Universitat Autònoma de Barcelona

2020/2021

Biomolecular Spectroscopy

Code: 100905
ECTS Credits: 6

Degree	Type	Year	Semester
2500252 Biochemistry	OT	4	0

The proposed teaching and assessment methodology that appear in the guide may be subject to changes as a result of the restrictions to face-to-face class attendance imposed by the health authorities.

Contact

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Use of Languages

Principal working language: catalan (cat)
Some groups entirely in English: No
Some groups entirely in Catalan: No
Some groups entirely in Spanish: No

Teachers

Josep Bartomeu Cladera Cerda
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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

- 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.
- Manage information and the organisation and planning of work.
- Read specialised texts both in English and ones 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. Apply spectroscopic and microscopic techniques to localise specific molecules in cells and determine enzyme activity.
2. Collaborate with other work colleagues.
3. Describe in detail the biophysical methods used to reveal the dynamic structure and properties of DNA and chromatin.
4. Describe in detail the spectroscopy and diffraction techniques used to establish the structure of biomolecules and of the supramolecular complexes of living matter.
5. Describe the scientific and technical principles underpinning knowledge of the structure and chemical properties of biomolecules.
6. Design experiments and understand the limitations of experimental approaches.
7. Explain in detail the biophysical methods used to reveal the dynamic structure and properties of proteins.
8. Identify scientific and technical advances in biophysics.
9. Interpret experimental results and identify consistent and inconsistent elements.
10. Manage information and the organisation and planning of work.
11. Read specialised texts both in English and ones own language.
12. Think in an integrated manner and approach problems from different perspectives.

Content

1. introduction

1.1 Interaction of electromagnetic radiation with matter.

1.2 Dispersion, absorption and emission.

2. Absorption spectroscopy in the ultraviolet and visible regions

2.1 Physical principles

2.2. Experimental design.

2.3. Absorption spectrophotometry.

2.4 Applications: study of proteins, nucleic acids and other biochemical chromophores.

2.5 Influence of the environment on the absorption spectrum: difference and derivative spectra.

3. Fluorescence spectroscopy and chemiluminescence

3.1. Physical basis: internal conversion, vibrational relaxation, radiative and non-radiative relaxation.

3.2. Experimental design: problems associated with measurements of fluorescence, strategies and components that allow to increase the sensitivity.

3.3. Time-resolved fluorescence: lifetime of the excited state, instruments for the measurement of the lifetime; biochemical applications.

3.4 Phenomena that can affect the fluorescent emission: effects of the environment and the solvent, collisional quenching of fluorescence, polarisation, formation of excited dimers (excimers), energy transfer.

3.5 Application to the structural analysis of macromolecular systems: intrinsic and extrinsic fluorophores, accessibility, rotational diffusion, measurement of distances.

3.6. Analytical applications in Biochemistry and Molecular Biology.

3.7 Application to studies of cell biology: fluorescence microscopy, flow cytometry.

3.8. Physical basis and applications of other emission phenomena: chemiluminescence and bioluminescence.

4. Nuclear magnetic resonance (NMR) Spectroscopy

4.1. Introduction. Basic principles of the resonance phenomenon: nuclear spin, resonance condition, macroscopic magnetization and vector model, rotating frame.

4.2. Experimental design and hardware: magnet, excitation andreception coils, radiofrequency pulse, RMN signal-free induction decay (FID) and Fourier transform. Signal to noise ratio.

4.3. Parameters characterizing the NMR spectrum of a biological sample. Resonance area, Chemical shift, Multiplicity. Relaxation: T2 relaxation time (spin echo concept) and T1 relaxation time. Nuclear Overhauser effect and hyperpolarization.

4.4. Magnetic resonance imaging basic principles. Magnetic field gradients and the concept of selective excitation. K-space. Image contrast in MRI. Single/multivoxel magnetic resonance spectroscopy and metabolic profiles

4.5. NMR Biomedical Applications. Morphological and functional information. . Biomedical applications in preclinical and clinical studies.

5. Positron Emission Tomography (PET).

5.1 Introduction. Basic physical principles. Annihilation process. Photon detection. Attenuation.

5.2. Experimental design. Detection system. Image reconstruction.

5.3 Radiotracers:postiron emitters. Radio-drugs: properties and metabolic activity. Cyclotron.

5.4. Applications in oncology, neurology and cardiology. Specific labeled compounds for receptor activity measurments.

6. Infrared spectroscopy and microscopy

6.1 The interaction of infrared radiation with molecules. Vibrational modes.

6.2 Michelson's interferometer. Principles, experimental design and Fourier transform. The interferogram. Apodization.

6.3 Practicalities: aqueous solutions. FTIR advantages.

6.4 Resolution enhancement mathematical techniques: derivation, deconvolution and curve-fitting.

6.5 Proteins. Vibrational bands, the amide bond and protein's secondary structure. Difference spectroscopy.

6.6 Lipids and membranes. Thermotropic studies.

6.7 Infrared microscopy and the use of synchrotron light.

6.8. Research case studies using infrared spectroscopy and microscopy.

7. Circular dichroism (CD).

7.1 Principles. Optical activity. Elipticity. CD spectrum.

7.2 Instrumentation.

7.3 Protein secondary structure. Examples.

"***Unless the requirements enforced by the health authorities demand a prioritization or reduction of these contents."***

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.

"The proposed teaching methodology may experience some modifications depending on the restrictions to face-to-face activities enforced by health authorities."

Activities

Title	Hours	ECTS	Learning Outcomes
Type: Directed			
Lectures	36	1.44	1, 3, 4, 5, 7, 8, 9, 11, 12
Type: Supervised			
Grup activity: preparation of a seminar about problems/scientific works	6	0.24	1, 2, 3, 4, 5, 7, 10
Laboratory work	9	0.36	1, 2, 3, 4, 5, 6, 7, 10

Tutorials	6	0.24	1, 3, 4, 5, 7, 8, 12
Type: Autonomous			
Individual study	55.5	2.22	1, 3, 4, 5, 7, 10, 8, 9, 11, 12
Problems/scientific works	30	1.2	1, 2, 3, 4, 5, 7, 10, 8, 9, 11, 12

Assessment

The evaluation is based on five elements:

(1) Oral presentation of problems/scientific works in class (group seminar): maximum 1 point (10%).

(2) Preparation of reports of problems/scientific works (group report): maximum 1 point (10%).

(3) Laboratory work: maximum 1 points (10%).

(4) Evaluation of participation in teory classes: maximum 2 points (20%).

(5) Final exam: maximum 5 points (50%).

Each block of the subject (1- UV / VIS, Fluorescence, chemiluminescence; 2- NMR, PET; 3- Infrared, CD) will be evaluated independently according to the 5 previous elements. The approved block will be considered as long as the grade 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 retake process will consist in a written exam on the course theory contents (point number 5 of the assessment activities described above).
Attendance to practical sessions is mandatory. Students missing more than 20% of programmed sessions will be graded as "No Avaluable.
"Student's assessment may experience some modifications depending on the restrictions to face-to-face activities enforced by health authorities."

Assessment Activities

Title	Weighting	Hours	ECTS	Learning Outcomes
Assessment of laboratory work	10%	0.75	0.03	1, 2, 3, 4, 5, 6, 7, 10, 8, 9, 11, 12
Assessment of problems/scientific works	10%	0.75	0.03	1, 3, 4, 5, 6, 7, 8, 9, 11, 12
Assessment of the presentation of problems/scientific works	10%	0.75	0.03	1, 2, 3, 4, 5, 6, 7, 10, 8, 9, 11, 12
Evaluation assistance/participation teory lectures	20%	1.5	0.06	6, 10, 8, 9, 12

Final exam	50%	3.75	0.15	1, 3, 4, 5, 6, 7, 8, 9, 11, 12

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.