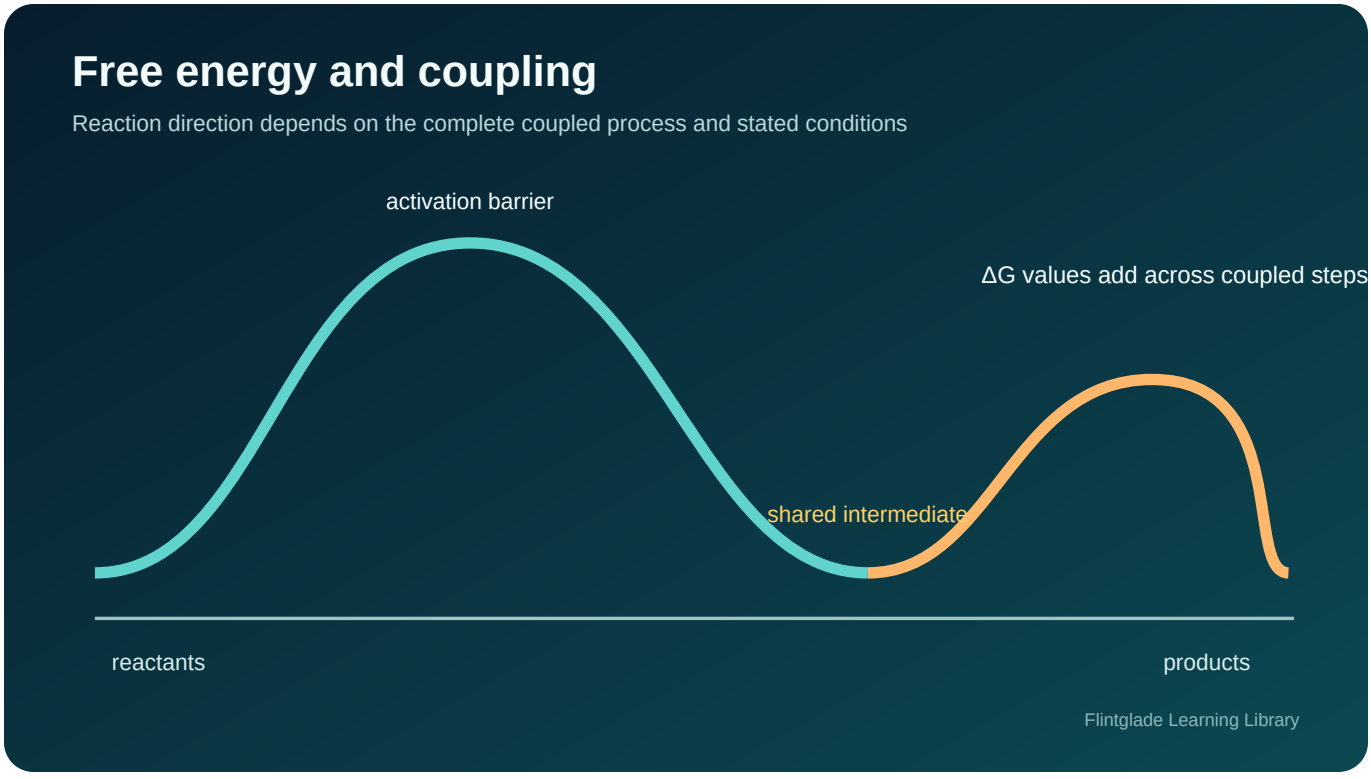
Metabolism and Mechanisms: Rapid Review

Flintglade Learning Library · General Biochemistry

Audience: Learners refreshing central pathway logic, carriers, regulation, and electron-flow patterns.

MCAT connections: This guide can assist study of 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

- Recall pathway inputs, outputs, and control logic
- Match carrier chemistry to transformations
- Check a proposed mechanism for chemical consistency

Navigate this guide

SECTION 1: CORE FACTS — ORGANIZED BY DEPTH

TIER 1 — FOUNDATIONAL PATTERNS

- The three irreversible glycolytic enzymes are hexokinase, PFK-1, and pyruvate kinase. These are the regulated steps. These are the steps bypassed in gluconeogenesis.
- PFK-1 is the committed step of glycolysis. Not hexokinase. G6P can go to glycogen or pentose phosphate pathway. F-1,6-BP is committed to glycolysis.
- F-2,6-BP is NOT a glycolytic intermediate. It is a regulatory molecule. It activates PFK-1 and inhibits FBPase-1. It is made by PFK-2 and destroyed by FBPase-2, which are two domains on the same bifunctional enzyme.
- Insulin → dephosphorylates bifunctional enzyme → PFK-2 active → F-2,6-BP rises → glycolysis ON. Glucagon → phosphorylates bifunctional enzyme via PKA → FBPase-2 active → F-2,6-BP falls → gluconeogenesis ON.
- Net glycolysis yield per glucose: 2 ATP, 2 NADH (aerobic) or 2 ATP, 0 NADH (anaerobic, because fermentation consumes the NADH).
- Gluconeogenesis costs 4 ATP + 2 GTP + 2 NADH per glucose (from 2 pyruvate).
- The sole purpose of fermentation is NAD^+ regeneration. Not ATP production. NAD^+ is needed for GAPDH to keep running.
- Mammals make lactate from pyruvate. Yeast make ethanol from pyruvate (via acetaldehyde). Ethanol is NOT a fate of pyruvate in humans.

- Aldolase cleaves F-1,6-BP between C3 and C4. C1-C3 → DHAP. C4-C6 → GAP. For carbon label tracking, C4 of glucose becomes C1 of GAP, which becomes the carboxyl carbon of pyruvate, which is lost as CO_2 in PDH.
- Hexokinase conformational change excludes water to prevent wasteful ATP hydrolysis. The phosphorylation traps glucose inside the cell because G6P cannot cross GLUT transporters.
- Glucokinase (liver) has high KM (~10 mM). At low blood glucose, it is inactive → glucose passes through liver to brain. A decrease in KM would trap glucose inappropriately.
- TIM prevents methylglyoxal formation by stabilizing the enediol intermediate. Without TIM, ATP yield is halved (only one GAP proceeds through payoff phase).
- Glucose-6-phosphatase exists only in liver and kidneys. Muscle cannot release free glucose into blood.
- Phosphoglycerate mutase requires catalytic 2,3-BPG to phosphorylate its active-site histidine, and Mg^{2+} for phosphoryl transfer.
- PDH is irreversible. Once pyruvate → acetyl-CoA, those carbons cannot become glucose again. Animals cannot convert acetyl-CoA to glucose.
- PDH does not run anaerobically because NAD^+ cannot be regenerated without the electron transport chain.
- Insulin activates PDH phosphatase → PDH ON. High ATP/acetyl-CoA/NADH activates PDH kinase → PDH OFF (phosphorylated).
- When PDH is off, pyruvate goes to gluconeogenesis (via pyruvate carboxylase → OAA) or to lactate (fermentation).
- Cholesterol buffers membrane fluidity in both directions. Restricts movement at high temp, disrupts packing at low temp.
- Higher unsaturated:saturated ratio → more fluid membrane → faster FRAP recovery.
- Transporters are integral membrane proteins. GLUT4 crosses the membrane 12 times.
- Na^+-glucose symporter = secondary active transport (symport). Na^+/K^+-ATPase = primary active transport. GLUT = facilitated diffusion (passive).
- In brush border cells: [glucose]cell > [glucose]lumen AND [glucose]cell > [glucose]capillary.
- ATP hydrolysis is favorable due to: relief of electrostatic repulsion, increased resonance stabilization of products, increased hydration of products, (sometimes) increased entropy.
- Keq = k_forward / k_reverse. Ka = 10^(-pKa). Reverse of acid dissociation: K = 10^(pKa). Sequential equilibria multiply: K_overall = $K_1 \times K_2$.

- On a reaction coordinate diagram: peaks = transition states (unstable, cannot isolate), valleys between peaks = intermediates (real molecules, transient). If K < 1, products are higher energy than reactants.
- RT at 298 K = 2.48 kJ/mol. RT at 310 K = 2.58 kJ/mol. ZFΔψ for +1 ion at -50 mV = -4.825 kJ/mol.

TIER 2 — CONNECTED PATTERNS THAT ARE EASY TO MIX UP

- ΔG°' tells you where equilibrium lies. ΔG' tells you what actually happens in the cell. Positive ΔG°' does NOT mean a reaction cannot proceed — it can if products are continuously removed (reaction coupling via shared intermediates).
- Malate dehydrogenase: ΔG°' = +29.7 kJ/mol but proceeds because citrate synthase consumes OAA, keeping [OAA] extremely low.
- The aldolase reaction has ΔG°' = +23.8 kJ/mol but is near equilibrium in the cell because GAP is continuously consumed downstream.
- GAPDH does NOT use ATP. The oxidation of the aldehyde (by NAD^+) provides the energy for acyl phosphate formation through a covalent thioester intermediate with an active-site cysteine.
- An aldehyde → acyl phosphate requires NAD^+ (no ATP). A carboxylate → acyl phosphate requires ATP (no NAD^+). The logic: aldehydes can be oxidized to release energy. Carboxylates are already fully oxidized and need external energy.
- 1,3-BPG and PEP can phosphorylate ADP because their phosphoryl-transfer potential is higher than ATP's. This is substrate-level phosphorylation.
- Phosphoryl-transfer potential hierarchy: PEP (-61.9) > 1,3-BPG (-49.4) > creatine-P (-43.1) > ATP (-30.5) > G6P (-13.8). Higher in the list can donate phosphate to anything lower.
- PEP's high phosphoryl-transfer potential comes from the enol → keto tautomerization of pyruvate after phosphate leaves. The tautomerization is highly exergonic.
- Creatine phosphate can drive ATP synthesis during exertion (when [ATP] is low, ΔG' < 0 forward) AND ATP can regenerate creatine phosphate at rest (when [ATP] is high, ΔG' < 0 in reverse). Same reaction, different directions, both spontaneous — because ΔG' depends on concentrations, not just ΔG°'.
- Catabolic pathways use NAD^+/NADH. Anabolic pathways use $NADP^+$/NADPH.
- The OAA detour in gluconeogenesis: pyruvate → OAA (pyruvate carboxylase, biotin + ATP) → PEP (PEPCK, GTP). The CO_2 added by pyruvate carboxylase is the same CO_2 removed by PEPCK. It is a thermodynamic handle — decarboxylation of OAA drives PEP formation.

- F-1,6-BP is a feed-forward activator of pyruvate kinase. It decreases KM (shifts T → R) but does not change kcat. ATP inhibits pyruvate kinase (increases KM, shifts R → T). All three curves (no effector, +F-1,6-BP, +ATP) reach the same Vmax.
- Citrate inhibits PFK-1 (signals abundant biosynthetic precursors). Alanine inhibits pyruvate kinase (signals abundant amino acids). Acetyl-CoA activates pyruvate carboxylase (signals: send carbons toward gluconeogenesis).
- Loss of PFK-2 (or its phosphatase domain, or its kinase domain) shifts the glycolysis/GNG balance. Think through which domain is lost, what happens to [F-2,6-BP], and what that does to PFK-1 and FBPase-1.
- For transport: $\Delta G = RT \ln(c_2/c_1) + ZF\Delta\psi$. For uncharged solutes, drop the $ZF\Delta\psi$ term. For multi-ion coupling, multiply single-ion ΔG by the stoichiometric number.
- To find the maximum concentration ratio from coupled ion transport: set total energy available = RT ln(ratio) and solve.
- Sphingolipids have a sphingosine backbone (not glycerol). Cerebrosides = one sugar. Gangliosides = oligosaccharide.
- Carbon labeling: 50% of lactate molecules carry a label when 100% of glucose molecules are labeled at one position, because aldolase splits the sugar into two trioses and only one carries the label at the relevant position.

TIER 3 — USEFUL EXTENSIONS

- Glycerol enters glycolysis as DHAP: glycerol → glycerol-3-P (ATP, kinase) → DHAP (NAD^+, oxidation).
- Sucrose = glucose + fructose (α-1,2 linkage). Net fermentation yield from sucrose: 4 ATP, 0 NADH.
- Entner-Doudoroff pathway: 1 ATP net per glucose (instead of 2 for glycolysis). If it shows up as a novel pathway, apply the same redox/cofactor reasoning you would to any unfamiliar sequence.
- Serine from 3-PG: oxidation (NAD^+) + transamination + dephosphorylation. Net ATP from glucose → 2 serine = 0 (2 ATP invested, 2 ATP from payoff phase up through 3-PG, nothing else).
- Hydropathy plot: peaks above threshold = transmembrane helices. 12 peaks = 12-pass transmembrane protein.
- Metabolic pathways must be overall irreversible. The regulated (committed) step ensures this.
- All phosphoryl transfers require Mg^{2+}.

SECTION 2: MECHANISM AND COFACTOR QUICK REFERENCE

THE FIVE PDH COFACTOR ROLES

(a) TPP → provides an electron sink for decarboxylation of pyruvate (E1)

(b) Lipoamide → reduced by the hydroxyethyl-TPP intermediate; shuttles the acetyl group between active sites via its flexible arm (E2)

(c) FAD → used catalytically in re-oxidation of E3 active-site cysteines (bridges thiol-disulfide chemistry to hydride chemistry)

(d) NAD^+ → used stoichiometrically to re-oxidize $FADH^-$ back to FAD; produces NADH as the final electron-carrying product (E3)

(e) CoA → accepts the acetyl group from acetyl-lipoamide via transesterification (E2)

Sequence check: TPP → lipoamide → CoA → FAD → NAD^+.

COFACTOR CARRIER ROLES (GENERAL, NOT PDH-SPECIFIC)

Cofactor	What it carries
TPP	Aldehydes / activated aldehyde fragments (2C units)
Lipoamide	Acyl groups + electrons (dual carrier)
Biotin	CO_2 (from bicarbonate)
NAD^+/FAD	Electrons (hydride for NAD^+; flexible $1e^-$ or $2e^-$ for FAD)
CoA	Acyl groups (as thioesters)
ATP	Phosphoryl groups

KEY DEFINITIONS FOR MECHANISM DRAWING

Nucleophile: Electron-rich, donates electrons to form bonds. Examples: thiolates (S^-), alkoxides (O^-), carbanions (C^-), enolates, TPP ylide, amines, water.

Electrophile: Electron-poor, accepts electrons. Examples: carbonyl carbon (C=O), phosphorus in phosphate, protons (H^+), disulfide sulfur atoms.

Carbanion: Carbon with a lone pair / negative charge. Unstable unless stabilized by an adjacent electron sink.

Enolate: A carbanion stabilized by resonance with an adjacent C=O. Negative charge shared between carbon and oxygen. Forms when a base removes an α-hydrogen (H on carbon next to carbonyl).

Enamine: A carbanion stabilized by resonance with an adjacent C=N (imine/Schiff base). Equivalent of an enolate but with nitrogen instead of oxygen as the electron sink.

Schiff base (imine): C=N bond formed between a substrate carbonyl and a lysine amine (with loss of water). Protonated form (iminium, $C{=}N^{+}H$) is a stronger electron sink than C=O.

Thioester: R–CO–SR'. Same oxidation state as carboxylic acid. High energy because sulfur donates poorly into carbonyl (weak resonance stabilization). Carbonyl carbon is more electrophilic than in an ester.

Acyl phosphate: R–CO–O–PO_3^{2-}. Mixed anhydride. Very high energy. Product of GAPDH.

Tetrahedral intermediate: Formed when a nucleophile attacks a carbonyl carbon. Four groups on the central carbon (was three, trigonal planar). Collapses by expelling a leaving group and reforming C=O.

Hemithioacetal: Tetrahedral intermediate from thiol attacking an aldehyde. Has –SR, –OH, –H, –R on the central carbon.

Ylide: The TPP C2 carbanion. Nucleophilic. Stabilized by adjacent N^{+} and S.

General acid (H–B^{+} or H–A): Donates a proton. Arrow goes FROM the H–A bond TO the atom being protonated.

General base (B: or:B): Accepts a proton. Arrow goes FROM the bond being broken TO the base.

Hydride (H^{-}): A hydrogen atom with both bonding electrons. Transferred from NADH C4 to a substrate carbonyl carbon, or from $FADH^{-}$ N5 to NAD^{+} C4.

MECHANISM TYPES — ORDERED BY conceptual sequence

TPP Decarboxylation (illustrated across multiple examples)

When: C–C bond cleaved adjacent to a carbonyl + CO_2 lost + no adjacent carbonyl to form enolate on the carbanion carbon → TPP needed.

Sequence:

1. Base removes C2–H from thiazolium → ylide (nucleophilic carbanion)
2. Ylide attacks substrate carbonyl carbon → new C–C bond; C=O electrons pushed onto O
3. C–C bond to carboxylate breaks → CO_2 leaves; electrons flow INTO thiazolium ring (the electron sink — draw arrow toward N^{+})
4. Product diverges:

- Released as aldehyde (pyruvate decarboxylase): protonate carbanion, break C–C to TPP
- Transferred to lipoamide (PDH E1): carbanion attacks lipoamide S, opens disulfide, oxidizes fragment to thioester
- Attacks second substrate (acetolactate synthase): carbanion attacks another carbonyl, new C–C bond forms

5. TPP regenerated

Do not forget: Ylide formation step. Arrow showing electrons going INTO the ring after CO_2 loss. General acid/base at every proton transfer. TPP regeneration at the end.

GAPDH-type (Aldehyde → Acyl Phosphate via NAD^+)

When: Aldehyde substrate, acyl phosphate product, NAD^+ present, no ATP.

Sequence:

1. Base deprotonates Cys–SH → Cys–S^- (thiolate nucleophile)
2. Thiolate attacks aldehyde carbonyl carbon → hemithioacetal (tetrahedral intermediate)
3. NAD^+ accepts hydride from C–H of hemithioacetal → thioester forms + NADH leaves; fresh NAD^+ binds
4. Pi attacks thioester carbonyl carbon → tetrahedral intermediate → Cys–S^- departs as leaving group → acyl phosphate product + free enzyme

Do not forget: No ATP anywhere. NADH must leave and fresh NAD^+ must bind between steps 3 and 4. The thioester is the energy-storing intermediate.

Disulfide Exchange (Lipoamide ↔ E3 Cysteines)

When: Thiols converting to disulfide (or reverse). Always involves FAD downstream.

Sequence:

1. Base deprotonates one lipoamide –SH → thiolate
2. Thiolate attacks one S of E3 cystine (disulfide) → S–S of cystine breaks → mixed disulfide + free cysteine thiol
3. Base deprotonates second lipoamide –SH → thiolate
4. Second thiolate attacks first lipoamide S in mixed disulfide → lipoamide S–S reforms → second cysteine released as thiol
5. Result: lipoamide re-oxidized, E3 cysteines reduced → now FAD re-oxidizes the cysteines (separate step)

Do not forget: Two sequential exchanges. Each one: thiolate attacks sulfur, other sulfur leaves. General base for each deprotonation.

LDH / Hydride Transfer (Ketone ↔ Alcohol)

When: Carbonyl (C=O) reduced to alcohol (C–OH), or reverse.

Reduction direction (pyruvate → lactate):

1. NADH C4–H (hydride) attacks C2 carbonyl carbon of pyruvate → C=O electrons pushed onto O (alkoxide)
2. General acid ($H–B^+$) protonates alkoxide → lactate formed, NAD^+ regenerated

Oxidation direction (lactate → pyruvate):

1. General base removes proton from C2–OH of lactate
2. C–H hydride transfers from C2 to NAD^+ C4 → C=O reforms → pyruvate formed, NADH produced

Do not forget: General acid for protonation in reduction direction. Two arrows for the hydride step (C–H to NAD^+ + C=O electrons to O, simultaneously).

PEPCK (β-Keto Acid Decarboxylation + Phosphoryl Transfer)

When: OAA → PEP + CO_2. No TPP (carbanion stabilized as enolate by adjacent carbonyl).

Sequence:

1. C3–C4 bond of OAA breaks → CO_2 leaves → carbanion at C3 → electrons delocalize into C2 carbonyl → enolate (negative charge on C2 oxygen)
2. Enolate oxygen (nucleophile) attacks γ-phosphorus of GTP (electrophile) → PEP formed + GDP leaves

Do not forget: No TPP. The enolate oxygen is the nucleophile for phosphoryl transfer. Two separate events (decarboxylation then phosphorylation).

Schiff Base / Aldolase (C–C Cleavage via Enamine)

When: C–C bond cleaved in substrate with a ketone that forms a Schiff base with active-site lysine.

Sequence:

1. Lysine $–NH_2$ attacks substrate C2 carbonyl → tetrahedral carbinolamine → water lost → Schiff base (C=N–Lys), protonated to iminium ($C=N^+H–Lys$)
2. C3–C4 bond breaks → GAP departs → electrons flow into $C2=N^+$ bond → enamine formed (stabilized carbanion)
3. Enamine protonated at C3 by general acid
4. Water attacks iminium C2 → C–N bond breaks → DHAP released with C2 ketone restored → free lysine regenerated

Do not forget: Schiff base formation first. The enamine is the stabilized carbanion. Lysine must be regenerated. No external cofactor needed.

FAD Regeneration ($FADH^- \rightarrow FAD$ via NAD^+)

When: Reduced FAD ($FADH^-$) must be re-oxidized.

Sequence:

1. $FADH^-$ donates hydride from N5 to NAD^+ C4 → FAD regenerated + NADH produced

One arrow. Conceptually important (explains why FAD exists in PDH) but mechanistically simple.

Biotin Carboxylation (Pyruvate Carboxylase)

When: CO_2 added to substrate from HCO_3^-. ATP consumed. Carbon count increases.

Sequence:

1. ATP γ-phosphoryl transferred to bicarbonate → carboxyphosphate + ADP
2. Biotin N1 attacks carboxyphosphate carbonyl → N-carboxybiotin + Pi
3. Base deprotonates pyruvate C3 → enolate; enolate C3 attacks carboxybiotin carbonyl → C–N bond breaks → OAA + free biotin

Transesterification (Acetyl-Lipoamide → Acetyl-CoA)

When: Acyl group moves from one thiol partner to another.

Sequence:

1. Base deprotonates CoA–SH → $CoA–S^-$
2. $CoA–S^-$ attacks thioester carbonyl → tetrahedral intermediate
3. Lipoamide S^- departs (leaving group) → C=O reforms → acetyl-CoA product

Standard nucleophilic acyl substitution.

Phosphoglycerate Mutase (His Ping-Pong)

When: Phosphate moves from one position to another on same substrate (3-PG → 2-PG).

Sequence:

1. Phospho-His donates phosphate to C2–OH of 3-PG → 2,3-BPG + free His
2. C3 phosphate of 2,3-BPG transfers to His → 2-PG + phospho-His regenerated

Requires catalytic 2,3-BPG and Mg^{2+}.

Enolase (Dehydration)

When: Water removed from substrate. Not redox.

Sequence:

1. Base removes C2–H → carbanion stabilized by C1 carboxylate
2. General acid protonates C3–OH → water leaves → C2=C3 double bond forms → PEP

UNIVERSAL MECHANISM RULES (FAST REFERENCE)

- Every arrow: tail at electron source, head at electron destination
- Nucleophile attacks carbonyl → ALWAYS show two simultaneous arrows (nuc→C and C=O→O)
- Leaving group departs from tetrahedral intermediate → ALWAYS show two arrows (C–LG→LG and O→C=O reforms)
- SHOW EVERY PROTON TRANSFER with ":B" or "H–B$^+$" and a curved arrow
- Label intermediates: "ylide," "enolate," "enamine," "Schiff base," "thioester," "hemithioacetal," "tetrahedral intermediate"
- Regenerate catalysts: TPP ylide, free lysine, FAD, phospho-His
- Check: did a carbon change oxidation state? If yes, a redox cofactor (NAD$^+$ or FAD) must be present. If no, no redox cofactor.
