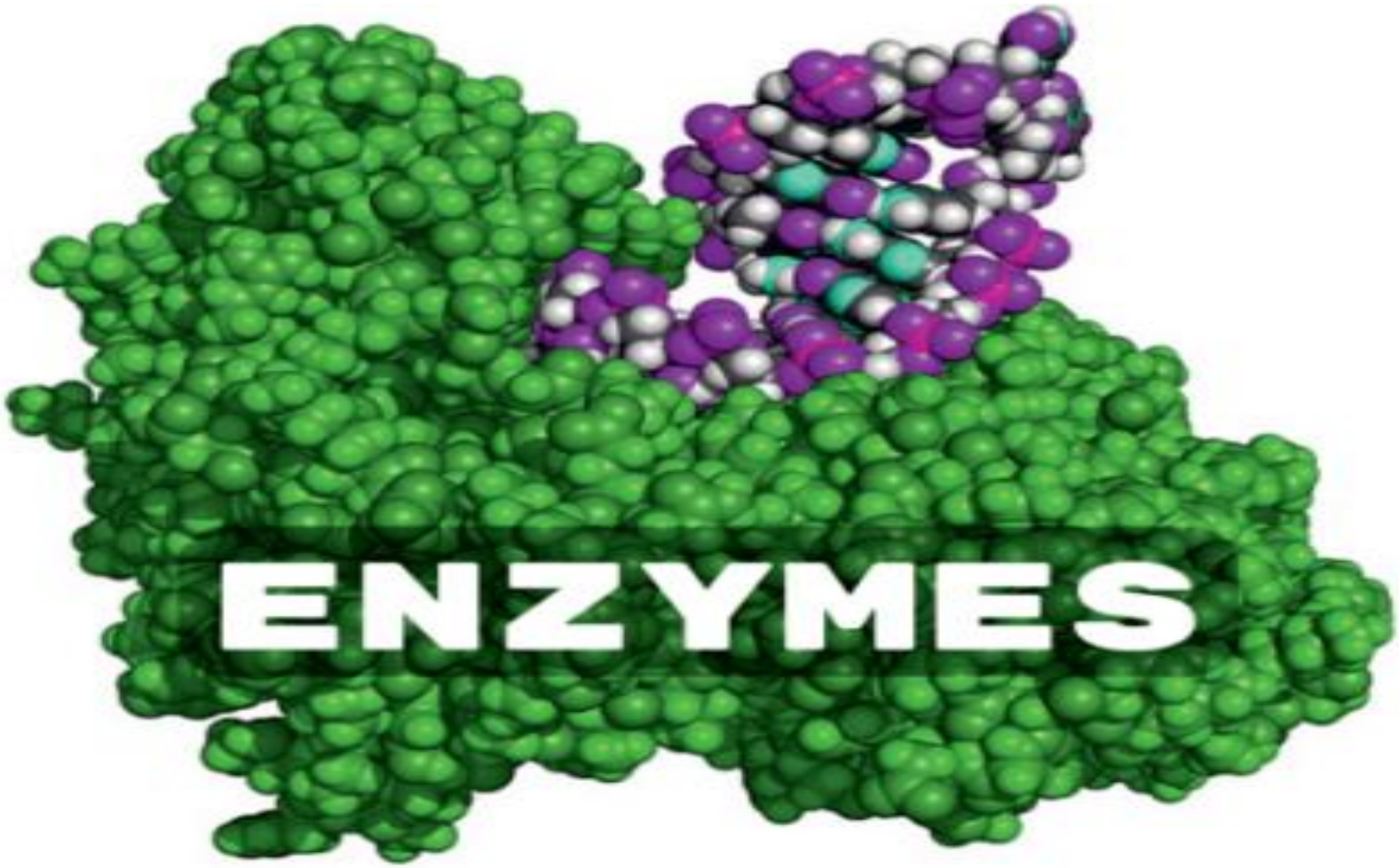
UNIT THREE

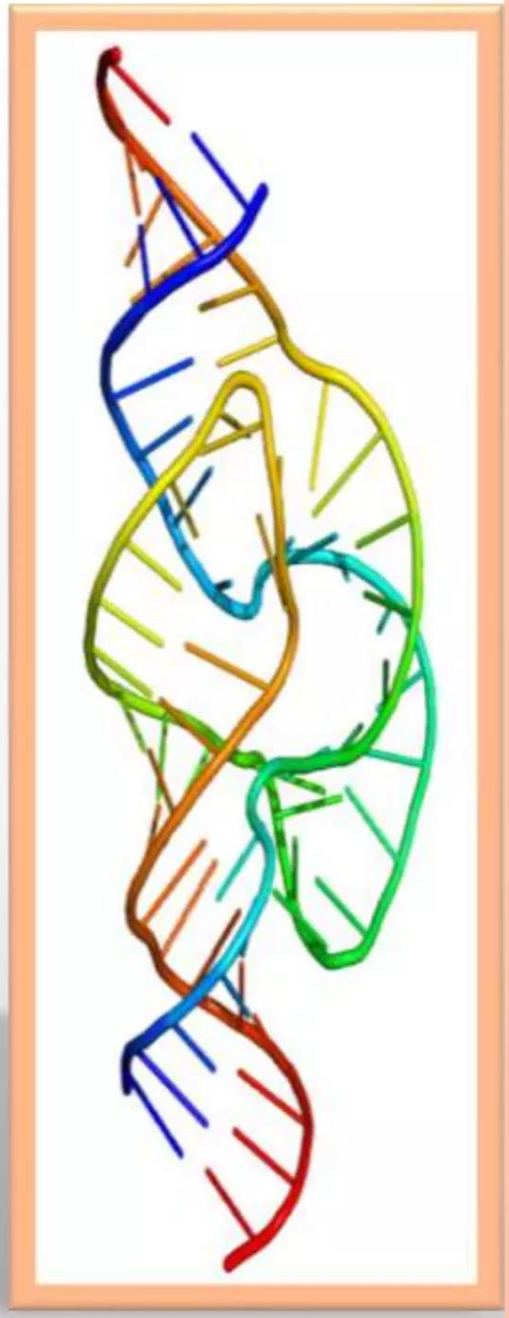


Unit learning outcomes

- After the successful completion of this unit, the student will be able to:
- Explain enzymes; their properties, factors affecting their activities, functions,
- mechanisms of action, regulation, their industrial applications and kinetics
- Demonstrate proteins and their structures

Introduction

- Enzymes are *biological catalysts* that speed up the rate of the biochemical reaction.
- Most enzymes are three dimensional *globular proteins* (tertiary and quaternary structure).
- Some special RNA species also act as enzymes and are called *Ribozymes* e.g. hammerhead ribozyme.



Hammerhead enzyme

Define enzymes
(Enzymes as Biological Catalysts)

- **Enzymes** are proteins that increase the rate of reaction by lowering the energy of activation
- They catalyze nearly all the chemical reactions taking place in the cells of the body.
- Not altered or consumed during reaction.
- Reusable

3.1. What are enzymes?

- Enzymes are protein molecules.
- Act as biological catalysts (biocatalysts) and
- **Accelerate rate of chemical reactions** by lowering activation energy.
- **Activation energy** is the minimum amount of energy required for the reactant to be converted to products.
- All enzymes are **proteins** made up of chains of amino acids linked together by **peptide** bonds.

Cont'd

- **All cells** contain different enzymes which engage in tremendous biochemical activity called metabolism.
- **Metabolism** is the process of chemical and physical changes, including:

✓ the breakdown **(catabolism)** and

✓ synthesis **(anabolism)** of molecules.

- ***The metabolic processes in the cells require enzymes to catalyze many biochemical reaction types at a rates fast enough to sustain life.***
- **Enzymes act upon molecules (substrates), convert them into products of different molecules, and remain unchanged**

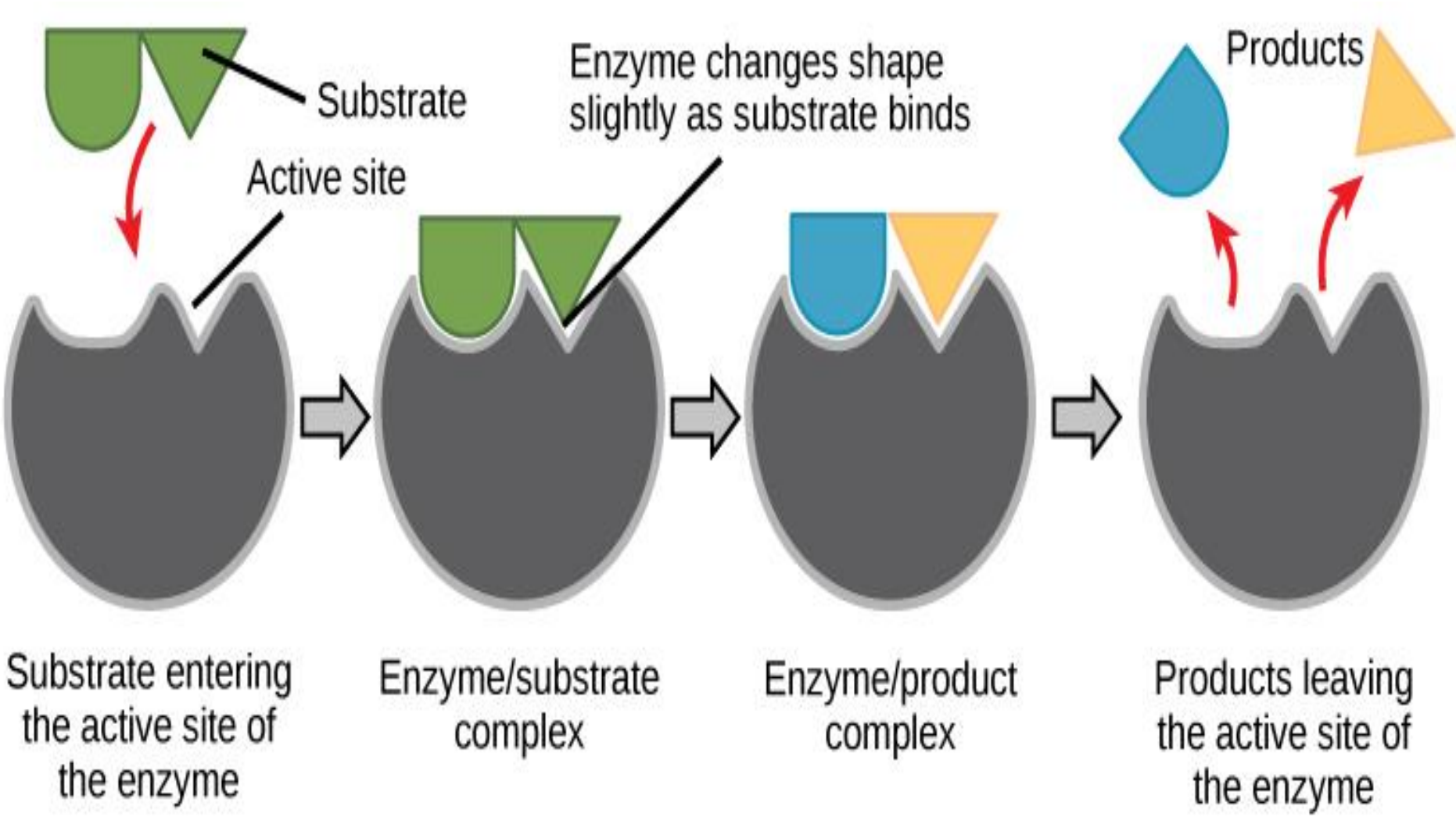


Figure 3.1 Enzymatic reactions

- The catalytic cycle of an enzyme

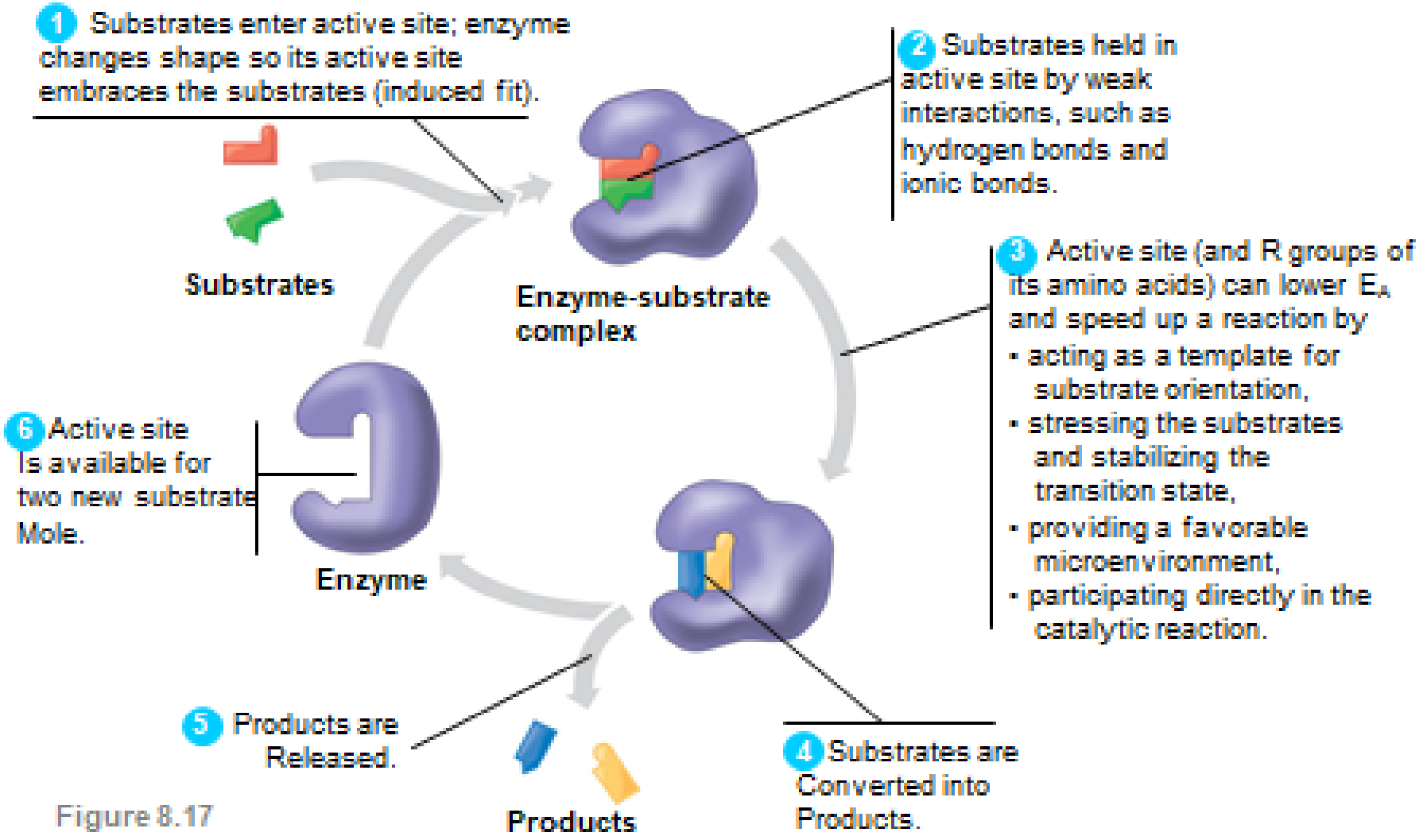


Figure 8.17

3.2 Properties and functions of enzymes

- The general properties of enzymes are the nature of both their physical and chemical properties.
- Enzymes **accelerate the reaction rates.**
- **They neither affect the nature of products formed nor undergo any changes by the reaction catalyzed.**

- A **catalyst** is a substance that speeds up a reaction; the reaction itself is unaltered.
- There is no overall change to:

✓ **the nature of the products**

✓ **the energy change that takes place during the reaction**

✓ **the catalyst itself**

A. The physical properties of enzymes

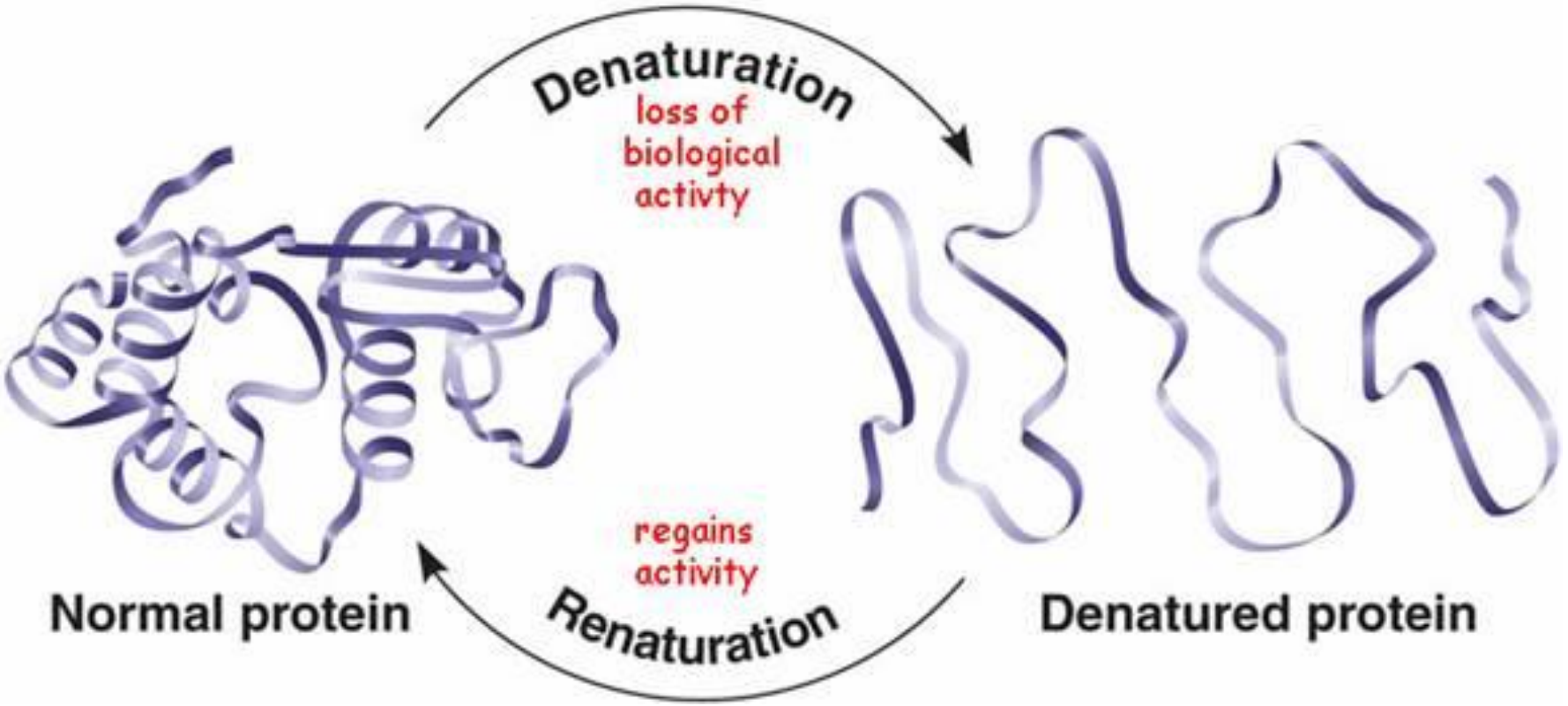
- The physical properties of enzymes include
- ✓ ***Denaturation***
- ✓ ***Solubility***
- ✓ ***Colloids***
- ✓ ***Biocatalysts***
- ✓ ***Precipitation***
- ✓ ***Molecular weight, and***
- ✓ ***Enzyme activity***.

Denaturation

- is the process of breaking the intra and inter-molecular non-covalent bonds that ***distort the shape and active site of the enzymes.***

Enzymes are denatured by:

- ✓ High heat (above 40ºC),
- ✓ Alternation in the pH (too low or too high),
- ✓ Heavy metals and
- ✓ High salt concentrations,
- ✓ Solvents and
- ✓ Other reagents.



- **Solubility** is the property of enzymes that allow them to be dissolved in water, salt (NaCl), diluted glycerol and alcohol causing denaturation

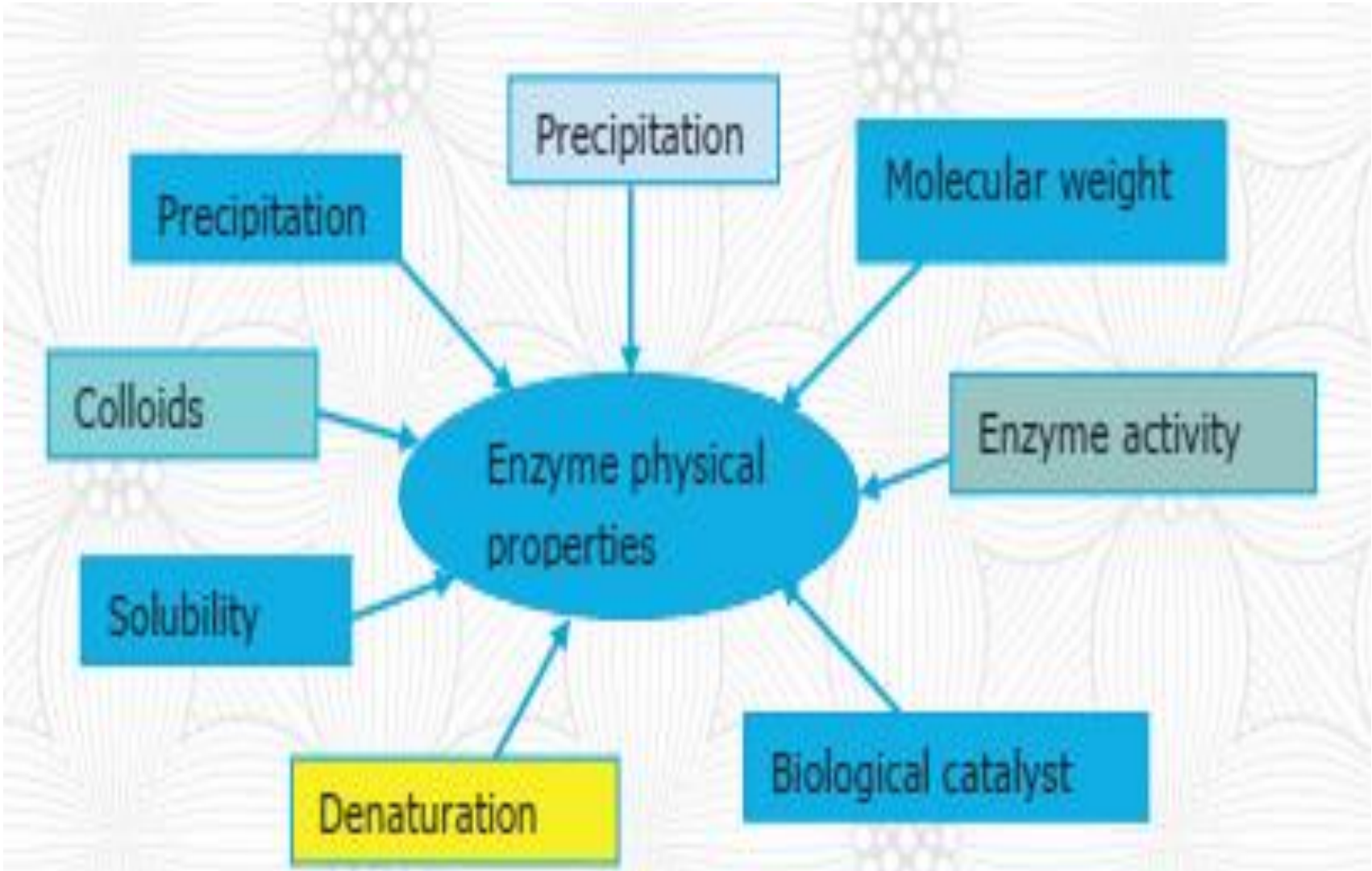


Figure 3.2 Physical properties of Enzymes

The colloidal

- Nature of enzyme is the tendency of having little or no dialysis cross the semipermeable membrane due to the large size or high molecular weight.

The biocatalyst property

- is the activity of enzymes in which very small quantities or a small amount of enzyme is enough to convert a large quantity of substrate and remain unchanged after the reaction.

Enzyme precipitation

- is the separation of enzymes for analysis using different aqueous or ethanol solvents.

Molecular weights

- of enzymes are large protein biomolecules that hold polypeptide chains of various amino acid sequences in enzymes having a high molecular weight.

Enzymatic activity

- is the general catalytic properties of an enzyme.
- It depends on factors such as temperature, pH, and enzyme concentration and substrate concentration.
- Enzymes show the highest activity at optimum temperature and pH that a low concentration of enzymes and substrates slows down the enzymatic reaction.

B. Chemical properties of enzymes

- Enzyme chemical properties are **sensitivity, regulations, specificity, catalysis and reversibility** reactions .

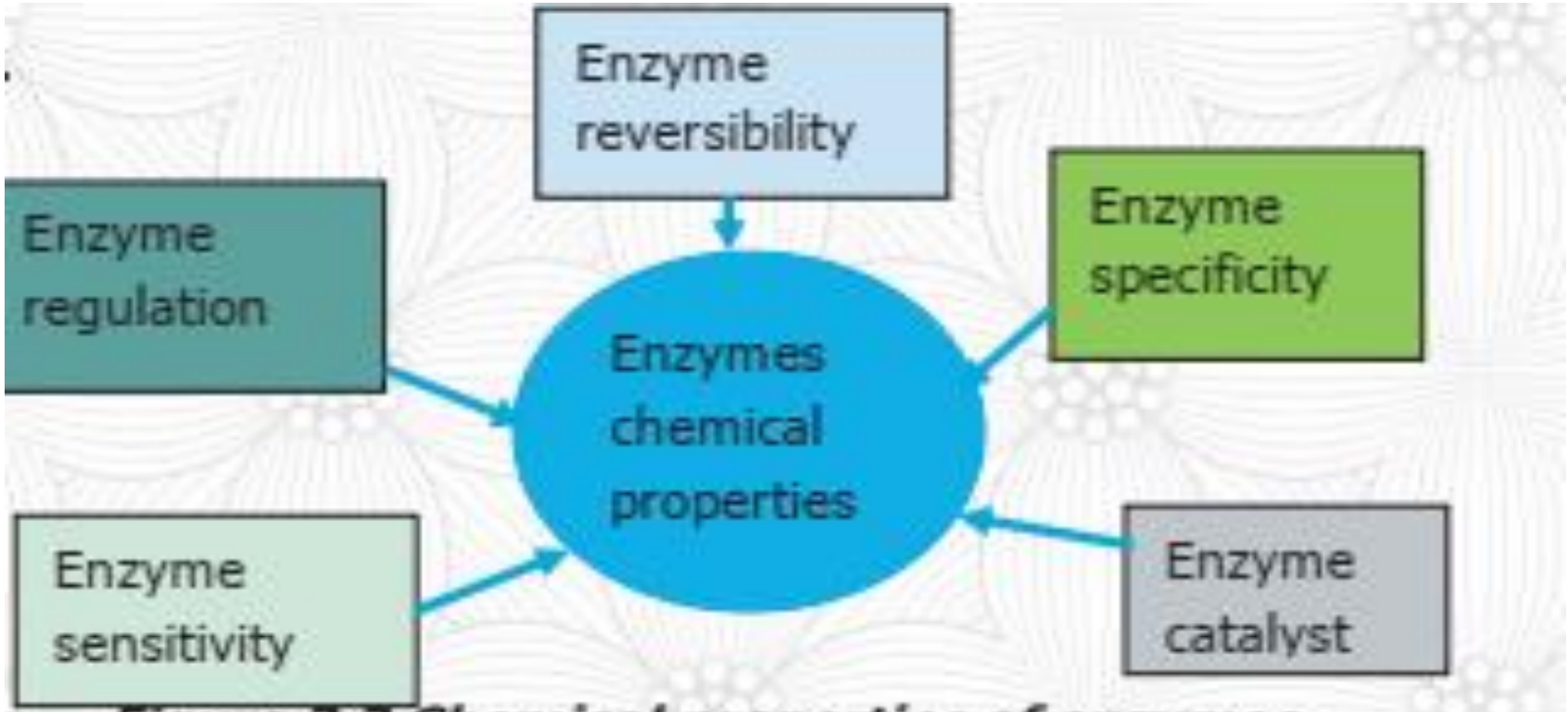


Figure 3.3 Chemical properties of enzymes

Heat and **PH Sensitivity**

- ✓ is an enzymatic reaction to heat (temperatures) and pH (acidity and basicity) activated at optimum levels.

Regulation

- ✓ is the process of controlling the activity of enzymes by activator and inhibitor molecules.

Catalysis

- ✓ is the process of the acceleration of a chemical reaction by a catalyst.
- ✓ Enzymes are biological catalysts that possess high catalytic efficiency.

- They can transform about 100-10,000 substrates per second.
- The reactions catalyzed by the enzymes show a 10^3- 10^8 times faster reaction rate in comparison to the non-catalyzed reactions.

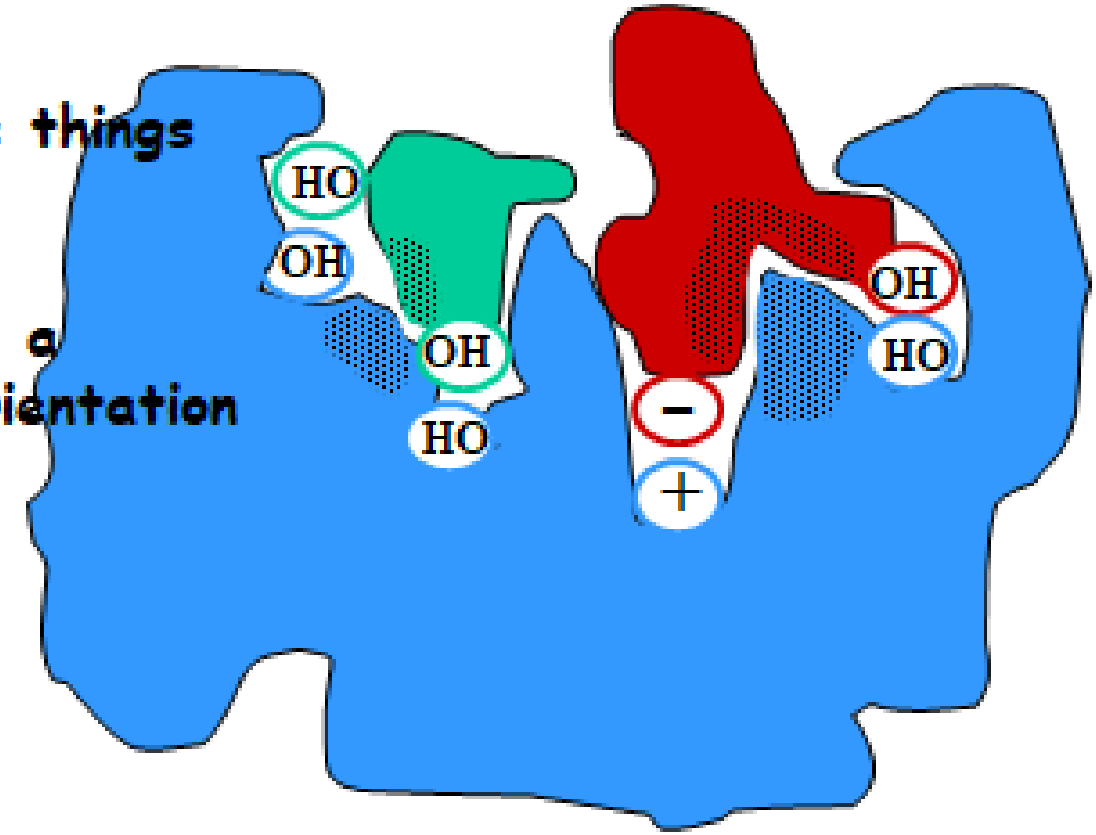
Reversibility

- is the ability of enzymatic biomolecules to catalyze various metabolic (anabolic and catabolic) reactions.
- ✓ It is the reaction to synthesize (build up new molecules or products) and
- ✓ decompose (breaks down different products)
- in which enzymatic reactions catalyze biochemical reactions in both forward and reverse directions.

ENZYMES:

Bind ONLY specific things

Bind them ONLY in a
Specific 3-D Orientation



SPECIFICITY is the Key to Enzyme Action

Enzyme specificity

- ✓ The ability of an enzyme to select a specific substrate from a range of chemically similar compounds is known as specificity.
- ✓ Since the enzyme and substrate exhibit complementary structural and conformational properties, specificity is a molecular identification process.
- ✓ **A completely specific enzyme would have only one substrate.**

- **Bond specificity**
- ✓ is a relative specificity of enzymes, which indicates that enzymes are specific for a bond.
- **Group specificity**
- ✓ is a structural specificity of enzymes, which describes that enzymes are specific for a group.
- **Substrate specificity**
- ✓ is the feature of enzymatic activity where an enzyme acts only on a particular substrate.
- **Optical specificity**
- ✓ is when enzymes act on the substrate optical configuration.
- **Co-factor specificity**
- ✓ is the enzymatic specificity to the substrate and co-factors.

3.2.2 The function of enzymes

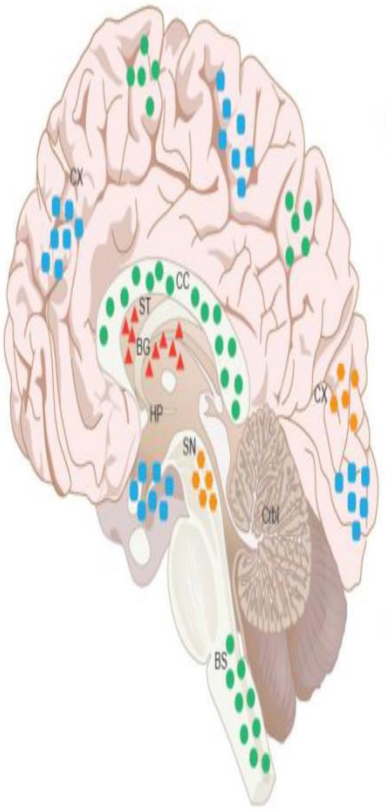
- Enzymes help speed up chemical reactions in the human body.
- They are essential for respiration, digesting food, the liver, muscle, and nerve function.
- Each cell in the human body contains thousands of enzymes that provide help in facilitating chemical reactions within each cell.
- The **turn over number** of molecules is the number of **substrates** converted by one enzyme molecule per second at saturated (fully occupied) active sites.

- Enzymes are markers of the states of various diseases like ***myocardial infraction, jaundice, pancreatitis, cancer and neurodegenerative disorders*** etc.

JAUNDICE

- Yellowish discoloration of skin, sclera and mucous membrane resulting from deposition of bilirubin .
- It is a sign of liver disease or less often a hemolytic disorder or disorder of bilirubin metabolism





Alzheimer's disease
Cortex
Hippocampus
Donepizil
Rivastigmine

Parkinson's disease
Substantia Nigra
Cortex
Levodopa+Carbidopa
Pergolide
Bromocriptine

Huntington's disease
Striatum
Basal Ganglia
Reserpine
Phenothiazines

Multiple sclerosis
Basal Ganglia
Brain Stem
Tysabri
Interferones
Glatiramer Acetate



Parkinson disease (PD) is a brain condition that causes problems with movement, mental health, sleep, pain and other health issues.

- There is no cure, but therapies and medicines can reduce symptoms.
- Common symptoms include tremors, painful muscle contractions and difficulty speaking.
- **Pancreatitis** is the redness and swelling (inflammation) of the pancreas. This happens when digestive juices or enzymes attack the pancreas.

❖**Each enzyme has an active site with a unique shape that speeds up metabolism or chemical reactions in our bodies and builds substances in all living things.**

- Examples of enzymes:

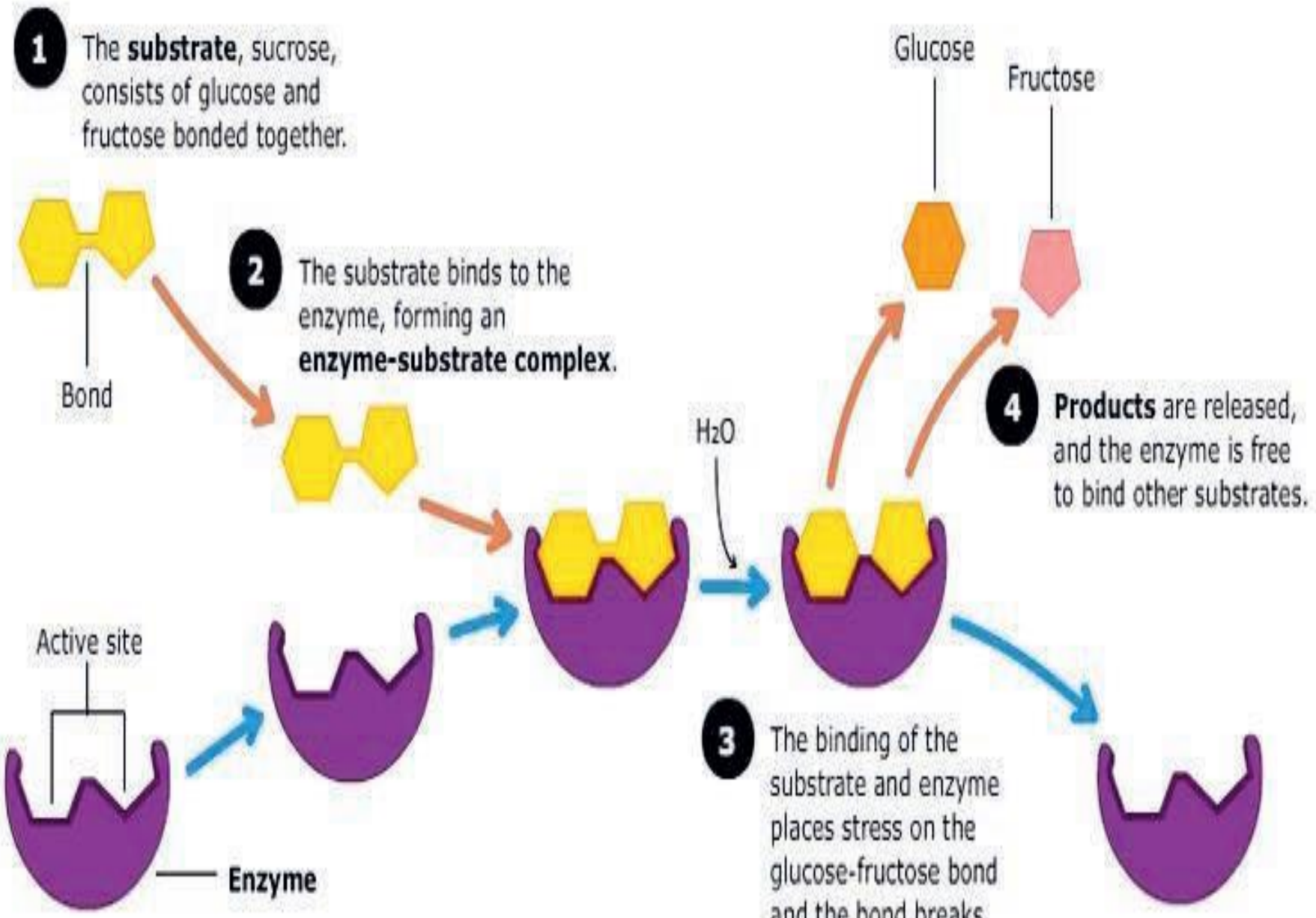
✍ **Sucrase** breaks down a sugar called sucrose.

✍ **Lactase** breaks down lactose, a kind of sugar found in milk products.

✍ **Carbohydrase** breaks down carbohydrates into sugars.

✍ **Lipase** breaks down fats into fatty acids.

✍ **Protease** breaks down protein into amino acids


1 The substrate, sucrose, consists of glucose and fructose bonded together.
Bond
2 The substrate binds to the enzyme, forming an enzyme-substrate complex.
Active site
Enzyme
H2O
3 The binding of the substrate and enzyme places stress on the glucose-fructose bond and the bond breaks.
Glucose
Fructose
4 Products are released, and the enzyme is free to bind other substrates.

- Enzymes perform their function by lowering a reaction's **activation energy**.
- Activation energy is the energy required to start a reaction.
- The lower the activation energy, the faster a reaction happens .

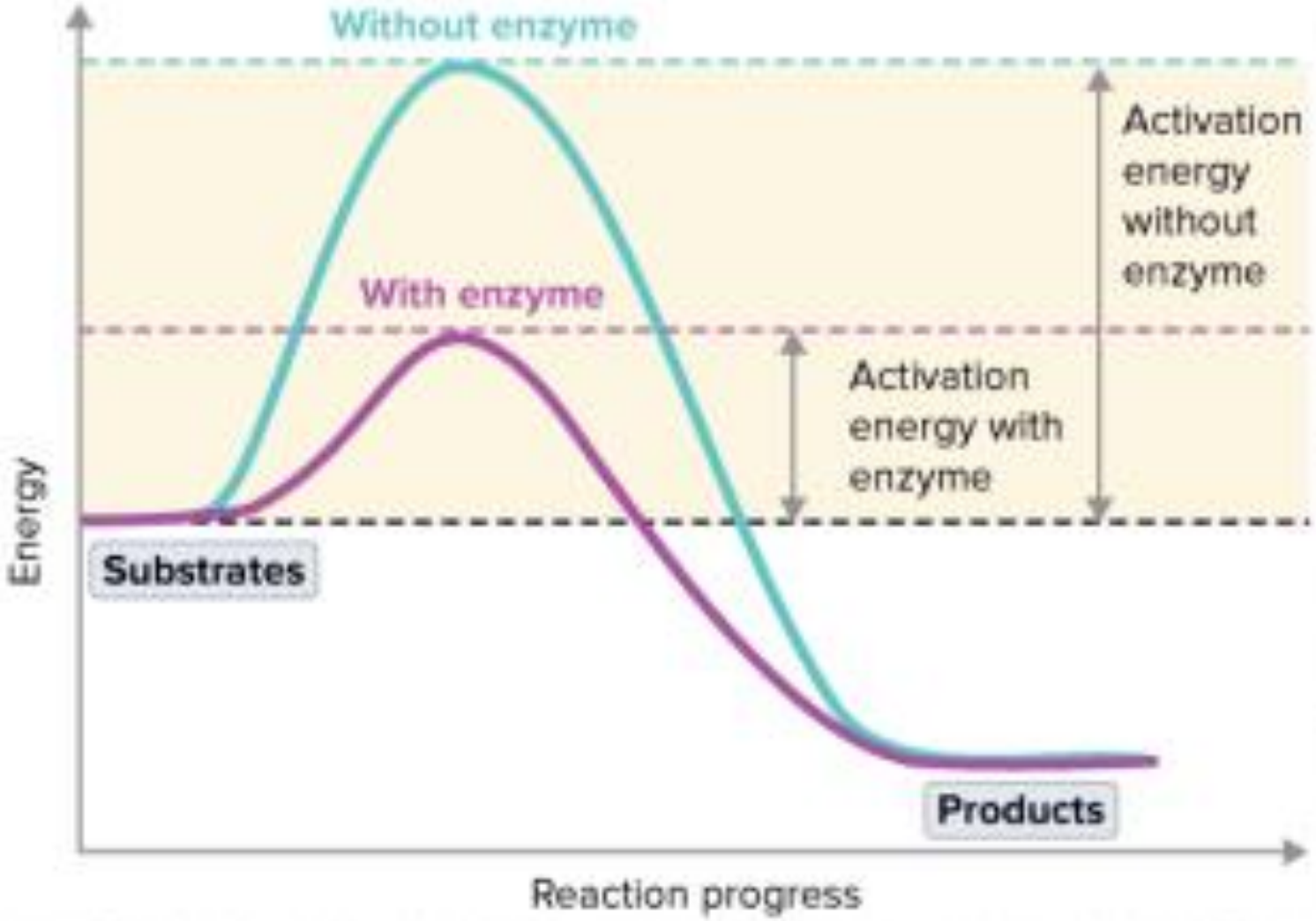


Figure 3.5 Enzymatic reactions between glucose and oxygen

Table 3.1 Some of enzymes in the body and their functions

Enzyme	Function
Lipases	Split fats found in the blood, gastric juices, pancreatic secretions, intestinal juices, adipose (fatty) tissues and participate in digestions.
Amylase	Amylase exists in saliva and helps in changing starches into sugars.
Maltase	Maltase exists in foods such as potatoes, pasta and beer and saliva breaks the sugar maltose into glucose.
Trypsin	Found in the small intestine, breaks proteins down into amino acids.
Lactase	Found in the small intestine, breaks lactose, the sugar in milk, into glucose and galactose.
Helicase	Unwinds DNA
DNA Polymerase	An enzyme responsible for forming new copies of DNA in the form of nucleic acids molecules
Acetyl cholinesterase	Breaks down the neurotransmitter acetylcholine in nerves and muscles

3.3 Protein structures

- Protein structure is a polymer of amino acids joined by peptide bonds with three-dimensional arrangements of atoms in amino acid chain molecules.
- The protein complex macromolecules have four structural levels:

1. Primary structure

2. Secondary structure

3. Tertiary structure

4. Quaternary structure

The primary structure of proteins

- The primary structure of a protein is defined as the sequence of amino acids linked together to form **a polypeptide chain.**
- Each amino acid is linked to the next amino acid through peptide bonds created during the protein biosynthesis process.
- The two ends of each polypeptide chain are known as the **amino terminus (N-terminus)** and the **carboxyl terminus (C-terminus).**
- ***Twenty different amino acids can be used multiple times in the same polypeptide to create a specific primary protein structure sequence.***

- **Humans require 20 amino acids out of which 10 amino acids are synthesized in the human body, and the rest 10 amino acids are obtained from diets.**
- **Cells use 20 different standards of L-α-amino acids containing basic amino acids and acidic carboxyl groups for protein construction.**

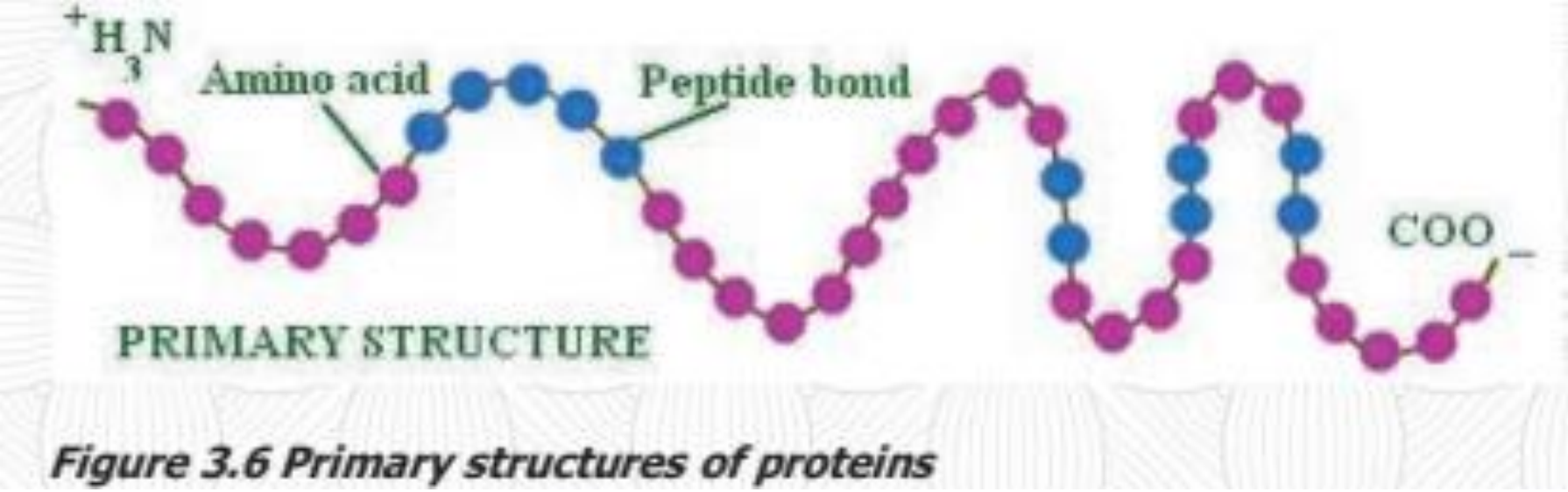


Figure 3.6 Primary structures of proteins

2. The secondary structure of proteins

- The most common types of secondary structures are:
- ✓ the α helix and
- ✓ β pleated sheet.
- Both structures are held in shape by **hydrogen bonds**, which form between the **carbonyl O of one amino acid** and **the amino H** of another.

2.1 The α – Helix

- is a right-handed coiled strand and the side-chain substituents of amino acid groups extend to the outside and form hydrogen bonds with oxygen (C=O) in the strand with the hydrogen of each (N-H) group of four amino acids to make the structure stable.

2.2 β–pleated sheet

- The hydrogen bonding in the ß-sheet is between the inter-strands and intra-strands in which the sheet conformation of the ß- sheet consists of pairs of strands lying side by-side.
- All peptide chains stretch out to nearly maximum extension, laid side by side and held together by intermolecular hydrogen bonds forming pleated folds of drapery (Figure 3.7).

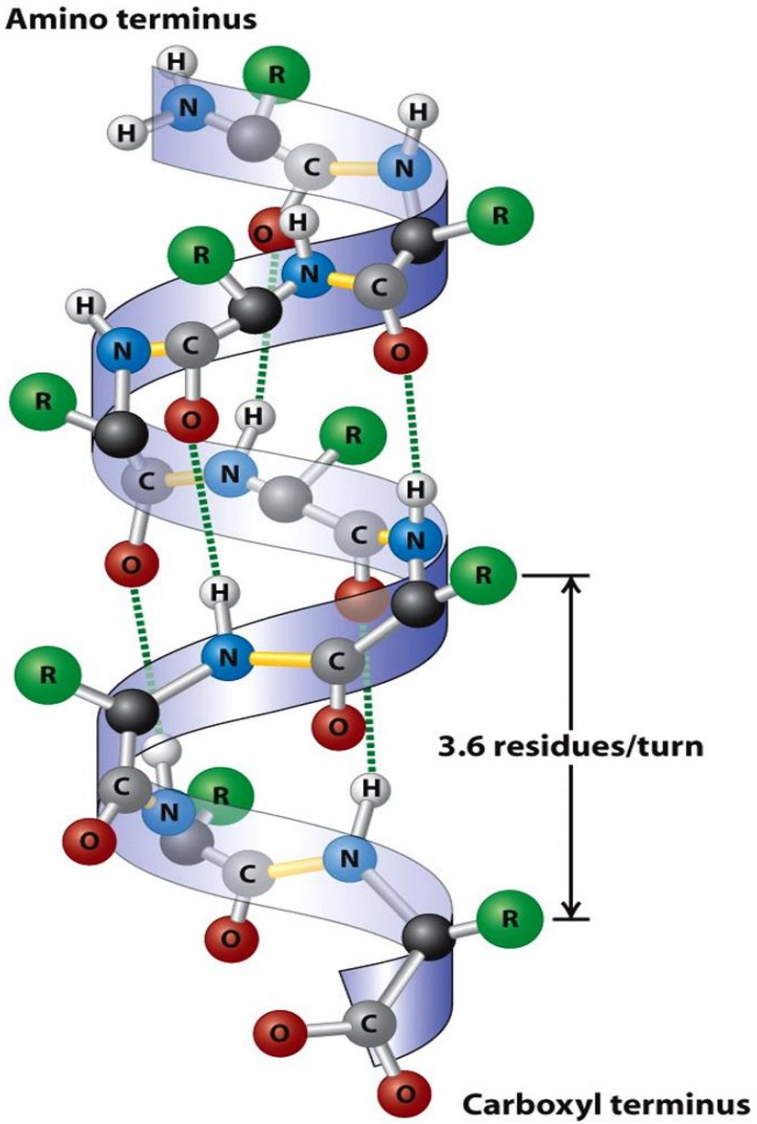
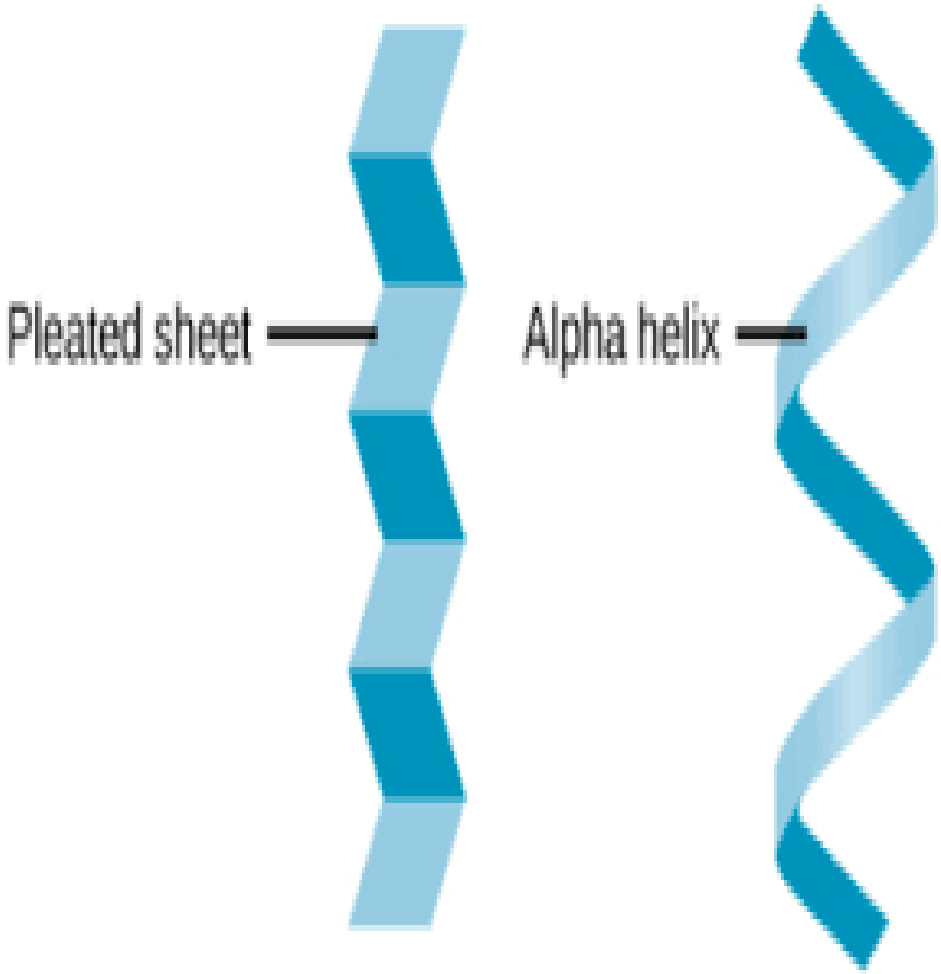


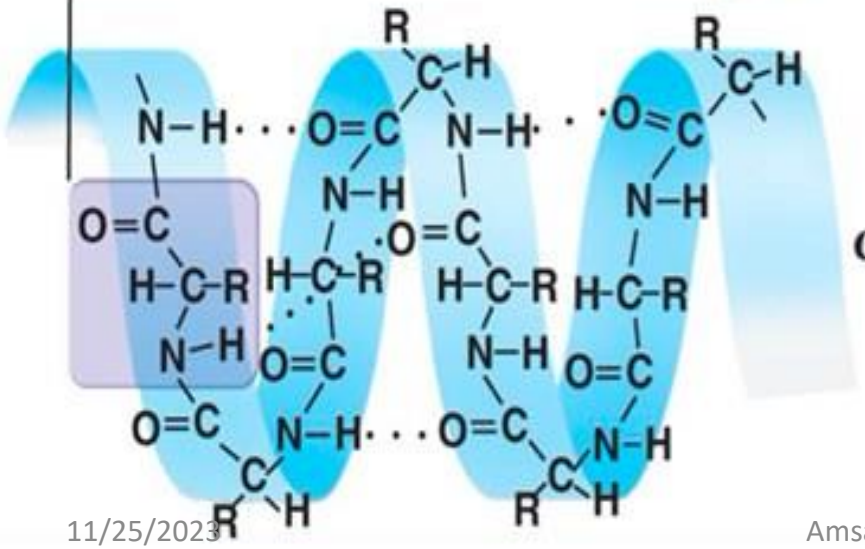
Figure 3-4
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company



Secondary Structure

β pleated sheet

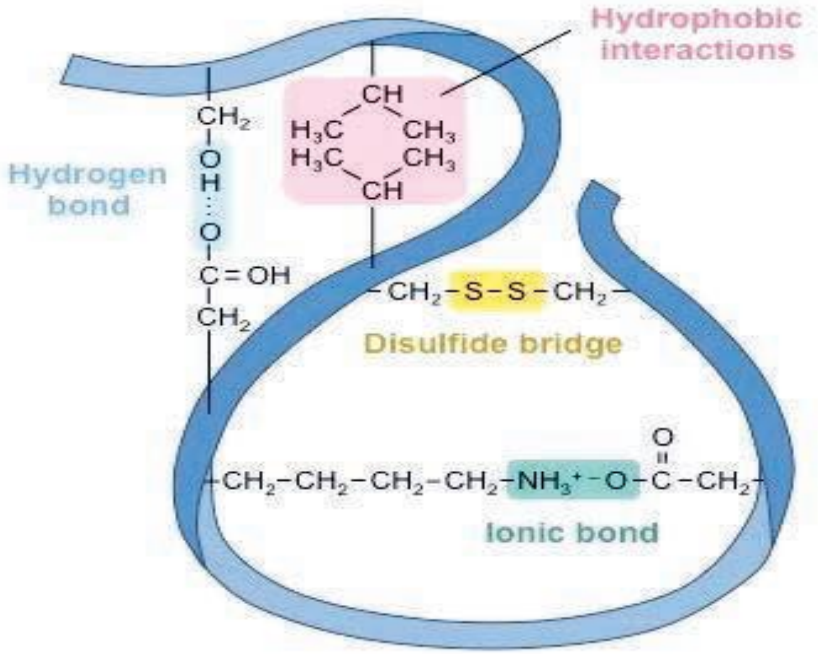
Examples of amino acid subunits



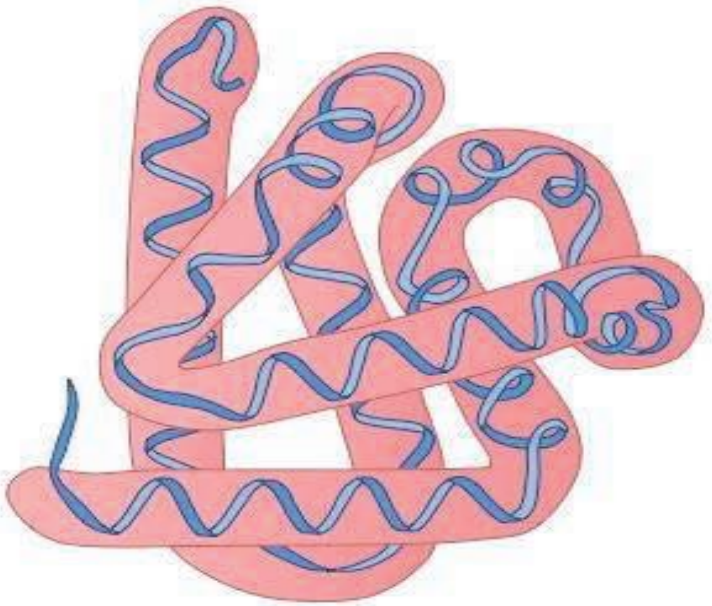
α helix

3. The tertiary structure of proteins

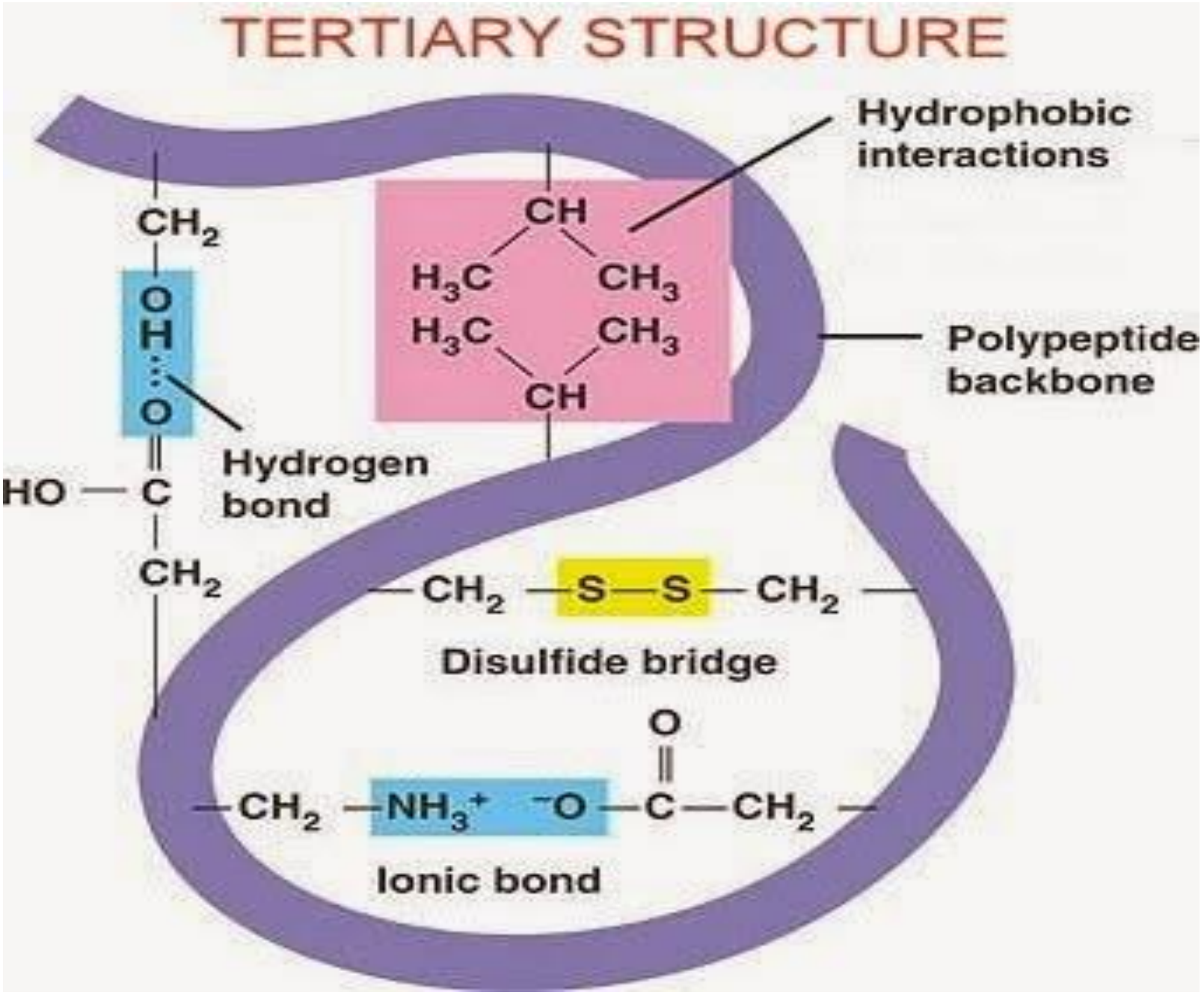
- is the three dimensional shape of protein molecules that bend and twist to achieve the maximum stability or the lowest energy state.
- It is fashioned by many stabilizing forces due to the bonding interactions between the side-chain groups of amino acids



Types of side chain interactions



Overall 3D shape (3° Structure)


TERTIARY STRUCTURE
Hydrophobic interactions
Polypeptide backbone
Hydrogen bond
Disulfide bridge
Ionic bond

4. The quaternary structure of proteins

- is the arrangement of multiple folded protein subunits in a multi-subunit complex.
- It is the association of several protein chains or subunits into closely packed arrangements with their own primary, secondary, or tertiary structures and held together by the hydrogen bonds

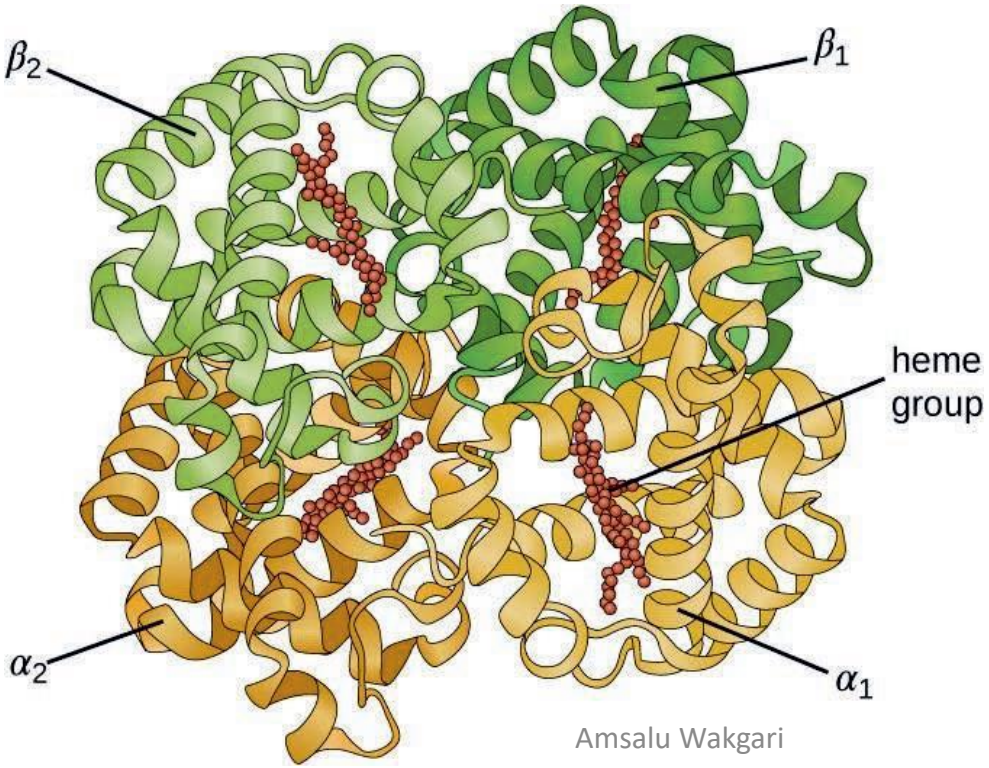
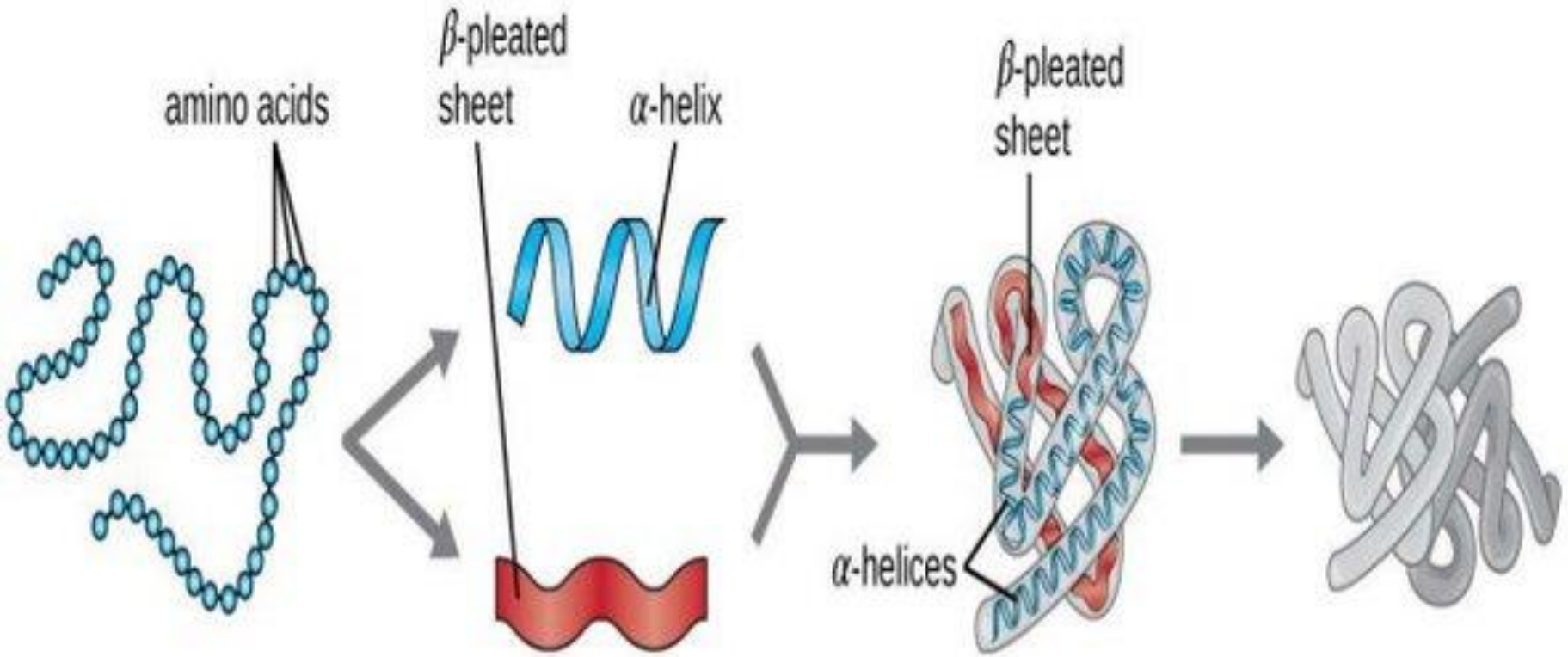


Table 3.2 Summary on protein structures

Proteins	Structures
Primary	The basic linear sequence level of amino acids comprising one polypeptide chain
Secondary	The next level up of proteins with folding regions into the specific structural patterns within one polypeptide chain
Tertiary	The next level up is the three-dimensional arrangement of all the amino acids in a single polypeptide chain.
Quaternary	All polypeptide chains are held together by a specific spatial arrangement and interactions.



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

3.4 Enzyme substrate models

- Enzyme substrate models are models for enzyme substrate interaction describing that the shapes of the active site and the substrate complement to fit into the binding **active site** perfectly.
- **3.4.1 Enzyme-substrate binding models**
- There are two different of enzyme-substrate binding models that we will look at in this section:
- the lock and key model and
- the induced fit model.

1. Enzyme lock and key model

- The lock and key model is when enzyme active sites fill-in with a substrate to interact through **non-covalent** interactions.
- The model explains on how the enzymes must bind to substrates before they catalyze a chemical reaction.
- Once the reaction progresses to the transition state and forms products, the **active site** will not be able to accommodate changes.

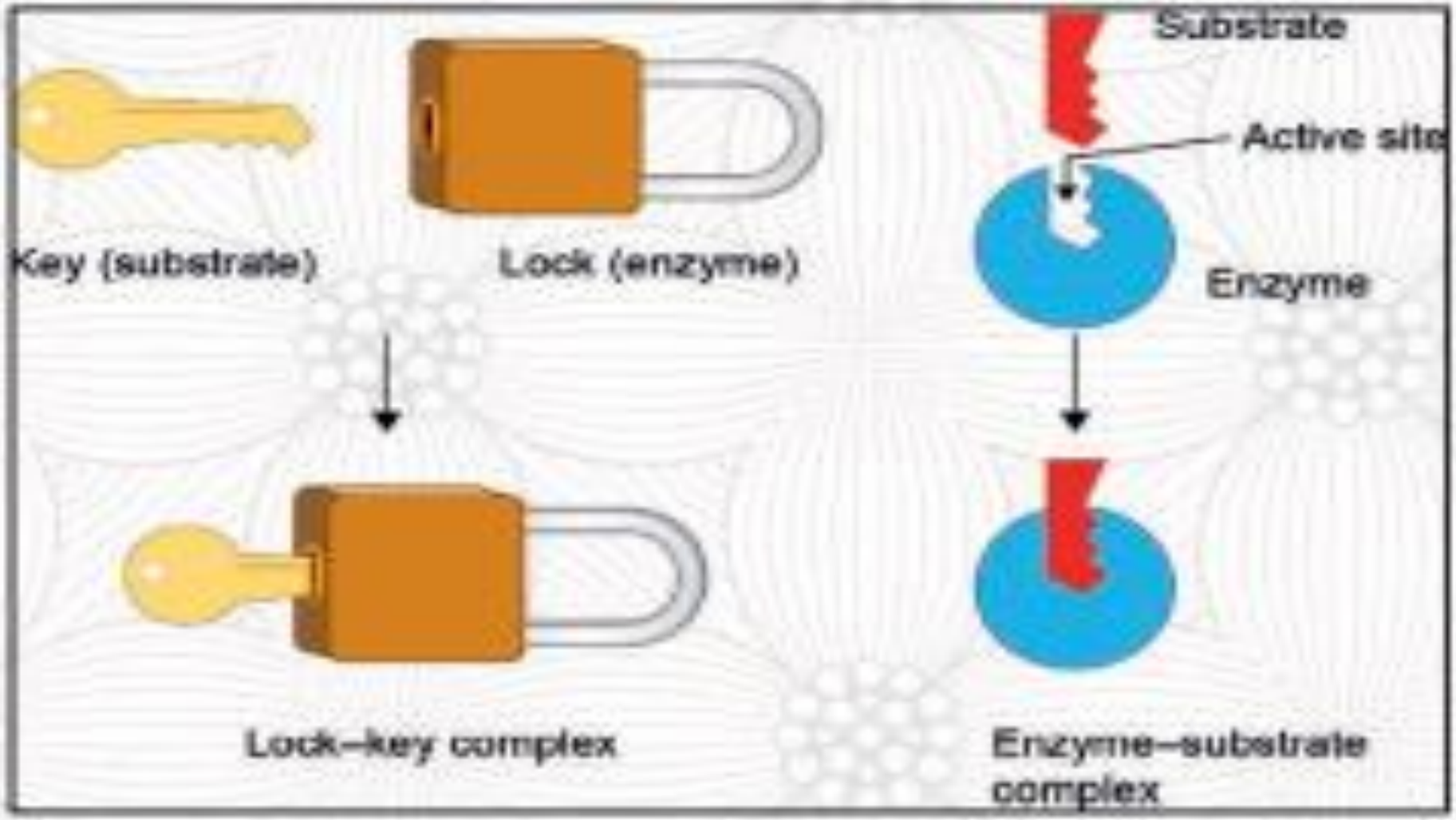


Figure 3.10 Lock and Key model

2. Enzyme induced fit model

- is the active site of an enzyme that elicits responses to the binding substrate by inducing the substrate to take up transition even when the **active site** is not perfect to perform the required product.
- Enzymes change shapes by induced fit upon substrate binding to form enzyme substrate complexes.
- The amino acid side chains that make up the active site mold into the precise positions enable the enzyme to perform its catalytic functions.
- The concept of induced fit states that when a substrate binds to an enzyme:

✓ it brings about a change in the shape of the enzyme,

✓ which either enhances or suppresses the activity of the enzyme.

- Upon binding, the inducing process enables to elicit required energy for the reaction to move by putting the active site under strain and making the transition stable (Figure 3.11)

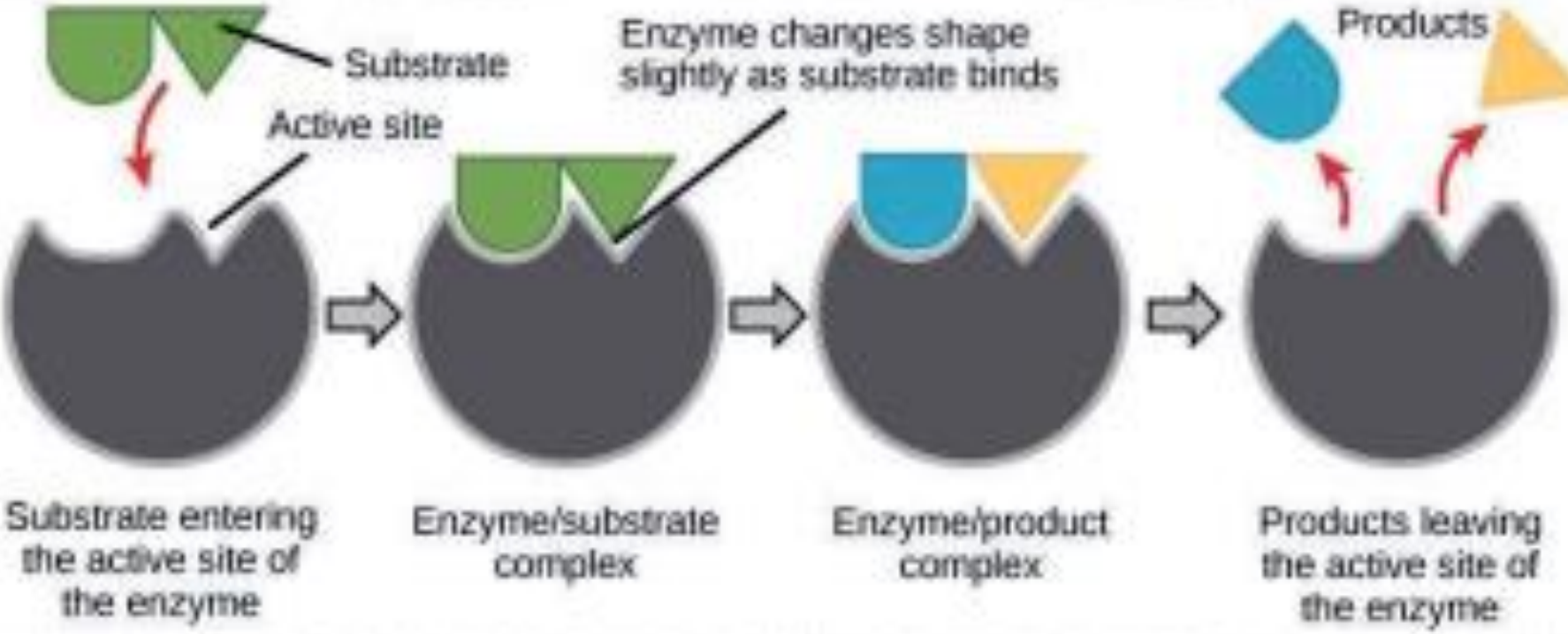
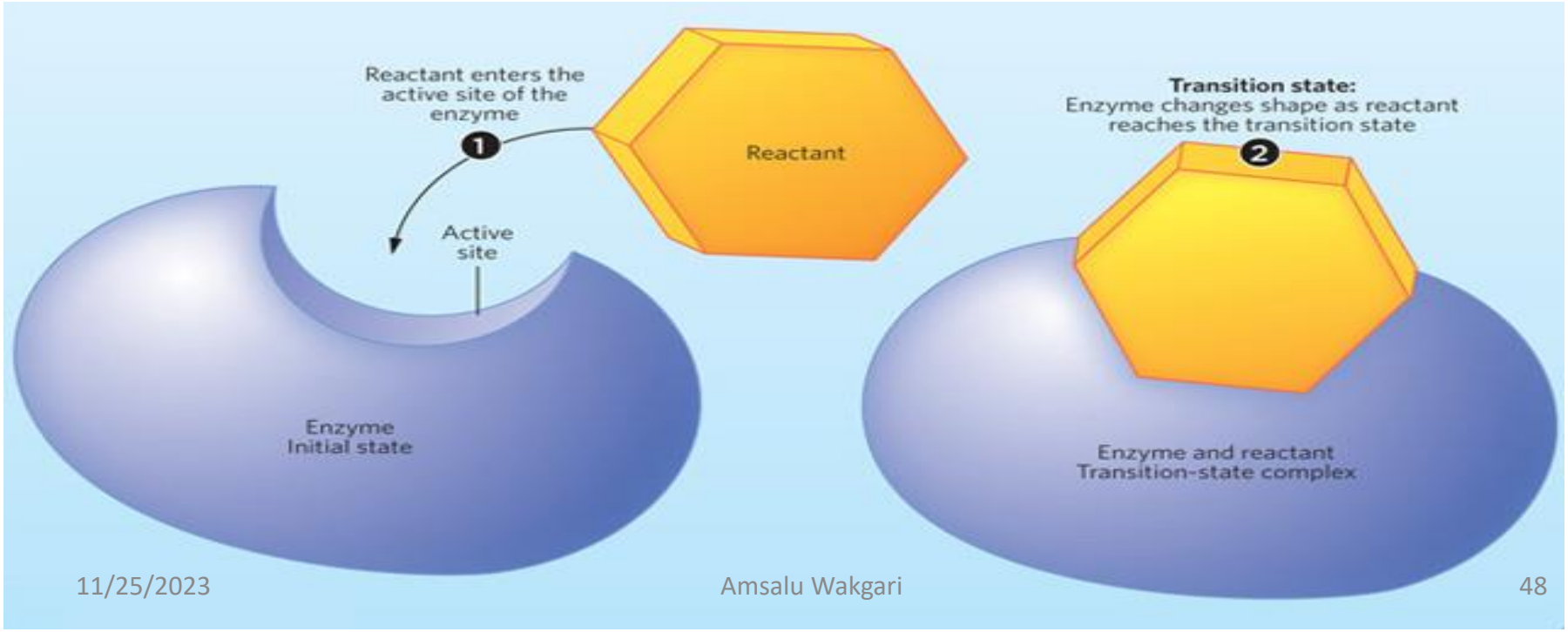


Figure 3.11 Induced fit enzyme model

3.4.2 Enzymatic transition state

- An enzymatic transition state is the reaction rates of elementary chemical reactions and assumes chemical equilibrium between reactants and activated transitions.
- It describes how the chemical reactions are taking place qualitatively in the activated enzyme-substrate complex of absolute reaction rates.



- The reactive state of substrate binding catalysis is corresponding to the maximum reaction activated and its state of transition (Figure 3.12). Where:

✓E = Enzyme

✓**S** = Substrate

✓**ES** = Enzyme substrate combined

✓**ES*=** Enzyme substrate complexes

✓**EP**= Enzyme product,

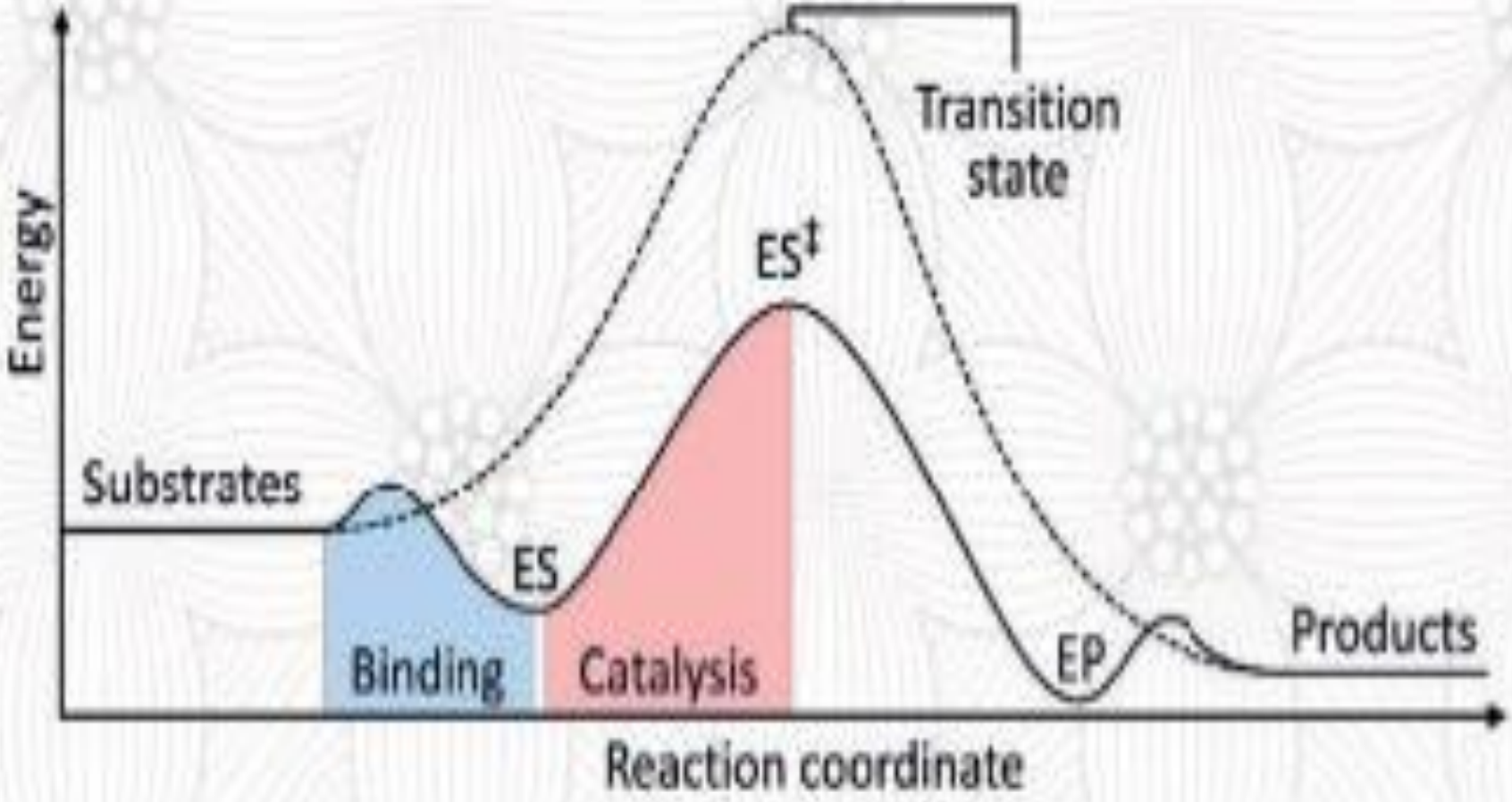


Figure 3.12 Enzymatic transitional states

3.5 Enzyme regulation

- Enzyme regulation is a control system for enzymatic activities in which enzymes are turned “on” or “off” depending on the organisms need.
- It is adapting enzymatic activities by other molecules or metabolic cells to either increase or decrease the activities.
- A regulatory enzyme is the one in a biochemical pathway through which it responds to the presence of certain other biomolecules and regulates the pathway activity.
- It requires an extra activation process to pass through some modifications and functions.
- Regulatory enzymes are of two types,
  - ✓ allosteric enzymes and
  - ✓ covalently modulated enzymes,

1. Allosteric enzymes

- are enzymes that have additional binding sites for effector molecules other than the active site that cause conformational changes, **leading to changes of catalytic properties.**
- Allosteric enzymes contain two binding sites called **active site/catalytic site and allosteric site/regulatory site** for binding effectors and substrates respectively.
- **Effectors** are small molecules (inhibitor or activator) modulating the enzyme activity and function through reversible non-covalent binding of a regulatory metabolite in the allosteric site or non-active site.
- Effectors lead to conformational changes in a concrete part of the enzyme that affect the overall conformation of the active site, causing modifications in the activity of the reaction.



Figure 3.13 Allosteric enzyme

2. Genetic and covalent modification

- The genetic and covalent modification modifies the protein surface and facilitates intracellular delivery.
- Genetic modification of enzymes is to improve the properties of enzymes and gain active and inactive forms.
- Covalent modulated enzymes are active and inactive forms of the enzymes altered due to covalent modification of structures catalyzed by other enzymes.
- Covalent modifications are enzyme-catalyzed alterations of synthesized proteins by the addition or removal of chemical groups.

- Modifications can target a single type of amino acid or multiple amino acids and will change the chemical properties of the site.
- Enzyme regulation occurs by the addition or elimination of some molecules attaching to the enzyme protein.
- **Phosphorylation** is the addition of phosphate groups to proteins. It is the most frequent regulatory modification mechanism in our cells (Figure 3.14)

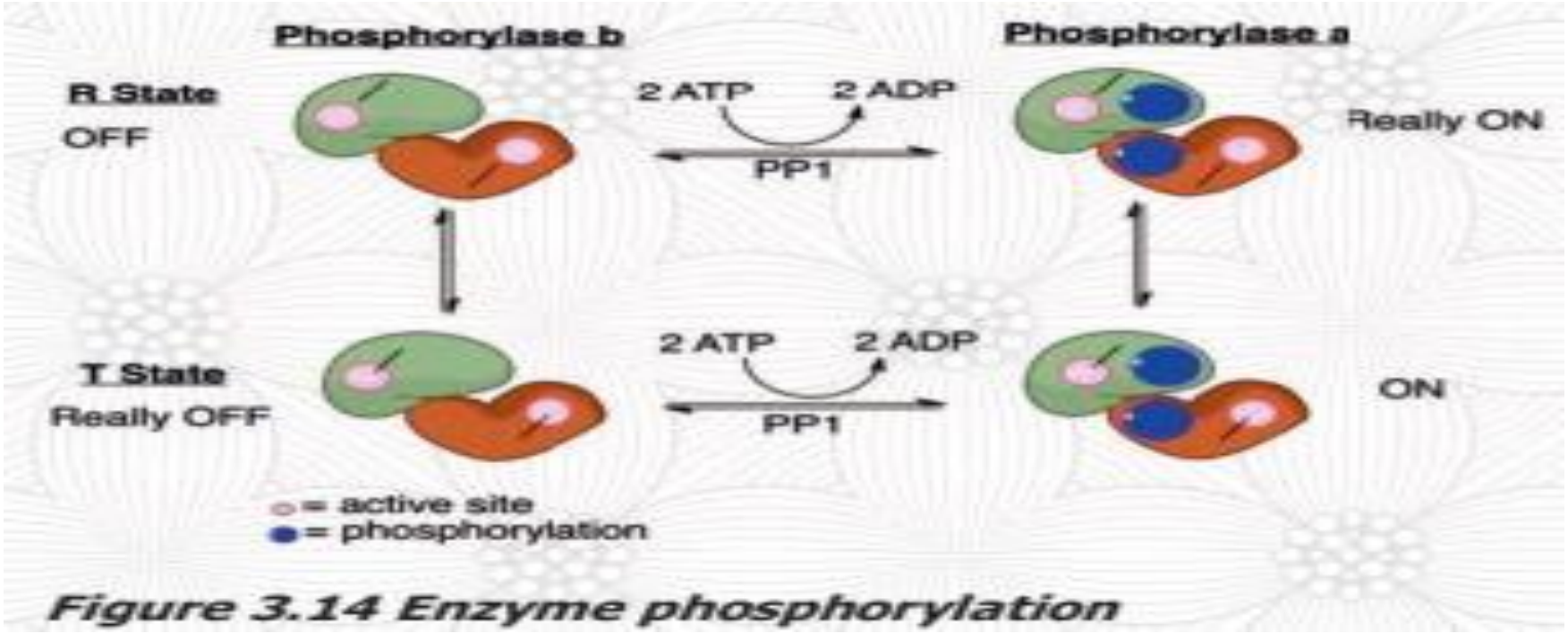


Figure 3.14 Enzyme phosphorylation

3. Enzyme inhibition

- **Inhibitors** are substances that bind to enzymes and prevent them from forming enzyme–substrate complexes and, as a result, **stop, or slow down, the reaction.**
- There are two main types of inhibitors:
- ✓ **irreversible inhibitors**, and
- ✓ **reversible inhibitors**.
- Irreversible inhibitors bind **strongly to enzymes**, usually by a covalent bond, permanently altering the structure of the enzyme molecule and **inactivating it**.
- The painkiller **aspirin** is an example of an irreversible inhibitor.

- It binds with the enzyme **cyclo-oxidase-2,** which is an important enzyme in producing **prostaglandins** which give the sensation of pain.
- **Reversible inhibitors** bind to enzymes only weakly and the bond that holds them **breaks easily** releasing the inhibitor.
- This allows the enzyme to become active again.
- There are two main kinds of reversible inhibitors:

✓ **competitive inhibitors**, and

✓ **non-competitive inhibitors**

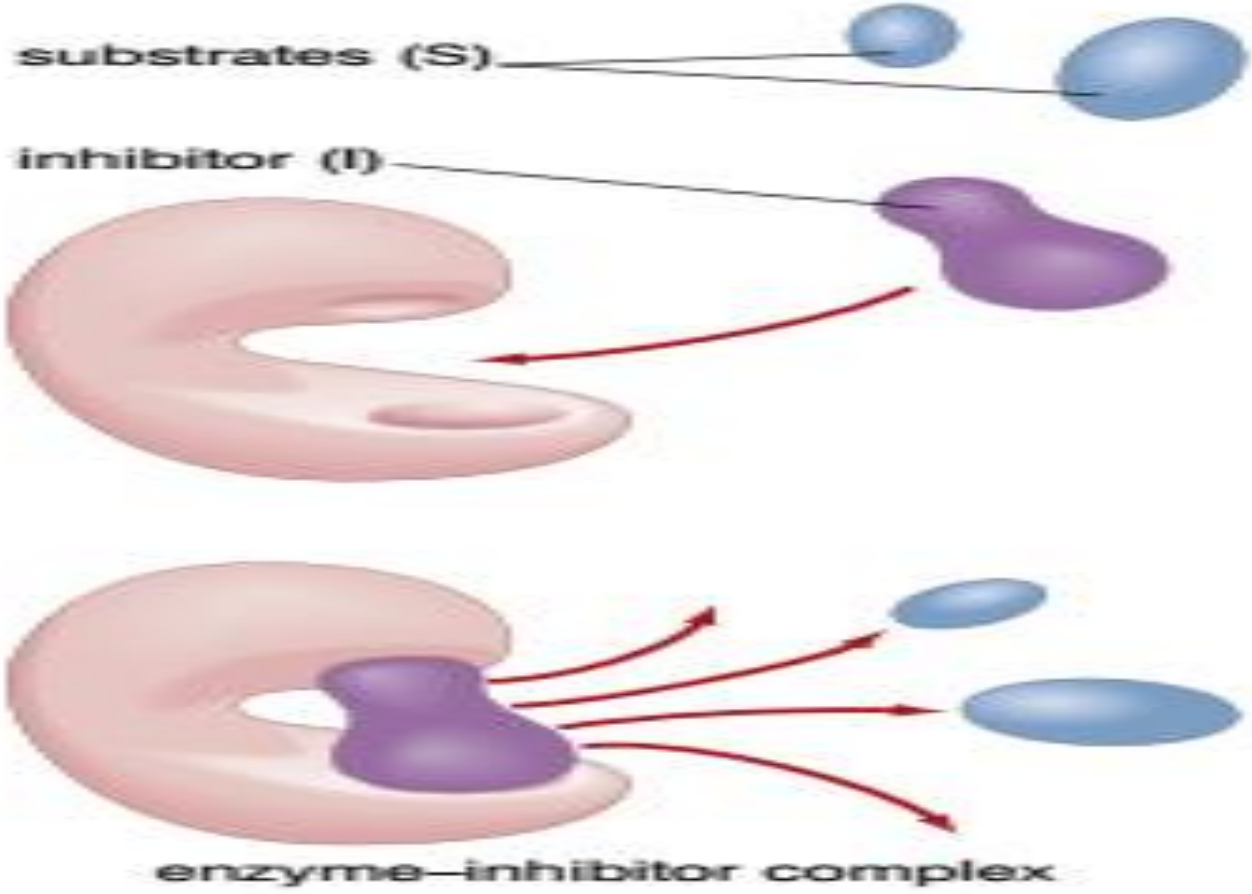


***Figure 3.20** Competitive inhibition*

Competitive inhibitors

- Competitive inhibitors have molecules with shapes that are complementary to **all, or part, of the active site of** an enzyme.
- They are often **similar in shape to the substrate molecules**.
- They can bind **with the active site** and **prevent substrate molecules from binding.**
- The binding is **only temporary** and the **competitive inhibitor is quickly released.**
- A competitive inhibitor blocks the active site so substrate molecules cannot bind.

- The overall effect on the rate of reaction depends on the **relative concentrations of substrate** and **inhibitor molecules**.
- Each molecule of competitive inhibitor can inhibit (temporarily) **one enzyme molecule** – but only if it can **collide with the enzyme** molecule and bind with the active site.
- To do this, it must **compete with the substrate molecules for the active site** – hence the name, **competitive inhibitor.**
- If there were 99 substrate molecules for every inhibitor molecule, then 99% of the collisions would be between enzyme and substrate and the reaction would proceed at 99% of the maximum rate.
- If the ratio were 90 substrate molecules to ten inhibitor molecules, there would be 10% inhibition and the reaction rate would fall to 90% of maximum.

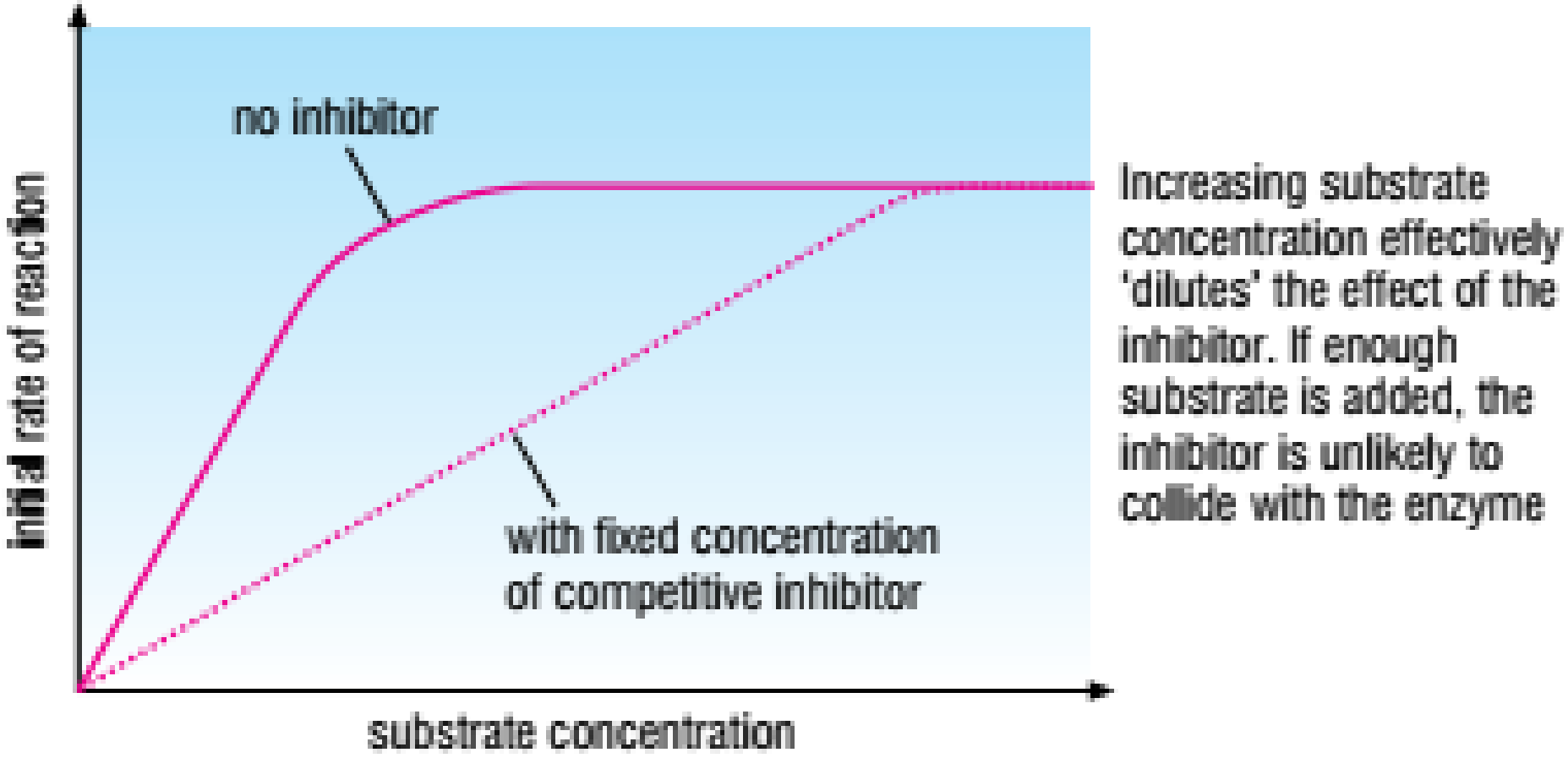


Figure 3.21 Effect of substrate concentration on inhibition by a competitive inhibitor

- The painkiller **ibuprofen** acts as a competitive inhibitor of the enzyme cyclo-oxidase-2, competing with the precursors of prostaglandins, which are the substrate of cyclo-oxidase-2.
- The **metabolic poison cyanide** acts as a competitive inhibitor of the enzyme **cytochrome oxidase,** an important enzyme in the release of energy in respiration.

Non-competitive inhibitors

- Do not compete for the active site.
- Instead, they bind to another part of the enzyme called **the allosteric site.**

- This produces a **conformational change** in the part of the enzyme molecule that includes the **active site.**
- Because of this, the active site is a different shape and can no longer bind with the substrate to catalyse the reaction.

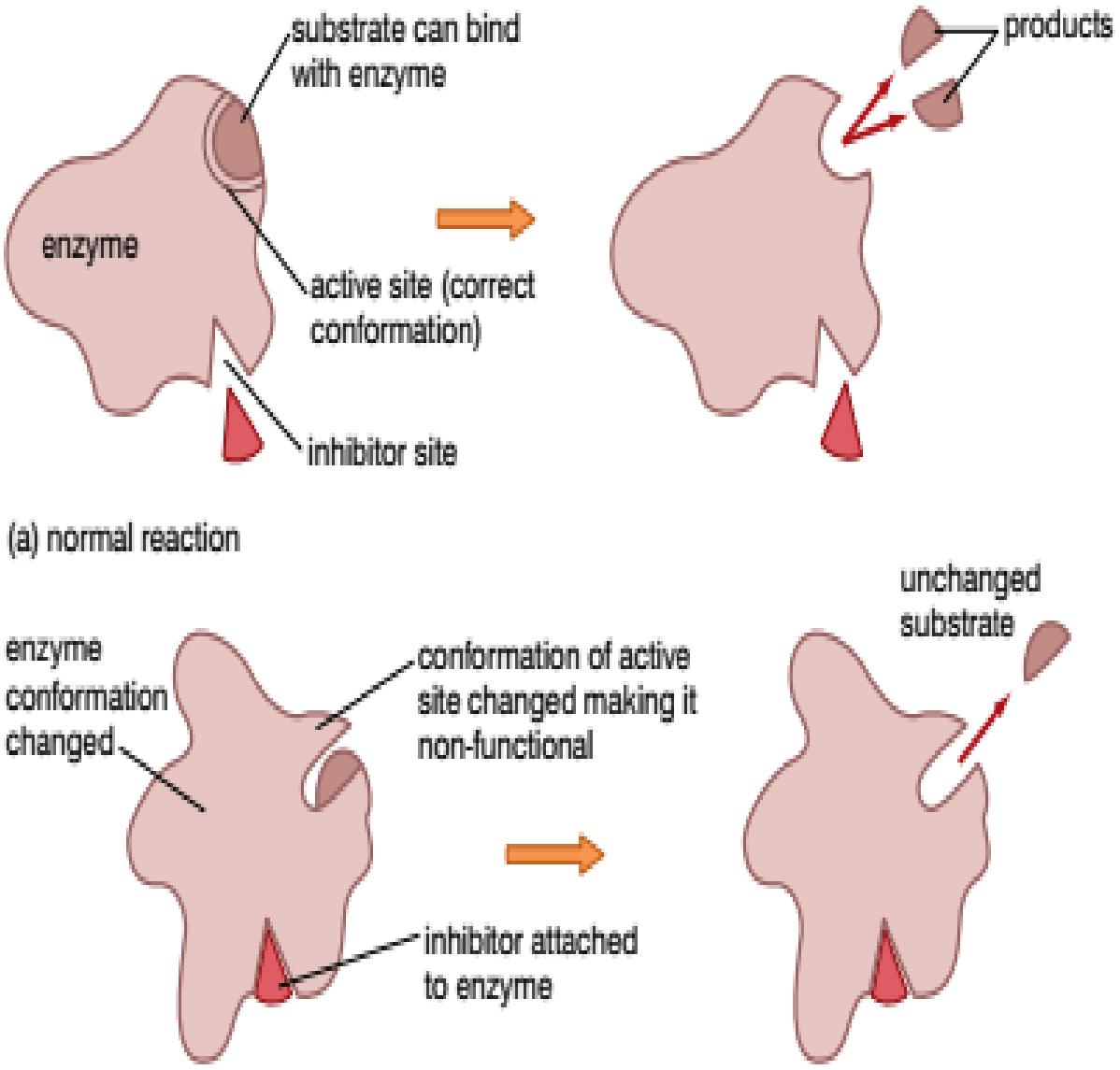


***Figure 3.22** Non-competitive inhibition*

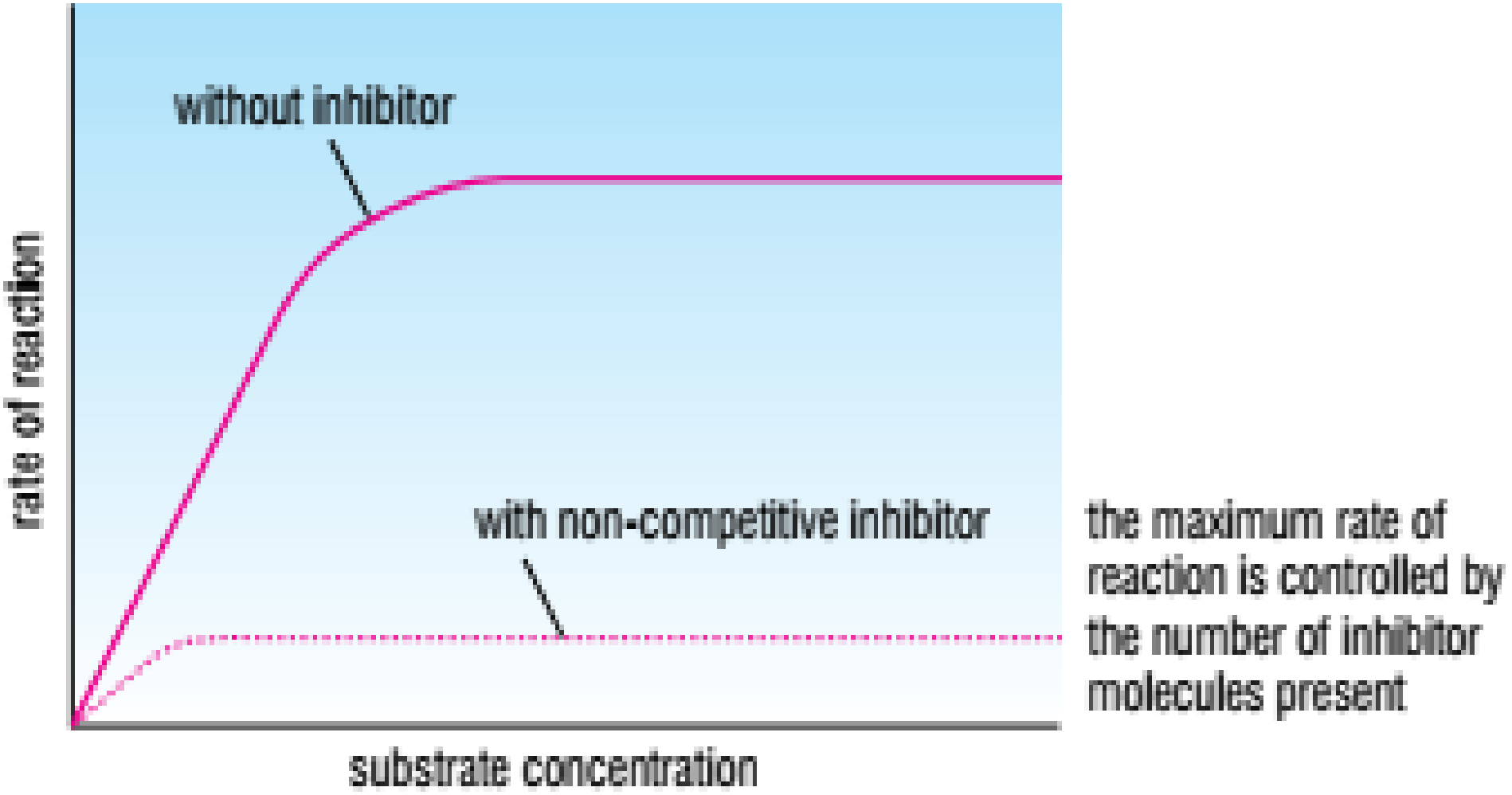


Figure 3.23 The effect of substrate concentration on a noncompetitive inhibitor

- The effectiveness of a non-competitive inhibitor is in no way affected by the **concentration of the substrate**.
- Suppose there are enough inhibitor molecules to bind with the allosteric sites of 80% of the enzyme molecules.
- 80% of the enzyme molecules will be inhibited irrespective of the number of substrate molecules (as the two are not competing for the same site) and the reaction rate will drop to 20% of maximum.
- ***Non-competitive inhibitors are particularly important in regulating metabolic pathways in cells.***

How do inhibitors control enzyme activity in living cells?

Allosteric regulation or end-product inhibition *when an end product inhibits the enzyme controlling the first stage of a reaction sequence*

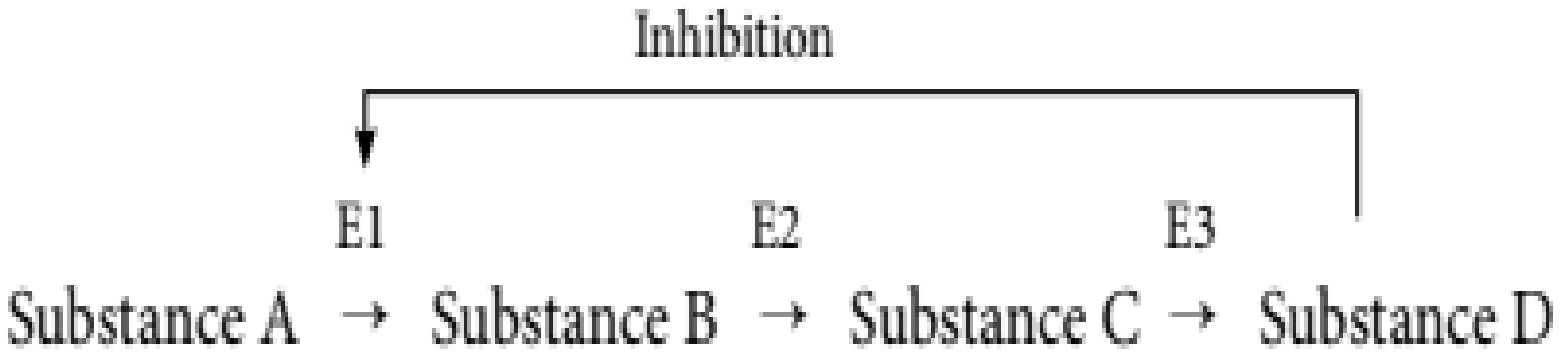
Many substances are produced in cells as a result of a metabolic pathway (a series of reactions), which can be represented as:

$$\text{Substance A} \xrightarrow{E1} \text{Substance B} \xrightarrow{E2} \text{Substance C} \xrightarrow{E3} \text{Substance D}$$

E1, E2 and E3 are enzymes catalysing the reactions.

All the reactions in this sequence are enzyme controlled. Therefore, inhibition of any of these enzymes will interrupt the process. However, the main function of this pathway is to produce substance D for use by the cell. If the requirement for substance D in the cell decreases, then the concentration of D will increase. This is at

- This is at least inefficient (producing something that is not being used) and may be potentially harmful because high concentrations could be toxic.
- Such reaction sequences are often controlled by **end-product inhibition**.
- The end product (D) inhibits the enzyme controlling the first stage of the reaction sequence, as shown in the diagram.



❖ **Substance D** normally acts as a **noncompetitive inhibitor**, which prevents the enzyme **E1** from catalysing the reaction that converts **A to B.**

- As a result, the entire reaction sequence is halted.
- When the inhibition is removed, the metabolic pathway can start again.
- Sometimes, **a decrease in the concentration of D** is sufficient to **remove the inhibition**.

Cont'd

- In other cases, the inhibitor must be removed by a substance known as an **activator**.
- Enzymes are often kept inactive in a cell by an **inhibitor** and are activated by a **specific activator** only when they are required.
- Figure 3.24 shows an example of a metabolic pathway controlled by end-product inhibition.
  In this pathway, the amino acid threonine is converted to another amino acid, isoleucine.
- Isoleucine acts as a non-competitive (allosteric) inhibitor.

- High concentrations of isoleucine inhibit more and more of the enzyme catalysing the first reaction in the pathway, until the pathway is completely inhibited.

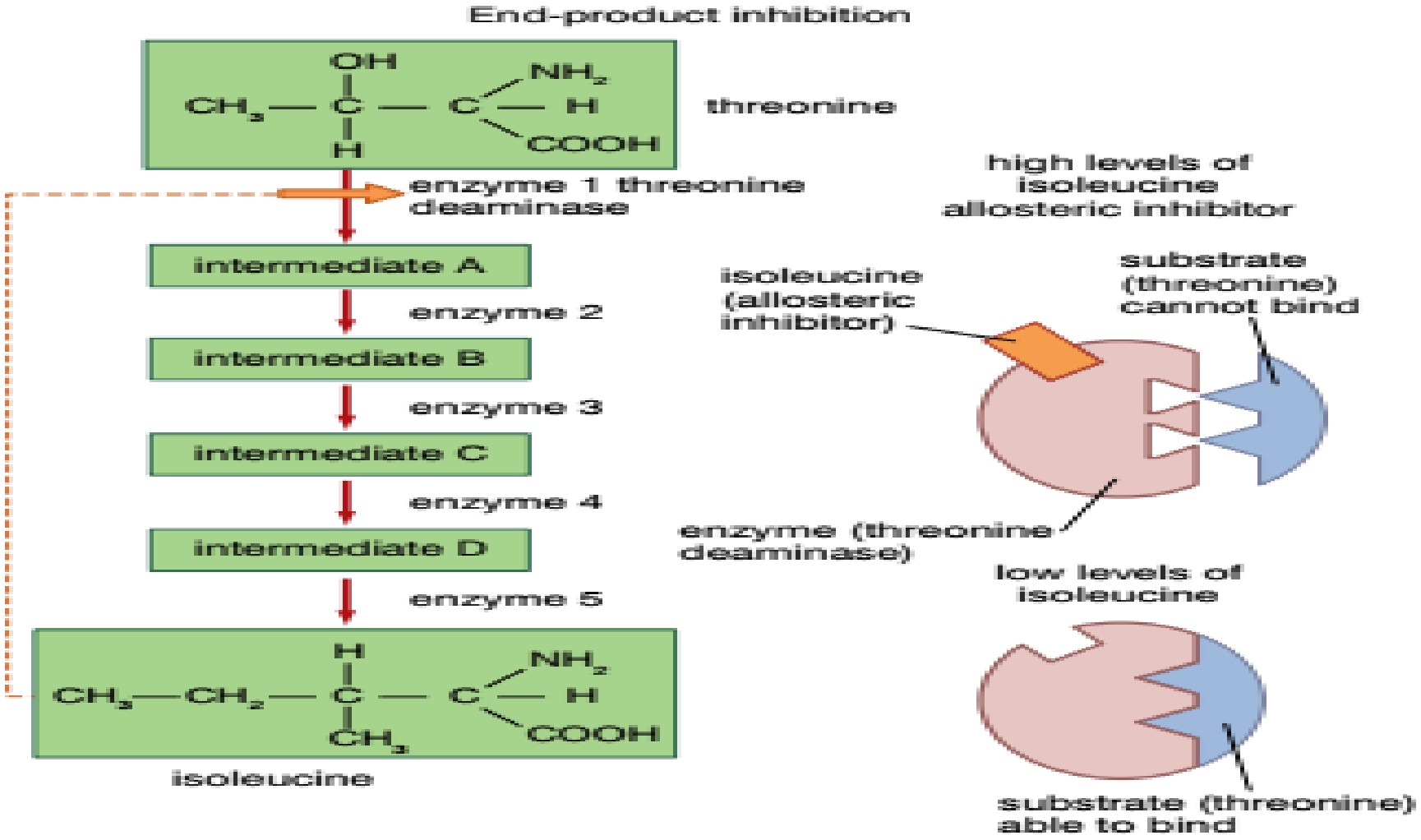


Figure 3.24 A metabolic pathway controlled by end-product inhibition

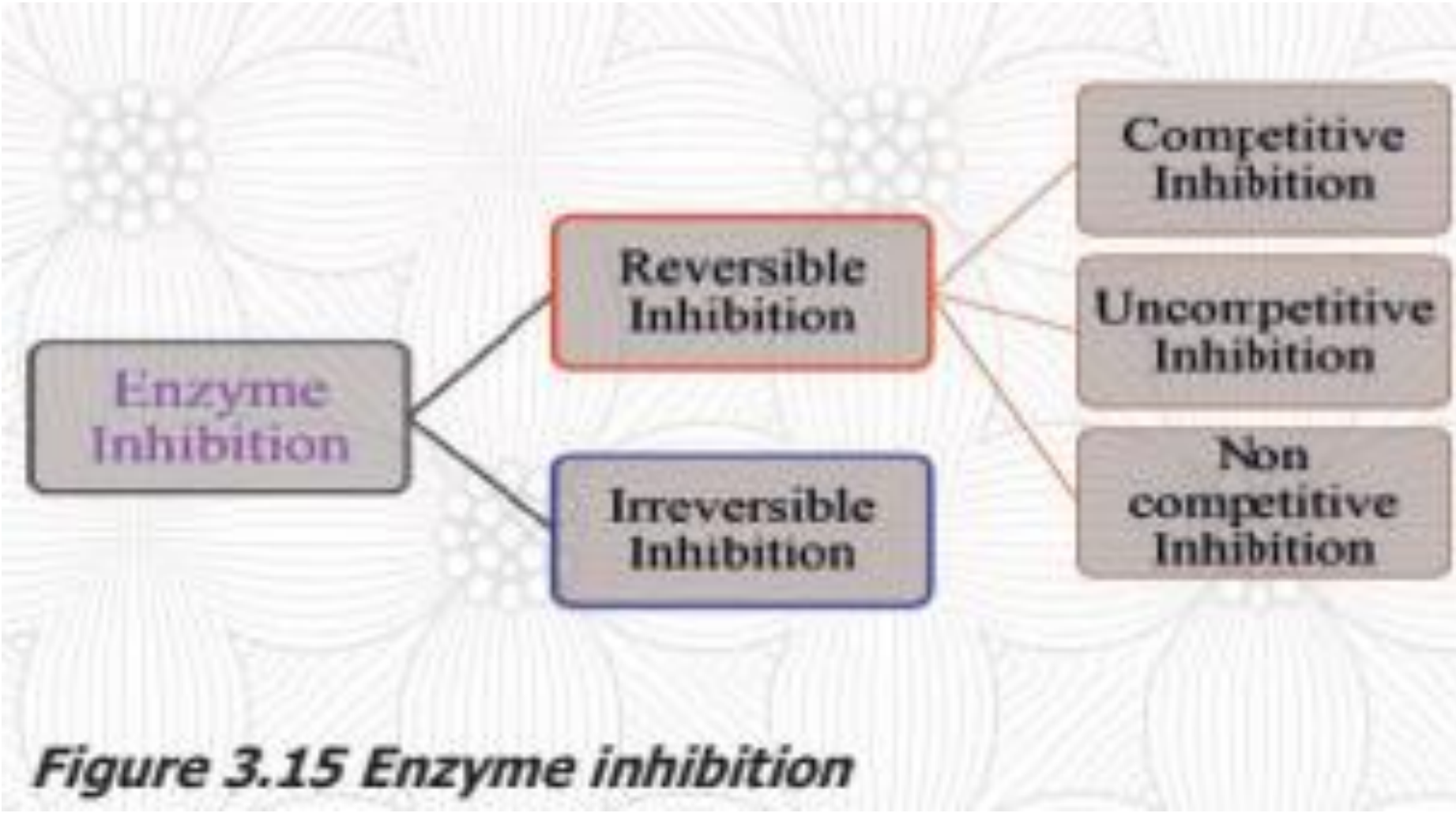
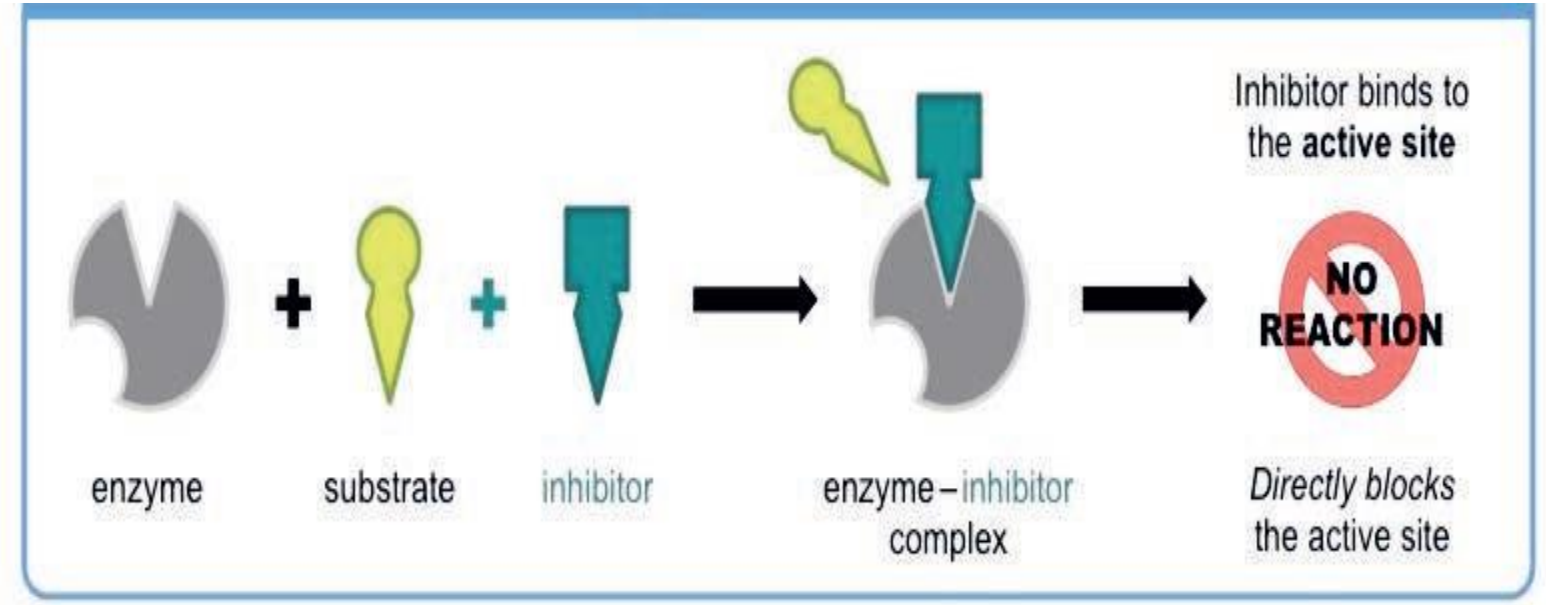


Figure 3.15 Enzyme inhibition

1. Competitive inhibitor

- is a molecule that blocks the binding of the substrate to the active site.



2. **Noncompetitive inhibitor** binds to the enzyme already bound the substrate and decreases the efficacy of the enzyme.

3. **Uncompetitive inhibitor** binds only to the enzyme substrate complex, but not to the free enzyme.

It occurs in reactions with two or more substrates or products and slows enzyme reactions by binding the substrate to each other (Figure 314).

3.6 Types of enzymes

- Enzymes that catalyze chemical reactions can be classified into various types.
- Enzyme types are based on how enzymes that bind specific molecules together to form new molecules and enzymes that break specific molecules apart into separate molecules.

3.6.1 Enzyme structural classification

- The structural classification of enzymes deals with the separation of an enzyme into **simple proteins** (**active**) and conjugated proteins **(holoenzymes).**
- Then, the conjugated protein part (holoenzyme) is divided into the non-protein part **(cofactor)** and the protein part **(apoenzyme: inactive)** groups.
- Finally, the non-protein part (cofactor) separates into the firmly attached metal ion **(prosthetic group)** and the loosely attached vitamin B complex **(coenzyme)** groups (Figure 3.17).

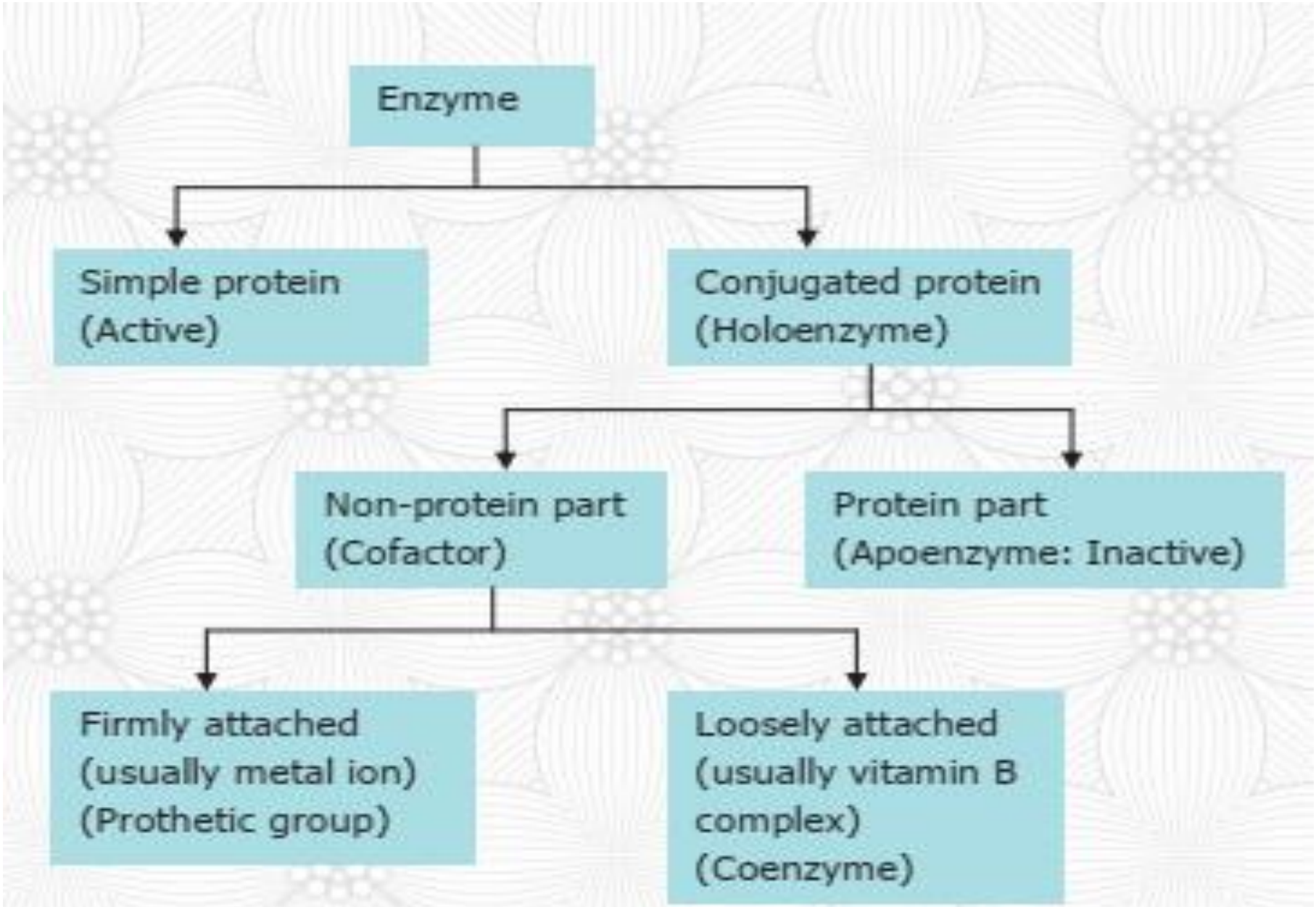


Figure 3.17 Structural classifications of enzymes

Why do some enzymes need cofactors?

- Sometimes an ***active enzyme*** isn't just a single molecule, but is made from **two molecules**,
- neither of which has enzymic activity without the other.
- The two parts are the apoenzyme and the cofactor.
- We can define these in the following way:
- **Apoenzyme** – a protein that combines with a cofactor, to form an active enzyme.
- The protein is inactive on its own.
- **Cofactor** – a small non-protein particle essential for the activity of some enzymes.
- The cofactor combines with the apoenzyme to produce an active enzyme.
- ***HOLOENZYME*** - Where an active enzyme molecule comprises an apoenzyme and a cofactor, the whole is sometimes referred to as the ***HOLOENZYME.***

❖Cofactors include:

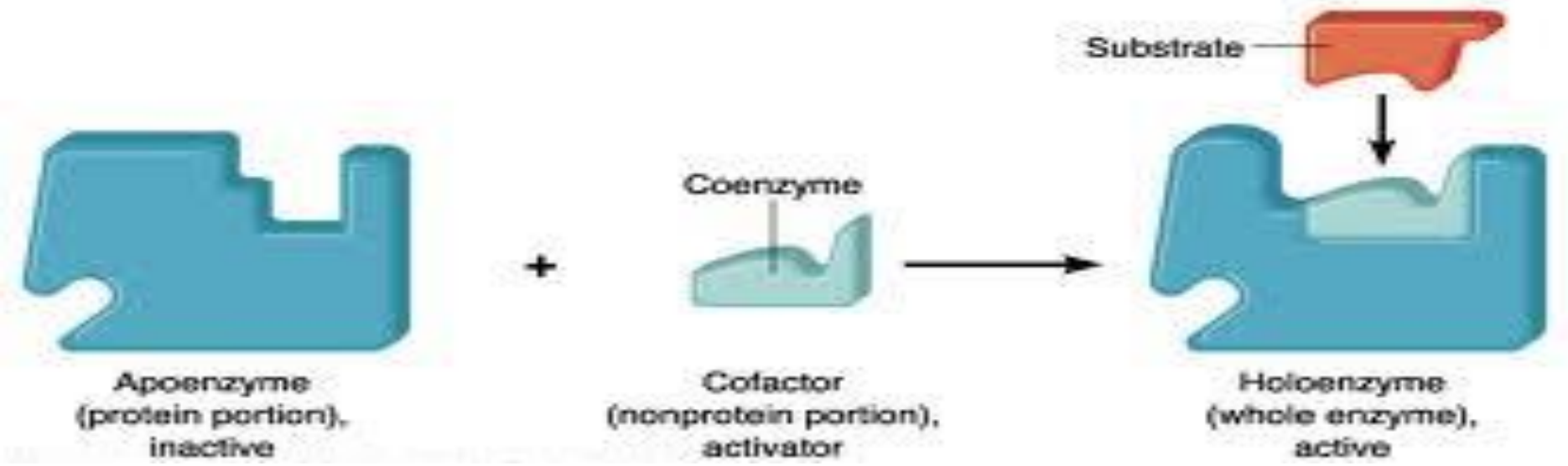
- coenzymes
- mineral ions

❖Coenzymes are organic molecules and many are derived from vitamins.

❖They bind with the enzyme to give catalytic activity.

APOENZYME and HOLOENZYME

- **The enzyme without its non protein moiety is termed as apoenzyme and it is inactive.**
- **Holoenzyme is an active enzyme with its non protein component.**



3.6.2 Basic classification of enzymes

- Enzymes are composed of six classes based on what and how they react, the types of reactions they catalyzed, and the end suffix "**-ase**".

The followings are basic classes of enzymes.

1. **Oxidoreductases** are a class of enzyme that catalyzes oxido-reduction reactions.

- It catalyzes the transfer of electrons from one molecule (oxidant) to other molecule (reductant) reactions in the following pattern:
- $\mathbf{A_{-}} + \mathbf{B} \rightarrow \mathbf{A} + \mathbf{B_{-}}$ where A is the oxidant and B is the reductant.

- **2. Transferase** is an enzyme that transfers functional groups like methyl from one donor molecule to acceptor molecule.
- **3. Hydrolases** are enzymes that catalyze the hydrolysis of various bonds.
- **4. Lyases** are enzymes that cleave bonds by other means rather than hydrolysis or oxidation in which two or more substrates are involved in one reaction.
- **5. Isomerases** are a general class of enzymes that convert molecules from one isomer to another isomer.
- **6. Ligases** are enzymes that catalyze the joining of two molecules with concomitant hydrolysis of the di-phosphate bond in **ATP.**

Enzyme Type	EC	Reaction	Examples
Oxidoreductase	EC1	Oxidation reactions involve the transfer of electrons from one molecule to another	Lipoxidases Dehydrogenases Glucose oxidase
Transferase	EC2	They catalyze the transfer of groups of atoms from one molecule to another	Aminotransferase Transaminase
Hydrolase	EC3	Hydrolysis reactions involve the cleavage of substrates by water	Lactase Proteases Trypsin
Lyase	EC4	Catalyze the addition of groups to double bonds or the formation of double bonds via the removal of groups	Pectate lyases Decarboxylase Hydratases
Isomerase	EC5	Catalyze the transfer of groups from one position to another on the same molecule	Topoisomerase Glucose isomerase
Ligase	EC6	They catalyze the joining of two molecules to form a new bond	Glutathione synthase Aminoacyl tRNA synthetase
Translocases	EC 7	Catalyze the movement of ions or molecules across membranes or their separation within membranes	Ubiquinone reductase ATP synthase Ascorbate ferrireductase

3.7 Factors affecting enzyme action

- There are varieties of factors that affect the activity of enzymes:

✓ temperature,
✓ pH,
✓ inhibitors,
✓ activators,
✓ radiation,
✓ water,
✓ enzyme,
✓ substrate, and
✓ end-product concentrations (Figure 3.18)

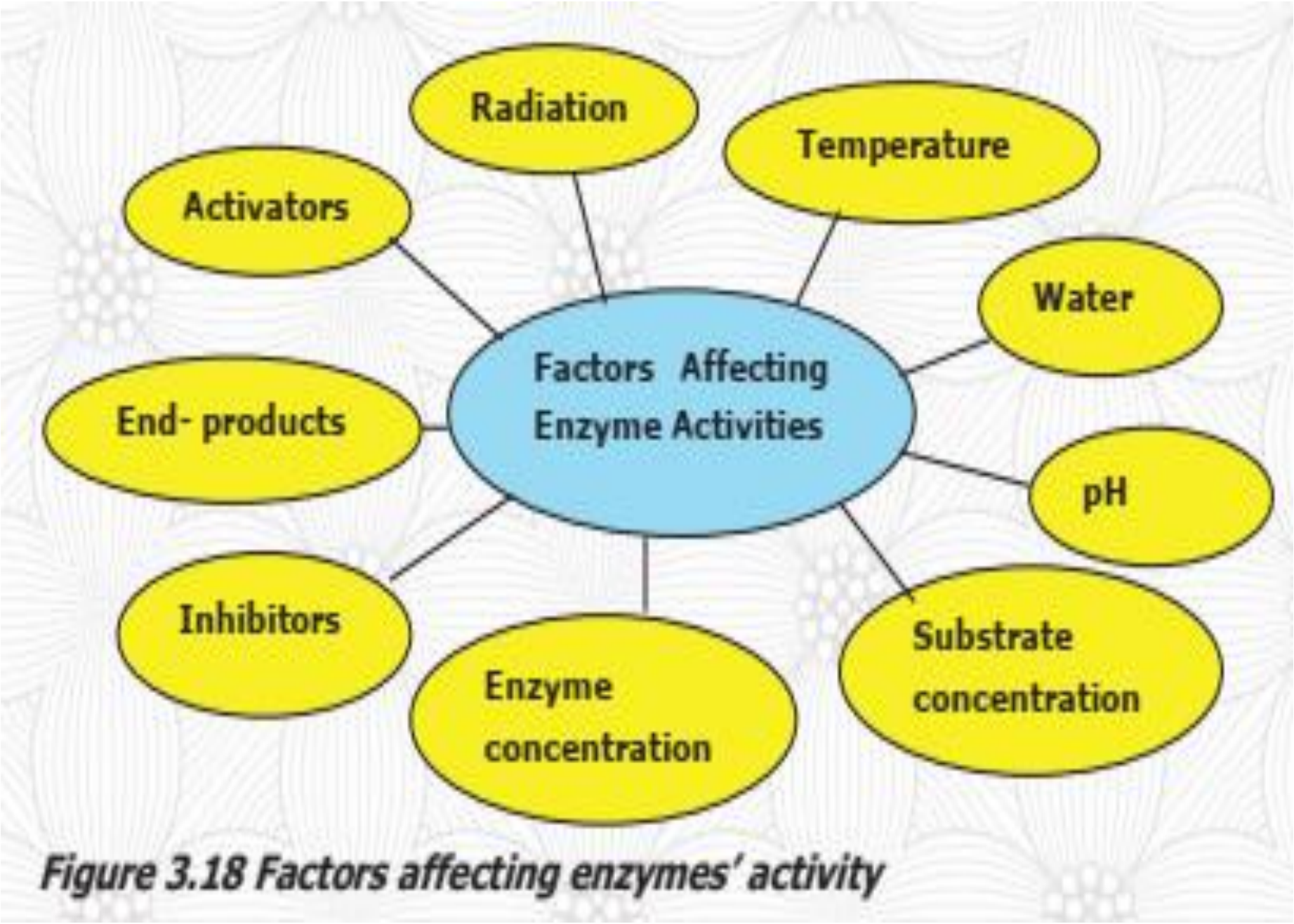


Figure 3.18 Factors affecting enzymes' activity

How are enzymes affected by pH and by temperature?

- **Temperature affects enzyme action in two ways:**

✓ a higher temperature gives the enzyme molecules (and their substrate molecules) more kinetic energy; they move around faster and form more enzyme–substrate complexes

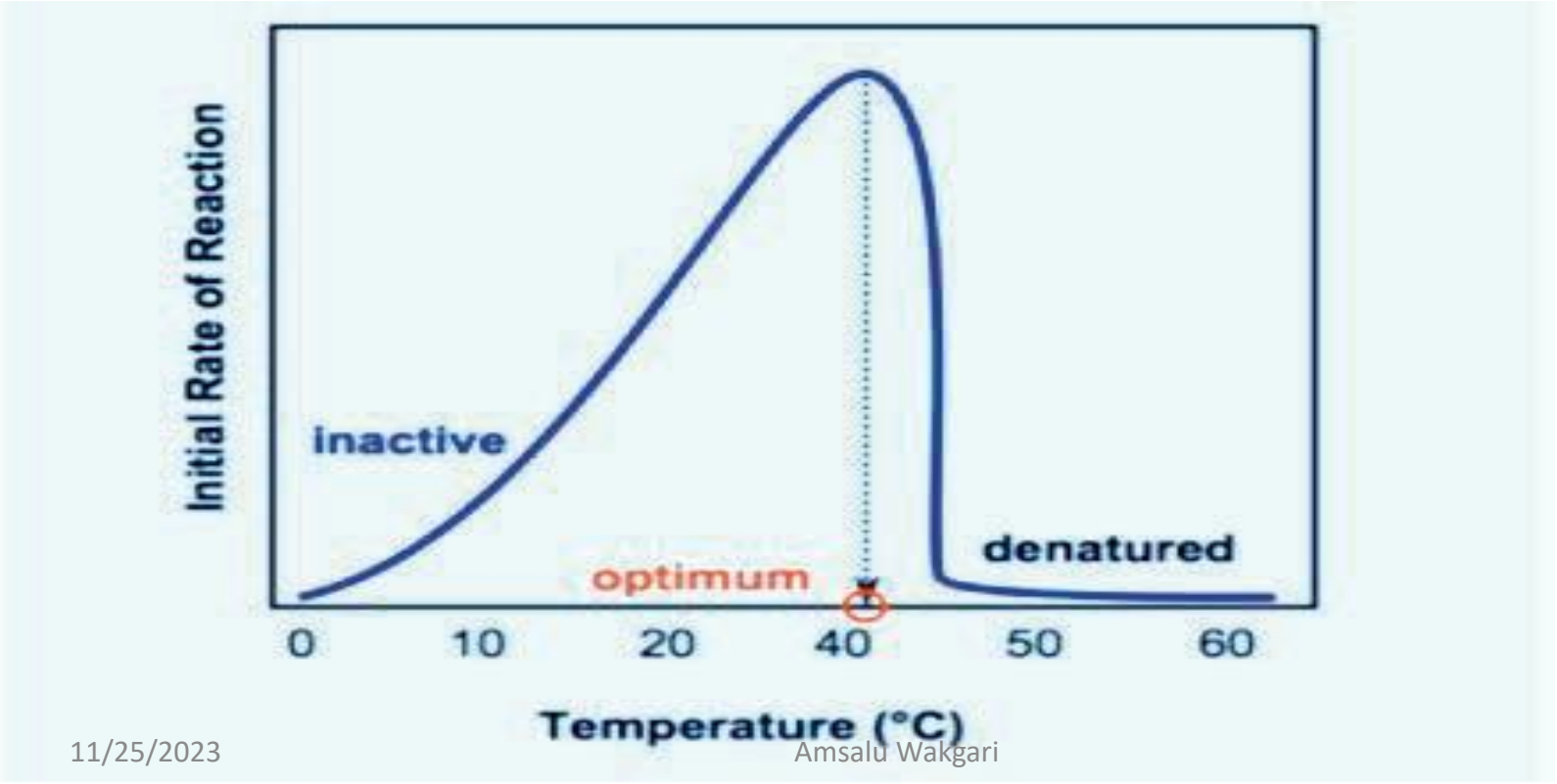
✓ a higher temperature affects the chemical bonds holding the tertiary structure of the enzyme in place **(particularly those in the active site)**; as more and more of these bonds break, the shape of the active site changes and it can no longer bind with its substrate

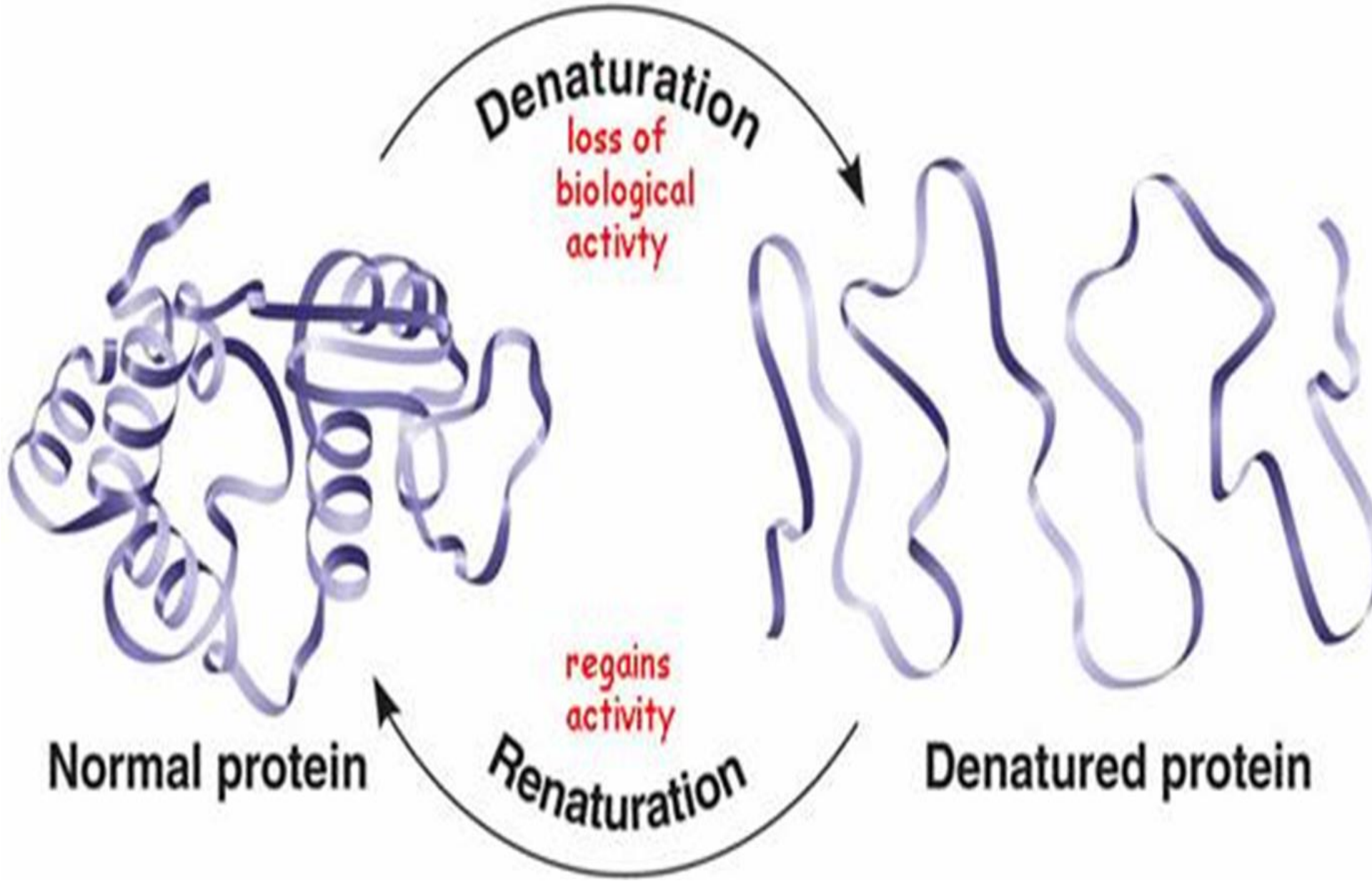
PH

- pH affects the enzyme molecule in a similar way to high temperatures.
- A pH that is too low (too acid) or too high (too alkaline) will cause charges on the active site to alter and cause the active site to lose its conformation.
- The substrate cannot bind and so the reaction is no longer catalysed.

3.7.1 Description on factors affecting enzymatic actions

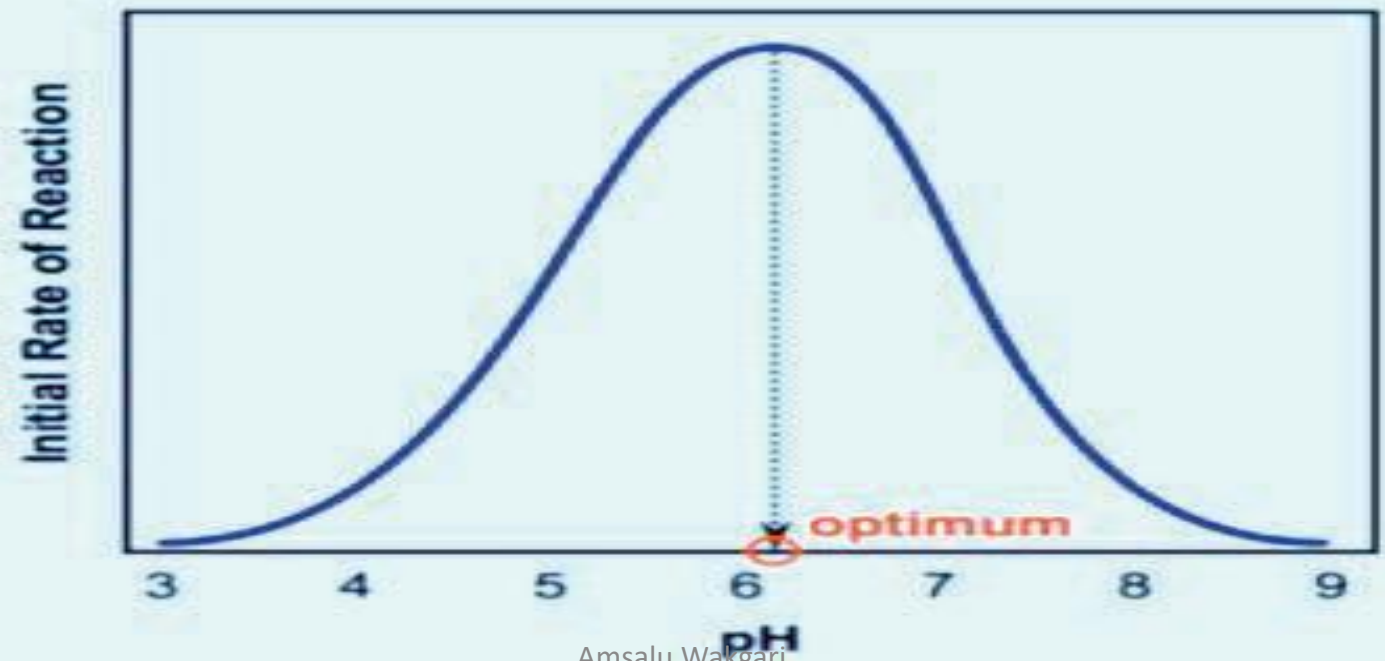
- **Temperature:** while all enzymes work best within the specific ranges of optimum temperatures, low or high temperature causes an enzyme to lose its activity and ability to bind a substrate and denatured.
- Once enzymes denatured, they cannot be renatured.




Denaturation
loss of
biological
activty
regains
activity
Renaturation
Normal protein
Denatured protein

PH:

- enzymes function at optimum pH (the potential of hydrogen ions) that ranges from too low (strong acid) to too high (too alkaline) pH.
- Such extreme temperatures cause an
- enzyme to lose its ability to bind into a substrate.



- **Substrate concentrations:** enzymes require a maximum limit of substrate concentration to bind.

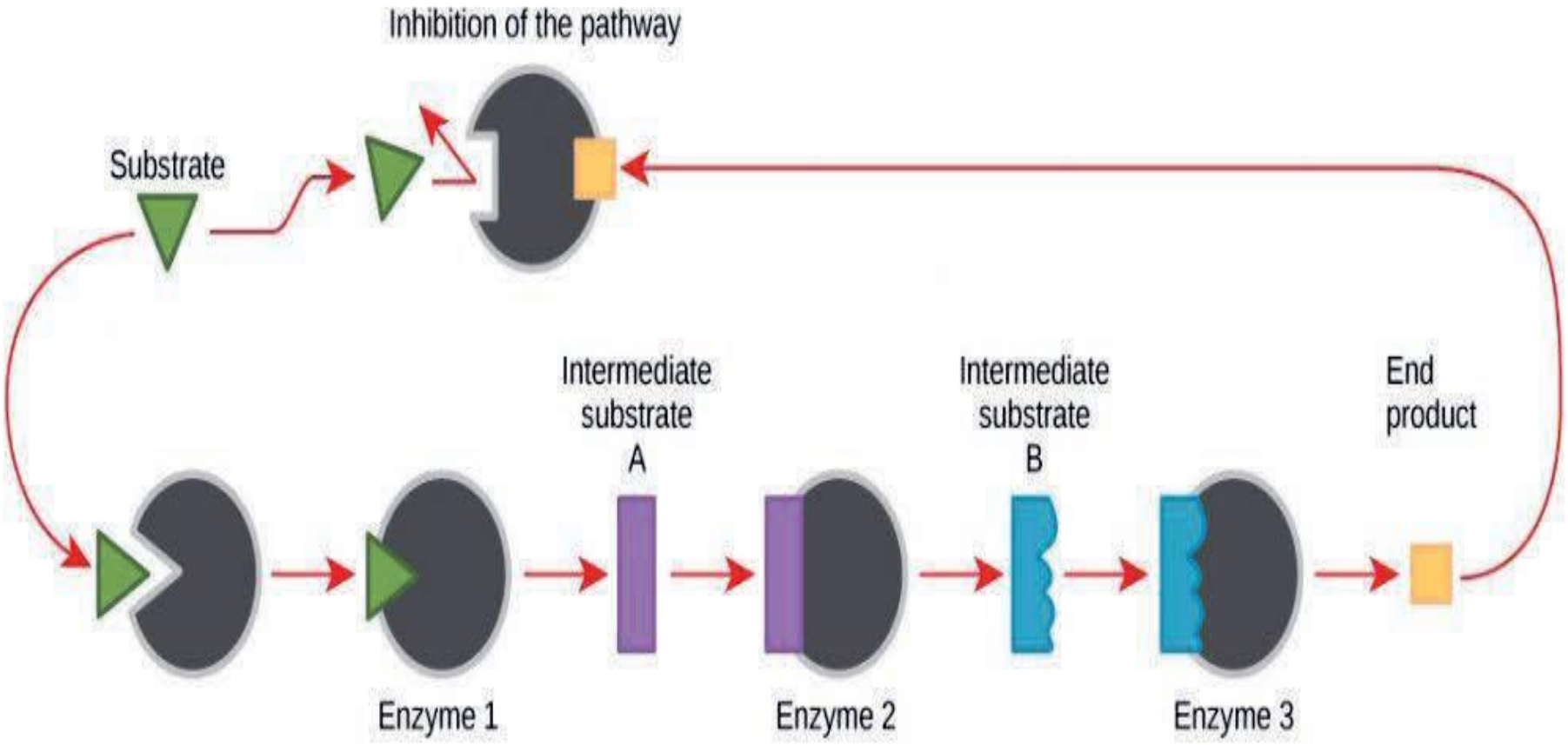
1. **Radiation** damages enzyme activities by reducing in enzymatic efficiency and creating disorders in the macromolecules.

2. **Water:** affects the performance of enzymes' activity beyond its optimum level.

3. **End product (Feedback) inhibition** is a cellular control mechanism in that the end product inhibit enzyme's activity.

In feedback inhibition, the end product binds to the allosteric site of the enzyme and change the structure of the active site. This prevents the enzyme to perform its activity. Due to feedback inhibition, a cell is able to know whether the amount of a product is enough for its subsistence or not.

Figure 3.21 Feedback inhibition



Example: The drug Tipranivir used to treat HIV blocks the activity of a viral genome enzyme to make more copies as a reversible inhibitor.

3.8 Enzyme kinetics

- Enzyme kinetics describes the rates of chemical reactions that are catalyzed by enzymes and the binding affinities of substrates, inhibitors and the maximal catalytic rates achieved.
- Enzyme kinetics explains that enzymes speed up reactions by lowering the activation energy of the reactants and turning them into products.
- Hence, the concentration of enzyme and substrates determines the rate of the reactions or production volumes per unit time.

- One of the most known models of **enzyme kinetics** is the **Michaelis-Menten** formula that takes a form of **equation** describing the rate of enzymatic reaction by relating the reaction rate, rate of formation of product to the concentration of substrate.
- The model explains the relationship between;

✓ the rate of an enzyme-catalyzed reaction [V_1],

✓ the concentration of substrate [S] and

✓ two constants, V_{max} and K_m with the following equation.

$$v_1 = \frac{V_{max}[S]}{\{K_m + [S]\}}$$

Where:

V_1 = the initial velocity/rate reaction

V_{max} = the maximal velocity/maximum rate of reaction

[S] = the substrate concentration

K_M = substrate concentration at half-maximal velocity (Michaelis constant).

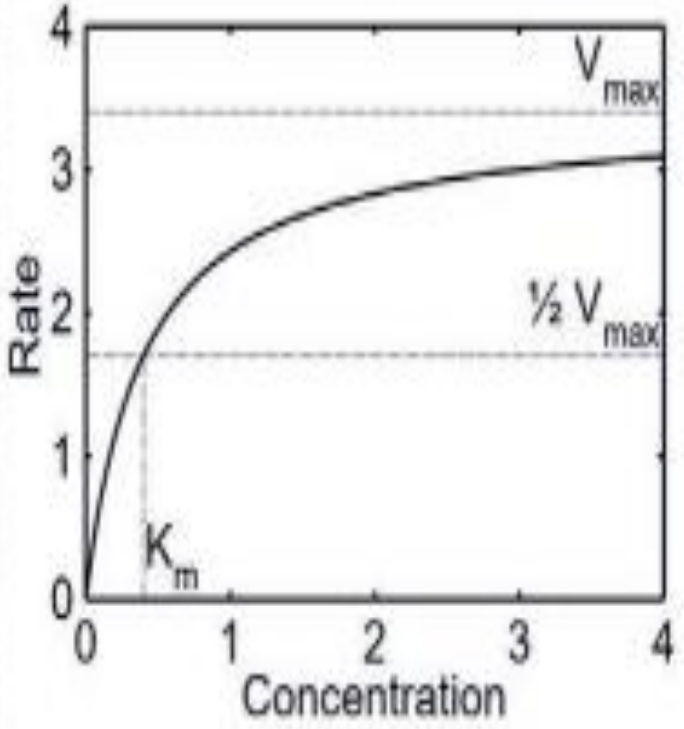


Figure3.22 Enzyme kinetics as a function of concentration

3.9 Application of enzymes in industries and their benefits

- Application of enzymes is the use of enzymatic biochemical reactions for chemical conversion process that are driving forces of great change for productivity of various industries.
- Enzyme protein catalytic activity is efficient enough (100s to 1000s) of times higher than that of inorganic catalyst.

3.9.1 Uses of enzyme application

- The application of enzymes are widely used in food, feed, textile, papermaking, leather and detergents, pharmaceutical and other industrial productions.

Examples:

1. Enzymes break down larger complex molecules into simpler molecules in our body where they can be used to fuel our digestive systems and cellular respirations.

2. Most enzymes used for food industry were extracted from the internal organs of animals and plants, but now most enzymes are obtained by microbial fermentation.

3. Enzymes cause billions of chemical reactions to happen at lightning speed inside the cells of our body.

- 4. Enzymes improve the utilization of feed rate of starch, protein, and minerals and degrade the anti-nutritional factors in animal feed, prevent animal indigestion and improve feed digestibility.
- 5. In the pharmaceutical industry, enzymes are used in drugs, antibiotics, household products to speed up chemical reactions and synthesis.
- 6. Enzymes are powerful tools in sustaining a clean environment in several ways.
- 7. Washing powders are enzymes used to break down protein, starch and fat stains on clothes

Table 3.3 the applications and functions of enzymes

Cellulases	Biofuel industry	Breakdown cellulose into sugars and ferment to produce cellulosic ethanol
Ligninase	Biofuel industry	Pre-treatment of biomass for biofuel production
Protease, Amylase, Lipase	Biological detergent	Protease removes protein, amylase removes starch, and lipase removes fat or oil stains from laundry and dishware
Mannanase	Biological detergent	Remove food stains from the common food additive guar gum
Betaglucanase	Brewing industry	Improve beer filtration
Papain	Culinary uses	Tenderize meat for cooking
Rennin	Dairy industry	It hydrolyses protein in the manufacture of cheese
Trypsin	Food processing	Manufacture hypo-allergenic baby foods
Cellulases, Pectinase	Food processing	Clarify fruit juices.
Nuclease, DNA Ligase, Polymerase	Molecular biology	Uses restriction digestion and polymerase chain reaction to create recombinant DNA

3.10 Malting in Ethiopian tradition

- Malting (sprouting) is a widely applied traditional technology.
- It is the process of **steeping**, **germinating** and **drying grain** to convert it into **malt.**
- Malting is the limited controlled germination of grains in moist air, which results: in the mobilization of amylases, proteases and other enzymes that hydrolyze and modify the grain components and its structure

3.10.1 Steps of modern malting

- There are three steps to modern malting,
- ✓ Steeping
- ✓ Germinating and
- ✓ Kilning.

1. Steeping

- Steeping is the process of cleaning the grain kernels and bringing it to life with water and oxygen by immersing it in the water and air for a specified time period.
- The water activates naturally the existing enzymes in grains and stimulates the production of enzymes in which water temperature and aeration are vital for producing high quality malt.
- Although the process can vary depending on the grain type and size, malting occurs over a period of 24–48 hours.

- The **steeping** will be complete when the barley has reached a sufficient moisture level to allow a uniform breakdown of starches and proteins.
- So starch and protein hydrolyzing enzymes are activated during steeping

2. Germinating

- Germinating is to continue the process with the growth and modification of the grain.
- Rootlets emerge from the kernel to the outside of the grain and within the outer husk and a shoot or acrospires grows.

- **Modification** is the breakdown of protein and carbohydrates, resulting in the opening up of the seeds' starch reserves within four to six days as **Green Malt**.
- The control of temperature and moisture levels with regulated airflow and the uniformity of water spray enables achieve a high quality and consistent germination process.
- Malting is partly an art and partly a science that can be gauged in the degree of modification with the eyes, sense of smell and hands.

3. Kilning (Heating)

- ✓ Kilning is the heating treatment of germinated grain to dry the green malt and prevent from further germination.
- ✓ Removing moisture from the germinated grain is initially for **withering**.
- ✓ Additional drying further reduces the moisture content and prepares the malt for flavor and color development.
- ✓ The kilning process achieves enzymatic activity and friability, a wide ranges of malt colors and flavors and distinctive ales and lagers.

3.10.2 Why is malting for?

- **Malting** aims to convert or modify the **physical structure of the barley grain** and **allow synthesis** or **activation of a series of enzymes** to produce malt for uses in the subsequent purposes (brewing, distilling or food production).
- Barley is the most common cereal used for the production of malt because of its high starch-to-protein ratio and adhering husk that contribute to the economic yield, ease of processing and production.
- The most common enzymes used in the malting process are **beta-glucanase**, **alphaamylase, protease** (breaks down proteins) and **beta-amylase.**

- The main purpose of malting is to produce enzymes such as:
- the **α-amylase** and
- **β- amylase** useful for modifying and converting grains' **starches** into simple sugar (monosaccharide), complex sugar (disaccharides) and malt sugar (maltose) and higher sugars called **maltodextrines.**
- Then after, the yeast uses these monosaccharides to to produce alcohol by alcoholic fermentation.

Traditional malting for local alcohol production

- Traditional malting is the process of sprouting barley grains for the production of enzymes (α-amylase and β-amylase**)** to process fermentation drinks such as Tella.
- The steps of traditional malting process include:

1. Soaking barley grains (steeping)
2. Germinating (sprouting)
3. Kilning (Heating) the malt

- The most commonly used grains for malting are barley, maize, millet, sorghum and the like.
- However, barley is the most preferable grain to produce local drinks in local malting (Figure 3.23).



Figure 3.23 Malted barley

- The main purpose of malt production is to produce alcoholic beverages drinks for consumptions and income generation to support the livelihood of the people.
- Malting requires raw materials like barley for fermentation of alcoholic drinks like Tella.
- People in Ethiopia produces local drinks like Booka, Cheka, Keribo, Korefe, Shameta, Borde and Teji in different occasions (holidays, wedding ceremonies, and celebrations).

3.11 Renowned Biochemists in Ethiopia

- A biochemist is a scientist who studies the chemical processes transformations in living organisms including DNA, proteins and cell parts.
- A biochemist also conducts research on how certain chemical reactions happen in cells, tissues and organisms and record the effects of products in food additive and medicines.
- The study of biochemistry deals with all aspects of the immune systems, expressions of genes, isolation, analysis and synthesis of products.

- It also concerned with studying mutations that leads to cancers, scientific procedures used to manage and monitor laboratory works.
- Biochemists also conduct research in the field of agriculture, in the interactions between herbicides with plants.
- They also examine the relationships between or among compounds that determine the ability to inhibit growth and evaluate the toxicological effects.

Our world is built on biology and once we begin to understand it, it then becomes a technology. …