**COURSE (TITLE): STRUCTURAL ANALYSIS OF BIOMOLECULES- BIOMOLECULAR STRUCTURE DETERMINATION BY NMR AND X-RAY**

**LECTURER: YEAR and SEMESTER: I year and II semester**

**CREDITS (CFU): 8**

**SECTOR (SDS): CHIM 03**

**ACADEMIC YEAR:**

**ASSESSMENT: Oral**

**LOCATION: Department of Environmental, Biological and Pharmaceutical Science and Technologies, Via Vivaldi 43 Caserta**

**COURSE OBJECTIVES/OUTCOMES:**

The aim of this course is to introduce the theory and practice for the structural characterization of biomolecules by using NMR spectroscopy and X-ray diffraction techniques, including the required data acquisition, processing steps and required computer software. At the end of the course the student will learn how to obtain NMR and X-ray data to characterize the structural proprieties of biomolecules. Moreover, the student will learn the most advanced NMR methodologies for studying Protein-protein and Protein-ligand interactions. During the course the student will be involved in several training activities on the spectrometers located in the NMR laboratory of the Department of Environmental, Biological and Pharmaceutical Science and Technologies in order to fully understand the procedure for NMR data acquisition.

**SYLLABUS (overview)**

In the first part of this course (NMR) the principles of how obtain and evaluate complex spectra and structure elucidation are provided. Theoretical basis of bio-NMR spectroscopy are given such as: the vector model, relaxation, nuclear Overhauser effect, scalar and dipolar coupling, chemical shift. NMR signal assignment and multidimensional NMR spectroscopy is introduced. Basics of NMR structure calculation will be described, including data collection, resonance assignment, collection of structural restraints. The most advanced NMR techniques for studying protein-protein and protein-ligand interactions will be illustrated such as: STD, transfer NOE, WaterLOGSY, Chemical Shifts perturbation.

In the second part of this course (X-ray) the main theoretical and practical concepts for structural characterization of biomolecules are given such as: preparing crystals, preliminary characterization, data collection, solution of the phase problem, refinement and structure

**SYLLABUS (Detailed description):**

**Fundamentals of modern NMR spectroscopy:** Spin magnetization; Vector model; Rotating frame of reference; Pulses (Soft and Hard); Fourier transformation; Spectral processing; NMR parameters: Chemical Shifts, J scalar couplings; integration; Nuclear Overhauser Effect (NOE); Dipolar coupling; Concepts of the Multidimensional NMR experiments; Analysis of pulse sequences 1D and 2D experiments (homo- and hetero-nuclear): 1D 1H; 2D TOCSY; 2D NOESY,2D ROESY, 2D COSY;2D 1H-15N HSQC; 1H-13C HSQC; Spin-lock; correlation time; constant time; Relaxation theory: Correlation function; Longitudinal Relaxation rate (R1); Transverse Relaxation rate(R2); Mechanisms inducing relaxation: Chemical Shifts Anisotropy; Dipolar Coupling; Experiments for measuring relaxation parameters: Inversion recovery (2D 1H-15N HSQC) R1,

Spin-echo (2D 1H-15N HSQC) R2, heteronuclear [1H]-15N NOE. Parts of the NMR spectrometer; the superconducting electromagnet, the sample probe, the pneumatic unit, the preamplifier, the console, the computer. Introduction of the basic operations for acquiring NMR spectra: loading the sample, locking, tuning and shimming, setting acquisition and processing parameters.

**Biomolecular NMR spectroscopy:** Use of NMR for biomolecular structure elucidation; introduction of 3D triple resonance experiments; spectral assignments experiments: examples HNCO; CBCA(CO)HN; CBCANH; HNCA; Overhauser effect Example 13C/15N-resolved NOESY; software for processing and analysis of spectra: SPARKY, CARA, NMRDRAW; protocols and software for structure calculation: Cyana, TALOS, PALES; Analysis and validation of the calculated structures: PROCHECK; PSVS and PROCESS server

Residual dipolar coupling measurements Example: IPAP-HSQC; aligned media (polyacrylamide gel (Compressed and Stretched), Pf1 Phage); Principles of TROSY Spectroscopy; Relaxation measurements, Models of motions, Protein dynamics: Example 15N T1; 15N T2; T1ρ; Het [1H]-15N NOE.

**NMR in Drug Discovery:** Physical effects of protein-ligand interactions relevant for NMR: thermodynamics of ligand binding; kinetics of ligand binding; Exchange regime (Fast, Intermediate, slow); chemical environment and interactions; Brownian motion (rotational and translational diffusion); NMR observable parameters associated with basic physical effects; How ligand binding effects influence the NMR signal; Protein observed chemical shift titrations: Chemical Shifts Mapping Example Heteronuclear HSQC detected titrations; Ligand observed methods: Ligand observed relaxation rates, chemical shifts, transverse and longitudinal rates, translational diffusion, magnetization transfer (Saturation Transfer Difference NMR STD, WaterLOGSY, transfer NOE).Example of Protein-ligand studies observing Protein or Ligand

**Biocrystallography:** Introduction, role of biocrystallography in macromolecular structural chemistry; Properties of crystals. Symmetry; Symmetry elements. Space groups; Reciprocal lattice; Unitary Cell - Miller Indices; Classification of crystalline lattices; - The 14 lattices of Bravais; X-ray diffraction; The Bragg Law; Diffraction Conditions; Structural Factors; X-ray sources; X-ray generators; Automatic diffractometers; Data collections; Crystallization of Macromolecules; Crystallization techniques of small and large molecules; crystal quality evaluation; use of polarized microscope; The diffraction data; Determination of the elementary cell; Calculation of the density of a crystal and of the number of molecules contained in the unit cell of a macromolecular crystal; Diffracted intensity; Scattering factor; Resolution and refinement of crystallographic structures; Problem of the phase; Data collection methods; Solving the phase problem; The method of multiple Molecular replacement; Other methods used to solve the problem of the phase: "trial and error" methods; "direct methods"; methods of Patterson; Crystallographic refinement; Index of disagreement R.

**TEXTBOOKS:**


1) **Understanding NMR Spectroscopy, 2nd Edition.**
2) **Protein NMR Spectroscopy: Practical Techniques and Applications.**
3) **BioNMR in Drug Research (Methods and Principles in Medicinal Chemistry).**
4) **Fundamentals of Crystallography I- International Union of Crysyallography.**
5) **Practical Crystallography - Pergamon Press.**

**ADDITIONAL READING:**
**https://www.cis.rit.edu/htbooks/nmr/**

**<u>Servers, software and tutorials</u>**
**http://psvs-1_5-dev.nesg.org/**
**http://www.prosess.ca/**
**https://spin.niddk.nih.gov/bax/software/NMRPipe/doc1/**
**https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/**
**https://spin.niddk.nih.gov/bax/software/PALES/**
**https://www.cgl.ucsf.edu/home/sparky/**
**http://cara.nmr.ch/doku.php**