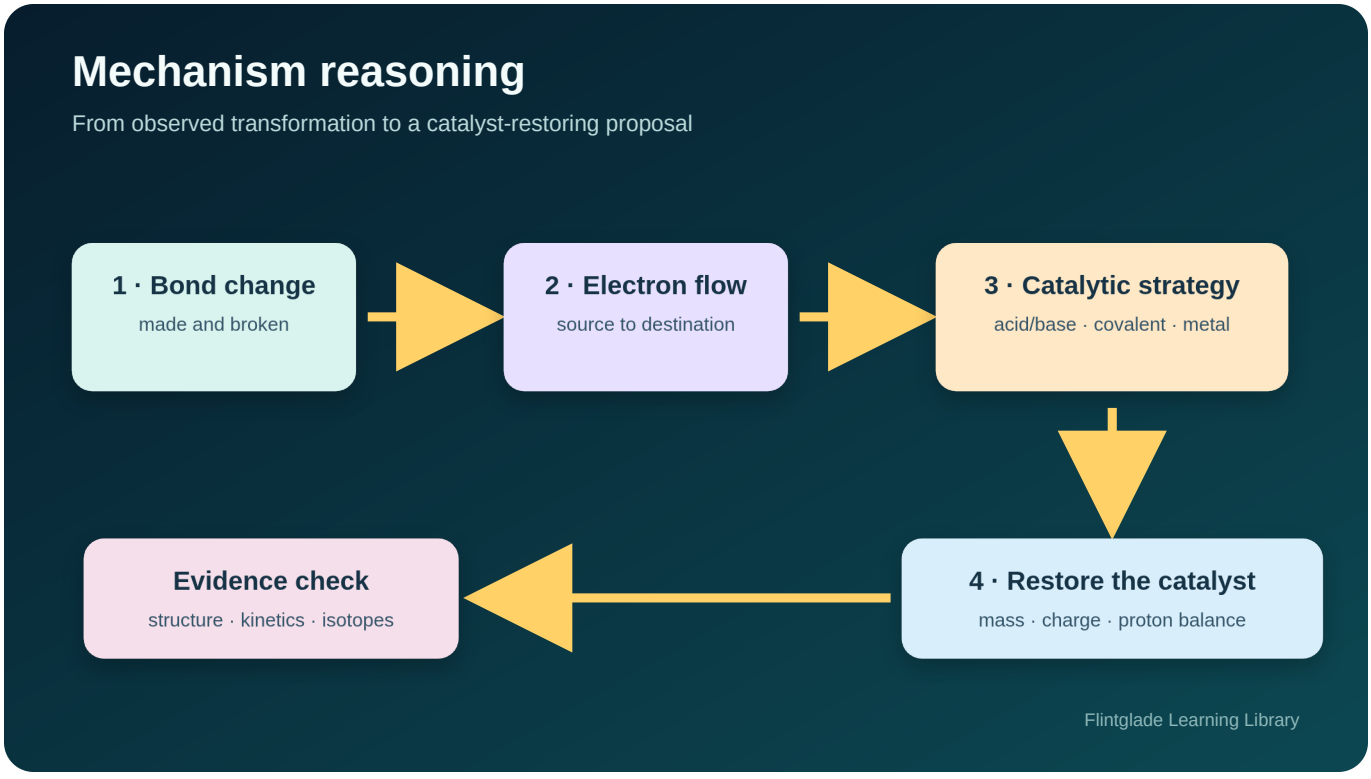
Metabolic Chemistry: Reaction Types and Carriers

Flintglade Learning Library · General Biochemistry

Audience: Intermediate learners connecting recurring organic transformations with metabolic carrier systems.

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 common metabolic transformations
- Recognize how activated carriers channel reactivity
- Use thermodynamic and mechanistic evidence together

Navigate this guide

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1. Oxidation State of Carbon and Fuel Value

The energy available from a carbon-containing molecule is directly related to how reduced its carbon atoms are. Carbon has a range of oxidation states from −4 (methane, CH_4) to +4 (CO_2). The more reduced (more hydrogenated) a carbon is, the more electrons it can donate through oxidation, and thus the more free energy can be extracted.

Oxidation state ladder (most reduced → most oxidized):

Molecule	Oxidation State of C	ΔG of combustion (approx.)
CH_4 (methane)	−4	−890 kJ/mol

Molecule	Oxidation State of C	ΔG of combustion (approx.)
CH_3OH (methanol)	−2	−727 kJ/mol
HCHO (formaldehyde)	0	−571 kJ/mol
HCOOH (formic acid)	+2	−255 kJ/mol
CO_2	+4	0 (fully oxidized)

Practical consequence: Fats are better fuels than carbohydrates on a per-gram basis because fatty acid carbons are overwhelmingly in the −2 state (–CH_2–), while sugar carbons average around 0 (each carbon carries roughly one oxygen). This is why fats yield ~9 kcal/g versus ~4 kcal/g for carbohydrates.

How to assign oxidation state to a carbon:

- Each C–H bond counts as −1.
- Each C–C bond counts as 0.
- Each C–O (or C–N, C–S) bond counts as +1.
- Each C=O bond counts as +2.

Sum these for any given carbon to obtain its formal oxidation state.

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2. General Principles of Metabolism

Metabolism is the totality of enzyme-catalyzed chemical reactions in a living organism. Several core principles govern its logic:

a) Catabolism vs. Anabolism: Catabolism is the degradative, oxidative arm — large molecules are broken into smaller ones, releasing free energy that is captured in ATP and reduced coenzymes (NADH, $FADH_2$). Anabolism is the biosynthetic, reductive arm — small precursors are assembled into complex macromolecules, consuming ATP and reducing equivalents (NADPH).

b) Metabolic pathways are irreversible in vivo: Even though most individual steps may be near equilibrium, every pathway contains at least one thermodynamically irreversible step (large negative ΔG) that commits flux in one direction. This makes the overall pathway irreversible.

c) Convergence and divergence: Catabolism is convergent — many different fuels funnel into a few common intermediates (pyruvate, acetyl-CoA, TCA cycle intermediates). Anabolism is divergent — a few building blocks are elaborated into thousands of distinct products.

d) Energy coupling: Thermodynamically unfavorable reactions are driven forward by coupling to favorable ones, most commonly ATP hydrolysis.

e) Regulation occurs at committed steps: Flux through a pathway is controlled at irreversible, rate-limiting enzymes (often the first committed step) via allosteric regulation, covalent modification, or changes in enzyme concentration.

f) Compartmentalization: Eukaryotic cells segregate opposing pathways into different compartments (e.g., fatty acid oxidation in the mitochondrial matrix vs. fatty acid synthesis in the cytosol), preventing futile cycling.

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3. Recurring Motifs in Metabolic Pathways

Despite the diversity of metabolic transformations, a small set of chemical motifs appears again and again:

a) Phosphoryl transfers: ATP donates a phosphoryl group to activate substrates or to drive reactions. Kinases transfer phosphoryl groups to substrates; phosphatases remove them.

b) Oxidation–reduction: Electrons are shuttled by carrier coenzymes (NAD^+/NADH, FAD/$FADH_2$, $NADP^+$/NADPH). Dehydrogenases catalyze these transfers.

c) Carbonyl chemistry (C–C bond making and breaking): Formation and cleavage of C–C bonds almost always proceed through one of two nucleophilic mechanisms: carbanion/enolate chemistry or Schiff base/TPP-mediated chemistry.

d) Acyl-group transfers: Thioester intermediates (e.g., acetyl-CoA, acyl-ACP) activate acyl groups for transfer.

e) Isomerizations: Intramolecular rearrangements (e.g., aldo–keto isomerization of glucose-6-phosphate to fructose-6-phosphate) reposition functional groups.

f) Eliminations and additions: Dehydrations create double bonds; hydrations add water across them.

Recognizing these motifs lets you predict mechanism even for unfamiliar enzymes.

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4. Recognizing Oxidation–Reduction Reactions in Metabolism

An oxidation–reduction (redox) reaction involves the transfer of electrons from one species to another. In organic/biological chemistry, this often manifests as the transfer of hydrogen atoms ($H = H^+ + e^-$).

Rules for spotting a redox reaction in metabolism:

- If a substrate gains C–H bonds (becomes more reduced), the reaction is a **reduction** of that substrate (and something else was oxidized).
- If a substrate loses C–H bonds or gains C–O bonds, the reaction is an **oxidation** of that substrate.

- If NAD^+, FAD, or $NADP^+$ appear as a cosubstrate and get converted to their reduced forms (NADH, $FADH_2$, NADPH), the other substrate was **oxidized**.
- If NADH, $FADH_2$, or NADPH appear and get converted to their oxidized forms, the other substrate was **reduced**.

Classic examples:

Reaction	Type
Lactate + NAD^+ → Pyruvate + NADH + H^+	Oxidation of lactate (alcohol → ketone)
Pyruvate + NADH + H^+ → Lactate + NAD^+	Reduction of pyruvate (ketone → alcohol)
Succinate + FAD → Fumarate + $FADH_2$	Oxidation (single bond → double bond)
Acetaldehyde + NADH → Ethanol + NAD^+	Reduction (aldehyde → alcohol)

Formal check: Count the oxidation state of the reactive carbon before and after. If it increases, oxidation occurred; if it decreases, reduction occurred.

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5. ΔG vs. ΔG°′ and Their Relationship

This is one of the most critical quantitative relationships in bioenergetics.

ΔG°′ (standard free energy change): The free energy change measured under *standard biochemical conditions*: all reactants and products at 1 M concentration, pH 7.0, 25 °C, 1 atm pressure. This is a constant for any given reaction — a property of the reaction itself.

ΔG (actual free energy change): The free energy change under the *actual cellular conditions*, where concentrations are almost never 1 M. This is what determines whether a reaction actually proceeds spontaneously in vivo.

The master equation connecting them:

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

where:

$$Q = \frac{[\text{Products}]}{[\text{Reactants}]}$$

- R = 8.314 J/(mol·K)
- T = temperature in Kelvin (310 K for physiological)
- Q = mass action ratio (actual concentration ratio)

Key implications:

1. Even if ΔG°′ is positive (unfavorable), the reaction can be driven forward (ΔG < 0) if the cell maintains [Products]/[Reactants] ≪ 1 — i.e., products are rapidly consumed.

2. Even if ΔG°′ is very negative, the reaction can stall if products accumulate such that Q becomes very large.

3. At equilibrium, ΔG = 0, and Q = K'eq, so:

$$\Delta G^{\circ\prime} = -RT \ln K'_{eq}$$

Worked example: For the reaction A → B, suppose ΔG°′ = +13.8 kJ/mol.

At equilibrium: K'eq = e^(−13800/(8.314 × 298)) ≈ 0.0038, meaning [B]/[A] = 0.0038 at equilibrium.

But suppose in the cell [A] = 10^{-2} M and [B] = 10^{-5} M:

ΔG = 13,800 + (8.314)(298) ln(10^{-5}/10^{-2}) ΔG = 13,800 + (2479)(−6.91) ΔG = 13,800 − 17,130 = −3,330 J/mol = **−3.3 kJ/mol**

The reaction proceeds spontaneously despite a positive ΔG°′ because the cell holds [B] far below its equilibrium value.

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6. Coupled Reactions

When two reactions are coupled, their free energies are additive. This allows a thermodynamically unfavorable reaction (positive ΔG) to be driven forward by pairing it with a highly favorable reaction (large negative ΔG), provided they share a common intermediate.

Example — Glutamine synthesis:

1. Glutamate + NH_3 → Glutamine + H_2O — ΔG°′ = +14.2 kJ/mol (unfavorable alone)
2. ATP → ADP + P_i — ΔG°′ = −30.5 kJ/mol

The coupled mechanism:

- Step A: Glutamate + ATP → γ-Glutamyl phosphate + ADP (phosphorylated intermediate)
- Step B: γ-Glutamyl phosphate + NH_3 → Glutamine + P_i

Overall: Glutamate + ATP + NH_3 → Glutamine + ADP + P_i — ΔG°′ = −16.3 kJ/mol

The key insight: the two reactions are not merely happening simultaneously in solution — they are mechanistically linked through a shared covalent intermediate (γ-glutamyl phosphate) on the enzyme's active site. Without the enzyme physically coupling them, the ATP hydrolysis would simply release heat without driving the synthesis.

General principle: For coupling to work, the sum of ΔG values must be negative, AND the reactions must share a common intermediate (often a phosphorylated or adenylated species).

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7. How ATP Powers Otherwise Unfavorable Reactions

ATP hydrolysis is highly exergonic under both standard and physiological conditions:

- $\Delta G^{\circ\prime} = -30.5$ kJ/mol
- ΔG (in vivo) ≈ −50 to −54 kJ/mol (because the cell maintains [ATP]/[ADP][P_i] far from equilibrium)

ATP drives unfavorable reactions by one of three mechanisms:

a) Phosphoryl group transfer: ATP phosphorylates the substrate, creating a high-energy phosphorylated intermediate that is more reactive. The unfavorable step then occurs spontaneously on this activated intermediate. Example: glucose + ATP → glucose-6-phosphate + ADP (hexokinase), which traps glucose in the cell.

b) Pyrophosphate cleavage (adenylation): ATP is cleaved to AMP + PP_i, and the AMP moiety is transferred to the substrate, forming an adenylated intermediate. The subsequent release of PP_i and its hydrolysis by inorganic pyrophosphatase ($PP_i \rightarrow 2\ P_i$, $\Delta G^{\circ\prime} = -33.5$ kJ/mol) makes the overall process essentially irreversible. This is equivalent to spending 2 ATP equivalents. Example: amino acid activation in translation (aminoacyl-tRNA synthetases).

c) Conformational coupling: ATP binding and hydrolysis drive conformational changes in proteins (motor proteins, transporters), enabling mechanical work or active transport against concentration gradients. Example: Na^+/K^+-ATPase, myosin, chaperonins.

Why ATP, specifically? ATP is kinetically stable (high activation energy for non-enzymatic hydrolysis) but thermodynamically unstable — this means it doesn't spontaneously hydrolyze in solution at appreciable rates, but enzymes can tap its energy on demand. The cell's ATP/ADP ratio is maintained far from equilibrium (~10^4 to 10^5 in some estimates when you include [P_i]), making the actual free energy of hydrolysis even more negative than the standard value.

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8. Phosphoryl-Transfer Potential

The phosphoryl-transfer potential of a compound is a measure of its tendency to transfer a phosphoryl group to water (i.e., to be hydrolyzed). It is quantified by the standard free energy of hydrolysis, $\Delta G^{\circ\prime}$ of hydrolysis.

More negative $\Delta G^{\circ\prime}$ of hydrolysis = higher phosphoryl-transfer potential.

A compound with a higher phosphoryl-transfer potential can transfer its phosphoryl group to a compound with a lower one. This establishes a thermodynamic hierarchy.

Why are some phosphorylated compounds "high energy"?

The large negative $\Delta G^{\circ\prime}$ of hydrolysis arises from one or more of these factors:

1. **Charge repulsion (electrostatic strain):** Multiple negative charges are forced into close proximity on the phosphorylated compound (e.g., in ATP, three negatively charged phosphoryl groups are packed together). Hydrolysis relieves this strain.
2. **Resonance stabilization of products:** The products of hydrolysis (e.g., P_i, carboxylate) enjoy greater resonance stabilization than the reactant. For example, in acyl phosphates, the phosphoryl group competes with the carbonyl for the lone pair on the bridging oxygen; hydrolysis frees both groups to resonate independently.
3. **Solvation effects:** The hydrolysis products are better solvated (stabilized by water) than the intact compound.
4. **Tautomerization:** In some cases (e.g., PEP), the product can tautomerize to a much more stable form (enol → keto tautomerization of pyruvate).

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9. Molecules with High Phosphoryl-Transfer Potential

The following table ranks biologically important phosphorylated compounds by their standard free energy of hydrolysis:

Compound	$\Delta G^{\circ\prime}$ of hydrolysis (kJ/mol)	Key driving force
Phosphoenolpyruvate (PEP)	−61.9	Enol → keto tautomerization of pyruvate
1,3-Bisphosphoglycerate (1,3-BPG)	−49.4	Resonance stabilization (acyl phosphate)
Acetyl phosphate	−43.1	Resonance stabilization
Phosphocreatine	−43.1	Resonance stabilization (guanidinium)
Pyrophosphate (PP_i)	−33.5	Charge relief, solvation
ATP → ADP + P_i	**−30.5**	Charge relief, resonance, solvation
ATP → AMP + PP_i	**−45.6**	(includes PP_i hydrolysis: −32.2 + −33.5)
Glucose-1-phosphate	−20.9	—
Glucose-6-phosphate	−13.8	Simple ester
Glycerol-3-phosphate	−9.2	Simple ester

ATP sits in the middle of this hierarchy. This is profoundly important — it means ATP can accept phosphoryl groups from compounds above it (PEP, 1,3-BPG, phosphocreatine) and donate phosphoryl groups to compounds below it (glucose, glycerol). This intermediate position makes ATP the universal phosphoryl-group shuttle of the cell.

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10. Molecules That Function as Energy Currency

Several molecules serve as mobile carriers of free energy in the cell, not just ATP:

Primary energy currencies:

- **ATP (adenosine triphosphate):** The dominant energy currency. Carries energy in its phosphoanhydride bonds. Used for mechanical work, transport, biosynthesis.
- **GTP (guanosine triphosphate):** Energetically equivalent to ATP ($\Delta G^{\circ\prime} \approx -30.5$ kJ/mol). Used specifically in signaling (G proteins), translation (elongation factors), and specific biosynthetic reactions (gluconeogenesis — PEP carboxykinase).
- **NADH and $FADH_2$:** Carry high-energy electrons harvested from fuel oxidation to the electron transport chain, where their reoxidation drives ATP synthesis via oxidative phosphorylation.
- **NADPH:** Carries reducing equivalents specifically for anabolic (biosynthetic) reductions — fatty acid synthesis, cholesterol synthesis, nucleotide synthesis, glutathione reduction.
- **Acetyl-CoA:** An "activated" two-carbon unit. The thioester bond to CoA is a high-energy linkage ($\Delta G^{\circ\prime}$ of hydrolysis ≈ -31.4 kJ/mol). Feeds into the TCA cycle or fatty acid synthesis.
- **Phosphocreatine:** A high-energy phosphagen that buffers ATP levels in tissues with high, fluctuating energy demands (skeletal muscle, brain). Creatine kinase rapidly regenerates ATP from ADP + phosphocreatine.

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11. How Certain Reactions Control Flux Through a Pathway

Metabolic flux is controlled at reactions that are far from equilibrium in the cell (large negative ΔG, essentially irreversible). These are the regulatory nodes of the pathway.

Characteristics of flux-controlling reactions:

1. **Large negative ΔG in vivo** (not near equilibrium) — these steps are committed and irreversible under physiological conditions.
2. **Catalyzed by regulated enzymes** — subject to allosteric control, covalent modification (phosphorylation/dephosphorylation), or transcriptional regulation.
3. **Often the first committed step** of a pathway — controlling entry prevents wasteful accumulation of intermediates.

Mechanisms of regulation at these steps:

- **Allosteric regulation:** End products inhibit the committed step (feedback inhibition). Pathway activators (often substrates or indicators of energy charge) stimulate the enzyme. Example: PFK-1 is inhibited by ATP and citrate (energy-replete signals) and activated by AMP and fructose-2,6-

bisphosphate (energy-depleted signals).

- **Covalent modification:** Kinases and phosphatases toggle enzymes between active and inactive states. Example: glycogen phosphorylase is activated by phosphorylation (phosphorylase a) and inactivated by dephosphorylation (phosphorylase b).
- **Substrate availability:** The actual concentrations of substrates and products determine Q and thus ΔG, modulating flow even at near-equilibrium steps.
- **Enzyme concentration:** Transcriptional induction/repression changes the amount of enzyme over hours to days.

Near-equilibrium reactions (ΔG ≈ 0) do NOT control flux — they simply equilibrate rapidly in response to changes imposed by the regulated steps. Their flux is determined by the supply of substrate, which is set by the upstream irreversible steps.

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12. Six General Types of Biochemical Reactions

Virtually all metabolic reactions fall into one of six categories:

Type 1: Oxidation–Reduction

Transfer of electrons, often as hydride ions (H^-) or hydrogen atoms. Coenzymes: NAD^+, $NADP^+$, FAD, FMN.

Example: Malate + NAD^+ → Oxaloacetate + NADH + H^+ (malate dehydrogenase)

Type 2: Group Transfer

A functional group is transferred from one molecule to another. Subcategories include phosphoryl transfer (kinases), acyl transfer (acyltransferases), and glycosyl transfer (glycosyltransferases).

Example: Glucose + ATP → Glucose-6-phosphate + ADP (hexokinase — phosphoryl group transfer)

Type 3: Hydrolysis

Cleavage of a bond by addition of water. This is formally a special case of group transfer (transfer to water) but distinguished because of its prevalence and thermodynamic importance.

Example: ATP + H_2O → ADP + P_i (ATPase) **Example:** Peptide bond hydrolysis by proteases.

Type 4: Carbon–Carbon Bond Formation or Cleavage

Making or breaking C–C bonds. These reactions typically involve carbanion (enolate) intermediates or coenzyme-stabilized carbanion equivalents (TPP, biotin, CoA).

Example (formation): Citrate synthase — Acetyl-CoA + Oxaloacetate → Citrate **Example (cleavage):** Aldolase — Fructose-1,6-bisphosphate → DHAP + G3P

Type 5: Isomerization

Intramolecular rearrangement. Includes epimerizations, cis-trans isomerizations, and intramolecular group transfers (mutases).

Example: Glucose-6-phosphate ⇌ Fructose-6-phosphate (phosphoglucose isomerase — aldo-keto isomerization) **Example:** Methylmalonyl-CoA → Succinyl-CoA (methylmalonyl-CoA mutase — carbon skeleton rearrangement)

Type 6: Ligation Coupled to ATP Hydrolysis

Formation of a covalent bond (C–C, C–O, C–N, C–S) that is driven by the simultaneous cleavage of ATP (or another NTP). These are the ligases/synthetases.

Example: Pyruvate + CO_2 + ATP → Oxaloacetate + ADP + P_i (pyruvate carboxylase — C–C bond ligation) **Example:** Amino acid + tRNA + ATP → Aminoacyl-tRNA + AMP + PP_i (aminoacyl-tRNA synthetase — C–O ligation)

Classification heuristic: Ask: (1) Did electrons move? → Redox. (2) Did a group transfer to another molecule? → Group transfer. (3) Did a group transfer to water? → Hydrolysis. (4) Was a C–C bond made or broken without ATP? → C–C bond formation/cleavage. (5) Did the molecular formula stay the same but the structure change? → Isomerization. (6) Was a new bond made AND ATP consumed? → Ligation coupled to ATP.

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13. Activated Carriers in Metabolism

"Activated carriers" are coenzymes that carry specific chemical groups in an activated (high-energy) form, ready for transfer to substrates. They are the workhorses of group-transfer biochemistry.

NAD^+ and $NADP^+$ — Electron (Hydride) Carriers

What they carry: A hydride ion ($H^- = 2e^- + H^+$).

Chemistry: The nicotinamide ring accepts a hydride at the C4 position, converting from the aromatic (oxidized) form to the non-aromatic (reduced) form. This is a 2-electron reduction.

$NAD^+ + 2H \rightarrow NADH + H^+$

Division of labor:

- **NADH** feeds electrons into the electron transport chain (catabolism — energy generation).
- **NADPH** donates electrons for reductive biosynthesis (anabolism). The extra phosphate on the 2′-OH of the adenosine ribose allows enzymes to distinguish between the two pools.

FAD (and FMN) — Electron Carriers

What they carry: One or two electrons (can do single-electron transfers, unlike NAD^+).

Chemistry: The isoalloxazine ring system can accept electrons in two sequential one-electron steps (FAD → FADH• → $FADH_2$) or as a net two-electron transfer. This makes flavins uniquely suited to interface between obligate two-electron donors and one-electron acceptors (e.g., in Complex II of the ETC).

FAD is typically tightly bound or covalently attached to its enzyme (it is a prosthetic group, not a freely diffusing cosubstrate like NAD^+).

TPP (Thiamine Pyrophosphate) — Activated Aldehyde Carrier

What it carries: An "activated aldehyde" — a two-carbon (hydroxyethyl) unit stabilized as a carbanion on the thiazolium ring.

Chemistry: The C2 carbon of the thiazolium ring is acidic (pK_a ~17–19 in enzyme active sites, effectively much lower due to enzyme stabilization). It is deprotonated to generate a nucleophilic ylide/carbene. This carbanion attacks the carbonyl carbon of an α-keto acid, leading to decarboxylation and formation of a resonance-stabilized carbanion intermediate (the "active aldehyde"). This intermediate can then:

1. Be protonated and released as an aldehyde (simple decarboxylation — pyruvate decarboxylase).
2. Be oxidized and transferred to CoA (oxidative decarboxylation — pyruvate dehydrogenase).
3. Attack another carbonyl to form a new C–C bond (transketolase).

Key reactions requiring TPP:

- Pyruvate dehydrogenase (pyruvate → acetyl-CoA)
- α-Ketoglutarate dehydrogenase (α-KG → succinyl-CoA)
- Branched-chain α-keto acid dehydrogenase
- Transketolase (pentose phosphate pathway)
- Pyruvate decarboxylase (in yeast/ethanol fermentation)

Biotin — CO_2 (Carboxyl Group) Carrier

What it carries: An activated CO_2 moiety, covalently linked as N-carboxybiotin.

Chemistry: Biotin is covalently attached to a lysine residue of its enzyme via an amide bond (forming biocytin), creating a long flexible tether that can swing between two active sites. In a two-step mechanism: (1) ATP-dependent carboxylation of biotin by bicarbonate; (2) transfer of the $–COO^-$ group from carboxybiotin to the substrate.

Key reactions requiring biotin:

- Pyruvate carboxylase (pyruvate → oxaloacetate)
- Acetyl-CoA carboxylase (acetyl-CoA → malonyl-CoA — committed step of fatty acid synthesis)
- Propionyl-CoA carboxylase

Acetyl-CoA (Coenzyme A) — Acyl Group Carrier

What it carries: Acyl groups, most commonly the acetyl (2-carbon) group.

Chemistry: The thioester bond between the acyl group and the sulfhydryl of CoA is a high-energy linkage ($\Delta G^{\circ\prime}$ hydrolysis ≈ −31.4 kJ/mol). This is because the thioester does not enjoy the resonance stabilization that oxygen esters do — the larger, less electronegative sulfur atom overlaps poorly with the carbonyl π system. The result: the carbonyl carbon remains highly electrophilic, and the α-carbons are more acidic, making the acyl group "activated" for both:

- **Acyl transfer:** nucleophilic acyl substitution (e.g., citrate synthase)
- **Condensation:** α-carbon deprotonation to form an enolate, followed by C–C bond formation (e.g., Claisen condensation in fatty acid synthesis)

Lipoamide — Acyl Group and Electron Carrier

What it carries: Both acyl groups and electrons — it is a dual-function carrier.

Chemistry: Lipoamide is the amide of lipoic acid attached to a lysine residue (forming lipoyllysine), creating a long swinging arm (~14 Å). The dithiolane ring undergoes reversible oxidation/reduction:

- **Oxidized form:** Cyclic disulfide (S–S).
- **Reduced form:** Dihydrolipoamide (two free –SH groups).

In the pyruvate dehydrogenase complex:

1. The hydroxyethyl group from TPP is transferred to oxidized lipoamide, simultaneously oxidizing it to an acetyl group and reducing the disulfide to two thiols → acetyl-dihydrolipoamide.
2. The acetyl group is then transferred to CoA.
3. Dihydrolipoamide is reoxidized by dihydrolipoamide dehydrogenase (E3), which passes electrons to FAD → NADH.

Lipoamide thus serves as the critical relay between TPP-mediated decarboxylation and CoA-mediated acyl transfer.

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14. Carbon–Carbon Bond Formation and Cleavage in Metabolism

C–C bond-making and breaking reactions are the essence of constructive and degradative metabolism. They are mechanistically demanding because carbon is not intrinsically a good nucleophile or electrophile in most contexts.

General principle: A C–C bond is formed when a nucleophilic carbon attacks an electrophilic carbon. The challenge is generating a carbon nucleophile (carbanion equivalent). Biology uses three main strategies:

Strategy 1: Enolate/Enol Chemistry

An α-carbon adjacent to a carbonyl is deprotonated (or tautomerizes to the enol form), generating a nucleophilic carbon that attacks an electrophilic carbonyl carbon (aldol condensation, Claisen condensation).

Examples:

- **Aldol condensation:** Aldolase (FBP → DHAP + G3P, reverse = aldol addition)
- **Claisen condensation:** Citrate synthase (acetyl-CoA enolate attacks OAA carbonyl)
- **Retro-Claisen:** Thiolase (cleavage of acetoacetyl-CoA → 2 acetyl-CoA)

Strategy 2: TPP-Stabilized Carbanions

TPP generates a nucleophilic carbanion equivalent from α-keto acids via decarboxylation (see Section 13).

Strategy 3: Biotin-Mediated Carboxylation

CO_2 (electrophilic carbon) is added to an enolate or carbanion substrate via carboxybiotin.

How to recognize C–C formation/cleavage in a reaction: Count the carbons in reactants and products. If the carbon count of any single molecule increases, a C–C bond was formed. If a molecule fragments into two carbon-containing pieces, a C–C bond was cleaved.

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15. Metabolic Reactions Requiring TPP

TPP is required whenever a metabolic reaction involves the cleavage of a bond adjacent to a carbonyl of an α-keto acid (the C–C bond between the carbonyl carbon and the carboxyl group). This results in decarboxylation and formation of the TPP-bound "active aldehyde."

Complete list of major TPP-dependent reactions:

Enzyme	Reaction	Pathway
Pyruvate dehydrogenase (E1)	Pyruvate → Acetyl-CoA + CO_2	Pyruvate → TCA link
α-Ketoglutarate dehydrogenase (E1)	α-Ketoglutarate → Succinyl-CoA + CO_2	TCA cycle
Branched-chain α-keto acid DH	Branched-chain keto acids → acyl-CoA + CO_2	BCAA catabolism
Pyruvate decarboxylase	Pyruvate → Acetaldehyde + CO_2	Ethanol fermentation
Transketolase	Transfers 2-C unit between sugar phosphates	Pentose phosphate pathway

Pattern recognition rule: If you see an α-keto acid being decarboxylated, TPP is involved.

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16. Proposing Mechanisms for C–C Bond Reactions Involving Enolates

The enolate mechanism is the dominant strategy for C–C bond chemistry in metabolism when TPP is not involved.

The General Enolate Mechanism

Step 1 — Enolization: A base (enzyme residue) abstracts the α-proton from a carbon adjacent to a carbonyl, generating a resonance-stabilized enolate (carbanion delocalized onto the carbonyl oxygen).

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 O O⁻
‖ |
–C–C–H → –C=C (enolate, nucleophilic at Cα)
|
(α-H)
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Step 2 — Nucleophilic attack: The enolate carbon attacks an electrophilic carbonyl carbon of a second substrate.

Step 3 — Protonation/elimination/hydrolysis to give the final product.

Aldol Condensation/Cleavage

Forward (condensation — C–C bond formation): An enolate (nucleophile) attacks the carbonyl carbon of an aldehyde (electrophile) to form a β-hydroxy carbonyl (aldol).

Reverse (retro-aldol — C–C bond cleavage): The aldol is cleaved by the reverse: the C–C bond breaks heterolytically, regenerating the enolate and the aldehyde.

Example — Aldolase (glycolysis):

Fructose-1,6-bisphosphate ⇌ DHAP + Glyceraldehyde-3-phosphate

Retro-aldol direction (glycolysis):

1. Schiff base forms between FBP ketone and active-site Lys (Class I aldolase).
2. C3–C4 bond breaks: G3P leaves as the aldehyde product.
3. Remaining enamine (stabilized carbanion equivalent) tautomerizes.
4. Schiff base hydrolyzes, releasing DHAP.

Claisen Condensation/Cleavage

Same principle, but the electrophile is a thioester carbonyl instead of an aldehyde. The product is a β-keto thioester.

Example — Citrate synthase:

1. Acetyl-CoA is deprotonated at the methyl carbon → enolate of acetyl-CoA.
2. This enolate (nucleophilic carbon) attacks the si face of the carbonyl of oxaloacetate.
3. The resulting citryl-CoA intermediate undergoes hydrolysis of the thioester bond, releasing CoA and driving the reaction irreversibly forward.

Framework for proposing any enolate-based C–C mechanism:

1. Identify the nucleophile (the enolizable carbon — look for an α-H next to a carbonyl or thioester).
2. Identify the electrophile (a carbonyl carbon).
3. Draw the enolization.
4. Draw the nucleophilic attack.
5. Show any subsequent steps (hydrolysis of thioester, protonation, etc.).

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17. Proposing Mechanisms for TPP-Catalyzed C–C Reactions

The General TPP Mechanism

Step 1 — Ylide formation: The C2–H of the thiazolium ring is deprotonated, generating the nucleophilic TPP ylide (carbene).

```
N⁺–C: (ylide/carbene at C2)
| ‖
S
```

Step 2 — Nucleophilic attack on substrate: The ylide C2 attacks the carbonyl carbon of the α-keto acid substrate.

Step 3 — Decarboxylation: The C–C bond between the carbonyl and the carboxylate breaks, releasing CO_2. The resulting carbanion is stabilized by delocalization into the thiazolium ring (the positively charged nitrogen acts as an electron sink). This is the "active aldehyde" intermediate (also called the hydroxyethyl-TPP or α-carbanion/enamine).

```
N⁺–C–C(OH)R → electrons delocalize into the ring
| |
S
```

Step 4 — Fate of the active aldehyde: This is where different TPP enzymes diverge:

a) Simple decarboxylation (pyruvate decarboxylase): The carbanion is protonated, and the product aldehyde is released from TPP.

- Pyruvate → Acetaldehyde + CO_2

b) Oxidative decarboxylation (pyruvate dehydrogenase complex): The hydroxyethyl-TPP carbanion attacks the disulfide of lipoamide, transferring the two-carbon unit as an acetyl group to dihydrolipoamide, and regenerating TPP.

- Pyruvate → [hydroxyethyl-TPP] → [acetyl-dihydrolipoamide] → Acetyl-CoA

c) C–C bond formation (transketolase): The carbanion attacks the carbonyl of a new aldehyde acceptor substrate, forming a new C–C bond and transferring the 2-carbon (glycolaldehyde) unit.

- Xylulose-5-P → [glycolaldehyde-TPP] + G3P → then attack on ribose-5-P → sedoheptulose-7-P

Framework for Proposing Any TPP Mechanism

1. **Form the ylide** (deprotonation of C2).
2. **Attack the α-keto acid** (nucleophilic addition of C2 to the substrate carbonyl).
3. **Decarboxylate** (loss of CO_2, stabilized carbanion on TPP).
4. **Determine the fate of the carbanion:**

- Protonation → free aldehyde (decarboxylase)
- Transfer to lipoamide → acyl group → CoA (oxidative decarboxylation)
- Attack new carbonyl → new C–C bond (transferase)

5. **Regenerate TPP.**

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18. Summary Table — Activated Carriers at a Glance

Carrier	Group Transferred	Key Bond Type	Vitaminic Precursor
$NAD^+/NADH$	Hydride ($H^- = 2e^- + H^+$)	C–H	Niacin (B_3)
$NADP^+/NADPH$	Hydride (H^-, for biosynthesis)	C–H	Niacin (B_3)
$FAD/FADH_2$	Electrons (1 or 2 at a time)	N–H	Riboflavin (B_2)
TPP	Activated aldehyde (2C unit)	C–C	Thiamine (B_1)
Biotin	CO_2	N–C	Biotin (B_7)
Coenzyme A	Acyl groups	Thioester (C–S)	Pantothenate (B_5)
Lipoamide	Acyl groups + electrons	Disulfide (S–S)	Lipoic acid

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19. Quick-Reference: Reaction Classification Decision Tree

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Given a metabolic reaction, classify it:

1. Do electrons (H⁻, H atoms) transfer between molecules?
→ YES → Oxidation-Reduction
→ NO ↓

2. Does a chemical group transfer from one molecule to another?
→ YES → Is the acceptor water?
→ YES → Hydrolysis
→ NO → Group Transfer
→ NO ↓

3. Is a C–C bond formed or broken?
→ YES → Is NTP (ATP/GTP) consumed to drive it?
→ YES → Ligation coupled to ATP hydrolysis
→ NO → C–C bond formation/cleavage
→ NO ↓

4. Does the molecular formula stay the same but the structure change?
→ YES → Isomerization
→ NO → Reconsider; the reaction may combine types.
```

This document covers the learning goals for this topic with mechanistic depth. To build fluency, practice proposing enolate and TPP mechanisms on novel substrates — the transferable framework matters more than memorizing specific examples.