

Department of Chemistry, FCT-UNL

Title

REQUIMTE/CQFB, Bioinorganic Chemistry Lab; and CNC (Centro de Neurociências de Coimbra), Universidade de Coimbra



requimte
rede de química e tecnologia



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B.Sc. degree in
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Objectives

- To clone and heterologously express *Marinobacter hydrocarbonoclasticus* cytochrome c_{552} and the soluble portion (NorC) of Nitric Oxide Reductase (NOR), in their isotope enriched forms (^{13}C and ^{15}N). Both are heme containing proteins.
- To determine the backbone and side-chain resonance assignment using standard solution state NMR pulse programs.
- To characterize the interaction interface between the two proteins (and other putative partners) using NMR experiments, with and without the aid of paramagnetic relaxation probes (using specific lanthanide chelates).

Methodology

- Cloning and over-expression using standard techniques on *E. coli* cells (PCR, gene ligation, transformation, expression tests in LB and M9 media); purification using standard HPLC techniques (ion affinity, size exclusion).
- Determination of backbone and side-chain resonance assignments using standard 2D and 3D NMR pulse sequences for ^{13}C , ^{15}N and ^1H .
- Determination of interaction interfaces by NMR titrations and local relaxation parameters (R_1 , R_2 , steady-state NOE); use of lanthanide probes (DOTAM, DOTP, HPDO3A) to determine specific patches and orientations.

Expected Results

- Cytochrome c_{552} and NOR are expected to interact in such a way as their metal centers are able to transfer electrons between themselves (distance up to 20 Å).
- NMR chemical shifts and peak volumes upon titration will allow estimation of dissociation constant and local relaxation/ mobility parameters.
- Use of *in silico* docking software (e.g. BiGGER) will help predict and refine the orientation of the probable complexes.

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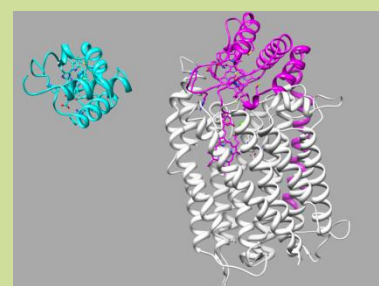


Fig. 1 – *Marinobacter hydrocarbonoclasticus* cytochrome c_{552} (left, blue ribbon) and homology model off NOR (right, white and purple ribbon)

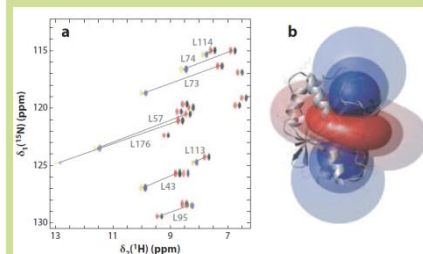


Fig. 2 – Left: Typical 2D NMR (HSQC) spectrum, and effects of paramagnetism. Right: Typical PCS isosurfaces induced by paramagnetic species.

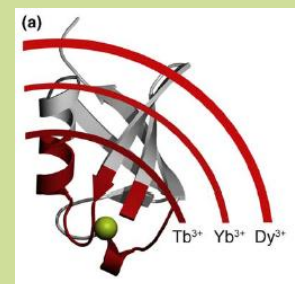


Fig. 3– Schematic representation of the far-reaching paramagnetic effects of lanthanide ions, used in interaction and structural studies in NMR.