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FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Chemistry Department

Structural studies on Mo-enzymes and chaperones

Macromolecular Crystallography Laboratory

xtal Crystallography Group @ REQUIMTE

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requimte rede de química e tecnologia

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2010 - 2012: Master in Biotechnology FCT/UNL (18 out of 20).

2006 - 2010: Graduated in Applied Chemistry FCT/UNL (15 out of 20).

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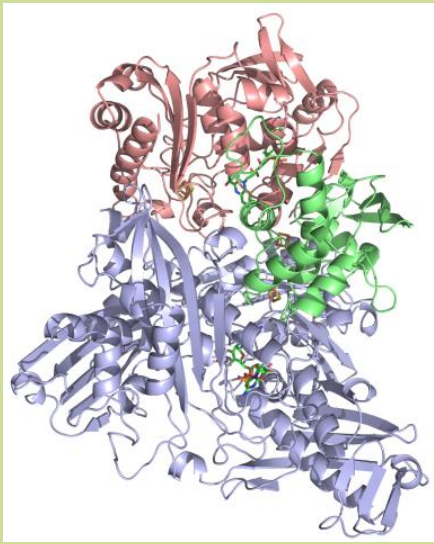
Objectives

Molybdenum is found in the active site of a diverse group of enzymes that are present in almost all forms of life. For Mo-enzymes of the xanthine oxidase family, like the Periplasmic Aldehyde Oxidase from *Escherichia coli*, PaoABC, the final step in protein production is the insertion of the matured cofactor (Moco).
The insertion of Moco into the target apo-enzymes requires specific cofactor storage/binding proteins. PaoD is one of this proteins, responsible for the production of mature and fully loaded Mo-enzyme PaoABC.
The main objective of this work is to structurally characterize a Moco-binding protein and its chaperone. The gathered information will provide insight on the functioning of the Mo enzymes maturation machinery.

PaoABC crystal

Methodology

In order to understand how the molybdenum cofactor (Moco) is inserted into apo-enzymes we intend to solve the crystal structures of PaoABC and proteins involved in Moco binding (PaoD, XdhC and YqeB) using **X-ray Crystallography**.
Soaking and co-crystallization experiments will be performed with Moco in order to obtain crystals of the chaperone/Moco complex and enlighten this interaction. In addition, solution studies using **Saturation Transfer Difference** (STD) NMR will also be attempted in order to characterize the protein-Moco specific interactions.
The characterization of the interaction between the PaoABC and its Moco binding partner, PaoD will be also structurally characterized by **Small Angle X-ray Scattering** (BioSAXS) methodologies using synchrotron radiation.

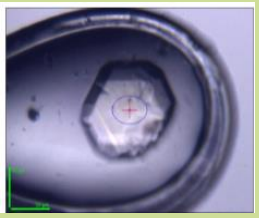


Overall structure of PaoABC

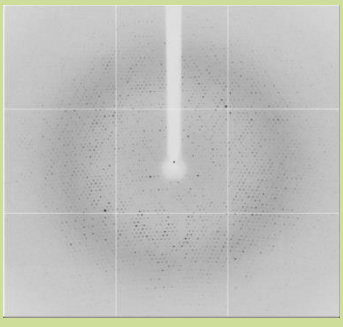
Expected Results

The crystallographic structure of the Mo-enzyme PaoABC has already been solved at 1.8 Å of resolution. In order to understand the interaction between this enzyme and its chaperone we expect to solve the structure of PaoD and homologous (XdhC and YqeB). Recently, small crystals of native PaoD have been obtained but since no model is available for any protein of this family, phase determination is a challenging task.
Using Bio-SAXS, the structural analysis of the complex PaoABC-PaoD will be performed in order to try to understand how the chaperone delivers the MCD cofactor and assist PaoABC folding. Bio-SAXS data has already been collected for PaoABC alone, providing a low molecular model of the protein in solution.

Ref.: Cardoso, A.R., *et al.* Biochemical, Stabilization and Crystallization Studies on a molecular chaperone (PaoD) involved in the maturation of molybdoenzymes (*in prep.*)



PaoD crystal



Diffraction pattern of PaoD crystal

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