

A Perfect pH-it: Investigating the Relationship Between pH and Enzyme Structure

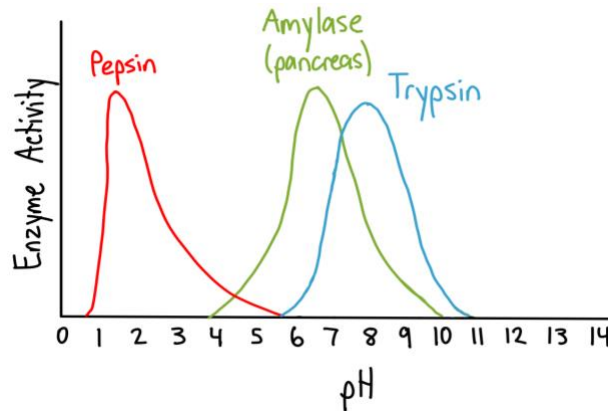
Target Audience: This activity is intended for introductory biochemistry students who are familiar with enzyme activity but are beginning to consider structure-function relationships in biological structures, prominently regarding amino acids.

Estimated Time: 10-20 minutes

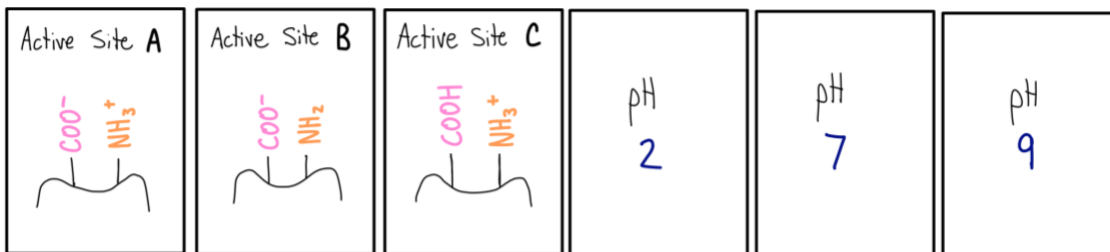
Big Inquiry Question: How does pH affect the activity of different enzymes through structure-function relationships?

Learner activity:

1. Take a minute to observe the provided graph comparing the activity of different enzymes at various pH's, then use the data it provides to answer the following questions:



- a) Estimate the optimum pH for each enzyme. Is the environment acidic, basic, or neutral at each optimum pH?
 - b) Predict what will happen to the activity of the trypsin enzyme if you move +2 pH units up the scale from its optimum pH. How about -2 pH units down the scale?
2. You are now provided 3 cards of simplified optimal active sites of some unknown enzymes, and 3 cards of different pH's. Each active site contains one acidic amino acid **Asp** (COO^-/COOH) and one basic amino acid **Lys** ($\text{NH}_2/\text{NH}_3^+$). ****NOTE:** Simplified amino acid structures are used for educational purposes only, to demonstrate how pH-dependent (de)protonations can affect an enzyme's active site; these are not the true biological structures of the provided enzymes' active sites.



- a) Determine the protonation state of Asp and Lys on each active site.
 - b) Match each active site card with the pH card you think best supports each active site's ability to bind with its specific substrate. Discuss with a partner how each environment (pH) creates the desired/matching active site.
 - c) Now based on which pH supports each enzyme's activity, refer back to the original enzyme graph provided and identify which active site belongs to each enzyme.
3. Earlier you were asked to discuss the effects of moving ± 2 units on the pH scale for one of the given enzymes. After investigating how pH change may affect an enzyme's active site, could you predict what would happen to the structure of an enzyme itself with a major change in pH (ex. ± 5 units)? Explain your thinking.

Instructor Guide:

Purpose: To help students infer the relationship between amino acid structure and enzymatic function, based on the (de)protonation effects of various pH environments.

Materials: To complete this activity, the only materials required are a few sheets of paper and a writing utensil to create cards (drawn out in the Learner Activity section) that can be matched with one another.

Facilitations Notes: Have the instructor create the cards and enzyme graph prior to facilitating the activity during class time. To begin the activity, have students perform the observation section individually to enforce everyone to make their own predictions about the graph. After they are done, form small groups of 2-3 students and distribute one set of cards to each group for the main activity. Have them work together to match the cards and answer the rest of the questions in the activity collaboratively.

Expected Responses:

1. a) Pepsin optimum pH: 1.5 - 1.6 (acidic)
 Amylase (pancreas) optimum pH: 6.7 - 7.0 (neutral)
 Trypsin optimum pH: 7.8 - 8.7 (basic)
 - b) The enzyme will be much less effective with a ± 2 pH change as it moves away from its optimum pH. The reaction may happen much slower as it doesn't catalyze as well due to weaker enzyme substrate interactions that arise from changes in the active site shape.
2. a) b) c)

	State of each amino acid when active site is working optimally		pH
	Asp	Lys	
Active Site A (Amylase)	Deprotonated	Protonated	7
Active Site B (Trypsin)	Deprotonated	Deprotonated	9

Active Site C (Pepsin)	Protonated	Protonated	2
---------------------------	------------	------------	---

- b) continued: Acidic environments have high $[H^+]$ concentration so can donate hydronium to the amino acids (protonation); basic environments have a low $[H^+]$ concentration so steal a hydronium from the amino acids (deprotonation). The (de)protonation of these amino acids result in slight conformational changes within the active site, allowing the substrate with a well-defined shape to bind easiest at the enzyme's optimum pH.
3. With extreme pH changes, the amino acids that compose the tertiary structure of an enzymatic protein may also be (de)protonated, which could lead to denaturation of the enzyme as the structural ionic and hydrogen bonds may be broken, causing a change in its shape. This would result in inactivity of the catalytic enzyme as the entire enzyme-substrate binding concept is built on specificity of enzymes to only bind with substrates of particular shapes that match their active site conformation.

Safety Notes: Beware that scissors are sharp when cutting out the paper cards.

Accessibility Notes: Allow students to share their thinking any way that works best for them (writing, speaking, or creating additional visual aids). Have the facilitator present the original graph with accessibility in mind, using a large display and verbal cues to reinforce the concept.

SDG 4 Connection: This activity contributes to quality education as it supports scientific literacy by displaying the importance of comprehending fundamental science concepts (such as pH) in order to infer their effects onto higher level content (like amino acid structure and enzyme activity). The *dual coding* learning strategy presented by this activity, encapsulates how to build scientific literacy by linking verbal information taught in lecture with visual elements that require students to observe, identify, and expand their thinking through collaborative discussion and tactile tasks. Tailoring material into multiple forms of media is highly beneficial to active learning as it allows you to create a big picture idea of the concept through auditory, visual, and physical elements.

References:

1. Clark, J., & Harvey, D. (2025, June 29). *10.7: The effect of pH on enzyme kinetics*. Libretexts.
[https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Physical_Chemistry_for_the_Biosciences_\(LibreTexts\)/10%3A_Enzyme_Kinetics/10.07%3A_The_Effect_of_pH_on_Enzyme_Kinetics](https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Physical_Chemistry_for_the_Biosciences_(LibreTexts)/10%3A_Enzyme_Kinetics/10.07%3A_The_Effect_of_pH_on_Enzyme_Kinetics)
2. Pandey, N., Tiwari, A., Begam, T., & Pal, N. (2026). Effect of pH, temperature, and concentration on enzyme activity: a comprehensive review. *Int. J. of Pharm. Sci.*, 4(1), 2420-2424.
<https://doi.org/10.5281/zenodo.18344642>

I consent to sharing this resource publicly under a CC BY-NC-SA license.