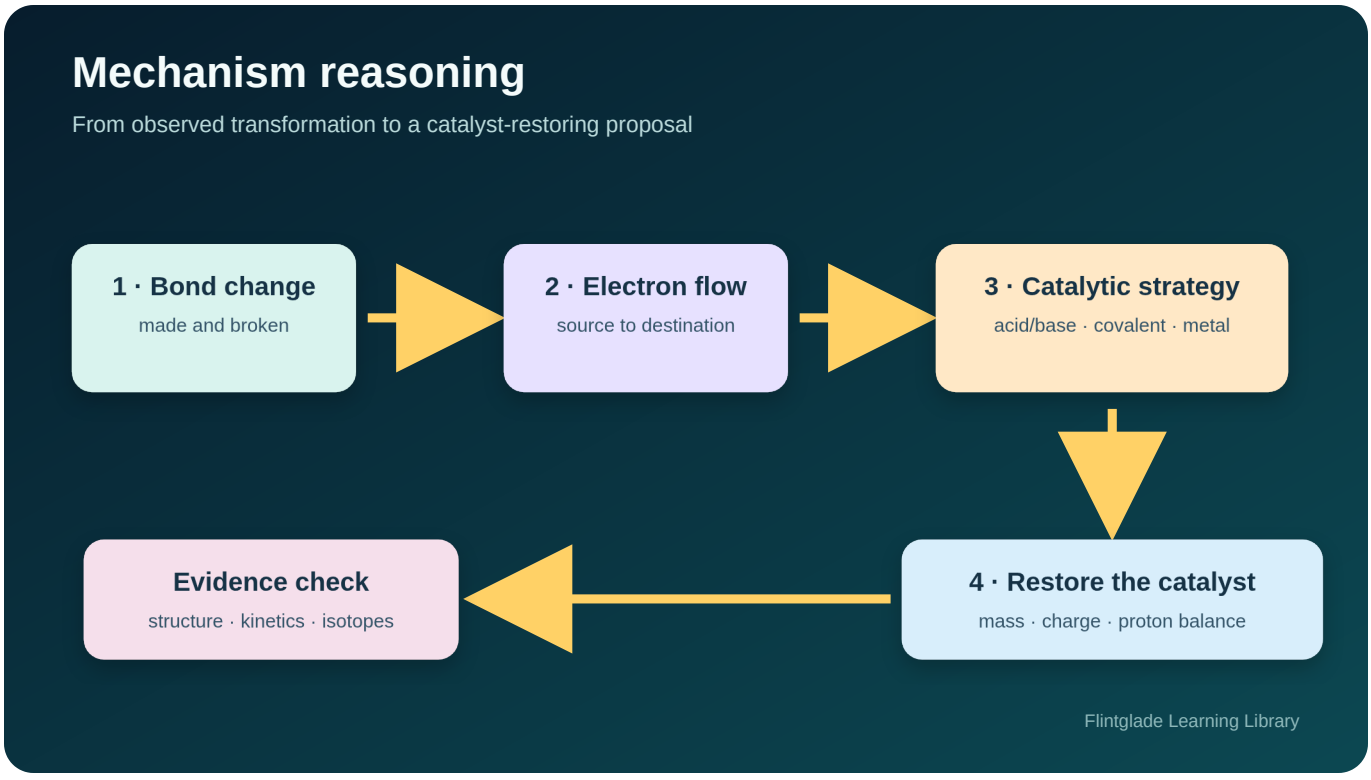
Reasoning from Biochemical Structure to Mechanism

Flintglade Learning Library · General Biochemistry

Audience: Learners who know basic organic chemistry and want a repeatable method for interpreting biochemical transformations.

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

- Classify transformations from bond and oxidation-state changes
- Infer likely carrier chemistry without relying on recall alone
- Construct plausible mechanisms with explicit electron flow

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The Complete Logic Map for Cofactor Identification, Reaction Classification, and Mechanism Construction

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PART I: THE COFACTOR IDENTIFICATION FRAMEWORK

A reliable reasoning pattern presents a chemical transformation and asks you to identify a cofactor that is not shown. The answer is never arbitrary — it follows directly from the chemistry of the transformation. What follows is an exhaustive treatment of every decision pathway, the chemical logic that governs it, the visual and structural cues that trigger each decision, and the edge cases that could trip you up.

Decision 1: Is a C–C Bond Being Cleaved Adjacent to a Carbonyl, with Loss of CO_2?

If yes → TPP is required.

The Chemical Logic

When a carboxyl group ($-COO^-$) is removed from a molecule as CO_2, the carbon that was bonded to the carboxyl group loses its bonding partner. If that carbon is adjacent to a carbonyl (meaning the substrate is an α-keto acid, a β-keto acid, or a similar structure where the carboxyl carbon is directly bonded to a carbon bearing a C=O), then removal of CO_2 generates a carbanion at the α-carbon. Carbanions are intrinsically unstable — a naked negative charge on carbon with no resonance stabilization would have an extraordinarily high energy. TPP solves this problem by providing a pre-formed nucleophile (the C2 ylide of the thiazolium ring) that bonds covalently to the substrate before

decarboxylation occurs. After CO_2 departs, the electrons that formed the C–C bond flow into the thiazolium ring, where the positively charged nitrogen and the adjacent sulfur atom delocalize and stabilize the negative charge. Without this electron sink, the decarboxylation would not occur at a biologically meaningful rate.

How to Recognize It

Look at the substrate and the product. If the product has one fewer carbon than the substrate and CO_2 is released as a byproduct, you are dealing with a decarboxylation. Now examine where the carboxyl group was attached. If it was attached to a carbon that is α to a carbonyl (the carbon immediately adjacent to a C=O group), TPP is required. The most common substrates are α-keto acids: pyruvate ($CH_3–CO–COO^-$), α-ketoglutarate ($^-OOC–CH_2–CH_2–CO–COO^-$), and oxaloacetate ($^-OOC–CH_2–CO–COO^-$). In each case, the carboxyl group being lost is directly bonded to the keto carbon.

There is an important subtlety: β-keto acid decarboxylations do NOT require TPP. When a carboxyl group is removed from a β-keto acid (where the carboxyl is two carbons away from the carbonyl, with one carbon between them), the resulting carbanion is stabilized by the existing carbonyl through enolization — the electrons flow into the adjacent C=O as an enolate, which is a perfectly adequate electron sink without any cofactor assistance. This is why the decarboxylation of oxaloacetate in the PEPCK reaction does not require TPP — OAA is a β-keto acid (the carboxyl at C4 is β to the keto at C2, and the carboxyl at C1 is the one that gets removed, but the C3 carbanion intermediate is stabilized by the C2 ketone through enolization). Similarly, the decarboxylation of acetoacetate does not require TPP. The rule is specifically about α-keto acid decarboxylations where the carbanion has no other means of stabilization.

All the Variants You Might See

Pyruvate decarboxylase (yeast fermentation): Pyruvate → acetaldehyde + CO_2. TPP required. The hydroxyethyl-TPP intermediate is protonated and released as free acetaldehyde.

Pyruvate dehydrogenase E1: Pyruvate → (hydroxyethyl-TPP intermediate) → acetyl group transferred to lipoamide. TPP required. The intermediate is not released free; it attacks the lipoamide disulfide.

α-Ketoglutarate dehydrogenase (TCA cycle): α-Ketoglutarate → succinyl-CoA + CO_2. TPP required. Mechanistically identical to PDH — the E1 component uses TPP for decarboxylation, and the subsequent steps involve lipoamide, CoA, FAD, and NAD^+.

Acetolactate synthase (valine biosynthesis): Two pyruvates → acetolactate + CO_2. TPP required. After the first pyruvate is decarboxylated on TPP (generating hydroxyethyl-TPP), the carbanion attacks the carbonyl of a second pyruvate molecule, forming a new C–C bond. The product is released and TPP is regenerated. This is a TPP-mediated C–C bond formation, not just cleavage.

Transketolase (pentose phosphate pathway): Transfers a 2-carbon fragment from a ketose sugar to an aldose sugar. TPP required. The mechanism is analogous: TPP ylide attacks the ketose carbonyl, C–C bond cleavage releases an aldose product, and the 2-carbon fragment on TPP then attacks a

different aldose acceptor, forming a new C–C bond.

Novel-substrate pattern: If you see any reaction where a molecule loses CO_2 and the departing carboxyl was α to a carbonyl, the answer is TPP. It does not matter if you have never seen the specific substrate before. The chemical logic is universal.

The Critical Edge Case: C–C Cleavage Without CO_2 Loss

TPP is also required when a C–C bond adjacent to a carbonyl is cleaved without loss of CO_2, as long as a carbanion would be generated that has no other means of stabilization. A representative example shows a β-hydroxy-α-keto thioester being cleaved to produce an aldehyde and an acetyl-CoA fragment. The C–C bond being broken is adjacent to a carbonyl, and the resulting carbanion at the α-carbon requires TPP stabilization. The recognition cue is the same: C–C bond cleavage adjacent to a carbonyl → check if the resulting carbanion needs an electron sink → if yes, TPP.

Decision 2: Is an Aldehyde Being Oxidized to an Acyl Phosphate (Without ATP)?

If yes → NAD^+ is required (and no ATP is needed).

The Chemical Logic

An aldehyde (R–CHO) is at a lower oxidation state than a carboxylic acid (R–COOH) or its equivalent (an acyl phosphate, R–CO–OPO_3^{2-}). Oxidizing the aldehyde to the carboxylic acid oxidation state is thermodynamically favorable — it releases free energy. This released energy can be captured and used to form a high-energy bond (the acyl phosphate) without requiring ATP. The oxidation is performed by NAD^+, which accepts a hydride (H^-) from the substrate, becoming NADH.

The mechanism that couples the oxidation to acyl phosphate formation involves a covalent enzyme intermediate. An active-site cysteine thiol attacks the aldehyde carbon, forming a hemithioacetal. NAD^+ oxidizes this hemithioacetal to a thioester (now at the carboxylic acid oxidation state). The thioester is a high-energy intermediate. Inorganic phosphate (Pi) then attacks the thioester carbonyl, displacing the cysteine and forming the acyl phosphate product. The energy of oxidation is trapped in the thioester and then transferred to the acyl phosphate bond. No ATP participates at any point.

How to Recognize It

You are given a reaction where the substrate contains an aldehyde functional group and the product is an acyl phosphate (a phosphorylated carboxylic acid, with the structure R–C(=O)–O–PO_3^{2-}). The transformation involves both an increase in oxidation state (aldehyde → carboxylic acid equivalent) and the formation of a phosphorylated product. The key recognition cue is: the starting material has a C–H bond on the carbonyl carbon (aldehyde), and the product has a C–O–P bond at that same carbon (acyl phosphate). This combination — oxidation plus phosphorylation in a single step — signals the GAPDH-type mechanism requiring NAD^+ and Pi, but not ATP.

The Specific Examples

GAPDH (glycolysis reaction 6): GAP (glyceraldehyde-3-phosphate, an aldehyde) → 1,3-BPG (an acyl phosphate). NAD^+ required. No ATP consumed.

Fucose metabolism reaction 4: An aldehyde intermediate is oxidized to an acyl phosphate. NAD^+ required. The same GAPDH-type logic applies.

Any novel substrate with aldehyde → acyl phosphate: The cofactor is NAD^+. The driving force is the oxidation. No ATP needed.

The Contrast That Makes This Pattern Clear

This reaction type pairs naturally with a phosphoryl transfer reaction (Decision 5 below) as a comparison. The comparison pattern is: "Here are two reactions. One requires ATP; the other does not. Which one requires ATP? Does either require an alternate cofactor?" Reference mapping: the aldehyde → acyl phosphate does not require ATP (it requires NAD^+), and the carboxylate → acyl phosphate does require ATP. Understanding why is the entire point: the aldehyde can be oxidized, releasing energy that drives phosphorylation; the carboxylate is already oxidized and cannot release energy this way, so external energy (ATP) must be supplied.

Decision 3: Is a Carboxylate Being Added to a Substrate Using Bicarbonate (HCO_3^-)?

If yes → Biotin and ATP are both required.

The Chemical Logic

Biotin is the biological carrier of activated CO_2. Carbon dioxide by itself is a poor electrophile, and simply adding CO_2 to a substrate without activation would be thermodynamically unfavorable. The cell solves this problem in two steps. First, ATP is used to activate bicarbonate: the γ-phosphoryl group of ATP is transferred to bicarbonate, forming carboxyphosphate (an unstable mixed anhydride between carbonic acid and phosphoric acid) and ADP. This activation step consumes ATP and is the energetic driving force. Second, the activated CO_2 from carboxyphosphate is transferred to the N1 nitrogen of the biotin ring, forming carboxybiotin (N-carboxybiotin). Biotin is covalently attached to the enzyme via a long, flexible linker (a lysine side chain), which allows it to swing from the site where it was carboxylated to the active site where it delivers the CO_2 to the substrate.

How to Recognize It

If a reaction involves the addition of a $–COO^-$ group (a carboxylate) to a substrate, and the source of that carboxylate is bicarbonate or CO_2, then biotin and ATP are required. The most recognizable cue is an increase in carbon count where the added carbon is at the carboxylic acid oxidation state.

Alternatively, if you are told that bicarbonate is a substrate, that alone is sufficient to invoke biotin + ATP.

The Specific Examples

Pyruvate carboxylase (gluconeogenesis): Pyruvate (3C) + HCO_3^- + ATP → Oxaloacetate (4C) + ADP + Pi. Biotin carries the CO_2 from bicarbonate to pyruvate, adding a carboxylate at C3 to form OAA. This reaction is activated by acetyl-CoA (allosteric activator).

Acetyl-CoA carboxylase (fatty acid synthesis): Acetyl-CoA (2C) + HCO_3^- + ATP → Malonyl-CoA (3C) + ADP + Pi. Biotin carries CO_2 to the methyl carbon of acetyl-CoA.

Propionyl-CoA carboxylase: Propionyl-CoA (3C) + HCO_3^- + ATP → Methylmalonyl-CoA (4C). Same mechanism.

Important Distinction: Biotin Carboxylation vs. Non-Biotin Carboxylation

Not all reactions that add CO_2 to a substrate require biotin. If the CO_2 is added by a mechanism that does not involve bicarbonate activation (for example, if CO_2 is fixed by a different chemical strategy), biotin may not be needed. However, in this guide, every carboxylation reaction that uses bicarbonate as the CO_2 source requires biotin + ATP. The reverse reaction — decarboxylation (removal of CO_2) — does not require biotin. Decarboxylation is thermodynamically favorable and does not need activation energy from ATP.

The Connection to Gluconeogenesis

The requirement for biotin in pyruvate carboxylase is directly connected to an integration example. If the lysine residue that serves as the attachment site for biotin is mutated, biotin cannot bind to the enzyme. Without biotin, CO_2 cannot be transferred from the activation site to the substrate binding site. Pyruvate carboxylase is completely nonfunctional, and gluconeogenesis cannot proceed because the first bypass reaction (pyruvate → OAA) is blocked.

Decision 4: Is a Disulfide Being Formed from Two Thiols (or Vice Versa)?

If yes → FAD is required.

The Chemical Logic

The interconversion of a dithiol (two –SH groups) and a disulfide (an S–S bond) is a two-electron oxidation-reduction. Forming a disulfide from two thiols is oxidation (electrons are removed from sulfur); breaking a disulfide into two thiols is reduction (electrons are added to sulfur). This type of electron transfer chemistry — involving sulfur-based redox — cannot be performed by NAD^+ because

NAD^+ exclusively accepts hydride ions (H^-), and the thiol-disulfide exchange mechanism does not generate or consume hydride ions. The electrons move through nucleophilic displacement at sulfur, not through hydride transfer.

FAD is the cofactor uniquely suited for this chemistry because the isoalloxazine ring of FAD can accept electrons from non-hydride sources. In the pyruvate dehydrogenase complex, the reduced dithiol of dihydrolipoamide transfers its electrons to the E3 active-site cysteines through thiol-disulfide exchange. The reduced E3 cysteines then reduce FAD through a covalent mechanism involving the C4a position of the isoalloxazine ring. The resulting $FADH^-$ (the anionic hydroquinone) can then donate a hydride to NAD^+, bridging the mechanistic gap between thiol-disulfide chemistry and the nicotinamide-based hydride chemistry of NAD^+.

How to Recognize It

Look for the presence of sulfur in the reaction. If you see two –SH groups (thiols) being converted to an S–S bond (disulfide), or vice versa, FAD is involved. In the context of this guide, the primary examples occur in multienzyme complexes where lipoamide participates.

A worked example may show reduced lipoamide (or dihydrolipoamide, shown as two free –SH groups on a five-carbon chain attached to the enzyme) being converted to oxidized lipoamide (shown as a cyclic disulfide with a five-membered ring containing the S–S bond). This transformation requires FAD.

You may also see the process framed from the perspective of the E3 enzyme: two cysteine residues in the reduced (thiol) form being oxidized to a cysteine disulfide. This is the same chemistry, and FAD is required.

The Subtle Connection

FAD is used catalytically in this context (it is regenerated within each cycle), while NAD^+ is used stoichiometrically (it is consumed, producing NADH as a product that must be regenerated by the electron transport chain). For catalytic-versus-stoichiometric classification, FAD in the PDH complex is catalytic, and the answer for NAD^+ is stoichiometric. The distinction matters because it affects accounting: one molecule of NAD^+ is consumed per molecule of pyruvate oxidized, but FAD cycles back to its oxidized form and is not depleted.

An Important Extension: FAD in Non-Disulfide Oxidations

FAD is also used in reactions where a C–C single bond is oxidized to a C=C double bond (as in succinate dehydrogenase, which converts succinate to fumarate in the TCA cycle). In these reactions, FAD accepts two hydrogen atoms (2H, equivalent to $2e^- + 2H^+$), becoming $FADH_2$. The distinction from NAD^+ is that FAD can accept hydrogen atoms from a C–H bond on each of two adjacent carbons (removing H from both C–H bonds to form C=C), while NAD^+ only accepts a single hydride from one carbon. This reaction type is less prominent in the worked examples but is worth knowing for the TCA cycle.

Decision 5: Is a Poor Electrophile (Carboxylate) Being Phosphorylated?

If yes → ATP is required as a phosphoryl donor.

The Chemical Logic

A carboxylate ($R–COO^-$) is at the highest common oxidation state for a carbon atom in organic molecules. It cannot be oxidized further to provide energy for bond formation (unlike an aldehyde, which can be oxidized to a carboxylic acid equivalent). Therefore, if you need to form a high-energy phosphorylated carboxylate (an acyl phosphate), the energy must come from an external source. ATP provides this energy by donating its γ-phosphoryl group to the carboxylate oxygen.

The phosphorus center of ATP is electrophilic (surrounded by electron-withdrawing oxygen atoms), and the carboxylate oxygen acts as the nucleophile in a phosphoryl transfer reaction. The product is an acyl phosphate ($R–C(=O)–O–PO_3^{2-}$) and ADP. This is fundamentally different from the GAPDH-type mechanism because no oxidation occurs — the carbon's oxidation state does not change. All the energy for forming the acyl phosphate bond comes from ATP hydrolysis.

How to Recognize It

The substrate is a carboxylate (has a $–COO^-$ group, no C–H bond on the carbonyl carbon), and the product is the corresponding acyl phosphate or phosphorylated derivative. There is no change in oxidation state at any carbon. The reaction is purely a group transfer (phosphoryl transfer from ATP to the carboxylate). If you see a carboxylate being converted to a phosphorylated product, the answer is ATP.

The Specific Examples

Glutamate → glutamate-5-phosphate (proline biosynthesis): The side-chain carboxylate of glutamate (a poor electrophile) is phosphorylated by ATP. This is a phosphoryl transfer reaction.

Comparison pair: Whenever you see two reactions side by side, one converting an aldehyde to an acyl phosphate and the other converting a carboxylate to an acyl phosphate, the aldehyde reaction uses NAD^+ (no ATP) and the carboxylate reaction uses ATP (no NAD^+).

Decision 6: Is an Amino Group Being Transferred Between Molecules?

If yes → Pyridoxal phosphate (PLP) is required.

The Chemical Logic

Transamination reactions transfer an amino group from an amino acid to an α-keto acid, converting the amino acid to a new α-keto acid and the original α-keto acid to a new amino acid. PLP (the active form of vitamin B_6) is the cofactor for all aminotransferases. It forms a Schiff base with the substrate amino acid, facilitating the transfer. PLP mechanisms provide an additional connection alongside TPP and NAD^+ chemistry; transamination reactions appear in the context of alanine formation from pyruvate and in serine biosynthesis.

How to Recognize It

If a reaction shows an amino group ($–NH_3^+$ or $–NH_2$) moving from one molecule to another, PLP is the cofactor. The presence of α-ketoglutarate as a co-substrate (being converted to glutamate) or glutamate as a co-substrate (being converted to α-ketoglutarate) is a strong cue for a transamination reaction.

Decision 7: Is a Substrate Being Activated as a Thioester for Acyl Transfer?

If yes → Coenzyme A (CoA) is the acyl carrier.

The Chemical Logic

CoA activates carboxylic acids for group transfer by forming a thioester bond (R–C(=O)–S–CoA). The thioester is a high-energy bond because sulfur is less effective than oxygen at donating its lone pair electrons into the carbonyl through resonance. This means the carbonyl carbon of a thioester is more electrophilic than that of an ordinary ester, making it more reactive toward nucleophilic attack. The hydrolysis of a thioester releases approximately as much energy as the hydrolysis of ATP's phosphoanhydride bond.

How to Recognize It

If a reaction involves the transfer of an acyl group (R–C(=O)–) from one molecule to another, and one of the molecules is or becomes a CoA derivative (R–CO–SCoA), then CoA is the cofactor. Common acyl-CoA species include acetyl-CoA, succinyl-CoA, malonyl-CoA, and fatty acyl-CoAs. The "SCoA" group in a structure is the visual cue. If you see a transesterification (exchange of one thioester for another, such as acetyl-lipoamide → acetyl-CoA), CoA is the accepting partner.

Decision 8: Is Mg^{2+} Required?

If phosphoryl transfer is occurring → Mg^{2+} is likely required as a cofactor.

The Chemical Logic

All phosphoryl transfer reactions in metabolism require a divalent metal ion, almost always Mg^{2+}, to proceed efficiently. The role of Mg^{2+} is to coordinate the negatively charged oxygen atoms of the phosphate groups, neutralizing some of the charge repulsion and properly orienting the phosphoryl group for nucleophilic attack. Without Mg^{2+}, the electrostatic repulsion between the incoming nucleophile and the negatively charged phosphate oxygens would be prohibitive.

How to Recognize It

Any reaction involving ATP as a phosphoryl donor, any kinase reaction, and the phosphoglycerate mutase reaction all require Mg^{2+}. The phosphoglycerate mutase mechanism provides a concrete example in which Mg^{2+} is the additional cofactor.

Decision 9: Is NADH Being Used as an Electron Donor (Reduction)?

If a carbonyl is being reduced to a hydroxyl → NADH (or NADPH) is the electron donor.

The Chemical Logic

NADH donates a hydride (H^-) to the electrophilic carbonyl carbon of the substrate. A general acid in the active site simultaneously protonates the developing alkoxide oxygen. The net result is the conversion of a ketone (C=O) to an alcohol (C–OH) — a two-electron reduction. The NADH is oxidized to NAD^+ in the process.

How to Recognize It

If a substrate's carbonyl group (C=O, specifically a ketone or aldehyde) is converted to a hydroxyl group (C–OH), and no other changes occur at that carbon, the reaction is a reduction requiring NADH (or NADPH in biosynthetic contexts). The lactate dehydrogenase reaction (pyruvate → lactate) is the prototypical example: the C2 ketone of pyruvate is reduced to the C2 hydroxyl of lactate by NADH.

The Distinction Between NAD^+ and NADH as the "Required Cofactor"

To decide whether a cofactor is required, pay attention to the direction of the reaction. If the reaction is an oxidation (substrate loses electrons, e.g., alcohol → ketone or aldehyde → carboxylic acid equivalent), the required cofactor is NAD^+ (the oxidized form, which accepts the electrons). If the reaction is a reduction (substrate gains electrons, e.g., ketone → alcohol), the required cofactor is NADH (the reduced form, which donates the electrons). Getting this backwards is a common error.

Decision 10: Is the Reaction a Schiff-Base-Mediated C–C Bond Cleavage or Formation?

If yes → No external cofactor is required (the Schiff base is formed with an active-site lysine).

The Chemical Logic

Aldolase and transaldolase use a Schiff base (covalent imine linkage between the substrate carbonyl and the ε-amino group of an active-site lysine) to stabilize carbanion intermediates during C–C bond cleavage or formation. The protonated imine nitrogen serves as an electron sink, stabilizing the developing negative charge on the α-carbon during retro-aldol cleavage. This is an intrinsic enzymatic mechanism — no exogenous cofactor is consumed or recycled. For a Schiff-base-mediated reaction, the answer is "no additional cofactor is required" (the Schiff base is part of the enzyme mechanism, not a separate cofactor).

However, be careful: if the C–C bond cleavage generates a carbanion that is NOT α to the Schiff base carbonyl (i.e., the carbanion is in a position where the Schiff base cannot stabilize it), then TPP or another stabilizing cofactor may be needed. The Schiff base is effective only when the carbanion is generated at the carbon directly bonded to the imine carbon (the α-carbon).

PART II: REACTION CLASSIFICATION SIGNIFIERS

How to Identify a Redox Reaction

A redox reaction involves a change in the oxidation state of one or more atoms in the substrate. The most reliable method for identifying redox in organic biochemistry is to track the oxidation state of carbon by counting bonds to oxygen (or nitrogen/sulfur) versus bonds to hydrogen.

Oxidation indicators: A C–H bond is replaced by a C–O bond. A C–OH (alcohol) becomes a C=O (carbonyl). An aldehyde (one C=O, one C–H) becomes a carboxylic acid or equivalent (two bonds to oxygen on the same carbon). A thiol (C–SH or S–H) becomes a disulfide (S–S). Whenever electrons leave the substrate (or hydrogen atoms leave without their electrons), the substrate is oxidized.

Reduction indicators: A C=O bond is replaced by a C–OH bond (with addition of hydrogen). A disulfide (S–S) is broken into two thiols (S–H). Whenever electrons are added to the substrate (or hydrogen atoms are added with their electrons), the substrate is reduced.

Not-redox indicators: Phosphoryl transfer (the phosphorus changes partners but no carbon changes oxidation state). Isomerization (atoms rearrange but oxidation states do not change — check carefully though, because some apparent isomerizations like the phosphoglycerate mutase reaction are truly not redox, while others might involve internal redox). Hydrolysis (a bond is cleaved by water, but no oxidation state change occurs). Dehydration (loss of water, as in enolase, is not redox — the carbon oxidation states do not change despite the loss of H_2O).

A common trap: The decarboxylation of OAA by PEPCK is NOT a redox reaction. CO_2 departs at the same oxidation state it was in the carboxylate, and the remaining enolate has the same oxidation state at its carbons. The overall reaction involves C–C bond cleavage and phosphoryl transfer, but no change in carbon oxidation states. By contrast, the decarboxylation of pyruvate by the PDH complex IS an oxidation, because the two-carbon fragment is simultaneously oxidized (from the hydroxyl oxidation state of hydroxyethyl-TPP to the thioester oxidation state of acetyl-lipoamide). The difference is that in PDH, lipoamide accepts electrons (it is reduced from disulfide to dithiol), proving that the substrate was oxidized. In PEPCK, no electron acceptor is involved.

How to Identify a Phosphoryl Transfer vs. An Acyl Transfer

Phosphoryl transfer: The electrophilic center is a phosphorus atom. A phosphoryl group ($–PO_3^{2–}$) moves from a donor to a nucleophilic acceptor (typically an oxygen atom on the substrate). The phosphorus atom has four oxygen substituents and is electrophilic due to the electron-withdrawing effect of those oxygens. Examples: hexokinase (ATP → glucose), PFK-1 (ATP → F6P), pyruvate kinase (PEP → ADP), PEPCK (GTP → enolate of pyruvate).

Acyl transfer: The electrophilic center is a carbonyl carbon (C=O) that is bonded to a leaving group (such as a thiolate from CoA, or a phosphate from an acyl phosphate). The acyl group (R–C(=O)–) moves from one partner to another. Examples: transesterification in PDH (acetyl-lipoamide → acetyl-CoA), phosphoglycerate kinase (acyl phosphate of 1,3-BPG → phosphoryl transfer to ADP — this one is technically a phosphoryl transfer from the perspective of the phosphate moving to ADP, even though the substrate is an acyl phosphate).

The distinction that matters for cofactor identification: To distinguish phosphoryl transfer from acyl transfer, look at what is moving and what the electrophilic center is. If a phosphate group moves from one molecule to another, it is a phosphoryl transfer. If an acyl group (R–C(=O)–) moves from one partner to another, it is an acyl transfer. The proline biosynthesis reaction (ATP + glutamate → ADP + glutamate-5-phosphate) is a phosphoryl transfer because the phosphate moves from ATP to glutamate. The electrophile is the phosphorus of ATP, and the nucleophile is the carboxylate oxygen of glutamate.

How to Identify Whether a Reaction is Thermodynamically Favorable, Unfavorable, or Near Equilibrium

Favorable reactions ($\Delta G^{\circ\prime} << 0$): Reactions coupled to ATP hydrolysis or other strong driving forces. Decarboxylations (loss of CO_2 is entropically favorable and generally exergonic). Reactions that form resonance-stabilized products. The three irreversible steps of glycolysis (hexokinase, PFK-1, pyruvate kinase) are all strongly favorable.

Unfavorable reactions ($\Delta G^{\circ\prime} >> 0$): Reactions that form high-energy bonds without a coupled energy source. Aldolase ($\Delta G^{\circ\prime} = +23.8$) is the classic example — C–C bond cleavage in an unfavorable direction under standard conditions. These reactions proceed in the cell because product removal drives them ($\Delta G' \approx 0$).

Near equilibrium ($\Delta G^{\circ\prime} \approx 0$ or $\Delta G' \approx 0$): Most of the "middle" reactions of glycolysis. Phosphoglucose isomerase, aldolase (in vivo), TPI, phosphoglycerate mutase, and enolase all operate near equilibrium in the cell. The hallmark is that these steps are freely reversible and use the same enzyme in both glycolysis and gluconeogenesis.

A thermodynamic judgment: When you see a reaction that involves both a thermodynamically favorable component (e.g., decarboxylation) and a thermodynamically unfavorable component (e.g., thioester formation), and both happen in the same step, the overall reaction is likely near equilibrium. The favorable component drives the unfavorable one. A worked example illustrates this directly: "Reaction 1 involves decarboxylation (favorable) coupled to thioester formation (unfavorable). Do you predict it is favorable, unfavorable, or near equilibrium?" The answer is near equilibrium, because the two opposing thermodynamic forces roughly cancel.

PART III: CONNECTION MAP — WHAT LINKS TO WHAT

The NAD^+ Supply Chain

NAD^+ is consumed by GAPDH (glycolysis), by LDH in reverse (if lactate is being oxidized to pyruvate), and by the PDH complex. Under aerobic conditions, NADH is re-oxidized to NAD^+ by the electron transport chain. Under anaerobic conditions, the only way to regenerate NAD^+ is fermentation (LDH or alcohol dehydrogenase). If NAD^+ is not regenerated, glycolysis halts at reaction 6 (GAPDH), and if the cell is relying solely on glycolysis for ATP, energy production stops entirely. This is why fermentation is essential under anaerobic conditions — its sole purpose is NAD^+ regeneration.

The ATP / Energy Charge Network

ATP is the central node connecting catabolic regulation to anabolic regulation. High [ATP] (high energy charge) inhibits PFK-1, pyruvate kinase, and PDH — shutting down catabolism. High [ATP] also activates pyruvate carboxylase, favoring gluconeogenesis. Low [ATP] (high [AMP]) does the reverse: activates PFK-1, activates pyruvate kinase, inhibits FBPase-1, and keeps PDH active. The energy charge of the cell is approximately ([ATP] + 0.5[ADP]) / ([ATP] + [ADP] + [AMP]). When this value is high, anabolism is favored; when low, catabolism is favored.

The F-2,6-BP Regulatory Circuit

F-2,6-BP is the most potent single regulator of glycolysis vs. gluconeogenesis in the liver. It activates PFK-1 (glycolysis on) and inhibits FBPase-1 (gluconeogenesis off). Its concentration is controlled by the bifunctional enzyme PFK-2/FBPase-2. Insulin → dephosphorylation of bifunctional enzyme → PFK-2 active → F-2,6-BP rises → glycolysis on. Glucagon → phosphorylation of bifunctional enzyme by PKA → FBPase-2 active → F-2,6-BP falls → gluconeogenesis on. Any mutation or modification that locks either domain (kinase or phosphatase) in a permanently active or inactive state will shift the glycolysis/gluconeogenesis balance accordingly.

The PDH – TCA – ETC Connection

PDH produces acetyl-CoA and NADH. The TCA cycle oxidizes acetyl-CoA, producing more NADH and $FADH_2$. The ETC oxidizes NADH and $FADH_2$, regenerating NAD^+ and FAD while producing ATP. If any downstream component is blocked (no oxygen for ETC), reduced cofactors accumulate, and both the TCA cycle and PDH stall. This is why PDH does not run anaerobically even though it does not directly use oxygen.

The Pyruvate Crossroads

Pyruvate connects glycolysis to: (1) the TCA cycle via PDH (irreversible, aerobic only), (2) lactate via LDH (anaerobic NAD^+ regeneration), (3) oxaloacetate via pyruvate carboxylase (gluconeogenesis), (4) alanine via transamination (amino acid metabolism), and (5) ethanol via pyruvate decarboxylase and alcohol dehydrogenase (yeast only). The fate of pyruvate is determined by the metabolic state of the cell: energy charge, oxygen availability, hormonal signals, and the availability of biosynthetic precursors.

The Cori Cycle Connection

During intense anaerobic exercise, muscle cells produce lactate from pyruvate (LDH, regenerating NAD^+ for continued glycolysis). Lactate is exported into the blood, travels to the liver, and is oxidized back to pyruvate (by LDH running in reverse, producing NADH). The liver then uses this pyruvate for gluconeogenesis, producing glucose that is exported back into the blood for the muscle to use. The net effect is that the liver expends ATP (6 NTP equivalents per glucose via gluconeogenesis) to recycle the muscle's metabolic waste (lactate) back into usable fuel (glucose). The energy cost is borne by the liver, not the muscle.

PART IV: HOW TO APPROACH DRAWING EVERY MECHANISM TYPE

This section provides a systematic protocol for constructing each mechanism type. For each type, the approach is: identify the reaction, recall the chemical logic, and then execute the electron-flow arrows in the correct sequence.

Mechanism Type 1: TPP-Catalyzed Decarboxylation (and Variants)

Setup Protocol

Before drawing anything, identify three things: (1) the substrate (an α-keto acid or similar), (2) what happens after decarboxylation (is the product released as an aldehyde, transferred to lipoamide, or used to attack another substrate?), and (3) whether general acids or bases are needed.

Step-by-Step Construction

Step 1 — Draw the TPP ylide. Draw the thiazolium ring of TPP with the positive nitrogen, the sulfur, and the C2 carbon. Show a base (B:) abstracting the C2 proton, generating the C2 carbanion. Draw the curved arrow from the C–H bond to the base. The resulting species has a lone pair on C2, stabilized by the adjacent N^+ and S. Label this the ylide.

Step 2 — Nucleophilic attack on substrate. Draw the substrate (e.g., pyruvate: $CH_3–CO–COO^-$). The TPP C2 carbanion attacks the electrophilic carbonyl carbon (the keto carbon, C2 of pyruvate). Draw the curved arrow from the C2 lone pair of TPP to the carbonyl carbon of the substrate. Simultaneously, the π electrons of the substrate C=O are pushed onto the oxygen (draw arrow from C=O bond to oxygen). The product is a tetrahedral addition compound with TPP covalently bonded to the former keto carbon, and the oxygen now bearing a negative charge (or protonated by a general acid, $H\text{-}B^+$).

Step 3 — Decarboxylation. The C–C bond between the keto carbon and the carboxylate carbon breaks. Draw the curved arrow from this C–C bond toward the carboxylate, pushing the electrons into the C=O bond of CO_2 (or simply show CO_2 departing). Simultaneously, the electrons that previously formed the C–C bond flow in the opposite direction — into the thiazolium ring. Draw the curved arrow from the C–C bond toward the TPP ring, showing the electrons being delocalized into the C2–N bond of the thiazolium. This generates the resonance-stabilized carbanion intermediate (hydroxyethyl-TPP or analogous species). The positive nitrogen stabilizes the negative charge.

Step 4 — Diverge based on reaction type.

If pyruvate decarboxylase (yeast): A general acid ($H\text{-}B^+$) protonates the carbanion α-carbon. The C–C bond between TPP and the product breaks (draw arrow from this bond toward the product carbon). Acetaldehyde is released. TPP is regenerated as the ylide (or as the protonated form, ready to be re-deprotonated).

If PDH E1: Draw oxidized lipoamide (the cyclic disulfide) approaching the hydroxyethyl-TPP carbanion. The carbanion attacks one sulfur of the lipoamide disulfide (draw arrow from carbanion to sulfur). The S–S bond breaks (draw arrow from S–S bond to the other sulfur, generating a thiolate). The two-carbon fragment is now bonded to lipoamide sulfur as a thioester (acetyl-lipoamide), having been oxidized from the hydroxyl to the acyl oxidation state. A general acid protonates the thiolate. TPP is regenerated.

If acetolactate synthase: The hydroxyethyl-TPP carbanion attacks the carbonyl carbon of a second pyruvate molecule (draw arrow from carbanion to the keto carbon of the second pyruvate). The second pyruvate's C=O electrons are pushed onto oxygen. A new C–C bond is formed. A general acid protonates appropriately. The product is released from TPP (draw arrow from the TPP–product C–C bond toward the product). TPP is regenerated.

Common Errors to Avoid

Do not skip the ylide formation step. Do not forget to show CO_2 leaving. Do not forget to show the general acid/base catalysis for protonation and deprotonation events. Do not forget to regenerate TPP at the end.

Mechanism Type 2: GAPDH-Type Oxidative Phosphorylation

Setup Protocol

Identify the aldehyde substrate, confirm that NAD^+ and Pi are involved, and note that no ATP participates.

Step-by-Step Construction

Step 1 — Cysteine attack on aldehyde. Draw the active-site cysteine (Enz–S–H). A general base (B:) deprotonates the cysteine thiol. The thiolate (Enz–S^-) attacks the aldehyde carbonyl carbon of the substrate. Draw the curved arrow from the sulfur lone pair to the carbonyl carbon, and simultaneously push the C=O π electrons onto the oxygen. The product is a hemithioacetal (tetrahedral intermediate with –S–Enz, –OH, –H, and –R on the central carbon).

Step 2 — Oxidation by NAD^+. NAD^+ accepts a hydride from the hemithioacetal. Draw the curved arrow from the C–H bond of the hemithioacetal to the C4 of the nicotinamide ring of NAD^+. Simultaneously, the electrons from the C–O bond (the hydroxyl) reform the C=O double bond (draw arrow from the oxygen lone pair back to the C–O bond to re-form the carbonyl). The product is a thioester (Enz–S–C(=O)–R) and NADH. A fresh NAD^+ binds to the enzyme.

Step 3 — Phosphorolysis. Inorganic phosphate (Pi, as HPO_4^{2-}) attacks the thioester carbonyl carbon. Draw the curved arrow from an oxygen lone pair on Pi to the carbonyl carbon. The C=O π electrons are pushed onto oxygen (tetrahedral intermediate). Then the C–S bond breaks as the cysteine thiolate departs (draw arrow from the C–S bond to the sulfur). The product is the acyl phosphate (1,3-BPG) and the free enzyme cysteine.

Common Errors to Avoid

Do not use ATP anywhere in this mechanism. Do not forget the NADH departure and fresh NAD^+ binding between steps 2 and 3. Do not confuse the order — oxidation must occur before phosphorolysis, because the thioester intermediate is what makes the phosphorolysis thermodynamically feasible.

Mechanism Type 3: Lactate Dehydrogenase (NAD^+/NADH Hydride Transfer)

Setup Protocol

Identify the direction of the reaction. In fermentation (glycolysis context), NADH reduces pyruvate to lactate. In gluconeogenesis/Cori cycle context, NAD^+ oxidizes lactate to pyruvate. Draw the mechanism in the stated direction.

Step-by-Step Construction (Reduction Direction: Pyruvate → Lactate)

Step 1 — Hydride delivery. Draw NADH with its nicotinamide ring, showing the C4–H bond. Draw pyruvate with its C2 carbonyl (C=O). The hydride (H^-) transfers from C4 of NADH to the C2 carbonyl carbon of pyruvate. Draw the curved arrow from the C4–H bond of NADH to the C2 of pyruvate. Simultaneously, the C=O π electrons are pushed onto the oxygen (draw arrow from C=O bond to oxygen, generating an alkoxide).

Step 2 — Protonation. A general acid (H-B^+, representing His195 or a similar residue) donates a proton to the alkoxide oxygen. Draw the curved arrow from the H–B bond to the oxygen. The product is L-lactate (C2 now bears –OH and –H), and NADH has been oxidized to NAD^+. Label the general base that formed (B:).

For the Reverse Direction (Lactate → Pyruvate)

Reverse all arrows. A general base (B:) deprotonates the C2 hydroxyl of lactate. The electrons flow from the C–O bond into a C=O double bond (regenerating the ketone). Simultaneously, the hydride on C2 transfers to NAD^+ at its C4. The product is pyruvate and NADH.

Mechanism Type 4: FAD Regeneration from $FADH^-$ (PDH E3)

Setup Protocol

This mechanism shows how $FADH^-$ (the resonance-stabilized anionic hydroquinone of FAD) is re-oxidized to FAD by transferring a hydride to NAD^+.

Step-by-Step Construction

Step 1 — Draw $FADH^-$. Show the isoalloxazine ring in its reduced form: N5 bears a hydrogen, and the ring has the anionic hydroquinone electronic configuration. (The N1 position bears a negative charge that is delocalized throughout the ring.)

Step 2 — Hydride transfer to NAD^+. The hydride at N5 of $FADH^-$ transfers to C4 of NAD^+. Draw the curved arrow from the N5–H bond of $FADH^-$ to C4 of the nicotinamide ring. The electrons from the N5–H bond flow to the nicotinamide ring. Simultaneously, the electrons in the FAD ring rearrange to restore the fully oxidized conjugated system (FAD is regenerated). NAD^+ becomes NADH.

Common Errors to Avoid

Do not confuse $FADH^-$ (anionic, one-electron reduced relative to $FADH_2$) with $FADH_2$ (fully reduced, neutral). In the PDH complex, the species formed after dithiol oxidation is specifically $FADH^-$, which then donates a hydride to NAD^+.

Mechanism Type 5: Disulfide Exchange (Lipoamide ↔ Enzyme Cysteines)

Setup Protocol

This mechanism occurs in the E3 step of the PDH complex. Dihydrolipoamide (two free thiols) transfers its electrons to a cystine (disulfide) in the E3 active site.

Step-by-Step Construction

Step 1 — First thiol-disulfide exchange. A general base (B:) deprotonates one thiol of dihydrolipoamide, generating a thiolate. The thiolate attacks one sulfur of the E3 cystine disulfide (draw arrow from thiolate sulfur to one sulfur of the disulfide). The S–S bond of the cystine breaks (draw arrow from S–S bond to the other cysteine sulfur, generating a cysteine thiolate). The result is a mixed disulfide between lipoamide and one cysteine, and one free cysteine thiol.

Step 2 — Second thiol-disulfide exchange. The second thiol of lipoamide is deprotonated by a general base. This second thiolate attacks the sulfur of the lipoamide that is part of the mixed disulfide (draw arrow from second thiolate to the mixed-disulfide sulfur). The lipoamide S–S bond forms (draw arrow showing the cysteine sulfur departing as a thiolate). The result is oxidized lipoamide (disulfide reformed, ready for next catalytic cycle) and both E3 cysteines in the reduced (dithiol) form.

Step 3 — FAD re-oxidizes the cysteines. This is where the FAD chemistry (from Mechanism Type 4) begins. The reduced E3 cysteines transfer their electrons to FAD through a covalent C4a adduct mechanism, generating $FADH^-$, which then donates a hydride to NAD^+.

Mechanism Type 6: Schiff Base (Aldolase-Type) C–C Bond Cleavage

Setup Protocol

Identify the substrate (F-1,6-BP for aldolase, S-7-P for transaldolase), the cleavage site (which C–C bond is broken), and the two products.

Step-by-Step Construction

Step 1 — Schiff base formation. The C2 ketone of the substrate reacts with the ε-amino group of an active-site lysine. The nitrogen lone pair attacks the carbonyl carbon (draw arrow from N to C). Water is lost (the –OH departs with assistance from a general acid). The product is a protonated Schiff base

(iminium ion, C=N^+H–Lys).

Step 2 — Retro-aldol cleavage. The C3–C4 bond breaks. The electrons from this bond move toward C3, forming a carbanion at C3, but this carbanion is immediately stabilized as an enamine because the electrons delocalize into the C2=N bond of the Schiff base. Draw the curved arrow from the C3–C4 bond toward C3, and simultaneously show the C4 fragment departing as an aldehyde (GAP). A general base may assist by deprotonating as needed.

Step 3 — Enamine hydrolysis (product release). The enamine (the Schiff base-stabilized carbanion equivalent) is protonated at C3 by a general acid (H-B^+). Then water hydrolyzes the Schiff base: the oxygen of water attacks the C2=N carbon (draw arrow from water oxygen to the iminium carbon). The C–N bond breaks (draw arrow from C–N bond to nitrogen). The product (DHAP) is released, and the free lysine amino group is regenerated.

Mechanism Type 7: PEPCK (Decarboxylation-Phosphorylation of OAA)

Setup Protocol

The substrate is oxaloacetate (a β-keto dicarboxylic acid). The cofactor is GTP. The products are PEP, CO_2, and GDP.

Step-by-Step Construction

Step 1 — Decarboxylation. The C4 carboxyl group (the β-carboxyl) of OAA departs as CO_2. Draw the curved arrow from the C3–C4 bond toward C3, and from the C=O of the carboxylate toward the departing CO_2. The electrons from the C–C bond flow into the C3–C2 system, forming an enolate at C3 (the C3 oxygen bears a negative charge, stabilized by resonance with the C2 carbonyl). This decarboxylation is thermodynamically favorable because it converts a β-keto acid to an enolate — the electron sink provided by the C2 carbonyl makes this carbanion stable without TPP.

Step 2 — Phosphoryl transfer from GTP. The enolate oxygen (C3 oxygen bearing the negative charge) acts as a nucleophile and attacks the γ-phosphorus of GTP. Draw the curved arrow from the enolate oxygen lone pair to the phosphorus atom. The P–O bond between the γ-phosphate and the β-phosphate of GTP breaks (draw arrow from this bond to the bridging oxygen, which departs with GDP). The product is PEP (with the phosphate esterified at the enol oxygen) and GDP.

The Key Insight

No TPP is required because the carbanion (enolate) formed by decarboxylation is stabilized by the adjacent carbonyl through normal enolate resonance. The carboxyl being removed is β to the carbonyl (two carbons away, with one carbon between them), and the resulting enolate has the negative charge on the oxygen of the remaining carbonyl. This is a fundamentally different situation from α-keto acid decarboxylation, where the carbanion is at the α-carbon with no resonance stabilization except that provided by TPP.

Mechanism Type 8: Biotin-Dependent Carboxylation (Pyruvate Carboxylase)

Setup Protocol

The substrates are pyruvate, HCO_3^-, and ATP. The product is oxaloacetate.

Step-by-Step Construction

Step 1 — Bicarbonate activation. The γ-phosphoryl group of ATP is transferred to one oxygen of bicarbonate (draw arrow from the bicarbonate oxygen to the γ-phosphorus, and from the γ–β phosphoanhydride bond to the β-phosphorus oxygen). The product is carboxyphosphate (an unstable mixed anhydride) and ADP.

Step 2 — Biotin carboxylation. The N1 nitrogen of the biotin ureido ring attacks the carbonyl carbon of carboxyphosphate (draw arrow from N to C). The C–O bond to phosphate breaks (phosphate departs). The product is N1-carboxybiotin and Pi.

Step 3 — Carboxyl transfer to pyruvate. The biotin arm swings to the second active site. A base deprotonates the C3 (methyl) of pyruvate, generating a carbanion (stabilized as an enolate by the C2 carbonyl). The carbanion at C3 attacks the carbonyl carbon of the carboxyl group on carboxybiotin (draw arrow from C3 to the carboxyl carbon). The C–N bond between the carboxyl and biotin breaks (draw arrow from C–N bond to nitrogen). The product is oxaloacetate (pyruvate with a new $–COO^-$ at C3) and regenerated biotin.

Mechanism Type 9: Phosphoglycerate Mutase (Phospho-Histidine Ping-Pong)

Setup Protocol

The substrate is 3-PG. The product is 2-PG. The enzyme has a pre-phosphorylated histidine (phospho-His) and requires catalytic 2,3-BPG and Mg^{2+}.

Step-by-Step Construction

Step 1 — Phosphoryl transfer from His to substrate. The phosphoryl group on the phospho-histidine is transferred to the C2 hydroxyl of 3-PG. Draw the arrow from the C2 oxygen to the phosphorus of phospho-His, and from the P–N bond (phosphorus to histidine nitrogen) to nitrogen (histidine departs). The product is 2,3-BPG and free (unphosphorylated) histidine.

Step 2 — Phosphoryl transfer from substrate back to His. The C3 phosphate of 2,3-BPG is now transferred to the histidine nitrogen. Draw the arrow from the histidine nitrogen to the C3 phosphorus, and from the C3 P–O bond to the oxygen (C3 oxygen departs). The product is 2-PG and regenerated phospho-histidine.

Why catalytic 2,3-BPG is required: The enzyme needs the histidine to be phosphorylated to start the cycle. Catalytic 2,3-BPG serves as the initial phosphoryl donor that phosphorylates the histidine during enzyme activation. Without it, the histidine would never be phosphorylated, and the enzyme would be inactive.

Mechanism Type 10: Transesterification (Acetyl-Lipoamide → Acetyl-CoA)

Setup Protocol

This is a straightforward thioester exchange. The acetyl group moves from one sulfur (lipoamide) to another (CoA).

Step-by-Step Construction

Step 1 — CoA thiol attacks thioester. A general base deprotonates the CoA thiol (CoA–SH → CoA–S^-). The CoA thiolate attacks the carbonyl carbon of acetyl-lipoamide (draw arrow from CoA sulfur to the carbonyl carbon). The C=O π electrons are pushed onto oxygen (tetrahedral intermediate).

Step 2 — Lipoamide thiolate departs. The C–S bond to lipoamide sulfur breaks (draw arrow from C–S bond to lipoamide sulfur). The tetrahedral intermediate collapses back to a carbonyl. The product is acetyl-CoA and dihydrolipoamide (with the free thiol on the sulfur that was bonded to the acetyl group, plus the other thiol that was already free).

Mechanism Type 11: Enolase (Dehydration of 2-PG to PEP)

Setup Protocol

This reaction removes water from 2-PG. It is not redox. No cofactor is consumed (Mg^{2+} stabilizes the enolate intermediate but is not consumed).

Step-by-Step Construction

Step 1 — Proton abstraction. A general base (B:) removes the proton from C2 of 2-PG (the carbon bearing the phosphate ester and the hydroxyl). Draw the curved arrow from the C2–H bond to the base. The electrons delocalize into the C1 carboxylate, forming a stabilized carbanion (enolate at C2–C1).

Step 2 — Hydroxide departure. The C3–OH bond breaks. A general acid (H-A) protonates the departing hydroxide (draw arrow from H-A to the oxygen). The electrons from the C–O bond form the C2=C3 double bond of the enol. Water departs. The product is PEP, with the phosphate still esterified at C2.

General Rules for All Mechanisms

Always draw general acids and bases. Every protonation event must show a general acid (H-B^+ or H-A) donating the proton, with a curved arrow from the H–A bond to the atom being protonated. Every deprotonation must show a general base (B: or A^-) accepting the proton, with a curved arrow from the bond being broken to the base. The guide explicitly states that complete reasoning requires each of these steps.

Curved arrows always flow from electron-rich to electron-poor. The tail of the arrow starts at the source of electrons (a lone pair, a bond) and the head points to the destination (an electrophilic atom, or to an atom that will bear the electrons as a lone pair).

Show all intermediates. Do not jump from substrate to product in one step if multiple bond-making and bond-breaking events occur. Each elementary step should show one or two bond changes (one bond breaking and one forming is standard for most steps).

Regenerate the catalyst. For TPP mechanisms, the last step must show TPP being released in its ylide (or protonated) form, ready for another cycle. For Schiff base mechanisms, the last step must show the free lysine being regenerated. For FAD mechanisms, FAD must be regenerated from $FADH^-$.

Check oxidation states. After drawing the mechanism, verify that any oxidation or reduction you claimed is actually reflected in the electron flow. If NAD^+ appears, confirm that a hydride was transferred to it. If a thioester was formed, confirm that the substrate carbon went from the aldehyde oxidation state to the carboxylic acid oxidation state.

Label everything. Write "TPP ylide," "Schiff base," "thioester intermediate," "enolate," "carbanion," "$FADH^-$," etc. at each stage. This makes the electron flow unambiguous and easier to verify.

End of Analytical Decision Framework