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 2 sessions/week

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
- Protein Data Bank search and computer graphics

2013 August				
Monday	Tuesday	Wednesday	Thursday	Friday
5	**6**	**7** 9 -12 Noon Mob Intro 12-1 Lunch 1-2 Safety Training 2-5 XRF	**8** 9-12 Noon XRF 12-1 Lunch 1-5 PBF	**9** 10-5 In'tl Orient. *Ghosh only* Domestic students work on residency documents
12 9-12 Noon PBF 12-1 Lunch 1-5 PEF	**13** 9-12 Noon PBF 12-1 Lunch 1-5 PEF	**14** 9-12 Noon XRF 12-1 Lunch 1-5 PEF	**15** 9-12 Noon XRF 12-1 Lunch 1-5 PEF	**16** 9-12 Noon PBF 12-1 Lunch 1-5 PEF
19 9-12 Noon PBF 12-5 Lunch and do errands	**20** 8-5 Grad Student Orientation	**21** 9-12 Noon XRF 12-1 Lunch 1-5 Computer	**22** 9-12 Noon XRF 12-1 Lunch 1-5 Computer	**23** 9-12 Noon PBF 12-5 Lunch and do errands
26 CLASSES START 2 -5 PEF	**27** 12:30-1:45 Biopolymers 2 -5 PEF	**28** 2 -5 PEF	**29** 12:30-1:45 Biopolymers 2 - 5 PEF	**30** 3-5 Resp. Conduct Research

Session: We will meet for 3 hours per session and two sessions per week with 15 minute break. The session will consist of two 20 minutes lecture followed by two 60 minutes of lab-work or hands on training which will be followed by 10 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.

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 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]
- Kevin Cowtan's Crystallography Teaching Materials
- Cambridge University Crystallography Course
- *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. [13pcxrd_mod_0.pdf, 13pcxrd_mod_1a.pdf, and 13pcxrd_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. [13pcxrd_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 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. [13pcxrd_mod4a.pdf, 13pcxrd_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. [13pcxrd_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; 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.

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, space groups, lattices, and unit cell

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] | Data Collection Module [pdf]

7. Linux operating system and data processing

Presentation | Data Processing Module [pdf]

8. Protein Data Band Data processing and visualization

Presentation [pdf] | Chimera Manual [pdf]

Updated: July 15, 2013.