Case Teaching Notes

for

"Rough Games and the Brain: The Structure and Function of Proteins"

by

Peggy Brickman, Department of Plant Biology, University of Georgia

Introduction / Background

In this one-hour case study, students learn about the chemical nature of protein molecules—in particular how the constituent parts of proteins (amino acids) contribute to protein three-dimensional structure and folding. Several important human diseases, such as cystic fibrosis, BSE (Mad Cow disease), an inherited form of emphysema, Alzheimer's and even many cancers are believed to result from protein miss-folding. Students often view these as "old-people" diseases, but may be unaware that many athletes suffer from dementia and have problems much like Alzheimer's at a very early age due to concussions suffered in their sport, a condition called Chronic Traumatic Encephalopathy (CTE). This case focuses on the efforts made by fans and ex-players to highlight the serious problem of head trauma and the resulting CTE symptoms caused by protein-filled plaques seen in many pro-football players, boxers, and even wrestlers. As students learn about what causes CTE, they begin to see the need to understand protein composition, folding and how amino-acid composition and folding affect protein function.

Objectives

- Compare the subunits of proteins with the subunits of carbohydrates, lipids, or DNA.
- Understand that a polypeptide is a linear polymer of amino acids linked together by peptide bonds that then folds into a 3-dimensional shape using largely hydrogen bonds but some covalent bonds as well.
- Apply knowledge of the polar and non-polar bonds found in phospholipid membrane bilayers and R groups of amino acids, to predict the type of R group that would be found in a protein inside a cell compared to one embedded in the cell membrane.
- Explain how shape relates to protein function.

Misconceptions

- That all protein molecules are identical, like all starch molecules.
- Proteins don't change shape after they are created.

Prerequisite Concepts

- Hydrogen and covalent bonds—the effect polar and non-polar covalent bonds make on a molecule's response to water. Students should have the ability to recognize a polar bond and predict if a molecule containing that bond would be hydrophilic, as well as a non-polar bond and hydrophobic.
- Composition of carbohydrates, lipids (in particular phospholipids), proteins, and nucleic acids from pre-class reading from a chapter on biological macromolecules.

Classroom Management

The case takes about 15–20 minutes of student discussion punctuated with mini-lectures lasting 10 minutes, so the entire case is completed by the end of a 50-minute lecture session (see "Teaching the Case" below). This case is designed for use in a one-semester introductory biology course that is taken primarily by freshmen and sophomores to fulfill a general education requirement but could be used by any introductory biology course or for a review in an anatomy and physiology course. The case has students use their understanding of the molecular bonds found in amino acids and proteins to predict the effect of substituting one amino acid for another in how a protein folds.

Teaching the Case

Part I: The Case and Chronic Traumatic Encephalopathy

Slides 1–3: The case opens with a 2-minute introduction of the story: that of Chris Nowinski's quest to expose the serious link between contact-sport brain trauma and Chronic Traumatic Encephalopathy. In

particular, Nowinski contacted the relatives of wrestler Chris Benoit when he read of his suicide and asked his father, Michael, to allow him to have his brain examined for signs of decay.

Slide 4–6: The case then leads into a brief description of what occurs during brain trauma, in particular that it is known to cause a small protein, called ß-amyloid precursor protein, to be cut into pieces called ß-ameloid. Over time, neurofibrillary tangles containing tau protein also accumulate. ß-ameloid functions to trigger axon growth and signaling of neurons, inflammatory response, and gene expression. Nowinski hired University of Pittsburgh forensic pathologist, Dr. Bennet Omalu, to look for signs of chronic brain traumatic encephalopathy in Benoit's brain. Omalu concluded that Water's brain had enough damage to resemble the brain of an 80 year-old Alzheimer's patient. According to Chris Nowinski, "the Alzheimer's comparison by Dr. Omalu was a poor choice of words that has created many misunderstandings. He was only referring to the accumulation of abnormal tau, which exists in both diseases, but in different locations in the brain. While there are many differences between the two diseases, the biggest is that nearly all the NFL cases (and Chris Benoit) do not have beta amyloid. Beta amyloid changes have been noted in acute trauma, but has not been shown to be connected to chronic trauma" (personal communication, 2009).

Slide 7: The purpose of this slide is to give students a chance to express what they think about the problem of connecting cause and effect with biological diseases and to give them a chance to talk about the story with their peers. Many students, like many Americans, are fans of wrestling and football particularly because of its violent nature and may not have thought about the consequences. Players, fans, owners of the NFL, and colleges and high schools all have a vested interest in determining if brain trauma is causing depression and CTE. In this slide students are asked to indicate what evidence they would need to see linking concussions to CTE and depression. Give them a minute or two to talk about it, and then ask them to volunteer a few answers. Students will hopefully come up with exam ples like the correlation found on the next slide between number of concussions and symptoms and not just look to see if players have a higher rate of CTE than the general population. If they do, it provides an excellent opportunity to talk about controls. Students may even come up with some experiments that could be done in lab animals that also provide an opportunity to talk about experimental design.

Slide 8: Evidence that you can show students after they have given their examples on the previous slide is from a study of 2,500 former NFL players by the Center for the Study of Retired Athletes at UNC that found that cognitive impairment, Alzheimer's-like symptoms, and depression rose proportionately with the number of concussions they had sustained. The UNC data, on symptoms and "early-Azheimer's" is likely representative of CTE cases, not Alzheimer's cases. Of the 11 football players that have been examined, 9 had CTE, one had mixed CTE/Alz, and one was healthy.

After reading this to the students I would ask them what the NFL might counter about these individuals. Hopefully, they will come up with the answer of looking at their personal lives or families and what other things might be linked to depression and CTE and how strong that link is compared to this data.

Slide 9: The case now focuses more on Alzheimer's and the effects of both ß-ameloid and tau protein and the role proteins and shape can play in this disease. ß-ameloid plaques were named after amylose, or starch, because they were first seen in brain sections stained with iodine (known to bind to starch). This led to a debate for years about whether or not the contents of these large brown regions were carbohydrate, protein, or lipid, an important issue in understanding what was causing the disease. This opens up to the first learning objective of the case: to compare the subunits of proteins with the subunits of carbohydrates, lipids, or DNA.

Slide 10: Clicker Question #1 is a review of the compositional differences between carbohydrates, lipids, and proteins that students should have read about in preparation for class. This question tests students' initial level of understanding from their reading about the differences in the subunits found in carbohydrates, lipids, proteins, and DNA.

Part II: Proteins and Amino Acids

Slide 11: After answering the question, students are told that in 1984 scientists purified the protein (28 amino-acids long) from tangles of fibrils seen in Alzheimer's patient's brains and in 1987 the gene for the protein was cloned, and it was shown to be much larger in size than the small fragments.

Slide 12: Most dementia and Alzheimer's occurs in the elderly (98% are over 65), and most did not suffer brain trauma, so there must be other causative agents.

5% of Alzheimer's is inherited and people with this type of Alzheimer's tend to get the disease much earlier (average age 51.) 15% of these familial mutations are in the ß-APP gene, resulting in changes to the amino acids.

Slide 13–14: Structure of amino acids and differences between amino acids.

Slide 15: Covalent bonds link amino acids together to form polypeptides.

Slide 16: Comparison of the color-coded amino acids sequence for three different proteins: insulin, ß-amyloid, and the ß-amyloid mutation in some familial Alzheimer's to demonstrate that the differences between proteins stem from the types and arrangement of their amino acids. Students can be reminded that the R groups are what differ and one difference is that they can be hydrophilic or hydrophobic and that this difference changes the attraction of hydrophilic side chains with water, and they will examine this in the next section.

Part III: How Proteins Differ from Other Macromolecules

Slide 17–18: Proteins have many different functions and they can have these different functions because of the variety of different amino acid subunits (scrabble analogy). If the monomers of molecules were imagined as tiles in scrabble it becomes easy to comprehend why different carbohydrates pretty much have the same function. One string of glucose will be much like any other string of glucose. But, with proteins, it is like playing scrabble with many different tiles—you can make infinitely many different combinations of those tiles to create words, just like you can create infinitely many different combinations of amino acids to create different proteins.

Slide 19: Clicker Question #2 is a review of students' comprehension of the previous four slides and requires them to compare and contrast proteins to the other macromolecules.

Part IV: Proteins Fold into Active Shape

The focus of the case now turns to the second learning objective: to understand that a polypeptide is a linear polymer of amino acids linked together by peptide bonds that then folds into a 3-dimensional shape using largely hydrogen bonds but some covalent bonds as well.

Slide 20: Using the color coding, this slide shows what such a mutation would look like in the whole protein, and defines this difference as a change in the primary structure of the protein.

Slide 21–23: But why would a change in the primary structure affect the whole protein? Folding. This slide talks about the importance of H-bonds with a great animation that can be found at: http://www.stolaf.edu/people/giannini/flashanimat/proteins/hydrophobic%20force.swf and continues with the tertiary and quaternary structure.

Slide 24: Clicker Question #3: Tests students' comprehension of the last 3 slides. The protein produced by the mutation differs in the order of amino acids along the chain of polypeptides.

Part V: ßAPP Proteins and Alzheimer's Disease

Attention now turns to the third learning objective: to apply knowledge of the polar and non-polar bonds found in phospholipid membrane bilayers, in aqueous environments, and in the R groups of amino acids to predict the type of R group that would be found in a protein inside a cell compared to one embedded in the cell membrane.

Slide 25: ß-amyloid precursor protein (ßAPP) is found in the phospholipid outer cell membranes of neurons.

Slide 26: Clicker Question #4: What type of amino acid would you NOT expect to find in the trans-membrane portion of ß-amyloid precursor protein (ßAPP)? This question is meant to test students' comprehension of the difference between hydrophobic and hydrophilic bonds in amino acids. They must also remember that the center of the phospholipid bilayer is hydrophobic. The intra-membrane region is highly hydrophobic because of the presence of the fatty acid tails of the phospholipids.

Slide 27–28: Diagrams the proteolytic cleavage of the ß-amyloid precursor protein (ßAPP) into ß-amyloid. Inform students that ß-amyloid doesn't tend to clump by itself, rather it starts to stick to existing clumps, and then these begin damaging nerve cells. It takes decades to start to see clumps, and then more to see damage as a result of clumps. This explains why most people who get Alzheimer's are old. An excellent video of the process is available at: http://www.healthscout.com/animation/68/7/main.html or http://www.usatoday.com/news/health/2007-06-11-alzheimers-cover_N.htm.

Slide 29: Clicker Question #5: Tests students' comprehension of the last 2 slides. Students have to put together what they answered in the last clicker question about hydrophobic and hydrophilic R groups to predict what would make the ß-amyloid clump together in an

aqueous environment. The area of the protein is one that was once in the interior of the membrane so it should be hydrophobic because the intra-membrane region is highly hydrophobic due to the presence of the fatty acid tails of the phospholipids. It is probably clumping to other hydrophobic molecules that are also not attracted to water, so by replacing one hydrophobic amino acid with another that is even more hydrophobic it would be even stickier.

Slide 30: Answer to the last clicker question with a real example in familial APP mutation which is a replacement of the small hydrophobic amino acid valine with the much larger, more hydrophobic R group in phenylalanine.

Slide 31: Clicker Question #6: This question is meant to assess the understanding of the difference between covalent bonds like peptide bonds responsible for maintaining a protein as a linear polymer of amino acids compared to the mostly hydrogen bonds responsible for the 3-dimensional shape. Alzhemed® was designed to be more attractive to the basic R-groups on ß-amyloid fragments than the fragments are to each other, and thus would prevent their aggregation by disrupting H-bonds between R groups.

Slide 32: Clicker Question #7: Asks students to express their opinion of what the NFL should do about this issue.

Slide 33: Starts to wrap up the case on the positive note that Chris Nowinski's efforts have gone a long way to help raise public awareness of the dangers of sports concussions. The NFL has not admitted any relationship, but has advocated two initiatives: (1) League and players union created a fund to help pay medical expenses of players suffering from Alzheimer's disease; and (2) new guidelines that include obligatory neuropsychological testing and a "whistle-blower system" for anonymous reports of any coach's attempt to override the wishes of concussed players or medical personnel.

Slide 34: Clicker Question #8: Describes a common mutation in another gene, APOE-e4, which is found in about 40 percent of people with AD; APOE-e4 lowers the age of onset and thus increases risk. (Having this gene form does not mean that a person will definitely develop AD; it only increases risk.) Students are tested on the final learning objective: explaining the relationship between protein structure and function.

Slide 35: Ends the case with a wrap-up of the fact that most of us will get Alzheimer's, too, even if we never get a concussion. For students' own interests, this slide describes some methods of prevention of Alzheimer's.

Slide 36: This slide gives further reading.

Answer Key

Answers to the questions posed in the case study are provided in a separate answer key. The answer key also includes the answers to the pre-case assessment questions. Those answers are password-protected. To access the answers for this case, go to **the key**. You will be prompted for a username and password. If you have not yet registered with us, you can see whether you are eligible for an account by reviewing our **password policy and then apply online** or write to **answerkey@sciencecases.org**.

References

Benoit's Story from: http://www.sciencedaily.com/releases/2007/09/070905224343.htm

McKee, Ann C., Cantu, R., Nowinski, J, Hedley-Whyte T., Genetics of Alzheimer's at: http://ghr.nlm.nih.gov/condition=alzheimerdisease

Role of APOE: http://www.medscape.com/viewarticle/484811_1

Excellent source of animation of damage to the brain found in Alzheimer's can be found at several web sites, for example: http://www.healthscout.com/animation/1/7/main.html#transcript & http://www.usatoday.com/news/health/2007-06-11-alzheimers-cover_N.htm

Excellent source of animation for hydrophobic and hydrophilic interactions in protein folding at: http://www.stolaf.edu/people/giannini/flashanimat/proteins/hydrophobic%20force.swf

Acknowledgements: I appreciate the helpful comments and suggestions given by Eric Ribbens. This material is based upon work supported by the NSF under Grant No. DUE-0618570. Any opinions, findings, conclusions, or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the NSF.