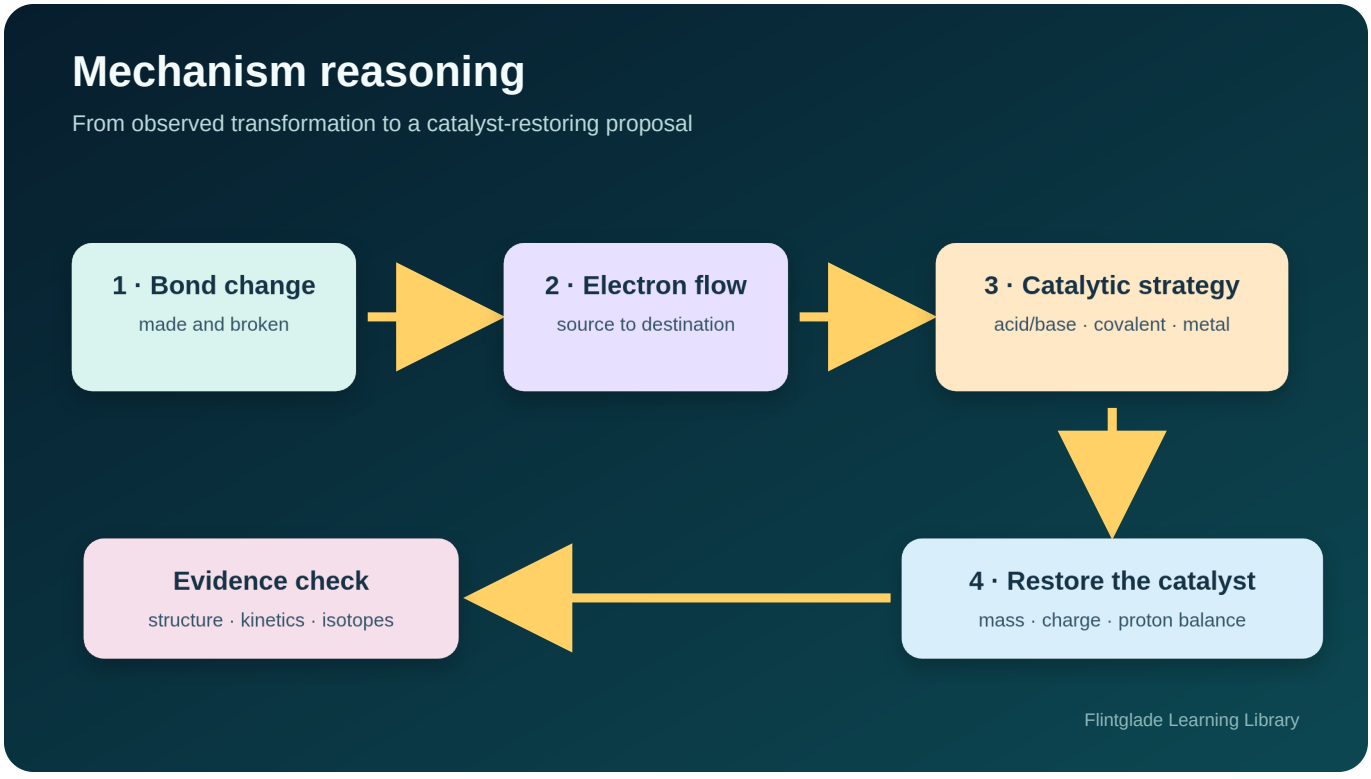
# Enzyme Mechanisms: A Quick Reasoning Map

*Flintglade Learning Library · General Biochemistry*

**Audience:** Learners who want a concise checklist for analyzing catalytic residues, intermediates, and electron flow.

**MCAT connections:** This guide can assist study of enzymes and kinetics 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

- Choose between covalent and direct-attack pathways
- Assign acid, base, nucleophile, and stabilizing roles
- Audit curved arrows and catalyst regeneration

## Navigate this guide



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## THE FORK: Every analysis starts here.

**Covalent intermediate (Path A)?** → Enzyme residue attacks substrate. **Direct attack (Path B)?** → Water/hydroxide attacks substrate.

How to decide: **Is Ser, Cys, or Thr a catalytic residue?**

- Yes → Path A.
- No → Path B.

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## PATH A — Covalent Intermediate

Nucleophile = enzyme residue (Ser-$O^-$ / Cys-$S^-$ / Thr-$O^-$)

**Phase 1 (Acylation):** Base pulls $H^+$ off nucleophile → nucleophile attacks C=O → tetrahedral intermediate (oxyanion stabilized) → C–N breaks, base donates $H^+$ to leaving group → acyl-enzyme intermediate

**Phase 2 (Deacylation):** Water enters → base pulls $H^+$ off water → $OH^-$ attacks acyl-enzyme C=O → second tetrahedral intermediate → C–O/S bond to enzyme breaks, base returns $H^+$ to enzyme residue → enzyme regenerated

**Counts:** 2 tetrahedral intermediates. 1 covalent intermediate.

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## PATH B — Direct Attack (no covalent intermediate)

Nucleophile = water activated by metal or general base

**Single phase:** Metal lowers water pKa (and/or base pulls $H^+$ off water) → $OH^-$ attacks C=O → tetrahedral intermediate (metal or Arg stabilizes oxyanion) → bond breaks, acid protonates leaving group → products released, water re-coordinates

**Counts:** 1 tetrahedral intermediate. 0 covalent intermediates.

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## ROLE ASSIGNMENT — 4 roles, fill each one.

| Role | What it does | Usual suspects |
|---|---|---|
| **Nucleophile** | Attacks electrophilic carbon | Ser, Cys, Thr (Path A) / $OH^-$ from water (Path B) |
| **General base** | Pulls $H^+$ to activate nucleophile | His, Glu, Asp, Lys |
| **General acid** | Donates $H^+$ to leaving group | Same residue that was the base (it toggled) |
| **Stabilizer** | Neutralizes oxyanion charge | Oxyanion hole (backbone NH), $Arg^+$, $Zn^{2+}$, Asp (positions His) |

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## CURVED ARROW RULES — 5 rules, no exceptions.

1. Arrows go FROM electron-rich TO electron-poor. Always.
2. Nucleophile attacks C=O: one arrow to carbon, one arrow from C=O to oxygen. Always two arrows simultaneously.
3. Proton transfer: arrow from acceptor's lone pair to $H^+$, arrow from X–H bond back to X.
4. Leaving group departs: arrow from C–LG bond to leaving group, arrow from oxygen lone pair reforms C=O.
5. Mark all formal charges on every intermediate.

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## TRIGGER KEY — pattern recognition

**Residues you see → What to do**

| You see... | Pathway | Nucleophile | Base | Stabilizer |
|---|---|---|---|---|
| Ser + His + Asp | A | Ser | His | Asp holds His; oxyanion hole |
| Cys + His + (Asp or Arg) | A | Cys | His | Asp holds His, or Arg stabilizes oxyanion |
| Thr + His + Asp | A | Thr | His | Asp holds His; oxyanion hole |
| $Zn^{2+}$ + His/Cys ligands + Glu | B | Water → $OH^-$ | Glu | $Zn^{2+}$ stabilizes oxyanion |
| Asp + Asp (or Glu + Glu) | B | Water → $OH^-$ | One Asp/Glu | Other Asp/Glu is acid |
| Glu + Arg (no Ser/Cys/Thr) | B | Water → $OH^-$ | Glu | Arg stabilizes oxyanion |

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## BOND TYPE → PRODUCTS

| Bond | Where found | Electrophile | Leaving group becomes |
|---|---|---|---|
| Peptide (C–N) | Proteins | Carbonyl C | Free amine ($–NH_3^+$) |
| Glycosidic (C–O) | Sugars | Anomeric C | Free –OH on sugar |
| Phosphodiester (P–O) | DNA/RNA | Phosphorus | Free –OH + phosphate |
| Ester (C–O) | Fats | Carbonyl C | Free alcohol (–OH) |

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## Reasoning Pitfalls — Quick Checklist before finishing

- [ ] Did I show **how the nucleophile was activated** (deprotonation step)?
- [ ] Did I show **both phases** if Path A (acylation AND deacylation)?
- [ ] Did I **protonate the leaving group** (show where $H^+$ comes from)?
- [ ] Are my arrows pointing **nucleophile → electrophile** (not backwards)?
- [ ] Did I **stabilize every oxyanion** (oxyanion hole, metal, or Arg)?
- [ ] Did I use a covalent intermediate **only for Path A**, not Path B?

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## SPECIAL SCENARIOS

**Transpeptidase (like SrtA):** Path A, but Phase 2 uses R–$NH_2$ instead of water. Everything else identical.

**Burst kinetics:** Path A where Phase 1 is fast, Phase 2 is slow. First product released quickly (burst), then steady-state rate limited by deacylation.

**Ser→Thr mutation:** Same Path A mechanism. Slower because: (1) methyl group adds steric hindrance, (2) secondary alcohol has higher pKa → harder to deprotonate.

**Substrate analog with nitrophenol:** Same mechanism as real substrate. Nitrophenol leaving group turns yellow when released → measurable by UV absorbance.