Positions for Masters Students

We have two active projects in the group of Biomolecular NMR:

a) Structural and Biochemical characterization of novel Fe^{2+} metabolic pathways

b) Structural proteomics of thermostable protein libraries by NMR

For more information look in www.itqb.unl.pt/~matzman

In those projects students will be trained in biochemical and biophysical methods in addition to advanced NMR methods. The students will have the choice to specialize in molecular biology and heterologous protein expression or in more computationally intensive tasks, such as protein structure and dynamics determination.

Some of the scientific problems that we are working on in our lab are: the cloning of new proteins, protein biochemistry, protein structure elucidation and dynamics studies, Protein-protein interactions, Metal binding to proteins, paramagnetic NMR, study of large proteins and solid state NMR.

The projects are interdisciplinary and students from many different backgrounds can apply.
Student with a strong background in chemistry, biochemistry or biophysics are welcome. The prior knowledge of NMR is not required but it is welcome.

Please contact Manolis Matzapetakis at : matzman@itqb.unl.pt for more information.

Description of projects follows in the next pages:

Structural and Biochemical characterization of novel Fe^{2+} metabolic pathways

State of the Art

Iron is an essential element since it is an integral component of many proteins and enzymes. In order to ensure its adequate supply, bacteria have devised complex and elaborate mechanisms to harvest and transport it. While a lot is known about transport of Fe^{3+} much less is known about the metabolic pathways of Iron in the ferrous state (Fe^{2+}) in bacteria.

Genome analysis has revealed operons potentially related to ferrous iron transport but little is known about the structure and function of the small proteins that they encode. These proteins are thought to bind Fe^{2+} for either transport or gene regulation. Such types of iron transporters have not been adequately characterized in spite of their importance. Apart from the obvious scientific interest in such systems, it is also possible that pharmaceutical applications can be explored by targeting these metabolic pathways in pathogenic bacteria.

The goal of the project is to isolate and characterize the proteins of those operons. We will investigate Fe^{2+} binding characteristics and structurally characterize them in the apo and metallated forms. The functional characterization of the system will involve the study of the way these proteins interact with each other and with other biologically relevant Fe^{2+} binding proteins in order to understand the pathway that iron follows. A variety of methods will be used, such as molecular biology, chromatography and spectroscopy, with NMR playing a key role in this project.

Project description

The first stage of this project is to isolate the genes that compose the *E.coli* Fe^{2+} operon using PCR. These genes will be inserted into expression plasmids for protein over-expression. The expression conditions will be optimized for rich and minimum (defined) media that is suitable for isotopic labelling of the proteins. The proteins will then be purified and biochemically characterized. Their folding, stability and biophysical properties will be determined using spectroscopy and denaturation titrations. The binding of various metals will then be investigated to determine their binding affinity. Spectroscopy and pH titrations will help define the metal binding site in the protein. These titration will also be monitored by NMR and site directed mutagenesis will be used to investigate the effect of certain residues in the metal binding.

Isotopically labeled proteins (^{13}C ^{15}N) will be used for structural studies by NMR employing multidimentional heteronuclear experiments for resonance assignment. Structures will be calculated using distance restrains and residual dipolar couplings. The interaction of proteins will mainly be studied by simple NMR monitored titrations.

Techniques

During this project students will be trained in the use of basic molecular biology techniques such as PCR and DNA gel electrophoresis. They will extensively use bioinformatics tools for sequence alignment and prediction of protein properties. During protein purification, they will use various electrophoretic (SDS- and native PAGE) and chromatographic techniques (FPLC, HPLC). During protein characterization the students will gain experience in various spectroscopic techniques such as UV-visible, CD and NMR. Interested students will have the opportunity to have in-depth trained in biomolecular NMR for the structural characterization of the proteins. They will learn how to setup modern NMR experiments at the 800 MHz NMR of ITQB and travel to European facilities to perform additional experiments. During the analysis of the data they will be trained in the most modern computational methods used in NMR analysis.

Structural proteomics of thermostable protein libraries by NMR

State of the Art

Proteins are adapted to function at specific conditions and their stability is related to those environmental conditions they are expected to face. Outside those limits, due to stress or because of mutations, proteins tend to lose their original structure, often irreversibly. The understanding of the factors that make proteins more or less stable is crucial for medicinal but also industrial purposes. One way to learn more about protein stability is to study proteins from hyper-thermophilic organisms. These organisms are known to live at extreme temperatures reaching 95 ºC.

In the framework of collaboration with the Protein Biochemistry, Folding and Stability Laboratory at ITQB we will perform a proteomic based study of such hyper-stable proteins. Recent protein screening methods have identified large numbers of previously unknown proteins that are over-expressed during thermal stress. We will over-express, isolate and characterize these proteins with a variety of methods such as UV, CD, fluorescence and NMR. Then we will structurally characterize them using NMR. The structure, stability relationship will be analyzed to gain insight into the factors that make these proteins so stable.

Project description

The goal of the student in this project will be to identify, express and structurally characterize these proteins by NMR and biophysical methods.

During this project we will have a secondary goal of implementing efficient manual and automated protocols for structure elucidation using NMR that will pave the way for a larger scale proteomics and functional proteomics initiative.

Techniques

The plasmids for the first set of proteins are available. The proteins will be overexpressed in *E.coli* and the protocols will be optimized for expression in rich and minimum media.

They will also extensively use bioinformatics tools for sequence alignment and prediction of protein properties. During protein purification, they will use various electrophoretic (SDS- and native PAGE) and chromatographic techniques (FPLC, HPLC). During protein characterization the students will gain experience in various spectroscopic techniques such as UV, CD and NMR. Interested students will have the opportunity to have in-depth training in biomolecular NMR for the structural characterization of the proteins. They will learn how to setup modern NMR experiments at the 800 MHz NMR of ITQB and travel to European facilities to perform additional experiments. During the analysis of the data they will be trained in the most modern computational methods used in NMR analysis.