Academic Year/course: 2022/23

68456 - Molecular Biotechnology: instrumental techniques

Syllabus Information

Academic Year: 2022/23
Subject: 68456 - Molecular Biotechnology: instrumental techniques
Faculty / School: 100 - Facultad de Ciencias
Degree: 626 - Máster Universitario en Biofísica y Biotecnología Cuantitativa/Biophysics and Quantitative Biotechnology
ECTS: 6.0
Year: 01
Semester: Second semester
Subject Type: Optional
Module:

1. General information

1.1. Aims of the course

Students will learn about standard and advanced biophysical techniques (methodologies and protocols) employed in diverse tasks: biomolecular target characterization (e.g., protein structure-function relationship) and validation, screening validation and optimization, target engagement and hit confirmation, and drug optimization, among other issues. Special emphasis will be placed on the description of the basic concepts for each experimental technique, the advantages and disadvantages of each technique, and in appropriate data analysis procedures. The students will acquire specific capabilities and skills for a future career in Molecular Biophysics and Structural Biology within different fields, biomedicine and biotechnology (pharmaceutical, health, food, cosmetics, cleansing?).

These approaches and objectives are aligned with the following Sustainable Development Goals (SDGs) of the United Nations Agenda 2030 (https://www.un.org/sustainabledevelopment/es/), so that the acquisition of the learning outcomes of the subject provides training and competence to contribute to some extent to their achievement: Goal 3: Good Health and Well-being, Goal 4: Quality Education, Goal 6: Clean Water and Sanitation, Goal 7: Affordable and Clean Energy, Goal 9: Industry, Innovation and Infrastructure.

1.2. Context and importance of this course in the degree

This course will allow the students to learn about:
- Thermodynamics of macromolecular equilibria: conformational and binding equilibria.
- Basic and advanced concepts and methodologies in spectroscopy.
- Basic and advanced concepts and methodologies in calorimetry.
- Basic and advanced concepts and methodologies in mass spectrometry, surface plasmon resonance, and ultracentrifugation.
- Characterization of protein conformational landscape and interactions with ligands.
- Basic concepts on screening validation, target engagement and hit validation.
- Methodologies for drug optimization.
- Basic and advanced data analysis.

1.3. Recommendations to take this course

To benefit from this course, it is advisable to have experience on lab work on protein purification and isolation, as well as basic knowledge on protein structure. Basic knowledge on spectroscopic and calorimetric techniques, as well as basic data analysis (statistics and inference) is also desirable. Minimum English level is required (B2 or equivalent).

2. Learning goals

2.1. Competences

Basic and general competences:
CG01 ? Gather, order, critically analyze, interpret, and synthesize information from different types of sources, evaluating its reliability.
CG03 - Learn efficiently through autonomous study and acquire a significant degree of independence.
CG04 - Apply the knowledge acquired and solve problems in new or unfamiliar environments within broader (or multidisciplinary) contexts related to the area of ??study.
CG05 - Formulate, analyze, evaluate, and compare new or alternative solutions for different problems.
CG06 - Be able to work in multidisciplinary and international teams.
CG07 - Develop the capacity for criticism and self-criticism.
CG08 - Make decisions taking into account social, ethical and legal responsibilities.
CG09 - Be able to develop a project, participating in the stages of bibliographic search, planning of experiments, obtaining results, interpretation, and dissemination of the same.
CB6 - Possess and understand knowledge that provides a basis or opportunity to be original in the development and/or application of ideas, often in a research context.
CB7 - Apply the acquired knowledge and their ability to solve problems in new or unfamiliar environments within broader (or multidisciplinary) contexts related to their area of ??study.
CB8 - Integrate knowledge and face the complexity of formulating judgments based on information that, being incomplete or limited, includes reflections on social and ethical responsibilities linked to the application of their knowledge and judgments.
CB9 - Communicate the conclusions and the knowledge and ultimate reasons that support them to specialized and non-specialized audiences in a clear and unambiguous way.
CB10 ? Acquire the learning skills that allow to continue studying in a way that will be largely self-directed or autonomous.

Transversal competences:
CT01 - Properly manage the resources and time available for solving a problem or developing a project.
CT02 - Communicate own conclusions and the knowledge and ultimate reasons that support them - to specialized and non-specialized audiences in a clear and unambiguous way.
CT03 - Transmit information orally, written or graphically using appropriate presentation tools and with the limitations imposed by time or space.
CT04 - Communicate fluently in English (understanding scientific texts, writing reports, talks, colloquia ...

Specific competences acquired by the student:
CE01 - Understand the intimate relationship between the structure of a biomolecule (folding, stability, etc.) and its function (interaction, activity, etc.), within its physiological context and when it is used in other contexts for research or biotechnological purposes.
CE02 - Design target protein stabilization strategies and identify suitable protein engineering techniques to modify or combine pre-existing protein functions.
CE03 - Identify bioactive compounds by performing massive molecular binding screening assays: formulate basic equations and material balances characteristic of the ligand protein binding equilibria, perform coupling assays between a target and a library of ligands for the identification of substances with predetermined biological activities and adapt the assay to high throughput formats to screen large libraries of candidate molecules.

2.2. Learning goals

The structural and functional information derived from the different experimental techniques is relevant across different fields with broad application; not only in Structural Biology, but also in Biochemistry and Molecular and Cell Biology, with applications in Biomedicine and Biotechnology.

To know the application of different instrumental techniques of habitual use, mainly of a spectroscopic and biophysical nature, for the study of the relationship between the structure and function of biomolecules (e.g., antibodies, enzymes ...) in relevant fields of Biotechnological and Biomedical research.
Be able to select the most appropriate method for each particular case. Describe, quantify, analyze, integrate, critically evaluate the results obtained through the use of these techniques in biological terms and, consequently, make decisions.

2.3. Importance of learning goals

This is not a compulsory course within the master, but it will allow extending the scope and deepen on the knowledge covered by other courses.

3. Assessment (1st and 2nd call)

3.1. Assessment tasks (description of tasks, marking system and assessment criteria)

Problems and practical cases (30/100): The resolution of these exercises constitutes an individual or group work of the students. Students must submit a report at the end of each session following the guidelines and the presentation format that will be marked. The grades and the corrected exercises themselves will be made available to students for review. These types of controls are framed within the concept of continuous evaluation, which will allow monitoring of the learning process.
Written Test (60/100): The written test will be constituted by questions that require short answers (limited response tests) or that require a broad development of the subject (essay tests or free and open answer). The first will allow a broad sampling of the student's knowledge on the subject, and the second will allow assessing their ability to express themselves, to present and sustain arguments, and to make critical judgments. The written test will be based on the program of programmed learning activities.
Seminars (10/100): Preparation of report, presentation and public defense of a work on a subject related to the subject. The report will be done individually or in groups of 2 students. The work will be exposed and defended by each group of students in seminar-type sessions. The time available for the exhibition and defense of the topic during the seminar sessions will be 10-15 minutes. It will be assessed if the work follows a coherent structure and provides an appropriate bibliography. During the presentation the clarity and order in the exhibition will be assessed, and the maturity in the debate.

4. Methodology, learning tasks, syllabus and resources

4.1. Methodological overview

The methodology followed in this course is oriented towards the achievement of the learning objectives. The master classes will generally use computer screen projections (PowerPoint), including small animations, videos and off-line navigation. Blended learning methodologies will be used to exchange information with the student and to advice on the presentation of their individual work. Students are expected to participate actively in the class throughout the semester. Classroom materials will be available via Moodle. These include a repository of the lecture notes used in class, the course syllabus, as well as other course-specific learning materials.
The problems and cases will be distributed through the mixed learning platforms and after the theoretical presentation; they will be resolved and discussed in class. The blackboard will be used above all. The student will be instructed on how to design experiments, present data and results, and how to organize the discussion of them through the proposal of theoretical-practical cases. This part of the subject requires team work and individual student, as well as search and discussion of information, and resolution of specific problems. These activities will allow the student to acquire the necessary skills and abilities to analyze and solve experimental problems related to the techniques of the subject, design experiments (and/or applications) independently and describe, quantify, analyze and critically evaluate the results obtained.
The preparation of seminars and individual works will instruct students in the search for relevant information on the Internet, the use of databases, scientific bibliography and online applications. The use of original

scientific material will be encouraged by the students (scientific publications, patents) and their interpretation for the presentation of information to a specialized public and the general public. This activity will help students in the practice of knowing how to communicate conclusions -and the knowledge and ultimate reasons that sustain them- in a clear and unambiguous way.
The discussion of a relevant research or technological development topic that has shown significant progress in recent years in workshops and debates will allow students to express their opinions on the subject in question, as well as to propose alternatives to the solutions presented to them.
Further information regarding the course will be provided on the first day of class.

4.2. Learning tasks

This is a 6 ECTS course organized as follows:
? Lectures (24 hours).
? Problems and practical cases (22 hours).
? Assignments (2 hours).
? Debates and workshop (12 hours).
? Autonomous work (90 hours).
Teaching and assessment activities will be carried out, as long and as much as possible, "in person". Whenever, due to safety regulations related to the COVID19 crisis, this is not possible, "synchronous" activities (i.e., allowing real-time interactions among teachers and students, even by using videoconferencing tools) will be preferred and fostered over "asynchronous" ones.

4.3. Syllabus

The course will address the following topics:
1. Proteins: Conformation and function. Macromolecular ensembles. Conformational and functional landscape. Basic chemical equilibrium concepts: conformation and binding. Statistical physics formalism: macromolecular microstates and macrostates. Linkage relationships.
2. UV-Visible absorption spectroscopy: instrumentation and applications. Chromophores in biomolecules. Determination of concentrations, kinetic parameters, and reduction potentials. Differential spectroscopy: equilibrium of complex formation and ligand binding. Stability and folding, and denaturation curves of biomolecules. Absorption spectroscopy in quality control, Biotechnology and Biomedicine.
3. Circular dichroism spectroscopy. Chromophores in biomolecules. Identification of different components in CD spectra of biological samples; allocation of secondary structures. Characterization of the folded state of proteins. Stability and quality control of biomolecules.
4. Emission spectroscopy. Fluorophores in biomolecules. Fluorescence anisotropy. Förster resonance energy transfer (FRET). Fluorescence correlation spectroscopy (FCS). Single particle fluorescence techniques. Identification of different components in spectra of biological samples. Calculation of reaction rates and interaction constants. Denaturation curves and characterization of the folded state of proteins. Fluorescent probes in the study of ligand binding and conformational changes of biological structures. Fluorescent proteins. Image techniques. Fluorescence in quality control, Biotechnology and Biomedicine.
5. Spectroscopy and fast kinetic techniques. Kinetic spectrometry induced by laser pulse and rapid mixing with stopped flow. Pre-stationary state: kinetic and interaction parameters.
6. Dispersion of light. Static light scattering. Dynamic light scattering. Polydispersion Determination of hydrodynamic radius, radius of gyration and molecular weight from measurements of light scattering. Detection of molecular aggregates. Study of the assembly and/or aggregation of macromolecules. Use of light scattering techniques in the biotechnology industry.
7. Differential scanning calorimetry (DSC). Protein stability and foundations of DSC. Deployment of proteins according to processes of two or more states. Determination of thermodynamic stability indexes in proteins. Determination of interaction affinity through the stabilizing effect of a ligand. Dependence on protein stability with respect to intrinsic and extrinsic factors. Use of DSC in formulation and quality control in the biotechnology industry.
8. Isothermal titration calorimetry (ITC). Interactions in proteins and foundations of ITC. Determination of interaction parameters in simple proteins (single binding site) and complex proteins (more than 1 binding site).

Cooperative interaction phenomena: homotropic and heterotropic interactions. Dependence of the interaction parameters with respect to intrinsic and extrinsic factors. Use of ITC in formulation and quality control in the biotechnology industry. Use of ITC in the development of bioactive molecules.
9. Mass spectrometry. Mass spectrometers and their components: ionization sources and analyzers. Mass spectrometry in tandem MS/MS. Interpretation of mass spectra. Applications in proteomics and metabolomics. Image mass spectrometry.
10. Optical biosensors based on surface plasmon resonance (SPR) phenomenon. SPR phenomenon and system components. Interaction of biomolecules in real time. Design of a SPR experiment: preparation and selection of the surface of the biosensor, strategies for immobilization of the ligand, injection of the sample, obtaining the sensorgram, regeneration of the interaction surface. Applications: specificity studies, calculation of concentrations, interaction kinetics and affinity studies.
11. Equipment for specific tasks. Development of instrumental equipment dedicated to specific tasks.

Laboratory sessions
Session 1. Protein unfolding: denaturation process followed by spectroscopy and calorimetry.
Session 2. Protein-ligand interaction: spectroscopy and calorimetry.
Session 3. Ligand-induced protein stabilization: Thermal-shift assay through spectroscopy and calorimetry.

4.4. Course planning and calendar

Further information concerning the timetable, classroom, office hours, assessment dates and other details regarding this course will be provided on the first day of class or please refer to the "Facultad de Ciencias? website.
This course will take place during the second semester.
Lab practical classes, written jobs, oral presentations and written tests will be announced and planned after each set of theoretical lessons.

4.5. Bibliography and recommended resources

http://psfunizar10.unizar.es/br13/egAsignaturas.php?codigo=68456