Federal State Autonomous Educational Institution of Higher Education "Moscow Institute of Physics and Technology (National Research University)"

APPROVED
Head of Landau Phystech-School of Physics & Research
A.V. Rogachev

Work program of the course (training module)

course:	Electron Microscopy of Biological Objects/Электронная микроскопия биологических объектов
major:	Applied Mathematics and Physics
specialization:	General and Applied Physics/Общая и прикладная физика
	Landau Phystech-School of Physics & Research
	Chair of Biophysics
term:	1
qualification:	Master

Semester, form of interim assessment: 2 (spring) - Exam

Academic hours: 30 AH in total, including:
lectures: 15 AH.
seminars: 15 AH.
laboratory practical: 0 AH.

Independent work: 30 AH.

Exam preparation: 30 AH.

In total: 90 AH, credits in total: 2

Author of the program: I.V. Manukhov, doctor of biological sciences

The program was discussed at the Chair of Biophysics 01.02.2021

Annotation

This course will provide an overview of the methods used for structural characterization and analysis of protein and drug interactions. An overview of general principles of the organization of proteins and nucleic acids will also be given.

1. Study objective

Purpose of the course

the formation and improvement of the students' competencies in the field of modern ideas about the cryo-electron microscopy method - a modern and dynamically developing experimental method that is actively gaining popularity for research in the field of structural biology, which makes it possible to determine the structure of macromolecular complexes with a resolution close to atomic.

Tasks of the course

1) Acquaintance of students with the methods used for the structural characterization and analysis of interactions between proteins and drugs, an overview of general principles of organization of proteins and nucleic acids.

2) Acquaintance of students with fundamental principles underlying cryo-electron microscopy, including theoretical training:

a. principles of operation of electron microscopes and an overview of the modern world instrument infrastructure;

b. introduction to Fourier transforms and principles of image formation;

c. stages and features of sample preparation;

d. data collection strategies and basic image processing workflows for all three main methods of modern cryo-electron microscopy: tomography, single-particle analysis, and two-dimensional electron crystallography.

2. List of the planned results of the course (training module), correlated with the planned results of the mastering the educational program

Mastering the discipline is aimed at the formation of the following competencies:

Code and the name of the competence	Competency indicators
UC-1 Use a systematic approach to critically analyze a problem,and develop an action plan	UC-1.1 Systematically analyze the problem situation, identify its components and the relations between them
	UC-1.2 Search for solutions by using available sources
	UC-1.3 Develop a step-by-step strategy for achieving a goal, foresee the result of each step, evaluate the overall impact on the planned activity and its participants
UC-4 Use modern communication tools in the academic and professional fields, including those in a foreign language	UC-4.1 Exchange business information in oral and written forms in Russian and at least one foreign language
	UC-4.3 Present the results of academic and professional activities at various academic events, including international conferences
	UC-4.4 Use modern ICT tools for academic and professional collaboration
Gen.Pro.C-1 Gain fundamental scientific knowledge in the field of physical and mathematical sciences	Gen.Pro.C-1.1 Apply fundamental scientific knowledge in the field of physical and mathematical sciences
	Gen.Pro.C-1.2 Consolidate and critically assess professional experience and research findings
Gen.Pro.C-2 Acquire an understanding of current scientific and technological challenges in professional settings, and scientifically formulate professional objectives	Gen.Pro.C-2.1 Assess the current state of mathematical research within professional settings
	Gen.Pro.C-2.2 Assess the relevance and practical importance of research in professional settings
	Gen.Pro.C-2.3 Understand professional terminology used in modern scientific and technical literature and present scientific results in oral and written form within professional communication

Gen.Pro.C-3 Select and/or develop approaches to professional problem-solving with consideration to the limitations and specifics of different solution methods	Gen.Pro.C-3.1 Analyze problems, plan research strategy to achieve solution(s), propose, and combine solution approaches
	Gen.Pro.C-3.2 Employ research methods to solve new problems and apply knowledge from various fields of science (technology)
	Gen.Pro.C-3.3 Gain knowledge of analytical and computational methods of problem-solving, understand the limitations of the implementation of the obtained solutions in practice
Gen.Pro.C-4 Successfully perform a task, analyze the results, and present conclusions, apply knowledge and skills in the field of physical and mathematical sciences and ICTs	Gen.Pro.C-4.1 Apply ICT knowledge and skills to find and study scientific literature and use software products
	Gen.Pro.C-4.2 Apply knowledge in the field of physical and mathematical sciences to solve problems, make conclusions, and evaluate the obtained results
	Gen.Pro.C-4.3 Justify the chosen method of scientific research
Gen.Pro.C-5 Undertake professional training, achieve professional growth, and become a team leader in a professional sphere, tolerant of social, ethnic, religious, and cultural differences	Gen.Pro.C-5.1 Tolerate social, ethnic, religious, and cultural differences in teamwork
	Gen.Pro.C-5.2 Manage a small professional team
Pro.C-1 Assign, formalize, and solve tasks, develop and research mathematical models of the studied phenomena and processes, systematically analyze scientific problems and obtain new scientific results	Pro.C-1.1 Locate, analyze, and summarize information on current research findings within the subject area
	Pro.C-1.3 Apply theoretical and/or experimental research methods to a specific scientific task and interpret the obtained results
Pro.C-3 Use research and testing equipment (devices and installations, specialized software) in a selected subject field	Pro.C-3.1 Understand the operating principles of the equipment and specialized software
	Pro.C-3.2 Conduct an experiment (simulation) using research equipment (software)
	Pro.C-3.3 Evaluate the accuracy of the experimental (numerical) results

3. List of the planned results of the course (training module)

As a result of studying the course the student should:

know:

1) The principles of operation of cryo-electron microscopes, features and advantages of modern cryo-electron microscopes;
2) Features of the preparation and conduction of experiments on cryo-electron microscopy: heterogeneity of samples and problems associated with biological samples; transmembrane proteins and features of their study; tomography and analysis of individual particles; the use of electronic tomography to study the structure of proteins; analysis of helical structures;
3) Basics of 3D reconstruction. Modern software and algorithms for image analysis and 3D reconstruction (cisTEM, RELION, SPHIRE, cryoSPARC, etc.);
4) Databases EM. Validation of data and reconstructions obtained on their basis.

be able to:

1) Apply knowledge based on modern ideas about the method of cryo-electron microscopy to solve fundamental professional problems;
2) Creatively use in scientific activity knowledge about the principles of operation of electron microscopes and modern world instrumental infrastructure;
3) Highlight and systematize the main ideas in scientific texts;
4) Critically evaluate any incoming information, regardless of the source;
5) Generate new ideas and methodological solutions;
6) Carry out the design of their scientific activities;
7) Present your scientific results in oral reports.

master:

1) Methods of theoretical and experimental research;
2) Skills of search (including using information systems and databases), processing, analysis and systematization of information;
3) Skills of critical analysis and assessment of modern scientific achievements.

4. Content of the course (training module), structured by topics (sections), indicating the number of allocated academic hours and types of training sessions

4.1. The sections of the course (training module) and the complexity of the types of training sessions

№	Topic (section) of the course	Types of training sessions, including independent work			
		Lectures	Seminars	Laboratory practical	Independent work
1	Structure of proteins	2			2
2	Structure of nucleic acids	2			2
3	Drug-protein interactions	2			2
4	Introduction into drug fragment design and virtual screening	2			2
5	Main biophysical methods for the study of proteins	2			2
6	Sample preparation in EM	1	1		2
7	Electron microscope	1	1		2
8	Image formation	1	1		2
9	Single particle analysis	1	1		2
10	Tomography	1	1		2
11	Hands-on Seminars		10		10
AH in total		15	15		30
Exam preparation		30 AH.			
Total complexity		90 AH., credits in total 2			

4.2. Content of the course (training module), structured by topics (sections)

Semester: 2 (Spring)

1. Structure of proteins

Levels (primary, secondary, tertiary), of protein organization, kinds of proteins (soluble vs membrane), domains.

2. Structure of nucleic acids

Double helix, RNA vs DNA, sugar puckering, forms of DNA.

3. Drug-protein interactions

Affinity, specificity, thermodynamics of binding.

4. Introduction into drug fragment design and virtual screening

Drug development pipeline, lead discovery and validation, Lipinski rules, ADMET.

5. Main biophysical methods for the study of proteins

Short introduction and comparison of X-ray crystallography, NMR, cryo-electron microscopy, etc.

6. Sample preparation in EM

Types of grids, girds preparation, assembly, major problems and troubleshooting.

7. Electron microscope

Basic anatomy, guns, lenses, columns, detectors.

8. Image formation

Amplitudes and phases, contrast transfer function.

9. Single particle analysis

Pipeline and practical aspects.

10. Tomography

Pipeline and practical aspects.

11. Hands-on Seminars

Preparation of grids, negative staining
Working with the electron microscope
Data analysis and processing. Part 1
Data analysis and processing. Part 2
Model building and refinement

5. Description of the material and technical facilities that are necessary for the implementation of the educational process of the course (training module)

Cryo-electron microscopy platform: cryo-electron 300 kV microscope FEI Polara G2, equipment for efficient and reproducible sample preparation, including the FEI Vitrobot Mark IV vitrification system, computing power for the analysis and processing of electron microscopy data and subsequent modeling and refinement of structures.
Computing power for processing diffraction and spectroscopic measurement data. There are local computer clusters for computer simulation. The resources of the MIPT data center, which provides a shared virtual server (including dedicated servers and access to a shared computing cluster), are constantly available. There is access to a computing CPU cluster at the Center for Free Electronic Laser Science (CFEL, Hamburg, Germany) with 132 CPU cores, as well as a system of priority access to computations during the experiment.

6. List of the main and additional literature, that is necessary for the course (training module) mastering

Main literature
Methods in Enzymology, Volume 481

Additional literature

Electronic sources only:
doi.org/10.1042/BST20180267
doi: 10.3389/fmolb.2018.00074
doi:10.1002/1873-3468.12757
doi.org/10.1007/s00109-018-1640-y

7. List of web resources that are necessary for the course (training module) mastering

Not used

8. List of information technologies used for implementation of the educational process, including a list of software and information reference systems (if necessary)

When preparing and conducting lectures, the Internet is used.
In addition, Libre Office is used, as well as the Ink Scape graphics package.

9. Guidelines for students to master the course

A student studying the discipline must, on the one hand, master the general conceptual apparatus, and on the other hand, must learn to apply theoretical knowledge in practice.
As a result of studying the discipline, the student must know the basic definitions and concepts, be able to apply the knowledge gained to solve various problems.
Successful completion of the course requires:
- attendance of all classes provided for by the curriculum for the discipline;
- keeping a synopsis of classes;
- student's intense independent work.

Independent work includes:
- reading recommended literature;
- study of educational material, preparation of answers to questions intended for independent study;
- solving problems offered to students in the classroom;
- preparation for the performance of tasks of the intermediate certification.

An indicator of mastery of the material is the ability to answer questions on the topics of the discipline without a synopsis.
It is important to achieve an understanding of the material being studied, not its mechanical memorization. If a student finds it difficult to study certain topics, questions, he/she should seek advice from a teacher.
Intermediate control of students' knowledge is possible in the form of solving problems in accordance with the topic of classes.

SUPPLEMENT

Assessment funds for course (training module)

major:	Applied Mathematics and Physics
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term:	1
qualification:	Master

Semester, form of interim assessment: 2 (spring) - Exam

Author: I.V. Manukhov, doctor of biological sciences

1. Competencies formed during the process of studying the course

Code and the name of the competence	Competency indicators
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Pro.C-3 Use research and testing equipment	Pro.C-3.1 Understand the operating principles of the equipment and specialized software

Pro.C-3 Use research and testing equipment (devices and installations, specialized software) in a selected subject field	Pro.C-3.2 Conduct an experiment (simulation) using research equipment (software)
	Pro.C-3.3 Evaluate the accuracy of the experimental (numerical) results

2. Competency assessment indicators

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7) Present your scientific results in oral reports.

master:

1) Methods of theoretical and experimental research;
2) Skills of search (including using information systems and databases), processing, analysis and systematization of information;
3) Skills of critical analysis and assessment of modern scientific achievements.

3. List of typical control tasks used to evaluate knowledge and skills

Not provided.

4. Evaluation criteria

Checking questions:

Please describe the hydrophobic effect in the context of soluble and membrane proteins

Please explain why DNA and not RNA is used as the genetic storage material

Please describe the main steps in the development of a new drug

What are the main advantages / disadvantages of X-ray crystallography, NMR and cryo-EM for the structural analysis?

Why do scientists aim to create the drugs with very high affinities?

Checking tasks:

Please describe the basic anatomy of an electron microscope

What are the main bottlenecks of single particle analysis?

What is CTF? Why is it used in EM?

Describe main steps in data processing and analysis

Describe the principle of the negative staining

Examples of exam question papers:

Question paper 1.

1. Please describe the hydrophobic effect in the context of soluble and membrane proteins
2. Please describe the basic anatomy of an electron microscope Question paper 2.
1. Please explain why DNA and not RNA is used as the genetic storage material
2. What are the main bottlenecks of single particle analysis?

Question paper 3.

1. Please describe the main steps in the development of a new drug
2. What is CTF? Why is it used in EM?

Question paper 4.

1. What are the main advantages / disadvantages of X-ray crystallography, NMR and cryo-EM for the structural analysis?
2. Describe main steps in data processing and analysis

Question paper 5.

1. Why do scientists aim to create the drugs with very high affinities?
2. Describe the principle of the negative staining

Assessment “excellent (10)” is given to a student who has displayed comprehensive, systematic and deep knowledge of the educational program material, has independently performed all the tasks stipulated by the program, has deeply studied the basic and additional literature recommended by the program, has been actively working in the classroom, and understands the basic scientific concepts on studied discipline, who showed creativity and scientific approach in understanding and presenting educational program material, whose answer is characterized by using rich and adequate terms, and by the consistent and logical presentation of the material;

Assessment “excellent (9)” is given to a student who has displayed comprehensive, systematic knowledge of the educational program material, has independently performed all the tasks provided by the program, has deeply mastered the basic literature and is familiar with the additional literature recommended by the program, has been actively working in the classroom, has shown the systematic nature of knowledge on discipline sufficient for further study, as well as the ability to amplify it on one’s own, whose answer is distinguished by the accuracy of the terms used, and the presentation of the material in it is consistent and logical;

Assessment “excellent (8)” is given to a student who has displayed complete knowledge of the educational program material, does not allow significant inaccuracies in his answer, has independently performed all the tasks stipulated by the program, studied the basic literature recommended by the program, worked actively in the classroom, showed systematic character of his knowledge of the discipline, which is sufficient for further study, as well as the ability to amplify it on his own;

Assessment “good (7)” is given to a student who has displayed a sufficiently complete knowledge of the educational program material, does not allow significant inaccuracies in the answer, has independently performed all the tasks provided by the program, studied the basic literature recommended by the program, worked actively in the classroom, showed systematic character of his knowledge of the discipline, which is sufficient for further study, as well as the ability to amplify it on his own;

Assessment “good (6)” is given to a student who has displayed a sufficiently complete knowledge of the educational program material, does not allow significant inaccuracies in his answer, has independently carried out the main tasks stipulated by the program, studied the basic literature recommended by the program, showed systematic character of his knowledge of the discipline, which is sufficient for further study;

Assessment “good (5)” is given to a student who has displayed knowledge of the basic educational program material in the amount necessary for further study and future work in the profession, who while not being sufficiently active in the classroom, has nevertheless independently carried out the main tasks stipulated by the program, mastered the basic literature recommended by the program, made some errors in their implementation and in his answer during the test, but has the necessary knowledge for correcting these errors by himself;

Assessment “satisfactory (4)” is given to a student who has discovered knowledge of the basic educational program material in the amount necessary for further study and future work in the profession, who while not being sufficiently active in the classroom, has nevertheless independently carried out the main tasks stipulated by the program, learned the main literature but allowed some errors in their implementation and in his answer during the test, but has the necessary knowledge for correcting these errors under the guidance of a teacher;

Assessment “satisfactory (3)” is given to a student who has displayed knowledge of the basic educational program material in the amount necessary for further study and future work in the profession, not showed activity in the classroom, independently fulfilled the main tasks envisaged by the program, but allowed errors in their implementation and in the answer during the test, but possessing necessary knowledge for elimination under the guidance of the teacher of the most essential errors;

Assessment “unsatisfactory (2)” is given to a student who showed gaps in knowledge or lack of knowledge on a significant part of the basic educational program material, who has not performed independently the main tasks demanded by the program, made fundamental errors in the fulfillment of the tasks stipulated by the program, who is not able to continue his studies or start professional activities without additional training in the discipline in question;

Assessment “unsatisfactory (1)” is given to a student when there is no answer (refusal to answer), or when the submitted answer does not correspond at all to the essence of the questions contained in the task.

5. Methodological materials defining the procedures for the assessment of knowledge, skills, abilities and/or experience

The course is graded at an exam. The questioning starts with a random task assigned to each student and time given for completion of the task. No aids are allowed. The student then proceeds to a chat with the examiner, at which he/she presents his/her solution to the assigned task. The examiner then asks the student several questions that evenly cover the course content. A final grade is assigned based on the quality of answers and demonstrated level of understanding.