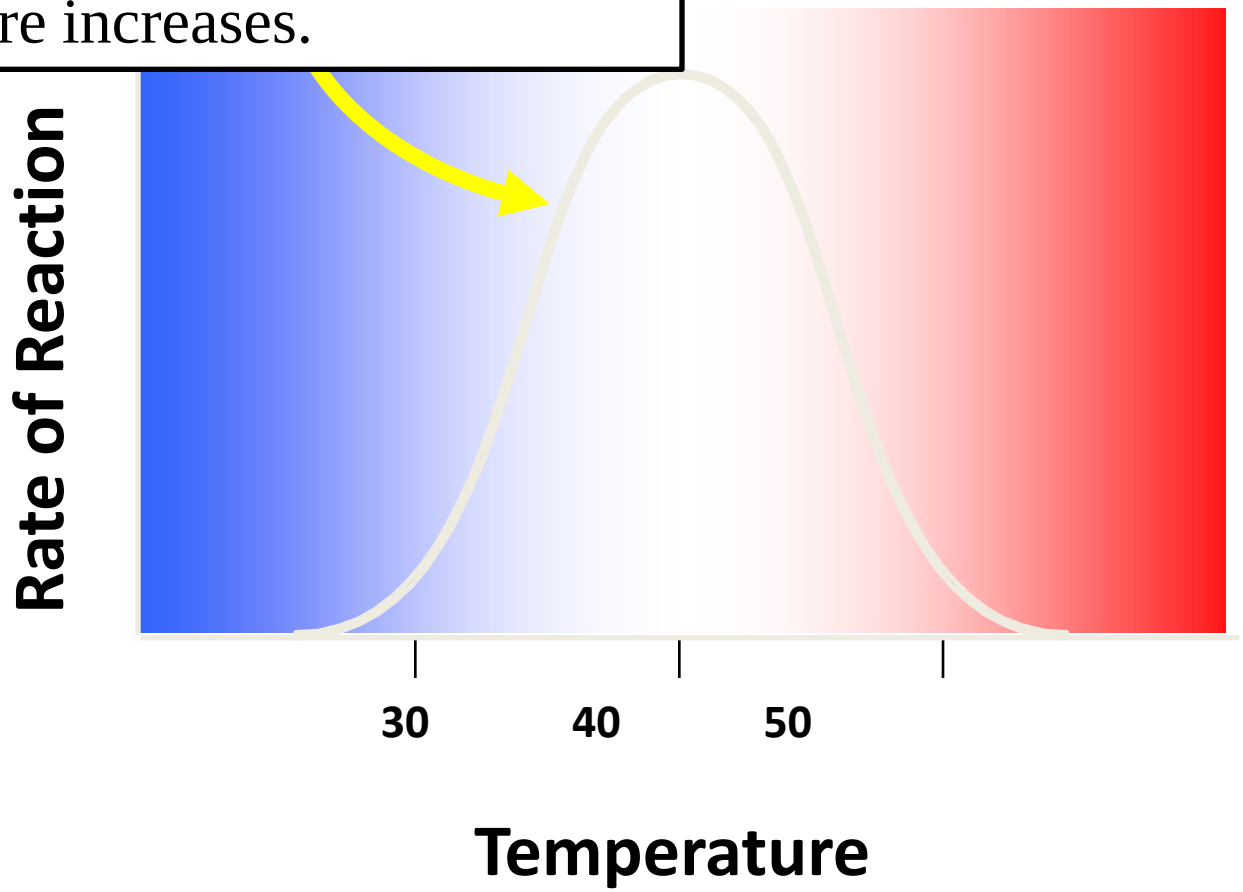


Even when adding more enzymes, there isn't any more available substrate to create product at a faster rate

Effect of Temperature on Enzyme Activity

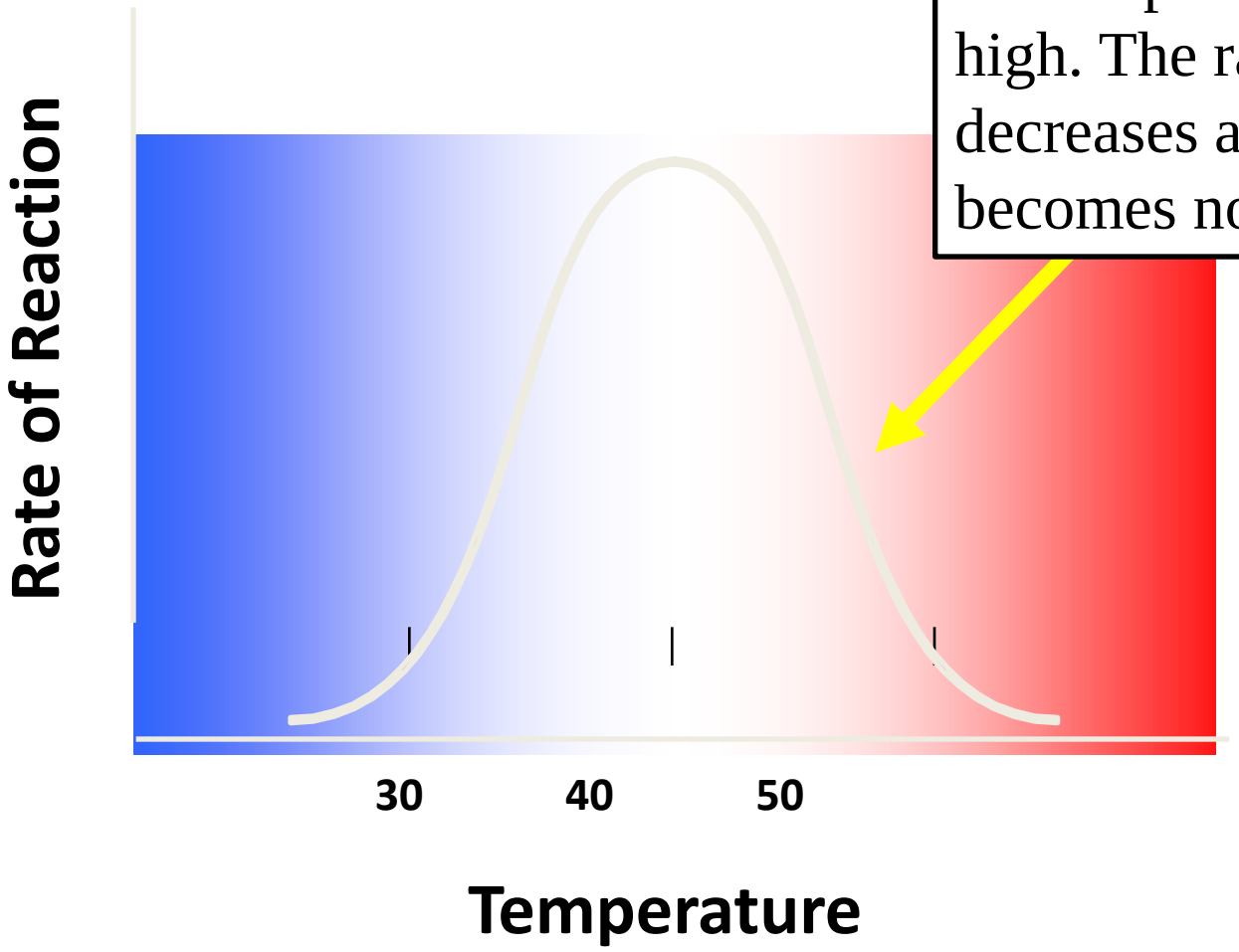


Increasing the temperature causes more collisions between substrate and enzyme molecules. The rate of reaction therefore increases as temperature increases.



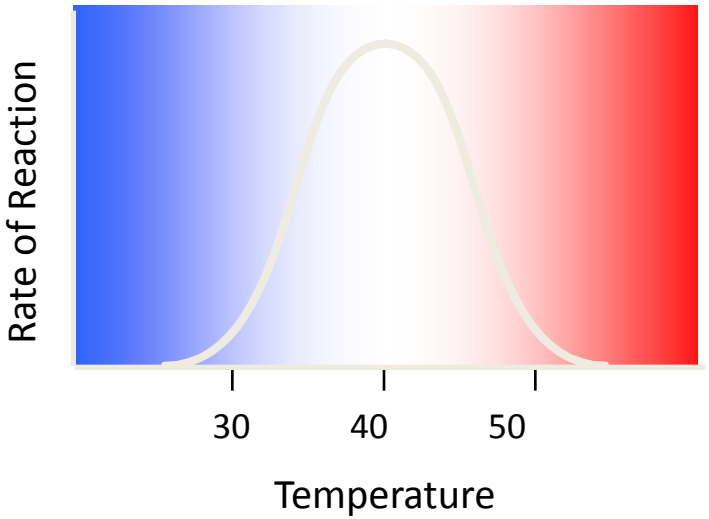
Effect of Temperature on Enzyme Activity

Enzymes denature when the temperature gets too high. The rate of reaction decreases as the enzyme becomes nonfunctional.



Temperature:

- Higher temperature causes more collisions between the atoms, ions, molecules, etc. It therefore increases the rate of a reaction – "Turnover Rate". More collisions increase the probability that substrate will bind with the active site of the enzyme.
- Above a certain temperature (40 ^{o} c) activity begins to decline because the enzyme begins to denature (unfold).
- The rate of chemical reactions therefore increases with temperature but then decreases.



Temperature Effect:

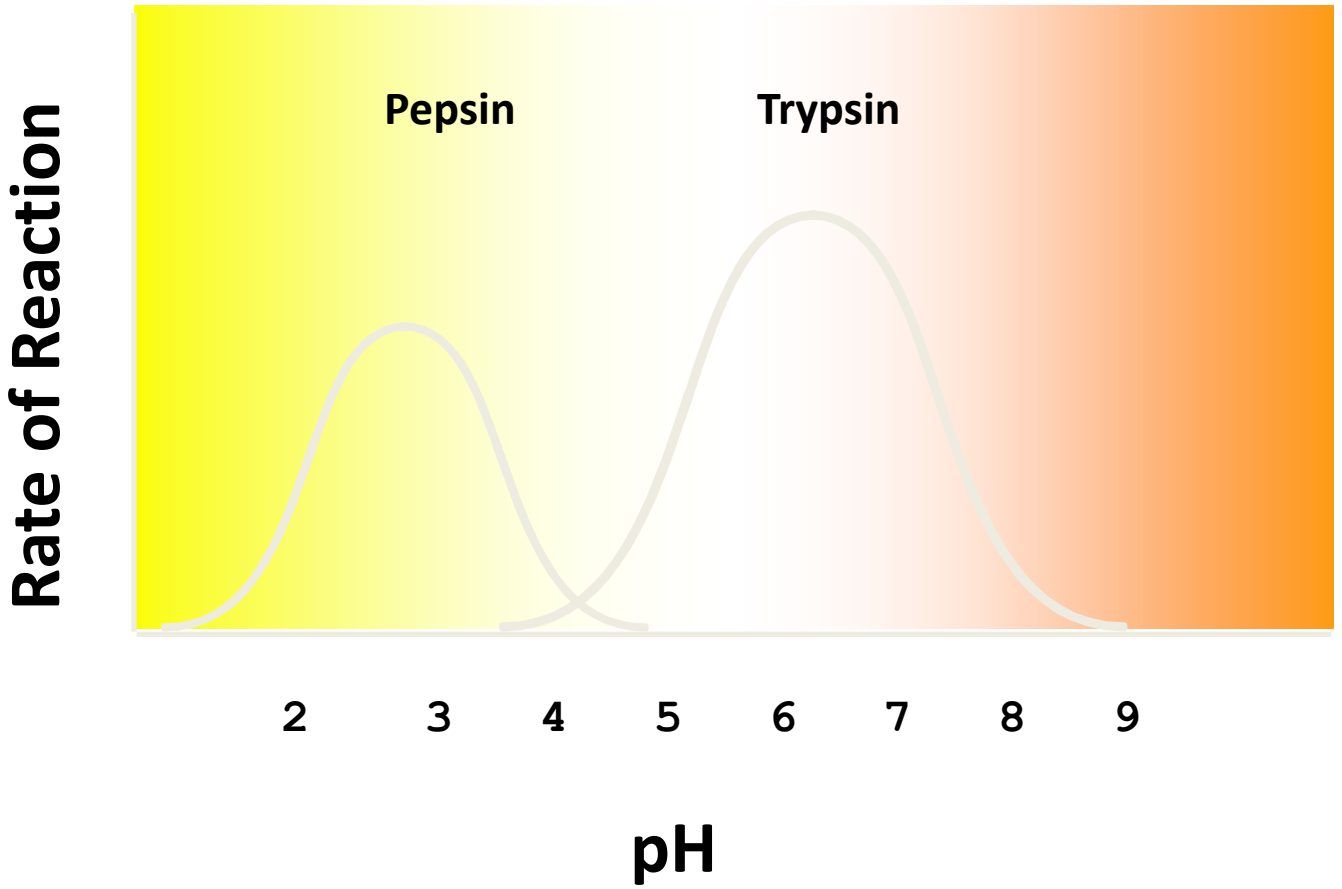
- Optimum temp: The temp at which the enzyme activity is maximum. And this optimum temp for many enzymes between 35 -- 4 5 ^{0} c
- Storage of enzymes at 5^{0} c or below is the most suitable
- Some enzymes lose activity when frozen
- At zero temp : The enzyme is inactive but not destructed

Denaturation

- If the hydrogen bonds within an enzyme are broken, the enzyme may unfold or take on a different shape. The enzyme is denatured.
- A denatured enzyme will not function properly because the shape of the active site has changed.
- If the denaturation is not severe, the enzyme may regain its original shape and become functional.
- The following will cause denaturation:
  - Heat
  - Changes in pH
  - Heavy-metal ions (lead, arsenic, mercury)
  - Alcohol
  - UV radiation

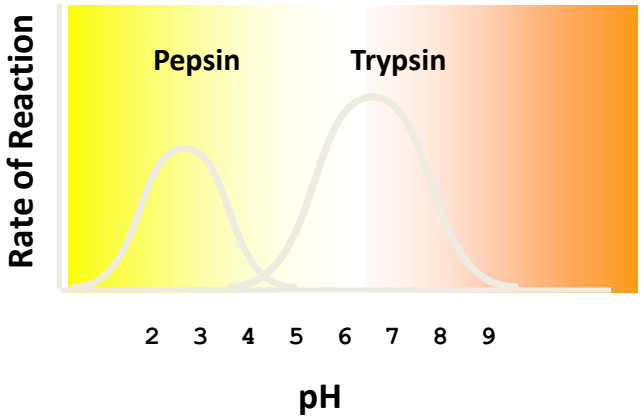
Effect of pH on Enzyme Activity

Each enzyme has its own optimum pH.



PH Effect:

- **Each enzyme has an optimal pH. Pepsin, an enzyme found in the stomach, functions best at a low pH. Trypsin, found in the intestine, functions best at a neutral pH.**
- **A change in pH can alter the ionization of the R groups of the amino acids. When the charges on the amino acids change, hydrogen bonding within the protein molecule change and the molecule changes shape. The new shape may not be effective.**
- **The diagram shows that pepsin functions best in an acid environment. This makes sense because pepsin is an enzyme that is normally found in the stomach where the pH is low due to the presence of hydrochloric acid. Trypsin is found in the duodenum (small intestine), and therefore, its optimum pH is in the neutral range to match the pH of the duodenum.**



Physical Agents:

- Physical agents that cause denaturation of proteins affect the enzyme activity
- Example: X- ray , UV light , Ultrasonic vibrations, repetitive freezing and thawing in addition to other physical factors

Activators:

- Co-enzymes , Cofactors, metal ion activators (Ca ion activate thrombokinase , and Cl ion activate salivary amylase)
- Types of activators
- 1- Allosteric activation : the binding of an activator molecule to the allosteric site (any site other than the active site) can alter the shape of the enzymes as to make it more likely to bind to its substrate
- 2- Cooperativity : A substrate molecule binding to one active site may stimulate the catalytic powers of a multisubunit enzyme by affecting the other active sites ., Example : Hemoglobin binding to one oxygen promotes the pickup of additional oxygen molecules (hemoglobin is not an enzyme , but the concept of cooperativity is shown here)

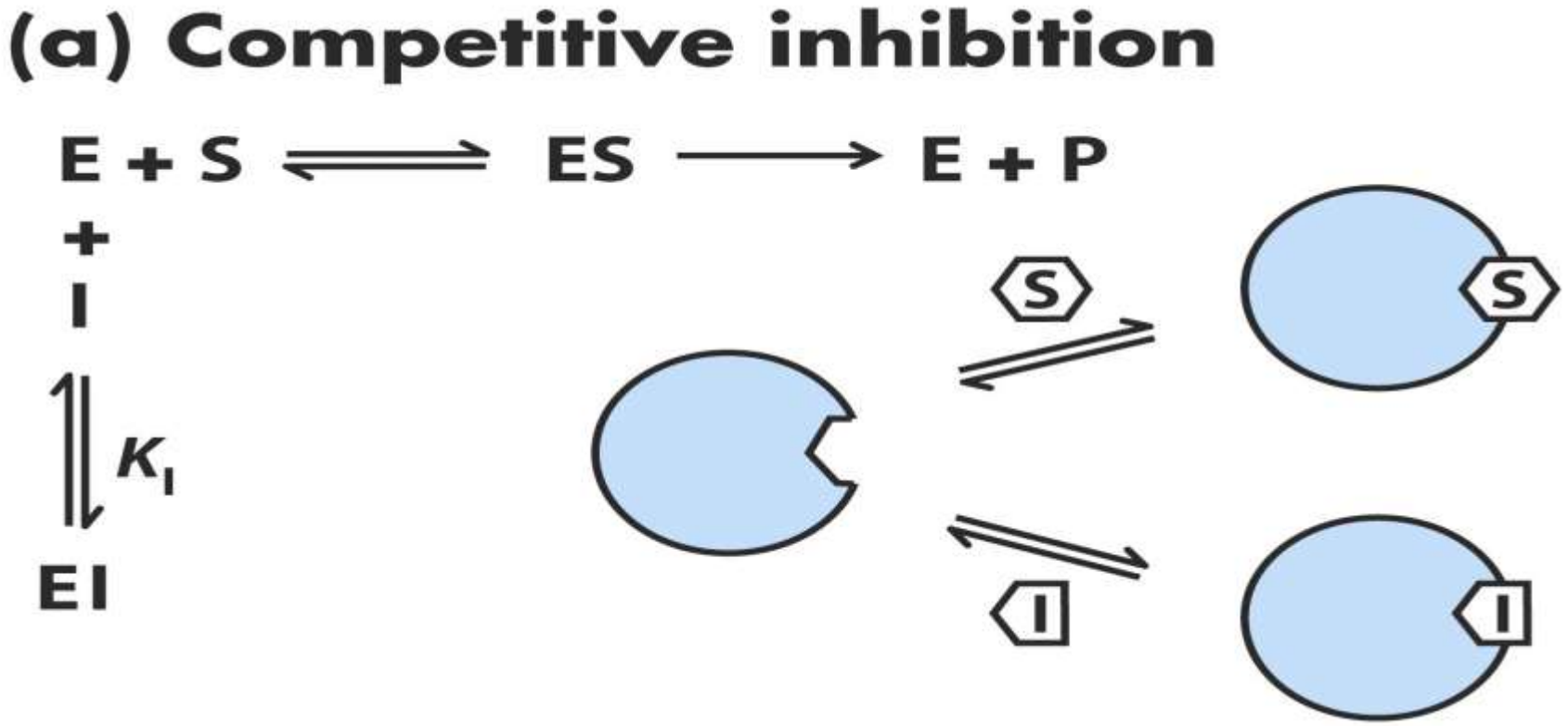
Inhibitors:

- Inhibitors are chemicals that reduce the rate of enzymic reactions.
- The are usually specific and they work at low concentrations.
- May decrease or even abolish enzyme activity. Many drugs and poisons are inhibitors of enzymes in the nervous system.

Competitive inhibitor:

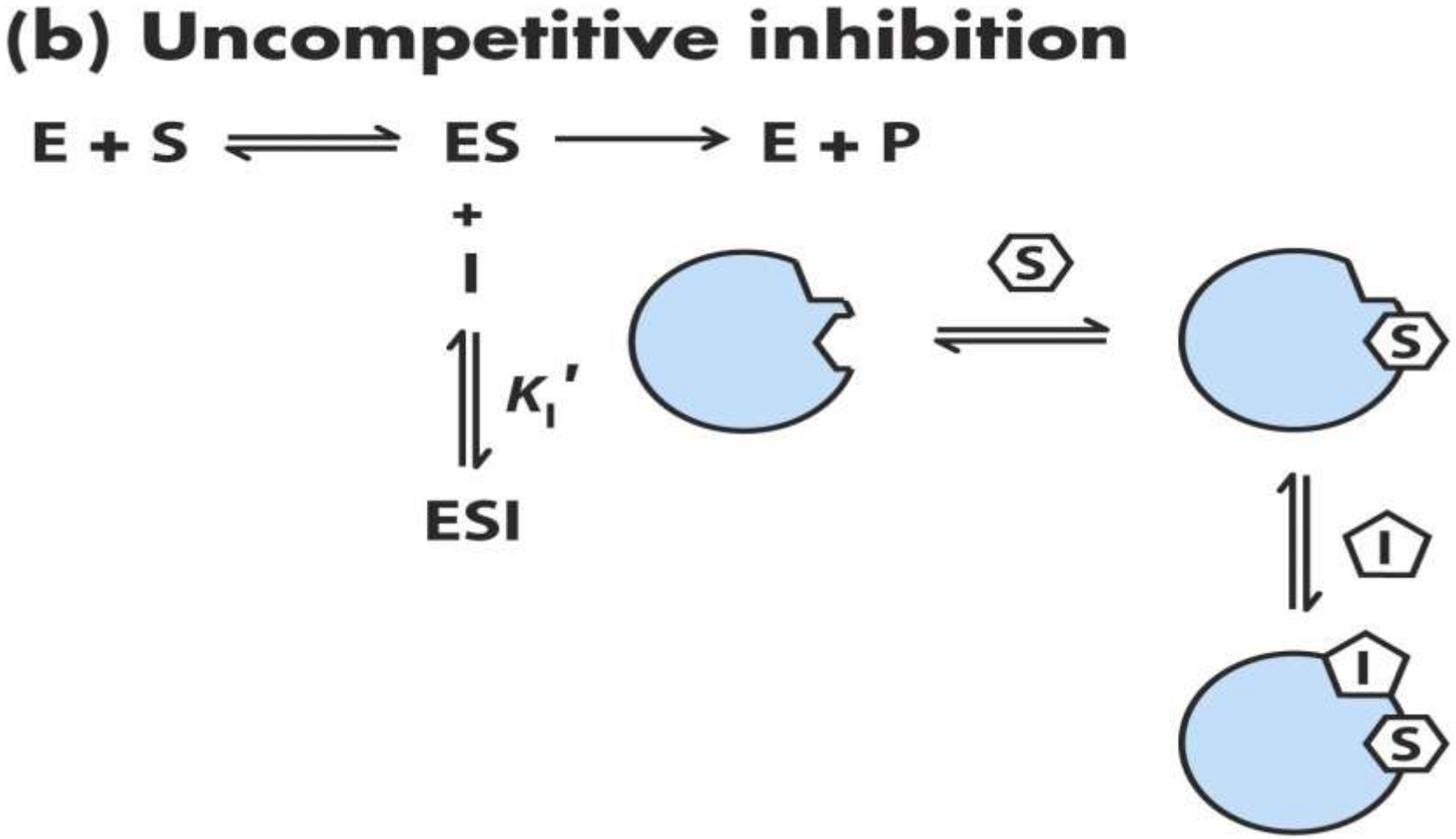
reduces velocity by effectively reducing the number

of *available* active sites



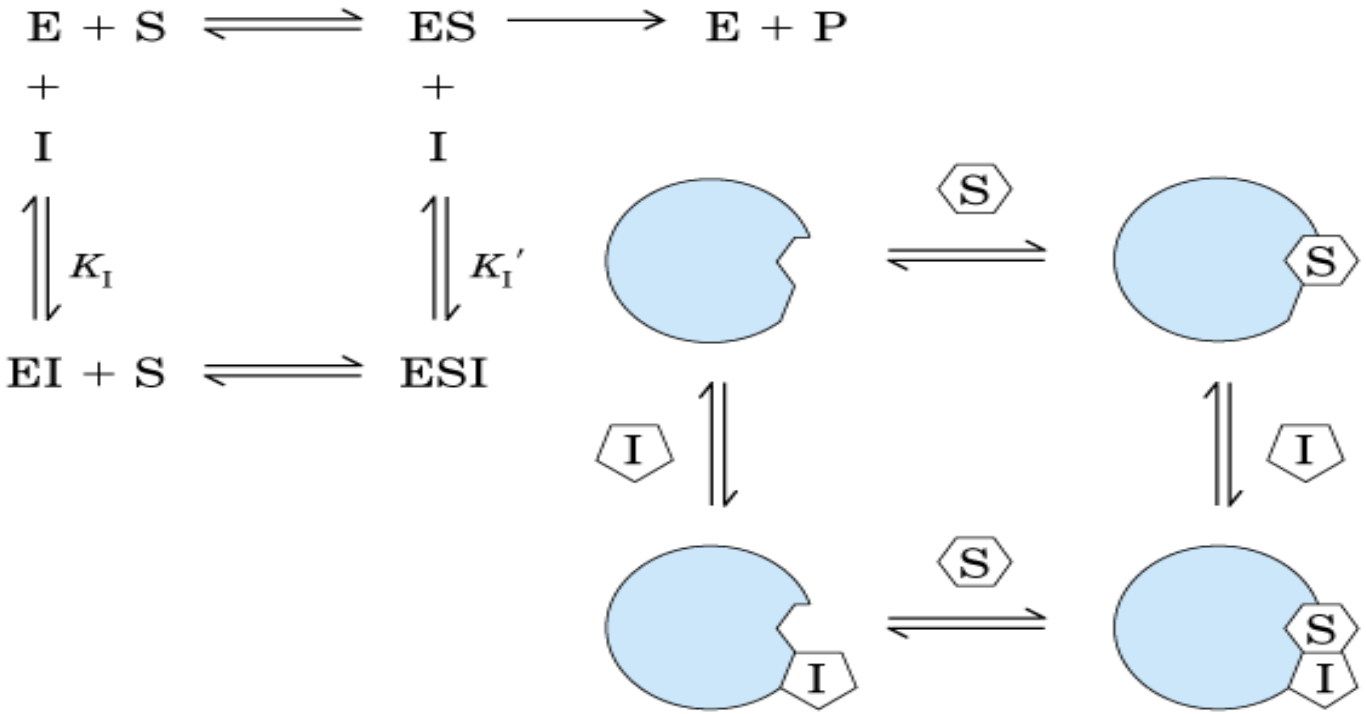
Reversible Inhibitors – Uncompetitive

Uncompetitive I binds only to the ES complex, and cannot bind (*detectibly*) to the free enzyme E.



A mixed inhibitor(noncompetitive inhibition)

- **binds to a site other than the active site**, and
- **reduces the rate of product formation**, and
- **can bind to either E or to ES, not with the same affinity**



(c) Mixed inhibition

Irreversible inhibition

- **Irreversible inhibitors:** Combine with the functional groups of the amino acids in the active site, irreversibly.

Examples: nerve gases and pesticides, containing organophosphorus, combine with serine residues in the enzyme acetylcholine esterase.

Enzymes kinetics

Lec 11

By: Inaam Ahmed ameen

Enzyme kinetics

Enzyme kinetics

Enzyme kinetics show the number & order of the individual steps by which enzymes transform substrates in to products more over the catalytic mechanism of a given enzyme

- Deals with quantitative measurement of the rates of enzyme-catalyzed reactions

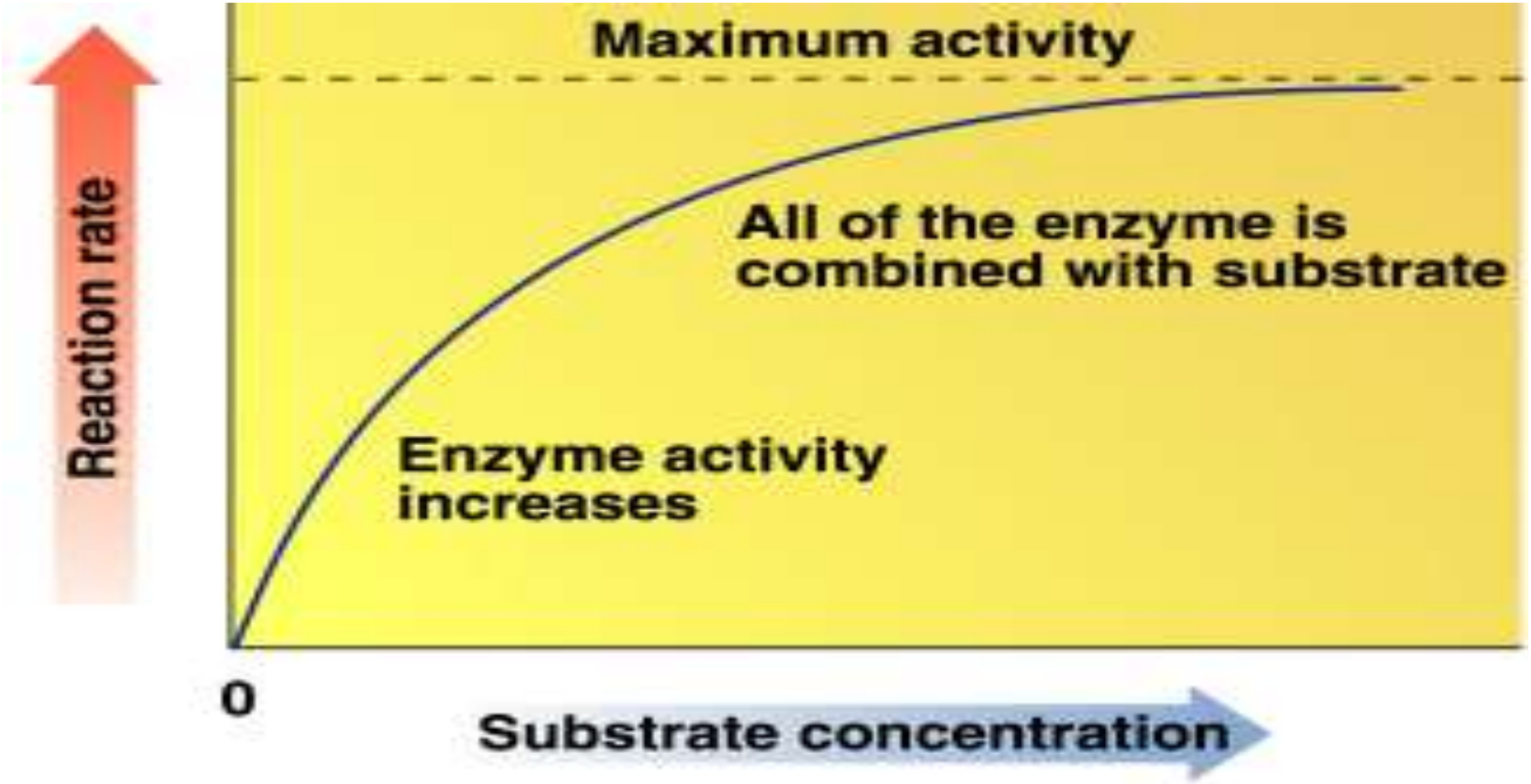
- In addition to the factors affecting the rates of enzyme-catalyzed reactions

Applied enzyme kinetics represents the principal tool by which scientists identify and indicate therapeutic agents that selectively inhibit the rates of specific enzyme catalyzed processes

Substrate Concentration and Reaction Rate

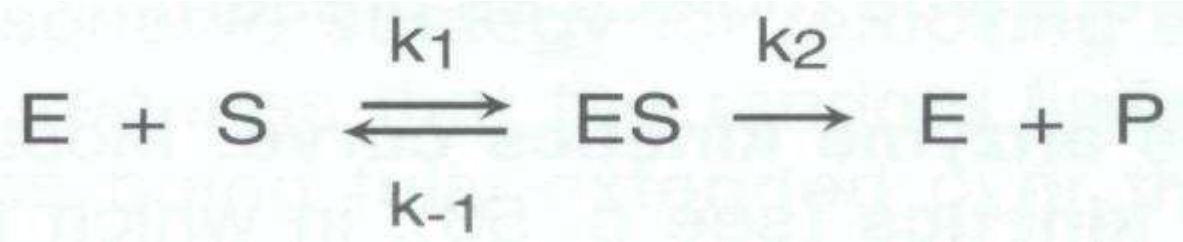
- The rate of reaction increases as substrate concentration increases (at constant enzyme concentration)
- The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time.
- velocity is usually expressed as μ mol of product formed per minute.
- The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity is reached .
- The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.

SUBSTRATE CONCENTRATION AND REACTION RATE



The Michaelis - Menten Model

- Michaelis and Menten proposed a simple model that accounts for most of the features of enzyme-catalyzed reactions.
- In this model, the enzyme reversibly combines with its substrate to form an ES complex that subsequently breaks down to product, regenerating the free enzyme



The Michaelis- Menten equation

- The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration

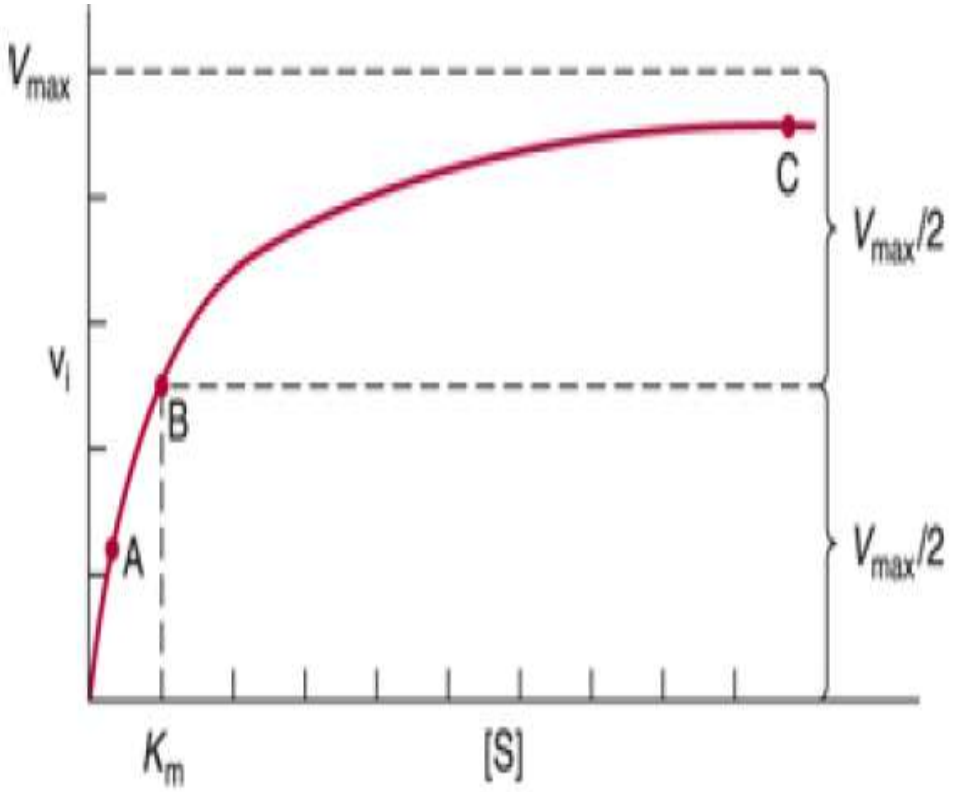
$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$

Vmax = maximum velocity **vo = initial velocity,**
Km = (k-1 + k2)/k1

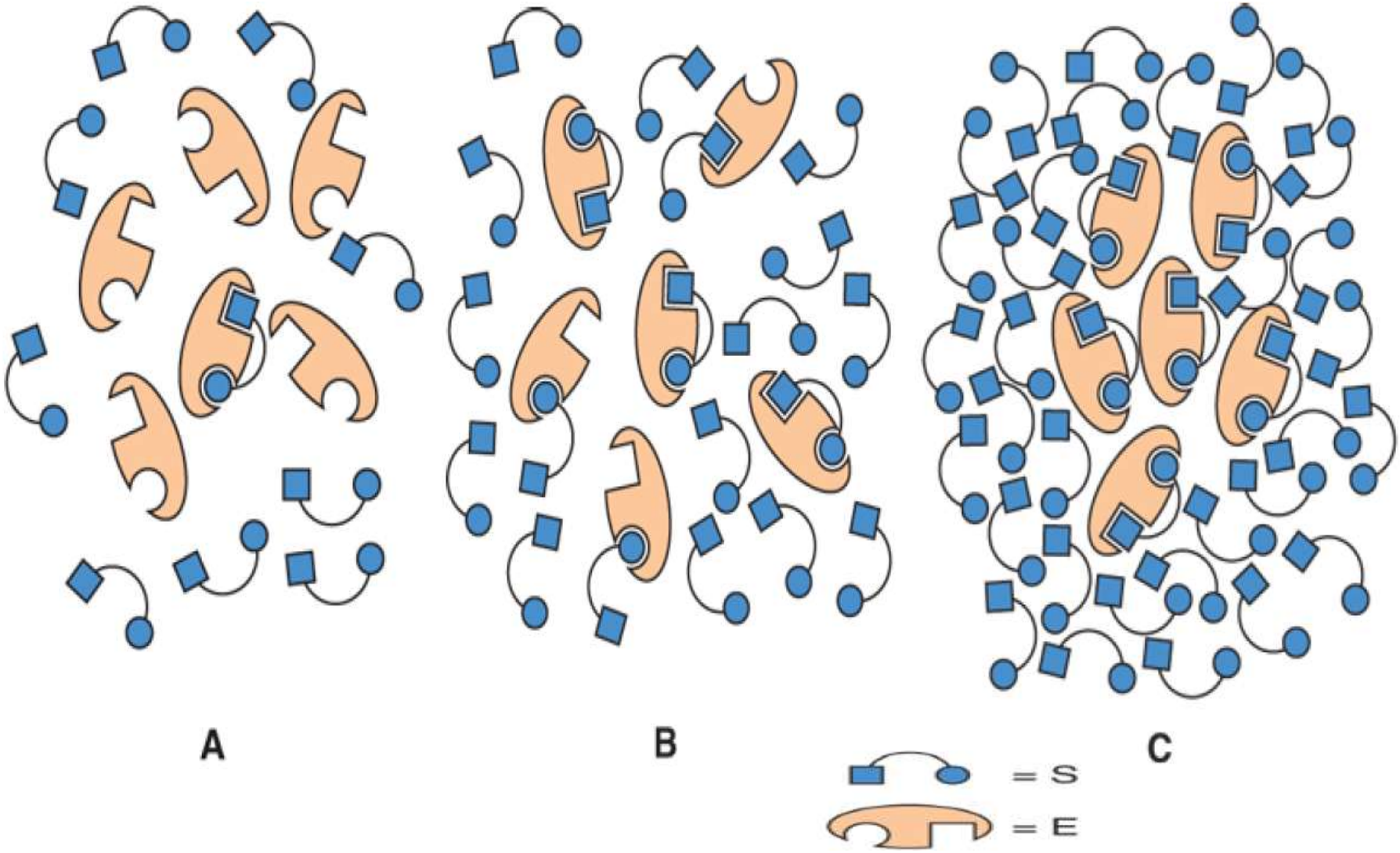
To determine the maximum speed of an enzymatic reaction, the substrate concentration is increased until a constant rate of product formation is achieved. This is the maximum velocity (Vmax) of the enzyme. In this state, all enzyme active sites are saturated with substrate.

The Michaelis-Menten constant (Km)

- The Michaelis-Menten constant (Km) = is the substrate concentration required for an enzyme to reach one half its maximum velocity.
- Each enzyme has a characteristic Km for a given substrate.
- Most enzymes show Michaelis-Menten kinetics, in which the plot of initial reaction velocity ,against substrate concentration [S], is hyperbolic



Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction



parameters of Michaelis-Menten kinetics

The initial velocity V^0 = the rate of the reaction is measured as soon as enzyme & substrate are mixed , at that time the concentration of product is very small & therefore the rate of back reaction can be ignored thus the dependence of initial reaction velocity on S & Km can be evaluated under three conditions :

. 1. [S] << Km point A

- When [S] is much less than Km , the term km+ [S] will be equal to Km only :
- Replacing Km + [S] by km reduce the equation to:

$$Vi = \frac{Vmax\,[S]}{Km + [S]} = \frac{Vmax\,[S]}{Km} = \left[\frac{Vmax}{Km}\right][S]$$

$$\left[\frac{Vmax}{Km}\right] \text{ is constant} \longrightarrow Vi = k[S]$$

2. [S] = Km point B

$$Vi = \frac{Vmax\,[S]}{Km + [S]} = \frac{Vmax\,[S]}{2\,[S]}$$

$$Vi = \frac{Vmax}{2}$$

3. [S] >> Km point C

- When **[S]** is much grater than Km , the term km+ **[S]** will be equal to **[S]** only :
- Replacing Km + **[S]** by **[S]** reduce the equation to:

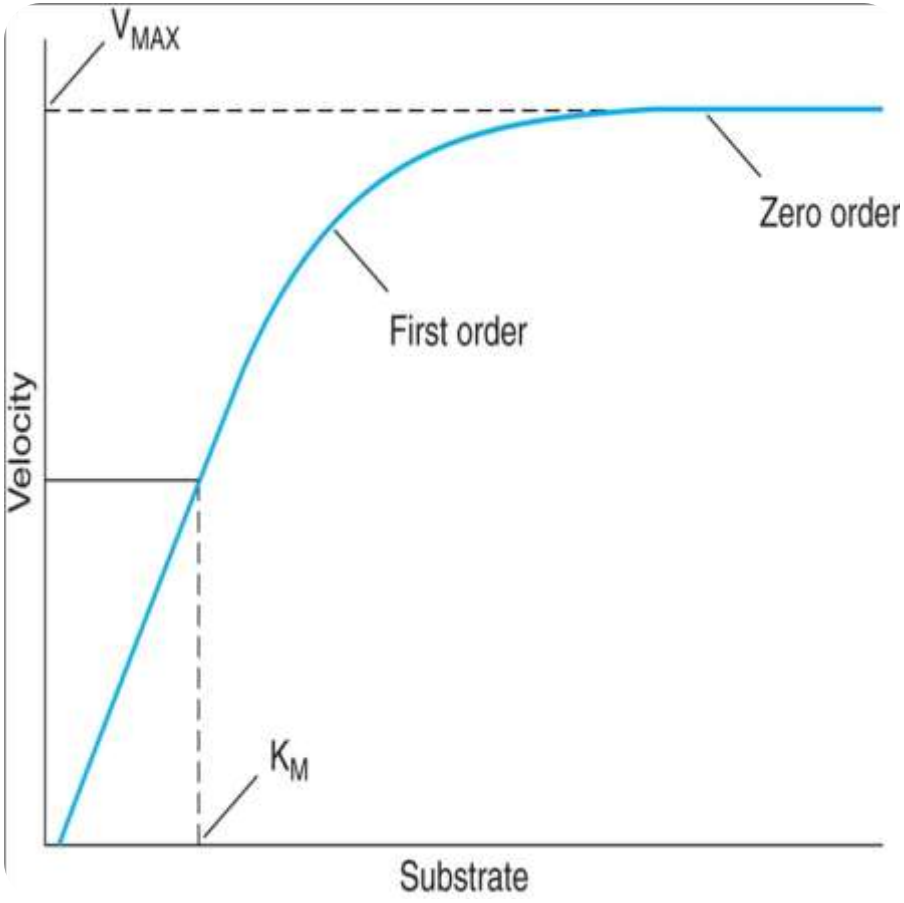
$$Vi = \frac{Vmax\,[S]}{Km + [S]} = \frac{Vmax\,[S]}{[S]} = Vmax$$

order of reaction

- When [S] is much less than Km

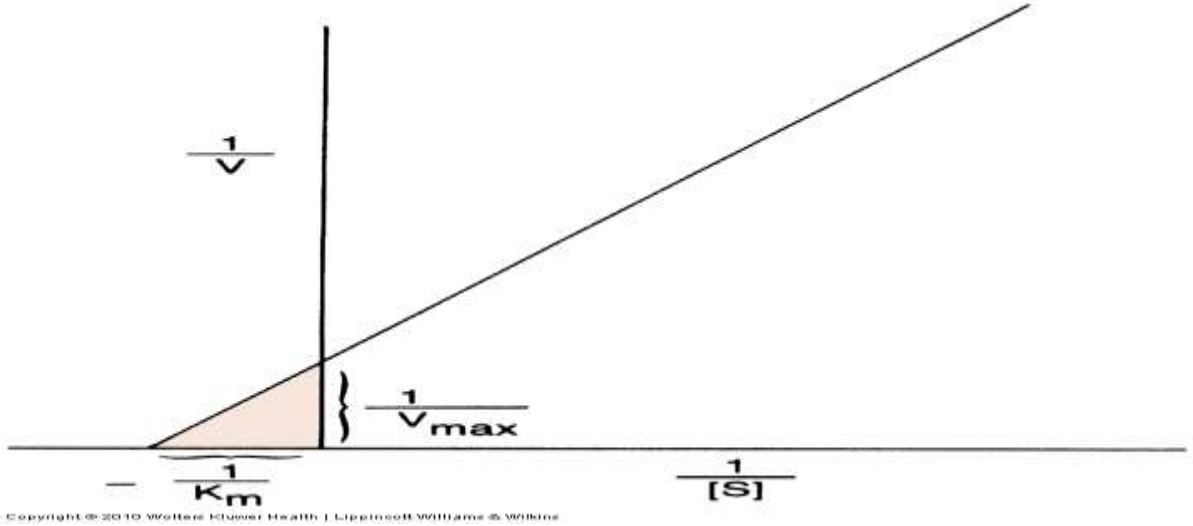
  the velocity of the reaction is approximately proportional to the substrate concentration & the rate of reaction is then said to be **first order with respect to substrate.**
- **In** First order kinetics the Rate is directly proportional to substrate concentration

- When [S] is much greater than Km the velocity is constant and equal to Vmax & the rate of reaction is then independent of substrate concentration, and is said to be **zero order with respect to substrate concentration**
- **In** Zero order kinetics Level is reached depends only on enzyme concentration



Lineweaver-Burk Plot:
the reciprocal of the Michaelis-Menten plot

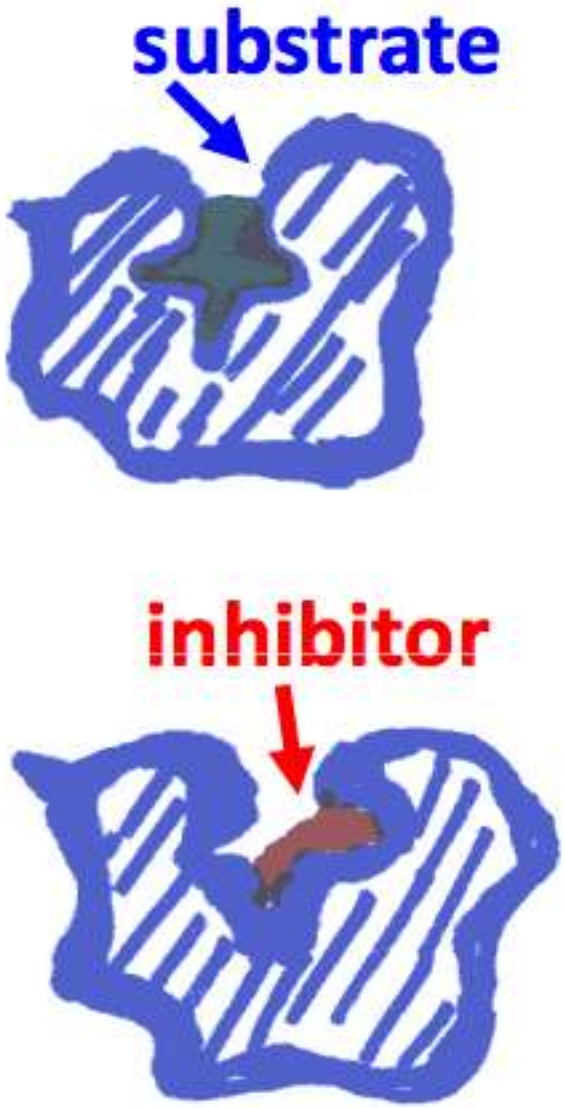
- When Vi is plotted against [S], it is not always possible to determine when Vmax has been achieved, because of the gradual upward slope of the hyperbolic curve at high substrate concentrations.
- However, if 1/Vi , is plotted versus 1/[S], a straight line is obtained .
- This plot, the **Lineweaver-Burke plot (also called a double-recipro-cal plot) can be used to calculate Km & Vmax** and as well as to determine the mechanism of action of enzyme inhibitors.

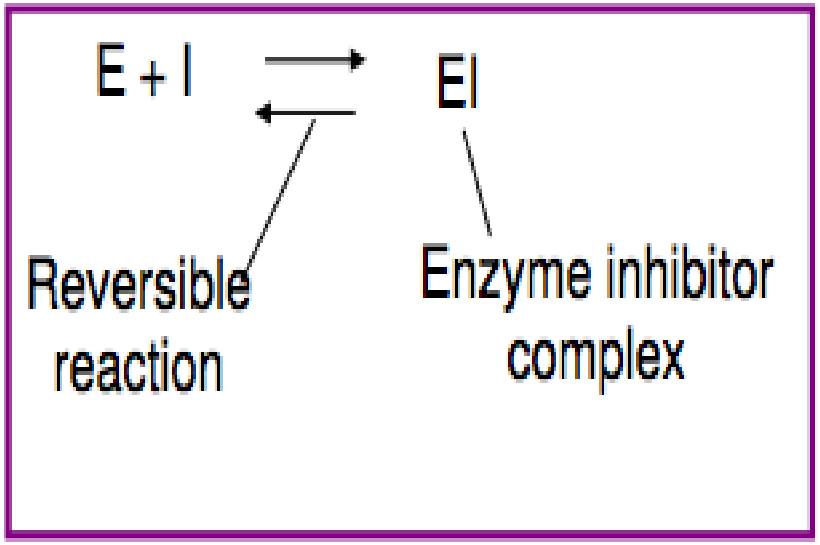


Inhibition of enzyme activities

- **Inhibitor: any molecule which acts directly on an enzyme to lower its catalytic rate is called an inhibitor.(not denaturation)**
- **Some enzyme inhibitors are normal body metabolites.**
- **Other may be foreign substances such as drugs or toxins.**

Competitive inhibition





I

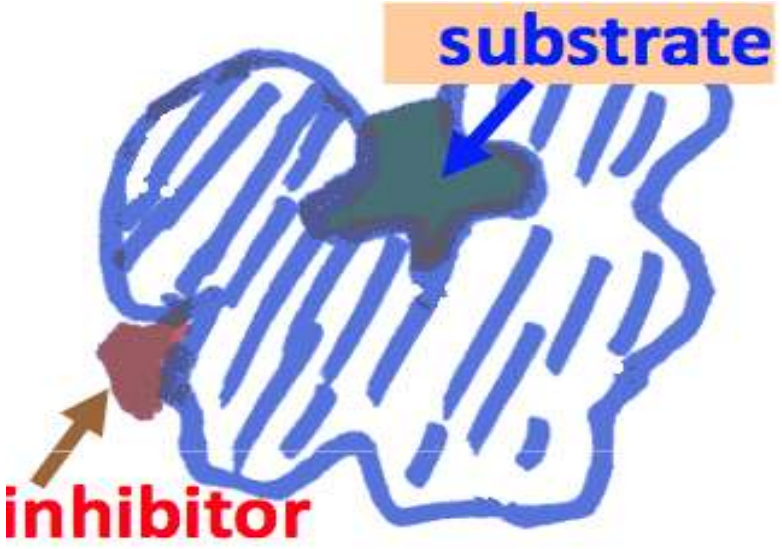
Non-competitive inhibitors

- They bind reversibly to the enzyme.
- They bind non-covalently to the enzyme.
- They bind to a site on the enzyme other than the active site .
- It can bind to the free enzyme and the ES complex.
- This action results in a conformational change in the protein that affects a catalytic step and hence decreases the enzyme activity.
- E + I ⟷ EI
- ES + I ⟷ ESI
- Both the EI and the

ESI complex are inactive

And cannot produce the

product.



Equations Define the Kinetics of Enzyme-Catalyzed Reactions

1. The Michaelis-Menten Equation

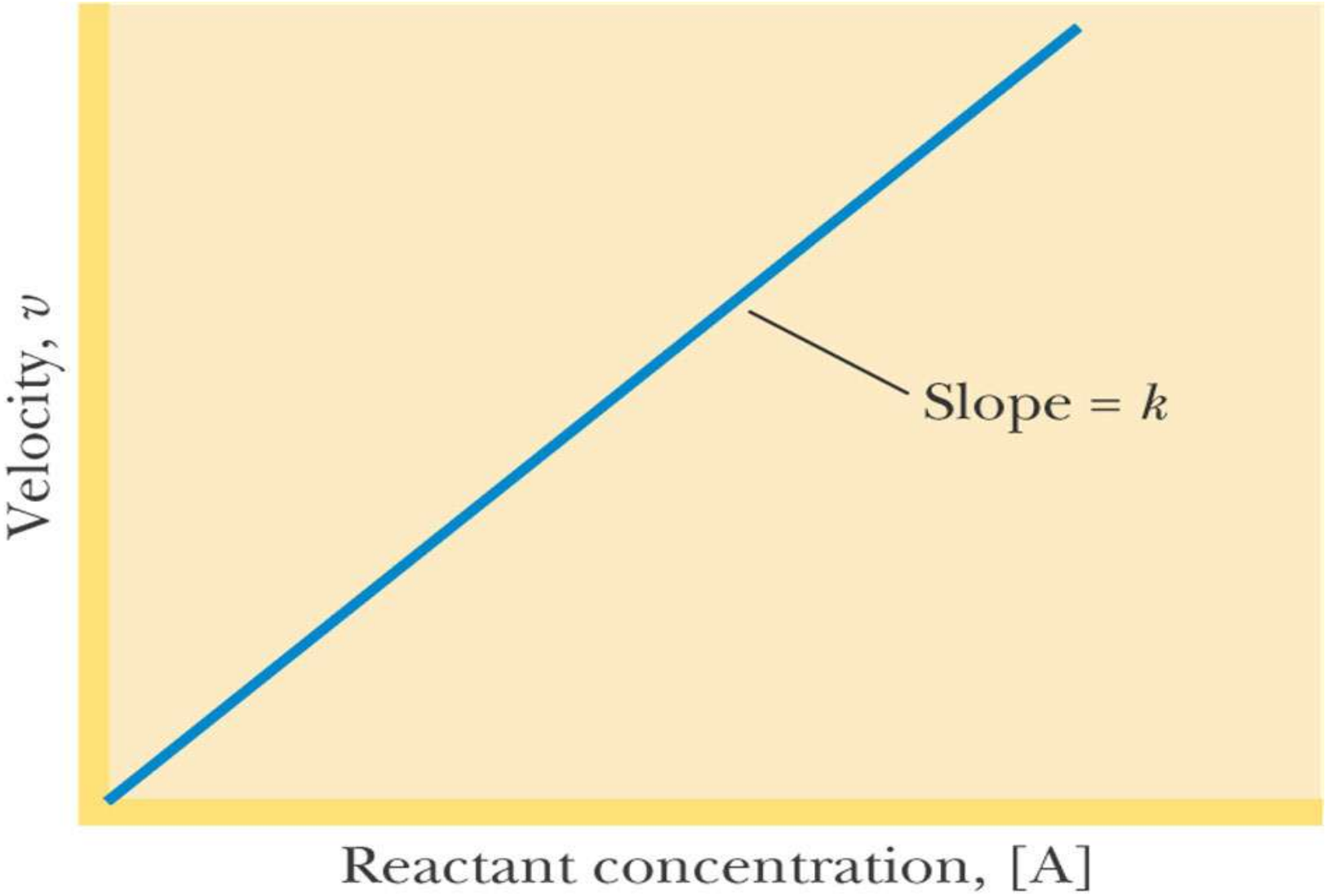
$$v = \frac{V_{max}\,[S]}{K_m + [S]}$$

2. The Lineweaver-Burk double-reciprocal plot

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

3. Hanes-Woolf plot

$$\frac{[S]}{v} = \left(\frac{1}{V_{max}}\right)[S] + \frac{K_m}{V_{max}}$$


Velocity, v
Slope = k
Reactant concentration, [A]

The Michaelis-Menten Equation derivatization

- Louis Michaelis and Maud Menten's theory
- It assumes the formation of an enzyme-substrate complex (ES)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

- At equilibrium

$$k_{-1}[ES] = k_1[E][S]$$

And

$$K_s = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1}$$

$$E + s \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

- The steady-state assumption

  ES is formed rapidly from E + S as it disappears by dissociation to generate E + S and reaction to form E + p

$$\frac{d[ES]}{dt} = 0$$

- That is; formation of ES = breakdown of ES

$$k_1 [E] [S] = k_{-1}[ES] + k_2[ES]$$

$$k_1\,[E]\,[S] = k_{-1}[ES] + k_2[ES] = (k_{-1} + k_2)\,[ES]$$

$$[ES] = \left(\frac{k_1}{k_{-1} + k_2}\right)[E]\,[S]$$

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

K_m is Michaelis constant

$$K_m\,[ES] = [E]\,[S]$$

$$K_m\,[ES] = [E]\;\;[S]$$

Total enzyme, $[E_T] = [E] + [ES]$

$$[E] = [E_T] - [ES]$$

$$K_m\,[ES] = ([E_T] - [ES])\,[S] = [E_T]\,[S] - [ES]\,[S]$$

$$K_m\,[ES] + [ES]\,[S] = [E_T]\,[S]$$

$$(K_m + [S])\,[ES] = [E_T]\,[S]$$

$$[ES] = \frac{[E_T]\,[S]}{K_m + [S]}$$

$$[ES] = \frac{[E_T]\,[S]}{K_m + [S]}$$

The rate of product formation is

$$v = k_2\,[ES]$$

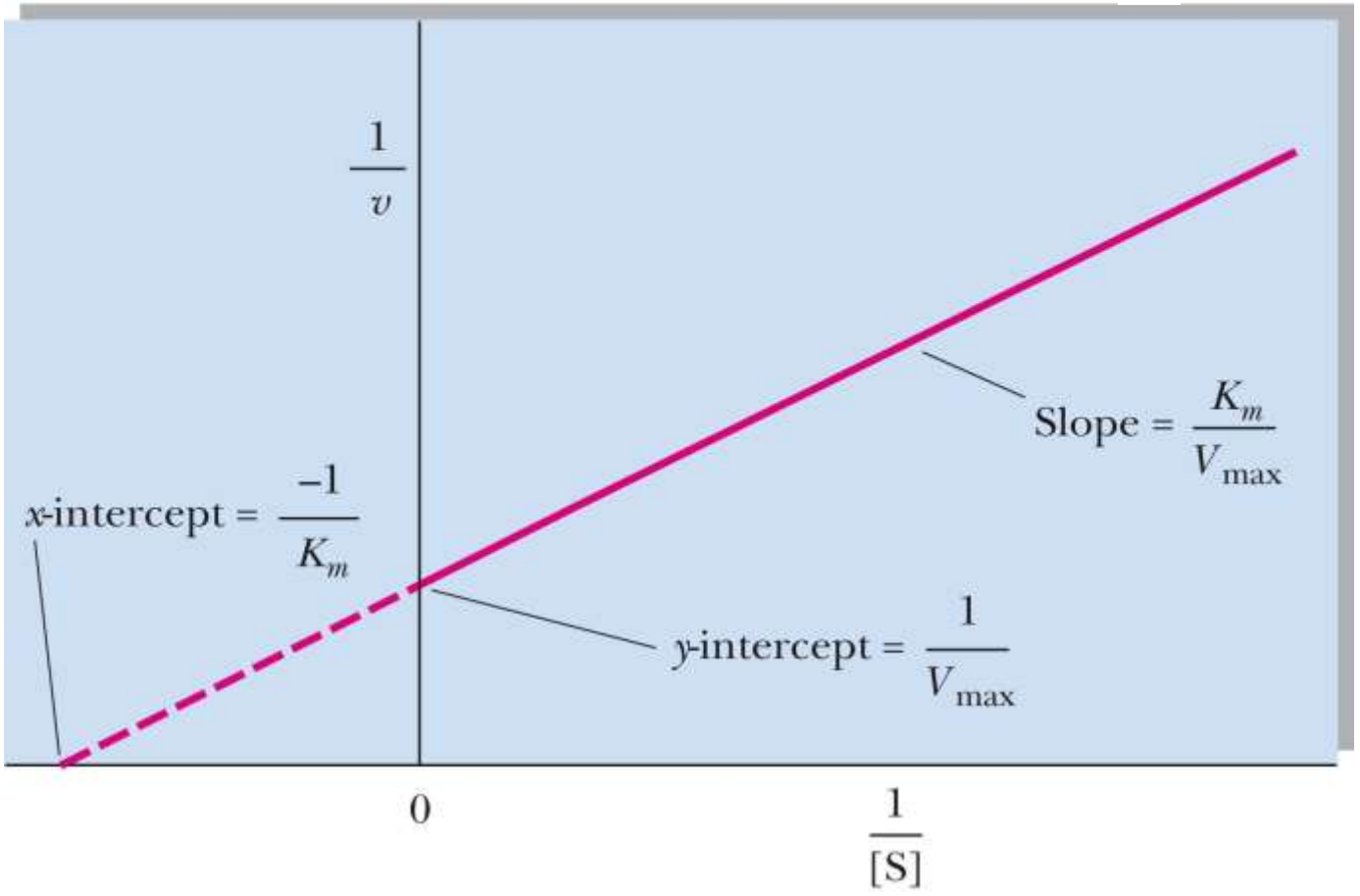
$$v = \frac{k_2\,[E_T]\,[S]}{K_m + [S]}$$

$$V_{max} = k_2\,[E_T] \qquad v = \frac{V_{max}\,[S]}{K_m + [S]}$$

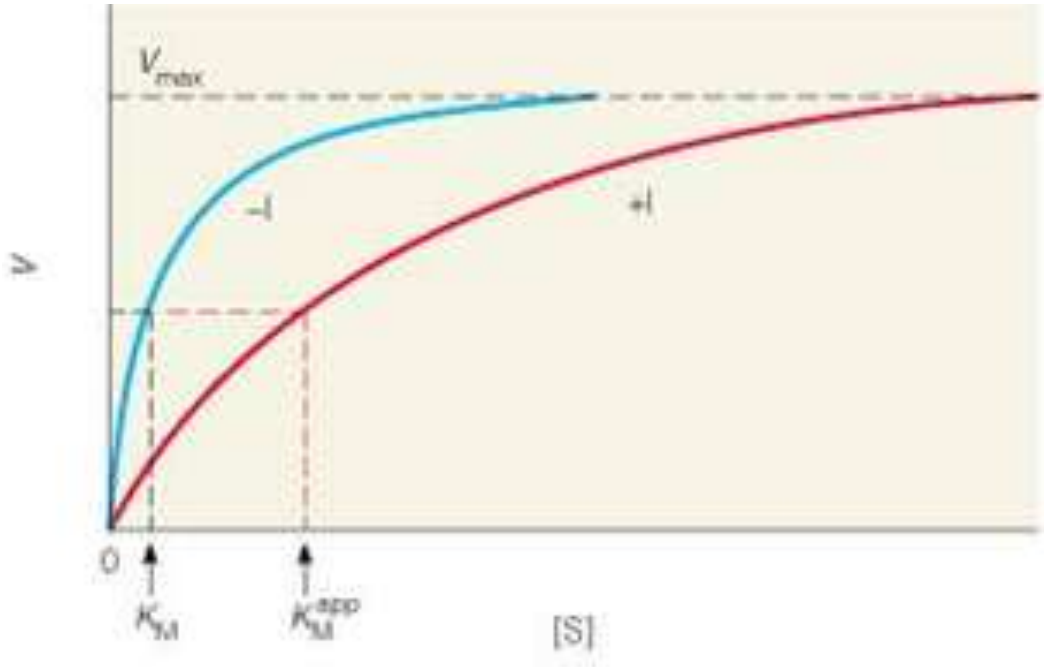
Linear Plots of the Michaelis-Menten Equation

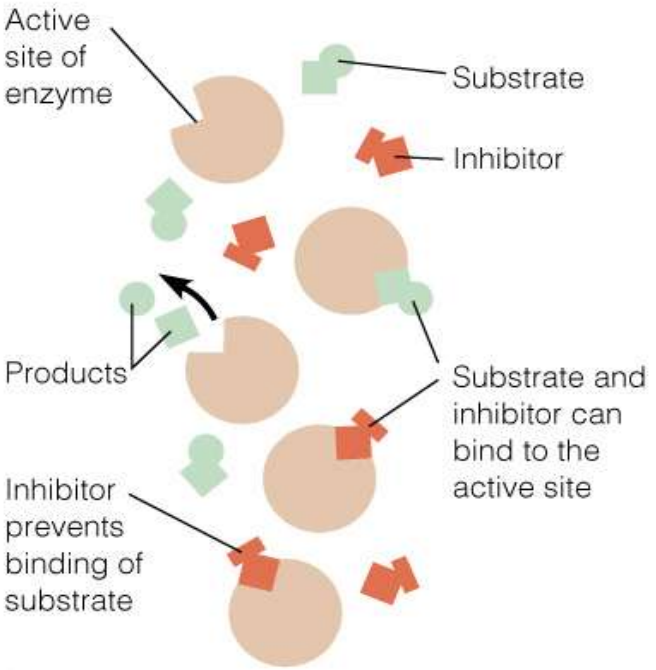
- Lineweaver-Burk plot
- Hanes-Woolf plot

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

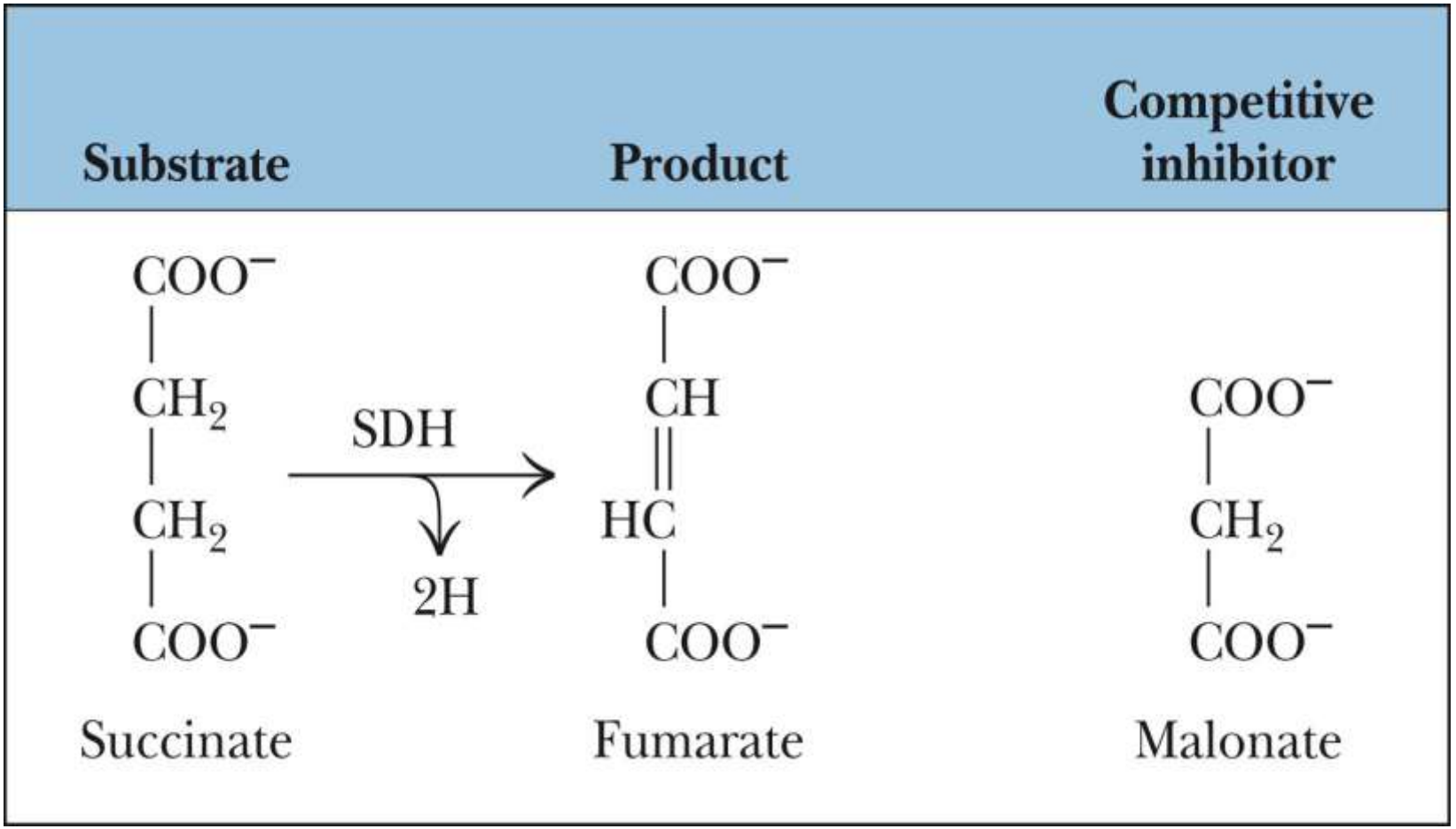


Competitive inhibition



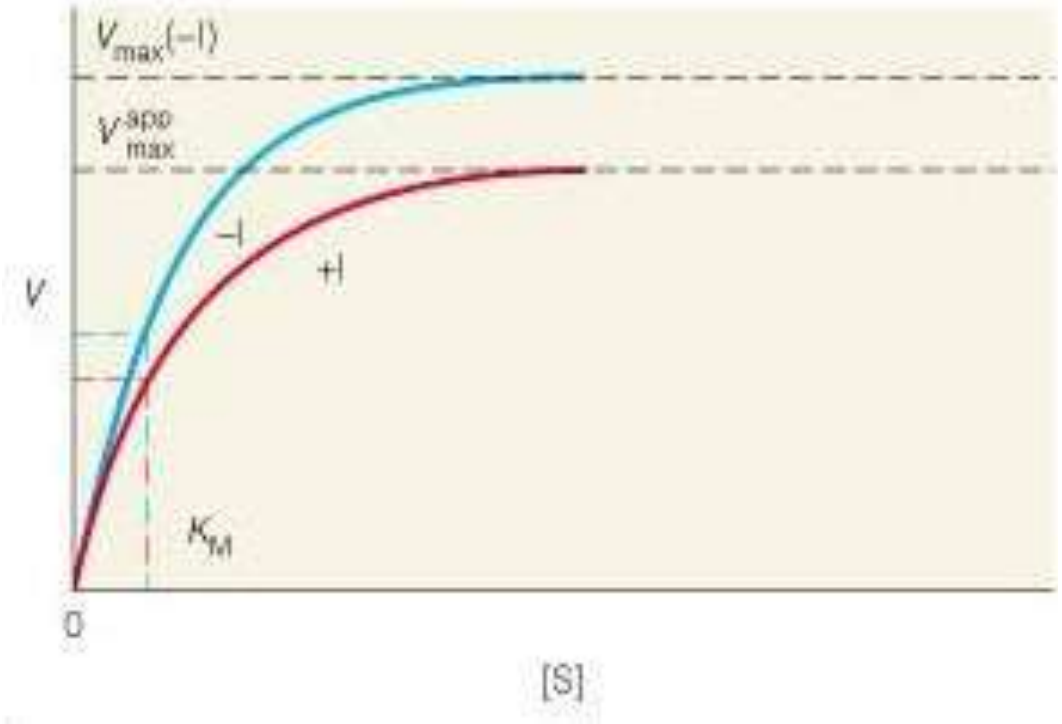


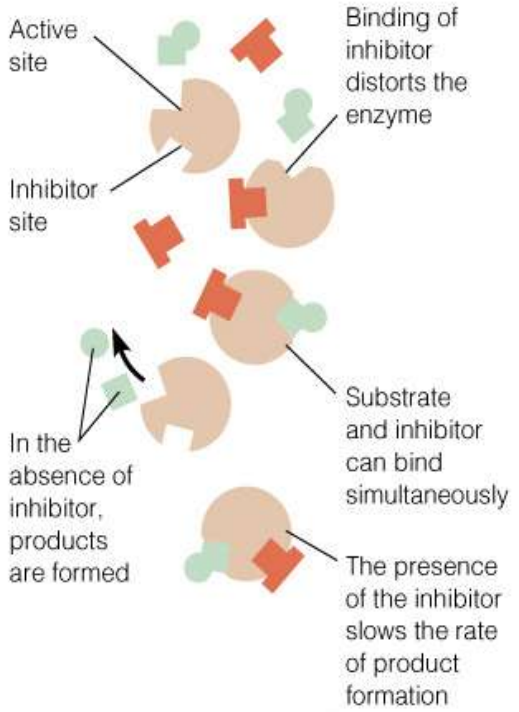
- Michaelis-Menten plot of the reaction velocity (v) against substrate concentration [S] of normal enzyme activity (-I) compared to enzyme activity with a competitive inhibitor (+I). Adding a competitive inhibitor to an enzymatic reaction increases the K_m of the reaction, but the V_{max} remains the same.



Structures of succinate, the substrate of **succinate dehydrogenase (SDH**), and malonate, the competitive inhibitor. Fumarate (the product of SDH action on succinate) is also shown.

Noncompetitive inhibition





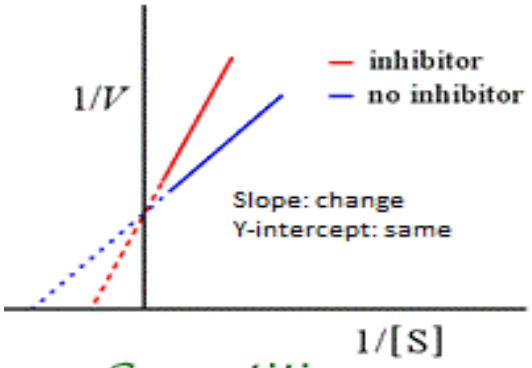
- **Noncompetitive Inhibitors Lower *V* max But Do Not Affect *K* m**

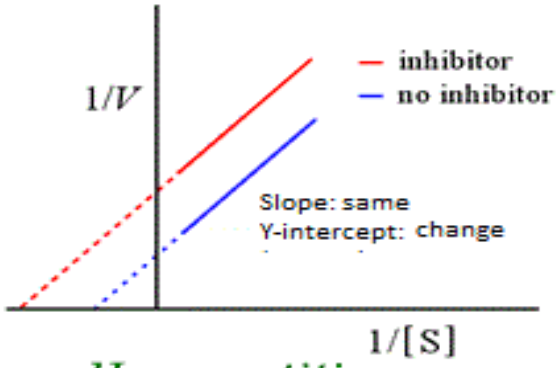
- a Noncompetitive inhibitors are usually structurally unrelated to the substrate, and bind to an **allosteric site** on the enzyme (i.e., one other than the active site) Thus, both the substrate and inhibitor theoretically can bind to the enzyme at the same time. The inhibitor, however, usually **distorts the active sites**, thus altering conformation of their catalytic residues (which further reduces effectiveness of the **ES** complex). Since the effect of a noncompetitive inhibitor cannot be reversed by increasing substrate concentrations, $\mathbf{K_m}$ remains unchanged; however,
- there is a reduction in the pparent $\mathbf{V_{max}}$ ($\mathbf{V_{max}}'$)

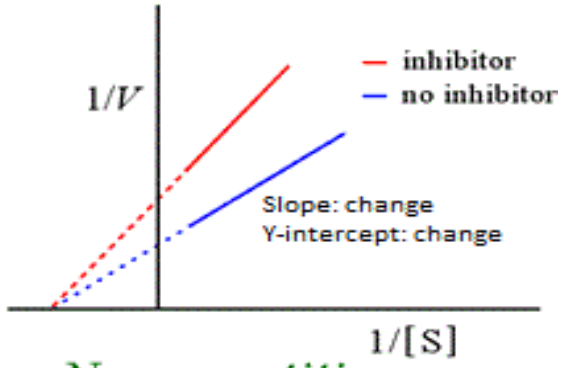
Non competitive inhibitor

Inhibitor	Enzyme inhibited
Heavy metals – Ag^{2+} ,Hg^{2+} , Pb^{2+}	Binding with cysteinyl SH gr of E
Pepstatin	Pepsin
Soyabean trypsin inhibitor	Trypsin
Ethanol or narcotic drugs	Acid phosphatase

Lineweaver-Burk plots for enzyme inhibition



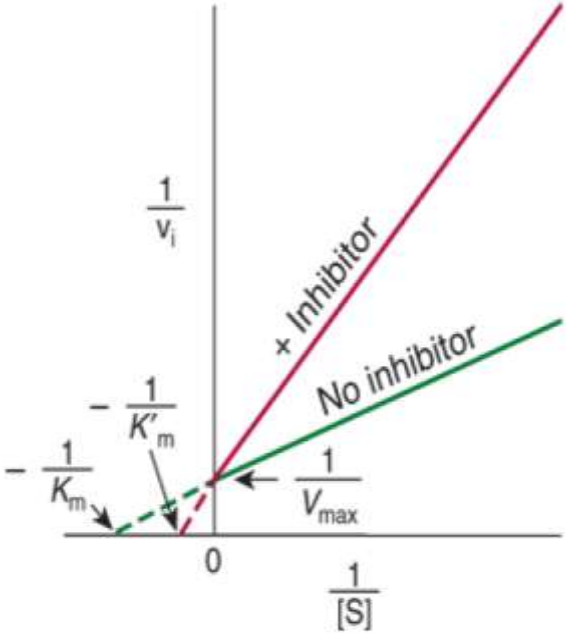


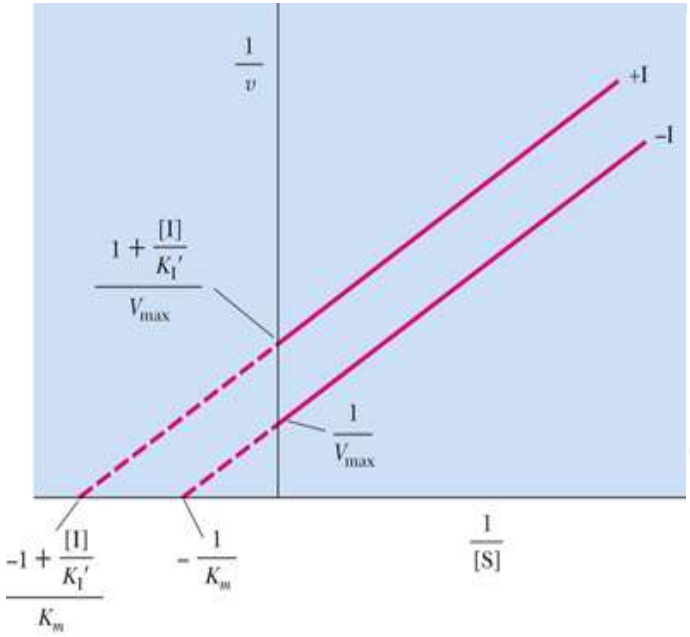


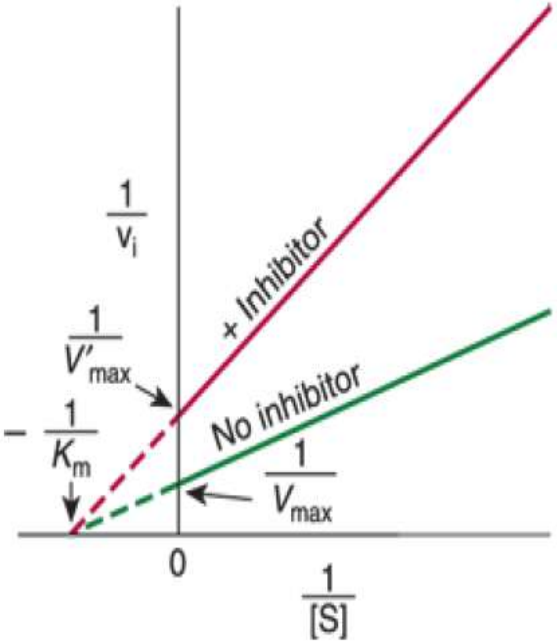
Competitive inhibition
K_M increased
V_{max} unaffected

Uncompetitive inhibition
K_M reduced
V_{max} reduced

Noncompetitive (Mixed) inhibition
K_M unaffected
V_{max} reduced

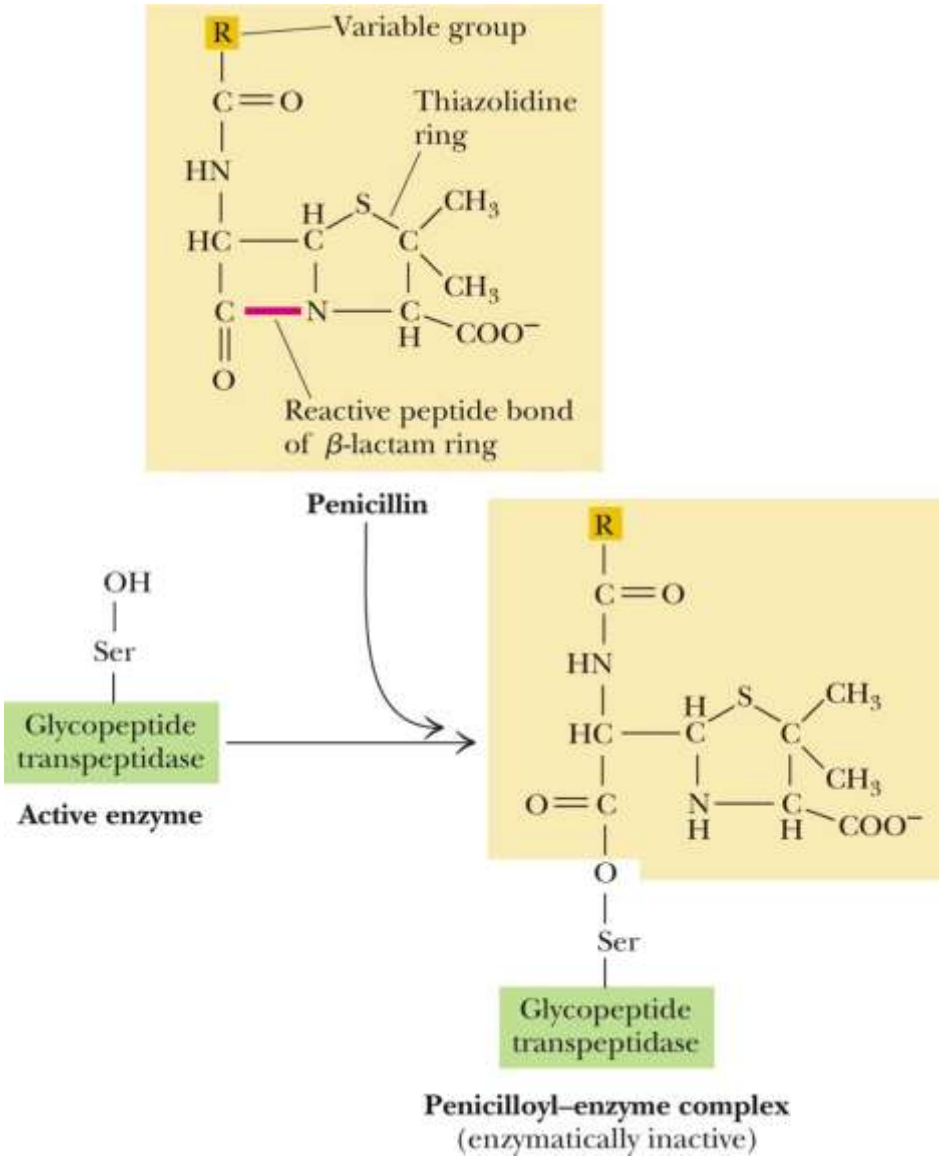






Irreversible inhibition

- Irreversible inhibition occurs when substances combine covalently with enzymes so as to inactivate them irreversibly.
- Suicide substrates are inhibitory substrate analogs proposed, via normal catalytic actions of the enzyme, a very reactive group is generated. This reactive group then forms a covalent bond with a nearby functional group within the active site of the enzyme, thereby causing irreversible inhibition
- Almost all irreversible enzyme inhibitors are toxic substances, either natural or synthetic. Such as penicillin



Penicillin is an **irreversible inhibitor** of the enzyme *glycoprotein peptidase,* which catalyzes an essential step in bacterial cell wall synthesis.

REGULATORY ENZYMES

By: Inaam A.Ameen

Regulatory enzymes

- In metabolic processes, groups of enzymes work together in successive pathway such as the multi-reaction conversion of glucose to lactate in skeletal muscle.
- In such enzyme systems the reaction product of the first enzyme becomes the substrate for the next enzyme, and so on.
- In each metabolic pathway, there is at least one enzyme that adjusts the rate of the overall sequence because it catalyzes the slowest or rate limiting reaction.
- These enzymes(regulatory enzymes) increased or decreased catalytic activity in response to certain signals.

- Regulatory enzymes have certain special properties:

  Their kinetics do not obey the Michaelis-Menten equation
  Their v versus [S] plots yield sigmoid- or S-shaped curve
  A second-order (or higher) relationship between v and [S]
  Substrate binding is cooperative

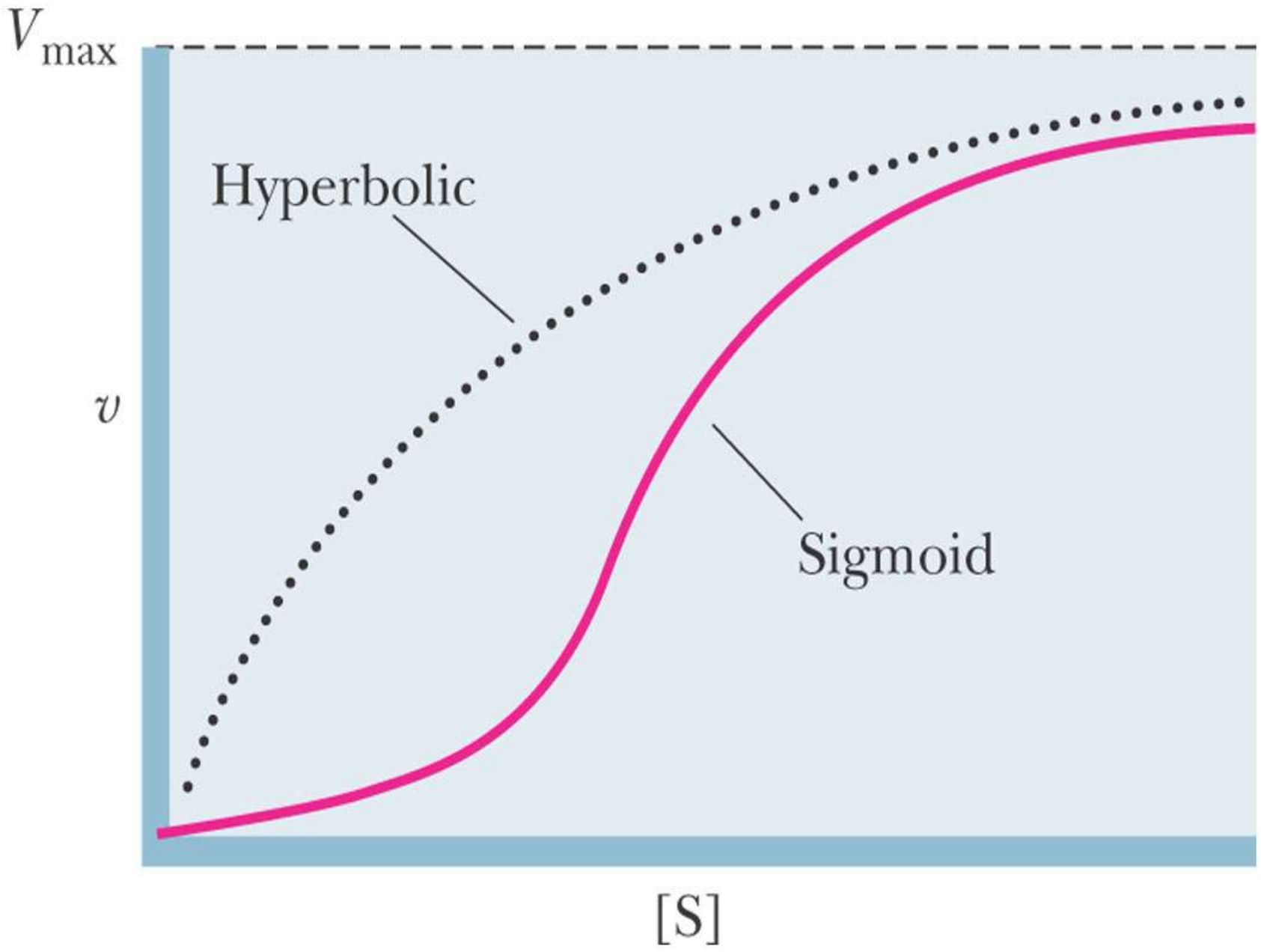
Reaction order

- Reaction order: the number of reactant molecules need to come together to generate a product

A unimolecular $S \longrightarrow P$ reaction is first order

A bimolecular $2S \longrightarrow P$ reaction is second order

A bimolecular $S1 + S2 \longrightarrow P$ is second order, first order in S1 and first order in S2.



Classification of regulatory enzymes:

1-Allosteric enzymes:

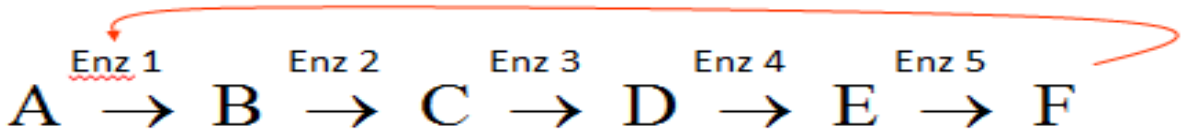
function through reversible, non-covalent binding of regulatory compounds called allosteric modulators which are in general small metabolites or cofactors

2-Enzymes regulated by reversible covalent modification

Both classes of regulatory enzymes tend to be multi- subunit proteins, and in some cases the regulatory site(s) and the active site are on separate subunits

2 types of modulators (inhibitors or activators)

- ***Negative modulator (inhibitor)***
  - –binds to the allosteric site and inhibits the action of the enzyme
  - –usually it is the end product of a biosynthetic pathway - end-product (feedback) inhibition



- ***Positive modulator (activator)***
  - –binds to the allosteric site and stimulates activity
  - –usually it is the substrate of the reaction

Amodulators of allosteric enzymes acts as activators as:

Homotropic modulator:

For which substrate and modulator are identical, the effect is similar to that of oxygen binding to the nonenzymatic protein hemoglobin, binding of the substrate causes conformational changes that affect the subsequent activity of the other sites on the protein

Heterotropic modulators:

When the modulator is a molecule other than the substrate

Allosteric enzymes

- The properties of allosteric enzymes are significantly different(structurally) from those of simple non regulatory enzymes
- In addition to the active sites, allosteric enzymes generally have one or more regulatory or allosteric sites for binding the modulator
- Just as an enzymes active site is specific for its substrate, each regulatory site is specific for its modulator.
- Enzymes with several modulators generally have different specific binding sites for each.
- In homotropic enzymes, the active site and regulatory site are the same, (the same binding site on each subunit may function as both the active site &the regulatory site)
- .

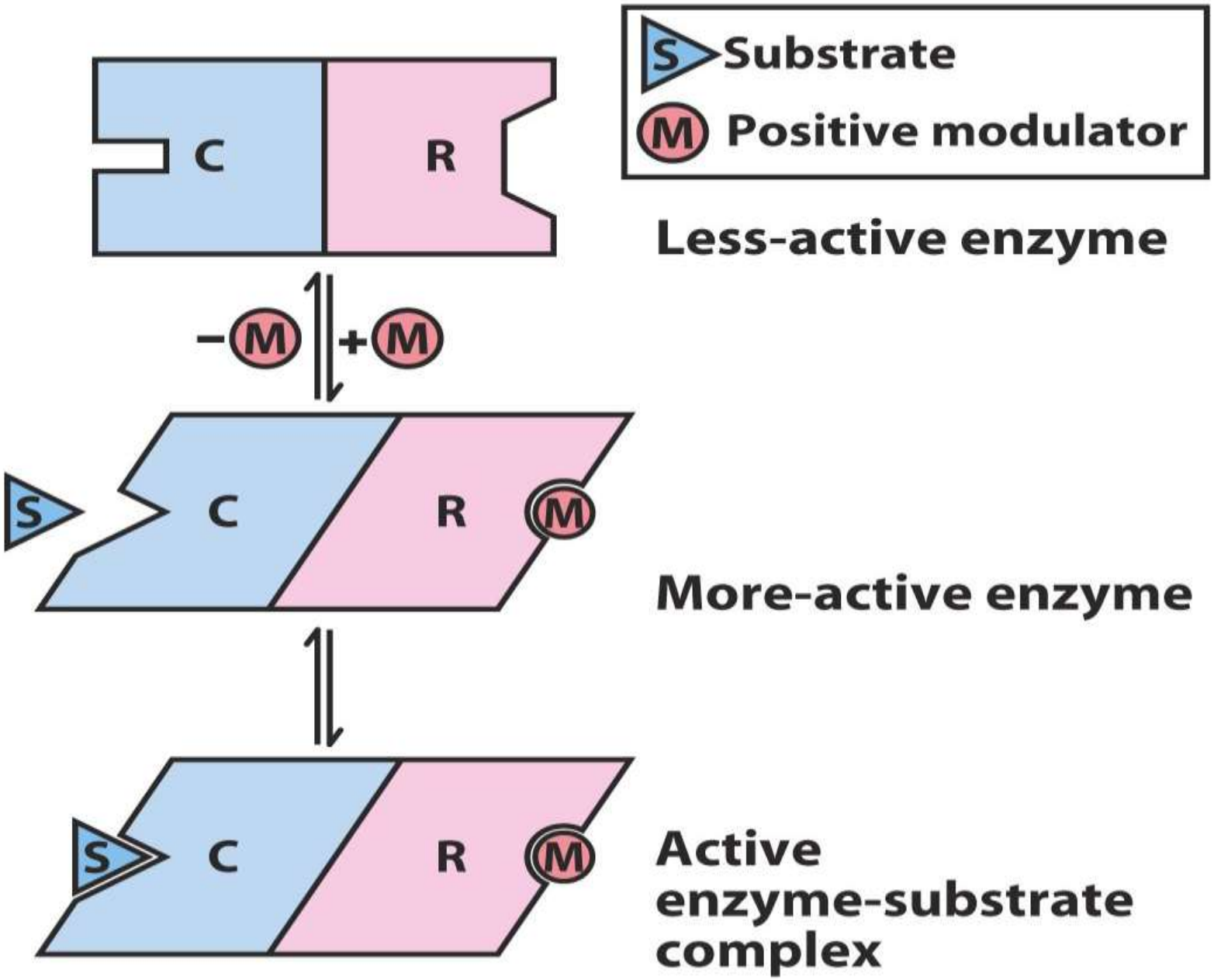
Allosteric enzymes continue •

- The substrate can be a positive modulator(an activator) because the subunits act cooperatively; the binding of one molecule of substrate to one binding site alters the enzyme conformation and enhances the binding of the subsequent substrate molecules

- Allosteric enzymes are generally larger and more complex than nonallosteric enzymes Most have two or more polypeptide chains or subunits.

- Aspartate transcarbamoylase, which catalyzes the first reaction in the biosynthesis of pyrimidine nucleotides has 12 polypeptide chains organized in to catalytic and regulatory subunits.

Allosteric Effectors – Bind to Allosteric Site



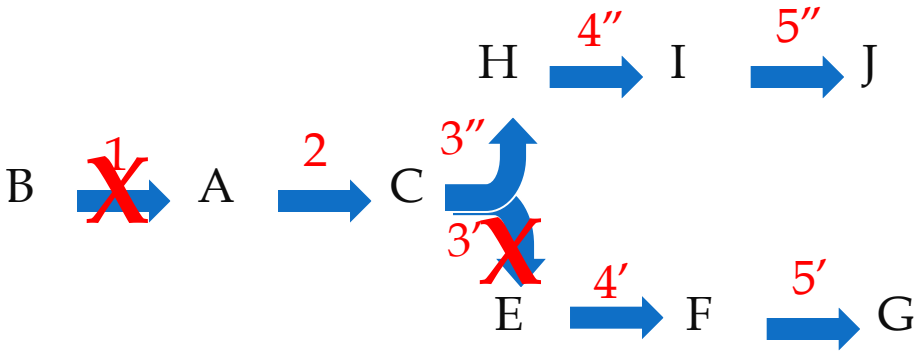
The regulatory step in many pathways is catalyzed by an allosteric enzyme

- In some multi enzyme systems, the regulatory enzyme is specifically inhibited by the end product of the pathway

- Whenever the concentration of the end product exceeds the cell requirements
- When the regulated enzyme reaction is slowed, all subsequent enzymes operate at reduced rates as their substrates are depleted.

Regulation of Enzyme Activity (biochemical regulation)

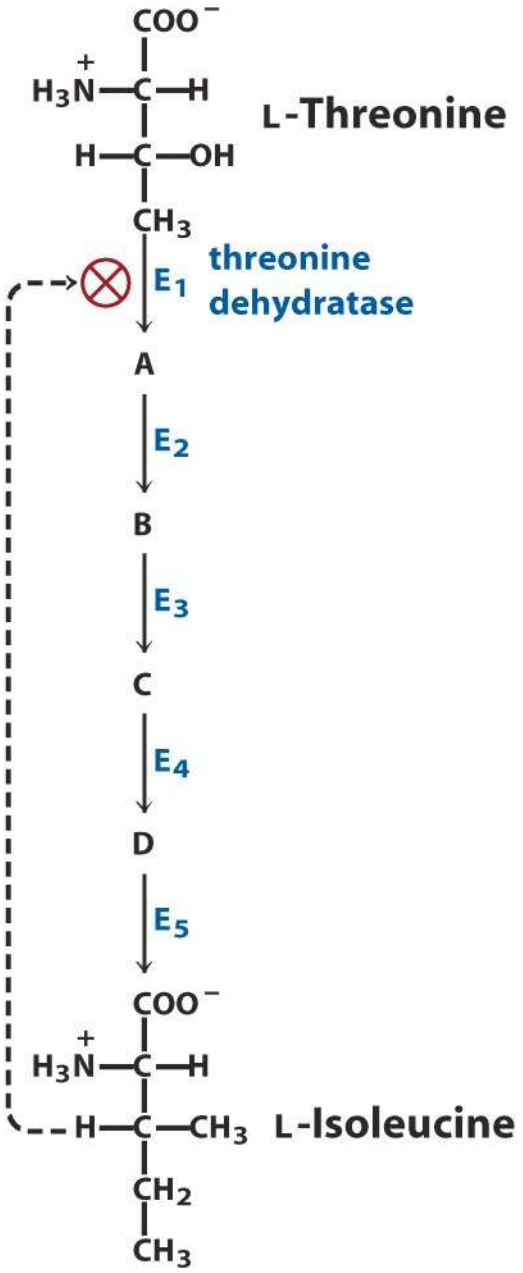
- 1st step of a biosynthetic pathway or enzymes at pathway branch points often regulated by feedback inhibition.



- Feedback inhibition may ocurrs at different points of branched biochemical pathway

- The rate of production of the pathways end product is thereby brought into balance with the cells needs. This type of regulation is called feedback inhibition
  - Example of allosteric feedback inhibition was bacterial enzyme system that catalyzes the convertion of L-threonine to L-isoleucine in five steps:
  - In this system the first enzyme threonine dehydratase, is inhibited by isoleucine(the product of the last reaction of the series). This is example of heterotropic allosteric inhibition.

Feedback Inhibition is the Classic Form of Allosteric Inhibition



Kinetic properties of allosteric enzymes deviate from Michaelis-Menten behavior:

- Allosteric enzymes show relationship between V_0 and [S] that differ from Michalis -Menten kinetics
- They do exhibit saturation with the substrate when [S] is sufficiently high, but for some allosteric enzymes, when V_0 is plotted against [S] a sigmoid saturation curve results, rather than the hyperbolic curve typical of nonregulatory enzymes.
- Although we can find a value of [S] on the sigmoid saturation curve at which V_0 is half maximal, we can not reffer to it Km because the enzyme dose not follow the hyperbolic michalis -Menten relation ship. Instead the symbol$[S]_{0.5}$ or $K_{0.5}$ is often used to represent the substrate concentration giving half maximal velocity of the reaction catalyzed by an allosteric enzyme.

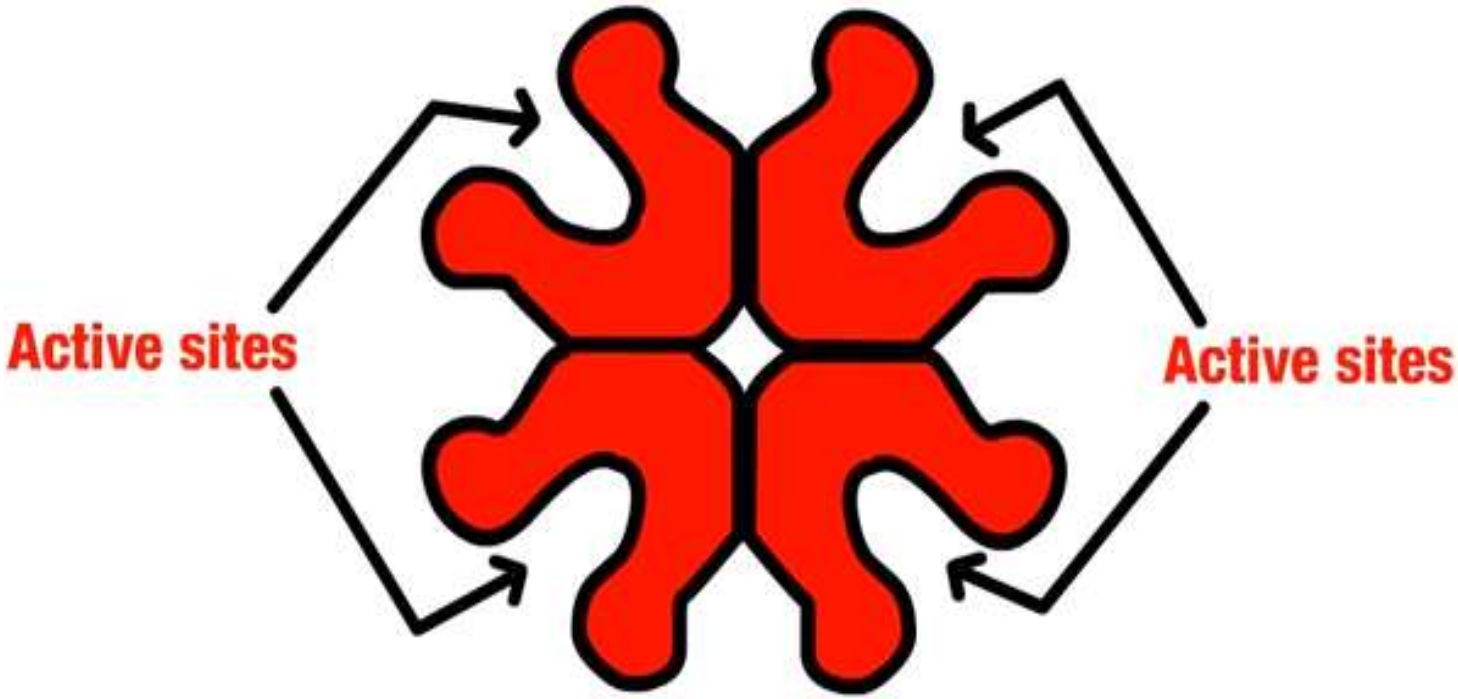
Kinetics of allosteric enzymes

- Sigmoid kinetic behavior represent cooperative between multiple protein subunits that is mean changes in the structure of one subunit are translated into structural changes in adjacent subunits(this effect is mediated by non-covalent interactions at the subunit-subunit interfaces, example: O_2 binding to hemoglobin

- Homotropic allosteric enzymes generally have multiple subunits, the same binding site on each subunit may function as both the active site and the regulatory site

- The substrate can be a positive modulator(an activator) because the subunits act cooperatively(the binding of one molecule of substrate to binding site alters the enzymes conformation and enhances the binding of subsequent substrate molecules(Sigmoid model).

Subunits of enzyme acts cooperatively

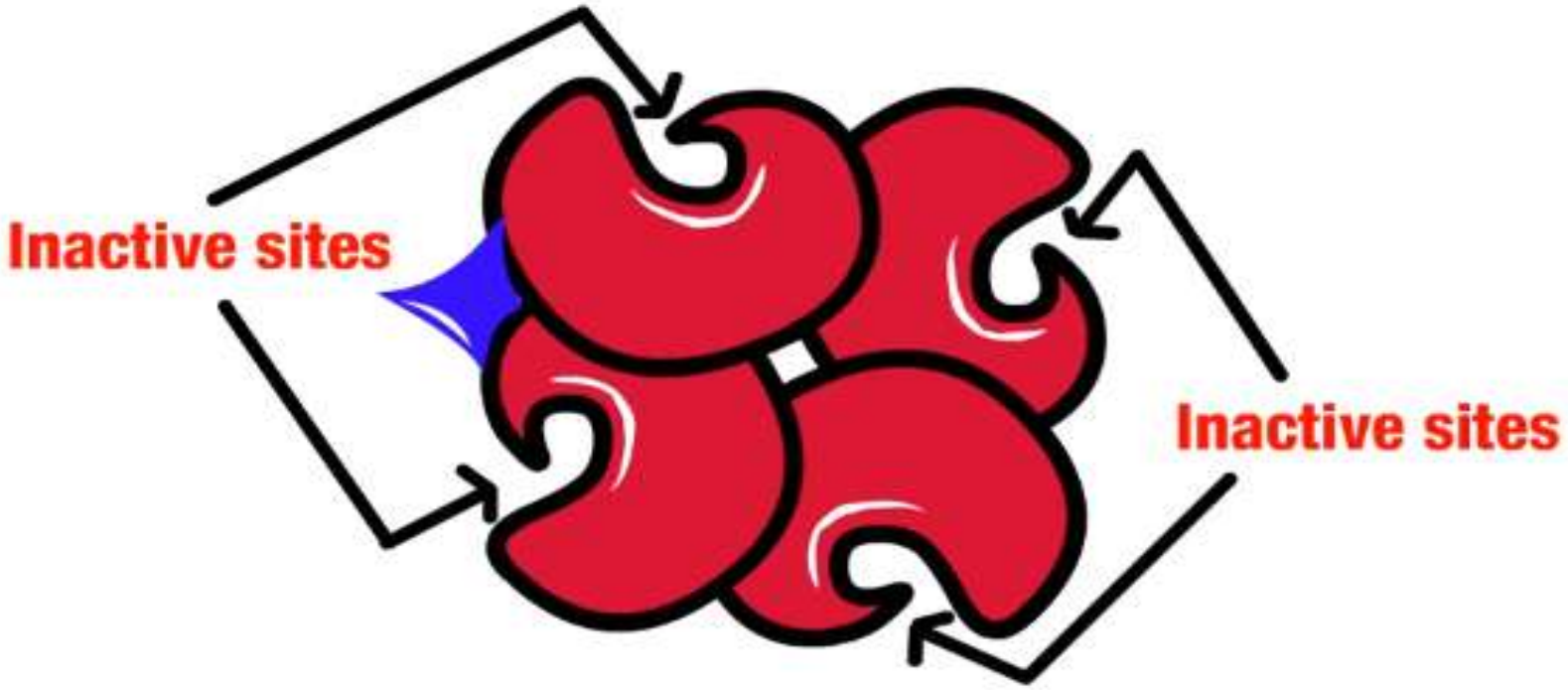


Allosteric enzymes are constructed from two or more polypeptide units, each having its own active site.

Inactive state

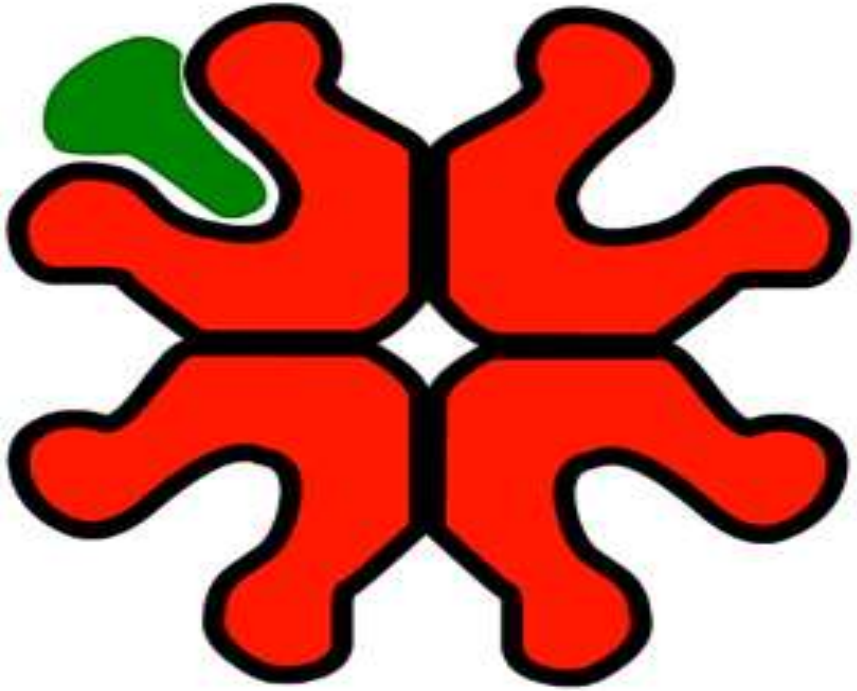
The enzyme oscillates between two states.
The active state and the inactive state.

Inhibitor modulator



An inhibitor stabilizes the inactive form.
A single activator or inhibitor will affect the active sites of all the subunits.

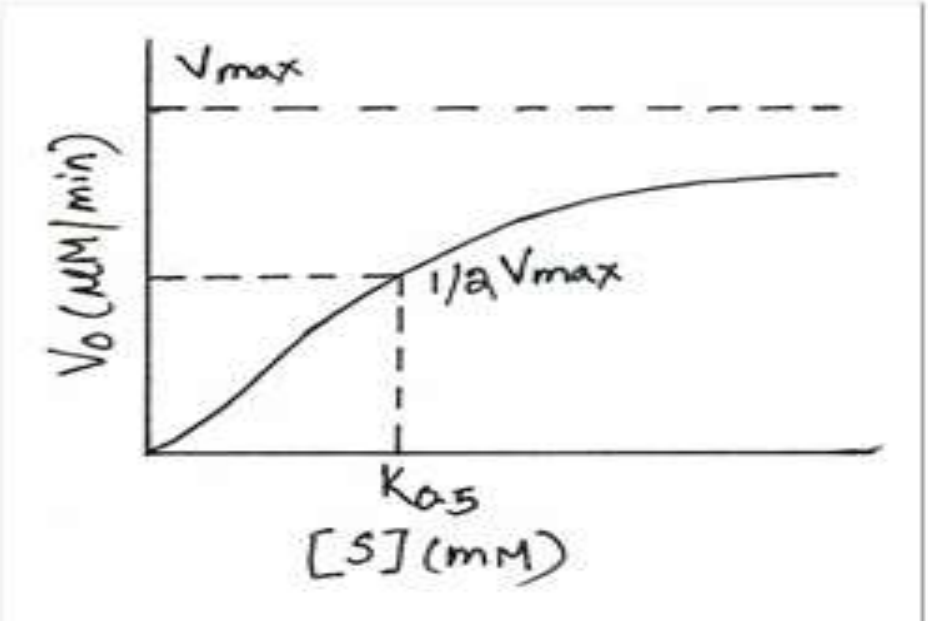
Activator(substrate) modulator



Cooperativity is the binding of one substrate molecule to an active site causing the remaining subunits to assume their active state.

- The substrate can be a positive modulator(an activator) because the subunits act cooperatively(Sigmoid model).

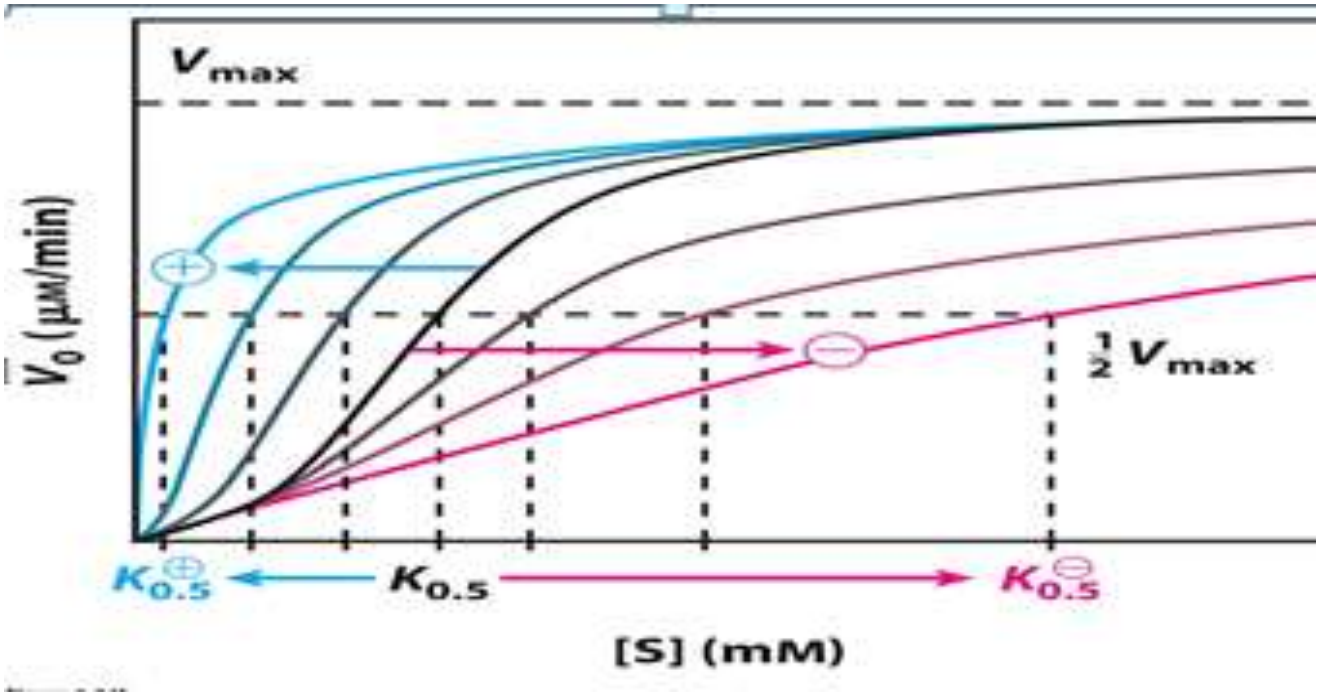
- One characteristic of sigmoid kinetics that small changes in the concentration of modulator can be associated with large changes in activity



- The sigmoid curve of a homotropic enzyme in which the substrate also serves+ve modulator(activator)

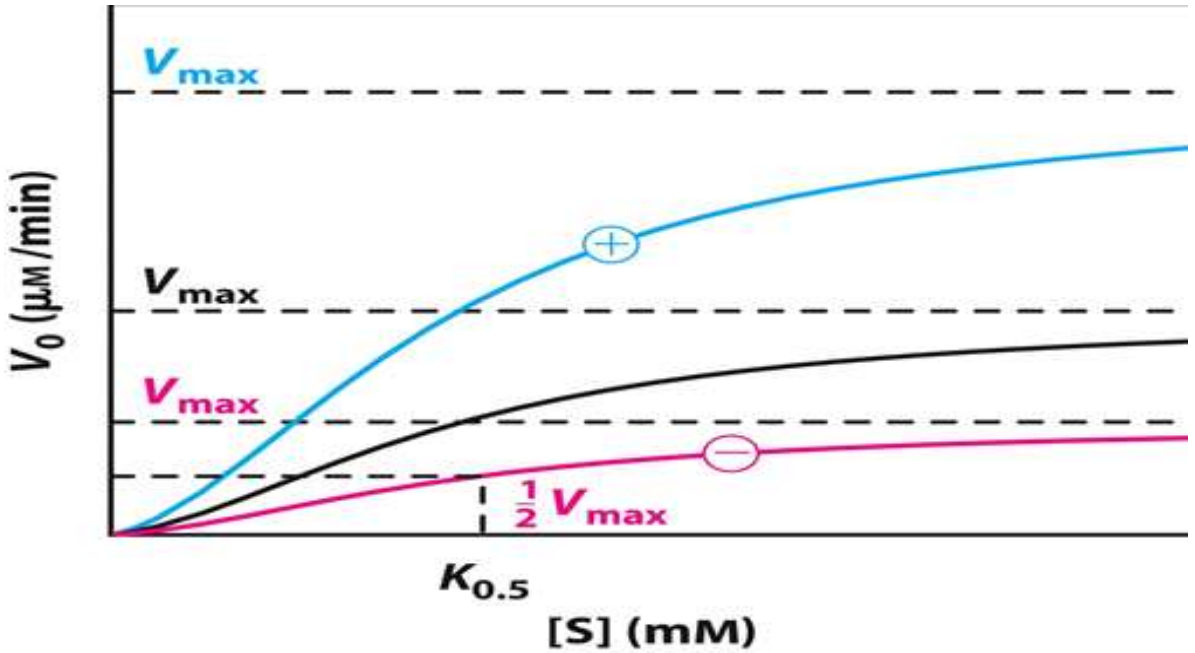
For heterotropic allosteric enzymes, with a modulator that is a metabolite other than the substrate itself, it is difficult to generalize about the substrate saturation curve.

An activator may cause the curve to become more nearly hyperbolic, with a decrease in $k_{0.5}$ but no change in V_{max} , thus resulting in an increased reaction velocity at a fixed substrate concentration(V_0 is higher for any value of [S].



The effects of a +ve and _ve modulators on an allosteric enzyme in which $k_{0.5}$ is altered without a change in V_{max} (the central curve show the substrate activity relationship without a modulator)

Other heterotropic allosteric enzymes respond to activator by an increasein V_{max} with little change in $k_{0.5}$



A less common type of modulation in which V_{max} is altered and $k_{0.5}$ is nearly constant

So heterotropic allosteric enzymes therefore show different kinds of responses in their substrate activity curves, because some have activating modulators, some have inhibitory modulators ,and some have both.

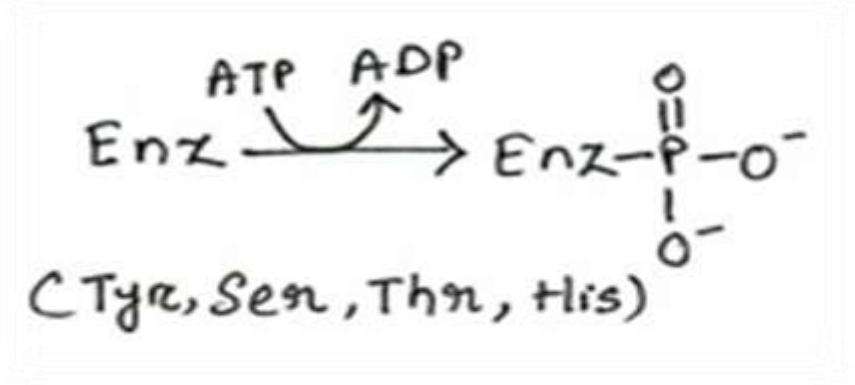
Reversible covalent modification:

- In which activity is modulated by covalent modification of enzyme molecule. modifying groups include
- phosphoryl,adenylyl,uridylyl,adenosine diphosphate ribosyl, and methyl groups. These groups are generally covalently linked to and removed from the regulatory enzyme by separate enzymes.

- Example: regulation of glycogen phosphorylase activity by covalent modification.

Phosphorylation

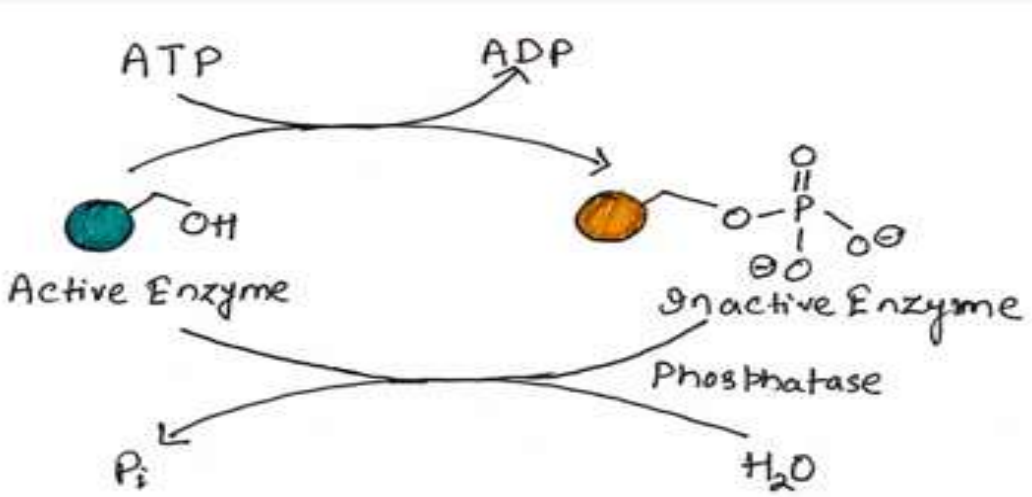
- Phosphorylation is the most common type of regulatory modification.
- Some enzymes are phosphorylated on a single amino acid residue while others are phosphorylated at multiple sites.
- To serve as an effective regulatory mechanism, phosphorylation must be reversible. In general, phosphoryl groups are added and removed by different enzymes, and the processes can therefore be separately regulated.



phosphorylation of enzymes

The attachment of phosphoryl groups to specific amino acid residues of a protein is catalyzed by protein kinases; removal of phosphoryl groups is catalyzed by protein phosphatases.

The phosphoryl groups are attached to serine, thereonine, or tyrosine residues, thus introducing a bulky charged group into the enzyme



Phosphorylation and dephosphorylation of enzyme

- **Glycogen phosphorylase** is found in glycogen granules, this enzyme is typically an allosteric enzyme.it binds inorganic phosphate cooperatively.
- **Glycogen phosphorylase** generates glucose-1-phosphate which is isomerized into glucose-6-phosphate and enters the glycolytic pathway to produce ATP. This end product ATP is a feed back inhibitor of glycogen phosphorylase. Glucose-6-phosphate is an allosteric inhibitor of the enzyme. ATP and glucose-6-phosphate produce a negative effect on the cooperativity of substrate binding.
- **AMP** is also an allosteric effector of glycogen phosphorylase. It competes for the same allosteric binding site as ATP but stimulates glycogen phosphorylase by having a positive effect on the cooperativity of substrate binding.

- Increase in the cellular concentration of AMP is an indicator that the energy status of the cell is low and more ATP via glycolysis needs to be produced. The reciprocal changes of ATP and AMP concentrations combined with their competition for the allosteric binding site with opposite effects provide a mechanism for rapid and reversible control over glycogenolysis

- **Regulation by covalent modification is slower than allosteric regulation**
- **Require one enzyme for activation and one enzyme for inactivation**

Enzyme Regulation by Covalent Modification

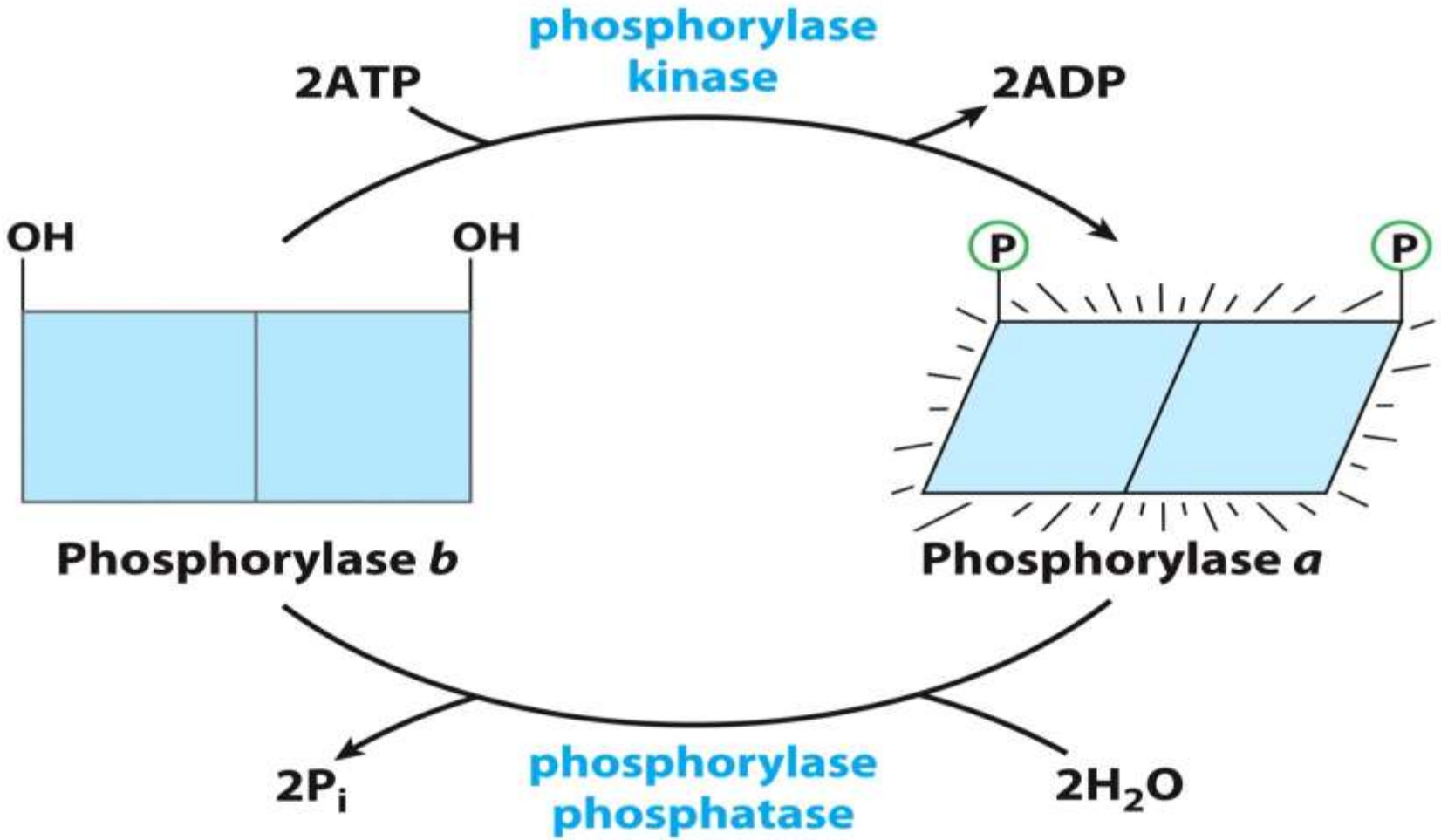


Figure 6-36
Lehninger Principles of Biochemistry, Sixth Edition

- Muscle glycogen phosphorylase is a dimer of two identical subunits (842 residues)
- Each subunit contains a pyridoxal phosphate cofactor covalently linked (Lys-680)
- An active site
- An allosteric effector site near the subunit interface
- A regulatory phosphorylation site (Ser-14)
- A glycogen binding site
- A tower helix (residues 262 to 278)

Multisubstrate Reactions

- In many enzymatic reactions two(and sometimes more than two),different substrate molecules bind to the enzyme and participate in the reaction .

For example, in the reaction catalyzed by hexokinase, ATP and glucose are the substrate molecules, and ADP and glucose 6-phosphate are the products

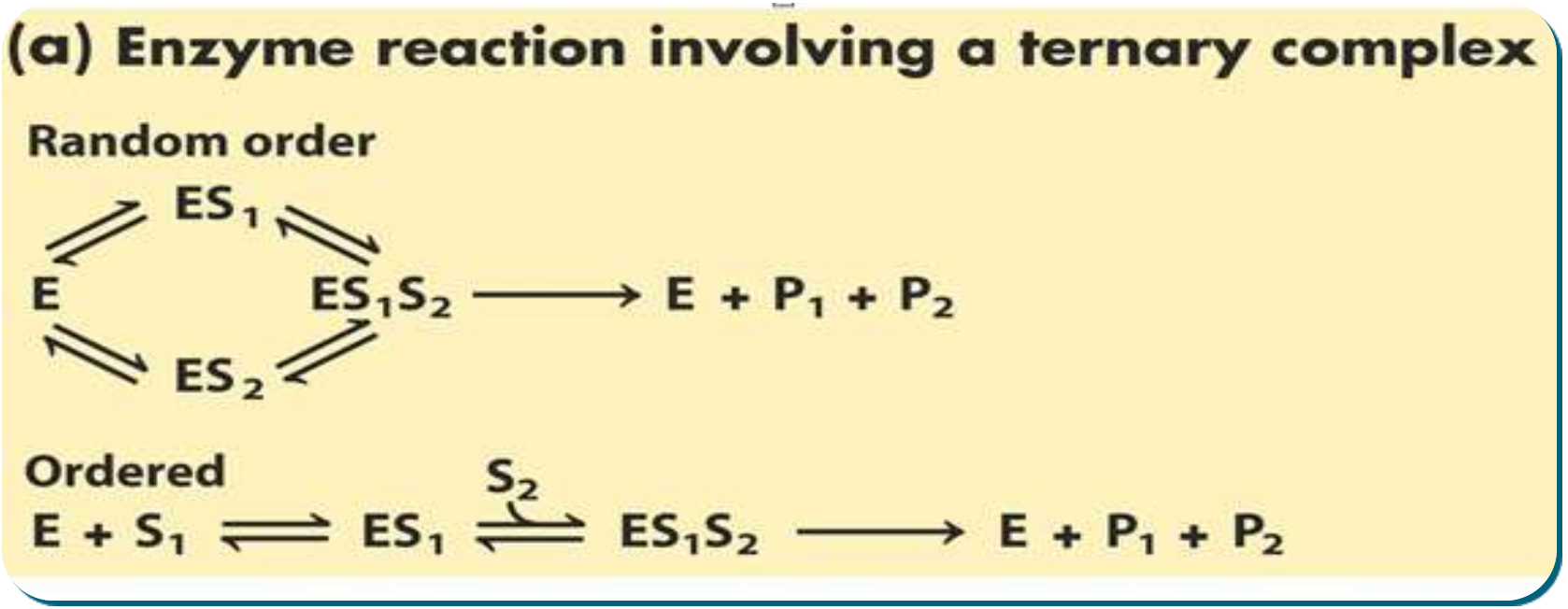
D-Glucose + ATP → Glucose-6-phosphate (G6P) + ADP

The rate of such substrate reactions can also be analyzed by Michaelis-Menten approach. Hexokinase has a charactristic km for each of its substrates.

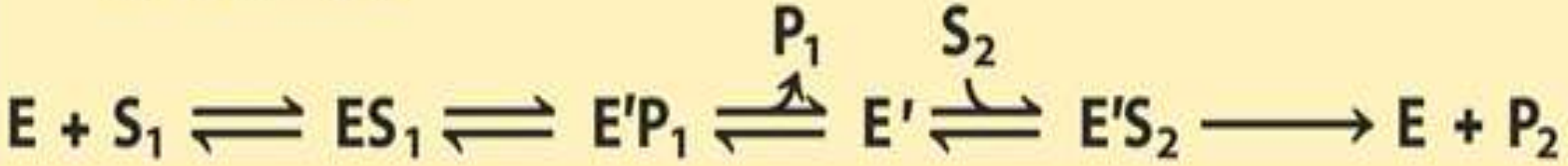
Enzymatic reactions with two substrates usually involve transfer of an atom or a functional group from one substrate to the other.

Multisubstrate reactions proceed by one of several different pathways

In some cases, both substrates are bound to the enzyme concurrently at some point in the course of the reaction, forming anon-covalent ternary complex



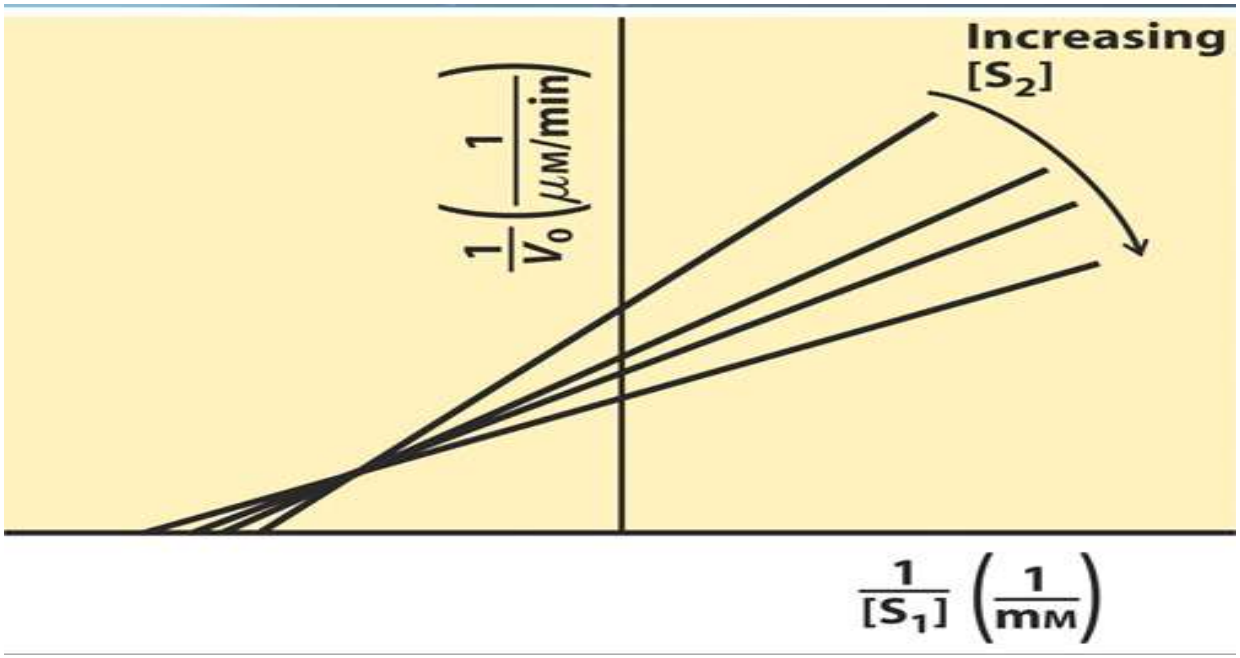
(b) Enzyme reaction in which no ternary complex is formed



- In such pathway, no ternary complex is formed, in which the first substrate is converted to product and dissociates before the second substrate binds. An example of this is the ping-pong or double-displacement mechanism

Steady_state kinetics:

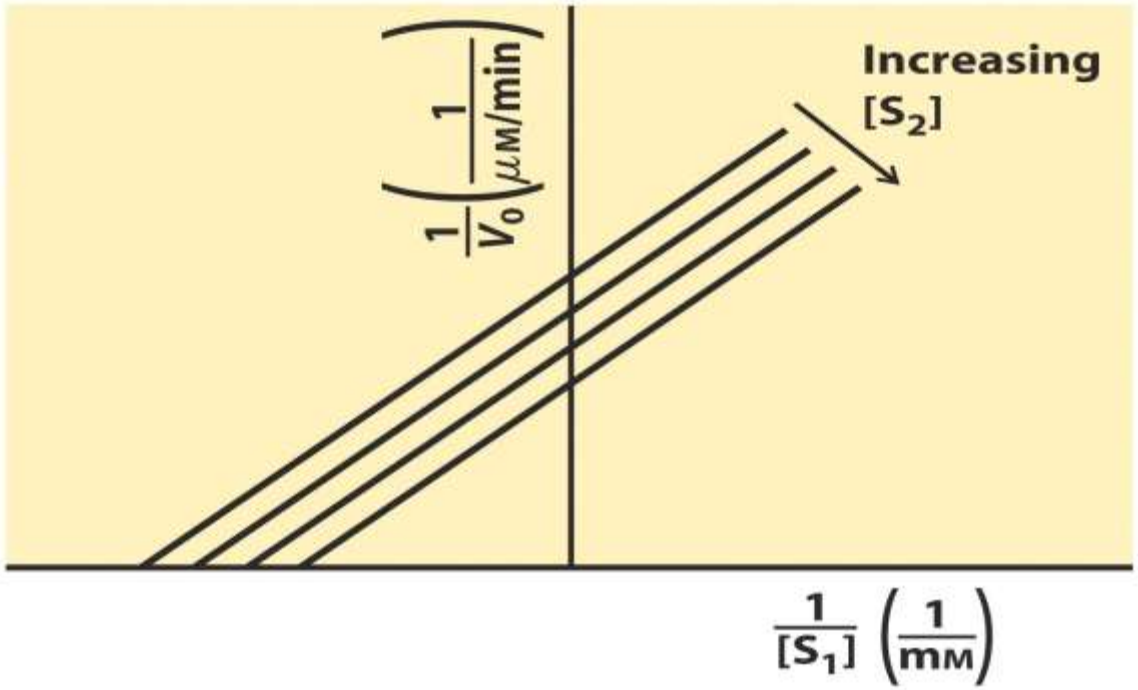
- Steady-state kinetics can often help distinguish among these possibilities:



- The reaction is repeated for several values of $[S_2]$, generating several separate lines.
- Intersecting lines indicat e that a ternary complex is formed in reaction.

Lineweaver Burke Plot – Enzymes Without Ternary Complexes

- Parallel lines indicate a ping pong or double – displacement pathway



- Big difference from substrates forming a ternary complex with the enzyme

Presteady state kinetics can provide evidence for specific reaction steps

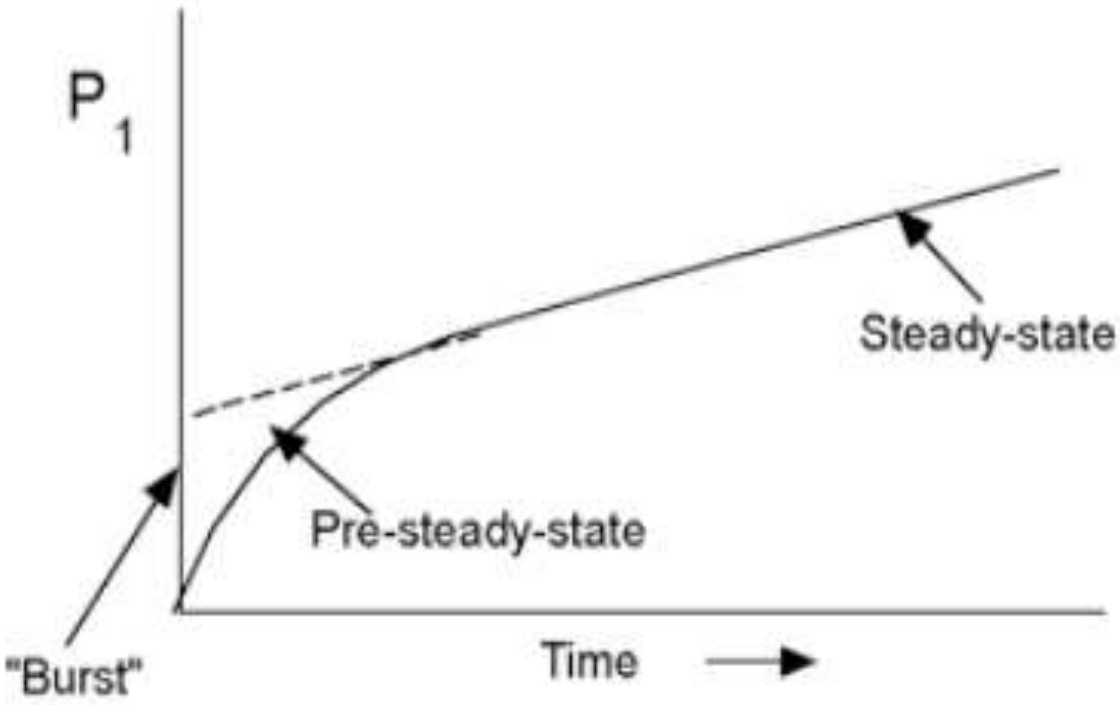
- A complete description of an enzyme-catalyzed reaction requires direct mesurment of the rates of individual reaction steps.

- Reaction conditions are adjusted to facilitate the measurment of events that occur during reaction of a single substrate molecule.

- Because the pre-steady state phase is generally very short, this often requires specialized techiques for very rapid mixing and sampling.

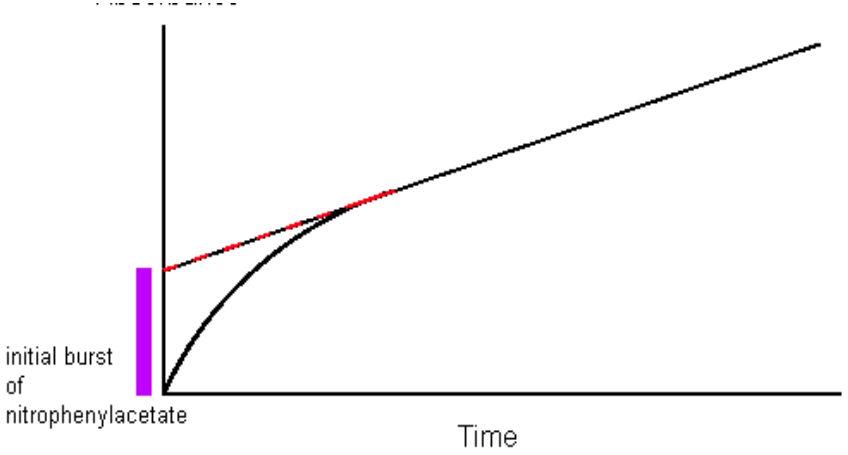
- Reaction rates and equilibria are related to the free energy occur during the reaction measuring the rate of individual reaction steps shows how energy is used by specific enzyme, which is an important component of the over all reaction mechanism.

Presteady state kinetics



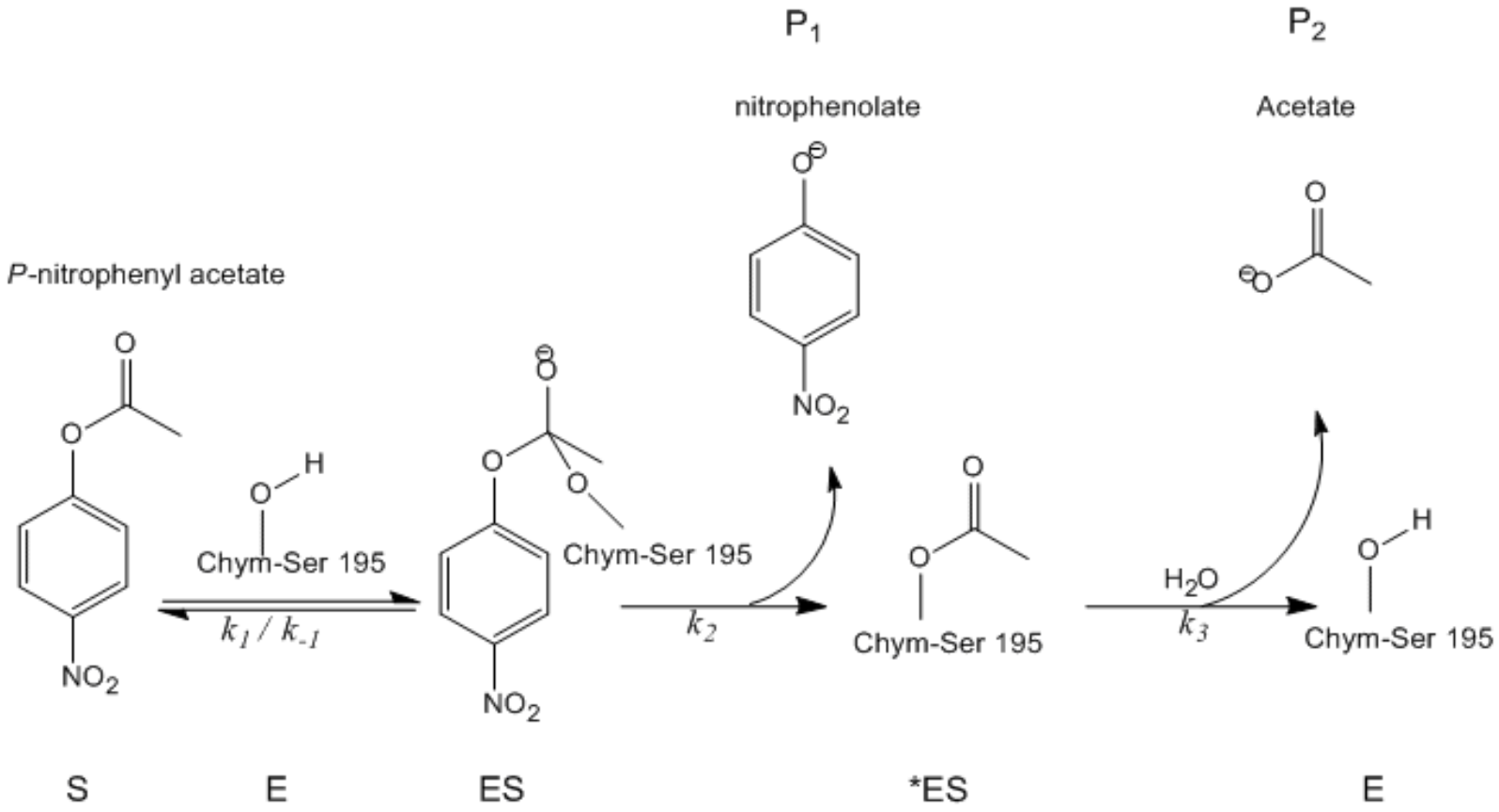
The kinetics of chymotrypsin catalyzes the hydrolysis of p-nitrophenol acetate

- The first step, the initial burst of nitrophenolate , is the fastest. The attack of nitrophenylacetate substrate chymotrypsin immediately cleaves the nitrophenolate moiety and leaves the acetate group attached to chymotrypsin.



- The second step has been assumed to involve the hydrolysis of the acetate group from the inactivated chymotrypsin to regenerate the original enzyme.

The hydrolysis of p-nitrophenolacetate



Isoenzymes

- Are enzymes that catalyze the same reaction but have different molecular forms.
- They do not have the same physical properties of genetically determined differences in amino acid sequence.
- For this reason, isoenzymes may contain different numbers of charged amino acids and therefore separated from each other by electrophoresis.
- Isoenzymes have different properties such as sensitivity to particular regulatory factors or substrate affinity that adapt them to specific tissues or environments.

Lactate dehydrogenase(LDH)

- Found in the cells of many body tissues specially the heart, liver, red blood cells, kidneys, skeletal muscle, brain and lungs.
- When a disease or injury affects the cells that contain LDH, the cell lyse, and LDH spilled in to the blood stream, where it is identified in higher than normal levels.
- The LDH is a measure of total LDH(which is not specific indicator of any one disease affecting any one organ).

Lactate dehydrogenase

- LDH occurs in 5 possible forms(isoenzymes) in the blood serum :
- Actually the five separate fractions make up the total LDH.
- LDH_1 comes mainly from heart
- LDH_2 from the reticuloendothelial system
- LDH_3 comes from lungs &other tissues
- LDH_4 from kidney, placenta and pancreas
- LDH_5 mainly from the liver and striated muscle

Clinical importance of LDH

Acute myocardial infarction

LDH_1 and LDH_2

Acute liver damage

LDH_4 and LDH_5

Clinical importance of LDH

- Normally, LDH_2 makes up the greatest % of total LDH.
- With myocardial injury, the serum LDH level rise within 24_48hr, after the myocardial infraction.
- Peaks in 2 to 3 days & return to normal in 5-10 days
- LDH level is useful for delayed diagnosis of patients with MI

Creatine phosphokinase(creatine kinase)

- Creatine phosphokinase (CPK) is found mostly in the heart muscle, skeletal muscle and brain.
- Serum CPK levels are elevated when injury occurs to these muscles or nerve cell.
- CPK levels in plasma can rise within 6hr after damage.
- Electrophoresis is performed to detect three CPK isoenzymes:
- CPK-BB(CPK_1)
- CPK-MB(CPK_2)
- CPK-MM(CPK_3)

CPK-MB(CPK_2)

- CPK-MB isoenzyme part appears to be specific for myocardial cells.
- CPK-MB levels rise from3-6hrs after infraction occurs, with peak levels at 12-24hrs and returns to normal at 12-48hrs after infraction.
- Sever injury to skeletal muscle can be significant enough to raise the CPK-MB iso enzyme above normal.
- A relative index is calculated to determin whether myocardial injury has occurred.
- The ratio of CPK-MB / total CPK is calculated to determine this index.
- An index > 2.5 is highly determine myocardial injury
- An index < 2.5 is indeterminant for myocardial injury

CpK-BB mostly found in the brain & lung, injury to either of these organs (like in cerebreovascular accident, pulmonary infarction) is associated with elevated levels of this isoenzyme.

CPK-MM isoenzyme normally comprises almost all of the circulatory total CPK enzymes in healthy people, so when the total CPK level is elevated as a result of increased CPK-MM, this would indicate injury or disease of skeletal muscle is present like in myopathies, vigorous exercise.

Alkaline phosphate(ALP)

- ALP found in many tissues, the highest concentrations are found in the liver, biliary tract epithelium and bone.
- The intestinal mucosa and placenta also contain ALP.
- Measurements of this enzyme is important for determining liver and bone disorders.
- ALP levels are greatly increased in both extrahepatic and intrahepatic obstructive biliary disease and cirrhosis.
- Other liver abnormalities such as hepatic tumors, hepatotoxic drugs, and hepatitis causes lesser elevation in ALP levels.

Alkaline phosphate(ALP)

- Bone is the most frequent extrahepatic source of ALP, new bone growth is associated with elevatedALP levels, which explains why ALP levels are high in adolescents.
- Pathologic new bone growth occurs with osteoblastic metastate eg: breast, prostate tumors.
- Paget's disease, hyperparathyrodism and normal growing bones are sources of elevated ALP level as well.
- Isoenzymes of ALP are used to distinguish between liver and bone diseases.
- ALP_1 is from liver and ALP_2 is from bone.