Biomolecular Spectroscopy

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
Silvia Lope Piedrafita

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 well-founded scientific and technical knowledge to take advantage of all the possibilities of the different spectroscopies. This course will study in depth of 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; 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.

Skills
- 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 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. 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 one's 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.


5. Infrared spectroscopy and microscopy

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


5.3 Practicalities: aqueous solutions. FTIR advantages.

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

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

5.6 Lipids and membranes. Thermotropic studies.

5.7 Infrared microscopy and the use of synchrotron light.

5.8. Research case studies using infrared spectroscopy and microscopy.


6.2 Instrumentation.

6.3 Protein secondary structure. Examples.

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.

**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.

### Activities

<table>
<thead>
<tr>
<th>Title</th>
<th>Hours</th>
<th>ECTS</th>
<th>Learning outcomes</th>
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<tr>
<td><strong>Type: Directed</strong></td>
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<td></td>
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<tr>
<td>Lectures</td>
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<td>1.44</td>
<td>1, 3, 4, 5, 7, 8, 9, 11, 12</td>
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<td><strong>Type: Supervised</strong></td>
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<td>Grup activity: preparation of a seminar about problems/scientific works</td>
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<tr>
<td>Laboratory work</td>
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<td>Tutorials</td>
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<td><strong>Type: Autonomous</strong></td>
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<td>Individual study</td>
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### Evaluation

The evaluation is based on four 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. Final exam: maximum 7 points (70%).

To pass the course the sum of the scores must be ≥ 5 points (maximum 10 points).

Students who have completed evidence of learning with a weight less than 50% will be mark as "not evaluated".
The date for the review of the exams will be announced with a minimum of 2 days in advance.

**Evaluation activities**

<table>
<thead>
<tr>
<th>Title</th>
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<th>Hours</th>
<th>ECTS</th>
<th>Learning outcomes</th>
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<td>Assessment of laboratory work</td>
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<td>Assessment of problems/scientific works</td>
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<td>0.75</td>
<td>0.03</td>
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<td>Assessment of the presentation of problems/scientific works</td>
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<td>0.03</td>
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<td>Final exam</td>
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<td>0.21</td>
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**Bibliography**


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