Aix*Marseille université

Ecole Doctorale des Sciences Chimiques ED250

Contrats Doctoraux **2017**

Structure-reactivity relationship and engineering of a copper-containing metalloenzyme

Laboratory: Institut des Sciences Moléculaires de Marseille (iSm2, UMR CNRS 7313)

Team: BiosCiences

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Context:

The histidine brace is a rare motif found in metalloenzymes / metalloproteins for which a *N*-terminal histidine residue coordinates a metal ion in a bidentate fashion (*N*-terminal amine and nitrogen of the side chain imidazole group) (Figure 1). For instance, it is observed in the active site of two copper monooxygenases: the dinuclear particulate methane monooxygenase (pMMO) [1] and the recently discovered family of mononuclear lytic polysaccharides monooxygenases (LPMOs) [2]. pMMO and LPMOs catalyze the monooxygenation of strong C-H bonds of methane (104 kcal.mol^{-1}) and the glycosidic bonds of recalcitrant polysaccharides (95-100 kcal.mol^{-1}), respectively. The catalytic mechanism of these copper-containing enzymes is still the subject of intense research. It is postulated that the unique primary amine ligation to copper is responsible for the formation of highly reactive copper-oxygen species required for the activation of the substrate unactivated C-H bond.

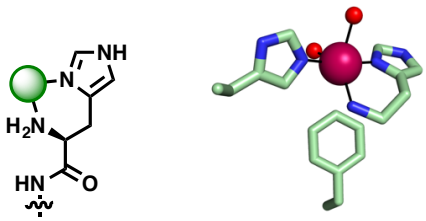

Figure 1: Metal coordination by the histidine brace motif and active site structure of a chitin-active bacterial LPMO

Project:

LPMOs are fungal or bacterial copper metalloenzymes that participate in the degradation of recalcitrant polysaccharides (such as cellulose or chitin) in synergy with glycoside hydrolases. From a molecular point a view, they catalyze the oxidative cleavage of polysaccharide glycosidic bonds [2]. The active site copper ion is coordinated by the *N*-terminal histidine brace and another histidine side chain (Figure 1). In this project, we aim at understanding the structure-reactivity relationships of the histidine brace coordination motif of LPMOs, and engineer new biocatalysts based on the LPMO scaffold with alternative histidine brace reactivities.

In the first part of the project, we will probe the molecular determinants of the histidine brace reactivity by site-directed mutagenesis (of the first and second spheres of coordination). Mutants will be characterized with a wide range of biochemical and biophysical techniques to study their ability to bind copper or other metals, to activate dioxygen and their substrate, etc… This will contribute to understanding how the active site copper ion of LPMOs activate dioxygen and perform the highly energetic oxidation of their substrate.

In the second part of the project, we will engineer the LPMO active site to perform other metal-catalyzed reactions. This strategy (enzyme engineering) has already been successfully

developed for other metalloenzymes such as cytochrome P450s and related hemeproteins (for the creation of C-C, C-N, C-S, C-Si bonds) [3]. In our case, the mutants will be screened for alternative metal-catalyzed activities and better biocatalysts will be developed. Additionally, the scope of the newly LPMO engineered reactions (substrate scope, steric and electronic effects of the substrates, stereoselectivity) will be studied. This will allow the development of new metalloenzymes with potential use in biocatalysis.

The PhD candidate will join the BiosCiences group at iSm2 (Marseille). This group has a strong expertise in the study of metalloenzyme mechanisms and the development of new biocatalysts [4]. The PhD student will evolve in a multidisciplinary environment (chemistry / biology / biophysics) and will become familiar with a wide range of experimental techniques used in this project at the interface of chemistry and biology.

Keywords: chemical biology, bioinorganic chemistry, metalloenzyme, enzyme mechanism, engineereing, biocatalysis

Candidate profile and procedure:

Applicants (master degree or equivalent) with strong background in molecular chemistry, physical chemistry, or biochemistry are strongly encouraged to apply. Background / experience in biochemistry would be beneficial but is not mandatory for this project.

Applicants are invited to send their CV, a cover letter, their transcripts of academic records, and the contact information for at least two references to Christophe Decroos (christophe.decroos@univ-amu.fr) before April 24th.

References:

[1] Rosenzweig AC. (**2017**) A tale of two methane monooxygenases. *J. Biol. Inorg. Chem.* 22(2-3):307-319.
[2] Walton PH *et al*. (**2016**) On the catalytic mechanisms of lytic polysaccharide monooxygenases. *Curr. Opin. Chem. Biol.* 31:195-207.
[3] Arnold FH. (**2015**) The nature of chemical innovation: new enzymes by evolution. *Q. Rev. Biophys.* 48(4):404-410.
[4] Decroos C *et al.* (**2014**) Compromised structure and function of HDAC8 mutants identified in the Cornelia de Lange Syndrome spectrum disorders. *ACS Chem. Biol.* 9(9):2157-2164. Lazarides *et al*. (**2013**) Visible light-driven O_2 reduction by a porphyrin-laccase system. *J. Am. Chem. Soc.* 135(8):3095-3103. Sallmann M *et al*. (**2015**) A structural and functional model for the 1-aminocyclopropane-1-carboxylic acid Oxidase. *Angew. Chem. Int. Ed.* 54(42):12325-12328. Concia AL *et al.* (**2017**) Copper complexes as bioinspired models for Lytic Polysaccharide Monooxygenases. *Inorg. Chem.* 56(3):1023-1026.