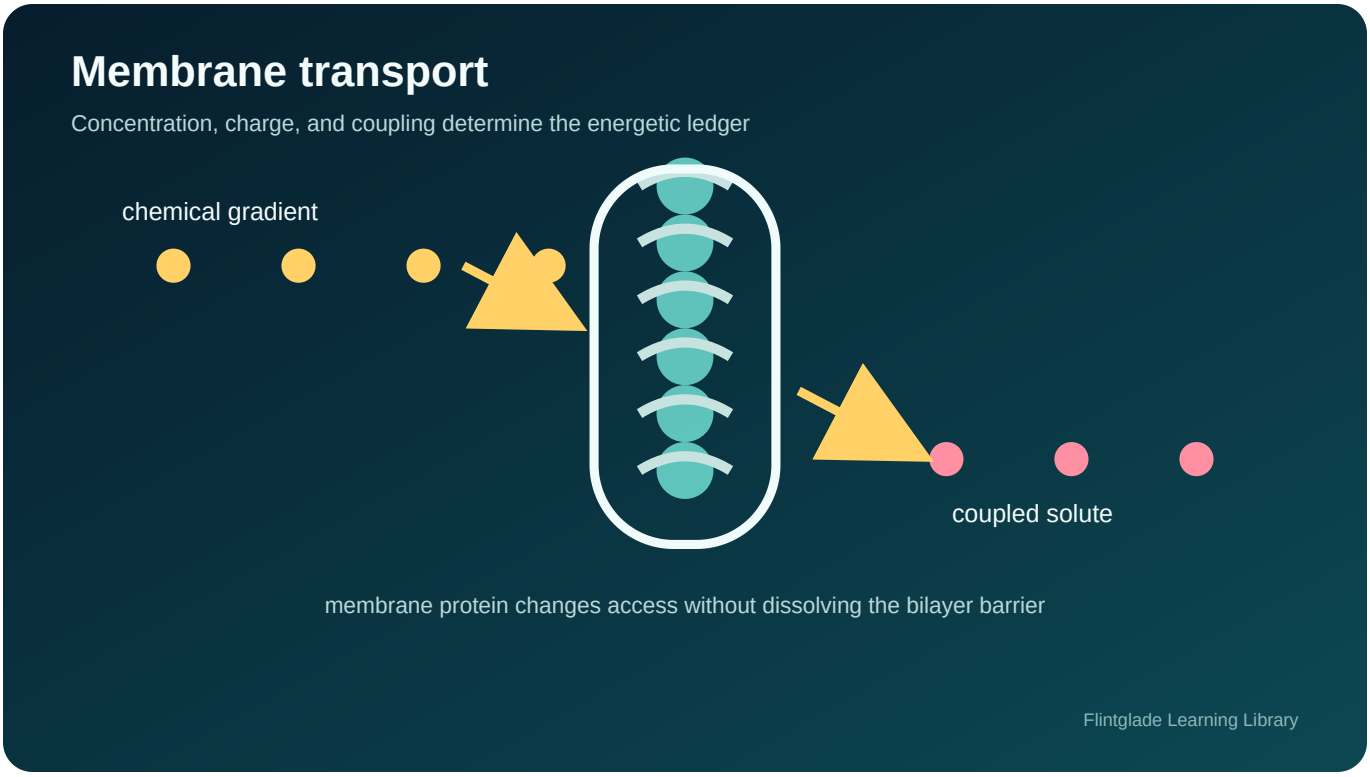
Biomolecules and Catabolism: Core Concepts

Flintglade Learning Library · General Biochemistry

Audience: Readers building an integrated foundation in membranes, transport, metabolism, and energy conversion.

MCAT connections: This guide can assist study of amino acids and proteins, energetics and metabolism while keeping general biochemistry as its primary frame.



About this edition: This standalone edition organizes the material for independent reading with clear navigation, reusable reasoning frameworks, and print-ready presentation.

Learning goals

- Relate molecular structure to biological function
- Trace carbon and energy through central metabolism
- Explain transport and membrane behavior with physical principles

Navigate this guide

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A Foundational Knowledge Base

Topic: Lipids and Membrane Structure

12.1 Fatty Acids — Structure, Naming, and Physical Properties

A fatty acid is a long-chain hydrocarbon terminated at one end by a carboxylic acid group. The hydrocarbon chain is almost always unbranched, and the total number of carbon atoms is almost always even, because fatty acids are built from two-carbon acetyl units during biosynthesis. Fatty acids are named by two numbers separated by a colon: the first number is the total carbon count, and the second is the number of carbon–carbon double bonds. If double bonds are present, their positions are specified using the delta (Δ) notation, which counts from the carboxyl carbon as carbon 1. For example, oleic acid is designated 18:1, Δ^9, meaning it contains 18 carbons and one double bond between carbons 9 and 10.

A fully saturated fatty acid has no double bonds and adopts an extended, all-trans zigzag conformation. This allows saturated chains to pack tightly against one another through extensive van der Waals contacts along the entire length of the chain. Tight packing means strong intermolecular interactions, which translates to a high melting point. Stearic acid (18:0) melts at about 70°C, meaning it is a solid at physiological temperature.

An unsaturated fatty acid contains one or more double bonds. In biological systems, these double bonds are nearly always in the cis (Z) configuration. A cis double bond introduces a rigid kink of approximately 30 degrees into the hydrocarbon chain. This kink disrupts the regular packing

geometry, preventing neighboring chains from aligning as closely. Fewer van der Waals contacts means weaker intermolecular forces, which translates to a lower melting point. Oleic acid (18:1, Δ^9) melts at about 16°C, and linolenic acid (18:3, $\Delta^{9,12,15}$) melts at about -11°C. The more double bonds a fatty acid has, the more disrupted its packing, and the lower its melting point. Chain length also matters: shorter chains have fewer van der Waals contacts and therefore lower melting points than longer chains of the same saturation.

The fatty acids most commonly encountered in this guide are: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1, Δ^9), linoleic acid (18:2, $\Delta^{9,12}$), α-linolenic acid (18:3, $\Delta^{9,12,15}$), and arachidic or eicosanoic acid (20:0). When constructing structures, it is essential to draw each cis double bond with the correct geometry — the two hydrogen atoms on the double-bond carbons are on the same side, and the chain bends at that point.

12.2 Triacylglycerides

A triacylglyceride (also called a triglyceride or triacylglycerol) consists of a glycerol backbone with three fatty acids attached via ester bonds. Glycerol is a three-carbon molecule with a hydroxyl group on each carbon. Each hydroxyl reacts with the carboxyl group of a fatty acid in a condensation reaction, releasing water and forming an ester linkage. The three positions on glycerol are designated sn-1 (top), sn-2 (middle), and sn-3 (bottom) using the stereospecific numbering convention.

To draw a triacylglyceride, you place the glycerol backbone vertically. At each of the three positions, draw the ester bond (the carbonyl oxygen points away from the chain, and the ester oxygen connects to the glycerol carbon), and then extend the appropriate fatty acid chain. For a saturated chain, the hydrocarbon extends in a straight zigzag. For each cis double bond, the chain bends. The order of the fatty acids on the glycerol backbone can vary; a given structure may specify which fatty acid goes where, or you may place them in any order if unspecified.

Triacylglycerides are the primary storage form of metabolic fuel in animals. They are highly reduced molecules, meaning they contain a large number of C–H bonds and very few C–O bonds, which makes them energy-dense. They are also hydrophobic, which means they can be stored without associated water, further increasing energy density per unit mass compared to carbohydrates like glycogen.

12.3 Glycerophospholipids

A glycerophospholipid has the same glycerol backbone as a triacylglyceride, but with a critical structural difference. Fatty acids are esterified at the sn-1 and sn-2 positions, while the sn-3 position carries a phosphate group linked to a polar head group through a phosphodiester bond. This architecture makes glycerophospholipids amphipathic: the two fatty acyl tails are hydrophobic, and the phosphorylated head group is hydrophilic.

The identity of the head group determines the specific type of glycerophospholipid. Phosphatidylethanolamine has ethanolamine ($HO–CH_2–CH_2–NH_2$) as the head group. Phosphatidylcholine has choline ($HO–CH_2–CH_2–N^+(CH_3)_3$). Phosphatidylserine has serine. Phosphatidylinositol has the cyclic sugar alcohol inositol. When drawing these molecules, the

phosphate connects to the sn-3 oxygen of glycerol on one side and to the hydroxyl of the head group alcohol on the other side, forming the phosphodiester bridge. The phosphate group carries a negative charge at physiological pH, contributing to the overall polar character of the head group region.

12.4 Sphingolipids

Sphingolipids are built on a different backbone than glycerophospholipids. Instead of glycerol, the backbone is sphingosine, an 18-carbon amino alcohol with a trans double bond between C4 and C5, a hydroxyl at C1 and C3, and an amino group at C2. A fatty acid is attached to the amino group at C2 via an amide bond, producing a molecule called a ceramide. Different head groups attached at the C1 hydroxyl produce different sphingolipid classes.

Cerebrosides have a single monosaccharide (such as glucose or galactose) attached at C1. Gangliosides have a more complex oligosaccharide chain attached at C1, often containing sialic acid residues. Sphingomyelin has a phosphocholine head group at C1, making it structurally analogous to phosphatidylcholine but with a sphingosine backbone instead of glycerol. Sphingolipids are abundant in the nervous system, particularly in the myelin sheath.

12.5 The Lipid Bilayer

The amphipathic nature of glycerophospholipids drives their spontaneous self-assembly into a lipid bilayer in aqueous environments. The hydrophobic tails of two layers of lipid molecules face inward, sequestered from water, while the hydrophilic head groups face outward into the aqueous environment on both sides of the membrane. This arrangement is thermodynamically favorable because it minimizes the entropic cost of ordering water molecules around exposed hydrophobic surfaces — the driving force is the hydrophobic effect.

The bilayer is not a static structure. It is a two-dimensional fluid in which individual lipid molecules can move laterally (within the plane of the membrane) rapidly and freely. However, transverse movement (flipping from one leaflet to the other, called "flip-flop") is extremely rare without the assistance of enzymes called flippases, floppases, or scramblases, because it requires the polar head group to traverse the hydrophobic interior.

12.6 Membrane Fluidity and Its Determinants

The fluidity of a lipid bilayer refers to the ease with which lipid molecules can move laterally within the membrane. Fluidity is essential for proper membrane function: membrane proteins must be able to diffuse laterally to interact with their partners, and the membrane must be flexible enough to accommodate the conformational changes of integral proteins like transporters and channels.

Three structural features of the lipid components determine fluidity. First, the degree of unsaturation of the fatty acyl chains: more unsaturated (more cis double bonds) means more kinks, less packing, and greater fluidity. Second, the chain length: shorter chains interact less strongly through van der Waals forces, resulting in higher fluidity. Third, the presence of cholesterol.

Cholesterol is a steroid lipid with a rigid four-ring structure, a short hydrocarbon tail, and a small polar hydroxyl head group. It inserts into the bilayer with its hydroxyl group near the head group region and its rigid ring system alongside the upper portions of the fatty acyl chains. Cholesterol has a dual effect on fluidity that depends on temperature. At high temperatures, where the membrane would otherwise be very fluid, the rigid steroid ring restricts the movement of neighboring acyl chains, decreasing fluidity. At low temperatures, where the membrane would otherwise be rigid and potentially gel-like, cholesterol disrupts the regular crystalline packing of saturated chains, preventing the phase transition to a gel state and thus increasing fluidity. The net effect is that cholesterol acts as a fluidity buffer, narrowing the range of temperatures over which the membrane undergoes a sharp phase transition.

12.7 FRAP — Experimental Evidence for Membrane Fluidity

Fluorescence Recovery After Photobleaching is an experimental technique that directly measures the lateral diffusion rate of membrane components. The procedure involves labeling membrane lipids or proteins with a fluorescent dye, then using an intense focused laser to irreversibly bleach the fluorophores in a small, defined region of the membrane. Immediately after bleaching, that region appears dark. Over time, unbleached fluorescent molecules from surrounding regions diffuse laterally into the bleached zone, and bleached molecules diffuse out, causing the fluorescence in the bleached region to recover.

The rate of recovery is a direct measure of lateral diffusion speed, which is a proxy for membrane fluidity. A more fluid membrane will show faster recovery (steeper initial slope on a plot of fluorescence intensity versus time), while a less fluid membrane will show slower recovery. In a plot, both eventually approach the same plateau, but the more fluid membrane reaches it sooner. A membrane with a high ratio of unsaturated to saturated fatty acids will recover faster than one with a low ratio, because the unsaturated chains pack less tightly and permit faster lateral diffusion.

12.8 Membrane Proteins

Membrane proteins are classified based on how they associate with the bilayer. Integral membrane proteins are embedded within the lipid bilayer, with one or more segments spanning the hydrophobic core. The transmembrane segments are typically alpha-helices composed of hydrophobic amino acid residues. A protein like the GLUT4 glucose transporter crosses the membrane twelve times, meaning it has twelve transmembrane helices. On a hydropathy plot, which graphs the average hydrophobicity of amino acid residues along the protein sequence, each transmembrane helix appears as a peak above the threshold line. A twelve-pass transmembrane protein would therefore show twelve distinct hydrophobic peaks.

Peripheral membrane proteins do not penetrate the bilayer. They associate with the membrane surface through electrostatic interactions and hydrogen bonds with the polar head groups of lipids or with exposed regions of integral proteins. They can be removed from the membrane by changes in pH or ionic strength, without disrupting the bilayer itself.

The fluid mosaic model describes the membrane as a two-dimensional fluid lipid bilayer in which integral proteins are embedded like icebergs floating in a sea, free to diffuse laterally. This model, supported by experiments including FRAP and freeze-fracture electron microscopy, captures the essential features of membrane architecture: the lipid bilayer provides the structural barrier, and proteins embedded within it carry out the functional roles of transport, signaling, and catalysis.

12.9 Thermal Adaptation of Membrane Composition

Organisms that live at different temperatures adjust their membrane lipid composition to maintain appropriate fluidity — a principle called homeoviscous adaptation. Bacteria isolated from cold environments (such as glacial lakes at 4°C) will have membranes enriched in unsaturated and polyunsaturated fatty acids with shorter chain lengths, because these features prevent the membrane from becoming too rigid at low temperatures. Bacteria from hot environments (such as geothermal springs at 40°C) will have membranes enriched in saturated fatty acids with longer chain lengths, which prevents the membrane from becoming excessively fluid and unstable at high temperatures. If the lipid compositions were swapped, the cold-adapted organism's membranes would be dangerously fluid at 40°C (leading to membrane instability and cell death), and the heat-adapted organism's membranes would be dangerously rigid at 4°C (preventing proper protein function and solute transport).

Topic: Solute Transport

13.1 Membrane Permeability

The lipid bilayer is a selective permeability barrier. Small, nonpolar molecules such as O_2, CO_2, and N_2 can diffuse freely across the membrane because they can dissolve in and pass through the hydrophobic interior. Water, despite being polar, can also cross the bilayer to a limited extent due to its small size, though aquaporin channels greatly increase the rate. However, ions (Na^+, K^+, Cl^-, H^+, Ca^{2+}), large polar molecules (glucose, amino acids), and charged molecules (ATP, phosphorylated intermediates) cannot cross the lipid bilayer at any appreciable rate without the assistance of membrane proteins. This impermeability to charged and polar species is what makes the membrane a useful barrier for establishing and maintaining concentration gradients, electrical potentials, and compartmentalized chemistry.

13.2 Passive Transport

Passive transport is the movement of a solute across a membrane down its concentration gradient — from high concentration to low concentration. This process is thermodynamically favorable (negative ΔG) and does not require an external energy source. Simple diffusion is passive transport without any protein involvement, and it applies only to small nonpolar molecules. Facilitated diffusion is passive

transport mediated by a membrane protein — either a channel or a carrier (transporter). The protein provides a pathway through the membrane that accommodates the polar or charged solute, but the driving force is still the concentration gradient.

The GLUT family of glucose transporters mediates facilitated diffusion of glucose. GLUT4, for example, allows glucose to enter muscle and adipose cells by moving down the glucose concentration gradient. The transporter is an integral membrane protein that crosses the bilayer twelve times and undergoes conformational changes to alternately expose the glucose-binding site to one side of the membrane and then the other. Importantly, facilitated diffusion reaches a maximum rate (saturation) when all transporter molecules are occupied, which distinguishes it kinetically from simple diffusion.

13.3 Active Transport — Primary

Active transport is the movement of a solute against its concentration gradient — from low concentration to high concentration. This is thermodynamically unfavorable and requires an input of free energy. In primary active transport, the energy comes directly from the hydrolysis of ATP or another high-energy molecule.

The Na^+/K^+-ATPase is the paradigmatic primary active transporter. It pumps 3 Na^+ ions out of the cell and 2 K^+ ions into the cell per molecule of ATP hydrolyzed. Both ions are moved against their respective concentration gradients. The resulting low intracellular Na^+ concentration and high intracellular K^+ concentration are essential for establishing the membrane potential and for driving secondary active transport processes.

The H^+/K^+-ATPase in stomach parietal cells is an antiporter that uses ATP hydrolysis to pump H^+ ions into the stomach lumen (creating the acidic environment needed for digestion) while simultaneously importing K^+ ions into the cell. Because both ions are moved against their gradients by the direct action of ATP hydrolysis, this is primary active transport. In an antiporter, the two solutes move in opposite directions through the same protein.

13.4 Active Transport — Secondary

In secondary active transport, the movement of one solute against its gradient is driven not by ATP directly, but by the simultaneous movement of a second solute down its gradient. The free energy stored in the gradient of the second solute (which was established by a primary active transporter) is the energy source.

The Na^+–glucose symporter (SGLT1) in intestinal brush border cells is the canonical example. Na^+ ions flow from the intestinal lumen (high $[Na^+]$) into the cell (low $[Na^+]$) down their electrochemical gradient. This favorable flow of Na^+ is coupled to the simultaneous transport of glucose from the lumen into the cell against the glucose concentration gradient. In a symporter, both solutes move in the same direction. The low intracellular $[Na^+]$ that drives this process is maintained by the Na^+/K^+-ATPase on the basolateral membrane, which continuously pumps Na^+ out of the cell. Thus, the ultimate energy source for glucose uptake is still ATP, but the coupling is indirect.

In the brush border cell model, glucose accumulates inside the cell to a concentration higher than in either the intestinal lumen or the blood capillaries. On the basolateral side, glucose exits the cell into the capillaries via a GLUT transporter (facilitated diffusion, moving down the gradient from cell to capillary). The cell thus serves as a concentrating relay: active uptake on one side, passive release on the other.

13.5 Thermodynamics of Solute Transport

The free energy change associated with transporting a solute across a membrane depends on whether the solute is charged or uncharged. For an uncharged solute, the free energy depends only on the concentration gradient. The equation is $\Delta G = RT \ln(c_2/c_1)$, where c_2 is the concentration on the side the solute is moving to, and c_1 is the concentration on the side it is moving from. If $c_2 < c_1$ (moving down the gradient), ΔG is negative and the process is spontaneous. If $c_2 > c_1$ (moving against the gradient), ΔG is positive and energy input is required.

For a charged solute (an ion), there is an additional electrical contribution from the membrane potential. The total free energy of transport is $\Delta G = RT \ln(c_2/c_1) + ZF\Delta\psi$, where Z is the charge on the ion (positive for cations, negative for anions), F is Faraday's constant (96.5 kJ mol^{-1} V^{-1}), and $\Delta\psi$ is the membrane potential (defined as the voltage on the destination side minus the voltage on the origin side, or equivalently, $\psi_{inside} - \psi_{outside}$, which is typically negative in animal cells, around -50 mV).

When a cation moves from outside to inside a cell with $\Delta\psi$ = -50 mV, the $ZF\Delta\psi$ term is negative (favorable), because the cation is attracted to the negatively charged interior. When the same cation moves from inside to outside, the $ZF\Delta\psi$ term is positive (unfavorable). For a transport process driven by ATP hydrolysis, the magnitude of ΔG for ATP hydrolysis under cellular conditions (typically about -50 to -55 kJ/mol, though the standard value of -30.5 kJ/mol is often used in worked calculations when no other convention is stated) must equal or exceed the total ΔG of transporting all the ions in the given stoichiometry.

In coupled transport problems, the energy released by the downhill flow of one ion can be calculated and then set equal to the energy required for the uphill transport of a second solute, allowing calculation of the maximum concentration ratio achievable. For example, in the Na^+–glucose symporter, two Na^+ ions flow downhill per glucose transported uphill, so the total energy available is twice the single-ion ΔG for Na^+, and this energy determines the maximum $[glucose]_{in}/[glucose]_{out}$ ratio.

13.6 Why Phosphorylation Traps Metabolites Inside Cells

When glucose enters a cell via a GLUT transporter, it can freely exit through the same transporter because transport is reversible. However, the first reaction of glycolysis — phosphorylation by hexokinase — converts glucose to glucose-6-phosphate, adding a bulky, doubly-charged phosphate group. Glucose-6-phosphate is not a substrate for GLUT transporters (the transporter's binding site is specific for uncharged glucose), and the negative charges prevent passive diffusion through the lipid

bilayer. The metabolite is therefore trapped inside the cell. This "trapping" is the fundamental reason why the first step of glycolysis consumes ATP: the investment ensures that the substrate cannot escape before it is metabolized.

Topic: Principles of Metabolism

15.1 Oxidation State and Fuel Value

The usefulness of a carbon-containing molecule as a metabolic fuel is directly related to how reduced it is — that is, how many electrons its carbon atoms can donate when oxidized. The oxidation state of a carbon atom increases (becomes more oxidized) as it forms more bonds to electronegative atoms like oxygen and fewer bonds to hydrogen. A methane carbon (CH_4) is fully reduced; a carbon dioxide carbon (CO_2) is fully oxidized. Fatty acids are more reduced than carbohydrates (they have a higher ratio of C–H bonds to C–O bonds), which is why fats yield more energy per gram than sugars.

To assess the oxidation state of a carbon, count bonds to oxygen versus bonds to hydrogen. An aldehyde carbon (one C=O, one C–H) is at a higher oxidation state than an alcohol carbon (one C–OH, one C–H). A carboxylic acid carbon (one C=O, one C–OH) is at the highest common oxidation state for organic molecules. An ester or thioester has the same oxidation state as a carboxylic acid. A thiol (C–SH) is at the same oxidation state as an alcohol (C–OH). These comparisons are essential for identifying which reactions in a metabolic pathway are oxidations, which are reductions, and which involve no change in oxidation state.

15.2 General Principles of Metabolic Pathway Organization

Metabolic pathways are sequences of enzyme-catalyzed reactions that transform a starting molecule into a final product through a series of intermediates. Several organizing principles govern these pathways.

First, metabolic pathways are thermodynamically favorable overall. The sum of the free energy changes for all steps in the pathway must be negative under cellular conditions. Individual steps may be thermodynamically unfavorable, but they are driven by coupling to favorable steps — usually through shared intermediates.

Second, every metabolic pathway has at least one irreversible step that commits substrate to that pathway. This is called the committed step, and it is typically the first step that is unique to the pathway (not shared with other pathways). The committed step is usually the primary site of regulation.

Third, catabolic pathways (degradative, fuel-oxidizing) converge: many different fuels are broken down into a small number of common intermediates (pyruvate, acetyl-CoA, TCA cycle intermediates). Anabolic pathways (biosynthetic) diverge: a few common precursors are used to build many different complex molecules.

Fourth, catabolic and anabolic pathways are not simply the reverse of each other. They use different enzymes at the irreversible steps, may occur in different cellular compartments, and use different electron carriers (catabolism uses NAD^+/NADH; anabolism uses $NADP^+$/NADPH).

15.3 Free Energy — Standard vs. Cellular Conditions

The standard free energy change, ΔG°', is the free energy change for a reaction when all reactants and products are at 1 M concentration (the biochemical standard state also specifies pH 7 and 25°C). ΔG°' is a constant for any given reaction and is related to the equilibrium constant by ΔG°' = -RT ln Keq. A negative ΔG°' means the equilibrium favors products; a positive ΔG°' means the equilibrium favors reactants. However, ΔG°' does not tell you whether a reaction will actually proceed in a living cell, because cellular concentrations are almost never at 1 M.

The actual free energy change, ΔG', is the free energy change under the actual cellular concentrations of reactants and products. It is calculated by the equation ΔG' = ΔG°' + RT ln Q, where Q is the mass-action ratio (the ratio of product concentrations to reactant concentrations at the current moment, not at equilibrium). ΔG' is the quantity that determines whether a reaction is spontaneous in the cell. If ΔG' < 0, the reaction proceeds spontaneously in the forward direction. If ΔG' > 0, the reaction proceeds spontaneously in the reverse direction. If ΔG' = 0, the reaction is at equilibrium.

A reaction with a positive ΔG°' can have a negative ΔG' if the cellular concentrations of products are kept very low relative to reactants — that is, if Q is much less than Keq. This is precisely how thermodynamically unfavorable reactions are driven forward in metabolism: the product of the unfavorable reaction is consumed by a subsequent favorable reaction, keeping its concentration low and maintaining a favorable Q. This principle is called reaction coupling through shared intermediates, and it is the single most important thermodynamic concept in metabolism.

The classic example is malate dehydrogenase, which oxidizes malate to oxaloacetate with a ΔG°' of +29.7 kJ/mol. Despite this highly unfavorable standard free energy, the reaction proceeds readily in the cell because oxaloacetate is immediately consumed by citrate synthase in a highly favorable condensation reaction. The continuous removal of oxaloacetate keeps its concentration extremely low, driving the malate dehydrogenase reaction forward.

15.4 Reaction Coupling and ATP

When two reactions share an intermediate, they are coupled: the overall ΔG°' for the coupled process is the sum of the individual ΔG°' values. If the sum is negative, the overall process is thermodynamically favorable under standard conditions.

ATP hydrolysis (ATP + H_2O → ADP + Pi) has a ΔG°' of approximately -30.5 kJ/mol. This large negative value means that ATP hydrolysis can be coupled to otherwise unfavorable reactions to make them favorable. If a biosynthetic reaction has ΔG°' = +10 kJ/mol, coupling it to ATP hydrolysis gives an overall ΔG°' of +10 + (-30.5) = -20.5 kJ/mol, which is favorable. In practice, this coupling is achieved

mechanistically — ATP does not simply hydrolyze in solution and somehow push the other reaction along. Instead, the enzyme uses ATP as a co-substrate, often transferring the phosphoryl group to the other substrate to create a reactive (activated) intermediate.

The large negative $\Delta G^{\circ\prime}$ of ATP hydrolysis arises from four factors. First, electrostatic repulsion: the three negatively charged phosphate groups in ATP are closely packed, and separating them into ADP and Pi relieves this repulsion. Second, increased resonance stabilization: ADP and Pi each have more resonance structures available than the corresponding groups in ATP. Third, increased hydration: the products (ADP and Pi) interact more favorably with water than ATP does. Fourth, in some contexts, increased entropy: one molecule becomes two (though this factor varies depending on whether the reaction also consumes or releases water and protons).

15.5 Phosphoryl-Transfer Potential

The phosphoryl-transfer potential of a compound is a measure of its tendency to transfer its phosphate group to water (or another acceptor). It is quantified by the $\Delta G^{\circ\prime}$ of hydrolysis of its phosphate ester or anhydride bond. The more negative the $\Delta G^{\circ\prime}$ of hydrolysis, the higher the phosphoryl-transfer potential and the stronger the compound's "desire" to give up its phosphate group.

ATP occupies a middle position in the phosphoryl-transfer hierarchy. Compounds with higher phosphoryl-transfer potential than ATP — such as phosphoenolpyruvate (PEP, $\Delta G^{\circ\prime} = -61.9$ kJ/mol), 1,3-bisphosphoglycerate ($\Delta G^{\circ\prime} = -49.4$ kJ/mol), and creatine phosphate ($\Delta G^{\circ\prime} = -43.1$ kJ/mol) — can spontaneously transfer their phosphate group to ADP to synthesize ATP. This is the basis of substrate-level phosphorylation: in glycolysis, the high-energy phosphorylated intermediates 1,3-BPG and PEP donate their phosphate groups to ADP, producing ATP. Compounds with lower phosphoryl-transfer potential than ATP — such as glucose-6-phosphate ($\Delta G^{\circ\prime} = -13.8$ kJ/mol) — cannot phosphorylate ADP; instead, ATP must donate a phosphate to make them.

The phosphoryl-transfer potential of PEP is exceptionally high because the phosphate group locks the molecule in the unstable enol form. Upon hydrolysis, the phosphate departs and the enol spontaneously tautomerizes to the much more stable keto form (pyruvate). This tautomerization releases a large amount of energy, which contributes to the very negative $\Delta G^{\circ\prime}$ of PEP hydrolysis. The phosphoryl-transfer potential of 1,3-BPG is high because the acyl phosphate bond is an anhydride between a carboxylic acid and phosphoric acid, and both products of its hydrolysis (3-PG and Pi) are stabilized by resonance and hydration.

The important quantitative application of phosphoryl-transfer potential is determining the concentration ratio of products to reactants needed to keep a substrate-level phosphorylation reaction spontaneous under cellular conditions. This involves combining the $\Delta G^{\circ\prime}$ values for the relevant hydrolysis reactions, setting $\Delta G' \leq 0$, and solving for the concentration ratio using the relationship $\Delta G' = \Delta G^{\circ\prime} + RT \ln Q$.

15.6 Activated Carriers — Cofactors in Metabolism

Metabolism relies on a set of small molecules and coenzymes that serve as carriers of specific chemical groups. These activated carriers are recycled by metabolic enzymes and are essential for shuttling groups between reactions.

NAD^+ and NADH: Nicotinamide adenine dinucleotide is the principal electron carrier in catabolic reactions. NAD^+ is the oxidized form; it accepts a hydride ion (one proton and two electrons, H^-) from a substrate, becoming reduced to NADH. The hydride is accepted at the C4 position of the nicotinamide ring. The reaction involves two-electron chemistry exclusively. NAD^+ functions stoichiometrically in catabolism — each molecule of NAD^+ is consumed and must be regenerated (either by the electron transport chain aerobically or by fermentation anaerobically) for catabolism to continue. $NADP^+$ and NADPH are structurally identical except for an additional phosphate group on the 2' hydroxyl of the adenosine ribose. NADPH is the electron donor used in anabolic (biosynthetic) reactions.

FAD and $FADH_2$: Flavin adenine dinucleotide is a versatile electron carrier that can perform both one-electron and two-electron transfers, which distinguishes it from NAD^+. The reactive portion is the isoalloxazine ring system. FAD can accept a hydride (two electrons + one proton) to become $FADH_2$, or it can accept a single electron to become the semiquinone radical FADH·. This flexibility allows FAD to mediate electron transfers between molecules that operate by different mechanisms. In the pyruvate dehydrogenase complex, FAD serves as an intermediary between the dithiol/disulfide chemistry of lipoamide and the hydride chemistry of NAD^+. The key mechanistic point is that reduced lipoamide (a dithiol) cannot directly reduce NAD^+ because it is not a hydride source — it transfers electrons through disulfide exchange chemistry. FAD can accept these electrons and then deliver a hydride to NAD^+. FMN (flavin mononucleotide) is the same isoalloxazine ring with only a ribitol phosphate (no adenine nucleotide).

Thiamine Pyrophosphate (TPP): TPP is the cofactor for all reactions involving the cleavage of a carbon–carbon bond adjacent to a carbonyl group, typically with loss of CO_2 (decarboxylation). The reactive center is the C2 carbon of the thiazolium ring. In the resting state, C2 bears a hydrogen. A base in the active site removes this proton, generating a carbanion (the ylide) that is stabilized by the adjacent positively charged nitrogen atom and the sulfur atom in the ring. This carbanion is a potent nucleophile.

The general mechanism proceeds as follows. The TPP ylide attacks the carbonyl carbon of the substrate (such as the α-keto carbon of pyruvate), forming a covalent adduct. The carbon–carbon bond between the α-carbon and the carboxylate is then cleaved, releasing CO_2. The electrons that formed the C–C bond flow into the thiazolium ring, which acts as an electron sink because the positively charged nitrogen can delocalize the negative charge. The resulting intermediate is a resonance-stabilized carbanion — in the case of pyruvate, this is called hydroxyethyl-TPP. What happens next depends on the specific enzyme: the carbanion can be protonated and released as an aldehyde (pyruvate decarboxylase in yeast), transferred to lipoamide with simultaneous oxidation (pyruvate dehydrogenase E1), or used as a nucleophile to attack another carbonyl substrate (acetolactate synthase, transketolase).

The recognition rule for when TPP is required is straightforward: if a reaction involves cleavage of a C–C bond adjacent to a carbonyl (especially loss of CO_2 from an α-keto acid), TPP is needed to stabilize the carbanion that would otherwise be unstable.

Biotin: Biotin is the carrier of activated CO_2. It is covalently attached to enzymes (biotinylated enzymes) via an amide bond between the valeric acid side chain of biotin and a specific lysine residue in the enzyme. In carboxylation reactions (such as pyruvate carboxylase), bicarbonate (HCO_3^-) is activated by ATP to form carboxyphosphate, which then reacts with biotin to form carboxybiotin (N-carboxybiotin). The carboxybiotin swings on its long, flexible tether (the lysine side chain) from the site where CO_2 was attached to the active site where the carboxyl group is transferred to the substrate (pyruvate → oxaloacetate). Biotin-dependent reactions always require ATP.

Coenzyme A (CoA): CoA carries acyl groups as thioesters. The reactive portion is the terminal thiol group (–SH), which forms a thioester bond with the acyl group (R–C(=O)–S–CoA). Thioesters are high-energy bonds — their hydrolysis releases a similar amount of free energy as ATP hydrolysis. The high energy of the thioester bond is due to the fact that sulfur does not donate its lone pair electrons into the carbonyl as effectively as oxygen does (less resonance stabilization compared to ordinary esters), making the carbonyl carbon more electrophilic and the bond easier to break. Acetyl-CoA is the most important acyl-CoA in central metabolism.

Lipoamide (Lipoic Acid): Lipoamide is unique among cofactors because it functions as both an electron carrier and an acyl-group carrier simultaneously. It is covalently attached to a lysine residue of the E2 enzyme in multienzyme complexes (like the pyruvate dehydrogenase complex), forming a flexible arm (lipoyllysine) that can swing between the active sites of different enzymes in the complex.

In its oxidized form, lipoamide contains a cyclic disulfide (a five-membered ring with two sulfur atoms connected by an S–S bond). The disulfide is electrophilic — a nucleophilic carbanion (like hydroxyethyl-TPP) can attack one of the sulfur atoms, opening the ring. This reaction simultaneously transfers the two-carbon fragment from TPP to lipoamide (forming acetyl-lipoamide, a thioester) and reduces one of the sulfur atoms from the disulfide to a thiol. The acetyl group is then transferred to CoA (transesterification), leaving dihydrolipoamide (both sulfurs in the thiol/reduced form). Dihydrolipoamide must be re-oxidized to the disulfide form to complete the catalytic cycle, which is accomplished by disulfide exchange with a pair of cysteines in the E3 active site, followed by FAD-mediated electron transfer to NAD^+.

15.7 Classification of Metabolic Reactions

Every reaction in metabolism can be classified into one of six general types.

Oxidation-reduction reactions involve the transfer of electrons, usually as hydride ions (H^-) or hydrogen atoms. The oxidized and reduced forms of the substrate differ in their oxidation state at one or more carbon atoms. Oxidation increases the number of bonds to oxygen (or sulfur or nitrogen) or decreases the number of bonds to hydrogen. Reduction is the reverse. These reactions require an electron carrier cofactor: NAD^+, $NADP^+$, FAD, or FMN.

Group transfer reactions involve the transfer of a chemical group from a donor to an acceptor. The most common are phosphoryl transfers (from ATP to a substrate, or from a high-energy phosphorylated intermediate to ADP), acyl transfers (from a thioester to another thiol or to a hydroxyl), and amino-group transfers (transamination).

Hydrolysis reactions involve the cleavage of a bond by the addition of water. Phosphatases catalyze the hydrolytic removal of phosphate groups. Peptidases cleave amide bonds.

Carbon–carbon bond formation and cleavage reactions involve the making or breaking of C–C bonds. These include aldol condensations and retro-aldol cleavages (as in aldolase), Claisen condensations (as in citrate synthase), and decarboxylations (loss of CO_2 from a carboxyl group). C–C bond cleavage adjacent to a carbonyl requires TPP if the carbanion generated would otherwise be unstable.

Isomerization reactions involve the rearrangement of atoms within a single molecule without any change in molecular formula. Examples include the conversion of glucose-6-phosphate to fructose-6-phosphate (aldose-ketose isomerization by phosphoglucose isomerase) and the conversion of DHAP to GAP (triose phosphate isomerase).

Ligation reactions coupled to ATP hydrolysis involve the joining of two molecules using the free energy of ATP (or another NTP) cleavage. Examples include pyruvate carboxylase (which joins CO_2 to pyruvate using ATP) and aminoacyl-tRNA synthetases.

15.8 Phosphoryl Transfer vs. Acyl Transfer — A Critical Distinction

This distinction is used repeatedly and deserves special attention. In a phosphoryl transfer reaction, a phosphate group is transferred from a donor (usually ATP) to a nucleophilic acceptor (a hydroxyl or carboxylate group on the substrate). The electrophilic center is the phosphorus atom of ATP. This type of reaction is required when the substrate lacks an inherent driving force for forming a phosphorylated product — for example, when a carboxylate (a poor electrophile, already at a high oxidation state) must be phosphorylated.

In an acyl transfer reaction (also called substrate-level phosphorylation when the acyl phosphate is formed in situ), the driving force comes from a redox reaction that occurs within the same enzymatic step. The classic example is GAPDH: the aldehyde group of GAP is oxidized to a thioester by NAD^+ (a favorable reaction), and the energy of this oxidation is captured by forming the thioester with an active-site cysteine. Inorganic phosphate then attacks the thioester to form the acyl phosphate (1,3-BPG). No ATP is consumed because the oxidation provides the thermodynamic driving force.

The decision rule is: examine the starting functional group. If it is an aldehyde that can be oxidized, NAD^+ provides the energy and no ATP is needed. If it is a carboxylate that is already fully oxidized, ATP must provide the energy for phosphorylation.

15.9 Enolate Chemistry in Metabolism

Many metabolic C–C bond formation and cleavage reactions proceed through enolate intermediates. An enolate is a resonance-stabilized carbanion formed by deprotonation of a carbon adjacent to a carbonyl group (the α-carbon). The negative charge is delocalized between the α-carbon and the carbonyl oxygen. Enolates are nucleophilic at the α-carbon and can attack electrophilic carbon centers (such as carbonyl carbons of aldehydes or ketones) to form new C–C bonds in aldol-type reactions.

In enzyme-catalyzed reactions, the enolate may be stabilized as an enamine by forming a Schiff base (imine) between the carbonyl of the substrate and the ε-amino group of an active-site lysine. The Schiff base nitrogen, when protonated, serves as an electron sink analogous to the carbonyl oxygen but with a positive charge that is even more effective at stabilizing developing negative charge during C–C bond cleavage. This is the mechanism used by aldolase and transaldolase.

15.10 Pathway Regulation — General Principles

The flux through a metabolic pathway is controlled primarily at the steps catalyzed by enzymes that operate far from equilibrium — the irreversible steps with the most negative ΔG' values. These enzymes are subject to regulation because their reactions are essentially one-directional under cellular conditions, making them natural control points.

The most common regulatory mechanisms are allosteric regulation (binding of an effector molecule at a site other than the active site, changing the enzyme's activity), covalent modification (typically phosphorylation/dephosphorylation, which can activate or inactivate an enzyme), and changes in enzyme concentration (through transcriptional regulation or protein degradation, which operates on a longer timescale).

In reciprocally regulated pathways like glycolysis and gluconeogenesis, the regulatory effectors activate one pathway while simultaneously inhibiting the other. This ensures that the cell does not waste energy by running both pathways at high flux simultaneously — a situation called a futile cycle.

Topic: Glycolysis and Gluconeogenesis

16.1 Overview of Glycolysis

Glycolysis is the pathway by which one molecule of glucose (a six-carbon sugar) is converted into two molecules of pyruvate (a three-carbon α-keto acid), with the net production of two molecules of ATP and two molecules of NADH. Glycolysis occurs in the cytosol of all living cells, requires no oxygen, and is the most ancient and universal pathway of glucose catabolism.

The pathway consists of ten enzyme-catalyzed reactions divided into two phases. The first five reactions constitute the investment phase (also called the preparatory phase), during which two molecules of ATP are consumed to phosphorylate and cleave the six-carbon sugar into two three-carbon units. The last five reactions constitute the payoff phase, during which each three-carbon unit generates two ATP and one NADH, for a total of four ATP and two NADH per glucose. The net yield is therefore two ATP and two NADH per glucose.

16.2 The Investment Phase — Reactions 1 through 5

Reaction 1 — Hexokinase: Glucose → Glucose-6-phosphate. This reaction consumes one ATP. Hexokinase transfers the γ-phosphoryl group from ATP to the C6 hydroxyl of glucose. The reaction is thermodynamically irreversible under cellular conditions (ΔG' is very negative). Hexokinase undergoes a large conformational change upon binding glucose — the enzyme closes around the substrate in an induced-fit mechanism. This conformational change is functionally essential because it excludes water molecules from the active site. If water were present, it could attack the γ-phosphoryl group of ATP instead of the glucose hydroxyl, resulting in wasteful ATP hydrolysis rather than productive phosphoryl transfer.

The product, glucose-6-phosphate, carries a doubly negative charge from the phosphate group. This charge prevents the molecule from crossing the membrane via GLUT transporters or by passive diffusion, effectively trapping glucose inside the cell. In the liver, a specialized isozyme called glucokinase (hexokinase IV) performs this reaction. Glucokinase has a much higher KM for glucose (approximately 10 mM versus 0.1 mM for hexokinase in other tissues). This means glucokinase is only significantly active when blood glucose levels are high. At low blood glucose, the liver's glucokinase is relatively inactive, allowing glucose to pass through the liver and reach the brain and other glucose-dependent tissues.

Reaction 2 — Phosphoglucose isomerase: Glucose-6-phosphate → Fructose-6-phosphate. This is an aldose-to-ketose isomerization. G6P (an aldose, with an aldehyde at C1 in the open-chain form) is converted to F6P (a ketose, with a ketone at C2). The reaction is near equilibrium (ΔG' ≈ 0) and is fully reversible. The purpose of this isomerization is to set up the symmetrical cleavage of the six-carbon sugar in reaction 4: converting the aldose to a ketose places the carbonyl at C2, which means the subsequent aldol cleavage between C3 and C4 will produce two three-carbon phosphorylated fragments.

Reaction 3 — Phosphofructokinase-1 (PFK-1): Fructose-6-phosphate → Fructose-1,6-bisphosphate. This reaction consumes the second ATP. PFK-1 transfers a phosphoryl group from ATP to the C1 hydroxyl of F6P. This is the committed step of glycolysis — the first step unique to the glycolytic pathway (G6P and F6P can also enter other pathways such as the pentose phosphate pathway or glycogen synthesis). PFK-1 is the most important regulatory enzyme in glycolysis. The reaction is thermodynamically irreversible.

Reaction 4 — Aldolase: Fructose-1,6-bisphosphate → Glyceraldehyde-3-phosphate (GAP) + Dihydroxyacetone phosphate (DHAP). This is a retro-aldol cleavage that splits the six-carbon sugar bisphosphate into two three-carbon sugar phosphates. The C3–C4 bond is cleaved. Carbons 1, 2, and 3

of the fructose become DHAP (with the ketone at C2 and phosphate at C1). Carbons 4, 5, and 6 become GAP (with the aldehyde at C1, corresponding to C4 of fructose, and phosphate at C3, corresponding to C6 of fructose).

The standard free energy of this reaction is highly positive ($\Delta G^{\circ\prime}$ = +23.8 kJ/mol), meaning that under standard conditions the equilibrium overwhelmingly favors the substrate. However, in the cell, the products GAP and DHAP are continuously consumed by subsequent reactions, keeping their concentrations low and driving the reaction forward ($\Delta G' \approx 0$).

The mechanism of aldolase involves a Schiff base. The C2 ketone of the substrate forms a covalent imine linkage with the ε-amino group of an active-site lysine residue. The protonated imine nitrogen acts as an electron sink during the retro-aldol C–C bond cleavage, stabilizing the developing negative charge on the enamine intermediate. After GAP departs, the enamine is hydrolyzed back to the free ketone (DHAP) and the free lysine.

Reaction 5 — Triose phosphate isomerase (TIM or TPI): Dihydroxyacetone phosphate → Glyceraldehyde-3-phosphate. Only GAP continues down the glycolytic pathway; DHAP must be converted to GAP by this isomerase. The reaction is a ketose-to-aldose isomerization (the reverse of reaction 2, but on a three-carbon sugar). TIM is a kinetically perfect enzyme — its rate approaches the diffusion-controlled limit, meaning that every encounter between enzyme and substrate results in product formation. The reaction proceeds through an enediol intermediate (a cis-1,2-diol on the double bond between C1 and C2).

TIM performs a critical protective function: DHAP can spontaneously decompose to methylglyoxal (a toxic compound) by elimination of the phosphate group from the enediol intermediate. TIM prevents this by catalyzing the isomerization so quickly and by stabilizing the enediol intermediate in a conformation that minimizes orbital overlap with the phosphate leaving group, thereby suppressing the elimination pathway.

Without TIM, only one of the two three-carbon fragments produced by aldolase (GAP) would enter the payoff phase, cutting the ATP yield of glycolysis in half.

16.3 The Payoff Phase — Reactions 6 through 10

After reaction 5, both three-carbon units are in the form of GAP. Each molecule of GAP undergoes reactions 6 through 10, so the yields from these reactions are doubled per molecule of glucose.

Reaction 6 — Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): GAP + NAD^+ + Pi → 1,3-Bisphosphoglycerate (1,3-BPG) + NADH. This is one of the most mechanistically important reactions in all of metabolism. It accomplishes two things simultaneously: the oxidation of an aldehyde to a carboxylic acid equivalent (a thermodynamically favorable process) and the formation of a high-energy acyl phosphate bond (a thermodynamically unfavorable process). The coupling of these two events through a covalent enzyme intermediate is the key.

The mechanism proceeds as follows. An active-site cysteine thiol attacks the aldehyde carbon of GAP, forming a covalent hemithioacetal intermediate. NAD^+ then oxidizes this hemithioacetal to a thioester (which is at the same oxidation state as a carboxylic acid), accepting a hydride and becoming NADH. The thioester is a high-energy intermediate — its hydrolysis would release substantial free energy. Instead of hydrolysis, inorganic phosphate attacks the thioester carbonyl carbon, displacing the cysteine thiol and forming the acyl phosphate bond in 1,3-BPG. The critical point is that no ATP is consumed; the energy for forming the acyl phosphate comes from the oxidation.

Reaction 7 — Phosphoglycerate kinase: 1,3-BPG + ADP → 3-Phosphoglycerate (3-PG) + ATP. The high-energy acyl phosphate group of 1,3-BPG is transferred to ADP, producing ATP. This is the first substrate-level phosphorylation in glycolysis. The name "kinase" refers to the reverse reaction (the enzyme could phosphorylate 3-PG using ATP), but the forward direction is strongly favored in glycolysis because 1,3-BPG has much higher phosphoryl-transfer potential than ATP.

Reaction 8 — Phosphoglycerate mutase: 3-Phosphoglycerate → 2-Phosphoglycerate (2-PG). This enzyme moves the phosphate group from C3 to C2. The mechanism involves a phosphorylated histidine residue in the active site. The phospho-histidine donates its phosphate to the C2 hydroxyl of 3-PG, creating a transient 2,3-bisphosphoglycerate (2,3-BPG) intermediate. Then the C3 phosphate of 2,3-BPG is transferred back to the histidine, regenerating the phospho-histidine and producing 2-PG. Catalytic amounts of 2,3-BPG are required to initially phosphorylate the histidine — without this priming, the enzyme is inactive. Mg^{2+} is required as a cofactor for this phosphoryl transfer.

Reaction 9 — Enolase: 2-Phosphoglycerate → Phosphoenolpyruvate (PEP) + H_2O. This is a dehydration reaction that removes water from 2-PG to create the enol phosphate PEP. The dehydration itself is not a particularly energetically significant reaction, but it transforms a relatively low-energy phosphate ester (at C2 of 2-PG) into an extremely high-energy enol phosphate (PEP). The phosphoryl-transfer potential of PEP is the highest of any common metabolite because hydrolysis of the phosphate releases the enol, which immediately tautomerizes to the much more stable keto form (pyruvate), and this tautomerization is highly exergonic.

Reaction 10 — Pyruvate kinase: PEP + ADP → Pyruvate + ATP. The phosphoryl group of PEP is transferred to ADP, producing the second ATP per three-carbon unit (fourth ATP per glucose). This is the second substrate-level phosphorylation. The reaction is highly exergonic ($\Delta G^{\circ\prime}$ = -31.4 kJ/mol) and irreversible under cellular conditions. After phosphoryl transfer, the enol form of pyruvate spontaneously tautomerizes to the keto form, which is thermodynamically much more stable, providing additional driving force for the reaction.

16.4 Net Yield and Stoichiometry

The overall equation for glycolysis is: Glucose + 2 NAD^+ + 2 ADP + 2 Pi → 2 Pyruvate + 2 NADH + 2 ATP + 2 H_2O.

The investment phase consumes 2 ATP (reactions 1 and 3). The payoff phase produces 4 ATP (reactions 7 and 10, each occurring twice). The net is 2 ATP. The payoff phase also produces 2 NADH (reaction 6, occurring twice). Under aerobic conditions, each NADH can generate approximately 2.5

ATP through oxidative phosphorylation. Under anaerobic conditions, NADH must be re-oxidized by fermentation, and no additional ATP is obtained from it.

When metabolites enter glycolysis at points other than glucose, the ATP accounting changes. If glucose-6-phosphate enters (bypassing hexokinase), only one ATP is consumed in the investment phase, giving a net yield of 3 ATP. If GAP enters (bypassing the entire investment phase), no ATP is consumed, and 2 ATP are produced — but only per one molecule of GAP, not two. If DHAP enters, it must first be converted to GAP by TIM (no ATP cost), then proceeds through the payoff phase for 2 ATP. Glycerol enters by first being phosphorylated to glycerol-3-phosphate (costs 1 ATP) and then oxidized to DHAP (costs 1 NAD^+), entering glycolysis at the DHAP stage.

16.5 Fermentation

Under anaerobic conditions, cells face a critical problem: the NADH produced by GAPDH in glycolysis cannot be re-oxidized by the electron transport chain (which requires O_2 as the final electron acceptor). Without regeneration of NAD^+, glycolysis will halt because reaction 6 requires NAD^+ as a substrate. Fermentation solves this problem by using pyruvate (or a derivative of pyruvate) as an electron acceptor to re-oxidize NADH back to NAD^+.

In mammalian lactic acid fermentation, pyruvate is directly reduced to lactate by lactate dehydrogenase (LDH). NADH donates a hydride to the C2 carbonyl of pyruvate, converting the ketone to a hydroxyl group and producing L-lactate and NAD^+. The mechanism involves hydride delivery from the C4 of the nicotinamide ring of NADH to the si face of the pyruvate carbonyl, with simultaneous protonation of the developing alkoxide by a general acid in the active site (His195 in the actual enzyme, though in simplified notation this is represented as $H\text{-}B^+$).

In yeast alcoholic fermentation, the process requires two steps. First, pyruvate is decarboxylated by pyruvate decarboxylase (which requires TPP) to produce acetaldehyde and CO_2. The TPP mechanism here follows the standard pattern: ylide formation, nucleophilic attack on the α-keto carbon, decarboxylation with electron delocalization into the thiazolium ring, and protonation/release of acetaldehyde with TPP regeneration. Second, acetaldehyde is reduced to ethanol by alcohol dehydrogenase, with NADH providing the hydride and being oxidized to NAD^+.

The sole purpose of fermentation is NAD^+ regeneration. Fermentation does not produce additional ATP beyond the 2 ATP from glycolysis. Lactate and ethanol are effectively waste products — they are metabolic dead ends in the cells that produce them (though lactate can be transported to the liver for gluconeogenesis via the Cori cycle).

16.6 Fates of Pyruvate

Pyruvate sits at a critical metabolic crossroads and can follow several different paths depending on the metabolic needs and oxygen status of the cell.

Under aerobic conditions, pyruvate is oxidatively decarboxylated to acetyl-CoA by the pyruvate dehydrogenase complex. This is an irreversible, committed entry into the citric acid cycle and oxidative metabolism. Under anaerobic conditions in mammals, pyruvate is reduced to lactate by lactate dehydrogenase, regenerating NAD^+. Under anaerobic conditions in yeast, pyruvate is decarboxylated to acetaldehyde and then reduced to ethanol. When blood glucose is low, pyruvate can be carboxylated to oxaloacetate by pyruvate carboxylase, entering gluconeogenesis. Pyruvate can also undergo transamination with glutamate to produce alanine, connecting carbohydrate metabolism to amino acid metabolism.

Ethanol is not a fate of pyruvate in humans — this is a yeast-specific pathway. This is an important distinction that has been explicitly emphasized.

16.7 Gluconeogenesis — Overview and Bypass Reactions

Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors. It occurs primarily in the liver (with a minor contribution from the kidneys) and is essential for maintaining blood glucose levels during fasting, starvation, and prolonged exercise. The main precursors are lactate (from anaerobic glycolysis in muscle), alanine (from protein degradation), and glycerol (from fat mobilization).

Seven of the ten glycolytic reactions are near-equilibrium and are freely reversible. These same enzymes catalyze the reverse reactions in gluconeogenesis. However, three glycolytic reactions are irreversible (hexokinase, PFK-1, and pyruvate kinase), and these must be bypassed by different enzymes in gluconeogenesis. The bypass reactions are thermodynamically favorable in the gluconeogenic direction.

Bypass of pyruvate kinase (PEP → pyruvate): Two enzymes, two NTPs.

The conversion of pyruvate back to PEP cannot be accomplished in a single step because the reverse of the pyruvate kinase reaction is thermodynamically impossible under cellular conditions (it would require $\Delta G' >> 0$). Instead, two enzymes are used.

Pyruvate carboxylase converts pyruvate to oxaloacetate (OAA) by adding CO_2 from bicarbonate. This reaction requires biotin (which carries the activated CO_2) and ATP (which activates bicarbonate to carboxyphosphate). Pyruvate carboxylase is allosterically activated by acetyl-CoA, which signals that the cell has ample acetyl-CoA for the TCA cycle and that carbons can be diverted toward glucose synthesis.

Phosphoenolpyruvate carboxykinase (PEPCK) converts oxaloacetate to PEP by removing the CO_2 that was just added (decarboxylation) and simultaneously phosphorylating the enolate using GTP. The decarboxylation is highly favorable and provides the thermodynamic driving force to form the high-energy enol phosphate bond in PEP. The CO_2 added by pyruvate carboxylase and removed by PEPCK serves no net synthetic purpose — it is a thermodynamic "handle" that makes the overall conversion energetically feasible by providing a favorable decarboxylation step.

The PEPCK mechanism proceeds through an enolate intermediate: the β-carboxyl group of OAA departs as CO_2, generating an enolate (pyruvate enolate). The γ-phosphate of GTP is then transferred to the enolate oxygen, forming the enol phosphate PEP and GDP.

Bypass of PFK-1 (F-1,6-BP → F6P): Fructose-1,6-bisphosphatase (FBPase-1) catalyzes the hydrolytic removal of the C1 phosphate group from F-1,6-BP, producing F6P and inorganic phosphate. This is a simple hydrolysis, not a reversal of the kinase reaction — no ATP is synthesized. FBPase-1 is allosterically inhibited by AMP and by F-2,6-BP.

Bypass of hexokinase (G6P → glucose): Glucose-6-phosphatase catalyzes the hydrolytic removal of the phosphate from G6P, releasing free glucose that can be exported from the cell via GLUT transporters into the bloodstream. This enzyme is present only in the liver and kidneys — not in muscle or brain. This is why only the liver and kidneys can release free glucose into the blood via gluconeogenesis. Muscle cells lack glucose-6-phosphatase and cannot export glucose; they use G6P internally for glycolysis or glycogen synthesis.

16.8 Energetics of Gluconeogenesis

The synthesis of one molecule of glucose from two molecules of pyruvate requires: 4 ATP (2 for pyruvate carboxylase, 2 for phosphoglycerate kinase running in reverse), 2 GTP (2 for PEPCK), and 2 NADH (2 for the reverse of GAPDH). The total cost is 6 NTP equivalents and 2 NADH per glucose, or equivalently 4 ATP + 2 GTP + 2 NADH. Gluconeogenesis is energetically expensive, which is why it is tightly regulated and only runs when the cell has sufficient energy (ATP) to power it.

When starting from lactate, the conversion of lactate to pyruvate by LDH produces 1 NADH per lactate. Two lactates provide 2 NADH, which exactly matches the 2 NADH consumed in gluconeogenesis. Therefore, gluconeogenesis from lactate has a net NAD^+ requirement of zero.

16.9 Carbon Label Tracking Through Glycolysis and Gluconeogenesis

Understanding where a labeled carbon ends up requires knowing how aldolase splits the six-carbon sugar. Fructose-1,6-bisphosphate has its carbons numbered 1 through 6 from top to bottom in the standard Fisher projection. Aldolase cleaves between C3 and C4. The C1–C3 fragment becomes DHAP (ketone at C2, phosphate at C1). The C4–C6 fragment becomes GAP (aldehyde at C1, which was C4 of fructose; hydroxyl at C2, which was C5; phosphate at C3, which was C6).

After TIM converts DHAP to GAP, the two GAP molecules are chemically identical but retain different labels. Each GAP goes through reactions 6–10, where the aldehyde (C1 of GAP) becomes the carboxyl group (C1 of pyruvate, also called C3 of the original pyruvate numbering where C1 = methyl, C2 = carbonyl, C3 = carboxyl). In the pyruvate dehydrogenase reaction, the carboxyl carbon (C1 of pyruvate by this convention, or C3 if you number from the methyl end) is lost as CO_2, and the remaining two carbons become the acetyl group of acetyl-CoA.

In gluconeogenesis, the label tracking reverses. Lactate labeled at C1 (the carboxyl) becomes pyruvate labeled at C1. Through pyruvate carboxylase and PEPCK, the label stays at the same carbon (the carboxylate-derived position). Through the reverse glycolytic steps, GAP and DHAP are formed, and the aldolase condensation joins them into F-1,6-BP. The label from the GAP-derived triose ends up on C4 of glucose, and the label from the DHAP-derived triose ends up on C3 of glucose (these positions correspond to the carbons adjacent to the C3–C4 cleavage site).

16.10 Reciprocal Regulation of Glycolysis and Gluconeogenesis

Glycolysis and gluconeogenesis share seven enzymes but use different enzymes at the three irreversible steps. Regulation is concentrated at these non-shared steps, particularly at the PFK-1/FBPase-1 pair and the pyruvate kinase/pyruvate carboxylase-PEPCK pair.

Regulation at PFK-1/FBPase-1 — the F-2,6-BP axis:

Fructose-2,6-bisphosphate is a potent allosteric activator of PFK-1 and a potent inhibitor of FBPase-1. It is not a glycolytic intermediate — it is a regulatory molecule synthesized and degraded by a single bifunctional enzyme that has both a kinase domain (PFK-2) and a phosphatase domain (FBPase-2) on the same polypeptide.

When blood glucose is high, insulin signaling leads to dephosphorylation of the bifunctional enzyme, which activates the PFK-2 kinase domain and inactivates the FBPase-2 phosphatase domain. PFK-2 converts F6P to F-2,6-BP, which accumulates and activates PFK-1, stimulating glycolysis, while simultaneously inhibiting FBPase-1, suppressing gluconeogenesis.

When blood glucose is low, glucagon signaling activates protein kinase A (PKA), which phosphorylates the bifunctional enzyme. Phosphorylation activates the FBPase-2 phosphatase domain and inactivates the PFK-2 kinase domain. FBPase-2 hydrolyzes F-2,6-BP, lowering its concentration. Without F-2,6-BP, PFK-1 loses its activator (glycolysis slows) and FBPase-1 loses its inhibitor (gluconeogenesis accelerates).

Energy charge regulation:

ATP inhibits PFK-1 (the cell has enough energy, no need for more glycolysis). AMP activates PFK-1 (the cell needs energy, glycolysis should speed up). AMP inhibits FBPase-1 (low energy means the cell should not be spending energy on gluconeogenesis). These effects ensure that at high energy charge, glycolysis is suppressed and gluconeogenesis can proceed; at low energy charge, glycolysis is activated and gluconeogenesis is suppressed.

Metabolite signaling:

Citrate is an allosteric inhibitor of PFK-1. High citrate levels indicate that the TCA cycle is producing more intermediates than needed — both energy production and biosynthetic precursor supply are adequate. Under these conditions, glycolysis is not needed. Alanine (which is just one transamination step from pyruvate) inhibits pyruvate kinase, signaling that amino acid precursors are abundant and pyruvate production can slow down.

Feed-forward activation:

Fructose-1,6-bisphosphate allosterically activates pyruvate kinase (the last irreversible step of glycolysis). This is feed-forward regulation: when flux through the early steps of glycolysis is high, F-1,6-BP accumulates and pre-activates the downstream enzyme, ensuring that intermediates do not pile up. This regulation affects KM (F-1,6-BP decreases KM for PEP by shifting the T⇌R equilibrium toward the more active R state) but does not affect kcat (because F-1,6-BP binds at an allosteric site, not in the active site, and does not change the intrinsic catalytic rate of the R-state enzyme).

ATP allosterically inhibits pyruvate kinase. On a substrate saturation curve, the presence of ATP shifts the curve to the right (higher apparent KM, lower apparent affinity for PEP — T-state stabilization), while F-1,6-BP shifts the curve to the left (lower apparent KM, higher apparent affinity — R-state stabilization). All three curves (no effector, +F-1,6-BP, +ATP) converge at the same maximum velocity because the allosteric effectors alter the T⇌R equilibrium but not the catalytic capacity of the R state.

Glucokinase KM and its physiological significance:

The liver-specific glucokinase (hexokinase IV) has a high KM of approximately 10 mM, which is close to the normal blood glucose concentration. This means that at normal blood glucose, glucokinase operates well below its Vmax, and its activity is highly sensitive to changes in glucose concentration. When blood glucose rises (after a meal), glucokinase activity increases proportionally, and the liver traps more glucose for glycogen synthesis and glycolysis. When blood glucose falls, glucokinase activity drops sharply, allowing glucose to pass through the liver to reach the brain. If the KM were decreased (higher affinity for glucose), the liver would continue trapping glucose even at low blood glucose levels, potentially depriving the brain and other tissues of essential fuel. Additionally, glycolysis would be inappropriately active at a time when gluconeogenesis is what the body needs to maintain blood sugar.

Topic: The Pyruvate Dehydrogenase Complex

17.1 The Metabolic Significance of the PDH Complex

The pyruvate dehydrogenase complex occupies one of the most critical junctions in all of metabolism. It catalyzes the irreversible oxidative decarboxylation of pyruvate to acetyl-CoA, permanently committing the carbons of glucose-derived pyruvate to oxidation in the citric acid cycle. Once pyruvate is converted to acetyl-CoA, those carbons cannot be used to synthesize glucose — animals cannot convert acetyl-CoA back to pyruvate or to any gluconeogenic intermediate. This irreversibility makes the PDH complex a point of no return and explains why its regulation is so stringent.

The overall reaction is: Pyruvate + CoA + NAD^+ → Acetyl-CoA + CO_2 + NADH. The reaction involves the sequential action of three enzymes (E1, E2, and E3) and five cofactors (TPP, lipoamide, CoA, FAD, and NAD^+). Each cofactor has a specific and non-interchangeable role.

17.2 The Three Enzymes

E1 — Pyruvate dehydrogenase (pyruvate decarboxylase component): E1 uses TPP to catalyze the oxidative decarboxylation of pyruvate. The TPP ylide attacks the α-keto carbon of pyruvate, forming an adduct. Decarboxylation occurs, with CO_2 departing and the electrons flowing into the thiazolium ring as an electron sink. This generates the hydroxyethyl-TPP intermediate, which carries the two-carbon fragment derived from pyruvate in a reduced (hydroxyl) state.

E2 — Dihydrolipoyl transacetylase: E2 has lipoamide covalently attached via a lysine residue (lipoyllysine). The lipoamide arm swings into the E1 active site, where the hydroxyethyl group from hydroxyethyl-TPP attacks the disulfide bond of the oxidized lipoamide. This simultaneously transfers the two-carbon fragment to lipoamide, oxidizes it from a hydroxyl to a thioester (acetyl-lipoamide), reduces one sulfur of the lipoamide from disulfide to thiol, and regenerates the TPP ylide. The lipoamide arm then swings to the E2 active site, where the acetyl group is transferred from the sulfur of lipoamide to the thiol of CoA in a transesterification reaction, producing acetyl-CoA. This leaves lipoamide in the fully reduced (dihydro) form with two free thiol groups.

E3 — Dihydrolipoyl dehydrogenase: E3 re-oxidizes the dihydrolipoamide back to its oxidized disulfide form, regenerating the cofactor for the next catalytic cycle. The electrons from dihydrolipoamide are transferred through a pair of cysteine residues in the E3 active site (via disulfide exchange chemistry), then from the reduced cysteines to the tightly-bound FAD (which accepts the electrons, becoming $FADH^-$), and finally from $FADH^-$ to NAD^+ (which accepts a hydride, becoming NADH). The FAD is used catalytically — it is regenerated at each cycle — while NAD^+ is used stoichiometrically.

17.3 Why FAD is Necessary as an Intermediary

This distinction deserves emphasis because it reveals the underlying chemical logic. Dihydrolipoamide is a dithiol — its two sulfur atoms are each bonded to hydrogen. When this dithiol is re-oxidized to a disulfide, the electrons are transferred through a thiol-disulfide exchange mechanism (a sulfur attacks a sulfur in a nucleophilic displacement). This is fundamentally different from hydride transfer. NAD^+ specifically accepts a hydride ion ($H^- = 2e^- + H^+$) at the C4 of the nicotinamide ring. There is no direct chemical pathway for a thiol-disulfide exchange to deliver a hydride to NAD^+.

FAD bridges this mechanistic gap. FAD can accept electrons from the thiol-disulfide exchange mechanism (through the covalent C4a-thiol-FAD chemistry at the E3 active site), becoming reduced to $FADH^-$ (the anionic hydroquinone form, which is resonance-stabilized). $FADH^-$ can then donate a hydride to NAD^+, regenerating FAD and producing NADH. Without FAD, the re-oxidation of dihydrolipoamide and the reduction of NAD^+ could not be mechanistically coupled.

17.4 The Cofactor Matching Framework

Each of the five cofactors has a specific, observable role:

TPP provides the electron sink for the decarboxylation of pyruvate. It stabilizes the carbanion that would otherwise be an unstable intermediate after CO_2 departs.

Lipoamide is the oxidant that accepts the hydroxyethyl group from hydroxyethyl-TPP. It is reduced by this transfer (its disulfide is opened to a thiol), and it simultaneously oxidizes the two-carbon fragment to a thioester.

CoA accepts the acetyl group from acetyl-lipoamide in a transesterification reaction (exchange of one thioester for another).

FAD is used catalytically in the re-oxidation of the pair of cysteines in the E3 active site. It cycles between FAD and $FADH^-$ within each catalytic cycle and is regenerated.

NAD^+ is used stoichiometrically to re-oxidize $FADH^-$ to FAD, producing NADH as a final product.

17.5 Regulation of the PDH Complex

The PDH complex is regulated by two interrelated mechanisms: allosteric regulation and covalent modification (phosphorylation/dephosphorylation).

Product inhibition (allosteric): Acetyl-CoA and NADH, the two products of the reaction, inhibit the complex. High concentrations of acetyl-CoA or NADH signal that the products are being produced faster than they are consumed (the TCA cycle is saturated or downstream pathways are backed up), and the complex slows down.

Energy charge regulation (allosteric): ATP inhibits the complex. High ATP signals that the cell has sufficient energy and does not need to oxidize more pyruvate. AMP activates the complex (low energy charge, need more oxidation).

Substrate/precursor activation (allosteric): Pyruvate, CoA, and NAD^+ (the substrates of the reaction) activate the complex by feed-forward and mass-action effects.

Covalent modification: The E1 subunit of the PDH complex has a specific serine residue that can be phosphorylated by pyruvate dehydrogenase kinase (PDH kinase). Phosphorylation of this serine inactivates E1, shutting down the entire complex. Dephosphorylation by pyruvate dehydrogenase phosphatase reactivates E1.

PDH kinase is activated by high concentrations of acetyl-CoA, NADH, and ATP — the same signals that indicate the cell does not need more oxidation. PDH kinase is inhibited by pyruvate, CoA, NAD^+, and ADP — signals that the cell has substrate available and needs more oxidation.

PDH phosphatase is activated by insulin. Insulin signals high blood glucose, which means the cell should be metabolizing glucose. Activating the phosphatase removes the inhibitory phosphate from E1, turning on the PDH complex so that pyruvate from glycolysis is efficiently converted to acetyl-CoA for the TCA cycle. Glucagon signals the opposite metabolic state (fasting, low blood glucose), and under glucagon signaling, PDH kinase is more active, keeping the complex phosphorylated and inactive, diverting pyruvate toward gluconeogenesis instead.

17.6 Why PDH Does Not Run Under Anaerobic Conditions

The PDH complex does not directly use molecular oxygen, yet it does not function under anaerobic conditions. The reason is that PDH produces NADH, and under anaerobic conditions, there is no electron transport chain activity to re-oxidize NADH back to NAD^+. Without NAD^+ regeneration, NAD^+ is rapidly depleted, and the PDH reaction (which requires NAD^+ as a stoichiometric substrate) cannot proceed. Additionally, the accumulation of NADH would allosterically inhibit the complex and activate PDH kinase, leading to phosphorylation and inactivation. The same logic applies to the citric acid cycle, which also produces NADH and $FADH_2$ and also stalls when these cofactors cannot be re-oxidized.

17.7 Alternative Fates of Pyruvate When PDH Is Inhibited

When PDH is inactivated (by phosphorylation or product inhibition), pyruvate accumulates and must be directed elsewhere. The two principal alternative fates are gluconeogenesis (pyruvate → oxaloacetate via pyruvate carboxylase, then → PEP → glucose) and fermentation (pyruvate → lactate in mammals, or pyruvate → ethanol in yeast). Both of these pathways consume pyruvate without requiring the PDH complex.

17.8 The Citric Acid Cycle — Functional Summary

The primary catabolic purpose of the citric acid cycle (also called the TCA cycle or Krebs cycle) is to oxidize the acetyl group of acetyl-CoA completely to two molecules of CO_2, capturing the released electrons as reduced cofactors. Each turn of the cycle produces 3 NADH, 1 $FADH_2$, and 1 GTP (equivalent to 1 ATP). These reduced cofactors are the main energy currency produced by the cycle; they feed into the electron transport chain for oxidative phosphorylation, which generates the vast majority of ATP in aerobic organisms. The TCA cycle also provides critical biosynthetic precursors (oxaloacetate for gluconeogenesis, α-ketoglutarate for amino acid synthesis, succinyl-CoA for porphyrin biosynthesis, citrate for fatty acid synthesis), making it an amphibolic pathway — one that serves both catabolic and anabolic functions.

Supporting Foundations: Thermodynamics and Enzyme Kinetics

Thermodynamic Calculations

The relationship $\Delta G' = \Delta G^{\circ\prime} + RT \ln Q$ is the single most important quantitative tool in this guide. R is the gas constant in units compatible with kJ: 8.314×10^{-3} kJ mol^{-1} K^{-1}. T is the absolute temperature in Kelvin (typically 298 K for standard conditions or 310 K for physiological conditions, depending on the stated convention). Q is the mass-action ratio of actual concentrations: [products]/[reactants]. When multiple reactants or products are involved, their concentrations are multiplied together (each raised to the power of its stoichiometric coefficient, though all coefficients in these examples are 1).

To find the concentration ratio at which a reaction becomes non-spontaneous (the threshold of spontaneity), set ΔG' = 0 and solve for Q. To find the actual ΔG' given concentrations, compute Q from the given concentrations, take the natural log, multiply by RT, and add to ΔG°'. To find the ΔG°' of a coupled reaction, add the ΔG°' values of the component reactions (being careful to reverse the sign of ΔG°' if the reaction is reversed).

For hydrolysis-based coupling: if compound X is hydrolyzed with ΔG°' = -A, and compound Y is synthesized from its components with ΔG°' = +B, then the coupled reaction (X drives Y synthesis) has ΔG°' = B - A (or more precisely, -A + B). When a hydrolysis ΔG°' is provided but the synthesis direction is needed, reverse the sign of that component reaction.

Enzyme Kinetics — KM, kcat, and Mutant Analysis

KM is the substrate concentration at which the reaction velocity is half of Vmax. It is an approximate measure of the enzyme's affinity for its substrate: a lower KM indicates higher affinity (the enzyme reaches half-maximal velocity at a lower substrate concentration). KM is not a true dissociation constant except in the specific case where the rate of product formation is much slower than the rate of substrate release (the rapid equilibrium assumption), but for practical purposes in this guide, it is treated as an affinity measure.

kcat (the turnover number) is the maximum number of substrate molecules converted to product per enzyme molecule per unit time when the enzyme is saturated with substrate. It reflects the intrinsic catalytic efficiency of the enzyme.

When analyzing the effects of active-site mutations on kinetic parameters, the key is to determine whether the mutated residue is involved in substrate binding, transition-state stabilization, or both. If a residue contacts the substrate but is not involved in the chemical transformation, its mutation will primarily affect KM (binding) without significantly affecting kcat (catalysis). If a residue stabilizes the transition state during the chemical step, its mutation will decrease kcat. If a residue does both (as is often the case for residues that hydrogen-bond to the reactive functional group of the substrate), both KM and kcat will be affected.

For allosteric enzymes, the substrate saturation curve is sigmoidal rather than hyperbolic, reflecting cooperative binding. Allosteric activators shift the curve to the left (lower apparent KM, the enzyme becomes more responsive to substrate at lower concentrations) by stabilizing the R (active) state. Allosteric inhibitors shift the curve to the right (higher apparent KM) by stabilizing the T (inactive) state. In the MWC (concerted) model, allosteric effectors do not change the Vmax — they only change the T⇌R equilibrium, affecting the apparent KM but not the intrinsic catalytic rate of the R state.

End of Core Knowledge Reference