Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2021-2022

Laboratory/Institute: Laboratoire de Chimie et Biologie des Métaux **Director:** Vincent ARTERO
Team: Metals and Organs (Met&Or) **Head of the team:** Aurélien DENIAUD

Name and status of the scientists in charge of the project: Aurélien DENIAUD, MCU UGA, Sabine HEDIGER, DR CNRS (Laboratoire CEA/MEM)
HDR: yes x **no** ☐
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Program of the Master's degree in Biology:

☐ Immunology, Microbiology, Infectious Diseases x Structural Biology of Pathogens
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Title of the project: Bio-orthogonal chemistry as a tool towards structural studies in mammalian cells by DNP-enhanced NMR

Objectives (up to 3 lines)

In this project, a bio-orthogonal chemistry approach will be developed to engineer in human cells Atox1, a protein central for copper trafficking. The methodology will be optimized to tag a polarizing agent on Atox1 to allow its structural investigation in-cell by hyperpolarized solid-state NMR.

Abstract (up to 10 lines)

Structural information of a target protein in its cellular environment is fundamental to understand its functions and interactions *in vivo*. This goal can be reached thanks to bio-orthogonal chemistry, which enables the specific engineering of the protein of interest by the introduction of an unnatural amino acid at a specific position and its further modification in living cells by click chemistry. Our aim is to use this strategy on Atox1, a major protein involved in copper trafficking in mammalian cells. Atox1 will be site-specifically labelled with a polarizing agent required to perform ultimately dynamic nuclear polarization-enhanced solid-state NMR (DNP-NMR) in mammalian cells. This hyperpolarization NMR technique has the potential to extract specific structural information inside the cellular environment, to better understand Atox1 copper binding and transfer to partner proteins. The development of the methodology is challenging and we plan to achieve in the framework of this project the first steps towards in-cell DNP-NMR.

Methods (up to 3 lines): Mammalian cell culture, molecular biology, bio-orthogonal chemistry, protein purification and modification, dynamic nuclear polarization-enhanced solid-state nuclear magnetic resonance

Up to 3 relevant publications of the team:

- Lelièvre P, Sancey L, Coll JL, Deniaud A, Busser B. *The Multifaceted Roles of Copper in Cancer: A Trace Metal Element with Dysregulated Metabolism, but also a Target or a Bullet for Therapy*, **Cancers**. 2020; 12 (12), 3594.
- I. Marin-Montesinos, D. Goyard, E. Gillon, O. Renaudet, A. Imberty, S. Hediger, G. De Paëpe, *Selective high-resolution DNP-enhanced NMR of Biomolecular binding sites*, **Chem. Sci.**, 2019, 10, 3366-3374.
- D. Gauto, O. Dakhlaoui, I. Marin-Montesinos, S. Hediger, G. De Paëpe, *Targeted DNP for biomolecular Solid-State NMR*, **Chem. Sci.**, 2021, 12, 6223.

Requested domains of expertise (up to 5 keywords): Molecular and cellular biology, biochemistry and/or NMR