

Crystallization and Diffraction

Practical course in Protein Crystallization and X-Ray Diffraction

Session: Lecture: 60 min; Lab: 90 min; Q&A: 30 min; 3 weeks 2 sessions/week

GOALS

The practical course in crystallization and x-ray diffraction is intended to be a precursor to a graduate level macromolecular crystallization special topics course taught every other year. In this course, we will cover basic concepts of crystals, symmetry, crystallization, x-ray generation, radiation safety, x-ray diffraction, cryo safety, cryogenic technique, data collection, data processing, Linux o/s, computer graphics, data visualization, and model building. 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:

- Protein crystallization and crystal symmetry
- Cryogenic safety and cryo-crystallography
- Need for crystals, cryogenic temperatures, and X-Rays
- X-Ray generation and safety
- X-Ray diffraction and data collection
- Linux operating system and running programs
- Computer graphics and model visualization

SCHEDULE (dates are tentative)

Session: We will meet for 3 hours per session and two sessions per week with 15 minute break. The session will consist of two 30 minutes lecture followed by two 45 minutes of lab-work or hands on training which will be followed by 15 minutes of Question and Answer session.

Course: The total course length is expected to last four 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.

August 2012						
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
			1	2	3	4
5	6	7	8 XRF: 10-12	9 XRF: 9-12	10	11
12	13	14 XRF: 9-12	15 XRF: 9-12	16	17	18
19	20	21	22 XRF: 9-12	23 XRF: 9-12	24	25
26	27	28	29	30	31	

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

RESOURCES

Required Background

To successfully complete this course you should be

- under-grad (junior/senior) in Chemistry, Physics, Biology or related subjects
- grad in Chemistry, Physics, Biology, or related subjects

Required Materials

To successfully complete this course, you will need some basics in

- wet-lab
- computer usage
- knowledge of macromolecules

Resources

- <http://www.ruppweb.org/Xray/101index.html> [On-line]
- *Crystallography made Crystal Clear* by Gale Rhodes, 2nd Ed, 2000, Academic Press, NY.[Book]

CONTACT INFORMATION

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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.

[[12pcxrd_mod_0.pdf](#), [12pcxrd_mod_1a.pdf](#), and [12pcxrd_mod_1b.pdf](#)]

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. [[12pcxrd_mod_1b.pdf](#), [CrystallizationModule.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.

[[CrystallizationModule.docx](#)]

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

In this session we will cover topics pertaining to why we need 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. [[12pcxrd_mod4a.pdf](#), [12pcxrd_mod4b.pdf](#)]

5. Cryogenic safety and cryo-crystallography

In this session, we will start off 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. [[12pcxrd_mod4a.pdf](#), [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-temp crystal diffraction data will be collected. [[2012_XRF_SOP.pdf](#)]

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.

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.

The session will be wrapped up with overview of the course.

MANUALS AND HANDOUTS

0. Introduction, syllabus, and schedule

[Presentation](#) [pdf]

1. Symmetry, lattices, crystals, and crystallization

[Presentation](#) [pdf]

2. Crystals and protein crystallization set-up

[Presentation](#) [pdf] | [Crystallization Module](#) [pdf]

3. Crystallization observation and optimization

[Presentation](#) [pdf] | [Crystallization Module](#) [pdf]

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

[Presentation](#) [pdf]

5. Cryogenic safety and cryo-crystallography

[Presentation](#) [pdf] | [Cryo Crystallography](#) [pdf]

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

[Presentation](#) [pdf]

7. Linux operating system and computer graphics

[Presentation](#) | [Chimera Manual](#) [pdf]

8. Data processing and visualization

[Presentation](#) [pdf]