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

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Host laboratory: 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 shell that assembles at the surface of the spore from several dozens of distinct protein species. Despite its critical role, the assembly dynamics and architecture of the coat remain largely elusive, partly 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 provided first structural insights into early coat assembly in *B. subtilis*, showing the emergence of five layers of distinct composition and architecture (Bauda et al., *Nat Commun* 2024). Building on this breakthrough, the proposed M2 project aims to **describe the architecture 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** (~2.5 h after sporulation initiation) before transitioning into a **2D matrix** (~4.5 h after sporulation initiation).

The project is at a pivotal stage to conduct the **structural characterization of SpoIVA polymers at the surface of *B. subtilis* spores using cryo-FIB-ET**. Specifically, the M2 internship will focus on:

1. **Optimizing the cell vitrification and FIB milling protocol** for sporulating *B. subtilis* cells.
2. **Collecting and processing preliminary high-resolution cryo-ET datasets** on *B. subtilis* cryo-FIB lamellae to generate first insights into the structure of SpoIVA filaments and 2D matrix.

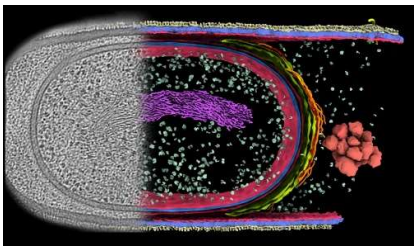

Illustration of data that will be generated during the M2 internship. Composite image showing a developing spore of *B. subtilis* observed by cryo-ET (left) and the segmentation of various cellular components (right): spore chromosome in violet, membranes in pink, cell wall in blue, surface glycans in light yellow, coat layers in yellow/orange/red, storage granules in salmon, ribosomes in light green.

Key assets of this project are the demonstrated feasibility of visualizing SpoIVA assemblies via cryo-FIB-ET (Bauda et al., 2024), and the synergy between the two groups involved: the MEM group, which has expertise and privileged access to cryo-FIB/SEM and electron microscopes, and the PG group, which specializes in bacterial sporulation. This work will lay the foundation for a PhD project focused on dissecting the assembly dynamics of the SpoIVA layer in relation to key protein partners using cryo-FIB-ET, biophysics, bacterial genetics and cell phenotyping.

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.