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

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Title of the M2 research internship:
Characterization of a proteasome interactant at the ribosome interface

Project summary:
The project objective is to understand how the ribosome and proteasome cellular machineries cooperates within cells to avoid the accumulation of protein aggregates or to recycle stalled ribosomes. By eliminating disabled cytosolic protein, the proteasome systems is essential to maintain a functional proteome. This function is essential for extremophilic microorganims thriving in deep sea vents. We recently demonstrated that under stress, a significant part of the proteasome of P. abyssi interacts with the polyribosomes. Interactomics and proteomics allowed to identify a novel proteasome-binding proteins in the native ribosomal fraction. This unassigned protein shows some homologies to GTPases The purified recombinant protein apparently forms a large complex apparently made of 6 subunits. The project aims is to characterize in vitro its interaction with the reconstructed proteasome complex using various biophysical tools (Biacore, SecMALS and AUC). Its interactions with native polysomes and proteasomes will be assayed by using pull downs experiments. Finally assays will be designed to study the degradation of defective translational products by the archaeal proteasome and to demonstrate the role of the novel partner in this process. Preliminary crystallisation conditions have been obtained and will be optimized to obtain the X-ray structure of the protein complex. Samples containing protein in complex with the proteasome will be prepared to screen for Cryo Electron Microscopy experiments.

Keywords:
intracellular proteolysis, intracellular dynamics of supramolecular complexes, integrated structural biology

Relevant publications of the team:

Mahieu, E., J. Coves, G. Kruger, A. Martel, M. Moulin, N. Carl, M. Hartlein, T. Carlomagno, B. Franzetti & F. Gabel, (2020) Observing Protein Degradation by the PAN-20S Proteasome by Time-Resolved Neutron Scattering. Biophys J 119: 375-388.

Structural Insight into Ubiquitin-Like Protein Recognition and Oligomeric States of JAMM/MPN(+) Proteases. Shiyun Cao, Sylvain Engilberge, Eric Girard, Frank Gabel, Bruno Franzetti, Julie A Maupin-Furlow. Structure (London, England : 1993), 2017, ⟨10.1016/j.str.2017.04.002⟩

Basbous H, Appolaire A, Girard E, Franzetti B. Characterization of a glycyl-specific TET aminopeptidase complex from Pyrococcus horikoshii. J Bacteriol. 2018.

Cao, S., S. Engilberge, E. Girard, F. Gabel, B. Franzetti * & J.A. Maupin-Furlow, (2017) Structural Insight into Ubiquitin-Like Protein Recognition and Oligomeric States of JAMM/MPN+ Proteases. Structure 25: 823-833 e826.

Ibrahim, Z., A. Martel, M. Moulin, H.S. Kim, M. Hartlein, B. Franzetti* & F. Gabel, (2017) Time-resolved neutron scattering provides new insight into protein substrate processing by a AAA+ unfoldase. Scientific reports 7: 40948.

Colombo, M., Girard, E., and Franzetti, B. * (2016) Tuned by metals: the TET peptidase activity is controlled by 3 metal binding sites. Scientific reports 6, 20876.

Appolaire, A., M.A. Dura, M. Ferruit, J.P. Andrieu, A. Godfroy, S. Gribaldo & B. Franzetti*, (2014) The TET2 and TET3 aminopeptidases from Pyrococcus horikoshii form a hetero-subunit peptidasome with enhanced peptide destruction properties. Mol Microbiol 94: 803-814.