



Master Research Projects 2010/2011

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[Coordination and Supramolecular Chemistry](#) - Rita Delgado

Master Students Research Project

Peptide-based dual probes for targeted molecular imaging of tumor angiogenesis

Noninvasive imaging of the expression of endothelial cell surface markers or adhesion-promoting molecules in vivo represents an opportunity for identifying early signs of tumor angiogenesis, arthritis and inflammation as well as atherosclerosis. We are particularly interested in E-selectin, a cell adhesion molecule which is induced on the surface of endothelial cells in response to inflammatory cytokines. E-selectin is upregulated in proliferating endothelial cells and its overexpression was colocalized with dividing microvascular endothelial cells in tissues with active angiogenesis. Therefore, imaging of the E-selectin expression in vivo can be an attractive approach to detect tumor angiogenesis and inflammation-mediated diseases.

In collaboration with the group of Dr. Jessica Gätjens we intend to develop peptidic bimodal probes that will allow in vivo mapping of E-selectin expression by combined Ultrasound (US)/Magnetic Resonance (MR) and Near-Infrared (NIR)-fluorescence imaging and allow for translation in between these different complementing imaging modalities.

The proposed Master research project will involve:

- The solid-phase synthesis (using an automatic peptide synthesizer), the purification (by reverse-phase HPLC) and the characterization (by mass spectroscopy: ESI-MS or MALDI-MS) of the peptide conjugates containing a NIR fluorophore and a reactive group for subsequent coupling to both imaging media (US and MR). This work will be carried out in ITQB.
- The coupling of the pure peptide conjugates to both imaging media, superparamagnetic iron oxide nanoparticles (USPIOs) for MR and gas-filled microbubbles for US, and posterior characterization of the new particles with different analytical methods tailored to the specific contrast agent (i.e. light microscopy, transmission electron microscopy, fluorescence spectrometry, powder XRD, ICP OES and ICP-MS measurements). This work will be carried out in the Medical Faculty of the RWTH-Aachen University (Germany).
- Preliminary “in vitro” studies of their binding affinities and their specific targeting properties. This task will also be developed in Germany.

Supervisors: Dr. Olga Iranzo (oiranzo@itqb.unl.pt)

Dr. Jessica Gätjens (jgaetjens@ukaachen.de)

Area: Chemistry

Location: ITQB (Bioinorganic Chemistry and Peptide Design Laboratory) – Oeiras

Medical Faculty, RWTH-Aachen University (Dept Experimental Molecular Imaging) -
Aachen, Germany

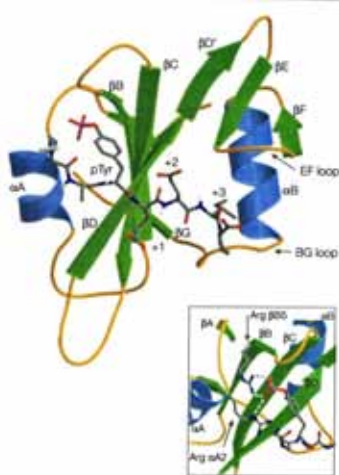
Master Students Research Project

Peptidomimetics for phosphoproteome analysis

The phosphoproteome consists of the entire complement of phosphorylated proteins (p-Pr) in cells. Phosphoproteomics may prove to be valuable in unravelling p-Pr as markers that are potential drug targets or of predictive value in disease therapeutics. As the stoichiometry of phosphorylation is very low and the event is highly dynamic, phosphoproteome analysis usually comprises a first step of enrichment. Despite progress on the development of enrichment methods, IMAC (Immobilized Metal Affinity Chromatography) using Fe^{3+} , Zr^{4+} or Ti^{4+} remains the commonest method with its associated low selectivity. The need of proteomic methods compatible with large-scale analysis is a trigger for the development of selective and robust p-Pr binding molecules.

Peptidomimetics are peptide-based structures that can be designed and engineered to target specific molecules. This proposed research project focuses in the development of peptidomimetics with potential to bind to phosphorylated proteins and will be carried out in the framework of a collaboration project with Dr. Ana Cecília Roque (PTDC/EBB-BIO/102163/2008).

This proposal will involve:



(i) In silico studies of the specific interactions with phosphorylated proteins and the design of the peptidomimetics binding to the targeted phosphorylated moieties (molecular modeling).

(ii) Solid-phase synthesis of the peptidomimetics using standard Fmoc solid-phase chemistry and an automated peptide synthesizer.

(iii) Subsequent purification by reverse-HPLC and characterization by mass spectroscopy (ESI-MS or MALDI-MS) and circular dichroism spectroscopy (CD).

(iii) Preliminary screening of the interactions between the peptidomimetics and phosphorylated proteins (protein quantification methods; fluorescence microscopy; high-throughput screening methods).

Supervisors: Dr. Olga Iranzo (oiranzo@itqb.unl.pt)

Dr. Ana Cecília Roque (cecilia.roque@dq.fct.unl.pt)

Area: Chemistry - Biotechnology

Location: ITQB – UNL (Bioinorganic Chemistry and Peptide Design Laboratory)

REQUIMTE (Departamento de Química, FCT – UNL)

Enantioselective Organocatalysis: Synthesis and Application of Novel Organocatalysts.

Organocatalysis can be utilised for the construction of enantiopure complex organic molecules, thus providing an alternative or a complement to organometallic and enzymatic catalysts, with a strong potential for green chemistry and industrial applications. The term "organocatalysis" describes the acceleration of chemical reactions through the addition of a substoichiometric quantity of a small organic molecule. One of the most described organic catalysts is proline, an aminoacid. However, most new organocatalysts are not general, i.e., they work very well in one or a few reaction types but do not give satisfactory results in a different transformation. Therefore, there is a need to develop new organocatalysts which are easily recycable and possess enhanced catalytic properties.

The aim of this project is to develop new organocatalysts from tartaric acid, which is a very abundant and cheap chiral starting material and is available in both enantiomeric forms. All new organocatalysts synthesised will be tested in several kind of reactions. The structure of the catalysts will be changed in order to increase their reactivity and stereoselectivity and to study the mechanistic aspects of the catalytic cycle.

Local: ITQB- UNL, Oeiras, Bioorganic Chemistry Laboratory

Supervisor: Dr. Rita Ventura (rventura@itqb.unl.pt)

Synthesis of compatible solutes with protein thermostabilisation properties.

Halotolerant and moderately halophilic microorganisms accumulate compatible solutes to face fluctuations in the water activity of their environment. Hyperthermophiles (thriving optimally above 80°C) isolated from marine sources also use the same general strategy. Protein stability, often viewed as a direct consequence of its three dimensional fold, is highly influenced by its dynamic behaviour, i.e. its internal motions in relation to its structure, which ultimately decide in what conditions the structure collapses or unfolds. With this in mind, to understand the stabilisation phenomenon, one should also study the changes in the dynamical behaviour of a protein upon solute addition.

The aim of this project is to synthesise new solutes, with minor but significative structural modifications when compared with alpha-glucosyl-D-glycerate and alpha-mannosyl-D-glycerate, the last one a well known and studied natural solute.

The natural solutes are 1,2-*cis* glucosides, and for the construction of an alpha-glucosidic bond there is not a general method and studies for improving the anomeric selectivity of this challenging glycosylation reaction will be performed.

Task 1. Synthesis of the glycosidic donor.

Task 2. Synthesis of the glycosidic acceptor when needed.

Task 3. Glycosylation reaction – study of several conditions.

Task 4. Selective cleavage of the protecting groups.

Task 5. Structural analysis and characterisation of the products obtained throughout the synthesis.

Local: ITQB- UNL, Oeiras, Bioorganic Chemistry Laboratory

Supervisor: Dr. Rita Ventura (rventura@itqb.unl.pt)

Synthesis of DPD and new analogues, the precursor of AI-2, the bacterial signalling molecule for inter-species communication.

The bacterial signal molecule called autoinducer-2 (AI-2) is well known for its ability to mediate inter-species communication regulating important bacterial group behaviours such as biofilm formation, virulence, and antibiotic production.

In this project our aim is to synthesize the precursor of AI-2, 4,5-dihydroxy-2,3-pentanedione (DPD). The lack of an easy and inexpensive method for synthesizing this compound has been a major drawback in the advance of these novel research areas aiming to understand the molecular mechanisms of bacterial communication.

The new strategy will allow the preparation of labelled DPD and new analogues by introducing and varying the substituents in different positions of the molecule. The labelled DPD constitutes an important reagent for the ongoing elucidation of the biochemical fate of this molecule at the cellular level. The new derivatives will hopefully provide potent agonists and antagonists of AI-2 and will also contribute for a better understanding of the influence of the new groups added, the nature of these groups, their stereochemistry on the response of bacteria, and which properties are important for the recognition of the molecule by the different receptors.

Local: ITQB- UNL, Oeiras, Bioorganic Chemistry Laboratory

Supervisor: Dr. Rita Ventura (rventura@itqb.unl.pt)

Research Project for Master Students

Field: Organometallic Chemistry/Homogeneous Catalysis

Supervisor: Beatriz Royo (broyo@itqb.unl.pt)

Institution: Instituto de Tecnologia Química e Biológica – Homogeneous Catalysis Lab

Duration: 1 year

Research project: Sustainable catalysis based on N-heterocyclic carbene metal complexes

Sustainable catalysis based on N-heterocyclic carbene metal complexes

The development of sustainable, more efficient and selective organic synthesis is one of the fundamental research goals in chemistry. In this respect, catalysis is a key technology for both industrial and academic research. The reactivity and selectivity of the catalysts are widely influenced by the choice of the central metal and the surrounded ligands. N-heterocyclic carbenes (NHCs) are an important class of carbenes that have attracted a lot of attention due to their use as ligands in many catalytic reactions catalyzed by transition metals.

Our research group is engaged with a project dealing with the functionalization of NHCs, their coordination to transition metals and study of their catalytic applications. The present project aims at preparing new organometallic complexes containing NHC ligands, their fully characterization and catalytic applications [1-4].

References:

- [1] A. P. da Costa, J. A. Mata, B. Royo, E. Peris (2010) *Organometallics* DOI: 10.102/om100090c.
- [2] V. V. K. M. Kandepi, A. Pontes da Costa, E. Peris, B. Royo (2009) *Organometallics* 28, 4544.
- [3] A. P. da Costa, M. Sanaú, E. Peris, B. Royo (2009) *Dalton Trans.* 28, 4544.
- [4] A. P. da Costa, M. Viciano, M. Sanaú, S. Merino, J. Tejada, E. Peris, B. Royo (2008) *Organometallics* 50, 949.

Projecto de tese de mestrado para alunos de 2ºciclo da área de Química

Área: Química Organometálica/ Catálise Homogénea

Orientador: Beatriz Royo (broyo@itqb.unl.pt)

Local de Realização: Instituto de Tecnologia Química e Biológica – Laboratório de Catálise Homogénea

Duração aproximada: 1 ano lectivo

Tema do projecto: Catálise sustentável baseada em complexos metálicos com carbenos N-heterocíclicos

Catálise sustentável baseada em complexos metálicos com carbenos N-heterocíclicos

O desenvolvimento sustentável de sínteses orgânicas mais eficientes e selectivas é um dos objectivos fundamentais da investigação química. Neste contexto, a catálise é uma tecnologia-chave tanto para a indústria como para o meio académico. A reactividade e selectividade dos catalisadores é amplamente influenciada pela escolha do centro metálico e pelo desenho dos ligandos adjacentes. Os carbenos N-heterocíclicos (NHCs) constituem uma importante classe de carbenos que têm atraído cada vez mais atenção pelo seu uso como ligandos em um grande número de reacções catalisadas por metais de transição.

Nosso grupo de investigação está envolvido num projecto que lida com a funcionalização de ligandos N-heterocíclicos, sua coordenação a metais de transição e o estudo das suas aplicações catalíticas [1-4]. O presente projecto visa a síntese de novos compostos organometálicos contendo ligandos NHC a sua completa caracterização e estudo da sua reactividade.

Referências:

- [1] A. P. da Costa, J. A. Mata, B. Royo, E. Peris (2010) *Organometallics* DOI: 10.102/om100090c.
- [2] V. V. K. M. Kandepi, A. Pontes da Costa, E. Peris, B. Royo (2009) *Organometallics* 28, 4544.
- [3] A. P. da Costa, M. Sanaú, E. Peris, B. Royo (2009) *Dalton Trans.* 28, 4544.
- [4] A. P. da Costa, M. Viciano, M. Sanaú, S. Merino, J. Tejada, E. Peris, B. Royo (2008) *Organometallics* 50, 949.

Research Project for Master Students

Synthetic and Mechanistic Studies on Metal Carbonyls for therapeutic delivery of CO

Carbon monoxide (CO) is an endogenous mediator that plays important roles in mammalian physiology. Inhalation of CO doses well below toxic levels has been shown to prevent inflammation, thrombosis, oxidative stress and apoptosis and has been shown to have therapeutic, curative effects on a wide variety of diseases like rheumatoid arthritis, multiple sclerosis, cerebral malaria and many, many others. (see Ryter and Otterbein, 2004)

To circumvent the fact that inhaled CO - despite its obvious potential - is difficult to use in the clinical setting (because it is a gas and because it binds strongly to hemoglobin after inhalation), several efforts have been undertaken in recent years to generate molecules which deliver CO in a more specific way to diseased tissues. A plethora of CO-Releasing Molecules (CO-RMs) has been produced by various groups and used in animal proof-of-concept studies (see Alberto & Motterlini, 2007). Although various CO-RMs have been shown to reproduce and/or improve on the therapeutic effects of CO seen in inflammatory and other diseases there is still a need to prepare new CO-RM which possess improved physical-chemical and “drug-like” properties that make them more biocompatible and more efficient for pharmaceutical, clinically-acceptable test and use.

The work will be coordinated with that currently taking place in the laboratories of the company Alfama Lda which possesses the world leading technology, expertise and intellectual property in the CO-RM field.

The proposed research plan will contemplate:

- synthesis and chemical characterization (IR, NMR, MS) of some transition metal carbonyl complexes bearing pharmacologically acceptable ligands involving inert atmosphere techniques;
- characterization of the pharmacologically relevant parameters for the new compounds (solubility, stability water and to biological media, lipophilicity (logP), distribution (log D7.4))
- characterization of the CO releasing profile in vitro in chemical and biological media.
- mechanistic investigation of the CO releasing mechanisms promoted by chemicals and proteins necessary to establish structure-activity relationships.

Supervisor: Carlos C. Romão

Area: Chemistry, Pharmacology

Location: ITQB, Laboratory of Organometallic Chemistry

Alberto and Motterlini. Chemistry and biological activities of CO-releasing molecules (CORMs) and transition metal complexes. Dalton Transactions 2007, 17, 1651

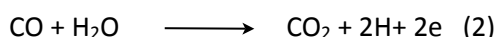
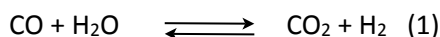
Ryter and Otterbein. Carbon monoxide in biology and medicine. Bioessays 2004, 26, 270-280

Research Project for Master Students

Metal Activation of CO for Energy Production

Early studies on the chemistry of transition metal carbonyls, that is, complexes of CO, showed that in certain conditions coordinated CO is activated to react with H₂O.

Depending on the reaction system two types of processes may occur:



Equation (1) is called the water-gas shift reaction which can be catalyzed by homogeneous and heterogeneous catalysts. However, the kinetic and thermodynamic parameters of this reaction have largely prevented its more widespread use for the production of H₂ (see Kolb et al, 2005 and refs therein).

On the contrary, equation (2) has been adopted by CO-trophic organisms to feed on CO and produce energy or Hydrogen by processing the protons and electrons produced by different enzymes than those that catalyze equation (1), e.g. Hydrogenase.

There are a number of organometallic systems capable of reacting according to eq (2). In these reactions, the electrons and protons produced may be used to produce either H₂ or to generate an electrical current when associated or supported on appropriate electrodes (see Kim et al, 2004 and Rodriguez-Rivera et al, 2005).

The aim of this project is to use the new generation of water solubilizing ligands to produce metal carbonyl complexes that may undergo reaction (2) under homogeneous conditions and/or facilitate their support to modified electrodes.

This work will be done in collaboration with one of the leading Electrochemistry laboratories in Portugal.

The proposed research plan will contemplate:

- synthesis and chemical characterization (IR, NMR, MS) of some transition metal carbonyl complexes bearing water solubilizing ligands involving inert atmosphere techniques;
- study of the reaction of these complexes with water in search of conditions of catalytic turnover;
- characterization of the electrochemical behavior of the complexes including, when feasible the preparation of electrochemical devices driven by the above reactions..

Supervisor: Carlos C. Romão and Dr. Ana Melato (PhD)

Area: Chemistry, Electrochemistry, Green Chemistry, Sustainable Energy

Location: ITQB, Laboratory of Organometallic Chemistry

Kim et al. Powering fuel cells with CO via aqueous polyoxometalates and gold catalysts. *Science* 2004, 305, 1280-1283

Rodriguez-Rivera et al. Hydrogenation of benzene using aqueous solution of polyoxometalates reduced by CO over gold catalysts. *J Am Chem Soc* 2005, 127, 10790-10791

Kolb et al. Water-gas shift reaction in micro-channels - Results from catalyst screening and optimisation. *Catalysis Today* 2005, 110, 121-131

Fluorescent Nanoparticles for probing biological systems

The work involves the synthesis of compatible water soluble functionalised nanoparticles for the visual (fluorescence) of their behaviour in biological systems such as plant cells or parasites. Typical biocompatible groups will be proteins, sugars, and antibodies. The student(s) will be expected to synthesise functionalised ligands based upon lipoic acid and attach the respective biocompatible groups which will have affinity for a particular part of the target biological system. The particles will be observed using fluorescence microscopy within the system.

Local: ITQB/UNL

Orientation: Chris Maycock.

Criteria: Good student interested in interdisciplinary studies.

Contact: maycock@itqb.unl.pt



Master Research Projects 2010/2011

Biological Chemistry Division

- *Energy Transduction by Complex I from Respiratory Chains* [[pdf.](#)]
[Biological Energy Transduction Laboratory](#) - Manuela M. Pereira
- *Structural and functional investigation of type II NADH:quinone oxidoreductases* [[pdf.](#)]
[Biological Energy Transduction Laboratory](#) - Manuela M. Pereira
- *Functional characterization of haem-copper oxygen reductases* [[pdf.](#)]
[Metalloproteins and Bioenergetics Laboratory](#) - Miguel S. Teixeira, Manuela M. Pereira
- [Genomics and Stress Laboratory](#) - Claudina Rodrigues-Pousada
- *Bactérias que produzem electricidade: Estudos de estrutura e função de proteínas envolvidas neste processo* [[pdf.](#)]
[Inorganic Biochemistry and NMR Laboratory](#) - Ricardo O. Louro
- [Macromolecular Crystallography Unit](#) [[pdf.](#)] - Maria Arménia Carrondo
- *Structural studies on Superoxide reductases* [[pdf.](#)]
[Macromolecular Crystallography Unit](#) - Célia V. Romão, Tiago Bandeiras and Pedro Matias
- *Aspectos Moleculares da Neurodegeneração a Esclerose Lateral Amiotrófica: efeito de mutações e factores celulares na formação de amiloide da proteína SOD1* [[pdf.](#)]
[Protein Biochemistry, Folding & Stability Laboratory](#) - Cláudio M. Gomes Fulfilled
- *Modelação molecular/Bioinformática estrutural da hemaglutinina do vírus influenza* [[pdf.](#)]
[Protein Modelling](#) - Cláudio M. Soares
- *Modelação molecular/Bioinformática estrutural de ABC transporters* [[pdf.](#)]
[Protein Modelling](#) - Cláudio M. Soares
- *Analysis of protein-ligand interactions by NMR spectroscopy* [[pdf.](#)]
[Molecular Interactions and NMR](#) - Patrick Groves
- *New methods for the analysis of protein-ligand interactions by NMR spectroscopy* [[pdf.](#)]
[Molecular Interactions and NMR](#) - Patrick Groves
- *Determining the pH structural switch in calbindin D28k* [[pdf.](#)]
[Molecular Interactions and NMR](#) - Patrick Groves
- *Characterization of a new EF-hand protein from Medicago truncatula* [[pdf.](#)]
[Molecular Interactions and NMR](#) - Patrick Groves



Master Research Projects 2010/2011

- *Yeast two-hybrid at low pH* [[pdf.](#)]
[Molecular Interactions and NMR](#) - Patrick Groves
- *Biological hydrogen production by anaerobic bacteria* [[pdf.](#)]
[Bacterial Energy Metabolism](#) - Inês Cardoso Pereira
- *New Enzymes for Biotechnological Applications* [[pdf.](#)]
[Microbial & Enzyme Technology](#) - Lígia O. Martins
- *Structural and Biochemical characterization of novel Fe 2+ metabolic pathways* [[pdf.](#)]
[Biomolecular NMR](#) - Manolis Matzapetakis
- *Structural proteomics of thermostable protein libraries by NMR* [[pdf.](#)]
[Biomolecular NMR](#) - Manolis Matzapetakis

Biological Energy Transduction

Manuela M. Pereira

Project 1

Energy Transduction by Complex I from Respiratory Chains

Complex I from respiratory chains couples the NADH:quinone oxidoreduction to charge translocation across the membrane, which contributes to the build up of an electrochemical potential. The dissipation of this potential through the ATP synthase is used for the synthesis of ATP. Complex I deficiencies have been shown to be implicated in several pathologies, namely neurodegenerative diseases such as Parkinson and Dystonia disorders.

The present proposed project will address the functional role of the membrane subunits of complex I, namely the Na^+/H^+ antiporter modules, Nqo12, 13 and 14 by investigating their proton and sodium transports. Specifically we aim 1- at functionally and structurally characterizing the three different modules; 2- at investigating the operation of these modules individually or integrated in a consortium as the related Mrp antiporters seem to operate; 3-at identifying the architectural features (single amino acid residues or secondary structure motives) responsible for sodium and/or proton conduction; and 4- kinetically studying the ion transports. In particular, these modules expressed in *E.coli* will be reconstituted in liposomes or planar membranes in order to determine the kinetic parameters of Na^+ and H^+ translocations, the Na^+/H^+ stoichiometry and Na^+ binding constants. Addressing these questions will definitively contribute to the knowledge of complex I, a fundamental enzyme in bioenergetics and thus in all metabolism.

Project 2

Structural and functional investigation of type II NADH:quinone oxidoreductases

Type II NADH:quinone oxidoreductases (NDH-II) or alternative NADH dehydrogenases are membrane associated enzymes involved in respiratory chains. In opposite to the other respiratory complexes, which are transmembrane oligomeric enzymes having several prosthetic groups, NDH-II are non-transmembrane monomeric enzymes with a molecular mass around 50 kDa and having FAD as the only prosthetic group. NDHs-II have been suggested to be used in gene therapy correcting NADH:quinone oxidoreductase activity in pathologies with malfunctioning complex I, such as neurodegenerative disorders. The project aims to investigate NDH-II catalytic mechanism and intermediates, and the interaction with the substrates. It is also a goal to recognize the structural elements/motives determinants for catalysis and substrate interaction. A multidisciplinary approach will be performed using a wide range of biochemical and biophysical techniques.

Metalloproteins and Bioenergetics Laboratory

Miguel S. Teixeira, Manuela M. Pereira

Project 3

Functional characterization of haem-copper oxygen reductases

Haem copper oxygen reductases are the widest spread enzymes involved in aerobic respiratory chains, in eukarya, bacteria and archaea. However, both the catalytic mechanism for oxygen reduction and its coupling to proton translocation remain to be fully understood.

With this project we expect to contribute to the elucidation of the electron-transfer, catalytic and proton translocation mechanisms in haem copper oxygen reductases, exploring the natural diversity of this family of enzymes. A multidisciplinary approach will be performed using a wide range of biochemical and biophysical techniques.

Projecto de investigação

Área Científica: Bioquímica

Tema: Bactérias que produzem electricidade: Estudos de estrutura e função de proteínas envolvidas neste processo.

Descrição: Existe uma crescente preocupação com o bem estar do nosso planeta que se manifesta numa forte motivação para o desenvolvimento de métodos alternativos de produção de energia associados a uma menor pegada ecológica.

Foram descritos recentemente organismos de sedimentos marinhos e de água doce capazes de produzir corrente eléctrica directamente a partir do seu metabolismo bioenergético. O trabalho proposto visa a purificação e caracterização de proteínas essenciais para esta actividade. Na execução do plano de trabalhos serão aplicadas técnicas microbiológicas para os crescimentos em “batch”, técnicas cromatográficas para purificação de proteínas e técnicas espectroscópicas para a sua caracterização funcional. O grupo tem financiamento estável e uma composição multidisciplinar que proporciona um ambiente estimulante para desenvolver investigação inovadora.

Duração: 2 semestres (Tese de mestrado, correspondente aos 60 ECTS do 2º Ciclo)

Local de execução: Grupo de Bioquímica Inorgânica e RMN, ITQB-UNL, Oeiras

Público-Alvo: Alunos de mestrado em áreas Bioquímica, Química, Biologia, Biotecnologia ou afins.

Orientação científica: Doutor Ricardo O. Louro e Doutora Catarina M. Paquete

Requisitos: Estudantes interessados neste trabalho com forte motivação para a investigação e para a aprendizagem de multiplas técnicas devem enviar candidatura por correio electrónico (breve CV incluindo indicação do curso e das disciplinas realizadas com a respectiva classificação, e nomes e contactos de dois professores que estejam disponíveis para dar referencias) para: <louro@itqb.unl.pt>

Mais informações sobre a actual orientação científica do grupo pode ser obtida na página: www.itqb.unl.pt/~louro/

Nowadays, **Macromolecular crystallography (MX)** is one of the most important research areas providing essential information to the rationalization of cellular molecular mechanisms and with practical application in other research fields, such as rational drug design, bio- and nanotechnological areas. As an example, while studying a protein target known to be essential in the development of a particular disease, the extensive analysis of the protein crystal structure will provide molecular clues for the development of a specific drug therefore defining novel therapeutic strategies. MX can also be important in protein design, where starting from a known protein structure, a modified version is obtained by making calculated structural variations aiming the enhancement of a specific function. The Macromolecular Crystallography Unit at ITQB is organized into four different Laboratories: Structural Genomics, Structural Biology, Industry and Medicine Applied Crystallography and Membrane Protein Crystallography. Different projects are currently underway in our Unit (<http://xtal.itqb.unl.pt>) and the methodologies used can be summarized into five stages: gene cloning, protein expression, purification and crystallization, structure determination, refinement and analysis. For some projects, the target proteins are cloned, expressed and purified within the Unit, whereas for others the purified protein is supplied by other research Laboratories, either from ITQB or outside, within the framework of collaboration projects. Our main goal is to determine the crystal structures of the protein targets under study, in order to understand the molecular processes in which those proteins are key players.

Macromolecular Crystallography Unit

Master Project – ITQB 2010

Supervisors: Dr. Célia V. Romão, Dr. Tiago Bandejas and Dr. Pedro Matias

Title: *Structural studies on Superoxide reductases*

Abstract: A Master project is proposed to carry out structural studies of superoxide reductases, an important biological system involved in the protection against oxidative damage in prokaryotes.

Introduction:

Although dioxygen (O_2) molecule is relatively inert, it can be rapidly converted into reactive species, through the formation of a one-electron reduction product, superoxide anion (O_2^-). This species can be further reduced in a one-electron cascade reduction, forming two reactive species: hydrogen peroxide (H_2O_2) and the hydroxyl anion (OH^-). The adaptation of ancient microbes to aerobic habitats involved the evolution of enzymes responsible for scavenging reactive oxygen species (by-products of aerobic metabolism) [1]. In anaerobic organisms, the presence of these enzymes is also known, since they may have to deal transiently with oxygen.

The best known enzymatic systems are the superoxide dismutases (SODs) ($O_2^- \rightarrow H_2O_2$) and peroxidases ($H_2O_2 \rightarrow H_2O$). Besides these systems, another type of protein has been studied in recent years, the superoxide reductases (SORs), which detoxify the superoxide anion through its reduction to hydrogen peroxide. The two types of enzymes, SODs and SORs, operate by distinct mechanisms. Although they share a common step, the O_2^- reduction, SOD enzymes are also able to catalyse the oxidation of O_2^- , whereas SOR enzymes are not [2], and the reasons for these different reactivities are still unclear. Using Macromolecular Crystallography we aim to study SOR enzymes from different prokaryotes. These studies will contribute to elucidate their mechanism of reaction.

This work will involve the collaboration with Prof. Miguel Teixeira, leader of the Metalloenzymes and Molecular Bioenergetics Group.

[1] Imlay J.A. (2008) Cellular Defenses against Superoxide and Hydrogen Peroxide *Annu. Rev. Biochem.* **77**, 755–76

[2] Pinto A.F., Rodrigues J.V., Teixeira M. (2010) Reductive elimination of superoxide: Structure and mechanism of superoxide reductase *Biochem Biophys Acta* **1804**, 285-97.

Applications should be sent by email to Dr. Célia Romão (cmromao@itqb.unl.pt).

Aspectos Moleculares da Neurodegeneração na Esclerose Lateral Amiotrófica: efeito de mutações e factores celulares na formação de amiloide da proteína SOD1

Várias doenças neurodegenerativas envolvem a formação de depósitos de proteína agregada (amilóide) que resultam de alterações na estrutura proteica, causadas por factores genéticos (ex: mutações) ou alterações celulares. A esclerose lateral amiotrófica (ELA) é uma doença neurodegenerativa à qual está associada a formação de depósitos da proteína SOD1 (Superóxido Dismutase 1) que se acumulam nos neurónios motores. Mutações no gene SOD1 resultam numa proteína instável com maior propensão para formar amilóide, mas outros factores celulares desempenham um papel igualmente importante dado que nem todos os pacientes ELA com agregados proteicos de SOD1 possuem mutações neste gene. Estes factores celulares adversos incluem níveis celulares anormais do ião zinco, cuja desregulação em doenças neurodegenerativas é conhecida, ou por exemplo uma alteração no funcionamento dos chaperões moleculares, como a chaperonina GroEL.

Este projecto visa contribuir para compreender os mecanismos através dos quais a proteína SOD1 forma agregados amilóide, um aspecto que permanece ainda por esclarecer. Para o efeito propomo-nos analisar o processo de formação de fibras amilóide pela proteína SOD1 normal e em variantes SOD1 com mutações pontuais identificadas em pacientes ELA. O efeito do ião zinco no mecanismo de amiloidogénese será também estudado, assim como a interacção entre a SOD1 e a chaperonina GroEL, de modo a avaliar o efeito de factores não genéticos no processo. As metodologias previstas são diversas, cobrindo várias técnicas moleculares e bioquímicas (ex: expressão e purificação proteica, métodos electroforéticos, análise proteica); métodos espectroscópicos de análise estrutural (ex: Fluorescência marcadores detecção de amilóide); interacções proteína-proteína (ex: interacção com GroEL); estudos de citotoxicidade celular (ex: avaliação da toxicidade das fibras produzidas in vitro em células).

Este trabalho será levado a cabo no ITQB (Oeiras), no laboratório *Protein Biochemistry Folding and Stability* sob a orientação do Dr. Cláudio Gomes, com a co-orientação da Dra. Sónia Leal. Os potenciais interessados devem enviar e-mail para gomes@itqb.unl.pt solicitando mais informações ou para agendar uma visita informal ao laboratório para um contacto mais directo com o tipo de trabalho proposto.

Publicações recentes do grupo: <http://www.itqb.unl.pt/~gomes/publications.html>

Dr. Cláudio M. Gomes (gomes@itqb.unl.pt)
Protein Biochemistry Folding and Stability Laboratory (www.itqb.unl.pt/pbfs)
Instituto Tecnologia Química e Biológica – Universidade Nova de Lisboa (Oeiras)

Proposta de Tese de Mestrado

Tema:

Modelação molecular/Bioinformática estrutural de *ABC transporters*

Local de trabalho:

Grupo de Modelação de Proteínas, Instituto de Tecnologia Química e Biológica
Universidade Nova de Lisboa, Oeiras

Orientador:

Prof. Cláudio M. Soares

Plano de trabalhos sucinto:

Os *ABC transporters* são uma família de proteínas que utilizam a energia da hidrólise de ATP para transportar substratos através de membranas. Os membros desta família intervêm num grande número de processos fisiológicos que vão desde o transporte de nutrientes até a exportação de medicamentos nas células cancerígenas. Mutações destas proteínas estão associadas ao aparecimento de doenças, como por exemplo a fibrose cística.

Apesar da grande diversidade de substratos transportados, todos os membros desta família são formados por uma unidade básica funcional constituída por dois domínios catalíticos (NBDs) e dois domínios transmembranares (TMDs). Presumivelmente, a ligação/hidrólise de ATP produz alterações conformacionais nos NBDs, rearranjos esses que são posteriormente transmitidos aos TMDs, permitindo assim o transporte unidireccional dos substratos. Contudo e apesar de toda a informação disponível acerca desta família, ainda existem muitas questões por esclarecer, tais como a identificação das alterações conformacionais que ocorrem durante a hidrólise de nucleótidos ou o mecanismo de transmissão de energia entre os vários domínios.

O trabalho do/a estudante consistirá em investigar, através da utilização de metodologias de Simulação Molecular e Bioinformática Estrutural, o funcionamento desta família de proteínas, nomeadamente esclarecer os detalhes atómicos associados ao processo de ligação/hidrólise dos nucleótidos e identificação das alterações conformacionais que ocorrem durante o ciclo de transporte.

Pretende-se um/a estudante muito motivado/a para trabalhar numa área de investigação em grande expansão e num grupo dinâmico e muito competitivo. Motivação para trabalhar com metodologias de modelação molecular e com meios informáticos é importante para o sucesso do trabalho. No entanto, não é necessária experiência nestas metodologias.

O Grupo de Modelação de Proteínas do ITQB:

O Laboratório de Modelação de Proteínas desenvolve investigação na área da simulação física de proteínas, tentando compreender processos biológicos utilizando meios computacionais, ou em colaboração com grupos de investigação experimental. O objectivo final destas investigações é a compreensão da Vida ao nível molecular e atómico, pela simulação dos seus mais pequenos componentes. O trabalho do Laboratório de Modelação de Proteínas centra-se no estudo de proteínas envolvidas em cadeias transportadoras de electrões, e em processos com interesse biotecnológico e biomédico.

São possíveis trabalhos noutras temáticas de interesse para o grupo de Modelação de Proteínas.

Para mais informações sobre o nosso trabalho visite a nossa *home page*:

<http://www.itqb.unl.pt/labs/protein-modelling>

<http://www.itqb.unl.pt/research/biological-chemistry/protein-modelling>

Ou contacte:

Prof. Cláudio M. Soares

Instituto de Tecnologia Química e Biológica

Universidade Nova de Lisboa

Av. da República – EAN,

2780-157 Oeiras

Tel: 214469610

e-mail: claudio@itqb.unl.pt

Proposta de Tese de Mestrado

Tema:

Modelação molecular/Bioinformática estrutural da hemaglutinina do vírus influenza

Local de trabalho:

Laboratório de Modelação de Proteínas, Instituto de Tecnologia Química e Biológica
Universidade Nova de Lisboa, Oeiras

Orientação:

Orientador: Prof. Cláudio M. Soares, ITQB-UNL

Co-orientador: Dr. Bruno L. Victor, ITQB-UNL

Plano de trabalhos sucinto:

Epidemias e pandemias virais são problemas que cada vez mais afectam a humanidade. O vírus influenza é o exemplo clássico de vírus permanentemente emergentes responsáveis pelas infecções virais mais devastadoras do século XX e agora XXI. Temos como exemplos a famosa Gripe Espanhola e mais recentemente a Gripe das Aves e a Gripe A.

O vírus influenza apresenta no seu envelope membranar uma glicoproteína denominada por hemaglutinina (HA). Esta proteína está envolvida nos passos iniciais da infecção do vírus, através da promoção da sua fusão com a célula. Apesar da sua importância no ciclo de vida deste vírus, os actuais conhecimentos estruturais acerca da sua acção bem como os mecanismos associados a esta são ainda limitados. A HA é composta por duas cadeias polipeptídicas, a HA1 e a HA2. A cadeia HA1 contém regiões que são utilizadas para ligação a receptores à superfície da célula contendo ácido siálico. Após a internalização do vírus em endossomas, o baixo pH induz dramáticas alterações conformacionais na cadeia HA1. Estes movimentos permitem que o péptido de fusão, localizado no N-terminal da cadeia HA2, fique exposto, promovendo a fusão das membranas do vírus e da célula.

O péptido de fusão é o caso mais simples que podemos utilizar para perceber o processo de fusão que ocorre na infecção de um vírus a uma célula. No entanto, também há outras regiões da proteína envolvidas nessas alterações conformacionais, nomeadamente a região conhecida como o péptido dobradiça. O objectivo deste trabalho é a simulação da hemaglutinina e dos seus péptidos importantes, com vista à compreensão do mecanismo de fusão e consequente infecção pelo vírus influenza.

Pretende-se um estagiário/a muito motivado para trabalhar numa área de investigação em grande expansão e num grupo dinâmico e competitivo. Motivação para trabalhar com metodologias de modelação molecular e com meios informáticos é importante para o sucesso do trabalho. No entanto, não é necessária experiência prévia nestas metodologias.

O Laboratório de Modelação de Proteínas do ITQB:

O Laboratório de Modelação de Proteínas desenvolve investigação na área da simulação física de proteínas, tentando compreender processos biológicos utilizando meios computacionais, ou em colaboração com grupos de investigação experimental. O objectivo final destas investigações é a compreensão da Vida ao nível molecular e atómico, pela simulação dos seus mais pequenos componentes. O trabalho do Laboratório de Modelação de Proteínas centra-se no estudo de proteínas envolvidas em cadeias transportadoras de electrões, e em processos com interesse biotecnológico e biomédico.

São possíveis trabalhos noutras temáticas de interesse para o grupo de Modelação de Proteínas.

Para mais informações sobre o nosso trabalho visite a nossa *home page*:

<http://www.itqb.unl.pt/labs/protein-modelling>

<http://www.itqb.unl.pt/research/biological-chemistry/protein-modelling>

Ou contacte:

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Molecular Interactions and NMR

Instituto de Tecnologia Química e Biológica - Universidade Nova de Lisboa

Av. Da Republica, Estação Agronómica Nacional, 2780-157 OEIRAS PORTUGAL

Tel: 21 446 93 00, Email: pgroves@itqb.unl, mpalczewska@itqb.unl.pt

A brief overview of our research interests are given in the Institute's webpages (link to:

<http://www.itqb.unl.pt/research/biological-chemistry/molecular-interactions-and-nmr>)

The group's main focus is on method development for structural and systems biology, with a personal interest in calcium-binding, EF-hand proteins. Our project proposals cover a wide range of topics and there is something suitable for chemists, biochemists, biologists, biotechnologists...

A) Analysis of protein-ligand interactions by NMR spectroscopy

BACKGROUND: We offer training in NMR spectroscopy and NMR methods, in particular Diffusion NMR and Saturation Transfer Difference spectroscopy –commonly used by the pharmaceutical industry for drug discovery and design [1-3]. The project can be expanded to involve protein expression and purification as part of the sample preparation and/or docking and molecular modeling of the NMR data. We are open to collaboration where you “bring your own samples” from another laboratory.

WORKPLAN: (i) Protein expression and purification will involve cloning and selection, expression and purification. (ii) NMR spectroscopy methods including sample preparation, parameter optimization, processing and analysis. (iii) Docking of ligand structures with existing protein structures. Verification of structures with NMR data.

SUPERVISION: Supervised by P.Groves. Part (i) will be co-supervised by M. Palczewska.

References:

1. M. Politi, J. Alvaro-Blanco, P. Groves, A. Prieto, J.A. Leal, F.J. Cañada and J. Jiménez-Barbero “Screening garlic water extract for binding activity with Cholera Toxin B pentamer by NMR. An old remedy giving a new surprise”, *Eur. J. Org. Chem.*, 2006, 2067-73.
2. A. Bastida, A. Hidalgo, J.L. Chiara, M. Torrado, F. Corzana, J.M. Cañadillas, P. Groves, E. Garcia-Junceda, J. Jimenez-Barbero and J.L. Asensio “Exploring the use of conformationally locked amino-glycosides as a new strategy to overcome bacterial resistance”, *J. Am. Chem. Soc.*, 2006, 126, 100-16.
3. F. Chevalier, J. Lopez-Prados, P. Groves, S. Perez, M. Martín-Lomas and P.M. Nieto “Structure and dynamics of the conserved protein GPI anchor core inserted into detergent micelles”, *Glycobiol.*, 2006, 16, 969-980.

B) New methods for the analysis of protein-ligand interactions by NMR spectroscopy

BACKGROUND: Although we have many established NMR protocols suitable for the project described above, we still need to develop new tools to solve some of the problems brought to us by our collaborators. Examples of new and modified protocols are given in [4-8]. Future challenges include the use of DNA or in situ membrane proteins present in live mammalian cells as target molecules in STD experiments, as well as the application of the methods in [4] to investigate protein-protein interactions.

WORKPLAN: The student will first work on a short project involving a model target-ligand system to learn the established NMR methods before starting to develop new methodology on a model system. The project is mostly a dry lab project requiring computer skills.

SUPERVISION: Supervised by P.Groves.

References:

4. K.E. Kövér, P. Groves, J. Jiménez-Barbero and G. Batta “Molecular recognition and screening using STD NMR: ¹⁵N-group selective STD NMR experiment to study intermolecular interactions in heavily overlapped spectra”, *J. Am. Chem. Soc.*, 2007, 129, 11579-82.
5. P. Groves, K.E. Kövér, S. André, J. Bandorowicz-Pikula, G. Batta, M. Bruix, R. Buchet, A. Canales, F.J. Cañada, H-J. Gabius, D.V. Laurents, J.R. Naranjo, M. Palczewska, S. Pikula, E. Rial, A. Strzelecka-Kiliszek, and J. Jiménez-Barbero “Effect of temperature in Saturation Transfer Difference NMR experiments”, *Magn. Reson. Chem.*, 2007, 45, 745-8.
6. K. Fehér, P. Groves, G. Batta, J. Jiménez Barbero, C. Muhle-Goll, K.E. Kövér “Application of isotope edited and filtered STD NMR experiments for ligands with overlapping signals”, *J. Am. Chem. Soc.*, 2008, 130, 17148-53.
7. J.P. Ribeiro, M. Palczewska, S. André, F.J. Cañada, H-J. Gabius, J. Jiménez-Barbero, B. Mellström, J.R. Naranjo, D.J. Scheffers, P. Groves “Diffusion nuclear magnetic resonance spectroscopy detects substoichiometric concentrations of small molecules in protein samples.” *Anal. Biochem.* 2010, 396, 117-23.
8. P. Groves, M. Webba da Silva “Rapid Stoichiometric Analysis of G-Quadruplexes in Solution.” *Chem. Eur. J.* 2010, in press.

C) Determining the pH structural switch in calbindin D28k

BACKGROUND: Closely related calretinin and calbindin D28k are neuronal proteins that offer protection against intracellular calcium insults. We believe these proteins also contain a pH switch that turns them into dual sensors, only becoming activated in the presence of elevated concentrations of both calcium and protons.

WORKPLAN: (i) to take the established expression clones to express and purify a small, 87 residue calbindin domain in isotope-labeled form. (ii) to biochemically characterize the proteins to verify their pH properties are the same as the full-length proteins. (iii) to prepare two sets of samples at pH 7.5 and pH 6.5. (iv) to acquire and process NMR data using established methods. (v) to assign the NMR data, using published data as a guide. (vi) to model the NMR data. (vii) to verify the resulting 3D structures.

SUPERVISION: Overall supervision will be given by P. Groves. M. Palczewska will co-supervise parts (i-iii).

References:

9. M. Palczewska, G. Batta, P. Groves, S. Linse, and J. Kuźnicki "Localization of the Ca(2+)- and H(+)-dependent hydrophobic properties of calretinin", *Protein Sci.*, 2005, 14, 1879-87.

D) Characterization of a new EF-hand protein from *Medicago truncatula*

BACKGROUND: The lab of Prof. Julie Cullimore (INRA, Toulouse) has discovered a new EF-hand protein related to the signaling pathway between symbiotic rhizobia and legumes, leading to nitrogen fixation. Our task is to express and purify this protein, its deletion mutants and to characterize the interaction of these proteins with a peptide derived from the interacting protein by spectroscopic and biochemical methods.

WORKPLAN: (i) design, cloning and expression of a series of His-tagged deletion mutants. (ii) Purification of the proteins. (iii) Spectroscopic analysis of mutants by fluorescence, circular dichroism, NMR.

SUPERVISION: Overall supervision will be given by M. Palczewska. Co-supervision will be provided by P. Groves.

References: 7, 1, 4, 5 and:

10. M. Palczewska, P. Groves, A. Ambrus, A. Kaleta, K. E. Kövér, G. Batta and J. Kuźnicki "Structural and biochemical characterization of neuronal calretinin domain I-II (residues 1-100); comparison to homologous calbindin D28k domain I-II (residues 1-93)", *Eur. J. Biochem.*, 2001, 268, 6229-6237.

E) Yeast two-hybrid at low pH

BACKGROUND: Yeast two-hybrid (YTH) techniques are at the forefront of systems biology efforts to define protein-protein interactors. A recent paper describes the conditions where the intracellular pH of yeast can be lowered and maintained at pH 6.8. In principal, these conditions allow us to screen protein-protein interactions at low pH.

In this project, we will set up a defined library to compare protein-protein interactions at the two different pH. The library will include neuronal calbindin D28k and three known binding partners. We expect at least one of them will only interact at low pH. A second test system from Dr D.J. Scheffers' lab will test the reverse case, i.e. an interaction at pH 7.4 that will be absent at pH 6.8, namely the interaction between MinC and FtsZ from a Gram-positive bacterium, *Bacillus subtilis*.

WORKPLAN:

(i) design, cloning and expression of two bait proteins fused with DB domain and a variety of target proteins and/or its fragments as a AD fusion. (ii) Setting up YTH system and testing it in physiological and low pH. (iii) Screening the interaction of two bait proteins and their predicted targets using YTH system in physiological and low pH conditions.

SUPERVISION: Overall supervision will be given by M. Palczewska. Co-supervision will be provided by P. Groves.

References: 9, 10, 11

11 Scheffers DJ "The effect of MinC on FtsZ polymerization is pH dependent and can be counteracted by ZapA." *FEBS Lett.* 2008, 582:2601-8.



Instituto de Tecnologia Química e Biológica
Universidade Nova de Lisboa

MASTERS PROJECT

BIOLOGICAL HYDROGEN PRODUCTION BY ANAEROBIC BACTERIA

Area: Microbiology/Biochemistry

Laboratory: Bacterial Energy Metabolism

Supervisor: Dr.^a Inês Cardoso Pereira

Hydrogen is a promising energy resource/energy carrier as future alternative to fossil fuels. Nowadays, hydrogen is mainly produced from carbon-containing non-renewable sources, in processes that are not sustainable or environmentally friendly. Thus, development of a sustainable hydrogen production system is of the utmost importance. Biological production of hydrogen is a very interesting alternative as it requires a very low energy input and is sustainable if using waste or renewable substrates. In this project we propose to study an anaerobic sulfate-reducing bacterium, *Desulfovibrio vulgaris* Hildenborough, as an alternative hydrogen producer under fermentative conditions, to replace methanogenic organisms in the second step of anaerobic digestion processes. Sulfate-reducing bacteria are notorious for expressing very high levels of hydrogenases (Hases), the enzymes responsible for hydrogen production and/or consumption. The genome of *D. vulgaris* has been sequenced and encodes for six different Hases. Some of these are hydrogen-producing and others are hydrogen-consuming. The project will comprise the study of conditions to optimize the hydrogen producing ability of *D. vulgaris* wild type as well as deletion mutants of the hydrogen-consuming Hases. This work will develop expertises in several areas such as: Microbial Technology, Microbiology, Biochemistry, Bioinformatic analysis and others.

For more information contact:

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New Enzymes for Biotechnological Applications

Brief description

Industrial biotechnology includes the practice of using cells or enzymes acting on renewable feedstocks to generate useful bioproducts. It is expected to have an increasing impact in several industries and it will enable economies to become less dependent on fossil fuels. Recently, we have focused our research towards the study of enzymatic bioconversions of lignin-based compounds. Lignin is a complex aromatic biopolymer found in the cell walls of vascular plants, which make it an interesting renewable source for aromatic chemicals. However this polymer is highly recalcitrant towards degradation. In nature, lignin is primarily degraded by fungal secreted enzymes, such as peroxidases and laccases, which are capable of oxidizing lignin substructures. This proposal focuses on the characterization and engineering of bacterial lignolytic enzymes. We will undertake a multidisciplinary approach to understand the key structural and functional determinants of lignolytic enzymes. For example, through site directed mutagenesis we will examine how replacing key amino acid residues affects enzyme properties. Studies will be undertaken in order to get deep insight over the general mechanistic pathways of substrate degradation. Our system for improving enzyme properties towards industrial application is to resort to directed evolution techniques, followed by robotic high-throughput screening.

Supervisor

Lígia O. Martins, lmartins@itqb.unl.pt, <http://www.itqb.unl.pt>

Duration

6-12 meses

Place

Laboratório de Tecnologia Microbiana e Enzimática, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras.

Students

1 or 2

Biomolecular NMR @ itqb

Positions for Masters Students

The following two projects are available at the group of Biomolecular NMR:

- a) Structural and Biochemical characterization of novel Fe²⁺ 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.

Applications should be send by email to Dr. Manolis Matzapetakis at : matzman@itqb.unl.pt

**<http://www.itqb.unl.pt/research/biological-chemistry/biomolecular-nmr>
<http://www.itqb.unl.pt/~matzman/>**

Description of projects follows in the next pages:

Biomolecular NMR @ itqb

Structural and Biochemical characterization of novel Fe²⁺ 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 Fe³⁺ much less is known about the metabolic pathways of Iron in the ferrous state (Fe²⁺) 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 Fe²⁺ 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 Fe²⁺ 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 Fe²⁺ 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* Fe²⁺ 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 (¹³C ¹⁵N) will be used for structural studies by NMR employing multidimensional heteronuclear experiments for resonance assignment. Structures will be calculated using distance restraints 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.

Applications should be send by email to Dr. Manolis Matzapetakis at : matzman@itqb.unl.pt

Biomolecular NMR @ itqb

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 °C.

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.

Applications should be send by email to Dr. Manolis Matzapetakis at : matzman@itqb.unl.pt



Master Research Projects 2010/2011

Biology Division

- *Single cell studies of the action of antibiotics* [\[pdf.\]](#)
[Bacterial Cell Biology Laboratory](#) - Mariana Gomes de Pinho
- *Papel da cápsula de streptococcus pneumoniae no reconhecimento do peptidoglicano pelo sistema imunitário inato do hospedeiro* [\[pdf.\]](#)
[Bacterial Cell Surfaces and Pathogenesis Laboratory](#) - Sergio R. Filipe
- *Cell Signaling in Drosophila Project* [\[pdf.\]](#)
[Cell Signaling in Drosophila Laboratory](#) - Pedro Domingos
- *Glycobiology Laboratory Project* [\[pdf.\]](#)
[Glycobiology Laboratory](#) - Júlia Costa

Single cell studies of the action of antibiotics

Mariana Gomes de Pinho, mgpinho@itqb.unl.pt

Brief description: Microbiology has traditionally focused on studies at the population level, which look at the average behavior of cells. However, in recent years, the availability of tools to study individual cells, has allowed a new understanding of the existence and significance of cellular heterogeneity. Importantly, even cells from isogenic populations, growing in the same conditions, can exhibit phenotypic variation.

We will use as a model organism the Gram positive bacteria *Staphylococcus aureus*, an extremely versatile pathogen capable of causing from minor infections to life threatening ones. We want to determine if different cells from isogenic populations of *S. aureus* strains have an heterogeneous response to the presence of antibiotics due to (i) biochemical and morphologic differences arising from cells being at different stages of the cell cycle (ii) stochastic fluctuations in the expression of genes required for the stress response of *S. aureus* to the presence of cell wall active antibiotics.

In this master project we aim to determine if there is a dependence of the killing action of antibiotics on the cell cycle stage.

Variations in susceptibility/tolerance to antibiotics over the bacterial cell cycle can be due to variations of the internal biochemical parameters, or to variations in the morphology of the cells which can affect, for example, the access of antibiotics to their targets and/or the susceptibility of the targets to the antibiotics. We will use dyes which specifically label dead cells to determine if there are stages of the cell cycle during which the cell is either more tolerant or more susceptible to death by the action of different classes of antibiotics (namely antibiotics that inhibit either cell wall synthesis or DNA replication).

Projectos de tese para alunos 2º Ciclo da área da Biologia (Biologia Celular e Biotecnologia, Biologia Molecular e Genética, Microbiologia Aplicada)

1. Tema do projecto

Papel da cápsula de *Streptococcus pneumoniae* no reconhecimento do peptidoglicano pelo sistema imunitário inato do hospedeiro

2. Identificação do orientador

Sérgio Raposo Filipe, ITQB/UNL

3. Plano do projecto (tema e enquadramento geral)

Tema enquadrado no projecto “Contribuição da cápsula para a actividade inflamatória do peptidoglicano bacteriano” financiado pela Fundação para a Ciência e Tecnologia com a referência PTDC/SAU-MII/75696/2006.

Uma característica essencial aos organismos superiores, desde o mais pequenos dos insectos aos mamíferos, para a sua própria sobrevivência é a capacidade de induzir uma resposta imunitária inata quando invadidos por um microrganismo. Dos vários componentes detectados pelo hospedeiro e assim capazes de induzir uma resposta inflamatória, o peptidoglicano é a molécula comum a quase todas as bactérias.

As bactérias gram-negativas têm uma camada fina de peptidoglicano, rodeada por uma membrana exterior constituída por fosfolípidos e LPS. Por outro lado, as bactérias gram-positivas têm uma camada espessa de peptidoglicano, o componente principal da sua parede celular, ao qual se associam de um modo covalente ou não-covalente outros polissacarídeos e proteínas. Um destes polissacarídeos é a cápsula que é um factor essencial de virulência em *Streptococcus pneumoniae*. Iremos usar como modelo de estudo esta bactéria gram-positiva que está associada a uma mortalidade anual nos EUA de valor semelhante à mortalidade causada por SIDA, cancro da mama e cancro da próstata.

Resumidamente pretendemos:

- Construir mutantes de *S. pneumoniae* em genes que codificam proteínas envolvidas no metabolismo da cápsula.
- Identificar o efeito da ausência destas proteínas no processo da síntese da cápsula.
- Purificar paredes e respectivos peptidoglicanos das diferentes estirpes capsuladas de *S. pneumoniae* e respectivos mutantes.
- Analisar a afinidade para a superfície celular dos diferentes mutantes de *S. pneumoniae* de uma proteína modelo capaz de detectar peptidoglicano bacteriano, PGRP-SA, usando SDS-Page e microscopia de fluorescência.

Esperamos no fim do projecto determinar se a cápsula de pneumococos poderá interferir com a capacidade de o hospedeiro detectar o peptidoglicano bacteriano.

4. Duração aproximada

1 ano lectivo.

5. Local de Realização

Laboratório de Patogénese e Superfícies Bacterianas - ITQB/UNL

6. Número de alunos por projecto

Um (1).

Proposal for a Masters degree internship (Projecto Tese de Mestrado)

Supervisor: Pedro Domingos (domingp@itqb.unl.pt)

Starting date – September 2010

Duration – 9 to 12 months

Number of positions - 1

Summary

The aim of our research is to understand the molecular mechanisms that regulate specification, differentiation and degeneration of the photoreceptors, the cells that sense light in the visual system, using *Drosophila* as our biological model. Our most recent work focuses on the protective role of the Unfolded Protein Response (UPR), a cellular signaling pathway activated by the presence of unfolded proteins in the Endoplasmic Reticulum (ER), against photoreceptor degeneration in a *Drosophila* model for Autosomal Dominant Retinitis Pigmentosa. We use the tools of modern genetics, cell biology and imaging to pursue the signaling mechanisms that regulate cell death/cell protection in our biological model system.

Publications:

1) Rasheva, V.I. and **Domingos, P.M. (2009)** “Cellular responses to endoplasmic reticulum stress and apoptosis”, in press in **Apoptosis**. Review Article

2) **Domingos PM**, Steller H. (2007) Pathways regulating apoptosis during patterning and development. **Curr Opin Genet Dev**. Aug;17(4):294-9. Review Article.

3) Ryoo HD*, **Domingos PM***, Kang MJ, Steller H. (2007) Unfolded protein response in a *Drosophila* model for retinal degeneration. **EMBO J**. Jan 10;26(1):242-52. *equal contribution

Glycobiology Laboratory

Júlia Costa

Carbohydrates from mammalian cells play several functional roles in cell adhesion and recognition, cell development, glycoprotein folding among others. The fucosylated carbohydrate structure Lewis X (Le X,

Galbeta4[Fucalpha3]GlcNAc) is abundant in the brain. Le X expression in neurons is temporally and spatially regulated and it seems to be involved in neuron adhesion and neurite outgrowth, however, the molecules that participate in the process have not been identified and the mechanisms underlying these roles have not been elucidated. In the central nervous system, Le X expression also identifies stem cells and specific progenitor cells. In brain, Le X is synthesized by fucosyltransferase

9 (FUT9). This is an alpha3 fucosyltransferase expressed at higher levels in brain tissue. The knock-out mouse FUT9^{-/-} showed disappearance of Le X in the brain, concomitant with behavior alterations.

Previous work from the laboratory has identified the expression of Le X specifically in differentiated human NT2N neurons in vitro most likely synthesized by FUT9. A specific neuron glycoprotein or proteoglycan Le X-carrier of 460 kDa was also identified. Furthermore, incubation with antibody anti-Le X led to the inhibition of neurite outgrowth and impairment of neuron adhesion. Le X was found in the exocytotic compartment defined by the tetanus neurotoxin-insensitive vesicle-associated membrane protein (TI-VAMP), which is involved in neurite outgrowth, of NT2N neurons and also of rat hippocampus neurons in culture.

Our aim is to characterize molecules that are associated with the Le X motif and to identify the corresponding lectin receptors, and, therefore, to elucidate mechanisms that underlie the functional role of Le X in neurite outgrowth and neuron adhesion. The results might have impact in the improvement of brain plasticity and brain repair.

<http://www.itqb.unl.pt/research/biology/glycobiology>



Master Research Projects 2010/2011

Plant Sciences Division

- *Chromosome cohesion and DNA double strand break damage repair* [[pdf.](#)]
[Disease and Stress Biology Laboratory](#) - José C. Nunes
- *Molecular Analysis of Class III HD-Zip transcription factors and role in plant vascular development* [[pdf.](#)]
[Forest Biotechnology Laboratory](#) - Célia Miguel
- *Functional studies of genes involved in maritime pine embryogenesis* [[pdf.](#)]
[Forest Biotechnology Laboratory](#) - Célia Miguel
- *Small RNAs in Quercus suber cork tissue* [[pdf.](#)]
[Forest Biotechnology Laboratory](#) - Célia Miguel
- *Produção de proteínas recombinantes em culturas de células vegetais* [[pdf.](#)]
[Plant Cell Biology Laboratory](#) - Rita Abranches
- *Caracterização citológica do fungo Hemileia vastatrix responsável pela doença da ferrugem alaranjada da folha do cafeeiro* [[pdf.](#)]
[Plant Cell Biology Laboratory](#) - Rita Abranches, Pedro Talhinhos
- *Identification and functional characterization of rice transcription factors that regulate photosynthesis-related genes under salt stress* [[pdf.](#)]
[Plant Genetic Engineering Laboratory](#) - Nelson Saibo
- *Análise proteómica do efeito de agentes antioxidantes num modelo celular de neurodegeneração* [[pdf.](#)]
[Disease and Stress Biology laboratory](#) - Cláudia Nunes dos Santos e Marta Alves
- *Implementação do modelo celular de Parkinson em células SK-N-MC por exposição crónica à rotenona. Estudo do efeito de antioxidantes naturais* [[pdf.](#)]
[Disease and Stress Biology laboratory](#) - Cláudia Nunes dos Santos e Lucélia Tavares
- *DROUGHTYRUS - Caracterização das respostas fisiológicas de espécies de Lathyrus ao deficit hídrico* [[pdf.](#)]
[Plant Cell Biotechnology](#) - Carlota Vaz Patto e Susana Araújo
- *NUTICAEVOLVE - Evolução da diversidade genética na adaptação a longo prazo de germoplasma exótico de milho num projecto Português de conservação e melhoramento participativos* [[pdf.](#)]
[Plant Cell Biotechnology](#) - Carlota Vaz Patto

Master thesis - 2010 / 2011

“ Chromosome cohesion and DNA double strand break damage repair “

Contact details

Thesis supervisor: José António Melo da Costa Nunes

email: jcnunes@itqb.unl.pt

URL: <http://sites.google.com/site/costanunescohesin/>
<http://www.itqb.unl.pt/labs/disease-and-stress-biology/group-members>

Goals:

To further characterise Arabidopsis cohesins.

Cohesins are required for chromosome cohesion and DNA double strand break damage repair.

MSc thesis:

- Chromosome cohesion and DNA damage repair -

Cohesins are critical for the maintenance of genome integrity due to their role in chromosome segregation during cell division. Cohesins are also involved in DNA double strand break (dsb) damage repair. Chromosome mis-segregation and DNA dsb damage faulty repair, can lead to chromosome rearrangements, chromosome fragmentation, as well as other genome aberrations which can trigger cancer, cell death, etc.

In Arabidopsis there are four cohesin genes, one of which has been shown to be responsive and required to DNA double strand break damage repair (da Costa Nunes *et al.*, 2006).

During this project, the expression of some cohesion genes in wild type and mutant background plants will be monitored. Their response to different DNA damaging agents will be characterised. Yeast may also be used to further characterise Arabidopsis cohesins protein functions.

The student will get training on plant handling, genetic analysis and molecular biology techniques. The facilities and equipment required to carry out the experiments are available in ITQB.

References: da Costa Nunes *et al.*, 2006

(<http://jxb.oxfordjournals.org/cgi/reprint/erj083v1>)

The **student** should be familiar with molecular biology techniques and genetics, and preferably have good knowledge of the English language.

Thesis will be carried out in: Instituto de Tecnologia Química e Biológica (ITQB),
Universidade Nova de Lisboa

Encontram-se abertas 3 candidaturas a estágios de Mestrado em *Biologia Celular e Biotecnologia* (ano lectivo 2010/2011), no Grupo Forest Biotech (ITQB/IBET, Oeiras). Pretendem-se candidatos com uma forte motivação e interesse em Investigação nas áreas de Biologia Molecular e Biotecnologia em Plantas.

Os Projectos de Mestrado (ver em baixo) terão a duração máxima de um ano. Os candidatos deverão enviar uma carta de apresentação, acompanhada de Cv, do qual deverão constar grau académico, classificação final e ano de conclusão.

Projectos de Mestrado em *Biologia Celular e Biotecnologia* (ano lectivo 2010/2011)

Projecto 1

Tema do Projecto: Molecular Analysis of Class III HD-Zip transcription factors and role in plant vascular development

Sumário do projecto: In eukaryotes, transcription of protein-coding genes is controlled by complex networks of transcription factors. In *Arabidopsis* model plant, the transcription factors family Class III homeodomain-leucine zipper (HD-Zip III) genes have been reported to regulate vascular development. In trees, the role of these transcription factors is still largely unknown. The aim of this project is to characterize the function of these genes in the provascular and vascular organization in the woody poplar plants using different molecular and cell biology approaches. The transcriptional regulation of vascular development in plants is a competitive area of study, with implications in the control of important developmental processes such as wood formation.

The experimental work will involve:

- *In vitro* and greenhouse growth of poplar and *Arabidopsis* plants;
- Over-expression and silencing of HD-Zip III genes in poplar and *Arabidopsis* plants;
- Confocal microscopy for analysis of vascular development

Orientador – Célia Miguel

Duração e carga horária - 6 meses a 1 ano, 35 horas semanais

Local de realização – Forest Biotech Laboratory, ITQB/IBET, Oeiras
(cmiguel@itqb.unl.pt)

Número de estagiários – 1

Projecto 2

Tema do Projecto: Functional studies of genes involved in maritime pine embryogenesis

Sumário do projecto: Most studies of plant embryogenesis have been conducted in angiosperms like the model plant *Arabidopsis* (Willemson e Scheres 2004, Annu Rev Genet 38:587–614). Despite the similarities in the embryogenesis of angiosperms and gymnosperms, the evolutionary divergence resulted in unique characteristics in the development of the embryo in both groups of plants. However, comparative molecular studies of embryo development in angiosperms and gymnosperms are still scarce (Cairney et al. 2006, Plant Mol Biol 62:485-501). In this project, the previously initiated functional

characterization of genes involved in the embryogenesis of the gymnosperm species *Pinus pinaster* will be continued using somatic embryogenesis as an experimental system.

The experimental work will involve:

- Preparation of constructs for manipulating gene expression in somatic embryos;
- Genetic transformation of embryogenic cultures;
- Phenotype characterization and molecular analyses of transformants

Orientador –Célia Miguel

Duração e carga horária - 6 meses a 1 ano, 35 horas semanais

Local de realização – Forest Biotech Laboratory, ITQB/IBET, Oeiras
(cmiguel@itqb.unl.pt)

Número de estagiários – 1

Projecto 3

Tema do Projecto: Small RNAs in *Quercus suber* cork tissue

Small RNA molecules of about 20–30 nucleotides have emerged as powerful regulators of gene expression and genome stability [Moazed D, Nature (2009) 457, 413-420]. They function by guiding sequence-specific gene silencing at the transcriptional and/or post-transcriptional level. MicroRNAs (miRNAs) represent one of the major classes of small regulatory RNAs in plants and since the first discovery of miRNAs in *Arabidopsis* in 2002 [Reinhart BJ et al, Genes Dev. (2002) 16, 1616–1626], more than 700 plant miRNAs have been identified using different strategies.

Microarray miRNA expression profiling revealed the deep conservation of many plant miRNA families, with at least eight families conserved since before the emergence of seed plants [Axtell M and Bartel DP, Plant Cell (2005) 17, 1658–1673]. However, a majority of plant microRNA families have a different degree of divergence between species. It is believed that the diversity is necessary for plants to regulate various specific processes. Cork cambium (or phellogen) from cork oak is a lateral meristem that produces cork (phellem) with a unique composition and structure. This project will consist in the analysis of cork cambium/phellem regarding small RNAs content, in order to identify candidates conferring the tissue specificity to produce cork. The experimental work will involve:

- collection of phellem tissues from previously geo-identified cork oak trees
- optimization of protocols for small RNA isolation
- identification and expression analysis of specific small RNAs

Orientadores: Inês Chaves e Célia Miguel

Duração e Carga horária: 6 meses a 1 ano (35 horas semanais)

Local de realização: Forest Biotech Lab (ITQB/IBET)

Número de estagiários – 1

PRODUÇÃO DE PROTEÍNAS RECOMBINANTES EM CULTURAS DE CÉLULAS VEGETAIS

Orientadores: Ana Sofia Pires, Rita Abranches

No nosso laboratório utilizamos plantas transgénicas e culturas de células vegetais para produção de proteínas recombinantes com aplicação na indústria farmacêutica e alimentar. O trabalho a realizar envolve o estabelecimento e caracterização de culturas celulares de plantas (*Arabidopsis thaliana* e *Medicago truncatula*) para produção de proteínas humanas, e optimização de processos de purificação da proteína para posterior caracterização e realização de ensaios de actividade biológica. Existem 2 lugares disponíveis para desenvolvimento de tese de mestrado no âmbito deste projecto.

Tema do projecto

Caracterização citológica do fungo *Hemileia vastatrix* responsável pela doença da ferrugem alaranjada da folha do cafeeiro.

Orientação: Sílvia Tavares, Rita Abranches

A infecção causada pelo fungo *Hemileia vastatrix* denominada ferrugem alaranjada da folha do cafeeiro é a principal doença do cafeeiro Arábica (*Coffea arabica*) provocando perdas que rodam os 20% a nível mundial. A doença atinge praticamente todos os países produtores de café diminuindo consideravelmente o rendimento obtido na produção do grão, quer pela perda directa na quantidade produzida quer pelo elevado custo dos fungicidas aplicados que continuam a ser o método mais corrente de controlo da doença. O projecto tem como objectivo principal o estudo da interacção entre o cafeeiro e *Hemileia vastatrix*, começando pela caracterização citológica e em concreto pela determinação do cariótipo de *Hemileia vastatrix*. Em fungos patogénicos tem sido descrito o papel importante na patogenicidade que cromossomas supranumerários, que existem em apenas alguns indivíduos de uma determinada espécie, possuem em estirpes especialmente virulentas, daí a importância da determinação do cariótipo que é totalmente desconhecido para o fungo *Hemileia vastatrix*. Para tal vão ser utilizadas técnicas de microscopia de fluorescência, tais como visualização dos cromosomas com DAPI ou a técnica de FISH (Fluorescence In Situ Hybridization), assim como a técnica de separação electroforética em gel de agarose PFGE.

Identification and functional characterization of rice transcription factors that regulate photosynthesis-related genes under salt stress

Salt stress reduces photosynthetic efficiency, thus limiting plant growth and productivity. In rice, the transcription level of important genes associated with photosynthesis (*Rubisco Activase*, *Sedoheptulose-Bisphosphatase*, *Chloroplast ATP synthase* and *Oxygen evolving enhancer 2*) is down regulated under high salinity and their over-expression can improve abiotic stress tolerance. Transcription factors (TFs), which may control various downstream genes, clearly play an important role controlling photosynthetic-related gene expression under salt stress. However, little is known concerning the identity and function of these TFs. Using the “yeast one-hybrid” system, we aim to identify TFs that regulate this process. Functional analysis of the novel TFs will be performed using a transgenic and/or mutant approach. These results will help to understand fundamental mechanisms of salinity tolerance in rice especially regarding transcriptional regulation of the photosynthetic response. This understanding may eventually be used to improve photosynthetic capacity of crop plants and reduce productivity losses under stress environments.

Orientador: Nelson Saibo

saibo@itqb.unl.pt

TÍTULO: Análise proteómica do efeito de agentes antioxidantes num modelo celular de neurodegeneração

LOCAL: Disease and Stress Biology laboratory, I.T.Q.B.

ORIENTADORES: Cláudia Nunes dos Santos e Marta Alves

INTRODUÇÃO:

A neurodegeneração é um processo multifactorial, já que inclui um conjunto complexo de reacções oxidativas que levam à morte neuronal, incluindo o mau funcionamento da via ubiquitina/proteassoma com formação dos corpos inclusos, estruturas comumente observadas nestas patologias. A terapia destas patologias requer um cocktail de fármacos com propriedades plurifarmacológicas, em vez de um único fármaco. Contudo, a progressão ou atraso da neurodegeneração ainda não foi alcançada. O estudo de como os antioxidantes naturais, fornecidos pela dieta, modelam a expressão proteica deverá permitir a identificação de novos biomarcadores e dos mecanismos moleculares pelos quais a nossa dieta pode exercer um efeito potencialmente protector contra as doenças neurodegenerativas.

O presente plano de trabalho insere-se num projecto mais amplo que visa estudar os mecanismos celulares e bioquímicos subjacentes aos modos de acção de antioxidantes naturais e que serão investigadas a vários níveis: (i) viabilidade celular e apoptose; (ii) produção de ROS; (iii) tióis totais (níveis de GSH e GSSG); (iv) medição dos hidroperóxidos lipídicos por substâncias reactivas ao ácido tiobarbitúrico; (v) maquinaria endógena celular antioxidante e (vi) alterações redox nas proteínas.

O objectivo final deste trabalho será o de avaliar os efeitos dos antioxidantes no metabolismo proteico do modelo celular de neurodegeneração usando uma abordagem proteómica. Para o efeito decorrerá a optimização do procedimento da obtenção do proteoma de células SK-N-MC. Esta optimização será fundamental para a subsequente avaliação das alterações na expressão proteica e estado de oxidação quando os neuroblastomas são incubados na presença de polifenóis antioxidantes num modelo de neurodegeneração induzido por peróxido de hidrogénio.

PLANO DE TRABALHO:

O material biológico utilizado para este estudo é um neuroblastoma humano (linha celular SK-N-MC). O plano de trabalho inclui várias fases:

Fase 1 - Obtenção de um padrão reprodutível para o proteoma das células SK-N-MC.

Fase 2 - Implementação do modelo de neurodegeneração induzido por H₂O₂ em células SK-N-MC

Fase 3 - Monitorização da produção de espécies reactivas de oxigénio no modelo de neurodegeneração com e sem pre-incubação de polifenóis.

Fase 4 – Monitorização do estado de oxidação das proteínas (avaliação dos grupos carbonilo) no modelo de neurodegeneração com e sem pre-incubação de polifenóis.

Fase 5 – Avaliação das alterações do proteoma no modelo de neurodegeneração induzido por peróxido de hidrogénio com e sem pre-incubação de polifenóis.

CONTACTOS: Cláudia Nunes dos Santos (csantos@itqb.unl.pt, 214469651) e Marta Alves (malves@itqb.unl.pt, 214469653)

TÍTULO: Implementação do modelo celular de Parkinson em células SK-N-MC por exposição crónica à rotenona. Estudo do efeito de antioxidantes naturais

LOCAL: I.T.Q.B.

ORIENTADORES: Cláudia Nunes dos Santos e Lucélia Tavares

INTRODUÇÃO:

A doença de Parkinson (PD) é uma doença neurodegenerativa, para a qual não existe tratamento efectivo. Envolve reacções oxidativas, que conduzem à disfunção do sistema da ubiquitina/proteassoma (UPS), com a formação de agregados proteicos (inclusões proteicas citoplasmáticas, contendo ubiquitina e alfa-sinucleína), conhecidos por corpos de Lewis. Têm sido publicados numerosos trabalhos sobre a participação da via da ubiquitina/proteassoma (via da Ub) nas doenças neurodegenerativas que afectam o Homem, como sejam a doença de Parkinson, a doença de Alzheimer e o próprio envelhecimento. A acumulação de corpos inclusos, que incluem agregados de conjugados de ubiquitina-proteína, característico destas patologias, permanece um mistério. Por outro lado, tem sido referido que alguns compostos fenólicos com propriedades antioxidantes, como as catequinas do chá e o resveratrol do vinho, têm um efeito benéfico sobre aqueles estados patológicos, embora não tenha ainda sido estabelecida qualquer relação com a via da Ub.

O presente plano de trabalho insere-se num projecto mais amplo que visa estudar o efeito de polifenóis antioxidantes de plantas sobre a via da ubiquitina/proteassoma na doença de Parkinson, uma inter-relação ainda não estudada, mas muito prometedora já que esta via é muito sensível ao estado oxidativo da célula. Para o efeito, é necessário implementar e validar o modelo celular de Parkinson in vitro. O modelo celular é estabelecido pela exposição crónica e sistemática a uma neurotoxina, a rotenona, que induz a maioria das características da doença de Parkinson, incluindo a formação das inclusões citoplasmáticas contendo agregados de α -synucleína.

PLANO DE TRABALHO:

O material biológico em estudo envolve uma linha celular SK-N-MC em que foi induzida a patologia parkinsoniana por tratamento prévio com uma neurotoxina durante 4 semanas.

Fase 1 Implementação do modelo de neurodegeneração induzido por exposição crónica com rotenona em células SK-N-MC durante 4 semanas.

Fase 2 – Validação do modelo por monitorização das principais alterações bioquímicas que ocorrem em células da doenças de Parkinson:

- 2.1-Imunodeteção em membrana da acumulação de agregados de α -synucleína e ubiquitina
- 2.2- Confirmação do aumento da oxidação de proteínas, por detecção da presença de grupos carbonilo (“oxiblotting”).
- 2.3-Imunocitoquímica dos agregados de α -synucleína e ubiquitina

CONTACTOS: Cláudia Nunes dos Santos (csantos@itqb.unl.pt, 214469651) e Lucélia Tavares (ltavares@itqb.unl.pt, 21 4469653)

PROJECTOS DE MESTRADO 2010/11

Tema do Projecto: ***DROUGHTYRUS** - Caracterização das respostas fisiológicas de espécies de *Lathyrus* ao deficit hídrico.*

Orientador/Contact Person:

Doutora Carlota Vaz Patto (ITQB): cpatto@itqb.unl.pt

Doutora Susana Araújo (IICT): saraujo@itqb.unl.pt

Objectivo do Projecto:

Pretende-se averiguar a existência de uma potencial função do aminoácido não-proteico ODAP nas respostas do deficit hídrico em leguminosas do género *Lathyrus*.

Plano Detalhado do Projecto:

As leguminosas do género *Lathyrus* têm grande potencial agronómico não só como espécies produtoras de grão mas também como forrageiras. Dentro deste género, salienta-se o chícharo (*Lathyrus sativus*) considerada como a principal fonte de proteína vegetal das populações de regiões áridas, como as zonas marginais da Ásia e África, por ser a única espécie capaz de crescer em tais condições. No entanto, esta espécie acumula uma neurotoxina (um aminoácido não proteico: ODAP) cuja acumulação em organismos animais resulta em paralisias irreversíveis. Embora alguns estudos tenham demonstrado uma relação entre o teor em ODAP e as respostas ao deficit hídrico, ainda existe um desconhecimento da sua verdadeira função nas plantas.

Neste trabalho pretendemos:

- Caracterizar e comparar as respostas fisiológicas de várias espécies do género *Lathyrus* (entre 5 a 7 espécies), em especial da *Lathyrus sativa*, ao deficit hídrico. Nos indivíduos provenientes de cada tratamento (deficit hídrico e bem irrigados) serão efectuados ensaios sumários de caracterização das relações hídricas e determinação dos parâmetros fotoquímicos, como forma de estimar a resistência diferencial das espécies ao stress hídrico.

- Paralelamente serão colhidas amostras para quantificação dos teores de ODAP. Desta forma pretendemos averiguar se existe uma relação entre o estado

hídrico destas plantas e a acumulação de ODAP, identificando potenciais funções deste aminoácido não-proteico nas respostas ao deficit hídrico.

Este trabalho irá complementar os trabalhos de investigação actualmente a decorrer no Laboratório de Biotecnologia de Células Vegetais do ITQB com base no projecto europeu LEGRESIST.

(http://www.genxpro.info/science_and_technologies/Legresist/)

Duração e Carga Horária: equivalente um ano lectivo (de Setembro a Junho)

Local de Realização: Laboratório de Biotecnologia de Células Vegetais do ITQB
(<http://www.itqb.unl.pt/~BCV>)

Número de Mestrandos por Projecto: 1

Tese de Mestrado para ano lectivo 2010-2011

Tema do Projecto: NUTICAEVOLVE - *Evolução da diversidade genética na adaptação a longo prazo de germoplasma exótico de milho num projecto Português de conservação e melhoramento participativos*

Orientador: Doutora Carlota Vaz Patto (cpatto@itqb.unl.pt)

Plano Detalhado:

Através de vários séculos de selecção natural e humana, foi se desenvolvendo em Portugal uma grande diversidade de germoplasma de milho (*Zea mays*), traduzida por um elevado numero de variedades tradicionais. As alterações climáticas evidenciam a importância da conservação desta biodiversidade. No entanto, especialmente a selecção humana pode provocar uma redução na diversidade genética existente. Este projecto avaliará o efeito da selecção participativa na diversidade genética de uma variedade de milho resultante do cruzamento de germoplasma nacional e exótico.

A diversidade genética será monitorizada ao longo de várias gerações:

1. Desde o processo de génese de uma população de milho sintética (NUTICA) resultante do policruzamento de 77 linhas puras de milho americanas e portuguesas.
2. Até às fases iniciais do processo de adaptação deste germoplasma às condições de produção nacionais através de selecção/melhoramento participativa (North Carolina Design I matting e Programa VASO).

Para tal recorrer-se-á à utilização de marcadores moleculares do tipo SSR (Simple Sequence Repeat) ou microsátélites seleccionados a partir da base de dados MaizeGDB-Maize Genetics and Genomics database (<http://www.maizegdb.org/>). Serão aplicadas técnicas de genotipagem molecular baseadas em PCR e electroforese de fragmentos com a utilização de um sequenciador automático. Serão utilizados vários programas informáticos de análise multivariada para o tratamento dos dados obtidos na caracterização molecular destes materiais e avaliação da evolução da diversidade ao longo das diferentes gerações de recombinação e selecção.

Este estudo insere-se no âmbito do projecto europeu **SOLIBAM – “Strategies for Organic and Low-input Integrated Breeding and Management”** (FP7-KBBE-2009-3) co-financiado pela Comissão Europeia. FP7 Framework Programme.

Duração e Carga Horária: equivalente um ano lectivo (de Setembro a Junho)

Local de Realização: Laboratório de Biotecnologia de Células Vegetais do ITQB (<http://www.itqb.unl.pt/~BCV>)

Número de Alunos por Projecto: 1



Master Research Projects 2010/2011

Technology Division

- Evaluation of bioactive extracts as promising natural chemotherapeutic agents- an in vitro approach [[pdf.](#)]
[Nutraceuticals and Delivery](#) - Catarina Duarte
- Development of particulate systems for the delivery of ursodeoxycholic acid (and conjugates): strategies to overcome the BBB [[pdf.](#)]
[Nutraceuticals and Delivery](#) - Catarina Duarte
- Preparation of novel gelatine-based drug delivery systems – application to model anti-inflammatory drug [[pdf.](#)]
[Nutraceuticals and Delivery](#) - Catarina Duarte
- Pharmacokinetics and Biopharmaceutical Analysis Laboratory - Ana Luísa Simplício
- *Desenvolvimento de ELISA para diagnóstico de Theileria spp* [[pdf.](#)]
[Biomolecular Diagnostics Laboratory](#) - Abel Oliva

Evaluation of bioactive extracts as promising natural chemotherapeutic agents- an in vitro approach

Cancer is one of the most leading causes of death worldwide. According to a recent report by the World Health Organization (WHO), the disease accounted for 7.9 million deaths in 2007 and the projection is that this number will increase up to 18 million in 2020. In particular, colorectal cancer is the third most common form of cancer in men (after prostate and lung) and the second in women (after breast). Diet and lifestyle are pointed to be major risk factors for developing this type of cancer.

Epidemiological data suggests that the ingestion of bioactive compounds from fruits and vegetables, such as phytochemicals, may contribute to reduce the incidence of cancer in humans. The mechanisms by which these compounds inhibit tumorigenesis include inhibition of tumour cell mediated protease activity, attenuation of tumour angiogenesis, and induction of cell cycle arrest and promotion of apoptosis. In addition, it has been reported that combining natural compounds with chemotherapeutic drugs is a promising strategy to enhance the inhibition of tumour survival.

The aim of this project is to evaluate the anticancer properties of bioactive extracts in order to develop a promising natural chemotherapeutic agent. The study will be performed using human HT29 colon cancer cells, which is a widely used model for *in vitro* colorectal cancer studies.

In a first approach, the candidate will evaluate the antiproliferative activity of the bioactive extracts on HT29 cell growth using MTT, BrdU and Cy-Quant assays. The effect of incubation time as well as the concentration of the bioproducts will be studied in order to determine the effective dose values.

In an effort to characterize the mechanism of action of the bioactive products, cell cycle analysis and induction of apoptosis in HT29 cells will be carried out by FACs (Flow Cytometry Analysis), and the generation of reactive oxygen species will be monitored using the DCFH fluorimetric assay.

Finally, the anticancer effectiveness of the bioproducts will be compared with drugs such as doxorubicin and 5-fluoracil. Moreover, studies with drug-resistant HT29 cancer cells will be performed in order to evaluate the bioproducts potential in overcoming the main drawback of chemotherapy.

Development of particulate systems for the delivery of ursodeoxycholic acid (and conjugates): strategies to overcome the BBB.

Ursodeoxycholic acid (UDCA) and its conjugated species have antiapoptotic, antioxidant and anti-inflammatory effects in nerve cells indicating their therapeutic potential for central nervous system (CNS) disorders. However, although UDCA evidenced to reduce neurological injuries in animal models the concentrations achieved in brain tissue after its administration are much lower than those in serum. This fact is mainly due to the low permeability of the blood-brain barrier (BBB) to drugs, a challenging problem in drug delivery development.

Thus, in order to overcome this challenging, this project aims to develop unconjugated and conjugated UDCA delivery systems (UDS) able to circumvent obstacles presented by the BBB to these potential neuroprotective compounds, and therefore increasing their efficacy for CNS disorders. For this purpose, clean and mild supercritical fluid processes will be used which represent a viable option in particle engineering with relevant advantages, namely minimization of organic solvent, use of environmentally benign non toxic materials, and production of smaller particles with controllable morphology and narrow size distribution.

As first approach the candidate will carry out fundamental studies that will determine: i) solubility of UDCA species in the SCF phase (CO₂); ii) solubility of the biomaterials selected as carriers systems in compressed CO₂; and iii) melting temperature curves determined by a visual method.

Afterwards, the UDCA particulate delivery systems (UDS) will be produced from gas-saturated solutions (SCF as solute), or by supercritical anti-solvent precipitation processes (SCF as antisolvent). In both cases, rapid decompression will produce very high supersaturations leading to the formation of particles with a narrow size distribution.

The UDS will be physically characterized in terms of morphology, particle size (PS), particle size distribution (PSD) and solid state characterization.

Measurements of PS and PSD will be performed by photon correlation spectroscopy – PSC and morphology, such as shape and occurrence of aggregation phenomena, by scanning electron microscopy- SEM - and/or by transmission electron microscopy – TEM.

The evaluation of UDS solid-state as the degree of crystallinity (of polymers) and the evaluation of lipid modifications will be assessed by differential scanning calorimetry - DSC and by X-ray scattering, respectively. The coexistence of additional colloidal structures such as micelles and liposomes will be evaluated by nuclear magnetic resonance – NMR.

Preparation of novel gelatine-based drug delivery systems – application to model anti-inflammatory drug

The combination of the chemical versatility of an ionic liquid with the morphological flexibility of gelatine has recently generated a new gelatine-based biomaterial, Ionjelly®. The excellent tuneable solvent power of ionic liquids mixed with the biocompatibility and bioavailability of a natural biopolymer like gelatine, makes this innovative material an excellent candidate for developing new drug delivery systems, an important potential application that remains unexplored.

In this work, supercritical fluid (SCF) technology will be explored for producing novel gelatine-based drug delivery systems. The work plan starts with the development of new Ionjelly® materials. Several non-toxic ionic liquids (ILs) will be synthesized in order to develop innovative biocompatible materials. A relevant breakthrough of this research work will be the opportunity to explore the use of '*Third Evolution of Ionic Liquids*', in which a specific biological activity is introduced through one of the ions, making of the ionic liquid the active principle ingredient (API). Then, the behaviour of Ionjelly® materials will be studied in scCO₂ which will be fundamental to determine the best SCF methodology to apply for the efficient preparation of the delivery systems. Finally, drug delivery systems will be prepared and characterized by Differential Scanning Calorimetry (DSC), Scanning Electronic Microscopy (SEM) and Transmission Electron Microscopy (TEM). In addition delivery systems will also be evaluated for drug load and release profiles in simulated gastric and intestinal fluids.

Título do trabalho: Desenvolvimento de ELISA para diagnóstico de *Theileria spp* (Development of ELISA protocol for *Theileria spp* diagnostic)

Plano do projecto (tema e enquadramento geral)

O trabalho está focado na detecção de anticorpos contra a parasita *Theileria spp* em amostras de sangue ovina, com o intuito de obter uma imunoreacção eficiente e reprodutível para uso em diagnóstico clínico. Os pontos a desenvolver no trabalho serão:

- Implementação e caracterização da imunoreacção, a partir de antígeno recombinante e anticorpos anti-IgG.
- Optimização do protocolo e caracterização do limite de detecção
- Teste de ELISA com amostras de campo de soros ovinos

Interesse científico: Um método de diagnóstico baseado em ELISA para o diagnóstico de uma doença endémica em Portugal é de maior importância. O teste de ELISA é uma ferramenta de diagnóstico largamente estendida em laboratórios para diagnóstico clínico. Na medida que ainda não há um perfil epidemiológico no país, é clara a utilidade de um teste com estas características. Assim, os trabalhos conducentes ao desenvolvimento de um protocolo de ELISA para diagnóstico clínico possuem elevado interesse para a formação prática e o futuro profissional do aluno de mestrado.

Duração aproximada:

- 12 meses (trabalho experimental, trabalho de pesquisa bibliográfica e escrita da tese)

Identificação do orientador:

- Doutor Abel González Oliva – Biomolecular Diagnostic Lab – ITQB-UNL
- Doutora Ana Domingos - Unidade de Tecnologia de Proteínas e Anticorpos Monoclonais (UTPAM), IHMT-UNL

Local de Realização:

- Biomolecular Diagnostic Laboratory – ITQB-UNL Av. da República EAN 2780 -157 Oeiras
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Perfil pretendido: Bioquímica, Biotecnologia, Biologia ou afins.

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