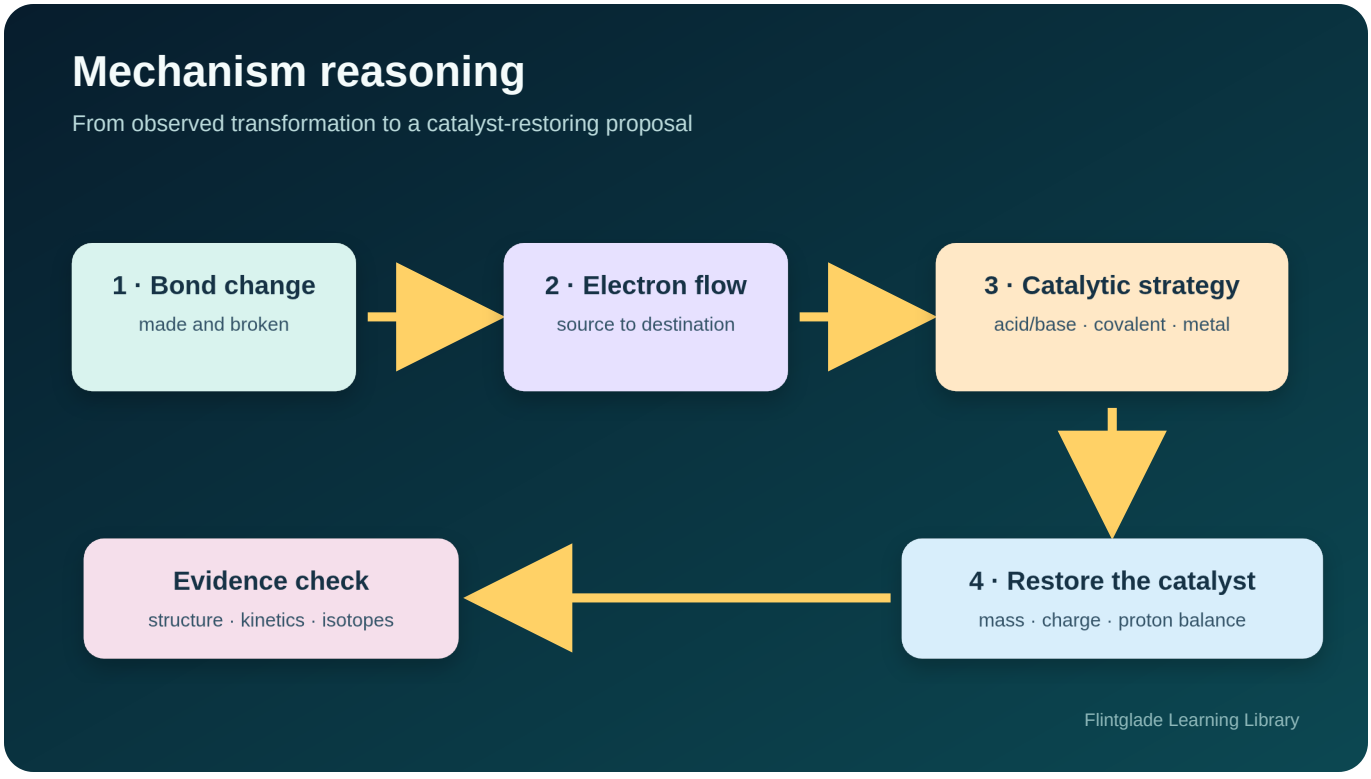
Enzyme Mechanisms from First Principles

Flintglade Learning Library · General Biochemistry

Audience: Readers new to curved-arrow reasoning who want a guided route into catalytic mechanisms.

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

- Use the vocabulary of electron flow accurately
- Assign catalytic roles to residues and metals
- Build hydrolysis mechanisms one chemically justified step at a time

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Part 0: The Words You Need Before Anything Else

Before we can talk about how enzymes break bonds, we need to define every piece of vocabulary that the framework depends on. Nothing here is optional — every term shows up later.

What is an enzyme?

An enzyme is a protein (a large molecule made of amino acids chained together) that speeds up a specific chemical reaction in a living cell. It does this by lowering the **activation energy** of the reaction — the minimum energy needed for the reaction to proceed. The enzyme is not consumed or permanently changed by the reaction. It participates, then returns to its original state, ready to do it again.

What is a mechanism?

A mechanism is the step-by-step description of exactly how a chemical reaction happens at the level of individual atoms and electrons. It answers: which bonds break, which bonds form, in what order, and what pushes each step forward.

What is hydrolysis?

Hydrolysis literally means "water-splitting." It is a type of reaction where a bond in a molecule is broken by the addition of water (H_2O). One fragment of the original molecule receives the -OH from the water, and the other fragment receives the -H. The net result: one molecule becomes two, and one

water molecule is consumed.

What is a bond?

A chemical bond is a pair of electrons shared between two atoms, holding them together. When we say "break a bond," we mean those shared electrons are redistributed — they end up belonging to one atom or are used to form a new bond elsewhere.

What is an electrophile?

"Electrophile" means "electron-lover." An electrophilic atom is one that is relatively electron-poor — it has a partial positive charge (written $\delta+$) or a full positive charge. Because it lacks electron density, it attracts electrons. In hydrolysis, the carbon atom of the bond being broken is almost always the electrophile. Why? Because that carbon is bonded to an electronegative atom (like oxygen or nitrogen) that pulls electron density away from it, leaving the carbon slightly positive.

What is a nucleophile?

"Nucleophile" means "nucleus-lover." A nucleophilic atom is one that is relatively electron-rich — it has a lone pair of electrons (a pair of electrons not involved in any bond) or a negative charge. Because it has excess electron density, it donates electrons to an electrophile. In enzyme mechanisms, the nucleophile is the atom that attacks the electrophilic carbon to start the reaction.

What is a lone pair?

Atoms like oxygen and nitrogen have electrons that are not shared in bonds with other atoms. These unshared pairs are called lone pairs. They sit on the atom and are available to form new bonds. When we draw a curved arrow starting from a lone pair, it means those electrons are being used to form a new bond.

What is a curved arrow?

In mechanism drawings, a curved arrow shows the movement of a pair of electrons. The arrow starts where the electrons currently are (a lone pair, or a bond) and points to where the electrons are going (forming a new bond, or landing on an atom). This is the universal language of organic chemistry mechanisms. Every curved arrow represents exactly two electrons moving.

What is a proton transfer?

A proton is a hydrogen atom that has lost its single electron — it is H^+, just a bare positive charge. A proton transfer is when H^+ moves from one atom to another. The atom losing the proton is acting as an acid (proton donor). The atom gaining the proton is acting as a base (proton acceptor). This is critical because many enzyme mechanism steps involve shuffling protons between residues and substrates.

What is a general acid?

A general acid is an amino acid residue in the enzyme that donates a proton (H^+) to the substrate during the reaction. This is different from the solvent (water) donating a proton. The word "general" distinguishes it from "specific acid catalysis," where the proton comes from the surrounding solution. In enzyme mechanisms, the general acid often donates H^+ to the leaving group to help it depart.

What is a general base?

A general base is an amino acid residue in the enzyme that accepts a proton (H^+) from the substrate or from water during the reaction. By pulling away a proton, the general base makes something more nucleophilic. For example: if a general base pulls H^+ off of water, the water becomes hydroxide (OH^-), which is a far better nucleophile than water itself.

What is a leaving group?

When a bond breaks, the fragment that departs is called the leaving group. In hydrolysis of a peptide bond, for example, the amine portion (-NH-) departs as the leaving group when the C-N bond breaks. Leaving groups depart more easily if they are stable on their own — and a general acid can help by donating a proton to the leaving group, neutralizing any developing negative charge and making departure more favorable.

What is a catalytic residue?

Enzymes are made of amino acids. Most of the amino acids in an enzyme provide structural support — they hold the protein in the right shape. But a small number of amino acids (typically 2–4) are directly involved in the chemical reaction happening at the active site. These are called catalytic residues. They are the ones doing the chemistry: attacking bonds, donating protons, accepting protons, or stabilizing charges.

What is the active site?

The active site is the specific pocket or cleft on the enzyme where the substrate (the molecule being acted upon) binds and where the chemical reaction occurs. The catalytic residues are located here.

What is pKa?

The pKa of a group tells you how tightly it holds onto its proton. A low pKa means the group gives up its proton easily (it is acidic). A high pKa means the group holds its proton tightly (it is basic — you'd need strong conditions to remove the proton). This matters because whether a residue is protonated or deprotonated at physiological pH (around 7.4) determines what role it can play. For example, if a residue's pKa is 6, then at pH 7.4 it has already given up its proton and exists in its deprotonated, negatively charged form — which might make it a good nucleophile.

What is an intermediate?

An intermediate is a molecular species that forms during the reaction but is neither the starting material nor the final product. It exists temporarily. In enzyme catalysis, intermediates are often unstable and short-lived, which is why the enzyme must stabilize them.

What is a tetrahedral intermediate?

In many hydrolysis reactions, the electrophilic carbon starts out bonded to three things in a flat, triangular arrangement (this is called "trigonal planar" geometry — think of a Mercedes-Benz logo). When a nucleophile attacks this carbon, the carbon is now bonded to four things, and its geometry changes to a three-dimensional shape with four corners — a tetrahedron. This species is called the tetrahedral intermediate. It is higher in energy than the starting material, which is why the enzyme has to stabilize it (see oxyanion hole below).

What is an oxyanion hole?

When the tetrahedral intermediate forms, the oxygen that was originally double-bonded to the carbon (the carbonyl oxygen) picks up a negative charge — it becomes an oxyanion (an oxygen with a negative charge). The enzyme has a pre-built pocket of hydrogen-bond donors (typically backbone -NH- groups) positioned to donate hydrogen bonds to this negatively charged oxygen, stabilizing it. This pocket is called the oxyanion hole. Without it, the tetrahedral intermediate would be too unstable to form, and the reaction would be extremely slow.

What is a covalent intermediate?

In some enzyme mechanisms, one of the enzyme's own residues forms a temporary covalent bond with part of the substrate. This enzyme-substrate species is called a covalent intermediate (also called an acyl-enzyme intermediate in the case of serine proteases). The enzyme must then break this covalent bond in a second step — usually by having water attack — to regenerate the free enzyme.

Part 1: The Four Steps of the Framework

Now that every term is defined, here is the general strategy for proposing a mechanism for any hydrolytic enzyme you have never seen before.

Step 1: Identify the catalytic residues and assign each one a role.

When an example presents a novel enzyme, it will tell you which amino acid residues are in the active site. Your job is to assign a role to each residue. The possible roles are:

Nucleophile — the residue that directly attacks the electrophilic carbon of the bond being broken. Common nucleophilic residues: Serine (Ser, via its -OH), Cysteine (Cys, via its -SH), Threonine (Thr, via its -OH). Water or hydroxide can also serve as the nucleophile if activated by a metal or base.

General base — the residue that accepts a proton to activate the nucleophile. Common general bases: Histidine (His), Glutamate (Glu), Aspartate (Asp). A general base pulls a proton off the nucleophile, making it more reactive. For example, His pulling H^+ off Ser's -OH converts Ser-OH into Ser-O^- (an alkoxide), which is a much stronger nucleophile.

General acid — the residue that donates a proton to the leaving group to help it depart. Often the same residue that acted as a general base earlier in the mechanism — it accepted a proton in Step 1, and now donates it in a later step. His commonly serves this dual role.

Electrostatic stabilizer (positioning residue) — a residue that doesn't directly participate in bond-making or bond-breaking, but holds another catalytic residue in the correct orientation or charge state. Classic example: Asp in the Ser-His-Asp triad. The Asp forms a hydrogen bond with His, which positions His correctly and stabilizes the positive charge that develops on His when it accepts a proton.

Metal activator — a metal ion (most commonly Zn^{2+}) that activates water by lowering its pKa. Water normally has a pKa of about 15.7, meaning it almost never gives up a proton at pH 7.4. But when water coordinates to Zn^{2+}, the metal's positive charge pulls electron density away from the water, weakening the O-H bond and dropping the pKa to around 7. At pH 7.4, a significant fraction of the metal-bound water is now hydroxide (OH^-) — a potent nucleophile.

How to assign roles when you see a list of residues:

Here is the decision process. Look at what you are given and match patterns:

- If you see **Ser** + **His** + **Asp** (or Ser + His + Glu): this is the classic catalytic triad. Ser = nucleophile, His = general base (and later general acid), Asp = electrostatic stabilizer. Expect a covalent intermediate.
- If you see **Cys** + **His** + **Asp**: same triad logic, but Cys replaces Ser as the nucleophile (Cys uses its sulfur atom instead of oxygen).
- If you see **Zn^{2+}** + **Glu** (or Zn^{2+} + His ligands + Glu/Asp): metal-activated mechanism. Zn^{2+} activates water, Glu/Asp may help deprotonate it. No covalent intermediate with the enzyme — hydroxide directly attacks.
- If you see **Ser** + **Tyr** + **Lys** (an unusual variant): same logic as the classic triad, adapted. Ser = nucleophile, Lys = general base (deprotonates Ser), Tyr = stabilizer (may donate to oxyanion hole). Expect a covalent intermediate.
- If you see **two Asp residues** (as in HIV protease): one Asp acts as a general base to activate water, the other acts as a general acid to protonate the leaving group. No covalent intermediate.
- If you see **Glu** alone near a glycosidic bond: one Glu acts as a nucleophile or general base, another Glu acts as a general acid. This is the lysozyme-type pattern.

Step 2: Identify the bond being broken.

The description will either explicitly tell you or describe the substrate in a way that makes it clear. The common bond types in hydrolytic enzymes are:

Peptide bond (C-N): Found in proteins. The carbon is the carbonyl carbon (C=O), and it is bonded to a nitrogen. Hydrolysis breaks this C-N bond, producing a carboxylate on one fragment and a free amine on the other. Enzymes that cleave peptide bonds are called proteases.

Glycosidic bond (C-O): Found in carbohydrates (sugars). This is the bond that links one sugar ring to another, going through an oxygen atom. The electrophilic center is the carbon bonded to that bridging oxygen. Enzymes that cleave glycosidic bonds are called glycosidases.

Phosphodiester bond (P-O): Found in DNA and RNA. A phosphorus atom is bonded to oxygen atoms, linking two nucleotides. Here the electrophilic center is phosphorus, not carbon. Enzymes that cleave these are nucleases or phosphodiesterases.

Ester bond (C-O): Found in fats (lipids). A carbonyl carbon is bonded to an oxygen that links to the rest of the molecule. Enzymes that cleave ester bonds include lipases and esterases.

Identifying the bond tells you where the nucleophilic attack will occur and what the leaving group will be.

Step 3: Apply the universal hydrolysis pattern.

Every hydrolytic enzyme mechanism, regardless of the specific enzyme, follows one of two general paths. Here is how to determine which path applies and what happens in each.

Decision: does a covalent enzyme intermediate form, or not?

Ask: is there a residue acting as the nucleophile (Ser, Cys, Thr)? If yes → covalent intermediate pathway. If the nucleophile is water (activated by a metal or a general base) → direct attack pathway.

Path A: Covalent Intermediate Pathway (Ser/Cys/Thr nucleophile)

This proceeds in two phases. Phase 1 is called "acylation" (forming the covalent intermediate). Phase 2 is called "deacylation" (breaking the covalent intermediate with water).

Phase 1 — Acylation (forming the enzyme-substrate covalent bond):

1. The general base (e.g., His) pulls a proton off the nucleophilic residue (e.g., Ser-OH → Ser-O^-). The electrostatic stabilizer (e.g., Asp) holds the general base in position and stabilizes its newly acquired positive charge.

2. The now-activated nucleophile (Ser-O^-, the alkoxide) attacks the electrophilic carbon of the bond being broken. In the curved arrow notation: an arrow starts from the lone pair on Ser's oxygen and points to the electrophilic carbon.

3. As the nucleophile attacks, the electrophilic carbon cannot have five bonds (carbon's maximum is four). So the double bond between the carbon and its carbonyl oxygen breaks — those electrons shift fully onto the oxygen, giving it a negative charge. The geometry around the carbon changes from flat (trigonal planar) to three-dimensional (tetrahedral). This is the tetrahedral intermediate. The negatively charged oxygen (the oxyanion) is stabilized by the oxyanion hole.

4. The tetrahedral intermediate collapses. The bond between the carbon and the leaving group (e.g., the nitrogen of the peptide bond) breaks. Those electrons go to the leaving group. The general base (which now holds an extra proton from Step 1) donates that proton to the leaving group, helping it depart as a neutral species rather than a charged one.

5. Result of Phase 1: the leaving group has departed, and the remaining fragment of the substrate is covalently bonded to the enzyme's nucleophilic residue. This is the acyl-enzyme intermediate. The general base has returned to its original (deprotonated) state.

Phase 2 — Deacylation (water breaks the covalent intermediate):

6. A water molecule enters the active site.

7. The general base (His, now deprotonated again) pulls a proton off the water, converting it to hydroxide (OH^-).

8. The hydroxide attacks the electrophilic carbon of the acyl-enzyme intermediate. Same curved arrow logic: arrow from the lone pair on oxygen of hydroxide to the carbonyl carbon.

9. A second tetrahedral intermediate forms, again stabilized by the oxyanion hole.

10. The tetrahedral intermediate collapses. This time, the leaving group is the enzyme's own nucleophilic residue (Ser-O^-). The bond between carbon and Ser's oxygen breaks. His donates its proton back to Ser's oxygen, regenerating Ser-OH.

11. The second product is released. The enzyme is back to its original state — ready for another round.

Path B: Direct Attack Pathway (water/hydroxide as nucleophile, no covalent intermediate)

This is simpler — there is only one phase.

1. Water is positioned in the active site, coordinated to a metal ion (Zn^{2+}) or near a general base (Glu, Asp).

2. The metal ion (by withdrawing electron density) or the general base (by pulling a proton off the water) converts water into hydroxide. If both are present, they work together: the metal lowers the pKa, and the base pulls the proton.

3. The hydroxide attacks the electrophilic carbon (or phosphorus) of the bond being broken. Curved arrow: from the lone pair on hydroxide's oxygen to the electrophilic center.

4. A tetrahedral intermediate forms. If a metal is present, it may also coordinate with the oxyanion of this intermediate, stabilizing the negative charge (doing the same job as an oxyanion hole, but using electrostatic attraction from the metal instead of hydrogen bonds from backbone amides).

5. The tetrahedral intermediate collapses. The leaving group departs. A general acid (which may be the same Glu/Asp that acted as a general base, having picked up a proton during step 2) donates a proton to the leaving group.

6. Products are released. Water returns to the metal coordination site or the base re-deprotonates. The enzyme resets.

Step 4: Show electron flow with curved arrows.

Here are the rules for drawing the curved arrows correctly:

Rule 1: Every arrow starts from an electron-rich source. This is either a lone pair on an atom, or an existing bond (which represents a pair of electrons). The arrowhead points to the electron-poor destination — either an atom that will accept those electrons or a bond that will form.

Rule 2: When a nucleophile attacks an electrophilic carbon, draw one arrow from the nucleophile's lone pair to the carbon, and simultaneously draw a second arrow from the C=O double bond up to the oxygen. These two arrows happen at the same time (concerted) — the new bond forms as the old double bond breaks. You cannot show one without the other, because carbon cannot have five bonds.

Rule 3: When a proton is transferred, draw an arrow from the lone pair of the atom accepting the proton to the proton itself, and another arrow from the bond between the proton and its donor atom to the donor atom. The first arrow means "I'm grabbing this proton," and the second means "the bond holding the proton breaks and the electrons stay on the donor."

Rule 4: When the tetrahedral intermediate collapses and the leaving group departs, draw an arrow from the bond between the carbon and the leaving group to the leaving group. This means: the electrons in that bond go with the leaving group. Simultaneously, draw an arrow from the lone pair now on the oxygen back down to reform the C=O double bond.

Rule 5: Every intermediate species you draw should have correct formal charges marked. If an oxygen gained an extra electron pair, it has a negative charge. If a nitrogen lost a lone pair (donated it to form a bond), check whether it has a positive charge. Charges matter — they make the electron bookkeeping visible.

Part 2: The Two Major Enzyme Archetypes in Detail

Archetype 1: The Serine Triad (Ser-His-Asp)

This is the mechanism used by chymotrypsin, trypsin, elastase, subtilisin, and many other proteases and esterases.

The setup:

- Ser (serine): provides the -OH that will become the nucleophile. In its normal state, the oxygen of Ser-OH is not a good enough nucleophile to attack a carbonyl carbon at useful speed. It needs to be activated.
- His (histidine): acts as the general base. Histidine's imidazole ring has a nitrogen with a lone pair that can accept a proton. His will pull the proton off Ser-OH, creating Ser-O^- (the alkoxide ion), which is a powerful nucleophile.
- Asp (aspartate): the negatively charged carboxylate side chain of Asp forms a hydrogen bond with the other nitrogen on His's imidazole ring. This does two things: (a) it orients His perfectly so its basic nitrogen faces Ser's -OH, and (b) when His picks up Ser's proton and becomes positively charged (His-H^+), Asp's negative charge stabilizes that positive charge, preventing the proton from bouncing back to Ser.

The full mechanism, step by step:

(Acylation)

1. The substrate binds in the active site. The carbonyl carbon of the peptide bond to be cleaved is positioned near Ser's -OH.
2. His abstracts (removes) the proton from Ser's -OH. Curved arrow: from His's nitrogen lone pair to the proton on Ser's oxygen. A second arrow from the O-H bond to Ser's oxygen (the electrons from that bond stay on the oxygen, giving it a negative charge). Asp stabilizes the resulting His-H^+.
3. Ser-O^- attacks the carbonyl carbon. Arrow from Ser-O^- lone pair to the carbonyl carbon. Simultaneously, the C=O π-bond electrons shift onto the carbonyl oxygen, giving it a negative charge.
4. The tetrahedral intermediate exists. The former carbonyl oxygen is now an oxyanion (O^-). It is stabilized by hydrogen bonds from the oxyanion hole (backbone NH groups of Gly and Ser residues in chymotrypsin specifically).
5. The C-N bond of the peptide breaks. Arrow from the C-N bond to the nitrogen. His-H^+ donates its proton to the departing nitrogen (arrow from the N-H bond on His to nitrogen of the leaving group, plus arrow from the leaving group nitrogen's lone pair to the proton). This protonation makes the amine a better leaving group.
6. The amine fragment departs. Ser is now covalently bonded to the remaining carbonyl fragment — this is the acyl-enzyme intermediate. His is back to its original deprotonated state.

(Deacylation) 7. Water enters the active site and is positioned near His and the acyl-enzyme carbonyl. 8. His abstracts a proton from water (same arrow pattern as Step 2, but on water instead of Ser). This generates hydroxide ion. 9. Hydroxide attacks the carbonyl carbon of the acyl-enzyme intermediate. Same arrow pattern as Step 3. 10. A second tetrahedral intermediate forms, again stabilized by the oxyanion hole. 11. The C-O bond between the acyl fragment and Ser's oxygen breaks. Ser-O^- departs as the leaving group. His-H^+ donates the proton back to Ser, regenerating Ser-OH. The C=O double bond reforms. 12. The carboxylate product is released. The enzyme is fully regenerated.

Recognition cues for a Ser-triad mechanism:

- The words "serine protease" or "serine hydrolase"
- A catalytic triad of Ser, His, and either Asp or Glu
- An oxyanion hole mentioned or implied
- A covalent (acyl-enzyme) intermediate is expected
- Two tetrahedral intermediates form (one in acylation, one in deacylation)

Archetype 2: Metal-Activated (Zn^{2+}) Mechanism

This is the mechanism used by carbonic anhydrase, carboxypeptidase A, thermolysin, and matrix metalloproteinases.

The setup:

- Zn^{2+}: a metal ion held in place by three amino acid side chains (typically three His residues, or two His and one Glu/Asp, depending on the enzyme). The fourth coordination position is occupied by a water molecule. Zn^{2+} has a +2 charge. This positive charge pulls electron density away from the water molecule bound to it, weakening the O-H bonds. This lowers water's pKa from ~15.7 to ~7, meaning at physiological pH, about half of the metal-bound water has already lost a proton and exists as hydroxide (Zn^{2+}-OH^-).
- Glu (or Asp): positioned near the Zn^{2+}-bound water. Acts as a general base to pull a proton off the water, fully converting it to hydroxide. In some enzymes, this residue also acts as a general acid later.

The full mechanism (using carbonic anhydrase as the template — this enzyme hydrates CO_2, but the principles apply to any Zn^{2+} hydrolase):

For a generic Zn^{2+} protease (like carboxypeptidase A, which cleaves peptide bonds):

1. The substrate binds. The carbonyl oxygen of the peptide bond coordinates to Zn^{2+} (the metal pulls electron density from the carbonyl oxygen, making the carbonyl carbon even more electrophilic). A water molecule also sits coordinated to Zn^{2+}.
2. Glu (acting as a general base) abstracts a proton from the Zn^{2+}-bound water. Arrow from Glu's carboxylate oxygen lone pair to the proton on water; arrow from the O-H bond to the water oxygen. The water is now hydroxide (Zn^{2+}-OH^-).

3. The hydroxide attacks the electrophilic carbonyl carbon. Arrow from hydroxide's lone pair to the carbonyl carbon. The C=O double bond electrons shift onto oxygen.
4. A tetrahedral intermediate forms. Zn^{2+} stabilizes the developing negative charge on the former carbonyl oxygen (electrostatic stabilization — the positive charge of the metal ion attracts and stabilizes the negative charge on the oxyanion). This is the metal-ion equivalent of the oxyanion hole.
5. The C-N bond breaks. The nitrogen of the leaving group picks up a proton — this proton may come from Glu (which picked one up in Step 2), or from solution. Arrow from the C-N bond to nitrogen.
6. The two product fragments are released. A new water molecule coordinates to Zn^{2+}, and the enzyme is reset.

Key differences from the Ser-triad mechanism:

- No covalent intermediate with the enzyme. Hydroxide attacks the substrate directly, and the products are released without any enzyme-substrate covalent bond forming.
- The metal ion plays the role that the oxyanion hole plays in serine proteases — stabilizing the negative charge on the tetrahedral intermediate.
- The metal ion also plays an activating role that His plays in serine proteases — but instead of deprotonating a residue, it lowers the pKa of water itself.

Recognition cues:

- A metal ion (Zn^{2+}, Mn^{2+}, or similar) in the active site
- Residues listed as metal ligands (His, Cys, Glu, Asp coordinating to the metal)
- No Ser or Cys listed as a nucleophile
- The description includes water activation
- Only one tetrahedral intermediate (no acyl-enzyme intermediate)

Part 3: A Decision Flowchart for Novel Mechanisms

When analyzing a novel enzyme mechanism, follow this exact sequence:

Question 1: Is there a metal ion in the active site?

- Yes → You are likely in the metal-activated pathway (Path B). The metal activates water. Go to the Zn^{2+} archetype and adapt it.
- No → Continue to Question 2.

Question 2: Is there a Ser, Cys, or Thr identified as a catalytic residue?

- Yes → You are likely in the covalent intermediate pathway (Path A). That residue is your nucleophile. Look for a nearby His or Lys to serve as the general base, and an Asp or Glu to stabilize. Go to the Ser-triad archetype and adapt it.
- No → Continue to Question 3.

Question 3: Are there two acidic residues (Asp-Asp or Glu-Glu or Asp-Glu)?

- Yes → One acts as a general base (activates water), the other acts as a general acid (protonates the leaving group). This is the aspartic protease pattern (like HIV protease). No covalent intermediate. Water attacks directly.
- No → Re-read the description. The available context should provide enough information to identify the catalytic strategy. Look for any residue that could serve as a nucleophile or any indication of how water is activated.

Question 4: What bond is being broken?

- Match the bond type to the expected products.
- Peptide bond → one fragment gets -COO^-, the other gets -NH_3^+ (at physiological pH)
- Glycosidic bond → one sugar gets -OH where the bond was
- Phosphodiester bond → the nucleophile attacks phosphorus (not carbon); products include a free hydroxyl and a phosphate
- Ester bond → one fragment gets -COO^-, the other gets -OH

Question 5: Draw the mechanism.

- Use the appropriate Path (A or B) from Step 3 above.
- Label every catalytic residue and its role.
- Show every curved arrow.
- Show every proton transfer explicitly — do not skip any.
- Mark formal charges on all intermediates.
- Label the tetrahedral intermediate(s) and identify what stabilizes them.
- If Path A: clearly label the acyl-enzyme intermediate and show both the acylation and deacylation phases.

Part 4: Common reasoning pitfalls and How to Avoid Them

Trap 1: Forgetting the second half of the mechanism. A common incomplete drawing shows the nucleophilic attack and tetrahedral intermediate, then stops. If the mechanism involves a covalent intermediate (Path A), you must show the deacylation phase: water attacks, second tetrahedral intermediate forms, enzyme regenerates. If you stop after acylation, you have only drawn half the mechanism.

Trap 2: Not showing how the nucleophile is activated. Ser-OH does not attack on its own at meaningful rates. You must show His (or equivalent) pulling the proton off Ser before the attack. Similarly, water does not attack on its own — you must show the metal or general base activating it. If your mechanism starts with "nucleophile attacks," you have skipped the activation step.

Trap 3: Ignoring the leaving group's protonation. After the tetrahedral intermediate collapses and the leaving group departs, the leaving group typically needs a proton to become a neutral, stable species. You must show where that proton comes from (usually from His or Glu that picked one up earlier). If your leaving group departs as an anion (e.g., $-NH^-$) without being protonated, you've made an error.

Trap 4: Drawing arrows in the wrong direction. Electrons flow from nucleophile to electrophile. Arrows always start at the electron-rich source and end at the electron-poor destination. If you draw an arrow starting at the electrophilic carbon and pointing toward the nucleophile, the arrow is backwards and the mechanism is wrong.

Trap 5: Forgetting the oxyanion hole or metal stabilization. Every tetrahedral intermediate has a developing negative charge that must be stabilized. If you don't mention or draw the oxyanion hole (for Ser-triad enzymes) or the metal ion stabilization (for Zn^{2+} enzymes), you are missing a critical part of the catalytic strategy.

Trap 6: Confusing which path uses a covalent intermediate. Serine/cysteine nucleophile → covalent intermediate. Metal-activated water/hydroxide → no covalent intermediate. If you draw an acyl-enzyme intermediate for a Zn^{2+} enzyme, that is incorrect. If you show water directly attacking the substrate in a serine protease without first forming the acyl-enzyme intermediate, that is also incorrect.

Part 5: Quick-Reference Summary Table

Feature	Ser-Triad (Path A)	Metal-Activated (Path B)
Nucleophile	Ser-O^- (or Cys-S^-)	Zn^{2+}-OH^- (metal-activated hydroxide)
How nucleophile is activated	His pulls H^+ off Ser	Metal lowers water pKa; Glu may also pull H^+
General base	His	Glu or Asp
Electrostatic stabilizer	Asp (supports His)	Zn^{2+} (stabilizes oxyanion)
Oxyanion stabilization	Oxyanion hole (backbone NH groups)	Metal ion (Zn^{2+}) electrostatic attraction
Covalent intermediate?	Yes (acyl-enzyme)	No
Number of tetrahedral intermediates	Two (one in acylation, one in deacylation)	One
General acid (protonates leaving group)	His (donates H^+ it accepted earlier)	Glu (donates H^+ it accepted earlier)
Enzyme regeneration	His re-protonates Ser in deacylation	New water coordinates to Zn^{2+}