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ITQB at a glance

Operating since 1989

Research Institute
of Universidade Nova de Lisboa
since 1992

Laboratório Associado
since 2001
ITQB / IGC / IBET

Director: Peter Lindley

5 Research Divisions

CHEMISTRY
BIOLOGICAL CHEMISTRY
BIOLOGY
PLANT SCIENCES
TECHNOLOGY

52 Laboratories

141 PhD holders

179 PhD students

21 PhDs awarded in 2005

103 Ongoing Research Projects

195 Papers published in 2005

Foto: David Marçal

Introduction by the Director

This is the last occasion that I shall have the opportunity to write the foreword to the Annual Report and Plan for the ITQB. My term of office as Director will terminate later this year and then I shall return to England. The last few years in Portugal have provided me with a large number of pleasant experiences and I have had the privilege to meet many people who it is an honor to call friends. Portugal is a very beautiful country with a very varied coastline and an interior ranging from the agricultural to the rugged mountains typified by the Serra de Estrela. Like the climate the Portuguese are warm and sunny and I have really enjoyed your kind hospitality and, of course, your cuisine and excellent wines, particularly those from Bairrada and Alentejo. However, from the financial and scientific viewpoints my stay has been less enjoyable and in my usual forthright, but I hope relatively diplomatic manner, I must make a few comments on these points.

The Director seeks inspiration and finance!

Before I came to the ITQB, I was assured that although Portugal was not one of the richer countries in Europe, the financial situation looked reasonably stable for the next few years. If only we had all known what was about to happen! Of course it turned out that almost immediately the ITQB was faced with financial difficulties and the last few years have been very difficult indeed. For a foreigner accustomed to a stable and reliable annual budget, the financial situation has been, from time to time, almost impossible to comprehend. It is not so much the size of the budget, but the fact that the majority of it has come as “soft” money in an irregular and often seemingly random fashion. I am now very well aware of the problems faced by the Fundação para a Ciência e a Tecnologia and its relationships with the Government of the day, and with the European Union. I would like to take this opportunity, once again, to record my thanks for the help and support provided by Professor Ramôa Ribeiro (former President of the FCT) and his colleagues, whenever they have been able. However, if resources are not forthcoming in a regular and planned manner, then it is almost impossible to undertake proper management and to make realistic plans for the future; the most common effect of a “stop-go” budget is a waste of resources. Further, I am still left with the feeling that the distinction between scientific excellence and mediocrity (or worse) in this country is not fully recognized and that this works to the severe disadvantage of an Institute as prestigious as the ITQB and its Laboratório Associado. To the funding bodies I will give the following message, *“If a scientific organization is going to execute research at the national or international level in the Life Sciences, a field that must surely be at the forefront of strategic importance to Portugal, then it must be resourced properly. The true costs of pursuing research in Molecular Biology and associated Biomedical fields must be recognized by the Government and resources allocated accordingly in a regular and systematic manner.”*

The Laboratório Associado contract initiated in 2000 specified the phased recruitment of 25 young scientists and 9 technicians to undertake research in the five scientific themes, Biologically Active Molecules, Molecular Medicine and Veterinary Studies, Developmental Biology in Animals and Plants, Biological Risk and Improvement of Plants and Forest.

During the various financial crises there was a strong temptation to save money by not adhering to the contract, but I made a very firm decision that this would not be in the best interests of the Laboratório Associado and that a full recruitment should take place as specified. This strategy was not supported by everyone at the ITQB, but I believe that it has enabled us to survive the difficult years and also build a firm platform for future success. Hopefully the year 2006 and subsequent years will see some stabilization of the budget since the financing of the Laboratório Associado component has been transferred to the PIDDAC. I am also told that the Laboratório Associado will be able to recruit around 30 more staff over the next five years, the second phase of funding, and this will enable some new fields of research and the strengthening of existing ones. Of particular interest are areas involving Neurosciences, Biomathematics, Animal Cell Technology, Membrane Proteins and Protein Complexes, the Use of Ionic Liquids for Chemical and Biological Processes, and the involvement of the Laboratório Associado in the various National Services.

I am often asked why I agreed to become the Director of the ITQB and the Laboratório Associado. There is no simple answer, but the desire to help young scientists, personal vanity, and the nature of the Laboratório Associado itself, all played a part. The Laboratório Associado is probably unique in Portugal with its combination of two, essentially fundamental research institutes covering many important aspects of the Life Sciences and related areas, and an institute that is designed to provide an interface with industry. I have always believed a statement made by Sir George Porter, when he was the Director of the Royal Institution in the UK which roughly translates as, “the only difference between fundamental and applied research is that fundamental research has yet to find an application”. I am still firmly convinced that although the individual components of the Laboratório Associado have great merit, the combination of them is far more effective and powerful and will provide the best contribution to the development of Science and Technology in Portugal and Europe. This is because it offers the flexibility to move, as appropriate, between academic and applied research. This does not imply that everyone must move towards applied research and that fundamental research has little, or no, place in the scientific program. It provides the flexibility to go from a scientific discovery towards a product that could help to improve the quality of life and/or the environment. This path is available for those scientists who wish to take it. I firmly believe that the Laboratório Associado is the future for the ITQB and links and collaborations within it should continue to be developed and strengthened.

From the organizational viewpoint I have not been able to achieve all the objectives that I set out to undertake at the beginning of my term of office. This has been mainly due to financial problems, but the unwillingness of some staff to adapt to new circumstances has also played a role. When I first arrived at the ITQB, I formed the impression that there was a very uneven distribution of resources and support throughout the Institute. Whilst I fully appreciate that Laboratories performing excellent science need to be rewarded, I have always taken the view that the strength of any institution or device is only as good as its weakest component. I therefore adopted a strategy whereby there was a more equitable distribution of resources and the weaker and newer laboratories were given more support than previously with the aim that they in turn would become stronger and more effective to the benefit of the Institution as a whole. Of course, those who were relatively well supported saw this as a diminution of their privileges, but I firmly believe that overall the ITQB now has a far stronger and healthier organization. The Infra-structure Support (ISC) and Safety and Floor Coordination Committees (SFCC) are operating effectively

and give both scientific and administrative staff the opportunity to participate in the overall organization of the Institute. The ISC in particular has made the different components of the administrative staff aware of common problems and the response of the administrative staff to finding solutions and giving more effective and efficient infra-structure support has been very good. With regards to safety, a crucial aspect of the organization of any Institute, the ITQB has made major advances. There is now an external store for bulk solvents and potentially dangerous chemicals and all staff are more aware of good laboratory practice and overall safety issues. Externally, the one way traffic systems and the refurbishment of the entry and exit roads at the rear of the building coupled with a significant increase in the car parking capacity have helped to make the environment safer. However, safety is an issue that needs regular and constant attention, particularly as new staff come on site.

From the scientific viewpoint, I was fortunate to inherit a strong program in the Life Sciences and related areas. My strategy has been to try and strengthen this program whenever and wherever possible and, in particular, through the use of Laboratório Associado positions to bring in young scientists to give a firm basis for future growth and development. In a similar manner to the infra-structure support, this strengthening has been designed to help the weaker groups and increase the overall, already high, standard of scientific endeavor. The Division of Chemistry has caused major concerns, since the Division lacks the size and critical mass to cover all the areas that are considered necessary for a modern Department of Chemistry. However, the Division undertakes high class research in key areas such as chemical synthesis (both organic and organometallic), and resources have therefore been concentrated in these areas, since they also provide very strong support to the other Divisions. Among several initiatives in the overall ITQB program, the development of the structure and function of membrane proteins and their complexes, gives me considerable satisfaction. This is a genuinely inter-disciplinary field of key importance to cell biology. As indicated above I hope that further developments will take place in areas such as Biomathematics and Animal Cell Technology. The ITQB now has nearly 50 individual laboratories within the five Divisions of Chemistry, Biology, Biological Chemistry, Technology and Plant Sciences, compared with just over 30 in 2002. This organization gives considerable flexibility in being able to change the research emphasis as scientific trends change and yet, inter-Laboratory and inter-Division collaborations ensure that the overall science program is focused on the themes defined in the Laboratório Associado contract to the mutual benefit of the ITQB and the Laboratório Associado.

The overall scientific program has also been considerably strengthened by a concerted refurbishment and acquisition of equipment program. This has been very hard to achieve bearing in mind the financial difficulties and yet the ITQB has been spending on average over 300 K€ per annum on this program. This ought to continue but, of course, this year has also seen the beginning of the release of funds under the National Re-equipment Program, initiated in 2001. The Laboratório Associado, due the foresight of my predecessor and his colleagues at IBET and the IGC, has been remarkably successful in this program and can expect to receive some 7 million Euros over the years 2005-2007. In particular, the ITQB has acquired new EPR Spectroscopy equipment, a Biacore for studying protein-protein interactions, and substantial participation in two of the National Networks covering Mass Spectrometry and NMR. This funding has been a long time in coming, and needs to be provided at more frequent intervals, but the equipment should enable the Laboratório Associado to maintain its position at the forefront of

research in the Life Sciences in Portugal and Europe. With respect to the Networks, it must be remembered that future support from the Government regarding maintenance and operational costs may only be forthcoming if the networks are organized for the common good of all interested scientific parties in the country.

The scientific program has also involved resurrecting the teaching for PhD students. It is now mandatory for all first year PhD students to attend a course that is designed to explain the techniques and methods that are available to undertake research in the Life Sciences (CBS – Chemical and Biological Sciences). Many of the staff participate and overall the students have been very receptive. Thanks are due to Luís Paulo Rebelo, Cláudio Soares and Adriano Henriques for helping to restructure this program. In the future the ITQB will offer a set of courses under the general umbrella of "Functional Biology" at the Bologna stage 2 level and CBS will be merged within this program. The main objective in this initiative is to attract high quality students into the research program to undertake PhD degrees and post-doctoral activities.

Another aspect on which I must comment is the public awareness of science and technology. Portugal is fortunate to have an active Ciência Viva organization and I have tried to ensure that ITQB plays an appropriate part. Thus, ITQB now has an annual Open Day when the local community is encouraged to come and find out how we spend their taxes. There are also a number of other activities designed to bring the public attention to our scientific program and objectives. I was particularly pleased and honored that as result of this activity the ITQB was awarded a Gold Medal by the Oeiras Camâra and I hope that this activity can be sustained in the future.

It is my firm belief that the ITQB and the Laboratório are in very good scientific shape and that the future will bring more high quality Science and Technology. Some evidence for this statement is that in 2005 the ITQB and IBET produced nearly 200 papers in peer reviewed international journals and that 21 doctorate degrees were awarded to researchers in the Laboratório Associado. In addition, the ITQB received an increase of over 10% in its Orçamento de Estado budget, the largest increase within the Universidade Nova de Lisboa. Let's hope that in 2006 this momentum can be maintained. I am confident that my successor will inherit an excellent, well organized scientific institute at the forefront of Portuguese and European science. I would like to take this opportunity to wish my successor a very successful period of office and hope that you will all give as much support as possible to whoever is appointed.

I must end these introductory remarks by thanking all the people who have made my stay in Portugal so enjoyable, educational and productive. There are far too many to mention individually, but Manuel Carrondo (Chief Executive Officer, IBET) and António Coutinho (IGC) have given me every support in the development of the Laboratório Associado. Maria Arménia Carrondo was my Vice-Director at the ITQB for nearly three years and had to suffer my strange foreign ways and sense of humor and also to pass on the responsibility to Miguel Teixeira and Cláudio Soares, the current Vice-Directors. To Maria da Glória Leitão, Fernando Jorge Tavares, Madalena Pereira, João Rodrigues and Henrique Campas Nunes and all their colleagues in the infra-structure support sections, a very warm thanks for all your hard work and patience. To the secretaries, Mafalda, Cristina, Rosina, Fátima and Xana, many, many thanks for keeping the Institute going and for putting up with my "jokes" and introduction to the Anglo-Saxon language. To all the scientists, technicians, students etc., thank you for making the ITQB and the Laboratório

Associado such an excellent place to undertake science. Keep up the good work in the future. To the Rector and his colleagues at the Universidade Nova de Lisboa, thank you for accommodating a foreigner into your activities.

Last, but certainly not least, a heartfelt thank you to Ana Sanchez and her team for helping to produce this Annual Report and Plan.

My best wishes to all of you for the future of ITQB and the Laboratório Associado,

Peter F. Lindley, 9th February 2006
Director, ITQB-UNL [1].

This Annual Report and Plan 2004/5 contains material from the Laboratório Associado involving the ITQB, IBET and IGC. The relevant web addresses of these three Institutes can be found as follows;

Instituto de Tecnologia Química e Biológica	http://www.itqb.unl.pt/
Instituto de Biologia Experimental e Tecnológica	http://www.ibet.pt/
Instituto Gulbenkian de Ciência	http://www.igc.gulbenkian.pt/

[1] At the time of writing this foreword, part of the Orçamento de Estado budget has been frozen so that despite a 10.3% increase in 2006, the ITQB is actually receiving less per month than in 2005. In addition, the ITQB has not been advised regarding the settlement of the Laboratório Associado contract for 2006-10.

ORGANIZATION OF THE INSTITUTE

DIRECTORATE

Peter F. Lindley	Director
Mª Arménia Carrondo	Vice-Director (until May 2005)
Cláudio Soares	Vice-Director (from June 2005)
Miguel Teixeira	Vice-Director

SCIENTIFIC ADVISORY COMMITTEE

(Scientific Advisory Committee of the Scientific Council)

Directorate	Peter Lindley (Director) Mª Arménia Carrondo (Vice-Director until May 2005) Miguel Teixeira (Vice-Director) Cláudio Soares (Vice-Director from June 2005) Ana Maria Portocarrero (Secretary to the SAC until Feb.05) Fátima Madeira (Secretary to the SAC from March 2005)
Chemistry Division	Carlos Romão Eurico de Melo
Biology Division	Adriano Henriques Helena Santos
Biological Chemistry Division	Cláudio Soares Claudina Rodrigues-Pousada
Technology Division	Luis Paulo Rebelo Teresa Crespo
Plant Sciences Division	Cândido Pinto Ricardo Margarida Oliveira
Substitutes	Cecília Arraiano Júlia Costa
Ex-Officio Members	António Xavier Manuel Nunes da Ponte Manuel Carrondo

INTERNATIONAL ADVISORY COMMITTEE

Professor Sir Thomas L. Blundell, FRS, Biochemistry Department, 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-Institut für Bioanorganische Chemie, Mulheim, Germany.

INFRA-STRUCTURE SUPPORT COMMITTEE

Mª Arménia Carrondo - Vice-Director (Chairman until May 2005)
Cláudio Soares - Vice-Director (Chairman from June 2005)
Peter Lindley – Director
Miguel Teixeira – Vice-Director
Mafalda Mateus – Secretary (until March 2005)
Rosina Gadit – Secretary (from April 2005)
Maria da Glória Leitão (until August 2005), Madalena Pereira, Fernando Tavares, João Rodrigues – Administrative, Accounting & Economato
Ana Sanchez – External Affairs
Lurdes Conceição – Academic Services
Carlos Frazão, Carlos Cordeiro, Daniel Branco - Computing & Networks
Susana Lopes - Library
Manuela Regalla – Washrooms and Services
Ana Coelho – Analytical Services
Rosário Mato - ITQB I
Christopher Maycock – Chemistry Building
Henrique Campas Nunes, Nuno Monteiro – Safety/ Workshop & Maintenance

SAFETY AND FLOOR COORDINATION COMMITTEE

Abel Oliva - Chairman
Peter Lindley - Director
Mª Arménia Carrondo - Vice-Director (until May 2005)
Mafalda Mateus - Secretary
Henrique Campas Nunes, Alexandre Maia – First Floor
Fernando Tavares, Nuno Lopes – Second Floor
Lígia Saraiva Teixeira, Inês Cardoso Pereira – Third Floor
Abel Oliva, Luís Paulo Rebelo – Fourth Floor
Teresa Crespo, Vitória San Romão – Fifth Floor

Cândido Pinto Ricardo, Margarida Oliveira – Sixth Floor
Carlos Romão, Rita Delgado (until November 2005), António Lopes, Ana Luísa Simplício – Seventh Floor
Rosário Mato, Ricardo Louro – ITQB-I
Christopher Maycock, Rita Ventura – Chemistry Building
Beatriz Royo – Chemical Management
Cecília Arraiano and Adriano Henriques – Radioactive Sources
Teresa Crespo, Júlia Costa – Biological Hazards
Helena Santos, MD - Medicine and Health
Helena Matias - Safety Adviser
Henrique Campas Nunes, Nuno Monteiro – Workshops & Maintenance
Isabel Ribeiro - IBET representative
António Cunha, João Clemente – Pilot Plant Representative

STEERING COMMITTEE OF THE ANALYTICAL SERVICES

Miguel Teixeira	Chairman and Vice-Director
Cristina Lopes	Secretary
Pedro Lamosa	Biology Division
Cláudio Gomes	Biological Chemistry Division
Carlos Romão	Chemistry Division
Philip Jackson	Plant Sciences Division
Paula Alves	Technology Division
Ana Coelho	Mass Spectrometry
Conceição Almeida	Elemental Analysis
Manuela Regalla	Protein Sequencing
Paula Chicau	Amino Acid Analysis
Isabel Bento	Small Molecule X-ray Crystallography
João Carita	Fermentation and Protein Expression Laboratory
Helena Matias	NMR
António Ferreira	IBET representative

INFRA-STRUCTURE SUPPORT SERVICES

ADMINISTRATIVE AND ACCOUNTING SERVICES

Head: Maria da Glória Figueira Gonçalves Reis Leitão (until August 2005)

Administrative Department Coordinators

Fernando Jorge Dias Tavares
Maria Alexandra Ferreira Lopes Pinto dos Santos
Maria Madalena Albuquerque Marques Pereira

Personnel Section
Ana Luísa da Silva Teixeira Cruz
Goretti Anjos Gomes da Rocha
Helena Isabel Gomes Cordeiro Rodrigues
Maria Cristina Pereira Pinto

Mailing and Archive
Ana Sofia Marques de Sousa Mendes (from March to April 2005, part- time)
Artur Elias dos Santos Freitas
Felicidade Jesus da Silva Rei (until Sptember 2005)
Claúdia Sofia de Carvalho Lopes (from May 2005)

Accounting
Ana Cristina Afonso Silva
Ana Mónica Adriano Vieira
César Paulo da Mata Simões (until September 2005)
Isabel Maria Soares Palma Mestre
Nuno Miguel Nobre Lopes

Treasury section
Ana Dores dos Santos Freire
Anabela dos Santos Bernardo Costa

Stores
Ana Isabel Soeiro Jesus Francisco dos Santos
Bruno Alexandre Lucas Gouveia
Carlos Eduardo Branco de Matos Aires Martins
João Augusto Lourenço Rodrigues
Ricardo Manuel Pereira Pinto

Secretariat
Ângela Mafalda Faria Baptista Mateus
Isabel Cristina Respício Valente Almeida Lopes
Maria de Fátima da Costa Madeira
Rosina Faruk Gadit

EXTERNAL AFFAIRS
Head: Ana Maria Beirão Reis de la Fuente Sanchez Fuzeta da Ponte
Luís Manuel Ramalho Morgado

ACADEMIC AND PROJECTS OFFICE
Head: Maria de Lurdes Madaleno Conceição
Ana Cristina Porfírio Amaral
Ana Sofia Marques de Sousa Mendes (from May to August 2005)
Ana Maria Cerveira e Castro da Silveira Portocarrero
Isabel Maria Coelho Gonçalves Guerreiro Murta

WORKSHOP AND MAINTENANCE

Head : Henrique José Vaz de Campas Nunes
Alexandre Saturnino Largo Maia
Aníbal José Neves Ribeiro
António Veiga Ramalho
António Miguel Diogo Rodrigues
Elias Louro
João Carlos Zanão Simões
José Costa
José Luís Pereira Liberato
Luís Miguel Sousa Gonçalves
Nuno Miguel de Jesus Soares
Rui Hélder Amor Pereira Dias
Walter Peres
Nuno Monteiro (Power management)

WASHROOMS FOR EQUIPMENT

Coordinator: Manuela Regalla
Ana Cristina Martins Barreiros
Carmen Popula Pereira de Jesus Fernandes
Isilda Marques Martins Gueifão
Maria Alice Rosa Ferreira
Maria Eugénia Ferreira Pereira dos Santos
Pilar da Conceição Lobo da Costa Campos
Sónia Cristina Capucho Serrano

COMPUTER SYSTEMS SUPPORT

Scientific Coordinator: Carlos Frazão
Carlos Manuel dos Santos Cordeiro
Daniel Feliciano Branco
Maria Isabel da Costa Baía
Maria Manuel Isaías Paulo Rato
José Miguel de São Bento Figueiredo Loureiro
Miguel Paulo Vinhas Pires Bento Ribeiro

ANALYTICAL SERVICES

Ana Maria de Jesus Bispo Varela Coelho - Mass Spectrometry
Maria da Conceição Lucas Carvalho Pereira de Almeida - Elemental Analysis
Maria Manuela Sobral Martins Alberto Regalla - Protein Sequencing
Paula Maria Gonçalves de Oliveira Roldão Chicau - Amino Acid Analysis
Isabel Bento - Small Molecule X-ray Crystallography

FERMENTATION AND PROTEIN EXPRESSION LABORATORY

João Nuno Carichas Carita

LIBRARY

Susana Lopes

EVALUATION PANELS

BIOLOGICAL CHEMISTRY REVIEW

Professor Thomas Blundell, University of Cambridge, Department of Biochemistry, UK
Professor Robert Crichton, Université de Louvain-la-Neuve, Unité de Biochimie, Belgium
Professor Pedro Moradas Ferreira, Universidade do Porto, Grupo de Microbiologia Celular e Aplicada do IBMC, Portugal

CHEMISTRY DIVISION REVIEW

Professor Karl Wieghardt, MPI Mulheim, Germany
Professor Mike Hursthouse, School of Chemistry, University of Southampton, UK
Professor João Fraústo da Silva, Instituto Superior Técnico, Unversidade Técnica de Lisboa, Portugal

BIOLOGY DIVISION REVIEW

Professor Staffan Normark, Swedish Foundation for Strategic Research, Microbiology and Tumor Biology Center, Karolinska Institute, Sweden
Professor Arsélio Pato Carvalho, Universidade de Coimbra, Centro de Neurociências de Coimbra, Departamento de Zoologia, Portugal
Professor Wolfgang Hillen, University of Erlangen, Institute of Microbiology, Germany

HEALTH SCIENCES REVIEW

Professor Fernando Lopes da Silva, Swammerdam Institute of Life Sciences, Section Neurobiology, Amsterdam, Netherlands
Professor João Monjardino, Imperial College of Science Technology and Medicine, Department of Medicine, UK
Professor Fernando Tomé, INSERM U.523, Institut de Myologie, France

TECHNOLOGY DIVISION REVIEW

Professor Joachim Klein, Technishe Universitat Braushweig, Institute of Macromolecular Chemistry, Germany
Professor Daniel Wang, Massachusetts Institute of Technology, Department of Chemical Engineering, USA
Professor Manuel José Magalhães Gomes Mota, Vice-Reitor da Universidade do Minho, Portugal

BIOLOGY AND CHEMISTRY REVIEW

Professor Sven-Olof Enfors, Royal Institute of Technology, Department of Biotechnology, Sweden
Professor Eric Derouane, Faculdade de Ciências e Tecnologia da Universidade do Algarve, Departamento de Química e Bioquímica, Portugal
Professor Josef Van Beeumen, University of Gent, Laboratory of Protein Chemistry and Protein Engineering, Belgium

PLANT SCIENCES DIVISION REVIEW

Professor Pat Heslop-Harrison, Department of Biology, Faculty of Medicine & Biological Sciences, University of Leicester, UK
Professor Evert Jacobsen, Wageningen UR, Laboratory of Plant Breeding, Netherlands
Professor Serafim Tavares, Instituto de Investigação da Floresta e do Papel, Portugal

ITQB RELEVANT STATISTICS

The Instituto de Tecnologia Química e Biológica (ITQB) is an institute of the Universidade Nova de Lisboa devoted to research in the life sciences and associated technologies. Its mission is also to provide advanced training in these areas.

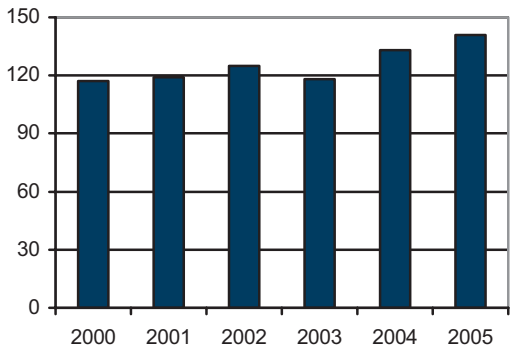
RESEARCHERS

Since 1989, ITQB operates as an open institute with the participation of researchers from other institutions and universities. The permanent research or teaching staff is limited and a number of researchers have been hired for 5-year periods under the Laboratório Associado contract. The majority of researchers at ITQB are supported through PhD or post-doctoral scholarships.

Currently, research at ITQB is supported by over 300 researchers. About half of the researchers at ITQB hold a PhD Degree, and around two thirds of these are women.

PhD holders	**141**
Laboratory Heads	52
ITQB	14
LA	16
Other Institutions	22
Post Doctoral Fellows	62
Other PhD s	28

PhD holders over the years:



PhD holders by gender: Female 88 / Male 53

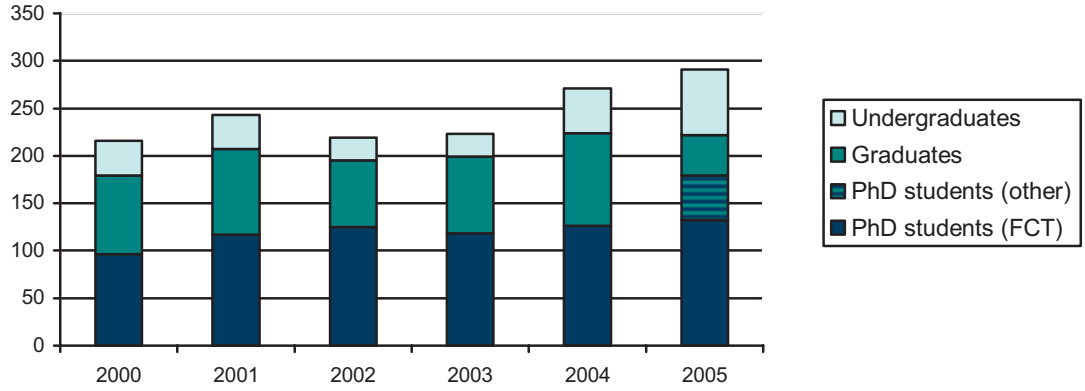
Average age of a PhD holder: 41,16 years
(The average age of a Post-Doc research at ITQB is 35 years)

The quality and diversity of the research and the researchers at ITQB make it an exceptional centre for higher science education in Portugal. Graduate students at ITQB, mostly PhD students, are integrated into ITQB's research groups where they undergo their training in scientific research. ITQB also welcomes students in the final year of their degrees for short periods of training in scientific research.

PhD students	**179**
FCT fellowships	132
Other sources of funding*	47
Other graduates (BI)	**43**
Undergraduates	**69**

*This figure includes PhD students (25) whose PhD fellowship from FCT has finished.

Graduate students and undergraduates at ITQB over the years:



In previous years, only FCT PhD fellows were counted as PhD students and all others were included in the figure Graduates. For comparison, in 2005, the PhD students with other sources of funding are accounted separately.

Researchers' Funding

The permanent research staff at ITQB is limited to a small number, currently 18. Additionally 20 researchers are supported through Laboratório Associado contracts (see LA section). Most researchers at ITQB are either staff members of other academic institutions (40 researchers) or are grantees. Many PhD students and Post-doc fellows resort to the Fundação para a Ciência e a Tecnologia (FCT) for funding. At this moment ITQB has 178 FCT grantees (132 PhD students and 46 post-docs).

In 2005, the approval rate of PhD and Post-doc scholarships from FCT was 64% (39 out of 61 applications accepted) and 76 % (22 out of 29 applications accepted), respectively. This clearly demonstrates the capacity of ITQB to attract and train high quality researchers.

RESEARCH

ITQB has presently 52 Laboratories that are organized into five Research Divisions - Chemistry, Biology, Biological Chemistry, Plant Sciences and Technology. In many cases the allocation of a particular Laboratory to a Division is an organizational convenience and collaboration between Divisions is strongly encouraged. The number of Laboratories and their relatively small size gives considerable flexibility so that it is easy to change research emphasis as external forces dictate. The diversity of expertise contributes to the multidisciplinary atmosphere that makes this Institute unique in the country.

Projects

Research at ITQB is mainly supported by contracted projects with R&D funding agencies. Currently ITQB coordinates 77 research projects and further participates in 26 more. The total 103 ongoing projects are mainly funded by Fundação para a Ciência e a Tecnologia (FCT), but there are other additional sources of funding. The list of all funded projects currently running at ITQB is given in the Research Output section.

Ongoing projects:

84 projects Fundação para a Ciência e a Tecnologia
7 projects Re-equipment Program FCT
3 projects Fundação Calouste Gulbenkian
7 projects European Commission
2 projects Rockfeller University

In the last call for projects from FCT, in 2004, ITQB submitted 80 projects and achieved an overall approval rate of 49 %.

The rate of approval of FCT funded projects in the last call by project area and a comparison of the success rate of ITQB and the national percentages is shown below.

Rate of Approval of FCT Projects by ITQB:

Thematic Area	Approved/Submitted	Success rate ITQB	Success rate Portugal
Ciênc. Agrárias e Florestais	7/13	54%	25,31%
Ciênc.e Engenharia do Ambiente	1/2	50%	no data available
Ciênc. Biológicas - Biol. Celular e Molecular	4/14	29%	25,45%
Ciênc. Biológicas - Biol. Microbiana	5/7	71%	24,13%
Ciênc. Biológicas - Proteínas Biologia Estrutural	5/12	42%	24,95%
Engº Bioquímica e Biotecnologia	4/7	57%	22,55%
Ciências e Engenharia dos Materiais	0/1	0%	25,40%
Ciência Animal e Ciências Veterinarias	0/2	0%	20,71%

CONT.

Engenharia Química	1/2	50%	28,50%
Química e Bioquímica	6/8	75%	35,63%
Ciênc. Saúde - Medicina Molecular	0/3	0%	29,64%
Ciênc. Saúde - Epidemiologia e Saúde Publica	2/3	67%	no data available
Ciênc. Saúde - Infecção e Microbiologia	4/6	67%	38,13%

Publications

In 2005, ITQB researchers published 195 papers in peer reviewed journals. It must be emphasised that research at ITQB results often from internal collaborations between the small research groups and this is reflected in the authorship of published work. In 2005, about 1/5 of the papers were published by researchers from more than one of ITQB's research Laboratories.

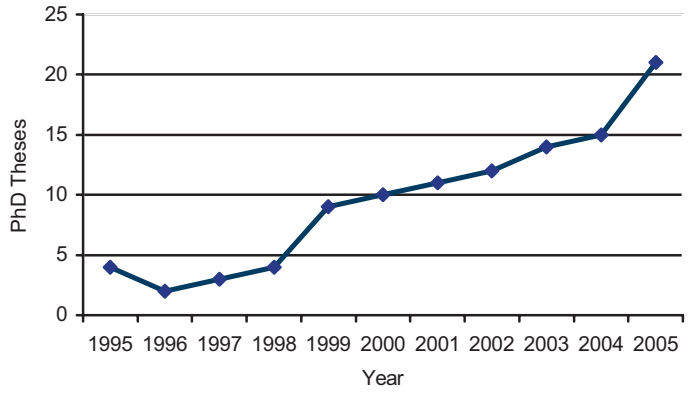
Some 73 papers have already been published in 2006 or are now in press.

ADVANCED EDUCATION

As an academic institution, ITQB awards PhD degrees in Chemistry, Biology, Biochemistry and Chemical Engineering. So far, ITQB has awarded 106 PhD degrees.

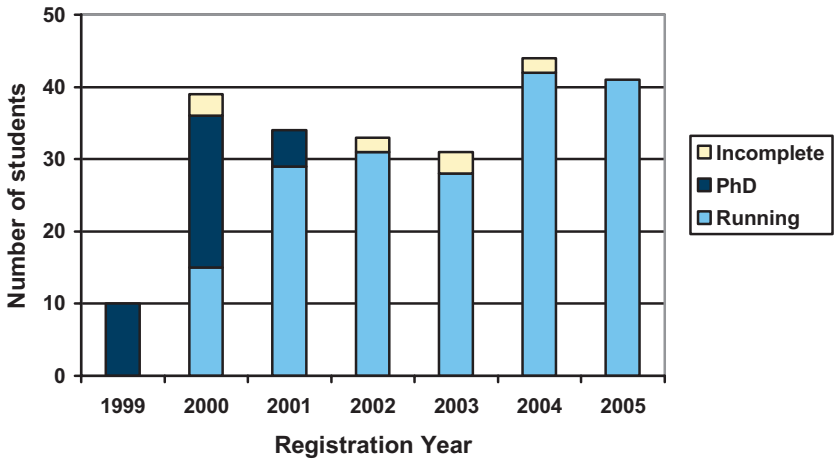
In 2005, 21 PhD theses were awarded at ITQB: 13 in Biology, 7 in Biochemistry, 1 in Chemical Engineering.

PhD thesis awarded at ITQB over the years:



In 2005, 41 graduates registered at ITQB as PhD students. At the moment there are 202 registered PhD students at ITQB. About 50 of these students do research at other institutions that cannot award academic degrees and resort to ITQB for that purpose.

PhD students over the years according to their registration year:



The number of students who don't complete their PhD is very small, and any premature fall out usually reflects lack of funding (some students register before) or in some cases, moving of groups to other institutions.

Graduate students at ITQB are also provided with formal elements of training through post-graduate courses, Master courses and through the ITQB PhD program in Chemical and Biological Sciences (CBS).

In 2005/6, ITQB coordinates the Masters Degree in Medical Microbiology, a collaborative Masters Course between ITQB, the Instituto de Higiene e Medicina Tropical, Faculdade de Ciências Médicas and Faculdade de Ciências e Tecnologia from Universidade Nova de Lisboa. For this period, 21 Master students are registered at ITQB.

Complementing the research training of PhD research, ITQB offers an educational program that aims to provide young scientists with a broader view of science methodologies and their applications. The CBS PhD program at ITQB was introduced in 2002 and is mandatory for first year PhD students. In 2005, 22 PhD students attended this PhD program.

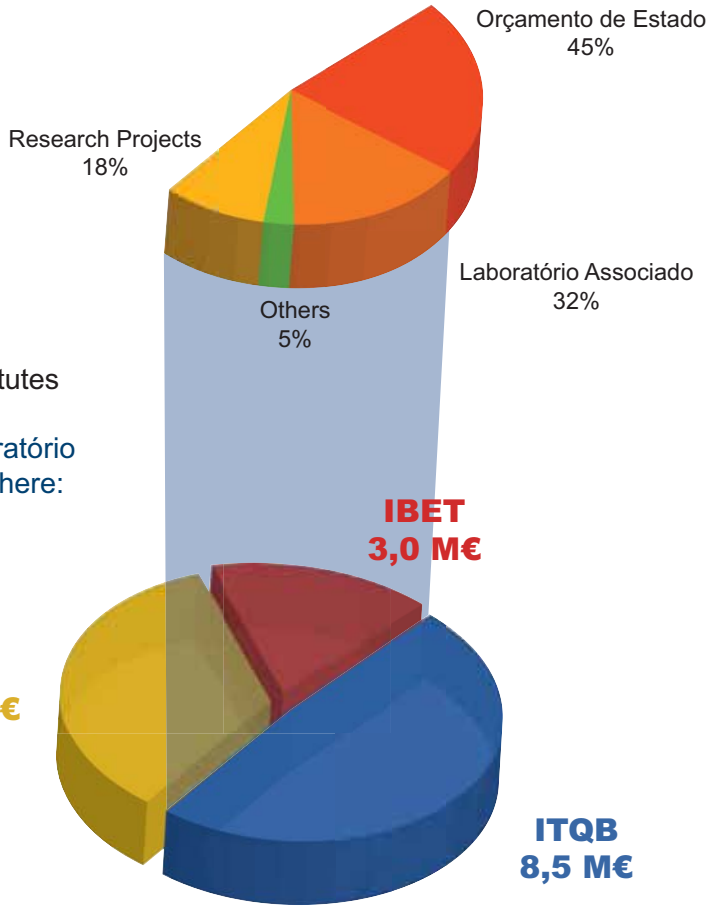
BUDGET

ITQB Budget

ITQB has two main sources of revenue; Orçamento de Estado and the national science funding agency, Fundação para a Ciência e a Tecnologia (FCT). The contribution from the Orçamento de Estado, through Universidade Nova de Lisboa, represents less than half of the overall ITQB budget. FCT accounts for two sources of financial support, through the Laboratório Associado contract and through project funding. Additional sources for research projects include the European Commission, the Fundação Calouste Gulbenkian, the Rockfeller University and international cooperation projects.

Some 5 % of the total budget represents additional sources of funding including revenues from Masters Course, the sale of analytical services, rental of rooms and facilities, etc.

The overall budget of ITQB for 2005 was around 8,5 M € distributed as follows:



Laboratório Associado

overall budget of the three institutes

The budget for the whole Laboratório Associado in 2005 is depicted here:

Considering that Gulbenkian is a private foundation and IBET is a non-profit making institution supported by Industry, then for every Euro invested by the Government, another Euro is invested by non-governmental sectors. Surely, that represents value for money.

ROLL OF HONOUR

Claudina Rodrigues-Pousada,
head of the Stress and Genomics Laboratory
>Diplôme of Honorary Lifetime member of the Cell Stress Society International (CSSI)

Maria Manuela Chaves,
head of the Plant Molecular Ecophysiology Laboratory
>elected for the Fellowship Committee of FEBS.

Maria Arménia Carrondo,
head of the Macromolecular Crystallography Laboratory
> "Medalha de Honra do Município" by the Municipality of Vila Nova de Famalicão, July 2005.
>distinguished by Editorial Verbo in the publication "Annualia 2005/2006"

Ana Rocheta Gomes,
post-doc researcher in the Laboratory of Molecular Genetics
>First prize, for the best poster presented in the session Clinical Microbiology and Health Biotechnology at the MICRO'05 - BIOTEC'05 Congress, Póvoa do Varzim, 30 November-3 December, 2005
Evolution of methicillin-resistant *Staphylococcus aureus* epidemic clonal lineages (authors: Ana Rocheta Gomes, Henrik Westh and Hermínia de Lencastre)

Paula Fareleira,
collaborator of the Laboratory of Cell Physiology and NMR
>"Lidel Prize" for poster in the area of "Environmental Microbiology and Biotechnology" at the "MICRO05/BIOTEC'05", Póvoa do Varzim, 30 November-3 December, 2005
Stress resistance of rhizobial strains from dry environments in Southern Portugal (authors: Fareleira P., Matos N., Ferreira E.M., Marques J.F. and Santos H.)

Cláudia Serra,
PhD student in the Laboratory of Microbial Development
>First prize, for the best poster presented in the session Cell Physiology, Biochemistry and Molecular Biology at the "MICRO05/BIOTEC'05", Póvoa do Varzim, 30 November-3 December, 2005
Regulatory circuits involved in the initiation of spore development in undomesticated *Bacillus subtilis* (authors: Cláudia Serra, Teresa M. Barbosa, and Adriano O. Henriques)

Animal Cell Technology Laboratory

>2005 Scientific Prize from APDF (Associação Portuguesa de Doutorandos em França) for excellent collaboration project between the CIRAD (France) and ITQB/IBET (Portugal). Project: ”Optimization of a vaccine against Heartwater”

Joanna Lachwa,

post-doc researcher in the Molecular Thermodynamics Laboratory

>2005 Christopher J. Worlmald Prize

J. Łachwa and L.P.N. Rebelo First evidence for closed-loops and other lcst-containing phenomena in binary and quasi-binary ionic liquid solutions “Thermodynamics 2005“, Sesimbra, Portugal, April 6-8, 2005

Mariana Pereira,

graduate student in the Plant Cell Biology Laboratory

>American Club Award 2005

Vesna Najdanovic-Visak,

PhD student in the Processes with Supercritical Fluids Laboratory

>Award Royal Society of Chemistry - “Hot Article”

V. Najdanovic-Visak, L.P.N. Rebelo, M. Nunes da Ponte Liquid-liquid behaviour of ionic liquid + 1-butanol + water and high pressure co2 – induced phase changes Green Chemistry (2005), 7, 443.

L.P.N. Rebelo,

head of Molecular Thermodynamics Laboratory

>Award Ionic Liquids Today, Issue 2/05 – “Hot Article”

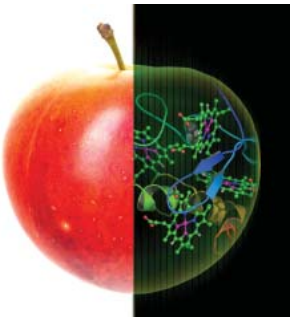
L.P.N. Rebelo, J.N. Canongia Lopes, J.M.S.S. Esperança. E. Filipe On the critical temperature, normal boiling point and vapor pressure of ionic liquids Journal of Physical Chemistry B (2005), 109, 6040.

ITQB GOES PUBLIC

ITQB is an institution devoted to research and advanced training in the area of the Life Sciences and associated technologies. As part of its mission, ITQB also engages in promoting public understanding of science and scientific research.

In fact, ITQB considers it an obligation to communicate its research to society, not only because society has the right to know how its money is being spent - science relies on funded projects and fellowships, most of the time through public money - but also because research has often outcomes with implications in people's lives.

During 2005, ITQB was involved in many activities dedicated to bring science closer to the public. Some of these activities were integrated in the project *Oeiras vive a Ciência*, a collaboration between three research institutions in Oeiras (ITQB, IGC and EAN) supported by the City Council; i) the initiative *Ciência vai ao cinema*, aimed at triggering science curiosity and discussion with the exhibition of movies related to scientific areas, such as genetics, ecology, chemistry or mathematics, followed by a debate with invited specialists; ii) with *Fábrica da Ciência*, with the idea to take science away from science institutions and bring it closer to the public, researchers from the three institutes were at Fábrica da Pólvora for a whole day with exhibits, experiments and games to show how science is present in so many things around us; iii) included in this project was also the distribution of paper meal trays depicting games and curiosities covering chemistry, physiology, mathematics, among others.



As part of the celebration of the World Year of Physics, ITQB promoted a one day symposium on the 17th November devoted to the important role of physics in biology, specially in structural biology. In the symposium *Ver a vida com a Física*, scientists from the European Synchrotron Research Facility (ESRF), the Institute Laue-Langevin and the Partnership for Structural Biology explained to a group of more than 80 students how life's molecules can be seen with radiation.

ITQB is an associate member of Ciência Viva, the National Agency for Scientific Culture, and as such is actively involved in activities promoted by this agency. In the Summer of 2005, ITQB received a dozen of high school students eager to learn about the daily routines of scientific research for short training periods in the laboratories, as part of the initiative *Ocupação Científica de Jovens nas Férias*. During the Science and Technology week, between 21 and 27 of November, ITQB also organized scientific activities in different laboratories mainly dedicated to students.

As traditionally happens, in 2005 ITQB received many visits from both high-schools and universities, which represent valuable opportunities to show the quality of the research undergoing at ITQB and to motivate young students to pursuit scientific careers.

In 2005, a new section in the webpage of ITQB dedicated to Science and Society was created. Here it is possible to learn about ongoing and past activities, to access the school visits program and to find many links to interesting scientific sites.

Foto: Luis Morgado

In January 2005, ITQB held the first edition of a very successful Science Open Day dedicated to local families. This event has already been described in last year's Annual Report.

This year, on a cold Saturday in January, ITQB presented an improved and enlarged version of the Open Day. The response of the public was fantastic and ITQB received 1898 visitors on that day.

Visitors could learn about the different research divisions at ITQB by visiting the five stands located at entrance level where many different activities were displayed; from meeting different fungi and bacteria to measuring the stress of a plant, or seeing the yogurt's bacteria, finding out that proteins have to fold just in the right way, and witnessing a rainbow colored chemical reaction, are only a few examples of what was on display.

The younger members could further explore science in their own corner performing experiments with household reagents and playing with molecules and cells made of dough.

This year, visitors were taken on a laboratory tour to see where researchers spend their day. Because research at ITQB is actually leading to real applications in many fields, this year a special exhibit entitled "From gene to drug" was presented.

Throughout the day, researchers were available for talking with the public and every hour, a special topic was presented in the auditorium to stimulate discussion.

As in the previous edition, the enthusiasm of both visitors and scientists was noteworthy and this made this open day a very special day.

Foto: Nuno Faria



LABORATÓRIO ASSOCIADO

LABORATÓRIO ASSOCIADO at Oeiras

1.INTRODUCTION

The Laboratório Associado at Oeiras is a partnership between;

Instituto de Tecnologia Química e Biológica (ITQB),
Instituto de Biologia Experimental e Tecnológica (IBET),
Instituto Gulbenkian de Ciência (IGC)

The contract between the Fundação para a Ciência e a Tecnologia and the Instituto de Tecnologia Química e Biológica initiating the Laboratório Associado was signed in November 2000 and the first scientific staff were appointed in July 2001. The length of the contract was originally fixed at 10 years, but a financial plan was only allocated for the first 5 years. The major features of the research strategy for the second period of 5 years are as given below and concomitant financial support has been requested from the Fundação para a Ciência e a Tecnologia.

The Laboratório Associado wishes to continue and strengthen its five existing scientific themes, namely Biologically Active Molecules, Molecular Medicine and Veterinary Studies, Developmental Biology in Animals and Plants, Biological Risk and Improvement of Plants and Forest, and to complement these by the addition of special scientific initiatives including Neurosciences, Computational Biology, Animal Cell Technology, Ionic Liquids for Chemical and Biological Processes, and Membrane Proteins and Protein Complexes. It also wishes to develop its role in providing national services to Portuguese scientists in Nuclear Magnetic Resonance Spectrometry, Mass Spectrometry, Resonance Raman Spectrometry and X-ray Crystallography (both macromolecular and small molecule). In this plan, of strategic importance to the national and European development of research in the Life Sciences and related areas, it is inherent that the existing 25 scientific and 9 technical positions are retained. In addition further scientific and technical positions will be necessary and extra funding will be needed to support the concomitant increase in the infra-structure and additional equipment.

2.THE LABORATÓRIO ASSOCIADO

The combination of the three institutions, two of which focus predominantly on fundamental research in the Life Sciences and related areas and the third providing an interface with Industry is unique in Portugal, and probably, in Europe. Together the three institutes comprise some 500 researchers of which 200 are holders of the PhD Degree. The Laboratório Associado has the following characteristics;

- 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 shared services of high technology, many of which provide national facilities.
- An interface between academia and industry.
- 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, and bioinformatics.

-Strong graduate teaching programmes, some of which are also “open” (e.g. PGDB), attracting PhD students from both Portugal and abroad, whilst “placing” them for Thesis work in Laboratories all over the world.

A fundamental objective throughout the research programme is the understanding of organisms and their interactions with the environment, at the higher levels of organisation. This involves a Systems Biology approach, focused on the organisms and closer to physiopathology, development and evolution, but encompassing the wider systems of biological ecology and spreading to the means of production. The predictable consequences of the understanding of the behaviour of biologically complex systems and their mechanisms are enormous and of great importance. Reaching this understanding requires the use of new techniques, some of which are still in the developmental stage. These “new” competences need to be used in an interdisciplinary manner, and will open the possibility of participation of new partners (new institutions of R&D, new enterprises, different countries). The scientific competences of the Laboratório Associado are precisely the hard nucleus (Genome-Proteome, Systems Biology) of these developments. They will allow the exploitation of this knowledge with an impact on the areas of Molecular Design with Pharmaceutical or Agrochemical applications, of Agroforestry Biotechnology, of Food Quality including Genetically Modified Organisms, of Genetic Resistance to Drugs and also Infections and Developing Diseases inserted in a modern Clinical Epidemiology, and of Development Biology and Molecular Medicine.

3. MISSION

The mission of the Laboratório Associado is to perform the highest quality fundamental research that the available resources permit within the Life Sciences and related areas. A second important function is to transfer the science and technology to an industrial and economic base by pursuing applications of the research to improve the quality of life and/or the environment wherever appropriate and feasible. In pursuing these mission objectives the Laboratório Associado serves as a Centre of Excellence for the Life Sciences, both in Portugal and abroad. It provides advanced training for research workers at the pre- and post-doctoral levels, acts as an impartial consultant in areas of public interest such as the genetic manipulation of organisms, and provides a database of scientific knowledge and technological expertise within the Life Sciences.

4. RESEARCH PROGRAMME, CURRENT PROGRAMME AND NEW INITIATIVES

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. These common research themes are listed below and utilize the full complementarity of the Laboratório Associado, namely the fundamental research in Chemistry, Biology, Biological Chemistry, Technology and Plant Sciences of

the ITQB, the fundamental research in Biomedical Sciences and Developmental Biology of the IGC, and the technological, industrial interface of the IBET. The Laboratório Associado will continue to pursue these objectives during the period 2006-2010. The current program has five themes;

a. Biologically active molecules (including viral vectors and cells).
b. Medicine and molecular veterinary studies
c. Developmental biology in animals and plants
d. Biological risk
e. Improvement of plants and forests

The Laboratório Associado intends to continue and expand this programme during the period 2006-2010 and a special focus will be placed on the following areas:

Neurosciences

Neurosciences can be expected to undergo significant growth over the next 10 years in biomedicine. However, modern neurosciences, particularly in the most active field of behavior, are poorly represented in Portugal. The diversity of biological themes being practiced at the LA provides ideal conditions to develop a neurosciences research program that would be well embedded in the whole of the biological activities in the LA. Indeed, this initiative has received strong support from the Scientific Boards of the IGC and to this end, renovation work adapting existing laboratories for this purpose has already been approved and will soon be completed. Indeed, the first groups in this area have been installed. Furthermore, contacts have been established with a number of young Portuguese scientists abroad, in order to plan their return and installation in the most productive conditions. There is no doubt that the LA is in a very competitive position at the European level, provided that it will be able to offer 5 year contracts (LA positions) to such scientists. The quality and international reputation of these scientists is very high and both positions and a structured programme will be required to attract them bact to Portugal.

Computational Biology

Computational Biology is not entirely new at the Laboratório Associado. Thus, both the IGC and the ITQB have maintained service and research activities in Bioinformatics (the Portuguese node of the EMBnet and the respective public service to the community), an user-directed educational program in collaboration with the Faculdade de Ciências da Universidade de Lisboa – PGBIONF), as well as other topical courses in this area. Moreover, several research groups in Mathematical and Systems Biology, Bioinformatics (e.g. gene ontology), Theoretical Epidemiology, and Molecular Modeling and Simulation have been hosted at the Laboratório Associado since its foundation. It is now felt that investments should be made in formal education programs for young scientists in this large area of modern biomedical science. To that end, a structure has been created, a Program in Computational Biology, designed with in house and external teachers (Portuguese and foreign), and an agreement negotiated with the Ministry and the FCT. This structure includes a private innovation-driven multinational company (Siemens), in order to launch an experimental PhD Programme that will admit 12 students per year for the next 4 years.

In terms of research, one of the Laboratório Associado positions that were filled in 2005 by open international competition was attributed to Biomathematics. New infrastructures were also put in place to co-locate all the groups at the Laboratório Associado in this topic. It is to be expected that the interaction between the research scientists, the

PhD programme teachers and students with both the ongoing wet-research at the LA (gene expression patterns, in vivo confocal microscopy, etc.) and the competences and interests of Siemens, may lead to new ways to explore in this area. Topics that might benefit from this initiative are imaging and image quantification, novel technologies to score diagnostic tests in complex diseases, data-base mining (both DNA and amino acid sequences) among others.

Animal Cell Technology

The scientific interests at the Animal Cell Technology Laboratory are related with research and development for complex biopharmaceuticals (vaccines, gene therapy vectors and cells for therapy). The unifying themes are, (i) bioprocess development, in particular for the production of novel complex vaccines (e.g. adenovirus, virus like particles, VLP's, and marker vaccines) and of retrovirus and adenovirus for gene therapy, (ii) the development of alternative culture systems for the growth of primary brain cells and stem cells in bioreactors, and (iii) systems biology approaches.

Another major research interest is to understand viral (Adenovirus and Baculovirus) infection kinetics. This knowledge is of particular interest to gain further insights on the effect of infection on specific viruses, virus like particles, recombinant proteins and/or bacteria productivity and stability. A large project has been undertaken to compare the kinetics of rotavirus-like particles (RLP's) synthesis using multigene and single gene baculovirus infection and the effects of stoicheometry and thermodynamics of protein aggregation leading to RLPs and their impact upon process development of a safe rotavirus vaccine production.

More recently these activities have been extended to include stem cell differentiation control and storage. Studies on (i) cell handling and cell programming using surface interaction leading to differentiated cells for medical therapies and (ii) the development of human hepatic reliable in vitro models derived from stem cells that can be used by the pharmaceutical industry to replace experimental animals in investigations on human drug metabolism, are ongoing within the scope of two European funded projects, CellPROM and VITROCELLOMICS, respectively.

The fusion of previous knowledge, acquired using primary brain cell cultures as tools for metabolic studies, will be bridged to stem cell work pursuing stem cell differentiation to neuronal cells and to develop in vitro models for neuroscience research. The major objective is to create reliable culture systems where it will be possible to mimic several pathologic situations, namely stroke (linking to Neurosciences).

Ionic Liquids for Chemical and Biological Processes

Both the scientific and industrial communities are currently witnessing an explosion in the field of ionic liquids which impacts wealth of a number of distinct areas, including chemistry, physics, environmental sciences and the life sciences. Ionic liquids are low-temperature molten salts, and thus liquid at room-temperature. Their behaviour, chemical and physical properties are easily coarse- and fine-tuned (tailored) by the proper manipulation of an almost infinite number of cation-anion combinations. They are extraordinary solvents so that they are, for example, to stabilize proteins, enhance enzymatic reactions, reduce microbial activity, and dissolve plastics. Moreover, they are absolutely involatile under standard conditions, and therefore, environmentally friendly - a key characteristic that has driven the recent burgeoning of interest in these salts as alternative, clean media for chemical and biochemical reactions, novel composites,

separations and extractions, fuel cells.

The Laboratório Associado has a group that is already internationally recognized as being in the forefront of research in this area. This group will be nurtured into a broader, more competitive team able to keep Portugal among the top-ten countries in the world with respect to the characterization and use of these salts.

Membrane Proteins and Complexes

The importance of membranes is unquestionable; the origin of the first cells and thus of the first living beings is intimately related with the advent of membranes. Membranes not only provide a boundary for cells, but also allow compartmentation and separation of enzymatic reactions, provide the control of fluxes, allow cell to cell communication and energy transduction. These last three processes are performed and controlled by proteins. Thus, membrane proteins, which are some 30-40% of the total proteins in a cell, are essential and indispensable for life as it is nowadays. A good knowledge of any organism, from bacteria to humans and of essential processes for life is only possible through a solid study of membrane proteins. Only a thorough structural and functional characterization of these proteins will enable the understanding of phenomena such as metabolite import to different cells, signaling, energy conservation and aging. Such an understanding will certainly influence quality of life by knowledge, allowing diseases control and development of treatment strategies. A number of teams in the Laboratório Associado are undertaking research involving either structure-function studies on important membrane proteins and protein complexes or on the nature of the membranes themselves and how they permit ions and other molecules to permeate through them. It is intended to strengthen this key area of research.

National Services

The Laboratório Associado is involved in a number of national services including Nuclear Magnetic Resonance Spectrometry, Mass Spectrometry, X-ray Crystallography, Imaging and the Animal House Facility at the IGC. Indeed the Laboratório will receive in the region of 7.0 million € as as result of the recent National Re-Equipment bid, much of which is oriented towards the National Services. However, this money is for central equipment and in order to provide genuine National Services in an effective and efficient manner, fully utilizing this major capital investment, it will be necessary to invest in both staff and peripheral equipment. With respect to the NMR service a team is being created that will have a service manager to organize day-to-day operations, a developmental scientist to ensure that the full potential of the 800 MHz high field machine is reached, at least one more support scientist and two technically oriented staff to help with fault diagnosis and sample and experiment preparation.

5. RECRUITMENT OF STAFF OVER THE PERIOD 2001-5

The Laboratório Associado staff appointed during the first five years of the contract are given below. As far as possible the appointments have been made according to the contract. In classifying the area of research, it should be remembered that many of the scientists undertake studies that traverse the boundaries between one or more themes.

A.Scientific Staff.

a.Biologically Active Molecules.

Name	Date	Status	Unit
Ricardo Saraiva Louro	16/10/ 01	IA	ITQB
Daniel Murgida †	29/10/ 01	IA	ITQB
António Baptista	01/05/03	IA	ITQB
Margarida Archer Frazão	01/07/ 03	IA	ITQB
Cláudio Moreira Gomes	01/12/03	IA	ITQB
Beatriz Cantabrana	01/12/03	IA	ITQB
Manuela Pereira	01/07/05	IA	ITQB
Maria Rita Ventura	01/10/05	IA	ITQB

† Daniel Murgida obtained a position at the Technical University in Berlin early in 2002.

b.Medicine and Veterinary Studies.

Name	Date	Status	Unit
Miguel Che Soares	01/07/01	IP	IGC
Paula Marques Alves	11/09/01	IA *	IBET
Carlos Penha Gonçalves	01/08/03	IA	IGC
Sérgio Raposo Filipe	01/10/04	IA	ITQB
José Bártholo Leal ‡	01/01/06	IA	IGC
Karina Bivar Xavier ‡	01/01/06	IA	ITQB
Mariana Gomes de Pinho ‡	01/01/06	IA	ITQB

* Promoted to IP on the 1st December 2005.
‡ Appointment delayed due to freezing of posts in 2004/5.

c.Developmental Biology in Animals and Plants.

Name	Start Date	Status	Affiliation
Moises Mallo Perez	01/07/01	IP	IGC
Jorge Albino Carneiro	26/06/02	IP	IGC
Henrique Teotónio	01/11/05	IA	IGC
Ana Sofia Coroadinha	01/012/05	IA	IBET

d.Biological Risk.

Name	Start Date	Status	Affiliation
Ana Martins †	01/09/01	IA	ITQB
Rosário Mato Labajo	17/12/01	IA	ITQB
Maria Fátima Lopes	16/06/02	IA	IBET
Ana Rute Neves	01/12/03	IA	ITQB
Ana Simplício	01/05/04	IA	IBET

† Resigned from the LA on the 31/08/03.

e.Improvement of Plants and Forests.

Name	Start Date	Status	Affiliation
Margarida Rocheta	02/10/01	IA	IBET
Philip Jackson	16/07/02	IA	ITQB
Rita Abranches	01/12/03	IA	ITQB

B.Technical Staff.

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

Name	Start	Institute	Responsible
Sandra Diniz da Silva	09/10/01	IBET	M. Carrondo
Cátia Maria Morgado Peres	16/10/01	IBET	M. Carrondo
Marta Aires de Sousa Ferreira	17/12/01	ITQB	H. de Lencastre
Ana Cabral Couto Lopes Nóvoa	21/01/02	IGC	A. Coutinho
Paula Isabel Loução Alves	19/03/02	IBET	M. Carrondo
Paulo David Dias Almeida	26/06/02	IGC	A. Coutinho
Sandra Isabel Coimbra Miranda	11/09/02	IBET	M. Carrondo
Sandra Maria Monteiro	01/02/03	IBET	M. Carrondo
Elisabete Andrade Alves Pires	01/07/03	ITQB	A.V. Coelho

Publications Laboratório Associado 2005
(46 papers published)

Abranches, R., S. Marcel, E. Arcalis, F. Altmann, P. Fevereiro and E. Stoger (2005). “Plants as bioreactors: A comparative study suggests that Medicago truncatula is a promising production system.” Journal of Biotechnology 120(1): 121-134.

Abranches, R., R. W. Shultz, W. F. Thompson and G. C. Allen (2005). “Matrix attachment regions and regulated transcription increase and stabilize transgene expression.” Plant Biotechnology Journal 3(5): 535-543.

Bandeiras, T. M., M. M. Pereira, M. Teixeira, P. Moenne-Loccoz and N. J. Blackburn (2005). “Structure and coordination of Cu-B in the Acidianas ambivalens aa(3) quinol oxidase heme-copper center.” Journal of Biological Inorganic Chemistry 10(6): 625-635.

Boucontet, L., N. Sepúlveda, J. Carneiro and P. Pereira (2005). “Mechanisms controlling termination of V-J recombination at the TCR gamma locus: Implications for allelic and isotypic exclusion of TCR gamma chains.” Journal of Immunology 174(7): 3912-3919.

Campino, S., S. Bagot, M. L. Bergman, P. Almeida, N. Sepulveda, S. Pied, C. Penha-Goncalves, D. Holmberg and P. A. Cazenave (2005). “Genetic control of parasite clearance leads to resistance to Plasmodium berghei ANKA infection and confers immunity.” Genes and Immunity 6(5): 416-421.

Carapuco, M., A. Novoa, N. Bobola and M. Mallo (2005). “Hox genes specify vertebral types in the presomitic mesoderm.” Genes and Development 19(18): 2116-2121.

Carneiro, J., T. Paixão, D. Milutinovic, J. Sousa, K. Leon, R. Gardner and J. Faro (2005). “Immunological self-tolerance: Lessons from mathematical modeling.” Journal of Computational and Applied Mathematics 184(1): 77-100.

Coimbra, P., M. H. Gil, C. M. M. Duarte, B. M. Heron and H. C. de Sousa (2005). “Solubility of a spiroindolinonaphthoxazine photochromic dye in supercritical carbon dioxide: Experimental determination and correlation.” Fluid Phase Equilibria 238(1): 120-128.

de Sousa, M. A., T. Conceição, C. Simas and H. de Lencastre (2005). “Comparison of genetic backgrounds of methicillin-resistant and -susceptible Staphylococcus aureus isolates from Portuguese hospitals and the community.” Journal of Clinical Microbiology 43(10): 5150-5157.

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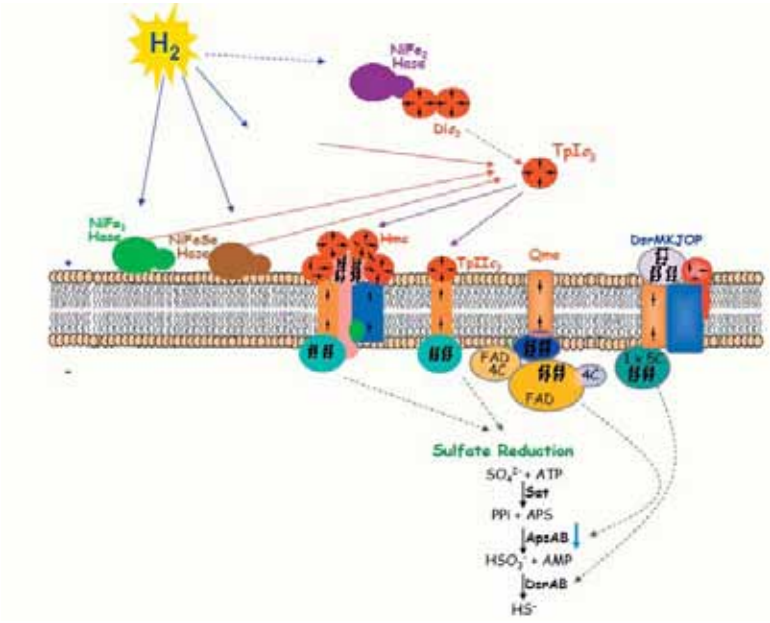
Structure-function studies on Type II cytochromes c3 from *Desulfovibrio sp.*

Ricardo O. Louro : Inorganic Biochemistry and NMR Laboratory

Bacteria of the *Desulfovibrio* genus are anaerobic organisms found in a wide variety of environments either natural, man made, or even in the gastrointestinal tract of mammals which display a versatile metabolism.

The type II cytochrome c_3 is located on the periplasmic side of a transmembrane electron transfer complex involved in the anaerobic respiratory processes of *Desulfovibrio* sp. This membrane associated protein has been isolated from two species, *D. vulgaris* Hildenborough and *D. africanus*, and characterized using biochemical techniques. Purification and characterization of the intact membrane complex from *D.vulgaris*, shows the presence of a diverse array of metal containing centres including *c*-type hemes, *b*-type hemes, and Fe-S centres. The detailed characterization of the four haems of the isolated cytochrome and of the type I cytochrome c_3 which is the proposed upstream physiological partner is being conducted using NMR techniques. The work on type II cytochrome c_3 has been performed in collaboration with the Structure and Function of Metalloproteins Group and the Microbial Biochemistry Group [1].

During 2005 collaboration was established with the group of Dr. J. Zhou from the Environmental Sciences Department of the Oak Ridge National Laboratory, in Tennessee, USA. The study of the transcription levels of the genes coding for the proteins involved in the bioenergetic metabolism of *Desulfovibrio vulgaris* Hildenborough was performed using whole genome arrays developed at the Oak Ridge laboratory. The analysis of these data is on-going and is expected to enable the assignment of the multiple gene products involved in the bioenergetic metabolism of *D. vulgaris* Hildenborough to their specific respiratory pathways, and therefore to allow the determination of the physiological role of the transmembrane complex that incorporates the type II cytochrome c_3.



Cartoon of the versatile respiratory chains of *Desulfovibrio vulgaris* Hildenborough showing the putative pathways leading to type II cytochrome c_3.

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Prediction of pKa values in proteins using implicit conformational reorganization

António M. Baptista: Molecular Simulation Laboratory

The solution pH is an important factor for the biological function of proteins, as clearly illustrated by the existence of optimum pH ranges for enzyme activity or structural stability. pH acts by modulating the state of certain protein ionizable groups (amino and carboxyl termini, and the side-chains of His, Asp, Glu, etc), often directly involved in the catalytic mechanism of enzymes. The determination of the ionization states at a particular pH may seem a trivial task, because pK_a values for these ionizable groups can be found in any standard handbook. Unfortunately, things are not that simple, for three main reasons. Firstly, the typical environment inside a protein cannot usually stabilize a charged group as well as water, meaning that the pK_a of a buried group tends to be different from the value in solution (the one found in handbooks). Secondly, ionizable groups interact with each other and may reciprocally affect the equilibrium between their charged and neutral forms (*e.g.*, it is difficult to have two positively charged His residues near each other). Thirdly, the situation is further complicated by the existence of ionization-conformation couplings, whereby a particular ionization state may favor some conformational state, and vice-versa. Therefore, although groups at the protein surface are expected to exhibit pK_a values similar to the solution ones, the pK_a values for buried groups have to be found by experiment (*e.g.*, NMR) or theoretical calculations.

Poisson-Boltzmann (PB) methods are one of the most reliable approaches to predict pK_a values in proteins. These methods model the structural reorganization induced by electrical changes (*e.g.*, addition or removal of charges) by using dielectric constants; the higher the structural reorganization, the higher the dielectric constant. Thus, a high dielectric constant is usually assigned to the highly reorganisable water region and a lower value to the structurally more constrained and less reorganisable protein region; this intends to implicitly model the ionization-conformation coupling mentioned above. Some authors take this relation even further and claim that buried groups require a lower dielectric constant (around 4) than the solvent-exposed ones, which has actually became a widespread idea. However, the relation between the dielectric constant and the reorganization extent is borrowed from macroscopic electrostatics and should not be pushed too far at the molecular level. This Laboratory has made an extensive study of this issue, [1], using eight proteins with a total of 96 experimentally determined pK_a values. Our results indicate no specific trend for the buried groups. On the contrary, the same dielectric constant (around 20) gives the best predictions for buried and exposed groups alike. The overall prediction error is around 0.8 pH units, which is reasonably close to typical experimental errors. The study conclusively shows that there is generally no benefit from using lower dielectric constants for buried groups, and that theoretically unjustified interpretations of PB methods lead to bad modeling and consequently to bad prediction of pK_a values in proteins. A more detailed account of structural reorganization necessarily requires its inclusion in an explicit way, as we have recently shown [2].

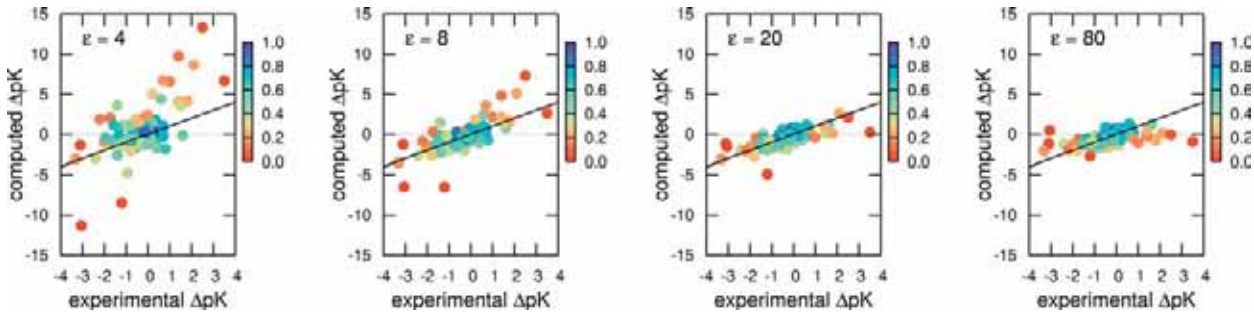

Figure: Computed versus experimental pK_a shifts (with respect to the solution values) for all 96 ionizable groups, using different dielectric constants (ε). The color gradient indicates the relative solvent exposure of the group (fully buried = red, fully exposed = blue).

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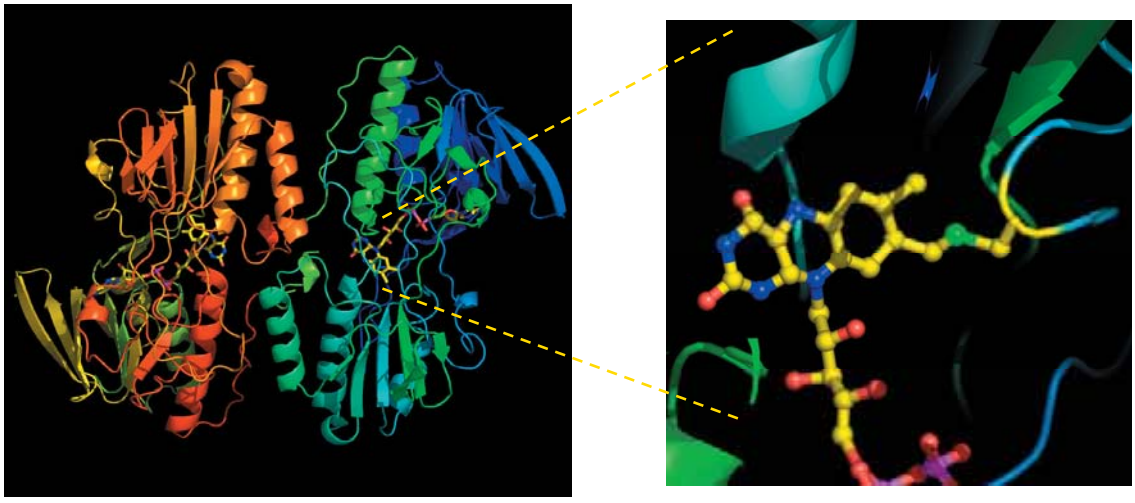
The 3D structure of a bifunctional protein

Margarida Archer : Membrane Protein Laboratory

The structure of NADH:quinone oxidoreductase type II (NDH-2) isolated from the membrane fraction of *Acidianus ambivalens*, a hyperthermoacidophilic archaeon capable of growing at 80^{o} C and pH 2.0, has been determined. NDH-2 was solubilized with the detergent n-dodecyl-β-D-maltoside. The NADH:quinone oxidoreductases, constitute one of the electron entry points into membrane-bound respiratory chains, oxidizing NADH and reducing quinones. Type-II NDHs are functionally unable to translocate protons and are typically constituted by a single ~50 kDa subunit lacking iron-sulfur clusters and containing one flavin as the sole redox center.

The structure analysis has led to further investigations which showed that this enzyme also has a sulfide:quinone oxido-reductase (SQR) activity. Biological sulfide oxidation is a reaction occurring in all three domains of life. One enzyme responsible for this reaction in many organisms has been identified as sulfide:quinone oxidoreductase (SQR). Hydrogen sulfide, the most reduced form of inorganic sulfur, occurs in hydrothermal vents, as well as in sediments. Although H_2S is toxic for most organisms, it may serve as electron donor via the quinone pool.

The enzyme NDH-2 performs two different functions *in vitro*, since it is able to transfer electrons either from NADH or sulfide to the quinone pool. The NDH/SQR structure reveals that it belongs to the glutathione reductase family of flavoproteins.



Secondary structure representation of NDH/SQR with FAD cofactor - crystallographic dimer

Zoomed view near FAD

Protein folding and disease: conformational stability of frataxin clinical mutants

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

Proteins play a critical role in the cell, so it is inevitable that incorrectly folded proteins will lead to a malfunction of the processes in which they are involved, resulting in many circumstances in disease. The number of pathologies which are known to result from protein misfolding is very large, and includes those that result from the accumulation of amyloid deposits and those that lead to inadequate protein conformations as a result of mutational changes.

The Laboratory is currently addressing the molecular basis of the neurodegenerative disorder Friedreich's Ataxia (FRDA), which results from a deficiency in frataxin, a putative iron chaperone. Due to the presence of a high number of GAA repeats in the coding regions of both alleles of the frataxin gene, protein expression is impaired. However, some FRDA patients are heterozygous for the expansion, containing a deleterious point mutation on the other allele, [1]. In collaboration with Annalisa Pastore (NIMR, UK), the Laboratory is aiming at understanding the consequences of these point mutations in frataxin folding, which might contribute to the elucidation of the cellular role of frataxin, whose biological function still remains unclear.

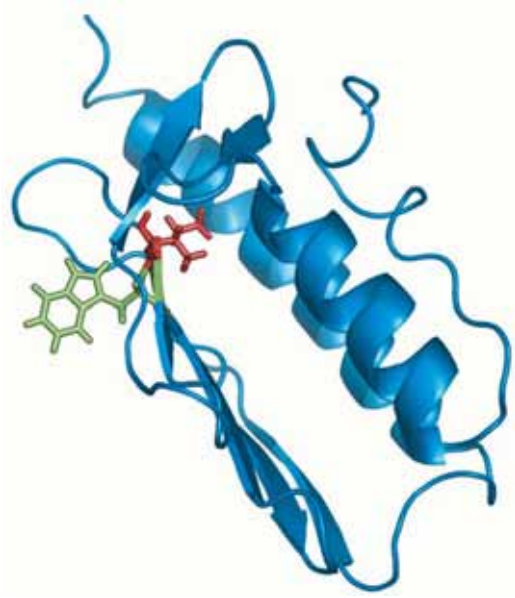


Figure 1: Human frataxin structure [2], highlighting the clinically relevant mutated positions: I154F (red) and W155R (green).

This year, a detailed comparison of the conformational properties of wild type and two clinically relevant frataxin mutants, I154F and W155R (Fig. 1) has been performed. It has been shown that FRDA mutants are destabilised as evidenced by a reduced thermodynamic stability (Fig. 2) and a higher tendency towards proteolysis. The I154F mutation has the strongest effect, in agreement with the fact that this residue contributes to the hydrophobic core formation. The W155R modification alters a residue which is likely to be involved in protein-protein interactions. Nevertheless it results in a destabilised protein as it introduces electrostatic repulsions within the protein surface. It is however interesting to note that both mutants remain folded at room temperature, and that destabilization and reversible unfolding becomes evident only under stressing conditions (thermal or chemical).

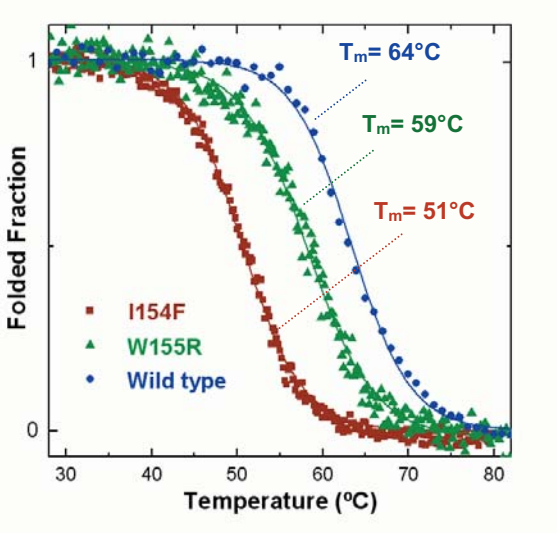


Figure 2: Thermal denaturation curves of wild type and clinical mutant forms of human frataxin

The mechanism determining the pathology in heterozygous cases may involve two scenarios; either a lower concentration of 'functional' protein is available in the cell, since as a result of a higher destabilization the folding efficiency could be impaired, or the mutant proteins are more efficiently degraded, as evidenced from an increased rate of proteolytic degradation in comparison to the wild type form. Frataxin mutants which result in milder forms of the disease are also particularly interesting in the molecular understanding of this disease, and are currently under investigation in our Laboratory.

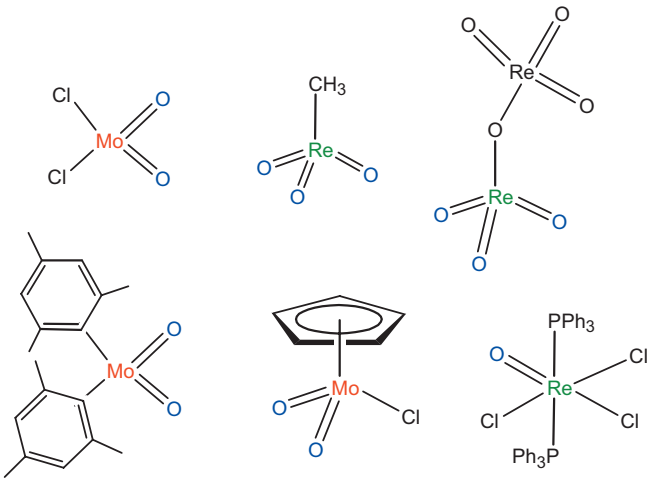
[1] Cossee et al. (1999) Ann Neurol 45, 200-6 [2] Musco et al. (2000) Structure Fold Des 8, 695-707

High valent oxo-rhenium and –molybdenum complexes as catalysts for hydrosilylation of carbonyl groups

Beatriz Royo: Homogeneous Catalysis Laboratory

The Laboratory has demonstrated the excellent efficiency of high valent oxo-molybdenum(VI) and –rhenium(VII) and (V) complexes to activate Si-H bonds. The use of oxo-metal complexes as catalysts for oxidation and oxygen transfer reactions has been extensively studied but their catalytic activity in a reducing reaction is extremely rare. We have found that the dioxomolybdenum(VI) complexes MoO_2Cl_2, $MoO_2(acac)_2$, $CpMoO_2Cl$, $MoO_2(mes)_2$ and the polymeric organotin-oxomolybdates $[(R_3Sn)_2MoO_4]$ catalyze the hydrosilylation of aldehydes and ketones with dimethylphenylsilane.[1] The wide scope molybdenum oxide-mediated hydrosilylation was established with a variety of aldehydes and ketones. A new mechanism has been found for metal oxo catalyzed hydrosilylation reactions of carbonyl groups in which radical intermediates are involved. Radical scavenging experiments have shown that the hydrosilylation reaction proceeds by a free-radical pathway. A variety of oxorhenium(VII) and (V) complexes also catalyze the hydrosilylation reaction. It has been found that Re_2O_7, CH_3ReO_3, $CpReO_3$, $ReO_2Cl(DMSO)_2$, $CH_3ReO_2(RC{\equiv}CR)$ and $ReOCl_3(PPh_3)_2$ are effective catalysts for the reduction of carbonyl groups.[2]

Catalysts for the hydrosilylation of carbonyl groups



[1] A. C. Fernandes, R. Fernandes, C. C. Romão and B. Royo, *Chem. Commun.*, **2005**, 213.
[2] B. Royo and C. C. Romão, *J. Mol. Catal. A: Chemical*, **2005**, *236*, 107.

Electron Paramagnetic Resonance studies of the iron-sulphur centres from complex I of *Rhodothermus marinus*

Manuela M. Pereira : Metalloproteins and Bioenergetics Laboratory

Complex I of aerobic respiratory chains catalyses the NADH:quinone oxidoreductase reaction coupled to charge translocation across the membrane, which contributes to the membrane electrochemical potential that is the driving force for ATP synthesis. The bovine complex is composed of more than 40 subunits and has a molecular mass of ~1MDa, while the bacterial counterpart is formed generally by 13 or 14 subunits with ~500kDa, the so called minimal functional unit.

Rhodothermus marinus, our model system, is a thermohalophilic Gram negative bacterium, containing a type I NADH:quinone oxidoreductase (complex I). Its purification has been optimised, yielding large amounts of pure and active protein. The large amounts of protein have enabled a thorough and exhaustive characterisation by EPR spectroscopy made for the first time in an intact complex I[1]. This characterisation was performed at different temperatures and microwave powers, using NADH, NADPH and dithionite as reducing agents. A minimum of two $[2Fe\text{-}2S]^{2+/1+}$ and four $[4Fe\text{-}4S]^{2+/1+}$ centres were observed in the purified complex. Redox titrations monitored by EPR spectroscopy have made possible the determination of the reduction potentials of the iron-sulphur centres; with the exception of one of the $[4Fe\text{-}4S]^{2+/1+}$ centres, which has a lower reduction potential; all the other centres have reduction potentials of -240 $\pm$ 20 mV, pH 7.5.

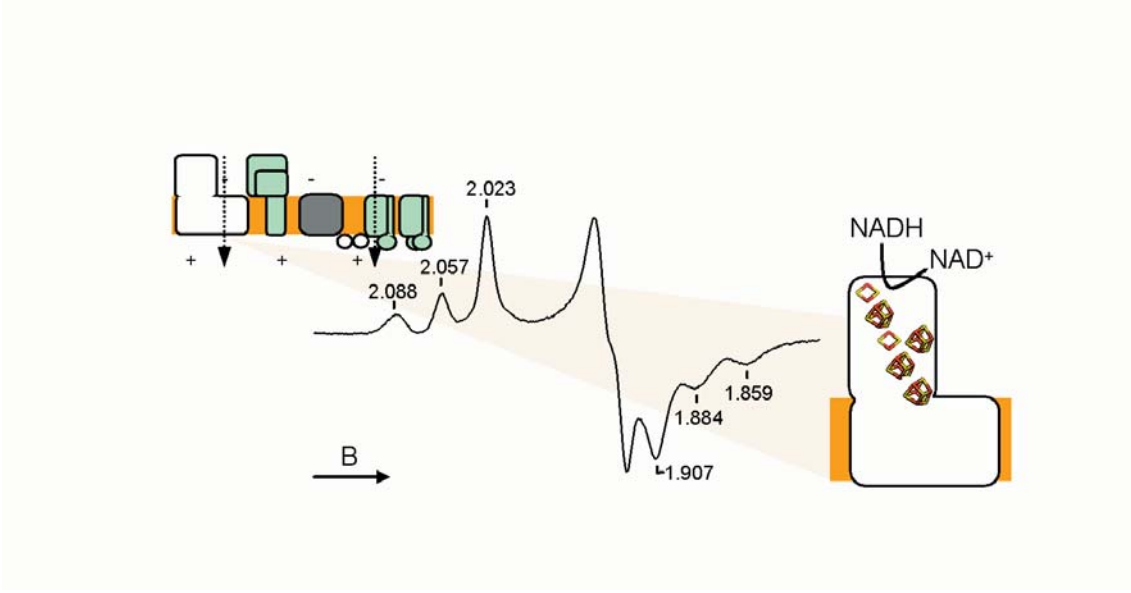


Figure – schematic representation of *Rhodothermus marinus* respiratory chain and its complex I. EPR spectrum of *R. marinus* complex I reduced with 10 mM NADH plus dithionite, at 10 K and 2.4 mW

[1] Fernandes, A. S., Sousa, F. L., Teixeira, M. and Pereira, M. M. (2005) Electron Paramagnetic Resonance studies of the iron-sulphur centres from complex I of *Rhodothermus marinus*, *Biochemistry*, *in press*.

Synthesis of organic coumpounds of biological importance

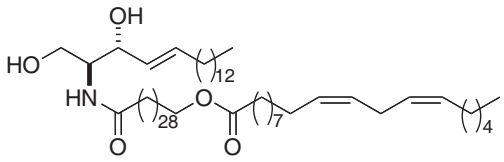
Rita Ventura : Biorganic Chemistry Laboratory

The synthesis of ceramide Cer1 (Figure 1) has been undertaken in conjunction with Micro-heterogeneous Systems Laboratory. This ceramide is important for studies of permeation of the stratum corneum and other properties of the skin. It is not commercially available and requires a long multi-step synthesis. Due to the long chains of fatty acids, apparently easy reactions turn out to be rather complicated. Modifications to some methods described in the literature are being studied in order to improve yields and avoid side reactions.

The synthesis of new solutes for thermostabilisation of proteins has continued, as part of the project HotSolutes FP6-2002-SME-1. The most recent solutes were α-glucosyl-D-glycerate and α-glucosyl-D-glyceramide (Figure 2) on a large scale for testing by the other groups involved in this European project. Studies of the synthesis of glycerophosphoinositol, a solute very difficult to obtain from its natural source, and CDP-inositol for the Cell Physiology and NMR Laboratory have been started, (Figure 3).

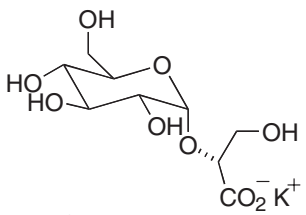
Studies of glycosylation reactions between several glycosyl donors derived from glucose and mannose with different steroids furnished by the Faculdade de Farmácia, Universidade de Coimbra, are also being studied in order to synthesise natural products and derivatives with possible antitumor activity. Steroids are large and complex molecules and this has a different influence on anomeric selectivity compared with other alcohols studied previously.

Figure 1.

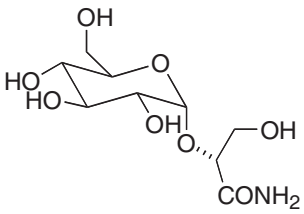


Cer1

Figure 2.

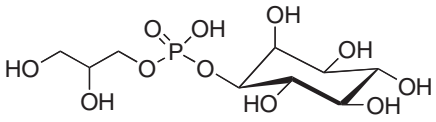


α-D-Glucosyl-D-glycerate

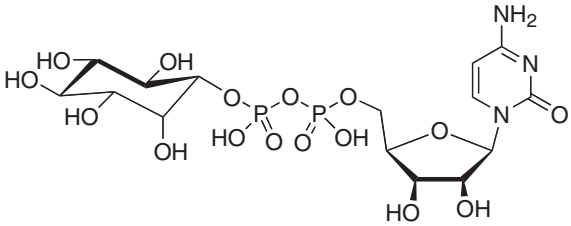


α-D-Glucosyl-D-glyceramide

Figure 3.



Glycerophosphoinositol

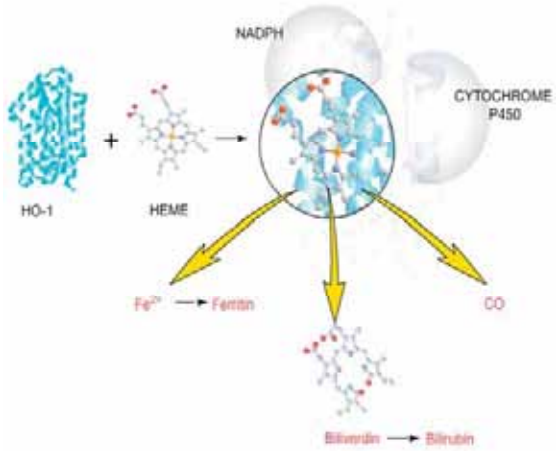


CDP-inositol

Control of Inflammation

Miguel Soares : IGC Inflammation Laboratory

Inflammation, originally defined as *rubor, calor, tumor and dolor* (*Aulus Conelius Celsus; 30AD*) is a beneficial host response to injury characterized by the migration of circulating white cells and soluble molecules from blood into tissues. While absolutely required to achieve microbial clearance inflammation must be controlled so that it is "shut off" as soon as microbial infections have been cleared. One of the mechanisms that control inflammation relies on the expression of "protective genes". By definition these are genes that when expressed in non-lymphoid cells, dampen inflammation, preventing irreversible injury and restoring homeostatic cellular/tissue function. When inflammation is not controlled it can become pathologic leading to the development of "inflammatory diseases".



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.

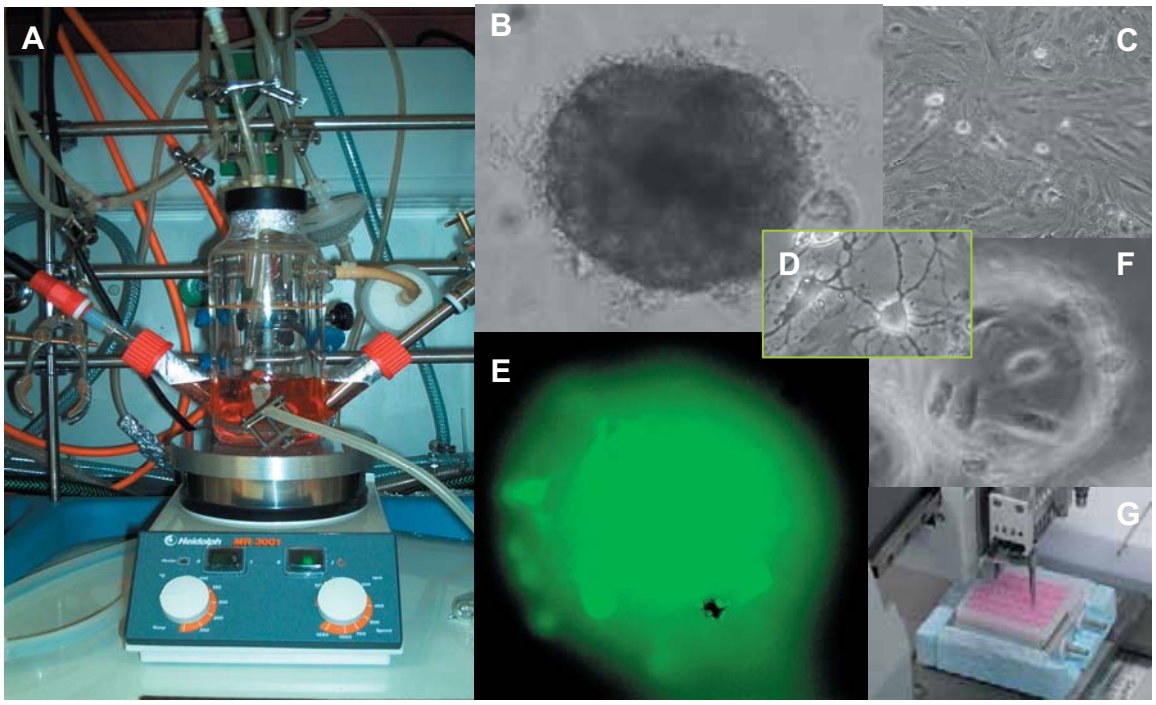
The term "inflammatory diseases" defines those pathologic conditions of infectious or non-infectious origin in which the common underlying cause of pathology is the development of acute or chronic inflammation resulting from inadequate expression of one or several "protective genes". Based on this definition "inflammatory diseases" should regroup apparently disparate pathologic conditions, including atherosclerosis, multiple sclerosis, severe sepsis and/or severe acute malaria.

Over the past two years the Laboratory has tested the hypothesis that the pathogenesis of these "inflammatory diseases" is indeed driven by defective expression of "protective genes". This hypothesis has been tested specifically for the protective gene heme oxygenase-1 (HO-1) (see Figure). If indeed HO-1 expression controls the pathogenesis of inflammatory diseases then the pathologic outcome of inflammatory diseases should be significantly exacerbated when HO-1 expression is inhibited or deleted. It has been found that this is the case in mice in which the expression of HO-1 has been deleted by homologous recombination. Expression of HO-1 and/or administration of one or several of the end-products of heme degradation by HO-1 suppresses the pathogenesis of lipid-mediated atherosclerosis in apolipoprotein E deficient mice exposed to a high cholesterol diet, the experimental autoimmune encephalomyelitis in mice (an experimental model of multiple sclerosis), the cecal ligation and puncture in mice (an experimental model of severe sepsis), and severe malaria following *plasmodium* infection in mice (an experimental model of severe acute malaria). The interpretation of this data is that, indeed, there seems to be a common mechanism that regulates the pathogenesis of inflammatory diseases, namely the expression of protective genes. This suggests that modulation of such common denominators may be used therapeutically to overcome the pathogenesis of these diseases.

Human Adult Pancreatic Stem Cells: Designing novel propagation strategies and cryo-preservation tools

Paula M Alves : IBET Animal Cell Technology Laboratory

Currently stem cells are expanded and differentiated on static surfaces, *i.e.* culture dishes and T-flasks. Due to the increasing demand of stem cells for therapy purposes, these systems are limited not only in terms of scaleability, but also in terms of control of culture parameter conditions (*e.g.* Oxygen and pH), relevant issues also for reproducibility. In order to design and improve novel systems for expansion, differentiation and cryo-preservation of Adult Pancreatic Stem Cells several issues have been addressed. The possibility of culturing stem cells in scaleable systems (*e.g.* spinners, stirred vessels, bioreactors) has been evaluated. Human Adult Pancreatic Stem Cells (PSLCs) are anchorage dependent cells, thus immobilization systems using micro-carriers and/or aggregates are required for cell culturing in non-static conditions. Preliminary results have shown that PSLCs can adhere and grow on micro-carriers. Moreover, it has been observed that, when inoculated in stirred vessels, PSLC aggregates are naturally formed. The aggregates grow in size during the culture. Analytical techniques to monitor aggregate formation and maintenance, both at the level of cell expansion and differentiation, for characterization of these aggregates are currently under development. Development of automation systems for cryo-preservation and thawing of PSLCs is also undergoing in collaboration with IBMT (Fraunhofer, Germany).

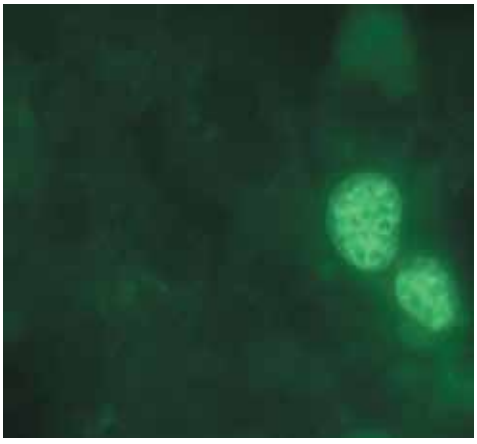


(A) Small scale bioreactor for propagation/differentiation of PSLCs (B) Human Adult Stem cells growing as aggregates. (C) Undifferentiated and (D) differentiated PSLCs. (E, F) Propagation of PSLC's in micro-carriers. (G) Automation of cryo-preservation

Genetics of Malaria Liver Stage

Carlos Penha-Gonçalves : IGC Disease Genetics Laboratory

Malaria constitutes one of the major world-scale health problems being responsible for 1-2 million deaths every year, mainly in tropical Africa where it is endemic. *Plasmodium* sporozoites are injected into the mammalian host by infected mosquitoes and migrate to the liver. The liver stage is likely to be an ideal target for the control of malaria because it represents an initial infection step when numbers of parasites and pathology effects are still reduced. Susceptibility to malaria shows a genetic component that has led to a search for host genetic factors that confer resistance to plasmodial infection. Mouse models of malaria infection have been particularly useful in the study of the genetics of malaria resistance. In this study the Laboratory has looked for mouse genetic determinants of resistance to liver stage infection by *Plasmodium berghei*. A genetic factor (Berl1 locus) has been identified, conferring resistance to parasite proliferation in the liver and located within a 17 cM region on mouse chromosome 17 and within a 10Mb region adjacent to the MHC locus. It has been demonstrated that genetic resistance conferred by this locus develops during malaria liver stage by reproducing the resistance phenotype after infection by intra-hepatic injection. In addition, it has been shown that the resistance phenotype arises from limited parasite proliferation that becomes apparent by 32h post-infection. The Laboratory has also been able to show that the proliferation impairment is reproduced in hepatocyte primary cultures infected "*in vitro*" with *P.berghei* sporozoite. These results indicate that Berl1 is a genetic factor that limits *Plasmodium berghei* proliferation inside the hepatocytes. The current aim is to identify the gene that underlies the genetic resistance conferred by the Berl1 locus.



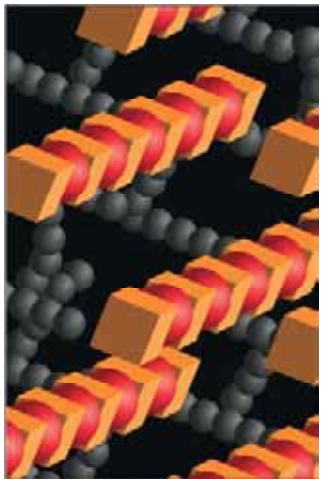
Legend: Malaria parasites inside liver cells. The Figure shows mouse hepatocyte primary culture, after 40 hours infection with GFP-fluorescent Plamodium Berghei sporozoites.

Biological activity of bacterial cell wall and its components

Sérgio R. Filipe : Microbial Pathogenesis and Cell Biology Laboratory

The Laboratory is interested in how pathogenic bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae* divide and, in particular, in the process of bacterial cell wall synthesis. The assembly of the bacterial cell wall is carried out by an extremely complex mechanism that allows incorporation of new building blocks without weakening of the cell wall. Perturbation of this process by antibiotics or other stresses may result in a cell wall that it is not strong enough to protect bacteria from the incredible osmotic pressure to which it is subjected. On the other hand in some bacteria, there is a mechanism of cell wall turnover in order to recycle its building blocks or eventually to control the release of cell wall fragments to the exterior. These cell wall fragments may be recognized by the host immune system and allow triggering of an inflammatory response with the aim of removing the bacterial agent. The Laboratory has recently determined that muropeptides from *S. aureus* and *S. pneumoniae*, the smallest components of the bacterial cell wall, can induce an innate immune response in Drosophila flies.

The Laboratory is fully committed to inquire about the molecular interactions between inflammatory muropeptide components and their host receptors. It is also interested in the complex mechanisms associated with the cell wall metabolism and whether any perturbation in this process may have an impact on the ability of bacteria to evade host detection.



Representation of *S. aureus* peptidoglycan

Evolution of the modular organization of cellular systems

José B. Pereira-Leal: IGC Computational Genomics Laboratory

Genome projects have provided complete lists of the building blocks of living systems, but not the knowledge about how they are regulated, interact or assemble into functional units. The Laboratory is interested in understanding the organizational principles that bring all the components together in a cell (genes, proteins, metabolites, *etc*.), and what were the evolutionary mechanisms driving that cellular organization. The work is based on computational approaches (Computational Biology; Bioinformatics).

Biological systems such as cells are organized in a modular way. Individual functions within the cell are accomplished by groups of molecules working together. Much of the work has focused on protein complexes and pathways, which are types of functional modules in the cell (Figure 1). Using the former it has been shown that functional modules, like individual genes, can duplicate as a whole and that this is a mechanism of functional specialization[1]. More recently an evolutionary mechanism has been uncovered based on duplication of self-interactions that drives the origin as well as specialization of protein complexes. The Laboratory also collaborates with experimental groups and has recently participated in a study that uncovered new components of the Fanconi anemia tumor-suppressor pathway[2].

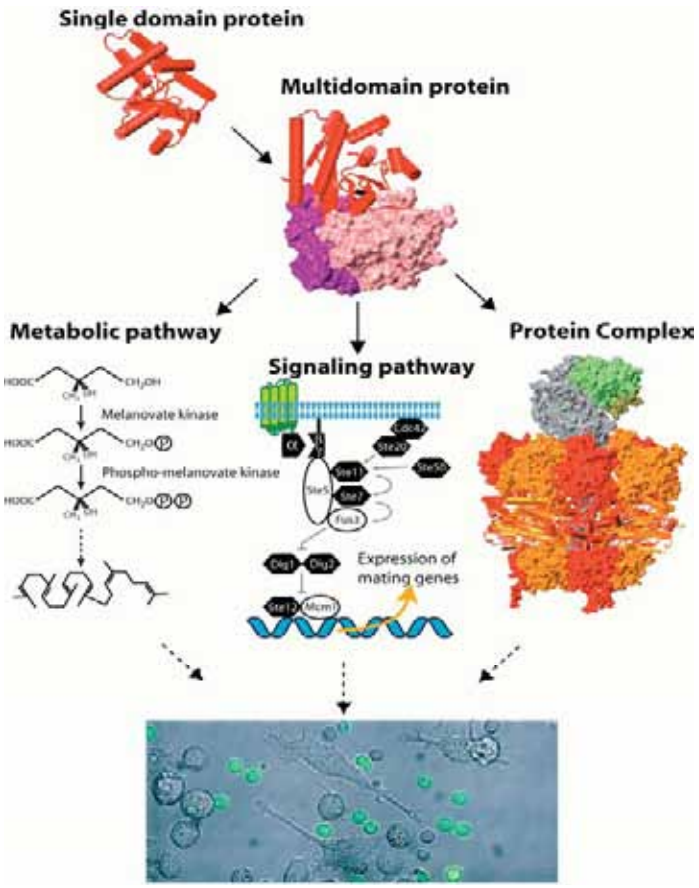


Figure 1 – Levels of modularity, from protein structures, to complexes and pathways to cell types

On a smaller scale, the Laboratory studies the role of modularity on the evolution of proteins. Protein domains are the modules from which proteins are constructed (see Figure 1). At this level a mechanism has recently been proposed for the evolution of proteins composed of multiple domains[3] and proteomes of pathogenic Trypanosomes have been analyzed in terms of structural domain composition[4].

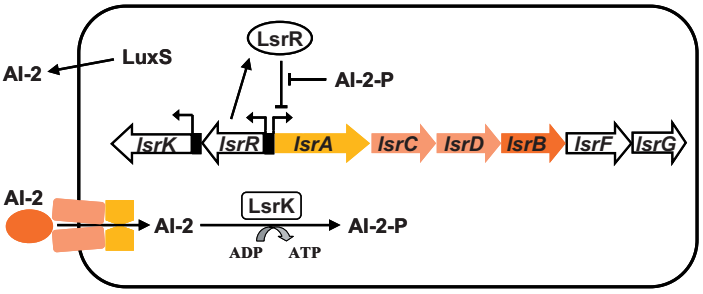
1 Pereira-Leal, & Teichmann (2005) *Genome Res.* 15(4):552-9
2 Mosedale *et al.* (2005) *Nature Struct. Mol. Biol.* 12(9):763-71
3 Vogel, Teichmann & Pereira-Leal, JB (2005) *J. Mol. Biol.* 346(1):355-65
4 El-Sayed *et al*. (2005) *Science.* 309(5733):404-9

Interference with inter-species communication in bacteria

Karina B. Xavier : Bacterial Signaling Laboratory

Bacteria use chemical signal molecules called autoinducers to communicate with one another by a process called quorum sensing. This process enables a population of bacteria to regulate behaviors which are only productive when many bacteria act in concert as a group, similar to what happens with multi-cellular organisms. Behaviors regulated by quorum sensing are often crucial for successful bacterial-host relationships, both symbiotic and pathogenic. While most quorum sensing autoinducers are species-specific, one autoinducer called autoinducer-2 (AI-2), is produced and detected by a wide variety of bacteria and is proposed to allow inter-species communication.

In this Laboratory a biochemical, genetic, and chemical approach is being used to study the AI-2 systems promoting bacterial inter-species communication. By studying the molecular mechanisms underlying AI-2 quorum sensing in the enteric bacterium *Escherichia coli* it has been shown that, in *E. coli,* AI-2-regulates a transport system that internalizes and degrades the AI-2 signal. Specifically, at high population densities, *E. coli* uses this system to remove AI-2 produced by itself and also AI-2 produced by other species present in the same co-culture. AI-2 internalization by *E. coli* has the consequence of interfering with other species' ability to use AI-2 to regulate their group behaviours by quorum sensing. The characterization of the *E. coli* AI-2 system will be pursued, as will investigations on novel AI-2 signalling systems in other bacteria to understand how bacteria use inter-species cell-cell communication to coordinate population-wide behaviors in consortia and in microbial-host interactions.



***E. coli* transports AI-2 by the Lsr operon.** In *E. coli,* AI-2 is produced by LuxS and released to the extra-cellular medium. At a particular treshold concentration, AI-2 is internalized by the Lsr transporter, and intercellular AI-2 is phosphorylated by LsrK. AI-2-P induces *lsr* transcription by inactivating LsrR, the repressor of the *lsr* operon.

The *E. coli* AI-2 internalization process represents the first example of interference with AI-2-mediated quorum sensing. Understanding the natural strategies organisms use to interfere with other species' ability to communicate, such as in *E. coli*, will give models for the design of clinical and biotechnological strategies intended to manipulate bacterial behaviours. Such studies will, in turn, lead to the development of new therapies to control functions regulated by quorum sensing, such as virulence, and also to develop biotechnological applications to control industrial scale production of beneficial bacterial products, like antibiotics or recombinant proteins.

K. B. Xavier and B. L. Bassler. 2005. Interference with AI-2-mediated bacterial cell-cell communication. *Nature.* 437: 750-753.

K. B. Xavier and B. L. Bassler. 2005. Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. *J. Bacteriol.* 187: 238-48.

Localization of the cell wall synthetic machinery in *Staphylococcus aureus*

Mariana Gomes de Pinho : Laboratory of Bacterial Cell Division

Staphylococcus aureus is a gram-positive bacteria that causes a large variety of infections and is one of the major causes of hospital-acquired infections nowadays. This problem is worsened by the increasing number of hospital strains which are resistant to beta-lactam antibiotics, as well as to other classes of antibiotics. Beta-lactam antibiotics inactivate the so-called penicillin-binding proteins (PBPs) which are responsible for the last stages of peptidoglycan synthesis. Peptidoglycan is the main component of the bacterial cell wall, a structure essential for survival, which protects the cells from osmotic pressure and is responsible for its morphology.

The mechanism of beta-lactam resistance in methicillin resistant *S. aureus* (MRSA) strains involves the acquisition of a foreign protein – PBP2A – which has very low affinity for beta-lactams and is therefore capable of cell wall synthesis in the presence of high concentrations of antibiotic. The Laboratory is interested in understanding how the machinery responsible for cell wall synthesis works, which are its components, and how does it find its proper place in the cell. Also of great interested is to learn if proper localization of PBPs is involved in antibiotic resistance mechanisms.

It has also been recently shown that PBP2, one of the native PBPs from *S aureus*, localizes to the division septum (Fig 1). However, this localization is lost upon addition of beta-lactam antibiotics. Interestingly, in the presence of the resistance protein PBP2A, the native PBP2 can remain at its proper place even when beta-lactams are present in the medium. This finding has led to the suggestion that besides its well known role in peptidoglycan biosynthesis, PBP2A has role in recruiting members of the cell wall synthetic machinery to the division septum in the presence of beta-lactams. The Laboratory is currently studying the localization of the known components of the cell wall synthetic machinery, as well as trying to identify new ones.

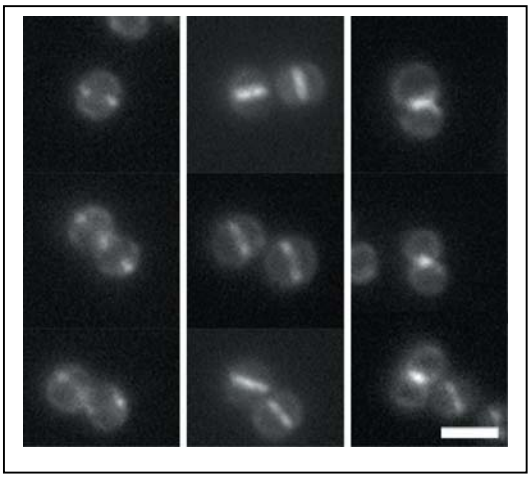


Fig. 1. Localization of PBP2 during the cell cycle. When the septum starts to form, GFP-PBP2 can be seen as two spots, that correspond to a ring around the future division site (LH panel). This pattern then changes to a line across the cell, indicating that PBP2 is localized along the entire closed septum (Centre panel). This pattern is maintained even as the two daughter cells start to separate (RH panel). Scale bar 2 μm.

An antisense transcript for the Hoxb3 gene

Moises Mallo : IGC Neural Crest and Gene Modification Laboratory

Genome-wide expression analyses and functional annotation of the mouse and human genomes has revealed the existence of antisense transcripts at a frequency much higher than anticipated. The functional relevance of these transcripts, which in most cases do not code for a protein, is still unknown. The Laboratory has discovered the existence of an antisense transcript for the mouse Hoxb3 gene (see figure), subsequently referred to as Hoxb3AS.

The Hoxb3AS transcript has 4 exons, separated by 3 quite large introns and contains a polyA tail. The 5' exon is located close to the Hoxb2 gene (located 5´with respect to Hoxb3) and the 3' exon overlaps the last exon of the Hoxb3 sense transcript. The polyadenylation signal is located within the intron of the Hoxb3 gene. A database search has revealed that a similar transcript exists in humans, although with small structural differences with respect to that found in the mouse. Expression analysis of this transcript during mouse development has shown that Hoxb3AS is expressed in a spatial domain that is complementary to that of the Hoxb3 sense transcript, suggesting that it might play a role in the regulation of Hoxb3 gene expression. In addition, its expression is dynamic throughout development, which is indicative of the existence of precise regulatory mechanisms for its expression.

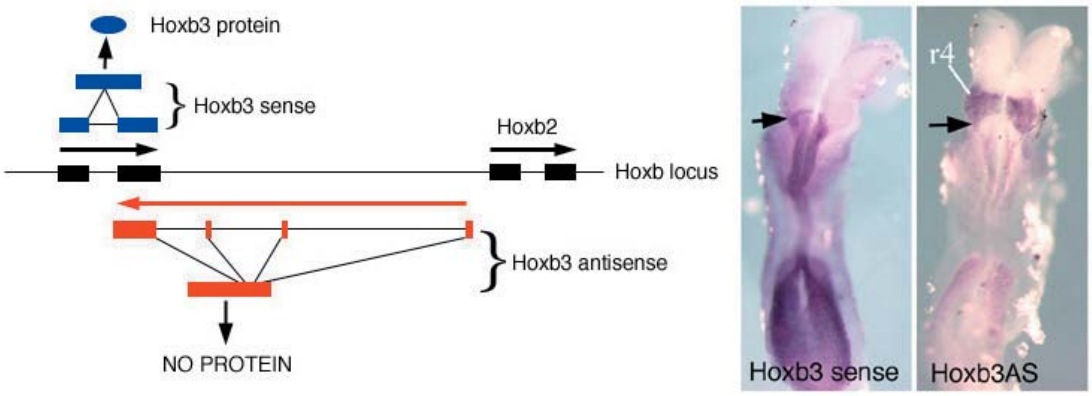


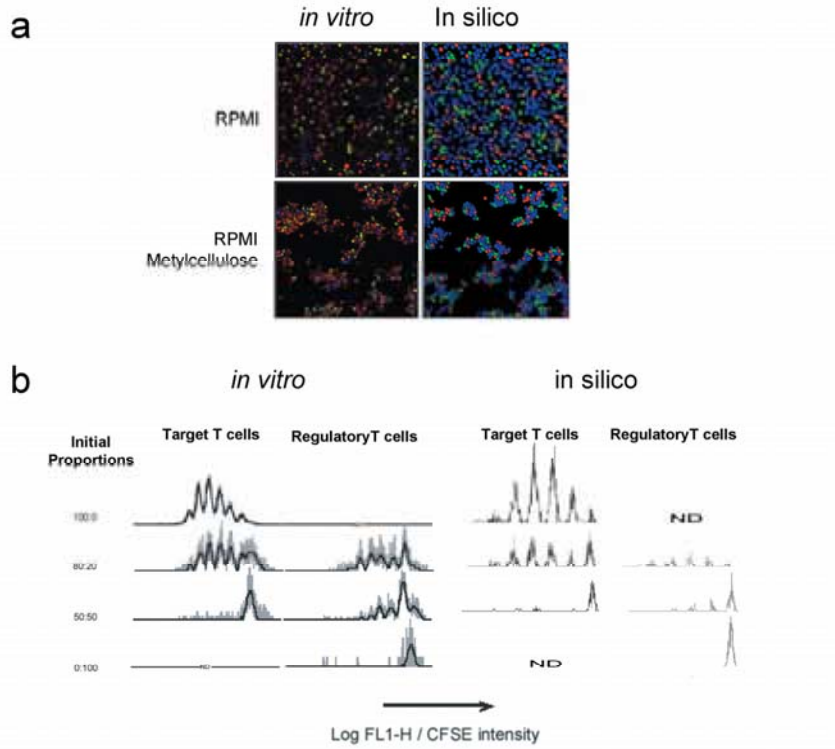
Figure 1. Structure and expression of the sense and antisense transcripts of Hoxb3. The Hoxb3 antisense transcript (in red) spans the intergenic region between Hoxb2 and Hoxb3 and is partially overlapping with the latter. It runs in an opposite direction to the Hoxb2 and Hoxb3 genes and does not code for a protein. *In situ* hybridization analysis (day 8 mouse embryos shown in the pictures) shows that the Hoxb3 antisense transcript is expressed in a domain adjacent to that of the Hoxb3 gene, with very sharp limits (indicated by the arrow). The fourth rhombomere is indicated by r4, a major area of expression of the Hoxb3 antisense transcript.

The specific characteristics of Hoxb3AS and our extensive knowledge of the functional role of Hoxb3 in mouse development and physiology, makes this system a good model to approach the role of antisense transcription in the genome and of its mechanisms of action.

The Role of Regulatory CD4 T Cells in Natural Tolerance

Jorge Carneiro : IGC Theoretical Immunology Laboratory

Regulatory CD4 T cells, that express FoxP3 and are enriched in the CD25 pool of healthy individuals, have gained increasing relevance in Immunology. Many lines of evidence indicate that these cells play a key role in the development of natural tolerance and in the prevention of autoimmune pathologies, by controlling the activation and proliferation of other autoreactive lymphocytes. The functional significance of these cells has broadened as they have been shown to control the collateral damage of efficient responses against pathogens, to be able to modulate the immune response impinging on memory generation, and to prevent rejection of transplants. Despite their key role in orchestrating the immune system there are many questions about regulatory T cell immunobiology. One fundamental question is how do regulatory T cells interact and suppress other cells? To gain insight into the suppression mechanism the Laboratory has developed an individual cell-oriented simulation describing the motion, aggregation, interactions and life-cycle of different cell types. Different hypotheses about the interaction mechanisms can be assessed using these simulations by comparing the *in silico* predictions with the results of *in vitro* cell cultures both in terms of aggregation patterns imaged by confocal microscopy, and proliferation profile assessed by CFSE-delabeling. These results are illustrated in the figure that compares real simulated and simulated cell aggregation patterns (panel A) as well as real and simulated CFSE-delabeling profiles (panel B) during in vitro cell cultures of regulatory T cells (green), target T cells (red), and antigen presenting cells (blue). These simulation tools might be useful not only to simulate interactions of cells of the immune system but also to simulated developmental patterns.



Reverse Evolution at the Molecular Level with *Drosophila melanogaster*

Henrique Teotónio : IGC Laboratory of Evolution

The Laboratory seeks to understand whether or not the evolutionary trajectories during reverse evolution follow similar patterns when considering phenotypes or genotypes. Genotypic changes are being followed that have occurred during reverse experimental evolution at several candidate loci. This work involves the characterization of patterns of gene diversity and linkage disequilibrium at the candidate loci for populations that have different evolutionary (laboratory) histories. During 2005 the characterization of molecular diversity was continued together with the linkage disequilibrium in one of the control populations (a population that has been throughout its history in the ancestral environmental conditions), by DNA sequencing approximately 5 kb in four different regions of normal recombination frequencies, for more than 20 chromosomes each. Preliminary analysis shows that the population that is being used is similar to diverse population genetic statistics to described natural populations. A search for genotype polymorphisms will now be conducted at regular intervals in populations that have undergone reverse evolution.

A second project involves the experimental evolution of outcrossing rates in Caenorhabditis elegans. Whilst a selfing mode of reproduction has an intrinsic reproductive advantage relative to outcrossing, many species either show only outcrossing or a mixed breeding system when they reproduce. Several hypotheses have been proposed that rely on the expression of beneficial or deleterious mutations and their fate in selfing or outcrossing populations. At the phenotypic level adaptive changes are being characterized that genetically defined populations undergo when subject to increased mutagen exposure on the one hand and adaptation to alternative novel environments on the other. Already it has been found that males, which in this species are a proxy for outcrossing, are maintained at higher levels when the mutational load of the populations is higher than under natural conditions. The patterns of genetic variation among wild isolates have also been characterized for phenotypes related to outcrossing, such as the rates of X-chromosome non-disjunction, male mating ability, and competitive performance in different environments.

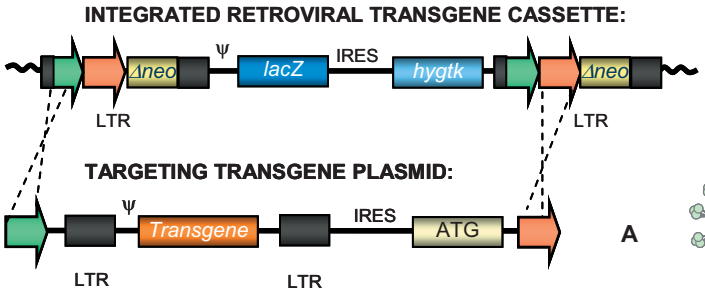
Development of Packaging Cell lines for the production of Retroviral Gene Therapy Vectors

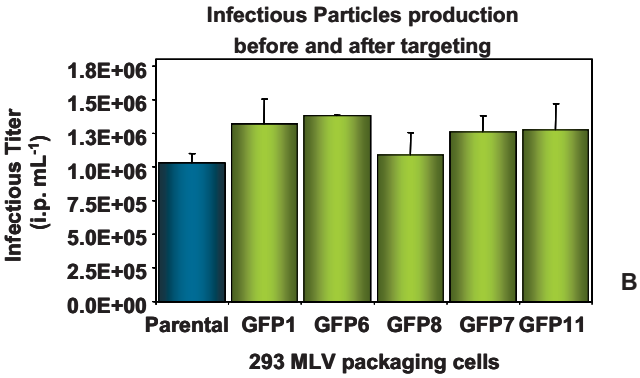
Ana Sofia Coroadinha: IBET Animal Cell Technology Laboratory

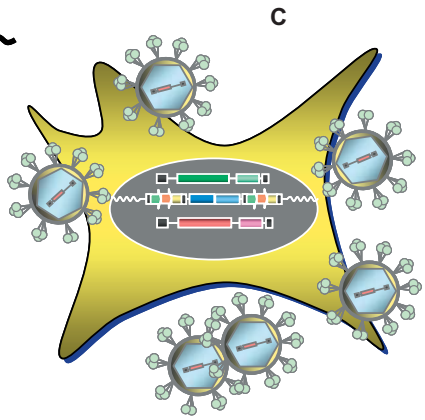
Gene therapy is the treatment or prevention of diseases by gene transfer and has been regarded by many as a potential revolution in medicine. The clinical experience obtained so far suggests that gene therapy has the potential to treat a broad range of both inherited and acquired human diseases. Recombinant murine leukemia virus (MLV) derived vectors were one of the first viral vehicles used for human gene therapy, where it proved to be an effective vector. Since then, recombinant retroviruses have been extensively used in hundreds of clinical trials for the treatment of several types of diseases as cancers, infectious diseases and monogenic disorders.

Currently, retroviral vector producer cell lines must be established for the production of each therapeutic gene. This is done by transfection of a packaging cell line with the gene of interest. In order to find a high titer retroviral vector producer clone, exhaustive clone screening is necessary, as the random integration of the transgene gives rise to different expression levels.

The Laboratory established a pioneering MLV packaging cell line using a human cell line in which the transgene construct containing the therapeutic gene is introduced using a tag and target cassette exchange system. In a first phase the chromosomal *loci* of the producer cell line are tagged and screened for high gene expression with a reporter gene and in a second phase it can be targeted and reused for the expression of a therapeutic gene. The tagged retroviral packaging cell line represents a break-through in the current technology, constituting a high advantage since it has a predictable titer and can be rapidly used for different therapeutic applications.







Fiigures **A**) Tag and Target methodology for the cassette exchange: schematic representation of the tagging construct integrated in the cell and the targeting plasmid. **B**) Retroviral vector production titers before (Parental) and after Targeting (GFP). **C**) Schematic representation of a Retroviral Gene Therapy Producer Cell Line.

Coroadinha *et al.* The use of recombinase Cassette E:
Cell Lines: predictability and efficiency in transgene replacement. J.Biotechnol. (in press)

Molecular epidemiology of multidrug-resistant *Enterococci* at the hospital setting

Rosario Mato : Laboratory of Microbial Epidemiology

The research is focused on the molecular epidemiology and genotypic characterization of nosocomial multidrug-resistant *Enterococcus faecalis*, *E. faecium* and other enterococcal species isolated from infection products and colonization sites collected in Lisbon hospitals. The molecular typing methods used for comparison of the enterococcal strains includes; antimicrobial resistance, pulsed-field electrophoresis profiling (PFGE), polymerase chain reaction, multiple loci sequencing, determination of virulence factors such as *esp* genes related with the capacity of epidemicity, and the identification of mobile elements involved in the acquisition of virulence traits. In parallel, a surveillance study is being carried out in collaboration with a neonatology intensive care unit (NICU). This study will allow the achievement of an improved knowledge of the risk factors associated with infection and the enterococcal carriage stage in high-risk patients. This study started in January 2005 and is included in the project *"Infection and colonization by multidrug-resistant Enterococci recovered from high risk newborns in neonatal intensive care units (NICU). Epidemiological surveillance and infection control"*, financed by Fundação Calouste Gulbenkian Contract Nº 65882, July 2004.

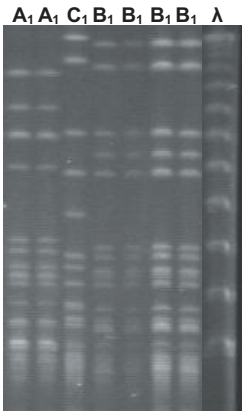


Fig. 1. PFGE of HLGR enterococci major clones colonizing newborns in the NICU.

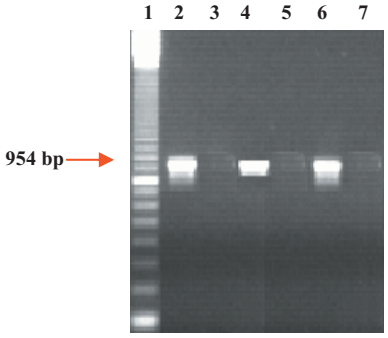


Fig. 2. PCR detection of *esp* genes in vancomycin-resistant *E. faecium* strains, isolated from infection products.

References.

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Miller M. B., S. L. Allen, M. E. Mangum, A. Doutova, and P. H. Gilligan. 2004. Prevalence of Vancomycin-Resistant *Enterococcus* in Prenatal Screening Cultures. J. Clin. Microbiol. **42**(2): 855-857.

MLST as a tool for population analysis in *Enterococcus*

Maria de Fátima Silva Lopes : IBET Stress by Antibiotics and Virulence of Enterococci Laboratory

"*Bacterial genomes are increasingly viewed in terms of the integration of accessory and dispensable genetic elements into a conserved genomic core. This duality provides both the evolutionary stability that is required for the maintenance of essential functions and the flexibility that is needed for rapid exploitation of new niches*", Edward J. Feil (1). This premise is the starting point for the analysis of genomes. One of the methods for genome analysis is MLST (multi-locus sequence typing), developed primarily as an epidemiologic tool, which provides information on the nucleotide divergence of the core genomes from large population samples, allows clonal assignments to be made to given strains, and estimates the rate of homologous recombination and phylogenetic analysis to be carried out (1). This approach allows the construction of unambiguous international databases and thus functions as a powerful resource for global epidemiologic studies, recognition and tracking of worldwide inter-hospital spread of virulent, epidemic and multi-resistant clones (2). The resulting sequence type (ST) of a strain can be examined using web-based tools, enabling any strain that has been characterized using this method to be compared with all previously characterized strains. A small group of enterococcal strains belonging to the species *E. faecalis*, isolated from different environments, namely dairy products, infections in humans and infections in pets, were analysed by MLST. Each strain was subsequently ascribed a ST and this information was used to look for groups and phylogenetic relations between the analysed strains. Although preliminary, the results obtained indicate that there is clonal dissemination in different environments, a result that was confirmed with PFGE. Either there was transference of strain from food to humans or a parallel evolution of the two strains took place. Further studies are in course to shed more light into these worrisome findings.

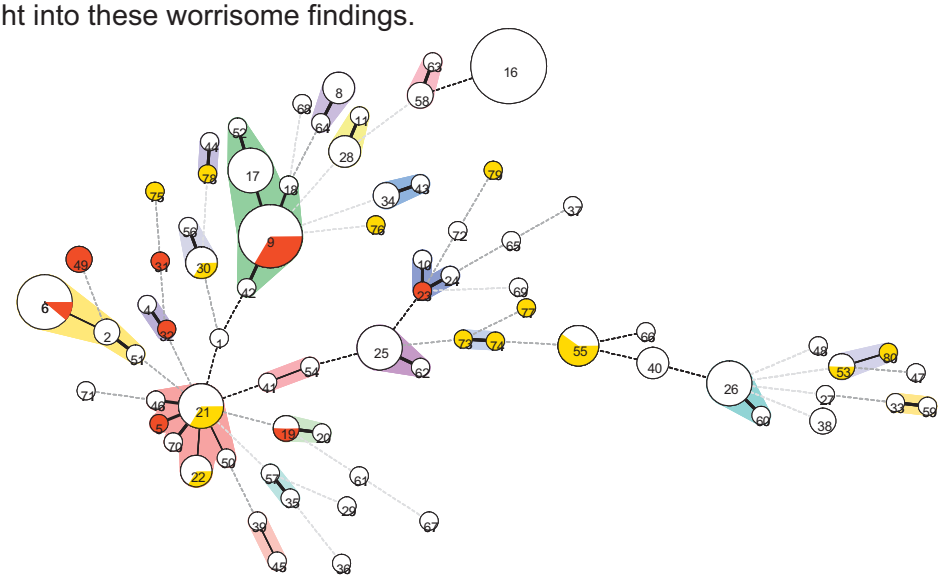


Figure. MST (minimum-spanning tree) with *E. faecalis* strains from the MLST database (we wish to thank Professor Rob Willems for his help and colaboration). In yellow and red are the VSE and VRE Portuguese representatives from our Laboratory.

1. Feil, E. J. (2004) "Small change: keeping pace with microevolution", Nature Reviews, 2:483-495.
2. Homan, W. L., Tribe, D., Poznanski, S., Li, M., Hogg, G., Spalburg, E., van Embden, J. D. A., Willems, R. J. L. (2002) "Multilocus sequence typing scheme for *Enterococcus faecium*", J. Clinical Microbiol., 40:1963-1971.

Natural sweetening of food products by engineering *Lactococcus lactis* for glucose production

Ana Rute Neves, Physiology of Lactic Acid Bacteria & *in vivo* NMR Laboratory

Lactic acid bacteria (LAB) are used worldwide in the production of fermented dairy products. *Lactococcus lactis* is generally recognized as a model organism, characterized by the simplicity of its metabolism and the availability of a wide range of genetic tools as well as the availability of a complete genome sequence. In this work it is shown that sweetening of food products by natural fermentation with *L. lactis* can be achieved by a combined metabolic engineering and transcriptome analysis approach. Production of glucose from lactose to be used as a natural sweetener requires engineering the catabolism of glucose. A strain that ferments only the galactose moiety should be impaired in glucose transport and phosphorylation. Hence, a double mutant carrying specific deletions of the glucose PTS (*ptnABCD*) and glucokinase (*glk*) was made. However, this strain could still grow on glucose. Determination of enzymatic activities coupled to *in vivo* NMR studies of glucose metabolism suggested the presence of a second PTS system with a clear preference for β-glucose. A DNA microarray experiment revealed up-regulation of the genes coding for the cellobiose-PTS (*ptcBAC*). A strain was constructed in which glucose metabolism was completely disrupted by deletion of the genes coding for glucokinase (*glk*), $EII^{man/glc}$ (*ptnABCD*), and the newly discovered glucose-PTS EII^{cel} (*ptcBAC*) (Fig. 1). After introducing the lactose metabolic genes, the deletion strain could ferment only the galactose moiety of lactose, whilst the glucose moiety accumulated extracellularly (Fig. 1). Additionally, less lactose remained in the medium after fermentation. The resulting strain can be used for *in situ* production of glucose, circumventing the need to add sweeteners as additional ingredients to dairy products. Moreover, the enhanced removal of lactose achieved by this strain could be very useful in the manufacture of products for lactose intolerant individuals.

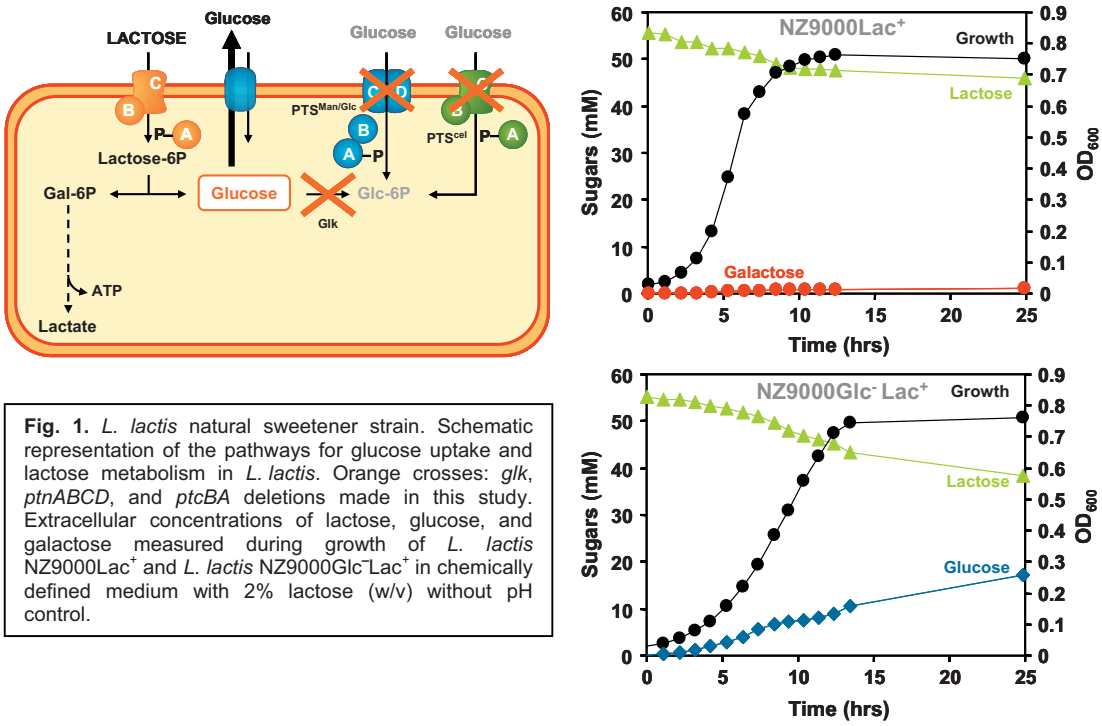


Fig. 1. *L. lactis* natural sweetener strain. Schematic representation of the pathways for glucose uptake and lactose metabolism in *L. lactis*. Orange crosses: *glk*, *ptnABCD*, and *ptcBA* deletions made in this study. Extracellular concentrations of lactose, glucose, and galactose measured during growth of *L. lactis* NZ9000Lac$^+$ and *L. lactis* NZ9000Glc$^-$Lac$^+$ in chemically defined medium with 2% lactose (w/v) without pH control.

In Vitro/in Vivo Correlation

Ana Luísa Simplício : IBET Pharmacokinetics and Biopharmaceutical Analysis Laboratory

In vitro/in vivo correlations (IVIVC) refer to the relationship between the behaviour of a pharmaceutical formulation in laboratory tests, namely its dissolution rate, and its *in vivo* bioavailability.

In vitro dissolution (figure 1) has been extensively used as a quality control tool for solid oral dosage forms (such as tablets) but in most cases it is not known how well it predicts the *in vivo* performance of the product [1]. However, in order to reduce unnecessary human testing, investigation of IVIVC is becoming increasingly important in drug development. In addition, the industry has also recognized the importance of IVIVC in reducing the number of clinical trials and bureaucracy necessary if post-approval changes have to be introduced in the original formulation. Once established, the IVIVC enables *in vitro* dissolution tests to be used as a surrogate for *in vivo* bioequivalence.

In certain cases, aspects other than the dissolution rate have to be considered, such as the absorption mechanism (figure 2) and metabolism, especially if the biotransformation of the pharmaceutical ingredient has a direct effect in its bioavailability or on the pharmaceutical outcome. This happens, for example, in the case of prodrugs and soft drugs. In such cases, the knowledge of the mechanisms of transformation and the development of biorelevant dissolution tests, that include the simulation of the metabolism, may be necessary.

In addition to dissolution tests, other *in vitro* tests, such as cellular and non cellular model assays, have been routinely used for prediction of oral bioavailability. One of these assays, the PAMPA model (parallel artificial membrane permeability assay) is increasingly gaining importance due to its simplicity and because it can give to high throughput. This model (figure 3) uses a lipid filled membrane to simulate the lipid bilayer of various cell types, including intestinal epithelium cells. The permeability of the test compounds through this artificial membrane is therefore used for the prediction of passive absorption through the biological membranes. For the prediction of active transport it is necessary, however, to use cellular models such as the caco-2 bioavailability model.

This laboratory is currently developing biorelevant dissolution tests that will be used in the establishment of *in vitro/in vivo* correlations as well as implementing *in vitro* bioavailability models for the prediction of bioavailability of model prodrug systems previously developed [2]. Capillary electrophoresis and/or HPLC methods are used for quantification of the active compounds and their metabolites.

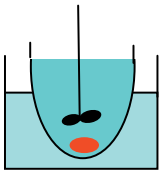


Figure 1: Dissolution test

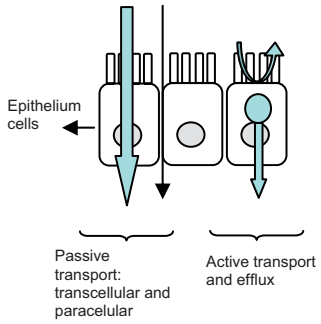


Figure 2: Mechanisms of drug absorption

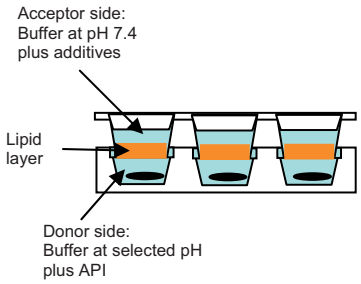


Figure 3: PAMPA assay

[1] Uppoor, V., Journal of Controlled Release 702 (2001) 127-132
[2] Gilmer J.F., Simplício A.L., Clancy J., European Journal of Pharmaceutical Sciences 24 (2005) 315-323

Quercus species identification using retrotransposon, SSR and ITS sequences

Margarida Pedro Rocheta : IBET Forest Biotechology Laboratory

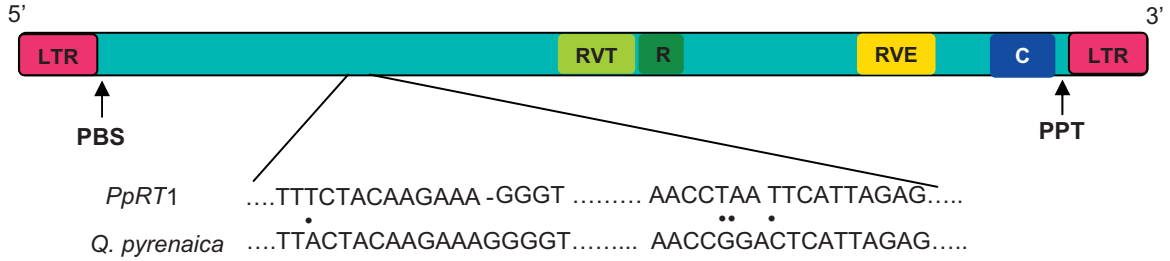
Mediterranean countries are rich in endemic minor species belonging to the genus *Quercus*, which plays an outstanding role in the dynamics of temperate and Mediterranean vegetation types. Along numerous areas of the Mediterranean basin, distinct oak populations come into contact generating hybrid zones which hold interest not only because of the evolutionary processes they reveal, but also because distinct plant communities grow under the different canopies. The development of molecular markers has provided new tools for further investigation of the genetic relationship and interspecific variability of oaks. However, the first studies were discouraging because molecular markers appeared to be less discriminating than observations of the phenotype. Retrotransposon and microsatellites could be markers of choice for discrimination between related oak species at the population level.

Several retrotransposons have been sequenced and were found to display a high degree of heterogeneity and insertional polymorphism, both within and between species. Because retrotransposon insertions are irreversible, they are considered particularly useful in phylogenetic studies. Several retrotransposons have been shown to be highly polymorphic for insert location within plant species. These properties have been exploited in several molecular marker systems for genetic analysis in a wide range of plant species.

Retrotransposons appear to replicate in the same manner as retroviruses with transcription of an RNA intermediate initiating and terminating within long terminal direct repeats (LTRs) that flank an interior coding region. How these transposable elements have achieved their apparent ubiquitous distribution in eucaryote genomes without a mechanism of dispersal remains speculative.

A *Quercus pyrenaica* retrotransposon sequence (770 bp) has been identified with 91% similarity with ***PPRT*1** (***P****inus* ***p****inaster* **r**etrotransposon **1**) a *gypsy*-like retrotransposon.

Typical organization of *a gypsy*-like retrotransposon



LTR, Long terminal repeat; **RVT**, Reverse transcriptase; **R**, RNAse H; **RVE**, integrase; **CH**, Chromatin organization modifier. **PBS**, primer binding site a sequence complementary to the 3' sequence of a particular tRNA species. **PPT**, polypurine tract.

The *Quercus pyrenaica* retrotransposon sequence is being used to classify a controversial form of *Quercus* tree in Arrábida. In addition, it is intended to use microsatellites and ITS sequences. The last class of markers, particularly for plant systematics, has developed into a ubiquitous tool for the identification of closely related species.

Cell Wall Proteins in Eucalyptus Wood Development

Phil Jackson: Plant Cell Wall Laboratory

Cell wall proteins are likely to play a central role in the development of wood characteristics and quality. Recently, The Laboratory has begun to look at several classes of cell wall proteins in eucalyptus wood (Fig. 1A). Using homology based cloning of eight major wall protein families, 42 sequences have been cloned, 36 of which are new additions to the data-base. These genes were seen to be differentially expressed during the development xylem or phloem in wood-forming tissues (B). Other analyses demonstrate that many of the genes show altered expression in different wood developmental programmes, such as that which occurs during the adaptive formation of tension and opposite wood (C). These initial results are now indicating potentially important genes in eucalyptus wood formation.

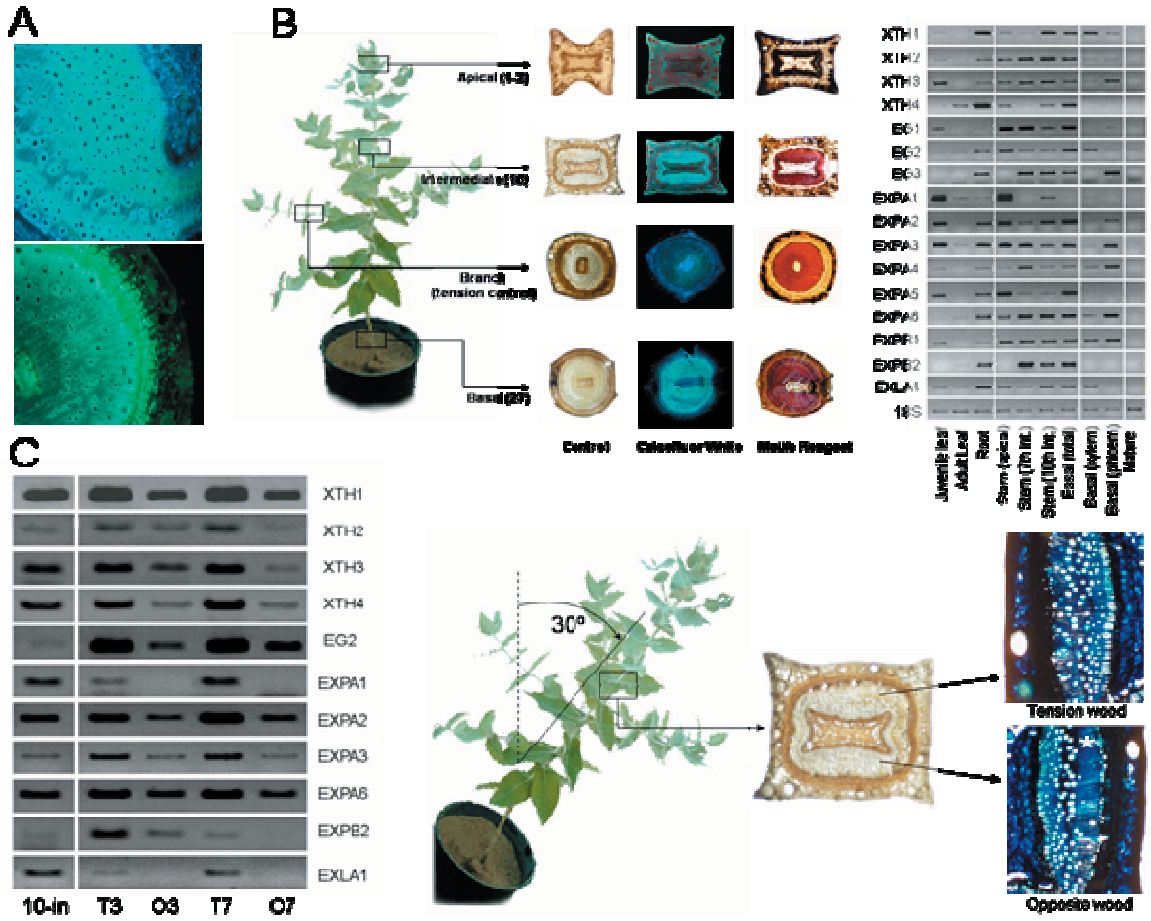


Fig. 1. Cell Wall proteins in Eucalyptus wood formation. A) Eucalyptus woody tissues. B) Semi-quantitative PCR analyses show many genes are differentially expressed during eucalyptus development and C) alteration to wood developmental programmes, such as the formation of opposite and tension woods. Note the outer layer formed in opposite wood (*) is denser due to heavier deposition of cellulose and is relatively free from larger metaxylem elements.

The production of high-value molecules in plants: fundamental and applied aspects of transgene expression

Rita Abranches : Plant Cell Biology Laboratory

Molecular Farming - the large scale production of recombinant proteins in plants – is a growing field of research since it became clear that plants offer many advantages over other currently used production systems. The Plant Cell Biology Laboratory has focused its research on the use of plant-based systems for the production of valuable recombinant proteins. The target products range from important biopharmaceuticals to animal feed additives. Plant based systems, including both transgenic plants and cell suspension cultures, are being used. In the past year the model legume, *Medicago truncatula*, has been proposed as a promising production system [1]. Using this plant, as well as other models such as *Arabidopsis* and tobacco, a multidisciplinary approach is being taken to dissect the pathway from gene to RNA and finally to the recombinant protein. Elucidating the processes involved in this pathway will enable their manipulation to produce high value molecules in a cost-effective manner.

A challenge in this field is to integrate knowledge obtained from a wide variety of sources – molecular and cell biology, biochemistry, transformation technology among others – with applications both at the biotechnology and basic research levels. In the laboratory, these transgenic plant and cell lines are being used as tools to study chromatin organization and regulation of gene expression in the nucleus. The Laboratory is also trying to understand the correlation between protein levels of production and their properties and the intracellular location of the recombinant protein. It has been observed that cell lines which produce a recombinant protein at different levels show different subcellular structures. Of particular interested is the question of whether it is the structure of the cell that determines the subcellular fate of the recombinant protein, or whether it is the level of expression of the protein that determines the cell morphology. This knowledge will help devise strategies for subcellular targeting of recombinant proteins to increase yields, improve storage, and facilitate purification.

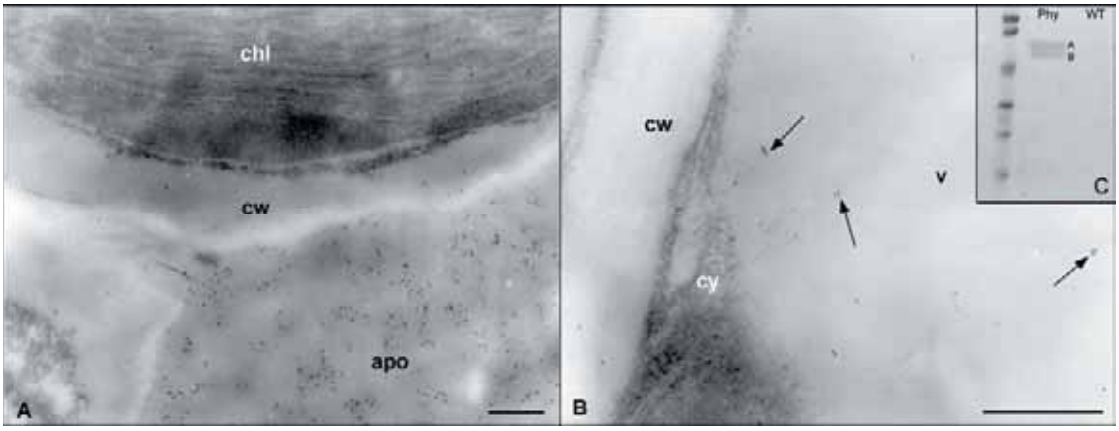


Figure 1 – A and B) Subcellular localization of recombinant phytase in *M. truncatula* leaves. Phytase was detected with a rabbit antiserum followed by a gold-labeled secondary antibody. Both the apoplast (A, apo) and the vacuole (B, v) are labeled. cw, cell wall. chl, chloroplast. cy, cytoplasm. Bars: 0.5 µm. C) SDS-PAGE showing purified phytase from *M. truncatula* leaves. The upper band corresponds to the phytase found in the apoplast while the lower band corresponds to the vacuolar form. These results were confirmed by glycosylation analyses.

[1] Abranches, R.; Marcel, S.; Arcalis, E.; Altmann, F.; Fevereiro, P. and Stoger, E. (2005). Plants as bioreactors: a comparative study suggests that *Medicago truncatula* is a promising production system. Journal of Biotechnology 120, 121-134.

CHEMISTRY DIVISION

CHEMISTRY DIVISION : Objectives and Activities

This year has seen the creation of two new laboratories within the Chemistry Division, which will reinforce existing areas and also cement links between the Chemistry and Biological Divisions. The **Colloids, Polymers and Surfaces Laboratory**, headed by Dr. António Lopes, will cover a wide range of activities including the surface aspects of colloidal and polymeric materials and the development of materials for medical applications. The **Bioorganic Chemistry Laboratory**, headed by Dr. Rita Ventura, has the task of affecting the synthesis of natural and unnatural products of biological interest, usually in collaboration with other groups. Also new synthetic methodologies will be developed for the synthesis of small molecules with, for example, enzyme-like activity. These activities will help bring together the Chemistry Division and the biological sciences both here at the ITQB and outside.

The established laboratories have continued to carry out high quality research under stringent economic conditions, which are not propitious to productivity.

Hydrosilylation is a very important reaction for the reduction of unsaturated functional groups. The range of metal compounds known to promote this reaction has been extended by the **Homogeneous Catalysis Laboratory** lead by Dr. Beatriz Royo. It has been found that oxides of Molybdenum and Rhenium, which are normally oxidising agents, are efficient catalysts for the hydrosilylation of aldehydes and ketones. **Organometallic Chemistry** has continued with its collaborations. Previous activity is now carried out in collaboration with the University of Aveiro and two other universities in Lisbon, IST and FCUL. Activity at the ITQB is now restricted to Bio-organometallic Chemistry and includes Alfama Inc. which is a company created to study the biological applications of non-toxic organometallic compounds which contain carbon monoxide. This has increased considerably the funding available in this research area and should make an impact on health care. Medical and environmental problems are also being solved by the **Coordination and Supramolecular Chemistry Laboratory** headed by Prof. Rita Delgado. Cyclic polydentate compounds which complex anions and cations are very important in everyday life from analytical techniques to the preservation of food. Recent developments have been the discovery of bis-ferrocenyl compounds which can act as sensors for both anionic and cationic species. The iron redox potentials obtained by cyclic volammetry are different depending upon the species complexed. This is very useful for the analysis of effluents for example. The use of radioactive isotopes for the diagnosis or treatment of cancers is now very common and one of the major problems is the removal/retention of these materials. The design of ligands which complex metal ions and are retained in specific regions of the human body is very important. Several new ligands have been prepared which help to understand and solve these problems. An understanding of the mechanisms by which the skin is able to control water loss and the intake of xenobiotics is very important for many reasons. The stratum corneum is a complex layer of the skin composed of a specific mixture of compounds with differing polarities. How the composition of this layer affects its structure and properties and the effect of pH is one of the main topics under study by the **Microheterogeneous Systems Laboratory** under the leadership of Prof. Eurico de Melo. These studies use an array of analytical techniques including calorimetry, X-ray WAXS/SAXS diffraction, fluorescence spectroscopy and NMR. Another enthralling topic is the pH of the water pools in water-in-oil emulsions. These micro-systems do not obey the rules for macro-systems and the methods for their study are correspondingly complex. Access to complex organic molecules is an important aspect of multidisciplinary research and the **Organic Synthesis Laboratory** attempts to furnish these molecules to other groups within the Institute. A waiting list exists and in the future this service will also be partially undertaken by the Bioorganic Chemistry Laboratory. Asymmetric synthesis is the primary aim of the Organ-

ic Synthesis Laboratory and some approaches to natural products have been attempted. Small asymmetric molecules for the synthesis of enantiopure compounds are essential and a new project involving the preparation of chiral azabicycloalkanones using readily available chiral amines is just starting. The aziridines which are diastereomers can then be converted to chiral cycloalkanones after removal and recovery of the chiral amine. The control of stereochemistry during chemical transformations is far from being understood and much work has to be done.

The chemistry division is suffering from a lack of funding by the FCT and this manifests itself mainly in the attribution of grants for students. Chemistry is a work intensive experimental science and the productivity of the Division is seriously under threat if greater emphasis is not given to this essential science. The present system for the selection of candidates is not adequate and a general overhaul is essential.

CHEMISTRY DIVISION

Head of Division: Christopher David Maycock

Laboratory: Colloids, Polymers and Surfaces

Head of Laboratory: António Lopes
Research Team:

Marijana Blesic	PhD Student (Lab. of Molecular Thermodynamics)
José Filipe Almeida	PhD Student
Andreia Sousa	Undergraduate Student
Filipa Pereira	Undergraduate Student
Maria Helena Marques	Undergraduate Student
Mónica Prata	Undergraduate Student
Rosa Monteiro	Undergraduate Student
Vera Mira	Undergraduate Student

Publications 2005: 14,15
Highlight: 75

Laboratory. Homogeneous Catalysis

Head of Laboratory: : Beatriz Royo (see also Laboratório Associado section)
Research Team:

Patricia M. Reis	Post-Doc
André Filipe Pontes da Costa	Graduate Student (BI)
Elena Más Marza	PhD Student (MEC)

Publications 2005: 65, 160
Highlight: 47

Laboratory: Organometallic Chemistry

Head of Laboratory: : Carlos Romão
Research Team:

João Seixas	PhD Student (ITQB/Alfama)
Carlota Veiga de Macedo	PhD Student
Ana Cristina Fernandes	Professora Auxiliar da Univ. Lusofona

Publications 2005: 3, 5, 65, 66, 76, 82, 101,113, 140, 141, 142, 160, 184
Highlight: 76

Laboratory: Organic Synthesis

Head of Laboratory: : Christopher David Maycock
Research Team:

Rita Ventura	Post-Doc (up to October 2005)
Hovsep Avedissian	Post-Doc
Jorge Wahnon	PhD Student
Sofia Miguel	Graduate student (Industry)
Li Li	Graduate student (Industry)
Leonardo Mendes	Graduate student (Industry)
Filipa Siopa	Graduate Student (CRAFT)

David Quintino	Graduate Student (CRAFT)
Eva Lourenço	Undergraduate Student

Publications 2005: 20, 21
Highlight: 77

Laboratory: Microheterogeneous Systems

Head of Laboratory: : Eurico Melo
Research Team:

Rute Mesquita	PhD Student
Sofia de Souza	PhD Student
Helena Lameiro	PhD Student

Highlight: 78

Laboratory: Co-ordination and Supramolecular Chemistry

Head of Laboratory: : Rita Delgado
Research Team:

Xiuling Cui	Post-Doc
Patrícia dos Santos Antunes	PhD Student
Krassimira Passos Guerra	PhD Student
Feng Li	PhD Student
Sílvia Cristina Ferreira de Carvalho	PhD Student
Luís Miguel Lima	PhD Student

Publications 2005: 12, 46, 48, 49, 58, 102, 107, 108, 112
Highlight: 79

Laboratory: Bioorganic Chemistry

Head of Laboratory: : Rita Ventura (see also Laboratório Associado section)

Highlight: 49

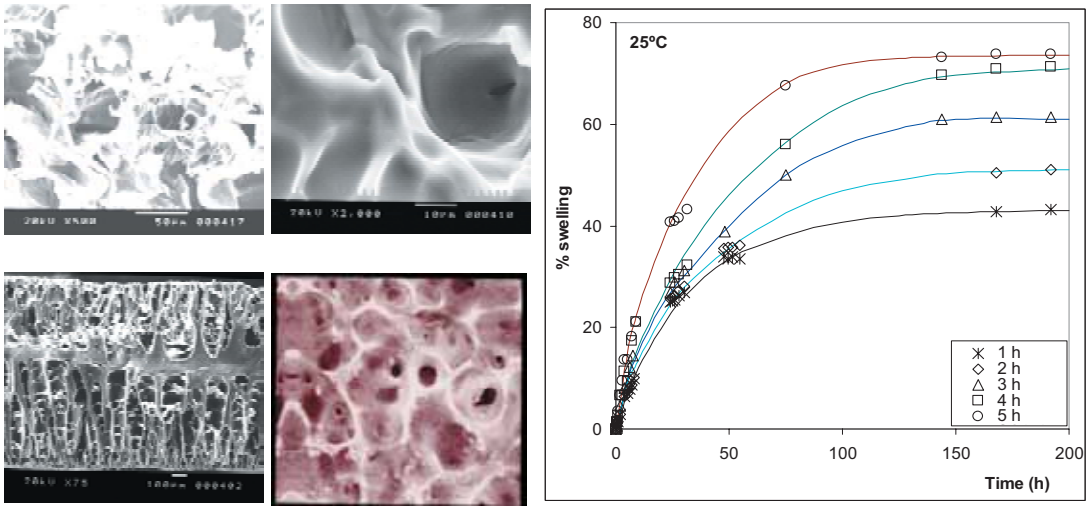
Hydrogels for Drug Delivery

António Lopes : Colloids, Polymers and Surfaces (CoPoS) Laboratory

During the last decade industrial demand has turned to more biocompatible, non-toxic and biodegradable materials. The combination of one or more biosurfactant(s) and one or more biopolymer(s) finds many technological applications in detergents, cosmetics, paints, paper coatings, food, pharmaceuticals or pesticide formulations. Besides the emulsification/flocculation properties which each component possesses, they are employed together to achieve some synergistic effects - colloidal stability, structuring properties, rheological control, or the control of the kinetics of release of encapsulated drugs. In this regard polymeric matrices, namely hydrogels (entangled or cross-linked networks of polymer-based structure with swelling and entrapment capabilities) possess a high potential for biomedical-oriented applications. The Laboratory has focused on the development of new hydrogels for biomedical applications, namely for allergy, burn and pain treatments.

Natural-occurring water soluble polysaccharides such as dextran, pollulan, cellulose, or chitosan, have been hydrophobically modified in order to give rise to hydrogel systems with the desirable rheological properties. The “modification” (usually the cross-linking or the branching with hydrophobes) keeps the polymer biocompatible in order to be used in pharmaceutical, cosmetic or food technology. After the synthesis is optimized a search will be made for the best way to incorporate drugs into the gel, such as simple flocculation / coacervation, entrapment, or chemical linkage (pro-drug).

After the drug is incorporated studies will be undertaken on the stability of the formulation and the release properties of the drug will be modeled. In the case of burn treatment the Laboratory is exploring the best strategies, not just for fluid compatibility and fluid absorption, but also for skin regeneration.

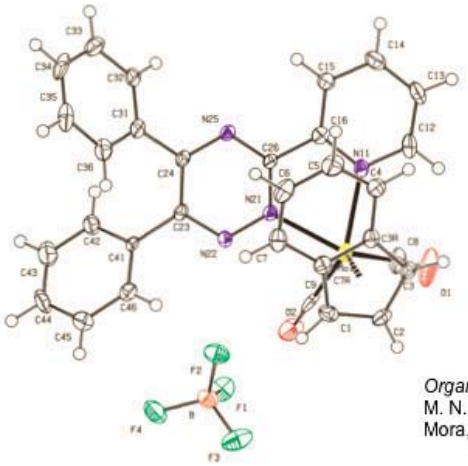


Hydrogel structures as seen with SEM (scanning electron microscopy) and a swelling pattern as a function of time of reaction.

A new type of anti-tumoral organometallic compound

Carlos Romão : Organo-metallic Chemistry Laboratory

Transition metal complexes have been successfully used as anti-tumoral drugs, and, in particular, cis-platinum and analogue molecules. The need to circumvent the use of highly toxic heavy metals has led to the discovery of a new type of anti-tumoral compound based on Molybdenum (**Mo**), which is essentially a non-toxic metal. These molecules, based on the $[(Indenyl)Mo(CO)_2]^+$ cation, such as the one shown in the figure, are the first of the anti-tumorals to carry carbon monoxide, a recently discovered anti-inflammatory active agent.



These molecules, based on the $[(Indenyl)Mo(CO)_2]^+$ cation, like the one shown, are the first of anti-tumorals to carry CO, a recently discovered anti-inflammatory active principle.

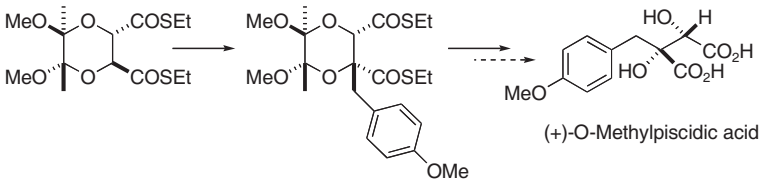
Organometallic Molybdenum Compounds for Treating Cancer, M. N. Matos, C. Pereira, S. Rodrigues, M. J. P. da Silva, M. Mora, P. Alves, C. A. Reis , C. C. Romão, WO2005/087783

Progress on the Synthesis of Natural Products

Christopher David Maycock : Organic Synthesis Laboratory

The energies of the Laboratory have been divided between the synthesis of natural and unnatural compatible solutes for projects financed by the European Union (CRAFT, Stab and Prof. Helena Santos) and the FCT (Helena Santos) and our interest in attacking the problems of natural product synthesis. The former involves the synthesis of complex mono and dialkylated phosphates, the formation of sugar glycosides and, specifically, stereochemical control necessary for the synthesis of alfa-glucosides which are particularly difficult. A synthetic glucosyl glucosyl glycerate has been shown to be identical to a natural solute isolated from thermophilic bacteria and this constitutes a proof of the structure. These compounds have been distributed to several partners in the European project and the results on the stabilisation of proteins and genetic material have been encouraging. Progress has now been made on the synthesis of inositol phosphates although a stage involving an enantiomer resolution is a necessity. Progress has also been made on the synthesis and use of aziridines as protecting and directing agents. This project is now financed by the FCT and future efforts will be supported by a Postdoctoral fellow from India. An increased understanding has been reached with respect to the directing effects in azabicycloheptane systems and material has been synthesized with opposite stereochemical attributes. Some cyclohexane based natural products have also been constructed.

The stereoselective alkylation of chiral dioxanes derived from tartaric acid has produced a rapid total synthesis of a natural highly functionalised diacid and several natural analogues. The production dialkylated tartaric acid derivatives is now possible. The technique involves chiral memory technology whereby the protecting group retains the memory of the starting compound, even though the stereocentres of the original tartrate are destroyed. The scope and limitations of this reaction have also been determined.



A Concise Stereoselective Synthesis of (+)-*O*-Methylpiscidic acid

The acid catalysed rearrangement of allylic alcohols has also been achieved. Many allylic rearrangements of secondary and tertiary alcohols in the presence of Lewis acids give complex mixtures of products. This method is not useful for synthetic purposes. It has been found that the yield of expected rearranged product is dependent upon the substitution pattern on the alicyclic system. Steric effects are very important in determining the product and this was obvious when secondary allylic alcohols were treated with acid. Silicon halides have been shown to particularly mild for this process and several basic functional groups are left untouched by these reagents. It is hoped to demonstrate the utility of this process and to carry out a synthesis of a natural product. It is also hoped to determine stereo-preferences for these rearrangements which may or may not involve carbo-cations. Many natural compounds of chemical/biological interest are not readily available except via synthesis. In collaboration with Prof. Eurico de Melo and Rita Ventura attempts are underway to synthesize ceramide Cer1, a very large and complex molecule. The multi-step syntheses published in the literature are proving very difficult to reproduce.

There are also three ongoing projects with the pharmaceutical industry for the efficient synthesis of proven medications. The discovery of new synthetic routes provides the possibility of patents and the development of the industry in the area of raw value added materials.

Collaboration with the FCT/UNL with respect to the synthesis of chiral ligands for metal catalysed organic transformations is also ongoing.

Research in Microheterogeneous Systems and Soft Interfaces

Eurico Melo : Micro-heterogeneous Systems Laboratory

Mammals have a system that prevents uncontrolled body water loss and intake of xenobiotics through the skin. This barrier is located in the uppermost layer of the skin, the stratum corneum (SC), Figure 1. It is constituted by layers of flattened dead cells, the corneocytes, which are filled with keratin, riveted together by desmosomes, and the whole surrounded by a lipid matrix. In the last couple of decades evidence has been accumulated attributing a major role to the lipids, located in the intercellular space, in the barrier function of the skin. The lipid matrix involving the corneocytes is constituted by ceramides, cholesterol and saturated fatty acids, in the molar percentages of 38, 33 and 13% respectively; the remaining 16% represents "minor" components. The research has focused on three aspects related with the structure and composition of the SC lipid matrix; (i) how the relative percentages of ceramide, cholesterol and fatty acid composition affect the structure of the mixture (Figure 2), (ii) what is the influence of the pH on the structure and thermotropic properties of a lipidic mixture similar to that found in the SC, and (iii) how cholesteryl oleate, a "minor" component that has been found in molar percentages as high as 16%, interacts with the lipid mixture. Research involving these subjects has been undertaken using techniques such as differential scanning calorimetry, small and wide-angle X-ray diffraction, fluorescence emission anisotropy and magic-angle spinning ^{13}C Nuclear Magnetic Resonance in collaboration with Dr. J. Hamilton from the Boston Medical School and Dr. M.J. Capitán from IEM-CSIC, Madrid.

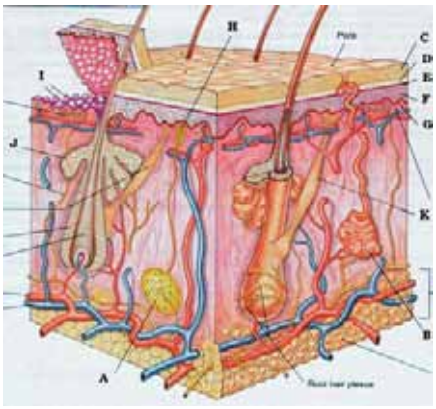


Figure 1. The skin with its several layers and components. C is the stratum corneum, the layer studied in our group.

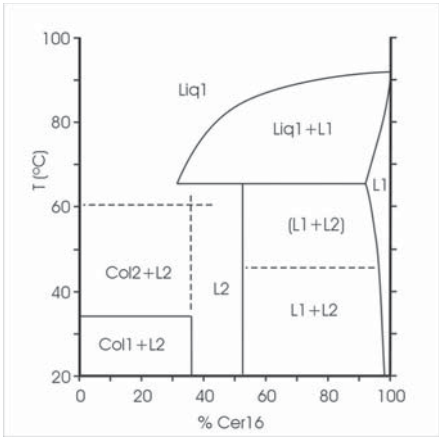


Figure 2. Preliminary phase diagram for the system Cer16/Chol where data from DSC, SAXS and WAXS is combined.

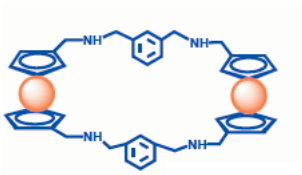
Another study involves the pH definition near soft interfaces. This theme is related with the behavior of the SC lipids, which are subject to a pH varying between 7.4 and 5.5, but it has a more general importance for reactions taking place near soft interfaces in biology and chemistry. The approach involves the use of the same fluorescent pH-sensitive chromophore for the determination of bulk and interface pH in micro-compartments. In future developments, it is intended to study the pH and interface potential in membranes with controlled composition.

Molecular Recognition of Anions and Radiopharmaceuticals for Bone Pain Therapy

Rita Delgado : Coordination and Supramolecular Chemistry Laboratory

Phthalate sensors

Many anions are pollutants of the environment, as is the case of phthalate. Therefore, the design of novel synthetic receptors displaying anion selectivity is an emergent research area. In this context, the Laboratory is interested in developing molecular sensors and compounds capable of removing certain anions from the environment. Among the receptors that have been synthesized and studied, $fc_2bz_2N_4$ exhibits an interesting behaviour for phthalate anions. Indeed, when protonated, it presents the strongest binding and the largest cathodic shift of the ferrocene oxidation peak in the presence of phthalate when compared to related anions, see Fig 1(b). However, the super-molecule formed has a 2:1 stoichiometry, Fig. 1(a); kinetic constraints for the formation of the receptor/substrate entity are also observed.



$fc_2bz_2N_4$

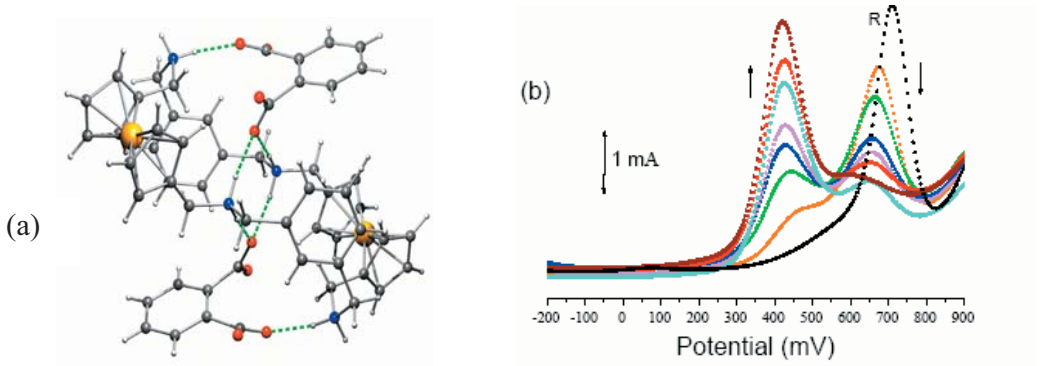
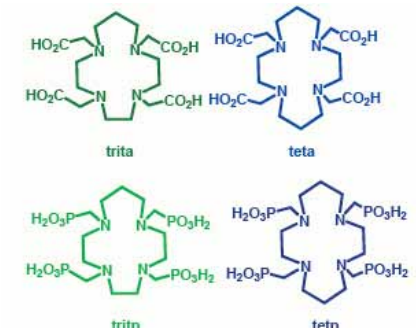


Fig. 1 Macrocyclic compound containing two ferrocene units, **$fc_2bz_2N_4$** (L). (a) Structure of $[H_4L(ph)_2]$ showing the overall disposition of the macrocyclic receptor and the anions in the super-molecule in the R:2S ratio (b) Square-wave voltamograms for the successive addition of 0.5 to 4 equivalents of ph^{2-} (R is the initial voltamogram of the receptor. (X. Cui, R. Delgado, H. M. Carapuça, M. G. B. Drew, V. Félix, *Dalton Trans.*, 2005, 3297-3306.)

Tetra-azamacrocycles containing methylphosphonate and acetate arms complexes for diagnostic and/or therapeutical radiopharmaceuticals using metal radionuclides



Biodistribution studies in mice demonstrated that $^{153}Sm/^{166}Ho$–trita complexes have a fast tissue clearance with more than 95% of the injected activity excreted after 2h, a value that is comparable to the best complexes found till now. In contrast, the ^{153}Sm–tetra complex has a significantly lower total excretion. $^{153}Sm/^{166}Ho$–tritp complexes are retained by the bone, particularly ^{166}Ho–tritp that presents 5-6% (% I.D./g) bone uptake and also a high rate of total excretion. Thus, the studies carried out support the potential interest of $^{153}Sm/^{166}Ho$–trita complexes for therapy when conjugated to a biomolecule and the potential usefulness of the ^{166}Ho-tritp complex in bone pain palliation.

R. Delgado, J. Costa, K. P. Guerra, L. M. P. Lima, *Pure App. Chem.,* 2005, 77, 569-579. F. Marques, L. Gano, M. P. Campello, S. Lacerda, I. Santos, L. M. P. Lima, J. Costa, P. Antunes, R. Delgado, *J. Inorg. Biochem.,* 2006, *in press*.

BIOLOGICAL CHEMISTRY DIVISION

BIOLOGICAL CHEMISTRY DIVISION: Objectives and Activities

The research objectives of the Biological Chemistry Division aim at an integrated contribution to the understanding of structure-function relationships of proteins and enzymes, with a special focus on metalloproteins. The various Laboratories have developed complementary specialised expertise, from molecular genetics and microbial physiology to methods such as NMR, EPR, X-ray Crystallography and computational methods. The research undertaken at this division is highly interdisciplinary, with various biological themes involving several laboratories. The main subjects are: i) molecular mechanisms of biological energy conservation, namely by soluble and membrane-bound electron-transfer metalloenzymes/complexes; ii) anaerobic bacterial respiration; iii) protein stability and folding; iv) mechanisms of response to metal, metalloids, oxidative and nitrosative stress, at the level of both regulatory and enzymatic pathways v) proteins related to human health and pharmacological targets; vi) multi-copper enzymes involved in several biotechnological processes and vii) global genomic approaches to study signalling pathways. The biological targets are several aerobic and anaerobic bacteria/archaea (including extremofiles), some of them being human pathogens, and the eukaryotic micro-organism *Saccharomyces cerevisiae*.

In 2004 and 2005 the Division was expanded with the appointment of new leaders within the Associated Laboratory programme in the topic “Biological Active Molecules” and an Invited Assistant Professor. The FCT re-equipment program has allowed the acquisition of a new EPR instrument, and the Division has just obtained an ATR-FT-IR instrument; it will also benefit from the acquisition under the same program of a Biacore, which will allow the study of protein-protein interactions, and of a CD spectrometer. The installation of a high field NMR spectrometer will also considerably enhance the breadth of biological problems that can be addressed by the groups working in this Division. These new techniques will allow an extension of the studies carried out using up-do-date instrumentation.

An F6P integrated project “BIORENEW” was recently approved for financing by the EU that will allow the pursuing of multidisciplinary studies of bacterial laccases currently undertaken within the Division. Collaborations with Pharmaceutical companies Schering AG Berlin and Merck KgaA in Darmstadt, have been pursued and established through IBET, involving the Macromolecular Crystallography Laboratory. One PhD student, sponsored by Schering is under a co-supervision of ITQB/IBET and Schering. Aiming at a higher internationalisation of the graduating activities of the Division, two European-label PhD students are currently finishing their PhD s, in collaboration with the University of Rome, and another is finishing his PhD in collaboration with the ESRF.

The main scientific achievements and objectives of this Division during 2005 are given below.

The **Structure and Function of Metalloproteins Laboratory** is directed towards the study of structure-function relationships, with particular emphasis on the determination of the structural basis for cooperativity in electron-proton coupling and its role in the mechanisms of energy transduction, which lead to oxidative phosphorylation. This work is focused on soluble proteins with multiple redox centres, primarily haem groups. In addition to their redox activity, haems commonly bind diatomic molecules such as CO and NO. A new project funded by FCT links these strands in the study of cytochrome c” from M. methylotrophus. Although this is a monohaem cytochrome, it provides an excellent model for many subtle effects at the atomic level, including a negative redox-Bohr effect in which the normal electrostatic interaction between the charge of the haem and a nearby ionisable group is reversed by a change in conformation. This effect may be

crucial to understanding the mode of action of cytochrome c oxidase. Studies of tetra-haem proteins from sulfate reducing organisms are also continuing with new structures in solution determined by NMR (see group highlight), the integration of data from diverse experimental techniques into a single biophysical model, and studies of the interactions with physiological partner proteins.

The **Molecular Simulation Laboratory** is oriented towards the development and application of theoretical/computational methods for the simulation of (bio)molecular systems, with an emphasis on electrostatic processes. During this last year, a major reassessment of methods for computing anomalous pKas in proteins was undertaken, analysing the extent to which conformational reorganization can be treated implicitly. This conformational reorganization was also investigated explicitly using other in-house methods (linear-response approximation and constant-pH molecular dynamics). The subjects investigated with these methods included; the pH-induced helix-coil transition of poly-lysine and its dependence on ionic strength, the identification of biologically relevant conformers of the endogenous analgesic neuropeptide kyotorphin, and the conformation-protonation coupling in arginine-glutamate repeats and their eventual role in dentatorubal-pallidoluysian atrophy. Similar studies of cow serum proteins are also under way in collaboration with the University of Milan, Italy.

The research developed in the **Genomics and Stress Laboratory** studies the mechanisms by which gene expression is reprogrammed under stress conditions. Indeed, cells from bacteria to man when subjected to environmental changes activate a myriad of transcription factors. Studies are being focused on the yeast Yap family of 8 b-Zip transcription factors. So far, the best studied is Yap1, the major oxidative stress regulon which has been shown to contain two redox centres. However, Yap1 is also involved with Yap8 in arsenic compound (As) detoxification. The way this cross-talk occurs is being investigated. Besides the stress-imposed nuclear localization of both factors and the binding of As to the Yap8 cysteine residues, it has also been shown that this protein forms heterodimers with Yap1 and that both bind TGATTAATAATCA in target gene promoters. Yap1 factor also binds TATTACTAATC in a similar manner to Yap2 –Yap4. The regulation of Yap4/Yap6 is also being examined together with their function under hyperosmolarity (see high-light). More recently, data have been obtained showing that the yap7 mutant is sensitive to diethyl-maleimide, a drug which interferes with the cell cycle. Aiming towards the functional analysis of the transcription factors which do not display a clear phenotype a “super-mutant” in all the members of the Yap family is under construction. The complete genome sequence of D. gigas is being performed and the systematic deletion of genes encoding proteins involved in nitrosative stress and metabolism has been implemented.

The **Protein Biochemistry, Folding and Stability Laboratory** aims at establishing associations between protein conformational stability, biological function and intrinsic structure determinants, combining molecular, biophysical and structural techniques. One of the interests concerns the study of proteins involved in human disorders that result from protein destabilisation, for example due to a mutational change. That is the case of frataxin in some patients suffering from the neurodegenerative disease Friedreich’s ataxia, which is under study in the laboratory (see group highlight). An interest has also been continued on the relationship between metal centres and protein folding, driving at the elucidation of the role of metals as fold determinants and nucleation points. This year studies have been reported on two iron-sulfur proteins, ferredoxin and rubredoxin,

and the characterisation of non-native, molten-globule like forms of metalloproteins is being pursued. It is hoped to gain insights into intermediate forms of the folding process and metal cluster assembly. Finally, a new proteomics project has been initiated, with the objective of identifying and characterising a subset of hyper-stable proteins from thermophilic organisms.

The **Protein Modelling Laboratory** continued to focus many efforts on studying redox proteins. Together with external collaborators the mechanistic features of haem-copper oxidases have been studied. In collaboration with the Resonance Raman Laboratory studies have been undertaken on the association between redox proteins and Self-assembly monolayers, as a mimic of supramolecular associations. Redox-driven conformational changes were also studied, using cytochrome c3 as a model system, using MD simulations, which, once properly applied can replace costly experiments. In collaboration with the Microbial Biochemistry Laboratory, the structure of a [NiFeSe]-hydrogenase was derived by comparative modelling methods, and compared with structurally related hydrogenases. The subject of enantioselectivity in non-aqueous solvents was further studied this year, with emphasis on the effects of hydration. Together with external collaborators the small peptide kyathorphin has been studied using simulation methods in order to understand its conformational behaviour in solution and correlate it with its biological activity.

The **Microbial Biochemistry Laboratory** is interested in the study of anaerobic microorganisms. Current research topics include the study of proteins involved in the respiratory electron transfer pathways of sulfate reducing microorganisms, as well as the role and impact of sulfide-producing bacteria among the human colonic flora. Recent research achievements include i) the isolation and characterisation of a membrane respiratory complex, strictly conserved in all sulfate-reducing organisms, that includes two novel and unusual metaloproteins; ii) characterisation of a membrane-bound [NiFeSe] hydrogenase and its physiological role; iii) study of expression conditions of three periplasmic hydrogenases in a Desulfovibrio spp. iv) study of hydrogen metabolism in the human pathogen Bilophila wadsworthia. A study in collaboration with the Molecular Genetics of Metalloproteins Laboratory and Hospital de Santa Maria is ongoing to investigate the prevalence of specific species of sulfide-producing bacteria in stools of healthy children and children with inflammatory bowel diseases.

The **Molecular Genetics of Metalloproteins** Laboratory focuses on the analysis of the bacterial responses to nitrosative stress and on the mechanisms that confer oxygen resistance to anaerobic organisms. Recent research highlights include: i) the use of DNA microarrays which showed that nitrosative stress modifies around 4% of the entire E. coli genome, triggering the induction of enzymatic systems such as detoxification enzymes, iron-sulphur cluster assembly systems, DNA repairing enzymes and stress response regulators; ii) the study of the E. coli nitric oxide sensor, NorR, that mediates the nitric oxide induction of flavorubredoxin, a NO reductase enzyme that has previously been shown to have a key role in nitrosative detoxification, and iii) the very recent discover of a new gene, ytfE, with an important function in assembly of proteins containing iron-sulphur clusters. These studies are now being extended to several pathogenic bacteria.

The **Inorganic Biochemistry and NMR Laboratory** initiated work on three research projects funded by Fundação para a Ciência e Tecnologia devoted to the characterization of respiratory proteins of strict- and facultative anaerobes that have been identified

as prime candidates for bioremediation applications, namely *Desulfovibrio*, *Desulfuromonas* and *Shewanella*. These projects fit the general aim to characterize, in detail, a broad range of proteins with key functions in the anaerobic metabolism of these organisms, both at the level of intermediaries in electron transfer chains and at the level of terminal reductases. Some of these proteins are associated with the cell membrane, and the core expertise of the group in NMR spectroscopy of diamagnetic and paramagnetic proteins was complemented with that of other groups in the division such as the Structure and Function of Metalloproteins and the Microbial Biochemistry Laboratories, and collaborations with groups overseas at Oak Ridge and Oxford.

The main goals of the **Metalloproteins and Bioenergetics Laboratory** are the understanding at the molecular level of biological mechanisms, in prokaryotes for i) energy conservation by membrane-bound metalloprotein complexes, ii) detoxification of the superoxide anion in anaerobes, iii) iron storage in anaerobes and iv) detoxification of nitric oxide. Towards these goals, the mechanism of superoxide reduction by superoxide reductases was further clarified by a combination of stopped flow and pulse radiolysis experiments. A complete spectroscopic and thermodynamic (redox) characterisation of the enzymes involved in NO reduction in *E. coli* was achieved, an essential point for the continuing study of the catalytic and electron transfer mechanisms. The haem-copper oxygen reductase from *Acidianus ambivalens* (a B-type enzyme) was further investigated, using EXAFS spectroscopy, which allowed the determination of the structure of the copper centre in the catalytic site in both the oxidized and reduced forms. Also, by studying the redox behaviour of this enzyme using surface enhanced Resonance Raman spectroscopy (in collaboration with P. Hildebrandt and D. Murgida, invited professors at ITQB), it was possible to unequivocally prove that the haem at the catalytic centre has a reduction potential higher than that of the low-spin haem, in contrast with the mitochondrial-like enzymes, which suggests a less sophisticated mechanism of electron/proton coupling in the B-type enzymes.

The **Macromolecular Crystallography Laboratory** aims at solving the structures of macromolecules by X-ray Diffraction using state-of-the art methods and based on the advantages of synchrotron radiation. The work developed in the laboratory is conducted under two approaches. The first is based on internal and external collaborations which provide pure protein samples. The second includes the production of proteins on site followed by the crystallographic study of their structures. The main results during 2005 under the first approach include the structural characterization of a sulfur oxygenase reductase (an initial enzyme in the biological assimilation of elemental sulphur), the helicase RuvBL1 (helicases are pivotal enzymes involved in DNA metabolism that use the energy of ATP hydrolysis to unwind nucleic acids), a ribonuclease II (a key enzyme involved in RNA maturation, turnover and quality control), a UDP-glucose pyrophosphorylase (involved in several polysaccharide formation pathways), an aliphatic amidase (which hydrolizes a small range of short aliphatic amides), and a ferritin from the hyperthermophilic archaeon and anaerobe. The main results of the second approach include the study of 1,3-propanediol dehydrogenase and the carbonate dehydratase and SurE survival protein. These studies are the Laboratory's contribution to SPINE, the European Union Structural Genomics project.

In the **Membrane Protein Crystallography Group** the main interest focuses on the structural characterization of membrane-bound proteins. This work is done in collaboration with the Metalloproteins and Bioenergetics and Microbial Biochemistry Laboratories.

In particular, proteins and protein complexes, which are constituents of the respiratory chain of the hyperthermoacidophilic archaeon *Acidianus ambivalens*. Knowing the 3D structure of the selected membrane proteins will provide new insights into the mechanisms of electron transfer and catalysis of these novel membrane-bound respiratory complexes. Recently, the structure of a type II NADH:quinone oxidoreductase (NDH-2) isolated from the same organism, has been determined. This enzyme contains FAD as a redox cofactor and constitutes one of the electron entry points into membrane-bound respiratory chains, oxidizing NADH and reducing quinones. Structure analysis has led to further investigations which have shown that this enzyme also has a sulfide:quinone oxido-reductase (SQR) activity. Collaboration with a Portuguese pharmaceutical company, BIAL, has continued with the 3D structure determination of methyltransferase in complex with novel compound, with potential application in Parkinson's Disease therapy. Another successful project, together with University of the Algarve, has been the structural analysis of cinammomin with different ligands, an elicitin secreted by a phytopathogenic fungus.

The main research focus of the **Microbial and Enzyme Technology Laboratory** is to explore the structure-function relationships of enzymes, with particular emphasis on prokaryotic *laccases*.The bacterial *CotA-laccase* from *Bacillus subtilis* has been used as a model bacterial laccase and a tool for biotechnology. *Laccases* are biocatalysts that can replace harsh chemicals that cause environmental contamination in a variety of industrial processes. Following the 3D structure determination, mechanistic and engineering studies are underway aimed at understanding functional and structural determinants of *laccase* activity and stability (see highlight). This knowledge will allow the rational design of optimized enzymes that better fit environmental or industrial applications.

BIOLOGICAL CHEMISTRY DIVISION

Head of Division: Maria Arménia Carrondo

Laboratory: Molecular Simulation

Head of Laboratory: : Antonio M. Baptista (see also Associate Laboratory Section)

Research Team:

Miguel Machuqueiro	Post-Doc
Vitor H. Teixeira	PhD student (Lab. of Protein Modelling)
Sara Campos	Graduate student

Publications 2005: 119, 134, 156, 181

Highlight: 44

Laboratory: Structure and Function of Metalloproteins

Head of Laboratory: : António V. Xavier

Research Team:

Antonio Pinho de Aguiar	Assistant Professor at IST
David L. Turner	Visiting Professor
Teresa Catarino	Assistant Professor at FCT
Miguel Pessanha	PhD
Vitor Manuel	PhD Student
Patrícia Pereira	PhD Student (Lab. of Microbial Biochemistry / Lab. Inorganic Biochemistry and NMR)
Ricardo Pires	PhD Student (Lab. of Microbial Biochemistry)
Catarina Paquete	PhD Student
Ricardo Lopes	Junior Researcher
Pedro Quintas	Junior Researcher
Isabel Pacheco	Technician

Publications 2005: 161, 185

Highlight: 93

Laboratory: Genomics and Stress

Head of Laboratory: : Claudina Rodrigues-Pousada

Research Team:

Isabel Solange Oliveira	PhD ,Visiting Scientist
Regina Menezes	Post-Doc
Maria Manuela Parreira Broco	PhD Student (until November 2005)
Tracy Nevitt	PhD Student (until December 2005)
Rute Rodrigues	PhD Student
Catarina Amaral	PhD Student
Jorge Pereira	PhD Student
Liliana do Nascimento	Master Student (ITQB/U.Aveiro-until Dec. 05)
Rute Felix	Research Student (until October 2005)
Patrícia Machado	Research Student (until October 2005)
Ana Marques	Undergraduate Student

Publications 2005: 26, 27, 159

Highlight: 94

Laboratory: Protein Biochemistry, Folding and Stability

Head of Laboratory: : Cláudio M. Gomes (see also Associate Laboratory Section)

Research Team:

Sónia S. Leal	PhD Student
Vesna Prosinecki	PhD Student
Ana R. Correia	PhD Student
Bárbara Henriques	Graduate Student
Rita Rocha	Undergraduate Student
Hugo Botelho	Undergraduate Student
Catarina Silva	Undergraduate Student

Publications 2005: 91, 99, 105

Highlight: 46

Laboratory: Protein Modelling

Head of Laboratory: : Cláudio M. Soares

Research Team:

Paulo J. Martel	Assistant Professor Universidade Algarve
Carlos A. Cunha	Post-Doc
Vitor H. Teixeira	PhD Student (Lab. of Molecular Simulation)
Bruno L. Victor	PhD Student
Nuno M. Micaelo	PhD Student.
Ana Sofia Oliveira	Research student
Diana Lousa	Master student
Rita Guedes	Teaching assistant FFUL

Publications 2005: 98, 104, 114, 119, 164, 156, 181, 185

Highlight: 95

Laboratory: Microbial Biochemistry

Head of Laboratory: : Inês Cardoso Pereira

Research Team:

Ana Filipa Valente	PhD student
Patrícia Pereira	PhD student (Lab. Structure and Function of Metalloproteins /Lab. Inorganic Biochemistry and NMR)
Sofia Venceslau	Graduate student
Sofia da Silva	Graduate student
Rita Lino	Undergraduate student
Tiago Granja	Graduate student
Inês Martins	Graduate student

Publications 2005: 114, 185

Highlight: 96

Laboratory: Microbial and Enzyme Technology

Head of Laboratory: : Lígia Martins
Research Team:

Luciana Pereira	Post-Doc
Paulo Durão	PhD Student
André Fernandes	PhD Student
Diana Mateus	Undergraduate Student

Publications 2005: 23, 195
Highlight: 97

Laboratory: Molecular Genetics of Metalloproteins

Head of Laboratory: : Lígia M. Saraiva
Research Team:

Cláudia Almeida	Research technician
Marta C. Justino	PhD Student
Susana Lobo	PhD Student
Vera Gonçalves	Graduate Student (BIC)
Lígia Nobre	PhD Student

Publications 2005: 91, 96, 97, 118, 157, 176
Highlight: 98

Laboratory: Macromolecular Crystallography

Head of Laboratory: : Maria Arménia Carrondo
Research Team:

Macromolecules with Industrial and Medical Applications Group

Group Leader: Pedro Matias, Senior Research assistant

Susana Gonçalves	Graduate Student (ITQB/PSB)
Sabine Gorynia	PhD student (Schering AG Berlin/IBET)

Crystallographic Structure-function Group

Group Leader: Carlos Frazão, Senior Research Assistant

David Aragão	PhD student
Joana Raquel Rocha	Graduate Student
Jorge Andrade	Graduate Student

Host-Pathogen Interactions Group

Group Leader: Francisco Enguita, SPINE Post-doc

Ana Maria Gonçalves	Post-Doc FCT
David Marçal	PhD Student
Mário Correia	PhD Student
Ana Rêgo	Graduate Student

Membrane Proteins Group

Group Leader: Margarida Archer, Research Assistant (see also Associate Laboratory Section)

Tiago Bandeiras	Post-Doc IBET
Maria Luísa Rodrigues	PhD Student / Post-doc

Diana Plácido	PhD Student
José Brito	Graduate Student
Tânia Oliveira	Graduate Student

Other Members:

Colin McVey	Post-Doc FCT
Isabel Bento	Post-Doc
Daniele de Sanctis	Post-Doc SPINE
Célia Romão	Post-Doc (Lab. Metalloproteins and Bioenergetics)
Ricardo Coelho	Technician

Publications 2005: 13, 23, 45, 59, 74, 114, 115, 158, 165, 183
Highlight: 99

Laboratory: Metalloproteins and Bioenergetics

Head of Laboratory: : Miguel Teixeira
Research Team:

Manuela M. Pereira	LA Researcher
Andreia S. Fernandes	Assistant Prof. UALG
Ana P. Melo	Post-Doc / Prof. Auxiliar Universidade Lusofona
Smiljia Todorovic	Post-Doc
Célia V.Romão	Post-Doc
João B. Vicente	PhD Student (Universiy of Rome "La Sapienza"/ITQB)
Filipa L. Sousa	PhD Student
João V. Rodrigues	PhD Student
Andreia F. Veríssimo	PhD Student
Ana P. Baptista	PhD Student
Ana P. Refojo	PhD Student
Maxime Cuypers	PhD Student (ESRF/ITQB)
Francesca M. Scandurra	PhD Student (Universiy of Rome "La Sapienza"/ITQB)

Publications 2005: 17, 67, 97, 99, 118, 157, 176, 182, 185, 187
Highlight: 100

Laboratory: Inorganic Biochemistry and NMR

Head of Laboratory: : Ricardo O. Louro (see also Associate Laboratory Section)
Research Team:

Patricia M. Pereira	PhD Student
Bruno Fonseca	Undergraduate Student
Miguel Pessanha	PhD Student (until September 2005)
Carlos A. Salgueiro	Professor (moved to FCT-UNL)

Publications 2005: 161
Highlight: 43

Novel Techniques for obtaining NMR structures of Haem proteins

António Xavier : Structure and Function of Metallo-proteins Laboratory

Understanding the cooperative effects that are fundamental for electroprotonic energy transduction processes requires detailed measurements of the microscopic thermodynamic parameters for the functional centres, and structural studies to reveal how interactions are controlled at the atomic level [1]. X-ray crystallography is the most convenient method for obtaining structural information about biological macromolecules, but it has significant limitations. One problem is that proteins involved in energy transduction are typically highly sensitive to the environmental conditions, such as solution potential and pH, and these are difficult to control or measure in crystals. Another difficulty is that interactions are often mediated by electrically charged groups such as lysine side chains near the protein surface or with high solvent exposure, and their flexibility may make them invisible in the crystal structure. NMR allows the structures of small to medium sized proteins to be determined in solution under conditions which can be controlled and made physiologically relevant. NMR also has its limitations, not least that relatively large amounts of material are needed. The method is still very labor-intensive, as crystallography was 30 years ago, and NMR structures are generally less well-resolved. However, the ability to observe flexible side chains and even characterize their dynamic behavior, plus the ability to follow changes in the structure as a function of pH or solution potential, makes the effort worthwhile.

The development of techniques for determining protein structures by NMR has been an important aspect of research in this Laboratory for several years. Paramagnetic metalloproteins are a particular challenge and the use of new sources of information such as electron-nuclear dipolar shifts and Fermi contact interactions have been pioneered to improve the resolution of structures. The figure below shows the NMR structures of an oxidized tetrahaem protein and a mutant designed to probe the control of haem-haem interactions. Since there was only a small quantity of the mutated protein available, the structure was determined only in the region of the change (shown in blue) using a novel technique of 'grafting' [2].

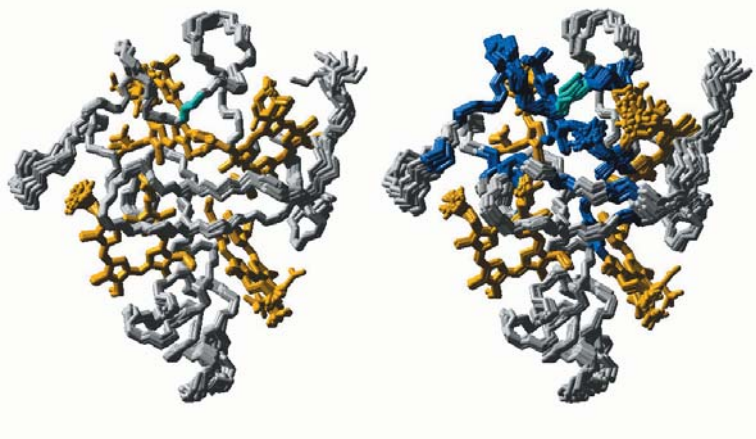


Figure : The backbone and haems from the NMR structures of the wild-type (left) and K45Q (right) ferricytochrome c_3 from *D. vulgaris* Hildenborough. The calculation of the K45Q structure was performed with data from regions coloured blue grafted onto data from the wild-type protein. Gln 45, the mutated residue, is shown in cyan.

1. AV Xavier (2004) *Biochim. Biophys. Acta-Bioenergetics* **1658**, 23-30
2. AC Messias, AP Aguiar, L Brennan, CA Salgueiro, LM Saraiva, AV Xavier, DL Turner (2006) *Biochim. Biophys. Acta-Bioenergetics,* submitted.

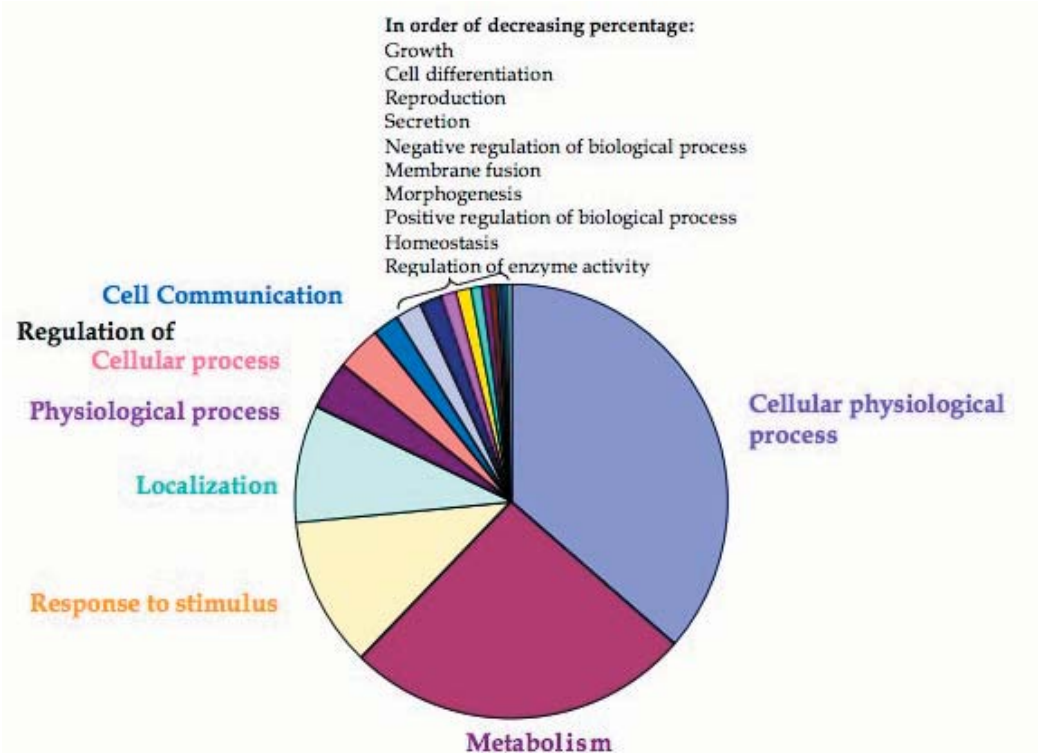
Genomic approaches to study Yap4 under hyperosmolarity

Claudina Rodrigues-Pousada : Genomics and Stress Laboratory

The Yap family of 8 b-Zip transcription factors in yeast has been shown to be involved in controlling gene expression under stress conditions provoked by H_2O_2, N-ethyl maleimide, cadmium, arsenic metalloid and hyperosmolarity among others. Previously it has been shown that *yap4* mutants show an osmo-sensitive phenotype [1]. *YAP4* is induced under these conditions and is accompanied by strong protein phosphorylation at a threonine (T_{192}) and a serine (S_{196}). Global micro-array analyses performed under 0.4M NaCl have shown that the absence of Yap4 affects the expression of 123 genes from which only 80 belong to the set of osmo-induced genes. Yap4 responsiveness can be divided into, 1) genes whose expression is largely dependent on Yap4, 2) genes whose expression is partially regulated by this factor, and 3) genes repressed by Yap4 upon exposure to hyperosmolarity. Furthermore validation experiments have confirmed that Yap4 is required for fine-tuning the expression of many of these targets. Parallel studies on Yap4 protein-protein interactions have recently revealed that Yap4 can interact with Yap6 through heterodimer formation and also with Bur2, the cyclin for the cyclin-dependent kinase Bur1, known to regulate components of the general transcriptional machinery.

[1] (Rodrigues-Pousada *et al* FEBS J., 2005, 272:2639-2647)

Gene ontology of potential Yap4 target genes

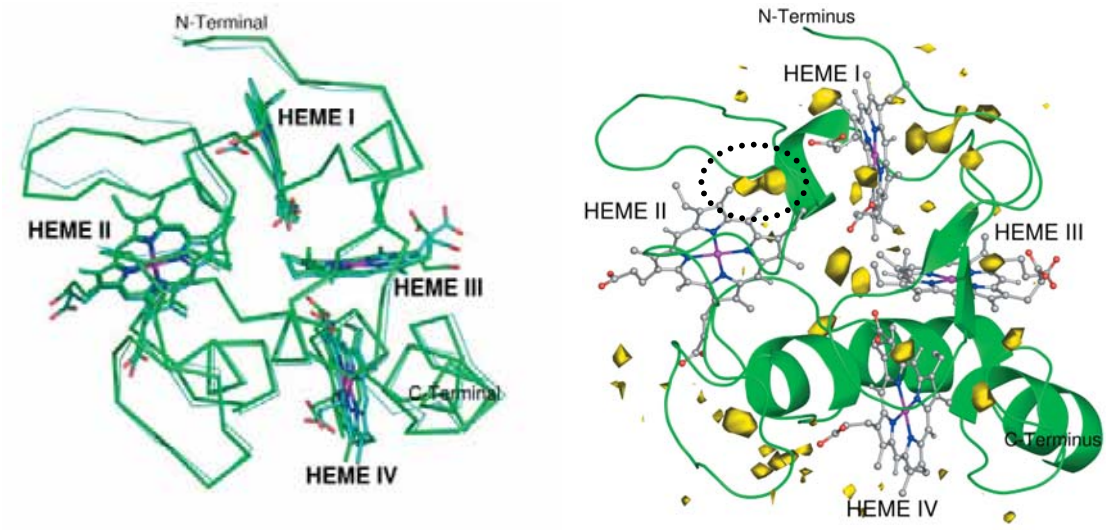


Developing simulation methods to study conformational changes in redox proteins

Cláudio M. Soares : Protein Modelling Laboratory

The study of conformational changes in proteins is one of the most important aspects in Structural Biology, given that these conformational changes mediate many biological phenomena. Proteins experience conformational changes upon certain stimuli, performing their function. Despite its importance, the study of conformational changes in proteins is rather difficult, given that many of these changes are transient, fast, and difficult to be captured by experimental techniques (usually X-ray crystallography or NMR). The use of molecular dynamics simulation (MD) holds much promise to investigate these problems in a very controlled manner and with reduced material and human resources. However, technical and methodological difficulties have reduced the widespread use of these methodologies.

Over the years, the Laboratory has focused considerable attention on conformational changes in proteins occurring upon redox changes. In many cases these conformational changes are small, requiring high resolution experimental techniques and extreme care in the interpretation of the results. The early attempts to characterise redox-driven conformational changes with MD simulation on a simple model system, the tetrahaem cytochrome c_3, gave less than satisfactory results. Recently a new parameterisation was applied, by using a better force field and by deriving a new set of charges from the oxidised and reduced states of the haems. With this new set-up 10 simulations were performed in both the fully oxidised and fully reduced states, spanning 4 ns each. This large number of simulations is a necessity in order to obtain statistically significant results, which cannot be obtained with one simulation for each state. The results (see figure) are in agreement with experimental results for this system, showing that these simulation methodologies can be used as an alternative to time consuming experiments, providing that proper care and statistical significance is achieved. Moreover, these methods can go beyond what can be obtained experimentally, as shown in the figure, where differences in water density in certain zones of the protein can be seen between different redox states. Reduction of the four haems prompts localised changes in the protein backbone, mostly around some loops (see figure A).



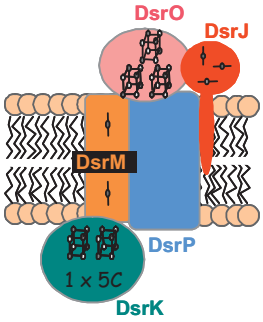
A **B**

Figure – **A.** The average structure of the fully oxidised state (tick green lines) is compared with the average structure of the fully reduced state (thin blue lines). **B.** The oxidised structure is rendered with contoured zones of higher water density (in yellow), with a zone which is absent from the fully reduced state encircled by a dotted line.

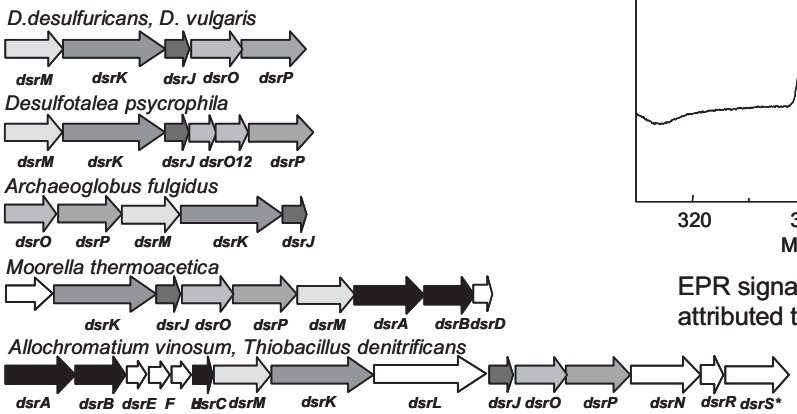
Reference: Oliveira, ASF, Teixeira, VH, Baptista, AM, Soares, CM (2005) "Reorganization and conformational changes in the reduction of tetrahaem cytochromes.", *Biophys. J.*, **89**, 3919-3930.

DsrMKJOP from *Desulfovibrio desulfuricans*: a membrane-bound complex involved in sulfate respiration

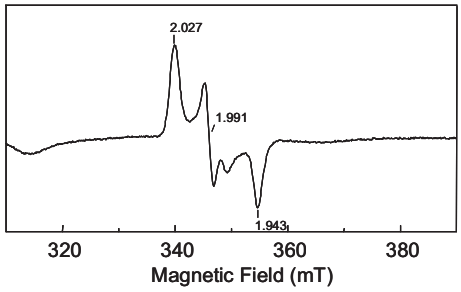
Inês Cardoso Pereira : Microbial Biochemistry Laboratory.



The respiratory chain of the anaerobic sulfate-reducing organisms is still poorly understood. In particular, the role of membrane-associated quinones and redox proteins is not clear. Only two membrane redox complexes (Qmo and Dsr) are strictly conserved in the genomes of sulfate-reducing organisms reported to date. The QmoABC complex was first isolated and characterised by this Laboratory in 2003. This year the isolation of the DsrMKJOP complex from *D. desulfuricans* was reported. The complex has hemes of the *c* and *b* types and several iron-sulfur centers. The corresponding genes in the genome of *D. vulgaris* indicate that *dsrM* encodes an integral membrane cytochrome *b*, *dsrK* encodes a protein homologous to the HdrD subunit of heterodisulfide reductase, *dsrJ* encodes a tri-heme periplasmic cytochrome *c*, *dsrO* encodes a periplasmic FeS protein and *dsrM* another integral membrane protein. DsrJ belongs to a novel family of multiheme cytochromes *c* and its three hemes have different types of coordination, one bis-His, one His/Met, and the third a very unusual His/Cys coordination. The His/Cys-coordinated heme is only partially reduced by dithionite. About 40% of the hemes are reduced by menadiol, and no reduction is observed upon treatment with H_2 and hydrogenase, irrespective of the presence of cytochrome c_3. The Dsr complex displays an EPR signal with similar characteristics to the catalytic $[4Fe4S]^{3+}$ species observed in heterodisulfide reductases. The Dsr complex is present in all prokaryotic genomes that contain a dissimilatory sulfite reductase (DsrAB) and several lines of evidence indicate that it is involved in electron transfer to the sulfite reductase.



The *dsr* gene locus in several organisms



EPR signal in oxidised DsrMKJOP attributed to a $[4Fe4S]^{3+}$ cluster

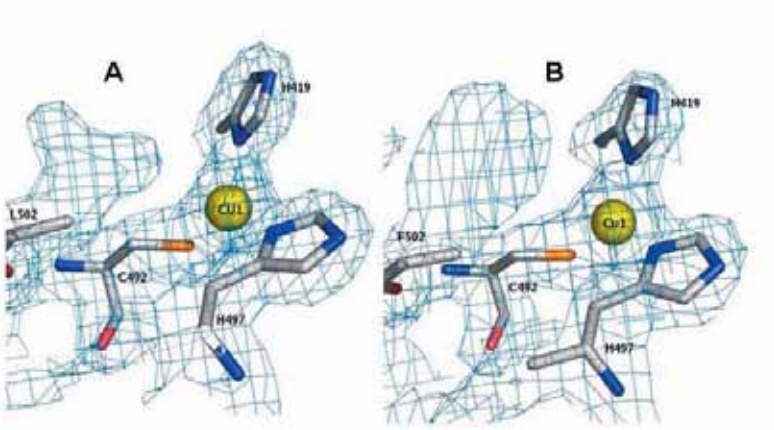
Pires, R.H., Venceslau S., Morais F., Teixeira M., Xavier A.V. and Pereira I.A.C. (2005) Characterization of the *Desulfovibrio desulfuricans* ATCC 27774 DsrMKJOP complex - a membrane-bound redox complex involved in sulfate respiration, *Biochemistry*, DOI: 10.1021/bi0515265

Tailoring catalytic sites of laccases

Ligia Martins : Microbial and Enzyme Technology

Laccases (multi-copper oxidases) are potential biocatalysts for diverse biotechnological applications mainly due to their high relative non-specific oxidation capacity, the lack of a requirement for cofactors, and the use of readily available oxygen as an electron acceptor. Recently, the Laboratory has undertaken a multidisciplinary study of the CotA-laccase from *Bacillus subtilis* as a model bacterial laccase system. Such studies aim at clarifying, at the molecular level, the catalytic activity and stability of these enzymes and the design of laccases that better match biotechnological applications by protein engineering techniques.

The role of the T1 Cu site within the multi-copper oxidases is related to the long-range intramolecular electron transfer, shuttling the electrons from the reduced substrate to the trinuclear center, where O_2 is reduced to water. The catalytic rate-limiting step in laccases is considered to be the oxidation of substrate at the T1 site, most probably controlled by the redox potential difference between this site and the trinuclear site. The conserved coordinating amino acids for this site are two histidines and a cysteine, and the natural variations occur in the so-called axial position with a single interaction from a Met being the most common arrangement. Fungal laccases have the non-coordinating Phe or Leu at this position and these may contribute, at least in part, to the high E^{o} observed in these enzymes, although other elements of the protein matrix are known to affect this important parameter of the T1 Cu center.



Detail of the type 1 Cu center showing the mutated residues M502L (A) and M502F (B)

Site-directed mutagenesis has been used to replace Met-502 in CotA-laccase by the residues leucine and phenylalanine. Structural comparison of M502L and M502F mutants with the Wt CotA shows that the geometry of the T1 copper site is maintained for both mutants as well as the overall fold of tertiary structure. Thus, an increase in the redox potential of both mutants by as much as 100mV has been attributed to the weakening of the T1 Cu coordination. Nevertheless, no direct correlation has been found between the redox potentials determined for the M502L and M502F enzymes and the oxidation rates of several non-phenolic and phenolic substrates tested, as lower turnover rates were calculated for both mutants when compared with the Wt enzyme. Furthermore, the mutations in the axial ligand have a profound impact on the thermodynamic stability of the enzyme. The results indicate that copper depletion is a key event in the inactivation and thus in the thermodynamic stability of CotA-laccase. It has been shown that subtle rearrangements in the coordination sphere of the T1 copper resulted in major loss of function regarding the catalytic as well as the overall stability of the enzyme, launching new questions regarding our understanding of the structure and function of the oxidative copper site of the blue multi-copper oxidases.

Durão, P., Bento, I., Fernandes, A.T., Melo, E.P., Lindley, P.F. and Martins, L.O. “Perturbations of the T1 copper site in the CotA-laccase from *Bacillus subtilis*: structural, biochemical, enzymatic and stability studies”. *Submitted.*

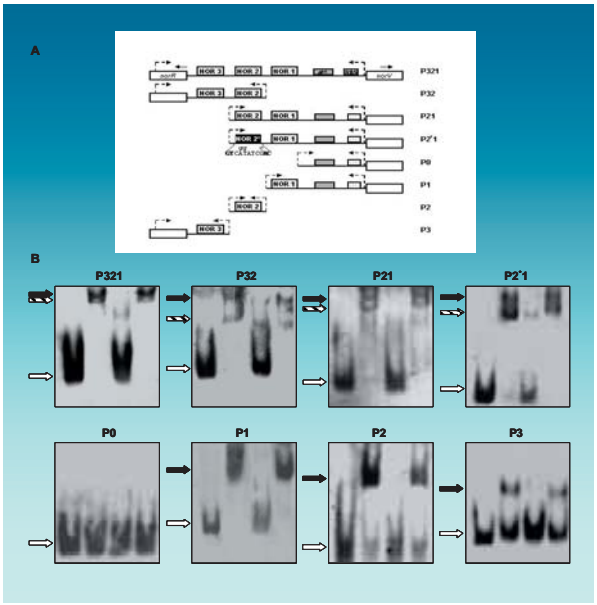
Understanding the nitrosative defence mechanisms of Escherichia coli

Lígia M. Saraiva : Molecular Genetics of Metalloproteins Laboratory

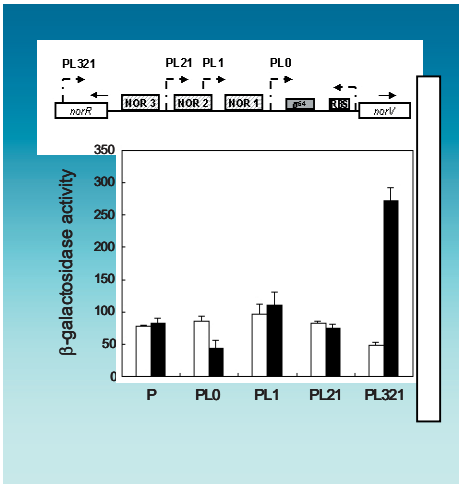
The increase in antibiotic resistance and the lack of efficient alternative pharmacological treatments are the factors responsible for the high prevalence of infectious diseases of difficult therapy and show the crucial importance of the study of the mechanisms for resistance of bacteria against the human immune system. Nitric oxide (NO) generated by the eukaryotic NO synthases is one of the key weapons to fight pathogens, as judged by the release of high fluxes of NO and reactive nitrogen species by eukaryotic macrophages upon infection and by the decreased resistance observed in eukaryotes in which the generation of NO was blocked. However, our understanding of how pathogens respond to nitrosative stress is still insufficient. In this laboratory the nitrosative defence mechanisms of *Escherichia coli* have been studied and it has been shown that flavorubredoxin, a NO reductase, has a key role in NO detoxification. The *E. coli* nitric oxide sensor, NorR, that mediates the nitric oxide induction of flavorubredoxin has now been studied. Recombinant NorR was produced and the purified NorR binds specifically to DNA fragments of the flavorubredoxin promoter region, either containing one, two or three NorR-binding sites. Protein crosslinking experiments indicated that when bound to the flavorubredoxin promoter NorR is in the trimer form. Furthermore, the simultaneous binding to all three NorR-affinity sites was shown to be essential for the NorR-mediated nitric oxide promoter activation of the flavorubredoxin gene. These type of studies is now being extended to other bacterial pathogens.

Activation of flavorubredoxin gene promoter requires triple-binding of NorR

EMSA analysis of the interaction of NorR with DNA fragments containing segments of the norVW promoter region.



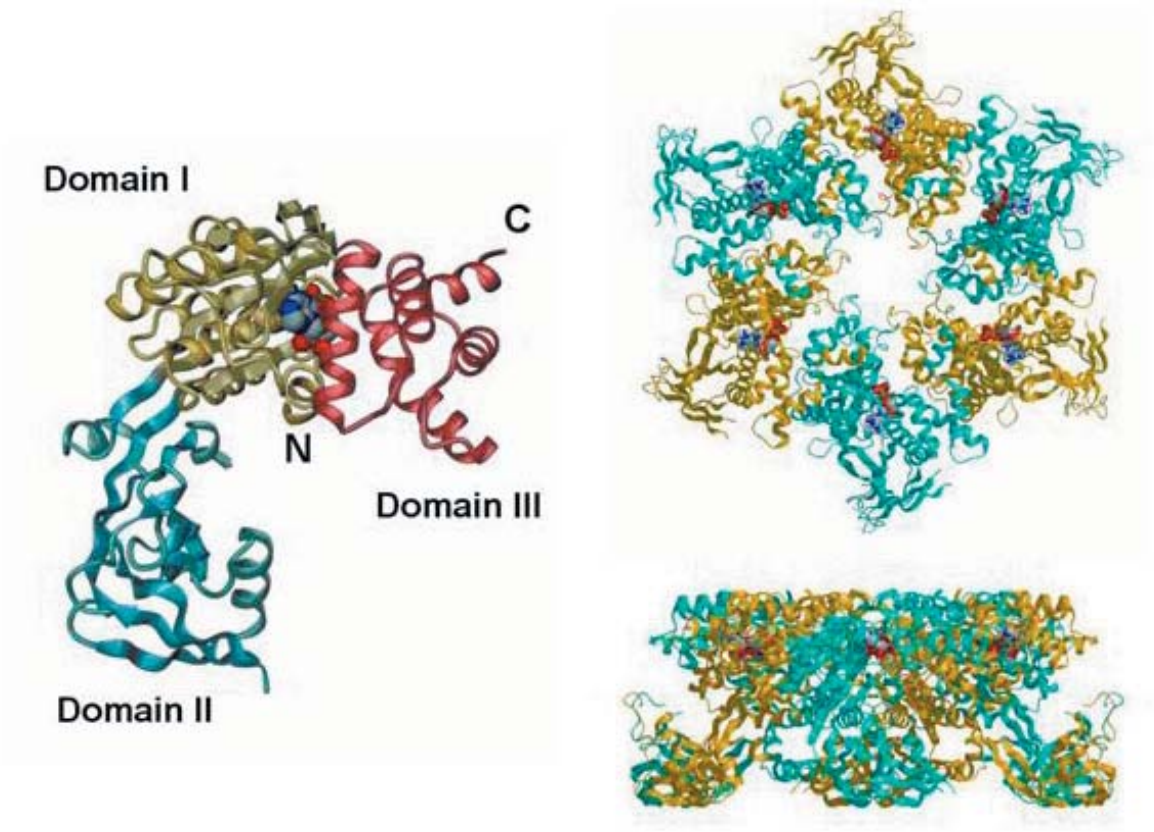
Activation of the flavorubredoxin gene promoter requires triple-binding of NorR.



Crystal structure of the human RuvB-like helicase RuvBL1

Maria Arménia Carrondo: Macromolecular Crystallography Laboratory
Pedro Matias: Macromolecules with Industrial and Medical Applications Group

RuvBL1 is an evolutionary highly conserved helicase related to the AAA^+-family of ATPases (ATPase associated with diverse cellular activities) and plays important roles in essential signalling pathways like c-Myc and Wnt, in transcription, in DNA repair and apoptosis. Helicases are pivotal enzymes involved in DNA metabolism that use the energy of ATP hydrolysis to unwind nucleic acids. The first three-dimensional structure of a human helicase was solved at a resolution of 2.2 Å and shows an hexameric arrangement with bound ADP. The RuvBL1 monomer consists of three domains, of which the first and the third are involved in ATP binding and hydrolysis. Structural homology suggests that the second domain, which is unique to this kind of helicases, is a novel DNA binding domain. Our biochemical results show that RuvBL1 binds ssDNA and dsDNA in a sequence-independent manner, with higher affinity for ssDNA. The RuvBL1/ADP complex structure illuminates how RuvBL1 may couple nucleotide hydrolysis to hexamerization and suggests how conformational changes might drive DNA unwinding.

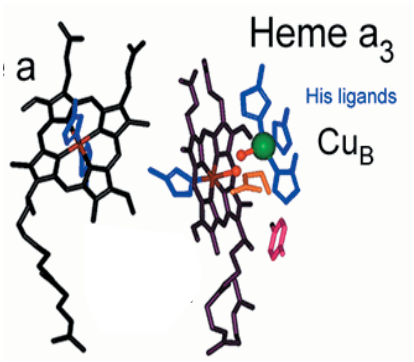


Legend: Ribbon diagram of the RuvBL1 monomer showing its domain structure on the left. Top and side views of the RuvBL1 hexamer on the right. Adjacent monomers are coloured cyan and gold. The bound ADP molecules are represented as spheres.

Spectroscopic characterization of *Acidianus ambivalens* aa_3 quinol oxidase

Miguel Teixeira : Metalloproteins and Bioenergetics Laboratory

Aerobic organisms contain membrane-bound respiratory chains, which couple oxygen reduction to water, by oxygen reductases, with ion translocation, creating an electrochemical potential difference that drives ATP synthesis by the ATP synthase. One of our model systems is the *Acidianus ambivalens* aa_3 quinol oxidase, a member of the haem-copper superfamily, which is characterized by having in subunit I a low-spin haem and a binuclear center composed by a high-spin haem and a copper ion, Cu_B. The coordination environment of the Cu_B center of this enzyme was investigated by FTIR and EXAFS spectroscopy[1] (Figure 1), in different oxidation states. The oxidized state was best simulated by 3 histidines and an O scatterer, possibly a water or hydroxide anion. On reduction, the site became 3-coordinate, and there was no evidence for heterogeneity of binding of the coordinated histidines. The Cu_B centers in both the oxidized and reduced enzymes also appeared to contain substoichiometric (0.2 mol equivalents) of non-labile chloride ion.



In order to address the redox behavior of the aa_3 oxygen reductase, the enzyme was immobilized on detergent coated silver electrode and studied by potential dependent surface-enhanced resonance Raman (SERR) spectroscopy[2]. The midpoint potentials of hemes *a* and a_3 were unambiguously determined for the first time: 320 ± 20 mV and 390 ± 20 mV. In contrast to type A enzymes, *a* to a_3 intra-protein electron transfer in this enzyme is already guaranteed by the order of the midpoint potentials at the onset of enzyme reduction and, therefore, does not require a complex network of cooperativities to ensure exergonicity. In the immobilized state, conformational transitions of the a_3-Cu_B active site, which are essential for proton translocation, are drastically slowed down compared to solution, most likely due to the interfacial electric field. These results suggest that the membrane potential may play an active role in the regulation of the enzymatic activity of aa_3 quinol oxidase (Figure 2).

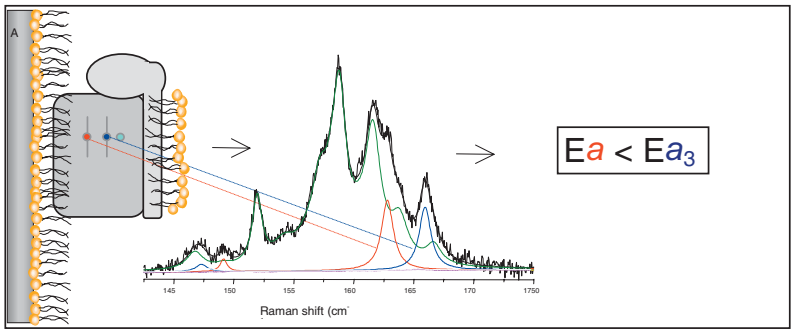


Figure 2 – Schematic representation of the immobilized aa_3 quinol oxidase. SERR spectrum of the immobilized aa_3 quinol oxidase in 1425-1750 cm^{-1} region, at -3 mV. The experimental and overall simulated spectra are shown in black lines. The different colors represent the individual spectral components. Red: ferrous heme *a*. Blue: ferrous heme a_3. Red: ferric heme a_3. Green: all other non-distinct bands in the 1425-1750 cm^{-1} region.

1 Bandeiras, T. M., Pereira, M. M., Teixeira, M., Moenne-Loccoz, P. and Blackburn, N. J. (2005) Structure and Coordination of Cu_B in the *Acidianus ambivalens* aa_3 Quinol Oxidase Heme-Copper Center. *J. Biol. Inorg. Chem.*, 10, 625-635.

2 Todorovic, S., Pereira, M. M., Bandeiras, T. M., Teixeira, M., Hildebrandt, P. and Murgida, D. H. (2005) Reversal of the midpoint potentials of hemes *a* and a_3 in the quinol oxidase of *Acidianus ambivalens*. *JACS*, 127, 13561-13566.

Mutant Studies of the CotA Laccase from *Bacillus subtilis*

Peter F. Lindley & Isabel Bento : Director's Laboratory

The cotA protein is a laccase isolated from the bacteria, *Bacillus subtilis.* In a similar manner to other multicopper oxidases it couples the oxidation of substrates with the reduction of oxygen to water. The oxidation of substrates takes place at a mononuclear type 1 copper centre whereas the reduction of oxygen occurs at a trinuclear copper centre. This trinuclear centre is comprised of two type 3 and one type 2 copper ions. It is well known that the electrons captured at the mononuclear type 1 copper centre are channeled through a cysteine residue, C492, and two histidine residues (H491 and H493) that coordinate to the type 3 copper ions. The dioxygen binds to these copper ions and is subsequently reduced to two molecules of water. However, the path that allows the entry of protons into the trinuclear centre is still unclear. It has been suggested that a glutamate residue, E498, may play an important role in this process (Bento *et. al.*, 2005). To assess the importance of this residue in this regard, it has been mutated to a leucine residue and the structure of the mutant determined by X.-ray crystallographic methods. Surprisingly, the analysis of electrons density maps (2FoFc and FoFc) has shown that the mutant protein lacks the entire trinuclear centre. The mutation seems to have a drastic effect, destabilizing the trinuclear centre and causing complete copper depletion within it. However, the mononuclear type 1 copper centre seems unaffected and has an environment typical of the wild type protein.

a) b)

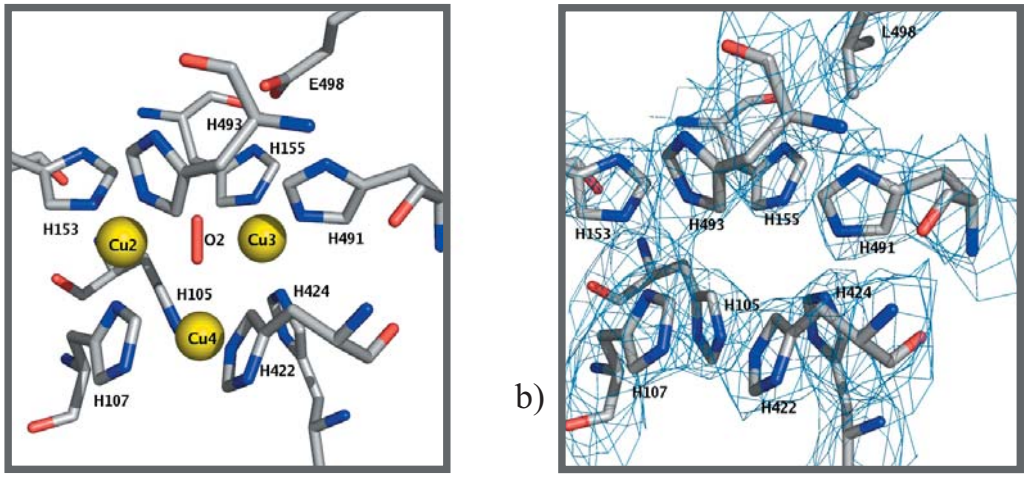


Figure 1. Details of the trinuclear centre and its environment;
a) the native cotA protein,
b) the cotA mutant E498L (2FoFc map contoured at 1.2σ).

BIOLOGY DIVISION

BIOLOGY DIVISION : Objectives and Activities

The main areas of research in this Division cover a wide range of topics from microbial genetics, pathogenesis and epidemiology to microbial physiology, from questioning how gene expression takes place to how glycoproteins are produced in mammalian cells. Most of the expertise in this Division is focused on bacteria and archaea, but two of the teams use eukaryotic cells as the frame work to better understand their biologic questions. Problems such as how can bacteria induce disease in the human host, resist to antibiotics or evade the host immune system are being addressed by different research Laboratories in the Biology division.

The division has recently (2004-2005) increased the number of independent research groups from 6 to the current number of 11. A number of young scientists whose research was taking place in different countries such as the UK, Netherlands and the USA have been hired to form independent research laboratories. Their collaboration with the existing groups at ITQB will allow the strengthening of the research in fundamental areas such as pathogenic bacteria metabolism, bacterial cell wall synthesis, bacterial cell-cell communication and bacterial cellular and developmental biology.

Studies performed at the **Laboratory of Molecular Genetics** (H. de Lencastre) on **molecular mechanisms** responsible for **antibiotic resistance** and on **molecular epidemiology** represent a highly visible research area and most studies have direct social impact. The Laboratory has been the centre of multinational projects 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. Extensive studies are also ongoing to understand the mechanism of epidemic behaviour and evolution of multidrug resistant clones of *Staphylococcus aureus* in hospitals and in the community. Previous studies at the Laboratory have demonstrated that expression of the beta-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 several auxiliary genes has been put under the control of inducible promoters in order to test the effect on the resistant phenotype. This novel experimental system has been analysed for mRNA expression profiles using DNA microarrays.

The **Microbial Development Laboratory** (A Henriques) studies spore development in the model bacterium *Bacillus subtilis*. Spore development involves the metamorphosis of the rod-shaped vegetative cell into a dormant, highly resistant spore. Research focuses on the process by which the cells abandon medial cell division during growth, to switch to a mode of asymmetric (polar) division, which gives rise to dissimilar progeny cells, the mother cell and the smaller prespore. The Laboratory is also interested in the alterations in the mechanics of chromosome segregation that accompany this switch. It also investigates the mechanisms by which the programs of gene expression in the two sporangial compartments are coordinated and kept in register with the course of morphogenesis.

The main area of interest in the **Microbial Genetics Laboratory** (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*. The *B. subtilis*

AraR transcription factor represses at least thirteen genes and plays an important role in sugar utilization. The functional domains of AraR required for DNA binding, dimerization, and effector-binding, have recently been mapped by random and site-directed mutagenesis, deletion and construction of chimeric LexA-AraR fusion proteins. In vivo analysis has identified particular amino acids required for dimer assembly, formation of the nucleoprotein complex and composition of the sugar-binding cleft. This work presents a structural framework for the function of AraR and provides insight into the mechanistic mode of action of this modular repressor. Moreover, a successful characterization of a new endo-arabinanase (YxiA) capable of releasing arabinosyl oligomers and arabinose from plant cell walls, has been achieved. The genetic and biochemical studies on hemicellulases will continue in 2006.

The **Cell Physiology & NMR Laboratory** (H. Santos) uses *in vivo* and *in vitro* NMR as the main experimental tool to study microbial physiology. *Lactic Acid Bacteria* and hyperthermophilic microorganisms are the two major objects of research. *In vivo* NMR is used to measure on line the dynamics of intracellular pools of glycolytic intermediates in *Lactococcus lactis* strains to provide reliable *in vivo* data to be used as guidelines for efficient metabolic engineering strategies. A more fundamental goal is to characterize central metabolism and regulatory networks in *L. lactis* taking advantage of global approaches. The Laboratory maintains close collaboration with groups with expertise in mathematical modelling for the integration of the data at multi-level organization. A second research interest of the Laboratory is the physiology of hyperthermophiles. The main objectives of this research line are genetic and biochemical characterization of biosynthetic pathways of compatible solutes, 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, and the identification of novel compatible solutes. In 2005 the Laboratory participated in an EU-project aiming at finding novel applications for solutes from hyperthermophiles. One more patent was filed in 2005 via IBET.

The main research area in the **Control of Gene Expression Laboratory** (C. M. Arraiano) has been to elucidate the process of RNA degradation. RNase II is an exonuclease often essential for growth and mutations in its gene have been linked with abnormal chloroplast biogenesis, mitotic control and cancer. The RNase II family of enzymes is present in the three kingdoms of life. Structural and functional studies have been performed on *Escherichia coli* RNase II and a mutant protein has been characterized. This year, it has also been shown that *Escherichia coli* RNase R can be very important for mRNA decay in stationary phase and the developmentally regulated TAZMAN in Drosophila has been discovered and studied. In 2005 it has been shown that ribonuclease deficient strains can increase the production of penicillin amidase, an enzyme important in the production of synthetic penicillins. In addition, research has also been focused on the role of polyadenylation. Very important results have been obtained that show that Poly (A)-polymerase I links transcription with mRNA degradation via S proteolysis. This unexpected finding makes the first connections between the RNA decay apparatus and proteolysis and opens an exciting chapter extending the research on RNA degradation to other important fields.

Protein transport and glycosylation in mammalian cells is the main topic in the Laboratory coordinated by J. Costa. During the year 2005, the **Laboratory of Glycobiology** has continued to develop research in the areas of neurodegenerative diseases,

namely Alzheimer's disease and Amyotrophic Lateral Sclerosis, neuronal differentiation and ovarian carcinoma. Most striking has been the finding that His and Ala concentrations decrease whereas Asn concentration increases in the plasma of ALS patients. Furthermore, α2-macroglobulin, (α2-M), increases during disease progression for some patients. The results obtained suggest that diet supplementation with His and Ala and modulation of α2-M might have some beneficial effects on the course of ALS. The Laboratory has continued to develop work on the elucidation of the glycosylation role in ovarian carcinoma within the context of the European STREP Project "Signalling and Traffic". The Laboratory has also participated in the European Project "Cell Programming by Nanoscaled Devices" in collaboration with the Animal Cell Technology Laboratory. A soluble recombinant form of a human cell adhesion glycoprotein from insect cells has been obtained that can be used for studies on cell differentiation. In 2006, these lines of research will be continued.

The main interest of the new laboratory of **Microbial Pathogenesis and Cell Biology** (S. Filipe) is the relationship of Gram-positive pathogens and their hosts, namely the role of cell wall synthesis and turnover in the process of host colonization and infection. The objective is to better understand the bacterial cell wall metabolism from pathogenic bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae* and its contribution to the ability of bacteria to trigger an inflammatory response in different hosts (Drosophila flies, mice and rabbits). The assembly of the bacterial cell wall is carried out by an extremely complex mechanism that allows incorporation of new building blocks without weakening of the cell wall. The Laboratory is interested in this process and whether there is a mechanism of cell wall turnover in order to recycle the building blocks or eventually to control the release of cell wall fragments.

The laboratory of **Physiology of Lactic Acid Bacteria & in vivo NMR** (A. R. Neves) was established in January 2004. LAB are a group of Gram positive bacteria with habitats ranging from milk to specific parts of the human body. A main feature of these organisms is the conversion of carbohydrates through glycolysis to lactate. The main research interests in the Laboratory concern the physiological and biochemical analysis of metabolic pathways and their regulation in the model organism *Lactococcus lactis* and the closely related human pathogen, *Streptococcus pneumoniae*. Focus is given to the elucidation of the regulatory mechanisms underlying sugar uptake, and the link between the glycolytic pathway and formation of sugar nucleotides, biosynthetic precursors of capsule and cell wall polysaccharides. Different methodologies, such as genetic engineering tools, in vivo NMR studies to obtain information on the dynamics of intracellular pools, and DNA microarrays are currently used.

Also recently established, the **Microbial Epidemiology Laboratory** (R. Mato) studies the epidemiology of multi-antibiotic resistant *enterococci* in the hospital setting. The research is centred around four main objectives; a) Evaluation of the prevalence of nosocomial infection and/or colonization by clinical relevant *enterococcal* species, particularly in high-risk groups such as newborns in neonatal intensive care units, and haematological malignancy patients, b) Application of typing methodologies to the characterization and identification of the clonal types circulating at the hospital setting, c) Detection of virulence genetic determinants associated with glycopeptide-resistant *enterococcal* strains (GRE), and (d) Surveillance studies of GRE isolated from infection and carriage in high-risk patients, as well as the identification of the risk factors that contribute to the infection and colonization by GRE.

The **Bacterial Cell Biology Laboratory** (M. Pinho) was formally established in early 2006 and focuses on studies of cell division in Gram-positive pathogens. Cell division is a complex process which involves duplication of the mass of the cell, replication and segregation of the chromosomes and synthesis of the septum for the separation of the two daughter cells. Currently this laboratory is working principally on the process of cell wall (CW) synthesis which is required for the synthesis of the septum, with the aim of identifying and localizing all the components of the CW synthetic machinery. CW synthesis is also the target of beta-lactam and glycopeptide antibiotics. Resistance to these and other classes of antibiotics among pathogenic bacteria is a problem of high clinical relevance and attempts are being made to elucidate the resistance mechanisms to antibiotics that target CW synthesis.

Interference with inter-species communication in bacteria are the topics of the **Bacterial Signaling Laboratory** (Karina B. Xavier) a Laboratory that will be formally established in March, 2006. Bacteria use chemical signaling molecules called autoinducers to communicate with one another by a process called quorum sensing. This process enables a population of bacteria to regulate behaviors which are only productive when many bacteria act in concert as a group similar to what happens with multi-cellular organisms. Behaviors regulated by quorum sensing are often crucial for successful bacterial-host relationships, both symbiotic and pathogenic. While most quorum sensing autoinducers are species-specific, one autoinducer called autoinducer-2 (AI-2), is produced and detected by a wide variety of bacteria and is proposed to allow inter-species communication. In this laboratory a biochemical, genetic, and chemical approach will be used to study the AI-2 systems promoting bacterial inter-species communication.

The Divison had a prominent role in several Grants for equipment, which culminated this year with the purchase of a surface plasmon resonance biosensor and negotiations for the installation of a National NMR facility at the IQTB. In addition an ionic chromatographer for monosaccharide and oligosaccharide analysis will be purchased in 2006 through a Re-equipment Grant. These are important additions to the portfolio of equipment installed in the Laboratório Associado.

The Division has also been active in several teaching and science-communication initiatives, including the Dia Aberto, the CBS PhD program of the ITQB, and the Masters Course in Medical Microbiology (MMM), a joint effort with the IHMT, FCM, FCT-UNL In particular, the present (2nd) edition of the MMM course is being coordinated by researchers of the Division. Importantly, four Master Degree students have conducted research leading to their Dissertations within Laboratories of the Biology Division. One of the courses of the Master Degree course in Clinical Microbiology from Faculdade de Medicina de Lisboa was taught at the Laboratory of Molecular Genetics or the 4rd year.

Most of the Laboratories of the Divison were highly successful in their applications for Projects at the National (FCT and FCG) and International (NIH, European Union).

BIOLOGY DIVISION

Head of Division: Hermínia de Lencastre

Laboratory: Microbial Development

Head of Laboratory: : Adriano O. Henriques

Research Team:

Rita Zilhão	PhD
Teresa Barbosa	PhD
Mónica Serrano	PhD
Anabela Isidro	PhD
Teresa Costa	PhD student
Gonçalo Real	PhD student
Luísa Côrte	PhD student
Cláudia Serra	PhD student
Joana Santos	Master student

Publications 2005: 19, 124, 152, 153, 168, 178, 195
Highlight: 113

Laboratory: Lactic Acid Bacteria & In Vivo NMR

Head of Laboratory: : Ana Rute Neves (see also Laboratório Associado section)

Research Team:

Rute Castro	PhD Student (Lab. of Cell Physiology and NMR)

Main Collaborators: Prof. Helena Santos, ITQB
Prof. Oscar Kuipers, RUG, The Netherlands

Publications 2005: 129
Highlight: 63

Laboratory: Control of Gene Expression

Head of Laboratory: : Cecília Maria Arraiano

Research Team:

Monica Amblar	Post-Doc
Patrick Freire	Post-Doc
Sandra Viegas	PhD student
Ana Barbas	PhD student
José Andrade	PhD student
Francisco Mesquita	Master student
Ana Rita Furtado	Master student
Inês Heinrichson	Undergraduate student

Publications 2005: 11, 28, 188
Highlight: 114

Laboratory: Cell Physiology and NMR

Head of Laboratory: : Helena Santos

Research Team:

Pedro Miguel Lamosa	Post-Doc
Clélia Neves Afonso	Post-Doc
Claudia Sanchez	Post-Doc
Mª Margarida Moreira dos Santos	Post-Doc
Nuno Miguel Formiga Borges	Post Doc
Paulo Lemos	Post-Doc (ITQB/ FCT-UNL)
Rasmus Larsen	Post-Doc
Tiago Quininha Faria	Post-Doc
Luís Maria Lopes da Fonseca	PhD Student
Luís Pedro Gafeira Gonçalves	PhD Student
Filipa Maria Lage Silva Cardoso	PhD Student
Carla Alexandra Duarte Jorge	PhD Student
Paula Cristina Lima Gaspar	PhD Student
Rute de Almeida Ferreira de Castro	PhD Student (Lab. Lactic Acid Bacteria & In Vivo NMR)
Marta Viseu Rodrigues	PhD Student
Melinda Carmen Noronha	PhD Student (ITQB/IST)
Tiago Vasconcelos Moreira Pais	PhD Student
Cátia Maria Machado	PhD Student (ITQB/STAB-VIDA)
João Manuel Beirão Cavalheiro	Research Technician
Ana Lúcia Serafim de Carvalho	Research Student
Ana Isabel Mingote	Research Technician
Carla Patrícia Almeida	Research Technician
Catarina Isabel Ferreira da Silva	Research Student

Publications 2005: 6, 72, 125, 129, 130, 137, 161, 173, 191
Highlight: 115

Laboratory of Molecular Genetics

Head of Laboratory: : Hermínia de Lencastre

Research Team:

Alexander Tomasz	Adjunct Full Professor:
Ana Madalena Ludovice	Invited Assistant Professor FCT/UNL
Ilda Santos Sanches	Associate Professor FCT/UNL (collaborator)
Mario Ramirez	Assistant Professor FM/UL (collaborator)
Isabel Couto	Assistant Professor IHMT/UNL (collaborator)
Ana R. Gomes	Post-Doc
Duarte C. Oliveira	Post-Doc
Raquel Sá-Leão	Post-Doc
Marta Aires de Sousa	Post-Doc
Rita Sobral	PhD Student
Susana Gardete	PhD Student
M. Inês Crisóstomo	PhD Student
Maria Miragaia	PhD Student
Sandro Pereira	PhD Student
Nuno Faria	PhD Student

Sónia Nunes	Graduate student
Natacha Sousa	Graduate student (until July 31)
Carla Simas	Graduate student (until March 31)
Nelson Frazão	Graduate student
Teresa Crathorne	Graduate student
Catarina Milheiriço	Graduate student
Teresa Conceição	Graduate student
Helena de Deus	Graduate student
Alexandra Simões	Master Student
Maria Teresa Maia	Visiting Scientist
Akos Toth	Visiting Scientist
Carlos Eduardo Parente	Visiting Scientist
Isilda Gueifão	Laboratory Assistant
Manuela Nogueira	Administrative Assistant

Publications 2005: 31, 32, 43, 44, 52, 60, 64, 75, 81, 85, 116, 122, 132, 149, 174, 194
Highlight: 116

Laboratory: Microbial Genetics

Head of Laboratory: : Isabel de Sá-Nogueira
Research Team:

Irina Franco	PhD Student
José M. Inácio	PhD Student
Ana S. M. Antunes	Graduate Student (until February 2005)
Maria João Faria	Graduate Student

Highlight: 117

Laboratory of Glycobiology

Head of Laboratory: : Júlia Costa
Research Team:

Vanessa Morais	PhD student
Angelina Palma	PhD student
Catarina Brito	PhD student
Catarina Gomes	PhD student
Eda Machado	Graduate student
Cristina Escrevente	Graduate student
Ricardo Gouveia	Graduate student

Publications 2005: 92, 93, 138
Highlight: 118

Laboratory: Microbial Cell Biology

Head of Laboratory: : Mariana Gomes Pinho (see also Laboratório Associado section)
Research Team:

Ana Maria Jorge	Graduate student
Pedro Matos	Undergraduate student

Publications 2005: 146, 167
Highlight: 56

Laboratory: Microbial Epidemiology

Head of Laboratory: : Rosario Mato (see also Laboratório Associado section)
Research Team:

Renato Hélder Pires	Graduate student

Publications 2005: 75, 116, 132, 174
Highlight: 61

Laboratory: Microbial Pathogenesis and Cell Biology

Head of Laboratory: : Sergio Filipe (see also Laboratório Associado section)
Research Team:

Luís Alves Pacheco	Graduate student
Magda Pereira Atilano	Graduate student

Publications 2005: 71
Highlight: 57

Laboratory: Bacterial Signaling

Head of Laboratory: : Karina Xavier (see also Laboratório Associado section)

Highlight: 55

Prespore chromosome segregation during asymmetric cell division in *Bacillus subtilis*

Adriano O. Henriques : Microbial Development Laboratory

The rod-shaped *Bacillus subtilis* switches from a medial mode of vegetative cell division, to a polar division at the onset of spore development, the latter producing a smaller prespore and a larger mother cell. In preparation for asymmetric division, two copies of the chromosome in pre-divisional cells are remodeled into an axial filament that extends from pole to pole. The RacA protein is required for axial filament formation, and also serves as a kinetochore-like function by binding to a centromere-like element located close to the origin of chromosome replication (*oriC*). Centromere-bound RacA recognizes the topological marker protein DivIVA at the cell poles, and promotes polar anchoring of the chromosomes. Polar septation then ensues, trapping about 30% of the chromosome into the newly formed prespore, while segregation of the remaining 70% requires assembly of foci of the SpoIIIE DNA translocase in the center of the septal plate.

Cell division and chromosome segregation are closely regulated to ensure that each new cell receives a copy of the genome (1). The Laboratory has shown that a mutant for cell division protein DivIB, able to divide both medially and asymmetrically, is impaired in prespore chromosome segregation (2). Firstly, the polar retention of DivIVA is perturbed, as is that of RacA, with the consequence that *oriC* is not efficiently trapped into the forespore (Fig. 1A). Second, SpoIIIE spreads along the septum rather than assembling as foci (Fig. 1B). Proper assembly of SpoIIIE may require DivIVA-mediated polar sequestration of the MinCD inhibitor complex. It has been found that MinCD is mislocalized in mutant and that deletion of *minD* restores SpoIIIE foci assembly and DNA translocation activity. Hence, DivIB functions in two stages of prespore chromosome segregation. This suggests that DivIB is a key factor in coordinating asymmetric cell division with accurate segregation of the prespore chromosome.

1- Real, G., S. Autret, E. J. Harry, J. Errington, and A. O. Henriques. 2005. Cell division protein DivIB influences the Spo0J/Soj system of chromosome segregation in *Bacillus subtilis*. *Mol. Microbiol.*, **52**:349-367. 2 - Real, G., and A. O. Henriques. 2005. Prespore chromosome segregation during asymmetric cell division in *Bacillus subtilis*. *In preparation*.

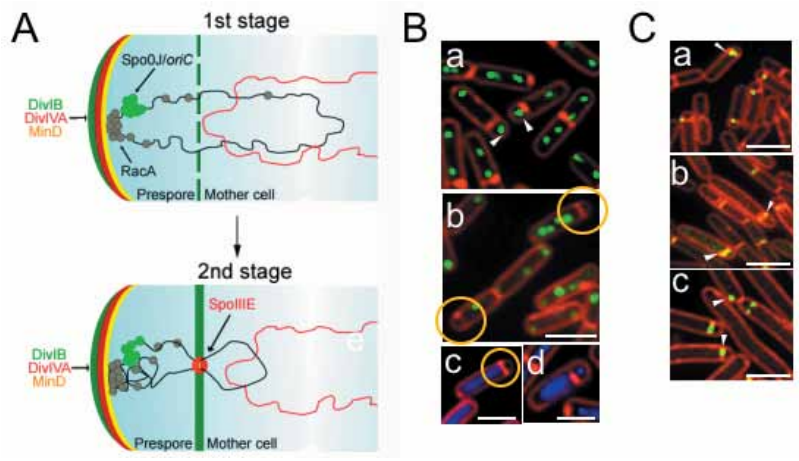
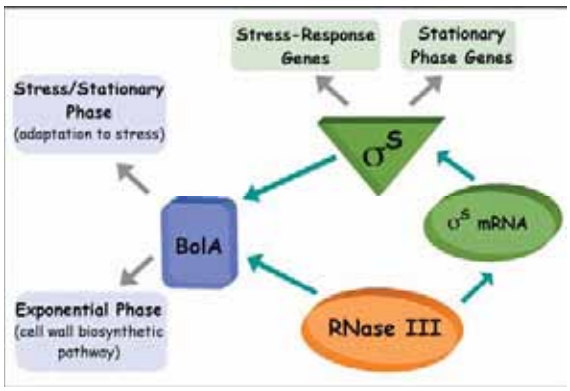


Figure – Panel A: the figure shows the localization of *oriC* in developing cells of a wild type strain (a,b), and in cells of the *divIB ftsL* mutant (c,d) as revealed by Spo0J-GFP, a protein known to bind to *oriC*. Note the absence of DNA (blue) in prespores of the mutant (d,e,f). Panel B: in the wild type, the SpoIIIE DNA translocase assembles as foci in the middle of the asymmetric septum, from where it exports the portion of the prespore chromosome that remains in the mother cell following polar septation (a); in the *divIB ftsL* mutant, SpoIIIE is misassembled across the septum (b), but deletion of the *minD* gene restores SpoIIIE foci assembly (c) and the DNA translocation function.

Impact of RNA Degradation Mechanisms on General Stress Responses

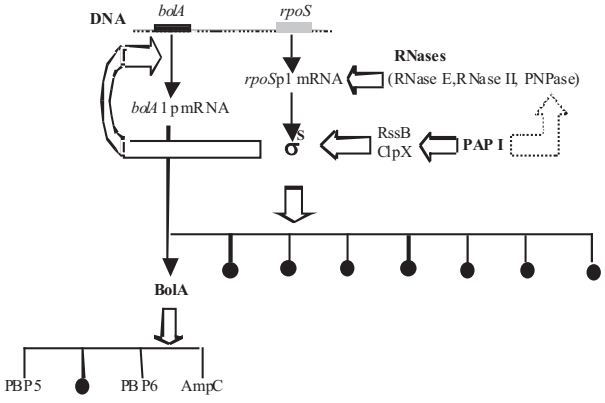
Cecília Maria Arraiano : Control of Gene Expression Laboratory

Bacteria rapidly adapt to changes in growth conditions. *bolA* is a σ^S-dependent *E. coli* morphogene involved in the general cellular adaptation to stress and cell division. Endoribonuclease RNase III is a mRNA degradation enzyme that cleaves double stranded RNA. RNase III has been shown to act as a post-transcriptional modulator of *bolA* expression under carbon starvation conditions. RNase III also regulates positively *bolA1p* mRNA levels and stabilities. Furthermore RNase III has been shown to be necessary for the normal expression of the transcription factor σ^S, ensuring normal levels of *rpoS* mRNA and σ^S protein under glucose starvation. Since σ^S controls a complex regulon of stress-response genes, RNase III has been proposed as possible modulator of bacterial cell response to stress.



In the stationary phase, *bolA1p* mRNA levels were reduced 2-5 fold in a poly (A)-polymerase I (PAPI) mutant, consistent with the significant 3-fold reduction in σ^S protein levels in the same strain. Furthermore, fusions with the *rpoS* gene, analysis of the stability of σ^S and the levels of RssB indicate that the absence of PAPI enhances RssB-mediated σ^S proteolysis specifically in starved cells. The fact that PAPI induces higher cellular levels of a global regulator is a novel finding of wide biological significance. PAPI could work as a linker between transcription and mRNA degradation with the ultimate goal of adapting and surviving to growth-limiting conditions.

These results connect the degradation machinery with transcription and proteolysis and unravel a new role for post-transcriptional regulation mechanisms in adaptation and survival to famine growth conditions.



1 Freire, P., Amaral, J.D., Santos, J.M. and Arraiano, C.M. Adaptation to Carbon Starvation: RNase III ensures normal expression levels of *bolA1p* mRNA and σ^S **Biochimie** *in press*

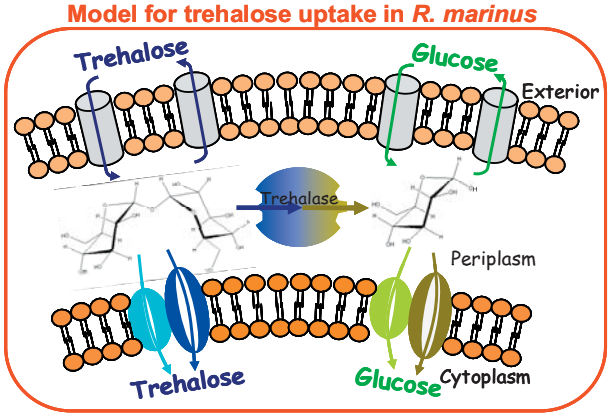
2- Santos, J.M., Freire, P. Mesquita,F.S., Mika F. , Hengge R. and Arraiano C.M. Poly (A)-polymerase I links transcription with mRNA degradation via σ^S proteolysis. **Molecular Microbiology** *in press*

Model for trehalose uptake and discovery of a highly thermostable trehalase in the thermophilic bacterium *Rhodothermus marinus*

Helena Santos: Cell Physiology and NMR Laboratory

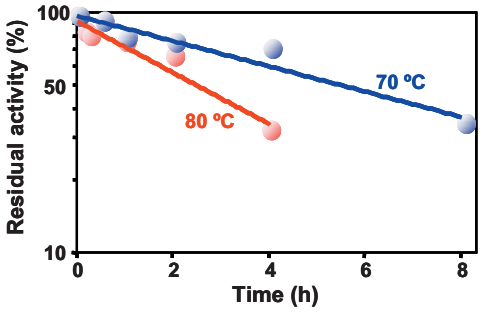
Rhodothermus marinus is a thermophilic bacterium able to grow between 54ºC and 77 ºC, isolated from marine hot springs in the Azores islands. In response to elevated temperature or salinity *R. marinus* accumulates compatible solutes such as mannosylglycerate, mannosylglyceramide, glucose and trehalose that play a role in osmo- and thermoprotection. Solutes are either synthesised *de novo* or, when available, taken up from the medium. *R. marinus* was unable to transport mannosylglycerate, but trehalose is transported and the Laboratory is studying the transport system in detail. *R. marinus* possesses two transport systems for trehalose: one has high affinity for this sugar but low capacity and the other has medium affinity and high capacity. Surprisingly, trehalase has a very high activity in the periplasmic space of *R. marinus*, which actually plays an important role in the uptake of trehalose. In fact, through the action of this enzyme, trehalose is converted to glucose which is then transported to the cytoplasm *via* specific transporters. Therefore, the action of trehalase maximizes the uptake of trehalose by the cell. To fully describe the internalization of trehalose account had to be taken not only of the transport of trehalose itself, but also the activity of trehalase assessed in living cells, the transport of the resulting glucose, and the diffusion of glucose out of the periplasm. Given the complexity of this system a mathematical model was developed. The experimental results were best fitted with a model that considers the existence of two kinetically distinct trehalose transporters and two glucose transport systems. At trehalose concentration lower than 2 µM, this sugar is exclusively taken up through the high affinity/low capacity trehalose transporter. In conditions where the concentration of trehalose is well above the µM range the net uptake of trehalose is considerably enhanced due to the involvement of trehalase and assistance via the glucose transport systems.

Model for trehalose uptake in *R. marinus*



The trehalase of *R. marinus* is the most thermostable trehalase ever reported.

The trehalase gene was identified and the recombinant protein fully characterized. The enzyme has a half-life of 6 h at 70 ºC, and of 2.5 h at 80 ºC. This is the most thermostable trehalase reported thus far. This enzyme may find application, in biosensors for determination of trehalose in complex mixtures, *e.g*, clinical or food samples.



Population structure(s) of Staphylococcus aureus

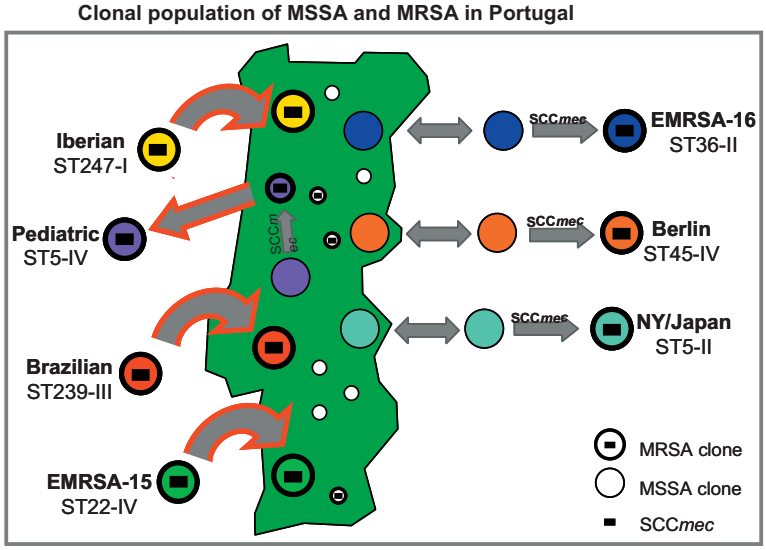
Hermínia de Lencastre: Molecular Genetics Laboratory

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prevalent and important multi-resistant human pathogens, and causes serious nosocomial and community acquired infections. It is now clear that methicillin-resistance has emerged by the introduction of a mobile genetic element called staphylococcal chromosomal cassette (SCC) *mec* into at least five distinct successful methicillin-susceptible *S. aureus* (MSSA) lineages originating a relatively small number of pandemic MRSA clones.

The nosocomial prevalence of MRSA in Portugal is close to 50%, which is one of the highest in Europe. In order to understand the origins of the dominant MRSA clones in Portuguese hospitals, the genetic backgrounds of nosocomial MRSA with MSSA isolates from the same hospitals and from the community where they were located have been compared. The findings suggest that the three major MRSA clones identified in Portuguese hospitals during the last 15 years have not originated from the introduction of SCC*mec* into dominant MSSA backgrounds present in the Portuguese nosocomial or community environment but were probably imported from abroad. (1)

Overall these and other studies suggest the international spread of *S. aureus* lineages within both the nosocomial and the community environments. However, it seems that so far there has been little spreading of MRSA clones between those two settings. In sharp contrast to the situation in Portugal, strict infection control measures introduced during the 1970s have kept the incidence of MRSA infections extremely low in Denmark. Nevertheless, MRSA infections began to appear in the community in the late 1990s associated with a single clonal type carrying a highly virulent toxin. This community MRSA clone was rarely found in nosocomial infections which belonged to a large number of clonal types, including some pandemic MRSA clones. The mechanism of spread of the ST80-IV clone in the Danish community is not known, (2).

Clonal population of MSSA and MRSA in Portugal



The three major MRSA clones identified in Portuguese hospitals during the last 15 years were probably imported from abroad.

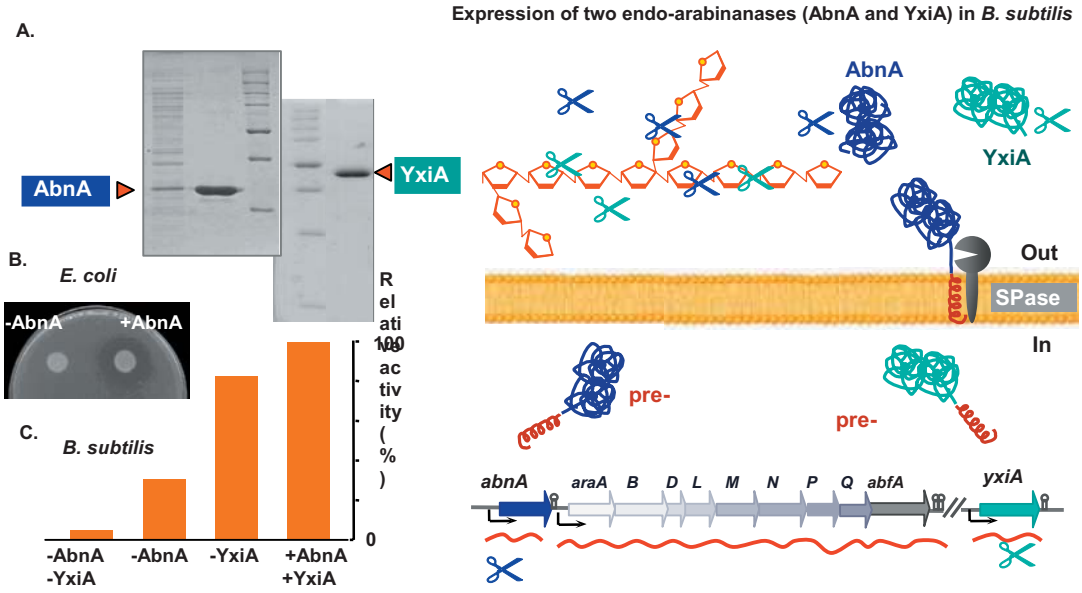
Aires de Sousa, M, T. Conceição, C. Simas, and H. de Lencastre. 2005. Comparison of genetic backgrounds of MRSA and MSSA isolated in Portuguese hospitals and in the community. J. Clin. Microbiol 43:5150-7.

Faria, N., D. C. Oliveira, H. Westh, D. L. Monnet, A. R. Larsen, R. Skov, and H. de Lencastre. 2005. Epidemiology of emerging methicillin-resistant *Staphylococcus aureus* in Denmark: a nationwide study in a low prevalence country. J. Clin. Microbiol, 43:1836-42.

Hemicellulases from Bacillus subtilis

Isabel de Sá Nogueira : Microbial Genetics Laboratory

Bacillus subtilis produces hemicellulases capable of releasing arabinosyl oligomers and arabinose from plant cell walls. The polysaccharides containing arabinose residues, found as constituents of plant cell walls, are homoglycans, arabinans, or heteroglycans, such as arabinoxylans and arabinogalactans. The two major enzymes that hydrolyse arabinan, generally named arabinases, are α-L-arabinofuranosidases (EC 3.2.1.55; AF) and endo-1,5 α-L-arabinanases (EC 3.2.1.99; ABN). The Laboratory has found that *B. subtilis* synthesizes two arabinanases encoded by the *abnA* and *yxiA* genes. The full-length *abnA* and *yxiA* coding regions have been expressed in *Escherichia coli* (A). AbnA (33 kDa) and YxiA (46 kDa) accumulated in the periplasmic space and were correctly processed by the *E. coli* signal peptidase, as confirmed by amino acid sequencing. The substrate specificity of purified AbnA and YxiA, physic-chemical properties and kinetic parameters have been determined. Functional analysis studies in *E. coli* and *B. subtilis* have revealed that both AbnA and YxiA contribute to extracellular degradation of arabinan (B). Furthermore, the hydrolytic activity assayed in different *B. subtilis* mutants indicates that AbnA and YxiA are the two major endo-arabinanases responsible for extracellular degradation of arabinan (C).



Characterization of plasma from patients suffering from Amyotrophic Lateral Sclerosis

Júlia Costa: Laboratory of Glycobiology

Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disorder characterized by a progressive loss of motor neurons (MN) in the motor cortex, brainstem and spinal cord. In 90-95 % of patients there is no apparent genetic linkage (sporadic ALS - SALS), but in the remaining 5-10 % of cases the disease is inherited in a dominant manner (familial ALS - FALS). The aetiology of the disease is unknown but several molecular approaches have led to the identification of genes that are differentially expressed in ALS. Among these are genes involved in antioxidant systems, regulation of MN function, lipid metabolism, protease inhibition and in inflammation and apoptosis processes. In ALS, neuro-inflammatory changes occur, as observed in other neurodegenerative diseases such as Alzheimer's disease. In particular, there is accumulation of reactive microglia and macrophages in the degenerating spinal cord. In ALS, MN present proteasome dysfunction, caspases 1 and 3 activation, Golgi fragmentation, neurofilament disorganization, impairment of axonal transport, protein aggregation and oxidative stress (Figure 1). Surrounding astroglial cells have in some cases impaired glutamate transport that can be toxic for the MN.

No biological marker has been identified in ALS either for diagnosis or to measure disease progression. Plasma analysis can be useful in the identification of amino acids or proteins that are deregulated in ALS. The Laboratory has characterised biochemically the plasma of a group of ALS patients, and compared this with healthy controls and patients with other chronic neuromuscular disorders associated with muscle atrophy. The most striking findings are the decrease in His and Ala concentrations and increase of Asn in the plasma of the ALS patients, as well as increased proteolytic processing of the acute-phase protease inhibitor α2-macroglobulin (α2-M). Furthermore, α2-M (intact subunit and proteolytic product) increased during disease progression for some patients. The results obtained suggested that diet supplementation with His and Ala and modulation of α2-M might have some beneficial effects on the course of ALS.

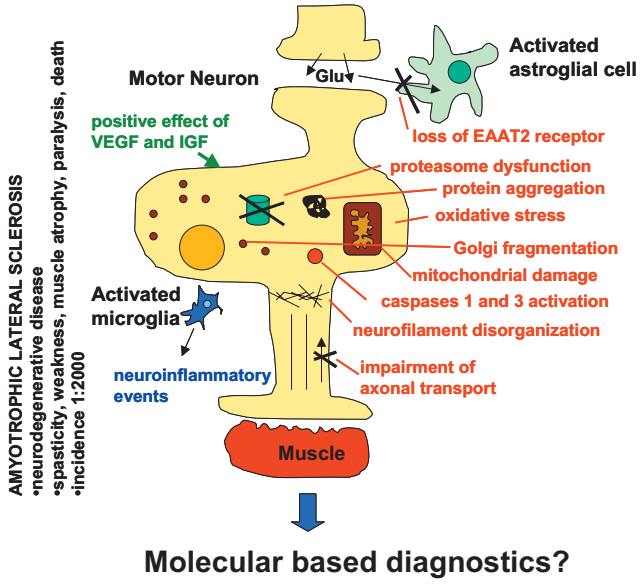


Figure 1 Cellular events associated with amyotrophic lateral sclerosis.

Palma, A., de Carvalho, M., Barata, N., Evangelista, T., Chicau, P., Regalla, M., Costa, J. (2005) *Amyotrophic Lateral Sclerosis and other Motor Neuron Disorders* 6, 104-110

PLANT SCIENCES DIVISION

PLANT SCIENCES DIVISION: Objectives and Activities

The Plant Sciences Division comprises 9 laboratories that perform basic research in plant development and response to stress. Studies of a more applied nature are also being carried out, in close association with IBET, aiming to solve problems of agronomic or industrial relevance to Portugal in important crops such as, rice, maize, lupin, grapevine, olive tree, almond, pine, cork oak and Eucalyptus. During 2005 the active involvement of the Division in the National Platform of Plant Genomics greatly contributed to the admission of Portugal in the ERA-NET Plant Genomics. The specific activities of the several laboratories are outlined below.

The **Plant Biochemistry Laboratory** centers its activity on the stress responses of plants by studying proteins and changes in metabolism. Making use of 2-D electrophoresis proteomic analysis has been applied to investigate; wounding in *Medicago truncatula* and potato slices, cork formation in cork oak stems, B deficiency in *Medicago* and *Arabidopsis*, fruit development in grapevine under drought, and light stress in *Chlamydomonas*. Protease inhibitors and protein phosphorylation during early stress responses continue to be studied. The biochemistry of lupin seed development has been analyzed. Using ^{13}C-NMR the metabolite profiles of cork oak under drought, pasture plants under salt stress and olive trees during a one year cycle, were monitored to obtain information on the metabolic alterations associated with stress. These studies will be continued during 2006.

The **Disease and Stress Biology Laboratory** studies the ubiquitin/proteasome pathway from plant cells. Given the permanent surveillance of the cells by proteolytic systems, which continuously monitor mature proteins for chemical damage, it is expected that arsenite (As), as well as any condition that alters the cellular redox state, results in an increased/decreased supply of protein substrates to the ubiquitin pathway by inducing/reducing oxidative damage imposed on the cell proteins. Three groups of studies were undertaken in *Lemna minor* exposed to As stress; expression levels of the components and intermediates of the ubiquitin/proteasome pathway, the influence of the cell redox state on the response of the proteome to arsenite, and the supply of protein substrates to the ubiquitin/proteasome pathway dependent upon the redox state of the cells. Considering that many neurodegenerative diseases that affect man (Parkinson and Alzheimer diseases and even ageing) accumulate inclusion bodies, essentially composed of ubiquitin-protein conjugates, a study will be initiated in 2006 on the effect of a variety of plant phenolic compounds, exhibiting antioxidant activity, on the ubiquitin/proteasome pathway of cultured primary neuronal cells with chemically induced neurodegeneration and brains from control, Parkinsonian and aged mice.

The **Plant Cell Biotechnology Laboratory** has been using different molecular tools to identify or to transform genotypes of plants of agronomic interest to improve them against different types of stress (biotic or abiotic) or to select appropriate germplasm for defined needs, such as resistance against diseases or wood quality for pulp and paper or cultivar discrimination. Different crops have been the object of study (legumes, maize, olive tree, grapevine, maritime pine and eucalyptus). New areas have started to be explored, such as the phylogenetics of specific plant groups. Finally, public intervention related to the adoption of transgenic crops has been maintained. During 2006 it is intended to continue developing strategies to transform *Medicago truncatula* towards drought tolerance, integrating knowledge from different genomic approaches. In addition, the efforts to develop associations between molecular markers and defined phenotypes for wood quality in maritime pine and eucalyptus will be continued. The Laboratory will continue to

be involved in the understanding of the genetic diversity and the genetic structure of the Portuguese *cultivars* of important crops such as maize, olive tree and grapevine.

The **Plant Cell Wall Laboratory** has completed in 2005 proteomic studies of cell wall proteins during leaf wounding in *Medicago*. An interesting aspect of this work has been the identification of wound-responsive apoplastic proteins intimately regulated by reactive oxygen species (ROS)–related signalling. In more recent work, the use of NADH oxidase inhibitors allowed the identification of further wall proteins apparently involved in ROS metabolism, ROS signal propagation in the cell wall during either normal growth, or in post-oxidative burst phases of stress. During 2006, these studies will be extended to Arabidopsis to enable further comparative studies with a range of known *Arabidopsis mutants* deficient in stress-related ROS signaling.

The **Plant Developmental Genetics Laboratory** has continued work on the role of Arabidopsis homologues of DIVARICATA, a gene encoding a MYB transcription factor required for normal petal growth in *Antirrhinum*. *Arabidopsis* NASC mutants in two DIV-like genes has no obvious morphological phenotype, even though they carry insertions in coding regions and express no detectable levels of mRNA. A double mutant, which will be compared to wild type with respect to growth, was therefore constructed to test for redundancy. In parallel, transgenic *Arabidopsis* lines expressing the Antirrhinum DIV gene were obtained. These lines may be of use for testing whether DIV can complement possible defects of double mutant *Arabidopsis* plants. Additional research relating to Genetic controls of plant growth is reported in the highlights.

The **Plant Genetic Engineering Laboratory** focuses on the improvement of important Portuguese crops making use of biotechnological tools. The current efforts are mainly directed towards almond and rice, but other crops are also studied, such as fig trees. Molecular biology strategies are used for plant characterisation, marker-assisted selection, gene search for the study of important agronomical processes, tissue culture, and genetic engineering as tools for crop improvement and gene functional studies. A strong focus is at present on rice, aiming to understand the role of transcription factors in rice adaptation to abiotic stress and for breeding Portuguese varieties supported by marker-assisted selection. Work in almond is presently mainly focused on gene functional analyses through genetic engineering. In collaboration with other laboratories work is also being conducted on protein analyses to evaluate the potential impact on human health of genetically modified foods and of fungi inhabiting human environments.

The **Plant Molecular Ecophysiology Laboratory** has developed in 2005 basic studies relating with mechanisms (physiological, biochemical and molecular) that govern plant resistance to environmental stress, namely drought, and mostly applied to *Vitis vinifera* and *Quercus suber*. Thermal imaging data obtained in *V. vinifera* and *Q. suber* during joint field investigations with the University of Dundee in the Summers of 2004 and 2005, is now being utilized to develop and test mathematical models for predicting leaf stomatal conductance. Research in aquaporins has been further developed. Different PIP and TIPS aquaporins have been cloned from grapevine berries and the Laboratory managed to obtain cell suspensions that will be used to study stress effects. Cooperation with a group of the Department of Biology, Universidade do Minho, was developed for the study of water transport across membranes. In July 2005, a POCI project was started in cooperation with the Biochemistry Laboratory of ITQB and with ISA on the metabolic regulation in leaves and berries following the imposition of drought in grapevines; this

project will last until 2008. In February and March 2006, Prof. Fernando Broetto (Universidade Estadual de São Paulo), will address the chilling effects on two contrasting genotypes of Eucalyptus globules, earlier characterized for drought resistance.

The **Plant Cell Biology Laboratory** is working with plants and suspension cell cultures of the model systems *Medicago truncatula*, *Arabidopsis* and tobacco, aiming to establish and characterise plant-based systems for the production of valuable recombinant proteins (e.g., biopharmaceuticals for treatment of human diseases and additive enzymes for animal feed). These transgenic systems are also being used as tools to understand fundamental aspects of the biology of the cell, particularly chromatin organization in different plant species and its role in the regulation of (trans)gene expression. This task will be facilitated once complete access to the genome sequence of several plants is achieved and the Laboratory becomes acquainted with many genes and proteins involved in RNA related factors and chromatin-mediated gene regulation. A project just initiated aims to unravel the processes that affect recombinant protein synthesis, accumulation and stability, and to identify ways of controlling these processes for production. A careful dissection of the pathway from the gene to the production and sorting of stable functional recombinant proteins, (which includes tracking the gene, epigenetic patterns, mRNA localization and stability, and sorting of functional recombinant products) will provide insights into the nuclear role in transgene expression and consequent controlled manipulation, and also help optimize plant expression systems for large-scale production of recombinant molecules. In addition, the know-how of such basic processes in transgenic plants may have a social impact on public concern about the use of genetically modified organisms for further applications, potential benefits vs. risks and environmental safety.

The **Forest Biotech Laboratory** focuses mainly on maritime pine, aiming to support pine production for reforestation, according to a breeding program that seeks to obtain plants better adapted to the Portuguese edaphoclimatic conditions. However, fundamental studies on the basic aspects of plant development, gene functional analyses, genetic stability, and genetic diversity in pine are also being conducted. Methods for cryopreservation and genetic transformation of maritime pine have been established and the genetic stability of the cultures analyzed. Transcribed *copia-retroelement* sequences in in vitro propagated material (somatic embryogenesis cultures and emblings, as well as other vegetatively propagated material) are being identified with the aim of inferring their activity under *in vitro* conditions (as the stress imposing conditions). Retrotransposon-based markers (SSAP) are presently being used to investigate genetic stability in vitro plants. Molecular work on *Quercus* species, mainly *Q. suber*, has also been started in the Laboratory.

PLANT SCIENCES DIVISION

Head of Division: Cândido Pinto Ricardo

Laboratory: Plant Biochemistry

Head of Laboratory: : Cândido Pinto Ricardo

Research Team:

Ana Isabel Faria Ribeiro	PhD
Ana Paula Ferreira Regalado	PhD
José António Pires Passarinho	PhD
Carla Maria Alexandre Pinheiro	Post-Doc
Ana Sofia Correia Fortunato	PhD Student
Inês Maria Silva Almeida Chaves	PhD Student
Marta Alexandra Marques Alves	PhD Student
Rita Maria de Brito Francisco	PhD Student

Publications 2005: 22, 33, 34, 144, 145, 148
Highlight: 129

Laboratory: Plant Developmental Genetics

Head of Laboratory: : Jorge Almeida

Research Team:

Lisete Galego	PhD

Publications 2005: 8
Highlight: 130

Laboratory: Plant Molecular Ecophysiology (LEM)

Head of Laboratory: : Maria Manuela C. C. F. Chaves

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Olga Grant	Post-Doc (Until May 2005)
Miguel Costa	Post-Doc (ISA/ITQB)
Maria Fernanda Galud	Post-Doc
Ana Rodrigues	Technical Assistant [(ISA) / Master Student (ITQB)]
Elisabete Vieira da Silva	PhD Student
Lukasz Tronina	PhD Student
Tiago Santos	PhD Student (ISA/ITQB)
Raquel Vale	PhD Student (ISA/ITQB)
André Pestana	PhD student (ISA/ITQB)

Collaborators:

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Maria Lucília Rodrigues	Investigator at ISA

Publications 2005: 33, 36, 47, 53, 54, 55, 86, 87, 145, 164
Highlight: 131

Laboratory: Plant Genetic Engineering

Head of Laboratory: : M. Margarida Oliveira

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Jayamani Palaniappan	Post-Doc
Maria Helena Teixeira Raquel Gonçalves	Post-Doc
Ana Maria Beirão Reis Sánchez	Post-Doc
Madalena Cristina Rocha Martins	Post-Doc (until June 2005)
Cristina Maria Neves Silva	PhD Student (until April 2005)
Ana Paula Martins Farinha	PhD Student (ITQB/CSIC-Barcelona)
Ana Margarida Santos	PhD Student
Tiago Lourenço	PhD Student
Sónia Negrão	PhD Student
Rita Batista	PhD Student INSA/ visiting scientist
Milene Costa	Researcher
Pedro Barros	Undergraduate / Graduate Researcher
Ossama Kodad	Visiting scientist (Oct. - Dec. 2005)

Publications 2005: 22, 40, 83, 84, 162, 171
Highlight: 132

Laboratory: Forest Biotech

Head of Laboratory: : M. Margarida Oliveira / C. Pinto Ricardo
(See Laboratories: “Plant Genetic Engineering” and “Plant Biochemistry”)

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Célia Maria Romba Miguel	Post-Doc
Sónia Cláudia Morgado Gonçalves	PhD Student / Post-Doc
Susana Isabel Lopes Tereso	PhD Student / Post-Doc
Liliana Maria Bota Marum	PhD Student
Jorge Cordeiro	Researcher (until Nov. 2005)
Ana Filipa Gonçalves Milhinhos	Graduate student
Marta Andreia Horta Simões	Undergarduate/Graduate researcher
Tânia Chaves	Undergraduate/Graduate (until Nov. 05)

Publications 2005: 83, 84, 89
Highlight: 133

Laboratory: Plant Cell Biotechnology

Head of Laboratory: : Pedro Fevereiro

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Susana Neves	Post-Doc
Vitória Gemas	Post-Doc
Changhe Zhang	Post-Doc

Jingsi Liang	PhD Student
Jorge Paiva	PhD Student
Sofia Duque	PhD Student
Susana Araújo	PhD Student
Isabel Raposo	Master Student
Catarina Fonseca	Postgraduate Student
Matilde Cordeiro	Graduate Student
Leonor Tomaz	Lab Technician

Publications 2005: 1, 7, 30, 35, 39, 56, 80, 90, 131
Highlight: 134

Laboratory: Plant Cell Wall

Head of Laboratory: : Phil Jackson (see also Associate Laboratory Section)
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Luis Filipe Sanchez Goulão	Post-Doc
Nelson Alexandre de Cruz Soares	PhD Student
Ada Dorotea Vatulescu	PhD Student
José Mario Ribeiro	PhD Student

Highlight: 66

Laboratory: Disease and Stress Biology

Head of Laboratory: : Ricardo Boavida Ferreira
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Zhenjia Chen	Post-Doc
Cláudia Nunes Santos	PhD Student
Ana Sofia Caeiro	PhD Student
Cristina Branco Price	PhD Student
Regina Luz Freitas	PhD Student
António Jorge Oliveira	Undergraduate Student

Publications 2005: 25
Highlight: 135

Laboratory: Plant Cell Biology

Head of Laboratory: : Rita Abranches (see also Associate Laboratory Section)
Research Team:

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Stefanie Rosa	PhD Student
Mariana Pereira	PhD Student
Marina Pedro	Graduate Student
Nuno Almeida	Undergraduate Student

Publications 2005: 1, 2
Highlight: 67

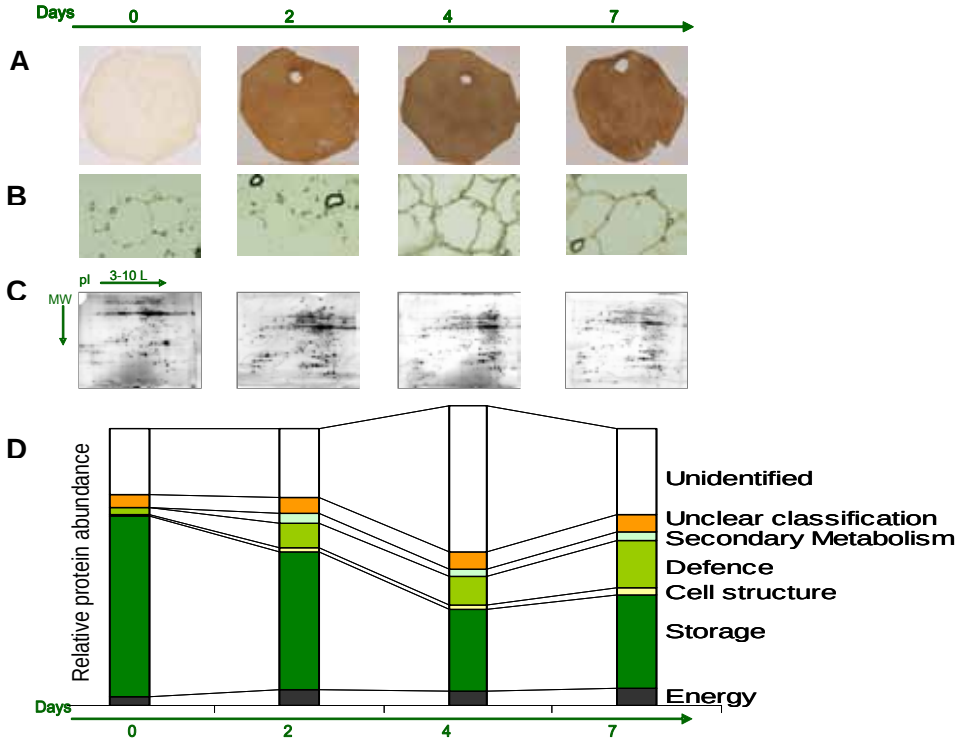
Proteomics of Wound-healing Mechanisms of Potato

Cândido Pinto Ricardo: Plant Biochemistry Laboratory

Due to their sedentary nature, plants are permanently under attack at the exposed surface layers. Consequently, to coat the external organs they have developed resistant epidermis/periderms, rich in cutin/suberin. Furthermore, plants have evolved very strong biochemical mechanisms that respond to wounding, contributing to the protection of organ integrity.

Potato (*Solanum tuberosum* L.) is a very important food crop consumed worldwide and adequate conservation of the tubers is of great economical relevance. Tuber ability to rapidly reconstruct the periderm, upon damage, is fundamental to guarantee long term storage. Excised potato tuber slices, when aged under moist conditions (A), reconstruct their periderm and are therefore a good model system to study the wound-healing biochemical processes. With that purpose, the Laboratory has been analyzing the proteins that are formed sequentially in aged slices, as they are the agents of the transformations that are taking place. Suberization is certainly of great significance since suberin deposition acts as a physical barrier against dehydration and microbial attack. Sudan IV, that stains red the polyaliphatic components of cell walls, evidenced suberin deposition from the 4th day after wounding (B).

The polypeptide patterns (C) obtained by two-dimensional gel electrophoresis were compared by image analysis, revealing the increase in protein complexity up to the 5th day from wounding. From an initial number of 179 polypeptide spots (at day 0) a total of 1121 spots could be visualized during the 7 days of the experiments. Polypeptides that were associated with the wound-healing process were excised from the gels and subsequently identified by peptide mass fingerprint (MALDI-TOF MS). A great diversity of proteins could be identified that were grouped into the several classes established in accordance with the physiological processes where they are implicated. A significant decrease in storage proteins was detected. These were probably utilized for the synthesis of proteins of the classes that increased during slice ageing, namely, those related to pathogenesis and stress defence mechanisms, secondary metabolism, energy metabolism, cell structure and some others of unclear classification (D).

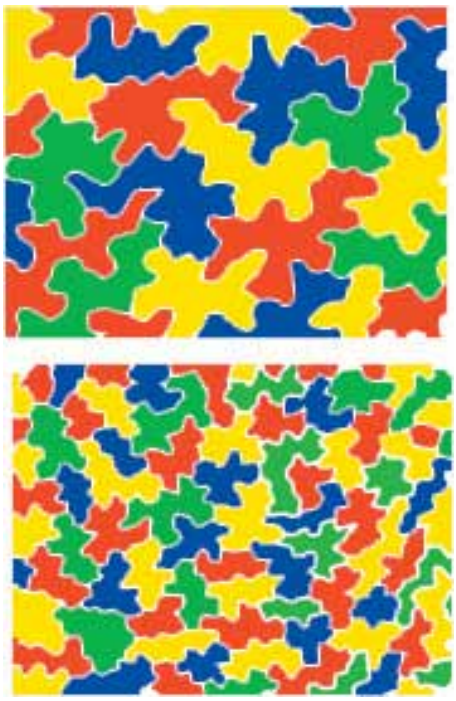


Genetic control of plant size

Lisete Galego and Jorge Almeida : Plant Developmental Genetics Laboratory

How the size of plants and of their organs is determined can be investigated by examining mutants in which normal growth is impaired. Dwarfs are one such type of mutant, characterized in general by abnormally short internodes but normal leaves and flowers. The Laboratory has identified a novel recessive mutant in *Antirrhinum* which, unlike dwarves, shows a generalized reduction in size relative to the wild type (shorter internodes, thinner stems and smaller leaves, fig. 1). In addition, mutant plants initiate leaves at a reduced rate, and completely fail to flower. Genetic analysis indicates that these apparently multiple effects are caused by mutation at a single locus, termed *par*.

Figure 1. Wild type (left) and *par* mutant (right)



Analysis of mature leaves shows that the size of epidermal cells in the mutant is reduced by more than 3-fold relative to that in wild type (fig.2). However, this accounts for only part of the difference in the final sizes of leaves, indicating that cell numbers must also be reduced in the mutant. This suggests that the *par* mutant is defective in both cell growth and division.

The mutant phenotype indicates that the normal *PAR* gene is a key determinant of shoot apical meristem behaviour and of organ growth. Insight into these fundamental aspects of Plant development might therefore come from further studies on *PAR*. Such studies could lead to applications in Plant breeding, a field in which size matters.

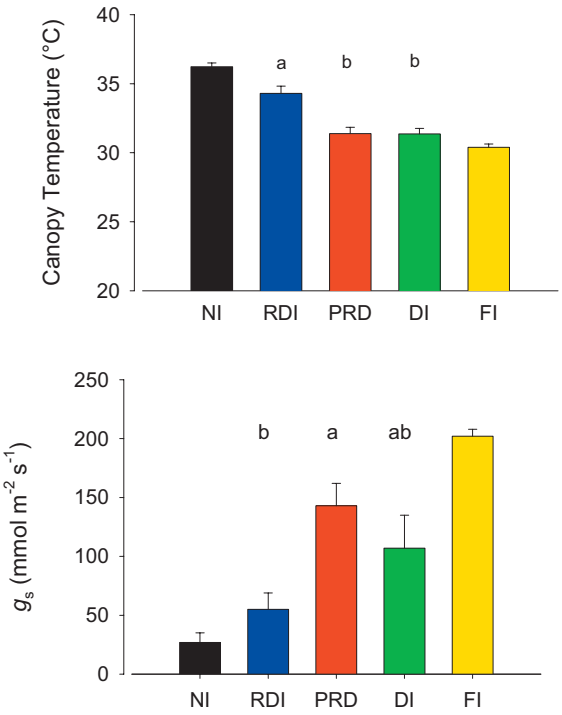
Figure 2. Epidermal cells in leaves of wild type (top) and of *par* mutant plants.

Thermal imaging successfully identifies water stress in field-grown grapevines

Manuela Chaves : Plant Molecular Ecophysiology Laboratory

Mean global temperatures are expected to rise over the next few decades, evaporation rates will increase, arid regions will expand, and thus water availability will be a major limitation to plant growth in the future (Houghton *et al.*, 2001). As a result, irrigation will become an increasingly common practice. However, an increase in the area under irrigation will only be possible if the quantity of water used per unit area is reduced, *i.e*. if plant water-use efficiency can be improved.

Deficit drip-irrigation strategies (DI) have been used to save water in viticulture and simultaneously improve wine quality (Dry *et al.*, 2001). Regulated Deficit Irrigation (RDI) aims to manipulate grapevine vegetative and reproductive growth by withholding or applying less than the full vineyard water use (as in a full irrigation system, FI) at specific periods of the growing season. Partial Rootzone Drying (PRD) is an alternative technique where only part of the root system is wet, allowing control of vegetative growth and transpiration, as a result of hormonal signals generated in dehydrating roots and transported to the shoot. PRD prevents the severe water stress periods that can occur in RDI season or in non-irrigated grapevines, NI (see Fig).



The Laboratory has found that temperatures of leaves or canopies can be used as indicators of stomatal closure (gs) in response to soil water deficit. In two years of field experiments with *Vitis vinifera* L. (cvs Castelão and Aragonês), it has been found that thermal imaging can distinguish between irrigated and non-irrigated canopies, and even subtle differences between deficit irrigation treatments. Canopy temperature is more sensitive to treatment than the temperature of individual leaves. A model to derive stomatal conductance from leaf temperature and *vice versa* is under construction.

Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA. 2001. Climate change 2001: the scientific basis. Cambridge University Press.

Dry PR, Loveys BR, McCarthy MG, Stoll M. 2001. Strategic irrigation management in Australian vineyards. *Journal International des Sciences de la Vigne et du Vin* **35,** 129-139.

Genomic tools to identify genes involved in adventitious shoot induction in almond

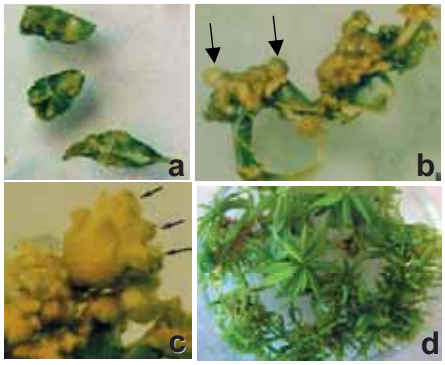
M. Margarida Oliveira : Plant Genetic Engineering Laboratory.

Rosaceae is perhaps the most important family of fruit trees, with a wide variety of species producing fruits commercialised all over the world. The main sub-groups of this family include the *Maloideae* (apple, pear, quince) and *Prunoideae* (peach, apricot, plum, cherry, almond). In most cases the function of genes isolated from *Rosaceae* has been estimated from comparisons with genes characterised in model species. This is due to the difficulty in obtaining mutants or genetically transformed plants that can be used to silence or over-express the gene under study and infer about its role. The use of genetic engineering in woody fruiting species is often hampered due to their reduced regeneration plasticity. In almond, the limiting step in transformation is not DNA transfer to the host plant cells, but plant regeneration from those cells.

Until very recently, adventitious organogenesis (Fig. 1) was mostly studied from a histological or physiological perspective, observing morphological changes in the tissues or obtaining empirical data based on trial and error experiments. Evaluating such a developmental process demands an extensive dissection at the gene expression level, to gain insight into molecular mechanisms determining “when” and “where” organogenesis is going to happen.

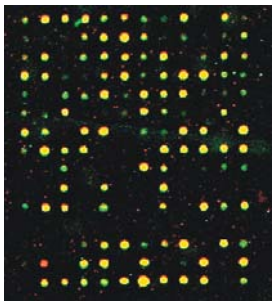
The Laboratory has targeted “de novo” shoot induction in almond using genomic tools. Two main strategies have been used; a candidate gene approach for the almond Knotted-1 and CDKA:1 (putative markers of organogenesis events) and a transcriptomic approach with micro-array technology (Fig. 2), using two suppression subtractive hybridisation (SSH) libraries constructed from two defined time frames of organogenesis (*Early* and *Late* induction stages), to screen and discover novel markers. Several candidate genes have been identified and studied by Real-time PCR expression analyses. Almond Knotted-1 gene was found to strongly increase expression after day 10, just before meristemoids become visible and it may be a good marker for regeneration. Micro-arrays have also revealed a number of interesting genes for further studies (Fig. 3). The molecular data of regeneration events may pinpoint solutions to increase regeneration yield and genetic transformation efficiency.

1



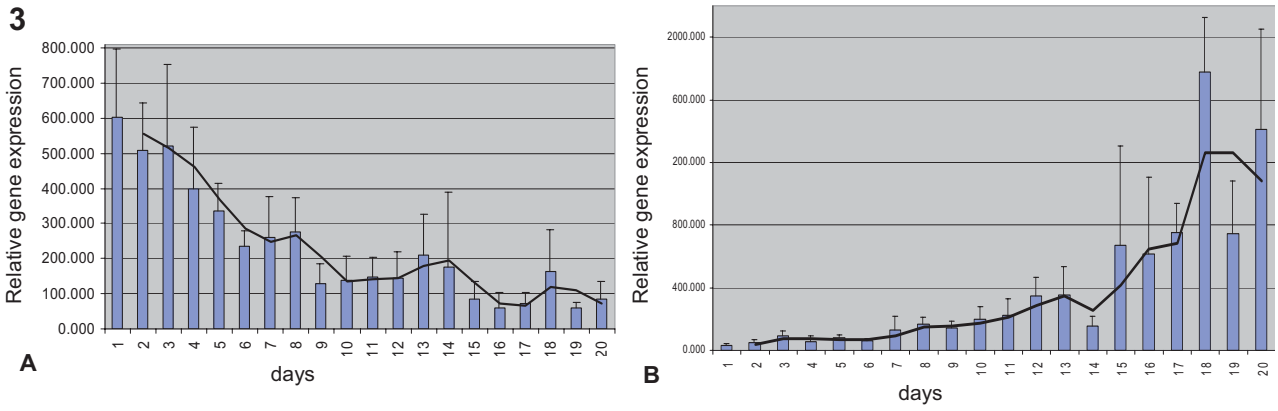
Almond leaves (a) induced to regenerate adventitious shoots under appropriate culture conditions. Globular structures (b) are produced from meristemoids, and shoot buds (c) emerge from them. Regenerated shoots (d) are isolated and sub-cultured on micropropagation medium.

2



Almond cDNA micro-array analysing genes differentially expressed in early (green) and late (red) organogenesis. The micro-array was prepared using robotic printing of cDNAs (labeled green or red) obtained from suppression subtractive hybridization libraries, built from early or late stages of shoot induction. Yellow spots indicate genes with no differential expression. Micro-arrays were prepared at USDA-ARS, Lubbock, Texas (USA) with the collaboration of Dr. Mel Oliver.

3

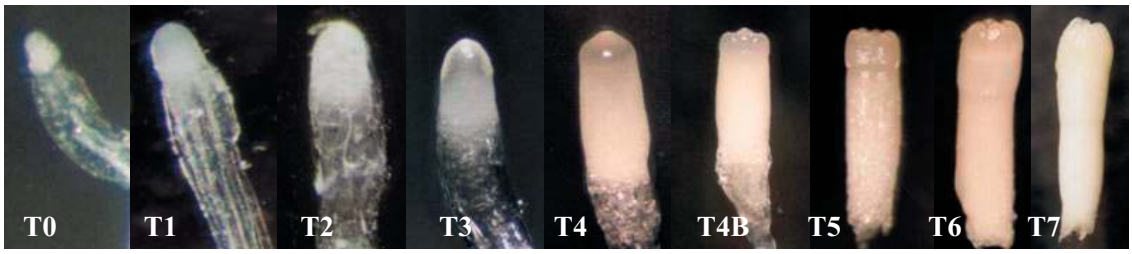


Two candidate genes identified from micro-arrays, for Early (A) and Late (B) organogenesis, as confirmed from their expression profiles (assessed by quantitative RT-PCR) along the 20 days of induction (**A** – *Auxin down regulated gene*, with and expression 3-fold higher in the first 8 days of induction; **B** – *β-1-3 glucanase gene*, with an expression more than 6-fold higher in days 15-20 as compared to 1-14).

Characterization of a Rab GTPase differentially expressed during pine embryogenesis

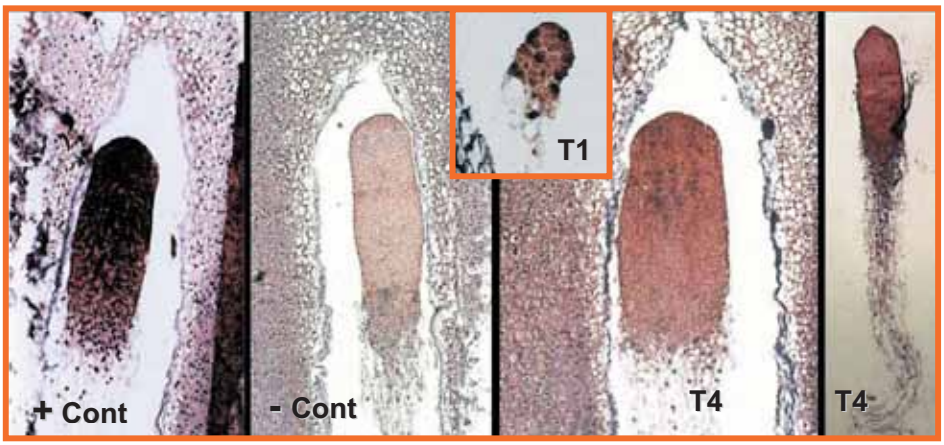
M. Margarida Oliveira and C. Pinto Ricardo : Forest Biotechnology Laboratory

Embryogenesis is a complex process that requires regulation of genes within the embryo proper and neighbouring seed tissues surrounding the embryo. Molecular studies of expression and characterization of genes involved in early and middle stages of zygotic embryogenesis of *Pinus pinaster* have been conducted. Using differential display over nine stages of embryo development, a GTPase transcript belonging to the Rab family was identified as up-regulated in early stages of embryo development.



Stages of embryo development (T0-T7) used for gene expression analysis

Rab-related small GTP-binding proteins are known to be involved in the regulation of the vesicular transport system in eukaryotic cells. The deduced PpRab1 protein of 202 amino acid residues contains the G1-G5 conserved domains of the GTPase superfamily and the double cysteine motif in the COOH-terminal. Phylogenetic analysis shows that, within the Ras superfamily of GTPases, PpRab1 is more closely related to the Rab family and within this, the PpRab1 protein was found to cluster with *Arabidopsis* subfamily AtRABE, whose members are known to regulate ER-to-Golgi membrane trafficking steps. Expression analysis of the *PpRab1* transcript by real-time PCR revealed that *PpRab1* expression is at a constitutively high level in early stages of zygotic embryo development, and then decreases as the embryo matures. The *PpRab1* transcript is not embryo-specific and an increase in *PpRab1* expression level is observed when seeds are germinated and collected at successive time points of development. *In situ* analysis reveals an expression signal in early zygotic and somatic embryos. Functional studies are underway to clarify PpRab1 protein role during embryogenesis.



Spatial localization of *PpRab1* transcript in maritime pine embryos by *in situ* RT-PCR

Transgenic plants to avoid drought

Pedro Fevereiro : Plant Cell Biotechnology Laboratory

Several genetic engineering approaches have been endeavored, with varied levels of success, aiming to increase the various osmoprotectant substances in plants. One of these is trehalose, a disaccharide whose accumulation is considered to confer protection against multiple abiotic stresses.

The *Arabidopsis thaliana* AtTPS1 gene was used to produce transgenic tobacco plants that accumulate the protein trehalose-6-phosphate synthase (fig 1). Three lines showing different levels of expression were used to verify their ability to withstand water deficit. The photosynthetic response to water withdrawal, using the modulated chlorophyll *a* fluorescence showed that one of the lines is more tolerant to drought.

At a soil water content below 12-15 % the Fv/Fm values of B5H plants decrease less than those observed in WT or B1F plants (fig 2). The quantum yield of leaves adapted to darkness (Fv/Fm) reflects the potential quantum efficiency of photosystem II and is a sensitive indicator of the plant photosynthetic performance. It seems that, when subjected to advanced drought stress, B5H plants have better levels of photosynthetic performance when the soil water content is less than 20 %. Under high actinic light the effective quantum yield of PSII (ϕPSII) in both transgenic lines seems to maintain higher effective quantum yields than the wild type. ϕPSII is a measurement of the efficiency of PSII photochemistry. It is concluded that the efficiency of PSII photochemistry is higher in the transgenic lines than in wild type plants. This seems to indicate that transgenic plants have been developed with an increased resistance to drought.

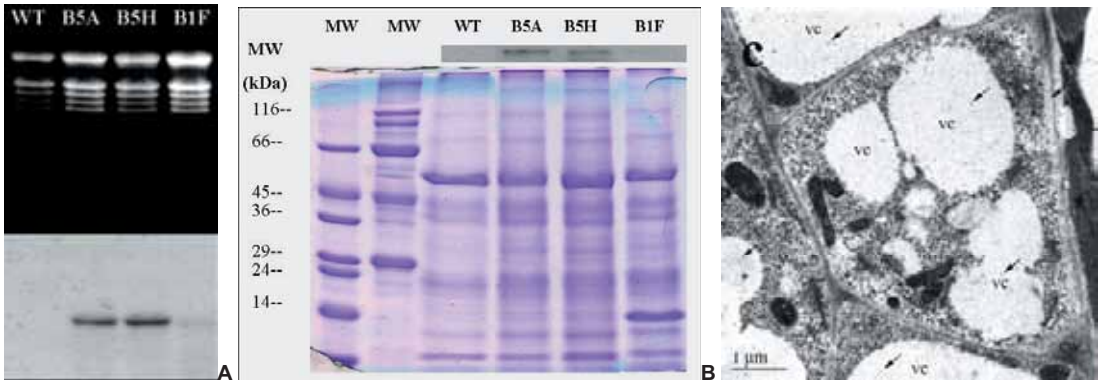


Figure 1 - A - northern blot analysis of AtTPS1 gene expression in the T2 generation of transgenic tobacco plant lines. B - western blot analysis of T2 generation transgenicplant lines. C Immunolocalization of *At*TPS1 in tobacco phloem cells

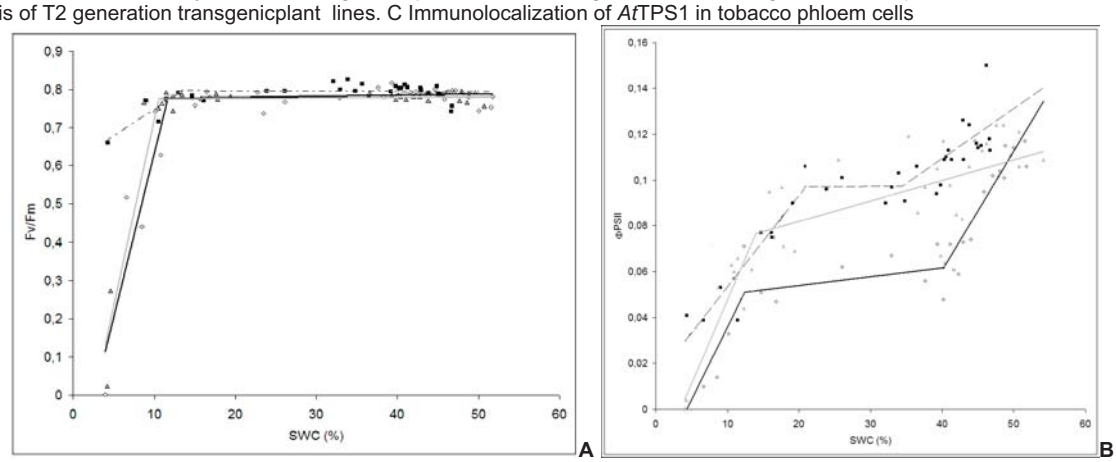


Figure 2 – A - Quantum yields of a leaf adapted to darkness (Fv/Fm) of Wild Type (◊), B5H (■) and B1F (▲) plants. B - Effective Quantum Yield of Photosystem (Φ_{PSII}) at an actinic light of 1850 µmol photons.m^{-2}.s^{-1} of Wild Type (◊), B5H (■) and B1F (▲) plants.

The supply of protein substrates to the ubiquitin/proteasome pathway depends upon the redox state of *Lemna minor* cells

Ricardo Boavida Ferreira : Disease and Stress Biology Laboratory.

Stresses are typically oxidative in nature and usually lead to enhanced levels in the activity of the ubiquitin/proteasome pathway, as judged by increments in the cellular pool of its intermediates. Given the permanent surveillance of the cells by the proteolytic systems, which continuously monitor mature proteins from chemical damage, it is expected, from a theoretical point of view, that arsenite (As), as well as any condition that alters the cellular redox state, results in an increased/decreased supply of protein substrates to the ubiquitin pathway by inducing/reducing oxidative damage imposed on the cell proteins.

Using As stress in *Lemna minor* as a model system, the effects on the levels of ubiquitin-protein conjugates were assessed by using a variety of compounds that influence the cell redox state, either by altering [inducing (*N*-acetylcysteine, NAC) or inhibiting (buthionine sulfoximine, BSO)] glutathione synthesis, acting as reactive oxygen species scavenger (dimethylsulfoxide, DMSO, and NAC) or simply by their antioxidant activity (glutathione, GSH, and dithiothreitol, DTT).

As a whole, the data obtained highlight the important role played by the ubiquitin/proteasome pathway in the response of *Lemna* to As. The great intensification of the response, as judged by a high amount of ubiquitin conjugates, when GSH levels are depleted, suggests the participation of this thiol in As detoxification. Unlike reducing agents, scavengers for reactive oxygen species minimized the As-induced accumulation of conjugates. In particular, NAC was capable of dramatically reducing the ubiquitin conjugates to levels well below the control. The results obtained with GSH, NAC and DMSO hint that reactive oxygen species may be the true inducers of the ubiquitin-protein conjugates, presumably via an increased availability in protein substrates.

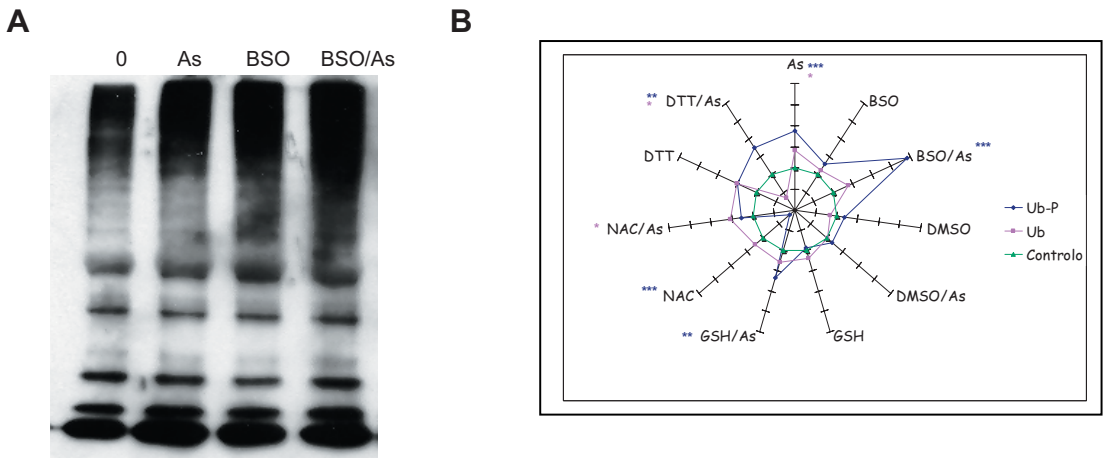


Fig. 1. Involvement of the Ub/proteasome pathway in the response of *Lemna minor* to arsenite. Plants of *L. minor* were exposed to 50 μM $NaAsO_2$, with or without a previous treatment with a variety of compounds that influence the cell redox state. Samples were collected and the total protein fraction extracted, fractionated by SDS-PAGE, blotted onto a membrane and probed with anti-Ub antibodies (**A**). The levels of free Ub (—■—) and of the high molecular mass Ub-protein conjugates (—◆—) were determined by image semi-quantitative analysis with Scion Image program (**B**). Data are shown as the percentage of pixels (arbitrary unit) measured relative to the control (100%). The mark (*) indicates a significant difference against the control value (—▲—) at $p<0.05$ for the Ub-protein levels. No significant differences at $p<0.05$ were observed for the free Ub levels. The results are the averages of three independent experiments and are represented as the mean ± standard deviation

TECHNOLOGY

TECHNOLOGY DIVISION

TECHNOLOGY DIVISION: Objectives and Activities

The Technology Division encompasses Engineering Sciences related to chemical and biochemical systems as well as some components in Microbial and Enzyme Technologies related to foods, 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.

In the **Animal Cell Technology Laboratory**, research and development has been conducted for complex biopharmaceuticals (vaccines, gene therapy and cells for therapy and regenerative medicine). Three unifying themes are present: (i) process integration (up and downstream), (ii) kinetics of infection (adenovirus and retrovirus in mammalian, including human cell lines, baculovirus in insect cells) (iii) systems biology approaches. Highlights for 2005-2006 include modelling RLP production, infection, gene expression and assembly (a collaboration with Prof Rui Oliveira at FCT-UNL), cell handling and cell programming using surface interaction leading to differentiated cells for medical therapies (CellPROM), and studies on the relationship between retroviral vector (RV) membranes and RV stability.

The **Physiology of Environmentally Conditioned Microbiota Laboratory**, has developed its major research interests, contributing to a deeper understanding of the microbial phenomena which sustain the well being of people and of the environment. Research has followed 3 main themes; 1) *Oenococcus oeni* surviving mechanisms, which are correlated to the mechanisms of biogenic amine formation in wine and the understanding of key environmental factors which may control and influence their formation, 2) The dynamics of specific fungi communities and the identification of key environmental/ stress factors which control the diversity of the active population, and 3) environmental mycology. With respect to the second theme, most of the work during 2005 has focused on the development and optimisation of phenotypic and molecular methods to analyse the diversity of the fungi species. Moreover, the main mechanisms used by these fungi to transform chlorophenol to chloroanisole are being analysed following the accumulation of the main reaction intermediates produced under specific environmental conditions. The third theme Environmental Mycology, under the responsibility of Cristina Silva Pereira, is the most recent addition to the research portfolio. Several aspects were pursued initially to build a strong basis of fundamental data and technical expertise. Studies analyzed fungi biodegradability of different substrates, aiming to correlate this data with fungi bioremediation of persistent organic pollutants, and also to understand some of the process behind fungi adaptation to extreme, ionic based, environments.

The **Nutraceuticals and Controlled Delivery Laboratory**, conducts research on the development of clean processes, involving alternative green technologies for the isolation of bioactive concentrates from natural sources, development of new functional foods/nutraceuticals, and preparation of improved delivery systems, and exploiting new solvent systems such as supercritical fluids. Laboratory and pilot plant high-pressure equipment are being used to extract and isolate products with high added-value and application in the food, cosmetic and pharmaceutical industries. The main activities are very interdisciplinary, hence several collaborations with different research groups with expertise in distinct areas have been established. In collaboration with the Animal Cell Biotechnology Laboratory, Instituto Gulbenkian Ciência and Faculdade de Farmácia, UL, the biological properties of isolated natural extracts are assessed at a cellular level in

appropriate cell lines searching for protection responses to environmental stresses and cell death signals. Anti-inflammatory response, immuno induction and/or suppression, and anticholesterolemic effects are also studied.

In the field of materials science and particle engineering, the impregnation, encapsulation and micro/nano-scale particle formation using non-conventional techniques, namely methods that use supercritical carbon dioxide, are being developed. Polymeric and lipid-based formulations with specific properties are being prepared, so that they can act as effective pharmaceuticals/nutraceuticals delivery systems with application in the pharmaceutical industry.

Alternative clean processes for the impregnation of some polymeric matrixes (namely contact lenses) with ophthalmic active drugs have been developed and patented. A new semi pilot-scale apparatus for particle formation by rapid crystallisation using supercritical CO2 as an anti-solvent (SAS method) has been constructed. Experiments on the preparation of microspheres impregnated with bioactive compounds are being performed using anti-inflammatory drugs and ethylcellulose, methylethylcellulose, PEG and PLA as carriers.

The **Microbiology of Transformation and Conservation of Olives and Other Plant Products Laboratory**, directs its activities towards table-olive fermentation, according to INIAP priorities. Two main lines of research are pursued in this laboratory, (1) technological aspects of table olive manufacturing; and (2) study and applications of *Lactobacillus* metabolites. Both lines are related since *Lactobacillus* strains are of utmost importance in table-olive fermentation processes, and some strains are bacteriocin producers. Their ecological importance has been evaluated by testing the use of these strains as starters. The influence of olive composition, technological aspects, and metabolites from different bacterial populations on bacteriocin production has been evaluated as part of the overall knowledge about table olive fermentation, which will help to control the process. The next objective is to control the production of bacteriocins by altering physical parameters or by slightly adjusting brine composition. Routine methods to screen bacteriocin producers have been established. Some bacteriocin producers were selected for further studies, mainly based on the stability and antimicrobial spectra of their bacteriocins. Some of these compounds were found to be active against bacteria that carry multiple resistances to antibiotics. Methods for the purification and characterization of bacteriocins and other inhibitory compounds have been developed.

During the current year the Laboratory expects to develop studies on obtaining a probiotic or derived component anti-*Helicobacter*, from *Lactobacillus*: *Lactobacillus* spp. Efforts will be made to determine the active compounds. Live bacterial strains and respective active extracts will be patented, aiming at future applications in the treatment of *H. pylori* infections. A second project deals with the valorisation of wastewaters trough the recovery of valuable compounds, such as β-hydroxytyrosol (for applications in the pharmaceutical industriy) and manitol (to be used as an additive in food industries).

The research of the **Mass Spectrometry Laboratory** can be divided in two fields;

(1) studies on the composition and characterization of protein and protein complexes. The Laboratory has been involved in projects that include the use of MS methodologies for protein identification and for the characterization of post-translational modifications. Some studies on the composition of MALDI matrices with the aim of improving the ionisation process have been performed. MS based methods have also been used in protein-protein interactions studies, namely H/D exchange and plasmon resonance.

(2) studies on chemical systems with particular relevance to the food and pharmaceu-

tical industries. The work has included the development and optimisation of MS methods for the characterization of particular types of chemical compounds, namely macrocycle-guest complexes, organo-metallics, and ionic liquids, and for the quantitative determination of antioxidants.

The **Microbiology of Man-Made Environments Laboratory** has continued its research in aspects related to food safety, especially on the impact of the presence of certain genus of bacteria in traditional food products. The study of microbial community evolution, related to food products and water, has been one of the main areas of activity with the development and implementation of new laboratory tools to be applied in waste water remediation models and in food models. The work of the Microbiology Laboratory of the Good Laboratory Practices Unit of IBET continues working on GMO (genetically modified organisms) detection and quantification for the Portuguese government and private companies. A strong investment in terms of development and validation of bioassays for the pharmaceutical industry has been carried out by the Good Laboratory Practices Unit.

The **Biosensors/Biomolecular Diagnostics Laboratory** works on the development of molecular diagnostics, mainly for veterinary applications (ELISA; Reverse Line Blot, immunosensors) as well as in the development of devices for bioprocess monitoring. Diagnostic ELISA (*Theileria* sp, *Babesia* sp) as well as optical immunosensors -based on the cy5 fluorophore- have been developed (*Brucelosis* sp, *Anaplasmosis* sp.). With respect to the development of veterinary diagnostics, studies for identification of antigenic proteins of *Theileria uilenbergi*, *Babesia bovis* and *B. bigemina* have been performed, followed by the characterisation and purification of these proteins. In addition, these parasites have been cultured in erythrocyte cells, in order to produce biological material without limitations. These parasite cultures are the subject of microfluidic experiments in microchips, taking advantage of di-elecrophoretic and capacitive spectroscopic techniques (in collaboration with LEISTER and EPFL/Switzerland) for cell handling and manipulation. For bioprocess monitoring a fibre optic sensor for measurement of dissolved oxygen is under development, in collaboration with the Optoelectronic group, INESC/Porto, as well as a device for measurement of cellular density in fermentors, in collaboration with the UNICAM/UNL.

The **Antibiotic Stress and Virulence of *Enterococci* Laboratory**, has found incomplete VanA and VanB operons in many *enterococccal* isolates from environments differentially stressed by the use of vancomycin, in particular, and antibiotic therapy, in general. By using MLST, implemented in the laboratory for *E. faecalis* during the 2005, studies will be continued through 2006 aimed at obtaining a picture of the course of these operons amongst enterococcal communities around the world. Vancomycin tolerance will continue to be studied in the Laboratory isolates using a proteomic approach, to be complemented with microscopic and biochemical studies. Simultaneously, characterization of the state of antibiotic resistance in isolates that are continuously being sent by different environmental sources, will be continued. The virulence factor gelatinase, present in half of the isolates, is regulated by a two-component regulatory system coded by the fsr operon. The role of this operon in virulence of non-clinical isolates and the way it is regulated will also continue to be a subject of future studies, by constructing mutants and using *C. elegans* as the model for virulence tests. The regulation at the RNA level will also be a part of this study.

The **Processes in Supercritical Fluids Laboratory** has performed research on Clean Technologies using high-pressure carbon dioxide. The main strategy has been to combine carbon dioxide with liquid solvents or liquid chemical reactants to obtain enhanced extraction and purification capacities or control of reaction rates or selectivities.

The **Molecular Thermodynamics Laboratory** has focused major attention during 2005 on the new emerging area of Ionic Liquids as potential substitutes to common volatile organic compounds. The major achievement has been the discovery that these liquid salts can be distilled without decomposition. This finding has opened a whole new vista regarding these compounds. For 2006 extensive studies on the interaction between ionic liquids and aqueous macromolecular solutions will be performed aiming at understanding the influence of ILs on aggregation and micellar phenomena. Solubility studies of polymers in ionic liquid media will be initiated. Another family of "green" solvents – perfluoroalkanes and pefluoralcohols - will also be deeply investigated.

The **Pharmacokinetics and Biopharmaceutical Analysis Laboratory** is involved in the study of the bioavailability and metabolism of pharmaceutical compounds and in the development and application of models for establishing *in vitro/in vitro* correlations. The target compounds are mainly prodrugs and work is undertaken in cooperation with the Portuguese pharmaceutical industry and academic partners either from within or outside ITQB. The Laboratory is also devoted to the development of analytical separation methods, particularly capillary electrophoresis and liquid chromatography, for biopharmaceutical compounds.

The aim of the **Biomathematics Laboratory** is the development of new methodologies for the quantitative analysis of biological systems, from the storage and description of biological data, to its modelling, simulation and analysis. In the Life Sciences there is an increasing need for quantitative modelling and descriptions of the biological and biochemical phenomena that surround us, in order to understand living organisms as a whole and not simply as the sum of their individual parts. In addition, the efficient development of novel drugs and biotechnological products crucially depends on the capability to collect and analyze the vast amount of information involved in such endeavours. The research activities of the group comprise non-linear time series analysis, biological sequence analysis, biofilm modelling, molecular epidemiology, gene and metabolic regulatory networks and biological clocks. Besides collaborations with groups at ITQB, such as the Molecular Genetics and the Cell Physiology and NMR Laboratories, research is conducted within the framework of various projects funded by the European Union, and in collaboration with several national and international institutions such as Instituto de Medicina Molecular (IMM), Instituto de Engenharia de Sistemas e Computadores (INESC), The University of Texas MD Anderson Cancer Center, National Center for Scientific Computing of Brazil, Universitat Politècnica de Catalunya (UPC), Universidade de São Paulo (USP), Technical University of Delft (TU Delft) and Pontífica Universidade Católica do Rio de Janeiro (PUC-Rio).

The **Pilot Plant** develops activities divided into three different categories; support for other research groups, providing production of biological material in larger quantities, and research and development and contract services. During 2005 the construction of a production area for operation according to Good Manufacturing Practices (GMP) has been initiated, and will be equipped with bioreaction and purification equipment providing a complete service in the scope of new biopharmaceuticals development, from the R&D

stage to the production of batches for preclinical and clinical trials. The research and development work will be performed mainly in the bioreaction area. The development of hybrid models for bioprocesses applied to bioreaction process control and optimisation, in collaboration with Sebastião Feyo Azevedo (ISR/FEUP) and Rui Oliveira (FCT/UNL) is one of the main areas of research, being the model development performed using a *Pichia pastoris* strain expressing a recombinant antibody fragment. Development of new vaccines for strangles in collaboration with António Almeida (FF/UL), and the development of starter cultures of table olive fermentation in collaboration with Cidália Peres (INIAP), Maria Amália Peito (INETI), Apafna, and Probeira has continued. Contract services, namely with Schering, Merck and LETI has been an important part of the work performed, with the collaboration of several ITQB Laboratories, namely the Animal Cell BioTechnology, Macromolecular Crystallography and Mass Spectrometry Laboratories and the GLP Services.

TECHNOLOGY DIVISION

Head of Division: Luis Paulo Rebelo

Laboratory: Bionsensors

Head of Laboratory: Abel Oliva
Research Team:

Hélder Cruz	Post-Doc (part time)
Joana Paiva Miranda	PhD Student
Marta Silva Gomes	PhD Student
Elisabete Nascimento	M.Sc. Student
José Valério Palmeira	M.Sc. Student
Óscar Silvestre	Graduate Student
Miriam Azevedo	Undergraduate Student

Publications 2005: 95
Highlight: 151

Laboratory: Pharmacokinetics and biopharmaceutical analysis

Head of Laboratory: Ana Luísa Simplício (see also Associate Laboratory Section)
Research Team:

Hugo Serra	PhD student

Publications 2005: 79
Highlight: 64

Laboratory: Mass Spectrometry

Head of Laboratory: Ana Maria Varela Coelho
Research Team:

Alexandre Campos	Post-Doc (Inst. de Biologia Molecular, Barcelona, Jul 2005)
Gonçalo Conde da Costa	PhD Student (ITQB/IGC)
Judite Maria Marques Dias	PhD Student (ITQB/INSA-Inst Ricardo Jorge, until Nov. 05)
Patrícia Gomes Alves	PhD Student (ITQB/INSA-Inst Ricardo Jorge)
Sérgio Mota	PhD Student (ITQB/IHMT-UNL)
Marta Lavoura Mendes	Master Student (ITQB/IHMT-UNL)
Catarina Ferraz Franco	Graduate Student
Gonçalo Graça	Undergraduate Student (until October 2005)
Cíntia Penque Vicente	Undergraduate Student (until July 2005)
André Lopes	Undergraduate Student (since May 2005)
Elisabete Pires	Technician

Publications 2005: 150, 172, 185
Highlight: 152

Laboratory: Nutraceuticals and Delivery

Head of Laboratory: Catarina Duarte

Research Team:

Ana Rita Cruz Duarte	PhD Student
Ana Raquel Sampaio de Sousa	PhD Student
Ana Alexandra Figueiredo Matias	PhD Student
Ana Vital Morgado Nunes	PhD Student
Ana Teresa Serra	PhD Student
Mariana Sousa Costa	PhD Student
Carlos Tiago Cravo	Graduate Student (BIC)

Publications 2005: 42, 62, 63, 172

Highlight: 153

Laboratory: Food Microbial Technology

Head of Laboratory: Cidália Peres

Research Team:

Mª Dulce Brito	Researcher
Amélia Delgado	PhD Student
Cristina Serrano	Research Technician
Luís Catulo	Technician
Luísa Reis	Lab Technician

Publications 2005: 38, 56, 57

Highlight: 154

Laboratory: Antibiotic Stress and Virulence of Enterococci

Head of Laboratory: Fátima Lopes (see also Associate Laboratory Section)

Research Team:

Paulo Marujo	Post-Doc
Tânia Ribeiro	PhD Student
Frédéric Gaspar	PhD Student
Vera Pinto	Master Student
Marta Ruivo	Master Student
Neuza Teixeira	Master Student

Publications 2005: 110

Highlight: 62

Laboratory: Biomathematics

Head of Laboratory: Jonas Almeida

Research Team:

Antonio Maretzek	PhD , System administrator
Andreas Bohn	Post-Doc
João Xavier	Post-Doc
Susana Vinga Martins	Post-Doc
Francisco Pinto	PhD

Sara Garcia	PhD
João André Carriço	Graduate Student
Helena Deus	Undergraduate Student
Pedro Eleutério	Undergraduate Student
Dominick Beck	Visiting Undergraduate Student

Publications 2005: 9, 10, 32, 75, 77, 78, 88, 116, 117, 120, 123, 133, 143, 147, 170, 174, 177, 190, 192, 193
Highlight: 155

Laboratory: Molecular Thermodynamics

Head of Laboratory: Luís Paulo N. Rebelo
Research Team:

José Canongia Lopes	Prof. Aux. Convidado (since August 2005)
Joanna Łachwa	Post-Doc
José Esperança	Post-Doc
Zoran Visak	Post-Doc
Marijana Blesic	PhD Student
Joana Trindade	Undergraduate Student

Publications 2005: 37, 50, 51, 103, 109, 127, 128, 154, 155, 179
Highlight: 156

Laboratory: Analytical Chemistry

Head of Laboratory: Luís Vilas Boas and Rosário Bronze
Research Team:

Valentim Ribeiro de Almeida	Post-Doc
Antero Ramos	Research Technician
Maria Nubélia Bravo	PhD Student
Rodrigo Daniel Feliciano	PhD student
Ludovina Galego	PhD student
Alberto Alexandre Mosqueiro	Master Student
José Alexandre Dias	Master Student

Publications 2005: 150, 172
Highlight: 157

Laboratory: Animal Cell Technology

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

Pedro E Cruz	PhD , senior researcher
Ana Sofia Coroadinha	PhD , researcher
Helena Vieira	Post-Doc
Maria João Barbosa	Post-Doc
Cláudia Istrate	PhD Student
Isabel Marcelino	PhD Student
Marlene Carmo	PhD Student

Sónia Santos	PhD Student
Teresa Rodrigues	PhD Student
Tiago Ferreira	PhD Student
Rita Malpique	PhD Student
António Roldão	PhD Student
Leonor Norton	PhD Student
Maria Candida Mellado	PhD Student
Teresa Serra	PhD Student (Laboratory of Nutraceutics & Delivery)
Catarina Esteves	Master Student
Cristina Peixoto Lisboa	Research Technician (Purification)
Marcos Sousa	Research Technician (Bioreaction)
Maria do Rosário Clemente	Research Technician (Cell Bank & Analyticals)
Ana Carina Silva	Research Student
Margarida Serra	Research Student
Ana Isabel Amaral	Research Student
Sofia Leite	Research Student
Ana Catarina Pereira	Research Student
Ana Lúcia Ferreira	Research Student
Ricardo Perdigão	Undergraduate Student
Ana Teixeira	PhD student (ITQB /FCT-UNL)
Carla Portugal	Post-Doc (ITQB/FCT-UNL)

Publications 2005: 68, 69, 70, 72, 100, 111, 124, 135, 139, 163, 175, 180, 189
Highlight: 158

Laboratory: Processes with Supercritical Fluids

Head of Laboratory: Manuel Nunes da Ponte
Research Team:

Joana Fonseca	Post-Doc
Vesna Najdanovic-Visak	Post-Doc
Ana Serbanovic	PhD Student

Publications 2005: 24, 37, 50, 103, 121, 127, 128, 155, 169
Highlight: 159

Laboratory: Microbiology of Man-maid Environments

Head of Laboratory: Maria Teresa Crespo
Research Team:

Gilda Carvalho	Post-Doc
Vanessa Pereira	Post-Doc
Helena Isabel dos Santos	PhD Student
Cristina Isabel Pereira	PhD Student
Frédéric Gaspar	PhD Student
Paula Isabel Alves	Research Technician

Publications 2005: 110
Highlight: 160

GLP Unit

Fernanda Spínola Rodrigues
Cátia Morgado Peres
Sandra Marina Martins
Susana Tenedório

Laboratory of Physiology of Environmentally Conditioned Microbiota

Head of Laboratory: Maria Vitória Gonçalves San Romão
Research Team:

Cristina Silva Pereira	Post-Doc (Sept 2004)
Sónia Vitorino	PhD Student
Maria do Carmo Basílio	PhD Student
Ana Paula Marques	PhD Student
Mariana Carvalho	PhD Student
Ricardo Gaspar	Graduated Student
Isabel Martins	Graduated Student
Maria Cristina Jorge Leitão	Research Technician

Publications 2005: 106
Highlight: 161

Pilot Plant

Head: António Cunha
Pilot Plant Team:

Rui Gomes	M.Sc. (Fermentation)
Sandra Monteiro	M.Sc. (Fermentation)
Filipe Pinto	B.Sc. (Fermentation)
Monica Thomaz	B.Sc. (Purification)
Sandra Miranda	B.Sc. (Purification)
Lídia Gonçalves	PhD (Quality Assurance)
João Clemente	B.Sc. (Measurement & Control)
Catarina Azevedo	Secretariat
Sofia Dias	B.Sc. Maintenance
Manuel Robalo	Maintenance
José Costa	Maintenance
Helena Gonçalves	Maintenance

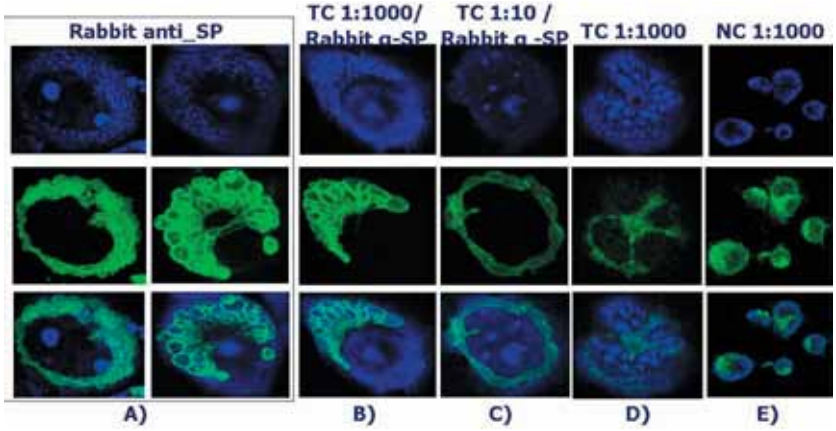
Publications 2005: 100, 124, 135, 180

Veterinary Biomolecular Diagnostics

Abel Oliva : Laboratory of Bionsensors

Ticks are the most important ectoparasites of livestock in tropical and sub-tropical areas, and are responsible for severe economic losses both through the direct effects of blood sucking and indirectly as vectors of pathogens and toxins. The major losses caused by ticks are due to their ability to transmit protozoan (e.g. *Babesia*, *Theileria*), rickettsial and viral diseases of livestock, which are of great economic importance world-wide.

The Biosensor/Biomolecular Diagnostic Laboratory develops applications for veterinary diagnostics, namely in the area of veterinary tick-borne diseases, as a part of its continuous specialization on the use of biosensing and rapid biomolecular identification of parasites and infection diseases. The parasites *Theileria sp*., *Babesia sp*. and *Anaplasma sp*., which are the main objects of study in the laboratory, are widely distributed in tropical and subtropical regions, affecting ruminants on farms and herds in many countries.



Immunofluorescence staining after sequential incubations of *Theileria sp* infected macrophages.
A) Positive control: incubation with rabbit anti-SP **B)** Sequential incubation: incubation with serum from *Theileria sp.* (China) infected animals (TC) **C)** Sequential incubation: 1st incubation with serum from *Theileria sp.* (China) infected animals **E)** Negative control: one incubation with serum from non-infected animals.

A recently completed European Project, dealing with the study of a newly described Chinese *Theileria sp*. parasite, allowed the Laboratory to study the disease and to develop diagnostic methods. During this project different technologies were developed and applied, namely erythrocyte cell culturing for the continuous production of parasite material; biochemical techniques for the identification of antigenic proteins (SDS-PAGE, Western-blots, immunofluorescence, 2-D gel electrophoresis); PCR and PCR-based technology (Reverse Line Blotting) for the detection of parasite DNA sequences in blood samples or tick extracts; recombinant DNA methodology needed to produce standardised antigens for the diagnosis of tick-borne diseases; and finally an ELISA test based on a recombinant protein, for rapid diagnostic. These diagnostic tools are used for epidemiological surveys in the endemic region (North-Western China) and will contribute to improve animal production and welfare by providing effective control measures.

Identification of antigenic proteins of a Theileria species pathogenic for small ruminants in China: characterization of the immune response and assessment of diagnostic potential (2004) J.Miranda , B.Stumme, D. Beyer, H. Cruz, A.G. Oliva, D. Wicklein, H. Yin, L. Jianxun, J.S. Ahmed, U. Seitzer. *Ann NYAcad Sci,* 1026:1-4

Protein identification by mass spectrometry is used to understand human diseases

Ana Maria Varela Coelho : Mass Spectrometry Laboratory

Mass spectrometry is a powerful analytical method used to determine with high accuracy the mass of a broad type of chemical compounds. In order to perform protein identification, a prior separation and digestion of the protein with a specific protease has to be accomplished. The mass spectrum of the peptide digest allows the determination of the peptide masses. The list of masses is used to search a protein sequence database. This strategy, known as *peptide mass fingerprinting,* together with two-dimensional gel electrophoresis of proteins (2D), was used to compare the proteome profiling of human airway nasal cells from cystic fibrosis (CF) patients *versus* control individuals[1] (in collaboration with D. Penque, INSA-Inst Ricardo Jorge) and to determine the influence of the hepatitis delta virus RNA expression in human liver cells proteome (in collaboration with C. Cunha, IHMT-Univ Nova de Lisboa).

Some of the proteins identified from the 2D gels of nasal brushing samples collected from non-CF individuals are ubiquitously expressed confirming the epithelial nature of this tissue. Comparison of the nasal cells protein profile with the control evidenced a set of proteins that are differentially expressed. These included proteins related to CF chronic inflammation and others involved in oxidative stress injury. Variations in expression levels were found for proteins probably implicated with changes in cytoskeleton organization of CF airway cells and for some mitochondrial proteins suggesting an altered mitochondrial metabolism in CF. Furthermore, differences in the expression level were also found for other proteins that have not been related so far to CF lung disease. The identified proteins can be potential biological markers for cystic fibrosis lung disease and this might be useful as a screening tool in diagnosis and prognosis of this pathology.

The hepatitis delta virus (HDV) is a human virus pathogen that infects liver cells in association with the hepatitis B virus and increases the risk and severity of fulminant disease. The HDV genome consists of a ssRNA molecule that encodes a unique protein, the delta antigen. Due to its simplicity the HDV is highly dependent on host cellular factors and is an excellent model for the study of virus-host interactions. Human hepatoma cell line (Huh7) was transfected with constructs encoding genomic and anti-genomic RNA. Transfected Huh7 cells and the original cell line when analysed in 2D gels displayed different expression patterns. Several of these identified proteins, hnRNps, 26S proteosome related proteins and transcriptional factors were found to be of great interest in understanding the HDV infection process[2].

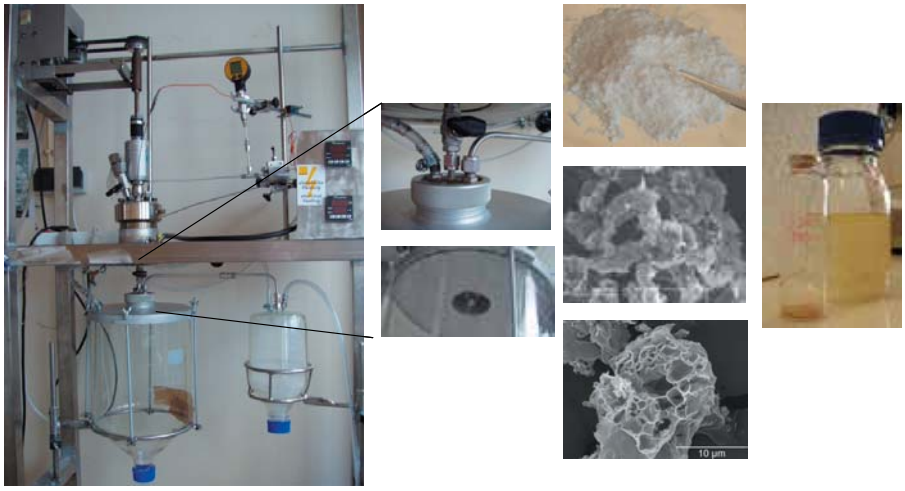
Peptide mass fingerprinting procedure: gel spot pickin proteolytic digestion and mass spectra acquisition.

[1]M Roxo-Rosa; G da Costa; TM Luider; BJ Scholte; AV Coelho; MD Amaral; D Penque "Proteomic analysis of nasal cells from Cystic Fibrosis (CF) patients and non-CF control individuals: search for novel biomarkers of CF lung disease", *Proteomics*, in press

[2]Mendes ML "Influence of the HDV genomic and antigenomic RNA expression in human liver cells proteome" (2006) Master thesis, IST

Development of Improved Delivery Systems for Food Supplements

Catarina Duarte: Nutraceuticals and Delivery Laboratory



High-pressure apparatus for PGSS, micro/nano lipid particles and sun flower oil enriched with hydrophilic natural extract

An innovative supercritical CO_2 process has proven to be efficient for the preparation of improved delivery systems for food supplements, [1, 2].

The use of natural compounds, especially those extracted from plants for the preservation or addition of supplements in foods is nowadays widely in use, since the plant matrices possess several active principles with strong biological activities. One problem that still remains is the limited quantities that can be incorporated in the food matrices when using conventional incorporation processes. Additionally, the stability and bioavailibility of the additives should be guaranteed.

Lipid matrices have been proven to be very good carriers with more convenient properties for increasing the bioavailability and the shelf life of the active compounds. Up to date, lipid micro/nanoparticulated delivery systems were always prepared using quite drastic operational conditions for the active substances, such as organic solvents and thermal stress.

PGSS (particles form gas-saturated solutions) is a supercritical fluid technology that can be carried out under normal conditions. It has minimal detrimental effects on the nutraceutical materials and avoids multi-step processes. A new semi-pilot scale apparatus for particle formation using the PGSS technique has been constructed. This clean technique has been successfully applied to produce lipid particles from GRAS (generally recognized as safe) compounds. The particles were loaded with hydrophilic bioactive natural extracts and incorporated in commercially available sun flower oil. Tests have shown that the solubilization of the lipid based delivery systems containing the active compound was much higher than when trying to incorporate directly the natural extract alone. When comparing with traditional emulsification techniques, less stirring time and temperature were needed to incorporate the same amount of active compound and the shelf life of the final product was significantly improved.

1 A.R. Sampaio de Sousa, Marilyn Calderone, Elisabeth Rodier,Jacques Fages, Catarina M.M. Duarte, Solubility of carbon dioxide in three lipid-based biocarriers, *J. Supercritical Fluids*, accepted, October 2005.

2 A.R. Sampaio de Sousa, A. A. F. Matias, Catarina M.M. Duarte, Development of lipid particles using supercritical carbon dioxide, 10th European Meeting on Supercritical Fluids, December 2005, Colmar - France

Cidália Peres : Microbiology of Man-Made Environments

(Ecology and Microbiota) Laboratory

A *Lactobacillus* strain that is known to produce multiple bacteriocins has been studied in order to isolate and identify the various antimicrobial compounds produced; this work is still in progress. The screening of bacteriocin production has been continued from lactic strains from the collection of table olives. A simple protocol has been developed to test, more efficiently, a large quantity of strains. Antagonistic properties have been determined against Gram-negative bacteria and moulds (isolated from olive brines).

Fig. Inhibition of moulds by olive LAB bacteriocins

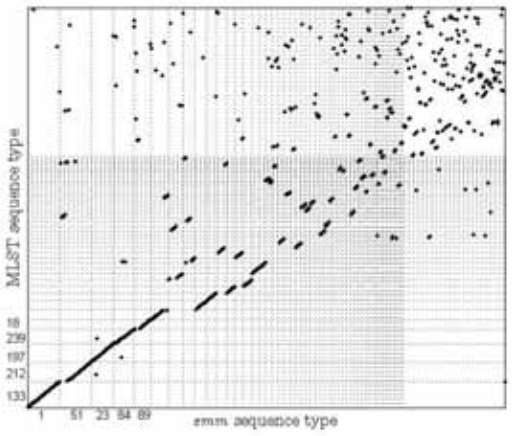
The inhibition of some potential human pathogens as well as antibiotic resistant *Enterococcus'* strains have indicated potential healthcare applications and . prospective studies in this field will be a feature of the future research program.

The inhibition of the vegetative growth of moulds (of genus *Penicillium*, *Aspergillus* and *Crysonilia*) by some bacteriocins have been observed. This inhibition is an important technological feature in table-olive manufacture. Only a few bacteriocins are reported to be active against eukaryotic organisms. Further data will be acquired to try to confirm these reports and to determine the nature and mechanisms of the inhibition.

Molecular Epidemiology: new methodology for typing methods comparison

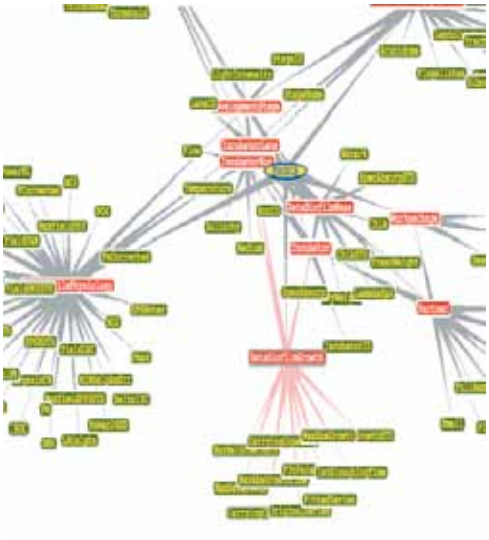
Jonas S. Almeida & João Carriço : Biomathematics Laboratory

A large number of methods are available to type microbial pathogens. The choice of which method to use in a given study is sometimes guided by the perception that newer methods are better than older ones. The studies that correlate the results obtained by different methodologies rely solely on a qualitative comparison of the groups defined by each methodology. Although this is useful to obtain insights into the relationships among the isolates under study, it provides little information on the global congruence between typing methodologies. The Laboratory has proposed a framework of measures for the quantitative assessment of correspondence between different typing methods as a first step to the global mapping of type equivalences.



Carriço, J.A.P[P] ;C. Silva-Costa[P]; J. Melo-Cristino[P]; F. R. Pinto[P]; H. de Lencastre[P]; J.S.AlmeidaP; M.RamirezP[P]. A common framework for relating multiple typing methods illustrated using macrolide- resistant *Streptococcus pyogenes*, submitted

Project PHOBIA: an integrative analysis of biofilm heterogeneous data

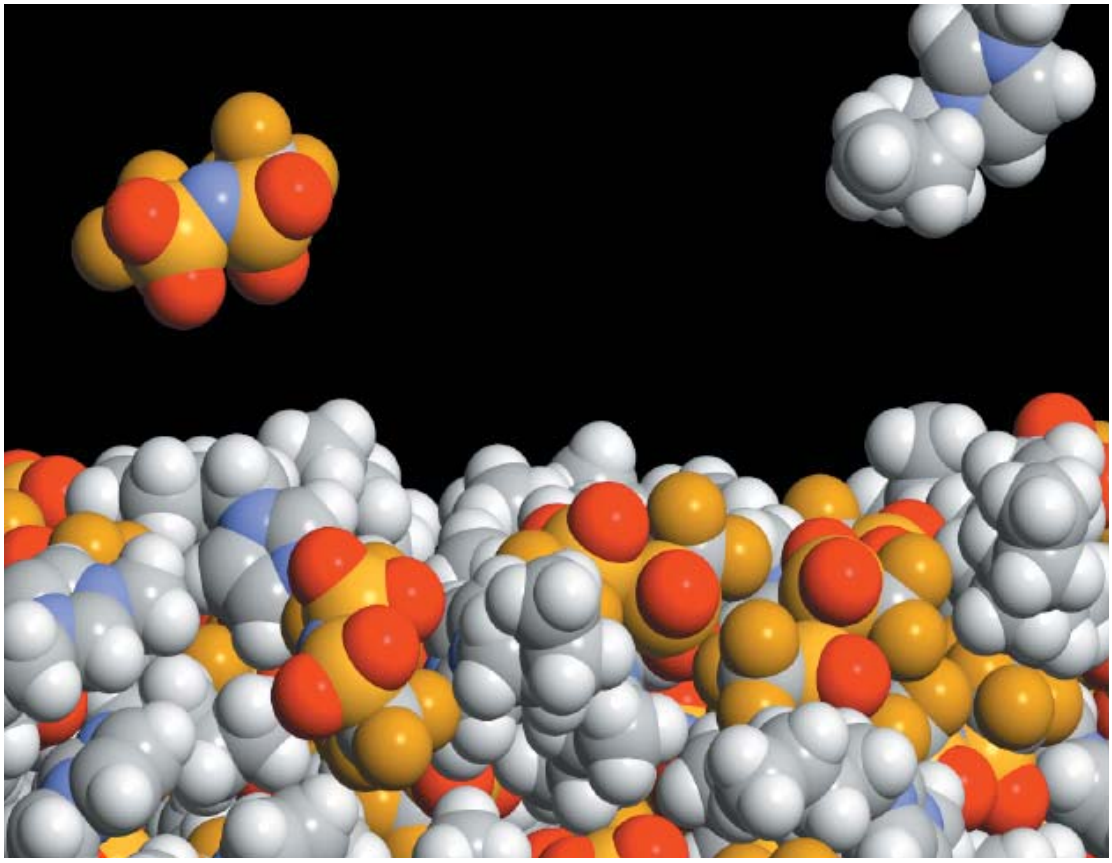


Computational approaches to integrate, analyze and model the entirety of heterogeneous data of phototrophic biofilms, collected by five laboratories, all over Europe are being developed, aiming to extract the essential information about biofilm growth, physiology and structure and predict its dynamics.

A novel type of universal database, S3DB, developed by the Laboratory, is used to accommodate the different types of data while allowing an interface with data analysis tools, such as Artificial Neural Networks.
[S3DB project homepage: www.s3db.org]

Salts in the Gas-phase

Luis Paulo Rebelo : Molecular Thermodynamics Laboratory



Ionic liquids have universally been thought to be involatile. In contrast to this scientific belief, the Laboratory has shown that this is in fact incorrect. Many common, thermally-stable ionic liquids are easily transferred to the gas phase at moderate temperatures providing reduced pressure is applied. Hence, they are distillable. Nonetheless, at standard, lower temperatures they exert undetectable pressure in the gas phase, and, thus, they keep their environmentally friendly (to the atmosphere) status – one of the key factors that has underpinned their increasing popularity.

There are now both immediate implications and far reaching new science to be exploited. The Laboratory has demonstrated significant enrichment upon separative distillation of, for example, binary mixtures of ionic liquids – a powerful method for reaching high-purity levels is now available. High-temperature crystallization can also be envisaged. Ionic liquids can now potentially participate in gas-phase processes, from which they have always been excluded.

J. Phys. Chem B (2005), **109**, 6040 : *Nature* (2005) – in press

Chemical and sensory properties of food components

Maria Rosario Bronze & Luís Vilas Boas : Analytical Chemistry Laboratory

Foods are essential for the survival of human beings but they are also important for their health and well-being. There are chemical compounds in foods with important contributions to our health helping to prevent some diseases (for instance antioxidants) or contributing to our preference for foods (*eg*. key aroma compounds).

Separation techniques are very useful to study the chemical composition of foods and can help us to understand how the technological processes can lead to foods with different characteristics. Chemical compositions of fruit juices, wines [1], vinegars, table olives, olive oil, tea, can give clues about components that may be responsible for effects such as sensory characteristics (colour, taste, flavour) or other biological effects in the human body. These methods are also useful to give information about compounds that are present in residues from related industries [2].

Chromatography using several detectors (figure 1) and hyphenation with mass spectrometry are powerful tools for the identification and quantification of compounds.

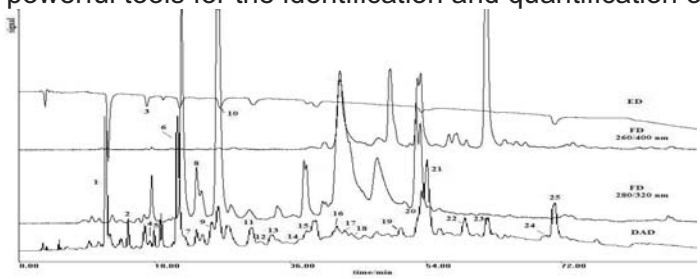


Figure 1: Chromatograms obtained in the analysis of a Muscatel wine using different detectors: diode array (DAD), fluorescence (FD) and electrochemical (ED)

Further study of the quality of products has to take into account the perception of properties by humans. Some techniques such as olfactometry use instrumental detectors in parallel with human noses in order to conclude the contribution of compounds to the sensory properties of products; for some compounds the human nose is more sensitive than the analytical instruments currently available.

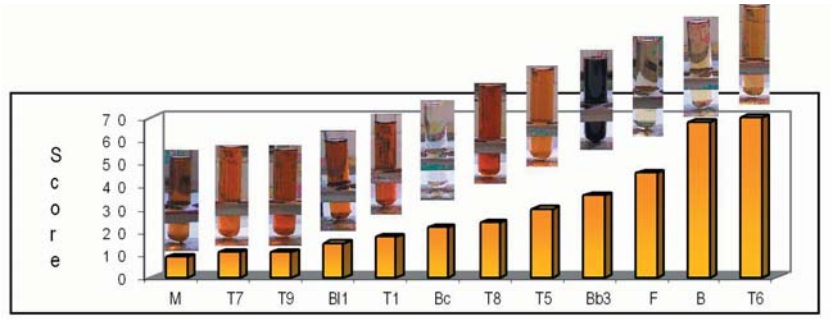


Figure 2. Preferences of a sensory panel evaluating the colour of wine vinegars

The Laboratory studies the chemical composition of food products and their important with respect to both sensory and biological properties. Chromatography (gas and liquid) and electrophoresis are the techniques mainly used.

[1] M.N. Bravo, S.Silva, A.V. Coelho, L. Vilas Boas, M.R. Bronze, Analytica Chimica Acta, accepted for publication

[2] S. Silva, A.A. Matias, A. Nunes, C. Duarte; A.V. Coelho, M.R. Bronze, Ciência Téc. Vitiv. 20(1), 17-33, 2005

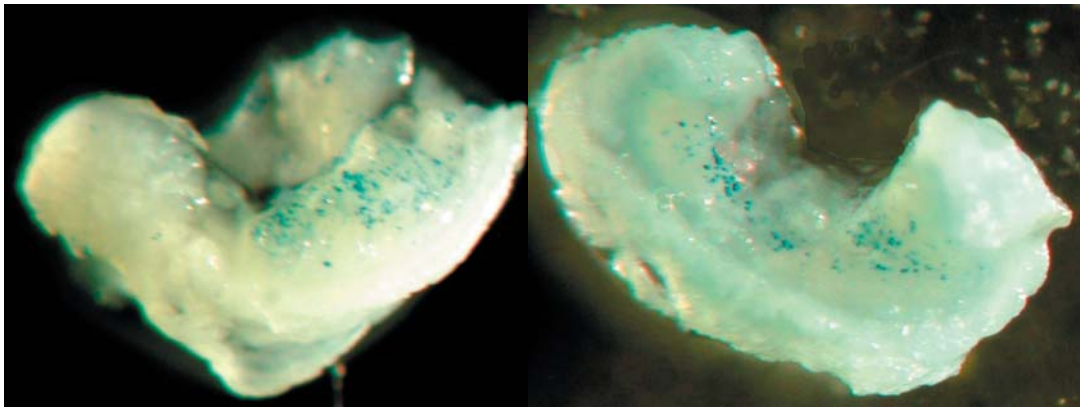
Stability of retroviral vectors for gene therapy clinical applications

Manuel Carrondo, Paula M Alves & Pedro E. Cruz :
Animal Cell Biotechnology Laboratory

Recombinant retroviruses are one of the most commonly used vectors for gene therapy. However, these vectors have a low stability, exhibiting half-lives in the range of 2 to 8 hours at 37ºC. The negative impact of a low stability is reflected not only in the dramatic reduction of the concentration of infectious particles but also in their efficacy in clinical applications. Several factors that affect the stability of the vectors have been studied including production temperature, pH and additives. Nevertheless, it is not yet understood how all these factors affect the constitution of the vectors with respect to their stability.

In an attempt to understand the complex processes involved in the inactivation of retroviral vector a multidisciplinary approach was followed. In particular, the physico-chemical properties of retroviral vector membranes were studied in a work involving the use of several specific technologies such as Electron Paramagnetic Resonance (EPR) to evaluate membrane rigidity, Differential Scanning Calorimetry (DSC) to study the conformation of the membrane proteins and Electron Microscopy (EM) to observe vector integrity. The efficacy of the vectors in transducing cells with clinical relevance was assessed in collaboration with the Hadassah Medical School of the Hebrew University (Israel).

Transduction studies using HCT 116 cells and tri-dimensional organ cultures of mouse skin showed that vectors produced at 37ºC have higher stability and thus higher transduction efficiency in gene therapy relevant cells as compared to vectors produced at 32ºC. Overall, vectors produced at 37ºC show an increased stability at temperatures below 4ºC. Since vector membrane physico-chemical properties are affected in response to changes in culture temperature, such changes, along with alterations in medium composition, can be used prospectively to improve the stability and the transduction efficiency of retroviral vectors for therapeutic purposes.[1] The ACT lab is a partner of CLINIGENE - European Network for the Advancement of Clinical Gene Transfer and Therapy, a European Network of Excellence starting March 2006, being responsible for the coordination of its retrovirus platform.



Mouse skin micro-organs infected with retroviral vectors

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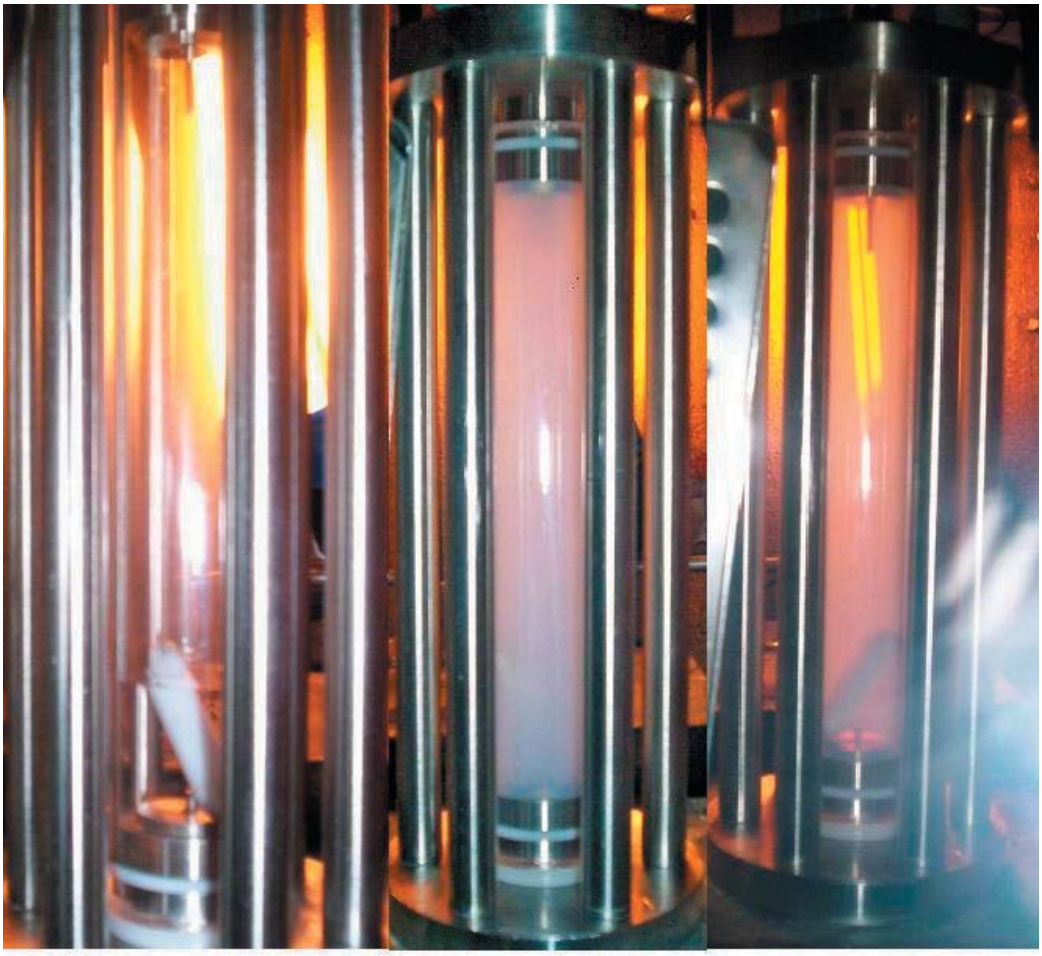
Processes with Super-critical Fluids

Manuel Nunes da Ponte : Super-critical Fluids Laboratory

High pressure CO_2 dissolves in large amounts in terpenes. The Laboratory has benn exploring these “gas-expanded” liquid solutions to perform chemical reactions. In 2005, it was discovered that the selectivity of the hydrogenation reactions of terpenes is very sensitive to the pressure of the carbon dioxide added to the mixture, especially when the mixture is close to the critical pressure. In the figure, a sequence of three pictures shows the visual effects observed when a mixture of CO_2 and α-pinene goes through the critical region.



Snapshots of the two-phase system, liquid α-pinene and gaseous CO_2. Before, near and at the mixture's critical point in the left, middle and right hand panels, respectively. CO_2 pressure is increasing from left to right at constant temperature.

Peptide catabolism in food microorganisms: a mechanism of survival?

Maria Teresa Crespo : Microbiology of Man-Made Environments (Food and Environment) Laboratory

Lactic Acid Bacteria (LAB) are responsible, partially or in some cases totally, for the production and final organoleptic properties of traditional cheeses, meat products and wine that are part of the daily diet in rural areas of Portugal, as well as, fashionable food products in urban centres. These products have an enormous social impact in rural areas due to the creation of jobs and to the settlement of population in the interior areas of the country.

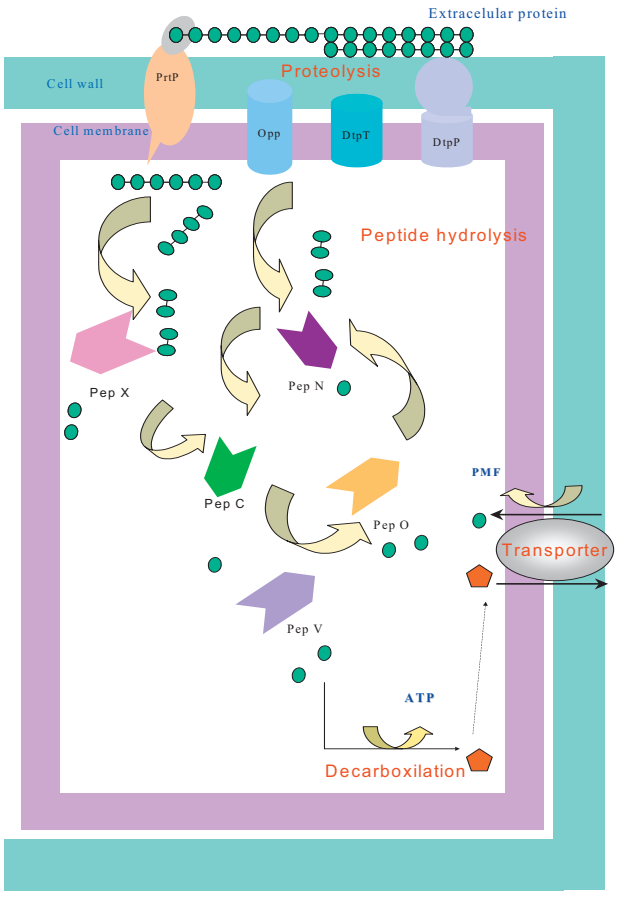


Figure 1- The model of the proteolytic system proposed for *Lactococcus lactis*.

LAB is a group of genera that include among others *Lactococcus*, *Lactobacillus* and *Oenococcus*. The enzymatic activities of this group, responsible for the unique characteristics of those food products, are partially known. In fact, work has already been performed on some of the enzymes and enzymatic pathways that lead to production of the chemical compounds responsible for the organoleptic properties, such as malolactic activity, lipolytic activity and proteolytic activities, to mention only a few examples.

In this context genes involved in the peptidolytic activities of *Lactobacillus* and *Enterococcus* have been identified, namely dipeptidase V, aminopeptidase X and peptidses C and O.

Commonly the major energy source for the creation of a proton motrice force (Δp) in fermenting bacteria is ATP hydrolysis catalyzed by the proton translocating ATP synthase. However, *Lactobacillus* and *Enterococcus* have evolved additional ways to generate a ΔpH, namely, (i) fermentation end-product efflux down the concentration gradient, coupled to the translation of protons out of the cell; (ii) electrogenic precursor/product antiport such as arginine/ornithine, agmatine/putrescine, histidine/histamine. These pathways are the focus of the research currently being undertaken in the Laboratory.

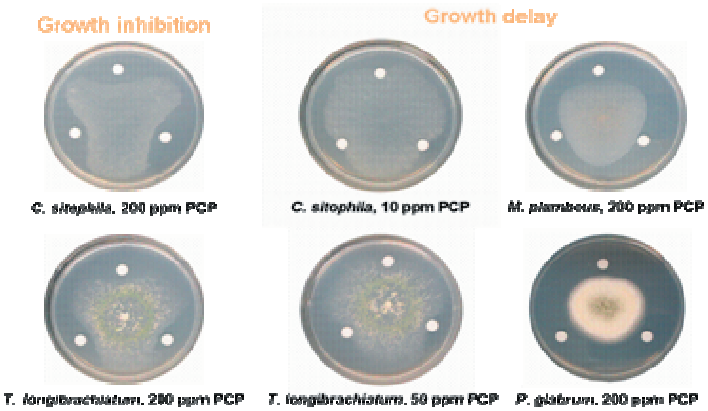
Degradation of Fungal Polychlorophenols.

Vitória San Romão: Microbiology of Man-made Environments Laboratory.

The appearance of chloroanisols and other metabolites known to impart defects in bottled wines are responsible for severe loses in the Portuguese cork stopper industry. These compounds have been found to be related to fungi activity over the cork slabs. Their production is intensified when the direct precursor (polychlorinated phenols) are available as contaminants of cork tissues. Polychlorinated phenols have a wide spectrum of biocidal properties that have led to their extensive use as an ingredient of pesticides and wood preservatives. The mechanisms that lead to the degradation of polychlorinated phenols, and subsequently to the formation of chloroanisol by some of the cork colonising moulds is being studied to clarify 'cork taint' occurrence in wine. Additionally, the occurrence of other degradation intermediates has been investigated.

The importance of polychlorinated phenol degradation increases when their properties are considered. PCP can lead to unpredictable physiologic responses, causing chromosomal aberrations and carcinoma formation in humans. They are being considered as priority toxic substances both by the EC commission and the US Environmental Protection Agency. The natural attenuation of these compounds through microbial degradation involves some classes of depolymerising enzymes which are also involved in lignin degradation. The high surface-to-cell ratio of filamentous fungi, together with the natural overlap between the capability of fungi towards biodegradability and bioremediation, make fungi better degraders under certain niches, such as contaminated soils. In this regards the potential of fungi is unquestionable. However, the mechanisms by which indigenous moulds metabolise polychlorinated phenols are not sufficiently studied to guarantee a safe use in the environment. Fungi metabolism of PCP detoxification is an important natural process that may be further explored for controlling PCP on-site attenuation (bioremediation).

All the studied fungi *(Penicillium olsoni*, *P. glabrum*, *Trichoderma longibrachiatum* and *Chrysonilia sitophila)* are able to grow in the presence of 2,4,6-trichlorophenol (2,4,6-TCP) and pentaclorophenol (PCP) as the main carbon source, metabolising those compounds. *P. glabrum* cultures showed the lowest 2,4,6-TCP and PCP consumption yield while *T. longibrachiatum* can degrade these compounds more efficiently.



Comparison of chromatographic profiles of fungi culture media fortified with polychlorophenols (with control experiments) shows that there are some chromatographic peaks not observed in control media for *C. sitophila*, *P. glabrum* and *P. olsoni*. The identification of the relevant compounds detected is ongoing.

The profile showed by *P. olsoni* is somewhat different from the rest (a new peak appears at 18.13 min, in presence of PCP). The diverse behaviour of this species indicates an additional direction for future work.

RESEARCH OUTPUT

Publications ITQB 2005

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PATENTS 2005

Organometallic Molybdenum Compounds for Treating Cancer,
M. N. Matos, C. Pereira, S. Rodrigues, M. J. P. da Silva, M. Mora, P. Alves, C. A. Reis, C. C. Romão, PCT/PT2004/000004 - WO2005/087783. Filed via IBET.

ITQB Publications 2005 - Journal Impact Factors

Abbreviated Journal Title	ISSN	2004 Total Cites	Impact Factor	ITQB Articles
NAT BIOTECHNOL	1087-0156	18169	22.355	1
MICROBIOL MOL BIOL R	1092-2172	4807	17.037	1
FEMS MICROBIOL REV	0168-6445	3426	8.703	1
CURR OPIN MICROBIOL	1369-5274	3548	8.182	1
EMBO REP	1469-221X	4411	7.567	1
J ALLERGY CLIN IMMUN	0091-6749	19416	7.205	1
J AM CHEM SOC	0002-7863	231890	6.903	3
J BIOL CHEM	0021-9258	405017	6.355	4
MOL MICROBIOL	0950-382X	27182	5.959	2
PLANT PHYSIOL	0032-0889	37237	5.881	1
BIOINFORMATICS	1367-4803	11390	5.742	2
CLIN CANCER RES	1078-0432	23585	5.623	2
CLIN INFECT DIS	1058-4838	23917	5.594	1
J MOL BIOL	0022-2836	62325	5.542	2
PROTEOMICS	1615-9853	3540	5.483	2
GENE THER	0969-7128	9020	4.977	1
KIDNEY INT	0085-2538	29257	4.790	1
GLIA	0894-1491	5224	4.781	1
BIOPHYS J	0006-3495	30826	4.585	3
PROG BIOPHYS MOL BIO	0079-6107	1923	4.551	1
PROTEINS	0887-3585	10582	4.429	1
BIOCHEM J	0264-6021	48161	4.278	2
ANTIMICROB AGENTS CH	0066-4804	28261	4.216	3
MOL PHYLOGENET EVOL	1055-7903	5394	4.213	1
J BACTERIOL	0021-9193	52553	4.146	4
BIOCHEMISTRY-US	0006-2960	96809	4.008	4
CHEM COMMUN	1359-7345	53341	3.997	4
FEBS LETT	0014-5793	54417	3.843	4
J PHYS CHEM B	1520-6106	46122	3.834	5
APPL ENVIRON MICROB	0099-2240	46407	3.810	3
J NEUROSCI RES	0360-4012	11031	3.727	1
BIOL CHEM	1431-6730	4886	3.598	1
ENVIRON SCI TECHNOL	0013-936X	34474	3.557	1
BBA-BIOENERGETICS	0005-2728	5176	3.503	1
GREEN CHEM	1463-9262	2011	3.503	2
INORG CHEM	0020-1669	51887	3.454	1
J CLIN MICROBIOL	0095-1137	35117	3.439	7
J EXP BOT	0022-0957	8583	3.366	2
J CHROMATOGR A	0021-9673	41467	3.359	1
J BIOL INORG CHEM	0949-8257	2141	3.300	2
FEBS JOURNAL/EUR J BIOCHEM	0014-2956	32354	3.260	4
BMC GENOMICS	1471-2164	389	3.250	1
ORGANOMETALLICS	0276-7333	27459	3.196	2

MICROBIOL-SGM	1350-0872	8969	3.114	1
PLANTA	0032-0935	10711	3.113	2
THEOR APPL GENET	0040-5752	12422	2.981	2
DALTON T	1477-9226	23422	2.926	3
BIOCHEM BIOPH RES CO	0006-291X	64346	2.904	4
DEV DYNAM	1058-8388	5073	2.868	1
RAPID COMMUN MASS SP	0951-4198	7070	2.750	1
PLANT BIOTECHNOL J	1467-7644	144	2.738	1
NEW J CHEM	1144-0546	5653	2.735	1
PEDIATR INFECT DIS J	0891-3668	7523	2.735	1
TETRAHEDRON	0040-4020	37427	2.643	1
J PHYS CHEM A	1089-5639	27189	2.639	1
INT J FOOD MICROBIOL	0168-1605	6038	2.490	1
TETRAHEDRON LETT	0040-4039	67752	2.484	2
TETRAHEDRON-ASYMMETR	0957-4166	11137	2.386	1
CLIN MICROBIOL INFEC	1198-743X	1911	2.361	1
PHYS REV E	1063-651X	42737	2.352	2
EUR J INORG CHEM	1434-1948	5520	2.336	3
J BIOTECHNOL	0168-1656	4029	2.323	7
J MOL CATAL A-CHEM	1381-1169	7426	2.316	4
RES MICROBIOL	0923-2508	1819	2.301	1
J SUPERCRIT FLUID	0896-8446	1560	2.275	1
ANN BOT-LONDON	0305-7364	5521	2.262	1
J MICROBIOL METH	0167-7012	2622	2.146	1
BBA-PROTEINS PROTEOM	1570-9639	754	2.113	1
PHYTOCHEMISTRY	0031-9422	19126	2.101	1
GENOME	0831-2796	3590	2.100	1
PHYS CHEM CHEM PHYS	1463-9076	8572	2.076	1
FUNCT PLANT BIOL	1445-4408	588	2.075	2
EUR J PHARM SCI	0928-0987	1685	1.949	1
VET MICROBIOL	0378-1135	4239	1.930	2
J ORGANOMET CHEM	0022-328X	20543	1.905	2
CATAL LETT	1011-372X	5841	1.904	1
INT J DEV BIOL	0214-6282	1919	1.888	1
FEMS MICROBIOL LETT	0378-1097	11734	1.840	1
MICROB DRUG RESIST	1076-6294	926	1.807	4
BIOTECHNOL PROGR	8756-7938	3540	1.635	2
POLYHEDRON	0277-5387	8929	1.586	1
INORG CHIM ACTA	0020-1693	11626	1.554	1
PURE APPL CHEM	0033-4545	7463	1.449	2
PLANT SCI	0168-9452	4526	1.389	1
BIORESOURCE TECHNOL	0960-8524	3347	1.387	1
J CHEM ENG DATA	0021-9568	5412	1.368	2
FLUID PHASE EQUILIBR	0378-3812	4543	1.356	1
AMYOTROPH LATERAL SC	1466-0822	364	1.219	1

AGR ECOSYST ENVIRON	0167-8809	2862	1.207	2
MOL ECOL NOTES	1471-8278	731	1.175	1
CR CHIM	1631-0748	311	1.156	1
J CHEM THERMODYN	0021-9614	2735	1.144	2
FOOD CONTROL	0956-7135	557	1.132	1
FOOD MICROBIOL	0740-0020	1347	1.105	1
J PLANT PHYSIOL	0176-1617	3548	1.054	1
COMP FUNCT GENOM	1531-6912	233	1.038	1
J CARBOHYD CHEM	0732-8303	957	1.017	1
BIOTECHNOL LETT	0141-5492	3338	0.849	2
STRUCT CHEM	1040-0400	424	0.833	1
EUPHYTICA	0014-2336	3287	0.797	1
BIOL PLANTARUM	0006-3134	903	0.744	1
RADIAT PROT DOSIM	0144-8420	2347	0.680	1
WATER SCI TECHNOL	0273-1223	10132	0.586	2
DNA SEQUENCE	1042-5179	367	0.578	1
VITIS	0042-7500	733	0.566	2
J AGRON CROP SCI	0931-2250	381	0.496	1
CYTOTECHNOLOGY	0920-9069	599	0.438	1
FIBER INTEGRATED OPT	0146-8030	174	0.420	1
ACTA CRYSTALLOGRAPHICA F				2
CIÊNCIA E TÉCNICA VITIVINICOLA				1
IONIC LIQUIDS				3
SILVA LUSITANA				1
TOTAL				**195**

Oral Presentations at Scientific Meetings

Aires de Sousa, M., C. Simas, T. Conceicao and H. de Lencastre (2005) Comparison of genetic backgrounds of MRSA and MSSA isolated in Portuguese hospitals and in the community. 15th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Copenhagen, Denmark, April 2-5.

Almeida, A. M., A. Bernardes da Silva, J. Marques da Silva, E. Villalobos, S. S. Araújo, L. A. Cardoso, J. M. Torné, M. P. Fevereiro and D. Santos (2005) Sucrose and soluble reducing sugar contents in trangenic tobacco plants expressing a trehalose synthesis gene of plant origin at two different levels. IX Congresso Luso-Espanhol de Fisiologia Vegetal, Évora, Portugal, September 19-21.

Alves, P. M. (2005) Animal Cell Technology for R&D. Symposium "Biotechnology: from science to start-ups", Lisboa, Portugal, October.(Invited lecture).

Alves, P. M. (2005) Rational Improvement of complex vaccines: the case for Rotavirus like particles. Micro'05 Biotec'05, Póvoa do Varzim, Portugal, December.(Invited lecture).

Amblar, M. (2005) mRNA decay in bacteria: study of ribonucleases. CINVESTAV, Mexico City, Mexico, November.

Archer, M. (2005) Cristalografia de Proteínas- Aplicações na indústria farmacêutica. III Jornadas Nacionais de Bioquímica, Univ. Beira Interior, Covilhã , Portugal, March 4-6.

Archer, M. (2005) NADH dehydrogense: the bumpy road from crystal to structure. Workshop "Membrane Proteins: the rocky road from gene to structure", ITQB, Oeiras, Portugal, September 19-24.

Arraiano, C. M. (2005) Challenging the Dogma: RNA Dictates the Rules. Congresso Nacional de Microbiologia e Bioquímica "MICROBIOTECH'05", Póvoa do Varzim, Portugal, December.(Plenary Conference).

Arraiano, C. M. (2005) RNAs, RNases and Post-Transcriptional control of Gene Expression. Max Plank Institute for Infectious Biology, Berlin, Germany, April.

Arraiano, C. M. (2005) Unsuspected connections between bacterial polyadenylation, transcription and the proteolytic machinery. RNA turnover Conference of EU MAMBA: Centre of Excellence for Multi-scale Biomolecular Modelling, Informatics and Applications, Warsaw, Poland, October.

Baptista, A. A. (2005) Binding and electrostatics: insights from a redox protein complex. Receptors: from silico to laboratory, Università degli Studi di Milano, Italy, June 17.

Barbas, A. (2005) RNase II family of enzymes Structural and Functional Analysis. RNA 2005, IMM, Lisbon, Portugal, December.

Basílio, M. C., R. Gaspar, C. Silva Pereira and M. V. San Romao (2005) Geographical similarity analysis of cork colonising Penicillium glabrum isolates using DNA fingerprinting. One Day Meeting - Trends in Micology, Braga, Portugal.

Bento, I. (2005) Dioxygen reduction by multi-copper oxidases; a structural perspective. Dalton Discussion 8: Metals - Centres of Biological Activity, Nottingham, UK, September 7-9.

Borges, N. (2005) The role of compatible solutes in osmoadaptation and thermoadaptation of the thermophilic bacterium *Rhodothermus marinus*: biosynthesis and regulation. Micro'05 Biotec'05, Póvoa do Varzim, Portugal, November 30 – December 3.(Invited lecture).

Carrondo, M. A. (2005) Binding of dioxygen to iron and copper in metalloenzymes: structural overview. 1st European Conference on Chemistry for Life Sciences, Understanding the chemical mechanisms of life, Rimini, Italy, October 4-8.

Carrondo, M. A. (2005) Sulphate respiration from hydrogen in Desulfovibrio bacteria: a structural biology overview. "Structure of Energy Transducing Systems in Prokariotes" Priority Program 1070, Final Meeting, Naurod (Wiesbaden), Germany, April 3-5.

Carrondo, M. J. T. (2005) Animal Cell Biotechnology. 1as Jornadas de Bioquímica da Universidade do Algarve, Faro, Portugal, October.(Invited Lectures).

Carrondo, M. J. T. (2005) Biotecnologia: a aplicação à Terapia Genética. Simpósio Farmacêutico Da Investigação à Terapêutica – Eixos de Desenvolvimento e Realidades Actuais, Lisbon, Portugal, October.(Invited lecture).

Carrondo, M. J. T. (2005) Rotavirus-like Particles production: Baculovirus DNA replication, mRNA synthesis and protein production. 1st European Rotavirus Biology Meeting, Paris, France, April.(Invited lecture).

Carvalho, S. (2005) Molecular Recognition Studies with a Hexaazamacrocycle and anions. 6th Conference on Inorganic Chemistry, Funchal, Portugal, March 31 - April 2.

Chaves, M. M. (2005) Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. WUEMED Workshop 'Improving Water-Use Efficiency in MEDiterranean agriculture: what limits the adoption of new technologies?, Rome, Italy, September 29-30.(Invited lecture).

Coelho, A. V. (2005) Protein characterization by mass spectrometry. 3rd Annual Meeting of Portuguese Proteomic Network-ProCura-Functional genomics and proteomics, Cascais, Portugal.

Crespo, M. T. B. (2005) Cálculo de incertezas em ensaios de biologia molecular - o exemplo da quantificação de OGM. Workshop Relacre "Incertezas em análise microbiológica", Lisboa, Portugal.

Crespo, M. T. B. (2005) Food safety versus food satisfaction – a microbiological evaluation. Micro'05-Biotec'05, Póvoa do Varzim, Portugal.

Cruz, P. E. (2005) Influence of retroviral vector membrane properties upon vector stability and transduction efficiency. 4th Euregenethy International Forum, Paris, France, June.(Invited lecture).

de Lencastre, H. (2005). 6th Annual Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA) Meeting, Charlottesville, VA, USA, March 8-9.

de Lencastre, H. (2005) Methicillin and vancomycin resistance in Staphylococcus aureus, mechanisms and evolution. SGM 156th Meeting, Edinburgh, UK, April 4-7.

Duarte, A. R. C., C. Cravo, A. Nunes and C. M. M. Duarte (2005) "Green" impregnation of biopolymers for application in food packaging. 10th European Meeting on Supercritical Fluids, Colmar, France, December.

Duarte, R. (2005) Supercritical fluid impregnation of different polymeric matrices for controlled drug delivery. Nutraceutical and food processing Meeting, Oeiras, Portugal, July.

Enguita, F. J. (2005) Structural genomics of the human pathogens Klebsiella pneumonia and Campylobacter jejuni. SPINE Congress 2005, Montecatini Terme, Italy, August 31 - September 1.

Ferraz Franco, C., P. I. Alves, P. Faroleira, M. T. B. Crespo and A. V. Coelho (2005) Identification and characterization of soil bacterium using mass spectrometry approach: fingerprinting Sinorhizobium cell surface proteins by MALDITOF mass spectrometry. 3rd Annual Meeting of Portuguese Proteomic Network-ProCura-Functional genomics and proteomics, Cascais, Portugal.

Fevereiro, P. (2005) Avanços da Biotecnologia em Portugal: um caso de estudo sobre a aceitabilidade de produtos transgénicos no continente europeu e seus reflexos na América Latina. IV Simpósio Latino-Americano de Produtos Transgênicos Porto Alegre, Porto Alegre, Brasil, September 26-29.

Fevereiro, P. (2005) Biotecnologia em Portugal. Dias da Biotecnologia (Universidade Lusófona), Lisboa, Portugal, May 30 - June 3.

Fevereiro, P. (2005) Ethics and the use of plants: the ways and the ends. Workshop "Plants for the Future" ICAT, Lisbon, Portugal, June 16-17.

Fevereiro, P. (2005) Melhoramento e fitossanidade da oliveira. Jornada de Reflexão Sobre a Investigação em Olivicultura, Departamento de Olivicultura – Estação de Melhoramento de Elvas Herdade do Reguengo, Elvas, Portugal, January 25.

Fevereiro, P. (2005) Organismos geneticamente modificados. IV Congresso de Nutrição e Alimentação, Porto, Portugal, May 19 - 20.

Fevereiro, P. (2005) Plant biotechnology to cope with water deficit: are we progressing? IX Congresso Luso-Espanhol de Fisiologia Vegetal, Évora, Portugal, September 19-21.

Filipe, S. (2005) Requirements of Staphylococcus aureus peptidoglycan structure allowing detection by the Drosophila Toll pathway. Gordon Research Conferences: Staphylococcal diseases, Salve Regina University, Rhode Island, USA., August 21-26.(Invited Lectures).

Franco, I. S., L. J. Mota, C. M. Soares and I. d. Sá-Nogueira (2005) A comprehensive mutagenesis of the Bacillus subtilis transcription factor AraR: identifying amino acids important for nucleoprotein complex assembly and effector-binding. 3rd Conference on Functional Genomics of Gram-positive Microorganisms, San Diego, USA.

Freire, P., J. M. Santos, H. L. A. Vieira, A. R. Furtado and C. M. Arraiano (2005) BolA, an interesting model to study bacterial growth and morphology. Workshop Cryo Methods in Analytical and immuno-Electron Microscopy, Oncology Portuguese Institute, Lisbon, Portugal, May.

Gaspar, P., A. R. Neves, M. J. Gasson, C. A. Shearman and H. Santos (2005) A food-grade approach to engineer mannitol production in Lactococcus lactis: the effect of manipulating NAD+ regeneration downstream of pyruvate. Symposium on Lactic Acid Bacteria: genetics, metabolism and applications, The Netherlands, August 28 - September 1.

Gomes, M. S. and A. Oliva (2005) Overview of Portugal situation regarding regional susceptibility to Babesia sp. Babesia World Summit, Buenos Aires, Argentina, December 1-3.

Gonçalves, S., J. Cairney, M. Oliveira and C. M. Miguel (2005) PpRab1, a Rab-related small GTP-binding protein is expressed predominantly in early embryogenesis and seedlings of Pinus pinaster. IUFRO Tree Biotechnology 2005, Pretoria, South Africa, November 6-12.

Grant, O. M. (2005) Thermal imaging successfully identifies water stress in field-grown grapevines. International Gesco Meeting, Geisenheim. Germany, August.

Henriques, S. T., J. Costa and M. A. R. B. Castanho (2005) Uptake of β-galactosidase mediated by pep-1 into large unilamellar vesicles and HeLa cells is driven by membrane potential. 30th FEBS Congress-9th IUBMB Conference, Budapeste, Hungary.

Lachwa, J. and L. P. N. Rebelo (2005) First evidence for closed-loops and other LCST-containing phenomena in binary and quasi-binary ionic liquid solutions. Thermodynamics 2005, Sesimbra, Portugal, April 6-8.

Martins, L. O., I. Bento, C. Pinheiro, M. A. Carrondo and P. F. Lindley (2005) Bacterial laccases: Biochemical and Structural Studies. Biotechnology for Pulp and Paper Manufacture: From Tailor-made Biocatalysts to Mill Applications, Baiona, Spain, April 26-29.

Martins, L. O., I. Bento, C. Pinheiro, M. A. Carrondo and P. F. Lindley (2005) Biochemical and Structural Studies on the bacterial CotA-laccase from *Bacillus subtilis*. International Conference on Enzyme Technology, RELATENZ'2005, Varadero, Cuba, September 20-23.

Matias, A. (2005) New Nutraceuticals: new challenges new opportunities. Bioproducts for Food, the 6th Rothamsted International Biomarket, UK, November.

Matias, P. M. (2005) The activation process of D.desulfuricans ATCC 27774 [NiFe] Hydrogenase. IUCR 20 - XX Congress of the International Union of Crystallography, Florence, Italy, 23-31 August.

Miranda, J., E. Nascimento, H. J. Cruz, H. Yin, A. V. Coelho, J. Ahmed and A. Oliva (2005) Identification and Characterization of Merozoite Theileria sp. (China) Antigens. 8th Congress of the Society of Tropical Veterinary Medicine, Hanoi, Vietnam, June 26 - July 1.

Nunes, S., N. G. Sousa, N. Frazão, R. Sá-Leão and H. de Lencastre (2005) Prevalence and diversity of serotype 16F in Streptococcus pneumoniae isolated from healthy children in Lisbon. Micro'2005-Biotec´05, Póvoa de Varzim, Portugal, November 30 - December 3.

Oliveira, D. C. and H. de Lencastre (2005) Clonal evolution and SCCmec in community-acquired MRSA. 15thEuropean Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Copenhagen, Denmark, April 2-5.

Oliveira, D. C., C. Milheirico, S. Vinga and H. de Lencastre (2005) Assessment of allelic variation in the ccrAB locus in MRSA strains. Gordon Research Conference on Staphylococcal Diseases, Salve Regina University, Newport, RI, USA, August 21-26.

Oliveira, M. M. (2005) As plantas transgénicas no melhoramento de plantas. VIII Jornadas Tecnológicas de Engenharia Química (JORTEC 2005), Univ. Nova de Lisboa, Portugal, April 12.

Oliveira, M. M. and V. Cordeiro (2005) Prunus collections in Portugal – the almond situation. VII Meeting of the ECP/GR Working Group on Prunus, Larnaca, Cyprus, December, 1-3.

Oliveira, M. M., C. Silva and A. M. Sanchez (2005) Molecular basis of flowering in fruit trees. V Congresso Ibérico de Ciências Hortícolas, Porto, Portugal, May 25.

Paiva, J. P., J. M. Gion, E. Eveno, P. Garnier-Gére, D. Pot, F. Canton, G. Le Provost, J. Brach, F. Canovas, P. Fevereiro and C. Plomion (2005) Expression profiling of wood forming tissues in maritime pine Plant & Animal Genomes XIII Conference, San Diego, CA, USA, January 15-19.

Palma, A., T. Feizi, J. Costa, Y. Zhang, G. Brown and W. Chai (2005) Generation of oligosaccharide arrays by the neoglycolipid technology for the assignement of β-glucan receptor, Dectin-1. Glupor6 - III Iberian Carbohydrate Meeting, Coimbra.

Pereira, I. A. C. (2005) The cytochrome c3 family – one structural module but multiple functions. LeGall Symposium, University of Georgia, Athens, USA, May.

Peres, C. (2005) Tecnologia da fermentação da azeitona de mesa: tecnologias tradicionais, processos melhorados e novos produtos. Jornadas de Produtos Vegetais: Conservação e Inovação, Castelo Branco, Portugal, June 1.

Pinho, M. G. (2005) Localization of the cell wall synthetic machinery in S. aureus. Gordon conferences on "Staphylococcal Diseases", Newport, USA, August 21-26.(Invited lecture).

Real, G. and A. O. Henriques (2005) Requirement for cell division protein DivIB for prespore chromosome segregation during asymmetric cell division in *Bacillus subtilis*. International Conference on Functional Genomics of Gram-Positive Microorganisms, La Jolla, CA, June 12-16.

Rebelo, L. P. N. (2005) Can ionic liquids still surprise us? 21st European Conference on Applied Thermodynamics, Jurata, Poland, June 1-5.(Plenary lecture).

Rebelo, L. P. N. (2005) Novel findings and new challenges in the thermodynamics of ionic liquids. 1st International Congress on Ionic Liquids (COIL) Salzburg, Austria, June 19-22.(Plenary lecture).

Ricardo, C. P. P. (2005) Proteomic studies at the Plant Biochemistry Laboratory (ITQB). 3rd Annual Meeting of Procura (Portuguese Proteomics Network), Cascais, Portugal, November 10-12.(Invited lecture).

Ricardo, C. P. P. (2005) Proteomics studies of abiotic stress responses in plants. IX Congresso Luso-Espanhol de Fisiologia Vegetal, Évora, Portugal, September 18-21

Rodrigues-Pousada, C. (2005) Integrating Yap8 and Yap1 role in response to metalloids. 2nd International Congress on Cell Stress Response in Biology and Medicine, Tomar, Portugal, September 24-28.

Rodrigues-Pousada, C. (2005) Yap8 and Yap1 under stress conditions. Gordon Conferences "On STRESS PROTEINS IN GROWTH, DEVELOPMENT & DISEASES", Salve Regina University, New England, USA, July 7-22.

Roldão, A., H. L. A. Vieira, C. Estevao, P. E. Cruz, M. J. T. Carrondo, P. M. Alves and R. Oliveira (2005) Modeling rotavirus-like particle production: baculovirus DNA replication, mRNA synthesis and protein production. ChemPor 2005 - 9th International Chemical Engineering Conference, Coimbra, Portugal, September 21-23.

Roldão, A., H. L. A. Vieira, C. Estevao, P. E. Cruz, R. Oliveira, M. J. T. Carrondo and P. M. Alves (2005) Modeling integrated production of Rotavirus like particles in the Baculo insect cell system. 7th PEACE conference on Protein Expression in Animal Cells, Crete, Greece, Semptember 18-22

Romão, C. C. (2005) Organometallic Oxo-complexes in Oxygen Transfer Catalysis. Transmediterranean Symposium on Organometallic Chemistry and Catalysis-RENACOM2005, Marrakech, Marrocos.(Invited lecture).

Sá-Leão, R. (2005) Comparison of carriage and disease pneumococcal isolates. 4th PREVIS Meeting, Carcavelos, Portugal, October 15.

Sá-Leão, R. (2005) Epidemiology of pneumococci in Europe: clonal diffusion and antibiotic resistance. French Symposium on the Epidemiology of Pneumococci, Paris, France, March 22.

Sá-Leão, R. (2005) Genetic backgrounds of DRPn and DSPn recovered from colonization sites – Portuguese day care centers. 3rd PREVIS Meeting, Stockholm, Sweden, May 13-14.

Sá-Leão, R., A. S. Simões, S. Nunes, N. G. Sousa, N. Frazão and H. de Lencastre (2005) Population structure of non-typeable Streptococcus pneumoniae. 7th European Meeting on the Molecular Biology of the Pneumococcus, Braunschweig, Germany, May 8-11.

Sá-Leão, R., A. S. Simões, S. Nunes, N. G. Sousa, N. Frazão and H. de Lencastre (2005) Prevalence of non-typeable Streptococcus pneumoniae in carriage and insights into its population structure. 15th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Copenhagen, Denmark, April 2-5.

Sampaio de Sousa, A. R., A. A. F. Matias and C. M. M. Duarte (2005) Development of lipid particles using supercritical carbon dioxide. 10th European Meeting on Supercritical Fluids, Colmar, France, December.

Santos, A. M., M. Oliver, A. M. Sanchez and M. M. Oliveira (2005) Adventitious shoot regeneration in Prunus dulcis – a molecular approach to the regeneration process. COST 843, Stara Lesna, Eslovaquia, June 28 - July 1.

Santos, A. M., M. Oliver, A. M. Sanchez and M. M. Oliveira (2005) Shoot regeneration in Prunus dulcis – a molecular approach to the regeneration process. International Symposium on Biotechnology of Temperate Fruit Crops and Tropical Species, Daytona Beach, Florida, USA, October 10-14.

Santos, H. (2005) Biosynthesis and regulation of chemical chaperones in microorganisms from hot, marine environments. 2nd Iinternational Meeting on Stress Responses in Biology and Medicine, Tomar, Portugal, September 24-28.(Invited lecture).

Santos, H. (2005) Biosynthesis, regulation and applications of compatible solutes of microorganisms from hot, marine environments. International Symposium on Extremophiles and their Applications, Tokyo, Japan, November 29 – December 2.

Santos, H. (2005) Insights into sugar metabolism and its control in lactic acid bacteria - the input from in vivo NMR studies. Symposium on lactic acid bacteria: genetics, metabolism and applications, Egmond aan Zee, The Netherlands, August 28 – September 1.(Plenary lecture).

Santos, H. (2005) Lessons from extreme environments on protein stabilization: novel osmolytes from hyperthermophiles. Annual meeting of the American Chemical Society, Session on “Biocatalysis and Biotransformations Under Extreme Conditions”, San Diego, USA, March 13-17.(Invited lecture).

Santos, H. (2005) Metabolism of Desulfovibrio gigas and rubredoxin stabilization by osmolytes from hyperthermophiles: case studies of my collaboration with Jean LeGall. Metals in Microbial Metabolism, LeGall Symposium, Athens, Georgia, USA, May 6.(Invited lecture).

Santos, J. M., P. Freire, F. Scheller, R. Hengge and C. M. Arraiano (2005) Bacterial polyadenylation links transcription and mRNA degradation. EMBO-Workshop on “Mechanisms and Regulation of mRNA turnover”, Arolla, Switzerland, August.

Schyns, G., S. Potot, Y. Geng, T. A. Barbosa, A. O. Henriques and J. B. Perkins (2005) Novel Bacillus subtilis mutations support the riboswitch mechanism of thiamine gene regulation. Wind River Conference on Prokaryotic Biology, Estes Park, CO, June 7-11.

Schyns, G., S. Potot, Y. Geng, T. A. Barbosa, A. O. Henriques and J. B. Perkins (2005) Novel Bacillus subtilis mutations support the riboswitch mechanism of thiamine gene regulation. International Conference on Functional Genomics of Gram-Positive Microorganisms, La Jolla, CA, June 12-16.

Serra, C., T. M. Barbosa and A. O. Henriques (2005) Sporulation in undomesticated Bacillus subtilis. Bacell Meeting, Paris, France, April 12-15.

Serrano, M., J. Santos, C. P. Moran and A. O. Henriques (2005) Interactions in the pathway of G-containing RNA polymerase activation during development in *Bacillus subtilis*. International Conference on Functional Genomics of Gram-Positive Microorganisms, La Jolla, CA, June 12-16.

Silva Pereira, C., M. C. Basílio, R. Gaspar, G. Soares and M. V. San Romao (2005) Ligninolytic cork colonising fungi consortium and its ability to degrade PCP. Exploitation of fungi. Annual scientific meeting of the Brithish Mycological Society, University of Manchester, UK.

Silva Pereira, C., S. Vitorino, C. Basílico, R. Gaspar and M. V. San Romao (2005) The potential of cork colonising fungi consortia. SEGH CONFERENCE, Paisley, UK.

Simplício, A. L. (2005) Applications of capillary electrokinetic separation methods. 4º Encontro Nacional de Cromatografia, Évora, Portugal, December 12-14.

Souza, S. and E. P. Melo (2005) Thermotropism of ceramide/cholesterol/ionized fatty acid systems in excess water. Thermal Analysis and Calorimetry: throughbred or hybrid? Thermal Methods Group of the Royal Society of Chemistry, Norwich, UK, April.

Teixeira, A., C. Alves, P. M. Alves, M. J. T. Carrondo and R. Oliveira (2005) Hybrid Metabolic Flux Analysis/ Artificial Neural Networks Modelling of Bioprocesses. HIS05 proceedings, Rio de Janeiro, Brasil, November, 6-9.

Teixeira, A., A. Cunha, J. Clemente, P. M. Alves, M. J. T. Carrondo and R. Oliveira (2005) Dynamic modeling and optimisation of a mammalian cells process using hybrid grey-box systems. ICANNGA05 proceedings, Coimbra, Portugal, March 21-23.

Xavier, A. V. (2005) Molecular motors - Energy transducers in biological chemistry. Academia das Ciências de Lisboa, Lisbon, Portugal, May.

Xavier, A. V. (2005) Structural, functional, and thermodynamical characteristics of electroprotonic energy transducers. International Symposium on Metals in Biology and Medicine, Seville, Spain, April.

Xavier, A. V. (2005) Thermodynamic and choreographic constraints of energy transducer. Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Lisbon, Portugal, December.

Scientific events organized by ITQB members

ISA-ITQB Seminar Series 2005
Rita Abranches / Ricardo Ferreira, organizers

"Identification of proteins using mass spectrometry data – practical course"
ITQB, Oeiras, January 2005. Ana M. V. Coelho, organizer (in collaboration with Thermo Finnighan)

Workshop organized on behalf of Rede Nacional de Espectrometria de Massa
ITQB, Oeiras, May 2005. Ana M. Varela Coelho, organizer (in collaboration with FCUL and Univ Aveiro)

Workshop "Membrane Proteins: the rocky road from gene to structure"
ITQB, 19-24 September 2005. Professor So Iwata (IC London, UK) / Margarida Archer (ITQB, Oeiras, Portugal), organizers

Meeting of European STREP Project "Signaling and Traffic"
7-8 October, ITQB. Julia Costa, organizer

FEBS Forum for Young Scientists
Visegràd, Hungary, Budapest June 30th – 2nd July, 2005 as a satellite meeting of the FEBS congress C. Rodrigues-Pousada, organizer

IX Portuguese- Spanish Congress on Plant Physiology
Évora. 18-21 Sep 2005. M M Chaves, President of the Congress.

2nd International congress in Cell Stress Responses in Biology and Medicine
September 24th-28th, Tomar, Portugal. C. Rodrigues-Pousada, organizer

INTERDROUGHT-II - The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress
Rome, Italy, September 24-28, 2005. Manuela Chaves, member of the International Steering Committee

6th International Meeting of the Portuguese Carbohydrate Group, 3rd Iberian Carbohydrate Meeting
Coimbra, September 2005. Júlia Costa, member of the Scientific Committee

International Symposium on "Biotechnology of Temperate Fruit Crops and Tropical Species"
Daytona Beach, Florida. 10-14 October 2005. Margarida Oliveira, member of the Scientific Committee

4th PREVIS Meeting
Carcavelos, Portugal, October 14-15. Herminia de Lencastre, organizer.

6th Short course of the Portuguese Biophysical Society "From cellular transport to neurosciences: a biophysical perspective"
14-16 of October 2005, Santarém, Portugal. Cláudio Soares, organizer

International Conference on "Plant Genomics and Biotechnology: Challenges & Opportunities"
Raipur, India. 26-28 October 2005. Margarida Oliveira, member of the Scientific Committee

3rd Annual Meeting of Portuguese Proteomic Network-Procura
Cascais, November 2005. Ana M. V. Coelho, organizer

Preventive and remediation strategies for continuous elimination of poly-chlorinated phenols from forest soils and ground waters -Project meeting
Participating Countries: Tunisia, Portugal, Morocco, England and Italy (NATO PROGRAMME FOR SECURITY THROUGH SCIENCE FOR PEACE PROPOSAL). Vitória S. Romão, organizer

PhD Theses defended at ITQB in 2005

Susana de Almeida Mendes Vinga Martins
25.02.2005
Biological sequence analysis by vector maps: alignment-free comparison of DNA and proteins
Supervisor: Jonas Almeida
Biology

Tiago Miguel Guerra Miranda Bandeiras
29.03.2005
Archaeal Respiratory Chains - Coupling the sulfur to oxygenic metabolism
Supervisor: Miguel Teixeira
Biochemistry

Cristina Maria Neves da Silva
31.03.2005
Mechanisms underlying flowering in Almond (Prunus dulcis): a molecular perspective
Supervisor: Margarida Oliveira
Biology

Alexandre Marnoto de Oliveira Campos
01.04.2005
Analysis of Protein Expression in Olea europaea L. , a Proteomic Approach
Supervisor: Pedro Fevereiro
Biology

Susana Isabel Lopes Claro Tereso
18.04.2005
In vitro culture system for clonal propagation and genetic transformation of maritime pine (Pinus pinaster Sol.)
Supervisor: Margarida Oliveira
Biology

Maria Manuel Machado Lopes Sampaio
06.06.2005
Engineering Escherichia coli for the Synthesis of Mannosylglycerate, a solute widely distributed in (hyper)thermophiles
Supervisor: Helena Santos
Biochemistry

Patrick de Oliveira Freire
20.06.2005
Characterization of the gene bolA and its function in cell morphology and survival
Supervisor: Cecília Arraiano
Biology

Isabel Maria Teiga Zilhão
24.06.2005
Nuclear, Plastidial and Mitochondrial Polymorphisms in Portuguese Olive Cultivars
Supervisor: Pedro Fevereiro
Biology

Tiago Quininha Faria
08.07.2005
Understanding Protein Stabilization by Solutes from Hyperthermophiles: Effect of Mannosylglycerate on Protein Unfolding Thermodynamics and Enzyme Activity
Supervisor: Helena Santos
Biochemistry

Miguel Pedro Januário Pessanha
26.09.2005
Structural, thermodynamic and functional characterization of multihaem cytochromes isolated from Shewanella and Geobacter species
Supervisor: António Xavier
Biochemistry

Ana Sofia de Sousa Valente Coroadinha
06.10.2005
Improved Strategies for the Developement of Retroviral Gene Theraphy Vectors: from the producer cell to the viral vector
Supervisor: Manuel Carrondo
Chemical Engineering

André Martinho de Almeida
21.10.2005
Using the TPS1 gene from Arabidopsis thaliana to increase abiotic stress tolerance in plants
Supervisor: Manuel Pedro Fevereiro
Biology

Maria Manuela Parreira Broco
21.11.2005
Desulfovibrio gigas Flavoredoxin. A flavin reductase involved in thiosulfate metabolism
Supervisor: Claudina Rodrigues Pousada
Biology

Sara Cristina de Almeida Pinto Garcia
02.12.2005
Phase Space Reconstruction of Multivariate Time Series from Molecular and Field Biology
Supervisor: Jonas Almeida
Biology

Francisco Rodrigues Pinto
05.12.2005
Gene regulatory networks – tools for an enhanced understanding
Supervisor: Jonas Almeida
Biochemistry

Mónica Rodrigues Fortunato Hilário
12.12.2005
Cerebral networks for memory strategies and Dissociation of new and old memories in posterior cingulate
Supervisor: António Xavier/Joy Hirsch
Biology

Teresa Parente Madureira de Vasconcelos Costa
13.12.2005
Assembly of the Bacillus subtilis spore coat
Supervisor: Adriano O. Henriques
Biology

Gonçalo Bruno Ferreira Real Rodrigues Carvalho
15.12.2005
Cell division and chromosome segregation in Bacillus subtilis
Supervisor: Adriano O. Henriques
Biology

Sónia Cláudia Morgado Gonçalves
16.12.2005
Embryo development in maritime pine – a differential gene expression approach
Supervisor: Célia Miguel
Biology

Maria Luísa de Ornelas Sieuve de Azevedo Soares Rodrigues
19.12.2005
Crystallographic studies on β-cinnamomin, catechol-O-methyltransferase and cytochrome c nitrite reductase
Supervisor: Mª Arménia Carrondo/Margarida Archer
Biochemistry

Tracy Laura Nevitt Gonçalves
20.12.2005
Towards understanding the functional role of Yap4 in the yeast response to hyperosmolarity
Supervisor: Claudina Rodriques Pousada
Biochemistry

ONGOING RESEARCH PROJECTS

Project nº	Project title	Project Leader	Duration
Projects funded by FCT:			
BME/35109/99	Analysis of an intercellular signalling pathway coupling gene expression to morphogenesis in Bacillus subtillis	Adriano Henriques	2000-2005
BIO/36203/99	Bola confers resistance to antibiotics: study of this new target focusing on the control of cell division and protein production	Cecilia Arraiano	2001-2005
BSE/34794/99	Dynamic invariance in biological systems	Jonas de Almeida	2001-2005
QUI/42902/01	Structural and functional mapping of a novel multihaem cytochrome: a model for fumarate reductases	Carlos A. Salgueiro	2002-2005
QUI/37521/01	Understanding the intrinsic stability mechanisms of hyperthermophilic metalloproteins	Cláudio Gomes	2002-2005
QUI/38269/01	Biphasic hydrogenation and oxidation of super-critical carbon dioxide	Manuel N.Ponte	2002-2005
QUI/43435/01	Binding of diatomic molecules to haem proteins	Teresa Catarino	2002-2005
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	2002-2005
BCI/38861/01	Modelling biomembrane rafts: characterization of the physical-chemical properties of lipid bi-layer 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 novel 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	2002-2006
AGR/39095/01	Coat protein mediated resistance against almond llarviruses: strategy and risk assessment	Margarida Oliveira	2002-2005
AGR/38507/01	Adventitious organogenesis in almond: an histological and molecular approach	Ana Sanchez	2003-2006
BIO/43105/01	Molecular basis of the functioning of immobilised redox enzymes in bioelectronic devices	Daniel Murgida	2003-2006
BME/37406/01	Unravelling the acrobic respiratory chain of the “anaerobic” sulfate reducing bacteria	Miguel Teixeira	2003-2006
BME/37480/01	Genes involved in bioenergetic mechanisms in the sulphate reducing bacterium Desulfovibrio gigas	Claudina R. Pousada	2003-2006
CBO/39099/01	Structure and function of the centrosomal proteins HsMob in cell division	Mª Arménia Carrondo	2003-2006
MGI/40878/01	In search of new molecular targets for the development of novel therapeutic strategies for cystic fibrosis	Ana Coelho	2003-2005

Project nº	Project title	Project Leader	Duration
BME/44597/02	Reduction of nitric oxide in prokaryotes: new metabolic routes	Miguel Teixeira	2003-2006
BME/45810/02	Increasing realism in protein modelling: including pH and redox effects into molecular dynamics simulations	António Baptista	2003-2006
EQU/35437/99	Green processing with ionic liquids coupled to supercritical CO2 extraction or membrane pervaporation	Luis Paulo Rebelo	2003-2006
ESP/44782/02	Anaerobic metabolism of the human pathogen Bilophila wadsworthia	Inês Cardoso Pereira	2003-2006
QUI/45090/02	Structure, tropisms and molecular dynamics of the stratum corneum lipid matrix. A study in model systems	Eurico de Melo	2003-2006
QUI/45758/02	Thermodynamics of metalloprotein folding and stability	Cláudio Gomes	2003-2006
ESP/46428/02	Structural characterization of MrkD protein from Klebsiella pneumoniae: implications in the epithelial adhesion properties of the microorganism	Francisco Enguita	2003-2006
BME/45122/02	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	2003-2006
QUI/47866/02	Oxidative Phosphorylation in sulfate respiration	António V. Xavier	2003-2006
BCI/48647/02	Dissection of a checkpoint linking chromosome 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 lactic acid bacteria for the construction of strains important for food processing by the dairy industry	Cecilia Arraiano	2003-2006
BIO/48333/02	Global experimental approaches to model central metabolism in L. lactis: modulation of the levels of key-enzymes	Helena Santos	2004-2007
CBO/43952/02	Clinical, neurophysiological and neurochemical studies in amyotrophic lateral sclerosis	Júlia Costa	2003-2006
AGG/41359/02	Reforestation with cork oak: genetic variability and seed storage biology	C.Pinto Ricardo	2003-2006
QUI/49114/02	Macrocyclic compounds selective for heavy metals poisoning: Cd(II), Hg(II), and Pb(II)	Rita Delgado	2003-2006
BIO/42331/02	New compatibles solutes from thermophiles and hyperthermophiles: screening, identification and physiological role	Helena Santos	2003-2006
AGG/46371/02	Searching for rhizobial strains with improved skills to thrive in arid lands	Helena Santos	2004-2007
MGI/47382/02	Folding, processing and function of normal and mutant cystic fibrosis transmembranar conductance regulator: structural implications	Cláudio Soares	2003-2006
BIA-MIC/55106/04	The role of RNase and its homologues in the control of gene expression: structural and functional studies	Cecilia Arraiano	2005-2008

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QUI/555862/04	Rhenium, molybdenum and tungsten oxo complexes: new class of catalysts for reduction reactions	Beatriz Royo	2005-2008
BIA-PRO/55621/04	Structural characterization of membrane proteins of the respiratory chain of a thermoacidophilic organism	Margarida Frazão	2005-2008
AGR/55651/04	Role of defense-related genes during the establishment of root-nodule symbioses between higher plants and nitrogen-fixing bacteria	Ana Ribeiro	2005-2008
QUI/55690/04	Characterisation of membrane bound cytochrome involved in the anaerobic respiration in sulphate reducing bacteria	Ricardo Louro	2005-2008
SAU-IMI/55729/04	Structure function studies of murine Toll-like receptors: activation of the innate immune response	Mª Armenia Carrondo	2005-2008
BIA-BCM/55792/04	Transgenic plants as models to study regulation of transgene expression and recombinant protein deposition	Rita Abranches	2005-2008
BIA-BCM/56063/04	Transcription factors regulating abiotic stress response in rice (Orysa sativa): a transgenic approach to improve tolerance, and search for novel players	Nelson Saibo	2005-2008
SAU-IMI/56088/04	Interactions between proteins in adjacent sister cells that signal the activation of RNA polymerase in response to cellular morphogenesis	Ligia Saraiva Teixeira	2005-2008
BIA-BCM/56493/04	Organizations of the staphylococcal cell wall synthetic machinery	Mariana Pinho	2005-2008
SAU-IMI/56501/04	Role of bacterial cell wall on the host innate immune response	Sérgio Filipe	2005-2008
QUI/56569/04	Molecular recognition of phthalate and phthalic acid esters pollutants by ditopic receptors by cascade dicopper systems	Rita Delgado	2005-2008
AGR/56658/04	Molecular markers for Portuguese pine wood quality	Pedro Fevereiro	2005-2008
BIO/56659/04	Improving tolerance to water stress in legumes using the model Medicago truncatula	Pedro Fevereiro	2005-2008
CVT/56811/04	The role of small non-coding RNAs and RNases on the pathogenicity de Salmonella	Cecilia Arraiano	2005-2008
BIO/57083/04	Nano-Engineering of bacterial laccases	Ligia Martins	2005-2008
AGR/57157/04	Functional characterization of genes related to nitrogen metabolism in genetically modified maritime pine	Susana Tereso	2005-2008
BIA-PRO/57263/04	Structural determinants of protein stabilization by compatible solutes from hyperthermophiles: in search of guidelines solute improvement	Helena Santos	2005-2008
AMB/57374/04	Bioremediation of PCP by the co-metabolism of cork endogenous moulds	Cristina Silva Pereira	2005-2008
SAU-ESP/57841/04	Staphylococcus aureus and Staphylococcus epidermidis: links between hospital and community	Herminia de Lencastre	2005-2008

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AGR/57994/04	Maize “broa” quality attributes: Identifying genes that affect the technological ability for bread production.	Carlota Vaz Pato	2005-2008
SAU-ESP/58030/04	Epidemiology of multidrug resistant enterococci in a Lisbon Hospital – Surveillance study in malignancy ward	Rosario Mato Labajos	2005-2008
BIA-PRO/58374/04	Complexes I from the respiratory chains of the thermohalophilic bacterium Rhodothermus marinus and of the Cyanobacterium Synechocystis sp PCC6803, model systems of the mitochondrial and chloroplastidial complexes I	Manuela Pereira	2005-2008
BIA-MIC/58416/04	Regulation of cell wall synthetic genes and enzymes in B-lactam resistant Staphylococcus aureus	Herminia de Lencastre	2005-2008
BIO/58465/04	Screening hyperthermophilic proteomes for hyperstable proteins	Cláudio Gomes	2005-2008
BIA-PRO/58608/04	Heme-copper oxygen reductases – mechanisms of electron/proton transfer and oxygen reduction	Miguel Teixeira	2005-2008
BIO/58652/04	Characterization of metal and sulphur respiratory chains in a marine organism targeted for bioremediation applications	Ricardo Louro	2005-2008
BIA-PRO/58722/04	Characterization of CymA: a focal protein in anaerobic respiration by Shewanella	Ricardo Louro	2005-2008
QUI/58985/04	Cytochrome c: A model protein to probe thermodynamic and choreographic constraints in electroprotonic energy transducers	António V. Xavier	2005-2008
AGR/59079/04	Understanding defence responses of grapevine to drought stress-metabolic regulation at the leaf and berry levels	Manuela Chaves	2005-2008
BIA-MIC/59310/04	Strategies of life adaptation to hot environments: heat and osmotic stress responses in the extremely thermophilic bacterium Rhodothermus marinus	Helena Santos	2005-2008
QUI/59824/04	Studies on quinine-protein interaction in complexes of respiratory chains	Manuela Pereira	2005-2008
AGR/60236/04	Molecular characterization of a microbial hemicellulolytic system	Isabel Sá Nogueira	2005-2008
BIA-MIC/60320/04	Transcriptional control of the mecA gene, the central element of methicillin-resistance in staphylococci.	Duarte de Oliveira	2005-2008
BIA-BCM/60855/04	Interactions between proteins in adjacent sister cells that signal the activation of RNA polymerase in response to cellular morphogenesis	Adriano O. Henriques	2005-2008
BIA-MIC/61140/04	Mechanisms of repression by AraR, a key regulator of carbohydrates utilization in Bacillus subtillis	Isabel Sá Nogueira	2005-2008
EQU/61550/04	Process integration of supercritical fluid extraction and membrane separation to recover “Vegetal” squalene from olive oil residues	Rui Ruivo	2005-2008
QUI/62794/04	Studies on the synthesis and applications of 2-Oxoaza [x.1.0] bicycles	Christopher Maycock	2005-2008

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AGR/61980/04	Constraints to carbon gain by tree age in Eucalyptus globules (Labill.) stands.	Manuela Chaves	2005-2008
BIO/57193/04	Rationalization of cutinase enantioselectivity in nonaqueous media	Cláudio Soares/ Isabel Sá Nogueira	2005-2008
QUI/56585/04	Mechanism and kinetics of protein stabilization by osmolytes.	Helena Santos	2005-2008
AMB/56075/04	Metabolism and characterization of mixed cultures in wastewater processes for simultaneous removal of nitrogen and phosphorus	Helena Santos	2005-2008
QUI/55985/04	Synthesis, structure and reactivity of transition metal complexes with potential application in oxidative catalysis	Carlos Romão	2005-2008
SAU-IMI/55112/04	Gene expression changes during hepatitis delta virus infection I. Analysis of the cellular proteome.	Ana Coelho	2005-2008

Projects funded by FCT, under the Re-Equipment call:

REEQ/122/AGR/2005	Interaction at the molecular level between vines and fungi	Ricardo Ferreira	2005-2006
REEQ/336/BIO/2005	Structure, Dynamics and functions of proteins	Miguel Teixeira	2005-2006
REEQ/374/BIO/2005	Plant development under environmental controlled conditions to study the response to biotic and abiotic stresses, at the genomic, physiological, biochemical and structural levels	Cândido Pinto Ricardo	2005-2006
REEQ/392/BIO/2005	A platform for protein expression profiling, cell mapping, and the analysis of protein-protein interactions	Adriano O. Henriques	2005-2006
REEQ/834/BIO/2005	Study of plant response to stress using Thermography and Fluorescence Imaging	Manuela Chaves	2005-2006
REDE/1504/REM/2005	National Facility for Mass Spectrometry	Ana Coelho	2005-2006
REDE/1517/RMN/2005	National Facility for High-Field Nuclear Magnetic Resonance	Helena Santos	2005-2006

Projects funded by FCG:

55068/02	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
61052/03	Creation of a reference collection of antimicrobial resistant gram-positive bacteria serving the national and international scientific and clinical communities	Herminia Lencastre	2004-2006
65882/04	Infection and colonization by multidrug-resistant Enterococci recovered from neonatal intensive care units. Epidemiological surveillance and infection control	Rosario Labajos	2004-2007

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Projects funded by European Commission			
QLK3-CT-2002-01938	Phototrophic biofilms and their potential applications: towards the development of a unifying concept (PHOBIA)	Jonas Almeida	2002-2005
QLG2-CT-2002-00988	Structural proteomics in Europe (SPINE)	Mª Arménia Carrondo	2002-2006
LSHM-CT-2003-503413	Molecular mechanisms of resistance, virulence and epidemicity in Streptococcus pneumoniae (PREVIS)	Hermínia de Lencastre	2004-2006
C O O P - C T - 2003-508644	New applications for compatible solutes from extremophiles (HOTSOLUTES)	Helena Santos	2004-2007
LSHG-CT-2004-503228	Signalling and membrane trafficking in transformation and differentiation (SIGNALLING AND TRAFFIC)	Júlia Costa	2004-2007
INCO-CT-2004-509163	Water resources strategies and drought alleviation in western Balkan agriculture (WATER-WEB)	Manuela Chaves	2004-2007
RICA 505977	European macromolecular crystallography infrastructure network 2 (MAX-INFO 2)	Mª Arménia Carrondo	2004-2009
Projects funded by Rockefeller University			
	Evolution and acquisition of drug resistance in MRS	Sérgio Filipe	2005-2008
	Pathogen – specific drug targets for weaponized bacteria	Adriano O. Henriques	2005-2008

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