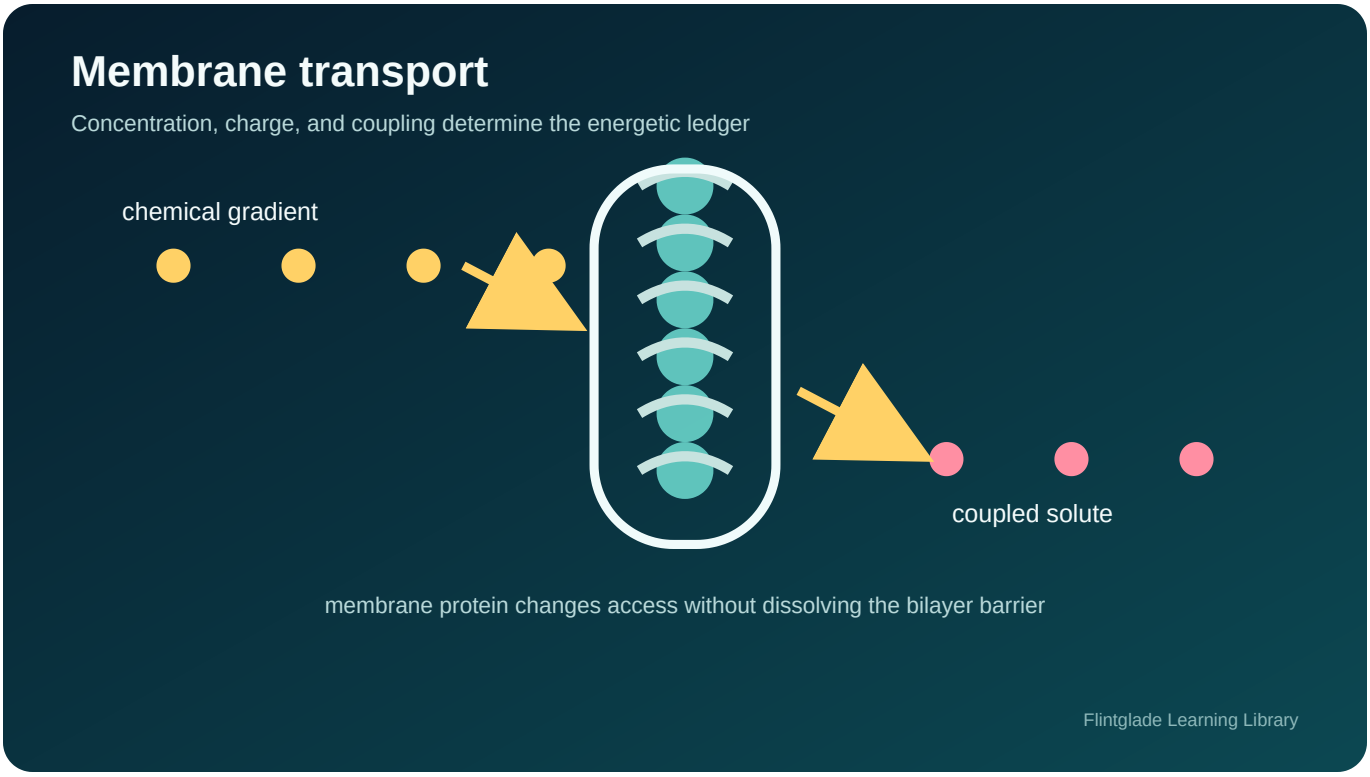
Biomolecules, Membranes, and Metabolism

Flintglade Learning Library · General Biochemistry

Audience: Intermediate learners connecting lipid structure, transport, central metabolism, and catalytic 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

- Connect membrane composition to transport behavior
- Explain the logic and regulation of central metabolic pathways
- Interpret carrier-dependent transformations mechanistically

Navigate this guide

- FOUNDATIONAL CONCEPT ARCHITECTURE
- CONCEPT ORGANIZATION
- INTEGRATED SYNTHESIS GUIDE
- STEP 4: EXTENSION CONCEPTS

A CONNECTED MAP OF BIOMOLECULES, TRANSPORT, AND METABOLIC CHEMISTRY

FOUNDATIONAL CONCEPT ARCHITECTURE

Tier Classification

Tier I — Conceptual Foundations (Principles)

These are the governing axioms. The applications in this guide draw on one or more of these.

1. **Thermodynamic reality vs. standard-state fiction.** $\Delta G^{\circ\prime}$ tells you where equilibrium lies. $\Delta G'$ tells you what actually happens in the cell. The entire regulatory logic of metabolism depends on this distinction.
2. **Oxidation state determines fuel value.** The more reduced a carbon, the more electrons it can donate, the more ATP it can ultimately generate.
3. **Membrane architecture is a consequence of lipid chemistry.** Fluidity, permeability, and transport all derive from the physical properties of fatty acyl chains and their packing geometry.
4. **Pathway regulation is reciprocal and hierarchical.** Glycolysis and gluconeogenesis cannot run simultaneously at high flux in the same tissue. The regulatory logic ensures this through allosteric effectors, covalent modification, and hormonal signaling.
5. **Cofactors are chemical tools with specific mechanistic roles.** TPP stabilizes carbanions after decarboxylation. NAD^+ accepts hydrides. FAD performs one- and two-electron chemistry. Biotin carries CO_2. Lipoamide shuttles acyl groups and electrons. CoA carries acyl groups as thioesters.

Tier II — Quantitative Mastery (Thermodynamics/Kinetics)

These require mathematical execution.

1. $\Delta G' = \Delta G^{\circ\prime} + RT \ln Q$ — calculating actual free energy under cellular conditions
2. $\Delta G_{transport} = RT \ln(c_{in}/c_{out}) + ZF\Delta\psi$ — electrochemical gradient calculations
3. Phosphoryl-transfer potential arithmetic — coupling hydrolysis free energies to determine spontaneity
4. Keq from $\Delta G^{\circ\prime}$ and vice versa
5. KM/kcat interpretation from mutant analysis (supported by earlier foundations)
6. ATP yield accounting under anaerobic conditions from various entry points

Tier III — Mechanistic Proficiency (Mechanisms/Substrates)

These require drawing or verbally articulating electron flow.

1. TPP-catalyzed decarboxylations (pyruvate dehydrogenase E1, acetolactate synthase, and novel substrates)
2. Schiff-base (imine) mechanisms (aldolase, transaldolase)
3. GAPDH-type oxidative phosphorylation via covalent thioester intermediate
4. Lipoamide oxidation/reduction and transesterification to CoA
5. FAD regeneration from $FADH^-$ (hydride transfer to NAD^+)
6. Biotin-dependent carboxylation (pyruvate carboxylase)
7. PEPCK mechanism (decarboxylation coupled to phosphoryl transfer from GTP)
8. Lactate dehydrogenase (hydride transfer from NADH to pyruvate)
9. Phosphoglycerate mutase (phospho-His ping-pong)
10. Enolate-mediated C–C bond formation/cleavage (general class)

First Principles by Topic

Topic (Lipids & Membranes): Lipid structure dictates membrane behavior. The degree of unsaturation, chain length, and head group chemistry determine melting point, fluidity, and bilayer organization. Cholesterol buffers fluidity bidirectionally.

Topic (Solute Transport): Membranes are selectively permeable barriers. Moving a charged or polar solute against its gradient costs free energy; this cost is quantifiable via the Nernst-type equation. Primary active transport hydrolyzes ATP directly. Secondary active transport couples uphill movement of one solute to the downhill movement of another.

Topic (Metabolism Principles): Metabolism is organized into coupled reaction sequences where thermodynamically unfavorable steps are driven by favorable ones through shared intermediates. ATP is the universal phosphoryl-group donor, but its role is always contextual — it can drive phosphoryl transfers, acyl transfers (via activation), and ligations. Cofactors are "activated carriers" with specific chemical logic.

Topic (Glycolysis & Gluconeogenesis): Glycolysis is a 10-step pathway that converts glucose to 2 pyruvate with a net yield of 2 ATP and 2 NADH. Gluconeogenesis reverses this using 4 NTPs and 2 NADH per glucose, bypassing the three irreversible glycolytic steps. The two pathways are reciprocally regulated primarily at PFK-1/FBPase-1 via F-2,6-BP, ATP/AMP ratios, and hormonal signaling.

Topic (PDH & TCA entry): The pyruvate dehydrogenase complex is the irreversible commitment of carbon to oxidation. It integrates five cofactors across three enzymes and is regulated by energy charge, product inhibition, feed-forward activation, and covalent modification (phosphorylation).

CONCEPT ORGANIZATION

RECURRING REASONING PATTERNS

Archetype 1: "Identify the missing cofactor and explain why" (CORE PATTERN)

- A transformation is presented without a cofactor; identify the missing carrier from the chemistry.
- **Decision tree:**
- Is a C–C bond being cleaved adjacent to a carbonyl, with loss of CO_2? → **TPP**
- Is an aldehyde being oxidized to an acyl phosphate? → **NAD^+** (via covalent thioester intermediate, as in GAPDH)
- Is a carboxylate being added to a substrate using HCO_3^-? → **Biotin** + **ATP**
- Is a disulfide being formed from two thiols (or vice versa)? → **FAD**
- Is a poor electrophile (carboxylate) being phosphorylated? → **ATP** as phosphoryl donor

Archetype 2: "Draw a complete mechanism with curved arrows" (CORE PATTERN)

- mechanism families: TPP decarboxylation/C–C cleavage (widely reusable), LDH reduction, PEPCK decarboxylation-phosphorylation, FAD/$FADH^-$ regeneration, lipoamide–cysteine disulfide exchange, Schiff-base aldol chemistry
- **Critical notation requirement:** General acid/base roles must be explicitly labeled (H-A/A^- or B:/H-B^+). Complete notation includes every protonation and deprotonation event.

Archetype 3: "ΔG' calculation under cellular conditions" (CORE PATTERN)

- Standard pattern: Given $\Delta G^{\circ\prime}$ values for two half-reactions (hydrolysis), combine them, then use $\Delta G' = \Delta G^{\circ\prime} + RT \ln Q$ to find the concentration ratio that maintains spontaneity.
- Variants include: finding the [1,3-BPG]/[3-PG] ratio, the [glut-5-P]/[glut] ratio, and the maximum glucose concentration ratio achievable by Na^+-glucose symport.

Archetype 4: "ATP/NADH yield accounting" (CORE PATTERN)

- Must trace entry points (G6P, DHAP, GAP, fructose, glycerol) into glycolysis and account for ATP consumed and produced.
- Under anaerobic conditions: NADH produced by GAPDH is consumed by fermentation → net NADH = 0, net NAD^+ = 0.
- Entner-Doudoroff variant: different stoichiometry (1 ATP net per glucose).

Archetype 5: "Cofactor matching in PDH" (CONNECTED PATTERN)

- Five cofactors map to five roles: TPP = electron sink for decarboxylation; lipoamide = reduced by hydroxyethyl-TPP; FAD = re-oxidation of E3 cysteines; NAD^+ = stoichiometric re-oxidation of $FADH^-$; CoA = accepts acetyl from acetyl-lipoamide via transesterification.

Archetype 6: "FRAP and membrane fluidity" (CONNECTED PATTERN)

- Higher unsaturated:saturated ratio → more fluid → faster fluorescence recovery. Must sketch or describe the recovery curve.

Archetype 7: "Regulation / mutation analysis" (CONNECTED PATTERN)

- Key applications: F-2,6-BP bifunctional enzyme, citrate inhibition of PFK-1, glucokinase KM significance, biotin attachment site in pyruvate carboxylase, pyruvate kinase allosteric regulation, insulin/glucagon signaling to PDH.

Archetype 8: "Carbon label tracking" (CONNECTED PATTERN)

- Requires knowing that aldolase cleaves F-1,6-BP such that C1-C3 → DHAP and C4-C6 → GAP. After TPI equilibration, the label distributes. Through PDH, the carboxyl carbon of pyruvate (C3 of glucose = C1 of pyruvate) is lost as CO_2.

Archetype 9: "Phosphoryl transfer vs. acyl transfer distinction" (CONNECTED PATTERN)

- Key logic: If the reactant is a poor electrophile (carboxylate), ATP must activate it via phosphoryl transfer. If the reactant is already a good electrophile (aldehyde → thioester), NAD^+ provides the driving force for acyl phosphate formation without ATP.

Archetype 10: "Electrochemical gradient / transport calculation" (CONNECTED PATTERN)

- Uses $\Delta G = RT \ln(c_2/c_1) + ZF\Delta\psi$ for charged species. For Na^+-glucose symport, the energy from ion flow is coupled to concentrative glucose uptake.

Concept Map — core and extension

Core coverage:

- ΔG vs. ΔG°' calculations (core coverage)
- TPP mechanisms — central mechanism type
- Cofactor identification from reaction inspection (core coverage)
- Glycolysis structures, enzymes, and ATP yield (core coverage)
- PDH cofactor roles and regulation (core coverage)
- Reciprocal regulation of glycolysis/GNG (core coverage)
- Membrane fluidity and FRAP (core coverage)
- Solute transport with electrochemical gradient math (core coverage)

SUPPORTING COVERAGE:

- Lipid structure drawing (triacylglyceride, glycerophospholipid)
- LDH mechanism
- PEPCK mechanism
- Carbon labeling
- Entner-Doudoroff pathway
- Serine biosynthesis / valine biosynthesis

ADDITIONAL COVERAGE:

- "Explain how channels selectively move ions" (core coverage) — extension topic
- "Describe the relationship between oxidation state and fuel value" (core coverage) — integrated implicitly rather than treated as a standalone topic
- "Explain the primary catabolic purpose of the citric acid cycle" (core coverage)
- Hydropathy plots — included as an extension

INTEGRATED SYNTHESIS GUIDE

Topic: LIPIDS & MEMBRANE STRUCTURE

Master Framework

Biological membranes are lipid bilayers whose physical properties emerge from the chemical structures of their constituent lipids. The three structural variables that govern membrane behavior are: (1) fatty acyl chain length, (2) degree of unsaturation (number and geometry of double bonds), and (3) head group identity.

12.1 Fatty Acid Nomenclature and Structure

A fatty acid is a hydrocarbon chain terminated by a carboxylate group. It is specified by two numbers: total carbons and number of double bonds. The notation 18:2, $\Delta^{9,12}$ means 18 carbons, 2 double bonds at positions 9 and 12 (counting from the carboxyl end). All naturally occurring unsaturated fatty acids

in membranes have *cis* (Z) double bonds, which introduce a ~30° kink in the chain.

Key fatty acids:

- Palmitic acid: 16:0 (saturated)
- Oleic acid: 18:1, Δ^{9}
- Linoleic acid: 18:2, $\Delta^{9,12}$
- α-Linolenic acid: 18:3, $\Delta^{9,12,15}$
- Eicosanoic acid (arachidic): 20:0

12.2 Triacylglycerides and Glycerophospholipids

Triacylglyceride (TAG): A glycerol backbone esterified at all three hydroxyl positions to fatty acids. To draw one: place glycerol vertically with three –OH groups. Each fatty acid attaches via an ester bond (loss of H_2O). The sn-1, sn-2, and sn-3 positions are conventionally assigned top to bottom.

Glycerophospholipid: A glycerol backbone with fatty acids at sn-1 and sn-2, and a phosphodiester-linked head group at sn-3. The head group determines the lipid class:

- Phosphatidylethanolamine (PE): head group = ethanolamine ($HOCH_2CH_2NH_2$)
- Phosphatidylcholine (PC): head group = choline
- Phosphatidylserine (PS): head group = serine

Construction example: Draw a PE with palmitic (16:0) at sn-1 and linoleic (18:2, $\Delta^{9,12}$) at sn-2. The phosphoethanolamine group connects at sn-3 via a phosphodiester linkage.

Sphingolipids (extension): The backbone is sphingosine rather than glycerol. Cerebrosides have a single monosaccharide attached; gangliosides have an oligosaccharide.

12.3 Lipid Bilayer Self-Assembly

Glycerophospholipids are amphipathic: the hydrocarbon tails are hydrophobic, the head group is hydrophilic. In aqueous solution, they spontaneously form bilayers to minimize the contact of hydrocarbon chains with water — this is driven by the hydrophobic effect (entropy of water).

12.4 Membrane Fluidity

Governing principle: Anything that disrupts tight van der Waals packing of acyl chains increases fluidity.

- **Unsaturation increases fluidity.** Cis double bonds introduce kinks → less packing → lower melting point → more fluid.
- **Shorter chains increase fluidity.** Fewer van der Waals contacts per molecule.
- **Cholesterol buffers fluidity.** At high temperatures, cholesterol's rigid steroid ring restricts chain movement → decreases fluidity. At low temperatures, cholesterol disrupts crystalline packing → increases fluidity. Net effect: cholesterol narrows the phase transition.

FRAP (Fluorescence Recovery After Photobleaching): The experiment: Label membrane lipids with a fluorescent probe. Bleach a defined region with intense light. Monitor the recovery of fluorescence as unbleached lipids diffuse laterally into the bleached zone.

- Faster recovery = higher fluidity = more lateral diffusion
- A bilayer with unsaturated:saturated ratio of 2.9 recovers faster than one with ratio 0.8
- On a plot of fluorescence intensity vs. time: both curves start at the same bleach point and recover toward the same plateau, but the high-unsaturation curve has a steeper initial slope and reaches the plateau sooner.

Reusable reasoning chain: Higher unsaturated:saturated → more cis kinks → less packing → more fluid → faster FRAP recovery.

Thermal adaptation: Bacteria in cold environments (4°C) need more unsaturated and shorter-chain fatty acids to maintain fluidity. Bacteria in hot environments (40°C) need more saturated and longer-chain fatty acids to prevent excessive fluidity and membrane instability.

12.5 Membrane Proteins

- **Integral proteins** span the bilayer (one or more transmembrane α-helices or β-barrels). Transporters are integral proteins.
- **Peripheral proteins** associate with the membrane surface via electrostatic/hydrogen bonding interactions.

Hydropathy plots: A transmembrane helix of ~20 residues will appear as a peak above the hydrophobicity threshold line. A transporter like GLUT4 that crosses the membrane 12 times will show 12 such peaks.

Topic: SOLUTE TRANSPORT

Master Framework

Biological membranes are permeable to small nonpolar molecules (O_2, CO_2, N_2) and water, but essentially impermeable to ions and polar molecules. Transport of these species requires protein-mediated mechanisms, classified by energy source.

13.1 Passive vs. Active Transport

Passive transport (facilitated diffusion): Movement down the concentration gradient. No energy input. Mediated by channels or carriers (e.g., GLUT transporters for glucose). The driving force is the free energy stored in the concentration gradient itself.

Active transport: Movement against the concentration gradient. Requires energy input.

- **Primary active transport:** Energy comes directly from ATP hydrolysis (e.g., Na^+/K^+-ATPase, H^+/K^+-ATPase).
- **Secondary active transport:** Energy comes from the downhill flow of one ion driving the uphill transport of another solute.
- **Symport:** Both solutes move in the same direction (e.g., Na^+-glucose symporter in brush border cells).
- **Antiport:** Solutes move in opposite directions (e.g., H^+/K^+-ATPase pumps H^+ out and K^+ in).

13.2 Free Energy of Transport — The Quantitative Core

For an uncharged solute:

$$\Delta G_{transport} = RT \ln \left(\frac{c_{in}}{c_{out}}\right)$$

For a charged solute (electrochemical gradient):

$$\Delta G_{transport} = RT \ln \left(\frac{c_{in}}{c_{out}}\right) + ZF\Delta\psi$$

Where:

- $R = 8.314 \times 10^{-3}$ kJ mol^{-1} K^{-1} (note the units — this is the high-value form)
- $F = 96.5$ kJ mol^{-1} V^{-1}
- Z = charge on the ion (+1 for Na^+, K^+, H^+)
- $\Delta\psi$ = membrane potential (sign convention: inside minus outside; typically -50 mV for most cells)

Worked example (Na^+-glucose symport):

Given: $[Na^+]_{in}$ = 12 mM, $[Na^+]_{out}$ = 145 mM, $\Delta\psi$ = -50 mV (inside negative), T = 310 K, 2 Na^+ per glucose.

Step 1: Calculate ΔG for transport of 1 mol Na^+ from outside to inside.

$\Delta G_{Na} = RT \ln(c_{in}/c_{out}) + ZF\Delta\psi = (8.314 \times 10^{-3})(310) \ln(0.012/0.145) + (1)(96.5)(-0.050) = (2.577)(-2.491) + (-4.825) = -6.42 + (-4.825) = -11.25$ kJ/mol

Step 2: Two Na^+ ions → 2 × 11.25 = 22.5 kJ available to drive glucose transport.

Step 3: Maximum glucose concentration ratio: 22.5 = RT ln([glucose]_in/[glucose]_out) 22.5 = (2.577) ln(ratio) ln(ratio) = 8.73 ratio = e^8.73 ≈ 6185

Worked example (H^+/K^+-ATPase):

Given: $\Delta G'_{ATP}$ = -43 kJ/mol, $pH_{stomach}$ = 1, $pH_{cytosol}$ = 7, $\Delta\psi$ = +50 mV (for this example, inside negative means the proton moves from negative to positive side), 1:1:1 ratio.

The total energy from ATP hydrolysis must equal the sum of the energies required to transport H^+ and K^+:

$+43 = RT \ln(10^{-1}/10^{-7}) + ZF\Delta\psi_H + RT \ln([K^+]_{in}/[K^+]_{out}) + ZF\Delta\psi_K$

Working through the algebra yields $[K^+]_{in}/[K^+]_{out} \approx 34$.

13.3 Brush Border Cell Glucose Transport (model system)

The brush border cell model integrates three transport systems:

1. **Apical (lumen-facing) side:** Na^+-glucose symporter — secondary active transport. Glucose moves from lumen into cell against its gradient, driven by Na^+ moving down its gradient.
2. **Basolateral side:** GLUT (glucose uniporter) — facilitated diffusion. Glucose moves from cell into capillary down its gradient.
3. **Basolateral side:** Na^+/K^+-ATPase — primary active transport. Maintains the low intracellular $[Na^+]$ that drives the apical symporter.

Concentration relationships:

- [glucose]_cell > [glucose]_lumen (active concentration by symporter)
- [glucose]_cell > [glucose]_capillary (glucose leaves cell by facilitated diffusion down gradient)

13.4 Why Phosphorylation Traps Metabolites

The phosphorylation of glucose by hexokinase adds a 2^- charge to the molecule. This charged species cannot traverse the GLUT transporters (which are designed for neutral glucose) and cannot passively cross the lipid bilayer. The metabolite is effectively "trapped" inside the cell. This is the chemical logic behind the first step of glycolysis being an ATP-consuming phosphorylation — it is a metabolic investment that prevents substrate loss.

Topic: PRINCIPLES OF METABOLISM

Master Framework

Metabolism is a network of enzyme-catalyzed reactions organized into pathways. Each pathway has a defined logic: catabolic pathways oxidize fuel to generate ATP and reducing equivalents; anabolic pathways consume ATP to build complex molecules. The central quantitative tool is free energy accounting.

15.1 ΔG°' vs. ΔG' — the central distinction

ΔG°' (Standard Free Energy Change): The free energy change when all reactants and products are at 1 M concentration (or 1 atm for gases), pH 7, 25°C. This is a property of the reaction itself.

ΔG' (Actual Free Energy Change): The free energy change under the actual cellular concentrations of reactants and products. This determines whether a reaction is spontaneous in vivo.

$$\Delta G' = \Delta G^{\circ\prime} + RT \ln Q$$

Where Q = [products]/[reactants] at actual cellular concentrations.

Critical insight used repeatedly: A reaction with ΔG°' >> 0 can still proceed spontaneously (ΔG' < 0) if the product is continuously removed by a subsequent favorable reaction. This is the principle of **reaction coupling through shared intermediates.**

Worked example (malate dehydrogenase): ΔG°' = +29.7 kJ/mol, yet the reaction proceeds readily. The product (oxaloacetate) is consumed by citrate synthase (a highly favorable condensation with acetyl-CoA), keeping [OAA] extremely low, which drives the mass-action ratio Q far below 1, making ΔG' < 0.

Quantitative template:

To find the ratio [B]/[C] for enzyme 2 (B → C, ΔG°' = +16.7, ΔG' = -1.2): ΔG' = ΔG°' + RT ln([C]/[B]) -1.2 = +16.7 + RT ln([C]/[B]) RT ln([C]/[B]) = -17.9 Since -17.9 < 0, ln([C]/[B]) < 0, therefore [C]/[B] < 1, meaning [B] > [C].

15.2 Reaction Coupling and Shared Intermediates

Two reactions are coupled when the product of one is the reactant of the other. The overall ΔG°' is the sum of the individual ΔG°' values. If the overall sum is negative, the coupled process is spontaneous under standard conditions.

ATP coupling: ATP hydrolysis (ΔG°' = -30.5 kJ/mol) can be coupled to an otherwise unfavorable reaction. The net ΔG°' = ΔG°'_unfavorable + (-30.5). If this sum is negative, the overall process is favorable.

Example (proline biosynthesis):

- Phosphorylation of L-glutamate: ΔG°' = +10.3 kJ/mol
- ATP hydrolysis: ΔG°' = -30.5 kJ/mol
- Overall: +10.3 + (-30.5) = -20.2 kJ/mol (favorable)
- Therefore ΔG°' for hydrolysis of L-glutamate-5-phosphate = -40.8 kJ/mol (by Hess's Law rearrangement)

15.3 Phosphoryl-Transfer Potential

Phosphoryl-transfer potential is the tendency of a phosphorylated compound to transfer its phosphate group to water (or another acceptor). It is quantified by the ΔG°' of hydrolysis. The more negative the ΔG°' of hydrolysis, the higher the phosphoryl-transfer potential.

Transfer-potential hierarchy: | Compound | ΔG°' of hydrolysis (kJ/mol) | |---|---| | PEP | -61.9 | | 1,3-BPG (acyl phosphate) | -49.4 | | Creatine phosphate | -43.1 | | ATP (→ ADP + Pi) | -30.5 | | G6P | -13.8 |

The transfer rule: A compound with higher phosphoryl-transfer potential (more negative ΔG°' of hydrolysis) can spontaneously phosphorylate a compound with lower potential. This is why PEP, 1,3-BPG, and creatine phosphate can all drive ATP synthesis via substrate-level phosphorylation.

Quantitative pattern:

Given ΔG°' values for hydrolysis of 1,3-BPG and ATP, find the ratio of [1,3-BPG]/[3-PG] needed to keep the phosphoglycerate kinase reaction spontaneous when [ATP]/[ADP] = 4.

ΔG°'_overall = (-49.4) + (+30.5) = -18.9 kJ/mol

Set ΔG' = 0 (threshold of spontaneity): 0 = -18.9 + (0.008314)(298) ln([ATP][3-PG] / [ADP][1,3-BPG]) 18.9 / 2.478 = ln(4 × [3-PG]/[1,3-BPG]) 7.63 = ln(4 × [3-PG]/[1,3-BPG]) e^7.63 = 2056 = 4 × [3-PG]/[1,3-BPG] [3-PG]/[1,3-BPG] = 514 [1,3-BPG]/[3-PG] = 1.9×10^{-3}

Interpretation: Only 1 molecule of 1,3-BPG is needed per 514 molecules of 3-PG to keep the reaction spontaneous. This demonstrates how thermodynamically favorable the reaction is.

15.4 ATP Hydrolysis — Why It Is Favorable

Four factors contribute to the large negative ΔG°' of ATP hydrolysis:

1. **Relief of electrostatic repulsion** — the closely spaced negative charges on the phosphoanhydride are separated upon hydrolysis
2. **Increased resonance stabilization** — ADP and Pi each have more resonance forms than ATP
3. **Increased hydration** — the products are better solvated than the reactant
4. **Increased entropy** — one molecule becomes two (in some cases; concentration effects also matter)

15.5 Cofactors as Activated Carriers

Cofactor	What it carries	How it works
NAD^+/NADH	Hydride ($2e^- + H^+$)	Accepts hydride at C4 of nicotinamide ring; 2-electron chemistry
FAD/$FADH_2$	Electrons ($1e^-$ or $2e^-$)	Isoalloxazine ring; can do 1-electron or 2-electron transfers
TPP	Aldehydes / activated aldehyde equivalents	C2 carbanion (ylide) attacks carbonyls; stabilizes carbanions after decarboxylation
Biotin	CO_2	Carboxybiotin carries activated CO_2 from bicarbonate to substrate
CoA	Acyl groups	Thiol forms thioesters; high-energy bond stores acyl-transfer potential
Lipoamide	Acyl groups + electrons	Cycles between disulfide (oxidized) and dithiol (reduced); swinging arm shuttles between active sites
ATP	Phosphoryl groups	Phosphoanhydride bond hydrolysis provides driving force

15.6 Reaction Classification

Six types:

1. **Oxidation-reduction:** Change in oxidation state (NAD^+/NADH, FAD/$FADH_2$)
2. **Group transfer:** Phosphoryl, acyl, or glycosyl transfer

3. **Hydrolysis:** Cleavage by addition of water
4. **C–C bond formation/cleavage:** Includes aldol reactions, decarboxylations, Claisen condensations
5. **Isomerization:** Intramolecular rearrangement (e.g., G6P ↔ F6P)
6. **Ligation coupled to ATP hydrolysis:** Joining two molecules using ATP energy (e.g., pyruvate carboxylase)

Critical distinction — phosphoryl transfer vs. acyl transfer:

- **Reaction i:** Aldehyde → acyl phosphate. This is an acyl transfer that does NOT require ATP. The oxidation of the aldehyde (by NAD^+) generates the energy needed to form the high-energy acyl phosphate bond. This is exactly the GAPDH mechanism.
- **Reaction ii:** Carboxylate → acyl phosphate. A carboxylate is a poor electrophile. ATP must be used as a phosphoryl donor to activate the carboxylate. This IS a phosphoryl transfer requiring ATP.

The generalized rule: If the starting functional group is already at a high oxidation state (carboxylate) and cannot be oxidized further, then external energy (ATP) is required. If the starting functional group can be oxidized (aldehyde → carboxylic acid equivalent), the oxidation provides the driving force.

15.7 Metabolic Regulation — First Principles

1. **Regulated enzymes catalyze irreversible steps** — specifically, the step with the most negative $\Delta G'$ under cellular conditions. This is typically the first committed step of the pathway.
2. **Regulation avoids waste** — if products are abundant, the pathway shuts down.
3. **Catabolic pathways are inhibited by ATP and activated by AMP** — because the purpose of catabolism is ATP production.
4. **Feedback inhibition** — end products inhibit early enzymes (e.g., ATP inhibits PFK-1).
5. **Feed-forward activation** — early intermediates activate later enzymes (e.g., F-1,6-BP activates pyruvate kinase).

Which enzyme is regulated? In a hypothetical pathway $A \rightarrow B \rightarrow C \rightarrow D \rightarrow E$, the enzyme with the most negative $\Delta G'$ (most thermodynamically favorable, hardest to reverse) is most likely regulated. This is typically the first step of the pathway, preventing wasteful commitment of substrate.

15.8 TPP Mechanism — Verbal Walkthrough (Generalized)

TPP (thiamine pyrophosphate) participates in all reactions that involve the cleavage of a C–C bond adjacent to a carbonyl, typically with loss of CO_2 (oxidative or non-oxidative decarboxylation).

The general TPP catalytic cycle:

Step 1 — Ylide formation: A base in the active site deprotonates C2 of the thiazolium ring, generating a nucleophilic carbanion (the ylide).

Step 2 — Nucleophilic attack: The TPP C2 carbanion attacks the electrophilic carbonyl carbon of the substrate (e.g., the α-keto carbon of pyruvate or α-ketoglutarate). This forms a tetrahedral adduct (the "addition compound") with a new C–C bond between TPP and the substrate.

Step 3 — Decarboxylation: The C–C bond between the α-carbon and the carboxylate is cleaved. CO_2 departs. The electrons that previously formed that bond are delocalized into the thiazolium ring, which acts as an electron sink. This generates the resonance-stabilized carbanion (the "hydroxyethyl-TPP" intermediate in the case of pyruvate). The positive nitrogen of the thiazolium ring stabilizes the negative charge that would otherwise reside on the α-carbon.

Step 4 — Divergence based on reaction:

- *In pyruvate decarboxylase (yeast fermentation):* The hydroxyethyl-TPP intermediate is protonated by a general acid and released as acetaldehyde, regenerating the TPP ylide.
- *In pyruvate dehydrogenase (E1):* The hydroxyethyl-TPP intermediate reduces the disulfide bond of lipoamide (the hydroxyethyl group is oxidized to an acetyl group as it is transferred to the sulfur of lipoamide). TPP is regenerated.
- *In acetolactate synthase (valine biosynthesis):* The hydroxyethyl-TPP carbanion attacks a second molecule of pyruvate (C–C bond formation). The product is released and TPP is regenerated.
- *In transketolase:* The TPP-bound 2-carbon fragment attacks an aldose sugar acceptor.

Step 5 — TPP regeneration: In all cases, the product departs and the TPP ylide is regenerated, ready for another catalytic cycle.

15.9 Enolate-Mediated C–C Bond Formation/Cleavage (Generalized)

The aldolase reaction and its reverse are the paradigm.

Aldol cleavage (glycolysis direction): The substrate (F-1,6-BP) forms a Schiff base (imine) with an active-site lysine. The imine nitrogen acts as an electron sink. The C3–C4 bond is cleaved heterolytically: the C4–C6 fragment departs as GAP (an aldehyde), and the remaining C1–C3 fragment is stabilized as an enamine (the Schiff base equivalent of an enolate). Hydrolysis of the Schiff base releases DHAP and regenerates the free lysine.

Aldol condensation (reverse/gluconeogenesis direction): The DHAP-Schiff base enamine acts as a carbon nucleophile, attacking the aldehyde carbonyl of GAP to form the new C–C bond.

The Schiff base is essential because: It provides an electron sink (the protonated imine nitrogen) that stabilizes the developing negative charge on the enamine intermediate during C–C bond cleavage. This is illustrated in another example, where the analysis asks how the enzyme stabilizes the high-energy intermediate — the answer is Schiff base formation.

Topic: GLYCOLYSIS & GLUCONEOGENESIS

Master Framework

Glycolysis is a 10-step pathway that converts one molecule of glucose (6C) into two molecules of pyruvate (3C), with a net production of 2 ATP and 2 NADH. It occurs in the cytosol of all cells.

Gluconeogenesis is the synthesis of glucose from non-carbohydrate precursors (primarily pyruvate, lactate, and certain amino acids). It occurs primarily in the liver and kidneys. It requires 4 ATP + 2 GTP + 2 NADH per glucose synthesized.

16.1 The Ten Reactions of Glycolysis

Phase 1 — Investment Phase (Reactions 1–5): 2 ATP consumed

#	Enzyme	Substrate → Product	Notes
1	**Hexokinase**	Glucose → G6P	ATP → ADP. Irreversible. Induced-fit conformational change excludes water to prevent ATP hydrolysis.
2	Phosphoglucose isomerase	G6P → F6P	Near equilibrium. Aldose → ketose isomerization.
3	**PFK-1**	F6P → F-1,6-BP	ATP → ADP. Irreversible. Committed step. Major regulatory point.
4	Aldolase	F-1,6-BP → GAP + DHAP	$\Delta G^{\circ\prime} = +23.8$. Near equilibrium in vivo (product removal). Schiff base mechanism.
5	Triose phosphate isomerase (TIM/TPI)	DHAP → GAP	Near-perfect enzyme (diffusion-limited). Prevents methyl glyoxal formation by stabilizing the enediol intermediate.

Phase 2 — Payoff Phase (Reactions 6–10): 4 ATP + 2 NADH produced

#	Enzyme	Substrate → Product	Notes
6	**GAPDH**	GAP + NAD^+ + Pi → 1,3-BPG + NADH	Oxidation + acyl phosphate formation via covalent thioester intermediate. No ATP required.
7	Phosphoglycerate kinase	1,3-BPG + ADP → 3-PG + ATP	Substrate-level phosphorylation.
8	Phosphoglycerate mutase	3-PG → 2-PG	Requires phospho-His intermediate and catalytic 2,3-BPG. Cofactor: Mg^{2+}.
9	Enolase	2-PG → PEP + H_2O	Dehydration. Creates the high-energy enol phosphate.
10	**Pyruvate kinase**	PEP + ADP → Pyruvate + ATP	Irreversible. Substrate-level phosphorylation. Allosterically regulated.

Net equation: Glucose + 2 NAD^+ + 2 ADP + 2 Pi → 2 Pyruvate + 2 NADH + 2 ATP + 2 H_2O

16.2 ATP Yield from Various Entry Points (Anaerobic Conditions)

Under anaerobic conditions, NADH produced by GAPDH must be re-oxidized by fermentation, so the net NADH = 0.

Entry Point	ATP Consumed	ATP Produced	Net ATP
Glucose	2 (HK + PFK)	4 (PGK + PK, ×2)	**2**
G6P	1 (PFK only)	4	**3**
F6P	1 (PFK only)	4	**3**
F-1,6-BP	0	4	**4**
DHAP (→ GAP)	0	2 (only 1 GAP)	**2**
GAP	0	2	**2**
Fructose (in adipose, via HK → F6P)	2 (HK + PFK)	4	**2**
Sucrose (glucose + fructose)	4	8	**4**
Glycerol (→ DHAP via kinase + oxidation)	1 ATP, 1 NAD^+	2	**1 ATP** (net)

Serine biosynthesis from glucose: Glucose → 2 GAP → 2 × (GAP → 3-PG): produces 2 ATP (from 1,3-BPG → 3-PG step ×2) But 2 ATP consumed in investment phase. 3-PG → serine: no ATP produced or consumed (it's oxidation + transamination + dephosphorylation). Net: **0 ATP** for 2 molecules of serine from 1 glucose.

16.3 Fermentation

Purpose: Regenerate NAD^+ so that glycolysis can continue under anaerobic conditions. Glycolysis requires 2 NAD^+ per glucose; without regeneration, glycolysis halts.

Mammalian (lactic acid) fermentation: Pyruvate + NADH → Lactate + NAD^+ (catalyzed by lactate dehydrogenase)

Yeast (alcoholic) fermentation: Pyruvate → Acetaldehyde + CO_2 (pyruvate decarboxylase, requires TPP), then Acetaldehyde + NADH → Ethanol + NAD^+ (alcohol dehydrogenase)

LDH Mechanism (verbal): NADH delivers a hydride (H^-) from its C4 position to the C2 carbonyl carbon of pyruvate. Simultaneously, a general acid ($H\text{-}B^+$) in the active site protonates the C2 oxygen (converting the ketone to a hydroxyl). The product is L-lactate, and the cofactor is oxidized to NAD^+.

16.4 Fates of Pyruvate

In humans:

1. **Acetyl-CoA** — via pyruvate dehydrogenase complex (aerobic, irreversible)
2. **Lactate** — via lactate dehydrogenase (anaerobic)

3. **Oxaloacetate** — via pyruvate carboxylase (gluconeogenesis, requires biotin + ATP)
4. **Alanine** — via transamination

NOT in humans: **Ethanol** (yeast only). A worked example illustrates this.

16.5 Key Mechanisms in Glycolysis

GAPDH (Reaction 6) — The covalent intermediate strategy:

Step 1: An active-site cysteine thiol attacks the aldehyde C1 of GAP, forming a hemithioacetal (tetrahedral intermediate). Step 2: NAD^+ oxidizes the hemithioacetal to a thioester, accepting a hydride. NADH departs and a fresh NAD^+ binds. Step 3: Inorganic phosphate (Pi) attacks the thioester carbonyl, displacing the cysteine thiol and forming the acyl phosphate product (1,3-BPG).

Chemical logic: The oxidation of the aldehyde to a thioester (thermodynamically favorable) is coupled to the formation of a high-energy acyl phosphate bond (thermodynamically unfavorable). The covalent enzyme intermediate (thioester) captures the oxidation energy so it can drive the phosphorylation. No ATP is required.

Phosphoglycerate Mutase (Reaction 8): A phospho-histidine in the active site donates its phosphate to the C2 hydroxyl of 3-PG, forming the 2,3-BPG intermediate. Then the C3 phosphate of 2,3-BPG is transferred back to the histidine, yielding 2-PG and regenerating the phospho-His. Catalytic 2,3-BPG is required to initially phosphorylate the histidine. Mg^{2+} is required as a cofactor for phosphoryl transfer.

TIM — prevention of methyl glyoxal: TIM catalyzes DHAP → GAP via an enediol intermediate. The enzyme's active site architecture (specifically, a loop closure) prevents the enediol from undergoing β-elimination of the phosphate, which would produce the toxic byproduct methyl glyoxal + Pi.

16.6 Gluconeogenesis — The Three Bypass Reactions

The three irreversible glycolytic reactions (HK, PFK-1, PK) must be bypassed because their ΔG' values are so negative that they cannot be reversed by simply raising substrate concentrations.

Bypass 1 (Pyruvate → PEP): Two enzymes, 2 NTPs

Pyruvate carboxylase: Pyruvate + HCO_3^- + ATP → Oxaloacetate + ADP + Pi

- Requires biotin as a CO_2 carrier
- Activated by acetyl-CoA (allosteric)
- ATP drives the attachment of CO_2 (from bicarbonate) to biotin; biotin then transfers CO_2 to pyruvate

PEPCK: Oxaloacetate + GTP → PEP + CO_2 + GDP

- The decarboxylation of OAA is thermodynamically favorable and drives the formation of the high-energy enol phosphate bond in PEP

- GTP donates the phosphoryl group

Chemical logic of the OAA detour: Direct conversion of pyruvate to PEP would require +31.4 kJ/mol (the reverse of the highly favorable pyruvate kinase reaction). Instead, the cell "invests" 2 NTPs and uses a decarboxylation as a thermodynamic driving force. The CO_2 added by pyruvate carboxylase is the same CO_2 removed by PEPCK — it serves as a thermodynamic handle, not a net chemical change.

PEPCK Mechanism (verbal example): Step 1: The β-carboxyl group of OAA departs as CO_2. The electrons from the C–C bond are delocalized into the enolate of the resulting pyruvate enolate intermediate. Step 2: The γ-phosphate of GTP is transferred to the enolate oxygen, forming PEP and GDP.

Bypass 2 (F-1,6-BP → F6P): FBPase-1 (fructose-1,6-bisphosphatase) catalyzes hydrolysis of the C1 phosphate.

Bypass 3 (G6P → Glucose): Glucose-6-phosphatase (liver and kidney only — not in muscle!) catalyzes hydrolysis of the C6 phosphate. This is why gluconeogenesis only produces free glucose in liver and kidneys.

Energetic cost of gluconeogenesis (2 pyruvate → 1 glucose):

- 2 ATP (pyruvate carboxylase, ×2)
- 2 GTP (PEPCK, ×2)
- 2 NADH (reverse of GAPDH, ×2)
- Total: **4 NTPs** + **2 NADH**

16.7 Carbon Label Tracking

The aldolase split determines label fate: Glucose C1-C2-C3 → DHAP (which becomes GAP via TPI) Glucose C4-C5-C6 → GAP

After TPI equilibration, both triose phosphates are equivalent. But tracking a specific label:

- Glucose C4 → GAP C1 (the aldehyde carbon) → 1,3-BPG C1 → 3-PG C1 → 2-PG C1 → PEP C1 → Pyruvate C1 (carboxyl)
- In PDH: Pyruvate C1 (carboxyl) is lost as **CO_2**. Pyruvate C2 and C3 become the acetyl group of acetyl-CoA.
- Label at C3 or C4 of glucose → **CO_2** after PDH (50% of lactate molecules will be labeled if both GAP and DHAP are considered).

Worked example: Fructose labeled at C4. After glycolysis: C4 of fructose = C4 of glucose (same position in F-1,6-BP) → same tracking → C1 of pyruvate (carboxyl). In lactate: the carboxyl carbon retains the label. But only 50% of lactate molecules carry the label (because the other triose phosphate, derived from C1-C3, has the label at a different position — actually, in fructose, C4 goes to GAP C1, and through the pathway becomes pyruvate C1 = lactate C1). Since the other pyruvate (from DHAP → GAP) has C3 of fructose at its C1 position (unlabeled), 50% of lactate molecules carry the label.

16.8 Reciprocal Regulation of Glycolysis and Gluconeogenesis

The central regulator: Fructose-2,6-bisphosphate (F-2,6-BP)

F-2,6-BP is NOT a glycolytic intermediate. It is a regulatory molecule synthesized and degraded by the bifunctional enzyme PFK-2/FBPase-2.

- **PFK-2 (kinase domain):** F6P + ATP → F-2,6-BP + ADP. Active when blood glucose is high (insulin signaling, dephosphorylated enzyme).
- **FBPase-2 (phosphatase domain):** F-2,6-BP → F6P + Pi. Active when blood glucose is low (glucagon signaling, PKA phosphorylates the bifunctional enzyme).

Effects of F-2,6-BP:

- Potent allosteric **activator** of PFK-1 → stimulates glycolysis
- Potent allosteric **inhibitor** of FBPase-1 → suppresses gluconeogenesis

Loss of phosphatase domain: F-2,6-BP cannot be degraded → F-2,6-BP accumulates → PFK-1 remains activated → glycolysis continues even when it shouldn't → gluconeogenesis is suppressed.

PFK-2 denatured: No F-2,6-BP is produced → PFK-1 loses its activator → glycolysis decreases → FBPase-1 loses its inhibitor → gluconeogenesis increases.

Additional regulatory effectors:

Effector	Target	Effect	Signal
ATP	PFK-1	Inhibits	High energy charge
AMP	PFK-1	Activates	Low energy charge
AMP	FBPase-1	Inhibits	Low energy charge
Citrate	PFK-1	Inhibits	Abundant biosynthetic precursors
Alanine	Pyruvate kinase	Inhibits	Abundant amino acid precursors
F-1,6-BP	Pyruvate kinase	Activates	Feed-forward: high glycolytic flux
ATP	Pyruvate kinase	Inhibits	High energy charge
Acetyl-CoA	Pyruvate carboxylase	Activates	Signals need for GNG
ADP	Pyruvate carboxylase, PEPCK	Inhibits	Low energy charge → GNG requires energy

Glucokinase KM significance: Liver glucokinase has a high KM (~10 mM) compared to hexokinase in other tissues (~0.1 mM). When blood glucose is low, glucokinase activity is minimal → glucose passes through the liver → reaches the brain and other tissues. When blood glucose is high, glucokinase becomes active → liver traps glucose for glycogen synthesis or glycolysis. A decrease in KM would cause the liver to trap glucose even at low blood glucose, depriving the brain.

Pyruvate kinase allosteric curves:

- No effector: sigmoidal curve (cooperative, allosteric enzyme)
- - F-1,6-BP (activator): curve shifts LEFT (lower apparent KM, higher affinity, R-state stabilized). Same Vmax.
- - ATP (inhibitor): curve shifts RIGHT (higher apparent KM, lower affinity, T-state stabilized). Same Vmax.

All three curves converge at the same Vmax because allosteric effectors shift the T⇌R equilibrium but do not change the intrinsic catalytic rate of the R state.

Topic: PYRUVATE DEHYDROGENASE COMPLEX & TCA ENTRY

Master Framework

The pyruvate dehydrogenase complex (PDH) catalyzes the irreversible oxidative decarboxylation of pyruvate to acetyl-CoA, linking glycolysis to the citric acid cycle. It is a massive multienzyme complex containing three enzymes (E1, E2, E3) and five cofactors.

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

17.1 The Five Cofactors and Their Roles

Cofactor	Enzyme	Role
TPP	E1 (pyruvate dehydrogenase)	Electron sink for decarboxylation of pyruvate; stabilizes the carbanion after CO_2 loss
Lipoamide	E2 (dihydrolipoyl transacetylase)	Reduced by the hydroxyethyl-TPP intermediate (accepts the 2-carbon fragment as an acetyl group on its sulfur); shuttles the acetyl group between E1 and E2 active sites via a flexible "swinging arm"
CoA	E2	Accepts the acetyl group from acetyl-lipoamide in a transesterification reaction
FAD	E3 (dihydrolipoyl dehydrogenase)	Used catalytically to re-oxidize the cysteine disulfide in the active site of E3 (which was reduced during lipoamide re-oxidation)
NAD^+	E3	Used stoichiometrically to re-oxidize $FADH^-$ back to FAD

Cofactor-role matching: (a) TPP — **e** (electron sink for decarboxylation) (b) lipoamide — **b** (reduced by hydroxyethyl-TPP) (c) FAD — **a** (re-oxidation of E3 cysteines) (d) NAD^+ — **d** (stoichiometric re-oxidation of $FADH^-$) (e) CoA — **c** (transesterification from acetyl-lipoamide)

17.2 Mechanistic Details

E1 — TPP-mediated decarboxylation: Follows the general TPP mechanism (Section 15.8). The hydroxyethyl-TPP intermediate attacks the disulfide bond of lipoamide (electrophilic sulfur). This simultaneously oxidizes the 2-carbon fragment from hydroxyl to thioester oxidation state and reduces one sulfur of lipoamide from disulfide to thiol.

E2 — Transesterification: The acetyl group on acetyl-lipoamide (a thioester) is transferred to the thiol of CoA in a transesterification reaction (thioester exchange). This regenerates reduced (dihydro) lipoamide with two free thiol groups.

E2→E3 — Disulfide exchange: Step 1: One thiol of dihydrolipoamide attacks one sulfur of the E3 active-site cystine (disulfide). This forms a mixed disulfide between lipoamide and the enzyme, releasing one cysteine thiol. Step 2: The second thiol of lipoamide attacks the mixed disulfide, displacing the second cysteine thiol. Lipoamide is now re-oxidized (disulfide restored); both E3 cysteines are now in the thiol (reduced) form. Step 3: The reduced E3 cysteines must be re-oxidized. This is where FAD enters.

E3 — FAD-mediated cysteine re-oxidation: Step 1: A base deprotonates one cysteine thiol. The thiolate attacks the FAD isoalloxazine ring (C4a position), forming a covalent C4a-thiol-FAD adduct. Step 2: The second cysteine thiol attacks the first sulfur, forming the disulfide bond and releasing $FADH^-$ (the FAD hydroquinone anion, resonance-stabilized). Step 3: $FADH^-$ transfers a hydride to NAD^+, regenerating FAD and producing NADH.

Why FAD is needed: Reduced lipoamide (dihydrolipoamide) has two thiols. Thiols can transfer electrons via disulfide exchange (2-electron, non-hydride chemistry), but they are NOT a source of hydride. NAD^+ specifically accepts hydride (H^-). Therefore, direct electron transfer from dithiol to NAD^+ is mechanistically impossible. FAD bridges this gap: it can accept electrons from thiols (via the cysteine disulfide exchange described above) and then donate a hydride to NAD^+.

17.3 Regulation of PDH

Allosteric regulation:

- Inhibited by: Acetyl-CoA (product), NADH (product), ATP (high energy charge)
- Activated by: Pyruvate (substrate, feed-forward), CoA, NAD^+, AMP

Covalent modification:

- **Pyruvate dehydrogenase kinase:** Phosphorylates E1 → INACTIVATES PDH. Activated by high [ATP], [acetyl-CoA], [NADH].
- **Pyruvate dehydrogenase phosphatase:** Dephosphorylates E1 → ACTIVATES PDH. Activated by **insulin** (signals high blood glucose → need to oxidize glucose → need PDH active).

Worked example: Insulin activates the phosphatase, turning ON PDH. This makes sense: high blood glucose → insulin secretion → glucose enters cells → glycolysis produces pyruvate → PDH should be active to convert pyruvate to acetyl-CoA for the TCA cycle.

Worked example: When PDH kinase is activated (PDH is OFF), pyruvate cannot be converted to acetyl-CoA. Alternative fates: (1) gluconeogenesis (pyruvate → OAA → glucose), (2) fermentation (pyruvate → lactate or ethanol).

Why PDH doesn't run anaerobically: PDH produces NADH. Under anaerobic conditions, the electron transport chain (ETC) cannot oxidize NADH back to NAD^+. Without NAD^+ regeneration, NADH accumulates, and PDH (and the TCA cycle) stall due to lack of the oxidized cofactor. Additionally, acetyl-CoA would accumulate with no way to be oxidized.

17.4 The Citric Acid Cycle — Entry Point Logic

The primary catabolic purpose of the TCA cycle is to oxidize the acetyl group of acetyl-CoA to 2 CO_2, generating 3 NADH + 1 $FADH_2$ + 1 GTP per turn. These reduced cofactors feed the electron transport chain for oxidative phosphorylation.

Supporting Foundations: ENZYME KINETICS & MUTANT ANALYSIS

Lactate Dehydrogenase Active Site Analysis

KM interpretation: NADH has KM = 1.5 μM; pyruvate has KM = 2 mM. Lower KM = higher affinity. Therefore NADH binds with higher affinity.

Arg109Gln mutation:

- KM,NADH: **No change.** Arg109 does not contact NADH in the active-site model.
- KM,pyruvate: **Increase** (lower affinity). Arg109 H-bonds to the C2 oxygen of pyruvate. Loss of this interaction weakens substrate binding → higher [S] needed for half-maximal velocity → increased KM.
- kcat: **Decrease.** Arg109 stabilizes the transition state during hydride transfer to C2 of pyruvate. Loss of this electrostatic stabilization raises the activation energy → slower catalysis.

Arg171Gln mutation:

- Greater effect on **KM** than kcat. Arg171 H-bonds to the C1 carboxylate oxygens (involved in binding, not catalysis). C2 is where the chemistry occurs; C1 contacts are primarily for substrate positioning.

STEP 4: EXTENSION CONCEPTS

The following extensions apply the same core chemistry to additional pathways:

1. **Entner-Doudoroff pathway analysis:** Applies general metabolic reasoning—redox identification, cofactor assignment, and ATP accounting—to a novel pathway.

2. **Serine biosynthesis from 3-PG:** Requires tracing glycolytic intermediates into a branching biosynthetic pathway and accounting for ATP consumed upstream.

3. **Valine biosynthesis via acetolactate synthase:** A TPP mechanism applied to a non-PDH context: condensation of two pyruvate-derived units.
4. **Inositol-1-phosphate cyclase (extension example):** NAD^+ used catalytically — oxidation followed by reduction at a different position. Requires understanding that NAD^+ can serve a catalytic (not just stoichiometric) role when it oxidizes one position and then re-reduces another in the same substrate.
5. **Phosphoglycerate mutase mechanism:** Requires knowledge of the phospho-histidine ping-pong mechanism and the role of catalytic 2,3-BPG. This extends the core mechanism map.
6. **Transaldolase mechanism:** Schiff-base mediated transfer of a 3-carbon fragment from S-7-P to GAP, yielding E-4-P and F-6-P. Application of the general aldolase/Schiff-base mechanism to the pentose phosphate pathway.
7. **Glycerol entry into glycolysis:** Glycerol → glycerol-3-phosphate (ATP, kinase) → DHAP (NAD^+, oxidation) → enters glycolysis. Requires knowing that glycerol must be phosphorylated and oxidized.
8. **Creatine phosphate as an ATP buffer:** The same reaction can be spontaneous in both directions depending on cellular conditions. During exertion, [ATP] is low → phosphocreatine drives ATP synthesis. At rest, [ATP] is high → ATP drives phosphocreatine regeneration. This is a direct application of $\Delta G' = \Delta G^{\circ\prime} + RT \ln Q$.
9. **Hormonal regulation (insulin/glucagon) of glycolysis/GNG:** Connects insulin and glucagon signaling to the PDH regulation and F-2,6-BP sections.
10. **Lactate dehydrogenase kinetics / active-site mutant analysis:** Applies enzyme-kinetics reasoning to active-site mutations.

APPENDIX A: QUANTITATIVE FORMULA REFERENCE

Thermodynamics:

- $\Delta G' = \Delta G^{\circ\prime} + RT \ln Q$
- $\Delta G^{\circ\prime} = -RT \ln K_{eq}$
- $\Delta G = \Delta H - T\Delta S$ (less commonly illustrated in this guide)
- $R = 8.314 \times 10^{-3}$ kJ mol^{-1} K^{-1}

Electrochemical transport:

- $\Delta G_{transport} = RT \ln(c_2/c_1) + ZF\Delta\psi$
- $F = 96.5$ kJ mol^{-1} V^{-1}

Enzyme kinetics (supporting foundations):

- $v = V_{max}[S] / (K_M + [S])$

- 1/v = (KM/Vmax)(1/[S]) + 1/Vmax

ATP/NADH accounting principle: Count every ATP consumed (negative) and produced (positive) from the entry point to the exit point. Under anaerobic conditions, all NADH produced must be consumed by fermentation → net NADH = 0.

APPENDIX B: COMMON REASONING PITFALLS

1. **Confusing ΔG°' with ΔG'.** ΔG°' is a constant for a given reaction. ΔG' depends on actual concentrations. A positive ΔG°' does NOT mean the reaction cannot occur in vivo.

2. **Forgetting general acid/base notation in mechanisms.** Every protonation and deprotonation event must be labeled. Use $H\text{-}B^+/B$: for general base catalysis and $H\text{-}A/A^-$ for general acid catalysis.

3. **Miscounting ATP in yield calculations.** The investment phase costs 2 ATP per glucose, and the payoff phase produces 2 ATP per triose phosphate (×2 = 4 total). Always check the entry point — G6P skips the hexokinase step, so only 1 ATP invested.

4. **Misidentifying redox reactions.** Check whether the oxidation state of any carbon changes. If a C–H bond is replaced by a C–O bond, it's oxidation. If a C=O bond is replaced by a C–OH bond (with hydrogen added), it's reduction. If only bond connectivity changes without oxidation state change (e.g., isomerization, phosphoryl transfer), it's NOT redox.

5. **Confusing F-2,6-BP with F-1,6-BP.** F-1,6-BP is a glycolytic intermediate. F-2,6-BP is a regulatory molecule that controls the flux through glycolysis and GNG. They are different molecules.

6. **Assuming PDH is a glycolytic enzyme.** PDH is NOT part of glycolysis. It sits between glycolysis and the TCA cycle. It is a separate, regulated complex.

7. **Sign convention for membrane potential.** $\Delta\psi$ is typically stated as "inside negative" (-50 mV). For a cation moving inward: $ZF\Delta\psi = (+1)(96.5)(-0.050) = -4.825$ kJ/mol (favorable, as cations are attracted to the negative interior).
