Universitat de Lleida

DEGREE CURRICULUM

PROTEOMICS AND PROTEIN ENGINEERING

Coordination: VILLORBINA NOGUERA, GEMMA

Academic year 2023-24

PROTEOMICS AND PROTEIN ENGINEERING 2023-24

Subject's general information

Subject name	PROTEOMICS AND PROTEIN ENGINEERING
Code	101618
Semester	ANUAL CONTINUED EVALUATION
Typology	

Degree	Course	Character	Modality
Bachelor's Degree in Biotechnology	3	COMPULSORY	Attendance-based

Course number of credits (ECTS): 10.5

Type of activity, credits, and groups

Activity type	PRALAB			PRAULA		TEORIA
Number of credits	1.7	0.3	0.7	0.4	0.4	7
Number of groups	4	8	3	2	1	1

Coordination	VILLORBINA NOGUERA, GEMMA
Department	ENVIRONMENT AND SOIL SCIENCES AND CHEMISTRY
Teaching load distribution between lectures and independent student work	105 contact hours 203 hours of student work
Important information on data processing	Consult this link for more information.
Language	Catalan English, slides and extra documentation
Distribution of credits	0.47 Master lesson 1.45 Practical cases 0.70 Seminars 1.67 Laboratory practices 0.21 Informatics practices

PROTEOMICS AND PROTEIN ENGINEERING 2023-24

Teaching staff	E-mail addresses	Credits taught by teacher	Office and hour of attention
LARA AYALA, ISABEL	isabel.lara@udl.cat	3,1	
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VILLORBINA NOGUERA, GEMMA	gemma.villorbina@udl.cat	7,4	

Subject's extra information

It would be convenient to have passed the following subjects:

101600 General and Organic Chemistry

101607 Biochemistry

101617 Instrumental Techniques

PERSONAL PROTECTIVE EQUIPMENT (PPE) for the practical sessions

It is **MANDATORY** that students have the following personal protective equipments (PPE) in the course of teaching practices.

Laboratory coat UdL unisex
Safety glasses
Chemical protection gloves

The PPE can be purchased at UdL's ÚDELS store

Center for Cultures and Cross-Border Cooperation - Cappont Campus

Carrer de Jaume II, 67 low

25001 Lleida

http://www.publicacions.udl.cat/

For more information, check the product listings: http://www.biotecnologia.udl.cat/en/pla-formatiu/equipament.html

For other protection equipment (for example, caps, respiratory masks, etc.), they will depend on the type of practice to be performed. In this case, the responsible professor will inform if the use of these specific PPE is necessary.

Not carrying the PPE described or not complying with the general security regulations detailed below will mean that the student can not access the laboratories or have to leave the same.

GENERAL SAFETY RULES IN LABORATORY PRACTICES

- Maintain the place of performance of clean and tidy practices. The work table must be free of backpacks, folders, coats ...
- In the laboratory you can not come with shorts or short skirts.
- Bring closed and covered shoes during the performance of the practices.

- Bring long hair always collected
- Keep the cords fit to protect against spills and spills of chemical substances.
- Do not carry wide bracelets, pendants or sleeves that can be trapped by the equipment, assemblies ...
- Avoid wearing contact lenses, since the effect of chemicals is much greater if they are introduced between the contact lens and the cornea.
- Do not eat or drink in the laboratory
- Smoking is prohibited within laboratories
- Wash your hands whenever you have contact with a chemical and before leaving the laboratory.
- Follow the teacher's instructions and consult any questions about security

Learning objectives

Knowledge objectives

The student that exceeds the subject must:

It is intended that they learn the following knowledge to pass the subject:

1. Structural-function proteins relations
2. Methods of structural characterization of proteins
3. Main routes to obtain and modify proteins
4. Basic biochemical concepts

Capacity objectives

The student that exceeds the subject must be able to:

1. Solve problems related to the synthesis of proteins
2. Understand and discuss scientific articles related to proteins
3. Determine the three-dimensional structure of the proteins
4. Use the existing computer tools for structural study of proteins
5. Design new proteins and implement them to search new solutions

Competences

The graduate in Biotechnology have to:

General skills

- GC 1 Be able to selectively search for and use sources of information necessary to achieve the training objectives.
- GC 4 Knowing and adequately use the scientific and technical vocabulary of the different areas of Biotechnology.
- GC 5 Work in the laboratory applying criteria of quality and good practice.
- GC 7 Use the scientific method to analyze data and design experimental strategies with biotechnological applications.
- GC 11 Acquire criteria for choosing the most appropriate analytical techniques for each specific practical case.

Specific skills (according to the Plan of Studies document)

- CE 26 Be able to use experimental techniques for molecular, cellular and physiological analysis.
- CE 27 To know how to apply techniques for the analysis of molecular structures and for the detection and quantification of metabolites and macromolecules.
- CE 28 To know how to apply the techniques of omic analysis and interpretation of the results.
- CE 34 Be able to design the protocol of a specific biotechnological process with the necessary practical requirements to carry it out and its evaluation parameters.
- CE 35 To know how a biotechnology laboratory works and be able to work in it.

Subject contents

THEORETICAL CONTENTS:

Chapter 1. The peptide bond and polypeptide sequence (5h)

Proteins, peptides and their functions to beings alive. Stereochemistry of the peptide bond. Natural peptides. Chemical reactivity of peptides. Structural and functional implications of the polypeptide sequence. Determination of the protein sequence. Chemical synthesis of peptides; combinatorial libraries

Chapter 2. Proteins structuring levels. The three-dimensional proteins structure (5h)

Main types of secondary structures: amino acids that participate. Supersecondary structures and structural motifs. Domains. Tertiary structure. The quaternary structure: protomers and subunits. Advantages of quaternary structures adoption. Factors that govern the quaternary structure. Relative arrangement of protomers in space. Structure-function relations in some oligomeric forms.

Chapter 3. Structure-function of proteins. Examples (4h)

Enzymatic proteins: chymotrypsin, lysozyme, carboxypeptidase. Proteins that bind to nucleic acids: α-turn-α motif, zinc fingers, leucine zipper. Molecular motors: myosin and actin; kinases, dineins. Membrane proteins. Structure of immunoglobulins.

Chapter 4. Post-translation functional modifications (3h)

Types of post-translation modifications and functional implications. Transport and association. Proteolysis limited: pre-proteins, zymogens. Cascading activation. Some systems regulated by limited proteolysis: blood clotting, digestive proenzymes. Evolution of zymogens. Modifications by phosphorylation, acetylation, glycosylation. Modifications for oxidative damage. Degradation and protein replacement in vivo: ubiquitination. Structure and function of the proteosoma.

Chapter 5. Folding and conformational dynamics (4h)

Protein denaturation; kinetic and energetic bases of transconformation and denaturation; folding *in vitro*. Fluctuations, flexibility and conformational dynamics in native proteins. Protein molecular dynamics. Protein folding *in vivo*: molecular chaperones. Conformational pathologies

Chapter 6. Protein-ligand interaction (1h)

Forces affecting protein-ligand association. Methods to study the interaction. Design of drugs based on the structure.

Chapter 7. Purification and characterization of proteins (8h)

Previous goals and considerations. Quantification methods. Stages and monitoring of the purification process. Fractionation and separation: usable properties and general methods. Characterization of the purified protein. Immunological methods: obtaining and using polyclonal and monoclonal antibodies. Immunoblot, immunoprecipitation, immunoassays, immunotherapy.

Chapter 8. Determination of the three-dimensional proteins structure (6h)

Methodologies used for structural characterization of proteins. Analysis in films and in dissolution: IR, DC, RPE, CDE. Analysis in crystals: X-ray and ME. NMR spectroscopy.

Chapter 9. Artificial production of proteins (5h)

Expression systems and their control. Expression vectors and hosts usable for the recombinant protein production. Inclusion bodies. Fusion proteins and affinity tags. Maximization of expression levels. Production of recombinant protein in eukaryotic hosts: strategies and advantages.

Chapter 10. Targeted mutagenesis and protein redesign (5h)

Objectives and methods. Examples and applications of protein engineering in the analysis of its structure, stability, and functionality. Modification and improvement of the properties of proteins.

Chapter 11. Introduction to the global analysis of biological systems (1h)

Concept of genome, transcriptome, proteome and metabolome. Complexity of the proteome. Objectives and areas of proteomics

Chapter 12. Protein electrophoresis (3h)

Basic principles of electrophoresis: native electrophoresis, BN-PAGE, denaturant and isoelectric focusing. Types of detergents. Separation of proteins in two-dimensional gels: Staining and detection methods of. Electrophoresis in non-conventional supports: capillary electrophoresis and microfluidic systems

Chapter 13. Metalloproteins (3h)

Characteristics of protein-metal union. Concept of free metal and metallochaperone. Main forms of iron coordination in metalloproteins. Spectroscopic tools for the study of metalloproteins. Biocatalyst and biomimetic chemistry based on metalloproteins

Chapter 14. Identification of proteins by mass spectrometry (3h)

Mass spectrometry in proteomics: principles, sources of ionization and detectors. Identification of proteins by footprint of peptide masses. Identification of peptides by mass spectrometry in tandem.

Chapter 15. Gel-free proteomics (3h)

Definition, workflow and advantages of gel-free proteomics. *Top-down*, *bottom-up* and *shot-gun* proteomics. Instruments and modes of operation in tandem mass spectrometry. Quantitative strategies in gel-free proteomics.

Chapter 16. Clinical Proteomics (2h)

Interest in proteomics in the clinic. Concept of biomarker. Biomarkers in plasma. MALDI imaging. MALDI biotyper.

Chapter 17. Characterization of protein complexess (4h)

Basic biochemical principles of the interaction between proteins. Techniques based on affinity capture: immunoprecipitation and related strategies. Use of cross-linkers to characterize complexes and their architecture. Analysis of binary interactions *in vivo*: *double hybrid*, FRET, *protein fragment complementation*. Separation of complexes in a native way. SPR Spectroscopy Interaction data bases.

Chapter 18. Identification of post-translational modifications (3h)

Detection of post-translational modifications by fragment analysis: neutral loss and precursor ion scanning. Phosphoproteomics. Glycoproteomics Ubiquitinómica. Modifications related to oxidative damage.

Chapter 19. Applications of nanotechnology to proteomics (protein chips) (3h)

General principles and immobilization strategies. Types of protein chips: analytical, functional, antigen and reverse phase. Production systems: PISA, NAPPA, DAPA. Particle arrays in suspension.

PRACTICE ACTIVITIES:

Practical cases. Discussion of selected articles. They deal topics related to the subject, prepared by the teacher. **(4h)**

Seminars. Exhibition and discussion of selected articles. They deal topics related to the subject, prepared by the students. **(3h)**

Computer session A. Identification of proteins by peptide fingerprint. Use of MASCOT **(2h)**

Computer session B. Identification of proteins through MS/MS ion search and use of peptide atlas **(2h)**

Laboratory session I. Solid-phase synthesis of a tripeptide. **(8h)**

Laboratory session II. Extraction and SDS-PAGE of total soluble proteins **(4h)**

Laboratory session III. Determination of the structure of the synthesized tripeptide by NMR. **(2h)**

Laboratory session IV. Determination of the stability of myoglobin for circular dichroism. **(3h)**

Laboratory session V. Two-dimensional protein electrophoresis **(6h)**

Methodology

Methodology

- Master classes, alternating face-to-face and non face-to-face sessions
- Practical cases sessions and seminars in small groups
- Laboratory sessions with the aim of knowing the laboratory safety procedures and the techniques useful for the subject
- Alternative activities will be carried out for all those activities that cannot be carried out normally due to the current situation

Development plan

Activity type	Description	Classroom activity student		Non-contact activity student		Evaluation	Total time	
		Aims	Hours	Student work	Hours	Hours	Hours	ECTS
Master class	Master class (Classroom, large group + Videoconference)	Explanation of the main concepts	**60**	Study: Gain, understand and synthesize knowledge	**131**	**5**	**196**	**6.47**
Problems and cases	Practice session (Classroom, large group + Videoconference)	Resolution of problems and cases	**14**	Learn to solve problems and cases	**28**	**2**	**44**	**1.45**
Seminar	Practice session (middle group)	Conducting discussion activities	**3**	RSolve problems and cases. To argue	**17**	**1**	**21**	**0.70**
Laboratory	Laboratory session (Small group)	Practice development: understanding phenomena, measuring…	**24**	Study and write a report	**25**	**1.5**	**50.5**	**1.67**
Computer room	Computer classroom session (middle group)	Practice development: understanding phenomena, measuring ...	**4**	Study and write a report	**2**	**0.5**	**6**	**0.21**
Total			**105**		**203**	**10**	**318**	**10.5**

Evaluation

To pass the subject you must pass each activity with a 5

Activity type	Evaluation activity		Percentage grading
	Procedure	Number	(%)
Master class	Written tests of the theoretical program of the subject	4	**70**
Practice cases	Case studies deliveries or written tests	1	**8**
Seminar	Written or oral tests	1	**10**
Laboratory	Delivery of reports. Written or oral tests.	6	**8**
Computer session	Delivery of reports. Written or oral tests.	2	**4**
Total			**100**

Alternative assessment

If alternative assessment is used, the following activities must be carried out in order to pass the course:

- Carry out the practical laboratory sessions and submit the report 20%
- Take the final exam on the last day of the course 80%

Bibliography

Basic bibliography

- Michael M. Cox and George N. Philips, Jr., **Handbook of Proteins. Structure, Functions and Methods, Vol.1 i 2** (2007) John Wiley & Sons Ltd.

- Whitford, D.; **Protein Structure and Function** (2005) John Wiley & Sons Ltd.
- Kessel, A.; Ben-Tal, N.; **Introduction to Protein Structure, Function and Motion** (2011) CRC Press, Taylor & Francis Group.
- Walsh, G.; **Proteins. Biochemistry and Biotechnology** (2002) John Wiley & Sons Ltd.
- Gómez-Moreno C i Sancho J. **Estructura de Proteínas** (2003) Ariel Ciencia
- Walsh, C. T.; **Posttranslational Modification of Proteins: Expanding Nature's Inventory** (2006) Englewood, Col. : Roberts and Co. Publishers
- Twyman, R. M., **Principles of Proteomics** (Advanced Text Series) (2004) Garland Scince/BIOS Scientific Publishing
- Simpson, R. J., **Proteins and Proteomics. A Laboratory Manual** (2003) Cold Spring Harbor Laboratory Press
- Van Holde, M., **Bioquímica** (2002) Ahern. Interamericana/McGrawHill
- Palzkill, T., **Proteomics** (2002) Kluwer Academic cop
- Kannicht, C., **Posttranslational modifications of proteins** (2002) Humana Press cop.
- Liebler, D.C., Yates, J. R., **Introduction to proteomics tools for the new biology** (2002) Humana Press cop
- Campbell, M., Heyer, L.J., **Discovering genomics, proteomics, and bioinformatics** (2003) Benjamin Cummings cop

Complementary bibliography

- Buckel, P. (ed), **Recombinant Protein Drugs** (2001), Birkhäuser Verlag, Basel
- Cleland J.L. & CraiK C.S., **Protein Engineering. Principles and Practice**. (1996) John Wiley & Sons Ltd.
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- Perutz M., **Protein Structure. New Approaches to Disease and Therapy**. (1992). Freeman W.H. and Co., New York.
- Schultz, G.E. i Schirmer, R.H. **Principles of Protein Structure** (1979) Springer Verlag
- Sternberg M.J.E. **Protein Structure Prediction**. (1996) IRL- Oxford University Press.
- Wrede P. Schneider G., **Concepts in Protein Engineering and Design**. (1994) Walter de Gruyter.