2015

Cryo Crystallography

Practical Course in X-Ray Diffraction: Module 3 + 4 (Protein Cryo Crystallography)

In this module, the students will learn cryo sample preparation and cryo crystallographic data collection. This will involve harvesting a protein crystal from a crystal-tray using a CryoLoop, equilibrating the crystal in cryo protectant, flash cooling it in a cryogen like gaseous and/or liquid nitrogen, mounting the crystal in a goniometer and collecting x-ray diffraction data at cryogenic temperature of 100°K.

2015 Fall IMB Boot camp
8/19/2015

Cryo Crystallography

Practical Course in X-Ray Diffraction: Module 3+4 (Protein Cryo Crystallography)

Practical Course: When, What, Where?

Facility	X-Ray Crystallography Facility
Date	Wednesday, August 19, 2015
Event	Practical Course in X-Ray Diffraction Module 3+4
Venue	Kasha Lab Room Numbers 410 and 412

Module Objectives: Learn to perform cryo crystallographic experiment

Target	In-coming graduate students
Main Objective	The main objective is to learn how to harvest a protein crystal from a tray, flash cool it in a cryogen, mount the crystal in a goniometer and collect x-ray diffraction data
Other Objectives	Learning about cryogens, cryogenic safety, flash cooling using liquid and gaseous cryogens
	Learning about cryo protection, cryo buffers, and crystal handling
	Mounting a crystal in x-ray diffraction machine, aligning and collecting x-ray diffraction data

Resources: Book and web links

Subject	Resource
Book	Rhodes, G. (2006). *Crystallography made crystal clear*. Academic Press, New York.
Book	Blow, D. (2002). *Outline of Crystallography for Biologists.* Oxford University Press.
Web	UCLA Michael Sawaya's Notes on Cryo Crystallography: http://people.mbi.ucla.edu/sawaya/m230d/Data/data.html
Web	Elspeth Garman Oxford England Lab's Help Tools: http://www.biop.ox.ac.uk/www/garman/lab_tools.html

Contact: X-Ray Facility Director

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Cryo Crystallography

Materials: List of chemicals and items required

Chemicals Required:

Ethylene Glycol (Liquid)	Poly Ethylene Glycols (Solid & Liquid)	Sucrose (Solid)
Lysozyme Crystal	Ammonium Sulfate (Solid)	Sodium Chloride (Solid)

Other Items Required:

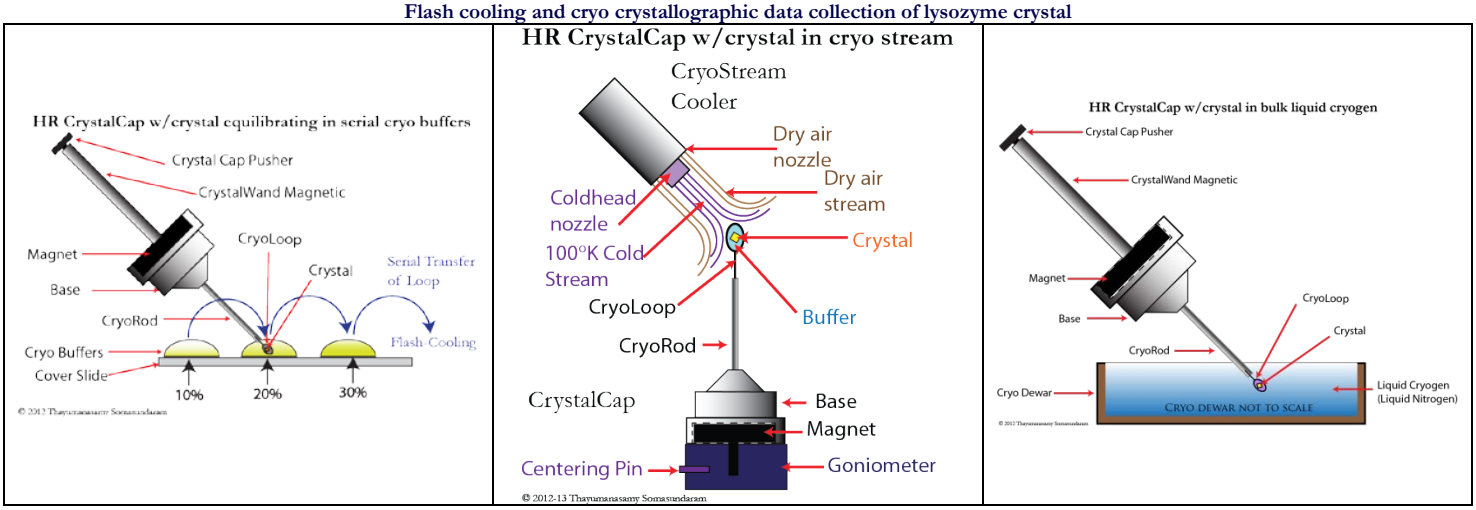
Cryo Storage Dewar (10 L)	Cryo Foam Dewar (Short)	Cryo Foam Dewar (Tall)
CryoLoop®	CryoRod®	CrystalCap®
CrystalCap Magnetic®	Cryo Tongs°	CrystalWand®
Cryo Vial	Vial Clamp	Cover Slide
Forceps	Bottle Caps	

Items for Crystal Set-up and diffraction:

Goniometer	Goniometer Magnet	Goniometer Wrench
Cryo Stream Blocker	Pipette Tips	Gilson® Pipettes
Optical Microscope	X-Ray Equipment	Crystal Scoring Sheet

How to do cryo crystallographic experiment

Flash cooling and cryo crystallographic data collection of lysozyme crystal



The students will learn how to harvest a single-crystal (in this module the crystal of an enzyme lysozyme) grown using a technique called *hanging drop vapor-diffusion crystallization*. In this technique the crystals will grow in a drop of buffer hanging under the VDX plate's cover slip. A CryoLoop mounted on a CrystalCap will be used to pick up the crystal. The crystal will then be equilibrated in a cryo buffer. Then using either bulk liquid nitrogen or gaseous nitrogen maintained at 100°K (-173.15°C), the lysozyme crystal in the CryoLoop will be flash-cooled in milliseconds. The students will then mount the flash cooled crystal on goniometer attached to x-ray diffraction equipment and center the crystal. The students will then start x-ray diffraction data collection.

Cryo crystallography of protein crystal involves several steps and each step will be explained in detail next.