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

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Host laboratory:
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Title of the M2 research internship:
Bio-orthogonal chemistry to study the trafficking of the Atox1 copper chaperone in mammalian cells

Project summary:
The ability to specifically engineer a protein of interest in living cells offers a wide range of possible studies. A way that emerged within the last ten years is the use of bio-orthogonal chemistry strategies to incorporate a non-canonical amino acid (ncAA) at a specific position in the protein of interest. The development of cyclo-octyne ncAA enables the use of click chemistry to couple these ncAA to tetrazine-conjugated molecules such as fluorophores. Besides, this type of click chemistry reactions can be performed in vitro but also in living cells. In this master project, this strategy will be used to specifically fluorescently label Atox1. Atox1 is a small protein, crucial for copper (Cu) homeostasis in Human. Atox1 is a Cu chaperone that chelate Cu with high affinity upon its cellular entry. Cu-bound Atox1 traffics through the cell for delivery of Cu to target proteins. Since Cu is at the same time an element essential for life and heavily toxic for the cell in its free form, these trafficking processes are finely orchestrated and there is a need to better understand them. This goal will be achieved thanks to super resolution fluorescence microscopy. This imaging approach will enable to decipher Atox1 localization and dynamics in different conditions and at tens of nanometer resolution. This analysis will be performed in Cu normal, deprived and overloaded conditions to mimic different situations encountered by human cells in healthy and disease conditions. Besides, Atox1 has been shown to play a role in intracellular trafficking and excretion of platinum complexes used in cancer therapy. Therefore, the methodology developed in this project will enable to study this question. Finally, bio-orthogonal chemistry will be used to engineer Atox1 with a polarizing agent to explore a novel in cell NMR approach enabling to obtain molecular information within the cellular context.

Keywords:
bio-orthogonal chemistry, copper homeostasis, super resolution fluorescence microscopy

Relevant publications of the team:

Tardillo Suárez V, Gallet B, Chevallet M, Jouneau PH, Tucoulou R, Veronesi G*, Deniaud A*. Submitted to J. of Structural Biology.

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