Structural Biology Methods

Faculty of Sciences

Fall 2022

Lectures

3/0/0. 3+2 cr. Concluded by an exam.

Course instructor

Mgr. Pavel Plevka PhD, Ing. Tibor Füzik PhD, Mgr. Jiří Nováček PhD

Course supervision

prof. RNDr. Zdeněk Glatz, CSc. (ÚBioch Chem PřF MU)

Contact person: Mgr. Pavel Plevka PhD

Provider: CEITEC

Study pre-requisites

This course is aimed at students of general biology, molecular biology and genetics, specialised biology, biochemistry interested in understanding macromolecular structures and macromolecular structure determination methods.

Limitations to signing-up for the course

The course is offered to all students.

This course is specifically directed towards:

- Analytical biochemistry (PřF, N-BCH)
- Biochemistry (PřF, N-BCH)
- Biomolecular chemistry (PřF, N-BCH)
- Genomics and proteomics (PřF, N-BCH)
- Chemoinformatics and bioinformatics (PřF, N-BCH)

Course objectives:

This course aims to provide understanding of theoretical background and practical aspects of X-ray crystallography (5 lectures) and cryo-electron microscopy (cryo-EM) (7 lectures). Both X-ray crystallography and cryo-EM use the concepts of reciprocal space and Fourier transform to allow calculation of electron density maps that show shapes of macromolecules. Cryo-EM part of the course will benefit from the mathematical concepts introduced in preceding X-ray crystallography lectures. Students will learn methodological approaches allowing determination of macromolecular structures with atomic details.

By the end of this course, students should be able to do the following :

- **Understanding of principles allowing the use of X-ray crystallography and cryo-EM.**
- **Undestanding of theory behind:**
  - Interaction of waves with objects
  - Approaches to resolve phase problem in crystallography
  - Use of electrons to display objects with high magnification and fine detail

- Calculation of three-dimensional reconstruction from two-dimensional projections of an object

- **Use of structural biology approaches to study molecular processes necessary for the existence of life.**

Students will learn skills that will allow them to apply for jobs with local high-tech companies such as Tescan and FEI. Knowledge gained in the course can be used in both basic and applied research to study structures of macromolecules, their complexes, viruses, and cells.

Course requirements and grading

Grade will be based on the final exam. The exam will be a mixture of multiple choice and essay questions.

Academic Integrity

Students are required to adhere to the following the rules of academic integrity (as described in „Disciplinární řád pro studenty"). You are prohibited from (among other things): cheating, lying, stealing, and deceit in any of their diverse forms (such as use of ghost-written papers, the use of substitutes for taking examinations, the use of illegal cribs, plagiarism, and copying during examinations).

Violations of academic integrity will result in sanctions. These sanctions range from taking off points from the particular part of the grade to assigning „Failed" as the final grade in the class. The decision about the sanction for academic dishonesty is entirely at the discretion of the instructor. All cases of academic dishonesty will be reported to the „Disciplinární komise PřF MU" (disciplinary commitee).

Course Format

In this course, you will be acquiring knowledge in two main ways: in the lectures and by home study (which means by reading the assigned materials and working on assignments and homeworks). Reading the material will give you a base of factual knowledge. It will also introduce you to the main theoretical points. The lectures then will reinforce your understanding of the material. The lectures will also introduce you to concepts and theories that are not covered by the readings. Class sessions will also be a space for discussions.

Class rules

Before coming to class, please turn off anything that beeps or rings.

Please, help create a nice learning environment for yourself and your classmates. Reading any material that is not related to the class, texting, or checking the internet during the class is rude and will not be tolerated.

Please refrain from eating during class. Having something to drink is fine.

If you have any question regarding the material being discussed in class, do not hesitate to ask. If you ask such question, it will help to clarify the issue not only for you but for your peers as well!

In class discussions, be respectful of other students' opinions. You have the right to hold and express whatever opinion on the discussed topic, no matter how unacceptable they may seem to your classmates. They can disagree with you by they must respect your

right to hold your opinion. Likewise, you must respect their right to express their views freely.

Course Outline

Remember, you are required to read the readings *before* the day for which they are assigned!

This syllabus is subject to change upon notification. All updates will be announced on the course www. It is your responsibility to check for updates to the course outline.

Week 1: Introduction, crystals, symmetry, and the Theory of X-Ray Diffraction

- Introduction to the course
- Crystallizing a Protein
- Crystals and symmetry I.
- Asymmetric Unit; Point Groups; Crystal Systems; Characterization of the Crystals; Matthew's coefficient
- Waves and Their Addition; Argand diagram; Exponential Terms
    - **Read chapters 1, 2, 3, 4, and 16 from "Principles of Protein X-Ray Crystallography".**

Week 2: The Theory of X-Ray Diffraction by a Crystal

- A System of Two Electrons; Scattering by an Atom; Scattering by a Unit Cell; Scattering by a Crystal; Diffraction Conditions; Reciprocal Lattice and Ewald Construction
- The Temperature Factor; Calculation of the Electron Density ρ(x y z); Comparison of F(hkl) and F($\bar{h}\bar{k}\bar{l}$); Symmetry in the Diffraction Pattern; Integral Reflection Conditions for Centered Lattices; Intensity Diffracted by a Crystal; The Polarization Factor; Scattering by a Plane of Atoms; Choice of Wavelength, Size of Unit Cell, and Correction of the Diffracted Intensity
    - **Read chapters 4 from "Principles of Protein X-Ray Crystallography".**

Week 3: Isomorphous Replacement Method and Phase Improvement

- The Patterson Function; Convolution; The Isomorphous Replacement Method; Effect of Heavy Atoms on X-ray Intensities; Determination of the Heavy Atom Parameters from Centrosymmetric Projections; Parameters of Heavy Atoms Derived from Acentric Reflections; The Difference Fourier Summation
- Anomalous Scattering; The Anomalous Patterson Summation; Refinement of the Heavy Atom Parameters Using Preliminary Protein Phase Angles; Protein Phase Angles; The Remaining Error in the Best Fourier Map; The Single Isomorphous Replacement Method;
- The OMIT Map With and Without Sim Weighting; Solvent Flattening; Non-crystallographic Symmetry and Molecular Averaging; Histogram Matching; wARP: Weighted Averaging of Multiple-Refined Dummy Atomic Models; Further Considerations Concerning Density Modification
    - **Read chapter 5, 6, 7, and 8 from "Principles of Protein X-Ray Crystallography".**

Week 4: Anomalous Scattering in the Determination of the Protein Phase Angles and the Absolute Configuration and Molecular Replacement I.

- Protein Phase Angle Determination with Anomalous Scattering; Improvement of Protein Phase Angles with Anomalous Scattering; The Determination of the Absolute Configuration; Multiple- and Single-Wavelength Anomalous Diffraction (MAD and SAD);
- The Rotation Function;
    - **Read chapters 9 and 10 from "Principles of Protein X-Ray Crystallography".**

Week 5: Molecular Replacement II., Laue Diffraction, Refinement of the Model Structure, The Combination of Phase Information, Checking for Gross Errors and Estimating the Accuracy of the Structural Model.

- The Translation Function; AMORE; Rigid-Body Refinement;
- The Accessible Region of Reciprocal Space; The Multiple Problem; Unscrambling of Multiple Intensities; The Spatial Overlap Problem; Wavelength Normalization;
- The Mathematics of Refinement; The Principle of the Fast Fourier Transform Method; Specific Refinement Methods;
- Phase Information from Isomorphous Replacement; Phase Information from Anomalous Scattering; Phase Information from Partial Structure Data, Solvent Flattening, and Molecular Averaging; Phase Information from SAD
- *R*-Factors; The Ramachandran Plot; Stereochemistry Check; The 3D–1D Profile Method; Quantitative Estimation of the Coordinate Error in the Final Model;
    - **Read chapters 10, 12, 13, 14 and 15 from "Principles of Protein X-Ray Crystallography".**

Week 6: Electron Microscopy of Macromolecular Assemblies I.

- Principle of the Transmission Electron Microscope; The Weak-Phase Object Approximation;
    - **Read chapter 2 from "Three-Dimensional Electron Microscopy of Macromolecular Assemblies".**

Week 7: Electron Microscopy of Macromolecular Assemblies II.

- The Contrast Transfer Theory; Determination of the Contrast Transfer Function;
    - **Read chapter 2 from "Three-Dimensional Electron Microscopy of Macromolecular Assemblies".**

Week 8: Electron Microscopy of Macromolecular Assemblies III.

- Computational Correction of the Contrast Transfer Function;
    - **Read chapter 2 from "Three-Dimensional Electron Microscopy of Macromolecular Assemblies".**

Week 9: Multivariate Data Analysis and Classification of Images I.

- Alignment Methods; Averaging and Global Variance Analysis; Resolution; Validation of the Average Image;
- Theory of Correspondence Analysis; Classification
  - **Read chapters 3 and 4 from "Three-Dimensional Electron Microscopy of Macromolecular Assemblies".**

Week 10: Multivariate Data Analysis and Classification of Images II.

- Theory of Correspondence Analysis; Classification; Principal Component Analysis
  - **Read chapters 3 and 4 from "Three-Dimensional Electron Microscopy of Macromolecular Assemblies".**

Week 11: Three-Dimensional Reconstruction

- General Mathematical Principles; The Rationales of Data Collection: Reconstruction Schemes; Overview of Existing Reconstruction Techniques; Reference-Based Methods and Refinement; Contrast Transfer Function and Fourier Amplitude Correction; Resolution Assessment
  - **Read chapter 5 from "Three-Dimensional Electron Microscopy of Macromolecular Assemblies".**

Week 12: Symmetry and a-symmetry of macromolecular objects

- Symmetry, Rotation Matrices, Employing Symmetry in Reconstruction Process, Masking
  - **Read chapter 7 from "Three-Dimensional Electron Microscopy of Macromolecular Assemblies".**

Literature – 14 copies of each of the books are available from MUNI Campus library

- Jan Drenth. (2007) "**Principles of Protein X-Ray Crystallography**", 3rd edition; ISBN: 978-0-387-33334-2; JSpringer Science+Business Media, LLC, 233 Spring Street, New York, NY 10013, USA
- Joachim Frank. (2010) "**Three-Dimensional Electron Microscopy of Macromolecular Assemblies: Visualization of Biological Molecules in Their Native State**", 3rd edition; ISBN-13: 9780195182187; Oxford Scholarship Online

Learning suggestions

- Read the readings before the day for which they are assigned. This will help you to acquire knowledge gradually in small chunks.
- When reading summarize for yourself the main points from each paragraph. You can write these little summaries in the margins.
- Download the powerpoint slides and the audio recordings and use them for home-studying.
- Last but not least, you are very welcome to come to my office hours or to contact me if you have any questions.

Information for students with disabilities

Students with disabilities are encouraged to contact me if they have any special needs. Your privacy will be respected.

Course caveat:

In the event of a major campus emergency, course requirements, deadlines and grading percentages are subject to changes that may be necessitated by a revised semester calendar or other circumstances beyond the instructor's control.

Lecturing approach

The course will be presented as a series of lectures presented with the aid of PowerPoint slides.

Additional comments

The course is offered every second year.

Lectures take place every week unless stated otherwise.