

Master in Chemistry

Sujet de stage de Master 2 (1 page max.)

Laboratoire : Institut de Biologie Structurale

Directeur : Winfried Weissenhorn

Intitulé de l'équipe : METALLO

Responsable : Yvain Nicolet

Nom et Qualité du Responsable du Stage : Yvain Nicolet

HDR oui non

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Parcours de Master 2 (*Rayer la/les mention(s) inutile(s)*) :

Chemistry for Life Sciences (CLS)

~~Polymers for Advanced Technologies (PTA)~~

~~Organic Synthesis (SOIPA)~~

Titre du sujet : *Structural characterization of the protein DarE involved in the synthesis of the antibiotic darobactin*

Objectifs visés du stage (5 lignes max) :

The goal of this internship is to address the structure-function relationships of the DarE protein involved in the post-translational modification of a short peptide to produce darobactin. The radical-based chemistry involved is unique and would inspire chemists for future use in syntheses. We aim at understanding the molecular mechanism of the enzyme combining structural biology and theoretical calculations.

Intérêts pédagogiques et compétences visées (5 lignes max) :

The candidate will learn how to purify and crystallize a metalloprotein sensitive to oxygen. He/she will also be in charge of improving protein expression. If possible, He/she will also learn how to collect X-ray diffraction data at synchrotron beamlines and how to solve a crystal structure. He/she will also learn how to work in a collaborative project involving several groups from Europe and abroad.

Résumé :

Darobactin has been recently identified as a potent antibiotic against Gram negative bacteria. It corresponds to a small peptide that is ribosomally synthesized and subsequently modified by DarE to form two macrocycles. DarE belongs to the radical SAM enzyme superfamily and uses radical-based chemistry to make C-C bonds between non-activated sp^2 and sp^3 carbons, in the one hand and to create an ether bridge between two tryptophan side chain sp^2 carbons on the other hand. Such modifications confer stability and antibiotic properties to the 7-mer peptide. The presence of FeS clusters renders the protein highly sensitive to oxygen and all the experiments will be performed under anaerobic environment. The project is a collaboration with groups in Germany and in the US, who will perform the functional characterizations and provide substrate.

Approches & matériels utilisés (5 lignes max) :

The protein will be overproduced and purified using standard techniques (including affinity chromatography), but all the experiments will be performed under anaerobic conditions using our set of gloveboxes to work from gen to structure. The candidate will then seek for crystals using both our highthroughput setup and manual approaches to optimize crystallization conditions, and if possible will collect diffraction data and solve the X-ray structure of the protein in complex with its substrate.

Domaines de compétences souhaitées du candidat (3 lignes max):

Biochemistry, structural biology, organic chemistry.

Dates du stage : 4/01/2021 – 30/06/2021