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

Supervisor(s):
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Host laboratory:
IBS, NMR group
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Title of the M2 research internship:
Structural and Functional investigation of ClpX/ClpP proteolytic machine in action

Project summary:
Caseinolytic proteases (ClpP – 300 kDa) are large, cylindrical serine proteases, present in bacteria. Although ClpPs are capable of degrading small peptides by themselves in vitro, association with their cognate disassembly chaperone ClpX is required in vivo to degrade larger peptides and proteins. ClpX is an energy-dependent AAA+ ATPase, forming a ring of six subunits (MW 270 kDa), able to unfold and translocate client protein to ClpP cleavage catalytic centers. Two rings of ClpX bind to ClpP to form a complex assembly of 0.8 MDa. ClpP/X assembly is an elaborated macromolecular machine involved in degradation of proteins and thus, cell homeostasis. ClpP/X assembly also plays an active role in survival and virulence of pathogenic bacteria.
The objective of the project is to understand the mechanism of the ClpP/X machine, by obtaining detailed structural insights and kinetic information on the mechanisms of these ATP-fueled proteolytic machinery 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 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. These results will be combined with cryoEM studies, in order to obtain high resolution structural models of various intermediate states of ClpP/X functional cycle in presence of ATP and in interaction with client protein. This integrated structural biology project offer an unique opportunity to study, at atomic resolution, the structure and the mechanism of dynamics molecular machine in the heat of action.

Keywords:
NMR, cryoEM, molecular machine

Relevant publications of the team:

Mas G, Guan J-Y, Crublet E, Colas Debled E, Moriscot C, Gans P, Schoehn G, Macek P, Schanda P, Boisbouvier J. Structural Investigation of a Chaperonin in Action Reveals How Nucleotide Binding Regulates the Functional Cycle. Science Adv. 4, eaau4196 (2018).

Gauto D, Estrozi L, Schwieters C, Effantin G, Macek P, Sounier R, Sivertsen AC, Schmidt E, Kerfah R, Mas G, Colletier JP, Güntert P, Favier A, Schoehn G, Schanda P, Boisbouvier J. Integrated NMR and cryo-EM atomic-resolution structure determination of a half-megadalton enzyme complex. Nature Communication. doi: /10.1038/s41467-019-10490-9 (2019)

Törner R., Kupreichyk T, Gremer L, Colas Debled E, Fenel D, Gans P, Willbold D, Schoehn G, Hoyer W, Boisbouvier J. Structural Basis for the Inhibition of IAPP Fibril Formation by the Hsp60 Co-Chaperonin Prefoldin. Submitted to Nature Communication. doi: 10.1101/2021.10.20.465084