Curso Académico: 2023/24

68456 - Técnicas Instrumentales en Biotecnología Molecular

Información del Plan Docente

Año académico: 2023/24 Asignatura: 68456 - Técnicas Instrumentales en Biotecnología Molecular Centro académico: 100 - Facultad de Ciencias Titulación: 626 - Máster Universitario en Biofísica y Biotecnología Cuantitativa / Master in Biophysics and Quantitative Biotechnology Créditos: 6.0 **Curso: 01** Periodo de impartición: Segundo semestre Clase de asignatura: Optativa Materia:

1. Información básica de la asignatura

The objectives of this 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 common mathematical foundations underlying all techniques and 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 in biomedicine and biotechnology (pharmaceutical, health, food, cosmetics, cleansing...). 1 - Possess and understand knowledge that provides a basis or opportunity to be original in the development and / or

application of ideas, often in a context of research

2 - Students should be able to apply acquired knowledge and problem-solving skills in environments new or little known within broader (or multidisciplinary) contexts related to their area of study
3 - Students are able to integrate knowledge and confront the complexity of making judgments from information that, incomplete or limited, includes reflections on the social and ethical responsibilities associated with the application of their knowledge and judgments

4 - That the students know how to communicate their conclusions and the last knowledge and reasons that support them to specialized public and non-specialized services in a clear and unambiguous manner

5 - Students have the learning skills that allow them to continue studying in a way that will be largely self-directed or autonomous.

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 8: DECENT WORK AND ECONOMIC GROWTH. GOAL 9: INDUSTRY, INNOVATION AND INFRASTRUCTURE.

2. Resultados de aprendizaje

Students will achieve the following results:

1 - Order, analyze critically, interpret and synthesize information

2 - Obtain information from different types of sources and evaluate their reliability

3 - Learn efficiently through autonomous study and acquire a significant degree of independence

4 - Apply acquired knowledge and solve problems in new or unfamiliar environments within broader (or multidisciplinary) contexts related to the area of study

5 - Formulate, analyze, evaluate and compare new or alternative solutions to different problems

6 - Be able to work in multidisciplinary and international teams

7 - Develop capacity for criticism and self-criticism

8 - Make decisions taking into account social, ethical and legal responsibilities

9 - Be able to develop a project, participating in the stages of bibliographic search, experiment planning, obtaining results, interpreting, and disseminating them

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.

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.

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.

3. Programa de la asignatura

The course will address the following topics:

Theory sessions (30 hours of lectures):

1. Biological Macromolecules

- 1.1. Conformation and function.
- 1.2. Macromolecular ensembles. Conformational and functional landscape.
- 1.3. Statistical physics formalism: macromolecular microstates and macrostates.
- 1.4. Basic chemical equilibrium concepts: conformation and binding.
- 2. UV-Visible absorption spectroscopy.
 - 2.1. Chromophores in biomolecules.
 - 2.2. Determination of concentrations, kinetic parameters, and reduction potentials.
 - 2.3. Differential spectroscopy: equilibrium of complex formation and ligand binding.
 - 2.4. Stability and folding, and denaturation curves of biomolecules.
 - 2.5. Absorption spectroscopy in quality control, Biotechnology and Biomedicine.

3. Circular dichroism spectroscopy.

- 3.1. Chromophores in biomolecules. Quirality.
- 3.2. Identification of different components in CD spectra of biological samples.
- 3.3. Assessment of secondary structures.
- 3.4. Characterization of the folded state of proteins.
- 3.5. Stability and quality control of biomolecules.

4. Emission spectroscopy.

- 4.1. Fluorophores in biomolecules.
- 4.2. Fluorescence emission.
- 4.3. Fluorescence anisotropy.
- 4.4. Förster resonance energy transfer (FRET).
- 4.5. Fluorescence correlation spectroscopy (FCS).
- 4.6. Single particle fluorescence techniques.
- 4.7. Identification of different components in spectra of biological samples.
- 4.8. Calculation of reaction rates and interaction constants.
- 4.9. Protein stability and characterization of the folded state of proteins.

4.10. Fluorescent probes in the study of ligand binding and conformational changes of biological structures.

- 4.11. Fluorescent proteins.
- 4.12. Image techniques.

4.13. Fluorescence in guality control, Biotechnology and Biomedicine.

5. Spectroscopy and fast kinetic techniques.

- 5.1. Kinetic spectrometry induced by laser pulse and rapid mixing with stopped flow.
- 5.2. Pre-stationary state: kinetic and interaction parameters.

6. Light scattering.

- 6.1. Static light scattering.
- 6.2. Dynamic light scattering.
- 6.3. Polydispersion.
- 6.4. Determination of hydrodynamic radius, radius of gyration and molecular weight.

6.5. 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.

- 7.1. Protein stability and foundations of DSC.
- 7.2. Assessment of thermodynamic stability in proteins.
- 7.3. Protein unfolding analysis.
- 7.4. Determination of interaction affinity through the stabilizing effect of a ligand.
- 7.5. Dependence on protein stability with respect to intrinsic and extrinsic factors.
- 7.6. Use of DSC in formulation and quality control in the biotechnology industry.

8. Isothermal titration calorimetry.

- 8.1. Interactions in proteins and foundations of ITC.
- 8.2. Determination of interaction parameters in simple and complex proteins.
- 8.3. Cooperative interaction phenomena: homotropic and heterotropic interactions.
- 8.4. Dependence of the interaction parameters with respect to intrinsic and extrinsic factors.
- 8.5. Use of ITC in formulation and quality control in the biotechnology industry.
- 8.6. Use of ITC in the development of bioactive molecules.

9. Mass spectrometry.

- 9.1. Mass spectrometers and their components: ionization sources and analyzers.
- 9.2. Mass spectrometry in tandem MS/MS.
- 9.3. Interpretation of mass spectra.
- 9.4. Applications in proteomics and metabolomics, and quality control.
- 9.5. Image mass spectrometry.

10. Optical biosensors based on surface plasmon resonance.

- 10.1. SPR phenomenon and system instrumental components.
- 10.2. Interaction of biomolecules in real time.
- 10.3. Experimental design: practical and technical aspects.
- 10.4. Applications: specificity studies, calculation of concentrations, interaction kinetics and affinity studies.

Practical sessions (12 hours of practical classes):

- Session 1. Spectroscopic techniques.
- Session 2. Calorimetric techniques.
- Session 3. Interpretation and data analysis.
- Session 4. Interpretation and data analysis.

Problems sessions (12 hours of practical classes):

There will be 2-hour sessions for discussing problems and exercises.

Seminar sessions (6 hours of student's presentations):

Students will explain a subject related to the subject topic.

4. Actividades académicas

The activities offered to the student to help him/her achieve the expected results are:

- This is a 6 ECTS course organized as follows: Lectures (30 hours, 1.2 ECTS). Problems and practical cases (24 hours, 1 ECTS).
- Debates and workshop (6 hours, 0.24 ECTS).
- Autonomous work (90 hours, 3.6 ECTS).

5. Sistema de evaluación

Student must demonstrate that he/she has achieved the expected learning outcomes by means of the following assessment activities:

Problems and Practical Classes (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 vill 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 course. 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.