FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

MSc in Biochemistry
Dissertation Project – 2nd Cycle

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Scientific area: Biochemistry

TITLE: **Structure and Functional Studies of S-formylglutathione hydrolase from Mouse *(Mus musculus)***

BACKGROUND

S-formylglutathione hydrolase (SFGH) is an enzyme belonging to glutathione thiolesterase that hydrolyzes S-formylglutathione to glutathione and formate. The enzyme SFGH is present in the liver of mouse or any animal, which is regulating detoxification pathway conserved in all living systems. The sequence alignment of SFGHs from both prokaryotic and eukaryotic organisms with site directed mutagenesis experiment results has proved that SFGHs are serine hydrolases, which consist of Ser-Asp-His residues conserved at the catalytic triad. To this date, amongst all the mammals, only one SFGH structure from humans has been solved and characterized. It has 90% identity with mouse SFGH. In this situation, the structural characterization of SFGH assumes paramount importance for developing new drugs for indigestion or other liver diseases such as jaundice, cirrhosis, hepatitis, etc., and it will be benifical for the animal model testing.

OBJECTIVES

Aim of this project divided in to three major parts.

1. The cloned SFGH gene will be transformed with BL28 competent cells, buffer optimization for the homogenity, purification and crystallization to be carried out.
2. The data collection of SFGH will be carried out using our home source in our department. The SFGH structure solution and refinement to be determined by the software CCP4i and Phenix.
3. Biochemical characterization of native SFGH to be performed with suitable substrates

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PROJECT DESCRIPTION

This project includes the bufer optimization for getting homogenous protein, protein purification will be carried out by the affinity and gel filtration columns. The purity of the compound to be analysed using native and SDS pages. The crystallization of SFGH to be screened for the available crystal screening kits using Robotic crystallization methods. The screened crystal conditions are to be applied in hanging drop vapor diffusion methods to get the optimum size of crystal with good diffraction quality. The suitable crystals are to be selected for X-ray data collection using the home source x-ray machine, solving the structure of proteins using different programs and validation of refined protein structure followed by interpretation across the three dimensional structure in the electron density maps. The active site residues are to be analysed more based on its stability towards the interactions. The enzymatic assay of SFGH will be performed against with p-nitrophenyl acetate derivatives using the UV fluorimeter. The selected student will be involved in all the experiments of this project, which are mentioned above.

TIMELINE (use fill tool for the cells)

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1	SFGH gene transformation, optimization of buffer for the purification and crystallization of purified protein.									
Task 2		X-ray diffraction data collection								
Task 3				3D structure determination and analysis towards the interaction for explaining active site stability						
Task 4					Enzymatic assay analysis for PNP derivatives					
Task 5										
Task 6										
Thesis								Thesis writing		