Exercise 2

Molecular Structure

Keywords: polarity, hydrophobic, hydrophilic, building blocks, macromolecules, side chains, protein folding

Introduction:

The purpose of this lab exercise is to improve your understanding of the structure of, and interactions between, molecules that are important for life. Some of these molecules are small, like water. Other molecules, like proteins, are very large and are referred to as macromolecules. Many macromolecules are polymers made of repeating units of smaller molecules called building blocks. The specific composition of building blocks, the types of bonds that connect them and the order in which they are connected are important determinants of the three-dimensional structure.

In this exercise, you will first explore the chemical nature of water that is the solvent in which biological molecules are found. Water’s chemical nature strongly influences how biological molecules interact. The models you will view include water, small biological molecules, building blocks and macromolecules. Some of the models are to scale allowing you to compare their size. As you complete the exercise, pay close attention to the types of chemical groups on each biological molecule and the three-dimensional structure. These features are critical to biological reactions and molecular interactions that make life possible.

The models will be available for viewing at least through the end of the week, so if you do not have time to complete this exercise by the end of lab, you can return to finish during a time when lab is not in session. Even if you complete the exercise, you may wish to return to look at the models again once we complete our discussion of biological molecules in class.

Principles:

Review the appropriate sections of the textbook and your class notes before coming to lab. Additional theoretical information is provided with each of the exercises.

Procedures:

Complete the activities on the handout that will be provided in lab. The topics covered are:

1. Water Basics
2. Molecules of Life
3. Assembly of Macromolecules
4. Amino Acid Structure
   a. Amino Acid Monomers
   b. Amino Acid Properties
5. Protein Secondary Structure
   a. Secondary Structure in Protein Folding
   b. Alpha Helices & Beta Pleated Sheets – A Closer Look
6. Protein Folding
7. Enzyme Structure & Function
   a. Substrate binding
   b. Enzymes in Action (optional)
Activities, Background & Instructions

Throughout the exercises, you will notice that most of the models use cpk colors. In cpk coloring, each element is represented by a different color. For our purposes, the most important ones to recognize are:

<table>
<thead>
<tr>
<th>Atom</th>
<th>Carbon</th>
<th>Hydrogen</th>
<th>Oxygen</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
<th>Sodium</th>
<th>Chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Gray</td>
<td>White</td>
<td>Red</td>
<td>Light blue</td>
<td>Orange</td>
<td>Blue to Purple</td>
<td>Green</td>
</tr>
</tbody>
</table>

Note: slight variations in convention exist, so the shades of colors may vary between model representations.

Activity 1. Water Basics

1. Water molecules interact with each other by forming hydrogen bonds. That is, the positive hydrogens are attracted to and “stick to” the negative oxygens. This phenomenon is called cohesion, which means attraction between like molecules. Hydrogen bonds are different from the covalent bonds that hold together the hydrogen and oxygen in single water molecule.

   Use the Water Kit® to:

   - Examine the way two water molecules interact. The magnets simulate the polar nature of the water molecule. Unequal sharing of electrons in the covalent bond between hydrogen and oxygen pulls more electrons to the oxygen, making it slightly negative and leaving the hydrogens slightly positive.

   - Hydrogen bonds are formed when the slightly positive hydrogen is aligned close enough to the slightly negative oxygen. One water molecule can form hydrogen bonds with four other water molecules — 1 through each of the hydrogens and 2 through the oxygen.

   - Compare the difference in effort it takes to pull 2 water molecules apart with that needed to pull 1 of the hydrogen atoms from the oxygen of a single water molecule. Simulate the constantly changing bonding patterns by putting the water models in a clear container and shake. Hydrogen bonds are roughly 20 times weaker than covalent bonds. They rapidly form, break and re-form. Each bond lasts only a few trillionths of a second as the water molecules constantly form new bonds with a succession of partners.

2. Sodium ions and chloride ions are held together tightly by electrostatic forces, forming crystalline salt — an ordered crystal. When salt is added to water, sodium and chloride ions interact with water. Positive hydrogen forms an electrostatic interaction with the chloride ions (green), and negative oxygen forms an electrostatic interaction with sodium ions. When enough water molecules surround the sodium-chloride crystal, the ions are pulled apart and salt is dissolved.

   Use the Water Kit® to show how water can dissolve salt, by forming a “shell” of water molecules around the negatively charged chloride ion with their hydrogens atoms directed toward the chloride. The magnets in the hydrogen atoms will snap onto the chloride’s magnets. Form another “shell” around the sodium, with the oxygen atoms directed toward the sodium and the magnets in the oxygen will snap onto sodium’s magnets.

* The Water Kit® activity can demonstrate the principle behind hydration, but it can’t simulate the true chemical reaction. For example to approximate a physiological salt solution (~0.1M), the ratio of water molecules to ions should be roughly 600 water molecules for every sodium chloride ion pair, since the concentration of water is 55.6M.
Find the ethyl group in your water cup (i.e. -CH$_2$CH$_3$). Notice: none of the hydrogens on the ethyl group have a magnet, so they do not stick to the oxygens on the water. Depending on how it was left the last time it was used, it may have a hydrogen or a hydroxyl group attached to it. For the next two steps, you will need to switch out the hydrogen atom and the hydroxyl group on the ethyl group to observe the difference between ethane and ethanol.

To make ethane, insert the hydrogen that has a post into the hole of the second carbon.

![Ethane model](image)

3. Many chemical compounds are made of carbon atoms covalently bonded to hydrogens. Oily substances are made of carbon-hydrogen bonds and are non-polar. In a carbon-hydrogen bond the electrons are equally shared between the carbon and the hydrogen, and the bond is not charged (also called non-polar). As a result, carbon-hydrogen bonds don’t have charges to form hydrogen bonds with water, so water and oil don’t hydrate (mix). If you shake the molecules, you will see tiny droplets as the water tries to create open spaces to for non-polar oils or various organic solvents.

*Use the Water Kit* to show the lack of hydrogen bonds with ethane — a simple two carbon molecule — and how a water “cage” forms around ethane.

Now make ethanol: Locate the hydrogen that is surrounded by three tiny triangular bumps. That will be the hydrogen with the post. Remove the hydrogen along with the post and insert the exposed knob of the hydroxyl group into the hole on the ethane model.

![Ethanol model](image)

4. Although sugar is made of many carbon and hydrogen atoms, it also has several hydroxyl (OH) groups. These OH groups act in a similar way to water. The groups are polar with a negative oxygen and positive hydrogen. As a result, OH groups in sugar form hydrogen bonds with water and dissolve in the same way as salt dissolves.

*Use the Water Kit* to show how ethanol dissolves in water. While not a sugar, ethanol illustrates how the presence of a hydroxyl group enables hydrogen bonding — hydration. Note: the rest of the ethanol molecule is non-polar.

**Activity 2. Molecules of Life**

*Before going to one of the stations* at the back of the room, make a sketch as specified in the worksheet. Then use the models to answer the questions in the worksheet.
Activity 3. Assembly of Macromolecules

In pairs, work on the assembly of a phospholipid or a nucleic acid, as designated by your instructor. Once complete, you will present to the class, a few points about the molecule (see the end of the section for each macromolecule). Your instructor will score you on your ability to accurately present the points, so be prepared.

Assembly of a Phospholipid

**Basic Assembly**
For assembly and disassembly, snap together the following parts as shown.

Before leaving lab, disassemble your macromolecule, account for all of the pieces based on the list of contents available in lab, and place the pieces in their storage containers.

Your kit will have components to build one of the phospholipids indicated below. One partner should build the head, the other should build the tails, then connect the two. The hydrocarbon tails will likely take less time to build, so that partner can help with the head when they complete the tails. Ultimately both group members must understand the assembly to be able to present their model to the class.

**Phospholipid Heads**

* Shown in back or gray, indicates the negatively charged oxygen so no hydrogen should be attached
**Phospholipid Tails**

Note the double bonds found in some of the hydrocarbon tails do not have free rotation like a single bond and form a kink in the chain.

The chemical structures for reference are available on the next page.

**Points to present about your model:**

Point out the following on your model to the class:

- Phospholipids are composed of a 3-carbon backbone (that comes from glycerol or serine), 2 hydrocarbon chains (that come from fatty acids that react with the glycerol), a phosphate, and in some cases an additional polar group. Point out each of these parts.
- Point out the hydrocarbon tails and phospholipid heads, indicating which is polar and which is nonpolar.
- Demonstrate the atoms that make these structures polar or nonpolar.
- Demonstrate the flexibility of the hydrocarbon chains that provide fluidity in biological membranes. Show the free rotation of a single bond and the lack of rotation of a double bond.
- Indicate the kink in a tail that results from a double bond.
- **Optional:** Your instructor may want groups to show how their phospholipids would interact in a phospholipid bilayer.
**Assembly of a Nucleic Acid**

**The “Dynamic DNA” Model**

The model is based on X-ray crystallographic structures and is built to scale (approximately 80,000,000 times actual size). For clarity in the model, hydrogen atoms are not shown except for the hydrogens forming the hydrogen bonds between the base pairs (shown in white) and the insertable hydrogen of the hydroxyl group on the 3’ carbon of the sugar at the end of each DNA strand.

**Nucleotides, Nucleic Acids & their Components**

Nucleotides are the monomers of polynucleotides. There are two classes of polynucleotides (also known as nucleic acids): DNA & RNA. Nucleotides are composed of a 5-carbon sugar (deoxyribose or ribose), 1-3 phosphates, and a nitrogenous base (A, C, G, T, or U). Compare the different types of components of a nucleotide using the illustrations on the next page.

Notice: the parts are shown without a hydrogen capping the oxygen for simplicity.
5-C Sugar

Notice that the ring is numbered with numbers that have a prime to distinguish the sugar ring from the rings of the nitrogenous bases.

Phosphate

Nitrogenous base:

The nitrogenous bases come in two types: purines and pyrimidines. The purines are larger and are composed of two fused rings: a 6-membered and 5-membered ring. The pyrimidines only have a single 6-membered ring. Notice that the ring is numbered with simple numbers.
A Nucleotide

Assemble the components required to make four nucleotides.

Base Pairs

Form a base pair between the G and C, as well as between the A and T. Notice the similarities and differences.

Phosphodiester Bond

Take your two base pairs and form phosphodiester bonds to connect the nucleotides.

Note: Even though we have illustrated the hydrogen bonding before the phosphodiester bond, what you have done does not accurately demonstrate DNA synthesis. You may want to think about the differences.

With only two base pairs, you will not be able to see the characteristic major and minor groove of DNA. After you present your model to the class, assemble your base pairs with other models so you can visualize these grooves.
Major & Minor Groove

As you assemble the base pairs together, use a metal rod to support the helix so you can wind and unwind it. When wound, you can see the major and minor grooves (see below). After the base pairs are assembled into a 12 base pair strand, your instructor can then show this to the class before you disassemble the parts for storage.

Points to present about your model:

Point out the following on your model to the class:

- Point out the 5-carbon sugar, phosphate, and nitrogenous base. Indicate which sugar is represented and how you can tell.
- Demonstrate the hydrogen bonding between complementary base pairs indicating how many hydrogen bonds formed. Show the difference between an A-T and a G-C base pair.
- Show the location of the phosphodiester bond. Demonstrate the difference in strength between the phosphodiester bonds and the bonds between complementary base pairs.
- Even though your strand is short, demonstrate the different ends of the polynucleotide strand (only 2 nucleotides long). Point out the antiparallel nature of DNA using your model.
- Trace the backbone to illustrate the repeating nature: sugar-phosphate-[repeat]. Show the location of the base pairs within the double helix.
- Point out the major and minor grooves of double stranded DNA.
- After assembly or the 12 base pairs, assist your instructor in demonstrating the major and minor grooves of the double helix.
**Activity 4. Amino Acid Structure**

**Activity 4A. Amino Acid Monomers**

1. Construct two separate amino acids using the Molymod® atoms and covalent bonds. Identify the following components: amino group, carboxyl group, the R group or sidechain, alpha carbon, carboxyl carbon, nitrogen. *(See labeled diagram and parts below).*

2. Compare the two amino acids that have been built. Are they similar? How might two amino acids be different?

   Amino acids are similar because they share the same “core” structure. Amino acids are different because the composition of the “R group” is different for each of the 20 amino acids.

   A second way that amino acid structures may be different is their stereochemistry. The arrangement of atoms around the alpha-carbon may be “right-handed” or “left-handed” to form a D-amino acid or an L-amino acid. The L-amino acids are the naturally occurring form used to make proteins. If you hold the hydrogen atom attached to the alpha carbon in your fist, then move from the carboxyl group to the amino group to the R group in a CLOCKWISE direction, you have an L-amino acid. If you trace the path in a COUNTERCLOCKWISE direction, it is a D-amino acid. An L-amino acid is shown in the illustration.

3. Two amino acids can be chemically linked by a reaction called “condensation” or “dehydration synthesis” to form a dipetide bond linking two amino acids. A chain of amino acids linked together by peptide bonds is called a **polypeptide**. Using the two amino acids built in step 1, create a dipetide. Answer the questions in the worksheet.
Activity 4B. Amino Acid Properties

Amino Acids - Building Blocks of Proteins

Introduction
Proteins are more than an important part of your diet. Proteins are complex molecular machines that are involved in nearly all of your cellular functions. Each protein has a specific shape (structure) that enables it to carry out its specific job (function).

A core idea in the life sciences is that there is a fundamental relationship between a biological structure and the function it must perform. At the macro level, Darwin recognized that the structure of a finch’s beak was related to the food it ate. This fundamental structure-function relationship is also true at all levels below the macro level, including proteins and other structures at the molecular level. For two examples of proteins and their functions, see the photos and outlines at the right.

In this activity, you will explore the structure of proteins and the chemical interactions that drive each protein to fold into its specific structure, as noted below.

- Each protein is made of a specific sequence of amino acids. There are 20 amino acids found in proteins.
- Each amino acid consists of two parts — a backbone and a side chain. The backbone is the same in all 20 amino acids and the side chain is different in each one.
- Each side chain consists of a unique combination of atoms which determines its 3D shape and its chemical properties.
- Based on the atoms in each amino acid side chain, it could be hydrophobic, hydrophilic, acidic (negatively charged), or basic (positively charged).
- When different amino acids join together to make a protein, the unique properties of each amino acid determine how the protein folds into its final 3D shape. The shape of the protein makes it possible to perform a specific function in our cells.
Chemical Properties Circle & Amino Acid Chart

Hydrophobic and Hydrophilic Properties
What do you think hydrophobic means? Separate the word ‘hydrophobic’ into its two parts — hydro and phobic. Hydro means water and phobia means fear or dislike, so hydrophobic side chains don’t like water. Hydrophobic side chains are also referred to as non-polar side chains.

Now can you guess what hydrophilic means? Philic means likes or attracted to, so hydrophilic side chains like water. Hydrophilic side chains are also referred to as polar side chains.

Acidic (Negatively Charged) and Basic (Positively Charged) Properties
Can you think of acids you have around your house? Lemon and fruit juices, vinegar and phosphoric acid (in dark sodas) are common household acids. Acids taste sour and are typically liquids.

Can you think of bases you have around your house? Tums®, baking soda, drain cleaner and soap are common bases. Bases taste bitter and can be a liquid or solid.

The colored areas on the Chemical Properties Circle, the color coding on the Amino Acid Side Chain Chart, the key below and the colored clips show the chemical properties of side chains.

KEY
Hydrophobic Side Chains are Yellow
Hydrophilic Side Chains are White
Acidic Side Chains are Red
Basic Side Chains are Blue
Cysteine Side Chains are Green

Directions
Select any side chain and a colored clip that corresponds to the property of the side chain. Insert the side chain into the clip.

Place each amino acid side chain attached to its clip on the bumper near its name and abbreviations. You will need to consult the Amino Acid Side Chain Chart in your kit to find the name of each side chain, so you can position it correctly on the circle.
After each side chain has been correctly positioned on the circle, look at the colored spheres in each side chain. Scientists established a CPK coloring scheme (see chart below) to make it easier to identify specific atoms in models of molecular structures.

**KEY**
- Carbon is Gray
- Oxygen is Red
- Nitrogen is Blue
- Hydrogen is White
- Sulfur is Yellow

**Folding a 15-Amino Acid Protein**

1. Unwind the 4-foot mini toober (foam-covered wire) that is in your kit. Place a blue endcap on one end and the red endcap on the other end. The blue endcap represents the N-terminus (the beginning) of the protein and the red endcap represents the C-terminus (the end) of the protein (see photo on next page).

2. Choose 15 side chains from the chemical properties circle as indicated in the chart shown right.

   Mix the side chains together and place them (in any order you choose) on your mini toober.

3. You may want to use a ruler to place your side chains on your mini toober.

   Beginning at the N-terminus of your mini toober, measure about three inches from the end of your mini toober and slide the first colored clip with its side chain onto the mini toober. (See photo.) Place the rest of the clips three inches apart until all are attached to the mini toober.
The sequence of amino acid side chains that you determine when placing them on the mini toober is called the primary structure of your protein. As a general rule the final shape of a protein is determined by its primary structure. Remember that protein folding happens in the watery environment of the cell.

4. Now you can begin to fold your 15-amino acid protein according to the chemical properties of its side chains. Remember all of these chemical properties affect the protein at the same time.

**Photo A — Hydrophobic Side Chains**
Start by folding your protein so that all of the hydrophobic (non-polar) side chains are buried on the inside of your protein, where they will be hidden from polar water molecules.

**Photo B — Acidic & Basic Side Chains**
Fold your protein so the acidic and basic (charged) side chains are on the outside surface of the protein. Place one negative (acidic) side chain with one positive (basic) side chain so that they come within one inch of each other and neutralize each other. This positive-negative pairing helps stabilize your protein.

**Note:** As you continue to fold your protein and apply each new property listed below, you will probably find that some of the side chains you previously positioned are no longer in place. For example, when you paired a negatively charged side chain with a positively charged one, some of the hydrophobic side chains probably moved to the outer surface of your protein. Continue to fold until the hydrophobic ones are buried on the inside again. Find a shape in which all the properties apply simultaneously.

**Photo C — Cysteine Side Chains**
Fold your protein so that the two cysteine side chains are positioned opposite each other on the inside of the protein where they can form a covalent-disulfide bond that helps stabilize your protein.

**Photo D — Hydrophilic Side Chains**
Continue to fold your protein making sure that your hydrophilic (polar) side chains are also on the outside surface of your protein where they can hydrogen bond with water.

The final shape of your protein when it is folded is called the tertiary structure.
**Activity 5. Protein Secondary Structure**

**Activity 5A. Secondary Structure in Protein Folding**

In the previous protein folding activity, you created a hypothetical 15-amino acid protein and learned that basic principles of chemistry determine how each protein spontaneously folds into its characteristic 3-dimensional shape. You learned that the sequence of amino acids in a protein (from N-terminus to C-terminus) is called its primary structure. The final folded, 3D shape of your protein is called the secondary structure.

In this second protein-folding activity, you will learn about the secondary structure of proteins. This secondary structure consists of alpha helices and/or beta sheets. Proteins commonly contain a combination of alpha helices and beta sheets. Proteins can be described as a series of alpha helices and beta sheets, joined by loops of less regular protein structure.

An **alpha helix** is a compact right-handed helix, with 3.6 amino acids per turn of the helix. The amino acid side chains are bonded to the alpha carbon of each amino acid and radiate outward from the helix. The alpha helix is stabilized by hydrogen bonds – weak bonds between the amino nitrogen of one amino acid (x), and the carbonyl oxygen of another amino acid (x+4) located four side chains further along the chain.

A **beta sheet** is an extended, zig-zag structure in which individual strands are positioned parallel or anti-parallel to each other to form flat sheets in proteins. Since the amino acid side chains are bonded to the alpha carbons of each amino acid, they are alternately orientated above and below the plane of the sheet. The beta sheet is stabilized by hydrogen bonds between the amino nitrogen of one amino acid and the carbonyl oxygen of another amino acid in an adjacent beta strand.
Folding a Toober Model of a Zinc Finger

In this activity, you will fold a model of the first of three zinc fingers of the Zif268 protein. Zinc finger proteins regulate the transcription of DNA into mRNA — by binding to DNA and attracting RNA polymerase. A zinc finger protein contains two cysteine amino acids and two histidine amino acids which simultaneously bind to a single zinc atom. These four amino acids are contained within a 30 amino acid sequence that folds into a two-stranded beta sheet and short alpha helix. Many zinc finger proteins (like zif268) are composed of three consecutive fingers with similar features (motifs) which bind to a nine base pair sequence of double-stranded DNA.

The primary structure of this zinc finger is below.

![Primary Structure Image]

The side chains of the seven circled amino acids in the above sequence will be included in the model you fold.

1. **Primary Structure**
   
   Map the positions of the seven amino acids on your mini toober. Since the toober is 48 inches long and the zinc finger is 28 amino acids long, each amino acid occupies 1.7 inches of toober. Using a ruler, measure the distances shown below and add the appropriate side chains to the mini toober at each position.

2. **Secondary Structure**
   
   Fold the toober into its secondary structure. The first 13 amino acids (the first 22 inches from the N-terminus) should be folded into a 2-stranded beta sheet. This can be made by creating a zig-zag structure that is bent in the middle as shown in the photos below. Add the plastic hydrogen bonds connectors to your model as shown in the far right photo below.

![Secondary Structure Photos]

The last 15 amino acids of the zinc finger exist as a compact, right-handed alpha helix. This can be made by wrapping the mini toober around your finger or an empty paper towel tube to create three full turns as shown in the photos below. Loosen the loops and add the hydrogen bond connectors as shown in the far right photo.
Activity 5B. Alpha Helices & Beta Pleated Sheets – A Closer Look

Compare the models of alpha helices and beta pleated sheets and answer the questions on your worksheet. Below are images of the models you will explore.

Alpha Helices

Beta Pleated Sheets

Activity 6. Protein Folding

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

1. Begin folding your 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 on the next page.
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 side chains to create an enzyme active site.

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

5. The three amino acid side chains that make up your enzyme’s active site interact with a substrate to catalyze a specific chemical reaction. This requires that the side chains 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. Now, holding the protein near one end, jiggle it gently, then more vigorously, to simulate the thermal motion that would occur as the temperature was increased.

6. Now carefully remove the connectors that were stabilizing your folded protein (see photo J). Once again, jiggle your protein. Notice that without the stabilizing effect of the hydrogen bonding in your protein’s secondary structure, the normal thermal motion experienced by proteins can quickly disrupt the proteins conformation, even that of the active site.

7. At higher temperatures, the protein would completely unfold (denature). Simulate this by 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, as shown below.

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.
Activity 7. Enzyme Structure & Function

Activity 7A. Substrate Binding

Refer to the worksheet for questions regarding your Enzyme and Substrate.

1. Construct a generic substrate as follows, referencing the photos as you do.
   a. Join the 4-hole sphere with the 2-hole sphere and post.
   b. Connect one yellow functional group to the 4-hole sphere and the second yellow functional group to the 2-hole sphere and post.
   c. Randomly connect the other functional groups to the remaining holes in the spheres.

   Important: 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.

   

![Substrate Construction](image)

   The color coding in this exercise represent the chemical properties of groups or atoms:
   - Blue – positively charged (basic) group
   - Red – negatively charged (acidic) group
   - White – polar hydrophilic group
   - Yellow – nonpolar hydrophobic group

2. Assemble the enzyme by placing the metal clips with the different colored dots along the entire length and in random order on the 6-foot toober.

3. 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. Blue should pair with red; white with white, yellow with yellow.

4. Assess the specificity of your substrate by swapping substrates with a partner. Observe the fit, or lack thereof, between your enzyme and your partner’s substrate.

5. Shake the enzyme structure, taking note of the overall stability of the design.
6. Refold the enzyme adding secondary structure (alpha helices and/or beta sheets) to the design as in the example shown below. 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. Consider the stability of the enzyme now, with secondary structure components, compared with before.

7. 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.

**Activity 7B. Enzymes in Action**

**This exercise is optional.** An instruction sheet will be available in lab along with the models.
Activity 1. Water Basics

Answer all questions in your own words and be sure to include information from what you learned in class and lab, as appropriate, to answer the questions completely. Your answers should include concepts such as polarity, charges, and bonds/interactions between atoms and molecules.

1. What holds the water molecules together in a cup of water? Explain.

2. How does the salt sodium chloride dissolve in water?

3. Why will oily substances not dissolve in water?

4. Sugar has many carbons. Why does it dissolve in water?
Activity 2. Molecules of Life

1. **Before going to one of the stations** at the back of the room, in the space below, sketch the following to scale: a water molecule, a phospholipid, a phospholipid bilayer. You can use the typical circular polar head with 2 hydrophobic tails for the phospholipids.

2. Now compare the size of the drawings you made to the molecular models at the station. The models are to scale so they are showing you the relative size of these molecules. Your drawings will be a different size than the models, but was the relative size of your water, phospholipid and phospholipid bilayer accurate?

3. Note the relative size of the various monomers (water, monosaccaride, amino acid, nucleotide, phospholipid) to their polymers (ice, glycogen/starch/cellulose protein, DNA, a phospholipid bilayer). Briefly describe how they differ.
4. Indicate two side chains that might position themselves on the interior of the protein, where they are shielded from water?

Activity 3. Assembly of Macromolecules
In lieu of written questions, each group will orally describe several characteristics of their models to the class.

Activity 4. Amino Acid Structure
4A. Amino Acid Monomers
1. What are the products of the condensation reaction? Be sure to list all of the products.

2. Identify the following components of the dipeptide: amino groups, amino terminal end, carboxyl groups, carboxyl terminal end, carbonyl group, peptide bond, R-groups or sidechains, alpha carbon, carbonyl carbon. Sketch and label the parts of your dipeptide below.

4B. Amino Acid Properties
1. Compare the amino acid side chains: Do you see similarities or patterns? Explain.
2. Hydrophobic side chains are composed primarily of __________________________ atoms.

3. Acidic side chains contain two __________________ atoms. This is called a carboxylic acid functional group.

4. Basic side chains contain __________________ atoms. This is called an amino functional group.

5. Hydrophilic side chains all have some combination of __________________ atoms.
   Although these atoms are found mainly in hydrophilic amino acid side chains, name an amino acid that is not hydrophilic that represents an exception to this rule: ______________________________________________________________

6. Models help us to better understand the items they represent. The toober represents the protein backbone. So what specifically do the clips represent?

Activity 5. Protein Secondary Structure

5A. Secondary Structure in Protein Folding

1. How many different amino acids that are genetically encoded are found in most proteins?

2. Compare the structure of your protein toober with two other protein toobers. Even though you each used the same 15 amino acid tacks, the structures are likely different. Why is this and how does this relate to protein structure and the wide variety of functions that proteins perform?
3. Protein folding is a spontaneous process that is determined by the primary amino acid sequence of the protein and the chemical environment. Do you think that the final shape of a protein will be a high energy or low energy state? Why?

5B. Alpha Helices & Beta Pleated Sheets – A Closer Look

1. Look at the models of alpha helices. What is the difference between the two models? Hint: something is omitted from one of the models and replaced by a green dot. The same is true of the beta pleated sheet models.

2. Describe the location of the amino acid side chains...

   ... in an alpha helix:

   ... in beta pleated sheets:

3. Look at one of the DNA models, either in the Molecules of Life or Dynamic DNA. Where are the nitrogenous bases located in this helix? How does that compare to the location of the side chains in an alpha helix? Think about the function of DNA. If the nitrogenous bases were in the same orientation as they are in an alpha helix, would this work? Explain.
**Activity 6. Protein Folding**

1. What does the toober represent in this model?

2. Describe the kinds of bonds and interactions that are present in a protein’s secondary and tertiary structure that contribute to the stability of the structure.

3. Consider the structural stability of the enzyme when the hydrogen bonds were removed. Nevertheless, this is a model and the hydrogen bonds in real proteins are not plastic connectors. In actuality, hydrogen bonds are weak bonds. Are they important in maintaining the structural stability of proteins? Explain.

**Activity 7. Enzyme Structure & Function**

**7A. Substrate Binding**

1. Why did blue pair with red, while white was with white and yellow with yellow? What did this represent?

2. Refer to the Amino Acid Side Chain Chart. What would be an appropriate example of an amino acid (residue) for each of the four different colors of metal clips? List examples of each below.
3. 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? Explain.

4. Did your partner’s substrate fit in your enzyme easily and correctly without significantly altering the structure of the enzyme? Did you expect it would? Explain your observations.

5. Consider what happened when you shook the enzyme. Explain why structural stability may be a desirable characteristic of enzyme structure.

6. How did the structural stability of the enzyme compare when secondary structure was added compared to the enzyme without these elements?

7. When you made subtle changes in the shape of the enzyme, did the contact points change when you changed the enzyme’s structure? Were the changes expected? Explain.