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

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Host laboratory:

Lab : IBS

Host group/team:

Methods & Electron Microscopy group ; Pneumococcus group

Title of the M2 research internship:

In situ **cryo-FIB-tomography study of coat assembly during bacterial sporulation**

Project summary:

Bacterial spores exhibit remarkable resistance to environmental stresses due to specialized molecular structures that shield their cellular content. This resilience is advantageous when spores serve beneficial purposes, such as in probiotics (e.g., *Bacillus subtilis*), but poses significant challenges in public health, food safety, and biowarfare when associated with pathogenic species (e.g., *Bacillus cereus*, *Clostridium difficile*).

A key determinant of spore resistance is the **coat**, a robust extracellular shell composed of four proteinaceous layers, built through intricate interactions among ~80 different proteins. Despite its critical role, the assembly and architecture of the coat remain largely elusive due to its prolonged (>7-h) and complex formation process.

Using **cryo-electron tomography (cryo-ET)** on spore lamellae generated by **cryo-FIB/SEM** (cryo-focused ion beam milling coupled to scanning electron microscopy), we recently provided first structural insights into early coat assembly in *B. subtilis* (Bauda et al., *Nat Commun* 2024). Building on this breakthrough, the proposed M2 project aims to unravel the **molecular mechanisms driving the polymerization of the innermost coat layer**, which is formed by SpoIVA - the most conserved and functionally essential coat protein across all spore-forming bacteria, including pathogens. SpoIVA is an ATPase that polymerizes into a scaffold-like exoskeleton, anchoring the rest of the coat. Preliminary **cryo-FIB-ET** data suggest a dynamic polymerization process, where SpoIVA first assembles into **filaments** (~2.5h after sporulation initiation) before transitioning into a **2D matrix of unknown architecture** (~4h after sporulation initiation). To complement these *in situ* observations, we have successfully reconstituted SpoIVA filaments and 2D crystals *in vitro*. However, *in vitro* studies alone cannot fully capture the polymerization mechanism, which is regulated by various cellular partners.

The project is at a pivotal stage to conduct a comprehensive **structural and cellular analysis of SpoIVA polymerization using cryo-FIB-ET**. Specifically, the M2 internship will focus on:

1. **Transferring the established FIB milling protocol** (Bauda et al., 2024) from the CEA FIB/SEM to the IBS FIB/SEM equipment.
2. **Collecting high-resolution cryo-FIB-ET datasets** on *B. subtilis* sporulating cells to unravel the structure of SpoIVA filaments and 2D matrix.

A key advantage of this project is the availability of an optimized sample preparation protocol and the demonstrated feasibility of visualizing SpoIVA assemblies via cryo-FIB-ET. In addition, it involves a collaboration between a group with expertise in biology and a group with expertise in, and privileged access to, instrumentation: a synergy that is essential to the project. This work will lay the foundation for a **PhD project** focused on dissecting the **assembly dynamics of the two structured inner coat layers** formed by SpoIVA and CotE using cryo-FIB-ET and cryo-PALM super-resolution microscopy (architecture at different sporulation stages, interaction with and role of partners, …).

Keywords:

Cryo-FIB/SEM, cryo-electron tomography, integrated structural and cellular microbiology, bacterial sporulation, cell envelope, macromolecular assemblies.

Relevant publications of the team:

Bauda E, **Gallet B**, … **Morlot C** (2024). Ultrastructure of macromolecular assemblies contributing to bacterial spore resistance revealed by in situ cryo-electron tomography. *Nat. Commun*. 15(1):1376.

Shimakawa G, Demulder M, Flori S, …, **Gallet B**, et al. (2024). Diatom pyrenoids are encased in a protein shell that enables efficient CO2 fixation. *Cell* 187(21):5919-5934.e19.

Rodrigues CDA, … **Morlot C** (2016). A ring-shaped conduit connects the mother cell and forespore during sporulation in B. subtilis. *Proc. Natl. Acad. Sci.USA* 113(41):11585-11590.