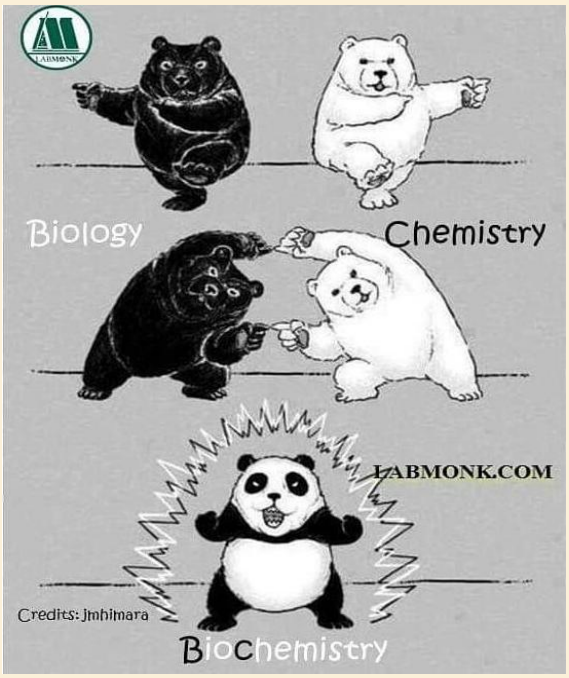
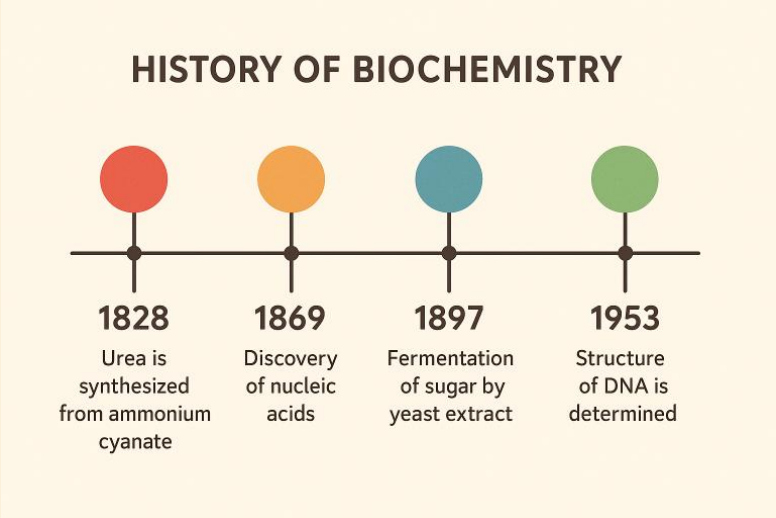
Hey guys! Hope your summers are going well so far. We are glad that you chose this course and we hope to provide you with all the resources so that you can explore the topic of Biochemistry and Biochemical Engineering very well. This module introduces you to the topic and terminology of Biochemistry and the basics which you will need to understand the further modules. So, let's kickoff with –



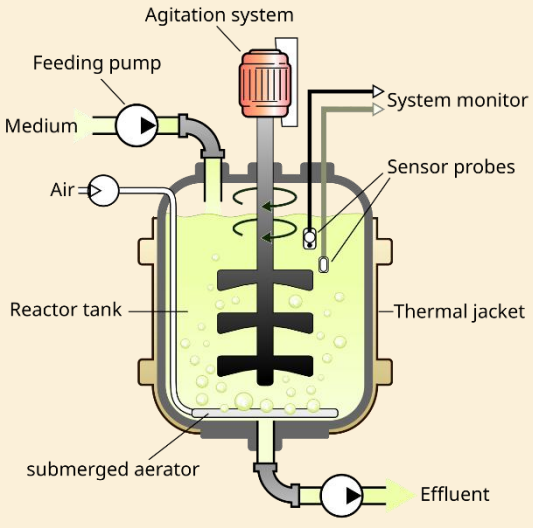
An Introduction and Brief History of Biochemistry

Biochemistry is the study of the chemical substances and processes in living things. It combines biology and chemistry to determine how molecules such as proteins, lipids, carbohydrates, and nucleic acids work to enable life. These molecules are involved in processes like metabolism, energy generation, cell communication, and genetic transmission. Enzymes, which are biochemical catalysts, facilitate the acceleration of chemical reactions in the body. The origin of biochemistry goes back to the 18th and 17th centuries when researchers started investigating physiological functions such as digestion and fermentation. The big breakthrough came in 1828 when Friedrich Wöhler prepared a sample of urea from an inorganic substance, refuting the belief that organic molecules could be formed by living organisms alone. The late 19th and early 20th centuries saw the

discovery of enzymes, vitamins, and hormones, as well as the mapping of metabolic pathways. In 1953, the discovery of the DNA double helix by Watson and Crick marked a turning point in molecular biology. Today, biochemistry continues to evolve with advances in genomics, biotechnology, and medicine, playing a vital role in understanding and improving life at the molecular level. Closely associated with biochemistry is the discipline of biochemical engineering, which uses biological and chemical principles to design large-scale pharmaceutical, biofuel, food, and industrial enzyme-producing processes. Biochemical engineers optimize and design bioreactors and fermentation systems, translating scientific discoveries into practical, real-world solutions in health, agriculture, and industry.



Fermentation Bioreactor



Continuous stirred-tank type Bioreactor

Introduction to Microbiology

Microbiology is the study of microscopic organisms, including bacteria, viruses, fungi, protozoa, and algae-very much a study of their structure, function, reproduction, and interaction with other life forms and the environment. Microorganisms are important in the cycling of nutrients, the making of diseases, biotechnology, and maintaining ecological equilibrium. An appreciation of microbiology has a bearing on medicine, agriculture, food production, and the environmental science process.

Microbiology is intimately linked with biochemistry in that the life processes of microbes-metabolism, respiration, and genetic expression-are governed by biochemical reactions. Many important biochemical discoveries such as enzyme action, genetic coding, and metabolic pathways were first made in microorganisms because they are much simpler and grow faster. For example, bacteria such as E. coli are particularly suitable for studies concerning gene expression and protein synthesis.

The relation between microbiology and biochemical engineering is equally important. Biochemical engineers usually apply the powers of microbes for production processes, for example, industrial production of ethanol by yeast or production of human insulin, some antibiotics, and enzymes by genetically modified bacteria. Growing microorganisms in bioreactors under optimal controlled conditions allows the engineers to scale up biochemical reactions for commercial purposes. Therefore, microbiology provides the biological background, biochemistry introduces the molecular mechanisms, and biochemical engineering uses the mechanisms practically for in vitro implementation. You will need some concepts of Microbiology in the further modules.

Next, we cover Biomolecules briefly.

Carbohydrates

Functions

- Cells are coated with dense and complex chains of carbohydrates.
- Secreted proteins often have extensive carbohydrate decorations **essential to their functions**.
- The extracellular environment in eukaryotes is **rich in carbohydrate secretions**.
    - These are central to **cell survival** and **intercellular communication**.
- Carbohydrates, carbohydrate-containing proteins, and specific carbohydrate-binding proteins:
    - Are **required for cell interactions** that allow formation of tissues.
    - **Form the basis of human blood groups**.

How is this possible?

- Carbohydrates have **tremendous structural diversity**, which enables their many biological functions.

Respect for Carbohydrates

- Their recognized importance has spawned two major fields of study:
    - **Glycobiology**:
        - Study of carbohydrate synthesis and structure.
        - Focuses on how carbohydrates are **attached to and recognized by other molecules** (like proteins).
    - **Glycomics**:
        - Study of the **glycome** — all the carbohydrates and carbohydrate-associated molecules a cell produces.

A Brief Detour – Proteomes

- Proteome is an entire set of proteins produced or modified by an organism, cell, tissue, or system under specific conditions.

A Chart of Isomers

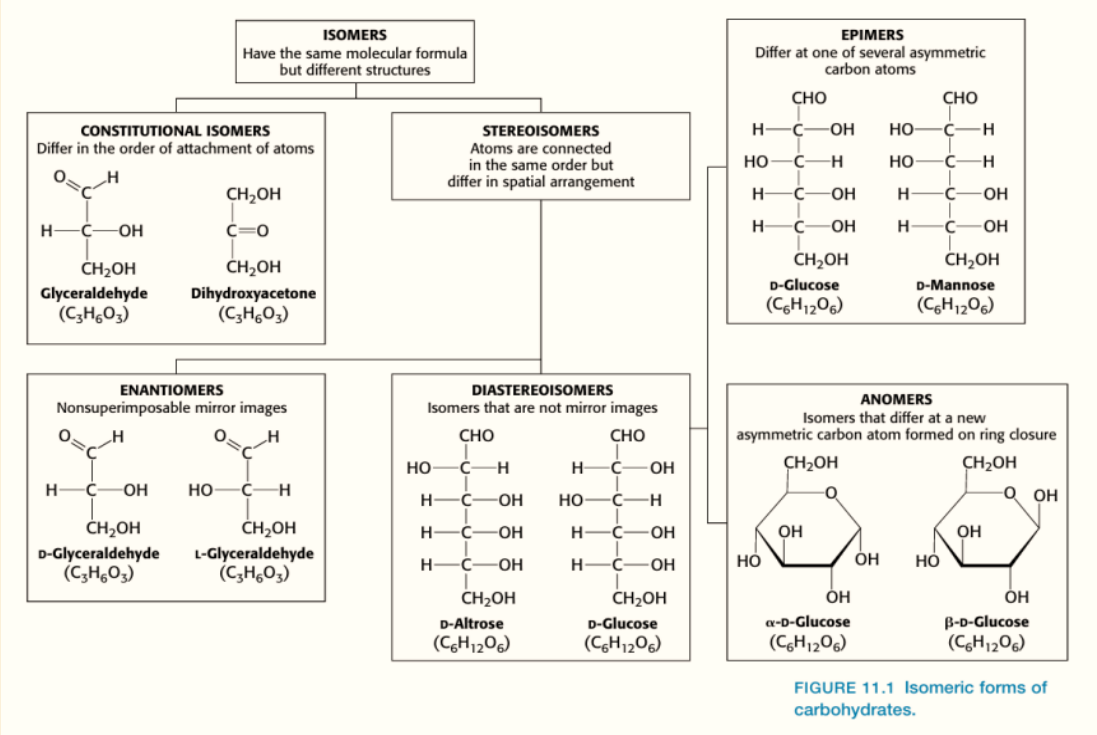


FIGURE 11.1 Isomeric forms of carbohydrates.

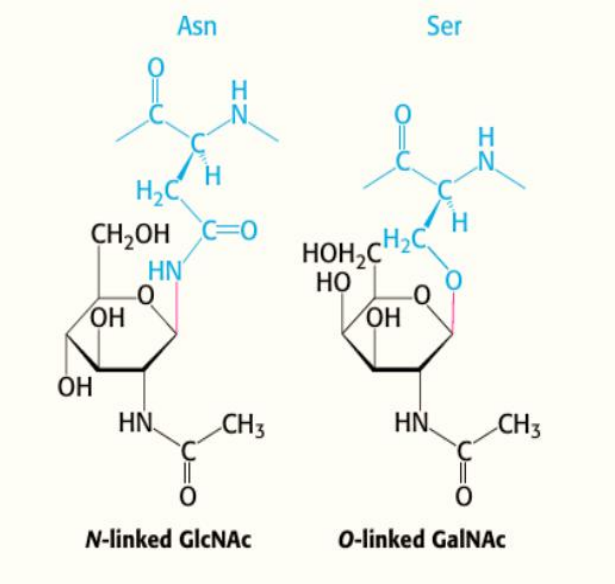
Glycoprotein – Carbohydrate & Protein Linkage

- A carbohydrate group **can covalently attach to a protein** to form a glycoprotein.
- About **50% of the proteome** consists of glycoproteins.
- Three classes of glycoproteins:
    1. Glycoproteins:
        - Components of **cell membranes**.
        - Involved in **cell adhesion** and **sperm–egg binding**.
        - The protein component is **largest by weight**.

2. Proteoglycans:
    - **Much higher carbohydrate content** than glycoproteins.
    - Function as **structural components** and **lubricants**.
3. Mucins (Mucoproteins):
    - Predominantly **protein**.
    - Key component of **mucus**.
    - Act as **lubricants**.

Glycosidic Bonds

- Sugars in glycoproteins are linked via:
    - **N-linkage**: To the **amide nitrogen** of **asparagine**.
    - **O-linkage**: To the **oxygen atom** of **serine or threonine** side chains.



Lipids

Lipids are hydrophobic organic compounds essential for energy storage, insulation, membrane structure, and signaling. They include triglycerides, phospholipids, waxes, and steroids.

Structure & Absorption:

- **Triglycerides**: Made of glycerol + 3 fatty acids.
- **Fatty Acids**: Even-chain hydrocarbon chains (14–24C), may be saturated or unsaturated.
- **Absorption**:
    - **Short-chain (≤12C)**: Absorbed into the portal vein → liver.

- **Long-chain**: Form chylomicrons → lymphatic system → blood → tissues → liver (as remnants).

Essential Fatty Acids (EFAs):

- **Omega-6**: Linoleic acid (LA), arachidonic acid (AA).
- **Omega-3**: ALA, EPA, DHA – crucial for cardiovascular and brain health.
- **Intake Recommendations**: Vary by age, sex, pregnancy/lactation.

Lipids in Biological Membranes

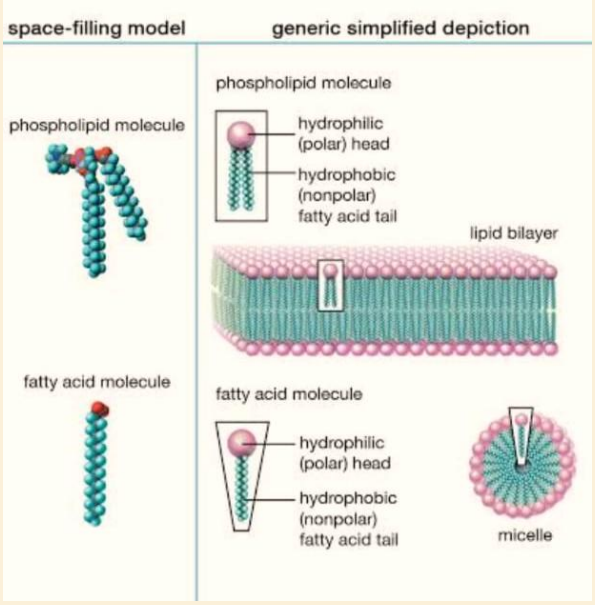
Lipids form dynamic, semi-permeable bilayers crucial for cellular function.

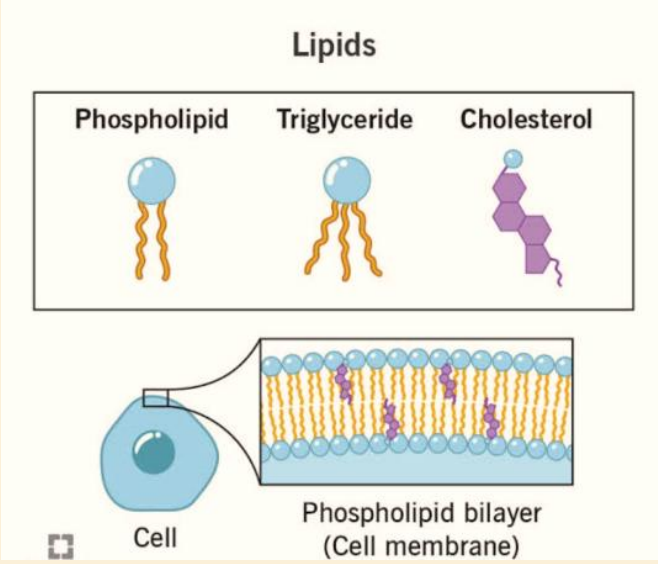
Phospholipids:

- Key types: PC, PE, PG, PI, PS.
- **sn1**: Usually saturated FAs; **sn2**: PUFAs like AA, DHA.

Physical Properties:

- **Polymorphism**: Lipids adopt bilayer, hexagonal (HII), or micellar forms.
- **Molecular Shape**: Dictates membrane structure (cylindrical vs. cone-shaped).
- **Cholesterol**: Modulates membrane fluidity and phase behavior.
- **Membrane Permeability**: High to water, low to most ions.





Lipid Nanoparticles (LNPs)

LNPs are advanced drug/gene delivery systems with broad biomedical applications.

A. Liposomes:

- Bilayer vesicles that encapsulate hydrophilic or hydrophobic drugs.
- Used in approved drugs (e.g., Doxil, Epaxal).
- **Benefits**: Improved solubility, reduced toxicity, longer circulation.
- **Challenges**: Immune responses (e.g., anti-PEG antibodies), toxicity from cationic lipids.

B. SLNs & NLCs:

- **SLNs**: Solid lipid core, introduced in 1991.
- **NLCs**: Improved version with higher drug loading, stability, and controlled release.

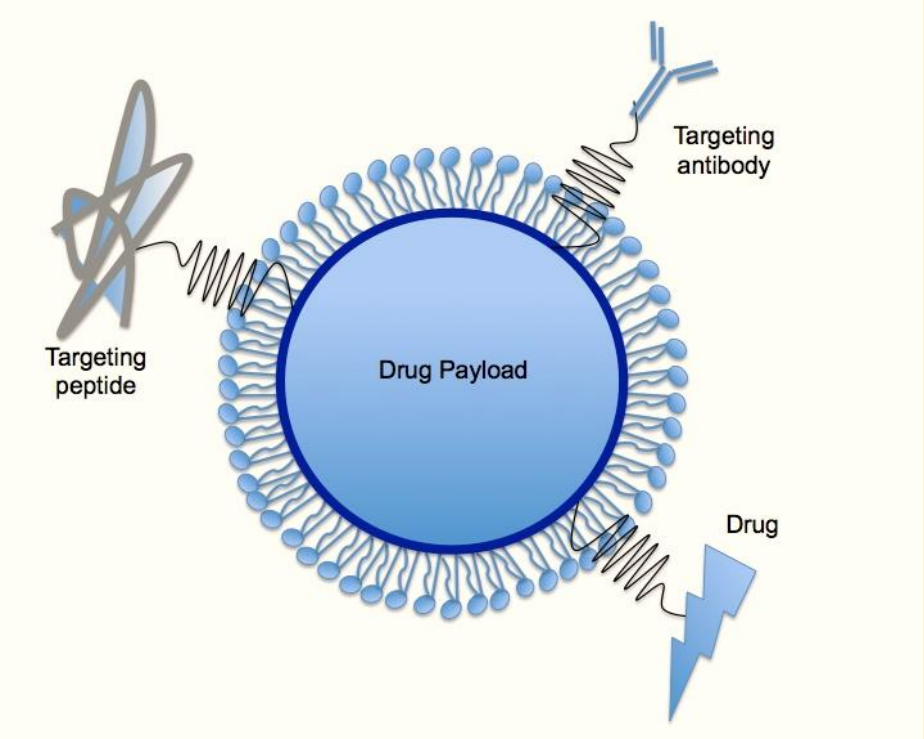
C. Targeted & Triggered Delivery:

- **Targeting**: Ligands (e.g., folate, antibodies) guide LNPs to specific cells.

- **Stimuli-Responsive Release**: Triggered by heat, pH, light, or gas production for localized therapy.
- **Nanoreactors**: LNPs carry enzymes or prodrugs to tumors for in situ therapeutic generation.

D. Other Uses:

- Imaging (e.g., radiolabeled, echogenic liposomes), gene delivery, agriculture, cosmetics.



A lipid-based nanoparticle that can be used for drug-delivery

Dietary Lipids and Health

Saturated Fatty Acids (SFA):

- Lowering SFA intake reduces LDL-C and cardiovascular risk.
- Best replaced with PUFA, MUFA, and soluble fiber.

Unsaturated Fats:

- **PUFA Sources**: Corn, soybean, sunflower oil.
- **MUFA Sources**: Olive oil, nuts, avocado.
- **Nuts & Seeds**: Improve lipid profiles due to healthy fats and fiber.

Other Factors:

- **Fiber**: Improves cholesterol.
- **Moderate Alcohol**: Raises HDL-C but may increase triglycerides.

Key Takeaways

- Lipids are essential for structural, metabolic, and signaling roles.
- Omega-3 and -6 intake influences inflammation and heart health.
- LNPs offer cutting-edge delivery of therapeutics and diagnostics.
- Dietary fat quality—not just quantity—greatly affects health outcomes.

Proteins

Amino Acids – The Building Block of Proteins

Proteins are the workhorses of the cell. Every function that a living cell performs—whether it's catalyzing reactions, replicating DNA, sensing signals, transporting molecules, or building structural components—is enabled by proteins. But despite this vast diversity of roles, all proteins are composed of just **20 standard amino acids**. This is remarkably efficient: the biological world has essentially evolved a 20-letter alphabet that can write an infinite variety of functional "words" (proteins).

At the molecular level, each of these amino acids has a similar **basic structure**. They all share a **central (α) carbon atom** that is **tetrahedral**, meaning it has four different groups attached to it. These four groups are:

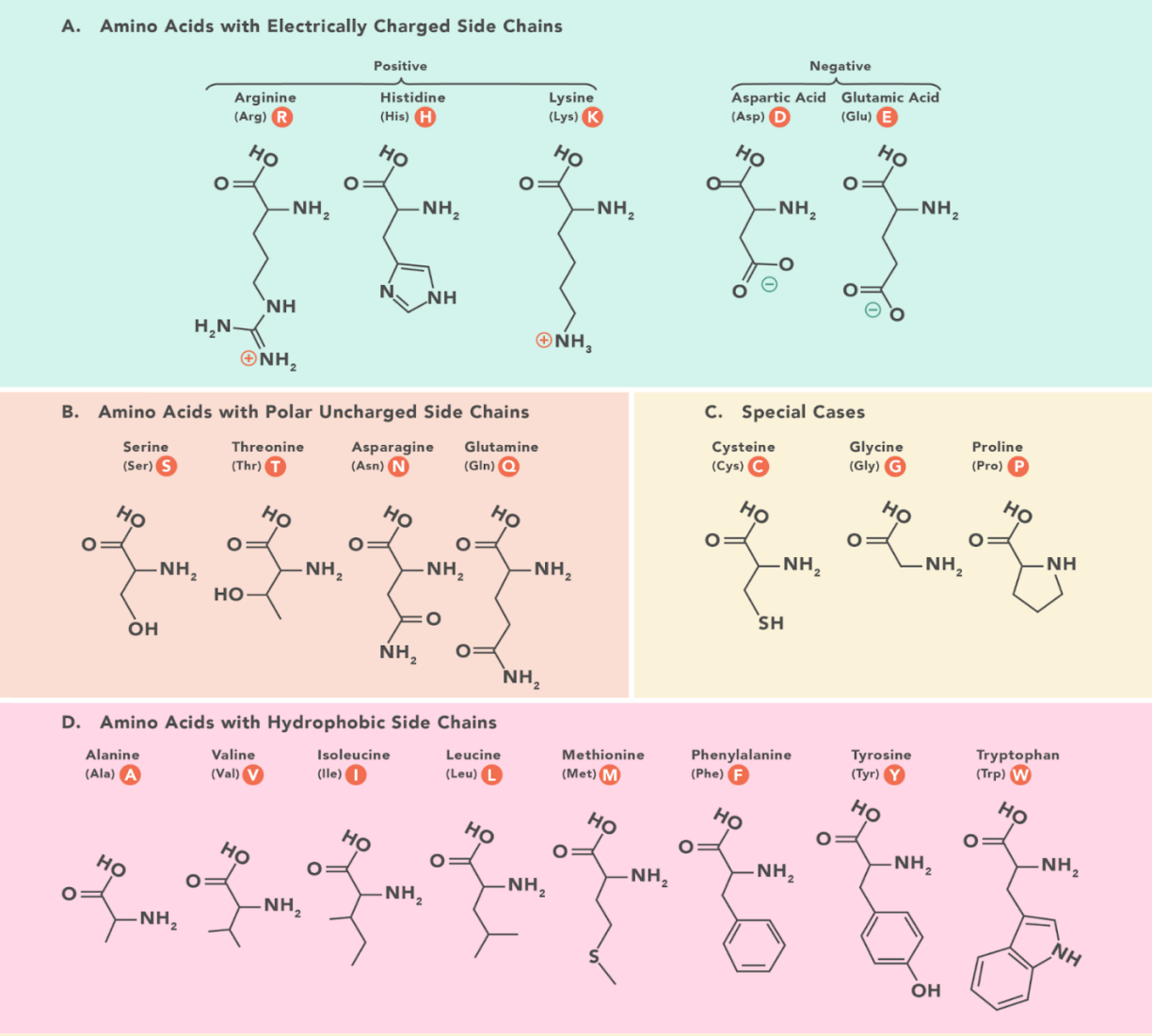
(1) an **amino group** ($-NH_2$, which is protonated to $-NH_3^+$ at physiological pH),

(2) a **carboxylic acid group** (–COOH, deprotonated to $–COO^-$ at physiological pH),

(3) a **hydrogen atom**, and

(4) a **variable side chain**, also called the **R group**. The **R group** determines the chemical nature of the amino acid—its size, shape, polarity, and reactivity.

Because of these four different groups, the central carbon atom (except in glycine) is a **chiral center**. This means the amino acid can exist in two **stereoisomeric forms**, L and D, which are mirror images of each other. In proteins, only the **L-form** is used. This stereochemical consistency is thought to be a result of evolutionary selection and is critically important because the 3D folding of proteins depends on the spatial orientation of these amino acids. Amino acids are **zwitterions** at neutral pH—molecules with both positive and negative



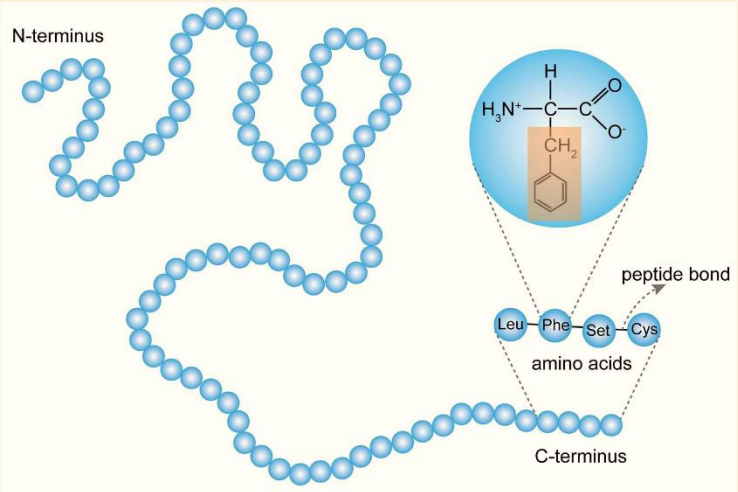
charges but an overall neutral charge. The amino group is protonated, while the carboxylic acid is deprotonated. This unique property affects their **solubility, reactivity**, and **behavior in peptide bond formation**.

Primary Structure: Amino Acids Are Linked by Peptide Bonds to Form Polypeptide Chains

Once we have a collection of amino acids, they must be **linked together** to form functional proteins. This linking occurs through a specific chemical reaction: the **formation of a peptide bond**. A peptide bond is formed when the **carboxyl group (–COOH)** of one amino acid reacts with the **amino group (–NH_2)** of another, releasing a molecule of **water** (H_2O). This type of reaction is known as a **condensation** or **dehydration** reaction. The resulting bond—**CO–NH**—is known as a **peptide bond** or **amide bond**.

What makes this bond unique is its **partial double bond character**. This means that the bond between the carbon and nitrogen in the peptide linkage is not a free single bond—it has **resonance** between a single and a double bond due to electron delocalization. As a result, this bond is **planar** and **does not rotate freely**. creating **rigidity** in the backbone of the protein.

Peptide chains are **directional**, meaning they have two chemically distinct ends. One end has a free **amino group** (called the **N-terminus**), and the other has a free **carboxyl group** (**C-terminus**). By convention, amino acid sequences are written from the N-terminus to the C-terminus



Peptides longer than about 50 amino acids are typically called **polypeptides**, and when they fold into a functional shape, they are called **proteins**.

Importantly, the **primary structure**—the linear sequence of amino acids—is **coded by genes**. A mutation in a gene leads to a change in the primary structure, which may alter the final protein's shape and function, often with devastating effects (e.g., sickle cell anemia, cystic fibrosis).

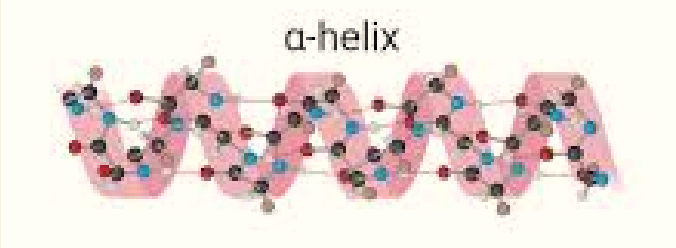
Secondary Structure: Polypeptide Chains Can Fold into Regular Structures Such as the Alpha Helix, the Beta Sheet, and Turns and Loops

After the amino acids are joined into a linear chain, this chain begins to fold locally into specific, **repeating structures** driven by **hydrogen bonds** between backbone atoms. These local folding patterns are known as **secondary structures**, and the two most common types are the **alpha helix (α-helix)** and the **beta sheet (β-sheet)**.

Alpha Helix

The α-helix is a **right-handed spiral** in which the backbone coils tightly, with side chains projecting outward. Each carbonyl oxygen (C=O) of residue *i* forms a **hydrogen bond** with the amide hydrogen (N–H) of residue *i + 4*, stabilizing the structure.

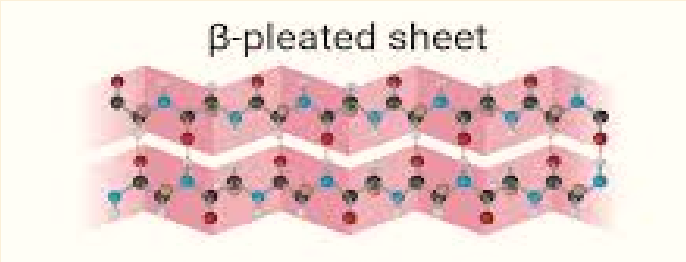
Certain amino acids favor or disfavor α-helix formation. **Alanine** is a strong helix-former. **Proline** is a known **helix breaker**. **Glycine**, being extremely flexible, also tends to destabilize helices.



Beta Sheet

In β-sheets, the polypeptide backbone is **fully extended**, not coiled. Strands align side-by-side and form **inter-strand hydrogen bonds**. If the strands run in opposite directions, it's an **antiparallel β-sheet**; if they run in the same direction, it's a **parallel β-sheet**. The **side chains alternate** above and below the sheet, making it a useful scaffold in protein interiors or surfaces.

Beta sheets are often twisted rather than perfectly flat. These sheets can be **pure (all strands same direction)** or **mixed** (both orientations).

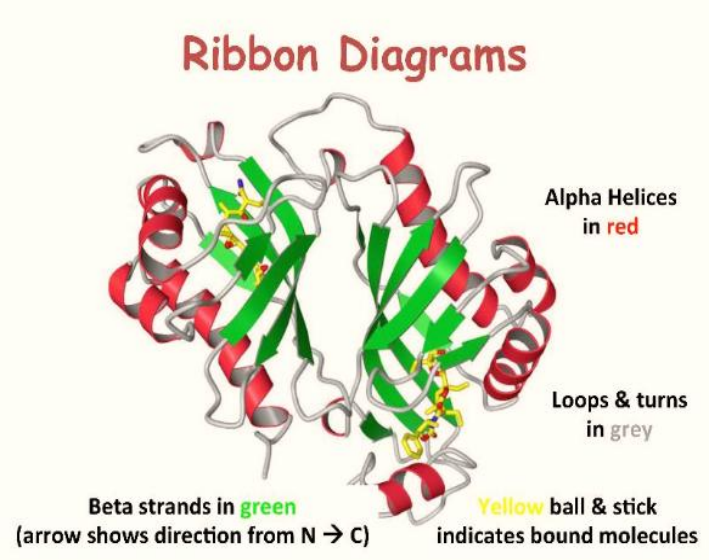


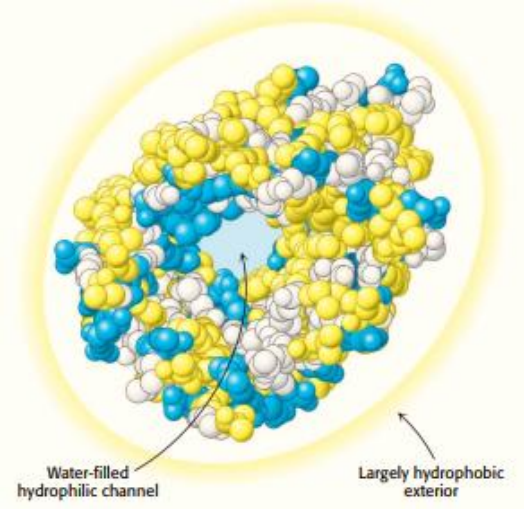
Tertiary Structure: Water-Soluble Proteins Fold into Compact Structures with Nonpolar Cores

The **tertiary structure** of a protein refers to the **overall 3D shape** of a single polypeptide chain, including all its secondary structure elements and the spatial relationships among its side chains. This structure is **unique and specific**, and it's the final folded form that is **biologically active**.

One of the strongest forces driving tertiary folding is the **hydrophobic effect**. Water is a polar solvent, and it excludes nonpolar molecules. As a result, **nonpolar side chains (like valine, leucine, phenylalanine)** cluster together in the **interior** of the protein, while **polar and charged side chains** are generally found **on the surface**, where they can interact with water. This **sequestration of hydrophobic residues** is energetically favorable and is the main reason proteins fold.

Hydrogen bonds, **ionic interactions**, **disulfide bonds**, and **van der Waals forces** further stabilize the tertiary structure.





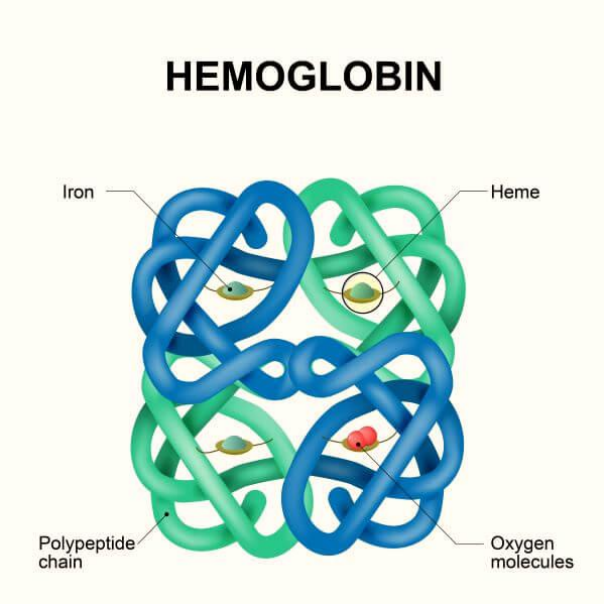
'Inside out' amino acid distribution in porin: The outside of porin (which contacts hydrophobic groups in membranes) is covered largely with hydrophobic residues, whereas the centre includes a water-filled channel lined with charged and polar amino acids.

Quaternary Structure: Polypeptide Chains Can Assemble into Multi-subunit Structures

Some proteins consist not of one but **multiple polypeptide chains**, each called a **subunit**. The **quaternary structure** refers to the **spatial arrangement** and interactions among these subunits.

These subunits can be:

- **Homomeric**: All subunits are identical (e.g., lactate dehydrogenase)
- **Heteromeric**: Subunits are different (e.g., hemoglobin: 2 alpha + 2 beta)



The forces that bind subunits together are similar to those in tertiary structure—**noncovalent interactions** such as hydrogen bonding, hydrophobic effects, ionic interactions, and sometimes **disulfide bridges**. This assembly is **not random**; it's required for **function**, **stability**, or **regulation**.

Hemoglobin, for example, uses **allosteric regulation**—its quaternary structure changes when oxygen binds, enhancing cooperative binding across subunits.

The Amino Acid Sequence of a Protein Determines Its Three-Dimensional Structure

This section underscores a profound principle of molecular biology: **the primary sequence of a protein determines its 3D structure**, which in turn determines its **function**.

Each protein's sequence is encoded in **DNA**, transcribed into **mRNA**, and translated by ribosomes into a linear amino acid chain. As soon as this chain emerges, it begins to fold.

Any **mutation** (change in amino acid) can disrupt folding, rendering the protein dysfunctional or even toxic. Diseases such as **Alzheimer's**, **Parkinson's**, and **Huntington's** involve protein misfolding and aggregation.

Understanding how sequence leads to structure is the heart of **structural biology**, and the foundation for modern **protein engineering**, **drug design**, and **synthetic biology**.

Enzymes:

Highly specific catalysts. They are very good at catalyzing one specific reaction. Mostly Globular proteins.

Vitamins:

Organic compounds required in the diet in small amounts to perform specific biological functions for normal maintenance of optimum growth and health of the organism.

Nucleic Acids:

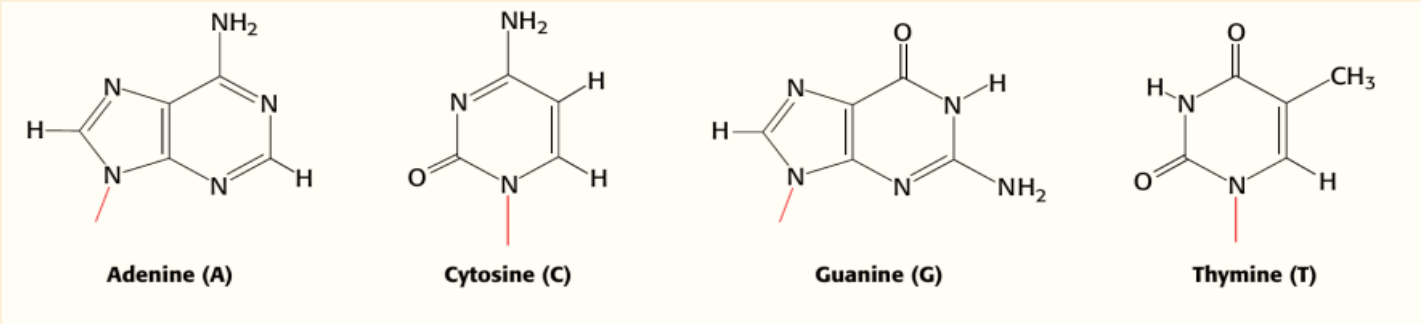
Molecules responsible for heredity. They are known for passing down characteristics from one generation to the next.

Hormones:

Intercellular messengers. If there is excess secretion of one compound then an appropriate hormone is sent to the site of action to reduce production and the opposite also happens.

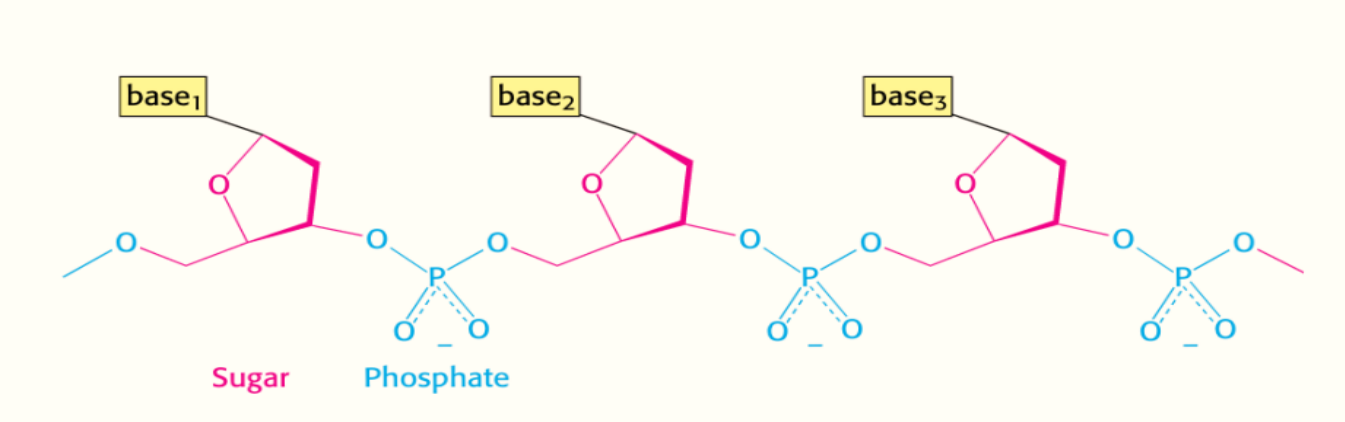
DNA

DNA is a linear polymer made up of four different types of monomers. It has a fixed backbone from which protrude variable substituents, referred to as bases. The backbone is built of repeating sugar– phosphate units. The sugars are molecules of deoxyribose from which DNA receives its name. Each sugar is connected to two phosphate groups through different linkages. Moreover, each sugar is oriented in the same way, and so each DNA strand has



directionality, with one end distinguishable from the other. Joined to each deoxyribose is one of four possible bases: adenine (A), cytosine (C), guanine (G), and thymine (T).

These bases are connected to the sugar components in the DNA backbone through the bonds shown in black in below figure. All four bases are planar but differ significantly in other respects. Thus, each monomer of DNA consists of a sugar–phosphate unit and one of four bases attached to the sugar. These bases can be arranged in any order along a strand of DNA.



The above figure depicts a single strand of DNA.

Now, let's take two strands of DNA and the structure they form.

α – Helix structure of two DNA strands:

Most DNA molecules consist of not one but two strands. In 1953, James Watson and Francis Crick deduced the arrangement of these strands and proposed a three-dimensional structure for DNA molecules. This structure is a double helix composed of two inter twined strands arranged such that the sugar–phosphate backbone lies on the outside and the bases on the inside.

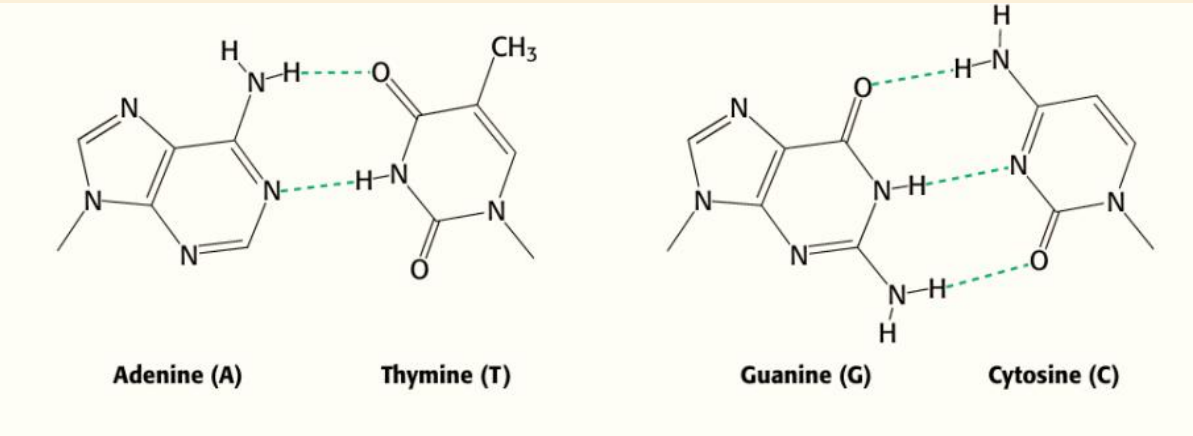
The key to this structure is that the bases form specific base pairs (bp) held together by hydrogen bonds:

• Adenine pairs with Thymine (A–T)

• Guanine pairs with Cytosine (G–C)

Hydrogen bonds are much weaker than covalent bonds such as the carbon-carbon or carbon– nitrogen bonds that define the structures of the bases themselves. Such weak bonds are crucial to biochemical systems; they are weak enough to be reversibly broken in

biochemical processes, yet they are strong enough, particularly when many form simultaneously, to help stabilize specific structures such as the double helix.



Q:

For a double strand DNA, one strand is given below:

The amount of energy required to split the double strand DNA into two single strands is _____ kcal mol−1.

[Given: Average energy per H-bond for A-T base pair = 1.0 kcal mol^{-1}, G-C base pair = 1.5 kcal mol^{-1}, and A-U base pair = 1.25 kcal mol^{-1}. Ignore electrostatic repulsion between the phosphate groups.]

Ans:41 kcal mol^{-1}

Now, why do we need Chemistry?

By understanding the **chemical nature** of how bases can form hydrogen bonds, scientists were able to explain **how genetic information is stored, duplicated, and passed on**—a foundational insight into **molecular biology and genetics**. In many similar ways, chemical concepts such as types of chemical bonds; the structure of water, the solvent in which most biochemical processes take place; the First and Second Laws of Thermodynamics; and the principles of acid–base chemistry led to many important biological implications.

Chemical Concept	**Biological Implication**
Type of Chemical Bonds	Explains structure, shape and stability
Thermodynamics	Explains energy flow, reaction spontaneity
Acid-Base reactions	pH affects **enzyme function, membrane potential,** and **metabolic pathways.**
Properties of Water	Explains solubility, molecular interactions

So, yes, everything is about structure and stability here, without understanding thermodynamics, you wouldn't know **why DNA strand hybridization releases heat, or why some reactions happen spontaneously while others require energy input.**

That's all for Module 1. For your ease, we have compiled all the references in the next page, where you can explore about these topics in an even greater depth. Hope you enjoyed this module and are excited for the next one.

Happy Learning !!!

REFERENCES

#1: Biochemistry by Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto Jr., Lubert Stryer

#2: A Biochemistry Playlist by the YouTube Channel - Prof. Dave Explains

#3: A playlist by MIT OpenCouseWare

#4: Visualisation of DNA

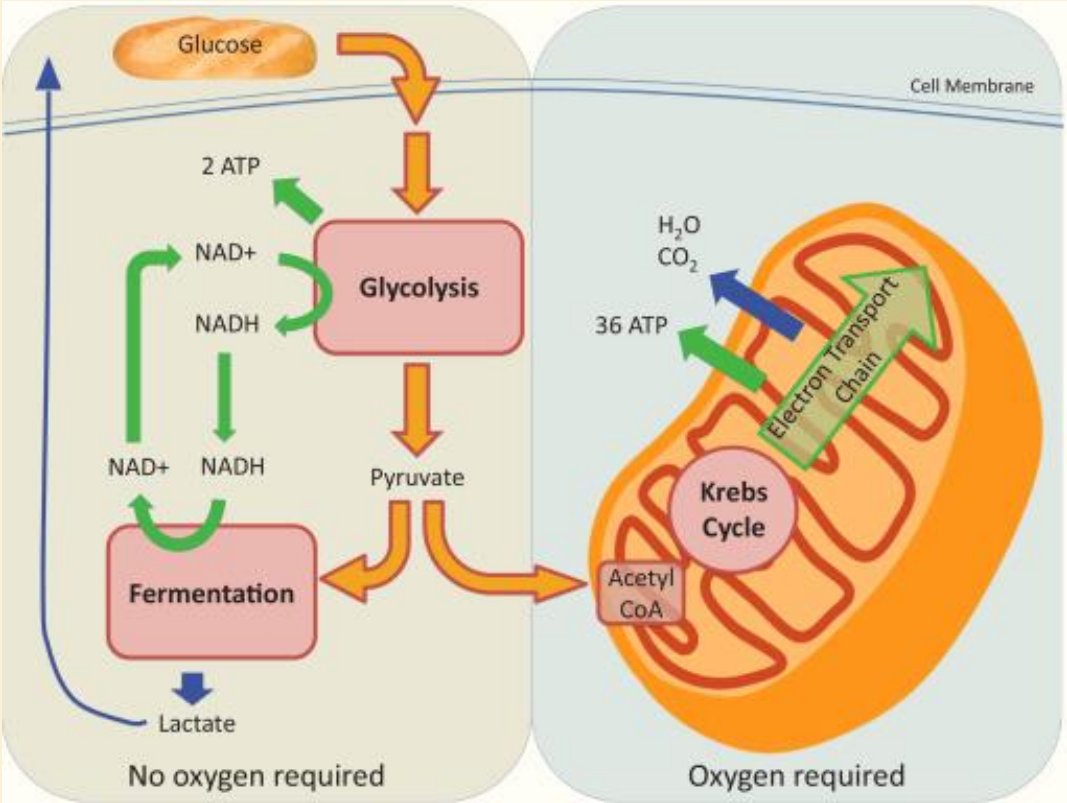
Hey Guys! Hope you all enjoyed Module-1 and Quiz-1. Module-2 covers Metabolism and energy aspects along with Biosynthesis of vital Biomolecules and Response to Environmental Changes.

Metabolism

Metabolism is the system of the chemical reactions that take place within a living organism for sustaining life. Metabolic pathways into catabolic and anabolic types. Catabolic pathways liberate energy by degrading complex molecules into simpler ones, whereas anabolic pathways use energy in order to build up complex biomolecules.

ATP (adenosine triphosphate) is the primary cellular energy currency. Hydrolysis of ATP is thermodynamically favorable and tends to drive other nonspontaneous reactions. Energy derived from the oxidation of carbon fuels, like glucose and fatty acids, is applied to build up ATP, which is utilized to energize cellular processes.

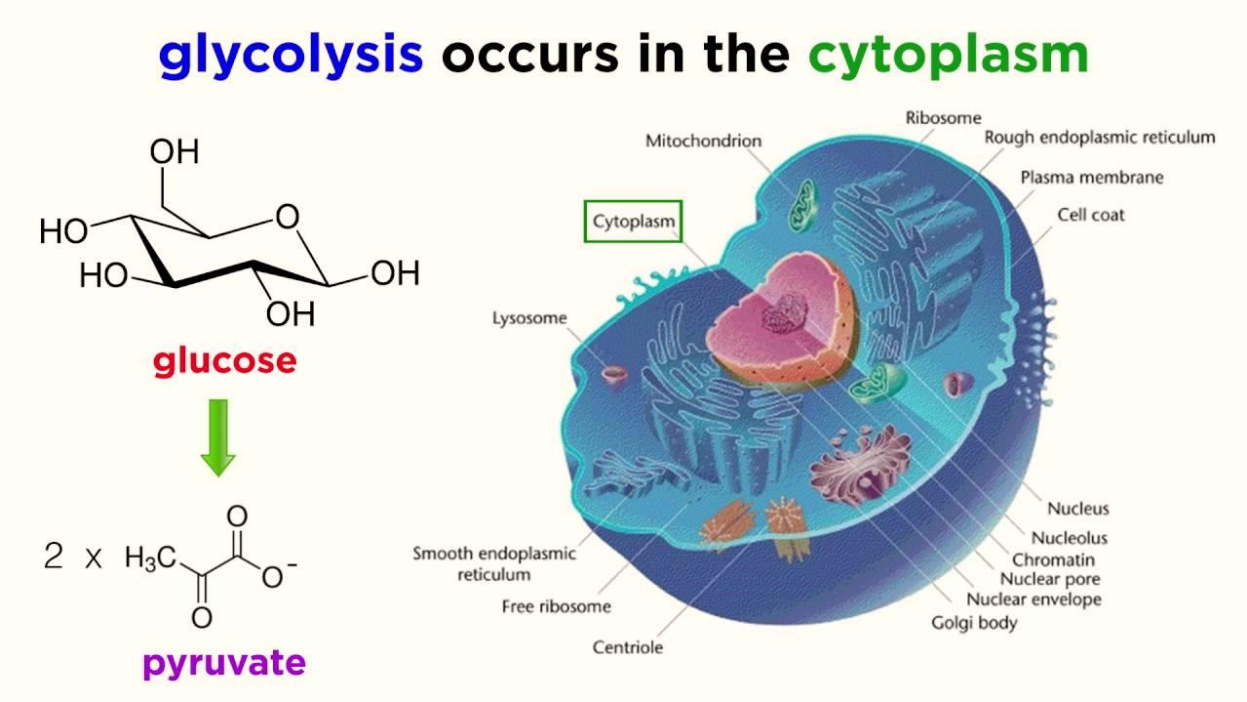
At the center of these processes are molecules called activated carriers, such as NADH, $FADH_2$, and CoA, which temporarily retain energy or chemical groups. These carriers tend to be vitamin-derived and are critical to energy transfer in the cell. Control of metabolism includes mechanisms such as allosteric control, covalent modification, and compartmentalization of enzymes. These regulatory characteristics guarantee that metabolic reactions take place only when necessary.



Glycolysis and Gluconeogenesis

Glycolysis is a ten-step process that breaks glucose down into pyruvate. It produces a small amount of ATP and NADH. When there is no oxygen, pyruvate turns into lactate or ethanol to regenerate NAD^+. This allows glycolysis to keep going. In the presence of oxygen, pyruvate undergoes further oxidation through the citric acid cycle.

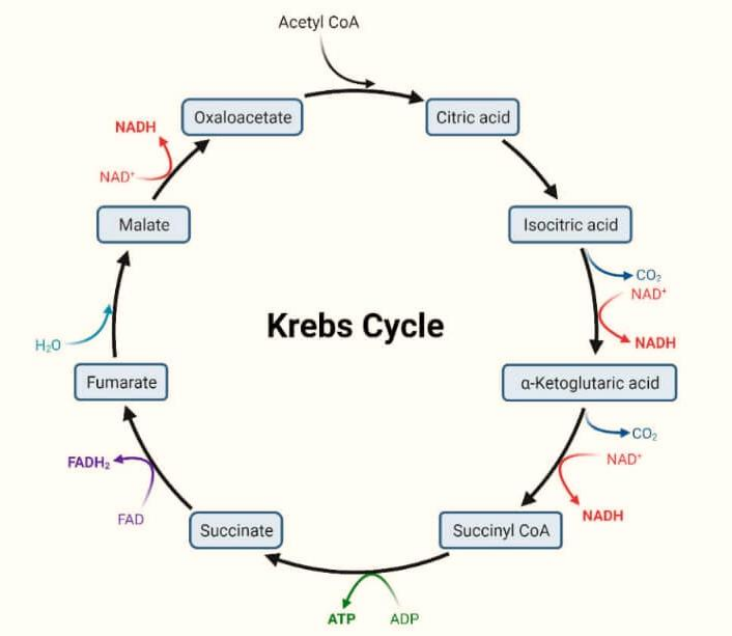
When glucose levels are low, the body makes it through gluconeogenesis, mainly in the liver and kidneys. This process uses non-carbohydrate sources like lactate, glycerol, and some amino acids. Glycolysis and gluconeogenesis are closely regulated. They are often controlled in opposite ways to avoid unnecessary cycles.



Krebs Cycle (Citric Acid Cycle)

The citric acid cycle is central to aerobic metabolism. It takes place in the mitochondrial matrix and finishes oxidizing acetyl-CoA from carbohydrates, fats, and proteins. This cycle produces high-energy electron carriers, NADH and $FADH_2$, along with GTP, which are used in later energy-producing reactions.

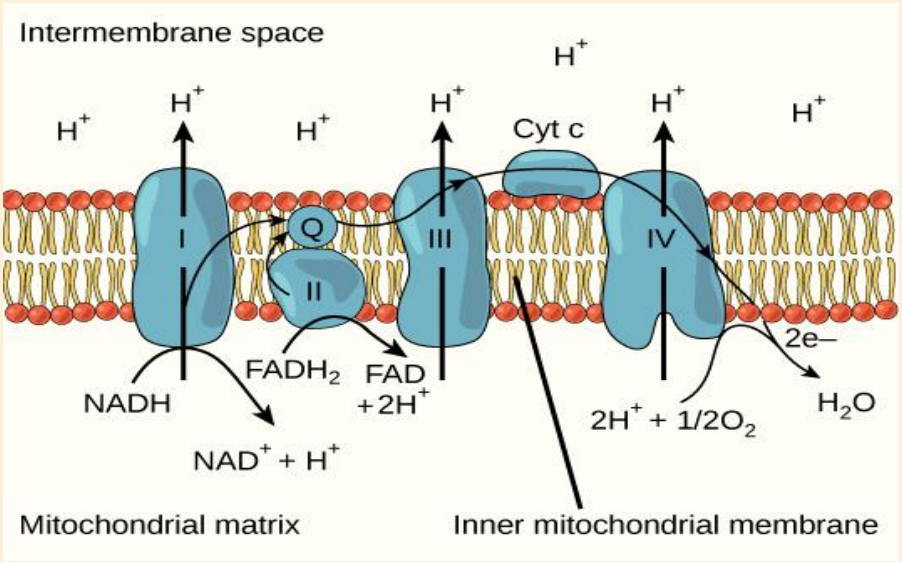
Besides generating energy, the cycle also supplies intermediates for biosynthetic pathways. Its activity depends on the availability of substrates and the energy level of the cell. This regulation helps maintain a balance between energy production and the needs for building new molecules.



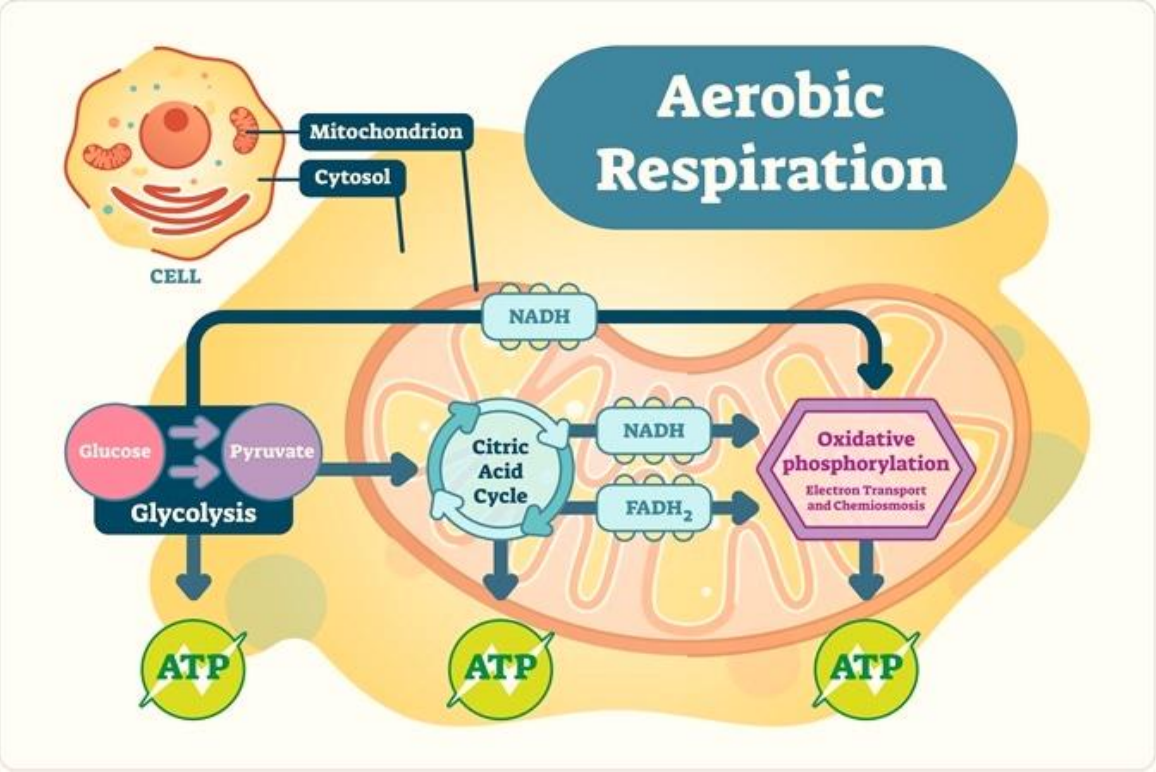
Oxidative Phosphorylation

Oxidative phosphorylation is a metabolic pathway in which the electron transport chain, embedded in the inner mitochondrial membrane, transfers electrons from NADH and $FADH_2$ to oxygen to generate ATP. This transfer is coupled to the pumping of protons across the membrane, generating a proton gradient.

ATP synthase uses the energy stored in this gradient to synthesize ATP in a process known as chemiosmosis. Oxidative phosphorylation is the most efficient way cells generate ATP. Dysfunction in this system is linked to several diseases and the aging process.



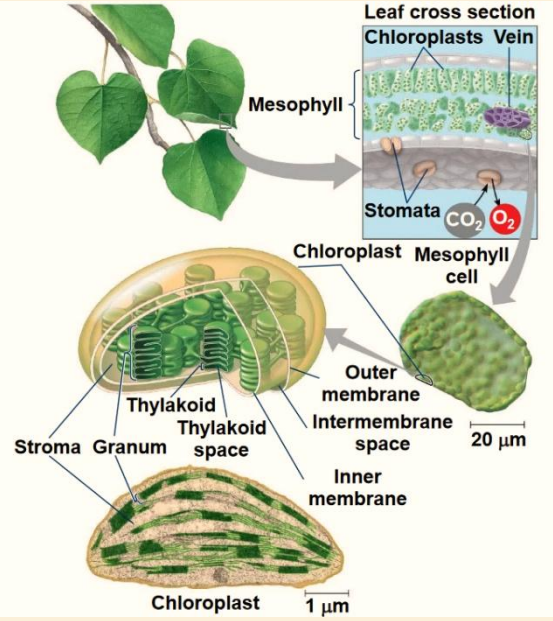
Aerobic Respiration involves Glycolysis, Citric Acid Cycle and Oxidative Phosphorylation as depicted in the picture below.

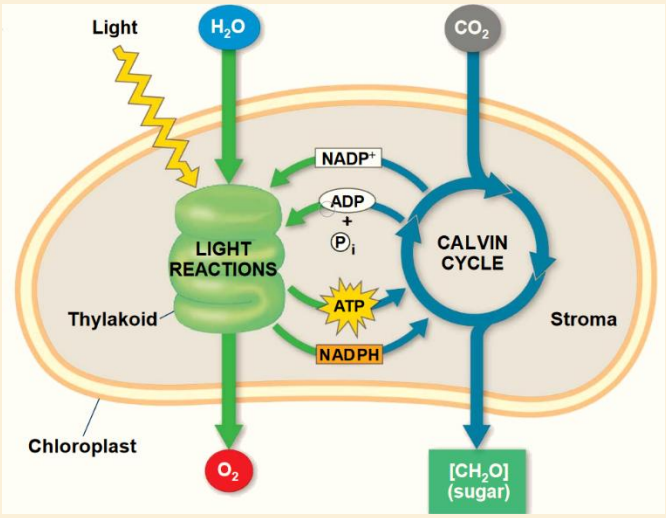


Photosynthesis – Light Reaction

In plants and photosynthetic organisms, pigments like chlorophyll in the thylakoid membranes of chloroplasts capture light energy. This energy splits water molecules, releasing oxygen and transferring electrons through photosystems I and II.

These reactions produce ATP and NADPH, which are used in the Calvin cycle to fix carbon dioxide. The creation of a proton gradient across the thylakoid membrane allows ATP production, similar to what happens in mitochondria.





Photosynthesis – Dark Reaction (The Calvin Cycle)

The Calvin cycle occurs in the chloroplast stroma. It uses ATP and NADPH from the light reactions to turn CO_2 into carbohydrates. The enzyme Rubisco starts this process by fixing carbon.

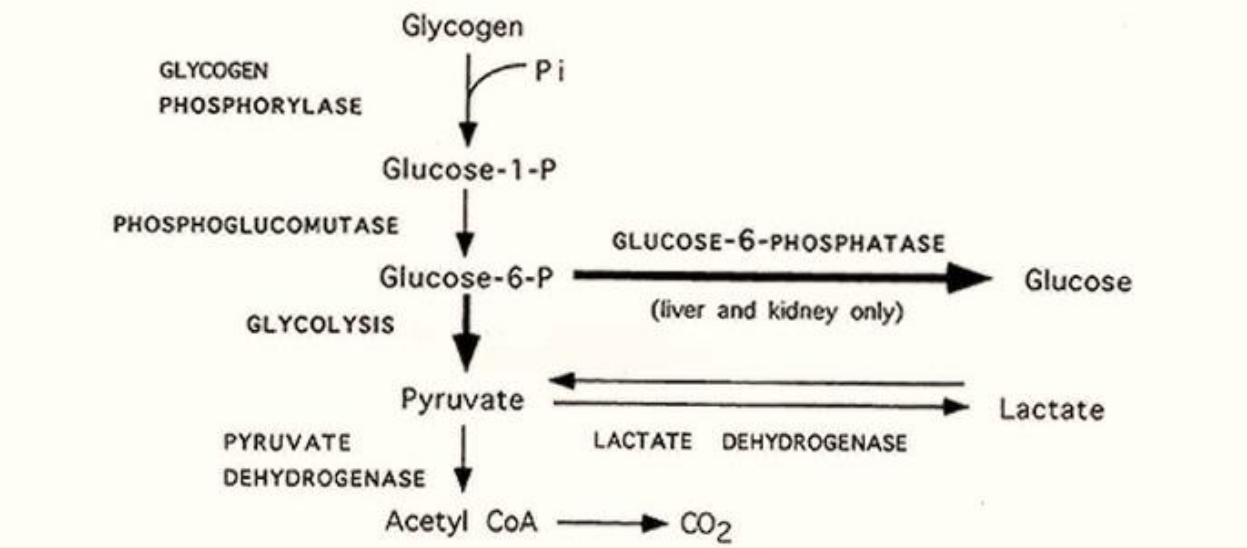
$$CO_2 + NADPH + ATP \rightarrow [CH_2O] + NADP^+ + ADP + P_i$$

Pentose Phosphate Pathway

The Pentose Phosphate Pathway functions in the cytoplasm. It has two main purposes: providing NADPH for biosynthetic reactions and producing ribose-5-phosphate for making nucleotides and nucleic acids. This pathway also helps handle oxidative stress by keeping glutathione in its reduced form.

Glycogen Metabolism

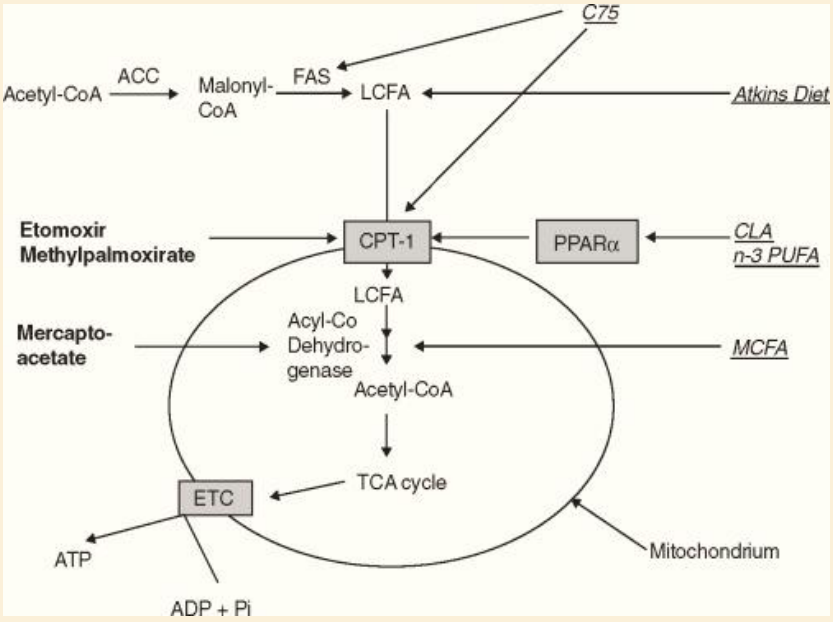
Glycogen is a type of carbohydrate that stores glucose. The body controls its production and breakdown to make sure energy is available. Glycogen phosphorylase breaks down glycogen when we are fasting or exercising. In contrast, glycogen synthase helps store extra glucose after we eat. Hormones like insulin, glucagon, and epinephrine are important for controlling glycogen metabolism. They help different tissues respond well to energy needs.



Fatty Acid Metabolism

Fatty acids are an energy-dense fuel source. They are broken down via β-oxidation in mitochondria to generate acetyl-CoA, NADH, and $FADH_2$. These products enter the citric acid cycle and oxidative phosphorylation to produce ATP.

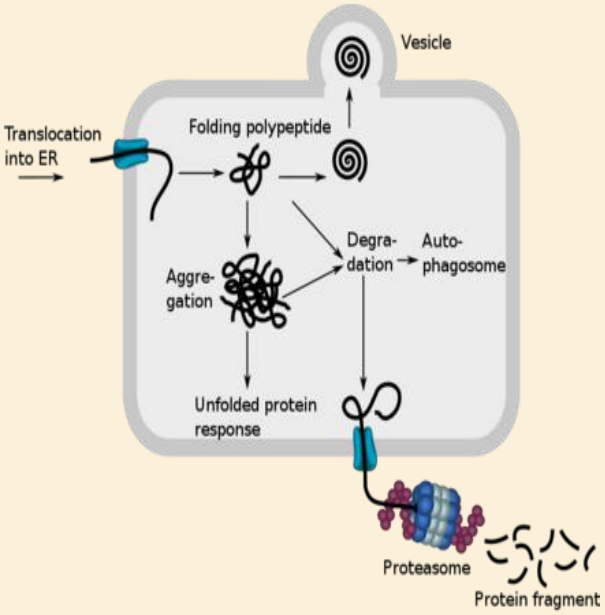
In contrast, fatty acid synthesis occurs in the cytosol and involves the formation of malonyl-CoA. It is highly regulated by hormonal signals, particularly insulin and glucagon, and is active during times of energy surplus.

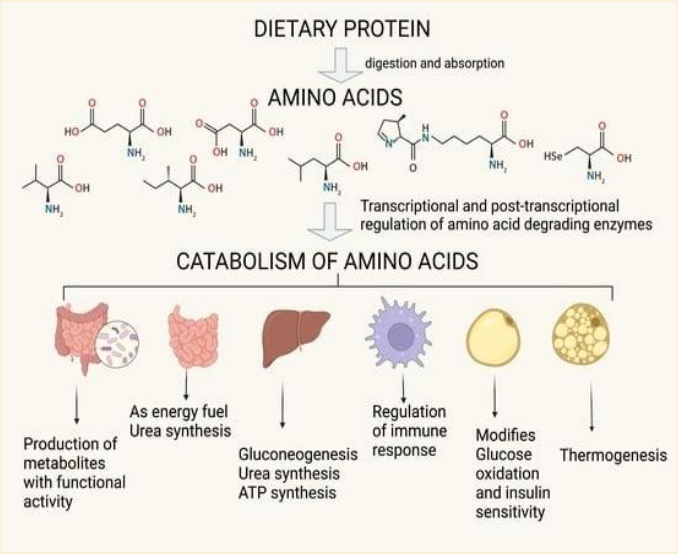


Protein Turnover and Amino Acid Catabolism

Proteins are always being broken down and built up. Breakdown happens through the proteasome and lysosomal pathways, which recycle amino acids for creating new proteins or producing energy.

When amino acids are used for energy, their amino groups are removed and turned into urea in the liver. The remaining carbon structures then enter main metabolic pathways. Genetic or enzyme issues in these processes can cause disorders like phenylketonuria (PKU).





The Biosynthesis of Amino Acids

How Are Amino Acids Made in the Cell?

Amino acids are made through **biosynthetic pathways** that use:

1. **Carbon skeletons** from metabolic intermediates
2. **Nitrogen atoms** from ammonia (NH_4^+) or other amino acids
3. **Energy** (ATP, NADPH) to drive certain reactions

STEP 1: Get the Nitrogen

- Nitrogen enters as **NH_4^+ (ammonium)**.

- It gets incorporated into **glutamate** and **glutamine**:
    - **Glutamate**: made from α-ketoglutarate + NH_4^+
    - **Glutamine**: made from glutamate + NH_4^+

STEP 2: Build the Carbon Backbone

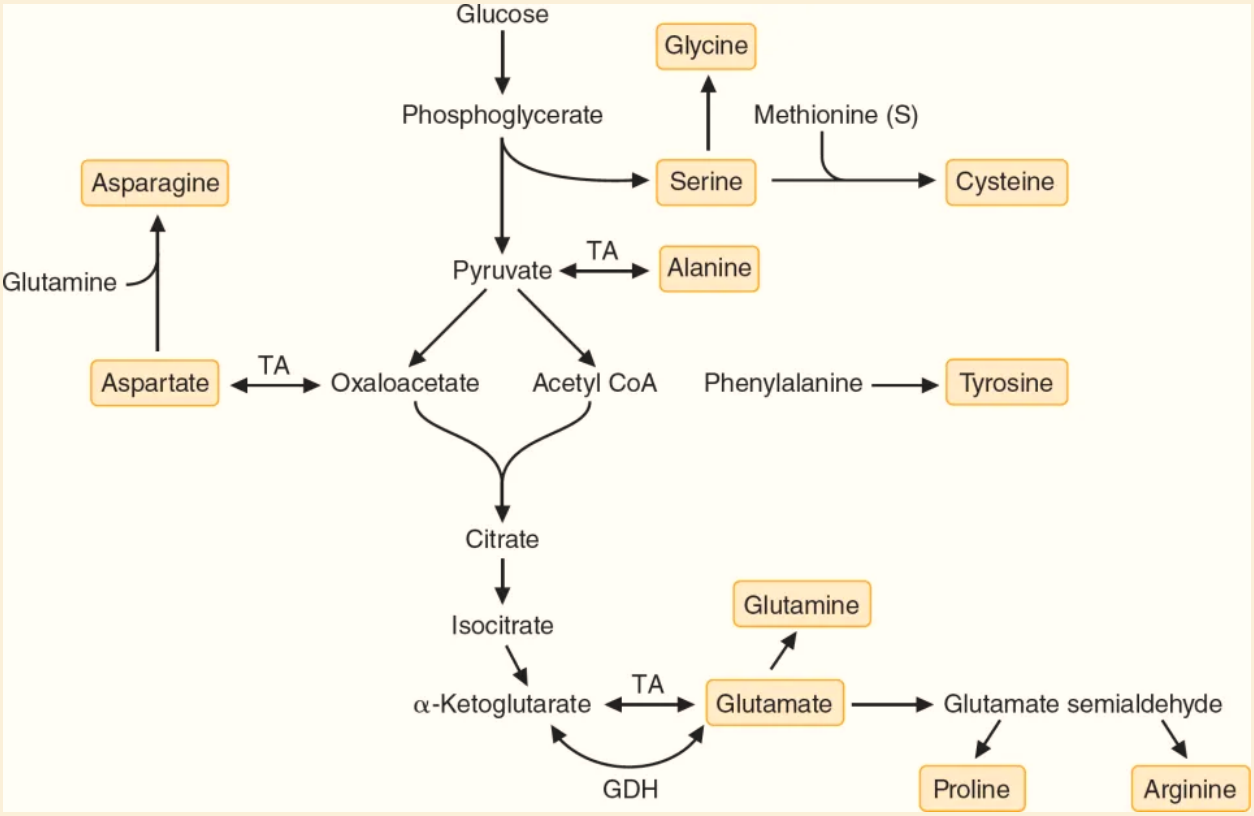
- The carbon parts come from **intermediates** of major pathways:

Pathway	**Intermediate**	**Amino Acids Formed**
Citric Acid Cycle	α-ketoglutarate	Glutamate, Glutamine, Proline, Arginine
Citric Acid Cycle	Oxaloacetate	Aspartate, Asparagine, Methionine, Lysine, Threonine
Glycolysis	Pyruvate	Alanine, Valine, Leucine, Isoleucine
Glycolysis	3-Phosphoglycerate	Serine, Cysteine, Glycine
Pentose Phosphate	Ribose-5-phosphate	Histidine
Glycolysis + Pentose Phosphate	PEP + Erythrose-4-phosphate	Phenylalanine, Tyrosine, Tryptophan

STEP 3: Add the Amino Group

- Most amino acids are made by **transamination**:
    - The amino group from glutamate is **transferred** to a keto acid.

Enzymes used: **Aminotransferases**
Coenzyme: **Pyridoxal Phosphate (PLP)**



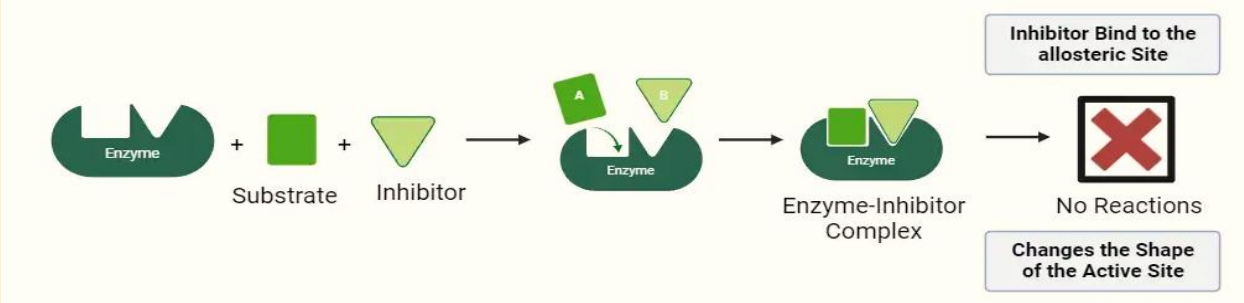
Feedback Inhibition in Amino Acid Biosynthesis

What's Feedback Inhibition?

Feedback inhibition is when the **final product** of a metabolic pathway **inhibits** the **first enzyme** in that pathway (usually at the committed step).

This stops the pathway from running unnecessarily and **prevents waste** of:

- ATP
- Raw materials (like nitrogen and carbon



Why It Matters in Amino Acid Biosynthesis

Making amino acids is expensive. If too much of one amino acid is made:

- Other pathways may suffer.
- The cell may run low on nitrogen, energy, or carbon.

So, the cell uses **feedback inhibition** to:

- Balance production
- Match demand
- Prioritize critical pathways

The Biosynthesis of Lipids and Steroids

Lipids include:

1. Fatty acids
2. Triacylglycerols (TAGs) – energy storage fats
3. Phospholipids – membranes
4. Cholesterol – membranes and hormone precursor

Fatty Acid Synthesis

Where?

- In the **cytoplasm** of **liver and adipose** cells

Starting Point:

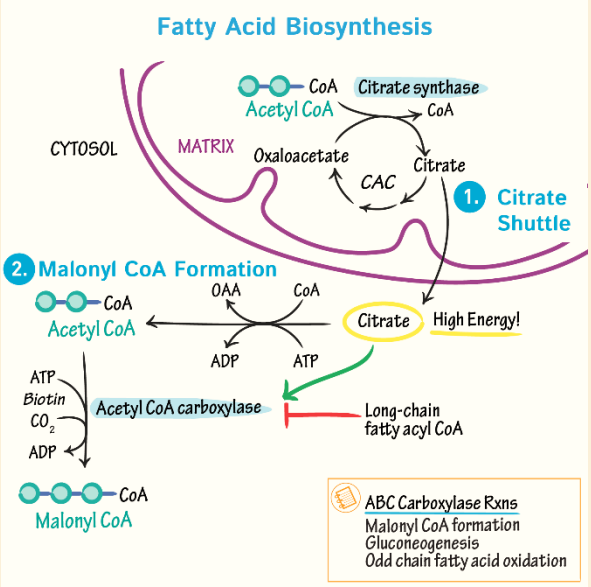
- **Acetyl-CoA** (comes from carbohydrates via glycolysis)

Steps of Fatty Acid Synthesis

1. **Acetyl-CoA + CO_2 → Malonyl-CoA**
    - Enzyme: **Acetyl-CoA carboxylase** (ACC)
    - *This is the committed step and highly regulated*

2. **Fatty acid synthase (FAS)** complex takes:
    - 1 Acetyl-CoA (starter unit)
    - Multiple Malonyl-CoA (extender units)
    - Adds 2-carbon units per cycle
3. **Produces Palmitate (16:0)**
    - Can be further elongated/desaturated in ER

NADPH is the reducing power for the synthesis.



Formation of Glycerol 3-Phosphate

- **Source**: Lactate is converted to **DHAP** (via gluconeogenesis)
- DHAP is reduced to **Glycerol 3-phosphate**
- This happens in **liver or adipose tissue**

TAG Breakdown and Reassembly (Adipose Tissue or Diet)

- TAGs (from **fat stores** or **diet**) are broken down into:
    - **Glycerol**
    - **Free fatty acids**
- Glycerol can be:
    - Sent to the **liver** to re-enter lipid metabolism
    - Converted to DHAP → used for energy or gluconeogenesis

Phospholipid Synthesis

Phospholipids are made from **phosphatidate** (same as TAGs initially), but:

- Instead of adding a fatty acid, the pathway adds a **head group** (like choline, serine, ethanolamine)

Cholesterol Is Synthesized from Acetyl CoA in Three Stages

Cholesterol synthesis happens in **three major stages**:

Stage 1: Formation of Mevalonate

- 3 Acetyl-CoA → **HMG-CoA**
- HMG-CoA is reduced by **HMG-CoA reductase** → **Mevalonate**
    - This is the **rate-limiting step**

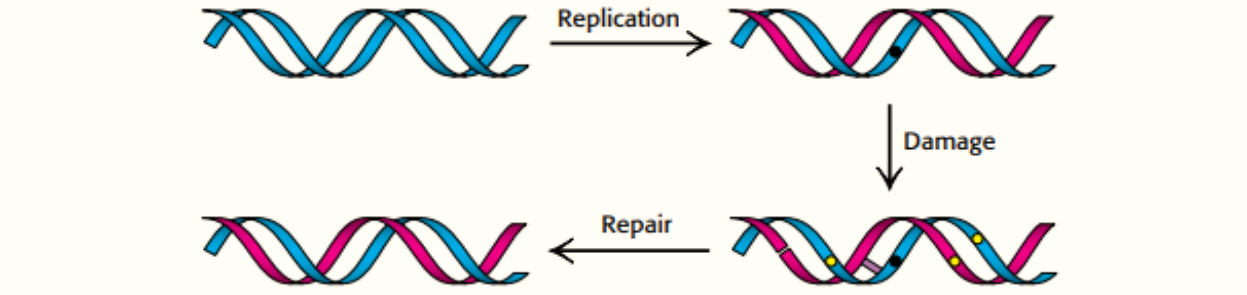
Stage 2: Formation of Activated Isoprenes

- Mevalonate → **Isopentenyl pyrophosphate (IPP)** (activated 5-carbon units)
- Through **phosphorylation** and **decarboxylation**

Stage 3: Assembly of Squalene and Cyclization

- 6 IPP units → **Squalene** (30 carbon chain)
- Squalene → **Lanosterol** (first ringed structure)
- Lanosterol → **Cholesterol** (after several reactions)

DNA Replication Repair and Recombination



In the image above, some errors (shown as a black dot) may arise in the replication processes. Additional defects (shown in yellow), including modified bases, cross-links, and single- and double-strand breaks, are introduced into DNA by subsequent DNA-damaging reactions. Many of the errors are detected and subsequently repaired.

DNA Replication

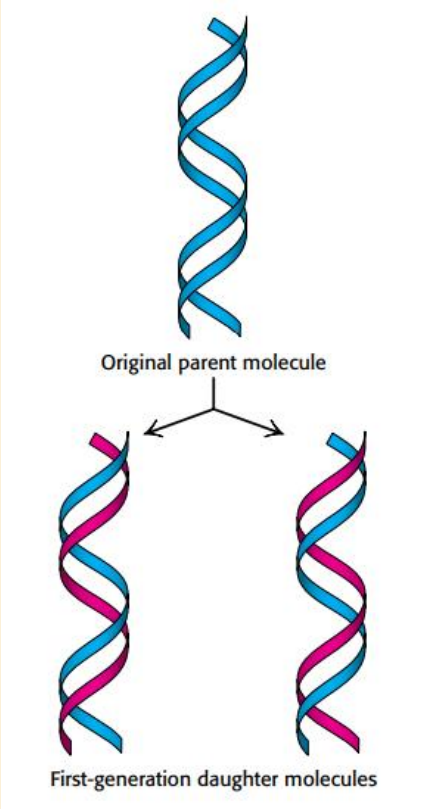
DNA replication is the process by which a cell copies its entire genome before cell division. It ensures genetic continuity between generations.

Key Features

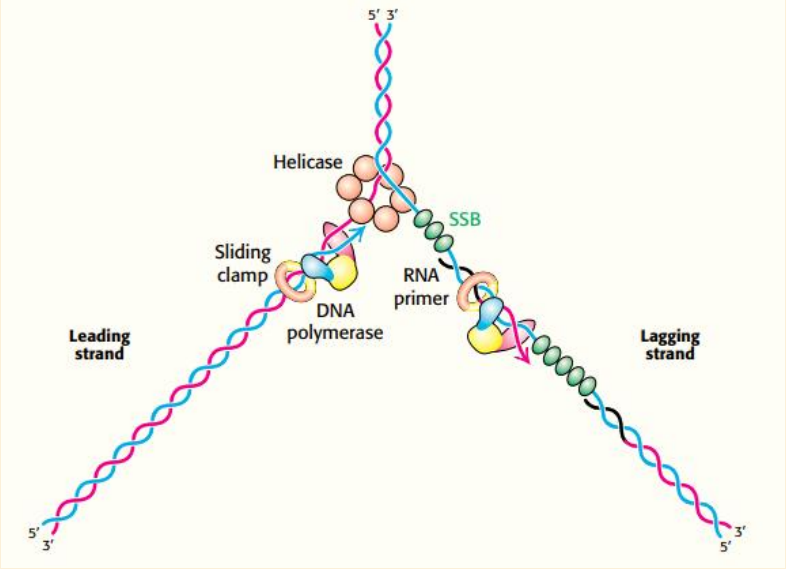
- **Semiconservative**: Each daughter DNA has one parental and one new strand.
- **Bidirectional**: Replication begins at an origin and proceeds in both directions.
- **Semi-discontinuous**: Leading strand synthesized continuously; lagging strand in Okazaki fragments.

Eukaryotic Replication

- Multiple origins per chromosome
- DNA polymerase α (with primase), δ, and ε
- Telomerase extends telomeres (ends of linear chromosomes)



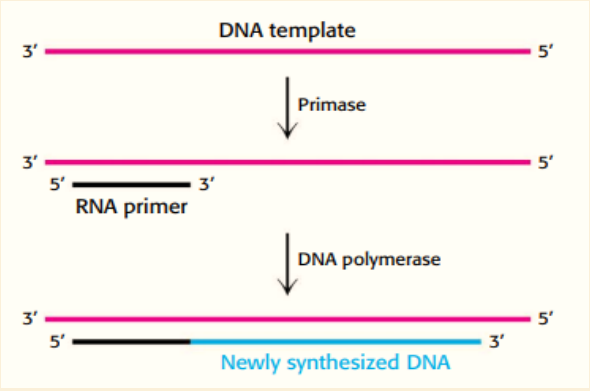
Each strand of one double helix (shown in blue) acts as a template for the synthesis of a new complementary strand (shown in red).



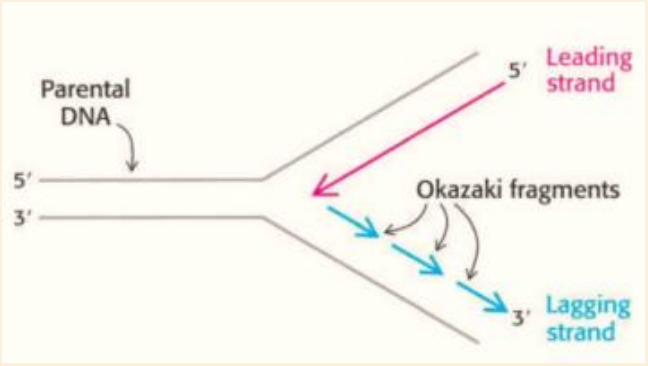
A schematic view of the arrangement of DNA polymerase III and associated enzymes and proteins present in replication of DNA. The helicase separated the two strands of the parent double helix, allowing DNA polymerases to use each strand as a templated for DNA synthesis. Abbreviation: SSB, single-stranded-binding protein.

Enzymes and Proteins

Enzyme/Protein	Function
Helicase	Unwinds DNA helix
Single-strand binding proteins (SSBs)	Prevent reannealing of DNA strands
Topoisomerase	Relieves supercoiling ahead of replication fork
Primase	Synthesizes RNA primers
DNA Polymerase	Adds nucleotides in 5'→3' direction; requires primer
DNA Ligase	Seals nicks between Okazaki fragments
Sliding Clamp	Increases DNA polymerase processivity
Clamp Loader	Loads sliding clamp onto DNA



DNA replication is primed by a short stretch of RNA that is synthesized by primase, an RNA polymerase. The RNA primer is removed at a later stage of replication



At a replication fork, both stands are synthesized in the 5’ → 3’ direction. The leading strand is synthesized continuously, whereas the lagging strand is synthesized in short pieces termed as Okazaki fragments.

DNA

Repair

DNA is constantly damaged by:

- UV light (causes thymine dimers)
- Chemicals (e.g., deaminating agents)
- Replication errors

Without repair, mutations accumulate, leading to diseases like cancer.

Repair Type	Damage Repaired	Mechanism Overview
Direct Repair	Pyrimidine dimers (by photolyase), methylated bases	Reverses damage directly
Base Excision Repair (BER)	Single damaged base (e.g., uracil in DNA)	DNA glycosylase removes base, endonuclease cuts backbone
Nucleotide Excision Repair	Bulky lesions (e.g., thymine dimers)	Excision of ~30 nt segment around damage
Mismatch Repair (MMR)	Replication errors	Detects and replaces incorrect base on new strand
Double-Strand Break Repair	Ionizing radiation, replication fork collapse	Homologous recombination or non-homologous end joining (NHEJ)

Genetic Recombination

1. Homologous Recombination (HR)

- Exchange of DNA between similar sequences
- Steps:
    1. Double-strand break
    2. Resection to form 3′ overhangs

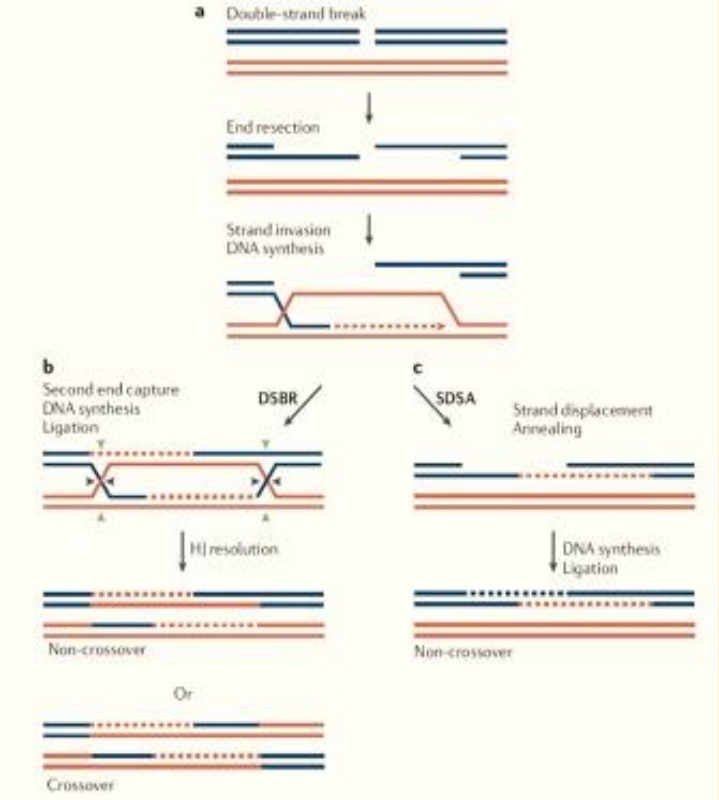
3. Strand invasion
4. Branch migration and resolution

Functions:

- Accurate repair of double-strand breaks
- Genetic diversity during meiosis

2. Non-Homologous End Joining (NHEJ)

- Quick, error-prone repair of double-strand breaks
- Involves **Ku proteins** and **DNA ligase IV**



3. Site-Specific Recombination

- Occurs at particular DNA sequences (e.g., in viruses or immune system)
- Requires specific recombinases (e.g., Cre, integrase)

4. Transposition

- Mobile genetic elements (transposons) move within the genome
- Can be:
    - **Cut-and-paste** (DNA transposons)
    - **Copy-and-paste** (retrotransposons via RNA intermediate)

Protein Synthesis

Protein synthesis involves **translating** the nucleotide sequence of an mRNA molecule into the amino acid sequence of a protein. This process occurs in three major steps:

Component	Function
mRNA	Carries the genetic code from DNA
Ribosomes	Machinery that reads mRNA and synthesizes protein
tRNA	Delivers specific amino acids to the ribosome
Aminoacyl-tRNA synthetase	Charges tRNAs with the correct amino acids
Initiation factors, Elongation factors, Release factors	Help in different phases of translation

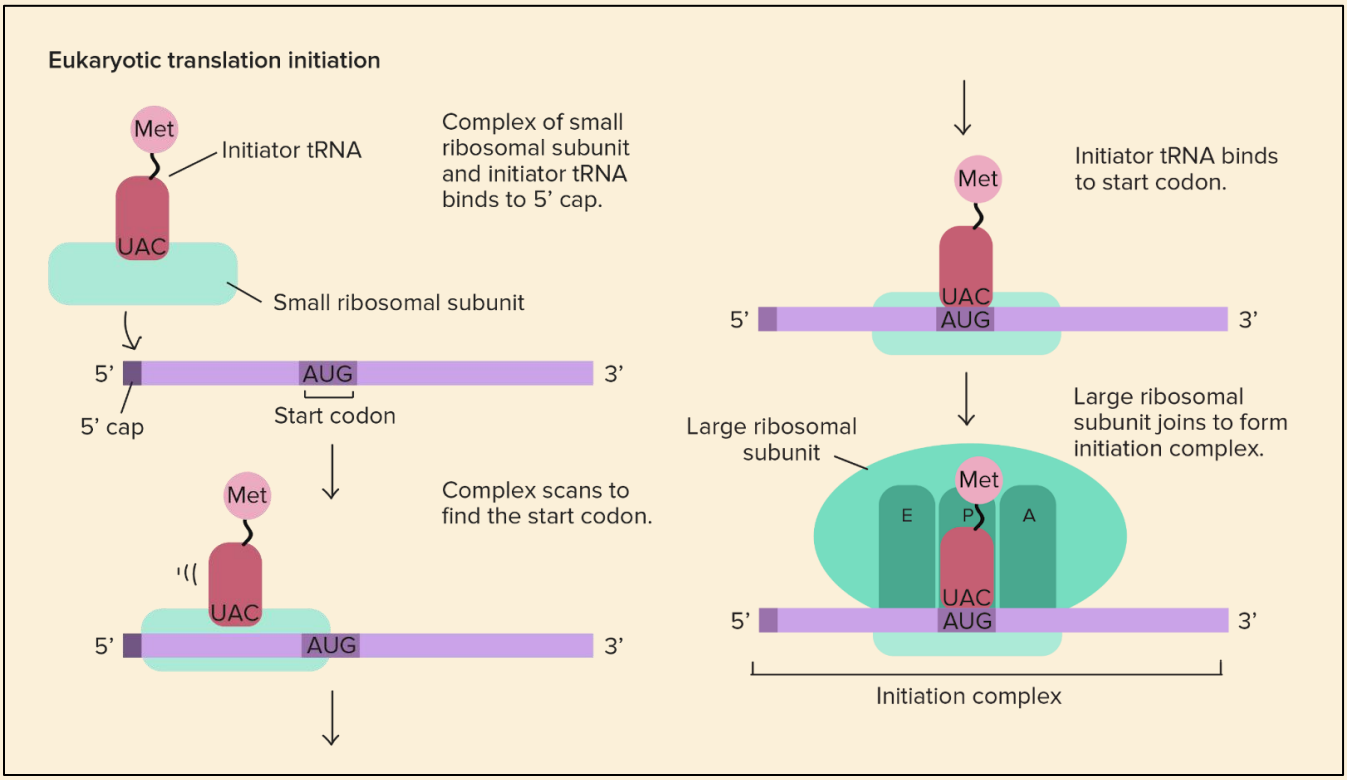
Stages of Protein Synthesis

1. Initiation

- **Prokaryotes**:
    - Small ribosomal subunit (30S) binds to the **Shine-Dalgarno sequence** on mRNA.
    - **Initiator tRNA** carrying *formylmethionine (fMet)* pairs with the start codon (AUG).
    - Large subunit (50S) joins to form 70S initiation complex.

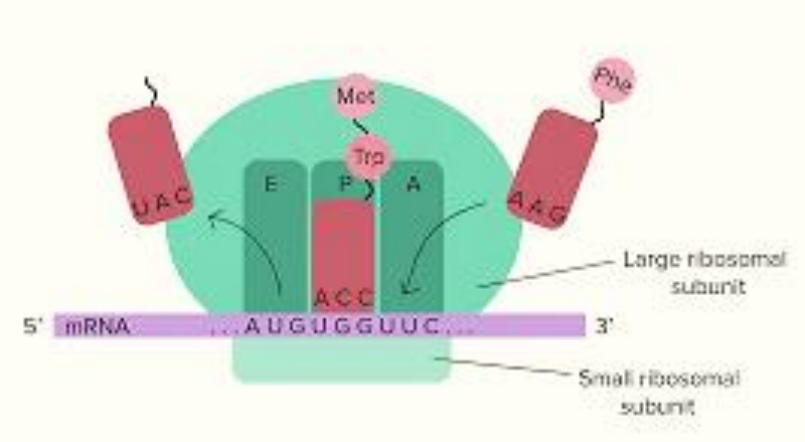
- **Eukaryotes**:
    - 40S subunit binds the **5' cap** of mRNA and scans for the **AUG** start codon.
    - Initiator tRNA carries *methionine (Met)*.

- Large subunit (60S) joins to form 80S ribosome.



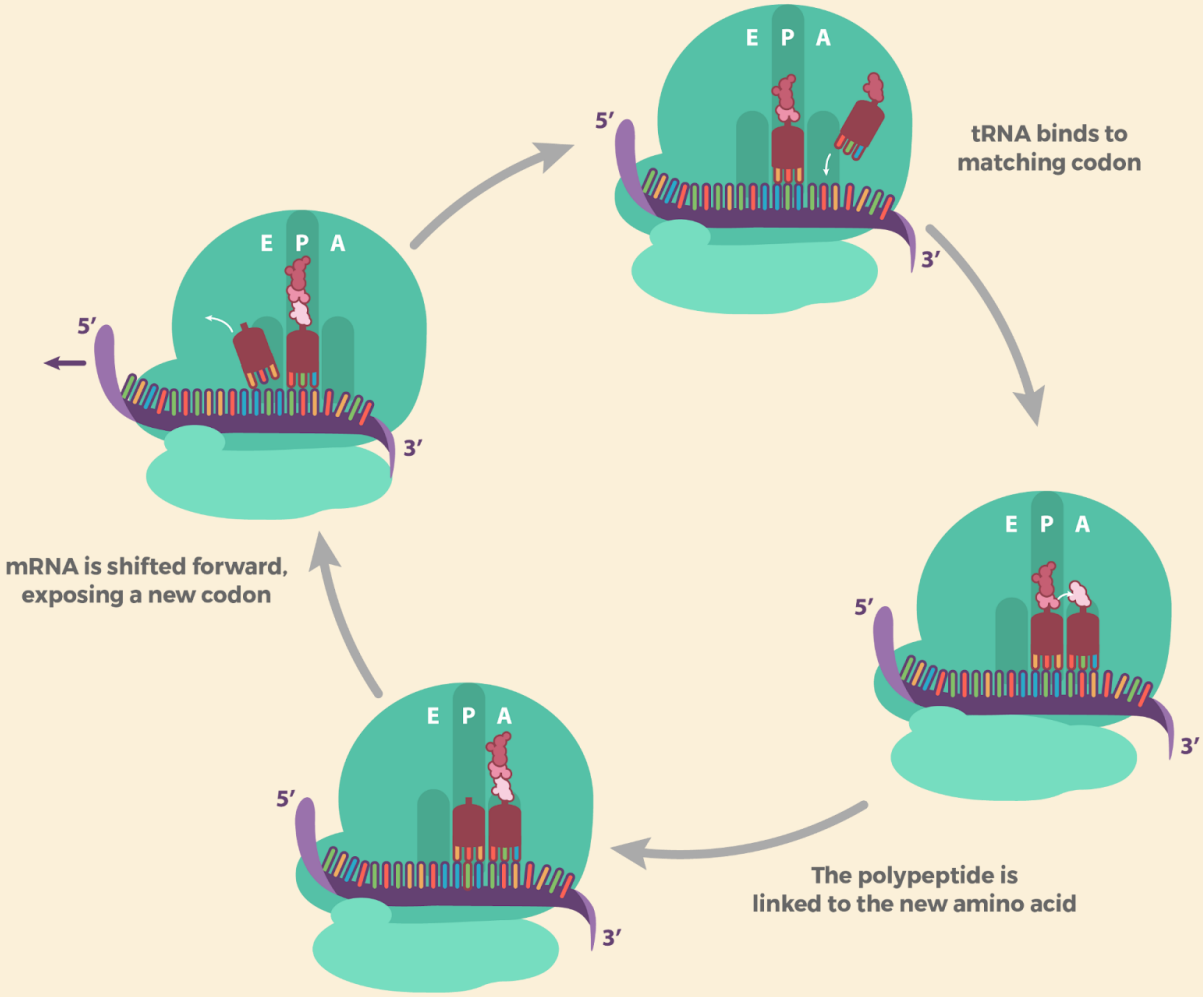
The ribosome has 3 sites:

- **A (Aminoacyl)** site: accepts incoming tRNA
- **P (Peptidyl)** site: holds the growing peptide chain
- **E (Exit)** site: tRNA exits



2. Elongation

1. **Aminoacyl-tRNA** enters A site.
2. **Peptidyl transferase** (an rRNA-based enzyme) forms a peptide bond between the amino acid in the P site and the new one in the A site.
3. **Translocation**: Ribosome moves forward; tRNA in P site moves to E site and exits; new tRNA enters A site.



3. Termination

- Stop codon (UAA, UAG, or UGA) is encountered.
- No tRNA matches stop codons.
- **Release factors (RF1, RF2 in prokaryotes)** bind and release the polypeptide.
- Ribosome dissociates.

Differences: Prokaryotic vs Eukaryotic Translation

Feature	**Prokaryotes**	**Eukaryotes**
Ribosome	70S (30S + 50S)	80S (40S + 60S)
Start Codon Recognized By	fMet-tRNA	Met-tRNA
mRNA Recognition	Shine-Dalgarno sequence	5’ cap and scanning mechanism
Transcription & Translation	Coupled	Separated (nucleus vs cytoplasm)

Inhibitors of Translation

These are **clinically important** for antibiotics and drugs:

Inhibitor	**Target**	**Organism Affected**
Streptomycin	30S subunit	Prokaryotes
Tetracycline	A-site binding	Prokaryotes
Chloramphenicol	Peptidyl transferase	Prokaryotes
Erythromycin	Translocation (50S)	Prokaryotes
Puromycin	Premature chain termination	Both
Cycloheximide	Eukaryotic ribosomes	Eukaryotes

RESPONSE TO ENVIRONMENTAL CHANGES

Sensory Systems

Smell, taste, vision, hearing, and touch are based on **signal- transduction pathways** activated by signals from the environment. These sensory systems function similarly to the signal-transduction pathways for many hormones. These intercellular signaling pathways appear to have been **appropriated and modified to process environmental information.**

Smell

- The sense of smell, or **olfaction**, is remarkable in its specificity; it can, for example, **discern stereoisomers of small organic compounds as distinct aromas.**
- An outstanding feature of the olfactory system is its ability to detect a vast array of odorants.
- Each olfactory neuron expresses **only one type of receptor** and connects to a particular region of the olfactory bulb.
- Odors are decoded by a combinatorial mechanism: each odorant activates a number of receptors, each to a different extent, and most receptors are activated by more than one odorant.

Taste

- We can detect only five tastes: bitter, sweet, salt, sour, and umami.
- Bitter, sweet, and umami tastants are experienced through **7TM receptors** acting through a special G protein called **gustducin.**
- Salty and sour tastants act **directly through membrane channels.**
- Salty tastants are detected by passage though **Na+ channels**, whereas sour taste results from the effects of **hydrogen ions** on a number of types of channels.
- The end point is the same in all cases—membrane polarization that results in the transmission of a nerve impulse.

Sight

- Two classes of photoreceptor cells exist: **cones**, which respond to bright lights and colors, and **rods**, which respond only to dim light.
- Color vision is mediated by three distinct 7TM photoreceptors that employ 11- cis -retinal as a chromophore and absorb light in the blue, green, and red parts of the spectrum.

Hearing

- The immediate receptors for hearing are found in the **hair cells of the cochleae**, which contain bundles of stereocilia.
- When the stereocilia move in response to sound waves, cation channels will open or close, depending on the direction of movement.
- The mechanical motion of the cilia is converted into current flow and then into a nerve impulse.

Touch

- Touch, detected by the skin, senses pressure, temperature, and pain.
- Specialized nerve cells called **nociceptors** transmit signals that are interpreted in the brain as pain.
- The **capsaicin receptor**, also called VR1, functions as a cation channel that initiates a nerve impulse.

The Immune System

There are two types of immune system – **innate** and **adaptive** immune system.

The innate immune system targets feature common to many different pathogens but misses those pathogens lacking these features.

The adaptive immune system is both more specific and wide-reaching. It follows the principles of evolution; an enormously diverse set of potentially useful proteins is generated which is then subjected to intense selection so that only the useful ones remain.

The immune system must meet two tremendous challenges in the identification of pathogens:

(1) To produce a system of receptors diverse enough to recognize a wide array of potential pathogens

(2) To distinguish invaders and their disease-causing products from the organism's own products (i.e., self- versus no self-recognition).

To meet these challenges, the innate immune system evolved the ability to recognize structural elements such as specific **glycolipids** or forms of nucleic acid.

As mentioned before, the adaptive immune system makes an enormous number of proteins to detect structural elements of foreign organisms however this has the potential to create anti-bodies and T-

cells which recognize and attack cells or molecules normally present in our body – a situation which can result in auto-immune diseases.

In principle, the immune system is capable of generating antibodies and T-cell receptors that bind to self-molecules—that is, molecules that are normally present in a healthy and uninfected individual organism. Selection mechanisms prevent such self-directed molecules from being expressed at high levels. The selection process includes both positive selections, to enrich the population of cells that express molecules that have the potential to bind foreign antigens in an appropriate context, and negative selection, which eliminates cells that express molecules with too high an affinity for **self-antigens**. Autoimmune diseases such as insulin-dependent diabetes mellitus can result from the amplification of a response against a self-antigen.

Molecular Motors

Cells are not static but are bustling assemblies of moving proteins, nucleic acids, and organelles. This motion is enabled by two elements: molecular-motor proteins and complex networks of filamentous proteins termed the cytoskeleton.

A few interesting points:

- The fundamental biochemical mechanisms that produce contractions in our muscles are the same as those that propel organelles along the cytoskeleton.
- Many of the proteins that play key roles in converting chemical energy into kinetic energy are members of the same protein family, the **P-loop NTPases**
- These molecular motors are homologous to proteins including the G proteins in **protein synthesis, signaling, and other processes.**

Eukaryotic cells contain three major families of motor proteins: myosins, kinesins, and dyneins.

- **Myosin**, first characterized on the basis of its role in muscle, moves along filaments of the protein actin. Each molecule of muscle myosin consists of two copies each of a heavy chain with a molecular mass of 220 kDa, an essential light chain, and a regulatory light chain. The human genome encodes more than **40 distinct myosins**; some function in muscle contraction, and others participate in a variety of other processes.
- **Kinesins**, which have roles in protein, mRNA, and vesicle transport as well as **construction of the mitotic spindle** and **chromosome segregation,** are generally dimers of two polypeptides. The human genome encodes more than 40 kinesins.
- **Dyneins** power the motion of cilia and flagella, and a general cytoplasmic dynein contributes to a variety of motions in all cells, including vesicle transport and various transport events in

mitosis. Dyneins are enormous, with heavy chains of molecular mass greater than 500 kDa. The human genome encodes approximately **10** dyneins.

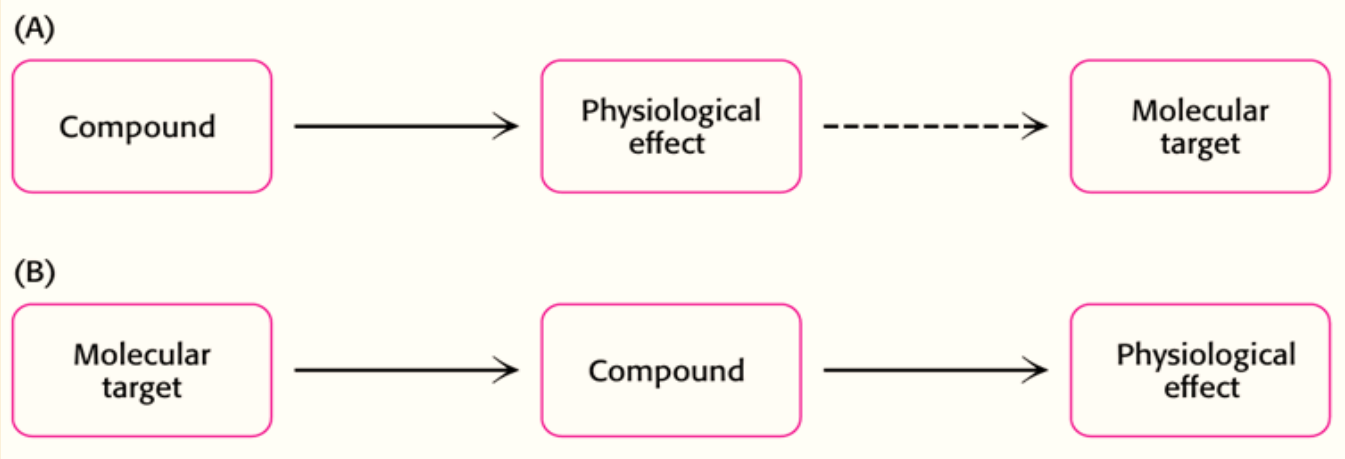
Drug Development

The development of drugs can be said to be the most important interface between biochemistry and medicine. In most cases, drugs act by binding themselves to proteins like enzymes, receptors and transporters and inhibit or module their activities. Thus, knowledge of these molecules and the pathways in which they participate is crucial to drug development.

An effective drug however is not simply a potent modulator of its target protein. After all, the drug has to survive its journey from the mouth (if administered orally) long enough till it reaches its target. Also, to prevent any other unwanted physiological effects, drugs must not modulate the properties of biomolecules other than the intended target.

Drugs have been discovered majorly by two approaches:

- The first approach identifies a substance that has a **desirable physiological consequence** when administered to a human being, to an appropriate animal, or to cells. In this approach, a biological effect is known before the molecular target is identified. The mode of action of the substance is only later identified after substantial additional work.
- The second approach begins with a **known molecular target**. Compounds are sought, either by screening or by designing molecules with desired properties, that bind to the target molecule and modulate its properties.



Before compounds can be given to human beings as drugs, they must be extensively tested for safety and efficacy. Clinical trials are performed in stages: first testing safety, then safety and efficacy in a small population, and finally safety and efficacy in a larger population to detect rarer adverse effects. Largely due to the expenses associated with clinical trials, the cost of developing a new drug has been estimated to be more than $800 million (or 6900 crore rupees). Even when a drug has been approved for use, complications can arise. With regard to infectious diseases and cancer, patients often develop resistance to a drug after it has been administered for a period of time because variants of the disease agent that are less susceptible to the drug arise and replicate, even when the drug is present.

That's all for Module-2. Hope you find this module interesting. Feel free to ask any doubts in the WhatsApp group or DMs (whichever you are comfortable with).

As usual, we have attached some references in the next page for even greater understanding of the topics.

HAPPY LEARNING !!!

REFERENCES

#1: Biochemistry by Jeremy M. Berg, John L. Tymoczko, Gregory J. Gatto Jr., Lubert Stryer

#2: A Biochemistry Playlist by the YouTube Channel - Prof. Dave Explains

#3: A playlist by MIT OpenCouseWare

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The Kinetics of Enzyme-Catalyzed Reactions

The Enzyme-Substrate Complex and Enzyme Action

Enzymes are remarkable biological catalysts that enable life by accelerating biochemical reactions by many orders of magnitude. Their high specificity arises from their three-dimensional structure, allowing selective binding and transformation of substrates into products.

Mechanism: $E + S \rightleftharpoons ES \rightarrow E + P$

This three-step model underpins most enzymatic reactions:

- Binding of substrate to enzyme (formation of the enzyme-substrate complex, ES)
- Conversion to product
- Release of product and regeneration of enzyme

The **active site** is typically a cleft or pocket formed by the tertiary structure, involving residues that engage the substrate through:

- **Hydrogen bonds** (e.g., between amino and carbonyl groups)
- **Ionic interactions** (charged amino acid side chains)
- **Hydrophobic interactions** (nonpolar side chains)

Lock-and-Key Model: Suggests the active site is rigid and only fits the correct substrate.

Induced Fit Model: Recognizes that enzyme structure is dynamic—substrate binding induces conformational changes enhancing catalytic efficiency.

Transition State Theory: Enzymes stabilize the high-energy transition state intermediate (‡), reducing the activation energy (G‡) and enhancing the forward rate without shifting equilibrium.

Simple Enzyme Kinetics with One and Two Substrates

Michaelis-Menten Kinetics

The most foundational model in enzyme kinetics, developed by Michaelis and Menten in 1913, assumes:

- A steady-state concentration of ES ($\frac{d[ES\}}{dt} = 0$)
- Irreversible conversion to product (no back reaction)

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

Where:

- v is the rate of product formation
- $V_{max} = k_{cat}[E]_0$, the maximum rate when all enzyme is saturated
- $K_m = \frac{k_{-1}+k_2}{k_1}$ reflecting the affinity of enzyme for substrate
- k_{cat} (turnover number): number of substrate molecules converted per enzyme per second

Catalytic efficiency: k_{cat}/ K_m

- High values indicate efficient enzymes (diffusion-limited enzymes like catalase, superoxide dismutase)

Evaluation of Parameters

Experimental analysis often involves transforming the Michaelis-Menten equation into linear forms:

- **Lineweaver-Burk**: $\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$
- **Eadie-Hofstee**: $v = -K_m \cdot \frac{v}{[S]} + V_{max}$
- **Hanes-Woolf**: $\frac{[S]}{v} = \frac{[S]}{V_{max}} + \frac{K_m}{V_{max}}$

Each has pros and cons—Lineweaver-Burk is sensitive to small errors in low [S] regions, while Hanes-Woolf spreads data more evenly.

Reversible Reactions, Two-Substrate Reactions, and Cofactor Activation

- **Reversible Reactions:** When product accumulates and reversibly binds to enzyme: $E + P \rightleftharpoons$

  $EP \rightarrow ES \rightarrow E + S$ The equation becomes: $v = \frac{V_f[S] - V_r[P]}{K_{m,f}(1 + \frac{[P]}{K_{m,r}}) + [S]}$

- **Two-substrate reactions:**
- ***Sequential (Ordered or Random)***: $A + B + E \rightarrow EAB \rightarrow E + P + Q$
- ***Ping-pong***: One product leaves before second substrate binds.
- **Cofactors**: Enzymes such as dehydrogenases require NAD+, FAD, or ATP for activity.

Determination of Elementary-Step Rate Constants

Relaxation Kinetics

Relaxation kinetics techniques analyze how an enzyme system returns to equilibrium after a small perturbation, such as a temperature or pressure jump. These are especially useful for reversible reactions.

For a simple reversible reaction: $A+B \rightleftharpoons C$ Relaxation time τ is related to the forward and reverse rate constants: $\frac{1}{\tau} = k_f + k_r$

This approach allows determination of rate constants when direct observation of intermediates is challenging.

Some Results of Transient-Kinetics Investigation

Transient kinetics captures rapid events before steady-state is reached. Commonly studied with stopped-flow or quenched-flow techniques, this method is used to measure pre-steady state bursts and to observe intermediates.

Example: In burst kinetics of serine proteases, a rapid initial product formation indicates a fast acylation step followed by slower deacylation.

Mathematical tool: Fit observed time courses to exponential terms for extracting rate constants.

Other Patterns of Substrate Concentration Dependence

Substrate Activation and Inhibition

Some enzymes exhibit substrate activation where binding of one molecule enhances binding or activity at another site. Others experience inhibition at high substrate concentrations (substrate inhibition).

Rate expression with inhibition: $v = \frac{V_{max}[S]}{K_m + [S] + \frac{[S]^2}{K_i}}$

Multiple Substrates Reacting on a Single Enzyme

Complex enzymes may catalyze different reactions depending on the substrate. This requires kinetic modeling that accounts for mutually exclusive or cooperative substrate binding.

Modulation and Regulation of Enzymatic Activity

The Mechanisms of Reversible Enzyme Modulation

Regulation can occur by non-covalent allosteric effectors or reversible covalent modifications.

- **Allosteric sites:** Effectors bind here to change enzyme conformation.
- **Phosphorylation:** Alters enzyme activity in response to signaling.

Example: Glycogen phosphorylase is activated by phosphorylation in response to adrenaline.

Analysis of Reversible Modulator Effects on Enzyme Kinetics

Allosteric enzymes often display sigmoidal (S-shaped) velocity vs [S] plots.

Hill equation: $\theta = \frac{[S]^n}{K_d + [S]^n}$ or $v = \frac{V_{max}[S]^n}{K_{0.5}^n + [S]^n}$

Where *n* is the Hill coefficient (degree of cooperativity).

Other Influences on Enzyme Activity

The Effect of pH on Enzyme Kinetics in Solution

pH affects ionization states of amino acids at active and binding sites. The enzyme may be active only when specific residues are charged.

Bell-shaped curve: Reflects that both protonated and deprotonated forms are necessary for activity.

Enzyme Reaction Rates and Temperature

Temperature increases molecular collisions but also causes denaturation above a threshold.

Arrhenius Equation: $k = Ae^{-Ea/RT}$

Q10 rule: Rate approximately doubles for every 10°C rise within physiological range.

Enzyme Deactivation

Mechanisms and Manifestations of Protein Denaturation

Denaturation is loss of structure and function due to extreme conditions. Can be reversible (e.g. urea removal) or irreversible (e.g. heating).

Molecular effects:

• Disruption of hydrogen bonds, hydrophobic core, disulfide bridges

Deactivation Models and Kinetics

Commonly modeled by first-order kinetics: $E(t) = E_0e^{-kdt}$

Where k_d is the deactivation rate constant.

Mechanical Forces Acting on Enzymes

Industrial reactors cause stress via stirring, shear, or interfacial adsorption. These can unfold proteins or cause aggregation.

Strategies for Enzyme Stabilization

- I**mmobilization** on beads or membranes
- **Additives** like glycerol or trehalose
- **Genetic engineering** to introduce disulfide bridges or increase hydrophobic core

Enzyme Reactions in Heterogeneous Systems

Enzymes can function in heterogeneous media such as immobilized systems, emulsions, or membranes.

These systems improve reuse, stability, and separation but pose diffusion challenges.

Factors affecting kinetics:

- Surface area
- Enzyme loading
- Mass transfer limitations

Immobilized Enzyme Kinetics:

Why Immobilization?

Yes, why do we need an immobilized enzyme and why learn about it's kinetics and what's an immobilized enzyme anyways?

Immobilization of an enzyme means that it has been confined or localized so that it can be reused continuously. There are several reasons why this is desirable: for processing with isolated enzymes, we need to retain an enzyme in immobilized form in the reactor. An immobilized enzyme can be fixed in position near other enzymes participating in a catalytic sequence, thereby increasing efficiency of multi-step conversions.

Now let's have a look at the actual kinetics.

First is the simplest case, enzyme is immobilized only on the external surfaces of slab support. Thus, only the mass transport from the bulk solution to the support surface and the reaction at that position needs to be considered.

One model for this, is the Nernst Diffusion layer which gives the following equation.

$$N_s = k_s(s_0 - s)$$

Where:

Ns - moles per unit time per unit area(flux)

S – substrate concentration at interface

S_0 – substrate concentration in bulk fluid

K_s – mass transfer coefficient

Note : k_s increases with increasing liquid flow rate through a packed column enzyme reactor. It depends on other hydrodynamic conditions as well, which shall not be discussed here and can be read through the provided reference.

In steady state, substrate cannot accumulate at catalyst interface. Thus, rate of substrate supply by mass transfer must equal the rate of substrate consumption by the reaction at the interface.

Assuming that Michaelis-Menten kinetics applies at the surface, and letting denote surface reaction rate.

$$k_s(s_0 - s) = \bar{v} = \frac{v_{max} s}{K_m + s}$$

The number of parameters required to specify the system can be reduced from four (k_s, s_0 , v_{max}, K_m) to two (Da and) by introducing dimensionless variables:

$$x = \frac{s}{s_0} \qquad \mathrm{Da} = \frac{v_{max}}{k_s s_0} \qquad \kappa = \frac{K_m}{s_0}$$

Thus, the equation reduces two:

$$\frac{1-x}{\mathrm{Da}} = \frac{x}{\kappa + x}$$

Da, also known as Damkohler number should be emphasized:

$$\mathrm{Da} = \frac{v_{max}}{k_s s_0} = \frac{\text{maximum reaction rate}}{\text{maximum mass-transfer rate}}$$

There are two special cases of this reaction, namely

Reaction-limited regime – max mass-transfer rate >> max reaction rate

Diffusion-limited regime – max reaction rate >> max transfer rate

With a bit more algebraic manipulation we get,

$$x = \frac{\beta}{2}\left(\pm \sqrt{1 + \frac{4\kappa}{\beta^2}} - 1 \right)$$

where

$$\beta \equiv \mathrm{Da} + \kappa - 1$$

By tradition in chemical engineering, the influence of mass transfer on the overall reaction process is represented using the effectiveness factor , which is defined physically by:

$$\eta = \frac{\text{observed reaction rate}}{\text{rate which would be obtained with no mass-transfer resistance, i.e., surface concentration } s = \text{bulk concentration } s_0}$$

For our problem we have, after substituting values,

$$\eta = \frac{x/(\kappa + x)}{1/(\kappa + 1)}$$

For Da approaching zero, i.e., very slow reaction relative to maximum transfer rate show that x must approach unity and so for this condition(reaction-limited case):

$$\eta = 1 \qquad \bar{v} = \frac{v_{max} s_0}{K_m + s_0}$$

Many experimental reactors designed to operate in the reaction-limited regime have been developed for ordinary heterogeneous catalysts. They all share the common concept of high fluid flow rates near the catalyst to minimize mass-transfer resistance (larger k_s, smaller Da). A few drawbacks of said system is that fluid forces might cause partial or complete denaturation of the attached enzymes and relative motion of catalyst particles may give enzyme loss by particle-particle abrasion.

Now again by doing a bit of mathematical manipulation (which I suggest you try out), we can arrive at equation for diffusion-limited case. (Da->, finite)

$$\eta = \frac{1 + \kappa}{\mathrm{Da}} \qquad \bar{v} = k_s s_0$$

Thus, when Da is very large is first order in bulk substrate concentration and totally independent of the intrinsic rate parameters v_{max} and K_m. Thus, studies aimed at determining activity retention or denaturation rates of immobilized enzymes should be conducted as close as possible to reaction-limited regime.

Analysis of Intraparticle Diffusion and Reaction:

Now, enzymes are typically immobilized on the internal surfaces of porous systems or entrapped in matrices through which substrates can diffuse. Unfortunately, this part cannot be covered here but I do recommend checking out section 4.4.2 of Bailey, the reference used for creating this module, it's an interesting derivation.

Effects of Inhibitors, Temperature and pH on Immobilized Enzyme catalytic Activity and Deactivation:

The effect of pH on enzyme kinetics were previously discussed in this same module, but when an enzyme is immobilized, it might affect some of its intrinsic properties. This requires careful study using methods like **Nernst Diffusion Layer** (The one discussed above). After all, immobilization might mean different rate constants and even alterations to the form of the equation needed to describe the effects of reaction parameters on immobilized enzyme intrinsic activity.

To understand the effect of mass transfer processes, let's take a first order reaction.

Firstly, the kinetics of the reaction in reaction-limited regime will be same as actual kinetics. On the other hand, in diffusion-limited regime, the apparent overall kinetics will still be first order, but the apparent rate constant will be the square root of the intrinsic rate constant.

Thus, apparent activation energy will be ½ of the true activation energy. Similarly, a change in pH which reduces kinetic rate constant by 4 will only reduce it by 2 under diffusion-limited condition.

Thus, one must be very careful when studying effects of reaction parameters on the kinetics of immobilized enzyme-catalyzed reactions. Adequate care is required so as to distinguish the kinetic parameters altered due to mass-transfer effects and hence are different from the actual kinetic parameters of the enzyme itself.

Bioprocess Engineering and Cell Culture Briefing

1. Cell Growth Kinetics and Modeling

Cell growth in bioprocesses follows distinct phases: lag, log (exponential), deceleration, stationary, and death. Understanding and modeling these phases are critical for optimizing product yield and process efficiency.

Growth Phases:

Lag Phase: A period of adaptation where cells synthesize new enzymes and increase in mass and volume, but not in number. This phase can be prolonged by low inoculum volume, poor inoculum condition, or nutrient-poor medium. Multiple lag phases,

known as diauxic growth, can occur if a medium contains multiple carbon sources that are utilized sequentially (e.g., glucose then xylose). To avoid diauxic growth, consistent culture medium composition, highly active inoculum in the exponential phase, and a large inoculum volume (5-10% of new medium volume) are recommended.

Log (Exponential) Phase: Characterized by rapid cell doubling, where the growth rate is independent of nutrient and substrate concentration. Carbon sources are actively utilized, and products are formed.

Stationary Phase: Occurs when nutrients are exhausted (substrate concentration approaches zero) and waste or secondary metabolic products accumulate. The growth rate equals the death rate, resulting in no net increase in cell population. Cells may remain metabolically active, producing secondary metabolites.

Death Phase: Follows the stationary phase, marked by a decline in viable cell numbers due to nutrient exhaustion and accumulation of toxic by-products.

Modeling Cell Growth and Metabolism:

Monod Equation: A widely used model to describe specific growth rate (μ) as a function of limiting substrate concentration (S): $\mu = \mu m\ S / (Ks + S)$.
[μm: Maximum specific growth rate, S: Concentration of limiting substrate, Ks: Half-saturation constant (substrate concentration at which μ is half of μm)]

In high substrate concentration ($S >>> Ks$), growth occurs at maximum rate ($dX/dt = \mu mX$).
In low substrate concentration ($S <<< Ks$), growth is linearly dependent on substrate concentration ($dX/dt = \mu m\ S\ X / Ks$).

Modified Monod Kinetics for CHO Cells: For CHO cells, a Monod-type equation can be used, but it's important to consider a threshold substrate concentration [S]t below which growth is not observed: $\mu = \mu max\ [S]^* / (Ks + [S]^*)$, where $[S]^* = [S]o - [S]t$.

Studies on CHO cells show that kinetic parameters like μmax and Ks can vary with temperature and cell type (naive vs. recombinant). For example, naive CHO cells at 37°C had $\mu max = 0.050\ h^{-1}$ and Ks = 1.023 g/L, while at 33°C, $\mu max = 0.038\ h^{-1}$ and Ks = 0.286 g/L. Recombinant CHO cells showed less temperature dependence, with $\mu max \approx 0.040\ h^{-1}$ and Ks ≈ 0.664 g/L at both 33°C and 37°C.

Yield Coefficients: Quantify the efficiency of converting substrate to biomass or product.
Yx/s: Grams of cells produced per gram of substrate consumed.
Yp/s: Grams of product produced per gram of substrate consumed.
Yp/x: Grams of product produced per gram of cells produced.

For *B. sacchari* on xylose, the xylose to PHA yield was approximately 0.37 gP(3HB)/gxyl under both nitrogen and phosphorus limitation.
For CHO cells, Yx/s was observed to be a function of substrate concentration, ranging from 0.27–1.08 × 10^7 cells/mL for naive cultures and 0.72–2.79 × 10^6 cells/mL for recombinant cultures. (Using simple models to describe the kinetics of growth, glucose consumption, and monoclonal antibody formation in naive and infliximab producer CHO cells)

[Specific Product Formation Rate (qp): Can be growth-associated (qp = Yp/x * μg), non-growth-associated (qp = β = constant, often during stationary phase), or mixed-growth-associated (qp = α μg + β - Luedeking-Piret equation).]

Monoclonal antibody (mAb) production in CHO cells can be described by a Luedeking–Piret model: d[mAb]/dt = αd[X]/dt + β[X], with specific values for α and β. (Using simple models to describe the kinetics of growth, glucose consumption, and monoclonal antibody formation in naive and infliximab producer CHO cells)

Hybrid Cybernetic Approach (HCM): Combines physiological models (logistic growth, external metabolite mass balances) with metabolic models (Elementary Mode Analysis, EMA) to dynamically describe cell behavior. This approach can segregate viable cells into growth and stationary metabolic states, each with different specific rates of consumption/production.

2. Bioreactors and Bioprocess Design

Bioreactors are crucial for providing optimal environmental conditions for microbial growth and product formation. Their design and operation are influenced by the organism and desired product.

Bioreactor Types: Generally categorized into lab-scale or industrial-scale, with variations including photobioreactors for light-dependent organisms.

Key Parameters and Control:

Agitation: Essential for mixing nutrients, oxygen, and cells, and for temperature distribution. Improper agitation can lead to shear stress on cells.

Temperature: Directly impacts metabolic rates and overall mass transfer.

pH: Controlled by acid/base addition.

Dissolved Oxygen (DO): Maintained above a certain saturation level (e.g., 40%) by varying agitation speed or gas flow.

Oxygen Mass Transfer: Overall Mass Transfer Coefficient (KLa): A critical parameter characterizing oxygen transport from the gas to the liquid phase. NA = KLa (C* - CL), where NA is oxygen transfer rate, C* is oxygen saturation concentration, and CL is dissolved oxygen concentration. The liquid side often presents the greatest resistance to mass transfer, so KL ≈ kL.

Factors Affecting KLa: Gas hold-up (volume fraction of gas in total volume), superficial gas velocity, power input from agitator (which influences interfacial area), and temperature. Smaller bubbles and higher gas hold-up generally lead to higher mass transfer rates.

3. Metabolic Engineering and Advanced Analysis Techniques

Metabolic engineering aims to modify cellular metabolism for improved product yield or desired phenotypes. Advanced techniques like 13C Metabolic Flux Analysis (MFA) and Elementary Mode Analysis (EMA) are vital tools.

13C Metabolic Flux Analysis (MFA): Uses labeled tracers (e.g., 13C) to track metabolic pathways and determine intracellular flux distribution.

Applications: Identifying metabolic engineering targets. For instance, inhibiting PDH kinase in CHO cells reduced lactate production and glycolytic fluxes while maintaining TCA cycle flux and specific productivity (qP), leading to higher volumetric titer and viable cell density (VCD) due to reduced lactate and delayed glucose depletion.

Future Directions: Mini Bioreactors: High-throughput systems like AMBR mini bioreactors provide industrially relevant scale-down models (over 10,000L commercial scale) and allow for multiple replicates and parallel tracer studies, overcoming cost and representativeness issues of larger or smaller systems.

Expansion of Metabolic Models: Increasingly accounting for compartmentalization within eukaryotic cells (cytosol and mitochondria) to accurately determine intracellular flux landscapes. Methods for isolating subcellular compartments and combining their labeling data with compartmentalized models enhance understanding, such as identifying cytosolic isocitrate dehydrogenase as a major source of cytosolic citrate.

Elementary Mode Analysis (EMA): A method to decompose a metabolic network into elementary flux modes (EMs), which are minimal sets of reactions that can operate in a steady state.
Polar Space Analysis Predictors (PSYA & EMPA): A novel approach that transforms the traditional Cartesian yield solution space of EMs into a polar coordinate system (using modules and angles). This "polar space" offers expanded visualization, better discrimination of EMs with specific traits, and allows for the identification of "cluster-paths" of EMs with similar characteristics.

Genetic Modification and Metabolic Engineering Prediction: EMPA (Elementary Mode Perturbation Analysis) uses the polar solution space to predict the impact of genetic modifications. It operates on the assumption that cells minimize metabolic change while adapting, searching for the closest EM that maximizes or minimizes a particular reaction. This theoretically provides a path with the lowest overall metabolic modification rate while achieving a desired change in a specific reaction.

Application Example: EMPA analysis successfully identified attractive modification targets to reduce lactate production in CHO-S cells by increasing mitochondrial pyruvate transport (v39). This perturbation was found to reduce lactate production (after a two-fold increase in v39 flux yield), increase TCA flux (up to 15-fold), and affect glutamine transport and related reactions, consistent with previous reports.

4. Nutrient Limitation Strategies

Nutrient limitation is a common strategy to enhance product accumulation, particularly for secondary metabolites or storage compounds like polyhydroxyalkanoates (PHAs).

***Burkholderia sacchari* and PHA Production:** *B. sacchari* LMG19450 is a promising microbial platform capable of producing poly(3-hydroxybutyrate) [P(3HB)] from xylose and other carbohydrates.

Nitrogen (N) vs. Phosphorus (P) Limitation: N-limited fed-batch experiments resulted in higher specific cell growth (around 0.19 1/h) and substrate consumption rates (around 0.24 1/h) compared to phosphorus-limited conditions.
Phosphorus limitation, while leading to higher P(3HB) concentration (g/L) and overall cell dry weight (CDW), required approximately 20 hours longer to achieve an equivalent P(3HB) content compared to nitrogen limitation. This suggests that pre-adapting seed cultures to diminished phosphorus could avoid this delay.

Yields: The residual biomass yield from nitrogen (YXr/N) for *B. sacchari* was around 7.5 g/g, similar to *E. coli* and *P. putida*. The YXr/P for *B. sacchari* was considerably higher (around 80 g/g) than for *E. coli* (33 g/g), suggesting lower phosphorus amounts in *B. sacchari* biomass or possible polyphosphate accumulation.

Phosphate Transport Genes: Genome analysis of *B. sacchari* revealed the presence of a complete *pst* locus (high-affinity phosphate uptake system) and polyphosphate kinase genes, indicating a sophisticated system for phosphate acquisition and storage.

5. Sterilization in Bioprocesses

Sterilization is a critical step to eliminate transmissible agents (fungi, bacteria, viruses, spores) from media and equipment, ensuring purity and preventing contamination in biological cultures.

Methods of Sterilization:

Physical Sterilization:

Heat Sterilization: Moist Heat: Boiling (for metallic devices), Pasteurization (for milk, alternative heating and cooling), Autoclaving (steam under pressure, 115°C for 60 min or 121°C for 20 min at 15psi, highly efficient and can kill bacterial spores).

Dry Heat and Radiation Sterilization

Chemical Sterilization: Uses agents like ethylene oxide, ozone, chlorine bleach, glutaraldehyde, formaldehyde, hydrogen peroxide, peracetic acid.

Mechanical Methods:

Filtration: Using filters like Seitz filters (asbestos or other materials, pad-like, thicker than membrane filters, may absorb solution) or sintered glass filters (glass, brittle, do not absorb liquids). Candle filters (made of diatomous mud with minute pores) trap microbes. In summary, the provided sources emphasize the importance of kinetic modeling, advanced analytical techniques like 13C MFA and EMA, and strategic nutrient management (e.g., limitation) in optimizing cell culture bioprocesses for improved product yield and efficiency, particularly in industrially relevant cell lines like CHO cells and promising microbial factories like *Burkholderia sacchari*. The increasing use of mini bioreactors and compartmentalized metabolic models further highlights the trend towards more rigorous and high-throughput bioprocess development.

That's it for Module – 3, hope you liked it. Do check out the videos in reference and stay tuned for Module – 4.
Happy Learning!!!

REFERENCES

#1: Biochemical Engineering Fundamentals by Bailey and Ollis
#2: https://youtu.be/8PWF5OeB7Ec?si=Hyk3qw51HwnP9BQC – Michaelis-Menten Model
#3: **https://youtu.be/sL_iEOuvK80?si=gAvBWYE7IngMRkV6** – Enzyme Immobilization

Introduction to Bioreactors: The Heart of Biochemical Processes

A bioreactor is a sophisticated vessel designed to provide a controlled and optimal environment for the growth of microorganisms or cells and for the biochemical reactions they mediate. These controlled environments are the cornerstone of the biotechnology, pharmaceutical, food, and beverage industries, enabling the large-scale production of a vast array of products, from life-saving antibiotics and vaccines to everyday items like yogurt and beer.

Drawing inspiration from the principles outlined in "Introduction to Biochemical Engineering" by Bailey and Ollis, this document will delve into the fundamental concepts of bioreactors. We will explore their core components, examine various types with illustrative examples, and discuss the critical operational parameters that govern their performance.

At its core, a bioreactor meticulously manages key factors to maximize the productivity of the biological system it houses. These factors include temperature, pH, nutrient and oxygen levels, and mixing. By providing this precisely controlled environment, bioreactors allow for the cultivation of high concentrations of cells, leading to efficient and consistent production of desired products.

The design and operation of bioreactors are central to biochemical engineering. A well-designed bioreactor not only ensures the viability and productivity of the cultured cells but also facilitates the recovery of the final product. The choice of a specific bioreactor type depends heavily on the characteristics of the cells being used (e.g., whether they are fragile or robust, aerobic or anaerobic) and the specifics of the desired bioprocess.

This introduction will serve as a gateway to understanding the diverse world of bioreactors, providing a foundation for further exploration into their design, operation, and application in various biotechnological endeavors.

The Anatomy of a Bioreactor: Core Components and Their Functions

While bioreactors come in various designs, they all share a set of fundamental components that are crucial for creating and maintaining the optimal conditions for biological processes. These components work in concert to ensure sterility, provide necessary nutrients and gases, and remove waste products.

1. The Vessel:

The main body of the bioreactor, the vessel, is typically a cylindrical container made of glass for laboratory-scale operations or stainless steel for industrial applications. The material choice is critical to ensure it can be sterilized, is non-toxic to the cultured organisms, and can withstand the pressures and temperatures of the process. Its geometry is also a key design consideration, influencing mixing and mass transfer.

2. Agitation System:

To ensure a homogeneous environment, an agitation system is employed to mix the contents of the bioreactor. This promotes uniform distribution of cells, nutrients, and oxygen, and helps to maintain a consistent temperature. In stirred-tank bioreactors, this is achieved through an impeller driven by a motor. The type of impeller (e.g., Rushton turbine, marine propeller) is chosen based on the viscosity of the culture medium and the shear sensitivity of the cells.

3. Aeration System (Sparger):

For aerobic processes, a continuous supply of oxygen is essential. The aeration system, or sparger, introduces sterile, compressed air or a specific gas mixture into the bioreactor. The sparger's design, often a porous ring or a single orifice, is crucial for creating small bubbles, which increases the surface area for efficient gas transfer into the liquid culture.

4. Temperature Control System:

Biological processes are highly sensitive to temperature. A temperature control system, typically a jacket surrounding the vessel or internal coils, is used to add or remove heat to maintain the optimal temperature for cell growth and product formation.

5. pH Control System:

The pH of the culture medium can change due to cellular metabolism. A pH control system, consisting of a pH probe and pumps for adding acid or base, is used to monitor and maintain the pH at a predetermined set point.

6. Probes and Sensors:

To monitor and control the bioprocess, bioreactors are equipped with various probes and sensors to measure critical parameters such as temperature, pH, dissolved oxygen concentration, and cell density.

7. Inlet and Outlet Ports:

These ports allow for the sterile addition of nutrients, media, and other supplements, as well as the removal of samples for analysis and the harvesting of the final product.

A Tour of Bioreactor Types: From Stirred Tanks to Photobioreactors

The choice of bioreactor is dictated by the specific requirements of the bioprocess, including the type of cells being cultured, the desired product, and the scale of operation. Here, we explore some of the most common types of bioreactors.

Stirred-Tank Bioreactor (STR)

The stirred-tank bioreactor is the most widely used type of bioreactor in the industry. Its versatility and robust design make it suitable for a wide range of applications, including microbial fermentations and animal cell culture.

- Design and Operation: An STR consists of a cylindrical vessel with a top-driven or bottom-driven agitator. The agitator, equipped with impellers, provides efficient mixing and aeration. Baffles are often installed to prevent the formation of a vortex and improve mixing efficiency.
- Advantages: Excellent mixing and mass transfer, good temperature control, and scalability.
- Disadvantages: High shear stress due to the impeller can damage fragile cells. High energy consumption.

Airlift Bioreactor

Airlift bioreactors are a gentler alternative to stirred-tank bioreactors, making them ideal for culturing shear-sensitive cells like plant and animal cells.

- Design and Operation: These bioreactors use the injection of gas to both aerate and mix the culture. They are typically divided into two zones: a "riser" where gas is introduced, and a "downcomer." The density difference between the gas-liquid mixture in the riser and the liquid in the downcomer drives the circulation of the culture.
- Advantages: Low shear stress, low energy consumption, and simple design with no moving parts inside the vessel.
- Disadvantages: Less efficient mixing compared to STRs, and potential for foaming.

Specialized Bioreactors for Diverse Applications

Packed Bed Bioreactor

Packed bed bioreactors are used for processes involving immobilized enzymes or cells.

- Design and Operation: The bioreactor is filled with solid particles (the "packing") to which the biocatalysts (enzymes or cells) are attached. The nutrient medium flows through the packed bed, allowing for a continuous process.
- Advantages: High concentration of biocatalyst, leading to high reaction rates. Reduced risk of catalyst washout.
- Disadvantages: Potential for clogging and channeling of the liquid flow. Difficult to control temperature and pH uniformly throughout the bed.

Photobioreactor

Photobioreactors are designed for the cultivation of photosynthetic organisms, such as algae and cyanobacteria, which require light for growth.

- Design and Operation: These bioreactors are constructed from transparent materials to allow for maximum light penetration. They come in various configurations, including tubular, flat-panel, and column designs, all aimed at optimizing light exposure to the culture.
- Applications: Production of biofuels, pigments, and high-value compounds from microalgae.
- Challenges: Light limitation at high cell densities, and difficulty in scaling up.

Membrane Bioreactor (MBR)

Membrane bioreactors integrate a membrane filtration unit with a biological reactor. This combination allows for the retention of the biomass within the reactor, leading to higher cell concentrations and a more efficient process.

- Design and Operation: The membrane can be submerged within the bioreactor or placed in an external loop. It acts as a barrier, separating the cells from the treated effluent.
- Applications: Primarily used in wastewater treatment for producing high-quality effluent that can be reused. Also used in cell culture applications to retain cells and continuously remove waste products.
- Advantages: High effluent quality, smaller footprint compared to conventional wastewater treatment plants.

Taming the Process: Key Operational Parameters in Bioreactors

The success of any bioprocess hinges on the precise control of several key operational parameters within the bioreactor. These parameters directly influence the growth rate of the cells, the yield of the desired product, and the overall efficiency of the process.

1. Temperature:

As one of the most critical parameters, temperature affects the rate of all biochemical reactions, including enzyme activity and cell growth. Most microorganisms have a narrow optimal temperature range for growth. Maintaining this temperature is crucial for maximizing productivity. For example, most mammalian cell cultures are maintained at a physiological temperature of 37°C, while fermentations with E. coli are often carried out at the same temperature.

2. pH:

The pH of the culture medium affects the activity of extracellular enzymes, the transport of nutrients across the cell membrane, and the overall metabolic activity of the cells. Maintaining a stable pH within the optimal range for the specific organism is essential. For instance, the optimal pH for most bacterial cultures is around 7.0, while fungi often prefer slightly acidic conditions.

3. Dissolved Oxygen (DO):

For aerobic organisms, the concentration of dissolved oxygen is a critical factor that can limit cell growth and product formation. The solubility of oxygen in water is low, so a continuous supply of oxygen is necessary. The DO level is monitored and controlled by adjusting the aeration rate and/or the agitation speed.

4. Substrate Concentration:

The concentration of essential nutrients, such as the carbon source (e.g., glucose), nitrogen source, and trace elements, directly impacts cell growth and product synthesis. In batch cultures, the initial substrate concentration is a key determinant of the final cell density. In fed-batch and continuous cultures, the feed rate of the substrate is carefully controlled to maintain optimal conditions.

5. Agitation and Aeration Rates:

In stirred-tank bioreactors, the agitation speed influences mixing, mass transfer, and the level of shear stress experienced by the cells. The aeration rate determines the amount of oxygen supplied to the culture. These two parameters are often manipulated together to achieve the desired dissolved oxygen level while minimizing cell damage.

6. Sterility:

Maintaining a sterile environment is paramount in most bioprocesses to prevent contamination by unwanted microorganisms. Contamination can compete for nutrients, produce inhibitory substances, or contaminate the final product. Bioreactors are sterilized before use, and all additions (media, air, etc.) are also sterilized.

Instrumentation And Control:

We have seen in the previous module, that the activity and useful lifetime of an enzyme catalyst or cell population depends directly on the catalyst environment. Accordingly, in order to develop and optimize biological reactor and in order to operate them most efficiently, the state of the environment must be monitored and controlled and the response of catalyst to the environment must also be determined.

To achieve these goals we need:

- Measurement
- Analysis of Measured Data
- Control

This brings us to instrumentation and control.

Physical And Chemical Sensors for the Medium and Gases:

One of the major goals of bioreactor data analysis is estimation of cell properties based on the available physiochemical measurements of the gas streams and the medium.

Sensors Of Physical Environment:

Major physical parameters that influence cellular functions and process economics and which can also be monitored continuously are temperature, pressure, agitator shaft power, impeller speed, broth viscosity, gas and liquid flow rates, foaming and volume or mass of reactor contents. It's a lot I know, but nowadays we have sensors for everything, even for measuring pH.

Of course, while all of these exist, for small laboratory experiments, we commonly measure only temperatures and air-feed flow rates. For larger fermenters, we also measure pressure management and regulation.

About the sensors:

The most widely used temperature sensor is the thermistor, a semiconductor device which exhibits changing resistance as a function of temperature. Although temperature-resistance relationship is nonlinear (as you might remember from JEE), this doesn't pose too much of an issue over the small range of temperature for most fermentations (25-45 degree C). Other temperature sensors include platinum resistance sensor, thermometer bulbs and thermocouples.

Pressure monitoring is important during sterilization. Pressure also influences gas monitoring gas solubility. Usually in fermentation reactors, diaphragm gauges are used to monitor pressure.

To monitor power input, there are ways like:

- Hall effect wattmeter
- Torsion Dynamometer

Online devices for measuring broth viscosity are not well developed. A possible strategy is measurement of power consumption at several different impeller (like a stirrer in simpler words) speeds. A dynamic method has also been proposed in which shaft power input is monitored during and after a brief (less than 30 seconds) shutdown in agitator power drive.

Medium Chemical Sensors:

Electrodes which can be repeatedly steam-sterilized in place are now available for pH, redox potential (E_h) and dissolved oxygen and CO_2 partial pressures. The most widely used and reliable among these is the pH electrode. On the next page is a schematic of said electrode. Electrodes for in situ sterilization must include a housing to provide pressure balance during sterilization or pressurized bioreactor operation.

The influence of pH on enzyme catalysis kinetics was covered in the previous module and was relatively simple, but the relation between redox potential and cell activity is not so simple. One application of measuring redox potentials is monitoring low contents of dissolved oxygen in anaerobic processes. The various types of dissolved oxygen probes are of galvanic (potentiometric) or polarographic types.

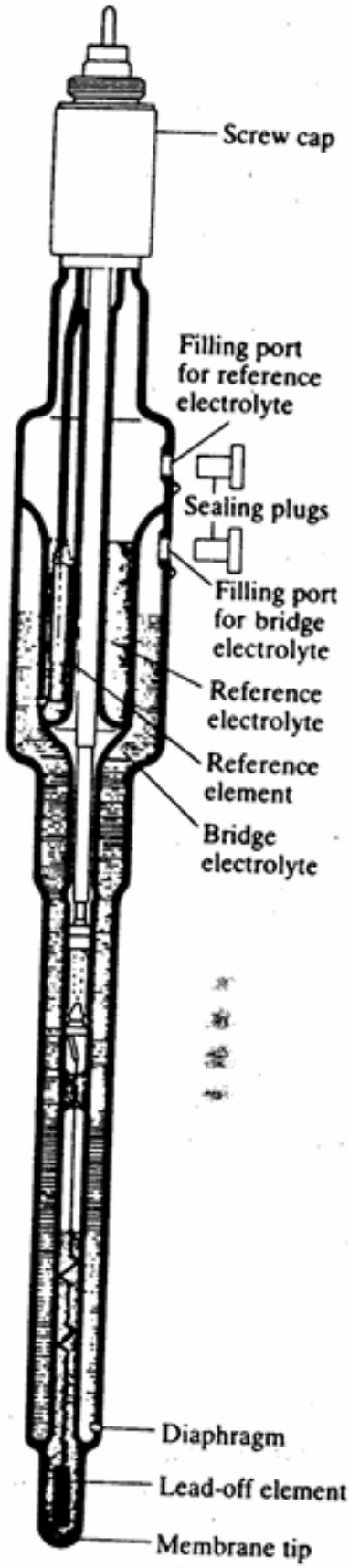

Figure 10.2 Schematic diagram of a combination pH electrode designed for autoclave steam sterilization. *(Ingold type 465. Illustration courtesy of Ingold Electrodes Inc.)*

Gas Analysis:

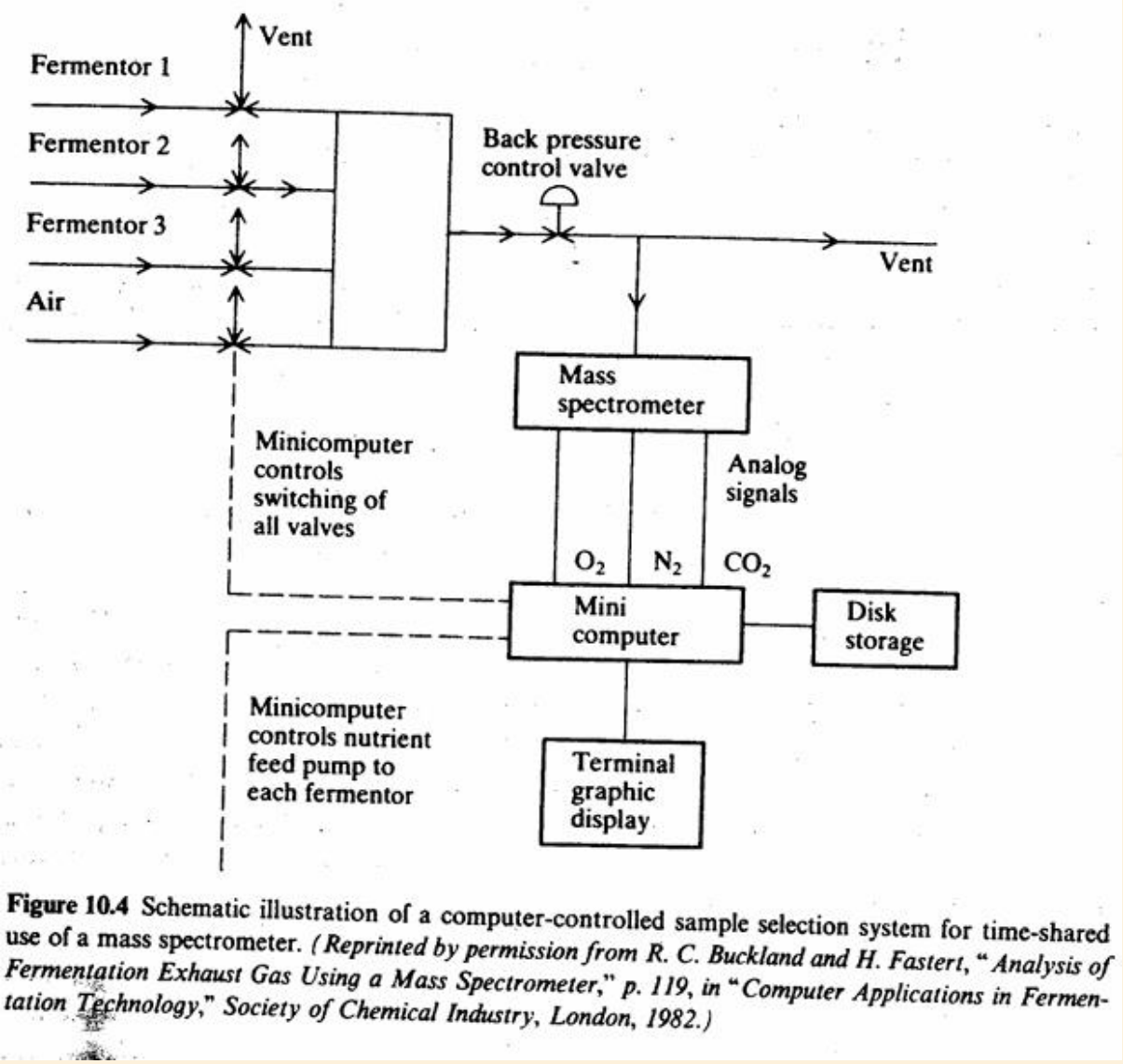

Figure 10.4 Schematic illustration of a computer-controlled sample selection system for time-shared use of a mass spectrometer. *(Reprinted by permission from R. C. Buckland and H. Fastert, "Analysis of Fermentation Exhaust Gas Using a Mass Spectrometer," p. 119, in "Computer Applications in Fermentation Technology," Society of Chemical Industry, London, 1982.)*

Mass spectrometry (MS) is enjoying increasing popularity for monitoring gas stream composition. Lower-priced instruments are making MS more accessible for research applications, and reliable, robust process instruments have made mass spectrometry more practical for industrial application. MS instruments offer rapid response times (< 1 min), high sensitivity (around 10^{-5} M detection limit), capability to analyze several components essentially simultaneously, linear response over a broad concentration range, and negligible calibration drift. Because of the expense of MS instruments, it is often desirable to interface the analyzer to several bioreactors and use a computer-controlled switching manifold to cycle sample streams from different reactors into the MS . As indicated in the above schematic diagram (only three fermentors are shown, but a single mass spec can support up to 30), the same computer may also be used for process control.

The mass spectroscopy is the method, so what does it measure? – concentration of CO_2. The concentration of CO_2 in the exhaust gas indicates the respiratory or fermentative activity of the organisms and hence is one of the most useful and widely applied measurements in monitoring and controlling bioreactors and hence why we measure it.

Insights Into Biochemical Industry:

In the industry, the same processes taking place in labs are scaled up to mass-manufacture goods. Major industries involve design, construction and advancement of unit processes that involve biological organisms (fermentation) or organic molecules(mostly enzymes).

Biochemistry has a lot of significance in industries like :

- Biotechnology
- Food Industry
- Pharmaceuticals

What the industry actually does

- Makes things like medicines, biofuels, enzymes, and food ingredients.
- Uses living cells, bacteria, or enzymes in factories to produce chemicals.

How things are made

- Scientists use fermentation, bioreactors, and purification methods.
- Lab research is turned into large-scale production.

Who works there and what they do

- Biochemical engineers, industrial microbiologists, and quality control specialists.

What problems the industry is solving

- Making cleaner fuels (biofuels).
- Producing medicines more cheaply or sustainably.
- Replacing plastics with biodegradable materials.

Latest trends and technology

- Stuff like synthetic biology, CRISPR, or green chemistry.
- AI and data science are starting to be used in bioprocesses(AI is everywhere!).

SO yes module 4 is over, this is an extremely short module especially about the industry, this is because we want you to focus on the problem statement for you project/report which you will be doing this week.