ITQB

and the Laboratório Associado

on the Oeiras Campus

Chemistry

Biology

Biological Chemistry

Plant Sciences

Technology

Laboratório Associado

2003|2004

ANNUAL REPORT and PLAN

Index

INTRODUCTION BY THE DIRECTOR

The period April 2003 to the present has been a time of highly contrasting fortunes for ITQB and the Laboratório Associado. From the research viewpoint, the laboratories have continued to be very successful and productive as is clearly illustrated by the Scientific Highlights section of this report. Once again we have tried to explain what we are trying to do, and why it is important, in terms that the non-specialist can understand, or at least, achieve a superficial insight. This is a difficult task in many research fields, but we will keep trying in this regard so that all the readers of this report can judge the importance of our science and technology as a contribution to improvements in the quality of life and the national economy.

Our scientists have participated in a large number of scientific meetings both in Portugal and abroad, giving plenary lectures, invited lectures and many other oral and poster presentations. In addition we have hosted a number of prestigious meetings on the Oeiras Campus including that of the Portuguese Society of Genetics. This meeting, organised by Prof. Margarida Oliveira and her colleagues, was held on two days and attracted some 180 registered participants. Meetings had been held between Portuguese and Spanish scientists interested in Genetics on a regular basis in the past, but had been allowed to lapse on the Portuguese side. This meeting therefore helped to regenerate interest in the field of genetics in Portugal. In conjunction with our colleagues at the Estação Agronómica Nacional and Estação Florestal Nacional an open day symposium was also held in order to bring together the diverse skills and interests on the Oeiras Campus. This meeting resulted in the creation of five joint projects that have been given a modest amount of seed money in order that they can grow and become the basis for applying for funds from the national funding bodies. We also hosted a science week as part of the initiative organised by Ciência Viva. In the future we hope to hold an “Open Day” so that the citizens of Oeiras will have the opportunity to come and find out what is happening in our Laboratories. The communication of science and technology to the public at large, and especially the politicians, industrialists and venture capitalists, is an essential part of our culture and operations.

ITQB and the Laboratório Associado have also hosted a number of prestigious visitors over the last few months including the Secretary of State for Science and Higher Education and the President of the Fundação para a Ciência e a Tecnologica. The

Secretary of State now has a special responsibility for our affairs and we hope to see more of him and, in particular, to discuss the development of biotechnology and biomedicine in Portugal and the lead role that the Laboratório Associado could play. Several Laboratório Associado Events have taken place including lectures by Prof. Helen Saibil (Birkbeck College, London) on "The Chaperonin Folding Machine", Drs. Siobhan Yeats and Ashok Chakravarty (European Patents Office, Munich) on "The European Patent System and Patenting Biotechnology", Prof. Anders Liljas (Lund, Sweden) on "How do Translation Factors Catalyse Protein Synthesis", Prof. Matthias Wilmanns (EMBL, Hamburg) on "Emerging life science activities at PETRA/DESY", Prof. Ed Hough and Dr. Ole Andersen (Tromso, Norway, on "Autosomal Recessive Diseases: the role of phenylalanine hydroxylase and lysosomal α – mannosidase" and a workshop on "The use of radioisotopes in Laboratorial work" organised by Sérgio Gulbenkian (IGC) and Abel Oliva (ITQB).

I am pleased to report that on the positive side of our affairs, the various committees that were set up at the beginning of 2003 are working well. In particular the Safety Committee under the stewardship of Abel Oliva has worked prodigiously to improve our awareness of safety matters. We now have the emergency red telephone system fully installed and working complete with other evacuation measures on every floor of the ITQB-II building. A recent evacuation practice was undertaken satisfactorily and this exercise will be a regular feature of our overall safety measures. My own personal point of satisfaction in this regard is the completion of the external store for bulk solvents, hazardous chemicals and waste. The construction of this building was long overdue, but we now have a store that is virtually earthquake proof, hydrogen bomb proof and certainly adequate for its purpose! It is a pity about the cost! The Infra-structure Support Committee under the stewardship of the Vice-Director, Prof. Mª Armenia Carrondo continues to find ways of improving the quality of support that the administration can give to the scientists and a new initiative is a sub-committee responsible for common analytical services. The Science Advisory Committee, elected by the Scientific Council, has been an invaluable source of advice to the Director, in planning for the future, in advising on the various financial problems (see below), in helping to select new staff, and in many other important matters. Do not forget that the members of these committees are representatives of the ITQB Divisions and all Division members have the right to ask about the business conducted by these committees and to have their views put forward for discussion.

The last few months have seen scientific reviews of the Chemistry and Biological Chemistry Divisions. For Chemistry the review panel comprised Profs. Karl Wieghardt (Mulheim, chairman), Mike Hursthouse (Southampton) and João Fraústo da Silva (Lisbon). For Biological Chemistry Prof. Sir Tom Blundell, FRS (Cambridge, chairman) was joined by Robert Crichton (Louvain la Neuve) and Pedro Moradas Ferreira (Porto). Both panels commented on the excellent quality of the science being undertaken, often at the level of world class, and made useful and exciting proposals for future developments in the science programme. However, both panels noticed the lack of support in terms of the provision of modern equipment. It is over 10 years since Portugal financed a major equipment bid in science and technology and the one that is imminent is long overdue. Modern science demands sophisticated equipment and operational support and Portugal must focus its funding to ensure that its centres of excellence are adequately equipped. Later this year reviews will also be conducted for the remaining Divisions, Biology, Technology and Plant Sciences. The Laboratório Associado may be the subject of a special review.

The downside of the year has clearly been the financial situation and since November 2003, the Laboratory has been brought to an almost standstill. This has had very deleterious effect on many of the groups, although our publication rate should improve next year, since writing papers is one of the few tasks that can be undertaken with minimum funds! The problem is quite simple. The Orçamento de Estado budget is insufficient to cover all the infra-structure costs and normally about 25% of the Laboratório Associado funds have to be used as a supplement. However, the Laboratório Associado funds arising from the EU POCTI and POSI programmes have been blocked for reasons which are not relevant to this introduction. Non-payment of these funds causes the ITQB and the Laboratório Associado considerable problems, and there is no “fall-back” position since the ITQB is financially autonomous from the Universidade Nova de Lisboa. The President of the FCT and his colleagues have made strenuous efforts to help this situation, but there is a limit to what can be done by the FCT and this limit has been reached. Sympathy has also been expressed by the Secretary of State for Science and Higher Education, the Rector of the UNL and our colleagues on the IBET Management Board. I thank them all for their help and advice, but as a measure of almost desperation, I have now written to the Minister of State and Finance and the Prime Minister. If these eminent politicians cannot solve our short term problems and help us to plan for the longer term future, I see little hope for science and technology in Portugal. It is ironic that at a time when the Government claims to be increasing the science and technology budget, the ITQB and the

Laboratório Associado are in such awful financial straits. I sincerely hope that by the time this introduction appears, the immediate crisis will have been solved and planning for the long term will be well underway. In the meantime the Laboratories must not loose heart, but try to maintain the highest standards of science that circumstances permit. I firmly believe that excellence will always be rewarded over mediocrity and that ITQB and the Laboratório Associado will continue to make significant contributions to Portuguese science and technology for a long time to come. ITQB is a centre of excellence and its young scientists are the entrepreneurs and wealth creators of the next decade. The Government must give then proper support.

I would like to thank numerous people for giving me support over the last few months and in particular my Vice Director, Prof. Mª Arménia Carrondo. My fellow Directors at the Laboratório Associado, Profs, Manuel Carrondo (IBET) and António Coutinho (IGC) have also been very supportive and the Management Board of IBET has offered both financial and political advice. The Rector of the UNL has always lent a sympathetic ear and Profs. Fernando Ramôa Ribeiro and João Paulo Crespo have tried to do whatever they can within their own financial restraints, even though my diplomacy has not always been at the highest level. However, my main thanks are due to all the staff, scientists, technicians, administrators at ITQB at the Laboratório Associado. You provide the real reason for me being in Portugal. Your enthusiasm, expertise and the quality of the science being produced by the Laboratories make the battle for resources worthwhile.

This Annual Report[1] and Plan has many contributors including the Heads of Divisions, Carlos Romão, António Xavier, Helena Santos, Luis Paulo Rebelo and Candido Pinto Ricardo and the individual members of the groups and laboratories. Once again Margarida Martinez has achieved a miracle and brought it all together, on time and with the minimum of fuss. I hope that all the readers will appreciate what the ITQB and its Laboratório Associado are trying to do and that those politicians involved in funding science and technology will devise a mechanism to ensure a profitable and successful future.

Peter Lindley
Director, ITQB-UNL

[1] The operation and research interests of the ITQB are intimately linked to those of IBET. This Annual Report and Plan covers many aspects of the scientific activities of IBET.

ORGANIZATION OF THE INSTITUTE

DIRECTION

- Peter F.Lindley - Director
- Maria Arménia Carrondo - Vice-Director

MANAGEMENT

-	Margarida Senna Martinez	- Advisor to the Director and Head of the Planning and Academic Division
-	Maria da Glória Reis Leitão	- Head of the Administrative Department
-	Henrique Campas Nunes	- Head of Maintenance and Workshop Services

SCIENTIFIC ADVISORY COMMITTEE
(Coordinating Committee of the Scientific Council)

Management
- Peter Lindley (Director)
- Maria Armenia Carrondo (Vice-Director)
- Margarida Martinez (Secretary to the SAC)

Chemistry Division
- Carlos Romão
- Eurico Melo

Biology Division
- Adriano Henriques
- Helena Santos

Biological Chemistry Division
- Claudio Soares
- Miguel Teixeira

Technology Division
- Luis Paulo Rebelo
- Teresa Crespo

Plant Sciences Division
- Cãndido Pinto Ricardo
- Margarida Oliveira

Substitutes
- Júlia Costa
- Cecilia Arraiano

Ex-Officio Members
- António Xavier
- Manuel J.J.T.Carrondo
- Manuel Nunes da Ponte

INTERNATIONAL ADVISORY COMMITTEE

- Professor Sir Thomas L. Blundell, FRS, Department of Biochemistry, University of Cambridge, UK
- Professor Joachim Klein, Institute of Macromolecular Chemistry, Universität Braunschweig, Germany
- Professor Chris Leaver, Department of Plant Sciences, University of Oxford, UK Professor Staffan Normark, Swedish Foundation for Strategic Research and Department of Bacteriology, Karolinska Institute, Sweden
- Professor Karl Wieghardt, Max-Planck Institute für Bioanorganische Chemie, Mulheim, Germany

INFRA-STRUCTURE SUPPORT COMMITTEE

- Maria Armenia Carrondo - Vice-Director (Chairman)
- Peter Lindley - Director
- Alexandra Lopes - Secretary
- Rosário Mato - ITQBI
- Henrique Campas Nunes (Nuno Monteiro) - Safety; Workshop & Maintenance
- Maria da Glória Leitão (Madalena Pereira; Fernando Tavares) - Administrative & Accounting
- Carlos Frazão (Carlos Cordeiro; Daniel Branco) - Computing & Networks
- Margarida Senna Martinez (Lurdes Conceição) - Planning and Academic
- Miguel Teixeira (Susana Ferreira) - Library
- Manuela Regalla - Protein & Amino-Acid Analysis
- Ana Coelho Mass Spectroscopy

SAFETY AND FLOOR COORDINATION COMMITTEE

- Abel Oliva (Chairman)
- Peter Lindley (Director)
- Maria Armenia Carrondo (Vice-Director)
- Mafalda Mateus (Secretary)
- Henrique Campas Nunes (Valter Peres) – First Floor
- Maria da Glória Leitão (Madalena Pereira; Fernando Tavares) – Second Floor
- Margarida Senna Martinez (Ana Maria Portocarrero) – Third Floor
- Luís Paulo Rebelo – Fourth Floor
- Teresa Crespo– Fifth Floor
- Cândido Pinto Ricardo (Margarida Oliveira) – Sixth Floor
- Carlos Romão (Rita Delgado) – Seventh Floor
- Rosário Mato (Ricardo Louro) – ITQB I
- Cecília Arraiano (Adriano Henriques) – Radioactive Sources
- Teresa Crespo (Júlia Costa) – Biological Hazards
- Helena Santos, MD - Medicine and Health
- Margarida Senna Martinez (Ana Maria Portocarrero) – Planning and Academic
- Isabel Ribeiro -IBET representative
- António Cunha – Pilot Plant Representative
- Henrique Campas Nunes (Nuno Monteiro) – Workshops & Maintenance

RESEARCH STATISTICS

RESEARCHERS

The following tables indicate the numbers of Ph.D. holders and graduate students, respectively, undertaking research at the ITQB during the years 1999-2003.

Ph.D. Holders	Group Heads	Post Doctoral Fellows	Other Ph.D. Holders	TOTAL
1999	33	32	47	112
2000	35	36	46	117
2001	33	39	47	119
2002	34	37	54	125
2003	34	49	35	118

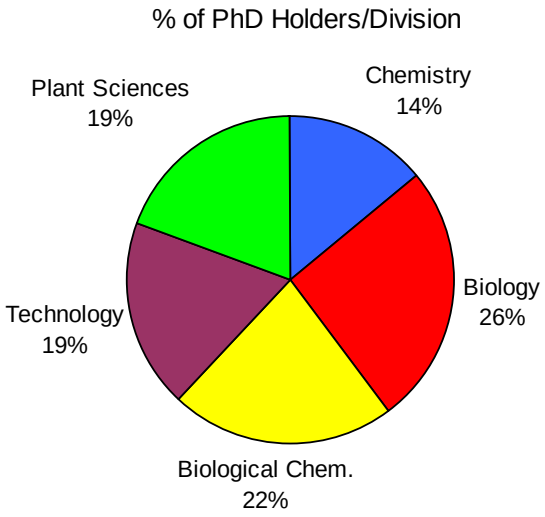
Students	Ph.D. Students	Other Graduates	Total Graduates	Under-graduates
1999	77	85	162	37
2000	96	83	179	37
2001	117	90	207	36
2002	125	70	195	24
2003	118	81	199	24

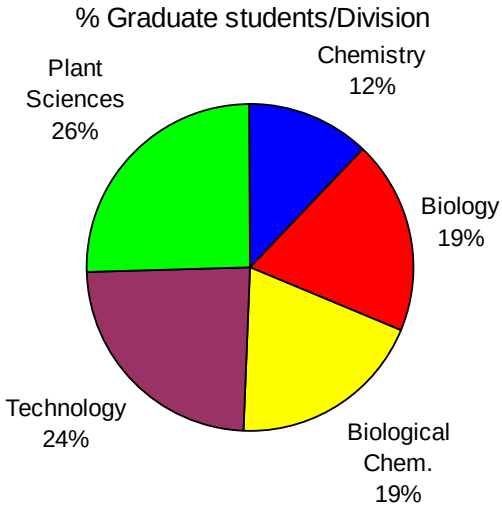
In the lower table the category “other graduates” refers to researchers working on short term contracts within research projects and who frequently progress to become Ph.D students.

Post-doctoral researchers constitute some 40% of the 118 Ph.D. holders in 2003. Some 60% of the Ph.D. holders and 70% of the students are female. Females also represent 45% of the group leaders and 80% of the Post-doc fellows.

The Portuguese Foundation for Science and Technology (FC&T) has been the main source of Ph.D. and Post-doc Fellowships over the past few years, although the contribution from the EC, namely through the Marie Curie Programme is slowly growing.

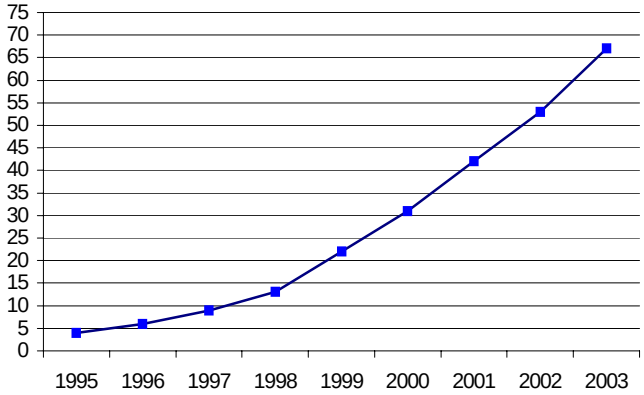
Ph.D. holders and Graduate Students are distributed amongst the ITQB Research Divisions as shown in the following pie-charts.



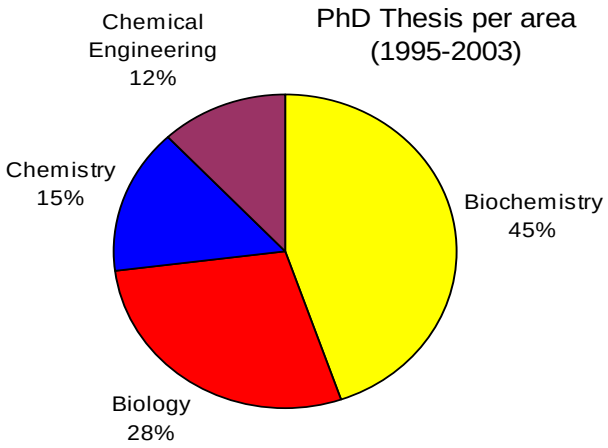


The average number of trainees per Ph.D. holder is 1.7

The Ph.D. theses completed at ITQB between 1995 and 2003 are shown in the graph below. In 2003, 14 theses were successfully submitted and examined.



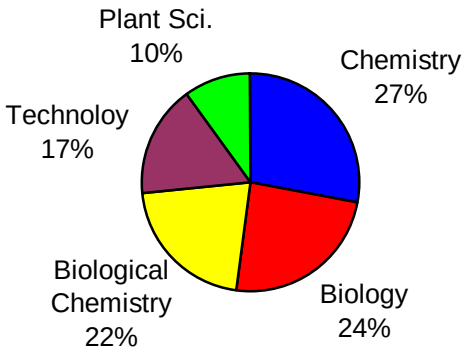
ITQB awards Ph.D. degrees through the Universidade Nova de Lisboa in the scientific areas of Chemistry, Biochemistry, Biology and Chemical Engineering. The figure shows the distribution of degrees amongst these four areas for the period 1995-2003.



PUBLICATIONS

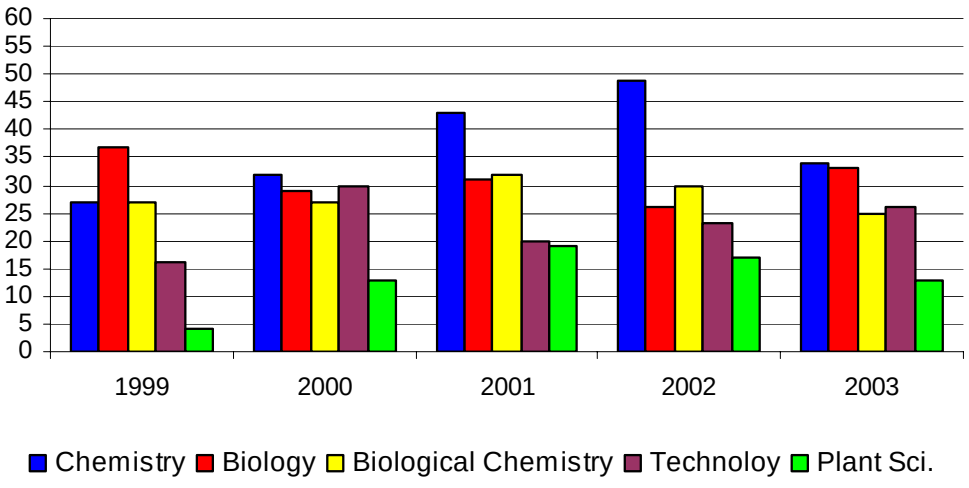
The complete list of publications by ITQB researchers during 2003/2004 is given in the "Research Output" section. The number of papers published in refereed journals was 131, for 2003 alone. Some 60 papers have been either published or are in press in 2004.

Overall contribution to total number of ITQB papers (1999-2003)



An ad-hoc division of papers among the 5 ITQB research areas yields the following results, although the exact figures have to be treated with caution since many publications span more that one area (papers were attributed to a specific area according to the principal authors):

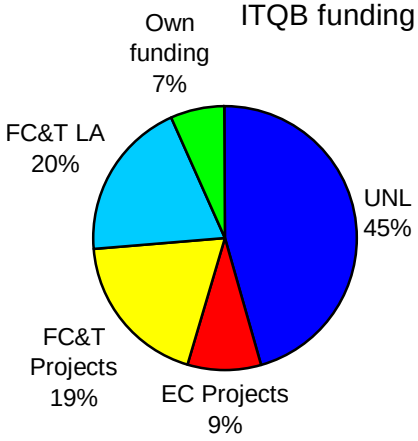
Number of papers per Research Division



BUDGET

The ITQB budget has two main sources of revenue: Universidade Nova de Lisboa, and FC&T (Laboratório Associado contract and project funding). European Commission (EC) project contracts also make an important contribution, although on a smaller scale. The different contributions to the budget are plotted as percentages of an overall budget of 7,6 M€.

ITQB funding 2003

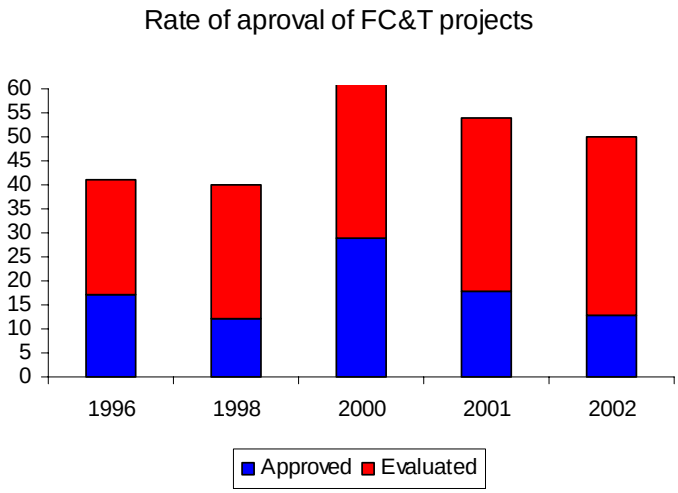


However, additional contributions should be considered in order to calculate the overall value-for-money of the ITQB operations. Most of the researchers at ITQB are paid by external sources, either salaries paid by other institutions to senior researchers, or fellowships awarded to graduate students and Post-Docs. The contribution of IBET, in particular, to projects led by ITQB researchers, is also a sizeable amount and represents the part of ITQB activities dedicated to collaboration with industry, and mostly paid by private sources. Taking these considerations into account, a ball park calculation for the overall budget of the ITQB operations would be some 12 M€. In the estimation of this figure, external salaries have been calculated as a fraction (from 0.3 to 0.7) of the salaries of senior researchers who perform research at ITQB, but have teaching or other duties in other institutions and are paid by them. Equipment value and depreciation has been calculated on the basis of a 20 year lifetime and an initial investment of 20 M€. The cost of Ph.D. and Post Doc fellowships granted by FC&T and paid directly to fellows is also included. The figure of 12 M€ is therefore likely to be an under estimate.

One point that should be emphasized is that some **45 % of the total budget** is obtained competitively. However, the present budget is clearly unsatisdactory (see the Director's introduction) with too much reliance on "soft money" and insufficient "hard money" to cover the basic infra-strcuture. The Orçamento de Estado contribution must be significantly increased if the ITQB is to remain competitive at a national and European level. The "soft money" contribution through the Laboratório Associado contribution must akso be maintained and protected.

RESEARCH PROJECTS

The main sources of project funding have been FC&T and the European Commission. All projects involving collaboration of ITQB researchers with Industry are organized through IBET, and they are therefore accounted separately. FC&T calls for projects were issued in 1996, 1998, 2000, 2001 and 2002. The number of projects (total submitted and approved) with ITQB as co-ordinating institution is given in the adjacent Figure.



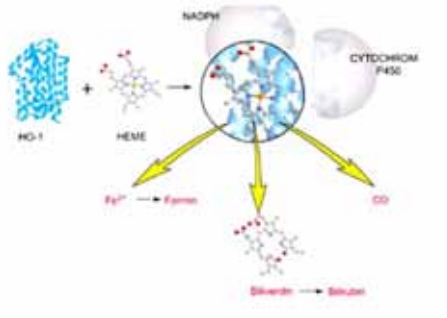
The capability of ITQB to submit competitive projects every year is a necessary condition for the success of the Institute.

The average rate of approval for ITQB projects on the period 1996-2001 was 55%. This rate of success is clearly higher than the average approval rate for FC&T project calls. Although it varies widely, depending on evaluating panel/scientific area and year/call, results can be averaged from the FC&T database (data referring to the 2000 and 2001 calls). A comparison is shown in the following table for three of ITQB's main scientific areas.

Project rate of approval (%)	National average	ITQB
Chemistry	52%	76%
Molecular and Structural Biology	39%	72%
Chemical Engineering & Biological Engineering	30%	64%

THE LABORATÓRIO ASSOCIADO

A. Constitution.



The Laboratório Associado is a partnership between the **Instituto de Tecnologia Química e Biológica** (ITQB, Director Professor Peter Lindley), the **Instituto de Biologia Experimental e Tecnológica** (IBET), a private non-profit institution (CEO, Professor Manuel J. T. Carrondo) and the **Instituto Gulbenkian de Ciência** (IGC) of the Calouste Gulbenkian Foundation (Director, Professor António Coutinho).

ITQB belongs to the Universidade Nova de Lisboa and is devoted to advanced training and research in the areas of Biology and Chemistry and associated Technologies. It is an open institution, with a small number of paid staff and the majority of researchers belonging to other institutions or being financed by grants. The research at ITQB covers a vast interdisciplinary area with an emphasis in Chemistry and Biology.

IBET is a private non-profit organisation. It is a platform for University-Industry collaboration in the area of Biotechnology. Its pilot plant infra-structure is certified as "current Good Manufacturing Practices" and three of its services (Chemical Analysis, Microbiology, Molecular Biology and Field Trials) are certified "Good Laboratory Practices". These constitute a unique group at the national level. It has a long track-record of collaborative Research and Development in areas such as Biopharmaceutical Products, Vaccine Development, Medical and Veterinarian Diagnosis, Production of purified protein for research of Structure-Function relationships, Starter Cultures of Micro-organisms, and Supercritical Fluid Extraction. IBET also has an important role as a base for start-up companies.

IGC activities are focused on biomedical research, particularly those related to the genetic basis of development and evolution of complexes systems, namely, Developmental Biology and Evolution, Genetic Susceptibility of Complex Diseases (diabetes, lupus and autism) and Infections, Molecular and Cellular Mechanisms of Response to Stress, Control of Cellular Cycle and Genetic Expression, Immunity Tolerance and the development of new Strategic Vaccines.

Within the ITQB and IBET there are around 320 researchers of which 130 are PhD holders whereas the IGC has some 130 researchers and 60 of them are PhD holders.

Together the three institutions have;

- an open structure with the participation of researchers of various national and foreign institutions,
- a scientific programme based on the strong interaction of small groups of researchers in different scientific areas,
- a large network of services of high technology, some of which could, and should, become national facilities.
- A set of complementary skills that cover a wide area of the Life Sciences and related disciplines. The areas of competence include; the structural and functional characterisation, design and production of biologically active molecules; micro-organism physiology and genetics; analysis and manipulation of complex biological systems; bioinformatics.

B. Research Objectives.

The Laboratório Associado focuses its research efforts on a number of themes relevant to the study of human disease, the improvement of the quality of life and the environment, and basic research.

1. Biologically Active Molecules

This theme includes the fields of Genomics and Proteomics; there are diversified competences in these areas in the partner institutions with contributions from groups in Chemistry, Biological Chemistry, Biochemistry and Molecular Biology, and from high-tech services. Within this thematic line, the Laboratório Associado has undertaken;

- the establishment of a reference laboratory of national/international scope in analysis, sequencing and determination of protein structure.
- the development of pilot plant fermentation for the production of biological molecules for clinical trials and others, by expression in micro-organisms or animal cells.

These infra-structures enable the Laboratory to give support to the scientific community as a whole in the following tasks;

- the use of spectroscopic methods for the study of the dynamics of protein structure and function,
- the production of adequate quantities of biological molecules for fundamental and applied research,
- legislation, certification and spread of Good Manufacturing Practices (GMP),
- the manufacture of biological molecules, either natural or man-made, in prokaryote or eukaryote cells, and their purification,
- the transfer of knowledge and technology to the chemical and pharmaceutical industries including contractual matters and,
- the initiation of “start-up” enterprises in these areas.

2. Medicine and Molecular Veterinary Studies

Modern biomedicine has an increasing impact on practically all Medicine and Veterinary domains, particularly in the more prevalent diseases of a degenerative nature in Man (cancer, cardiovascular and neurological diseases, and ageing itself) and in infectious diseases, both in Man and domestic animals. The transfer of knowledge to clinical practice has been very fast, *e.g.* for methods of diagnosis and in public health and in the genetic tracking of groups with high susceptibility to certain diseases. One of the most problematic consequences in the delay in developing these areas in Portugal is the absence, or small number, of medical biotechnology firms.

Biomedical sciences are likely to be one of the keys to economic success in the new millennium. In Portugal, such a phenomenon has not yet happened. A key competence of the Laboratório Associado will be to promote the “transfer” of the new technologies to economic activities such as molecular diagnosis and the genetic search for the more

predominant diseases and their susceptibility ("DNA chips", "genome wide scans", "single nucleotide polymorphism", *etc*.). This will involve;

- Development of an internal service of DNA sequencing, bio-informatics and genotyping, with external consulting capability;
- Development of know-how in preparing and reading "DNA chips".

In this thematic line, the Laboratório Associado is committed the following tasks;

- Support of the scientific community in relation to DNA sequencing,
- Elaboration of studies and support in the regulation and certification of the use of DNA tests, and support with the certification and control of these in other public and private institutions, as requested by the respective authorities. Advising the establishment of the needs for regulation in the use of genetic material,
- Development of processes and products for diagnosis and vaccines, in collaboration with firms in this area of business, with public services or in cooperation with developing countries,
- Support for the establishment of good practices in the management of new technologies of molecular diagnosis and genetic search – DNA chips, genome-wide scans,
- Establishment, organisation and management of a "gene data bank" for the Portuguese population, namely of carriers of complex diseases and their respective families and contributing to the preparation of rules, regulations and legislation about the genetic research on complexes diseases.

3. Developmental Biology in Animals and Plants

Developmental Biology is the biology of the post-genomic era, at the fundamental research level. However, it should find answers to practical problems of great economic interest, encompassing replacement therapeutics of human tissues and organs in medicine, and *in vitro* reproduction and plant improvement. For several reasons, Portugal has developed poorly in this area. Some competence has been established in a research group at the Faculty of Medicine of Lisbon and at the ITQB/IGC. Within the Laboratory there are now several groups studying animal and plant development and this represents a significant increase in investment over the last few years. The Laboratório Associado has an important role in mobilising, instructing and acting as a catalyst for a growing interest in this area, through the formation of a group of competencies in Developmental Biology and the development of a service for genetic manipulation of mice and transgenic mice.

In this thematic line, the Laboratório Associado is;

- Providing support to certification, survey and control, according to current international regulations, of facilities for small rodents. This concerns the regulations for good scientific practices, as well as the well-being of the animals. Advice is also given to the public administration on these matters.
- Supporting the scientific community, through the supply of specific breeds of rats and small transgenic mice.

- Training people with diverse skills in Developmental Biology, who can offer technical counselling services to the public administration on the preparation of rules and legislation about matters such as manipulation and use of embryos, “therapeutic cloning”, genetic manipulation of somatic cells (gene therapy) and germ-line cells (transgenics).

4. Biological Risk

This area involves implementing a national reference laboratory and services for the analysis of genetically modified organisms; and the molecular typing of bacteria resistant to antibiotics. The laboratories involved operate in accordance with the authorities in the areas of Food Security and Public Health, developing internationally accepted and validated methods and advising on regulation issues.

The Laboratório Associado contributes to the following tasks;

- Certification of protocols for the analysis of genetically modified organisms and dissemination of good practices,
- Support in drafting regulations concerning food and environmental security related with genetically modified organisms,
- The study of the dissemination of contamination by antibiotic resistant bacteria in populations,
- Providing support to the elaboration of regulations related to good practices in the use of antibiotics,
- Providing support to the certification and validation of methods for searching for antibiotic resistant bacteria in sick-wards, healthcare centres and hospitals.

5. Improvement of Plants and Forest

This thematic line includes the expression, isolation and characterisation of genes (sequencing, expression analysis and regulation). Molecular markers are used for the characterisation of plant varieties and also as aids in traditional plant improvement, and in the genetic engineering of plants for qualitative characteristics. It also includes extraction, purification and characterisation of proteins and vegetable enzymes, and physiological studies associated with the response to stress situations and propagation of selected genotypes. The plants under study at the Laboratório Associado are eucalyptus and pine trees, with possible extension to cork oaks. The Laboratório Associado is at the crossroads of a network of several laboratories – academic, state laboratories and private enterprises, namely from the pulp and paper industry.

The Laboratório Associado contributes to the following fields;

- The support and maintenance of germoplasms, gene embryogenics and micropropagation processes, in collaboration with public and private plant nurseries, and in the development of growing forest programmes or the introduction of new species or agricultural techniques (*e.g*. vines, rice and almonds).
- Providing support in the improvement of cork oak, pine tree and eucalyptus species, by methods of Molecular Biology.

C. Research Staff

The scientists are listed in the five research themes described above, although the research of many of these scientists traverses the boundaries between one or more themes.

1. Biologically Active Molecules.

Ricardo Louro	(16th October 2001)	ITQB
António Baptista	(1st May 2003)	ITQB
Margarida Archer	(1st July 2003)	ITQB
Claúdio Gomes	(1st December 2003)	ITQB
Beatriz Royo	(1st December 2003)	ITQB

2. Medicine and Veterinary Studies.

Miguel Soares	(1st July 2001)	IGC
Paula Alves	(11th September 2001)	IBET
Carlos Gonçalves	(1st August 2003)	IGC

3. Developmental Biology in Animals and Plants.

Moises Mallo Perez	(1st July 2001)	IGC
Jorge Carneiro	(26th June 2002)	IGC

4. Biological Risk.

Ana Martins	(1st September 2001)	ITQB*
Rosário Mato	(17th December 2001)	ITQB
Maria de Fátima Lopes	(16th June 2002)	IBET
Ana Rute Neves	(1st December 2003)	ITQB
Sergio Filipe	(1st July 2004)	ITQB**

5. Improvement of Plants and Forests.

Margarida Rocheta	(2nd October 2001)	IBET
Philip Jackson	(16th July 2002)	ITQB
Rita Abranches	(1st December 2003)	ITQB

* Ana Martins resigned from the Laboratório Associado on the 31st August 2003. She is being replaced by Ana Luisa Simplicio (from 1st May 2004) who will undertake research within the area of Biological Risk within the auspices of IBET.

** Sergio Filipe will join the Laboratório Associado from Oxford in 2004

According to the Laboratório Associado contract there are 8 vacancies, 4 each in 2004 and 2005, distributed 4 to ITQB, 2 to IBET and 2 to IGC

Nine technicians have also been recruited as specified in the Laboratório Associado Contract.

D. The Laboratório Associado "Think Tank".

The Laboratório Associado is a complex organization involving three separate entities, the ITQB, the IBET and the IGC. Each institute has evolved its own style of operation and research and bringing elements of them together to form a genuine Laboratório Associado with joint projects and a common research philosophy has not been a straightforward task. Although much progress has been made since the inception of the Laboratório Associado in 2001, the Directors feel that there is room for significant development to enable it to fulfill its full potential as a centre of excellence in the Life Sciences and related fields. As a consequence a "Think Tank" has been instituted to assist the Directors in the task of focusing the skills and expertise possessed by the three Institutes comprising the Laboratório Associado. Professor João Fraústo da Silva (IST and the Cultural Centre of Belém) has agreed to chair the Think Tank and various eminent persons from academia (both Science and the Social Sciences) and industry are being invited to participate. A full list of members will be reported in next year´s Annual Report and Plan. The Think Tank will meet at least twice a year and will advice the Directors of the three institutes regarding ways of making the Laboratório Associado more effective and also helping to formulate and plan new research initiatives and concomitant methods of resourcing.

E. Selected Research Highlights of Investigators.

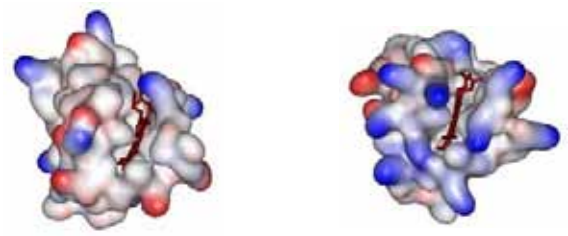
The research programme being undertaken by the scientists and technicians is extensive and wide ranging within the objectives listed in section B. In order to give a comprehensive overview, but avoiding excess detail, each of the groups has prepared a research highlight. Full reports of the activities of individual investigators are available on request to Peter Lindley, overall Director of the Laboratório Associado. The highlights are given in the order of appointment of the scientists listed in section C.

Cytochrome c_7 a haem protein with sulfur reductase activity

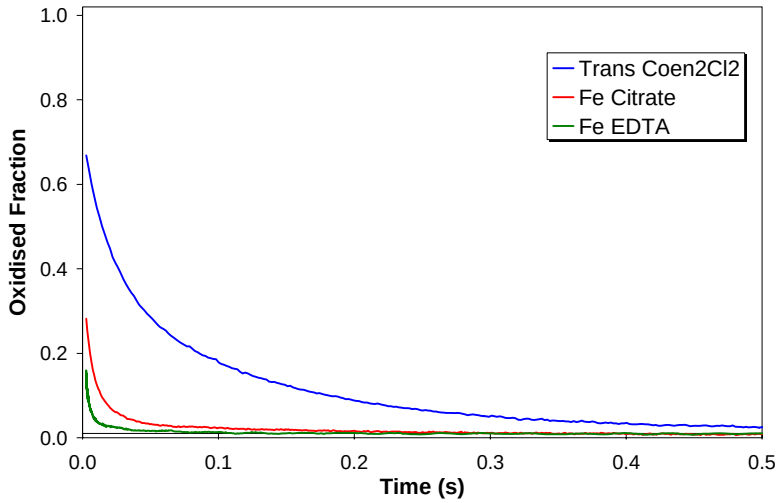
Metalloprotein Laboratory : Investigator, **Ricardo Louro**

Cytochrome c_7 is a tri-haem cytochrome originally found in the sulfur reducing bacterium *Desulfuromonas acetoxidans* which has recently been classified as a member of the *Geobacteriaceae* family, and has been implicated in sulfur and metal respiration in several organisms.

The equilibrium thermodynamic data obtained by analysing the spectroscopic data (NMR and UV/visible) obtained for cytochrome c_7 was used for interpreting the transient kinetic traces of reduction of this protein with sodium dithionite. The results showed that, surprisingly, dithionite reacts faster with haem I, and not with haem IV, as expected on the basis of electrostatic complementarity[1].



Haem I Haem IV



Preliminary kinetic studies on the reactivity of this cytochrome with several metal complexes shows that rates are faster as the driving force increases, and also as the charge of the metal complex becomes more negative. We expect that ongoing kinetic modelling work will allow for a detailed description of the interaction with these model electron acceptors that mimic physiological acceptors as well as potential targets in remediation of contaminated environments.

1 Correia IJ (2002) *Eur. J. Biochem.* 269, 5722-5730

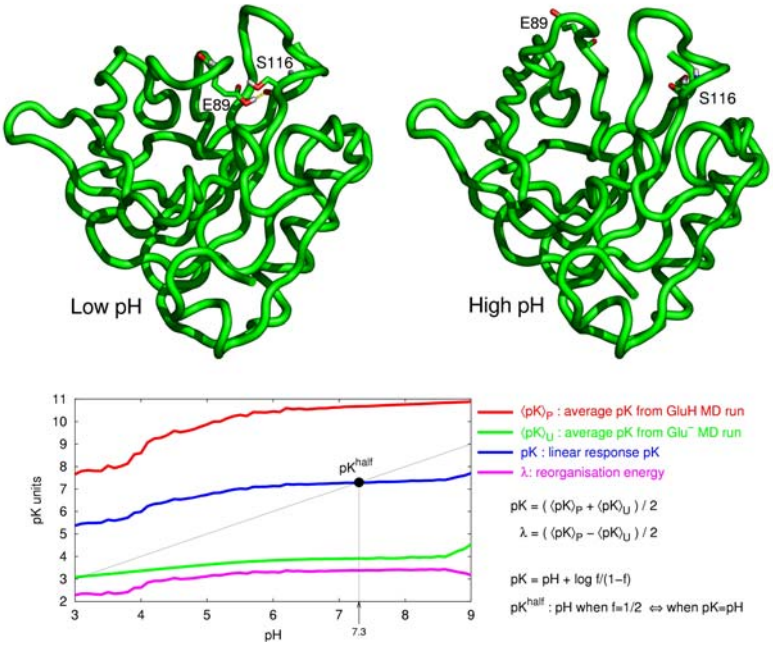
Deprotonation of Glu89 triggers the lid opening in beta-lactoglobulin: molecular dynamics and p*K*a calculations using linear response.

Molecular Simulation Laboratory : Investigator, **António Baptista.**

Bovine beta-lactoglobulin (BLG) is the most abundant protein of cows milk, but its role is not yet fully elucidated. It can bind a variety of small hydrophobic molecules (fatty acids, cholesterol, retinol, sex hormones, *etc*.) and may play a role as a carrier of those ligands. BLG contains a "lid" at the entry of a hydrophobic cavity where the ligands can bind. The opening/closing of this lid seems to be mediated by Glu89, which displays a highly anomalous titration behaviour (p*K*a = 7.3). Our study aimed at elucidating the role of Glu89, using both apo- and holo-structures of the protein.

Our molecular dynamics (MD) simulations show that Glu89 works as a "lock". When Glu89 is at neutral (low pH), its carboxylic proton establishes a persistent hydrogen bond with the carbonyl oxygen of Ser116, thereby keeping the lid in the closed position. When Glu89 is charged (high pH), the hydrogen bond is lost and the side-chain becomes well exposed to the solvent, allowing the lid to remain open.

The calculation of the p*K*a of Glu89 poses a serious problem to traditional methods based on continuum electrostatics (CE), since these methods rely on the use of a rigid protein structure. Furthermore, calculations using either an open- or closed-lid structure give very different results, since they fail to capture the coupling between the protonation of Glu89 and the lid position. In order to overcome this problem we developed a new method, based on MD, CE, and a linear response approximation (LRA). This method could predict the exact p*K*a value for Glu89, 7.3. Furthermore, it allowed us to compute the reorganisation (free) energy of this process, which is a measure of the coupling between the protonation of Glu89 and the lid motion.



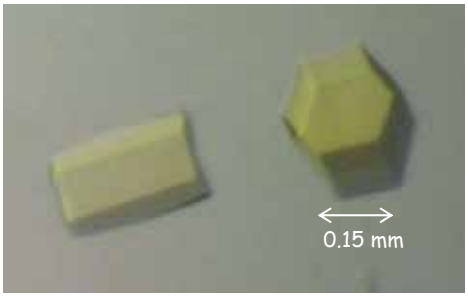
Structural Studies on Membrane Proteins.

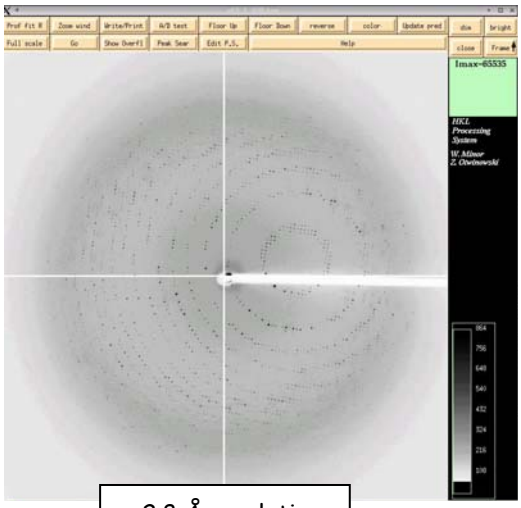
Membrane Protein Laboratory : Investigator, **Margarida Archer Franco Frazão**

Proteins that are either associated with membranes or integral membrane proteins constitute around 30% of the total number of proteins expressed by any genome. They have crucial roles in many processes including the transport of small molecules and ions across membranes and signaling pathways. However, they are technically difficult to handle and characterize. The aim of this laboratory is to obtain expertise in the field of structure-function studies of membrane proteins.

We have recently obtained good quality crystals of an NADH dehydrogenase type II, isolated from the *Acidianus ambivalens*. This associated membrane enzyme (NADH: caldariella quinone oxidoreductase) is a novel variant of type II, containing covalently bound flavin as its only redox cofactor. The organism from which NADH dehydrogenase is isolated is a hyperthermoacidophilic archaeon, showing one of the simplest respiratory chains known so far. The determination of the three-dimensional structure using single crystal X-ray methods should help to elucidate the electron transfer mechanism within the respiratory chain.

NADH dehydrogenase was solubilized with n-dodecyl-δ-D-maltoside and crystallized using ammonium phosphate as precipitant. X-ray data have been collected at the European Synchrotron Radiation Facility at Grenoble, France. The resolution of the data is 2.8 Å and structural studies are now in progress.





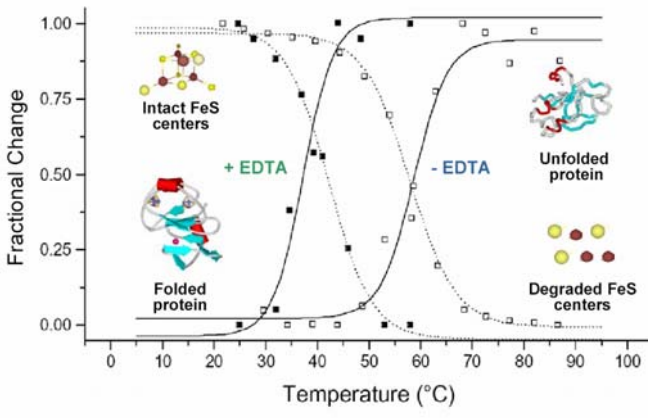
~ 2.8 Å resolution

An intrinsically hyperstable metalloprotein from a hyperthermophilic organism: insights into the role of cofactors on protein stability.

Protein Biochemistry, Folding and Stability Laboratory : Investigator, **Cláudio M. Gomes**

Proteins are optimally functional if they assume a given three-dimensional structure, which is determined by their amino acid sequence. This functional conformation corresponds to the so-called folded state. Failure to maintain this structure results in either total or partial loss of function. Ultimately, an unstructured protein is said to be unfolded. The energetics of protein stability can be described in terms of the difference in the free energy between the folded and unfolded states. Several factors contribute cumulatively to determine the maintenance of a particular structure but curiously the free energy of unfolding is very low (5-15 Kcal/mol) indicating that proteins are only marginally stable. Among the factors that favour the folded state are local interactions, secondary structure elements, packing and docking of domains, subunit association and combination with prosthetic groups or metals. The detailed study of the particular contributions of these factors greatly profits from the use of simple protein models, and even further if these proteins are from thermophiles, which are intrinsically stable at the high growth temperatures of the organisms from which they are isolated.

We have been studying the stability properties of ferredoxin (right) isolated from several hyper thermoacidophilic organisms, which grow optimally at ~80°C and pH ~2, in volcanic habitats (far right). These proteins contain two iron sulphur clusters that are involved in redox reactions and additionally a Zn centre, which is likely to have a structural role. Although this is a very abundant cytosolic protein its physiological function remains to be determined. However we found it to be extremely stable to thermal denaturation in neutral conditions. Incubation at 70°C up to 72 hours does not unfold the protein, nor incubation with 8M guanidinium hydrochloride, a very powerful protein denaturant. Accordingly, at pH 7, this protein has a melting temperature of ~125°C. Only by combining thermal and chemical denaturants or in alkaline conditions (pH=10) can the protein be totally unfolded.



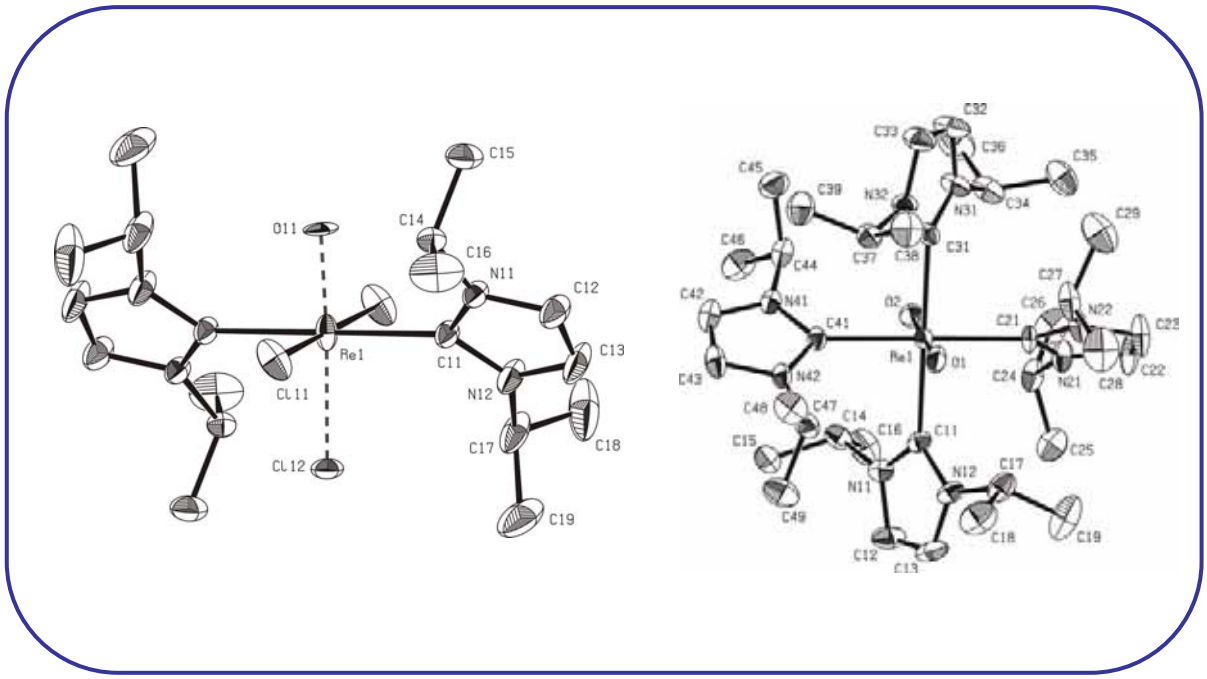
We are currently investigating the role of metal centres on the maintenance of the overall protein stability, as they are likely to play an important role in a protein with such a compact structure. In fact, this is demonstrated by the observation that disturbing the geometry of the metal centres during protein unfolding by the addition of the Fe chelator EDTA results in a significant decrease of the melting temperature from 60°C to 40°C, as shown on the figure on the right (solid line protein unfolding, dotted line, FeS centres degradation).

Gomes, C.M. et al. (1998) *J. Biol. Inorg. Chem.*, 3, 499-507; Moczygemba, C. et al. (2001) *Protein Sci. ,* 10: 1539-1548.; and Jones, K. et al. (2002) *J. Biol. Inorg. Chem.,* 10(8):1539-4.

Novel Rhenium carbenes: Synthesis and Catalysis

Homogeneous Catalysis Laboratory : Investigator, **Beatriz Royo.**

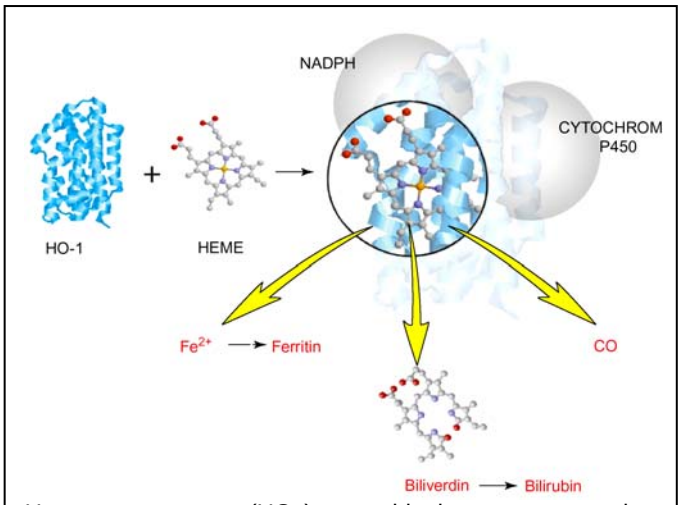
Acting as strong Lewis bases, N-heterocyclic carbenes (NHC) are promising alternatives for the well established phosphine ligands in organometallic catalysis. Although the use of this type of ligands in a large number of transition metal complexes has led to excellent catalytic performances, their application to metal oxo systems has been poorly investigated. Indeed, the first example of a metal-oxo-NHC derivative $MeReO_3$.NHC was published in1994 by Herrmann but no structural characterization was possible. We have synthesized and characterized by X-ray diffraction studies the first rhenium-oxo-NHC species. The catalytic activities of these complexes are currently under investigation.



The Molecular Basis of Inflammation.

Inflammation Laboratory : P. Investigator, **IGC, Miguel Soares.**

The molecular basis of inflammation, originally defined by Celsus (AD40) as '*rubor, calor, dolor, tumor*' (redness, heat, pain and swelling), is still today an intellectually challenging problem. Over many years now a multitude of laboratories have engaged in understanding the mechanisms that initiate inflammatory reactions. The reason for this is that inflammation, which occurs in most cases in association with microbial infections, is critical to initiate those immune responses that will ultimately lead to microbial clearance. Thus, understating how inflammatory reactions are triggered may lead to understanding on how to manipulate these in a manner that would increase the ability to clear microbial infections. However, it has become apparent in the last few years that when inflammatory responses are not tightly controlled they may cause more damage than the microbial infection themselves. This is clearly illustrated by the number of diseases considered to have an 'inflammatory' origin, i.e. sepsis, multiple sclerosis, rheumatoid arthritis and atherosclerosis. To avoid the development of these type of diseases must have reached a compromise in which inflammatory reactions are triggered in a manner that allows microbial clearance but that does not lead to tissue injury and disease**.** Our laboratory studies the molecular mechanisms that control inflammatory reactions in such a manner. We found some years ago that there are genes, that we refer to as "protective genes", that control inflammatory reactions, in a manner that avoids tissue injury and disease. One of such genes is the stress responsive gene heme oxygenase-1 (HO-1) (see box). In our laboratory we are trying to understand how the expression of HO-1 controls signal transduction pathways that regulate the expression of pro-inflammatory genes as well as apoptosis, two of the main events involved in the pathogenesis of inflammatory diseases. In the past year we found that expression HO-1 in blood vessels plays a critical role in controlling the progression of atherosclerosis. We found that this protective effect is associated with the ability of HO-1 to generate the gas carbon monoxide (CO), via the degradation of heme. CO acts directly on smooth muscle cells of the vessel wall to stop their proliferation thus stopping the progression of atheroscleosis. We are now in the process of elucidating the molecular mechanisms by which CO acts in such a manner to design strategies aimed to suppress the progression of this disease in a therapeutic manner. For more details on our lines of research access



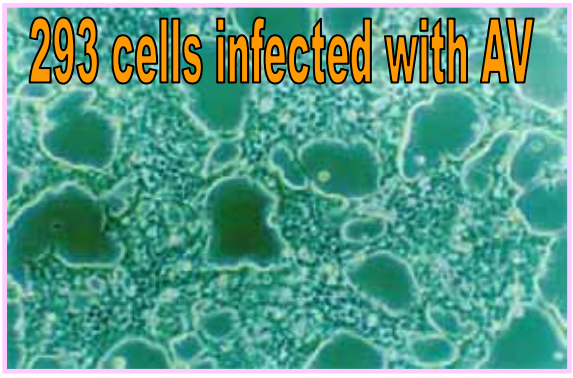
Heme oxygenases (HOs) are ubiquitous enzymes that catalyze the initial and rate-limiting steps in the oxidative degradation of heme to bilirubin. HOs cleave a mesocarbon of the heme molecule, producing equimolar quantities of biliverdin, iron, and carbon monoxide (CO). Biliverdin is reduced to bilirubin by bilirubin reductase, and the free iron released from heme is used in intracellular metabolism to induces the iron chelator ferritin, by which it is sequestered.

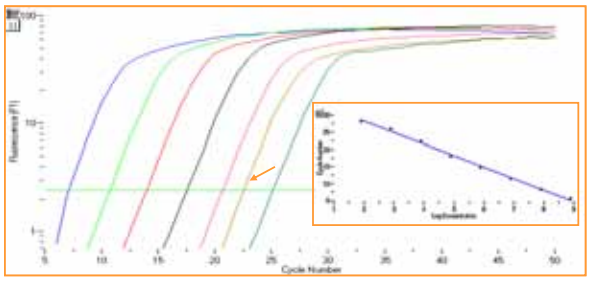
http://www.igc.gulbenkian.pt/fwd/msoares.html.

Integrated strategies for production, purification and storage of adenoviral vectors

Animal Cell Biotechnology Laboratory : Investigator, **Paula M. Alves**

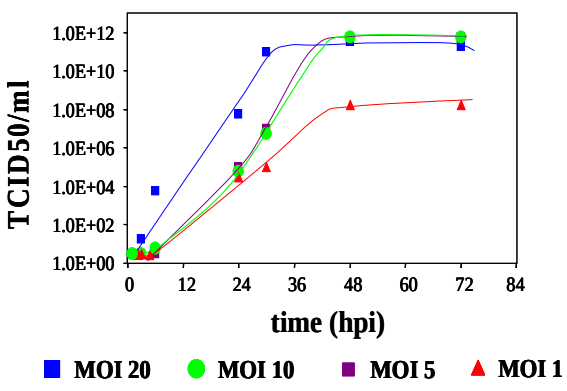
Recombinant adenovirus (AV) vectors are excellent vehicles for gene delivery. Their potential for vaccination purposes has been consolidated for over 10 years, being an attractive alternative for oral delivery due to their natural tropism to mucosal tissues. Moreover, AVs are currently used in 25% of the gene therapy clinical trials, mainly targeting cancer diseases. Consequently, the market requirements for AVs are constantly increasing causing a high demand for methodologies for the large scale production of concentrated vectors with warranted purity, safety and efficacy.
We have developed and optimized an integrated process for AV production for oral immunization against Rinderpest (RP) and Pest des Petits Ruminants (PPR). Critical issues were addressed to achieve maximum production yields of the AV with preserved infectivity. Biological and physical variables which could affect the uniformity of the final product such as medium composition, serum content, multiplicity of infection (MOI), optimal time of infection and harvest time were studied. Adenovirus downstream processing was also studied using ultracentrifugation methods as well as more easily scaleable methods such as anion exchange chromatography. Real Time PCR quantification protocols were implemented for AV quantification, leading to higher throughput and reproducibility and allowing the monitoring of the infection kinetics during the bioreaction process. Studies of AV stability leading to the development of efficient and biocompatible storage formulations containing novel additives (compatible solutes produced by hyperthermophiles organisms, discovered by Prof Helena Santos group at ITQB) were also performed. Our main objective is to predict end-product characteristics and to minimize batch to batch variations.
The first vaccine doses produced at 2 L scale were delivered at the middle of 2003 and are currently being tested in cattle at Prof Tom Barret Laboratory (IAH, Pirbright Laboratory, UK).

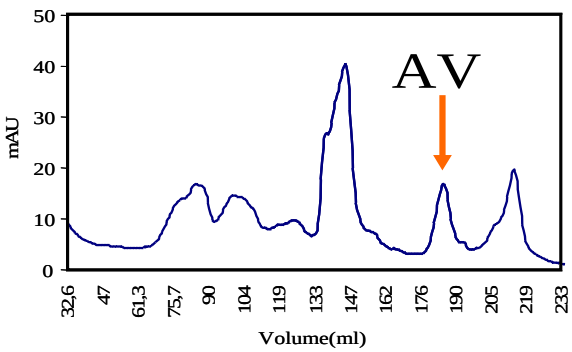




Q-PCR for the AV quantification

Effect of MOI on AV Production kinetics



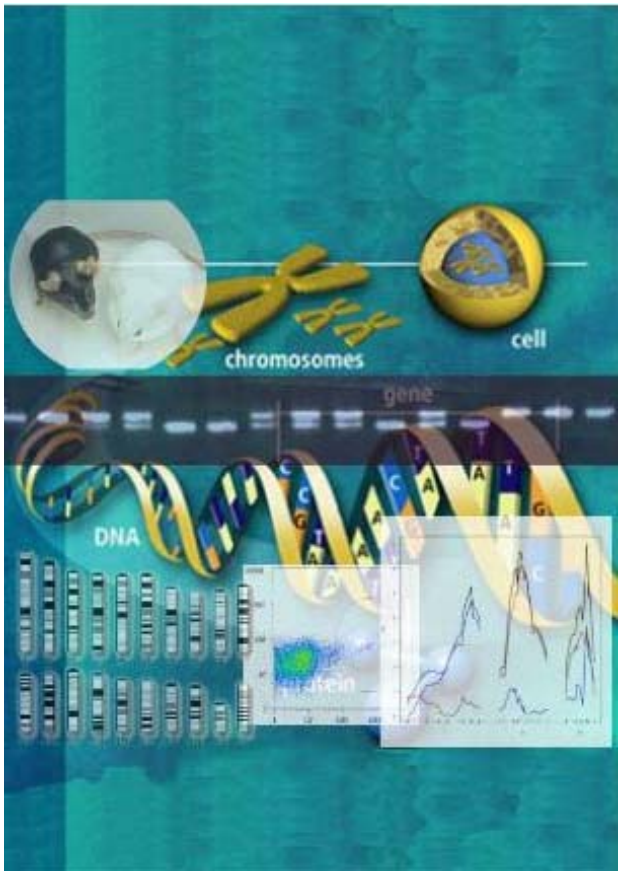


Chromatogram from a run of clarified AV

Disease Resistance and Susceptibility in human and murine models

Disease Genetics Laboratory; Investigator, **Carlos Penha Gonçalves**

The research program aims to unravel individual genetic factors involved in disease resistance or disease susceptibility both in humans and in murine models. The vast majority of common diseases have been shown to depend on multiple genetic factors. Our strategy is to combine the knowledge acquired in studying disease genetics in animal models with the expertise on recent tools enabling the genetic study of complex diseases in human populations. The research program is focused on Type 1 Diabetes and Malaria and will be develop in four distinct but convergent perspectives: mouse genetics, human genetics, statistical genetics and bioinformatics.



Development of a System to Produce Reversible Gene Inactivation in the Mouse.

Neural Crest and Gene Modification Laboratory : P. Investigator, **Moisés Mallo.**

The system is based on the introduction of additional transcriptional control elements into the gene locus of interest using a two step knock-in strategy. We have used the elements controlling transcription in the tetracycline resistance operon of *E. coli*. These elements are active in the eukaryotic genome and impose a dominant transcriptional block, which is suitable for controlled modulation (*i.e.* switching on or off), just by administration, or not, of the antibiotic doxycycline.

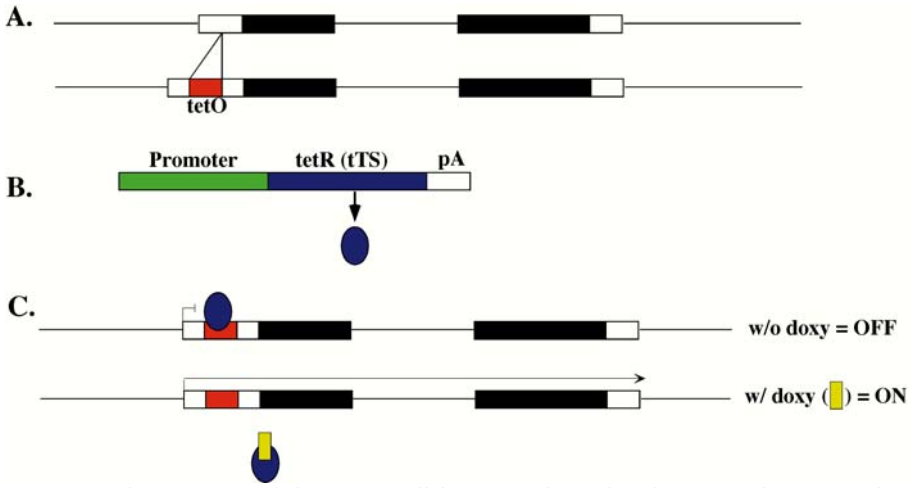


Figure 1. General strategy of the reversible gene inactivation. A. The gene locus is modified to introduce the tetO sequences (in red). B. tetR (or tTS), in blue, is expressed using a constitutive or a tissue specific promoter. C. In the absence of doxycycline (yellow box), tTS binds tetO and blocks transcription. When doxycycline is provided, tTS is removed from tetO and transcription resumes.

The main qualitative difference with currently used methods is that, while still producing complete inactivation, including both a spatial and temporally control on the inactivation process, it does not produce an irreversible structural change in the genomic locus, thus allowing reactivation of the target gene after a period of controlled inactivity. This novel technology is an important addition to the technologies that allow functional analyses of the mouse genome and the genesis of mouse models for disease.

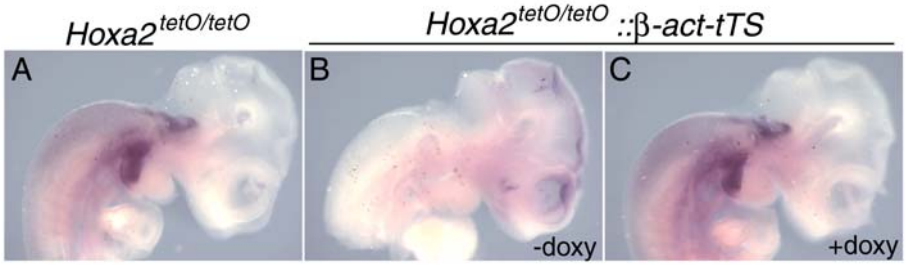


Figure 2. Reversible inactivation of the *Hoxa2* gene. In situ hybridization on E10.5 embryos using a probe against the *Hoxa2* gene. A. *Hoxa2*$^{tetO/tetO}$ embryos showing normal pattern for Hoxa2 expression. B. *Hoxa2* expression is blocked in *Hoxa2*$^{tetO/tetO}$*; -actin-tTS* embryos in the absence of doxycycline. C. *Hoxa2* expression is recovered in *Hoxa2*$^{tetO/tetO}$*; -actin-tTS* embryos growing on mothers that were kept with doxycycline in the drinking water.

[Mallo, M. et al. (2003) *Genomics* **81:**356-360]

Prevention of autoimmune diseases by suppression of pathogenic T cells.

Theoretical Immunology Laboratory (IGC) : Head of Laboratory, **Jorge Carneiro ;**

1. The increasing incidence of autoimmune and allergic diseases in countries with occidental life style has been a clear and worrying trend in the last 50 years. In these countries the incidences of autoimmune diseases is inversely correlated with the prevalence of infectious diseases. Hitherto, this puzzling observation has been a challenge to immunology. Recently, a population of regulatory CD4+CD25+ T cells has been identified that prevents autoimmune diseases by suppressing pathogenic T cells. We have shown on theoretical and experimental grounds that the persistence and expansion of this regulatory cell population is promoted by disease causing T cells. A recent mathematical analysis of the implications of this crosstalk mechanism for the ethiology of autoimmune diseases by Leon et al (J. Autoimmunity 2004) has shown that non-specific T cell stimulation in individuals undergoing diverse sub-clinical infections would result in a net re-enforcement of regulatory T cell populations. According to this model, the frequency of autoimmunity prone individuals in a genetically heterogeneous population should increase as the prevalence of infectious agents in the environment decreases (figure 1). The rational is that natural selection has minimized the risk of autoimmunity by adjusting the genes controling the growh dynamics of regulatory and pathogenic T cells in environments with higher prevalence of infectious agents. As infection incidence decreases the gene pool in the population becomes out of balance.

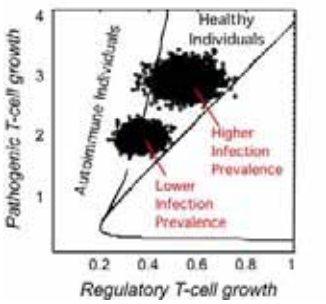


Figure 1

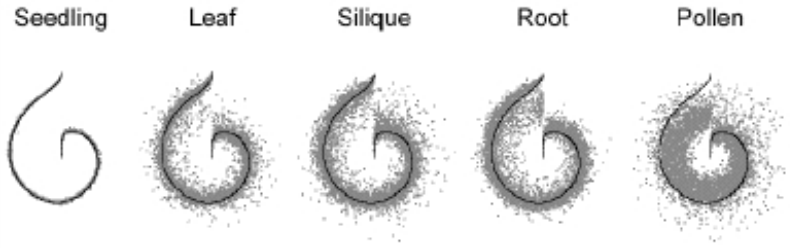


Figure 2

2. Comparison of profiles of gene expression in different tissues.

In recent years, gene-chip technology made it possible to measure simultaneously the expression thousands of genes in cellular tissues. How can we compare the expression of so many genes in different tissues? SnailView is a graphical representation (Becker et al. 2003) to visualize and compare profiles of gene expression in different tissues. Gene expression values of a given tissue are plotted in angular coordinates (angle, radius); the radius represents the gene expression in the tissue and the angle represents the gene rank according to expression in some reference tissue. Using this representation, subsets of genes that have the same expression pattern in a test tissue and a reference tissue can be found in a simple and computationally inexpensive way.

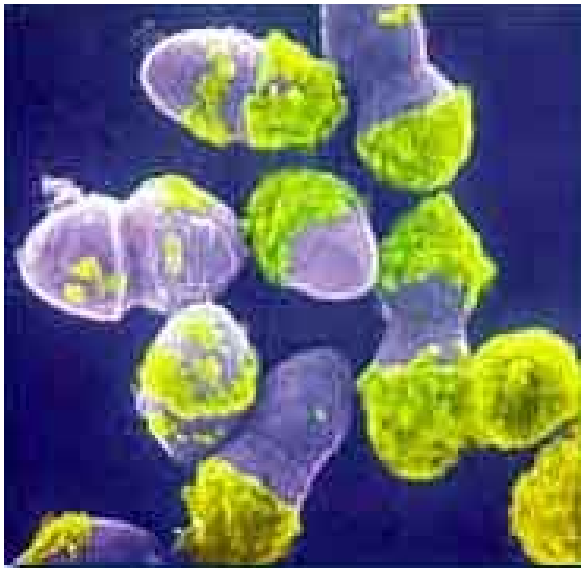
A SnailView comparison of the expression of 5999 genes in five tissues of Arabidopsis is illustrated in figure 2. The reference tissue is represented as a continuous black line, and the test tissues as gray dots. Pollen is strikingly different from the remaining tissues, which have relatively similar gene expression profiles. Snailview analysis software is available at: http://eao.igc.gulbenkian.pt/ti/index.html.

Epidemiology and virulence of *Enterococcus* spp.

Microbial Epidemiology Laboratory : Investigator, **Rosario Mato**

Enterococci are normal inhabitants of the gastrointestinal tract in human and animals, being considered relatively innocuous until the late of 1970s. Over the past two decades, the increasing incidence of high-level enterococcal resistance to penicillin, and aminoglycosides, in addition with the emergence of glycopeptide resistance strains, mainly vancomycin which usually is considered the treatment of last resort, present serious problems for physicians treating patients with nosocomial infections causes to these microorganisms. Enterococci are also capable of causing a variety of community-acquired infections.

What makes Enterococci interesting microorganisms?



1. Their remarkable ability to survive in adverse environmental conditions (for example in hospital environments), including the effects of many antimicrobial agents. This property must be due to: i) baseline intrinsic resistance to several commonly used antimicrobial agents, ii) acquired resistance *via* mobility of the resistance genes on plasmids and transposons, and chromosomal exchange, and iii) the transferability of resistance.

2. Their capacity to disseminate *via* person-to-person transmission from source patients or *via* contact with contaminated equipment.

3. The poor understanding about virulence factors, cellular receptors and colonization factors involved on the infection and colonization by Enterococci.

Gentamicin resistance in dairy enterococci.

Antibiotic stress and virulence of enterococci Laboratory:

Investigator, **Maria de Fátima Silva Lopes**

Figure legend: Electron microscope image (80000x amplification, JEOL JEM-100CX II microscope) of a broth culture of an enterococcal isolate from cheese (left); Example of an E-test plate for MIC determination (write).

Severe enterococcal infections are usually treated by using a combination of a cell-wall active agent and an amynoglycoside, often gentamicin. The appearance of an increasing number of high-level gentamicin resistant enterococci in the clinical environment makes it difficult to treat severe cases of endocarditis because synergism with cell-wall active agents no longer works. Therefore, gentamicin is one of the antibiotics of major concern at the moment. Enterococci isolated from Portuguese dairy products and clinical settings (hospitals and veterinarian clinics), together with reference strains from the genus *Enterococcus*, were screened for low-level and high-level gentamicin resistance. Four genes (*aac(6')-aph(2'')*, *aph(2'')-Ib*, *aph(2'')-Ic* and *aph(2'')-Id*), responsible for high-level gentamicin resistance and mid-level gentamicin resistance were searched using PCR. As opposed to the generalised idea that enterococci are intrinsically resistant to low levels of gentamicin, results revealed that many dairy enterococci are still not intrinsically resistant to gentamicin. High-level gentamicin resistance was not detected in any of the dairy isolates studied, although *aph(2'')-Ib* was found in one of these isolates. On the contrary, all clinical isolates studied were, as expected, intrinsically resistant to low levels of gentamicin and 15% of these clinical isolates proved to be high-level gentamicin resistant and the bifunctional gene *aac(6')-aph(2'')* was detected in four of these isolates. Although it seems not to be a problem at the present time, gentamicin resistance should be monitored in dairy enterococci, which already present genes encoding high-level gentamicin resistance.

Regulation and Control of Sugar Metabolism in *Lactococcus lactis*: Role of Hexose Transport in Glycolytic Dynamics

Physiology of Lactic Acid Bacteria and *in vivo* NMR Laboratory :

Investigator, **Ana Rute Neves.**

Lactic acid bacteria (LAB) are used worldwide in the production of fermented dairy products. *Lactococcus lactis* is generally recognized as a model organism, because of its simple metabolism, a small genome (sequence available) and genetic accessibility.

Blockage of glucose metabolism

For most metabolic pathways, the uptake of substrates represents the first step. Lactose, the milk sugar, is transported in *L. lactis* by a dedicated PTS and the internalized lactose 6-P is hydrolyzed by a phospho-δ-galactoside into galactose 6-P and glucose. One of our practical objectives is the natural sweetening of dairy products through fermentation with *L. lactis*. To block glucose metabolism we constructed a strain deficient in the mannose-PTS (the only PTS known to transport glucose) and glucokinase (intracellular phosphorylation of the sugar), but this strain could still grow on glucose. Resorting to DNA-microarrays analysis, a second PTS was identified and deletion of this gene in the double knockout mutant led to a glucose negative phenotype. Our data suggested an interaction between glucokinase and the mannose-PTS that will be investigated in 2004. To prove this interaction *glk* will be fused to GFP, and we will perform localization studies of glucokinase-GFP in *L. lactis* cells in collaboration with the group of A. Henriques (ITQB). Furthermore, a glucose-PTS deficient strain will be studied in an attempt to identify all the non-PTS glucose uptake systems. Our final goal is to obtain a global picture of glucose utilization in *L. lactis*.

Enhancement of galactose consumption

The intake of galactose by individuals with a deficiency in one the galactose metabolic enzymes results in a disease condition generally called galactosemia. Therefore it is desirable to obtain strains with improved capacity to utilize galactose. Overexpression of the genes involved in the galactose-specific part of the metabolic pathway used to convert this sugar did not result in improved galactose utilization. A bottleneck at the level of δ-phosphoglucomutase was identified and *pgmA* from *S. thermophilus* was overexpressed in *L. lactis*. In the resulting strain, galactose was used faster. Recently, we identified the gene coding for δ-PGM in *L. lactis* and the protein, which shows low homology with other δ-PGMs, is being characterized. *In vivo* NMR analysis of galactose mutants pinpointed galactose transporters other than *galP*. To gain insight into galactose utilization we will identify these transporters in 2004. A comprehensive analysis of metabolic parameters, including time dependent concentrations of relevant intracellular intermediate metabolites and coenzymes will be collected using *in vivo* ^{13}C-NMR techniques coupled to the supply of specifically labeled substrates.

Foreign pollen contamination evaluation in a clonal seed orchard using molecular markers.

Pinus Laboratory : **Investigator,** Margarida Rocheta.

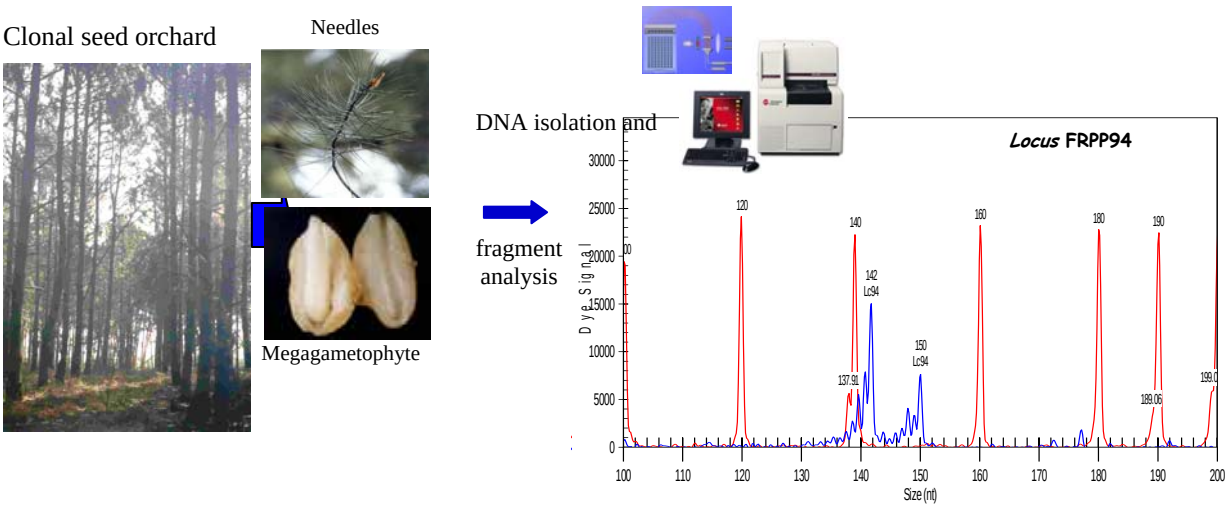
Estimation of a seed orchard's pollen contamination is fundamental in computing the decrease in the expected genetic gain due the contamination with foreign pollen from outside sources. The aims of this study were to identify the parental clones with a high pre-determined confidence level by fragment analysis, to estimate the pollen contamination (paternity analysis) and to study the mating system inside the clonal seed orchard.

SSR fragment Analysis: 3 *loci* were found to be sufficient to distinguish all the 60 clones except for 2 pairs that were found to have the same genotype.

Paternity Analysis: the minimum and maximum estimates of pollen contamination were 46% and 56%, respectively. These values could be explained by the presence of a *P. pinaster* stand at a distance less than 2 km and the existence of natural regeneration of this species within the orchard's isolation zone. Pollen contamination was higher in plots 1 and 3 than in the interior plot 2. In this study, only 20% of the clones contributed to the next generation, which may lead to a reduction in the seeds' genetic variability. However, an asymmetrical sampling from the orchard (3 plots) was made that might have biased the results and the effective number of clones participating in the next generation should probably be higher.

Mating System: the outcross pollination rate was found to be very high at 90.2%. The minimum estimate of biparental inbreeding was 21.2%, a low value if the number of seeds sampled and the orchard size are considered.

Future Research: This will focus on obtaining the allelic frequencies from outside the orchard to estimate the cryptic genetic flow and calculate the true genetic flow.

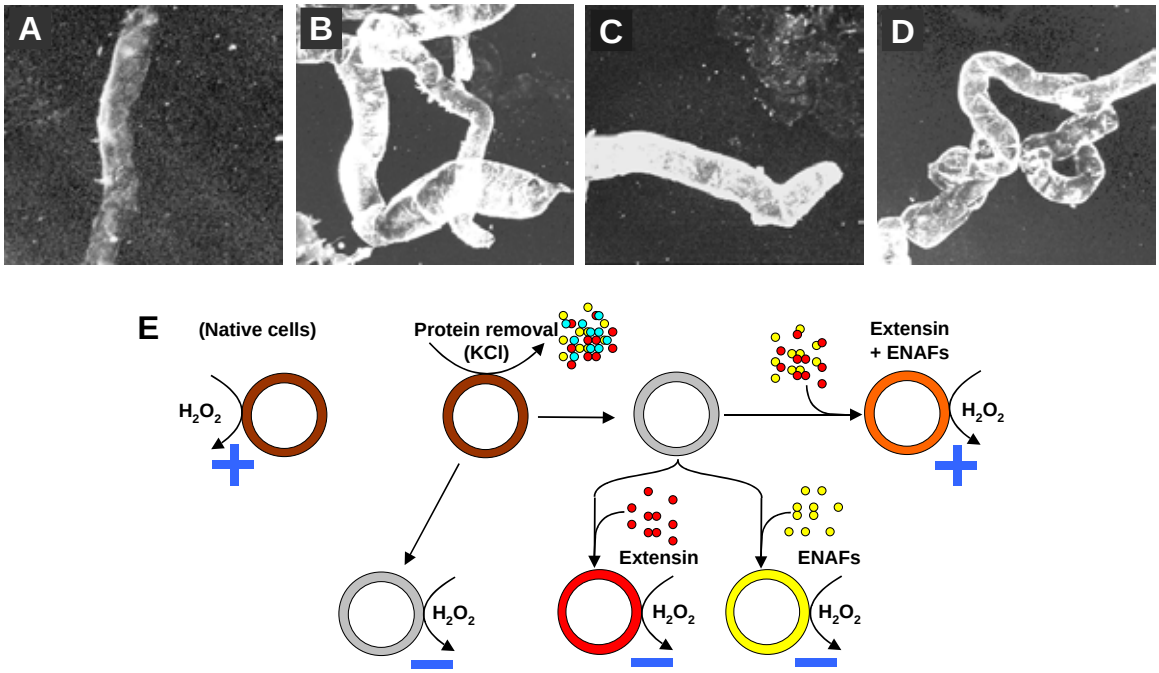


From the forest to the details of DNA sequence.

Extensin Network Associated Factors (ENAFs) in primary cell walls

Plant Cell Wall Laboratory : Investigator, **Philip Jackson**

Extensin is a major structural protein in plant primary cell walls. Peroxide-mediated formation of network extensin has been considered important for cell wall formation and to have direct effects on cell wall properties. We have devised the means to manipulate extensin network levels in primary cell walls (see also Fig. 1) and tested its effects on cell wall swelling and resistance to fungal, lytic enzymes. Contrary to consensus opinion, these independent assays both indicate that extensin network formation is essential for peroxide-mediated changes in primary cell wall properties, but full expression of effects requires the presence of additional cell wall proteins, named Extensin Network Associated Factors (ENAFs). Although the exact nature of ENAFs remains to be determined, these appear to be small, highly basic proteins that interact electrostatically with the cell wall matrix. Our results indicate an essential role for a new class of cell wall proteins in defining cell wall nanostructure and properties.

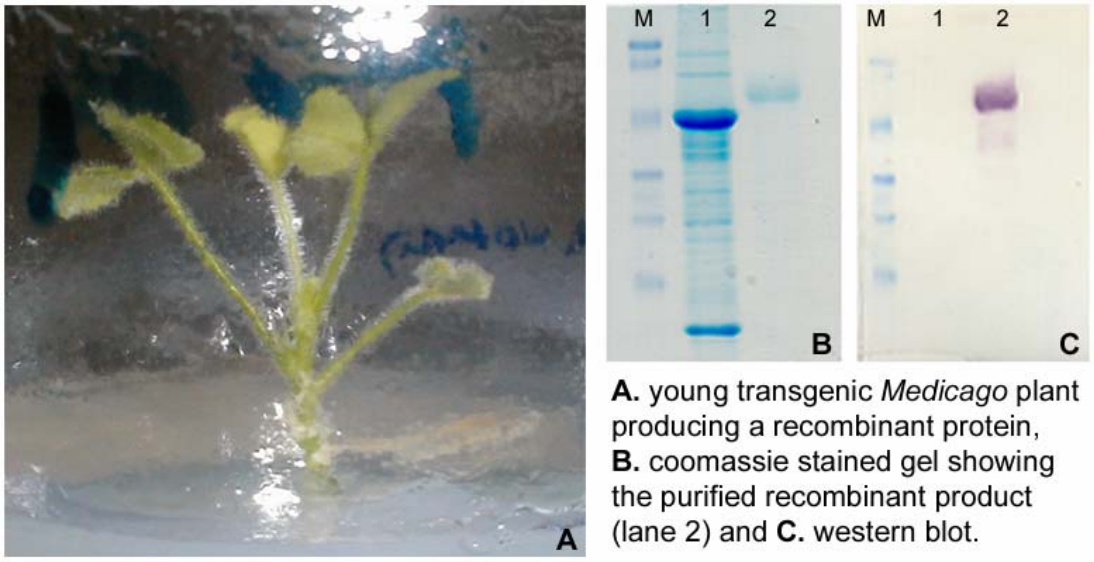


JIM11 (anti-extensin) immunolabelling of primary cell walls (A). Network extensin is formed after treatment with H_2O_2 (B). Extensin network also forms in cell walls containing total wall matrix proteins (C) or purified extensin and peroxidase (D) . A schematic presentation of methods and measurements of peroxide-mediated changes in cell wall properties (+ or negative) is presented in (E).

Plants as alternative systems for the production of recombinant proteins

Plant Cell Biotechnology Laboratory : Investigator, **Rita Abranches.**

This work was carried out in the Plant Cell Biotechnology Laboratory during 2003. It focuses on the use of plants as alternative systems for the production of recombinant proteins. Nowadays, plants are being engineered to improve human nutrition or to produce biopharmaceutical products that can either be purified or directly delivered by ingestion of the modified plant. However, much remains to be understood in the processes underlying the correct synthesis, processing and accumulation of recombinant proteins in plant tissues, as well as determining the most appropriate plant species for this purpose. In this project we are interested in the detailed characterization of recombinant products, including the implications of specific lycosylation and tissue specific differences in protein deposition in the model legume *Medicago truncatula.* For this, we have utilized a simple and efficient transformation-regeneration method in *Medicago truncatula*, using a new highly embryogenic line isolated in BCV.

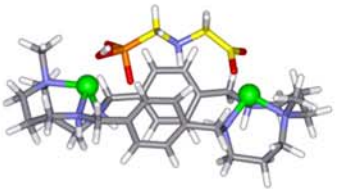


A. young transgenic *Medicago* plant producing a recombinant protein, **B.** coomassie stained gel showing the purified recombinant product (lane 2) and **C.** western blot.

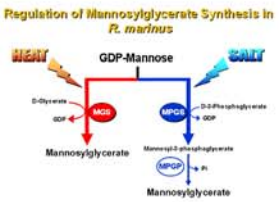
This figure shows a young *Medicago* plant transformed by *Agrobacterium* and regenerated via somatic embryogenesis. The recombinant product was purified from total protein extract of leaves. The gel and respective western blot indicate a high production of the protein of interest. Transient expression and analysis of the protein, by infiltration of plant leaves with *Agrobacterium*, was also performed to enable a relatively rapid verification of the functionality of the constructs.

Research in model organisms is important, but it is also essential to study their applications in crop plants, bridging the gap between fundamental and applied research. We are therefore interested in comparing the recombinant products from *Medicago truncatula* with the agronomically important species *Medicago sativa.*

Research Divisions

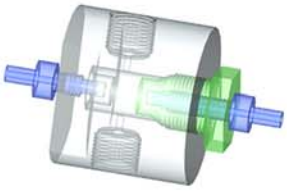


Chemistry



Biology

Biological Chemistry



Technology

Plant Sciences

RESEARCH DIVISIONS AND RESEARCH GROUPS

CHEMISTRY DIVISION – Laboratories and Staff

Laboratory: Organometallic Chemistry (Oxo-complexes/ ring slippage)
Head of Laboratory: Carlos C. Romão
Research Team:

Ana Cristina Silva Fernandes	Ph.D.
Marta Norton de Matos	Post-Doc
Ana Margarida Santos	Post-Doc
Marta Ramilo Abrantes	Ph.D.Student
Cláudia Cristina Lage Pereira	Ph.D.Student
Tiago Lourenço	Ph.D.Student
Zeljko Petrovski	Ph.D.Student
João Seixas	Undergraduate trainee

Laboratory: Organic Synthesis (Asymmetric synthesis)
Head of Laboratory: Christopher David Maycock
Research Team:

Maria Rita Bordalo Ventura	Post-Doc
Stefan Panev	Post-Doc
Jorge Wahnon	Ph.D.Student
Ana Cruz	Research Student (from Industry)
Soraia Santos	Research Student (from Industry)
Sofia Miguel	Research Student (from Industry)

Laboratory: Microheterogeneous Systems (Chemical kinetics/ mesophases)
Head of Laboratory: Eurico Melo
Research Team:

Antonio Manuel Gonçalves Lopes	Ph.D.
Rute Cristina da Silveira Mesquita	Ph.D. Student
Sofia Leite de Souza	Ph.D. Student
Maria Helena Lopes Lameiro	Ph.D. Student
Sónia Lopes	Research Student
Ana Paula Silva	Research Student

Laboratory: Theoretical and Inorganic Chemistry (DFT calculations / polynuclear complexes)
Head of Laboratory: Maria José Calhorda
Research Team:

Vitor Manuel Sousa Felix	Associate Professor
Maria João Pires da Silva	Master Student
Paulo Jorge Costa	Ph.D. student
Márcia Mora	Ph.D. student
Paulo Martinho	Ph.D. student
Clara Cabrita	Undergraduate
Nuno Bandeira	Undergraduate

Laboratory: Raman spectroscopy
Head of Laboratory: Ricardo Franco
Research Team:

Roberto Di Paolo	Post Doc
Smilja Todorovic	Post-Doc
Vena Prosinecki	Research Student

Laboratory: Co-ordination and Supramolecular Chemistry (Macrocyclic compounds / Supramolecular assemblies)
Head of Laboratory: Rita Delgado
Research Team:

Xiuling Cui	Post-Doc
Patrícia dos Santos Antunes	Ph.D. student
Krassimira Passos Guerra	Ph.D. student
Feng Li	Ph.D. student
Sílvia Ferreira de Carvalho	Research student
Luís Miguel Lima	Research student

CHEMISTRY DIVISION – Plan and Objectives.

The general scientific objectives of the Chemistry Division are the synthesis, characterization and identification of natural and synthetic molecules and chemical processes which have a relevance to Biology and related Life and Environmental issues. Although not comprehensively covering all the aspects of this extremely wide area, the Chemistry Division makes a significant impact in the following fields,

- Theory and modelling, of molecules and chemical reactions (Group of M. J. Calhorda , Theoretical and Inorganic Chemistry);
- Selective synthesis and catalysis of organic, inorganic and organometallic molecules of potential pharmaceutical interest (Groups of C. Maycock, Organic Synthesis; R. Delgado, Coordination and Supramolecular Chemistry; C. Romão, Organometallic Chemistry; Beatriz Royo, Catalysis)
- Characterization and kinetics of chemical processes in biological interfaces, membranes and aggregates by photophysical methods (Group of E. Melo, Microheterogeneous Systems);
- Raman spectroscopy of metalloenzymes and biologically relevant molecules and materials (Group of R. Franco, Raman Spectroscopy).

In addition, the Chemistry Division has an Analytical Service that covers elemental analysis, chemical analysis and speciation of water, food and biological samples providing in-house and external services. The group of Organometallic Chemistry houses a start-up company devoted to exploiting the therapeutic capability of organometallic compounds producing anti-cancer and CO delievering drugs. This work, carried out in collaboration with IGC researchers, emphasizes the scientific synergies within the Laboratório Associado.

Two groups left the Chemistry Division early in the year; P. Hildebrandt to the Technical University of Berlin and A. Maçanita to the Technical University of Lisbon, Instituto Superior Técnico. Some of the fields previously covered by Hildebrandt's group are now covered by the new group of R. Franco.

These changes prompted an internal exercise of reassessment of the objectives and possible restructuring of the Division. This exercise concluded the need to reinforce the alignment of the objectives and goals of the Division with those of strategic importance to ITQB in the broad Biology and Life Sciences areas. As such, the synthetic chemistry and catalysis operation is directing its studies towards pharmacologically important targets, including drug synthesis, and a stronger collaboration with Life Sciences

Divisions has been actively sought. However, to better interweave chemistry's biologically richer frontier with many of the biology groups and projects in ITQB, it was accepted that new groups should be recruited within the areas of Bio-organic Chemistry, namely in peptide and carbohydrate chemistry. This course of action was explicitly taken in the call for Laboratório Associado positions, held in mid 2003. Unfortunately, no suitably qualified applicants could be found in either of these areas. It was therefore decided to strengthen the existing position of the Division in the area of synthetic chemistry by initating a new group devoted to outstanding frontier problems in Catalytic Chemistry, particularly C-H activation. This group, led by Beatriz Royo, only started its establishment in December 2003, but its activities will strongly reinforce the overall potential of the Chemistry Division.

An active seminar programme was started within the Chemistry Division aiming at maximizing the capabilities of the Division and improving the scientific formation/information level of all its members.

The main needs for equipment refurbishing were identified with utmost priority being given to the replacement of the existing obsolete NMR spectrometer. Important steps were taken towards raising the quality of the analytical services, namely by the creation of the new X-ray service for small molecules, the enrolment of a NMR technician and the achievement of high quality and reliability standards in the elemental analysis service. The use of the Mass Spectrometry facilities installed from 2002 considerably improved the work of the groups in Synthetic Chemistry.

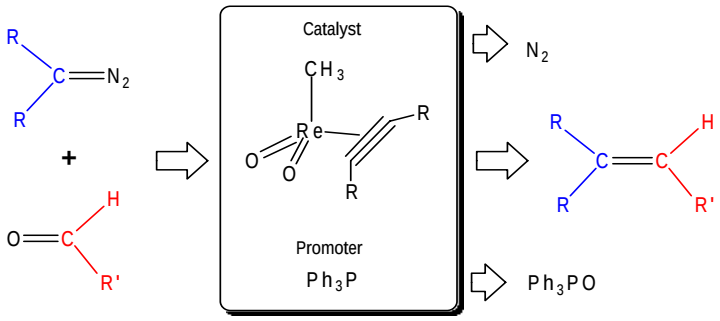
In December 2003, the Chemistry Division was evaluated by an external committee comprising Profs. Karl Wieghardt (Chairman; Max-Planck Institut für Bioanorganische Chemie, Mulheim, Germany), Michael Hursthouse (University of Southampton, UK) and João Fraústo da Silva (IST/Lisbon Technical University and Cultural Centre of Bélem). This exercise, launched by the Director of ITQB, was accepted by the Chemistry Division as an important tool to measure its own performance. The panel praised the high quality and competitiveness of most of the research produced within the Chemistry Division, acknowledged the effort put into the reorientation towards more biologically significant goals and the growing interaction with other Life Sciences groups within ITQB. Importantly, the evaluation Committee fully recognized and supported our on-going quest for a new NMR and X-ray equipment.

CHEMISTRY DIVISION – Highlights

New Catalysts for Oxygen Transfer Reactions.

Organometallic Chemistry Group : Head of Group, **Carlos Romão**

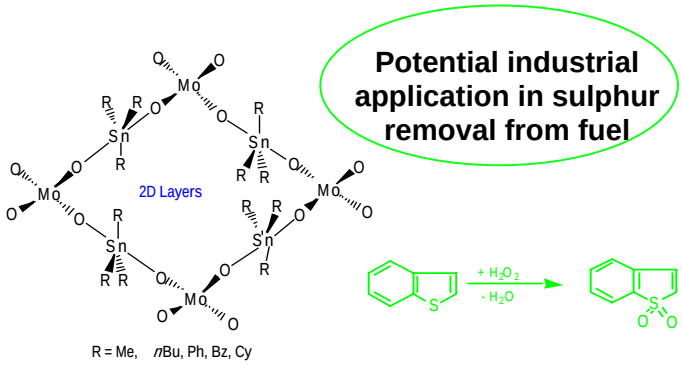
A catalytic version of the classical stoichiometric Wittig Reaction is highly desirable to avoid strongly basic conditions, thereby enlarging the number of complex molecules that may undergo the reaction and expensive/dangerous reagents. The number of known catalysts is still very small and mechanistic questions are still under debate.
We have prepared a new family of efficient catalysts for aldehyde olefination based on a Re(V)-dioxo fragment. The reaction features a new mechanism for the formation of the active species.



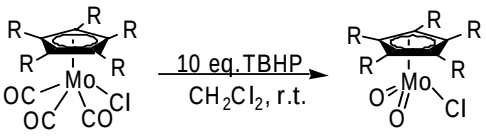
A. M. Santos et al., *J. Am. Chem Soc.* **2003**, *125*, 2414-2415

The elimination of sulfur containing compounds from gasolines and diesel oils is a major goal to achieve in order to reduce emission of noxious sulfur oxides to the environment. Hydrodesulfurization of crude oil brings these compounds to a low level but doesn't eliminate them completely. Oxidation of these compounds in the refined fuels to water soluble sulfones is a potentially very important method for their elimination. We established this possibility on commercially obtained diesel oil using a new family of polymeric Mo/Sn oxide catalysts and hydrogen peroxide as the oxidant.

Potential industrial application in sulphur removal from fuel



Organometallic oxo complexes have had a spectacular success in oxidation chemistry in the past decade, particularly in the case of $(CH_3)ReO_3$ (MTO). We have shown that the classic Cp"MoO_2Cl complexes are also very active in olefin epoxidation, even surpassing MTO. Quite conveniently, these complexes can be replaced by their much more stable precursors Cp'$Mo(CO)_3Cl$ without any change in catalytic activity.



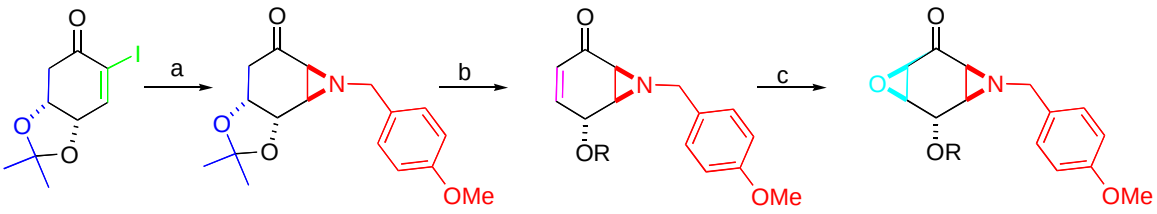
✓**Unusually high initial activities in epoxidation**
✓ **Equally active catalyst precursors**
✓ **Reusable in ionic liquids**

M. Abrantes et al., *Organometallics* **2003**, *22*, 2112-2118

Regio-selective Ring Opening in Strained Bicycles and Enantioselective Synthesis from Renewable and Sustainable Natural Resources.

Organic Synthesis Group : Head of Group, **Christopher D. Maycock**

The ring opening of the aziridine ring 2-oxo-x-azabicyclo [x.1.0] cycloalkanes in a region-selective manner and with elimination of an amine has allowed us to generate a series of α-substituted enones and this has been applied to the synthesis of the enantiomerically pure natural product bromoxone (see the scheme) which is also an important intermediate for the synthesis of a range of polyoxygenated cyclohexanes. This work was published in Organic Letters one of the principle journals in Organic Chemistry. Also completed was an enantioselective synthesis of one of the paraconic acids, which are an important class of natural lactonic acids. The method is general and starts from readily available tartaric acid such that the enantiomers of these compounds are available. This is a continuation of our work on the use of renewable resources for efficient enantioselective organic synthesis and we remain active in this internationally competitive area. Quinic acid is also an abundant chiral starting material which can be transformed into a wide range of intermediates for natural product synthesis, such as for complex dehydrodecalins. We have studied the cycloaddition reactions of 2-vinylcyclohexenones and have found that this apparently electron deficient system is a good substrate for 4+2 cycloadditions. The reactions are regio- and stereoselective and apparently remote group affect the π-face selectivity. Allylic 1,3-rearrangements are also possible in cyclohexenols. This provides a route to isomeric cyclohexenols and we have used this rearrangement we have converted into bicycle [4.2.1] systems which have a highly substituted 3,5-*cis*-disubstituted pyrrolidinone ring. This type of substitution exists in several natural products. Efforts are continuing to synthesise optically pure natural products using strategies discovered in our laboratory. The development of "green" methods and reagents for the functionalisation of molecules with special emphasis on industrial application is also a priority within the group.
Collaboration with other groups has continued and has resulted in publications and the discovery of some new biologically important compounds. Several new projects have been started which are entirely financed by industrial partners and we are part of the Stab directed CRAFT project which has been approved and financed by the EEC.



a) Addition of an amine to the iodoenone to form an aziridine

b) Elimination of acetone to form another enone

c) Stereoselective epoxidation controlled by aziridine nitrogen atom

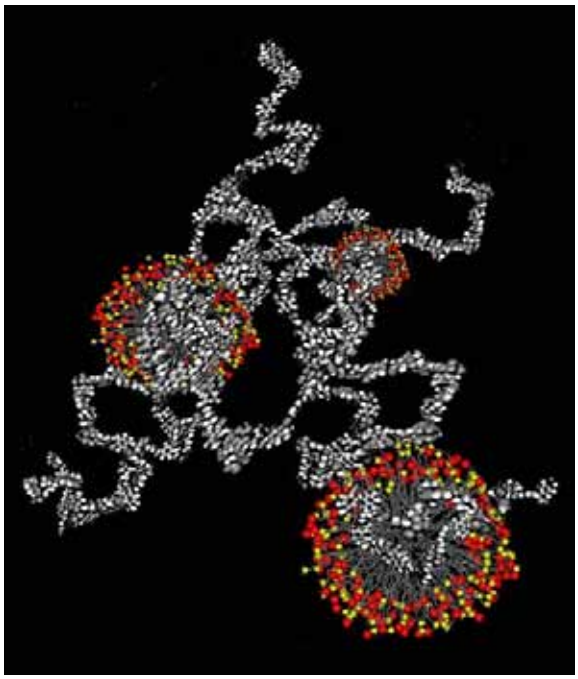
d) Reaction with acid (HX) to form α-substituted enone

Aziridine is acting as a protecting and stereodirecting group

Studies on the Stratum Corneum

Micro-heterogeneous Systems Group : Head of Group, **Eurico Melo**

The uppermost layer of the epidermis, the stratum corneum, is the main barrier for the penetration of chemical species through the skin ; it acts as a semi-permeable “varnish”. It is constituted by dead cells and corneocytes, interconnected by protein rivets, the desmosomes, all of it imbedded in a lipid matrix. Our studies refer to the properties of this lipid matrix that play the main role in the protection of our body from the environment. With this purpose we developed a lipidic model system consisting of the three main lipid components of the stratum corneum (ceramide, cholesterol and fatty acid). With this model we have found that the relative proportions in which the components are blended in human skin (and other mammals) is a kind of “magic mixture” (called an eutectic mixture) that displays unique thermal properties, namely it becomes homogeneous upon melting. Exactly what are the physiological consequences of this fact is beyond our expertise, but we can speculate that it avoids phase boundaries that are known to enhance the skin permeability.



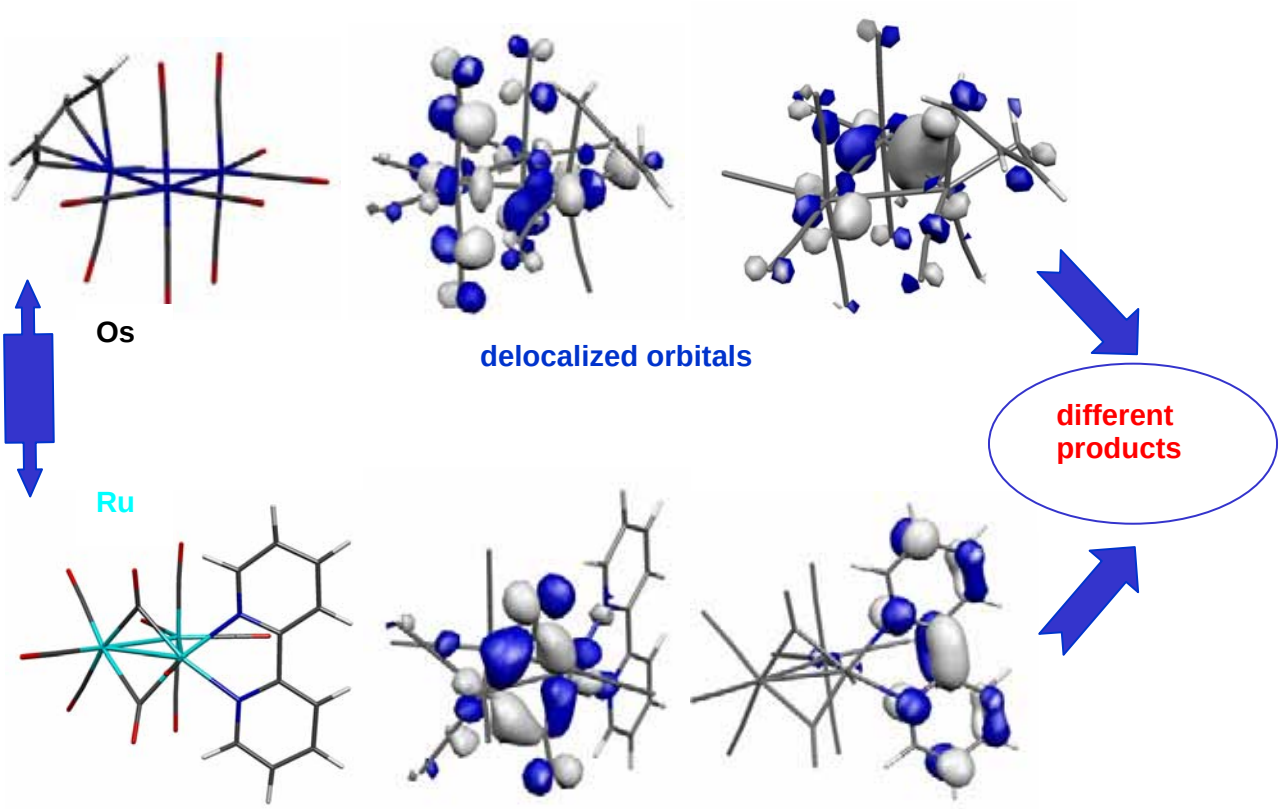
Polymer-amphiphile interaction in aqueous media

The transdermal route for drug delivery is very attractive but, due to the stratum corneum barrier, it is not feasible for most of the existing bioactives. In order to generalize it to other bioactives we have to allow for a high loading of a drug in the transdermic patch and simultaneously enhance the skin permeability to the drug. In this regard, we study mixtures of proteins with amphiphiles (lipids) and biocompatible hydrophobically modified polymers with lipids, both of which show particular characteristics due to the wide range of rheologic and thermotropic properties displayed. We have developed thermotropic reversible systems forming gel phases that exhibit thermal rheology enhancement at around 37 ºC. The systems also present a very convenient pH and ionic strength dependence that affects the phase boundaries, both in the dilute and semi-dilute regime. These polymer-lipid mixtures are useful not only as a base for patches for transdermal administration but also for oral pharmaceutical formulations.

Using DFT and TD-DFT calculations to understand photochemical reactivity of trinuclear transition metal clusters $M_3(CO)_{10}(L\text{-}L)$.

Theoretical and Inorganic Chemistry Group :
Head of Group, **Maria José Calhorda**

$Ru_3(CO)_{10}(L\text{-}L)$ and $Os_3(CO)_{10}(L\text{-}L)$ clusters, where L-L are bidentate ligands, exhibit different photochemical reactivities. Substitution of Os by Ru results in a different structure. Two carbonyl groups form bridges spanning one Ru-Ru bond, a situation electronically very unfavourable for Os clusters, where the preference for only terminal carbonyls holds, even in the presence of a good sigma donor L-L. As a consequence, HOMO and LUMO differ greatly. Electronic transitions, involving strong contributions from these orbitals, show different natures. Excitations at the corresponding wavelengths will result in the formation of different photoproducts.

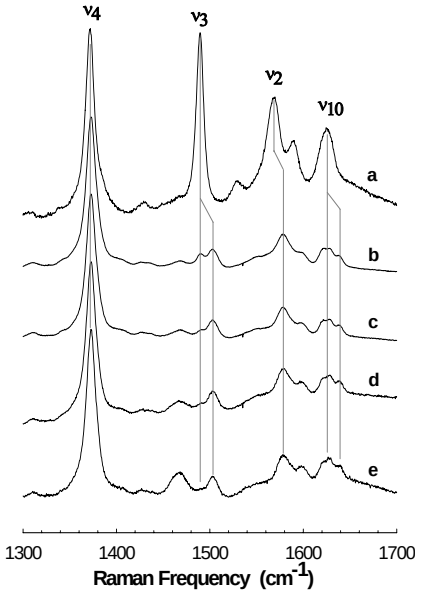


Os: F. W. Vergeer, P. Matousek, M. Towrie, P. J. Costa, M. J. Calhorda, F.Hartl (submitted)
Ru: F. W. Vergeer, M. J. Calhorda, P. Matousek, M. Towrie, F. Hartl
***Dalton Trans.* 4084-4099 (2003).**

Resonance Raman Studies of Ferrochelatase, the Last Enzyme of the Heme Biosynthetic Pathway and Other Studies.

Resonance Raman Group : Head of Group, **Ricardo Franco**

Ferrochelatase (EC 4.99.1.1) is the terminal enzyme in the biosynthesis of heme, catalyzing *in vivo* Fe^{2+} chelation into protoporphyrin IX to give heme. Resonance Raman (RR) data on the binding of free-base protoporphyrin IX and its metallated complexes (Fe(III), Fe(II) and Ni(II)) to active wild-type protein were obtained at varying ratios of porphyrin to protein as shown in the Figure; (a) ferric heme in buffer; and ferric heme titration of native wild-type ferrochelatase at ferric heme/protein ratios of (b)1.0 (same concentration of heme as in spectrum a); (c) 0.5; (d) 0.25; and (e) 0.1. The binding of ferric heme, a known inhibitor of the enzyme, leads to the formation of a low spin six-coordinate adduct. Ferrous heme, the enzyme's natural product, binds in the ferrous high spin five-coordinate state. Ni(II) protoporphyrin, a metalloporphyrin that has a low tendency toward axial ligation, becomes distorted when bound to ferrochelatase. Similarly for free-base protoporphyrin, the natural substrate of ferrochelatase, the RR spectra of porphyrin-protein complexes reveal a saddling distortion of the porphyrin. RR studies are on-going on ferrochelatase active-site mutants in order to elucidate the role of the protein active-site pocket residues on porphyrin binding and distortion.



Confocal Raman Microscopy Applied to Polymers

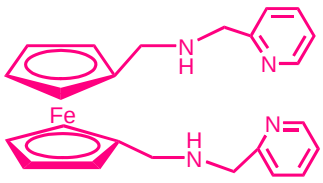
Confocal Raman microscopy was used to measure solute concentration gradients within membranes. First, the depth resolution of the system was obtained by using a sample of silicon. According to the optical system employed, the depth of the analyzed layer in the polymer was 9 m. The presence of cyclohexane in PDMS was studied from the Raman spectra with a step size of 10 m. The decreasing of the signal due to attenuation of the laser power within the membrane was taken into account following the Raman signal of the polymeric matrix.

Calorimetry Studies of Ferrochelatase

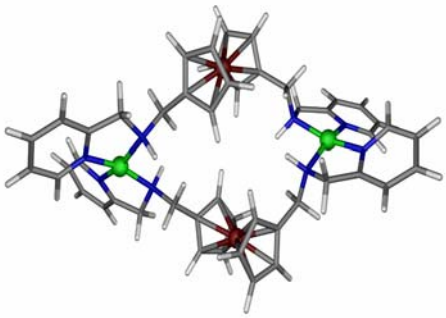
Calorimetric studies of wild-type murine ferrochelatase using Differential Scanning Calorimetry (DSC) revealed a single fully cooperative two state denaturation process with a transition temperature of 56 ºC. The Glu-289-Gln and Glu-289-Ala variants contain endogenous porphyrin in its as-isolated form and exhibited higher melting temperatures than the wild-type enzyme, an effect that was also observed in a 1:1 complex of ferrochelatase and mesoporphyrin. In contrast to the latter species or to wild-type ferrochelatase, both variants presented a non-cooperative denaturation process.

A new redox molecular sensor for copper, L1.

Coordination and SupramolecularChemistry Group : Head of Group, **Rita Delgado**



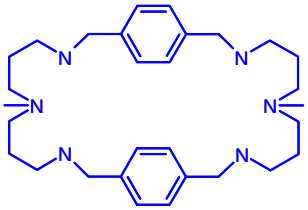
L^1



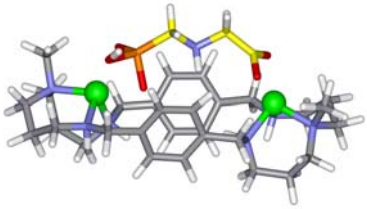
X-ray structure of $[\{CuL^1(H_2O)(NO_3)\}_2]^{2+}$

L^1 can selectively detect copper, in aqueous solutions, in the presence of other metal ions, such as Ni^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}, using cyclic voltammetry

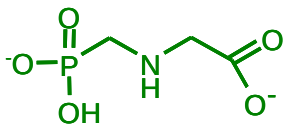
Receptors for anions



$Me_2[30]pbz_2N_6$



Cu···Cu 7.48(3) Å



Guest: pesticide

Several dianions can bridge the two copper centres of the dinuclear copper complex of $Me_2[30]pbz_2N_6$, such as indicated above, or co-ordinate directly to the hexaprotonated ligand by electrostatic interactions, hydrogen bonds and/or π-π interactions.

BIOLOGY DIVISION – Laboratories and Staff

Laboratory: Microbial Development (Intercellular signalling and cellular morphogenesis during *Bacillus subtilis* sporulation)
Head of Laboratory: Adriano O. Henriques
Research team:

Rita Zilhão	Ph.D.
Teresa Barbosa	Post-Doc
Rachele Isticato	Post-Doc
Mónica Paula Fernandes Serrano	Ph.D.Student
Gonçalo Bruno Rodrigues Carvalho	Ph.D.Student
Teresa Vasconcelos Costa	Ph.D Student
Luísa Côrte	Ph.D.Student
Claudia Serra	Research Student
Sérgio Pinto	Undergraduate Student
Filipe Vieira	Undergraduate Student

Laboratory: Control of Gene Expression (mRNA degradation / ribonucleases transcription)
Head of Laboratory: Cecília Maria Arraiano
Research team:

Ana Alexandra Cruz Varandas	Post-Doc
Mónica Amblar Esteban	Post-Doc
Helena Luísa Araújo Vieira	Post-Doc
Maria de Fátima Afonso Cairrão	Ph.D.Student
Patrick Oliveira Freire	Ph.D.Student
Sandra Cristina de Oliveira Viegas	Ph.D.Student
Paulo Emanuel Oliveira Marujo *	Ph D student
Ana Lúcia Mesquita Barbas **	Ph D student
José Eduardo Marques Andrade	Ph.D Student
Joana São José Dias Amaral	Research Student
Kathrin Franziska Wunder Baptista	Research Student
Rita Margarida Tavares	Trainee
Rui Pedro Albuquerque Freitas	Trainee

*in IBPC-Paris co-oriented Prof. Ph. Régnier
** in Biotecnol S.A. co-oriented Dr. Andrew Kelly

Laboratory: Cell Physiology and NMR (Microbial Physiology/Biochemistry of Hypertermophiles and Metabolic Engineering of Lactic Acid Bacteria)
Head of Laboratory: Helena Santos
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Claudia Sanchez	Post-Doc Student
Maria Margarida Moreira dos Santos	Post-Doc Student
Paulo Lemos	Post-Doc Student *
Tiago Quininha Faria	Ph. D. Student
Luís Maria Lopes da Fonseca	Ph. D. Student

Nuno Miguel Formiga Borges	Ph. D. Student
Maria Manuel Sampaio	Ph. D. Student
Luís Pedro Gafeira Gonçalves	Ph. D. Student
Filipa Maria Lage Silva Cardoso	Ph. D. Student
Carla Alexandra Duarte Jorge	Ph. D. Student
Paula Cristina Lima Gaspar	Ph. D. Student
Melinda Carmen Noronha	Ph. D. Student
Susana Margarida Gonçalves	Reseach Student
Tiago Moreira Pais	Research Student
Rute Ferreira de Castro	Research Student
Ana Isabel Mingote	Research Technician
Marta Viseu Rodrigues	Undergraduate Student

* co-supervision with FCT-UNL

Laboratory: Molecular Genetics (Antibiotic resistance/ infectious diseases)
Head of Laboratory: Hermínia de Lencastre
Research team:

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Ana R. Gomes	Post-Doc
Duarte C. Oliveira	Post-Doc
Raquel Sá-Leão	Post-Doc
Marta Aires de Sousa	Post-Doc
Rita Sobral	Ph.D. Student
Susana Gardete	Ph.D. Student
M. Inês Crisóstomo*	Ph.D. Student
Maria Miragaia	Ph.D. Student
Sandro Pereira	Ph.D. Student
Maria Daniela Brito	Graduate Student
Sónia Nunes	Graduate Student
Natacha Sousa	Graduate Student
Carla Simas	Graduate Student
Teresa A. Figueiredo	Graduate Student
Nelson Frazão	Graduate Student
Nuno Faria	Graduate Student
Ana Isabel Castro	Graduate Student
Ana Paula Couto	Master Degree Student
Catarina Milheiriço	Undergraduate student
Maria Cândida Lopes	Research Assistant
Isilda Gueifão	Laboratory Assistant

Collaborators

Ilda Santos Sanches, Ph.D.	Associate Professor FCT/UNL
Mario Ramirez, Ph.D.	Assistant Professor FM/UL
Isabel Couto, Ph.D.	Assistant Professor IHMT/UNL
António Brito-Avô, M.D.	Pediatrician
Joana Saldanha, M.D.	Pediatrician

Visiting Scientists
Maria Elena Meza
Oto Melter
Teresa Baptista

Laboratory: Microbial Genetics (Metabolism and regulation of carbohydrate utilization in *Bacillus subtilis* / control of gene expression)
Head of Laboratory: Isabel Sá-Nogueira
Research team:

Irina Franco	Ph.D.Student
José Manuel Inácio	Research Student
Vânia Brissos	Research Student
Rodrigo Saraiva Roda	Research Student

Laboratory: Glycobiology (Intracellular transport / Protein Glycosylation)
Head of Laboratory: Júlia Costa
Research team:

Vítor Sousa	Ph.D. student
Vanessa Morais	Ph.D.
Angelina Palma	Ph.D.
Catarina Brito	Ph.D.
Rui Almeida	undergraduate
Nuno Barata	undergraduate

BIOLOGY DIVISION – Plan and Objectives

The main subjects of research of the six laboratories in this Division are Microbial Genetics, Microbial Development, Microbial Physiology, Control of Gene Expression, and Glycobiology. Most of the expertise is focused on bacteria and archaea, but one of the teams studies protein glycosylation pathways in mammalian cells, and metabolic trafficking in primary cultures of brain cells has also been examined.

Studies performed in the Molecular Genetics Group I (H. de Lencastre) on **molecular mechanisms** responsible for **antibiotic resistance** and on **molecular epidemiology** represent a highly visible research area and many of the studies have a direct social impact. The Group has been the centre of multinational projects supported by the European Community – project EURIS (1999-2003) and its continuation project PREVIS (2004-2007) – the aim of which is to understand the molecular mechanisms of bacterial colonization and to test the efficacy of interventions that would lower the carriage rate of drug resistant and virulent strains of *Streptococcus pneumoniae* by healthy children attending day care centres. Day care centres represent a major ecological reservoir of *S. pneumoniae* and reduced carriage should also reduce both pediatric and adult disease by this dangerous pathogen. Extensive studies are also ongoing to understand the mechanism of epidemic behaviour and evolution of multi-drug resistant clones of *Staphylococcus aureus* in hospitals and in the community. Previous studies by the Molecular Genetics Group demonstrated that expression of the β-lactam resistant phenotype in *S. aureus* involves not only the resistance gene *mecA* but also a surprisingly large number of determinants in the genetic background of the bacteria, which appear to be part of a stress response pathway. The transcription of

three such auxiliary genes – *murE, murF* and *pbp2* – was put under the control of inducible promoters in order to test the effect on the resistant phenotype. This novel experimental system will be analyzed for mRNA expression profiles using DNA microarrays.

Research in the Mircrobial Development Group (A. O. Henriques) is centred on the analysis of the regulatory circuits coupling **gene expression to morphogenesis** during spore formation in *Bacillus subtilis*, and on the molecular mechanisms that govern **assembly of the spore surface layers**. Recent achievements are: *i*) the finding that normal expression of **cell division** gene *divIB* is required for the activation of a key regulatory protein governing entry into sporulation, via its control over the activity of **chromosome segregation** proteins Soj/Spo0J; *ii*) the functional interaction between a permease and a membrane protein translocase, defining a **cell-cell signalling** pathway controlling the prespore-specific activation of transcription factor sG at an intermediate stage of sporulation; *iii*) the discovery of an oxalate decarboxylase at the spore surface, or the elucidation of a hierarchical cascade of interactions among spore surface proteins CotH, CotG, and CotB, leading to the assembly of a cross-linked form of CotB. This Laboratory has also pursued the characterization of a collection of **gut-associated aerobic sporeformers**, mainly assessing properties important for the potential exploitation of selected strains as Probiotics.

The main area of interest in the Microbial Genetics Group II (I. Sá Nogueira) is the analysis of the mechanisms through which the cell senses nutrient availability and transmits that information to the level of gene expression. The research focuses on the mechanisms of transcriptional regulation that govern the expression of genes involved in carbohydrate metabolism in the Gram-positive model organism *B. subtilis*. More specific objectives include; structure-function analysis of the transcription factor AraR, the key-master regulator of arabinose utilization and uptake of galactose and xylose; genetic and biochemical characterization of extracellular degrading enzymes of arabinose containing polysaccharides, transport systems, and intracellular enzymes involved in further catabolism; elucidation of the mechanisms that underlie carbon catabolite repression of the arabinose regulon.

The main objective of the Control of Gene Expression Group (C. M. Arraiano) is to elucidate mechanisms that **control gene expression**. The focus is on post-transcriptional control of gene expression and **control of mRNA degradation,** a research topic of major current interest. *E. coli* is the primary model organism, but studies with *L. lactis* and eukaryotes (*Drosophila*) have also been performed. This team has recently shown that RNase R can be involved in the maturation of SsrA/tmRNA, an important small stable RNA involved in trans-translation (a protein quality control

system that involves protein tagging and ribosome rescue). Main specific objectives are; study of ribonucleases; RNA processing and RNA degradation; small RNAs; quality control; metabolism of the poly(A) tail in bacterial mRNAs; control of cell division in *E. coli* ; control of gene expression under stress and stationary phase.

The Biology Division includes one of the leading groups in the application of ***in vivo*** **NMR** techniques to study microbial metabolism (H. Santos). This methodology has been used to measure *on line* the intracellular pools of glycolytic intermediates in *Lactococcus lactis* strains. The aim is to provide reliable *in vivo* data to be used as guidelines for efficient **metabolic engineering** strategies in **dairy bacteria** that allow for the construction of strains with desired metabolic traits. The "applied goal" of these studies is to redirect carbon fluxes to the *in situ* production of metabolites with beneficial properties for human health (mannitol, trehalose) or the removal of undesired end-products (lactose, galactose). A more fundamental goal is to characterise central metabolism and regulatory networks in *L. lactis* taking advantage of global approaches.

Important information on the **physiology of hyperthermophilic microorganisms** has also been developed. In particular, five patents have been filed on the application of compatible solutes of hyperthermophiles (**hypersolutes**) for the preservation of bio-materials. The main objectives of this research line are; genetic and biochemical characterization of biosynthetic pathways of hypersolutes; identification of biochemical strategies for adaptation to hot environments; development of microbial cell systems for the production of hypersolutes; characterisation of the molecular basis for protein stabilisation by compatible solutes; identification and characterisation of novel compatible solutes; characterisation of transport systems for hypersolutes.

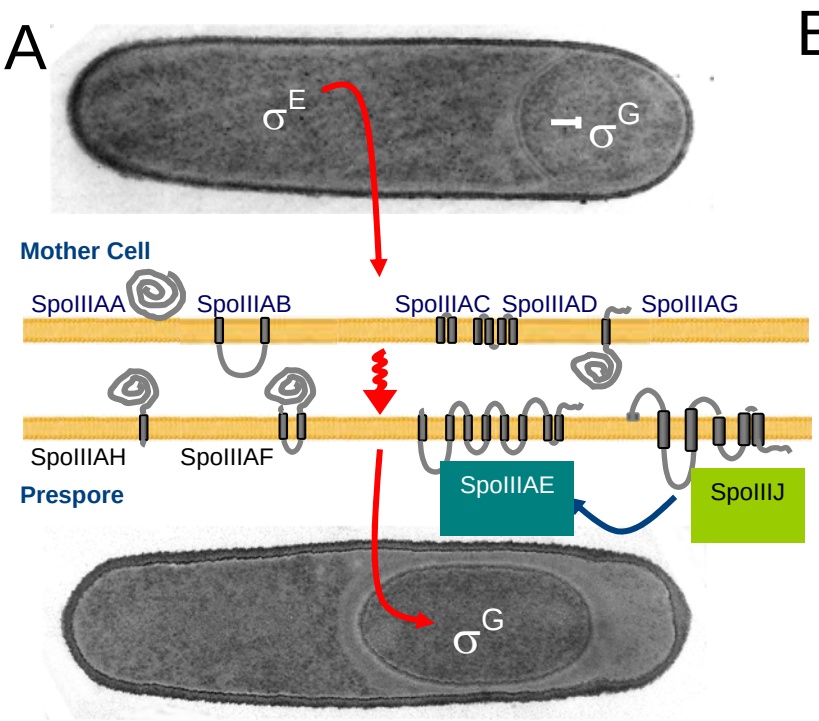
Protein transport and glycosylation in mammalian cells is the main topic of the Glycobiology Group (J. Costa). A stable expression system for insect cells has been used for the production of high amounts of a recombinant human glycosyltransferase, opening a good perspective for the production of recombinant glycoproteins of therapeutic interest and membrane proteins. In collaboration with the University of Pennsylvania, it has been found that the transmembrane domain and flanking regions of human nicastrin are important for mediating interactions with the gamma-secretase complex required for the production of Abeta, which is associated with Alzheimer's disease. Plans for future work include the study of, i) the role of nicastrin glycosylation for the targeting and activity of the gamma-secretase complex, ii) the proteome and glycome of amyotrophic lateral sclerosis and iii) glycosylation and neuronal differentiation.

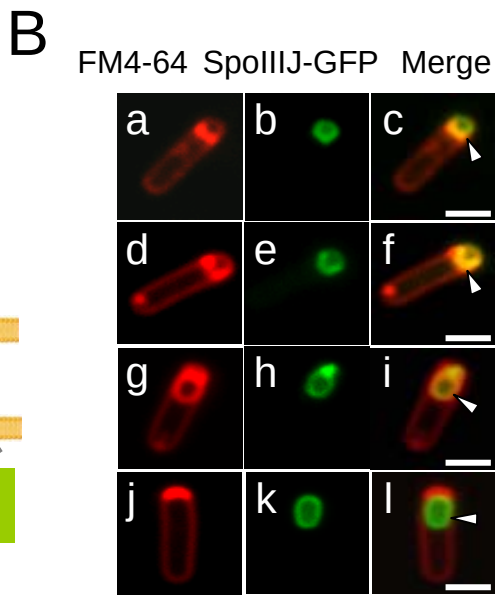
BIOLOGY DIVISION – Research Highlights

Activation of a transcription factor in response to cellular morphogenesis

Microbial Development Group ; Head of Group, **Adriano Henriques.**

Spore differentiation in *B. subtilis* starts with the asymmetric partitioning of the rod-shaped cell into a smaller prespore and a larger mother cell. Gene expression in the two cells is then governed by a cascade of compartment-specific RNA polymerase sigma (σ) subunits. Following asymmetric division, the two cells lie side by side (panel A, top), and in these cells the late prespore-specific regulator σ^G does not becomes active. Activation of σ^G is coupled to the complete engulfment of the prespore by the mother cell, a process akin to phagocytosis, whereby the septal membranes move towards the proximal cell pole, eventually fusing at the pole (panel A, bottom). It also requires expression of the *spoIIIA* operon, transcribed by σ^E in the mother cell, and which encodes eight membrane-associated proteins, and a membrane protein translocase coded for by the vegetative gene *spoIIIJ*. Synthesis of SpoIIIJ in the prespore is sufficient for the activation of σ^G (Serrano *et al*. 2003. *J. Bacteriol.*, 185:3905-3917). SpoIIIJ co-localizes with the prespore membranes, encircling the prespore at the time (engulfment completion) when σ^G becomes active (panel B). We found that production of SpoIIIAE, SpoIIIAF, SpoIIIAH in the prespore is also sufficient for σ^G activation (panel A, center). Moreover, we have found evidence that SpoIIIJ governs the insertion of SpoIIIAE into the prespore inner membrane. Thus, *spoIIIA* and *spoIIIJ* define a mother cell-to-prespore signaling pathway that promotes the activation of σ^G following engulfment completion. Previous work has suggested that σ^G is kept inactive prior to engulfment completion by the anti-sigma factor SpoIIAB, which prevents its interaction with core RNA polymerase. Our results challenge this view: *i*) in cells expressing a form of σ^G that is less efficiently bound by SpoIIAB, σ^G does not become active in the prespore (Serrano *et al*. 2004. *J. Bacteriol.*, *in press*); *ii*) in *spoIIIA* or *spoIIIJ* mutants, the fraction of σ^G found in association with core RNA polymerase does not differ from wild type cells. It follows that the *spoIIIA*/*spoIIIJ* pathway counteracts the activity of an as yet unidentified antagonist of σ^G-containing RNA polymerase.

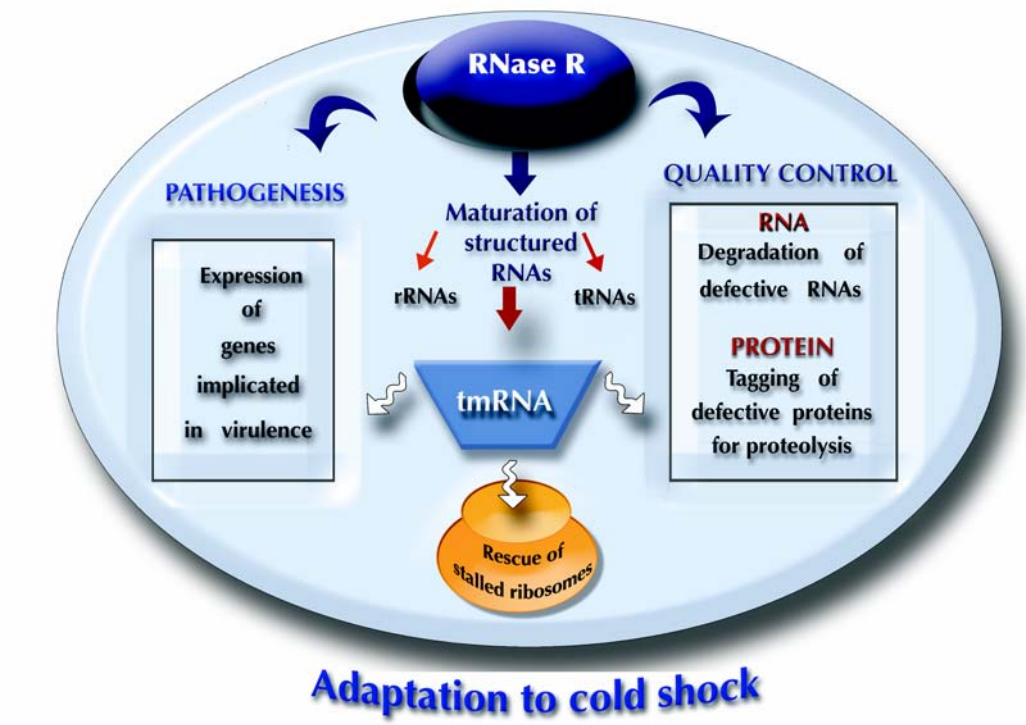




RNR family and RNA metabolism: RNA processing, small RNAs, and quality control.

Control of Gene Expression Group: Head of Group, **Cecília Maria Arraiano**

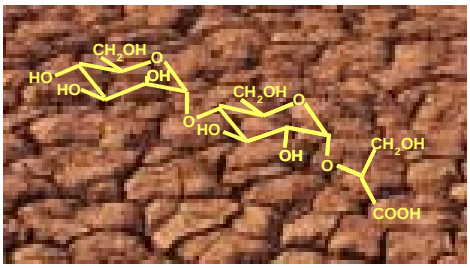
Ribonucleases (RNases) are essential elements in the regulation of post-transcriptional control of gene expression. *Escherichia coli* RNase II and RNase R are 3'-5' exoribonucleases that belong to the RNR family that has homologues widespread in most sequenced genomes. RNR-like enzymes are present in eukaryotic exosomes. The levels of RNase R can modulate virulence in *E. coli* and *Shigella* and RNase R has been shown to be a mediator of RNA quality control processes. RNase II is responsible for most of the hydrolytic activity in *E. coli.* We have constructed several RNase II mutants. We have demonstrated that the S1 domain is essential for the enzyme due to its involvement in binding. In addition, we have identified a key residue for activity that does not affect RNA binding. We have shown that RNase R can be involved in the maturation of SsrA/tmRNA, an important small stable RNA involved in trans-translation, a protein quality control system that involves protein tagging and ribosome rescue. Our results demonstrate that RNase R is a cold shock protein reaching a 7-8 fold induction after cold shock imposition and its expression is tightly regulated by temperature. RNase R was shown to contribute to growth at low temperatures, We have presented evidence that demonstrates the wide biological significance of RNase R regarding adaptation to cold shock and its involvement in RNA surveillance, protein quality control and pathogenesis.



Adaptation of extremophiles to high temperature: novel hot solutes and molecular mechanisms of protein stabilization

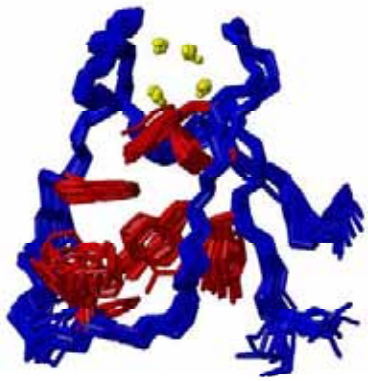
Cell Physiology & NMR Group : Head of Group**, Helena Santos**

A Novel Compatible Solute from Thermophiles

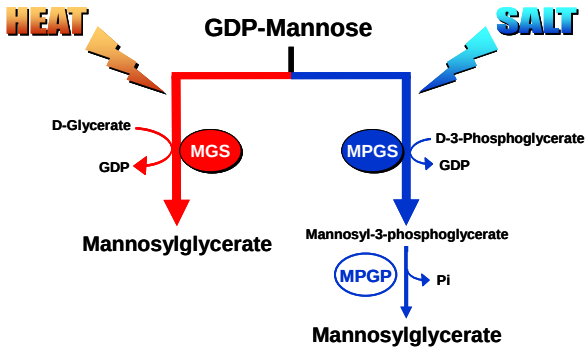


In 2003 we identified a novel compatible solute in the thermophilic bacterium *Petrotoga miotherma*, a bacterium able to grow at temperatures up to 65ºC, with salt concentrations ranging from 0.5 to 10% NaCl. The molecular structure of this new solute as determined by Nuclear Magnetic Resonance is α-mannopyranosyl α-glucopyranosyl glycerate. A European patent application covering the use of this solute in cosmetics as well as in the pharmaceutical industry has been filed via IBET. This solute is much more efficient than conventional osmolytes in the stabilization of enzymes against heating. To understand the molecular mechanisms leading to protein stabilization, a mutant of *D. gigas* rubredoxin with a deletion of the hairpin-loop region was constructed and the structure determined by NMR. Our results point towards the existence of at least two modes of action by which these solutes confer a higher stability to proteins; an overall increase in the rigidity of the native protein, more obvious at structured regions, and weak, preferential binding to specific sites. The reason for the pathway duality in the synthesis of mannosylglycerate in *Rhodothermus marinus* was elucidated. We found that the two-step and single-step pathways have specific roles in the adaptation of this thermophilic bacterium to osmotic stress and heat stress, respectively. This is the only example of pathway multiplicity being rationalized in terms of the need to respond efficiently to distinct environmental stresses.

NMR Structure of a Mutant of *D. gigas* Rubredoxin with a Deletion of the Loop Region



Regulation of Mannosylglycerate Synthesis in *R. marinus*



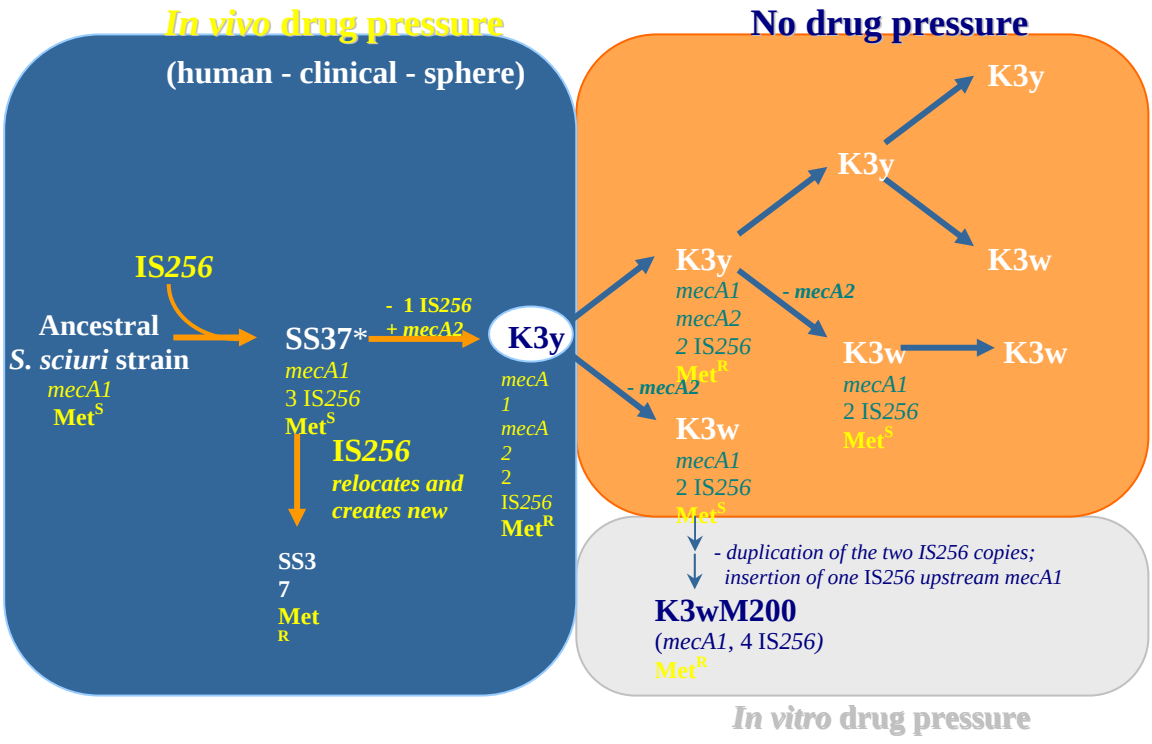
Development of methicillin resistance in clinical isolates of *Staphylococcus sciuri* by transcriptional activation of the *mecA* homologue native to the species.

Molecular Genetics Group : Head of Group, **Herminia de Lencastre**

Resistance to β-lactam antibiotics in staphylococci is conferred by PBP2A, a penicillin-binding protein with greatly reduced affinity to these antibiotics, encoded by the *mecA* gene. Studies on the origin of *mecA*, which is exogenous to *Staphylococcus aureus* and to other clinically relevant staphylococci, brought to light a genetic element closely related to it, carried by every strain of the animal commensal species *Staphylococcus sciuri*. However, despite the similarities between the *S. sciuri mecA* and the methicillin-resistant *S. aureus* (MRSA) *mecA*, the great majority of *S. sciuri* isolates showed no appreciable resistance to β-lactam antibiotics.

Characterization of the few *S. sciuri* methicillin-resistant strains, all isolated from humans, led us to identify three different mechanisms by which this bacterium can overcome the antibiotic action. The first two mechanisms are associated with increased transcription of the *S. sciuri* native *mecA* and production of a protein resembling PBP2A, either by single nucleotide alterations or by insertion of IS*256* in the promoter region of the gene. This is the first report of *S. sciuri* strains isolated *in vivo* carrying only a copy of the native *mecA* gene with the capacity to confer resistance to β-lactam antibiotics and shows that the *S. sciuri mecA* has the capacity to confer resistance to methicillin and other β-lactam antibiotics, provided that a more powerful promoter is acquired. These findings provide further suggestive evidence that the *S. sciuri mecA* homologue may have been the evolutionary precursor of the *mecA* determinant in methicillin-resistant *S. aureus*. The third mechanism corresponds to the acquisition of a second copy of *mecA*, of the MRSA-type. This second *mecA* was found to be unstable and is lost by some cells in the absence of drug selection.

Thus, the different *S. sciuri* strains characterized so far represent several stages in the development of resistance to β-lactams, ranging from drug-susceptible to fully resistant bacteria and illustrate the remarkable variety of strategies available to bacteria to acquire mechanisms of drug resistance in the *in vivo* environment. Further studies on the *mecA* elements carried by these strains may help us to understand the evolution and dissemination of this type of drug resistance among staphylococci.



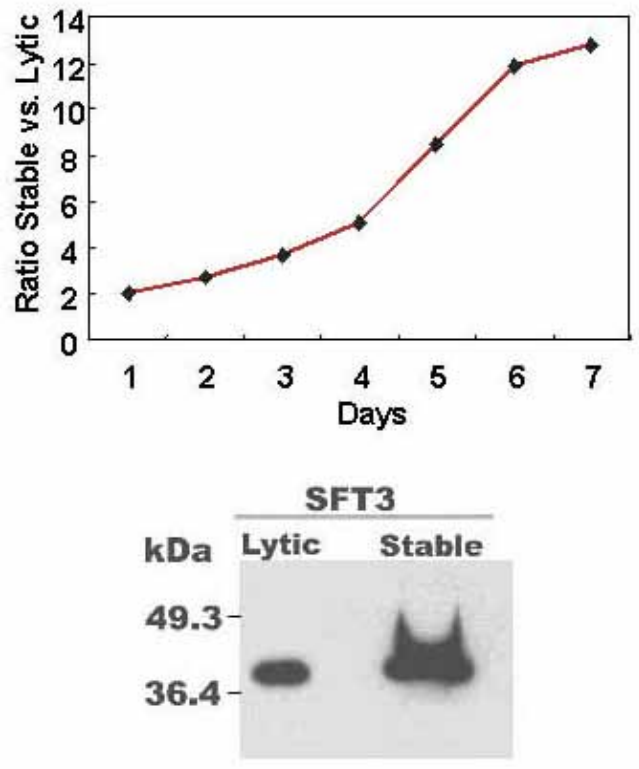
Putative evolutionary sequence of events for *S. sciuri* strains
(adaptated from I. Couto *et al*. 2003. J. Bacteriol., 185,

Recombinant Glycoproteins using Insect Cells.

Glycobiology Group ; Head of Group, **Julia Costa.**

The recombinant baculovirus insect cell system has been widely used for the expression of heterologous proteins available only at low concentrations from their original sources. This system has the advantage of producing high amounts of recombinant protein and performing the correct folding and post-translational modifications. However, for membrane proteins including Golgi glycosyltransferases or glycoproteins this system has shown serious limitations since there is a large accumulation of recombinant protein intracellularly. As an alternative to the lytic system, we have used a stable expression system in insect *Spodoptera frugiperda* Sf9 cells. We have stably expressed a soluble secretory form of fucosyltransferase III, using the expression vector pIB/V5-His-TOPO under the control of the OpIE2 promoter. We have found that this system produces approximately 13-fold more active enzyme than the baculovirus expression system, which might be due to a lower intracellular accumulation of the enzyme.

The stable system constitutes an advance for the expression of recombinant glycoproteins of therapeutic relevance and membrane proteins, such as receptors, in insect cells. Since it is a non-lytic system the proteins obtained will probably be more suitable for structure and function studies.

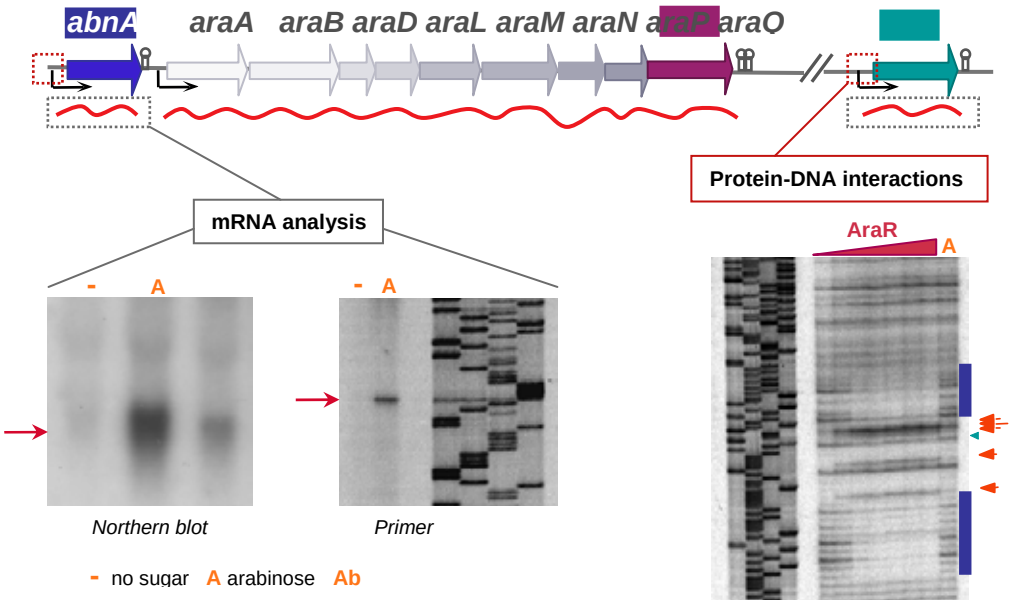


Comparison of the stable with the lytic baculovirus expression system for the production of recombinant soluble human fucosyltransferase III.

Transcriptional Regulation of Arabinan-Degrading Genes in *Bacillus subtilis*

Microbial Genetics Group : Head of Group, **Isabel de Sá-Nogueira**

Bacillus subtilis produces hemicellulases capable of releasing arabinosyl oligomers and arabinose from plant cell walls. Although many hemicellulases have been purified and characterized from both fungi and bacteria, including mesophilic and thermophilic *Bacillus sp.*, the knowledge concerning the regulation at the molecular level of hemicellulolytic genes is scarce. We characterized the transcriptional regulation of three arabinan-degrading genes that are clustered with genes encoding enzymes that further catabolise arabinose. The *abfA* gene comprised in the metabolic operon *araABDLMNPQ-abfA*, and the *xsa* gene located 23-kb downstream most probably encode α-l-arabinofuranosidases (EC 3.2.1.55). We have shown that the *abnA* gene, positioned immediately upstream from the metabolic operon, encodes an endo-α-1,5-arabinanase (EC 3.2.1.99). Furthermore, by *in vivo* RNA studies we inferred that *abnA* and *xsa* are monocistronic, and transcribed from σ^A-like promoters. Transcriptional fusion analysis revealed that the expression of the three arabinases is induced by arabinose and arabinan, and repressed by glucose. The induction by arabinose and arabinan is higher during early post-exponential growth, suggesting a temporal regulation. Moreover, the induction mechanism of these genes is mediated through negative control by the key regulator of arabinose metabolism, AraR. Thus, we analyzed AraR-DNA interactions by *in vitro* quantitative DNase I footprinting and *in vivo* analysis of single base pair substitutions within the promoter regions of *xsa* and *abnA*. The results indicate that transcriptional repression of the *abfA*, and *xsa* genes is achieved by a tightly controlled mechanism, while regulation of *abnA* is more flexible. We suggest that expression of genes encoding extracellular-degrading enzymes of arabinose-containing polysaccharides, transport systems, and intracellular enzymes involved in further catabolism is regulated by a coordinate mechanism triggered by arabinose via AraR.



Raposo, M. P., Inácio, J. M., Mota, L. J., and I. Sá-Nogueira. J. Bacteriol., *in*

BIOLOGICAL CHEMISTRY DIVISION – Laboratories and Staff

Laboratory: Structure and Function of Metalloproteins (Metalloproteins (Bioenergetics; Molecular Genetics; NMR; Sulfate Reducing Bacteria)
Head of Laboratory: António V. Xavier
Research Team:

António Bernardo Pinho de Aguiar	Ph.D..
Teresa Catarino	Ph.D.
Carlos Salgueiro	Ph.D.
Miguel Pessanha	Ph.D. Student
Vitor Manuel	Ph.D. Student
Catarina Paquete	Ph.D. Student
Patricia Pereira	Ph.D. Student
Rui Neves	Research Student
Rita Ribeiro	Research Student
Isabel Pacheco	Technician

Laboratory : Genomics and Stress (Gene Expression and Stress)
Head of Laboratory: Claudina Rodrigues-Pousada
Research team:

Isabel Solange Oliveira	Ph.D/Collaborator
Paulo Guerreiro	Ph.D./Collaborator
Regina Menezes	Post-doctoral student
Dulce Alexandra Azevedo	Ph.D.Student
Maria Manuela Parreira Broco	Ph.D.Student
Tracy Nevitt	Ph.D.Student
Rute Rodrigues	Ph.D Student
Catarina Oliveira**	Ph.D Student
Jorge Pereira	Research Student
Catarina Amaral	Research Student
Patricia Machado***	Undergraduate student
Rute Felix***	Undergraduate student

**Ph.D. in co-supervision with Prof. Carlos Faro (UC)
***Students collaborating in projects under progress in order to write their reports to obtain their graduation in Biochemistry/Biology

Laboratory: Protein Modelling (Molecular Mechanisms / Redox proteins)
Head of Laboratory: Cláudio M. Soares
Research Team:

Paulo J. Martel	Ph.D.
Carlos A. Cunha	Post-Doc
Vitor H. Teixeira	Ph.D. student
Bruno L. Victor	Ph.D. student
Nuno M. Micaelo	Ph.D. student.
Ana Sofia Oliveira	Research student

Laboratory: Microbial Biochemistry (Sulfate Reducing Bacteria / Anaerobic respiration)
Head of Laboratory: Inês A. Cardoso Pereira
Research Team:

Ana Filipa Valente	Ph.D. Student
Claudia Lomba Fernandes	Research Student
Sofia Venceslau	Undergraduate

Laboratory: Molecular Genetics of Metalloproteins
Head of Laboratory: Lígia Saraiva Teixeira
Research Team:

Cláudia Almeida - BIC student	Research Student
Marta C. Justino	Ph.D. Student
Susana Lobo	Undergraduate

Laboratory: Microbial and Enzyme Technology
Head of Laboratory: Lígia Oliveira Martins
Research Team: (New group)

Laboratory: Protein Crystallography (Metalloproteins/ Proteins with biomedical applications)
Head of Laboratory: Maria Arménia Carrondo
Research Team:

Pedro Matias	Ph.D.
Carlos Frazão	Ph.D.
Francisco Enguita*	Ph.D.
Peter Crowley	Post Doc
Ana Maria Gonçalves	Post-Doc
Isabel Bento	Post-Doc
David Aragão	Ph.D. student
David Marçal	Ph.D. student
Luísa Rodrigues	Ph.D. student
Diana Plácido	Ph.D. student
Tim Urich**	Ph.D. Student
Ricardo Coelho	Technician
Ana Rêgo	Research student
Rui Brito	Research student
Gonçalo Gato Lopes	Undergraduate

* SPINE Research Assistant (Structural Proteomics in Europe)
** EDS-SB Marie Curie Ph.D. student (in collaboration with Univ. Darmstadt)

Laboratory: Metalloproteins and Bioenergetics (Membrane- and metallo- proteins / aerobic respiration/ oxygen detoxification/ iron metabolism/ EPR)
Head of Laboratory: Miguel Teixeira
Research Team (October 2002)

Ana Margarida Carvalho Melo	Post-Doc
Manuela Alexandra Marques Pereira	Post-Doc
Andreia Isabel Ferreira Veríssimo	Ph.D. student
Andreia de Sousa Fernandes	Ph.D. student
João Filipe Bogalho Vicente	Ph.D. student
João Vieira Rodrigues	Ph.D. student
Tiago Bandeiras	Ph.D. student
Maria Filipa de Lima de Sousa	Research Student

BIOLOGICAL CHEMISTRY DIVISION – Plan and Objectives

The Biological Chemistry Division brings together a group of researchers with different expertises (Biochemists, Molecular Biologists, Microbiologists, X-ray Crystallographers, Theoreticians, Spectroscopists, and Kineticists) providing the most relevant techniques and methodologies necessary for the characterization of proteins. Much of the work is focused on the understanding of specific biological processes (carried out by both meso and thermophilic bacteria and archaea), such as: i) sulfate respiration; ii) nitrate respiration; iii) oxygen respiration, activation, and detoxification; iv) nitric oxide detoxification; and more recently, v) respiration of metal ions. Soluble and membrane-bound redox metalloproteins (enzymes), particularly those containing haem and non-haem iron, and/or other redox metal ions (e.g. Cu, Mo, and Ni) play crucial roles in these processes.

Metal ions are involved in some of the most fundamental chemical transformations of life. Metallo-proteins and in particular metallo-enzymes, are essential for many processes that have significance in health (signalling molecules and transport of metals and oxygen), agriculture (nitrogen fixation and denitrification), the environment (processes linking the bio- and geocycles), and the economy (biocorrosion). Of particular importance in this respect are reactions involving the activation of small molecules, which cannot be achieved, under mild conditions, in the chemical laboratory. Elucidation and modelling of these processes will have an enormous impact for the rational design of new catalysts able to carry out the same kind of chemistry that is performed so efficiently in Nature.

The global research programme is an integrated effort comprising the following tasks; cell growth, protein purification and characterisation (biochemical, structural, physicochemical and molecular genetics), with the aim to: i) determine the structure, function and mechanism of action of metal centres in proteins and enzymes; ii) understand the mechanisms involved in metalloenzymes biosynthesis, including the pathways of the incorporation of metal clusters into apoproteins; iii) study of gene expression and regulation; iv) develop the physical methods to investigate the molecular, electronic and magnetic structure of metal centres; and v) perform specific alteration of functional properties by engineering wild type metalloproteins.

Thus, the approach to study (metallo)proteins is strengthened by putting together information gathered by a wide range of techniques and methodologies (taking advantage of X-ray crystallography, Resonance Raman, NMR, EPR, fast kinetics,

molecular genetics, mass spectrometry, and a fermentation plant). High priority is given to the continuous development of specifically designed experimental and theoretical modelling methodologies.

Great effort has been put into the characterization of redox proteins (enzymes), including those involved in electroprotonic energy transduction steps leading to oxidative phosphorylation. In particular, modelling redox and (de)protonation processes in multihaem cytochromes has been one of the most important developments. Together with structural (X-ray and/or NMR) and detailed thermodynamic data for wild type and specific mutants, these studies are used to help establishing the molecular basis of cooperativity phenomena, both of soluble as well as transmembrane proteins.

The molecular genetics work includes the cloning, sequencing, transcriptional analysis of bacterial operons, and gene regulation, in particular of genes encoding for relevant metalloproteins (soluble and membrane-bound) from the *Desulfovibrio* sp., *E. coli*, *Rhodo- thermus marinus*, and thermophilic archaea such as *Acidianus ambivalens*, and *Archaeoglobus fulgidus*. It also includes the development of expression systems for overproduction of these metalloproteins (wild-type, truncated, specifically mutated, as well as isotopically enriched, whenever necessary). These studies are the result of an interdisciplinary approach, recently enlarged by new groups, which benefits from the collaboration of several groups of other Divisions of ITQB and international collaborations.

This methodology will be continued, together with the sequencing project of *D. gigas* genome, so that new (metallo)proteins will be characterized. Meanwhile, gene deletion has already been achieved for this bacterium. In conjunction with the structural and functional studies, this knowledge may be used to guide the design of proteins with new desired functions, and will be extended to pathogenic bacteria.

Some specific achievements in 2003 include:

- Considerable effort was dedicated to the study of transmembrane and membrane bound electron/proton transfer complexes. A novel respiratory complex from *Desulfovibrio* sp. was isolated and characterized, and novel mechanisms for proton transfer and electroprotonic energy transduction were proposed. Considerable success was also accomplished in the characterization of the molecular basis of electroprotonic energy transduction mechanisms performed by soluble and membrane-linked multihaem proteins, using X-ray crystallography, NMR, and modelling studies (including interactions with partner proteins).

- A novel pathogenic mechanism linked to the degradation of nitric oxide by flavorubredoxin proteins (bifunctional proteins that can work as NO and O_2 reductases, amply characterized at ITQB) was proposed. Further molecular genetic characterizations were achieved and theoretical studies for docking with partner proteins were performed.
- Novel methodologies were developed to study protein structure and dynamics in non-aqueous solvents.
- A membrane-linked hydrogenase was isolated from *Desulfovibrio gigas*, providing the missing link to consider the hydrogen cycle as a general bioenergetic mechanism when linked to sulfate respiration.
- Full characterization of an enzyme present in the spores of *Bacillus subtilis*: This is a multicopper oxidase involved in the biosynthesis of a pigment that protects the spore against UV radiation, for which biotechnology exploitation is now being thought.

BIOLOGICAL CHEMISTRY DIVISION – Research Highlights

Multihaem proteins from the anaerobic respiration of *Shewanella frigidimarina*

Structure and Function of Metalloproteins Group : Head of Group, António V. Xavier

Under anaerobic growth conditions, the facultative aerobe *Shewanella frigidimarina* produces a small *c*-type tetrahaem cytochrome. This protein is involved in the **respiration of iron** (Fig. 1 and 2) and shares 42% sequence identity with the tetrahaemic N-terminal domain of a soluble flavocytochrome (Figs. 2 and 3 Fcc_3) with fumarate reductase activity.

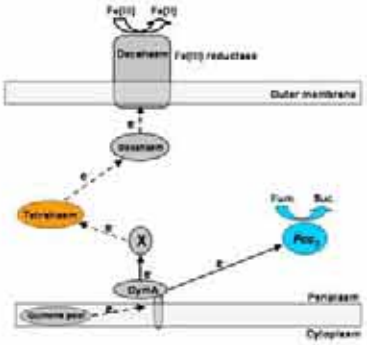


Fig. 1 - Electron transfer pathway of fumarate and Fe(III) reduction

The thermodynamic properties of the redox centres and of one ionizable centre in the tetrahaem cytochrome were determined using NMR and visible spectroscopy techniques. This study revealed unique features. The redox centres have negative and different redox- and positive redox-Bohr interactions [2], in particular involving haem III (Fig. 3 and 4).

Fig. 2 - Preliminar NMR solution structure of the reduced tetrahaem cytochrome c [1]

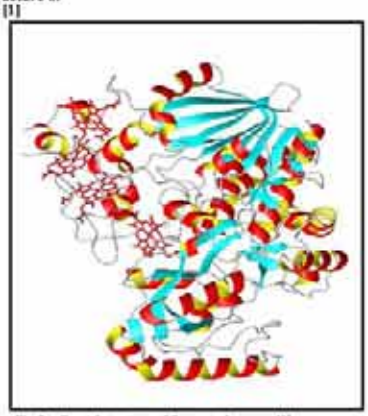


Fig. 3 - Crystal structure of flavocytochrome c_3 [3].

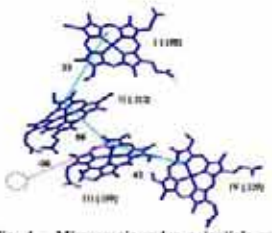


Fig. 4 – Microscopic redox potentials and redox interactions for tetrahaem cytochrome. Values are given in meV and the larger redox-Bohr interaction (between haem III and the ionisable centre) is also indicated.

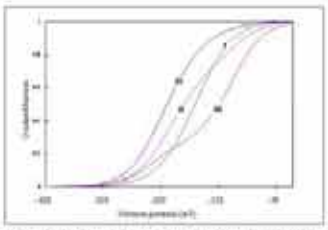


Fig. 5 - Oxidized fractions of the individual haems of the tetrahaem cytochrome at pH 7.0 [2].

NMR spectroscopy was tested for its potential to elucidate the oxidation profile of each of the four haem groups in Fcc_3, using the strategy developed previously to perform the thermodynamic characterization of small tetrahaem cytochromes (Figs. 6-8). Despite the large size of the protein (63.8 kDa), NOESY experiments can now be used to obtain the network of chemical exchange connectivities, between sequential oxidation stages [4].

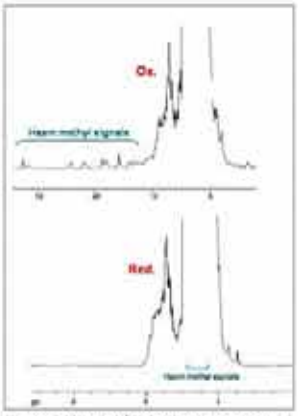


Fig. 6 – 500 MHz 1D ^{1}H NMR spectra obtained for Fcc_3 in the reduced (Red.) and oxidised (Ox.) forms at pH 7.0 and T=298K

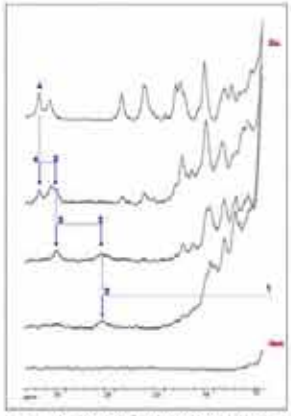


Fig. 7 – 500 MHz 1D ^{1}H NMR redox titration of Fcc_3 at T=298 K, in different redox stages from the fully reduced (bottom) to the fully oxidised (top).

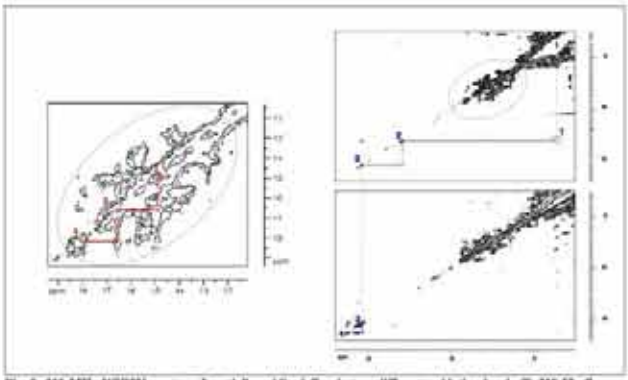


Fig. 8 500 MHz NOESY spectra of partially oxidised Fcc_3 in two different oxidation levels (T=298 K). Cross peaks resulting from slow intermolecular electronic exchange in the NMR time scale, are indicated for one methyl group from each haem in the four stages of oxidation (1, 2, 3 and 4).

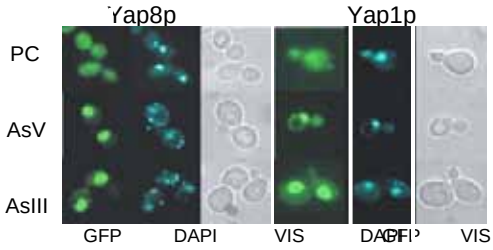
References
[1] Pessanha, M., Brennan, L., Xavier, A. V., Cuthbertson, P. M., Reid, G. A., Chapman, S. K., Turner, D. L., Salgueiro, C. A. (2001), *FEBS Lett.*, 489, 9-13.
[2] Pessanha, M., Louro, R. O., Correia, I. J., Rothery, E. L., Pankhurst, K. L., Reid, G.A., Chapman, S. K., Turner, D. L., Salgueiro, C. A. (2003), *Biochem. J.*, 370, 489.
[3] Taylor, P., Pealing S. L., Reid, G. A., Chapman, S. K., Walkinshaw, M. D. (1999), *Nature Struct. Biol.*, 6, 1108.
[4] Pessanha, M., Turner, D.L., Rothery, E.L., Pankhurst, K.L., Reid, G.A., Chapman, S.K., Xavier, A.V., Salgueiro, C.A. (2003) *Inorg. Chim. Acta* 356, 379-381.

Gene expression of *Saccharomyces cerevisiae* under stress conditions and the Genetics of *Desulfovibrio gigas*

Genomics and Stress Group : Head of Group, **Claudina R. Pousada**

1. Gene expression under stress conditions

The budding yeast *Saccharomyces cerevisiae* is able to respond to different situations imposed by the environmental stress by triggering different mechanisms. Yap4p, a transcription factor belonging to eight transactivators of the bZIP family, is highly induced upon osmotic stress and having been located under the HOG pathway. In order to understand its role under these conditions, several gene targets were identified using micro-array analysis. Several down-regulated genes encode proteins involved in glycerol and sugar metabolism indicating their potential role in osmotic stress. Other genes do not have any function assigned and thus they are being analysed for their function under these circumstances.
Yap8p, another member of the family above described, is activated under arsenic. For the first time it was shown that this activation takes place at the level of the nuclear translocation (see below) and at the level of its transactivation domain. The exportin Crm1 is responsible for the regulated Yap8p transport and the Cys^{132}, Cys^{137} and Cys^{274} are essential for both Yap8p nuclear distribution and transactivation function.



Yap8 and Yap1 nuclear localization under arsenic

2. Genetics of *Desulfovibrio gigas*

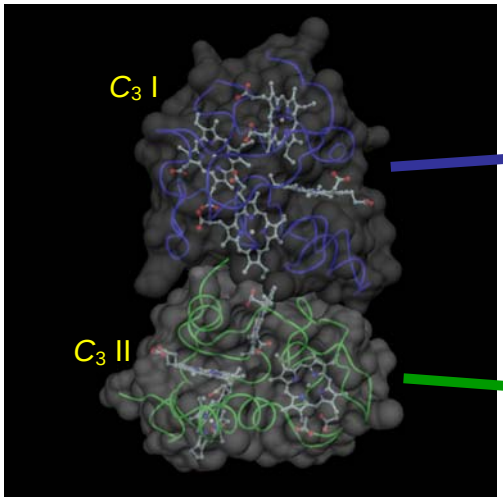
Contrary to other *Desulfovibrio* species, *D. gigas* was thought to contain only a periplasmic [NiFe] hydrogenase and therefore, it was proposed that in this bacterium a bioenergetic mechanism different from the hydrogen cycling hypothesis could be operating. Our study revealed the presence of a multi-subunit membrane-bound [NiFe] hydrogenase belonging to the Ech hydrogenase family in *D. gigas*. Sequence analysis of the predicted subunits suggests that this hydrogenase is bound to the cytoplasmic side of the membrane, in a situation ideal to constitute the missing link in the hydrogen cycling mechanism that is responsible for hydrogen formation in the cytoplasm.
We identified an operon with six coding regions for the multi-subunit membrane-bound [NiFe] hydrogenase in the genome of *Desulfovibrio gigas*. Sequence analysis of the deduced polypeptides reveals a high similarity to subunits of proteins belonging to the family of Ech hydrogenases. The operon is organised similarly to the operon coding for the Ech hydrogenase from *Methanosarcina barkeri*, suggesting that both encode very similar hydrogenases. Expression of the operon was detected by Northern blot and RT-PCR analyses, and the presence of the encoded proteins was examined by Western blotting. The possible role of this hydrogenase relating it with a potential function in the H_2 cycling as a mechanism for energy conservation in *D. gigas* was proposed. The present study provides therefore valuable insights into the open question of the energy conserving mechanism in *D. gigas.*

Modelling electron transfer thermodynamics in protein complexes: Interaction between two cytochromes c_3

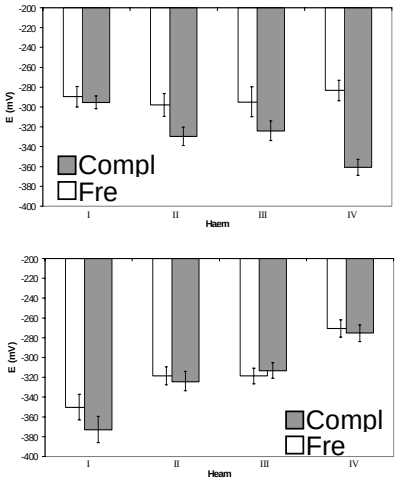
Protein Modelling Group : Head of Group, **Claudio Soares**

Electron transfer chains form the basis of metabolism in all branches of life. In many cases electron flow is controlled by redox proteins, which need to be in contact so that electrons are transferred between their redox centres. Despite being so important, protein electron transfer is poorly understood at the molecular level, mostly because redox protein interactions are elusive to experiments and hard to predict using modelling methods.

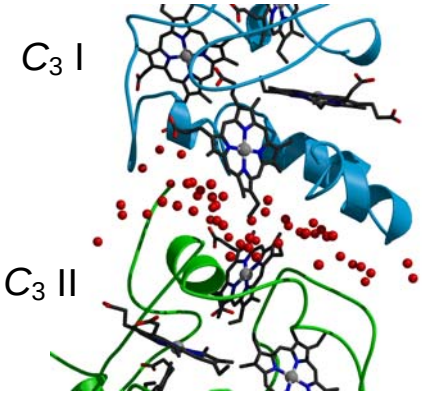
We have studied, using molecular simulation methods, electron transfer between two proteins that are known to interact in a redox chain in the bacterium *Desulfovibrio vulgaris* Hildenborough, the type I and type II tetrahaem cytochromes c_3. Various complexes were generated using rigid body docking techniques, and the two lowest energy models were relaxed using molecular dynamics simulations with explicit solvent. Poisson-Boltzmann and surface accessibility methods (MM/PBSA) allowed us to select the most probable one, which corresponds to an interaction between haem IV from the type I and haem I from the type II cytochrome c_3 (see figure). Thermodynamic calculations on this complex showed that complex formation induces changes in the reduction potential of both cytochromes c_3, but the changes are larger in the type I cytochrome c_3 (the largest one occurring on haem IV, of about 80 mV). These changes are sufficient to invert the global titration curves of both cytochromes, generating directionality in electron transfer from the type I to the type II cytochrome c_3 (which is what happens experimentally), a phenomenon of obvious thermodynamic origin and consequences, but also with kinetic implications. The existence of processes such as this occurring at complex formation may constitute a natural design of efficient redox chains.



A schematic representation of the complex between the two cytochromes



Redox potential of the heams before and after complex formation

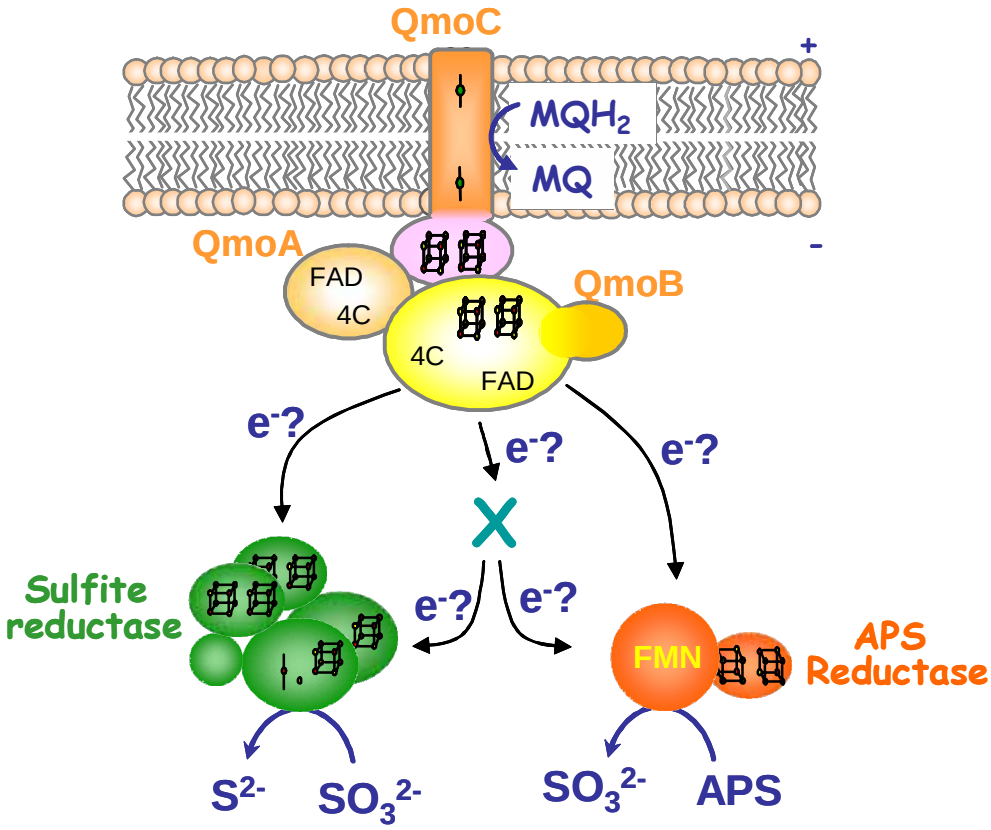


Water molecules (red spheres) in the interface between the two cytochromes

A novel membrane-bound respiratory complex from *Desulfovibrio desulfuricans* ATCC27774

Microbial Biochemistry Group : Head of Laboratory, **Inês Cardoso Pereira**

A membrane-bound redox complex from the sulfate-reducing bacterium *D. desulfuricans* ATCC27774 was isolated and characterized. This complex was named as Qmo for Quinone-interacting membrane-bound oxidoreductase complex, and is the first example of a new family of respiratory complexes found in the genomes of other anaerobic organisms such as *Archaeoglobus fulgidus* and *Chlorobium tepidum*. Qmo is formed by three subunits, and contains two hemes *b*, two FAD groups and several iron-sulfur centers. Cloning of the genes coding for the Qmo subunits revealed that they form a putative transcription unit and all have homology to subunits of heterodisulfide reductases. However, the membrane-bound subunit QmoC is unique in that it is the first example of a heme *b* subunit that also contains a hydrophilic domain with FeS centers. Both hemes *b* are reduced by menadiol, a menaquinone analogue, indicating a function for this complex in the respiratory electron-transport chain. This function is likely to involve electron transfer from the membrane menaquinone pool to the enzymes responsible for sulfate reduction in the cytoplasm (APS reductase and sulfite reductase).

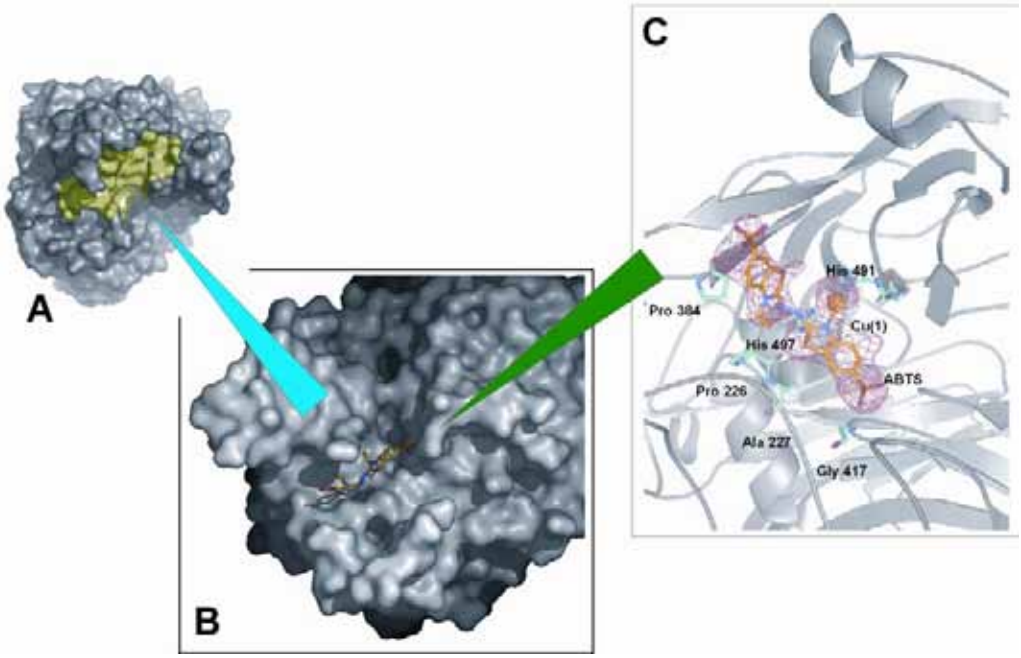


Pires RH,Lourenço AI, Morais, F, Teixeira M, Xavier AV, Saraiva LM, and Pereira I.A.C. *Biochim. Biophys. Acta/Bioenergetics* (2003) 1605, 1-3, 67-82

Structural Characterization of an Enzyme-Substrate Interaction. A Bacterial Case.

Macromolecular Crystallography Group : Head of Group, **Maria Armenia Carrondo**

Enzymes are proteins that can help the cells to produce energy, synthesize structures and maintain their basic functions. However some of these enzymes, due to their intrinsic characteristics or their catalytic properties, are potentially useful in our daily life. In particular, enzymes from microbial origin have become a useful tool for a wide range of industries. For example fungal lipases and proteases are used as cleaning adducts in washing powders and amylases are widely employed in animal foods.
The microbial enzyme described here is present in the spores of a bacterial cell, *Bacillus subtilis*. Bacterial spores are resistance forms that allow cells to survive in aggressive environments. Our case study, the CotA enzyme, is a “laccase”, an oxidase involved in the biosynthesis of a brownish pigment that protects the spore against UV radiation.
In our first crystallographic studies we were able to determine the structure of the CotA protein in its native form. Further studies allowed the determination of the structure of CotA enzyme complexed with a substrate (ABTS), which was strongly bound in the active centre of the enzyme and also to determine the specific atoms of the CotA enzyme interacting with the substrate.
Further studies are aimed at understanding how the enzyme acts as a biocatalyst so that it may be successfully applied in biotechnological processes.



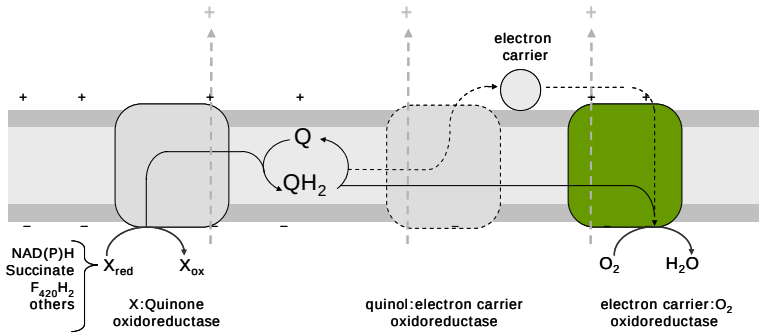
Panel A*:* Surface of the native CotA structure as determined by X-ray crystallography, showing the potential area for substrate binding (shadowed in light green). **Panel B:** structure of the CotA-substrate complex showing the position of the substrate molecule within the previously characterized substrate binding pocket. **Panel C:** details of the interactions of the enzyme with the substrate at the atomic level, showing the enzyme atoms involved in the substrate binding and recognition.

Publications:
1. F. J. Enguita, L. M. Martins, A. O. Henriques and M. A. Carrondo, (2003) *J. Biol. Chem.* **278**, 19416-19425.
2. F. J. Enguita, D. Marçal, L. O. Martins, R. Grenha, A. O. Henriques, P. F. Lindley and M. A. Carrondo *J. Biol. Chem.,* in press.

Oxygen Reductases

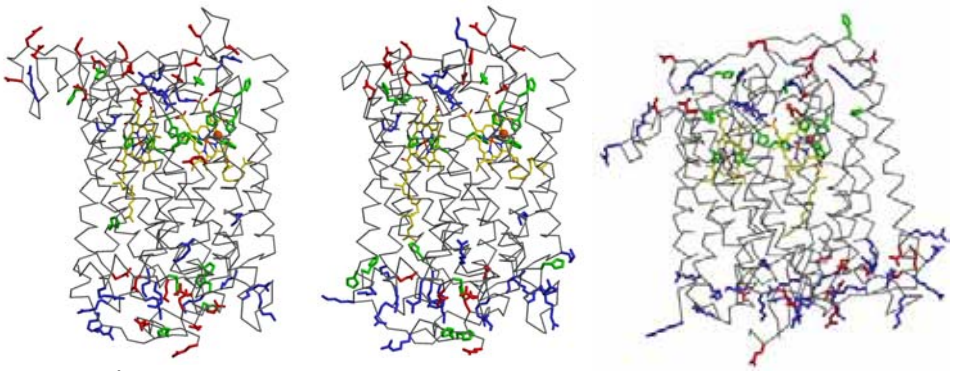
Metalloproteins and Bioenergetics Group : Head of Group, **Miguel Teixeira**

Oxygen reductases are the final enzymes in the aerobic respiratory chains catalysing the reduction of dioxygen to water. This reaction is associated with charge separation, both due to the chemical reaction and to the pumping of protons across the mitochondrial (in eukaryotes) or periplasmic (in prokaryotes) membranes, contributing to the establishment of the membrane potential, which allows the synthesis of ATP.



Schematic representation of an aerobic respiratory chain. Oxygen reductases are outlined in green.

Oxygen reductases are able to use cytochromes, quinols, High Potential Iron-sulfur Proteins (HiPIPs) and probably blue-copper proteins as electron donors. Most of these enzymes belong to the family of haem-copper oxygen reductases, characterised a subunit I with a haem-copper binuclear centre and a low-spin haem. Intra-protein proton conducting pathways are needed for the chemical reaction and for the translocated protons. Based on sequence and structural analyses, and site-directed mutagenesis, two proton channels were established for the mitochondrial-like oxygen reductases. However, the amino acid residues forming these channels are not conserved among the family members. Most importantly, many oxygen reductases do not contain ionisable amino acid residues in the putative proton pathways neither in alternative positions. The diversity of channels in haem-copper oxygen reductases exemplifies the plasticity of proton pathways that occurred throughout evolution, and strongly suggests a substantial role for water as the main proton carrier.



P. denitrificans aa_3 oxidase | *R. marinus* caa_3 oxidase | *T. thermophilus* ba_3 oxidase

The structure of *P. denitrificans aa*3 and *T. thermophilus ba*3 oxygen reductases (left and right, respectively) and the 3D model for *R. marinus caa*3 oxygen reductase (middle). The side chains represented are from amino acid residues predicted to be titrating at physiological pH. Tyr256 from *R. marinus caa*3 oxygen reductase, which is not titrating at physiological values of pH, is indicated in green for comparison. It can be observed that not all oxygen reductases have protonable amino acid residues between the membrane surface facing the cytoplasm and the catalytic centre. Thus, ionizable residues are not required for proton transfer, which has to rely on water channels

TECHNOLOGY DIVISION – Laboratories and Staff

Laboratory: Biosensors (Optical sensors / immuno-sensors)
Head of Laboratory: Abel Oliva
Research Team:

Helder Cruz	Ph.D.
Joana Miranda	Ph.D. student
Marta Gomes	Research Student
Óscar Silvestre	undergraduate
Elisabete Nascimento	undergraduate
José Vicente	undergraduate
Cristina Raposo	undergraduate

Laboratory: Biomathematics
Head of Laboratory: Jonas S. Almeida
Research Team:

João Carriço	Ph.D. student
Sara Garcia	Ph.D. student
António Maretzek	informatics consultant
Lukas Müller	research student
Rodrigo Oliveira	research student
Francisco Pinto	Ph.D. student
Sara Silva	research student
Susana Vinga	Ph.D. student

Laboratory: Microbiology of Man-Made Environments (Ecology / Microbiota)
Head of Laboratory: Cidália Peres
Research Team:

José J. Figueiredo Marques	Ph.D.
Maria Dulce Carneiro de Brito	Ph.D.
Amélia Maria Muralha Delgado	Research Student
Maria Cristina Jorge Leitão	Technician

Laboratory: Molecular Thermodynamics (Isotope Effects/ Polymer Solutions/ Metastable Liquids/ Sound Propagation in Dense Phases/ Ionic Liquids)
Head of Laboratory: Luís Paulo N. Rebelo
Research Team

Jerszy Szydlowski	Invited Professor (4 months)
Joanna Lachwa	Post-Doc (since Nov. 2003)
Isabel Mota Veiga	Ph.D. Student
Ricardo Gomes de Azevedo	Ph.D. Student
Zoran Visak	Ph.D. Student
Vesna Najdanovic-Visak	Ph.D. Student

Laboratory: Animal Cell Biotechnology (Biologicals for therapies and diagnosis / cell and gene therapy)

Heads of Laboratory: Manuel J.T. Carrondo and Paula M. Alves
Research Team:

Pedro Estilita Pereira Monteiro Cruz	Ph.D.
Helena Vieira	Post-Doc
Changhe Zhang	Post-Doc
Ana Sofia Coroadinha	Ph.D. Student
Cláudia Istrate	Ph.D. Student
Isabel Eloi Marcelino	Ph.D. Student
Marlene Isabel Rosa do Carmo	Ph.D. Student
Sónia Adelaide Queirós de Sá Santos	Ph.D. Student
Teresa Rodrigues	Ph.D. Student
Tiago Bruno Pereira Soares Ferreira	Ph.D. Student
Luis Maria Lopes da Fonseca	Ph.D. Student (co-supervision with Prof H. Santos)
Cristina Peixoto Lisboa	Technician
Marcos Filipe Quintino de Sousa	Technician
Maria do Rosário Clemente	Technician
Filipa Rosa	Research Student
Ana Carina Ferreira da Silva	Research Student
Célia Casado Veríssimo	Research Student
Joana Ribeiro	Research Student
Miguel Monteiro	Research Student
Hélio João dos Santos Crespo	Undergraduate Student
Ana Lúcia Ferreira	Undergraduate Student

Laboratory: Processes with Supercritical Fluids (Supercritical CO_2 / extraction and reaction)
Head of Laboratory: Manuel Nunes da Ponte
Research Team:

Catarina Duarte	Ph.D.
Anna Bannet	Post-Doc
Joana Fonseca	Post-Doc
Rui Ruivo	Post-Doc
Vesna Najdanovic-Visak	Ph.D. Student
Ana Serbanovic	Ph.D. Student
Ana Rita Duarte	Ph.D. Student
Ana Raquel Sousa	Ph.D. Student
Ana Alexandra Matias	Lab. Technician
Ana Morgado Nunes	Lab. Technician

Laboratory: Microbiology of Man-made Environments (Food & Environment)
Head of Laboratory: Teresa Crespo
Research Team:

Helena Isabel dos Santos	Ph.D. student
Ana Teresa Pires	Research student
Luís Raposo	Research student
Catarina Rodrigues	Research student
Susana Tenedório	Technician

GLP Section:

Cristina Isabel Pereira	Graduate technician/Ph. D. student
Fernanda Spínola Rodrigues	Graduate technician
Cátia Morgado Peres	Graduate technician
Paula Lopes Alves	Graduate technician

Laboratory: Microbiology of Man-Made Environments (Stress/wine quality)
Head of Laboratory: Maria Vitória San Romão (Stress, Wine quality)
Research Team

Giselle Soares	Pos-Doc (IBET/Amorim & Irmão)
Sónia Vitorino	Ph.D. student
Ana Paula Marques	Research student (IBET/Agro))
Fréderique Gaspar	Research student (IBET/FCT)
Maria do Carmo Basílio	Research student (IBET/Amorim & Irmão)
Vera Basto	Undergraduate
Ricardo Gaspar	Undergraduate
Maria Cristina Jorge Leitão	Technician

TECHNOLOGY DIVISION – Plan and Objectives

The Technology Division encompasses Engineering Sciences related to chemical and biochemical systems as well as some components in Microbial and Enzyme Technologies related to food, pharmaceuticals, fine chemicals, and the environment. The Division is one of the mainstays of the private-not-for-profit Institute, IBET. Within the functions contracted under the Laboratório Associado, the Technology Division has contributions in three of the five areas: Biologically Active Molecules, Human and Animal Health, Biological Risk Assessment.

At the **Animal Cell Biotechnology** Laboratory, Manuel Carrondo and Paula M Alves have conducted research and development for biopharmaceuticals (*rec* proteins, vaccines, and gene therapy biovectors) with two key themes; process integration (up – and downstream) and kinetics of infection (adenovirus and retrovirus in mammalian, including human, cell lines, baculovirus in insect cells or Erlichia, a ricketsial bacteria, in endothelial cells). Highlights for 2003/2004 include;

- Cell culture research studies to establish a scalable manufacturing culture process for the production of a Heartwater vaccine in a cost-effective manner. Complementary studies on bacteria inactivation strategies and vaccine formulation have also been undertaken.
- Development and establishment of the best production process for rotavirus-like particles (RLP's) using baculovirus infected cells; in particular fundamental studies on the kinetics of RLP's synthesis using multigene and single gene baculovirus infection.
- Development and optimization of an integrated process for adenovirus (AV) production for oral immunization against Rinderpest (RP) and Pest des petits ruminants (PPR). This includes studies of the stability of adenovirus-based vectors leading to the development of efficient and biocompatible storage formulations for AV recombinants.
- Development and optimization of the Retrovirus (RV) production processes including RV purification methods. Studies have also been performed on cell metabolism to understand the rate of RV production and decay, the correlation between RV properties and RV stability. Retrovirus degradation mechanisms with a view to devise improved RV storage conditions and processes for use of RV in gene therapy protocols have also been examined.

In 2004 the Animal Cell Biotechnology Laboratory will start working on a large project (CellPROM) of cell handling and cell programming using surface interactions leading to differentiated cells for medical therapies performed in a modular device.

The **Microbiology of Man-Made Environments: Stress, Wine Quality** Laboratory, headed by Maria Vitória San Romão, has several objectives for 2004. The influence of moulds on cork chemical and physical properties will be finished during the first months of 2004 (Cooperation with IGC and ISA) in the scope of a Project partially financed by Amorim & Irmão. The study of fungal interactions and the biochemical mechanisms in the synthesis of off-flavour compounds (chlorinated phenols and anisols) will continue. The study of chloroanisols formation in natural cork and agglomerated cork will also be continued. This work will be done in cooperation with Luís Vilas-Boas from the Analytical Chemistry Laboratory. The identification of cork moulds using molecular methods is on-going in cooperation with Rogério Tenreiro from FC-UL. Also in cooperation with Amorim & Irmão and the University of Strathclyde, Glasgow, it is expected to start a project aimed at investigating the poly-chlorinated phenols found in cork and water of oak forests that may be at the origin of anisols in cork stoppers, in order to study and develop strategies for bioremediation at forest level. The investigation of the enzymatic systems that allow *Chrysonilia sitophila* to grow on cork remains an objective of the group. The cellulolytic and lypolitic systems and respective regulation mechanisms will therefore continue to be investigated. Included in the wine quality section of the group, the study of the mechanisms of biogenic amines formation in wine will progress, namely the decarboxylase profiles of amino acids and the expression of the responsible genes will be investigated (histidine, tyrosine and arginine). This work is being done in cooperation with EVN and Rogério Tenreiro from FC-UL. The proteolityc system of *O. oeni* is being investigated in cooperation with T. Crespo and C. Arraiano.

The **Microbiology of Man-Made Environments** Group, formerly headed by José Figueiredo Marques and currently by Cidalia Peres, conducted studies on **Table Olive Fermentation - Stress and Antimicrobials of Lactic Acid Bacteria.** In particular, research was carried out on olive brine composition in order to define chemical profiles of different types of table olives. Studies on two bacteriocin (antimicrobial proteins) producers (*L.plantarum* 17.2b and *L. pentosus* B96) were pursued. Bacteriocin from *L. pentosus* B96 was identified as a plantaricin S analog while *L.plantarum* 17.2b was found to produce 2 different inhibitory peptides that are not fully characterized yet.

Studies on the effect of stress factors (temperature, osmolarity and acidity) on bacteriocin production were carried out by statistical factorial designs. Bacteriocin production was stimulated by stress, in both strains, although maximum production corresponded to different conditions.

The results that were obtained from brine composition were used to program studies on the effect of brine components and some metabolites (produced by yeasts and gram-negatives) on LAB (lactic acid bacteria) growth and bacteriocin production. In a first phase, metabolites (mainly acids and alcohols) were added to MRS. In a second phase prospective studies were carried out in real brines where bacteriocin production was detected, under environmental conditions that differ for each of the strains. These studies will continue in order to understand the role of bacteriocins in the fermentation process.

For 2004 it is planned to continue research efforts on the effect of brine composition and stress factors on bacteriocin production. These studies aim at understanding: (a) the role of bacteriocins in LAB survival; (b) the ecological importance of bacteriocin producers during olive fermentation. The development of suitable analytical methods to determine brine composition will be part of the studies on methodologies, necessary to implement a traceability system for table olives.

The **Microbiology of Man-Made Environments: Food and Quality** Group, headed by Maria Teresa Crespo will maintain Food Quality and Safety as the main line of research for 2004. Studies of the quality of traditional products will continue using as a model cheeses produced from ewe's raw milk. The microbial ecology of the raw materials, environments of production and other factors that can influence the dynamics of the populations will be the main focus of this area of work. Metabolic profiles and molecular biology methods will be used as laboratorial tools. One of the aspects of food safety that will be investigated is related to the presence of *Enterococcus* spp. in traditional food products. Studies on the detection and expression of virulence factors and antibiotic resistances in this genus will be continued. Another aspect that will be also assessed is the production of peptidases, amino acids and the corresponding biogenic amines in Lactobacillus also isolated from traditional fermented foods.

The **Biosensors** Laboratory (BL), headed by Abel Oliva, works in the development of optical immunosensors, mainly towards veterinary diagnostic applications and

bioprocess monitoring. Presently, the main activities are on the immobilisation of proteins on solid supports (membranes, CPG, sol-gel), identification of antigenic proteins for veterinary diagnostic and production of immuno-reagents, immunoassay design, ELISA protocols and Reverse Line Blot diagnostics. The development of instrumental aspect of the sensors, such as flow cell design, optical signal measurement and bioprocess-control will also be undertaken. The BL is an interdisciplinary group which has expertise in the development of applications for veterinary disease diagnostics, such as African swine fever, *Theileria sp.* and *Babesia sp.),* the environment (nitrite monitoring) and bioprocessing (measurement of cell density in bioreactors and dissolved oxygen in cell media). The BL participates in several international projects, namely an international collaboration project for *Babesia sp* diagnostics and an integrated project for cell manipulation, recently approved by the European Commission.

The **Processes in Supercritical Fluids** Laboratory under the leadership of Manuel Nunes da Ponte and Catarina Duarte has conducted research work on the development of Clean Technologies using Supercritical Carbon Dioxide, namely;

- Extraction from solids and fractionation of complex liquid mixtures,
- Chemical reaction (hydrogenation, oxidation with air),
- Induced phase changes in (ionic liquids, water and ethanol mixtures) solutions with applications to biphasic catalysis,
- Emulsions for protein transport,
- Impregnation of polymers with pharmaceuticals.

With respect to one of the on-going projects in the area of Delivery/Nutraceuticals, (water+ethanol) mixtures and supercritical CO_2 have been used to extract from food residues (wine and olive oil wastes) concentrates of antioxidants containing high-value biologically active substances, such as resveratrol or hydroxytirosol. Incorporation of the extracts in commercial products will be explored. In collaboration with the Animal Cell Biotechnology Laboratory and Instituto Gulbenkian Ciência, bioactivity tests and analysis of RNA expression are under study and will be continued during 2004. The use of emulsions (with several bio compatible emulsifiers) to transport different proteins in supercritical CO_2 will continue to be explored.

A lab-scale apparatus for sorption measurements of CO_2 into polymers was built. Two new semi pilot-scale apparatus for particle formation by rapid crystallisation using supercritical carbon dioxide are under construction. These two devices are based upon different techniques (SAS:supercritical antisolvent and PGSS: particles from gas

saturated solution) and will be developed to prepare delivery systems with interest to the pharmaceutical or food industries.

In the **Molecular Thermodynamics** Laboratory, Luis Paulo Rebelo and co-workers focused their major attention during 2003 on the new emerging area of Ionic Liquids as potential substitutes for common volatile organic compounds. Pioneering work was performed both in respect to their thermodynamic characterization in broad pressure and temperature ranges as well as to their solution behaviour. In the latter case, a huge co-solvent effect between water and alcohols was found in their solutions with ionic liquids. This fact can profitably be used as a switching device to trigger situations of total miscibility, partial miscibility, or almost complete phase separation. This effect may play an important role in the recovery of alcohols produced in fermentation processes using ionic liquids.

Studies of the thermodynamic response to metastable liquid regimes at large absolute negative pressures have continued during 2003 as well as sound-speed measurements in liquids up to 200 MPa using a novel, miniaturized, non-intrusive cell.

For 2004 it is foreseen that extensive studies on ionic liquids will be performed aiming at understanding the underlying interactions at a microscopic level. In particular, solubility studies of polymers and of metals in ionic liquid media are anticipated.

The goal of the **Biomathematics** Group at ITQB/UNL is the quantitative analysis of biological systems with special emphasis on the identification of unifying quantities and methodologies. This objective is pursued by multidisciplinary collaborations with other groups at ITQB and elsewhere.

From a methodological point of view, the claim for originality of this group is the use of the structure and behaviour of biological systems as a model for the development of data analysis techniques - Biology as a model for Mathematics. This inversion of Galileo's cornerstone formulation - *Mathematics is the language of Nature* - is at the core of the ongoing revolution in Biomathematics. It has been made possible by the advent of parallel processing, which enables simulation of the networked multi-agent nature of biological systems.

TECHNOLOGY DIVISION – Research Highlights

Veterinary Diagnostics

Biosensors Group : Head of Group, **Abel Oliva**

A larger demand in veterinary diagnostic methods has been observed in recent years due to the increase in public concern related to animal health, food safety and quality. Tick-borne diseases (TBD) are responsible for major depressions in livestock production and mortality in sub-Saharan Africa, Latin America, Asia and the Mediterranean region. New tools are needed for efficient control of TBD, including better surveillance and other control measures, as well as improved vaccines. The Biosensor Laboratory is working on the identification of antigenic proteins of a recently described *Theileira sp.* parasite. That causes a tick-borne disease that affects small ruminants in China, inducing several loses in a vast region of Asia. The identification and characterisation of the antigenic proteins are being performed by using SDS-Page, Western Blot, mass spectrometry and various types of chromatography.

The identification of different parasite species in blood samples is achieved by a recently established technique, reverse line blotting (RLB), which uses species specific molecular probe hybridization. RLB is currently used to validate other diagnostic methods under development. In order to avoid seasonal limitation of the amount of parasite antigens, development of parasite culture in erythrocyte cells has been implemented. In an alternative to the cell culture, cloning of the antigen cDNA is under consideration for recombinant expression of those proteins.

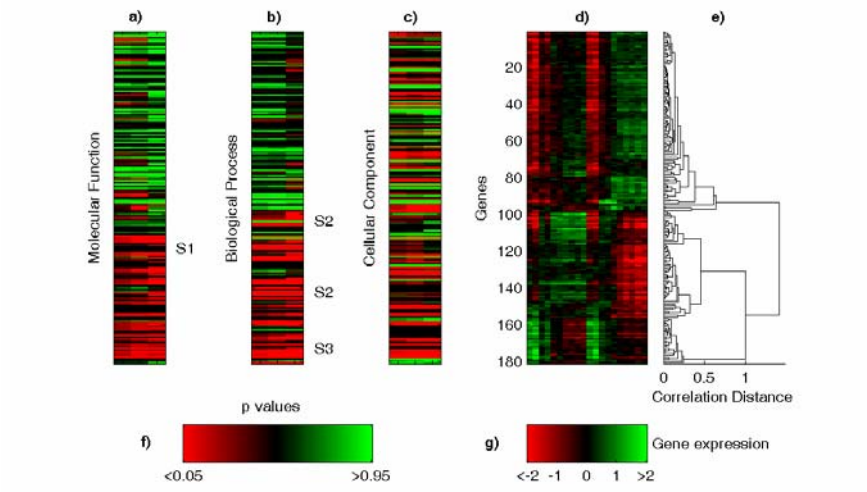
After the identification of the antigenic proteins, the isolated proteins will be used for the development of diagnostic tools, such as ELISA, rapid tests and optical immunosensors. Conjugation of specific antibodies with fluorophore molecules (e.g. cy5) is being studied in terms of its effect on antibody binding affinity and specificity. Recently approved EC projects on TBD diagnostic will support the continuity of this research leading to the improvement of Biosensor Laboratory competence in the field of veterinary medicine.

New information extraction techniques in gene sequence and expression analysis

Biomathematics Group : Head of Group, **Jonas S. Almeida**

Alignment-free metrics for sequence comparison were reviewed by the authors [1] and quantitatively compared. In order to complement the existing word composition methods, we also proposed a *novel W-metric* [2] between two proteins based in their amino acid frequencies. This metric was shown to be more accurate than other non-intensive computational methods in the identification of protein secondary structure from SCOP (Secondary Classification of Proteins) database. This result can help the pre-screening of sequence sets for structure prediction problems.

In the *gene expression* field, a *new method* was also developed to automatically incorporate information about gene function in the analysis of micro-array generated gene expression profiles [3]. It uses functional annotations normalized according to the Gene Ontology Consortium, being able to quantify similarities between every two annotation terms. This method enables visual detection of gene modules sharing expression patterns and functional annotations, and was further developed to attribute statistical significance for each gene membership to the referred modules, as shown in the following figure.



[1] S.Vinga and J.S.Almeida (2003) Alignment-free sequence comparison – a review. *Bioinformatics*, **19**: 513-523.

[2] S.Vinga, R.Gouveia-Oliveira and J.S.Almeida (2004) Comparative evaluation of word composition distances for the recognition of SCOP relationships. *Bioinformatics*, **20**: 206-215.

[3] Cowart, LA, Y Okamoto, FR Pinto, JL Gandy, J S Almeida (2003) Roles for Sphingolipid Biosynthesis in Mediation of Specific Programs of the Heat Stress Response Determined Through Gene Expression Profilling. *J Biol. Chem.* **278**(32):30328-38.

Table Olive Fermentation : Stress and Antimicrobials of Lactic Acid Bacteria

Microbiology of Man-made Environments Group : Head of Group, **Cidalia Peres**

Studies on two bacteriocin (antimicrobial proteins) producers (*L.plantarum* 17.2b and *L. pentosus* B96) have been undertaken as follows;

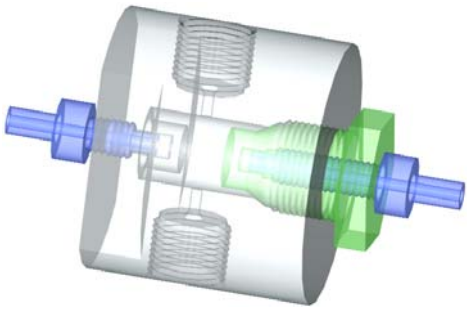
- Bacteriocin from *L. pentosus* B96 was identified as a plantaricin S analog while *L. plantarum* 17.2b was found to produce 2 different inhibitory peptides that are now under characterization.

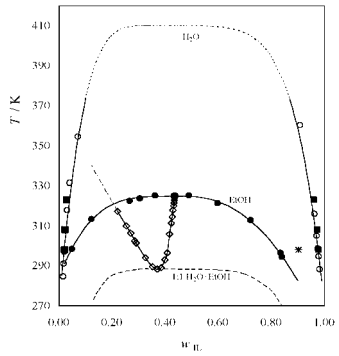
- Studies on the effect of stress factors (temperature, osmolarity and acidity) on bacteriocin production have been carried out by statistical factorial designs. Bacteriocin production was stimulated by stress, in both strains, although maximum production corresponded to different conditions.

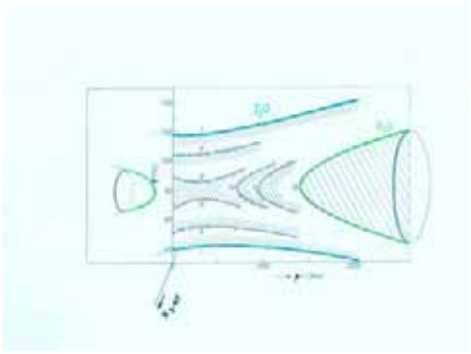
- The results that were obtained from brine composition were used to program studies on the effect of brine components and some metabolites (produced by yeasts and gram-negatives) on LAB (lactic acid bacteria) growth and bacteriocin production. In a first phase, metabolites (mainly acids and alcohols) were added to MRS. In a second phase prospective studies were carried out in real brines where bacteriocin production was detected, under environmental conditions that differ for each of the strains. These studies will be continued in order to understand the role of bacteriocins in the fermentation process.

A novel high-pressure ultrasonic cell for the determination of the speed of sound in liquids

Molecular Thermodynamics; Head of Group**: Luis Paulo Rebelo**







A novel high-pressure, ultrasonic cell of extremely reduced internal dimensions (~ $0.8 \cdot 10^{-6}$ m^3) and excellent precision for the determination of the speed of propagation of sound in liquids was conceived and built. It makes use of a non-intrusive methodology where the ultrasonic transducers are not in direct contact with the liquid sample under investigation. The new cell is used to carry out speed of sound measurements at pressures up to 160 MPa. A patent is filled (#102877). One can now determine isentropic (κ_s) and isothermal (κ_T) compressibilities, isobaric thermal expansivities (α_p) , isobaric (c_p) and isochoric (c_v) specific heat capacities, and thermal pressure coefficients (γ_v) in broad temperature and pressure ranges.

A surprising and extreme mixed solvent effect, both in its magnitude and direction, has been found in the phase diagram of the ternary mixture of ($[C_4mim][PF_6]$ + (water + ethanol)). For a molar ratio of 1:1 of water to ethanol, the co-solvent effect in the near-critical demixing temperature can be as large as 80 K. Therefore, one can immediately envisage many distinct strategies for obtaining the desired situations of total miscibility, partial miscibility, or almost complete phase separation. The ratio of water to ethanol content is the tool for fine-tuning those different situations. Theaforementioned effect may come to play an important role in the recovery of alkanols produced in fermentation processes using ionic liquids.

Liquid-liquid (L-L) phase splitting in mixtures of water (H/D) + 3-methylpyrydine (3-MP) at the limit of pure H_2O as solvent and under high tension are reported for the first time. The phase diagram is thus encountered at large absolute negative pressure regimes. These studies constitute the first to report phase transitions in non-polymeric fluid mixtures at negative pressures, and the values of tension achieved (≈ -350 bar) constitute a record for macroscopically-sized samples. This phenomenon corresponds to an impressive pressure shift of several hundred atmospheres upon (H/D) solvent isotopic substitution. It is shown that an entropic effect originated at the relatively large difference in molar volumes between water and 3-MP is responsible for the location of the L-L phase diagram in the low-concentration region of 3-MP. It is again another (but subtle) entropic effect that provokes the above-mentioned abnormally large shift in the phase diagram upon isotopic substitution.

J. Chem. Thermodyn. (2004) in press

J. Phys. Chem B (2003), **107**,

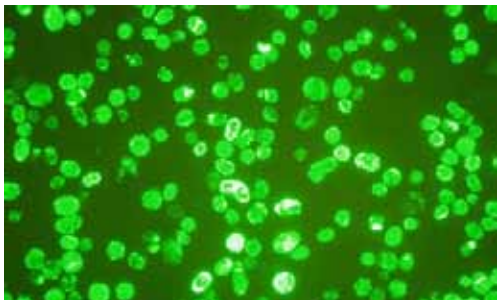
J. Phys. Chem. B (2003), **107**,

Production of Rotavirus-Like Particles
using Baculovirus-Insect Cells Expression System

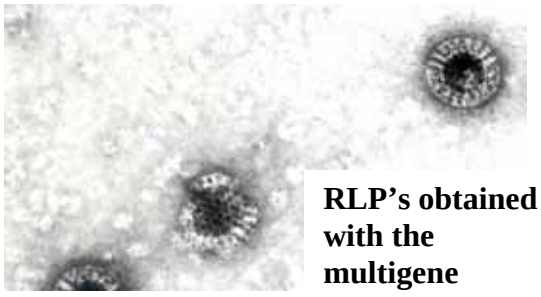
Animal Cell Biotechnology Group : Heads of Group,
Manuel J Carrondo and Paula Alves

Rotaviruses are the leading cause of severe infantile gastroenteritis worldwide, accounting for approximately one million deaths each year. Many alternatives have been exploited for immunization against this virus, but to date, none has been fully effective. The use of rotavirus-like particles (RLPs) is a promising alternative for immunisation as they offer several advantages that include safety, stability and high immunogenicity. Rotavirus RLP's are triple layered particles, composed of VP2, VP6 and VP7 proteins being especially problematic to obtain significant amounts of RLP's with the correct stoichiometry and proper assembling. One approach to produce RLP's is the co-expression of these 3 proteins that self-assemble in insect cells co-infected with recombinant single gene baculoviruses. However the co-infections involving 3 different viruses leads to variations of the infecting viruses' ratio and, as a consequence, the proteins synthesized in individual cells, and their assembly can be very variable. This problem has been addressed and an alternative strategy using a multigene Baculovirus vector (constructed by Prof. Polly Roy at the London School Univ. Hygiene and Tropical Med, UK) was evaluated. This approach allows the simultaneous expression of the proteins permitting a better particle homogeneity and consistency. Moreover it requires the maintenance of a single master seed baculovirus bank minimizing the production process complexity and costs. The dynamics of single gene co-infections *versus* multigene infection is being studied using real-time quantitative polymerase chain reaction (RT-PCR) and immunofluorescence techniques to further understand the kinetics of replication of baculovirus infections *in vitro.* Both production alternatives are being compared either in terms of the quality and the quantity of the particles obtained.

The first vaccine doses produced at 2 L scale using the multigene vector were delivered last December and are currently being tested in a gnotobiotic piglet model at Prof. Janice Bridge Laboratory (Royal Vet College, UK).



Monitoring of Baculovirus infection by immunofluorescence tecnhiques

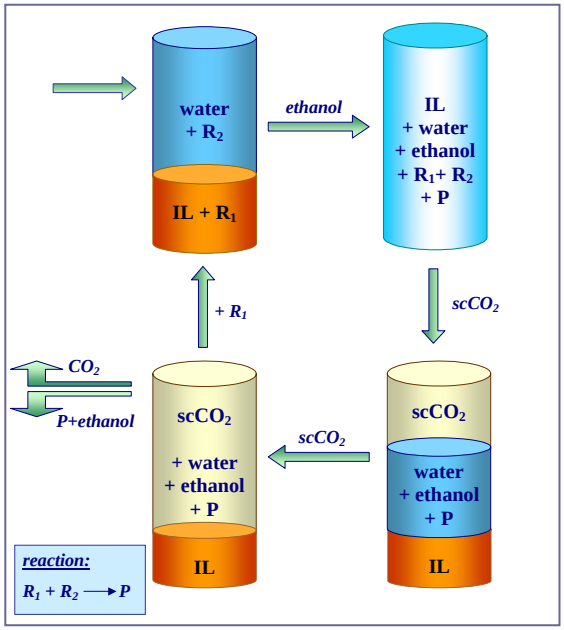


RLP's obtained with the multigene

(EM image is a courtesy of Dr. Sérgio Gulbenkian, Instituto Gulbenkian de Ciência, Portugal)

Supercritical Carbon Dioxide – induced Phase Changes in (Ionic Liquid, Water and Ehanol Mixture) Solutions: Application to Biphasic Catalysis

Supercritical Fluid Processes Group : Head of Group, **Manuel Nunes da Ponte**



Isophorone epoxidation: Monophasic reaction followed by supercritical CO_2 product recovery

The best of both worlds: Phase changes in ionic liquid (IL) + water systems, induced by addition of ethanol and supercritical carbon dioxide ($scCO_2$), allow reaction cycles to proceed as depicted, combining the high reaction rates of one-phase conditions with easy separation of products, characteristic of biphasic catalysis. The (usually slow) epoxidation of isophorone (**R_1**) by hydrogen peroxide (**R_2**), catalysed by sodium hydroxide, was rapidly carried out in these conditions, with complete recovery of the reaction product (**P**) by $scCO_2$ decompression.

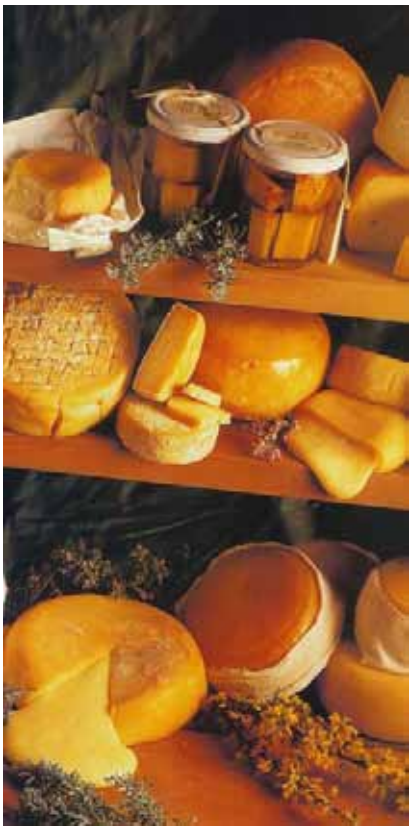
Enterococcus, a bacterial genus, in Portuguese traditional cheeses

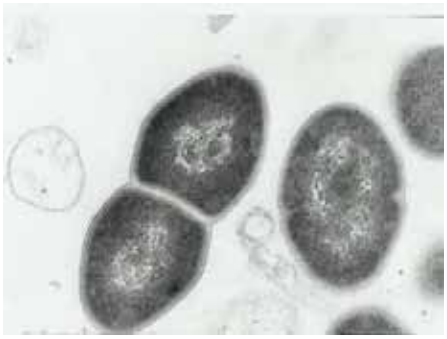
Microbiology of man-made environments Group :
Head of Laboratory, **Maria Teresa Crespo**

Cheeses produced from raw ewe's milk are part of the daily diet in rural areas of Portugal as well as fashionable foods product in urban centers. Cheeses are still produced in a traditional way in certain regions of Portugal by relying on colonization by microbial populations associated with raw materials, equipment and local environments. *Enterococcus*, a bacterial genus, are present in Portuguese traditional cheeses and their activty is related to the organoleptic properties of the final product.

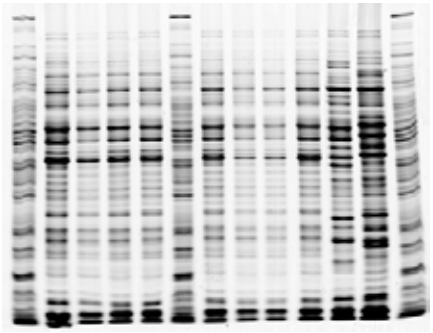
The purpose of the study was to assess the phenotypic and genotypic taxonomic congruence in order to allow species allocation in dairy enterococci. A total of 364 enterococci isolated from ewes'milk and cheese from four Portuguese Registered Designation of Origin areas and 25 type and reference strains of *Enterococcus* spp. were characterized by a taxonomical polyphasic approach involving 40 physiological and biochemical tests, whole-cell protein profiles, amplification of 16S-23S intergenic spacer regions (ITS-PCR) and subsequent restriction analysis (ARDRA). Ribotyping was also performed with reference strains and a subset of 146 isolates.

Reliable identification of enterococci from milk and cheese could be obtained by analysis of whole-cell protein profiles. ITS-PCR can be used to confirm *E. durans* and *E. faecium* and ARDRA further confirms *E. faecalis*. Results of diversity of dairy enterococci revealed *E*. *faecalis*, *E. durans*, *E. hirae* and *E. faecium* as the prevalent species, although species prevalence showed some degree of variation among the areas





Enterococcus spp.



Whole-cell protein profiles

Cork stoppers industry -- the role of cork moulds during the stabilization period for the final properties of cork:

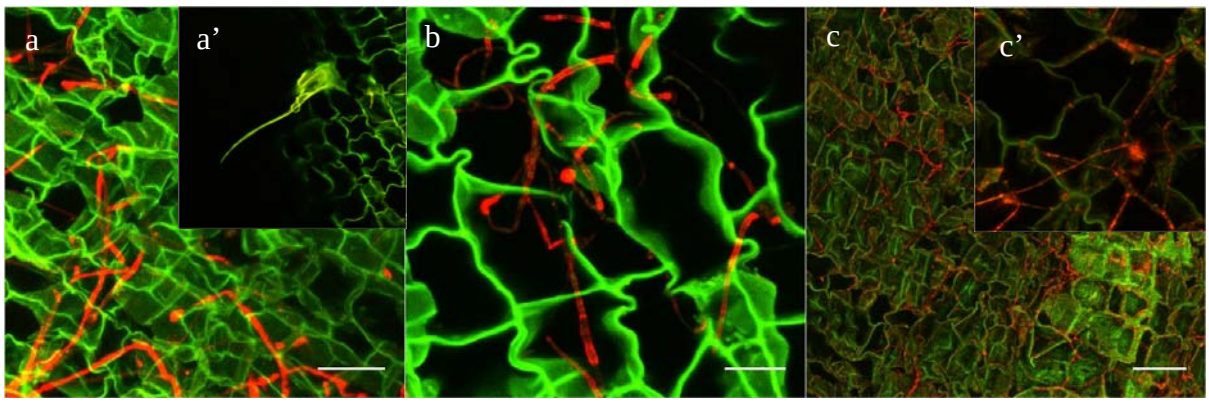
Microbiology of Man-Made Environments (Stress/wine quality) Group :
Head of Group, **Maria Vitória Gonçalves San Romão**

The aim of this work is to have a better understanding of the role of the cork mycobiota isolated from a Portuguese cork factory, and their interaction with the cork cells during the cork slabs stabilization process after boiling.

Fungal Penetration

Fluorescence detection of the cork slice samples was carried out to analyse the interaction between the fungal hyphae and the cork cells. Nevertheless the cork cells have auto-fluorescence in a wide range of wavelengths from the UV to the red, emitting higher intensity from 510 to 610 nm. This property of the cork disables the differentiation between the fungal hyphae and the cork cells when some of the most frequent fluorochromes were used. However, the differentiation was possible using the chitin-labeling wheat germ agglutinin (WGA) conjugated with Cy5, allowing fungal detection in a far red wavelength without the interference of the cork cells. The results show that all the selected fungi used in this experiment were able to grow on the surface of the cork slides. Using different magnifications one can also see how the fungi make a kind of a network.

Using the confocal microscopy technique, it was observed that the cork moulds can actually interact with the cork slides. The maximum projection of the stacks also shows the fungal mycelium growing around the cork cell walls, which are basically hollow inside, having only an irregular cell wall of about 1.0-1.5μm wide. Although the fungal hyphae attaches to the cork cell wall and grows on the cork slides, it could not be certified using this technique whether the fungal hyphae goes through the cell walls, perforating them, since no fungi was observe in the second cell wall.



Fluorescence detection of the transversal section obtained from the cork slice samples using confocal microscopy with a maxim projection algorithm. A) *Chrysonilia sitophila* and an example of bounding (a') and as we can see a channel bleed through due to the use of acridine orange – bar 40 μm. B) *Penicillium glabrum* – bar 20 μm. C) *Trichodema sp* and a higher magnification on which one can see the interaction – bar 50 μm. All the photographs were taken after 14 days of growth on a cork slice.

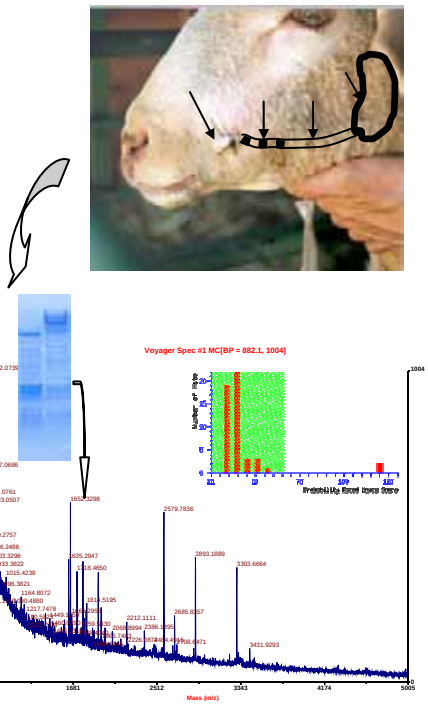
Effects of Phenolic Compounds in Taste Sensitivity ; Cell Kinetics, Chemical and Morphological Adaptations in the Oral Cavity.

Mass Spectrometry Group :

Head of Group, **Ana Maria de Jesus Bispo Varela Coelho**

Mammalian herbivores may have innate defensive mechanisms against dietary compounds, such as polyphenols, with a potential negative effect,. These mechanisms are thought to involve the production, mainly by the parotid glands, of particular kind of salivary protein, namely proline-rich proteins (PRPs), which have a high affinity to dietary tannins. In rats and mice, receiving a low tannin diet, the PRPs production is reduced or even nonexistent, being significantly and proportionally intensified with increased administered concentrations of these secondary compounds. However, some controversy still exists about the presence, the type(s) and the amounts of salivary proteins with high affinity to tannins, and their effects in feeding behaviour of several herbivore species.

The electrophoretic salivary proteins profile of regularly fed Balbc mice was characterized and identification of a large number of proteins performed by MALDI MS peptide mass fingerprint (PMF). This protein profile is changed after the administration of polyphenols, which seem to stimulate the production of proteins. The identification of these proteins by PMF is under way.



In domestic ruminants the production of salivary tannin-binding proteins, namely PRPs, is not clear. Since sheep and goats usually share the same pastures, but present different feeding behaviours, it is possible that they have some differences in their saliva composition. For both species the normal salivary protein profile was characterized by 1D electrophoresis and MALDI PMF identification. Significant differences were observed between the 2 species. The salivary response of these animals to dietary polyphenols will be studied by 2D electrophoresis followed by PMF identification.

In Search of New Molecular Targets for the Development of Novel Therapeutic Strategies for Cystic Fibrosis.

Cystic fibrosis (CF) is a mis-processing or mis-folding disease since most individuals with CF carry at least one F580del-CFTR allele whose product is synthesized but fails to reach the cell surface where it functions as a Cl^- channel. Proteomic tools, such as 2D electrophoresis and mass spectrometry, are now being applied to discover new molecular targets for the development of new therapeutic strategies to care and treat the CF. The 2D maps of nasal brushing samples isolated from different healthy individuals are reproducible and consistent. Identification by peptide mass fingerprinting (PMF) strategy of several proteins was performed. Significant changes in the protein profile of F580del-homozygous patients give evidence of qualitative or/and quantitative alterations, and may suggest novel mechanisms in CF pathology. Identification of these proteins by PMF is under way.

PLANT SCIENCES DIVISION – Laboratories and Staff

Laboratory: Plant Biochemistry (Stress proteins / extracellular proteins)
Head of Laboratory: Cândido Pinto Ricardo
Research team:

Ana Isabel Faria Ribeiro	Ph.D.
Ana Paula Ferreira Regalado	Ph.D.
José António Pires Passarinho	Ph.D.
Carla Maria Alexandre Pinheiro	Ph.D.Student
Inês Maria Silva Almeida Chaves	Ph.D. Student
Ana Sofia Correia Fortunato	Research Student
Sandra Marina Gonçalves Martins	Research Student
Isabel Tavares Lima Martins	Research Student
Rita Maria de Brito Francisco	Research Student
Marta Alexandra Marques Alves	Research Student

Laboratory: Plant Molecular Ecophysiology
Head of Laboratory: Maria Manuela C. C. F. Chaves
Research team:

João Maroco Domingos	Ph.D.
Maria Helena Cruz de Carvalho	Post-Doc
Alla Shvaleva	Post- Doc
Olga Grant	Post-Doc
Cláudia Rita de Souza	Ph.D. Student
Elisabete Vieira da Silva	Ph.D. Student
Lukasz Tronina	Ph.D. Student
Ana Rodrigues	Master Student (ITQB/ISA)
Raquel do Vale	Master Student (ISA/ITQB)
Collaborators:	
João Santos Pereira	Professor at ISA
Maria Lucília Rodrigues	Investigator at ISA

Laboratory: Plant Genetic Engineering (Gene search and molecular characterisation / Plant transformation and improvement)
Head of Laboratory: M. Margarida Oliveira
Research Team:

Ana Maria Beirão Reis Sánchez	Post-Doc
Madalena Cristina Rocha Martins	Ph.D.Student / Post-Doc
Maria Helena Raquel Gonçalves	Ph.D.Student
Ana Paula Martins Farinha	Ph.D. Student
Cristina Maria Neves Silva	Ph.D. Student
* Marta Wilton de Vasconcelos	Ph.D. Student
Ana Margarida Santos	Ph.D. Student
Tiago Lourenço	Ph.D. student
Ana Luísa Pereira	Ph.D. Student (FC-UL)
Rita Batista	Researcher (INSA)
* Maida Romera	Researcher
Sónia Negrão	Ph.D. Student/Researcher
David Sarmento	Researcher
* Ana Milhinhos	Undergraduate
* Sarah Silva	Undergraduate

(* students that moved to other Labs before the end of 2003)

Laboratory: Pinus
Coordination of Laboratory: M. Margarida Oliveira / C. Pinto Ricardo
(See Laboratories: "Plant Genetic Engineering" and "Plant Biochemistry"**)**
Research Team:

Célia Miguel	Post-doc
Margarida Rocheta	Post-doc
Susana Tereso	Ph.D. student
Sónia Gonçalves	Ph.D. student
Liliana Marum	Master
Jorge Cordeiro	Graduate
Catarina Estêvão	Graduate
Sandra Carvalho	Graduate

Laboratory: Plant Biotechnology (Plant enzyme purification and characterisation / Plant differentiation and molecular characterisation)
Head of Laboratory: Pedro Fevereiro
Research Team:

Dulce Maria Fernandes dos Santos	Post-Doc
Carlota Vaz Pato	Post-Doc
Susana Neves	Post-Doc
Vitória João Valente Gemas	Ph.D.Student
Ana Sofia Lopes Duque	Ph.D.Student
Ana Sofia Fernandes Pires	Ph.D.Student
Jorge Almiro Pinto Paiva	Ph.D.Student
Susana de Sousa Araújo	Ph.D.Student
André Almeida	Ph.D.Student
Jingsi Liang	Ph.D. Student
Isabel Raposo	Master Student
Leonor Tomaz	Technician

Laboratory: Plant Cell Wall
Head of Laboratory: Philip Jackson (See also Laboratório Associado Section)
Research Team:

Cristina Silva Pereira	Ph.D. Student
José Ribeiro	Ph.D. Student
Nelson Soares	Ph.D. Student
Ada Vatulescu	Ph.D. Student
Sara Vieira da Silva	Ph.D. Student

Laboratory: Plant Biochemistry (Ubiquitin / lectins)
Head of Laboratory: Ricardo B.Ferreira
Research Team:

Sara Monteiro	Post-Doc
Sam Cherian	Post-doc
Zenjhia Chen	Post-Doc
Maria Cláudia Nunes dos Santos	Ph.D. student
Ana Sofia Sénica Caeiro	Ph.D. student
Cristina Branco Price	Ph.D. student
Pedro Miguel Duarte Martins	Post-graduate student
Mariana Boavida Carvalho	Undergraduate student

PLANT SCIENCES DIVISION – Plan and Objectives

The Plant Sciences Division is composed of 8 laboratories which undertake basic research directed to the study of important problems of plant development and response to stress. Most of the laboratories also carry out research of a more applied nature, in close association with IBET, aiming to solve problems of agronomic or industrial relevance to Portugal and in respect to important crops such as, rice, maize, lupin, grapevine, olive tree, almond, pine, cork oak and *Eucalyptus*.

Plant Biochemistry Group I

Research is focused on proteins that are extracted from tissues, separated by electrophoresis either by SDS-PAGE or 2D-Electrophoresis, and identified by immuno-blotting or mass spectrometry. This is a primary step in the elucidation of their function in several plant processes such as;

- Apoplast characteristics (*Lupinus albus, Medicago truncatula, Arabidopsis thaliana)* and the responses of the cell compartment to wounding.
- Boron deficiency, in *L. albus.*
- Seed development in *L. albus* and stem responses under water deficit.
- Programmed cell death of tobacco BY-2 cells.
- Cork formation in cork oak tissues.
- Suberization of potato slices.

Metabolic changes in *L. albus* tissues due to water and B deficiency have also been analyzed by means of ^{13}C-NMR.

Plant Biochemistry Group II

Research is centred on the biotechnology of grapevine for increased resistance to fungal pathogens;

- A study was undertaken to determine the involvement and the levels of expression of the enzymatic components of the ubiquitin-mediated proteolytic pathway during the interaction grapevine and *U. necator* (powdery mildew agent).
- Grapevine moving particles (GMPs), showing a powerful antifungal activity, were recently detected in leaves. Their number appears to increase during infection. Research will continue to fully characterize these particles.
- Two major defense mechanisms have been characterized in grapevine; pathogenesis-related proteins (PR proteins) and phytoalexins. A transformation experiment is underway to express constitutively in leaves a thaumatin-like protein (a PR protein) and resveratrol (the major phytoalexin in this plant).

Plant Cell Biotechnology Group

The group has three main activities as follows;

- Genetic transformation to water stress tolerance (*Medicago truncatula*, maize and tobacco) using different genes (trehalose phosphate synthetase, DSP22 and arginine decarboxylase). Tobacco and *M. truncatula* homozygotic lines have been achieved and phenotypes will be analysed.
- Expression analysis at the transcriptional level (in association with pine wood qualities), using macro arrays, and at the protein level (to understand

Olive/*Pseudomonas savastanoi* interaction) using 2D-Electrophoresis and Mass spectrometry.
- Genetic diversity studies using different types of molecular markers; co-dominant (RAPDs and ISSRs) for olive and *Eucalyptus globulus* and dominant (SSRs) for grapevine cultivars, Portuguese near-isogenic maize lines and *Pinus pinaster* population.

Plant Cell Wall Group

Studies on this group include;
- Wound-responsive cell wall proteins in *Lupinus albus* and *Medicago truncatula* have been studied (in conjunction with Plant Biochemistry I).
- A project to identify wall proteins differentially regulated during eucalypt wood formation has been initiated.
- Extensin's contribution to cell wall hydration, resistance to lytic enzymes and polygalacturonase catalysed pectin release has been studied. Results indicate that the extensin network requires interaction with ENAFs (extensin network associated factors) to be effective.
- Transcriptional studies of extensin peroxidase in grapevine have been performed.
- To characterise the nature of the cross-link formed during extensin network formation, a small cross-linking peptide has been isolated and purified and is being characterised by MS-ESI to help elucidate the extensin cross-link.

Plant Developmental Genetics Group

Comparative studies of dorsoventral asymmetry of flowers in *Antirrhinum majus* (a species with asymmetric flowers) and *Arabidopsis* (symmetric flowers), have been undertaken in collaboration with the group of E. Coen (JIC, Norwich, UK). A specific objective is to analyse how a set of key genes determining floral asymmetry in *Antirrhinum* may interact in the context of *Arabidospis*. As a first step, constructs of one of these genes, *DIVARICATA*, were transformed into *Arabidopsis*. Transgenic plants are currently being analysed.

Plant Genetic Engineering Group

The main activities of the group are;
- Almond subtracted cDNA libraries have been constructed from leaves undergoing organogenesis and two putatively organogenic cDNAs extended to full-length.
- The stability and gene expression of constructs for virus resistance in almond have been monitored in model plants.
- Three abiotic stress-related transcription factors coding genes cloned in transformation vectors have been studied and large-scale rice transformation has been initiated (collaboration with S Datta, IRRI).
- Twelve almond genes related to flowering have been assigned to 7 linkage groups in the *Prunus* genetic map (collaboration with P Arus, Spain). New S-alleles have been isolated from 6 self-compatible *P. webbii*; only 2 ecotypes carry the Sf-allele thought to be the origin of SC almond. (collaboration with Ma RC, China).
- The first crosses have been conducted to breed rice varieties with blast resistance and higher productivity; molecular studies have been initiated.
- The potential allergenicity of approved GM foods has been analysed and found negative in the populations tested.

- Chemotypes of Azorean *Thymus caespititius* populations have been studied and molecular characterization is under way for comparison (collaboration with J Barroso, FCUL)

Plant Molecular Ecophysiology Group

The group interests include;
- Root-shoot chemical signalling and the systems that facilitate water transport (aquaporins) in *Vitis vinifera* under water scarcity conditions.
- Identification of key markers for drought resistance in *L. albus*, to be used in genetic improvement for drought prone areas.
- Study of plant stress responses by thermal imaging techniques. This information was related to defence mechanisms, namely leaf xantophylls.
- Study of water uptake and respiration patterns for Cork oak and Holm oak in a Montado ecosystem, using stable isotopes.
- Study of resistance mechanisms to water deficits in two contrasting *Eucalyptus globulus* clones – hydraulic and metabolic components in roots and leaves.

Pinus Laboratory

The activities of the Laboratory include the following;
- Somatic embryogenesis is being applied for clonal propagation of *Pinus pinaster*; more than 400 somatic embryo plants have been established in the greenhouse and will be transferred to a field trial during 2004.
- The expression patterns of 3 putatively identified genes differentially expressed during embryo development, with homologies to GTP-binding, Kelch motif and lipid transferases, are under study. Functional analysis of genes involved in nitrogen metabolism is being undertaken and one transgenic line has been obtained carrying an antisense PII gene.
- All genotypes established in Escaroupim clonal seed orchard were fingerprinted and foreign pollen contamination was evaluated using SSR markers. A complete *gipsy*-like retrotransposon sequence (5700bp) was determined and will be used to monitor retrotransposon activity during *in vitro* propagation of pine.

PLANT SCIENCES DIVISION – Research Highlights

Importance of *Lupinus albus* seed "glutelins" and glycoproteins for the discrimination of cultivars.

Plant Biochemistry Group : Head of Group, **Cândido Pinto Ricardo**

White lupin (*Lupinus albus)* is a valuable legume crop that can grow in poor soils and produces seed of high quality protein. Portugal has an important germplasm collection of this species with cultivars distributed from north (small-seeded, higher plant architecture and later flowering type) to south (large-seeded, shorter and early flowering type). We have examined the variability in the seed protein fractions of 17 cultivars from different regions, in order to disclose existing genetic relationships and to obtain information useful for a breeding program. Use was made of the Osborne protein fractionation methodology, the electrophoretic (SDS-PAGE) separation of the several protein fractions and the multivariate analysis of the electrophoretogram patterns. The major seed protein constituents were the globulins and the fraction extracted with NaOH (''glutelins'' not usually considered as proper components of legume seed proteins), were precisely the bands of "glutelins", together with those of glycosylated polypeptides (Fig.1), which contributed the most in the discrimination between lupin populations. These were thus grouped according to their clinal geographical distribution. It was also evident that the polypeptide patterns reflected microclimatic specificities related with altitude and temperature. In fact, one cultivar from the south but from high altitude (Monchique) was grouped with the north plant types adapted to colder and wetter weather, whereas two cultivars from a warmer north region (Terra Quente Duriense) were grouped with the southern types. The results show, in addition, that a general correlation exists between cultivar early flowering characteristics and higher amounts of both total seed protein and glutelins, and lower glutelin glycosylation. Since high seed protein content and quality and early flowering are important aims of lupin breeding programs, these findings on the seed protein fractions could stimulate and direct future research in lupin improvement. Lupin seed "glutelins" (or glutelin-like globulins) are mostly unknown and appear to be an interesting class of proteins that we intend to further study, making use of the powerful separation capacity of 2D-electrophoresis (Fig.2).

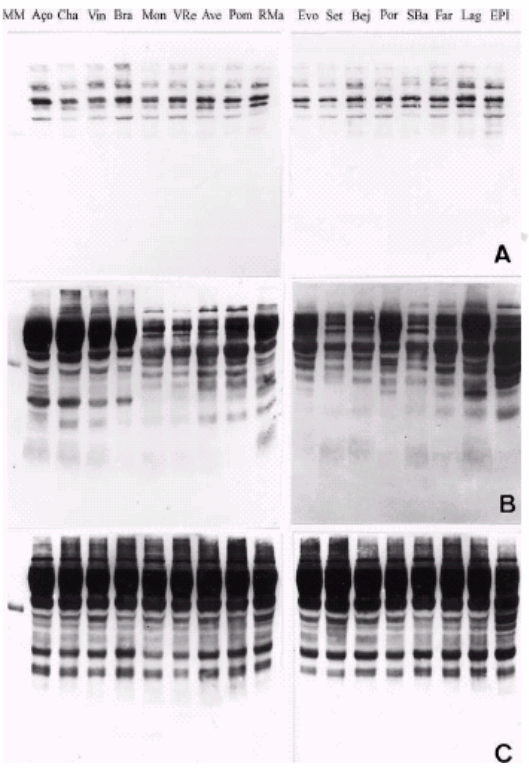
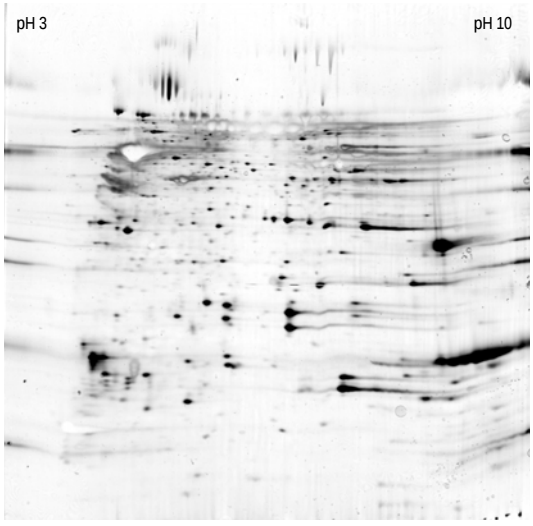


Fig.1. Glycoprotein electroblot patterns of seed albumins (A), globulins B (B) and glutelins (C)

Fig.2. 2D-electrophoresis of mature seed proteins



Self-incompatibility in Almond, *Prunus dulcis webbii*

Plant Genetic Engineering Group: Head of Group**, Margarida Oliveira**

Almond, *Prunus dulcis webbii*, is an economically important fruit tree grown in the Mediterranean basin. Like other Rosaceae, almond exhibits self-incompatibility (SI), a genetically determined characteristic that prevents self-fertilization or crossing between similar individuals. SI is determined by a single multi-allelic locus, the S locus, that encodes a protein with RNase activity on the pistil side. The S-RNases are thought to prevent the growth of incompatible pollen tubes. So far the pollen counterpart molecule in this process remains unknown. Since fertilization is essential for almond production, overcoming SI has been a major goal of almond breeders. Tuono is a self-compatible almond variety used in many breeding programs that is thought to have acquired its self-compatibility by introgression of this trait from a wild almond relative, *P. webbii*. *P. webbii* has been described as a self-compatible species.

A specific S-RNase allele, Sf, has been identified in Tuono and correlated with self-compatibility by segregation analysis. Also no RNase activity in the pistil could be attributed to the Sf allele. This work tested the theory behind the self-compatibility introgression from by isolating the Sf allele from *P. webbii*. Not only has this allele been successfully isolated and sequenced, but also, for each of the individuals tested two different S-alleles were cloned. The sequences of these new S-alleles were found to be homologous to other S-RNases from almond but also from different *Prunus* species, demonstrating once again that SI probably predates speciation in this genus. *P. webbii* was probably once a self-incompatible species and lost this ability in the course of evolution while maintaining at least part of the SI machinery. Although Sf seems to be a non-functional S-allele, it remains to be proven whether the other S- sequences cloned from *P. webbii* are also non-functional. If some individuals have fully functional S genes the breakdown of SI is probably due to other genes, linked, or not, to the S locus.

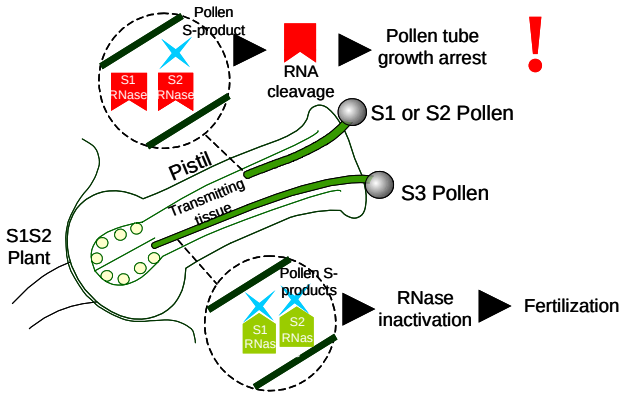


Figure 1 - Currently accepted model for RNase based SI mechanism
The diploid plant has two different pistil-expressing S-alleles, encoding two different S-RNases. The self or non-self pollen grains expressing the pollen S-counterparts of these pistil alleles have their growth arrested. Fertilization is only possible when there is no match between the pollen S-alleles and those expressed in the pistil.

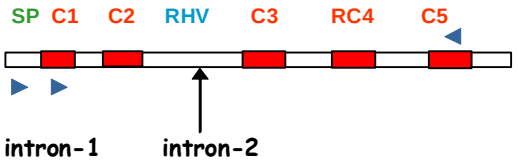


Figure 2 – Structure of *Rosaceae* S-RNase proteins
S-RNases possess five conserved domains (C1-C5) thought to be responsible for RNase activity. The protein is directed to the intercellular spaces of the transmitting tissue due the presence of a signal peptide; S-RNase specificity is probably located at the hypervariable region (RHV). S-RNase gene sequences is interrupted by two introns which also differ in size and sequence for different S-alleles. Blue arrows indicate position of primers.

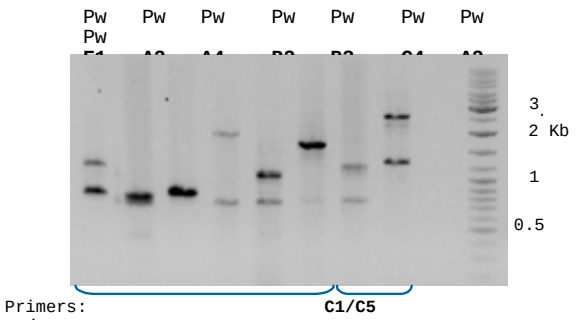


Figure 3 – Amplification of *Prunus webbii* S-alleles
Using primers against *Rosaceae* S-RNase conserved regions the S-alleles of six different ecotypes of *Prunus webbii* have been amplified. The obtained sequences were cloned and sequenced.

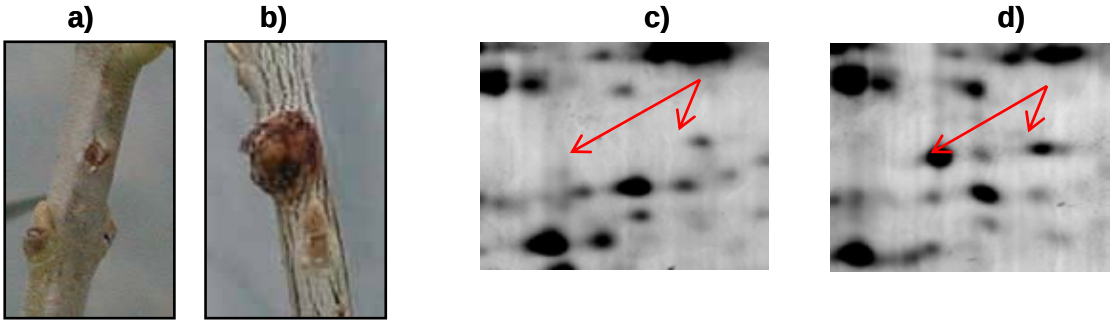
Identification of proteins differentially expressed in olive tissues infected with *Pseudomonas savastanoi* by 2D Electrophoresis and Peptide Mass Fingerprint.

Plant Biotechnology Group : Head of Group, **Pedro Fevereiro**

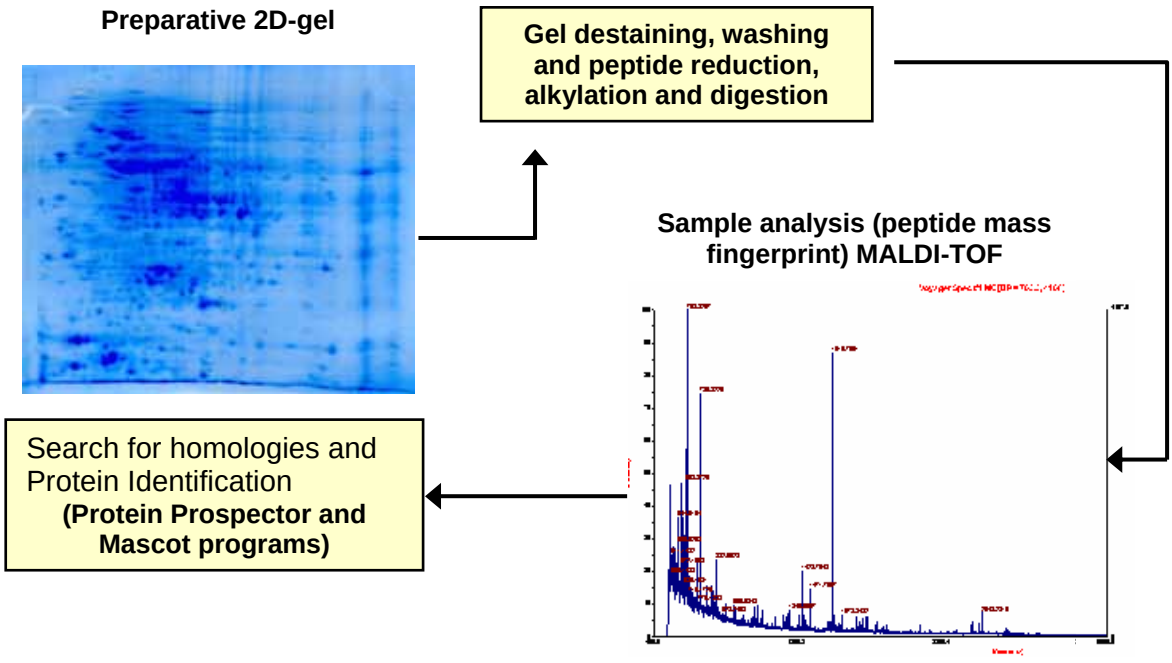
Olive tree (*Olea europaea* L.) can be infected by the bacteria *P. syringae* (subs*. savastanoi* Janse 1982) resulting in the formation of abnormal growths of plant tissues at wound sites. These outgrowths are known as knots and occur mainly in young twigs, branches and stems. The disease can cause serious losses in yield. The disease is reported in every country where *Olea europaea* grows. The fight against the disease implies the discovery of resistance genotypes, as well as the genetic basis of that resistance. The Portuguese cultivar Galega is known to have a tolerant phenotype in the field, having low to nearly absent disease symptoms of olive-knot disease.

Proteomics is a rapid emergent technology driven by the development of high-resolution two-dimensional gel electrophoresis (2DE) of proteins for the separation, detection and quantification of individual proteins, and mass spectrometry techniques (MS) together with sequence database searching for protein identification. Applying proteomics to plant-microbe interactions could bring some elucidation about the proteins involved in plant response, the biochemical pathways involved and their localisation in time and space.

In this study we aim to identify proteins that are differentially expressed during the initial stages of olive/*P. savastanoi* interaction.



Symptoms produced in olive plants after 8 weeks of infection with *P. savastanoi*. a) Control wound, b) knot; and differential protein expression in Galega stem tissues, after 7 days of infection c) control with distilled water, d) infection



Partial root dehydration leads to an improved water use efficiency.

Plant Molecular Ecophysiology Group : Head of Group, **Manuela Chaves**

It has been shown that roots that partially dehydrate have the ability to synthesize abscisic acid (ABA) which is then transported to the leaves via the xylem conduits. Once in the leaves, ABA induces stomatal closure, therefore preventing excessive leaf transpiration. This concept was applied to field growing grapevines (*Vitis vinifera* L) by watering the crop in alternate rows and therefore producing partial root zone drying (PRD). When we compared the sap flow data in PRD and DI vines (see Fig 1) we observed a significant decline in transpiration in PRD plants as compared to DI, in spite of plants of both treatments receiving the same amount of water. By decreasing transpiration without significantly affecting photosynthesis, water use efficiency is increased, as observed by the lower ^{13}C discrimination in the berries (Fig.2). This type of irrigation also led to diminished vegetative growth and increased cluster exposure, with potential benefits on the quality of production.

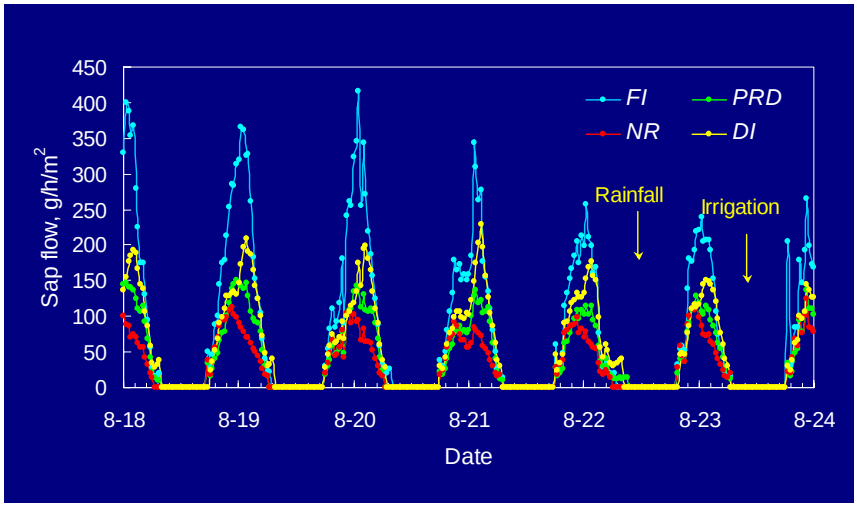


Fig 1: Sap flow rates (measured with sap flow meters on the left) in grapevines irrigated with 100% total crop evapotranspiration ETc (FI), 50% ETc (DI), 50% ETc with partial root drying (PRD)and non-irrigated grapevines (NI).

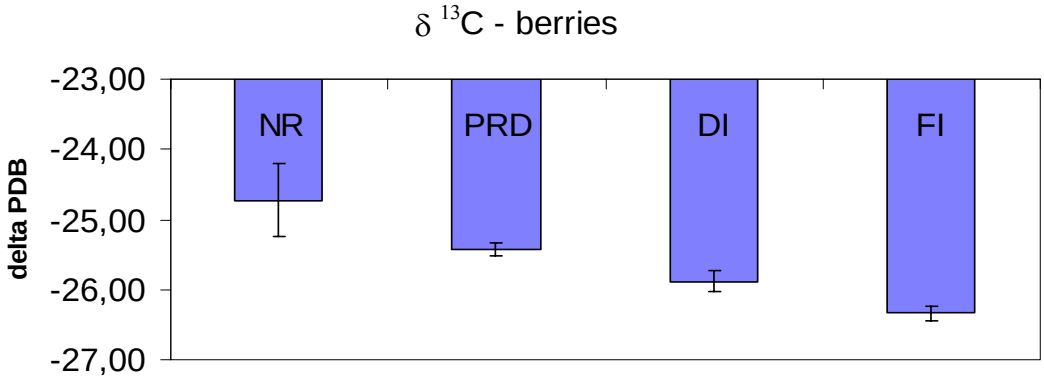


Fig 2: ^{13}C discrimination in berries of grapevines watered with 100% Etc (FI), 50% ETc (DI), 50% ETc with partial root drying (PRD)and non-irrigated grapevines (NI).

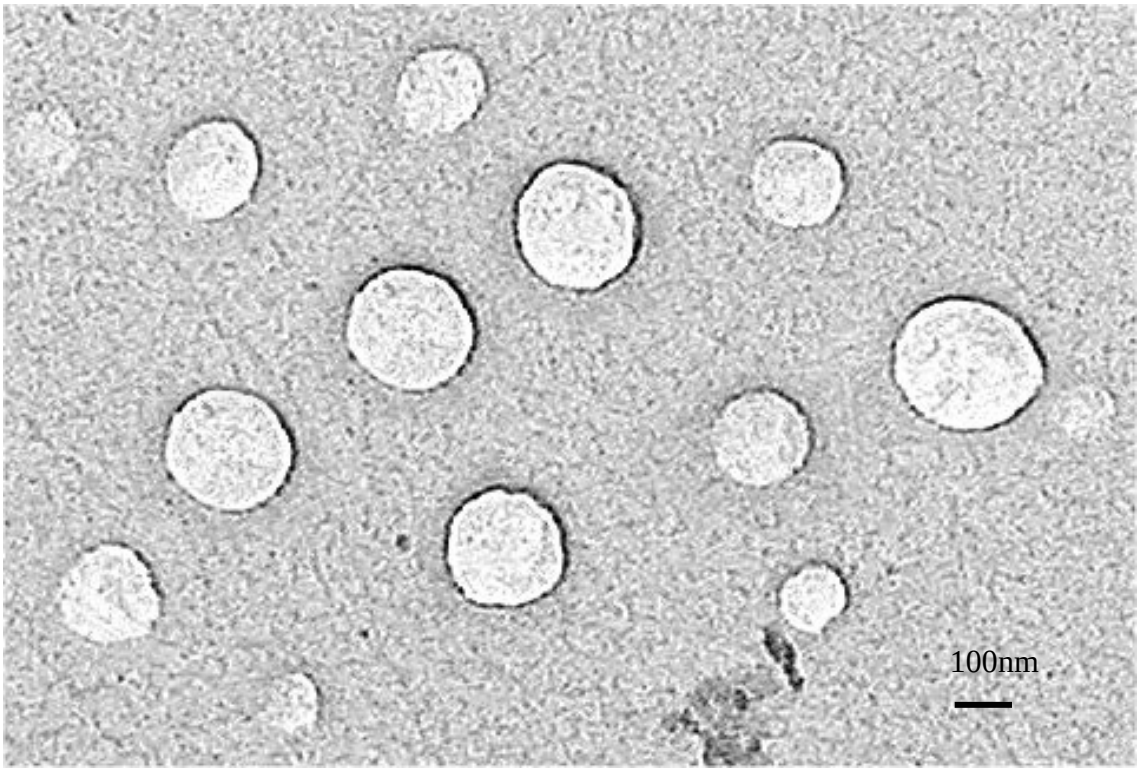
Grapevine Moving Particles in Vine Leaves

Plant Biochemistry Group II : Head of Group, **Ricardo Ferreira**

Two major pathogens are currently responsible for great losses and massive fungicide applications to vineyards around the world; *Uncinula necator* (the fungal causal agent of powdery mildew) and *Plasmopara viticola* (the oomycete that originates downy mildew). These pathogens co-evolved with the *Vitis* species in the Americas, where they are endemic. This probably explains why the American species of *Vitis* are inherently resistant to these pathogens.

The European *Vitis* species (*Vitis vinifera*), the single species responsible for the worldwide production of both grapes and wines, is naturally highly susceptible to these pathogens. This can easily be comprehended if we consider that *U. necator* and *P. viticola* are obligate pathogens that were introduced in Europe only in the 19th century. Therefore, before that date, *V. vinifera* evolved in the absence of these parasites. From the introduction of the pathogens in Europe, there was not enough time for *V. vinifera* to develop a resistance mechanism. Moreover, such development was blocked by the vegetative propagation of all the cultivars currently in use.

Grapevine moving particles (GMPs) have recently been detected in vine leaves. Their number per cell is very high for the American species and very low for their European counterparts. Also, their number in *V. vinifera* leaves appears to increase slightly during the course of an infection. They have the capacity to move inside the cells in which they occur and they maintain this capacity after being purified on sucrose gradients. They have a very powerful antifungal activity. We suggest that GMPs are partly or totally responsible for the difference in resistance observed between American and European *Vitis* species.



Structure of the purified particles as observed by transmission electron microscopy.

Genetics of Floral Symmetry.

Plant Developmental Genetics Group : Head of Group, **Jorge Almeida.**

Flowers are classified as actinomorphic or zygomorphic according to their symmetry. Actinomorphic, or radially symmetrical flowers, have two or more planes of symmetry whereas zygomorphic flowers have a single plane of bilateral symmetry. Another term for zygomorphy is dorsoventrality, after the observation that the plane of bilateral symmetry usually coincides with an axis of dorsoventral asymmetry of the flower. It is this view of zygomorphy, as a case of asymmetry, which has been adopted by developmental biologists who are interested in mechanisms generating differences. The theme of floral symmetry has also interested systematic botanists and evolutionary biologists as an instance of repeated evolution of a trait, since zygomorphy is thought to have arisen several times independently from an ancestral actinomorphic condition

Genes controlling dorsoventrality have been identified through studies with *Antirrhinum majus*, a species with zygomorphic flowers that is amenable to molecular genetic analysis. Two of these genes, *CYC* and *DICH*, encode related transcription factors required to establish asymmetry. These genes are expressed in dorsal regions of the flower and inhibit *DIV*, a determinant of ventral identity. This interaction has been suggested by the analysis of mutant combinations (Fig. 1). *div* Mutant plants have flowers in which the ventral region has lateral identity. In contrast, *cyc;dich* mutant plants have radially symmetrical flowers with all petals of the ventral type. This spread of ventral identity reflects the expansion of the domain of *DIV* activity, as suggested by the observation that inactivation of all three genes results in radially symmetrical flowers that are completely lateralized.

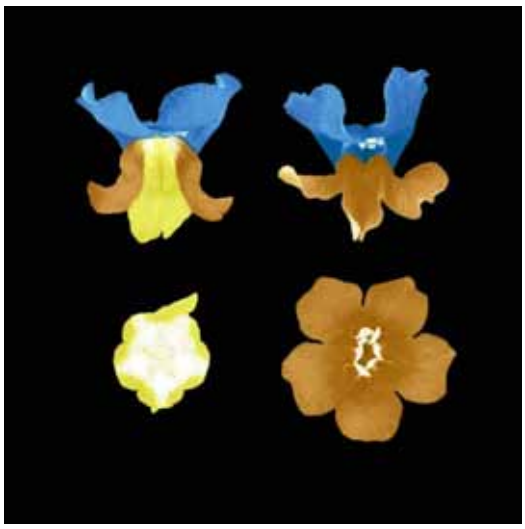
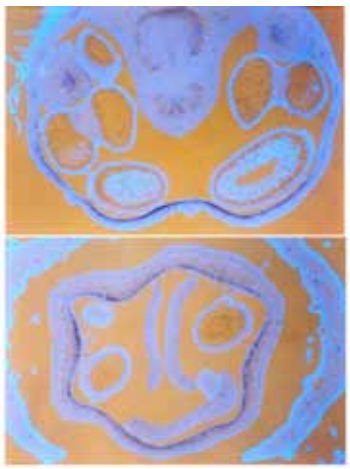


Fig. 1. The wild type *Antirrhinum* corolla has five petals of three types or identities according to their positions relative to a dorsoventral axis of the flower: Two dorsal (painted in blue in the top left flower), two lateral (brown) and one ventral (yellow). Top right: *div* mutant. Bottom left: *cyc;dich* double mutant. Bottom right: mutant for the three genes



The Plant Developmental Genetics group isolated *DIV* using a map-based strategy combined with transposon mutagenesis and showed that DIV is a protein of the MYB-family of transcription factors. Isolation of *DIV* allowed the interaction described above to be further dissected. *DIV* transcription is induced specifically in an epidermal layer of the ventral region of the corolla at a relatively late stage of development. In line with the view that *CYC;DICH* inhibit *DIV*, this induction occurs all around the corolla in *cyc;dich* double mutant flowers (Fig. 2).

Fig. 2. RNA-RNA in situ hybridization showing the expression of *DIV* (dark blue cells) in wild type (top) and radially symmetrical *cyc;dich* mutant buds (bottom). In each panel, dorsal is to the top and ventral is to the bottom.

RESEARCH SERVICES

Service: Analytical Chemistry
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Team:

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Valentim Almeida	Ph.D.
Rui Manuel Lopes Marques	Ph.D.
Cistina Maria Almeida	Ph.D. Student
Antero Ramos	Technician

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Head of Section: Maria Lucília Raimundo Gomes
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António Ferreira	Technician
António Pires	Technician
Sandra Silva	Technician
Mário Patrício	Technician

Service: Mass Spectroscopy
Head of Service: Ana Maria Varela Coelho
Team:

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Gonçalo Conde da Costa	Ph.D. Student
Elisabete Pires	Technician

Service: Small Molecules X-Ray Service

Isabel Bento	Post-Doc

Service: Amino Analysis and Protein Sequence Services
Team:

Manuela Regalla	Graduate Technician
Paula Chicau	Graduate Technician

Service: Elemental Analysis

Maria da Conceição ALmeida	Graduate Technician

Service: ITQB Fermentation Unit

João Nuno Carita	Graduate Technician

Pilot Plant: (IBET)
Head of Plant: António Cunha

Fermentation:

Lídia Gonçalves	Ph.D.
Rui Gomes	MSc
Sanda Monteiro	MSc
Filipe Pinto	Chemical Eng.

Purification:

Monica Thomaz	Chemical Eng.
Ruth Pinto	MSc
Sandra Miranda	Cehmical Eng.

Process Measurement and Control:

João Clemente	Electronic Eng.

Maintenance:

Sofia Dias	Mechanical Eng.

RESEARCH OUTPUT

PUBLICATIONS

Publications are organized by Research Divisions and the Laboratório Associado. The total number of publications of the ITQB researchers in 2003 on peer reviewed scientific journals was 131[2]. In the lists below, some papers repeat among the Research Divisions since they have contributions from researchers from more than one Division.

CHEMISTRY

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14. Cui X, Cabral MF, Costa J, Delgado R; Tetrazamacrocycle bearing quinoline pendent arms and its complexation properties; Inorg Chim Acta, 256, 133-141; (2003)

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2. Sampaio MM, Santos H, Boos W; Synthesis of GDP-mannose and mannosylglycerate from labeled mannose by genetically engineered *Escherichia coli* without loss of specific isotopic enrichment; Appl Environ Microb, 69 (1) 233-240; (2003)
3. Lemos PC, Serafim LS, Santos MM, Reis MAM Santos H; Metabolic pathway for propionate utilization by phosphorus-accumulating organisms in activated sludge: C-13 labeling and in vivo nuclear magnetic resonance; Appl Environ Microb, 69, 241-251; (2003)
4. Empadinhas N, Albuquerque L, Henne A, Santos H, da Costa MS; The bacterium *Thermus thermophilus*, like hyperthermophilic archaea, uses a two-step pathway for the synthesis of mannosylglycerate; Appl Environ Microb, 69, 3272-3279; (2003)
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11. Goncalves LG, Huber R, da Costa MS, Santos H; A variant of the hyperthermophile Archaeoglobus fulgidus adapted to grow at high salinity; FEMS Microbio Lett, 218, 239-244; (2003)
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14. Couto I, Wu SW, Tomasz A, de Lencastre H; Development of methicillin resistance in clinical isolates of Staphylococcus sciuri by transcriptional activation of the mecA homologue native to the species; J Bacteriol, 185, 645-653; (2003)

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5. Matos OC, Ricardo CP; Plant Screening for Light Activated Antifungal Activity; in *Plant Derived Antimycotics* Rai, MK, Mares, D (eds) The Harworth Press, Inc, pag 229-256, 2003
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8. Almeida P, Penha-Goncalves C; Long perfect dinucleotide repeats are typical of vertebrates, show motif preferences and size convergence; Molecular Biology Evolution, in press; (2004)
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12. Carmo M, Peixoto C, Coroadinha AS, Alves PM, Cruz PL, Carrondo MJT; Quantitation of MLV-based retroviral vectors using real-time RT-PCR; J Virological Methods, in press; (2004)

PATENTS

Diglycosyl glyceryl compounds for the stabilisation and preservation of biomaterials
H. Santos, P. Lamosa, C. Jorge & M. S. da Costa
International Patent Application (PCT/PT03/0000) submitted on 22/04/2003.

Identification of a bifunctional gene for mannosylglycerate synthesis and development of a high-scale-production heterologous system based on *Saccharomyces cerevisiae*
M. S. da Costa, N. Empadinhas, L. Albuquerque & H. Santos
Patente Internacional submitted in on 17/10/2003 (processo nº 3398007Y).

LIST OF PRIZES AND AWARDS

M. Abrantes, A. A. Valente, M. Pillinger, I. S. Gonçalves, J. Rocha, and C. C. Romão. Chem. Eur. J., 2003, 9, 2685-2695
"Sulfoxidation of benzothiophene with hydrogen peroxide catalyzed by organotin-oxomolybdate coordination polymers"
Awarded the selection for special frontispice annoucement in Chemistry-A European Journal.

Gonçalo Real, E. J. Harry, J. Erington, and A. O. Henriques. 2003.
"A link between chromosome structure and segregation and cell division at the onset of sporulation in *Bacillus subtilis*"
Meeting of the Portuguese Society for Microbiology, Tomar, November 29 – December 1 - Prize for the best poster presented at the Meeting (Gonçalo Real)

Marta C. Justino, Patrícia N. da Costa, João B. Vicente, Miguel Teixeira, Lígia M. Saraiva
"E. coli Flavorubredoxin, a novel type of Nitric Oxide Reductase"
SFRBM 2003 Travel Award, Society for Free Radical Biology and Medicine (SFRBM) 10th Annual Meeting of The Society For Free Radical Biology And Medicine, Seattle, Washington, USA, November 20-24, 2003 (Prize for one of the 12 best posters presented at the Meeting, attributed to Marta C. Justino)

Ana V Carvalhal, Sónia Sá Santos, Mathias Haury, Manuel J.T. Carrondo
"Cell growth arrest by nucleotides, nucleosides and bases as a tool for improved production of recombinant proteins"
Best Poster Presentation, 18th Meeting of the European Society for Animal Cell Technology, Granada, May 2003

Paula M Alves, Sónia Sá Santos, Luis L. Fonseca, Miguel AR Monteiro & Manuel J.T. Carrondo
"Alternative Approaches to Culture Brain Cells in Stirred Tank Bioreactors"
Best Poster Presentation, First World Congress on Regenerative Medicine, Leipzig, October 2003

Raquel Vale - award for the best Poster in the Plant section of the Society for Experimental Biology Main Meeting, March 31 – April 4, 2003. Southampton, UK (Lab Leader: M.Manuela Chaves)

2003 Prize for the Best Portuguese-French Scientific Collaboration (Prize awarded by Prof. Ramoa Ribeiro, President of the Associação Portuguesa de Doutorados em França, APDF) (awarded to Cecília Arraiano)

Invited visiting Professor at Department of Biochemical Sciences "A. Rossi Fanelli", University of Rome "La Sapienza" (Cláudio M. Gomes, 2003)

Appointed by the Portuguese Biophysical Society (SPB*f*) as the national candidate for the 2003 European Biophysical Society Award Prize. (Cláudio M. Gomes, 2003)

The 2004 European Medal for Bio-Inorganic Chemistry, awarded to Maria Arménia Carrondo and will be presented at EUROBIC-7 in Garmisch-Partenkirchen, Germany in August 2004.

Gordon Research Conference on Isotopes in Biological and Chemical Sciences (on its 50th Annyversary), Ventura, California, USA, Feb. 15-20, 2004. (Luis Paulo Rebelo was Invited as Plenary Lecturer)

Thermodynamics Applied for Environmental Purposes, Warsaw, Poland, November 13-16, 2003 (Luis Paulo Rebelo Invited as *Plenary Lecturer*)

ORGANIZATION OF SCIENTIFIC CONFERENCES AND SEMINARS

7th FIGIPS Symposium in Inorganic Chemistry, Lisboa, June 2003 (1 member of the Organizing Committee and sevral embers involved in different tasks during the actual Meeting) (Carlos C. Romão, Maria José Calhorda)

6º Encontro da Divisão de Catálise e Materiais Porosos, Évora, May 2003 (Carlos C. Romão was a member of the Scientific Committee)

5th International Meeting of the Portuguese Carbohydrate Group (Glupor 5), Covilhã, 2003 (Júlia Costa was a member of the Scientific Committee)

Congresso Nacional de Microbiologia - MICRO'03. Organized by H. de Lencastre. Other members of the Organizing Committee: I. Santos Sanches, I. Couto, A. M. Ludovice, R. Mato, Adiano Henriques. Hotel dos Templários, Tomar, Portugal. November 29 – December 2, 2003.

EURIS Final Management Meeting. Organized by H. de Lencastre. Lisbon, Portugal. December 5-6, 2003.

European Conference on Spore Biology. Bratislava, Slovac Republic, July 2004 (H. de Lencastre as a member of the organizing and Scientific Committees)

2nd Portuguese-Brazilian NMR Conference, Sintra (Portugal), 23-26 September 2003 (António V. Xavier)

Organization of the symposium "Control of Growth and Proliferation" – XXI International Conference on Yeast genetics and Molecular biology, Gothenburg, Sweden (Claudina Rodrigues-Pousada)

Collaboration in the Scientific Organization of the 1st Luso-Spanish workshop, Coruña (Claudina Rodrigues-Pousada)

Organization of the FEBS Executive Committee held in Carcavelos (Claudina Rodrigues-Pousada)

Co-organisation of the 5th Short course of the Portuguese Biophysical Society "Genomics and Proteomics: biophysical perspectives", Santarém, Portugal, October 2003. (Claudio Soares)

Workshop "Establishment of tick-colonies and tissue cell cultures for the immunizatiopn against tropical theileriosis" Oeiras/Lisbon – 31st March – 10th April 2003 (Abel Oliva)

International Meeting of the Project "Molecular and immunological characterisation of merozoite antigens and their encoding genes of a *Theileria* species highly pathogenic for small ruminants in China: application for the development of diagnostic tools and a vaccine" (ICA4-CT2000-30028). Oeiras / Portugal – April 10th 2003 (Abel Oliva)

ACS National Meeting – Symposium on Ionic Liquids, New York, NY, USA, September 7-11, 2003. (Luis Paulo Rebelo - Invited by the American Chemical Society to organize and preside a Session on Thermophysical Properties)

"Jorge Calado: 40 Years of Molecular Thermodynamics", Centro de Congressos, IST, Lisbon, Portugal, January 6, 2003. (Luis Paulo Rebelo, Manuel Nunes da Ponte members of the Promoting and Organizing Committee of the Conference)

I Encontro Nacional Sobre Microssatélites e Genética de Populações, INETI - Lisboa, 16-17 Junho (Pedro Fevereiro)

III Simpósio Nacional de Olivicultura, Membro da Comissão Científica ESACastelo Branco 29-31/Outubro (Pedro Fevereiro)

Biotec2003 – Membro da Comissão Científica – IPIMAR 6 a 8 de Dezembro (Pedro Fevereiro)

"EMBO practical course / Gulbenkian Biology course: The Molecular and Cellular Basis", IGC, ITQB, Oeiras (31 March-16 April, 2003) (Organizer: José Feijó, IGC/FCUL; Co- Organizer: M.M. Oliveira)

"XIII GREMPA Meeting on Almond and Pistachio" Mirandela, Trás-os-Montes (1-5 June 2003) (Organizers: M.M. Oliveira & Vitor Cordeiro)

"XXXI Jornadas Portuguesas de Genética", ITQB; Oeiras, Portugal (5, 6 Feb., 2004) Organisers: M. Mota, MM Oliveira, AM Sanchez, M Chaves, C Pinto Ricardo, C Arraiano, A Bessa, A Queiroz

Conference on 'Optimisation of water use by plants in the Mediterranean', Association of Applied Biologists, 24 – 27 March, Cala Bona, Majorca, Spain. Maiorca. (Member of Organizing Committee- M Manuela Chaves).

XIII Congresso Hispano-Luso de Fisiologia Vegetal , 16-19 Sep, Universidad Illes Baleares. Maiorca (Member of Organizing Committee- M Manuela Chaves).

Seminar: "O Progresso dos Conhecimentos em Biologia Vegetal". Celebration of the Centennial of "Sociedade de Ciências Agrárias de Portugal". Joint organization of C. Pinto Ricardo (ITQB) and Maria Manuela Chaves (Sociedade Portuguesa de Fisiologia Vegetal).ITQB, 11December 2003.

Ph.D. THESIS DEFENDED DURING 2003

NAME	DATE	SUPERVISOR	TITLE	AREA
Sofia Isabel Vaz Queiroz de Macedo	23-01-2003	Maria Arménia Carrondo	X-ray Structure Determination of Iron-Containing Proteins from *Desulfovibrio desulfuricans* ATCC 27774 in Different Redox States: Bacterioferritin and Hybrid Cluster Protein	Biochemistry
Ana Verónica de Sousa Uva Carvalhal	27-02-2003	Manuel Carrondo	Mammalian Cell Growth Arrest for Biopharmaceutical Production improvement	Chemical Eng.
Luis Jorge Camilo Maranga	28-02-2003	Manuel Carrondo	Engineering Challenges in the Production of VLPs - understanding baculovirus-insect cell interactions	Chemical Eng.
Nuno Miguel Deodato Fontes	04-04-2003	Susana Barreiros	Studies on critical factors affecting enzyme activity in nonaqueous media: supercritical fluids and organic solvents	Chemical Eng.
Marta Gibert Aires de Sousa Ferreira	04-07-2003	Hermínia Lencastre	Bridges From Hospitals to the Laboratory: Genetic Portraits of Methicillin-Resistent *Staphylococcus aureus* Clones	Biology
Isabel Maria Travassos de Almeida de Jesus Bento	14-07-2003	Maria Arménia Carrondo	Structural Studies of the Tetrahaem cytochrome c_3 and nine haem cytochrome c from *Desulfovibrio desulfuricans* ATCC 27774 in the oxidised and reduced state. Correlation with cooperativity effects	Biochemistry
António Carlos Silva Henriques	15-07-2003	Christopher Maycock	Síntese Assimétrica e Reacções de sulfóxidos e sulfonas de ditioacetais ciclícos	Chemistry
Joana de Almeida Pires da Fonseca	16-07-2003	Manuel Nunes da Ponte	Vapour Liquid Equilibrium Measurement and Emulsion Formation in $ScCO_2$	Chemical Eng.
Ricardo Castela Gomes de Azevedo	18-09-2003	Luís Paulo Rebelo	Termodinâmica de Líquidos Puros em Regimes Estáveis e Metaestáveis. Uma nova microcélula não-intrusiva para a monitorização da velocidade do som em condições (p,T) extremas	Chemical Eng.
Ilídio Joaquim Sobreira Correia	19-09-2003	António Xavier	Characterization of three multiheme cytochromes isolated from sulfidogenic bacteria	Biochemistry

NAME	DATE	SUPERVISOR	TITLE	AREA
Célia Maria Valente Romão	06-10-2003	Miguel Teixeira	Iron metabolism is sulfate reducing bacteria: the hemoferritin from *Desulfovibrio desulfuricans* ATCC 27774	Biochemistry
Marta Wilton Pereira Leite de Vasconcelos	14-10-2003	Mª Margarida Oliveira	A Biotechonological Strategy to Improve Iron Content in the Endosperm of Elite Rice Varieties	Biology
Helena Isabel Abrantes de Gouveia da Mota Veiga	07-11-2003	Luís Paulo Rebelo	Líquidos a pressões negativas: correlação entre propriedades de equilíbrio e propriedades em regimes de metaestabilidade	Chemical Eng.
Rita Alexandra da Luz Benasulin Santos Lemos	27-11-2003	Miguel Teixeira	Respiratory chains in prokaryotes. A novel complex in archaea and the oxygen respiratory chain from na "anaerobe"	Biochemistry
Maria de Fátima Afonso Cairrão	12-12-2003	Cecília Arraiano	Regulation of 3' to 5' exoribonucleases from the RNR family	Biology

VISITING SCIENTISTS

- Chantal Daniel, CNRS, Univ. Strasbourg, France (Host: Maria José Calhorda)
- Carlo Mealli, CNR, Florence, Italy (Host: Maria José Calhorda)
- Professor John Trojanowski and Professor Virginia Lee, University of Pennsylvania (Host Júlia Costa)
- Jerôme Etienne, MD, Ph.D., Professor, Hôpital Edouard Herriot, Lyon, France (Host: Hermínia de Lencastre)
- Alex Van Belkum, Ph.D. Professor, Erasmus Medical Centre Rotterdam, The Netherlands.m (Host: Hermínia de Lencastre)
- Rob J. L. Willems, Ph.D., Professor, National Institute of Public Health and the Environments (RIVM) Bethoven, The Netherlands (Host: Hermínia de Lencastre)
- Alexander Tomasz Ph.D., Professor and Head of the Laboratory of Microbiology and Infectious Diseases. The Rockefeller University, New York, USA (Host: Hermínia de Lencastre)
- Steven J. Projan, Ph.D., Director of Antibacterial Research, Wyeth-Ayerst Research, Pearl River, USA (Host: Hermínia de Lencastre)
- Simon M. Cutting, Ph.D., School of Biological Sciences, Royal Holloway University of London, UK. (Host: Adriano O. Henriques)
- Lynne E. MAQUAT, Ph.D., University of Rochester, Rochester, New York, U.S.A. April 2003 (Cecília Arrainao)
- Carsten Schwalb, Ph.D. Institute of Cell and Molecular Biology, University of Edinburgh, Scotland (Host: Carlos Salgueiro)
- Olivier Bensaúde, Ph.D., École Normale Supérieure, Paris (Claudina Rodrigues-Pousada)
- Michael Tuite, Ph:D., University of Kent (UK) (Claudina Rodrigues-Pousada)
- Robert Poole, Ph.D. University of Reading, UK (Host: Miguel Teixeira)
- Simon Andrews, Ph.D., University of East Anglia, UK (Host Miguel Teixeira)
- Jerry King, Ph.D., Los Alamos National Laboratory (Host: Manuel Nunes da Ponte / Catarina Duarte)
- Kenneth R. Seddon, Ph.D., The QUILL Centre, The Queen's University of Belfast
- "Ionic Liquids and Green Chemistry", October 2003 (Host: Luís Paulo Rebelo)
- Carlos A. Pereira, Ph.D., Univ. Strasbourg, França and Instituto Butantan, Brasil (Manuel J.T.Carrondo / Paula Alves)
- John Aunins, Ph.D., MERCK & CO, Philadelphia, EUA and Invited Researcher, ITQB/UNL, Portugal (Manuel J.T.Carrondo / Paula Alves)
- Hansjorg Hauser, Ph.D., GBF and Univ. Braunschweig, Germany (Manuel J.T.Carrondo / Paula Alves)
- Mike Jackson (Director for Program Planning & Coordination of the International Rice Research Institute) (8th July, 2003) (Host: Margarida Oliveira)
- Swapan K Datta (Head of Tissue Culture and Genetic Engineering Lab at the Ineternational Rice Research Institute) (14th October, 2003) (Host: Margarida Oliveira)

RESEARCH PROJECTS FUNDED BY PORTUGUESE AND FOREIGN AGENCIES

Projects funded by FC&T:

REFERENCE	TITLE	PROJECT LEADER ITQB	DURATION
QUI/10013/ 1998	Phase behaviour of the epicuticular wax layer in plants and its relation to cuticular transport: a model study.	Eurico de Melo	1999-2003
BIO/11070/ 1998	Development of lactic acid bacteria strains deficient in ribonucleasesfor food processing by the dairy industry	Cecilia Arraiano	2000-2003
BIO/12080/ 1998	Application of new phasing methods to explore structural diversity in the cytochrome family	Mª Arménia Carrondo	2000-2003
BIO/14314/ 1998	Structure-function relationships of serine hydrolases in non-conventional media	Susana Barreiros/ Claudio Soares	2000-2003
FCT 33499/99	Molecular characterisation of almond genes associated with self-fertility and late flowering	Margarida Oliveira	2000-2003
FCT 36212/99	Molecular analysis of arabinan degradation in Bacillus subtillis	Isabel Sa Nogueira	2000-2003
FCT 34872/99	Molecular evolution and molecular epidemiology of methicillin resistance in Stphylococcus aureus	Hermina de Lencastre	2000-2003
FCT 36562/99	Metabolism of sulfate reducing bacteria isolated from humans: implications for ulcerative colitis	Ines Cardoso Pereira	2000-2003
FCT 35877/99	Activity against mycobacterium tuberculosis and the mycobacterium avium complex, and immunomodulation by macrocyclic compounds	Rita Delgado	2000-2003
FCT 34842/99	Staphylococi of animal origin: molecular identification, antimicrobial resistance and virulence mechanisms	Hermina Lencastre	2000-2003
FCT 36245/99	Diagnosis , isolation and molecular epidemiology of CAEV: A goat retrovirus	Cecilia Arraino	2000-2003
FCT 34955/99	Thermdynamics of critical phenomena in binary liquid-liqid equilibria. Simulations, theory and experiment	Luis Paulo Rebelo	2000-2003

REFERENCE	TITLE	PROJECT LEADER ITQB	DURATION
FCT 32789/99	Molecular modelling of key redox proteins in bacterial metabolism: methodology and applications	Claudio Soares	2001-2004
FCT 33201/99	The role of extension peroxidase and extension deposition in plant development	Philip Jackson	2000-2003
FCT 34967/99	Interplay of the transcription factors encoded by yap gene family in stress response	Claudina Rodrigues-Pousada	2001-2004
FCT 34418/99	Charaterization of bacteriophage impact and dynamics in natural populations of Streptococcus pneumoniae	Mario Ramirez	2001-2003
FCT 35021/99	Structural, thermodynamic and kinetic bases for energy transduction in an anaerobe: Desulfovibrio desulfuricans ATCC 27774	Antonio V. Xavier	2001-2004
FCT 35109/99	Analysis of an intercellular signalling pathway coupling gene expression to morphogenesis in Bacillus subtillis	Adriano Henriques	2000-2003
FCT 35131/99	Molecular basis of protein thermostabilization by hypersolutes	Helena Santos	2001-2004
FCT 36155/99	The role of RNase II an its homologues in the control of gene expression: structural and functional studies	Cecilia Arraiano	2000-2003
FCT 36164/99	Structure-function relation of Arabinose binding proteins of Bacillus subtillis	Isabel Sa Nogueira	2000-2003
FCT 36191/99	Symbiotic nitrogen fixation: common features between actinorhizal and legume nodules	Ana Ribeiro	2001-2004
FCT 36560/99	Novel respiratory complexes: molecular basis for energy transduction	Miguel Teixeira	2000-2003
FCT 35699/99	Cytochrome c7: a haem protein with sulfur reductase activity	Ricardo Louro	2001-2003
FCT 35715/99	Towards the utilization of cell-reactors for the production of mannosylglycerate, an enzyme stabiliser from hyperthermophiles	Helena Santos	2001-2004
FCT 35719/99	Exploiting metabolic biodiversity: sugar metabolism in the hyperthermophile *Archaeoglobus fulgidus*	Helena Santos	2001-2004

REFERENCE	TITLE	PROJECT LEADER ITQB	DURATION
FCT 36203/99	Bola confers resistance to antibiotics: study of this new target focusing on the control of cell division and protein prodution	Cecilia Arraiano	2001-2004
FCT 36558/99	Mechanisms of iron and oxygen responses in anaerobic prokaryotes	Miguel Teixeira	2001-2004
FCT 35396/99	Non-symmetric macrocycles for metal complexes and supramolecular aggregates with pesticides	Rita Delgado	2001-2004
FCT 36127/99	Experimental and theoretical studies on group 5 and 6 metallocene ring-slippage processes	Carlos Romão	2001-2004
FCT 35413/99	Polymer-surfactant interactions in aqueous solutions: phase diagrams, calorimetry, rheology and dynamics of polymer chains	António Lopes	2001-2004
FCT 34794/99	Dynamic invariance in biological systems	Jonas de Almeida	2001-2004
FCT 33679/99	Photophysics and photochemistry of anthocyanins	João Carlos Lima	2001-2004
FCT 35429/99	Phase behavior and microscopic characterisation of micro/macro-emulsions in CO2. New strategies for polymerisation and enzymatic catalysis	Susana Barreiros	2001-2004
FCT 35679/99	Purification, Gene cloning and characterization of plant fucosyltransferases	Pedro Fevereiro	2001-2004
FCT 34701/99	Structure and function of eleicitins and their role in the infection of the cork oak by the fungus Phytophthora cinnamomin	Paulo Martel	2001-2004
BIO/35675/99	Production of PHB/PHV CoPolymers by mixed cultures	Helena Santos	2000-2003
CBO/35859/99	Radiopharmaceuticals for treatment of bone cancer pain and/or therapy based on macrocyclic lanthanide complexes	Rita Delgado	2002-2005
QUI/37726/01	Novel rhenium complexes for olefin metathesis and aldehyde olefination:synthesis, characterisation and catalytic studies	Betariz Royo Cantabrana	2002-2004

REFERENCE	TITLE	PROJECT LEADER ITQB	DURATION
QUI/42902/01	Structural and functional mapping of a novel multihaem cytochrome: a model for fumarate reductases	Carlos Alberto Salgueiro	2002-2005
QUI/37521/01	Understanding the intrinsic stability mechanisms of hyperthermophilic metalloproteins	Claúdio Moreira Gomes	2002-2004
QUI/38269/01	Biphasic hydrogenation and oxidation of supercritical carbon dioxide	Manuel N.Ponte	2002-2004
QUI/43435/01	Binding of diatomic molecules to haem proteins	Teresa Catarino	2002-2005
QUI/38884/01	Intramolecular complexes and nanoencapsulation of anthocyanins	António Maçanita	2002-2005
QUI/43323/01	Electron transfer mechanisms nad dynamics of a proton thruster: cytochrome c3	Peter Hildebrandt	2002-2004
QUI/43313/01	Approaches to the synthesis of optically pure natural compounds having a dehydrodecalin nucleus	Christopher Maycock	2002-2005
EQU/41644/01	Applications of static mixers in supercritical technology	Manuel N.Ponte	2002-2004
BCI/38861/01	Modelling biomembrane rafts: charaterization of the physical-chemical properties of lipid bilayer membranes with raft-like structures	Eurico de Melo	2002-2005
BCI/38631/01	Identification and characterization of proteins involved in Golgi organization from mammalian cells	Júlia Costa	2002-2005
ESP/41971/01	Group A streptococci in the community: molecular epidemiology and clinical relevance	Ilda Sanches	2002-2005
BME/38859/01	Structure-function studies on novl iron-cluster containing proteins	Carlos Frazão	2002-2005
BME/42377/01	Rnases and polyadenylation in the adjustment of bolA mRNA levels necessary for cells growth and survival	Cecilia Arraiano	2002-2004
AGR/39095/01	Coat protein mediated reistance against almond llarviruses: strategy and risk assessment	Margarida Oliveira	2002-2004
AGR/38507/01	Adventitious organogenesis in almond: an histological and molecular approach	Ana Sanchez	2003-2005
BIO/43105/01	Molecular basis of the functioning of immobilised redox enzymes in bioelectronic devices	Daniel Murgida	2003-2005
BME/37406/01	Unravelling the acrobic respiratory chain of the “anaerobic” sulfate reducing bacteria	Miguel Teixeira	2003-2005

REFERENCE	TITLE	PROJECT LEADER ITQB	DURATION
BME/37480/01	Genes involved in bioenergetic mechanisms in the sulphate reducing bacterium Desulfovibrio gigas	Claudina R. Pousada	2003-2005
BME/44597/02	Reduction of nitirc oxide in prokaryotes: new metabolic routes	Miguel Teixeira	2003-2005
BME/45810/02	Increasing realism in protein modelling: including pH and rdox effects into melecular dynamics simulations	António Baptista	2003-2005
CBO/39099/01	Structure and function of the centrosomal proteins HsMob in cell division	Mª Arménia Carrondo	2003-2005
EQU/35437/99	Green processing with onic liquids coupled to supercritical CO2 extraction or membrane pervaporation	Luis Paulo Rebelo	2003-2005
ESP/44782/02	Anaerobic metabolism of the human pathogen bilophila wadsworthia	Inês Cardoso Pereira	2003-2005
QUI/45090/02	Structure, tropisms and molecular dynamics of the stratum corneum lipid matrix. A study in model systems	Eurico de Melo	2003-2005
QUI/45758/02	Thermodynamics of metalloprotein folding and stability	Claudio Gomes	2003-2005
ESP/46428/02	Structural characterization of MrkD protein from Klebsiella pneumoniae: implications in the epithelial adhesion properties of the microorganism	Francisco Enguita	2003-2005
BME/45122/01	Energy transduction in a plant symbion from Sinerhizobium melitoli	Manuela Pereira	2004-2006
AGR/46671/02	Cell wall proteins with roles in xylogenic programmes in eucalyptus	Philip Jackson	2003-2005
QUI/47866/02	Oxidative Phosphorylation in sulfate respiration	António V. Xavier	2003-2005
BCI/48647/02	Dissection of a checkpoint linking chromoso segregation to asymmetric cell division at the onset of endospore development in Bacillus subtilis	Adriano O. Henriques	2003-2005
AGR/49306/02	Study of ribonucleases from lactid acuid bacteria for the construction of strains important for food processing by the dairy industry	Cecilia Arraiano	2003-2005
AGG/45073/02	Removal of contaminants from municipal solid waste incinerators fly ashes aiming their valorisation	Alexandra Ribeiro	2003-2005
POCTI BIO/48333/2002	Global experimental approches to medel central metabolism in L. lactis: modulation of the elvels of key-enzymes	Helena Santos	2004-2007
POCTI/BIO/42331/2001	New Compatible Solutes from Thermophiles and Hyperthermophiles: Screening, Identification and Physiological Role	Helena Santos	2003-2006

REFERENCE	TITLE	PROJECT LEADER ITQB	DURATION
CBO/43952/02	Clinical, neurophysiological and neurochemical studies in amyotrophic lateral sclerosis	Júlia Costa	2003-2005
AGG/41359/02	Reforestation woth corl oak: genetic variability and seed storage biology	C.Pinto Ricardo	2003-2005
QUI/49114/02	Macrocyclic ompounds selective for heavy metals poisoning: Cd(II), Hg(II), and Pb(II)	Rita Delgado	2003-2005
MGI/40878/01	In search of new molecular targets for the development of novel therapeutic strategies for cystic fibrosis	Ana Coelho	2003-2005
AGG/46371/02	Searching for rhizobial strains with improved skills to thrive in arid lands	Helena Santos	2004-2006
MGI/47382/02	Folding, procesing and function of normal and mutant cystic fibrosis transmembranar conductance regulator: structural implications	Claúdio Soares	2003-2005

Projects funded by FCG:

Title	Project Leader	Duration
Functional genomics and high resolution molecular typing of methicillin resistant *Staphylococcus aureus* (MRSA)	Herminia Lencastre	2002-2005
Towards eradication of drug-resistance bacterial disease in a tertiary hospital: mapping reservoirs and transmission routes of methicillin resistant *Staphylococcus aureus*	Herminia Lencastre	2002-2005

Projects funded by the European Comission:

REFERENCE	TITLE	PROJECT LEADER	DURATION
QLK2-CT-200/01020	European resistance Intervention study – Reducing resistance in respiratory tract pathogens in children	Herminia de Lencastre	2000-2003
QLK2-CT-2000-01404	Network for automated bacterial strain fingerprinting in Europe	Hermina de Lencastre	2000-2003
QLK3- CT-2000-00640	Exploiting new solutes from hyperthermophiles for the preservation of biomaterials: cell factories for production of hypersolutes.	Helena Santos	2001-2004
CRAFT, contrato nº COOP-CT-2003-58644	New Applications for Compatible Solutes from Extremophiles	Helena Santos	2004-2006
QLK5-CT-2001-01729	Spore probiotics: an alternative to antibiotics in animal husbandry	Adriano Henriques	2001-2004
QLK3-CT-2002-01938	Phototrophic biofilms and their potential applicatioons: towards the development of a unufying concept	Jonas Almeida	2002-2005
QLG2-CT-2002-00988	Structural proteomics in Europe	Mª Arménia Carrondo	2002-2005

RESEARCH PROJECTS OF ITQB RESEARCHERS AT IBET

REFERENCE	PROJECT LEADER	TITLE	OTHER PARTICIPANTS
POCTI/AGR/33434/99	Cândido Pereira Pinto Ricardo	Studies for improvement of rice produced in portugal and mozambique	
POCTI/BIO/35679/99	Júlia Carvalho Costa	Purification, gene cloning and characterization of plant fucosyltransferases	ITQB-UNL ISA-UTL
POCTI/BIO/35687/99	Manuel José Teixeira Carrondo	Cell cycle control for better biotechnological methods in therapeutic protein production	
POCTI/BIO/35695/99	Manuel José Teixeira Carrondo	Stabilization of retroviruses for gene therapy clinical applications	Genethon Gesellschaft für Biotechnologische Forschung, GBF
POCTI/AGR/36239/99	Manuel Pedro Salema Fevereiro	Study of the Resistance of Portuguese Olea Europaea L. to Psudomonas Savastanoi	Centro de Genética e Biologia Molecular - UL Centro de Ciências do Ambiente - UM
POCTI/BME/38306/2001	Margarida Archer	Structural analysis of methyltransferase complexed with novel inhibitors for rational drug design	BIAL - Portela & C^{a}., SA
POCTI/AGR/38940/2001	Maria Vitória San Romão	Study of the mechanism of chloroanisol formation on cork stoppers	
POCTI/BIO/39214/2001	Paula Maria Marques Alves	Metabolic trafficking between neurones and astrocytes under stress conditions implicated in neurological disorders: 13C-NMR and flux analysis	
POCTI/AGR/39371/2001	Maria de Fátima Silva Lopes	Enterococci in food: antibiotics and safety	
POCTI/AGR/39613/2001	Maria Teresa Crespo	Characterisation of peptides catabolism system of meat and wine lactic acid bacteria	

REFERENCE	PROJECT LEADER	TITLE	OTHER PARTICIPANTS
POCTI/AGR/46283/2002	Célia Rodrigues Miguel	Monitoring retrotransposon activity during in vitro propagation of maritime pine	
POCTI/BIO/46515/2002	Paula Maria Marques Leal Sanches Alves	Integrated strategy for production, purification and storage of adenovirus	
EC	Manuel J.T.Carrondo	Bionose for animal cells, electronic noses for non invasive quality, safety and in process control of industrial animal cell culture processes	2000-2003
EC (ICA4-CT-2000-30027)	Manuel J.T.Carrondo	Development of Oral Vaccines, Role of Wildlife in The Maintenance of these Diseases	2001-2004
EC (8 ACP TPS 040)	Manuel J.T.Carrondo	Mass production *of Cowdria ruminantium* to produce a cost effective veterinary vaccine against heartwater	2001-2004
EC (QLRT-2001-01249)	Manuel J.T.Carrondo	Novel strategies for a safe rotavirus vaccine	2003-2005
EC. (QLK3-CT-2002-01949)	Manuel J.T.Carrondo	Integrated Strategies for efficient therapeutic retrovirus based on modular cell lines	2003-2005
IBET –Agro 33-201	Vitória San Romão	Formação de aminas biogénicas no vinho – caracterização de produtos oncológicos existentes no mercado	EVN - IBET
PIDDAC - 704/2001	Vitória San Romão	Compounds related to cork taint in wine. Validation of analytical methods and study of the mechanisms involved in their formation	EVN - IBET
(ADDAV) 2000-2004 INCO-DEV	Abel Oliva	Molecular and immunological characterization of merozoite antigens and their encoding genes of a Theileria species highly pathogenic for small ruminants in China: application for the development of diagnostic tools and a vaccine	

REFERENCE	PROJECT LEADER	TITLE	OTHER PARTICIPANTS
INCO-DEV	Abel Oliva	Molecular characterization of Latin American and Mediterranean *Babesia bovis* and *B. bigemina* strains and its application for the development of improved control strategies	(MEDLABAB). 2004-2007
(ICTTD-3) 2004-2008 CA	Abel Oliva	Intergrated Consortium on Tick and Tick-borne Diseases	
(CellPROM)– IP	Abel Oliva	Cell Programming by Nanoscaled Devices.	2004-2009
QLRT-2001-02380-DECARBOXYLATE)	Helena Santos	Fermentation of Food Products: Optimised Lactic Acid Bacteria Strains with Reduced Potential to Accumulate Biogenic Amines	2003-2006
QLK1-CT-2000-01376)	Helena Santos	Increase in Nutritional Value of Food Raw Materials by Addition, Activity, or *In Situ* Production of Microbial Nutraceuticals	2000-2003

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