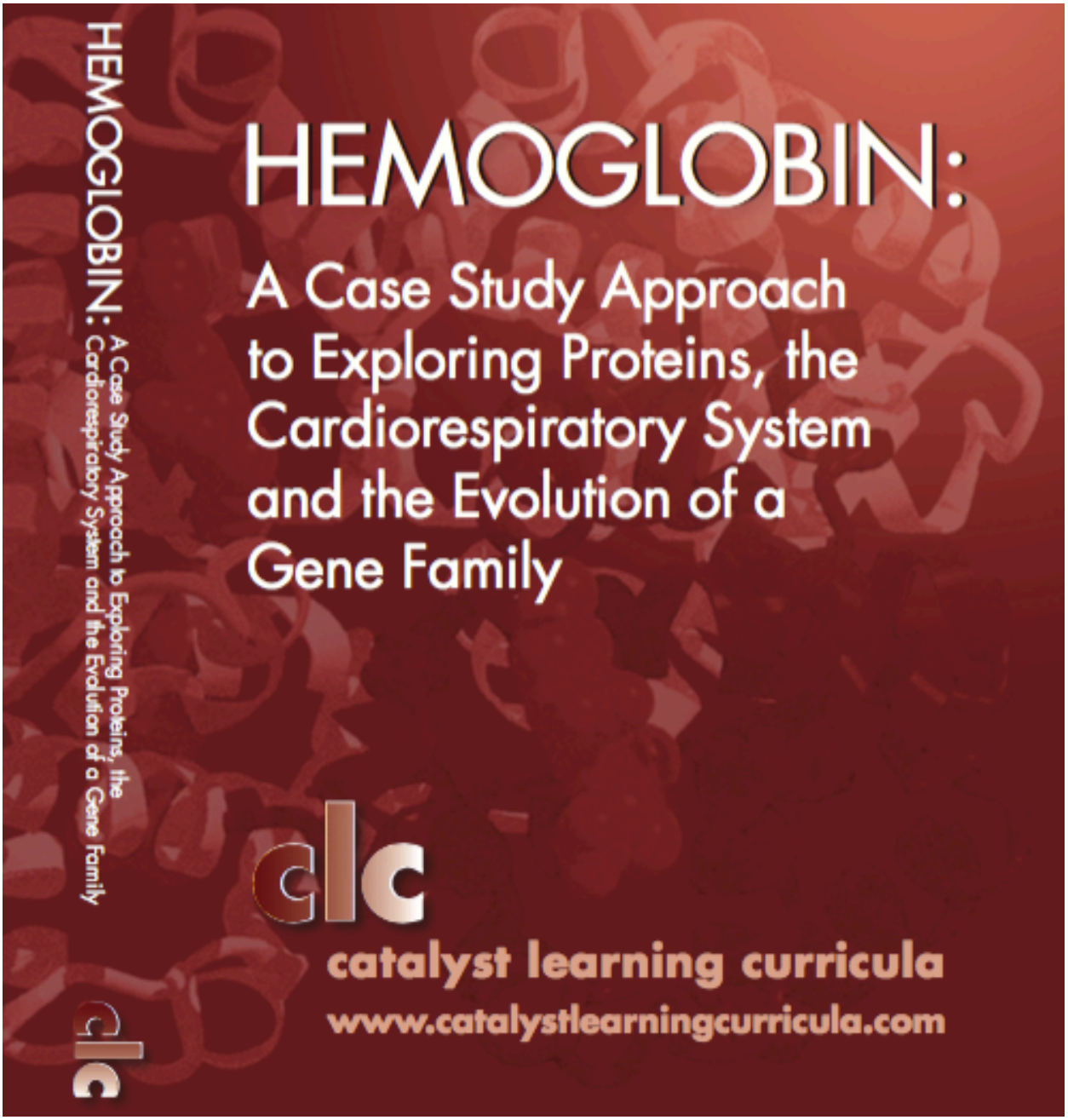
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# HEMOGLOBIN:

**24 experiential, higher-order thinking activities for Advanced Placement* (AP) or International Baccalaureate** (IB) high school or college students, covered in 30-42 class periods**

The external environment of early Earth underwent incredible changes that dramatically affected the evolution of the 2-billion-year-old globin family of proteins. This allosteric molecule accepts and releases oxygen, depending on the presence of competitive and non-competitive inhibitors, in a complex dance that is finely regulated by pH, pressure, and changes in concentration and conformation. Feedback mechanisms at the cellular level present a clear example of homeostasis. Every organism's respiratory anatomy and physiology is tailored to the challenges of acquiring oxygen in its environment. In addition, the globin genes are regulated pre- and post-expression, conferring differences in fitness for each individual. This study of blood protein includes interesting histories such as hemophilia in the royal families of Europe, blood typing, transfusions and the co-evolution of mutant forms of this molecule that have contributed to the survival of people in tropical regions of the world. Hemoglobin provides a perfect example for teaching the diverse structure and function of proteins. Molecular analysis of the gene and phylogenetic comparisons of gene sequences elucidate the history of this important molecule through taxa and through time.

**This case study was specifically designed to provide complete, stand-alone coverage of the following topics required by the AP College Board and the IB program:**

Structure and Function of Amino Acids
Biochemistry of Allosteric Proteins
Comparative Evolution of Respiratory Anatomy and Physiology
Molecular Analysis of the Beta-globin Gene
Bloodstream Components and Blood Types
Cell Respiration and Other Energy Utilization Processes
Homeostatic Regulation of Oxygen
Phylogenetic Comparison of Globin Protein and DNA Sequences
Evolution of a Gene Family
Gene Mutation and Duplication Events
Sickle Cell Anemia and the Heterozygote Advantage
Hemophilia and Pedigree Analysis
Genetic Engineering of Clotting Factors
Causes and Consequences of Cardiovascular Disease

**This case study also offers partial coverage of the following required topics:**

Early Earth and the Origin of Life
Bioethics

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# Hemoglobin: A Case Study for Advanced Science
## Table of Contents

(This sample contains Activity 1 and 12 only, which do not correspond with the page numbers listed below. The full curriculum can be purchased at the Catalyst Learning Curricula website)

## Activity One: Modeling the Structure and Function of Proteins

**Teaching Time:** 1 class period of 50 minutes

Objectives:

a) For students to create a human model of an amino acid and a polypeptide in a class simulation.
b) For students to recognize how the backbone and the side chains of a polypeptide influence protein folding.
c) For students to visualize the primary, secondary, tertiary, and quaternary structures of a folded protein.
d) For students to simulate the function of an enzyme.
e) For students to experience the physical causes of denaturation due to temperature and pH.

Materials:

For each student: 1 chenille stick; 20-25 colored beads and a paper cup or bowl; 2 name tag stickers with at least 3x6cm of writing space (stickers that come in packages of five assorted colors work best, but you can use plain white stickers as well); 1 black marker; paper towels. For the class: 1 sheet of red dot stickers; 3 sheets of white dot stickers; 1 average-sized sink sponge for every 4 students; 1 black permanent marker; 5-6 chenille sticks (aka pipe cleaners).

Procedure:

1. Prior to class, create one shirt sticker for each student with the letters C, N, or R written on them. There should be two C stickers for every N and R sticker created. Because there is only one letter written on each sticker, large stickers can be cut in half, thirds or quarters, if necessary or desired.
2. In addition, create one shirt sticker for each student, plus about six extra stickers, with the full name of the following amino acids (one per sticker) and the properties of each amino acid's side chain noted next to the name. If you have stickers in five assorted colors, choose one color to represent each property. For example:
    - Yellow: all the nonpolar amino acids
    - White: polar amino acids
    - Red: negatively charged amino acids
    - Blue: positively charged amino acids
    - Green: sulfur-containing amino acids

| Name of the amino acid: | Property symbols to add to the sticker: |
|---|---|
| Glycine | N, sm |
| Alanine | N, sm |
| Isoleucine | N, sm |
| Leucine | N, sm |

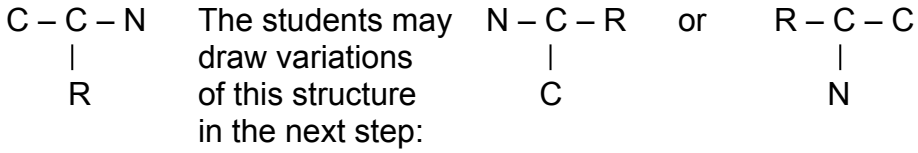
| | |
|---|---|
| Valine | N, sm |
| Methionine | N |
| Proline | N |
| Phenylalanine | N, lg |
| Tryptophan | N, lg |
| Tyrosine | N, lg |
| Serine | P, sm |
| Threonine | P, sm |
| Asparagine | P |
| Glutamine | P |
| Arginine | + |
| Histidine | + |
| Lysine | + |
| Aspartic Acid | - |
| Glutamic Acid | - |
| Cysteine | S |

| | | |
|---|---|---|
| N = nonpolar | sm = small molecule | + = positive charge |
| P = polar | lg = large molecule | - = negative charge |
| S = sulfur group | | |

3. Cut the sink sponges into 2 cm cubes and write “OH” on half of them and “H” on the other half, creating enough pieces to give one of each kind to each student. Once the marker has dried, and immediately prior to class, wet the sponges with water such that they drip when squeezed.
4. Also prior to class, build at least one 3-D model of an amino acid and place it on your desk. (You can make models of amino acids using organic model kits or supplies such as toothpicks and marshmallows.) In addition, have an image to project, a handout, or the reference page in the students’ textbook ready with a list of each of the twenty amino acids so students can relate the human models they are building to the 3-D model and the 2-D drawings of similar structures. Being able to visualize relative size, particularly items on a nano scale, is a cognitive skill that is constantly utilized in science—having several different examples of amino acids and polypeptides will help students develop this skill.
5. When the students arrive to class, have them move the tables, chairs, and/or desks against the walls. Give each a sticker with the letter C, N or R written on it.
6. Ask the students to stand up and, using the information on their shirt stickers, create a monomer of a protein, grouping themselves based on their stickers. If the students are not sure how to proceed, ask them the following questions to draw out what they know:
    a. What is a monomer of a protein? *(A single amino acid.)*
    b. What do you think the letters on the stickers represent? *(C stands for carbon; N stands for nitrogen; and R stands for R-group, or residue, or side chain of an amino acid—tell the students that textbooks and scientists use these different names for the R-group interchangeably.)*

c. What are the components of a protein monomer or amino acid? *(Each monomer has an amine group, a carboxyl group, and a side chain or R-group.)*

7. Allow the students to use what they have learned from the discussion questions above to form amino acids. Expect them to hold hands, link elbows, or place hands on elbows or shoulders to demonstrate the bonds between atoms. Do not direct them or give them too many hints, but instead, encourage them to try any formation and tell them if they are getting "hotter" or "colder." Or, you could give them more specific feedback such as, "You have the correct number of components, but not the right types of components," or "You have the correct number and types of components, but they are not bonded correctly," or "The shape of an amino acid is not linear (or circular)." The students may struggle, but if you make sure they feel comfortable taking guesses and using their resources (such as their textbook or computers), they will discover the structure on their own through trial and error or through peer tutoring. Eventually the students should form a "T-shaped" structure with C–C–N connected in a line and the R-group branching off the central carbon:




8. When one group forms the correct structure, quietly praise them, pointing out the aspects of the structure that are essential (i.e., "Great, you have a T-shaped structure with the R-group stemming from the central carbon"). Then ask them to draw their shape on the board. (Some groups will find translating the model from 3-D to 2-D challenging since it can be drawn in different rotated forms.) Continue to give groups feedback as needed until they have all correctly formed and drawn a monomer.
9. Ask one student to come to the board and be a note-taker. Take a moment to go over what the students have discovered and what information can be added to the models. Use these guiding questions to help create notes:
   a. Are all the drawings on the board the same structure? *(Take a moment to explain how the molecules/drawings can be rotated in space.)*
   b. What atoms are missing from the models you have created and drawn? *(The nitrogen atom should be surrounded by either two or three hydrogen atoms and the end carbon should be bonded to an oxygen atom with a double bond and an OH group with a single bond.)*
   c. Which part of this structure is the "amino" portion of the amino acid? *(The nitrogen end with the additional hydrogen atoms.)*
   d. What do we call this type of functional group? *(–$NH_2$ or –$NH_3$ is called an amine group.)*
   e. Which part of this structure is the "acid" of the amino acid? *(The –COOH end.)*
   f. What do we call the functional group that has –COOH? *(A carboxyl group.)*

g. Is this molecule polar or nonpolar? *(When ignoring the properties of the side chain, the backbone of each amino acid is polar since there is no symmetry in the $NH_2$–CHR–COOH pattern.)*
h. Which end of the molecule could be termed the electropositive pole and which portion of the molecule could be termed the electronegative pole? *(Draw an electropositive symbol near the amine group and an electronegative symbol near the carboxyl group.)*

10. Ask the students if they would like the note-taker to add any information to make the notes easier to understand or more complete, and then give the students time to copy the notes. Using a note-taker to model note-taking skills allows students to learn from what they see and to suggest improvements for the note-taker. This can improve all of the students' study skills. If you ask one student to take notes while the other students are performing an activity, it allows the note-taker an opportunity to observe what is happening and summarize it for the group. Change out the note-taker every time the activity changes direction so no student sits out for multiple activities.
11. Ask the students if they are satisfied with the models they created or if they would now like to show the position of the hydrogen and oxygen atoms that were not represented in the human models they created. If so, offer the students a sheet of white dot stickers with which to represent the hydrogen atoms and a sheet of red dot stickers with which to represent the oxygen atoms, and allow each group to recreate their amino acid with the additional atoms. Now ask the students to reassemble in monomers, this time interacting with different classmates than they did during the first simulation.
12. Give one wet sponge labeled "H" and one wet sponge labeled "OH" to each amino acid group. (Keep paper towels at hand for cleanup.) Ask the students where the extra hydrogen atoms are on the amino acid (at the $–NH_3$ end), and then tell the students to pass the wet "H" sponge to the person who represents the amine group. Ask the students where the "OH" group is located on the amino acid (at the –COOH end), and then tell the students to pass the wet sponge labeled "OH" to the person who represents the carboxyl group.
13. Ask the students to stay connected as a monomer but to interact with another amino acid to form a dipeptide. As the students are moving toward another amino acid, ask if the amine group would bind to another amine group or to a carboxylic acid group. If the students are not sure of the arrangement, ask them how long a protein chain would be if amine groups joined with other amine groups (or carboxyl groups with other carboxyl groups) and they should conclude that the only way to form chains of more than two amino acids would be to require opposite ends to bond with one another.
14. As the human monomers join to form dipeptides, ask the students who are forming a new bond between the two amino acids to squeeze their sponges as their hands meet and tell you what is happening. *(Water is released.)* Again, you will want to keep paper towels handy. Ask the students if they know what type of reaction has just taken place. *(The formation of a peptide bond is a dehydration reaction.)* Ask the note-taker to draw a dipeptide and label the reaction appropriately. The students in the simulation may want to help guide the note-

taker since they are currently away from their notes and may have particular concepts they want to recall later when they go back to their desks.

15. Ask the students to stay connected to their amino-acid partners and slowly move to form two polypeptides, one on each side of the room. However, this time, as they bond with other amino acids, ask each monomer to narrate the reaction with appropriate scientific terminology (i.e., “Our dipeptide is joining another dipeptide using a dehydration reaction. The peptide bond between our amino acids allows the release of water and a polypeptide is formed”).
16. Tell the students that they have just exemplified the primary structure of a protein by connecting their monomers of amino acids in a particular linear, sequential order. Give each amino acid group a name (glycine, alanine, serine, lysine, histidine, etc.) and then ask them to tell you the primary structure of their particular polypeptide starting from the N-terminus (where the last person represents an amine group) and ending at the C-terminus (where the last person represents a carboxyl group). Ask the note-taker to take notes as needed on the definition of the primary structure.
17. The students should now be divided into two polypeptides. Ask each polypeptide to stretch out while remaining attached to the others in its group, such that the polypeptides are elongated across the room. Point out the position of the side chains, and tell the students that if they were a molecule in space, the side chains would move freely around the carbon bond until they found the most stable position. (The side chains are most stable when not too cramped due to size/space, not repulsed by charge or polarity, etc.) For the next simulation, ask the R-groups to position themselves so they are on alternating sides of the polypeptide from one end to the other.
18. While remaining connected to the other members of their amino acid and to the other amino acids in their polypeptide, ask the R groups to slowly pull away from the backbone of the polypeptide chain, gently dragging the central carbon (and therefore the other members of the amino acid) with them until they have formed a zigzag structure that stretches across the room. Ask the students if they know the name of this structure. *(The students have formed a beta-pleated sheet.)* Ask the students who are slightly electronegative to identify themselves. *(The students who represent carboxyl groups should identify themselves as electronegative.)* Ask the students who are slightly electropositive to identify themselves. *(The students who represent amine groups should identify themselves as electropositive.)* Ask the students if they can hypothesize why polypeptides often form beta-pleated sheets spontaneously. *(The slightly positive amine groups are drawn to the slightly negative carboxyl groups of the neighboring amino acids, so the beta-pleated sheet adds stability to the polypeptide chain.)*
19. Explain that the beta-pleated sheet is called a secondary structure, which can be found in most polypeptides. Clarify that secondary structures may be numerous or few within a polypeptide, but they arise due to the polar nature of the C–C–N backbone of the amino acid and are stabilized by hydrogen bonds.
20. Twist a chenille stick around your finger until it has three complete coils. Gently slide it off and show the students the shape. Tell the students that this is another

form that spontaneously emerges due to the attraction of the poles of the C–C–N backbone within a single polypeptide chain. Ask the students if they know the name of this secondary structure *(alpha helix).* Challenge the students to go back to their notes and draw the location of each C–C–N of a polypeptide strand in a diagram that shows the attraction between carboxyl and amine groups within the spiral. Give the students a hint while they are making their drawings: tell them it takes four amino acids to make one complete turn of the helix. They can check their work using their textbook or a projected image you provide, or they can work in groups or as a class.

21. Allow a moment for the students to catch up on their notes or clarify any questions they might have about primary or secondary structure. Allow the note-taker to leave the board and join in the next activity.
22. Give each student an amino-acid sticker to wear, asking them to cover their previous sticker with the new one. Distribute two wet sponge cubes to each student—one with "H" written on it and one with "OH" on it. Tell the students they now represent an amino acid with the properties noted by the abbreviations. Ask the class if anyone can guess what each of the abbreviations means. (See key above, in which N=nonpolar, P=polar, etc.) If they do not know, encourage them to use their textbook or the Internet.
23. Ask the students why one sponge says "H" and the other says "OH". *(The excess hydrogen (H) atoms are on the amine group at one end of each amino acid monomer, and the extra hydroxyl group (OH) is part of the carboxylic acid end.)*
24. Ask the students to each use their right hand to represent the amine group and their left hand to represent the carboxylic acid group, placing the sponges in the correct hand as needed. (The "H" sponge will go in the right hand of the amine group, and the "OH" sponge will go in the left hand of the carboxylic acid group.)
25. Split the class in half, and ask the two groups of students to each form a polypeptide with the other amino acids in their group, narrating the process quietly to themselves using the appropriate scientific terminology. When all students have joined hands and narrated the dehydration reaction, they will have formed two separate linear polypeptide chains. Ask the students what macromolecule they just formed. *(A polypeptide.)*
26. Ask the students what level of protein structure these chains represent? *(The students have simulated the primary structure of a polypeptide since all of the amino acids are now arranged sequentially in a linear chain.)* You may want to reinforce the concept of primary structure by asking a student to tell you the primary structure of their polypeptide. They should be able to read the sequence of amino acids in order from the N-terminus to the C-terminus.
27. Assign a new note-taker.
28. Ask the students to now consider the properties of their side chains. (These properties are summarized in the abbreviations listed on their stickers.) Ask the students to consider how the properties of their side chain might influence how that particular amino acid relates to the other amino acids in the polypeptide chain and to act accordingly. If the students are unsure how to proceed, you may ask the following questions:

a. What do you think the plus sign on a sticker indicates about that amino acid's properties? *(It indicates that the side chain of this amino acid has a positive charge.)*
b. What is the positive side chain going to be attracted to? *(It will be attracted to negatively charged atoms, molecules, or regions.)*
c. What will it be repulsed by? *(It will be repulsed by other positively charged atoms, molecules, or regions.)*
d. Repeat the above questions with the negative sign, the P, NP, and S. *(The nonpolar side chains are attracted to other nonpolar molecules or side chains and repulsed by polar substances, while the polar side chains will be attracted to other polar molecules or side chains and repulsed by nonpolar substances. The sulfur-containing amino acid [cysteine is the only amino acid containing a sulfur atom] will be able to form covalent disulfide bonds or salt bridges with other cysteine amino acids, an important property that lends a tremendous degree of stability to a folded protein chain. Large or small side chains will need the appropriate amount of space when interacting with other parts of the peptide chain.)*

29. Ask the students if they would like the note-taker to add anything to the notes.
30. While considering the properties of their own amino acid's side group, the students should remain linked in a primary structure sequence but move toward or away from the other members in their chain according to the interactions that they think would take place. Do not give the students any hints, but you should observe side chains with opposite charges moving toward each other; those with like charges should move away from each other; nonpolar side chains should move toward one another but away from polar side chains; polar side chains should move toward one another but away from nonpolar side chains; large side chains will move so that they have more space; and disulfide bridges will form between cysteine amino acids.
31. Ask the students what level of protein structure these chains represent now that they have moved into a specific position in response to the properties of their side chain? *(This folding pattern represents the tertiary structure of a polypeptide.)*
32. Ask the students how they knew where and how to move. They should be able to describe what attractions or repulsions directed their movement. If they do not mention the actions described in step 30 above, take a moment to review the properties and the resulting molecular interactions of the side chains.
33. Students will often figure out how to fold and be able to describe what they did and why, even if they need to consult their resources. However, they often will forget to place the nonpolar side chains to the interior of the folded structure and press the polar side chains on the exterior of the folded polypeptide. Unless a protein is a membrane-spanning protein, it will usually fold the nonpolar side chains into the interior of the polypeptide because the surrounding environment in most cellular spaces is aqueous and therefore polar. You can remind the students of this using questions such as, "Do you think the interior of a cell, the cytoplasm, is a watery/polar environment or an oily/nonpolar environment?" or "Some proteins fold their polar side chains on the interior, however the majority of

proteins fold their nonpolar side chains to the interior. Why do you think this is so?"

34. Ask the two polypeptide chains to interact with one another according to the properties of their side chains. Remind them to move slowly as they approach each other so they can maintain the folded arrangement of their tertiary structure.
35. When they have arrived at a comfortable arrangement, ask the students what level of protein structure these chains represent? *(The quaternary structure of a polypeptide.)*
36. Ask the students to summarize what dictates the folding pattern of the secondary structure *(the polar properties of the C–C–N backbone*), tertiary structure (*the properties of the side chains within the polypeptide)*, and quaternary structure *(the properties of the side chains between two or more polypeptides)*. Ask the students if they want to suggest any additional information the note-taker may have missed.
37. Assign a new note-taker. (The current note-taker can take the new note-taker's shirt sticker and their place in the polypeptide chain so that the chains are not disrupted.)
38. Ask the students if they think a protein will fold in the same way every time. Listen to their responses and ask them to back up their ideas. *(Primary structures of proteins will fold in the same pattern every time if the environmental conditions are held constant. If the environment becomes more acidic, basic, polar, nonpolar, or charged, then the folding pattern will change as the environment affects the interacting properties of the side chains.)* You can emphasize this point by telling the students that this protein has been inserted into the cell membrane and is now surrounded by the hydrophobic tails of the phospholipids. The protein they have formed should move the nonpolar side chains to the exterior of the molecule, while the polar side chains should be enclosed in the interior.
39. As a challenge, you can ask the students what would happen if you changed one amino acid in the middle of the 3-D structure they've simulated. *(They should recognize that the secondary, tertiary, and quaternary structures might all be affected by a single amino acid change in the primary structure.)* Ask them to describe a specific substitution that would NOT likely alter the folding structure of this particular protein *(such as the substitution of a small nonpolar amino acid in the place of another small nonpolar amino acid on the periphery of the protein)*. Ask the students to now think of a substitution that would dramatically alter the folding pattern of the protein *(such as the substitution of a charged amino acid for an amino acid of the opposite charge that is located at a place in the folding where several amino acids are interacting)*.
40. To check how much the students understand about the folding structure and the function of polypeptides, tell them that they represent a polypeptide that acts as an enzyme. An enzyme is a specialized protein that makes (anabolic) or breaks (catabolic) a particular type of molecule, called a substrate. Look at the interior of the folded protein the students have created and find a place where you could wiggle in between three or four amino acids. Point out this location within the polypeptide, and tell the students that this is the active site of the enzyme—the

place where the substrate will sit to undergo a chemical reaction that will break the substrate into two smaller molecules (or where two reactants are joined together). From the extra stickers you made before class, choose three or four that match the properties of the amino acids in the active site of this enzyme and place them on your shoulders and hips. For example, if the active site has a negatively charged amino acid, a nonpolar amino acid, and a positively charged amino acid, place one charge of each type on your shoulders and place a nonpolar sticker on one hip, so that you can wiggle into the active site and have the right "mates" to help your body line up to the amino acids in that pocket of the active site.

41. Tell the students you are now the substrate that fits into the active site of this enzyme. Wiggle into the active site of the enzyme and ask the students how this molecule will be able to line up properly. The students should be able to direct you to line up with opposite charges attracting and polarities seeking similar side chains. Tell the students that to fit into this active site perfectly, you must twist a bit due to the interactions of the side chains with your molecular structure. As you twist, make a popping sound and tell them this twisting helps initiate the catabolic reaction that breaks you into two pieces. (Enzymes lower activation energy so that this is more likely to occur.) Now that you are broken into two pieces, you can leave the active site of the enzyme.
42. Ask the amino acids nearby if they were affected by the reaction that just took place or if they are "okay" and ready to take on a new substrate. They should respond that when you left, they were the same as they were before you entered, since the enzyme is not used for anything other than an optimization of the occurrence of the reaction. If you like, you can discuss things further, telling the students that an enzyme might hold two molecules in close proximity to one another so they can bond to form a larger molecule. Point out that the enzyme is not changed by the reaction.
43. Ask the students if there is any information they'd like for the note-taker to add to the board.
44. Now, tell the students that you are no longer a substrate (or product), but instead you are a mad scientist and you're increasing the temperature under the beaker that contains the protein that they're representing. Tell the students to continue holding hands in their primary structure, and ask them to show you what atoms do when they're suspended in space. *(They move.)* Tell the students that the heat makes the particles in their atoms move at a higher speed, so they will become more active as the heat is increased. Tell the students that you are turning the heat up even more, so now their atoms are moving so vigorously that some of the bonds between the amino acids are actually breaking. Ask the students when they have seen this type of disruption occur in a protein. *(Melted or burned hair, a cooked egg, cooked pieces of meat or cheese, etc.)* Ask them to explain how heat affected their particular protein chain. *(They should recognize that heat breaks the hydrogen bonds between the side chains of the tertiary molecular interactions, it affects the coils and pleats of the secondary structure, and finally, if its temperature is high enough, it will begin to break the*

*covalent bonds between sulfur groups and between amino acids, causing the protein to denature permanently.)*

45. Challenge the students to think of other ways a protein might be denatured. *(It may denature if it is placed in a strongly acidic or basic solution, due to disruption of secondary, tertiary, and quaternary structures.)*
46. Ask the students to demonstrate what might happen if the pH in the environment surrounding a polypeptide changes significantly: Have the students return to the original quaternary shape they held prior to denaturation to model the peptide bonds. Tell them that you are a mad scientist again and you're pouring an acid—with an excess of positively charged hydrogen ions—into the beaker that contains the protein they represent. Walk around the polypeptide, telling the negatively charged atoms that they are now surrounded by positively charged hydrogen ions. Ask the students how they think the protein will be affected. *(They should be able to tell you that the negatively charged side chains will now be attracted to the positive hydrogen ions, distorting the folded protein's shape. The positively charged side chains will now be repulsed by the hydrogen protons in the surrounding environment, so they will move toward the interior of the polypeptide away from the acid solution. Likewise the polar residues will move out into the solution, further distorting the overall structure.)* Ask the students what would happen if you, the mad scientist, had used a basic solution with an excess of negatively charged hydroxide ions ($OH^-$) instead. *(The excess of negatively charged ions would be attracted to the positively charged side chains, disrupting the bonding pattern of these side chains as well as hydrogen bonding throughout the molecule.)*
47. Allow the students to release their peptide bonds and go back to their desks to jot down in their notebooks the main points of this activity.
48. Explain that there are many different types of proteins, some with one chain (with only tertiary structure) and others with multiple protein chains (with quaternary structure). Let the students know that proteins are very diverse and classified by the job they perform for a cell or a system (for example, catabolic or anabolic enzymes, transport proteins, cell surface proteins, etc.). Ask the students if they can name or guess some of the different functions of proteins in a bacterium, fungus, animal, or plant.
49. Tell the students that hemoglobin is a protein composed of four polypeptide chains, two identical alpha chains, and two identical beta chains. It is not an enzyme but a carrier protein that is capable of transporting oxygen and carbon dioxide needed for cell respiration. Let the students know that there are several different types of oxygen-binding molecules used by different types of organisms, used at different points of development or used in different locations within the same organism. Let them know they will be exploring these different molecules to become familiar with the specific and unique properties that any one protein can have.
50. In conclusion, ask the students to respond to an individual assessment question on paper: "Your stomach produces a mixture of digestive juices that has an acidity of around pH 2, and your internal temperature is approximately 98.6 °F. Explain how the chemical and physical environment of the stomach helps it to

successfully fulfill its function." You may want to collect and grade the students' responses to this individual assessment question, to discover how much of the information from the hands-on learning activity was retained and processed.

## Activity Twelve: Tracking Mutations and Gene Duplication Events

**Teaching Time:** 2-3 class periods of 50 minutes each

Objectives:

a) For students to understand how repeated sequences of DNA are added to the genome of an individual or a species.
b) For students to create a sequential, visual tutorial for one type of gene duplication process.
c) For students to understand how gene duplication processes impact the evolution of a population over time.
d) For students to consider the implications that gene duplication processes have on origin of life hypotheses.

Materials:

For each student: 1 pair of scissors. For each group of 4-5 students to share: 4m yarn in one color; 2m of yarn in a second color; 1m yarn in a third color; tape; marker; 1 piece of blank, white copy paper; 1 device that can take and store photos and support an application for creating time-lapse photography videos; assorted props for the creation and performance of skits that depict gene duplication processes.

Procedure:

1. Prior to this activity, use a digital device (such as a smart phone or tablet) to download a time-lapse photography app. You can search for such an app using single words or combinations of: time lapse, chrono lapse, stop motion, stop frame, stop animation, etc. Explore one or more of the apps to determine which one you like best, and learn how it works so you can advise your students and help them navigate it easily. There are several free applications for Apple® and Android® operating systems that work well and offer a range of functions. As long as the students are able to take pictures and arrange them in a series that can be shown as a rapid-succession slideshow, the application should be suitable. The ideal would be to find a free application that allows voiceover narration to be added to the slideshow. If you do not have a class set of computers or tablets and you plan to ask students to use their own devices, choose one application for each type of operating system that your students are likely to have (for example, an app for Apple® OS and an app for Android® OS). It is very likely that your students will have devices that can run apps (and they may be very eager to use their tablets or phones during class).
2. In this activity, the students will first review what they know about genetic mutations by creating a compare-and-contrast chart to synthesize their prior knowledge. The students will learn how to use the time-lapse photography program as they are led through the modeling experience to learn how one

type of gene duplication occurs. They will then be asked to work in groups to create a time-lapse slideshow using props and models of their own choosing to depict the creation of duplicate genes from aneuploidy, polyploidy, transposons or reverse transposons.

3. Follow these steps to begin a review of the ways in which DNA mutations may occur:
    a. Ask the students to make a list of the different processes that can result in a mutation of DNA *(deletion, insertion, point mutation, translocation, duplication, nondisjunction, etc.).*
    b. Ask the students to share their list with a neighbor and expand it if possible. You may allow them to use resources such as their textbook or the Internet if you find their lists are incomplete, or you can ask pairs of students to join together so that groups of four can share and brainstorm additional ideas. You may also want to have identified ahead of time a target number of terms that you expect them to know. Circulate, telling pairs or groups that they have "four out of the seven" (from your list), to encourage them and give them a goal.
    c. Ask the pairs of students to each list characteristics that can be used to distinguish one type of mutation from another. *(Such as the location, the number of base pairs involved, the starting and ending products, the timing, etc.)*
    d. If they have not already done so, have each pair of students join with another pair of students and ask the resulting groups to each create a compare-and-contrast chart that includes characteristics they have listed so mutations can easily be distinguished one from another in a visual format. Asking the students to create a study chart such as this is good practice, so give them time to try different ideas and share their best results with the rest of the class. Some groups may create charts that depict a higher level of analysis, such as one that features subgroups. (For example, duplications, translocations, and tandem repeats can be considered subgroups of insertions.) Or they may depict mutations that can be classified in several categories. (For example, a translocation can be classified as both an insertion and a deletion.)
    e. When the students are finished, tell them that in the next activity they'll focus on duplications, as there are several different processes that can result in a duplication of genetic material.
4. Again, have the students work in pairs or groups of 3-4 members, depending on the availability of electronic devices that can support the time-lapse photography application. (Each pair or group will need one device.) Show the students how to find the application you would like to use and ask those who have a capable device to download the application for group use.
5. To learn how to use the time-lapse photo application, the students will be guided through one type of duplication event. The steps below use unequal crossing-over as the teaching topic; however, the topic of aneuploidy could be used instead. If you choose to review (or teach) the alteration of the

genome that occurs with aneuploidy, ask the students to simulate the process of meiosis (as described below), but ask them to depict an incomplete separation of the chromosomes during either anaphase 1 (homologous pairs do not separate correctly) or anaphase 2 (sister chromatids do not separate correctly) so that nondisjunction results. If nondisjunction occurs during anaphase 1, the resulting gametes (that start with a diploid number of 2n=2) will have 3 chromosomes, 3 chromosomes, 1 chromosome, and 1 chromosome. If nondisjunction occurs during anaphase 2, the resulting gametes (that start with a diploid number of 2n=2) will have 3 chromosomes, 2 chromosomes, 2 chromosomes, and 1 chromosome. The point of this activity is to teach the students how to use the time-lapse photo application, help them understand the self-guided portion of the assignment, and provide a review of meiosis—all of which is groundwork for their understanding how gene duplication has allowed for the emergence of the globin family of proteins.

6. Give each person a pair of scissors and each group of students ~4m of yarn of one color, ~2m of yarn in a second color, ~1m of yarn in a third color, tape, and a marker. Ask the students to cut the ~4m piece of yarn into 34 pieces that are each exactly 10cm long, and cut the ~2m piece of yarn into 34 pieces that are each exactly 5cm long. Tell the students that the yarn pieces will be used to represent chromosomes of two different lengths. (This is why it's important that all of the pieces be of the exact two lengths prescribed.) Ask the students to cut a 50cm piece and a 25cm piece from the third color of yarn. These segments will be used to represent the cell membrane and the nuclear membrane.
7. Tell the students that they will use the yarn-chromosomes they have just cut to create a simulation of a cell in the process of meiosis. Each 10cm piece of yarn will represent chromosome 1 and each 5cm length will represent chromosome 2 in a cell that has a diploid number of 2 (2n=2).
    a. Ask the groups to each clear a space on their table (or some other flat surface with no markings) and form a circle with the 50cm length of cell membrane yarn.
    b. Ask the students to place two long chromosomes and two short chromosomes in the center of the circle and use the 25cm piece of yarn to make a circle that represents the nuclear envelope.
    c. Ask the students to create a small sign ~3cm x 10cm that says "Meiosis: Prophase 1" and place it beneath the cell they have created.
    d. Ask each group to take a photo of the cell they have created, making sure the cell and sign fit entirely within the frame.
    e. Ask the students to move the chromosomes in their model very slightly and take another picture, framing the cell the same way they did in the first photo.
    f. Ask the students to move the chromosomes again and take another photo, and then place a duplicate version of one of the small chromosomes into the nucleus and take another photo.

g. They can then place a duplicate of the large chromosome into the nuclear envelope and take another photo and then repeat steps f. and g. with the second short and long chromosomes.
h. They can move the chromosomes ever so slightly 2-3 more times, taking a photo after each move (the resulting series of photos, when played in a slideshow, will give the impression of constant motion), until both of the long chromosomes and both of the short chromosomes are near one another.
i. The students should then move one leg of each chromosome pair so they are overlapping to represent the crossing over state. They should then take another photo, still attempting to frame the cell the same way they have in previous shots.
j. Ask the students to explain what happens during crossing over, and after a clear explanation has been given, ask the students to use the short homologous pair to depict this occurrence, taking at least 4 photos as they simulate the process with small movements of the yarn. The students can cut out the segment that will be exchanged and tape the segment into the matching location on the opposite homolog, taking photos to document each step of the process. Remind the students that there should be nothing in any of the photos except the yarn and tape props that are representing the parts of the cell.
k. Tell the students that for long chromosome pairs, the normal process of crossing over has not resulted in an equal exchange of genetic information. Ask the students to cut out a small section of yarn on one of the long chromosomes at a place in which the legs of chromosome 1 are crossing over. On the matching location for the homologous pair, ask the students to cut out a part of the chromosome that is longer than the section removed from the other chromosome. Ask the students to allow the long chromosomes to exchange genetic information by taping the segments onto the leg of the homologous chromosome, even though the segments are different lengths. A photo should be taken at each step of the exchange.
l. One of the chromosome 1 homologs should now be noticeably longer than the other, while the homologous chromosome 2 pair should be the same length. The rest of the cell division process should continue normally, but the final gametes will result in one cell with less information than would be expected, while another will have more genetic information than a normal haploid cell. (And two other gametes will have exactly the amount of genetic information expected for a haploid produced from a diploid cell.)
m. Ask the students to continue to simulate the process of meiosis, moving the yarn chromosomes slowly through each phase, changing the signs (that they create for each phase) and taking several photos to make the chromosomes appear to move across the cytoplasm.
n. Point out to the students that if they use more photos rather than fewer, and if they take several photos to show the movement of the

chromosomes even when a significant event is not happening, the resulting series of photos, when played in a slideshow, will resemble a video animation. The chromosomes will appear to be dancing and moving by themselves; the process will appear to be dynamic and fluid. However, if the students take very few photos or if the chromosomes underwent big moves between photos, the animation effect won't be as smooth or as captivating.

o. When the students have finished simulating and documenting with photos the entire process of meiosis, teach them how to set the parameters for showing the photos in a rapid slideshow. In most of the free apps, you will be able to set the total time period or the amount of time between photos. Some apps allow for the addition of music, narration, and other enhancements. If narration is possible, ask your students to narrate the process, pointing out the important features and using scientific terms in context.
p. Allow each group to share what they have created with at least one other group. The more slideshows the students see, the more ideas they will have for how they can improve their own stop-animation video.
q. Ask the students what would have to happen next for the mutation that occurred to be passed along in the general population. Tell the students that their slideshow will not be considered complete until they have shown how a mutation can result in evolution. Remind them that evolution does not occur in a single individual (as has been simulated by this slideshow of an error in one individual's meiotic division), but must be contributed to the gene pool to have an impact on the population through multiple generations.
r. Ask the groups to plan how they could show this mutation impacting evolution (the change in the population over multiple generations). Give them enough time to plan how they would depict their ideas, what props they would need, and how they could enhance their procedure to end up with a captivating and accurate video.
s. You may choose to have the students finish this photo-video sequence for the topic of unequal crossing over (or for aneuploidy), or you may choose to go on to step 8, immediately below (in which you give them a new topic from the ones listed and ask them to, instead, incorporate their improvements into the time-lapse photo series they create on their own).

8. The students are now ready to create a time-lapse photo-video depicting one of the following gene duplication processes:
   a. Transposons
   b. Retrotransposons

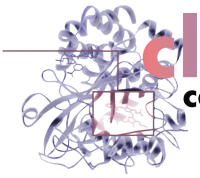
   Neither of these topics concern meiosis; they are errors that occur during transcription and translation. Therefore the students will have to create a different setting and use different props to depict the steps, location, and items involved in these types of duplication events. The students will very likely need to research the process they are going to depict (and thus will get

a review of transcription and translation), and it will be helpful if they are allowed time overnight to gather the supplies they need to create their stop-animation series, if the activity will be performed in the classroom.

9. Give each group a copy of the grading rubric you will use. Following is a rubric you may use, or that you may want to revise to reflect your priorities:

| **Gene Duplication Grading Rubric** | **Points possible** | **Your grade** |
|---|---|---|
| The gene duplication process was depicted accurately. | 3 | |
| The gene duplication process was narrated using appropriate scientific terms. | 3 | |
| There was a clear depiction of at least two potential impacts resulting from the way in which the duplicated gene was reinserted. | 6 (3 each) | |
| The impact of this type of gene duplication on the evolution of a population was accurately depicted. | 3 | |
| The impact of this type of gene duplication on the evolution of a population was accurately narrated using scientific terms. | 3 | |
| The photos were clear and it was easy to understand what was occurring. | 3 | |
| All group members contributed equally in the planning and implementation of this project. | 3 | |
| **Total points:** | 24 | |

10. Point out the essential aspects of the grading rubric so the students understand what is expected of them before they begin the project. Remind them that other props can be used (clay or moldable materials, things from home, etc.), but no humans or living organisms should be in any photo. You may decide to set a minimum number of photos and/or a minimum/maximum amount of text that can be used in the photos. If recorded narration is not an option, you may require the students to present their stop-animation video with one or more group members narrating the process.
11. When the projects are complete, allow class time for the students to share what they have created, so everyone can see the two processes depicted in several different ways.
12. As an application of the knowledge students have gleaned from their projects, ask the students to work in pairs to discuss the following question: Retrotransposon activity accounts for 42% of the human genome. It has long been debated whether the first self-replicating life was dependent on DNA, RNA, or proteins for heredity. How does the frequent occurrence of gene duplication due to retrotransposon events support or refute this debate?
13. Ask the students to write an argument for how transposons and reverse transposons might support or contradict the RNA World Hypothesis.

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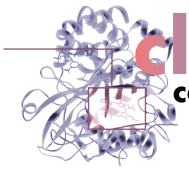


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