

2017 IMB X-RAY BOOT CAMP

Fall 2017 | 3 Week Course

Crystallization and Diffraction

Syllabus and Calendar

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CRYSTALLIZATION AND DIFFRACTION

Practical course in Protein Crystallization and X-Ray Diffraction

Session: Lecture: 40 min; Lab: 120 min; Q&A: 20 min; 3 weeks 6 sessions

GOALS

The practical course in crystallization and x-ray diffraction is a precursor to a graduate level macromolecular crystallography special class taught every other year. In this course, we will cover concepts of crystals, symmetry, crystallization, x-ray generation and safety, x-ray diffraction, cryo safety and technique, data collection and processing, Linux o/s, computer graphics, and data visualization. In this practical course, more emphasis will be given to hands-on training and lab work than the full-fledged graduate level course.

At the end of this course, you will learn basic concepts in:

- Symmetry, space groups, lattice, and unit cell
- Need for crystals, cryogenic temperatures, and X-Rays
- Crystallization set-up, harvesting and loading crystals
- Cryogenic safety and cryo-crystallography
- X-Ray generation and safety
- X-Ray diffraction and data collection
- Linux operating system and running programs
- Computer graphics and data analysis

CALENDER

August 2017 MOB Boot Camp Tentative Schedule				
Monday	Tuesday	Wednesday	Thursday	Friday
07-Aug	08-Aug	09-Aug	10-Aug	11-Aug
				International Student Orientation
14-Aug	15-Aug	16-Aug	17-Aug	18-Aug
				International Student Orientation
21-Aug	22-Aug	23-Aug	24-Aug	25-Aug
8-12: Solution Prep	Graduate Student Orientation	8-12: Data Collect	8-12: Data Process	8-12 Data Analysis
1-5: Cryo Prep				1-5 Visualization
28-Aug	30-Aug	31-Aug	01-Sep	02-Sep
Classes Start				

XRF Sessions are in purple font (8-12: AM session and 1-5: PM session)

COURSE

Session: We will meet for three hours per session for six sessions. The six sessions will be spread over three weeks. The session will consist of 20 minutes lecture followed by 60 minutes of lab-work or hands on training followed by 10 minutes of Question and Answer session.

Course: The total course length is for three weeks with two sessions per week. We expect the course will have 4-5 students per session and all lab supplies will be provided to the students.

SYLLABUS

1. Symmetry, lattices, crystals, and crystallization
2. Crystals and protein crystallization set-up
3. Crystallization observation and optimization
4. X-Rays, radiation safety and X-Ray generation
5. Cryogenics safety and cryo-crystallography
6. X-Ray Diffraction set-up and data collection
7. Linux operating system and computer graphics
8. Data processing and visualization and wrap-up

Sessions highlighted in blue will involve more hands-on training time and sessions highlighted in green will involve more lecture time than indicated in the original description.

RESOURCES

Requirements

- Wet lab bench work experience
- Computer skills using Windows/Mac/Linux operating systems
- Basic knowledge about macromolecules and their properties

Resources

- <http://www.ruppweb.org/Xray/101index.html> [On-line]
- [Kevin Cowtan's Crystallography Teaching Materials](#)
- [Cambridge University Crystallography Course](#)
- *Crystallography made Crystal Clear* by Gale Rhodes, 2nd Ed, 2000, Academic Press, NY
- A folder containing all the required materials will be given to the students on the first day

CONTACT INFORMATION

Contact

- Dr. Thayumana Somasundaram
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- URL: biophysics.fsu.edu/soma/projects/core-facilities-workshop/

DETAILED SYLLABUS

1. Symmetry, lattices, crystals, and crystallization

In this session, we will cover basic ideas of symmetry: point groups, space groups, symmetry elements, Bravais lattices, and chiral molecules and their symmetry. Then we will study about crystals of macromolecular materials. We will compare and contrast crystal growth techniques. The lab portion of the session will involve learning about hanging-drop and sitting-drop vapor diffusion experiments. We will also cover Commercial Crystallization Screens. [2017-Mod-0_Introduction.pptx, 2017-Mod-1_Symm+Charzn.pptx, 2017-Mod-2_Crystlzn.pptx]

2. Crystals and protein crystallization set-up

In this session, we will start to learn about macromolecular crystals and their characteristics. We will learn about de novo crystallization. We will also learn about commercial screens available for proteins, nucleic acids from various vendors. We will also learn about crystal screening services available for testing multiple conditions.

The lab portion of the session will involve preparing two separate protein solutions (lysozyme, glucose isomerase), buffers, and precipitating agents. We will also be setting up hanging-drop vapor diffusion crystallization trays. [2017-Mod-2_Crystlzn.pptx, 2017-Mod-1+2_Crystallization.docx]

3. Crystallization observation and optimization

In this session, we will optically observe and score the crystal trays we have set-up during the last session. Based on the original scores modified crystallization conditions will be prepared and additional trays will be set-up for future observation and harvesting.

We will also crystal seeding (micro and macro), crystal manipulation, and mounting. Previously prepared crystals will be mounted into glass capillaries, and cryo loops. [2017-Mod-1+2_Crystallization.docx]

4. X-Rays, radiation safety, and X-Ray generation

In this session, we will cover topics pertaining to need for X-Ray radiation, why we need to carry out X-Ray diffraction to obtain atomic resolution images of macromolecules. We will cover basics of X-Ray safety. How X-Rays are produced both at home and at a synchrotron station.

The lab portion of the session will involve tour the home X-Ray Diffraction laboratory and exploring various components involved in collecting the data. [2017-Mod-3_X-Ray+Cryo_Safety.pptx]

5. Cryogenic safety and cryo-crystallography

In this session, we will start with safety dealing with liquid nitrogen. We will then move on to the need for low-temperature (cryo-crystallography) crystallography. The advantages and disadvantages of low-temp work will be discussed.

During the lab portion of the session, we will learn how to mount a crystal in a cryo loop. We will also learn how to flash-cool the crystal in a loop under bulk liquid nitrogen bowl and low-temp flowing nitrogen gas. [2017-Mod-3_X-Ray+Cryo_Safety.pptx, 2017-Mod-3+4_CryoCrystallography.docx]

6. X-Ray diffraction set-up and data collection

In this session crystals in glass capillaries cryo loops will be mounted on to the X-Ray diffraction machine. Crystal alignment and detector specific adjustments will be taught. In the lab portion of the session data from ambient or low-temperature crystal, diffraction data will be collected.

[2017_XRF_SOP.pdf; XRayView demonstration]

7. Linux operating system and computer graphics

In this session, basic Linux operating system commands and procedures will be introduced. Introduction to computer graphics and macromolecular graphics programs will be introduced.

[Chimera, PDB]

8. Data processing and visualization

Using a shared account data collected during the previous session will be visualized, processed, and simple interpretation of the data will be carried out. [Phenix: Xtriage, Molecular Replacement, Refinement].

MANUALS AND HANDOUTS

0. Introduction, syllabus, and schedule

Presentation [pptx]

1. Symmetry, space groups, lattices, and unit cell

Presentation [pptx]

2. Crystals and protein crystallization set-up

Presentation [pptx] | Crystallization Module [pdf]

3. Crystallization observation and optimization

Presentation [pptx] | Crystallization Module [pdf]

4. X-Rays, radiation safety, and X-Ray generation

Presentation [pptx]

5. Cryogenic safety and cryo-crystallography

Presentation [pptx] | Cryo Crystallography [pdf]

6. X-Ray diffraction set-up and data collection

Presentation [pdf] | Data Collection Module [pdf]

7. Linux operating system and data processing

Presentation | Data Processing Module [pdf]

8. Protein Data Band Data processing and data visualization

Presentation [pdf] | Chimera Manual [pdf] Phenix [pdf]

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