3D Molecular Designs
...where molecules become real ™

Substrate Specificity Field Test Kit©

6-Foot Mini Toober Activity

Student Handout

Big Idea

The interaction between the substrate and enzyme is highly specific. Even a slight change in shape of either the substrate or the enzyme may alter the efficient and selective ability of the enzyme to catalyze the reaction.

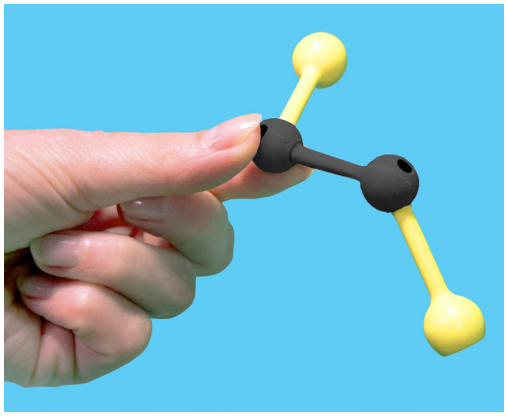
Introduction

Human cells have more than 100,000 different proteins, many of which are enzymes. There are between five million and two trillion molecules in a typical human cell. Each enzyme will catalyze a reaction with only a small subset of all these molecules in the cell. *How does the enzyme know with which molecules (substrates) to react?*

Assembling the Substrate

1. Follow the directions below and on the next page to construct the substrate.

1 a. Join the 4-hole sphere with the 2-hole sphere and post.



1 b. Connect one yellow functional group to the 4-hole sphere and the second yellow functional group to the 2-hole sphere and post.

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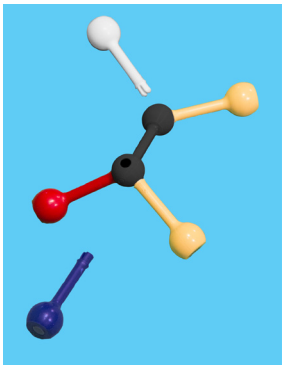
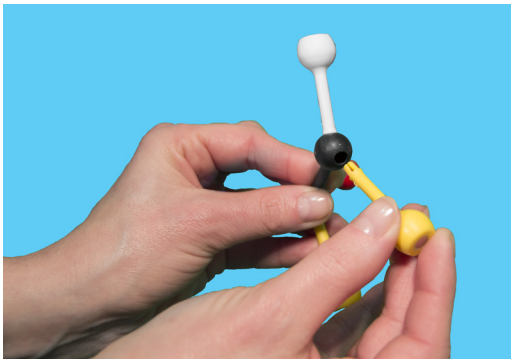
Assembling the Substrate Continued

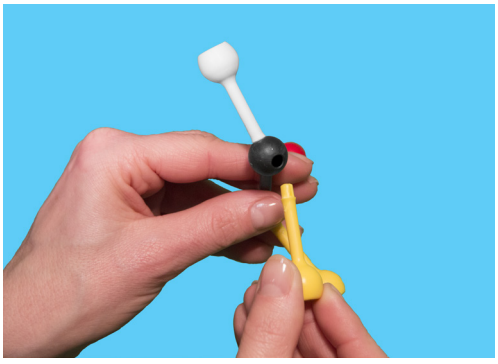
1 c. Randomly connect the other functional groups to the remaining holes in the spheres.



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.



yes



no

The structure you have assembled represents a generic substrate. The colored pieces represent the properties of various functional groups or atoms:

a. Blue – positively charged group
b. Red – negatively charged group
c. White – polar hydrophilic group
d. Yellow – nonpolar hydrophobic group

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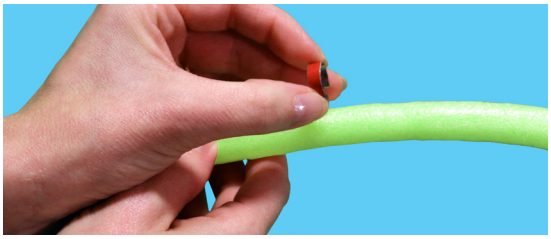
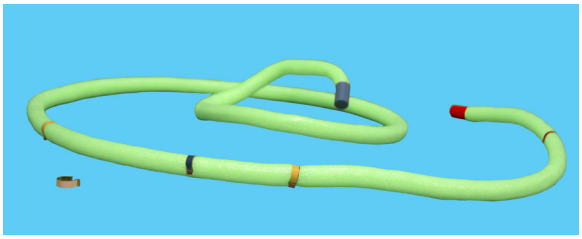
2. Sketch the structure of the substrate you have constructed in the space below. Label each of the groups in your substrate using the designations:

a. blue - positive
b. red - negative
c. white - polar
d. yellow - hydrophobic

Assembling the Enzyme

3. Place the metal clips along the entire length and in random order on the 6-foot mini toober.





What does the toober represent in your model?

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The colored bands on each of the metal clips provide 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:

a. Blue – positively charged (basic) side chain
b. Red – negatively charged (acidic) side chain
c. White – polar hydrophilic side chain
d. Yellow – nonpolar hydrophobic side chain

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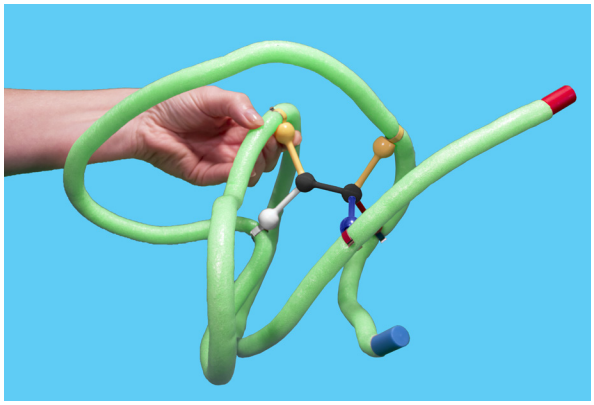
Constructing the Enzyme-Substrate Complex

4. 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.



4 a. Which part of the substrate should match to the yellow metal clips?

4 b. Which part of the substrate should match to the white metal clip?

4 c. Which part of the substrate should match to the red metal clip? Explain your answer.

4 d. Which part of the substrate should match to the blue metal clip? Explain your answer.

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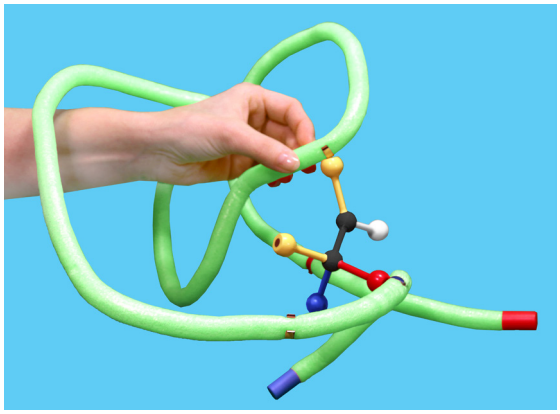
5. Use the Amino Acid Side Chain Chart© found on the last page of this handout to choose an appropriate example R group (residue) for each of the metal clips on the alpha carbon backbone of the toober. Sketch and label these structures below.

6. Shake the enzyme structure, taking note of the overall stability of the design.



6 a. Explain why structural instability may not be a desirable characteristic of enzyme structure.

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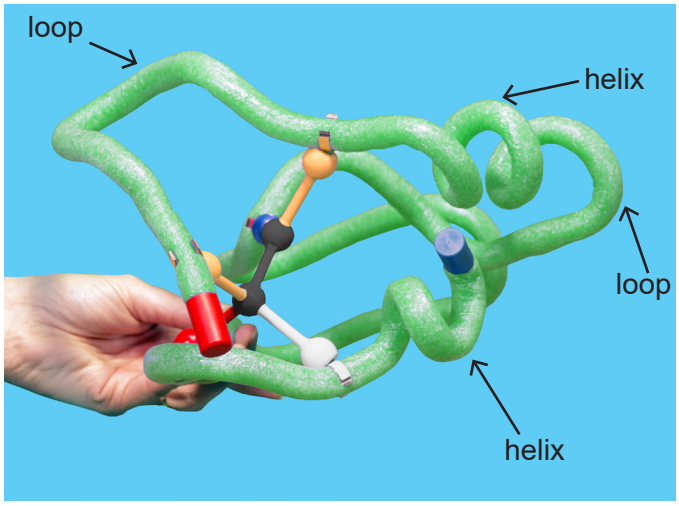
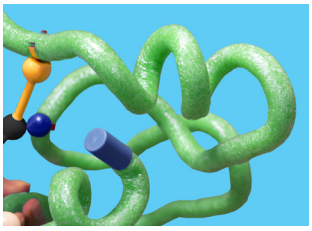
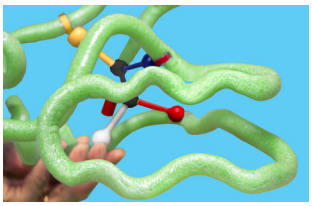




helix



beta sheet

7. Refold the enzyme adding secondary structure (alpha helices and/or beta sheets) to the design. Please note that the five metal clips should NOT be incorporated within the secondary structures but rather in loops found between the secondary structures. Shake the new enzyme structure you have created. What do you notice about the stability of the enzyme's design with the inclusion of secondary structure?

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8. Sketch (or photograph), label and discuss the N-terminus, C-terminus, side chain properties and secondary structures in the enzyme you have engineered.

9. Take note of the location of the side chains (metal clips) that comprise the active site of your enzyme. Is it necessary for the side chains to be adjacent to each other in order to form an active site? Provide evidence for your answer from your model.

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10. Label the types of interactions between the substrate and the enzyme as one of the following and define each type. These are some of the same interactions that occur when proteins fold into their tertiary structures.

a. Salt bridge –

b. Hydrophilic interactions –

c. Hydrophobic interactions –

d. Disulfide bond –

11. Did all of the above interactions occur between your enzyme and substrate. Why or why not?

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_____
_____
_____

12. Compare your enzyme-substrate complex to others in the class. Identify similarities.

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13. What differences can be observed between the enzyme-substrate complexes?

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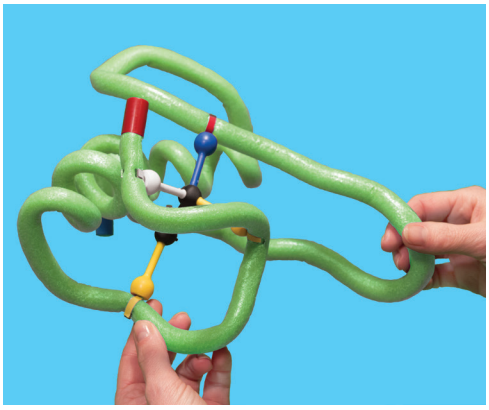
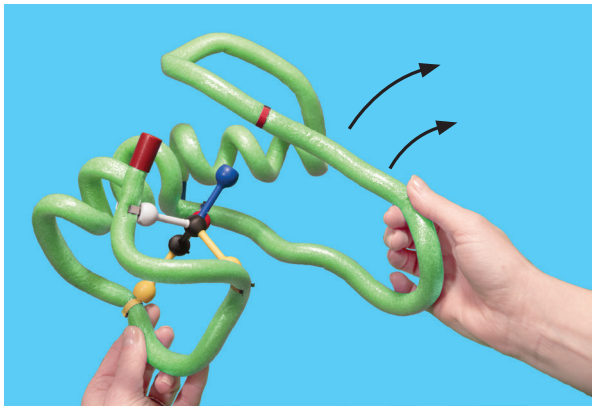
14. Subtle changes in the 3-dimensional shape of the enzyme can potentially have a significant impact on the strength of binding of the substrate in the active site. Move the N-terminus end, C-terminus end or “loops” found in your enzyme and observe the effect slight changes may have on the substrate binding.





14 a. How did the contact points change when you changed the enzyme’s structure?

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14 b. Explore changing the shape of other parts of the enzyme. What changes in the enzyme shape caused the greatest disruption to binding the substrate?

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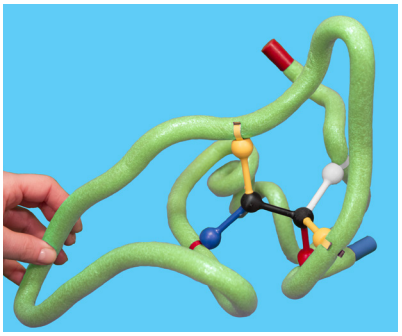
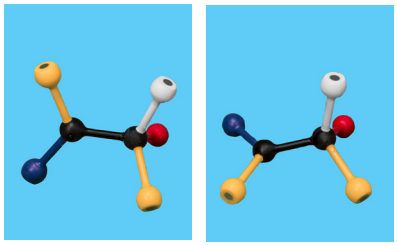
Investigating Substrate Bond Rotation

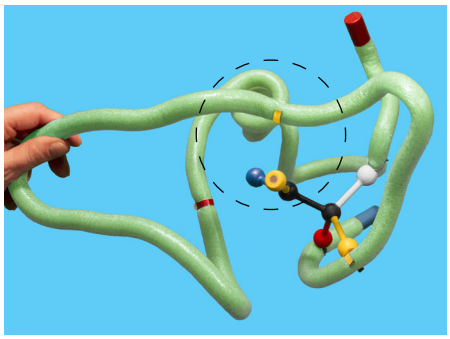
15. In the past it was thought that both enzymes and substrates were rigid structures with the substrate (the key) fitting perfectly into the active site (the lock). The lock and key model implies that the enzyme has an optimum substrate, whereas all other substrates fit less perfectly. We now know that some enzymes can catalyze a reaction on a range of different substrates. The induced fit model proposes that the substrate induces the active site to take on an ideal shape to accommodate it. This new model of enzyme action explains why some enzymes can catalyze a wide range of substrates. Additionally, the substrate is not a passive spectator in the process. Bond rotation may occur to fix the substrate in a particular conformation allowing for catalysis. Gently remove the substrate from the enzyme. Rotate the functional groups around the movable bond between the two black spheres and try to dock the new configuration back into the active site.



Original



Example of bond rotation



Redocking with bond rotation

15 a. Describe how well the substrate binds in the active site after rotating the bond.

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15 b. What might be the purpose for holding the substrate in a particular conformation?

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15 c. Return the substrate to its original conformation.

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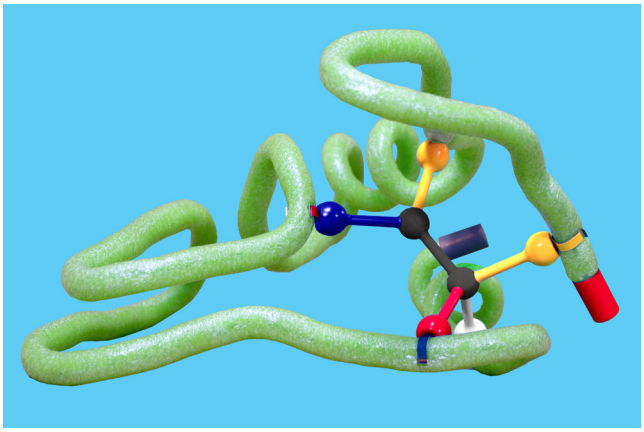
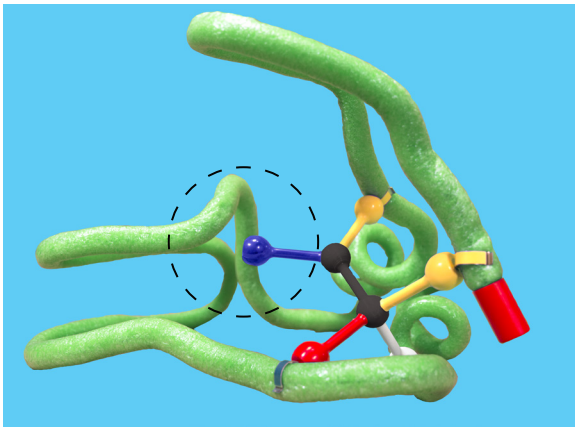
Creating Variant Forms of the Enzyme

Imagine that a mutation in the DNA has caused a variant form of the enzyme.





16. Remove one of the clips from the active site to simulate this scenario.

16 a. Describe what effect the loss of one of the active site side chains has on the strength of binding to the substrate.

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16 b. Return the substrate to its original conformation.

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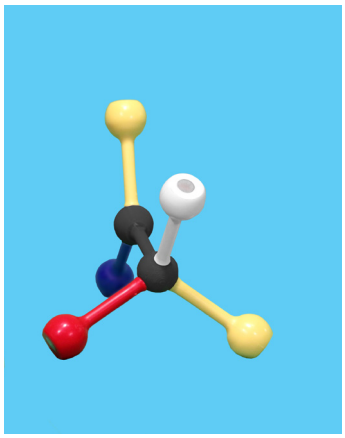
Exploring Enzyme-Substrate Specificity - Stereochemical Specifity

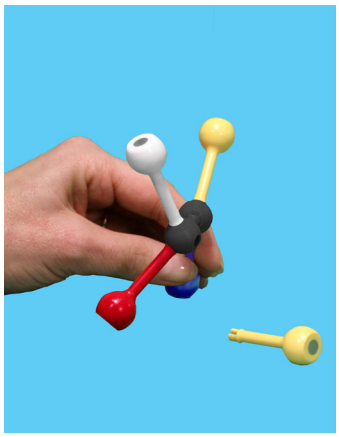
Specificity is a molecular recognition mechanism that operates through the **structural and conformational complementarity** - or **fit** - between an enzyme and its substrate. There are several types of specificity. You will model two types of specificity.

17. To determine if your enzyme has **stereochemical specificity (optical specificity)**, gently remove the substrate from the enzyme you have constructed without altering the structure of the enzyme. Exchange two of the groups on the 4-hole sphere to create an enantiomer (mirror image) of your substrate.

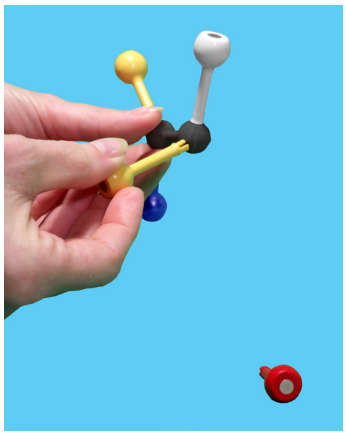
Constructing an Enantiomer



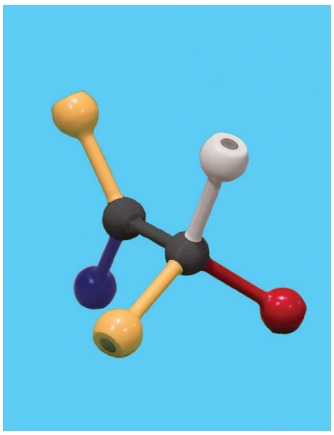
Original



Remove two functional groups



Swap the two functional groups



Mirror

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17 a. Sketch (or photograph) the original substrate and enantiomer you have created.

17 b. Test the new structure to see how well it fits into the active site.

17 c. Sketch (or photograph) the enantiomer bound to the enzyme.

17 d. Describe differences in how the enantiomer binds to your enzyme.

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17 e. Was your enzyme “stereospecific”? Explain your answer.

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17 f. Write a definition of stereochemical specificity.

_____

_____

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17 g. Optional challenge: Search to find a real life example of stereochemical specificity. Describe the results of your search.

_____

_____

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_____

17 h. Return your substrate to the original form.

Exploring Enzyme-Substrate Specificity - Absolute Specificity

18. To determine if your enzyme has **absolute specificity (substrate specificity)**, trade your original substrate for a substrate that is neither the same as your original substrate nor an enantiomer of your original substrate.

18 a. Sketch (or photograph) the new substrate structure.

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18 b. Test the new substrate to see if it will fit into your enzyme's active site. Sketch (or photograph) the new substrate binding to your enzyme's active site.

18 c. Describe your results.

18 d. Write a definition of absolute specificity.

18 e. Optional challenge: Search to find a real life example of absolute specificity. Describe the results of your search.

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Evaluating the Model

19. George E. P. Box, a British mathematician and a professor of statistics at the University of Wisconsin, has said, "All models are wrong; some models are useful." While models help us to build our conceptual understanding of a topic, they are a simplification of reality. Answer the following questions to evaluate the model you have just used.

19 a. What new information have you discovered by using this model? In other words, how is the model "useful"?

_____

_____

_____

19 b. What are some limitations of this model? In other words, how is the model "wrong"?

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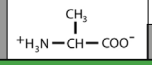
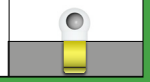
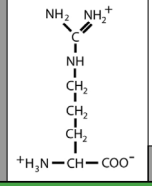
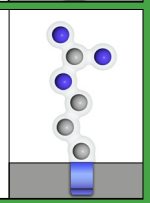
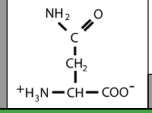
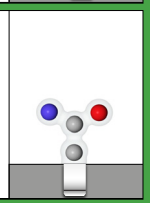
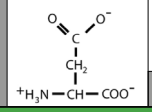
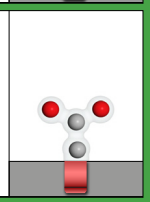
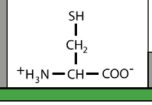
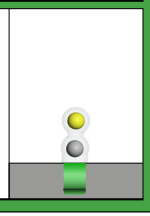
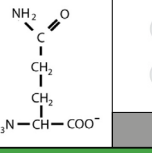
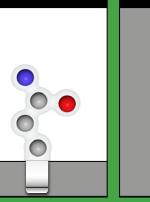
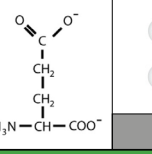
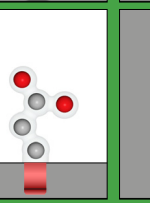
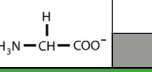
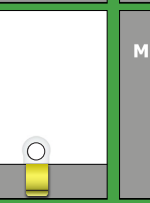
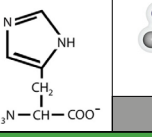
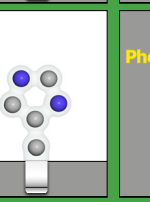
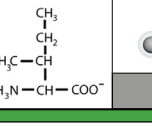
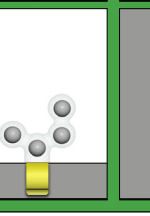
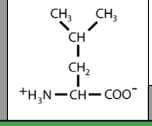
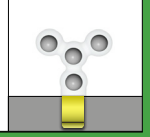
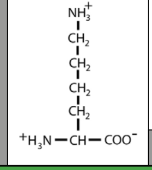
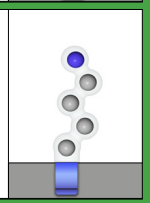
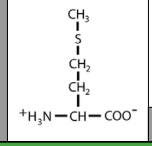
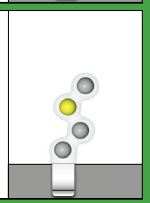
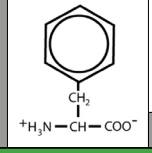
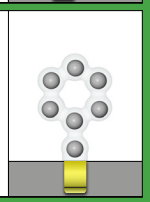
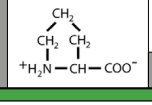
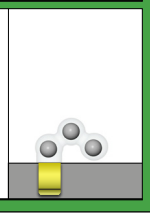
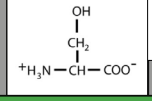
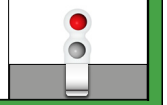
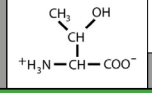
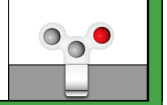
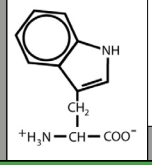
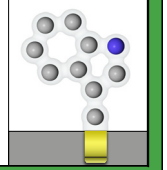
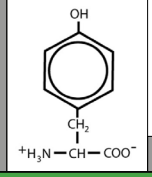
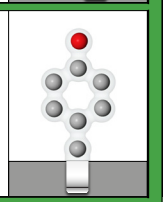
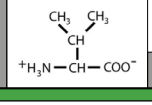
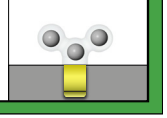
Amino Acid Side Chain Chart©

Name	Amino Acid	Side Chain
Alanine Ala A		
Arginine Arg R		
Asparagine Asn N		
Aspartic Acid Asp D		
Cysteine Cys C		
Glutamine Gln Q		
Glutamic Acid Glu E		
Glycine Gly G		
Histidine His H		
Isoleucine Ile I		
Leucine Leu L		
Lysine Lys K		
Methionine Met M		
Phenylalanine Phe F		
Proline Pro P		
Serine Ser S		
Threonine Thr T		
Tryptophan Trp W		
Tyrosine Tyr Y		
Valine Val V		

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Atom Color Key

Carbon
Oxygen
Nitrogen
Sulfur

Negative Charge

Aspartic Acid
Glutamic Acid

Cysteine

Positive Charge

Lysine
Arginine

Amino Acid Property Key

Amino acid clip color and name color indicate property

Clips attach to mini toobers in 3D Molecular Designs' kits

Hydrophobic

Tryptophan
Phenylalanine
Alanine
Leucine
Valine
Proline
Glycine
Isoleucine

Hydrophilic

Methionine
Tyrosine
Histidine
Glutamine
Serine
Threonine
Asparagine