SCIENCESPRINGDAY

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

REQUIMTE/CQFB - Chemistry Department

The membrane nitric oxide reductase - electron transfer complex and catalytic cycle/center

BioProt and Microbial Stress and Bioremediation Groups
Supervisors: Isabel Moura and Sofia R. Pauleta
REQUIMTE/CQFB, Dpto. Química, Faculdade de Ciências e Tecnologia, Universidade N[illegible]

Susana Ramos

(Post Doctoral Fellow)

2012 – Post Doctoral Researcher (FFCT/UNL)
2011 – Ph.D. in Biochemistry, specialty Physical Biochemistry (FCT/UNL)
2005 – Degree in Biochemistry (FCT/Ualg)

Objectives

Nitric Oxide Reductase (NOR) is a membrane enzyme of the denitrification pathway, that catalyses the two electron/two proton reduction of NO to N_2O, contributing to the proton gradient (Fig. 1).This enzyme also catalyses the four electron/four proton reduction of O_2 to H_2O.

The aim of this project is to gain insights into the catalytic center of *Marinobacter hydrocarbonoclausticus* NOR. This *c*NOR (class I) has two subunits: NorC (electron transfer subunit) and NorB (catalytic subunit) (Fig. 2).

The specific issues that still need to be elucidated are the following:

1) The magnetic coupling in the binuclear Fe center; **2)** The coordination of the heme b_3; **3)** Electron transfer complex pathway with cytochrome c_{552}.

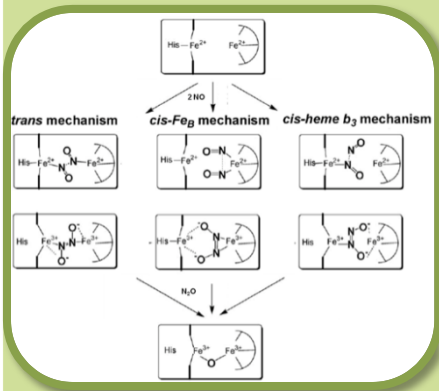

Fig. 1 – Putative mechanism of NOR (*Nat. Proc. Rep.*, 2007, 24, 610-620).

Methodology

To elucidate the catalytic mechanism of *Ma. hydrocarbonoclasticus* NOR and the electron transfer complex with its redox partner, cytochrome c_{552}, several approaches will be used:

- Purification of NOR from *Ma. hydrocarbonoclasticus* membranes and cytochrome c_{552} from soluble extract
- Heterologous production of NOR catalytic subunit, NorB, and of NorC
- Spectroscopic characterization of the NOR active center (UV-visible, EPR, and Mössbauer spectroscopies)
- (Fast) Kinetic studies on the diiron center and electrochemical studies
- Backbone resonance assignment of rNorC – NMR
- Characterization of the electron transfer complex between cytochrome c_{552} and rNorC - *ab-initio* docking calculation and heteronuclear NMR experiments

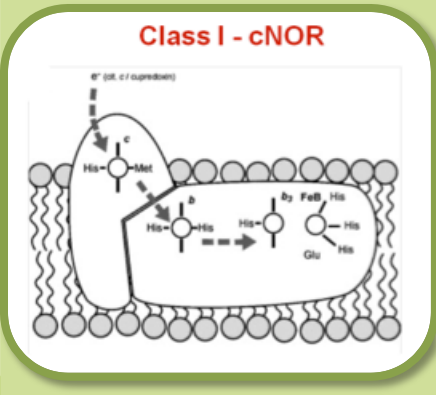

Fig. 2 – Schematic representation of Class I NOR (cNOR) (*J. Inorg. Biochem.*, 2006, 100, 2087-2100; *BBA*, 2012, 1817, 680-687).

Expected Results

It is expected that all the experiments conducted will unraveled the biological relevance of NOR catalytic center, the mechanism of NO reduction, substrate affinity, reduction potentials of the centers, as well as, their coordination during catalysis.

In the case of rNorC, heterologous protein production will allow labeling of rNorC with ^{13}C and/or ^{15}N in order to perform NMR experiments for backbone resonance assignment.

Mössbauer and EPR data will enable the detection of the postulated diiron mixed valence species, as there is a gap between the midpoint reduction potential of the heme b_3 and FeB.

Site directed mutagenesis on residues proposed to be involved in the catalysis will be used to assess the proposed mechanism of NO reduction.

Funding:
This project is funded by Fundação para a Ciência e Tecnologia [Project PTDC/QUI-BIQ/116481/2010 (IM) and SFR/BPD/91288/2012 (SR)]

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA