

Internship Proposal Master 2 Research – 2027

Mechanisms of extracellular vesicle production by the ESCRT machinery in a fungal phytopathogen

Keywords : extracellular vesicles (EV), phytopathogenic fungus *Botrytis cinerea*, ESCRT machinery (Endosomal Sorting Complexes Required for Transport), biomimetic systems and fluorescence lifetime imaging microscopy (FLIM)

Applicant profile : We are looking for a highly motivated candidate with knowledge and skills in microbiology, biochemistry, and microscopy. Applicants should be able to work effectively in a team and possess strong communication and writing abilities. The internship will begin in January 2027 and will last for six months.

Project description : Fungal **extracellular vesicles (EVs)** are nanometric particles surrounded by a lipid bilayer and carrying diverse biomolecules. They play a key role in intercellular communication and are involved in numerous pathologies in animals, plants, and fungi. Despite their importance, the mechanisms governing **their formation, release, and passage through the thick fungal cell wall** remain largely unknown. **This project aims to uncover the molecular mechanisms controlling the formation and release of fungal EVs, focusing on membrane tension and on the role of the ESCRT machinery** (Endosomal Sorting Complexes Required for Transport), a protein complex essential in mammals for membrane budding and the biogenesis of multivesicular bodies. Our hypothesis is that **the regulation of fungal membrane tension conditions EV formation, and that the tension of the EVs themselves influences their ability to cross the cell wall**. To test this hypothesis, we will combine the generation of deletion mutants of the ESCRT protein Snf7 in the fungus, quantitative analysis of purified EVs by NTA (Nanoparticle Tracking Analysis), which measures their size and concentration, with measurement of membrane tension using FLIM (Fluorescence Lifetime Imaging Microscopy) in living cells, in order to monitor membrane dynamics associated with EV production and release. **The internship is part of an interdisciplinary, multiscale approach combining membrane dynamics in living cells, EV isolation and characterization, and in vitro biomimetic systems.** The ultimate goal is to determine **the role of the ESCRT protein Snf7** in EV formation, release, and membrane mechanics, by linking membrane tension measurements obtained by FLIM to variations in EV quantity and size distribution revealed by NTA.

Presentation of the Research Team and partner: The research team ECCP (Effectors of Cellular Communication at the fungal-Plant interface, <https://bioger.versailles-saclay.hub.inrae.fr/equipes/eccp-effectors-of-cellular-communication-at-the-fungal-plant-interface>) is focusing on fungal diseases in plants. The main objectives of our group are to understand (1) the mode of action and plant targets of fungal effectors and (2) the mechanisms of communication between fungi and plants. Guillaume Dupuis, a partner in the project, is a senior lecturer at Université Paris-Saclay. His team at ISMO in Orsay has strong expertise in cell biology and in the imaging of intracellular structures using super-resolution microscopy.

Methods used during the internship :

- Microbiology: growth of mutant strains of phytopathogenic fungi
- Biochemistry: purification and characterization of EVs using nanoparticle tracking analysis (NTA)
- Microscopy: fluorescence lifetime imaging microscopy (FLIM)

References :

- 2022 - Rutter, B. D. *et al.* The development of extracellular vesicle markers for the fungal phytopathogen *Colletotrichum higginsianum*. *J. Extracell. Vesicles*
- 2026 - Kumakura, N. *et al.* Dihydroxyhexanoic acid biosynthesis controls turgor in pathogenic fungi. *Science*

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