Master Research Projects 2017

ITQB NOVA, Oeiras
Contact: Lígia O. Martins,
Tel: 214469534,
E-mail: lmartins@itqb.unl.pt
http://met.itqb.unl.pt/

Project: X-ray studies of a suitable enzyme for medical diagnosis biosensors

Areas: Biochemistry/Enzymology/Biotechnology

Supervisor: Carlos Frazão, Structural Biology, Macromolecular Crystallography Unit; **Co-supervisor:** Lígia O. Martins

Objectives:

The aim of this proposal is to determine the crystal structure of bacterial pyranose 2-oxidase from *Arthrobacter siccitolerans* (AsP2Ox) recently identified and characterized in our laboratory [1] as well as of engineered variants showing improved enzymatic efficiency. This is expected to give insights into the catalytic mechanism performed by AsP2Ox and contribute to answering the long-standing question in protein science of *"how function and structure are related"* and guide further improvement of the enzyme properties.

Background:

Pyranose 2-oxidases (P2Ox, pyranose:oxygen 2-oxidoreductase; EC 1.1.3.10) are enzymes particularly interesting for biotechnological applications in clinical chemistry, industrial process monitoring and in synthetic carbohydrate chemistry. They do not only employ molecular oxygen, a cheap and clean oxidant, but are active with both the α- and β-anomers of D-glucose, and not exclusively with β-D-glucose, as it is the case for glucose oxidase, to generate oxidized organic products and H_2O_2. P2Ox have proved useful in the manufacture of diagnostic kits, biofuel cells, and as biosensors of 1,5-anhydroglucitol in plasma for diagnosis of diabetes mellitus. Additionally they have been successfully utilized in the biotransformation of carbohydrates into building blocks, bulk sweeteners, vitamins' precursor, rare sugars, fine chemicals and new drugs.

In this proposal X-ray studies will be followed to solve the crystal structure of the bacterial pyranose 2-oxidase from *Arthrobacter siccitolerans* (AsP2Ox)[1] and of engineered variants obtained by protein engineering approaches. The enzyme, the first of bacterial origin to be characterized, shows some striking features as compared with known fungal counterparts; it is a 64-kDa monomer and contains a non-covalently bound flavin adenine dinucleotide (FAD) cofactor. The outcome of this study will expand the understanding of bacterial oxidoreductases with importance in biotechnological and diagnostic applications.

1. Mendes, S., Banha, C., Madeira, J., Santos, D., Miranda, V., Manzanera, M., Ventura, M. R., van Berkel, W. J. H., and Martins, L. O. (2016) Characterization of a bacterial pyranose 2-oxidase from *Arthrobacter siccitolerans*, *J. Mol. Cat. B: Enzymatic*, 10.1016/j.molcatb.2016.1011.1005.