

2015

Crystallization of a Protein

Practical Course in X-Ray Diffraction: Module 1 & 2 (Protein Crystallization Set-up)

In this module, the students will learn how to grow lysozyme single-crystals from lysozyme solution with sodium chloride as precipitating agent using a technique called hanging drop vapor-diffusion method. They will then evaluate the number, shape, and size of the crystals using an optical microscope.



Crystallization of a Protein

Practical Course in X-Ray Diffraction: Module 1 & 2 (Protein Crystal Set-up and observation)

Practical Course: When, What, Where?

Facility X-Ray Crystallography Facility

Date	Friday, August 07 Wednesday August 19, 2015
Event	Practical Course in X-Ray Diffraction Module 1 & 2
Venue	Kasha Lab Room Numbers 410, 412 and 312

Module Objectives: Learn to grow a protein crystal

Target In-coming graduate students

Main Objective	The main objective is to learn how to grow a protein crystal at ambient temperature using hanging-drop vapor diffusion method from a protein solution
Other Objectives	Preparation of buffer solution, and protein, precipitant solutions (including adjusting the pH) from laboratory chemicals Preparing and setting-up VDX plates with protein solutions of different concentrations (on cover slips) and precipitant solutions of different concentrations (in the wells) Viewing (after 3-4 days) the VDX plates under light microscope and recording the presence and nature of the protein crystals obtained

Resources: Book and web links

Subject Resource

Book	Ducruix, A. & Giege, R. (1992). <i>Crystallization of Nucleic Acids and Proteins, A Practical Approach</i> , edited by A. Ducruix & R. Giege. Oxford University Press
Book	McPherson, A. (1999). <i>Crystallization of Biological Macromolecules</i> . Cold Spring Harbor Press, Cold Spring Harbor.
Web	<i>Crystal Growth Techniques</i> from Hampton Research: http://hamptonresearch.com/documents/growth_101/2.pdf
Web	<i>Hanging Drop Vapor Diffusion Crystallization</i> from Hampton Research: http://hamptonresearch.com/documents/growth_101/3.pdf

Contact: X-Ray Facility Director

Instructor Thayumanasamy Somasundaram

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Version: 20150714; Original version: 20120628

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Crystallization of a Protein

Materials: List of chemicals and items

Chemicals Required:

Sodium Acetate (Solid)	Sodium Hydroxide (Pellets)	Hydrochloric Acid (11 N solution)
Lysozyme (Powder)	Sodium Chloride (Solid)	

Other Items Required:

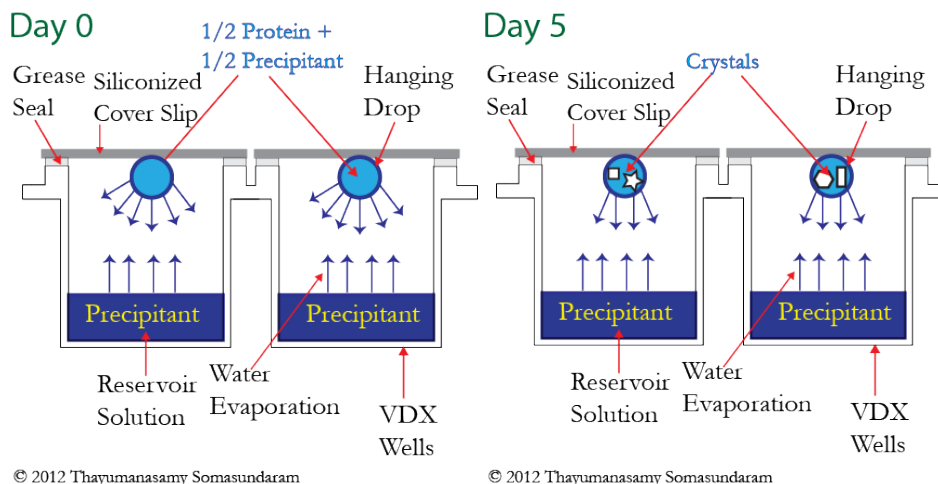
pH Meter	Gilson® Pipettes and Pipette Tips	Electronic Scale
Magnetic Stirrer	Stir Bars	Spatula
Eppendorf® Tubes	Glass Rods	Falcon® Tubes (15 and 50 mL)
Glass Beakers	Measuring Cylinders	Aluminum Foil
Syringes w/ Luer-Loc® (1-5 mL)	Acrodisc® Syringe Filters	Parafilm®-M
Plastic Dropping Pipets	Deionized Water	Vortex Mixer

Items for Crystallization Set-up and examination:

VDX® Plates (Pre-Greased)	Siliconized Cover slips	10 μ L Pipette and Pipette Tips
1 mL Pipette and Pipette Tips	Markers	Tapes
Optical Microscope	Crystal Scoring Sheet	Crystal Scoring Reference Sheet

Growing Single Crystals of a Protein

Hanging drop vapor diffusion crystallization of lysozyme



The students will learn how to grow protein single-crystals (in this module the protein is lysozyme, an enzyme) from a solution of lysozyme in sodium acetate buffer using sodium chloride as a precipitating agent. The students will grow the single crystals using a technique called *hanging drop vapor-diffusion crystallization*. The method involves hanging few microliters of protein + precipitant solution on a glass cover slip directly over few milliliters of precipitant solution in a reservoir on a VDX plate. Due to the concentration difference between the protein and precipitant solutions, more water vapors will diffuse from the protein solution toward the precipitant, rendering the protein solution super-saturated. This will lead, over three to four days, to the formation of protein single crystals. So after 4 or 5 days the students will transfer VDX plates to a light microscope and look for the presence of single crystals of protein in the cover slip. The students will then score for number, shape, and size of the crystals.