3-D Molecular Designs

. . . where molecules become real ™

Amino Acid Starter Kit

Key for Student Handout 3

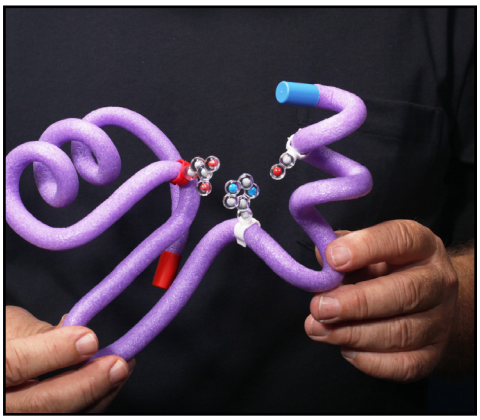
Understanding an Enzyme Active Site

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.

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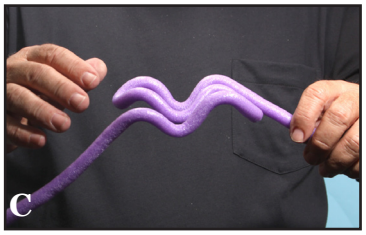
Modeling an Active Site

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.


A


B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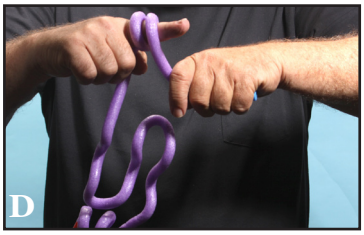
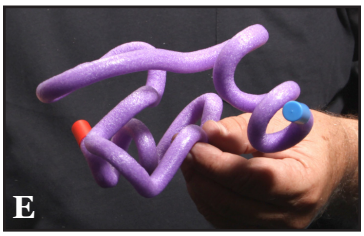
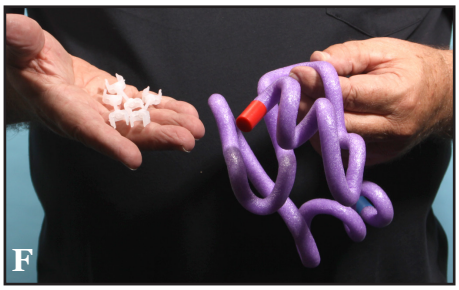

D

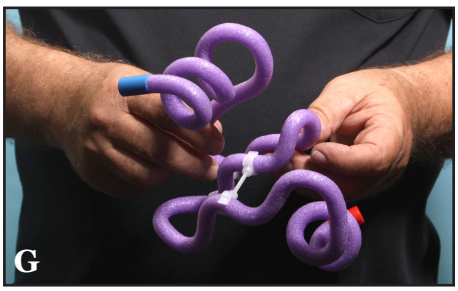
2. Fold the beta sheet and the alpha helices into a compact, globular shape. See photo E.


E

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

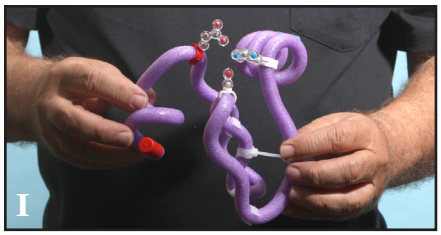
   These connectors stabilize your protein's structure in the same way that hydrogen bonds, which are present in alpha helices and beta sheets, stabilize the structure of a real protein. You now have a stable 3D structure – upon which you can precisely place three specific amino acid sidechains to create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


F


G

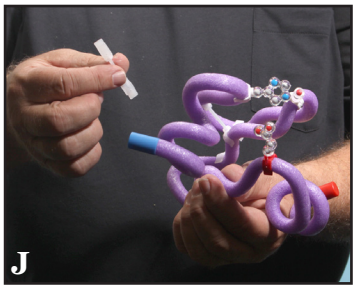
Modeling an Active Site (continued)

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.

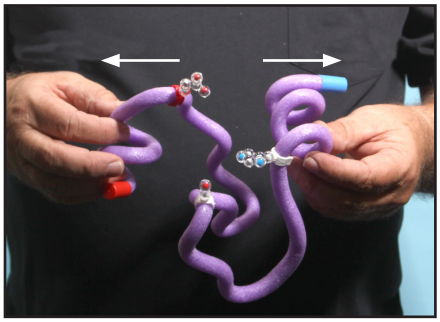


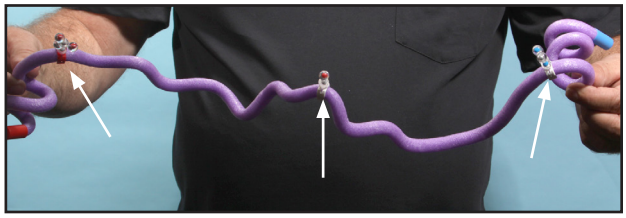
5. The three amino acid sidechains that make up your enzyme's active site interact with a substrate to catalyze a specific chemical reaction. This requires that the sidechains be precisely positioned in 3D space. Examine you protein, noting how its secondary and tertiary structure combines to provide 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.

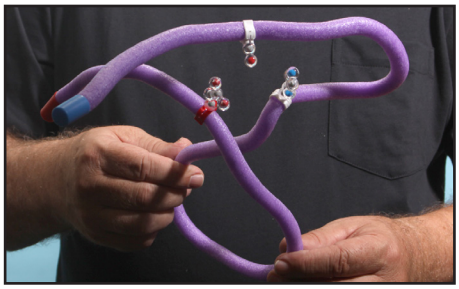


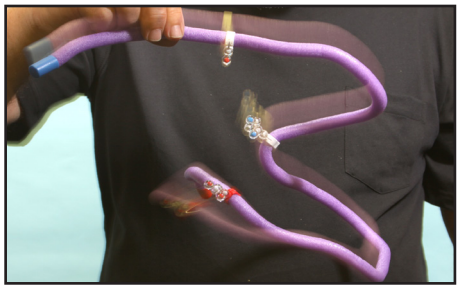


The 3 active site amino acids — that were close together in a folded enzyme — are now far apart in the linear sequence of the protein.

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.

The protein's secondary structure (both alpha helices and beta sheets) are stabilized by **hydrogen bonds** — between the polar nitrogen and carbonyl oxygen atoms of the protein's backbone.

The protein's tertiary structure is stabilized by a variety of bonds and interactions between the amino acid sidechains that make up the protein. Bonds that stabilize the protein include: **hydrogen bonds** between polar sidechains and **electrostatic bonds** between oppositely-charged sidechains (acidic and basic sidechains). **Hydrophobic interaction** between hydrophobic sidechains — as they try to minimize their interaction with water — is another major force that stabilizes a protein's tertiary structure.

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

The surprising thing about an enzyme active site is that the three amino acids — that were positioned very close together in the 3D shape of the protein — are actually very far apart in the linear sequence of the amino acids that make up the protein. The protein has to fold into its 3D shape for the sidechains that make the active site to come together, so they can perform their function.

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

Teaching Points

Although most enzymes consist of 200 or more amino acids, the active site of an enzyme is made up of only 2 to 3 amino acids that are precisely positioned in 3D space. In this activity, your students will be asked to think about how all the other amino acids in the enzyme create a compact, stable scaffold upon which the 2-3 active site amino acids can be positioned. This activity will also demonstrate the role of protein secondary structure in achieving this stable scaffold. In addition your students may be surprised to discover that the three active site amino acids in this example are very far apart from each other in the linear sequence of amino acids that make up the protein.

Key Points

Enzyme active sites are composed of a small number (2-3) of amino acids that are precisely positioned in 3D space such that their sidechains create the chemistry needed to catalyze a reaction.

Protein secondary structure (alpha helices and beta sheets) provides that stable scaffolding upon which the critical active site amino acids can be precisely positioned in 3D space.

The 2-3 amino acids that come together in 3D space to create an enzyme active site are very far apart in the linear sequence of the amino acids that make up the protein.