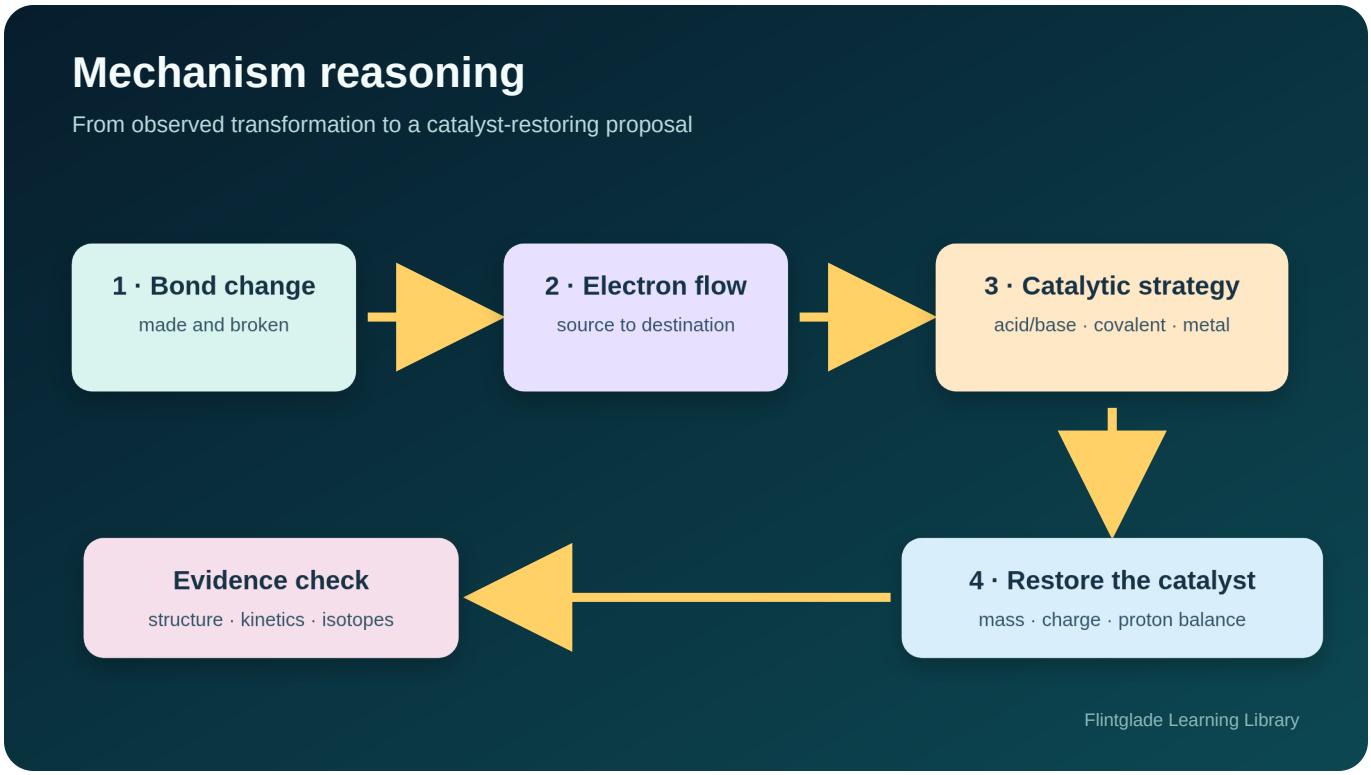
# Reading Redox Reactions and Cofactors

*Flintglade Learning Library · General Biochemistry*

**Audience:** Learners who want to identify oxidation, reduction, and carrier roles from molecular structures.

**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

- Track oxidation-state changes in biochemical structures
- Distinguish hydride, hydrogen-atom, and single-electron transfer
- Choose plausible carrier families while respecting context

## Navigate this guide



## What This Analysis Is Asking

Unfamiliar structures can look intimidating. The goal is to **read a reaction** and identify:

1. What *type* of chemical transformation occurred
2. Whether electrons moved (redox or not)
3. What cofactor would be needed to do that job

These are three separate skills, and each has a learnable, repeatable logic.

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## PART 1: Is It Redox? — The Carbon Oxidation State Method

This is the central skill for reaction analysis. Forget about memorizing which reactions are redox. Instead, **learn to see oxidation state changes by inspection.**

### The Oxidation State Ladder for Carbon

Carbon's oxidation state in organic molecules follows a simple ladder based on what's bonded to it. Each rung represents a two-electron change:

```
 MOST REDUCED MOST OXIDIZED
Alkane → Alcohol/Amine → Aldehyde/Ketone → Carboxylic Acid → CO₂
(C–H) (C–OH, C–NH₂) (C=O with H/C) (C=O with OH/O⁻) (O=C=O)
-4 → -2 to 0 → 0 to +1 → +2 to +3 → +4
```

**You don't need to calculate exact oxidation numbers.** Instead, use this shortcut:

## The Bond-Counting Shortcut

For any carbon atom, count its bonds to electronegative atoms (O, N, S) vs. bonds to H:

- **More bonds to H** = more reduced
- **More bonds to O/N/S** = more oxidized
- **Each C–H → C–O swap** = a 2-electron oxidation
- **Each C=O appearing where C–H was** = oxidation

**Practical rule:** Look at the carbon(s) that changed. Did they gain bonds to oxygen (or lose bonds to hydrogen)? That's oxidation. Did they gain bonds to hydrogen (or lose bonds to oxygen)? That's reduction.

## When It's NOT Redox

If no carbon changes its bonding pattern with respect to H vs. O/N/S, it's not redox. This includes:

- **Decarboxylation alone** ($CO_2$ leaves, but the remaining carbon doesn't change oxidation state)
- **Hydration/dehydration** (adding/removing $H_2O$ across a double bond — the carbons that gain –OH also gain –H, so the net oxidation state doesn't change)
- **Thiolysis/thioester exchange** (CoA swapping — sulfur comes and goes, but carbons stay at the same oxidation level)
- **Isomerization** (nothing enters or leaves the molecule)
- **Phosphoryl transfer** (P–O bond changes, no carbon oxidation change)
- **Aldol cleavage/condensation** (C–C bond breaking/forming without oxidation state change)

## When It IS Redox

If a carbon atom ends up with **more bonds to O or fewer bonds to H** than it started with, oxidation occurred. The common patterns:

| Transformation | Redox? | Direction |
|---|---|---|
| R–$CH_2$–$CH_2$–R → R–CH=CH–R | YES | Oxidation (lost 2 H's, C=C formed) |
| C–OH → C=O | YES | Oxidation (alcohol → ketone/aldehyde) |
| C=O → COOH | YES | Oxidation |

| Transformation | Redox? | Direction |
|---|---|---|
| R–CH=CH–R → R–$CH_2$–$CH_2$–R | YES | Reduction |
| C=O → C–OH | YES | Reduction (ketone → alcohol) |
| $CO_2$ leaving (decarboxylation only) | NO | — (the carbon that leaves was already fully oxidized; the carbon left behind doesn't change) |
| $H_2O$ adding across C=C | NO | — (one C gains OH, the other gains H — net zero) |
| Thioester exchange (R–S–X → R–S–Y) | NO | — (sulfur substitution, no C oxidation change) |

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# PART 2: The Five Reaction Types You'll See Over and Over

Biochemistry reuses the same handful of organic reaction types across every pathway. Isoleucine degradation, fatty acid β-oxidation, citric acid cycle intermediates — they all draw from the same playbook. Learn the playbook, and every new pathway becomes a remix of familiar moves.

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## REACTION TYPE A: Oxidative Decarboxylation (α-Keto Acid → Acyl-CoA + $CO_2$)

**What's happening overall:** An α-keto acid loses $CO_2$ and gets oxidized, with the remaining fragment attached to CoA as a thioester.

**Why it matters:** This is a *multi-step* process catalyzed by a dehydrogenase complex. A worked example may break it into sub-reactions and ask about each one individually.

**The sub-steps (this is critical when decomposing the complex):**

| Sub-step | What happens | Redox? | Cofactor |
|---|---|---|---|
| Decarboxylation | $CO_2$ is released; remaining carbon forms hydroxyethyl adduct on TPP | **NOT redox** | **TPP** |
| Oxidative transfer to lipoamide | Hydroxyethyl is oxidized to acyl level and transferred to lipoamide's sulfur | Oxidation | **Lipoamide** |
| Transfer to CoA | Acyl group moves from lipoamide-SH to CoA-SH | NOT redox | **CoA-SH** |
| Lipoamide regeneration | Dihydrolipoamide is reoxidized to lipoamide | Oxidation | **FAD → $NAD^+$** |

**The reasoning pitfalls:** When only the decarboxylation sub-step is shown (α-keto acid → TPP intermediate + $CO_2$), it's tempting to call it redox because the overall process IS oxidative. But the decarboxylation sub-step itself is NOT redox. The carbon that leaves as $CO_2$ was already at +4. The

carbon left behind stays at the same oxidation state (it was a ketone carbon, and it becomes the hydroxyethyl carbon on TPP — still at the alcohol/aldehyde oxidation level). The oxidation happens in the NEXT sub-step.

**Worked example 1:** The α-keto acid derived from isoleucine undergoes decarboxylation. $CO_2$ leaves, TPP captures the fragment. **Not redox. Cofactor: TPP.** ✓

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## REACTION TYPE B: Acyl-CoA Dehydrogenation (Saturated → α,β-Unsaturated)

**What's happening:** Two hydrogens are removed from adjacent carbons (Cα and Cβ), creating a C=C double bond (a trans/E-enoyl-CoA).

```
 H H H
 | | |
—C—C—SCoA → —C=C—SCoA + FADH₂
 | | |
 H (rest) (rest)
```

**Redox?** YES — **oxidation.** Two C–H bonds are broken, and no C–O bonds replace them. The electrons go to a cofactor. This is a net removal of 2H from the substrate.

**Cofactor: FAD**, not $NAD^+$. Here's the reason:

**Why FAD and not $NAD^+$?** This is one of the most important cofactor-assignment rules:

> **FAD handles saturated → unsaturated (C–C → C=C).** The redox potential of this transformation is not negative enough to reduce $NAD^+$. FAD has a more positive (less negative) reduction potential, making it the appropriate electron acceptor for this relatively modest oxidation. Think of it thermodynamically: FAD accepts electrons from "weaker" donors.

> **$NAD^+$ handles alcohol → carbonyl (C–OH → C=O).** This oxidation has a more negative reduction potential, sufficient to reduce $NAD^+$.

This FAD vs. $NAD^+$ distinction is arguably the most reusable cofactor rule in all of biochemistry.

**Worked example 2:** The branched acyl-CoA from isoleucine degradation has a C=C double bond introduced between Cα and Cβ. Two hydrogens removed. **Oxidation. Cofactor: FAD.** ✓

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## REACTION TYPE C: Hydration of an Enoyl (C=C + $H_2O$ → β-Hydroxy)

**What's happening:** Water adds across the C=C double bond. One carbon gets –OH, the adjacent carbon gets –H.

```
 H OH H
 | | |
–C=C–SCoA + H₂O → –C––C–SCoA
 | | |
(rest) (rest) H
```

**Redox?** NO. One carbon gained a bond to oxygen (+OH), but the other carbon gained a bond to hydrogen (+H). These cancel out. Net oxidation state change across the molecule = zero. This is simply an addition reaction.

**Cofactor:** None needed. The enzyme (enoyl-CoA hydratase) just provides the right geometry and acid/base catalysis. Water is the reactant, not a redox cofactor.

**Worked example 3:** Water adds across the double bond of the enoyl-CoA to give a β-hydroxyacyl-CoA. **Not redox. No cofactor.** ✓

**The intuition check:** Hydration and dehydration are NEVER redox. If $H_2O$ is being added or removed across a double bond, both an O–H and a C–H bond are made (or broken) simultaneously. The oxidation effects cancel.

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## REACTION TYPE D: β-Hydroxy → β-Keto Oxidation (Alcohol → Ketone)

**What's happening:** A hydroxyl group (–OH) on a carbon is converted to a carbonyl (C=O). A hydrogen is removed from that carbon.

```
 OH H O
 | | ‖
–C––C–SCoA → –C––C–SCoA + NADH + H⁺
 | | | |
(rest) H (rest) H
```

**Redox?** YES — **oxidation.** The carbon bearing the –OH loses an H, and the C–OH becomes C=O. That carbon went from alcohol oxidation state to ketone oxidation state. Two electrons removed.

**Cofactor: $NAD^+$.** This is the classic alcohol → carbonyl oxidation. The reduction potential is sufficient to reduce $NAD^+$. The electrons flow: substrate → $NAD^+$ → NADH.

**Why $NAD^+$ and not FAD?** Recall the rule: C–OH → C=O is a "stronger" oxidation (more negative E°') than C–C → C=C. $NAD^+$ is the appropriate acceptor for this higher-energy electron pair. FAD would not be reduced efficiently by this substrate.

**Worked example 4:** The β-hydroxyacyl-CoA is oxidized to the β-ketoacyl-CoA. Hydroxyl becomes a ketone. **Oxidation. Cofactor: $NAD^+$. ✓**

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### REACTION TYPE E: Thiolysis (β-Keto Cleavage by CoA-SH)

**What's happening:** CoA-SH attacks the β-keto group, cleaving the C–C bond between Cα and Cβ. The result is two fragments, each bearing a CoA thioester.

```
 O  O  O  O
 ‖  ‖  ‖  ‖
–C——C–SCoA + HSCoA → –C–SCoA + CH₃–C–SCoA
|  (acetyl-CoA)
(rest)
```

**Redox?** NO. Look at the carbons involved in the cleavage. The β-keto carbon was at ketone oxidation state before and becomes a thioester carbon after — but thioester carbons (C=O bonded to S) are at approximately the same oxidation level as ketone carbons. No net electron transfer. This is a C–C bond cleavage driven by the stability of the thioester products, not by redox chemistry.

**Cofactor:** None needed. CoA-SH is a *substrate*, not a cofactor. It's consumed in the reaction (appears in the product). The enzyme (thiolase) just provides the active site.

**Worked example 5:** The β-ketoacyl-CoA is cleaved by CoA-SH into propionyl-CoA and acetyl-CoA (for isoleucine's specific degradation). **Not redox. No cofactor. ✓**

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## PART 3: The Cofactor Assignment Framework

This is the second core skill. Once you've identified the reaction type, cofactor assignment is almost automatic if you know the rules.

### The Master Cofactor Table

| Reaction Type | Cofactor | Why |
|---|---|---|
| **Decarboxylation of α-keto acid** | **TPP** | TPP's carbanion (ylide) attacks the carbonyl, stabilizes the carbanion after $CO_2$ loss via electron-sink mechanism |
| **C–C → C=C** (dehydrogenation to form double bond) | **FAD** | Modest reduction potential; FAD's two-electron/two-proton ring system is suited for this. Enzyme-bound. |

| Reaction Type | Cofactor | Why |
|---|---|---|
| **C–OH → C=O** (alcohol → ketone/aldehyde) | **$NAD^+$** | Higher reduction potential; $NAD^+$ accepts hydride ($H^-$) from the substrate |
| **Hydration / dehydration** | **None** | No electron transfer — acid/base catalysis only |
| **Thiolysis / thioester exchange** | **None** | CoA-SH is a substrate. Nucleophilic acyl substitution, not redox. |
| **Carboxylation ($CO_2$ fixation)** | **Biotin** | Biotin carries activated $CO_2$ as carboxybiotin |
| **Amino group transfer** | **PLP ($B_6$)** | PLP forms Schiff base with amino acid, enables transamination |
| **1-carbon transfer (formyl, methyl, etc.)** | **THF or SAM** | THF carries 1C units at various oxidation states; SAM for methyl transfers |
| **Acyl group activation** | **CoA-SH** | Thioester linkage activates the acyl group for subsequent reactions |
| **Oxidative decarboxylation (full complex)** | **TPP, lipoamide, CoA, FAD, $NAD^+$** | All five are needed across the sub-steps of the dehydrogenase complex |

## The FAD vs. $NAD^+$ Decision Rule (Expanded)

This comes up constantly, so it deserves extra attention.

**Ask: "What is the substrate donating?"**

| Substrate transformation | Electron acceptor | Product |
|---|---|---|
| Saturated C–C → C=C double bond | **FAD** | $FADH_2$ |
| Alcohol (C–OH) → Carbonyl (C=O) | **$NAD^+$** | NADH |
| Aldehyde (CHO) → Acid (COOH) | **$NAD^+$** | NADH |
| Amine ($C–NH_2$) → Imine (C=NH) | **FAD** (often) | $FADH_2$ |

**The thermodynamic basis:** $NAD^+$/NADH has a more negative standard reduction potential ($E°' = -0.32$ V) than FAD/$FADH_2$ ($E°' \approx -0.22$ V, though this varies when enzyme-bound). Electrons "flow" from more negative to more positive potential spontaneously. The C–OH → C=O oxidation generates electrons at a potential negative enough to reduce $NAD^+$. The C–C → C=C oxidation generates electrons at a less negative potential — not enough to efficiently reduce $NAD^+$, but sufficient for FAD.

**Mnemonic (if you want one): FAD is satisfied with less.** It accepts electrons from "weaker" oxidations (single bond → double bond). **$NAD^+$ demands more.** It requires the "stronger" oxidation (alcohol → carbonyl) to be reduced.

Another way: **FAD** = **Flat** (creates a flat C=C double bond). **NAD** = **Nab the OH** (removes H from an alcohol to form C=O).

### The "Is CoA a Cofactor?" Trap

A common classification error is to list "CoA" as a cofactor. Be careful:

- **CoA-SH as a SUBSTRATE:** When CoA appears in the product (as acyl-SCoA) and is consumed, it's a substrate, not a cofactor. Thiolysis consumes CoA-SH → it's a substrate. **Don't list it as a cofactor.**
- **CoA-SH as a COFACTOR:** In the oxidative decarboxylation complex, CoA-SH acts catalytically within the multi-enzyme complex (it's recycled). In that specific context, it's considered a cofactor of the complex.
- **rule of thumb:** For a single isolated reaction where CoA-SH is clearly a reactant → it's a substrate. For the overall dehydrogenase complex → it's a cofactor.

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## PART 4: The β-Oxidation Pattern — Your Secret Weapon

Here's the deepest insight from these examples: **isoleucine degradation (after the initial decarboxylation) IS β-oxidation.** The same four-reaction spiral appears in:

- **Fatty acid β-oxidation** (the classic version)
- **Branched-chain amino acid degradation** (isoleucine, leucine, valine — after decarboxylation)
- **Parts of the citric acid cycle** (succinate → fumarate → malate → oxaloacetate mirrors this pattern)

The cycle is always:

```
 Step 1: C–C → C=C (oxidation, FAD)
Step 2: C=C + H₂O → C–OH (hydration, no cofactor)
Step 3: C–OH → C=O (oxidation, NAD⁺)
Step 4: C–C cleavage (thiolysis with CoA-SH, not redox, no cofactor)
```

**If you recognize this four-step pattern, you can analyze the redox and cofactor roles for ANY pathway that uses it — even one you've never seen before.** The pathway itself need not be familiar: recognizing the β-oxidation pattern is enough. Same reactions, different substrate.

### The Citric Acid Cycle Parallel

The TCA cycle contains this same pattern in disguise:

| TCA Step | Equivalent β-ox Step | Redox? | Cofactor |
|---|---|---|---|
| Succinate → Fumarate | C–C → C=C | Oxidation | FAD (via succinate dehydrogenase) |
| Fumarate → Malate | C=C + $H_2O$ → C–OH | Not redox | None |

| TCA Step | Equivalent β-ox Step | Redox? | Cofactor |
|---|---|---|---|
| Malate → Oxaloacetate | C–OH → C=O | Oxidation | $NAD^+$ |

Same logic, same cofactors, same redox calls. Once you see the pattern, it becomes automatic.

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# PART 5: Quick-Recognition Decision Trees

## Decision Tree 1: Is It Redox?

```
Look at the carbon atoms that changed
│
├── Did any carbon GAIN bonds to O (or LOSE bonds to H)?
│ ├── YES, without compensating H gain → OXIDATION
│ └── YES, but another carbon gained H simultaneously
│ (like hydration: one C gets OH, adjacent C gets H)
│ → NOT REDOX (changes cancel)
│
├── Did any carbon LOSE bonds to O (or GAIN bonds to H)?
│ └── YES, without compensating O gain → REDUCTION
│
├── Did CO₂ leave?
│ └── Check the REMAINING carbons, not the CO₂ carbon
│ (CO₂ was already fully oxidized at +4)
│ Did the remaining carbon change?
│ Usually NO → NOT REDOX
│
├── Did a thioester exchange happen? (–SCoA swaps, –S–Enz swaps)
│ └── NOT REDOX (sulfur substitution, same oxidation state)
│
└── None of the above?
└── NOT REDOX
```

## Decision Tree 2: Which Cofactor?

```
Identify the reaction type
│
├── Decarboxylation of an α-keto acid?
│ └── TPP
│
├── Oxidation: C–C → C=C? (single bond to double bond)
```

```
│ └── FAD
│
├── Oxidation: C–OH → C=O? (alcohol to carbonyl)
│ └── NAD⁺
│
├── Hydration or dehydration? (H₂O added/removed across double bond)
│ └── No cofactor
│
├── Thiolysis? (C–C bond cleaved by CoA-SH attack on β-keto)
│ └── No cofactor (CoA-SH is a substrate)
│
├── Carboxylation? (CO₂ being fixed)
│ └── Biotin (+ ATP for activation)
│
├── Transamination? (amino group moving between molecules)
│ └── PLP
│
└── Reoxidation of dihydrolipoamide?
└── FAD (then FAD passes electrons to NAD⁺)
```

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# PART 6: Worked Examples for independent study

## Reaction 1: α-Keto acid → Acyl-enzyme intermediate + $CO_2$

**Step 1 — What type?** $CO_2$ is released from an α-keto acid. This is a decarboxylation. Specifically, TPP attacks the α-keto carbonyl, and $CO_2$ departs, leaving a hydroxyethyl-TPP adduct.

**Step 2 — Is it redox?** The $CO_2$ carbon was already at +4 (fully oxidized) — it didn't change. The remaining α-carbon: it was a carbonyl carbon in the keto acid (bonded to C=O and $COO^-$), and it becomes the hydroxyethyl carbon on TPP (bonded to OH, H, and TPP). Count: it went from 3 bonds to O → 1 bond to O + 1 bond to H. That's actually a *reduction* of that carbon? No — the carboxylate carbon that left as $CO_2$ was the one with those oxygen bonds. The α-carbon itself stays roughly the same. **Not redox.** ✓

**Step 3 — Cofactor?** Decarboxylation of an α-keto acid → **TPP.** ✓

## Reaction 2: Acyl-CoA → Enoyl-CoA (C=C introduced)

**Step 1 — What type?** Two hydrogens are removed from Cα and Cβ of the acyl-CoA, forming a trans double bond.

**Step 2 — Is it redox?** Two C–H bonds broken, C=C formed. Carbons lost H without gaining O. That's a net loss of electrons from the substrate. **Oxidation.** ✓

**Step 3 — Cofactor?** C–C → C=C = **FAD.** ✓

### Reaction 3: Enoyl-CoA → β-Hydroxyacyl-CoA

**Step 1 — What type?** $H_2O$ adds across the C=C double bond. One carbon gets –OH, the other gets –H.

**Step 2 — Is it redox?** One carbon gained O (from OH), another gained H. Changes cancel. **Not redox.** ✓

**Step 3 — Cofactor?** Hydration → **No cofactor.** ✓

### Reaction 4: β-Hydroxyacyl-CoA → β-Ketoacyl-CoA

**Step 1 — What type?** The –OH on the β-carbon becomes a C=O. The H on that carbon is removed.

**Step 2 — Is it redox?** C–OH → C=O. Carbon lost an H and gained a bond to O. Net: more oxidized. **Oxidation.** ✓

**Step 3 — Cofactor?** Alcohol → ketone = **$NAD^+$.** ✓

### Reaction 5: β-Ketoacyl-CoA + CoA-SH → Two Acyl-CoA fragments

**Step 1 — What type?** C–C bond cleaved by nucleophilic attack of CoA-SH on the β-keto carbonyl. Thiolysis.

**Step 2 — Is it redox?** The β-keto carbon goes from C=O (ketone) to C=O bonded to S (thioester). Same oxidation level. The α-carbon doesn't change either. **Not redox.** ✓

**Step 3 — Cofactor?** CoA-SH is consumed (appears in product). It's a substrate. **No cofactor.** ✓

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## PART 7: Common reasoning pitfalls

**Trap 1: "Oxidative decarboxylation means the decarboxylation step is an oxidation."** No. The OVERALL process is oxidative, but the decarboxylation sub-step ($CO_2$ leaving via TPP) is not itself redox. The oxidation occurs in the subsequent transfer to lipoamide. If an analysis isolates the decarboxylation step, call it "not redox."

**Trap 2: "Hydration involves oxygen, so it must be redox."** No. In hydration, one carbon gains –OH (oxidizing effect) and the adjacent carbon gains –H (reducing effect). They cancel. Net change = zero. Hydration is NEVER redox.

**Trap 3: "CoA is a cofactor."** Only in the context of the dehydrogenase complex where it's recycled. In a simple thiolysis reaction, CoA-SH is consumed → it's a substrate. Read the transformation carefully.

**Trap 4: Mixing up FAD and $NAD^+$.** Use the rule: FAD handles C–C → C=C (the "weaker" oxidation). $NAD^+$ handles C–OH → C=O (the "stronger" oxidation). These are not interchangeable.

**Trap 5: Forgetting that thioester exchange is not redox.** When an acyl group transfers from one sulfur to another (lipoamide → CoA, or CoA → another CoA), no oxidation state change occurs. Sulfur acts as a nucleophile, not a redox agent, in these substitutions.

**Trap 6: Calling a reaction "reduction" when the substrate is oxidized.** Remember: the analysis asks about THE REACTION (from the substrate's perspective). If the substrate loses electrons, the reaction is an OXIDATION — even though the cofactor is being reduced. Always describe the reaction from the perspective of the organic substrate.

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## Summary: The Five Instincts for Reaction Identification

1. **Track carbon oxidation states by counting bonds to O vs. H.** More bonds to O (or fewer to H) = oxidation. Reverse = reduction. Changes that cancel (hydration) = not redox.
2. **Recognize the β-oxidation spiral.** FAD oxidation → hydration → $NAD^+$ oxidation → thiolysis. This four-step sequence recurs across fatty acid degradation, branched-chain amino acid catabolism, and sections of the TCA cycle. Learn it once, apply it everywhere.
3. **FAD handles C–C → C=C. $NAD^+$ handles C–OH → C=O.** This is non-negotiable and comes from reduction potential differences. It will never be reversed.
4. **TPP = decarboxylation of α-keto acids.** Whenever you see an α-keto acid losing $CO_2$, TPP is doing the work. The mechanism (carbanion stabilization via the thiazolium ylide) is unique to TPP.
5. **"No cofactor" is a valid and common outcome.** Hydration, thiolysis, and many isomerizations don't require cofactors. The enzyme provides acid-base catalysis and geometric control, but no organic cofactor or metal ion is needed for the chemistry itself. Don't force a cofactor where none belongs.