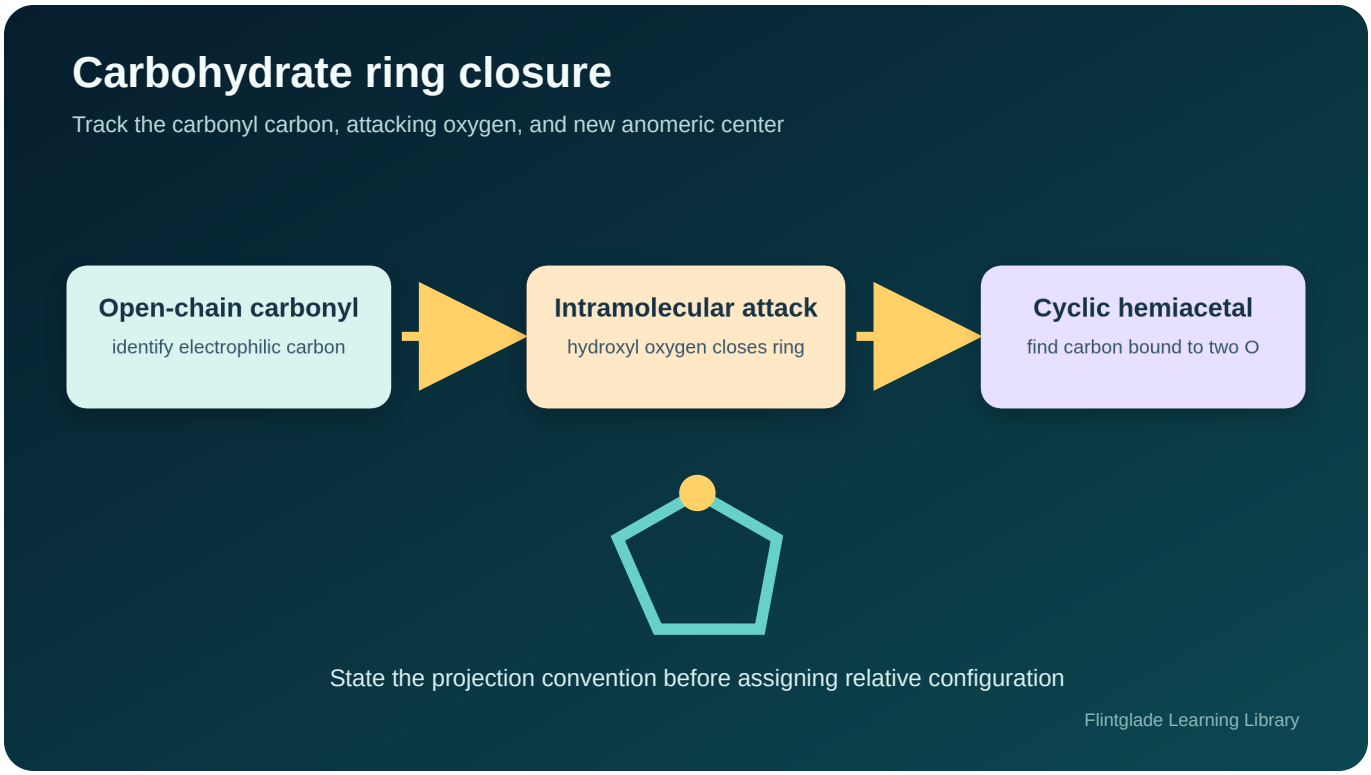
Proteins, Enzymes, and Carbohydrates

Flintglade Learning Library · General Biochemistry

Audience: Beginning and intermediate learners connecting molecular structure to folding, catalysis, and carbohydrate chemistry.

MCAT connections: This guide can assist study of amino acids and proteins, enzymes and kinetics, 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

- Explain the forces that shape protein structure
- Interpret enzyme kinetics and catalytic strategies
- Convert among common carbohydrate representations with explicit conventions

Navigate this guide

- Topic: Protein Structure, Folding & Thermodynamics
- Topic: Enzyme Kinetics
- Topic: Catalytic Mechanisms
- Topic: Enzyme Regulation
- Topic: Carbohydrates
- Summary of Key Equations

Topic: Protein Structure, Folding & Thermodynamics

Objective: Describe the forces that stabilize protein structure

Protein structure is maintained by a combination of covalent and noncovalent forces. Understanding these is central to understanding why proteins fold and how they function.

1. Covalent bonds — Disulfide bonds Disulfide bonds (–S–S–) form between the thiol groups of two cysteine residues. They are the only covalent cross-links that routinely stabilize tertiary and quaternary structure. They are especially common in extracellular proteins (e.g., antibodies, insulin) because the oxidizing environment outside the cell favors their formation.

2. Electrostatic interactions (ion pairs / salt bridges) Oppositely charged side chains (e.g., Lys^+ and Asp^-) can form salt bridges. In the low-dielectric interior of a protein, these interactions can be quite strong (~40–200 kJ/mol in vacuum), but in aqueous solution they are partially screened by water. A buried salt bridge can contribute significantly to stability; a surface-exposed one contributes less because both charges are well-solvated when the protein is unfolded.

3. Hydrogen bonds Hydrogen bonds (~2–20 kJ/mol each) form between a donor (N–H, O–H) and an acceptor (C=O, N, O). Backbone NH and C=O groups form the regular hydrogen-bond patterns that define secondary structure (α-helices and β-sheets). Side chains such as Ser, Thr, Asn, Gln, Tyr, His, Trp, and backbone atoms all participate. Individually weak, but there are hundreds in a typical protein, making them collectively essential.

4. Van der Waals interactions (London dispersion forces) These arise from transient dipole–induced dipole attractions between atoms in close proximity. Each interaction is very weak (~2–4 kJ/mol) but the tightly packed interior of a protein contains thousands of atom–atom contacts, so the cumulative contribution is large. Optimal packing of the hydrophobic core is critical.

5. The hydrophobic effect (the dominant driving force) When a protein folds, nonpolar side chains (Ala, Val, Leu, Ile, Phe, Trp, Met, Pro) are buried away from water. This releases ordered water molecules from around the hydrophobic groups back into the bulk solvent, increasing the entropy of the water. The hydrophobic effect is considered the single largest driving force for folding. It is primarily an entropic effect at room temperature (ΔS of water increases when structured "cages" around nonpolar groups are disrupted).

Objective: Identify amino acids involved in intramolecular interactions that stabilize protein structure

Hydrophobic core packing (van der Waals + hydrophobic effect): Ala, Val, Leu, Ile, Phe, Trp, Met, Pro — nonpolar side chains that cluster in the protein interior.

Salt bridges / electrostatic interactions: Positively charged: Lys (ε-amino), Arg (guanidinium), His (imidazolium, when protonated). Negatively charged: Asp (carboxylate), Glu (carboxylate). Example: a buried Arg–Asp salt bridge in the interior of a protein.

Hydrogen bonds: Polar uncharged: Ser, Thr (hydroxyl), Asn, Gln (amide), Tyr (hydroxyl), Trp (indole NH), Cys (thiol, as H-bond donor), His (imidazole). Charged residues (Lys, Arg, Asp, Glu) also form hydrogen bonds. The backbone NH and C=O groups of every residue participate in H-bonds in secondary structures.

Disulfide bonds: Cys–Cys. Two cysteine residues that are brought into proximity by folding can be oxidized to form a covalent –S–S– link.

Aromatic stacking: Phe, Tyr, Trp, His — aromatic rings can stack in edge-to-face or offset-parallel geometries, contributing van der Waals and electrostatic (quadrupole) interactions.

Cation–π interactions: A positively charged group (Lys or Arg) interacting with the π-electron cloud of an aromatic ring (Phe, Tyr, Trp). These are surprisingly strong (5–80 kJ/mol).

Objective: Summarize the process of protein folding

Protein folding is the process by which a linear polypeptide chain acquires its unique three-dimensional native structure.

Key features of the folding process:

1. **Levinthal's paradox:** If a 100-residue protein randomly sampled all possible conformations, it would take longer than the age of the universe to find the native state. Yet proteins fold in milliseconds to seconds. This tells us folding is not a random search.

2. **The folding funnel (energy landscape model):** Folding is best described as a funnel-shaped energy landscape. The top of the funnel represents the unfolded ensemble (high energy, high entropy — many conformations). As the chain progresses down the funnel, it loses conformational entropy but gains stabilizing interactions, decreasing its free energy. The bottom of the funnel is the native state (lowest free energy). The funnel can have local minima (kinetic traps) and the surface is rugged, but overall the thermodynamic gradient guides folding.

3. **Nucleation–condensation and framework models:** Early folding events may involve the rapid formation of secondary structural elements (helices, turns) that serve as nuclei around which the rest of the structure organizes. Hydrophobic collapse — the rapid burial of nonpolar residues — often occurs early and dramatically narrows the conformational search.

4. **Chaperones:** In the crowded cellular environment, partially folded proteins can aggregate. Molecular chaperones (e.g., Hsp70, chaperonins like GroEL/GroES) assist folding by binding exposed hydrophobic regions, preventing aggregation, and providing an isolated environment for folding. They do NOT provide folding information — the sequence dictates the structure (Anfinsen's dogma).

5. **Anfinsen's experiment:** Christian Anfinsen showed that reduced and denatured ribonuclease A could spontaneously refold to its active native conformation when denaturing agents were removed. This proved that all the information needed for folding is encoded in the amino acid sequence.

Objective: Apply the laws of thermodynamics to understand protein folding

First Law (conservation of energy): Energy is neither created nor destroyed during folding. The enthalpy changes in the system (protein + solvent) are balanced by heat exchange with the surroundings.

Second Law (entropy of the universe must increase): For folding to be spontaneous, the total entropy of the universe (system + surroundings) must increase, or equivalently, the Gibbs free energy of the system must decrease ($\Delta G < 0$).

The Gibbs free energy equation:

$\Delta G_{folding} = \Delta H_{folding} - T\Delta S_{folding}$

For folding to be spontaneous: $\Delta G_{folding} < 0$.

Dissecting the contributions:

The *protein chain* loses conformational entropy upon folding ($\Delta S_{chain} < 0$ — unfavorable). Thousands of backbone and side-chain rotations become restricted.

The *water* gains entropy when hydrophobic side chains are buried ($\Delta S_{water} > 0$ — favorable). Ordered water "cages" (clathrate-like structures) around nonpolar groups are released.

The net ΔS_{total} of the system depends on the balance: at physiological temperature, the favorable ΔS_{water} typically outweighs the unfavorable ΔS_{chain}.

ΔH contributions include the formation of hydrogen bonds, van der Waals contacts, and salt bridges in the folded state. However, the unfolded state also makes H-bonds (with water), so the net ΔH of folding is often small and can be either slightly favorable or unfavorable.

The net result: $\Delta G_{folding}$ is typically only about −20 to −65 kJ/mol (−5 to −15 kcal/mol) — marginally stable. This marginal stability is biologically important: it allows proteins to be flexible enough to function and to be degraded when no longer needed. The native state sits in a shallow free-energy minimum.

Temperature dependence: At very low temperatures, the hydrophobic effect weakens (ΔS_{water} contribution decreases), and cold denaturation can occur. At high temperatures, the $-T\Delta S_{chain}$ term dominates and the protein unfolds (thermal denaturation). There is an optimal temperature range where $\Delta G_{folding}$ is most negative.

Topic: Enzyme Kinetics

Objective: Explain the difference between ΔG and ΔG°, and use the relationship to determine ΔG, ΔG°, and/or the ratio of reactants to products under cellular conditions

Standard free energy change (ΔG°): ΔG° (or $\Delta G^{\circ\prime}$ in biochemistry, at pH 7) is the free energy change when reactants and products are all at their standard-state concentrations (1 M for solutes, 1 atm for gases, pH 7 for biochemical standard state). It is a fixed thermodynamic constant for a given reaction at a given temperature.

$$\Delta G^\circ = -RT \ln K_{eq}$$

where K_{eq} is the equilibrium constant. If $K_{eq} > 1$, ΔG° is negative (products favored at equilibrium). If $K_{eq} < 1$, ΔG° is positive (reactants favored).

Actual free energy change (ΔG): ΔG is the free energy change under the actual conditions in the cell — meaning whatever concentrations of reactants and products happen to exist at that moment. It determines whether a reaction will proceed spontaneously under those specific conditions.

$\Delta G = \Delta G^\circ + RT \ln Q$

where Q is the reaction quotient: Q = [products]/[reactants] (with appropriate stoichiometric exponents).

Key distinctions:

- ΔG° is a constant; ΔG depends on actual concentrations.
- A reaction with a positive ΔG° can still proceed forward in the cell if concentrations are maintained such that Q is small enough to make ΔG negative.
- At equilibrium, $\Delta G = 0$ (not $\Delta G^\circ = 0$), and $Q = K_{eq}$.

Practical application: Many biosynthetic reactions have positive ΔG° values, yet they proceed in cells because the cell maintains reactant/product ratios far from equilibrium (using coupled reactions, compartmentalization, or rapid removal of products).

Objective: Describe how catalysts affect the rate of a reaction

A catalyst accelerates a reaction by lowering the activation energy ($\Delta G^\ddagger$) — the energy barrier between reactants and the transition state. Key principles:

1. **A catalyst does NOT change the thermodynamics.** ΔG and ΔG° are unaffected. The equilibrium position is unchanged. Both forward and reverse reactions are accelerated equally.
2. **A catalyst provides an alternative reaction pathway** with a lower activation energy. For enzymes, this involves specific binding of substrates and stabilization of the transition state.
3. **A catalyst is not consumed** by the reaction. It participates transiently and is regenerated at the end of each catalytic cycle.
4. **A catalyst speeds up the attainment of equilibrium** but does not shift where equilibrium lies.
5. **Magnitude of rate enhancement:** Enzymes typically accelerate reactions by factors of 10^6 to 10^{17}. Even modest reductions in $\Delta G^\ddagger$ produce enormous rate increases because the rate depends exponentially on the activation energy (Arrhenius equation: $k \propto e^{(-\Delta G^\ddagger/RT)}$).

Objective: Explain the relationship between the transition state and the active site, and list characteristics of active sites

Transition state and the active site:

The transition state is the highest-energy species along the reaction coordinate — a fleeting, unstable arrangement of atoms at the point of bond breaking/forming. Enzymes work primarily by binding and stabilizing the transition state more tightly than the substrate or products.

The active site is complementary in shape, charge, and polarity to the transition state rather than to the substrate in its ground state. This preferential binding of the transition state lowers ΔG‡ and accelerates the reaction. Linus Pauling first articulated this idea; transition-state analogs (molecules that mimic the transition state) are potent enzyme inhibitors, confirming this model.

Characteristics of active sites:

1. **Three-dimensional cleft or pocket** formed by residues from different parts of the polypeptide chain (sometimes from different subunits). It constitutes only a small fraction of the total enzyme volume.
2. **Specific substrate binding** via multiple weak noncovalent interactions (H-bonds, van der Waals, electrostatic, hydrophobic) — collectively producing high specificity.
3. **Microenvironment distinct from bulk solvent:** The active site is often relatively nonpolar, excluding water. This can enhance electrostatic interactions and facilitate catalysis.
4. **Precisely positioned catalytic residues:** Amino acid side chains (and sometimes cofactors) are arranged to perform acid-base catalysis, covalent catalysis, metal-ion catalysis, or electrostatic stabilization.
5. **Induced fit:** Many enzymes undergo a conformational change upon substrate binding (Daniel Koshland's induced-fit model), closing the active site around the substrate, excluding water, and optimally positioning catalytic groups.

Objective: Define reaction velocity

Reaction velocity (v) is the rate at which substrate is converted to product in an enzyme-catalyzed reaction. It is typically expressed as the amount of product formed (or substrate consumed) per unit time:

$v = -d[S]/dt = d[P]/dt$

Units: usually μmol/min or $mol \cdot L^{-1} \cdot s^{-1}$ (M/s).

In practice, we measure the **initial velocity (v_0)** — the rate measured at the very beginning of the reaction, when $[P] \approx 0$. This is important because at early time points the reverse reaction is negligible, product inhibition has not yet occurred, and $[S] \approx [S]_0$, so conditions are well defined.

Objective: Explain how reaction velocity is measured and how velocities characterize enzyme activity

Measuring velocity: One monitors either the disappearance of substrate or the appearance of product over time. Common methods include spectrophotometric assays (e.g., measuring NADH absorbance at 340 nm), fluorometric assays, radioactive labeling, or coupled enzyme assays.

The initial velocity (v_0) is obtained from the slope of the [P] vs. time curve at time ≈ 0 (the linear portion).

Using velocities to characterize enzyme activity: By measuring v_0 at many different substrate concentrations [S] and plotting v_0 vs. [S], we obtain a saturation curve. This curve reveals the kinetic parameters (V_max, K_M) that define the enzyme's catalytic behavior.

At low [S]: v increases roughly linearly with [S] (first-order behavior). At high [S]: v approaches V_max asymptotically (zero-order behavior — the enzyme is saturated).

A Lineweaver-Burk (double reciprocal) plot — $1/v_0$ vs. 1/[S] — linearizes the Michaelis-Menten equation and allows graphical determination of K_M and V_max from the axis intercepts.

Objective: Describe the Michaelis-Menten model and the assumptions used to derive the Michaelis-Menten equation

The model:

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

The enzyme (E) binds substrate (S) reversibly to form an enzyme–substrate complex (ES), which then breaks down irreversibly to release product (P) and regenerate free enzyme.

Rate constants:

- k_1: $E + S \rightarrow ES$ (association)
- k_{-1}: $ES \rightarrow E + S$ (dissociation)
- k_2 (also called k_cat): $ES \rightarrow E + P$ (catalytic step)

Key assumptions:

1. **Steady-state assumption (Briggs-Haldane):** After a brief initial transient, the concentration of ES reaches a steady state where its rate of formation equals its rate of breakdown: $d[ES]/dt = 0$ $k_1[E][S] = (k_{-1} + k_2)[ES]$

2. **Initial velocity conditions:** [S] >> [E]_total, so [S] is essentially constant. Product concentration is negligible, so the reverse reaction ($P \rightarrow S$) does not occur.

3. **Free substrate approximation:** Because [S] >> [E], the amount of S bound in ES is negligible compared to total [S]. Therefore [S]_free ≈ [S]_total.

Derivation leads to:

$v_0 = V_{max} \cdot [S] / (K_M + [S])$

where:

- $V_{max} = k_{cat} \cdot [E]_{total}$ (the maximal velocity when all enzyme is saturated)

- $K_M = (k_{-1} + k_2)/k_1$ (the Michaelis constant)

Objective: Differentiate between V_max, k_cat, and K_M

V_max (maximal velocity): The reaction rate when every enzyme molecule is saturated with substrate (all enzyme is in the ES form). V_max depends on both the catalytic rate constant AND the total enzyme concentration: V_max = k_cat × [E]_total. It is not an intrinsic property of the enzyme alone — doubling the enzyme concentration doubles V_max.

k_cat (catalytic constant / turnover number): k_cat = V_max / [E]_total. It is the number of substrate molecules converted to product per enzyme molecule per unit time when the enzyme is fully saturated. It IS an intrinsic property of the enzyme. Units: s^{-1} (or min^{-1}). Example: carbonic anhydrase has k_cat ≈ 10^6 s^{-1}.

K_M (Michaelis constant): K_M is the substrate concentration at which v_0 = V_max/2. It has units of concentration (M). Operationally, a low K_M means the enzyme reaches half-maximal velocity at a low [S] — the enzyme has high apparent affinity for the substrate. A high K_M means more substrate is needed.

If $k_2 << k_{-1}$, then $K_M \approx k_{-1}/k_1 = K_d$ (the dissociation constant), and K_M reflects true binding affinity. But in general, K_M is a kinetic parameter, not a pure equilibrium binding constant.

Objective: Evaluate the efficiency and specificity of an enzyme based on kinetic parameters

Catalytic efficiency: k_cat/K_M

The ratio k_cat/K_M (also called the specificity constant) is the best measure of how efficiently an enzyme converts substrate to product under physiological conditions (where [S] << K_M). It has units of $M^{-1}s^{-1}$.

When [S] << K_M, the Michaelis-Menten equation simplifies to: $v_0 \approx (k_{cat}/K_M) \cdot [E] \cdot [S]$

So k_cat/K_M is effectively a second-order rate constant for the reaction of free enzyme with free substrate.

Diffusion-controlled limit: The upper limit for k_cat/K_M is set by the rate at which E and S can diffuse together in solution, approximately 10^8–10^9 $M^{-1}s^{-1}$. Enzymes that approach this limit (e.g., triosephosphate isomerase, carbonic anhydrase, acetylcholinesterase) are called "catalytically perfect" — every encounter between enzyme and substrate leads to product.

Specificity: When an enzyme acts on two different substrates (A and B), the ratio of their k_cat/K_M values predicts which substrate is preferred. If $(k_{cat}/K_M)_A > (k_{cat}/K_M)_B$, then substrate A is the preferred substrate.

Objective: Distinguish between reversible and irreversible inhibitors

Irreversible inhibitors: These bind to the enzyme covalently or with such extreme affinity that the inhibition is essentially permanent. The enzyme molecule is permanently inactivated. Examples include nerve agents (DIPF reacting with the serine of acetylcholinesterase), aspirin (acetylates COX), and suicide inhibitors (mechanism-based inhibitors that are converted by the enzyme into a reactive species that covalently modifies the active site). Irreversible inhibitors reduce the effective [E]_total; they lower V_max proportionally and cannot be overcome by adding more substrate.

Reversible inhibitors: These bind noncovalently through weak interactions (H-bonds, hydrophobic, electrostatic) and can dissociate from the enzyme. The inhibition can be overcome (at least partially) by adding more substrate or by dilution. There are three major types: competitive, uncompetitive, and noncompetitive (mixed).

Objective: Describe how competitive, uncompetitive, and noncompetitive inhibitors alter kinetics

Competitive inhibition:

- The inhibitor (I) binds only to free enzyme (E), typically at the active site, competing directly with substrate.
- Effect on kinetics: K_M(apparent) increases (more S needed to reach half-V_max). V_max is unchanged (at sufficiently high [S], the substrate outcompetes the inhibitor).
- Lineweaver-Burk: Lines intersect on the y-axis (same 1/V_max); the slope increases.
- K_M(app) = K_M(1 + [I]/K_I)

Uncompetitive inhibition:

- The inhibitor binds only to the ES complex (not to free E). It locks the substrate on the enzyme in a dead-end complex (ESI).
- Effect on kinetics: Both V_max(app) and K_M(app) decrease by the same factor. The ratio V_max/K_M is unchanged.
- Lineweaver-Burk: Parallel lines (same slope); the y-intercept and x-intercept both shift.
- V_max(app) = V_max / (1 + [I]/K_I')
- K_M(app) = K_M / (1 + [I]/K_I')

Noncompetitive (mixed) inhibition:

- The inhibitor binds to both free enzyme (E) and the ES complex, at a site distinct from the active site.
- Pure noncompetitive: K_I = K_I' (equal affinity for E and ES). V_max(app) decreases; K_M is unchanged. Lineweaver-Burk: Lines intersect on the x-axis.
- Mixed noncompetitive: K_I ≠ K_I'. Both V_max and K_M are altered. Lines intersect to the left of the y-axis (but not on an axis).
- Cannot be overcome by increasing [S] because the inhibitor can still bind to ES.

Topic: Catalytic Mechanisms

Objective: Describe the various catalytic strategies used by enzymes

Enzymes use several strategies, often in combination:

1. Acid-Base Catalysis (General Acid-Base Catalysis) Amino acid side chains donate or accept protons during the reaction, stabilizing charged intermediates. In general acid catalysis, a proton is donated by a group other than water (e.g., protonated His, Glu, Asp). In general base catalysis, a proton is abstracted by a group other than hydroxide (e.g., deprotonated His). Many enzymes use both simultaneously ("concerted acid-base catalysis"). Key residues: His (pKa ~6, excellent proton shuttle), Glu, Asp, Lys, Cys, Tyr.

2. Covalent Catalysis A nucleophilic residue on the enzyme (often Ser, Cys, His, or Lys) forms a transient covalent bond with the substrate, creating a covalent enzyme-substrate intermediate. This provides an alternative, lower-energy reaction pathway. Examples: serine proteases (acyl-enzyme intermediate via active-site Ser), chymotrypsin, trypsin.

3. Metal Ion Catalysis Metal ions can:

- Orient substrates for reaction (template effect).
- Stabilize negative charges on intermediates (electrostatic catalysis / charge shielding).
- Participate in oxidation-reduction reactions (e.g., Zn^{2+} in carbonic anhydrase generates a hydroxide nucleophile; Mg^{2+} in kinases coordinates phosphate groups).
- Generate powerful nucleophiles (e.g., Zn^{2+}-bound water has a much lower pKa than free water).

4. Electrostatic Catalysis (Transition State Stabilization) The enzyme active site provides a pre-organized electrostatic environment that preferentially stabilizes the charge distribution of the transition state. Oxyanion holes (backbone NH groups that stabilize negatively charged tetrahedral intermediates in proteases) are a classic example.

5. Proximity and Orientation Effects (Entropy Reduction) By binding substrates in the correct orientation and in close proximity, the enzyme converts an intermolecular reaction into an effective intramolecular reaction. This enormously reduces the entropic cost of bringing reactants together. The effective concentration of reactants in the active site can be equivalent to many molar.

6. Desolvation / Exclusion of Water Upon substrate binding, water is excluded from the active site. This enhances electrostatic interactions (charges interact more strongly in a low-dielectric environment) and prevents water from competing as a nucleophile when it is not desired.

Objective: Give examples of specific features of enzyme active sites and describe their role in catalysis

The Catalytic Triad (Serine Proteases — chymotrypsin, trypsin, elastase): Ser195, His57, Asp102 work in concert. Asp102 orients and polarizes His57, which acts as a general base to abstract a proton from Ser195, making Ser195 a powerful nucleophile that attacks the substrate's peptide carbonyl carbon. The oxyanion hole (backbone NH of Gly193 and Ser195) stabilizes the tetrahedral oxyanion intermediate via hydrogen bonds.

Oxyanion Hole: Backbone amide NH groups positioned to donate hydrogen bonds to the negatively charged oxygen of the tetrahedral intermediate. This is electrostatic/transition-state stabilization.

Specificity Pocket (S1 pocket in chymotrypsin): A hydrophobic pocket adjacent to the scissile bond that accommodates the side chain of the residue being cleaved. In chymotrypsin, it is large and hydrophobic (selects for Phe, Trp, Tyr). In trypsin, an Asp at the bottom creates a negative charge (selects for Lys, Arg). In elastase, bulky residues partially fill the pocket (selects for small side chains like Ala, Gly).

Lysozyme active site: Glu35 (general acid, protonates the leaving group oxygen) and Asp52 (stabilizes the oxocarbenium ion intermediate through electrostatic interaction). The enzyme also distorts the sugar residue in the D subsite toward the half-chair conformation (substrate strain), which resembles the transition state.

Carbonic anhydrase: Zn^{2+} ion coordinated by three His residues and a water molecule. The zinc lowers the pKa of the bound water from ~15 to ~7, generating a hydroxide nucleophile at physiological pH.

Objective: Describe how enzymes achieve substrate specificity

1. **Shape complementarity:** The active site is a precise three-dimensional cavity whose shape matches the substrate. Steric exclusion prevents larger or differently shaped molecules from binding productively.
2. **Chemical complementarity:** Charge, polarity, and hydrogen-bonding capability of the active site match those of the correct substrate. Incorrect substrates cannot form the full complement of favorable interactions.
3. **Induced fit (Koshland model):** The enzyme undergoes a conformational change upon binding the correct substrate, bringing catalytic groups into optimal alignment. Incorrect substrates do not induce the proper conformational change, so catalysis does not occur. This provides an additional layer of selectivity beyond the initial lock-and-key complementarity.
4. **Specificity pockets:** Defined binding pockets select for particular side chains (e.g., the S1 pocket of serine proteases described above).

5. **Transition-state complementarity:** The active site is most complementary to the transition state. Only substrates whose transition states match the active site geometry will be efficiently catalyzed.

Objective: Discuss experimental approaches used to elucidate catalytic mechanisms

1. **X-ray crystallography and cryo-EM:** Determine three-dimensional structures of enzyme, enzyme-substrate complexes, and enzyme-inhibitor complexes at atomic resolution. Transition-state analogs bound in the active site reveal what the transition state looks like.
2. **Site-directed mutagenesis:** Systematically replace active-site residues (e.g., Ser → Ala) and measure the effect on k_cat and K_M. If mutating a residue dramatically reduces k_cat, that residue is likely involved in catalysis. If it increases K_M, it is likely involved in binding.
3. **pH-rate profiles:** Plotting k_cat (or k_cat/K_M) vs. pH reveals the pKa values of ionizable groups essential for catalysis. This identifies which residues must be protonated or deprotonated for activity.
4. **Kinetic isotope effects:** Substituting ^{2}H (deuterium) or ^{18}O for protium or ^{16}O and measuring the change in rate constant. A significant kinetic isotope effect indicates that bond breaking/forming at that atom is part of the rate-limiting step.
5. **Chemical modification and affinity labeling:** Chemically modify specific residues (e.g., DIPF labels the active-site Ser) and test for loss of activity.
6. **Intermediate trapping:** Use rapid-quench or freeze-quench techniques to capture and identify covalent intermediates (e.g., the acyl-enzyme intermediate in serine proteases).
7. **Mechanism-based inhibitors (suicide substrates):** These compounds are processed by the enzyme's normal mechanism but generate a reactive intermediate that covalently inactivates the enzyme, confirming the proposed mechanism.

Objective: Given experimental data, propose chemically-reasonable catalytic mechanisms for hydrolytic enzymes

General approach for proposing a mechanism:

1. **Identify the bond being broken.** In hydrolytic enzymes, this is typically an ester, amide (peptide), glycosidic, or phosphodiester bond.
2. **Identify the nucleophile.** This could be water/hydroxide (direct hydrolysis) or an active-site residue (covalent catalysis) such as Ser, Cys, Asp, or Glu.

3. **Identify acid-base catalysts.** Look for His, Glu, Asp near the scissile bond. Determine which is the general base (abstracts a proton to activate the nucleophile) and which is the general acid (donates a proton to the leaving group).
4. **Identify stabilization of the transition state/intermediate.** Look for oxyanion holes, metal ions, or electrostatic interactions that stabilize the tetrahedral intermediate (for ester/amide hydrolysis) or the oxocarbenium ion intermediate (for glycosidic bond hydrolysis).
5. **Write the mechanism step by step** using curved arrows to show electron flow. For a two-step mechanism (acyl-enzyme pathway): (a) nucleophilic attack on the carbonyl carbon by the enzyme nucleophile, forming a tetrahedral intermediate, collapse to form the acyl-enzyme + leaving group; (b) water attacks the acyl-enzyme (deacylation), forming a second tetrahedral intermediate, which collapses to release product and regenerate the enzyme.
6. **Use experimental data:** The identity of catalytic residues (from mutagenesis), the pH optimum, the detection of intermediates (from rapid kinetics), and the structure (from crystallography) should all be consistent with the proposed mechanism.

Example — Serine protease mechanism (two-step): Step 1 (Acylation): His57 (general base) activates Ser195, which attacks the peptide C=O, forming a tetrahedral intermediate (stabilized by oxyanion hole). The intermediate collapses, breaking the C–N bond; His57 (general acid) donates a proton to the departing amine leaving group. Result: acyl-enzyme. Step 2 (Deacylation): Water enters. His57 (general base) activates water, which attacks the acyl-enzyme carbonyl. A second tetrahedral intermediate forms (stabilized by oxyanion hole), then collapses, releasing the carboxylic acid product and regenerating Ser195.

Topic: Enzyme Regulation

Objective: Describe the characteristics of allosteric enzymes and the shape of their v vs. [S] curves

Allosteric enzymes differ from simple Michaelis-Menten enzymes in several important ways:

1. **Quaternary structure:** They are almost always multi-subunit (oligomeric) proteins.
2. **Multiple active sites:** Each subunit typically has its own active site.
3. **Cooperative substrate binding:** Binding of substrate to one subunit influences binding at other subunits (positive cooperativity), similar to O_2 binding to hemoglobin.
4. **Sigmoidal (S-shaped) kinetics:** Instead of the hyperbolic v vs. [S] curve predicted by Michaelis-Menten kinetics, allosteric enzymes show a sigmoidal curve. At low [S], the enzyme is relatively inactive (T state). As [S] increases past a threshold, activity rises steeply (transition to R state),

then plateaus at V_max.

5. **Regulatory sites distinct from the active site:** Allosteric enzymes have binding sites for regulatory molecules (effectors/modulators) at locations other than the active site (hence "allosteric" — "other site").

6. **Concerted (MWC) model:** The enzyme exists in an equilibrium between a low-activity T (tense) state and a high-activity R (relaxed) state. All subunits switch states simultaneously. Substrates and activators shift the equilibrium toward R; inhibitors shift it toward T.

7. **Not described by simple K_M:** Instead of K_M, the substrate concentration producing half-maximal velocity is called K_0.5 (it is analogous but not identical to K_M). The Hill coefficient (n_H) quantifies cooperativity: n_H > 1 indicates positive cooperativity; n_H = 1 is non-cooperative (hyperbolic); n_H < 1 is negative cooperativity.

Objective: Define feedback inhibition

Feedback inhibition is a regulatory strategy in which the end product of a metabolic pathway inhibits an enzyme early in the same pathway, usually the first committed step. This prevents the unnecessary accumulation of intermediates and product, conserving cellular resources.

Example: In the pyrimidine biosynthesis pathway, CTP (the final product) inhibits aspartate transcarbamoylase (ATCase), the enzyme catalyzing the first committed step (aspartate + carbamoyl phosphate → N-carbamoylaspartate). When CTP levels are high, the pathway slows down. When CTP levels drop, inhibition is relieved and flux through the pathway increases.

Feedback inhibition is rapid, reversible, and precisely tuned to cellular needs.

Objective: Describe the regulatory mechanism used by ATCase

Aspartate transcarbamoylase (ATCase) from E. coli is the classic example of an allosteric enzyme.

Structure: ATCase has 12 subunits: 6 catalytic subunits (organized as 2 catalytic trimers, c_3) and 6 regulatory subunits (organized as 3 regulatory dimers, r_2). The holoenzyme is c_6r_6. Catalytic subunits contain the active site; regulatory subunits bind allosteric effectors.

Allosteric regulation:

- **CTP (inhibitor, feedback inhibition):** CTP binds to the regulatory subunits and shifts the T ⇌ R equilibrium toward the T (inactive) state. This decreases the enzyme's affinity for substrates (increases K_0.5) without significantly changing V_max. The sigmoidal curve shifts to the right.
- **ATP (activator):** ATP binds to the regulatory subunits and shifts the equilibrium toward the R (active) state. This increases substrate affinity (decreases K_0.5). The sigmoidal curve shifts to the left, approaching a more hyperbolic shape. ATP signals that purine nucleotides are abundant and that the cell can afford to make pyrimidines, maintaining nucleotide balance.

Conformational change: In the T → R transition, the two catalytic trimers rotate relative to each other and move apart along the three-fold axis. This opens up the active sites and increases substrate affinity.

Substrate cooperativity: Aspartate binds cooperatively: binding of aspartate to one active site favors the T → R transition, increasing the affinity of the remaining active sites (homotropic effect).

Objective: Differentiate between homotropic and heterotropic effects, and explain how they modify the T ⇌ R equilibrium

Homotropic effects: The effector molecule is the same as the substrate. Substrate binding to one subunit affects substrate binding at other subunits. In ATCase, aspartate is a homotropic activator — binding of aspartate to one catalytic subunit promotes the T → R transition, increasing the affinity of the remaining subunits for aspartate. This produces the sigmoidal v vs. [S] curve and is the basis of cooperativity.

Heterotropic effects: The effector molecule is different from the substrate. It binds at a regulatory site, not the active site. In ATCase:

- CTP is a heterotropic inhibitor: shifts the equilibrium toward T.
- ATP is a heterotropic activator: shifts the equilibrium toward R.

How they modify the T ⇌ R equilibrium: In the MWC (concerted) model:

- Activators (homotropic or heterotropic) preferentially bind to and stabilize the R state, pulling the equilibrium from T toward R. The R state has higher substrate affinity and higher catalytic activity.
- Inhibitors preferentially bind to and stabilize the T state, pulling the equilibrium from R toward T.

The key insight is that the T ⇌ R equilibrium is a molecular switch, and allosteric effectors "dial" the switch in one direction or another.

Objective: Define isozymes and explain their purpose in metabolism

Isozymes (isoenzymes) are different enzymes that catalyze the same chemical reaction but differ in amino acid sequence, kinetic properties, regulatory properties, tissue distribution, or subcellular localization. They are encoded by different genes.

Purpose: Isozymes allow the same reaction to be regulated differently in different tissues or metabolic contexts.

Classic example: Lactate Dehydrogenase (LDH) LDH exists as a tetramer of two types of subunits: H (heart) and M (muscle). Five isozymes exist (H_4, H_3M, H_2M_2, HM_3, M_4). The H_4 isozyme (predominant in heart) has a low K_M for lactate — it efficiently converts lactate → pyruvate for oxidation in the

heart. The M_4 isozyme (predominant in skeletal muscle) favors pyruvate → lactate conversion during anaerobic glycolysis. This allows each tissue to have kinetic properties appropriate to its metabolic needs.

Other examples: Hexokinase (I–IV) and glucokinase (hexokinase IV) in liver vs. other tissues. Glucokinase has a much higher K_M for glucose, allowing the liver to sense and respond to high blood glucose levels after a meal.

Objective: Describe the activity of protein kinases and phosphatases

Protein kinases catalyze the transfer of the γ-phosphoryl group from ATP to a specific amino acid side chain on a target protein:

Protein + ATP → Protein-PO_4^{2-} + ADP

Common phosphorylation sites: serine, threonine (Ser/Thr kinases), or tyrosine (Tyr kinases). Histidine kinases exist in prokaryotes. Kinases are highly specific — a given kinase recognizes particular consensus sequences on its target proteins.

Protein phosphatases catalyze the hydrolytic removal of the phosphoryl group:

Protein-PO_4^{2-} + H_2O → Protein + P_i

Phosphatases reverse the action of kinases. Different phosphatases have different specificities (e.g., PP1, PP2A for Ser/Thr; PTP1B for Tyr).

Together, kinases and phosphatases form a "switch" that toggles proteins between phosphorylated and dephosphorylated states, each with potentially different activity, conformation, or binding properties.

Objective: Explain why phosphorylation is an effective means of regulating enzyme activity

1. **Introduction of charge:** The phosphoryl group carries approximately two negative charges at physiological pH. This introduces a significant electrostatic perturbation that can alter protein conformation, create or disrupt binding interfaces, or change active-site geometry.
2. **Versatile hydrogen bonding:** The phosphoryl group can form up to three hydrogen bonds (more than any amino acid side chain), creating new interaction surfaces.
3. **Thermodynamic commitment:** ATP hydrolysis makes phosphorylation thermodynamically favorable ($\Delta G^\circ \approx -30.5$ kJ/mol for ATP hydrolysis). This ensures that the kinase reaction is essentially irreversible under cellular conditions. Dephosphorylation by a phosphatase is required to reverse it, providing a separate point of regulation.

4. **Amplification through signaling cascades:** A single activated kinase can phosphorylate many substrate molecules, which can in turn activate other kinases (e.g., MAP kinase cascade), amplifying the signal enormously.

5. **Speed and reversibility:** Phosphorylation/dephosphorylation cycles operate on timescales of seconds to minutes — fast enough to respond to extracellular signals (hormones, growth factors) in real time.

6. **Specificity:** Different kinases recognize different consensus sequences, allowing precise targeting of specific enzymes in specific pathways.

7. **Flexibility:** Phosphorylation can either activate OR inhibit an enzyme, depending on where the phosphoryl group is placed and how the protein responds conformationally. Example: glycogen phosphorylase is activated by phosphorylation; glycogen synthase is inhibited by phosphorylation.

Objective: Define zymogen and proenzyme

A zymogen (proenzyme) is an inactive precursor form of an enzyme. It becomes active only after proteolytic cleavage removes a peptide segment (the pro-peptide or activation peptide). This is an irreversible activation mechanism — once the peptide is cleaved, it cannot be reattached without synthesizing a new zymogen molecule.

Examples:

- Trypsinogen → trypsin (cleaved by enterokinase in the duodenum)
- Chymotrypsinogen → chymotrypsin (cleaved by trypsin)
- Pepsinogen → pepsin (autocatalytic in acidic conditions of the stomach)
- Blood clotting factors (e.g., prothrombin → thrombin)
- Caspases (procaspases → active caspases in apoptosis)

Objective: Explain why zymogen activation is important physiologically and how it differs from other regulatory mechanisms

Physiological importance:

1. **Protection from self-digestion:** Digestive proteases (trypsin, chymotrypsin, pepsin, elastase) are synthesized in the pancreas or stomach lining. If they were active inside the cell, they would digest the very tissue that produces them. Zymogens ensure that these enzymes are only active in the correct compartment (e.g., the intestinal lumen or stomach lumen).

2. **Rapid, spatially controlled activation:** Blood clotting requires an immediate, localized burst of enzyme activity at the site of injury. The coagulation cascade is a series of zymogen activations — each active protease activates the next zymogen, amplifying the signal rapidly. Activation is

confined to the wound site by local cofactors.

3. **Temporal control:** In apoptosis, procaspases are activated only when the cell receives appropriate death signals, ensuring that the irreversible cell-death program is not triggered prematurely.

How it differs from other regulatory mechanisms:

1. **Irreversibility:** Unlike phosphorylation (reversible), allosteric regulation (reversible), or reversible inhibitor binding, zymogen activation is a one-way process. The cleaved peptide cannot be reattached. The enzyme must be degraded and a new zymogen synthesized to reset the system. This makes it appropriate for one-time or irreversible processes (digestion, clotting, apoptosis).

2. **Proteolytic (not conformational):** Other mechanisms change the enzyme's conformation without altering its covalent structure. Zymogen activation permanently alters the primary structure by removing a peptide.

3. **Amplification cascades:** Zymogen cascades (as in coagulation) provide enormous amplification — one molecule of the initiating protease can ultimately generate millions of molecules of the final product (fibrin clot). This is distinct from allosteric regulation, which fine-tunes existing activity rather than creating explosive bursts.

4. **Not responsive to metabolite levels:** Allosteric regulation and feedback inhibition adjust enzyme activity based on real-time metabolite concentrations. Zymogen activation is triggered by specific signals (tissue damage, pH change, death signals) and is not designed for fine metabolic tuning.

Topic: Carbohydrates

Objective: Explain the difference between an aldose and a ketose

Monosaccharides are classified by the type of carbonyl group they contain:

Aldose: The carbonyl group is at C-1 (an aldehyde). Examples: glucose (an aldohexose — 6 carbons, aldehyde), ribose (an aldopentose — 5 carbons, aldehyde), glyceraldehyde (an aldotriose — 3 carbons, aldehyde).

Ketose: The carbonyl group is at C-2 (a ketone). Examples: fructose (a ketohexose — 6 carbons, ketone), ribulose (a ketopentose — 5 carbons, ketone), dihydroxyacetone (a ketotriose — 3 carbons, ketone).

Aldoses and ketoses can be interconverted by isomerization. For example, glucose (aldose) ⇌ fructose (ketose) via the enzyme phosphoglucose isomerase in glycolysis.

Both aldoses and ketoses cyclize in solution to form ring structures (pyranoses or furanoses), as described below.

Objective: Identify the anomeric carbon in any monosaccharide and the reducing end of any polysaccharide

Anomeric carbon: When a monosaccharide cyclizes (forming a hemiacetal or hemiketal), the carbonyl carbon (C-1 in aldoses, C-2 in ketoses) becomes a new chiral center called the anomeric carbon. It is the only carbon in the ring bonded to TWO oxygen atoms (the ring oxygen and the –OH or –OR group).

How to find it: look for the carbon bonded to two oxygens in the cyclic form.

Reducing end: The reducing end of a polysaccharide is the end with a free anomeric carbon (one that is not involved in a glycosidic bond to another sugar). This free anomeric carbon can open to expose a free aldehyde (or ketone) group, which can reduce oxidizing agents (hence "reducing"). The other end, where the anomeric carbon IS involved in a glycosidic bond, is the nonreducing end. In a branched polysaccharide like glycogen, there is only one reducing end but many nonreducing ends.

Objective: Draw the structure of a monosaccharide in both open-chain and cyclic forms

Open-chain form (Fischer projection for glucose):

For D-glucose (an aldohexose), the open-chain form shows C-1 as an aldehyde (CHO), with hydroxyl groups on C-2 through C-5 in specific orientations (R/S configurations that define D-glucose as distinct from its diastereomers), and C-6 as a primary alcohol (CH_2OH).

Cyclic form (Haworth projection):

Glucose cyclizes when the C-5 hydroxyl attacks the C-1 aldehyde, forming a six-membered ring (pyranose) with a hemiacetal linkage. This creates the anomeric carbon at C-1.

Key conversion rules (Fischer → Haworth for D-sugars in a pyranose ring):

- Groups on the RIGHT in the Fischer projection go DOWN in the Haworth projection.
- Groups on the LEFT go UP.
- The terminal –CH_2OH at C-6 goes UP for D-sugars.
- The anomeric –OH at C-1 can be either DOWN (α anomer) or UP (β anomer) in a Haworth drawing of a D-sugar.

Chair conformations (more realistic than Haworth): D-glucose preferentially adopts the $^4C_1$ chair, placing most large substituents in the equatorial position (β-D-glucopyranose has ALL substituents equatorial — explaining why glucose is the most common monosaccharide in nature).

Objective: Draw a mechanism showing how to convert between pyranose and furanose forms

The interconversion goes through the open-chain form as an intermediate:

Step 1 — Ring opening (pyranose → open chain): The ring oxygen is protonated (acid catalysis). Electrons from the ring oxygen help break the C–O bond, opening the ring and regenerating the carbonyl (aldehyde at C-1 for an aldose). The hemiacetal/hemiketal is hydrolyzed.

Mechanistically: the anomeric –OH is protonated or the ring oxygen is protonated, the C–O bond breaks, and the aldehyde reforms.

Step 2 — Ring closure (open chain → furanose): Instead of the C-5 hydroxyl attacking C-1 (which would reform the pyranose), the C-4 hydroxyl attacks C-1, forming a five-membered ring (furanose). The hydroxyl oxygen acts as a nucleophile, attacks the electrophilic carbonyl carbon, and a new hemiacetal is formed. Proton transfer completes the reaction.

In solution, there is a dynamic equilibrium among the open-chain form, the α-pyranose, the β-pyranose, the α-furanose, and the β-furanose. For glucose, the pyranose forms dominate (>99%), and the open-chain form is present at <0.01%.

Objective: Draw a mechanism showing how to convert between α and β anomers (mutarotation)

Mutarotation is the interconversion of α and β anomers through the open-chain form.

Mechanism:

Step 1 — Ring opening: General acid catalysis protonates the ring oxygen (or the anomeric –OH acts as the leaving group). The C–O bond at the anomeric center breaks, forming the open-chain aldehyde (or ketone). This is the reverse of hemiacetal formation.

Step 2 — Ring closure on the opposite face: The hydroxyl (e.g., C-5 –OH) attacks the re or si face of the carbonyl carbon. Attack from one face gives the α anomer; attack from the other face gives the β anomer. A general base deprotonates the attacking hydroxyl, and a general acid protonates the resulting alkoxide.

The result is that the anomeric –OH "flips" from axial (α, for D-glucopyranose in standard chair) to equatorial (β), or vice versa.

Mutarotation can be observed by watching the change in optical rotation of a freshly dissolved sugar solution over time (e.g., pure α-D-glucose has [α] = +112°; it equilibrates to +52.7° as the β anomer forms).

Objective: Describe how monosaccharides are linked to form complex carbohydrates

Monosaccharides are linked by **glycosidic bonds** — a type of covalent bond formed between the anomeric carbon of one sugar and a hydroxyl group of another sugar (or another molecule).

O-glycosidic bonds: The anomeric carbon is linked through an oxygen atom to another sugar. This is the most common type.

N-glycosidic bonds: The anomeric carbon is linked through a nitrogen atom (as in nucleosides/nucleotides, where the sugar is linked to a nitrogenous base).

Nomenclature: The bond is designated by (1) the anomeric configuration (α or β), (2) the number of the anomeric carbon, and (3) the number of the hydroxyl group on the other sugar. For example:

- Maltose: α-1,4 glycosidic bond (α-D-glucopyranosyl-(1→4)-D-glucopyranose).
- Lactose: β-1,4 glycosidic bond (β-D-galactopyranosyl-(1→4)-D-glucopyranose).
- Sucrose: α,β-1,2 glycosidic bond (both anomeric carbons participate — this is why sucrose is a nonreducing sugar).

Polysaccharides are formed by chains of repeating glycosidic bonds:

- Starch (amylose + amylopectin): α-1,4 links (linear stretches) + α-1,6 links (branch points in amylopectin).
- Glycogen: Similar to amylopectin but more highly branched.
- Cellulose: β-1,4 links throughout (cannot be digested by most animals due to lack of β-glucosidase).

Objective: Draw a mechanism showing how to form or hydrolyze a glycosidic bond

Formation (condensation/dehydration):

The anomeric –OH of sugar 1 is activated (e.g., converted to a nucleotide-sugar like UDP-glucose by a glycosyltransferase). The hydroxyl of sugar 2 acts as a nucleophile and attacks the anomeric carbon. A leaving group departs (e.g., UDP). A proton is transferred, water (or the nucleotide) is released, and the glycosidic bond is formed.

In non-enzymatic terms: the anomeric carbon (electrophilic due to the adjacent ring oxygen) is attacked by the hydroxyl nucleophile. General acid catalysis protonates the departing –OH (making it water, a better leaving group). The new C–O bond forms with either retention or inversion of

configuration at the anomeric center.

Hydrolysis (cleavage by water):

General acid catalysis: An acid catalyst (e.g., a Glu residue in lysozyme) donates a proton to the glycosidic oxygen, making it a better leaving group. The C–O bond breaks, generating an oxocarbenium ion intermediate (a positively charged, planar species at the anomeric carbon — this is the transition state analog). Water (or a carboxylate acting as a nucleophile) attacks the oxocarbenium ion. A proton is lost, and the two monosaccharide products are released.

Retention vs. inversion: Retaining glycosidases use a two-step (double-displacement) mechanism with a covalent glycosyl-enzyme intermediate: first, a nucleophilic Asp/Glu attacks the anomeric carbon (forming the intermediate and releasing the first product with inversion), then water attacks the intermediate (second inversion = net retention). Inverting glycosidases use a single-step mechanism: water attacks directly from the opposite face (single displacement = inversion).

Objective: Explain the role of glycosyltransferases in catalyzing the formation of glycosidic bonds

Glycosidic bond formation is thermodynamically unfavorable in isolation ($\Delta G^\circ > 0$ for condensation in aqueous solution). Cells overcome this by activating the sugar donor.

Glycosyltransferases catalyze glycosidic bond formation using **activated sugar donors** — typically nucleotide sugars (e.g., UDP-glucose, GDP-mannose, CMP-sialic acid). The nucleotide diphosphate (e.g., UDP) serves as an excellent leaving group, making the reaction thermodynamically favorable (driven by the hydrolysis of the high-energy nucleotide-sugar bond and subsequent hydrolysis of the released pyrophosphate).

Features of glycosyltransferases:

1. They are highly specific for both the donor sugar (including its anomeric configuration) and the acceptor hydroxyl group.
2. They determine the stereochemistry (α or β) of the newly formed glycosidic bond.
3. They define the position of linkage (e.g., $1 \rightarrow 4$ vs. $1 \rightarrow 6$).
4. They work in the ER and Golgi apparatus (for glycoprotein and glycolipid biosynthesis) or in the cytoplasm (e.g., glycogen synthase, which uses UDP-glucose to extend glycogen chains).

Because each glycosidic linkage requires a specific glycosyltransferase, the structure of complex carbohydrates is determined by which glycosyltransferases are expressed in a given cell — NOT by a genetic template (unlike DNA or protein synthesis).

Objective: Explain the biochemical roles of different types of glycoconjugates

Glycoconjugates are molecules in which carbohydrates are covalently attached to proteins or lipids.

1. Glycoproteins: Proteins with one or more covalently attached oligosaccharide chains. Two major types:

- **N-linked glycosylation:** Oligosaccharide attached through GlcNAc to the amide nitrogen of an Asn side chain (consensus sequence: Asn-X-Ser/Thr, where X ≠ Pro). Processing occurs in the ER and Golgi.
- **O-linked glycosylation:** Oligosaccharide attached through GalNAc (or other sugars) to the hydroxyl of Ser or Thr. Added in the Golgi.

Roles of glycoprotein carbohydrates:

- Protein folding and quality control (calnexin/calreticulin cycle in the ER).
- Protein stability and protection from proteolysis (the "sugar coat" shields the surface).
- Cell-cell recognition and signaling (e.g., selectins on endothelial cells bind to specific carbohydrate structures on leukocytes during inflammation).
- Immune recognition (blood group antigens A, B, O are determined by specific glycosyltransferases).

2. Proteoglycans: Proteins with very large, highly charged glycosaminoglycan (GAG) chains (e.g., heparan sulfate, chondroitin sulfate, keratan sulfate). GAGs are long unbranched polysaccharides of repeating disaccharide units, often heavily sulfated.

Roles:

- Structural components of the extracellular matrix (cartilage, connective tissue).
- Form hydrated gels that resist compression (e.g., aggrecan in cartilage).
- Act as co-receptors for growth factors (e.g., heparan sulfate proteoglycans present FGF to its receptor).
- Lubrication (hyaluronate in synovial fluid).

3. Glycolipids: Carbohydrates attached to lipids (sphingolipids). Found in the outer leaflet of the plasma membrane.

Roles:

- Cell-cell recognition (e.g., gangliosides in neural tissue).
- Signal transduction.
- Pathogen binding (many bacteria, viruses, and toxins bind to glycolipids; e.g., cholera toxin binds GM1 ganglioside).
- Blood group antigens (the A, B, and O antigens are glycolipids on red blood cells as well as glycoproteins).

4. Peptidoglycan: A polymer of sugar chains (alternating NAG and NAM) cross-linked by short peptides. It forms the bacterial cell wall, providing structural rigidity and osmotic protection. It is the target of antibiotics like penicillin (which inhibits the transpeptidase that cross-links the peptide bridges).

5. GPI anchors (glycosylphosphatidylinositol): Complex glycolipid structures that anchor certain proteins to the extracellular surface of the plasma membrane.

Summary of Key Equations

Parameter	Equation	Notes
Gibbs Free Energy	$\Delta G = \Delta H - T\Delta S$	Spontaneous if $\Delta G < 0$
Standard vs. Actual	$\Delta G = \Delta G^\circ + RT \ln Q$	Q = [products]/[reactants]
At Equilibrium	$\Delta G^\circ = -RT \ln K_{eq}$	$\Delta G = 0$ at equilibrium
Michaelis-Menten	$v_0 = V_{max}[S]/(K_M + [S])$	Hyperbolic saturation
Turnover Number	$k_{cat} = V_{max}/[E]_{total}$	Units: s^{-1}
Catalytic Efficiency	k_{cat}/K_M	Units: $M^{-1}s^{-1}$; max ~10^8–10^9
Michaelis Constant	$K_M = (k_{-1} + k_2)/k_1$	[S] at v = V_max/2
Competitive Inhibition	$K_{M(app)} = K_M(1 + [I]/K_I)$	V_max unchanged
Uncompetitive Inhibition	$V_{max(app)} = V_{max}/(1 + [I]/K_I')$	K_M also decreases