Enzymes: Catalysis, Kinetics & Regulation

BCH 100 — Introductory Biochemistry · Dr. Radi

By the end of this unit, you can…

- Explain how enzymes accelerate reactions by lowering activation energy without changing ΔG
- Describe the active site and induced fit, classify enzymes, and interpret Michaelis–Menten and Lineweaver–Burk plots
- Distinguish the modes of regulation (inhibition, allostery, covalent modification, zymogens) and walk the chymotrypsin catalytic-triad mechanism

Today's route

1. Enzymes – Catalysis & the Active Site
2. The Six Classes of Enzymes
3. Michaelis–Menten Kinetics
4. The Lineweaver–Burk Plot
5. Enzyme Inhibition
6. Allosteric Regulation & Feedback
7. Covalent Modification & Zymogens
8. Chymotrypsin – the Catalytic Triad in Action

1 · Enzymes — Catalysis & the Active Site

"Explain how enzymes speed reactions by lowering activation energy, and how the active site grips the substrate."

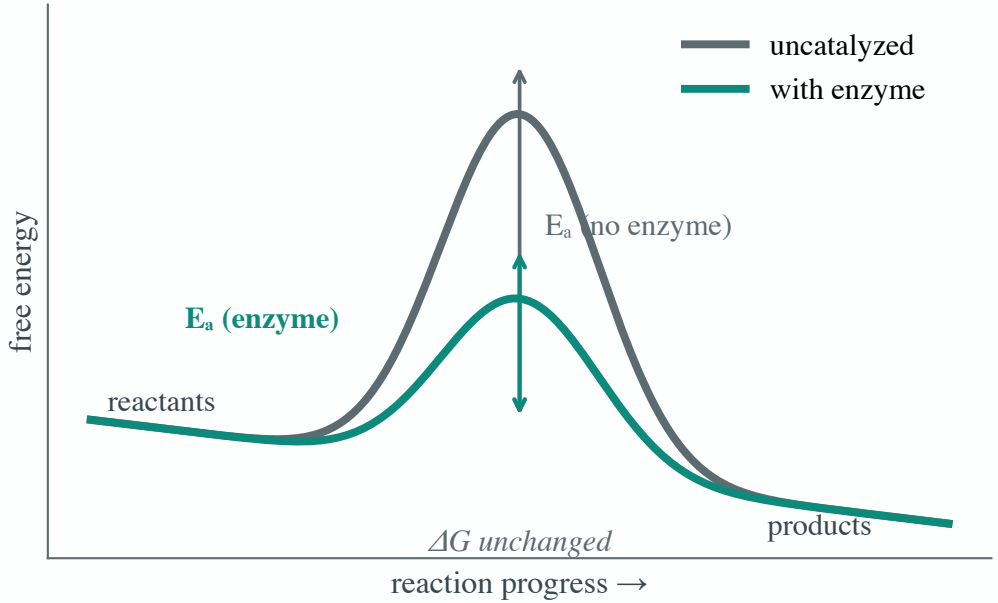
What an enzyme does

Enzymes are the cell's **catalysts** — almost always proteins. One can speed a reaction up by **a million-fold or more**, come out **unchanged** at the end, and it is exquisitely **specific** for its substrate.

enzyme changes the rate, not the ΔG

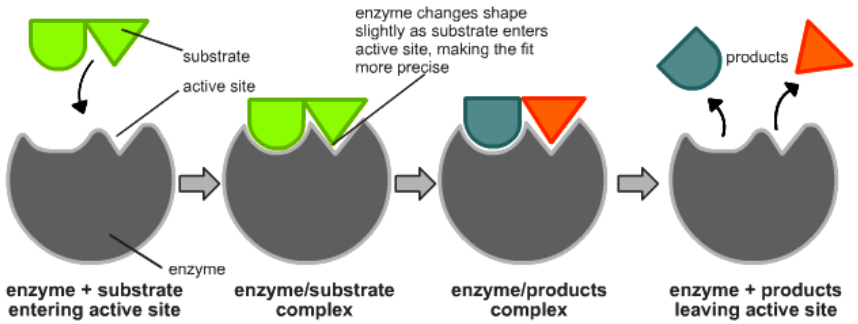
it can't make an unfavorable reaction favorable — only faster

It lowers the activation energy



Every reaction has an energy hill — the **activation energy (E_a)** — between reactants and products. An enzyme doesn't flatten the valley (**ΔG is unchanged**); it carves an **easier path over the hill**, stabilizing the shaky **transition state** so far more molecules make it across.

The active site & induced fit



The substrate binds a small, precisely-shaped pocket — the **active site**. It's not a rigid lock: as the substrate settles in, the enzyme **closes around it** (**induced fit**), squeezing it toward the transition state. That snug grip is where the specificity — and the catalysis — comes from.

Induced-fit diagram: TimVickers, Wikimedia Commons, public domain

2 · The Six Classes of Enzymes

"Sort any enzyme into one of the six classes by the reaction it catalyzes."

Six classes of enzymes

1. **Oxidoreductases**
   move electrons — oxidation & reduction (dehydrogenases)
2. **Transferases**
   move a functional group from one molecule to another (kinases)
3. **Hydrolases**
   split a bond by adding water (proteases, lipases)
4. **Lyases**
   add or remove groups to form/break double bonds (no water)
5. **Isomerases**
   rearrange atoms within one molecule
6. **Ligases**
   join two molecules together — powered by ATP

The Enzyme Commission sorts **every** enzyme into **six classes** – by the **reaction it catalyzes**, not by what it acts on.

Naming them

Most enzyme names tell you the class: they end in **–ase** and often name the substrate or reaction — **lactase** splits lactose, **DNA polymerase** builds DNA, **alcohol dehydrogenase** removes hydrogens.

substrate/reaction + –ase → the enzyme's name

a few keep old names: pepsin, trypsin, chymotrypsin (all proteases)

💡 Quick check: an enzyme that adds water to break a bond is a **hydrolase**; one that joins two molecules using ATP is a **ligase**.

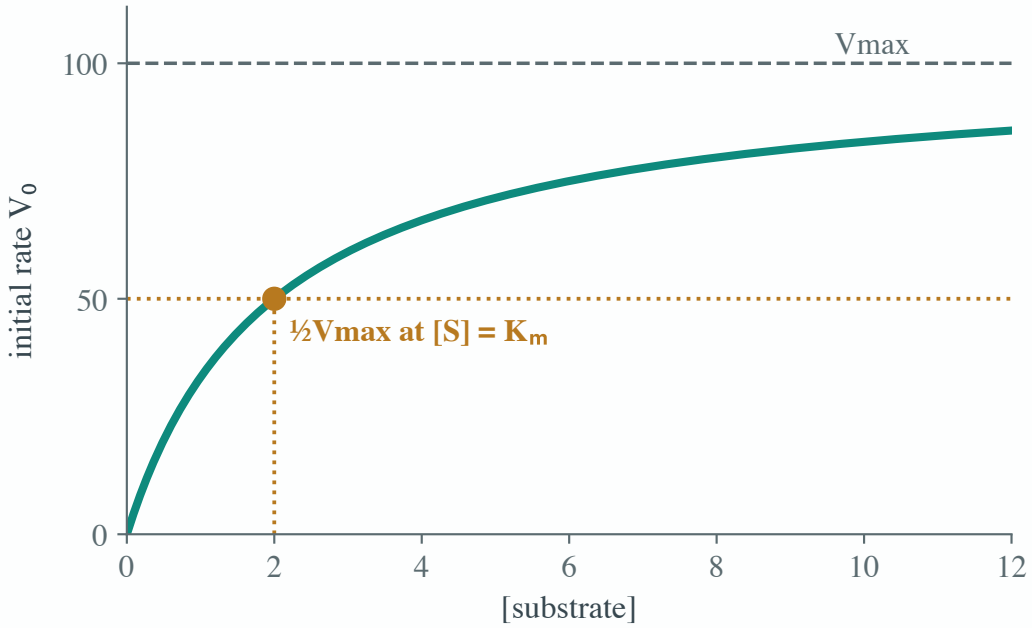
3 · Michaelis–Menten Kinetics

"Read an enzyme's saturation curve and interpret Vmax and K_m."

How fast does an enzyme go?

Measure the **initial rate (V_0)** at different substrate levels. At **low [S]** the rate climbs steeply — plenty of free enzyme. At **high [S]** it flattens out: every enzyme is busy, so the enzyme is **saturated** and can't go faster.

The Michaelis–Menten curve



That saturating shape is described by two numbers. **Vmax** is the top speed, when every active site is full. **K_m** is the substrate concentration that gives **½ Vmax** — a handy stand-in for how **tightly** the enzyme grabs its substrate.

What Vmax and K_m tell you

- **Vmax** — flat-out speed; more enzyme (or a faster **k_{cat}**) raises it.
- **K_m** — the [S] at half-speed. **Low K_m = high affinity** (the enzyme grabs substrate even when it's scarce).

💡 This is why alcohol "tolerance" varies: the **K_m** of your alcohol dehydrogenase helps set how fast you clear a drink.

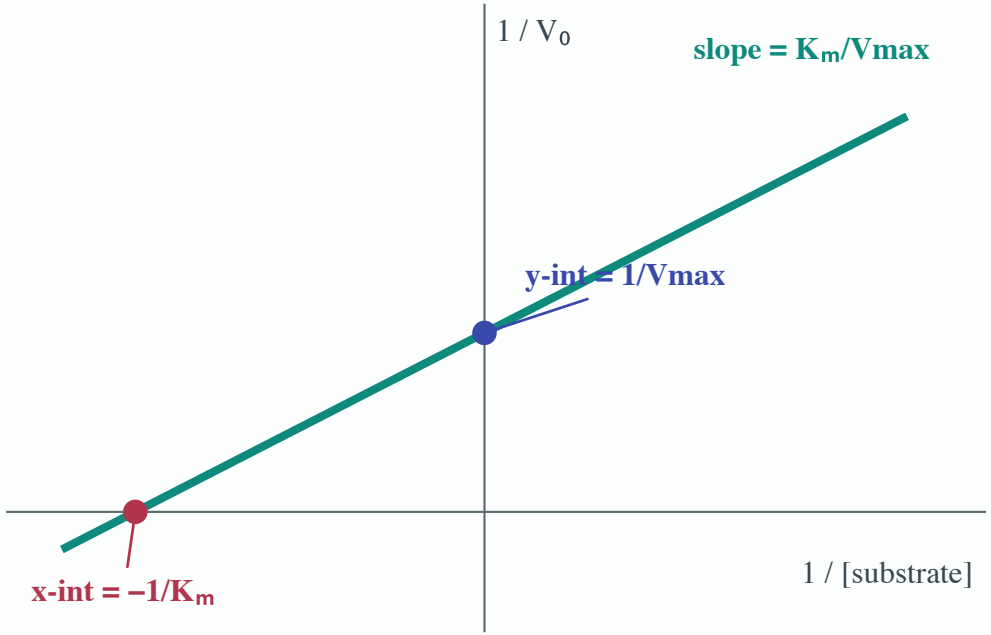
4 · The Lineweaver–Burk Plot

"Linearize the Michaelis–Menten curve to read Vmax and K_m straight off a line."

Straightening the curve

The Michaelis–Menten curve is a **hyperbola** — and Vmax is that ceiling you only **approach**, so it's hard to pin down by eye. The fix: take the **reciprocal of both sides**. A hyperbola flips into a **straight line**, and a line is easy to read exactly.

The Lineweaver–Burk plot



Plot **1/V_0** against **1/[S]** and everything falls on one line: the **y-intercept is 1/Vmax**, the **x-intercept is −1/K_m**, and the **slope is K_m/Vmax**. Read all three constants straight off — **lines are so much easier to interpret!**

💡 It's also how you spot inhibitors: each type of inhibitor moves these intercepts in its own tell-tale way.

5 · Enzyme Inhibition

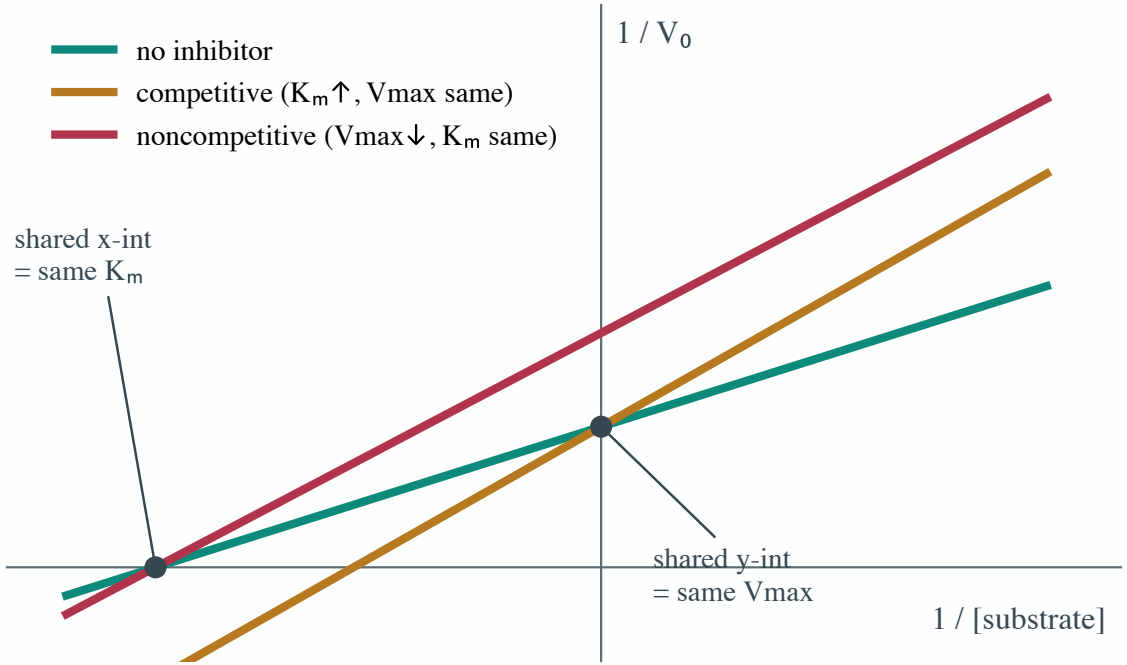
"Tell competitive from noncompetitive inhibition by how each moves Vmax and K_m on a Lineweaver–Burk plot."

Slowing an enzyme down

An **inhibitor** is any molecule that makes an enzyme run slower. The two classic reversible types differ only in **where they bind**:

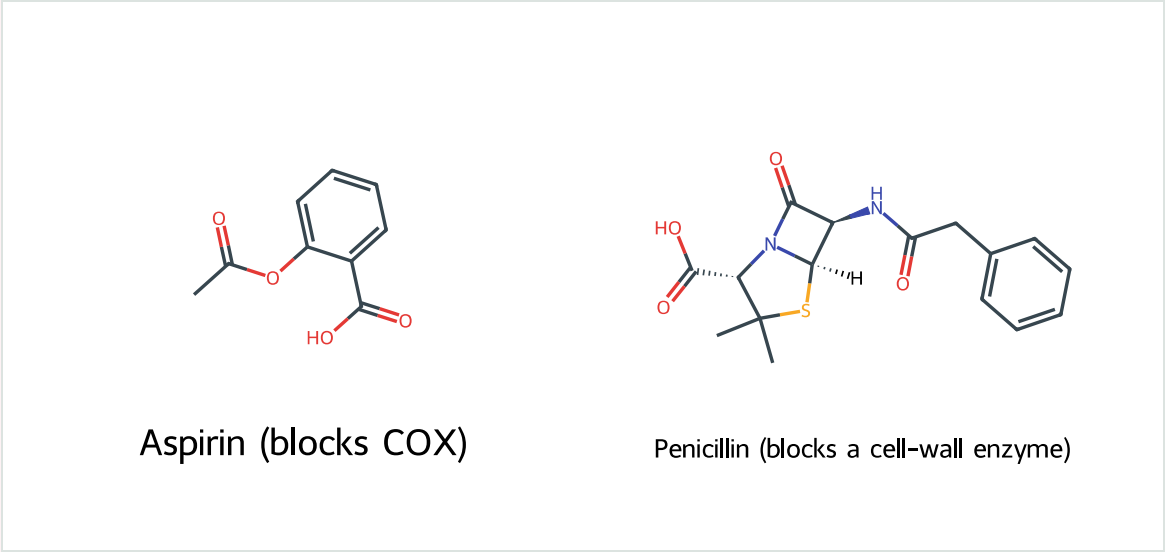
- **Competitive** — binds the **active site**, fighting the substrate for the same spot. Pile on more substrate and you **out-compete** it.
- **Noncompetitive** — binds **somewhere else** and bends the enzyme out of shape. More substrate **can't rescue** it — the spare enzyme is just broken.

Reading it on a Lineweaver–Burk



The double-reciprocal plot diagnoses the type at a glance. **Competitive** lines share the **y-intercept** — same **Vmax**, but **K_m rises** (you need more substrate). **Noncompetitive** lines share the **x-intercept** — same **K_m**, but **Vmax falls**.

In the body: most drugs are inhibitors



Blocking one enzyme is how a huge share of medicine works. **Aspirin** shuts down **COX** so you make less of the prostaglandins behind pain and fever; **penicillin** jams the enzyme bacteria use to **build their cell wall**, so they burst. Both bind **irreversibly** — the enzyme never comes back.

💡 Same trick, more examples: **statins** block cholesterol synthesis (competitive), **methotrexate** blocks DNA-precursor synthesis in chemo.

6 · Allosteric Regulation & Feedback

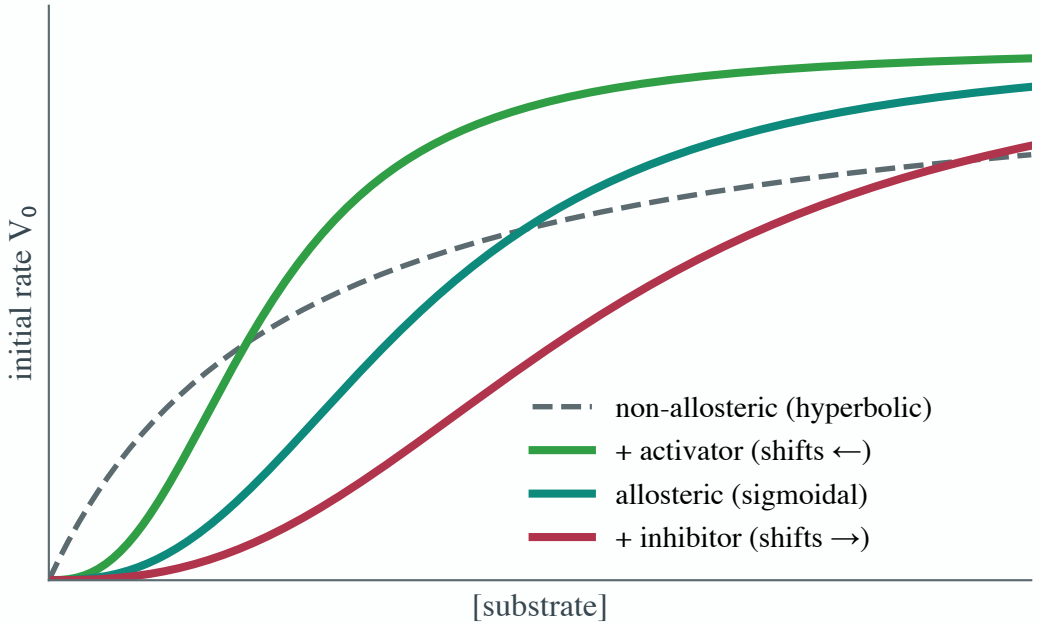
"Read a sigmoidal kinetic curve and explain how allosteric effectors and feedback inhibition tune a pathway."

Regulators that never touch the active site

Allosteric enzymes have a second pocket — the **allosteric site**. When a regulator binds there, the whole enzyme **changes shape**, and every active site feels it. These enzymes usually have **multiple subunits** that bind substrate **cooperatively**, just like hemoglobin binds O_2.

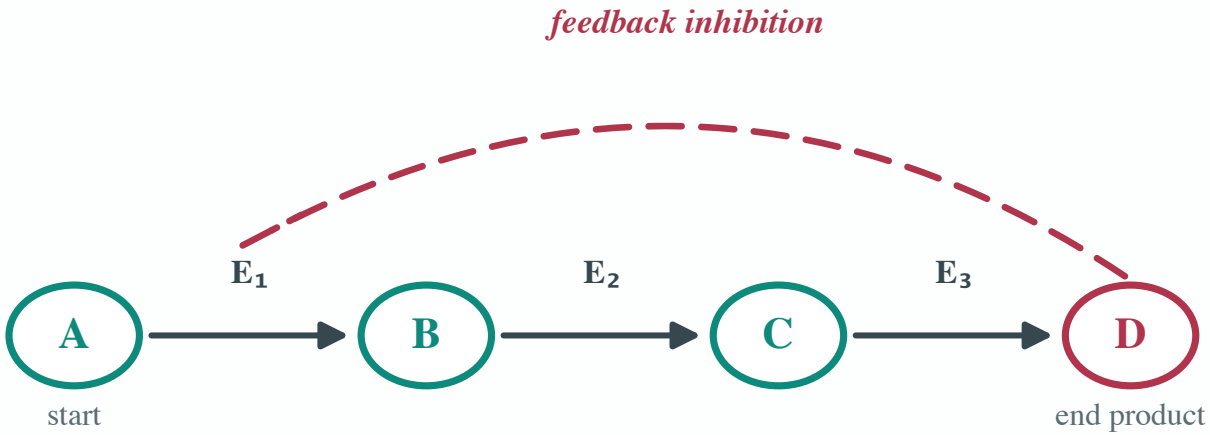
bind the allosteric site → shift the shape → change activity at all active sites

The sigmoidal curve



Cooperativity bends the Michaelis–Menten hyperbola into an **S-shape** – flat, then a steep switch-like turn-on. An **activator** slides the curve **left** (active at lower substrate); an **inhibitor** slides it **right**. That steepness makes the enzyme a **sensitive switch**, not a gentle dial.

Feedback inhibition



The smartest place to regulate a pathway is the **first committed step**. So the **end product** loops back and shuts off the **first enzyme** — **feedback inhibition**. When you have plenty of D, you stop spending A to make more. Simple, fast, and it keeps the cell from wasting materials.

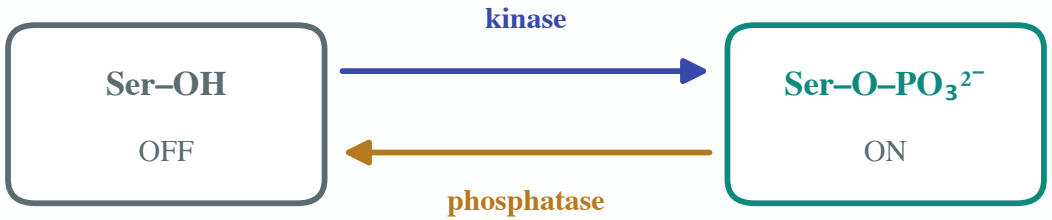
💡 Textbook case: the amino acid **isoleucine** feedback-inhibits the very first enzyme that starts building it from threonine.

7 · Covalent Modification & Zymogens

"Contrast a reversible phosphorylation switch with the one-way activation of a zymogen."

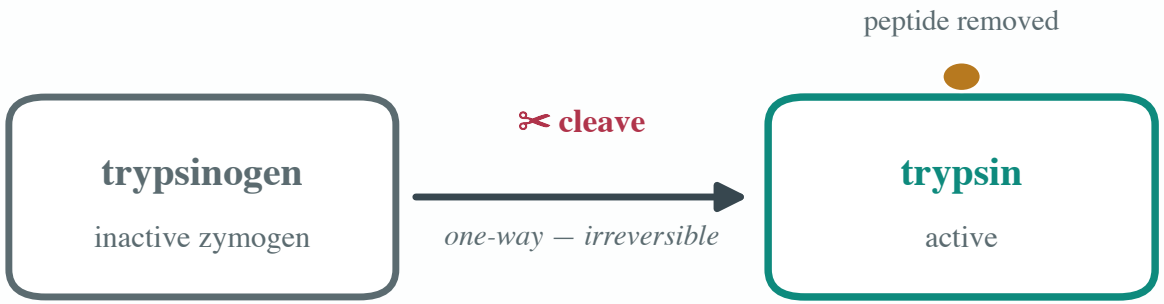
A reversible switch: phosphorylation

Attach a **phosphate** to a Ser, Thr, or Tyr and the enzyme's activity flips. A **kinase** adds it (spending ATP); a **phosphatase** takes it back off — so the switch runs **both ways**, on demand.



💡 This is how adrenaline gets you moving: it switches on **glycogen phosphorylase** to break down glycogen and free up glucose.

A one-way switch: zymogens



Some enzymes are made as **inactive precursors** — **zymogens**. A single **proteolytic cut** snips out a piece and locks them **on for good**. There's no going back — you can't glue the peptide back on — so it's perfect for a **commit-once** signal.

In the body: safety and cascades

Your **digestive enzymes** are built as zymogens so they don't **digest the pancreas that makes them** — they're only cut open once they reach the gut. When that safety fails and **trypsin activates early**, the pancreas starts digesting itself: **pancreatitis**.

zymogen → one cut → active enzyme

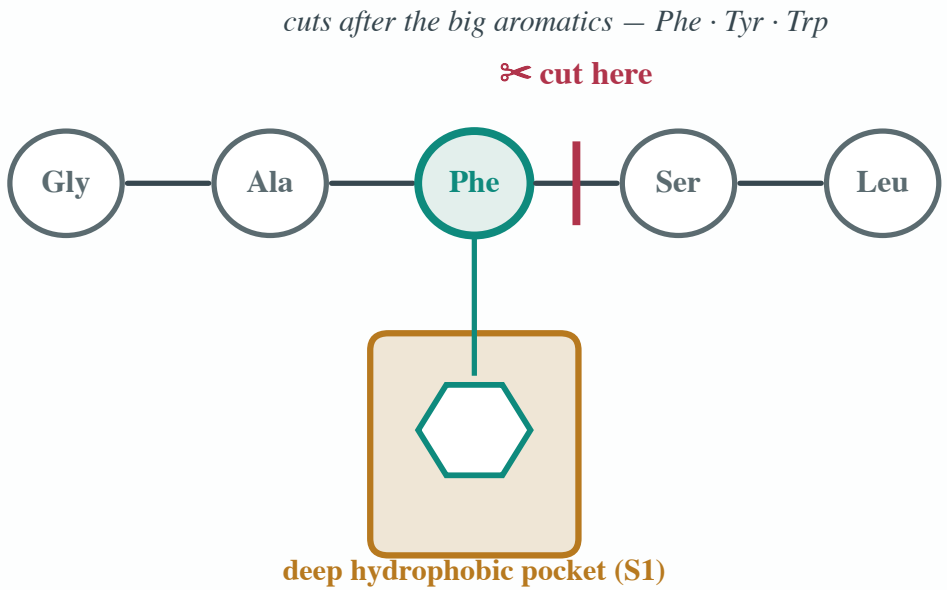
blood clotting is a whole cascade of these one-way cuts, each zymogen switching on the next

8 · Chymotrypsin — the Catalytic Triad in Action

"Take chymotrypsin apart: the Ser–His–Asp triad, the oxyanion hole, and the two-step acylation/deacylation cut."

Meet chymotrypsin: the gut's scissors

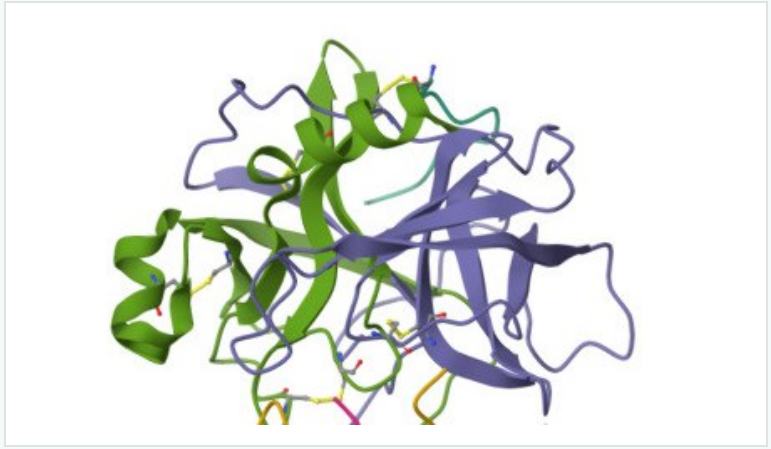
Chymotrypsin is a **serine protease** — it snips peptide bonds using one supercharged **serine**. And it's picky: it prefers to cut on the **carbonyl side of the big aromatics** — **Phe, Tyr, Trp** — whose fat rings drop into a deep pocket.



💡 Built in the pancreas as an inactive zymogen (**chymotrypsinogen**) — one snip switches it on, so it can't digest you on the way out.

The real enzyme

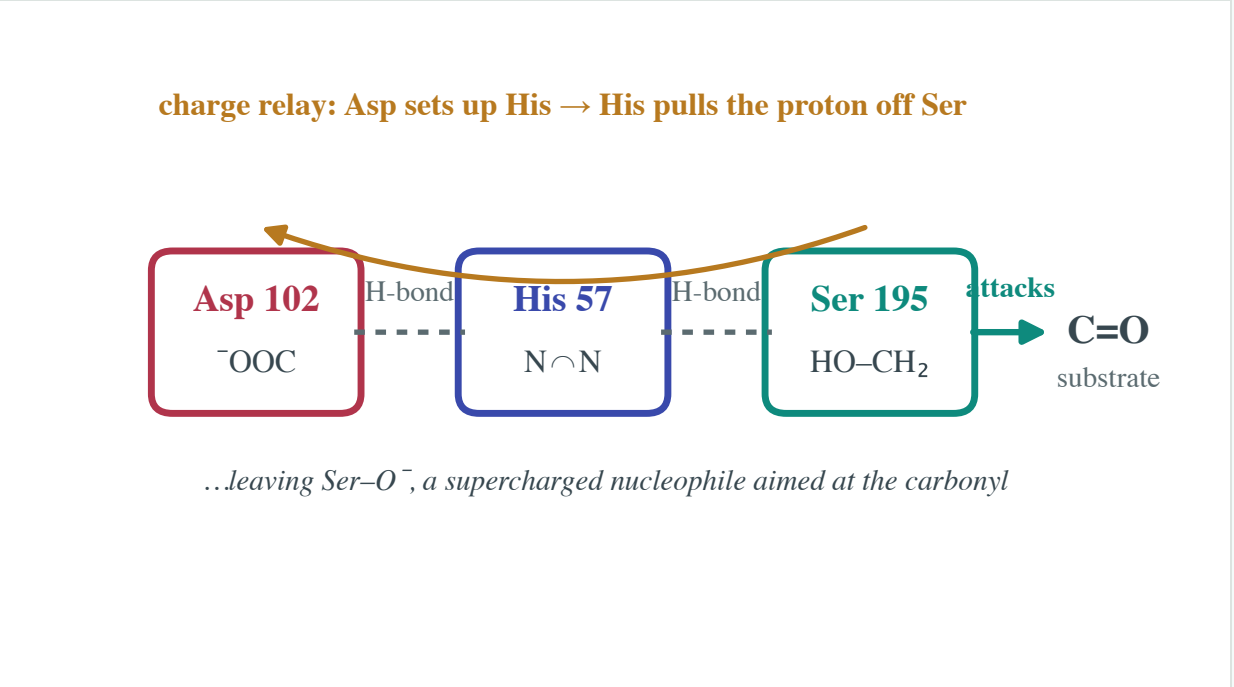
Here's chymotrypsin itself — two **β-barrel domains** packed together, with the **active site** tucked into the cleft between them. Everything we're about to unpack sits right there, at the bottom of that groove.



Structure: RCSB PDB, PDB 4CHA (rcsb.org)

The catalytic triad: Ser–His–Asp

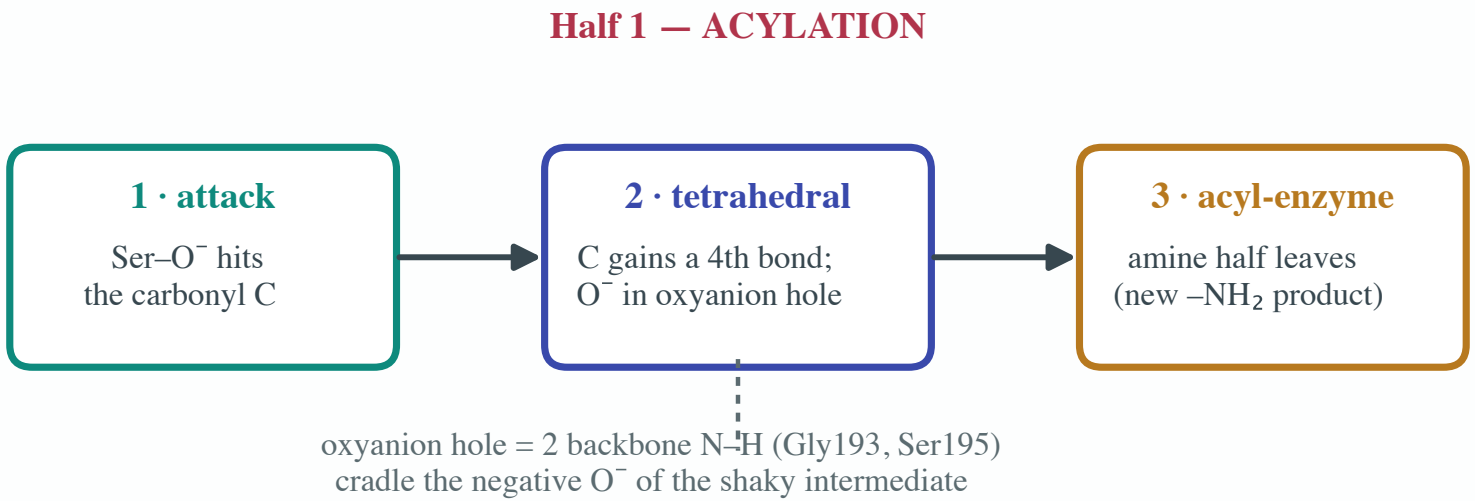
Three residues, far apart in sequence but folded shoulder-to-shoulder, make a **charge relay**: **Asp102** props up **His57**, and His57 rips the proton off **Ser195** — turning its sleepy –OH into a **fierce nucleophile**.



💡 **KNOW THE TRIAD:** Ser 195 · His 57 · Asp 102 — Ser does the attacking.

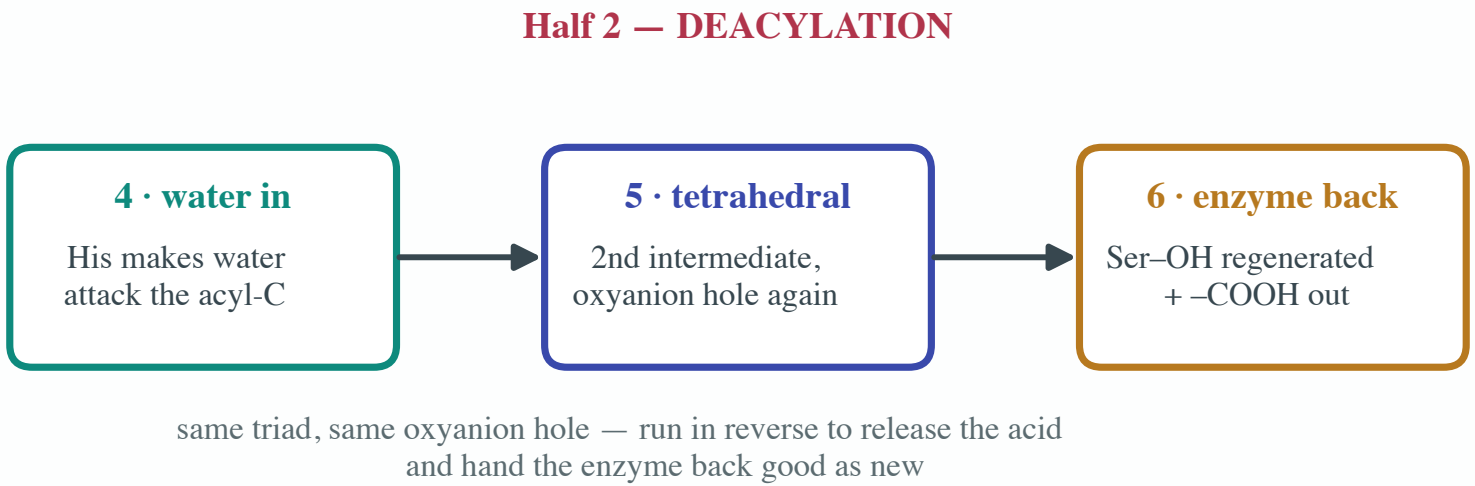
Half 1: acylation

Ser–O^- slams into the carbonyl carbon → a shaky **tetrahedral intermediate** whose negative oxygen is hugged by the **oxyanion hole**. The peptide breaks, the **first product** (the new amino end) walks off, and the enzyme is left wearing the acyl piece.



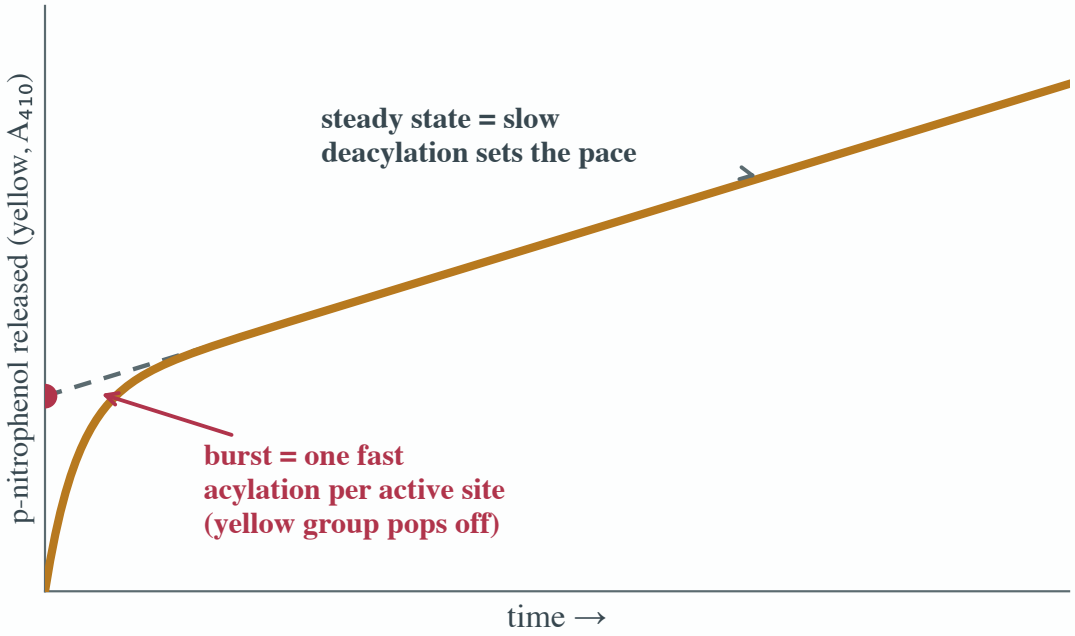
Half 2: deacylation

Now **water** finishes the job. His57 pulls a proton off it so it can attack the **acyl-enzyme** — a second tetrahedral intermediate in that same oxyanion hole — and the **second product** leaves as a **–COOH**. Ser–OH is reborn, ready to cut again.



Watch the burst — how we assay it

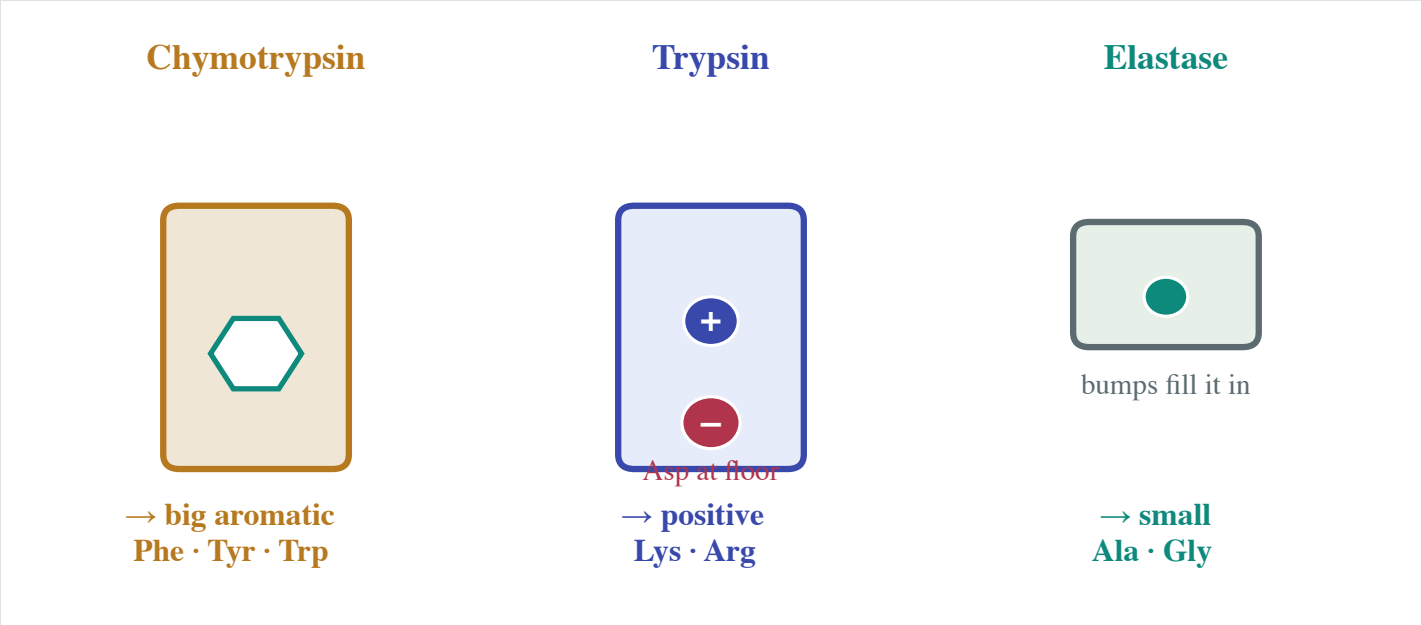
Feed chymotrypsin a fake substrate that spits out a **yellow dye** when cut, and track the color over time. You get a fast **burst** — one quick acylation per active site — then a **slow, steady** climb. That two-speed shape is the smoking gun for the **acyl-enzyme intermediate**.



💡 Assay = measure how fast the yellow (A_{410}) builds up. Faster color = more active enzyme.

In the body: one machine, many jobs

Swap the pocket and you get a **whole protease family** — trypsin cuts after **Lys/Arg**, elastase after **tiny** residues — same triad, same mechanism. They run digestion, **blood clotting**, and immune defense. They're also a target: **nerve agents** and **DFP** glue that catalytic Ser shut for good — irreversible poisoning.



Can you…?

- □ explain how enzymes accelerate reactions by lowering activation energy without changing ΔG?
- □ describe the active site and induced fit, classify enzymes, and interpret Michaelis–Menten and Lineweaver–Burk plots?
- □ distinguish the modes of regulation (inhibition, allostery, covalent modification, zymogens) and walk the chymotrypsin catalytic-triad mechanism?

If any box stays empty, the practice site has a drill for it.
🧪