UFR CHIMIE & BIOLOGIE Université Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2026-2027

Laboratory/Institute: Institut de Biologie Structurale
Director: Winifred WEISSENHORN
Team: NMR of Large Assemblies group
Head of the team: Jérôme Boisbouvier

Name and status of the scientist in charge of the project:
Jérôme BOISBOUVIER, CNRS Research Director

HDR: yes ☒ no ☐

Address: 71 avenue des Martyrs
Phone: 04 57 42 86 71
e-mail: jerome.boisbouvier@ibs.fr

Program of the Master's degree in Biology:

☐ Microbiology, Infectious Diseases and Immunology ☒ Biochemistry & Structure
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Title of the project:

Development and application of a novel biochemical approach for the atomic-scale study of large assemblies by nuclear magnetic resonance

Objectives (up to 3 lines):

The objective is to apply a novel biochemical method to produce site-specifically labeled human ClpX, ClpP, and its biomolecular assembly ClpXP using cell-free protein synthesis.

Abstract (up to 10 lines):

Nuclear magnetic resonance (NMR) is an ideal method for studying proteins, providing valuable insights into their conformation, dynamics, and function at the atomic scale. However, NMR presents significant interconnected challenges in resolution, sensitivity, and signal assignment for large biomolecular assemblies. To overcome these obstacles, we have developed an innovative isotope labeling method involving cell-free technology and reduced codon protein synthesis to integrate site-specific isotopic markers at the residue(s) of interest. The approach holds much promise for application to medically relevant proteins, such as human ClpXP. This >300 kDa unfoldase-protease complex plays a central role in human mitochondrial protein control. Although its dysregulation directly contributes to various malignancies, we lack insight into the dynamics of this assembly due to its large size. Therefore, we will apply our innovative labeling method to ClpXP to gain deeper insights into their structural and allosteric functional properties.

Methods (up to 3 lines):

The student will learn protein expression (bacterial, cell-free), large-scale RNA transcription (run-off, plasmid), column purification (ion exchange, affinity, size exclusion), DNA extraction, enzymatic activity assays (aminoacylation, malachite green), 2D NMR / methyl-NMR, and RNA/protein/plasmid design.

Up to 3 relevant publications of the team:

1. Arthur Giraud, Lionel Imbert, Adrien Favier, Faustine Henot, Francis Duffieux, et al. Enabling site-specific NMR investigations of therapeutic Fab using a cell-free based isotopic labeling approach: application to

anti-LAMP1 Fab. *Journal of Biomolecular NMR*, **2024**, 78 (2), 73-86.

2. Elena-Real et al. Site-specific introduction of alanines for the nuclear magnetic resonance investigation of low-complexity regions and large biomolecular assemblies. *ACS Chemical Biology*. **2023**, 18, 2039-2049.

3. Subunit-specific isotope labeling of heteromeric complexes using cell-free protein expression : application to the 760 kDa ClpXP molecular machine. RSC Chemical Biology, **2026**, doi: 10.1039/D5CB00259A

Requested domains of expertise (up to 5 keywords):

Protein expression, biotechnology, molecular biology, RNA, NMR