Scuola Superiore di Catania

Corso Specialistico

Classe delle Scienze Umanistiche e Sociali

Ambito Scienze e Tecnologie

a.a. 2022-2023

Physico-chemical Techniques for the Study of Biomolecular Interactions

Tecniche fisico-chimiche per lo studio delle interazioni biomolecolari

Language: English

Prerequisites:

Basic knowledge of physics as well as analytical, inorganic and organic chemistry.

The course will present modern tools and strategies for the characterization of the interactions occurring among systems of biological/biochemical relevance through the use of different but often complementary techniques such as NMR, absorbance/fluorescence spectroscopy and mass spectrometry.
Module #1: NMR
The aim of the module is to provide knowledge on the application of Nuclear Magnetic Resonance (NMR) Spectroscopy in the field of protein-ligand interactions.
Module #2: Absorbance/fluorescence
The students will acquire knowledge on how to use absorbance and fluorescence experiments to study the details of the binding mechanisms of small molecules to biosubstrates such as DNAs, RNAs and proteins. The experiments will consider both thermodynamic (complex systems characterisation, titrations, melting tests) and kinetic aspects (fast reaction techniques).
Module #3: Mass spectrometry
The aim of the module is to provide concepts and strategies for understanding mass spectrometry based chemical and structural proteomics for investigating protein the structure and interaction of biomolecules.

Course Content
NMR basic principles and applications in ligand-protein interaction
Nuclear Magnetic Resonance (NMR) Spectroscopy is an indispensable analytical technique that has been successfully applied to investigate the structure, dynamics and interactions of biologically significant small and large

macromolecules and their complexes in the solution state. Structural information pertaining to the interactions between biological macromolecules and ligands is of potential significance for the understanding of molecular mechanisms in key biological processes. NMR spectroscopy-based techniques are versatile due to their ability to examine weak binding interactions and for a rapid screening of the binding affinities of ligands with proteins at atomic resolution. This module will provide a brief introduction on the basic principles of the technique followed by an overview of the various homo- and heteronuclear 2D NMR methods that are currently used to unambiguously identify and assign the NMR spectra of ligands and proteins. Several examples and practical applications will be provided as well.

- Fluorescence and absorbance spectroscopies: methods and tools to get information on the thermodynamics and kinetics of the binding of small molecules to biosubstrates

Basic principles on how to prepare an absorbance or fluorescence experiment
Fluorescence and absorbance titrations: the case of nucleic acids – experimental aspects
Fluorescence and absorbance titrations: the case of nucleic acids – data analysis
Fluorescence and absorbance titrations: the case of proteins – experimental aspects
Fluorescence and absorbance titrations: the case of proteins– data analysis
Other tests/experiments to be carried out by either absorbance or fluorescence spectroscopy
Kinetic studies on the abovementioned biomolecular systems, with a particular focus on DNA

- Mass spectrometry-based tools in modern structural biology

Mass spectrometry based chemical and structural proteomics comprises several advanced analytical techniques that has been successfully applied to investigate the structure, dynamics, and interactions of biologically significant small and large macromolecules and their complexes in solution. Those methods only require very little amount of sample and can be applied to in vitro and in vivo systems, without altering the natural biomolecule environment. On top of that, they are not limited by neither the protein size nor by the sample complexity and can provide structural information on small proteins as well as on megadalton protein complexes. Due to its unique characteristics and advantages, mass spectrometry based chemical and structural proteomics has matured as an essential toolkit in structural biology, drug discovery, and biochemistry.

Didactic methodology
Face-to-face lessons and examples of data analysis through proper software.

Exam
Written test on the topics discussed during the course.