UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Internship project Master 2

Year 2017-2018

Laboratory/Institute: IBS
Director: W Weissenhorn
Team: metalloproteins unit
Head of the team: Y Nicolet

Name and status of the scientist in charge of the project: M Cherrier **HDR: yes** ☐ **no** ☑
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Program the Master's degree in Biology:

☐ Neurosciences and Neurobiology ☐ Immunology, Microbiology, Infectious Diseases
☑ Integrative Structural Biology ☐ Physiology, Epigenetics, Development, Differentiation

Title of the project: Structural study of metalloproteins in anaerobic conditions using electron microscopy

Objectives (up to 3 lines):

The goal of this M2 proposal is to develop, with the candidate, the tools necessary to do the structural study of metalloproteins complexes sensitive to oxidation by oxygen by cryo electron microscopy.

Abstract (up to 10 lines):

Many metalloproteins, especially the one possessing an iron-sulfur cluster [Fe-S], are involved in key cell processes, such as photosynthesis, respiration, or oxygen transport. But they are often sensitive to degradation by oxygen, and must be studied in a strict anaerobic environment. Their structural analyses are usually performed using X-ray crystallography, but in the last few years, electron microscopy (EM) has become a more and more powerful technique. We would like to study, for the first time, metalloproteins sensitive to oxygen using this technique. We plan to study different metalloproteins complexes: first the FeFe-hydrogenase from the sulfate reducing bacterium *Desulfovibrio fructosovorans* and components of the assembly machineries for the active site of nitrogenase and for the biogenesis of [Fe-S] clusters in mammals.
This project has two sides: on the one hand a methodological development to prepare EM samples under anaerobic conditions and on the other hand a fundamental research on these biological systems.

Methods (up to 3 lines):

The protein complexes will be purified and characterized in anaerobic conditions. Techniques to prepare negative stain and cryo electron microscopy grids will have to be adapt in our glove boxes. Reconstruction of the proteins will be calculated using specialized software.

Up to 3 relevant publications of the team:

- Rohac R, *et al* (2016) Carbon–sulfur bond-forming reaction catalysed by the radical SAM enzyme HydE. *Nature Chemistry* **8(5)**: 491-500.
- Pagnier A, *et al* (2016) CO and CN^- syntheses by [FeFe]-hydrogenase maturase HydG are catalytically differentiated events. *Proceedings of the National Academy of Sciences* **113**: 104-109.
- Marinoni E, *et al* (2012) $(IscS\text{-}IscU)_2$ complex structures provide insights into Fe_2S_2 biogenesis and transfer. *Angewandte Chemie International Edition* **51**(22) : 5439-5442.

Requested domains of expertise (up to 5 keywords):

Biochemistry; Structural Biology; Bioinformatic