

## Key Teaching Points for the Substrate Specificity Kit®

### Overall Student Learning Objective: What Factors Influence Substrate Specificity?

Students will:

- Construct a model of a substrate and examine its chemical properties.
- Engineer an enzyme active site specific to the substrate constructed.
- Explore different types of specificity including stereochemical specificity and absolute specificity.
- Discover how subtle changes in enzyme structure – either in the active site or elsewhere in the enzyme’s structure – can potentially have a significant impact on substrate binding in the active site.

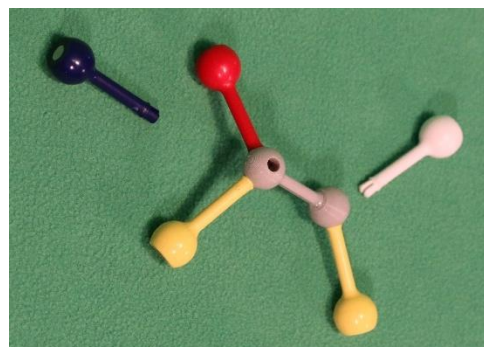
★ Complete lesson guide coming soon at:

<http://www.3dmoleculardesigns.com>

### Assemble the Substrate

Build the substrate following these specifications:

- Join the 4-hole sphere with the 2-hole sphere and post.
- Connect one yellow functional group to the 4-hole sphere and the second yellow functional group to the 2-hole sphere and post.
- Randomly connect the other functional groups to the remaining holes in the spheres.
- Please note: When connecting or disconnecting the functional groups with the spheres, align the pegs and holes straight into each other. Bending the pieces at an angle to connect or disconnect them disfigures the pieces and permanently loosens the connection between the functional groups and the spheres.



### Assemble the Enzyme

Construct a model of the enzyme by placing the metal clips along the entire length and in random order on a 3-foot or 6-foot mini toober.

The toober represents the alpha carbon backbone of the enzyme. The colored band on each of the metal clips provides information about the properties of specific amino acid side chains that, when folded into a complex three-dimensional shape, will comprise the active site of the enzyme. The color coding for these bands is as follows:

- Blue** – positively charged (basic) side chain
- Red** – negatively charged (acidic) side chain
- White** – polar hydrophilic side chain
- Yellow** – nonpolar hydrophobic side chain



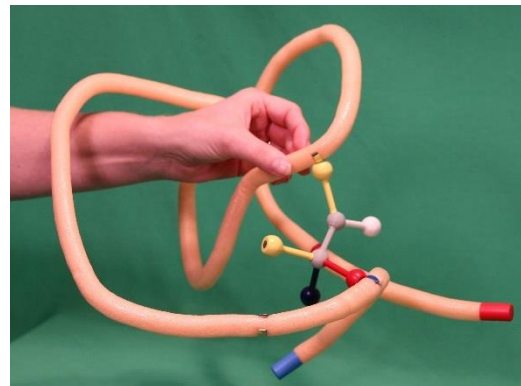
## Assemble the Enzyme-Substrate Complex

---

### Construct the enzyme-substrate complex by folding the toober around the substrate.

Be sure to keep the basic principles of chemistry in mind when engineering your enzyme's structure.

1. Which part of the substrate should match to the yellow metal clip?
2. Which part of the substrate should match to the white metal clip?
3. Which part of the substrate should match to the red metal clip? Explain your answer.
4. Which part of the substrate should match to the blue metal clip? Explain your answer.
5. Trade your substrate for a substrate that is not the same as your original substrate. Test the new substrate to see if it will fit into your enzyme's active site. Explain how your enzyme is specific to the substrate you have created.
6. Using your own substrate again, rotate the functional groups around the movable bond between the two gray spheres and try to dock the new configuration back into the active site. What do you propose as the purpose for holding the substrate in a particular conformation?



You may wish to have your students incorporate protein secondary structures when engineering the shape of their enzymes.

7. How does the introduction of alpha helices and beta sheets impact the enzyme's structure?

