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

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Title of the M2 research internship:
Biochemical and Structural characterization of newly identified Uranium-binding proteins from plants

Project summary:
Uranium (U) is a natural element that is mainly redistributed in the environment due to anthropogenic activities. Contamination of soils and water by U and its absorption by plants represent a significant health risk for human beings. The aim of the team project is to gain insight into the molecular mechanisms governing the fate of U in plants. This includes the characterization of U transporters as well as the identification of U-targeted cellular proteins.
The goal of the present student project is the biochemical and structural characterization of U-binding proteins from plants that we recently identified using metalloproteomic approaches (Immobilized U affinity chromatography and conventional chromatographic and electrophoretic techniques, coupled to ICP-MS for metal profiling, and tandem mass spectrometry for protein identification). These proteins can be either the cellular targets of toxicity or actors of detoxification mechanisms. Upon the 91 U-binding candidate proteins identified, 2 from the most promising ones will be further characterized in this project. These proteins were chosen because of their known capacity to bind metals and of their amino acid composition (U binds preferentially to acidic residues). Recombinant U-binding proteins will be produced in Escherichia coli, purified and their ability to bind U and other metals will be checked. Metal binding assays in vitro by ICP-MS and tryptophan fluorescence titration will be used to determine the stoichiometry of metal binding and the dissociation constant for the complexes, respectively. Depending on the nature of the identified targets the effect of uranyl binding will be performed by thermal stability and circular dichroism analyses (structural proteins) or activity measurements (enzymes). To gain insight into the mechanisms of U binding to targets, the 3D structures determination of U-protein complexes will be initiated by either X-ray crystallography (preferred approach), solution-state NMR spectroscopy (small proteins) or alternatively by cryo-electronic microscopy (large complexes).

Keywords:
uranium, protein, structure

Relevant publications of the team:
Cobessi D., Dumas R., Pautre V., Meinguet C., Ferrer J.-L., and Alban C. (2012) Biochemical and Structural Characterization of the Arabidopsis Bifunctional Enzyme DTB Synthetase – DAPA Aminotransferase. Evidence for Substrate Channeling in Biotin Synthesis The Plant Cell, 24(4): 1608-1625.

Berthet S., Villiers, F., Alban, C. Serre, N. Martin-Laffon, J., Figuet, S., Boisson, A.-M. Bligny, R., Kuntz, M., Finazzi, G., Ravanel, S. and Bourguignon, J. (2018) Arabidopsis thaliana plants challenged with uranium reveal new insights into iron and phosphate homeostasis. New Phytologist, 217: 657–670

Sarthou M., Revel B., Villiers F., Alban C., Bonnot T., Gigarel O., Boisson A.-M., Ravanel S. and Bourguignon J. (2020) Development of a metalloproteomic approach to analyse the response of Arabidopsis cells to uranium stress. Metallomics, 12: 1302-1313