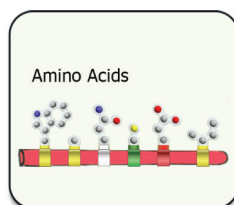


Student Handout 2

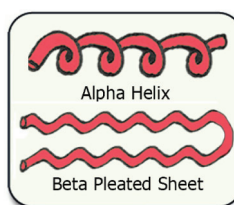
Secondary Structure

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 its **tertiary structure**.

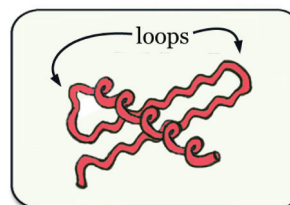
In this second protein-folding activity, you will learn about the **secondary structure** of proteins. This secondary structure consists of alpha helices and/or beta sheets. Proteins commonly contain a combination of alpha helices and beta sheets. Proteins can be described as a series of alpha helices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



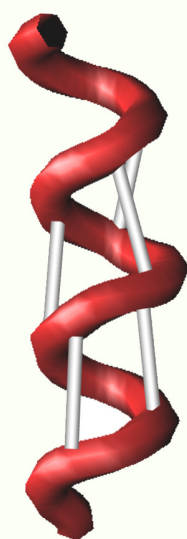
Primary Structure



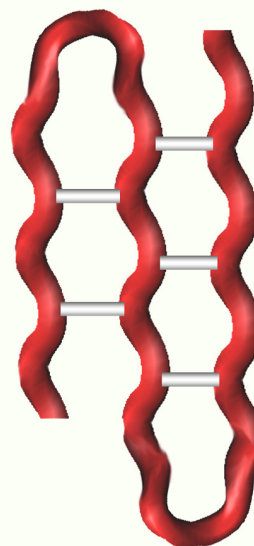
Secondary Structure



Tertiary Structure



An **alpha helix** is a compact right-handed helix, with 3.6 amino acids per turn of the helix. The amino acid sidechains 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 sidechains 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 sidechains 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 the 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.

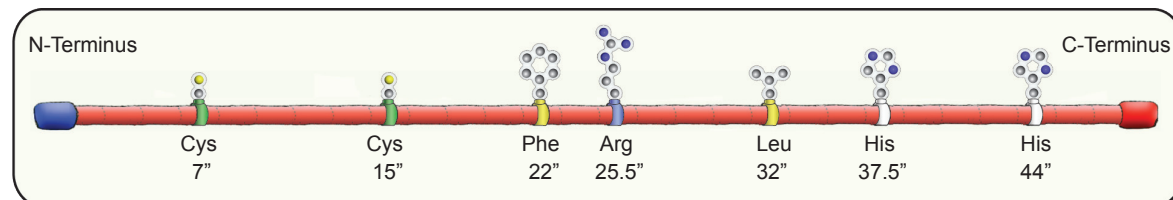
The primary structure of this zinc finger is below.

N-Terminus P Y A (C) P V E S (C) D R R (F) S (R) S D E (L) T R (H) I R I (H) T G C-Terminus

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

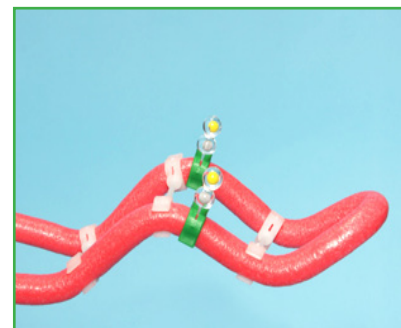
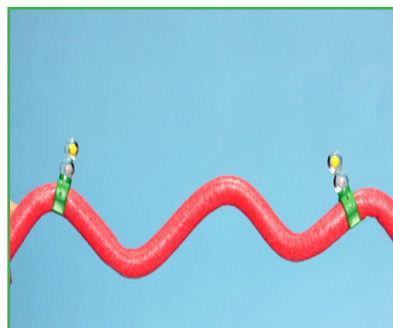
1. Primary Structure

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 sidechains to the mini-toober at each position.



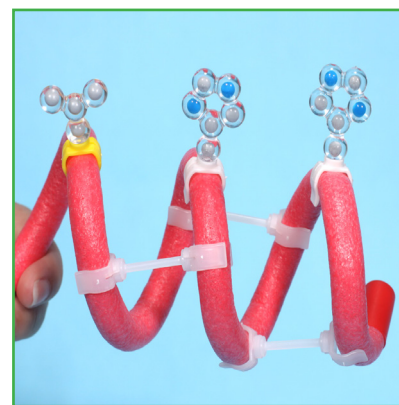
2. Secondary Structure

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.



Folding a Toober Model of the Zinc Finger (continued)

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.



Your mini-toober should look similar to the one shown below.



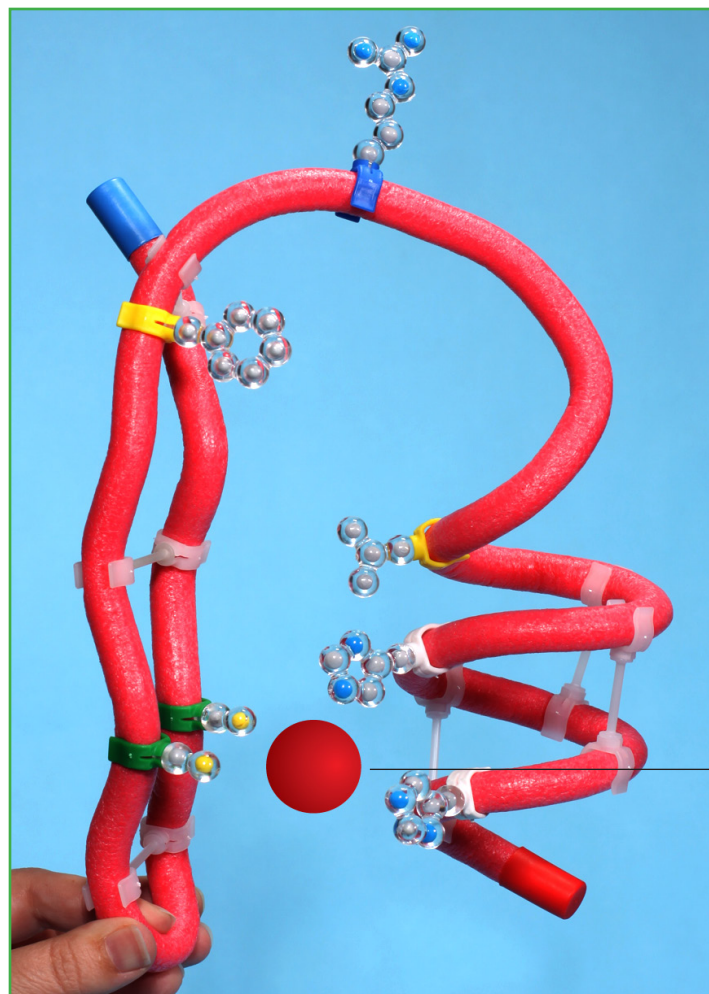
Folding a Toober Model of the Zinc Finger (continued)

3. Tertiary Structure

Fold the beta sheet and alpha helix into the final tertiary structure of the zinc finger.

In its final tertiary structure, the seven sidechains will be positioned such that:

- The two cysteine and two histidine sidechains will be oriented to simultaneously bind to a single zinc atom (not included) in the center of the structure (see photo).
- The two hydrophobic amino acid sidechains phenylalanine and leucine will be orientated toward the inside of the structure.
- The positively-charged arginine sidechain will be exposed at the top of the alpha helix, where it is available to bind to the negatively-charged phosphate backbone of DNA.



As a folding guide, you can either use the photo shown below or the interactive Jmol image of a zinc finger on website (www.3dmoleculardesigns.com/resources.php).

Note: As you fold your mini-toober, you may need to rotate the sidechains around the mini-toober to make them adopt to the desired final shape.

The zinc ion (not included with the kit) binds simultaneously to the two histidines and two cysteines.



Folding a Toober Model of the Zinc Finger (Questions)

1. Both alpha helices and beta sheets are stabilized by hydrogen bonds.

- Which atoms share the hydrogen in these weak bonds?

- Are these backbone atoms or sidechain atoms?

2. Describe the secondary structural elements that comprise a zinc finger:

3. How is a zinc atom involved in the stabilization of the zinc finger motif?

4. Zinc fingers often bind to DNA. How might the arginine sidechain (positively-charged) shown on your model be involved in DNA binding?

• **Optional Activity** - Zinc Finger Jmol (see AASK Lessons on website)