



## **CHEM599: X-ray Crystallography for Biochemists**

**Units:** 4

**Term:** Spring 2025

**Time:** M,W 2:00-3:20 pm

**Location:** GFS 223

**Instructor:** Vadim Cherezov

**Office:** MCB 318

**Office Hours:** by appointment

**Contact Info:** Email: [cherezov@usc.edu](mailto:cherezov@usc.edu)

Phone: (213) 821-1464

### **Course Overview**

Advanced techniques and methods in X-ray crystal structure determination of biological macromolecules with emphasis on practical work.

### **Course Description**

The importance of single-crystal X-ray diffraction in structural biology is evident, with over 200,000 crystal structures deposited in the Protein Data Bank (PDB). This success can be attributed to significant breakthroughs and advancements in recombinant protein expression, stabilization and crystallization, developments of new X-ray sources, crystallographic hardware and software used for X-ray structure determination, allowing the determination of crystal structures for challenging macromolecules and their complexes, previously deemed too difficult to crystallize. This course aims to familiarize students with prevalent crystallization approaches, crystallographic software tools, and the essential techniques and methods necessary for handling biomolecular structure determinations.

The course will integrate lectures with hands-on practical work, allowing students to engage in data processing, structure solutions, and structure refinements using original X-ray diffraction datasets. Additionally, the utilization of structural databases and AI tools, such as AlphaFold, in research will be explored during the course.

### **Course Goals and Learning Objectives**

The objective of this course is to acquaint you with the techniques and methodologies involved in X-ray structure determination, particularly focusing on biological macromolecules like proteins and nucleic acids. You will gain proficiency in the use of contemporary crystallographic software tools, including the CCP4 suite, Phenix, SHELX, Coot, and Pymol. Additionally, you will learn the processes of accessing and utilizing the Protein Data Bank (PDB) and other relevant crystallographic databases.

This course is designed to be interactive and practical. You will engage in the process of crystallizing a test protein, work with genuine X-ray diffraction datasets, and proceed to solve and refine the crystal structure. Furthermore, you will delve into advanced subjects, including challenging structures, low resolution, structural disorder, twinning, and pseudo-symmetry.

After successfully completing this course, you will be able to:

- Design a macromolecular structure determination project.
- Prepare protein for crystallization, set up crystallization trials, detect initial crystal hits, optimize crystal growth and harvest crystals for data collection.
- Select an appropriate strategy for diffraction data collection.

- Process diffraction data sets, solve, refine and validate the structure.
- Visualize, manipulate and use crystal structures for gaining mechanistic insights into protein function and generation of new hypotheses.

## **Required Readings and Supplementary Materials**

### **Required:**

Rhodes, G. 2016. Crystallography Made Crystal Clear. A guide for users of macromolecular models. 3rd Edition, Academic Press.

### **Recommended:**

Rupp, B. 2009. Biomolecular Crystallography. Principle, practice and application to structural biology. 1st Edition, Garland Science.

### **Supplementary Textbooks and References:**

- McPherson, A. 2016. Introduction to Macromolecular Crystallography. 2nd ed., Wiley-Blackwell.
- McPherson, A. 1999. Crystallization of Biological Macromolecules. 1st ed., Cold Spring Harbor Laboratory Press.
- Branden, C, and Tooze, J. 1999. Introduction to Protein Structure. 2nd ed. Garland Science
- Bergfors, TM. 2009. Protein Crystallization. 2nd ed., International University Line.
- Petsko, GA, and Ringe, D. 2003. Protein Structure and Function (Primers in Biology). 1st ed., Sinauer Associates, Inc.
- Hahn, T. 2005. International Tables for Crystallography Volume A: Space-group symmetry, 5th ed., Springer.

## **Reasons for Taking this Course**

Macromolecular Crystallography is one of the most successful technique in structural biology that provides three-dimensional structural information about biological molecules at atomic resolution. This structural information is critical for understanding the mechanisms of action of biological macromolecule and designing specific ligands to modulate their function.

For a long time, X-ray crystallography remained a highly specialized technique that was inaccessible to the average undergraduate or even the postgraduate. With the development of more and more sophisticated diffractometers, the steady rise in computing power, and the advances in crystallographic computer software, macromolecular X-ray crystallography has become a very powerful method to gain insight and understanding structure and function of biological molecules. While X-ray diffraction is now seen as a routine method, it is essential to have a good grasp of the process of crystal structure determination and be able to evaluate crystallographic results and data.

## **Course Notes**

This course is a letter grade. We will use Brightspace as the course website. Lecture slides will be posted on Brightspace before each lecture.

## **Technological Proficiency and Hardware/Software Required**

Given the highly hands-on nature of this course, it is essential to have access to a personal computer or laptop. Familiarity with both Windows and/or macOS is expected. All the software utilized throughout the course is either freely available or covered by academic licenses.

**Prerequisite(s):** NO

## Description and Assessment of Assignments

*Homework:* The homework assignments will involve practical problems and exercises corresponding to various stages of the structure determination process. Homework is expected to be submitted at the beginning of the class on the specified day. Late submissions will incur a penalty of one point for each day, up to 10 days past the deadline. Grading of homework will typically be completed within one week of the due date.

*Exams:* The midterm and final exams are closed book tests for which you are only required to bring a calculator, a ruler, and a pen. The final exam covers the course material studied during the entire semester (that is, it is a cumulative test) and uses a combination of multiple choice and free response questions. Students who are not able to attend the exam must notify the instructor before the test.

**Date of the Midterm Exam:** February 26, 2025

**Date of the Final Exam:** May 12, 2025

## Grading Breakdown

Your final grade will be based on one midterm, one final exam, and homework assignments, as follows:

Assessment Tool (assignments)	Points	% of Grade
Homework	450	50
Midterm exam	50	20
Final exam	100	30
<b>TOTAL</b>	<b>600</b>	<b>100</b>

## Grading Scale

Course final grades will be determined using the following scale

A	94-100
A-	89-93
B+	84-88
B	79-83
B-	74-78
C+	69-73
C	64-68
C-	59-63
D+	54-58
D	49-53
D-	40-48
F	39 and below

## Academic Integrity

The University of Southern California is foremost a learning community committed to fostering successful scholars and researchers dedicated to the pursuit of knowledge and the transmission of ideas. Academic misconduct is in contrast to the university's mission to educate students through a broad array of first-rank academic, professional, and extracurricular programs and includes any act of dishonesty in the submission of academic work (either in draft or final form).

This course will follow the expectations for academic integrity as stated in the [USC Student Handbook](#). All students are expected to submit assignments that are original work and prepared specifically for the course/section in this academic term. You may not submit work written by others or "recycle" work prepared for other courses without obtaining written permission from the instructor(s). Students suspected of engaging in academic misconduct will be reported to the Office of Academic Integrity.

Other violations of academic misconduct include, but are not limited to, cheating, plagiarism, fabrication (e.g., falsifying data), knowingly assisting others in acts of academic dishonesty, and any act that gains or is intended to gain an unfair academic advantage.

Academic dishonesty has a far-reaching impact and is considered a serious offense against the university. Violations will result in a grade penalty, such as a failing grade on the assignment or in the course, and disciplinary action from the university itself, such as suspension or even expulsion.

For more information about academic integrity see the [student handbook](#) or the [Office of Academic Integrity's website](#), and university policies on [Research and Scholarship Misconduct](#).

Please ask your instructor if you are unsure what constitutes unauthorized assistance on an exam or assignment or what information requires citation and/or attribution.

### **Policy on using Artificial Intelligence (AI)**

The use of AI tools (e.g., ChatGPT, image generation tools, AlphaFold) in this class is encouraged. Learning to use AI is an emerging skill, and I welcome the opportunity to meet with you to provide guidance with these tools during office hours or after class. Keep in mind the following:

- AI tools are permitted to help you brainstorm topics or revise work you have already written.
- If you provide minimum-effort prompts, you will get low-quality results. You will need to refine your prompts to get good outcomes. This will take work.
- Proceed with caution when using AI tools and do not assume the information provided is accurate or trustworthy. If it gives you a number or fact, assume it is incorrect unless you either know the correct answer or can verify its accuracy with another source. You will be responsible for any errors or omissions provided by the tool. It works best for topics you understand.
- AI is a tool, but one that you need to acknowledge using. Please include a paragraph at the end of any assignment that uses AI explaining how (and why) you used AI and indicate/specify the prompts you used to obtain the results what prompts you used to get the results. Failure to do so is a violation of academic integrity policies.
- Be thoughtful about when AI is useful. Consider its appropriateness for each assignment or circumstance. The use of AI tools requires attribution. You are expected to clearly attribute any material generated by the tool used.

### **Course Content Distribution and Synchronous Session Recordings Policies**

USC has policies that prohibit recording and distribution of any synchronous and asynchronous course content outside of the learning environment.

Recording a university class without the express permission of the instructor and announcement to the class, or unless conducted pursuant to an Office of Student Accessibility Services (OSAS) accommodation. Recording can inhibit free discussion in the future, and thus infringe on the academic freedom of other students as well as the instructor. ([Living our Unifying Values: The USC Student Handbook](#), page 13).

Distribution or use of notes, recordings, exams, or other intellectual property, based on university classes or lectures without the express permission of the instructor for purposes other than individual or group study. This includes but is not limited to providing materials for distribution by services publishing course materials. This restriction on unauthorized use also applies to all information, which had been distributed to students or in any way had been displayed for use in relationship to the class, whether obtained in class, via email, on the internet, or via any other media. ([Living our Unifying Values: The USC Student Handbook](#), page 13).

### **Additional Policies**

Student attendance and active participation in the class work is expected. Electronic communication devices (phones, etc) must be turned off or silenced and placed away during lectures.

## Tentative Course Schedule: A Weekly Breakdown

	<b>Topics/Daily Activities</b>	<b>Readings/Preparation</b>	<b>Deliverables</b>
<b>Week 1</b>	An overview of macromolecular crystallography. General outline of the crystal structure determination process.	Rhodes: Chapter 1 and lecture slides	
<b>Week 2</b>	Protein crystallization. Properties of protein crystals, crystallization methods.	Rhodes: Chapter 3 and lecture slides	Homework 1
<b>Week 3</b>	Diffraction data collection. Geometric principles of diffraction. X-ray sources. Oscillation method. Serial crystallography.	Rhodes: Chapter 4 and lecture slides	
<b>Week 4</b>	Fundamentals of crystallography. Fourier transform. From diffraction data to electron density.	Rhodes: Chapter 5 and lecture slides	
<b>Week 5</b>	X-ray diffraction data processing I. Indexing and Integration.	Lecture slides	Homework 2
<b>Week 6</b>	X-ray diffraction data processing II. Scaling and Merging.	Lecture slides	Homework 3
<b>Week 7</b>	Structure solution methods.	Rhodes: Chapter 6 and lecture slides	<i>Midterm Exam</i>
<b>Week 8</b>	Structure solution I. Molecular replacement.	Lecture slides	Homework 4
<b>Week 9</b>	Structure solution II. Isomorphous replacement.	Lecture slides	Homework 5
<b>Week 10</b>	Structure solution III. Anomalous scattering.	Lecture slides	Homework 6
<b>Week 11</b>	Map inspection and model building.	Rhodes: Chapters 7.1 – 7.4 and lecture slides	Homework 7
<b>Week 12</b>	Structure refinement.	Rhodes: Chapters 7.5 – 7.7 and lecture slides	Homework 8
<b>Week 13</b>	Difficult structures. Low resolution. Disorder. Twinning. Pseudo-symmetry.	Lecture slides	
<b>Week 14</b>	Structure analysis, validation and presentation.	Rhodes: Chapter 8 and lecture slides	
<b>Week 15</b>	Protein Data Bank. What structures can and can't tell us. Future outlook of macromolecular crystallography.	Rhodes: Chapter 11 and lecture slides	Homework 9
<b>FINAL</b>			<i>Final Exam</i>

## Statement on University Academic and Support Systems

### **Students and Disability Accommodations:**

USC welcomes students with disabilities into all of the University's educational programs. [The Office of Student Accessibility Services](#) (OSAS) is responsible for the determination of appropriate accommodations for students who encounter disability-related barriers. Once a student has completed the OSAS process (registration, initial appointment, and submitted documentation) and accommodations are determined to be reasonable and appropriate, a Letter of Accommodation (LOA) will be available to generate for each course. The LOA must be given to each course instructor by the student and followed up with a discussion. This should be done as early in the semester as possible as accommodations are not retroactive. More information can be found at [osas.usc.edu](https://osas.usc.edu). You may contact OSAS at (213) 740-0776 or via email at [osasfrontdesk@usc.edu](mailto:osasfrontdesk@usc.edu).

### **Student Financial Aid and Satisfactory Academic Progress:**

To be eligible for certain kinds of financial aid, students are required to maintain Satisfactory Academic Progress (SAP) toward their degree objectives. Visit the [Financial Aid Office webpage](#) for [undergraduate-](#) and [graduate-level](#) SAP eligibility requirements and the appeals process.

### **Support Systems:**

[Counseling and Mental Health](#) - (213) 740-9355 – 24/7 on call

Free and confidential mental health treatment for students, including short-term psychotherapy, group counseling, stress fitness workshops, and crisis intervention.

[988 Suicide and Crisis Lifeline](#) - 988 for both calls and text messages – 24/7 on call

The 988 Suicide and Crisis Lifeline (formerly known as the National Suicide Prevention Lifeline) provides free and confidential emotional support to people in suicidal crisis or emotional distress 24 hours a day, 7 days a week, across the United States. The Lifeline consists of a national network of over 200 local crisis centers, combining custom local care and resources with national standards and best practices. The new, shorter phone number makes it easier for people to remember and access mental health crisis services (though the previous 1 (800) 273-8255 number will continue to function indefinitely) and represents a continued commitment to those in crisis.

[Relationship and Sexual Violence Prevention Services \(RSVP\)](#) - (213) 740-9355(WELL) – 24/7 on call

Free and confidential therapy services, workshops, and training for situations related to gender- and power-based harm (including sexual assault, intimate partner violence, and stalking).

[Office for Equity, Equal Opportunity, and Title IX \(EEO-TIX\)](#) - (213) 740-5086

Information about how to get help or help someone affected by harassment or discrimination, rights of protected classes, reporting options, and additional resources for students, faculty, staff, visitors, and applicants.

[Reporting Incidents of Bias or Harassment](#) - (213) 740-2500

Avenue to report incidents of bias, hate crimes, and microaggressions to the Office for Equity, Equal Opportunity, and Title for appropriate investigation, supportive measures, and response.

[The Office of Student Accessibility Services \(OSAS\)](#) - (213) 740-0776

OSAS ensures equal access for students with disabilities through providing academic accommodations and auxiliary aids in accordance with federal laws and university policy.

[USC Campus Support and Intervention](#) - (213) 740-0411

Assists students and families in resolving complex personal, financial, and academic issues adversely affecting their success as a student.

[Diversity, Equity and Inclusion](#) - (213) 740-2101

Information on events, programs and training, the Provost's Diversity and Inclusion Council, Diversity Liaisons for each academic school, chronology, participation, and various resources for students.

[USC Emergency](#) - UPC: (213) 740-4321, HSC: (323) 442-1000 – 24/7 on call

Emergency assistance and avenue to report a crime. Latest updates regarding safety, including ways in which instruction will be continued if an officially declared emergency makes travel to campus infeasible.

[USC Department of Public Safety](#) - UPC: (213) 740-6000, HSC: (323) 442-1200 – 24/7 on call

Non-emergency assistance or information.

[Office of the Ombuds](#) - (213) 821-9556 (UPC) / (323-442-0382 (HSC)

A safe and confidential place to share your USC-related issues with a University Ombuds who will work with you to explore options or paths to manage your concern.

[Occupational Therapy Faculty Practice](#) - (323) 442-2850 or [otfp@med.usc.edu](mailto:otfp@med.usc.edu)

Confidential Lifestyle Redesign services for USC students to support health promoting habits and routines that enhance quality of life and academic performance.