Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

Supervisor(s):
Jérôme Boisbouvier, jerome.boisbouvier@ibs.fr

Host laboratory:
IBS

Host group/team:
NMR of large biomolecular assemblies

Title of the M2 research internship:
Structural and Functional investigation of ClpX/ClpP proteolytic machine in action

Project summary:
Caseinolytic proteases (ClpP - 14*22 = 308 kDa) are large barrel-shaped serine proteases found in bacteria. Although ClpPs are capable of degrading small peptides on their own, their association with ClpX, an unfoldase, is required to degrade larger peptides and proteins. ClpX is an energy-dependent AAA+ ATPase, forming a ring of six subunits (ClpX - 6*44 = 264 kDa), capable of unfolding and translocating the client protein into the ClpP barrel pore. Two rings of ClpX bind to the ClpP barrel to form a 0.8 MDa complex. The ClpP/X assembly is an elaborate macromolecular machine that participates in protein degradation and thus cellular homeostasis. The ClpP/X assembly also plays an active role in the survival and virulence of pathogenic bacteria. In particular, it participates in the regulation of cell division by degrading its main regulator, a tubulin-like protein called FtsZ. The objective of the project is to understand the mechanism of the ClpP/X machine, by obtaining detailed structural and kinetic information on the mechanisms of these ATP-driven proteolytic machines under functional conditions. To achieve this goal, we will integrate state-of-the-art high-field nuclear magnetic resonance spectroscopy and cryo-electron microscopy methods. Solution NMR spectroscopy combined with in-house developed cell-free production and isotope labeling methods will be used to observe this large machinery as it processes substrate proteins such as FtsZ. These results will be combined with cryoEM studies to obtain high resolution structural models of various intermediate states of the ClpP/X functional cycle in the presence of ATP and in interaction with the client protein. This integrated structural biology project offers a unique opportunity to study, at atomic resolution, the structure and mechanism of the dynamic molecular machine in the heat of action.

Keywords:
NMR, cryoEM, molecular machines

Relevant publications of the team:
Henot, Rioual, Favier, Macek, Crublet, Josso, Brutscher, Frech, Gans, Loison, Boisbouvier “Visualizing the Transiently Populated Closed-State of Human HSP90 ATP Binding Domain”. Nature Communications (2022). doi: 10.1038/s41467-022-35399-8

Törner, Kupreichyk, Gremer, Colas Debled, Fenel, Gans, Willbold, Schoehn, Hoyer, Boisbouvier “Structural Basis for the Inhibition of IAPP Fibril Formation by the Co-Chaperonin Prefoldin”. Nature Communications (2022). doi: 10.1038/s41467-022-30042-y

Gauto, Estrozi, Schwieters, Effantin, Macek, Sounier, Sivertsen, Schmidt, Kerfah, Mas, Colletier, Güntert, Favier, Schoehn, Schanda, Boisbouvier “Integrated NMR and cryo-EM atomic-resolution structure determination of a half-megadalton enzyme complex“. Nature Communications (2019). doi: /10.1038/s41467-019-10490-9

Mas, Guan, Crublet, Colas Debled, Moriscot, Gans, Schoehn, Macek, Schanda, Boisbouvier “Structural Investigation of a Chaperonin in Action Reveals How Nucleotide Binding Regulates the Functional Cycle”. Science Advances (2018). doi: 10.1126/sciadv.aau4196

Macek, Kerfah, Boeri Erba, Crublet, Moriscot, Schoehn, Amero,Boisbouvier “Unraveling Self-Assembly Pathways of the 468 kDa Proteolytic Machine TET2”. Science Advances (2017). doi: 10.1126/sciadv.1601601