

LECTURE: PROTEIN, AMINO ACIDS, ENZYMES, AND KINETICS (BIOT274 IVC)



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Lecture: Protein, amino acids, enzymes, and kinetics (Biot274 @ IVC)

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1: Chemistry

Learning Objectives

- Explain the significance of biochemistry in understanding life processes.
- Describe the origin of elements and their role in biochemical structures.
- Differentiate between atoms, molecules, and compounds.
- Understand the octet rule and how it influences chemical bonding.
- Identify and compare different types of chemical bonds and their biological significance.
- Recognize the importance of key biochemical elements (C, H, O, N) in sustaining life.

Definition: Term

- **Biochemistry** - The study of the chemical processes and substances occurring within living organisms.
- **Atom** - The smallest unit of matter that retains the properties of an element.
- **Molecule** - A combination of two or more atoms bonded together.
- **Compound** - A molecule composed of different types of atoms.
- **Element** - A pure substance made entirely of one type of atom.
- **Atomic Number** - The number of protons in an atom's nucleus.
- **Mass Number** - The total number of protons and neutrons in an atom's nucleus.
- **Isotope** - Variants of an element with different numbers of neutrons.
- **Octet Rule** - The principle stating that atoms are most stable when they have eight electrons in their outer shell.
- **Covalent Bond** - A chemical bond where atoms share electrons.
- **Ionic Bond** - A bond formed by the transfer of electrons, creating charged ions.
- **Hydrogen Bond** - A weak attraction between a hydrogen atom and an electronegative atom.
- **Van der Waals Forces** - Weak intermolecular forces due to temporary dipoles.
- **Hydrophilic** - Molecules that are attracted to water.
- **Hydrophobic** - Molecules that repel water.

Why Study Biochemistry?

Biochemistry is the study of the molecules and chemical processes that make up life. It helps us understand how life originates, functions, and sustains itself. Have you ever wondered what you and a distant star have in common? The answer is **stardust**! Everything around us, including our bodies, is made from elements that were formed in the cores of stars billions of years ago (**Figure 1.1**). When these stars exploded, they scattered their elements across the universe, eventually forming planets and life. This means that the atoms in your body were once part of a star. But how does this stardust become **life**? Let's break it down step by step.

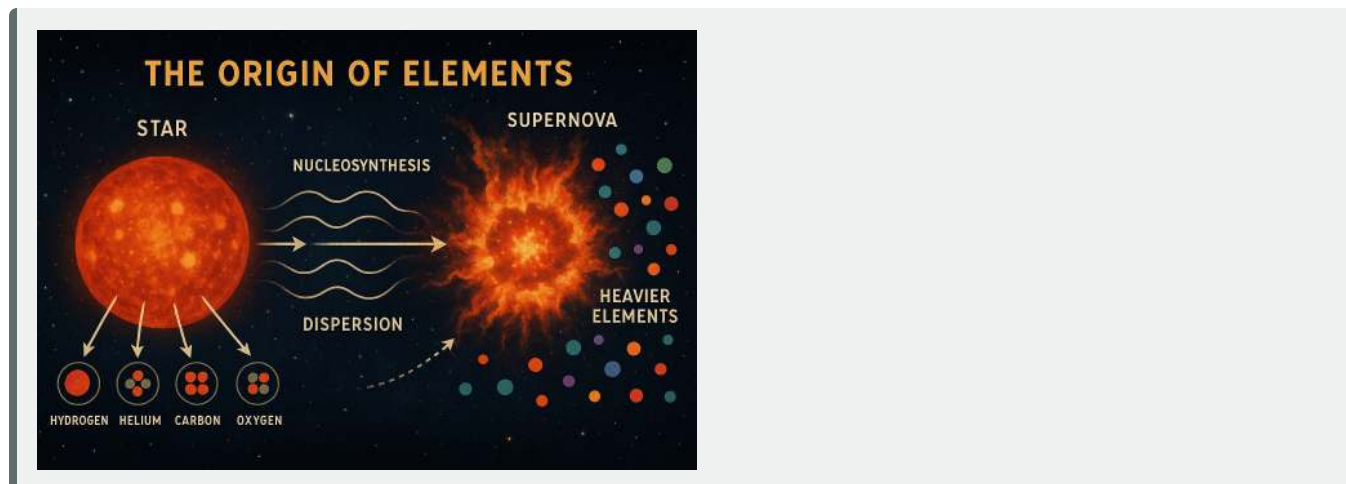


Figure 1.1: The Origin of Elements. A visual representation of nucleosynthesis in stars, showing how elements are formed and dispersed through supernovae. Image generated via ChatGPT

An **atom** is the smallest unit of matter that retains the properties of an element. Atoms interact to form **molecules** and **compounds**, which are essential for life. A **molecule** is combination of two or more atoms (e.g., O_2 – oxygen molecule), whereas a **compound** is a molecule composed of different types of atoms (e.g., H_2O – water). An atom can also be referred to as an element. An **element** is a substance made entirely of one type of atom. Each element has a unique number of protons, called the **atomic number** (Figure 1.2). For example, **Hydrogen (H)** has an atomic number of 1, which means it has **1 proton**, whereas **Carbon (C)** has an atomic number of 6, which means it has **6 protons**. The **mass number** is the total of protons and neutrons in an atom's nucleus. Different atoms have different masses due to their varying numbers of neutrons.

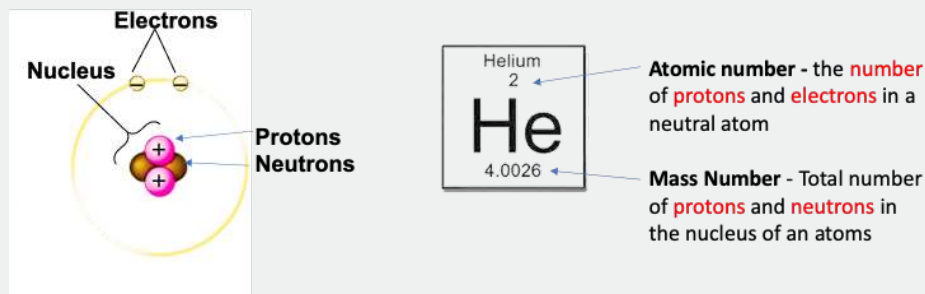


Figure 1.2: **Atomic Structure**. Diagram of an atom with labeled protons, neutrons, and electrons, including electron shells. Image of the atomic structure featuring Helium atom.

Isotopes are atoms of the same element that differ in the number of neutrons (Figure 1.3). While isotopes have the same chemical properties, they have different mass numbers. For example, Carbon has two stable isotopes: **Carbon-12 (^{12}C)** and **Carbon-13 (^{13}C)**, but it also has an unstable isotope, **Carbon-14 (^{14}C)**, which undergoes radioactive decay. Some isotopes are **radioactive** because their nuclei are unstable. These **radioisotopes** decay over time, releasing energy. Scientists use this decay to measure the age of fossils and rocks. For example, Carbon-14 decays to Nitrogen-14 in 5730 years (Figure 1.4), whereas Potassium-40 decays to Argon-40 in 1.29 billion years. This process is like a ticking clock, helping us understand the history of our planet and evolution.

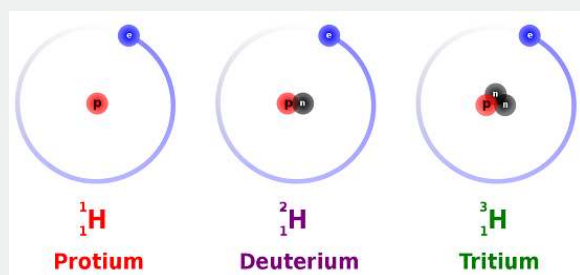


Figure 1.3: Figure The three most stable isotopes of hydrogen: protium ($A = 1$), deuterium ($A = 2$), and tritium ($A = 3$). (CC SA-BY 3.0; Balajijagadesh via Wikipedia).

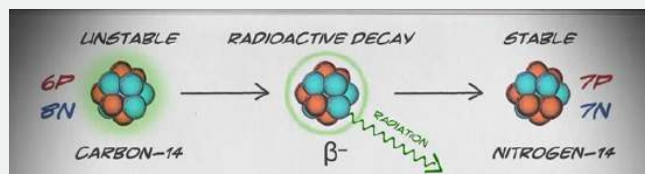


Figure 1.4: A **radioactive isotope** of carbon (carbon-14) has six protons and eight neutrons. It decays to a stable isotope of nitrogen (nitrogen-14), which has seven protons and seven neutrons. Radioactive decay releases radiation. (The particular type

of radiation that occurs in this example is called beta minus decay, β^- .) Carbon-14 decays at a predictable rate, with half of it decaying every 5730 years. Because carbon is abundant in organisms, this predictable decay rate is commonly used for dating fossils—image from [CDC](#) (public domain).

Introduction to the Octet Rule

The **octet rule** states that atoms are most stable when they have eight electrons in their outermost shell (valence shell). This is why atoms tend to gain, lose, or share electrons—to achieve a full outer shell and reach a stable state, like the **noble gases**. The octet rule is a fundamental principle in chemistry that helps explain why atoms interact the way they do. It states that atoms are most stable when they have a full outer shell of electrons, typically eight for most elements. This drives atoms to either share, lose, or gain electrons to achieve a stable electronic configuration, similar to noble gases, which are naturally inert. For example, sodium (Na) has one valence electron, making it highly reactive as it seeks to lose that electron and become a stable Na^+ ion. On the other hand, chlorine (Cl) has seven valence electrons and needs one more to complete its outer shell, making it eager to gain an electron and become Cl^- . When sodium donates its electron to chlorine, they form an ionic bond, creating table salt (NaCl). This concept is crucial in understanding how molecules form and function in biological systems. In human biochemistry, ions like Na^+ , K^+ , and Cl^- play key roles in nerve signal transmission, muscle contraction, and maintaining fluid balance. **Sodium-potassium pumps (Na^+/K^+ ATPase)** work because sodium and potassium ions achieve stability by losing or gaining electrons, allowing nerve impulses to travel through neurons. **Calcium (Ca^{2+}) ions** are essential for muscle contractions, as they move across membranes to trigger responses in muscle cells. **Oxygen (O_2)**, which we breathe, forms a stable molecule by sharing electrons between two oxygen atoms in a double bond.

The octet rule helps explain why certain elements are essential for life and why imbalances in these ions can lead to health problems such as electrolyte disorders.

Take a look at the Periodic Table on Figure 1.2 and determine the number of valence electrons for the elements below:

- Sodium (Na)** has 1 valence electron and will tend to lose it to become Na^+ .
- Chlorine (Cl)** has 7 valence electrons and will tend to gain 1 to become Cl^- .
- Oxygen (O)** has 6 valence electrons and will tend to gain 2 to become O^{2-} .

	1	2	3						4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19														
	H	He	Li						Be	B	C	N	O	F	Ne	Na						Mg	Al	Si	P	S	Cl	Ar										
1	1		3						4	5	6	7	8	9	10	11						12	13	14	15	16	17	18										
	H		Li						Be	B	C	N	O	F	Ne	Na						Mg	Al	Si	P	S	Cl	Ar										
	1.008		6.939						9.012	10.81	12.01	14.01	16.00	18.99	20.18	22.99						24.31	26.98	28.09	30.97	32.07	35.45	39.95										
2			11						12	metals						nonmetals						metalloids						5						6	7	8	9	10
			Na						Mg		B						C	N	O	F	Ne	Al						Si	P	S	Cl	Ar						
			22.99						24.31		10.81						12.01	14.01	16.00	18.99	20.18	26.98						28.09	30.97	32.07	35.45	39.95						
3			19						20	21						22	23	24	25	26	27	28	29	30	31	32	33	34	35	36								
			K						Ca	Sc						Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr								
			39.10						40.08	44.96						47.88	50.94	52.00	54.94	55.85	58.93	58.69	63.55	65.38	69.72	72.64	74.92	78.96	79.90	83.80								
4			37						38	39						40	41	42	43	44	45	46	47	48	49	50	51	52	53	54								
			Rb						Sr	Y						Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe								
			85.47						87.62	88.91						91.22	92.91	95.96	(98)	101.07	102.91	106.4	107.87	112.41	114.82	118.71	121.76	126.91	131.29									
5			55						56	57-70						71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86							
			Cs						Ba	La						Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn								
			132.91						137.33	138.91						174.97	178.49	180.95	183.84	186.21	190.23	192.22	195.08	196.97	200.59	204.38	207.2	208.98	(209)	(210)	(222)							
6			87						88	89-102						103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118							
			Fr						Ra	Ac						Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	Nv	Lr	Ts	Og							
			(223)						(226)	(227)						(232)	(231)	(238)	(237)	(244)	(243)	(247)	(247)	(251)	(252)	(257)	(258)	(260)	(261)	(264)								
7			57						58	59	60	61	62	63	64	65	66	67	68	69	70	71						72	73	74	75	76						
			La						Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	57-70						71	72	73	74	75	76					
			138.91						140.12	140.91	144.24	(144.91)	150.36	151.96	157.25	158.93	162.50	164.93	167.26	168.93	171.96	173.04	(138.91)						(140.12)	(140.91)	(144.24)	(144.91)	(150.36)					
			69						90	91	92	93	94	95	96	97	98	99	100	101	102	103						104	105	106	107	108	109					
			Ac						Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	(69)						(71)	(72)	(73)	(74)	(75)	(76)					
			(227)						(232)	(231)	(238)	(237)	(244)	(243)	(247)	(247)	(251)	(252)	(257)	(258)	(260)	(261)	(227)						(232)	(231)	(238)	(237)	(244)	(243)				

Figure 1.5: The Periodic Table is shared under a [CC BY-NC-SA 4.0](#) license and was authored, remixed, and/or curated by LibreTexts.

Types of Chemical Bonds and Their Importance in Biochemistry and Medicine

Chemical bonds are the forces that hold atoms together in molecules, and they determine the structure and function of biological macromolecules such as proteins, DNA, and enzymes. The three main types of chemical bonds are **covalent**, **ionic**, and **hydrogen bonds**, each with distinct properties. **Covalent bonds** involve the sharing of electrons between atoms, forming strong and stable

connections. These are essential in organic molecules, like the carbon-carbon bonds in DNA and proteins. Within covalent bonding, there are **polar covalent bonds**, where electrons are shared unequally, and **nonpolar covalent bonds**, where electrons are shared equally (**Figure 1.6 and 1.7**). The strength of covalent bonds ensures that **enzymes** can perform complex reactions without breaking apart easily. Many **drugs** target covalent interactions in enzymes, like **aspirin**, which forms a covalent bond with an enzyme to reduce inflammation. This difference plays a role in molecular polarity, which affects solubility and interactions with other molecules.

- **Nonpolar Covalent Bonds:** Electrons are shared equally (e.g., **O₂, H₂, CH₄**).
- **Polar Covalent Bonds:** Electrons are shared unequally, leading to partial charges (e.g., **H₂O, NH₃**).

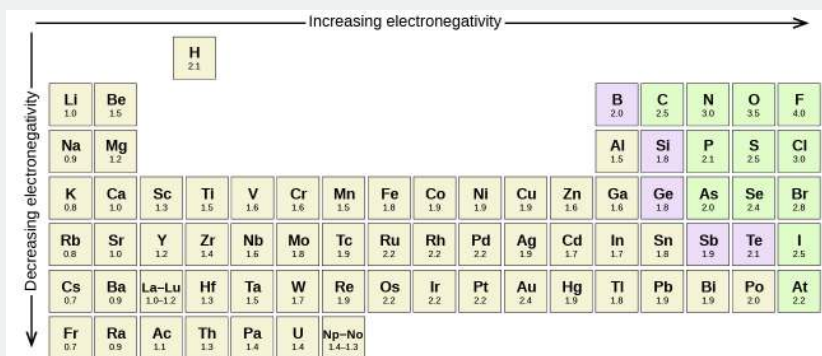


Figure 1.6: Pauling scale electronegativities of elements. Licensed [CC BY-SA 4.0](https://creativecommons.org/licenses/by-sa/4.0/).

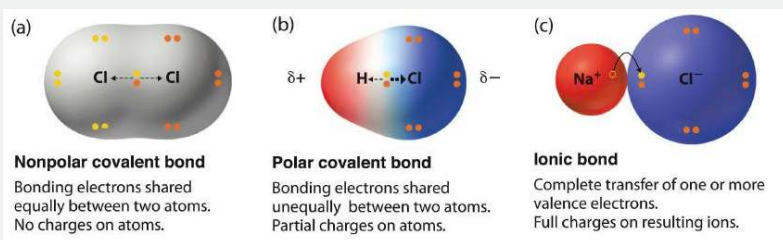


Figure 1.7: The Electron Distribution in a Nonpolar Covalent Bond, a Polar Covalent Bond, and an Ionic Bond Using Lewis Electron Structures. Electron-rich (negatively charged) regions are shown in blue; electron-poor (positively charged) regions are shown in red. (CC BY-SA-NC)

Ionic bonds, on the other hand, form when electrons are completely transferred from one atom to another, creating positively and negatively charged ions. These bonds are weaker in aqueous solutions because water molecules can surround and separate the ions. In medicine, this is significant because many drugs are formulated as salts (such as sodium or potassium salts) to improve solubility in the bloodstream. **Na⁺ and Cl⁻ form salt (NaCl)**, which dissolves in water to help maintain fluid balance in cells. **Calcium ions (Ca²⁺)** are crucial in **blood clotting, muscle contractions, and nerve transmission**. Many medications work by interacting with **ionic sites** on proteins, affecting how enzymes function.

Hydrogen bonds are weak but critical for biological function. They occur between molecules with polar covalent bonds, such as water molecules, and play a key role in maintaining the three-dimensional structures of proteins and nucleic acids (**Figure 1.8**). In drug studies, hydrogen bonding is essential because it determines how a drug interacts with its target protein. Many drugs function by mimicking natural molecules and forming hydrogen bonds with enzymes or receptors, enhancing their effectiveness. For example, DNA base pairing relies on hydrogen bonding between adenine and thymine and cytosine and guanine. The double helix structure of DNA is stabilized by hydrogen bonds between base pairs (A-T and G-C). The shape of enzymes depends on hydrogen bonding, which determines how they interact with substrates. Many drugs form hydrogen bonds with their target molecules, improving their effectiveness and specificity. Any disruption to these bonds can lead to mutations, which can have serious consequences in diseases like cancer.

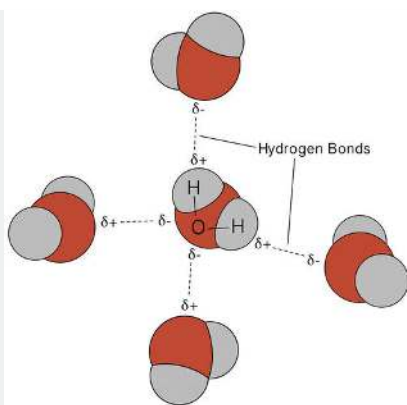


Figure 1.8: Multiple hydrogen bonds occur simultaneously in water because of its bent shape and the presence of two hydrogen atoms per molecule. Image by Allison Soult, Ph.D. (Department of Chemistry, University of Kentucky).

Among the various types of chemical bonds and interactions, **Van der Waals interactions** are usually the least taught in basic chemistry courses. However, these weak forces play a critical role in biological systems, drug interactions, and even the physical abilities of certain animals. Van der Waals forces are weak attractions that occur due to **temporary shifts in electron density**, creating momentary partial charges in otherwise **nonpolar molecules (Figure 1.9)**. This temporary charge can influence nearby molecules, leading to subtle but significant **conformational changes** in proteins, enzymes, and other biomolecules. A useful analogy to understand Van der Waals interactions is to think of a **magnet pulling all electrons to one side** of a molecule. Even though the molecule may not normally have a charge, this temporary shift creates a weak attraction between it and a neighboring molecule. These **induced dipoles** allow nonpolar molecules, which typically do not interact strongly, to exhibit weak attractive forces. While individually weak (with bond strengths between 0.4-4 kJ/mol), these forces become significant when many such interactions occur together, contributing to molecular **stability and shape**. One of the most famous real-world examples of Van der Waals interactions is how **geckos climb walls**. Geckos have microscopic hair-like structures, called **setae**, on their feet (**Figure 1.10**). These setae branch out into even finer structures that maximize contact with surfaces, allowing the gecko to take advantage of Van der Waals forces. Even though these interactions are weak, the sheer number of tiny contacts between the gecko's feet and a surface generates enough attraction to support the animal's weight—allowing it to cling to walls and even walk upside down on ceilings.

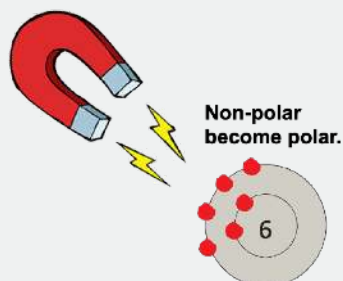


Figure 1.9: A molecule, represented as the magnet, that can contribute to shifting electrons of another molecule.



Figure 1.10: Here is an image of a gecko climbing a wall using Van der Waals forces. Image generated via ChatGPT.

In biochemistry and medicine, Van der Waals interactions contribute to the **binding specificity** between drugs and their targets. Many drugs fit into enzyme active sites using a combination of hydrogen bonds, ionic interactions, and Van der Waals forces. These weak forces also help proteins fold into their correct functional shapes, ensuring biological activity. If these interactions are disrupted—such as in protein misfolding diseases like Alzheimer's—serious health consequences can occur. Therefore, despite often being overlooked, Van der Waals interactions play a vital role in both **molecular stability and biological function**.

Hydrophilic vs. Hydrophobic Interactions in Enzyme and Drug Studies

Hydrophilic and hydrophobic interactions also play a crucial role in the structure and function of biological molecules. **Hydrophilic** molecules, which have a charge or polar groups, interact well with water and dissolve easily in aqueous environments. In contrast, **hydrophobic** molecules, which are nonpolar, tend to repel water and cluster together to minimize contact with it (**Figure 1.11**). This distinction is vital in biochemistry because the way molecules interact with water determines their function in the body.

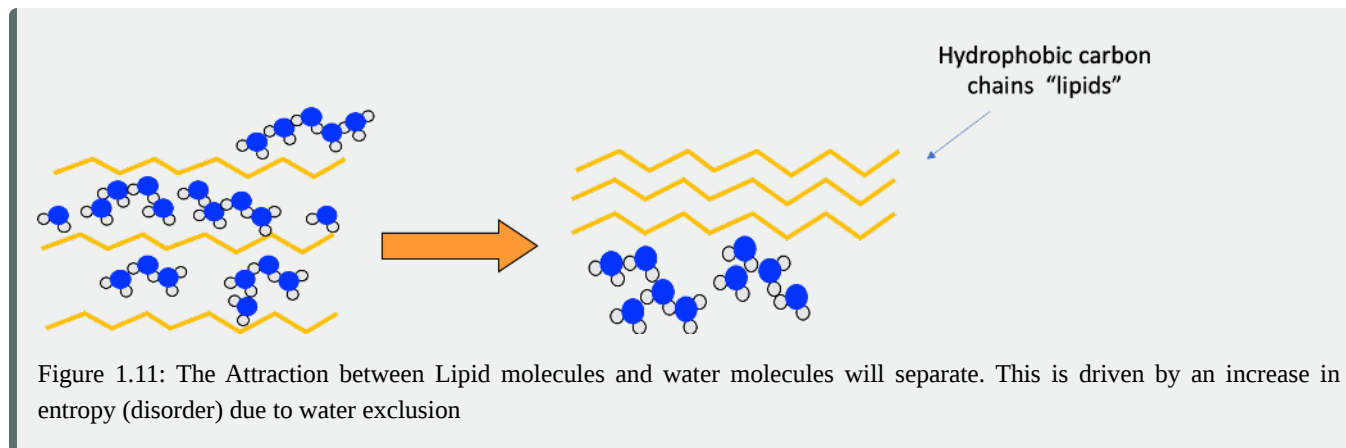


Figure 1.11: The Attraction between Lipid molecules and water molecules will separate. This is driven by an increase in entropy (disorder) due to water exclusion

In enzymes and drug development, hydrophilic and hydrophobic properties influence how drugs are absorbed, distributed, and interact with their targets. For instance, cell membranes are composed of **phospholipids**, which have amphipathic properties—meaning they have both hydrophilic heads and hydrophobic tails. This structure forms the lipid bilayer, creating a selective barrier that controls what enters and exits the cell. Drugs designed to cross cell membranes must often have hydrophobic properties to penetrate the lipid layer, while others need to be hydrophilic to dissolve in blood plasma. Understanding these interactions allows scientists to modify drug structures for better delivery and effectiveness. Additionally, proteins fold in a way that places hydrophobic amino acids inside, away from water, while hydrophilic amino acids remain on the outside. This folding is crucial for enzyme function, and any disruption—such as in genetic diseases or misfolded proteins in Alzheimer's—can have severe consequences.

Comparison of Bond Strengths and Their Importance in Drug Development

Understanding bond strength is crucial in drug design, as the type of bonds formed between a drug and its biological target determines **how strongly and how long the drug remains active** in the body. Different types of chemical bonds—covalent, ionic, hydrogen, hydrophobic interactions, and Van der Waals forces—play distinct roles in drug effectiveness, metabolism, and elimination. The stronger the bond, the more stable the interaction; however, in some cases, transient and reversible interactions are more desirable to allow for controlled biological effects.

Covalent bonds are the strongest, with bond energies ranging from **100-1000 kJ/mol**. These bonds form when two atoms share electrons, making them nearly irreversible in biological conditions. Drugs that form covalent bonds with their targets often have **long-lasting effects** because breaking the bond requires enzymatic intervention or protein turnover. One of the most well-known covalent inhibitors is **Aspirin (Acetylsalicylic Acid)**, which **irreversibly inhibits cyclooxygenase (COX-1 and COX-2)** enzymes. By forming a covalent bond with a serine residue in the active site, aspirin permanently disables the enzyme, reducing inflammation and pain until new enzymes are synthesized. Another example is **Clopidogrel (Plavix)**, an antiplatelet drug that covalently binds to the **P2Y12 receptor** on platelets, preventing clot formation in heart disease patients. Similarly, some **cancer drugs, like Ibrutinib**, use covalent bonding to target specific proteins, ensuring prolonged inhibition of cancer cell signaling.

Ionic bonds, with bond strengths around **20 kJ/mol**, occur due to the attraction between positively and negatively charged molecules. Many drugs exploit ionic interactions to bind to their targets while still allowing for **reversible binding**, which is critical for regulated biological activity. For instance, **Atorvastatin (Lipitor)**, a cholesterol-lowering drug, binds ionically to **HMG-CoA reductase**, the enzyme responsible for cholesterol synthesis. This interaction effectively blocks the enzyme's function but allows for **controlled drug clearance**, preventing excessive cholesterol reduction. **Beta-lactam antibiotics, like Penicillin**, also use ionic interactions to bind to bacterial enzymes (transpeptidases), disrupting cell wall synthesis and killing bacteria.

Hydrogen bonds, which range in strength from **12-30 kJ/mol**, are vital for increasing drug selectivity and specificity. These bonds form between a hydrogen atom and electronegative elements like **oxygen or nitrogen**, stabilizing drug-protein interactions. A classic example is **Remdesivir**, an antiviral drug designed to treat **COVID-19**. It forms multiple hydrogen bonds with the **RNA-dependent RNA polymerase (RdRp)** enzyme of SARS-CoV-2, mimicking natural nucleotides and disrupting viral replication. Similarly, **Oseltamivir (Tamiflu)**, an anti-influenza drug, relies on hydrogen bonding to fit snugly into the active site of **neuraminidase**, preventing viral spread.

Hydrophobic interactions, though weaker than covalent and ionic bonds, are essential in drugs that interact with **cell membranes or hydrophobic protein pockets**. These interactions occur due to water exclusion, forcing nonpolar molecules together, with bond strengths typically less than **40 kJ/mol**. Drugs like **Tamoxifen**, used in breast cancer treatment, bind to **estrogen receptors** via hydrophobic interactions, blocking estrogen signaling and slowing cancer growth. Similarly, **Statins (such as Simvastatin and Atorvastatin)** fit into the **hydrophobic active site of HMG-CoA reductase**, reducing cholesterol synthesis.

Van der Waals forces, the weakest of all at **0.4-4 kJ/mol**, occur due to temporary dipole-induced attractions. Although weak, these interactions contribute significantly to **drug-receptor binding stability** and fine-tuning molecular fit. One example is **Benzodiazepines (like Diazepam/Valium and Alprazolam/Xanax)**, which bind to **GABA-A receptors** through a combination of hydrogen bonds and Van der Waals forces. These interactions help modulate receptor activity, producing anxiolytic (anti-anxiety) effects. Another example is **HIV protease inhibitors (such as Ritonavir and Lopinavir)**, which use Van der Waals interactions to snugly fit into the hydrophobic pocket of **HIV protease**, preventing viral replication.

A deep understanding of bond strength is essential in drug design because different **therapeutic goals require different types of binding interactions**. Covalent inhibitors offer long-lasting effects but must be used cautiously to avoid off-target toxicity. Ionic and hydrogen bonds allow for strong, yet reversible interactions, ensuring drug effectiveness while preventing excessive buildup. Hydrophobic and Van der Waals forces help improve drug specificity, allowing precise molecular targeting. By fine-tuning these interactions, pharmaceutical scientists can develop drugs with **optimal binding, selectivity, and duration of action**, leading to more effective and safer treatments for diseases.

Bond Type	Strength (kJ/mol)
Covalent Bonds	100 - 1000
Ionic Bonds	~20
Hydrogen Bonds	12 - 30
Hydrophobic Interactions	< 40
Van der Waals Forces	0.4 - 4

Table 1.1: Bond Strength Comparison between covalent, ionic, hydrogen, hydrophobic interactions, and Van der Waals forces

The Importance of Key Elements in Biochemistry and Human Health

The periodic table serves as a fundamental tool in chemistry, organizing elements based on their properties. However, in biochemistry, only a select group of elements play a direct role in sustaining life. These essential elements—carbon (C), hydrogen (H), oxygen (O), nitrogen (N)—form the building blocks of biomolecules, regulate cellular functions, and maintain homeostasis in the human body. Understanding these elements is critical to grasping the biochemical processes that govern health and disease.

Carbon (C) is often referred to as the backbone of life because of its unparalleled ability to form stable covalent bonds with other elements. Its versatility allows it to create long chains, rings, and highly complex biomolecules such as glucose, proteins, fats, and DNA. Every organic molecule that makes up the human body—carbohydrates, lipids, nucleic acids, and proteins—relies on carbon's ability to form multiple strong bonds. Without carbon, the structure and function of life-sustaining molecules would not be possible.

Hydrogen (H) plays an equally critical role in biological systems, particularly in maintaining pH balance and energy transfer. Hydrogen is a major component of water (H_2O), the most abundant molecule in the human body, and is also present in biological acids and bases. It participates in cellular respiration by driving the electron transport chain, which ultimately produces ATP, the primary energy currency of cells. Moreover, hydrogen bonding is responsible for stabilizing the double-helix structure of DNA, ensuring the proper replication and function of genetic material.

Oxygen (O) is essential for survival, as it drives cellular respiration, the process by which cells extract energy from glucose. In the mitochondria, oxygen serves as the final electron acceptor in the electron transport chain, allowing ATP to be synthesized. Without oxygen, aerobic respiration cannot occur efficiently, leading to decreased energy production and potential cell death. Oxygen also plays a role in immune responses and is a key component of many biologically significant molecules, including hemoglobin, which transports oxygen in the blood.

Nitrogen (N) is another indispensable element in biochemistry, forming the backbone of amino acids, proteins, and nucleotides. It is present in DNA and RNA, where it forms the nitrogenous bases (adenine, thymine, cytosine, guanine, and uracil) that encode genetic information. Additionally, nitrogen is crucial for neurotransmitter production, influencing mood, cognition, and overall brain function. Without nitrogen, life-sustaining molecules such as enzymes, hormones, and structural proteins could not be synthesized.

In summary, these key elements—C, H, O, N—are foundational to life. Their interactions govern the structure and function of biomolecules, drive metabolic processes, and maintain physiological balance. Understanding their roles provides insight into how the human body functions at a molecular level and highlights the delicate biochemical equilibrium required for health and survival. Understanding biochemistry helps us grasp how life works at a molecular level. From the atoms in our bodies to the reactions that keep us alive, everything follows the laws of chemistry. The next time you drink water, eat food, or breathe, remember—you're interacting with the fundamental forces of biochemistry, made possible by **stardust**!



Key Takeaways:

- Life is built from elements originating from stars, emphasizing the universality of chemistry in biology.
- Atoms form molecules and compounds through various types of bonds, which influence biological function.
- The octet rule explains atomic stability and drives interactions leading to molecular formation.
- Different types of bonds—covalent, ionic, hydrogen, and Van der Waals—play essential roles in biochemistry and medicine.
- Essential elements (C, H, O, N) are fundamental to biological molecules, metabolic pathways, and life processes.

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2: Macromolecules

Learning Objectives

- Understand the structure and bonding properties of carbon and its importance in forming complex molecules essential for life.
- Recognize the role of functional groups in determining the properties and reactivity of organic molecules.
- Identify the major classes of biological macromolecules, including carbohydrates, lipids, proteins, and nucleic acids, and their biological functions.
- Learn how the structure of nucleic acids and proteins is related to their function in cells.
- Explore the importance of carbon-based molecules in metabolic processes, drug development, and cellular functions.

Definition: Term

- **Carbon Backbone:** The central structure formed by carbon atoms that can bond with other atoms to form complex molecules.
- **Covalent Bond:** A strong bond formed by the sharing of electrons between atoms, especially important in carbon chemistry.
- **Functional Groups:** Specific atoms or clusters of atoms within molecules that determine their chemical behavior and interactions (e.g., hydroxyl, carboxyl, amino).
- **Monosaccharides:** Simple sugar molecules that serve as the basic building blocks of carbohydrates (e.g., glucose, fructose).
- **Polysaccharides:** Complex carbohydrates formed by linking monosaccharides (e.g., starch, glycogen, cellulose).
- **Triglycerides:** Lipids consisting of one glycerol molecule and three fatty acid chains, serving as an energy storage form.
- **Amino Acids:** Organic compounds that link together to form proteins, each with distinct side chains (R groups).
- **Nucleotides:** The building blocks of nucleic acids, consisting of a nitrogenous base, a sugar, and a phosphate group.
- **Dehydration Synthesis:** A reaction in which water is removed to form larger molecules (e.g., forming a polysaccharide from monosaccharides).
- **Hydrolysis:** A reaction in which water is added to break down larger molecules (e.g., breaking down a polysaccharide into monosaccharides).

Macromolecules: The Building Blocks of Life

The Importance of Carbon

Carbon is found in all living things! It is the ultimate "Lego" of life because it is lightweight and has four bonding sites, allowing it to form complex and stable molecules. Imagine building with Legos—carbon atoms act like the base pieces that connect to different structures. Because of its ability to form four bonds, carbon creates a strong and flexible backbone for molecules essential to life.

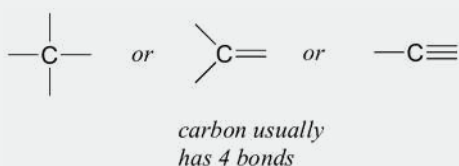
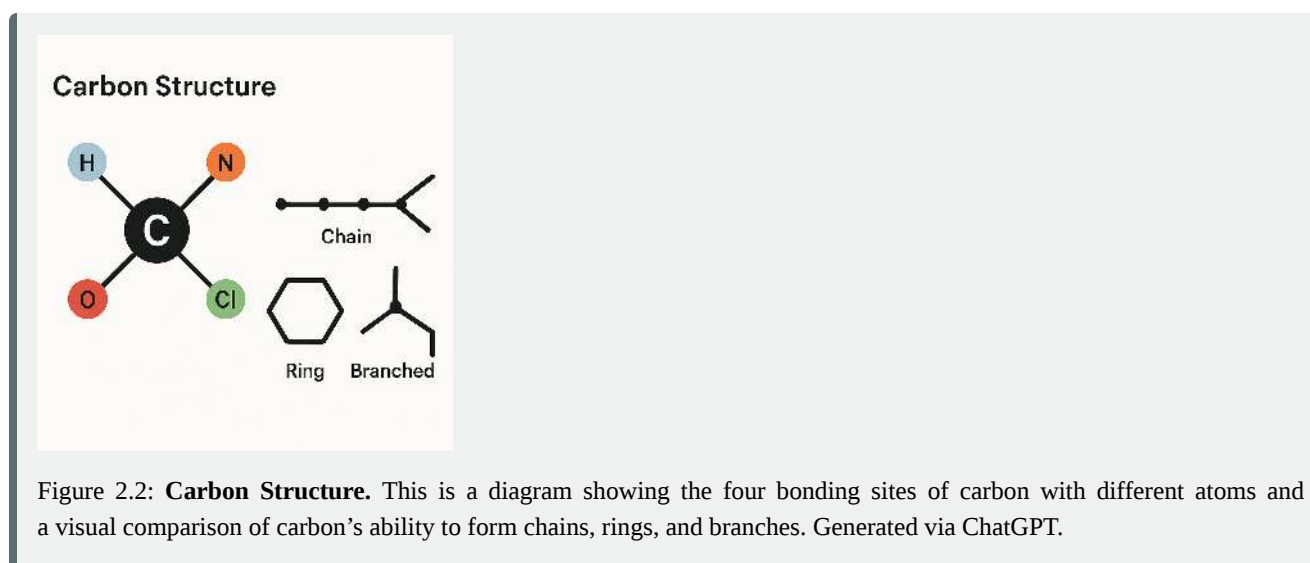


Figure 2.1: Carbon has four bonding sites, allowing for multiple potential complex binding. Image by [CC BY-NC-SA 4.0](https://creativecommons.org/licenses/by-nc-sa/4.0/) license

Carbon is a unique element due to its **four valence electrons** (electrons in its outer shell) and its ability to form **four covalent bonds** with other atoms (**Figure 2.1**). The electron configuration of carbon is $1s^2 2s^2 2p^2$, meaning it needs **four more electrons** to

complete its octet (stable electronic configuration). This results in carbon forming **strong, stable covalent bonds** with a variety of elements, including hydrogen, oxygen, nitrogen, sulfur, and phosphorus. Below are some advantages that carbon has:

- **Versatility in Bonding:** Carbon can bond with four different atoms or groups, allowing for an immense diversity of molecular structures. Carbon's ability to form varied structures leads to a vast array of organic molecules, each with unique chemical and physical properties.
- **Formation of Stable Molecules:** Carbon forms **single, double, or triple bonds**, influencing molecular geometry and reactivity. Linear chains provide flexibility, rings offer stability (e.g., aromatic rings), and branching influences solubility and function (**Figure 2.2**).
 - **Straight Chains:** Carbon atoms can link in a linear fashion, forming molecules like fatty acids and polymers.
 - **Branched Structures:** Some molecules, such as isoprenoids, have branching patterns that alter their chemical properties.
 - **Rings:** Carbon atoms form cyclic structures (e.g., benzene, glucose), which are fundamental in biomolecules like sugars, steroids, and nucleotides.
- **Diversity of Organic Compounds:** The four bonding sites enable carbon to create small molecules (like methane, CH_4) and massive macromolecules (like DNA and proteins). Shape and structure determine how molecules interact with each other in biological systems.
- **Polarity and Solubility:** By bonding with different atoms, carbon-containing molecules can be hydrophilic (water-



r-soluble) or hydrophobic (water-insoluble), which is crucial for biological functions.

Carbon's structural flexibility and bonding properties make it **the foundation of life and biochemistry**. Carbon-based molecules are central to metabolism, the set of chemical reactions that sustain life. One key molecule, ATP (adenosine triphosphate), acts as the primary energy currency of the cell, driving essential biological processes. Additionally, carbon chains and rings contribute to the structure of enzyme active sites. These active sites are responsible for binding specific substrates and catalyzing biochemical reactions efficiently, making enzymes indispensable for cellular function.

Many pharmaceutical compounds are carbon-based molecules, including antibiotics, painkillers, and chemotherapy agents. The ability of carbon to form diverse structures allows for the development of a wide range of therapeutic drugs targeting specific biological pathways. Carbon's structural versatility enables drugs to interact precisely with biological targets, improving their effectiveness while minimizing side effects. Furthermore, modifying the carbon skeleton of drug molecules can enhance their solubility, stability, and bioavailability, optimizing their therapeutic potential.

Overall, carbon's **four bonding sites, chain/ring/branching abilities, and structural diversity** make it the backbone of life and biochemistry. Its versatility is essential for **biological macromolecules, metabolism, and drug development**, reinforcing why carbon-based chemistry is central to science, medicine, and industry.

Functional Groups: The "Equipment" of Molecules

Carbon skeletons can be modified by functional groups, which change the molecule's properties (**Table 2.1**). Just like in a video game, where equipping your character with different gear changes their abilities, functional groups affect how molecules fold, bind, and interact with each other. This flexibility allows biological molecules to perform various functions.

Functional groups are specific **groups of atoms** within molecules that determine their **chemical properties and reactivity**. These groups influence how molecules interact with each other and participate in biochemical reactions. In organic chemistry and biochemistry, functional groups play a critical role in **defining molecular behavior**, such as solubility, acidity, polarity, and ability to form hydrogen bonds. Because they dictate how molecules function in biological systems, functional groups are essential in **enzymatic reactions, metabolism, and drug interactions**.

Common Functional Groups and Their Effects on Molecules

1. Hydroxyl Group (-OH):

- The hydroxyl group consists of an oxygen atom bonded to a hydrogen atom (-OH). It is **polar** due to the oxygen's high electronegativity, making molecules containing hydroxyl groups **hydrophilic** (water-soluble). Hydroxyl groups are commonly found in **alcohols, carbohydrates, and nucleotides**. In biological molecules, hydroxyl groups enable hydrogen bonding, which is essential for **protein folding, enzyme-substrate interactions, and DNA stability**.

2. Carboxyl Group (-COOH):

- The carboxyl group is a combination of a **carbonyl (-C=O)** and **hydroxyl (-OH)** group attached to the same carbon. It is found in **amino acids, fatty acids, and carboxylic acids**. Carboxyl groups are **acidic**, meaning they can donate a hydrogen ion (H^+) in solution, influencing pH levels in biological systems. This property is crucial in **protein structure and enzyme function**, as pH changes can alter molecular shape and reactivity.

3. Amino Group (-NH₂):

- The amino group consists of a nitrogen atom bonded to two hydrogen atoms (-NH₂). It is **basic**, meaning it can accept a proton (H^+) and become **positively charged (-NH₃⁺)** in physiological conditions. Amino groups are a fundamental part of **amino acids**, which form proteins. They contribute to **hydrogen bonding, protein folding, and enzyme catalysis**, making them essential for biological activity.

4. Phosphate Group (-PO₄³⁻):

- The phosphate group consists of a phosphorus atom bonded to four oxygen atoms, often carrying a negative charge. Phosphate groups are crucial in **energy transfer and storage** in biological systems, as seen in **ATP (adenosine triphosphate)**, the main energy carrier of cells. They also play a significant role in **DNA and RNA structure**, cell signaling, and enzyme regulation. The presence of a phosphate group increases molecule **solubility and reactivity**, which is why it is widely used in metabolism.

5. Carbonyl Group (-C=O):

- The carbonyl group contains a **carbon atom double-bonded to an oxygen atom**. It is found in **ketones and aldehydes**, which are present in **sugars, steroids, and metabolic intermediates**. Carbonyl groups influence **polarity, reactivity, and hydrogen bonding**, making them key in **metabolic pathways and enzyme function**.

6. Sulfhydryl Group (-SH):

- The sulfhydryl group consists of a **sulfur atom bonded to a hydrogen atom**. It is found in **cysteine**, an amino acid that helps form **disulfide bonds (-S-S-)**, which are crucial for **protein stability and structure**. Disulfide bridges are essential in **enzyme function and cellular signaling**.

Functional groups are the **active sites** of biological molecules, determining how they interact with **enzymes, receptors, and other biomolecules**. Enzymes recognize specific functional groups in substrates, allowing them to catalyze chemical reactions efficiently. For example, enzymes that break down carbohydrates recognize hydroxyl and carbonyl groups, while those involved in protein digestion target amino and carboxyl groups. In drug development, functional groups influence **drug solubility, absorption, and target interaction**. Many drugs mimic or modify natural functional groups to enhance **bioavailability and efficacy**. For instance, aspirin contains a carboxyl group that interacts with enzymes to reduce inflammation, and many antibiotics contain hydroxyl and amine groups that disrupt bacterial processes.

Overall, Functional groups are **the foundation of molecular interactions** in biology and chemistry. They dictate the properties of biomolecules, determine how enzymes function, and play a vital role in drug action. Understanding functional groups is essential for **biochemistry, medicine, and pharmaceuticals**, as they influence the structure, stability, and reactivity of life's most important molecules.

Functional Group	Structure	Properties
Hydroxyl		Polar
Methyl		Nonpolar
Carbonyl		Polar
Carboxyl		Charged, ionizes to release H ⁺ . Since carboxyl groups can release H ⁺ ions into solution, they are considered acidic.
Amino		Charged, accepts H ⁺ to form NH ₃ ⁺ . Since amino groups can remove H ⁺ from solution, they are considered basic.
Phosphate		Charged, ionizes to release H ⁺ . Since phosphate groups can release H ⁺ ions into solution, they are considered acidic.
Sulfhydryl		Polar

Table 2.1: This is a chart displaying common functional groups (hydroxyl, carboxyl, amino, phosphate, etc.) and their effects on molecules.

Functions of Carbohydrates

Carbohydrates provide energy and structural support to cells (**Table 2.2**). They are made of monomers called monosaccharides (such as glucose, fructose, and galactose). When these monomers link together, they form polysaccharides (such as starch, glycogen, cellulose, and peptidoglycan) (**Figure 2.3**). Carbohydrates are essential biological molecules that serve as a primary source of **energy**, play a role in **structural support**, and contribute to **cell signaling and molecular recognition**. They are composed of **carbon (C)**, **hydrogen (H)**, and **oxygen (O)** in a typical ratio of **1:2:1**. Carbohydrates are classified based on their complexity into **monosaccharides, disaccharides, and polysaccharides**, each serving different functions in biological systems.

Monosaccharides → Disaccharides → Polysaccharides

1. Monosaccharides (Simple Sugars)

- Basic units of carbohydrates consist of a single sugar molecule.
- Examples: **Glucose, Fructose, Galactose**

2. Disaccharides (Two Sugar Units)

- Formed by the condensation of two monosaccharides through **dehydration synthesis**.
- Examples: **Sucrose (Glucose + Fructose), Lactose (Glucose + Galactose), Maltose (Glucose + Glucose)**

3. Polysaccharides (Complex Carbohydrates)

- Long chains of monosaccharides linked together, serving as energy storage or structural components.
- Examples: **Starch, Glycogen, Cellulose, Chitin, Peptidoglycan**

Comparison of Starch, Glycogen, and Cellulose

Property	Starch (Plants)	Glycogen (Animals)	Cellulose (Plants)
Structure	Linear & branched (Amylose & Amylopectin)	Highly branched	Linear, rigid
Function	Energy storage in plants	Energy storage in animals	Structural support in plants
Linkages	$\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$	$\alpha(1 \rightarrow 4)$ and $\alpha(1 \rightarrow 6)$	$\beta(1 \rightarrow 4)$
Digestibility	Easily digested	Easily digested	Humans cannot digest it

Table 2.2: Comparison between different polysaccharides

Energy Storage Functions of Carbohydrates

1. Starch (Plants' Energy Reserve)

Starch is the primary storage carbohydrate in plants, found in seeds, tubers, and roots (e.g., potatoes, rice). A baked potato or French fries are rich in starch, which the body digests into glucose for energy. Rice and bread are also common starch sources. Starch can also consists of two forms:

- **Amylose:** Linear chains of glucose molecules linked by $\alpha(1 \rightarrow 4)$ bonds.
- **Amylopectin:** Branched structure with $\alpha(1 \rightarrow 6)$ linkages, making it easier to break down for quick energy.

2. Glycogen (Animals' Energy Reserve)

Glycogen is the primary carbohydrate storage molecule in animals, stored mainly in the **liver and muscles**. It has a highly branched structure, allowing rapid hydrolysis for quick energy release during **exercise or fasting**. Beef liver contains glycogen, which provides quick energy when needed. However, most glycogen in animals breaks down after slaughter, so it's not a major dietary source.

Carbohydrates in Cell Structure

1. Cellulose (Plants & Algae)

Cellulose is the most abundant **organic polymer** on Earth, forming the structural component of plant **cell walls**. It consists of **$\beta(1 \rightarrow 4)$ glucose linkages**, which create long, unbranched fibers that provide **rigidity and strength** to plants. Some structural carbohydrates, like cellulose and chitin, can also serve as an energy source for certain organisms. For example, some bacteria, including those in our gut, and herbivores have specialized enzymes to break down cellulose, allowing them to digest plants. Also, celery and kale have tough, fibrous textures because of cellulose, which provides structure to plant cell walls. Even though we can't digest cellulose, it acts as dietary fiber, helping digestion. Humans **cannot digest cellulose** because we lack the enzyme **cellulase**, which breaks **$\beta(1 \rightarrow 4)$ glycosidic bonds**. However, some bacteria in our gut microbiome can partially break down cellulose, producing **short-chain fatty acids** that provide some energy. These gut microbes help with **digestion, gut health, and immune function**.

2. Chitin (Insects & Fungi)

Chitin is a modified carbohydrate forming the **exoskeleton** of insects, crustaceans, and the **cell walls of fungi**. It consists of **N-acetylglucosamine** (a derivative of glucose), giving it extra strength and durability. Mushrooms like shiitake and portobello contain chitin in their cell walls, giving them a slightly chewy texture. Also, the crunchy shell of shrimp or crabs is made of chitin.

3. Peptidoglycan (Bacteria)

Peptidoglycan is a structural carbohydrate found in **bacterial cell walls**, consisting of sugar chains cross-linked by amino acids. This structure gives bacteria **shape and protection** from environmental stress. Some antibiotics, like penicillin, target peptidoglycan to weaken bacterial cell walls and kill bacteria.

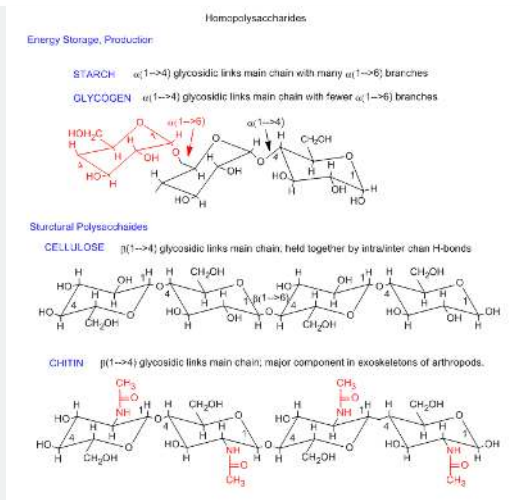


Figure 2.3: Image of starch, glycogen, cellulose, and Chitin. Image from CC BY-NC 3.0 license

Hydrolysis vs. Dehydration Synthesis (Figure 2.4)

1. Dehydration Synthesis (Condensation Reaction)

- Monosaccharides (simple sugars) join together to form disaccharides or polysaccharides by **removing water (H_2O)**, or releasing a water molecule to form a polysaccharide.
- Example: **Glucose + Fructose $\rightarrow$ Sucrose + H_2O**

2. Hydrolysis (Breaking Down Carbohydrates)

- Polysaccharides are broken down into monosaccharides by **adding water (H_2O)**, reversing dehydration synthesis. Think of cotton candy—when it touches your tongue, it dissolves quickly due to hydrolysis!
- Example: **Sucrose + H_2O $\rightarrow$ Glucose + Fructose**

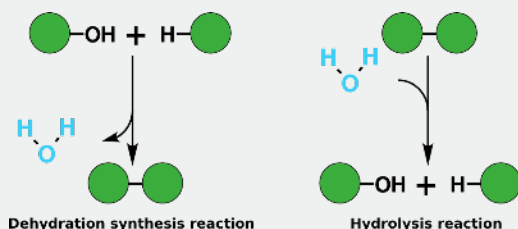


Figure 2.4: Polymer formation. Dehydration reactions join monomers. Hydrolysis splits polymers.

Carbohydrates are **critical in biochemistry** because they provide **energy, structural integrity, and cellular communication**. Many **enzymes** specialize in metabolizing carbohydrates, such as **amylase (breaks down starch)**, **lactase (breaks down lactose)**, and **cellulase (not found in humans but present in some bacteria and fungi)**. In **drug development**, carbohydrates play a role in **vaccine design, targeting cancer cells, and antiviral therapies**. For example, heparin (a polysaccharide) is used as a **blood thinner**, and some antibiotics (e.g., streptomycin) contain sugar molecules that enhance their function.

Overall, Carbohydrates are **essential macromolecules** involved in **energy storage, structural support, and biochemical signaling**. Their diversity in structure—ranging from simple sugars to complex polysaccharides—allows them to perform multiple biological roles. Understanding their functions is crucial in **biochemistry, nutrition, medicine, and biotechnology**.

Lipids: Structure, Function, and Importance

Lipids are an essential class of biological macromolecules that play crucial roles in **energy storage, cellular structure, hormone production, and protection**. Unlike proteins and carbohydrates, lipids are **hydrophobic (water-repelling)**, making

them ideal for forming barriers like cell membranes. There are several types of lipids, including **triglycerides**, **phospholipids**, **steroids**, and **waxes**, each with unique functions and biological significance.

1. Triglycerides: The Body's Energy Reserves

Triglycerides are composed of **one glycerol molecule** and **three fatty acid chains** (Figure 2.5). These fatty acids can be either **saturated** (solid at room temperature) or **unsaturated** (liquid at room temperature). Triglycerides serve as the **primary energy storage molecules** in animals and plants. When the body requires energy, specialized enzymes called **lipases** break down triglycerides into **glycerol and fatty acids**, which can then be converted into ATP through cellular respiration.

Triglycerides are primarily stored in **adipose tissue**—the body's fat deposits found under the skin (subcutaneous fat) and around organs (visceral fat). In animals, they are stored in **white adipose tissue (WAT)** for long-term energy storage, while **brown adipose tissue (BAT)** is responsible for generating heat, particularly in newborns and hibernating animals. Small amounts of triglycerides are also stored in **muscle cells**, where they can be quickly broken down for energy during exercise.

Fatty acids in triglycerides can be classified as **saturated** or **unsaturated**, affecting their physical properties and health effects (Table 2.3).

- **Saturated fatty acids** have **no double bonds** in their hydrocarbon chains, making them **solid at room temperature**. They are commonly found in **animal fats (butter, lard, red meat)** and **tropical oils (coconut oil, palm oil)**. High consumption of saturated fats is linked to an **increase in LDL (low-density lipoprotein) cholesterol**, which can contribute to heart disease.
- **Unsaturated fatty acids** contain **one or more double bonds**, making them **liquid at room temperature**. They are found in **olive oil, avocados, nuts, and fatty fish (salmon, tuna, mackerel)**. These fats are beneficial because they **increase HDL (high-density lipoprotein) cholesterol**, which helps remove excess cholesterol from the blood.

Cholesterol is transported through the bloodstream by **lipoproteins**, mainly **LDL ("bad" cholesterol)** and **HDL ("good" cholesterol)**. LDL can deposit cholesterol in artery walls, leading to **plaque buildup (atherosclerosis)**, **high blood pressure**, and **increased risk of heart attack or stroke**. HDL, on the other hand, helps remove excess cholesterol and transport it to the liver for excretion, reducing cardiovascular risk.

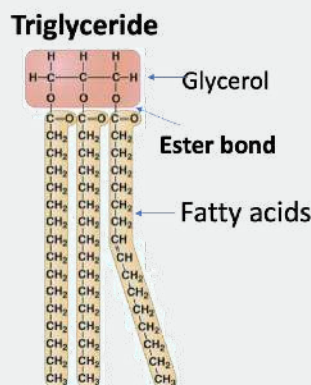


Figure 2.5: Image of Triglyceride.

Type	Structure	Sources	Effects on Health
Saturated Fatty Acids	No double bonds (solid at room temp)	Butter, red meat, cheese, coconut oil	Raises LDL ("bad" cholesterol)
Unsaturated Fatty Acids	One or more double bonds (liquid at room temp)	Olive oil, nuts, avocados, fish	Raises HDL ("good" cholesterol), lowers LDL

Table 2.3: Saturated vs. Unsaturated Fatty Acids

2. Phospholipids: The Cell Membrane Barrier

Phospholipids are crucial structural components of **cell membranes**, forming the **phospholipid bilayer** that separates the inside of the cell from its external environment (**Figure 2.5**). A phospholipid molecule consists of a **hydrophilic (water-loving) phosphate head** and **two hydrophobic (water-repelling) fatty acid tails**. When exposed to water, phospholipids arrange themselves into a bilayer, with the **hydrophobic tails facing inward** and the **hydrophilic heads facing outward**, creating a **selectively permeable membrane** that regulates the movement of substances in and out of cells.

This bilayer acts as the **“skin” of our cells**, providing structure while also allowing **communication, transport of molecules, and protection**. Proteins embedded within the membrane function as **receptors, channels, and transporters**, enabling essential nutrients to enter and waste products to exit. Without phospholipids, cells would not be able to maintain their internal environment, ultimately leading to cell death.

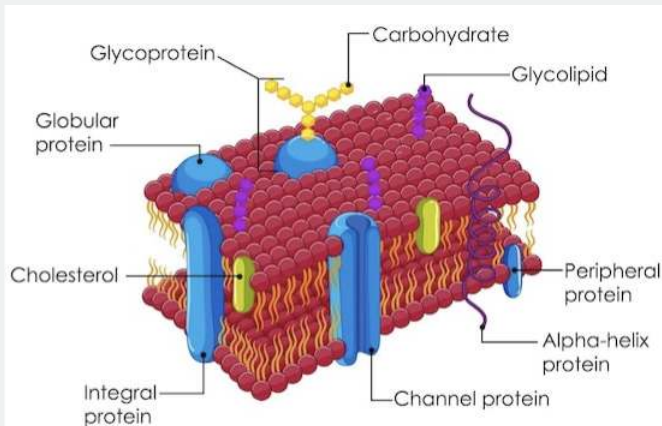


Figure 2.5: Illustration of the Human cell membrane structure from <https://www.freepik.com/author/brgfx>

3. Steroids: Cholesterol and Hormones

Steroids are lipids with a distinctive **four-ring carbon structure**. **Cholesterol** is the most well-known steroid, serving as the **precursor for important hormones**, including **testosterone, estrogen, cortisol, and vitamin D** (**Figure 2.6**). While cholesterol is essential for various bodily functions, excessive levels of LDL cholesterol can contribute to **plaque buildup in arteries**, increasing the risk of **hypertension, heart attacks, and strokes**.

When LDL cholesterol accumulates in blood vessels, it forms **plaques that narrow arteries**, restricting blood flow. This leads to **high blood pressure** because the heart must work harder to pump blood through narrowed pathways. If a plaque ruptures, it can cause a **blood clot**, potentially leading to a **heart attack (if in coronary arteries)** or a **stroke (if in the brain)**.

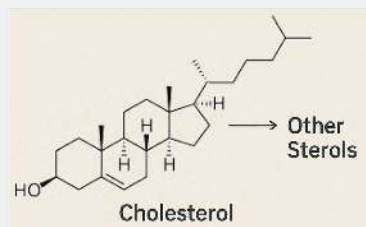


Figure 2.6: Image of Cholesterol leading to other precursors. Generated via ChatGPT

Despite its risks, cholesterol is vital for:

- **Vitamin D production**, which helps in calcium absorption and bone health.

- **Sex hormone synthesis**, including **testosterone and estrogen**, which regulate reproduction and secondary sexual characteristics.
- **Maintaining cell membrane fluidity**, allowing cells to function properly.

Some steroid hormones, such as **testosterone and estrogen creams**, can be **absorbed through the skin** and enter the bloodstream, making transdermal hormone therapy possible. Steroid hormones also **regulate gene expression**, turning specific genes on or off to control various biological functions. Anabolic steroids, synthetic derivatives of **testosterone**, are sometimes used by athletes to **increase muscle mass, strength, and endurance**. However, their misuse can lead to severe health issues, including **heart disease, liver damage, mood disorders (e.g., "roid rage"), and infertility**.

4. Waxes: Nature's Waterproofing Agent

Waxes are composed of **long-chain fatty acids** linked to **alcohols or hydrocarbons**, making them highly **hydrophobic** and ideal for **waterproofing surfaces** in plants and animals. Wax can be found in:

- **Humans: Earwax (cerumen)** is produced in the ear canal to trap dust, dirt, and microorganisms, preventing infections. The skin and hair also have a thin layer of **sebum**, a waxy secretion that helps retain moisture and protect against pathogens.
- **Plants:** The **waxy cuticle** on leaves and fruits prevents **water loss** and protects against environmental damage.
- **Animals:** Bees produce **beeswax**, used to build honeycombs, while some birds secrete a waxy coating to waterproof their feathers.

Overall, Lipids are essential to life, playing diverse roles in **energy storage, cellular structure, hormone signaling, and protection**. While some fats, such as **unsaturated fats and phospholipids, contribute to good health**, excessive **saturated fats and LDL cholesterol** can lead to severe health problems. Understanding lipids is critical in fields like **biochemistry, medicine, nutrition, and pharmacology**, as they influence everything from **cell function to disease prevention and drug development**.

Proteins: Structure, Function, and Importance

Proteins are made of **20 different amino acids (Figure 2.7)**. These amino acids act like letters in a language—just as you can form countless sentences using a limited alphabet, proteins can take on millions of forms with different functions. Proteins are among the most essential macromolecules in living organisms, responsible for **structural support, enzymatic activity, transport, signaling, and defense mechanisms**. These molecules are made up of **amino acids**, which are linked together in specific sequences to form unique structures that determine their function. The shape of a protein is critical for its role in biological systems, and understanding protein structure is fundamental in **biochemistry, medicine, and drug development**.

All proteins are made up of **amino acids**, which share a common structure shown in **Figure 2.8**.

- A **central carbon (α -carbon)**
- A **hydrogen atom (H)**
- An **amino group ($-NH_2$)**
- A **carboxyl group ($-COOH$)**
- A **variable side chain (R-group)** that determines the properties of the amino acid.
 - The **R-group** (side chain) varies between different amino acids, giving each one unique chemical properties. Some are **hydrophilic (water-attracting), hydrophobic (water-repelling), acidic, or basic**, influencing how they interact and fold into functional proteins.

AMINO ACID		
Nonpolar, aliphatic R groups	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{H} \end{array}$	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_3 \end{array}$
	Glycine	Alanine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH} \\ \quad \\ \text{CH}_3 \quad \text{CH}_3 \end{array}$	Valine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH} \\ \quad \\ \text{CH}_3 \quad \text{CH}_3 \end{array}$	Leucine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{S} \\ \\ \text{CH}_3 \end{array}$	Methionine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{H}-\text{C}-\text{CH}_3 \\ \\ \text{CH}_2 \\ \\ \text{CH}_3 \end{array}$	Isoleucine
Polar, uncharged R groups	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2\text{OH} \end{array}$	Serine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{H}-\text{C}-\text{OH} \\ \\ \text{CH}_3 \end{array}$	Threonine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{SH} \end{array}$	Cysteine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_2\text{N}^+-\text{C}-\text{H} \\ \quad \\ \text{H}_2\text{C} \quad \text{CH}_2 \end{array}$	Proline
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{C} \\ \\ \text{H}_2\text{N}=\text{O} \end{array}$	Asparagine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{C} \\ \\ \text{H}_2\text{N}=\text{O} \end{array}$	Glutamine
AMINO ACID		
Positively charged R groups	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{NH}_3^+ \end{array}$	Lysine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{NH} \\ \\ \text{C}=\text{NH}_2^+ \\ \\ \text{NH}_2 \end{array}$	Arginine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{C}-\text{NH}^+ \\ \quad \\ \text{CH} \quad \text{N} \end{array}$	Histidine
Negatively charged R groups	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{COO}^- \end{array}$	Aspartate
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \\ \\ \text{COO}^- \end{array}$	Glutamate
Nonpolar, aromatic R groups	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_5 \end{array}$	Phenylalanine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{C}_6\text{H}_4\text{OH} \end{array}$	Tyrosine
	$\begin{array}{c} \text{COO}^- \\ \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ \\ \text{CH}_2 \\ \\ \text{C}_8\text{H}_6\text{N} \end{array}$	Tryptophan

Figure 2.7: Types of amino acids: There are 21 common amino acids commonly found in proteins, each with a different R group (variant group) that determines its chemical nature. The 21st amino acid, not shown here, is selenocysteine, with an R group of $-\text{CH}_2-\text{SeH}$. Shared under a [CC BY-SA](#) license

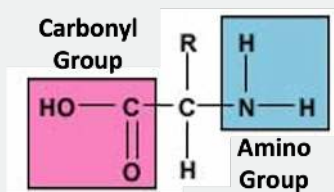


Figure 2.8: Image of an Amino Acid.

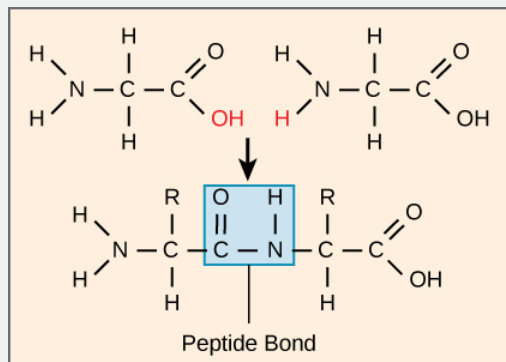


Figure 2.9: Peptide bond formation: Peptide bond formation is a dehydration synthesis reaction. The carboxyl group of one amino acid is linked to the amino group of the incoming amino acid. In the process, a molecule of water is released. Shared under a [CC BY-SA](#) license

Proteins fold into complex three-dimensional structures, which are categorized into **four levels** of organization.

1. The **primary structure** of a protein is the **linear sequence of amino acids** linked together by **peptide bonds** (Figure 2.9). This sequence is encoded by DNA and determines the protein's final shape and function. Even a single mutation in this sequence can alter the protein's activity, sometimes leading to diseases like **sickle cell anemia**.
2. The **secondary structure** forms when the amino acid chain **folds into patterns**, stabilized by **hydrogen bonds** between nearby amino and carboxyl groups. The most common patterns are:
 - **Alpha-helix (α -helix)** – A spiral shape stabilized by hydrogen bonds. Found in structural proteins like **keratin (hair, nails)**.
 - **Beta-pleated sheet (β -sheet)** – A folded sheet-like structure. Found in strong proteins like **silk**.
3. The **tertiary structure** refers to the overall **three-dimensional folding** of a protein due to interactions between **R-groups**. This structure determines the **function of enzymes, antibodies, and transport proteins**. For example, the enzyme **lysozyme** folds in a way that allows it to break down bacterial cell walls. These interactions include:
 - **Hydrogen bonding** (helps stabilize the structure)
 - **Ionic bonds** (between charged R-groups)
 - **Hydrophobic interactions** (hydrophobic side chains group together inside the protein, away from water)
 - **Disulfide bridges** (strong covalent bonds between sulfur-containing amino acids like cysteine)
4. Some proteins consist of **multiple polypeptide chains** (subunits) that join together to form a functional unit. This is called the **quaternary structure**. Examples include:
 - **Hemoglobin** (a protein in red blood cells that carries oxygen, composed of four subunits).
 - **Collagen** (a structural protein in skin and connective tissues, made of three intertwined chains).

Proteins must **fold correctly** to function properly. If a protein misfolds, it can lead to diseases such as **Alzheimer's, Parkinson's, and cystic fibrosis**, where misfolded proteins aggregate into harmful plaques or fibers. In **drug development**, understanding protein structure is crucial. Scientists use **X-ray crystallography and cryo-electron microscopy** to determine protein structures and design drugs that fit into their active sites, like a **lock and key mechanism**. Many drugs work by **binding to specific proteins** to either **activate or inhibit** their function. For example:

- **Antibiotics** target bacterial enzymes, preventing them from functioning.
- **Cancer drugs** block proteins that promote uncontrolled cell growth.
- **Insulin** (a hormone protein) regulates blood sugar levels in diabetes treatment.

Overall, proteins are vital molecules in all living organisms, involved in nearly every biological process. Their **structure determines their function**, and even slight changes in folding can have profound effects on health. Understanding protein structure is essential for **biochemistry, medicine, and drug design**, helping researchers develop new treatments for diseases. Through techniques like **protein engineering and drug targeting**, scientists continue to explore the incredible potential of proteins in biotechnology and healthcare.

Nucleic Acids: Structure, Function, and Biological Importance

Nucleic acids are **biological macromolecules** that store and transmit genetic information and are essential for cellular functions. The two main types are **DNA (deoxyribonucleic acid)** and **RNA (ribonucleic acid)**. These molecules are composed of **nucleotides**, which serve as the building blocks of nucleic acids. In addition to genetic storage, nucleotides play vital roles in energy transfer (ATP), catalysis (ribozymes), and molecular recognition (aptamers).

Each nucleotide consists of three main components as shown in Figure 2.10.

1. A **five-carbon sugar (pentose)**:
 - **Deoxyribose** in DNA
 - **Ribose** in RNA
2. A **phosphate group**, which links nucleotides together in a chain via **phosphodiester bonds**.
3. A **nitrogenous base**, which determines the identity of the nucleotide:
 - **Purines (double-ring structures): Adenine (A), Guanine (G)**
 - **Pyrimidines (single-ring structures): Cytosine (C), Thymine (T) in DNA, Uracil (U) in RNA**

Table 2.4 describes the differences between DNA and RNA. The sugar-phosphate backbone forms the structural framework of DNA and RNA, while the nitrogenous bases pair together to encode genetic information. The **absence of the 2'-OH group in deoxyribose** makes DNA less reactive and more resistant to hydrolysis. This stability is crucial for long-term genetic storage, as DNA must remain intact over generations. RNA, with its extra **hydroxyl (-OH) group at the 2' position**, is more chemically reactive and can catalyze biochemical reactions (ribozymes). This makes RNA ideal for transient roles like **messenger RNA (mRNA) and transfer RNA (tRNA)**. DNA also exists as a **double-helix**, where two strands wind around each other in an **antiparallel fashion** (5' → 3' direction of one strand runs opposite to the other) (**Figure 2.11**). The **3D structure of DNA** is crucial for processes like **replication, transcription, and epigenetic modifications**, influencing gene expression and cellular function. The helical structure is stabilized by the **hydrogen bonds** between complementary base pairs.

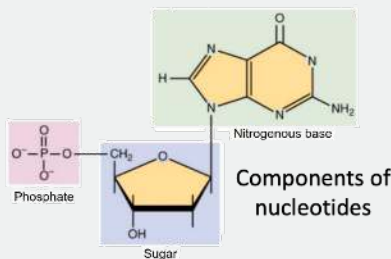


Figure 2.10: Image of a Nucleic Acid.

Feature	DNA (Deoxyribonucleic Acid)	RNA (Ribonucleic Acid)
Sugar	Deoxyribose (lacks 2'-OH)	Ribose (has 2'-OH)
Strands	Double-stranded (helix)	Single-stranded
Bases	A, T, C, G	A, U, C, G
Stability	More stable, less reactive	More reactive (due to 2'-OH)
Function	Long-term genetic storage	Involved in protein synthesis, gene regulation, catalysis

Table 2.4: Differences between DNA and RNA.

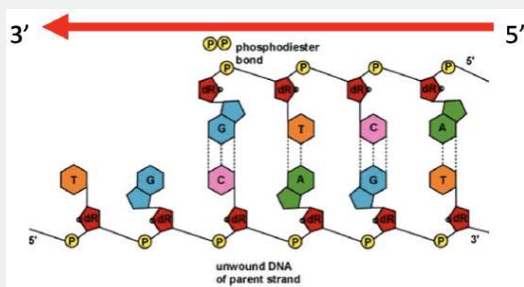


Figure 2.11: DNA built from a 5' to 3' direction

In DNA and RNA, nitrogenous bases pair through **hydrogen bonding** following **Chargaff's base-pairing rules**:

- **Adenine (A) pairs with Thymine (T) in DNA and Uracil (U) in RNA → 2 hydrogen bonds**
- **Cytosine (C) pairs with Guanine (G) → 3 hydrogen bonds**

The additional hydrogen bond in **C-G pairs** makes them **stronger** than **A-T pairs**, which is critical in maintaining DNA stability. Organisms in **high-temperature environments** (e.g., **thermophilic bacteria**) have a higher proportion of **C-G base pairs**, as they prevent DNA strands from denaturing. At the **ends of eukaryotic chromosomes**, DNA sequences called **telomeres** serve as protective caps, preventing loss of essential genetic information during replication. Telomeres consist of **repetitive nucleotide sequences** (e.g., **TTAGGG in humans**) and do not code for proteins. During DNA replication, the **lagging strand** cannot be fully copied, leading to progressive shortening of chromosomes. **Telomerase**, an enzyme active in stem cells and cancer cells, extends telomeres by adding repeated sequences, counteracting this loss. Without telomerase, telomeres gradually shorten, leading to **cell aging (senescence)** and eventual cell death. The repetitive **G-rich sequences in telomeres** can form **G-quadruplex structures**, stabilized by **hydrogen bonds**. These structures help **protect chromosome ends** from degradation and fusion with neighboring chromosomes.

Nucleotides also serve other functions as well. For example, ATP, which is a **nucleotide derivative** that acts as the primary **energy currency of the cell**. It consists of **Adenine base, Ribose sugar, and Three phosphate groups**. When ATP is hydrolyzed into **ADP (Adenosine Diphosphate)** and **inorganic phosphate (Pi)**, it releases energy used for cellular functions such as **muscle contraction, active transport, and enzyme activity**.

- **Reaction:** $\text{ATP} \rightarrow \text{ADP} + \text{Pi} + \text{Energy}$

Other Functions of Nucleotides

- **rRNA (Ribosomal RNA):** A structural and catalytic component of ribosomes, essential for protein synthesis.
- **tRNA (Transfer RNA):** Carries amino acids to ribosomes during translation.
- **Aptamers:** Short, single-stranded DNA or RNA sequences that bind specifically to target molecules (used in diagnostics and therapeutics).
- **Coenzymes (e.g., NAD⁺, FAD, Coenzyme A):** Derived from nucleotides, these molecules assist in **metabolic reactions** like cellular respiration.

Overall, Nucleic acids are **fundamental to life**, storing genetic information, regulating gene expression, and facilitating biological reactions. DNA's **stability and structure** allow for long-term genetic storage, while RNA's **versatility** enables various cellular functions. Understanding these molecules is essential in **biochemistry, medicine, and drug development**, as it aids in advancements like **gene therapy, RNA-based vaccines, and targeted cancer treatments**.

Post-Lecture Learning Objectives

- **Understand Carbon's Versatility:** Recognize how carbon's ability to form various bonding patterns (straight chains, branched chains, rings) leads to a wide variety of organic molecules.
- **Analyze the Role of Functional Groups:** Understand the role functional groups play in modifying the behavior of molecules in biological systems.
- **Link Structure to Function in Biological Molecules:** See how the structure of molecules such as carbohydrates, lipids, proteins, and nucleic acids influences their function in cells.
- **Explore the Relationship Between Structure and Disease:** Learn how misfolded proteins or genetic mutations related to carbon-based molecules contribute to diseases like Alzheimer's, cancer, and sickle cell anemia.
- **Understand the Biochemical Role of ATP:** Identify ATP as the energy currency of cells and explain how it's used in biochemical reactions.

Definition: Term

- **ATP (Adenosine Triphosphate):** A nucleotide that serves as the primary energy carrier in cells, used in processes like muscle contraction and active transport.
- **Polysaccharides:** Large carbohydrate molecules made from many monosaccharides (e.g., starch, glycogen, cellulose) that store energy or provide structural support.
- **Phospholipids:** Molecules that form the lipid bilayer of cell membranes; consist of a hydrophilic head and hydrophobic tails, enabling selective permeability.

- **Steroids:** Lipids with a four-ring carbon structure, like cholesterol, which are precursors for hormones and maintain membrane fluidity.
- **Protein Structure Levels:** The four levels of protein structure (primary, secondary, tertiary, and quaternary) determine the shape and function of proteins.
- **Denaturation:** The process by which proteins lose their shape and function, often due to temperature or pH changes.
- **Nucleotides:** The building blocks of nucleic acids, containing a sugar, phosphate group, and nitrogenous base, also involved in energy transfer (e.g., ATP).

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3: Water and Buffer

Learning Objectives

- Define pH and explain how it is measured.
- Understand the logarithmic nature of the pH scale.
- Explain how pH influences biological systems and biochemical reactions.
- Describe the role and mechanism of buffer systems in maintaining pH homeostasis.
- Differentiate between strong and weak acids/bases and their dissociation behavior.
- Identify major biological buffer systems and their importance in the human body.

Definition: Term

- **pH:** A measure of hydrogen ion (H^+) concentration in a solution.
- **pKa:** The pH at which a weak acid is half dissociated; indicates acid strength.
- **Buffer:** A solution that resists pH changes by neutralizing added acids or bases.
- **Titration Point:** The stage in a titration where the amount of added acid or base fully neutralizes the solution.
- **Buffer Zone:** The range of pH values where a buffer effectively stabilizes pH.
- **Strong Acid/Base:** A substance that fully dissociates in water, drastically altering pH.
- **Weak Acid/Base:** A substance that only partially dissociates, helping to maintain pH balance.

Water: The Solvent of Life

Water is often referred to as the "**solvent of life**" because of its unparalleled ability to dissolve a wide range of substances essential for biological functions. This property makes water an indispensable component of cells, tissues, and bodily fluids. In biological systems, water serves as a medium for chemical reactions, facilitates nutrient transport, and aids in the removal of waste products.

Hydrogen bonding is a crucial interaction in water and many biological molecules. A hydrogen bond forms when a hydrogen atom covalently bonded to an electronegative atom (such as oxygen or nitrogen) experiences an attraction to another electronegative atom. These bonds are weak individually but collectively play a significant role in determining the properties of water and biological macromolecules. For example, the hydrogen bonds between water molecules give it a high surface tension, allowing insects like water striders to walk on water. In DNA, hydrogen bonds hold the two strands of the double helix together, ensuring genetic stability. The three-dimensional shape of macromolecules like proteins and nucleic acids is heavily influenced by hydrogen bonding and interactions with water. For example, the secondary and tertiary structures of proteins depend on hydrogen bonds, which stabilize alpha-helices and beta-sheets. In DNA, hydrogen bonds between base pairs maintain the double-helical structure necessary for genetic function.

Water is a **polar molecule**, meaning it has a partial positive charge on the hydrogen atoms and a partial negative charge on the oxygen atom. This polarity allows water molecules to attract one another through hydrogen bonds (**Figure 3.1**). Additionally, water can interact with other polar substances, enabling it to dissolve ionic compounds like sodium chloride (table salt) and hydrophilic molecules such as sugars and amino acids. A **polar covalent bond** occurs when electrons are shared unequally between atoms, resulting in partial charges. Water molecules exhibit this property, allowing them to engage in interactions with other polar substances. For instance, in protein folding, the interactions between polar groups help establish the molecule's final three-dimensional shape, which is crucial for function (**Figure 3.2**). Similarly, in cell membranes, the arrangement of phospholipids—where the polar heads interact with water while nonpolar tails remain shielded—helps maintain structural integrity.

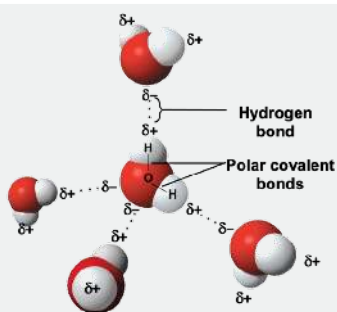


Figure 3.1: Water molecules are polar covalently bound and are interacting with other water molecules due to their partial charges.

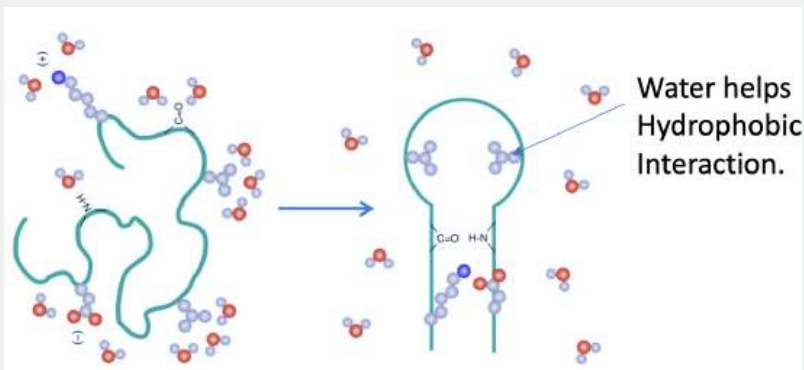


Figure 3.2: Water molecules interact with protein structures, helping the fold for the final three-dimensional structure.

Water's polarity also leads to hydrophobic interactions, where nonpolar molecules (such as lipids) do not dissolve in water. Instead, they cluster together to minimize their exposure to water. This property is crucial in biological systems:

- **Cell Membranes:** Phospholipid bilayers form because the hydrophobic tails face inward, avoiding water, while the hydrophilic heads interact with the aqueous environment.
- **Fat Metabolism:** Nonpolar fats do not mix with water and require emulsifiers (like bile salts) for digestion. If you get tar or grease on your hands, water alone will not wash it away because these substances are nonpolar. However, vegetable oil can dissolve the tar, as it is also nonpolar, allowing it to be wiped away.

Oxygen and **nitrogen** are **highly electronegative** elements that frequently participate in hydrogen bonding. These elements are found in many biological molecules such as proteins, nucleic acids, and carbohydrates. Their presence in these molecules allows for critical interactions, influencing molecular structure, stability, and function. For example, the presence of oxygen in carbohydrates allows them to readily dissolve in water, making them easily transportable in the bloodstream. Water plays an essential role in many biochemical reactions. It is involved in both the reactants and products of numerous metabolic processes. Two key reactions include:

- **Hydrolysis:** Water is used to break down macromolecules. For example, during digestion, polysaccharides are broken into simple sugars by adding water.
- **Dehydration Synthesis:** Water is removed to link smaller molecules into larger macromolecules. For instance, amino acids bond to form proteins by releasing water molecules.

Water has an exceptionally **high heat capacity** due to the energy required to break and form hydrogen bonds. This property makes water effective in regulating temperature in biological systems. Water helps buffer temperature changes, preventing sudden fluctuations that could harm cellular processes. Large bodies of water absorb heat during the day and release it slowly at night, stabilizing local climates.

Cohesion refers to water molecules sticking together due to hydrogen bonding, while **adhesion** refers to water molecules sticking to other surfaces (**Figure 3.3**). These properties are vital in biological processes. **In Plants**, Cohesion and adhesion enable

capillary action, allowing water to travel from roots to leaves through xylem vessels. Without these forces, plants would not be able to transport water against gravity, leading to dehydration and death. **In Animals**, Surface tension (a result of cohesion) helps organisms like insects walk on water. Additionally, cohesion is essential in blood circulation, where it assists in maintaining blood pressure and the movement of fluids in capillaries. **In Biochemistry**, Adhesion helps water interact with biomolecules, aiding in solubility and transport. For example, adhesion allows water to form hydration shells around ions and proteins, facilitating biochemical reactions inside cells. **In Drug Studies**, Many pharmaceutical drugs rely on water solubility for effective absorption in the body. Adhesion allows drugs to dissolve in biological fluids, ensuring proper distribution and efficacy. Cohesion helps in the consistency of solutions used for injections or intravenous fluids.

Overall, Water's unique properties, including its solvent capabilities, hydrogen bonding, high heat capacity, and cohesive and adhesive nature, make it indispensable for life. Whether facilitating biochemical reactions, maintaining cellular structures, or stabilizing ecosystems, water plays a fundamental role in sustaining biological functions across all forms of life.

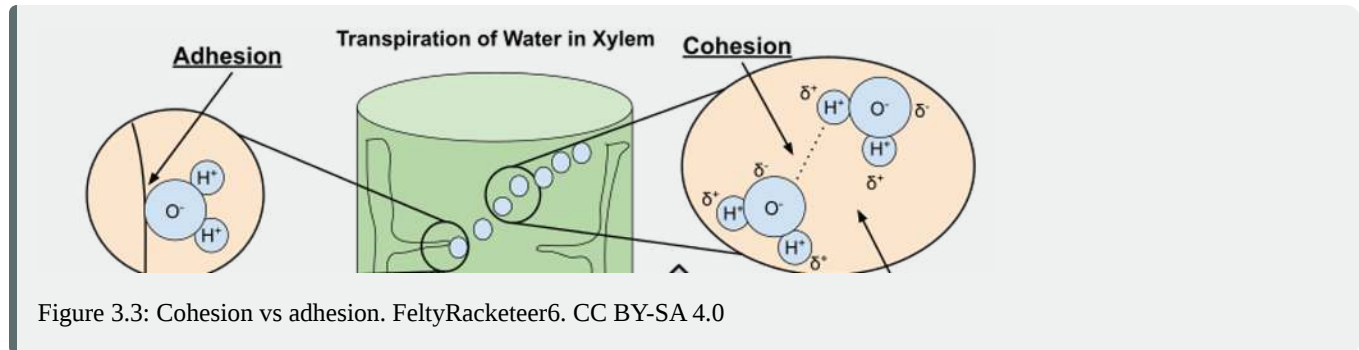


Figure 3.3: Cohesion vs adhesion. FeltyRacketeer6. CC BY-SA 4.0

Understanding pH and Its Measurement

pH is a measure of how acidic or basic a solution is, based on the concentration of hydrogen ions (H⁺) (**Figure 3.4**). It is calculated using the logarithmic scale $\text{pH} = -\log_{10}[\text{H}^+]$, meaning that a change of one unit represents a **ten-fold** difference in hydrogen ion concentration. For example:

- A solution with a **pH of 3** has **100 times** more H⁺ ions than a solution with a **pH of 5**.
- A shift from **pH 6 to pH 7** means the solution has **10 times fewer H⁺ ions**, becoming less acidic.
- A shift from **pH 10 to pH 13** indicates a **1,000-fold decrease in H⁺ ions**, making the solution significantly more basic.

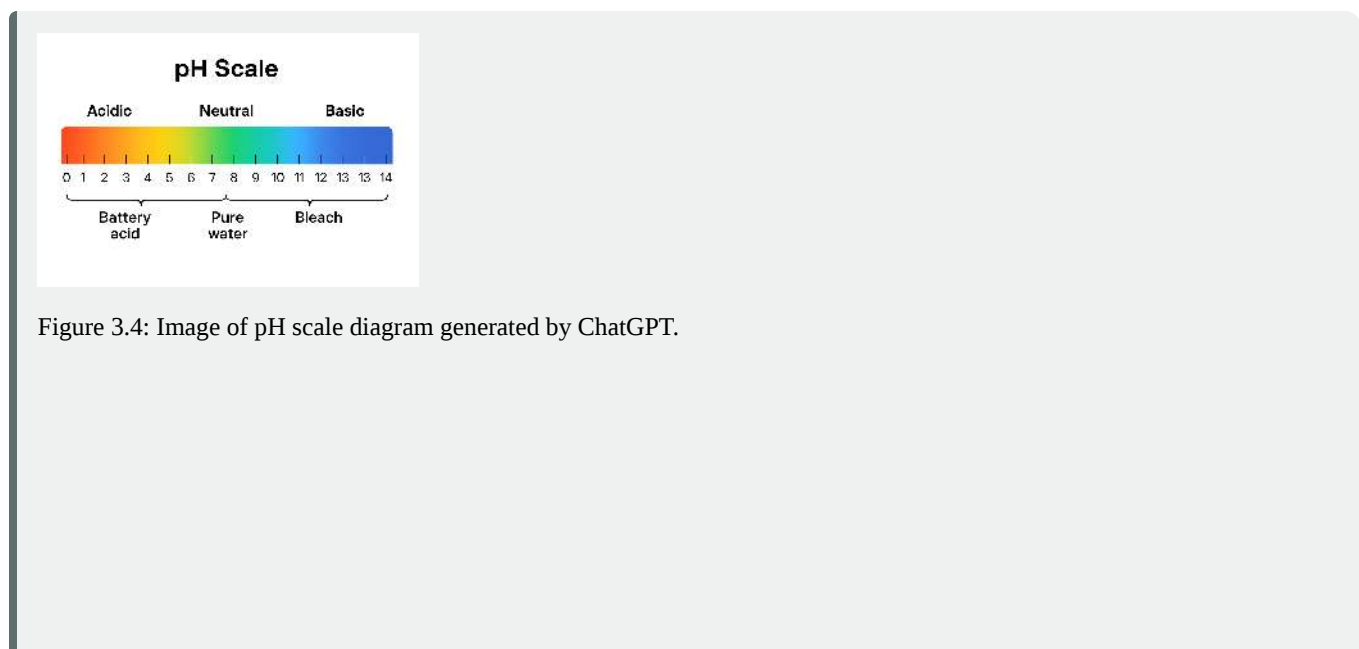


Figure 3.4: Image of pH scale diagram generated by ChatGPT.

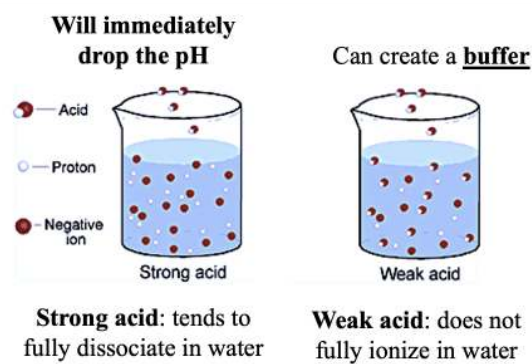


Figure 3.5: Image of strong vs weak acid making buffers.

Most life forms thrive within a **narrow pH range**. For instance, human blood maintains a pH around **7.35-7.45**. Deviations beyond this range can be harmful, as biochemical reactions depend on pH stability. Like temperature, pH extremes can lead to **protein denaturation**—a process where proteins lose their three-dimensional structure and functionality. At extreme acidic or basic pH values, the hydrogen or hydroxide ions interfere with the hydrogen bonds and ionic interactions that maintain the structure of proteins. This disruption unfolds proteins, rendering them inactive and potentially leading to cellular damage or death. For example:

- **Stomach enzymes**, such as pepsin, function optimally at very low pH (~2) but would denature at neutral pH.
- **Blood proteins**, such as hemoglobin, require a strict pH range (~7.4) to function correctly; even small deviations can impair oxygen transport.

To prevent drastic pH changes, biological systems rely on **buffers**, which stabilize pH by absorbing excess hydrogen (H^+) or hydroxide (OH^-) ions. Buffers help maintain homeostasis, ensuring that enzymatic and biochemical reactions occur under optimal conditions. One key property of a buffer is its **pKa**, which indicates the strength of an acid or base. The **pKa** value is the pH at which half of the acid or base molecules are dissociated into their ionized form.

- **Strong acids** ($pK_a < 3$) fully dissociate in water and drastically lower pH (e.g., HCl).
- **Weak acids** (pK_a 3-7) partially dissociate, creating buffer systems (e.g., acetic acid, $pK_a \sim 4.76$).
- **Weak bases** (pK_a 7-11) only partially accept protons (e.g., ammonia, NH_3 , $pK_a \sim 9.3$).
- **Strong bases** ($pK_a > 11$) readily accept protons and sharply increase pH (e.g., NaOH).

A **buffer** consists of a weak acid and its conjugate base (or a weak base and its conjugate acid) (**Figure 3.5**). It resists pH changes by either donating or accepting protons (H^+) in response to fluctuations in the environment. For example, when Acetic acid (CH_3COOH) partially ionizes in water: $CH_3COOH \rightleftharpoons CH_3COO^- + H^+$. If an acid (H^+) is added, the acetate ion (CH_3COO^-) binds to the excess protons, forming more acetic acid and preventing a pH drop. If a base (OH^-) is added, acetic acid donates protons to neutralize it, preventing a pH increase. This balance allows buffer solutions to resist pH changes when small amounts of acids or bases are introduced.

The **buffer zone** refers to the range of pH values over which a buffer solution can effectively maintain pH stability (**Figure 3.6 and 3.7**). This range is typically **± 1 pH unit around the pK_a** of the buffer system. Within this zone, the weak acid and its conjugate base are in equilibrium, allowing them to neutralize small amounts of added acid (H^+) or base (OH^-) without significantly altering the overall pH. The **titration point** (also known as the **equivalence point** or **tilting point**) occurs when the amount of added strong acid or base completely reacts with the buffer components, surpassing the buffer zone and leading to a rapid drop in pH or a drastic increase in pH. At this stage, the buffering capacity is overwhelmed, and the system can no longer resist shifts in pH.

Luckily, our body employs **multiple buffer systems** to maintain different pH environments in various biological fluids. Each buffer system works within its optimal buffer zone to ensure stability in its respective environment. This redundancy is crucial because different physiological processes generate acids and bases at various rates. Proteins, such as hemoglobin and albumin,

have amino acid side chains that can accept or donate protons, stabilizing pH. The **histidine** residue in hemoglobin, for example, plays a crucial role in buffering blood pH. By having multiple buffer systems in place, the body can efficiently regulate pH across multiple biological compartments. Each system operates within a specific buffer zone to regulate pH in different compartments:

- **Blood (pH 7.35-7.45)** – The **bicarbonate buffer system ($\text{H}_2\text{CO}_3/\text{HCO}_3^-$)** maintains blood pH by balancing CO_2 exhalation with carbonic acid formation. This system works efficiently because CO_2 can be expelled through respiration, shifting the equilibrium as needed. The bicarbonate buffer system is the primary regulator of blood pH, maintaining it around 7.4. It operates based on the following equilibrium: $\text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$. If pH drops (more acidic), bicarbonate (HCO_3^-) absorbs H^+ , shifting the reaction toward carbonic acid (H_2CO_3), which then decomposes into CO_2 and H_2O , expelled through respiration. If pH rises (more basic), carbonic acid releases H^+ to balance the system.
- **Intracellular fluids (pH ~6.8-7.2)** – The **phosphate buffer system ($\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$)** stabilizes pH inside cells where metabolic reactions produce acids. The phosphate buffer system is crucial for intracellular pH regulation. It works via the equilibrium: $\text{H}_2\text{PO}_4^- \rightleftharpoons \text{HPO}_4^{2-} + \text{H}^+$. This buffer is essential in urine and cellular fluids, helping to maintain pH stability in metabolic processes.
- **Urine (pH ~4.5-8.0)** – The **phosphate and ammonia buffer systems** help excrete excess H^+ , adjusting urine pH depending on dietary and metabolic conditions.
- **Stomach (pH ~1.5-3.5)** – The gastric environment relies on hydrochloric acid (HCl) for digestion, but **mucosal bicarbonate buffering** protects the stomach lining from damage.

Overall, understanding pH and buffer systems is essential in biology, medicine, and biotechnology. Buffers prevent harmful pH fluctuations, ensuring biochemical reactions occur efficiently. Whether in blood regulation, enzyme function, or pharmaceutical applications, buffer systems are indispensable for maintaining life's delicate balance.

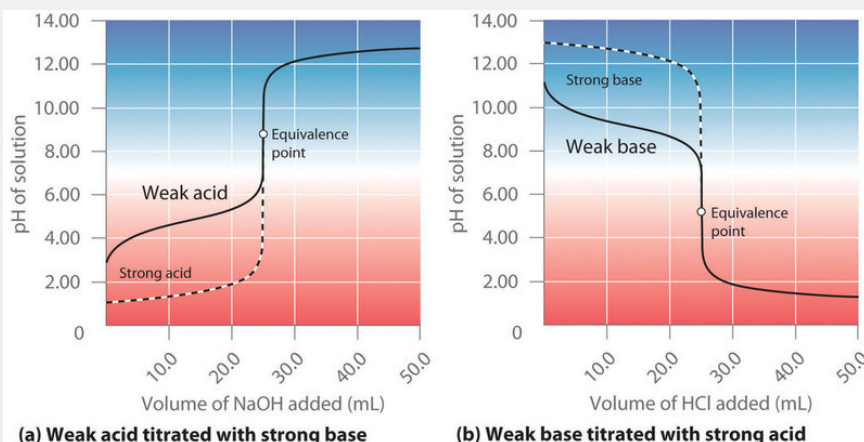


Figure 3.6: Comparison of the buffer zone of a weak base to that of a weak acid. Shared under a CC BY-NC-SA 4.0 license.

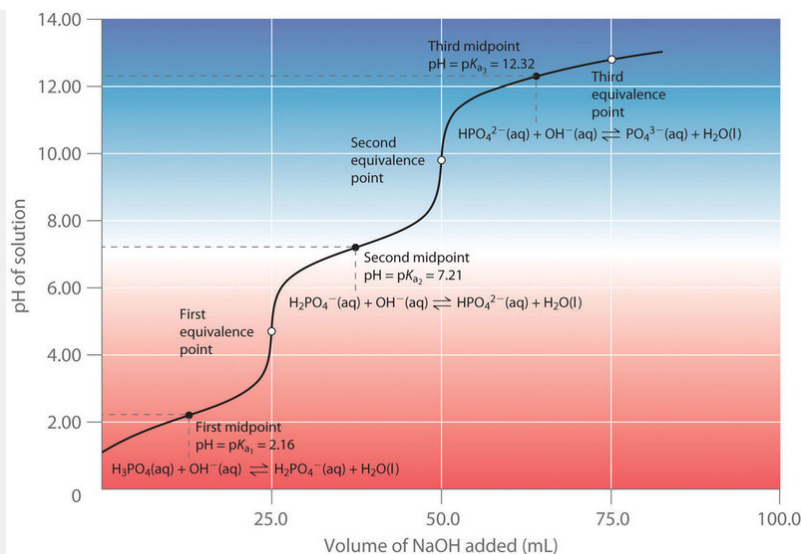


Figure 3.7: Polyprotic titration curve for phosphoric acid, showing multiple buffer zones. Shared under a CC BY-NC-SA 4.0 license.

Key Takeaways

- pH is a logarithmic scale representing H^+ concentration.
- Biological processes depend on a stable pH environment.
- Buffers prevent drastic pH changes, maintaining homeostasis.
- The body uses multiple buffers to regulate pH in different physiological compartments.

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4: Photosynthesis and Plant Adaptation

Learning Objectives

- Understand the fundamental role of photosynthesis in sustaining life on Earth.
- Identify different organisms capable of photosynthesis, including plants, algae, and bacteria.
- Explain the photosynthesis equation and the stages involved in the process.
- Recognize adaptations that enable photosynthesis in extreme environments.
- Explore plant defense mechanisms and how genetic modifications (GMOs) impact agriculture.

Definition: Term

- **Photosynthesis** - The process by which photoautotrophic organisms convert light energy into chemical energy.
- **Photoautotrophs** - Organisms that can synthesize their own food using light energy (e.g., plants, algae, cyanobacteria).
- **Chloroplast** - The organelle in plant and algal cells where photosynthesis occurs.
- **Chlorophyll** - The primary pigment that absorbs light energy for photosynthesis.
- **Light-Dependent Reactions** - The first stage of photosynthesis, occurring in the thylakoid membranes, where light energy is used to produce ATP and NADPH.
- **Calvin Cycle (Light-Independent Reactions)** - The second stage of photosynthesis, occurring in the stroma, where ATP and NADPH are used to convert CO₂ into glucose.
- **C3, C4, and CAM Plants** - Different plant types adapted to various environmental conditions based on their photosynthetic mechanisms.
- **Kleptoplasty** - A process in which certain animals (e.g., Eastern Emerald Elysia) retain chloroplasts from algae to perform photosynthesis.
- **Genetically Modified Organisms (GMOs)** - Organisms whose genetic material has been altered to enhance traits such as pest resistance or drought tolerance.

Photosynthesis: The Engine of Life on Earth

Photosynthesis is the biochemical process through which photoautotrophic organisms, such as plants, algae, and certain bacteria, convert light energy into chemical energy stored in organic compounds. It is the foundation of life on Earth, providing both the oxygen we breathe and the organic molecules that form the basis of the food chain. Photosynthesis is not limited to plants. Other species capable of this process include:

- **Algae:** Single-celled or multicellular organisms that thrive in aquatic environments, playing a crucial role in oxygen production.
- **Cyanobacteria:** Also known as blue-green algae, these bacteria are among the earliest photosynthetic organisms and were responsible for oxygenating Earth's atmosphere billions of years ago.
- **Purple and Green Sulfur Bacteria:** Unlike plants, these bacteria use hydrogen sulfide instead of water in their photosynthetic processes, producing sulfur instead of oxygen.

The Photosynthesis Equation

The Photosynthesis equation represents the conversion of carbon dioxide and water into glucose and oxygen using light energy. The process occurs in two main stages: the **light-dependent reactions** and the **Calvin cycle** (light-independent reactions). Photosynthesis can be summarized by the following equation:



Other Photosynthetic Organisms

Despite the vast presence of sunlight, less than 1% of the solar energy reaching Earth is converted into organic compounds through photosynthesis, making it a highly inefficient but essential process.

One fascinating example of an animal that can perform photosynthesis is the Eastern Emerald Elysia (*Elysia chlorotica*), a type of **sea slug** (Figure 4.1). This slug consumes algae and incorporates the algae's chloroplasts into its own cells in a process called

kleptoplasty. By doing so, it can photosynthesize like a plant, using sunlight to produce energy. Another example is the **Spotted Salamander** (*Ambystoma maculatum*), which has a symbiotic relationship with algae. The algae live inside the salamander's eggs and perform photosynthesis, providing oxygen and possibly nutrients to the developing embryos.



Figure 4.1: Other organisms can undergo photosynthesis, including a sea slug.

While we do not perform photosynthesis, humans indirectly benefit from the sun in various ways. One key biological process that utilizes sunlight is the conversion of **cholesterol into vitamin D**. When ultraviolet (UV) light from the sun strikes the skin, it triggers a reaction that converts 7-dehydrocholesterol into pre-vitamin D₃, which then transforms into active vitamin D. **Vitamin D** plays a crucial role in human health (Figure 4.2), offering a wide range of benefits beyond its well-known function in **calcium metabolism** and **bone health**. One of its most significant roles is in supporting the **immune system**. Vitamin D enhances the pathogen-fighting effects of monocytes and macrophages, key components of the body's defense mechanism. It has been shown to reduce the risk of acute respiratory infections and may lower the severity of illnesses such as influenza and COVID-19. By regulating immune responses, vitamin D helps **prevent excessive inflammation**, which is linked to severe outcomes in many infections.



Figure 4.2: Benefits of Vitamin D.

Beyond immunity, vitamin D is vital for **mental health and mood regulation**. Low levels of vitamin D have been associated with an increased risk of depression and seasonal affective disorder (SAD). This is likely due to its influence on serotonin, a neurotransmitter that plays a crucial role in mood stability. Ensuring adequate vitamin D levels may help improve mental well-

being and reduce the risk of mood disorders. Vitamin D also contributes to **cardiovascular health**. It helps regulate blood pressure by influencing the renin-angiotensin system, which controls fluid balance and vascular resistance. Additionally, its anti-inflammatory properties may reduce the risk of heart disease and stroke by preventing chronic inflammation in blood vessels. Studies suggest that maintaining optimal vitamin D levels can support overall cardiovascular function and reduce the likelihood of hypertension and arterial plaque buildup.

In relation to metabolic health, vitamin D plays a role in **diabetes prevention and management**. It enhances insulin sensitivity, helping to regulate blood sugar levels and prevent insulin resistance, a key factor in the development of type 2 diabetes. Some research suggests that vitamin D supplementation may reduce the risk of developing diabetes, particularly in individuals with prediabetes or those at high risk of metabolic disorders. Emerging evidence also suggests that vitamin D may help **protect against certain types of cancer**. It has been found to slow the growth of cancer cells and reduce the risk of developing cancers such as colon, breast, and prostate cancer. One way it achieves this is by promoting apoptosis, the programmed death of malignant cells, which helps prevent the uncontrolled cell proliferation characteristic of cancer.

Muscle health is another area where vitamin D plays a crucial role. A deficiency in vitamin D has been linked to muscle weakness, particularly in older adults. It is essential for muscle protein synthesis and neuromuscular function, reducing the risk of falls and fractures. By maintaining muscle strength, vitamin D helps improve mobility and overall physical performance, particularly in aging populations. Additionally, vitamin D may help **protect against autoimmune diseases** by modulating the immune system and reducing inflammation. Research has shown that it may lower the risk of autoimmune conditions such as multiple sclerosis, rheumatoid arthritis, and type 1 diabetes by preventing the immune system from attacking healthy tissues. This immunomodulatory function makes it an essential nutrient for maintaining immune balance and preventing chronic inflammatory diseases.

Vitamin D also plays a role in **skin health**. Its anti-inflammatory properties can help manage conditions like psoriasis and eczema, providing relief from symptoms such as redness and irritation. Furthermore, vitamin D contributes to wound healing and skin barrier maintenance, which are essential for protecting against infections and maintaining overall skin integrity. **Cognitive function and brain health** are yet another area where vitamin D demonstrates its importance. Studies suggest that adequate vitamin D levels are associated with a lower risk of dementia and cognitive decline. It may protect neurons and reduce neuroinflammation, which are crucial factors in neurodegenerative diseases such as Alzheimer's. By maintaining brain function, vitamin D contributes to long-term cognitive health and mental clarity.

Overall, since vitamin D is primarily synthesized in the skin through exposure to sunlight, individuals with limited sun exposure, darker skin tones, or those living in northern latitudes may struggle to maintain adequate levels. In such cases, dietary sources such as fatty fish, fortified dairy products, and vitamin D supplements can help ensure sufficient intake. Given its wide-ranging health benefits, maintaining optimal vitamin D levels is essential for overall well-being and disease prevention.

Role of Chloroplasts and Chlorophyll

Photosynthesis takes place inside **chloroplasts**, specialized organelles found in plant and algal cells. These organelles contain **thylakoid membranes**, where the light-dependent reactions occur, and a liquid-filled space called the **stroma**, where the Calvin cycle takes place. **Chlorophyll** is the primary pigment responsible for capturing light energy. It absorbs light most efficiently in the blue and red wavelengths while reflecting green, which is why plants appear green.

The process of photosynthesis is divided into two main stages: the light-dependent reactions and the light-independent reactions, also known as the Calvin cycle. These two processes work together to convert solar energy into chemical energy and ultimately produce glucose, which serves as the primary source of energy for plants and, indirectly, for all other organisms that rely on them for food.

The **light-dependent reactions** occur in the **thylakoid membranes** of the chloroplasts and require direct sunlight to proceed (**Figure 4.3**). When sunlight strikes chlorophyll molecules, it excites their electrons, leading to the initiation of a chain of biochemical reactions. This excitation of electrons drives the splitting of water molecules (H_2O), releasing **oxygen (O_2) as a byproduct**, while **hydrogen ions (H^+)** contribute to the formation of **ATP (adenosine triphosphate)** and **NADPH (nicotinamide adenine dinucleotide phosphate)**. These two molecules serve as energy carriers, storing the energy harnessed from sunlight and delivering it to the next stage of photosynthesis. The **oxygen produced during this stage is released into the atmosphere**, playing a crucial role in sustaining life on Earth.

Unlike the light-dependent reactions, the **light-independent reactions (Calvin cycle)** do not require direct sunlight and occur in the **stroma** of the chloroplast. Instead of using light energy, this stage relies on the ATP and NADPH generated from the previous reactions to **convert carbon dioxide (CO₂) into glucose (C₆H₁₂O₆)**. This process begins with **carbon fixation**, where CO₂ is captured and incorporated into organic molecules. These molecules are then transformed into glucose using the stored energy from ATP and NADPH. Finally, the cycle regenerates key molecules required for continued carbon fixation, ensuring that the process can repeat as long as ATP, NADPH, and CO₂ are available.

In summary, the **light-dependent reactions** harness sunlight to produce the energy carriers **ATP and NADPH**, along with oxygen as a byproduct, while the **light-independent reactions (Calvin cycle)** use those energy carriers to synthesize glucose from carbon dioxide. Both stages are essential for the plant's survival and contribute to the overall energy flow within ecosystems.

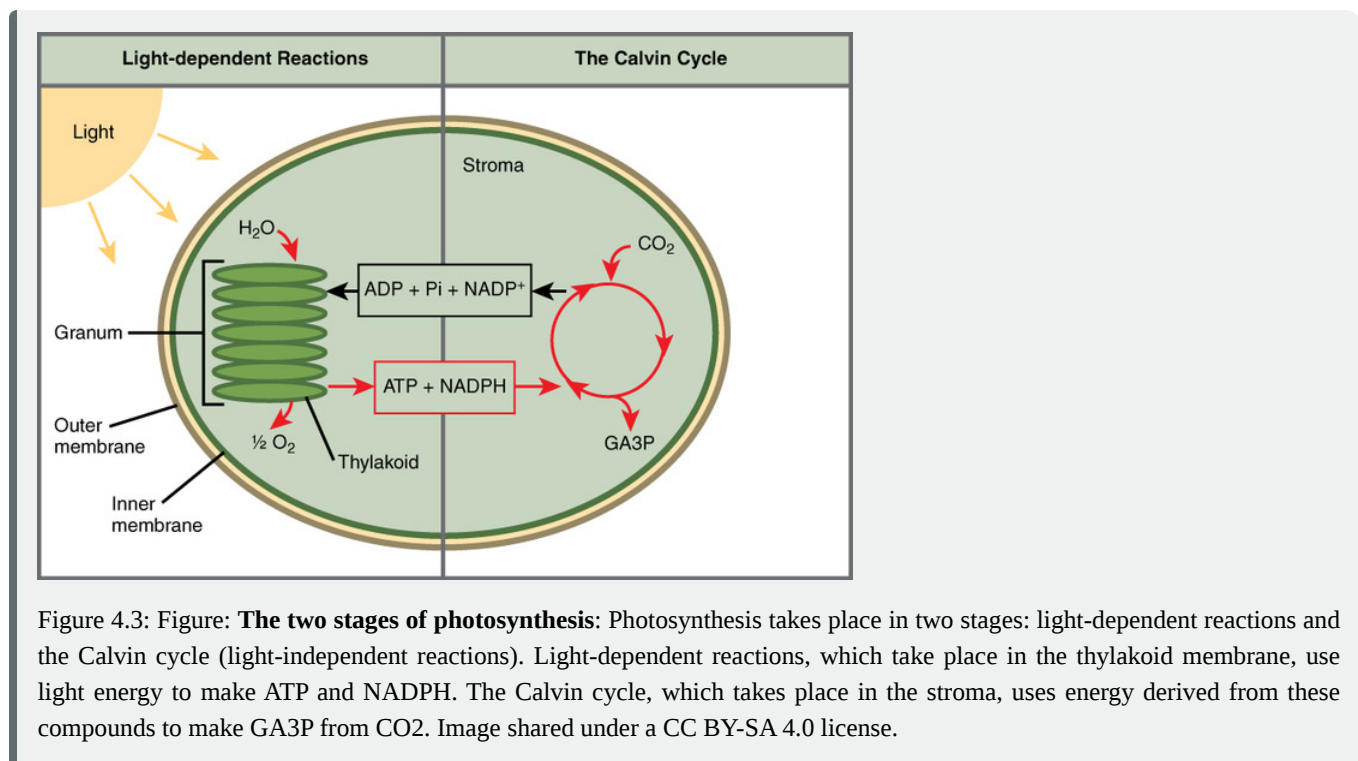
Here are the summary of the mechanism for photosynthesis:

1. Light-Dependent Reactions

- **Light absorption and electron excitation:** When chlorophyll absorbs sunlight, its electrons become excited, initiating the conversion of light energy into chemical energy.
- **Water splitting:** The absorbed energy is used to split water molecules (H₂O), producing oxygen (O₂) as a byproduct, and releasing hydrogen ions (H⁺).
- **ATP and NADPH formation:** The hydrogen ions help form **ATP (adenosine triphosphate)** and **NADPH (nicotinamide adenine dinucleotide phosphate)**, which store energy for the next stage of photosynthesis.

2. The Calvin cycle

- **Carbon Fixation:** CO₂ is captured and incorporated into organic molecules.
- **Energy Utilization:** ATP and NADPH are used to convert these molecules into glucose (C₆H₁₂O₆).
- **Regeneration:** The cycle regenerates molecules needed for continuous CO₂ fixation.



Products of Photosynthesis

The **products of photosynthesis** are essential for sustaining life on Earth, as they serve as the foundation of the food chain. The primary product is **glucose (C₆H₁₂O₆)**, which plants use for energy storage and metabolic functions. Some glucose is immediately consumed in cellular respiration, while excess glucose is converted into **starch**, a long-term energy storage molecule found in

roots, tubers, and seeds. Additionally, photosynthesis generates **sucrose**, a transportable sugar that moves through the plant to supply energy where needed. Another vital product is **oxygen (O₂)**, which is released as a byproduct of water splitting during the light-dependent reactions. This oxygen replenishes Earth's atmosphere and supports the respiration of most living organisms. Furthermore, plants use glucose to produce **cellulose**, a structural component of cell walls that provides rigidity and strength, allowing plants to grow upright and form complex structures. Photosynthesis produces several essential compounds:

- **Sucrose:** A transportable form of sugar that moves through the plant.
- **Starch:** A storage form of energy, stored in roots, tubers, and seeds.
- **Cellulose:** A structural component of plant cell walls, providing rigidity and strength.

Adaptations to Extreme Environments

Since plants cannot move to escape harsh conditions, they have evolved various adaptations. Plants have evolved various **adaptations to extreme environments** to survive in harsh conditions (**Figure 4.4**). In temperate climates, **C₃ plants** like trees, rice, and potatoes dominate, utilizing a straightforward photosynthetic pathway. However, in hotter and drier environments, **C₄ plants**, such as corn and sugarcane, have developed a more efficient system that minimizes water loss by **storing CO₂ in specialized cells**. This adaptation allows them to continue photosynthesis even when stomata, the small pores in leaves, are closed to conserve water. **CAM plants**, such as cacti and euphorbia, take this adaptation further by opening their stomata at night to capture CO₂, which they store until daylight for photosynthesis. This strategy drastically reduces water loss and enables survival in arid conditions. Many desert plants also exhibit structural adaptations like **thick cuticles, reduced leaf surface area, or needle-like leaves**, which further minimize water loss.

1. C₃ Plants (Trees, Rice, Potatoes)

- **Stomata closure:** To reduce water loss, stomata (leaf pores) close in hot, dry conditions, limiting CO₂ intake.
- **Reduced photosynthesis:** With less CO₂ available, sugar production decreases.

2. C₄ Plants (Corn, Sugarcane)

- **Carbon fixation occurs separately from the Calvin cycle:** These plants store CO₂ in specialized cells, creating a “carbon dioxide tank” that enables photosynthesis even when stomata are closed.
- **Enhanced drought resistance:** This adaptation allows C₄ plants to survive in high-temperature environments where C₃ plants would struggle.

3. CAM Plants (Cacti, Euphorbia)

- **Night-time CO₂ fixation:** These plants open their stomata at night to take in CO₂ and store it for daytime use.
- **Water conservation:** Stomata remain closed during the day to prevent water loss.
- **Structural adaptations:** Many have needle-like leaves to minimize surface area and reduce dehydration.

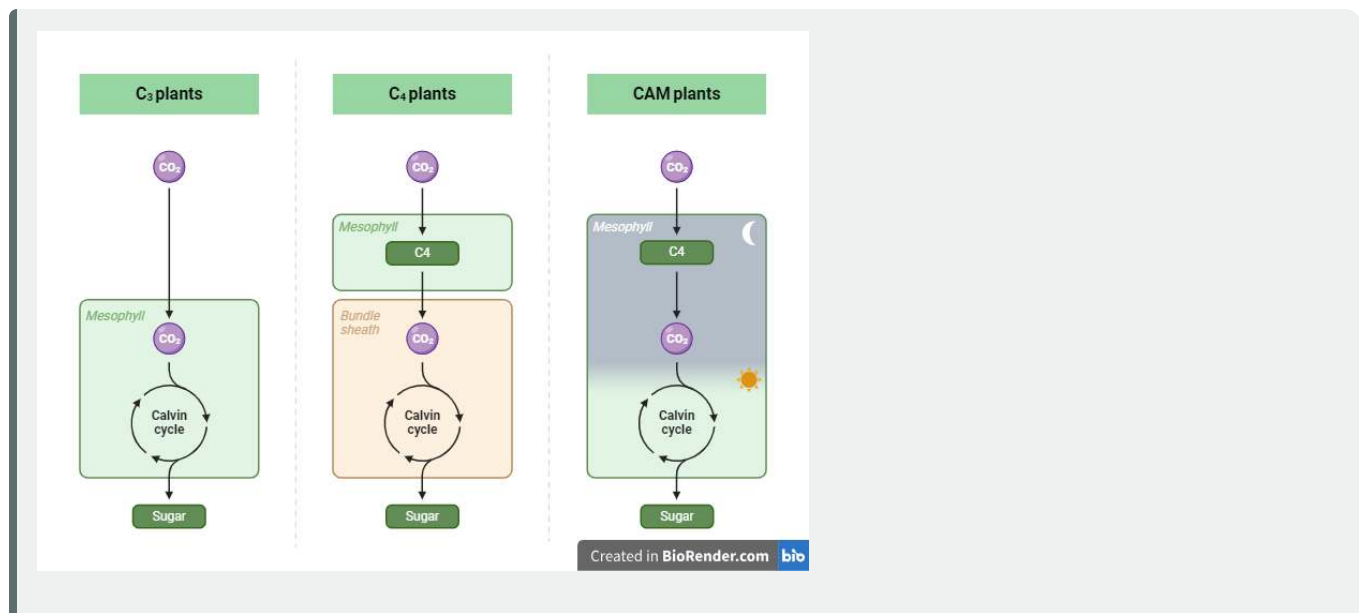


Figure 4.4: A comparison of carbon fixation in C3, C4 and CAM plants. (Lugano, G. (2023) [BioRender](#).)

Plant Defense Mechanisms

Plants face constant threats from herbivores, microbes, and insects. To defend themselves against herbivores, insects, and microbial threats, plants have developed **sophisticated defense mechanisms**. **Physical barriers**, such as tough bark, thick waxy cuticles, and silica deposits in leaves, make it difficult for herbivores to consume plant tissue. Some plants, like cacti, use **thorns and spines** to deter animals, while others have developed **trichomes**, tiny hair-like structures that can release irritants or toxins, as seen in stinging nettles. In addition to physical defenses, plants produce **chemical compounds** to deter predators or attract beneficial organisms. For example, certain plants release **toxins, alkaloids, or bitter compounds**, making them unpalatable or harmful to herbivores. Spinach and rhubarb contain **raphides**, tiny needle-like structures that cause irritation when eaten, discouraging consumption. Some plants, like tobacco, produce **nicotine**, which acts as a natural insecticide.

- **Physical Barriers:** Tree bark contains lignin, making it tough to chew, while leaves have a waxy cuticle that deters insects and microbes.
- **Defensive Structures:** Thorns, spines, and trichomes (hair-like structures) physically deter herbivores. Some trichomes even release irritants, like the stinging nettle.
- **Chemical Defenses:** Many plants produce toxins or deterrents. For example, spinach and rhubarb contain needle-like raphides that cause irritation when eaten.

Genetic Modifications and GMOs

Modern biotechnology has allowed scientists to enhance plant traits through **genetic modifications (GMOs)**, improving resistance to pests, diseases, and environmental stress. One of the most well-known examples is **BT crops**, which have been genetically engineered to produce a protein from *Bacillus thuringiensis* that is toxic to specific insect pests while being harmless to humans and other animals. This innovation reduces the need for chemical pesticides, leading to **higher crop yields and less environmental damage**. Another major advancement is the development of **herbicide-resistant crops**, such as glyphosate-resistant soybeans and corn, which enable farmers to control weeds more effectively without harming the crops. However, overuse of herbicides has led to the evolution of resistant weed species, creating new agricultural challenges. In addition to pest and herbicide resistance, genetic modifications have played a crucial role in **crisis prevention**, such as saving the Hawaiian papaya industry from the devastating ringspot virus through the introduction of a virus-resistant GMO variety. Looking ahead, scientists are working on **nitrogen-fixing crops** that can capture atmospheric nitrogen, reducing the need for synthetic fertilizers and minimizing environmental pollution.

- **BT Crops:** Scientists have introduced genes from *Bacillus thuringiensis* into crops such as corn and cotton, allowing them to produce a protein toxic to specific insect pests while remaining harmless to humans and other animals. This reduces pesticide use and increases crop yields.
- **Herbicide-Resistant Crops:** Some plants are genetically modified to withstand glyphosate, a common herbicide, allowing farmers to control weeds more efficiently. However, overuse of glyphosate has led to resistant weed strains, posing new challenges.
- **Crisis Prevention:** The Hawaiian papaya industry was nearly wiped out by the ringspot virus in the 1990s. The introduction of a genetically modified virus-resistant papaya saved the industry.
- **Environmental Benefits:** Scientists are developing nitrogen-fixing crops that can absorb nitrogen from the air, reducing the need for synthetic fertilizers, which contribute to water pollution and climate change.

Conclusion

Photosynthesis is a fundamental process that sustains life on Earth. Through complex biochemical pathways, plants and other photoautotrophs harness sunlight to convert inorganic molecules into the organic compounds needed for survival. Additionally, plant modifications through genetic engineering, such as GMOs, offer new strategies for improving crop resilience and environmental sustainability, ensuring food security for a growing global population.

Post-Lecture Reflection

- Objective 1
- Explain how photosynthesis supports ecosystems by producing oxygen and organic molecules.
- Differentiate between light-dependent and light-independent reactions.
- Discuss adaptations of plants in extreme environments (C3, C4, CAM mechanisms).
- Identify physical and chemical plant defense mechanisms.
- Analyze the benefits and challenges of genetic modifications in agriculture.

Key Takeaways

- Photosynthesis is a crucial process that not only sustains plant life but also supports life across ecosystems by providing oxygen and organic matter.
- The light-dependent reactions capture solar energy to produce ATP and NADPH, while the Calvin cycle utilizes these molecules to synthesize glucose.
- Some plants have evolved specialized mechanisms (C4 and CAM pathways) to survive in hot and arid environments by improving water-use efficiency.
- Plants defend themselves using physical barriers (thorns, waxy cuticles) and chemical deterrents (toxins, alkaloids, nicotine).
- GMOs provide agricultural benefits, such as increased crop yields and resistance to pests, but they also raise environmental and ethical concerns.

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5: Anaerobic and Aerobic Respiration

Learning Objectives

- **Understand the Purpose and Importance of Cellular Respiration:** Recognize how cells convert energy stored in food molecules into adenosine triphosphate (ATP), the primary energy currency.
- **Identify the Stages of Cellular Respiration:** Familiarize yourself with the three main stages—Glycolysis, Krebs Cycle (Citric Acid Cycle), and Electron Transport Chain—and their roles in energy production.
- **Comprehend the Role of Oxygen:** Learn how oxygen functions as the final electron acceptor in the electron transport chain, facilitating ATP synthesis.
- **Recognize the Influence of Lifestyle Factors:** Explore how exercise and dietary habits like fasting impact mitochondrial function and overall cellular respiration efficiency.

Definition: Term

- **Adenosine Triphosphate (ATP):** The primary energy carrier in cells, providing energy for various biochemical processes.
- **Glycolysis:** The anaerobic process occurring in the cytoplasm that breaks down glucose into pyruvate, yielding a net gain of two ATP molecules.
- **Krebs Cycle (Citric Acid Cycle):** A series of enzymatic reactions in the mitochondrial matrix that further oxidize acetyl-CoA, producing electron carriers NADH and FADH₂, and a small amount of ATP.
- **Electron Transport Chain (ETC):** A sequence of protein complexes in the inner mitochondrial membrane that transfer electrons from NADH and FADH₂ to oxygen, driving the production of ATP through oxidative phosphorylation.
- **Mitochondrial Biogenesis:** The process by which new mitochondria are formed within the cell, often stimulated by physical exercise.
- **Mitophagy:** The selective degradation of damaged or dysfunctional mitochondria, maintaining cellular health and energy efficiency.
- **Oxidative Stress:** An imbalance between the production of reactive oxygen species (ROS) and the cell's antioxidant defenses, potentially leading to cellular damage.

Cellular Respiration

Cellular respiration is a fundamental biochemical process by which living organisms convert energy stored in food molecules into adenosine triphosphate (ATP), the primary energy currency of the cell. This process is essential for powering various cellular activities, including muscle contraction, nerve impulse transmission, and biosynthesis of macromolecules. While glucose is the primary substrate for cellular respiration, other macromolecules such as fats and proteins can also be utilized to produce ATP. Notably, nucleic acids like DNA and RNA are not used as energy sources, as preserving their integrity is crucial for genetic stability.

Cellular respiration involves a series of metabolic pathways that systematically extract energy from food molecules. The cellular respiration equation illustrates that one molecule of glucose combines with six molecules of oxygen, resulting in the production of six molecules of carbon dioxide, six molecules of water, and energy in the form of ATP. Oxygen plays a critical role in this process due to its strong electronegativity, which allows it to serve as the final electron acceptor in the electron transport chain, facilitating the release of electrons from nutrient molecules and driving ATP synthesis.

The overall chemical equation for aerobic cellular respiration is: $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + \text{Energy}$

Stages of Cellular Respiration

Cellular respiration consists of three main stages: Glycolysis, the Krebs Cycle (Citric Acid Cycle), and the Electron Transport Chain.

- **Glycolysis (Figure 5.1)**
 - Glycolysis is an anaerobic process, meaning it does not require oxygen, and occurs in the cytoplasm of the cell. The primary objective of glycolysis is to break down one molecule of glucose (a six-carbon sugar) into two molecules of pyruvate (each

containing three carbons). This process results in a net gain of two ATP molecules and the formation of two molecules of nicotinamide adenine dinucleotide (NADH), which are used in later stages of respiration.

- In the absence of oxygen, cells can undergo fermentation to process pyruvate. For example, in yeast cells, pyruvate is converted into ethanol and carbon dioxide, a process exploited in baking and alcohol production. In muscle cells during strenuous exercise, when oxygen is scarce, pyruvate is reduced to lactic acid, leading to temporary muscle fatigue.

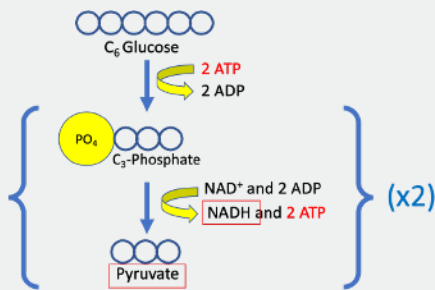


Figure 5.1: Simplified mechanism of Glycolysis. Image made by Victor Pham from Irvine Valley College, Ph.D.

• Transition Reaction (Pyruvate Oxidation) (Figure 5.2)

- When oxygen is present, pyruvate undergoes oxidation in the mitochondria to form acetyl-CoA, carbon dioxide, and NADH. This step serves as a bridge between glycolysis and the Krebs Cycle, preparing the carbon molecules for further energy extraction.

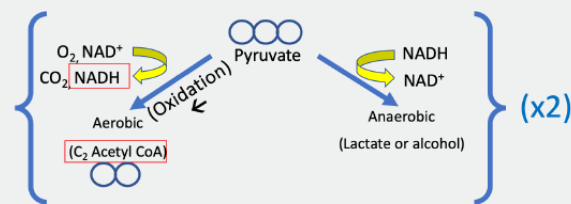


Figure 5.2: Simplified mechanism of Transition Reaction. Image made by Victor Pham from Irvine Valley College, Ph.D.

• Krebs Cycle (Citric Acid Cycle) (Figure 5.3)

- The Krebs Cycle takes place in the mitochondrial matrix and is central to aerobic respiration. Each acetyl-CoA molecule enters the cycle and undergoes a series of enzymatic reactions that release two molecules of carbon dioxide, generate three NADH molecules, one flavin adenine dinucleotide (FADH₂) molecule, and one guanosine triphosphate (GTP), which is often converted to ATP. Since each glucose molecule produces two acetyl-CoA molecules, the cycle turns twice per glucose molecule, resulting in a total yield of six NADH, two FADH₂, and two ATP molecules.

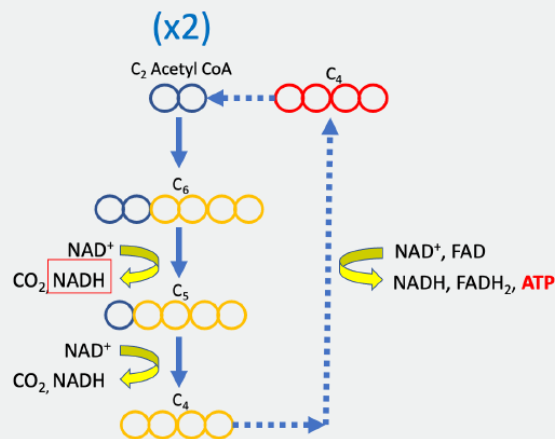


Figure 5.3: Simplified mechanism of Krebs Cycle. Image made by Victor Pham from Irvine Valley College, Ph.D.

• Electron Transport Chain (ETC) and Oxidative Phosphorylation (Figure 5.5)

- The final stage of cellular respiration occurs in the inner mitochondrial membrane, where the electron transport chain is located. Here, electrons from NADH and FADH₂ are transferred through a series of protein complexes, leading to the pumping of protons into the intermembrane space and creating an electrochemical gradient. Oxygen serves as the terminal electron acceptor, combining with electrons and protons to form water. The proton gradient drives the synthesis of ATP through a process known as oxidative phosphorylation. This stage produces the majority of ATP during cellular respiration, approximately 26 to 28 ATP molecules per glucose molecule, depending on the efficiency and conditions within the cell.

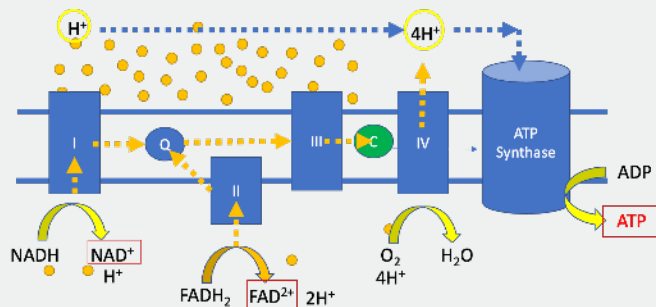


Figure 5.5: Simplified mechanism of Electron Transport Chain. Image made by Victor Pham from Irvine Valley College, Ph.D.

Total ATP Yield

The complete oxidation of one glucose molecule through cellular respiration can yield a maximum of about 30 to 32 ATP molecules. The exact number can vary based on factors such as the shuttle systems used to transport electrons into the mitochondria and the efficiency of the proton gradient. This includes:

- **Glycolysis:** Net gain of 2 ATP molecules.
- **Krebs Cycle:** 2 ATP molecules (one per cycle turn).
- **Electron Transport Chain:** Approximately 26 to 28 ATP molecules.

The Regeneration of NAD⁺ and FAD⁺

The **Electron Transport Chain (ETC)** is often understood primarily as an ATP-producing process, but its **fundamental biochemical goal is to regenerate NAD⁺ and FAD⁺** by oxidizing NADH and FADH₂. The production of ATP occurs as a **consequence** of this regeneration rather than being the sole driving purpose of the ETC. Without the constant oxidation of NADH to NAD⁺ and FADH₂ to FAD⁺, earlier metabolic pathways such as **glycolysis, the Krebs cycle, and fatty acid oxidation** would

cease because these pathways require NAD^+ and FAD^+ as electron acceptors. Since cells have a **limited supply of NAD^+ and FAD^+** , they must continuously recycle them to sustain metabolism. The ETC facilitates this by transferring electrons from NADH and FADH_2 to oxygen, which serves as the **final electron acceptor**, ultimately producing water. During this process, protons are pumped across the mitochondrial membrane, creating a gradient that drives ATP synthesis through **oxidative phosphorylation**. However, while ATP production is a critical outcome, the **primary necessity is the regeneration of NAD^+ and FAD^+** to keep metabolic processes running.

When oxygen is unavailable, the **Electron Transport Chain shuts down**, preventing the oxidation of NADH to NAD^+ . This creates a **bottleneck** where NAD^+ levels drop, halting glycolysis since it requires NAD^+ to accept electrons during the breakdown of glucose. To compensate, cells initiate **lactic acid fermentation** as an alternative method to regenerate NAD^+ . In this process, **pyruvate is reduced to lactic acid**, converting NADH back to NAD^+ . This ensures that **glycolysis can continue** despite the absence of oxygen, providing a small but critical ATP supply. Thus, the accumulation of lactic acid is not a random byproduct but a **necessary metabolic adaptation** to sustain energy production under anaerobic conditions. In essence, the body prioritizes NAD^+ regeneration over ATP production when oxygen is scarce, reinforcing the idea that the **true metabolic function of the ETC is to recycle NAD^+ and FAD^+ , with ATP generation as a secondary consequence**.

Influence of Lifestyle Factors on Cellular Respiration

Lifestyle choices significantly influence mitochondrial function, thereby impacting overall cellular respiration and energy production. Two key factors in this regard are regular **physical exercise** and **dietary patterns** such as intermittent fasting. Understanding the mechanisms, biochemical pathways, and health implications of these factors can provide insights into optimizing mitochondrial health.

1. Engaging in regular **physical activity** induces several adaptations that enhance mitochondrial function. Exercise stimulates the **creation of new mitochondria** within muscle cells, a process known as **mitochondrial biogenesis**. This adaptation increases the capacity for oxidative phosphorylation, thereby improving endurance and metabolic efficiency. Physical activity also promotes the selective degradation of damaged or dysfunctional mitochondria through **mitophagy**, ensuring a healthy mitochondrial population. This process is crucial for maintaining cellular energy balance and preventing the accumulation of defective mitochondria that can lead to cellular dysfunction. Finally, regular exercise enhances the **antioxidant** capacity of cells, reducing oxidative stress and protecting mitochondria from damage caused by reactive oxygen species (ROS). This protection helps maintain mitochondrial integrity and function over time. The mitochondrial adaptations resulting from regular exercise contribute to:
 - **Improved Metabolic Health:** Enhanced mitochondrial function leads to better glucose uptake and insulin sensitivity, reducing the risk of type 2 diabetes.
 - **Cardiovascular Benefits:** Efficient energy production supports heart function and reduces the risk of cardiovascular diseases.
 - **Increased Endurance and Muscle Performance:** A higher density of healthy mitochondria improves muscle endurance and overall physical performance.
2. **Intermittent fasting (IF)** and **caloric restriction** have been shown to positively influence mitochondrial health through several mechanisms. Fasting triggers **mitophagy**, facilitating the removal of damaged mitochondria and promoting mitochondrial quality control. This process is vital for maintaining a functional mitochondrial network. During fasting, cells switch from utilizing glucose to fatty acids and ketone bodies for energy. This **metabolic reprogramming** enhances mitochondrial efficiency and resilience. Fasting reduces oxidative stress by decreasing ROS production and enhancing antioxidant defenses, thereby protecting mitochondria from oxidative damage. The effects of fasting on mitochondrial function contribute to:
 - **Neuroprotection:** Improved mitochondrial function in neurons may protect against neurodegenerative diseases and support cognitive health.
 - **Enhanced Longevity:** By promoting mitochondrial health and reducing metabolic stress, fasting has been associated with increased lifespan in various organisms.
 - **Metabolic Health Improvement:** Fasting can lead to better lipid profiles and reduced risk factors for metabolic syndrome.

Pharmacological Interventions

Certain pharmacological agents have been explored for their potential to mimic the mitochondrial benefits of exercise and fasting. While these agents show promise, their use should be under medical supervision due to potential side effects and varying efficacy.

- **Metformin:** Commonly used for type 2 diabetes, metformin activates AMP-activated protein kinase (AMPK), promoting mitochondrial biogenesis and enhancing insulin sensitivity.
- **Resveratrol:** A polyphenol found in grapes, resveratrol activates sirtuin 1 (SIRT1) and PGC-1 α , key regulators of mitochondrial biogenesis and function.
- **Rapamycin:** An mTOR inhibitor, rapamycin has been shown to extend lifespan in animal models, potentially through effects on mitochondrial function and autophagy.

Conclusion

In conclusion, the lifestyle interventions such as regular exercise and intermittent fasting play a crucial role in maintaining and enhancing mitochondrial health. These practices induce beneficial adaptations that improve metabolic efficiency, reduce disease risk, and promote overall well-being. While pharmacological approaches offer additional avenues for supporting mitochondrial function, they should complement, not replace, healthy lifestyle choices.

Reflection

- **Evaluate the Efficiency of Cellular Respiration:** Assess how effectively cells convert energy from various macromolecules into ATP and the factors influencing this efficiency.
- **Analyze the Impact of Oxygen Availability:** Consider how the presence or absence of oxygen affects the pathways and outcomes of cellular respiration.
- **Reflect on Lifestyle Choices:** Contemplate how personal habits, such as exercise routines and dietary patterns, can enhance or impair mitochondrial function and energy metabolism.

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6: Amino Acids and Protein structures

Pre-Lecture

Learning Objectives

- Understand the basic structure and chemical properties of amino acids.
- Recognize the importance of amino acids in protein synthesis and structure.
- Grasp the process of peptide bond formation and the concept of dehydration synthesis.
- Understand how temperature affects proteins, including denaturation and reversible aggregation.
- Learn the significance of stereochemistry in amino acids, including D- and L-isomers.
- To understand the classification and properties of the 20 natural amino acids.
- To learn the significance of amino acid R-groups in determining protein structure, function, and interactions.
- To comprehend the biochemical implications of amino acid classifications, particularly acidic, basic, polar, and nonpolar groups, and their behavior in biological systems.

Definition: Term

- **Amino Acid:** Organic compounds containing an amino group ($-\text{NH}_2$) and a carboxyl group ($-\text{COOH}$), essential for protein synthesis.
- **Alpha Carbon (α -Carbon):** The central carbon atom in an amino acid that is bonded to an amino group, a carboxyl group, a hydrogen atom, and a variable R group.
- **R Group (Side Chain):** The group that differentiates each amino acid, determining its properties (polar, nonpolar, acidic, basic).
- **Amino Group ($-\text{NH}_2$):** A basic group in amino acids that can act as a hydrogen donor.
- **Carboxyl Group ($-\text{COOH}$):** An acidic group in amino acids that can act as a hydrogen acceptor.
- **Peptide Bond:** The covalent bond formed between the amino group of one amino acid and the carboxyl group of another, releasing water (H_2O) in the process (dehydration synthesis).
- **Dehydration Synthesis:** A chemical reaction in which two molecules are joined by the removal of water.
- **Denaturation:** The process where proteins lose their structure due to heat or other factors, resulting in the loss of their biological function.
- **Chirality:** The property of a molecule where it can exist in two mirror-image forms, like left and right hands, as seen in amino acids (D- and L-forms).
- **L-form Amino Acids:** The form of amino acids predominantly found in biological systems.
- **Polypeptide:** A chain of amino acids linked by peptide bonds, which folds into functional proteins.
- **Secondary Structure:** The folding of a polypeptide chain into structures such as alpha helices or beta sheets.
- **Tertiary Structure:** The three-dimensional shape of a protein formed by interactions between the side chains (R groups) of amino acids.
- **Quaternary Structure:** The organization of multiple polypeptide chains into a functional protein complex.
- **Acidic Amino Acids:** Amino acids with a negatively charged side chain due to the presence of an additional carboxyl group (e.g., aspartic acid, glutamic acid).
- **Basic Amino Acids:** Amino acids with a positively charged side chain due to the presence of nitrogen-rich groups (e.g., lysine, arginine, histidine).
- **Polar Amino Acids:** Amino acids with hydrophilic (water-loving) side chains that can form hydrogen bonds (e.g., serine, threonine, asparagine, glutamine).
- **Nonpolar Amino Acids:** Amino acids with hydrophobic (water-fearing) side chains that are typically found in the interior of proteins (e.g., glycine, tyrosine, tryptophan, methionine).
- **Hydrogen Bond:** A weak interaction between a hydrogen atom and an electronegative atom (such as oxygen or nitrogen).
- **Hydrophobic Effect:** The tendency of nonpolar substances to aggregate in aqueous environments, helping stabilize protein structures.

- **pKa:** The pH at which a given functional group is half protonated and half deprotonated, influencing amino acid charge and behavior in proteins.

Note

- **Read the section on amino acid classifications** and make note of any terms that are unclear.
- **Review basic biochemistry concepts** such as pH, protonation, and ionization, as these will be important in understanding the behavior of amino acids in biological systems.
- **Prepare to discuss the role of amino acid side chains** in protein structure and function. Consider how the environment (pH, solubility) influences amino acid interactions.

Structure of Amino Acids

Amino acids are the fundamental building blocks of proteins, each composed of a central (**alpha**) **carbon** atom bonded to four distinct groups: an **amino group** ($-\text{NH}_2$), a **carboxyl group** ($-\text{COOH}$), a **hydrogen** atom, and a variable **R group** or side chain that dictates the specific chemical properties of the amino acid (**Figure 6.1**). The **amino group** ($-\text{NH}_2$) is basic and acts as a hydrogen donor. It plays a critical role in peptide bond formation and enhances the solubility of amino acids in aqueous environments. The **carboxyl group** ($-\text{COOH}$), on the other hand, is acidic and functions as a hydrogen acceptor. It is essential for acid-base interactions and also participates in forming peptide bonds by reacting with the amino group of another amino acid. The **central (alpha) carbon** has a tetrahedral geometry, enabling the existence of stereoisomers—D and L forms—of amino acids. Among these, the L-forms are predominant in biological systems. Lastly, the **R group (side chain)** is the defining feature that varies between amino acids and determines their polarity, charge, reactivity, and role in protein folding and biochemical interactions.

- **Amino Group ($-\text{NH}_2$):** Acts as a hydrogen donor and is basic.
- **Carboxyl Group ($-\text{COOH}$):** Acts as a hydrogen acceptor and is acidic.
- **Central (Alpha) Carbon:** Allows for tetrahedral configuration and for stereoisomerism, leading to D and L forms. L-forms are predominant.
- **R Group (Side Chain):** Defines the properties, including its polarity, charge, and participation in reactions.

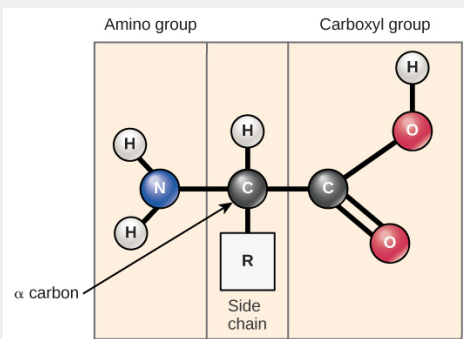


Figure 6.1: Amino Acids is shared under a CC BY-SA license by LibreTexts.

Effects of Temperature on Proteins (Figure 6.2)

When **proteins are chilled**, such as when storing enzymes or other protein-based solutions in the refrigerator or on ice, the **molecular movements slow down** due to decreased kinetic energy. Imagine placing a bottle of milk or an egg white solution in the fridge—everything still looks the same, and the proteins in the solution are still intact. This is because **cold temperatures help preserve** the protein structure by preventing the breakdown of the bonds that maintain their shape. However, in some cases, proteins might **aggregate**—or clump together—when stored for too long or under certain buffer conditions. This clumping is usually reversible. For instance, some lab enzymes form a cloudy suspension when cold but regain clarity and function once gently

warmed to room temperature. A practical example would be **cold storage of insulin**, which must be kept refrigerated to maintain its active structure, but must not be frozen, as freezing can cause irreversible aggregation.

Now compare this with what happens when you **heat proteins**. Think about cooking an egg: when you crack a raw egg into a hot frying pan, the **egg white**, which is mostly made of a protein called **albumin**, turns from clear and runny to white and solid. This change is a **visible example of protein denaturation**. As the temperature rises, the albumin proteins unfold (lose their secondary and tertiary structures) and then **tangle together in a new, disordered way**, which is what causes the egg white to firm up. These changes are **irreversible**—you cannot uncook an egg or restore the original structure of albumin. The same principle applies in biology: if human body temperature rises too high (such as in **heat stroke or high fever**), critical enzymes may denature, losing their shape and function, which can lead to **cellular dysfunction and even death** if not reversed quickly.

- **Chilling Proteins:** Cold temperatures slow down enzymatic activities and metabolic reactions but generally do not cause denaturation.
- **Heating Proteins:** High temperatures cause proteins to denature by breaking hydrogen bonds, and disrupting hydrophobic interactions, and altering the protein's secondary, tertiary, and quaternary structures. This irreversible process results in the loss of biological function.

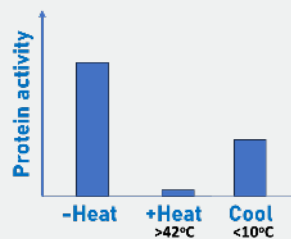


Figure 6.2: Bar graph of Protein activities under different temperature conditions

Amino Acid Structures and Classification

Each amino acid has a **central carbon atom**—called the **alpha carbon**—and this carbon is **tetrahedral**, meaning it forms four single bonds with four different groups: an amino group (-NH_2), a carboxyl group (-COOH), a hydrogen atom (H), and a variable **R group** (also called the side chain). Because all four groups are different, this carbon is **chiral** (except in glycine, where the R group is another hydrogen). This chirality means amino acids can exist in **two mirror-image forms** called **D (dextro)** and **L (levo) isomers**, similar to how your left and right hands are mirror images of each other but not superimposable. In nature, especially in proteins, almost all amino acids are found in the **L-form**. This is not just a coincidence—it's because the **enzymes** that make proteins (like ribosomes during translation) and the enzymes that break down or modify proteins are **designed to recognize only L-amino acids**. This selective use of L-amino acids is a fundamental characteristic of life on Earth, and it helps maintain the proper structure and function of proteins.

Now, when amino acids come together to form proteins, they are connected by **peptide bonds**. A **peptide bond** is a special covalent bond that forms when the **carboxyl group (-COOH)** of one amino acid reacts with the **amino group (-NH_2)** of another. During this reaction, a molecule of **water (H_2O)** is removed—a process called **dehydration synthesis**—and the result is a strong bond linking the two amino acids together. This chain can grow longer and longer to form **polypeptides**, which fold into functional **proteins**.

Finally, the **20 natural amino acids** that are used to build proteins can be grouped based on the characteristics of their R groups (side chains) (**Figure 6.3**). These groups are: (1) **Acidic** (have a negatively charged carboxylic acid group, like glutamic acid and aspartic acid), (2) **Basic** (have positively charged side chains, like lysine, arginine, and histidine), (3) **Polar uncharged** (have side chains that can form hydrogen bonds, like serine and threonine), and (4) **Nonpolar** (have hydrophobic side chains, like leucine, valine, and phenylalanine). These classifications help predict how amino acids behave in water, how they interact within a protein, and how proteins fold into their final functional shapes.

- **D and L-Amino Acids:** L-form predominates in biological systems because enzymes that synthesize and process proteins are specific to this configuration.
- **Peptide Bonds:** This bond forms between the carboxyl group of one amino acid and the amino group of another, releasing a molecule of water.
- **The 20 Natural Amino Acids:** Categorized based on their side-chain properties into acidic, basic, polar, and nonpolar groups.

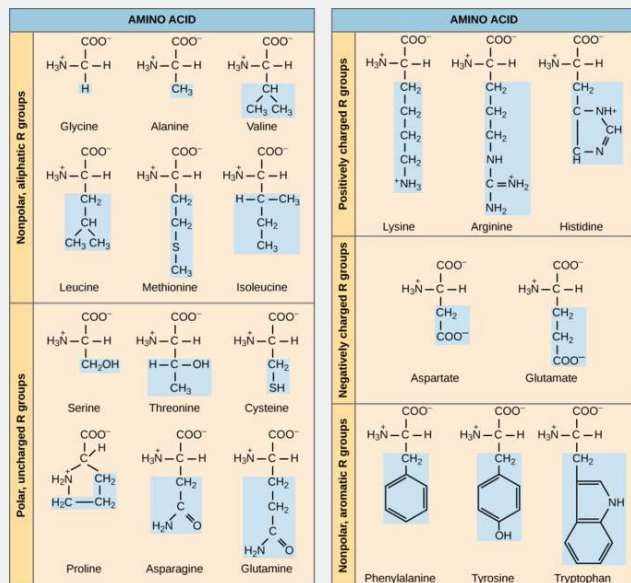


Figure 6.3: Types of amino acids: There are 21 common amino acids commonly found in proteins, each with a different R group (variant group) that determines its chemical nature. The 21st amino acid, not shown here, is selenocysteine, with an R group of -CH₂-SeH. Shared under a CC BY-SA license by LibreTexts.

Acidic Amino Acids

Acidic amino acids are a unique group of amino acids that contain **extra carboxyl (-COOH) groups** in their side chains. These additional carboxyl groups can **donate protons (H⁺)** when in a solution, especially at **physiological pH (~7.4)**, which causes them to become **negatively charged**. This negative charge is important because it affects how these amino acids interact with other molecules. Their ability to release a proton and carry a negative charge makes them excellent participants in **ionic (electrostatic) interactions**—a critical feature in maintaining protein structure and function, especially when interacting with positively charged groups like metal ions or basic amino acids.

Two key acidic amino acids are **aspartic acid** (abbreviated as **Asp**, one-letter code **D**) and **glutamic acid** (**Glu**, one-letter code **E**) (Figure 6.4). Aspartic acid has a **shorter side chain** (just one carbon between the alpha carbon and the carboxyl group), which makes it more compact and often places it in **enzyme active sites**, where it can directly participate in **catalysis** or **substrate binding**. Because of its negative charge, aspartic acid is often involved in forming **salt bridges**—strong ionic bonds with positively charged residues like lysine or arginine—which help stabilize protein structure or enzyme-substrate complexes.

Glutamic acid, on the other hand, has a **longer side chain** (two carbons before the terminal carboxyl group), giving it slightly more flexibility. It plays a prominent role in **cell signaling and metabolism**, especially in the **nervous system**, where it acts as a key **excitatory neurotransmitter**. In proteins, glutamic acid contributes to **protein folding** and **binding** by interacting with positive charges or forming hydrogen bonds. It also plays a role in **protein-protein interactions**, helping stabilize complexes or guiding enzymes to their targets.

In summary, both aspartic acid and glutamic acid are essential for the structural integrity and function of proteins due to their **negative charges**, **ability to donate protons**, and involvement in **electrostatic interactions**, **enzyme function**, and **signaling pathways**.

Both acidic amino acids contain carboxyl ($-\text{COOH}$) side chains, which donate protons at physiological pH, making them negatively charged.

- **Aspartic Acid (Asp, D):** Often found in enzyme active sites, where it participates in acid-base catalysis and forms ionic interactions.
- **Glutamic Acid (Glu, E):** Plays a role in protein structure and interactions via ionic bonding; its free form (glutamate) functions as a key neurotransmitter in the brain.

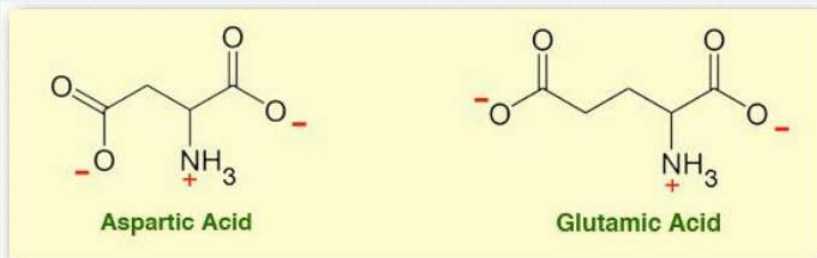


Figure 6.4: Carboxyl amino acids. Shared under a CC BY-NC-SA 4.0 license.

Basic Amino Acids

Basic amino acids are characterized by their side chains that contain **nitrogen-rich functional groups** capable of accepting protons, making them **positively charged at or near physiological pH (around 7.4)**. This positive charge plays a crucial role in how these amino acids interact with negatively charged molecules such as DNA, phosphate groups, or acidic amino acids. The three main basic amino acids are **lysine**, **arginine**, and **histidine**, and each has a unique chemical structure and specific biological functions.

Lysine (Lys, K) has a long, flexible aliphatic side chain ending in a primary amine group ($-\text{NH}_3^+$ at physiological pH), which is **highly basic** and remains positively charged in most biological environments. This allows lysine to form **strong ionic interactions and hydrogen bonds**, making it vital in **protein-protein interactions**, particularly in protein complexes and structural motifs. Additionally, lysine residues are **frequent targets of post-translational modifications**, especially **acetylation**, **methylation**, and **ubiquitination**, which play a central role in regulating **gene expression**. For instance, in **histones**, which help package DNA, lysine residues on their tails are often **acetylated** to loosen DNA-histone binding, allowing genes to become more accessible for transcription.

Arginine (Arg, R) has a side chain containing a **guanidinium group**, which is a planar, highly basic group that can form **multiple hydrogen bonds** and **strong electrostatic interactions**. This makes arginine especially useful in **binding negatively charged molecules** like DNA, RNA, and phosphate groups. It is also essential in **metabolic pathways** such as the **urea cycle**, where it helps eliminate excess nitrogen from the body. Additionally, arginine is the **precursor for nitric oxide (NO)**, a critical signaling molecule involved in **vasodilation**, immune responses, and neurotransmission. The ability of the guanidinium group to stabilize multiple interactions makes arginine-rich regions crucial for **binding specificity and regulation**.

Histidine (His, H) is unique among the basic amino acids because of its **imidazole side chain**, which has a **pKa close to physiological pH (~6.0–6.5)**. This means that histidine can **switch between a protonated (positively charged) and unprotonated (neutral) state** under small pH changes. As a result, histidine acts as a **versatile proton donor or acceptor**, making it an essential residue in **enzyme active sites** where proton transfer is needed during catalysis. This property is why histidine is frequently involved in **acid-base catalysis** and in stabilizing reaction intermediates.

One of histidine's most important applications in molecular biology is in **protein purification** using **Ni-NTA (Nickel-Nitrilotriacetic Acid) affinity chromatography**. This technique exploits the fact that the **imidazole ring of histidine has lone pair electrons** that can coordinate with **nickel ions (Ni^{2+})**. In this method, proteins are engineered with a **histidine tag (His-tag)**—usually a string of six or more histidines (6x-His tag)—on either the N- or C-terminus. When the tagged protein mixture is passed through a Ni-NTA resin, the **histidine residues bind tightly to the nickel ions** immobilized on the column, allowing the tagged protein to stick while other proteins wash through. The protein of interest is then **eluted by adding imidazole to the buffer**, which

competes with the histidine residues and displaces them from the nickel. This method is widely used because it's **specific, efficient, and gentle**, preserving protein structure and function.

In summary, **lysine, arginine, and histidine** are essential **basic amino acids** that support protein structure, biochemical reactions, and molecular interactions. **Histidine's imidazole group** is especially versatile, not only in catalysis but also in biotechnology through its critical role in **Ni-NTA purification**.

These amino acids have side chains that accept protons, making them positively charged at physiological pH.

- **Lysine (Lys, K):** Important for protein-protein interactions and histone modifications.
- **Arginine (Arg, R):** Participates in the urea cycle and nitric oxide synthesis.
- **Histidine (His, H):** Has an imidazole group that acts as a proton donor/acceptor in enzymatic reactions.



Figure 6.5: Amine amino acids. Shared under a CC BY-NC-SA 4.0 license.

Polar Amino Acids

Polar amino acids have side chains that contain **electronegative atoms**—such as oxygen or nitrogen—which make them **hydrophilic** (water-loving) and capable of forming **hydrogen bonds** with water and other polar molecules. These interactions are essential for **protein solubility, protein folding, and biochemical reactions**, especially in aqueous environments like the cytoplasm or extracellular fluid. Unlike acidic or basic amino acids, polar amino acids do not carry a net charge at physiological pH, but their side chains can **participate in bonding** that stabilizes protein structures and supports molecular recognition.

Two important polar amino acids are **serine (Ser, S)** and **threonine (Thr, T)**. Both of these amino acids have **hydroxyl (-OH) groups** in their side chains. These -OH groups are highly reactive and play a critical role in **hydrogen bonding** and **post-translational modifications**, particularly **phosphorylation**. In phosphorylation, a **phosphate group (PO₄³⁻)** is enzymatically added to the hydroxyl group, often regulating protein activity, signaling pathways, and protein-protein interactions. For example, serine and threonine residues in enzymes or receptors are often phosphorylated to **activate or deactivate** their function, making them key players in **cell signaling cascades** such as those driven by kinases (like in insulin signaling or MAPK pathways).

Asparagine (Asn, N) and **glutamine (Gln, Q)** are also polar but differ because their side chains contain **amide groups (-CONH₂)** instead of hydroxyls. These amide groups are **polar but uncharged**, and they are excellent at forming **hydrogen bonds**, making Asn and Gln crucial for maintaining **protein stability and hydration**. They are often found on the **surface of proteins**, where they can interact with water, or in the **core of protein structures**, where they participate in **hydrogen bonding networks** that stabilize secondary and tertiary structure. They are also involved in **molecular recognition** and **protein-DNA or protein-RNA interactions**, thanks to their ability to form specific and directional hydrogen bonds.

To help students **easily remember the structures** of asparagine (Asn) and glutamine (Gln), it helps to compare them to their **acidic counterparts, aspartic acid (Asp, D) and glutamic acid (Glu, E)**. Asp and Glu both have **carboxylic acid (-COOH)** groups in their side chains, which can **lose a proton** and become negatively charged. Asn and Gln are structurally similar—but instead of a carboxylic acid group, they have an **amide group (-CONH₂)** in the same position. In other words, if you "convert" the -OH part of the carboxyl group into an -NH₂ group, you go from Asp to Asn or from Glu to Gln. This structural similarity makes them easy to pair and memorize:

- **Asp (D) → Asn (N)** = 1-carbon chain ending in -COOH vs. -CONH₂
- **Glu (E) → Gln (Q)** = 2-carbon chain ending in -COOH vs. -CONH₂

Polar amino acids contain side chains with electronegative atoms like oxygen or nitrogen, enabling them to form hydrogen bonds with water, other amino acids, or biomolecules, which is essential for protein structure and function.

- **Serine (Ser, S) & Threonine (Thr, T):** Contain hydroxyl (-OH) groups that can form hydrogen bonds and serve as common sites for phosphorylation, playing key roles in cell signaling and regulation.
- **Asparagine (Asn, N) & Glutamine (Gln, Q):** Contain amide side chains that enable them to participate in hydrogen bonding, stabilizing protein structures and mediating molecular interactions.

Nonpolar Amino Acids

Nonpolar (hydrophobic) amino acids have side chains that lack electronegative atoms and are composed mainly of **hydrocarbon groups**—making them **water-insoluble** and **nonpolar**. These residues are crucial in **protein folding**, because they tend to cluster together away from water in the **interior of proteins**, creating a **hydrophobic core**. This clustering helps proteins achieve their proper three-dimensional shape and stability. They also play a major role in **membrane-associated proteins**, where nonpolar side chains interact with the lipid bilayer's hydrophobic tails.

Let's start with **glycine (Gly, G)**, the **smallest** of all amino acids. Unlike other amino acids that have a distinct R group, glycine's side chain is simply a **hydrogen atom**. Because of this, the alpha carbon in glycine is **not chiral**, and the amino acid is **extremely flexible**. It can fit into **tight spaces** within protein structures—especially in **turns, loops, and bends** of polypeptide chains. For example, glycine is often found in **beta-turns** and **collagen triple helices**, where steric hindrance would prevent larger side chains from fitting. This makes glycine essential for regions of proteins that need to **twist or fold sharply**, adding flexibility and mobility to the overall structure. In protein folding, glycine is like the **swivel joint** that allows proteins to **bend or make tight turns**. For example, when a protein needs to form a **sharp U-turn** (like in a beta turn), glycine is often there to make that turn smooth and sharp without creating a clash. For example, in collagen, one of the most abundant proteins in your body, every third amino acid is glycine. That's because collagen forms a triple helix, and without glycine's small size and flexibility, the helix would be too bulky and unstable.

Next, **tyrosine (Tyr, Y)** and **tryptophan (Trp, W)** are often confusing because they contain **polar functional groups**—a **hydroxyl (-OH)** group on tyrosine and an **indole nitrogen (-NH)** group on tryptophan. However, these amino acids are still considered **predominantly nonpolar** because the **bulk of their side chains is made up of large aromatic (ring) systems**. These rings are **hydrophobic** and dominate the molecule's behavior in aqueous environments. Their polar groups can form **hydrogen bonds**, but the **electron-rich aromatic rings** favor interactions with **other hydrophobic or aromatic regions** in proteins, like in **pi-pi stacking**. This duality makes Tyr and Trp important in **enzyme active sites** and **membrane proteins**, where both **hydrophobic packing** and **limited hydrogen bonding** are needed. Overall, these two amino acids are **aromatic**, meaning they have **large ring structures** in their side chains. These rings are made mostly of **carbon and hydrogen**, which makes them **nonpolar and hydrophobic** overall. However, they each contain a small **polar group** that can still form hydrogen bonds that are important for many biological functions. For example, Tryptophan is involved in the synthesis of serotonin, a neurotransmitter. Its indole ring is important for passing through cell membranes, thanks to its hydrophobic character. Also, in proteins embedded in membranes, like G-protein-coupled receptors (GPCRs), you often find tyrosine and tryptophan at the membrane-water boundary—they anchor the protein and interact with both the hydrophobic membrane interior and the aqueous exterior.

Methionine (Met, M) is another nonpolar amino acid that contains a **sulfur atom** in its side chain. This sulfur is part of a **thioether group**, where the sulfur is bonded to a **methyl group (-CH₃)**. The methylation of sulfur **masks its polarity**, making methionine's side chain **nonpolar and hydrophobic**, despite the presence of a sulfur atom. This allows methionine to participate in **hydrophobic interactions** rather than forming hydrogen bonds or reacting with polar molecules. Again, Methionine has a **thioether** group in its side chain, which means it contains a **sulfur atom** bonded to a **methyl group (-CH₃)**. Normally, sulfur might add polarity, but in methionine, the **methyl group “shields” the sulfur**, making the entire side chain **nonpolar**.

An important feature of methionine is that it is **the first amino acid incorporated into proteins during translation** in nearly all organisms. In eukaryotes and archaea, this is typically **initiator methionine (Met)**, while in prokaryotes, it is **N-formylmethionine (fMet)**. The reason for this is **evolutionary and functional**. The **AUG codon**, which codes for methionine, serves as the **universal**

start codon in the genetic code. Translation machinery (ribosomes and initiation factors) has evolved to recognize AUG as the signal to **start translation**. Over time, methionine became locked into this role, likely because it is chemically stable, relatively neutral (nonpolar but not too bulky), and does not interfere with the early folding of the nascent polypeptide. After translation, methionine is often **cleaved off by enzymes** if it's not needed in the final protein structure. Thus, its role as a starter is **more functional than structural**, driven by the **evolution of translation systems** rather than its chemical properties alone. So methionine is like the “**green light**” that tells the ribosome to **start building a protein**, and it doesn't cause drama—it gets things going and then sometimes even **steps aside** (many proteins have their initial methionine cleaved off after translation).

In summary, nonpolar amino acids like **glycine, tyrosine, tryptophan, and methionine** contribute to **protein shape, stability, and localization** in unique ways—from adding flexibility to forming hydrophobic cores to initiating translation. Understanding their properties helps explain how proteins fold and function in complex biological systems.

These amino acids have side chains that are largely nonpolar, contributing to hydrophobic interactions that drive protein folding, stability, and membrane association.

- **Glycine (Gly, G):** The smallest amino acid, glycine contributes flexibility to protein structures and is often found in tight turns or loop regions due to its minimal steric hindrance.
- **Tyrosine (Tyr, Y) & Tryptophan (Trp, W):** Tyrosine and tryptophan have large aromatic side chains that contribute to hydrophobic interactions. Although predominantly nonpolar, their hydroxyl (-OH) and indole nitrogen (-NH) groups allow them to also engage in hydrogen bonding, making them amphipathic.
- **Methionine (Met, M):** Methionine contains a nonpolar thioether side chain (-S-CH₃) that contributes to hydrophobic interactions, particularly in the protein interior.

Nomenclature of Peptides

Amino acids are the fundamental building blocks of peptides and proteins, each of which is structured around a central carbon, called the **alpha (α) carbon**, which is bonded to four different groups: a **hydrogen atom**, an **amino group (-NH₂)**, a **carboxyl group (-COOH)**, and a distinctive **R group** (or side chain). The **R group** is what differentiates one amino acid from another and defines its chemical properties (such as being nonpolar, polar, acidic, or basic). When amino acids are linked together in a chain, they form **peptides**, with the **N-terminus** (the amino group side) on one end and the **C-terminus** (the carboxyl group side) on the other. This specific orientation is critical because it determines the peptide's structure and function.

The process of peptide bond formation begins when the **amino group of one amino acid** reacts with the **carboxyl group of another**, releasing a molecule of water (a condensation reaction). The bond that forms between the two amino acids is called a **peptide bond**, and the result is a **dipeptide** (two amino acids linked together) (**Figure 6.4**). As more amino acids join the chain through similar peptide bonds, the structure grows into a **polypeptide**. The directionality of the polypeptide chain is always from the **N-terminus** to the **C-terminus**, meaning that proteins are always synthesized in this order by **ribosomes** during protein translation.

In terms of **drawing amino acids**, the **N-terminus** is usually drawn on the left, and the **C-terminus** is drawn on the right. Each amino acid's structure includes the central carbon (α-carbon) attached to:

- The **amino group (-NH₂)** on the left side (N-terminus).
- The **carboxyl group (-COOH)** on the right side (C-terminus).
- The **hydrogen atom** and the **R group** (side chain) are attached to the α-carbon.

When drawing a **peptide**, you would typically draw the **backbone** of the chain (the repeating sequence of **N-C-C** between each amino acid) and then show the **side chain (R group)** sticking out from each α-carbon (**Figure 6.5**). The **R group** plays an important role in the final shape of the protein because it dictates how the peptide interacts with other molecules. As the chain folds into its **three-dimensional shape**, the **R groups flip back and forth**, orienting themselves in ways that minimize or maximize interactions with other molecules, especially water. **Hydrophobic** side chains tend to hide inside the protein's core, away from water, while **hydrophilic** side chains may be exposed on the outside to interact with water or other molecules.

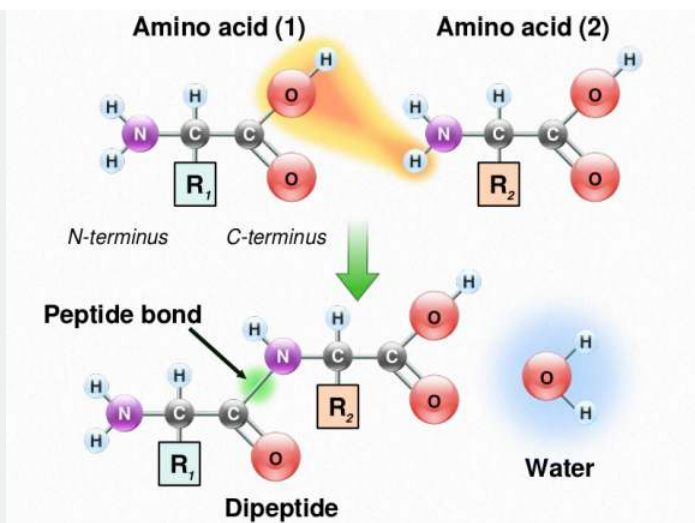


Figure 6.4: Formation of a peptide bond. Shared under a CC BY-NC-SA 4.0 license.

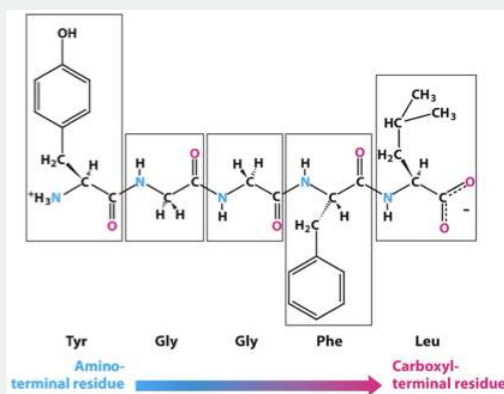


Figure 6.5: Nature way of writing amino acids. They are read from the amino (N) terminus to the carboxyl (C) terminus.

The sequence of amino acids in a peptide is critical because it dictates the **secondary**, **tertiary**, and even **quaternary** structures of the protein, which ultimately determine its **biological function**. The **N-terminus to C-terminus** directionality is not just an arbitrary naming convention but reflects the **sequence** in which the amino acids are **coded by DNA** and then **assembled** by the ribosome during **translation**. For example, if a protein's amino acid sequence is **Serine-Glycine-Leucine**, the **serine** would be at the N-terminus and the **leucine** would be at the C-terminus.

The process of protein folding occurs after the peptide chain is synthesized. As the chain folds, the **R groups** of the amino acids influence how the chain bends, twists, and forms **secondary structures** (such as **alpha helices** or **beta sheets**). These secondary structures are stabilized by interactions between the side chains, such as **hydrogen bonds**, **ionic bonds**, or **hydrophobic interactions**. As the protein folds into its **tertiary structure**, the R groups continue to play a critical role, driving the protein toward its functional, **three-dimensional shape**. For example, in a protein like **hemoglobin**, the **specific sequence of amino acids** from the N-terminus to the C-terminus determines how the protein folds and how it can bind to oxygen. **Mutations** in this sequence can cause the protein to fold incorrectly, as seen in **sickle cell disease**, where a single amino acid change in the sequence causes hemoglobin to form an abnormal shape, leading to health problems.

1. **Naming Peptides:** The **N-terminus** (amino group) is always at the start of the peptide chain, and the **C-terminus** (carboxyl group) is at the end. This directionality is fundamental because it reflects the sequence in which amino acids are added during protein synthesis.
2. **Drawing Amino Acids:** The general structure of each amino acid is **N-terminus – α -carbon – C-terminus**, with the **R group** attached to the α -carbon. When drawing a peptide, you represent the **backbone** of the chain (N-C-C) and show the **R groups**

attached to the α -carbons.

3. **The Role of R Groups:** The **R groups** flip back and forth as the polypeptide folds into its final three-dimensional shape, affecting how the protein interacts with other molecules, its stability, and its function.
4. **Function and Sequence:** The sequence of amino acids in a polypeptide (from N-terminus to C-terminus) determines the **folding** and **function** of the protein, with small changes in the amino acid sequence potentially leading to significant biological consequences.

By understanding these concepts and being able to draw and name amino acids and peptides, students will gain a strong foundation in how proteins are built, how their structures determine their function, and how small changes in the amino acid sequence can lead to profound changes in protein behavior and cellular processes.

Post-Lecture Learning Objectives

- Review and reinforce the importance of amino acids in protein structure and function.
- Examine how peptide bonds form and their significance in protein synthesis.
- Explore the effects of temperature on protein stability and functionality.
- Understand how protein folding determines its biological activity.
- Apply concepts of chirality and stereoisomerism to protein synthesis and function.
- To integrate knowledge about the 20 natural amino acids into a broader understanding of protein structure, function, and interactions.
- To reinforce how the properties of amino acid side chains influence protein folding, stability, and function in biological systems.

Key Definitions

- **Protein Folding:** The process by which a polypeptide chain assumes its functional three-dimensional structure, influenced by the interactions between R groups.
- **Protein Denaturation:** The irreversible loss of a protein's structure, and thus its function, due to high temperatures or chemical agents.
- **Peptide Bond Formation:** The covalent bond linking amino acids, formed by dehydration synthesis between the amino group of one amino acid and the carboxyl group of another.
- **Structure-Function Relationship:** The idea that the specific sequence and structure of amino acids in a protein determine its functional role in the body (e.g., how a protein folds to perform specific tasks).
- **Aggregation:** The clumping together of proteins, typically due to improper storage conditions like extreme cold or high temperature, which can sometimes be reversible (e.g., enzymes in cold storage).
- **Hydrophobic and Hydrophilic Interactions:** These interactions between amino acid side chains determine the folding of proteins, with hydrophobic amino acids typically hidden in the protein's interior and hydrophilic ones on the exterior.
- **Stereoisomerism in Amino Acids:** The presence of D- and L-isomers of amino acids, with L-isomers being used predominantly in protein synthesis due to enzyme specificity.
- **Acidic amino acids** have negatively charged side chains (e.g., aspartic acid and glutamic acid), critical for ionic interactions in protein structure.
- **Basic amino acids** carry positive charges due to their nitrogenous side chains (e.g., lysine, arginine, histidine), enabling interactions with negatively charged molecules and DNA.
- **Polar amino acids** can form hydrogen bonds, stabilizing protein structures, and are often involved in biochemical reactions, particularly phosphorylation (e.g., serine and threonine).
- **Nonpolar amino acids** are hydrophobic and contribute to the stability of the protein's interior (e.g., methionine, glycine, tyrosine).
- The R-groups play a critical role in protein folding by determining how amino acids interact with each other (hydrophobic effect, hydrogen bonding, ionic interactions).
- Nonpolar amino acids tend to cluster in the protein's core, while polar or charged amino acids are often found on the protein's surface, interacting with the surrounding aqueous environment.

- **Histidine** is versatile in catalysis due to its ability to accept or donate protons, making it essential in enzyme active sites.
- **Glutamine and Asparagine** are involved in protein-protein interactions due to their ability to form hydrogen bonds, stabilizing structures or aiding in molecular recognition.
- **Methionine** is unique for its role as the initiator of protein synthesis, marking the start of translation.

Key Takeaways

- Amino acids form the foundation of proteins, and their unique properties (amino group, carboxyl group, and R group) are essential for peptide bond formation and protein folding.
- Protein function is heavily influenced by its three-dimensional structure, which is determined by the sequence of amino acids.
- The ability to understand protein denaturation, folding, and the role of temperature is crucial in many biological processes, including enzyme activity and disease states like heat stroke or protein misfolding disorders.

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7: Enzymes and Kinetics

Learning Objectives

- Define Gibbs Free Energy (ΔG) and explain its role in determining reaction spontaneity.
- Differentiate between exergonic and endergonic reactions.
- Explain how ATP hydrolysis can drive non-spontaneous reactions through energy coupling.
- Understand how environmental factors (e.g., temperature, concentration) influence ΔG .
- Relate spontaneity (thermodynamics) to enzyme catalysis (kinetics).
- Describe the basic structure of enzyme-catalyzed reactions.
- Define key rate constants involved in enzyme kinetics (k_1 , k_{-1} , k_2).
- Understand the concept of the enzyme-substrate complex and its role in catalysis.
- Interpret the meaning of the dissociation constant (K_d) and its relationship to binding affinity.
- Recognize the relevance of enzyme binding affinity in drug design (e.g., Imatinib for BCR-ABL).
- Apply the **Michaelis-Menten equation** to describe and predict enzyme behavior.
- Analyze enzyme efficiency using **K_m** and **V_{max}** .
- Interpret kinetic data using a **Lineweaver-Burk plot**.
- Differentiate between **competitive** and **non-competitive** enzyme inhibition.
- Connect kinetic parameters (K_m , V_{max} , K_d , k_1) to real-world clinical and pharmacological scenarios.
- Explain how enzyme kinetics guide the design and evaluation of drugs (e.g., HIV protease inhibitors, acetaminophen metabolism, doxycycline absorption).

Definition: Term

- **ΔG (Gibbs Free Energy)**: A thermodynamic value that predicts if a reaction is spontaneous.
- **Exergonic Reaction**: A reaction that releases energy ($\Delta G < 0$); spontaneous.
- **Endergonic Reaction**: A reaction that requires energy input ($\Delta G > 0$); non-spontaneous.
- **ATP Hydrolysis**: The breakdown of ATP to ADP + P_i , releasing energy ($\Delta G \approx -30.5$ kJ/mol).
- **Coupling Reactions**: Linking an unfavorable (endergonic) reaction with a favorable one to make it proceed.
- **Activation Energy ($\Delta G^\ddagger$)**: The energy barrier that must be overcome for a reaction to proceed.
- **Enzyme Catalyst**: A biological molecule that speeds up reactions by lowering activation energy.
- **Le Chatelier's Principle**: When a system is disturbed, it shifts to minimize the disturbance.
- **V_{max}** : The maximum velocity of an enzymatic reaction when the enzyme is saturated with substrate.
- **K_m** : Substrate concentration at half-maximal velocity (V_{max}); reflects binding affinity.
- **Enzyme (E)**: A biological catalyst that speeds up chemical reactions.
- **Substrate (S)**: The molecule upon which an enzyme acts.
- **Enzyme-Substrate Complex (ES)**: A transient complex formed when an enzyme binds its substrate.
- **k_1** : The rate constant for the forward reaction forming ES.
- **k_{-1}** : The rate constant for the reverse reaction, dissociating ES into E and S.
- **k_2 (or k_{cat})**: The rate constant for conversion of ES to product (P).
- **K_d (Dissociation Constant)**: A measure of enzyme-substrate affinity; calculated as k_{-1} / k_1 . A lower K_d indicates stronger binding.
- **Affinity**: The strength of binding between an enzyme and its substrate.
- **Catalysis**: The acceleration of a chemical reaction by a catalyst.

Spontaneity of a Reaction and Gibbs Free Energy (ΔG)

In chemistry, the term **spontaneity** refers to whether a reaction can proceed on its own, without needing continuous input of external energy. This is not about speed but about thermodynamic favorability. The primary tool for determining spontaneity is the **Gibbs Free Energy** change, denoted as ΔG . If ΔG is **negative**, the reaction releases energy into the surroundings and is called

exergonic—this means the reaction is **spontaneous** and will proceed naturally. On the other hand, if ΔG is **positive**, the reaction requires energy input, making it **endergonic**, and thus **non-spontaneous** under standard conditions.

- If ΔG is **negative**, the reaction **releases energy**—this is an **exergonic** reaction and is **spontaneous**.
- If ΔG is **positive**, the reaction **requires energy input**—this is an **endergonic** reaction and is **non-spontaneous**.

To illustrate this, consider a biological example: the conversion of **glucose** into **glucose-6-phosphate**, an essential first step in **glycolysis**. This reaction has a ΔG° of +18 kJ/mol, indicating that it is non-spontaneous (**Figure 7.1**). This is similar to trying to roll a ball uphill; the system needs an input of energy to push it forward. Cells, however, need this reaction to happen regularly as part of their energy metabolism. To drive this uphill reaction forward, nature uses a clever trick called **energy coupling**. The cell pairs this unfavorable reaction with a highly favorable one: the hydrolysis of ATP (adenosine triphosphate) into ADP (adenosine diphosphate) and inorganic phosphate (Pi). ATP hydrolysis has a ΔG° of −30.5 kJ/mol, meaning it releases a significant amount of energy—like a ball rolling down a steep hill. By combining the two reactions—glucose phosphorylation (+18 kJ/mol) and ATP hydrolysis (−30.5 kJ/mol)—the cell effectively **merges their energy changes**. The **net ΔG°** becomes −12.5 kJ/mol, making the overall reaction spontaneous. In simpler terms, this is like trying to buy a \$50 item when you only have \$20. You can't afford it—unless you also have a \$30 gift card. Combining your money and the gift card, you now have \$50, enough to buy the item. That's what happens in the cell: ATP acts like an energy currency or a gift card that allows you to “pay for” processes that wouldn't occur otherwise.

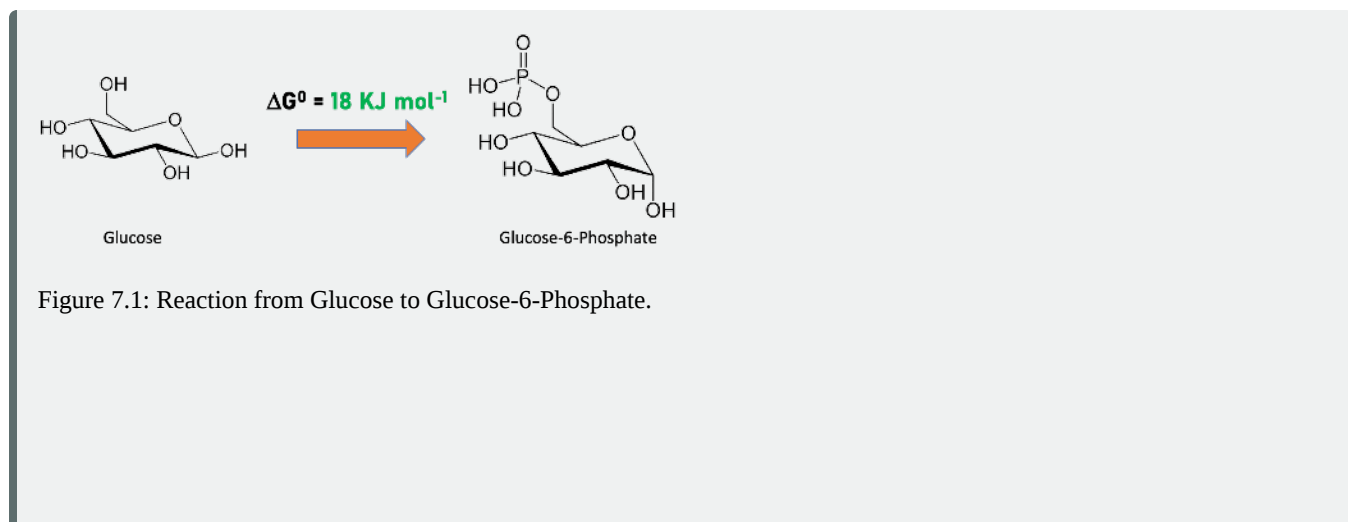
This concept of G-coupling is fundamental in biochemistry because many essential reactions—like protein synthesis, active transport of ions, and DNA replication—are inherently endergonic and would not proceed on their own. Cells rely on the free energy released from ATP hydrolysis or other high-energy compounds to push these processes forward. In this way, coupling allows biological systems to achieve thermodynamically unfavorable outcomes by linking them to favorable reactions. Without this mechanism, the machinery of life would come to a halt.

Example:

Consider this reaction (**Figure 7.2**):

- **Glucose → Glucose-6-Phosphate**, with $\Delta G^\circ = +18 \text{ kJ/mol}$
 - This is a **non-spontaneous** reaction under standard conditions. It won't proceed without external energy.
- **ATP → ADP + Pi** has $\Delta G^\circ = -30.5 \text{ kJ/mol}$.
 - Nature often drives non-spontaneous reactions forward by **coupling** them with highly **spontaneous reactions**, such as **ATP hydrolysis**.
- **Glucose + ATP → Glucose-6-Phosphate + ADP**
 - The ΔG° of the coupled reaction = +18 (from glucose phosphorylation) + (−30.5 from ATP hydrolysis) = **−12.5 kJ/mol**

Now, the net ΔG° is negative, so the **overall reaction becomes spontaneous**. This is how biological systems make “unfavorable” reactions proceed—by borrowing energy from ATP or other high-energy compounds.



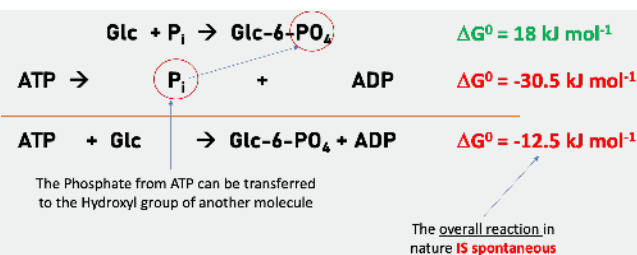


Figure 7.2: ΔG coupling reaction of Glucose to Glucose-6-Phosphate

Factors That Affect Reaction Spontaneity

The **spontaneity of a chemical reaction**—whether it can proceed on its own—is determined by the **Gibbs Free Energy change** (ΔG). But ΔG itself can be influenced by several **environmental and molecular factors**. One important factor is the **nature of the molecules involved**. Some chemical bonds are inherently unstable and prone to breaking, while others are very stable. For instance, the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen occurs spontaneously because the O–O bond in peroxide is weak and easily broken. In contrast, breaking the C–H bonds in methane requires a lot of energy, making the reaction non-spontaneous unless conditions are adjusted.

Environmental conditions such as temperature, pressure, and pH also affect ΔG . According to the Gibbs free energy equation ($\Delta G = \Delta H - T\Delta S$), increasing the **temperature (T)** can make a reaction more favorable if it **increases entropy (ΔS)**. That's why some reactions that are non-spontaneous at room temperature can become spontaneous when heated. For example, melting ice is non-spontaneous below 0°C , but it is spontaneous above it due to entropy increasing as solid turns into liquid.

Concentration of reactants and products is another key factor. According to **Le Chatelier's Principle**, increasing the concentration of reactants shifts the equilibrium to favor product formation, effectively making a reaction more favorable under certain conditions. For instance, in glycolysis, the accumulation of glucose and inorganic phosphate (P_i) helps push the formation of glucose-6-phosphate, even if the standard ΔG° is positive.

Lastly, and perhaps most powerfully, is the strategy of **reaction coupling**. As we've discussed, energetically unfavorable reactions (positive ΔG) can be "paid for" by coupling them to very favorable reactions (negative ΔG), such as ATP hydrolysis. This is a fundamental strategy in cells. Without it, many essential biosynthetic and metabolic processes could not proceed

1. **Nature of the Molecules** – Some bonds naturally break or form more easily due to inherent chemical stability.
2. **Conditions** – Temperature, pressure, and pH can shift ΔG . High temperatures often make endergonic reactions more feasible.
3. **Concentration** – High concentration of reactants can push reactions forward via Le Chatelier's Principle.
4. **Coupling Reactions** – As explained, unfavorable reactions can piggyback on favorable ones to proceed.

While ΔG tells us **if** a reaction can occur, it tells us nothing about **how fast** it will occur. That's where **reaction kinetics** and the **rate constant (k)** come into play. The **rate constant** is a numerical value that reflects how quickly a reaction occurs under a given set of conditions. It's influenced by factors like temperature, molecular orientation, and activation energy.

According to **collision theory**, for a reaction to happen, reactant molecules must collide. But not just any collision will do—the molecules must strike each other with the **correct orientation** and **sufficient energy** to overcome the **activation energy barrier** ($\Delta G^\ddagger$) (**Figure 7.3**). This energy barrier represents the "hill" the reactants must climb before they can be converted into products. The rate constant (k) increases when this barrier is lower or when collisions happen more frequently and effectively.

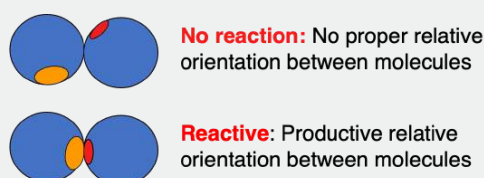


Figure 7.3: Collision Reaction for a substrate to become a product.

There are three main ways to **increase the rate of a reaction**:

1. **Increase substrate concentration**: Imagine a crowded room where more people are likely to bump into each other. Similarly, more substrate molecules mean a higher probability of effective collisions, which increases reaction rate.
2. **Increase temperature**: Heating the system makes molecules move faster, increasing the number and energy of collisions. This boosts the chances that collisions will overcome the activation barrier.
3. **Add enzymes**: Enzymes are biological catalysts that don't just increase the rate of reactions—they do so with incredible precision. Enzymes help orient the reactant molecules properly and stabilize the **transition state**, effectively lowering the activation energy ($\Delta G^\ddagger$). Think of an enzyme as a skilled dance partner who guides your movements perfectly so that the dance (reaction) happens smoothly and efficiently.

A dramatic illustration of how enzymes affect reaction rates is seen with the enzyme **urease**, which catalyzes the breakdown of urea into ammonium carbonate. Without urease, this reaction occurs **extremely slowly**—just one reaction every **3 million years**. That's practically geological time! But with urease, the reaction rate skyrockets to about **30,000 reactions per second**. This is because urease drastically lowers the **activation energy ($\Delta G^\ddagger$)** required, making the reaction occur almost instantly under biological conditions. This example demonstrates the enormous power of enzymes—not to change ΔG , or spontaneity, but to **change the speed at which equilibrium is reached**. They allow life to perform thousands of chemical reactions in a controlled and timely fashion. Without enzymes, metabolism would grind to a halt.

- The **activation energy ($\Delta G^\ddagger$)** is the energy needed to get reactants into a **transition state**, an unstable intermediate on the way to forming products (**Figure 7.4**).
- Even if a reaction has a negative ΔG (spontaneous), it might proceed slowly if $\Delta G^\ddagger$ is high.
 - Enzymes **lower $\Delta G^\ddagger$** , not ΔG , to speed up reactions.
- **ΔG** : Energy difference between reactants and products (predicts spontaneity).
- **$\Delta G^\ddagger$** : Energy needed to reach the transition state (predicts rate).

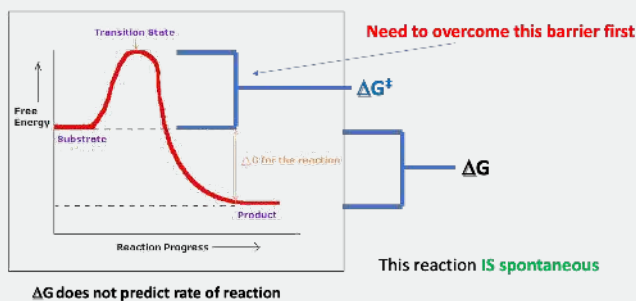


Figure 7.4: ΔG shows the energy difference between the reactant and the product, while $\Delta G^\ddagger$ is the Gibbs free energy of transition state.

Enzyme Specificity and Binding Models

One of the most fascinating features of enzymes is their **specificity**—the ability to select and act on a **particular substrate** among many similar molecules. This is crucial in the body because it ensures that reactions occur in a controlled and precise manner. For example, the enzyme **lactase** specifically acts on **lactose**, the sugar found in milk, and breaks it down into glucose and galactose. If lactase acted on many sugars indiscriminately, our metabolism would be chaotic and inefficient.

Enzymes can also show **stereospecificity**, meaning they only interact with one enantiomer (mirror image) of a molecule. A common example is how enzymes in our bodies typically recognize and process **L-amino acids**, not **D-amino acids**, which are rarely found in nature. This is highly relevant in drug design: some drugs have both L and D forms (called **chiral drugs**), but only

one form is effective or safe. The painkiller **ibuprofen**, for example, exists in two enantiomers, but only the **S-enantiomer** is active—this shows the importance of enzyme stereospecificity in how drugs work.

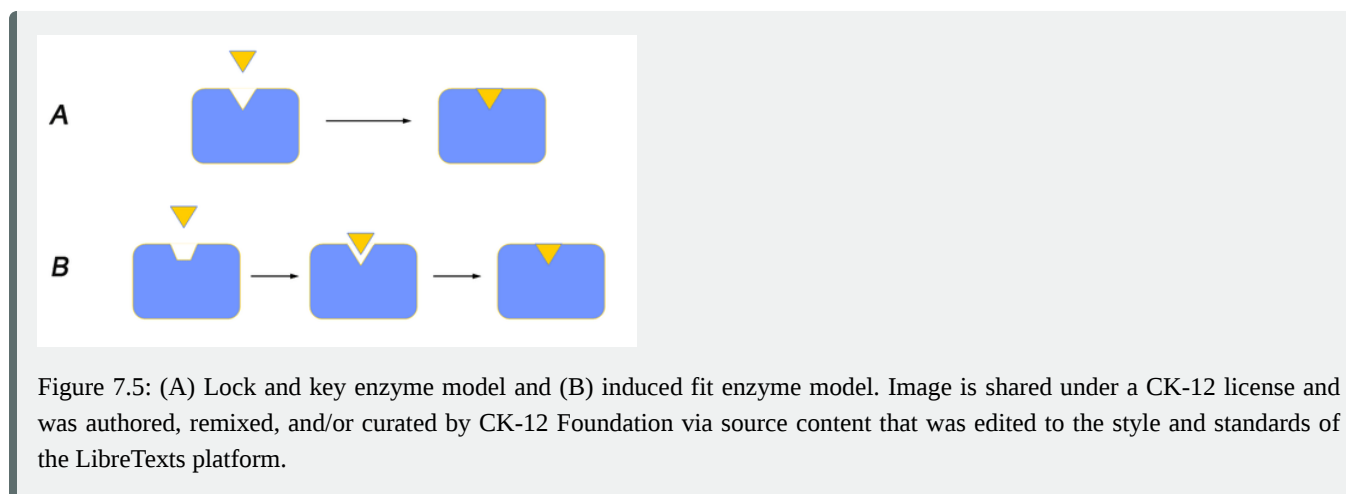
There are two primary models used to explain how enzymes bind to substrates: the **Lock and Key model** and the **Induced Fit model** (Figure 7.5). In the **Lock and Key model**, the enzyme has a **rigid active site** that matches the substrate's shape exactly, like a key fitting into a lock. This model explains high specificity, as only the correct “key” (substrate) will fit. A classic example is the enzyme **acetylcholinesterase**, which breaks down the neurotransmitter **acetylcholine**—the active site of this enzyme is so specific that it won't act on structurally similar molecules. However, most enzymes follow the **Induced Fit model**, which is more dynamic. In this model, the enzyme changes shape slightly to better accommodate the substrate once it starts to bind. This explains how enzymes can stabilize the **transition state**—the unstable, high-energy structure that forms during a reaction. A real-life example is **hexokinase**, an enzyme that adds a phosphate group to glucose during glycolysis. When glucose binds, hexokinase undergoes a conformational change that “clamps down” on the substrate, creating a snug fit and lowering the activation energy needed for the reaction.

Enzymes are highly specific and may recognize:

- Specific **substrates**
- Specific **stereoisomers** (e.g., only L-amino acids)

Binding models:

1. **Lock and Key:** Substrate fits enzyme perfectly (like a key in a lock).
2. **Induced Fit:** Enzyme molds around the substrate to fit better—this is **more common** and explains how enzymes stabilize the transition state.



Cofactors and Coenzymes

While many enzymes can function with just their amino acid side chains, others require **additional non-protein components** to carry out their catalytic activity. These helpers are broadly classified into **cofactors** and **coenzyme** (Figure 7.6).

Cofactors are typically **inorganic ions**, such as **magnesium (Mg^{2+})**, **zinc (Zn^{2+})**, **iron (Fe^{2+})**, or **heme groups**. These ions often help in stabilizing charges during the reaction or are directly involved in electron transfer. For instance, the enzyme **carbonic anhydrase**, which helps convert carbon dioxide and water into carbonic acid in your blood, uses **zinc** as a cofactor to hold water molecules in the correct position and polarize them for the reaction. Another example is **cytochrome P450**, a heme-containing enzyme family in the liver that helps detoxify drugs and toxins—many common drugs, including **statins** and **antidepressants**, are metabolized by this system.

Coenzymes, on the other hand, are **organic molecules**, often derived from **vitamins**, that act as temporary carriers of atoms or electrons during enzymatic reactions. For example, **NAD⁺** (**nicotinamide adenine dinucleotide**) and **FAD** (**flavin adenine dinucleotide**) are derived from **vitamin B3 (niacin)** and **vitamin B2 (riboflavin)**, respectively. They serve as **electron carriers** in

redox reactions, accepting and donating electrons as needed. A classic example is **alcohol dehydrogenase**, an enzyme in the liver that helps break down **ethanol** (alcohol). This enzyme uses **NAD⁺** to accept electrons from ethanol, converting it into **acetaldehyde**, a toxic intermediate that is further processed into acetic acid and eventually eliminated. This process is crucial in detoxifying alcohol from the body. Another well-known coenzyme is **Coenzyme A (CoA)**, derived from **vitamin B5 (pantothenic acid)**. CoA is essential for transferring **acyl groups**, especially in fatty acid metabolism. For example, during the breakdown of fats for energy, fatty acids are converted to **fatty acyl-CoA** before entering the mitochondria. This step is essential for producing ATP from fats.

Understanding enzyme specificity, binding models, and the role of cofactors/coenzymes is not just textbook knowledge—it's directly applicable to real-world biology and medicine. For instance, many **enzyme inhibitors** are designed as **drugs** to block specific enzymes. **ACE inhibitors** (like lisinopril) block the **angiotensin-converting enzyme**, reducing blood pressure. Their effectiveness relies on the drug mimicking the natural substrate of the enzyme, fitting into the active site based on these binding principles. Similarly, **vitamin deficiencies** can impair enzyme function because they limit coenzyme availability. A deficiency in vitamin B3, for example, results in **pellagra**, which manifests with symptoms like diarrhea, dermatitis, and dementia because many redox reactions in energy metabolism require **NAD⁺**.

Enzymes often need **helpers** to function:

- **Cofactors:** Inorganic (e.g., Zn^{2+} , Fe^{2+} , Mg^{2+} , or heme groups)
- **Coenzymes:** Organic molecules, often vitamins or derivatives (e.g., NAD^+ , FAD, CoA)
 - **Example:** Alcohol dehydrogenase uses NAD^+ to remove electrons from ethanol during detoxification.

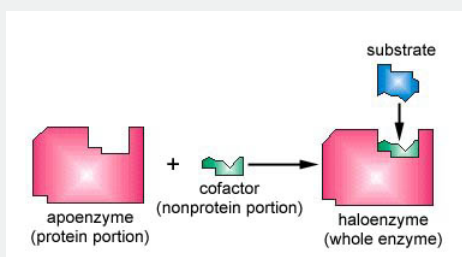
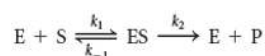


Figure 7.6: An apoenzyme and cofactor combine to form a holoenzyme. If the cofactor is an organic molecule, it is called a coenzyme. Image is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by Gary Kaiser via source content that was edited to the style and standards of the LibreTexts platform.

Enzyme Kinetics and Function

Enzyme kinetics is the study of how fast enzymatic reactions occur and what factors influence their speed. At the core of this understanding is a simplified reaction model involving an **enzyme (E)**, a **substrate (S)**, an **enzyme-substrate complex (ES)**, and the **product (P)**. The reaction begins with the enzyme binding the substrate to form the ES complex. This step is governed by the forward **rate constant k_1** . However, this binding is reversible, meaning the complex can also fall apart back into the enzyme and substrate, and this backward step is governed by the **rate constant k_{-1}** . Once the ES complex is formed, the reaction can proceed to convert the substrate into product, releasing the enzyme to act again. This final step is governed by another rate constant, k_2 , which reflects the speed at which the product is formed from the ES complex.

Basic enzyme kinetics reaction:



- **k_1** = rate constant for **formation** of ES from E + S
- **k_{-1}** = rate constant for **dissociation** of ES back into E + S
- **k_2** = rate constant for the **conversion** of ES into product (P)

- We often assume no product is present initially, so k_2 can be ignored in early analysis.

At the very **beginning of a reaction**, when almost no product has accumulated yet, we often simplify the kinetics by ignoring the reverse conversion of product back to ES. This allows us to focus on how effectively the enzyme binds the substrate and converts it into a product. One important concept that arises from this is the **dissociation constant**, denoted as K_d which is calculated as $K_d = k_{-1}/k_1 = [E][S]/[ES]$. This equation describes the equilibrium between free enzyme and substrate versus the bound complex. A low K_d value means the ES complex is very stable, indicating that the substrate binds tightly to the enzyme (**Figure 7.7**). Conversely, a high K_d value implies weak binding and a tendency for the complex to fall apart easily. This concept of K_d is critical when designing or evaluating drugs. For example, if a drug is designed to inhibit an enzyme involved in cancer cell growth, such as a kinase like BCR-ABL in chronic myeloid leukemia, the drug must bind very tightly to be effective. Imatinib (Gleevec) is a famous example of a drug that works because it has a very low K_d for BCR-ABL, allowing it to stay bound for a long time and block the enzyme's function. In contrast, a drug with a high K_d would quickly fall off the enzyme and require higher doses or might not be effective at all.

Dissociation Constant (K_d)

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

- **Low K_d** = Tight binding (substrate sticks well, strong affinity)
- **High K_d** = Weak binding (substrate doesn't bind well)
- **Example:** A drug with **low K_d** stays bound longer and may be more effective.

Building on this, enzyme kinetics also looks at how the **velocity** of the reaction changes depending on how much substrate is present. This is captured by the **Michaelis-Menten** equation: $V_0 = [V_{max}][S]/[K_m + S]$. This equation expresses the **initial velocity of the reaction (V_0)** as a function of substrate concentration ($[S]$). V_{max} is the maximum rate at which the enzyme can work, and K_m is the substrate concentration at which the reaction velocity is half of V_{max} . The value of K_m gives us a sense of **how much substrate is needed** to efficiently engage the enzyme (**Figure 7.8**). If K_m is low, it means the enzyme reaches half-maximal speed with a small amount of substrate, indicating high affinity. If K_m is high, more substrate is needed to get the enzyme working effectively, which points to low affinity. Consider enzymes involved in glucose metabolism. **Hexokinase**, which operates in most tissues, has a very low K_m for glucose, meaning it binds glucose tightly and works well even when glucose levels are low. This is important in the brain and muscles, where glucose is vital. **Glucokinase**, found in the liver, has a much higher K_m , meaning it only becomes active when glucose is abundant—after a meal, for example. This distinction allows the liver to store excess glucose without stealing it from more glucose-sensitive organs.

Michaelis-Menten Kinetics

$$V_0 = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

- V_{max} = Maximum velocity (when all enzymes are saturated)
- K_m = Substrate concentration at which reaction is at **half V_{max}**
 - **Low K_m** = Strong affinity (needs less substrate to reach half-max speed)
 - **High K_m** = Weak affinity

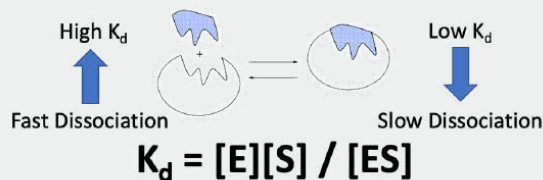


Figure 7.7: k_d is the dissociation constant at equilibrium.

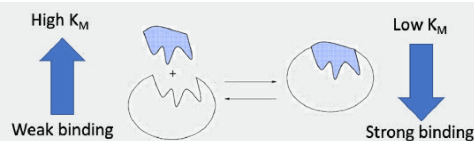


Figure 7.8: K_m defines the **affinity** of substrates.

The relationship between substrate concentration and reaction velocity creates a curved graph—a hyperbola—when you plot V_0 against $[S]$. But this shape can make it difficult to accurately calculate V_{max} and K_m (**Figure 7.9**). That's why biochemists use a transformed version of the equation, called the **Lineweaver-Burk plot**, which is simply a double reciprocal of the Michaelis-Menten equation (**Figure 7.10**). In this format, the graph plots $1/V_0$ on the Y-axis versus $1/[S]$ on the X-axis, resulting in a straight line. The slope of this line is K_m/V_{max} , the Y-intercept is $1/V_{max}$, and the X-intercept is $-1/K_m$. This linear transformation makes it easier to determine the kinetic constants from experimental data.

Lineweaver-Burk plot

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

This is the same as $y = mx + b$ (a straight line):

- **y-axis** = $1/V_0$
- **x-axis** = $1/[S]$
- **y-intercept** = $1/V_{max}$
- **x-intercept** = $-1/K_m$

In practical terms, these kinetics are used to evaluate how drugs interact with enzymes. If a new HIV protease inhibitor is being tested, researchers can use the Michaelis-Menten equation to determine how the drug affects K_m and V_{max} . A **competitive inhibitor** will raise the apparent K_m (because it competes with the substrate for binding), but won't change V_{max} . A **non-competitive inhibitor**, on the other hand, doesn't affect binding affinity (K_m remains the same), but it reduces V_{max} , because it prevents the enzyme from working even when the substrate is bound. Understanding enzyme kinetics in this way—through reaction rates, binding constants, and saturation behaviors—is fundamental not only to enzymology but also to pharmacology, biotechnology, and clinical biochemistry. Whether you're designing a drug, understanding a metabolic pathway, or evaluating enzyme deficiencies, these principles provide a detailed and powerful framework to interpret biological reactions.

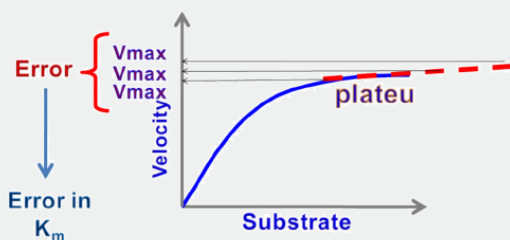


Figure 7.9: Michaelis-Menten graph.

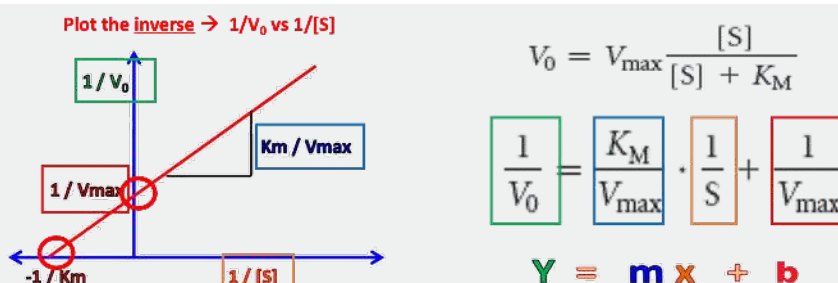


Figure 7.10: Lineweaver-Burk plot and its equation

Understanding the Application of Kinetics

Let's walk through each different examples and explain the enzyme kinetics in detail, so you can clearly understand the concepts like $V_{\max}$, K_m , and **rate constants (k_1)** with real-world context.

Example 1 - Understanding $V_{\max}$

- Hexokinase is an enzyme responsible for phosphorylating glucose to form glucose-6-phosphate—the very first step in glycolysis, which is the primary pathway cells use to extract energy from glucose. Different **isozymes** of hexokinase function in different tissues, and here, let's compare two: Hexokinase A with a **high $V_{\max}$** , and Hexokinase B with a **low $V_{\max}$** . $V_{\max}$ refers to the maximum rate of reaction when the enzyme is saturated with substrate. A **high $V_{\max}$** means the enzyme can process a large number of glucose molecules quickly when glucose is abundant. Therefore, **Hexokinase A is more active**, especially under high glucose conditions, because it can work at a faster rate. Conditions that affect $V_{\max}$ include the total concentration of enzyme present and whether the enzyme is saturated. $V_{\max}$ does **not** depend on how tightly the enzyme binds to the substrate (that's K_m) but rather how quickly it can catalyze the reaction once the substrate is bound. So if your body expresses more of Hexokinase A, or if you're in a tissue like muscle that demands fast glucose metabolism, you'll have a more "active" glucose metabolism.
- Imagine that glucose molecules are customers and hexokinase enzymes are cashiers. **Hexokinase A (high $V_{\max}$)** is like a **super-fast cashier**—they can scan and check out a lot of customers quickly. **Hexokinase B (low $V_{\max}$)** is a **slower cashier**—they still process everyone, but not as quickly. So, if there's a big rush of customers (high glucose), the fast cashier (Hexokinase A) is more efficient and clears the line quickly. This means **Hexokinase A is more active**, especially when there's lots of glucose. **Conditions affecting $V_{\max}$** would be like the number of cashiers you have. If you hire more (increase enzyme concentration), the total checkout speed ($V_{\max}$) increases

Example 2 - Understanding K_m

- Alcohol dehydrogenase (ADH)** is the enzyme that breaks down alcohol into acetaldehyde, a toxic compound that is then further broken down into harmless acetate. The K_m of an enzyme tells you how much substrate (in this case, alcohol) is needed for the enzyme to work at half its maximum speed. A **low K_m** means the enzyme has high affinity—it binds alcohol tightly and can start breaking it down even at low alcohol concentrations. Here, the **normal enzyme has a K_m of 0.746 mM**, while **Mutant A has a higher K_m (1.5 mM)** and **Mutant B has a lower K_m (0.2 mM)**. Since Mutant B binds alcohol most tightly, it is **more active at low alcohol concentrations**. Mutant A, with the highest K_m , is the **least efficient** and would require higher alcohol levels to function effectively. This directly ties into the **Asian Glow** phenomenon. Many East Asians have a variant of alcohol dehydrogenase (or the downstream enzyme ALDH) that either has altered K_m or $V_{\max}$, leading to inefficient metabolism of acetaldehyde. This causes it to build up, leading to facial flushing, nausea, and rapid heart rate after drinking even small amounts of alcohol.
- Think of alcohol as a Velcro ball and the enzyme as a Velcro paddle. A **low K_m** is like a paddle with **very sticky Velcro**—the ball (alcohol) sticks easily, even when there's not much of it. A **high K_m** is like a paddle with **worn-out Velcro**—you need to

throw more balls (higher alcohol concentration) before any of them stick. Thus, **Mutant B ($K_m = 0.2 \text{ mM}$)** is the stickiest (best binding), whereas **Mutant A ($K_m = 1.5 \text{ mM}$)** barely catches any balls—it's the weakest.

Example 3 - Understanding K_1

- **Acetaminophen (Tylenol)** is a common pain reliever that is generally safe at normal doses. It is metabolized by the liver, and a small portion is converted into a toxic metabolite called **NAPQI**. Normally, your body neutralizes this toxin quickly through a process involving glutathione. However, **alcohol consumption increases the k_1 rate constant** of liver enzymes, meaning the enzyme binds to acetaminophen **faster** and shunts more of it into the toxic pathway. This leads to higher levels of NAPQI. Alcohol also depletes the liver's glutathione reserves, further **reducing the body's ability to detoxify NAPQI**. As a result, if you take acetaminophen after drinking alcohol, you risk **severe liver damage** because the enzymes are working faster (higher k_1), more toxin is formed, and less is neutralized. That's why it's strongly recommended not to take acetaminophen after drinking or during a hangover.
- Imagine your liver is a factory with conveyor belts that handle acetaminophen. Normally, it processes the drug slowly and safely. Alcohol turns up the speed (**increases k_1**), making the conveyor belt run much faster. Now, more drug is being processed quickly, but some of it takes a **toxic side route**. With the belt moving faster, more drug is accidentally shunted to this dangerous path. Meanwhile, alcohol has also **used up your safety nets (glutathione)**. The result? **Toxic buildup** and potential liver damage. That's why **drinking alcohol before taking acetaminophen is dangerous**—it speeds up the wrong reaction pathway.

Sub Example 4 - Low K_1

- **Doxycycline** is an antibiotic that is also used in lower doses to treat acne due to its anti-inflammatory properties. It has a **high k_1** , meaning it is absorbed quickly and efficiently when taken on an empty stomach. However, this absorption is sensitive to interactions with **metal ions**, such as calcium, magnesium, and iron found in milk and multivitamins. When you consume milk or vitamins close to your doxycycline dose, the metal ions **bind to the drug and form a complex**—a process called **chelation**. This complex cannot be absorbed through the intestinal wall, effectively **lowering the k_1** and making the drug much less bioavailable. In practice, this means **doxycycline may not work as intended**, and bacteria or acne might not be treated effectively. That's why it's recommended to avoid milk and supplements for at least a couple of hours before and after taking doxycycline.
- Think of doxycycline as a **letter** trying to be mailed (absorbed into the body). The digestive system is like a **mailbox** that only accepts plain envelopes. If you drink milk or take vitamins, the **metal ions act like duct tape**, wrapping around the envelope. Now the mailbox (your body) **can't accept it**, and the letter just sits there. The chelation lowers the effective k_1 (rate at which doxycycline enters the bloodstream). So, the drug doesn't get absorbed properly and **won't work well**.

Example 5 - High K_1

- **Ibuprofen** is a non-steroidal anti-inflammatory drug (NSAID) commonly used for pain and inflammation. It has a **very high k_1** , meaning it is absorbed rapidly into the bloodstream when taken orally. However, this rapid absorption can come with a cost—it can irritate the stomach lining, especially when taken without food. When you take ibuprofen **on an empty stomach**, it reaches the stomach wall quickly and at high concentrations. Since ibuprofen also inhibits **COX-1**, an enzyme that protects the stomach lining by producing prostaglandins, this leads to reduced mucus protection and increased acid damage. As a result, the risk of **ulcers, bleeding, and irritation increases significantly**. Taking ibuprofen **with food** doesn't reduce the drug's effectiveness much but **slows down its absorption (lowers effective k_1)** and provides a buffer that protects the stomach lining. That's why doctors often recommend eating something before taking ibuprofen, especially for people who use it regularly.
- Think of it this way: Ibuprofen is like **hot sauce**—effective but intense. Your stomach lining is like **bare skin**. If you eat food first, it acts like a **shirt** that protects your skin. The hot sauce still gets absorbed (just a bit slower), but the stomach doesn't feel the burn. But if you take ibuprofen **on an empty stomach**, there's no protection. The hot sauce goes **straight on the skin**, causing **irritation, ulcers, or even bleeding**. That's why **food buffers and slows absorption (reduces the punch of k_1)** to protect you.

8: Inhibitors

Learning Objectives

- Describe how enzyme structure relates to function and drug binding.
- Differentiate between active sites and other binding pockets on enzymes.
- Explain the mechanisms of competitive, non-competitive, allosteric, and irreversible inhibition.
- Interpret kinetic changes ($V_{\max}$, K_m) associated with different types of enzyme inhibition.
- Analyze real-world examples of drug-enzyme interactions (e.g., aspirin, ibuprofen, ethanol).
- Understand how enzyme inhibition can be harnessed therapeutically.

Definition: Term

- **Active site:** The specific region on an enzyme where the substrate binds and the reaction occurs.
- **Binding pocket:** Any cavity or pocket in the enzyme where molecules (substrates, drugs, cofactors) may bind.
- **Competitive inhibitor:** A molecule that resembles the substrate and binds to the active site, competing with the substrate.
- **Non-competitive inhibitor:** A molecule that binds to an allosteric site on the enzyme and inactivates it regardless of substrate binding.
- **Allosteric site:** A non-active site location on an enzyme where molecules bind and alter enzyme activity.
- **Feedback inhibition:** A regulatory mechanism where the end product of a pathway inhibits an early step.
- **Irreversible inhibitor:** A molecule that binds covalently and permanently disables the enzyme.
- **K_m (Michaelis constant):** Substrate concentration required to reach half of $V_{\max}$. Reflects affinity.
- **$V_{\max}$:** The maximum rate of an enzymatic reaction when the enzyme is saturated with substrate.
- **K_i (Inhibition constant):** The concentration of inhibitor needed to bind 50% of enzyme; reflects inhibitor affinity.

Enzyme and Drug Targeting

The overall **three-dimensional (3D) structure** of enzymes determines their function. This structure often contains **natural cavities or pockets**, some of which serve as **active sites**—regions where substrates bind and reactions occur. Enzymes may also have **binding sites for co-factors or co-enzymes** (e.g., metal ions, vitamins like NAD^+ or FAD), and **protein interaction domains** where other proteins can dock to regulate activity. These binding pockets, particularly those not involved in catalytic activity, represent opportunities for **drug targeting**. These are referred to as **potential drug-binding sites**. If a small molecule can bind to one of these pockets, it can potentially alter or inhibit enzyme function—this is the core of rational drug design.

Competitive Inhibition: Outcompeted by Substrate

In **competitive inhibition**, an inhibitor molecule mimics the shape and chemical structure of the enzyme's natural substrate. Because of this similarity, the inhibitor can bind to the **active site** of the enzyme—but **only when the active site is free**, not when it is already occupied by the substrate (**Figure 8.1**). This means the inhibitor competes **directly** with the substrate for the same binding site on the enzyme (E). However, this inhibition is **reversible** and depends heavily on the relative concentrations of substrate and inhibitor. If the **substrate concentration increases**, it can **outcompete** the inhibitor by flooding the enzyme with more substrate molecules, thus **restoring enzyme activity** to near-normal levels. This concept is central to understanding how many drugs work: by temporarily blocking the active site, they reduce the enzyme's ability to catalyze its natural reaction, but without permanently disabling it.

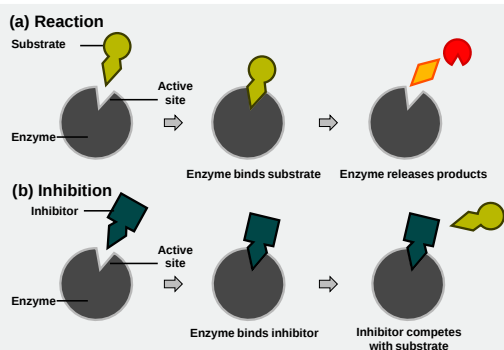


Figure 8.1: A competitive inhibitor is a molecule that binds to the active site of an enzyme without reacting, thus preventing the substrate from binding. Image shared under a CK-12 license and was authored, remixed, and/or curated by Tanesha Osborne via source content that was edited to the style and standards of the LibreTexts platform.

Imagine there's a couple **exclusive parking spots** in front of a popular coffee shop—this is the **active site** on the enzyme. Now imagine a **delivery truck** (the substrate) that needs to park there to **drop off important supplies** (the product). But there's a twist: there's also some **customers' car** (the competitive inhibitor)—and it **races to park in that same spot**. When the car is parked there, the **delivery truck can't park**, so the supplies don't get dropped off. This is exactly what a competitive inhibitor does—it **blocks access** to the active site by pretending to be the right substrate. Now, if **more and more delivery trucks show up**, eventually **one will catch the spot before the car does**. The higher the number of trucks (substrate), the **harder it is for the car to win the race**. This is why in competitive inhibition, if you **increase substrate concentration**, you can **outcompete the inhibitor and resume deliveries (enzyme activity)**. Also, the **maximum number of deliveries per hour ($V_{\max}$)** can still be reached—as long as you flood the area with enough trucks. But you'll need **more trucks than before** to maintain the same delivery speed, because the car keeps slowing things down. That's why **K_m increases**, reflecting the need for more substrate to get the job done.

One example of a fascinating and life-saving application of competitive inhibition is what occurs in **methanol poisoning**. Methanol is metabolized in the liver by **alcohol dehydrogenase (ADH)** into **formaldehyde and formic acid**, both of which are highly toxic to the optic nerve and can cause blindness or death. However, **ethanol**, the kind found in alcoholic beverages, is a **preferred substrate** for ADH. Ethanol has a **higher affinity** for the enzyme than methanol does. Therefore, administering ethanol to a person who has ingested methanol allows ethanol to **competitively inhibit** the metabolism of methanol. ADH becomes saturated with ethanol, preventing it from acting on methanol. As a result, unmetabolized methanol is slowly excreted by the kidneys without being converted into its toxic byproducts. This is a **therapeutic use of competitive inhibition** and highlights a critical medical principle: by understanding enzyme kinetics, we can **design interventions that manipulate biochemical pathways** to save lives. Modern treatments may also use **fomepizole**, a direct inhibitor of ADH, but the underlying principle remains the same—**block enzyme access to a dangerous substrate using a competitive agent**.

Another real-world and medically significant example of competitive inhibition involves the **Cyclooxygenase (COX)** enzyme. COX is responsible for converting arachidonic acid into **prostaglandins**, lipid compounds that mediate inflammation, fever, and pain. **Aspirin** and **ibuprofen**, two widely used pain-relieving drugs, target this enzyme—but in different ways. Aspirin acts as an **irreversible inhibitor**; it enters the active site and **covalently modifies** a serine residue, effectively **shutting down the enzyme** permanently (until new COX enzymes are synthesized). On the other hand, **ibuprofen** is a **reversible competitive inhibitor**. It structurally mimics arachidonic acid and temporarily binds to the same active site. Since this binding is non-covalent, ibuprofen competes with the substrate and is displaced when substrate concentrations increase. This makes ibuprofen a **dose-dependent** pain reliever—more effective at lower substrate levels.

The kinetic behavior of enzymes in the presence of a competitive inhibitor is distinct and predictable. **$V_{\max}$ (maximum velocity)** of the reaction **remains unchanged**. This is because, at **very high substrate concentrations**, the substrate is abundant enough to outcompete the inhibitor entirely, meaning the enzyme can still reach its full catalytic capacity. However, **K_m (Michaelis constant)**, which reflects the substrate concentration needed to reach half of $V_{\max}$, **increases**. This increase in **K_m** represents the fact that **more substrate is required** to achieve the same rate of reaction due to the competition from the inhibitor. The enzyme's

apparent affinity for the substrate decreases, not because the active site has changed, but because substrate molecules are getting blocked from binding.

These kinetic changes are reflected clearly in enzyme kinetics plots. In a **Michaelis-Menten plot**, which shows reaction rate (V) versus substrate concentration $[S]$, the curve representing the inhibited reaction **risers more slowly** compared to the uninhibited one (**Figure 8.2**). However, both curves eventually plateau at the **same $V_{\max}$** , since high substrate levels can displace the inhibitor. In contrast, the **Lineweaver-Burk plot**—a double-reciprocal graph of $1/V$ versus $1/[S]$ —displays the differences more starkly. In this plot, lines representing inhibited and uninhibited conditions **intersect at the same Y-intercept ($1/V_{\max}$)**, because $V_{\max}$ doesn't change. However, the **slope increases** and the X-intercept shifts closer to the origin, reflecting the **increased K_m** . The steeper the slope, the more the enzyme is inhibited at low substrate concentrations (**Figure 8.3**).

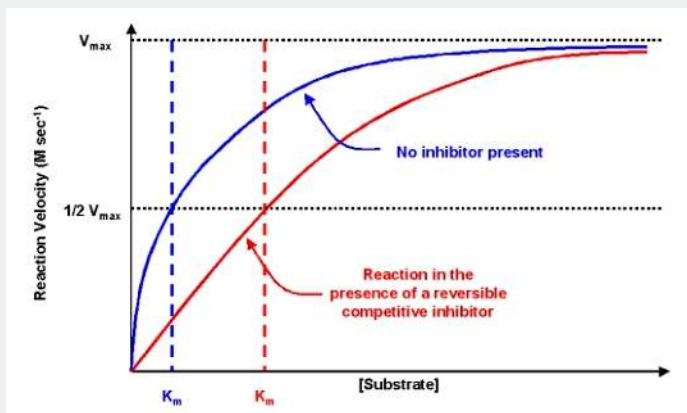


Figure 8.2: Effect of reversible competitive inhibitor shown in a Michaelis-Menten plot. Image is shared under a not declared license and was authored, remixed, and/or curated by Michael Blaber.

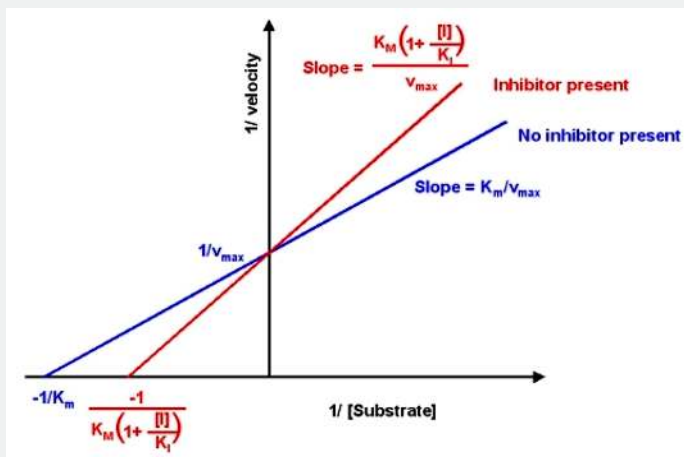


Figure 8.3: Lineweaver-Burk plot with reversible competitive inhibitor. Image is shared under a not declared license and was authored, remixed, and/or curated by Michael Blaber.

Let's now evaluate the **effectiveness of COX inhibitors** based on their inhibition constant, K_i (**This is the same as K_m but for inhibitors**), which represents the concentration of inhibitor needed to bind half the enzyme molecules. A **lower K_i** means the inhibitor has a **higher affinity** for the enzyme. For the COX enzyme, the **K_m for arachidonic acid (natural substrate)** is around **6 μM** . Aspirin has a **K_i of 100 nM (0.1 μM)**, while ibuprofen has a **K_i of 13 μM** . Aspirin's **K_i is much lower than the K_m** , indicating that it binds COX far more tightly than the natural substrate does. This makes it a **highly effective inhibitor**, especially since it also binds **irreversibly**, providing long-lasting inhibition. In contrast, ibuprofen's **K_i of 13 μM is more than twice the K_m**

of the substrate, suggesting that it is **less effective**, especially when substrate levels are high (e.g., during inflammation when arachidonic acid levels increase). Therefore, **ibuprofen may require higher doses to maintain effective inhibition**, and its efficacy is more limited by substrate competition.

Although aspirin is **pharmacologically potent**, its **irreversible mechanism** comes with **side effects**, most notably the **risk of gastrointestinal (GI) bleeding**. This is because COX enzymes also produce prostaglandins that protect the stomach lining. Blocking COX permanently with aspirin suppresses this protection, leading to **ulcers and bleeding**, especially with long-term use. Thus, while aspirin is a **powerful and effective COX inhibitor**, its **clinical use is limited** in certain populations (e.g., elderly, people with ulcers), and **alternatives like ibuprofen** (despite being less potent) may be preferred due to **better safety profiles**.

Kinetic Effects of competitive inhibitor:

- **$V_{\max}$ remains unchanged.**
- **K_m increases**, because more substrate is needed to reach half of $V_{\max}$ due to competition with the inhibitor.
- **Michaelis-Menten plot:** The curve rises more **slowly** in the presence of a competitive inhibitor, but still reaches the same $V_{\max}$.
- **Lineweaver-Burk plot:** The lines for inhibited and uninhibited reactions intersect at the **same Y-intercept ($1/V_{\max}$)** but have different slopes. The inhibited reaction shows a steeper slope (larger $K_m/V_{\max}$), indicating a higher K_m .

Non-Competitive Inhibition: Locking the Enzyme

In **non-competitive inhibition**, the inhibitor binds to an **allosteric site**—a location on the enzyme that is **not the active site** (Figure 8.4). Crucially, this binding can happen either when the enzyme is free (E) or when it has already formed the enzyme-substrate complex (ES). Once the inhibitor binds, it causes a **conformational change** in the enzyme that disrupts its catalytic ability. Even if the substrate is still present and has bound to the active site, the enzyme can no longer carry out its function. It's like having the right key in a lock (substrate bound to active site), but someone has poured glue into the mechanism (non-competitive inhibitor), so the lock can't turn. Importantly, increasing substrate concentration does **not reverse** this inhibition, because the inhibitor doesn't compete for the active site—it disables the enzyme through structural alteration. A portion of the total enzyme pool becomes **completely inactive**, no matter how much substrate is available.

Imagine an **automated vending machine** that sells snacks. Normally, you insert a coin (**substrate**), press the right button, and out comes a snack (**product**). Now, think of a **noncompetitive inhibitor** as someone coming by and **disconnecting the machine's internal wiring**—not the coin slot, but the control board inside. Even if you insert the right coin (even lots of coins), the machine is now **functionally broken**. It might still accept coins (substrate still binds), but nothing happens—**no snack is dispensed**. This is what happens in noncompetitive inhibition. The substrate may still bind to the enzyme's active site, but the enzyme has been "tampered with" from another location (the allosteric site), so the **reaction cannot proceed**, and the **enzyme is locked in an inactive state**. And just like in the vending machine analogy, **more coins won't fix it**—the issue isn't at the entry point, it's deeper in the machinery.

From a kinetic perspective, **$V_{\max}$ decreases** in non-competitive inhibition. This is because a fraction of the enzyme is effectively "locked" and removed from the pool of working catalysts. Since there are fewer functioning enzymes, the maximum rate at which the reaction can proceed is reduced. However, **K_m typically remains unchanged**, because the **affinity of the enzyme for its substrate is not affected**—the substrate can still bind just as well as before. But even when the ES complex forms, the reaction might not proceed if the inhibitor is also bound. In some complex or mixed inhibition scenarios, K_m may shift slightly, but in classic non-competitive inhibition, it remains the same.

In a **Michaelis-Menten plot**, the key signature of non-competitive inhibition is that the curve still rises with increasing substrate concentration but **plateaus at a lower $V_{\max}$** (Figure 8.5). This reflects the fact that part of the enzyme is permanently inactivated, limiting the maximum reaction rate. In the **Lineweaver-Burk plot**, which linearizes the Michaelis-Menten equation, the lines for inhibited and uninhibited reactions intersect on the **X-axis ($-1/K_m$)**, showing that K_m hasn't changed (Figure 8.6). However, the **Y-intercept ($1/V_{\max}$)** increases with inhibition, which reflects the lower maximum velocity. The slope of the line also increases, indicating reduced efficiency.

Kinetic Effects of competitive inhibitor:

- **$V_{\max}$ decreases** because a fraction of the enzyme is rendered completely inactive.
- **K_m typically stays the same**, but in some scenarios, it may be affected slightly.

- **Michaelis-Menten plot:** The curve plateaus at a lower $V_{\max}$.
- **Lineweaver-Burk plot:** The lines intersect on the X-axis ($-1/K_m$), showing same K_m but higher Y-intercept ($1/V_{\max}$). The inhibited reaction shows a steeper slope.

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Figure 8.4: Competitive vs Non-competitive inhibition. Image by CNX OpenStax, CC BY 4.0, via Wikimedia Commons

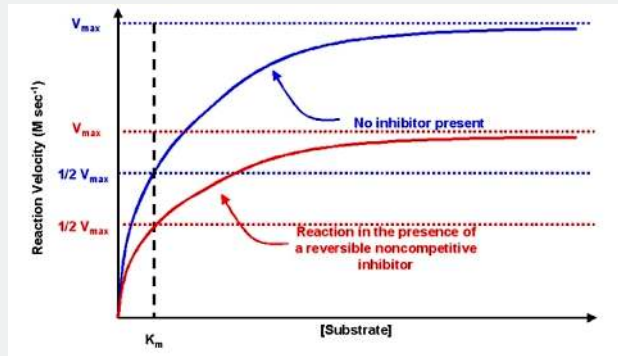


Figure 8.5: Effect of reversible noncompetitive inhibitor shown in a Michaelis-Menten plot. Image is shared under a not declared license and was authored, remixed, and/or curated by Michael Blaber.

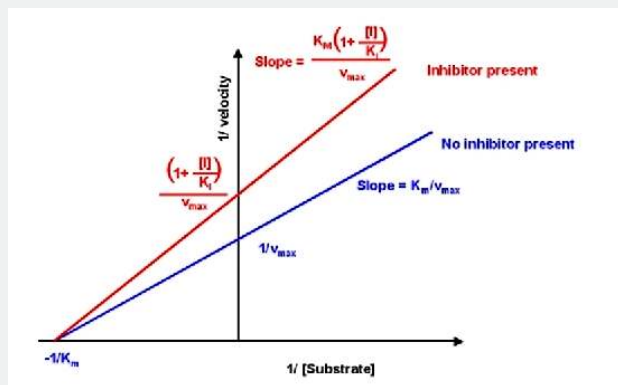


Figure 8.6: Lineweaver-Burk plot with noncompetitive inhibitor. Image is shared under a not declared license and was authored, remixed, and/or curated by Michael Blaber.

Feedback (Allosteric) Inhibition: Natural Braking System

Allosteric inhibition occurs when an inhibitor binds to an enzyme at a **regulatory site** (not the active site), causing a **shape change** that reduces the enzyme's ability to bind substrate or catalyze the reaction effectively. This is similar to non-competitive inhibition in location and mechanism, but allosteric inhibition is more often **reversible** and **regulated**, playing a central role in metabolic control and signal transduction. An easy way to picture it: imagine the enzyme is driving toward a product, and an allosteric inhibitor is a **backseat driver** yelling directions, causing the driver to hesitate, take a detour, or stop completely. Allosteric sites are often part of **multi-subunit enzymes**, allowing for fine-tuned regulation where binding of one molecule can influence the activity of multiple active sites—a key concept in **cooperative binding** (like in hemoglobin).

Allosteric inhibition is crucial in **feedback loops**, cell signaling, and maintaining balance within metabolic networks. It's like a **soft brake** that can slow or pause activity, but usually doesn't destroy the enzyme. **Feedback inhibition** is a beautifully efficient mechanism by which cells **self-regulate metabolic pathways**. Here, the **final product of a pathway** feeds back to **inhibit an earlier enzyme**—often the first committed step of the process. This prevents the cell from wasting resources by overproducing molecules it already has enough of. It's like a thermostat in a room: once the desired temperature (product level) is reached, the heater (enzyme activity) is turned off automatically. A well-known example is the **trp operon** in bacteria. When tryptophan levels

are sufficient, tryptophan acts as a **corepressor** by binding to a **repressor protein**, which in turn binds to the DNA and **blocks transcription** of the enzymes needed for its synthesis. This is a form of **allosteric inhibition** at the genetic level—feedback inhibition can occur both enzymatically and at the gene expression level. Feedback inhibition is typically **reversible and non-covalent**, allowing the system to **resume activity when product levels fall**, making it a **dynamic and adaptive** control mechanism.

Now picture a **factory production line** making toys. At the start of the line is a machine (the enzyme) that kicks off the production. Once the final product is made, there's a **smart manager** at the end of the line watching the output. If too many toys pile up, the manager sends a **signal back** to the first machine to slow down or temporarily stop—it's like switching a green light to a **red light**. That's **feedback inhibition**. The product itself acts as a **messenger**, binding to a regulatory (allosteric) site on the first machine and **telling it to stop working temporarily**. The machine isn't broken—it's just **instructed to pause** until production levels drop. Once there's demand again (products get used up), the manager removes the red light and the machine resumes. This analogy shows how **cells self-regulate** and avoid overproduction—a **reversible, adaptive mechanism**, like smart factory control.

Feedback inhibition

- A form of **end-product regulation** where the final product of a metabolic pathway inhibits an earlier enzyme in the pathway.
 - This maintains **homeostasis** and avoids wasteful overproduction
- This is a **reversible**.

8. Irreversible Inhibition: Permanent Shutdown

In **irreversible inhibition**, the inhibitor forms a **covalent bond** with the enzyme, often directly within the **active site**, permanently disabling its catalytic function. Unlike the other forms of inhibition, **this cannot be reversed**, and increasing the concentration of the substrate does **nothing** to restore activity. The enzyme is essentially **taken out of commission**, and the body must **synthesize new enzyme molecules** to replace it. A classic example is **aspirin**, which irreversibly inhibits **COX enzymes** by **acetylating a serine residue** in the active site. This permanently blocks the conversion of arachidonic acid to prostaglandins. While effective at reducing inflammation and pain, this permanent inhibition also means that **beneficial functions** of prostaglandins (like protecting the stomach lining) are also blocked, which can lead to **gastric side effects**.

Imagine you have a door with a **lock** (the enzyme's active site), and you regularly insert your **key** (substrate) to unlock it. But one day, someone comes along and **squirts superglue into the lock**. Now, it doesn't matter how many keys you try or how forcefully you turn them—the **lock is permanently damaged**, and the door won't open again until you **replace the entire lock**. This is how **irreversible inhibition** works. The inhibitor doesn't just temporarily get in the way—it **chemically modifies** the enzyme, often **covalently attaching** to a crucial part of the active site. The enzyme is **permanently deactivated**, and no amount of substrate will restore its function. The only fix is for the cell to **synthesize new enzyme molecules**. This is why drugs like **aspirin** are so powerful and long-lasting—they're essentially **enzyme assassins**, not just temporary blockers.

Irreversible inhibitors are often used in **drug design**, especially in antibiotics, cancer therapeutics, and nerve agents. Because of their **permanent effect**, they must be used with caution—they're like throwing away the key to a machine and welding the door shut.

Summary Table:

Inhibitor Type	Binds to	Effect on V_{max}	Effect on K_M	Reversible?	Example
Competitive	E only	No change	Increases	Yes	Ibuprofen
Non-Competitive	E or ES	Decreases	No change	Yes	Some enzyme toxins
Feedback (Allosteric)	Regulatory sites	Usually ↓	Often ↑ or ↓	Yes	Trp operon
Irreversible	Active site (covalent)	Decreases permanently	Usually unaffected	No	Aspirin

 Post-Lecture Objectives

- Illustrate the 3D structure-function relationship of enzymes in the context of drug binding.
- Compare kinetic graphs (Michaelis-Menten and Lineweaver-Burk) for different inhibition types.
- Explain why some drugs require high doses while others are potent at low concentrations.
- Evaluate a drug's effectiveness using K_m and K_i values.
- Critically assess the benefits and risks of using irreversible inhibitors like aspirin.

 Practice Activities

1. Label and interpret Michaelis-Menten and Lineweaver-Burk plots for each inhibition type.
2. Analyze COX inhibitors and predict side effects based on their mechanisms.
3. Propose a theoretical inhibitor for a key metabolic enzyme and justify its design based on binding site and type of inhibition.
4. Create a diagram of enzyme with labeled active and allosteric sites
5. What are the four Inhibition types with one real-world drug example each
6. Compare and contrast Michaelis-Menten and Lineweaver-Burk plot
7. Give one reflection on the ethical use of irreversible inhibitors in therapy

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9: Carbohydrates

Learning Objectives

- Identify the basic molecular formula and structural features of carbohydrates.
- Distinguish between monosaccharides, disaccharides, and polysaccharides.
- Recognize common examples of monosaccharides like glucose, fructose, and galactose.
- Describe the general function of carbohydrates in energy metabolism and structural roles.
- Understand the importance of glucose in the human body and how it is stored.
- Define the roles of structural carbohydrates such as cellulose, chitin, and peptidoglycan.
- Explain the chemical basis for carbohydrate classification and cyclization.
- Illustrate how glucose forms pyranose and identify the α - and β -anomers.
- Compare and contrast starch, glycogen, and cellulose in terms of structure and function.
- Describe why humans can digest α -linked polysaccharides but not β -linked ones.
- Explain how peptidoglycan supports bacterial cells and how antibiotics disrupt it.
- Apply the concept of carbohydrate stereochemistry (D- vs L- forms) in biological contexts.

Definition: Term

- **Carbohydrate:** Organic compound composed of C, H, and O (typically in the ratio $C_x(H_2O)_x$), functioning as energy sources or structural materials.
- **Monosaccharide:** A simple sugar with 3–7 carbon atoms; basic building block of carbohydrates.
- **Disaccharide:** Two monosaccharides linked together by a glycosidic bond.
- **Polysaccharide:** Long chain of monosaccharides used for energy storage or structural support.
- **Glucose:** A six-carbon aldose sugar; primary energy source in cells.
- **Starch:** Plant storage polysaccharide of glucose (amylose + amylopectin).
- **Glycogen:** Animal storage polysaccharide; more highly branched than starch.
- **Cellulose:** Structural polysaccharide of plants composed of β -1,4-linked glucose.
- **Chitin:** Structural polysaccharide of fungi/insects made from N-acetylglucosamine.
- **Peptidoglycan:** Bacterial cell wall polymer of NAG-NAM sugars cross-linked by peptides.
- **Hydrolysis:** Reaction that breaks glycosidic bonds by adding water.
- **Dehydration synthesis:** Reaction that forms glycosidic bonds by removing water.

Note

- Watch a short animation or video on the structure and classification of carbohydrates.
- Review diagrams of starch, cellulose, and glycogen for visual understanding.
- Skim a nutrition label and identify simple sugars vs. complex carbs.
- Prepare questions about why we can digest starch but not cellulose.

Introduction to Carbohydrates

Carbohydrates, as their name suggests (from “carbon” and “hydrate”), are organic molecules composed of carbon (C), hydrogen (H), and oxygen (O), typically in the ratio $C_x(H_2O)_x$. This general formula reflects the presence of one water molecule for every carbon atom, giving carbohydrates their distinctive name as “hydrates of carbon.” Chemically, they are classified as polyhydroxy **aldehydes** or **ketones**, or substances that yield these upon hydrolysis. Carbohydrates are among the most abundant and diverse biomolecules on Earth and are indispensable to life due to their broad range of functions. These include serving as a **primary source of metabolic energy**, acting as **structural building blocks** in cells and tissues, and participating in **cell recognition and signaling** processes. From the sweetness of cotton candy (pure sucrose) to the toughness of plant stems composed of cellulose, carbohydrates appear in nearly every aspect of life’s structure and function.

As the body's first and most accessible source of energy, **carbohydrates**—especially in the form of **glucose**—are fundamental to cell metabolism. **Glucose**, a six-carbon monosaccharide, is the key molecule that fuels nearly every cell. It is metabolized through the biochemical pathways of glycolysis, the citric acid cycle (Krebs cycle), and oxidative phosphorylation to generate ATP, the universal energy currency of the cell. In glycolysis, glucose is broken down into pyruvate, releasing a modest amount of ATP and NADH. The pyruvate is then further metabolized in the mitochondria, where high-energy electron carriers drive the production of a much larger ATP yield via the electron transport chain. This makes glucose an efficient and readily available energy source, especially for high-demand tissues like the brain and muscles.

Plants store excess glucose in the form of **starch**, a polysaccharide composed of α -1,4-linked glucose chains. **Starch** consists of two components: **amylose**, which is unbranched and helical, and **amylopectin**, which is highly branched and more readily digested. This energy reserve is stored in roots, seeds, and tubers—common in foods like potatoes, rice, and corn. When humans consume starchy foods, enzymes like salivary and pancreatic **amylase** break the starch down into glucose molecules, which are then absorbed into the bloodstream. In animals, including humans, glucose is stored as **glycogen**, a similar but more highly branched polysaccharide. **Glycogen** is primarily found in the liver, where it helps regulate blood glucose levels, and in muscle tissues, where it serves as an on-demand energy supply during physical activity. Because of its extensive branching (every 8–10 glucose units), glycogen can be rapidly broken down to release glucose when energy is urgently needed, such as during exercise or fasting.

Carbohydrates are the body's first line of energy.

- **Starch** is a plant-based storage polysaccharide made of glucose. It is stored in roots, seeds, and tubers—think of potatoes or rice.
- **Glycogen**, the animal equivalent of starch, is stored in liver and muscle tissues. During fasting or exercise, it is broken down to release glucose.

Beyond their role in energy metabolism, carbohydrates also serve critical **structural roles** in cells and tissues. One of the most important structural carbohydrates is **cellulose**, a linear polysaccharide composed of β -1,4-linked glucose units. Unlike starch and glycogen, **cellulose** molecules align side by side and form strong fibers due to hydrogen bonding, providing rigidity and strength to plant cell walls. Humans, however, lack the enzyme **cellulase** needed to hydrolyze the β -1,4 linkages in cellulose. As a result, cellulose cannot be digested and functions as dietary fiber, promoting digestive health by stimulating bowel movement and supporting gut microbiota. However, many herbivores, such as cows and termites, can digest cellulose thanks to symbiotic microorganisms in their digestive systems that produce cellulase.

A structurally similar but chemically distinct molecule is **chitin**, found in the exoskeletons of insects, crustaceans, and the cell walls of fungi. Like cellulose, chitin is composed of β -1,4-linked units, but instead of glucose, it consists of **N-acetylglucosamine**—a glucose derivative with an acetylated amino group. This gives chitin added strength and resilience, making it ideal for forming protective shells and rigid structural frameworks. Though also indigestible to humans, some organisms like fungi and certain marine bacteria can enzymatically degrade chitin and use it as a carbon source.

Another essential structural carbohydrate is **peptidoglycan**, unique to bacterial cell walls. It is a mesh-like polymer composed of alternating sugar units—**N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)**—linked by β -1,4-glycosidic bonds. These sugar chains are further cross-linked by short peptides, creating a strong, resilient structure that maintains bacterial shape and protects against osmotic stress. Importantly, the integrity of peptidoglycan is a major target for antibiotics. Enzymes like **lysozyme**, found in tears and saliva, can break β -1,4 bonds between NAG and NAM, compromising bacterial cell walls. Similarly, antibiotics like **penicillin** and **vancomycin** disrupt the cross-linking of peptidoglycan, leading to bacterial cell death. Thus, structural carbohydrates not only support cellular frameworks but also play roles in host-pathogen interactions and the development of antimicrobial therapies.

Certain carbohydrates are critical for maintaining the shape and strength of cells.

- **Cellulose**, found in the cell walls of plants and algae, gives structural integrity.
- **Chitin**, similar to cellulose, forms the exoskeleton of insects and the cell walls of fungi. It contains **N-acetylglucosamine** units instead of glucose.
- **Peptidoglycan**, exclusive to bacteria, provides rigidity to bacterial cell walls. It is made of alternating sugars—**N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)**.

In summary, carbohydrates are incredibly versatile biomolecules, playing indispensable roles as energy sources, structural materials, and signaling agents. Their diverse chemical structures—from simple sugars to complex polymers—allow them to participate in nearly every aspect of biology. Whether we are talking about the sugar in fruit, the starch in pasta, the cellulose in plant stems, or the glycogen in our liver, carbohydrates underpin many processes essential for life.

Monosaccharides vs Polysaccharides

Monosaccharides are the most basic units of carbohydrates, often referred to as **simple sugars**. These molecules consist of a single sugar unit and cannot be broken down further by **hydrolysis** into smaller carbohydrate units. They typically contain **three to seven carbon atoms**, and their general chemical formula is $(CH_2O)_n$, where n can range from 3 to 7. Monosaccharides are foundational building blocks for more complex carbohydrates like **disaccharides** and **polysaccharides**. They are highly soluble in water and have sweet tastes, which is why many simple sugars are commonly found in fruits and sweets.

Among the most important monosaccharides is **glucose**, a six-carbon sugar (hexose) and the primary source of energy for most cells. It is an **aldose**, meaning it contains an **aldehyde group** at carbon 1. Glucose plays a central role in metabolism and is the sugar measured in blood sugar tests. Another key aldose is **galactose**, which is structurally similar to glucose but differs in the orientation of one hydroxyl group. Galactose is commonly found as part of **lactose**, the disaccharide in milk, where it is linked to glucose. On the other hand, **fructose** is a **ketose**, containing a **ketone group** at carbon 2. It is found naturally in fruits and honey and is notably **sweeter than glucose**, which is why it is often used as a sweetener in processed foods and beverages. Despite these sugars having the same molecular formula ($C_6H_{12}O_6$), their structures and functions are distinct, a phenomenon known as **isomerism**.

Common examples of single sugar (monosaccharides):

- **Aldose**
 - **Glucose** – the primary cellular fuel.
 - **Galactose** – part of lactose (milk sugar).
- **Ketose**
 - **Fructose** – found in fruits; sweeter than glucose.

Polysaccharides, in contrast, are complex carbohydrates composed of **long chains of monosaccharide units** linked by **glycosidic bonds**. These macromolecules serve either as **energy storage** materials or **structural components** in cells, depending on their structure and the types of linkages between the sugars. In storage polysaccharides, the glucose units are typically connected by **α -glycosidic linkages**, which allow the chains to coil and be more easily broken down when energy is needed.

In **plants**, the primary storage polysaccharide is **starch**, which is made up of two components: **amylose**, a linear polymer of glucose units linked by **α -1,4-glycosidic bonds**, and **amylopectin**, which contains both α -1,4 linkages and **branch points** formed by **α -1,6-glycosidic bonds** every 20 to 25 glucose residues. Starch is stored in plastids and provides a reserve of energy that can be mobilized during seed germination or when photosynthesis is not possible. Common dietary sources of starch include potatoes, corn, and grains. In **animals**, the equivalent storage molecule is **glycogen**, which is structurally similar to amylopectin but **more extensively branched**, with α -1,6 branches occurring approximately every 8 to 12 glucose units. This high degree of branching allows glycogen to be rapidly broken down into glucose when energy is needed quickly, such as during physical exertion or fasting. Glycogen is stored mainly in the liver and skeletal muscles.

Polysaccharides also have **structural roles** in organisms. One prominent example is **cellulose**, a polymer of glucose linked by **β -1,4-glycosidic bonds**. These bonds result in straight, rigid chains that align parallel to each other, forming **fibers held together by hydrogen bonds**. This structural arrangement gives strength and rigidity to the **cell walls of plants and algae**. Unlike starch and glycogen, cellulose cannot be digested by humans because we lack the enzyme **cellulase** necessary to break β -1,4 linkages. As a result, cellulose serves as **dietary fiber**, aiding digestion by promoting bowel regularity.

Common examples of polysaccharides:

- **Storage polysaccharides:**
 - **Starch** (plants): composed of **amylose** (unbranched α -1,4-glucose) and **amylopectin** (branched α -1,4 and α -1,6-glucose).
 - **Glycogen** (animals): similar to amylopectin but more highly branched (~every 8–12 residues).

- **Structural polysaccharides:**
 - **Cellulose:** unbranched β -1,4-glucose; forms fibers.
 - **Peptidoglycan:** alternating NAG and NAM with β -1,4-linkages and peptide cross-links.

Another structural polysaccharide is **peptidoglycan**, which is found in the **cell walls of bacteria**. It consists of alternating units of **N-acetylglucosamine (NAG)** and **N-acetylmuramic acid (NAM)** joined by β -1,4-glycosidic bonds. These sugar chains are further **cross-linked by short peptide chains**, creating a strong and protective mesh that helps bacteria maintain their shape and resist **osmotic pressure**. Without this rigid support, bacterial cells would burst due to the influx of water from their surrounding environment, especially in hypotonic conditions. Peptidoglycan is also a target for many antibiotics: for example, **penicillin** inhibits the enzymes that cross-link the peptides, weakening the bacterial cell wall and leading to cell lysis.

To defend against bacterial invasion, the human body produces natural antimicrobial agents, one of which is **lysozyme**. Lysozyme is an enzyme found in **tears, saliva, mucus, and other secretions**, where it serves as a **first line of immune defense**. This enzyme specifically targets the **β -1,4-linkages** between NAG and NAM in the peptidoglycan structure. By cleaving these bonds, lysozyme disrupts the integrity of the bacterial cell wall, making the bacteria vulnerable to lysis. This is particularly effective against **Gram-positive bacteria**, which have thick, exposed layers of peptidoglycan. In contrast, **Gram-negative bacteria** possess an additional outer membrane that partially shields their thinner peptidoglycan layer, rendering them more resistant to lysozyme.

Beyond natural defenses, scientists have developed powerful **antibiotics** that exploit the unique structure of peptidoglycan. One well-known example is **penicillin**, a member of the **β -lactam antibiotic** family. Penicillin works by inhibiting the bacterial enzyme **transpeptidase** (also known as **penicillin-binding protein, PBP**), which is responsible for forming the peptide cross-links between NAM residues. By blocking this enzyme, penicillin prevents the final step in cell wall synthesis, leaving the bacterial wall **weak and incomplete**. As the bacterium grows and tries to divide, the compromised wall cannot hold the pressure, and the cell eventually **ruptures** and dies. Another potent antibiotic is **vancomycin**, often used as a last-resort treatment for **multi-drug-resistant Gram-positive infections**, such as MRSA (methicillin-resistant *Staphylococcus aureus*). Unlike penicillin, vancomycin does not inhibit an enzyme. Instead, it **physically binds to the peptide side chains** on the NAM residues, thereby **blocking the access of transpeptidase** to its substrate. This prevents the enzyme from cross-linking the peptidoglycan chains. The result is the same—an unstable cell wall that cannot support the bacterial cell under internal pressure.

Method to inhibit Peptidoglycan

- **Lysozyme:** an enzyme in human tears and saliva
- **Antibiotics** that target peptidoglycan synthesis
 - **Penicillin** blocks the **transpeptidase** enzyme needed for cross-linking.
 - **Vancomycin** binds to the peptide chains, preventing proper wall formation.

The assembly and breakdown of carbohydrates involve two important types of chemical reactions: **dehydration (condensation)** and **hydrolysis**. A **dehydration reaction** is how monosaccharides are linked together to form disaccharides and polysaccharides. In this process, two sugar molecules are joined, and a molecule of **water is removed**. For instance, two glucose molecules can undergo dehydration to form **maltose** and release a water molecule (glucose + glucose $\rightarrow$ maltose + H₂O). This is how larger carbohydrate structures are synthesized in cells.

Conversely, a **hydrolysis reaction** is the process by which complex carbohydrates are **broken down** into their simpler sugar units. In hydrolysis, a water molecule is **added back** to cleave the glycosidic bond between sugar units. This is the mechanism by which starch and glycogen are digested in our bodies. Enzymes like **amylase**, produced in saliva and the pancreas, hydrolyze the glycosidic bonds in starch to release glucose, which can then be absorbed into the bloodstream.

An easy way to visualize these two processes is to think of **dehydration** as building a **candy necklace**—each candy (monosaccharide) is added to the string with a bond, and water is removed to form that link. **Hydrolysis**, on the other hand, is like **cutting that necklace apart**—each time you snip a link, you need to add a drop of water to separate the beads (sugar units). Together, these two reactions regulate the formation and breakdown of carbohydrates, ensuring a steady supply of energy and structural materials when and where they are needed.

- **Dehydration (Condensation) Reaction:** Two monosaccharides are joined together, and a **water molecule is removed** in the process.
 - $\text{glucose} + \text{glucose} \rightarrow \text{maltose} + \text{H}_2\text{O}$.
- **Hydrolysis Reaction:** **Water is added** to split glycosidic bonds.
 - Starch is hydrolyzed to glucose in our digestive tract.

Cyclization and Stereochemistry of Sugars

Monosaccharides, especially those with **five or more carbon atoms**, rarely exist in their open-chain (linear) form when in aqueous (water-based) environments. Instead, they undergo an internal chemical reaction to form **cyclic (ring) structures**, which are more stable in solution. For **glucose**, which is a six-carbon sugar (an aldohexose), this ring formation occurs when the **aldehyde group at carbon 1 (C1)** reacts with the **hydroxyl group (-OH) on carbon 5 (C5)**. This intramolecular reaction forms a **hemiacetal**, resulting in either a **six-membered ring called a pyranose** (resembling the structure of pyran) or, less commonly, a **five-membered ring called a furanose** (like furan). In glucose, the six-membered **pyranose** form is by far the most common in nature. Once glucose forms a ring, something important happens: a new **chiral center** is created at carbon 1—the carbon that used to be the aldehyde. This carbon is now called the **anomeric carbon**. Because of its new tetrahedral configuration, it can orient the hydroxyl group (-OH) in two different ways in space, giving rise to two stereoisomers called **anomers**. These small differences may seem trivial, but they have a **huge impact** on how sugars behave biologically and chemically.

- In the **α -anomer**, the hydroxyl group attached to the anomeric carbon points **down** (on the opposite side of the ring relative to the CH_2OH group on C5).
- In the **β -anomer**, the hydroxyl group at the anomeric carbon points **up** (on the **same side** as the CH_2OH group on C5).

The difference between **α - and β -linkages** determines whether humans can digest certain polysaccharides. For instance, **starch** (in plants) and **glycogen** (in animals) are storage polysaccharides made of glucose units connected by **α -glycosidic bonds**, mostly **α -1,4 and α -1,6 linkages**. These α -linkages cause the glucose chain to coil into a helical, flexible structure that **can be easily broken down** by human enzymes such as **amylase**. This is why we can eat bread, rice, or potatoes and derive energy from them. In contrast, **cellulose** (found in plant cell walls) is composed of glucose units linked by **β -1,4-glycosidic bonds**. These β -linkages produce a straight, rigid structure that allows individual chains to align side-by-side and form strong hydrogen bonds, resulting in **fibers**. However, humans lack the enzyme **cellulase**, which is required to break these β -bonds. So, although cellulose is made of the same glucose units, it is **indigestible for us** and passes through our gut as **dietary fiber**.

Stereochemistry refers to the spatial arrangement of atoms in molecules that have the same molecular formula. Sugars like glucose have **multiple chiral centers**—carbons attached to four different groups—which means they can exist in many stereoisomeric forms. A specific type of stereoisomerism is seen in **enantiomers**, which are mirror images of each other (like your left and right hands). For monosaccharides, we categorize them as either **D-sugars** or **L-sugars** based on the orientation of the hydroxyl group on the chiral carbon **furthest from the carbonyl group** in the linear form (usually the second-to-last carbon). In nature, virtually **all sugars used by organisms are D-isomers**. For example, **D-glucose** is the form our enzymes recognize and metabolize. On the other hand, **L-glucose** is the mirror image of D-glucose and, despite looking similar, **cannot be used by our enzymes**, and therefore, it passes through the body unmetabolized. This is an excellent demonstration of how subtle differences in three-dimensional structure can determine **whether a molecule is biologically active** or completely inert.

- **Starch and glycogen** use **α -linkages** (flexible and digestible).
- **Cellulose** uses **β -linkages** (rigid and indigestible to humans).
- **D-sugars** are the ones actively used.
- **L-sugars** are typically not metabolized.

Comparison of Starch, Glycogen, and Cellulose

Feature	Starch	Glycogen	Cellulose
Source	Plants	Animals	Plants and algae
Linkages	α -1,4 and α -1,6 (amylopectin)	α -1,4 and α -1,6 (more frequent)	β -1,4
Structure	Helical (amylose) and branched	Highly branched	Linear, fibrous
Digestibility	Digestible by humans	Digestible	Indigestible (fiber)
Function	Energy storage	Energy storage	Structural support

Overall, understanding the structure of carbohydrates is fundamental to biochemistry because carbohydrates play essential roles not only as energy sources but also in modulating protein function, enzyme specificity, and cell signaling. Many proteins, especially those on the cell surface or secreted, are glycoproteins—proteins covalently bonded to carbohydrate chains—which influence protein folding, stability, solubility, and recognition by other molecules. Enzymes such as glycosidases and glycosyltransferases are highly specific for the stereochemistry and linkage types of carbohydrates (e.g., α -1,4 vs. β -1,4), meaning even small structural differences can dramatically affect catalytic activity or substrate binding. Carbohydrate structures also determine how immune cells distinguish self from non-self, how pathogens attach to host tissues, and how hormones like insulin regulate glucose uptake. In biotechnology and drug development, understanding carbohydrate structure is key for designing enzyme inhibitors, vaccines, and glycoengineered therapeutics. Therefore, mastering carbohydrate chemistry is not only about memorizing sugars—it's about unlocking how biological systems recognize, regulate, and respond to one another at the molecular level.

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10: Metabolism and Glycolysis

Learning Objectives

- Define metabolism and distinguish between catabolic and anabolic pathways.
- Describe the key steps and regulatory enzymes involved in glycolysis.
- Explain the roles of ATP, NAD⁺, and NADH in energy transfer and redox reactions.
- Identify how hormonal signals like glucagon regulate glycolysis during fasting.
- Compare and contrast hexokinase and glucokinase in terms of kinetics, tissue distribution, and physiological roles.

Definition: Term

- **Metabolism:** The sum of all chemical reactions occurring in a living organism to maintain life.
- **Catabolism:** Metabolic processes that break down molecules to release energy (e.g., glycolysis, beta-oxidation).
- **Anabolism:** Metabolic processes that build complex molecules using energy (e.g., protein synthesis, DNA replication).
- **ATP (Adenosine Triphosphate):** The primary energy carrier in cells, used to power nearly all cellular processes.
- **Glycolysis:** A ten-step metabolic pathway that breaks down glucose into pyruvate, yielding ATP and NADH.
- **Allosteric Enzyme:** An enzyme whose activity is regulated by binding of effectors at sites other than the active site.
- **Hexokinase:** A high-affinity enzyme that phosphorylates glucose in most tissues; inhibited by G6P.
- **Glucokinase:** A low-affinity glucose-phosphorylating enzyme found in the liver; not inhibited by G6P.
- **NAD⁺ / NADH:** A redox coenzyme pair; NAD⁺ accepts electrons (oxidized form), NADH donates electrons (reduced form).
- **Glucagon:** A hormone released during fasting that promotes glucose production and inhibits glycolysis in the liver.

Metabolism

Metabolism encompasses **all the chemical reactions** taking place in a living organism to sustain life. These reactions are not random but are highly coordinated and regulated by enzymes to ensure efficiency and responsiveness to the cell's needs. Every biological activity—whether it's muscle contraction, nerve signaling, cell division, or immune defense—relies on metabolism. Importantly, **metabolism** is not a single reaction but a complex network of thousands of interlinked pathways that work simultaneously. These pathways are broadly categorized into **catabolic** and **anabolic** processes. While **catabolism** is primarily concerned with **releasing energy**, **anabolism** focuses on **using energy** to build cellular structures and macromolecules. The balance between these two processes ensures the organism can both generate energy and use it for growth, repair, and adaptation.

Catabolism is the **energy-generating side** of metabolism. In catabolic reactions, large and complex biological molecules such as **carbohydrates, lipids, and proteins** are **broken down** into simpler molecules—like glucose, fatty acids, amino acids, carbon dioxide, and water. This breakdown process releases energy stored in chemical bonds, and much of that energy is captured in the form of **ATP**, the universal energy currency of the cell. One of the most important catabolic processes is **aerobic respiration**, which involves the complete oxidation of glucose in the presence of oxygen: $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O}$. This reaction is **highly exergonic**, meaning it releases a substantial amount of free energy—approximately **-686 kcal/mol** under standard conditions (ΔG°). This energy is not lost as heat but is instead used to synthesize ATP, which is then available for various energy-requiring cellular activities. For example, muscle contraction during exercise, ion pumping across membranes, or driving endergonic reactions in biosynthesis all depend on ATP produced through catabolic pathways like this.

In contrast to catabolism, **anabolism** represents the **constructive side of metabolism**. It involves the synthesis of complex macromolecules from simpler precursors. These reactions are typically **endergonic**—they require an input of energy to proceed. The energy for anabolism is primarily supplied by **ATP** and reducing equivalents like **NADPH**. Anabolic pathways are essential for processes such as **cell growth, tissue repair, and replication**, where new molecules must be built. Common examples include the synthesis of **proteins from amino acids**, **nucleic acids from nucleotides**, and **polysaccharides like glycogen from glucose**. For instance, when a cell divides, it must duplicate its entire genome (DNA replication) and synthesize many new proteins and lipids to form the daughter cells. These biosynthetic demands are powered by the energy captured during catabolic processes. Thus,

catabolism and anabolism are **interdependent**, and cells must carefully regulate the flow of resources and energy between these two sides of metabolism to remain healthy and functional.

- **Metabolism:** sum total of all chemical reactions
- **Catabolism:** involves the **breakdown of complex macromolecules**, which is stored in molecules like **ATP** (adenosine triphosphate).
- **Anabolism:** is the **synthesis of complex macromolecules**. This process **requires input of energy**.

Glycolysis: Glucose Catabolism

Glycolysis is a fundamental and ancient metabolic pathway that serves as the **starting point for glucose catabolism** in almost all living organisms. It takes place in the **cytoplasm** of the cell and does **not require oxygen**, making it especially important for cells in anaerobic environments or for tissues like muscle during intense activity when oxygen is scarce. The main objective of glycolysis is to break down **one molecule of glucose** (a six-carbon sugar) into **two molecules of pyruvate** (each containing three carbon atoms). In doing so, the pathway captures a small but critical amount of usable energy. Specifically, glycolysis yields a **net gain of two ATP molecules**, which serve as the immediate energy currency of the cell, and **two NADH molecules**, which are high-energy electron carriers that can later be used in oxidative phosphorylation (if oxygen is available) to generate additional ATP.

Although glycolysis consists of **ten enzyme-catalyzed reactions**, not all steps are equally important in terms of regulation. Out of the ten steps, **three are considered irreversible and highly regulated**. These three steps function as **metabolic checkpoints** and are catalyzed by **allosteric enzymes**, which are uniquely sensitive to the cell's energy status and the concentration of certain metabolites. The enzymes involved in these steps—**hexokinase**, **phosphofructokinase-1 (PFK-1)**, and **pyruvate kinase**—serve as control points where the rate of glycolysis can be turned up or down depending on the cell's needs. Because these steps have large negative ΔG values (free energy changes), they are essentially unidirectional under physiological conditions, meaning the reactions cannot easily reverse, making them ideal for regulation. This irreversible nature also commits the glucose molecule to continued breakdown once it enters the glycolytic pathway, thus playing a critical role in metabolic control. Overall, glycolysis not only provides quick energy but also serves as a feeder pathway for other essential metabolic routes, such as the Krebs cycle, anaerobic fermentation, and various biosynthetic processes.

Glycolysis is the central pathway for glucose metabolism

- **Net Total Products:**
 - 2 ATP (energy currency)
 - 2 NADH (electron carriers)
 - 2 Pyruvate (end products for further metabolism)

Three Allosteric Enzymes of Glycolysis

Allosteric enzymes play a **critical regulatory role** in metabolism, acting as molecular "switches" that help the cell respond rapidly to changing energy demands. What makes them unique is their ability to be regulated by **allosteric effectors**—small molecules that bind to specific regulatory sites on the enzyme, distinct from the active site. These effectors can either enhance (activators) or reduce (inhibitors) the enzyme's activity. This regulation allows cells to **fine-tune metabolic pathways** like glycolysis according to real-time energy needs. A helpful analogy is to think of the effector molecule as a **car key**—without the key, the enzyme (car) won't run. This regulatory mechanism ensures that enzymes aren't constantly active, which would lead to **wasteful energy use** and potentially harmful overaccumulation of intermediates. Instead, allosteric enzymes ensure that glycolysis is only active when needed, such as during exercise, fasting, or rapid cell growth.

Hexokinase catalyzes the **very first step of glycolysis**, phosphorylating glucose to form **glucose-6-phosphate (G6P)**. This reaction is crucial because it **traps glucose inside the cell**—phosphorylated sugars cannot easily cross the plasma membrane. It also marks glucose for metabolic use, committing it to glycolysis or other pathways like glycogenesis or the pentose phosphate pathway. The reaction is energetically favorable and is coupled with ATP hydrolysis:



Hexokinase is subject to **feedback inhibition** by its own product, G6P. When G6P levels build up—such as when glycolysis slows down—hexokinase activity is inhibited. This prevents the cell from taking in and phosphorylating more glucose than it can

handle, thereby **conserving energy** and avoiding toxic metabolite accumulation. In muscle cells, hexokinase has a **very low K_m** (~0.1 mM), meaning it has a **high affinity for glucose** and is almost always active under normal blood glucose levels (4–5 mM). This ensures that muscle tissues can **efficiently utilize glucose** for immediate ATP production during activity.

- **Function:** Catalyzes the phosphorylation of glucose to glucose-6-phosphate (G6P), **trapping glucose** and committing it to metabolism.
- **Allosteric Inhibition:** Inhibited by high levels of G6P (feedback inhibition).
 - **Relevance:** Prevents excessive accumulation of G6P when glycolysis slows down.

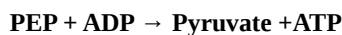
Phosphofructokinase-1 (PFK-1) controls the **rate-limiting step** in glycolysis, making it one of the most important regulatory points in metabolism. PFK-1 catalyzes the phosphorylation of **fructose-6-phosphate to fructose-1,6-bisphosphate**, a step that **commits the glucose molecule irreversibly to glycolysis**:



PFK-1 is exquisitely sensitive to the energy status of the cell. It is **inhibited by high levels of ATP**, which signal that the cell already has sufficient energy and does not need to break down more glucose. Additionally, **low pH**—caused by **lactic acid buildup** during anaerobic glycolysis—also inhibits PFK-1, protecting the cell from excessive acidification. On the other hand, **AMP** acts as a powerful **activator** of PFK-1. When ATP levels are low, AMP levels rise and signal an **energy-deficient state**, prompting PFK-1 to ramp up glycolysis. This feedback system ensures energy production is **matched to cellular demand**. For instance, during **intense exercise**, ATP is rapidly consumed, and AMP accumulates. This activates PFK-1, accelerating glycolysis to meet the muscle's **urgent energy needs**.

- **Function:** Catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate.
- **Inhibition:** High ATP (signals high energy) or low pH (indicating lactic acid accumulation).
- **Activation:** AMP (signals energy deficiency).

Pyruvate kinase catalyzes the final, ATP-generating step of glycolysis, converting **phosphoenolpyruvate (PEP)** into **pyruvate**:



This reaction is **highly exergonic** and essentially irreversible under physiological conditions. Pyruvate kinase plays a pivotal role in ensuring the **completion of glycolysis** and contributes to the net gain of ATP. Like the other key glycolytic enzymes, **Pyruvate kinase** is tightly regulated. It is **inhibited by high ATP**, indicating that the cell already has enough energy. Other inhibitors include **acetyl-CoA and alanine**, which signal that the cell has sufficient building blocks or energy for biosynthesis. **Pyruvate kinase** is also subject to **hormonal regulation**, especially in the **liver**. During **fasting**, the hormone **glucagon** (released by the pancreas when blood glucose is low) triggers the production of **cAMP**, which activates a kinase cascade that **phosphorylates and inactivates pyruvate kinase**. This ensures that glycolysis is slowed down in the liver, preserving glucose for **vital organs** like the brain and heart. At the same time, muscle tissues can continue glycolysis using their own, non-hormonally regulated **pyruvate kinase**. In energy-deprived states, **AMP can reactivate** pyruvate kinase, ensuring that ATP production continues as needed.

- **Function:** Converts **phosphoenolpyruvate (PEP)** to **pyruvate**.
- **Inhibition:** High ATP, acetyl-CoA, alanine, or **phosphorylation** (e.g., during fasting).
- **Activation:** AMP.

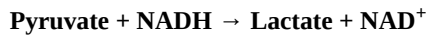
Together, hexokinase, PFK-1, and pyruvate kinase form the **three major control points of glycolysis**. Their allosteric regulation allows the cell to **finely adjust glucose metabolism** in response to energy supply, hormonal signals, and physiological conditions. This ensures that glycolysis remains a **flexible, efficient, and safe pathway** for managing the cell's energy economy.

The Role of NAD⁺ in Glycolysis and Anaerobic Metabolism

NAD⁺ (nicotinamide adenine dinucleotide) plays a **critical role in glycolysis**, serving as an essential electron carrier. During glycolysis, glucose is ultimately split into two molecules of **pyruvate**, and along the way, **two molecules of NAD⁺ are reduced to NADH**. These NADH molecules carry high-energy electrons that must be offloaded to regenerate NAD⁺, or glycolysis will stall due to a lack of oxidized coenzyme. Under **aerobic conditions**, when oxygen is readily available, the NADH produced during glycolysis is transported into the **mitochondria**, where it donates its electrons to the **electron transport chain (ETC)**. This

oxidation of NADH back to NAD⁺ enables glycolysis to continue while also supporting **oxidative phosphorylation**, which generates large amounts of ATP.

However, during **anaerobic conditions**, such as **vigorous muscle activity during intense exercise**, the oxygen supply may not meet the high metabolic demand. When oxygen is insufficient, the **ETC slows down or becomes inactive**, leading to a bottleneck in NAD⁺ regeneration. To resolve this, cells temporarily switch to an alternative pathway called **lactic acid fermentation** to regenerate NAD⁺ in the absence of oxygen. In this process, **pyruvate—the end product of glycolysis—is reduced to lactate by NADH, forming NAD⁺ in the process**:



This reaction is catalyzed by the enzyme **lactate dehydrogenase** and is vital for allowing glycolysis to persist when oxidative metabolism is impaired. Although this route is much **less efficient**, producing only **2 ATP per glucose molecule** compared to up to **36–38 ATP under aerobic respiration**, it is crucial for **short bursts of energy** and **cell survival in hypoxic conditions**. A practical example of this is during **sprint running or high-intensity interval training**, where muscle cells rapidly consume oxygen and switch to anaerobic glycolysis. The **accumulated lactate** can later be transported to the liver and **converted back into glucose via gluconeogenesis** once oxygen becomes available again. Thus, the ability to regenerate NAD⁺ through lactic acid fermentation represents a **metabolic adaptation** that sustains ATP production and preserves life under oxygen-limited circumstances.

Regulation during Fasting versus Overeating

During periods of **fasting or low blood glucose**, the body must carefully prioritize how glucose is used, preserving it for **vital organs** like the **brain, heart, and red blood cells**, which rely heavily on glucose as a fuel source. In this context, the **hormone glucagon**, secreted by the **alpha cells of the pancreas**, plays a central role in shifting liver metabolism from energy production to **glucose conservation and production**. When blood glucose levels drop, glucagon binds to its **specific G-protein-coupled receptor** on the surface of liver cells. This binding activates the enzyme **adenylate cyclase**, which catalyzes the conversion of ATP into **cyclic AMP (cAMP)**—a secondary messenger often described as the "battery" for activating various enzymes.

The rise in cAMP levels activates **protein kinase A (PKA)**, a key regulatory kinase. One of PKA's important targets in the liver is the enzyme **pyruvate kinase**, which normally catalyzes the final step of glycolysis—converting **phosphoenolpyruvate (PEP)** to **pyruvate** and generating ATP in the process. However, when PKA **phosphorylates pyruvate kinase**, the enzyme becomes **inactive**, effectively slowing down glycolysis. This inhibition ensures that PEP and pyruvate are **not funneled into energy production** but instead are **redirected toward gluconeogenesis**, the pathway by which the liver synthesizes **new glucose molecules** from non-carbohydrate sources like lactate, amino acids, and glycerol.

This hormonal regulation ensures that the liver does **not "burn" glucose** during times of scarcity but rather **exports glucose** into the bloodstream to maintain energy supply for tissues that are critically dependent on it. It is an elegant example of how hormonal signaling tightly coordinates metabolism to preserve life during nutrient deprivation.

During fasting or low blood glucose:

1. **Glucagon** (from the pancreas) signals the liver to **preserve glucose**:
2. Glucagon binds to its receptor → activates adenylylase → produces **cAMP**.
3. cAMP activates **protein kinase A (PKA)**.
4. PKA **phosphorylates pyruvate kinase**, inactivating it.
5. This slows glycolysis in the liver, diverting pyruvate toward **gluconeogenesis** instead of energy production.

Hexokinase vs Glucokinase

Hexokinase is the **primary glucose-phosphorylating enzyme** found in tissues with high and continuous energy demands, such as **skeletal muscle and the brain**. It has a **very low K_m (~0.1 mM)**, indicating a **very high affinity** for glucose. This means that even at **low physiological blood glucose levels (4–5 mM)**, hexokinase is **nearly saturated and operating at maximum activity**. Because of this high affinity, hexokinase ensures that **muscle and brain cells can consistently trap glucose** inside the cell and channel it into glycolysis or other metabolic pathways. Once glucose is phosphorylated into **glucose-6-phosphate (G6P)**, it

becomes trapped inside the cell due to its negative charge. However, to prevent excessive accumulation of G6P (which could be harmful or wasteful), **hexokinase is feedback-inhibited by G6P**. This means that when downstream glycolysis slows down or when there is enough metabolic fuel inside the cell, the enzyme automatically reduces its activity. This form of **self-regulation** ensures that energy is not wasted and that glucose uptake is tightly coordinated with the cell's energy demands.

In contrast, the **liver expresses a different isoform called glucokinase**, which behaves very differently from hexokinase. Glucokinase has a **much higher K_m ($\sim 8 \text{ mM}$)**, meaning it has a **low affinity for glucose** and only becomes significantly active when glucose levels in the blood are **elevated**, such as after eating **carbohydrate-rich meals**. Unlike hexokinase, **glucokinase is not inhibited by glucose-6-phosphate**, which allows it to process large quantities of glucose when available without shutting down. This property makes glucokinase function as a **glucose sensor** for the liver—it detects when blood glucose is high and activates glucose uptake and storage pathways like **glycogenesis** (glycogen synthesis) or **lipogenesis** (fat synthesis). However, at **normal fasting glucose levels ($\sim 4 \text{ mM}$)**, glucokinase remains **mostly inactive**, meaning the liver does **not compete** with more glucose-dependent tissues (e.g., the brain and muscles) for limited glucose resources. This selective inactivity is especially important during fasting, starvation, or exercise, when glucose must be prioritized for tissues that **lack alternative energy sources**, like neurons.

This difference in enzyme kinetics illustrates a **strategic metabolic design** in the body. Hexokinase's high affinity ensures that vital organs such as the brain and muscles have **uninterrupted access** to glucose regardless of blood sugar fluctuations. Meanwhile, glucokinase allows the liver to act as a **buffer and regulator** of blood glucose, only becoming active when **excess glucose is present**. This prevents unnecessary glucose uptake by the liver when it is needed elsewhere and helps maintain systemic glucose homeostasis. After a meal, when blood glucose can rise to $8\text{--}10 \text{ mM}$ or more, glucokinase kicks in to absorb the surplus, converting it into **glycogen or fat** for storage. This coordinated action between hexokinase and glucokinase ensures **efficient partitioning of glucose resources**, preventing hypoglycemia and supporting energy-demanding tissues at all times.

Hexokinase

- **$K_m = 0.1 \text{ mM}$** (very high affinity)
- Always active under normal glucose conditions ($4\text{--}5 \text{ mM}$).
- Inhibited by G6P.

Glucokinase (Liver)

Hexokinase

- **$K_m = 8 \text{ mM}$** (low affinity)
- Cannot be inhibited.
- Functions as a **glucose sensor**—only active when glucose is high.
 - This allows **to reserve glucose** for other organs like the brain and muscles during normal states.

Summary Points

Enzyme	Function	Inhibited By	Activated By
Hexokinase	Glucose $\rightarrow$ G6P	High G6P	Low G6P
PFK-1	Fruc-6P $\rightarrow$ Fruc-1,6-bisP	ATP, low pH	AMP
Pyruvate Kinase	PEP $\rightarrow$ Pyruvate	ATP, Acetyl-CoA, alanine, phosphorylation	AMP
Glucokinase	Glucose $\rightarrow$ G6P	None	High glucose only

Post-Lecture:

Key Takeaways

- Metabolism is an elegant balancing act between energy release and synthesis.
- Glycolysis is fast, flexible, and tightly regulated by energy status and hormonal signals.
- The body has evolved tissue-specific enzyme kinetics (e.g., hexokinase vs. glucokinase) to prioritize glucose use under different conditions.
- Even in oxygen-deprived states, cells can adapt to maintain survival through anaerobic pathways.

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11: Glycogenesis and Gluconeogenesis

Learning Objectives

- Define glycogenesis, glycogenolysis, and gluconeogenesis.
- Identify the tissues involved in glycogen storage and glucose production.
- Describe the purpose and physiological role of glycogen and gluconeogenesis in energy balance.
- Recognize key regulatory enzymes in glycogen metabolism and gluconeogenesis.
- Understand how hormones like insulin, glucagon, and epinephrine regulate glycogen and glucose metabolism.
- Predict metabolic changes in fasting versus fed states related to glycogen use and glucose synthesis.

Definition: Term

- **Glycogen:** A branched polymer of glucose used as a storage form of energy in animals, primarily in liver and muscle.
- **Glycogenesis:** The metabolic process of synthesizing glycogen from glucose.
- **Glycogenolysis:** The breakdown of glycogen to release glucose-1-phosphate.
- **Gluconeogenesis:** The generation of glucose from non-carbohydrate precursors such as lactate, amino acids, and glycerol.
- **Glycogen Synthase:** The key enzyme responsible for forming α -1,4-glycosidic bonds in glycogen.
- **Glycogen Phosphorylase:** The enzyme that cleaves glucose units from glycogen during glycogenolysis.
- **Debranching Enzyme:** A dual-function enzyme that removes branches in glycogen to allow continued degradation.
- **Glucose-6-Phosphatase:** Enzyme that converts glucose-6-phosphate into free glucose; active only in liver and kidney.
- **Pyruvate Carboxylase:** Biotin-dependent enzyme initiating gluconeogenesis by converting pyruvate to oxaloacetate.
- **Phosphoenolpyruvate Carboxykinase (PEPCK):** Converts oxaloacetate to phosphoenolpyruvate in gluconeogenesis.
- **Fructose-1,6-bisphosphatase:** Removes a phosphate group from fructose-1,6-bisphosphate in gluconeogenesis.
- **Insulin:** A hormone that promotes glycogen synthesis and inhibits gluconeogenesis.
- **Glucagon:** A hormone that stimulates glycogen breakdown and gluconeogenesis during fasting.
- **Epinephrine:** A stress hormone that mobilizes glycogen stores in muscle and liver.
- **Reciprocal Regulation:** Coordinated regulation where activation of one pathway (e.g., gluconeogenesis) inhibits the opposing one (e.g., glycolysis).

Glycogen Catabolism: Breaking Down Stored Glucose (Glycogenolysis)

Glycogen catabolism, also known as **glycogenolysis**, is the biochemical pathway by which stored glycogen is broken down to release glucose when energy is needed. Glycogen itself is a dense, highly branched polymer of glucose molecules, mainly stored in the **liver** and **skeletal muscles**. The liver acts as a glucose reservoir for the whole body, especially critical during fasting, while muscle glycogen is reserved locally for muscle contraction during physical activity. During times of fasting, intense exercise, or sudden energy demand (e.g., stress, trauma), hormones like **glucagon** and **epinephrine** stimulate glycogenolysis to ensure adequate glucose supply. In the **liver**, this glucose can be exported into the bloodstream to maintain normal blood sugar levels. In **muscles**, the glucose remains intracellular and fuels ATP production through glycolysis.

While **glycogenolysis** refers to the breakdown of stored **glycogen inside cells**, it's important to clarify a related process—the **digestion of dietary starch**, which begins in the digestive tract. When you chew starchy foods like bread or rice, **salivary amylase**, an enzyme secreted in the mouth, begins breaking down **starch** (a plant-based polysaccharide similar to glycogen but less branched). This is why starchy foods often taste sweet after prolonged chewing—some glucose and maltose are being released. In the small intestine, **pancreatic amylase** (secreted by the pancreas) continues this digestion, cleaving $\alpha(1 \rightarrow 4)$ glycosidic bonds in starch to form smaller sugars like maltose, maltotriose, and dextrins. While these enzymes do not break down **animal glycogen stored in our cells**, they illustrate the general concept of enzymatic carbohydrate degradation. Both starch and glycogen are polymers of glucose, but glycogen is more highly branched due to frequent **$\alpha(1 \rightarrow 6)$** linkages.

In cells, **glycogen phosphorylase** is the primary enzyme initiating glycogen breakdown. It catalyzes a reaction known as **phosphorolysis**, in which a molecule of **inorganic phosphate (Pi)**, not water, is used to cleave glucose residues from the non-

reducing ends of glycogen. This produces **glucose-1-phosphate (G1P)**. A major advantage of this reaction is that no ATP is consumed; instead, the glucose is immediately phosphorylated and ready to enter metabolic pathways. However, glycogen has a branched structure, and glycogen phosphorylase cannot remove glucose near branch points. That's where the **debranching enzyme complex** comes into play to ensure complete degradation of glycogen, even at its branch points. This includes two activities:

1. A **transferase activity** that shifts a block of three glucose residues to a nearby chain.
2. A **glucosidase activity** that hydrolyzes the remaining $\alpha(1 \rightarrow 6)$ -linked glucose at the branch point, releasing **free glucose** (unphosphorylated).

The **glucose-1-phosphate** generated by glycogen phosphorylase is not directly used in energy metabolism. Instead, it's converted into **glucose-6-phosphate (G6P)** by the enzyme **phosphoglucomutase**. This is a simple rearrangement of the phosphate group from carbon 1 to carbon 6.

- In **muscle cells**, G6P enters **glycolysis** to generate ATP on-site, especially during exercise.
- In the **liver**, G6P can be converted into **free glucose** by the enzyme **glucose-6-phosphatase**, which is present only in the liver and kidneys. This free glucose is then transported into the bloodstream to maintain blood glucose homeostasis—a critical function during fasting.
- **Example:** Imagine someone is running a marathon. Their muscles rely on glycogen breakdown to fuel glycolysis and generate rapid ATP. Meanwhile, their liver simultaneously breaks down glycogen to release glucose into the blood, ensuring that the brain and red blood cells continue to function properly.

Glycogen Anabolism: Storing Glucose (Glycogenesis)

Glycogenesis is the anabolic process of converting glucose into glycogen for storage. This occurs when there is **excess glucose**, such as after a carbohydrate-rich meal. The liver stores glycogen to regulate blood glucose, while muscles store it for use during activity. This process is essential to prevent **hyperglycemia** (high blood sugar) and to store energy efficiently. The process is **hormonally regulated**, with **insulin** playing a major role. **Insulin** is secreted in response to elevated blood glucose and promotes glucose uptake and storage in the liver and muscles.

The first step of glycogenesis involves the phosphorylation of glucose to form **glucose-6-phosphate (G6P)**, catalyzed by either **hexokinase** (in muscles) or **glucokinase** (in the liver). Then, **phosphoglucomutase** converts G6P into **glucose-1-phosphate (G1P)**. Next, G1P is **activated** by reacting with **UTP (uridine triphosphate)**, catalyzed by the enzyme **UDP-glucose pyrophosphorylase**. This reaction yields **UDP-glucose** and pyrophosphate (PPi), which is rapidly hydrolyzed to drive the reaction forward. The formation of UDP-glucose is crucial because it acts as an "activated" glucose donor—it has a high-energy bond that facilitates the transfer of glucose. The enzyme **glycogen synthase** then catalyzes the addition of glucose from UDP-glucose to a pre-existing glycogen primer (usually started by a protein called **glycogenin**). The glucose is added via $\alpha(1 \rightarrow 4)$ linkages. After a certain length, a **branching enzyme** introduces $\alpha(1 \rightarrow 6)$ branches every 8–12 glucose residues, making glycogen more soluble and allowing for rapid glucose release when needed.

- **Example:** After eating a large meal—say a plate of pasta and a banana—your blood glucose rises sharply. In response, the **pancreas releases insulin**, which signals liver and muscle cells to absorb glucose. Inside these cells, the glucose is converted to glycogen via the steps described. This prevents hyperglycemia and stores the glucose for future use. Later, during an overnight fast or the next workout, this stored glycogen will be mobilized back into glucose through **glycogenolysis**, providing energy without needing immediate food intake

Gluconeogenesis: Making Glucose from Non-Carbs

Gluconeogenesis is a **critical metabolic pathway** that allows the body to synthesize glucose from **non-carbohydrate precursors**. This pathway becomes essential during periods when dietary glucose is unavailable, such as **fasting**, **prolonged exercise**, **low-carbohydrate diets**, or **starvation**. Unlike glycogenolysis (which breaks down glycogen), gluconeogenesis generates **new glucose molecules** from substrates that are not carbohydrates—primarily **lactate**, **glycerol**, and certain **amino acids**. This process is not merely the reverse of glycolysis; it requires several bypass reactions due to the **irreversibility** of key glycolytic steps.

Gluconeogenesis is a tissue-specific process, mainly occurring in:

- **Liver (~90%):** The primary site under normal physiological conditions. The liver maintains **systemic glucose homeostasis**, especially during the early stages of fasting.
- **Kidneys (~10%):** The kidneys contribute more significantly during **prolonged fasting, starvation, or metabolic acidosis**. In acidosis, renal gluconeogenesis helps buffer blood pH by consuming protons (H^+) during amino acid metabolism.
- It's important to note that **muscle cells do not perform gluconeogenesis** because they lack the enzyme **glucose-6-phosphatase**, which is required to release free glucose into the blood. Instead, muscles send substrates like **lactate** and **alanine** to the liver for gluconeogenesis.

Some tissues in the body have an **absolute requirement** for glucose. The **brain** relies heavily on glucose, especially under normal, non-fasting conditions. Although the brain can adapt to using ketone bodies during prolonged starvation, glucose remains vital for certain neuronal functions. **Red blood cells (RBCs)** lack mitochondria, so they depend entirely on **anaerobic glycolysis**, which requires a constant glucose supply. **The renal medulla and the lens and cornea of the eye** also depend largely on glucose for energy. Gluconeogenesis ensures a **continuous supply of glucose**, especially when dietary intake is insufficient and glycogen stores are depleted. This is crucial to **prevent hypoglycemia**, which can impair brain function and, in severe cases, lead to coma or death.

- **Example: During fasting or overnight sleep**, glycogen stores in the liver begin to deplete. By the time you wake up, gluconeogenesis is already activated to maintain blood glucose levels. The liver converts **lactate** (from muscle), **glycerol** (from fat breakdown), and **alanine** (from muscle proteins) into glucose.
- **In prolonged exercise**, like marathon running or heavy endurance workouts, muscles produce **lactate** through anaerobic glycolysis. This lactate enters the **Cori cycle**, traveling to the liver where it's converted back into glucose and returned to the muscles—this cycle reduces muscle fatigue and supports ongoing activity.
- **In low-carb diets or ketogenic states**, the body restricts incoming carbohydrates, forcing the liver to ramp up gluconeogenesis. **Glycerol**, released from adipose tissue during fat breakdown, becomes a key substrate. Meanwhile, **glucogenic amino acids** (like alanine and glutamine) from muscle protein catabolism provide additional carbon skeletons for glucose production.

The Reverse of Glycolysis: Gluconeogenesis

Although glycolysis and gluconeogenesis seem like mirror-image pathways at first glance, they are **not simply reversible processes**. This is due to the presence of **three strongly exergonic steps** in glycolysis that have a large negative ΔG (free energy change), making them **thermodynamically irreversible** under normal physiological conditions. In gluconeogenesis, these steps are bypassed using **special enzymes** to allow the synthesis of glucose from pyruvate without violating energy principles.

Glucose-6-Phosphatase (Gluconeogenesis)

In glycolysis, the enzyme **hexokinase** catalyzes the first committed step: converting **glucose to glucose-6-phosphate (G6P)** by adding a phosphate from ATP. This reaction is highly favorable ($\Delta G \ll 0$), which helps trap glucose inside the cell and commit it to metabolism. However, this strong directionality means that the reverse reaction is not possible under normal conditions. To bypass this in **gluconeogenesis**, cells use **glucose-6-phosphatase**, an enzyme found primarily in **liver and kidney** cells. It removes the phosphate group from G6P, releasing free **glucose** into the bloodstream. This step is **essential for maintaining blood glucose levels**, especially during fasting. Muscles **lack glucose-6-phosphatase**, which is why they retain glucose for their own use and don't contribute to blood sugar regulation. For example, a person who's fasting overnight depends on liver glucose-6-phosphatase to generate free glucose from G6P for the brain and red blood cells.

Fructose-1,6-Bisphosphatase

PFK-1 is the **rate-limiting enzyme of glycolysis**, converting **fructose-6-phosphate (F6P) into fructose-1,6-bisphosphate (F1,6BP)** using ATP. This step commits glucose to full breakdown and is highly regulated—AMP and fructose-2,6-bisphosphate activate it, while ATP and citrate inhibit it. In **gluconeogenesis**, this irreversible step is bypassed by **fructose-1,6-bisphosphatase**, which hydrolyzes the phosphate group from F1,6BP to produce F6P. This step is **also regulated** but in the opposite way: it's **inhibited by AMP** and **activated by ATP and citrate**, reflecting the cell's energy status. For example, during prolonged exercise or fasting, high ATP levels (from fat oxidation) promote gluconeogenesis by activating fructose-1,6-bisphosphatase, helping the liver send glucose to tissues like the brain.

Pyruvate Carboxylase and PEP Carboxykinase (PEPCK)

The final irreversible glycolytic step involves **pyruvate kinase**, which converts **phosphoenolpyruvate (PEP)** into **pyruvate**, generating ATP in the process. This reaction is highly exergonic and is strongly regulated—inhibited by **ATP and alanine**, and in the liver, it is **inactivated by phosphorylation** during fasting (via glucagon signaling). To bypass this in gluconeogenesis, two enzymes are required: (1) **Pyruvate Carboxylase** converts **pyruvate into oxaloacetate (OAA)** inside the mitochondria. This reaction requires **biotin** as a coenzyme and **ATP** as an energy source. (2) **Phosphoenolpyruvate carboxykinase (PEPCK)** then converts **oxaloacetate into PEP**, using **GTP**. This step occurs either in the cytoplasm or mitochondria, depending on the tissue. Together, these two enzymes bypass the irreversible pyruvate kinase step and restore the carbon skeleton to the PEP level, allowing gluconeogenesis to continue upward toward glucose synthesis. For example, a **biotin deficiency** (e.g., due to excessive raw egg white consumption, which contains avidin) can impair pyruvate carboxylase activity, leading to poor gluconeogenesis and symptoms like hypoglycemia and lethargy.

Overall, these bypass reactions are not only necessary but also **thermodynamically favorable** in the direction of glucose synthesis. Each step is coupled to the **hydrolysis of high-energy phosphate bonds** (e.g., from ATP, GTP) to drive the reactions forward. Importantly, by using **different enzymes** and regulatory mechanisms, the cell can avoid a futile cycle (simultaneous glycolysis and gluconeogenesis), ensuring that these two pathways are **mutually exclusive depending on energy needs**.

Summary:

- Glycolysis = fast energy release
- Gluconeogenesis = glucose conservation and redistribution
- Regulation = based on energy status, hormones (insulin/glucagon), and tissue specificity

Hormonal Regulation: Balancing Blood Sugar

Maintaining stable blood glucose levels is essential for survival, as glucose is the primary energy source for the brain and red blood cells. This delicate balance is achieved by a finely tuned system of **hormonal regulation**, primarily involving **insulin**, **glucagon**, and **epinephrine**. These hormones act in opposition to either promote the **storage** or **release** of glucose depending on the body's energy state—fed, fasting, or under stress.

Insulin

Insulin is an anabolic hormone secreted by the **β -cells of the pancreas** in response to **high blood glucose levels**, such as after a carbohydrate-rich meal. Its primary role is to **reduce blood glucose levels** by promoting **glucose uptake** into tissues like muscle and fat and enhancing **storage** processes in the liver. One way insulin achieves this is by increasing the **expression and activity of glucokinase** in the liver. Glucokinase is the isoform of hexokinase that phosphorylates glucose to form **glucose-6-phosphate (G6P)**. This is a crucial first step for glucose to be retained in the cell and directed into metabolic pathways such as **glycolysis**, **glycogenesis**, or **lipogenesis**. Importantly, glucokinase has a high **K_m of ~8 mM**, meaning it is only active when glucose levels are high—making it perfectly suited to operate **after meals**, preventing blood sugar spikes. By increasing glucose uptake and phosphorylation, insulin facilitates the conversion of excess glucose into **glycogen (via glycogenesis)** and **fat (via lipogenesis)**. It also **inhibits gluconeogenesis and glycogenolysis**, ensuring that glucose is not being produced or released when it's already abundant. For example, after eating a bowl of rice, insulin is released, prompting the liver and muscles to store glucose as glycogen and adipose tissue to convert glucose into fat. This prevents hyperglycemia and sets aside energy for later use.

Glucagon

Glucagon is the major **catabolic hormone**, secreted by the **α -cells of the pancreas** in response to **low blood glucose levels**, such as during overnight fasting or between meals. Its main target is the **liver**, where it stimulates processes that **increase blood glucose**, namely **glycogenolysis** (glycogen breakdown) and **gluconeogenesis** (glucose synthesis from non-carbohydrate sources). One of the key mechanisms by which glucagon works is through the **cAMP signaling cascade**. When glucagon binds to its **G-protein-coupled receptor** on hepatocytes (Liver cells), it activates **adenylate cyclase**, which increases levels of **cyclic AMP (cAMP)**. This activates **protein kinase A (PKA)**, a key regulatory kinase that phosphorylates and modulates several metabolic enzymes.

One important target of PKA is **pyruvate kinase**, the enzyme that catalyzes the final step of glycolysis (conversion of phosphoenolpyruvate to pyruvate). PKA **phosphorylates and inhibits pyruvate kinase**, effectively slowing down glycolysis. This is crucial because, in a fasting state, the liver must preserve pyruvate and its precursors for **gluconeogenesis**, not burn them for energy. For example, during fasting or low-carb dieting, glucagon ensures that blood sugar remains stable by stimulating hepatic glucose production and limiting its breakdown within the liver.

Epinephrine (Adrenaline)

Epinephrine, also known as **adrenaline**, is released by the **adrenal medulla** during **acute stress, exercise**, or in response to fear—the classic "**fight or flight**" situations. Its effects are rapid and powerful, mobilizing energy stores to prepare the body for action. Epinephrine enhances **glycogenolysis** dramatically—up to **2000-fold**, especially in **skeletal muscle**. It binds to adrenergic receptors, activating a signaling cascade similar to glucagon's, leading to cAMP production and PKA activation. In muscle, this increases glycogen breakdown and glucose availability for **immediate ATP production via glycolysis**. Since muscle cells lack glucose-6-phosphatase, they do not export glucose but use it locally.

Epinephrine also **increases heart rate, constricts blood vessels (vasoconstriction)** to raise blood pressure, and **promotes water retention**, all of which prepare the body to respond quickly to stress. Unlike glucagon, epinephrine acts on **both liver and muscle**, and its effects are not just about maintaining blood glucose, but also about **rapid energy mobilization** for physical activity. For example, during a sprint or emergency situation, epinephrine floods the bloodstream, causing the heart to beat faster, sharpening focus, and driving the rapid breakdown of muscle glycogen to generate quick ATP for muscular action.

Summary:

- **Insulin** acts during the feeding to **store** glucose and suppress production.
- **Glucagon** acts during fasting to **produce and release** glucose.
- **Epinephrine** overrides both in acute stress, **mobilizing energy rapidly**.

Clinical Relevance

What happens if pyruvate kinase can't be phosphorylated?

Pyruvate kinase (PK) is a key glycolytic enzyme that catalyzes the final, irreversible step in glycolysis—converting **phosphoenolpyruvate (PEP) to pyruvate**, while producing ATP. In the **liver**, PK is hormonally regulated: during fasting, **glucagon** activates **protein kinase A (PKA)** via the cAMP pathway, which **phosphorylates and inactivates pyruvate kinase**. This shuts down glycolysis to favor **gluconeogenesis**, preserving glucose for the brain and red blood cells.

Now imagine a **mutation in PK** that **prevents phosphorylation** (e.g., mutation at the serine phosphorylation site). The enzyme remains **constitutively active**, even when fasting. This forces the liver to **keep burning glucose via glycolysis**, rather than preserving or producing it. The result? A paradoxical drop in blood sugar—**hypoglycemia**, especially during prolonged fasting or exertion. Clinically, such a scenario could mimic or exacerbate **fasting hypoglycemia syndromes**. Though rare, this situation parallels some metabolic disorders where glycolysis is inappropriately active. It also demonstrates the importance of **post-translational regulation**—here, phosphorylation of an enzyme—to adapt metabolism to hormonal cues.

What happens if glucokinase K_m is too low (e.g., 0.1 mM like hexokinase)?

Glucokinase is a specialized isoform of hexokinase found primarily in the **liver and pancreatic β -cells**. It has a **high K_m (~8 mM)** and **low affinity for glucose**, which makes it act like a glucose sensor—it only becomes active when glucose levels are high (after meals). This helps the liver **store excess glucose** and β -cells **regulate insulin release** appropriately.

Now consider a hypothetical or pathological mutation that **reduces the K_m** of glucokinase to **0.1 mM**, making it behave like **hexokinase**, which is always active. The liver would **begin phosphorylating glucose even at very low concentrations**, aggressively converting it into G6P and storing or using it. This can lead to **dangerous hypoglycemia**, especially **post-exercise**, when glucose is already low. A real-world parallel is seen in **glucokinase-activating mutations (GKAM)**, which are associated with **congenital hyperinsulinism**. These patients over-secrete insulin and aggressively clear glucose from the blood, resulting in **persistent hypoglycemia**. On the other hand, **glucokinase loss-of-function mutations** cause **MODY type 2 (Maturity-Onset Diabetes of the Young)**, characterized by mild fasting hyperglycemia.

When should you use a glucagon inhibitor?

In **type 2 diabetes mellitus (T2DM)**, especially in the early stages, patients often show **inappropriately elevated glucagon levels**, even in the feeding state. This leads to **increased hepatic gluconeogenesis and glycogenolysis**, contributing to **fasting and postprandial hyperglycemia**. Inhibiting glucagon signaling can help reduce **hepatic glucose output**. **Glucagon receptor antagonists** or drugs that interfere with **cAMP production** (like phosphodiesterase inhibitors) are being explored to **blunt glucagon's effect** on the liver. Another experimental target is **GCGR (glucagon receptor)** antagonists. For example, **volagidemab** is a monoclonal antibody targeting the glucagon receptor currently in clinical trials. Glucagon inhibitors are not yet widely approved, but they hold promise in patients with **poor glucose control despite high insulin** and those with excessive hepatic glucose production.

When should you use an insulin inhibitor?

While **insulin** is vital for glucose homeostasis, **excessive insulin secretion** can cause **life-threatening hypoglycemia**, particularly in rare tumors like **insulinomas** (insulin-secreting pancreatic neuroendocrine tumors). In such cases, pharmacological **insulin inhibitors** are used. A key example is **diazoxide**, which **opens K⁺ channels in β -cells**, **inhibiting depolarization and calcium influx**, thereby **reducing insulin release**. Diazoxide is used both in **insulinomas** and in **congenital hyperinsulinism** to manage dangerously low glucose levels. Other scenarios where insulin action might be temporarily dampened include **counter-regulatory support in hypoglycemia**, or experimental studies in **insulin-sensitizing therapies**. However, in clinical practice, **inhibiting insulin** is done cautiously and typically in very specific settings, as the risk of hyperglycemia and metabolic acidosis is high.

Post-Lecture Objectives

- Trace the steps of glycogen synthesis and degradation, including the enzymes involved.
- Describe how gluconeogenesis bypasses irreversible steps in glycolysis.
- Explain the hormonal signals that direct metabolic pathways during fasting and feeding.
- Apply knowledge of enzyme functions to predict symptoms of metabolic disorders like Von Gierke's or McArdle's disease.
- Contrast the roles of liver and muscle in glucose homeostasis.

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12: TCA cycle

Learning Objectives

- Describe the overall purpose and function of the TCA cycle within cellular metabolism.
- Identify the substrates, products, and enzymes involved in each step of the TCA cycle.
- Explain how Acetyl-CoA is formed and its central role as a metabolic hub.
- Discuss the significance of the TCA cycle in energy production (NADH, FADH₂, GTP) and CO₂ release.
- Recognize the clinical and biotechnological applications of TCA cycle enzymes.
- Analyze how mutations in TCA cycle enzymes (e.g., IDH1/2) contribute to disease and drug targeting.
- Integrate TCA cycle activity with amino acid and fatty acid metabolism.

Definition: Term

- **TCA Cycle (Krebs Cycle/Citric Acid Cycle):** A series of enzymatic reactions in the mitochondria that oxidize Acetyl-CoA to CO₂ and generate NADH, FADH₂, and GTP.
- **Acetyl-CoA:** A 2-carbon molecule derived from pyruvate, fatty acids, or amino acids; enters the TCA cycle to donate acetyl groups.
- **Substrate-level Phosphorylation:** ATP or GTP formation directly from a high-energy intermediate, not via the electron transport chain.
- **NADH / FADH₂:** Reduced electron carriers generated during the TCA cycle that donate electrons to the electron transport chain for ATP production.
- **Oncometabolite:** A metabolite like 2-hydroxyglutarate that promotes tumor growth by altering cellular metabolism and epigenetics.
- **Allosteric Regulation:** Enzyme activity modulation by binding of effectors at a site other than the active site.
- **Anaplerosis / Cataplerosis:** The replenishment (anaplerosis) and removal (cataplerosis) of intermediates from the TCA cycle for biosynthesis.
- **Ping-Pong Mechanism:** A kinetic model where one product is released before the second substrate binds; seen in some TCA enzymes like α-KGDH.

Note

- Review glycolysis and the conversion of pyruvate to Acetyl-CoA.
- Watch a short video animation of the TCA cycle to visualize intermediates and enzyme flow.
- Skim clinical case examples involving IDH mutations and Metformin use.
- Be prepared to relate the TCA cycle to amino acid and fatty acid metabolism.

TCA Cycle

The **TCA cycle** is a central metabolic pathway that **oxidizes Acetyl-CoA** into energy-rich molecules: **GTP**, **NADH**, and **FADH₂**, while releasing **CO₂** as a waste product. Importantly, **oxygen is not directly involved in the cycle itself**. Instead, **oxygen** is used later in the **electron transport chain** to accept electrons from NADH and FADH₂, which are produced during the TCA cycle. This process is part of **aerobic respiration**, and without oxygen to accept these electrons, the cycle and downstream ATP production would stall.

Acetyl-CoA

Acetyl-CoA is a **2-carbon molecule** derived from the oxidative decarboxylation of **pyruvate**, a product of glycolysis. It serves as the **entry molecule** for the TCA cycle. Acetyl-CoA is made up of: (1) An **acetyl group (CH₃CO-)**, which is a 2-carbon unit that is oxidized in the TCA cycle, and (2) **Coenzyme A (CoA)**, which is a large molecule derived partly from **pantothenic acid (Vitamin B5)** and **nucleotides**—yes, CoA is a nucleotide structure and acts like enzymes to recognize and transfer acetyl groups.

Sometimes you'll also see ADP components attached to CoA, emphasizing its nucleotide nature. This molecule also links carbohydrate, fat, and protein metabolism, because amino acids and fatty acids can also be converted into Acetyl-CoA.

Pathway Through the TCA Cycle

Step 1: Formation of Citrate

The TCA cycle begins with the condensation of **acetyl-CoA** (a 2-carbon molecule) and **oxaloacetate** (a 4-carbon dicarboxylic acid), forming a **6-carbon molecule called citrate**. This reaction is catalyzed by the enzyme **citrate synthase**, a key regulatory enzyme that operates through an ordered sequential mechanism, meaning oxaloacetate must bind first to induce a conformational change that allows acetyl-CoA to bind. This is an energetically favorable step and effectively "pulls" acetyl-CoA into the cycle.

From a protein biotech perspective, citrate synthase is often studied for **conformational changes and allosteric regulation**, making it a model for **protein folding and induced fit** mechanisms.

- **Acetyl-CoA (2C)** combines with **oxaloacetate (4C)** to form **citrate (6C)**.
 - This reaction is catalyzed by the enzyme **citrate synthase**.

Step 2: Isomerization of Citrate to Isocitrate

Citrate undergoes isomerization to form **isocitrate** through the action of **aconitase**, an iron-sulfur protein. This two-step reaction involves the reversible dehydration of citrate to **cis-aconitate**, followed by rehydration to isocitrate. Though it seems like a minor rearrangement, this conversion is crucial—it repositions the hydroxyl group on the molecule, making it a better target for subsequent oxidation. Aconitase is unique because its [4Fe-4S] cluster allows it to act as both a **catalyst** and a **sensor** of cellular oxidative stress, thus linking metabolism to **redox signaling**. In drug discovery, aconitase has been studied as a potential target in **neurodegenerative diseases** and **oxidative damage**, where disrupted iron metabolism is implicated.

- Citrate is rearranged to **isocitrate**,
 - This makes the molecule more reactive and ready for oxidative decarboxylation.
 - This process is catalyzed by **aconitase**.

Step 3: Oxidative Decarboxylation of Isocitrate

Isocitrate is oxidized and decarboxylated by **isocitrate dehydrogenase (IDH)** to form **α -ketoglutarate**, a 5-carbon molecule. This reaction also produces **NADH** and releases the first **molecule of CO₂**. IDH exists in **NAD⁺-dependent (mitochondrial)** and **NADP⁺-dependent (cytosolic and mitochondrial)** isoforms, each with distinct roles in metabolism and biosynthesis. Mutations in IDH1 and IDH2 isoforms are implicated in several **cancers**, particularly **gliomas and acute myeloid leukemia**, because they result in a neomorphic activity that produces the oncometabolite **2-hydroxyglutarate**, which disrupts epigenetic regulation.

- Isocitrate is oxidized and decarboxylated by **isocitrate dehydrogenase**, producing:
 - **α -ketoglutarate (5C)**
 - **CO₂**
 - **NADH**

Step 4: Oxidative Decarboxylation of α -Ketoglutarate

The next step is the oxidative decarboxylation of **α -ketoglutarate** into **succinyl-CoA**, catalyzed by the **α -ketoglutarate dehydrogenase complex (α -KGDH)**. This multi-enzyme complex functions similarly to the **pyruvate dehydrogenase complex** and uses **thiamine pyrophosphate (TPP)**, **lipoate**, **FAD**, **NAD⁺**, and **CoA** as cofactors. This reaction produces **another molecule of NADH** and the second **CO₂** of the cycle.

- α -Ketoglutarate is converted to **succinyl-CoA (4C)** by **α -ketoglutarate dehydrogenase**, producing:
 - **Another CO₂**
 - **Another NADH**

Step 5: Conversion of Succinyl-CoA to Succinate

The energy-rich **succinyl-CoA** is converted to **succinate** by **succinyl-CoA synthetase (or succinate thiokinase)**, coupled with the substrate-level phosphorylation of **GDP (or ADP) to GTP (or ATP)**. This is one of the few steps in metabolism that produces a high-energy phosphate bond directly without involving the electron transport chain.

- Succinyl-CoA is converted to **succinate**, catalyzed by **succinyl-CoA synthetase**.
- This step generates **GTP** (or ATP, depending on the tissue) via substrate-level phosphorylation.

Step 6: Oxidation of Succinate to Fumarate

Succinate dehydrogenase catalyzes the oxidation of **succinate** to **fumarate**, generating **FADH₂**. Unique among TCA enzymes, succinate dehydrogenase is embedded in the **inner mitochondrial membrane** and doubles as **Complex II** of the **electron transport chain (ETC)**. This dual role makes it a bridge between the TCA cycle and oxidative phosphorylation. Because FADH₂ donates electrons directly into the ETC, it yields less ATP than NADH. Succinate dehydrogenase is a hotspot for studying **flavoprotein chemistry**, **membrane-bound enzymes**, and **hereditary tumors** (e.g., mutations in SDH genes cause **parangliomas** and **pheochromocytomas**).

- Succinate is oxidized to **fumarate** by **succinate dehydrogenase**, producing:
 - **FADH₂**
- Note: Succinate dehydrogenase is part of both the TCA cycle and Complex II of the **electron transport chain**.

Step 7: Hydration of Fumarate to Malate

Fumarate is then hydrated to form **malate** via the enzyme **fumarase (fumarate hydratase)**. This is a simple **hydration reaction**, but it's clinically relevant—mutations in fumarase are linked to **hereditary leiomyomatosis and renal cell cancer (HLRCC)**. As such, fumarase is a **tumor suppressor protein**, and its deficiency causes a buildup of fumarate, leading to **epigenetic and signaling alterations**. Biotech applications include screening for fumarase inhibitors or stabilizers as part of **metabolic therapy** approaches.

- **Fumarase** hydrates fumarate to form **malate**.

Step 8: Oxidation of Malate to Regenerate Oxaloacetate

Finally, **malate** is oxidized to **oxaloacetate** by **malate dehydrogenase**, producing the third **NADH** of the cycle. This reaction is slightly **endergonic**, but it is driven forward by the rapid consumption of oxaloacetate in the citrate synthase reaction (step 1), demonstrating a beautiful example of **metabolic flux and equilibrium shifting**. Malate dehydrogenase is a key enzyme for studying **thermodynamics in enzymology**, and it's widely used in lab protocols for **coupled assays**, where it helps regenerate NAD⁺ for other enzyme reactions.

- **Malate dehydrogenase** oxidizes malate to regenerate **oxaloacetate**, producing:
 - **Another NADH**

Overall Outputs per Acetyl-CoA Molecule

From one turn of the TCA cycle (per **Acetyl-CoA**), the following high-energy products are generated:

- **3 NADH**
- **1 FADH₂**
- **1 GTP (or ATP)**
- **2 CO₂**

Each NADH yields approximately 2.5 ATP, and each FADH₂ yields about 1.5 ATP during oxidative phosphorylation. Thus, the TCA cycle indirectly drives **significant ATP production**. These reduced cofactors are essential in maintaining the **cell's redox state**, and their balance is crucial in **bioreactor design**, **mitochondrial disease therapy**, and **pharmacokinetics modeling**.

Application in Protein Biotechnology and Drug Discovery

Targeting TCA Enzymes in Cancer and Disease

The tricarboxylic acid (TCA) cycle plays a central role not only in energy metabolism but also in supporting the anabolic demands of rapidly proliferating cancer cells. Many tumors exhibit altered TCA cycle activity—a phenomenon often tied to the Warburg effect, where cells rely more heavily on glycolysis even in the presence of oxygen. A particularly striking example involves **mutations in isocitrate dehydrogenase (IDH)**, especially the IDH1 and IDH2 isoforms. These mutations are frequently found in cancers such as **gliomas** and **acute myeloid leukemia (AML)**. Mutant IDH enzymes gain a new (neomorphic) function—they reduce α -ketoglutarate to an abnormal metabolite called **2-hydroxyglutarate (2-HG)**. This oncometabolite interferes with DNA and histone demethylation, causing widespread epigenetic dysregulation and contributing to tumor progression. Understanding the structural and kinetic properties of these mutant enzymes enabled the development of **targeted inhibitors** such as **Ivosidenib (AG-120)** and **Enasidenib (AG-221)**. These small molecules bind specifically to the mutant IDH1 and IDH2 enzymes, respectively, suppressing 2-HG production and are now FDA-approved therapies for treating IDH-mutant AML. This illustrates how detailed biochemical knowledge of TCA cycle enzymes directly informs **precision oncology** and **rational drug design**.

Enzyme Kinetics in TCA Enzyme Studies

Enzyme kinetics offers powerful tools for understanding and manipulating the function of TCA enzymes. Through kinetic assays, researchers can explore **reaction mechanisms**, such as whether an enzyme operates through a **ping-pong mechanism** (as seen in α -ketoglutarate dehydrogenase) or a **ternary complex mechanism** (where all substrates must bind before the reaction proceeds). These kinetic characterizations inform drug design, especially when looking to inhibit enzymes in a **competitive, non-competitive, or uncompetitive** manner. For example, citrate synthase can be inhibited competitively by molecules mimicking acetyl-CoA, and the efficacy of such inhibitors can be tested using **Michaelis-Menten** or **Lineweaver-Burk plots**. Standard assays for TCA enzymes often involve measuring the production or consumption of **NADH** or **FADH₂**, which absorb strongly at **340 nm**, making them ideal for **colorimetric or spectrophotometric quantification**. These methods are foundational in **high-throughput drug screening platforms**, where hundreds of compounds can be tested for their effect on enzymatic rates, thereby identifying lead candidates for further development.

Metabolic Engineering in Biotechnology

In industrial biotechnology, the TCA cycle is often **manipulated or rewired** to optimize the production of valuable chemicals. For instance, overexpressing **citrate synthase** in certain microbial systems like **Aspergillus niger** or engineered **yeast strains** can boost the yield of **citric acid**, which is used widely as a food preservative, flavoring agent, and even in pharmaceuticals. Similarly, redirecting carbon flux through **succinate-producing branches** of the TCA cycle allows the generation of **succinic acid**, a building block for **biodegradable plastics (e.g., polybutylene succinate)** and other green chemicals. **Metabolic engineers** often introduce or overexpress genes encoding specific TCA enzymes, adjust cofactor balances (NADH/NAD⁺), and eliminate competing pathways to increase the efficiency of bioproduction. These engineered strains become biological factories, illustrating how deep biochemical understanding translates into practical, large-scale **bioproduction platforms**.

The centrality of the TCA cycle in cellular metabolism makes its enzymes and intermediates highly relevant in clinical pharmacology. Several drugs and toxic agents target different aspects of this pathway. For instance, **Ivosidenib**, as discussed, inhibits mutant **IDH1** to block the production of the oncometabolite 2-HG in cancer. **Metformin**, a first-line treatment for **type 2 diabetes**, indirectly affects TCA cycle function by inhibiting **Complex I** of the electron transport chain. This reduces **NADH oxidation**, thereby altering TCA flux and limiting hepatic gluconeogenesis. In toxicology, substances like **fluoroacetate** act as suicide inhibitors of **aconitase**, halting the TCA cycle and causing the accumulation of citrate, leading to cellular toxicity—a mechanism exploited in rodenticides. Similarly, **arsenite** is a potent inhibitor of **α -ketoglutarate dehydrogenase**, disrupting NADH production and inducing energy failure. Understanding these interactions is critical in both drug safety evaluation and the treatment of poisonings.

Amino Acid and Fatty Acid Metabolism

The TCA cycle acts as a **metabolic hub**, not just for carbohydrate metabolism, but also for the catabolism and biosynthesis of **amino acids and fatty acids**. Several amino acids are transaminated into TCA cycle intermediates: for example, **glutamate** is

converted to **α -ketoglutarate**, and **aspartate** can feed into **oxaloacetate**. These conversions are vital in nitrogen metabolism, neurotransmitter synthesis, and the urea cycle. In contrast, **fatty acids** are broken down through **β -oxidation** to yield **acetyl-CoA**, which enters the TCA cycle for complete oxidation. However, during prolonged fasting or in diabetes, excess acetyl-CoA from fat breakdown leads to **ketogenesis** instead of TCA entry. This dual role of the TCA cycle in **catabolic degradation (cataplerosis)** and **anabolic replenishment (anaplerosis)** is central in **metabolic engineering**, where maintaining balanced flux through TCA intermediates is crucial for sustained biosynthesis and cell health.

Post-Lecture Objectives

- Trace the full cycle of carbon atoms from Acetyl-CoA through the TCA cycle.
- Name the enzymes and reactions responsible for NADH, FADH₂, GTP, and CO₂ production.
- Explain the dual role of TCA intermediates in energy generation and biosynthesis.
- Identify points of regulation and their relevance in disease and drug targeting.
- Describe how TCA flux integrates with lipid and amino acid metabolism.

Key Takeaways

- The TCA cycle is not just a source of energy—it is a hub for biosynthetic precursors and therapeutic targeting.
- Alterations in TCA cycle enzymes are at the forefront of precision oncology and metabolic therapy.
- Understanding enzyme structure, kinetics, and regulation can drive innovations in biotechnology and medicine.

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13: Electron Transport Chain

Learning Objectives

- Explain the necessity of NAD^+ and FAD regeneration in cellular metabolism.
- Describe how electrons are transferred through the electron transport chain (ETC) and how this drives proton pumping.
- Define the proton motive force (PMF) and explain its role in ATP synthesis.
- Describe the structure and function of ATP synthase and the mechanism of chemiosmosis.
- Analyze the ATP yield from aerobic respiration and understand the factors that influence efficiency.
- Identify real-world biotechnological applications of redox balance, ETC components, and ATP production.
- Interpret the clinical significance of ETC dysfunction and its impact on diseases and therapeutic strategies.

Definition: Term

- **NAD^+/NADH :** Nicotinamide adenine dinucleotide; an essential electron carrier that cycles between oxidized (NAD^+) and reduced (NADH) states.
- **FAD/FADH_2 :** Flavin adenine dinucleotide; another electron carrier, typically bound to enzymes like Complex II.
- **Electron Transport Chain (ETC):** A series of protein complexes in the inner mitochondrial membrane that transfers electrons and pumps protons to generate a gradient.
- **Proton Motive Force (PMF):** The combined chemical and electrical gradient of protons across the inner mitochondrial membrane.
- **Chemiosmosis:** The movement of protons down their gradient through ATP synthase, leading to ATP production.
- **ATP Synthase (Complex V):** A rotary enzyme that uses PMF to synthesize ATP from ADP and P_i .
- **Oxidative Phosphorylation:** The synthesis of ATP driven by the ETC and chemiosmosis in the mitochondria.
- **Q-cycle:** A redox mechanism in Complex III that enhances proton pumping and electron efficiency.
- **Uncoupling Protein (UCP):** A mitochondrial protein that dissipates PMF to generate heat instead of ATP.
- **Cofactor Recycling:** Biochemical strategies (natural or engineered) that regenerate NAD^+ and FAD to maintain metabolic flux.

Regeneration of NAD^+ and FAD

In cellular metabolism, especially in **glycolysis** and the **tricarboxylic acid (TCA) cycle**, cofactors like NAD^+ and FAD act as electron acceptors. As these pathways proceed, NAD^+ is reduced to **NADH** , and FAD^{2+} to **FADH_2** . These reduced cofactors carry high-energy electrons to the **electron transport chain (ETC)**, where they are oxidized to be regenerated back into NAD^+ and FAD. This recycling is **essential** because the intracellular concentrations of NAD^+ and FAD are limited, and their depletion would arrest metabolic flux through glycolysis and the TCA cycle. For instance, each glucose molecule oxidized during glycolysis yields 2 NADH . If these NADH molecules are not reoxidized, glycolysis halts due to lack of available NAD^+ . Under aerobic conditions, the ETC restores NAD^+ and FAD by passing the electrons to oxygen, forming water. Under **anaerobic conditions**, alternative strategies like **lactate fermentation** (in muscle cells) or **ethanol fermentation** (in yeast) regenerate NAD^+ , but with significantly lower ATP yield and potential accumulation of metabolic byproducts.

In **Biotech industrial-scale bioreactors**, NAD^+/NADH balance is a key design parameter. Microbial systems engineered to produce proteins or biofuels often require **cofactor recycling modules**, such as overexpression of NADH oxidase or formate dehydrogenase, to maintain redox homeostasis. For example, **cofactor engineering** in *E. coli* or yeast can enhance yields of recombinant enzymes, antibiotics, or therapeutic proteins by ensuring uninterrupted glycolysis and energy generation.

The Proton Gradient and Mitochondrial Structure

The **mitochondrial inner membrane** is the site of a finely tuned electrochemical process driven by the ETC. As electrons pass through Complexes I, III, and IV, the energy released is used to **pump protons (H^+)** from the **mitochondrial matrix** into the **intermembrane space**, creating a steep **proton gradient**. This results in two key gradients: a **chemical gradient** (high $[\text{H}^+]$ or low pH in the intermembrane space) and an **electrical gradient** (positive charge outside relative to the inside), collectively known as

the **proton motive force (PMF)**. This PMF is essential not only for ATP production but also for **importing mitochondrial proteins**, **regulating calcium homeostasis**, and facilitating **heat generation** in **brown adipose tissue**, where **uncoupling proteins (UCPs)** dissipate the gradient to produce heat rather than ATP—a process relevant in thermogenesis and obesity studies.

In **protein biotechnology**, the PMF is exploited in **recombinant protein production systems**. For example, in *E. coli*, the proper folding and membrane insertion of mitochondrial or membrane proteins often require mimicking PMF conditions. Additionally, **drug delivery systems** targeting mitochondria (mito-targeted peptides or lipophilic cations like **triphenylphosphonium**) use the membrane potential component of the PMF to accumulate selectively in the organelle.

ATP Synthase and Chemiosmosis

ATP synthase (Complex V) is a remarkable rotary enzyme embedded in the inner mitochondrial membrane. It consists of two main parts: **F_o**, the membrane-embedded proton channel, and **F₁**, the catalytic domain that protrudes into the mitochondrial matrix. As protons re-enter the matrix through F_o, they cause rotation of a central shaft within the F₁ subunit, triggering conformational changes that allow the enzyme to bind ADP and Pi and catalyze their condensation into **ATP**. This process, known as **chemiosmosis**, is the direct coupling of the **proton gradient to ATP synthesis**. While a common simplification suggests "1 proton = 1 ATP," in reality, about **3 to 4 protons are required per ATP**, depending on the species and the structure of ATP synthase. This is due to the mechanical rotation mechanism and the number of c-subunits in the F_o rotor ring.

For **Biotechnological applications**, ATP synthase has been studied as a **nanomotor**, inspiring **bioengineering approaches to artificial molecular machines**. In synthetic biology, engineered ATP synthase variants are used to design **proton-driven ATP regeneration systems**, particularly in **cell-free protein synthesis platforms**, where maintaining ATP levels is critical for sustained transcription and translation.

Complex I: NADH Oxidation and Proton Pumping

Complex I (NADH:ubiquinone oxidoreductase) is the first and largest enzyme complex of the electron transport chain. It receives **two high-energy electrons** from NADH. These electrons are transferred through multiple **redox-active centers**, including **flavin mononucleotide (FMN)** and several **iron-sulfur (Fe-S) clusters**. The energy released during this electron transfer is used to pump **four protons (H⁺)** from the mitochondrial matrix into the intermembrane space, initiating the formation of the **proton motive force (PMF)**. The final step at Complex I is the reduction of **ubiquinone (Coenzyme Q)** to **ubiquinol (QH₂)**, which carries electrons to Complex III.

Mutations in nuclear or **mitochondrial DNA (mtDNA)** encoding Complex I subunits are linked to various **mitochondrial diseases**, including **Leigh syndrome** and **Parkinson's disease**. Neurons, which have high ATP demands, are particularly sensitive to Complex I dysfunction. The neurotoxin **rotenone**, a known Complex I inhibitor, is used in animal models to induce Parkinson-like symptoms for studying neurodegeneration. In protein biotechnology, Complex I is studied in **structural proteomics** and **drug screening platforms** using cryo-electron microscopy. Engineering **bacterial or yeast systems** to express individual Complex I subunits aids in dissecting redox mechanisms and screening **therapeutic agents** that restore or bypass defective electron transport.

Complex II: FADH₂ Oxidation (No Proton Pumping)

Complex II (succinate dehydrogenase) serves a dual role — it is part of both the **TCA cycle** and the **ETC**. Within the TCA cycle, it catalyzes the oxidation of **succinate to fumarate**, reducing **FAD** to **FADH₂**. This FADH₂ remains enzyme-bound and donates its electrons to **iron-sulfur clusters**, which then transfer them to **Coenzyme Q**. Unlike Complex I, **Complex II does not pump protons**, so its contribution to the proton gradient — and hence ATP yield — is lower. As a result, each FADH₂ molecule generates about **2 ATP** instead of 3.

Due to its dual role, Complex II is a **marker of mitochondrial integrity and metabolic flux**, often used in metabolic profiling of cancer cells. In **synthetic biology**, succinate dehydrogenase has been re-engineered in microbes for **metabolic pathway rerouting** — for instance, in *E. coli* engineered for succinate production. Mutations in Complex II subunits have been implicated in **hereditary paraganglioma** and **renal cell carcinoma**, revealing its relevance in tumorigenesis. These insights are exploited in **oncology drug development** and **metabolic pathway targeting**.

Coenzyme Q and Complex III: Electron Recycling and Q-Cycle

Coenzyme Q (ubiquinone) is a lipid-soluble molecule that shuttles electrons from **Complexes I and II** to **Complex III (cytochrome bc₁ complex)**. Complex III contains **cytochromes b and c₁**, as well as a **Rieske iron-sulfur protein**, all of which participate in the electron transfer. The Q-cycle at Complex III is a sophisticated process where **two electrons from QH₂** are split: one electron moves toward **cytochrome c**, while the other is recycled back to a second ubiquinone molecule, regenerating another QH₂. This **amplifies proton pumping efficiency**, and Complex III uses this mechanism to pump **4 additional protons** into the intermembrane space.

The Q-cycle is a **target for antimalarial and antiparasitic drugs**, such as **atovaquone**, which inhibits Complex III in *Plasmodium falciparum*. In research, **Coenzyme Q analogs** are used in artificial membrane systems to study **redox kinetics** and screen **electron transfer inhibitors** for drug development. In biotechnology, maintaining redox balance through intermediates like ubiquinone is crucial when using **engineered mitochondria** or **reconstituted respiratory chains** in vitro.

Cytochrome c and Complex IV: Final Electron Transfer to Oxygen

Cytochrome c is a small heme-containing protein that accepts one electron at a time from Complex III and delivers it to **Complex IV (cytochrome c oxidase)**. Complex IV is the final complex of the ETC and plays a pivotal role in cellular respiration by transferring electrons to **molecular oxygen (O₂)** — the terminal electron acceptor. Using **four electrons and four protons** from the mitochondrial matrix, Complex IV reduces one O₂ molecule to **two molecules of water (H₂O)**. Concurrently, it pumps **four more protons** into the intermembrane space, further contributing to the PMF.

Cyanide and **carbon monoxide** are potent inhibitors of Complex IV. By binding to the heme groups in cytochrome a₃, these toxins prevent oxygen binding, **halting ATP synthesis** and leading to **rapid cellular death**, particularly in high-demand tissues like the brain and heart. In biotechnology, cytochrome c is a model protein for studying **apoptosis**, as its release into the cytoplasm triggers **caspase activation**. Recombinant cytochrome c is used in **biochemical assays**, **protein folding studies**, and **biosensors** for oxidative stress detection.

ATP Yield and Energy Efficiency

From the complete aerobic oxidation of one glucose molecule, cells derive the following reducing equivalents:

- **10 NADH**: 2 from glycolysis, 2 from pyruvate dehydrogenase, 6 from the TCA cycle.
- **2 FADH₂**: from the TCA cycle via Complex II.

These feed electrons into the ETC and generate ATP:

- **1 NADH** → ~3 ATP → 10 × 3 = **30 ATP**
- **1 FADH₂** → ~2 ATP → 2 × 2 = **4 ATP**
- Total from oxidative phosphorylation: **~34 ATP**

When combined with ATP generated by **substrate-level phosphorylation**:

- **2 ATP from glycolysis**
- **2 GTP (equivalent to ATP) from TCA cycle**
- The theoretical total yield becomes **~38 ATP**, although the actual yield is **30–34 ATP**, considering losses due to:
 - **Shuttling electrons** into the mitochondria (e.g., via malate-aspartate or glycerol-phosphate shuttles)
 - **Proton leak** and thermogenic uncoupling
 - ATP cost of **transporting ADP/Pi and pyruvate** into the mitochondria

Industrial Relevance:

In large-scale **biomanufacturing**, optimizing ATP yield is critical for high-yield production of recombinant proteins, enzymes, or metabolites. Energy balance models are used in metabolic engineering to **maximize ATP-to-product ratios**, especially in microbial strains optimized for **biosynthesis of therapeutics or biofuels**.

Key Concepts

- **Regeneration of NAD^+ and FAD** is crucial for sustaining glycolysis and the TCA cycle. Under aerobic conditions, this is achieved through the ETC; under anaerobic conditions, fermentation is used.
- The **ETC** comprises Complexes I–IV and Coenzyme Q and Cytochrome c. Electrons are transferred stepwise, releasing energy used to pump protons into the intermembrane space.
- The resulting **proton gradient** creates an electrochemical potential (PMF) used by **ATP synthase** to drive **ATP production** via **chemiosmosis**.
- **ATP yield** varies due to losses in proton leak, substrate shuttling, and mitochondrial transport costs.
- In **biotechnology**, ETC components, redox control, and ATP synthase are manipulated to enhance bio-production of proteins, metabolites, or biofuels.
- Clinical relevance includes **neurodegenerative diseases**, **mitochondrial disorders**, and **pharmacological targeting** of ETC components.

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14: Lipid

Learning Objectives

- Classify different types of lipids and describe their chemical structures.
- Explain the function and metabolic significance of triglycerides, including their breakdown and role in gluconeogenesis.
- Differentiate between saturated, unsaturated, and trans fats based on structure and health impact.
- Describe the structure and function of waxes, steroids, and phospholipids in biological systems.
- Explain the role of lipids in membrane dynamics and transport mechanisms (diffusion, osmosis, active/facilitated transport).
- Analyze how lipid metabolism intersects with clinical conditions (e.g., diabetes, cardiovascular disease, and drug effects like Metformin).
- Interpret how membrane-targeting drugs and toxins affect cellular function.

Definition: Term

- **Lipid:** Hydrophobic or amphipathic molecule involved in energy storage, membrane formation, or signaling.
- **Triglyceride:** A lipid formed by glycerol and three fatty acids; main form of stored fat.
- **Lipolysis:** Breakdown of triglycerides into glycerol and free fatty acids.
- **β -oxidation:** Metabolic process that breaks down fatty acids to acetyl-CoA.
- **Gluconeogenesis:** Synthesis of glucose from non-carbohydrate precursors like glycerol.
- **Saturated Fat:** Fatty acid with no double bonds; solid at room temperature.
- **Unsaturated Fat:** Fatty acid with one or more cis double bonds; liquid at room temperature.
- **Trans Fat:** Artificially hydrogenated fat with trans double bonds; associated with health risks.
- **Waxes:** Esters of long-chain fatty acids and alcohols used for protection and waterproofing.
- **Steroid:** Lipid molecule with four fused rings; includes cholesterol and hormones.
- **Phospholipid:** Amphipathic lipid forming the bilayer of biological membranes.
- **Osmosis:** Passive movement of water across a semipermeable membrane.
- **Facilitated Transport:** Passive transport of molecules via membrane proteins.
- **Active Transport:** Energy-requiring transport against a concentration gradient.

Note

- Review structures of lipids and understand ester bonds.
- Read about lipid metabolism, especially triglyceride breakdown and glycerol's entry into glycolysis.
- Familiarize with the difference between types of fats (cis vs. trans) using dietary examples.
- Skim clinical implications of trans fats and Metformin-related lactic acidosis.
- Preview membrane structure and transport mechanisms (use animations if available).

Lipids

Lipids are a diverse group of biomolecules that are hydrophobic or amphipathic in nature. Their primary role in the body includes energy storage, forming biological membranes, insulation, and serving as signaling molecules. Unlike carbohydrates, lipids provide **more than twice the amount of energy per gram**—this is why they are the body's **preferred long-term energy storage molecules**, mainly stored in **adipose tissues**. Their hydrophobic property also makes them ideal for **barrier functions** in cells, forming the lipid bilayer of membranes that control what enters and exits a cell.

Triglycerides

Triglycerides are the most common form of stored fat in the body and serve as a **major energy reservoir**, particularly during periods of fasting or increased energy demand. Structurally, a triglyceride molecule consists of a **single glycerol backbone** esterified to **three fatty acid chains**. These ester bonds are formed through dehydration reactions and are energetically rich,

making triglycerides **highly efficient energy storage molecules**. In fact, gram for gram, triglycerides provide about **2.5 times more energy** than carbohydrates such as glycogen or starch. Due to their hydrophobic nature, triglycerides can be densely packed in adipose tissues **without water**, which further enhances their storage efficiency compared to hydrophilic glycogen.

When the body requires energy—during prolonged exercise, fasting, or low-carbohydrate intake—**triglycerides are broken down in a process called lipolysis**. The fatty acids released from triglycerides enter β -oxidation to produce acetyl-CoA, which then feeds into the TCA cycle. Meanwhile, **glycerol**, the three-carbon backbone of the triglyceride, is shuttled into glycolysis through a well-regulated enzymatic pathway. This involves the following steps:

1. **Glycerol is first phosphorylated by the enzyme glycerol kinase** to form **glycerol-3-phosphate**. This phosphorylation is essential for trapping glycerol within the cell and activating it for further metabolism.
2. **Glycerol-3-phosphate is then oxidized by glycerol-3-phosphate dehydrogenase**, producing **dihydroxyacetone phosphate (DHAP)**. This is an important glycolytic intermediate.
3. **DHAP can be isomerized into glyceraldehyde-3-phosphate (G3P)**, which directly enters glycolysis and contributes to the generation of ATP.

Through this pathway, glycerol derived from triglycerides becomes an important **gluconeogenic substrate** during times when glucose is scarce. In the liver, this mechanism helps maintain blood glucose levels. However, under certain conditions, such as **Type 2 Diabetes** or **excessive lipolysis**, there is a large influx of glycerol into the glycolytic pathway. This shifts metabolic equilibrium toward **increased production of pyruvate**, which under **anaerobic or hypoxic conditions** (such as in poorly oxygenated tissues), is converted into **lactate** rather than entering the TCA cycle. This results in the accumulation of lactic acid—a condition known as **lactic acidosis**.

A clinical correlate of this metabolic shift involves the use of **Metformin**, a first-line medication for managing Type 2 Diabetes. Metformin works by inhibiting hepatic gluconeogenesis, in part by blocking the activity of **glycerol kinase** by inhibiting **mitochondrial glycerol-3-phosphate dehydrogenase (mG3PD)**, thereby reducing the conversion of glycerol into glucose. While this helps lower blood glucose, inhibition of mG3PD also causes **glycerol-3-phosphate (G3P) to accumulate**, stalling the glycerol phosphate shuttle. This disrupts redox balance by **preventing NADH oxidation**, leading to an increase in the cytosolic **NADH/NAD⁺ ratio**. To restore NAD⁺, cells shift metabolism to convert **pyruvate to lactate**, which regenerates NAD⁺ but also leads to **lactate accumulation**. In patients with **renal impairment or tissue hypoxia**, this increases the risk of **metformin-associated lactic acidosis (MALA)**—a rare but serious complication.

Thus, triglyceride metabolism not only provides energy but also interfaces intricately with broader metabolic pathways like glycolysis and gluconeogenesis. Its dysregulation can have both metabolic and clinical consequences, making it a critical topic in both **biochemistry and clinical medicine**.

Saturated vs. Unsaturated vs. Trans Fats

Saturated fatty acids contain no double bonds between the carbon atoms in the hydrocarbon chain. Because their structure is completely linear, the molecules pack tightly together through van der Waals interactions. This tight packing contributes to **higher melting points**, making saturated fats **solid at room temperature**. This property is biologically significant in both animal fats and membrane structure. For instance, membranes enriched in saturated fatty acids are **less fluid and more rigid**, forming **stronger hydrophobic barriers**. From an energy perspective, saturated fats are more reduced and thus **contain more hydrogen atoms**, making them **energy-dense** when oxidized—an important trait for animals that store fat for energy during hibernation or long migrations. However, high dietary intake of saturated fats (commonly found in butter, cheese, and red meat) is linked to elevated LDL cholesterol and **increased cardiovascular risk**.

In contrast, **unsaturated fatty acids** have one or more **cis double bonds** within their hydrocarbon chains. These cis double bonds introduce **kinks or bends** in the fatty acid tail, which prevent tight packing and result in **greater membrane fluidity**. As a result, unsaturated fats are typically **liquid at room temperature** (e.g., oils). In biological membranes, the presence of unsaturated fatty acids helps maintain **membrane flexibility**, which is essential for functions like vesicle fusion, protein mobility, and cell signaling. These fats are also generally considered **cardioprotective**, particularly **monounsaturated** (e.g., oleic acid in olive oil) and **polyunsaturated** fats (e.g., omega-3 and omega-6 fatty acids). These are found in plant oils, nuts, seeds, and fatty fish, and are often emphasized in heart-healthy diets such as the Mediterranean diet.

Trans fats, meanwhile, are a product of **industrial hydrogenation**, a chemical process used to convert cis-unsaturated fats into trans-isomers. This process straightens the fatty acid tail, allowing it to **pack tightly like a saturated fat**, thus improving shelf life and texture in processed foods such as margarine, baked goods, and snacks. Although trans fats contain double bonds, their **trans configuration** eliminates the kink seen in cis forms, giving them a structure—and therefore a **physiological behavior**—similar to saturated fats. However, trans fats are particularly **problematic for health**. They interfere with essential fatty acid metabolism, **raise LDL (bad) cholesterol**, lower HDL (good) cholesterol, promote systemic inflammation, and are strongly linked to an increased risk of **atherosclerosis, heart disease, and stroke**. As a result, many countries have now **banned or restricted** their use in food production.

Trans fats are unhealthy because they disrupt several essential physiological processes. One of their most harmful effects is on cholesterol balance. Trans fats raise LDL ("bad" cholesterol) while lowering HDL ("good" cholesterol), leading to the buildup of cholesterol in arteries and increasing the risk of heart disease and stroke. This imbalance alone significantly raises cardiovascular risk even at small intake levels. Additionally, trans fats promote chronic inflammation by activating inflammatory signaling pathways like NF- κ B and increasing pro-inflammatory cytokines such as TNF- α and IL-6. Chronic inflammation is a common underlying factor in diseases like heart disease, diabetes, and cancer. Furthermore, trans fats are structurally rigid due to their trans double bonds, which makes cell membranes more rigid when incorporated. This rigidity impairs membrane fluidity, affecting the function of membrane proteins, nutrient transport, and cellular signaling.

Trans fats also interfere with insulin signaling, reducing insulin sensitivity and contributing to the development of type 2 diabetes. Unlike healthy fats, they promote the accumulation of visceral fat and may contribute to fatty liver disease. While they can be metabolized for energy, their breakdown is inefficient, and they may accumulate in tissues, leading to long-term effects. Due to these harmful impacts on heart health, metabolism, and inflammation, trans fats are strongly linked to increased risk of chronic diseases and mortality. For these reasons, many countries have banned or strictly regulated their use in food products.

Understanding the structural basis and biological consequences of different types of fatty acids is crucial in biochemistry, nutrition, and medicine. It explains not just how lipids function in membranes and energy metabolism, but also why certain fats are associated with **health risks or benefits**, guiding dietary and therapeutic decisions.

Waxes

Waxes are a specialized class of lipids composed of **long-chain fatty acids esterified to long-chain alcohols**. This combination results in a **highly hydrophobic, solid material** at room temperature. Unlike triglycerides, which serve primarily as energy stores, waxes are structured for **protection and insulation**, serving as **biological barriers** against water loss and environmental damage.

In humans and animals, waxes are secreted by specialized glands onto **skin, fur, and feathers** to create a **water-repellent coating**. For example, human **sebaceous glands** produce waxy lipids that lubricate and protect the skin, while **birds secrete wax from the uropygial gland** to coat their feathers—essential for waterproofing during flight and swimming. Similarly, marine mammals like whales and seals produce wax esters in their skin and ear canals for protection. In the plant kingdom, waxes are critical for survival in terrestrial environments. The **cuticle of leaves, stems, and fruits** is coated with a waxy layer composed of **cutin and wax esters**, which prevents **excessive water loss**, **reduces pathogen invasion**, and **reflects harmful UV radiation**. This adaptation is especially important in arid environments where water conservation is vital.

Commercially, waxes have numerous applications due to their **stability, hydrophobicity, and inert nature**. **Beeswax**, secreted by honeybees, is widely used in making candles, cosmetics, and ointments. **Carnauba wax**, derived from palm leaves, is found in polishes, automobile wax, and even pharmaceutical coatings. In the pharmaceutical and nutraceutical industries, wax coatings are used to **control the release rate of drugs or supplements** in pill form, ensuring **targeted delivery** and **timed absorption**.

Overall, waxes exemplify how lipid chemistry is tailored to specific biological and functional roles. Their long hydrocarbon chains and ester linkages provide the ideal material for **creating durable, water-resistant surfaces**, serving as both a natural adaptation and a foundation for human innovation.

Steroids

Steroids are a unique class of lipids characterized by a core structure made of four fused carbon rings: three six-membered rings and one five-membered ring. Unlike triglycerides or phospholipids, steroids do not contain fatty acid chains, yet they are still

considered lipids due to their nonpolar, hydrophobic nature. Their hydrophobicity allows them to embed within biological membranes and interact with lipid-based molecules, contributing to cellular structure and signaling.

Cholesterol is the most abundant and well-known steroid in the human body. It plays a crucial structural role in maintaining the integrity and fluidity of the plasma membrane. Cholesterol fits between phospholipid molecules in the membrane and helps regulate its consistency across different temperatures. At lower temperatures, it prevents phospholipids from packing too tightly, thereby maintaining fluidity. At higher temperatures, it restrains the movement of phospholipids, stabilizing the membrane and preventing it from becoming overly fluid or permeable. This dual role makes cholesterol essential for maintaining membrane function across a wide range of physiological conditions.

Beyond its structural role, cholesterol is also a vital biochemical **precursor**. It is the starting molecule for the synthesis of several key biological compounds, including steroid hormones such as **estrogen**, **progesterone**, and **testosterone**, which regulate reproductive functions and secondary sexual characteristics. Cholesterol also leads to the production of **corticosteroids** like **cortisol**, which modulate metabolism, stress responses, and immune function. In addition, ultraviolet light acts on cholesterol derivatives in the skin to initiate the synthesis of **vitamin D**, which is critical for calcium absorption and bone health. Thus, while often viewed negatively in the context of cardiovascular health, cholesterol is indispensable to many fundamental biological processes.

Phospholipids and Membrane

Phospholipids are essential building blocks of biological membranes. Each phospholipid molecule has a hydrophilic (water-loving) phosphate head and two hydrophobic (water-repelling) fatty acid tails. This amphipathic nature causes phospholipids to spontaneously form bilayers in aqueous environments, with the hydrophobic tails facing inward and the hydrophilic heads facing outward. This bilayer structure forms the foundation of all cellular membranes, creating a dynamic and semi-permeable barrier between the cell and its environment.

The semi-permeable membrane allows the selective passage of substances. Small nonpolar molecules like oxygen (O_2) and carbon dioxide (CO_2) diffuse freely through the lipid bilayer via **simple diffusion**, moving from areas of high concentration to low concentration without the need for energy. The rate of diffusion depends on several factors, including temperature (higher temperatures increase diffusion), concentration gradient (steeper gradients lead to faster diffusion), molecule size (smaller molecules diffuse faster), charge (nonpolar molecules cross more easily), and pressure differences across the membrane.

Osmosis is a specific type of diffusion involving water molecules. Water moves from regions of low solute concentration (hypotonic) to regions of high solute concentration (hypertonic) across a semipermeable membrane. In a medical context, if an IV solution lacks salts (making it hypotonic), water will enter the patient's cells, potentially causing them to swell and burst (lysis). On a global scale, the melting of freshwater glaciers into salty oceans changes osmolarity, affecting marine organisms that rely on stable osmotic conditions. These examples highlight the importance of osmotic balance in both health and environmental systems.

Facilitated transport is a passive process in which specific protein channels or carrier proteins help move substances like glucose or ions down their concentration gradient—from areas of high to low concentration. Because this process does not require energy (ATP), it is efficient for transporting essential molecules across the membrane when diffusion alone is insufficient.

Active transport, in contrast, moves substances against their concentration gradient—from low to high concentration—and requires ATP. This type of transport is crucial for maintaining concentration differences that cells rely on for function. A key example is the **sodium-potassium pump (Na^+/K^+ -ATPase)**, which maintains high potassium and low sodium concentrations inside cells. This gradient is vital for generating electrical signals in nerve cells and ensuring proper function of muscle and kidney tissues. Another important transporter is the **calcium pump (Ca^{2+} -ATPase)**, which keeps intracellular calcium levels very low. This tight regulation is essential for muscle contraction, neurotransmitter release, and other signaling events.

Biological membranes are not only structural barriers but also targets for certain drugs and toxins. Some antibiotics and microbial toxins exploit the lipid bilayer by forming **channels or pores** that disrupt ion gradients. **Ionophores**, for example, are antibiotics that shuttle ions like potassium or calcium across membranes, bypassing the cell's regulated transport systems. This uncontrolled ion flow disrupts membrane potential and leads to cellular dysfunction or death.

These properties have practical applications. Ionophore antibiotics are used in agriculture to control bacterial infections in livestock. In medicine and research, understanding how these compounds work helps scientists design targeted therapies—such as

using pore-forming toxins in cancer treatment to selectively destroy cancerous cells. Studying how membranes are disrupted also informs our understanding of antibiotic resistance and leads to the development of novel therapeutic strategies.

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15: Lipids Catabolism

Learning Objectives

- Describe how dietary triglycerides are digested, emulsified, and absorbed.
- Explain the role of bile salts and pancreatic enzymes in lipid digestion.
- Trace the journey of absorbed lipids from the intestine through the lymph and blood.
- Distinguish the roles of different lipoproteins (chylomicrons, LDL, HDL).
- Understand how triglycerides are stored and mobilized in adipose tissue.
- Describe the biochemical steps of fatty acid activation and β -oxidation.
- Explain how fasting triggers lipid utilization and gluconeogenesis via glucagon signaling.
- Quantify ATP yield from fatty acid oxidation and compare with glucose metabolism.

Definition: Term

- **Triglyceride:** A lipid molecule made of one glycerol backbone bonded to three fatty acids. It is the main form of fat in food and body fat stores.
- **Lipase (gastric, pancreatic):** Enzymes that break down triglycerides into free fatty acids and monoacylglycerols:
 - **Gastric lipase** acts in the stomach.
 - **Pancreatic lipase** acts in the small intestine and is essential for fat digestion.
- **Bile salts:** Amphipathic molecules made in the liver and stored in the gallbladder. They emulsify large fat droplets into smaller micelles, increasing surface area for enzyme action.
- **Micelle:** Tiny fat droplets formed by bile salts surrounding lipid molecules. Micelles transport digested fats across the intestinal lining for absorption.
- **Chylomicron:** A large lipoprotein particle formed in intestinal cells. It packages and transports triglycerides, cholesterol, and other lipids through the lymphatic system into the bloodstream.
- **Lipoprotein lipase (LPL):** An enzyme located on capillary walls of tissues (like muscle and fat). It breaks down triglycerides in chylomicrons into free fatty acids for cellular uptake and use/storage.
- **LDL and HDL:** Types of lipoproteins that transport cholesterol:
 - **LDL (Low-Density Lipoprotein):** Delivers cholesterol to tissues; high levels can contribute to plaque formation in arteries ("bad cholesterol").
 - **HDL (High-Density Lipoprotein):** Picks up excess cholesterol from tissues and returns it to the liver ("good cholesterol").
- **Hormone-sensitive lipase (HSL):** An enzyme in adipose tissue activated during fasting. It hydrolyzes stored triglycerides into free fatty acids and glycerol for energy use.
- **Fatty acyl-CoA:** A fatty acid linked to coenzyme A (CoA). This activated form is required for fatty acid transport into mitochondria and subsequent β -oxidation.
- **β -oxidation:** The stepwise breakdown of fatty acids in the mitochondria to produce acetyl-CoA, NADH, and FADH₂, which enter the TCA cycle and electron transport chain.
- **Acetyl-CoA:** A 2-carbon molecule formed from fatty acids, carbohydrates, or amino acids. It is the entry point into the TCA cycle for ATP production.
- **Gluconeogenesis:** The process of synthesizing glucose from non-carbohydrate sources like glycerol (from fats), lactate, or amino acids, especially during fasting.
- **Glucagon:** A hormone secreted by the pancreas during low blood glucose (fasting). It promotes lipolysis, gluconeogenesis, and ketogenesis to maintain energy supply.
- **cAMP signaling:** A molecular pathway activated by glucagon binding to its receptor. It uses cyclic AMP (cAMP) as a second messenger to activate enzymes like PKA, which regulates metabolism by phosphorylating target proteins (e.g., HSL).

Digestion of Fatty Foods

When you eat foods rich in fat—like burgers, fried chicken, pizza, or peanut butter—your body begins the process of digesting and absorbing these fats so they can be used for energy, hormone production, or storage. The primary form of fat in food is the **triglyceride**, which is made up of **three fatty acids attached to a glycerol backbone**. However, triglycerides are large, hydrophobic (water-fearing) molecules, and our digestive system is water-based. Therefore, the body needs special strategies to process and absorb them.

Digestion begins in the **stomach**, where an enzyme called **gastric lipase** starts the preliminary breakdown of triglycerides. However, this enzyme is not very efficient and acts mostly on short- and medium-chain triglycerides (like those found in milk). This step is minimal and mainly prepares the fats for further digestion. Most fat digestion happens in the **small intestine**, especially in the **duodenum**, where powerful enzymes and bile are released. The pancreas secretes an enzyme called **pancreatic lipase**, which is specialized in breaking down triglycerides. But before the enzyme can act, **bile salts** from the **liver and gallbladder** emulsify the fat droplets—breaking them into much smaller droplets called **micelles**. This emulsification increases the surface area for pancreatic lipase to work efficiently. Pancreatic lipase then hydrolyzes triglycerides into **2 free fatty acids** and **1 monoacylglycerol** (a glycerol with one fatty acid still attached). These smaller units are small and hydrophobic enough to be absorbed by **enterocytes**, the epithelial cells lining the small intestine.

Once absorbed into the enterocytes, these components are **re-esterified**—reassembled back into full triglycerides. But since triglycerides are still too hydrophobic to travel through the watery blood, they are packed into **chylomicrons**, which are large, spherical **lipoprotein particles** made of triglycerides, cholesterol, and proteins, which act as transport carriers.

Transport and Storage of Lipids

After their assembly into **lipoprotein**, triglycerides leave the intestinal cells and enter the **lymphatic system**—not the bloodstream right away. This is because **lipoprotein** are too large to enter the small blood capillaries. Instead, they are taken up by lymph vessels, which eventually dump their contents into the **thoracic duct**, which connects to the **subclavian vein**, allowing entry into the bloodstream.

Once in the bloodstream, **lipoprotein** delivers triglycerides to various tissues, including **Muscle cells**, which use the triglycerides for **energy** (especially during exercise or fasting), and **Adipose (fat) tissue**, which stores them for **later use** when energy is scarce. To facilitate this delivery, an enzyme called **lipoprotein lipase (LPL)**—anchored on the surface of capillaries—breaks down the triglycerides again into **free fatty acids**, which can then enter muscle or fat cells.

In fat cells, the fatty acids are again reassembled into triglycerides and stored in **lipid droplets**. These droplets can expand or shrink based on your body's energy demands. When you're in a fasted state, triglycerides in these droplets are broken down (lipolysis) to release fatty acids into the bloodstream for use.

Besides transporting triglycerides, **lipoproteins** are crucial for **cholesterol homeostasis**. Two types of lipoproteins are most relevant: LDL and HDL. **LDL** particles carry cholesterol **from the liver to peripheral tissues**. This is essential because cholesterol is a building block for cell membranes and steroid hormones. However, when LDL is **oxidized** (especially due to smoking, poor diet, or stress), it becomes dangerous. **Oxidized LDL** is taken up by immune cells called **macrophages**, which then become **foam cells**—lipid-laden cells that accumulate inside blood vessel walls. Over time, these form **plaques**, leading to **atherosclerosis** and increased risk of heart attack or stroke. HDL particles work in reverse—they **collect excess cholesterol** from tissues and blood vessels and bring it back to the **liver** for excretion. This protective action helps **clear the bloodstream** and prevents plaque buildup.

Statins like **Atorvastatin (Lipitor)** help lower LDL cholesterol. They work by **inhibiting HMG-CoA reductase**, an enzyme the liver uses to make cholesterol, and **upregulating LDL receptors** on liver cells, allowing the liver to **pull more LDL out of circulation**. This dual action **lowers blood LDL** levels and reduces the risk of cardiovascular diseases. If someone has high cholesterol and their doctor prescribes Lipitor, it helps reduce LDL in their blood and prevent artery plaque formation—protecting against heart disease.

Fatty Acid Activation

Before your cells can use **fatty acids** for energy, the fatty acids need to be **activated**. This process occurs in the **cytoplasm** and involves "tagging" the fatty acid with a molecule of **Coenzyme A (CoA)**, creating a compound called **fatty acyl-CoA**. This activation step is catalyzed by the enzyme **acyl-CoA synthetase** (also known as thiokinase), and it requires **energy from ATP**: **Fatty Acid + CoA + ATP → Fatty Acyl-CoA + AMP + PPi**

This reaction uses **2 high-energy phosphate bonds** because ATP is converted to **AMP** (not ADP), and pyrophosphate (PPi) is quickly broken down to drive the reaction forward, making it essentially irreversible.

Once formed, **fatty acyl-CoA** has two major fates:

1. **Biosynthesis**: It can be used to make structural lipids, such as **phospholipids**, **sphingolipids**, or **waxes**—important in membranes, skin, and signaling molecules.
2. **Energy production**: It can be transported into the **mitochondrial matrix** for **β-oxidation**, a catabolic process that breaks down fatty acids to produce ATP.

What About Glycerol? When triglycerides are broken down (especially during **fasting or prolonged exercise**), **glycerol** is released alongside fatty acids. Glycerol, being water-soluble, travels through the bloodstream to the **liver**, where it has two potential fates:

1. It can enter **glycolysis**, being converted into **pyruvate**, which can then become **acetyl-CoA** or **lactate**.
2. Or, it can enter **gluconeogenesis**, the pathway to generate **glucose**, helping maintain blood sugar levels during fasting.

β-Oxidation

Once inside the mitochondria, **fatty acyl-CoA** enters the **β-oxidation cycle**, which is the process that breaks down fatty acids **two carbons at a time**, producing **acetyl-CoA**, **NADH**, and **FADH₂** in each cycle. This process continues until the entire fatty acid chain is chopped into two-carbon units. Each round of β-oxidation includes **four key steps**, each catalyzed by a specific enzyme.

Step 1: Oxidation

- **Enzyme**: *Acyl-CoA dehydrogenase*
- **What happens**: A **double bond** is introduced between the **α (C2)** and **β (C3)** carbons of the fatty acid chain.
- **What's made**: **FADH₂** is produced as electrons are transferred to FAD.
 - This step contributes to the **electron transport chain**, eventually making **2 ATP per FADH₂**.

Step 2: Hydration

- **Enzyme**: *Enoyl-CoA hydratase*
- **What happens**: A **water molecule** is added across the double bond, introducing a **hydroxyl group (-OH)** on the β-carbon.
 - This sets up the β-carbon for further oxidation.

Step 3: Oxidation

- **Enzyme**: *Hydroxyacyl-CoA dehydrogenase*
- **What happens**: The hydroxyl group is **oxidized to a ketone**, forming a **β-ketoacyl-CoA**.
- **What's made**: **NADH** is generated as electrons are transferred to NAD⁺.
 - This NADH goes to the electron transport chain to produce about **3 ATP**.

Step 4: Thiolysis (Cleavage)

- **Enzyme**: *Thiolase*
- **What happens**: A **new CoA** molecule attacks the β-keto group, cleaving the molecule and releasing one **acetyl-CoA** and a **shortened fatty acyl-CoA** (2 carbons shorter).
 - The remaining fatty acyl-CoA enters another round of β-oxidation until completely converted into acetyl-CoA.

Let's do the math!

A 12-carbon fatty acid like *lauric acid* will undergo **5 cycles** of β -oxidation (because $12/2 - 1 = 5$), producing:

- **6 Acetyl-CoA** (remember that the final round gives 2 Acetyl-CoA) = **60 ATP**
 - (Each acetyl-CoA makes 3 NADH + 1 FADH₂ + 1 ATP in the TCA cycle → ~10 ATP total)
- **5 NADH = 15 ATP**
- **5 FADH₂ = 10 ATP**

Total = 10 + 15 + 60 = 85 ATP

But remember that there are three fatty acid tails, so $3 \times 85 = 255 \text{ ATP}$ in total! Wow!

- But remember: **Activation cost = 2 ATP per fatty acid**, so **subtract 6 ATP**. **Final Net ATP = 255 - 6 = 249 ATP**
- That's **nearly 10 times** the ATP produced by one molecule of glucose!

Unsaturated Fatty Acids

Unsaturated fats (like oleic acid or linoleic acid found in olive oil and nuts) have **pre-existing double bonds**, which **skip the first oxidation step** in β -oxidation. Because the first oxidation step produces **FADH₂**, skipping it means you **lose the ATP that FADH₂ would've generated**. So, for **each double bond** in an unsaturated fatty acid, you will miss out on **1 FADH₂ = 2 ATP lost**. **Thus**, A 12-carbon unsaturated fatty acid with one double bond yields: $85 \text{ ATP} - 2 \text{ ATP (for skipped FADH}_2) = \sim 83 \text{ ATP}$

Though unsaturated fats give slightly less ATP, they are **healthier** for cardiovascular health and have other metabolic benefits (like modulating inflammation and membrane fluidity).

Glucagon during Fasting

When you eat, your body releases **insulin**, which promotes storage of energy (like fat and glycogen). However, when you're **fasting**—for example, overnight or during a skipped meal—your blood glucose levels drop. In response, your pancreas (specifically the **α -cells**) secretes **glucagon**, the major "fasting hormone." Glucagon's job is to **preserve blood glucose** for critical organs like the brain and red blood cells. It does this by altering enzyme activity via **cAMP signaling pathways**.

Glucagon binds to the receptor on **liver cells**, activating the **adenylate cyclase-cAMP-PKA** signaling cascade. This results in **phosphorylation of enzymes**, switching off glucose-consuming processes and switching on glucose-producing ones.

- **Inhibition of Pyruvate Kinase:**
 - Pyruvate kinase is the final enzyme in **glycolysis**, converting phosphoenolpyruvate (PEP) to **pyruvate**. Glucagon triggers **PKA**, which **phosphorylates and inactivates** pyruvate kinase in the liver. This effectively **halts glycolysis**, preventing glucose breakdown when it's needed elsewhere.
- **Activation of Gluconeogenesis:**
 - Instead of breaking glucose down, the liver now starts making it. Glucagon induces **PEP carboxykinase (PEPCK)** and other gluconeogenic enzymes, enabling conversion of non-carbohydrate sources—like **glycerol, lactate, and amino acids**—into glucose. This newly synthesized glucose is **exported** to maintain blood sugar.

Another major effect of glucagon (and also **epinephrine** during stress) is the activation of **hormone-sensitive lipase (HSL)** in **adipocytes** (fat cells). This enzyme breaks down **triglycerides** stored in fat droplets into:

- **Free fatty acids (FFAs)** → released into the blood and taken up by organs (like liver and muscle) for **β -oxidation**
- **Glycerol** → travels to the **liver**, where it serves as a **gluconeogenic substrate**. Glycerol is converted into **glycerol-3-phosphate**, then into **dihydroxyacetone phosphate (DHAP)**—a glycolytic intermediate. From here, it enters **gluconeogenesis** to form glucose.

In tissues like the **liver and muscle**, the released **fatty acids** are taken up and converted into **fatty acyl-CoA** for **β -oxidation** in the mitochondria. This generates:

- **ATP** to meet energy demands
- **NADH and FADH₂**, which power the electron transport chain

- **Acetyl-CoA**, which can either:
 - Enter the **TCA cycle** (in energy-demanding tissues)
 - Be diverted in the **liver** to make **ketone bodies**, especially during prolonged fasting

Putting It All Together: Example

Let's say you've gone 12 hours without food (like overnight fasting). Here's what happens:

- **Glucagon rises**, insulin falls.
- In your **liver**, glucagon:
 - Turns **off glycolysis** (via pyruvate kinase inhibition)
 - Turns **on gluconeogenesis** (making glucose for the brain)
- In your **adipose tissue**, glucagon:
 - Activates **HSL**, which breaks down triglycerides
 - Releases **fatty acids** for energy and **glycerol** for gluconeogenesis
- Your **muscles** and **liver** use **fatty acids** for ATP, while the **brain uses glucose** (and eventually **ketone bodies** if fasting is prolonged).

Summary Table: Beta-Oxidation of a 12-Carbon Fatty Acid

Step	Output per round	Total (per chain)	Total (per triglyceride)
β -Oxidation rounds	5 rounds		
FADH ₂	1/round	$5 \times 2 = 10 \text{ ATP}$	$3 \times 10 = 30 \text{ ATP}$
NADH	1/round	$5 \times 3 = 15 \text{ ATP}$	$3 \times 15 = 45 \text{ ATP}$
Acetyl-CoA	$6 \times 10 \text{ ATP}$	60 ATP	$3 \times 60 = 180 \text{ ATP}$
Total		85 ATP	255 ATP
Activation cost	-2 ATP/chain	-6 ATP total	Final: ~249 ATP

Post-Lecture Objectives

- Diagram the full pathway from dietary triglyceride intake to cellular energy production.
- Identify where specific enzymes act in digestion and metabolism of lipids.
- Describe how lipoproteins enable fat transport in aqueous environments.
- Calculate ATP yield from β -oxidation of saturated vs. unsaturated fatty acids.
- Explain the molecular mechanism by which glucagon regulates metabolism during fasting.
- Predict metabolic shifts during prolonged fasting or starvation.

Key Takeaways

- Fat digestion primarily occurs in the **duodenum** with the help of **bile salts** and **pancreatic lipase**.
- Lipids are absorbed as **free fatty acids** and **monoacylglycerols**, reassembled into **triglycerides**, and packaged into **chylomicrons**.
- In the bloodstream, **LPL** hydrolyzes triglycerides for uptake by **muscle** (for energy) or **adipose tissue** (for storage).
- **Fatty acid activation** is ATP-dependent and necessary for mitochondrial entry and β -oxidation.
- **β -oxidation** is a 4-step cycle that generates **NADH**, **FADH₂**, and **acetyl-CoA**, feeding into the **TCA cycle** and **ETC** for maximal ATP yield.
- **Unsaturated fatty acids** yield slightly less ATP due to skipped FADH₂ production steps.

- During fasting, **glucagon** shifts metabolism by halting glycolysis, promoting gluconeogenesis, and activating lipolysis via **HSL**.

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16: Lipids Anabolism

Learning Objectives

- Explain the overall process and purpose of fatty acid anabolism (lipogenesis).
- Describe the role of **Acetyl-CoA Carboxylase (ACC)** and **Fatty Acid Synthase (FAS)** in fatty acid synthesis.
- Outline the four key steps involved in fatty acid elongation: **condensation**, **reduction**, **dehydration**, and **final reduction**.
- Discuss how hormonal regulation (insulin vs. glucagon) influences the direction of fatty acid metabolism.
- Apply your understanding of metabolic states (fed vs. fasting) to predict whether the body will favor lipogenesis or fatty acid oxidation.

Definition: Term

- **Lipogenesis:** The anabolic process of synthesizing fatty acids from acetyl-CoA in the cytoplasm.
- **Acetyl-CoA:** A central metabolic intermediate that serves as the building block for fatty acid synthesis.
- **Malonyl-CoA:** A three-carbon intermediate formed from acetyl-CoA, used to elongate fatty acid chains.
- **Acetyl-CoA Carboxylase (ACC):** The rate-limiting enzyme that catalyzes the formation of malonyl-CoA using biotin and ATP.
- **Fatty Acid Synthase (FAS):** A multifunctional enzyme complex that performs sequential reactions to elongate fatty acids.
- **Acyl Carrier Protein (ACP):** A domain of FAS that temporarily holds fatty acid intermediates during synthesis.
- **NADPH:** A reducing agent derived from the pentose phosphate pathway, used in biosynthetic reactions.
- **Condensation:** The step in fatty acid synthesis where acetyl-ACP and malonyl-ACP combine, releasing CO₂.
- **Hormone-sensitive lipase:** An enzyme that breaks down stored triacylglycerols into free fatty acids during fasting.
- **Carnitine Palmitoyltransferase I (CPT I):** Mitochondrial enzyme that transports fatty acids for β-oxidation, inhibited by malonyl-CoA.

Fatty Acid Anabolism (Lipogenesis)

Fatty acid anabolism, also known as **lipogenesis**, is the metabolic process through which the body synthesizes fatty acids from smaller molecular building blocks. This anabolic process primarily occurs in the **cytoplasm** of cells, particularly in **liver**, **adipose tissue**, and **mammary glands**. The building blocks for fatty acid synthesis are **acetyl-CoA** molecules, which are assembled **two carbons at a time** to produce long-chain fatty acids. This energy-intensive process requires **ATP**, **NADPH**, and **biotin** as cofactors. **Acetyl-CoA Carboxylase (ACC)** and **Fatty Acid Synthase (FAS)** are the two primary enzymes involved.

Acetyl-CoA Carboxylase (ACC)

The first committed step of lipogenesis is the **carboxylation of acetyl-CoA** to form **malonyl-CoA**, catalyzed by the enzyme **Acetyl-CoA Carboxylase (ACC)**. This enzyme uses **ATP** and **biotin** as a coenzyme to add a **carboxylic acid group** to acetyl-CoA, forming malonyl-CoA. This step is crucial because **malonyl-CoA serves as the donor of two-carbon units** in fatty acid elongation. In practical terms, when a person eats a carbohydrate-rich meal, glucose is metabolized into acetyl-CoA, and in the presence of insulin, ACC is activated to drive the synthesis of malonyl-CoA. This step is also **highly regulated** by nutritional and hormonal cues: insulin stimulates ACC, while glucagon and AMPK inhibit it via phosphorylation.

Once **acetyl-CoA** is converted into **malonyl-CoA**, **both** of these intermediates are loaded onto **Fatty Acid Synthase (FAS)**. Within this complex, the intermediates are temporarily attached to a domain called **Acyl Carrier Protein (ACP)**. This carrier domain acts like a molecular "arm," holding and transferring the growing fatty acid chain through the various enzymatic steps. The initial step involves attaching one acetyl group and one malonyl group to the ACP, forming **acetyl-ACP** and **malonyl-ACP**, respectively. This setup primes the system for elongation reactions.

Fatty Acid Elongation

The core of fatty acid synthesis involves a repeating cycle of four reactions: **condensation**, **reduction**, **dehydration**, and **another reduction**, each extending the carbon chain by two units.

1. **Condensation (Step 1): Fatty Acid Synthase** catalyzes the condensation of **acetyl-ACP** and **malonyl-ACP**, releasing **CO₂** and forming a β -keto intermediate called **acetoacetyl-ACP**. The CO₂ released in this step was originally added by ACC in the formation of malonyl-CoA. This decarboxylation helps drive the condensation reaction forward energetically. This is the step where the fatty acid chain begins to form.
2. **Reduction (Step 2):** The **β -ketone group** in acetoacetyl-ACP is reduced to a **β -hydroxyl group**, a reaction that consumes **NADPH** as a reducing agent. This reduction is analogous to reducing a ketone to an alcohol, and NADPH used here is typically sourced from the **pentose phosphate pathway**.
3. **Dehydration (Step 3):** The β -hydroxy group undergoes **dehydration**, resulting in the formation of a **double bond** between the α and β carbon atoms (α,β -unsaturated intermediate). This step helps stabilize the growing fatty acid chain and prepare it for the next reduction.
4. **Reduction (Step 4):** Finally, the double bond is **reduced again** by another molecule of **NADPH**, yielding a fully saturated four-carbon fatty acid intermediate. This product is now ready to go through **another round of elongation**, with a new malonyl-CoA providing two more carbon units.

These four steps repeat several times (typically **seven rounds**) until a **16-carbon saturated fatty acid** (palmitate) is produced.

Hormonal Regulation

The regulation of lipogenesis is tightly controlled by the **nutritional state** of the body and hormones such as **insulin** and **glucagon**. After a carbohydrate-rich meal, **insulin** is released, which activates lipogenesis through multiple mechanisms:

- **Glucokinase** is activated in the liver, promoting glycolysis and the conversion of glucose into **pyruvate** and then **acetyl-CoA**.
- Some **glucose-6-phosphate** is diverted to glycogen synthesis, while **glyceraldehyde-3-phosphate** serves as a precursor for **glycerol** synthesis (for triglyceride formation).
- Excess **acetyl-CoA** is used for fatty acid synthesis.
- Insulin also activates **phosphodiesterase**, which breaks down cAMP, thereby reversing the actions of glucagon and reducing PKA-mediated phosphorylation.

When fasting or under low glucose conditions:

- **Glucagon** is released and activates **cAMP-PKA signaling**, which **inhibits pyruvate kinase** in the liver, reducing glycolysis and glucose utilization.
- cAMP-dependent phosphorylation also **inhibits Acetyl-CoA Carboxylase**, thus blocking lipogenesis.
- Simultaneously, glucagon activates **hormone-sensitive lipase**, which breaks down stored **triacylglycerols** into **free fatty acids and glycerol**.
- **Glycerol** enters gluconeogenesis to help maintain blood glucose, while **fatty acids are diverted toward β -oxidation** to produce energy, not storage.

If a person is fasting for 24 hours, their liver is flooded with glucagon. As a result, **acetyl-CoA carboxylase is phosphorylated and inactivated**, preventing the formation of malonyl-CoA. At the same time, low malonyl-CoA levels remove inhibition on **carnitine palmitoyltransferase I (CPT I)**, allowing fatty acids to enter the mitochondria for β -oxidation. Hence, the body stops storing fat and shifts entirely toward **burning it** for ATP.

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