SCIENCESPRINGDAY

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Chemistry Department

Protein-ligand studies and inhibition mechanisms

Macromolecular Crystallography Group

xtal Crystallography Group @ REQUIMTE

http://xtal.dq.fct.unl.pt/

requimte rede de química e tecnologia

University of Sussex

Hugo Correia

(PhD Student, Supervisor: T. Santos-Silva, Co-supervisor: M. J. Romão)

Present, Ph.D. Student in Biochemistry @ FCT/UNL

2012, M.Sc. in Structural and Functional Biochemistry @ FCT/UNL

2010, B.Sc. in Biochemistry @ FCT/UNL

Objectives

A - Molybdenum enzymes - *Desulfovibrio gigas* Aldehyde Oxidoreductase (AOR):

- Determine the 3D structure with several ligands and inhibitors
- Analyze the results and extend these studies to other Aldehyde Oxidases

B - Tungsten enzyme - *Desulfovibrio gigas* Formate Dehydrogenase (Fdh):

- Determine the formate-reduced structure of the enzyme and analyze structural differences from the oxidized form

C - Serine proteases – urokinase-type Plasminogen Activator (uPA) and Trypsin:

- Determine the crystal structure of trypsin in complex with several uPA-specific inhibitors (boron compounds)
- Optimize uPA crystallization and perform similar studies

Methodology

A - AOR will be crystallized in complex with several substrates, inhibitors and analog compounds. EPR spectroscopy and kinetic assays will be used to complement the information obtained with the structures. Furthermore we will be extending these studies to *Rhodobacter capsulatus* Xanthine Dehydrogenase and to mouse and human Aldehyde Oxidases.

B - Crystallization of Fdh in the anaerobic chamber, under reducing conditions.

C - Inhibitor compounds specific to uPA are synthesized by our collaborators (Dr. John Spencer, University of Sussex, UK). Trypsin is used as a crystallographic model and its structure will be determined with these compounds. X-ray crystallography will also be performed using uPA.

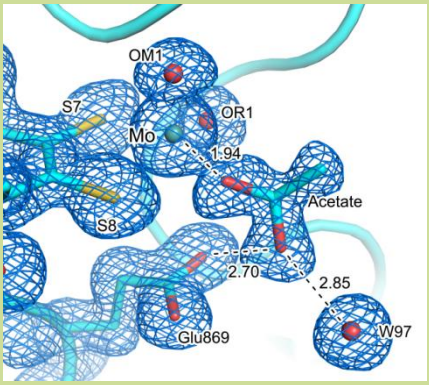
Expected Results

A – With atomic resolution data we have obtained several crystal structures where the position and orientation of substrates and inhibitors can be seen in detail contributing to a revised reaction mechanism of the enzyme, to be proposed soon (1).

B – Crystals of W-Fdh have already been prepared in the anaerobic chamber. Reduction process is currently under optimization for future data collection.

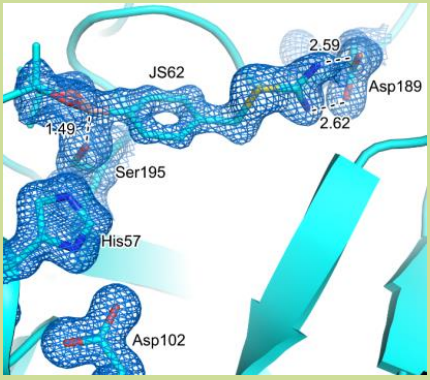
C - The crystal structure of trypsin with several inhibitors has been obtained and analyzed in detail. This data provides information upon inhibitor–protein interaction and contribute for the design of more specific and effective compounds (2).

Ref.: (1) Correia, et al, “Active and inactive forms of AOR”, in prep. (2) Correia, et al, “Investigation of selective uPA inhibitors”, in prep.



Acetate (reaction product) bound to AOR active site after crystal incubation with acetaldehyde (substrate).

Fdh crystals obtained under anaerobic conditions.



uPA inhibitor interacting with trypsin’s active pocket

Funding: This work was supported by Fundação para a Ciência e a Tecnologia through fellowship SFRH/BD/86796/2012, grant no. PEst-C/EQB/LA0006/2011 and by the projects PTDC/QUI/64733/2006 and PTDC/BIA-PRO/118377/2010.

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