MODELING PROTEIN SELF ASSEMBLY

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ABSTRACT

Understanding the structure and function of proteins is an important part of the standards-based science curriculum. Proteins serve vital roles within the cell and malfunctions in protein self assembly are implicated in degenerative diseases. Experience indicates that this topic is a difficult one for many students. We have found that the concept of protein self assembly can be effectively demonstrated using a hands-on investigative laboratory exercise and readily available resources. Complete methods and student guide will be presented so you can start protein synthesis right away!

Understanding the structure and function of proteins is an important part of the secondary school science curriculum. Proteins serve vital roles within the cell. Malfunctions in protein structure are being implicated in a wide variety of degenerative diseases. Research suggests that most proteins are synthesized in a modular fashion using specific combinations of structural and functional domains. Experience indicates that understanding protein domain structure and function is difficult for many students. We have found that the concept of protein domain function can be effectively demonstrated using a hands-on investigative laboratory exercise and readily available resources. While there are several laboratory exercises based on simulating protein synthesis using beads or models (e.g. Rode, 1995, Sprehn, 1993., Souter and Stitt, 1995, Templin and Fetters, 2002), it was our goal to design a lab demonstrating how domains within protein active sites interact to form a functional protein. It was also our goal to design a lab which was quick to prepare, fun, and interesting.

The purpose of this activity is to promote both content mastery and critical thinking through self-discovery. In the process, students will 1) improve their scientific reasoning and communication skills, 2) generate and test ideas about protein assembly and function 3) explain how abnormalities in protein assembly can cause disease 4) draw conclusions and communicate their reasoning. This activity is considered non-hazardous. However, regular safety procedures should be followed when using these materials.

Before beginning this activity, students should be familiar with protein structure and function. While some of the details of protein assembly may be more complex than required for your biology course, students will benefit from exploring the process at a basic level. Some important terms that might be presented in your discussion are listed in the Classroom Discussion section below. In preparation for this activity, you will want to obtain foam beads for each group of students to work with.

Protein facts

There are many types of proteins in the cell. Enzymes carry out the reactions in metabolism, structural proteins give cells and tissues their shape, and transport proteins move nutrients around the body. In order to perform these important functions, proteins must be folded correctly. Proteins are composed of amino acids. Each of the amino acids is linked to neighbouring amino acids by a covalent bond known a peptide bond. The peptide bond is a rigid and greatly limits the way in which an amino acid chain can fold. This gives each protein its characteristic shape or structure.

Research has shown that it is side chains that make amino acids different and results in each protein folding differently than others. The side chain is the only part of an amino acid that is unique for each of the twenty naturally occurring amino acids. For example, if a side chain is insoluble in water it is hydrophobic and most often found on the interior of a protein, shielded from water in the cytoplasm. If the side chain is soluble in water, it is hydrophilic, and can be on the surface of the protein where it interacts with water. The linear chain of amino acids must fold in a specific way to fashion the active protein. Given the importance of protein folding, it is not surprising that protein misfolding is involved in a large number of human diseases. A well known example of this is the most common mutation associated with cystic fibrosis. A genetic mutation results in loss of one amino acid from the protein. This causes an incorrectly folded, non-functional protein to be produced. The disease pathology results because the misfolded protein does not do its normal job, resulting in impaired cellular function.

More recently, scientists have found that protein misfolding is involved in a class of non-genetic disorders. When important structural proteins are misfolded, they tend to aggregate in large

deposits. These large deposits appear to be toxic to cells. They are hypothesized to cause the cellular damage seen in neurodegenerative diseases such as Alzheimer, Parkinson, Lou Gerhig's disease, and even mad cow disease. This exercise is designed to illustrate the importance of protein folding and how misfolding can lead to nonfunctional proteins and disease.

When good proteins go bad

The mechanism of protein domain function is modeled using a simple physical analogue and hands-on activity. Students work individually or in small groups depending on class size (Appendix 1). Each group is instructed to begin by obtaining one set of foam beads and 4 pipe cleaners. Students are told to observe their foam beads carefully. They represent amino acids that will be used to construct their protein model. While similar models are often used to show protein assembly and primary structure, these activities stop short of demonstrating the assembly of separate structural and functional units. In this activity, we emphasize how groups of amino acids form domains within the protein that create interaction or catalytic sites.

We tell students to arrange their beads into groups of 5 by size and shape. We tell them to be careful! Each group of beads is a separate unit or module that folds as a separate domain. Mistakes made at this point will lead to a dysfunctional protein that will not bind the ligand. Students are then told carefully thread their groups of foam beads onto one of the pipe cleaners. Next, we have them shape the pipe cleaner into a circle and twist the ends together to keep the circle from coming apart.

The teacher then supplies each group with a model that represents an important cellular molecule. We use round Styrofoam craft balls selected to fit the circle they have created. Students are told to observe how this molecule interacts with their protein and record their observations on

the Student Guide. We tell them this molecule is termed a ligand. Students see that the ligand fits within the properly folded ligand-binding site. When they have completed their observations, we have them construct 3 more proteins using groups of 5 beads at a time. We ask students not to shape these into a circle, rather, to keep these straight. We have them twist the ends to keep the beads from falling off.

Students are then asked observe how these proteins interact with the ligand and record their observations in the data table. The linear model will not bind the Styrofoam craft balls. Next, we have them lay the linear proteins side by side and twist their ends together. This creates a structure termed an aggregate sheet. It is this type of defective protein that creates the plaques observed in Alzheimers disease and Prion Diseases such as Creutzfeldt-Jakob Disease (CJD) and Mad Cow Disease. We then have students look up information in a reference book or on the internet regarding conditions associated with abnormalities in protein assembly. Case studies may also be used to further the simulation.

Teaching tips

You will want to monitor understanding by asking students to describe each step in the procedure. Provide feedback to insure there is comprehension before going on to the next step. You may wish to use simple inventory control, like labelling kits, to aid with materials management. Make sure to announce a clean-up policy at the start of the activity and stick with it. You may also select a member of each group to act as the materials manager. Emphasize the importance of the material manager role.

Classroom discussion

At this point in the exercise, define the following terms generated from the lesson, such as amino acid, assembly, protein, peptide, conformation, domain and any others that may have been used by yourself or the students. Encourage students to use these terms in subsequent investigations.

Evaluation and extension activities

To assess understanding, use open-ended questions that allow students to apply the concept of the protein domain function (Appendix 2). Students should be reminded that this activity is a simulation. The models used demonstrate the primary structure which is determined by the order of amino acids. The shape of the actual protein takes on a specific three dimensional shape based on hydrogen and covalent bonds that are not demonstrated by this activity.

Ideally, evaluation of inquiry activities should emphasize both content and process skills. For example, while the class is engaged in the activity, we observe each student's performance. We use portfolio-type research notebooks to collect products of individual student and group work such as lab worksheets, drawings, journals of observations, self-evaluations, and answers to assigned questions. We also observe students as they make presentations to the class, interact with peers, and use computers. We have them conduct research on the internet to find web sites that cover the gene they have identified and report their findings to the class. Always ask what other questions their results have brought to mind.

Conclusion

Research indicates that the effectiveness of instruction is enhanced when it incorporates materials that actively engage students in the generation of scientific explanations. To this end, the present

exercise allows students to model protein domain function using readily available resources. Students' comments indicate this hands-on experience to be beneficial. As one student responded when asked on a survey to comment about this laboratory exercise, "this activity helped me understand how cells create a protein that binds the ligand molecule. It also explains how errors lead to plaques in Alzheimers or prion diseases."

References

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Sprehn, J. (1993) Protein building blocks. *Science Teacher, 60*(7), 22-25.

Souter, N. and Stitt, R. (1995) An inexpensive polypeptide model. *School Science Review, 76*(276), 61-62.

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APPENDIX 1: STUDENT GUIDE

Name _____ Date _____

Purpose:
1) To improve your scientific reasoning and communication skills
2) To generate and test ideas about protein assembly and function
3) To explain how abnormalities in protein assembly can cause disease
4) To draw conclusions and communicate their reasoning.

Materials needed:
1 set of foam beads
4 pipe cleaners
1 model of an important cellular protein

Procedure:

1. Select a partner to work with. Begin by obtaining one set of foam beads and 4 pipe cleaners.
2. Observe your foam beads carefully. They represent amino acids that you will use to construct a protein model.
3. Next, arrange your beads into groups of 5 by size and shape. Make sure all of the beads in a group are the same size and shape.
4. Carefully thread your groups of foam beads onto one of the pipe cleaners. The groups of beads represent amino acid domains that make up the protein.
5. Now shape the pipe cleaner into a circle and twist the ends together to keep the circle from coming apart.
6. Your teacher will then supply you with a model that represents an important cellular molecule. Observe how this molecule interacts with your protein and record your observations in the chart below. This molecule is termed a ligand.
7. When you have completed your observations, construct 3 more proteins using groups of 5 beads at a time.
8. Do not shape these into a circle. Instead, keep them straight. Twist the ends to keep the beads from falling off.
9. Observe how these proteins interact with the ligand and record your observations in the chart below.
10. Next, lay the linear proteins side by side and twist their ends together. This creates a structure termed an aggregate sheet. It is this type of defective protein that leads to Alzheimers disease.
11. Make a sketch of your completed proteins in the chart below. Be prepared to share your ideas concerning your results in a class discussion.

Protein	Appearance	Interaction	Sketch

APPENDIX 2: APPLICATION QUESTIONS

1. Were your proteins exactly the same as any other group?
2. How do you account for the differences? Explain what might have caused the variation.
3. Define the following:
    a. Amino acid
    b. protein
    c. peptide
    d. conformation
    e. domain
    f. amino acid
4. Describe specific differences between real proteins and the way that they fold and the materials you used.
5. Explain how errors in protein domain function can lead to disease.
6. How are such conditions diagnosed?
7. Use a reference book or the internet to describe a specific human condition that is caused by an error in protein assembly or folding.