

INTERNSHIP PROPOSAL

Institute and Group: IBS, group Metalloproteins

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Research project title: Structure/Function Relationships of radical SAM enzymes involved in the biosynthesis of antibiotics.

5 Keywords to describe the project: Antibiotics, X-ray crystallography, Enzyme mechanism, Radical-based chemistry, Metalloproteins.

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

Looking for new antibiotics is a major challenge to fight multidrug resistant pathogenic bacterial strains. Over the last few years, our gradual understanding of the biosynthetic pathways of different classes of antibiotics has underscored the key role of a specific class of enzymes, which belong to the radical *S*-adenosyl-L-methionine proteins (rSAMp) family. These metalloproteins use radical-based chemistry to perform difficult and often unprecedented reactions. We thus propose to study the structure-function relationships of a rSAMp involved in the synthesis of antibiotic. The aims of this internship project are to i) implement an expression and purification protocol to produce suitable amounts of the enzyme under anaerobic conditions, ii) crystallize this protein and, possibly, iii) solve the structure of the protein. The master2 student will learn how to overexpress and purify a metalloprotein under anaerobic conditions. Since these proteins are sensitive to oxygen, they need to be manipulated in glove boxes. Then, using our automated crystallization robot, the student will search for initial crystallization conditions that he/she will subsequently manually improve to solve the structure of the protein by X-ray crystallography. Recommended background: practical knowledge in biochemistry and academic knowledge in organic chemistry.

Relevant publications of the team:

1. 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
2. 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
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 differentiated events" *Proc Natl Acad Sci U S A.* **113** 104-9