Protein Sequins Lab

Engage:

Each group of students needs a toober. Your task is to fold and twist the toober into a design that will allow you to hold a pen or pencil and 3 sheets of notebook paper and walk it across the room without dropping anything. You will have 5 minutes to come up with your design. You may not cut or tie the toober and the outer coating must remain on the wire inside without any of it being visible. You may use loops, folds and twists, but the coating must not be pinched or compressed from its original position. You have 5 minutes do not start any work until your teacher tells you to.

Look around at other groups designs, and be ready to discuss pros and cons of the designs you see. Does your design allow you to walk the full distance of the room without dropping anything? Now pair up with another group. Each group will be allowed to straighten one twist or fold on the other team's toober. Choose the one fold or twist to straighten that has the biggest effect on the task of carrying the objects. Perform the straightening and see the results. Now return the toober to its functional shape and choose a fold or twist that would not affect the functional shape of the toober and do the straightening.

Answer the following questions in your lab notebook or this paper:

1. Was everyone's design the same? What similarities in folding or twisting did you see in several other designs?

2. What fold or twist worked best (for your group or another groups design) to hold the pen or pencil?

3. What fold or twist worked best to hold the paper?

4. Do you think there is a fold or twist in every groups toober that if straightened will change its shape enough to make it nonfunctional? Is there one that does not make it nonfunctional in everyone's toober?

Explore:

Now we will make a structure with out toobers that has specific bends and twists based on some design rules. Take your toober and straighten it out. You will need 15 pieces of pipe cleaner with a sequin on them. You will need 15 pieces of pipe cleaner and sequins of the following colors: 2 blue, 2 red, 6 yellow, 3 white, and 2 green. Mix them up and randomly attach them to your toober evenly spaced 7-8 cm (about 3 inches) apart. Once you have them all twisted into place use the following rules to fold and twist your toober into a shape. Start at one end and move to the other end as you fold and twist.

1. Yellow pipe cleaner and sequins need to be on the interior of the structure as close together as you can.
2. White ones need to be on the outside of your structure and away from any yellow ones.
3. Red and blue ones will pair with each other one red to one blue in each pair so they are touching.
4. Green ones will pair up as well, touching each other.

Remember no tying the toober, or twisting too tight as to compress the foam coating. Look around at other group's toobers and notice any similarities and differences. Answer the following questions in your lab notebook or this paper:

1. How were the toobers of the different groups similar and different (2 of each)? Do you think there were any two alike? Were there groups with combinations that finished quicker, or could you choose a sequence of colors that would be easier to form into a shape?

2. Does each new rule lead to a new shape? Do the order of amino acids make a difference in the final structure?

3. Could you make a lot of different shapes with only the 4 different colored pipe cleaners with sequins in the 15 different locations? (How many? 15, 50, 500+) What if you had 20 different colors to choose from and could use 100 or more pipe cleaner sequins, would there be a lot of different shapes? (How many? 15, 50, 500+)

4. If your toober structure is a protein and the order of pipe cleaners and sequins are the amino acids, would the amino acid sequence cause the protein to be a specific shape? How many different levels of structure do proteins have if there are 4 "rules" for folding and twisting? Would a change in the order of the amino acids (pipe cleaners with sequins) change the shape of the protein they make up?

Explain: (Referenced: AASK Student Handout Amino Acid Building Blocks of Protein)

There are 20 Amino Acids and each one 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 determine its 3D shape and its chemical properties. 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. This activity will help you understand how the unique properties of each side chain contribute to the structure and function of a protein. They will walk you through the process of protein synthesis and folding of the resulting protein

Instructions:

1. From your instructor, obtain a strip of DNA that is already separated into codons. Copy your sequence into the chart below and perform transcription (DNA to mRNA; A-U, T-A, G-C, C-G) and translation (mRNA to Amino Acids; A-U, T-A, G-C, C-G) to form the protein corresponding to your piece of DNA. Be sure to include the number of the DNA sequence your received. Then using the codon chart fill in the amino acids

#?	DNA															
	mRNA															
	AA															

First Letter	Second Letter				Third Letter
	U	C	A	G	
U	phenylalanine	serine	tyrosine	cysteine	U
	phenylalanine	serine	tyrosine	cysteine	C
	leucine	serine	stop	stop	A
	leucine	serine	stop	tryptophan	G
C	leucine	proline	histidine	arginine	U
	leucine	proline	histidine	arginine	C
	leucine	proline	glutamine	arginine	A
	leucine	proline	glutamine	arginine	G
A	isoleucine	threonine	asparagine	serine	U
	isoleucine	threonine	asparagine	serine	C
	isoleucine	threonine	lysine	arginine	A
	methionine	threonine	lysine	arginine	G
G	valine	alanine	aspartate	glycine	U
	valine	alanine	aspartate	glycine	C
	valine	alanine	glutamate	glycine	A
	valine	alanine	glutamate	glycine	G

2. Using the table below choose the color of sequined pipe cleaner for each amino acid, for example Alanine is a yellow one. Then place your amino acids in the correct order from your chart make sure they are evenly spaced on your toober (7-8 cm apart). This represents your protein (chain of amino acids) in its unfolded state.

Amino Acid	Color	Reason
Lysine (lys) Arginine (Arg) Histidine (His)	Blue	Basic
Aspartic Acid (Asp) Glutamic Acid (Glu)	Red	Acidic
Glycine (Gly) Alanine (Ala) Valine (Val) Leucine (leu) Isoleucine (Ile) Methionine (Met) Phenylalanine (Phe) Tryptophan (Try) Proline (pro)	Yellow	Hydrophobic
Serine (ser) Threonine (thr) Tyrosine (tyr) Asparagine (Asn) Glutamine (Gln)	White	Polar
Cysteine (Cys)	Green	Cysteine (S-S)

3. Fold your protein, following the Protein Laws of Folding.

Toober Laws of Folding	
1. Blue and red will pair	Acidic groups and basic groups tend to form salt bridges. Opposites attract so pair up each red with a blue so they are touching
2. Yellow will fold in to the interior of the protein	Hydrophobic side chains are buried inside the protein to avoid water, fold the yellows as close together and as close to the center as possible
3. White will be exposed on the outside of the protein.	Polar (Hydrophilic) side chains have an affinity for water. All whites should be facing the outside and away from the yellow
4. Green will pair	Cysteines will form a disulfide bond to stabilize protein shape, so pair up each green with another green so they are touching

4. Answer the following questions in your lab book or this paper:
    1. Was the folding of this real protein structure easier than your random ordered one? Why or why not? Are proteins random orders of amino acids?

    2. Why do the chemical properties of the amino acids (acidic, basic, hydrophobic, etc.) help determine how it will cause the protein to fold? When you changed the shape of another groups toober in the beginning of this activity what does that represent in a protein?

    3. What is a change in the DNA sequence called? How does a change in the DNA sequence change the amino acid sequence? Are there changes in the DNA sequence that do not change the amino acid sequence?

    4. When you change environmental conditions of a protein we know from what we know about enzymes it makes them not work properly. What effect on the amino acid interactions that form the shape of a protein like an enzyme might a change in temperature or PH have? Why does the shape of a protein lead to its function?

Elaborate:

Sickle cell anemia is a disease caused by a single mutation in the gene that codes for beta-globin, a subunit of hemoglobin. Amino Acid #6, glutamic acid, is replaced with another amino acid at that position. The new amino acid is valine. This mutation leads to sticky deformed red blood cells.

1. Obtain your toober you saved or reconstruct one using your amino acid sequence from the last activity. (Remember your toober protein is fictional and not beta-globin. We can not reconstruct beta-globin because of its size and complexity, but we can use your toober protein as a model of the mutation that occurs with sickle cell anemia.)

2. Replace the glutamic acid (red) with valine (yellow) on your toober protein to simulate the sickle cell anemia mutation

3. Refold your mutated protein referring to the Protein Laws of Folding you used earlier.

4. Answer the following questions in your lab book or this paper:

   1. What shape or conformational change do you see in the protein? Why might the change in the shape of this protein change its function? Does this change in the amino acid order affect other bonds or how you might fold it if you started over from one end folding as you go?

   2. A point mutation is a change in a single nucleotide. How could the change from glutamic acid to valine have occurred (look at your original DNA sequence and determine what nucleotide (ATGC) was changed to get the mRNA code for valine not glutamic acid)?

   3. Look up the properties of glutamic acid and valine. Are they polar or non-polar? Hydrophilic or hydrophobic? Acidic, basic or neutral? How do these differences explain the conformational change in the protein? How could this change cause the effects of sickle cell anemia in a person with this mutation? Use your book or go to the following web site for more information on sickle cell anemia. (http://www.nhlbi.nih.gov/health/dci/Diseases/Sca/SCA_WhatIs.html)

Lab based on the "Toober" lab by the Center For Biomolecular modeling.