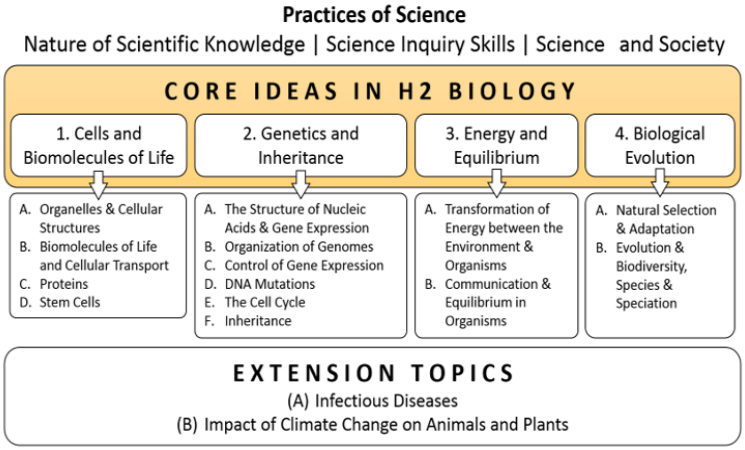
Before 1st lecture:
Pls complete and mark questions on pgs 5 and 6.

Tampines Meridian Junior College

JC1 H2/9744 Biology 2023

Core Idea 1B/1C/2A

2. Biomolecules of Life and Cellular Transport



SYLLABUS OVERVIEW		
No.	**Overarching Idea**	**Topics**
1	**Core Idea 1** The Cell and Biomolecules of Life	Cell – The Basic Unit of Life
2		Biomolecules of Life and Cellular Transport
3	**Core Idea 3** Energy and Equilibrium	Transformation of Energy – Photosynthesis and Cellular Respiration
4	**Core Idea 2** Genetics and Inheritance	Genetics and Inheritance (I) – The Cell Cycle
5		Genetics and Inheritance (II) – DNA Replication and Gene Expression
6		Genetics and Inheritance (III) – DNA Mutations and their Consequences
7		Genetics and Inheritance (IV) – Molecular Techniques in DNA Analysis
8		Genetics and Inheritance (V) – Organization of Genome & Control of Gene Expression in Eukaryotes *[Includes Core Idea 1D: Stem Cells]*
9		Genetics and Inheritance (VI) – Organization and Inheritance of Viral Genomes
10		Genetics and Inheritance (VII) – Organization of Genome & Control of Gene Expression in Prokaryotes
11		Genetics and Inheritance (VIII) - Inheritance
12	**Core Idea 3** Energy and Equilibrium	Communication and Equilibrium in Multicellular Organisms
13	**Core Idea 4** Biological Evolution	Biological Evolution
14	**Extension Topic A** Infectious Diseases	Immunity and Infectious Diseases
15	**Extension Topic B** Impact of Climate Change on Animals & Plants	Climate Change – Causes and Impacts on Animals and Plants

TOPIC SYNOPSIS

Core Idea 1: The Cell & Biomolecules of Life – entails the study of cells, which are the basic units of life.

The following questions should help you frame your learning:

- How are the structures of biomolecules related to their functions?
- How do cells regulate the movement of substances into and out of themselves; and what are the implications of such movements?

Biomolecules make up cells and cells regulate the movement of substances into and out of themselves and cellular processes through membranes

The different classes of biomolecules (sugars, lipids, proteins and nucleic acids) function as molecular building blocks for macromolecules to be assembled. Nucleic acids, which include DNA and RNA, are made from monomers known as nucleotides. Phospholipids, cholesterol, carbohydrates and proteins are important components in biological membranes.

Cells need to regulate the movement of substances into and out of themselves. Substances such as water, oxygen, glucose, minerals are important in the synthesis of new molecules and important cellular processes. According to the fluid mosaic model, cell membranes are selectively permeable due to the nature of the phospholipids and proteins from which they are made. The movement of different molecules depends on the nature of the substances through transport processes such as osmosis, diffusion and active transport. Membranes allow cells to create and maintain internal environments that are different from external environments.

Proteins, which are class of biomolecules, play a significant role in cells

Proteins play a variety of roles in cells including structural, transport, enzymatic and signalling functions. They are essential for biological processes and functions, such as chemiosmosis, protein synthesis, cell signalling, immunology and blood glucose homeostasis. Protein structure can be affected by temperature and pH. Enzymes are an important group of proteins that control many biological reactions. The functions of these proteins will be revisited in other core ideas.

LEARNING OUTCOMES

Core Idea 1B: Biomolecules of Life and Cellular Transport

This concept focuses on how the structures of biomolecules give rise to properties that allow these biomolecules to carry out their functions. One of these functions involves regulating transport of substances into and out of the cell. This regulation is afforded by the properties of the cell membrane, which comprise phospholipids and proteins. Regulation of the movements is important for several biochemical processes to occur.

Candidates should be able to:

g) Describe the structure and properties of the following monomers:
- **i)** α-glucose and β-glucose (in carbohydrates)
- **ii)** glycerol and fatty acids (in lipids)
- **iii)** amino acids (in proteins) (chemical formulae of specific R-groups of different amino acids are not required).

h) Describe the formation and breakage the following bonds:
- **i)** glycosidic bond
- **ii)** ester bond
- **iii)** peptide bond

i) Describe the structure and properties of the following biomolecules and explain how these are related to their roles in living organisms:
- **i)** starch (including amylose & amylopectin)
- **ii)** cellulose
- **iii)** glycogen
- **iv)** triglyceride
- **v)** phospholipid

j) Explain the fluid mosaic model and the roles of the constituent biomolecules (including phospholipids, proteins, glycolipids, glycoproteins and cholesterol) in cell membranes.

k) Outline the function of membranes at the surface of cells and membranes within the cell.

l) Explain how and why different substances move across membranes through simple diffusion, osmosis, facilitated diffusion, active transport, endocytosis and exocytosis.

Core Idea 2A: The Structure of Nucleic Acids and Gene Expression

The structure of DNA was proposed by Watson and Crick in 1953. With an understanding of DNA structure, experimental evidence supported the proposal that DNA replicates in a semi-conservative manner. The central dogma states that genetic information is encoded in the DNA and transferred to the mRNA during transcription. In addition to mRNA transcription, tRNA and rRNA are transcribed; tRNA is needed during translation while rRNA is a component of ribosomes. In eukaryotic transcription, pre-mRNA is synthesised and then processed to produce mature mRNA. Subsequently, through translation, the information on the mRNA is used to synthesise polypeptides, which are folded into functional proteins

a) Describe the structure and roles of DNA and RNA (tRNA, rRNA and mRNA). (Mitochondrial DNA is not required)

Core Idea 1C: Proteins

Proteins play a variety of roles in structural, transport, enzymatic and signalling functions. This concept focuses on the structure and properties of proteins and how temperature and pH may contribute to denaturation of proteins. The structure of a protein is related to its function.

m) Explain primary structure, secondary structure, tertiary structure and quaternary structure of proteins, and describe the types of bonds that hold the molecule in shape (hydrogen, ionic, disulfide bonds and hydrophobic interactions).

n) Explain the effects of temperature and pH on protein structure.

o) Describe the molecular structure of the following proteins and explain how the structure of each protein relates to the function it plays:
- **i)** haemoglobin (transport)
- **ii)** collagen (structural)
- **iii)** G-protein linked receptor (signalling)

(Details of the number of amino acids, types of secondary structures present are not required.)

p) Explain the mode of action of enzymes in terms of an active site, enzyme-substrate complex, lowering of activation energy and enzyme specificity using the lock-and-key and induced-fit hypothesis.

q) Investigate and explain the effects of temperature, pH, enzyme concentration and substrate concentration on the rate of an enzyme-catalysed reaction by measuring rates of formation of products (e.g. measuring gas produced using catalase) or rate of disappearance of substrate (e.g. using amylase, starch and iodine).

r) Describe the structure of competitive and non-competitive inhibitors with reference to the binding sites of the inhibitor.

s) Explain the effects of competitive and non-competitive inhibitors (including allosteric inhibitors) on the rate of enzyme activity.

Pre-lecture Exercise: How much do you remember concepts from 'O' level biology?

Recall that there are four classes of Biomolecules: (1) Carbohydrates, (2) Lipids, (3) Nucleic acids, and (4) Proteins.

Use the words in the helping box to fill in the blanks for the following statements. The words may be used more than once.

four	seven	acids	bases	carbon	catalyst	condensation	COOH
dipeptide	glycogen	hydrolysis	isomers	lipids	monosaccharides		peptide
poly	polymers	two	water	R-	substrate	polypeptide	organic
RNA	amino acids	DNA	enzymes	fatty	fructose	galactose	glucose
H	one	monomers	NH_2	oils			

Introduction

1. _____ compounds are made by living things and always contain carbon.
2. _____ forms the backbone of organic compounds.
3. _____ have the same molecular formula but different structures.
4. _____ is the most important inorganic compound.
5. _____ reactions break down polymers into monomers.
6. _____ release hydrogen ions in water and have a low pH.
7. _____ release hydroxide ions (OH) and have a high pH.
8. A _____ or dehydration reaction is a reaction that joins two monomers and releases a molecule of water.
9. A pH of ____ is neutral.
10. A substance that controls the rate of a reaction without being affected by the reaction is called a _____.
11. Carbon atoms can form _____ covalent bonds with other atoms.
12. Chains of monomers are called _____.
13. Oxygen atoms can form _____ bonds with other atoms.
14. Hydrogen atoms can form _____ bond with other atoms.
15. In hydrolysis reactions, _____ molecules combine with parts of the monomers.
16. The chemical catalysts of the body are called _____.
17. The molecule that an enzyme fits is called a _____.
18. Most organic molecules consist of basic units called _____ that are repeated over and over.
19. Stomach acid has a pH of ____.

Carbohydrates

20. _____ are single-molecule sugars.
21. _____saccharides are many-molecule sugars.
22. A polysaccharide found in animals is called _____.
23. Disaccharides are _____-unit sugars
24. Glucose, fructose, and galactose are examples of _____.
25. Lactose is made of glucose and _____.
26. Maltose is made of _____ and glucose.
27. Sucrose is made of glucose and _____.

Lipids

28. Cholesterol and triglycerides are examples of _____.
29. Fats are made of glycerol and _____ acids.
30. Lipids are made of fats, _____, waxes, and sterols

Nucleic acids

31. The two nucleic acids are _____ and _____.

Proteins

32. _____ bonds connect the amino group of one amino acid to the acid group of another amino acid.
33. A _____ is made of two amino acids joined together
34. A _____ is made of many amino acids joined together.
35. The acid group in an amino acid is the _____ group.
36. Each of the 20 kinds of amino acids has a different ___-group (or side chain).
37. Proteins are made of chains of _____.
38. The symbol for the amino group is _____.
39. The symbol for the hydrogen group is _____.

Ans: *1.Organic 2.Carbon 3.Isomers 4.Water 5.Hydrolysis 6.Acids 7.Bases 8.Condensation 9.Seven 10.Catalyst 11.Four 12.Polymers 13.Two 14.One 15.Water 16.Enzymes 17.Substrate 18.Monomers 19.Two 20.Monosaccharides 21.Poly 22.Glycogen 23.Two 24.Monosaccharides 25.Galactose 26.Glucose 27.Fructose 28.Lipids 29.Fatty 30.Oils 31.DNA and RNA 32.Peptide 33.Dipeptide 34.Polypeptide 35.COOH 36.R 37.Amino acids 38.NH2 39.H*

LECTURE OUTLINE

1. Introduction
- 1.1 Overview of the biomolecules of life
- 1.2 Basic biochemistry
- 1.3 Water
- 1.4 Macromolecules of life

2. Carbohydrates (*Campbell Biology 9th ed. page 115-123*)
- 2.1 Monosaccharides
  - 2.1.1 Glucose
- 2.2 Disaccharides
  - 2.2.1 Formation and breakage of the glycosidic bond
- 2.3 Polysaccharides
- 2.4 Examples of polysaccharides
  - 2.4.1 Starch (storage polysaccharide)
  - 2.4.2 Glycogen (storage polysaccharide)
  - 2.4.3 Cellulose (structural polysaccharide)
- 2.5 Checklist for carbohydrates

3. Lipids (*Campbell Biology 9th ed. page 120-123*)
- 3.1 Classification of lipids
- 3.2 Function of lipids
- 3.3 Examples of lipids
  - 3.3.1 Triglycerides
  - 3.3.2 Phospholipids
  - 3.3.3 Cholesterol
- 3.4 Checklist for lipids

4. Nucleic Acids (*Campbell Biology 9th ed. page 132-137*)
- 4.1 Structures of nucleic acid monomers
- 4.2 Formation of a polynucleotide
- 4.3 Macromolecular structure of deoxyribonucleic acid (DNA)
- 4.4 Macromolecular structure of ribonucleic acid (RNA)
  - 4.4.1 Types of RNA: Messenger RNA (mRNA), Ribosomal RNA (rRNA) & Transfer RNA (tRNA)
- 4.5 Checklist for nucleic acids

5. Proteins *(Campbell Biology 9th ed. page 123-132, page 152 - 160)*

- 5.1 Structure of amino acids
- 5.2 Types of bonding and interactions involved in structure of proteins
  - 5.2.1 Peptide bonds
  - 5.2.2 Disulphide bonds
  - 5.2.3 Ionic bonds
  - 5.2.4 Hydrophobic Interactions
  - 5.2.5 Hydrogen bonds
- 5.3 Structure of proteins
- 5.4 Examples of proteins
  - 5.4.1 Haemoglobin (globular protein, transport function)
  - 5.4.2 Collagen (fibrous protein, structural function)
  - 5.4.3 G-protein linked receptor (globular protein, signalling function)
- 5.5 Enzymes
  - 5.5.1 Metabolic reactions and pathways in a cell
  - 5.5.2 What are enzymes?
  - 5.5.3 Mode of action of enzymes
    - (a) Mechanism of enzyme action
    - (b) How enzymes lower the activation energy of a reaction
  - 5.5.4 Measuring the rate of an enzyme-catalysed reaction
  - 5.5.5 Factors affecting the rate of enzymatic reaction
    - (a) Effects of Substrate concentration
    - (b) Effects of Enzyme concentration
    - (c) Effects of pH
    - (d) Effects of Temperature
  - 5.5.6 Enzyme cofactors
  - 5.5.7 Enzyme Inhibition
    - (a) Competitive Inhibition
    - (b) Non-competitive Inhibition
    - (c) Competitive VS Non-competitive inhibition
  - 5.5.8 Regulation of metabolic pathways by cells
    - (a) Allosteric Enzymes
    - (b) End-product/Feedback Inhibition
- 5.6 Checklist for proteins

6. Cellular Transport *(Campbell Biology 9th ed. Page 171-187)*

- 6.1 Membrane Structure and Function
  - 6.1.1 Membrane Structure
  - 6.1.2 The fluid Mosaic Model
  - 6.1.3 Membrane components and their functions
  - 6.1.4 Functions of the membrane
- 6.2 Transport across the membrane
  - 6.2.1 Osmosis
  - 6.2.2 Simple diffusion
  - 6.2.3 Facilitated diffusion
  - 6.2.4 Active transport
  - 6.2.5 Bulk transport (Exocytosis and Endocytosis)
- 6.3 Checklist for Membrane Structure and Function

Annex A - A Brief Glossary of Biological Terminology

ANIMATIONS

Biological Molecules

Cyclisation of glucose:
https://www.youtube.com/watch?v=wFYsufJ9XMM

Formation of glycosidic bond (carbohydrates) and formation of triglyceride:
http://bcs.whfreeman.com/WebPub/Biology/hillis1e/Animated%20Tutorials/at0202/at_0202_carbs_lipids.html

Formation of glycosidic bond, peptide bond and phosphodiester bond:
https://learninglink.oup.com/access/content/cooper8e-student-resources/cooper8e-chapter-2-animation-1

Protein folding and structure:
https://www.youtube.com/watch?v=lijQ3a8yUYQ
http://www.bcconline.com/biol10rs/Pearson-Animations/protein_structure.swf

Protein denaturation (effect of temperature):
http://www.sumanasinc.com/webcontent/animations/content/proteinstructure.html

The chemical structure of DNA:
https://www.biointeractive.org/classroom-resources/chemical-structure-dna

RNA vs DNA structural comparison:
http://www.dnatube.com/video/1017/DNA-and-RNA-structural-comparison

Enzymes

Enzyme-controlled Biochemical Pathway
https://www.youtube.com/watch?v=9Vbm4__MMH4

Activation energy and enzymes
https://www.youtube.com/watch?v=k-3HG8CsTR0

How enzymes work
https://www.youtube.com/watch?v=aRSfPLp_I10

Coenzyme moulding the active site into shape (E.g. Vitamin b)
https://www.youtube.com/watch?v=jbltegi4sOk

Coenzyme transferring atoms and chemical groups (E.g. NAD^+)
https://www.youtube.com/watch?v=Kb-4uuCYLvE

Competitive VS Non-competitive inhibition
http://www.youtube.com/watch?gl=SG&hl=en-GB&v=PILzvT3spCQ

Allosteric enzymes
https://www.youtube.com/watch?v=WAZXqhtduFw

Feedback inhibition
https://www.youtube.com/watch?v=qHb7iieM2Ro

Cellular Transport

Fluidity of the cell membrane
https://www.youtube.com/watch?v=jM_xePC70Yo

How osmosis works
https://www.youtube.com/watch?v=-g-VJymtAf4

How facilitated diffusion works
https://www.youtube.com/watch?v=-ZofHcQ-k84

Phagocytosis
https://www.youtube.com/watch?v=qODxxmj9Uhc

How a pump works (using Na-K pump as an example)
https://www.youtube.com/watch?v=_bPFKDdWlCg

Endocytosis and exocytosis
https://www.youtube.com/watch?v=nXrU6AaPp88

A neutrophil (a type of white blood cell) chasing after a bacterium
https://www.youtube.com/watch?v=Z_mXDvZQ6dU

1. Introduction

1.1 Overview of the biomolecules of life

This topic gives an overview of the various biological molecules you will encounter in the 'A' Level syllabus.

- Living matter consists mainly of carbon, hydrogen, oxygen and nitrogen, with smaller amounts of sulfur and phosphorus. These elements all form strong covalent bonds, an essential characteristic in the architecture of complex organic molecules. Of all these elements, carbon is central to the organic molecules of life. The formation of four covalent bonds per carbon molecule with other atoms makes possible the great diversity of organic molecules. This forms the foundation of life at the molecular level.

- There are four main classes of biological molecules:
  (i) Carbohydrates (Section 2)
  (ii) Lipids (Section 3)
  (iii) Nucleic acids (DNA and RNA) (Section 4)
  (iv) Proteins (Section 5)

- Let us look at various properties of molecules first.

1.2 Basic Biochemistry

- **Charged molecule/particle (ion) -** An atom or molecule that has gained or lost one or more electrons, thus giving the molecule a net positive or negative charge (Fig. 1.1).

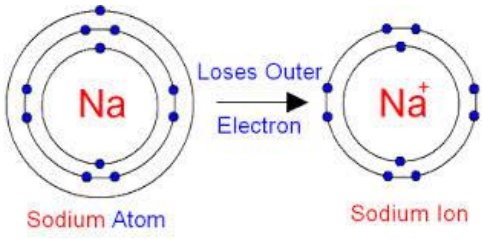


Fig. 1.1: An example of a charged atom, whereby there is a loss of 1 electron, resulting in an overall positive charge.

- **Ionic bond -** A **chemical bond** resulting from the attraction between oppositely **charged** ions (Fig. 1.2).

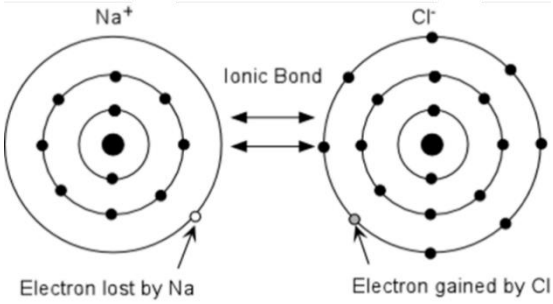


Fig. 1.2: An example of ionic bond (between sodium and chlorine).

- **<u>Covalent bond</u> -** A type of **strong chemical bond** in which two atoms *share* one or more pairs of valence electrons (Fig. 1.3).

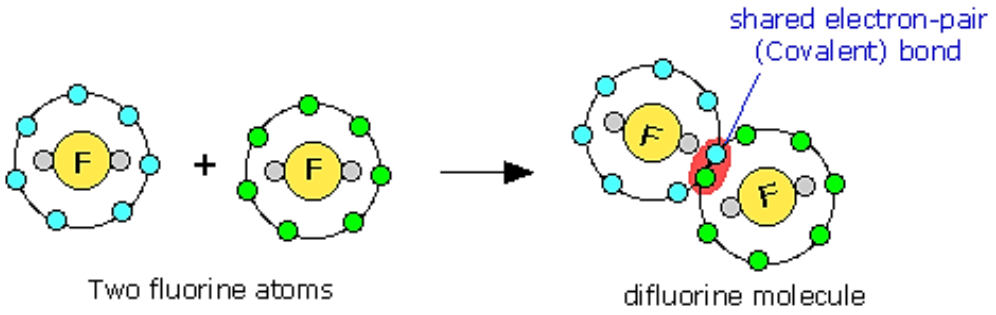


Fig. 1.3: An example of covalent bond; a fluorine atom with 7 electrons in the outer shell, will share with another fluorine atom with 7 electrons to form a closed shell (8 electrons).

- **<u>Polar</u> molecule** - A molecule with **uneven charges** in different regions of the molecule due to unequal sharing of electrons (Fig. 1.4).

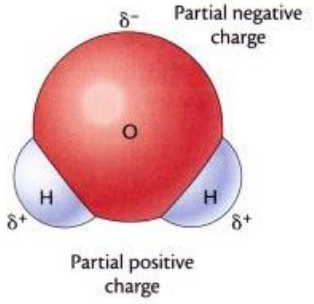


Fig. 1.4: Water is an example of a polar molecule. Water comprises two hydrogen (H) atoms joined to an oxygen (O) atom by covalent bonds. Due to differences in electronegativities between O atom and H atom, there is uneven distribution of charges, thus making water polar in nature.

- **<u>Hydrogen bond</u>** - A **weak chemical bond** that is formed when the **slightly positive atom (δ+)** of a **polar** molecule is attracted to a **slightly negative atom (δ-)** of another polar molecule / another region of the same molecule (Fig. 1.5).

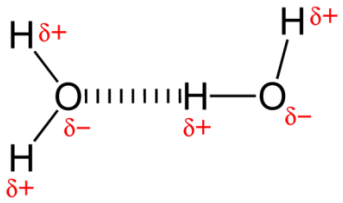


Fig. 1.5: An example of hydrogen bond between two water molecules. As water molecules are polar, the slightly positive H (δ+) of one water molecule is attracted to the slightly negative O (δ-) of *another* water molecule, thus forming a hydrogen bond**.**

- **<u>Hydrophilic</u>** *("hydro" = water, "philic" = like)* - the ability to form chemical interactions like **hydrogen bonds** with water (Fig. 1.6).
    - **Polar** and **charged** molecules are hydrophilic.

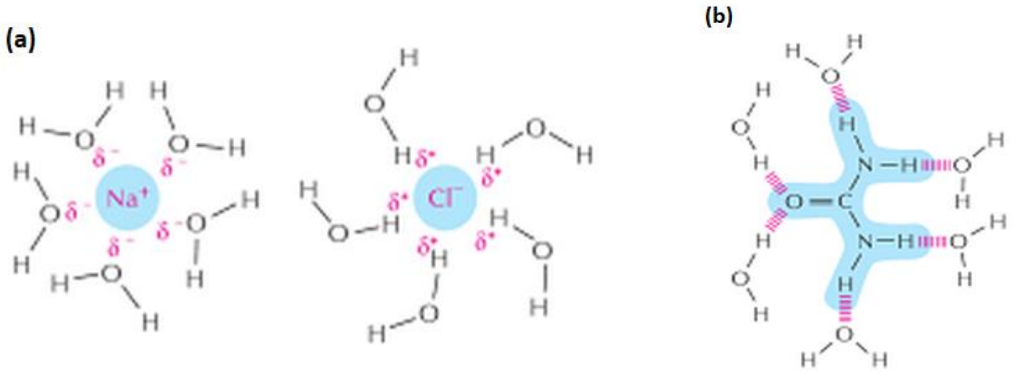


Fig. 1.6: (a) Ionic substances such as sodium chloride is hydrophilic and dissolve in water because polar water molecules are attracted to the positive charge (Na^+) or negative charge (Cl^-) of each ion. **(b)** Polar substances such as urea is hydrophilic and dissolve in water because their molecules form hydrogen bonds with the surrounding polar water molecules.

- **<u>Hydrophobic</u>** *("phobic" = hate)* - the **inability** to form chemical interactions like **hydrogen bonds** with water (Fig. 1.7).
    - **Non-polar** molecules are hydrophobic. These molecules form **<u>hydrophobic interactions</u>** with each other.

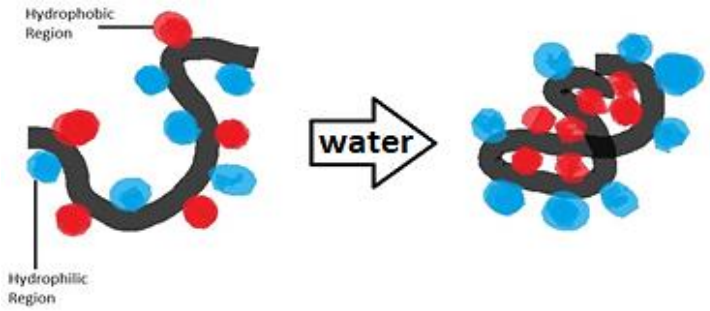


Fig. 1.7: Hydrophobic regions of a non-polar molecule forming hydrophobic interactions with each other when placed in water.

- **<u>Amphipathic</u>** – A term used to describe a molecule that has **both hydrophilic and hydrophobic regions** (Fig. 1.8).

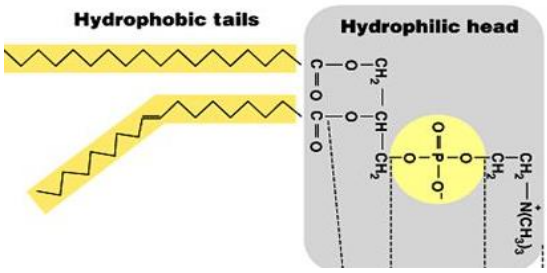


Fig. 1.8: An example of an amphipathic molecule. It has a hydrophilic head region and a hydrophobic tail region.

1.3 Water

- Water is the most abundant and important molecule in cells and tissues.
- Water has many functions:
    - Solvent for almost all other compounds in the cell (e.g. urea, proteins) by forming hydrogen bonds with polar groups or charges of many cellular constituents.
    - Reactant or product in thousands of different biochemical reactions (e.g. synthesis of proteins and ATP).
    - Important determinant of the biological structures of lipid bilayers, folded proteins, and macromolecules, which are all influenced by the aqueous environment they reside in.

1.4 Macromolecules of life

a) The macromolecules of life are polymers built from monomers.

- The macromolecules in three of the four classes of life's organic molecules – carbohydrates, proteins and nucleic acids are chain-like molecules called **polymers** (from the Greek *polys*, many, and *meros*, part).
- A polymer is a long molecule consisting of many **identical or similar building blocks** linked by **covalent** bonds.
- The repeating units that serve as the building blocks of a polymer are called **monomers** (from the Greek *monos*, single) (or sometimes referred to as '**residues'**). Some of the molecules that serve as monomers also have other functions of their own (e.g. glucose, amino acids).

b) The Synthesis and Breakdown of Polymers (carbohydrates, lipids, proteins).

- Although each class of polymer is made up of a different type of monomer, the chemical reactions by which cells make or break down polymers are similar. These reactions are usually catalysed by **enzymes**.
- Monomers are covalently bonded to each other, with the **loss of a water molecule**, by a **condensation** reaction: One monomer contributes a hydroxyl group (-OH), while the other provides a hydrogen (-H). This reaction is repeated as monomers are added (Fig. 1.9).

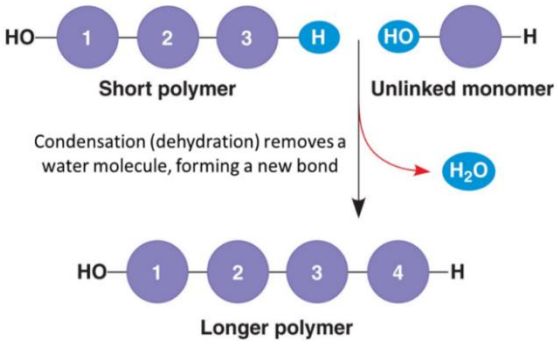


Fig. 1.9: Condensation reaction in the synthesis of a polymer

- Polymers are disassembled to monomers by **<u>hydrolysis</u>** *("hydro" = water, "lysis" = to break)* where the bond between monomers is broken by the **addition of a water molecule** (Fig. 1.10).

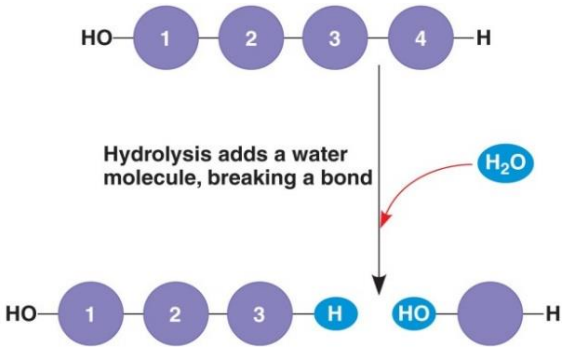


Fig. 1.10: Hydrolysis reaction.

- Biomolecules such as glucose can function as energy sources as **chemical energy** is **stored** in the **C-H and C-C bonds**. Energy is released when these bonds are broken by reactions e.g. hydrolysis.

- An example of hydrolysis and condensation reactions in our body is shown in Fig. 1.11.

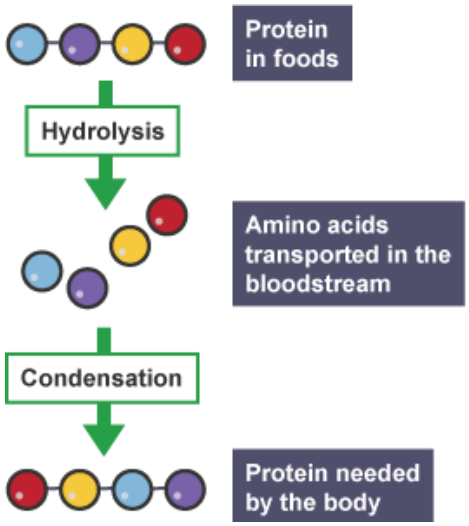


Fig. 1.11: Most of the organic material in the food we consume is in the form of polymers that are too large to be absorbed into our cells. In the digestive tract, various enzymes digest the polymers *via* hydrolysis reactions. The released monomers are then absorbed into the blood stream for distribution to all cells. The cells can then use condensation reactions to reassemble the monomers into new and different types of polymers that can perform specific functions required by the cell.

- In the study of biological molecules, you will need to take note of the following:

  - The **type of monomers**.
  - The type of chemical **bonds that hold the monomers** together.
  - **Other bonds** that holds the molecule in its three-dimensional configuration.
  - How the **<u>s</u>tructure** of the polymer is related to its **<u>p</u>roperty** which affects its biological **<u>f</u>unction(s)**. ["S → P → F"]
  - Knowledge of **where** these macromolecules are assembled.
  - What type of **enzymes** are involved in these reactions.

2. Carbohydrates

2.1 Monosaccharides

- Monosaccharides (from the Greek *sacchar*, sugar*)* are **single sugar units**.
- Consist of elements C, H and O. The general formula of monosaccharides is **$(CH_2O)_n$**.
- They are classified according to the **number of carbon atoms** such as trioses (3C), tetroses (4C), pentoses (5C), hexoses (6C) and heptoses (7C). Of these, pentoses and hexoses are the most common.
- The most common monosaccharide is glucose, which is of central importance in the chemistry of life as it serves as the *primary* energy source for respiration.

2.1.1 Glucose

a) Structure of glucose

- Glucose (**$C_6H_{12}O_6$**) is a **hexose** sugar. Glucose can exist in **linear** and **ring** forms (Fig. 2.1).

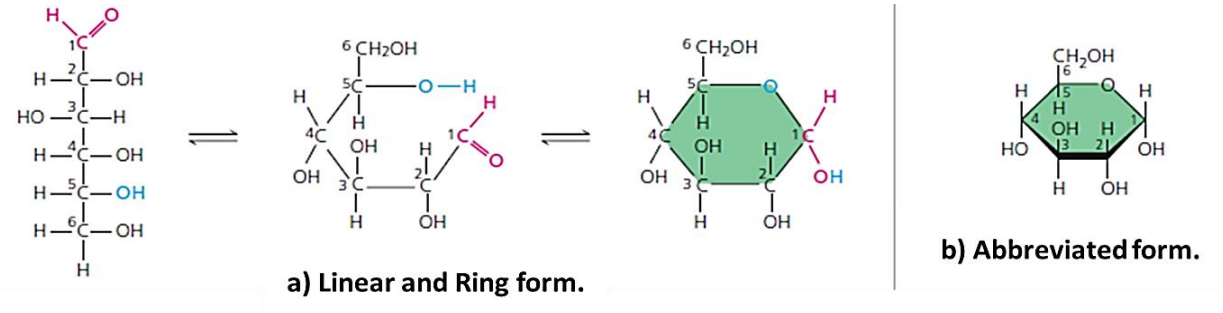


Fig. 2.1: Linear and ring forms of glucose. In glucose the first carbon atom (C1) forms a covalent bond with the oxygen atom on carbon number five (C5) to give a six-membered ring, known as a pyranose ring. The hydroxyl group attached to carbon atom five is oxidised. This result in carbon atom number six sticking out of the ring.

- Depending on how the ring closes, glucose can exist as α-glucose or β-glucose ring structures (isomers) (Fig. 2.2). This difference in position of the hydroxyl groups has important implications when they polymerise to form different polysaccharides (*Section 2.4*).

- For **<u>α-glucose</u>**, the **hydroxyl group (-OH)** of **carbon one (C1)** lies **<u>below</u>** the ring.
- For **<u>β-glucose</u>**, the **-OH group** of **carbon one (C1)** lies **<u>above</u>** the ring.

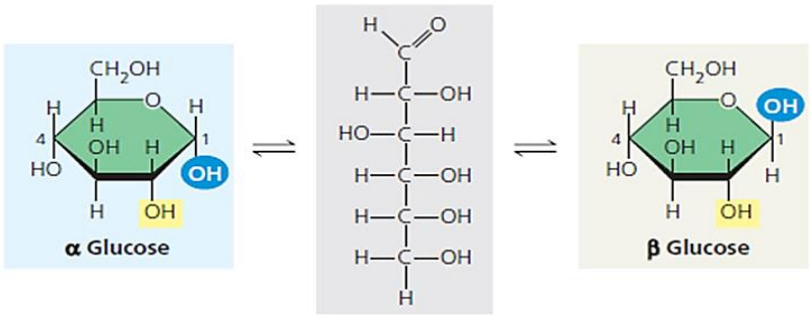


Fig. 2.2: Structure of α-glucose vs β-glucose.

b) Properties of glucose

- Glucose is **soluble in water**. This is because:
  (1) It has a relatively **small** molecular size.
  (2) It is a **polar** molecule as it has many hydroxyl groups. Hence glucose can form **hydrogen bonds** with water molecules and dissolve in it (Fig. 2.3).

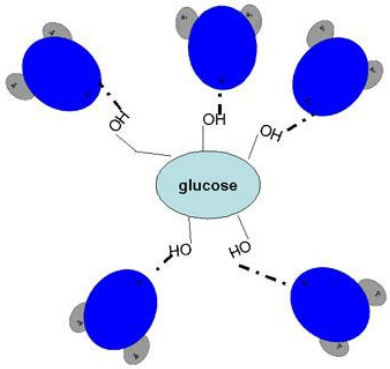


Fig. 2.3: Glucose forming hydrogen bonds with water molecules.

- Glucose is a **reducing sugar** because of its aldehyde group. Hence glucose gives a positive result (production of brick-red precipitate) with Benedict's test (Fig. 2.4).

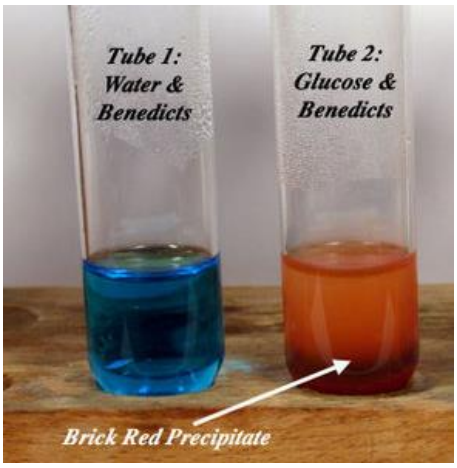


Fig. 2.4: Glucose giving a brick red precipitate with Benedict's solution.

Since glucose is soluble in water, what problems would arise if a cell stores excess glucose as an energy source? (Hint: recall osmosis)

2.2 Disaccharides

- When two monosaccharides are joined by a **glycosidic bond**, a **disaccharide** is produced. The type of **glycosidic bond** present depends on the monosaccharides involved.

Disaccharide	Component (monosaccharides)
Sucrose	Glucose + Fructose
Maltose	Glucose + Glucose
Lactose	Glucose + Galactose

(Note: Fructose and Galactose are isomers of Glucose)

2.2.1 Formation and Breakage of Glycosidic Bond

- **Glycosidic bonds** have specific names. For example, when the bond is formed between **carbon one atom** of one monosaccharide (e.g. α-glucose) and **carbon four atom** of **another** monosaccharide (e.g. α-glucose), it is known as an **α-1,4 glycosidic bond** (Fig. 2.5).

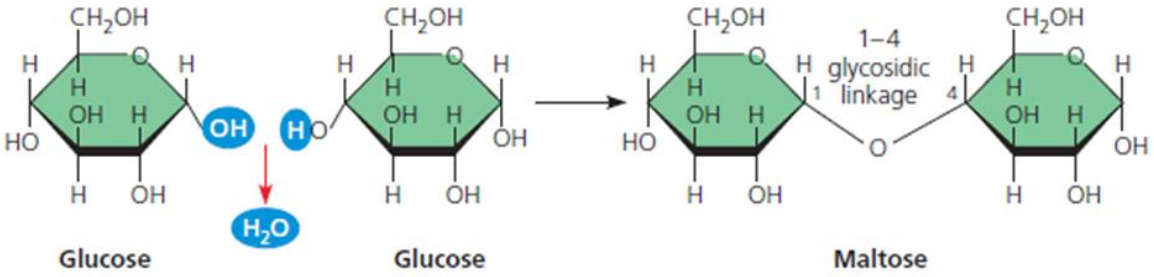


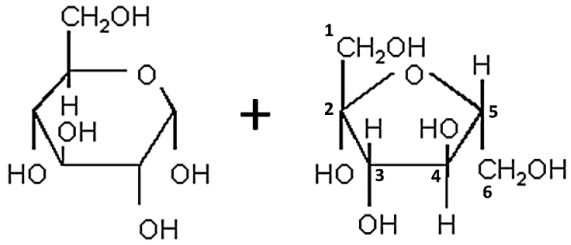
Fig. 2.5: The formation of a glycosidic bond between two monosaccharides *via* a condensation reaction.

- Glycosidic bonds between monosaccharides are formed by **condensation** reaction between two hydroxyl groups (one monosaccharides contributes a –OH, while the other contributes a –H). When a glycosidic bond is formed, it results in the **loss of a single water molecule.**

- Splitting a disaccharide into its constituent monosaccharides requires the **hydrolysis** of the glycosidic bond, involving the **addition of a water molecule**.

CONSOLIDATION PRACTICE

1. Name the type of glucose (α or β) shown below. Number its carbon atoms.
2. Draw the final product sucrose, in which the two original monosaccharides are held together by a glycosidic linkage between C1 of glucose and C2 of fructose.
3. Write down the name of the reaction that leads to the formation of sucrose.



glucose fructose

2.3 Polysaccharides

- Many monosaccharides combine by **condensation** reactions to give a **polysaccharide** linked by **glycosidic bonds**.
- General formula is $(C_6H_{10}O_5)_n$ where n = variable number of monosaccharide molecules linked together in the polysaccharides.
- The chains produced can be **branched** or **unbranched**.
- The chains may be **folded (coiled)**, thus making them **compact** and ideal for storage.
- The **large size** of the molecules make them **insoluble in water**, making them ideal as a storage or structural molecule as they **do not affect the water potential** of the cell and do not easily diffuse out of the cell.
- Upon **hydrolysis**, polysaccharides can be converted to their constituent monosaccharides ready for use in respiration.

2.4 Examples of polysaccharides

Based on the functions, polysaccharides can be divided into:

- Storage polysaccharides
  - serve as storage material, hydrolysed as needed to provide sugar for the cells
- Structural polysaccharides
  - serve as building material for structures that protect the cell or the whole organism

2.4.1 Starch (storage polysaccharide)

- **Starch** is found in most parts of the **plant** in the form of small **granules** (Fig. 2.6). They are visible in plant cells, notably in the **chloroplasts of leaves**, in storage organs such as potato tubers, and in seeds of legumes and cereals.

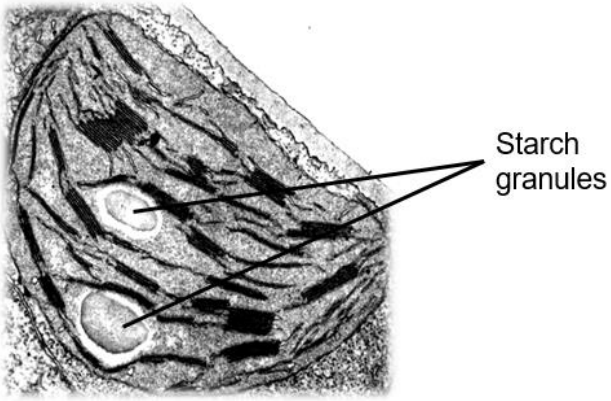


Fig. 2.6: Part of a plant cell with a chloroplast, where starch granules can be found.

- Its function is to reserve and store excess glucose produced during photosynthesis and is common in the seeds of some plants necessary for germination e.g. maize, where it forms the energy supply for germination.

- Starch is a polysaccharide with monomers of **α-glucose**.

- Starch is a mixture of two polymers: **amylose** and **amylopectin** (Fig. 2.8), which fit together to form a complex 3-dimensional structure. The **amylose helices are entangled** (20%) in the **branches of amylopectin** molecules (79%), with a remaining 1% comprising phosphates and fatty acids.

- Starch can be **hydrolysed** by incubating it with a dilute acid at 100°C, or by incubating it with enzymes at room temperate (Eg. amylase hydrolyses starch to maltose, and maltase hydrolyses maltose to glucose).

- **Iodine** test on starch will result in a **blue-black** colour (Fig. 2.7).

Fig. 2.7: Starch (tube on the left) gives a blue-black colour with iodine solution (brown solution).

STARCH (STRUCTURE)

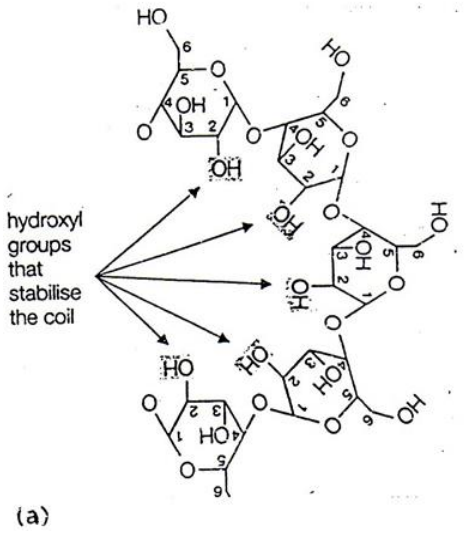
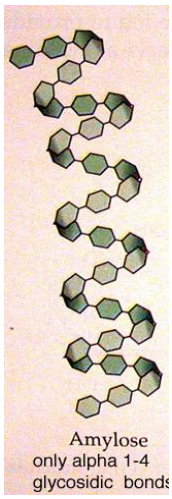
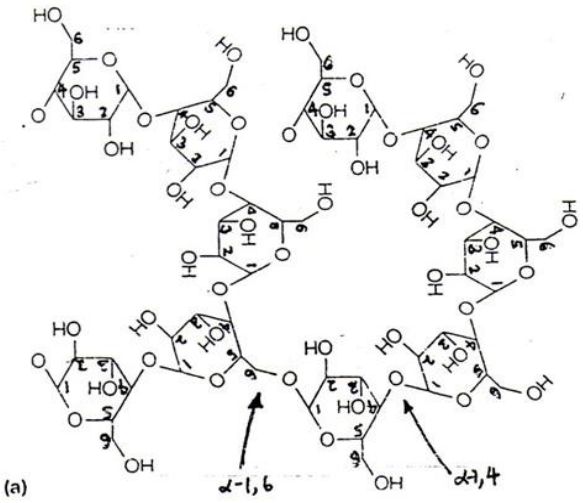
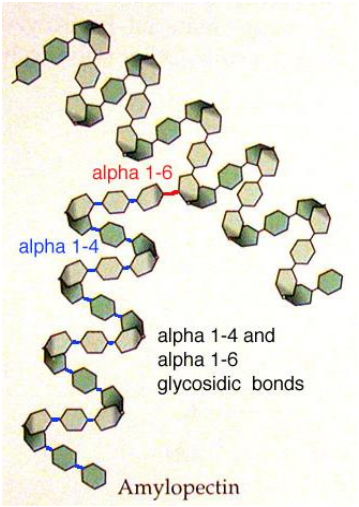
Amylose	Amylopectin
• Consists of 300 **α-glucose** monomers. • Monomers are held together by **α-1,4 glycosidic bonds**, making an angle of **109°** between two C-O bonds. • This causes the polymer to twist into a **helix**. • The **hydroxyl group on carbon-2 (C2)** of each glucose residue projects out into the middle of the spiral and forms **hydrogen bonds** with each other, stabilising the helical shape of the amylose polymer. • It forms a colloidal suspension when mixed with water. • With iodine, amylose stains dark blue.	• Consists of 1200 – 1500 **α-glucose** monomers. • Similar to amylose, monomers are held by **α-1,4 glycosidic bonds**, which causes the polymer to twist into a **helix**. This helical structure is also stabilised by **hydrogen bonds** between C2 of each glucose molecule. • In addition, amylopectin also has **α-1,6 glycosidic bonds**, which gives **branch points** at every 24-30 residues making a multi-branched, compact polymer. • Like amylose, amylopectin forms a colloidal suspension when mixed with water. • With iodine, amylopectin stains reddish brown.
 	 

Fig. 2.8: Comparison between structure of amylose and amylopectin.

2.4.2 Glycogen (storage polysaccharide)

- **Glycogen** is **stored** as free compact **granules** (Fig. 2.9) in liver and skeletal muscles (usually in close proximity to the smooth endoplasmic reticulum) in **animals**.

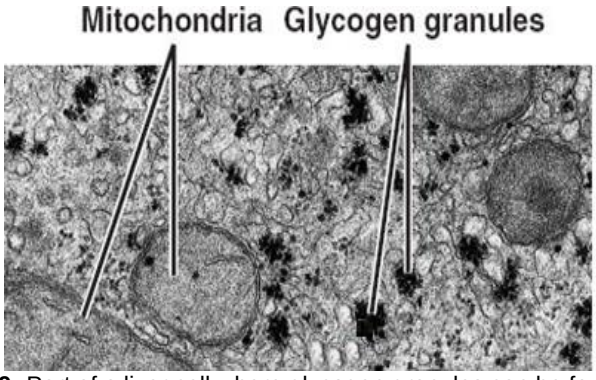


Fig. 2.9: Part of a liver cell where glycogen granules can be found.

- Glycogen functions to store excess glucose when blood glucose levels are high, and serves as a ready source of glucose for tissues in the body when blood glucose level decreases.

- For the Iodine test, the result is similar to that of amylopectin, i.e. reddish brown.

GLYCOGEN (STRUCTURE)

- A single glycogen polymer consists of approximately 54,000 **α-glucose** monomers.

- Bonds between monomers are **α-1,4 glycosidic bonds**, which cause the polymer to twist into a **helix**. This helical structure is stabilised by **hydrogen bonds** between glucose molecules (similar to amylose and amylopectin).

- In addition, glycogen also has **α-1,6 glycosidic bonds**, which gives **branch** points every 10-20 residues making a multi-branched, compact polymer. Glycogen is similar to amylopectin but more highly branched and is thus heavier and more compact (Fig. 2.10).

- Glycogen is relatively more soluble than amylopectin due to more branches in glycogen than in amylopectin.

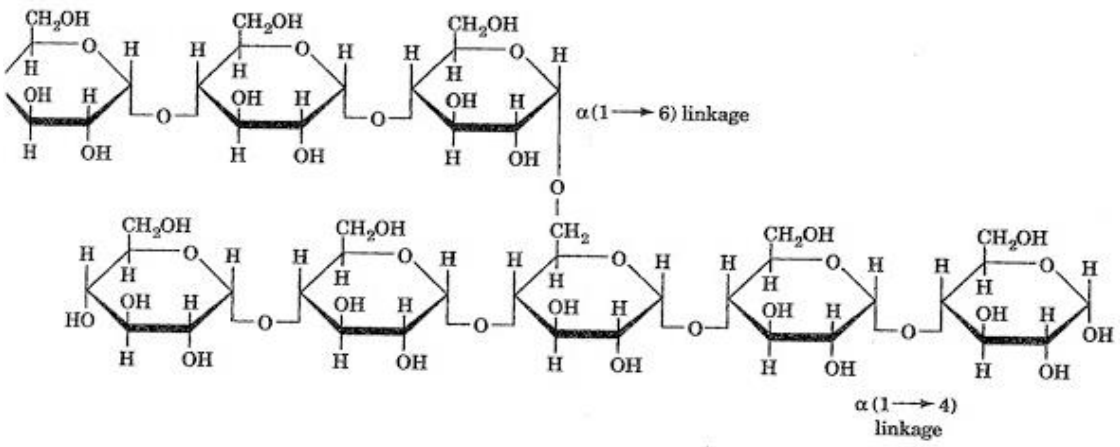


Fig. 2.10: The glycosidic bonds in glycogen.
Note that the α-1,4 glycosidic bonds make an angle of 109º between two C-O bonds to give a helical shape.

Relating Structure to Function (Starch and Glycogen)

	Structural feature(s)	**How structure contributes to property**	**Suited for function as an <u>ENERGY STORAGE molecule</u> because:**
1	• Consists several thousand α-glucose monomers → **Large size.**	**Insoluble in water**	➢ Can be stored in large quantities **without affecting the water potential of the cells.** (Note: the more small molecules such as glucose is present in the cell, the more concentrated the solution in the cell is, and more water will enter the cell by osmosis, which may lead to cell lysis).
2	➢ **α-1,4 glycosidic bonds** between glucose monomers are at an angle of **109°** between two C-O bonds → formation of **helices**. ➢ **α-1,6 glycosidic bonds** at every 24 – 30 glucose residues (for starch) and every 10 – 20 glucose residues (for glycogen) → form **branch points**. <u>For starch only</u> ➢ **Mixture of amylopectin and amylose** → forms a complex 3-dimensional structure**.**	**Compact** structure	• **Many glucose residues can be stored in a small volume** within the cell
3	• Residues are linked via **α-1,4 glycosidic bonds** in the helix and **α-1,6 glycosidic bonds** at branch points. <u>For glycogen only</u> • **α-1,6 glycosidic bonds (branch points)** every 10 to 20 glucose residues → **highly branched** structure **increases the number of terminal ends for enzymes to bind to.**	Can be **hydrolysed by enzymes** Allows **faster hydrolysis by enzymes at each branched end**	➢ **Glucose** can be released and **oxidised** during respiration to produce ATP ➢ Allows **glucose** to be **released at a fast rate** for **oxidation** during respiration to produce ATP

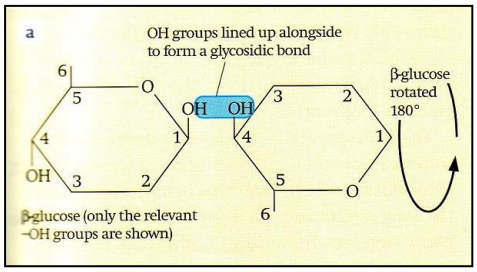
→: leads to / results in

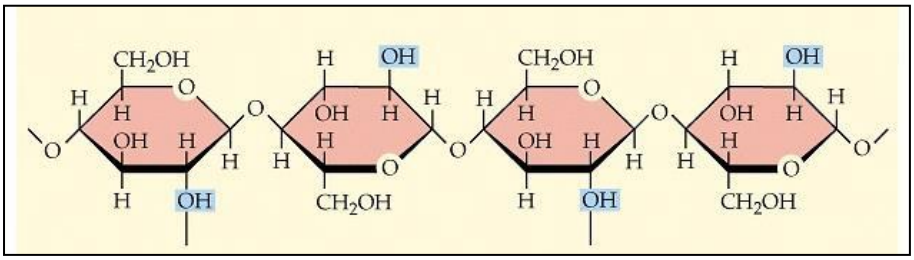
2.4.3 Cellulose (structural polysaccharide)

- It is a **component of cell wall in plants** and it is not easily broken down. The cell wall comprises many cellulose molecules in its entire structure.
- It functions to provide support and mechanical strength in addition to maintaining the cell shape.

STRUCTURE OF CELLULOSE

- A single cellulose polymer consists of approximately 10,000 **β-glucose** monomers.
- In β-glucose, the -OH group is above the ring at C1. Hence in order to form a bond with C4 of another β-glucose, one glucose molecule must be **rotated 180°** and is upside down **relative to the adjacent** molecule. This gives a **β-1,4 glycosidic bond** (Fig. 2.11) and forms a **straight** cellulose chain.





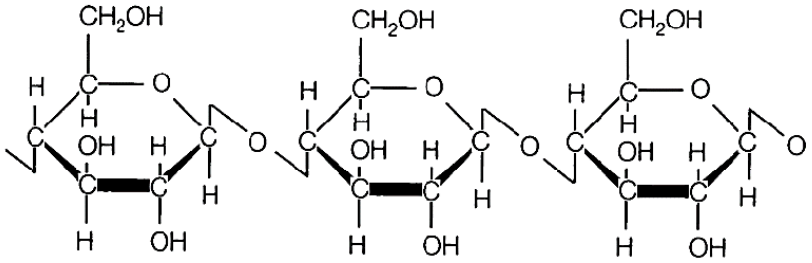


Fig. 2.11: The formation of glycosidic bond in cellulose. Every alternate glucose monomer is rotated 180°.

- **Hydroxyl (-OH) groups** project outwards from both sides of the chain. The hydroxyl groups of one chain form **<u>hydrogen bonds</u>** with hydroxyl groups of neighbouring chains, resulting in the formation of **cross-links** (Fig. 2.12).

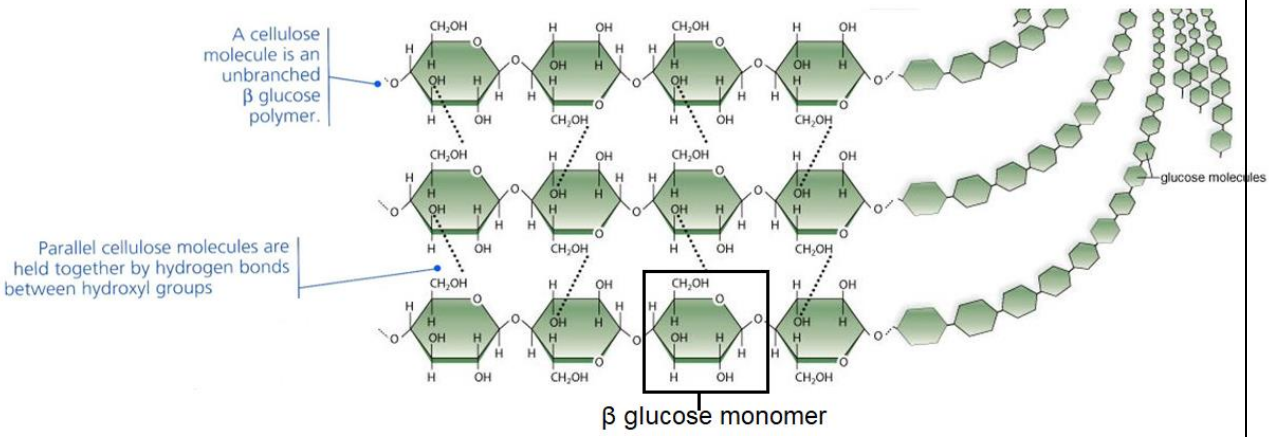


Fig. 2.12: The formation of cross links between adjacent chains of cellulose via hydrogen bonding.

- In the cell wall, groups of about 60 to 70 cellulose chains become tightly cross-linked to form bundles called **<u>microfibrils</u>** (10nm in diameter).

- Microfibrils are in turn held together **in bundles** called cellulose fibril or **<u>macrofibril</u>** (50nm in diameter) by **hydrogen bonding** (Fig. 2.13). The macrofibrils **run parallel to each other in each layer** but **at an angle to those in other layers** to form a lattice.

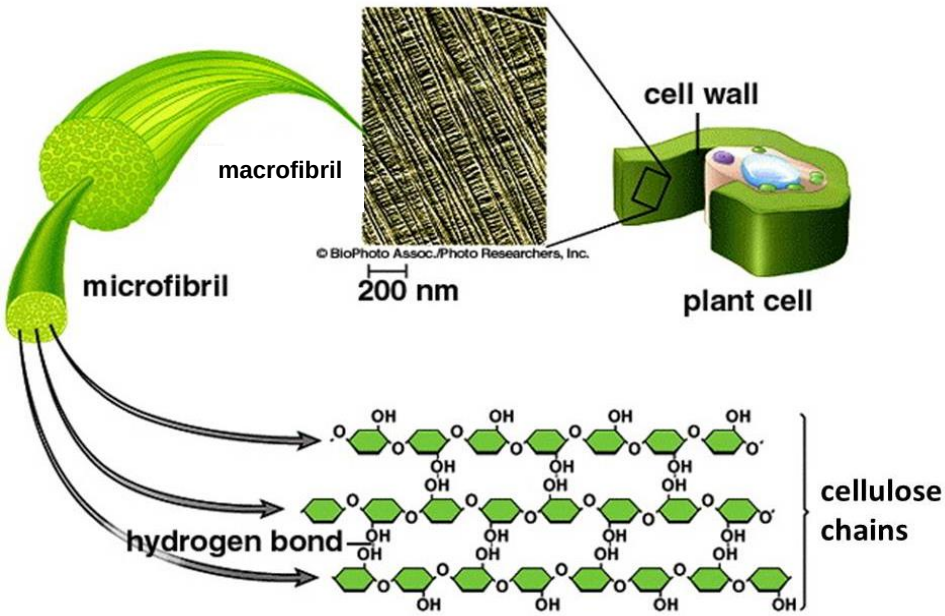


Fig. 2.13: The organisation of cell wall – from cellulose molecules to microfibril to macrofibril.

- Note that most plant cell walls contain a variety of different polysaccharides and proteins embedded in a gel-like organic matrix, which provides strength and flexibility. **In addition to cellulose**, other polysaccharides commonly found in the cell wall include **hemicellulose** and **pectin** (Fig. 2.14).

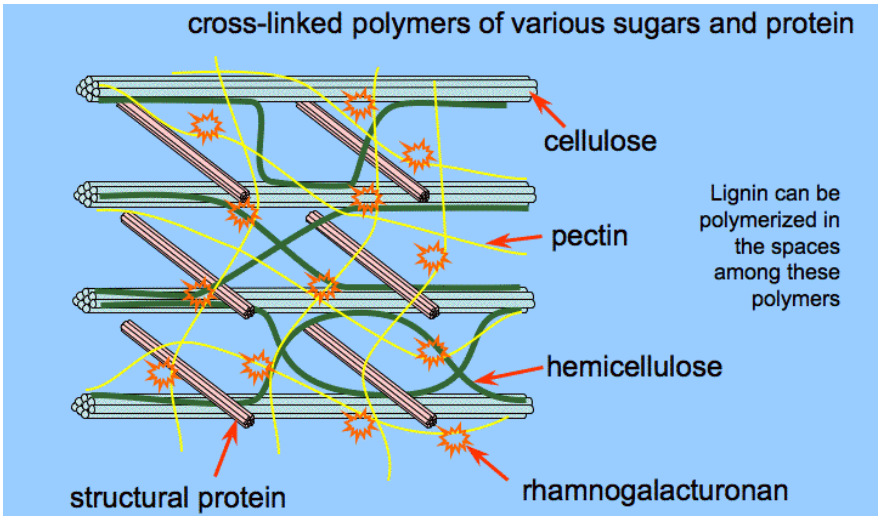


Fig. 2.14: The components of plant cell wall.

Relating Structure to Function (Cellulose)

	Structural feature(s)	How structure contributes to property	Suited for function to provide <u>STRUCTURAL SUPPORT</u> because:
1	• **Alternate β–glucose monomers** are **rotated 180°** → cellulose chains run **straight and parallel** to each other • **Hydroxyl groups project outwards from both sides** of a cellulose chain → forms **cross-links via hydrogen bonds** → forms **microfibrils** which further aggregate to form **macrofibrils**. • Macrofibrils run parallel in each layer but angled to those in other layers → lattice pattern.	**High tensile strength**	• Provides **mechanical strength to cell wall** and maintains the shape of the cell → **Prevents cell lysis** due to osmosis (when cell is in a solution of higher water potential)
2	• A single cellulose consists of approximately 10,000 β-glucose monomers → **Large size**	**Insoluble in water**	• Can remain in the location where it is formed

→: leads to / results in

CONSOLIDATION PRACTICE

Complete the table below showing the differences between starch, glycogen and cellulose.

Feature	Starch	Glycogen	Cellulose
Type of Monomer	α-glucose		
Orientation of monomers	No rotation of monomers		
Type of bonds between monomers	α-1,4 glycosidic bonds (in amylose) α-1,4 & α-1,6 glycosidic bonds (in amylopectin)		
Shape – Straight or Helical?	Helical		
Branching? (Y/N)	No - amylose Yes - amylopectin		
Function	Storage		
Location found	In plant cells		

2.5 Checklist for Carbohydrates

Are you able to confidently answer all the questions?

1. List the four classes of macromolecules.

2. Distinguish between monomer and polymer.

3. Distinguish among monosaccharides, disaccharides and polysaccharides.

4. Draw diagrams to illustrate condensation and hydrolysis of reactions in terms of the formation and breakage of glycosidic bonds, respectively.

5. Describe the structure of cellulose and how its structure confers great tensile strength to the cell wall.

6. Describe the structure of starch.

7. Describe the structure of glycogen.

8. Distinguish between the glycosidic linkages found in starch and cellulose. Explain why the difference is biologically important.

3. Lipids

- Lipids are the one class of biological molecules that does not include true polymers, and they are generally not big enough to be considered macromolecules. They are a large and diverse group of naturally occurring organic compounds that are grouped together due to their general **insolubility in water** and **solubility in nonpolar organic solvents** (e.g. ether, chloroform, acetone and benzene).

- Consist of only **C, H and O**, with no generalised formula. Characterised by low O, high H (e.g. $C_5H_{11}COOH$).

- Lipids consist mostly of non-polar hydrocarbon (C-H) regions, which accounts for their solubility behaviour.

- **Ethanol emulsion test** on lipids will give rise to a **cloudy white suspension** (Fig. 3.1).

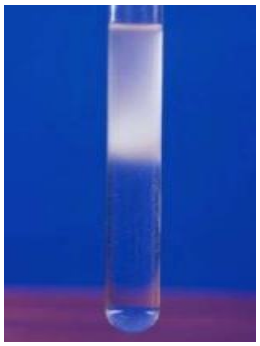


Fig. 3.1: Lipids give a cloudy white suspension with the ethanol emulsion test.

3.1 Classification of Lipids

- Lipids are varied in form and function. They can be generally classified into:
  (1) Simple lipids
  (2) Compound lipids
  (3) Steroids and sterols

  (1) Simple lipids

    - **Triglycerides (Fats and Oils)**
      - Formed from **fatty acids** that are joined to a **glycerol** molecule. At 20°C**,** solid state lipids are referred to “fats” while liquid state lipids are referred to “oils”.

    - **Waxes**
      - Formed from **fatty acids** that are joined to **high-molecular weight alcohols**, e.g. waxes in cuticles of leaves.

  (2) Compound lipids (lipid and non-lipid component)

    - **Phospholipids**
      - Formed from joining **fatty acids** and **one phosphate group** to a **glycerol** molecule. Non-lipid component is the **phosphate group.**

    - **Glycolipids** (details covered in Section 6.1)
      - Formed from joining **fatty acids** and a **carbohydrate chain** to a **glycerol** molecule. Non-lipid component is the **carbohydrate.**

(3) Steroids

- Lipids characterised by a **carbon skeleton consisting of 4 interconnected rings**. Examples of steroids: testosterone (Fig. 3.2) and cholesterol (Fig. 3.7).

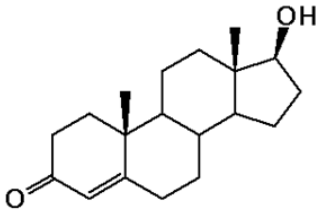


Fig. 3.2: Structure of testosterone.

3.2 Functions of Lipids

- Lipids serve as a good source of **energy**.
- They are also an important part of a healthy diet in the form of fat-soluble vitamins and essential fatty acids.
- Lipids also act as **insulating material** in the subcutaneous tissues and are also seen around certain organs.
- Lipids combined with proteins are **important constituents** of the cell membranes and mitochondria of the cell.

3.3 Examples of Lipids

3.3.1 Triglycerides

- Triglycerides are the commonest lipids in nature. They are generally called **fats** (solid at 20°C) or **oils** (liquid at 20°C).
- The structure consists of a **glycerol** and **three fatty acid chains** (Fig. 3.3).
  - **Glycerol** ($C_3H_8O_3$): An alcohol with three carbons, each bearing a **hydroxyl group (–OH)**.
  - **Fatty acid**: A **hydrocarbon chain** of varying number of carbon atoms (represented by R), with a **carboxyl group (COOH)** at one end of the molecule.
- The enzyme-catalysed **condensation reaction** between the **fatty acids** and the **glycerol** results in the formation of three **ester bonds** in a triglyceride molecule.

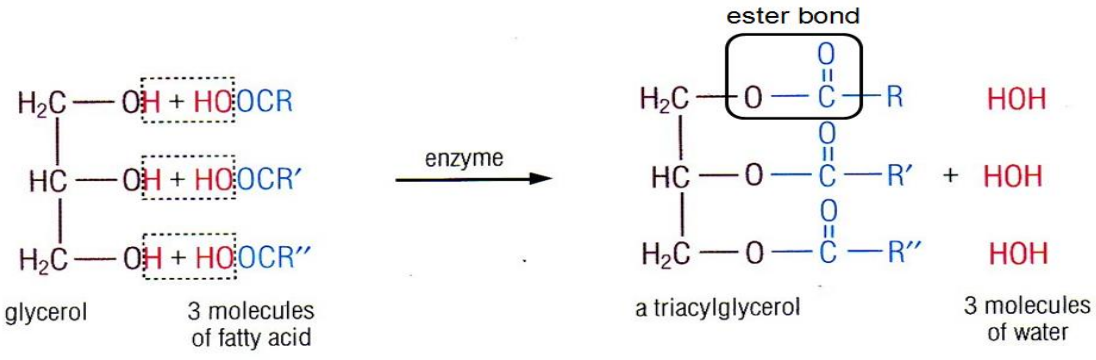


Fig. 3.3: The condensation reaction between one glycerol and three fatty acids (may or may not be the same) gives rise to a triacylglycerol/triglyceride/fat molecules and three water molecules.

- Fatty acids are **saturated** when there is an **absence of carbon-carbon double bond** in the hydrocarbon chain. This leads to a relatively **straight chain**. Molecules with **saturated fatty acids** tend to be more **closely packed,** with **stronger intermolecular attractions** hence higher melting / boiling points (Fig. 3.4).

- Fatty acids which are **unsaturated** have **one or more carbon-carbon double bonds**. Such fatty acids have "**kinks**" in its hydrocarbon chain which cause molecules to be **packed further apart**. The **intermolecular attractions are weaker**, hence, lower melting / boiling points (Fig. 3.4).

NOTE: The fatty acids on the triglyceride may be
1. different lengths (i.e. different number of carbon atoms)
2. saturated or unsaturated

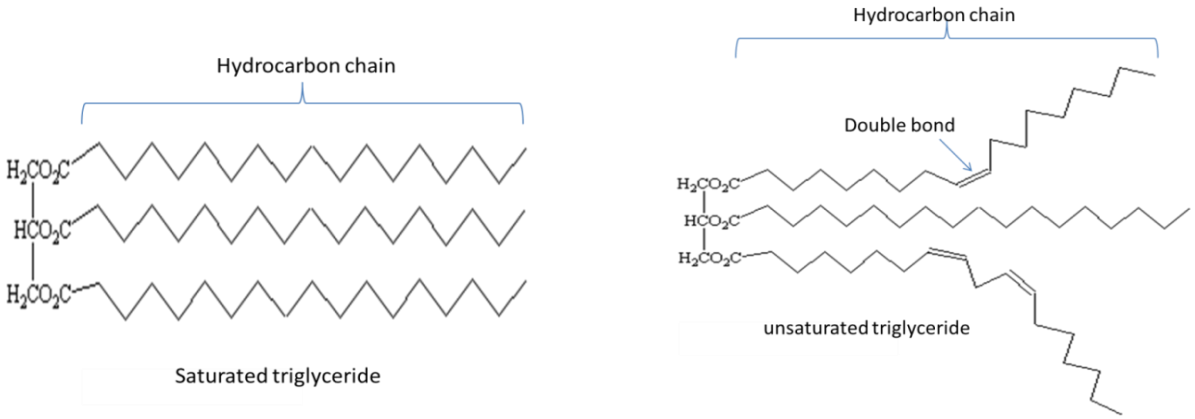


Fig. 3.4: (Left) Saturated vs (right) unsaturated triglycerides.

Relating Structure to Function (Triglyceride)

	Structural feature(s)	How structure contributes to property	Suited for function as an <u>ENERGY STORAGE</u> molecule because:
1	• Contain a **greater number of C-C and C-H bonds per unit mass** (that store chemical energy) than carbohydrates.	**Compact** as it yields 2X more energy than an equal mass of carbohydrates upon oxidation of the fatty acid chains. (E.g. 1g of triglyceride yields 38kJ while 1g carbohydrate yields only 17kJ of energy).	• Triglycerides are a **light-weight energy source** for animals that move by flight and seeds that are dispersed by wind or insects. *(Note: Carbohydrates are still the most direct source of energy in living things because they are metabolised quickly.)*
2	• Contain long and **non-polar/hydrophobic hydrocarbon chains**.	**Insoluble in water** as hydrocarbon chains are hydrophobic and triglycerides do not have polar functional groups → cannot form hydrogen bonds with water molecules.	• **Does not change the water potential of cells**
3	• Contain **more hydrogen atoms** (which yields metabolic water upon oxidation during respiration) than carbohydrates.	Triglycerides **release twice as much metabolic water** as compared to carbohydrates when oxidised.	• **Production of metabolic water**, which is important in animals living in hot and dry deserts (e.g. camel).

<u>Other functions:</u>

- Triglycerides are less dense than water, so they allow marine mammals (e.g. whales) to be **buoyant**.
- Triglycerides are poor conductor of heat, so they provide **thermal insulation** / prevent excessive heat loss for aquatic animals and animals living in cold climates (e.g. whales and polar bears).
- Triglycerides are important for **mechanical protection** as it surrounds delicate organs (e.g. heart and kidneys).
- Triglycerides are a **long-term energy store** as it is oxidised only after carbohydrates are depleted. This is important in hibernating animals living in cold climates.
- Triglycerides **reduce water loss** from the external surfaces of plants (cuticle) and animals (sebum).

If triglycerides are not soluble in water, suggest a possible solvent.

3.3.2 Phospholipids

- The general structure of a phospholipid consists of **two fatty acid chains**, **one phosphate group**, **one glycerol** and **one short organic molecule** (e.g. choline) (Fig. 3.5). The 2 molecules of fatty acids are linked to a glycerol molecule by two **ester bonds**. The phosphate group is attached to the third carbon of glycerol.

- Phospholipids have both **hydrophilic** and **hydrophobic** groups, thus **amphipathic** in nature. (Fig. 3.5):

    - Phosphate group: **charged (hydrophilic)**
    - Two fatty acids: **non-polar (hydrophobic)**
    - Additional groups (e.g. choline) – varies from membrane to membrane

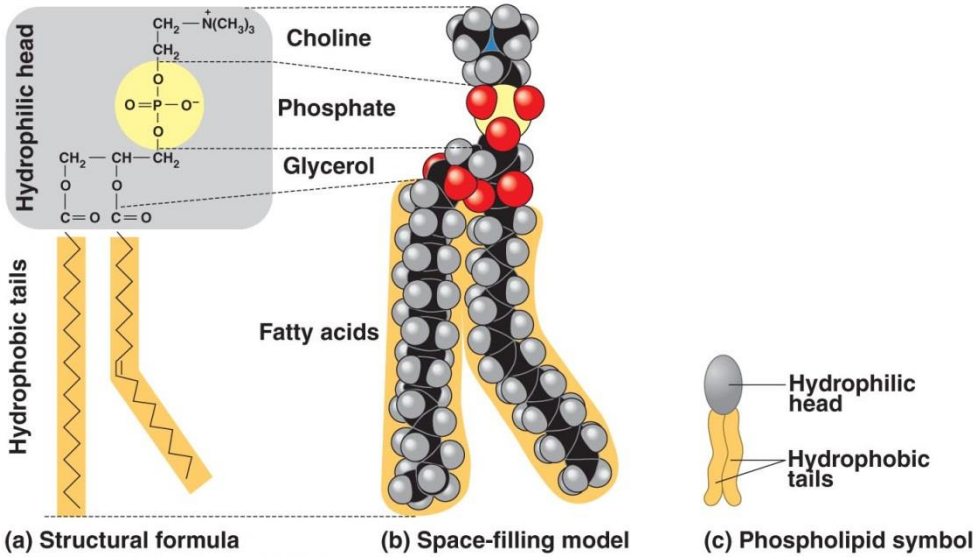


Fig. 3.5: An example of a phospholipid.

- Due to their amphipathic nature, phospholipids tend to aggregate together when placed in an aqueous environment (Fig. 3.6). This is largely due to the hydrophobic fatty acid tails of the phospholipids forming **hydrophobic interactions** with each other.

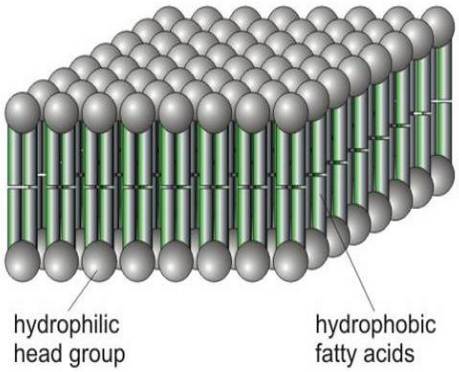


Fig. 3.6: Example of how phospholipids aggregrate - formation of a phospholipid bilayer.

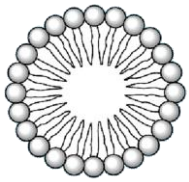
Relating Structure to Function (Phospholipid)

	Structural feature(s)	How structure contributes to property	Suited for function to FORM CELL MEMBRANES because:
1	• Phospholipids consists one **phosphate group,** and two **fatty acids**.	**Amphipathic**	➢ **Hydrophobic fatty acids** interact with each other and are shielded from the aqueous environment, while **hydrophilic phosphate groups** face outside and interact with the aqueous environments (i.e. external and internal environment of cell) → forms the **lipid bilayer** in cell membranes. ➢ Allows the **cell surface membrane** to be a **selectively permeable barrier** that regulates the type of molecules entering and leaving the cell *(details covered in Section 6.2)*.

Other function(s):

➢ Phospholipids also functions to form a layer around neurons (nerve cells), as it provides electrical resistance.

Q1) The diagram below shows a micelle, a structure formed by phospholipids in aqueous solution. Why do phospholipids form such a structure in aqueous solution?



Q2) How would phospholipid molecules respond differently when placed in a hydrophobic solution? (You may *sketch your answer!)*

3.3.3 Cholesterol

- Cholesterol is a **steroid** found in **cell membranes** of animals and transported in the blood plasma of animals.

- Cholesterol consists of a hydrocarbon chain, a ring structure region with 4 hydrocarbon rings and a hydroxyl group (Fig. 3.7). It has both **polar** (due to presence of –OH group) and **non-polar** properties (due to the hydrophobic hydrocarbon chain and steroid ring structure) and is considered an **amphipathic** molecule.

- In an aqueous environment, the hydrophilic hydroxyl group would interact with the aqueous environment, while the hydrophobic hydrocarbon chain and steroid ring structure would be shielded away from the aqueous environment and interact with other non-polar groups.

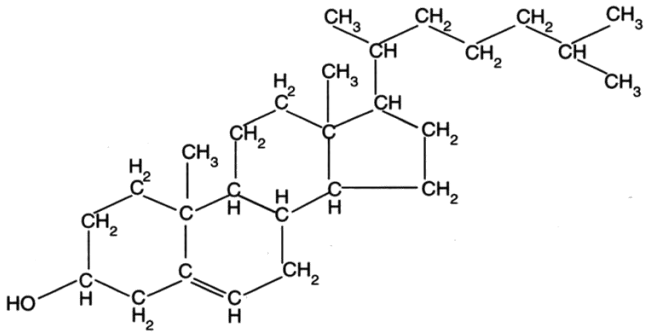


Fig. 3.7: Structure of cholesterol

Relating Structure to Function (Cholesterol)

Cholesterol is **amphipathic**, as it has both hydrophilic (OH group) and (largely) hydrophobic (steroid ring structure and hydrocarbon chains) groups**,** which allow cholesterol to:

- Regulate membrane fluidity (details covered in Section 6.1.3).

  1) **OH group** of cholesterol is **polar (hydrophilic)** and thus is able to **interact with** the **phosphate groups** of phospholipids.

  2) **Steroid ring structure and hydrocarbon chain** are **non-polar (hydrophobic)** and thus are able to interact with the **hydrophobic fatty acid chains** of phospholipids.

- Aid in the formation of bile salts.

- As a precursor molecule for the synthesis of Vitamin D, steroid hormones (e.g. estrogen, testosterone) and the hormones of the adrenal cortex.

3.4 Checklist for Lipids

Are you able to confidently answer all the questions?

1. Explain the meaning of the terms hydrophilic, hydrophobic and amphipathic.

2. Describe the formation of a triglyceride.

3. Distinguish between saturated and unsaturated fats.

4. Describe the main structural differences between a triglyceride and a phospholipid.

5. Describe the structure of cholesterol and its biological importance.

4. Nucleic Acids

- Nucleic acids are the genetic materials in which the **genetic information** of all living organisms are **encoded**.
- Nucleic acids are polymers that belong to a class of biological molecules.
- Nucleic acids are made of the elements **C, H, O, N and P**.
- Two types of nucleic acids exist in **eukaryotic** and **prokaryotic** cells as well as in **viruses** (Fig. 4.1):
  1. **Deoxyribonucleic acid (DNA)**
  2. **Ribonucleic acid (RNA)**

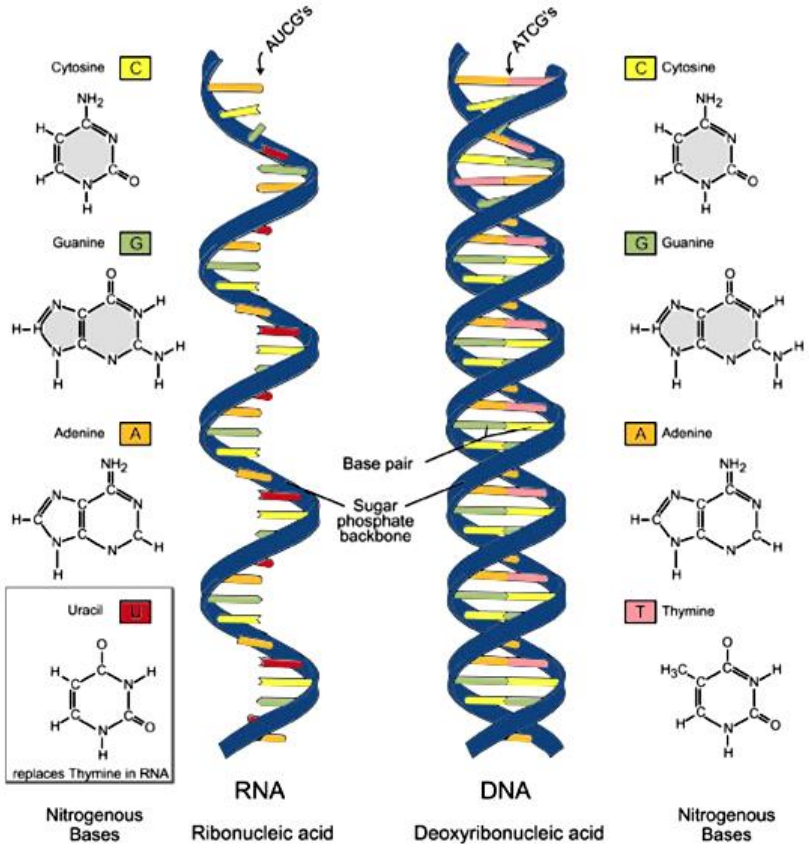


Fig. 4.1: RNA vs DNA

4.1 Structures of Nucleic Acid Monomers

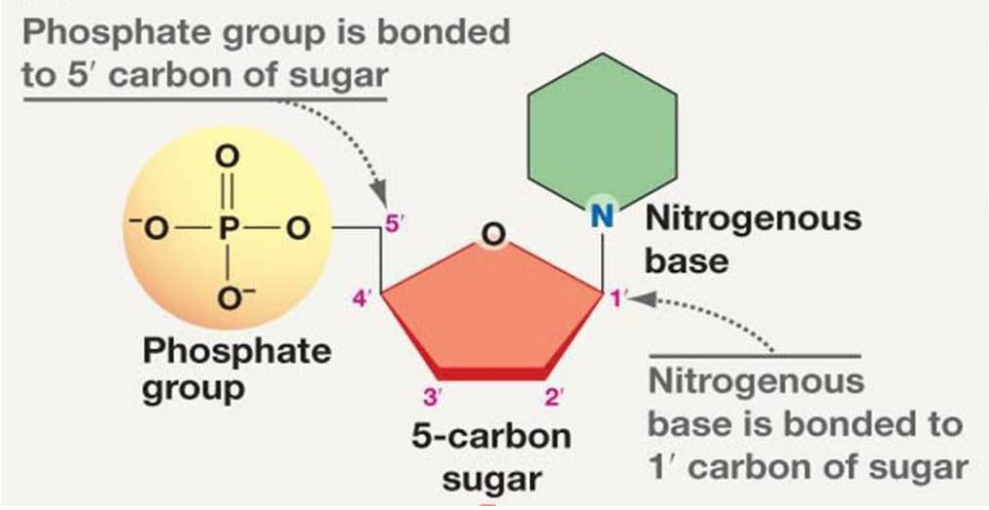


Fig. 4.2: The general structure of a nucleic acid monomer (nucleotide).

- The monomers of nucleic acids are called **nucleotides** (Fig. 4.2).

- **All nucleotides** consist of 3 components:
  - A **pentose** (5-carbon) **sugar** attached to a
  - A **nitrogenous base**, and a
  - A **phosphate group**

- DNA nucleotides are called **deoxyribonucleotides** while RNA nucleotides are called **ribonucleotides**.

  - Deoxyribonucleotides and ribonucleotides differ in their pentose sugar (Fig. 4.3) and the type of nitrogenous bases present.

1) Pentose sugars

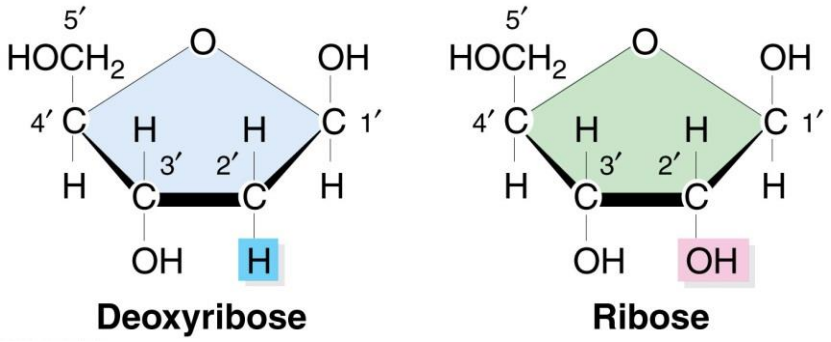


Fig. 4.3: A deoxyribose sugar found in DNA *(left)* and a ribose sugar found in RNA *(right)*. They differ only at the second carbon.

  - The sugar **deoxyribose** (Fig. 4.3) is present in **DNA** while the sugar **ribose** is present in **RNA.**

    - They are so called because the **-OH** (hydroxyl) group is found attached to **carbon 2** of **ribose**, while **-H** is found attached to **carbon 2** of **deoxyribose**.

2) Nitrogenous base

- The **nitrogenous base** is covalently bonded to **carbon 1** of the **deoxyribose / ribose** in a nucleotide (Fig. 4.2).

- There are **five** different nitrogenous bases in nucleic acids (Fig. 4.4), categorised into:
  (i) **Purines** (**2** rings) – Ade**n**i**n**e, Gua**n**i**n**e
  (ii) **Pyrimidines** (**1** ring) – Cytosi**n**e, Thymi**n**e (in DNA only), uracil (in RNA only).

** To remember the number of rings: count the number of 'n' in the names*
** To remember which are purines or pyrimidines: **C**ytosine, **U**racil and **T**hymine (**CUT**) are **Py**rimidines.*

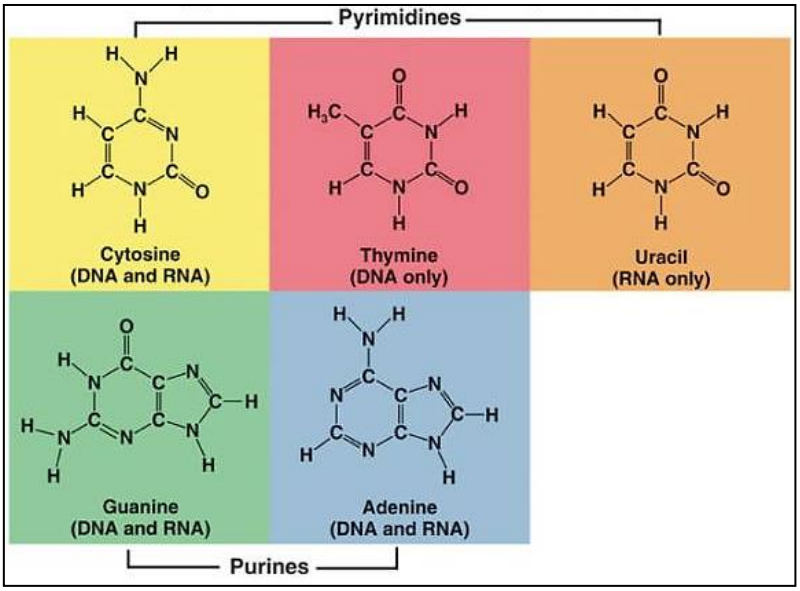


Fig. 4.4: Purines and pyrimidines.

- The bases found in **DNA** are **adenine**, **guanine**, **cytosine** and **thymine**.

- In **RNA**, the bases are **adenine**, **guanine**, **cytosine** and **uracil** (uracil replaces thymine).

3) Phosphate group

- The **phosphate** group is covalently bonded to **carbon 5** of the **deoxyribose** / **ribose** sugars (Fig. 4.2).

- Phosphate groups are **negatively charged** and therefore account for the **hydrophilic** and **water-soluble** nature of nucleic acids.

CONSOLIDATION PRACTICE

The symbols below represent the three main components of a nucleotide.

Nitrogenous base phosphate group pentose sugar

(i) Using the symbols, draw a nucleotide in the space below. Add lines to show the position of any bonds between the components, and number the five carbon atoms of the pentose sugar.

(ii) Complete the table below to describe two differences in structure between a deoxyribonucleotide and a ribonucleotide.

Feature	**Deoxyribonucleotide**	**Ribonucleotide**
1.		
2.		

Nucleosides, nucleoside diphosphates, nucleoside triphosphates

- Nucleo**s**ide = sugar + base
- Nucleo**s**ide <u>mono</u>phosphate = sugar + base + 1 phosphate group
- Nucleo**s**ide <u>di</u>phosphate = sugar + base + 2 phosphate groups
- Nucleo**s**ide <u>tri</u>phosphate = sugar + base + 3 phosphate groups
- Nucleo**t**ide = sugar + base + **at least** 1 phosphate group

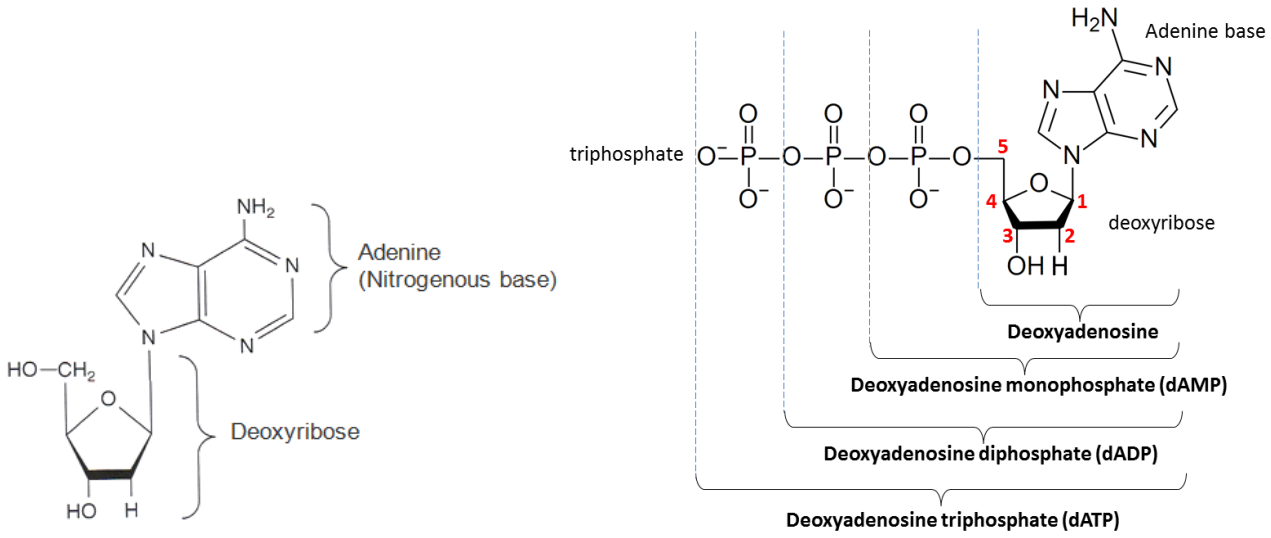
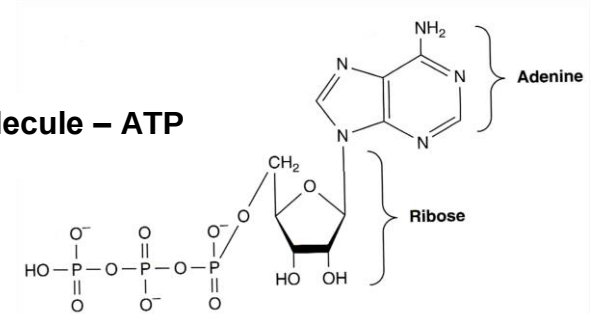


Fig. 4.5: The nucleoside – adenosine *(left)*. Deoxyadenosine triphosphate (dATP) *(right)*.

<u>Note:</u>
Ribonucleoside, ribonucleoside diphosphate and ribonucleoside triphosphate exist too. Familiar ribonucleoside triphosphates are **adenosine triphosphate** (**ATP**) and GTP, which are important in bioenergetics of the cell because of the large amounts of energy involved in adding or removing the terminal phosphate group.

The 'energy' molecule – ATP



4.2 Formation of a Polynucleotide by Phosphodiester Bond

- Many **free nucleoside triphosphates** join together to form a **polynucleotide** by a **condensation reaction** which occurs between the **5' phosphate group** of one free nucleoside triphosphate and the **hydroxyl group** (-OH) of the **3' carbon** of the previous nucleotide**,** forming a **<u>phosphodiester bond</u>**, which is a strong covalent bond (Fig. 4.6). Pyrophosphate (PPi) and water are released.

**Note that the direction of the reaction and attachment of nucleotides occurs only in the 5' to 3' direction (i.e. free nucleoside triphosphates are added only to the 3' end of the elongating chain)*

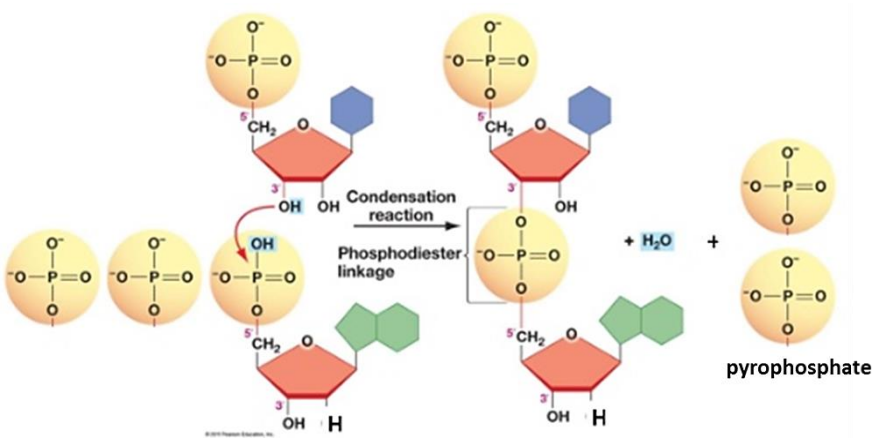


Fig. 4.6: An incoming nucleotide is being added to the free 3'-OH of a growing polynucleotide chain.

- This bonding of several nucleotides creates a **sugar-phosphate backbone** consisting of alternating sugar and phosphate groups with the bases projecting sideways from the sugars (Fig. 4.7).

- The **5' end** of a polynucleotide ends with a **phosphate group** attached to carbon atom 5 of the pentose sugar.

- The **3' end** of a polynucleotide ends with a **free hydroxyl group** on carbon atom 3 of the pentose sugar.

- The conventional way to write **nucleic acid sequences** is in the **5' to 3' direction,** for example: 5**'** AGGTTACCT 3'.

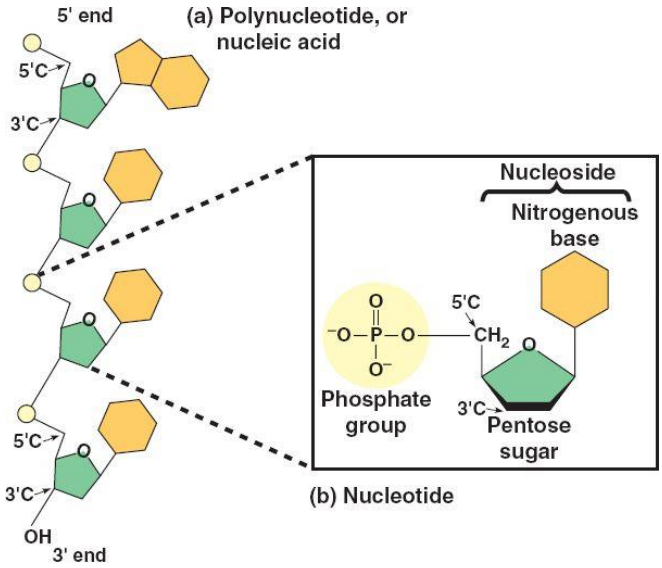


Fig. 4.7: Polynucleotide structure.

4.3 Macromolecular Structure of Deoxyribonucleic Acid (DNA)

- Basic unit of each DNA polynucleotide: **deoxyribonucleotides**.
- Each DNA **molecule** consists of **two polynucleotides** (Fig. 4.8) paired up and coiled around a central axis, forming a **double helix**.
- The two chains are **anti-parallel**, which means that their 5' to 3' orientations run in opposite directions.
- The **bases** of both chains lie **perpendicular to the axis** of the helix and are located **inside** the helix.
- **Complementary base-pairing** occurs between purine and pyrimidine bases (Fig. 4.8 and Fig. 4.9).
  - **A**denine pairs with **T**hymine through formation of **2 hydrogen bonds**.
  - **G**uanine pairs with **C**ytosine through formation of **3 hydrogen bonds**.
- Therefore the ratio of **A:T** and **C:G** is **1:1**, i.e., the ratio of **purines to pyrimidines** is **1:1**. This is known as **Chargaff's rule**.
- **All cells** of **same organism** of a species have **same amount of DNA**, except for gametes (egg and sperm cells).

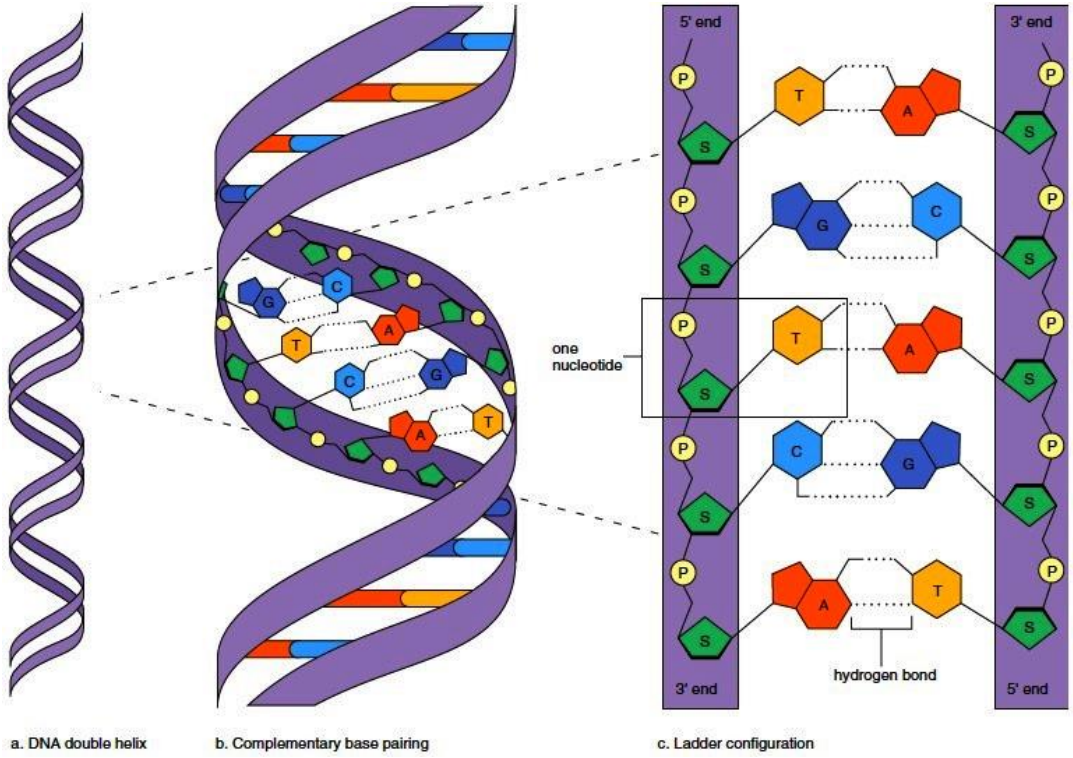


Fig. 4.8: DNA Structure

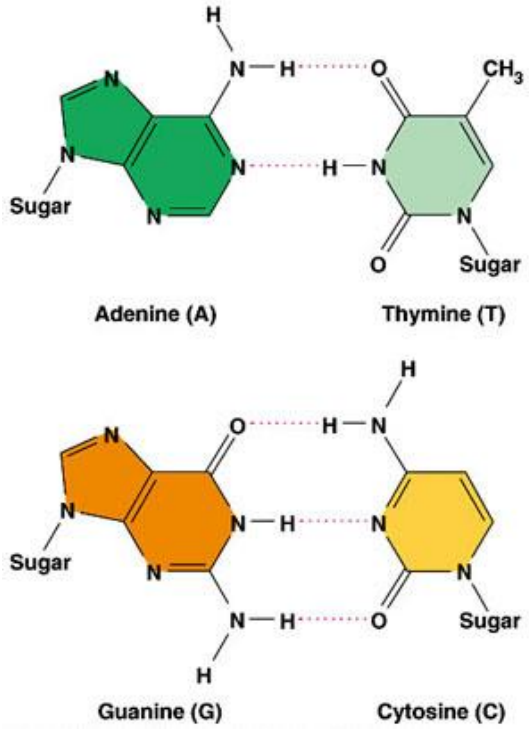


Fig. 4.9: Complementary base pairing via hydrogen bonding between purine and pyrimidines bases present on the two DNA strands that run antiparallel.
Notice how the polar groups are aligned to allow the formation of hydrogen bonds.

- The stability and integrity of the double helix is maintained by:
    - **Hydrogen bonds** between the **complementary bases**.
    - **Hydrophobic interactions** between the **stacked bases**.
    - Strong covalent **phosphodiester bonds** between **adjacent deoxyribonucleotides**.
- The double helix measures **2 nm** or **20 Å** in **diameter**, equal to the width of one purine (A,G) complementary base-paired with one pyrimidine (C,T) (Fig. 4.10 and 4.11).

  [Note: Å represents the Angstrom unit. 1 Å = 0.1 nm = 1×10^{-10} m]
- **Each complete turn** of the helix is **3.4nm** or **34 Å**, consisting of **10 base pairs per turn** in a double helix (Fig. 4.10, centre).
- In any segment of the molecule, alternating larger **major grooves** and smaller **minor grooves** are present along the axis (Fig. 4.10, right). **Proteins** can interact specifically with the exposed atoms in these grooves.
- **Function** of DNA: stores genetic information in the form of genes, which are unique sequences of deoxyribonucleotides that code for rRNA, tRNA and mRNA sequences. mRNA is then used to synthesise proteins, which carry out many cellular processes.
- DNA is also able to make more copies of itself *(Topic: DNA replication)* via complementary base pairing, without the loss of genetic information.

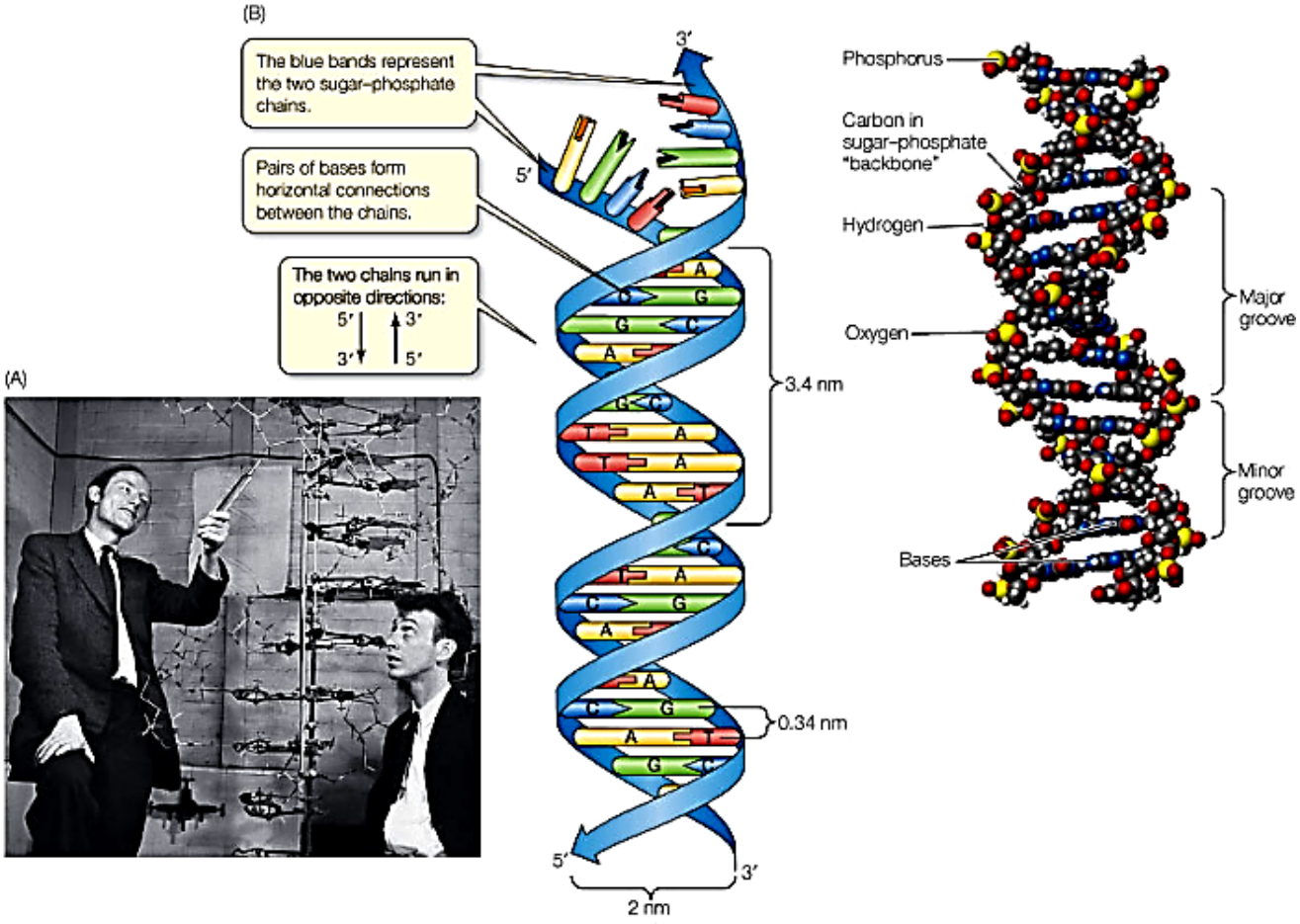


Fig. 4.10: Watson and Crick are scientists that discovered the three-dimensional structure of DNA. They are pictured here with their DNA model.

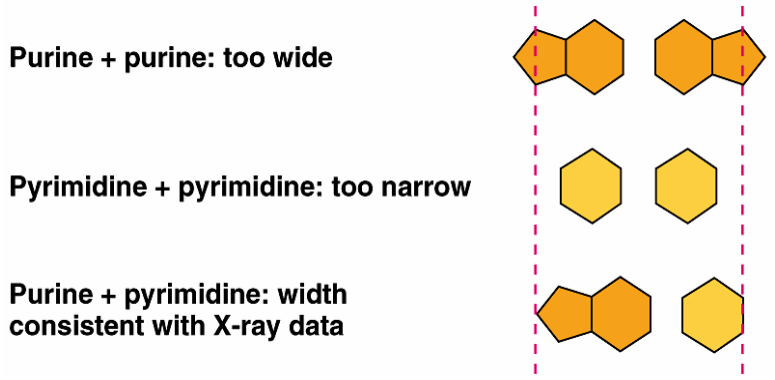


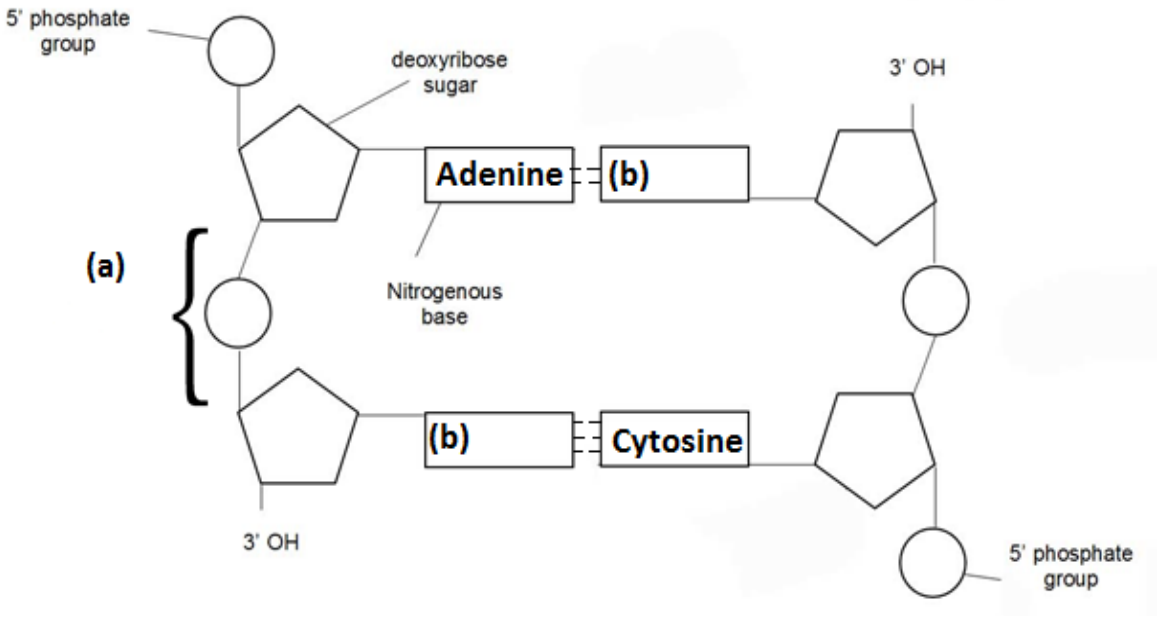
Fig. 4.11: A purine-pyrimidine base pair always results in a uniform diameter that is consistent with X-ray data. In contrast, a purine-purine pair is too wide while a pyrimidine-pyrimidine pair is too narrow to account for the 2nm diameter of the double helix.

CONSOLIDATION PRACTICE

In the space below, a short section of double stranded DNA molecule is shown.

In your diagram:

a) Name the covalent bond that links adjacent nucleotides.
b) Name the two missing nitrogenous bases in the diagram.
c) Label the 5' and 3' ends of the DNA molecule.
d) Circle the hydrogen bonds that hold the two strands together.



Discovery of the DNA Structure
(Source: http://www.dnai.org and www.nature.com)

James Watson and Francis Crick are given credit for developing the three-dimensional structural model of DNA used today. The stunning find made possible the era of "new biology" that led to the biotechnology industry and, most recently, the deciphering of the human genetic blueprint. However, they were not the first to discover DNA, or the first to ask the question, “How is hereditary information carried from one generation to the next?”

Throughout history, our understanding of science changes as small modifications are made to what is known. Each discovery makes it possible for the next step in our understanding of scientific concepts to occur. The Watson and Crick model of DNA structure would not have happened without previous contributions by other scientists:

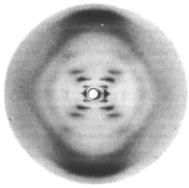
Friedrich Miescher – In 1869, Swiss Chemist Miescher first isolated nuclein (DNA and its protein scaffold) from white blood cells. The term “nuclein” was later changed to “nucleic acid” and eventually to “deoxyribonucleic acid (DNA)”.

Phoebus Levene – In 1919, Russian Biochemist Levene was the first to discover the order of the three major components of a single nucleotide (phosphate-sugar-base), and the carbohydrate component of DNA (deoxyribose) and RNA (ribose).

Oswald Avery – In 1944, Canadian-American physician Avery and his colleagues identified that hereditary units, or genes, are composed of DNA (“the transforming principle”).

Edwin Chargaff – In 1950, Austrian Biochemist Chargaff discovered that in all DNA, the amount of adenine (A) is usually similar to the amount of Thymine (T), and the amount of guanine (G) usually approximates the amount of cytosine (C). In other words, the total amount of purines (A+T) and the total amount of pyrimidines (C+T) are usually nearly equal. This discovery is later known as “Chargaff’s rule”.

Rosalind Franklin and Maurice Wilkins – In 1952, English researchers Franklin’s and Wilkins’ X-ray crystallography work (using X-ray diffraction to beam X-ray through DNA which yielded a shadow picture of the molecule’s structure) provided key information to the structure and dimensions of DNA: DNA’s phosphate groups are on the outside of the molecule, DNA could be a helical structure, and DNA has a uniform diameter along the length of the molecule (2nm).



James Watson and Francis Crick – In 1953, American Biologist Watson and English Physicist Crick used available information (e.g. Chargaff’s rule, X-ray data) and model building technique (e.g. using known molecular distances and bond angles) to decipher the three-dimensional structure of DNA. Using cardboard cut-outs representing the individual components of the four bases and other nucleotide subunits, Watson and Crick shifted molecules around until the complementary bases fit together perfectly, with each pair held by hydrogen bonds (based on the location of hydrogen atoms).

4.4 Macromolecular Structure of Ribonucleic Acid (RNA)

- Polynucleotides of RNA are structurally similar to DNA, except that RNA is usually **single-stranded**.
- Basic unit of each RNA polynucleotide: **ribonucleotides**.
- Similar to DNA, RNA nucleotides are bonded together via **5'-3' phosphodiester linkages** to form **polynucleotides**.
- Other than in some RNA viruses where the genetic material is double-stranded RNA, **RNA polynucleotides do not associate** to form a double-stranded helix.
- Some RNA polynucleotides e.g. tRNA and rRNA form **intra-molecular** hydrogen bonds between complementary bases (**A with U** and **G with C**) **within one strand** to form a 3D structure.
- There are mainly **three** types of RNA structures, each with different functions.

4.4.1 Types of RNA: mRNA, tRNA, rRNA

a) Messenger RNA (mRNA) – *Details of mRNA function will be covered in Topic 5 on Gene Expression*

- mRNA is a **single stranded** polyribonucleotide, containing the information **transcribed** from DNA (Fig. 4.12).
- **Function**: mRNA acts as a messenger that transfers genetic messages encoded in DNA from the nucleus to the ribosomes. It acts as a **template** for **translation** (protein synthesis).

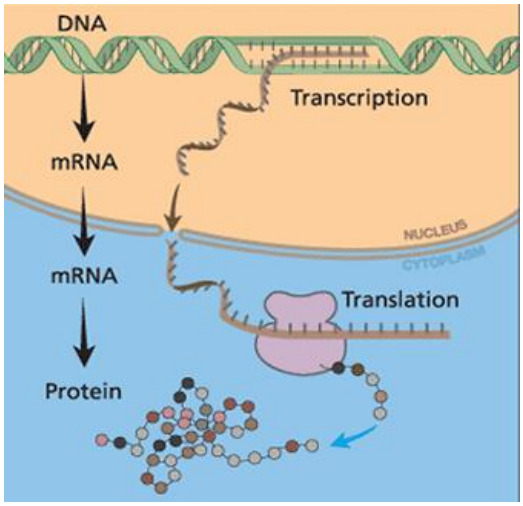


Fig. 4.12: The Central Dogma of Molecular Biology. The mRNA conveys information from the DNA, acting as a template for the synthesis of proteins.

- Bases are read in triplets known as **codons**. Each **codon** codes for an **amino acid** (Fig. 4.13).

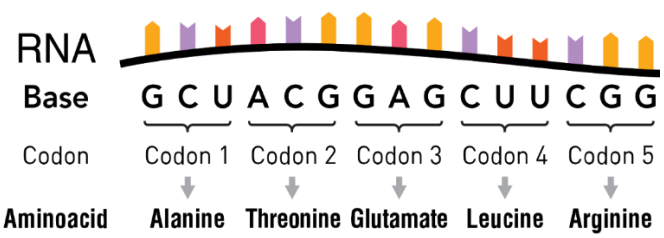


Fig. 4.13: Each mRNA codon codes for an amino acid.

b) Transfer RNA (tRNA) – *Details of tRNA function will be covered in Topic 5 on Gene Expression*

- Usually a single strand of RNA made up of ~70 to 90 ribonucleotides.
- tRNA has a specific sequence that allows folding of the single RNA strand unto itself by forming hydrogen bonds between complementary bases.
- Due to complementary base pairing occurring in some regions, 'hair-pin loops' form.
- Forms a **'clover leaf structure'** (Fig. 4.14(a)).

How does tRNA molecule form a 3-dimentional clover leaf structure? (Hint: see Fig. 4.14)

- **Function**: tRNA **transfers** specific **amino acids** from the cytoplasmic pool of amino acid to a **ribosome** during **translation of proteins**.
- It has dual recognition sites (Fig. 4.14a-c) for its involvement in the **protein synthesis** process:

(i) Amino acid attachment site

- ✓ The triplet CCA is always the site at the **3' end** of a tRNA.
- ✓ **Function**: Site where amino acid is attached to the tRNA by an enzyme.

(ii) Anticodon site

- ✓ Composed of 3 nucleotides.
- ✓ Each **anticodon** is **complementary** to a **corresponding codon** on the **mRNA** template.
- ✓ **Function**: allows the correct sequence of amino acids to be added to a growing polypeptide chain.

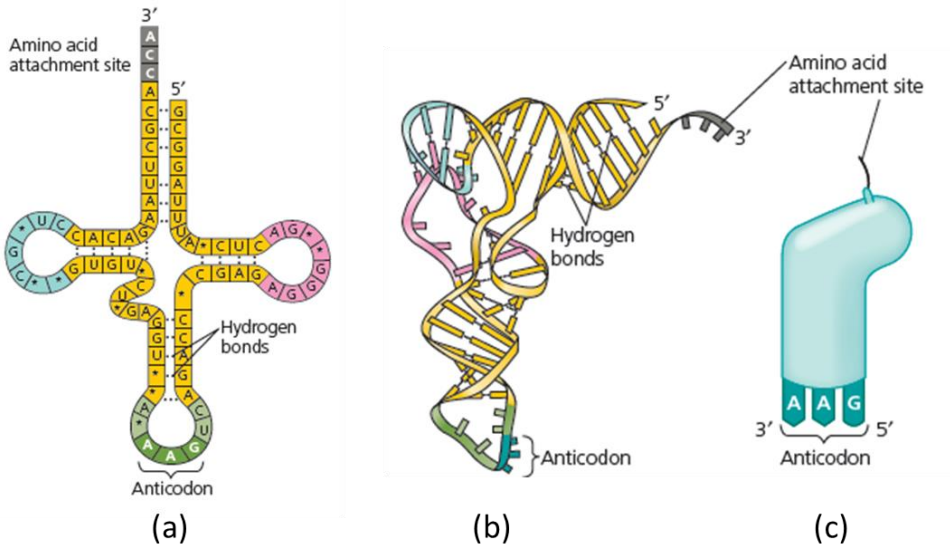


Fig. 4.14: (a) Intramolecular H-bonds in tRNA that maintain its 3D structure. **(b)** 3D depiction of how tRNA folds. **(c)** Simplest depiction of tRNA.

c) Ribosomal RNA (rRNA) – *Details of rRNA function will be covered in Topic 5 on Gene expression*

- rRNA, together with proteins, are the **components of ribosomes**.
- The assembly of **proteins** and **rRNA** produces
  - **80S** ribosomes in **eukaryotic cells**. Each ribosome comprises a **40S small subunit** and a **60S large subunit** (Fig. 4.15).
  - **70S** ribosomes can be found in **prokaryotic** cells. Each ribosome comprises a **30S small subunits** and a **50S large subunit**.

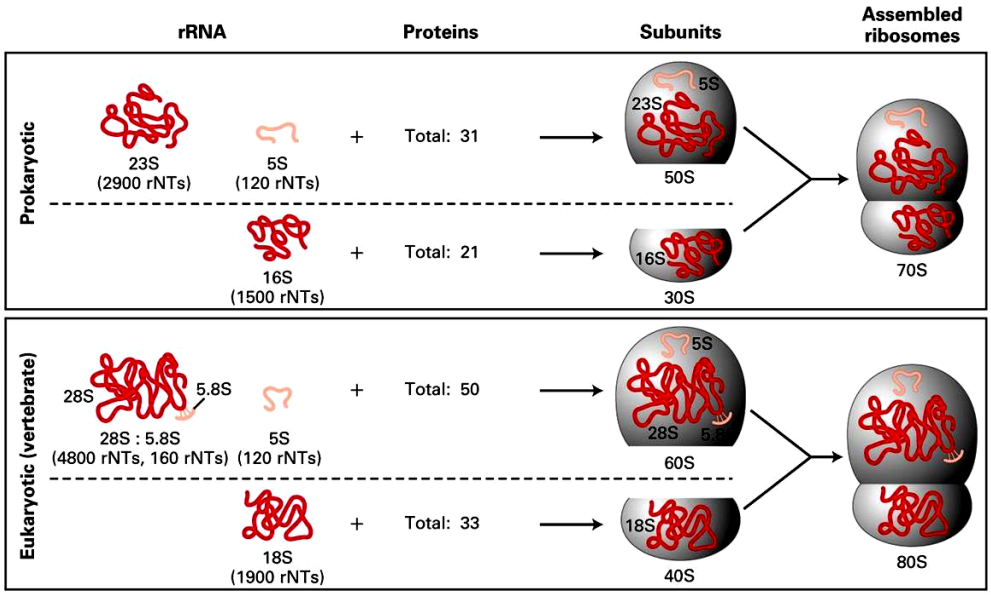


Fig. 4.15: The components of the prokaryotic 70S ribosome and the eukaryotic 80S ribosome

- The rRNA molecule folds into a 3D structure via **intramolecular H-bonding between bases A=U, C≡G** (Fig. 4.16).
- **Functions of rRNA:**
  - During translation, rRNA acts as an enzyme (ribozyme) providing **peptidyl transferase activity** to **catalyse the formation of peptide bonds** between the amino acids.
  - It has a structural role in helping to **orientate the ribosomal proteins into the correct position** within the ribosome.
  - rRNA from the large and small subunit interact, aiding in the **binding of the two subunits** during translation.
  - **Stabilises** the **interaction** between **mRNA** and **tRNA**.

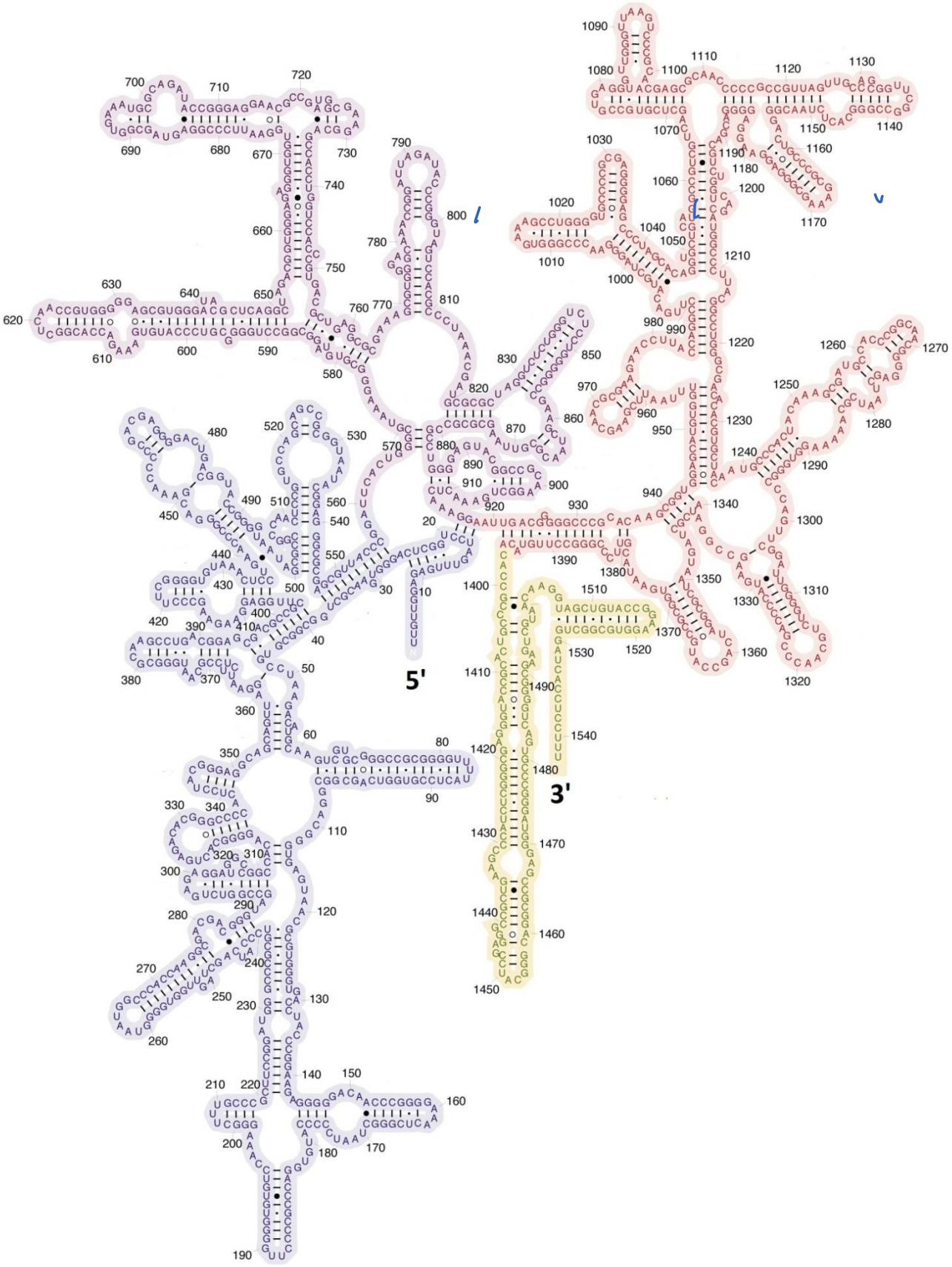


Fig. 4.16: A typical rRNA forming hydrogen bonds by complementary base pairing.

4.5 Checklist for Nucleic Acids

Are you able to confidently answer all the questions?

1. Describe the major components of a nucleotide.

2. Describe how nucleotides are linked to form a polynucleotide.

3. Distinguish between
   a) pyrimidine and purine

   b) nucleotide and nucleoside

   c) ribose and deoxyribose

   d) 5’ end and 3’ end of a polynucleotide

4. Describe the 3D structure of a DNA molecule.

5. Explain the base-pairing rule and explain its significance.

6. Explain the two key features that distinguish RNA from DNA.

7. Describe the structure and function of mRNA.

8. Describe the structure and function of tRNA.

9. Describe the structure and function of rRNA.

5. Proteins

- A protein consists of **one or more polypeptide(s)** typically folded into a **globular** structure or forming a **fibrous** structure (Fig. 5.1), facilitating a specific **biological function**.

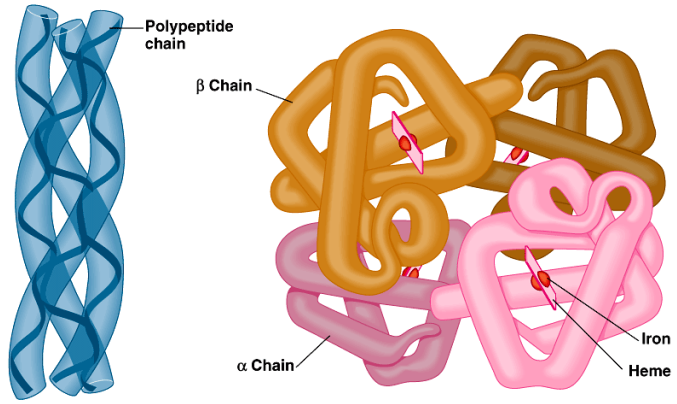


Fig. 5.1: (a) Collagen is an example of a fibrous protein while (b) haemoglobin is an example of a globular protein.

- A polypeptide is a single linear polymer chain of amino acids held together by **peptide bonds**.
- The **Biuret test** conducted on proteins will cause the biuret reagent to turn from **blue to violet**. This is due to the presence of peptide bonds (Fig. 5.2).

Fig. 5.2: Proteins (right) will cause the biuret reagent to turn from blue to violet.

- Many proteins are **enzymes** that **catalyse biochemical reactions** and are vital to metabolism.
- Proteins also have **structural** or **mechanical** functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system of scaffolding that **maintains cell shape**.
- Other proteins are important in **cell signalling**, **immune responses**, **cell adhesion**, and the **cell cycle**.

5.1 Structure of Amino Acids

- **Amino acids** are the monomers that make up proteins.
- There are over 500 amino acids; out of these, **only 20 of them are encoded by the genetic code** *(refer to Topic on "Gene expression").*
- All amino acids have a **central carbon atom** (α-carbon atom), which forms 4 bonds, each linking to an **amine** group ($-NH_2$), a **carboxylic** acid group (-COOH), a **hydrogen** atom (-H) and an **R group** (Fig. 5.3).
- The **R group** (also called the **side chain**) differs with each amino acid. The **physical and chemical properties** of the side chain **determine the unique characteristics** of an amino acid.
- When the R group of an amino acid is **polar or charged**, it is **more soluble in water** than one that has a **non-polar** R group (Fig. 5.4).

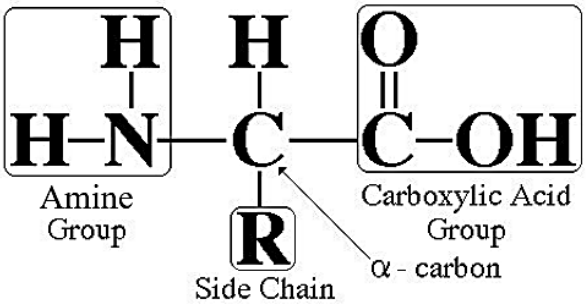


Fig. 5.3: Structure of an amino acid.

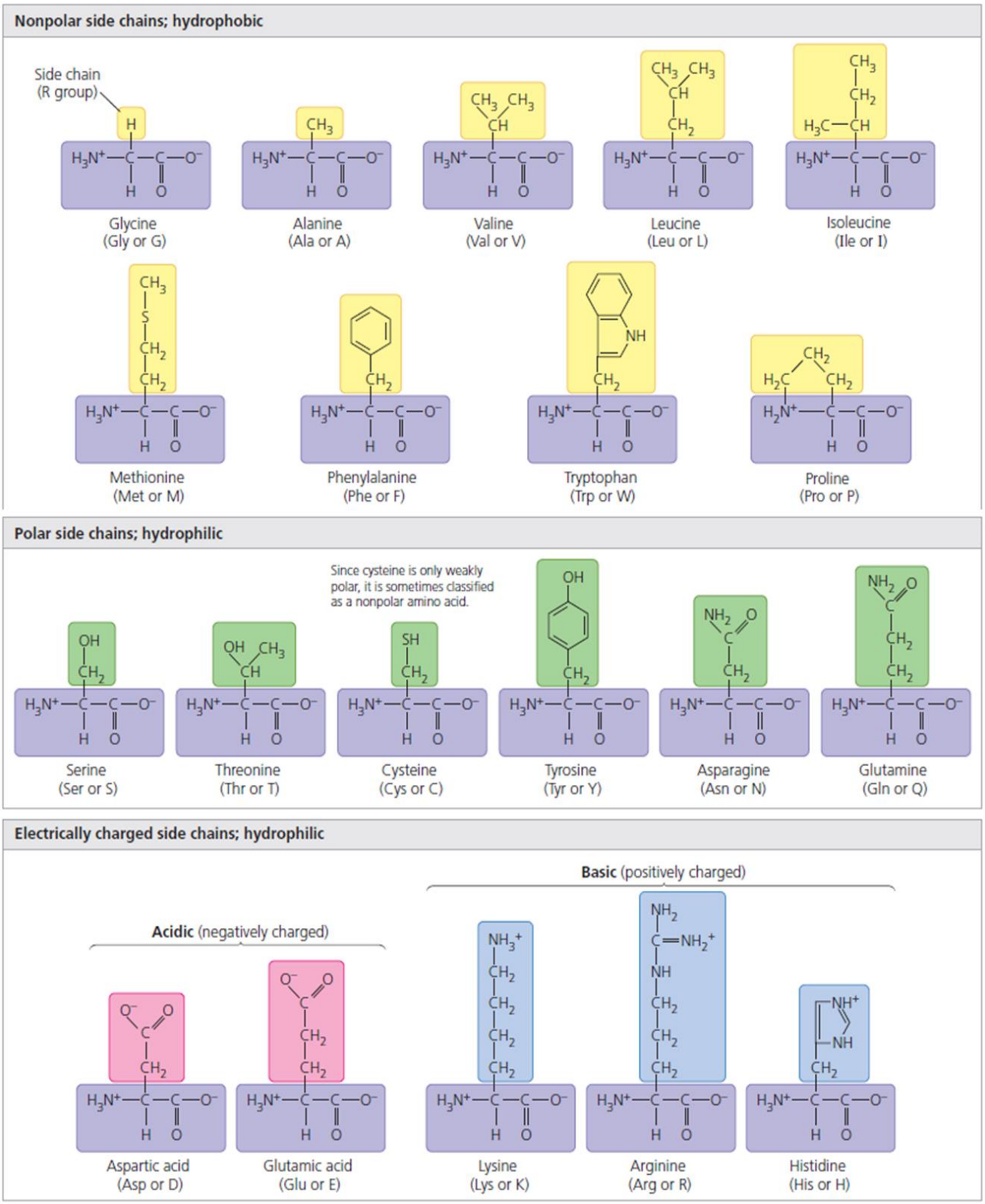


Fig.5.4: The 20 amino acids that are encoded by the genetic code, classified by their R groups/side chains. Note that the amino acids are depicted in the ionised form, the way they usually exist at the pH found in a cell. The R side chain may be as simple as a hydrogen atom (glycine) or it may be a carbon skeleton with various functional groups attached (e.g. glutamine).
(*Note: You are <u>not</u> required to remember the chemical formulae of each amino acid side chain.)

5.2 Types of bonds and interactions involved in structure of proteins

- The distinctive chemical properties of the amino acid side chains determine the types of bonds and interactions that can be formed between amino acids.

- The interactions between amino acids determine the structure of the protein. The **3-D structure** of a protein is also known as its **3-D conformation**.

5.2.1 Peptide Bonds

- Peptide bonds are strong **covalent bonds** formed between adjacent amino acids and are involved in the primary structure of proteins. These are the **strongest bonds** in the polypeptide chains.

- **Formation of a peptide bond** (Fig. 5.5)
    - One amino acid loses a **hydroxyl (-OH) group** from the **carboxylic acid group** while the other loses a **hydrogen atom** from its **amine group (-NH_2).**
    - The **carbon** atom of the first amino acid can thus bond with the **nitrogen** atom of the second. The bond formed is the **peptide bond**.
    - This is a **condensation** reaction, and a **water** molecule is formed as by-product.

- The **breakage** of the peptide bond occurs via a **hydrolysis** reaction, where a water molecule is required (Fig. 5.5).

- Regardless of the number of amino acids in the chain, one end of the chain has a free amino group (**N-terminus / amino end**) and the other end has a free carboxyl group (**C-terminus / carboxyl end**).

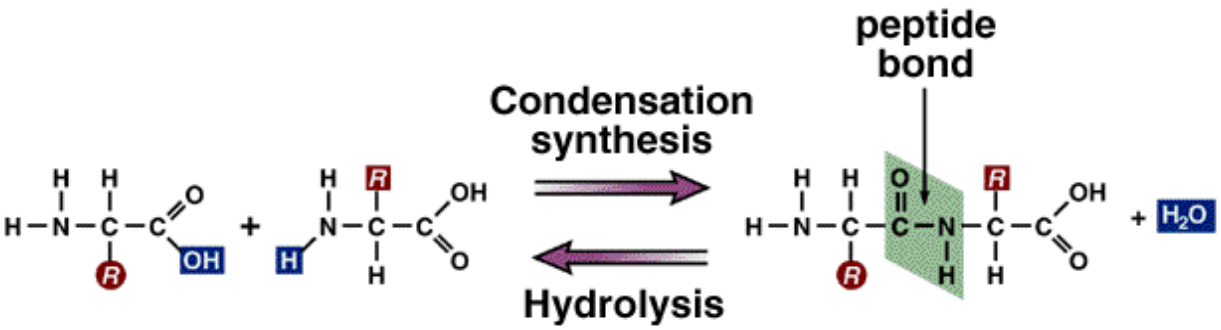


Fig. 5.5: Formation of a peptide bond via condensation.
Note that the resultant product is called a dipeptide as it is composed of two amino acid residues.

5.2.2 Disulfide bonds

- Disulfide bonds are **strong, covalent bonds** formed from oxidation of **sulfhydryl** (-SH) groups on the **R groups** of two **cysteine** amino acids (Fig. 5.6).

- These disulfide bonds can be broken by reducing agents (e.g. urea), even though they are the strongest of all the chemical bonds.

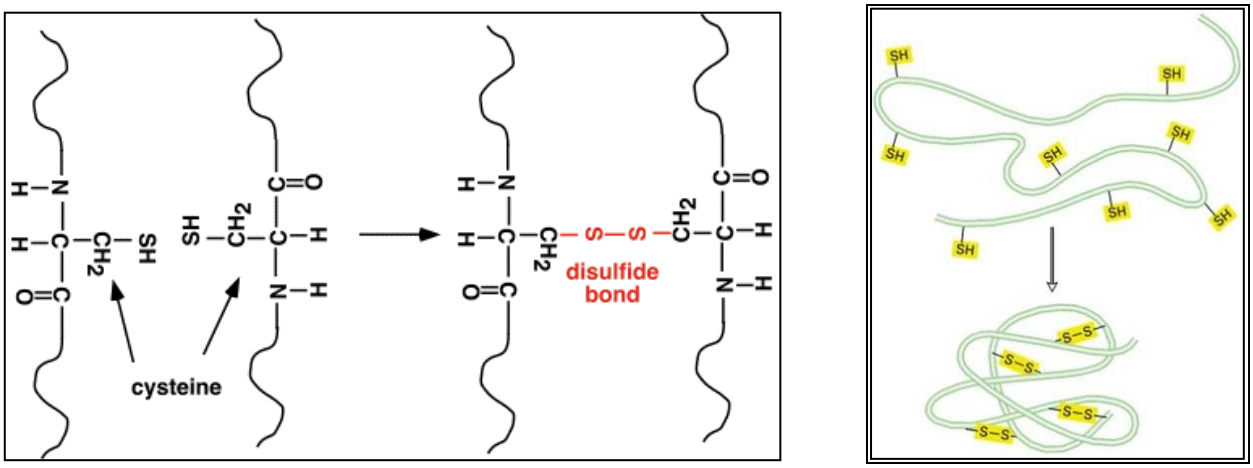


Fig. 5.6: (Left) The formation of a disufide bridge between two cysteine residues.
(Right) The disulfide bridges between cysteine residues spaced throughout the polypeptide help to stabilise the polypeptide into a globular 3D structure**.**

5.2.3 Ionic bonds

- Ionic bonds are **strong bonds** which are formed between **ionised / charged** amine (NH_3^+) and carboxylic groups (COO^-) present in **R groups** of amino acids (Fig. 5.7).

- Ionic bonds are formed at a suitable pH but can be broken if pH changes *(details will be covered in Enzymes lecture).*

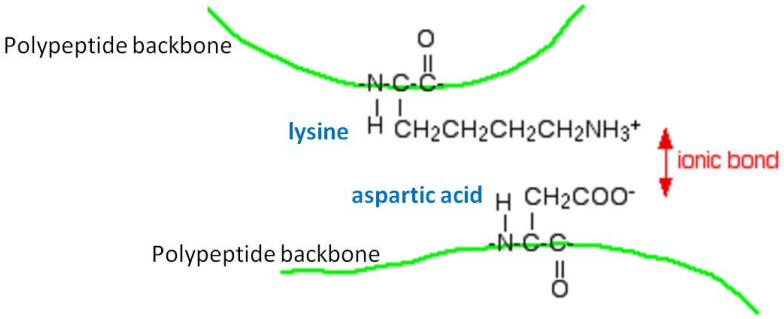


Fig. 5.7: An ionic bond formed between the R groups of aspartic acid and lysine.

TEST YOURSELF With reference to Fig 5.4, state one possible pair of amino acids that can form ionic bonds with each other. The first example is given.

Amino acid 1	Amino acid 2
Asp (-ve)	Lys (+ve)

5.2.4 Hydrophobic interactions

- As a polypeptide folds into its 3-dimentional shape, amino acids with **hydrophobic R groups** usually end up clustered at the core of the protein so that they are shielded from contact with the aqueous environment. These R groups form **hydrophobic interactions** with each other.

- Hence, in the aqueous cytoplasm of cells, many proteins assume a globular shape with their hydrophobic R groups located in the core region and the hydrophilic R groups (polar and charged) on the exterior surface to form hydrogen bonds with water molecules (Fig. 5.8).

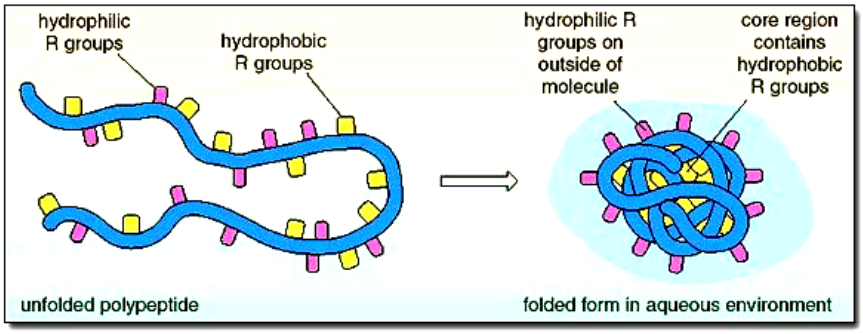


Fig. 5.8: Hydrophobic R groups tend to cluster into the core of the protein, away from the aqueous cytosolic environment in the cell. Hydrophilic R groups project out of the protein and interact with water.

5.2.5 Hydrogen bonds

- Hydrogen bonds are formed between polar groups.

- In proteins, **hydrogen atoms in amine (–NH) groups or hydroxyl (–OH) groups** form hydrogen bonds with **oxygen atoms in hydroxyl groups** (Fig. 5.9).

- Hydrogen bonds can stabilise the secondary structure and tertiary structure of proteins.
  a. **Secondary structure:** hydrogen bonds are formed between **C=O and N-H groups within the polypeptide backbone**.
  b. **Tertiary structure:** hydrogen bonds are formed between the **R-groups** of amino acids

- These bonds are **numerous** and stabilise the structure even though they are very **weak** on their own.

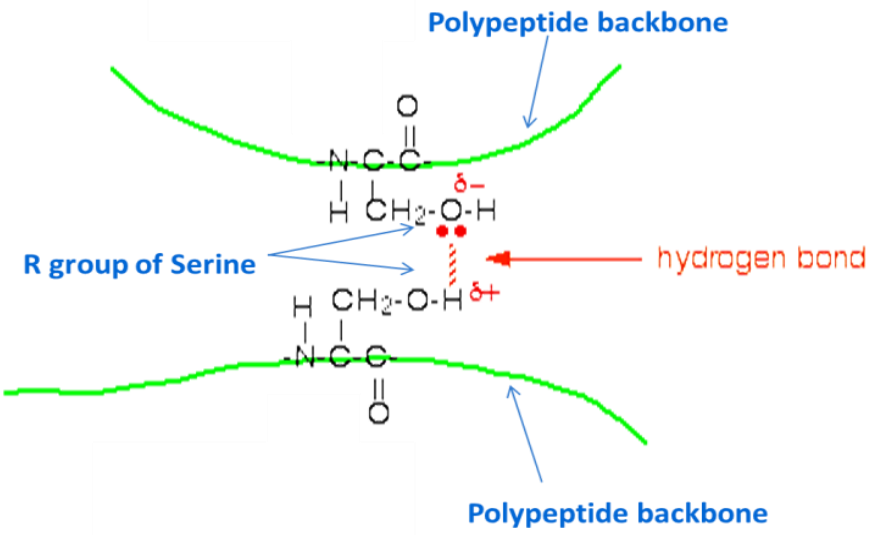


Fig. 5.9: Hydrogen bonding formed between the side chains of two serine residues.

TEST YOURSELF With reference to Fig 5.4, state one possible pair of amino acids that can form hydrogen bonds with each other. The first example is given.

Amino acid 1	Amino acid 2
Ser	Asn

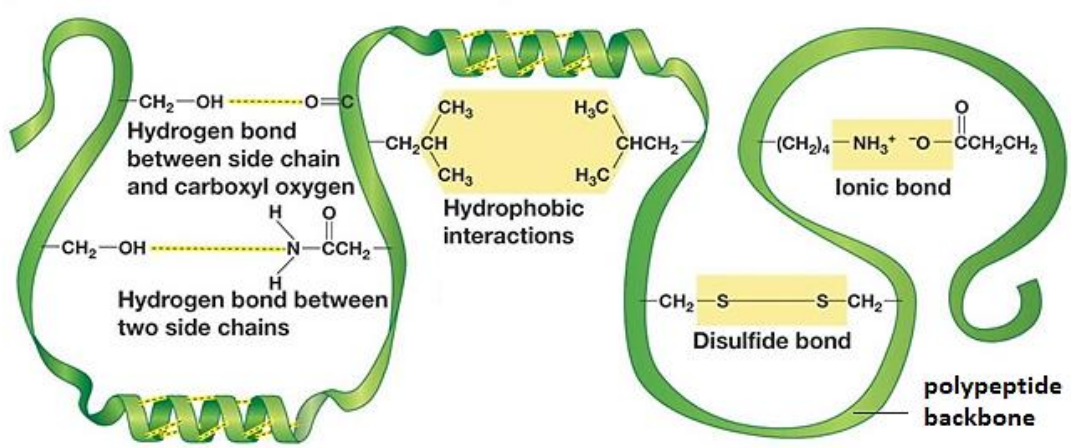


Fig. 5.10: Summary of the various types of bonds and interactions stabilising the 3D structure of proteins (Note: peptide bonds that form the polypeptide backbone are not shown).

5.3 Structure of proteins

- Nearly every dynamic function of a living being depends on proteins. Some proteins (enzymes) speed up chemical reactions, while others play a role in storage, transport, cellular communication, movement or structural support.

- A human has tens of thousands of different proteins, each with a unique **three dimensional structure that determines its specific function**.

- Most proteins share three *superimposed* levels of structure, known as **primary**, **secondary** and **tertiary structure**. A fourth level, **quaternary structure**, arises when a protein consists of two or more polypeptide chains (Fig. 5.11).

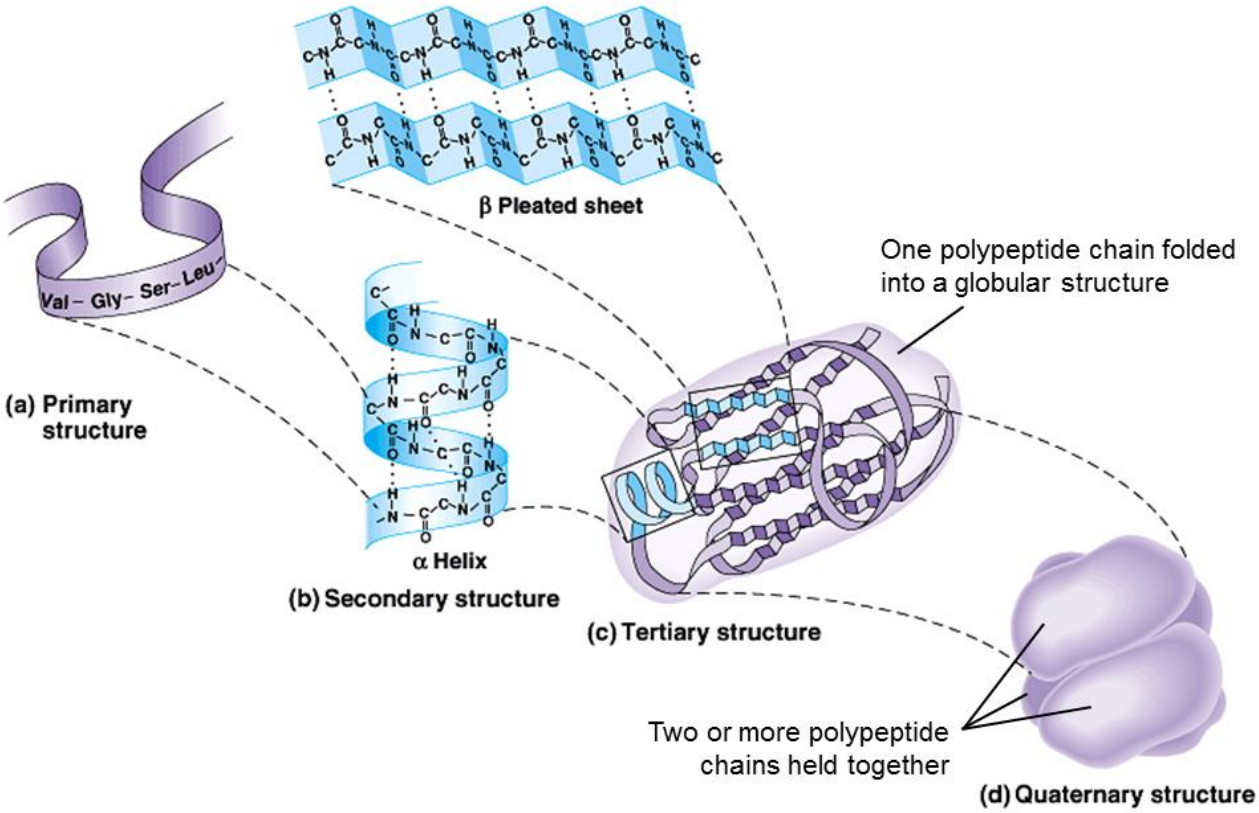


Fig. 5.11: The four levels of structure of proteins

a) Primary structure

- The primary structure of a protein is the **specific sequence**, **number** and **type** of **amino acids** held by **peptide bonds**. The primary structure is specific to every protein. The **number** of amino acids incorporated into the primary structure varies in many different proteins.

- The simple **linear** strand of amino acids is held together by peptide bonds in the polypeptide chain (Fig. 5.5).

- By convention, **amino acid number one** is the **one at the amino end** (also known as the **N-terminal**) while the **last amino acid** of the primary sequence is the **one at the carboxyl end** (also known as the **C-terminal**) (Fig. 5.12).

- The amino acid sequence and its R groups determine the types of interactions/bonds formed between regions of the polypeptide chain to form the secondary structure, tertiary structure and, if any, the quaternary structure. Hence, it determines the final **3D conformation** of a protein and thus dictates the **properties** and **biological function** of the protein.

- A **change** in one amino acid in the polypeptide chain may completely alter the **3D conformation** and hence the **properties** and **function** of the protein.

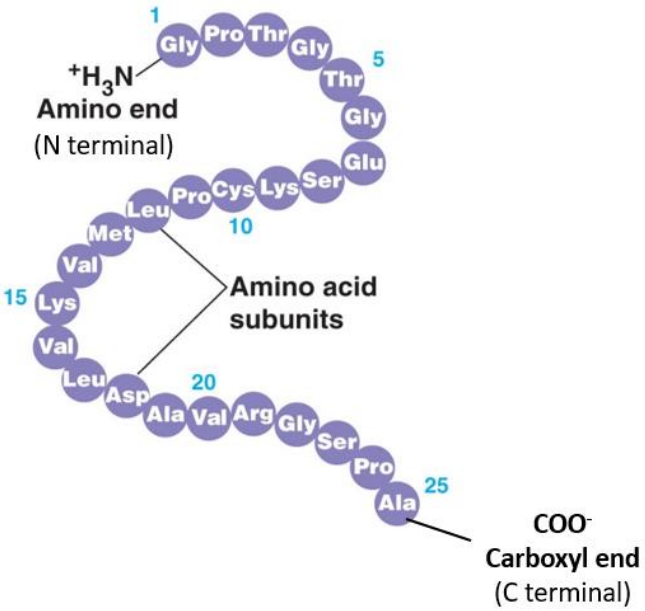


Fig. 5.12: The primary structure of a protein is the specific number, type and sequence of amino acids held by peptide bonds.

b) Secondary structure

- Most polypeptides have **segments of their chain repeatedly coiled or folded** in patterns that contribute to the protein's overall shape.

- These coils and folds, collectively referred to as the **secondary** structure, are stabilised by **<u>hydrogen bonding</u>** between **hydrogen atom of NH** of one amino acid and **oxygen atom of CO** of a neighbouring amino acid **within the polypeptide backbone**.

- There are two different types of secondary structures (Fig. 5.13 and 5.14):

  (1) α-Helix (Fig. 5.13)

  - **One** polypeptide chain coiled into an α-helix that is held in this shape by many **intra-molecular hydrogen bonds**.
  - **Hydrogen atom** of the **NH** group of one amino acid is **hydrogen bonded** to the **oxygen atom** of **C=O** group of another amino acid **4** places ahead of it **within the polypeptide backbone**.
  - The hydrogen bond is the result of unequal electron-sharing in the NH group which leaves the hydrogen atom slightly positive, and the CO group which leaves the oxygen atom slightly negative. Hence they attract each other.
  - There is **one complete turn** for **every 3.6 amino acids**, forming a stable structure.
  - Individually, each hydrogen bond is weak, but because they are repeated many times over a relatively long region of the polypeptide chain, the bonds can support a particular structure for that part of the protein.
  - E.g. Keratin is made up entirely of **α-helices** (keratin is found in hair, wool and nails).

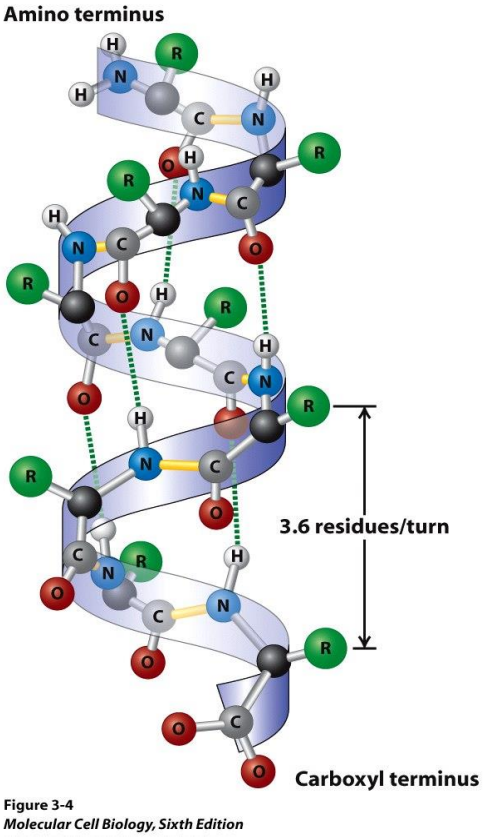


Fig. 5.13: An alpha helix. R groups points away from the helix. Dotted lines represent hydrogen bonding.

(2) β-Pleated Sheet (Fig. 5.14)

- Sections of a polypeptide chain can be folded to consist of **adjacent parallel** segments that run in either **opposite** or the **same** direction (Fig. 5.15).
- The segments are joined by **hydrogen bonds** between **hydrogen atom** of the **NH** group of one amino acid of one chain with **oxygen atom** of **C=O** group of another amino acid of an **adjacent segment** within the polypeptide backbone.
- β-pleated sheets make up the core of most globular proteins (e.g. enzymes) and dominate the structure of some fibrous proteins (e.g. silk protein of spider web).
- The β-pleated sheets give proteins **stability**, **high tensile strength** and **flexibility**, but not elasticity.

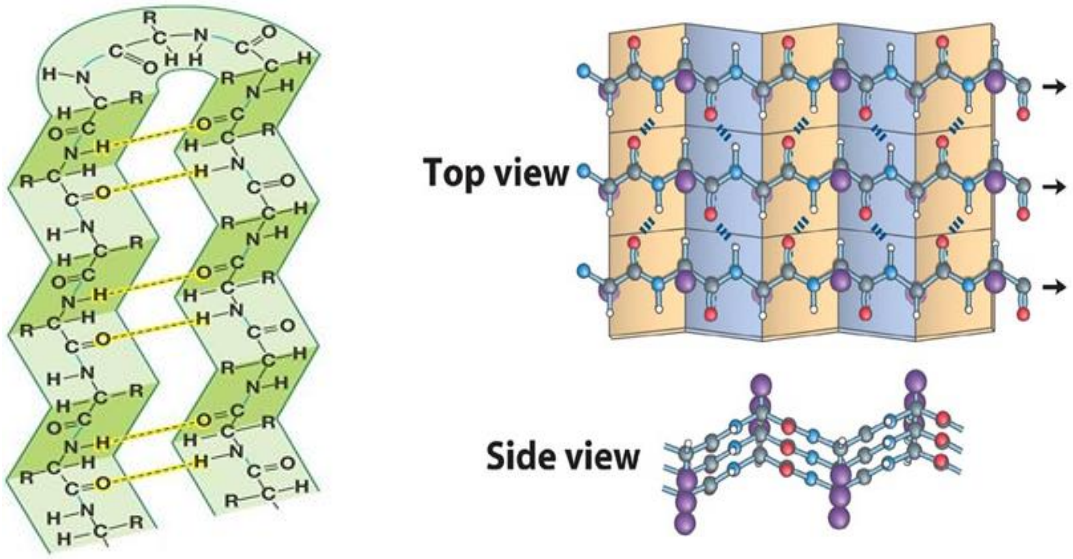


Fig. 5.14: (Left) Antiparallel and **(Right)** parallel β-pleated sheets.
Note the hydrogen bonding between the hydrogen atom of the NH group of one amino acid of one chain with the oxygen atom of C=O group of another amino acid of an adjacent segment.

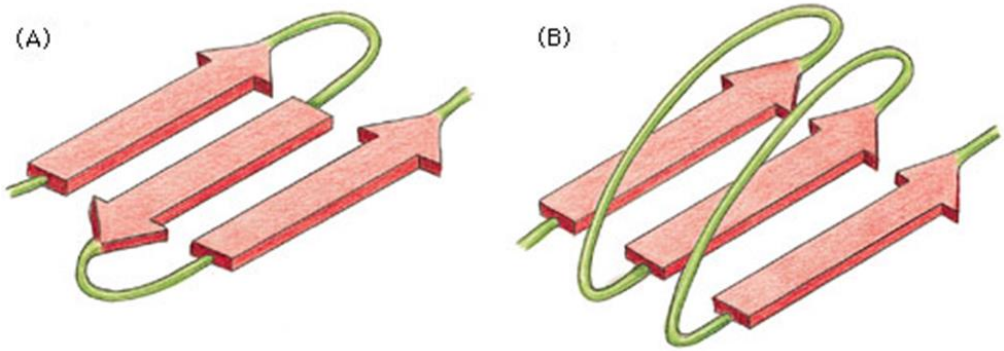


Fig. 5.15: Antiparallel (A) and parallel (B) β-pleated sheet

c) Tertiary structure

- When a **single** polypeptide chain (consisting of α-helices and/or β-pleated sheets) is further **coiled** and **extensively folded** to form a **compact and globular three-dimensional structure**, a tertiary structure results (Fig. 5.16, left).

- The tertiary structure is determined by the primary structure and is maintained by **disulfide bonds**, **ionic bonds**, **hydrophobic interactions**, and **hydrogen bonds** formed **between the R groups of amino acids** (Fig. 5.16, right).

- The majority of proteins have a globular tertiary structure. Examples of such are **enzymes**, **membrane bound receptors** and **antibodies**, among many others.

- The tertiary structure of these molecules is essential to their function as most of them have a **specific 3D conformation** (e.g. active site of enzymes is complementary in shape to its substrate).

- Disruption of the tertiary structure of the protein (e.g. by high heat) often results in the protein losing its function.

- The diversity of **globular** proteins is largely due to differences in their **tertiary** structure rather than secondary.

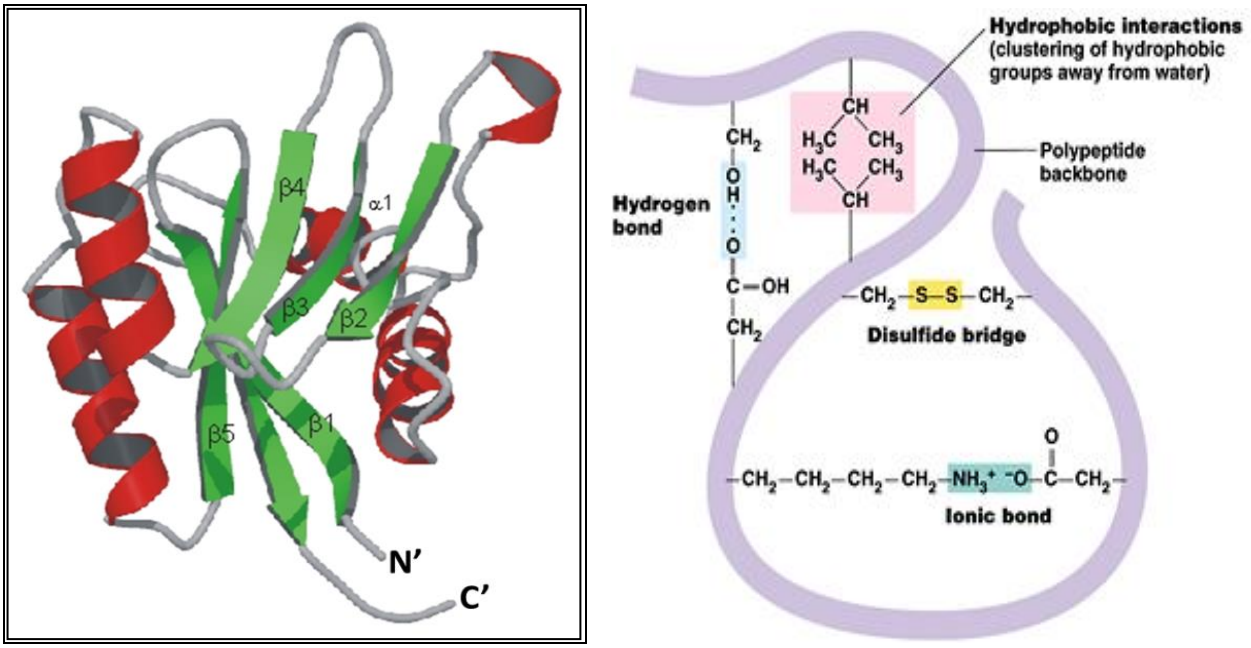


Fig. 5.16:
(Left) A globular protein consisting of both alpha helices and beta-pleated sheet (note that there is only one N and C termini, indicating that this protein is made up of only one polypeptide chain).
(Right) The various bonds and interactions between R groups that stabilise a tertiary protein.

d) Quaternary structure

- Proteins with quaternary structure are highly complex and consist of an aggregation of **two or more extensively coiled polypeptide chains** held together by combinations of **disulfide bonds**, **ionic bonds, hydrogen bonds and hydrophobic interactions** between the **R groups of amino acids**. For example, **haemoglobin** has **four** polypeptide chains linked by various bonds (Fig. 5.17a).

- Tertiary and quaternary structures are important because the **conformation** of the protein when **folded** is responsible for the biological activity of the protein.

Read through *Section 5.3*. Complete the table below summarising the type(s) of chemical bonds that are involved in maintaining the four levels of protein structure. The first example is given.

Level of Protein Structure	Type(s) of chemical bonds involved in maintaining the structure	Number of polypeptide chain(s)
Primary	Peptide bonds between amino acids	1
Secondary		
Tertiary		
Quaternary		

Heat/chemical denaturation can alter the structure of proteins. Why?

5.4 Examples of Proteins

- Nearly every dynamic function of a living being depends on proteins. Some proteins (enzymes) speed up chemical reactions (*Section 5.5*), while others play a role in storage, transport, cellular communication, movement or structural support.

- A human has tens of thousands of different proteins, each with a unique **three dimensional structure** that **determines its specific function**.

- Based on the shape of molecule, proteins can be classified into globular proteins or fibrous proteins. The differences between them are shown in the following table:

		Globular proteins (E.g. haemoglobin)	**Fibrous proteins** (E.g. collagen)
1	Amino acid sequence	**Wide variety** of amino acids arranged **irregularly**	Amino acid sequence tends to be **repetitive** and arranged in a **regular** manner
2	Level of structure that renders protein functional	**Tertiary structure** or quaternary structure (if present) gives rise to its functional 3D configuration.	**Secondary structure** or **quaternary structure**, depending on the protein. (In tropocollagen, microfibrils and fibres, the quaternary structure gives collagen high tensile strength) *Note: Fibrous proteins have little or no tertiary structure.*
3	Overall shape	Polypeptide chain is tightly folded to form a **spherical** shape.	Long, parallel tropocollagen molecules are cross-linked at intervals to form **long fibrils** (thus making them physically tough).
4	Solubility in water	Globular proteins are **soluble in water** to form colloidal solutions (not true solutions), due to hydrophilic R groups of amino acid residues facing outwards from their molecules.	Fibrous proteins are **insoluble in water**, due to large number of hydrophobic R groups of amino acid residues on their exterior.
5	Functions	Perform **metabolic functions**, such as enzymes, haemoglobin, antibodies, and some hormones (e.g. insulin).	Perform **structural functions**, such as collagen (in tendon, bone, connective tissues), keratin (in hair, nails), myosin (in muscles), and silk (in spider webs).

5.4.1 Haemoglobin (Globular protein, Transport function)

- An example of a **globular protein** with a **quaternary** structure is haemoglobin.

- It is the red, oxygen-carrying pigment found in the cytoplasm of red blood cells.

- Haemoglobin consists of **four** separate polypeptide chains (Fig. 5.17a):
  - Two **α-chains** (each chain a specific sequence of 141 amino acids).
  - Two **β-chains** (each chain a specific sequence of 146 amino acids).

***Note:** The β-chain does not refer to β-pleated sheets. It is just so named.

- Each polypeptide chain is first coiled into **α-helices** (**secondary structure**). The α-helices are in turn folded into the **tertiary structure** to yield a spherical **globular** protein that has a specific conformation that is held by **hydrogen bonds, ionic bonds** and **hydrophobic interactions** between **R groups** (tertiary structure).

- Amino acids with **hydrophobic** side-chains point inwards, holding the molecule in its correct three-dimensional shape. Amino acids with **hydrophilic** side-chains point outwards so that it is soluble in aqueous medium and hence a good transport protein for oxygen in blood.

- The four polypeptide chains are linked together by **ionic bonds, hydrogen bonds and hydrophobic interactions** between **R groups** (**quaternary structure**) (Fig. 5.17).

  (*Note: Haemoglobin **does not have disulfide bonds** in its structure.)

- **Each chain** carries a prosthetic (non-proteinaceous) **haem** group (Fig. 5.17a).

- The haem group contains **Fe^{2+}** which can **bind reversibly to a molecule of oxygen** and hence enhance the release of oxygen in metabolically active tissues such as muscle.

- Each haemoglobin molecule can bind **four** oxygen molecules since it has four haem groups.

- Collectively, the 4 polypeptides and their haem groups form a **specific conformation**, which allows it to carry out its function—to bind to oxygen molecules.

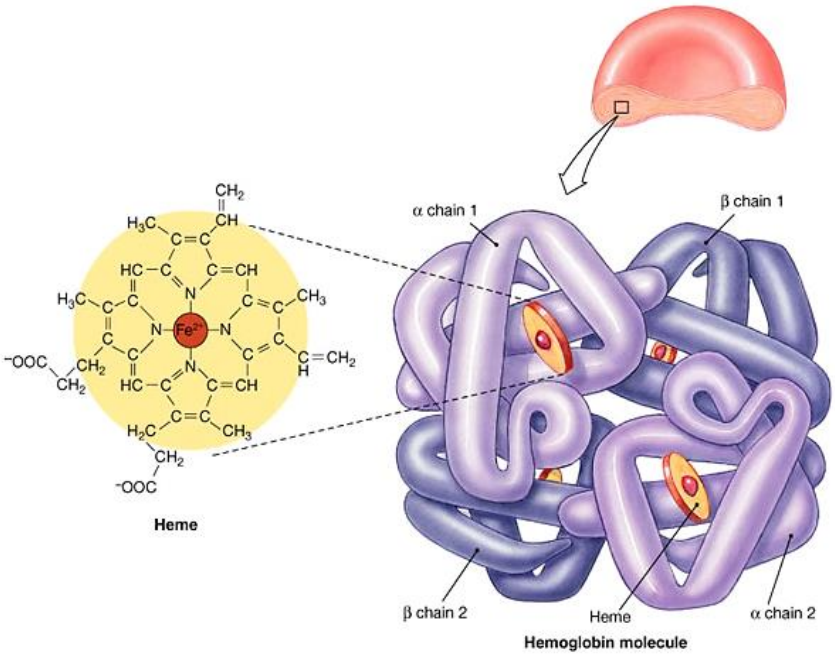


Fig. 5.17a: Structure of a haemoglobin molecule, showing the quaternary structure and haem group.

- Haemoglobin is an allosteric protein that undergoes **cooperative binding** with oxygen, a property that allows it to carry out its function (cooperativity in allosteric molecules is a concept that will also be taught in the topic on Enzymes).

- Allosteric proteins (including enzymes) change their conformation when a particular molecule binds to it. In the new shape, the protein's ability to bind to a second molecule is altered.

- When one oxygen molecule binds cooperatively to the first haem group of the haemoglobin tetramer (four polypeptide subunits), there will be a **conformational change** in its structure that increases haemoglobin's affinity for oxygen (Fig. 5.17b). The quaternary level structure of haemoglobin changes due to the movement of the alpha and beta subunits.

- Conversely, when an oxygen molecule is released from a haem group, the conformational change in the structure of haemglobin results in a lower affinity for oxygen.

- Cooperative binding enables haemoglobin to take up oxygen in regions of **higher oxygen concentration** (lungs) and release oxygen in regions of **lower oxygen concentration** (muscles) efficiently.

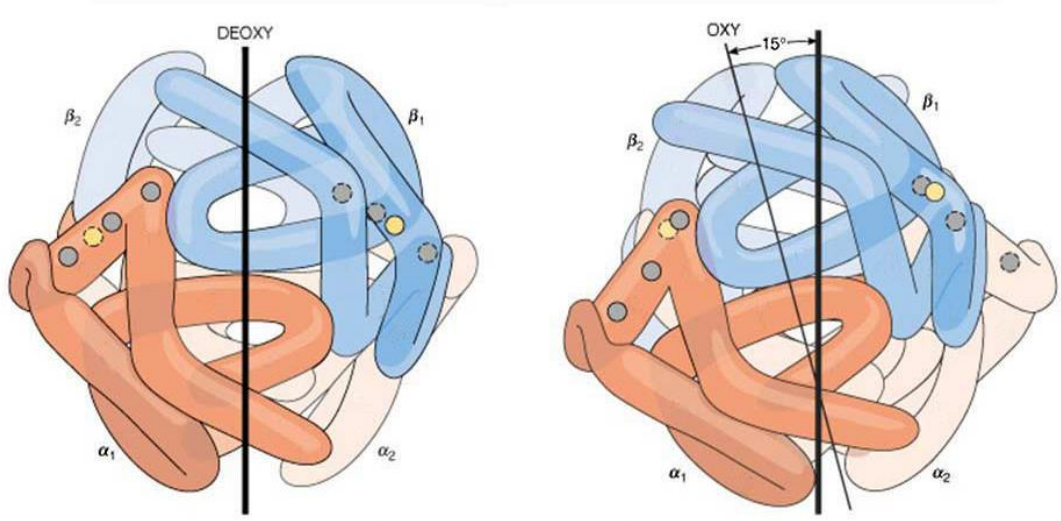


Fig. 5.17b: When oxygen binds to the haem group of deoxyhaemoglobin (left), the subunits undergo a conformational change to become oxyhaemoglobin (right) as indicated by the rotation of the axis by 15°.

- Thus, cooperative binding produces a **sigmoidal** (S-shaped) curve (Fig. 5.17c).

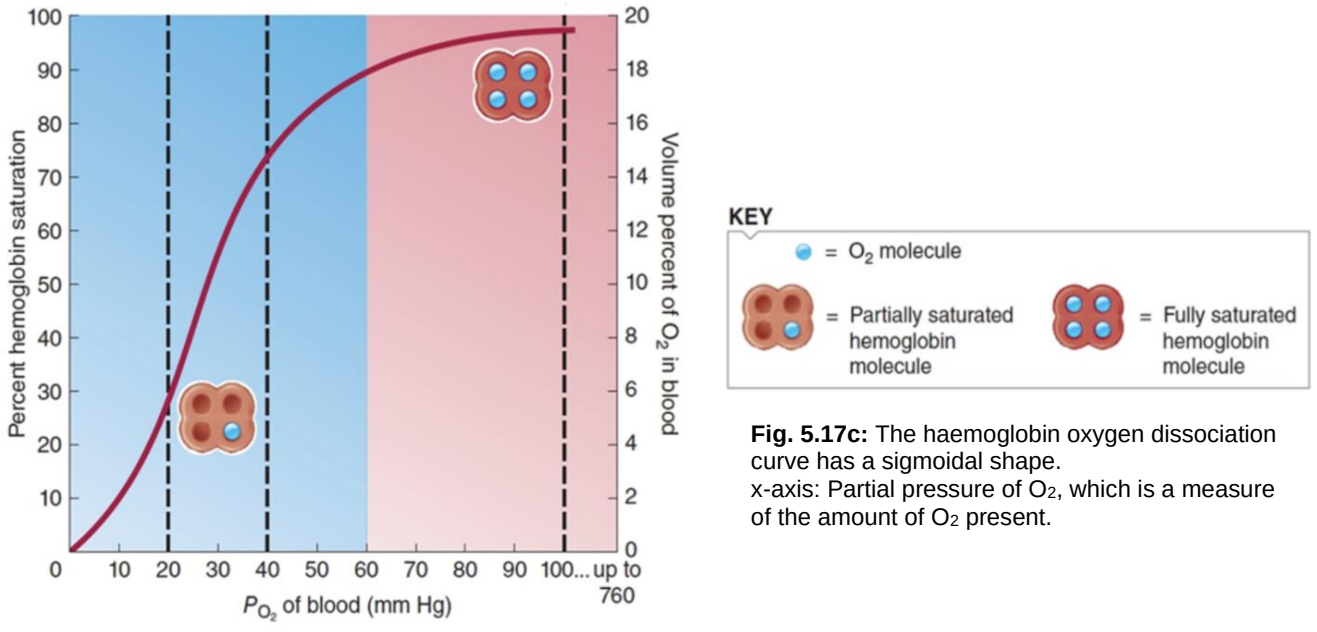


Fig. 5.17c: The haemoglobin oxygen dissociation curve has a sigmoidal shape.
x-axis: Partial pressure of O_2, which is a measure of the amount of O_2 present.

- A genetic disease known as **sickle cell anaemia** is caused by a change in a single amino acid in the polypeptide chains. This in turn changes the primary and secondary structure of the protein which results in a change in the tertiary/3-dimensional structure of the haemoglobin molecule and eventually results in the inability of red blood cells to carry oxygen. The affected red blood cells are sickle-shaped (Fig. 5.18).

  (*Knowledge of sickle cell anaemia is closely linked to Topic 6 on Gene Mutations)*

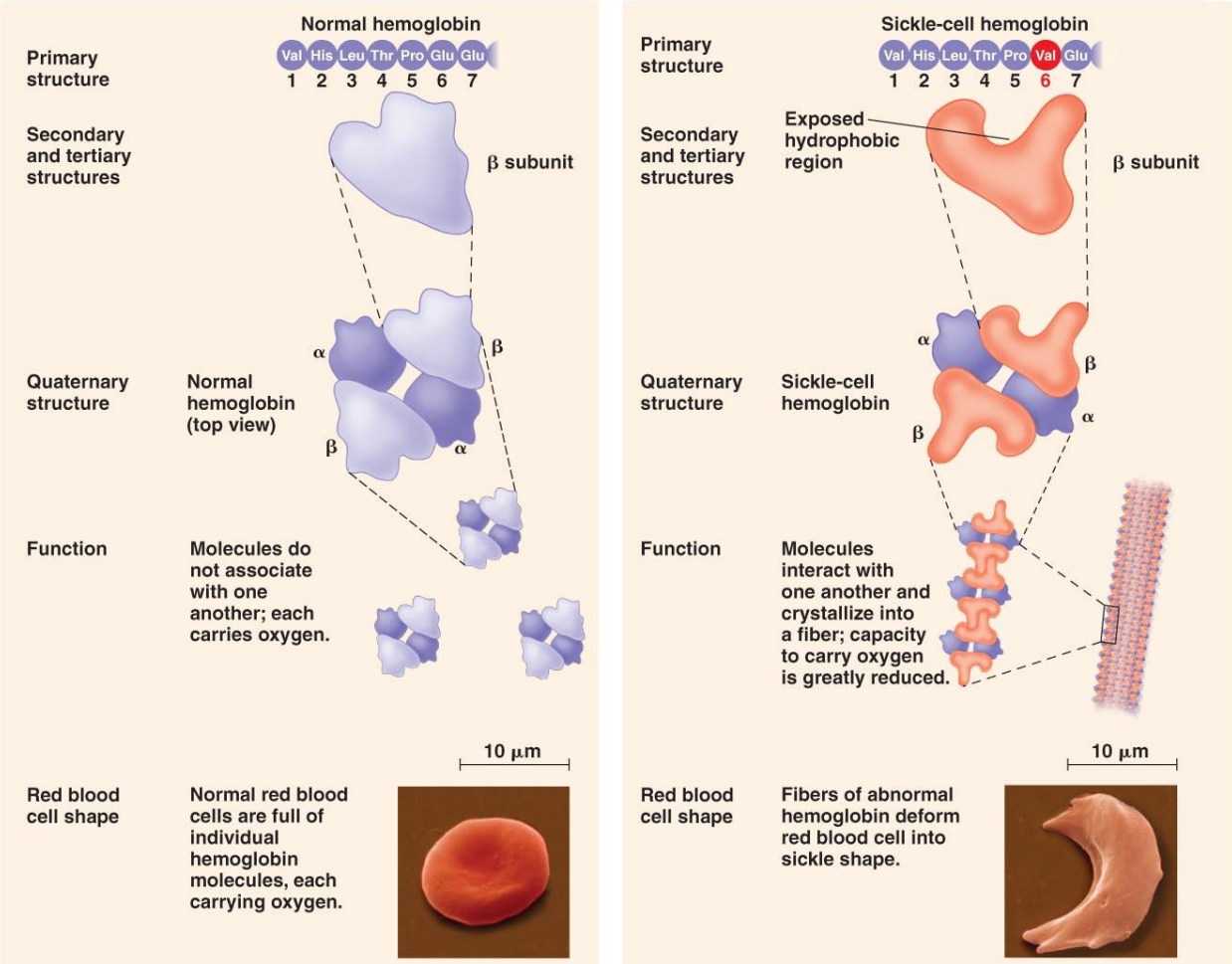


Fig. 5.18: In a medical condition known as sickle-cell anaemia, a single amino acid change leads to a change in tertiary structure of haemoglobin.

For more in depth understanding of haemoglobin:
https://pdb101.rcsb.org/motm/41

Animation on Haemoglobin: http://www.dnatube.com/video/274/Hemoglobin-Oxygen-Binding

Relating Structure to Function (Haemoglobin)

	Structural feature(s)	How structure contributes to property	Suited for Function to <u>TRANSPORT O_2</u> because:
1	• **Folding** of each polypeptide chain into a **globular subunit** held by hydrophobic interactions, hydrogen bonds and ionic bonds**.** → **Hydrophobic amino acid residues** of each subunit are hidden in the **interior** of the folded structure → **Hydrophilic amino acid residues** of each subunit are facing the **exterior** aqueous medium	Allows haemoglobin to be **soluble** in the aqueous cytoplasm of red blood cells	• Each red blood cell can **carry** many haemoglobin molecules and **transport** them around the body
2	• Each haemoglobin molecule is made up of **four subunits.**	Binding of one oxygen molecule to the first haem group of the haemoglobin tetramer leads to a conformational change that increases the other subunits' affinity for oxygen. **(Cooperative binding)**	• **More efficient** oxygen uptake of oxygen in regions of **higher oxygen concentration** (lungs) and release of oxygen in regions of **lower oxygen concentration** (muscles).
3	• **Each haemoglobin subunit** is bound to **one haem group.** • Each haem group contains **$Fe2^+$** that **binds weakly to oxygen.**	**Reversible binding** of oxygen to each haem group (four O_2 molecules per haemoglobin)	• **Oxygen** can be released in oxygen-deficient tissues

5.4.2 Collagen (Fibrous protein, Structural function)

- Collagen (Fig. 5.19) is a **fibrous** protein with a **quaternary** structure. It has **no tertiary structure**.

- Collagen is a protein found in skin, tendons, cartilage, bones, teeth and connective tissue of blood vessels to provide support for these structures.

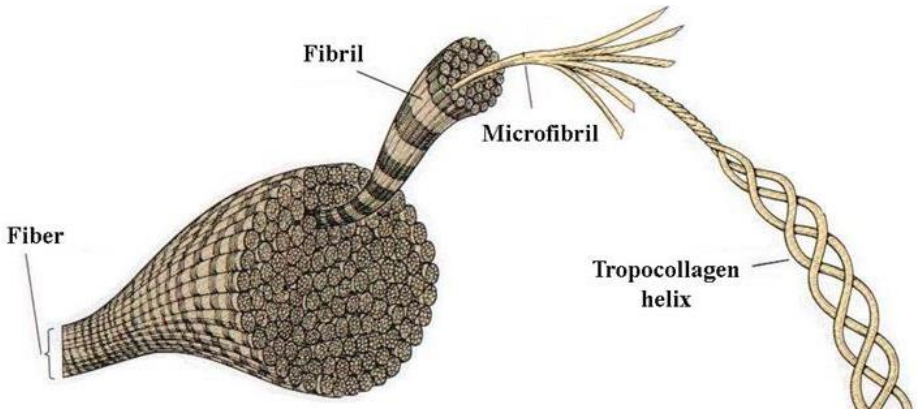


Fig. 5.19: An overview of the organisation of collagen.

Formation of tropocollagen

- Tropocollagen is the basic functional unit of collagen.
- A **tropocollagen** molecule consists of **three polypeptide chains** wound around each other to give a **triple helix** (Fig. 5.20).

- **Each collagen polypeptide chain** consists of about 1000 amino acid residues and is in the shape of a **loosely wound** left-handed **helix (<u>NOT</u> an α-helix).**

- Collagen consists of 2 modified amino acids – hydroxyproline and hydroxylysine. Hydroxylation of proline and lysine occurs in the rough endoplasmic reticulum.

- The sequence of amino acids is usually a repeat of
  - **Glycine – Proline – X**, or
  - **Glycine – X – Hydroxyproline**

  where X is any other amino acids except glycine (Fig. 5.21)

- The presence of **glycine** (R group is only a hydrogen atom) at **every third amino acid within each polypeptide chain** allows **close packing** of the triple helix to form a **tight coil** (Fig. 5.21).

- Because of their high content of glycine, proline, and hydroxyproline, collagen fibres are incapable of forming traditional secondary structures such as α-helices and β-sheets. Instead, collagen polypeptides intertwine to form the unique triple helix**,** with each of the three strands arranged in a helical fashion.

- The three strands in the triple helix are linked together by **<u>hydrogen bonds</u>** formed between **peptide N-H group** of glycine and **peptide C=O group** (within the polypeptide backbone) of other amino acids on the other chains.

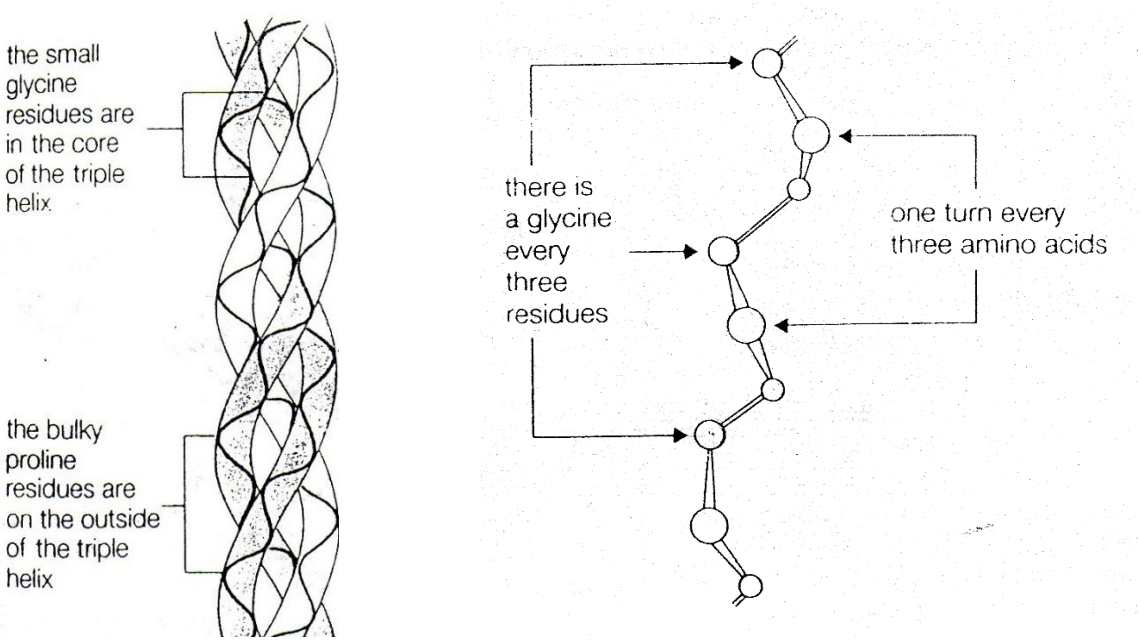


Fig. 5.20: The structure of tropocollagen.

13
-Gly-Pro-Met-Gly-Pro-Ser-Gly-Pro-Arg-
22
-Gly-Leu-Hyp-Gly-Pro-Hyp-Gly-Ala-Hyp-
31
-Gly-Pro-Gln-Gly-Phe-Gln-Gly-Pro-Hyp-
40
-Gly-Glu-Hyp-Gly-Glu-Hyp-Gly-Ala-Ser-
49
-Gly-Pro-Met-Gly-Pro-Arg-Gly-Pro-Hyp–
58
-Gly-Pro-Hyp-Gly-Lys-Asn-Gly-Asp-Asp-

Fig. 5.21: The sequence of part of a collagen polypeptide from amino acid no. 13 to 66. Note the general Gly-Pro-X and Gly-X-Hyp repeating nature. Glycine always appears at every 3rd residue.

Association of tropocollagen into collagen fibres

- Many **tropocollagen molecules** (each a complete triple helix) **lie parallel** such that adjacent molecules **overlap in a staggered manner** to form **fibrils** (Fig. 5.22).
- Fibrils are strong because **covalent crosslinks** are formed between **lysines and/or hydroxylysines** in the tropocollagen molecules lying next to each other (Fig. 5.22).
- Collagen **fibrils bundle** to form strong collagen **fibres** (a common feature of collagen found in bones).
- Because of the staggered nature of tropocollagen molecules within each fibril, collagen **fibre appears banded** (Fig. 5.23).

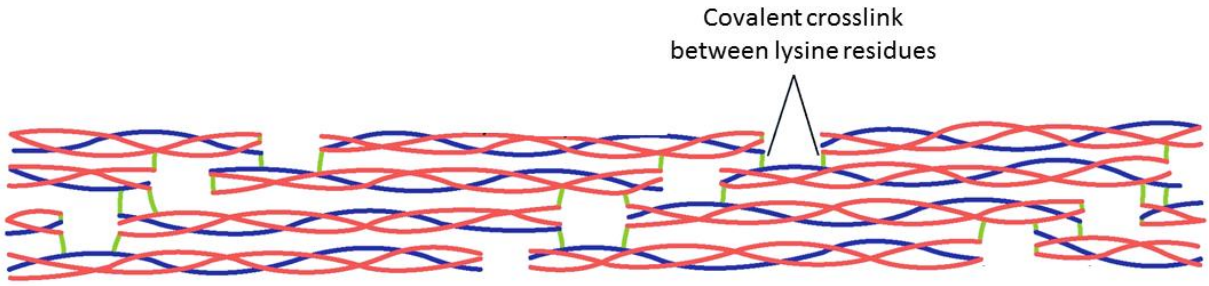


Fig. 5.22: shows the staggered ends and covalent cross-linking of tropocollagen in collagen fibrils. Such cross-links are formed between the lysine and/or hydroxylysine residues in chains lying next to each other.

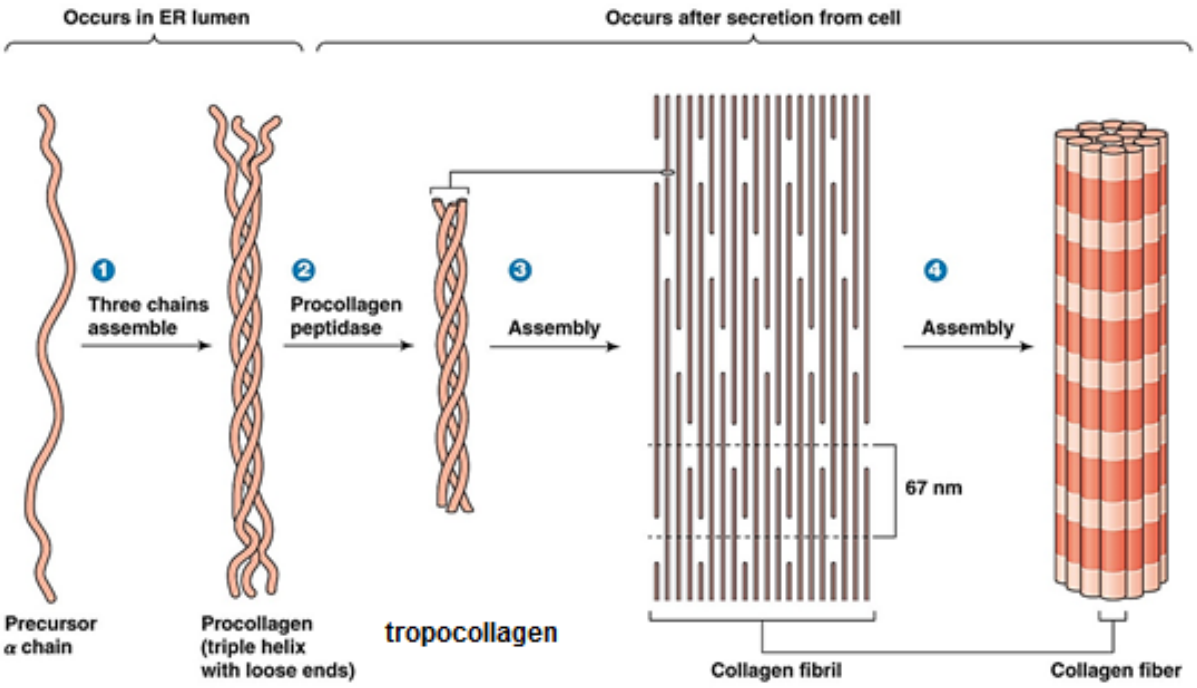


Fig. 5.23: The banded pattern of the collagen fibre is a result of the staggered nature in which the tropocollagen is packed.

Relating Structure to Function (Collagen)

	Structural Features	Contributes to Property	Suited for function to provide **STRUCTURAL SUPPORT because:**
1	**i. Within tropocollagen:** • **Repeating sequence** of **glycine-proline-X** (X: other amino acids except glycine) **or glycine-proline-hydroxyproline** within each polypeptide chain. • Thus, **every 3rd amino acid** is **glycine**, which is **small.** • Allows **close packing** of 3 polypeptide chains to form a **tightly coiled triple helix.** • The three polypeptide chains in the triple helix are linked together by **hydrogen bonds** between **peptide N-H group** of glycine and **peptide C=O groups** of other amino acids on the other chains. **ii. Within collagen fibres:** • Many **tropocollagen** molecules **lie parallel and overlap in a staggered manner** to form **fibrils**. • **Covalent cross-links** form between **lysines** and/or **hydroxylysines of parallel tropocollagen molecules** • Collagen **fibrils** bundle to form collagen **fibres**	**High tensile strength**	• Collagen can function as **strong connective tissue** such as **ligaments**, enabling it to **provide stability** to joints.
2	• **Large molecular size** of tropocollagen (each polypeptide chain consists about 1000 amino acid residues). • **Large numbers of hydrophobic amino acids** such as proline and hydroxyproline, which has large hydrophobic R groups that project towards the outside of the triple helix.	**Insoluble in water**	• Allows collagen to **stay in the location** where it is found to serve as structural protein.

Formation and Assembly of collagen fibre in the cell (Fig. 5.24) - FYI

(Recall: secretory pathway)

1. Synthesis of collagen polypeptides occurs at the **ribosomes** on the **rough endoplasmic reticulum.**

2. The collagen polypeptide is then threaded into the **rough endoplasmic reticulum lumen** where the loose winding of polypeptide and hydroxylation (adding of OH groups) of selected prolines and lysines occur. Some glycosylation (addition of saccharides) of selected hydroxylysines occur.

3. ER vesicles carrying the polypeptide bud off from the ER and travel towards the **Golgi apparatus** where glycosylation of selected hydroxylysines occur (most glycosylation occurs in the Golgi Apparatus).

4. Further assembly of 3 collagen polypeptides forms procollagen. Glycine molecule at every 3rd amino acid residue allows close packing of the molecule. This molecule is eventually packaged into a **secretory vesicle** which buds off from the Golgi apparatus.

5. The secretory vesicle moves towards the **cell surface membrane** and fuses with it (exocytosis).

6. Procollagen is thus secreted into the extracellular matrix.

7. At the extracellular matrix, polypeptides are cleaved to remove the N and C terminals to form tropocollagen.

8. Tropocollagen self-assemble into fibrils via cross-linking between lysine and/or hydroxylysine residues and overlapping of staggered ends.

9. Aggregation of collagen fibrils into bundles forms collagen fibres.

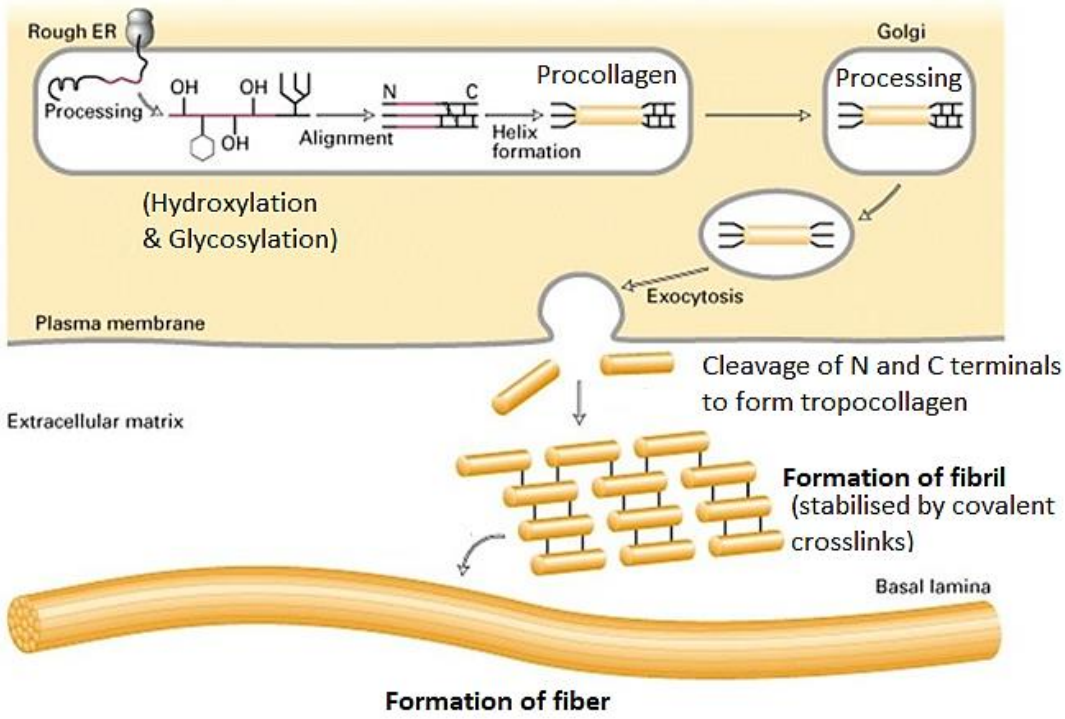


Fig. 5.24: Formation of collagen fibril in the cell and the subsequent assembly of fibrils into fibres in the extracellular matrix.

5.4.3 G-protein linked receptor (Globular Protein, Signalling function)

This will be covered in greater depth in Topic 12 on Communication.

- A G-protein linked receptor (GPLR) (Fig. 5.25a) consists of a **single polypeptide** that is folded into a **globular shape** and embedded in a cell's plasma membrane.
  - GPLR is able to form **hydrophobic interactions** with the phospholipid bilayer as it contains large numbers of non-polar amino acids.

- It is a **plasma membrane protein receptor** with **seven α-helices** spanning the cell membrane and specific loops between the helices that form **binding sites** for (Fig. 5.25a):

  - **Extracellular ligand/signal molecule**
  - **Intracellular G-protein** (a trimeric protein)

- G-protein linked receptor proteins are all remarkably similar in structure. However, these receptors vary in the binding sites for their signalling molecules and also for different types of G proteins inside the cell.
  - This is due to specific amino acids that form the binding sites on different GPLR.

- G-protein linked receptors are a large family of cell surface receptors that respond to a variety of external signals. When an external signalling molecule binds to a receptor, it causes a conformational change in the receptor. This change then triggers the interaction between the receptor and a nearby G protein (Fig. 5.25b). G-protein linked receptor-based signalling systems are extremely widespread and diverse in their functions, including roles in embryonic development and sensory reception (*linked to the topic of "Cell Signalling"*).

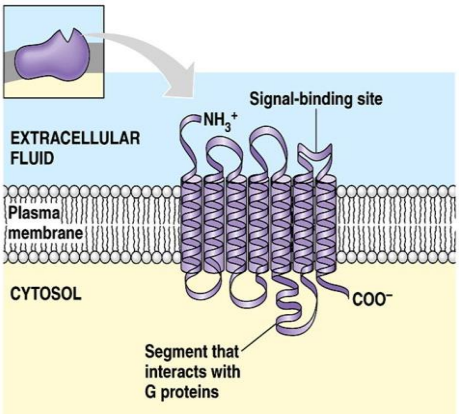


Fig. 5.25a: Structure of the G-protein-linked receptor

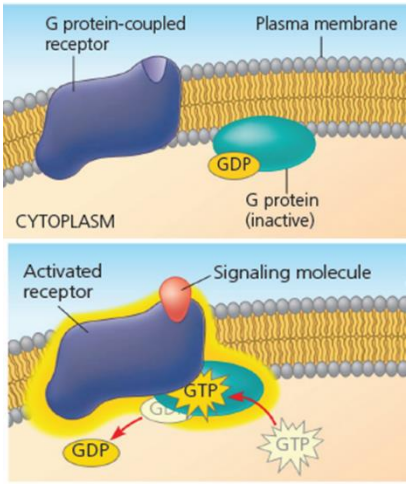


Fig. 5.25b: Activation of The G-protein-linked receptor

5.5 Enzymes

- One of the key features to the success of life on Earth was the development and the complexity of enzymes.

- Enzymes allowed **biochemical reactions** in most cells to **take place speedily** and **within a modest temperature range** (i.e. 0 – 50 ^{0}C). A few organisms (mainly bacteria) exist in extreme conditions outside this range.

- Enzymes act as **highly specific biological catalysts** that **speed up the rate of metabolic reactions**. ***(=> Function: Catalysis*)**

- Enzymes often **work consecutively to catalyse a chain of reactions**. In such a chain, the **product of one enzyme-catalysed reaction** becomes the **substrate** for the **next enzyme in the sequence**, and so on. Hence, enzymes of a cell play a part in the **control of metabolic pathways.** (**=> *Function: Regulation***)

5.5.1 Metabolic reactions and pathways in a cell

- **Metabolism** is the term to describe all the chemical reactions occurring in living organisms.

- A metabolic pathway begins with a specific molecule, which is then altered in a series of **defined steps**, resulting in a certain product. A specific enzyme (Fig.5.26) catalyses each step of the pathway.

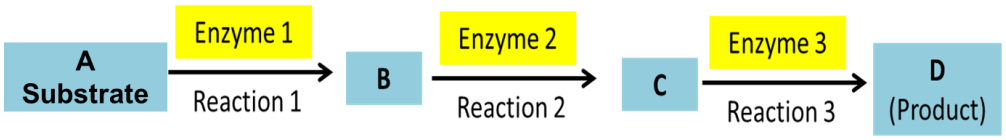


Fig. 5.26: A metabolic pathway comprising multiple smaller reactions

- Metabolic reactions are not performed in a single reaction. Instead, a **series of chemical reactions** constitute a metabolic pathway. Each reaction produces an **intermediate**, which is then used as a **substrate** for the next reaction, until the desired product is produced.

- Metabolic reactions occur in **small steps** because:

  - Large catabolic reactions would create unfavourable conditions, such as production of excessive heat, which is detrimental to the cells.

  - Energy derived from small catabolic reactions can be used more efficiently.

  - Substances can be partially broken down to provide raw materials for other reactions.

  - It allows the cell to **control** the **rate of reactions** and products made.

- A living system's **free energy** is energy that can do work under cellular conditions. The change in free energy (ΔG) during a biological process is related directly to enthalpy change (ΔH) and to the change in entropy (ΔS): $\Delta G=\Delta H-\Delta S$.

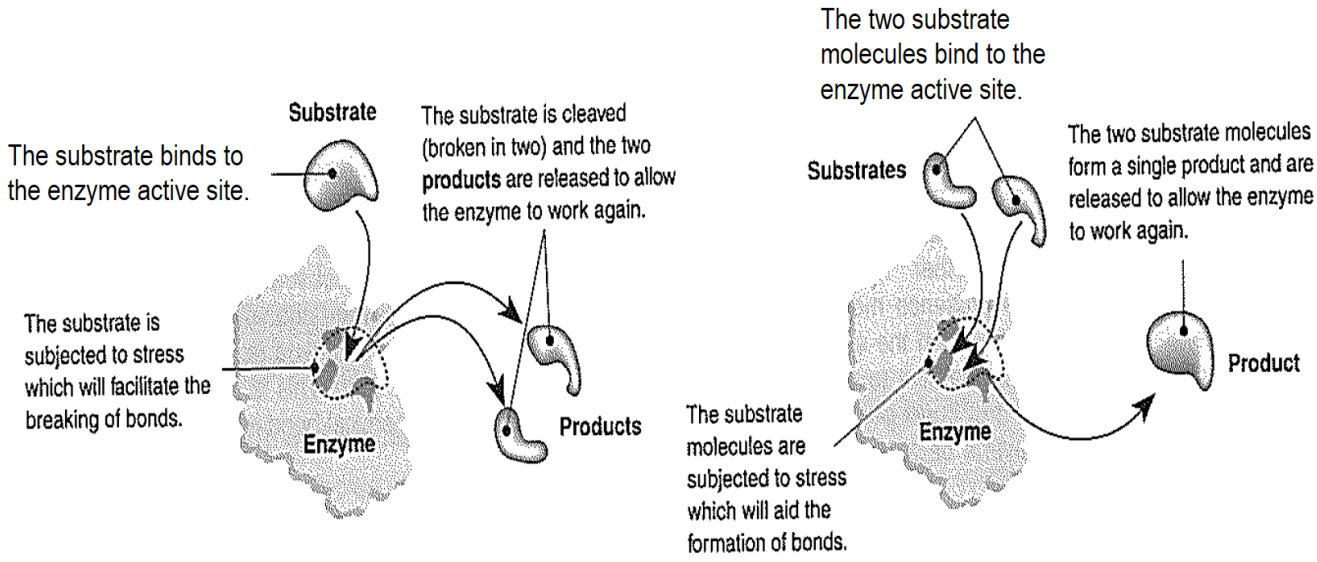
- **Two types** of metabolic reactions/pathways occur within the cell (Fig. 5.27 & 5.28):

  1) **Catabolic reactions/pathways** → involved in the **breakdown** of complex compounds into simpler molecules.

     - Such reactions **generally release energy** and are involved with using food stores and making energy available in cells.
     - These reactions are called exergonic reactions, and the products have less free energy than the reactants (-ΔG).

  2) **Anabolic reactions/pathways** → involved in the **synthesis** of complex compounds from simple molecules.

     - Such reactions **generally require energy** and are involved in the building up of structures, storage compounds and complex metabolites in the cell.
     - These reactions are called endergonic reactions, and they require an input of energy (+ΔG).



Catabolic reactions

Some enzymes can cause a single substrate molecule binds to the enzyme active site. Chemical bonds are broken, causing the substrate molecule to break apart to become two separate molecules.
Examples: *Hydrolysis of starch to maltose by amylase*

Anabolic reactions

Some enzymes can cause two substrate molecules to bind to the enzyme active site. Chemical bonds are formed, causing the two substrate molecule to form bonds and become a single molecule.
Examples: *protein synthesis*

Fig. 5.27: Catabolic (left) and anabolic (right) reactions.

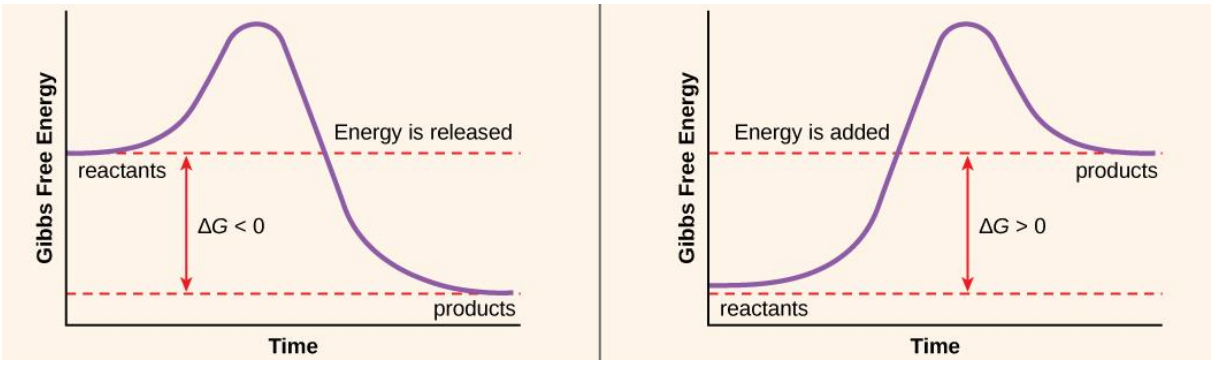


Fig. 5.28: Energy profile of a catabolic (left) and anabolic (right) reaction.

Activation energy

- During chemical reactions between molecules, chemical bonds are either broken or formed.
- However, before bonds are broken, reactant molecules must first absorb energy from its surroundings to contort to an unstable transition state, after which they can then form products. This initial investment of energy for starting a reaction is the **activation energy**.
- **Activation energy** is the **initial energy barrier that needs to be overcome** to start a chemical reaction to form products (Fig.5.29).
- Activation energy is the difference between the maximum energy (where reactants are at their transition state) and the energy of the reactants.
- This energy barrier can be overcome by heating, but…
  - High temperature would denature proteins and kill the cells.
  - Heat will speed up all reactions, not just those that are needed.
- Enzymes can overcome the energy barrier.
  - **Enzymes lower the activation energy** of chemical reactions (Fig.5.30) without denaturing proteins and killing the cells, thus
  - Speeding up the rate of reaction of any **specific** reaction.

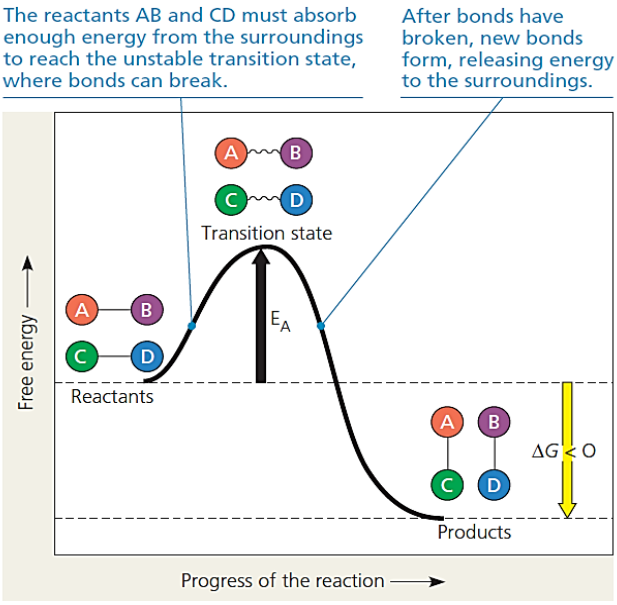


Fig. 5.29: Energy profile of a chemical reaction showing its activation energy (E_A)

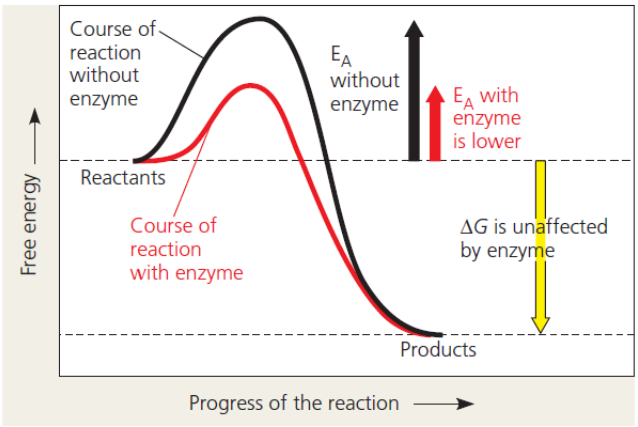


Fig. 5.30: Enzymes lower the activation energy (E_A) of a chemical reaction

5.5.2 What are enzymes?

Key Concept:

- Enzymes are **biological catalysts**, which **speed up the rate of chemical reactions** by **lowering the activation energy**.

Properties of Enzymes

a) Enzymes **speed up the rate of chemical reactions.**

- by **lowering the activation energy** (*Section 5.5.1*)

b) They are **highly specific**.

- The **specific 3D conformation of the enzyme** defines the **specific shape of its active site** which acts on specific substrate.
- Specific **shape of the active site** is **complementary to** the **shape of the substrate**

c) They **remain chemically unchanged** at the end of the reaction and can be **reused**.

- Hence, enzymes are only **required in small quantities**.

d) **Most** enzymes are **globular proteins**.

- However, some RNA molecules have enzymatic activity and are known as **ribozymes**.
- E.g. peptidyl transferase found in ribosomes, which functions to form peptide bonds between adjacent amino acids during translation (*refer to Gene expression*) is made up of rRNA.

Structural features of an enzyme molecule

- Enzymes are **large globular proteins** with **tertiary** (Fig. 5.31 left) and/or **quaternary** (Fig. 5.31 right) structures.

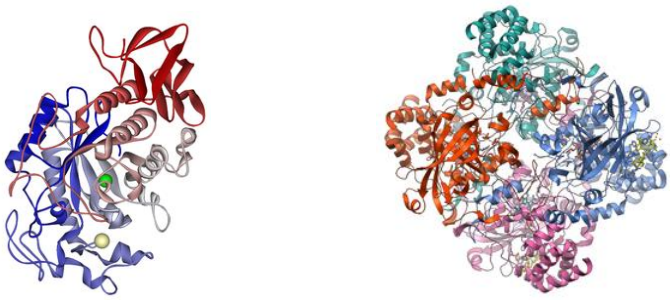


Fig. 5.31: Enzymes with tertiary structure (Left) and quaternary structure (Right)

- Enzymes are **macromolecules** with **specific overall 3-dimensional conformations**.

What determines the specific 3-dimensional conformation of the enzyme?

- A small portion of enzyme (about 3-12 amino acids) makes up the **active site**. The active site has **specific shape** that is **complementary to the shape** of its **substrate molecule**.

- The **active site** consists of **contact** and **catalytic residues** (Fig. 5.32), which are directly involved in enzyme action.

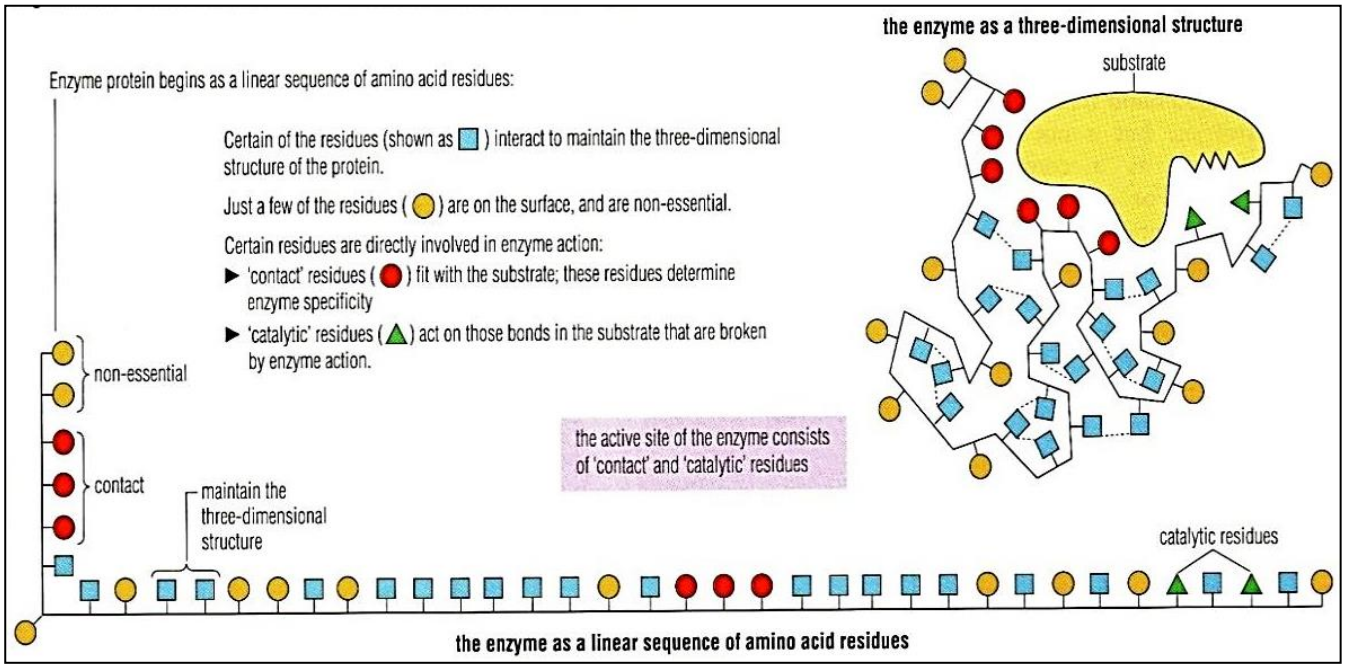


Fig. 5.32: Contact and catalytic residues at a protein's primary and tertiary structure

- Koshland (1963) suggested that an enzyme consists of essentially 4 categories of amino acids.

1. **Catalytic residues (catalytic site)** – these **make** and **break chemical bonds within the substrate(s) to form products.**

2. **Contact residues (binding site)** – these **recognise and bind** with the substrate and **hold the substrate in place** while catalysis is taking place.

   Take note:
   - The **catalytic** and **contact residues** together form its **active site**.
   - Active site is always found in a **cleft** or crevice in the **enzyme structure**.
   - The active site determines the **catalytic properties** and **specificity** of an enzyme.

3. **Structural residues** – these hold the active site in the correct shape so that it can function properly.

4. **Non-essential residues** – these have no specific function. They are often near the surface of an enzyme and can be removed or replaced without loss of function.

   - The **structural** and **non-essential residues maintain the specific globular structure** of the enzyme via **hydrogen bonds, ionic bonds, disulfide bonds and hydrophobic interactions between the R groups** of amino acids in the enzyme.

Explain how amino acids that are many residues away from each other in a polypeptide can form the active site.

How will (i) charged/polar amino acids and (ii) uncharged/non-polar amino acids be positioned in the enzyme molecule?

What is its implication in enzyme-substrate interaction?

For your information (No need to memorise)

- Enzymes are normally named by adding suffix "-**ase**" to the name of substrate on which it acts on or the reaction it catalyse.

- For example:
  - An enzyme that hydrolyses proteins is called protease.
  - An enzyme that hydrolyses lipids is called lipase.
  - Generally, enzymes can be divided into 6 categories according to the chemical reaction which they catalyse (note: these 6 groups can be further subdivided into smaller groups). (*Table 5.1*)

Enzyme	Reaction catalysed
Oxidoreductases	These enzymes catalyse biological oxidation and reduction by the transfer of hydrogen, oxygen or electrons from one molecule to another. Example: lactic acid + NAD $\xrightarrow{\textit{lactase dehydrogenase}}$ pyruvic acid + NADH + H^+
Transferases	These enzymes catalyse the transfer of a chemical group from one substrate to another. Example: glutamic acid + pyruvic acid $\xrightarrow{\textit{aminotransferase}}$ α-ketoglutaric acid + alanine (amino acid) (organic acid) (organic acid) (amino acid)
Hydrolases	These enzymes catalyse the formation of two products from a larger substrate molecule by a hydrolysis reaction. Example: sucrose + H_2O $\xrightarrow{\textit{sucrase}}$ glucose + fructose
Lyases	These enzymes catalyse the non-hydrolytic removal (or addition) of parts of a substrate molecule. Example: pyruvic acid $\xrightarrow{\textit{pyruvate decarboxylase}}$ ethanol + CO_2
Isomerases	These enzymes catalyse the internal rearrangement of molecules. Example: glucose-1-phosphate $\xrightarrow{\textit{phosphoglucomutase}}$ glucose-6-phosphate
Ligases	These enzymes catalyse the joining of two molecules with the simultaneous hydrolysis of ATP. Example: amino acid + specific tRNA + ATP $\xrightarrow{\textit{synthetase}}$ amino acid-tRNA complex + ADP + P_i

Table 5.1: Classification of enzymes by type of reactions

5.5.3 Mode of action of enzymes

(a) Mechanism of enzyme action

Key Concept:
- The **specificity** of enzyme action can be described using the ***lock and key hypothesis*** and the ***induced fit hypothesis***.
- **Substrate** binds at the **active site** of the **enzyme** and forms the **enzyme-substrate complex**.
- R groups of the amino acids, at the active site, **lower the activation energy barrier**.

- The specificity of enzyme action can be described using the

  1) **lock and key hypothesis** and
  2) **Induced fit hypothesis**.

Lock and key hypothesis (Fig. 5.34)

- First proposed by Emil Fischer in 1894.
- According to the hypothesis, the enzyme molecule has a fixed structure. The **specific substrate** molecule has a **shape that is complementary to the specific shape of the active site** of the enzyme and **fits exactly** into the active site.
- The substrate is imagined as the 'key', whose shape is complementary to the enzyme, which is the 'lock'.
- **Effective collision** of specific substrate (key) and active site of enzyme (lock) at their **correct orientation** forms the **enzyme-substrate complex**. This complex is **stabilised by ionic and hydrogen bonds** between the **contact residues** of the enzyme and substrate molecule.
- **Catalytic residues** catalyse the reaction by **acting on specific bonds within the substrate** to form products.
  - The R-groups of the catalytic residues are positioned in such a way that enables them to interact with and act on the bonds within the substrate molecules (Fig. 5.33).
  - Regions of the substrate molecule where bonds are to be broken or formed are positioned exactly with the catalytic R-groups at the active site of the enzymes.
  - The hydrogen and ionic bonds between the substrate and catalytic R-groups weaken the bonds within the substrate, causing the bonds within the substrate to break.
  - This lowers the activation energy barrier for the reaction to proceed. (Fig. 5.35)

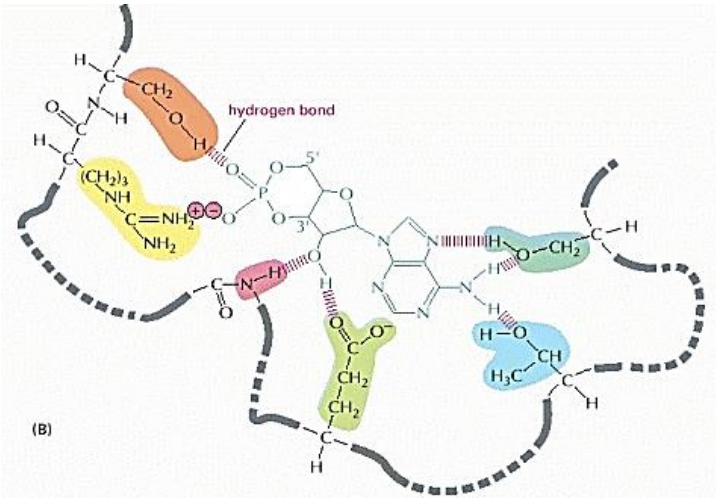


Fig. 5.33: The binding site of a protein, with catalytic R-groups of the active site shaded.

- When the reaction is completed, the **shape of the products** formed is **no longer complementary to the active site**. The products will then be released, freeing the active site for another substrate molecule.

- The **chemically unchanged** enzyme molecule is free to catalyse another reaction.

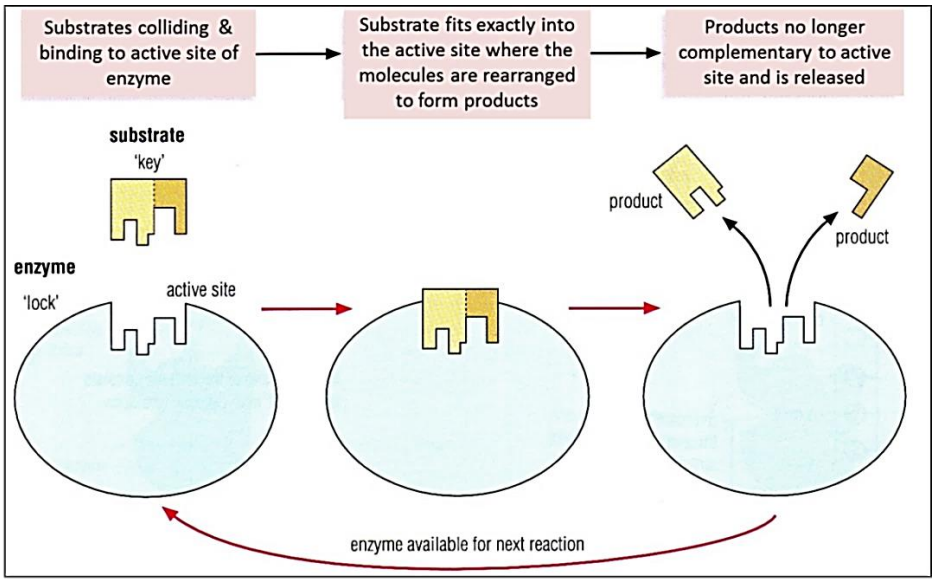


Fig. 5.34: Lock-and-key hypothesis for mechanism of enzyme action

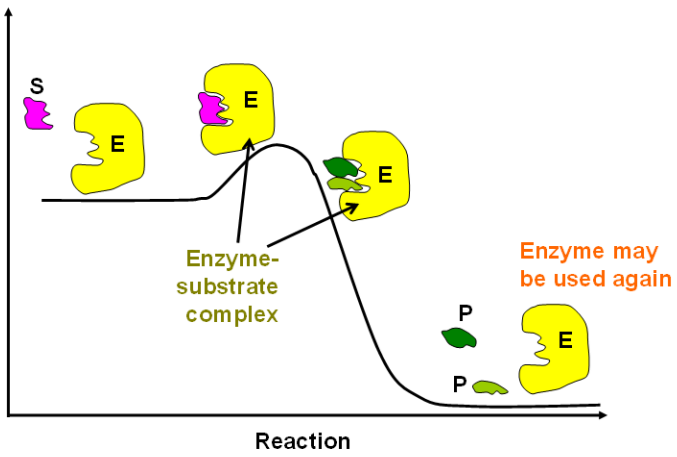


Fig. 5.35: Energy profile of an enzyme catalysed biochemical reaction incorporating the lock-and-key hypothesis

Induced Fit hypothesis (Fig. 5.36a and 5.36b)

- Proposed by Daniel Koshland in 1958, this is an improved hypothesis based on evidence that suggests that the structure of enzymes/proteins is flexible.
- This hypothesis suggested that the **active site** of the enzyme, while **still complementary to the substrate**, **may not necessarily have an exact shape** that fits the substrate from the start.
- Instead, the binding of substrate to the active site **induces a small <u>conformational change</u> in the enzyme**, which enables
    - The **substrate to fit more snugly into its active site** by forming bonds with contact residues.
    - The enzyme to carry out its **catalytic function more effectively** by catalytic residues.

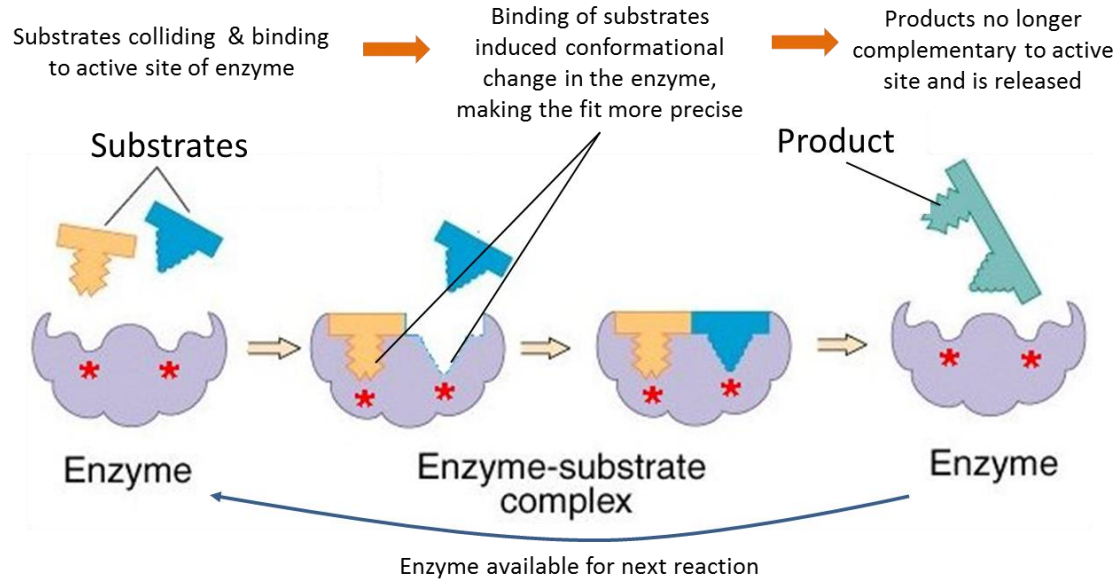


Fig. 5.36a: Induced-fit hypothesis of mechanism of enzyme action

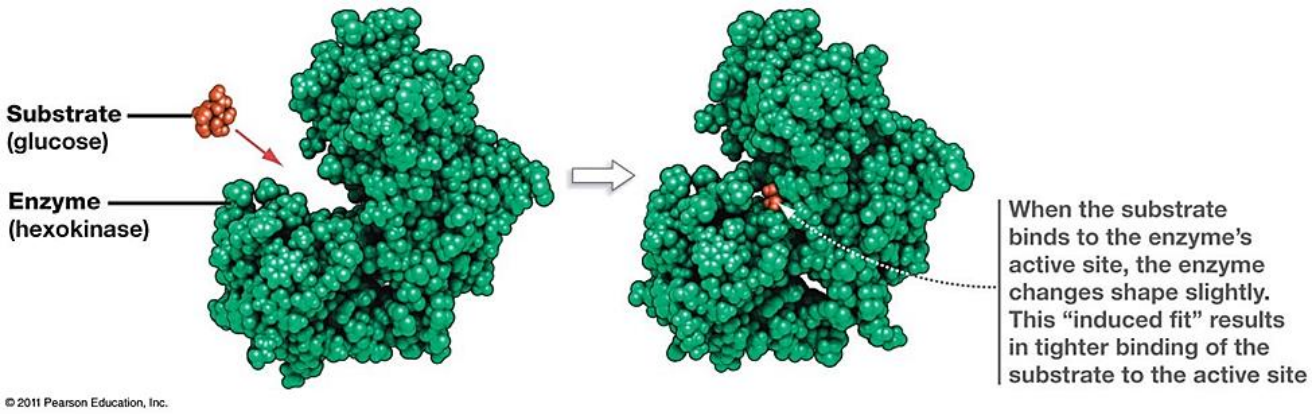


Fig. 5.36b: Hexokinase as an example for the induced-fit hypothesis

(b) How enzymes lower the activation energy of a reaction

- Enzymes are able to lower the activation energy of the reaction when they (*Fig. 5.37*):

  a) Hold their substrates in an arrangement that forces and **maintains the precise orientation of the substrates** at the **active site** for the anabolic/catabolic reaction to occur.

  - The precise orientation of the substrate in the active site of the enzyme ensures that the bonds are **exposed to the catalytic residues**.

  b) **Stress/Strain the bonds** within the substrates to **distort the substrate molecules** (for catabolic reactions).

  - This increases the likelihood of breaking the bonds

  c) **Increase the substrate reactivity** for anabolic/catabolic reactions.

  - When the R-groups of the amino acids of the enzyme are in close proximity to part of the substrate, they can:
    - **Change the charges** of the **substrate**
    - **Alter the distribution of the electrons within the bonds** of the substrate
    - Cause other **chemical changes**.

  d) Provide a **favourable microenvironment** for catalysis. For example, hydrophobic amino acids create a water-free zone in which non-polar reactants may react more easily.

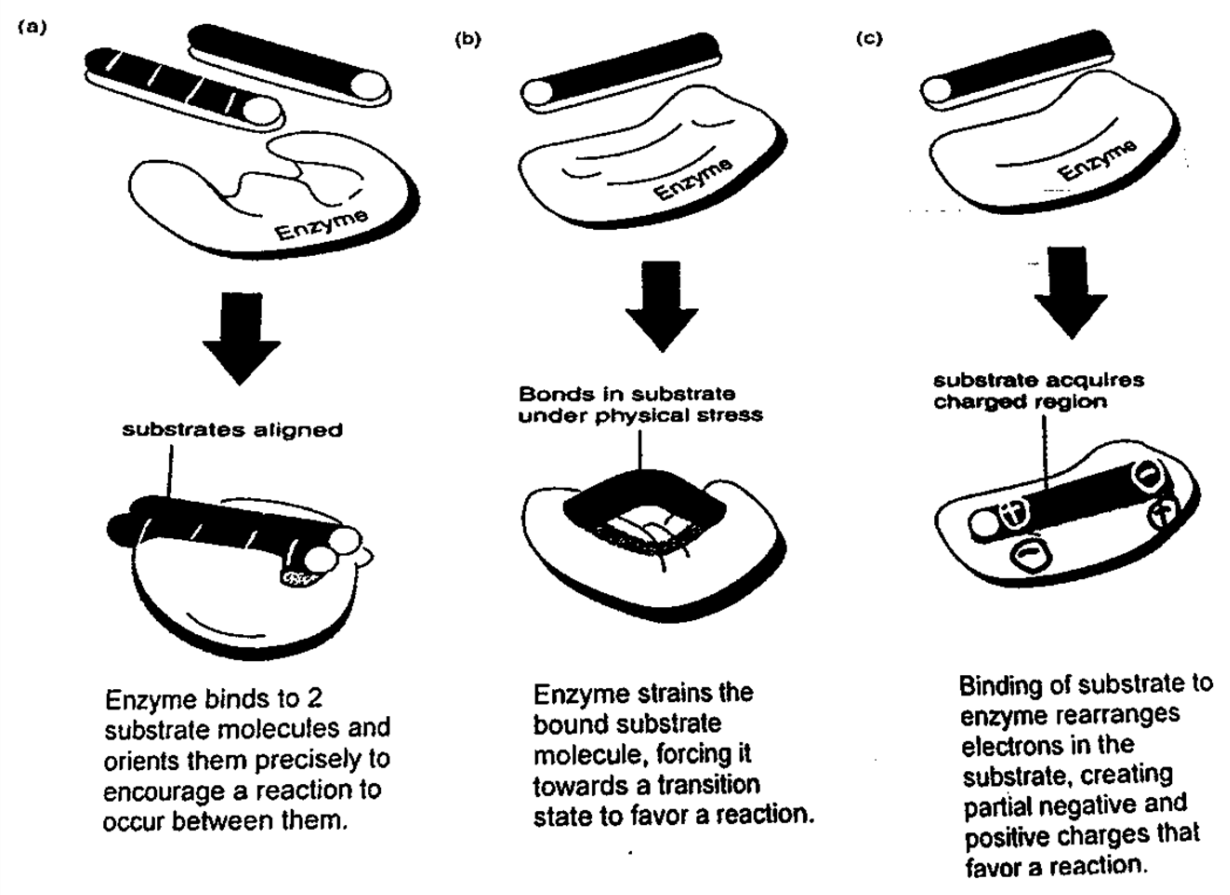


Fig. 5.37: Some general strategies by enzymes that lower the activation energy of a reaction

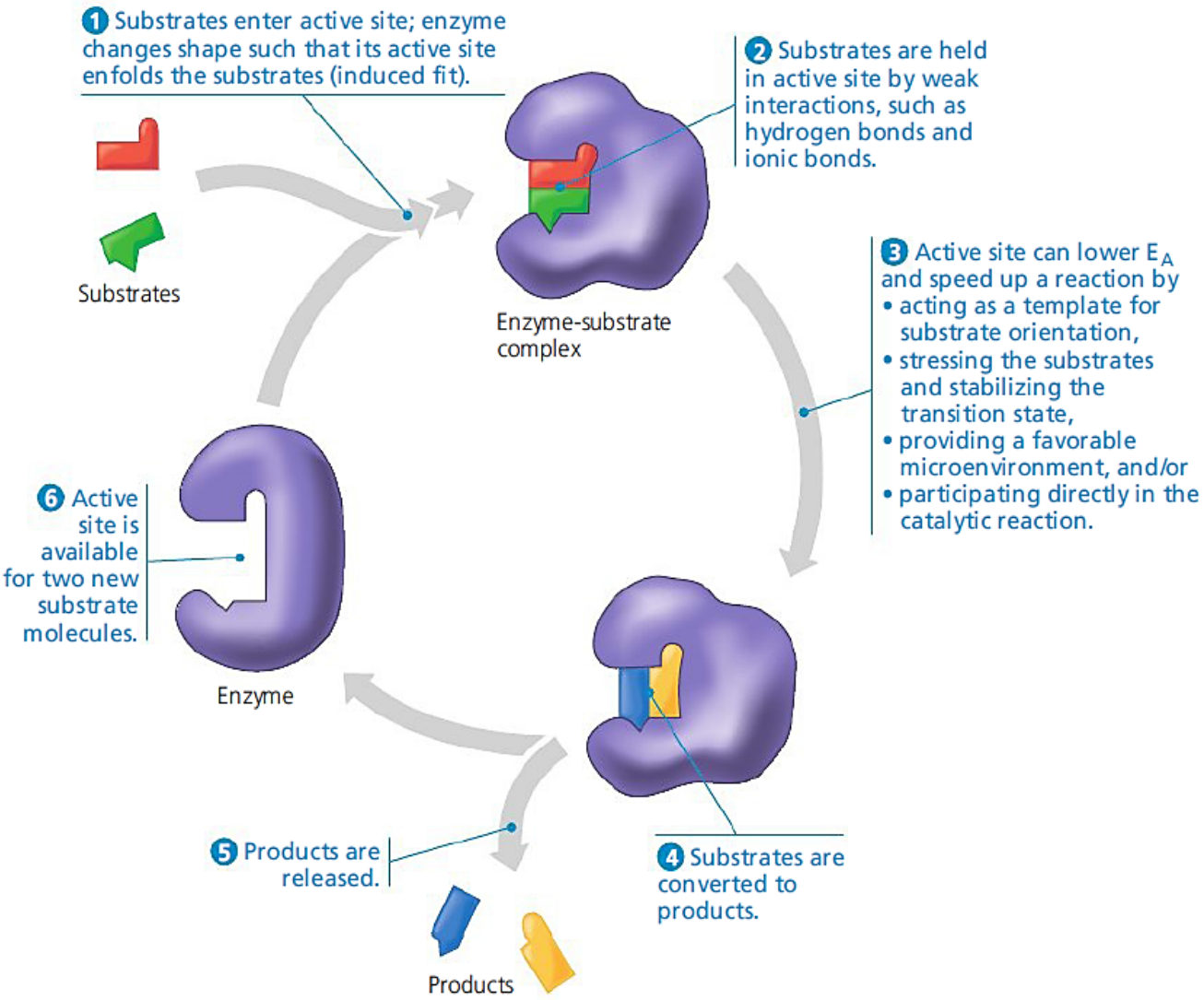


Fig. 5.38: The catalytic cycle of an enzyme

5.5.4 Measuring the rate of an enzyme-catalysed reaction

Key Concept:

- The **rate of an enzyme-catalysed reaction** can be determined by the **rate of formation of products** or the **rate of disappearance/change of substrate**.

- In a reaction with **fixed conditions and fixed substrate and enzyme concentrations**, the rate of reaction is the greatest initially. As the reaction progresses with time, the rate of reaction will slow down and eventually reach zero when all the substrates are changed into products (Fig. 5.39).

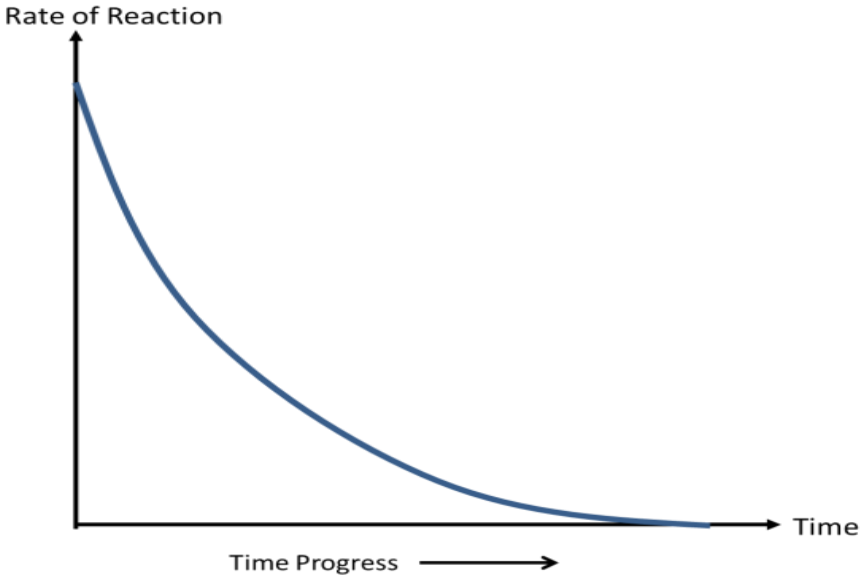


Fig 5.39: The rate of reaction as time progresses in a chemical reaction

- Consider this reaction: **A + B → C + D**

- As the reaction progresses, the concentration of products C and D will increase, while the concentration of substrates A and B will decrease.

- The rate of an enzymatic reaction can therefore be determined by

  1) **rate of formation of products** OR
  2) **rate of disappearance/change of substrate**

To calculate rate of reaction:

$$\text{Rate of reaction} = \frac{\text{amount of reactant used or amount of product formed}}{\text{Time taken}}$$

Measuring rate of reaction by rate of formation of products

- E.g. **Decomposition of hydrogen peroxide by catalase**

- **Hydrogen peroxide** (H_2O_2) is a **toxic by-product** produced by living organisms.

- **Catalase** is an enzyme found in living organisms that **catalyses the decomposition** of hydrogen peroxide into **water and oxygen gas**:

$$2H_2O_2\ (aq) \rightarrow 2H_2O\ (l) + O_2\ (g)$$

- The rate of reaction can be determined by measuring the **rate of formation of oxygen** (product) through:

  - Measuring the **volume of oxygen gas produced** using a gas syringe over a fixed time period; or

$$\text{Rate of oxygen formation } (cm^3\ s^{-1}) = \frac{\text{Volume of oxygen produced } (cm^3)}{\text{Time interval (s)}}$$

  - Measuring the **volume of water displaced by oxygen gas** over a fixed time period; or

$$\text{Rate of oxygen formation } (cm^3\ s^{-1}) = \frac{\text{Volume of oxygen produced } (cm^3)}{\text{Time interval (s)}}$$

  - Counting the **number of O_2 bubbles** formed over a fixed time period.

$$\text{Rate of oxygen formation } (s^{-1}) = \frac{\text{No of } O_2 \text{ bubbles produced}}{\text{Time interval (s)}}$$

- Example of how rate of formation of oxygen can be measured by volume of oxygen gas produced over fixed period of time. (Fig. 5.40)

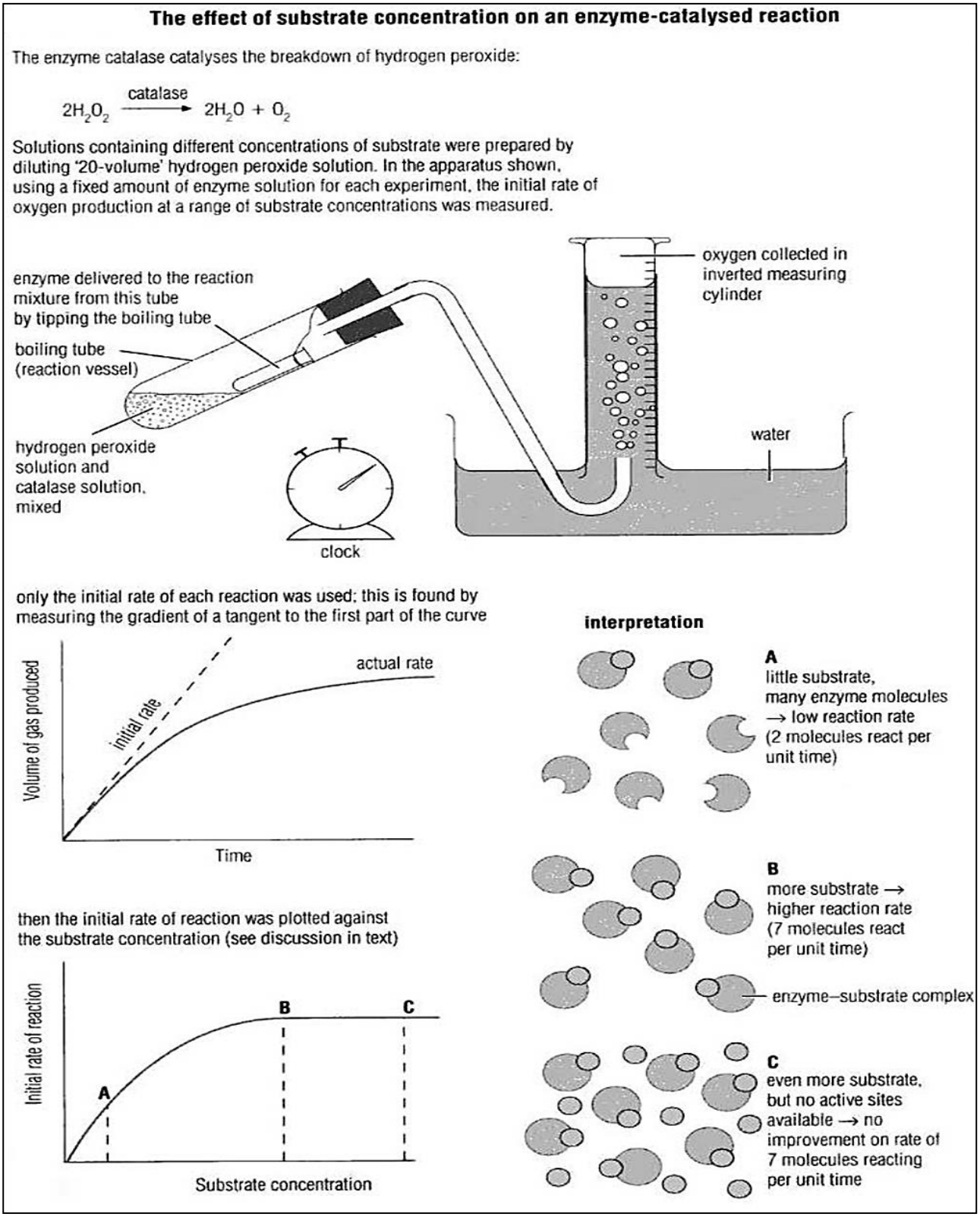


Fig.5.40: The rate of reaction measured by volume of oxygen gas produced over a period of time

Measuring rate of reaction by rate of disappearance/change of substrate

E.g. **Hydrolysis of starch to maltose by amylase**

- Amylase is an enzyme commonly found in saliva and germinating seeds, which catalyses the hydrolysis of starch (substrate) to form maltose (product).

Starch → Maltose

- The rate of reaction can be determined by measuring the **rate of disappearance of starch** using the **iodine test**.
    - The test for starch consists of adding a drop of potassium iodide (iodine) solution to the sample on a spotting tile. **Iodine forms complex with the starch present** to result in a **blue-black solution**.
    - The **deeper the colour,** the **greater the concentration of starch**.
    - As the reaction progress, less starch will be present, while the concentration of maltose increases. As the concentration of **starch decreases**, the iodine test will show a **progressively lighter blue colour**. When all the starch present is digested, the solution will remain **yellow** with potassium iodide solution.
    - Therefore, rate of reaction (s^{-1}) = $\frac{1}{\text{Time taken for colour change (s)}}$
- Results of the starch test can be quantified via:
    - **Colour standards of different known concentrations of starch solution** to estimate the concentration of starch left unhydrolysed at different time intervals; or
    - **Spectrophotometry** whereby the amount of light of a specific wavelength passing through (light transmission) increases as the concentration of unhydrolysed starch (which prevents light from passing through) decreases over time.
- Example of how rate of disappearance of starch (substrate) can be measured (*Fig. 5.41*).

Think!
*The rate of hydrolysis of starch to maltose can also be measured by **rate of production of maltose**. How is it possible?*

Why is this not the preferred method to measure the rate of the above reaction?

Fig. 5.41: The rate of reaction measured by disappearance of product (starch)

5.5.5 Factors affecting the rate of enzymatic reaction

- When a biochemical reaction is affected by more than one factor, the **rate is limited by the factor that is nearest to its minimum value**. This factor is known as the **limiting factor**.
- Any change in the concentration of the limiting factor will directly affect the rate of the biochemical reaction.
- Rate of enzymatic reaction can be affected by **substrate concentration, enzyme concentration, pH and temperature.**

DO YOU KNOW?

- In any experiment, one variable is usually changed to find out how it affects another variable, while keeping all the **other variables constant**.
- The **variable that is changed** is commonly referred to as the **independent variable** which is plotted on the **x-axis**.
- The **variable that is measured** as a result of the independent variable is referred to as the **dependent variable** which is plotted on the **y-axis**.

(a) Effects of substrate concentration (Fig. 5.42)

*** To study the effect of substrate concentration on enzyme activity, **all other factors are assumed to be constant (fixed enzyme concentration, constant pH and temperature).** ***

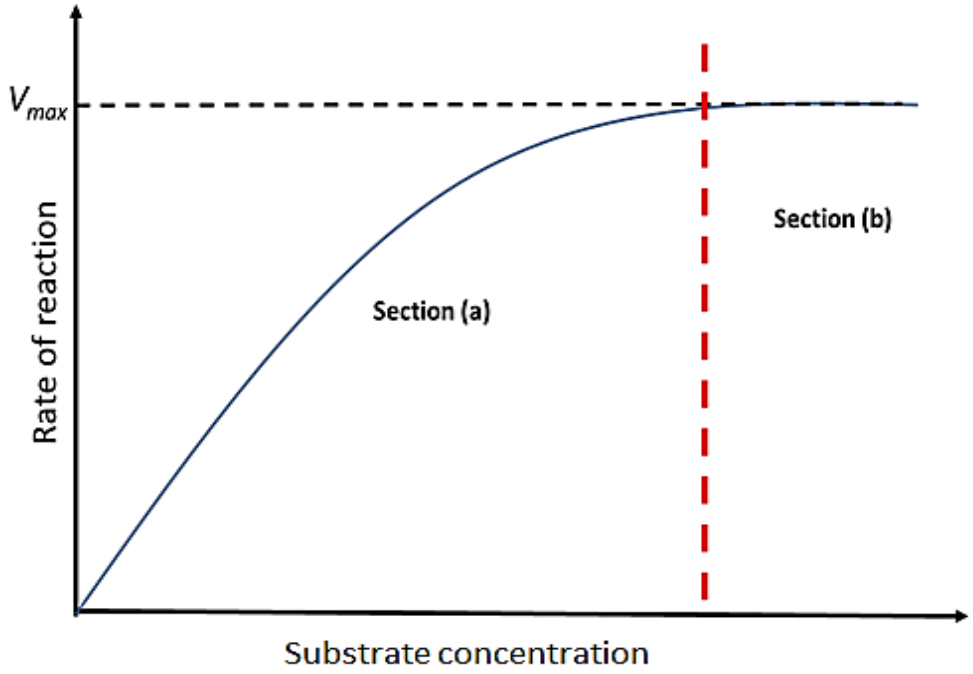


Fig. 5.42: Graph for effect of substrate concentration on rate of reaction

- As **substrate concentration increases**, the **rate of enzymatic reaction increases proportionally** [Fig. 5.42 section (a)]
    - *The **frequency of effective collisions between enzyme and substrate molecules increases**.*
    - *This results in an **increase in rate of formation of enzyme-substrate complexes** and hence **increases the rate of products formed**.*
    - *The rate of reaction is limited by **substrate concentration*** hence, substrate concentration is the **limiting factor**.

- *As substrate concentration increases beyond a point,* the ***rate of reaction*** will *plateau at **maximum rate of reaction** (**V_{max}**)* [Fig. 5.42 section (b)] , where
    - ***All active sites of all enzyme molecules are <u>saturated</u>** with substrate molecules.*
    - *Any free substrate molecules will have to wait until the product is released before it can bind to the active site.*
    - *The rate of reaction is now limited by **enzyme concentration** i.e. enzyme concentration is the limiting factor.*

(b) Effects of enzyme concentration (Fig. 5.43)

*** To study the effect of enzyme concentration on enzyme activity, **all other factors are assumed to be constant (fixed substrate concentration, constant pH and temperature).** ***

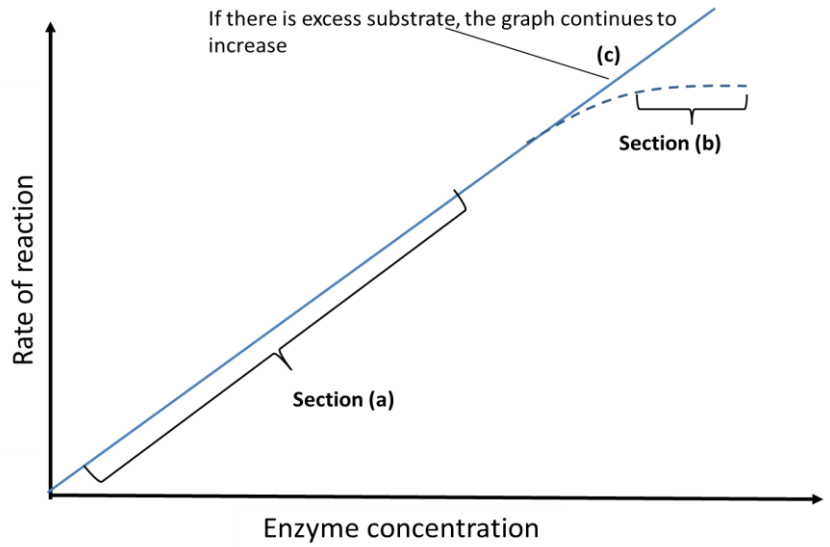


Fig. 5.43: Graph for effect of enzyme concentration on rate of reaction

- As **enzyme concentration increases**, the **rate of enzymatic reaction increases proportionally** [Fig.5.43 section (a)]
    - *The **frequency of effective collisions between enzyme and substrate molecules increases**.*
    - *This results in an **increase in rate of formation of enzyme-substrate complexes** and hence **increases in rate of products formed**.*
    - *The rate of reaction is limited by **enzyme concentration** i.e. enzyme concentration is the **limiting factor**.*

- *As enzyme concentration increases beyond a point,* the ***rate of reaction*** will *plateau at* the ***maximum rate of reaction*** **(V_{max})** [Fig. 5.36 section (b)], where
    - ***All available substrates** are now **bound to active sites** on enzymes.*
    - *Any further increase in enzyme concentration would not result in an increase in the rate of reaction.*
    - *The rate of reaction is now limited by **substrate concentration** i.e. substrate concentration is the **limiting factor**.*
- If there is **unlimited supply of substrates**, the rate of reaction will continue to increase [Fig. 5.43 section (c)].

(c) Effects of pH (Fig. 5.44)

*** To study the effect of pH on enzyme activity, **all other factors are assumed to be constant (fixed substrate concentration, fixed enzyme concentration and constant temperature).** ***

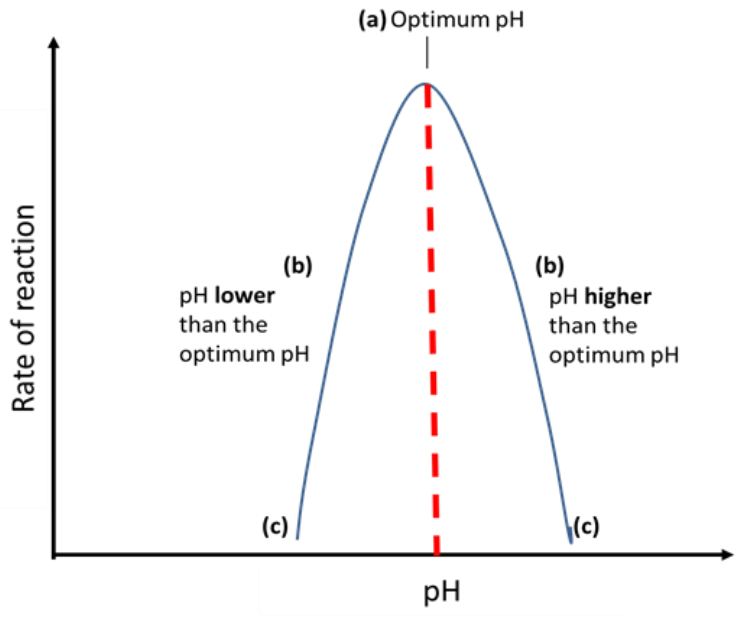


Fig. 5.44: Graph for rate of enzyme-catalysed reaction across a pH range

- At **optimum pH**, **maximum rate of reaction - V_{max}** occurs [Fig.5.44 section (a)]
    - *At this optimum pH, the **bonds which maintain the secondary** and **tertiary structures** of the enzymes are **intact**.*
    - *The conformation of the active site is **most ideal** for substrate binding.*
    - *Hence the **greatest number of enzyme-substrate complexes** is **formed per unit time**. Rate of reaction is at its maximum.*

- If the pH deviates from the optimum, enzyme activity decreases drastically [Fig.5.44 section (b)]
    - *At **pH higher or lower than optimum pH**, the concentration of hydrogen (H^+) and hydroxide (OH^-) ions in the solution would **change**. This results in reduced enzymatic activity as there is:*
        1. Change in **enzyme structure** due to **neutralisation** of the **charged/polar R-groups** of amino acids **in the enzyme**
            - This **disrupts** the **ionic and hydrogen bonds maintaining the secondary, tertiary and/or quaternary structure of the enzyme**.
            - The **specific conformation** of enzyme is disrupted.
            - This results in the **loss of the specific shape of the active site** and result in less effective substrate binding.
            - Less effective formation of enzyme-substrate complexes and hence, less products are formed per unit time.

2. Disruption of **bonds formation between active site of enzyme and substrate** due to **neutralisation** of the **charged/polar catalytic and contact R-groups** of amino acids in the active site of enzyme and charges on the substrate.
    - This **disrupts** the **ionic and hydrogen bonds between the active site of the enzyme and substrate**, resulting in
    - Less effective formation of enzyme-substrate complexes and hence, less products are formed per unit time.

Note: If pH **deviates only slightly** from optimum, the effects are normally **reversible**. Restoring pH to optimum would usually restore the optimum rate of reaction.

- At **extreme pH, no reaction** takes place [Fig.5.44 section (c)].
    - *The conformation of the enzyme is severely altered and is **denatured**. As the active site can no longer bind to the substrate, no enzyme-substrate complexes can form and so, no reaction takes place.*

- Enzymes works in a narrow range of pH. (Fig 5.45).

- **Different enzymes** have **different optimum pH** where rate of reaction is at the maximum (Fig. 5.45).

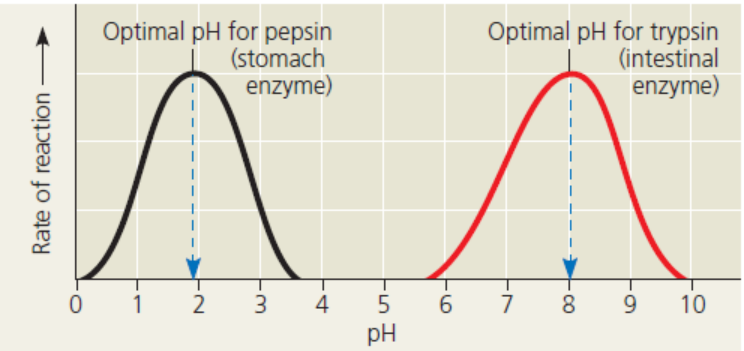


Fig. 5.45: Pepsin and trypsin work best at different pH

Why does a change in pH disrupt only ionic and hydrogen bonds, but not disulfide bonds and hydrophobic interactions between the amino acids?

(d) Effects of temperature (Fig. 5.46)

*** To study the effect of temperature on enzyme activity, **all other factors are assumed to be constant (fixed substrate concentration, fixed enzyme concentration and constant pH).** ***

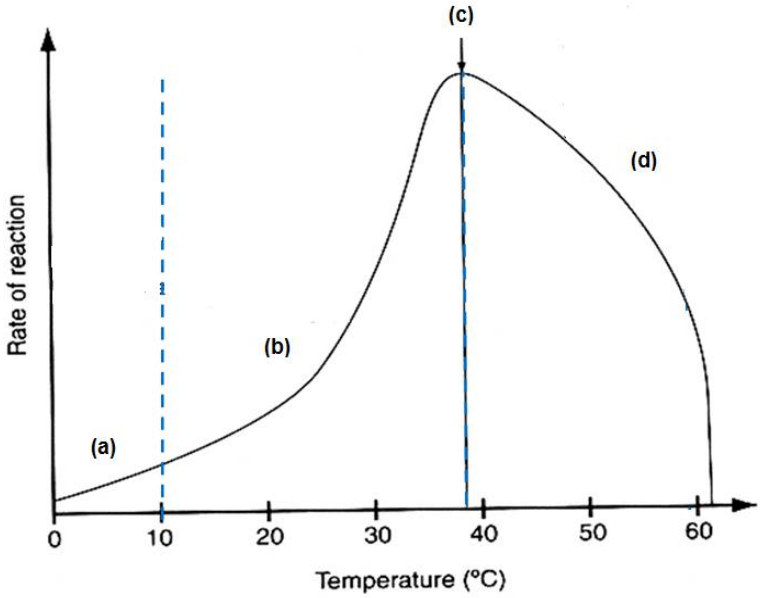


Fig. 5.46: Effect of temperature on enzyme activity

- At **very low temperature,** the **rate of enzyme reaction** is **very low**. [Fig.5.46 section (a)]
    - *The **kinetic energy of both substrate and enzyme molecules is low**, leading to a **low frequency of effective collision between substrate and enzyme molecules**.*
    - *This results in a **low /reduced rate of formation of enzyme-substrate complexes**, and hence **low rate of product formation**.*
    - *The enzymes are **inactivated** but can regain its catalytic ability when temperature is increased.*
- As **temperature increases**, the **rate of reaction increases gradually** till it reaches the optimum temperature ($\mathbf{V_{max}}$). [Fig.5.46 section (b)]
    - *There is an **increase in kinetic energy of both enzyme and substrate molecules**.*
    - *This leads to an **increase in frequency of effective collision between enzyme and substrate molecules**.*
    - *Rate of formation of enzyme-substrate complexes increases**, hence** increased rate of product formation**.***
- As **optimum temperature**, rate of reaction is at **its maximum** ($\mathbf{V_{max}}$). [Fig.5.46 section (c)]

Is kinetic energy of both enzyme and substrate molecules maximum at optimum temperature?

- **Beyond optimum temperature**, **the rate of reaction decreases steeply/sharply**. [Fig.5.46 section (d)]

    - *The excess heat **increases the kinetic energy** of the molecules even further, **leading to disruption of hydrogen bond, ionic bond and hydrophobic interactions** holding the secondary and tertiary structures of the enzymes.*

    - *The enzyme **loses its specific 3D conformation**, and hence **loses the specific shape of the active site**. The enzyme is **denatured.***

    - *The **shape of the active site** is **no longer complementary** to the **substrate**, hence, **rate of formation of enzyme-substrate complex decreases**, decreasing the rate of product formed.*

- Each enzyme has its own **optimum temperature** at which **maximum rate of reaction (V_{max})** occurs. (Fig. 5.47)

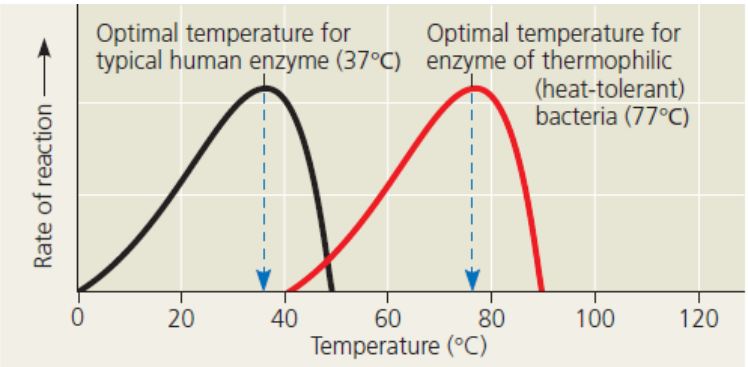


Fig. 5.47: Optimum temperature for two enzymes

- The **effect of temperature on the rate of enzymatic reaction** is expressed as the **temperature coefficient, Q_{10}**.

$$Q_{10} = \frac{\textbf{Rate of reaction at } (x+10)^{\circ}C}{\textbf{Rate of reaction at } x^{\circ}C}$$

    - For most enzymes, the Q_{10} value is approximately **2**. This means that **the rate of reaction doubles for every 10°C increase in temperature**. This Q_{10} value = 2 is only applicable to the temperature range below the optimum temperature.

5.5.6 Enzyme cofactors

- Many enzymes require **non-protein** components called **cofactors** for efficient catalytic activity. Cofactors may vary from simple inorganic ions to complex organic molecules.

- A catalytically active complex comprising the **enzyme and its cofactor** is known as a **holoenzyme**. The inactive enzyme that is not associated with its cofactor is known as **apoenzyme**. (Fig. 5.48)

- Cofactor may be bound to the enzyme tightly and permanently, or loosely and reversibly to the **active site,** or **allosteric site**.

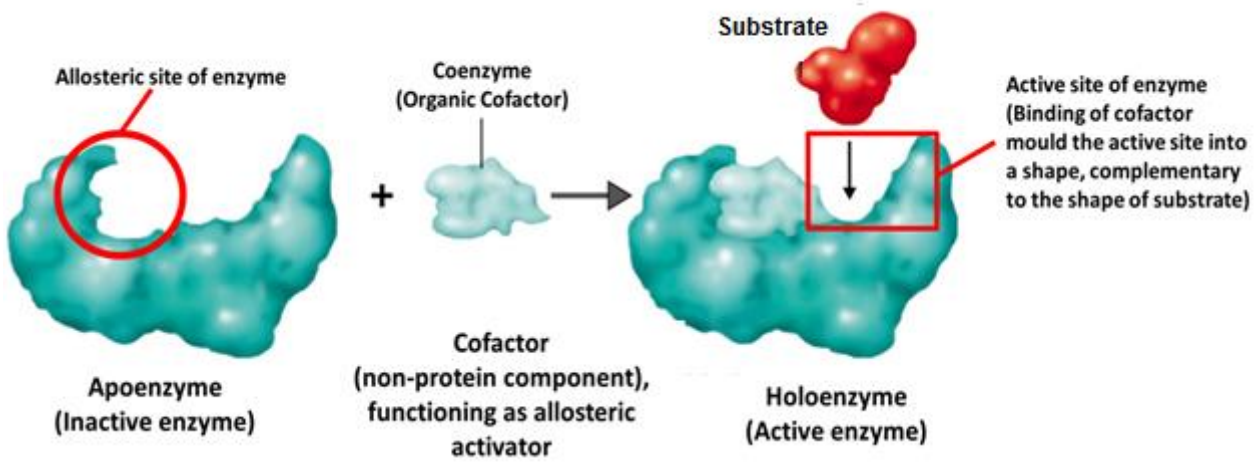


Fig. 5.48: An example of a cofactor binding to an inactive apoenzyme, to mould its active site into shape.

- There are three types of cofactors:

  1) **Inorganic ions** (enzyme activators)

     - They function to **mould the active site** of the enzyme **into shape** complementary to shape of substrate to **facilitate the formation of enzyme-substrate complex**, thereby increasing the rate of reaction catalysed by that particular enzyme (Fig. 5.48).

     - E.g. salivary amylase activity is increased by the presence of chloride ions (Cl^-).

     - E.g. Metal ions such as Zn^{2+} are required for catalytic activity of carboxypeptidase A.

  2) **Prosthetic groups**

     - Can be **inorganic or organic** in nature.

     - These are cofactors that are **tightly and permanently bound to the enzyme**.

     - E.g. Haem is an iron-containing prosthetic group, found in catalase, peroxidase, and in cytochrome *(refer to chapter on Respiration)* to function as an electron carrier.

  3) **Coenzymes**

     - These are **organic cofactors derived from vitamins**.

     - They are **loosely associated** with the enzyme during reaction,

     - E.g.Nicotinamide adenine dinucleotide (NAD) is an important coenzyme in respiration.

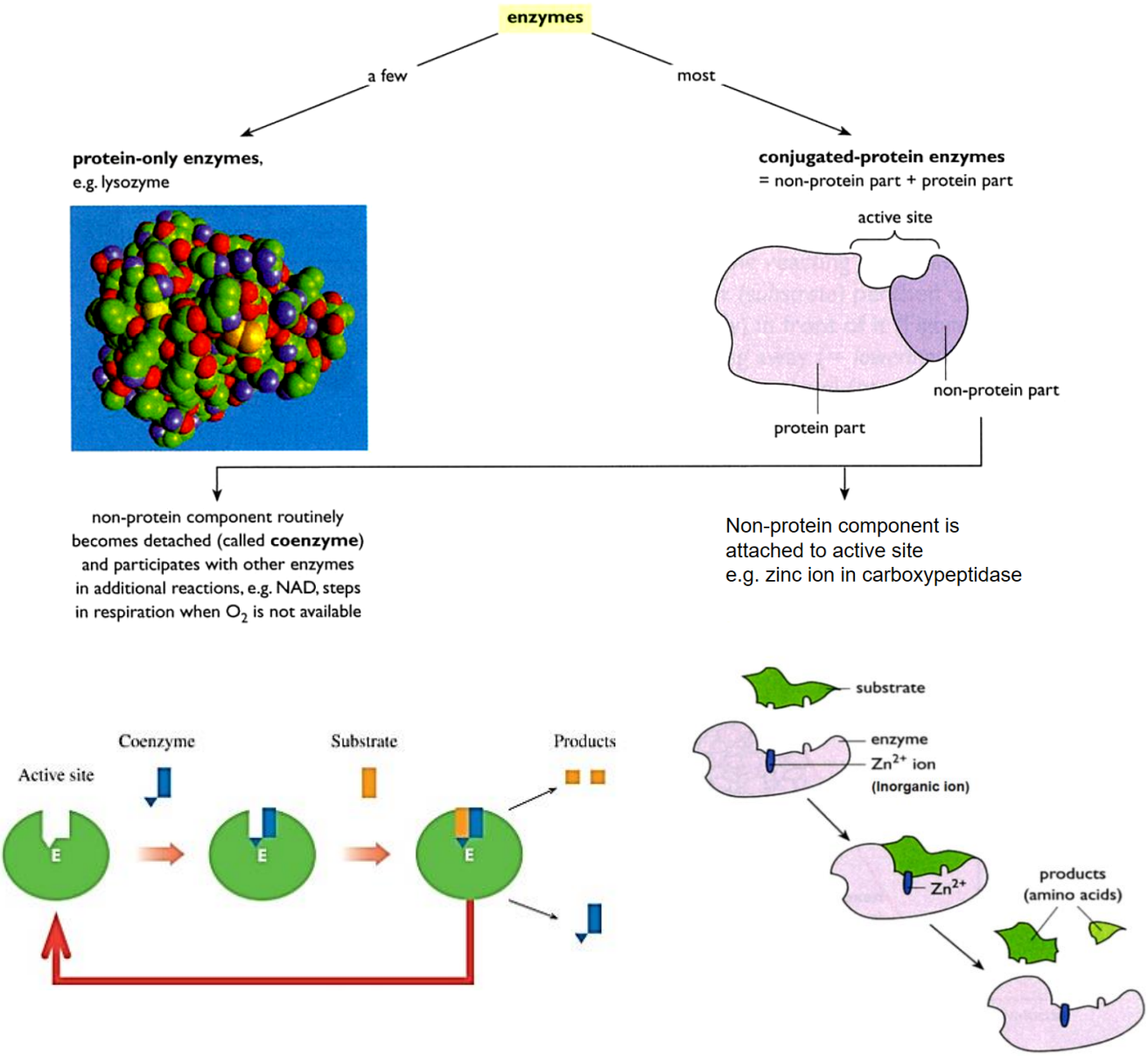


Fig. 5.49: Protein-only and conjugated protein enzymes

5.5.7 Enzyme inhibition

- An **inhibitor** is a molecule that **binds to** and **prevents an enzyme from catalysing** its reaction, and hence **reduces** the rate of reaction.

- The binding of an inhibitor to an enzyme forms an **enzyme-inhibitor complex** which prevents the enzyme from catalysing its reaction.

- The inhibitor may bind to the active site *or* a site away from the active site. There are two main groups of inhibitors:

  1) **Competitive inhibitors** which bind to the **active site** of the enzyme

  2) **Non-competitive inhibitors** which bind to a **site away from the active site**

- The binding of these inhibitors may be a reversible *or* irreversible process:

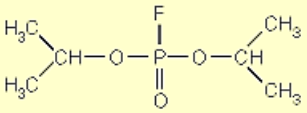
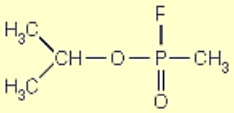
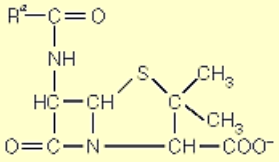
  1) The binding is **reversible** if the inhibitors

     - Have a **relatively loose association** by **hydrogen or ionic bonds** with the enzyme
     - Can **dissociate rapidly** from the enzyme under suitable conditions.

  2) The binding is **irreversible** if the inhibitors

     - **Bind permanently** through **strong covalent bonds** to the enzyme and stop the enzyme's activity

  3) Toxins and poisons are often *irreversible* enzyme inhibitors. Some examples are shown in Table 5.2.

Name	Formula[a]	Source	Mode of Action
Cyanide	CN^-	Bitter almonds	Reacts with enzyme metal ions (i.e., Fe, Zn, Cu); respiratory chain enzymes are primary targets (see Chapter 15)
Diisopropyl fluorophosphate (DFP)		Synthetic	Inhibits enzymes with active site serine, including acetylcholinesterase
Sarin		Synthetic (nerve gas)	Like DFP
Penicillin		From *Penicillium* fungus	Inhibits enzymes in bacterial cell wall synthesis (see Chapter 16)

[a]R = variable group; differs on different penicillins.

Table 5.2: Some examples of chemicals that are irreversible inhibitors of enzymes

(a) Competitive inhibitors

Key Concept:

- Competitive inhibitors are structurally similar to the substrate and bind to the active site of the enzyme.
- The effect of inhibition can be reduced by increasing the substrate concentration.

- **Competitive inhibitors** are **structurally similar** to **substrate** molecules, therefore complementary in shape to the enzyme active site.

- They **compete with the substrate molecules** for binding ***reversibly*** to the **active site** of the enzyme (Fig. 5.50) to form unreactive enzyme-inhibitor complexes.

- This **prevents** substrate molecules from binding to the active sites to **form enzyme-substrate complexes**. Hence, this **decreases the rate of the enzyme reaction**.

- Example of reversible competitive inhibition: Malonate competes with succinic acid for succinic dehydrogenase (an enzyme of the Krebs cycle in cellular respiration). (Fig. 5.51)

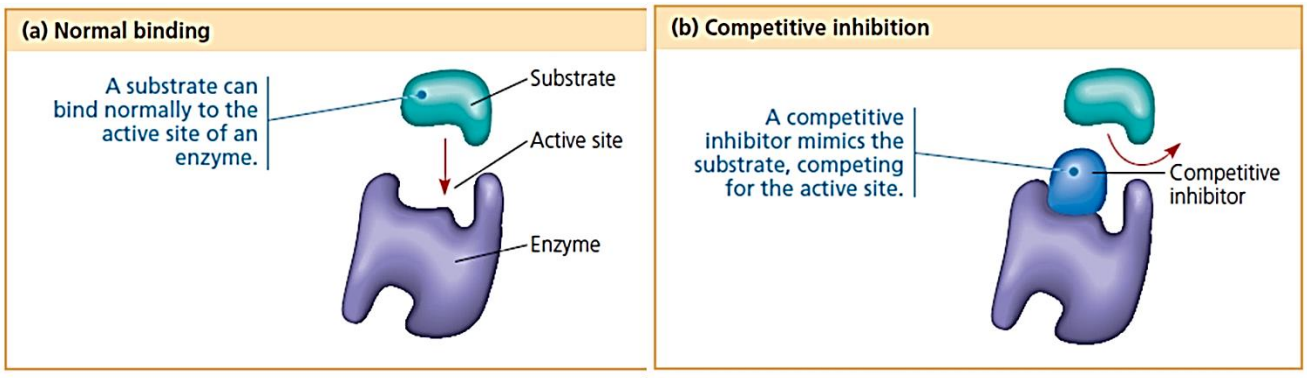


Fig. 5.50: Both the substrate and competitive inhibitor compete for an enzyme's active site

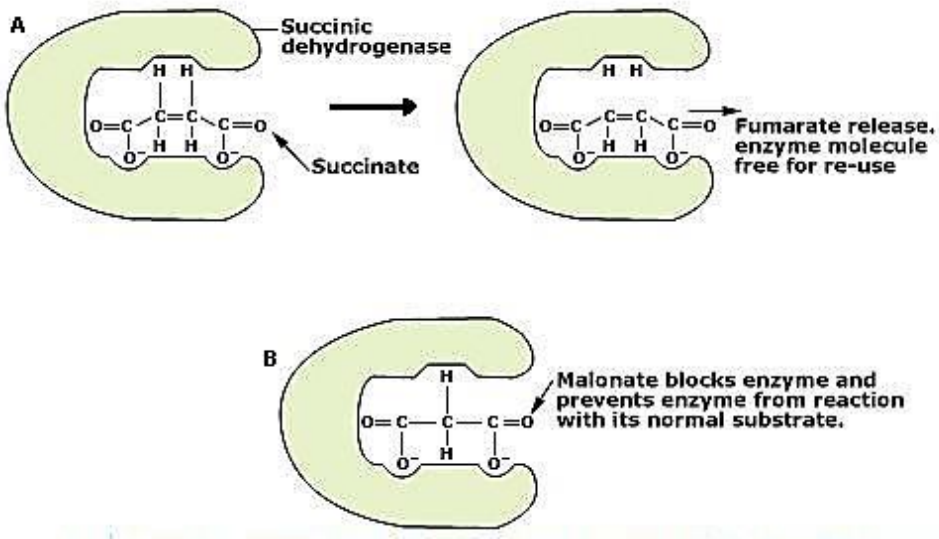


Fig. 5.51: An example of competitive inhibition in one of the reactions in the *Krebs* cycle of respiration.
A: Normal conversion of succinate to fumarate.
B: Succinate dehydrogenase is competitively inhibited by malonate, which binds to its active site but cannot be dehydrogenated.

- The **effect of competitive inhibitors can be reduced** by **increasing substrate concentration**.
.
- With an increase in the number of substrate molecules at the start of the reaction, the **probability of effective collisions between enzymes and substrate increases**.

- The substrate molecules thus **outcompete** the inhibitor molecules for the **active sites** of the enzymes.

- The **rate of reaction** increases to **reach its maximum value** (V_{max}), but **at a slower rate** and at **higher substrate concentration**. (Fig. 5.52)

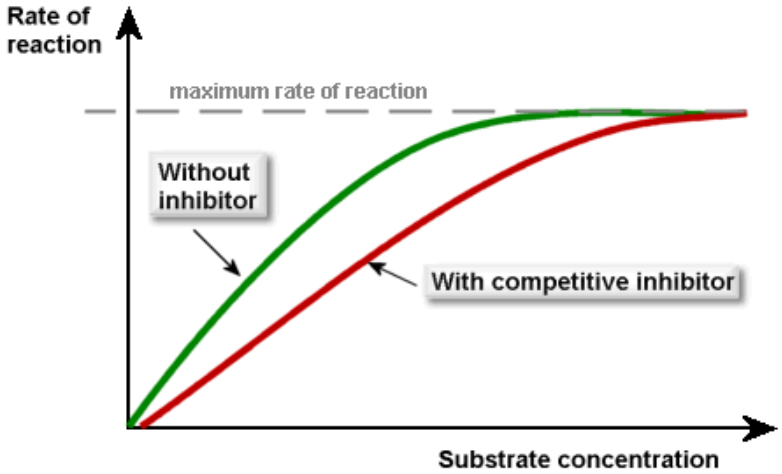


Fig 5.52: Rate of reaction with and without competitive inhibitor

(b) Non-Competitive Inhibitors

Key Concept:
- Non-competitive inhibitors have no structural similarity to the substrate and bind to a site other than the active site of the enzyme.
- The effect of inhibition cannot be reduced by increasing the substrate concentration.

- **Non-competitive inhibitors** have **no structural similarity/resemblance** to the **substrates**.

- Therefore they do not compete directly with the substrate to bind to the active site.

- Instead, they **bind** to the enzyme at a **site other than the active site** (Fig. 5.53a, 5.53b).

- This results in a **change in conformation of the enzyme** molecule, hence **altering the shape of the active site**, which is then

  1) **Unable to bind to the substrate** (Fig. 5.53a), or
  2) Can bind to the substrate but **cannot act on it**. (Fig. 5.53b)

- Example of irreversible non-competitive inhibition: Cyanide is known to inhibit many enzymes and acts by combining with metallic ions, which serve as prosthetic groups (e.g. Cu^{2+} of cytochrome oxidase).

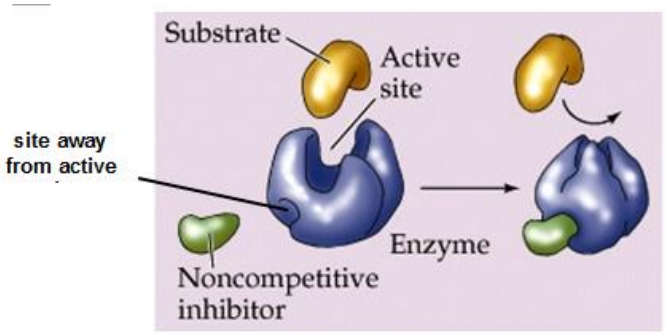


Fig. 5.53a: Non-competitive inhibitor prevents substrate from binding to enzyme's active site

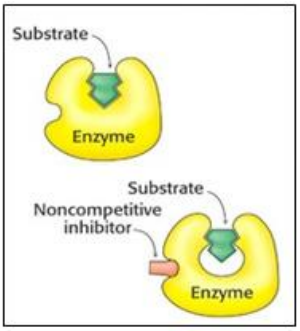


Fig. 5.53b: Non-competitive inhibitor allows substrate to bind to enzyme's active site but enzyme cannot act on substrate

- Thus, non-competitive inhibitors render a proportion of the enzyme molecules non-functional i.e. the **effective enzyme concentration is lowered**.
- Since non-competitive inhibitors do not compete with the substrate for the active site, **increasing the substrate concentration does not reduce the effect of inhibition**.
- Therefore, the **original maximum rate of reaction (V_{max}) can never be reached**, even at very high substrate concentrations (Fig. 5.54).

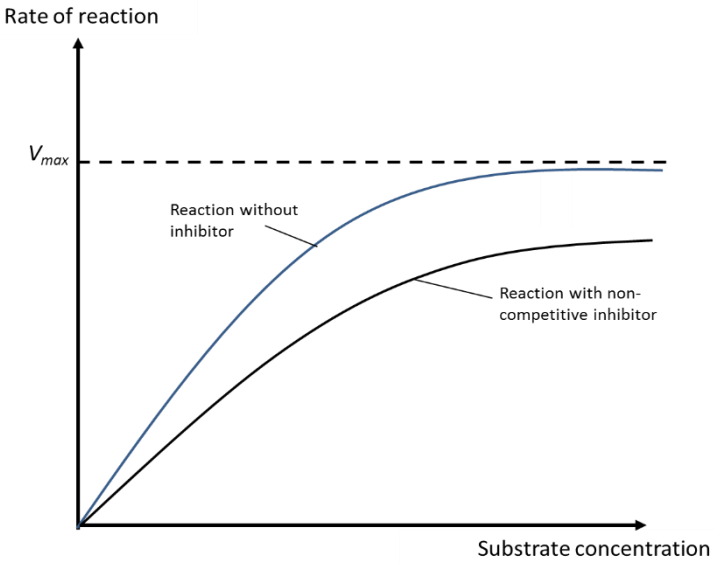


Fig. 5.54: Rate of reaction with and without non-competitive inhibitor

DID YOU KNOW?

~TOKYO GAS ATTACK IN 1995

Sarin gas was used in the attack, killing at least a dozen people, severly injuring 50, and causing temporary vision problem in more than a thousand people.

Other examples of non-competitive irreversible inhibition:

- **Sarin** forms an enzyme inhibitor complex with the amino acid serine at the active site of the enzyme **acetylcholinesterase**. The enzyme deactivates the transmitter substance acetylcholine, in nerve impulse transmission across a synapse. If acetylcholinesterase is inhibited, then acetylcholine accumulates. This results in the constant propagation of nerve impulses and therefore prolonged muscle contraction. This leads to **paralysis** and **eventually death**.
- Metabolic poisons like **DDT, parathion** and **malathion** (common pesticides used). These **affect enzymes of the nervous system**.

(c) Competitive VS Non-competitive Inhibition

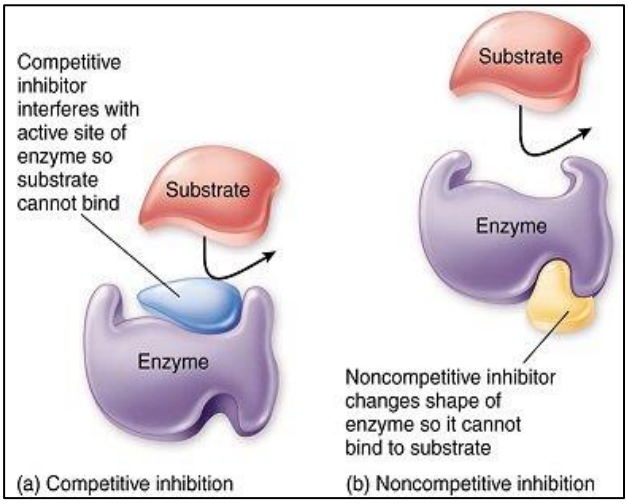


Fig. 5.55a: Competitive and non-competitive inhibition

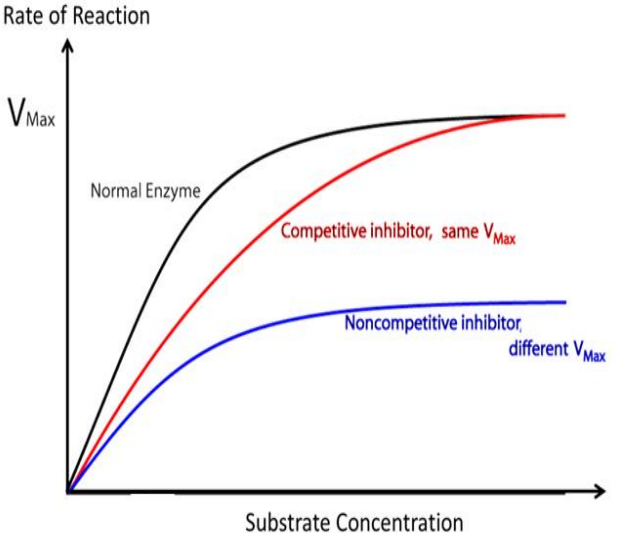


Fig. 5.55b: Rate of reaction without inhibitor, and with competitive and non-competitive inhibitors

Table 5.3: Differences between competitive and non-competitive inhibition

	Competitive Inhibition	Non-Competitive Inhibition
Structure of inhibitor	Structurally **similar to substrate**.	
Site of binding of inhibitor	Inhibitor competes with substrate for the **active site** of the enzyme.	
Action of inhibitor on shape of active site	Inhibitor **does not alter the shape** of the active site.	
Effect on active site function	Active site is still **functional** but is **blocked** when the inhibitor is attached to it.	
Effect of increasing[substrate]	**Inhibition is overcome** by increasing the concentration of substrate.	
Maximum rate of reaction	**Same V_{max}** can be attained at **higher substrate concentration**.	

5.5.8 Regulation of metabolic pathways by cells

- An organism must be able to regulate the catalytic activities of its enzymes so that it can coordinate its numerous metabolic processes, respond to changes in its environment, and grow and differentiate, all in an orderly manner.

- Thus, a cell has to control when and where its various enzymes are active. This is done by controlling enzyme synthesis (i.e. switching on/off the genes that encode specific enzymes) *(see topic: Organisation and Control of Gene expression)* or by **regulating the activity of enzymes once they are made** (*Section 5.5.8*).

(a) Allosteric Regulation

Key Concept:
- Allosteric inhibitors bind to an allosteric site (site other than the active site) of an enzyme and stabilises the inactive form of the enzyme. This reduces the rate of reaction.

- Allosteric regulation is the control of enzyme activity by the binding of a regulatory molecule to the allosteric site of an enzyme. The enzymes that are allosterically regulated are called **allosteric enzymes.**

- Allosteric inhibitors behave like **reversible non-competitive inhibitors** *(Section 5.5.7b)*.
    - This is because these regulatory molecules loosely **bind to the allosteric site** via **non-covalent interactions**, causing a change in the shape of the enzyme's active site.

- Features of allosteric enzymes: (Fig 5.56)
    - They usually **consist of two or more subunits** (**multimeric**) interacting with one another.
    - Each subunit is composed of **one polypeptide chain** and has its **own active site** and **allosteric site**.
    - The subunits are arranged in such a way that a **change in shape of one subunit** due to the binding of regulatory molecules at the allosteric site changes the **other subunits**.

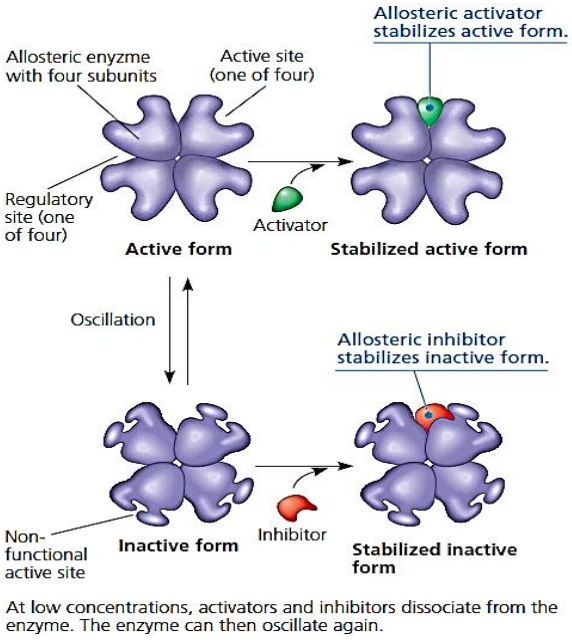


Fig. 5.56: An allosteric enzyme in its active and inactive forms

- The activity of allosteric enzymes can be regulated by (Fig. 5.57):

  - **Allosteric activation**
    - ❖ When regulatory molecules called **allosteric activators** bind to an **allosteric site** of the multimeric enzyme, **conformation change** of enzyme occurs, altering the shape of the **active site**.
    - ❖ The **active form** of the enzyme is stabilised**,** hence **increasing** the rate of reaction

  - **Allosteric inhibition**
    - ❖ When regulatory molecules called **allosteric inhibitors** bind to an **allosteric site** of the multimeric enzyme, **conformation change** of enzyme occurs, altering the shape of the **active site**.
    - ❖ The **inactive form** of the enzyme is stabilised, hence **reduces** the rate of reaction.

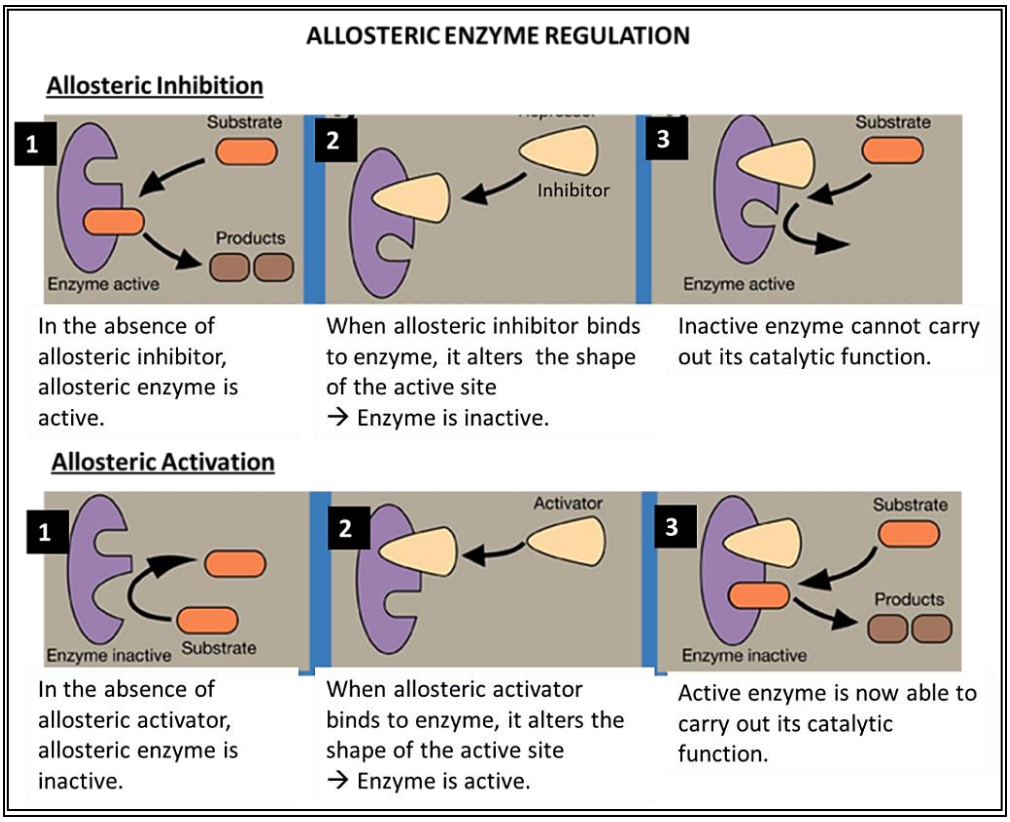


Fig. 5.57: The effects of an allosteric activator and allosteric inhibitor on an allosteric enzyme

- In some cases, **allosteric activation** can also occur in the presence of **just the substrate molecules** (without regulatory molecules) (Fig. 5.58).
    - The **substrate** binds to **one active site** in the multimeric enzyme, triggering a **conformational change** in the enzyme. All the other subunits are stabilised in the **active form**, hence the active sites of other subunits have an **increased affinity** for binding to its substrate molecules.
    - This is known as **cooperativity**. The subunits behave cooperatively with each other, with the activity of one subunit influencing the activity of the other subunits.

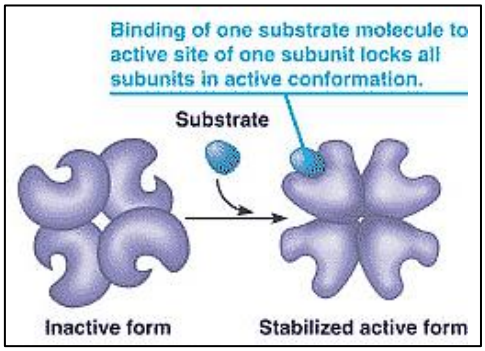


Fig. 5.58: Cooperativity between subunits of an allosteric enzyme

- A typical graph of multimeric allosteric enzymes is **sigmoidal** (S-shaped) (Fig. 5.59b). This is because:
    - At low substrate concentrations, the **inactive** form of the allosteric enzyme is favoured. Only a small number of enzymes are able to form enzyme-substrate complexes. Hence, the rate of enzyme-catalysed reaction is low.
    - However, as substrate concentration increases, the **binding of a substrate to the active site in one subunit** of an allosteric enzyme causes a **conformation change** in the enzyme that changes the conformation of all the other subunits to its **active form**. Hence, the rate of reaction thus increases steeply. The sigmoidal curve indicates **cooperative binding of substrate**.
    - When all active sites on the allosteric enzyme are occupied with substrate, a plateau is reached.

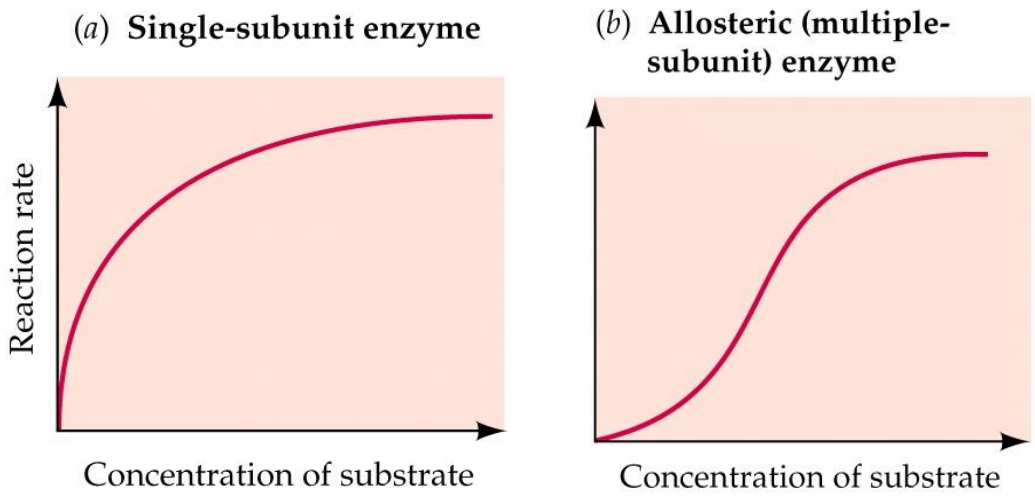


Fig 5.59: Graph for rate of reaction in a normal single-subunit enzyme-catalysed reaction (left) vs. sigmoidal graph for an allosteric enzyme-catalysed reaction

(b) End-Product / Feedback Inhibition

- Many enzymes in metabolic pathways are **allosterically controlled** by molecules that are also in the same pathway.

- **End-product / Feedback inhibition** occurs when the **metabolic pathway is switched off** / inhibited by the **pathway's final product** when its **concentration increases** and exceeds a certain level (Fig. 5.60).

- The final product acts as an **allosteric inhibitor** of an enzyme earlier in the pathway (usually the *first* enzyme).

    - This prevents the overproduction of the end-product and is therefore a form of **negative feedback**.

    - End-product inhibition is **self-regulatory**. As product is used up, the inhibition is lifted, and the pathway is switched on again.

- This adjusts the rate of synthesis of products according to the demands of the cells which helps to:

    - **Prevent wasting chemical resources and energy** by synthesising more products than required.

    - **Maintain a stable internal environment** (homeostasis) in living organisms.

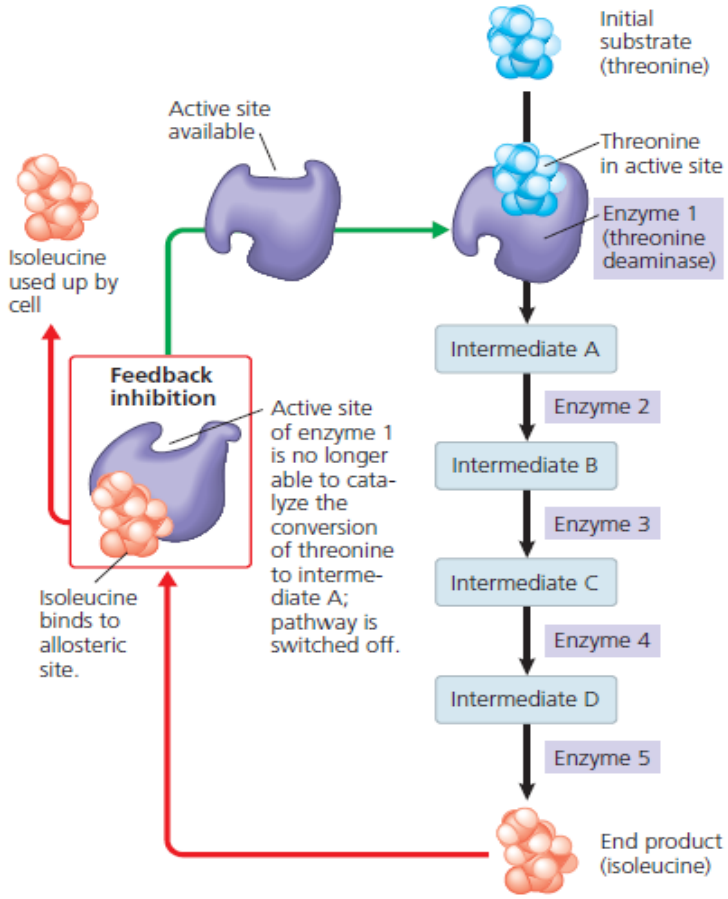


Fig. 5.60: Feedback inhibition in isoleucine production

Example of feedback inhibition on phosphofructokinase-1 (PFK-1) - For your information only

- Respiration is a process that produces ATP (an energy molecule in the cell) as a final product. PFK-1 is an enzyme that is involved in the glycolysis stage of respiration *(Topic: Cellular Respiration*).

- PFK-1 catalyses the conversion of fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate, by transferring a phosphate from ATP (Fig. 5.61).

- PFK-1 is a **tetrameric allosteric enzyme** that oscillates between its active and inactive forms.

- **ATP** is both a **substrate** and **allosteric inhibitor** of **PFK-1**. ATP can bind to the active site and stablise the active form. ATP, at high concentration, can also bind to the allosteric site to stabilise the inactive form (Fig. 5.62).

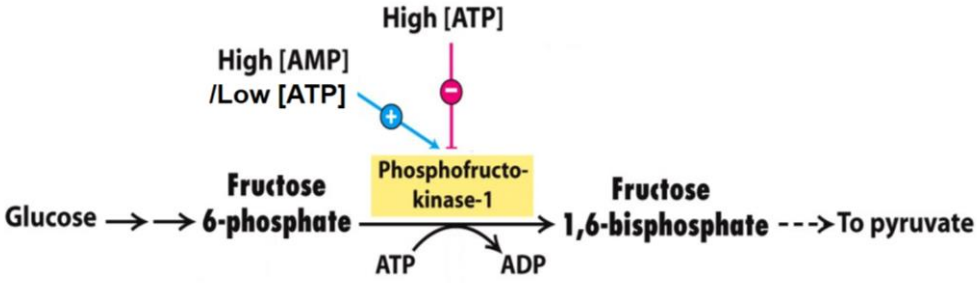


Fig. 5.61: Phosphofructokinase-1 catalyses one of the first reactions in glycolysis of respiration. AMP at high concentrations allosterically activates PFK-1, while ATP at high concentrations allosterically inhibit PFK-1.

- At **high ATP concentrations** (the final product of respiration), ATP acts as an **allosteric inhibitor of PFK-1.** ATP binds to an **allosteric site** on PFK-1 to stabilise its **inactive form**.
    - In the inactive form of PFK-1, the other substrate fructose 6-phosphate is then unable to bind to its active site. Thus this prevents the progression of glycolysis, and eventually prevents an overproduction of ATP (the final product of respiration).

- On the other hand, **high concentrations of AMP** (which indicate low levels of ATP in the cell) can reverse the allosteric inhibition of PFK-1 by ATP. AMP acts as an **allosteric activator** of PFK-1, where it binds to an **allosteric site** on PFK-1 to stabilise its **active form**.
    - In the active form of PFK-1, the other substrate fructose 6-phosphate is able to bind to the active site on PFK-1. Thus, the **feedback inhibition is lifted**.

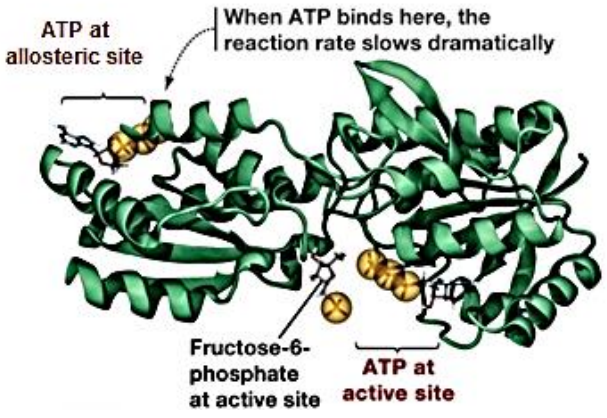


Fig. 5.62: Diagram shows one of the subunits in phosphofructokinase-1. Each subunit has two binding sites for ATP – an active site and a allosteric site. ATP is both a substrate and allosteric inhibitor.

5.6 Checklist for Proteins

Are you able to confidently answer all the questions?

1. Distinguish between a polypeptide and a protein.

2. Describe how a peptide bond is formed between two amino acids. Illustrate the reaction with diagrams.

3. List and describe the four components of an amino acid.

4. Explain how amino acids may be grouped according to the physical and chemical properties of the R groups.

5. Explain what determines the conformation of a protein and why it is important.

6. Explain how the primary structure of a protein is determined.
   (Cross reference to the topic on Gene Expression)

7. Name the two types of secondary protein structures and explain how their 3D shape is maintained.

8. Describe the various bonds that hold a globular protein in its 3D structure.

9. Describe the molecular structure of haemoglobin and relate its structure to its function.

10. Describe the molecular structure of collagen and relate its structure to its function.

11. Describe and explain the mode of enzymes in terms of active site, enzyme-substrate complex, lowering of activation energy and enzyme specificity.

12. Explain how enzymes lower the activation energy of a reaction.

13. Distinguish between the lock-and-key hypothesis and induced-fit hypothesis of the mechanism of enzyme action.

14. Describe and explain how the rate of formation of products in an enzyme-catalysed reaction (e.g. using catalase) may be measured.

15. Describe and explain how the rate of change of substrates in an enzyme-catalysed reaction (e.g. using amylase) may be measured.

16. Describe and explain the effect of temperature on the rate of enzyme-catalysed reactions.

17. Describe and explain the effect of pH on the rate of enzyme-catalysed reactions.

18. Describe and explain the effect of enzyme concentration on the rate of enzyme-catalysed reactions.

19. Describe and explain the effect of substrate concentration on the rate of enzyme-catalysed reactions.

20. Explain the effects of competitive and non-competitive inhibitors on the rate of an enzyme-catalysed reaction.

21. Explain how feedback inhibition can be used to regulate metabolic pathways in a cell.

6. Cellular Transport

6.1 Membrane Structure and Function

- The cell surface membrane is the edge of life, the boundary that separates the living cell from its surroundings and controls the traffic of molecules into and out of the cell it surrounds.
- Membranes also form various internal organelles to allow these organelles to take on specialised functions.
- The membrane constitutes various biomolecules to carry out different functions.

6.1.1 Membrane Structure

- All biological membranes have the **same basic structure.** The cell surface membrane and membrane of organelles (e.g. mitochondrion, chloroplast) comprise of a **phospholipid bilayer** (Fig. 6.1).
- The bilayer is approximately **7 to 10 nm** in width. Cell surface membranes are approximately 7.5 nm.
- The bilayer is formed as phospholipids are **amphipathic** molecules. The cell membrane comprises **phospholipids** arranged in **bilayers** with the **hydrophobic fatty acid chains** (or **hydrocarbons**) facing inwards and the **hydrophilic phosphate heads** facing the aqueous environment.

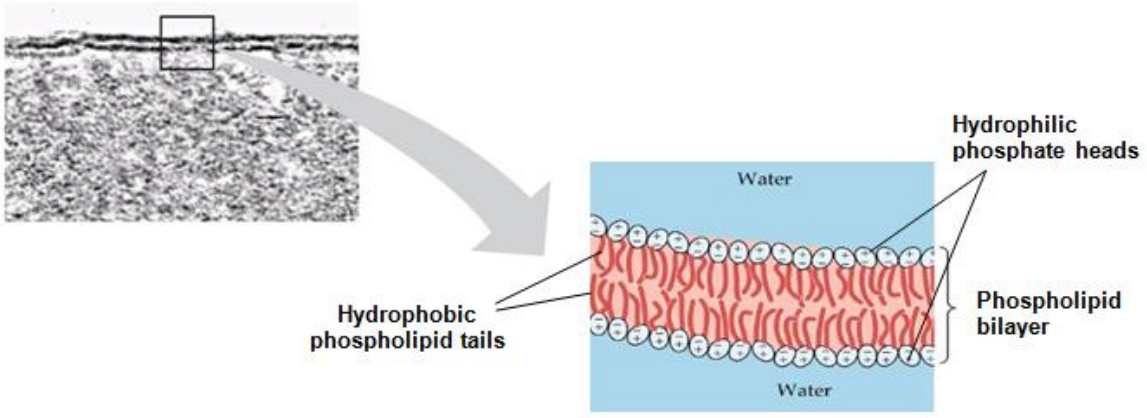


Fig. 6.1: A diagram and electron micrograph of the phospholipid bilayer

- Besides phospholipids, most other membrane constituents (e.g. proteins, cholesterol) are also **amphipathic** molecules. Hence the membrane is able to **reseal** itself when the bilayer is disrupted.
- The bilayer is **dynamic** and its components are constantly in motion (Fig. 6.2). Hence the term "**fluid mosaic model**" is used to describe membrane structure.

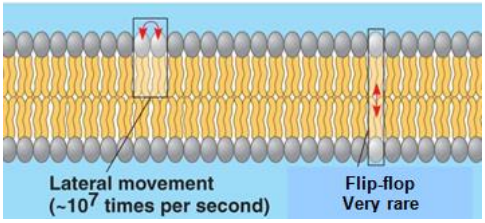


Fig. 6.2: The dynamic structure of the phospholipid bilayer

6.1.2 The Fluid Mosaic Model

- Proposed by Sanger and Nicholson in 1972, the fluid mosaic model describes the structure of biological membranes (Fig. 6.3).

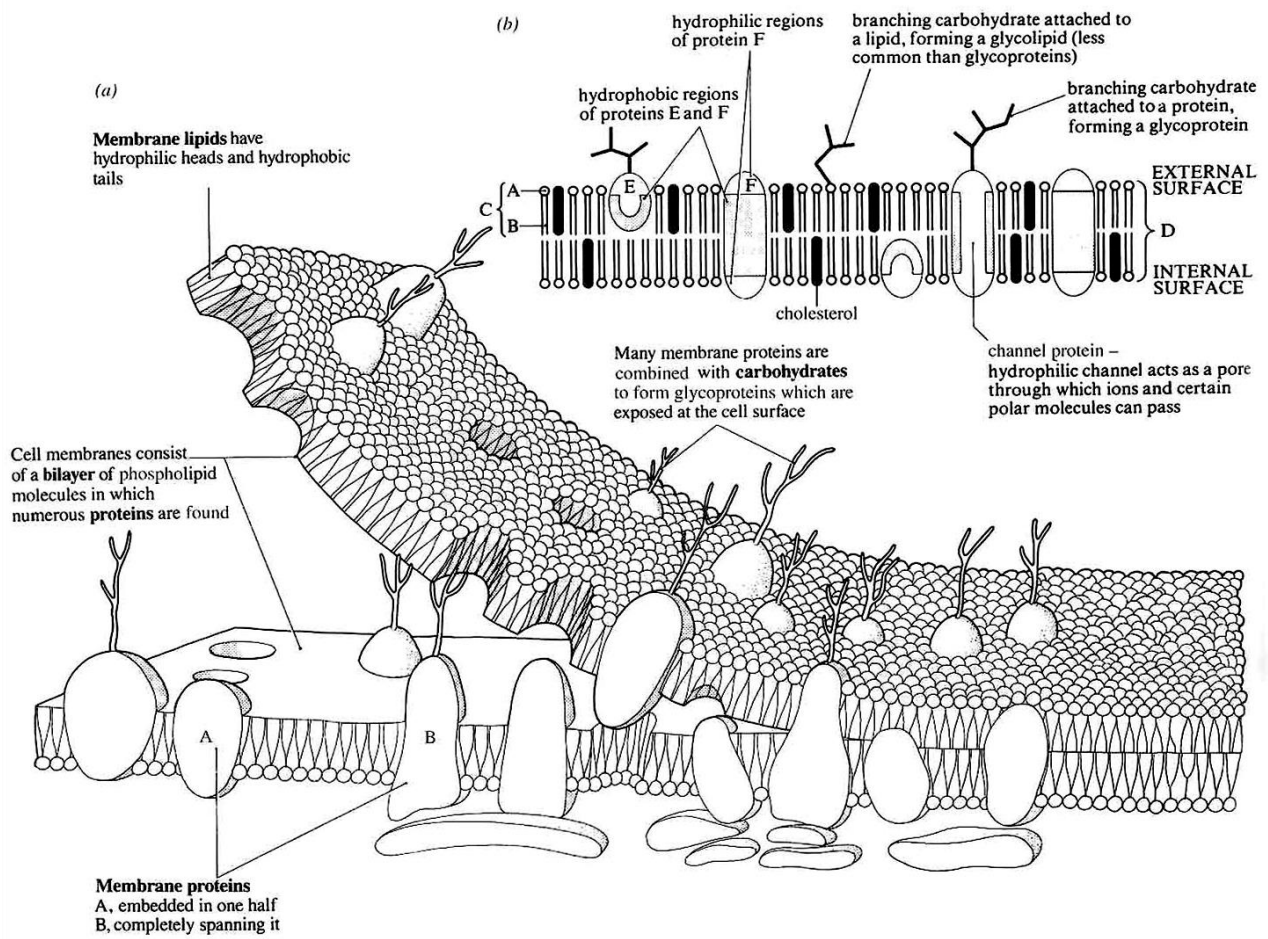


Fig. 6.3: An overview of membrane structure

- '**Fluid**' refers to the fact that individual **phospholipids** and **protein** molecules are in **constant motion** and are able to **move laterally** within the bilayer.

- '**Mosaic**' describes the **diverse and different types of proteins present** and the **random arrangement** in which these different protein molecules are scattered among the phospholipid molecules.

- Evidence of the fluid mosaic model is revealed from two experiments:

    - **Cell-fusion experiment** (Fig. 6.4), where a human cell and mouse cell is fused. Within an hour, the proteins from the two species become completely intermingled in the membrane of the hybrid cell. This revealed the **fluid** nature of the cell membrane.

    - **Freeze-fracture experiment** (Fig. 6.5), where the cell membrane is frozen and split along the middle of the bilayer. This revealed the **mosaic** nature of the cell membrane.

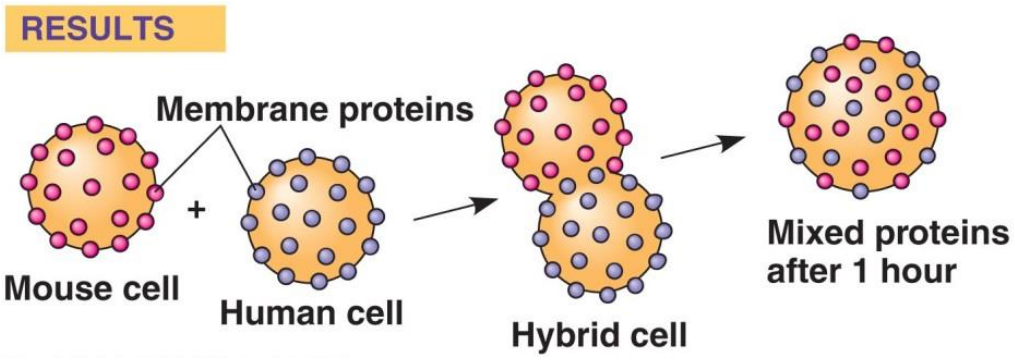


Fig. 6.4: The cell fusion experiment

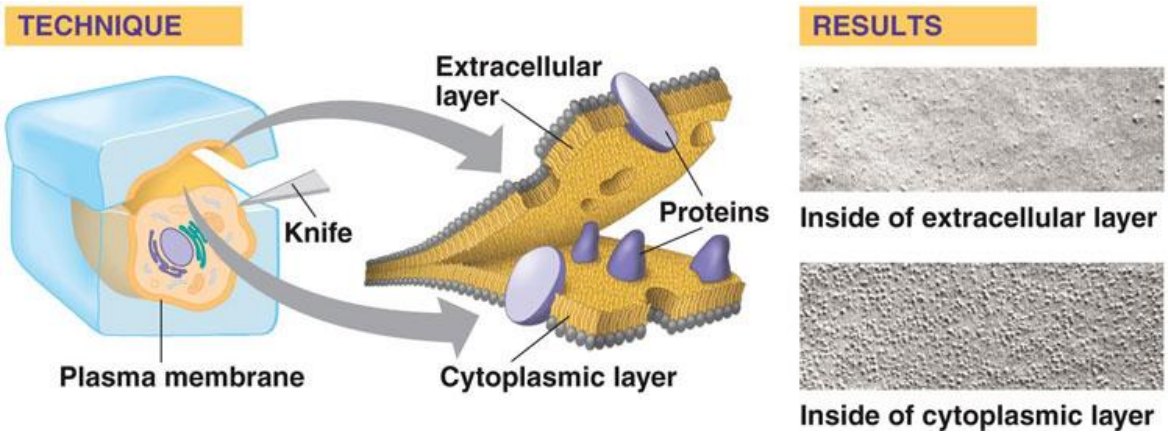


Fig. 6.5: The freeze-fracture experiment

6.1.3 Membrane components and their function

- The amount and types of components present in membrane varies between membranes of different subcellular locations (e.g. cell surface membrane vs. mitochondrial membrane).

- The amount and types of components present in membrane also varies between the two sides of the membrane as membranes are **asymmetric.**

- The membrane comprises of:

a) Phospholipid bilayer

- The cell membrane comprises **phospholipids** arranged in **bilayers** with the **hydrophobic fatty acid chains** facing inwards and the **hydrophilic phosphate heads** facing the aqueous environment.

- Function:

  - **Separates** the cell contents from the external environment, and allows for **compartmentalisation** (i.e. formation of organelles) within the cell where specialised biochemical reactions occur.

  - **Regulate membrane fluidity** and hence membrane **permeability** through:

    (i) The **degree of saturation of fatty acids**

    - **Saturated fatty acids** can be closely packed together, causing the membrane to be more rigid/ less fluid.

    - **Unsaturated fatty acids** have spaces between them due to the double bonds (C=C) that form kinks in the structure. This prevents the phospholipid molecules from packing close together, thus increasing membrane fluidity (Fig. 6.6).

    (ii) The **length** of **fatty acid chains**

    - **Shorter fatty acid chains increases the fluidity** of the membrane as the hydrophobic chains of the phospholipid molecules forming **hydrophobic interactions** with each other are **less extensive**.

    - **Longer fatty acid chains decreases the fluidity of the membrane** as the hydrophobic chains of the phospholipid molecules forming **hydrophobic interactions** with each other are **more extensive**.

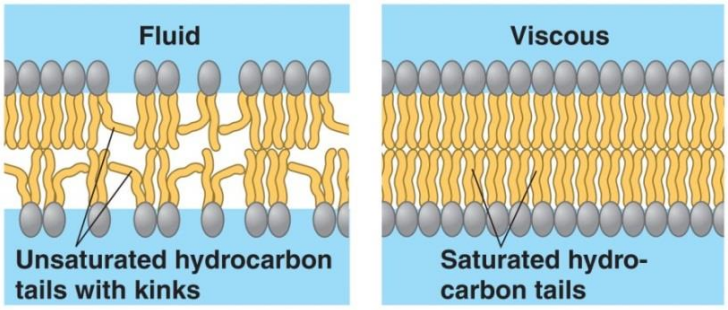


Fig. 6.6: Regulation of membrane fluidity

How would you expect the structure of the plasma membrane to differ in plants adapted to cold environments and plants adapted to warmer environments?

b) Cholesterol

- Cholesterol molecules are **amphipathic** (Fig. 6.7 left). They have a **hydrophilic region** made up of **hydroxyl group** that **interacts with the phosphate heads** of phospholipids and a **hydrophobic region** made up of the **ring structure** that **interacts with the fatty acid chains** of phospholipids. Hence they are wedged between the hydrophobic regions of phospholipid molecules (Fig. 6.7 right).

- Function:

  - To **regulate membrane fluidity** according to the environment. At high temperatures, presence of cholesterol prevents the membrane from becoming too fluid, while at low temperatures, presence of cholesterol prevents the membrane from becoming too rigid.

  - At relatively **high** temperatures (e.g. 38°C)
    - Cholesterol makes membrane **less fluid** by decreasing the mobility of hydrocarbon tails of phospholipids

  - At **lower** temperatures (e.g. 5°C)
    - Cholesterol **prevents close packing** of **phospholipids**
    - lowers the temperature required for the membrane to solidify

  - This is important as the fluidity of the membrane influences many of its functions. For example:
    - A completely frozen and therefore rigid membrane will be impermeable to even lipid-soluble, non-polar substances.
    - Membrane components (e.g. proteins) may not be able to perform their functions if a membrane is not fluid enough.
    - Fluidity is important in facilitating self-sealing (e.g. during endocytosis and exocytosis).

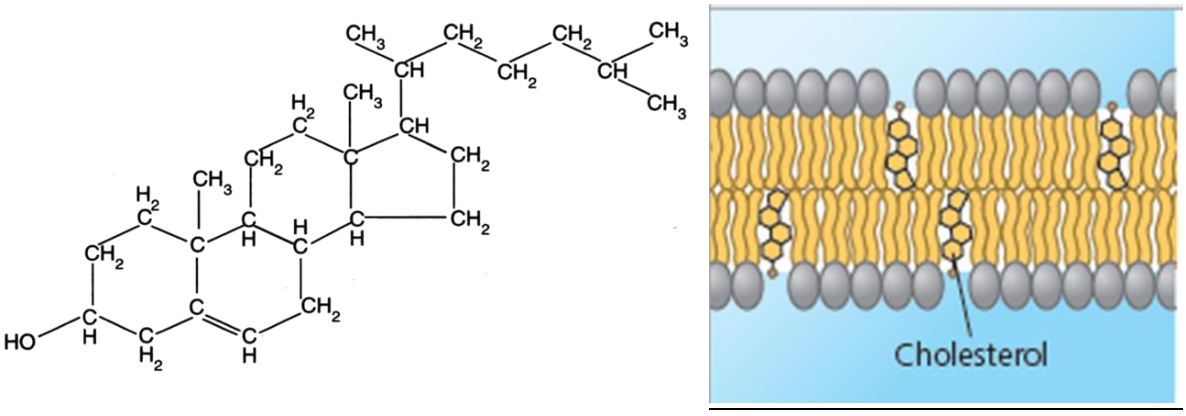


Fig. 6.7: Structure of Cholesterol (left) and interaction of cholesterol with phospholipids (right)

c) Proteins

1) **Peripheral** or extrinsic **proteins** (Fig. 6.8)

- Proteins that are not embedded in the lipid bilayer, but are usually bound to the membrane **indirectly by interactions with integral membrane proteins** or **directly by interactions with lipid polar head groups**.

  - They contain **charged** and **polar amino acid residues**, which are bound to the charged portion of lipids by **ionic bond** and **hydrogen bonds.**

2) **Integral** or intrinsic **proteins** (Fig. 6.8)

  - Proteins that are embedded in the lipid bilayer, either partly or completely span the membrane. Proteins that completely span the membrane are named **transmembrane proteins**.

  - They contain hydrophobic and hydrophilic amino acid residues. The hydrophobic amino acid residues interact with the fatty acid chains of the phospholipids, while the hydrophilic amino acid residues face the aqueous medium or interact with other hydrophilic amino acids. These **hydrophobic and hydrophilic interactions** hold the protein in place.

Functions of membrane proteins (*details in section 6.1.4*)

1. **Transport proteins** (only for transmembrane proteins)
   a. Channel/carrier – facilitated diffusion of hydrophilic molecules across the membrane
   b. Pump – active transport of hydrophilic molecules across the membrane

2. **Receptors** (only for transmembrane proteins) (*Topic 12: Communication*) – allows extracellular signals to be transmitted to the inside of the cell

3. **Enzymes** – catalyses biological reactions (e.g. adenyl cyclase converts ATP to cyclic AMP)

4. Allow cells to **adhere to one another** to **form tissues**

5. Allow cells to **recognize** one another as 'self', and to recognize foreign cells as 'non-self'.

6. Allows attachment to cytoskeleton and extracellular matrix to **provide structural support** to the cell

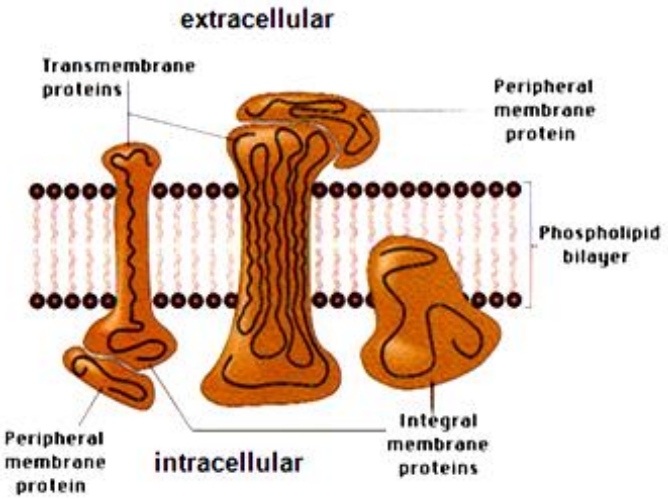


Fig. 6.8: The association of peripheral and integral proteins with the phospholipid bilayer

d) Carbohydrates (Fig. 6.9)

- Short oligosaccharides (<15 sugar units) that are found on the **external surface**.
- Oligosaccharides that are covalently bonded to lipids form **glycolipids**, while oligosaccharides that are covalently bonded to proteins form **glycoproteins.**
- Unlike peripheral proteins which could exist on either face of the membrane, both glycolipids and glycoproteins are normally only present on the extracellular face.
- Function: Serve as recognition sites for **cell-to-cell recognition** and **cell adhesion**.

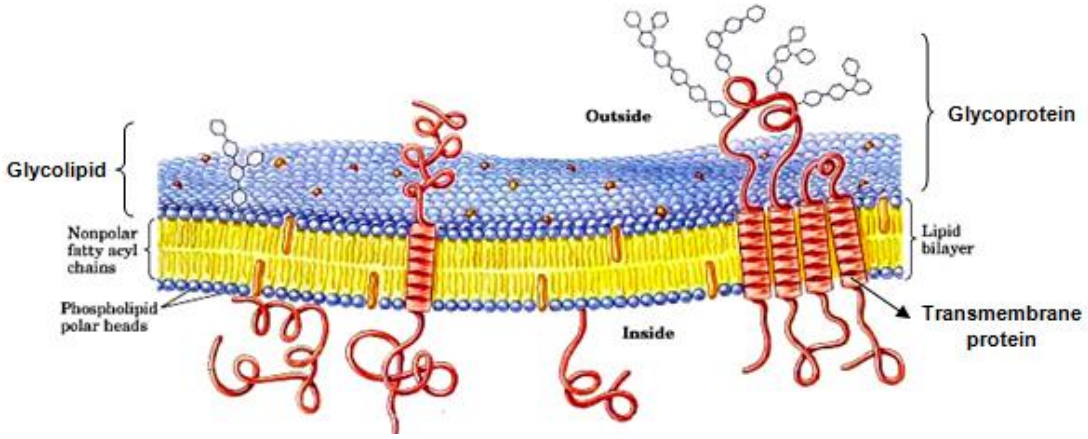
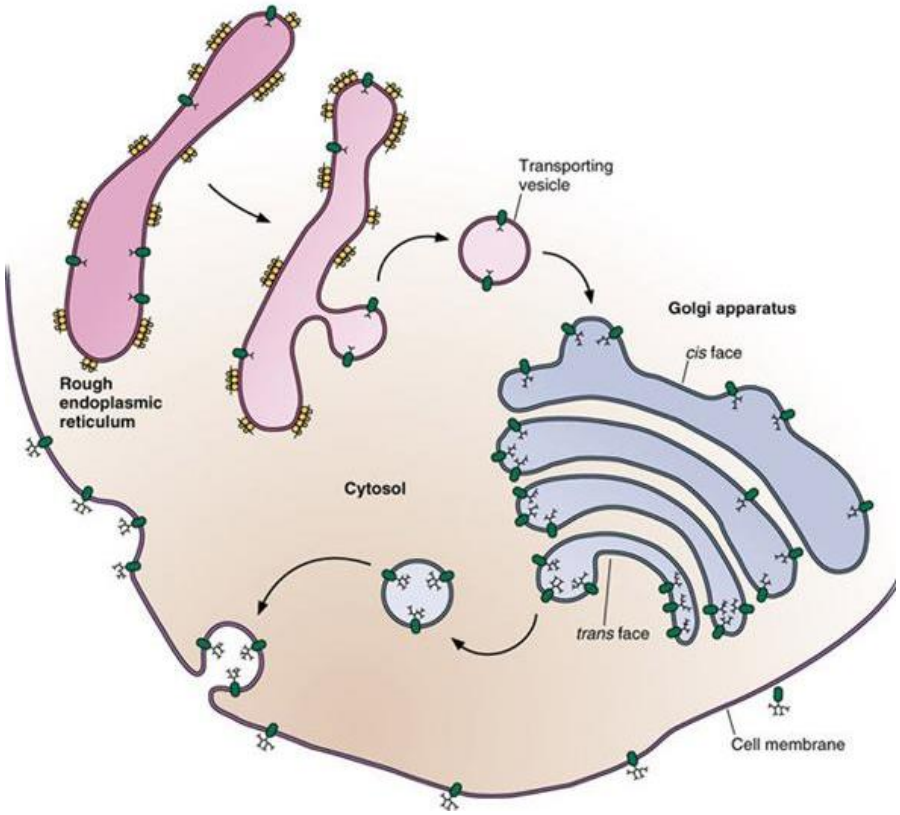


Fig. 6.9: A depiction of glycolipid, glycoprotein, as well as other components of the membrane

In the formation of glycoproteins and glycolipids, the short carbohydrate chains (oligosaccharides) are added to the proteins and lipids of the plasma membrane in ER or Golgi apparatus via ***glycosylation***.

This membrane containing the short carbohydrate chains forms transport vesicles that travel to the cell *surface* membrane.

On which side of the transport vesicle membrane (i.e. inner or outer) are the short carbohydrate chain found? Explain.



Explain:

6.1.4 Functions of the membrane

Function of membranes within the cell (i.e. intracellular membranes):

1) Form compartments (**compartmentalisation)** within the cell (i.e. formation of organelles).
(For details the function of these organelle membranes, please refer to Topic 1: Cell)

   Advantages of compartmentalization:

   - **Isolating enzymes and reactants** from the rest of the cell allows them to be in **high concentration**, **speeding up** the **rate of reactions**.
   - Localizing products within the organelle for use especially in a multi-step biochemical pathway (e.g. the intermediate molecules of Krebs cycle are kept within the mitochondrial matrix).
   - Attachment of specific proteins/enzymes within organelle membrane allows enzyme-catalyzed reactions to take place in a sequential manner in a metabolic pathway (Fig. 6.10)
   - Ensuring maintenance of optimal conditions (e.g. pH5 in lysosomes) for **specialized biochemical reactions** to occur
   - **Isolating harmful molecules** (e.g. free radicals such as superoxide in mitochondria) from the rest of the cell
   - Allowing organelles to move within cytoplasm where they are needed (e.g. mitochondria can be streamed to areas needing ATP)
   - Allowing **different/specialized metabolic pathway** to **occur at the same time** (e.g. Krebs cycle in the mitochondria and Calvin cycle in the chloroplast)

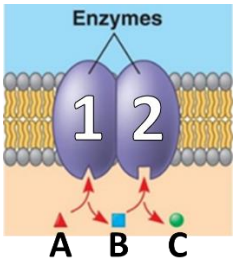


Fig. 6.10: Product of enzyme 1 (molecule B) is immediately available for enzyme 2 as a substrate.

2) Membranes are required for the **formation of transport vesicles** during intracellular transport.

Function of membrane at the surface of cell (i.e. cell surface membrane):

1) The cell surface membrane **separates cell contents from the external environment**, so that the cell can function independently of its environment.

2) **Control the exchange of substances across the membrane** as they are selectively permeable *(Section 6.2).* The selective permeability of the membrane depends on both the discriminating barrier of the lipid bilayer and the specific transport proteins present on the membrane.

Why is it important to control the exchange of substances between the cell and the external environment?

3) Enables the cell to **communicate** with the external environment. (*Topic 12: Communication)*

- Cells in a multicellular organism must communicate to coordinate their activities.
- This communication is achieved by cells releasing chemical messengers/signalling molecule (e.g. hormones) that are targeted for cells that may or may not be immediately adjacent.
- When the signal reaches the target cells, it is recognised by a specific receptor protein (i.e. transmembrane protein) that is embedded in the cell surface membrane (Fig. 6.11).
- Upon binding of the signalling molecule, the receptor protein undergoes a conformational change in shape, which relays/transduces the message to the inside of the target cell.

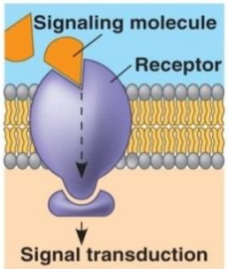


Fig. 6.11: A transmembrane receptor protein with a bound signalling molecule

Why do you think the signalling molecule is specific *only* to the target cells?

4) **Cell-to-cell recognition**

- The cell surface membrane comprises of glycoproteins and glycolipids. These glycoproteins (and to a lesser importance - glycolipids) are unique in different tissues of an individual and between different individuals, thus allowing them to serve as a form of cellular identification (Fig. 6.12).
- E.g. Glycoproteins are important in important in invoking immune responses. Foreign cells (eg. bacteria) that do not have host glycoproteins present on their cell surface membrane will be recognised and destroyed by white blood cells.

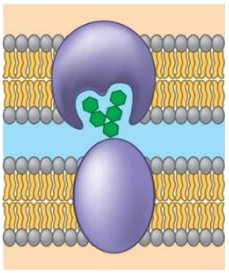


Fig.6.12: Glycoproteins allow cell-to-cell recognition between two adjacent cells.

5) Allows the **adhesion of cells** for tissue formation.

- Membrane proteins of adjacent cells may be joined together in various kinds of junctions (Fig. 6.13).

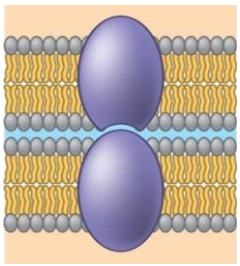


Fig. 6.13: Intercellular joining between two membrane-bound proteins of adjacent cells

- Examples of the various junctions between adjacent cells (Fig. 6.14) include:

(i) Tight junctions
- ❖ Plasma membrane of neighboring cells are tightly pressed against each other, forming continuous seal around the cell.
- ❖ This prevents extracellular fluids from moving across a layer of epithelial cells.

(ii) Gap junctions
- ❖ Consist of membrane proteins that surrounds a pore through which ions, sugar, amino acids and other small molecules may pass.
- ❖ This provides a cytoplasmic channel from one cell to an adjacent cell, allowing for cellular communication.

(iii) Desmosomes
- ❖ Function like rivets, fastening cells together into strong sheets.
- ❖ Desmosomes attach muscle cells to each other in a muscle. Some 'muscle tears' involves the rupture of desmosomes.

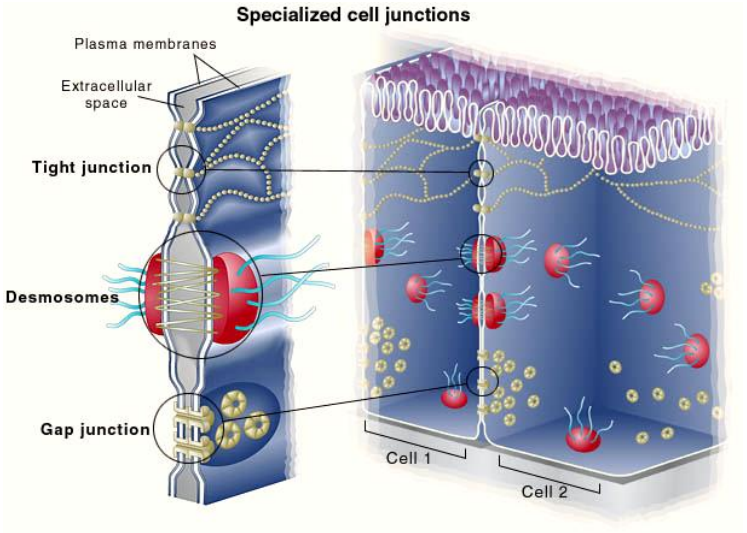


Fig. 6.14: Various junctions between adjacent cells

6) **Attachment to the cytoskeleton and extracellular matrix**

- Membrane proteins can be attached to the cytoskeleton (e.g. microfilaments) on the inside of the cell, and fibres of the extracellular matrix (collagen fibres interwoven with carbohydrate-containing protein molecules called proteoglycans) on the outside of the cell.

- This gives the cell membrane a stronger framework, thus providing **structural support** and helping to maintain cell shape (Fig. 6.15).

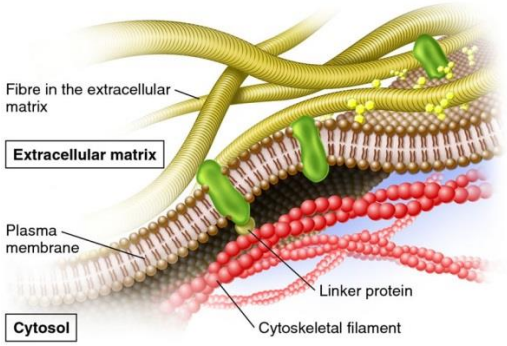


Fig. 6.15: Attachment of membrane proteins to the cytoskeleton and extracellular matrix (ECM)

6.2 Transport across the Membrane

- Gases and small hydrophobic molecules diffuse directly across phospholipid bilayer.
- Polar and charged molecules such as ions, sugars and amino acids cannot diffuse across the phospholipid bilayer.
- Water is a polar molecule, but because it is very small, a small percentage can still diffuse through the phospholipid bilayer.
- Charged and polar molecules require integral membrane proteins e.g. channels, carriers and pumps for the transport of molecules across the phospholipid bilayer.
- Types of integral membrane proteins for transport across membrane:

  1) A **channel protein** (ungated) forms an open hydrophilic pore across the membrane, allowing **facilitated diffusion** of any charged ions or polar molecule of the appropriate size and charge. An example of a channel protein is the aquaporin (water channel), which allows the facilitated diffusion of water, thus increases the rate of osmosis.

  2) A **carrier protein** (sometimes known as transporter) binds to specific molecules to be transported on one side of the membrane, and then undergo a **conformational change** (change in shape). This allows the molecule to pass through the membrane and be released on the other side. An example of a carrier protein is the glucose transporter, which is responsible for the uptake of glucose (main respiratory substrate).

  3) A **pump** ***utilises energy from ATP*** to move ions and other solutes ***against a concentration gradient*** via ***active transport*** process. An example of a pump is sodium-potassium pump that helps to restore the electrochemical gradient across the membrane, which is especially important in nerve and muscle cells.

	Channel	**Carrier**	**Pump**
No. of molecules moving across membrane per second	Allows multiple molecules to move simultaneously across membrane per second	Only binds to 1 or very few molecules at a time, hence, only allows 1 or few molecules to move across membrane per second	Only binds to 1 or very few molecules at a time, hence, only allows 1 or few molecules to move across membrane per second
Change in conformation	May or may not undergo conformational change	Undergoes conformational change	Undergoes conformational change
Use of ATP	Does not require ATP	Does not require ATP	Requires ATP
Concentration gradient	Transports substance down concentration gradient	Transports substances down concentration gradient	Transports substances against concentration gradient
Transport process	Facilitated diffusion	Facilitated diffusion	Active transport
Examples	Sodium ion channel	Glucose carrier	Proton pump

Table 6.1. Comparison between the different types of transport protein.

- Substances are transported across the cell surface membrane by various processes:

    1. Osmosis
    2. Simple diffusion
    3. Facilitated diffusion
    4. Active transport
    5. Bulk transport (exocytosis and endocytosis)

6.2.1 Osmosis

- These substances dissolved in water are termed **solutes**, and water is a **solvent**. Water molecules **cluster** around molecules of a solute.

- Because some water molecules cluster around a solute when it is dissolved, there are less 'free' water molecules which can diffuse to other areas. This effectively **lowers the concentration** of water.

- **Water potential** is a measure of the **tendency** of free water molecules to **diffuse** to one area to another. The **more free water molecules there are**, the **higher the water potential**.

- **Osmosis** is the **net movement** of **water molecules** from a region of **higher water potential** to a region of **lower water potential,** across a **selectively permeable membrane.**

    - There are three types of solution that a living cell can be placed in.

        (i) Hypertonic solution – Solution contains a higher concentration of solutes, so it has a lower water potential.

        (ii) Hypotonic solution – Solution contains a lower concentration of solutes, so it has a higher water potential.

        (iii) Isotonic solution – Concentration of solutes on both sides of the membrane is the same, so the net movement of water is zero.

- It is a **passive process** as no energy input is required. The energy for movement comes from the water potential.

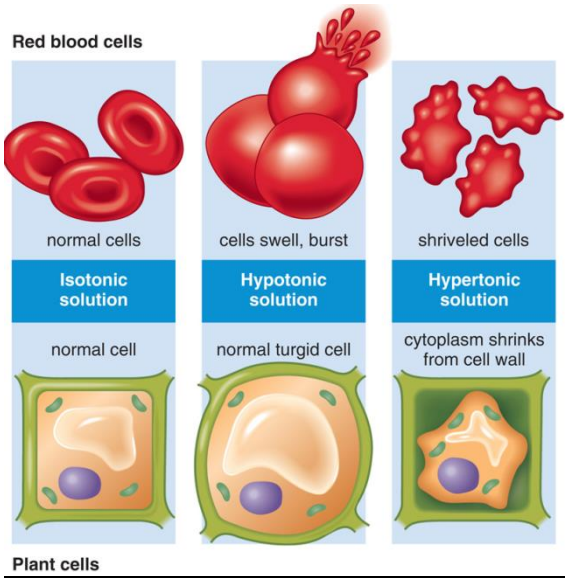


Fig. 6.16: Animal cells fare best in an isotonic environment while plant cells are generally healthiest in a hypotonic environment.

6.2.2 Simple diffusion

- **Particles possess kinetic energy, and move about randomly** until they are evenly distributed/ equilibrium is achieved.

- Diffusion is the **net movement** of particles from a region of high concentration to a region of low concentration, **down a concentration gradient**. It is a **passive process** as no energy input is required.

- Due to the presence of **hydrophobic region** of the membrane (made up of hydrophobic fatty acid chains of the phospholipids), substances that are **<u>non-polar or uncharged</u>** can diffuse directly across the phospholipid bilayer (Fig. 6.17).

- **<u>Polar (e.g. glucose) and charged (e.g. ions) molecules</u>** cannot diffuse across the phospholipid bilayer as the hydrophobic region of the membrane acts as a barrier (Fig. 6.17).

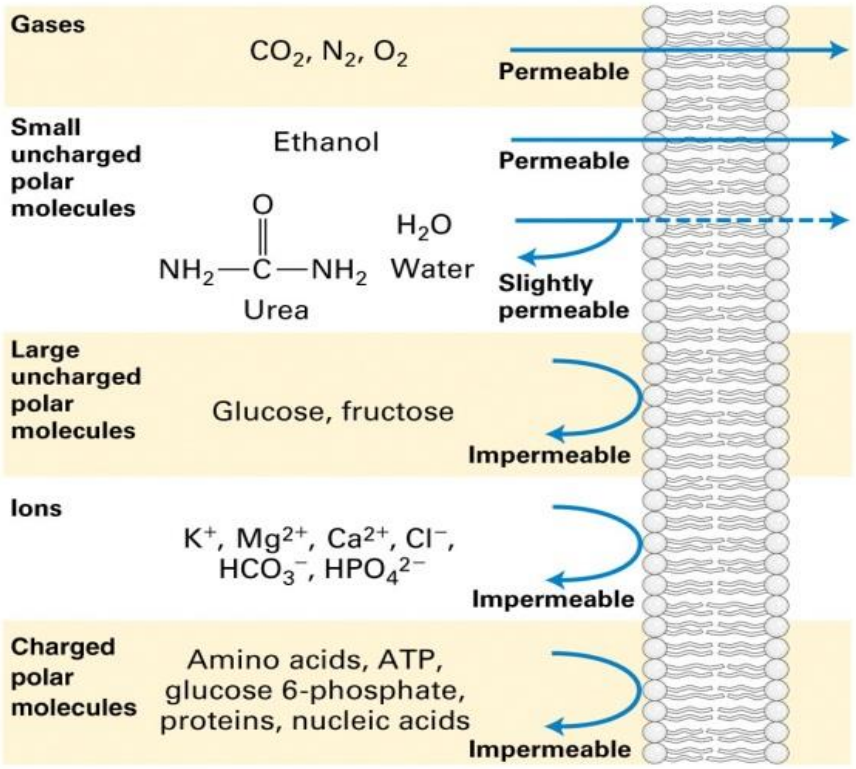


Fig. 6.17: An example of a nonpolar substance is oxygen gas.
An example of a charged substance is Na^+ ion, while an example of a polar substance is glucose.

6.2.3 Facilitated diffusion

- **Facilitated diffusion** is the **net movement** of particles **down a concentration gradient** via a transmembrane transport protein, which may be a channel protein or carrier protein (Fig. 6.18).

- **Polar** or **charged** substances are able to cross the membrane with the aid of these transport proteins, as these transport proteins shield these substances from the hydrophobic interior of the membrane and provide a **hydrophilic passage** for these substances to pass through.

- It is a **passive process** as no energy input is required.

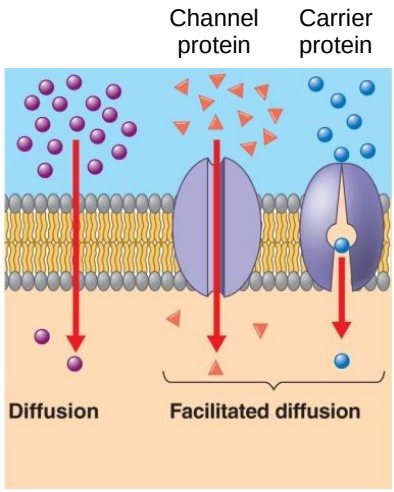


Fig. 6.18: Facilitated diffusion requires transport proteins, while simple diffusion does not require transport proteins.

What are some factors that affect the rate of facilitated diffusion?

6.2.4 Active transport

- Living cells are able to absorb certain substances even though these substances are of high concentration inside the cell than they are in the external environment. These substances are important for the cell to grow and survive.

- Active transport is the process of transporting substances **against its concentration gradient** from a region of lower concentration to a region of higher concentration.

- It is an **active process** as energy input from the **hydrolysis of adenosine triphosphate (ATP)** is required.

    - ATP hydrolysis is the reaction by which chemical energy that has been stored in the high-energy phosphate bond in ATP is released.

    - When ATP is hydrolysed to ADP, the phosphate group released from ATP is shifted to the transport protein.

    - This induces a **conformational change** in the transport protein, thus translocating the ion or molecule across the membrane.

- Active transport is performed by specific transmembrane **protein pumps** embedded in the membrane.

- Examples of protein carriers/pumps include:

    (i) **Proton pump** (Fig. 6.19) on lysosomal membranes.

    ❖ H+ from the cytosol is pumped into the lysosome to maintain its acidic pH. This is important as lysosomal enzymes are only active at acidic pH.

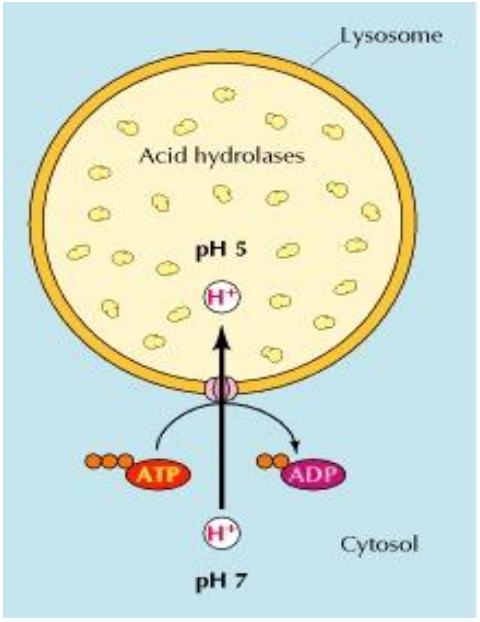


Fig. 6.19: A proton pump on lysosomal membrane moving protons against a concentration gradient, at the expense of ATP.

(ii) **Sodium-potassium pump** (Fig. 6.20) located on the cell surface membrane of all animal cells.

- ❖ An animal cell typically contains higher concentrations of potassium and lower concentrations of sodium inside the cell.
- ❖ The sodium-potassium pump uses the **energy** from the hydrolysis of **one ATP** molecule to pump **three sodium ions out** of the cell and **two potassium ions** that it pumps **into** the cell.
- ❖ This restores the electrochemical gradient across the membrane, which is especially important in nerve and muscle cells.

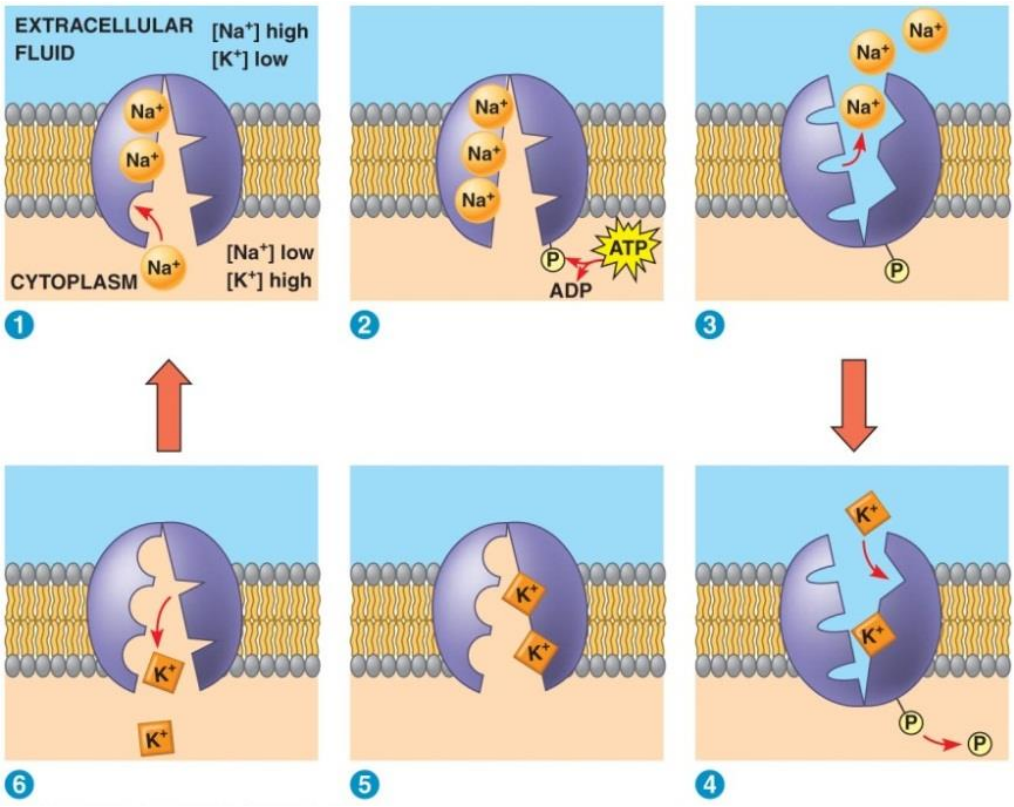


Fig. 6.20: The mechanism of action of a sodium-potassium pump.

6.2.5 Bulk transport (Endocytosis and Exocytosis)

- Certain particles are too large (E.g. polysaccharides or proteins) to diffuse through the membrane. Hence they cross the membrane by bulk transport, which is the transport of material into or out of a cell by enclosing it within a fluid-filled, membrane bound sac called **vesicles**.

- In bulk transport, materials are taken into cells by **endocytosis** (Fig. 6.21a), while materials are **secreted** out of the cells by **exocytosis** (Fig. 6.21b).

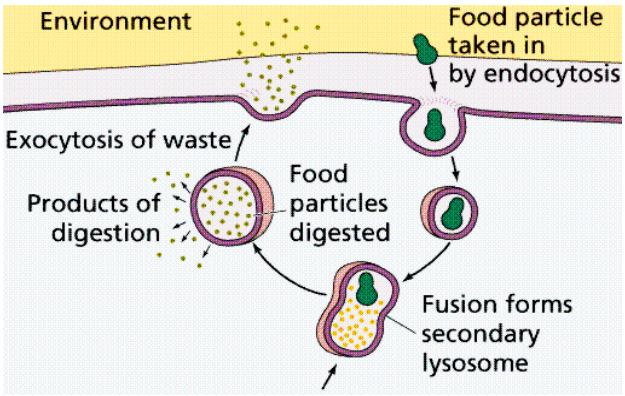


Fig. 6.21a: Endocytosis of food and exocytosis of waste

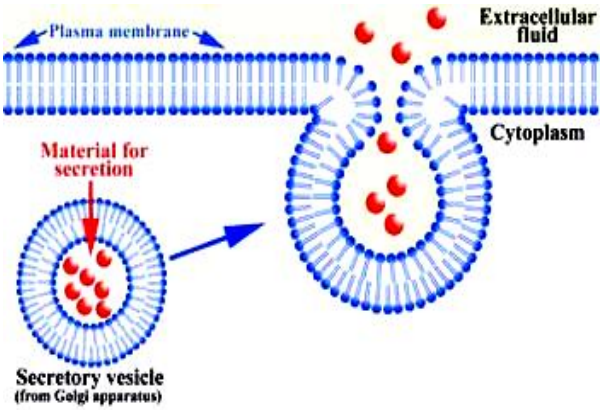


Fig. 6.21b: Secretion by exocytosis

- Both are **active processes** that require energy input. Energy is required for the movement of vesicles (e.g. to the cell surface membrane) along the **microtubules** of the cell cytoskeleton (Fig. 6.21c).

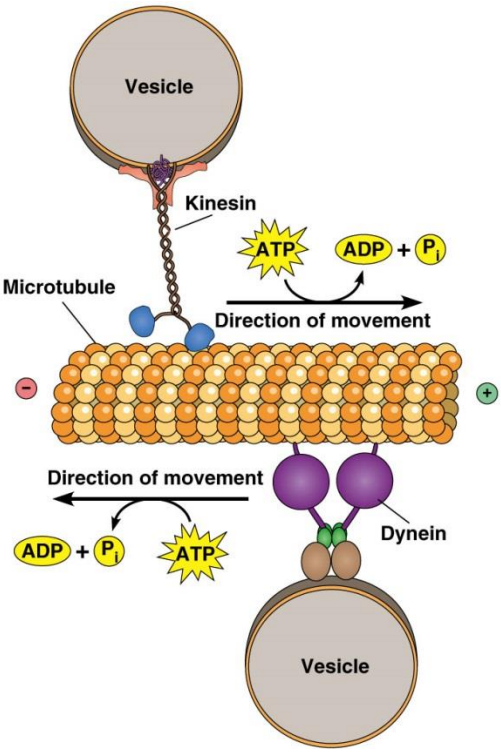


Fig. 6.21c: Movement of vesicles along microtubules occurs with the aid of motor proteins (kinesin and dynein). These motor proteins are powered by energy from ATP hydrolysis.

a) Endocytosis

- Endocytosis is the process by which a cell brings in macromolecules and particulate matter, by forming new vesicles from the cell surface membrane.

- During endocytosis, a small area of the cell surface membrane sinks inward to form an invagination (infolding). As the invagination deepens, it pinches inwards, forming a vesicle containing the material that had been outside the cell. This vesicle is called an endosome.

- There are 3 types of endocytosis (Fig. 6.22):

  (i) **Phagocytosis** ("cellular eating")

  - Process whereby a phagocytic cell engulfs a **solid particle** (eg. food, bacterium).
  - The cell surface membrane extends **pseudopodia** (**cytoplasmic extensions**) around it the particle. The pseudopodia fuse to form a large vesicle around the particle, known as a **phagosome**.
  - The contents of the vacuole are digested when the phagosome fuses with a primary lysosome to form a secondary lysosome.
  - Phagocytosis is a **specific** process (eg. amoeba ingests only particles of nutritional value, while phagocytic white blood cells engulf bacteria)

  (ii) **Pinocytosis** ("cellular drinking")

  - Process whereby a cell **invaginates** a region of the cell surface membrane, forming a **vesicle** around a small volume of **extracellular fluid.** It is not the fluid itself but the dissolved solutes that is needed by the cell.
  - This is a **non-specific** process.

  (iii) **Receptor-mediated endocytosis (RME)**

  - Process by which a cell can acquire **specific molecules** (e.g. cholesterol), even those that may be in low concentrations in the extracellular fluid.
  - The specific ligands/molecules (e.g. cholesterol-LDL) bind to proteins embedded on the cell surface membrane (e.g. LDL receptors). These proteins have binding sites that are specific to the ligands.
  - After binding, the receptor proteins cluster in regions of the membrane called **coated pits**, which are lined on their cytoplasmic side by a layer of coat proteins (e.g. clathrin).
  - Each coated pit forms a vesicle containing the ligand molecules.
  - After the ligand molecules are released from the vesicle, the vesicle (and receptors) is then recycled to the cell surface membrane.

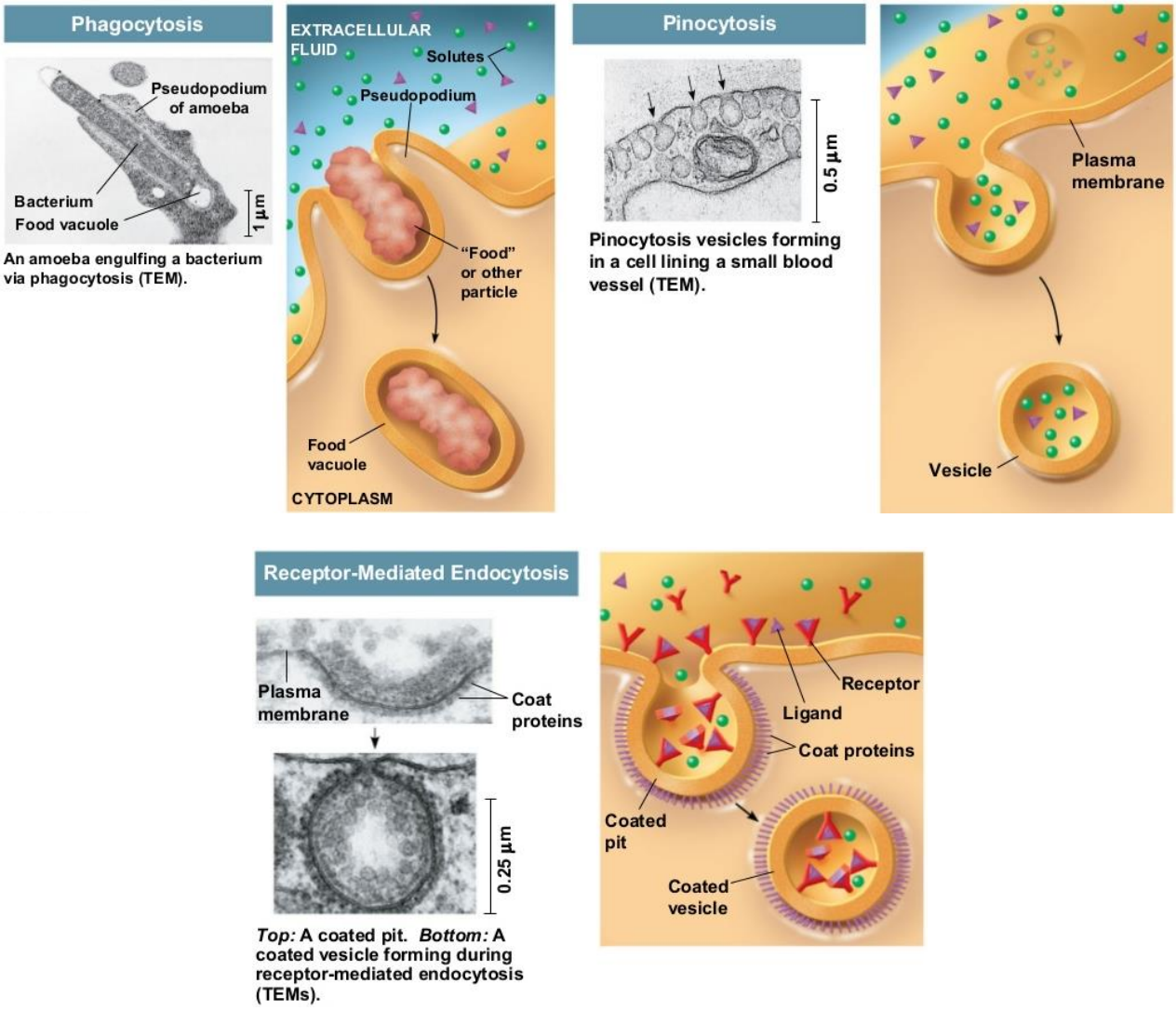


Fig. 6.22: The 3 main types of endocytosis

Additional Information

http://www.sumanasinc.com/webcontent/animations/content/organelles.html

Mitochondria and chloroplasts are organelles present in eukaryotic cells.
They are hypothesised to be derived from prokaryotic origin, due to the presence of 70S ribosomes and circular DNA.
Based on your knowledge on **endocytosis,** suggest a third feature of these two organelles that further support this hypothesis.

b) Exocytosis

- A **Golgi/ secretory vesicle** that buds off from the Golgi apparatus moves along the microtubules **towards** the cell surface membrane. The process whereby two phospholipid bilayers **fuse** to **release** the contents to the outside of the cell is known as **exocytosis**. The vesicle membrane then becomes part of the cell surface membrane (Fig. 6.23)

- Examples of exocytosis include
  - Secretion of insulin (hormone) by pancreatic beta cells.
  - Formation of plant cell walls (contain proteins and carbohydrates).

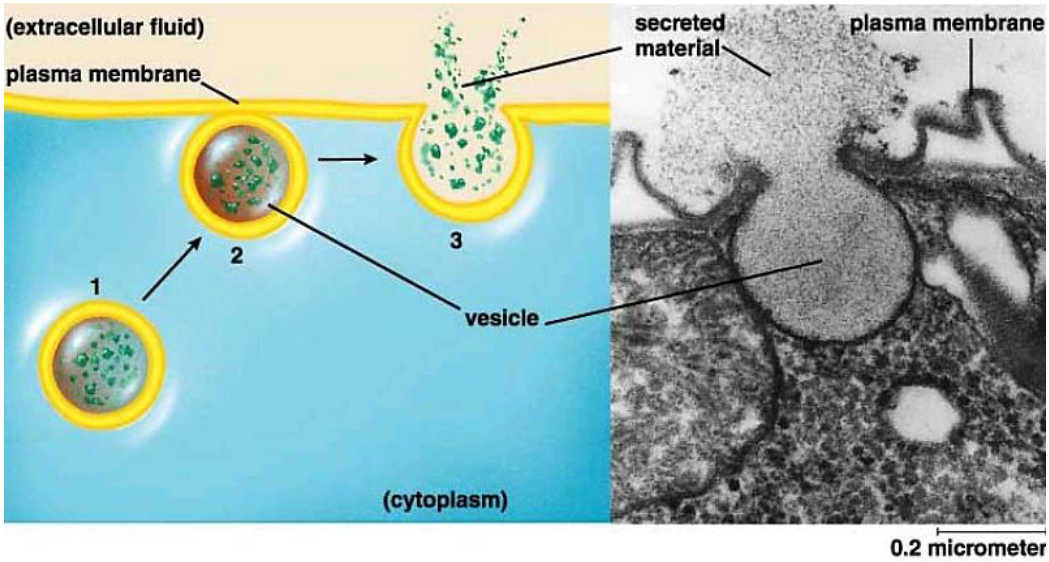


Fig. 6.23: The release of vesicle contents via exocytosis

6.3 Checklist for Membrane Structure and Function

Are you able to confidently answer all the questions?

1. Describe the fluidity of the components of a cell membrane and explain how membrane fluidity is influenced by temperature and membrane composition.

2. Explain how cholesterol resists changes in membrane fluidity with temperature change.

3. Distinguish between peripheral and integral membrane proteins.

4. List six major functions of membrane proteins.

5. Explain the role of membrane carbohydrates in cell-to-cell recognition.

6. Explain how hydrophobic molecules cross cell membranes.

7. Differentiate between active and passive transport across the membrane.

Glossary of Biological Terminology

- **-ase** Terms with suffix *–ase* are usually **enzymes**. The prefix usually describes the **substrate** of the enzyme. E.g. cellul***ase***, prote***ase***, lip***ase***, etc.
- **Glyco-** Terms with the *glyco-* prefix usually involve carbohydrates. E.g. ***glyco****gen*, ***glyco****lipid* (**lipid attached to a carbohydrate chain**), ***glyco****protein* (**protein attached to a carbohydrate chain**).
- **Hydro-** Terms with the *hydro-* prefix usually have something to do with water.
- **Oligo-** Means "short". E.g. ***oligo****saccharide*, **a short carbohydrate chain**.
- **-philic** Terms with suffix *–philic* usually means that the substance has an affinity to the substance named by the prefix. (E.g. *hydrophilic*; attracted to water.)
- **-phobic** Terms with suffix *–phobic* usually means that the substance is repelled by or tends to avoid the substance named by the prefix. (E.g. *hydrophobic*; repelled by water.)
- **Residues** Same meaning as the term *monomer* or building blocks.
- **Saccharide** Terms containing this word usually involve carbohydrates/sugars. E.g. mono***saccharide***, di***saccharide***.
- **Scientific naming conventions—numbering terms**: Mono—one, di/bi-two, tri—three, etc. E.g. ATP stands for **adenosine triphosphate**.
- **Activation energy**: The amount of energy that reactants must absorb before a chemical reaction will start; also called free energy of activation.
- **Active site**: The specific portion of an enzyme that binds the substrate by means of multiple weak interactions and that forms the pocket in which catalysis occurs.
- **Allosteric regulation**: The binding of a regulatory molecule to a protein at one site that affects the function of the protein at a different site.
- **Catalyst** *(kat'-uh-list)*: A chemical agent that increases the rate of a reaction without being consumed by the reaction.
- **Chemical bond**: An attraction between two atoms, resulting from a sharing of outer-shell electrons or the presence of opposite charges on the atoms. The bonded atoms gain complete outer electron shells.
- **Coenzyme**: An organic molecule serving as a cofactor. Most vitamins function as coenzymes in metabolic reactions.
- **Cofactor**: Any non-protein molecule or ion that is required for the proper functioning of an enzyme. Cofactors can be permanently bound to the active site or may bind loosely with the substrate during catalysis.
- **Enzyme**: A macromolecule serving as a catalyst, which is a chemical agent that changes the rate of a reaction without being consumed by the reaction.
- **Enzyme-substrate complex**: A temporary complex formed when an enzyme binds to its substrate molecule(s).
- **Feedback inhibition**: A method of metabolic control in which the end product of a metabolic pathway acts as an inhibitor of an enzyme within that pathway.
- **Metabolism**: The totality of an organism's chemical reactions, consisting of catabolic and anabolic pathways, which manage the material and energy resources of the organism.
- **NAD**: Nicotinamide adenine dinucleotide; a coenzyme that can accept an electron and acts as An electron carrier in the electron transport chain.
- **pH**: A measure of hydrogen ion concentration equal to –log [H+] and ranging in value from 0 to 14.

THE END