A study of the impact of protein crystallization on Advanced Placement Biology students' knowledge and attitudes

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Abstract

Students struggle grasping the structural complexity of proteins through lecture alone. This action research project used a pre/post-test with knowledge assessment to see if protein crystallization can have positive effects on Advanced Placement (AP) Biology student content knowledge and attitude towards the subject. Only minor increases in content area knowledge were observed, although the results were not statistically significant. There was no statistical difference in the attitudes of students towards science.

Rationale

Multiple studies have shown that students struggle with understanding complex systems (Chi, 2000; Feltovich et al., 1992). After I completed my fourth year teaching Advanced Placement (AP) Biology, it became clear to me that I have to take a different approach to teaching the structure of macromolecules- specifically the complexities of protein structure. Students have demonstrated in my classroom that they struggle with understanding these complex structures when they just hear about them in a lecture. Students attempt (and often succeed) at memorizing the levels of structure of proteins, but are not able to apply this memorized information to new, and complex problems. Pictures tend to be helpful while discussing proteins, and studies have shown that the human brain can take a two dimensional image, turn it into three dimensional (3-D) image, and is even capable of mentally rotating that same image (Shepard & Metzler, 1971). Additional studies show that learning is enhanced when 3-D objects are manipulated in virtual reality (Chan & Black, 2005 & 2006; Jang, Black & Jyung, 2010). With the advent of online protein databases that have manipulable, 3-D models of proteins it seems obvious to put them into use to help teach about the complexities of proteins.

This aforementioned mental modeling is a vital step to learning (Gentner & Steven, 1983). However, students also need conceptual frameworks which take abstract concepts and relate them to real-world situations (White, 1993). That being said, I feel that it is vital to not only use virtual models of the proteins to create a mental model, but also relate these structures to real-world clinical importance to create these conceptual frameworks. In addition to using these virtual methods, true, hands-on modeling of objects can be completed which has shown to benefit students by allowing them to understand unstated qualities about the object (Penner, Giles, Lehrer & Schauble, 1997).

The study of proteins (proteomics) is currently a dominant area of scientific research that is still blossoming (Wilkins, 2006). Because of this, I wanted to focus on proteins, but some of the techniques employed to study these proteins have reciprocity with the other types of macromolecules and fields of science. One of these techniques of study is x-ray crystallography, which is mentioned in most AP Biology textbooks in relation to the discovery of the structure of DNA, but rarely discussed in depth. I feel that it is vital to teach an overview of this topic because it is the bridge between making invisibly small (in relation to human eyesight) molecules, visible to a human.

This rationale has led me to develop a classroom project that incorporates the use of 3-D, virtual manipulation, real-life modeling, x-ray crystallography, and research into clinical implications in order to study proteins. Ideally, all of these areas can be explored; however, individual parts of it can be studied

separately of the others. The purpose of this project is to specifically describe the impact of protein crystallization on the students' understanding of proteins and their attitudes towards the topic.

Intervention

This activity fits in with the study of macromolecules during the AP Biology course. This section is filled with concepts that attempt to relate structure to function, and has a heavy emphasis on proteins. The objective of this activity is to allow students to learn about how proteins are studied in laboratories through protein crystallization.

This activity began with individual students being given a protein sample (lysozyme). It may be possible to use other proteins such as carbonic anhydrase, but this was not investigated. Students used a modified version of "Crystallization Hands On, Lysozyme Crystallization" (McKenna & McKenna, 2011) in order to crystallize the proteins given to them (methodology can be found in the appendix). Students then observed the resulting crystals under a dissection microscope. Examples of individual student work can be found in Figures 1-4.

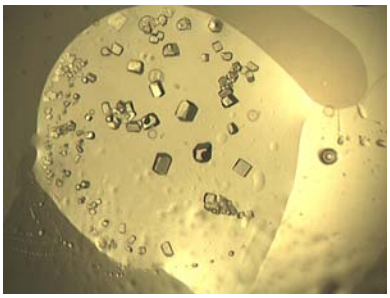

Figure 1. Student lysozyme crystallization.

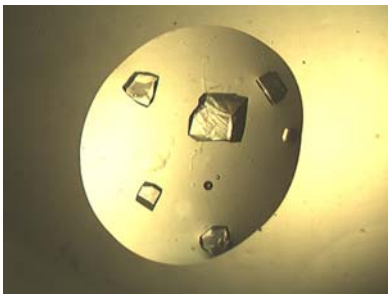

Figure 2. Student lysozyme crystallization.

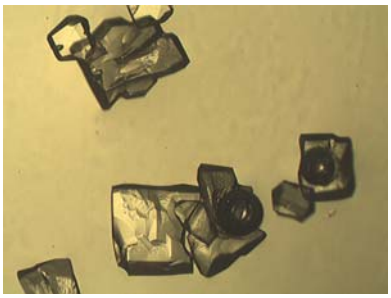

Figure 3. Student lysozyme crystallization.

Figure 4. Student lysozyme crystallization.

Extensions

Once crystallization is completed students can be given the x-ray diffractions of these proteins. Students would use the protein abbreviations (from pdb.org) to look up and observe these molecules which can be manipulated in 3-D. Additionally, this website can be used to research the function and structure of the proteins. Many 3-D models are available on this website and students can then use "Toobers" (http://3dmoleculardesigns.com/) to create a real model of some of the amino acids of the protein (each cm could represent one amino acid). Each amino acid can be labeled on the "toober" so students can see the primary sequence of the protein.

Once the model is complete, students could complete an online search for a clinical disorder related to their protein. A one page report including a description of the disorder, the mutation of the protein (if possible), and treatments should be required. The goal of this paper is to make sure students understand how every protein in the body can play a role in the health of the individual and that even small changes to them can have catastrophic effects.

Because of severe time and financial limitations, these extensions could not be competed during the initial implementation of this action research project. The project extensions are recommended, but it is not known if there is a positive correlation between their completion and students' content knowledge and attitude towards science.

Connections to Bench to Bedside Institute

Many of the concepts incorporated into this study and activity came directly from the Bench to Bedside Institute. We completed a crystallization activity that mimicked the same the crystallization activity that was competed in my classroom. I worked with the McKenna's laboratory in establishing procedures that can be used easily in the high school classroom. Additionally, the concepts of x-ray crystallography, pipetting, scientific method, and the use and availability of online protein databases were all incorporated throughout the Bench to Bedside Institute. One of the overarching concepts of the institute is the clinical importance of proteins in organisms. This project thoroughly explores this concept when all of the steps of the aforementioned activity are completed.

Data Collection and Analysis

AP Biology students completed a pre-survey (Box 1) before the crystallization was completed that encompasses their attitude towards science (first 16 questions) and also their general knowledge of proteins (last 4 questions). Students then completed a post-survey that mirrors the pre-survey to see if their attitude and knowledge changed. Only minor increases in content area knowledge were observed, although the results were not statistically significant. There was no statistical difference in the attitudes

of students towards science. Although not quantifiable, students had mixed reactions to the activity. Some thought the activity was "cool" and many were excited to actually see their crystals, and it seemed that some students just became frustrated with the repetition of using the pipettes.

Box 1: Student Survey.

Science Attitude/Content Knowledge Survey

Gender : M F Grade : 10 11 12

Please circle the letter of the response that best describes what you think about each statement.

	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1. I think being a scientist would be exciting.	A	B	C	D	E
2. I would rather listen to someone talk about science than read a science book.	A	B	C	D	E
3. I think science is important only at school.	A	B	C	D	E
4. I would rather use computers to learn about science than read a science book.	A	B	C	D	E
5. I learn more from doing experiments than from listening to the teacher' explanations.	A	B	C	D	E
6. Science is fun.	A	B	C	D	E
7. I like to use science equipment to study science better than reading science book.	A	B	C	D	E
8. I usually try my best in science class.	A	B	C	D	E
9. I like to figure out something without the teacher telling me how to do it.	A	B	C	D	E
10. We learn about important things in science class.	A	B	C	D	E
11. Science classes are exciting.	A	B	C	D	E
12. I am interested in many scientific ideas that are not taught in school.	A	B	C	D	E
13. I feel comfortable asking questions about science.	A	B	C	D	E
14. I know how to set up a science investigation.	A	B	C	D	E
15. I am good at science.	A	B	C	D	E
16. Scientists have interesting jobs.	A	B	C	D	E
17. I understand the three-dimensional structure of proteins.	A	B	C	D	E
18. I understand how a proteins structure will lead to its function.	A	B	C	D	E
19. I understand how scientists determine the shape of a protein.	A	B	C	D	E
20. I understand how the primary structure of a protein relates to clinical importance.	A	B	C	D	E

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Budget and Materials

The materials needed for protein crystallization are somewhat cost-restrictive (shown in Table 1. Reflects the quantity needed for 30 [groups of] students and does not include items the school already possessed). Methodologies used (with permission) for crystallization came from the University of Florida- McKenna Laboratory and pipettes came from CPET.

Table 1. Budget items and costs of each.

Item	Cost
Polyethylene Glycol 50%, 6K	$180
.5M Sodium Acetate, pH 4.5	$33
Lysozyme Kit	$132
Linbro Plates	$331
22mm Coverslips	$242
Microbridges	$72
Immersion Oil	$102

Modifications from the Original Proposal

When the original proposal was written, it was not known how much of the material would be provided through the University of Florida. After it was realized that only some of the material could be provided, cutbacks in the original project had to be made. Additionally, time restrictions in the AP Biology course made the completion of all parts of this activity impossible. It may be possible in future years to complete all parts of this project (including extensions) because of new course requirements and extended time with students in my classroom. All of these limitations resulted in the completion of the protein crystallization methodology with none of the extensions.

Insights from Action Research

I believe that the protein crystallization procedure is a fantastic addition to any classroom that wants to deeply explore proteins. It seems that "time constraints" is the most common restrictive force in classrooms today. My classroom is not the exception to this, and I am not sure if there is a possible solution for everyone. Next year, I will have some of my students for two hours, instead of one. Because of this, I believe that I can fully implement this activity into my course, and not have to rush through it.

I do not believe that I would drastically change anything done in this action research project. Minor adaptations/clarifications were made to the protein crystallization procedures after students completed the activity.

The action research process has been professionally helpful in that I gained a better insight into my students understanding of content knowledge, and their opinion of science. I feel that this is valuable because I can better adapt my classroom to fit individual student needs.

Dissemination

Information gained through the summer institute was disseminated throughout my AP Biology course and AICE Biology Exam preparation sessions. When teaching about protein structure and x-ray crystallography, concepts learned from this last summer were heavily emphasized and shared with students. Many students wanted to know more about how x-rays can be used to figure out molecular structure, and many were amazed at how scientists can look at diffraction and understand what it means. Also, after students completed crystallization by hand, it was fun to explain how technologically advanced machines now take the place of the scientist in many of the steps.

Many of my collogues wanted to know what I did over the summer, and were excited to hear about the program. The amount of quality summer programs for teachers is dwindling, and any new opportunities are typically explored by those interested. Students love to know what teachers are involved with when they are not at school, and many want to hear about the University of Florida. It is always a pleasure to explain the advancements that take place at this University, and what type of research is being completed there.

I have written a journal article before for "The American Biology Teacher" and am not opposed to submitting new work to any journal. I do not believe that this action research project is ready for publication yet, but am hoping that when the new AP Biology course requirements are implemented in my classroom next year all extensions of this project can be completed.

Appendix

Protein Crystallization Laboratory Methodology

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Purpose: To determine ideal lysozyme crystallization conditions.

Materials Supplied

Stock Solutions:

A- 4M NaCl

B- 0.5M Sodium Acetate, pH 4.5

C- 50% PEG 6K

D- ddH_2O

E- Lysozyme

Equipment/Supplies:

Linbro Plate

Cover Slips

Micro-bridges

Immersion oil and applicator

Pipette and tips

Notes:

- The volume of each well after the addition of solutions should be 1mL.
- Mix the solutions in the wells thoroughly with your pipettes.
- The lysozyme droplet should be 10uL (5uL of lysozyme + 5uL of the reservoir solution)

Lysozyme Crystallization Screening Conditions (Set up these wells BEFORE moving to "methodology")

Line 1- Volume (in uL) for Na Acetate, pH 4.5 (solution "B")

Line 2- Volume (in uL) for NaCl (solution "A")

Line 3- Volume (in uL) for PEG 6K (solution "C")

Line 4- Volume (in uL) for ddH_2O ("D")

	0.4M NaCl	0.8M NaCl	1.0M NaCl	1.2M NaCl	1.6M NaCl
Hanging Drop	"A1"	"A2"	"A3"	"A4"	"A5"
	100	100	100	100	100
	100	200	250	300	400
	200	200	200	200	200
	600	500	450	400	300
Sitting Drop	"B1"	"B2"	"B3"	"B4"	"B5"
	100	100	100	100	100
	100	200	250	300	400
	200	200	200	200	200
	600	500	450	400	300
Hanging Drop	"C1"	"C2"	"C3"	"C4"	"C5"
	100	100	100	100	100
	100	200	250	300	400
	500	500	500	500	500
	300	200	150	100	0
Sitting Drop	"D1"	"D2"	"D3"	"D4"	"D5"
	100	100	100	100	100
	100	200	250	300	400
	500	500	500	500	500
	300	200	150	100	0

Hanging Drop Method:

The hanging drop method vapor diffusion method is one of the most popular techniques for crystallization condition screening and optimization. It is economical and is a reasonably fast procedure to carry out.

Step 1: Apply a thin layer of oil around the rim of each well (reservoir) in the Linbro plate where you intend to set-up screening conditions (rows A-D, columns 1-5)

Step 2: Use your fingers to pick up the cover slips (hold them on the sides to prevent contamination from the oils on your skin) and lay out five of them on a clean surface.

Step 3: Pipette 5uL of lysozyme stock (solution "E") onto the center of each cover slip. Using a FRESH pipette tip, add 5uL of the solution from the first well (A1) to the first drop on the first cover slip. Gently pipette up and down to homogenize the mixture.

Step 4: Pick up the cover slip and invert, without losing the drop, over the first well. The oil will form a seal between the slip and the top of the well.

Step 5: Repeat steps 3 and 4 for wells A2 to A5.

Step 6: Repeat steps 1-5 for wells C1-C5.

Sitting Drop Method- Micro-Bridges

Sitting drop vapor diffusion is another popular method for crystallization condition screening and optimization. It allows you to use large volumes and is an even easier setup than the hanging drop.

Step 1: Same as "Step 1" above in the hanging drop method.

Step 2: Using your fingers, place a micro-bridge in wells B1-B5 and D1-D5.

Step 3: Pipette 5 uL of lysozyme stock ("E") into the depression at the center of each micro-bridge. Using a fresh tip, add 5uL of the precipitant from well B1 to the lysozyme in the micro-bridge in B1. Gently pipette up and down to homogenize the mixture.

Step 4: Pick up a clean cover slip and invert it over the well to create a seal between the slip and the top of the well.

Step 5: Repeat steps 3 and 4 for wells B2-B5.

Step 6: Repeat steps 1 to 5 for wells D1-D5.

Questions:

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- What do you observe in the wells after they have been setup?

- How has the salt concentration affected the formation of crystals?

- How has the PEG% affected the formation of the crystals?

- Does it matter if the drops or hanging or sitting?