

****There is also a video explaining some of Activity 2 & 3 that we did not get to in class!**

MATERIALS

- In your ziplock bag, you should have 1 pipe cleaner and 9 pony beads.
 - Please double check that you have...
 - 1 pipe cleaner
 - 5 yellow beads
 - 2 blue beads
 - 2 red beads
 - **Raise your hand if you are missing any materials.**

LEARNING OBJECTIVE

- 1) Compare and contrast the interactions driving the formation of secondary, tertiary, and quaternary structure of proteins.

PRECONCEPTIONS:

For Preconceptions, Activity 1, and Activity 2, please work individually. You will be able to talk to neighbors during iClickers and Activity 3!

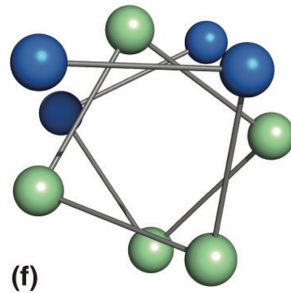
- 1) In the table below, define each level of protein structure and list the primary stabilizing noncovalent interactions involved:

Protein Structure	Definition	Primary stabilizing factors/noncovalent interactions
Primary	A sequence of amino acids; determines all other protein structures (ex: secondary, tertiary)	Peptide bonds
Secondary	The recurring backbone structural pattern of the protein; alpha helix/beta sheet	Hydrogen bonds between amino acids
Tertiary	Functional protein consisting of primarily hydrophobic R groups/residues/side chains	Hydrophobic core

ACTIVITY 1– Protein synthesis (primary protein structure)

- For this activity, the pipe cleaner represents a protein backbone, and the beads represent amino acids.
 - Each bead color represents an amino acid with the following properties:
 - Yellow = hydrophobic
 - Blue = positively charged
 - Red = negatively charged

- 1) Consider your hand a ribosome that is synthesizing a sequence of amino acids.
 - a) By stringing the beads onto the pipe cleaner, synthesize a sequence of amino acids that...
 - i) Is 9 amino acids long
 - ii) Allows for the formation of an amphipathic helix.
 - iii) Order: yellow yellow red red yellow blue blue yellow yellow
 - iv) [Aka: phobic phobic neg neg phobic pos pos phobic phobic]
 - b) **Requirements:**
 - i) **The first amino acid MUST be hydrophobic**
 - ii) **The third amino acid MUST be negatively charged**
- 2) Hint: The primary protein structure determines the secondary and tertiary structures! Think back to this ball and stick figure, and consider the noncovalent interactions between the amino acids. Think about what kinds of interactions would stabilize the structure to allow for helical formation, and how that would look in the amino acid sequence.



iClicker 1:

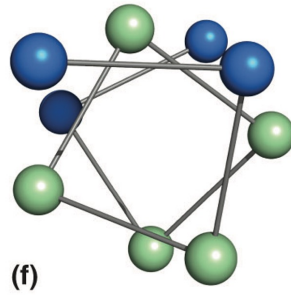
- Based on your model, what do you think the spaces in between each amino acid represent?
 - A) hydrogen bonds
 - B) ionic bonds
 - C) pi bonds
 - **D) peptide bonds**

ACTIVITY 2– Alpha helix formation (secondary protein structure)

iClicker 2:

- How many side chains will be in a single turn of an alpha helix and where do these point?
 - A) 3 to 4 and inward to the helical axis
 - **B) 3 to 4 and outward away from the helical axis**
 - C) 2 to 3 and inward toward the helical axis
 - D) 2 to 3 and outward away from the helical axis

- Consider this ball and stick figure when answering the iClicker:



- 1) Fold your amino acid sequence into an alpha helix structure by bending the pipe cleaner at each bead in a roughly 45° angle. Your alpha helix should...
 - a) Be stabilized by hydrogen bonds
 - b) Have about 3 (specifically 3.4) amino acids per turn
- 2) **Tip:** Curl the pipe cleaner around your fingers if you are having trouble getting the helix shape. Then, you can try to bend it at each bead, to get more of the box/triangle shape (depicted in the ball and stick figure in Activity 1).

Discuss in groups of four:

- What considerations did you make to ensure your side chains didn't repel? In other words, at what positions did you have to place the negative and positive charges given the initial requirements?
 - Positions 3 and 4 had to be negative which meant that positions 6 and 7 had to be positive.
- For an amphipathic protein (what you modeled), at which positions in the amino acid sequence did you have to put a hydrophobic residue to maintain helical structure?
 - Positions 1, 2, 5, 8, and 9

iClicker 3:

- If you made positions 6 and 7 (while keeping all other positions the same) a negative charge the helix would not form. What noncovalent interactions would be disrupted in this scenario?
 - A) Peptide bonds; the primarily stabilizing force of primary structure
 - B) Side chain hydrogen bonds; the primary stabilizing force of primary structure
 - C) Side chain hydrogen bonds; the primary stabilizing force of secondary structure
 - D) **Backbone hydrogen bonds; the primary stabilizing force of secondary structure**

[key]

- Each bead color represents an amino acid with the following properties:
 - Yellow = hydrophobic
 - Blue = positively charged
 - Red = negatively charged

ACTIVITY 3– Tertiary protein structure

- 1) Get into groups of 4! Using your alpha helices, construct a globular protein.
 - a) Hint: You should all have the same amino acid sequence (and therefore the same alpha helix structure). **Your helices MUST be stable!**

iClicker 4:

- What was the main consideration of the four helices that allowed you to form a tertiary protein?
 - A) The hydrophobic amino acids on each helix were all oriented on the same plane so that they ultimately faced *inside* the core of the tertiary structure
 - B) The hydrophobic amino acids on each helix were all oriented on the same plane so that they ultimately faced *outside* the core of the tertiary structure
 - C) The hydrogen bonds were oriented *within* amino acid side chains
 - D) The hydrogen bonds were oriented *between* amino acid side chains

NEW UNDERSTANDINGS:

- In the table below, list the interactions involved for each protein conformation:

Protein Conformation	Primary stabilizing factors/noncovalent interactions
Primary	Peptide bonds
Secondary	Hydrogen bonds between amino acids
Tertiary	Hydrophobic core

- Discuss with a neighbor: Explain the significance of the hydrophobic effect of R groups. How does the formation of the hydrophobic core stabilize the protein?
 - The formation of the hydrophobic core allows for the entropy of water to increase relative to the “unfolded” protein.
- Discuss with a neighbor: How do your answers *before* the activity compare to your answers *after* the activity? Which aspects of the model, if any, helped you understand the differences between those interactions?