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

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Title of the M2 research internship:
Visual Proteomics by high resolution cryo-EM of crude cell extracts

Project summary:
Cryo-electron microscopy (cryo-EM) is undergoing a rapid revolution, enabling atomic resolution structural insights into samples of ever greater complexity. One of the current bottlenecks of cryo-EM and other in vitro structural techniques is undoubtedly the sample preparation. In most cases it consists in isolating one protein complex of interest in a particular state before elucidating its structure. Here, we propose to partially skip the purification process and to work on semi-heterogeneous samples as close as possible to their native cellular state. Besides avoiding costly and lengthy purification, the advantages of working with non-purified samples are multiple: one can observe novel proteins which would not have been identified in a target-oriented approach, analyze complexes that are not amenable to over-expression, capture transient assemblies involving different interaction partners or different functional states, etc.

In the proposed internship project, the student will participate in fractionation of crude cell lysates, initiate analysis of fractions' contents by mass-spectrometry to identify most interesting targets, and image the obtained fractions by negative stain and cryo-EM. Dependent on the student profile, the project will then be oriented towards either (i) development and implementation of an effective data-processing pipeline for high resolution analysis of the acquired heterogeneous cryo-EM data or (ii) application of state-of-the-art image analysis tools for structural analysis of assembly intermediates of a complex macromolecular assembly.

Keywords:
cryo-EM, visual proteomics, cell extracts

Relevant publications of the team:
Desfosses A, Venugopal H, Joshi T, Felix J, Jessop M, Jeong H, Hyun J, Heymann JB, Hurst MRH, Gutsche I & Mitra AK (2019b) Atomic structures of an entire contractile injection system in both the extended and contracted states. Nat. Microbiol. 4: 1885–1894