



Laboratoire de Physiologie Cellulaire & Végétale

Unité Mixte de recherche UMR 5168 CNRS – CEA - UGA - UMR1417 INRAE

## **Understanding the molecular mechanisms of lipid transporters regulating lipid homeostasis in cells using time-resolved small-angle neutron scattering**

### **Job description**

A three-years PhD position held jointly between the Laboratoire de Physiologie Cellulaire et Végétale (LPCV, Grenoble, France) and the Institut Laue Langevin (ILL, Grenoble, France)

### **Summary of the project**

Plants need to adapt to environmental changes in order to grow and survive. Phosphate (Pi) is an essential nutrient that is often limited in soils. To adapt to low Pi, plants activate the degradation of phospholipids, the main components of cell membranes, and replace them with galactolipids synthesized in the chloroplast. The reduction in Pi levels in soil has been shown to trigger a significant remodeling of membrane lipids, involving the upregulation of genes involved in phospholipid degradation and galactolipid synthesis. This remodeling occurs in the membrane of several cell compartments and requires substantial transport of lipids to redistribute them between the different organelles. While the enzymes involved in lipid metabolism are well characterized, proteins and mechanisms involved in lipid transport during Pi starvation are less well understood. In the model plant *Arabidopsis thaliana*, we have identified a protein involved in lipid transfer that plays a role in this process: AtVPS13M1. We also showed that this protein has different binding affinity depending on lipid classes, raising fundamental questions about its implication in the regulation of lipid homeostasis during Pi-starvation stress in plants.

The objective of this project is to elucidate, at the molecular level, the mechanisms by which AtVPS13M1 modulates lipid remodeling. This will be achieved by analyzing its lipid transport capacity *in vitro* using time-resolved small-angle neutron scattering (TR-SANS) coupled with contrast match. Preliminary work showed that this system is suitable for analyzing lipid transfer between liposomes in a protein concentration dependent manner. The PhD candidate will use this setup to answer essential questions about AtVPS13M1, including the specificity of transfer of each lipid class or the importance of membrane structure, and will be involved in all steps of the project, including protein purification, preparation of deuterated lipids and TR-SANS experiments and analyses. The integration of TR-SANS outcomes with existing lipid binding and *in vivo* (i.e., lipidomics on mutants) data will serve as a fundamental element in enhancing our comprehension of the mechanisms through which AtVPS13M1 modulates lipid transport in plant cells, with a particular emphasis on its response to Pi starvation, a process essential for plant survival in low-Pi soils. Additionally, it will provide to the community a novel and straightforward approach to examine the real-time transport of natural lipids *in vitro*.

### **Associated references**

Leterme, S., Albriex, C., Brugière, S., Couté, Y., Dellinger, J., Gillet, B., Hughes, S., Castet, J., Bernard, A., Scheuring, D., et al. (2024). AtVPS13M1 is involved in lipid remodeling in low phosphate and is located at the mitochondria surface in plants. Preprint at BioRxiv, <https://doi.org/10.1101/2024.05.22.594332>.



## Laboratoire de Physiologie Cellulaire & Végétale

Unité Mixte de recherche UMR 5168 CNRS – CEA - UGA - UMR1417 INRAE

Leterme, S., Bastien, O., Aiese Cigliano, R.A., Amato, A., and Michaud, M. (2023). Phylogenetic and structural analyses of VPS13 proteins in Archaeplastida reveal their complex evolutionary history in Viridiplantae. *Contact* (Thousand Oaks). <https://doi.org/10.1177/25152564231211976>.

Leterme, S. and Michaud, M. (2022). Non-vesicular glycerolipids transport in plant cells. *Lipids in Plants and Algae: From Fundamental Science to Industrial Applications* 101, 121–189. <https://doi.org/10.1016/bs.abr.2021.07.001>.

Perez-Salas, U.; Garg, S.; Gerelli, Y.; Porcar, L. Deciphering Lipid Transfer between and within Membranes with Time-Resolved Small-Angle Neutron Scattering. In *Current Topics in Membranes*; Elsevier, 2021; Vol. 88, pp 359–412. <https://doi.org/10.1016/bs.ctm.2021.10.004>.

Garg, S.; Porcar, L.; Woodka, A. C.; Butler, P. D.; Perez-Salas, U. Noninvasive Neutron Scattering Measurements Reveal Slower Cholesterol Transport in Model Lipid Membranes. *Biophys J* 2011, 101, 370–377. <https://doi.org/10.1016/j.bpj.2011.06.014>.

### Methods

- Molecular biology (cloning, PCR...)
- Protein expression from different systems (cell free, insect cells...)
- Protein purification by affinity and size exclusion chromatography
- Protein characterization (SDS-PAGE, DLS, Mass spectroscopy...)
- Lipid purification and characterization from yeast cells
- Fluorescence assays for lipid transport/binding assessment
- TR-SANS experiments and data analysis

### Expected profile and skills

The project is an interdisciplinary project at the interface between biochemistry and biophysics. We are seeking for a highly motivated candidate with a Master degree in the field of molecular biology and biochemistry or biophysics. An experience with protein production and purification will be an advantage.

**For more information or to candidate (CV + motivation letter + references):**

**Morgane Michaud (LPCV): [morgane.michaud@cea.fr](mailto:morgane.michaud@cea.fr)**

**Lionel Porcar (ILL): [porcar@ill.fr](mailto:porcar@ill.fr)**