# Macromolecular Crystallographic Methods II

BIOC 669 / PHCO 669, Spring, 2024

The University of North Carolina at Chapel Hill



## Course Information

**Credit Hours:** 2

**Pre or Co-Requisites:** BIOC 666 or BIOC 675. BIOC 667 (Macromolecular Crystallographic Methods I) is recommended for crystallography students

**Target Audience:** Graduate students utilizing structural biology as a significant component of their research. The course focuses on the building and analysis of protein structures based on crystallographic or cryoEM data

**Meeting Pattern:** Twice a week, 1.5 hours per class

**Instructional Format:** In-person lecture and computational lab

**Classroom or Location:** Medical Research Building B, conference room

## Instructor Information

**Name:** Stuart Endo-Streeter

**Email Address:** stuart.endostreeter@unc.edu

**Office Location:** Genetic Medicine Building 4049G

**Office Hours:** TBD or by appointment

**Zoom Room ID:** By appointment

**Teaching Philosophy:** I believe in-person instruction is most effective in conveying information and concepts, where students and instructors can have productive conversations and questions are encouraged. Skills are most efficiently learned through examples and exercises, particularly when applied to complex tasks.



## Course Content

### Course Description

Protein x-ray crystallography and cryo-electron microscopy are two of the principle techniques used to experimentally determine the structures of macromolecules. While the data collection and initial processing steps are quite different, the fields converge at the model building, refinement, and analysis stage. This course addresses the fundamental concepts and best practices of how to build, refine, and analyze macromolecular structures with an in-depth treatment of the software and methodologies. Students will solve and build protein structures from provided data, or if available, their own, and analyze the quality of the structures using current best-practices and gold-standard metrics. This class picks up where BIOC 667 (Macromolecular Crystallographic Methods I) finishes. Classes will be twice a week; the first a lecture, the second a lab, where the lecture is put into practice.

### Course Texts & Materials

Outside reading of peer-reviewed literature assigned by the instructor. Access to a laptop computer, preferably running the Windows operating system or a stable Linux distribution. “Biomolecular Crystallography” by Bernhard Rupp is strongly recommended.

### Class Expectations

Assigned reading is expected to be completed before the relevant lecture. Assignments based on each week’s material will be given and expected by the listed due date.

### Course Goals & Student Learning Outcomes (SLOs)

At the end of the course students will be able to phase a protein structure or place an initial cryoEM model, build and refine that model to publication quality, and be able to assess the quality of published structures. They will understand the basics of current best-practices and how to implement them.



## Course Assignments & Assessments

### Assignment Descriptions *(all assignments are due via e-mail by 5 PM)*

#### Generating Kinemages and MolProbity reports

Use the MolProbity website to generate kinemages and quality metric reports on the assigned protein structures. Describe structure quality and what changes can be made to improve the model. Due Feb 28

#### Solve the phase problem of test data sets via molecular replacement and single-wavelength anomalous diffraction

Solve the phase problem for a lysozyme crystal via molecular replacement. Solve the phase problem for a test-data set via SAD. Report the solution metrics. Due March 6

#### Autobuild and manually build protein models into crystallographic or cryoEM density maps

Autobuild the majority of a protein into the corresponding crystallographic or cryoEM density map. Manually build at least twenty residues of a different protein into the corresponding crystallographic or cryoEM density map. Report the quality metrics of the each structure. Show the model fit to density of both sets of models. Due March 21

#### Calculate FoFo and omit maps and build the correct ligand into the density

Using a supplied crystallographic dataset, calculate both FoFo and omit maps and build the correct ligand from a list of supplied ligands. Will require the use of MolView and Elbow. Show the model fit to density and interatomic contacts. Due March 28

#### Perform several cycles of model building and refinement and report metrics

Build and refine part of the supplied structure in either crystallographic or cryoEM density maps. Perform several rounds of energy minimization refinement and report model statistics, methodology, and results. Due April 11

#### Final - Assess and report on an assigned structure

Assess the assigned structure. Structure factors and models will be supplied. Report on the model quality with specific examples of any concerns. Outside research is *not* allowed; students must draw their own conclusions. Due May 3, 2024. Group work is *not* allowed on the final

Each assignment is worth 10% of the final grade, the final is 25%, and participation and attendance are 25%

### Grading Scale & Schema

#### Late Work

Late work will not be accepted outside of university approved excuses, ie; death in the family, serious illness, etc. etc.

#### Grading Rubrics

Grades will be determined by attendance, participation, assignments, and the final.

#### Grading Scale

Undergraduate Graded

## Course Schedule

### Phasing and Building Macromolecular Models

| Class (Date/Week) | Topic(s) | Readings & Assignments Due |
| --- | --- | --- |
| Feb 19 | Macromolecular software;CCP4, PHENIX, Coot, KiNG, MolProbity | Curr. Opin. Struct. Biol 2013, 23:707-714FEBS J. 2008, 275:1-21Protein Sci. 2009, 18:2403-2409Protein Sci. 2017, 27:293-315Assignment: Kinemage & Molprobity, due Feb 28 |
| Feb 26 | Phasing and Molecular Replacement | Arch. Biochem. Biophys. 2016, 602:80-94Acta Cryst. 2007, D63:32-41Assignment: Solving the phase problem, due March 6 |
| March 3 | Auto- and manual model building | Acta Cryst. 2014, D70:144-154Acta Cryst. 2019, D75:1119-1128Nat. Protocols 2008, 3:1171-1179Assignment: Model building, due March 21.  |
| March 11-15 | Spring break |  |
| March 18 | Isomorphous difference density maps and ligand building | Assignment: FoFo and omit maps and ligand building, due March 28 |

### Model Refinement and Analysis

| Class (Date/Week) | Topic(s) | Readings & Assignments Due |
| --- | --- | --- |
| March 25 | Refinement – weights, parameters, workflows | Acta Cryst. 2018, D74:215-227 |
| March 28 | Well-being day |  |
| March 29 | University holiday |  |
| April 1 | Refinement – B-factors, occupancy, polishing | Acta Cryst. 2012, D68:468-477Cryst. Rev. 2013, 19:230-270Assignment: Model refinement, due April 11 |
| April 8 | Analysis and Assessment – MolProbity and metrics | Acta Cryst. 2018, D74:132-142Acta Cryst. 2018, D74:228-236 |
| April 15 | Analysis and Assessment – “When are you done and what makes a ‘good’ model?”  | IUCrJ 2014, 1:179-93Structure 2017, 25:1-12 |
| April 30 | Classes end |  |
| Take-home final | Assess and report on supplied structure | Take home final, due May 3 |



## Policy Statements

### Academic Policies

#### University Class Attendance Policy

University Policy: As stated in the University’s [Class Attendance Policy](https://catalog.unc.edu/policies-procedures/attendance-grading-examination/#text), no right or privilege exists that permits a student to be absent from any class meetings, except for these University Approved Absences:

1. Authorized University activities: [University Approved Absence Office (UAAO) website](https://uaao.unc.edu/sample-page/) provides information and [FAQs for students](https://uaao.unc.edu/faqs-for-students/) and [FAQs for faculty](https://uaao.unc.edu/sample-page/) related to University Approved Absences
2. Disability/religious observance/pregnancy, as required by law and approved by [Accessibility Resources and Service](https://ars.unc.edu/) and/or the [Equal Opportunity and Compliance Office](https://eoc.unc.edu/what-we-do/accommodations/) (EOC)
3. Significant health condition and/or personal/family emergency as approved by the [Office of the Dean of Students](https://odos.unc.edu/), [Gender Violence Service Coordinators](https://gvsc.unc.edu/), and/or the [Equal Opportunity and Compliance Office](https://eoc.unc.edu/what-we-do/accommodations/) (EOC).

Instructors may work with students to meet attendance needs that do not fall within University approved absences. For situations when an absence is not University approved (e.g., a job interview, illness/ flu or club activity), instructors are encouraged to work directly with students to determine the best approach to missed classes and make-up assessment and assignments.

#### Honor Code Statement

All students are expected to follow the guidelines of the UNC Honor Code. In particular, students are expected to refrain from “lying, cheating, or stealing” in the academic context. If you are unsure about which actions violate the Honor Code, please see me, or consult [studentconduct.unc.edu](https://studentconduct.unc.edu/).

#### Syllabus Changes

The instructor reserves the right to make changes to the syllabus including project due dates and test dates. These changes will be announced as early as possible.

#### Acceptable Use Policy

By attending the University of North Carolina at Chapel Hill, you agree to abide by the University of North Carolina at Chapel Hill policies related to the acceptable use of IT systems and services. The Acceptable Use Policy (AUP) sets the expectation that you will use the University’s technology resources responsibly, consistent with the University’s mission. In the context of a class, it’s quite likely you will participate in online activities that could include personal information about you or your peers, and the AUP addresses your obligations to protect the privacy of class participants. In addition, the AUP addresses matters of others’ intellectual property, including copyright. These are only a couple of typical examples, so you should consult the full [Information Technology Acceptable Use Policy](https://policies.unc.edu/TDClient/2833/Portal/KB/ArticleDet?ID=131247), which covers topics related to using digital resources, such as privacy, confidentiality, and intellectual property. Additionally, consult the [Safe Computing at UNC](https://safecomputing.unc.edu/) website for information about data security policies, updates, and tips on keeping your identity, information, and devices safe.

#### Data Security & Privacy

[UNC-Chapel Hill Privacy Statement](https://www.unc.edu/about/privacy-statement/): Sakai’s Discussion Forum, Assignments, DropBox, Gradebook, and Tests & Quizzes tools are designed to share FERPA-protected information privately between instructors and individual students.

#### Grade Appeal Process

If you have any concerns with grading and/or feel you have been awarded an incorrect grade, please discuss it with me as soon as possible. If we cannot resolve the issue, you may talk to our director of undergraduate studies or department chair.

### Services & Student Support Policies

#### Accessibility Resources & Services (ARS)

Accessibility Resources and Service (ARS – ars@unc.edu) receives requests for accommodations, and through the Student and Applicant Accommodations Policy determines eligibility and identifies reasonable accommodations for students with disabilities and/or chronic medical conditions to mitigate or remove the barriers experienced in accessing University courses, programs and activities. ARS also offers its Testing Center resources to students and instructors to facilitate the implementation of testing accommodations. Faculty and instructors with any concerns or questions about accommodations and/or their implementation, are invited to reach out to ARS to discuss.

#### Counseling & Psychological Services (CAPS)

UNC-Chapel Hill is strongly committed to addressing the mental health needs of a diverse student body. The [Heels Care Network](http://care.unc.edu) website is a placeto access the many mental health resources at Carolina. CAPS is the primary mental health provider for students, offering timely access to consultation and connection to clinically appropriate services. Go to their website <https://caps.unc.edu/> or visit their facilities on the third floor of the Campus Health building for an initial evaluation to learn more. Students can also call CAPS 24/7 at 919-966-3658 for immediate assistance.

#### Title IX Resources

Any student who is impacted by discrimination, harassment, interpersonal (relationship) violence, sexual violence, sexual exploitation, or stalking is encouraged to seek resources on campus or in the community. Reports can be made online to the EOC at <https://eoc.unc.edu/report-an-incident/> or by contacting the University’s Title IX Coordinator (Elizabeth Hall, titleixcoordinator@unc.edu) or the Report and Response Coordinators in the Equal Opportunity and Compliance Office (reportandresponse@unc.edu).  Confidential resources include Counseling and Psychological Services and the Gender Violence Services Coordinators (gvsc@unc.edu). Additional resources are available at [safe.unc.edu](https://safe.unc.edu/).

#### Policy on Non-Discrimination

The University is committed to providing an inclusive and welcoming environment for all members of our community and to ensuring that educational and employment decisions are based on individuals’ abilities and qualifications. Consistent with this principle and applicable laws, the University’s [Policy Statement on Non-Discrimination](https://eoc.unc.edu/our-policies/policy-statement-on-non-discrimination/) offers access to its educational programs and activities as well as employment terms and conditions without respect to race, color, gender, national origin, age, religion, genetic information, disability, veteran’s status, sexual orientation, gender identity or gender expression. Such a policy ensures that only relevant factors are considered, and that equitable and consistent standards of conduct and performance are applied. If you are experiencing harassment or discrimination, you can seek assistance and file a report through the Report and Response Coordinators (email reportandresponse@unc.edu or see additional contact info at [safe.unc.edu](https://safe.unc.edu/)) or the Equal Opportunity and Compliance Office at <https://eoc.unc.edu/report-an-incident/>.

#### Diversity Statement

I value the perspectives of individuals from all backgrounds reflecting the diversity of our students. I broadly define diversity to include race, gender identity, national origin, ethnicity, religion, social class, age, sexual orientation, political background, and physical and learning ability. I strive to make this classroom an inclusive space for all students. Please let me know if there is anything I can do to improve. I appreciate any suggestions.

#### Undergraduate Testing Center

The College of Arts and Sciences provides a secure, proctored environment in which exams can be taken. The center works with instructors to proctor exams for their undergraduate students who are not registered with ARS and who do not need testing accommodations as provided by ARS. In other words, the Center provides a proctored testing environment for students who are unable to take an exam at the normally scheduled time (with pre-arrangement by your instructor). For more information, visit <http://testingcenter.web.unc.edu/>.

#### Learning Center

Want to get the most out of this course or others this semester? Visit UNC’s Learning Center at <http://learningcenter.unc.edu> to make an appointment or register for an event. Their free, popular programs will help you optimize your academic performance.  Try academic coaching, peer tutoring, STEM support, ADHD/LD services, workshops and study camps, or review tips and tools available on the website.

#### Writing Center

For free feedback on any course writing projects, check out UNC’s Writing Center. Writing Center coaches can assist with any writing project, including multimedia projects and application essays, at any stage of the writing process. You don’t even need a draft to come visit. To schedule a 45-minute appointment, review quick tips, or request written feedback online, visit <http://writingcenter.unc.edu>.