UFR CHIMIE & BIOLOGIE Université Grenoble Alpes

Master in Chemistry

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

Laboratoire: Institute of Structural Biology (IBS)
Directeur: WEISSENHORN Winfried

Intitulé de l'équipe: Metalloproteines Responsable: NICOLET Yvain
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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)~~

Subject: ***Dynamics and mechanisms of peptide cyclization and hydroxylation in metalloproteins***

Objectifs visés du stage (5 lignes max) :
The objective of the internship is to characterize the dynamics of two concerted reactions, intrapeptide C–C crosslinking and β-hydroxylation, installed onto peptide substrates by a two-domain enzyme consisting of a radical SAM domain and a new family of α-ketoglutarate-dependent Fe oxygenase.

Intérêts pédagogiques et compétences visées (5 lignes max) :

1. Characterization of intermediate species to determine the reaction order via MS.
2. Characterization of intermediate organic radicals or inorganic species via EPR.
3. Enzyme kinetics and product characterization via HPLC.
4. Capacity to make decisions based on available results.

Résumé :

We have discovered a family of fused radical SAM and α-ketoglutarate (αKG)-dependent HExxH oxygenase domain proteins that catalyze cyclophane formation and β-hydroxylation on their peptide substrates. Their products are ribosomally synthesized and post-translationally modified peptides (RiPPs) that contain three C–C crosslinks between an aromatic amino acid and the β-carbon of another residue, installed by the radical SAM domain, and three β-hydroxylations, installed by the oxygenase domain. These C–C crosslinks are notoriously difficult to generate by conventional synthetic chemistry; thus, our goal is to understand the mechanism by which the enzyme achieves these transformations and to determine the order of the reactions. The work will focus on substrate mutants to probe reaction specificity, as well as on the characterization of the enzyme–substrate interaction and reaction intermediates using mass spectrometry and EPR spectroscopy.

Approches & matériels utilisés (5 lignes max) :

1. Protein and peptide purification
2. Anaerobic protein manipulation
3. In vitro assays characterized via HPLC
4. Mass spectrometry sample preparation
5. EPR sample preparation and data interpretation

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

1. Basic understanding of bio-organic and bio-inorganic chemistry.
2. Basic knowledge of mass spectrometry and EPR.

Dates du stage: 04/01/2027 – 30/06/2027