

INTERNSHIP PROPOSAL

Institute and Group: IBS / Metalloprotein unit

Supervisor: Cherrier Mickaël V.

Email: mickael.cherrier@ibs.fr

Phone: 04 57 42 87 58

Research project title:

Structural study of the Nitrogenase active site maturation using for the first time CryoElectron Microscopy in anaerobic conditions.

5 Keywords to describe the project: Nitrogenase ; Structural Biology ; CryoElectron Microscopy ; Anaerobic conditions ; Proteins complexes

Description of the project (aims, experimental techniques, recommended background):

Metalloproteins, are involved in key cell processes, such as photosynthesis, respiration, or oxygen transport. But due to the presence of transition metals and notably iron-sulfur cluster [Fe-S] containing proteins are often sensitive to degradation by oxygen, and must be studied under a strict anaerobic environment. Structural analyses are usually performed using X-ray crystallography, but in the last few years, electron microscopy has become a more and more powerful technique. We would like to study, for the first time, metalloproteins sensitive to oxygen using this technique. The M2 student will use the unique ability to freeze cryo electron microscopy grids under anaerobic conditions we are currently developing, to study a multiprotein complex ensemble responsible for the maturation of the nitrogenase active site. Nitrogenase is present in nitrogen-fixing organisms where it plays a key role in the global nitrogen cycle as it catalyzes the difficult reduction of dinitrogen to ammonia under ambient conditions. The selected student need to have strong knowledge in biochemistry and structural biology. Some knowledge in bioinformatics would be an added value.

Justification that the internship's subject fits with the general theme of GRAL:

The active site of the nitrogenase is synthesized by a highly complex multiprotein machinery. The study of the structure function relationships of this large complex falls into the GRAL Theme. Furthermore, development of new techniques such as protocols to prepare cryo-EM grids under anaerobic conditions is something that has not been reported so far in the world. Thus such technical challenge also falls into the GRAL Themes.

Relevant publications of the team:

Cherrier, M.V., Amara, P., Talbi, B., Salmain, M., and Fontecilla-Camps, J.C. (2018). "Crystallographic evidence for unexpected selective tyrosine hydroxylations in an aerated achiral Ru-papain conjugate." *Met. Integr. Biometal Sci.* 10, 1452–1459.

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., Zepieri, 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, 1320–1323.