

## Lesson Design

<b>Subject Area:</b> Biology		<b>Grade Level:</b> 9-12	
<b>Benchmark Period :</b> 1 <sup>st</sup> (in Fall)		<b>Duration of Lesson:</b> 1 class period/next day to record results	
<b>Standard(s):</b> ***as listed @ end of document			
<b>Big Ideas involved in the lesson:</b> Students will understand the real world application of science			
<b>As a result of this lesson students will:</b>			
<b>Know:</b> 1) Learn methods used by crystallographers to crystallize proteins for analysis 2) How to follow biochemical protocols 3) How to identify simple crystal structure			
<b>Understand:</b> 1) How a protein crystal can be used to develop new more effective medicines.			
<b>Be Able To Do:</b> 1) Pipette correct ratio of salt to lysozyme to create protein crystals. 2) Use a microscope to view protein crystals. 3) Analyze data to recommend the optimum mixture that produces the highest yield of crystals.			
<b>Assessments:</b> <b>What will be evidence of student knowledge, understanding &amp; ability?</b>		<b>Formative:</b> CFU  <b>Summative:</b> Response letter to drug company explain the results of their research.	<b>CFU:</b> 1. Journal reflection on creation of new drug. 2. Students answer orally question from power point presentation.
<b>Lesson Plan</b>			
<b>Anticipatory Set:</b> a. T. focuses students b. T. states objectives c. T. establishes purpose of the lesson d. T. activates prior knowledge		a. Students will view pictures to get them thinking about crystals. b. Students viewed objectives on power point slide. c. Students view treatment of macular degeneration. d. Students were asked questions about protein structure to access and determine prior knowledge.	
<b>Instruction:</b> <b>a. Provide information</b> ▪ Explain concepts ▪ State definitions ▪ Provide exs. ▪ Model <b>b. Check for Understanding</b> ▪ Pose key questions ▪ Ask students to explain concepts, definitions, attributes in their own words ▪ Have students discriminate between examples and non-examples ▪ Encourage students		<b>a.</b> • Students will read detailed protocol for laboratory lesson. • Students will have been able to view quick reference for lab protocol overview. • Peer assistant model critical lab protocols prior to lab beginning.  <b>b.</b> • Provide analysis and observation questions from demonstration to determine if students can distinguish between a positive or negative result. • Provide a picture of end result as reference for students to compare their work to. • Create an atmosphere where students have the feeling they are real researchers developing new medicines.	

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<p>generate their own examples</p> <ul style="list-style-type: none"> <li>▪ Use participation</li> </ul>	<ul style="list-style-type: none"> <li>• Establish roles for each group member to rotate through all lab procedures. Students will shuffle cards and randomly change roles through out the lab.</li> </ul>
<p><b>Guided Practice:</b></p> <ol style="list-style-type: none"> <li>a. Initiate practice activities under direct teacher supervision – T. works problem step-by-step along w/students at the same time</li> <li>b. Elicit overt responses from students that demonstrate behavior in objectives</li> <li>c. T. slowly releases student to do more work on their own (semi-independent)</li> <li>d. Check for understanding that students were <i>correct at each step</i></li> <li>e. Provide specific knowledge of results</li> <li>f. Provide close monitoring</li> </ol>	<ol style="list-style-type: none"> <li>a. Students will pipette water as peer assistant models technique.</li> <li>b. Choral initiated questions for coral response; tapping into new and prior knowledge.</li> <li>c. After introduction and demonstration students begin laboratory lesson on their group.</li> <li>d. Teacher and Peer Assistant ABWA and observe if correct results are being achieved and correct if necessary.</li> <li>e. Have students hold up samples to light to see if crystallization has occurred. Sample should sparkle.</li> </ol>
<p>What opportunities will students have to read, write, listen &amp; speak about science?</p>	<p>Read: Students will read background material about the medical use of their research. Students will read a detailed protocol for their laboratory lesson.</p> <p>Write: Students will write a response letter and comment on the outcome of their research.</p> <p>Listen: Throughout entire presentation; teacher led &amp; student</p> <p>Speak: Throughout entire presentation; teacher led &amp; student</p>
<p><b>Closure:</b></p> <ol style="list-style-type: none"> <li>a. Students prove that they know how to do the work</li> <li>b. T. verifies that students can describe the what and why of the work</li> <li>c. Have each student perform behavior</li> </ol>	<ol style="list-style-type: none"> <li>a. as per correct answers to Analysis questions &amp; crystallization of Lysozyme</li> <li>b. through correct following of protocols, student observation</li> <li>c. through correct following of protocols, student observation</li> </ol>
<p><b>Independent Practice:</b></p> <ol style="list-style-type: none"> <li>a. Have students continue to practice on their own</li> <li>b. Students do work by themselves with 80% accuracy</li> <li>c. Provide effective, timely feedback</li> </ol>	<ol style="list-style-type: none"> <li>a. as described in closure assignment with Response Letter</li> <li>b. Students exceed the rubric for Scientific Writing</li> <li>c. Return letters/Analysis questions as per rubric (within 1 week)</li> </ol>

<b>Resources:</b> materials needed to complete the lesson	See attached lab handout
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## California State Standards

### GRADES NINE THROUGH TWELVE—BIOLOGY/LIFE SCIENCES

#### Cell Biology

1.
  - b. *Students know* enzymes are proteins that catalyze biochemical reactions without altering the reaction equilibrium and the activities of enzymes depend on the temperature, ionic conditions, and the pH of the surroundings.
  - h. *Students know* most macromolecules (polysaccharides, nucleic acids, proteins, lipids) in cells and organisms are synthesized from a small collection of simple precursors.

#### Genetics

4.
  - e. *Students know* proteins can differ from one another in the number and sequence of amino acids.
  - f.\**Students know* why proteins having different amino acid sequences typically have different shapes and chemical properties.
5.
  - a. *Students know* the general structures and functions of DNA, RNA, and protein.
  - c. *Students know* how genetic engineering (biotechnology) is used to produce novel biomedical and agricultural products.

#### Investigation and Experimentation

1.
  - a. Select and use appropriate tools and technology (such as computer-linked probes, spreadsheets, and graphing calculators) to perform tests, collect data, analyze relationships, and display data.
  - b. Identify and communicate sources of unavoidable experimental error.
  - c. Identify possible reasons for inconsistent results, such as sources of error or uncontrolled conditions.
  - d. Formulate explanations by using logic and evidence.
  - g. Recognize the usefulness and limitations of models and theories as scientific representations of reality.
  - j. Recognize the issues of statistical variability and the need for controlled tests.
  - k. Recognize the cumulative nature of scientific evidence.
  - l. Analyze situations and solve problems that require combining and applying concepts from more than one area of science.
  - m. Investigate a science-based societal issue by researching the literature, analyzing data, and communicating the findings.

# LYSOZYME CRYSTALLIZATION EXPERIMENT

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### Background

Whenever atoms or molecules in a liquid state come together to form a solid, they can arrange in one of two ways. They can come together randomly to form an amorphous (disordered) structure, or they can associate into highly-ordered, three-dimensional structures called crystals. For example, when water freezes it can form an amorphous mass like the ice in your freezer, or it can form complex snowflakes or frost crystals. Other examples of crystals include diamonds (crystallized carbon) and quartz (crystallized silicon dioxide).

The word crystal comes from the Greek term "krystallos," meaning "clear ice." Crystals are formed when a chemical compound solidifies in such a way that its atoms are arranged in a symmetrical, three-dimensional pattern which is composed of regular, repeating geometrical units. Ideal crystals form perfect arrays; however, this perfection is very rare. For example, a nearly perfect one carat diamond may cost thousands of dollars, while smaller diamonds with imperfections are often used in saw blades and tool bits. Perfect crystals are a rarity, because they generally require very slow crystal development and the absence of contaminating substances and outside forces which could disrupt crystallization.

Under the proper conditions, crystals can be formed from chemical elements (such as diamonds from carbon), chemical compounds (quartz from silicon dioxide), salts (a solution of table salt left to evaporate), or even proteins. The crystallization of proteins is an important technique in biochemistry. Because protein crystals are highly-ordered arrays of a protein, the analysis of these crystals can give scientists important information about the chemical structure of the protein.

Protein crystals can be analyzed using a technique called X-ray *crystallography*, which takes advantage of a crystal's ability to diffract X-rays into a regular pattern called a *diffraction pattern*. Analysis of a protein crystal's diffraction pattern can help scientists determine the three dimensional structure of the protein. Once the structure of the protein is known, scientists can begin to speculate on its function and method of action within the cell. This branch of science which relates biological structure to function is known as *structural biology*. In addition to giving insights about the structure and function of proteins, structural biology also allows the development of drugs which can interact with specific regions of the protein. This is known as *rational drug design*.

In this experiment, we will grow crystals of the protein *lysozyme*. Lysozyme is an enzyme which degrades bacterial cell walls, and is found in abundance in human tears and chicken egg whites. It was one of the first proteins to be crystallized and used in X-ray diffraction experiments. We will prepare a saturated solution of lysozyme, and will cause the lysozyme to precipitate out of the solution by adding various concentrations of salt. In some samples, the lysozyme will precipitate quickly, leading to amorphous masses of solid lysozyme, and in other samples the lysozyme will take several days to precipitate. In general, the longer the protein takes to precipitate, the more highly ordered its structure will be. The objective of this experiment is to determine what concentration of salt is required to produce large, regular crystals. We will observe crystal formation under a microscope over a period of several days and record any observations.

## LYSOZYME CRYSTALLIZATION EXPERIMENT

### *EQUIPMENT AND SUPPLIES:*

Pipettes (5 mL or greater) and bulbs	Vinegar (5% Acetic Acid)
Graduated cylinders: 25 mL, 50 mL	Sodium acetate (Trihydrate)
One 250 mL Erlenmeyer flask	Lysozyme
4 - 5mL plastic receptacles	Analytical balance (at least 2)
One powder funnel	pH meter
4 microvials	methylene blue chloride
Deionized water	

### *PROCEDURE:*

#### **A) Prepare crystallization buffer (50 mL)**

*(has been prepared for group)*

1) In a 250 mL Erlenmeyer flask, combine the following and swirl until dissolved:

12.5 mL Vinegar

0.68 g Sodium Acetate

37.5 mL Deionized water

2) Check the pH of this solution with the pH meter. It should be approximately 4.2-4.5.

Household vinegar is a solution of 5% acetic acid. In this experiment, we will use acetic acid and sodium acetate as a buffering solution.

#### **B) Prepare saturated lysozyme solution (40 mg/mL)**

*(has been prepared for group)*

1) Weigh out 2.0 g of lysozyme

2) Using a funnel, slowly add the protein to the flask containing the crystallization buffer. Try to avoid getting the powder onto the walls of the flask.

3) Tap the funnel a few times to get the excess protein into the flask.

**Do not swirl the flask at this point.**

4) Allow the lysozyme to dissolve by letting it sit on the surface of the buffer. It may help to gently tap the flask a few times against a padded bench top, but **do not swirl** the solution at this point. The protein will take 5-10 minutes to dissolve. Wash, rinse and DRY the funnel for later use.

5) After the powder has dissolved, rotate or tilt the flask gently to incorporate any lysozyme powder on the sides of the flask. **BE CAREFUL NOT TO CREATE BUBBLES IN THE LYSOZYME SOLUTION.** Bubbles that form

in the solution indicate that the protein has become denatured, and may not crystallize properly. Once all of the lysozyme has dissolved, the buffer solution is saturated with lysozyme at a concentration of 2.0 g per 50 mL, or 40 mg/mL.

#### **C) Prepare supersaturated lysozyme solutions**

1) Label the 4 receptacles with the numbers corresponding to NaCl concentration assigned to your group (see below).

2) Into each of the microvials, carefully transfer 5.0 mL of the saturated protein solution. Into each

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receptacle, a different amount of sodium chloride will be added as assigned(see below).

3) For each sample, weigh out the correct amount of sodium chloride (see step 4; has been prepared). Inspect the funnel (if needed) to ensure that it is dry, then place it into the appropriate receptacle and **slowly** add the salt while swirling gently.

**\*\*NOTE (this part of the lab protocol is CRITICAL):** The salt must be added **very slowly** (a few crystals at a time) while **gently** swirling the receptacle, to avoid creating high local concentrations of salt. If salt begins to accumulate on the bottom of the receptacle, stop adding salt, but continue to swirl the receptacle until the salt dissolves. Again, **BE CAREFUL WHILE SWIRLING THE RECEPTACLE NOT TO CREATE BUBBLES IN THE LYSOZYME SOLUTION.** It may be easier if one person swirls the receptacle while someone else slowly adds the sodium chloride.

4) The concentrations of sodium chloride are as follows:

Rec. #	Sodium Chloride Concentration (%)	Mass of Sodium Chloride Added to Flask (g)
1	2.0	<b>0.100</b>
2	2.5	<b>0.125</b>
3	3.0	<b>0.150</b>
4	3.5	<b>0.175</b>
5	4.0	<b>0.200</b>
6	4.5	<b>0.225</b>
7	5.0	<b>0.250</b>
8	5.5	<b>0.275</b>

Did we mention to **BE CAREFUL WHILE SWIRLING THE FLASK NOT TO CREATE BUBBLES IN THE SOLUTION?** In some of the receptacles with higher concentrations of salt, precipitation of the lysozyme may begin to occur immediately (indicated by a cloudiness of the solution). Do not be concerned with this, but continue adding the salt until it is completely dissolved.

### **D) Transfer the solutions into the culture plate**

- 1) Label the 4 microvials with the # corresponding your groups assigned concentration.
- 2) **Gently** (did we say **gently?**) pipette 1.0 mL of solution 1 into microvial # \_\_\_\_, etc., until each microvial contains 1.0 mL of the appropriate solution.
- 3) Replace the lid and move the microvial tray into a cool, dry place where it will not be disturbed (under hood, if available).

### **E) Group differences**

Many factors affect the growth of crystals. One of these is the rate at which water is removed from the protein. In these experiments, water is removed by salt and evaporation. To better visualize your crystals, feel free to be creative with the things you try, but be sure to ask your facilitator before you do them. Also remember that at least one group should be the control and should do the experiment exactly as written. Make certain to accurately label each part of all apparatus used so you know exactly what is in it!

### **F) Record results**

For the next day, record any new observations in a table. Take note of how long it takes before you begin to see crystals in each sample. Also notice whether the crystals appear to be small, random aggregates, or large, organized crystalline structures. You may want to look at your crystals under a microscope for more detail. Draw the crystals you see the next day.

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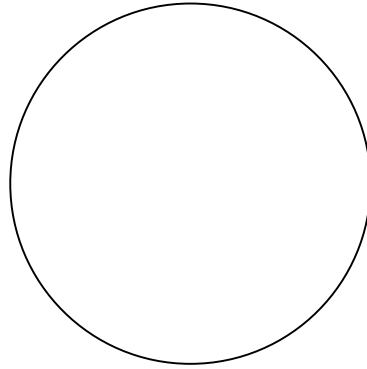
### G) Staining Crystal

For conformation of crystallized protein, we will use the staining technique demonstrated in lab.

### Lab Questions

#### Analysis and Observation:

1. How long did it take for the crystals to grow? Explain why it takes time for the crystals to form.
2. Describe the shape of the crystals that you grew. Make a sketch of them and note the microscope magnification.



3. What scientific problem could you state from the letter sent to you by Acme pharmaceutical?
4. Which ratio of NaCl to lysozyme produced the greatest yield of lysozyme crystals?
5. Explain how you verified the crystals you observed in your sample are the protein, lysozyme and not crystals of NaCl.

#### Conclusion:

6. Using the entire class data including all eight trials respond to Acme Pharmaceuticals with your conclusions and recommendations from your research. Be sure to include the problem researched, summary of your evidence and data, and a concluding statement citing your evidence to infer a possible solution to the problem.

Lesson Design

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*Dear STEM Team,*

*It is with great pleasure that we at **Acme W & P Drugs** offer to you and your colleagues this great and lucrative offer.*

*Currently our drug therapy for macular degeneration is being administered to our patients every thirty days but we want to improve the therapy where the same dose can be applied every six months.*

*We are looking to your research firm to create a crystalline form of the medication that will have a longer time released effect.*

*Best of luck,*

Dr. Edward Walton  
CEO Emeritus

Dr. Michael Page  
CEO Pro-tem

*“Changing the future, uuuuhhh, one day at a time”*