

# Student Handout 3

## Understanding an Enzyme Active Site

In the first protein folding activity, you learned that a protein begins as a linear sequence (primary structure) of amino acids that spontaneously folds into a compact 3D shape (tertiary structure) following basic principles of chemistry.

In the second activity, you learned that the 3D shape of a protein consists of stretches of alpha helices and/or beta sheets (secondary structure) connected by short turns of less regular protein structure.

In the space below, draw and label examples of primary, secondary and tertiary structures.



Proteins perform many different functions in cells. Some proteins function as structural supports for the cell's architecture. Others transport small molecules — such as oxygen or neurotransmitters — between cells.

### Enzyme Active Sites

In this third activity, you will explore enzymes — a major class of proteins. Enzymes bind a specific small molecule — a substrate — and then catalyze a chemical reaction that changes the substrate in some way. The active site of an enzyme is the **region** of the protein that is able to bind a specific substrate (usually a small molecule) and then catalyze the reaction.



### Modeling an Active Site

Imagine that your 4-foot mini-toober represents a protein consisting of 200 amino acids.

1. Begin folding your mini-toober into the shape of a protein by creating a three-stranded beta sheet and two short alpha helices. The beta sheet and alpha helices represent your protein's secondary structure. See photos A through D.



2. Fold the beta sheet and the alpha helices into a compact, globular shape. See photo E.



3. Use three connectors to stabilize the overall 3D shape of the folded protein. See photos F and G.

These connectors stabilize your protein's structure in the same way that hydrogen bonds, which are present in alpha helices and beta sheets, stabilize the structure of a real protein. You now have a stable 3D structure – upon which you can precisely place three specific amino acid sidechains to create an enzyme active site.



### Modeling an Active Site (continued)

4. Create an active site in a shallow crevice on the surface of your protein by adding three amino acid sidechains – a serine, a histidine and a glutamic acid – to your mini-toober in such a way that all three sidechains are within 2 cm of each other. See photos H and I.

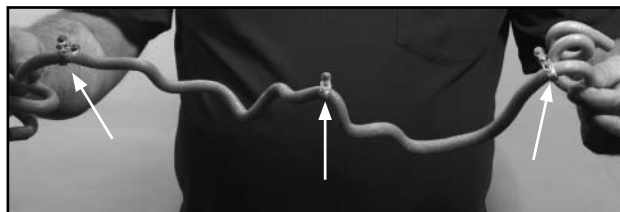
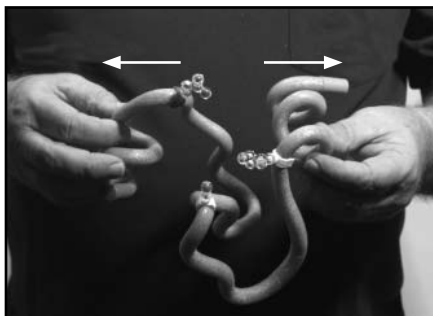


5. The three amino acid sidechains that make up your enzyme's active site interact with a substrate to catalyze a specific chemical reaction. This requires that the sidechains be precisely positioned in 3D space. Examine your protein, noting how its secondary and tertiary structure combines to provide a stable scaffolding, or framework, upon which the active site amino acids are precisely positioned relative to each other.

6. Now carefully remove the connectors that were stabilizing your folded protein. See photo J.



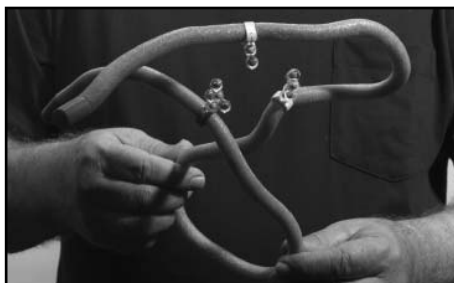
7. Holding your protein with one hand near the N-terminus end and the other near the C-terminus end, slowly move your hands away from each other – simulating the unfolding (denaturation) of your protein.



The 3 active site amino acids — that were close together in a folded enzyme — are now far apart in the linear sequence of the protein.

## Modeling an Active Site (continued)

Notice that without the stabilizing effect of the hydrogen bonding in your protein's secondary structure, the normal thermal motion experienced by proteins would cause them to unfold (denature).



- Describe the kinds of interactions (bonds) that are present in your protein's secondary and tertiary structure that contribute to the stability of this scaffolding.

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- Describe your observations of the distribution of the three active site amino acids in your enzyme?

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- **Optional Jmol Activity** - Active Site Jmol (see AASK Lessons on website)