



Course unit English denomination	Structural analysis of small- and macro-molecules by X-ray crystallography
SSD	CHEM-03A; CHEM-05/A
Teacher in charge (if defined)	Prof. Marco Baron Dr. Marzio Rancan Prof. Alessandro Dolmella Prof. Roberto Battistutta
Teaching Hours	24
Number of ECTS credits allocated	3
Course period	<u>09/2026</u>
Course delivery method	☐ In presence ☐ Remotely ☒ Blended
Language of instruction	English
Mandatory attendance	☒ Yes (75% minimum of presence) ☐ No
Course unit contents	The course will be organized in four different units. All the teaching units present theoretical and hands-on sessions: 1) <u>Data collection and data reduction</u> After a brief introduction on what is a single crystal and what is X-ray diffraction, the first unit of the course focuses on the single crystal X-ray diffraction experiment. Details will be provided about the set-up of the diffractometer and on the different steps of the diffraction experiments: - Selecting, mounting and centering the crystal; - Unit cell determination; - Data collection strategy; - Integration and corrections of the collected frames; - Space group determination. 2) <u>Small molecules crystal structure solution and refinement</u> After a brief overview of the main structure solution methods for small molecules, this section will focus on the structure refinement process using state-of-the-art and widely adopted software (e.g., SHELXL). Particular emphasis will be placed on: - Criteria to guide a good structure refinement; - The use of restraints, constraints and free variables; - Disorder and refinement of disorder; - The “squeeze” algorithm: why and when use it; - Placing hydrogen atoms in the model; - Twinning; - Absolute configuration determination.



3) Data validation and presentation

This unit of the course regards crystallographic data validation and presentation.

The parameters that indicate the goodness of the refined molecular model will be presented and discussed with focuses on the generation of the CIF file (crystallographic information file) and on the CIF validation process (the checkCIF process) according to the IUCr ([International Union of Crystallography](#)) recommendations.

Furthermore, the up-to-date software to analyze and present crystallography data (PLATON, ORTEP, Mercury) will be illustrated with hands-on examples.

4) Introduction to protein crystallography

An overview of the differences between small molecules and protein crystallography will be presented, in particular for:

- crystallization methods, properties of crystals, automated data collection at synchrotrons
- solving the phase problem: the advent of the AI in protein crystallography

In the second part, we will focus on model refinement and validation:

- from the first electron density to the final “validated” model; examples and practical demonstrations (with software “Phenix” and “Coot”)
- The PDB and the correct interpretation of the protein structures.

Learning goals

Knowledge:

Upon completion of this course, doctoral students will have acquired the following key knowledge objectives:

- A comprehensive understanding of fundamental concepts in crystallography, including the principles of diffraction and the instrumentation used in this analytical technique. This foundational knowledge will enable students to grasp the theoretical basis of crystallographic analysis.
- Proficiency in applying X-ray diffraction techniques to single crystals. Students will gain expertise in analyzing structures ranging from small molecules to biological macromolecules (proteins). This knowledge will be crucial for characterizing and understanding the structural properties of these molecular and supramolecular materials.
- A critical awareness of the current state-of-the-art in X-ray diffraction techniques, including recent advancements and limitations. Students will explore potential future developments in structural characterization methods that go beyond single-crystal X-ray diffraction. This forward-looking perspective will prepare them to adapt to and potentially contribute to emerging technologies in the field.

Through these objectives, students will develop a robust skill set in crystallographic analysis, enabling them to conduct advanced research in molecular and material sciences fields.

Skills:

The skills that a student will acquire through the course are:

- Crystal structure solution and refinement using different state-of-the-art software (Olex2, ShelXle, Phenix, Coot)
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- Data integration and correction using different state-of-the-art software (Apex6, Crysalis Pro)
- Integration and refinement of twinned data
- Data validation and presentation

Competencies:

The determination of the molecular structure of small molecules and macromolecules is fundamental to studying their properties. Single-crystal X-ray diffraction is the leading technique for studying the structural properties of complex and advanced materials. The course aims to provide students with advanced competences in structural determination and analysis of the final models using single crystal X-ray diffraction.

By the end of the course, the students will be able to:

- Critically analyze the results obtained in a structural determination using single crystal X-ray diffraction.
- Fully exploit the potential of this technique to apply it to their own research projects.

Teaching methods	Frontal teaching and hands on sessions
Course on transversal, interdisciplinary, transdisciplinary skills	☒ Yes ☐ No
Available for PhD students from other courses	☒ Yes ☐ No Students external to the PhD Course admitted upon evaluation of the CV by the teachers
Prerequisites (not mandatory)	max 3750 caratteri
Examination methods	Practical exam: refinement of a provided crystal structure.
Suggested readings	Slides/articles/books provided by the teachers
Additional information (not mandatory)	max 3750 caratteri