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FCT FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

Department of Chemistry

Mechanistic Studies of Molybdenum Enzymes

REQUIMTE
Group of Biophysical and Bioinorganic Chemistry

requimte
rede de química e tecnologia

PhD Program in Sustainable Chemistry

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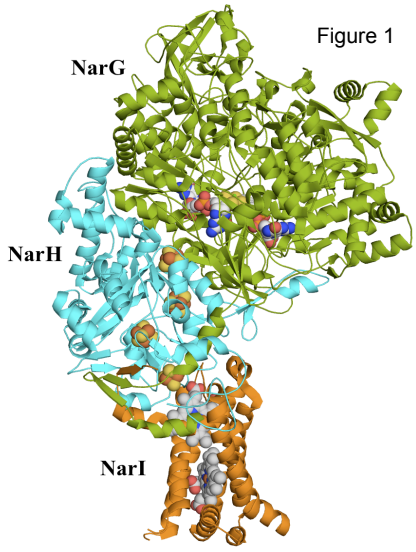
Objectives

Biochemical characterization of metalloenzymes, in particular molybdenum containing enzymes, from sulfate and nitrate reducing bacteria.

- Study of respiratory nitrate reductase (Figure 1 & 2) from *Marinobacter hydrocarbonoclasticus* 617, enzyme involved in bacterial respiration of nitrate catalysing the first step of denitrification.
- Study of aldehyde oxidoreductase (Figure 3 & 4) from *Desulfovibrio gigas*, highly promiscuous enzyme involved in energization and detoxification of the bacterial cell. It is also a model system for xanthine and aldehyde oxidase.

Figure 1



Methodology

- Protein Film Electrochemistry → redox enzymes were adsorbed on electrodes and the enzyme kinetics developed in the potential dimension
- Steady State Kinetics in solution → the kinetic parameters were determined in presence of substrates and inhibitors over a large variety of conditions
- EPR Spectroscopy → determination of ROS production throughout spin traps
- X-ray Crystallography → structural studies of enzyme-substrate intermediates and enzyme-inhibitors characterization
- NMR Spectroscopy → used to follow the enzymatic reaction and product formation

Figure 2

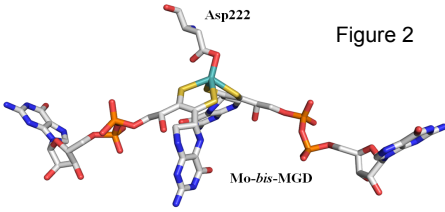
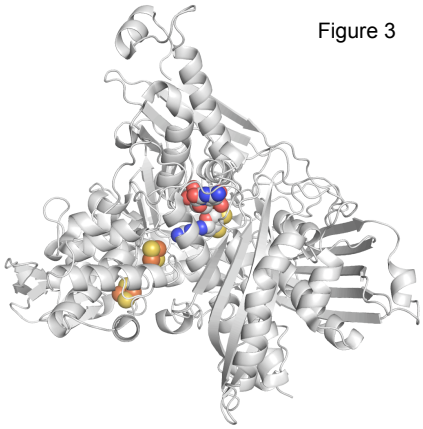


Figure 3

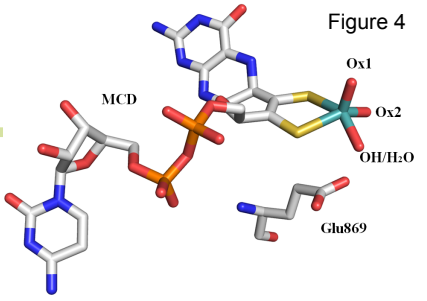


Expected Results

- Deep inside the catalytic mechanism of respiratory nitrate reductase, the chemistry underneath the substrate conversion and how the latter is coupled with ET.

- Shed light on the dynamics of activation / inactivation of aldehyde oxidoreductase. Identification of catalytic reaction intermediates throughout the use of new inhibitors and substrates .

Figure 4



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xtal Crystallography Group @ REQUIMTE

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