Protein Structure: Papier Mache

Name:_____

Case Study

At the University of Alberta, the Derda Research Group has developed a process to search for proteins called **ligands**. These ligands (**Fig. 1a**) bind to cell receptors (**Fig. 1b**) based on their shape. The 'lock and key' model is the traditional explanation for this. The ligand (key) turns the receptor (lock) which causes a change in the membrane of the cell. Either a channel is opened into the cell or the bond triggers a change inside of the cell. Either way, finding a ligand with a particular shape is critical because the cells in the body have variations in the shapes of their receptors. Being able to identify and then create a molecule that targets a specific type of cell is extremely useful for researchers – it allows them to target cells for treatment.

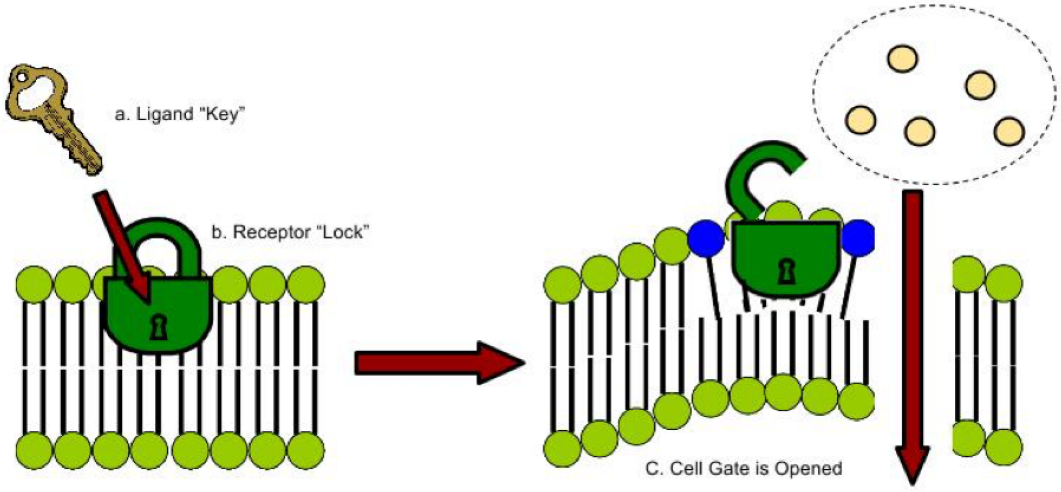

Figure 1.

Currently, almost everything in the body is accessed through the bloodstream. The vast majority of medicine is injected directly into the bloodstream or ingested (eaten) and then quickly absorbed into the bloodstream from the digestive system. This means that medicines have access to the entire body. Tylenol taken for back pain will affect the back- but it will also affect every other structure in the body with blood flow. Targeting a drug to one cell type can help to increase effectiveness, decrease required doses, and limit side effects.

The Derda group takes millions and millions of proteins with random variation in their amino acid sequences and tests how tightly they bind to the receptors that they are interested in. They dip the receptor molecule into a container will millions of protein variations and "fish" for proteins that bind well. When they remove the target receptor from the container, only the proteins with the best bonds will remain attached. Though

the specific chemistry of this process is very difficult to replicate in a high school environment, the importance of protein shape is not.
Today, you will be creating "random" proteins and exploring how secondary, tertiary, and quaternary structures affect their shape. Visualizing the process of protein formation is critical to understanding the complex nature of protein structure and function – and how everything a protein does relates back to its shape.

Protein Structure – Background Info

Proteins are one of the most complex macromolecules in biology. It's typical to think of proteins in association with the muscular system and, while they are critical for the creation of muscle fibres, the uses of proteins go far beyond this. Proteins are an integral part of every cell in the body – cell receptors, enzymes, hormones, antibodies, and many cellular structures depend on proteins. Proteins are some of the building blocks for these structures, but they also exist as the machinery that helps to create cell structures.

Though carbohydrates and lipids have variation in their makeup, proteins are unique because there is almost limitless possibility for their structure. In a protein, primary structure (**Fig 2a**) refers to the sequence of amino acids that make up a protein. There are 20 unique amino acids to work with and proteins usually contain hundreds of amino acid subunits. The possible combinations are immense.

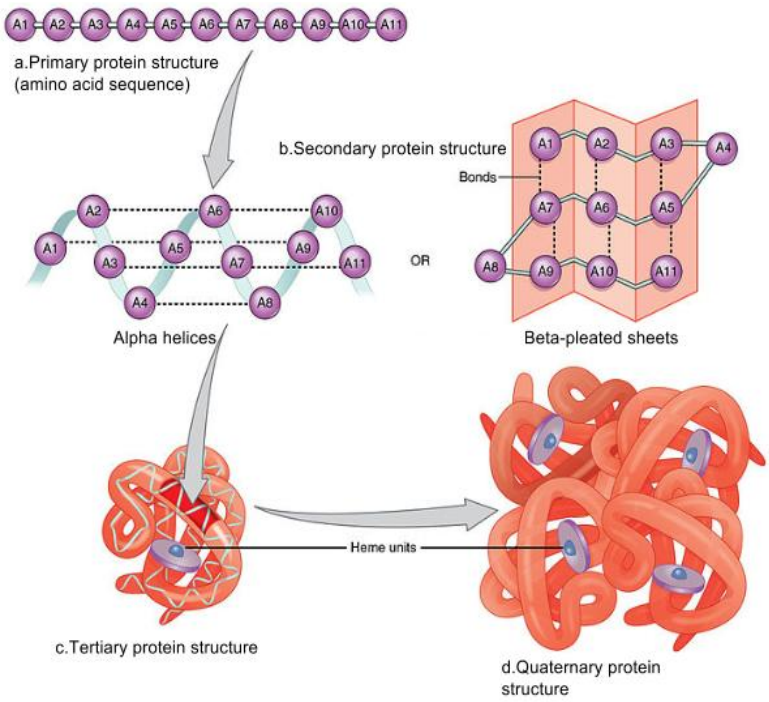

Figure 2.

After amino acids are connected (through peptide bonds), the sequence of amino acids doesn't change. The shape of the molecule, however, changes dramatically. Picture a beaded necklace with three or four hundred beads on it. How many different ways could it be folded, wrapped, tied, and twisted to form unique shapes? How are these changes determined? Secondary and tertiary structure determine these shape changes. Secondary structure (**Fig 2b**) is comprised of alpha helices and beta pleated sheets. These patterns are formed as a result of hydrogen bond interactions between amino acids that are located close to each other. Tertiary structure (**Fig 2c**) forms as amino acid substructures begin to twist and turn into a three dimensional shape. In very complex proteins, multiple subunits can be assembled together into a massive multi-protein structure. Hemoglobin (**Fig 2d**) is an excellent example of quaternary structure – it contains 4 subunits that have all come together to form a complicated but common cellular protein.

Materials

- Masking Tape (thin width is better)
- 1 - 1.5 meter section of wire
- Black Pen/Sharpie
- Camera (Cell phone cameras are acceptable)
- Papier mâché paste solution
- Newsprint or other light paper
- Margarine/Yogurt container
- One color of paint and a paintbrush (Day 2 only)

Procedure – Day 1

Part 1 - Primary Structure

As you progress through this lab procedure, there will be multiple camera icons. These are intended to give you a snapshot of what your project looks like after establishing each of the four levels of protein structure. At each of these stages, you will be required to take a picture of your project that will be included digitally in your write-up.

1. **Attach at least 50 pieces** of masking tape to create little flags along the length of your wire. These tape pieces need to be long enough to write amino acid names on and you should have about 1 cm between each flag.
2. **Label** each flag with one of the 20 amino acids from the amino acid chart below. You can use the 3 letter abbreviations or the full amino acid names if you choose. The rules for these amino acids are as follows:
    a. You may repeat amino acids, but try to randomize your pattern somewhat
    b. Add at least 10 molecules of **cysteine**

c. Add a total of 10 molecules of **proline**, **serine**, or **threonine** (combined). These requirements (b and c) will add molecules that are highly reactive and will make your end product more unique!

3.  **Document** your protein's primary structure on the student lab write-up sheet (attached). **Include any observations** or notes you might have about your protein so far.

Part 2 - Secondary Structure

4. Twist or bend the primary structure of your wire into either an **α helix**, **β pleated sheet**, or a combination of both (see **Figure 2**). An **α helix** structure usually contains about 3 or 4 amino acids per rotation. A **β pleated sheet** usually consists of 3-5 amino acids per sheet width. In reality, a protein's secondary structure elements are usually determined by the hydrogen bonds formed between amino acid side chains.
5.  **Document** your protein's secondary structure on the student lab sheet.

Part 3 - Tertiary Structure

6. To establish tertiary structure (3D shape), bend your wire 90^{o} inwards at each location of **proline**, **serine**, or **threonine**. If these bends interrupt your alpha helix or pleated sheet structure, do them anyways. This will cause your protein to begin to look globular.
7. **Cysteine** is a unique amino acid that contains sulfur. Disulphide bridges are very strong bonds that form when two cysteine molecules react with one another. Pair up cysteine amino acids and tape them together to simulate a bond. This will further develop your protein's tertiary structure.
8.  Document your protein's tertiary structure on the student lab sheet.
9. Before you move on to quaternary structure, team up with another student(s). Trade proteins and write-up sheets and do a **peer assessment on the rubric provided**. Remember to do your peer assessment on **THEIR WRITEUP**.

Part 4 - Quaternary Structure

10. With the group you traded with, determine a way to best integrate your proteins together. Try and find a good puzzle-piece type fit using the protein structures you have each individually created. Use tape to **connect your protein molecules together securely**.
11.  Document your protein's quaternary structure.
12. Working as a team with your new partner(s), cover the wire and tape structure you have created with **papier mâché** strips so that any unique three dimensional

aspects of your model are maintained. It's okay to have odd cavities or protrusions in your protein- these exist in the real world! Try to remove any excess liquid from your paper mache strips before wrapping them on your model- excess liquid makes your protein take longer to dry.

13. Once you have completely covered your protein and are satisfied with its shape and structure, place it on a paper towel to dry for 24 hours. Make sure you **label** your paper towel with your name.

Procedure – Day 2

14. Once your model is dry, identify two possible sites that look like they could act as binding sites for other molecules. These are called **active sites** because they are actively interacting with other molecules. Draw a border line around the perimeter of these sites with your sharpie and then **paint within the line**. You have now established where the active sites are likely to be on your protein.
15. **Name your protein**! Add the names of its creators underneath.
16.  Take one final **picture** of your finished product.
17. Complete the **extension questions** on the student write-up sheet.
18. **Hand in your model and your write-up**!

Part 5 - Student Write-Up

Protein Observations/Photos

Primary Structure - Observations:	**Secondary Structure - Observations:**
Photo:	**Photo:**
Tertiary Structure - Observations:	**Quaternary Structure - Observations:**
Photo:	**Photo:**

Peer Assessment Rubric

While evaluating your peers, keep in mind that an honest assessment of their work will lead to the most learning for everyone. Try to be impartial- not too lenient and not too strict!

Marked By: _____

Category	Score 3	1-2	0	Comments
Number of Flags	Model has 50+ flags	Model has 30-40 flags	Model has less than 30 flags	
Flags Labelled/Space Left	Flags are clearly labelled with randomized amino acid names. Spaces are left between flags	Flags are labelled, but are either messy, incomplete, or not random. Spaces may also not exist between	Flags are not labelled or touching one another consistently	
Specialized amino acids	At least 10 cysteine AND at least 10 proline/serine/threonine are present	One or both of the specialized amino acids are under represented	Student(s) have made no effort to include specialized amino acids	
Secondary Structure	Structure is covered in alpha helices/beta pleated sheets	Structure has occasional alpha helices/beta pleated sheets	Structure has no secondary structure	
Tertiary Structure	Wire is bent at ~90^{o} whenever proline/serine/threonine are present	Wire is bent occasionally at these locations (or bent randomly)	Wire is not bent at these locations or bent at random locations along the protein	

TOTAL: _____ / 15

Final Product - Observations: ***(/5)*** **(Teacher Evaluated)**	**Photo:**
Protein Name: _____ **Group Members**: (including those of the group you partnered with for quaternary structure)	

Now your protein model should be complete. Please complete the following questions with your original group before handing in your protein model. You are encouraged to complete them digitally so that your photos and work are all in one place.

Extension Questions: (**/15**)

1. What do the spaces between each tape flag represent? **/1**)
2. Why do proteins twist and fold themselves into 3 dimensional shapes? (**/2**)
3. Research a protein that functions as an enzyme in the body. What is this enzyme called? What does it do? What does it need to bind snugly in its active site?(**/3**)
4. Why does secondary structure form? How does secondary structure affect the overall shape of the protein? (**/2**)
5. In step #14, why did you choose the binding sites you did? Explain your logic. (**/2**)
6. *Your proteins, as with most molecules, are mostly hollow inside. Explain why we have paper mached our structures to make them 3D even though in reality they are largely hollow. (**/2**)
7. Take a look at some of the other proteins in the class. How do some of them differ from yours? Explain these differences in terms of the different levels of protein structure. (**/3**)

Item	Mark	Comments
Peer assessment	/15	
Extension Questions	/15	
Final Product	/5	
Total	**/35**	