VNIVERSITAT DE VALÈNCIA

Course Guide
33132 Macromolecular structure and enzymology

COURSE DATA

Data Subject	
Code	33132
Name	Macromolecular structure and enzymology
Cycle	Grade
ECTS Credits	7.5
Academic year	2024 - 2025

Study (s)

Degree	Center	Acad. year	Period
1109 - Degree in Biochemistry and Biomedical Sciences	Faculty of Biological Sciences	2	First term

Subject-matter

Degree	Subject-matter	Character
1109 - Degree in Biochemistry and Biomedical Sciences	8 - Bioquímica	Obligatory

Coordination

Name	Department
GOMAR ALBA, MERCE	30 - Biochemistry and Molecular Biology
SALGADO BENITO, JESUS	30 - Biochemistry and Molecular Biology
SANCHEZ DEL PINO, MANUEL MATEO	30 - Biochemistry and Molecular Biology

SUMMARY

Structure of macromolecules and enzymology is a compulsory subject of the second year of the degree in Biochemistry and Biomedical Sciences, to which correspond 7.5 ECTS credits taught in the first semester. This subject will allow to acquire knowledge about the characteristics of the structure of the main biological macromolecules, their stability and their specific interactions with each other and with other molecules, in order to reach understanding of structure-function relationships. Also, the student will address the study of biological catalysis, the mechanisms of enzymatic reactions, their kinetics and their regulation at the molecular level, including deduction and application of quantitative models used for the characterization of enzymatic reactions.

PREVIOUS KNOWLEDGE

Relationship to other subjects of the same degree

There are no specified enrollment restrictions with other subjects of the curriculum.

Other requirements

COMPETENCES (RD 1393/2007) // LEARNING OUTCOMES (RD 822/2021)

1101 - Degree in Biochemistry and Biomedical Sciences

- Conocer los principios de la estructura de las macromoléculas biológicas DNA, RNA y Proteínas, y de las fuerzas que las estabilizan.
- Relacionar la estructura de las macromoléculas con su función.
- Conocer las interacciones que se establecen entre diferentes tipos de macromoléculas biológicas.
- Conocer los mecanismos de las reacciones enzimáticas. Cinética enzimática.
- Conocer los principios de activación e inhibición enzimática: efectos alostéricos y cooperativos.
- Conocimiento y aplicación de los métodos experimentales y tecnología de enzimas. Análisis enzimático.
- Aplicación de los conocimientos sobre estructura tridimensional de proteínas al estudio de la función de máquinas moleculares transductoras de energía.

LEARNING OUTCOMES (RD 1393/2007) // NO CONTENT (RD 822/2021)

This subject aims to achieve the acquisition of concepts, skills or knowledge related to:

1. Chemical and physical principles that determine the conformation of macromolecules.
2. Molecular interactions that determine the properties and dynamics of the complexes that macromolecules form with each other or with small ligands.
3. Current models on folding mechanisms.
4. Methodologies that are used for the structural analysis of biological macromolecules.
5. Databases and computer programs used for the structural analysis of macromolecules.
6. The concept of enzyme and general characteristics of enzyme molecules.
7. Biological, medical and industrial importance of enzymes.
8. Quantitative study of the kinetics of reactions catalyzed by enzymes.
9. Mechanisms that underlie the enzymatic activity in general and understanding of some specific mechanisms of enzyme action.
10. Mechanisms of enzymatic regulation, especially in connection to reversible binding of ligands and through the phenomena of cooperativity and alosterism.
11. Common procedures used by scientists in the area of molecular biosciences to generate, and disseminate scientific information.
12. Experimental approaches and their limitations as well as the keys for the correct interpretation of

scientific results.

DESCRIPTION OF CONTENTS

1. Introduction

Structural Biology: relationship with other sciences. Working methods in Structural Biology. Physical interactions that determine the properties of Biomacromolecules.

2. Protein Structure (1)

Revision of the structure and classification of amino acids: hydrophobicity scales. The peptide bond. Properties of the peptide bond. Structural levels of proteins. Conformational restrictions of the peptide chain. Representation of Ramachandran. Secondary structure: alpha helix, beta sheet and turns. Determination of secondary structure. Prediction of secondary structures.

3. Protein Structure (II)

Supersecundary structures: reasons. Structural domains. Tertiary and quaternary structure of proteins: interactions. Structural classification of proteins. Fibrous proteins: alpha keratin, collagen and fibroin. Determination of structures by X-ray diffraction, nuclear magnetic resonance and electron microscopy. Prediction of tertiary structures.

4. Protein conformational stability

Concept of in vitro folding: hydrophobic effect. Native and denatured state. Thermodynamic stability of proteins. Folding of proteins. Transition states and intermediaries. Folding of proteins in vivo: molecular chaaperons and biogenesis. Intrinsically disordered proteins.

5. Wrong protein folding: amyloid fibers

Aggregation of proteins. Properties of amyloid fibers. Pathologies related to the defective folding of proteins: Alzheimer's, Parkinson's and spongiform encephalopathies (Prions).

6. Macromolecular complexes

Description. Cylindrical couplings: the proteasome and exosome RNA. The nuclear pore complex. Protein filaments: actin. Ribonucleoproteic complexes: the ribosome, the spliceosome and others.

7. Research on proteins and proteomes

Protein purification. Recombinant proteins: purification labels and heterologous expression systems. Proteomics: protein separation, identification and quantification.

8. Polysaccharides

Polysaccharides of biological interest. Structures of reserve polysaccharides. Polysaccharides with structural paper: cellulose, chitin, peptidoglycan and glycosaminoglycans. Glicoconjugats: proteoglicans, glycoproteins, glycolipids and lipopolisacàrids. Molecular recognition and cellular communication: the sugar code and the role of lectins. Glycomics.

9. Biological membranes

Membrane lipids. Lipid organization in aqueous solution: membrane dynamics, lipid movement and membrane structure. Folding and biosynthesis of membrane proteins. Structural examples: proton pumps, transporters, channels and GPCRs.

10. Chemical Composition of Nucleic Acids

Nitrogenous bases, nucleosides and nucleotides. Properties. Phosphodiester bonds. Pairings between nitrogenous bases: canonical and others. Other functions of nucleotides. (2 hours)

11. DNA secondary structure

Watson and Crick model of the double helix. Detailed DNA conformation and sequence dependency. DNA structural variability. Other types of double helix: DNA B, DNA A, DNA Z, DNA H, DNA G. Deformability and curvature. Triple helices: types. DNA denaturation and renaturation.(3,5 hours)

12. DNA topology

Topological properties of DNA: supercoiling: Concepts of binding index, twist and writhing. Constrained and unconstrained supercoiling. Topoisomerases. (2,5 hours).

13. DNA compaction in genomes

Organization of the bacterial chromosome. DNA packaging in eukaryotes: chromatin. Histones. Chromatin nucleosomal structure. Histone posttranslational modifications. Higher levels of organization. artificial DNAs. (2,5 hours)

14. The structure of RNAs

Types of RNA. Secondary and tertiary structure of RNAs. Prediction of the secondary structure. Riboswitches. Ribozymes. Aggregations in phase of liquid drops.(1,5 hours).

15. Interactions between nucleic acids and proteins

Protein-nucleic acid interactions. Surfaces and forces of interaction. Union to the major groove and the minor groove. Examples: Zn fingers, HTH domains, the TBP. Binding to DNA-Z. RNA binding proteins. (2,5 hours)

16. Protein-Ligand interactions

PL complexes. Ligand binding sites. Saturation isotherms of PL complexes (fraction and saturation functions). Cooperativity. Hill function. Graphic analysis (direct and linearized representations). Alosterism. Models of cooperativity / alosterism. Example of an allosteric system (hemoglobin): Molecular origin and graphical explanation. Allosteric effectors and their physiological role. Molecular variants of hemoglobin.

17. Enzymatic catalysis

Brief review of chemical kinetics for reversible reactions: Thermodynamics and kinetics. Rate constant. Reaction order. Integrated rate equations. The Arrhenius equation. Activation energy and its meaning. Transition state theory. Energy profiles. Reaction intermediaries. Catalysis. Biomolecular catalysis: Enzymes and ribozymes. The transition state hypothesis in enzymes. Active center. Models of the enzyme-substrate complex. Pauling and Wolfenden hypothesis. Types of enzymatic catalysis. Enthalpic and entropic contributions. Enzymatic cofactors. Classification of enzymes. Reaction mechanisms: Examples of peptidase enzymes and carbonic anhydrase.

18. Kinetics of monosubstrate reactions

Rate in successive reactions. Effective stages of the enzymatic reaction. Approximations necessary for the resolution of enzymatic velocity equations (concepts of pre-equilibrium and steady state). The Michaelis-Menten model: Detailed deductions using different approaches and with one or two intermediaries. Linearizations. Kinetic parameters and their meaning. Turnover number, transit time and the specificity constant. Kinetic perfection of enzymes. Experimental study of enzymatic activity. Obtaining and representing the Michaelis function. Use of coupled reactions. Effects of temperature and pH. Applications for the study of enzymatic activities.

19. Enzyme inhibition

Concept and types of inhibitors. Types of reversible inhibition and its quantitative study through graphic analysis. Special cases of reversible inhibition: Alternative substrates, inhibition by substrate and inhibition by product. Applications of enzymatic inhibition. Inhibition by chemical modification (group reagents). Penicillin. Inhibition of metalloproteases by coordination ligands. Suicide inhibitors.

20. Kinetics of multisubstrate reactions

Types and examples of multi-substrate reaction. Sequential mechanisms. Condition for a "random" mechanism. Examples of random reaction and ordered reactions. Double displacement reactions (examples). Quantitative study of bisubstrate reactions: Michaelis-Menten equation for the bisubstrate reaction (deduction and significance of kinetic parameters). Elucidation of the bisubstrate mechanism type through graphic analysis.

21. Molecular mechanisms of enzyme regulation

Types of regulation mechanisms. Regulation through the concentration of the substrate. Specificity by alternative substrates. Preference by enzyme (alternative routes). Regulation through the concentration of enzyme. Isoenzymes. Regulation by irreversible covalent modification: Cascades of digestion and coagulation of blood. Regulation by reversible covalent modification: Phosphorylation / dephosphorylation. Allosteric control. Examples of allosteric enzymes. Control mechanisms of metabolic pathways. Multienzyme complexes.

22. Practicals

1. Representation and Analysis of Protein Structures.

Databases of Structural Information. Structural data files and their representation. Representation and Analysis of Macromoleculecular Structures. Example: Analysis of the Structure of Myoglobin (study of structural properties and characteristics of a P-L complex).

2. Graphical Analysis for Studies of PL Complexes and Enzyme Kinetics.

Simulation / representation of mathematical models of PL and ES complexes. Resolution of enzyme problems with the help of a spreadsheet.

WORKLOAD

ACTIVITY	Hours	% To be attended
Theory classes	63,00	100
Classroom practices	8,00	100
Computer classroom practice	4,00	100
Development of group work	15,00	0
Study and independent work	30,00	0
Readings supplementary material	15,00	0
Preparation of evaluation activities	15,00	0
Preparing lectures	25,00	0
Preparation of practical classes and problem	2,00	0
Resolution of case studies	10,00	0
TOTAL	**187,00**	

TEACHING METHODOLOGY

The subject is structured in:
Theory class: In total 58 theory lectures, one hour each, will be given. The professor will present the most relevant contents of the subject, using audiovisual media. The documents used for projection during the classes will be made accessible in advance though the Virtual Classroom teaching platform.

Classes for problems and questions: 8 sessions, one hour each will be given, distributed along the course in between the theory classes. Usually, at the end of each of the sections of the syllabus. In these sessions the concepts presented in the theoretical sessions will be reinforced and the active participation of the students will be stimulated through the resolution of questions and problems. The lecturer will prepare a series of questions for each subject or thematic block, which will allow working individually (through personal preparation) and collectively (through the presentation and discussion of the by a group of students). In some cases, the teacher may request the delivery of some of the resolved questions, prior to particular sessions. The delivery will be made in electronic format through Virtual Classroom.

Practicals made in computer classrooms: The practicals will be performed in the computer rooms of the Faculty of Biology. It will be compulsory to attend this type of lectures. These will be completed in 2 sessions, counting a total of 4 hours.

Seminars: This activity will be organized jointly with the other subjects of the second year of the degree. The activity will consist in the preparation and presentation of a seminar with a duration of approximately 30 minutes by the students (in groups of two) and in their active participation in the discussion of the seminars. Students will complete the preparation and presentation of the seminar only once during the course. Approximately 6 of seminars of each course will have a direct connection with "Structure of macromolecules and enzymology" and will be supervised by professors of this subject. The seminar activities will be mandatory.

EVALUATION

Exams, for each of the 3 parts of the subject (Proteins, Nucleic Acids and Enzymology), will be made. On the other hand, the assessment of the practicals will depend on the grade obtained from the development of the activities carried out in Computer Classroom, for which it will be necessary to complete and give to the teacher a results form. The note corresponding to the Seminar will arise from the evaluation of its preparation and presentation.

The final grade of the subject will be obtained by adding to the exam score (a maximum of 9 pts out of a total of 10), the note from the activities in the computer room (up to 0.5 points) and the seminar grade (up to 0.5 points).

To pass the subject, each of the following four conditions must be fulfilled simultaneously (either in the first or in the second call):

1. You must attend the practical computer classes and prepare the corresponding results report, getting a note of at least 0.25 points.
2. You must have participated in the seminar activity, getting a grade of at least 0.25 points.
3. The overall mark of the theory exam (sum of the 3 parts) must be at least 4.5 points (half of its total value).
4. Each of the parts of the subject (Proteins, Nucleic Acids and Enzymology) must exceed 35% of its maximum value.

If any of the above conditions is not met, the subject will not be passed. In such a case, if it is a first call, the note of any of the theory parts may be maintained for the second call, but only if said parts have individually exceeded half of their maximum value. In any case, this option is only possible within the same academic year.

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