Fundamentals of Structural Biology (Les bases de la biologie structurale)

PAX9NBAD
Semester 9

Main objectives of the course

This course aims to show how fundamental concepts of the interaction between particles such as photons, electrons or neutrons and matter can be used to obtain structural and dynamics information on biological macromolecules, isolated, in complex or in their cellular context. After a short introduction on the recent approaches and methods in integrated structural biology, Electron Microscopy, X-ray diffraction and scattering, and Nuclear Magnetic Resonance will be developed. The complementarity between these methods to answer complex biological questions will also be addressed. This course will include practical work on advanced instrumentation and hands-on session on world-leading plateforms, including the ESRF synchrotron. Students will acquire competences in data acquisition and data processing as well as critical analysis of the results.

More specific aims in Electron Microscopy

Electron microscopy is a method which is currently considered as revolutionary in structural biology as the recent developments of (i) technologies for image collection and (ii) methodologies for image processing allow to determine structures of protein complexes that were previously extremely difficult to obtain. These developments lead to important discoveries on biological targets with fundamental and/or medical relevance. In this part of the course we will learn the composition of an electron microscope and see which central developments were recently done to improve the instrument. We will learn how to prepare a single particle electron microscopy sample (negative stain and cryo-EM) and we will define the different data collection strategies: tomography, random conical tilt, zero degree tilt. We will then see how to determine a structure by single particle electron microscopy (particle boxing, 2D alignment and classification, 3D reconstruction, resolution determination, model building).

At the end of the module, the student should be able to read and understand the methods which allow to determine a cryo-EM structure and understand the key biological points deciphered in a structure of a protein complex.

Pre-requisites:
Basic knowledge about proteins (primary, secondary, ternary, quaternary structures)
Basic knowledge about Fourier transforms

More specific aims in Nuclear Magnetic Resonance

Nuclear Magnetic Resonance (NMR) was established in the mid-80s as a powerful alternative method to X-ray crystallography for the structure of biomacromolecules. Developments in instrumentation to decrease sample needs and increase signal-to-noise ratios, overcome of size-limitations with intelligent data-acquisition schemes in combination with efficient isotopic labeling strategies, and implementation of innovative data processing algorithm, lead to significant advances in the extraction of structural and dynamical information on biological systems of increasing complexity. More recently NMR emerged as a key method to study intrinsically disordered biological systems, to offer structural information on

low-populated states in conformational exchange with highly populated ground-states, or to yield kinetic and thermodynamic information on macromolecular complexes of low affinity in parallel to structural information. In this part of the course, we will learn basic NMR principles and see which central developments were recently done to improve the instrumentation. We will learn how to manipulate spins through their interaction with oscillating magnetic field in order to recover key information for structure determination and its different steps. We will see how different interactions and their characteristic time-scales can be used to recover dynamic information on protein diffusion in solution, on protein folding and conformational exchange, and on protein shape and local dynamics. Applications of these methodologies to decipher challenging biological questions will be shown along the course.

Pre-requisites:
Very basic knowledge in quantum mechanics is advised.
Basic knowledge in protein and nucleic acid structure

More specific aims in X-ray diffraction and scattering (à compléter par Dominique)

Pre-requisites:

Proposal for three review articles that cover concepts detailed in this course

Electron Microscopy: Elmlund D, Le SN, Elmlund H, "High-resolution cryo-EM: the nuts and bolts" (2017) Current Opinion in Structural Biology, 46, 1-6.

Nuclear Magnetic Resonance: Marion D, "An introduction to biological NMR spectroscopy" (2013) Molecular and cellular Proteomics, 12, 3006-3025.

X-ray methods: Sayers Z, Avsar B, Cholak E, Karmous I, "Application of advanced X-ray methods in life sciences" (2017) Biochimica et Biophysica Acta, 1861, 3671-3685.