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X-ray Crystallography and Drug Design

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Ph.D. student (Structural Biochemistry) since January 2012

2006-2009 – B.Sc. in Cell and Molecular Biology (FCT/UNL)

2009-2011 – M.Sc. in Biotechnology (FCT/UNL)

Supervisors: Dr. Teresa Santos--Silva and Professor Maria João Romão

Objectives

My Ph.D. work is focused in the production of different protein-ligand complexes with potential pharmaceutical interest. In this sense, I am studying two types of compounds: CORMs (Carbon Monoxide Releasing Molecules – pro-drugs with possible anti-inflammatory, anti-apoptotic and anti-proliferative effects) and vanadium compounds which have an insulin-enhancing action and can be used as oral substitutes for the treatment of diabetes.
Both CORMs and V compounds interact with different proteins (namely hemoglobin, albumin and transferrin) and such interactions are essential to their function. Thus, I pretend to characterize in detail these interactions in order to help in the design of new, safer and more potent drugs.

Methodology

X-ray Crystallography can be considered the most powerful existing tool for protein structural characterization at the atomic level. In this sense, this is the main technique used in both projects and lysozyme, as a good crystallographic model, has been used.
With the mentioned proteins, soaking and co-crystallization experiments are going to be tried with CORMs and V compounds. In addition, other complementary structural methodologies (like Bio-SAXS – Small-Angle X-ray Scattering) will be performed.
Moreover, the protein-metal adducts, either derived from CORMs or V interactions, are also being characterized by different techniques such as FTIR, MS or ICP-AES. Urea gel electrophoresis is another simple technique that allows to determine if a metal binds to transferrin and is also going to be used for some of these compounds.

Expected Results

Emerging from an almost untouched area of research, these projects will strongly impact the rationale behind the design of therapeutically acceptable drugs. From these complementary methodologies, indications of the chemical stability and reactivity of CORMs with several proteins as well as structural features that modulate CORM-protein interactions are expected. The crystal structure of lysozyme covalently bound to ALF850, a Ru CORM, has already been obtained at atomic resolution (1).
The characterization of the interactions established between transferrin and different V compounds (2) should also provide indications on the transport and possibly delivery of V into cells. This information could be useful for the development of new anti-diabetes drugs.

(1) Santos, *et al*, JIB, 2012, 117, 285-291. (2) Santos, *et al*, JIB, 2013, 121, 187-195.

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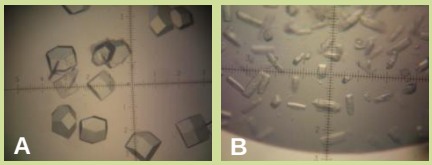
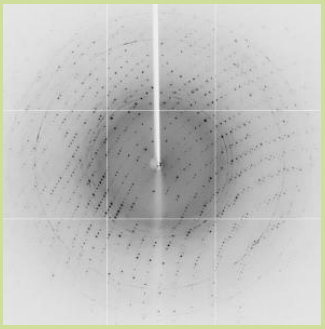
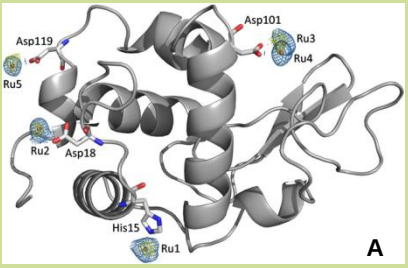
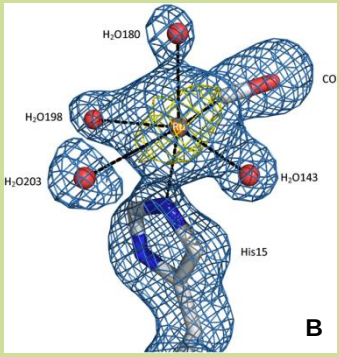


Lysozyme **(A)** & Transferrin **(B)** crystals



Diffraction pattern of lysozyme soaked with ALF850





Overall structure of lysozyme bound to ruthenium fragments derived from ALF850 **(A)** and the respective Ru–His15 adduct **(B)**