Grenoble Alliance for Integrated Structural & Cell Biology

INTERNSHIP PROPOSAL

Institute and Group: IBS-METALLO

Supervisor: Yvain Nicolet **Phone: +33457428603**

Email: yvain.nicolet@ibs.fr

Research project title: Radical-based chemistry and antibiotics: the case of radical *S*-adenosyl-L-methionine enzymes

Keywords: X-ray crystallography, Enzyme mechanism, Metalloproteins, Anaerobic conditions; Iron sulfur cluster.

Description of the project:

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 and of its interactions with its partner proteins. The aims of this internship project are to i) produce and purify suitable amounts of the enzyme under anaerobic conditions, ii) produce and purify its substrate, iii) characterize the interactions between the two partners under anaerobic conditions, iv) crystallize each protein separately and in complex, and, possibly, v) solve the X-ray structures. 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.

Justification that the internship's subject fits with the general theme of GRAL (3 lines):

Our project fulfils the objectives of GRAL as it aims at acquiring knowledge on the structure and function of proteins involved in the biosynthetic pathway of promising antibiotics against multidrug resistant bacterial pathogens.

Relevant publications of the team (3 max):

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 differenciated events" *Proc Natl Acad Sci* U S A. *113* 104-9