Internship project Master 2
Year 2017-2018

Laboratory/Institute: METALLO/IBS **Director:** W. Weissenhorn
Team: METALLO **Head of the team:** Yvain Nicolet
Scientist in charge of the project: Y. NICOLET (Group Leader) **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 and functional study of the FeFe-hydrogenase assembly machinery

Objectives (up to 3 lines):

Identification of the substrate of HydE, an essential component of the active site assembly machinery of the FeFe-hydrogenase. Identification of the missing components and development of an efficient in vitro assay for the whole process.

Abstract (up to 10 lines):

FeFe-hydrogenases, which catalyze the reversible reaction of oxidation of molecular hydrogen, have been widely studied in a joint effort to develop alternative and renewable energy sources. These enzymes use an organometallic active site composed of two iron atoms bound to cyanide and carbon monoxide ligands. These iron atoms are, in addition, bridged together by a unique azadithiomethyl ligand. This active is synthesized and inserted into the apo-hydrogenase by a dedicated machinery. In our laboratory, we have already solved the X-ray structures of two out of the three essential proteins, which compose this machinery. They correspond to proteins HydE and HydG that both belong to the widespread radical SAM protein superfamily. HydG converts L-tyrosine into CO and CN^-. While HydE is widely proposed to be involved in the synthesis of the azadithiomethyl ligand, neither its substrate, nor its exact function are known to date. We want to address this question using different techniques available in the laboratory and at IBS, combining structural biology and functional analyses.

Methods (up to 3 lines):

Anaerobic glove boxes, FPLC and HPLC-MS. Biochemistry (expression, purification…). Use of crude extracts and purified proteins, fractionation, X-ray crystallography and molecular docking.

Up to 3 relevant publications of the team:

Rohac R., Amara P., Benjdia A., Martin L., Ruffié P., Favier A., Berteau O., Mouesca J.M., Fontecilla-Camps J.C. and Nicolet Y. (**2016**) "Carbon-sulfur bond-forming reaction catalysed by the radical SAM enzyme HydE" *Nat. Chem. 8* 491-500
Sicoli G., Mouesca J.M., Zeppieri L., Amara P., Martin L., Barra A.L., Fontecilla-Camps J.C., Gambarelli S. and Nicolet Y. (**2016**) "Fine-tuning of a radical-based reaction by radical *S*-adenosyl-L-methionine tryptophan lyase" *Science 351* 1320-3
Pagnier A., Martin L., Zeppieri L., Nicolet Y. and Fontecilla-Camps J.C. (**2016**) "CO and CN- syntheses by [FeFe]-hydrogenase maturase HydG are catalytically differenciated events" *Proc Natl Acad Sci* U S A. *113* 104-9

Requested domains of expertise (up to 5 keywords):

Biochemistry, organic chemistry, structural biology.