FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

**MSc in Biochemistry**
**Dissertation Project – 2nd Cycle**

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TITLE: Structure based reaction mechanism studies on periplasmic nitrate reductase

## BACKGROUND

Nitrate reductases (NR) perform key roles in the metabolism of the nitrogen cycle where they are responsible for reducing nitrate to nitrite. Due to variable cell location, structure and function, they have been divided into periplasmic (Nap), cytoplasmic and membrane-bound (Nar) nitrate reductases.

The first crystal structure obtained for a NR was the monomeric NapA from *Desulfovibrio desulfuricans* in 1999. More recently, new crystallographic studies revealed the 3D structure of the heterodimeric NapAB from *Cupriavidus necator* with the highest resolution ever obtained for a nitrate reductase - 1.5Å. This new results allowed to clearly identify all the coordination elements of the molybdenum atom at the catalytic site of the enzyme. It was found that the 6$^{th}$ connecting element is a sulfur atom, rather than an oxygen as was previously established.

This has revolutionized the previously proposed reaction mechanism for nitrate reductase, which was based on the proximity of an oxygen atom to the Mo center. In order to establish a new reaction mechanism for NR, new crystallographic studies are necessary with the use of various ligands (substrates and inhibitors) which by interaction with the active site of the enzyme can show the most favorable means to the occurrence of the catalytic reaction.

## OBJECTIVES

The overall aim of the project is to establish the reaction mechanism for nitrate reductases. To do this new crystal structure of the *C.necator* NapAB enzyme with bound substrates and/or inhibitors must be obtained in combination with kinetic studies.

The purification of the NapAB protein will be important for the crystallization studies but also for other experiments such as Thermofluor assays (TF), ITC (Isotermal Titration Calorimetry) and time-resolved crystallography. The combination of this techniques will help to better characterize the protein-ligand complexes obtained.

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**PROJECT DESCRIPTION**

The project will be divided in the following tasks:

1 – Purification of a previously obtained NapAB batch from a 300L industrial growth. Size-exclusion chromatography and other Molecular Biology techniques will be used;

2 – Determination of the kinetic parameters of the enzyme. In this task ITC will also be used in parallel with the biochemical methods for comparison;

3 – Screen for the best crystallization conditions of the purified protein and preparation of complexes (with bound substrates and/or inhibitors) to obtain good diffracting crystals with the highest resolution as possible;

4 – Diffraction experiments performed in-house (X-ray machine of the Macromolecular Crystallography Laboratory, UCIBIO, FCT-UNL) and at several synchrotron sources (ESRF, Grenoble and SLS, Villigen) and data processing;

5 - Structural analysis of the 3D structures obtained and deposition in the PDB (Protein Data Bank);

6 – Characterization of the 3D structures complexes obtained using TF assays and time-resolved crystallography at PETRA III (Hamburg).

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