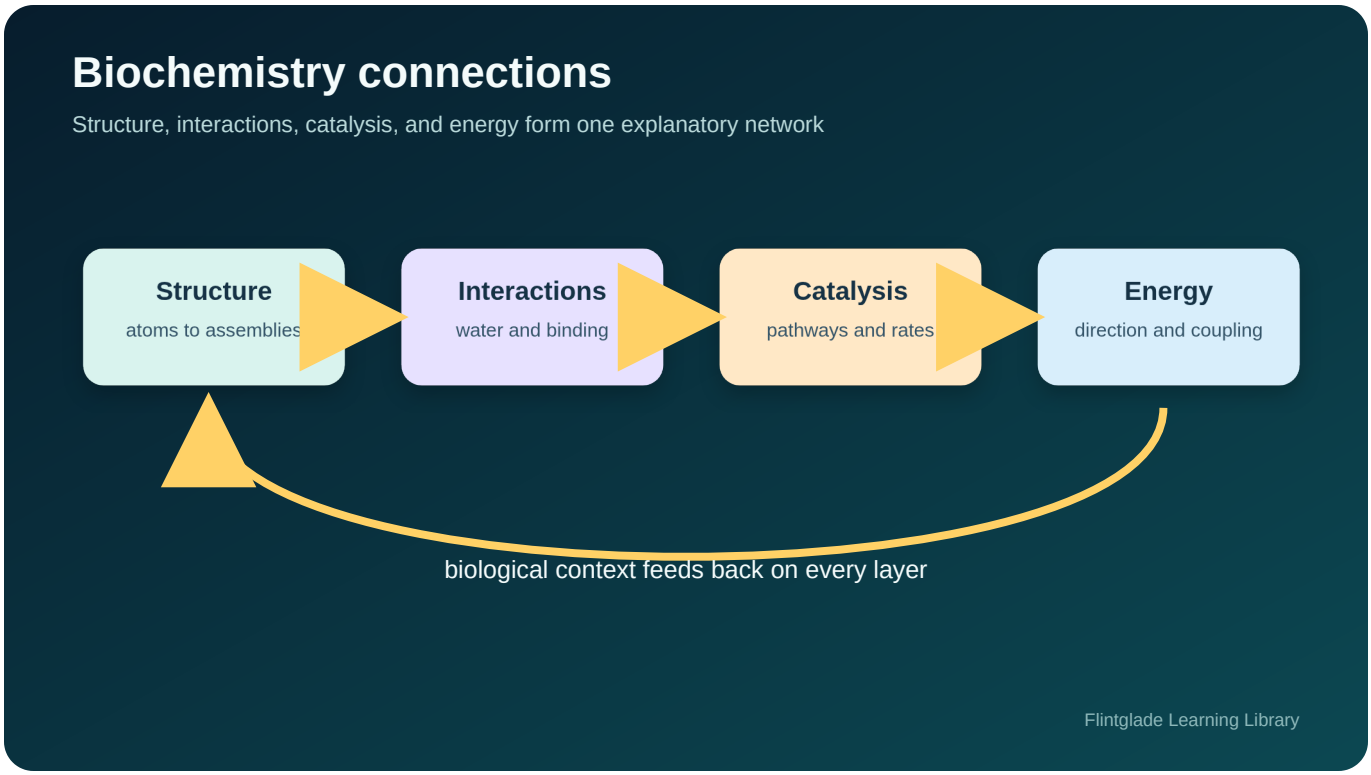
# Integrated Foundations of Biochemistry

*Flintglade Learning Library · General Biochemistry*

**Audience:** Independent learners seeking a broad, connected introduction from molecular forces through oxidative phosphorylation.

**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

- Build a connected mental model of structure, catalysis, and metabolism
- Distinguish thermodynamic direction from kinetic rate
- Follow matter, electrons, and free energy across biological systems

## Navigate this guide


- MOTIF 1: Noncovalent Interactions & The Aqueous Environment
- MOTIF 2: Acid-Base Equilibria & Buffers
- MOTIF 3: Thermodynamics & Free Energy
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- MOTIF 5: Protein Structure Hierarchy
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- MOTIF 15: Metabolic Logic & Reaction Types
- MOTIF 16: Glycolysis & Gluconeogenesis
- MOTIF 17: Pyruvate Dehydrogenase & Citric Acid Cycle
- MOTIF 18: Oxidative Phosphorylation (Sections 18.1–18.5)


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# MOTIF 1: Noncovalent Interactions & The Aqueous Environment

**Why this motif matters:** Every major topic in this guide — protein folding, membrane assembly, enzyme-substrate recognition, DNA stability, ligand binding — reduces to noncovalent interactions operating in water. If you understand these forces deeply, you can reason through many problems involving stability, specificity, or affinity.

### Types of Interactions (ranked by typical strength in biological systems)

**Covalent bonds** (~350–450 kJ/mol): Shared electron pairs. Not "noncovalent" but set the baseline. Peptide bonds, disulfide bonds, glycosidic bonds, phosphodiester bonds are all covalent.

**Ionic / Electrostatic interactions** (~20–40 kJ/mol in vacuum, much weaker in water): Full charge–charge attractions (e.g., $Lys^{+}\cdots Glu^{-}$ salt bridge in a protein interior). Strength depends critically on the dielectric constant of the medium — water's high dielectric (~80) screens these interactions heavily, which is why salt bridges buried in a hydrophobic protein core are much stronger than those on the surface.

**Hydrogen bonds** (~4–20 kJ/mol): Directional interaction between a hydrogen bond donor (electronegative atom bonded to H) and a hydrogen bond acceptor (electronegative atom with a lone pair). Key features: (1) they are directional — optimal geometry is linear; (2) they are distance-dependent; (3) in water, the net contribution to stability can be small because forming an intramolecular H-bond means breaking H-bonds to water. The *number* of H-bonds doesn't change much upon folding — what changes is *which* partners are involved. H-bonds are critical for specificity (e.g., base pairing in DNA, substrate recognition in active sites) more than raw stability.

**Van der Waals (vdW) interactions** (~2–4 kJ/mol each): Arise from transient, induced dipoles between atoms at close range. Individually weak but collectively significant because every atom participates. Optimal at the van der Waals contact distance — closer causes steric repulsion (Pauli exclusion), farther and the interaction drops off as $1/r^6$. The tight packing of protein interiors generates thousands of vdW contacts that collectively contribute substantial stabilization.

## Water Structure and the Hydrophobic Effect

Water is a polar molecule capable of forming up to four hydrogen bonds in a tetrahedral arrangement. Liquid water is a dynamic, flickering network of H-bonds that constantly break and reform.

**The hydrophobic effect** is the tendency of nonpolar molecules to aggregate in aqueous solution. This is the *dominant driving force* for protein folding, membrane assembly, and micelle formation. Crucially, the hydrophobic effect is NOT driven by attractive forces between nonpolar molecules — it is driven by the thermodynamics of water.

When a nonpolar solute is placed in water, surrounding water molecules form an ordered "cage" (clathrate-like structure) to maximize H-bonding while avoiding the nonpolar surface. This ordering *decreases entropy* ($\Delta S < 0$), which is thermodynamically unfavorable. When nonpolar molecules aggregate, the ordered water cages are disrupted and water molecules are released back into bulk solvent, *increasing entropy* ($\Delta S > 0$). This favorable entropy change is the primary thermodynamic driver.

**Temperature dependence:** At low temperatures, $\Delta H$ of the hydrophobic effect can be near zero or slightly positive (endothermic), meaning the process is *entirely entropy-driven*. At higher temperatures, the enthalpic contribution becomes more significant.

**Quantitative signature:** $\Delta G < 0$, $\Delta S > 0$ (favorable), $\Delta H \approx 0$ or slightly positive at 25°C. Large positive heat capacity change ($\Delta Cp > 0$) upon exposing nonpolar surface to water is a hallmark.

## Noncovalent Interactions in DNA Structure

The double helix is stabilized by: (1) hydrogen bonds between complementary base pairs (A=T has 2 H-bonds, G≡C has 3 H-bonds) — these provide *specificity* of base pairing; (2) base stacking interactions (vdW/London dispersion forces between the planar aromatic rings of stacked bases) — these are actually the *primary* stabilizing force, more so than H-bonding; (3) the hydrophobic effect — burial of the nonpolar base surfaces away from water.

DNA is *destabilized* by: (1) electrostatic repulsion between negatively charged phosphate groups on the backbone (counteracted by cations like $Mg^{2+}$ and histones); (2) the loss of conformational entropy upon forming the rigid double helix.

**DNA melting (denaturation):** The transition from double-stranded to single-stranded DNA is cooperative — once a few base pairs melt, neighboring pairs become destabilized (loss of stacking). This produces a sigmoidal melting curve (% denatured vs. temperature). The melting temperature (Tm) increases with GC content (three H-bonds vs. two) and with salt concentration (cations screen phosphate repulsion).

## Why Noncovalent, Reversible Interactions Matter

Biochemistry depends on *reversible* molecular recognition. If interactions were all covalent (irreversible), biological processes could not be regulated or turned off. Noncovalent interactions allow: signal transduction (ligand binding/release), enzyme-substrate turnover, oxygen loading/unloading by hemoglobin, membrane fluidity and dynamics, gene regulation (transcription factor binding/release). The weakness of individual noncovalent interactions is a feature, not a bug — specificity and regulation emerge from the collective, reversible action of many weak interactions.

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# MOTIF 2: Acid-Base Equilibria & Buffers

**Why this motif matters:** Ionization states dictate protein charge, enzyme activity, substrate recognition, and buffer capacity. The guide emphasizes quantitative problem-solving here — Henderson-Hasselbalch calculations, predicting ionization states, and understanding how local environment shifts pKa values.

## Definitions and Core Equations

**pH** = $-\log[H^+]$. A 1-unit change in pH = 10-fold change in $[H^+]$.

**Weak acid (HA):** Partially dissociates in water. $HA \rightleftharpoons A^- + H^+$. The equilibrium constant $K_a = [A^-][H^+]/[HA]$.

**pKa** = $-\log(K_a)$. The pKa is the pH at which the acid is 50% protonated and 50% deprotonated ($[HA] = [A^-]$). A lower pKa means a stronger acid (more readily donates a proton).

**Henderson-Hasselbalch equation:** $pH = pKa + \log([A^-]/[HA])$

This equation is the workhorse for buffer problems. Given any two of pH, pKa, $[A^-]$, [HA], you can find the third.

**Key relationships:**

- When pH < pKa: the protonated form (HA) predominates
- When pH > pKa: the deprotonated form ($A^-$) predominates
- When pH = pKa: $[HA] = [A^-]$ (50/50 mixture) — this is where buffering capacity is maximum
- A buffer is effective within approximately ±1 pH unit of its pKa

## Predicting Ionization States at a Given pH

For any ionizable group, compare pH to pKa:

- If pH < pKa → protonated form dominates
- If pH > pKa → deprotonated form dominates

For amino acids at physiological pH (7.4), using the standard pKa values:

- α-carboxyl (pKa ~2.0): deprotonated ($COO^-$)
- α-amino (pKa ~9.0): protonated ($NH_3^+$)
- Asp/Glu side chains (pKa ~4.1): deprotonated ($COO^-$)
- His (pKa ~6.0): mostly deprotonated (neutral imidazole), but close enough to be partially protonated — this is why His is such an effective catalytic residue
- Cys (pKa ~8.3): protonated (SH)
- Tyr (pKa ~10.0): protonated (OH)
- Lys (pKa ~10.4): protonated ($NH_3^+$)
- Arg (pKa ~12.5): protonated (guanidinium$^+$)

## pKa Shifts Due to Local Environment

A pKa can be shifted from its "normal" value when the ionizable group is placed in a non-standard environment. The rule: *any interaction that stabilizes the charged (deprotonated for acids, protonated for bases) form will lower the pKa; any interaction that stabilizes the neutral form will raise the pKa.*

Factors that shift pKa:

- **Nearby positive charge** → stabilizes the negative (deprotonated) form → lowers pKa
- **Nearby negative charge** → destabilizes the negative form → raises pKa
- **Burial in a hydrophobic environment** → destabilizes any charged form → raises pKa of acids (favoring neutral COOH), lowers pKa of bases (favoring neutral $NH_2$)
- **Hydrogen bonding** → can stabilize either form depending on geometry

Worked example: Asp at the N-terminus of an α-helix. The helix dipole places partial positive charge at the N-terminus and partial negative charge at the C-terminus. The positive environment stabilizes the deprotonated (negatively charged) Asp side chain, *lowering* its pKa.

### Buffers

A buffer resists changes in pH upon addition of acid or base. It consists of a weak acid and its conjugate base in comparable concentrations. Effective buffering range: pKa ± 1.

Buffer capacity depends on: (1) total concentration of the buffer components (higher = more capacity); (2) proximity of pH to pKa (maximum at pH = pKa).

In a buffer calculation, the total buffer concentration = [HA] + [$A^-$]. If you know total concentration and pH/pKa, you can solve for each component using Henderson-Hasselbalch.

### Polyprotic Systems (Amino Acids)

Amino acids have multiple ionizable groups and therefore multiple pKa values. The titration curve shows distinct buffering regions around each pKa.

**Isoelectric point (pI):** The pH at which the net charge on the molecule is zero. For a simple amino acid with no ionizable side chain: pI = (pKa1 + pKa2)/2. For amino acids with ionizable side chains, average the two pKa values that flank the zwitterionic (neutral) form.

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## MOTIF 3: Thermodynamics & Free Energy

**Why this motif matters:** This is the quantitative backbone of the entire subject. Every analysis of spontaneity, equilibrium, coupled reactions, phosphoryl-transfer potential, and metabolic flux depends on thermodynamic reasoning.

### The Laws of Thermodynamics

**First Law (Conservation of Energy):** Energy cannot be created or destroyed, only converted between forms. In biological systems, chemical bond energy is converted to mechanical work, heat, concentration gradients, etc.

**Second Law (Entropy):** The total entropy of the universe tends to increase. For a spontaneous process, ΔS_universe > 0. Living organisms create local order (decrease in entropy) at the expense of increasing entropy in their surroundings (usually by releasing heat).

### Gibbs Free Energy

**$\Delta G = \Delta H - T\Delta S$**

This is the master equation for predicting spontaneity at constant temperature and pressure.

- $\Delta G < 0$: spontaneous (exergonic)
- $\Delta G > 0$: nonspontaneous (endergonic)
- $\Delta G = 0$: at equilibrium

**ΔH (enthalpy):** Reflects bond energies. Negative ΔH means the products have lower bond energy (stronger/more bonds formed than broken) — energy is released to surroundings. In protein folding, ΔH_folding reflects the *net* of breaking interactions in the unfolded state (e.g., H-bonds with water) and forming new interactions in the folded state (intramolecular H-bonds, vdW contacts, electrostatic interactions). A negative ΔH_folding means the interactions formed during folding are enthalpically more favorable than those lost.

**ΔS (entropy):** Reflects disorder/freedom. In protein folding, there are competing contributions: (1) loss of conformational freedom in the polypeptide chain (ΔS < 0, unfavorable); (2) release of ordered water from hydrophobic surfaces (ΔS > 0, favorable — the hydrophobic effect). The sign of ΔS_folding tells you the relative magnitude of these opposing effects. A negative ΔS_folding indicates the conformational entropy loss outweighs the hydrophobic effect. A less negative ΔS_folding suggests a larger role for the hydrophobic effect.

## Standard Free Energy Change vs. Actual Free Energy Change

**ΔG°' (standard free energy change):** Measured under standard biochemical conditions (1 M concentrations, pH 7.0, 25°C, 1 atm). This is a *fixed property* of a reaction — it depends only on the nature of the reactants and products.

**ΔG (actual free energy change):** Depends on the actual concentrations in the cell.

**The relationship:** $\Delta G = \Delta G^{\circ\prime} + RT \ln([\text{products}]/[\text{reactants}])$

**Connection to equilibrium:** At equilibrium, $\Delta G = 0$, so: $\Delta G^{\circ\prime} = -RT \ln(K_{eq})$

Key implications:

- A reaction with $\Delta G^{\circ\prime} > 0$ can still proceed spontaneously ($\Delta G < 0$) if the ratio of products to reactants is sufficiently low
- Near-equilibrium reactions ($\Delta G \approx 0$) are readily reversible and respond to changes in metabolite concentrations
- Far-from-equilibrium reactions ($\Delta G << 0$) are essentially irreversible under cellular conditions and serve as control points for metabolic flux

## Coupled Reactions

Two reactions can be coupled through a shared intermediate. The overall ΔG°' is the sum of the individual ΔG°' values. A thermodynamically unfavorable reaction can be driven forward by coupling it to a favorable one (e.g., ATP hydrolysis).

Example from glycolysis: The aldolase reaction (ΔG°' = +23.8 kJ/mol) is driven forward because its product (G3P) is continuously consumed by downstream reactions. The actual ΔG in the cell is near zero.

### Activation Energy (ΔG‡)

**ΔG‡** is the free energy of activation — the energy barrier between reactants and the transition state. It determines the *rate* of a reaction, not whether it is spontaneous. Enzymes lower ΔG‡ without changing ΔG°'. An enzyme does NOT change the equilibrium position — it only changes how fast equilibrium is reached.

**Important distinction:** ΔG°' determines *if* a reaction will go; ΔG‡ determines *how fast* it goes.

### Quantitative Relationships

- $R = 8.314 \times 10^{-3}$ kJ $mol^{-1}$ $K^{-1}$
- At 298 K, RT = 2.48 kJ/mol
- $\Delta G^{\circ\prime} = -RT \ln(Keq)$: if Keq = 10, ΔG°' = −5.7 kJ/mol; if Keq = 100, ΔG°' = −11.4 kJ/mol
- A 10-fold change in Keq corresponds to ~5.7 kJ/mol change in ΔG°' at 298 K
- An enzyme that accelerates a reaction by $10^5$-fold stabilizes the transition state by ~28.5 kJ/mol

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## MOTIF 4: Amino Acids & Peptide Chemistry

**Why this motif matters:** Amino acids are the building blocks. Knowing their structures, properties, and ionization behavior is prerequisite for understanding protein structure, enzyme mechanisms, and purification strategies.

### The 20 Proteogenic Amino Acids

**Nonpolar/Hydrophobic:** Gly (G), Ala (A), Val (V), Leu (L), Ile (I), Pro (P), Phe (F), Trp (W), Met (M)

- Gly: smallest, most conformationally flexible, achiral
- Pro: cyclic side chain constrains backbone, is an α-helix breaker, often found in turns
- Phe, Trp: aromatic rings participate in stacking interactions and vdW contacts
- Met: thioether sulfur, not ionizable at physiological pH

**Polar/Uncharged:** Ser (S), Thr (T), Cys (C), Tyr (Y), Asn (N), Gln (Q)

- Ser, Thr: hydroxyl groups — targets for phosphorylation and nucleophilic catalysis
- Cys: thiol (−SH) can form disulfide bonds (covalent, stabilize extracellular proteins), can serve as nucleophile in catalysis (pKa 8.3)
- Tyr: phenolic hydroxyl, can be phosphorylated, pKa 10.0
- Asn, Gln: amide side chains, H-bond donors and acceptors but NOT ionizable

- **N-linked glycosylation** occurs on Asn residues (in the Asn-X-Ser/Thr sequon)

**Positively Charged at pH 7.4:** Lys (K), Arg (R), His (H)

- Lys: ε-amino group, pKa ~10.4 — almost always protonated at physiological pH
- Arg: guanidinium group, pKa ~12.5 — essentially always protonated
- His: imidazole ring, pKa ~6.0 — near physiological pH, making it uniquely suited for acid-base catalysis (can act as both proton donor and acceptor)

**Negatively Charged at pH 7.4:** Asp (D), Glu (E)

- Carboxylate side chains, pKa ~4.1 — deprotonated at physiological pH

## Peptide Bond Formation

Amino acids are linked by peptide bonds (amide bonds) formed by condensation (loss of water) between the α-carboxyl of one amino acid and the α-amino of the next. The peptide bond has partial double-bond character due to resonance, making it planar and restricting rotation. The six atoms of the peptide unit (Cα, C, O, N, H, Cα) lie in a single plane. Rotation IS allowed around the N-Cα bond (φ, phi) and the Cα-C bond (ψ, psi), though steric clashes limit the allowed values (Ramachandran plot).

**Convention:** Peptides are written from N-terminus (left) to C-terminus (right). The N-terminus has a free amino group; the C-terminus has a free carboxyl group.

## Stereochemistry

All proteogenic amino acids (except glycine) are L-amino acids. In a Fischer projection, the amino group is on the LEFT. In peptide structures, every Cα (except Gly) is a chiral center that must be drawn with correct S-configuration.

## Net Charge Calculations

To calculate net charge at a given pH: (1) identify all ionizable groups; (2) determine the protonation state of each using pH vs. pKa; (3) sum the charges. Groups that are protonated and carry a charge: amino groups ($NH_3^+$ = +1), His imidazolium ($HisH^+$ = +1), Arg guanidinium (+1), Lys ε-amino ($NH_3^+$ = +1). Groups that are deprotonated and carry a charge: carboxylates ($COO^-$ = −1), Cys thiolate ($S^-$ = −1), Tyr phenolate ($O^-$ = −1).

## Mass Spectrometry

**Tandem MS (MS/MS):** Peptides are ionized (typically by electrospray or MALDI), separated by m/z in the first mass analyzer, fragmented (usually at peptide bonds), then the fragments are separated in the second mass analyzer. The fragment ions form a "ladder" from which the sequence can be read. **b ions** contain the N-terminus; **y ions** contain the C-terminus. The mass of each amino acid residue is the mass of the amino acid minus water (18 Da).

For a y-ion: mass = sum of residue masses + 18 (for the terminal OH and H).

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# MOTIF 5: Protein Structure Hierarchy

**Why this motif matters:** Understanding the levels of structure and their stabilizing forces is foundational for predicting how mutations affect function, interpreting purification data, and reasoning about enzyme mechanisms.

## Primary Structure

The linear amino acid sequence, determined by the gene. Stabilized by covalent peptide bonds. Determines all higher levels of structure (Anfinsen's dogma). Also includes disulfide bonds (covalent cross-links between Cys residues).

## Secondary Structure

Regular, repeating local conformations of the polypeptide backbone stabilized by hydrogen bonds between backbone atoms (C=O and N–H groups).

**α-Helix:** Right-handed helix with 3.6 residues per turn. H-bonds form between the C=O of residue *n* and the N–H of residue *n+4*. Side chains project outward from the helix axis. The helix has a macrodipole — partial positive charge at the N-terminus (due to unbonded NH groups) and partial negative charge at the C-terminus (due to unbonded C=O groups). Negatively charged residues (Asp, Glu) are often found at the N-terminus to stabilize the helix dipole; positively charged residues at the C-terminus. The first four NH groups and last four C=O groups of the helix have no intrahelical H-bonding partners — capping residues can compensate. Pro is a helix breaker because its cyclic side chain lacks an amide H and introduces a rigid kink. Gly destabilizes helices due to excessive conformational flexibility.

**β-Sheet:** Extended polypeptide strands connected by H-bonds between backbone atoms of adjacent strands. Parallel sheets: strands run in the same direction (N→C); antiparallel sheets: strands alternate direction. Antiparallel sheets have straighter, stronger H-bonds. Side chains alternate above and below the plane of the sheet.

**Reverse turns (β-turns):** Short segments (usually 4 residues) that reverse the direction of the polypeptide chain. Often found at the surface of proteins. Gly and Pro are frequently found in turns — Gly for its flexibility, Pro for its ability to adopt the cis configuration of the peptide bond.

**Motifs (supersecondary structures):** Combinations of secondary structure elements. Examples: helix-turn-helix, β-α-β, coiled coil, leucine zipper. Similar motifs are often found in proteins with similar functions.

### Tertiary Structure

The overall three-dimensional shape of a single polypeptide chain. Stabilized by noncovalent interactions between side chains (and sometimes backbone atoms) that are distant in sequence but close in space: hydrophobic interactions (dominant driving force — nonpolar side chains pack in the protein interior), hydrogen bonds (side chain–side chain, side chain–backbone), electrostatic/ionic interactions (salt bridges), van der Waals contacts, and disulfide bonds (covalent, especially in extracellular proteins).

**Key principle:** In aqueous solution, globular proteins fold so that hydrophobic residues are buried in the interior (away from water) and hydrophilic residues are on the surface (in contact with water). This is the hydrophobic effect in action.

### Quaternary Structure

The arrangement of multiple polypeptide subunits (protomers) into a functional complex. Stabilized by the same noncovalent interactions as tertiary structure, but between subunits rather than within a single chain. Not all proteins have quaternary structure. Examples: hemoglobin ($\alpha_2\beta_2$ tetramer), ATCase.

### Domains

Compact, independently folding units within a single polypeptide chain. A single protein may have multiple domains with distinct functions (e.g., a catalytic domain and a regulatory domain).

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## MOTIF 6: Protein Folding & Stability

**Why this motif matters:** Understanding *why* and *how* proteins fold is explored through thermodynamic reasoning, experimental interpretation (Anfinsen, FRAP, denaturation curves), and disease connections.

### Anfinsen's Experiment (Ribonuclease A)

**Key conclusion:** The amino acid sequence (primary structure) contains all the information necessary for a protein to fold into its native, functional conformation. The native state represents the thermodynamic minimum — the lowest free energy state accessible to the polypeptide chain.

**Experimental details:** RNase A has four disulfide bonds (8 Cys residues). Denaturation with urea (disrupts noncovalent interactions) + β-mercaptoethanol (reduces disulfide bonds) → fully unfolded, inactive protein. Removal of denaturants → spontaneous refolding with full recovery of enzymatic activity.

**Scrambled RNase:** When reoxidized *in the presence of urea* (before noncovalent interactions can form correctly), random disulfide pairings form → "scrambled" RNase with only ~1-2% activity. Adding trace (catalytic) amounts of β-mercaptoethanol to scrambled RNase → allows disulfide reshuffling → protein samples conformational space → converges on native fold (global free energy minimum).

**Why only catalytic β-mercaptoethanol is needed:** β-mercaptoethanol acts as a thiol-disulfide exchange catalyst. It is regenerated in the reaction cycle — it reduces a wrong disulfide, the protein refolds, and a correct disulfide forms, releasing the β-mercaptoethanol to act again.

**Native vs. scrambled:** The native state is thermodynamically favored (lower ΔG). The scrambled forms are kinetically trapped, higher-energy states. β-mercaptoethanol provides the kinetic pathway (disulfide reshuffling) to escape these traps and reach the global minimum.

## Thermodynamics of Folding

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

For a stably folded protein, $\Delta G_{folding} < 0$ (but only marginally — typically −20 to −60 kJ/mol, which is the equivalent of a few H-bonds). This marginal stability is important: it allows for conformational flexibility, regulation, and eventual degradation.

**$\Delta H_{folding}$:** Reflects the net change in interactions. Breaking H-bonds and vdW contacts with water in the unfolded state (unfavorable), forming intramolecular H-bonds, vdW contacts, and electrostatic interactions in the folded state (favorable). If $\Delta H_{folding} < 0$, the interactions formed during folding are enthalpically more favorable than those broken. Important nuance: a more negative $\Delta H_{folding}$ does NOT necessarily mean "more or stronger bonds formed" — it could also mean fewer bonds were broken (i.e., fewer interactions existed in the unfolded state to begin with, as with cold-adapted proteins that may have fewer H-bonds in their unfolded state).

**$\Delta S_{folding}$:** Two major opposing contributions: (1) loss of conformational entropy (chains become ordered) → $\Delta S < 0$; (2) release of ordered water from hydrophobic surfaces (hydrophobic effect) → $\Delta S > 0$. If $\Delta S_{folding}$ is negative overall, conformational entropy loss dominates. A less negative $\Delta S_{folding}$ suggests greater contribution from the hydrophobic effect.

## Protein Denaturation

Unfolding can be induced by heat, extreme pH, detergents (e.g., SDS), chaotropes (e.g., urea, guanidinium chloride), or organic solvents. Denaturation disrupts noncovalent interactions; the protein loses its native conformation and (usually) its function.

Thermodynamic parameters upon denaturation:

- Nonpolar groups exposed to water → ordered water cages form → $\Delta S < 0$ (unfavorable)
- H-bonds between backbone/side chains broken, new H-bonds to water formed → $\Delta H$ can go either way depending on relative strengths

- The contribution of each type of interaction during denaturation is the reverse of its contribution during folding

### Cooperativity of Folding

Protein folding is highly cooperative — it is an "all-or-nothing" process. Intermediate states are rarely populated significantly. This is seen experimentally as a sharp, sigmoidal transition in denaturation curves (similar logic to DNA melting). Cooperativity arises because partial unfolding exposes hydrophobic surfaces, destabilizing the remaining structure.

### Molecular Chaperones

Some proteins require assistance to fold efficiently, especially under cellular conditions (high protein concentration → aggregation risk). Chaperones do NOT change the final folded structure — they prevent misfolding and aggregation by providing a protected environment for folding. The final structure is still dictated by the primary sequence.

### Protein Misfolding and Disease

Misfolded proteins can aggregate into amyloid fibrils (cross-β structure), which are associated with diseases: Alzheimer's (amyloid-β), Parkinson's (α-synuclein), prion diseases (PrP^Sc), sickle cell disease (HbS polymerization). Sickle cell is a single-point mutation (Glu6→Val in β-globin) that creates a hydrophobic patch on the surface, promoting polymerization of deoxy-HbS.

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## MOTIF 7: Ligand Binding & Cooperativity

**Why this motif matters:** The quantitative treatment of binding — Kd, fractional saturation, cooperativity, Hill plots — is developed in depth. This motif also connects to enzyme kinetics (KM is analogous to Kd under certain conditions) and allosteric regulation.

### Simple Binding (Myoglobin Model)

For a monomeric protein with a single binding site: $P + L \rightleftharpoons PL$

**Dissociation constant:** $K_d = [P][L]/[PL]$

**Fractional saturation:** $\theta = [L]/(K_d + [L])$

This produces a *hyperbolic* binding curve. When $[L] = K_d$, $\theta = 0.5$ (half-saturation). A lower Kd means higher affinity. Myoglobin follows this model for $O_2$ binding.

## Cooperative Binding (Hemoglobin Model)

Hemoglobin is an $\alpha_2\beta_2$ tetramer. Binding of $O_2$ to one subunit increases the affinity of the remaining subunits for $O_2$. This produces a *sigmoidal* binding curve. Hemoglobin exists in two quaternary states:

**T state (tense):** Low $O_2$ affinity. Stabilized by intersubunit salt bridges and electrostatic interactions. Predominates in the *absence* of $O_2$ (deoxyhemoglobin).

**R state (relaxed):** High $O_2$ affinity. Salt bridges are broken upon $O_2$ binding. Predominates when $O_2$ is bound.

**Structural basis of cooperativity:** When $O_2$ binds to the heme iron ($Fe^{2+}$), the iron moves into the plane of the porphyrin ring, pulling the proximal histidine (His, the histidine coordinated directly to the iron) with it. This movement is transmitted through the protein structure to the subunit interfaces, destabilizing T-state contacts and promoting the T→R transition.

## Two Models of Cooperativity

**Concerted (MWC) model:** All subunits switch between T and R simultaneously — no mixed states (e.g., no TR dimers). The equilibrium T ⇌ R is shifted by ligand binding. Ligand binds preferentially to R, pulling the equilibrium toward R. Predicts only positive cooperativity. This is the model that applies to allosteric enzymes like ATCase.

**Sequential (KNF) model:** Ligand binding to one subunit induces a conformational change in that subunit, which then influences neighboring subunits. Mixed states ARE allowed. Can explain both positive and negative cooperativity.

## Hill Plot Analysis

The Hill equation: $\log(\theta/(1-\theta)) = n \cdot \log[L] - n \cdot \log(p50)$

A Hill plot graphs $\log(\theta/(1-\theta))$ vs. $\log[L]$ (or $\log pO_2$). The slope in the linear region gives the Hill coefficient, **n**.

**Interpreting n:**

- n = 1: no cooperativity (hyperbolic binding, like myoglobin)
- n > 1: positive cooperativity (sigmoidal binding)
- n = number of binding sites: infinite cooperativity (theoretical maximum — never observed)
- For hemoglobin: n ≈ 2.8–3.0 (indicates strong positive cooperativity but less than the theoretical max of 4)

**At low [L]:** All subunits in T state, slope approaches 1 (binding is non-cooperative when only a few sites are occupied) **At high [L]:** All subunits in R state, slope approaches 1 **In between:** Cooperative transition, maximum slope = n

**p50:** The partial pressure at which θ = 0.5. On the Hill plot, it's the x-intercept (where log(θ/(1−θ)) = 0). Calculate: log(p50) = y-intercept/slope (from the linear regression), then p50 = 10^(that value).

### Regulators of Hemoglobin Function

**2,3-Bisphosphoglycerate (2,3-BPG):** Binds in the central cavity of deoxyhemoglobin (T state) between the two β subunits, forming electrostatic interactions with positively charged residues. Stabilizes the T state → decreases $O_2$ affinity → promotes $O_2$ release to tissues. At high altitude, red blood cells increase [2,3-BPG] to enhance $O_2$ delivery despite lower $O_2$ partial pressures. The small cost in loading efficiency in the lungs is outweighed by the large gain in unloading efficiency in the tissues.

**The Bohr Effect:** $CO_2$ and $H^+$ (low pH) both stabilize the T state, promoting $O_2$ release. In actively metabolizing tissues, $CO_2$ and $H^+$ are elevated → hemoglobin releases more $O_2$ precisely where it's needed most. In the lungs, high $pO_2$ and low $pCO_2$ → hemoglobin loads $O_2$. Molecular basis: $H^+$ protonates His residues at the subunit interface, stabilizing salt bridges in the T state. $CO_2$ reacts with N-terminal amino groups to form carbamate, adding negative charges that form new salt bridges stabilizing T.

**CO toxicity and the distal histidine:** Free heme binds CO ~25,000× more tightly than $O_2$ because CO prefers a linear Fe−C−O geometry (optimal orbital overlap). The distal histidine (His64 in myoglobin) sterically forces CO into a bent geometry, destabilizing it, while simultaneously forming a favorable H-bond with the naturally bent Fe−O−O of bound $O_2$. This reduces the CO/$O_2$ discrimination to ~200-fold. A His64→Ala mutation removes both effects → greatly increases CO susceptibility.

### Hemoglobin Mutations

**Sickle cell disease (HbS):** Glu6→Val in β-globin. Val is hydrophobic; the mutation creates a hydrophobic patch on the surface of deoxyhemoglobin that fits into a complementary pocket on another HbS molecule → polymerization → sickling of red blood cells. Only occurs in deoxy state (T state exposes the Val6 patch).

**Predicting mutation effects on isoelectric point:** Changing a charged residue to one with different charge shifts the pI. Lys(+) → Glu(−): net change of −2 charge units → pI decreases. On an isoelectric focusing gel, the mutant migrates further toward the positive electrode (lower pI band).

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## MOTIF 8: Enzyme Kinetics

**Why this motif matters:** Quantitative enzyme kinetics is central to this section — Michaelis-Menten calculations, interpreting Lineweaver-Burk plots, inhibitor classification, and connecting kinetic parameters to biological function.

## Reaction Velocity

**$v_0$ (initial velocity):** The rate of product formation (or substrate consumption) measured at the very beginning of the reaction, when [S] >> [P] and [S] ≈ $[S]_0$. Measured at the start to avoid complications from: product inhibition, reverse reaction, substrate depletion.

## The Michaelis-Menten Model

$E + S \rightleftharpoons ES \rightarrow E + P$ ($k_1$, $k_{-1}$, $k_2$)

**Key assumption (steady state):** d[ES]/dt = 0 — the rate of ES formation equals the rate of ES breakdown.

**Michaelis-Menten equation:** $v_0 = Vmax[S] / (KM + [S])$

**When $k_{-1} >> k_2$:** $KM \approx k_{-1}/k_1 = Kd$ (the dissociation constant for the ES complex). Under this condition, KM is a measure of substrate affinity. Lower KM = higher affinity.

## Key Kinetic Parameters

**Vmax:** Maximum velocity achieved at saturating [S]. Vmax = kcat[E]total.

**KM (Michaelis constant):** The substrate concentration at which $v_0$ = ½ Vmax. Units: concentration (M, mM, μM).

**kcat (turnover number):** The number of substrate molecules converted to product per enzyme molecule per unit time at saturation. kcat = Vmax/[E]total. Units: $s^{-1}$ (first order).

**kcat/KM (specificity constant or catalytic efficiency):** A second-order rate constant that measures efficiency at LOW substrate concentrations (when [S] << KM). Units: $M^{-1}$ $s^{-1}$. The upper limit is the diffusion-controlled rate (~$10^8$–$10^9$ $M^{-1}$ $s^{-1}$). An enzyme operating at this limit is said to have achieved "catalytic perfection."

## Behavior at Different [S]

- When [S] << KM: $v_0 \approx (kcat/KM)[E][S]$ — first order in [S], first order in [E], second order overall
- When [S] >> KM: $v_0 \approx Vmax = kcat[E]$ — zero order in [S], first order in [E]
- When [S] = KM: $v_0$ = ½ Vmax

To find [S] for any fraction of Vmax: set $v_0$ = fraction × Vmax, substitute into MM equation, solve for [S]. Example: for $v_0$ = ¼ Vmax → [S] = KM/3

## Lineweaver-Burk (Double Reciprocal) Plot

$1/v_0 = (KM/Vmax)(1/[S]) + 1/Vmax$

y-intercept = 1/Vmax; x-intercept = −1/KM; slope = KM/Vmax

**Effect of changing [E]:** Changes Vmax but NOT KM. On a Lineweaver-Burk plot, different [E] values give lines with the same x-intercept (same KM) but different y-intercepts (different Vmax) — this same relationship appears across the entire family of lines.

## Enzyme Inhibition

**Reversible Inhibitors:**

| Type | Binds to | Effect on Vmax | Effect on KM | L-B Plot Pattern |
|---|---|---|---|---|
| Competitive | E (active site) | No change | Increases (apparent) | Lines intersect on y-axis |
| Uncompetitive | ES only | Decreases | Decreases | Parallel lines |
| Noncompetitive (pure) | E and ES equally | Decreases | No change | Lines intersect on x-axis |
| Mixed | E and ES (unequally) | Decreases | Changes | Lines intersect left of y-axis |

**α factor:** $\alpha = 1 + [I]/KI$. For competitive inhibition: $K_{M,app} = \alpha K_M$, Vmax unchanged. For pure noncompetitive: $V_{max,app} = V_{max}/\alpha$, KM unchanged.

**Calculating KI:** Use $\alpha = V_{max}/V_{max,app}$ (for noncompetitive) or $\alpha = K_{M,app}/K_M$ (for competitive), then $K_I = [I]/(\alpha - 1)$.

**Irreversible inhibitors:** Form covalent bonds with the enzyme (e.g., TPCK with chymotrypsin — alkylates His57 in the active site). Cannot be overcome by increasing [S].

## Comparing Enzymes (Isozymes)

When comparing enzymes with different kinetic parameters, the fastest *in vivo* rate depends on *both* the kinetic parameters and the actual substrate concentration. Use: $v_0 = k_{cat}[S]/(K_M + [S])$ with the physiological [S]. An enzyme with high $k_{cat}/K_M$ but low physiological [S] may operate more slowly than one with lower $k_{cat}/K_M$ but much higher [S] (operating near Vmax).

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# MOTIF 9: Catalytic Strategies (Factual Reference)

**Note:** You have a detailed mechanism guide. This section covers factual context only — the concepts, not the arrow-pushing.

## General Principles

Enzymes accelerate reactions by stabilizing the transition state, thereby lowering $\Delta G^{\ddagger}$. They do NOT change $\Delta G^{\circ\prime}$ or Keq. Active sites are complementary to the *transition state*, not the substrate (transition-state stabilization > substrate binding).

## Characteristics of Active Sites

- Occupy a small fraction of the enzyme's total volume
- Three-dimensional cleft or crevice formed by residues that may be distant in primary sequence
- Substrates bind through multiple weak, noncovalent interactions
- Specificity depends on the precise arrangement of atoms in the active site
- Often contain bound water molecules that participate in catalysis

## Catalytic Strategies

**Acid-base catalysis:** Proton transfer to or from the substrate, often mediated by His (pKa ~6, ideal for proton transfer at physiological pH). General acid: donates a proton (H-A → $A^-$). General base: accepts a proton (B: → $H\text{-}B^+$).

**Covalent catalysis:** Transient covalent bond between enzyme and substrate (e.g., Schiff base with Lys in aldolase, acyl-enzyme intermediate in serine proteases). The covalent intermediate must be subsequently hydrolyzed to regenerate the enzyme.

**Metal ion catalysis:** Metal ions can stabilize negative charges on intermediates, generate nucleophiles (activating water), mediate redox reactions. Examples: $Zn^{2+}$ in carbonic anhydrase, $Mg^{2+}$ in kinases (coordinates phosphate groups of ATP).

**Electrostatic catalysis:** The active site provides a microenvironment with a low dielectric constant that stabilizes charged intermediates better than water would.

**Proximity and orientation effects:** The enzyme brings substrates together in the correct orientation, effectively increasing their local concentration.

**Transition-state stabilization:** Oxyanion holes (backbone NH groups that stabilize negatively charged tetrahedral intermediates in proteases), electron sinks (TPP thiazolium ring stabilizes carbanion intermediates).

## Serine Proteases (Chymotrypsin family)

Catalytic triad: Ser195, His57, Asp102. The mechanism proceeds through an acyl-enzyme intermediate. His57 acts as both a general base (in acylation) and a general acid (in deacylation). TPCK irreversibly inhibits chymotrypsin by alkylating His57. Substrate specificity determined by the S1 pocket (deep, hydrophobic in chymotrypsin → prefers bulky hydrophobic residues Phe, Trp, Tyr).

**Experiment with $H_2{}^{18}O$:** The $^{18}O$ label ends up in the *C-terminal* fragment (the "leaving group" in the first step is the C-terminal peptide, but the $^{18}O$ from water is incorporated during deacylation of the acyl-enzyme, ending up in the N-terminal fragment's new carboxyl group). For the LEFTY example: substrate LEFTY cleaved after Phe (chymotrypsin cleaves after aromatic residues). The acyl-enzyme forms with LEF. Water attacks the acyl-enzyme. The $^{18}O$ from $H_2{}^{18}O$ is incorporated into the new C-terminus of LEF. Therefore, the $^{18}O$ ends up in the carboxyl group of the N-terminal fragment (LEF) because water attacks the acyl intermediate.

### Ntn Hydrolases (PrpA Example)

Use an N-terminal nucleophilic residue (Thr, Ser, or Cys). The free α-amino group of the N-terminal residue acts as a general base to activate the side-chain hydroxyl/thiol as a nucleophile. Analogous to serine proteases but with a different architectural solution.

### EcoRV Endonuclease

Catalytic mechanism most similar to aspartyl proteases — both use metal ions and acidic residues to activate water as a nucleophile. NOT similar to chymotrypsin despite both being hydrolases.

### Aldolase

Schiff base (covalent) catalysis. A Lys residue forms a Schiff base with the substrate, stabilizing the carbanion/enamine intermediate during C-C bond cleavage (or formation in reverse). This is the "high-energy intermediate stabilization" relevant to this guide.

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## MOTIF 10: Enzyme Regulation

**Why this motif matters:** Regulation integrates enzyme kinetics with metabolic control. The guide emphasizes conceptual understanding of allosteric regulation, feed-forward/feedback loops, phosphorylation, and zymogens.

### Allosteric Enzymes

Allosteric enzymes have regulatory sites distinct from the active site. They typically have quaternary structure (multiple subunits). They do NOT follow Michaelis-Menten kinetics — their $v_0$ vs. [S] curves are sigmoidal (S-shaped), reflecting cooperative substrate binding.

**T state (tense):** Low activity, low substrate affinity **R state (relaxed):** High activity, high substrate affinity

The T ⇌ R equilibrium can be shifted by:

- Substrate binding (homotropic effect — substrate itself shifts the equilibrium toward R)
- Activators (heterotropic positive effectors — stabilize R state, decrease apparent KM, make the curve more hyperbolic)
- Inhibitors (heterotropic negative effectors — stabilize T state, increase apparent KM, make the curve more sigmoidal)

**Key distinction from competitive inhibition:** Allosteric inhibitors bind at a different site than the substrate. They shift the T ⇌ R equilibrium rather than competing for the active site.

**Effect on kinetic parameters:** For allosteric (K-type) regulation, Vmax is unchanged but apparent KM changes. Activators decrease KM (shift to R), inhibitors increase KM (shift to T). If the equilibrium is pushed so far toward R that cooperativity is lost, the curve becomes hyperbolic (this is what valine does to threonine deaminase).

## Aspartate Transcarbamoylase (ATCase)

The paradigm for allosteric regulation. Catalyzes the first committed step of pyrimidine biosynthesis. Has catalytic (C) subunits and regulatory (R) subunits.

- CTP (end product) = feedback inhibitor (binds R subunits, stabilizes T state)
- ATP = activator (binds R subunits, stabilizes R state)
- This is an example of feedback inhibition: the end product of the pathway inhibits an early enzyme

## Feedback Inhibition

A product of a metabolic pathway inhibits an enzyme earlier in the same pathway. This prevents wasteful overproduction. Isoleucine inhibits threonine deaminase (the first step of its own biosynthesis). The inhibitor is typically structurally dissimilar to the substrate — it binds at an allosteric site, not the active site.

## Homotropic vs. Heterotropic Effects

**Homotropic:** The substrate itself acts as an effector (cooperative binding is a homotropic effect — binding of substrate to one subunit increases affinity at other subunits). Always positive (activating).

**Heterotropic:** A molecule OTHER than the substrate acts as an effector. Can be positive (activator) or negative (inhibitor).

## Feed-Forward Activation

A metabolite produced earlier in the pathway activates an enzyme later in the pathway. Example: Fructose 1,6-bisphosphate (F1,6-BP) activates pyruvate kinase. When flux through glycolysis is high, F1,6-BP accumulates → activates pyruvate kinase → prepares the downstream enzyme for increased flux.

F1,6-BP is an allosteric activator: it stabilizes the R state, decreases KM, but does NOT affect kcat (because it does not bind in the active site — its structure is completely different from PEP).

## Isozymes

Different enzymes (different genes, different amino acid sequences) that catalyze the same reaction. They may have different kinetic parameters, regulatory properties, and tissue distributions. This allows the same reaction to be regulated differently in different tissues. Example: hexokinase (low

KM, inhibited by G6P, found in most tissues) vs. glucokinase (high KM, not inhibited by G6P, found in liver and pancreas).

### Phosphorylation as a Regulatory Strategy

Protein kinases transfer a phosphoryl group from ATP to Ser, Thr, or Tyr residues. Protein phosphatases remove the phosphate by hydrolysis.

**Why phosphorylation is so effective:**

1. Adds a large, doubly-negative group → can disrupt or create electrostatic interactions
2. Phosphate can form multiple H-bonds → additional noncovalent interactions
3. Reversible (transient) — phosphatases can quickly reverse the modification
4. Links regulation to the energy status of the cell (uses ATP)

Phosphorylation can activate or inactivate an enzyme depending on the specific protein. In the pyruvate dehydrogenase complex: phosphorylation by PDH kinase → inactivation; dephosphorylation by PDH phosphatase → activation.

### Zymogens (Proenzymes)

Inactive precursors of enzymes, activated by proteolytic cleavage. The cleavage is irreversible — this distinguishes zymogen activation from other regulatory mechanisms (which are typically reversible). Examples: digestive enzymes (trypsinogen → trypsin, chymotrypsinogen → chymotrypsin), blood clotting factors.

**Physiological importance:** Prevents premature activity that could damage the cell or tissue of origin (e.g., pancreatic proteases would digest the pancreas if active during synthesis).

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## MOTIF 11: Protein Purification & Analysis

**Why this motif matters:** Understanding purification logic is explored through experimental design questions — given a protein's properties, propose a purification strategy.

### Chromatography Methods

**Ion Exchange Chromatography:** Separates by charge. Cation exchange resins (e.g., CM-cellulose, carboxymethyl) have negative charges → bind positively charged proteins. Anion exchange resins (e.g., DEAE-cellulose) have positive charges → bind negatively charged proteins.

A protein will bind to an ion exchange column if it has the opposite charge to the resin at the column's operating pH. If the protein's pI < column pH, the protein is negatively charged and will bind to an anion exchanger (or pass through a cation exchanger). If pI > column pH, the protein is positively charged and binds a cation exchanger.

Elution: increasing salt gradient ($Na^+/Cl^-$ ions compete with protein for binding sites).

**Gel Filtration (Size Exclusion) Chromatography:** Separates by size. Beads have pores of defined size. Small proteins enter the pores (longer path → elute later); large proteins are excluded from pores (shorter path → elute first). No binding involved — purely based on hydrodynamic radius.

**Affinity Chromatography:** Separates by specific binding interactions. A ligand (e.g., substrate analog, antibody, metal ion) is covalently attached to the column matrix. Only the protein of interest binds; everything else washes through. Elution by: excess free ligand (competition), change in pH, or change in salt concentration. Most selective method.

### Ammonium Sulfate Precipitation (Salting Out)

Not chromatography, but a common initial step. Adding ammonium sulfate progressively strips hydration shells from proteins → hydrophobic patches on protein surfaces are exposed → proteins aggregate and precipitate. Different proteins precipitate at different ammonium sulfate concentrations (depending on their surface hydrophobicity). Less hydrophobic surface → higher [ammonium sulfate] required for precipitation.

### SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis. SDS denatures proteins and coats them with a uniform negative charge proportional to molecular weight. Separation is by size only (small proteins move faster through the gel). Used to assess purity — a pure protein gives a single band.

### Isoelectric Focusing

Proteins migrate through a pH gradient until they reach the pH = their pI, at which point they have zero net charge and stop moving. Separates strictly by pI. A single band = one protein species; multiple bands = multiple species (e.g., normal HbA + a mutant hemoglobin with altered pI).

### Mass Spectrometry

Proteins are digested into peptides (e.g., by trypsin), ionized, and separated by mass-to-charge ratio (m/z). Tandem MS (MS/MS) involves fragmenting individual peptides and analyzing the fragments. The mass difference between consecutive fragment ions reveals the amino acid identity.

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## MOTIF 12: Carbohydrate Chemistry

**Why this motif matters:** Structure-drawing questions involving monosaccharides, anomers, and glycosidic bonds are common applications. Understanding anomeric chemistry and glycoconjugate function is essential.

## Classification

**Aldoses:** Carbonyl group at C1 (aldehyde). Examples: glucose, galactose, mannose, ribose, xylose.

**Ketoses:** Carbonyl group at C2 (ketone). Examples: fructose, dihydroxyacetone, ribulose.

Number of carbons: triose (3C), tetrose (4C), pentose (5C), hexose (6C).

## Fischer Projections

Vertical chain with C1 at top. Horizontal bonds project toward the viewer; vertical bonds project away. **D-configuration:** highest-numbered chiral center has −OH on the RIGHT. **L-configuration:** −OH on the LEFT. Most biological sugars are D-sugars.

**Epimers:** Sugars that differ at exactly one chiral center. Galactose is the C4 epimer of glucose. Mannose is the C2 epimer of glucose.

## Cyclization

In solution, aldohexoses (≥5 carbons) predominantly exist as cyclic hemiacetals. The carbonyl carbon reacts with a hydroxyl group on the same molecule.

**Pyranose:** 6-membered ring (most common for hexoses). Formed by reaction of C1 with C5-OH (for aldohexoses).

**Furanose:** 5-membered ring. Formed by reaction of C1 with C4-OH (for aldoses), or C2 with C5-OH (for ketoses like fructose).

## The Anomeric Carbon

Upon cyclization, the carbonyl carbon (C1 for aldoses, C2 for ketoses) becomes a new chiral center called the **anomeric carbon**. The two possible configurations:

**α-anomer:** In a Haworth projection, the −OH on the anomeric carbon is *below* the ring (axial in chair, *trans* to the C6 hydroxymethyl group for D-sugars).

**β-anomer:** The −OH is *above* the ring (equatorial in chair, *cis* to C6 for D-sugars).

Anomers interconvert in solution through **mutarotation** — ring opens to the open-chain form, then recloses.

## Glycosidic Bonds

A glycosidic bond forms between the anomeric carbon of one sugar and a hydroxyl group of another molecule (sugar or non-sugar). Formation involves loss of water and is catalyzed by **glycosyltransferases**, which use activated sugar donors (e.g., UDP-glucose).

**Naming convention:** α or β (configuration at anomeric carbon), then the numbers of the two carbons involved. Example: β-1,4 glycosidic bond in lactose (Gal β-1,4 Glc). An α-2,6 bond between Neu5Ac and Gal means the anomeric C2 of Neu5Ac (it's a ketose-type derivative) connects to the C6-OH of Gal in the α configuration.

**Reducing end:** The end of a polysaccharide with a free (or potentially free) anomeric carbon. This carbon can open to the aldehyde form and act as a reducing agent.

### Glycoconjugates

**Glycoproteins:** Proteins with covalently attached oligosaccharide chains.

- **N-linked glycosylation:** Oligosaccharide attached to the amide nitrogen of Asn (in the Asn-X-Ser/Thr sequon, where X is any amino acid except Pro). The bond is a **β-N-glycosidic bond** to GlcNAc.
- **O-linked glycosylation:** Oligosaccharide attached to the −OH of Ser or Thr.

Functions: cell-cell recognition, protein folding and stability, signaling, blood type determination, pathogen binding.

**Sialic acids (e.g., Neu5Ac):** 9-carbon acidic sugars often found at the terminal position of glycan chains. Their negative charge (carboxylate) influences cell-surface charge and recognition. Sialidases (neuraminidases) cleave sialic acid residues — important in influenza virus release from host cells.

### Determining Hydroxyl Groups Available for Branching

In a disaccharide or glycan, free hydroxyl groups not involved in existing glycosidic bonds or the ring structure can potentially participate in additional glycoside bond formation to create branched structures. Count only the hydroxyls that are free (not already in a bond) and not the anomeric OH of the reducing end (which is already committed to ring closure or an existing bond).

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## MOTIF 13: Lipids & Biological Membranes

**Why this motif matters:** Membrane structure and dynamics are explored through FRAP experiments, fluidity predictions, and structural drawing.

### Fatty Acid Structure

Named by carbon count and number/position of double bonds. Example: palmitic acid = 16:0 (16 carbons, no double bonds); linoleic acid = 18:2 Δ9,12 (18 carbons, 2 cis double bonds at positions 9 and 12).

Saturated fatty acids: no double bonds, pack tightly, higher melting point. Unsaturated fatty acids: one or more cis double bonds → introduce kinks → disrupt packing → lower melting point, increase fluidity.

## Glycerophospholipids

Structure: glycerol backbone with two fatty acid chains esterified at C1 and C2 (sn-1 and sn-2), and a phosphate-linked head group at C3.

Head groups determine the type: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI).

To draw: glycerol backbone → ester bonds to fatty acid chains → phosphoester bond to phosphate → phosphoester bond to head group alcohol.

## Lipid Bilayers and Membrane Structure

**Self-assembly:** Amphipathic lipids spontaneously form bilayers in aqueous solution. Hydrophobic tails face inward; hydrophilic head groups face outward toward water. This is driven by the hydrophobic effect.

**Fluid mosaic model:** The membrane is a two-dimensional fluid in which lipids and proteins can move laterally. Proteins are embedded in (integral) or associated with the surface of (peripheral) the lipid bilayer.

**Integral membrane proteins:** Span the bilayer with transmembrane segments (typically α-helices with hydrophobic side chains). Require detergents (like SDS or Triton X-100) for extraction.

**Peripheral membrane proteins:** Associated with the membrane surface through electrostatic interactions or H-bonds. Can be removed by changes in pH or ionic strength.

## Membrane Fluidity

Fluidity depends on lipid composition:

- More unsaturated fatty acids → more fluid (cis double bonds disrupt packing → fewer vdW interactions between tails)
- Shorter fatty acid chains → more fluid (fewer vdW interactions)
- Cholesterol: at physiological temperatures, cholesterol *decreases* fluidity by intercalating between phospholipids and restricting their movement; at low temperatures, it *prevents* crystallization and maintains fluidity. Net effect: cholesterol stabilizes membrane structure by reducing fluidity at HIGH temperatures and maintaining fluidity at low temperatures.

### FRAP (Fluorescence Recovery After Photobleaching)

Experimental evidence for the fluid mosaic model. A patch of membrane is bleached (fluorescent lipids destroyed), and recovery of fluorescence is monitored as unbleached lipids diffuse laterally into the bleached area.

Rate of recovery reflects membrane fluidity:

- Higher ratio of unsaturated to saturated fatty acids → faster recovery (more fluid)
- On a plot of fluorescence intensity vs. time: after the bleach, intensity drops; then recovery. A more fluid bilayer shows a steeper recovery curve (faster return to pre-bleach fluorescence).

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## MOTIF 14: Membrane Transport

**Why this motif matters:** Transport thermodynamics and mechanism are explored through free energy calculations and classification of transport types.

### Impermeability of Membranes

Lipid bilayers are essentially impermeable to: ions (charged), large polar molecules (sugars, amino acids), and macromolecules. Small nonpolar molecules ($O_2$, $CO_2$, $N_2$) and small uncharged polar molecules (water, to some extent) can cross.

### Types of Transport

**Passive transport:** Movement DOWN the concentration (or electrochemical) gradient. $\Delta G < 0$. No energy input required.

- **Simple diffusion:** Direct passage through the bilayer (nonpolar molecules only)
- **Facilitated diffusion:** Through a channel or carrier protein. Still passive (downhill).

**Active transport:** Movement AGAINST the concentration (or electrochemical) gradient. $\Delta G > 0$. Requires energy input.

- **Primary active transport:** Energy comes directly from ATP hydrolysis (e.g., $Na^+/K^+$-ATPase, $H^+/K^+$-ATPase, $Ca^{2+}$-ATPase)
- **Secondary active transport:** Energy comes from the dissipation of an ion gradient that was itself created by primary active transport.

**Transporters:**

- **Uniporter:** Transports one solute in one direction
- **Symporter (cotransporter):** Transports two solutes in the SAME direction
- **Antiporter:** Transports two solutes in OPPOSITE directions (e.g., $H^+/K^+$-ATPase — $H^+$ out, $K^+$ in)

## Free Energy of Transport

For an uncharged solute: $\Delta G = RT \ln([C]in/[C]out)$

For a charged solute (electrochemical gradient): $\Delta G = RT \ln([C]in/[C]out) + zF\Delta\psi$

Where z = charge of the ion, F = Faraday's constant (96.5 kJ/mol·V), $\Delta\psi$ = membrane potential (inside minus outside, typically negative inside).

**Sign convention for membrane potential:** If inside is negative ($\Delta\psi = -50$ mV), moving a positive ion INTO the cell: $zF\Delta\psi = (+1)(96.5)(-0.050) = -4.825$ kJ/mol (favorable). Moving a positive ion OUT: +4.825 kJ/mol (unfavorable).

## Coupled Transport Calculation ($H^+/K^+$-ATPase Example)

The free energy from ATP hydrolysis ($\Delta G' = -43$ kJ/mol) powers the transport of $H^+$ and $K^+$ against their gradients. For a 1:1:1 stoichiometry, the total free energy cost of pumping both ions must be ≤ 43 kJ/mol. The cost of pumping $H^+$ (against pH gradient and membrane potential) + cost of pumping $K^+$ (against concentration gradient and membrane potential) = energy available from ATP.

## Ion Channels

Channels provide a hydrophilic pathway across the membrane. They are typically: highly selective (e.g., $K^+$ channels select for $K^+$ over $Na^+$ by a factor of ~10,000), gated (opened/closed by voltage, ligand binding, or mechanical force), and allow very high transport rates (approaching diffusion limit).

**Selectivity filter:** In $K^+$ channels, the filter mimics the hydration shell of $K^+$ with precisely spaced carbonyl oxygen atoms. $Na^+$ is too small to interact optimally with these oxygens, so it is excluded.

## Mitochondrial Transport (connecting to 18.5)

**ATP/ADP translocase:** Antiporter that exchanges cytoplasmic ADP for mitochondrial ATP across the inner mitochondrial membrane. This exchange is driven by the membrane potential (ATP has more negative charges than ADP, so exporting $ATP^{4-}$ while importing $ADP^{3-}$ is energetically favorable given the negative-inside potential).

**Mitochondrial transporters have a common tripartite structure:** Three repeated ~100-residue domains, each containing two transmembrane helices.

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# MOTIF 15: Metabolic Logic & Reaction Types

**Why this motif matters:** The guide emphasizes your ability to recognize reaction types, predict cofactor requirements, and understand metabolic logic (flux control, reaction coupling, activated carriers).

## Oxidation State and Fuel Value

The more reduced a carbon atom, the more energy it can yield upon oxidation. Fats are more reduced than carbohydrates → yield more energy per gram. $CO_2$ is fully oxidized (no more energy can be extracted).

**Recognizing oxidation and reduction:**

- Oxidation: loss of electrons, loss of H atoms, gain of O atoms, increase in C-O bonds, decrease in C-H bonds
- Reduction: gain of electrons, gain of H atoms, loss of O atoms, increase in C-H bonds
- Count the oxidation state of carbon: each bond to a more electronegative atom (O, N, S) = +1; each bond to H = −1; each bond to C = 0

## The Six General Reaction Types

1. **Oxidation-reduction:** Transfer of electrons (often via hydride or hydrogen atom transfer). Cofactors: $NAD^+$/NADH, FAD/$FADH_2$, FMN
2. **Group transfer:** Movement of a functional group from one molecule to another (e.g., phosphoryl transfer by kinases, amino transfer by transaminases)
3. **Hydrolysis:** Bond cleavage by addition of water (e.g., peptidases, esterases, glycosidases)
4. **Carbon-carbon bond formation/cleavage:** (e.g., aldol reactions by aldolase, decarboxylations, Claisen condensations by citrate synthase)
5. **Isomerization:** Rearrangement within a single molecule (e.g., phosphoglucose isomerase, triose phosphate isomerase)
6. **Ligation coupled to ATP hydrolysis:** Bond formation driven by NTP cleavage (e.g., pyruvate carboxylase, aminoacyl-tRNA synthetases)

## Activated Carriers (Cofactors)

**$NAD^+$/NADH:** Carries 2 electrons as a hydride ion. Used in catabolic oxidation reactions. The oxidized form ($NAD^+$) is the electron acceptor. Stoichiometric (used and regenerated, not a true catalyst).

**FAD/$FADH_2$:** Carries 2 electrons. Can accept electrons one or two at a time. Often tightly or covalently bound to enzymes (prosthetic group). Used when the reaction involves oxidation of C-C single bond to C=C double bond (e.g., succinate dehydrogenase).

**TPP (Thiamine Pyrophosphate):** Derived from vitamin $B_1$. The thiazolium ring provides an "electron sink" — stabilizes carbanion intermediates formed during decarboxylation of α-keto acids. Essential for: pyruvate dehydrogenase (E1), α-ketoglutarate dehydrogenase, transketolase. Used catalytically.

**Biotin:** $CO_2$ carrier. Covalently attached to enzyme via a long, flexible arm. Activates $CO_2$ for carboxylation reactions (e.g., pyruvate carboxylase: pyruvate → oxaloacetate). Requires ATP to activate $CO_2$.

**Coenzyme A (CoA):** Carries acyl groups as thioesters. The thioester bond is "high energy" — its hydrolysis is thermodynamically favorable ($\Delta G^{\circ\prime} \approx -31.4$ kJ/mol). This makes the acyl group "activated" for transfer. Acetyl CoA is the most important acyl-CoA carrier.

**Lipoamide:** Carries acyl groups and electrons. The lipoyl group swings between active sites in multienzyme complexes (E1, E2, E3 of pyruvate dehydrogenase). Exists in three forms: oxidized (disulfide), reduced (dithiol — dihydrolipoamide), and acetylated (acetyl-dihydrolipoamide).

## Phosphoryl-Transfer Potential

Molecules with a high phosphoryl-transfer potential can drive phosphorylation of other molecules. ATP is in the middle of the phosphoryl-transfer potential scale — it can both accept and donate phosphoryl groups.

Molecules with HIGHER phosphoryl-transfer potential than ATP (can phosphorylate ADP → ATP):

- Phosphoenolpyruvate (PEP): $\Delta G^{\circ\prime}$_hydrolysis = −61.9 kJ/mol
- 1,3-Bisphosphoglycerate: $\Delta G^{\circ\prime}$_hydrolysis = −49.4 kJ/mol

ATP: $\Delta G^{\circ\prime}$_hydrolysis = −30.5 kJ/mol

**Substrate-level phosphorylation:** Direct transfer of a phosphoryl group from a high-potential donor to ADP, catalyzed by a kinase (e.g., phosphoglycerate kinase, pyruvate kinase).

## Metabolic Flux Control

Reactions far from equilibrium (large negative $\Delta G$) serve as control points. These reactions are:

- Essentially irreversible under cellular conditions
- Catalyzed by regulated enzymes (subject to allosteric control, phosphorylation, etc.)
- The committed step (first irreversible step unique to a pathway) is often the primary control point

Near-equilibrium reactions respond passively to changes in metabolite concentrations and are NOT regulatory points.

## Recurring Motifs in Metabolic Pathways

- **Oxidation followed by group transfer** (e.g., oxidative decarboxylation in pyruvate dehydrogenase)
- **Substrate-level phosphorylation** to capture energy
- **Coupled reactions** to drive unfavorable transformations
- **Metabolic channeling** through multienzyme complexes
- **Use of activated carriers** to couple exergonic and endergonic processes

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# MOTIF 16: Glycolysis & Gluconeogenesis

**Why this motif matters:** A complete pathway model tracks every substrate, product, enzyme, and cofactor of glycolysis. The guide emphasizes structural drawing, energy accounting, carbon labeling, regulation, and the logic of gluconeogenesis bypass reactions.

## Glycolysis Overview

10 enzyme-catalyzed reactions that convert glucose (6C) to 2 pyruvate (3C) in the cytoplasm.

**Energy investment phase (steps 1-5):** 2 ATP consumed, one 6C molecule → two 3C molecules.

**Energy payoff phase (steps 6-10):** 4 ATP produced, 2 NADH produced.

**Net yield per glucose:** 2 ATP, 2 NADH, 2 pyruvate.

## The 10 Steps of Glycolysis

**Step 1 — Hexokinase:** Glucose + ATP → Glucose-6-phosphate + ADP. Phosphorylation traps glucose in the cell (phosphorylated sugars can't cross the membrane). Induced-fit mechanism: glucose binding causes conformational change that closes the active site, excluding water and preventing ATP hydrolysis without substrate. The conformational change in hexokinase prevents the hydrolysis of ATP.

**Step 2 — Phosphoglucose Isomerase:** Glucose-6-phosphate ⇌ Fructose-6-phosphate. Isomerization (aldose → ketose). Near equilibrium.

**Step 3 — Phosphofructokinase-1 (PFK-1):** F6P + ATP → Fructose-1,6-bisphosphate + ADP. The COMMITTED STEP of glycolysis. Irreversible. Most important regulatory point. Activated by: AMP, F2,6-BP. Inhibited by: ATP, citrate.

**Step 4 — Aldolase:** F1,6-BP ⇌ Dihydroxyacetone phosphate (DHAP) + Glyceraldehyde-3-phosphate (GAP). C-C bond cleavage (retro-aldol). $\Delta G^{\circ\prime}$ = +23.8 kJ/mol (unfavorable!) but driven forward by rapid consumption of GAP by downstream reactions. Schiff base mechanism (covalent catalysis with Lys).

**Step 5 — Triose Phosphate Isomerase (TPI):** DHAP ⇌ GAP. Isomerization. "Perfect enzyme" — kcat/KM near the diffusion limit. Equilibrium favors DHAP but pulled toward GAP by consumption in step 6.

**Step 6 — Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH):** GAP + $NAD^+$ + Pi → 1,3-Bisphosphoglycerate + NADH. Oxidation AND phosphorylation. The substrate (GAP, an aldehyde) is oxidized (GAP is the molecule oxidized, $NAD^+$ is reduced). The high-energy thioester intermediate is attacked by Pi to form the acyl phosphate (1,3-BPG). This captures oxidation energy as a high-phosphoryl-transfer-potential compound.

**Step 7 — Phosphoglycerate Kinase:** 1,3-BPG + ADP → 3-Phosphoglycerate + ATP. Substrate-level phosphorylation. The high phosphoryl-transfer potential of 1,3-BPG drives ATP synthesis.

**Step 8 — Phosphoglycerate Mutase:** 3-PG ⇌ 2-Phosphoglycerate. Isomerization (phosphoryl group migration).

**Step 9 — Enolase:** 2-PG ⇌ Phosphoenolpyruvate (PEP) + $H_2O$. Dehydration. Creates the high-energy enol phosphate bond of PEP.

**Step 10 — Pyruvate Kinase:** PEP + ADP → Pyruvate + ATP. Substrate-level phosphorylation. Irreversible. Regulated allosterically: activated by F1,6-BP (feed-forward activation); inhibited by ATP, alanine. F1,6-BP stabilizes the R state, decreasing KM for PEP but not affecting kcat (because F1,6-BP binds at an allosteric site, not the active site).

## Fates of Pyruvate

1. **Aerobic conditions:** Pyruvate → Acetyl CoA (pyruvate dehydrogenase complex) → citric acid cycle
2. **Anaerobic conditions (animals):** Pyruvate → Lactate (lactate dehydrogenase). Regenerates $NAD^+$ for glycolysis to continue.
3. **Anaerobic conditions (yeast):** Pyruvate → Acetaldehyde → Ethanol. Also regenerates $NAD^+$.
4. **Gluconeogenesis:** Pyruvate → Oxaloacetate → PEP → Glucose (reversal of glycolysis with bypass reactions).

## $NAD^+$ Regeneration

Glycolysis requires a continuous supply of $NAD^+$ (consumed in step 6). Under aerobic conditions, NADH is reoxidized by the mitochondrial electron transport chain. Under anaerobic conditions, fermentation (lactate or ethanol production) regenerates $NAD^+$.

## Carbon Labeling

When a 6C sugar enters glycolysis, it is split into two 3C fragments by aldolase (step 4). Because TPI interconverts DHAP and GAP, both 3C fragments ultimately pass through the GAP → pyruvate pathway. However, the two 3C fragments are NOT equivalent — they carry different carbons of the original glucose.

Glucose carbons 1,2,3 → one triose; carbons 4,5,6 → the other triose. After TPI equilibration:

- C1 of glucose → C3 of pyruvate
- C2 → C2 of pyruvate
- C3 → C1 of pyruvate (the carboxyl carbon)
- C4 → C1 of pyruvate
- C5 → C2 of pyruvate
- C6 → C3 of pyruvate

Fructose enters glycolysis after phosphorylation by hexokinase (in adipose tissue) to F6P, or by fructokinase (in liver) to F1P. A $^{13}C$ label at C4 of fructose ends up in only ONE of the two triose fragments, meaning only 50% of lactate molecules will carry the label.

## Fructose 2,6-Bisphosphate (F2,6-BP)

NOT a glycolytic intermediate. It is a potent allosteric regulator synthesized and degraded by the bifunctional enzyme PFK-2/FBPase-2. F2,6-BP activates PFK-1 (→ stimulates glycolysis) and inhibits FBPase-1 (→ inhibits gluconeogenesis). It is the single most potent activator of PFK-1.

## Gluconeogenesis

Essentially the reverse of glycolysis, but three steps of glycolysis are irreversible and must be bypassed:

**Bypass 1 (replaces pyruvate kinase):** TWO enzymes required.

- Pyruvate carboxylase: Pyruvate + $CO_2$ + ATP → Oxaloacetate. Requires biotin. Occurs in the mitochondrial matrix.
- PEPCK: Oxaloacetate + GTP → PEP + $CO_2$ + GDP. Occurs in the cytoplasm.

**Chemical logic of the OAA intermediate:** Direct conversion of pyruvate to PEP is too energetically costly ($\Delta G^{\circ\prime} >> 0$). Instead, carboxylation of pyruvate to OAA stores the energy from ATP hydrolysis. The subsequent decarboxylation of OAA is thermodynamically favorable and drives the formation of the high-energy enol phosphate bond in PEP. The $CO_2$ added in step 1 is the same $CO_2$ removed in step 2 — it serves as a transient activating group.

**Bypass 2 (replaces PFK-1):** Fructose-1,6-bisphosphatase (FBPase-1): F1,6-BP → F6P + Pi. Simple hydrolysis.

**Bypass 3 (replaces hexokinase):** Glucose-6-phosphatase: G6P → Glucose + Pi. Found ONLY in liver (and kidney), NOT in muscle. This is why muscle cannot export free glucose — it can only use glucose for its own glycolysis. Liver can release glucose into the blood for other tissues.

## Coordinated Regulation of Glycolysis and Gluconeogenesis

Reciprocal regulation ensures only one pathway operates at a time:

- High energy charge (high ATP/AMP ratio) → inhibits glycolysis (ATP inhibits PFK-1), activates gluconeogenesis
- Low energy charge → activates glycolysis (AMP activates PFK-1), inhibits gluconeogenesis
- F2,6-BP → activates glycolysis (activates PFK-1), inhibits gluconeogenesis (inhibits FBPase-1)
- Insulin → promotes glycolysis; glucagon → promotes gluconeogenesis
- Citrate → inhibits PFK-1 (signals that the citric acid cycle is saturated)

## Energetics

Gluconeogenesis is NOT simply the reverse of glycolysis. It costs 6 NTP equivalents (4 ATP + 2 GTP) to make one glucose from 2 pyruvate, whereas glycolysis yields only 2 ATP. The difference (4 NTP) is the energetic cost of making gluconeogenesis irreversible (thermodynamically favorable in the biosynthetic direction).

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# MOTIF 17: Pyruvate Dehydrogenase & Citric Acid Cycle

**Why this motif matters:** PDH is a mechanistic showcase (five cofactors, multienzyme complex) and a critical metabolic junction. The citric acid cycle integrates carbon oxidation with energy capture and biosynthetic precursor generation.

## Pyruvate Dehydrogenase Complex (PDC)

**Overall reaction:** Pyruvate + CoA + $NAD^+$ → Acetyl CoA + $CO_2$ + NADH

Three enzymes: E1 (pyruvate dehydrogenase), E2 (dihydrolipoyl transacetylase), E3 (dihydrolipoyl dehydrogenase).

**Five cofactors and their roles:**

| Cofactor | Enzyme | Role | Catalytic vs. Stoichiometric |
|---|---|---|---|
| TPP | E1 | Electron sink for decarboxylation of pyruvate; stabilizes the carbanion (hydroxyethyl-TPP intermediate) | Catalytic |
| Lipoamide | E2 | Oxidizing agent: accepts the hydroxyethyl group from TPP (is reduced in the process); then transfers the acetyl group to CoA | Catalytic |
| CoA | E2 | Accepts the acetyl group from acetyl-lipoamide in a transesterification reaction | Stoichiometric |
| FAD | E3 | Re-oxidizes dihydrolipoamide (the reduced form of lipoamide) back to the oxidized disulfide form; FAD is reduced to $FADH_2$ in the process; is then reoxidized by $NAD^+$ | Catalytic |
| $NAD^+$ | E3 | Terminal electron acceptor: re-oxidizes $FADH_2$ to regenerate FAD | Stoichiometric |

**Lipoamide's flexible arm** allows it to visit all three active sites (E1, E2, E3) in succession — a "swinging arm" mechanism.

**E3 mechanism:** Dihydrolipoamide (with two free −SH groups) reduces the E3 disulfide (pair of active-site cysteines) through a thiol-disulfide exchange reaction. This involves a series of nucleophilic attacks: thiolate of dihydrolipoamide attacks one sulfur of the enzyme disulfide → mixed disulfide

intermediate → second thiolate attacks to release the enzyme with reduced cysteines and re-oxidized lipoamide. A general base assists by deprotonating the thiols. The reduced cysteines on E3 are then reoxidized by FAD.

## Regulation of PDC

**PDH Kinase:** Phosphorylates E1 → INACTIVATES the complex. Activated by: acetyl CoA, NADH, ATP (signals that energy is abundant — no need to make more acetyl CoA).

**PDH Phosphatase:** Dephosphorylates E1 → ACTIVATES the complex. Activated by: insulin (signals high blood glucose → promote glucose oxidation), $Ca^{2+}$ (signals muscle contraction → need more energy).

**When PDH kinase is active (PDC inhibited), pyruvate fates:** gluconeogenesis (to replenish glucose/glycogen) or fermentation (to lactate or ethanol).

## The Citric Acid Cycle (Krebs Cycle / TCA Cycle)

**Purpose:** Oxidizes the acetyl group of acetyl CoA to 2 $CO_2$, capturing the energy as reduced cofactors (NADH, $FADH_2$) and GTP. Also provides biosynthetic precursors (amphibolic pathway).

**Location:** Mitochondrial matrix.

**Net reaction per acetyl CoA:** Acetyl CoA + 3 $NAD^+$ + FAD + GDP + Pi + 2 $H_2O$ → 2 $CO_2$ + 3 NADH + $FADH_2$ + GTP + CoA

## The 8 Reactions

**Step 1 — Citrate Synthase:** OAA + Acetyl CoA → Citrate + CoA. C-C bond formation (Claisen condensation). Highly exergonic (driven by hydrolysis of the thioester bond of acetyl CoA). The mechanism involves formation of a citryl-CoA intermediate, then hydrolysis. The enzyme prevents premature hydrolysis of the thioester or the formation of unwanted side products — "prevents undesirable reactions."

**Step 2 — Aconitase:** Citrate ⇌ Isocitrate. Isomerization via dehydration then rehydration (through cis-aconitate intermediate). The hydroxyl group is repositioned from C3 to C2.

**Step 3 — Isocitrate Dehydrogenase:** Isocitrate + $NAD^+$ → α-Ketoglutarate + $CO_2$ + NADH. Oxidative decarboxylation. First $CO_2$ lost. First NADH produced. Important regulatory step (activated by ADP, inhibited by ATP and NADH).

**Step 4 — α-Ketoglutarate Dehydrogenase Complex:** α-KG + CoA + $NAD^+$ → Succinyl CoA + $CO_2$ + NADH. Oxidative decarboxylation. Same mechanism and same 5 cofactors as pyruvate dehydrogenase complex (TPP, lipoamide, CoA, FAD, $NAD^+$). Second $CO_2$ lost. Irreversible.

**Step 5 — Succinyl CoA Synthetase:** Succinyl CoA + GDP + Pi → Succinate + GTP + CoA. Substrate-level phosphorylation. The thioester bond energy of succinyl CoA drives GTP synthesis. Mechanism involves a succinyl phosphate intermediate, then a phosphorylated His residue on the enzyme.

**Step 6 — Succinate Dehydrogenase:** Succinate → Fumarate + $FADH_2$. Oxidation (C-C → C=C). Uses FAD (not $NAD^+$) because the reaction is not sufficiently exergonic to reduce $NAD^+$. This is the only TCA enzyme embedded in the inner mitochondrial membrane (it's Complex II of the electron transport chain). Produces a trans double bond.

**Step 7 — Fumarase:** Fumarate + $H_2O$ → L-Malate. Hydration of a C=C double bond. Stereospecific: only L-malate is produced.

**Step 8 — Malate Dehydrogenase:** L-Malate + $NAD^+$ → OAA + NADH. Oxidation. $\Delta G°'$ is highly positive (+29.7 kJ/mol) but driven forward by the highly exergonic citrate synthase reaction (step 1), which continuously removes OAA.

## Regulation of the Citric Acid Cycle

Primary control points: isocitrate dehydrogenase (step 3) and α-ketoglutarate dehydrogenase (step 4). Both are inhibited by NADH and ATP (energy abundance), activated by ADP and $Ca^{2+}$. Citrate synthase (step 1) is inhibited by ATP and citrate.

The cycle is controlled at several points to match the rate of acetyl CoA oxidation to the cell's energy needs.

## Biosynthetic Functions (Anaplerosis)

The citric acid cycle provides precursors for biosynthesis: citrate → fatty acid synthesis; α-ketoglutarate → amino acid synthesis (glutamate family); succinyl CoA → heme synthesis; OAA → amino acid synthesis (aspartate family), gluconeogenesis.

When intermediates are withdrawn for biosynthesis, they must be replenished to keep the cycle running. The most important anaplerotic reaction is pyruvate carboxylase: pyruvate + $CO_2$ + ATP → OAA (requires biotin). This reaction is activated by acetyl CoA (signals that more OAA is needed to condense with the available acetyl CoA).

## Defects and Disease

Defects in citric acid cycle enzymes (e.g., succinate dehydrogenase, fumarase, isocitrate dehydrogenase) can contribute to cancer development through accumulation of oncometabolites that inhibit α-ketoglutarate-dependent dioxygenases involved in epigenetic regulation.

An enzyme in lipid metabolism (not in the cycle itself) can be hijacked to inhibit pyruvate dehydrogenase activity — specifically, by acetylation of lipoamide on E2, which prevents normal catalytic cycling.

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# MOTIF 18: Oxidative Phosphorylation (Sections 18.1–18.5)

**Why this motif matters:** The electron transport chain and ATP synthesis represent the culmination of catabolism. Understanding the physical principles — redox potentials, proton gradients, chemiosmotic coupling — ties together thermodynamics, membrane biology, and metabolic regulation.

## Overview

Oxidative phosphorylation occurs on the inner mitochondrial membrane. NADH and $FADH_2$ (produced by glycolysis, PDC, and the citric acid cycle) donate electrons to the electron transport chain (ETC). The free energy released by electron transfer is used to pump protons ($H^+$) across the inner mitochondrial membrane, creating an electrochemical gradient (proton-motive force). The protons flow back through ATP synthase, driving ATP synthesis.

## Mitochondrial Structure

**Outer membrane:** Permeable to small molecules and ions via porins.

**Inner membrane:** Highly impermeable. Contains the ETC complexes, ATP synthase, and transport proteins. Folded into cristae (increases surface area). The impermeability is essential — it maintains the proton gradient.

**Intermembrane space:** Between outer and inner membranes. Receives protons pumped by the ETC. Lower pH than the matrix (higher $[H^+]$).

**Matrix:** Contains: PDC, citric acid cycle enzymes, mitochondrial DNA, ribosomes.

**Endosymbiotic origin:** Mitochondria evolved from an ancestral α-proteobacterium engulfed by a primitive eukaryotic cell. Evidence: double membrane, own DNA and ribosomes, cardiolipin in inner membrane.

## Redox Potential and Electron Transfer

**Reduction potential (E°'):** Measures the tendency of a substance to accept electrons. More positive E°' = stronger oxidizing agent (greater tendency to accept electrons). Electrons flow spontaneously from lower to higher reduction potential (from more negative to more positive E°').

**$\Delta G^{\circ\prime} = -nF\Delta E^{\circ\prime}$** where n = number of electrons transferred, F = 96.5 kJ/mol·V.

The overall electron transfer: NADH → $O_2$. E°' for $NAD^+$/NADH = −0.32 V. E°' for $O_2/H_2O$ = +0.82 V. $\Delta E^{\circ\prime}$ = +1.14 V. $\Delta G^{\circ\prime}$ = −(2)(96.5)(1.14) = −220 kJ/mol. This large negative $\Delta G^{\circ\prime}$ drives proton pumping.

## The Electron Transport Chain

**Iron-sulfur clusters:** Common components of the ETC. Contain iron atoms coordinated by sulfur atoms from Cys residues and inorganic sulfide. They transfer electrons one at a time. Multiple types: [2Fe-2S], [4Fe-4S].

**Complex I (NADH-Q oxidoreductase):** NADH → FMN → Fe-S clusters → ubiquinone (Q). Pumps 4 $H^+$ per NADH. Accepts electrons from NADH (the entry point for electrons from NADH-producing reactions). NADH binds and transfers a hydride to FMN. Electrons pass through a series of Fe-S clusters to ubiquinone, reducing it to ubiquinol ($QH_2$).

**Complex II (Succinate-Q reductase = Succinate Dehydrogenase):** Succinate → FAD → Fe-S clusters → Q. Does NOT pump protons. This is the same enzyme as step 6 of the citric acid cycle. $FADH_2$ from succinate oxidation transfers electrons to ubiquinone.

**Ubiquinol ($QH_2$):** A mobile electron carrier in the lipid bilayer. Collects electrons from Complexes I and II and delivers them to Complex III.

**Complex III (Q-cytochrome c oxidoreductase):** $QH_2$ → Fe-S cluster → cytochrome $c_1$ → cytochrome c. Pumps 4 $H^+$ per pair of electrons (via the Q cycle). The Q cycle allows the two-electron carrier $QH_2$ to donate electrons to the one-electron carrier cytochrome c, while pumping additional protons.

**Cytochrome c:** Small, soluble, one-electron carrier protein in the intermembrane space. Contains a heme group. Transfers electrons from Complex III to Complex IV. Its structure has been remarkably conserved across more than a billion years of evolution.

**Complex IV (Cytochrome c oxidase):** Cytochrome c → CuA → heme a → heme $a_3$/CuB → $O_2$. Reduces $O_2$ to $H_2O$ (4 electrons needed: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$). Pumps 2 $H^+$ per pair of electrons (plus 2 $H^+$ consumed from matrix in water formation).

## Reactive Oxygen Species (ROS)

Toxic derivatives of molecular oxygen (superoxide $O_2^-$, hydrogen peroxide $H_2O_2$, hydroxyl radical ·OH). Generated when electrons leak from the ETC and reduce $O_2$ partially instead of completely. Scavenged by protective enzymes: superoxide dismutase ($O_2^- \rightarrow H_2O_2$), catalase and glutathione peroxidase ($H_2O_2 \rightarrow H_2O$).

## Electron Transfer Between Non-Adjacent Groups

Electrons can be transferred between groups that are not in direct contact, up to ~14 Å apart, via quantum mechanical tunneling. The rate depends on distance, driving force (ΔG), and reorganization energy.

## The Proton-Motive Force

**Proton-motive force (Δp)** has two components:

1. Chemical gradient: ΔpH (higher $[H^+]$ in intermembrane space)
2. Electrical gradient: Δψ (membrane potential, negative inside matrix)

$\Delta p = \Delta\psi - (2.303\ RT/F) \times \Delta pH$

Both components contribute to the free energy available for ATP synthesis. The electrical component (Δψ) typically dominates.

## ATP Synthase (Complex V)

A remarkable molecular machine that converts the proton-motive force into ATP.

**Structure:** Two main components:

- **$F_0$ (membrane-embedded):** Contains the c-ring (proton channel) and the a-subunit. Protons flowing through $F_0$ drive rotation of the c-ring.
- **$F_1$ (matrix-facing):** Contains the catalytic sites for ATP synthesis. Has three αβ pairs arranged around a central γ-subunit. The γ-subunit rotates with the c-ring.

**Binding change mechanism:** Each β subunit cycles through three conformations as γ rotates:

- O (open): releases ATP, binds ADP + Pi
- L (loose): binds ADP + Pi loosely
- T (tight): catalyzes ATP synthesis (ADP + Pi → ATP)

ATP synthesis itself requires minimal energy — the energy from the proton gradient is primarily used to *release* the tightly bound ATP from the T site. Approximately 3-4 $H^+$ flowing through ATP synthase drives the synthesis of 1 ATP.

**Rotational catalysis:** ATP synthase is literally a rotary motor — the world's smallest molecular motor. The rotation of the c-ring/γ-subunit drives the conformational changes in the β subunits.

## ATP Synthase and G Proteins Share Common Features

Both are P-loop NTPases that undergo conformational changes upon nucleotide binding and release. This represents a conserved evolutionary solution to coupling nucleotide chemistry with mechanical or signaling work.

## Mitochondrial Shuttles (Section 18.5)

The inner mitochondrial membrane is impermeable to NADH. Electrons from cytoplasmic NADH must enter mitochondria via shuttle systems:

**Malate-Aspartate Shuttle:** Transfers electrons from cytoplasmic NADH to mitochondrial $NAD^+$ (NADH → NADH equivalent). Each cytoplasmic NADH produces mitochondrial NADH → yields ~2.5 ATP. Used primarily in liver, kidney, heart.

**Glycerol-3-Phosphate Shuttle:** Transfers electrons from cytoplasmic NADH to mitochondrial FAD (NADH → $FADH_2$ equivalent). Lower yield: each cytoplasmic NADH produces mitochondrial $FADH_2$ → yields ~1.5 ATP. Used primarily in brain, skeletal muscle.

## ATP/ADP Translocase

An antiporter in the inner mitochondrial membrane. Exports $ATP^{4-}$ from the matrix and imports $ADP^{3-}$ from the cytoplasm. This exchange is driven by the membrane potential (net export of one negative charge per cycle is favored by the negative-inside potential). The entry of ADP into mitochondria is coupled to the exit of ATP.

**Mitochondrial transporters share a common tripartite structure** — three repeated domains, each ~100 residues with two transmembrane helices.

## Complete Oxidation of Glucose: ATP Yield

Per glucose molecule:

- Glycolysis: 2 ATP (net), 2 NADH (cytoplasmic)
- 2 Pyruvate → 2 Acetyl CoA: 2 NADH (mitochondrial)
- 2 turns of TCA cycle: 6 NADH, 2 $FADH_2$, 2 GTP

Electron transport/oxidative phosphorylation:

- Each mitochondrial NADH → ~2.5 ATP (10 NADH × 2.5 = 25 ATP)
- Each $FADH_2$ → ~1.5 ATP (2 $FADH_2$ × 1.5 = 3 ATP)
- 2 GTP → 2 ATP equivalent
- Cytoplasmic NADH: depends on shuttle used

**Total: ~30 ATP per glucose** (using the malate-aspartate shuttle for cytoplasmic NADH). This guide uses the ~30 ATP convention.

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*End of guide*