UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2021-2022

Laboratory/Institute: NMR group / IBS
Director: Pr. W. Weissenhorn
Team: NMR of large molecular assemblies
Head of the team: Dr. J. Boisbouvier

Name and status of the scientist in charge of the project: **HDR: yes ☒ no ☐**
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Program of the Master's degree in Biology:

☐ Immunology, Microbiology, Infectious Diseases ☒ Structural Biology of Pathogens
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Title of the project: *Structural investigation of* ClpX/ClpP proteolytic machinery in action

Objectives:

This project aims to study the mechanism of proteolytic machinery ClpP interacting with the co-chaperone ClpX by combining structural biology methods. We will use advanced Cell-Free and NMR approaches to investigate ClpP/X complex in action fuelled by ATP hydrolysis.

Abstract:

Caseinolytic proteases (ClpP) are large, cylindrical serine proteases (300 kDa) present in bacteria. Although ClpPs are capable of degrading small peptides by themselves, association with their cognate energy-dependent AAA+ disassembly chaperone ClpX (240 kDa) is required to degrade larger peptides and proteins. ClpPs play an active role in survival and virulence of pathogenic bacteria. The objective of the project is to understand the mechanism of the ClpP/X complex, by obtaining detailed structural insights and kinetic information on the mechanisms of these ATP-fueled proteolytic machinery under functional conditions. To achieve this goal, solution NMR spectroscopy associated with in house developed in vitro production and isotopic labelling methods, will be used to observe such large machinery while it is processing substrate proteins. The approach will be complemented by cryoEM to study interactions between ClpP/ClpX/ATP and client proteins.

Methods :

High Field NMR, Electron Microscopy, Cell-Free protein expression, Advanced isotopic labelling

Up to 3 relevant publications of the team:

• Macek *et al.* **Unraveling Self-Assembly Pathways of the 468 kDa Proteolytic Machine TET2**. *Science Advances* 3, e1601601 (2017) doi:/10.1126/sciadv.1601601
• Mas *et al.* **Structural Investigation of a Chaperonin in Action Reveals How Nucleotide Binding Regulates the Functional Cycle**. *Science Advances* 4, eaau4196 (2018) doi:/10.1126/sciadv.aau4196
• Gauto *et al.* **Integrated NMR and cryo-EM atomic-resolution structure determination of a half-megadalton enzyme complex.** *Nature Communication* 10, 2697 (2019) doi:/10.1038/s41467-019-10490-9

Requested domains of expertise (up to 5 keywords): Biochemistry, Structural Biology, Biophysics