Universitat Autònoma de Barcelona

2023/2024

Biomolecular Spectroscopy

Code: 100905
ECTS Credits: 6

Degree	Type	Year	Semester
2500252 Biochemistry	OT	4	0

Contact

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Teaching groups languages

You can check it through this link. To consult the language you will need to enter the CODE of the subject.
Please note that this information is provisional until 30 November 2023.

Teachers

Josep Bartomeu Cladera Cerdà

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Prerequisites

The students must have attained the learning skills of the courses: Basic Instrumental Techniques and Advanced Instrumental Techniques.

Objectives and Contextualisation

Much of the scientific knowledge of Nature is based on the study of various phenomena of absorption and emission that occur when electromagnetic radiation interacts with matter. In biosciences, spectroscopic techniques are used very often, but unfortunately many professionals are mere users that simply apply these techniques without having a well-founded scientific and technical knowledge to take advantage of all the possibilities of the different spectroscopies. This course will study in depth the scientific and technical foundations of the major spectroscopic techniques of interest for Biochemistry and Molecular Biology: absorption spectroscopy in ultraviolet and visible regions; fluorescence spectroscopy and chemiluminescence; nuclear magnetic resonance spectroscopy; positron emission tomography; spectroscopy in the infrared region; circular dichroism. In all cases, the instruments and analytical and structural applications in life sciences will be studied in detail.

Competences

- Act with ethical responsibility and respect for fundamental rights and duties, diversity and democratic values.
- Apply the principal techniques used in biological systems: methods of separation and characterisation of biomolecules, cell cultures, DNA and recombinant protein techniques, immunological techniques, microscopy techniques, etc.
- Clearly perceive current advances and possible future developments by reviewing scientific and technical literature in the area of biochemistry and molecular biology.
- Collaborate with other work colleagues.
- Define the structure and function of proteins and describe the biochemical and molecular bases of their folding, intracellular traffic, post-translational modification and replacement.
- Design experiments and understand the limitations of experimental approaches.
- Identify molecular structure and explain the reactivity of the different biomolecules: carbohydrates, lipids, proteins and nucleic acids.
- Interpret experimental results and identify consistent and inconsistent elements.
- Introduce changes in the methods and processes of the field of knowledge to provide innovative responses to the needs and demands of society.
- Manage information and the organisation and planning of work.
- Read specialised texts both in English and one's own language.
- Stay abreast of new knowledge of the structure, organisation, expression, regulation and evolution of genes in living beings.
- Think in an integrated manner and approach problems from different perspectives.
- Use analytical methodologies for assaying the biological activity of cellular components, especially enzymes, both in vitro and in vivo.

Learning Outcomes

1. Act with ethical responsibility and respect for fundamental rights and duties, diversity and democratic values.
2. Apply spectroscopic and microscopic techniques to localise specific molecules in cells and determine enzyme activity.
3. Collaborate with other work colleagues.
4. Describe in detail the biophysical methods used to reveal the dynamic structure and properties of DNA and chromatin.
5. Describe in detail the spectroscopy and diffraction techniques used to establish the structure of biomolecules and of the supramolecular complexes of living matter.
6. Describe the scientific and technical principles underpinning knowledge of the structure and chemical properties of biomolecules.
7. Design experiments and understand the limitations of experimental approaches.
8. Explain in detail the biophysical methods used to reveal the dynamic structure and properties of proteins.
9. Identify scientific and technical advances in biophysics.
10. Interpret experimental results and identify consistent and inconsistent elements.
11. Introduce changes in the methods and processes of the field of knowledge to provide innovative responses to the needs and demands of society.
12. Manage information and the organisation and planning of work.
13. Read specialised texts both in English and one's own language.
14. Think in an integrated manner and approach problems from different perspectives.

Content

BLOCK 1
1. Spectroscopy and infrared microscopy
1.1 The interaction of infrared radiation with molecules. Vibrational modes.
1.2. Michelson's interferometer. Principles, experimental design and Fourier transform. The interferogram. The apodization.

1.3 Practical aspects: spectra in aqueous suspension. Advantages of FTIR spectroscopy.
1.4 Mathematical techniques for band resolution: derivation, deconvolution and band adjustment.
1.5 Proteins. Vibrational bands associated with the amide bond and secondary structure of proteins. Difference spectroscopy.
1.6 Biological lipids and membranes. Thermotropic studies.

1.7 Infrared Microscopy and Synchrotron Light.

1.7.1 Studies by IR microscopy of cell cultures as models of human pathologies.

1.7.2 Studies by IR microscopy of brain tissues in animal models of Alzheimer's disease.

1.7.3 Studies by IR microscopy of human brain tissues in Alzheimer's disease.

2. Circular dichroism (CD).
2.1 Principles. Optical activity. Ellipticality. The spectrum of circular dichroism.
2.2 Instrumentation.
2.3 Secondary protein structure. Examples.

BLOCK 2
3. Ultraviolet and visible absorption spectroscopy
3.1. Physical principles and experimental design.
3.2. Absorption spectrophotometry.
3.3. Applications: study of proteins, nucleic acids and other biochemical chromophores.
3.4. Influence of the environment on the absorption spectrum: difference and derivative spectra.

4. Fluorescence and chemiluminescence spectroscopy
4.1. Physical bases: internal conversion, vibrational relaxation, emissive and non-emissive relaxation.
4.2. Experimental design: problems associated with fluorescence measurements, strategies and components that increase sensitivity.
4.3. Fluorescence resolved over time: lifetime of the excited state, measuring instruments, biochemical applications.
4.4 Phenomena that may affect fluorescent emission: effects of envelope and solvent, collisional quenching of fluorescence, polarization, formation of excited dimers (excimers), energy transfer.
4.5. Application to the structural analysis of macromolecular systems: intrinsic and extrinsic fluorophores, accessibility, rotational diffusion, distance measurement. Applications to Biochemical analysis, Molecular Biology and Cell Biology.
4.6. Physical bases and applications of other emitting phenomena: chemiluminescence and bioluminescence.
5. Positron emission tomography (PET)
5.1. Physical principles. Radioactive Decay. Annihilation process. Photon detection. Attenuation.
5.2. Experimental design. Detection system. Image reconstruction
5.3. PET radio tracers: Characteristics of positron emitting radionuclides. Properties of radiopharmaceuticals and metabolic activity. Cyclotron.
5.4. Applications in oncology, neurology and cardiology. Development of labeled compounds that measure the activity of specific receptors.

BLOCK 3
6. Nuclear magnetic resonance (NMR) spectroscopy
6.1. Introduction. Physical bases of the resonance phenomenon: nuclear spin, resonance condition. Radiofrequency pulse excitation, NMR signal detection (FID) and Fourier transform.
6.2. Experimental design, instrumental issues: magnet, coils for disturbing systems and their detection. Signal / noise quotient.
6.3. Parameters that characterize the NMR spectrum of a biological sample. Resonance area. Chemical displacement. Multiplicity. Relaxation: relaxation times T2 and T1.
6.4. Magnetic resonance imaging (MRI). Fundamentals. Magnetic field gradients and concept of selective excitation, concept of space k, contrast to MRI images. Single / multivoxel magnetic resonance spectroscopy and metabolic patterns.
6.5. Biomedical applications of NMR. Accessible information: morphological and functional anatomy. Applications to preclinical and clinical studies.

Methodology

Theory. The professors will explain much of the content of the course with the support of material that will be available to students in the Virtual Campus (VC). To be able to follow correctly the explanations, students should bring the VC material in class. The theory sessions address the conceptual parts of the course. Other parts of the course must be studied independently by students. The professors will indicate exactly which topics will have to be studied in this way and the material to be used.

The contents of the subject will be taught in three blocks: Block 1- UV / VIS spectroscopy, Fluorescence, chemiluminescence; Block 2- Nuclear Magnetic Resonance Imaging, Positron Emission Tomography (PET); Block 3- Infrared Spectroscopy / Microscopy, Circular Dichroism).

Problems. The professors will propose problems/scientific works related to the Spectroscopy of Biomolecules. The concrete way of developing each kind of problem/scientific work will be indicated in class or in the VC. Students will form small groups to solve and make oral and written presentations of proposed problems/scientific works.

Laboratory work. To acquire technical knowledge on the existing instruments related to spectroscopy, laboratory work will be done in various Scientific-Technical Services of the UAB: Laboratory of Luminescence and Spectroscopy of Biomolecules; Microscopy Service; Magnetic Resonance Service; Laboratory of Biophysics.

Annotation: Within the schedule set by the centre or degree programme, 15 minutes of one class will be reserved for students to evaluate their lecturers and their courses or modules through questionnaires.

Activities

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Lectures	36	1.44	2, 4, 5, 6, 8, 9, 10, 13, 14
Type: Supervised			
Grup activity: preparation of a seminar about problems/scientific works	6	0.24	2, 3, 4, 5, 6, 8, 12
Laboratory work	9	0.36	2, 3, 4, 5, 6, 7, 8, 12
Tutorials	6	0.24	2, 4, 5, 6, 8, 9, 14
Type: Autonomous			
Individual study	55.5	2.22	2, 4, 5, 6, 8, 12, 9, 10, 13, 14
Problems/scientific works	30	1.2	2, 3, 4, 5, 6, 8, 12, 9, 10, 13, 14

Assessment

Evaluation will be made according to the following items:
(1) Public presentation of problems / scientific papers in class (group assessment) and / or delivery of reports of problems / scientific papers (group assessment): maximum 2 points (20%).
(2) Evaluation of the participation in practices: maximum 1 point (10%)
(3) Evaluation of the participation in the classes of theoretical content: maximum 2 points (20%)
(4) Test of theoretical contents: maximum 5 points (50%)
There will be two partial tests of theoretical content:
1st partial test: Block 1
2nd partial test: Blocks 2 and 3
Each block of the subject (1- Infrared, CD; 2- UV / VIS, Fluorescence, chemiluminescence, PET; 3- NMR) will be evaluated independently according to the 5 elements above. The approved block will be considered as long as the mark is 5 points or higher (out of 10). To pass the course, each block must be passed separately.
The grade of the subject, if the three blocks are passed, will be the average of the grades of the 3 blocks.
Test review dates will be indicated at least 2 days in advance.
Students who have not passed the subject (grade less than 5 points out of 10 in any of the three blocks) must take the retake of the blocks not passed. To be able to take it, it must have been previously evaluated in a set of activities whose weight is equivalent to a minimum of two thirds of the total qualification of the subject. Therefore, students will obtain the grade of "Non-Assessable" when the assessment activities performed have a weighting of less than 67% in the final grade. The mark of the recovery will be the one of the theoretical exam weighted by the one of the other elements of evaluation.
Attendance at the practical sessions is mandatory. Students will obtain the grade of "Not Evaluable" when the absence is greater than 20% of the scheduled sessions.

Single evaluation

The single evaluation will consist of a single synthesis test that willassess the content of the entire theory program of the subject. The test will include multiple-choice questions and/or open-ended questions. The grade obtained in this synthesis test will account for 70% of the final grade of the subject.

The evaluation of practical activities and the public presentation of problems/scientific works in class (group evaluation) and/or the submission of reports on problems/scientific works (group evaluation) will follow the same process as continuous evaluation. The evaluation of participation in practical activities will account for 10% of the final grade of the subject, and the public presentation of problems/scientific works in class (group evaluation) and/or the submission of reports on problems/scientific works (group evaluation) will account for 20%.

The single evaluation test will take place on the same date as the final test of continuous evaluation, as indicated in the calendar, and the same recovery system as continuous evaluation will be applied.

To pass the subject, a minimum overall final grade of 5.0 points must be obtained.

Students who have not passed the subject through the single evaluation will have the opportunity to take a final recovery exam that will have the same characteristics as the recovery exam of the continuous evaluation.

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Assessment of laboratory work	10%	0.75	0.03	1, 2, 3, 4, 5, 6, 7, 8, 12, 9, 10, 11, 13, 14
Assessment of problems/scientific works	10%	0.75	0.03	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14
Assessment of the presentation of problems/scientific works	10%	0.75	0.03	1, 2, 3, 4, 5, 6, 7, 8, 12, 9, 10, 11, 13, 14

Evaluation assistance/participation teory lectures	20%	1.5	0.06	1, 7, 12, 9, 10, 11, 14
Final exam	50%	3.75	0.15	1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14

Bibliography

1. An Introduction to Spectroscopy for Biochemists. S.B. Brown, 1980. Academic Press.

2. Principles of Fluorescence Spectroscopy. J.R. Lakowicz, 1983. Plenum Press.

3. Biological Spectroscopy. I.D. Campbell i R.D. Dwek, 1984. Benjamin-Cummings.

4. NMR of Proteins and Nucleic Acids. K. Wüthrich, 1986. Wiley.

5. NMR in Medicine and Biology. Structure Determination, Tomography, in vivo Spectroscopy. K.H. Hausser i H.R. Kalbitzer, 1989. Springer-Verlag.

6. Espectroscopía *in vivo* por Resonancia Magnética Nuclear. J.M. García Segura, 1991. Eudema Universidad.

7. Fluorescence Spectroscopy. New Methods and Applications. O.S. Wolfbeis, 1993. Springer Verlag.

8. Biomolecular NMR Spectroscopy. J.N.S. Evans, 1995. Oxford University Press.

9. NMR and its Applications to Living Systems, 2nd Edition. D.G. Gadian, 1995. Oxford University Press.

10. Infrared Spectroscopy of Biomolecules. H.H. Mantsch i D. Chapman, 1996, Wiley-Liss.

11. Técnicas Instrumentales de Análisis en Bioquímica. J-M. García Segura y col., 1999, Editorial Síntesis, Madrid

12. Fluorescent and Luminiscent Probes for Biological Activity. W.T. Mason, 1999. Academic Press

13. Magnetic Resonance in Chemistry and Medicine. Ray Freeman, 2003. Oxford University Press.

14. Optical Spectroscopy in Chemistry and Life Sciences. Werner Schmidt, 2005.Wiley-VCH.

15. Spectroscopy for the Biological Sciences. Gordon G. Hammes, 2005. Wiley-Interscience.

16. Physical principles and techniques of protein chemistry. Sydney J. Leach Ed., 1973. Academic Press.

17. In vivo NMR Spectroscopy. Principles and Techniques. 2nd Edition. Robin A. de Graff, 2007. Wiley.

18. Fluorescence Applications in Biotechnology and Life Sciences. Ewa M. Goldys Ed., 2009. Wiley-Blackwell.

Scientific articles and web links will be indicated during the course.

Software

Software 'Paravision' MRI analysis

Software 'Topspin' for RMN analysis

Software 'Quasar' for FTIR and microspectroscopy analysis.