Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2025-2026

Laboratory/Institute: LPCV
Director: Eric Marechal
Team: MetalStress
Head of the team: Stéphane Ravanel

Name and status of the scientist in charge of the project: Jonathan Przybyla-Toscano, INRAE researcher

HDR: yes ☐ **no** ■

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Laboratory/Institute: IBS
Director: Winfried Weissenhorn
Team: Métalloprotéines
Head of the team: Yvain Nicolet

Name and status of the scientist in charge of the project: Mickaël V. Cherrier, UGA Assistant Professor

HDR: yes ■ **no** ☐

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Program of the Master's degree in Biology:

☐ Microbiology, Infectious Diseases and Immunology ■ Biochemistry & Structure
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Title of the project: Biochemical and structural characterization of Casparian strip membrane domain protein 1 (CASP1) from *Arabidopsis thaliana*

Objectives (up to 3 lines):

The objective of this project is to investigate the metal-binding capacity of AtCASP1 and to determine its three-dimensional structure in both native and metal-bound states.

Abstract (up to 10 lines):

The Casparian strip in plant roots acts as a selective barrier for ion uptake and relies on CASP membrane proteins for its formation. CASPs belong to the plant-specific MARVEL superfamily, an ancient and diverse group present across eukaryotes. Despite their biological importance, the biochemical properties of CASPs remain poorly understood. Recent ionomic and comparative studies in *Arabidopsis thaliana* suggest a potential role in metal homeostasis. The presence of conserved acidic and cysteine residues in CASPs points to possible metal-binding capabilities. To investigate this, we successfully expressed and purified the tagged protein corresponding to CASP1 from Arabidopsis in *Escherichia coli* using an optimized detergent-based purification protocol. The next step of the project will be to purify the untagged version of CASP1. Then, using

the recombinant proteins, the student will analyse several biochemical properties (oligomerization state and metal-binding capacity), and will contribute to resolving the 3D structures of both the apo- and holo-forms of CASP1.

Methods (up to 3 lines):

This internship will take place jointly between LPCV and IBS laboratories. This project will involve biochemical (protein purification under aerobic and anaerobic conditions, *in vitro* metal cofactor reconstitution, metal-binding assays, and mass photometry) and structural biology approaches (cryo-electron microscopy).

Up to 3 relevant publications of the team:

[1] J. Przybyla-Toscano, C. Chetouhi, L. Pennera, Y. Boursiac, A. Galeone, F. Devime, T. Balliau, V. Santoni, J. Bourguignon, C. Alban, S. Ravanel, New insights into uranium stress responses of Arabidopsis roots through membrane- and cell wall-associated proteome analysis, Chemosphere 370 (2025) 143873. https://doi.org/10.1016/j.chemosphere.2024.143873.

[2] M. Roland, J. Przybyla-Toscano, F. Vignols, N. Berger, T. Azam, L. Christ, V. Santoni, H.-C. Wu, T. Dhalleine, M.K. Johnson, C. Dubos, J. Couturier, N. Rouhier, The plastidial Arabidopsis thaliana NFU1 protein binds and delivers [4Fe-4S] clusters to specific client proteins, J Biol Chem 295 (2020) 1727–1742. https://doi.org/10.1074/jbc.RA119.011034.

[3] Broc M.‡, Cherrier M.V.‡, Uzel A., Arias-Cartin R., Arnoux P., Brasseur G., Seduk F., Guigliarelli B., Legrand P., Pierrel F., Schoehn G., Maté M.J., Martin L., Grimaldi S., Nicolet Y., Magalon A., Walburger A., "A scaffold for quinone channeling between membrane and soluble bacterial oxidoreductases." Nature Structural & Molecular Biology. 2025 (In Press).

‡: equal contribution

Requested domains of expertise (up to 5 keywords):

Biochemistry, structural biology, plant biology