Instituto de Tecnologia Quimica e Biológica

Annual Report and Plan

2006 | 2007

Index

Foreword

ITQB is a Research and Post-Graduation Institute from the Universidade Nova de Lisboa, which started its activities in 1989. At that time, ITQB was designed with a quite innovative and unique vision of what a Research Institute should be. Several key points, or leitmotifs, were introduced which are now as actual as they were then: a scientific multi and inter-disciplinary environment in chemistry, life sciences and associated technologies, which allowed to establish in Portugal a set of expertises which are (still) unique; the set-up of up-to-date scientific infrastructures and services to support research; a flexible scientific management, avoiding a negative "Departmentalisation" of its research objectives; the concept of an Open Institution, hosting researchers from any Portuguese or foreign Institution to develop their research projects at ITQB; the introduction, in its Statutes and Regulations, of a General Advisory Board, mainly composed by external members, supported by a Consultive Committee, which among other duties would act as a search committee for the ITQB Director. Latter, it was added an External Advisory Board, constituted only by foreign Scientists. Also since the very beginning ITQB was associated to the Instituto de Biologia Experimental e Tecnológica, IBET, which had as a mission to act as an interface to the economic world, transferring knowledge to the economic sector and fostering collaboration with industry.

It is remarkable that the Vision of its founders should now be part, in its basic aspects, of the recently proposed framework for the re-organization of the Portuguese Universities, a document that has just been approved by the Portuguese Council of Ministers.

Almost eighteen years have passed since ITQB started its activities. The Portuguese Scientific landscape has changed considerably, and is now closer to encompass with the best Institutions in Europe and worldwide. At the present, ITQB is one of the largest Research Institutions in Portugal, both by itself and, most importantly, through its association with the Instituto Gulbenkian de Ciência (IGC) and the IBET in the framework of the Associate Laboratory Contract.

Novel challenges are arising within the Universities and the System of Science and Technology in Portugal. It is vital to any (scientific) organization to continuously improve, change and question its own performance, i.e., to be dynamic, even utopic, to continuously create its future.

Aiming at the never ending objective of increasing the quality of the research performed at the Institute, the present Direction, which started its functions on the 1st of August of 2006, has proposed to the ITQB Scientific Council, in October 2006, a set of measures, both at the Scientific and Management levels, to act pro-actively and to respond to the new challenges. Several of these measures have already been implemented and others are in progress:

• To implement a profound change in the managerial structure of the Institute, making it more efficient, professional, and flexible, adjusting the Services to their function - the support of the Research Activities. Any organization needs professional management, and should follow the best practices of the private sector. An external audit was performed, which will now enable to proceed with a re-organization of the management services, both at the administrative and at the science planning levels. This, of course, will involve a change of part of the ITQB Bylaws, now made possible within the new general framework of University governance.

• To implement a culture of participation of all researchers in the activities of the Institute. Citing Benjamin Franklin "*Tell me and I forget, show me and I remember, involve me and I understand*". This is a never ending effort, if an Institution wants just to stay alive. Challenging people to have a

Foreword

"*corpus*" attitude (but not corporative), to discuss and share the scientific strategy of ITQB is a very crucial issue.

• To largely improve the flow of information, making all decisions clear and transparent to all members of the Institute, and to establish a minimal set of regulations needed in any organization, so that all procedures, obligations and rights are known to everyone.

• To involve the younger researchers in the Institute activities, by meeting with them, challenging them with the launch of several initiatives, such as a Mentoring and Career Development Programme and to develop the ITQB Alumni, for cross fertilization between ex- students and the Institute.

• To reinforce a culture of quality, by including a method for self-evaluation and creating a larger and renovated External Advisory Board.

• To introduce parameters/indicators of accountability at all levels.

• To establish an ethical perspective in the research and educational framework, not only in terms of research-based ethical issues, but also of an ethical behaviour at all levels.

• To completely re-structure the ITQB PhD course, now based mainly on Scientific Themes, allowing all new PhD students to get acquainted with the multidisciplinarity intrinsic both to the Institute and to a modern approach to the Life Sciences, and trying also to open their views in terms of Ethics, Entrepreneurship or Science Policy. The PhD course counts also with Professors external to ITQB, which should in the future lead to a multi- institutional Doctoral Program.

• To continue the re-organization of the Analytical Services, which now form a single unit together with IBET, under GLP rules, and subjected to technical audits.

• To reinforce activities in *Public Awareness of Science* on a "for all citizens" base while maintaining a strong commitment towards the education of the young students. In the present day's society, scientific knowledge is part of true citizenship and an imperative for social development. These activities should involve a close interaction with all the stakeholders in order to change the societal and political attitude towards science.

Many of these changes are already in progress. They (will) face internal and external resistance, but more than keeping it, this is a question of continuously improving the performance of the Institute, by creating a culture of merit, a new dynamics, finding the strength to face the still existing restrictive legal framework. Rather than waiting for others to do it for us, the challenge, let's say it again, is to force the future to happen now. In terms of the Theory of the Organizations, *through systems thinking, shared visions and individual commitment, organizations can rise to meet new challenges, in a cohesive manner.*

The discussion of the Scientific Strategy was the most recent challenge put to the ITQB Researchers; the discussion is still going on, and therefore I shall present you with my own perspective. This strategy has to start by recognizing the already established competences at the Institute, as a multidisciplinary institution mainly devoted to fundamental research in the Life Sci-

ences, putting together expertises in chemistry, biochemistry and biology, including a unique set of complementary tools for a deep molecular understanding of biological systems. In particular, ITQB is certainly the largest Portuguese centre in Bio-spectroscopy and in Structural Biology. The main research fields at the Institute are Microbiology, from molecular genetics, to physiology, metabolic engineering, protein chemistry, cellular development and pathogenesis; Computational biology, presently at the level of Biomolecular modelling; Plant physiology, genetics, development and biotechnology; Chemistry, namely of biologically relevant compounds, including pharmaceuticals, and of Ionic Liquids, on a fundamental and biologically relevant application levels; Technology of animal cells, development of vaccines and virology. In short, **Cell and developmental Biology, Structure and function of Proteins, Microbiology and Pathogenesis, Biotechnology of Animal cells and Plants**. What should be changed? In my view, increase the interaction of the Chemistry Laboratories with the Biological Sciences and introduce modern technologies, namely at the "single molecule" level; increase the research at the molecular level of pathogenesis (host-pathogen interactions); further develop Computational Biology, in Computational Genomics and "Systems" or "Integrative" Biology; increase the level of molecular complexity on the biological side, enabling to "close" the cycle between the molecular and the organism levels; re-enforce basic research in Plant Sciences; and finally, re-enforce the spectroscopic tools, essential at all levels of biological complexity, and the research and development in Mass Spectrometry. It should be emphasized that there is a very poor culture of instrumental development, in general in Portugal, but also at ITQB. All of these areas were contemplated in the *Concurso Internacional for PhD researchers* submitted by the three Institutions of the Associated Laboratory, in which we had a considerable success; the set of new positions we got through this proposal, will enable to implement several of the above listed changes, by choosing the best candidates to each position and, again, by a correct monitoring of the achievements of each new researcher, as well as their support and mentoring.

Would it be also advisable to install at the Institute more research fields, particularly at the Molecular Medicine level? I do not think so. ITQB is an Open Institution, in a very wide sense: those competencies already exist or are being developed at other Institutions including the ones within our Associate Laboratory. Therefore, what is now necessary is i) to foster a more coherent scientific management at the level of the LA, keeping the goals of each partner. The strength of the LA at Oeiras lays on strategically managing its intrinsic diversity; and ii) to foster the collaborations, at the level of *Consortia*, or *Networking*, with strategic Portuguese or Foreign Institutions, within the Universidade Nova de Lisboa but also with other Research Centres.

I will finish this already long Foreword with a positive vision for our future: ITQB and its Associated Laboratory have been continuously increasing their activities and scientific productivity. We have maintained our level of Excellence within Portugal, and this can be proved by using any type of analysis, bibliometric or others such as number of projects approved or PhD student and Posdoc grants. Now, the challenge is not to maintain this level, but rather to reach "Excellence" at the European Level, through the implementation of a culture of merit, of a good strategic management, but keeping also always in mind a citation I cherish by Max Perutz "*I rarely plan my research, it plans me*". This means that the scientific endeavour is not, by definition, predictable, and that it is through freedom in fundamental research that new discoveries are achieved, which may later be used by technology-oriented Institutes or by private companies for social development, if conditions are provided to strengthen transfer of knowledge leading to innovation. Also, by seeking novel or emergent scientific areas, i.e., by having a flexible structure that will allow to keep pace with the forefront of science.

Also, it is urgent to eliminate the "artificial" distinction between "Professors" and "Researchers"- in

Foreword

an Institution such as ITQB, and in spite of the still existing legal restrictions, all should behave as "Research Professors", with the corresponding rights and duties. It is also urgent to increase the number of permanent positions at ITQB – with its present size, nine permanent Professor positions is incompatible with ITQB missions. But "permanent" must necessarily be accompanied by fair and independent evaluation.
Finally, in a more general view of the University system, it is urgent to go back to what should be the Mission of the University – a place where culture and knowledge are generated, transferred to the students and to the society – and break the excessive "professionalization" or "specialization" that leads to a global loss of Culture (as the vital system of ideas at each time). To create again, in the good sense, a "School", open to the society, its changes, innovative, but leading to authentic and well informed citizens. This is also a political and sociological problem, that should be elaborated elsewhere.

This vision for ITQB will also be the best tribute we may pay to the memory of Antonio V. Xavier, the founder of the Institute. His own Vision for the development of Science in Portugal led to ITQB, a venture made only possible through very strong perseverance and strength to fight for convictions. António Xavier knew for sure that Portugal is a Country for good science.

As a final personnal note, I lost a life-long mentor. But did not loose his memory, his lessons, namely that only acting with strength, but also with honesty and idealism, we may continue to develop our Institution.

Miguel Sepúlveda Teixeira
Oeiras, 9 May 2007

"Science never gives up searching for truth, since it never claims to have achieved it. It is civilizing because it puts truth ahead of all else, including personal interests. These are grand claims, but so is the enterprise in which scientists share. How do we encourage the civilizing effects of science? First, we have to understand science. Scientia is knowledge."

"It is folly to use as one's guide in the selection of fundamental science the criterion of utility. Not because scientists despise utility. But because useful outcomes are best identified after the making of discoveries, rather than before".

John C. Polanyi. Nobel Prize for Chemistry, 1986

ITQB at a glance

Research Institute of Universidade Nova de Lisboa

137 PhD holders
154 PhD students
Director: Miguel Sepúlveda Teixeira

Laboratório Associado ITQB/IGC/IBET

600 researchers
11 biotechnology companies
14 active patents

Multisdisciplinary research

50 independent laboratories
99 ongoing research projects
207 papers published in 2006

Large Research Infrastructures

GLP Certified Analytical Services

Advanced Training

ITQB PhD program
Masters Course
20 PhDs awarded in 2006

Introduction

The Instituto de Tecnologia Química e Biológica (ITQB) is a scientific research and advanced training centre from Universidade Nova de Lisboa. Its mission is to develop scientifically recognized research in chemistry and the life sciences, considering all levels of complexity and its potential applications, so as to contribute to the understating of life's mechanisms and ultimately benefit the whole society. Its highly multidisciplinary nature and open atmosphere make ITQB a leading centre in advanced training for researchers in Portugal.

The origins of ITQB go back to 1986 when the concept of a new research centre was developed and took shape through a process led by Professor António V. Xavier. He was, at the time, the right person at the right moment and he was able to make the most out of the political and economical positive environment in Portugal. With the help of a group of political leaders that clearly understood the advantages of such a project to the development of the Portuguese Science and Technology, CTQB (Centro de Tecnologia Química e BiológicaI) was finally launched.

Operating since 1989, CTQB started its activities with a few research groups setting their laboratories at the Instituto Gulbenkian de Ciência (IGC), in Oeiras, nearby the new building future location.

Headed by Professor António V. Xavier since its foundation until 1999, this research centre became Instituto de Tecnologia Química e Biológica in 1993 when it was integrated in Universidade Nova de Lisboa.

In 1996, both ITQB and its associate institution IBET (Instituto de Biologia Experimental e Tecnológica) – a private, not-for-profit biotechnology institution - started to operate in the present site, in the campus of Estação Agronómica Nacional, in Oeiras. The new building, designed by the architect Gonçalo Byrne and awarded the Prémio Municipal de Arquitectura da Câmara Municipal de Oeiras in the same year, hosts most of the research groups and all administrative and support services; a few groups have remained in the previous location at IGC or otherwise use laboratory space from the Estação Agronómica.

The important contribution of ITQB in research and development is being maximized since 2001 when ITQB, IGC and IBET joined to form one of the first Laboratórios Associados in Portugal, a status attributed by the Minister of Science and Technology to scientific institutions recognized as excellent by international panels.

Since its foundation, ITQB has grown considerably in size and nowadays hosts 50 independent research groups, forming a scientific staff of more than 300 researchers. The researchers are assisted in their activities by the infrastructure support services that include around 60 people (see details in "Organization of the Institute")

The Institute has been supported over the years by the state budget and the Laboratório Associado contract but mainly through research grants awarded to its members in national or international calls from R&D funding agencies. The quality of the research conducted at ITQB is reflected by the ability of the researchers to attract such funds and is demonstrated in the more than 1000 papers published and over 9000 corresponding citations since 2001.

Over the years, as ITQB expanded, the organization of the Institute has evolved to its present structure. The Institute is headed by its director and two vice-directors assisted in all scientific matters by a representative committee of the Scientific Council which is formed by all PhD holders at ITQB for more than two years. The existence of an External Advisory Board constituted by renowned scientists from different areas and the regular internal and external evaluations to which the Institute is subjected assures that scientific excellence is the motto at ITQB.

António Xavier – the man who built ITQB

Professor António Xavier (1943-2006) became the President of the Comissão Instaladora of ITQB in 1986. He shaped the institution that is now ITQB, starting in 1989 with scientists invited to join him in laboratories loaned by the Gulbenkian Foundation, and oversaw the design, construction, and staffing of the new ITQB building. He stepped down after a decade of tireless effort and handed over ITQB as a fully operational research institute.

António Xavier finished his degree in Chemical Engineering at Instituto Superior Técnico, in 1969, and worked for a short time at IGC, Oeiras. He moved to the Inorganic Chemistry Laboratory, Oxford, where he finished his PhD in 1972 under the supervision of Prof. Robert J. P. Williams. His work pioneered the use of paramagnetic ions in NMR to determine the structure of biological molecules in solution. He returned to Portugal and was one of the founding members of the Universidade Nova de Lisboa. At the same time, António Xavier launched and directed the Molecular Biophysics Group at the Centro de Química Estrutural (Complexo Interdisciplinar I, at Instituto Superior Técnico). This group soon became one of the most productive in Portugal. His modern scientific attitude to life sciences was immediately apparent, and a multidisciplinary approach, using several biochemical and spectroscopic (NMR, EPR, UV-Visible) methods to tackle fundamental biological problems, was standard in the group. His continuous effort to provide the best possible conditions for research was also evident, with a never ending struggle for funding. He internationalised the research by establishing close collaborations with prestigious groups in France, England, and the United States and organising several International Conferences in Portugal in the area of Bioinorganic Chemistry. In 1986, he started a new battle - developing a unique research institute that became ITQB, bringing together a wide range of basic research in the fields of chemistry, biology, and biochemistry. As founding President of IBET (1989-1991), he also gave ITQB an interface with industry.

The best measure of António Xavier's success in building this institute is where ITQB stands now: a well equipped Research Institute, with a largely interdisciplinary environment, and which has always been classified as Excellent by external evaluation panels.

Organization of the Institute

DIRECTORATE

Miguel Sepúlveda Teixeira	Director
Cláudio M. Soares	Vice-Director

COORDINATION COMMITTEE OF THE SCIENTIFIC COUNCIL (CCSC)

Directorate	Miguel Teixeira (Director) Cláudio Soares (Vice-Director) Rosina Gadit (Secretary to the SC)
Chemistry Division	Chris Maycock (Head of Division) Carlos Romão (Eurico Melo)
Biology Division	Hermínia de Lencastre (Head of Division) Helena Santos (Adriano Henriques)
Biological Chemistry Division	Maria Arménia Carrondo (Head of Division) Inês Cardoso Pereira (Manuela Pereira)
Technology Division	Luis Paulo Rebelo (Head of Division) Teresa Crespo (Abel Oliva)
Plant Sciences Division	Cândido Pinto Ricardo (Head of Division) Margarida Oliveira (Phil Jackson)
IBET Representative	Manuel J.T. Carrondo (Paula Alves)

Organization of the Institute

INVITED AND VISITING PROFESSORS

Alessandro Giuffrè, Università di Roma “La Sapienza”, Italy	Bioenergetics
Alexander A. Konstantinov, Moscow State University, Russia	Bioenergetics
Alexander Tomasz, The Rockfeller University, USA	Microbiology
Daniel H. Murgida, Technische Universität Berlin, Germany	Raman spectroscopy
David Edward Onions, Invitrogen Corporation	Virology / Vectorology
David L. Turner, Univeristy of Southampton, UK	Structural NMR
Hansjörg Hauser, Gesellschaft für Biotechnologische Forschung GmbH.	Eukaryotic Molecular Biology
John G. Aunins, Merk Research Laboratories, West Point, USA	Bioprocess Engineering
Jonas Almeida, University of Texas, USA	Biomathematics
José Canongia Lopes, Instituto Superior Tecnico, UTL	Molecular Simulation
Kenneth R. Seddon, The Queen´s University of Belfast, UK	Ionic Liquids
M. Teresa Duarte, Instituto Superior Técnico, UTL	Small Molecule X-Ray Cristallography
Peter F. Lindley, Birkbeck College London, UK	Structural Biology
Peter G. Hildebrandt, Technische Universität Berlin	Raman Spectroscopy
Winchil L. Cláudio Vaz, Universidade de Coimbra	Biophysics
Winfried Boos, Universität Konstanz, Germany	Metabolic Engineering

EXTERNAL ADVISORY BOARD

Charles L. Cooney
Department of Chemical Engineering, Massachusetts Institute of Technology, USA

Gerard Canters
Leiden University, Gorlaeus Laboratories, Leiden Institute of Chemistry, Leiden, The Netherlands

Horst Vogel
Ecole Polytechnique Fédérale de Lausanne, Institut de Science Biomoléculaire, Switzerland

Geoffrey Cole
School of Biosciences, The University of Birmingham, UK

Joachim Klein
Inst. f. Technische Chemie, Technische Universität Braunschweig, Germany

Leslie Dutton
Department of Biochemistry and Biophysics, Johnson Foundation for Molecular Biophysics / Unniversity of Pensilvania, USA

Michael J. Kearsey
School of Biosciences, The University of Birmingham, Birmingham, UK

Pere Puigdoménech
Department de Genètica Molecular, Institut de Biologia Molecular de Barcelona, Spain

Peter J. Sadler
School of Chemistry, University of Edinburgh, UK

Staffan Normark
Microbiology & Tumor Biology Center (MTC), Karolinska Institutet, Stockholm, Sweeden

INFRA-STRUCTURE SUPPORT COMMITTEE

Cláudio M. Soares	Vice-Director (Chairman)
Miguel Teixeira	Director
Fátima Madeira	Secretary
Ana Rute Neves	ITQBI
Henrique C. Nunes	Safety; Workshop & Maintenance
Nuno Monteiro	Safety; Workshop & Maintenance
Madalena Pereira	Administrative & Accounting
Fernando Tavares	Administrative & Accounting
João Rodrigues	Economato
Carlos Frazão	Computing & Networks
Carlos Cordeiro	Computing & Networks
Daniel Branco	Computing & Networks
Lurdes Conceição	Academic Services
Ana M. Sanchez	External Affairs
Susana Lopes	Library
Manuela Regalla	Equipment Washrooms
Ana Coelho	Analytical Services

SAFETY AND FLOOR COORDINATION COMMITTEE

Abel Oliva	Chairman
Miguel Teixeira	Director
Claudio Soares	Vice-Director
Mafalda Mateus	Secretary
Henrique C.Nunes	First Floor
(Valter Peres)	First Floor
Madalena Pereira	Second Floor
(Fernando Tavares)	Second Floor
Ana Maria Portocarrero	Third Floor
Abel Oliva	Fourth Floor
(Luís Paulo Rebelo)	Fourth Floor
Teresa Crespo	Fifth Floor
Cândido Pinto Ricardo	Sixth Floor
(Margarida Oliveira)	Sixth Floor
Carlos Romão	Seventh Floor
(Rita Delgado)	Seventh Floor
Rosário Mato	ITQB I
(Marta Aires de Sousa)	ITQB I
Christopher Maycock	Chemistry Building
(Rita Ventura)	Chemistry Building
Cecília Arraiano	Radioactive Sources
(Adriano Henriques)	Radioactive Sources
Teresa Crespo	Biological Hazards
(Júlia Costa)	Biological Hazards
Helena Santos, MD	Medicine and Health
Ana Maria Portocarrero	Planning and Academic
Isabel Ribeiro	IBET representative
António Cunha	Pilot Plant Representative
Henrique C. Nunes	Workshops & Maintenance
(Nuno Monteiro)	Workshops & Maintenance

Organization of the Institute

INFRA-STRUCTURE SUPPORT SERVICES

ADMINISTRATIVE AND ACCOUNTING SERVICES

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

Mailling and Archive
Artur Elias dos Santos Freitas

Accounting & Treasury
Head: Fernando Jorge Dias Tavares
Accounting
Ana Cristina Afonso Silva
Ana Mónica Adriano Vieira
Isabel Maria Soares Palma Mestre
Nuno Miguel Nobre Lopes
Sónia Cristina Serra Ermida (from February 2006)
Treasury Section
Ana Dores dos Santos Freire
Anabela dos Santos Bernardo Costa

Stores
Maria Alexandra Ferreira Lopes Pinto dos Santos
Ana Isabel Soares 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
Cláudia Lopes
Isabel Cristina Respício Valente Almeida Lopes
Maria de Fátima Costa Madeira
Rosina Faruk Gadit

EXTERNAL AFFAIRS
Ana Maria Beirão Reis de la Fuente Sanchez
Luís Manuel Ramalho Morgado

ACADEMIC AND PROJECTS OFFICE
Head: Maria de Lurdes Madaleno Conceição
Ana Cristina Profírio Amaral
Ana Maria Cerveira e Castro da Silveira Protocarrero
Isabel Maria Coelho Gonçalves Guerreiro Murta

LIBRARY
Susana Lopes

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 Luis Pereira Liberato
Luís Miguel Sousa Gonçalves
Nuno Miguel de Jesus Soares
Nuno Monteiro (Power Managment)
Rómulo M. Dias Correia
Rui Hélder Amor Pereira Dias
Walter Peres

WASHROOMS FOR EQUIPMENT
Scientific Coordinator: Manuela Regalla
Ana Cristina Martins Barreiros
Carmen Popula Pereira de Jesus Fernandes
Helena Isabel Pinto Vilaranda (From June 2006)
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
Helena Matias – Nuclear Magnetic Resonance
João Pires – Nuclear Magnetic Resonance

FERMENTATION
João Nuno Carichas Carita

TEACHING LABORATORY
Teresa M. Baptista da Silva Cratorne

ITQB Relevant Statistics

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

RESEARCHERS

Since its foundation in 1989, ITQB operates as an open institute with the participation of researchers from other institutions and universities; permanent research or teaching staff is limited to 15 % of the total number of researchers holding a PhD degree. Since 2001, 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.

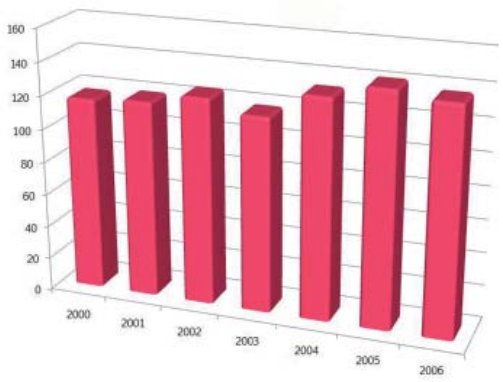
Currently, research at ITQB is supported by **327 researchers**, excluding undergraduates and visiting scientists.

PhD holders	**137**
Laboratory Heads	50
ITQB	28
Other Institutions	22
Post Doctoral Fellows	59
Other PhDs	28

PhD holder by gender: Female 83 / Male 54

Average age of a PhD holder: 41,4 years
(The average age of a Post-doc researcher at ITQB is 35 years)

PhD holders over the years:

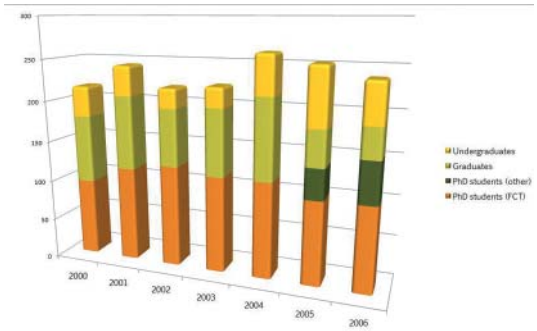


ITQB Relevant Statistics

The quality and diversity of the research and the researchers at ITQB make it an exceptional centre for science higher 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	**154**
FCT fellowships	104
Other sources of funding	50
Other Graduates (Science grantees)	**36**
Undergraduates	**48**

Graduate students and Undergraduates at ITQB over the years:



Before 2005, only FCT PhD fellows were counted as PhD students and all others were included in the figure Graduates. For comparison, in 2005 and 2006, the PhD students with other sources of funding are accounted separately.

Researcher's Funding

The permanent research staff at ITQB is limited to a small number, currently 21. Additionally 18 researchers are supported through Laboratório Associado contracts.
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 163 FCT grantees (104 PhD students and 59 Post-docs).

In 2006, the approval rate of PhD scholarships from FCT was 80% (51 out of 60 applications accepted). This clearly demonstrates the capacity of ITQB to attract and train high quality researchers.

RESEARCH

ITQB has presently **50 Laboratories** 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 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 68 research projects and further participates in 31 more. The total **99 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. In the last call for projects from FCT (2006) ITQB submitted 84 projects.
Additionally, ITQB researchers also coordinate and participate in a number of research projects where IBET is the host institution (not listed in this report)

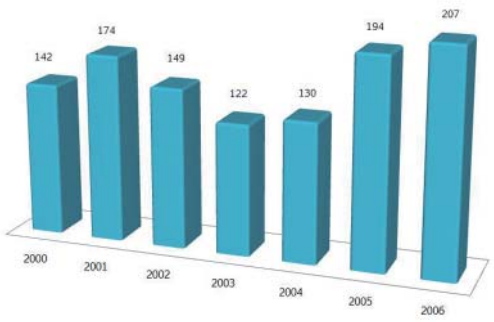
Ongoing projects:

77 projects Fundação para a Ciência e a Tecnologia
7 projects Re-equipment Program FCT
2 projects Fundação Calouste Gulbenkian
1 project Agência de Inovação
10 projects European Commission
2 projects Rockfeller University

Publications

In 2006, ITQB researchers published **207 papers** in peer reviewed international journals. Research at ITQB results often from internal collaborations between the several research groups and this is reflected in the authorship of published work (in 2006 this represents 1/5 of the papers). As of February 2007, 89 papers had already been published or been accepted for publication.

Publications in peer reviewed journals over the years:
(absolute number and ratio per PhD holder)



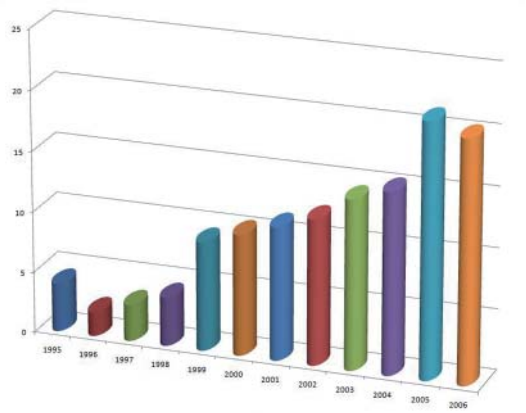
ITQB Relevant Statistics

ADVANCED EDUCATION

PhD Degrees

As an academic institution, ITQB awards PhD degrees in Chemistry, Biology, Biochemistry and Chemical Engineering. So far, ITQB has awarded **125 PhD degrees.**
In 2006, **20 PhD** theses were awarded at ITQB: 10 in Biology, 7 in Biochemistry, 1 in Chemical Engineering; 2 in Chemistry.

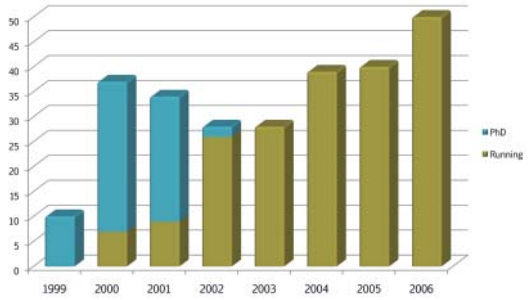
PhD thesis awarded at ITQB over the years:



At the moment there are 209 registered PhD students at ITQB. About 55 of these students do research at other institutions that cannot award academic degrees and resort to ITQB for that purpose. In 2006, 50 new Graduates registered at ITQB as PhD students.

PhD students over the years according to their registration year:

.



Formal education

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).

The **Masters Degree in Medical Microbiology**, is a collaborative Masters Course from Universidade Nova de Lisboa initiated in 2003 and also envolving the Instituto de Higiene e Medicina Tropical, Faculdade de Ciências Médicas and Faculdade de Ciências e Tecnologia. After the curricular activities in 2005/6, the students are now at the stage of preparing their dissertation. In 2006, **20 Master Students** were registered at ITQB.

Complementing the research training of PhD students, 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.

BUDGET

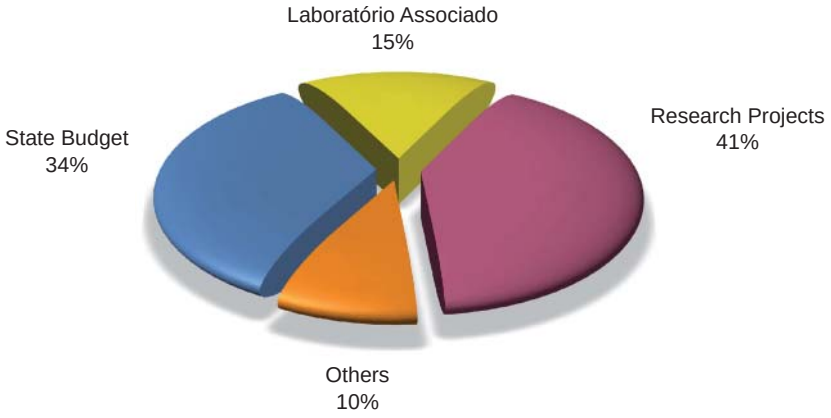
ITQB has two main sources of revenue; the State Budget, attributed by the Ministry of Science, Technology and Higher Education, and the national science funding agency, *Fundação para a Ciência e a Tecnologia* (FCT).

The contribution from the State Budget 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, both of competitive nature. In 2006, six projects under the national re-equipment call were also financed upon evaluation by FCT and these correspond to 3.1 M€.
Additional sources for research projects include the European Commission, the *Fundação Calouste Gulbenkian*, the Rockfeller University and international cooperation projects.

Some 10 % of the total budget represents additional sources of funding including revenues from Masters Courses, the sale of analytical services, rental of rooms and facilities, etc.

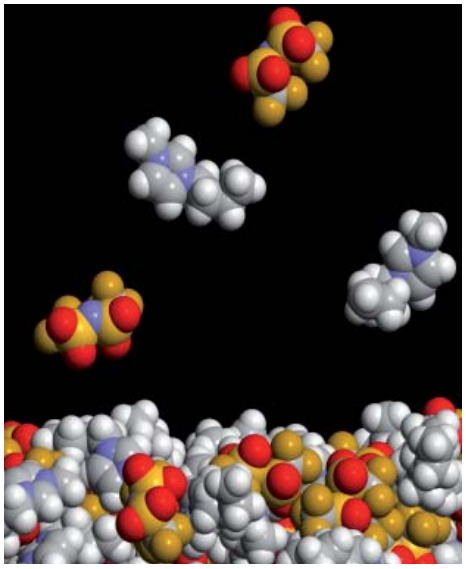
The overall budget of ITQB for 2006 was around 11,2 M € distributed as follows:

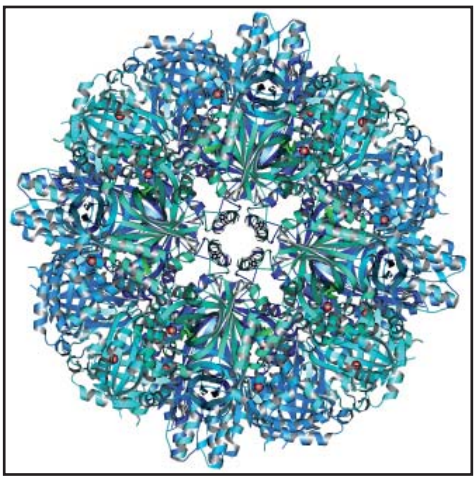


Highlights 2006

IONIC LIQUIDS: when a salt becomes a gas

In February 2006, the Laboratory of Molecular Thermodynamics, headed by Luis Paulo Rebelo, published a paper in Nature for their work with ionic liquids. Contradicting the previously accepted non-volatility of ionic liquids, the researchers experimentally demonstrated that, under the appropriate conditions of temperature and pressure, these relatives of table salt can be distilled and thus made purer. Besides showing the importance of evidences for supporting (or refuting) scientific theories, this research opens new avenues for the use of these green solvents.
This paper was featured in News and Views section of Nature, by Peter Wasserscheid, an authority in the subject of Ionic Liquids, and granted Luis Paulo Rebelo an interview broadcasted in Nature Podcast, a weekly audio show featuring highlights from this scientific journal.
See page 92.



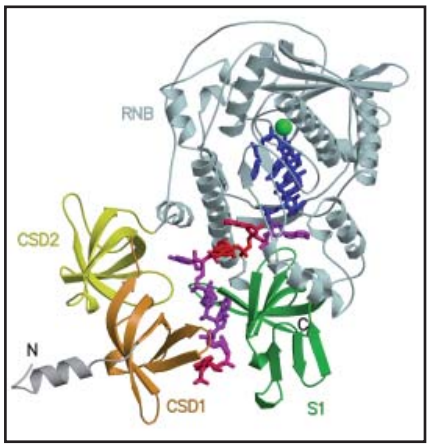


SOR: a nanoreactor in a protein

In the same month, a team led by Carlos Frazão, from the Protein Crystallography Laboratory, and in collaboration with the group of A. Kletzin (Darmstadt Technical University, Germany) published the structure of a sulfur oxygenase reductase (SOR) from a thermoacidophilic archaeon in Science.
Despite the importance of microbial oxidation of elemental sulfur in the global sulfur cycle, little is known about the mechanisms of this reaction. The spherical structure elucidated for SOR shows how the 24 monomers interact and suggests that the linear sulfur enters the sphere through the apolar channels. Once inside, the sulfur serves as a substrate for the disproportionation and oxygenation reaction that takes place in one of the 24 active sites.
Apart from the scientific significance of these findings the symmetry of the published structure makes it particularly appealing.
See page 53.

RNase II: killing the messenger

RNA's turnover plays a pivotal role in cell metabolism and elucidating its mechanisms provides clues on how cells regulate the fine tuning of gene expression. The action of RNA-degrading enzymes in bacteria has long been a research subject at ITQB.
In September 2006, as a result from collaborative efforts between the Laboratory of Control of Gene Expression (headed by Cecília Arraiano) and the Laboratory of Crystallography (headed by Maria Arménia Carrondo), Nature published the structure of the E. coli ribonuclease II. Two structures were obtained; the structure of the native RNase II and the structure of a mutant RNase II that holds to RNA molecules without degrading them. The comparison between both structures together with the extensive molecular and biochemical data already obtained showed how RNase II degrades its target.
See pages 53 and 61.



NMR National Facility

Through the National Program for Scientific Re-equipment, ITQB hosts three new NMR spectrometers (400, 500 and 800 MHz), including the highest field NMR spectrometer in Portugal. Together with the two already existing spectrometers (500 and 300 MHz), ITQB became the largest Portuguese NMR centre. This equipment is part of the National NMR Facility which will be officially launched on July 9, 2007 and will provide service for the portuguese scientific community. ITQB was the Principal Institution in this ambitious proposal (6.5 MEuros) that finally provides adequate support for the development of NMR in Portugal.

ITQB integrates MIT-Portugal Program

MIT|Portugal

In 2006, the Portuguese Government initiated a long-term collaboration with the Massachusetts Institute of Technology (MIT) focusing on basic research and education in four main areas. As one of the signing Associate Laboratories for the Bio-Engineering Systems focus area, ITQB will be involved in the joint research and educational projects contemplated in this protocol. Starting next September, ITQB researchers will already be lecturing in the PhD Program in Bioprocess Engineering.

Awards and Nominations

Ana Raquel Correia

PhD student (Protein Biochemistry Folding & Stability Laboratory)

↘Best Poster Award at the 8th European Bioinorganic Chemistry Conference. Aveiro, Portugal, 1-6 July 2006.
Correia A. R., Adinolfi S., Pastore A., Gomes C.M., (2006) "Structural and functional implications of clinical point mutations in frataxin, a putative iron chaperon involved in the neurodegenerative disorder Friedreich ataxia".

André T. Fernandes

PhD student (Microbial & Enzyme Technology Laboratory)

↘Prize for the best poster at the "Oxizymes in Oeiras. 3rd European Meeting in Oxizymes". Oeiras, Portugal, 7-9 September 2006.
"Enzymatic Properties, Conformational Stability and Model Structure of a Metallo-Oxidase from the Hyperthermophile *Aquifex aeolicus*"

Helena Santos

Head of the Cell Physiology & NMR Laboratory

↘Elected Vice-President of the "International Society for Extremophiles", September 2006.

↘Elected Member of the Editorial Board of the "FEBS Journal".

Maria Manuela Chaves

Head of Plant Molecular Ecophysiology Laboratory

↘Member of the Fellowships Committee of the Federation European Societies of Biochemistry (FEBS) from January 2006.

Awards and Nominations

Rita Abranches

Head of Plant Cell Biology Laboratory

↘Prize for best oral communication awarded at the XLI Congress of the Portuguese Society for Microscopy, Braga, Portugal. 14-15 December 2006.

Oral communication entitled: In situ methods to localize transgenes and transcripts in plant genomes: FISHing for answers".

Vesna Prosinecki

PhD student (Protein Biochemistry Folding & Stability Laboratory)

↘Best Poster Award at the International Conference on Proteomics PROTEOMLUX. 11-14 October 2006 (Luxembourg, Luxembourg)

Prosinecki, V., Botelho, H.M., Francese, S., Mastrobuoni, G., Moneti, G., Urich, T., Kletzin, A., Gomes, C.M. (2006) Mining for proteins with enhanced intrinsic thermal stability: a proteomic research on the hyperthermophile *Sulfurisphaera* sp.

Teresa Rodrigues

PhD student (Animal Cell Technology Laboratory)

↘Outstanding Poster Contribution (2nd prize) at European Downstream Technology Forum 2006, Goettigen, Germany.

Rodrigues T., Carvalho A., Roldão A., Carrondo M. J. T., Alves P. M., Cruz P. E. (2006) "Screening Anion-Exchange Chromatographic Matrices for Isolation of Onco-Retroviral Vectors".

Science and Society

Science thrives on communication.
By communicating research results to the scientific community and discussing them with their peers, scientists are able to build on their knowledge and proceed further. So, as an institution devoted to science, ITQB is highly committed to disseminate the results of its research projects by publishing papers in high quality scientific journals and by presenting lectures and posters at national and international meetings.

But science and scientists do not live isolated from the rest of society, they actually depend on society and its decisions, and research has often implications on people's lives, making it a duty for scientific institutions to bring science closer to society. Since its foundation, ITQB has considered it a mission to strengthen the interaction between scientists and the general public, particularly schools, and has been increasingly engaged in public awareness of science.

ITQB is aware of its responsibility towards society and seeks to convey its latest results through the media, by issuing press releases and by making its scientists available to talk to journalists about their work. In 2006, ITQB received considerable interest form the media especially after the publication of new discoveries on the properties of ionic liquids – considered green replacements for organic solvents – and the determination at atomic resolution of the molecular structure of important proteins in the microbial world.


Cientistas portuguesas
m o mundo
Portugueses desvendam estrutura de enzima que permite a vida nos vulcões

ITQB has an active science and society programme and collaborates with several institutions for implementing scientific activities that involve different sectors of the public.
Throughout 2006, ITQB has formally received the visit of almost 300 high-school students and their teachers who had the opportunity to visit research laboratories and talk directly with researchers, either about their work or about different aspects of a research career. The majority of our visitors found these visits extremely enlightening about the scientific research topics and environment.
During the summer, as traditionally happens for a number of years, high school students have also the opportunity, through a program coordinated by Agência Nacional para a Cultura Científica e Tecnológica Ciência Viva, to make short training periods at ITQB. In 2006, ITQB received 11 students in its research laboratories for periods ranging from two to four weeks. Both students and researchers truly cherished this successful initiative and new students will come again this year including four high school Spanish students.

Each year, in November, Portugal celebrates the National Science and Technology Week. This year,

ITQB, together with IGC, presented a different activity: a series of five scientific debates on topics that ranged from controversies on stem cells or transgenic plants to the myths and facts of chemistry. Most of the participants were high school students and the debates were very lively and presented an excellent opportunity to listen to what students have to say on such matters.

Once in a while, ITQB takes its research outside its walls and truly takes Science near to the public. In June 2006 during the summer fests, ITQB and IGC planned two whole days of scientific experiments for the public in the Municipal Garden of Oeiras that included playing with molecular and cellular models, extracting DNA from strawberry, using red cabbage as a pH indicator or seeing the effect of sun-block in yeast growth.

The most emblematic event in the science and society programme of ITQB is undoubtedly the ITQB Open Day. On a Saturday at the end of January, all the institute – including researchers and staff – organize themselves and provide a unique opportunity of interaction between researchers and the local (and not so local anymore) community. For the whole day, people are invited to visit ITQB 's laboratories, to debate a series of topics that even include scientific ice-creams, to experiment how scientists do their work, to discover why research is important even when no application is visible and to fully appreciate the new technologies that other times emerge from scientific research. The success of the previous editions (2005 and 2006) convinced us to continue this event in 2007 with an equal success, both in the number of visitors (1600), and in their comments and enthusiasm.

For more information about our Science and Society past and future activities, please visit our webpage.

Good Laboratory Practices Unit

The Good laboratory Practices Unit is certified by the INFARMED and integrates the Analytical Laboratory and the Microbiology Laboratory from IBET and the Protein Characterization and Mass Spectrometry Laboratory from ITQB
The GLP Unit has a Quality Assurance Unit (QAU) responsible for the maintenance of a Quality System as well as inspection of Studies, installations and processes. The GLP Unit has an Archive where all the documentation is kept.

Direction of GLP Unit: Maria Teresa Crespo | Maria do Rosário Bronze
Quality Assurance Unit: Ana Luisa Simplicio

Services provided:
Good Laboratory Practices Studies under OCDE certification
Implementation and validation of methods
Routine analysis

Analytical Laboratory (LA)

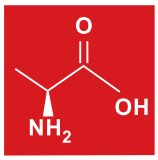
The laboratory has a long track record of providing services using chromatographic (HPLC and GC with several detectors) and electrophoretic methods for pharmaceutical, agro and chemical industry and academia.
António Ferreira, antoniof@itqb.unl.pt

Microbiology Laboratory (LM)

The laboratory has provided services for the pharmaceutical industry, for pharmaceutical formulas and API, agro-industry and academia. Services include in vitro potency assays, protein quantification, molecular biology analysis (GMOs in food and feed and other) and detection and quantification of impurities or contaminants in pharmaceutical
Fernanda Rodrigues, spinola@itqb.unl.pt

Protein Characterization and Mass Spectrometry Laboratory

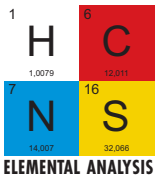
The laboratory performs development and validation of analytical methods as well as routine analysis for a broad range of chemical compounds, from small organic and organometalic compounds to peptides, oligosaccharides, nucleotides and proteins.



Mass Spectrometry
Molecular mass determination by mass spectrometry using MALDI and electrospray ionization, LC-MS and LC-MS/MS characterization, protein identification by peptide mass fingerprinting and detection of impurities or contaminants in pharmaceutical formulations.
Ana Varela Coelho, varela@itqb.unl.pt, labms@itqb.unl.pt

Amino Acids Analysis
Free or proteic amino acids analysis. Some less common amino acids such as hydroxyproline, hydroxylisine, glucosamine and galactosamine can also be quantified on request
Paula Chicau, chicau@itqb.unl.pt

Protein Sequencing
N-terminal and internal sequencing by Edman reaction
Manuela Regalla, mregalla@itqb.unl.pt



Elemental Analysis
Elemental analysis of solid samples to determine their quantitative composition carbon, nitrogen, hydrogen and sulphur
Conceição Almeida, salmeida@itqb.unl.pt

Other Services and Facilities available at ITQB

Small Molecule X-ray Crystallography

This X-ray crystallography facility is an analytical service that involves a close collaboration between three different institutions: ITQB, IST and ITN. X-ray diffraction by a single crystal is used to determine the three dimensional structure of small molecules.
The analysis can be complemented by advice on growing good diffracting crystals suitable for data collection, comprehensive analysis of the structural results and preparation of results and molecular illustrations, in colour or black & white, for publication.
Isabel Bento, bento@itqb.unl.pt

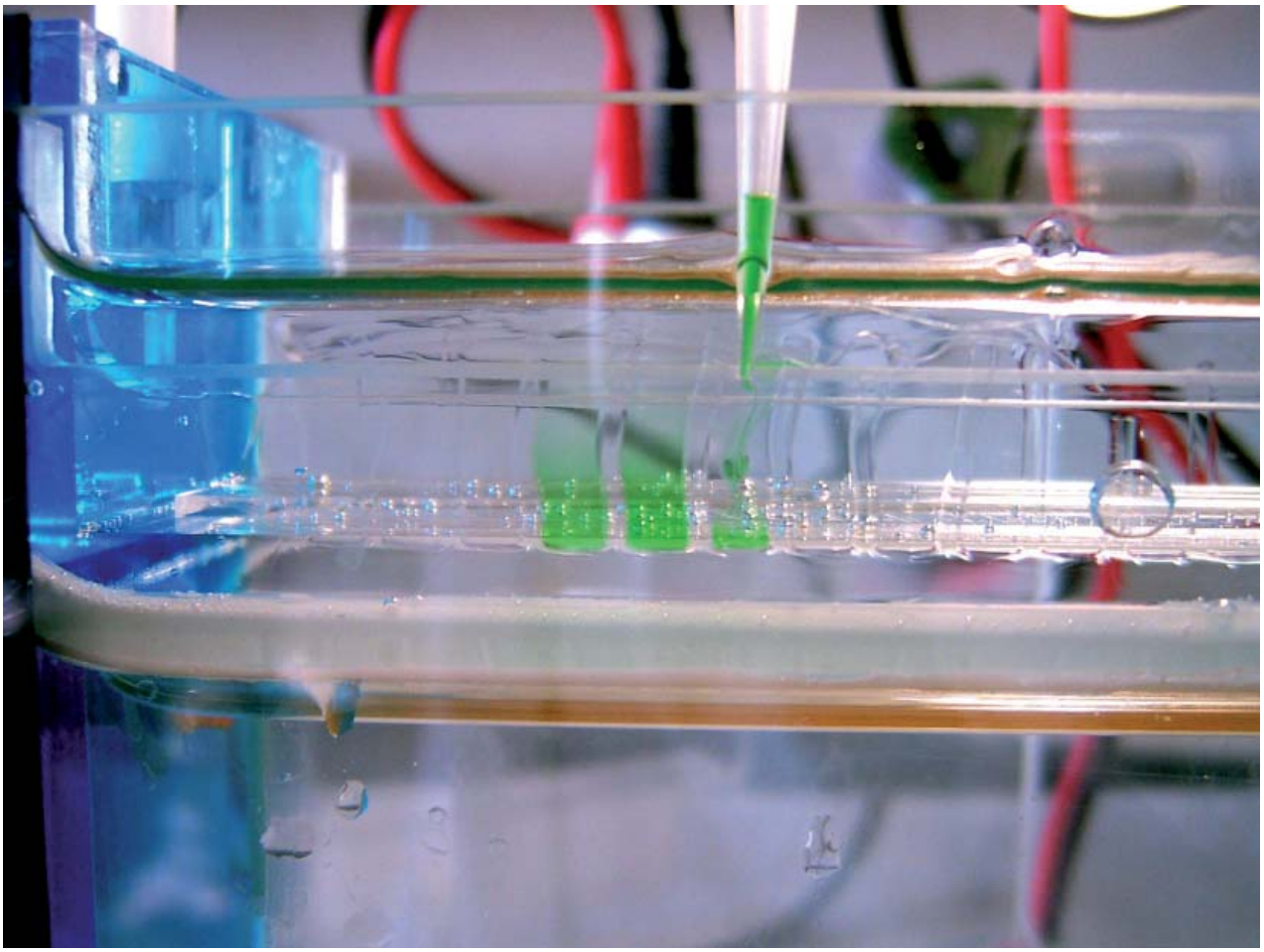
Nuclear Magnetic Resonance

ITQB hosts three new NMR spectrometers (400, 500 and 800 MHz), including the highest field NMR spectrometer in Portugal. Together with the two already existing spectrometers (500 and 300 MHz), ITQB became the largest Portuguese NMR centre. This equipment is part of the National NMR Facility which will be officially launched on July 9, 2007 and will provide service for the Portuguese scientific community. The ITQB NMR centre is headed by Helena Santos, full professor and coordinator of the Physiology and NMR Laboratory at ITQB.
Helena Matias, lenap@itqb.unl.pt; João Pires, jopires@itqb.unl.pt

Major equipment available at ITQB

ATR-FT- Infra Red
Circular Dichroism
Dynamic Light Scattering Particle Sizer / Instrument for Zeta Potential and Molecular Mass Determination
Electron Paramagnetic Resonance
Fluorescence Deconvolution Microscope
Fluorescence Recovery After Photobleaching (FRAP)
Greenhouse 300m^2
High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection
ITQB/IBET Pilot Plant
Mass Spectrometer (MALDI-TOF, LC-API-3D ion trap, LC-nanoESI-linear ion trap)
NMR spectrometers (300, 400, 2x500 and 800 MHz)
Resonance Raman Spectrometer
Room Temperature Time Resolved Phosphorescence
Steady State Fluorescence and Steady State Fluorescence Anisotropy
Surface Plasmon Resonance (Biacore)
Walk-in Plant Growth Chambers 2x6m^2 and Rooms 3x2m^2
X-ray Difractometer

Research Laboratories



"A preparar a corrida " **Pedro Barros,** PhD Student

Selected best photograph
ITQB from within - Internal photo competition held by the occasion of the Open Day 2007

Chemistry Division

The Chemistry Division at the ITQB consists of seven individual groups working in diverse areas of chemical research. These range from Organometallic chemistry and catalysis, through supramolecular chemistry organic and bioorganic chemistry to physical chemistry. Interests within these groups range from biomembrane studies, medical applications of inorganic complexes, organometallic pharmaceuticals and methods for the synthesis of natural and bioactive compounds. This small but highly diverse division provides a rich array of research at an international level with interdisciplinary collaborative projects within the institute, between the institutions which make up the Associated Laboratory and also with industry. Thus not only contributing to the nations research status but also to the economic well-being of its means of production.

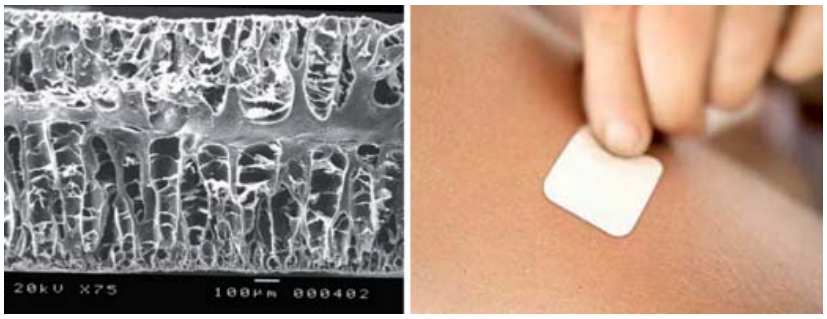
During 2006 some new projects have ben started within several of the groups of the division. Projects include the ability for oxometal complexes to carry out Si-H and H-H activation, new metal complexes for medical applications and green chemical processes. There are also new collaborative projects with the Biology Division and the synthesis of propharmaceuticals in collaboration with the Technology Division and IBET.

For the future we hope to see an increase in the number of students carrying out their doctoral studies in the chemistry division. This will depend to a large extent upon a national scientific policy which promotes subjects considered to be in the national economic interest.

António Lopes
Associate Professor, Universidade Lusófona de Humanidades e Tecnologias
PhD 1997 in Chemistry, Universidade Nova de Lisboa, ITQB

Colloids Polymers and Surfaces

As the name of the lab suggests we are dealing with surface/interface phenomena.
At a colloidal or polymeric level, and within the broad field of colloid and surface chemistry, research is largely concentrated on the surfactant self-assembly, with or without the presence of polymers (or proteins), developing solutions of tunable rheology, from gels to mesomorphic or smectic phases. Other studies involve the pH definition near the colloid interfaces and the permeation through the soft interfaces developed.
From the biochemical/biomedical point of view, we have been working with polymeric matrices, namely hydrogels (entangled or cross-linked networks of polymer-based structure with swelling and entrapment capabilities) which possess a high potential for biomedical-oriented applications. In this regard we have been developing matrices for drug delivery systems for the skin barrier and we have been focus on allergy, burn and pain treatments. These matrices are based upon crosslinked gels from chitosan and dextran (polysaccharides) due to their biocompatibility and biodegradability. After the drug is incorporated studies on the stability of the formulation and the release properties of the drugs are modeled.
Another multidisciplinary topic being covered is the extraction and identification of surface active agents from plant origin, including polyphenol families with antioxidant capabilities. Presently, most of our studies in this field are dealing with the cork tree, coffee and some Portuguese varieties of beans.
From the more environmental oriented point of view we have been working with the “green solvents” known as Room Temperature Ionic Liquids (RTIL) dispersed in bulky aqueous and non-aqueous solvents. Some of the studied RTIL’s, when dispersed in aqueous solutions, act as a new class of surfactants with unique abilities because along with the highly advantageous characteristic of these “green fluids” as bulk solvents, they can be nano-dispersed in water and some non-aqueous solvents. Here, one can combine two reagents of completely different natures (hydrophilic and hydrophobic) into a macroscopically homogeneous solution, being micellar or microemulsion states. Some very recent preliminary results suggest that in equimolar mixtures of SDS and long hydrocarbon chain RTIL’s, both mixed micelle behaviour and catalytic effects are present. These phenomena are currently under a deeper investigation.



Dextran hydrogel microstructure and patch for skin application

Polyphenol extracts from cork tree with possible application as antioxidants

Group Members

José Filipe Almeida	PhD student
Carla Antunes	PhD student
Marijana Blesic	PhD student
Rui Silva	Graduate

Selected Publications

Zanette D., Felippe A., Schweitzer B., Dal Bó A., Lopes A., (2006), “The absence of cooperative binding in mixtures of sodium cholate and poly(ethylene oxide) as indicated by surface tension, steady-state fluorescence and electrical conductivity measurements.”, Colloids and Surfaces A: Physicochem. Eng. Aspects 279 (1): 87–95

Schweitzer B., Felippe A. C., Dal Bó A., Minatti E., Zanette D. and Lopes A. (2006). “Sodium dodecyl sulfate promoting a cooperative association process of sodium cholate with bovine serum albumin.” Journal of Colloid and Interface Science 298(1): 457-466.

Lameiro M. H., Lopes A., Martins L. O., Alves P. M. and Melo E. (2006). “Incorporation of a model protein into chitosan-bile salt microparticles.” International Journal of Pharmaceutics 312(1-2): 119-130

Beatriz Royo
Auxiliary Investigator
PhD in 1992, Sussex University

Homogeneous Catalysis

Group Members

Patrícia Reis	Post-doc
André P. da Costa	Graduate
Mónica Viciano	PhD student

The catalysis of reductions by transition metals in high oxidation states is a new fresh area of chemistry. Our interest in the study of high-valent transition metals as catalysts leds us to explore this new pathway of catalytic activity. This year, we have explored the catalytic activity of a series of molybdenum(VI) dioxo complexes in the hydrosilylation of aldehydes and ketones. The mechanism of this reaction has been studied by experimental data and by means of density functional theory calculations. With the aim to develop the enantioselective version of the hydrosilylation reaction, we have synthesized novel dioxo-molybdenum(VI) and -tungsten(VI) complexes with the chiral binaphthol (H2BINOL) ligand. In addition, a novel catalytic system, HReO4(aqueous solution 75-80%)/silane for the reduction of carbonyl groups and for the dehydrogenative silylation of alcohols has been developed.
We have also demonstrated the capability of the high valent oxo complexes ReMeO3 and MoO2Cl2 to activate H2. These species are efficient catalysts for the hydrogenation of alkynes to alkenes using H2 (4 atm of pressure). Theoretical calculations for the elucidation of the mechanism of dihydrogen cleavage by MoO2Cl2 has been performed.
Another area of interest in our group is the use of N-heterocyclic carbenes as ligands for high-oxidation-state metal complexes. With strong sigma-donor properties, NHCs are well suited to stabilize high-oxidation-state metal complexes; however, the number of complexes that have been prepared to date is quite small and very little has been described concerning the oxidizing properties of these complexes. This year, we have synthesized NHC-containing Mo(VI) complexes and studied their reactivity towards water. We have also studied the influence of the modification of the steric and electronic properties of the NHC ligands in the stability and reactivity of the complexes obtained.

Schlenk containing a dioxo-Mo(VI) catalyst

A stainless steel autoclave for hydrogenation reactions

Selected Publications

Reis P M, Romão C C, Royo B (2006) Dioxomolybdenum(VI) complexes as catalysts for the hydrosilylation of aldehydes and ketones Dalton Transactions (15): 1842-1846.

Mas-Marza E, Reis P M, Peris E, Royo B (2006) Dioxomolybdenum(VI) complexes containing N-heterocyclic carbenes Journal of Organometallic Chemistry 691(12): 2708-2712.

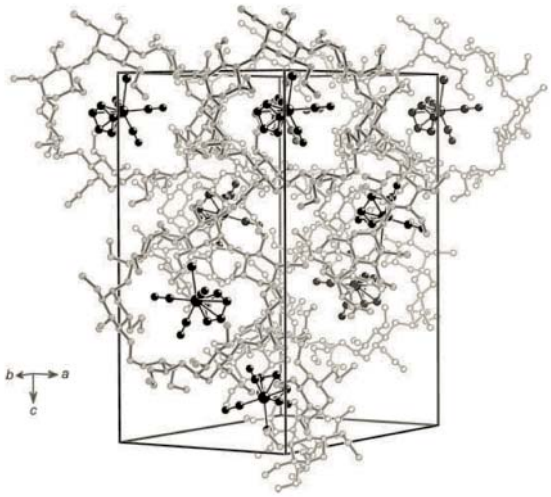
Carlos C. Romão
Full Professor
PhD 1979, Universidade Técnica de Lisboa (IST)

Organometallic Chemistry

Bridging Inorganic and Organic Chemistry, Organometallic Chemistry (OMC) strongly changed chemical synthesis by creating catalysts for many applications widely used in both large scale (refineries) and small scale (fine chemicals and pharmaceuticals) industries.
Moreover, organometallic compounds have found applications in a variety of fields including biomedical and pharmaceutical areas like therapy and diagnostics.

At ITQB, we have been exploring for some years the synthesis and catalytic chemistry of organometallic oxides of Molybdenum and Rhenium a relatively underdeveloped area of OMC. These oxo-complexes, contain metal-oxygen (M=O) bonds like those involved in many enzymes. Such compounds are able to accelerate (catalyze) the oxidation of organic molecules using environmentally safer oxidants like hydrogen peroxide (H2O2) and alkylhydroperoxides (ROOH). In 2006 our work covered mainly mechanistic aspects of these reactions in an effort to unify the understanding of the wealth of experimental results obtained in previous years.
In 2005 we had disclosed a new type of reactivity of metal-oxo compounds, namely their ability to catalyze reduction reactions. These processes are based on the capacity of the M=O bonds to break Si-H bonds, thereby transferring hydrogen atoms (reduction) to unsaturated organic molecules. Throughout 2006 we continued to explore these entirely new catalytic processes for the reduction of aldehydes, ketones, esters, amides, sulfoxides and N-oxides. Progress in this area includes the development of environmentally friendly processes where such reductions are carried out using water as solvent.

The development of organometallic compounds for therapeutical applications is the other main focus of our research. This led to the formation of the independent company, Alfama Inc., still operating in close collaboration with our research group within ITQB premises. The core of Alfama's proprietary technology is the development of CO releasing molecules for treatment of inflammatory diseases. Besides, we pursue the search for anti-tumoral molecules and the study of the interaction of organometallic molecules with the organism (e.g. with protein transporters) to improve their bio-compatibility and water solubility. Accordingly, encapsulation into soluble cyclic sugars (cyclodextrins) has received particular attention. It must be noted that very little is presently known about the behavior of organometallic compounds in vivo.



Unit cell contents, viewed in perspective towards the (1 7 0) plane, of the Monte Carlo optimised structural model of the inclusion compound TRIMEB•[CpMo(CO)3Cl]. TRIMEB and [CpMo(CO)3Cl] residues are represented with grey- and black-filled bonds, respectively. TRIMEB is a modified beta-cyclodextrin and these inclusion compounds can be used as catalysts or CO releasing anti-inflammatory drugs.

Group Members

Ana C. Fernandes	Assist. Prof.
Carla Reis	Assist. Prof.
Jan Honzicek	Post-doc
Jose Fernandes	Post-doc
João Seixas	PhD

Selected Publications

Pereira C. C. L., Costa P. J., Calhorda M. J., Freire C., Rodrigues S. S., Herdtweck E. and Romão C. C. (2006). "Ring slippage vs. charge transfer in the reductive chemistry of [IndMo(CO)2(alpha-diimine)]+cations." Organometallics 25(22): 5223-5234..

Braga S. S., Paz F. A. A., Pillinger M., Seixas J. D., Romão C. C. and Gonçalves I. S. (2006). "Structural studies of beta-cyclodextrin and permethylated beta-cyclodextrin inclusion compounds of cyclopentadienyl metal carbonyl complexes." European Journal of Inorganic Chemistry(8): 1662-1669.

Fernandes A. C. and Romão C. C. (2006). "A novel method for the reduction of sulfoxides and pyridine N-oxides with the system silane/MoO2Cl2." Tetrahedron 62(41): 9650-9654.

Christopher David Maycock

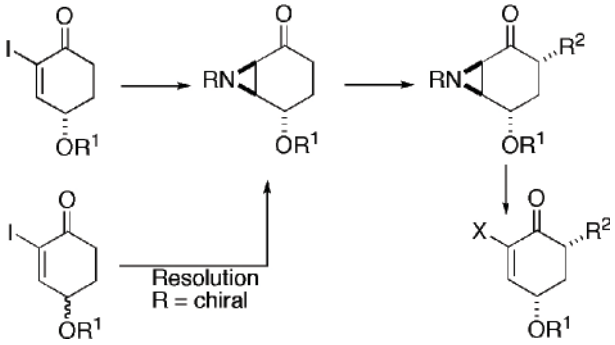
Associate Professor, Faculdade de Ciências, Universidade de Lisboa
PhD 1978 University of Newcastle upon Tyne, UK

Organic Synthesis

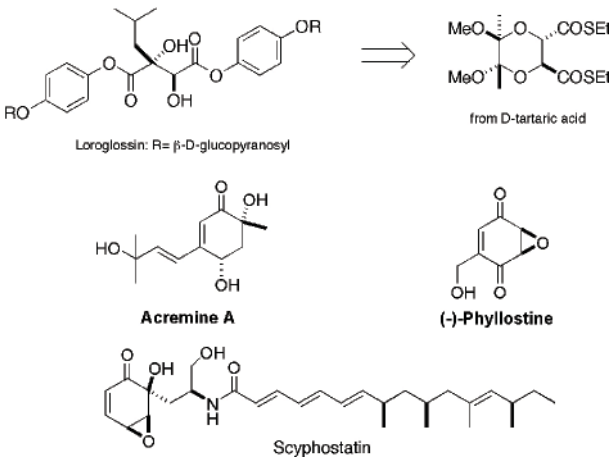
Group Members

Jorge Wahnon	PhD student
Hovsep Avedissian	Post Doc
Sofia Miguel	Graduate
Filipa Siopa	Graduate
David Quintino	Graduate
Justin Pine	Graduate
Eva Lourenzo	Undergraduate

Studies on the synthesis and uses of acylaziridines derived from enones have continued. These aziridines are prepared from simple primary amines anda suitable enone. Simple reaction of these aziridines with mild acids produces enones with alternative substitution. The aziridine group is thus a useful protecting group. We hope that these readily available compounds will allow us to resolve substituted cyclohexenones, to produce optically active starting materials for synthesis, using readily available optically pure amines. Further remote transformations will be studied in these conformationally semi-rigid systems in order to observe stereoselectivities. The synthesis of natural and totally synthetic compatible solvents has continued, in a Europe wide project, as well as the synthesis of biosynthetic intermediates. The alkylation of tartaric acid biacetals has lead to the synthesis of a group of natural products. Other chiral pool materials have also been studied for the improved synthesis of complex polyoxygenated cyclohexanes. Sustainable chemistry requires that alternative carbon sources are found and that the processes of synthesis are efficient. Carbon economy and clean catalytic processes are our aims.



Some acylaziridine chemistry



Targets for synthesis

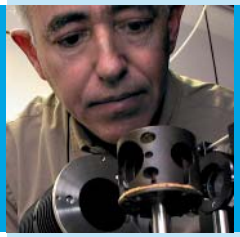
Selected Publications

Burke, A. J.; Maycock, C. D.; Ventura, M. R., "Stereoselective Alkylation of Tartrate Derivatives. A Concise Route to (+)-Piscidic Acid and Natural Analogues" Org. Biomol. Chem. 2006, 4, 2361.

Borges, N; Gonçalves, L. G.; Rodrigues, M. V.; Siopa, F.; Ventura, R.; Maycock, C.; Lamosa, P.; Santos, H. "The Biosynthetic Pathways of Inositol and Glycerol Phosphodiesters used by the Hyperthermophile Archaeoglobus Fulgidus in Stress Adaptation" Journal of Bacteriology 2006, 188, 8128.

(2R,4aS,7R,8aR)-Tetrahydro-2,9,9-trimethyl-5H-4a,7-methano-4H-1,3-benzoxathiin. ARTICLE – RN00697. For the Encyclopedia of Reagents for Organic Chemistry (EROS) and the on-line version (e-EROS) published by John Wiley and sons.

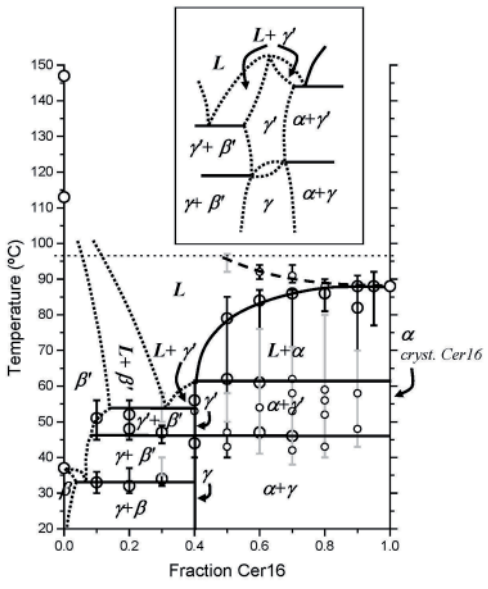
Eurico Melo
Assistant Professor at Instituto Superior Técnico, UTL
PhD 1986 in Chemical Engineering, Universidade Técnica de Lisboa (IST)



Micro-Heterogeneous Systems

At the Laboratory of Microheterogeneous Systems of ITQB - Chemistry Division, we are mainly involved with studies of the chemical equilibrium and the kinetics of reactions in lipidic mesophases. As a side activity we also characterize, when needed, the molecular structure and topology of the phases with which we intend to work. The objective of the research is the modelling of biological reactions that take place in biological membranes or at the soft-interfaces they define.
Studies in this area include the analysis of molecular diffusion and percolation in lipid lamellar phases using fluorescence recovery after photobleaching (FRAP), and singlet and triplet states emission quenching. Present work involves the structural characterization of lipid aggregates with high ceramide and cholesterol content, simulating the lipid matrix of the stratum corneum, and the organization of the lipids in mixtures of choline, sphingomyelin and cholesterol implicated in the formation of liquid ordered membrane domains, that are probably related to what is known as lipid “rafts”.

Recent advances (2006) in the field of the physical-chemical characterization of the lipid matrix of the stratum corneum, led to the characterization by wide-angle X-ray scattering of a complex between ceramide C16 and cholesterol, the first amphiphilic lipid-cholesterol complex fully characterized. The full binary phase diagram obtained for these lipids (see figure) allowed exploring the ternary mixture with fatty acid C16 and the pH behavior of the coexisting phases. Further studies involving systems that intend to simulate the stratum corneum lipid matrix were aimed at the examination of the possible solubility of cholesteryl oleate in these lipid mixtures.
In the field of pure photophysics, we were involved in an international project working with artificial light-harvesting antennae mimicking photosynthetic systems.



Phase diagram for the system Ceramide16/Cholesterol

Group Members

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

Selected Publications

LAMEIRO MH , MALPIQUE R, SILVA AC, ALVES PM, MELO E (2006) ”Encapsulation of adenoviral vectors into chitosan-bile salt microparticles for mucosal vaccination” Journal of Biotecnology, 126:152-162

MELO E, MARTINS J (2006) “Kinetics of bimolecular reactions in model bilayers and biological membranes. A critical review” Biophys. Chem. 123:77-94

Rita Delgado

Associated professor with "agregação" at Instituto Superior Técnico
PhD 1985 in Chemistry, Universidade Técnica de Lisboa (IST)

Coordination and Supramolecular Chemistry

Group Members

Krassimira Guerra	PhD student
Sílvia Carvalho	PhD student
Luís Lima	PhD student
Carla Cruz	PhD student
Pedro Mateus	Graduate
Ana Piloto	Graduate

The synthesis of new macrocyclic chelates useful for metalloradio-pharmaceuticals for diagnosis and treatment of tumours has been carried out. They are 12- to 14-membered derivatives of tetraazamacrocycles. The study of their chemical and biological properties were undertaken using as metallic radionuclides the ^{67}Cu, ^{153}Sm or ^{166}Ho. For instance, we have verified that the ^{153}Sm and ^{166}Ho complexes of the 13-membered macrocycle having methylphosphonate pendant arms (tritp) are retained by the bone and also they have a high rate of total excretion. The studies carried out support the potential interest of ^{153}Sm/^{166}Ho complexes of the 13-membered macrocycle with N-acetate pendant arms (trita) for therapy when conjugated to a biomolecule and the potential usefulness of the ^{166}Ho-tritp complex in bone pain palliation.[1]
A novel 13-membered benzodioxotetraaza macrocycle (13bzN$_4$) displayed an excellent affinity for copper(II), due to the good fit of copper(II) into its cavity, see Fig. 1. This chelator was labeled successfully with ^{67}Cu (yield > 98%) in mild conditions. On the basis of the studies undertaken, it is possible to advance that this ^{67}Cu chelate is promising for future applicability in nuclear medicine.

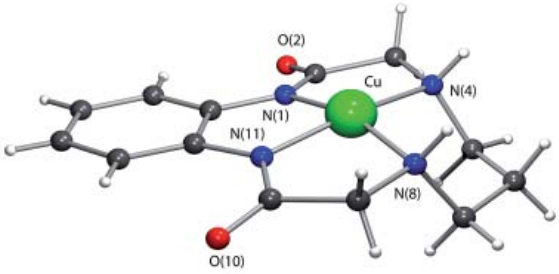


Fig.1- A molecular view of [Cu(13bzN4)] complex showing the overall structure of the complex. For clarity the atomic notation scheme of the carbon atoms is omitted.

The development of ditopic macrocycles and cryptands as receptors for the detection and the selective removal of pollutants, such as pesticides, phthalic acid esters and polycyclic aromatic hydrocarbons were undertaken. These receptors form homodinuclear complexes and can recognize a pollutant that bridges the metal centres, forming cascade species, see an example in Fig. 2. They can also selectively encapsulate the pollutants themselves forming supermolecules,[2,3] when complementarity (in shape, size and topology, and in number and type of interactions) between the receptor and the substrate structures is obtained.
Several families of receptors have been synthesized by a [2+2] condensation of different dicarboxaldehydes and diamines. The binding affinity between the substrate and the receptor has been studied by several techniques and the binding equilibrium constant determined. A very interesting receptor for substrates of extended aromatic systems, such as pyrene-1-carboxylate anion at low pH values (< 2.5), and high charged anions, such as benzenetricarboxylate at pH of about 5.5, was found.

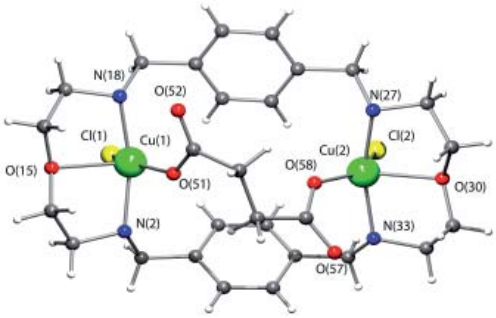


Fig.2- Molecular structure of the cascade complex [Cu$_2$L1(suc)2Cl] with succinate anion bridging the two copper(II) ions.

Selected Publications

Marques F, Gano L, Campello M. P, Lacerda S, Santos I, Lima L. M. P, Costa J, Antunes P, Delgado R (2006) "13- and 14-membered macrocyclic ligands containing methylcarboxylate or methylphosphonate pendant arms: Chemical and biological evaluation of their ^{153}Sm and ^{166}Ho complexes as potential agents for therapy or bone pain palliation", J. Inorg. Biochem. 100, 270-280.

Li F, Delgado R, Coelho A, Drew M. G. B, Félix V (2006) "New Dioxadiaza-, trioxadiaza- and hexaaza-macrocycles containing dibenzofuran units" Tetrahedron 62, 8550-8558.

Carvalho S, Delgado R, Fonseca N, Félix V (2006) "Recognition of Dicarboxylate Anions by a Ditopic Hexaazamacrocycle Containing Bis-p-xylyl Spacers" New J. Chem. 1, 247-257.

Rita Ventura
Auxiliary Investigator
PhD 1999 in Chemistry, Universidade Nova de Lisboa, ITQB

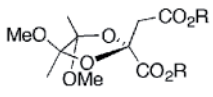
Bioorganic Chemistry

A new research line has just been started, which involves the synthesis of new organocatalysts from tartrate derivatives 1 and 2 (see figure). We plan to have candidate organocatalysts to test in several asymmetric reactions.

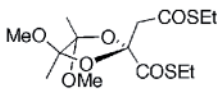
The synthesis of ceramide Cer1 3 (see figure) for the Eurico Melo research group (Micro-heterogeneous Systems) is still ongoing. Most steps of the synthesis have been optimised, however just 4 steps from the final product things are proving to be difficult. The cleavage of a silyl ether in the presence of an activated carboxylic acid, has failed. The protecting group strategy has had to be modified from the beginning of the synthesis.

The solute α-D-Glucosyl-(1,6)-α-D-Glucosyl-D-Glycerate 4 (see figure) has been synthesised on a large scale for the Helena Santos research group (Cell Physiology and NMR). A new glucosyl donor was used as well as a much simpler glycosylation method that has afforded the desired α anomeric selectivity and avoided the need for using expensive reagents.

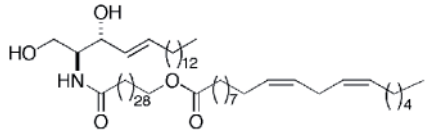
The dipeptide cystine-tyrosine 5 was also synthesised efficiently. Cystine is a symmetric aminoacid and attempts to desymmetrise it were unsuccessful (two carboxylic groups with the same reactivity) and the tyrosine-cystine-tyrosine tripeptide was formed instead of the desired product. This problem was overcome using a catalytic dissulfide exchange strategy, which can be applied for the synthesis of other asymmetric cystine-containing peptides.



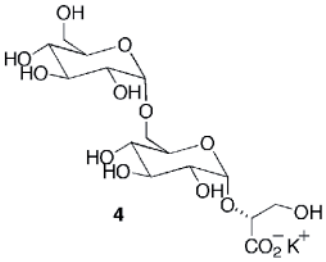
1
R=Me, i-Pr



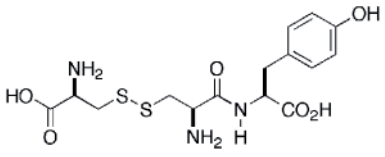
2



3



4



5

Group Members

Vera Lopes	Undergraduate
Teresa Marrafa	Undergraduate

Selected Publications

Burke A. J., Maycock C. D. and Ventura M. R. (2006). "Stereoselective alkylation of tartrate derivatives. A concise route to (+)-O-methylpiscidic acid and natural analogues." Organic & Biomolecular Chemistry 4(12): 2361-2363.

Borges N., Goncalves L. G., Rodrigues M. V., Siopa F., Ventura R., Maycock C., Larnosa P. and Santos H. (2006). "Biosynthetic pathways of inositol and glycerol phosphodiesters used by the hyperthermophile Archaeoglobus fulgidus in stress adaptation." Journal of Bacteriology 188(23): 8128-8135.

Biological Chemistry Division

The Biological Chemistry (BC) division has a strong focus on functional and structural characterisation of proteins, in particular metalloproteins, membrane proteins and proteins related to human health, and also an important program on cellular stress responses. The biological problems addressed range from biological energy conservation in aerobic and anaerobic microorganisms to mechanisms of oxidative and nitrosative stress responses used by pathogens to evade host immune defenses, and also responses to metal and metalloid stress. Other studies include protein folding and stability, protein modelling and development of theoretical/computational methods for the simulation of biomolecular systems. More applied studies target the structural characterisation of proteins with pharmacological or health importance, and of enzymes with biotechnological applications. The expertises within the division include Protein Chemistry (soluble and membrane proteins), Molecular Biology (cloning, sequencing, mutagenesis, transcriptional analysis, protein expression, protein-protein interactions, yeast two hybrid system), Protein Crystallography, Molecular Modelling/Structural Bioinformatics, Stopped Flow Kinetics, Redox Potentiometry, NO and O2 amperometry, and several spectroscopies: NMR, EPR, FT-IR, CD and Resonance Raman. The BC division also provides the ITQB with know-how on basic aspects of Biochemistry, Biophysics, and Spectroscopy.

In 2006 the BC division was comprised of 12 laboratories, 33 PhD researchers, 43 PhD students, 10 Graduate students and 8 Undergraduates. 25 national projects were coordinated by members of the BC division, which also participated in 4 EU projects. 4 PhD students defended their thesis. In 2006 50 papers were published in international journals, a considerable increase from the 35 published in 2005. Particularly noteworthy were 4 papers published in Science, Nature, EMBO J. and PNAS. The BC division is characterised by an extensive network of collaboration between its groups, which is reflected in the high number of papers involving several groups of the division (23). This characteristic, which has been a constant throughout the years, is a key point of its success. There are also important collaborations with groups from other divisions. Several meetings were organised by members of the division: “Meeting on Microbial Respiratory Chains”, 19-22 March in Tomar; “European Biological Inorganic Chemistry Meeting” (EUROBIC 8) 1-6 July in Aveiro; “3rd European Meeting of Oxizymes”, 7-9 September at ITQB, and “Biocrys2006”, 6-13 October at ITQB. In terms of novel equipment a liquid handling workstation, a Hamilton Microlab Starlet, was acquired for highthroughput screening, and there was an upgrade of the X-ray equipment, including a MICROSTAR rotating anode generator and a CCD area detector. The division will also profit considerably from the recent installation of the new 800, 500 and 400 MHz NMR spectrometers as part of the National NMR facility at ITQB.

In 2006 the BC division and the ITQB suffered a great loss with the death of Prof. António Xavier, founder of ITQB, and for a long time mentor and coordinator of the BC division. In this year Prof. António Xavier was awarded the EUROBIC medal for his contributions to the field of Biological Inorganic Chemistry and posthumously the honor medal from the Sociedad Española de Bioquimica y Biologia Molecular for his dedication and leadership in science.

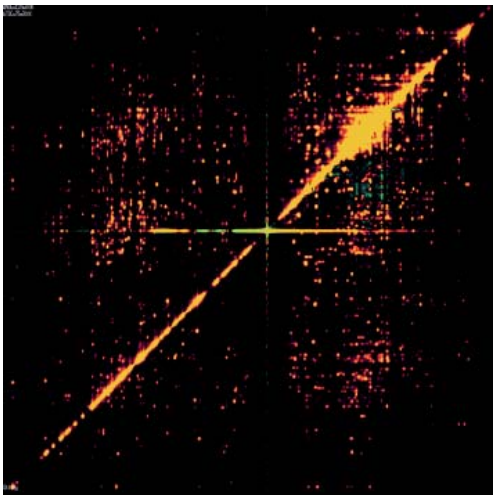
António Xavier
Full Professor
D.Phil 1972, Oxford University, UK

David Turner
Invited Full Professor
D.Phil 1977, Oxford University, UK

Structure and Function of Metalloproteins

After a long illness, António Xavier died on May 7th 2006. His death was a severe blow for his family, friends, and colleagues, and also a great loss for science in Portugal, at ITQB, and, of course, his research group. Professor Xavier's work continues: Catarina Paquete completed her PhD in December 2006, Pedro Quintas is applying for a PhD grant, and David Turner accepted responsibility for the ongoing research projects, as António Xavier wished.

Metalloproteins are essential to bioenergetics and these proteins commonly have multiple metal centres. The tetrahaem (four iron) cytochromes c3 from sulfate reducing bacteria have provided a challenging model for multicentre metalloproteins and we have developed the theory and experimental methods for a multidisciplinary approach to understanding their function. Redox titrations coupled with NMR allow the potentials of individual haems to be measured, as well as the interactions between them and with ionisable centres such as acidic groups. The structures of these proteins are then determined in solution at different pH values, both in the paramagnetic oxidized forms and the diamagnetic reduced forms, to elucidate the structural basis of interactions. Most recently, these studies have been extended to measuring the kinetic properties of individual haems. The reduction of the haems by sodium dithionite can be followed by the stopped-flow method, in which the protein solution is rapidly mixed with the reducing agent and the UV-visible spectrum is monitored over time. However, this measures the total amount of reduced haem and can not distinguish between the individual haems in the metalloprotein. That information can only be extracted by obtaining the detailed thermodynamic properties of the haems with the methods outlined above, measuring the rates at different pH values, and applying the Marcus theory of electron transfer. Now that we have succeeded in characterizing the thermodynamic and kinetic properties of the individual haems in an isolated cytochrome, we can move on to the crucial question of how cytochromes c3 transfer electrons and protons between the hydogenase that is in solution in the periplasm and clusters of proteins bound to the cell membrane.



Part of a 500 MHz NMR spectrum of the oxidised cytochrome c3 from Desulfovibrio desulfuricans ATC27774.
This is a two-dimensional NOE spectrum from which the distances between protons in the molecule can be measured so that the three dimensional structure of the protein can be determined.

Group Members

Teresa Catarino	PhD
António Aguiar	PhD
Vitor Paixão	PhD Student
Catarina Paquete	PhD Student
Pedro Quintas	Graduate
Tânia Mil-Homens	Graduate

Selected Publications

Structural evidence for a proton transfer pathway coupled with haem reduction of cytochrome *c*" from *Methylophilus methylotrophus* Enguita F.J., Pohl E., Turner D.L., Santos H., Carrondo M.A. J.Biol. Inorg. Chem., 11, 189-196 (2006).

Solution structures of tetrahaem ferricytochrome c(3) from *Desulfovibrio vulgaris* (Hildenborough) and its K45Q mutant: The molecular basis of cooperativity Messias A.C., Aguiar A.P., Brennan L., Salgueiro C.A., Saraiva L.M., Xavier A.V., Turner D.L. Biochim. Biophys. Acta – Bioenergetics, 1757, 143-153 (2006).

Functional properties of type I and type II cytochrome c3 from *Desulfovibrio africanus* Paquete C.M., Pereira P.M., Catarino T., Turner D.L., Louro R.O., Xavier A.V. Biochim. Biophys. Acta – Bioenergetics, 1767, 178-188 (2007).

António M. Baptista
Auxiliary Investigator
PhD in 1998, Universidade Nova de Lisboa, Portugal

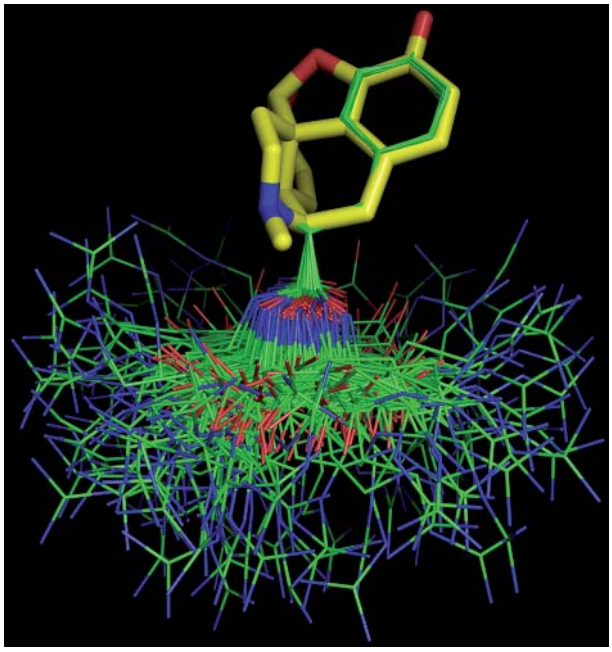
Molecular Simulation

Group Members

Miguel Machuqueiro	Post Doc
Vitor Teixeira	PhD student
Sara Campos	PhD student

The Molecular Simulation Laboratory uses theoretical and computational methods to study the atomic-level determinants of the properties of (bio)chemical molecules. The methods are based on physical principles (namely Statistical Thermodynamics) and intend to derive/simulate molecular behavior from those principles. We put a strong emphasis on the development of novel biomolecule-oriented methodologies, which are then applied to biologically interesting cases. Many of our studies focus on the study of processes with an electrostatic basis, such as the structural consequences of the uptake/release of protons and electrons in proteins. A major line of work is the inclusion in simulation methods of experimentally important parameters that are essentially electrostatic, such as pH, ionic strength and reduction potential of the solution. Other lines of work include the development of methods for characterizing protein structures using a small number of variables.

From the research done during 2006 we may highlight our study of kyotorphin, a small analgesic peptide that naturally exists in the human body. Kyotorphin has opioid-like effects and, although it is known that it does not bind to the morphine receptor, its receptor molecule has not yet been identified. Our study indicates that kyotorphin does not have a well-defined structure but rather a wide population of possible structural states. Furthermore, we found that at physiological pH the peptide is expected to vary its charge with some ease, which in turn strongly shifts the population of structural states. Somewhat surprisingly, the study also reveals that many of the structural states adopted by kyotorphin give an excellent match when fitted onto the structure of morphine (which is virtually rigid). Most strikingly, those well-fitted structures always have the morphine-like part of kyotorphin uncluttered and accessible for interaction. These results suggests that, despite being different, the kyotorphin and morphine receptors may have similar binding sites.



Best fits between the simulation-obtained structural states of kyotorphin (lines, green carbon atoms) and the structure of morphine (sticks, yellow carbon atoms).

Selected Publications

Machuqueiro M and Baptista AM (2006) Constant-pH molecular dynamics with ionic strength effects: protonation-conformation coupling in decalysine. Journal of Physical Chemistry B 110(6): 2927-2933.

Teixeira VH, Baptista AM and Soares CM (2006) Pathways of H2 towards the active site of [NiFe]-hydrogenase. Biophysical Journal 91:2035-2045.

Micaelo NM, Baptista AM and Soares CM (2006) Parameterization of 1-butyl-3-methylimidazolium hexafluorophosphate/nitrate ionic liquid for the GROMOS force field. Journal of Physical Chemistry B 110:14444-14451.

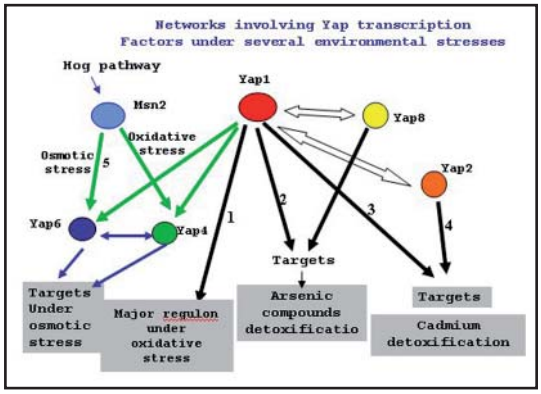
Cláudina Rodrigues-Pousada

Invited Full Professor

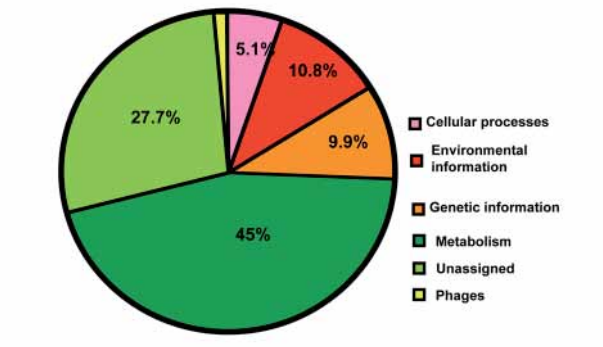
Doctorat d'Etat ès Sciences (1979), Biochemistry, Université Paris VII and Institut de Biologie Physico-Chimique

Genomics and Stress

1. Interplay between the transcriptional regulators:
Saccharomyces cerevisiae is continuously exposed to rapid and drastic changes in its external milieu. Cells must maintain their homeostasis, which is achieved through highly coordinated gene expression involving a plethora of transcription factors such as Hsf1, Msn2/Msn4, Skn7, as well as the yeast AP-1 proteins, the Yap family of b-ZIP proteins comprising eight members. In the last decade, it was shown that gene expression regulation under stress conditions does not involve a single transcription factor but the cooperation between several factors. In our lab we are studying the cross talk between the different Yap proteins, deciphering the respective targets and the mechanism of activation. We showed that both Yap4 and Yap6 are regulated by Msn2 and Yap1 under conditions of oxidative stress but are solely regulated by Msn2 under osmotic stress. The fact that these two proteins interact *in vivo* and have common targets indicats they may function as heterodimers. We showed that specific determinants of the Cd response are encoded within both Yap1 and Yap2 C-terminus, whereas those required for H_2O_2 response are only present in the Yap1 C-terminus. We identified Frm2 (a protein of the lipid peroxidation) as Cd-responsive Yap2 target indicating a possible role of this protein in regulating a metal stress response. In the context of arsenic stress we obtained strong evidence indicating the interplay between Yap1 and Yap8. We showed that, whereas Yap8 is the major regulator of this stress response by activating a specific extrusion system, Yap1 facilitates adaptation by regulating the vacuolar sequestration of this compound and by maintaining the redox equilibrium in the cytoplasm that is disturbed by arsenic stress. Indeed under these conditions the
Yap1 targets include several proteins belonging to antioxidant defences.



2. Deciphering *D. gigas* genome and trancriptional regulation under nitrosative stress *Desulfovibrio gigas* flavodiiron protein (FDP), rubredoxin:oxygen oxidoreductase (ROO), was proposed to be the terminal oxidase of a soluble electron transfer chain coupling NADH oxidation to oxygen reduction. The data obtained by us, show that D. gigas ROO acts also as NO reductase revealing a versatility which affords protection to D. gigas at the onset of both oxidative and nitrosative stresses. As we are finishing its genome, we already identified a family of about 16 genes encoding proteins which are putative regulators of ROO.



Functional categories found within the genome of Desulfovibrio gigas.

Group Members

Regina Menezes	Post Doc
Tracy Nevitt	Post Doc
Catarina Pimentel	Post Doc
Rute Rodrigues	PhD
Catarina Amaral	PhD
Jorge Pereira	PhD
Liliana Nascimento	PhD
Teresa Barata	Master Student
Cristina Alves	Master Student
Fábio Morais e Silva	Graduate

Selected Publications

Rodrigues R, Vicente JB, Felix R, Oliveira S, Teixeira M, Rodrigues-Pousada C. (2006) Desulfovibrio gigas flavodiiron protein affords protection against nitrosative stress in vivo. J Bacteriol. 2006 Apr;188(8):2745-51.

Machado, P., Félix, R., Oliveira, S., and Rodrigues-Pousada, C., (2006) and expression analysis of the cytochrome bd oxidase operon from Desulfovibrio gigas.Curr Microbiol. 52(4):274-81. J Bacteriol.188 (8):2745-51.

Nevitt T., and Rodrigues-Pousada C (2006) Stress Response in the Budding Yeastin the book "Stress Response in Biology and Medicine" (edited by Jurgen Radons and Gabriele Multhoff) Research Signpost

Cláudio M. Gomes
Auxiliary Investigator, Group Leader
PhD in Biochemistry 1999, Universidade Nova de Lisboa

Protein Biochemistry, Folding and Stability

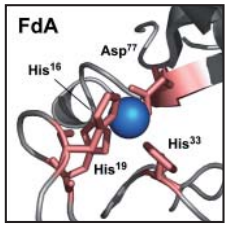
Group Members

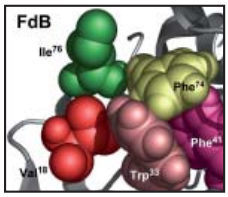
Sónia S. Leal	PhD student
Vesna Prosinecki	PhD student
Ana R. Correia	PhD student
Bárbara Henriques	PhD student
Hugo Botelho	PhD student
Catarina Silva	Undergraduate
Rita Rocha	Undergraduate

Our laboratory is interested in understanding the molecular determinants of protein structure and conformational stability. This topic has a direct impact on the protein folding problem and on the so-called conformational disorders. Studied models range from proteins bearing cross-linking interactions between a metal ion and the protein (such as zinc and calcium ions or iron-sulfur clusters), to human proteins whose conformational destabilization results in disease. Experimentally, we use a complementary set of tools, ranging from biochemical and molecular biology methodologies to biophysical, spectroscopic and proteomics techniques.

In recent years we have been increasingly engaged in the investigation of protein conformational disorders, especially the variants associated with late onset or milder clinical disease presentations. These are hypothesized to be more dependent on cellular environmental factors such as temperature, pH or molecular crowding, or to oxidative and nitrosative stress. In this theme, our overall goal is to obtain a structural and mechanistic picture of how disease-related genetic variability impacts on the cell, from protein-level alterations, to chaperon response, to global effects and oxidative stress. Current models under study include proteins involved in different disorders: e.g. frataxin (neurodegeneration), electron-transfer flavoprotein and the respective dehydrogenase (lipid metabolism) and phenylalanine hydroxylase (amino acid metabolism). For example, we are currently pursuing a conformational and functional characterization of a series of point mutations in the iron-chaperon frataxin, which are found in heterozygous individuals suffering from Friedreich's Ataxia (FRDA). Our recent work has shown that the studied mutant frataxin variants result in folded but conformationally destabilized proteins. We have noted a correlation between functional deficiency as iron-chaperons, and clinical expression of FRDA, and we are currently designing experiments to investigate the *in vivo* consequences of these mutations.

ETF (top) and Frataxin (bottom)





Alternative domain stabilisation in a ferredoxin zinc site

A long lasting interest of the laboratory concerns the study of the interplay between a protein and its metal centers and cofactors. Recent studies on this theme have elicited a natural protein design strategy within a zinc domain in ferredoxins, as we have shown that the enhanced thermal stability of a zinc-lacking ferredoxin isoform can be correlated with the efficient replacement of the structural metal site by a hydrophobic core. We have also carried out studies aiming at a structural characterization of the molten globule state of a ferredoxin model, with possible implications on the protein folding process and iron-sulfur centre assembly. During 2006 we have set up a proteomic approach allowing the selection of proteins with enhanced intrinsic conformational stability, in this case, in the context of a thermophilic organism. Interestingly, this approach highlighted not only proteins interesting for subsequent stability studies, but also key metabolic processes which may have themselves a 'thermophilic character'. More details and a complete publication list at www.itqb.unl.pt/pbfs

Selected Publications

Correia A. R., Adinolfi S., Pastore A. and Gomes C. M. (2006). "Conformational stability of human frataxin and effect of Friedreich's ataxia-related mutations on protein folding." Biochemical Journal 398: 605-611.

Prosinecki V., Botelho H. M., Francese S., Mastrobuoni G., Moneti G., Urich T., Kletzin A. and Gomes C. M. (2006). "A proteomic approach towards the selection of proteins with enhanced intrinsic conformational stability." Journal of Proteome Research 5(10): 2720-2726.

Rocha R., Leal S. S., Teixeira V. H., Regalla M., Huber H., Baptista A. M., Soares C. M. and Gomes C. M. (2006). "Natural domain design: Enhanced thermal stability of a zinc-lacking ferredoxin isoform shows that a hydrophobic core efficiently replaces the structural metal site." Biochemistry 45(34): 10376-10384.

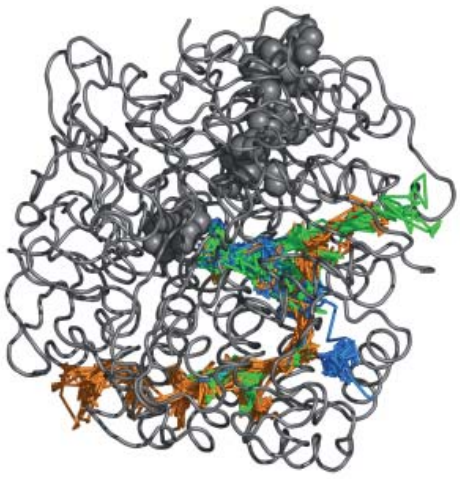
Cláudio M. Soares
Associate Professor
PhD 1994 in Theoretical Biochemistry, Universidade de Lisboa / Uppsala University

Protein Modelling

The Protein Modelling Laboratory works on molecular modelling of proteins using physical methods. Our areas of work range from basic research in modelling methodologies to applications with biotechnological and biomedical interest. Modelling redox proteins and redox chains is one of our research interests, and one of the most relevant examples in 2006 was the work, together with the Molecular Simulation Laboratory, on the mechanisms of [NiFe] hydrogenase, which catalyses the reversible molecular hydrogen cleavage into electrons and protons. Hydrogen permeation towards the internal active site was investigated by molecular dynamics simulations of the enzyme in solution. We were able to observe permeation events, highlighting the presence of channels. These channels lead hydrogen towards the Ni atom, and contain several bottlenecks, like a valine residue near the active site, which when mutated in silico to an alanine, showed improved hydrogen access. Another important aspect on the mechanism of [NiFe]-hydrogenase is the proton transfer process. Produced protons have to reach the surface of the enzyme and ionisable groups are required for an efficient proton transfer. Using continuum electrostatic and Monte Carlo simulations, we investigated the protonation events and we were able to propose proton transfer pathways.

Studying enzymes in non-aqueous solvents is another important area in our laboratory, with focus on the molecular mechanisms of enzyme hydration, enantioselectivity and catalysis. Ionic liquids are a new type of non-aqueous solvents with interesting properties for enzyme catalysis, but the molecular mechanisms present are largely unknown. In 2006 we published a parametrisation of two ionic liquids, which captures both the static and dynamic properties of these media. These liquids are currently being used in protein simulations, clarifying the behaviour of these systems at the molecular level.

ABC transporters constitute a new topic to our laboratory. Our first work on the subject was on the NBD1-NBD2 association in CTFR, the chloride transporter involved in cystic fibrosis, one of the most common genetic diseases. We built structural models for this association, responsible for ATP hydrolysis, and used them to understand the effect of mutations on disease causing genotypes. Right now we are studying the ATP-dependent “power stroke”, in order to understand molecular architecture and functionality of such transporters.



Three molecular dynamics simulation trajectories coloured in green, blue and orange, respectively, of molecular hydrogen permeation from the exterior towards the [NiFe]-hydrogenase active site (coloured in gray, together with the FeS centres). Teixeira et al. (2006). Biophysical Journal 91: 2035-2045

Group Members

Paulo Martel	Assist. Prof. UAlg.
Carlos Cunha	Post Doc
Vitor Hugo Teixeira	PhD student
Bruno Victor	PhD student
Nuno Micaelo	PhD student
Ana Sofia Oliveira	PhD student
Diana Lousa	PhD student
Zélia Ferreira	Graduate
João M. Damas	Undergraduate

Selected Publications

Micaelo N. M., Baptista A. M. and Soares C. M. (2006). “Parametrization of 1-butyl-3-methylimidazolium hexafluorophosphate/nitrate ionic liquid for the GROMOS force field.” Journal of Physical Chemistry B 110(29): 14444-14451

Roxo-Rosa M., Xu Z., Schmidt A., Neto M., Cai Z. W., Soares C. M., Sheppard D. N. and Amaral M. D. (2006). “Revertant mutants G550E and 4RK rescue cystic fibrosis mutants in the first nucleotide-binding domain of CFTR by different mechanisms.” Proceedings of the National Academy of Sciences of the United States of America 103(47): 17891-17896

Teixeira V. H., Baptista A. M. and Soares C. M. (2006). “Pathways of H_2 toward the active site of [NiFe]-hydrogenase.” Biophysical Journal 91(6): 2035-2045

Inês Cardoso Pereira
Auxiliary Investigator
PhD 1993, Oxford University, UK

Microbial Biochemistry

Group Members

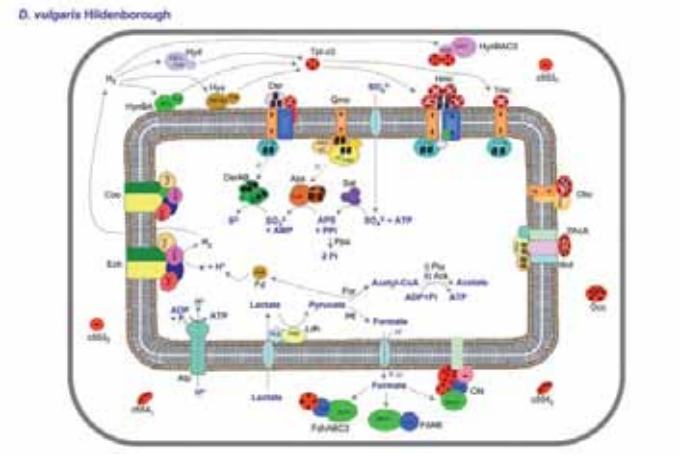
Luísa Rodrigues	Post Doc
Filipa Valente	PhD student
Sofia Silva	PhD student
Patrícia Pereira	PhD student
Sofia Venceslau	Graduate
Tiago Granja	Master Student
Rita Lino	Undergraduate

Microorganisms have a great impact in the chemistry of life in this planet, and also affect global climate. Their high biological diversity and range of environmental adaptations is associated with the fact that microbes explore many different metabolic strategies to sustain life. In anaerobic environments one of the possible strategies is that of Anaerobic Respiration, in which an organic or inorganic compound is used as terminal electron acceptor in place of oxygen. The Microbial Biochemistry group investigates the novel mechanisms and proteins used by a large group of ubiquitous bacteria that respire sulphur compounds (like sulfate and sulfite). These bacteria are implicated in a range of environmental and health issues, and are important research targets in the areas of Bioremediation, as well as Bio-Hydrogen production. By studying their respiratory metabolism and, in particular, several proteins of these bacteria, we aim to contribute to an informed exploitation of their biotechnological applications as well as to a better control of their biological activity, including potentially adverse health and environmental effects.

The respiratory chain of sulfate-reducing bacteria is very distinct from other organisms, and the mechanisms of energy conservation have not been clearly established. We have been studying the proteins involved in H2 oxidation, as well as membrane-associated protein complexes that may transfer electrons to the cytoplasmic reduction of sulfate. *Desulfovibrio vulgaris* Hildenborough, whose genome sequence is known, contains several hydrogenases (Hases) and membrane redox complexes that may participate in this process. In particular, there are four periplasmic-facing Hases, which may seem redundant. We have established that the expression of these Hases is affected by the availability of Ni and Se during growth, and that when both elements are available the main Hase present is the [NiFeSe] Hase. This Hase has a very high activity and is resistant to O2 inactivation, making this organism an interesting target for biological production of H2.

We have also isolated and characterised a new membrane complex from this organism, the Tmc complex that is associated with the TpIIc3 cytochrome. This complex has redox centers in the periplasm, membrane and cytoplasm, all of which are reduced in the presence of Hase/TpIc3, indicating that this complex may participate in the H2 to sulfate electron transport.

In collaboration with the Membrane Protein Crystallography group we obtained the structure for the NrfHA membrane-bound complex of nitrite reductase, which provided the first structural information for the family of cytochrome c quinol dehydrogenases, and which revealed that the menaquinol-interacting heme has very unusual coordination.



Schematic representation of D. vulgaris Hildenborough energy metabolism

Selected Publications

Valente FMA, Almeida CC, Pacheco I, Saraiva LM and Pereira IAC (2006) Selenium is involved in regulation of periplasmic hydrogenases gene expression in *Desulfovibrio vulgaris* Hildenborough, J. Bacteriol., 188: 3228-3235

Pereira PM, Teixeira M, Xavier AV, Louro RO and Pereira IAC (2006) The Tmc complex from *Desulfovibrio vulgaris* Hildenborough is involved in transmembrane electron transfer from periplasmic hydrogen oxidation, Biochemistry, 45: 10359-10367

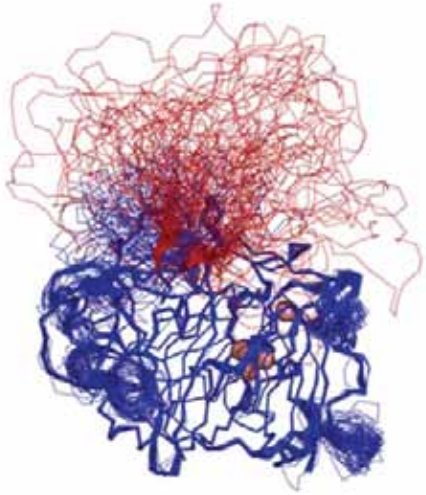
Rodrigues ML, Oliveira T, Pereira I A C and Archer M (2006) X-ray structure of the membrane-bound cytochrome c quinol dehydrogenase NrfH reveals novel heme coordination, EMBO J., 25: 5951–5960

Lígia O. Martins
Invited Assistant Professor
PhD 1994 in Biotechnology , Universidade Técnica de Lisboa, IST

Microbial and Enzyme Technology

The multicopper oxidases (MCO) constitute a family of enzymes with broad substrate specificity, which oxidise numerous aromatic phenols and amines. The one-electron oxidation of these substrates occurs concomitantly with a four-electron reduction of molecular oxygen to water. The laccases constitute a large subfamily of MCO and have a great potential in various biotechnological processes 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. A few MCO members are able to oxidize, with high specificity, lower valence metal ions such as Cu+ Fe2+ and Mn2+. These are thus designated as metallo-oxidases and prominent members such as human ceruloplasmin (hCp), yeast ferroxidase Fet3p and CueO from *Escherichia coli* are known to be critically involved in cellular metal homeostasis mechanisms.

The catalytic and stability characteristics of bacterial laccases at the molecular level are of considerable interest and, as a model system, the CotA-laccase from *Bacillus subtilis* has been extensively studied by our group. The main objectives of such studies are to dissect the catalytic mechanisms using protein engineering techniques and to design laccases that better match biotechnological applications. We have extended these studies to hyperthermophilic laccase-like enzymes. Our understanding of the structure-function relationships for the extremophilic enzymes is still limited, but their use offers new opportunities for biocatalysis as a result of their superior stability. The spectroscopic properties and biochemical characterization of the recombinant McoA (Multicopper oxidase from *Aquifex aeolicus*) revealed that this is copper-activated metallo-oxidase, with features typical of the well-known MCO. However, one aspect of McoA is the presence of a Met-rich segment that is absent in the “classic” MCO. McoA is a thermoactive and hyperthermostable enzyme with a three-domain thermal unfolding characterized by temperatures values at the mid-point ranging from 105 to 114ºC. The comparative study of CotA-laccase and McoA will contribute to identify the molecular basis for the enzyme stability and the specificity toward different substrates, key aspects of MCO that still remain to be elucidated.



Overlay of the 60 different models obtained in the final cycle of the comparative modelling procedure for McoA from *Aquifex aeolicus*. The protein is represented by a thin ribbon and the copper atoms are represented by salmon coloured spheres. The loop spanning residues Phe 321 to Val 363 is coloured in red, while the rest of the protein is coloured in blue. The segment P321-V363, containing 10 Gly and 12 Met, has no counterparts on the templates used and thus each model presents a different conformation. The kinetic analysis of a mutant enzyme from which this segment was deleted, McoAΔP321-V363, indicates that this Met-rich region occludes the substrate binding site, in agreement with possible conformations in the structural model, suggesting the involvement of this region in the catalytic mechanism of the enzyme.

Group Members

Luciana Pereira	Post-doc
André Fernandes	PhD student
Paulo Durão	PhD student
Rui Coelho	Undergraduate

Selected Publications

Durão P., Bento I., Fernandes A.T., Melo E.P., Lindley P.F., and Martins L.O. 2006. Perturbations of the T1 copper site in the CotA laccase from Bacillus subtilis: structural, biochemical, enzymatic and stability studies. Journal of Biological Inorganic Chemistry, 11:514-526.

Lameiro M.H., Lopes A., Martins L.O., Alves P.M., Melo E. 2006. Incorporation of a Model Protein into Chitosan-Bile salt Microparticle. International Journal of. Pharmaceutics 312: 119-130.

Lamosa P., Gonçalves L.G., Rodrigues M.V., Martins L.O, Raven N.D.H. and Santos H. 2006. Occurence of 1-Glycerol-1-myo-Inosityl Phosphate in Hyperthermophiles. Appl Envir. Microbiol. 72: 6169-6173

Lígia M. Saraiva
Auxiliary Investigator
PhD 1993 in Biochemistry, Universidade Nova de Lisboa

Molecular Genetics of Metalloproteins

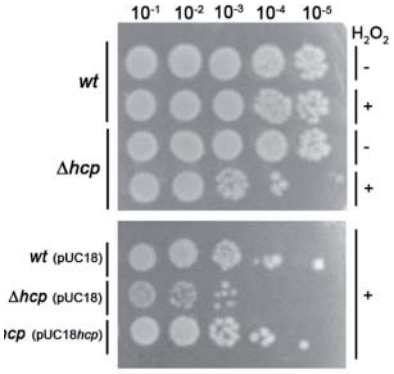
Group Members

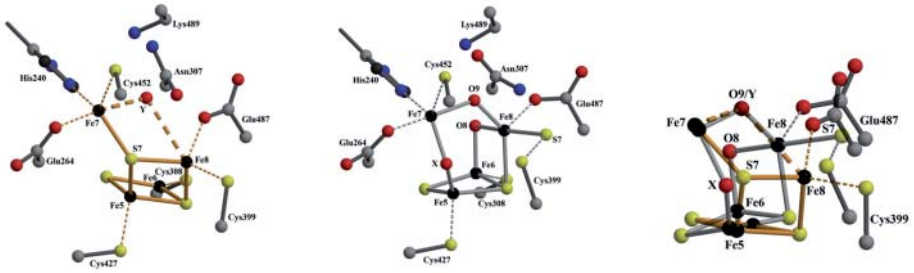
Marta C. Justino	PhD student
Susana Lobo	PhD student
Lígia Nobre	PhD student
Vera Gonçalves	PhD student
Joana Baptista	Undergraduate
Ana Filipa Tavares	Undergraduate
Cláudia Almeida	Technician

The Molecular Genetics of Metalloproteins Laboratory focuses on the analysis of the bacterial responses to nitrosative and oxidative stress and on the mechanisms that confer oxygen resistance to anaerobic organisms. Research highlights of the 2006 year include: i) the study of the NO detoxifying enzyme flavohemoglobin in *Staphylococcus aureus*; ii) the discovery of the physiological function of the Hybrid Cluster Protein of *Escherichia coli* and iii) the study of a newer gene, ytfE, involved in the assembly of proteins containing iron-sulphur clusters, a novel and so far unknown system.

The hybrid-cluster protein is involved in oxidative stress defense

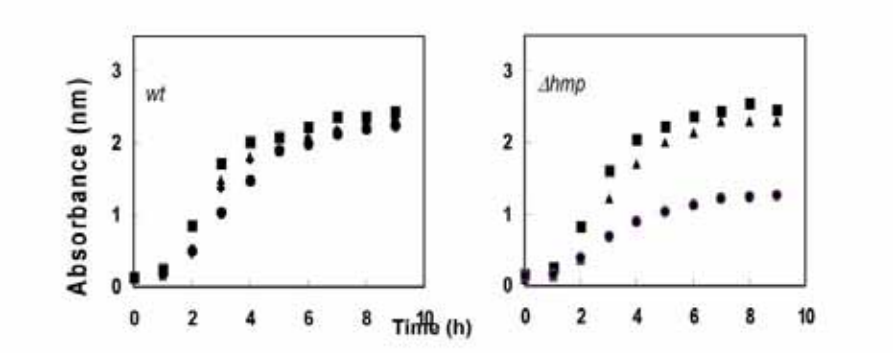
For a long time the physiological function of the Hybrid-cluster proteins, that contain a [4Fe-4S]$2^{+}/1^{+}$ or a [2Fe-2S]$2^{+}/1^{+}$ cluster and a novel type of hybrid cluster, [4Fe-2S-2O], remained unknown in spite of a detailed spectroscopic and structural characterization. We found that HCP is involved in oxidative stress protection and





Flavohemoglobin protects *Staphylococcus aureus* from nitrosative stress

The study of *S. aureus* flavohemoglobin revealed that, in contrast to other bacterial flavohemoglobins, this enzyme requires a microaerophilic environment to protect against nitrosative stress. Furthermore, the in vivo data corroborates that flavohemoglobin acts physiologically as a denitrosylase.



Selected Publications

Almeida CC, Romao CV, Lindley PF, Teixeira M and Saraiva LM (2006) The role of the hybrid cluster protein in oxidative stress defense. Journal of Biological Chemistry 281(43): 32445-32450

Justino MC., Almeida CC., Goncalves VL., Teixeira M and Saraiva LM (2006) Escherichia coli YtfE is a di-iron protein with an important function in assembly of iron-sulphur clusters. FEMS Microbiology Letters 257(2): 278-284

Gonçalves VL, Nobre LS, Vicente JB, Teixeira M, and Saraiva LM (2006) Flavohemoglobin requires microaerophilic conditions for nitrosative protection of Staphylococcus aureus FEBS Letters 580 (7):1817-21.

Margarida Archer
Auxiliary Investigator
PhD in 1999 in Universidade Nova de Lisboa, ITQB

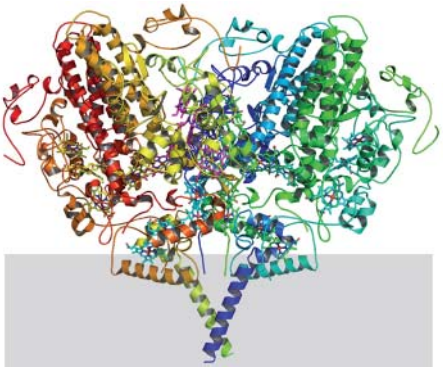
Membrane Protein Crystallography

Membrane proteins play crucial roles in essential cellular processes of all organisms. Although they comprise ca. 30% of the genomic information, little information is available on membrane protein structure Structural molecular biology plays a pivotal role in modern biology, both in the fundamental understanding of living things and in the design of new treatments for disease.

In our group, we are currently working on the structural characterization of membrane-bound proteins and complexes, namely those involved in respiration and metabolism. We have recently solved the X-ray structure of the membrane-bound cytochrome c nitrite reductase NrfHA complex (Rodrigues et al. EMBO J, 2006), which revealed novel heme coordination motifs and provided important insights into the menaquinol binding site and electron transfer flow within the complex. This structure represents an important contribution to the limited number of available structures of quinone- interacting membrane complexes Work in collaboration with the Microbial Biochemistry group at ITQB (Coordinator: Dr. Inês C. Pereira).

We have also solved the structure of Sulfide:quinone oxidoreductase, isolated from the membrane fraction of an hyperthermophilic archaea. The X-ray structure showed structural homology with gluthatione reductase family and revealed the presence of a disulphide group near the FAD cofactor, which prompted for a new functional characterization (manuscript in preparation). Work in collaboration with the Metalloproteins and Bioenergetics group at ITQB (Coordinator: Prof. Miguel Teixeira).

Besides respiratory and metabolic enzymes, we are also interest in membrane transport systems, which play crucial roles in essential cellular processes of all organisms. The Major Facilitator Superfamily (MFS) is one of the two largest families of membrane transporters and is found in Bacteria, Archaea, and Eukarya. MFS permeases usually comprise 12-14 transmembrane alpha helices. In general membrane transport systems are poorly understood, mostly because of the technical difficulties involved in isolating sufficient protein for elucidation of their structure-activity relationships. We have recently initiated a structural genomics approach on membrane transport proteins from Archaea, in collaboration with Prof. Peter Henderson (Leeds University, UK) and Dr. Arnulf Kletzin (Darmstadt University, Germany). Our main focus are MFS transporters involved in the uptake of sugars, nucleosides and amino acids; and those needed for efflux of antibiotics.



Representation of the overall structure of the membrane-bound cytochrome c nitrite reductase NrfHA complex

Protein crystals of NrfHA complex

Group Members

Mª Luísa Rodrigues	Post-doc
Meike Stelter	Post-doc
Diana Plácido	PhD student
José Brito	PhD student
Tânia Oliveira	PhD student

Selected Publications

Rodrigues ML, Oliveira TF, Pereira IAC and Archer M (2006). X-ray structure of the membrane-bound cytochrome c quinol dehydrogenase NrfH reveals novel haem coordination. EMBO Journal 25(24): 5951-5960

Rodrigues ML, Archer M, Martel P, Miranda S, Thomaz M, Enguita FJ, Baptista R, Melo E, Sousa N, Cravador A and Carrondo MA (2006) Crystal structures of the free and sterol-bound forms of β-cinnamomin" Bioch. Byophys Acta 1764: 110-121

Palma PN, Rodrigues ML, Archer M, Bonifacio MJ, Loureiro AI, Learmonth DA, Carrondo MA and Soares-da-Silva P (2006) Comparative study of ortho- and meta-nitrated inhibitors of catechol-O-methyltransferase: Interactions with the active site and regioselectivity of O-methylation." Molecular Pharmacology 70(1): 143-153

Maria Arménia Carrondo

Full Professor

PhD in Chemical Crystallography, Imperial College of Science and Technology, University of London, UK.

Macromolecular Crystallography

Group Members

Pedro Matias	Auxiliary Inv.
Carlos Frazão	Auxiliary Inv.
Isabel Bento	Post-doc
Colin McVey	Post-doc
Daniele deSanctis	Post-doc
Tiago Bandeiras	Post-doc
Mª. Luísa Rodrigues	Post-doc
Célia Romão	Post-doc
Rita Vargas	Post-doc
Ricardo Coelho	Technician
Susana Gonçalves	PhD student
Sabine Gorynia	PhD student
Jana Tatur	PhD student
Joana Rocha	PhD student
David Aragão	PhD student
Diana Plácido	PhD student
Tânia Oliveira	PhD student
José Brito	PhD student
Ana Rêgo	PhD student
David Marçal	PhD student
Miguel Lopes	Graduate
Catarina Silva	Graduate
S. Palnivelu	Graduate
Ana Ferreira	Undergraduate

The ITQB Macromolecular Crystallography (MX) Laboratory has contributed significantly to the 3D-structure determination by X-ray diffraction of proteins involved in catalytic and electron transfer processes, in human health and with biomedical applications. In addition, the research areas, include Proteins with Industrial and Medical Application, led by Dr. Pedro Matias, the Crystallographic Structure-Function Studies, led by Dr. Carlos Frazão, and involve a close collaboration with the Membrane Proteins Laboratory, coordinated by Dr. Margarida Archer (see previous page).

In 2006, the 3D structure of a Self-Compartmentalizing Sulfur Cycle Metalloenzyme was published in Science (see Figure 1). The first 3D structure of a RNAse II (see Figure 2) was determined and the structural determinants of its selectivity, processitivity and hydrolytic mechanism were elucidated and published in Nature. The crystal structure of the human AAA+ protein RuvBL1 was determined, allowing a structural understanding of its RNA/DNA-binding properties and surprisingly weak ATPase and helicase activities. The determination of the 3D-structure of native *B.subtilis* cotA incubated with $CuCl_2$, with H_2O_2 and in the reduced state allowed us to suggest a mechanism for the oxygen reduction to water for this type of multicopper oxidase enzymes. The 3D-structures of cotA mutants M502L and M502F showed that replacing the axial copper ligand metionine by a non-coordinating hydrophobic residue did not lead to major structural changes. Nevertheless, changes in the center's redox potential as well as in the activity and stability of the enzyme were observed. The three dimensional structure of the native Human ceruloplasmin protein was determined at 2.8 Å resolution, and showed a dioxygen moiety at the trinuclear copper cluster, as well as a $Ca2^+$ and a Na^+ centres with important structural and physiological roles. The crystal structure of the ferritin from the hyperthermophilic archaeal anaerobe *Pyrococcus furiosus* was determined, revealing that the 24-meric assembly exhibits the canonical 432 point-group symmetry. The structure of UgpG with a bound substrate was determined.

Access to synchrotron radiation for state of the art methods for data collection and structure solution is granted at ESRF in Grenoble, and through EU projects in EMBL in Hamburg and SLS in Villigen. Collaboration with Industry remains an important aspect of the research carried out at the ITQB MX Laboratory.

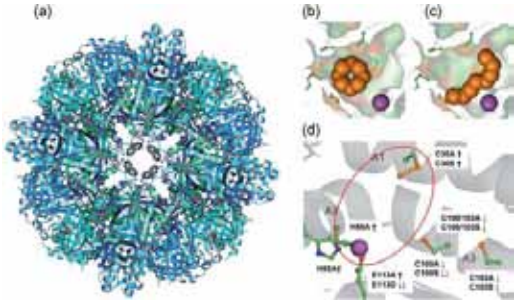


Figure1: Structure of Sulfur Oxygenase Reductase (SOR) from Acidianus ambivalens. (a) The SOR holoenzyme. Cartoon representation viewed along the crystallographic four-fold axis. The red spheres denote iron atoms. Note the protrusions at fourfold pseudo-symmetry axes, marked with bars. (b,c) modeling of putative substrates S8 sulfur and linear polysulfide into the catalytic pocket, with Fe represented as a purple sphere and catalytic cysteines in ball-and-stick; The inner surface of catalytic pocket is represented as semitransparent. (d) Effect of mutants on SOR activity: † zero activity, ↓reduced activity, ↓↓ strongly reduced activity. The core active site, composed of the iron site and the persulfide modified Css31, is highlighted with an ellipsoid.

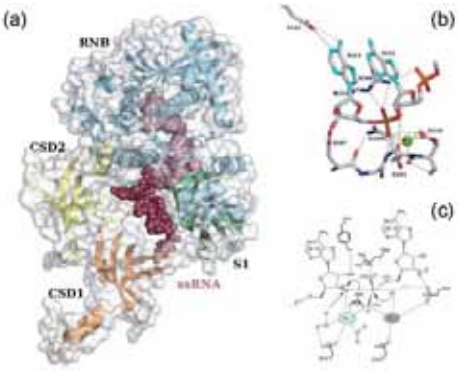


Figure2: RNA recognition by RNase II. (a) Structure of the RNase II D209N active site variant in complex with RNA (PDB code: 2ix1). The individual domains are labelled and the 13-mer ssRNA, which was bound to the 'as isolated' variant, is shown as crimson spheres. The magnesium ion found in the active site is represented as a green sphere. (b) The RNase II D209N mutant active site. (c) This figure represents the catalytic pocket containing the pentacoordinated phosphate in the (hypothetical) transition state.

Selected Publications

Frazão C, McVey CE, Amblar M, Barbas A, Vonrhein C, Arraiano CM and Carrondo MA (2006) Unravelling the dynamics of RNA degradation by RNAse II and its RNA-bound complex Nature 443(7107):110-114.

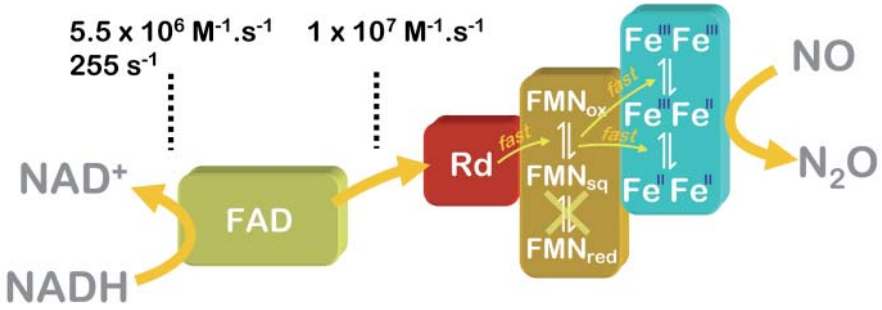
Urich T, Gomes CM, Kletzin A and Frazao C (2006) X-ray structure of a self-compartmentalizing sulfur cycle metalloenzyme Science 311(5763): 996-1000

Matias PM, Gorynia S, Donner P and Carrondo MA (2006) Crystal structure of the human AAA(+) protein RuvBL1 Journal of Biological Chemistry 281(50): 38918-38929.

Miguel Sepúlveda Teixeira
ITQB Associate Professor with Agregação
PhD in Chemistry, 1986, Universidade Nova de Lisboa

Metalloproteins and Bioenergetics

More than 50% of the predicted proteins are membrane-bound or metal containing, which just by itself shows the relevance of these types of proteins. Therefore, the research on this laboratory is focused on prokaryotic i) membrane-bound proteins, aiming at understanding at the molecular level the functional role of metalloproteins in energy transduction, and ii) metalloenzymes, namely those involved in novel mechanisms of oxygen and nitric oxide detoxification, quite relevant in host-pathogen interactions. The main target organisms are: sulfate reducers; facultative bacteria, e.g., E. coli and cyanobacteria, and extremophiles, which, mainly due to their phylogenetic distance from the most studied microbes, continuously reveal the presence of quite diverse and new proteins/enzymes, and allow a re-evaluation of several dogmas, by comparison with "canonical" enzymes, isolated from mitochondria and from the evolutionary related purple bacteria. These studies are now being extended to higher organisms, i.e., protozoa. The research involves a wide variety of complementary approaches: cell growth, protein chemistry, enzymatic assays, fast kinetics, spectroscopic studies (UV-Visible, Electron Paramagnetic Resonance, Resonance Raman), potentiometric and amperometric methods, reconstitution experiments in artificial liposomes, and a variety of ITQB and international collaborations for further complementary approaches. This reductionist approach, i.e., the in vitro studies of proteins, key players of the living world, is essential for the correct decoding of the genome sequences, and is being complemented to higher levels of organismal complexity by extensive collaborations with other laboratories. For 2006, several achievements were i) a detailed study of the molecular mechanism of 1Fe and 2Fe superoxide reductases and their physiological partners, which led to the proposal of a detailed mechanism for superoxide reduction to hydrogen peroxide; ii) the elucidation of the kinetics of the E. coli electron transfer chain that couples NADH oxidation to nitric oxide reduction to nitrous oxide; iii) a thorough and exhaustive characterisation by EPR spectroscopy coupled to potentiometric methods made for the first time in an intact complex I; iv) a comprehensive redox study of several oxygen reductases members of different families of the haem-copper superfamily was undertaken, using a novel theoretical methodology. The thermodynamic parameters obtained are important to understand the coupling mechanism of the redox and chemical processes during oxygen reduction and proton pumping. Surprisingly, we observed that there is not a common behaviour present among all the studied enzymes; v) a new sub-family of proton-sodium antiporters was identified.



Electron transfer mechanism of the nitric oxide detoxifying system from Escherichia coli, involving flavorubredoxin and its reductase partner, as inferred by a combination of redox and kinetics studies.

Group Members

Manuela Pereira	Auxiliary Inv.
Ana Melo	Assist. Prof. ULuso.
Andreia Fernandes	Assist. Prof. UAlg.
Smilja Todorovic	Post Doc
Célia Romão	Post Doc
Francesca Scandurra	PhD student
João Vicente	PhD student
Filipa Sousa	PhD student
Andreia Veríssimo	PhD student
Maxyme Cuypers	PhD student
Vera Gonçalves	PhD student
Ana Filipa Pinto	Undergraduate
Cláudia Almeida	Technician

Selected Publications

Rodrigues JV, Abreu IA, Cabelli D, Teixeira M. Superoxide reduction mechanism of *Archaeoglobus fulgidus* one-iron superoxide reductase Biochemistry. 2006 1;45(30):9266-78.

Almeida CC, Romão CV, Lindley PF, Teixeira M, Saraiva LM. The Role of the Hybrid-Cluster Protein in Oxidative Stress Defense. (2006) J Biol Chem, 281: 32445-32450

Electron Paramagnetic Resonance Studies of the Iron-Sulfur Centers from Complex I of *Rhodothermus marinus*. Fernandes AS, Sousa FL, Teixeira M, Pereira MM. (2006) Biochemistry Jan 24;45(3):1002-8.

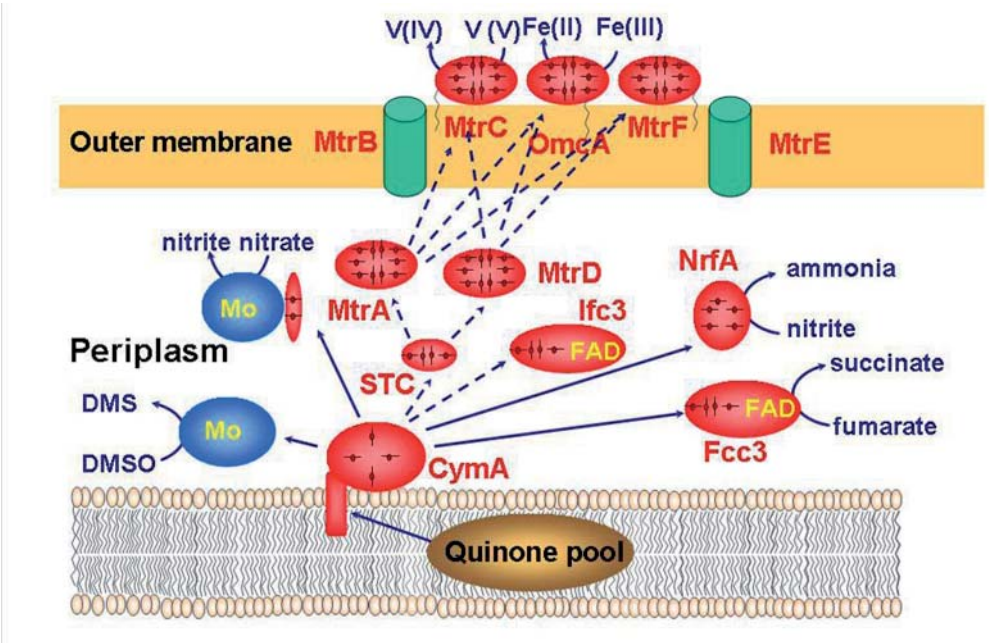
Ricardo O. Louro
Auxiliary Investigator
PhD 1998 in Biochemistry, Universidade Nova de Lisboa

Inorganic Biochemistry and NMR

Group Members

Catarina Paquete	Post Doc
Patrícia Pereira	PhD student
Ivo Saraiva	Graduate
Bruno Fonseca	Undergraduate
Isabel Pacheco	Technician

Organisms found in natural habitats that are capable of respiring metallic compounds have gained notoriety recently because of the novel approaches that they can provide for bioremediation of metal contaminated environments, biological electricity generation from sediment or waste water, and microbial based corrosion protection of sub-surface metallic structures.
The respiratory chains in these organisms must be able to deliver electrons to the outside of the cell wall, which requires that the proteins responsible for interacting with the solid substrate for electron transfer must be displayed at the cell surface, and participating in a novel arrangement and cellular location of the redox proteins committed to these respiratory networks. A large fraction of the proteins that have been identified as being involved in these respiratory networks are cytochromes but the structure and detailed functional mechanism of the majority of these proteins is not known.
This research group is currently engaged in the study of the structural bases for coupling the reduction of exogenous solid electron acceptors to energy conservation by several metal respiring organisms. This is being pursued in the framework of three research projects funded by the FCT, collecting structural and functional information on proteins from Shewanella oneidensis MR-1, *Desulfuromonas acetoxidans*, *Geobacter sulfurreducens* and *Desulfovibrio vulgaris* Hildenborough involved in the respiratory pathways of solid electron acceptors. NMR spectroscopy is the core technique used to obtain these data because in recent years the size and complexity of biological macromolecules that can be studied in detail by this technique has increased considerably. Therefore, it is uniquely suited for the collection of structural and dynamic information from the proteins under study.
Lack of detailed knowledge on the energy metabolism of metal-respiring organisms has been identified as a fundamental barrier to the optimisation of applications in microbial fuel cell technology and bioremediation. Establishing of the organization of the respiratory networks and detailed function of the proteins involved will provide the intellectual foundations for the optimization of biotechnological applications of these organisms or their proteins.



Cartoon of the cellular location of various proteins involved in anaerobic respiratory processes in Shewanella oneidensis MR-1

Selected Publications

Pessanha M, Morgando L., Louro RO, Londer YY, Pokkuluri PR, Schiffer M, Salgueiro CA (2006) Thermodynamic characterization of triheme cytochrome PpcA from Geobacter sulfurreducens: evidence for a role played in e-/H+ energy transduction, Biochemistry, 45, 13910-13917

Pereira PM, Teixeira M, Xavier AV, Louro RO, Pereira IAC (2006) The Tmc complex from Desulfovibrio vulgaris Hildenborough is involved in transmembrane electron transfer from periplasmic hydrogen oxidation, Biochemistry, 45, 10359-10367

Louro RO, Salgueiro CA (2006) Cytochromes of Shewanella respiratory pathways, in "Metal ions in Biology and Medicine" (MC Alpoim, PV Morais, MA Santos, AJ Cristovão, JA Centeno, P Collery, eds.) pp. 236-241, John Libbey Eurotext, Paris

Biology Division

Integrative Biology to Understand and Control Microbes

We are currently facing the comeback of old diseases previously controlled by the availability of antibiotics, and the appearance of new diseases produced by bacteria that have learned new strategies to infect humans and thus have become much more virulent. The main goal of the Biology Division is to provide solid knowledge on cellular processes in pathogens and model organisms. A secondary goal is to integrate this multi-level information as a means to control microbial infections or engineer non-pathogens for the synthesis of products with clinical or industrial importance.

To meet these goals different strategies are currently followed:

- Analysis of the evolution of different populations of pathogens in Portugal and worldwide to identify representative isolates which are most proficient, for example, in resisting antibiotics, causing disease or evading vaccination programs.

- Study of the mechanisms by which bacteria are able to resist antibiotics or to evade the host immune system. Studies aiming at identifying new targets that, if impaired, will contribute to the killing of bacteria by new or old antibiotics or to help the infected host immune system to overcome a bacterial infection.

- Studies of the mechanisms of bacterial cell growth and division and cellular morphogenesis during developmental programs.

- Study of the communication between bacteria, which may have a role in the ability of bacteria to colonize different hosts.

- Study of the regulatory mechanisms of gene expression in response to biological signals.

- Study of RNA metabolism in different systems with particular emphasis on RNases, mechanisms of RNA degradation and the role of small RNAs in the control of gene expression.

- Studies of sugar metabolism in pathogens and model bacteria, with emphasis on the metabolic networks and regulatory circuits that lead to products of interest, like the capsular polysaccharide essential for bacterial virulence, and nutraceuticals.

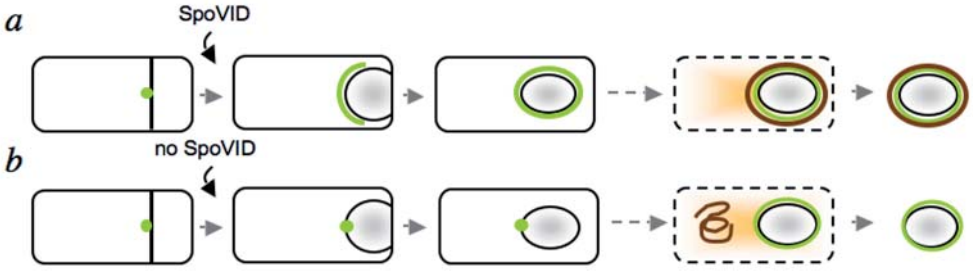
- Analysis of glycopeptides in bacterial infections. Use of chemical chaperones from hyperthermophiles to prevent protein aggregation associated with neurodegenerative diseases, such as Alzheimer´s disease or amyotrophic lateral sclerosis.

The Division has recently almost doubled the number of independent research groups and currently includes 11 Laboratories. Their collaboration with the existing groups at LA will allow the strengthening of research in fundamental areas such as pathogenic bacteria metabolism, bacterial cell wall synthesis, bacterial cell-cell communication and bacterial cellular and developmental biology. In addition, studies with eukaryotic cells in the areas of glycobiology and intracellular trafficking of glycoproteins associated with neurodegenerative diseases and ovarian carcinoma have been performed. The Division now expects to consolidate the existing groups as well as the interactions between them.

Adriano O. Henriques
Associate Professor
PhD 1993 in Cell Biology. Universidade de Coimbra, , Portugal.

Microbial Development

Bacterial spores are encased in a protein shield (or coat) that confers resistance against noxious chemicals and predation, protects the underlying cortex peptidoglycan layer from the action of lytic enzymes, and is a key sensor of the environment. The spore surface proteins are synthesized in the mother cell, one of the two compartments of the sporulating cell. They rely on morphogenetic proteins such as SpoVID and SafA for their targeting to the surface of the developing spore. In the absence of SpoVID, for instance, the surface proteins are misassembled as swirls throughout the mother cell cytoplasm, and the resulting spores, with an exposed cortex, are susceptible to lysozyme. Both SpoVID and SafA, orthologs of which are found in all *Bacillus* species, have LysM domains for peptidoglycan binding and localize to the cortex-coat interface. We have shown that a 13 amino acid region (region A) just downstream of the N-terminal LysM domain of SafA, is critical for its interaction with SpoVID. Lesions in region A impair the interaction of SafA with SpoVID in vitro, and while not affecting the accumulation of SafA in vivo, interfere with the localization of SafA around the developing spore, causing aberrant assembly of the coat and lysozyme sensitivity. A peptide corresponding to region A interacts with SpoVID, showing that residues within this region directly contact SpoVID. Since region A is highly conserved among SafA orthologs, this motif is likely to be an important determinant of coat assembly in the Bacillus group of spore-formers. Our results show that the function of SafA relies on the ability of the protein to reach its final cellular address trough a direct interaction with SpoVID. The localization of SpoVID in turn, relies on proper localization of another morphogenetic protein, SpoIVA, to the surface of the developing spore. Hence, the early stages in the attachment of the coat to the spore surface involve a cascade of protein-protein interactions among key morphogenetic proteins. These morphogenetic proteins have no counterparts in unrelated organisms and are likely to function in novel ways in the assembly of a multi-protein structure. Interference with their function will result in unstable spores, in what could be the basis for novel inactivation strategies of spores produced by pathogens such as *B. cereus* or *B. anthracis*.



Localization and function of coat morphogenetic proteins SpoVID and SafA. Sporulation is initiated by an asymmetric division that converts the sporangium into a smaller prespore and a larger mother cell. At this stage, SafA, which is produced in the mother cell, is targeted to the center of the septal plate (a). Next, the mother cell engulfs the prespore, and SafA migrates around the prespore fully encircling it. Tracking of the engulfment membranes by SafA requires a direct interaction with SpoVID (a). Following engulfment completion, assembly of the coat (brown circle) around the developing spore is completed, and following lysis of the mother cell, the free mature spore is resistant to lysozyme. In the absence of SpoVID (b), SafA is targeted to the septal plate but fails to migrate around the spore during engulfment. Following engulfment completion, the coat is deposited in the mother cell cytoplasm, and the released spores, with an exposed cortex, are sensitive to lysozyme treatment.

Group Members

Mónica Serrano	Post-doc
Gonçalo Real	Post-doc
Anabela Isidro	Post-doc
Luísa Côrte	PhD student
Cláudia Serra	PhD student
Carla Esteves	Master Student
Pedro Rodrigues	Master Student
Catarina Fernandes	Undergraduate
Ana Almeida	Volunteer

Selected Publications

Uyen, NQ, Tam, NMK, Hong, HA., Duc, LH, Hoa, TT, Serra, C, Henriques, AO, and Cutting, SM (2006) The intestinal life cycle of Bacillus subtilis and close relatives. Journal of Bacteriology 188(7): 2692-2700.

Costa, T, Isidro, AL, Moran CP Jr., and Henriques, AO (2006) The interaction between coat morphogenetic proteins SafA and SpoVID. Journal of Bacteriology 188(22): 7731-7741.

Thompson, LS, Beech, P, Real, G, Henriques, AO, and Harry, EJ (2006) A requirement for the cell division protein, DivIB, in polar cell division and engulfment during sporulation in Bacillus subtilis. Journal of Bacteriology 188(21):7677-7685.

Ana Rute Neves

Auxiliary Investigator

PhD 2001 in Biochemistry, Universidade Nova de Lisboa, ITQB

Physiology of Lactic Acid Bacteria and in vivo NMR

Group Members

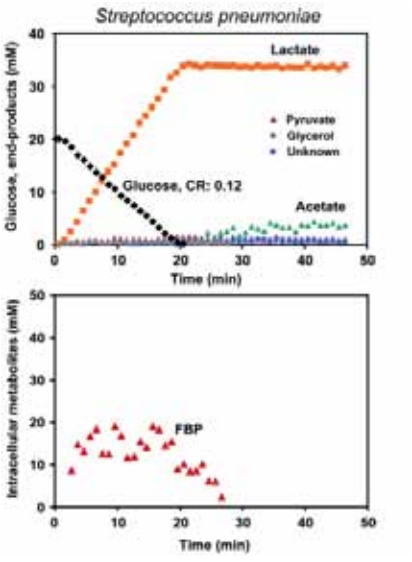
Rute Castro	PhD student
Sandra Carvalho	Graduate

Lactic acid bacteria (LAB) comprise a group of Gram-positive bacteria with habitats ranging from milk to specific niches in the human body. Some members have been used for thousands of years in food fermentations (e.g. *Lactococcus, Lactobacillus and Bifidobacteria*), whereas others are well-known for their ability to cause disease (e.g. *Streptococcus*). The key roles played at the industrial and clinical level have endowed LAB an enormous economical importance and drawn scientific attention to this group of microbes.

In our lab, we study metabolic and transcriptional regulation of sugar metabolism in human-associated LAB members, as well as the LAB model organism *Lactococcus lactis* (collaboration with H. Santos). Our main goal is to unravel the molecular mechanisms governing sugar utilization in the pathogen *Streptococcus pneumoniae* and in the probiotic *Bifidobacterium* breve. These are strictly fermentative organisms that rely on sugar fermentation for energy generation. Thus, the mechanisms governing sugar uptake and degradation are central to their physiology. Also, it is believed that mechanisms involved in carbon catabolite control are of utmost importance for fitness, survival and virulence in the host, since in the human body these bacteria are constantly challenged with variations in sugar composition. Several proteins involved in carbon catabolite control are unique to bacteria, and therefore, good targets for drug design.

In *S. pneumoniae*, we are also investigating the link between central carbon catabolite pathways and biosynthesis of capsule, which is recognized as a condition sine qua non of virulence.

For our studies we use analytical microbiological tools, molecular genetic tools, global transcriptome analysis, and in vivo Nuclear Magnetic Resonance (NMR) to probe biological processes directly in living cells.
During the last year we were able to establish medium and growth conditions that enable to collect time series data on intracellular metabolite pools during metabolism of glucose in *S. pneumoniae* cells by *in vivo* NMR (see figure). A comparison with *L. lactis* suggests different regulatory mechanisms at the level of pyruvate kinase and/or glucose transport. The application of this technique to genetically engineered strains of *S. pneumoniae* will extend our knowledge on the connection between sugar metabolism and virulence.



Dynamics of metabolite pools during the metabolism of glucose in Streptococcus pneumoniae R6 as monitored by in vivo 13C-NMR.

Selected Publications

Neves AR, Pool WA, Castro R, Mingote A, Santos F, Kok J, Kuipers OP and Santos H. (2006) The alpha-phosphoglucomutase of Lactococcus lactis is unrelated to the alpha-D-phosphohexomutase superfamily and encoded by the essential gene pgmH. Journal of Biological Chemistry. 281: 36864–36873.

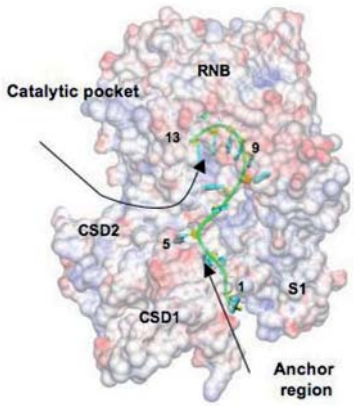
Cecília Arraiano
Principal Investigator with Agregação
PhD 1989 in Genetics, University of Georgia, Athens, USA

Control of Gene Expression

Many biological processes can not be fully understood without detailed knowledge of RNA metabolism. The analyses of RNA degradation have been difficult in all systems and, despite numerous studies, the process of RNA degradation is still poorly understood. Recent results appear to show that the similarities between mRNA decay in the pro- and eukaryotic systems are greater than were generally believed. It is important to study RNA metabolism in different systems, to allow universally conserved features to be recognized. Future work will involve the identification and study of the mechanism of action of more RNases, relating these RNases to RNA decay through the isolation of mutants, and assessing whether the various reactions are regulated. Taking this into account our research objectives are:

- Post-Transcriptional Control of Gene Expression
- Mechanism and Control of mRNA degradation.
- Characterization and Study of ribonucleases in the control of RNA Decay.
- Metabolism of the Poly(A) tail in Bacterial mRNAs
- Post-Transcriptional Studies in Escherichia coli Focusing on the Control of Cell Division
- Control of gene expression under stress and stationary phase.
- RNA processing in Lactic Acid Bacteria
- Small RNAs and Control of Gene Expression

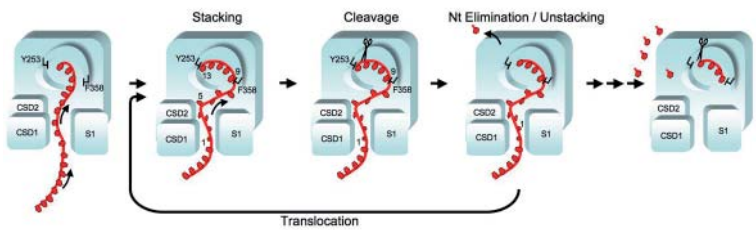
Ribonuclease II is a key exonuclease involved in the maturation, turnover and quality control of RNA. RNase II-family is ubiquitous in nature and mutations have been linked with abnormal chloroplast biogenesis, mitotic control and cancer. In eukaryotes, these enzymes are found in the ribonuclease complex called the exosome. In 2006 we have performed structural and functional studies on *Escherichia coli* RNase II and have characterized a mutant protein. In collaboration with the crystallography group of ITQB we have unravelled the dynamics of RNA degradation by RNase II and its RNA-bound complex.



A 3-dimensional model explaining the activity of these enzymes can now be proposed, opening an exciting new chapter in the comprehension of RNA maturation, selection and degradation, processes that ultimately control gene expression.

This year we have also shown that *Escherichia coli* RNase R, a member of the RNase II family, can be very important in stationary phase. In addition we showed that Poly (A)-polymerase I links transcription with mRNA degradation via sigma^S proteolysis. This unexpected finding makes important connections and extends the research on RNA degradation to other important fields.

RNase II and its RNA complex. Electrostatic potential of RNase II Asp209Asn, mapped on its semi-transparent solvent accessible surface, with docked RNA (phosphate backbone in green, ribose rings in yellow and bases in cyan). Picture from Frazão et al, Nature 2006



RNA degradation by RNase II. Proposed catalytic mechanism for RNase II showing the postulated second Mg (Mg II) and the attacking hydroxyl group (grey). e, Model for RNA degradation by RNase II. ssRNA (red) is threaded into the catalytic cavity and clamped between Tyr 253 and Phe 358. The additional stabilization of RNA inside the cavity drives the RNA translocation after each cleavage, up to a final 4-Nt fragment. Picture from Frazão et al, Nature 2006

Group Members

Mónica Amblar	Post-doc
Patrick Freire	Post-doc
Sandra Viegas	PhD student
Ana Barbas	PhD student
José Andrade	PhD student
Inês Guinote	PhD student
Ana Furtado	Master Student
Francisco Mesquita	Master Student
Margarida Matos	Master Student
Rute Matos	Master Student
Inês Heinrichson	Undergraduate

Selected Publications

Frazão C, McVey CE, Amblar M, Barbas A, Vonrhein C, Arraiano CM, Carrondo MA (2006). Unravelling the dynamics of RNA degradation by ribonuclease II and its RNA-bound complex. Nature. 443(7107):110-4.

Andrade JM, Cairrão F, Arraiano CM (2006). RNase R affects gene expression in stationary phase: regulation of ompA. Mol Microbiol. 60:219-28.

Santos JM, Freire P, Mesquita FS, Mika F, Hengge R, Arraiano CM (2006). Poly(A)-polymerase I links transcription with mRNA degradation via sigma^S proteolysis. Mol Microbiol. 60:177-88.

Helena Santos

Full Professor

PhD 1984 in Biophysics, Universidade Nova de Lisboa

Cell Physiology & NMR

Group Members

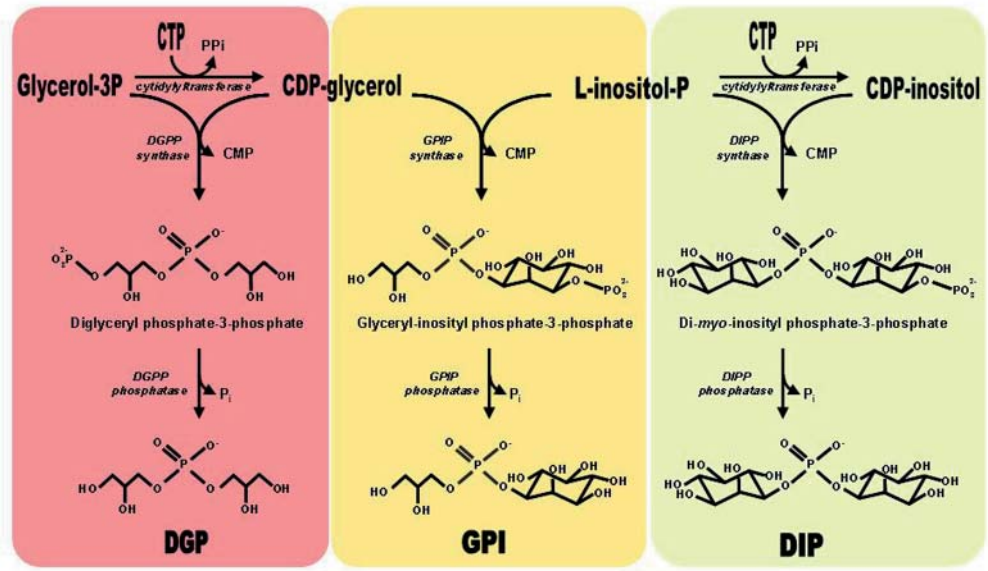
Pedro Lamosa	Post Doc
Clélia Neves	Post Doc
Claudia Sánchez	Post Doc
Margarida Santos	Post Doc
Nuno Borges	Post Doc
Tiago Faria	Post Doc
Luís Fonseca	Post Doc
Luís Gonçalves	PhD student
Tiago Pais	PhD student
Carla Jorge	PhD student
Paula Gaspar	PhD student
Rute Castro	PhD student
Marta Rodrigues	PhD student
Ana Carvalho	PhD student
Ana Mingote	Technician
Carla Almeida	Technician

The two major objects of research are Lactic Acid Bacteria (Systems Biology of Lactococcus lactis) and Hyperthermophilic Microorganisms (osmo- and thermo-adaptation in Bacteria and Archaea).

Physiology of LAB (in coll. with A. R. Neves, ITQB). In vivo NMR is used to measure on line the dynamics of intracellular metabolites and co-factors (Metabolomics) with the aim to provide reliable data to be used as guidelines for efficient metabolic engineering strategies. One goal is to characterize central metabolism and regulatory networks taking advantage of global approaches. The team collaborates with USA groups with expertise in mathematical modeling for the integration of the data at multi-level organization.

Physiology of hyperthermophiles. Main objectives: genetic and biochemical characterization of biosynthetic pathways of novel compatible solutes; regulation of biosynthesis; identification of strategies for adaptation to hot environments; development of microbial cell systems for the industrial production of hypersolutes; characterization of the molecular basis for protein stabilization by solutes; effects on protein structure and dynamics.

Scientific Highlight. A major achievement of our team in 2006 was the elucidation of the pathways for the synthesis of three compatible solutes from hyperthermophilic Bacteria and Archaea: diglycerol phosphate, di-myo-inositol phosphate (DIP) and the structural chimera glycerophosphoinositol (Figure). The genes and enzymes for the synthesis of DIP were identified. Furthermore, a labelling strategy using carbon-13 and NMR led to the establishment of the correct stereochemistry of this compound. This work was the result of a collaboration between our team and that of Christopher Maycock and Rita Ventura in the Chemistry Division.



Pathways for the synthesis of the compatible solutes di-myo-inositol phosphate (DIP), diglycerol phosphate (DGP) and glycero-phospho-myo-inositol (GPI) in hyperthermophilic Archaea and Bacteria. (Borges et. al., 2006)

Selected Publications

Borges N, Gonçalves LG, Rodrigues MV, Ventura R, Maycock C, Lamosa P and Santos H (2006) Biosynthetic pathways of inositol and glycerol phosphodiesters used for stress adaptation in *Archaeoglobus fulgidus*..Journal of Bacteriology 188: 8128-8135.

Neves AR, Pool WA, Castro R, Mingote A, Santos F, Kok J, Kuipers OP and Santos H (2006)The alfa-phosphoglucomutase of *Lactococcus lactis* is unrelated to the alfa-D-phosphohexomutase superfamily and encoded by rhe essential gene yfgH Journal of Biological Chemistry 281: 36864-36873.

Voit EO, Neves AR and Santos H (2006) The intricate side of systems biology Proceedings of the National Academy of Sciences, U.S.A., 103: 9452-9457.

Hermínia de Lencastre
Full Professor
PhD in Biology 1981 Universidade Nova de Lisboa

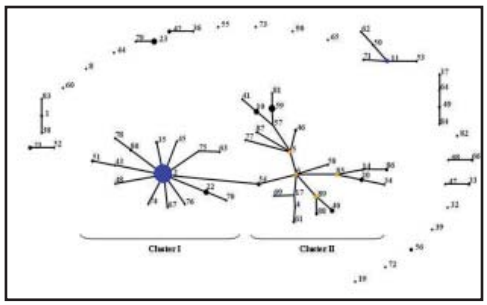
Molecular Genetics

Staphylococcus aureus, *Staphylococcus epidermidis* and *Streptococcus pneumoniae* are gram-positive pathogens, which are among the world leading causes of nosocomial and community-acquired infections. The emergence of antimicrobial resistance in these pathogens is a major drive for epidemiological surveillance and resistance mechanisms studies.

We recently found that methicillin-susceptible *S. aureus* strains from Cape Verde harbor the highly toxic leukocidin (PVL) and showed genetic backgrounds of pandemic methicillin-resistant *S. aureus* (MRSA) clones. The introduction of *mecA* in these backgrounds could pose a threat warranting continuing surveillance studies, namely in African countries.

The characterization of methicillin-resistant *S. epidermidis* (MRSE) isolates revealed the existence of genetic lineages disseminated worldwide and suggest that MRSE strains evolved quickly through recombination and transfer of genetic mobile elements such as SCC*mec* carrying *mecA*. Moreover, *S. epidermidis* may be functioning as a factory of new SCC*mec* types that can be transferred to other staphylococcal species, such as *S. aureus*. We have updated the previously published SCC*mec* multiplex PCR typing strategy for the proper identification and tracing of SCC*mec* types in staphylococci.

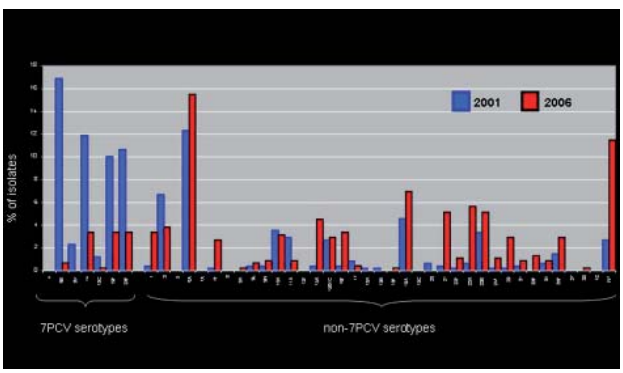
S. aureus has a remarkable ability to rapidly adapt to antibiotic pressure. The characterization of insertion and conditional mutants and DNA microarrays has provided insights not only on mechanisms of resistance to methicillin and vancomycin, but also on cell physiology and cell wall biosynthesis. Functional cooperativity and regulatory networks linking peptidoglycan biosynthetic genes and the methicillin and vancomycin resistance phenotype are being explored.



Application of eBURST algorithm to MLST data for the collection of *S. epidermidis* isolates.

To assess the impact of the seven-valent pneumococcal conjugate vaccine on carriage of pneumococci, a collection obtained from the nasopharyngeal flora of Portuguese children attending day-care centers is being characterized. Studies on the population structure of non-serotypeable *S. pneumoniae* showed that this group of isolates is quite diverse and is not related to capsulated pneumococci.

Penicillin resistance in pneumococci has been also studied and we recently identified a peptidoglycan O-acetylase that has a key role in the ß-lactam resistance mechanism.



Impact of the conjugate pneumococcal vaccine Prevenar on nasopharyngeal colonization.

Group Members

Alexander Tomasz	Full Professor
Ana Ludovice	Assist. Prof. FCT/UNL
M. Aires de Sousa	Research Associate
Ana R. Gomes	Post Doc
Duarte C. Oliveira	Post Doc
Raquel Sá-Leão	Post Doc
Susana Gardete	Post Doc
Maria Miragaia	Post Doc
M. Inês Crisóstomo	Post Doc
Rita Sobral	Post Doc
Sandro F. Pereira	PhD student
Nuno Faria	PhD student
Maria Luís Amorim	PhD student
Teresa Conceição	PhD student
Catarina Milheiriço	PhD student
Nelson Frazão	PhD student
Alexandra Simões	PhD student
Juliana Lamaro	PhD student
Liliana Pereira	Master Student
Sónia Nunes	Graduate
Pedro Arede	Graduate
Ana Tavares	Graduate
Bruno Correia	Graduate
Isilda Gueifão	Technician
Manuela Nogueira	Assistant

Selected Publications

Oliveira, D.C., C. Milheiriço, S. Vinga, and H. de Lencastre. (2006). Assessment of allelic variation on the ccrAB locus in methicillin-resistant *Staphylococcus aureus* clones. J. Antimicrob. Chemother. 58:23-30.

Gardete, S., H. de Lencastre and A. Tomasz. (2006). A link in transcription between the native pbpB and the acquired mecA gene in a strain of *Staphylococcus aureus*. Microbiology.152: 2549-58.

Gomes, A. R., H. Westh, and H. de Lencastre. (2006). Origins and evolution of methicillin-resistant *Staphylococcus aureus* (MRSA) clonal lineages. J. Antimicrob. Agents Chemother 50:3237-44.

Isabel M. G. de Sá-Nogueira

Associate Professor, Faculdade Ciências e Tecnologia, UNL
PhD 1991 in Biology-Molecular Biology, Universidade Nova Lisboa.

Microbial Genetics

Group Members

Irina Franco	PhD student
José Inácio	PhD student
Mª Isabel Correia	PhD student
Sónia Custódio	Res. student
Vera Augusto	Res. student
Mª João Faria	Res. student
Diogo Rombo	Undergraduate

The main area of interest in the laboratory of Microbial Genetics 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 *Bacillus subtilis.*

Currently we are carrying out two major research projects: the first focuses on mechanisms of gene regulation that involve the AraR transcription factor, the second project addresses the characterization of a hemicellulolytic system. AraR controls at least thirteen genes required for the extracellular degradation of polysaccharides, transport of oligomers and simple sugars, intracellular degradation and further catabolism. We are studying the AraR repressor-operator system for understanding protein-DNA interactions and allosteric mechanisms of gene regulation. Site-directed mutagenesis based on structure homology modeling is being used to characterize DNA binding, effector binding/response, and dimerization/oligomerization, in order to obtain a detailed structure-function correlation of AraR and gain insight into its mechanistic mode of action (see figure). Hemicellulases are enzymes that are important both for the global carbon cycle and for biotechnological applications. In addition to the design of efficient enzymatic systems for economic degradation of the plant cell wall, the remaining challenges in the field of hemicellulases include the elucidation of the regulatory mechanisms and the creation of novel enzyme functionalities.

We are addressing these questions through the genetic and biochemical characterization of an enzymatic consortium involved in the degradation of plant cell wall arabinose-containing polysaccharides, either in the soil or as part of the digestive tract of animals.

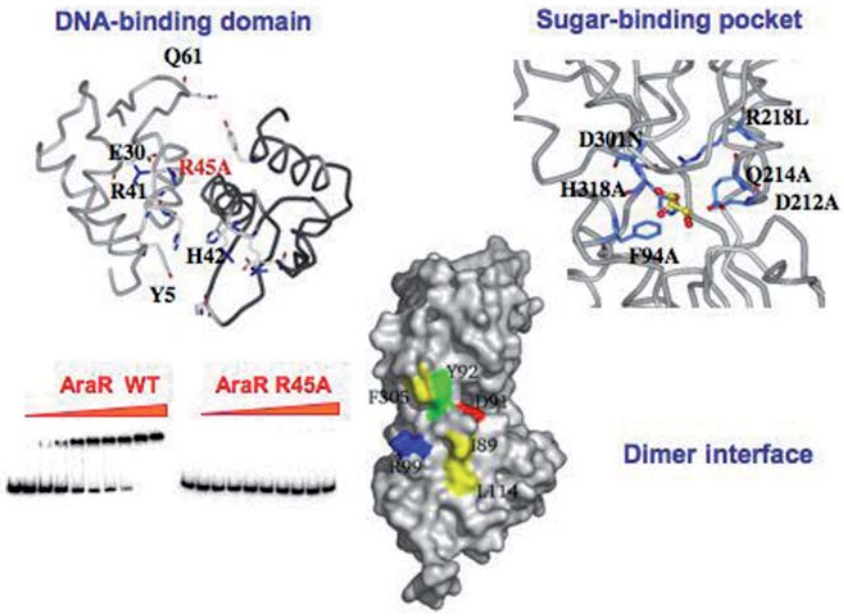


Figure- AraR modeling and site-directed mutagenesis. DNA-binding domain (N-terminal), close-in view of the sugar-binding pocket (C-terminal domain), dimer contact (C-terminal). AraR – DNA binding assays by EMSA.

Selected Publications

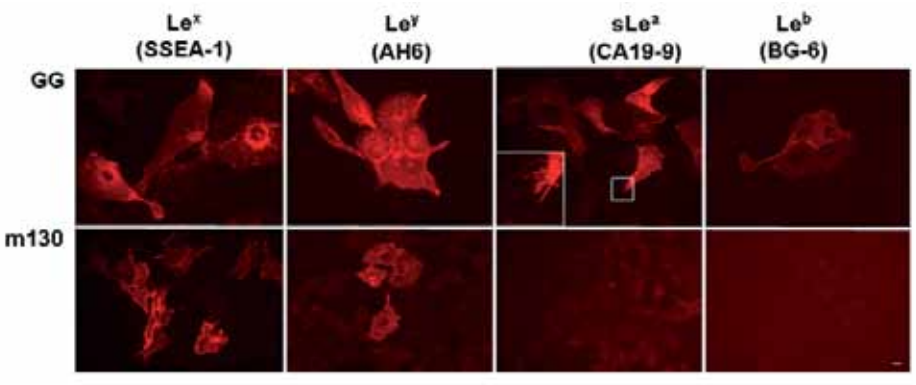
Franco I S, Mota L J, Soares C M, and de Sá-Nogueira I (2006) Functional domains of the Bacillus subtilis transcription factor AraR and identification of aminoacids important for nucleoprotein complex assembly and effector-binding. Journal of Bacteriology 188 (8): 3024-3036.

Júlia Costa
Principal Investigator
PhD 1994 in Biochemistry, Universidade de Lisboa

Glycobiology

We are studying the trafficking and glycosylation of several glycoproteins from mammalian cells.
In the last year, we have characterized and modified the glycosylation of ovarian carcinoma cells in order to understand how it might affect tumor cell growth, migration, spreading and invasion (Escrevente and Machado *et al.*, 2006). In this context, we have also studied the role played by N-glycans in the function of ADAM10, a sheddase that cleaves several cell surface proteins, including the cell adhesion glycoprotein L1, which induces tumor cell spreading.
In order to characterize protein glycosylation, we have implemented in the laboratory during the last year the technique of high performance anion exchange chromatography with pulsed amperometric detection, which yields a high resolution and high sensitivity in the subnanomolar range for carbohydrate analysis.
In the context of a collaboration, a student from the laboratory received training in the oligosaccharide microarray technology, which can be useful for ligand identification of carbohydrate-binding proteins (Palma *et al.*, 2006).

We have also performed studies in the field of neurodegenerative diseases, for which changes in protein trafficking are known to occur. First, we studied the biological role of N-glycans from nicastrin (NCT) in the trafficking and activity of the γ-secretase complex. NCT is a component of the γ-secretase membrane protein complex, which is required for the production of the Aβ peptide, the hallmark of Alzheimer's disease. It was found that N-glycosylation of human NCT was required for interaction with the lectins from the secretory pathway calnexin and ERGIC-53 (Morais et al., 2006). Furthermore, it was found that cellular localization of nicastrin determined the amount and the type of Aβ produced by the γ-secretase complex (V. Morais, PhD Thesis).
Another neurodegenerative disease has also been studied: Amyotrophic Lateral Sclerosis (ALS). We characterized the plasma of patients with ALS by proteomic techniques to identify biological markers. A set of proteins was identified, which could constitute a "signature" marker of the disease after proper validation (A. Palma, PhD thesis). Cellular models of ALS have been developed that exhibited pathological hallmarks of the disease, namely, Golgi fragmentation and aggregate formation.



Detection of adhesion fucosylated Lewis determinants (Lex, Ley, sLea, Leb) in GG and m130 ovarian carcinoma cells by immunofluorescence microscopy. Bar: 10 µm.

Group Members

Ana Catarina Brito	PhD student
Catarina Gomes	PhD student
Eda Machado	PhD student
Cristina Escrevente	PhD student
Ricardo Gouveia	PhD student

Selected Publications

Escrevente, C., Machado, E., Brito, C., Reis, C.A., Stoeck, A., Runz, S., Marmé, A., Altevogt, P., Costa, J. (2006) Expression of α3/4 fucosyltransferases and Lewis determinants in ovarian carcinoma tissues and cell lines. Int. J. Oncol. 29, 557-566.

Morais, V.A., Brito, C. Pijak, D.S., Crystal, A.S., Fortna, R.R., Li, T., Wong, P.C., Doms, R.W., Costa, J. (2006) N-Glycosylation of human nicastrin is required for full γ-secretase complex activity and for interaction with calnexin and ERGIC-53. Biochim. Biophys. Acta - Molecular Basis of Disease. 1762, 802-810

Palma, A., Feizi, T., Zhang, Y., Stoll, M. S., Lawson,A.M., Diaz-Rodriguez, E., Campanero-Rhodes, M.A., Costa, J., Gordon, S., Brown,G.D., Chai, W. (2006) Ligands for the β-glucan receptor, Dectin-1, assigned using "designer" microarrays of oligosaccharide probes (neoglycolipids) generated from glucan polysaccharides. J. Biol. Chem. 281, 5771-5779.

Karina B. Xavier

Auxiliary Investigator

PhD in 1999, Universidade Nova de Lisboa ,ITQB

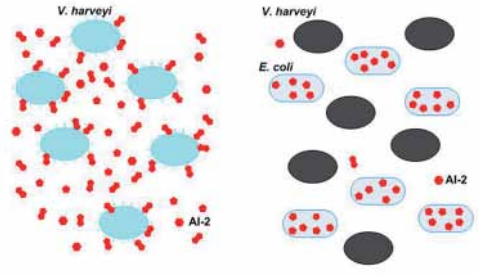
Bacterial Signalling

Group Members

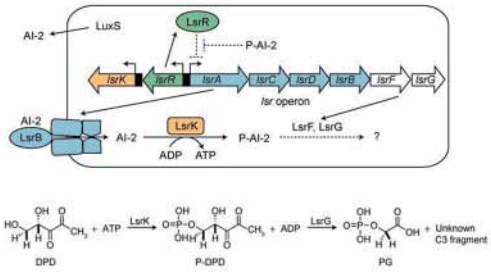
Michal Bejerano-Sagie	Post-doc
Catarina Pereira	PhD student
João Marques	Master Student

The bacterial signalling laboratory was formed in March, 2006. Our laboratory focuses on the molecular mechanisms bacteria use for cell-cell communication involving a process called quorum sensing. This process enables a bacterial population to regulate behaviours which are only productive when many bacteria act in concert as a group. Behaviours regulated by quorum sensing are often crucial for successful bacterial-host relationships; both symbiotic and pathogenic. In our laboratory we use a biochemical, chemical and genetic approach to study the molecular mechanisms underlying quorum sensing, with an emphasis on systems promoting bacterial inter-species communication. Our ultimate goal is to understand how bacteria use inter-species cell-cell communication to coordinate population-wide behaviours in consortia and in microbial-host interactions.

In previous work we have shown that enteric bacteria have the capacity of interfering with other species' ability to regulate group behaviours by manipulating a bacterial signal molecule which mediates bacterial inter-species communication. To continue deciphering the mechanisms of signalling interference in enteric bacteria we have started to study the metabolic pathway involved in destroying the *Escherichia coli* signal molecule. We have characterized the regulatory components and the first two enzymes used by these bacteria to deplete the signal in the intercellular environment. Additionally, we are investigating this mechanism of interference in other bacteria to assess the diversity of these processes in nature. Specifically, we have shown that *Sinorhizobium meliloti*, well known for its ability to fix nitrogen in symbiosis with legume hosts like alfalfa, uses an interference system similar to the system present in enteric bacteria. We are testing if S. meliloti uses this system to interfere with the quorum sensing systems of other bacteria in the rhyzosphere or if, alternatively, *S. meliloti* uses this system to sense the soil, where many different species co-exist, comparing it to the nodule, where only organisms from the *S. meliloti* species are present. We are also studying the function of this system in several *Bacillus* species, including laboratory strains and gut isolates. Determining the molecular mechanisms involved in these three groups of organisms will contribute to a better understanding of cell-cell communication in soil, in the gut, and during bacterial-host symbiotic interactions.



The E. coli mechanism of AI-2 interference involves the production of the AI-2 precursor 4,5-dihydroxy-2,3-pentanedione (DPD), a receptor protein, an uptake transport system, processing enzymes, and transcription regulators, including the kinase LsrK, the transcriptional repressor LsrR, and a protein of unknown function, LsrG. We have shown that LsrK phosphorylates DPD and the phosphorylated compound, termed P-DPD, binds to LsrR, an event that leads to induction of the lsr operon. Furthermore, LsrG is an enzyme that converts P-DPD to 2-phosphoglycolic acid (PG). Importantly, PG does not bind to LsrR therefore when DPD is cleaved production of PG results in transcriptional repression and termination of the AI-2 signaling cycle.



Enteric bacteria have the ability to produce but also to internalize the inter-species signal molecule, called AI-2, removing it from the environment. We have shown that enteric bacteria use this mechanism to terminate quorum sensing controlled behaviours of other species of bacteria in the vicinity. (A) Pure culture of the bioluminescent marine bacterium Vibrio harveyi. At high population densities V. harveyi senses high levels of the inter-species signal molecule (in red) and induces light production. (B) Co-culture of V. harveyi and the enteric bacterium Escherichia coli. E. coli has the ability to internalize this signal molecule produced by it-self and by V. harveyi, removing it from the extracellular medium. Consequently, V. harveyi is tricked, it senses low levels of the signal molecule and it is no longer able to measure its own cell densities and therefore does not induce its quorum sensing mechanism and does not emit light.

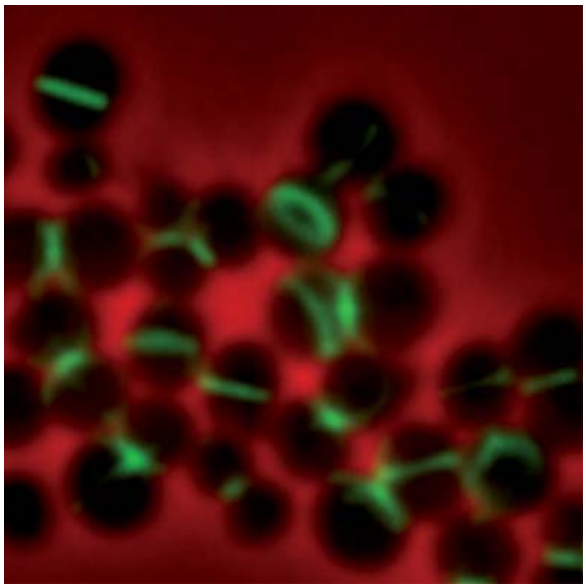
Mariana Gomes de Pinho
Auxiliary Investigator
PhD in 2001, Universidade Nova de Lisboa

Bacterial Cell Biology

Bacterial cells have revealed a surprising degree of protein organization. Many essential cellular processes are performed by higher order protein complexes, which are precisely regulated in time and space. An example of such processes is cell division, which has been studied in any detail only in a few model organisms.

Staphylococcus aureus is a Gram positive pathogen and the most common cause of antibiotic-resistant hospital-acquired infections. Besides its clinical relevance, *S. aureus* is also a very interesting model to study cell division because it has a different shape and mode of division from the traditional, widely used, model organisms *Escherichia coli* and *Bacillus subtilis*: it has round (coccoid) shaped cells and, more interestingly, divides in three consecutive perpendicular division planes over three division cycles, similarly to the first divisions of a fertilized egg.
For a bacterial cell to divide it has to double its mass, replicate its genome and synthesize a septum between the two daughter cells. It is this last process that is inhibited by a large number of antibiotics, such as beta-lactams or glycopeptides, which target cell wall synthesis.

In the Bacterial Cell Biology laboratory, which started in the beginning of 2006, the aim is to understand, at a molecular level, the organization and the temporal and spatial regulation of two fundamental steps of cell division - the segregation of the bacterial chromosome and the synthesis of the division septum, as well as to integrate this information for a better understanding of antibiotic resistance mechanisms in *S. aureus*.



Identification of the division septum as the place where cell wall synthesis takes place in *Staphylococcus aureus*. Labelling was done using a fluorescent derivative of the antibiotic vancomycin..

Group Members

Patricia Reed	Post Doc
Ana Jorge	PhD student
Pedro Matos	Master Student
Helena Veiga	Graduate

Rosario Mato

Auxiliary Investigator

PhD 1992 in Biology, Clinical Microbiology. Universidad Complutense, Madrid, Spain.

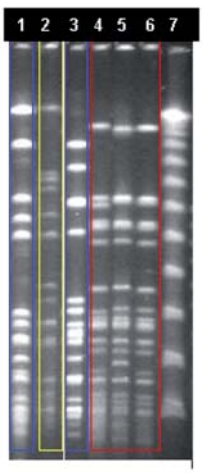
Group Members

Madalena Pereira	Master Student
Renato Pires	Graduate
Patricia Rodrigues	Graduate
Vanessa Verissimo	Graduate
Clara Cabral	Undergraduate

Enterococci considered commensal bacteria of animal and human gastrointestinal tract, are currently recognized as the second to third most common organism recovered from hospital-acquired infections, leading to high mortality rates, particularly in high risks patients. These bacteria possess a broad spectrum of intrinsic and acquired antibiotic resistances, capacity to accumulate genetic determinants (both virulence, and resistance to antimicrobial), and a highly effective gene transfer mechanism which enhances probably the enterococcal fitness, particularly in the hospital environment. As enterococci, beta-haemolytic streptococci (BHS) may vary from commensal to pathogens bacteria with capacity to infect a wide range of tissues sites, and causing fatal infections. In addition, streptococci are resistant to many of the antibiotics used at clinical practice, and possess virulence factors genes, some of them with certain homology with virulence determinants from enterococci. In both genera little is known about the pathogenesis that could explain their virulence and dissemination. These studies with streptococci are being carried out in collaboration with the Centro de Recursos Microbiológicos-CREM. Prof. I. Santos Sanches (FCT/UBL).
Colonization of asymptomatic carriers and high risk patients by these microorganisms may play an important role as source of dissemination and transfer of both antimicrobial resistant and virulence determinants genes in different settings such as hospitals and community. Epidemiological surveillance studies are crucial to elucidate these issues.

Focus on enterococci two epidemiological surveillance studies were carried out in two high risk wards:
- Neonatal intensive care unit (NICU). In collaboration with the Hospital Fernando Fonseca, Amadora. Project financed by Fundação Calouste Gulbenkian. Contract Nº 65882. July 2004.

- Haematological malignancies ward. In collaboration with the hospital Santo Antonio dos Capuchos e do Desterro, Lisboa. Project financed by Fundação para a Ciência e a Tecnologia. Ref. POCTI/SAU-ESP/58030/2004.

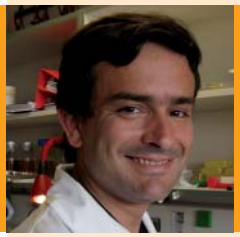


Representative's multidrug-resistant *E. faecalis* PFGE profiles. Blue box - PFGE dominants among *E. faecalis* from colonization at the NICU. Red box - PFGE dominant among *E. faecalis* from infection products, and colonization at the haematological malignancy unit. Yellow box - PFGE founds among *E. faecalis* from colonization and infection.

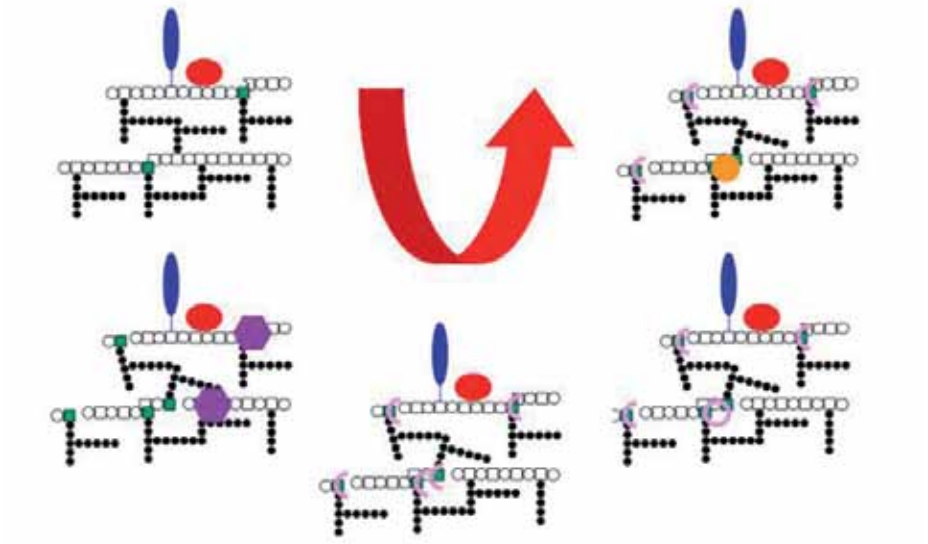
Sérgio Raposo Filipe

Auxiliary Investigator

PhD 2001 in Biology, Universidade Nova de Lisboa, ITQB



We are interested in understanding the synthesis of peptidoglycan, a large macromolecule from the cell wall that surrounds bacteria, which serves as an attachment site for extra cellular proteins, protects bacteria from the large osmotic forces and is involved in maintaining the bacterial shape.The synthesis of peptidoglycan is the target of different families of antibiotics and it seems to play an important role in detection of bacterial infection by different host immune systems. The synthesis of this macromolecule involves a significant number of proteins that allow incorporation of new material and elimination of old material, in a coordinated process that does not endanger the bacteria physical integrity and controls the amount of released material that may be detected by the infected host.The main interest of the laboratory of Bacterial Cell Surfaces and Pathogenesis 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. Using mainly *Staphylococcus aureus* and *Streptococcus pneumoniae* as bacterial model organisms, we aim to better understand the bacteria cell wall metabolism and to determine how muropeptides, the smallest components of the peptidoglycan can induce a similar innate immune in different host systems.During the year of 2006 and in collaboration with Dr. Petros Ligoxygakis, we have reported that GNBP1 (a Drosophila protein essential for the ability of the flies to trigger an inflammatory response against S. aureus infection) was able to hydrolyse Gram-positive peptidoglycan. A model has been proposed whereby GNBP1 presents a processed form of peptidoglycan which is sensed by PGRP-SA. A tripartite interaction between these two proteins and peptidoglycan is essential for downstream signaling.



Working model in which GNBP1 (purple) presents a processed form of peptidoglycan which is sensed by PGRP-SA (pink and orange for the activated form)

Group Members

James Yates	Post Doc
Magda Atilano	PhD student
Luis Filipe Ferreira	Master Student
Cláudio Alves	Graduate

Selected Publications

Payne B. T. I., van Knippenberg I. C., Bell H., Filipe S. R., Sherratt D. J. and McGlynn P. (2006). "Replication fork blockage by transcription factor-DNA complexes in Escherichia coli." Nucleic Acids Research 34(18): 5194-5202.

Possoz C.*, Filipe S. R.*, Grainge I. and Sherratt D. J. (2006). "Tracking of controlled Escherichia coli replication fork stalling and restart at repressor-bound DNA in vivo." EMBO Journal 25(11): 2596-2604. *Co-first Authors.

Wang L. H., Weber A. N., Atilano M. L., Filipe S. R., Gay N. J. and Ligoxygakis P. (2006). "Sensing of Gram-positive bacteria in Drosophila: GNBP1 is needed to process and present peptidoglycan to PGRP-SA." EMBO Journal 25(20): 5005-5014.

Plant Sciences Division

The Plant Sciences Division is composed of 9 Laboratories performing research that extends from cell biology and developmental genetics, through biochemistry, genetic engineering and biotechnology, to ecophysiology. The model-plants *Arabidopsis thaliana, Thellungiella halophila, Medicago truncatula, Oryza sativa* (rice) and *Antirrhinum majus*, are used with the purpose of elucidating basic aspects of development, biochemistry and responses to stress, and to give information that could support the study of economical relevant plants, such as, rice itself, maize, grain legumes, grapevine, olive, almond, pine, cork oak and eucalypt.

Transgenic *M. truncatula* is a model being used to study the biology of the plant cell, namely to investigate how transgene expression is influenced by higher-order chromatin structure, for instance, to understand how integration and epigenetic modifications influence expression and stability. In *A. majus*, detailed characterization of mutant alleles conferring variegated colour phenotypes has led to development of transposons as tools for systematically producing mutants important to investigate the genetic and molecular basis of flower colour variegation.

Since sustainable water use in dry areas of the Mediterranean region is crucial to secure food production, to understand the plant responses to drought and other abiotic stresses is of primary importance. So, the Laboratories of the Division are directing a special attention to drought and temperature stress, which are studied under different perspectives. In *M. truncatula*, genes/microRNAs are being analysed under drought and in *Lupinus albus* the early effects induced by this stress are under study. Proteomic approaches are being used to characterise proteins of the intercellular fluid and extracellular matrix of *M. truncatula* that respond to drought and wounding; the role of reactive oxygen species in the expression of those proteins are under study. In rice, the role of transcription factors (TF) in the plant performance under drought, cold and salinity is being studied. To characterise TF function, transgenic approaches are being used (over-expression and RNAi, using constitutive vs. inducible promoters) and mutant analyses. Thousands of transgenic plants have been generated and molecularly analysed. F2 progenies of selected ones were obtained and studied using transcriptomic, proteomic, cytogenetic and metabolomic strategies. Using Yeast-one-hybrid (Y1H) and Y2H screens new TFs are being identified and characterised.

On a smaller scale, this work is also being extended to almond, a Rosaceae fruit tree, since, despite the importance of abiotic stress response for productivity in fruit trees, little is known regarding the role of TFs in this process.
Concerning the understanding of grapevine productivity under environmental stress (drought, high light and temperature), the root-shoot signalling affecting photosynthetic carbon assimilation and the facilitated water transport (aquaporins) are being analysed. In domains of functional genomics, proteomics methodologies are being utilized to elucidate the events that control grape berry maturation under the effect of different irrigation regimes.

Forest tree biotechnology has been focused on developing tools for important forest species in Portugal as, e.g., maritime pine. The targeted tools have been those that can have an impact on the improvement of economically relevant traits. For maritime pine, the establishment of *in vitro* cloning, cryopreservation and genetic transformation methods are extremely useful for large scale vegetative propagation and for manipulating traits of interest. Simultaneously, these tools have been applied for the study of specific aspects of plant development such as molecular regulation of early embryogenesis and vascular differentiation, and nitrogen metabolism. The basic understanding of the mechanisms involved in such aspects of plant development and growth may provide potential strategies for further trait improvement. In *Eucalyptus globulus*, activities were largely directed towards identifying wall proteins associated with wood formation. Forty six genes were identified, and their differential transcription is being studied during the formation of opposite and tension wood. On-going work is looking at the histological distribution of some of the more important transcripts. In *Quercus suber*, seasonal stress impacts on

leaf compounds and secondary metabolism, namely on key enzymes of the phenylpropanoid pathway, are being investigated.

Concerning biotic stress, studies are being undertaken in grapevine, in order to determine the changes in gene and protein expression that occur during pathogenic fungal interactions (e.g., powdery mildew and grapevine wood diseases). A potent plant antifungal protein was already detected and a search for new antifungal compounds of plant origin is also being undertaken. For another fruit woody species, almond, markers are being developed to identify tolerance/sensibility to the *Fusicoccum* fungal disease, which is spreading in the Iberian Peninsula. Regarding rice, resistance to blast fungal disease is being introgressed in Portuguese landraces, together with a gene for higher yield, aiming to recover traditional varieties for commercial exploitation.

With a pharmaceutical perspective, transgenic *M. truncatula* is being used to integrate fundamental and applied aspects of Molecular Farming (large scale production of recombinant proteins in plants). Furthermore, plants of the Portuguese flora are being screened in a search for new polyphenolic compounds exhibiting in vivo antioxidant activity in a neuronal cell model.

Cândido Pinto Ricardo
Full Professor
PhD 1968 in Plant Biochemistry Cambridge University, UK

Plant Biochemistry

The Plant Biochemistry Laboratory centers its studies on the abiotic stresses of plants, particularly water deficit, temperature and mineral stress. Drought, one of the most serious world-wide problems, greatly affects crop plants, but even a transient water deficit can markedly reduce plant productivity. Salinity often comes together with drought, though mineral imbalances and deficiencies are important stresses in themselves that have dramatic effects on plants.
As plants of study we use the models *Arabidopsis, Tellungiella, Medicago* and rice but we are also interested in analysing the specific mechanisms of stress responses of plants of higher economic importance for Portugal, like grapevine, cork oak, olive, beans and lupin.
To study the plant metabolic alterations and adaptations induced by stress we make use of general biochemical techniques and of nuclear magnetic resonance, which can produce a general picture of the major metabolic changes that have occurred. Proteins, as the main mediators of metabolism, are receiving a high attention in our studies and for that we follow the methodologies of proteomics. For gene expression studies we use the DNA array technology (DNA-microarrays available commercially) and real-time RT-PCR.
Examples of studies we are presently undertaking are the following: comparative water deficit responses in *Arabidopsis, Tellungiella* and lupin; boron deficiency in *Arabidopsis, Medicago*, rice and lupin; salinity responses of olive; proteomics of berry development in grapevine under water deficit; phenolic metabolism of drought and temperature stressed cork oak.

Three weeks boron deficiency vs. control *Arabidopsis thaliana*.

Group Members

Carla Pinheiro	Post Doc
Inês Chaves	Post Doc
Rita Francisco	PhD student
Marta Alves	PhD student
Tiago Lourenço	PhD student
Carla Antunes	PhD student

Selected Publications

Alves M, Francisco R, Martins I and Ricardo CP (2006) Analysis of *Lupinus albus* leaf apoplastic proteins in response to boron deficiency. Plant and Soil 279 (1-2): 1-11

Balde JA, Francisco R, Queiroz A, Regalado AP, Ricardo CP and Veloso MM (2006) Immunolocalization of a class III chitinase in two muskmelon cultivars reacting differently to *Fusarium oxysporum* f. sp melonis. Journal of Plant Physiology 163(1): 19-25

Passarinho JAP, Lamosa P, Baeta JP, Santos H and Ricardo CPP (2006) Annual changes in the concentration of minerals and organic compounds of *Quercus suber* leaves. Physiologia Plantarum 127(1): 100-110

Célia Miguel
Auxiliary Investigator
PhD 1999 in Plant Biotechnology, Faculdade de Ciências da Universidade de Lisboa

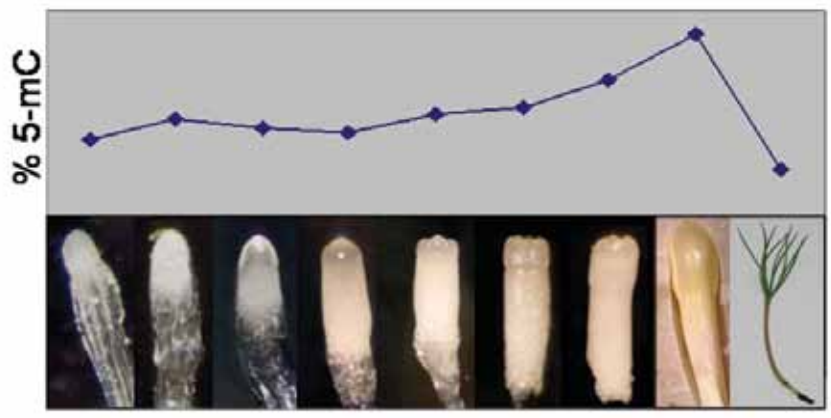
Forest Biotech (ITQB/IBET)

Group Members

Sónia Gonçalves	Post-doc
Susana Tereso	Post-doc
Liliana Marum	PhD student
Marta Simões	Graduate
Ana Milhinhos	Graduate

The Forest Biotechnology Lab is focused on developing tools with a potential impact on the gains obtained from breeding/production programs of forest tree species with the main effort being devoted to maritime pine. Simultaneously, we are interested in applying these tools for studying specific aspects of plant development and growth. Basic understanding of the mechanisms involved in such aspects may provide potential strategies for further trait improvement.

Gymnosperms, in which pines are included, separated from Angiosperms about 300 million years ago, and evolved different embryo development pathways, showing differences in morphology during seed development. Plant embryogenesis can be induced in vitro from somatic cells in a process called somatic embryogenesis. This process is of great interest worldwide for large scale and rapid production of genetically improved and uniform seedlings. Because of the experimental accessibility of somatic embryos, this system is also useful to investigate genes and factors which may regulate and affect unique characteristics of embryo development in Gymnosperms. We have established a somatic embryogenesis system for maritime pine, and we are now interested in the identification and characterization of genes that regulate specific stages of embryogenesis. A few genes identified by differential gene expression approaches were selected for further studies based on their expression patterns and are being characterized regarding their function during the early development of the pine embryo. Another closely related research line is focused on the analysis of genomic sequence stability along the several steps of the somatic embryogenesis process using microssatellites and retrotransposons, as a way of assessing true-to-typeness. Very low levels of variation were observed in genomic sequence among individuals of the same clone using the available molecular markers. On the other hand, higher levels of global DNA methylation were found in the embryos under artificial (*in vitro*) conditions as compared to the zygotic embryos. The relevance of these differences at the epigenetic level is being further explored.
We are also interested in using pine somatic embryogenesis and genetic transformation as tools for functional analysis, by over expression and/or RNAi approaches, of other genes that are putatively involved in important traits such as growth and vascular differentiation.



Global level of 5-methylcytosine measured along pine zygotic embryo development and germination

Selected Publications

Tereso S, Miguel C, Zoglauer K, Valle-Piquera C and Oliveira M M (2006) Stable *Agrobacterium*-mediated transformation of embryogenic tissues from *Pinus pinaster* Portuguese genotypes. Plant Growth Regulation 50(1): 57-68.

Tereso S, Miguel C, Maroco J and Oliveira M M (2006) Susceptibility of embryogenic and organogenic tissues of maritime pine (*Pinus pinaster*) to antibiotics used in *Agrobacterium*-mediated genetic transformation. Plant Cell Tissue and Organ Culture 87(1): 33-40.

Tereso S, Goncalves S, Marum L, Oliveira M, Maroco J and Miguel C (2006) Improved axillary and adventitious bud regeneration from Portuguese genotypes of *Pinus pinaster* Ait. Propagation of Ornamental Plants 6(1): 24-33.

Jorge Almeida
Associate Professor Instituto Superior de Agronomia, UTL
PhD 1989, John Innes Institute, University of East Anglia

Plant Genetics

To understand mechanisms by which phenotypic diversity is generated, we have investigated the genetic and molecular basis of flower color variegation in a cultivated variety of *Linaria*, a close relative of *Antirrhinum*. This variety, whose flowers are white with clonal patches of red cells (variegated), gives rise to a few progeny with fully red flowers (1-2% wild type revertants). By applying knowledge derived from the analysis of similar phenotypes in *Antirrhinum*, we have shown that variegation and genetic instability in Linaria is attributable to activity of a transposon (Transposon Linaria 1, Tl1) inserted in a gene that codes for an enzyme required for the synthesis of red anthocyanin pigment. Trapping of Tl1 in a gene that provides a ready assay for transposon movement may open the way for carrying out transposon-mutagenesis experiments in *Linaria*. This would in turn be of interest for studies on origins of morphological variations incorporating mutant comparisons between *Linaria* and *Antirrhinum*, a model species in which many basic plant developmental mechanisms have been uncovered.

Group Members

Lisete Galego	PhD
Hugo Tavares	Master Student

Variegated flower colour in Linaria. Note the red clonal patches on the white background.

Maria Manuela Chaves

Full Professor Instituto Superior de Agronomia, UTL
PhD 1986, Universidade Técnica de Lisboa

Plant Molecular Ecophysiology

Group Members

Alla Shvaleva	Post Doc
Maria Ortuño	Post Doc
Ana Rodrigues	PhD student
Lukasz Tronina	PhD student
Rita Francisco	PhD student
Tiago Santos	PhD student
Raissa Santos	Graduate

Our general interests concern the understanding of physiological and molecular mechanisms underlying plant responses to environmental stresses (drought, high light and temperature) as well as the genotypic differences in plant capacity to utilize external resources. During 2006 we studied:

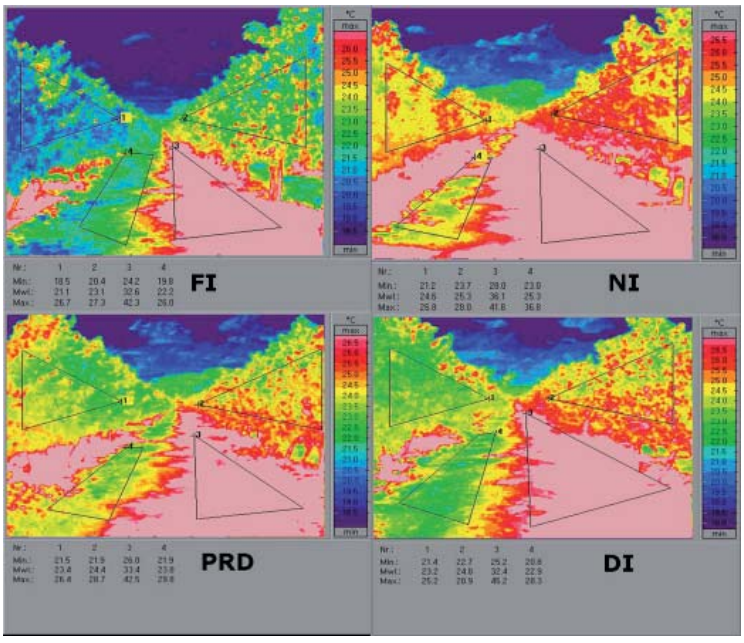
-Root-shoot chemical signalling affecting photosynthetic carbon assimilation, plant growth and yield under water scarcity.

-Molecular and biochemical events that control grape berry maturation under the influence of environmental stresses - a proteomic approach.

-Water acquisition as a main component of tree resistance to drought: root uptake patterns for Cork oak and Holm oak in a Montado ecosystem using stable isotopes.

We demonstrated in grapevine that large fluxes of water are not essential for optimal plant performance and that moderate water deficits, induced under deficit irrigation practices (namely with partial rootzone drying, PRD), might be used successfully in grapevine production to control sink-source relationships. This mild water deficit can maintain or even ameliorate fruit quality, while improving water use efficiency in relation to full irrigated crops. This effect seems to be due to a hormonal signal synthesised in roots under dehydration that is transported to the shoot, closing stomata, improving water status, and inhibiting meristematic activity, therefore decreasing photoassimilate consumption in vegetative growth. For the comparison of grape berry proteomes under different irrigation strategies 2-DE protein patterns were analyzed. Preliminary results in berry pulp revealed quantitative/qualitative changes of several protein spots at `veraison' under the drought conditions prevailing in non-irrigated treatment as compared with the irrigated ones.

Regarding water acquisition in the savannah-type Mediterranean system, we showed that in dry summers new roots formed in deeper soil layers, accompanying the interruption of shoot growth, and hydraulic lift from deeper to shallower roots are significant adaptation traits to counteract negative drought effects.



We optimised thermal imaging to evaluate grapevine stomatal opening and water status in four irrigation treatments: non-irrigated (NI); partial rootzone drying (PRD, 50% of the ETc was supplied to only one side of the root system); deficit irrigation (DI, 50% of the ETc was supplied, 25% to each side of the vine); full irrigation (FI, 100 % of the ETc was supplied). Canopy temperature is highest in NI vines due to stomatal closure, intermediate in PRD and DI and lowest in FI, where stomata are fully open.

Selected Publications

Leinonen I, Grant OM, Tagliavia CPP, MM Chaves, Jones HG (2006) Estimating stomatal conductance with thermal imagery. Plant, Cell and Environment 29, 1508–1518.

Shaleva A, Costa E Silva F, Breia E, Jouve L, Nausman JF, Almeida MH, Maroco JP, Pereira JS, Chaves MM (2006) Drought resistance mechanisms in two Eucalyptus globulus L. Tree Physiology, 26, 239-248.

Kurz-Besson C, Otino D, Lobo do Vale R, Siegwolf R, Schmidt M, Herd A, Nogueira C, Soares David T, Soares David J, Tenhunen J, Santos Pereira J, Chaves MM. (2006) Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance. Plant and Soil, 282, 361 – 378.

M. Margarida Oliveira
Assistant Professor with Agregação, Faculdade de Ciências de Lisboa
PhD 1993 in Biology, Universidade de Lisboa, Faculdade de Ciências

Plant Genetic Engineering

Transcriptional regulatory networks control all aspects of plant growth and development. The identification of upstream regulators is increasingly important to understand the plant response to abiotic stress. As a main focus in our lab we are presently applying an integrative approach to study the role of transcription factors (TFs) in plant abiotic stress tolerance, using rice as a model plant. To characterise TF function we are using transgenic approaches (for over-expression and RNAi, using constitutive vs. inducible promoters) and mutant analyses. Thousands of transgenic plants have been generated and molecularly analysed, and F2 progenies of selected ones were obtained. Transcriptomic (using the LA facility), proteomic, cytogenetic and metabolomic studies are being conducted on those. We are also identifying and characterising new TFs using Yeast-one-hybrid (Y1H) and Y2H screens.

On a smaller scale, this work is also being extended to almond, a Rosaceae fruit tree; despite the importance of abiotic stress response for productivity in fruit trees, little is known regarding the role of TFs in this process.

We are also comparing modern plant breeding (mutagenesis) with genetic engineering, in terms of modified expression at the whole genome level (transcriptomics).
Marker-assisted breeding is being used to introgress high production (semidwarfing) and disease resistance traits (2 blast resistance genes active against the Portuguese blast strains) in rice varieties of national interest. The advanced plants already obtained will be analysed for blast resistance and grain quality in 2007.

Additionally, profiting from an improved genetic transformation protocol we recently developed for almond, we are addressing the function of genes we previously isolated (using the candidate gene approach and microarrays) and putatively assigned as implicated in flowering or adventitious shoot induction. This is also being done using an heterologous system (Arabidopsis).
Although current efforts are mainly directed towards rice and almond, other plants are being addressed, such as maritime pine (collaboration with Forest Biotech Lab) and fig trees (collab. With Reg. Agricult. Serv. Trás-os-Montes). Also, in collaboration with other ITQB labs (Plant Biochemistry Lab and Physiology of Environmentally Conditioned Microbiota Lab), we are addressing, by protein analyses, the potential impact on human health of genetically modified foods and of fungi living in human environments.

Group Members

Nelson Saibo	Post-doc
Ana Paula Santos	Post-doc
Helena Raquel	Post-doc
Ana Sanchez	PhD
Tiago Lourenço	PhD student
Sónia Negrão	PhD student
Rita Batista	PhD student
Ana Paula Farinha	PhD student
Ana Santos	PhD student
Milene Costa	PhD student
Barbara Emmerich	PhD student
Duarte Figueiredo	PhD student
Tânia Serra	Graduate
Pedro Barros	Graduate

Selected Publications

Costa M, Miguel C, Oliveira MM (2006) An improved selection strategy and the use of acetosyringone in shoot induction medium increase almond transformation efficiency by 100-fold. Plant Cell Tissue and Organ Culture, 85(2): 205-209

Tereso S, Miguel C, Zoglauer K, Valle-Piquera C, Oliveira MM (2006) Stable Agrobacterium-mediated transformation of embryogenic tissues from Pinus pinaster Portuguese genotypes. Plant Growth Regulation 50(1): 57-68

Tereso S, Miguel C, Miranda C, Oliveira MM (2006) Susceptibility of embryogenic and organogenic tissues of maritime pine (Pinus pinaster) to antibiotics used in Agrobacterium-mediated transformation. Plant Cell Tissue and Organ Culture, 87: 33–40.

Manuel Pedro Salema Fevereiro

Assistant Professor Faculdade de Ciências de Lisboa with Agregação (UNL)
PhD 1992 in Cellular Biology, University of Lisbon

Plant Cell Biotechnology

Group Members

Carlota Vaz Patto	Post Doc
Dulce Santos	Post Doc
Changhe Zhang	Post Doc
Jorge Paiva	Post Doc
Vitória Gemas	Post Doc
Susana Araújo	PhD student
Sofia Duque	PhD student
Silvana Cardoso	PhD student
Jorge Cunha	PhD student
Matilde Cordeiro	PhD student
Inês Trindade	Master Student
Maria João Silva	Master Student
Mara Alves	Master Student
Nuno Almeida	Graduate
Leonor Tomaz	Technician

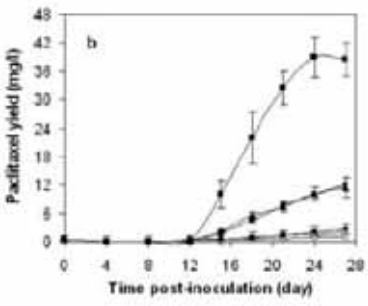
We use the model plant *Medicago truncatula* as a "platform" to study the response of legumes towards drought, and we derived an embryogenic line of this species and an efficient transformation protocol, that is now used to obtain different objectives, from the study of the legume/rhyzobia symbiosis to the production of recombinant proteins. During 2006 we developed a method to transform *Medicago* cells in suspension cultures, and we analysed the physiological response of transgenic lines of Medicago homozygous for the DSP22 protein from Craterostigma plantagineum and ADC (Arginine Decarboxilase) from *Avena sativa*, aiming to evaluate the physiology of the transgenic lines in face of water deprivation. Analogously a transgenic line of tobacco with the TPS from Arabidopsis thaliana was also studied and significant differences in water deficit responses were found.
We continue research to improve our knowledge on the molecular diversity of culture and wild olive trees and grapevine, using nuclear and mitochondrial SSRs.
Also studies to identify SNPs on expressing or on the promoter zones of expressional candidate genes associated to *Pinus pinaster* wood quality were undertaken.
Finally an in silico discovery of *Medicago truncatula* promoters presenting strong ABRE cis elements was developed leading to the isolation of 12 promoters for further study.
The technological ability for bread-making in maize is of enormous importance in maize production in Portugal, where the production of maize bread, commonly known as `broa', still has a great impact in the rural economy of the Central and Northern regions. To identify the genes controlling the bread making ability F2 seed, obtained from the selfing of F1 plants from contrasting ability maize cross, was sown and fresh leaf samples collected for DNA extraction. Plants were selfed to obtain the F3 progeny. DNA was extracted from the 237 F2 individuals and amplifications with SSR molecular markers were initiated to develop a linkage map to be used for location of bread making quality QTLs/genes.
SSRs were also applied to 3 different mass selection cycles of the open pollinated variety "Pigarro" (known by its bread making ability) to optimize the technique under Portuguese germplasm and to evaluate genetic diversity evolution through participatory plant breeding. It was demonstrate that an allele flow took place during this on-farm selection process but the level of genetic diversity was not significantly influenced.

Selected Publications

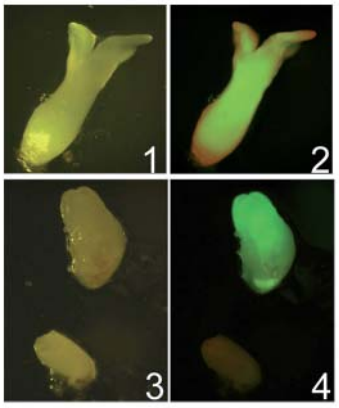
Araújo S., Duque A. S., Santos D. and Fevereiro P. (2006) "A2 - Transformation and regeneration through embryogenesis using the Jemalong genotype M9-10a in Agrobacterium tumefaciens-mediated transformation and in vitro plant regeneration of M. truncatula." in Medicago truncatula handbook. (S. R. N. Foundation).

Iantcheva A., Vlahova M., Atanassov A., Duque A. S., Araújo S., Santos D. F. d. and Fevereiro P. (2006) "Cell suspension cultures." in Medicago truncatula Handbook.

Vaz Patto M. C., Skiba B., Pang E. C. K., Ochatt S. J., Lambein F. and Rubiales D. (2006). "Lathyrus improvement for resistance against biotic and abiotic stresses: From classical breeding to marker assisted selection." Euphytica 147(1-2): 133-147



Paclitaxel yield of the chitosan-adapted and unadapted cell suspension cultures of *Taxus chinensis*. Symbols - (●) and (♦): adapted and unadapted cell suspension cultures treated by Ag+ (30 μM) at day 12 post-inoculation, respectively; (■) and (▲): adapted and unadapted cell suspension cultures treated by Methyl Jasmonate (100 μM) at day 12 post-inoculation, respectively; (🅰) and (♦): unelicited control of adapted and unadapted cell suspension cultures, respectively.

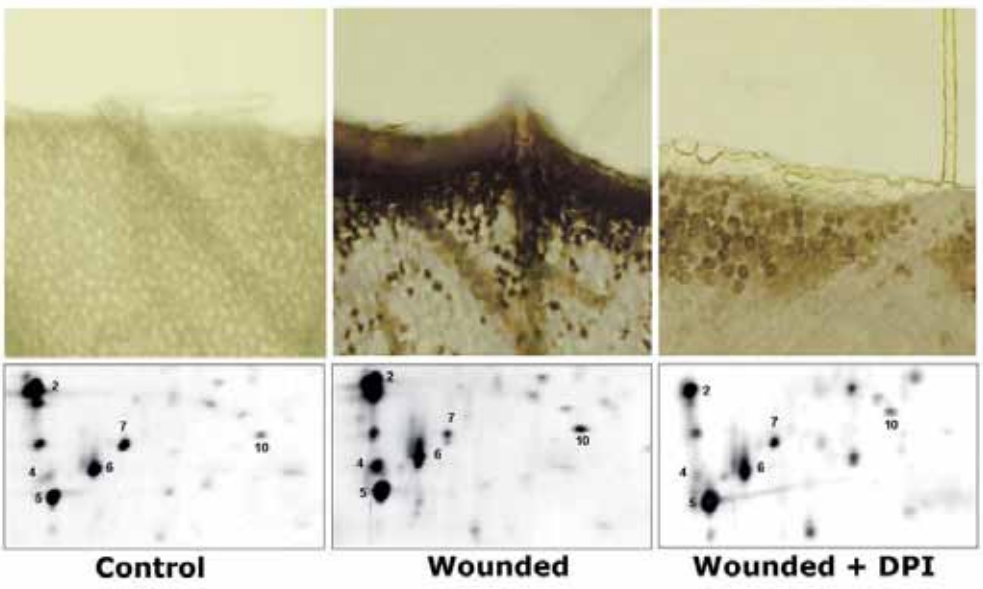


Transformation-regeneration of M. truncatula cv Jemalong M9-10a genotype using the bifunctional reporter gene construct pMP2482 containing fused gusA::intr and gfp genes: Selection of transformed embryos. (1) Pale yellow dicotyledonary stage somatic embryos developed in dark conditions observed under white light; (2) The previous dicotyledonary stage somatic embryos photographed under blue light showing green fluorescence (Green Fluorescence Protein expression); (3) Pale-yellow somatic embryos developed in dark conditions observed under white light; (4) The previous somatic embryos photographed under blue light; note clear differences between gfp+ (upper embryo) and gfp- (lower embryo) somatic embryos.

Phil Jackson
Auxiliary Investigator
PhD 1997 in Biochemistry, Universidade Nova de Lisboa, ITQB

Plant Cell Wall

The overall activities of the PCW group continues to probe the association of the extracellular matrix and extracellular proteins with a variety of processes in plant development and the response/adaptation to stress.
One major activity in 2006 was directed towards the role of apoplastic proteins in the response to plant wounding.
Plant wounding occurs via abiotic stress, such as hail and wind, but also biotic factors including herbivore feeding, and can cause considerable damage to economically important crops. Plants have developed the means to reduce the damage caused by such factors, not the least of which is the production of herbivore deterrents and rapid wound-healing to prevent facultative pathogen ingress. Despite the importance of these defensive responses, the underlying biochemical and molecular bases involved in their coordination remain poorly understood.
Our recent results indicate the apoplast as an important site for the expression of defensive measures to wounding, and have established reactive oxygen species (ROS)-signalling as one of the major regulatory pathways involved.
Proteomics and histochemical approaches were applied to study early (0-6 h) changes in the Medicago apoplast in response to wounding (see figure). Of the 110 proteins identified, 33 were shown to be wound-regulated. The use of a NAD(P)H oxidase inhibitor (DPI) and exogenous sources of reactive oxygen species (ROS) allowed us to associate wound-related changes in the expression of 15 apoplastic proteins with ROS signalling pathways. The results indicate that a major part of the early wound response in the apoplast is initiated by a O2- signal generated within 3 min of wounding, and is regulated by a downstream (30 min -1 h) signal of hydrogen peroxide proximal to the wound site.



The inhibition of ROS accumulation and ROS-signalling related changes in Medicago leaves by DPI. Upper panel: NTB staining of superoxide 3 min after wounding leaves (black staining) and its inhibition by the application of DPI. Lower panel: Wound-related changes in apoplastic proteins. A selected area of 2D electrophoretic gels is shown. Note the up-regulation of proteins 4 and 10 in response to wounding, and the inhibition of this response by DPI. Other proteins (5, 6) remain unaffected.

Group Members

Luis Goulão	Post Doc
Nelson Soares	PhD student
Ada Vatulescu	PhD student
José Ribeiro	PhD student

Selected Publications

Riberio MJ, Silva Pereira C, Soares N, Viera AM, Feijó JA and Jackson P (2006). The contribution of extensin network formation to peroxide-mediated increases in cell wall resistance to fungal, lytic enzymes. J. Expt. Bot. 57: 2025 - 2035.

Ricardo Manuel de Seixas Boavida Ferreira

Full Professor at Instituto Superior de Agronomia, UTL
PhD 1987 in Biochemistry, University of East Anglia, U.K

Disease and Stress Biology

Group Members

Cláudia Santos	Post-doc
Sara Monteiro	Post-doc
Ana Sofia Caeiro	PhD student
Cristina B. Price	PhD student
Regina Freitas	PhD student
Lucélia Tavares	Graduate
Vanessa Borrego	Graduate
David Barata	Undergraduate

New and nontoxic strategies to combat pathogenic fungi

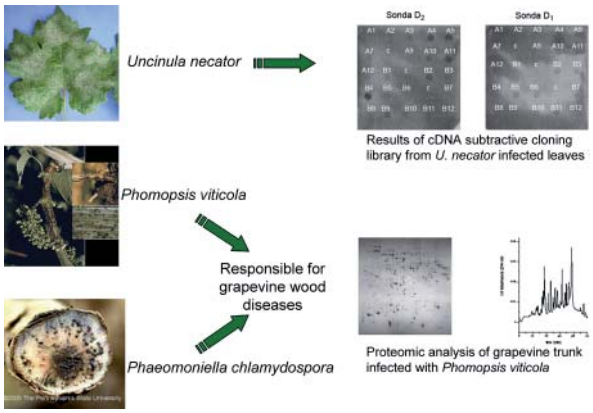
- Transcriptomic (suppressive subtractive hybridization), proteomic and metabolomic analyses of the interaction grapevine/pathogenic fungi. Three fungi will be studied: *Uncinula necator* (responsible for powdery mildew), *Phomopsis viticola* and *Phaeomoniella chlamydospora* (two fungi responsible for grapevine wood diseases);
- Search and development of new and non-toxic fungicides active against human and plant pathogens;
- Genetic engineering of grapevine and rose to express constitutively an antifungal protein.

Collaborations: José Vidal, Departamento de Biotecnologia, Universidade Politécnica de Madrid, Spain. Herma Koehorst-vanPutten, Laboratory of Plant Breeding, Wageningen University and Research Centre, Netherlands.

Biomedical applications of plant polyphenols

- Search for novel plant polyphenols exhibiting anti-oxidant activities in a range of plants, including several Portuguese endemic species;
- Screening of the polyphenols for neuroprotective and anti-proliferative activities using cultured neuroblastoma cells and for anti-fungal activity towards human and plant pathogens;
- Transcriptomic (microarrays) and proteomic analyses of polyphenol treatment in Parkinson's disease, using rotenone-treated cultured neuroblastoma cells;
- Effect of polyphenol treatment on the role played by the ubiquitin/proteasome pathway in Parkinson's disease, using rotenone-treated cultured neuroblastoma cells.

Collaborations: Paula Alves, Animal Cell Technology Laboratory, IBET/ITQB. Astrid Vicente, Centro de Biopatologia, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa. Catarina Duarte, Nutraceuticals and Controlled Delivery, IBET. Paul Jenö, Mass Spectrometry, Biozentrum, University of Basel, Switzerland.



Transcriptomic, Proteomic and Metabolomic Analyses of the Interaction Grapevine/Pathogenic Fungi

Search for novel plant polyphenols exhibiting anti-oxidant activities in a range of plants, including several Portuguese endemic species

Selected Publications

Ferreira R. B., Monteiro S., Freitas R., Santos C. N., Chen Z., Batista L. M., Duarte J., Borges A. and Teixeira A. R. (2006) Fungal pathogens: the battle for plant infection Critical Reviews in Plant Sciences 25(6): 505-524.

Monteiro S., Pereira M. A. P., Batista L. M., Loureiro V. B., Teixeira A. R. and Ferreira R. B. (2006) Electrophoretic analysis of the polypeptide composition during berry development Vitis 45(3): 149-150.

Santos C., Gaspar M., Caeiro A., Branco-Price C., Teixeira A. and Ferreira R. B. (2006) Exposure of Lemna minor to arsenite: Expression levels of the components and intermediates of the ubiquitin/proteasome pathway Plant and Cell Physiology 47(9): 1262-1273.

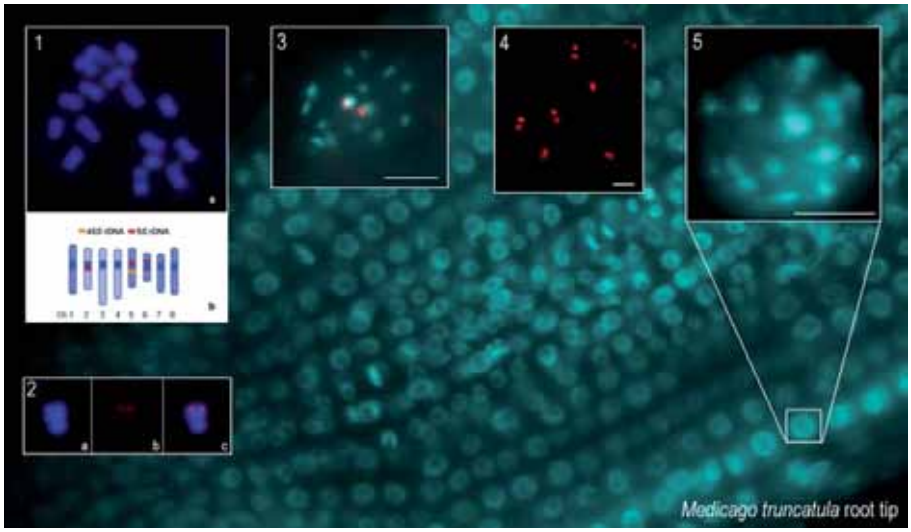
Rita Abranches
Auxiliary Investigator
PhD 2000 in Cell Biology, John Innes Centre / University of East Anglia, UK

Plant Cell Biology

The main objective of our current research is to integrate the fundamental and applied aspects of Molecular Farming - the large scale production of recombinant proteins in plants. We study the processes that influence recombinant protein expression, accumulation, and stability, and aim to identify new ways of controlling these processes to optimize production. In fact, one of the major challenges in plant biology is to generate transgenic plants that express foreign genes stably over generations. Therefore, during the past year we have focused on the use of transgenic plants for studying the mechanisms that control transgene expression, using the model legume *Medicago truncatula*.

The first step in assessing the long-term utility of a transgenic plant is the detailed analysis of the transgene locus. The activity state of a gene is determined not only by sequence specific regulatory factors but also by multi-protein complexes that directly or indirectly affect the structure of the chromatin surrounding the gene. We have used Fluorescence in situ hybridization (FISH) on both metaphase and interphase cells of transgenic *Medicago* plants, to study the relationship between higher order chromatin structure and the expression of transgenes. We have developed techniques for the visualization of transgene integration sites in *Medicago* plants expressing different levels of a recombinant protein – Phytase A from the fungus *Aspergillus niger*, which is used as an additive in animal feed.

We are also using these approaches to learn about nuclear organization and to study the dynamics of genes and chromatin. It has been postulated that the host genome size influences a species amenability to transformation. This may be related to the different patterns of euchromatin/heterochromatin in the nucleus, which varies between plant species. To investigate this phenomenon in greater detail we have initiated a comparative analysis of the *Medicago*, Arabidopsis and Rice genomes. We are comparing the distribution of markers of heterochromatin in order to understand the mechanisms that determine the packaging of repetitive sequences in different plant species.



Medicago truncatula metaphase chromosomes a) DAPI staining, b) schematic representation. 2: detection of transgenic DNA a) DAPI staining b) phytase gene visualized by FISH c) overlay. 3: Interphase nucleus showing ribosomal genes (detected by FISH) as two red dots. 4: a group of interphase nuclei where ribosomal genes are labeled in red. 5: interphase nucleus labeled with DAPI, showing brightly labeled blocks of heterochromatin. Background: Medicago root tissue section labeled with DAPI.

Group Members

Nuno Geraldo	Post-doc
Stefanie Rosa	PhD student
Ana Sofia Pires	Graduate
Teresa Cerqueira	Graduate
Mónica Silva	Undergraduate

Selected Publications

Santos AP, Wegel E, Allen GC, Thompson WF, Stoger E, Shaw P and Abranches R (2006) *In situ* methods to localize transgenes and transcripts in interphase nuclei: a tool for transgenic plant research. Plant Methods 2:18

Kohli A, Gonzalez-Melendi P, Abranches R, Capell T, Stoger E and Christou P (2006). The quest to understand the basis and mechanisms that control expression of introduced transgenes in crop plants. Plant Signaling & Behavior 1: 185-195.

Technology Division

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.

The Division spans its interests and activities from fundamental and applied research, to GLP Services, and to the Pilot Plant for fermentation, extraction, and purification.

The core scientific areas are centred on Vaccines and Gene Therapy as well as Microbiology of Man-Made Products and the Environment. Laboratories carrying out research on Mass Spectrometry, Biosensors/Diagnostics, Biomathematics, and Thermodynamics close the loop for integrated, multidisciplinary activities.

Brief accounts of 2006 highlights follow:

In 2006, the fundamental research component was rewarded with the first publication of the ITQB - as the leading institution - in *Nature* with an article on the burgeoning area of new alternative solvents – Ionic Liquids.

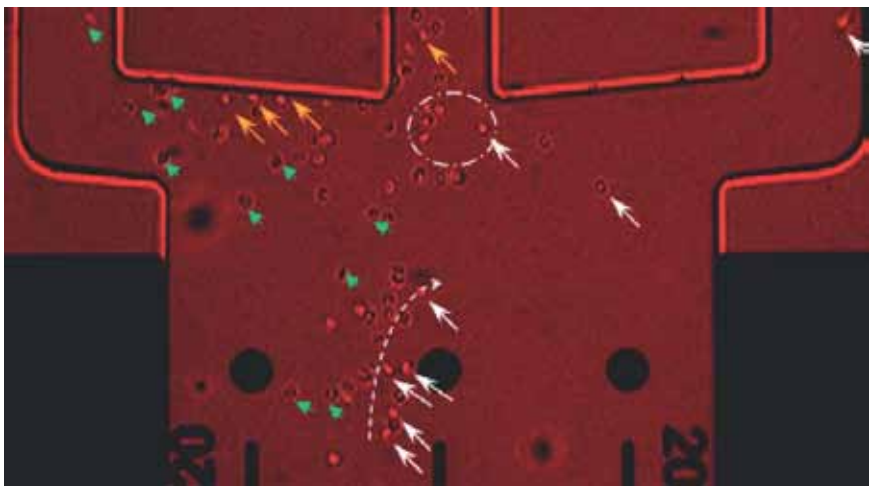
During 2006, the Pilot Plant Unit was challenged with its partial transformation to clean rooms and Good Manufacturing Practices (GMP) for the production of active biopharmaceutical ingredients for clinical trials in Phases I and II.

The GLP Services suffered a major re-organization by unifying the Analytical, Microbiological, and Mass Spectrometry laboratories in one Unit, encompassing both the services and a research and development perspective.

Abel González Oliva
Auxiliary Investigator
PhD in 1987 Universität Hohenheim/Germany

Biomolecular Diagnostics

The biomolecular diagnostic laboratory is constituted by a multidisciplinary team committed to develop new instrumental tools for practical applications in the field of disease diagnostic and bioprocess operation. Based on optical immunodetection, different applications have been developed towards new disease targets (e.g. African swine fever virus; *Brucella sp*.; and tick and tick-born disease parasites, like *Theileria sp*., *Babesia sp.*). Technologies like micro-fluidics and micro-fabrication, allied to the discovery and characterization of biomolecules and immunoreactions, allow the development of immunosensors, immuno-diagnostics and optical sensors using fiber optic technology. The laboratory main areas of expertise include immunological studies and characterization of antigens and antibodies for their use in diagnostics; development of ELISA and rapid tests based on immunoreactions; development of immobilization protocols for antibodies/antigens on solid supports; studies of biomaterials as support for immobilization; technology for the biosensor detection device and development of flow-cells and automation. Recently, microfluidic devices applied to cell handling and cell characterization has been introduced as a recent area of expertise in the group. Studies of impedance spectroscopy, di-electrophoresis and electrorotation techniques onto 20 - 40µm microchannels wide has been performed to explore the potential of individual cells manipulation, characterization of membrane, cytoplasm properties or diagnostic of *Babesia* in infected red blood cells.



Separation of ethidium bromide stained B. bovis iinfected red blood cells (RBC) and non- infected RBCs at 4MHz in a microfluidic chip (EPFL) using dielectrophoresis (DEP). White arrows correspond to iRBCs deviated by pDEP; green arrows to nRBCs being deviated by nDEP and orange arrows to iRBCs which were not deviated to the right outlet, as fluidic forces overcome electrical forces. Long white arrows defined the path taken by the cells.

Group Members

Marta Gomes	PhD student
Elisabete Nascimento	Master Student
José Palmeira	Master Student
Patricia Marques	Graduate
Tiago Silva	Graduate
Ana Rita Jorge	Graduate
Oscar Silvestre	Graduate

Selected Publications

Miranda J, Bakheit MA, Liu Z, Yin H, Yongjuan M, Guo S, Beyer D, Oliva A, Ahmed JS, Seitzer U (2006)Development of a recombinant indirect ELISA for the diagnosis of Theileria sp. (China) infection in small ruminants. - Parasitol Res. (2006) 98: 561–567

Silva M.G., Wilkowsky S., Echaide S., Farber M., Oliva A. (2006) Development of an immunosensor for the diagnosis of bovine Anaplasmosis. Ann N Y Acad Sci. 2006 Oct;1081:379-81

Miranda J., Nascimento E., Cruz H., Zweigarth E., Oliva A. (2006) Establishment of optimal conditions for long-term culture of erythrocytic stages of Theileria uilenbergi. Am J Vet Res. 2006 Nov;67(11):1908-13.

Ana Luísa Simplício
Auxiliary Investigator
PhD 2004 in Pharmaceutical Chemistry, Trinity College Dublin, Ireland

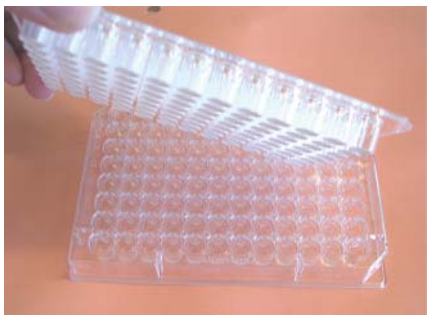
Pharmacokinetics and Biopharmaceutical Analysis
(ITQB/IBET)

Group Members

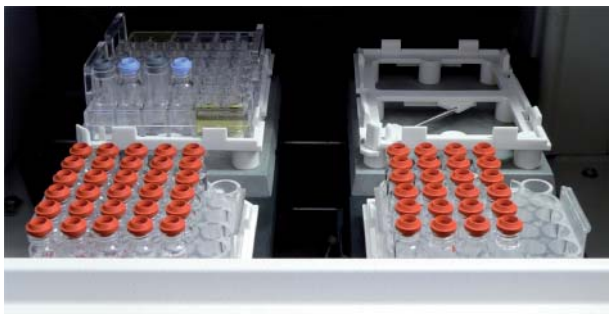
Rita Noronha	PhD
Hugo Serra	PhD student
Telma Mendes	Undergraduate
Vânia Capelo	Undergraduate
Joana Rodrigues	Undergraduate

In vitro - in vivo correlation (IV -IVC) deals with the relationship between an in vitro parameter (e.g. drug release) and an in vivo property (usually a pharmacokinetic parameter). In a true sense of correlation, in vitro measurement should predict in vivo performance of a drug. The most widely used IV -IVC in pharmaceutical science, which is applicable mainly to sparingly soluble, easily absorbed drugs (type II drugs), involves correlation between dissolution and absorption and it can also be used with high solubility drugs (type I). The main interest in studying this type of IV-IVC is its potential ability to reduce tests with humans and corresponding costs, during solid dosage form development. While still mainly in the field of research, due to difficult acceptance by the authorities, the increasing body of evidence of its usefulness is likely to change this picture soon and therefore we are already cooperating with industry partners. On the down side, correlations involving drug dissolution are not applicable to low permeability drugs (types III and IV). One of our research objectives is the establishment of new IV-IVC strategies for low solubility, low permeability drugs. For these, *in vitro* methods for permeability and absorption prediction may be necessary. We are currently using methodologies such as the Caco-2 and the PAMPA transport models with this purpose.

Such methods are also important in early drug development process in order to avoid later use of animal tests for compounds which are unlikely to be transported across biological membranes. Therefore, we also use these methods for evaluation of new compounds that we develop in the context of our second research objective: prodrugs. There are numerous reasons for the use of prodrug strategies but we have been working particularly on the preparation and evaluation of prodrugs with improved solubility in comparison with the parent compound. We have mainly been interested in the study of the pharmacokinetic properties of flavonoids and their derivatives.



We have also been cooperating with other groups inside ITQB and IBET in the development of separation methods involving chromatography and capillary electrophoresis.



Some In vitro and separation methodologies used at PABA laboratory: PAMPA and capillary electrophoresis

Selected Publications

Simplício A.L., Matias P., Gilmer J.F. and Clancy J.M., (2006) Chiral separation and identification of ß-aminoketones of pharmacological interest by HPLC and capillary electrophoresis Journal of Chromatography A, 1120, 89-93.

Duarte A.R.C., Gordillo M.D., Cardoso M.M., Simplício A.L. and Duarte C.M.M. (2006) Preparation of ethyl cellulose/methyl cellulose blends by supercritical antisolvent precipitation International Journal of Pharmaceutics 311, 50-54

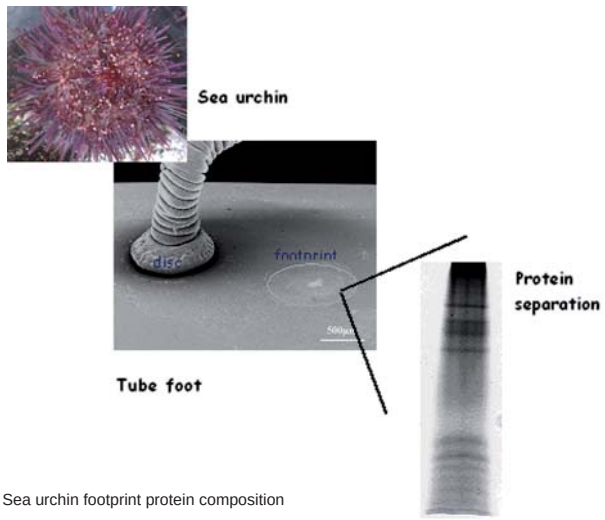
Ana Maria Varela Coelho
Assistant Professor, Universidade de Évora
PhD 1998 in Universidade de Évora
Degree in Biochemistry 1987, Faculdade de Ciências, Universidade de Lisboa

Mass Spectrometry

The information obtained with the powerful Mass Spectrometry techniques is fundamental for the structural characterization of chemical and biochemical species. Else than precise mass determination it is possible to perform controlled fragmentation of the molecular ions, which allows to get detailed structural information, like comparative identification of organic compounds, peptide and oligosacharide sequencing and characterization of post-translational modifications. More recently biological species have been analysed by Intact cell MALDI-TOF MS (ICM) which can be used to differentiate and identify bacterial species.
Our main research interests can be divided in 3 fields:
Studies on protein composition and characterization
Some of the studies performed involve the identification of differentially expressed proteins by an organism in different conditions. An example is the study on seasonal weight loss in domestic animals, namely in two breeds of sheep and rabbits that show different tolerance to SWL. Proteome map differences between breeds at two nutritional levels, control and underfed, in both species, have been observed.
Another project aims at identifying the biochemical composition of the poorly known adhesives of sea urchins. Sea urchins have hundreds of adhesive organs that are able to produce adhesive and de-adhesive secretions with which they attach and detach repeatedly from the substrate. The goal is to identify the proteins present in both secretions in order to find new adhesive and de-adhesive molecules for biomedical and technological applications. As far as the echinoid *P. lividus* is concerned, its footprints contain a low amount of proteins. Two of them have already been identified. The amino acid composition of the protein fraction of sea urchin footprints revealed a high percentage of cystein. These residues could be involved in intermolecular disulphide bonds reinforcing the cohesive strength of the adhesive

Studies using intact bacteria and mammalian cells
A new application was developed for the ICM-MS method with possible biotechnological industry application for monitoring viral proteins and viral particles production.

Studies on chemical systems, in particular with relevance to food and pharmaceutical industries. The projects include the development and optimization of MS methods for the characterization of particular types of chemical compounds, namely organo-metallics, and ionic liquids, and for the characterization of food industry residues.



Sea urchin footprint protein composition

Group Members

Alexandre Campos	Post-doc
Romana Santos	Post-doc
André Almeida	Post-doc
Gonçalo Costa	PhD student
Duarte Toubarro	PhD student
Patrícia Alves	PhD student
Sérgio Mota	PhD student
Elsa Lamy	PhD student
Catarina Franco	Graduate
Elisabete Pires	Technician
Catarina Pereira	Undergraduate

Selected Publications

Roxo-Rosa M., da Costa G., Luider T. M., Scholte B. J., Coelho A. V., Amaral M. D. and Penque D. (2006). "Proteomic analysis of nasal cells from cystic fibrosis patients and non-cystic fibrosis control individuals: Search for novel biomarkers of cystic fibrosis lung disease." Proteomics 6(7): 2314-2325

Gomes R. A., Miranda H. V., Silva M. S., Graca G., Coelho A. V., Ferreira A. E., Cordeiro C. and Freire A. P. (2006). "Yeast protein glycation in vivo by methylglyoxal - Molecular modification of glycolytic enzymes and heat shock proteins." FEBS Journal 273(23): 5273-5287.

Bravo M. N., Silva S., Coelho A. V., Boas L. V. and Bronze M. R. (2006). "Analysis of phenolic compounds in Muscatel wines produced in Portugal." Analytica Chimica Acta 563(1-2): 84-92

Catarina Duarte
Auxiliary Investigator
PhD 1997 in Physical Chemistry, Universidade Nova de Lisboa

Nutraceuticals and Controlled Delivery
ITQB/IBET

Group Members

Ana Raquel Sousa	PhD student
Ana Teresa Serra	PhD student
Ana Matias	PhD student
Mariana Costa	PhD student
Raquel Frade	Post-doc
Rodrigo Silva	Technician
Daniel Oliveira	Undergraduate
Nuno Fontes	Undergraduate

The Nutraceuticals and Controlled Delivery laboratory developed intensive research work centred on the development and characterization of bioactive concentrates from selected botanical sources, namely typical Portuguese apple and cherries varieties and residues from the wine and olive oil industries. Efficient clean processes were optimised for the isolation of the natural concentrates, to be considered generally regarded as safe for human comsumption. The selected fractions were characterised in terms of their polyphenolic content and quantified for their antioxidant capacity, relatively to standard antioxidants. The capacity for scavenging reactive oxygen species(ROS)- hydroxyl and superoxide radicals were accessed using an EPR (Electron paramagnetic resonance) method. The natural matrices were also analyzed for its ability in inhibiting AAPH-induced LDL oxidation. Cytotoxic was evaluated in Caco-2 (human-colonic cancer)cell line model and antiproliferation activities were studied using HT29 and MKN45 human colorectal and stomach cancer cells, respectively. As an attempt to investigate the effect on intestine inflammation, we looked at IL-8 levels in human colon carcinoma cells, HT-29, previously treated with the natural extracts.

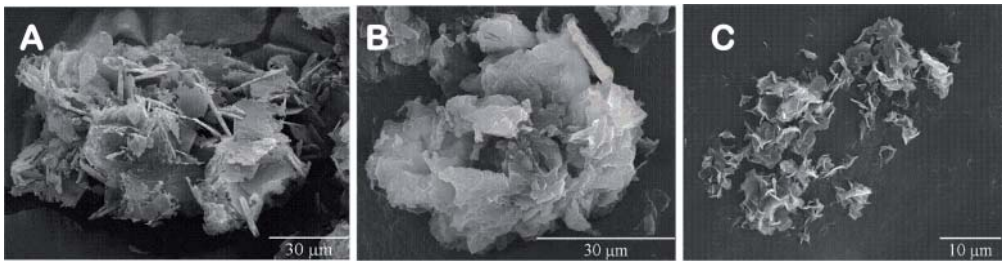
In the field of material science, supercritical fluid technology, namely PGSS® (Particles from Gas Saturated Solutions) technique, was explored to produce solid lipid low-micro particles as carriers or solubility enhancers for active substances. The carriers processed (precirol, gelucire, compritol, glyceryl monostearate) are generally regarded as safe compounds for human contact and, caffeine and chalcone, were used as model active substances. Caffeine was selected for being a hydrophilic substance, and thus a challenge to incorporate in lipid carriers, and also because of its therapeutical and cosmetical applications. Chalcone, on its turn, represents a group of compounds, with a wide array of pharmacological activities and exhibits very poor solubility. An alternative clean process, using supercritical carbon dioxide, was developed and applied for the impregnation of some polymeric matrixes with biological active drugs. Experiments on the preparation of matrices impregnated with bioactive compounds were performed using anti-inflammatory drugs.

Selected Publications

Duarte A. R. C., Casimiro T., Aguiar-Ricardo A., Simplicio A. L. and Duarte C. M. M. (2006). "Supercritical fluid polymerisation and impregnation of molecularly imprinted polymers for drug delivery." Journal of Supercritical Fluids 39(1): 102-106.

Duarte A. R. C., Costa M. S., Simplicio A. L., Cardoso M. M. and Duarte C. M. M. (2006). "Preparation of controlled release microspheres using supercritical fluid technology for delivery of anti-inflammatory drugs." International Journal of Pharmaceutics 308(1-2): 168-174.

Sampaio de Sousa A. R., Calderone M., Rodier E., Fages J. and Duarte C. M. M. (2006). "Solubility of carbon dioxide in three lipid-based biocarriers." Journal of Supercritical Fluids 39(1): 13-19. PDF



SEM pictures of Chalcone and composites processed by PGSS. A – Chalcone, , B – Chalcone + Gelucire 50/13 and C – Chalcone + Precirol ato5.

Cidália Peres
Principal Investigator with Habilitation (Agregation Eq.), INRB
PhD 2004 in Food Technology and Biotechnology (Eq.), INIA

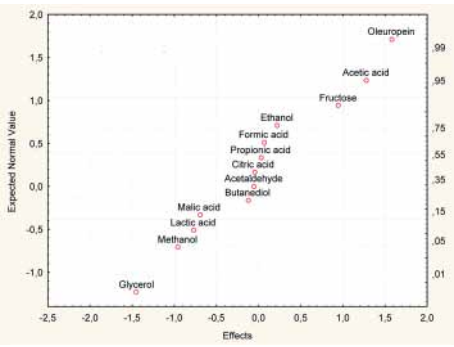
Food Microbial Technology

ITQB/IBET

The research follows two main lines, a) Characterization of traditional agricultural products: it has been studying the ecology of table-olive fermentation, a spontaneous process that relies on a succession of mixed populations. Traditional fermented olives are a rich source of novel, interesting lactic acid bacteria (LAB) strains. We have been isolating and characterizing some of them showing technologically important properties, such as: high salt tolerance; fair growth at temperatures lower than 15ºC; high yield in the conversion of substrate to lactic acid; production of bacteriocins; capability to degrade polyaromatic compounds. Strains, carrying one ore more of the above properties, were tested in inoculum's development studies. A resulting process is being scaled-up and promising results are in the pipeline for other specific industrial applications. Moreover, making use of specific properties of 'novel' strains may enhance the inherent value of Portuguese table-olives and enables a certain product diversification, which is important to maintain the microbial biodiversity of such fermented foods. b) Food Microbiology and Healthcare: an ecosystem of increasing importance is the gastrointestinal tract, in particular with respect to the use of probiotic LAB in the diet. It has been screening LAB for their probiotic capability, as well as for the production of anti-oxidants and antimicrobials. That is, it is gathering information to further evaluate if these living bacteria may improve the health of the table-olive's consumer, above the normal diet. If so, certain types of table-olives could be classified as symbiotic food products (combining probiotics and prebiotics) and also as functional foods. These aspects would further to increase the value of the traditional products of Mediterranean diet. It has been collected information for in-depth studies on the inhibitory effects of LAB towards *Helicobacter pylori*. Bacteriocins may play an important role in this context, mostly produced by members of the genus *Lactobacillus*. Since bacteriocin resistance mechanisms are distinct from those of antibiotics and not genetically transferred, healthcare applications have been considered. Existing know-how on the optimization of physical and nutritional factors for *in vitro* bacteriocin production and the development of mathematical models describing bacterial growth and bacteriocin production will be of valuable use for the development of these new biotherapeutic agents.

Group Members

Dulce Brito	Aux. Inv. INRB
Ana Isabel Cordeiro	Post Doc
Amélia Delgado	PhD student
Nadia Chammem	PhD student
Cristina Pintado	PhD student
Francisco Pinheiro-Alves	Master Student
Joana Canhoto	Graduate
Gonçalo Silva	Graduate
Sofia Fernandes	Undergraduate
Cristina Serrano	Technician
Luis Catulo	Technician
Luisa Reis	Assist. Technician



Expected normal values ofqualitative variables and their corresponding effects. Response was bacteriocin activity.

Probiotic aptitude test: growth of a lactic acid strain in MRS after exposure to peptic digestion conditions (bile salts, HCl and pepsin).

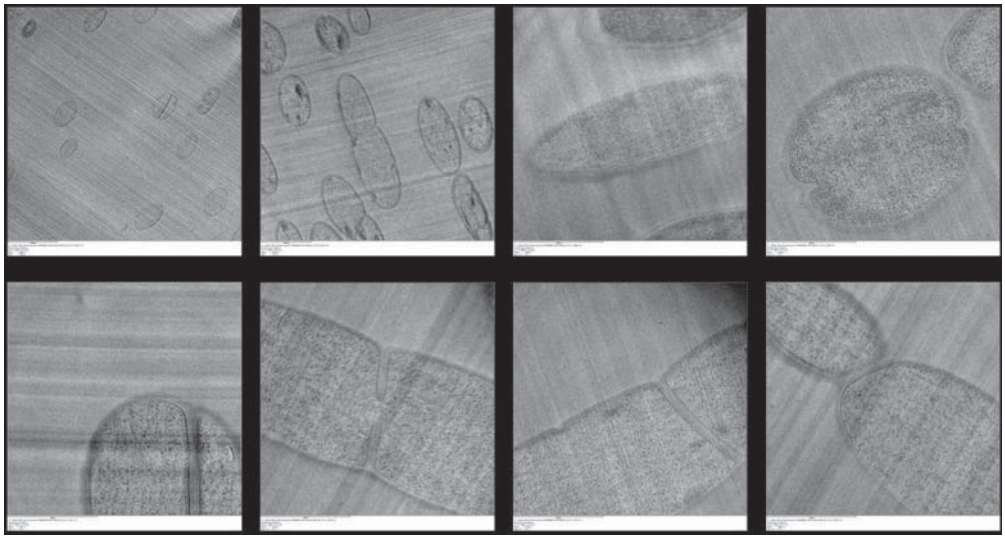
Maria de Fátima Silva Lopes
Auxiliary Investigator
PhD 1999 in Biochemistry, Universidade Nova de Lisboa, ITQB

Antibiotic Stress and Virulence of Enterococci
ITQB/IBET

Group Members

Paulo Marujo	Post-doc
Tânia Ribeiro	PhD student
Frederic Gaspar	PhD student
Teresa Braga	PhD student
Neuza Teixeira	Master Student
Marta Ruivo	Master Student
Daniela Matos	UnderGraduate
Daniela Pinto	Master Student
Renata Matos	Master Student
Vera Pinto	Master Student

Enterococcus is one of the genus of bacteria able to inhabit/survive in many different environments, namely outside and inside the human body. In a close correlation with the widespread use of antibiotics in both clinical practice and in agriculture, bacteria from the genus *Enterococcus* have turned into one of the major causes of nosocomial infections. We have collected enterococcal strains from different environments (human and animal infections, food, water and sand), identified and characterized them concerning both the antibiotic resistances and the presence of virulence factors. We have dedicated particular attention to the bacitracin resistance, which appears to be widely spread amongst all environments, despite the low use of this drug. Little is known on bacitracin resistance in enterococci. Another antibiotic resistance to which we have dedicated much attention is vancomycin resistance. We have collected and characterized some VRE strains from a Portuguese Hospital and found and insertion sequence (ISEFl) in the vanA operon, to which high variability was associated, as we have demonstrated earlier. Our studies revealed *E. faecium* as the multiresistant species, also associated with ampicillin and penicillin resistance. Using techniques such as PFGE and MLST, we were able to confirm that the same strain can colonize the human body and food and be responsible for nosocomial infections. Therefore, we are interested in understanding the factors (at all levels, namely DNA, RNA and protein) that enable the same strain to behave differently under diverse environments. We have chosen the gelatinase virulence factor to start this studies and we have proved, using an animal model (*C. elegans*), that food strains are as able to be virulent as clinical strains carrying the fsr-gelE operons. One of the factors that is accepted to be responsible for the increase in number of enterococcal caused infections is the fact that these bacteria are intrinsically resistant to many antibiotics. In order to find new targets for drugs that will enable us to fight multiresistant enterococcal strains, we have started studies intended to understand how the cell perceives the chemical signals (an antibiotic in a sub-lethal dose, for example). We are approaching this in three ways: at the proteomic level; at the transcriptomic level; and at the cell-wall/membrane level. In order to visualize the changes occurring at the membrane/cell-wall level we have complemented these studies with CEMOVIS (cry-electron microscopy of vitreous sections), a method allowing the observation of biological specimens in a closest-to native state.



Enterococcus faecalis cells visualized with CEMOVIS.

Selected Publications

Lopes M. F. S., Simoes A. P., Tenreiro R., Figueiredo Marques J. J. and Crespo M. T. B. (2006). "Activity and expression of a virulence factor, gelatinase, in fairy enterococci." International Journal of Food Microbiology 112: 208-214.

Zuber B., Haenni M., Ribeiro T., Minnig K., Lopes F., Moreillon P. and Dubochet J. (2006). "Granular layer in the periplasmic space of gram-positive bacteria and fine structures of Enterococcus gallinarum and Streptococcus gordonii septa revealed by cryo-electron microscopy of vitreous sections." Journal of Bacteriology 188(18): 6652-6660.

Jonas S. Almeida
Full Professor University of Texas, USA
PhD 1995, Universidade Nova de Lisboa

Biomathematics

The goal of the Biomathematics Group at ITQB is the quantitative analysis of biological systems with special emphasis on the identification of unifying quantities and methodologies. From a methodological point of view, the claim for originality of this group is the integrative use of data analysis techniques, allowing a tight yet flexible adaptation of the tools to the specific structure of a given biological system - integrative computation coined by the biological problem. The specific applications where we have been most active are in the fields of molecular epidemiology, reverse engineering of biological pathways, and microbial biofilms. The modus operandi of the group relies extensively on web-based, multidisciplinary collaborations with groups at the ITQB and elsewhere, and on the involvement in Graduate exchange programs.

Group Members

Andreas Bohn	Post Doc
João Carriço	PhD student
Francisco Pinto	PhD student
Helena F Deus	PhD student

Selected Publications

Carrico JA et al. (2006) Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant Streptococcus pyogenes. J Clin Microbiol. 44(7):2524-32.

Almeida JS et al. (2006) Data integration gets 'Sloppy'. Nature Biotechnology 24(9):1070-1071.

Luís Paulo N. Rebelo
Associate Professor with Agregação
Phd in 1989, Universidade Nova de Lisboa

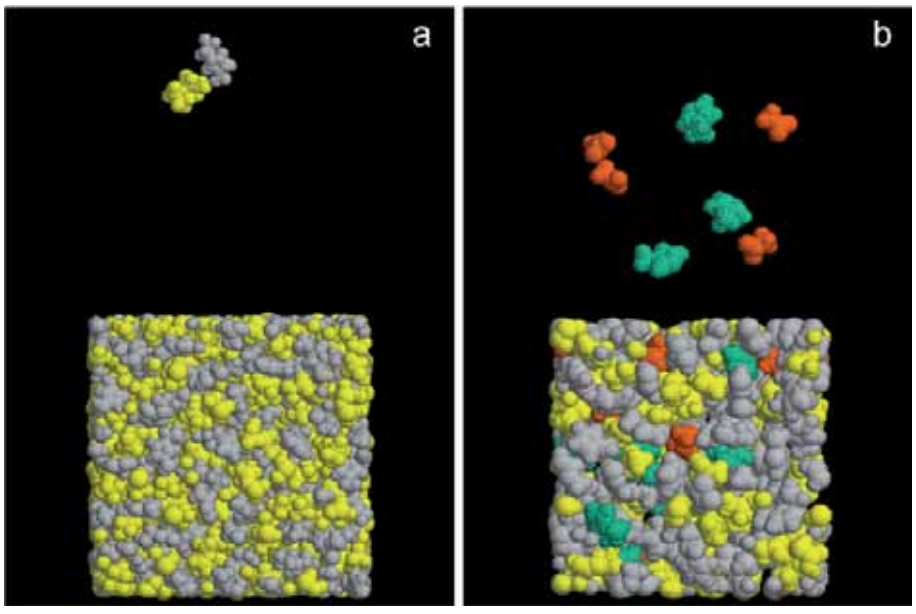
Molecular Thermodynamics

Group Members

José Lopes	Inv. Assist.Prof.
Zoran Visak	Post-doc
José Esperança	Post-doc
Marijana Blesic	PhD student
Joana Trindade	Undergraduate

Research interests are centred on the areas of Molecular Thermodynamics of Liquids and Liquid Solutions (theory and experiments), in particular, Isotope Effects, Polymer Solutions, Metastable liquids, Sound Propagation in Dense Phases, and since 2002, on the newly emerging area of Ionic Liquids. Regarding the latter, pioneering experimental work has been performed both in respect to their thermodynamic characterization in a broad range of pressures and temperatures as well as to their solution behaviour. Molecular Simulation began in 2006.

The recent years have witnessed the growing importance of ionic liquids – excellent solvents, which are liquid salts at or close to room temperature, and frequently studied as part of the important field of green chemistry. Their study has impacted a wealth of distinct areas, from chemistry to physics, from environmental sciences to the life sciences. Their general non-flammability, non-volatility, extraordinary solvent power, and easy recover from mixtures (recyclable) are the key characteristics that have driven the recent development of these salts as alternative clean media for chemical and enzymatic reactions, novel composites, separation and extraction processes, fuel cells, nuclear fuel reprocessing, and, more recently, biotechnology and pharmaceutical applications.



Thermal vaporization of two distinct classes of ionic liquids. Cations=grey, anions=yellow. (a) aprotic (1-methyl-3-ethylimidazolium bistriflamide, [C2mim][Ntf2]), versus (b) protic (methylimidazolium acetate, [Hmim][CH3COO]). In (b), the ionic species in the liquid phase are in equilibrium with the neutral molecules of methylimidazole (green) and acetic acid (orange).

Selected Publications

Earle M. J., Esperanca J. M. S. S., Gilea M. A., Canongia Lopes J. N., Rebelo L. P. N., Magee J. W., Seddon K. R. and Widegren J. A. (2006). "The distillation and volatility of ionic liquids." Nature 439(7078): 831-834

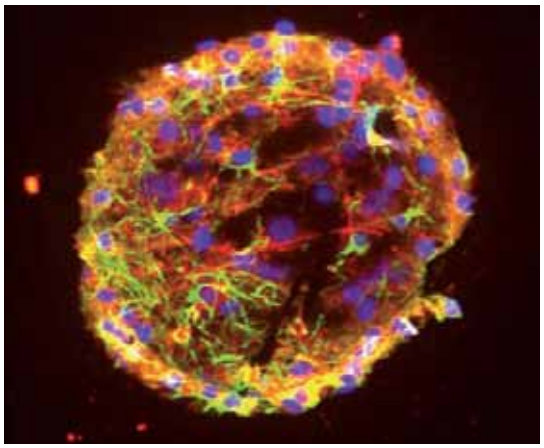
Lachwa J., Bento I., Duarte M. T., Lopes J. N. C. and Rebelo L. P. N. (2006). "Condensed phase behaviour of ionic liquid-benzene mixtures: congruent melting of a [emim][NTf_2] • C_6H_6 inclusion crystal." Chemical Communications(23): 2445-2447

Lachwa J., Szydlowski J., Makowska A., Seddon K. R., Esperanca J. M. S. S., Guedes H. J. R. and Rebelo L. P. N. (2006). "Changing from an unusual high-temperature demixing to a UCST-type in mixtures of 1-alkyl-3-methylimidazolium bis{(trifluoromethyl)sulfonyl} amide and arenes." Green Chemistry 8(3): 262-267

Manuel J.T. Carrondo
Full Professor, ITQB/IBET/FCT-UNL
PhD in 1979, Environmental Engineering, Imperial College, London
Paula M. Alves
Auxiliary Investigator, ITQB/IBET
PhD 2001 in Biochemistry, Universidade Nova de Lisboa, ITQB

Animal Cell Technology
ITQB/IBET

As befits a technological area, a number of knowledge competences have to be appropriately balanced: (i) the more "analytical" sciences of molecular biology, biochemistry and physiology of cells, viruses or tissues as well as the physico-chemical skills required for downstream processing of the products to be purified, (ii) the more "synthetic" physico-mathematical tools required for process integration and optimization, linking non-parametric systems biology and parametric bioprocess engineering modelling, keys for understanding complex phenomena like infection kinetics. Such competences are used to describe, design and control distinct systems, processes and/or applications: (i) Recombinant proteins as biopharmaceuticals: Using insect or mammalian cells for complex recombinant and fusion protein; Proliferation control and use of cell cycle synchronization, by genetic, chemical or physical means for improved recombinant protein production; Integrated process development for biopharmaceutical proteins (bioreactors, purification, formulation) and their hybrid control algorithms; (ii) Vaccine development: Virus like-particles production with Baculovirus infected insect cells for, eg., Rotavirus, single-gene co-infections versus multi-gene infections (intracellular dynamics, particle assembly/ disassembly); Recombinant adenoviral vectors production (bioprocess, formulation and storage); Mass production of endothelial cells for Cowdriosis Vaccine; (iii) Viral vectors for gene therapy Retrovirus, adenovirus and baculovirus as vectors; Fundamental studies on: molecular biology of viral assembly, using "cassette exchange" concepts for fast vector development and screening; viral degradation mechanisms and improvement of viral half life; Integrated strategies for vector production, preparation and storage, coupling with bioprocess engineering for developing hybrid control algorithms; (iv) Stem and primary cultures cell for cell therapy and tissue engineering: Embryonic and adult human and mice stem cells: expansion as undifferentiated cells; cryopreservation studies. In particular in 2006, a small scale bioreactor (250 ml) to culture aggregates of rat embryonic brain cells under fully controlled environment (pH, pO2, temperature and nutrients) was developed, being possible to mimic pathological conditions such as hypoxia and ischemia. Sampling throughout cultivation time enabled the characterization of aggregates cellular composition (assessed by western blot and immunofluorescence techniques) and metabolism.



Section of a brain cell aggregate (rat) after 19 days of culture in a fully controlled bioreactor. Staining: Astrocyte with Anti-GFAP(green); neurons with anti-Neurofilament-Light (red), nucleus with DAPI (blue).

Bioreactors DCU

Group Members

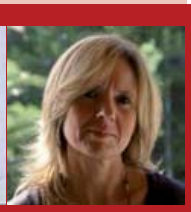
Pedro Cruz	Senior Researcher
Ana Coroadinha	Auxiliary Inv.
Helena Vieira	Post Doc
Maria Barbosa	Post Doc
Joana Miranda	Post Doc
Adriana Yokomizo	Post Doc
Isabel Marcelino	PhD student
Tiago Ferreira	PhD student
Cláudia Istrate	PhD student
Teresa Rodrigues	PhD student
Marlene Carmo	PhD student
Sónia Santos	PhD student
António Roldão	PhD student
Ana Teixeira	PhD student
Rita Malpique	PhD student
Leonor Norton	PhD student
Tiago Vicente	PhD student
Candida Mellado	PhD student
Ana Amaral	PhD student
Margarida Serra	PhD student
Carina Silva	PhD student
Sofia Leite	Graduate
Telma Lança	Graduate
Nuno Carinhas	Graduate
Liliana Cunha	UnderGraduate
Ana Mendes	UnderGraduate
Cláudia Queiroga	UnderGraduate
Ana Rodrigues	UnderGraduate
Daniela Ferreira	UnderGraduate
João Dias	Master Student
Cristina Peixoto	Technician
Marcos Sousa	Technician
Rosário Clemente	Technician

Selected Publications

Coroadinha AS, Ribeiro J, Roldao A, Cruz PE, Alves PM, Merten OW and Carrondo MJT (2006) Effect of medium sugar source on the production of retroviral vectors for gene therapy, Biotechnology and Bioengineering, 94(1): 24-36.

Marcelino I, Sousa MFQ, Verissimo C, Cunha AE, Carrondo MJT and Alves PM (2006) Process development for the mass production of Ehrlichia ruminantium, Vaccine, 24(10): 1716-1725.

Carmo M, Faria TQ, Falk H, Coroadinha A, Teixeira M, Merten OW, Geny-Fiamma C, Alves PM, Danos O, Panet A, Carrondo MJT and Cruz PE (2006)Relationship between retroviral vector membrane and vector stability Journal of General Virology 87: 1349-1356.



Luís Vilas Boas
Associated Professor at Universidade Técnica de Lisboa, IST
PhD 1974 in Chemistry, University of Kent at Canterbury

Maria do Rosário Bronze
Assistant Professor Faculdade de Farmácia, Universidade de Lisboa
PhD 1999 in Pharmaceutical Sciences, Universidade de Lisboa

Analytical Chemistry
ITQB/IBET

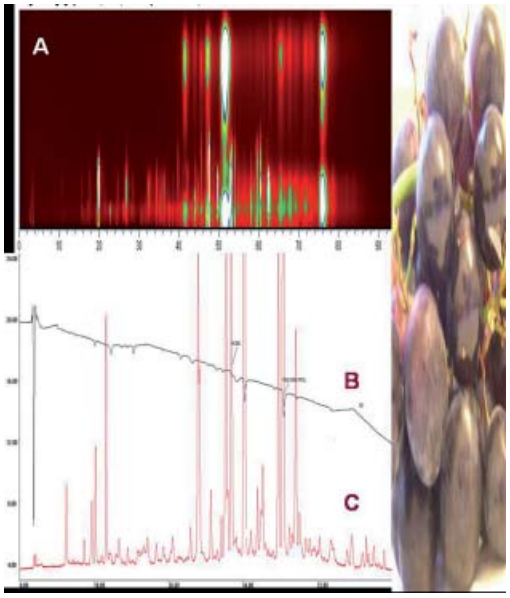
Group Members

Valentim Almeida	Post-doc
Ofélia Anjos	Post-doc
Nubélia Bravo	PhD
Raquel G. Oliveira	PhD
José A. Dias	Master Student
Antero Ramos	Technician
Rodrigo Feliciano	Technician

Food products are the source of nutrients necessary for survival, and play an important rule in human health and well-being, as some compounds may help to prevent some diseases (e.g. antioxidants), or may influence the consumers' preferences for foods (e.g. key aroma compounds).

The main goals of the work we performe at our laboratory are related with the study of the chemical composition of several food products including: fruits and fruit juices; wines and vinegars; table olives and olive oil; tea and coffee. The aim of our work has been mainly directed to analysis of volatile (e.g. terpenes, esthers) and non volatile compounds (e.g. phenolic and carotenoid compounds) in food products, in order to: characterize raw materials, determine changes in the chemical composition during processing and storage, and study the chemical composition of by-products from related food industries. We are looking for compounds that: may contribute to organoleptic characteristics of products; may be responsible for biological properties of foods that will be studied by other research groups. Different preparation techniques (liquid-liquid extraction, Solid Phase Microextraction, Solid Phase Extraction, Stir Bar Sorptive Extraction), separation techniques (e.g. chromatographic and electrophoretic) associated with different detectors (diode array, fluorescence and electrochemical), are used to study chemical composition of these samples and they help us to understand: how different products are in their chemical composition and which compounds may contribute more to the organoleptic properties.

Sometimes collaboration with other groups from ITQB and outside is necessary to complement some of the work we perform.
In the evaluation of quality of food products the perception of colour, taste, aroma, odour, flavour, texture, perceived by humans (tasters or consumers) is also of great interest, and so, different sensory tests are usually performed at our lab in order to find out relationships between chemical composition and sensory properties.
We work in collaboration with IBET and other groups from ITQB working on food products and by-products from food industries, non food products (e.g. cork and tobacco) and some projects are going on with food or food related industries.



Chromatograms of red grape skin extract: A) Absorbance density map in UV-vis (250-600 nm); B) Electrochemical detection (ED); C) Fluorescence (FD) at 300/390nm

Selected Publications

Bravo M.N., Silva S., Coelho A.V., Vilas Boas L., Bronze M.R. (2006) Analysis of phenolic compounds in Muscatel wines produced in Portugal, Analytica Chimica Acta,563 (1-2), 84-92.

Prado M. A., Boas L. E. V., Bronze M. R. and Godoy H. T. (2006). "Validation of methodology for simultaneous determination of synthetic dyes in alcoholic beverages by capillary electrophoresis." Journal of Chromatography A 1136(2): 231-236.

Maria Teresa Crespo
Senior Research Fellow at IBET, Director of Good Laboratory Practices Unit
PhD in Biological Engineering, Universidade Nova de Lisboa, FCT

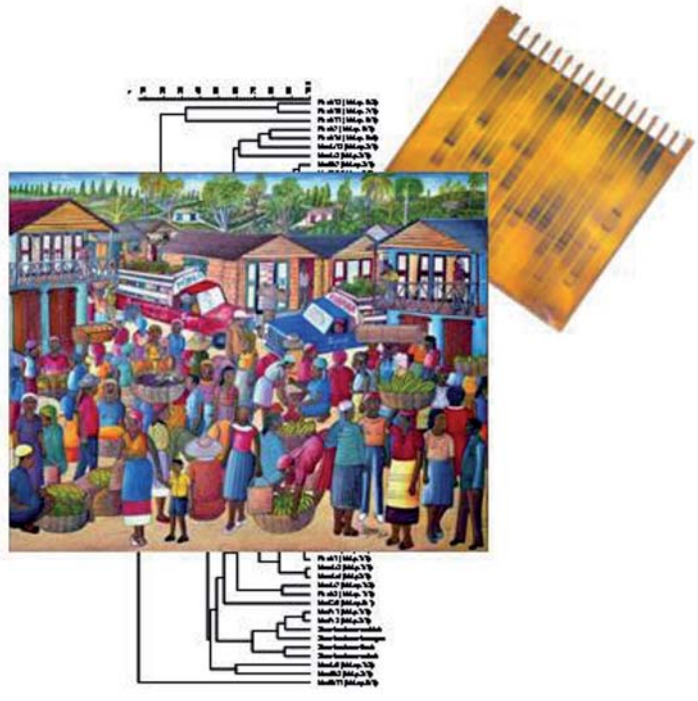
Microbiology of Man-Made Environments

ITQB/IBET

The main aim of the laboratory is the study microbial populations and isolated microbial strains in natural environments and mostly in environments created by man like food products, polluted waters or microbial/host pairs.

The dynamics of growth, survival and biochemical activity of microorganisms in these environments are the results of stress reactions in response to changing of physical and chemical conditions into the particular microenvironment that grow under the constraints imposed by spatial heterogeneity and the in situ cell-to-cell ecological interactions which often occurs at interfaces. The data is integrated in microbial and molecular biology tasks which are expected to evaluate which are the members of the microbial communities present, its dynamics and biochemical activity along the production process. Studies cases studied along the year were traditional cheeses with Registered Designation of Origin where data from molecular biology methods and biochemical methods, like use of C-sources by the population as a whole, allowed the proposal of new ways of early estimating the quality of a fermented food products (collaboration with FCT/UNL and EAN).

The study of the microbial populations responsible for pollutant removal using bioreactors that treat waste waters was approached during the year. Populations were studied as a whole using total DNA extraction, DGGE, cloning and sequencing strategies to identify the members of the population responsible for optimal bioreactor operation (collaboration with FCT/UNL).
A collection of rhizobial strains nodulating annual medics isolated from stressing environments in southeastern and central Portuguese locations (collaboration with EAN), was characterized by simultaneous detection of two highly conserved regions in S. meliloti, the nodbox 4 promoter and the mucR gene. Fingerpring of the isolates was also performed using M13 primer. Data is under analysis, as well as data, from mass spectrometry methodology, MALDI-TOF MS (collaboration with Mass Spectrometry Lab.) that will be used in taxonomical approaches.



Study of population dynamics. Painting - Leogane Market Day, Abner Dubic (1944).

Group Members

Vanessa Pereira	Post Doc
Gilda Carvalho	Post Doc
Cristina Pereira	PhD student
Helena I. Santos	PhD student
Frederic Gaspar	PhD student
Paula Isabel Alves	Technician

Selected Publications

Lopes M. F. S., Simoes A. P., Tenreiro R., Figueiredo Marques J. J. and Crespo M. T. B. (2006). “Activity and expression of a virulence factor, gelatinase, in fairy enterococci.” International Journal of Food Microbiology 112: 208-214.

Maria Vitória San Romão

Principal Investigator, INRB

PhD in 1987 Pharmacy Biochemistry, Universidade do Porto

Physiology of Environmentally Conditioned Microbiota

ITQB/IBET

Group Members

Cristina Silva Pereira	Pos-Doc
Ana Marques	PhD student
M. Carmo Basílio	PhD student
Mariana Carvalho	PhD student
Isabel Martins	Graduate
Helga Garcia	Graduate
Judite Duarte	Graduate
M. Cristina Leitão	Technician
Cátia Rodrigues	Undergraduate
Ana Rita Bento	Undergraduate
Mário Dias	Undergraduate

The group activity is essentially focused in IBET and ITQB-UNL priorities, having in mind EVNINIA main objectives. Actually, the activity is focused on the deep understanding of the microbial phenomena which sustain the well being of people and of the environment. Two areas are being developed:

1) Study of biogenic amines (BA) formation in wine by lactic acid bacteria (LAB), aiming to select regional LAB isolates able to perform malolactic fermentation in wine with no formation of BA or other unfavorable compounds; the selected isolates will be latter immobilized by PROENOL aiming their commercializing. This work is being done under the scientific supervision of Prof Rogério Tenreiro (ICAT-FCUL) having also the collaboration of UTAD.

2) Study of fungi communities. a) Fungi dynamics in a homogeneous community using the cork colonizing community as the study model. This work, aims to characterize the total fungal population present in cork along the cork stopper manufacturing process as to understand the relationship between the fungal population, special concerning the evolution of the forest endogenous population and its importance in determining the manufacturing places resident population. The taxonomic characterization of the fungal species is being used to solve species inter and intra genetic variety. This work is being done in cooperation with Amorim & Irmão company.

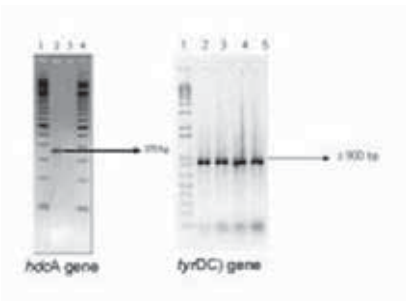
b) Fungi potential for applied and environmental mycology - Most species amongst the cork colonizing community are able to tolerate and degrade high concentrations of a model polychlorohalogenates pollutant: pentachlorophenol. Proteomic analysis will lead to the identification of the core of proteins associated with PCP fungi bioremediation. Degradation products are being studied by mass spectrometry and chromatography as to solve fungal PCP degradation pathway. Fungi potential for whole cell biocatalysis depends on the ability to overcome fungi biodegradation weakness. High recalcitrant plants tissues, enriched in lignin and suberin, are being used as model substrates for studying fungi biodegradation. Spectroscopic methods (FT-IR, solid state NMR) are being used to follow substrate compositional and structural alterations induced by fungi.

Institutional collaborations: Porf. Ana Gil (Universidade de Aveiro), Prof. P. Spencer-Philips (University of the West of England); Prof. A. Hursthouse (University of Paisley); Prof. V. Mazzoleni (Universta Cattolica del Sacro Cuore) and Dr. N. Moreno (IGC).

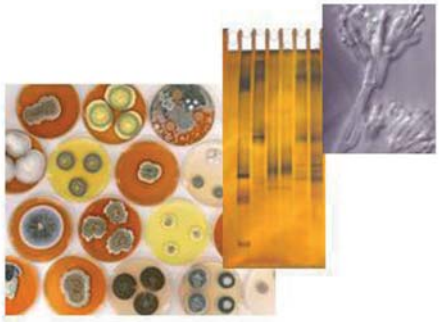
Pollution assessment studies using cork forest soil and ground-waters as a model to investigate pollution risk and remediation strategies will start at the beginning of 2007 (Preventive and remediation strategies for continuous elimination of poly-chlorinated phenols from forest soil and ground waters. NATO, Science for Peace Program coordinated by Cristina Silva Pereira).

Selected Publications

Pereira, CS, Soares, GAM, Oliveira, AC, Rosa, ME, Pereira, H, Moreno, N, and San Romao, MV. (2006) Effect of fungal colonization on mechanical performance of cork. Int Biodeter Biodegr; 57(4): 244-250



Characterization of some genes for biogenic amines production by lactic acid bacteria



Fungi diversity in cork slabs

Research Output

Opening of the academic year and awarding of doctoral diploma and insignia
Rectorate of the *Universidade Nova de Lisboa*, November 2006

From left to right: Tiago Faria, André Almeida, Francisco Pinho
Joana Miranda, Patrick Freire, Prof. Miguel S. Teixeira, Prof. Cláudio Soares, Tiago Bandeiras, Susana Vinga
Ana Sofia Coroadinha, Rute Rodrigues, Manuela Broco

Publications 2006

1. Aires-de-Sousa M., Boye K., de Lencastre H., Deplano A., Enright M. C., Etienne J., Friedrich A., Harmsen D., Holmes A., Huijsdens X. W., Kearns A. M., Mellmann A., Meugnier H., Rasheed J. K., Spalburg E., Strommenger B., Struelens M. J., Tenover F. C., Thomas J., Vogel U., Westh H., Xu J. and Witte W. (2006) "High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study." Journal of Clinical Microbiology 44(2): 619-621.

2. Aires-de-Sousa M., Conceicao T. and de Lencastre H. (2006) "Unusually high prevalence of nosocomial Panton-Valentine leukocidin-positive *Staphylococcus aureus* isolates in Cape Verde islands." Journal of Clinical Microbiology 44(10): 3790-3793.

3. Almeida C. C., Romao C. V., Lindley P. F., Teixeira M. and Saraiva L. M. (2006) "The role of the hybrid cluster protein in oxidative stress defense." Journal of Biological Chemistry 281(43): 32445-32450.

4. Almeida J. S., Chen C. M., Gorlitsky R., Stanislaus R., Aires-de-Sousa M., Eleuterio P., Carrico J., Maretzek A., Bohn A., Chang A., Zhang F., Mitra R., Mills G. B., Wang X. S. and Deus H. F. (2006) "Data integration gets 'Sloppy'." Nature Biotechnology 24(9): 1070-1071.

5. Alves M., Francisco R., Martins I. and Ricardo C. P. (2006) "Analysis of *Lupinus albus* leaf apoplastic proteins in response to boron deficiency " Plant and Soil 279(1-2): 1-11.

6. Amblar M., Barbas A., Fialho A. M. and Arraiano C. M. (2006) "Characterization of the functional domains of *Escherichia coli* RNase II." Journal of Molecular Biology 360(5): 921-933.

7. Andrade J. M., Cairrão F. and Arraiano C. M. (2006) "RNase R affects gene expression in stationary phase: regulation of ompA." Molecular Microbiology 60(1): 219-228.

8. Antonis A. F. G., Bruschke C. J. M., Rueda P., Maranga L., Casal J. I., Vela C., Hilgers L. A. T., Belt P. B. G. M., Weerdmeester K., Carrondo M. J. T. and Langeveld J. P. M. (2006) "A novel recombinant virus-like particle vaccine for prevention of porcine parvovirus-induced reproductive failure." Vaccine 24(26): 5481-5490.

9. Aragao D., Marques A. R., Frazao C., Enguita F. J., Carrondo M. A., Fialho A. M., Sa-Correia I. and Mitchell E. P. (2006) "Cloning, expression, purification, crystallization and preliminary structure determination of glucose-1-phosphate uridylyltransferase (UgpG) from *Sphingomonas elodea* ATCC 31461 bound to glucose-1-phosphate." Acta Crystallographica Section F-Structural Biology and Crystallization Communications 62: 930-934.

10. Balde J. A., Francisco R., Queiroz A., Regalado A. P., Ricardo C. P. and Veloso M. M. (2006) "Immunolocalization of a class III chitinase in two muskmelon cultivars reacting differently to *Fusarium oxysporum* f. sp melonis." Journal of Plant Physiology 163(1): 19-25.

11. Bardaji M., Calhorda M. J., Costa P. J., Jones P. G., Laguna A., Perez M. R. and Villacampa M. D. (2006) "Synthesis, structural characterization, and theoretical studies of gold(I) and gold(I)-gold(III) thiolate complexes: Quenching of gold(I) thiolate luminescence." Inorganic Chemistry 45(3): 1059-1068.

12. Bento I., Carrondo M. A. and Lindley P. F. (2006) "Reduction of dioxygen by enzymes containing copper." Journal of Biological Inorganic Chemistry 11(5): 539-547.

13. Bohn A., Lopes J. R., Diambra L. A. and Menna-Barreto L. S. (2006) "Delay model of circadian gene expression with two negative feedback loops." Biological Rhythm Research 37(5): 405-417.

14. Borges N., Goncalves L. G., Rodrigues M. V., Siopa F., Ventura R., Maycock C., Larnosa P. and Santos H. (2006) "Biosynthetic pathways of inositol and glycerol phosphodiesters used by the hyperthermophile *Archaeoglobus fulgidus* in stress adaptation." Journal of Bacteriology 188(23): 8128-8135.

15. Braga S. S., Gago S., Seixas J. D., Valente A. A., Pillinger M., Santos T. M., Goncalves I. S. and Romao C. C. (2006) "beta-cyclodextrin and permethylated beta-cyclodextrin inclusion compounds of a cyclopentadienyl molybdenum tricarbonyl complex and their use as cyclooctene epoxidation catalyst precursors." Inorganica Chimica Acta 359(15): 4757-4764.

16. Braga S. S., Paz F. A. A., Pillinger M., Seixas J. D., Romao C. C. and Goncalves I. S. (2006) "Structural studies of beta-cyclodextrin and permethylated beta-cyclodextrin inclusion compounds of cyclopentadienyl metal carbonyl complexes." European Journal of Inorganic Chemistry(8): 1662-1669.

17. Bravo M. N., Silva S., Coelho A. V., Boas L. V. and Bronze M. R. (2006) "Analysis of phenolic compounds in Muscatel wines produced in Portugal." Analytica Chimica Acta 563(1-2): 84-92

18. Brennan L., Alves P. M., Hewage C., Malthouse J. P. G. and McBean G. J. (2006) "Impact of the gliotoxin L-serine-O-sulphate on cellular metabolism in cultured rat astrocytes." Neurochemistry International 48(8): 739-745.

19. Brito J. A., Bandeiras T. M., Teixeira M., Vonrhein C. and Archer M. (2006) "Crystallisation and preliminary structure determination of a NADH : quinone oxidoreductase from the extremophile *Acidianus ambivalens*." Biochimica Et Biophysica Acta-Proteins and Proteomics 1764(4): 842-845.

20. Brizio A., Conceicao T., Pimentel M., Da Silva G. and Duarte A. (2006) "High-level expression of IMP-5 carbapenemase owing to point mutation in the -35 promoter region of class 1 integron among *Pseudomonas aeruginosa* clinical isolates." International Journal of Antimicrobial Agents 27(1): 27-31.

21. Burke A. J., Maycock C. D. and Ventura M. R. (2006) "Stereoselective alkylation of tartrate derivatives. A concise route to (+)-O-methylpiscidic acid and natural analogues." Organic & Biomolecular Chemistry 4(12): 2361-2363.

22. Cairrao N. and Arraiano C. M. (2006) “The role of endoribonucleases in the regulation of RNase R.” Biochemical and Biophysical Research Communications 343(3): 731-737.

23. Calhorda M. J., Costa P. J., Martinho P. N., Gimeno M. C., Laguna A., Quintal S. and Villacampa M. D. (2006) “Synthesis and ligand properties towards gold and silver of the ferrocenylamidobenzimidazole ligand.” Journal of Organometallic Chemistry 691(20): 4181-4188.

24. Cantelar E., Sanz-Garcia J. A., Di Paolo R. E., Munoz-Santiuste J. E. and Cusso F. (2006) “On-centered growth of periodically poled LiNbO3 : Er3+/Yb3+ crystals.” Ferroelectrics 334: 125-134.

25. Carmo M., Faria T. Q., Falk H., Coroadinha A. S., Teixeira M., Merten O. W., Geny-Fiamma C., Alves P. M., Danos O., Panet A., Carrondo M. J. T. and Cruz P. E. (2006) “Relationship between retroviral vector membrane and vector stability.” Journal of General Virology 87: 1349-1356.

26. Carrico J. A., Silva-Costa C., Melo-Cristino J., Pinto F. R., de Lencastre H., Almeida J. S. and Ramirez M. (2006) “Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant *Streptococcus pyogenes*.” Journal of Clinical Microbiology 44(7): 2524-2532.

27. Carvalho A. F., Costa-Rodrigues J., Correia I., Pessoa J. C., Faria T. Q., Martins C. L., Fransen M., Sa-Miranda C. and Azevedo J. E. (2006) “The N-terminal half of the peroxisomal cycling receptor Pex5p is a natively unfolded domain.” Journal of Molecular Biology 356(4): 864-875.

28. Carvalho A. S., Almeida R., Magalhaes A., Machado E., Marcos N., David L., Costa L., Costa J., Harduin-Lepers A. and Reis C. A. (2006) “Biosynthesis of sialylated Lewis antigens in human gastric carcinoma cells: Combined role of alpha2,3sialyltransferases and alpha3/4fucosyltransferases.” Glycobiology 16(11): 1124-1124.

29. Carvalho S., Delgado R., Fonseca N. and Félix V. (2006) “Recognition of dicarboxylate anions by a ditopic hexaazamacrocycle containing bis-p-xylyl spacers.” New Journal of Chemistry 30(2): 247-257.

30. Cerdan S., Rodrigues T. B., Sierra A., Benito M., Fonseca L. L., Fonseca C. P. and Garcia-Martin M. L. (2006) “The redox switch/redox coupling hypothesis.” Neurochemistry International 48(6-7): 523-530.

31. Chen Z. J., Liang J. S., Zhang C. H. and Rodrigues C. J. (2006) “Epicatechin and catechin may prevent coffee berry disease by inhibition of appressorial melanization of *Colletotrichum kahawae*.” Biotechnology Letters 28(20): 1637-1640.

32. Cherian S., Reddy M. P. and Ferreira R. B. (2006) “Transgenic plants with improved dehydration-stress tolerance: progress and future prospects.” Biologia Plantarum 50(4): 481-495.

33. Coimbra P., Duarte C. M. M. and de Sousa H. C. (2006) “Cubic equation-of-state correlation of the solubility of some anti-inflammatory drugs in supercritical carbon dioxide.” Fluid Phase Equilibria 239(2): 188-199.

34. Coroadinha A. S., Alves P. M., Santos S. S., Cruz P. E., Merten O. W. and Carrondo M. J. T. (2006) “Retrovirus producer cell line metabolism: implications on viral productivity.” Applied Microbiology and Biotechnology 72(6): 1125-1135.

35. Coroadinha A. S., Ribeiro J., Roldao A., Cruz P. E., Alves P. M., Merten O. W. and Carrondo M. J. T. (2006) “Effect of medium sugar source on the production of retroviral vectors for gene therapy.” Biotechnology and Bioengineering 94(1): 24-36.

36. Coroadinha A. S., Schucht R., Gama-Norton L., Wirth D., Hauser H. and Carrondo M. J. T. (2006) “The use of recombinase mediated cassette exchange in retroviral vector producer cell lines: Predictability and efficiency by transgene exchange.” Journal of Biotechnology 124(2): 457-468.

37. Coroadinha A. S., Silva A. C., Pires E., Coelho A., Alves P. M. and Carrondo M. J. T. (2006) “Effect of osmotic pressure on the production of retroviral vectors: Enhancement in vector stability.” Biotechnology and Bioengineering 94(2): 322-329.

38. Correia A. R., Adinolfi S., Pastore A. and Gomes C. M. (2006) “Conformational stability of human frataxin and effect of Friedreich’s ataxia-related mutations on protein folding.” Biochemical Journal 398: 605-611.

39. Costa J., Empadinhas N., Goncalves L., Lamosa P., Santos H. and da Costa M. S. (2006) “Characterization of the biosynthetic pathway of glucosylglycerate in the archaeon *Methanococcoides burtonii*.” Journal of Bacteriology 188(3): 1022-1030.

40. Costa M. S., Miguel C. and Oliveira M. M. (2006) “An improved selection strategy and the use of acetosyringone in shoot induction medium increase almond transformation efficiency by 100-fold.” Plant Cell Tissue and Organ Culture 85(2): 205-209.

41. Costa P. J., Calhorda M. J., Bossert J., Daniel C. and Romao C. C. (2006) “Photochemistry of methyltrioxorhenium revisited: A DFT/TD-DFT and CASSCF/MS-CASPT2 theoretical study.” Organometallics 25(22): 5235-5241.

42. Costa T., Isidro A. L., Moran C. P. and Henriques A. O. (2006) “Interaction between coat morphogenetic proteins SafA and SpoVID.” Journal of Bacteriology 188(22): 7731-7741.

43. Crisostomo M. I., Vollmer W., Kharat A. S., Inhulsen S., Gehre F., Buckenmaier S. and Tomasz A. (2006) “Attenuation of penicillin resistance in a peptidoglycan O-acetyl transferase mutant of *Streptococcus pneumoniae*.” Molecular Microbiology 61(6): 1497-1509.

44. Cruz P. E., Silva A. C., Roldao A., Carmo M., Carrondo M. J. T. and Alves P. M. (2006) “Screening of Novel Excipients for Improving the Stability of Retroviral and Adenoviral Vectors.” Biotechnology Progress 22(2): 568-576.

Publications 2006

45. De Sanctis D., Ascenzi P., Bocedi A., Dewilde S., Burmester T., Hankeln T., Moens L. and Bolognesi M. (2006) “Cyanide binding and heme cavity conformational transitions in *Drosophila melanogaster* hexacoordinate hemoglobin.” Biochemistry 45(33): 10054-10061.

46. Di Paolo R., Pereira P., Gomes I., Valente F., Pereira I. and Franco R. (2006) “Resonance Raman fingerprinting of multiheme cytochromes from the cytochrome c3 family.” Journal of Biological Inorganic Chemistry 11(2): 217-224.

47. Duarte A. R. C., Casimiro T., Aguiar-Ricardo A., Simplicio A. L. and Duarte C. M. M. (2006) “Supercritical fluid polymerisation and impregnation of molecularly imprinted polymers for drug delivery.” Journal of Supercritical Fluids 39(1): 102-106.

48. Duarte A. R. C., Costa M. S., Simplicio A. L., Cardoso M. M. and Duarte C. M. M. (2006) “Preparation of controlled release microspheres using supercritical fluid technology for delivery of anti-inflammatory drugs.” International Journal of Pharmaceutics 308(1-2): 168-174.

49. Duarte A. R. C., Gordillo M. D., Cardoso M. M., Simplicio A. L. and Duarte C. M. M. (2006) “Preparation of ethyl cellulose/methyl cellulose blends by supercritical antisolvent precipitation.” International Journal of Pharmaceutics 311(1-2): 50-54.

50. Duarte A. R. C., Martins C., Coimbra P., Gil M. H. M., de Sousa H. C. and Duarte C. M. M. (2006) “Sorption and diffusion of dense carbon dioxide in a biocompatible polymer.” Journal of Supercritical Fluids 38(3): 392-398.

51. Duque A. S., Pires A. S., Dos Santos D. M. and Fevereiro P. (2006) “Efficient somatic embryogenesis and plant regeneration from long-term cell suspension cultures of *Medicago truncatula* cv. Jemalong.” In Vitro Cellular & Developmental Biology-Plant 42(3): 270-273.

52. Durao P., Bento I., Fernandes A. T., Melo E. P., Lindley P. F. and Martins L. O. (2006) “Perturbations of the T1 copper site in the CotA laccase from *Bacillus subtilis*: structural, biochemical, enzymatic and stability studies.” Journal of Biological Inorganic Chemistry 11(4): 514-526.

53. Earle M. J., Esperanca J. M. S. S., Gilea M. A., Canongia Lopes J. N., Rebelo L. P. N., Magee J. W., Seddon K. R. and Widegren J. A. (2006) “The distillation and volatility of ionic liquids.” Nature 439(7078): 831-834.

54. Echaniz-Aviles G., Velazquez-Meza M. E., Aires-de-Sousa M., Morfin-Otero R., Rodriguez-Noriega E., Carnalla-Barajas N., Esparza-Ahumada S. and de Lencastre H. (2006) “Molecular characterisation of a dominant methicillin-resistant *Staphylococcus aureus* (MRSA) clone in a Mexican hospital (1999-2003).” Clinical Microbiology and Infection 12(1): 22-28.

55. Enguita F. J., Pohl E., Turner D. L., Santos H. and Carrondo M. A. (2006) “Structural evidence for a proton transfer pathway coupled with haem reduction of cytochrome c ‘’ from *Methylophilus methylotrophus*.” Journal of Biological Inorganic Chemistry 11(2): 189-196.

56. Escrevente C., Machado E., Brito C., Reis C. A., Stoeck A., Runz S., Marme A., Altevogt P. and Costa J. (2006) “Different expression levels of alpha 3/4 fucosyltransferases and Lewis determinants in ovarian carcinoma tissues and cell.” International Journal of Oncology 29(3): 557-566.

57. Esperanca J. M. S. S., Blesic M. and Rebelo L. P. N. (2006) “Densities and derived thermodynamic properties of ionic liquids. 3. Phosphonium-based ionic liquids over an extended pressure range.” Journal of Chemical and Engineering Data 51(1): 237-242.

58. Esperanca J. M. S. S., Visak Z. P., Plechkova N. V., Seddon K. R., Guedes H. J. R. and Rebelo L. P. N. (2006) “Density, speed of sound, and derived thermodynamic properties of ionic liquids over an extended pressure range. 4. [C(3)mim][NTf2] and [C(5)mim][NTf2].” Journal of Chemical and Engineering Data 51(6): 2009-2015.

59. Felix R., Rodrigues R., Machado P., Oliveira S. and Rodrigues-Pousada C. (2006) “A chemotaxis operon in the bacterium *Desulfovibrio gigas* is induced under several growth conditions.” DNA Sequence 17(1): 56-64.

60. Fernandes A. C. and Romao C. C. (2006) “A novel method for the reduction of sulfoxides and pyridine N-oxides with the system silane/MoO2Cl2.” Tetrahedron 62(41): 9650-9654.

61. Fernandes A. C. and Romao C. C. (2006) “Silane/MoO2Cl2 as an efficient system for the reduction of esters.” Journal of Molecular Catalysis a-Chemical 253(1-2): 96-98.

62. Fernandes A. S., Sousa F. L., Teixeira M. and Pereira M. M. (2006) “Electron paramagnetic resonance studies of the iron-sulfur centers from complex I of *Rhodothermus marinus*.” Biochemistry 45(3): 1002-1008.

63. Ferreira R. B., Monteiro S., Freitas R., Santos C. N., Chen Z., Batista L. M., Duarte J., Borges A. and Teixeira A. R. (2006) “Fungal pathogens: the battle for plant infection.” Critical Reviews in Plant Sciences 25(6): 505-524.

64. Fogg M. J., Alzari P., Bahar M., Bertini I., Betton J. M., Burmeister W. P., Cambillau C., Canard B., Carrondo M. A., Coll M., Daenke S., Dym O., Egloff M. P., Enguita F. J., Geerlof A., Haouz A., Jones T. A., Ma Q. J., Manicka S. N., Migliardi M., Nordlund P., Owens R. J., Peleg Y., Schneider G., Schnell R., Stuart D. I., Tarbouriech N., Unge T., Wilkinson A. J., Wilmanns M., Wilson K. S., Zimhony O. and Grimes J. M. (2006) “Application of the use of high-throughput technologies to the determination of protein structures of bacterial and viral pathogens.” Acta Crystallographica Section D-Biological Crystallography 62: 1196-1207.

65. Franco I. S., Mota L. J., Soares C. M. and de Sa-Nogueira I. (2006) “Functional domains of the *Bacillus subtilis* transcription factor AraR and identification of amino acids important for nucleoprotein complex assembly and effector binding.” Journal of Bacteriology 188(8): 3024-3036.

66. Frazao C., McVey C. E., Amblar M., Barbas A., Vonrhein C., Arraiano C. M. and Carrondo M. A. (2006) “Unravelling the dynamics of RNA degradation by ribonuclease II and its RNA-bound complex.” Nature 443(7107): 110-114.

67. Freire P., Amaral J. D., Santos J. M. and Arraiano C. M. (2006) “Adaptation to carbon starvation: RNase III ensures normal expression levels of bolA1p mRNA and sigma(S).” Biochimie 88(3-4): 341-346.

68. Freire P., Vieira H. L. A., Furtado A. R., de Pedro M. A. and Arraiano C. M. (2006) “Effect of the morphogene bolA on the permeability of the *Escherichia coli* outer membrane.” FEMS Microbiology Letters 260(1): 106-111.

69. Gardete S., de Lencastre H. and TomasZ A. (2006) “A link in transcription between the native pbpB and the acquired mecA gene in a strain of *Staphylococcus aureus*.” Microbiology-SGM 152: 2549-2558.

70. Gardete S., Wu S. W., Gill S. and Tomasz A. (2006) “Role of VraSR in antibiotic resistance and antibiotic-induced stress response in *Staphylococcus aureus*.” Antimicrobial Agents and Chemotherapy 50(10): 3424-3434.

71. Ge W. Z. and Li F. (2006) “2,6-Dichlorobenzaldehyde 2,4-dinitrophenyl-hydrazone.” Acta Crystallographica Section E-Structure Reports Online 62: O2888-O2889.

72. Geerlof A., Brown J., Coutard B., Egloff M. P., Enguita F. J., Fogg M. J., Gilbert R. J. C., Groves M. R., Haouz A., Nettleship J. E., Nordlund P., Owens R. J., Ruff M., Sainsbury S., Svergun D. I. and Wilmanns M. (2006) “The impact of protein characterization in structural proteomics.” Acta Crystallographica Section D-Biological Crystallography 62: 1125-1136.

73. Gomes A. R., Westh H. and de Lencastre H. (2006) “Origins and evolution of methicillin-resistant *Staphylococcus aureus* clonal lineages.” Antimicrobial Agents and Chemotherapy 50(10): 3237-3244.

74. Gomes I., Di Paolo R. E., Pereira P. M., Pereira I. A. C., Saraiva L. M., Penades S. and Franco R. (2006) “Spectroelectrochemistry of type II cytochrome c3 on a glycosylated self-assembled monolayer.” Langmuir 22(24): 9809-9811.

75. Gomes R. A., Miranda H. V., Silva M. S., Graca G., Coelho A. V., Ferreira A. E., Cordeiro C. and Freire A. P. (2006) “Yeast protein glycation *in vivo* by methylglyoxal - Molecular modification of glycolytic enzymes and heat shock proteins.” FEBS Journal 273(23): 5273-5287.

76. Goncalves V. L., Nobre L. S., Vicente J. B., Teixeira M. and Saraiva L. M. (2006) “Flavohemoglobin requires microaerophilic conditions for nitrosative protection of *Staphylococcus aureus*.” FEBS Letters 580(7): 1817-1821.

77. Gorynia S., Matias P. M., Goncalves S., Coelho R., Lopes G., Thomaz M., Huber M., Haendler B., Donner P. and Carrondo M. A. (2006) “Expression, purification, crystallization and preliminary X-ray analysis of the human RuvB-like protein RuvBL1.” Acta Crystallographica Section F-Structural Biology and Crystallization Communications 62: 61-66.

78. Grant O. M., Chaves M. M. and Jones H. G. (2006) “Optimizing thermal imaging as a technique for detecting stomatal closure induced by drought stress under greenhouse conditions.” Physiologia Plantarum 127(3): 507-518.

79. Grundmann H., Aires-De-Sousa M., Boyce J. and Tiemersma E. (2006) “Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat.” Lancet 368(9538): 874-885.

80. Guerra K. P., Delgado R., Drew M. G. B. and Felix V. (2006) “Bis- and tris-(3-aminopropyl) derivatives of 14-membered tetraazamacrocycles containing pyridine: synthesis, protonation and complexation studies.” Dalton Transactions(34): 4124-4133.

81. Heckmann A. B., Hebelstrup K. H., Larsen K., Micaelo N. M. and Jensen E. O. (2006) “A single hemoglobin gene in *Myrica gale* retains both symbiotic and non-symbiotic specificity.” Plant Molecular Biology 61(4-5): 769-779.

82. Henriques B. J., Saraiva L. M. and Gomes C. M. (2006) “Combined spectroscopic and calorimetric characterisation of rubredoxin reversible thermal transition.” Journal of Biological Inorganic Chemistry 11(1): 73-81.

83. Justino M. C., Almeida C. C., Goncalves V. L., Teixeira M. and Saraiva L. M. (2006) “*Escherichia coli* YtfE is a di-iron protein with an important function in assembly of iron-sulphur clusters.” FEMS Microbiology Letters 257(2): 278-284.

84. Kodad O., Sanchez A., Saibo N., Alonso J. M., Oliveira M. M. and Company R. S. I. (2006) “Cloning and sequencing of partial genomic DNA fragments corresponding to the S-11 and S-12 alleles of the Spanish almond cultivar ‘Marcona’.” Spanish Journal of Agricultural Research 4(4): 331-335.

85. Kohli A., Gonzalez-Melendi P., Abranches R., Capell T., Stoger E. and Christou P. (2006) “The quest to understand the basis and mechanisms that control expression of introduced transgenes in crop plants.” Plant Signaling & Behavior 1(4): 185 - 195.

86. Kordasti S., Istrate C., Banasaz M., Rottenberg M., Sjovall H., Lundgren O. and Svensson L. (2006) “Rotavirus infection is not associated with small intestinal fluid secretion in the adult mouse.” Journal of Virology 80(22): 11355-11361.

87. Kurz-Besson C., Otieno D., do Vale R. L., Siegwolf R., Schmidt M., Herd A., Nogueira C., David T. S., David J. S., Tenhunen J., Pereira J. S. and Chaves M. (2006) “Hydraulic lift in cork oak trees in a savannah-type Mediterranean ecosystem and its contribution to the local water balance.” Plant and Soil 282(1-2): 361-378.

88. Lachwa J., Bento I., Duarte M. T., Lopes J. N. C. and Rebelo L. P. N. (2006) “Condensed phase behaviour of ionic liquid-benzene mixtures: congruent melting of a [emim][NTf2]center dot C6H6 inclusion crystal.” Chemical Communications(23): 2445-2447.

89. Lachwa J., Morgado P., Esperanca J., Guedes H. J. R., Lopes J. N. C. and Rebelo L. P. N. (2006) “Fluid-phase behavior of {1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl) imide, [C(6)mim][NTf2], plus C-2-C-8 n-alcohol} mixtures: Liquid-liquid equilibrium and excess volumes.” Journal of Chemical and Engineering Data 51(6): 2215-2221.

Publications 2006

90. Lachwa J., Szydlowski J., Makowska A., Seddon K. R., Esperanca J. M. S. S., Guedes H. J. R. and Rebelo L. P. N. (2006) "Changing from an unusual high-temperature demixing to a UCST-type in mixtures of 1-alkyl-3-methylimidazolium bis{(trifluor omethyl)sulfonyl}amide and arenes." Green Chemistry 8(3): 262-267

91. Lameiro M. H., Lopes A., Martins L. O., Alves P. M. and Melo E. (2006) "Incorporation of a model protein into chitosan-bile salt microparticles." International Journal of Pharmaceutics 312(1-2): 119-130.

92. Lameiro M. H., Malpique R., Silva A. C., Alves P. M. and Melo E. (2006) "Encapsulation of adenoviral vectors into chitosan-bile salt microparticles for mucosal vaccination." Journal of Biotechnology 126(2): 152-162.

93. Lamosa P., Goncalves L. G., Rodrigues M. V., Martins L. O., Raven N. D. H. and Santos H. (2006) "Occurrence of 1-glyceryl-1-myo-inosityl phosphate in hyperthermophiles." Applied and Environmental Microbiology 72(9): 6169-6173.

94. Leinonen I., Grant O. M., Tagliavia C. P. P., Chaves M. M. and Jones H. G. (2006) "Estimating stomatal conductance with thermal imagery." Plant Cell and Environment 29(8): 1508-1518.

95. Li F., Delgado R., Coelho A., Drew M. G. B. and Felix V. (2006) "New dioxadiaza-, trioxadiaza- and hexaaza-macrocycles containing dibenzofuran units." Tetrahedron 62(36): 8550-8558.

96. Li F., Delgado R., Drew M. G. B. and Felix V. (2006) "Dioxadiaza- and trioxadiaza-macrocycles containing one dibenzofuran unit selective for cadmium." Dalton Transactions(45): 5396-5403.

97. Lopes J. N. C. and Padua A. A. H. (2006) "Using spectroscopic data on imidazolium cation conformations to test a molecular force field for ionic liquids." B 110(14): 7485-7489.

98. Lopes J. N. C., Gomes M. F. C. and Padua A. A. H. (2006) "Nonpolar, polar, and associating solutes in ionic liquids." Journal of Physical Chemistry B 110(34): 16816-16818.

99. Lopes J. N. C. and Padua A. A. H. (2006) "Molecular force field for ionic liquids III: Imidazolium, pyridinium, and phosphonium cations; Chloride, bromide, and dicyanamide anions." Journal of Physical Chemistry B 110(39): 19586-19592.

100. Lopes M. F. S., Simoes A. P., Tenreiro R., Figueiredo Marques J. J. and Crespo M. T. B. (2006) "Activity and expression of a virulence factor, gelatinase, in fairy enterococci." International Journal of Food Microbiology 112: 208-214.

101. Lopes S. C. D. N., Soares C. M., Baptista A. M., Goormaghtigh E., Costa Cabral B. J. and Castanho M. A. R. B. (2006) "Conformational and orientational guidance of the analgesic dipeptide kyotorphin induced by lipidic membranes: putative correlation toward receptor docking " Journal of Physical Chemistry B 110(7): 3385-3394.

102. Lou J. D., Cai X. X., Li F., Li L. and Gao C. L. (2006) "A new reagent, ferric (III) nitrate supported on graphite, for selective oxidation of alcohols under heterogeneous conditions." Synthesis and Reactivity in Inorganic Metal-Organic and Nano-Metal Chemistry 36(8): 599-601.

103. Lou J. D., Ma Y. C., Gao C. L. and Li L. (2006) "A new efficient oxidation of alcohols with Jones reagent supported on kieselguhr." Synthesis and Reactivity in Inorganic Metal-Organic and Nano-Metal Chemistry 36(5): 381-383.

104. Lou J. D., Pan L. L., Li L., Li F. and Gao C. L. (2006) "Selective oxidation of alcohols with potassium permanganate adsorbed on silica gel under solvent-free conditions." Synthesis and Reactivity in Inorganic Metal-Organic and Nano-Metal Chemistry 36(10): 729-731.

105. Lou J. D., Zhu L. H., Pan L. L., Li L., Li F. and Gao C. L. (2006) "Solvent-free oxidation of secondary alcohols with chromium trioxide." Synthesis and Reactivity in Inorganic Metal-Organic and Nano-Metal Chemistry 36(7): 585-587.

106. Lyashenko A. V., Bento I., Zaitsev V. N., Zhukhlistova N. E., Zhukova Y. N., Gabdoulkhakov A. G., Morgunova F. Y., Voelter W., Kachalova G. S., Stepanova E. V., Koroleva G. V., Lamzin V. S., Tishkov V. I., Betzel C., Lindley P. F. and Mikhailov A. M. (2006) "X-ray structural studies of the fungal laccase from *Cerrena maxima*." Journal of Biological Inorganic Chemistry 11(8): 963-973.

107. Lyashenko A. V., Zhukhlistova N. E., Gabdoulkhakov A. G., Zhukova Y. N., Voelter W., Zaitsev V. N., Bento I., Stepanova E. V., Kachalova G. S., Koroleva O. V., Cherkashyn E. A., Tishkov V. I., Lamzin V. S., Schirwitz K., Morgunova E. Y., Betzel C., Lindley P. F. and Mikhailov A. M. (2006) "Purification, crystallization and preliminary X-ray study of the fungal laccase from *Cerrena maxima*." Acta Crystallographica Section F-Structural Biology and Crystallization Communications 62: 954-957.

108. Machado P., Felix R., Rodrigues R., Oliveira S. and Rodrigues-Pousada C. (2006) "Characterization and expression analysis of the cytochrome bd oxidase operon from *Desulfovibrio gigas*." Current Microbiology 52(4): 274-281.

109. Machuqueiro M. and Baptista A. M. (2006) "Constant-pH Molecular Dynamics with Ionic Strength Effects: Protonation-Conformation Coupling in Decalysine." Journal of Physical Chemistry B 110(6): 2927-2933.

110. Marcelino I., Sousa M. F. Q., Verissimo C., Cunha A. E., Carrondo M. J. T. and Alves P. M. (2006) "Process development for the mass production of *Ehrlichia ruminantium*." Vaccine 24(10): 1716-1725.

111. Marques F., Gano L., Campello M. P., Lacerda S., Santos I., Lima L. M. P., Costa J., Antunes P. and Delgado R. (2006) "13- and 14-membered macrocyclic ligands containing methylcarboxylate or methylphosphonate pendant arms: Chemical and biological evaluation of their Sm-153 and Ho-166 complexes as potential agents for therapy or bone pain palliation." Journal of Inorganic Biochemistry 100(2): 270-280.

112. Martinho P. N., Quintal S., Costa P. J., Losi S., Felix V., Gimeno M. C., Laguna A., Drew M. G. B., Zanello P. and Calhorda M. J. (2006) "New polynuclear Mo-Fe complexes with ferrocenylamidobenzimidazole ligands." European Journal of Inorganic Chemistry(20): 4096-4103.

113. Mas-Marza E., Reis P. M., Peris E. and Royo B. (2006) "Dioxomolybdenum(VI) complexes containing N-heterocyclic carbenes." Journal of Organometallic Chemistry 691(12): 2708-2712.

114. Matias P. M., Gorynia S., Donner P. and Carrondo M. A. (2006) "Crystal structure of the human AAA(+) protein RuvBL1." Journal of Biological Chemistry 281(50): 38918-38929.

115. Matos O. C. and Ricardo C. P. (2006) "Screening of plants against fungi affecting crops and stored foods." in Natural occurring bioactive compounds. (M. K. Rai and C. Carpinella) Advances in Phytomedicine 3: 139-169.

116. McVey C. E., Amblar M., Barbas A., Cairrao F., Coelho R., Romao C., Arraiano C. M., Carrondo M. A. and Frazao C. (2006) "Expression, purification, crystallization and preliminary diffraction data characterization of *Escherichia coli* ribonuclease II (RNase II)." Acta Crystallographica Section F-Structural Biology and Crystallization Communications 62: 684-687.

117. Melo A. M. P., Felix N. A. M., Carita J. N., Saraiva L. M. and Teixeira M. (2006) "The Na^+/H^+ antiporter of the thermohalophilic bacterium *Rhodothermus marinus*." Biochemical and Biophysical Research Communications 348(3): 1011-1017.

118. Melo E. and Martins J. (2006) "Kinetics of bimolecular reactions in model bilayers and biological membranes. A critical review." Biophysical Chemistry 123(2-3): 77-94.

119. Melo M. J. P. d., Dias A. M. A., Blesic M., Rebelo L. P. N., Vega L. F., Coutinho J. A. P. and Marrucho I. M. (2006) "Liquid-liquid equilibrium of (perfluoroalkane plus alkane) binary mixtures." Fluid Phase Equilibria 242(2): 210-219.

120. Messias A. C., Aguiar A. P., Brennan L., Salgueiro C. A., Saraiva L. M., Xavier A. V. and Turner D. L. (2006) "Solution structures of tetrahaem ferricytochrome c(3) from *Desulfovibrio vulgaris* (Hildenborough) and its K45Q mutant: The molecular basis of cooperativity." Biochimica Et Biophysica Acta-Bioenergetics 1757(2): 143-153.

121. Micaelo N. M., Baptista A. M. and Soares C. M. (2006) "Parametrization of 1-butyl-3-methylimidazolium hexafluorophosphate/nitrate ionic liquid for the GROMOS force field." Journal of Physical Chemistry B 110(29): 14444-14451.

122. Miranda J., Bakheit M., Liu Z., Yin H., Mu Y., Guo S., Beyer D., Oliva A., Ahmed J. and Seitzer U. (2006) "Development of a recombinant indirect ELISA for the diagnosis of *Theileria sp.* (China) infection in small ruminants." Parasitology Research 98(6): 561-567.

123. Miranda J., Nascimento E., Cruz H., Hong Y. I. N., Coelho A. N. A., Ahmed J. S. and Oliva A. (2006) "Identification and characterization of Merozoite Antigens of a *Theileria species* highly pathogenic for small ruminants in China." Annals of the New York Academy of Sciences 1081(1): 443-452.

124. Miranda J. P. G., Bakheit M., Schneider I., Haller D., Ahmed J. S., Yin H., Oliva A. G. and Seitzer U. (2006) "Identification of homologous genes of *T. annulata* proteins in the genome of *Theileria sp.* (China)." Annals of the New York Academy of Sciences 1081(1): 468-470.

125. Miranda J. P. G., Nascimento E. M., Cruz H. J., Yin H., Zweygarth E. and Oliva A. G. (2006) "Establishment of optimal conditions for long-term culture of erythrocytic stages of *Theileria uilenbergi*." American Journal of Veterinary Research 67(11): 1908-1913.

126. Monteiro S., Pereira M. A. P., Batista L. M., Loureiro V. B., Teixeira A. R. and Ferreira R. B. (2006) "Electrophoretic analysis of the polypeptide composition during berry development." Vitis 45(3): 149-150.

127. Morais V. A., Brito C., Pijak D. S., Crystal A. S., Fortna R. R., Li T., Wong P. C., Doms R. W. and Costa J. (2006) "N-glycosylation of human nicastrin is required for interaction with the lectins from the secretory pathway calnexin and ERGIC-53." Biochimica/Biophysica Acta-Molecular Basis of Disease 1762(9): 802-810.

128. Mueller L., de Brouwer J., Almeida J., Stal L. and Xavier J. (2006) "Analysis of a marine phototrophic biofilm by confocal laser scanning microscopy using the new image quantification software PHLIP." BMC Ecology 6(1): 1.

129. Nafady A., Costa P. J., Calhorda M. J. and Geiger W. E. (2006) "Electrochemical oxidation of CoCp(CO)(2): Radical-substrate reaction of a 17 e(-)/18 e(-) pair and production of a unique dimer radical." Journal of the American Chemical Society 128(51): 16587-16599.

130. Najdanovic-Visak V., Rodriguez A., Visak Z. P., Rosa J. N., Afonso C. A. M., Ponte M. N. d. and Rebelo L. P. N. (2006) "Liquid Liquid behaviour of functionalized ionic liquids + 2 Propanol + Dichloromethane." Chimica Oggi/CHEMISTRY TODAY 24(6): 19.

131. Neves A. R., Pool W. A., Castro R., Mingote A., Santos F., Kok J., Kuipers O. P. and Santos H. (2006) "The alpha-phosphoglucomutase of *Lactococcus lactis* is unrelated to the alpha-D-phosphohexomutase superfamily and is encoded by the essential gene pgmH." Journal of Biological Chemistry 281(48): 36864-36873.

132. Neves C., Hand P. and Amancio S. (2006) "Patterns of B-type cyclin gene expression during adventitious rooting of micropropagated cork oak." Plant Cell Tissue and Organ Culture 86(3): 367-374.

133. Oliveira D. C., Milheirico C. and de Lencastre H. (2006) "Redefining a structural variant of staphylococcal cassette chromosome mec, SCCmec type VI." Antimicrobial Agents and Chemotherapy 50(10): 3457-3459.

Publications 2006

134. Oliveira D. C., Milheirico C., Vinga S. and de Lencastre H. (2006) “Assessment of allelic variation in the ccrAB locus in methicillin-resistant *Staphylococcus aureus* clones.” Journal of Antimicrobial Chemotherapy 58(1): 23-30.

135. Oliveira L., Henriques A. O. and Tavares P. (2006) “Modulation of the viral ATPase activity by the portal protein correlates with DNA packaging efficiency.” Journal of Biological Chemistry 281(31): 21914-21923.

136. Osorio M., Breia E., Rodrigues A., Osorio J., Le Roux X., Daudet F. A., Ferreira I. and Chaves M. M. (2006) “Limitations to carbon assimilation by mild drought in nectarine trees growing under field conditions.” Environmental and Experimental Botany 55(3): 235-247.

137. Palma A. S., Feizi T., Zhang Y., Stoll M. S., Lawson A. M., Diaz-Rodriguez E., Campanero-Rhodes M. A., Costa J., Gordon S., Brown G. D. and Chai W. (2006) “Ligands for the beta -glucan receptor, dectin-1, assigned using ‘designer’ microarrays of oligosaccharide probes (neoglycolipids) generated from glucan polysaccharides.” Journal of Biological Chemistry 281(9): 5771-5779.

138. Palma P. N., Rodrigues M. L., Archer M., Bonifacio M. J., Loureiro A. I., Learmonth D. A., Carrondo M. A. and Soares-da-Silva P. (2006) “Comparative study of ortho- and meta-nitrated inhibitors of catechol-O-methyltransferase: Interactions with the active site and regioselectivity of O-methylation.” Molecular Pharmacology 70(1): 143-153.

139. Passarinho J. A. P., Lamosa P., Baeta J. P., Santos H. and Ricardo C. P. P. (2006) “Annual changes in the concentration of minerals and organic compounds of *Quercus suber* leaves.” Physiologia Plantarum 127(1): 100-110.

140. Peixoto C., Ferreira T. B., Carrondo M. J. T., Cruz P. E. and Alves P. M. (2006) “Purification of adenoviral vectors using expanded bed chromatography.” Journal of Virological Methods 132(1-2): 121-126.

141. Pereira C. C. L., Costa P. J., Calhorda M. J., Freire C., Rodrigues S. S., Herdtweck E. and Romao C. C. (2006) “Ring slippage vs charge transfer in the reductive chemistry of [IndMo(CO)(2)(alpha-diimine)](+) cations.” Organometallics 25(22): 5223-5234.

142. Pereira C. S., Soares G. A. M., Oliveira A. C., Rosa M. E., Pereira H., Moreno N. and Romao M. V. S. (2006) “Effect of fungal colonization on mechanical performance of cork.” International Biodeterioration & Biodegradation 57(4): 244-250.

143. Pereira M. M., Sousa F. L., Teixeira M., Nyquist R. M. and Heberle J. (2006) “A tyrosine residue deprotonates during oxygen reduction by the caa3 reductase from *Rhodothermus marinus*.” FEBS Letters 580(5): 1350-1354.

144. Pereira P. M., Teixeira M., Xavier A. V., Louro R. O. and Pereira I. A. C. (2006) “The Tmc complex from *Desulfovibrio vulgaris* hildenborough is involved in transmembrane electron transfer from periplasmic hydrogen oxidation.” Biochemistry 45(34): 10359-10367.

145. Pessanha M., Morgado L., Louro R. O., Londer Y. Y., Pokkuluri P. R., Schiffer M. and Salgueiro C. A. (2006) “Thermodynamic characterization of triheme cytochrome PpcA from *Geobacter sulfurreducens*: Evidence for a role played in e(-)/H+ energy transduction.” Biochemistry 45(46): 13910-13917.

146. Petrovski Z., Valente A. A., Pillinger M., Dias A. S., Rodrigues S. S., Romao C. C. and Goncalves I. S. (2006) “Molybdenum(VI) oxides bearing 1,4,7-triazacyclononane and 1,1,1-tris(aminomethyl)ethane ligands: Synthesis and catalytic applications.” Journal of Molecular Catalysis a-Chemical 249(1-2): 166-171.

147. Pires R. H., Venceslau S. S., Morais F., Teixeira M., Xavier A. V. and Pereira I. A. C. (2006) “Characterization of the *Desulfovibrio desulfuricans* ATCC 27774 DsrMKJOP complex - A membrane-bound redox complex involved in the sulfate respiratory pathway.” Biochemistry 45(1): 249-262.

148. Pool W. A., Neves A. R., Kok J., Santos H. and Kuipers O. P. (2006) “Natural sweetening of food products by engineering *Lactococcus lactis* for glucose production.” Metabolic Engineering 8(5): 456-464.

149. Prado M. A., Boas L. E. V., Bronze M. R. and Godoy H. T. (2006) “Validation of methodology for simultaneous determination of synthetic dyes in alcoholic beverages by capillary electrophoresis.” Journal of Chromatography A 1136(2): 231-236.

150. Prinz M., Veiros L. F., Calhorda M. J., Romao C. C., Herdtweck E., Kuhn F. E. and Herrmann W. A. (2006) “Structural preferences of cyclopentadienyl and indenyl rings in iridium(I) carbene complexes.” Journal of Organometallic Chemistry 691(21): 4446-4458.

151. Prosinecki V., Botelho H. M., Francese S., Mastrobuoni G., Moneti G., Urich T., Kletzin A. and Gomes C. M. (2006) “A proteomic approach toward the selection of proteins with enhanced intrinsic conformational stability.” Journal of Proteome Research 5(10): 2720-2726.

152. Real G. and Henriques A. O. (2006) “Localization of the *Bacillus subtilis* murB gene within the dcw cluster is important for growth and sporulation.” Journal of Bacteriology 188(5): 1721-1732.

153. Reis P. M., Romao C. C. and Royo B. (2006) “Dioxomolybdenum(VI) complexes as catalysts for the hydrosilylation of aldehydes and ketones.” Dalton Transactions(15): 1842-1846.

154. Ribeiro J. M., Pereira C. S., Soares N. C., Vieira A. M., Feijo J. A. and Jackson P. A. (2006) “The contribution of extensin network formation to rapid, hydrogen peroxide-mediated increases in grapevine callus wall resistance to fungal lytic enzymes.” Journal of Experimental Botany 57(9): 2025-2035.

155. Rocha R., Leal S. S., Teixeira V. H., Regalla M., Huber H., Baptista A. M., Soares C. M. and Gomes C. M. (2006) “Natural domain design: Enhanced thermal stability of a zinc-lacking ferredoxin isoform shows that a hydrophobic core efficiently replaces the structural metal site.” Biochemistry 45(34): 10376-10384.

156. Rodrigues J. V., Abreu I. A., Cabelli D. and Teixeira M. (2006) “Superoxide reduction mechanism of *Archaeoglobus fulgidus* one-iron superoxide reductase.” Biochemistry 45(30): 9266-9278.

157. Rodrigues M. L., Archer M., Martel P., Miranda S., Thomaz M., Enguita F. J., Baptista R. P., Melo E. P., Sousa N., Cravador A. and Carrondo M. A. (2006) “Crystal structures of the free and sterol-bound forms of beta-cinnamomin.” Biochimica Et Biophysica Acta-Proteins and Proteomics 1764(1): 110-121.

158. Rodrigues M. L., Oliveira T., Matias P. M., Martins I. C., Valente F. M. A., Pereira I. A. C. and Archer M. (2006) “Crystallization and preliminary structure determination of the membrane-bound complex cytochrome c nitrite reductase from *Desulfovibrio vulgaris* Hildenborough.” Acta Crystallographica Section F-Structural Biology and Crystallization Communications 62: 565-568.

159. Rodrigues M. L., Oliveira T. F., Pereira I. A. C. and Archer M. (2006) “X-ray structure of the membrane-bound cytochrome c quinol dehydrogenase NrfH reveals novel haem coordination.” EMBO Journal 25(24): 5951-5960.

160. Rodrigues R., Vautier-Giongo C., Silva P. F., Fernandes A. C., Cruz R., Macanita A. L. and Quina F. H. (2006) “Geminate proton recombination at the surface of SDS and CTAC micelles probed with a micelle-anchored anthocyanin.” Langmuir 22(3): 933-940.

161. Rodrigues R., Vicente J. B., Felix R., Oliveira S., Teixeira M. and Rodrigues-Pousada C. (2006) “*Desulfovibrio gigas* flavodiiron protein affords protection against nitrosative stress in vivo.” Journal of Bacteriology 188(8): 2745-2751.

162. Rodrigues T., Carvalho A., Roldao A., Carrondo M. J. T., Alves P. M. and Cruz P. E. (2006) “Screening anion-exchange chromatographic matrices for isolation of onco-retroviral vectors.” Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 837(1-2): 59-68.

163. Rodriguez-Diaz J., Banasaz M., Istrate C., Buesa J., Lundgren O., Espinoza F., Sundqvist T., Rottenberg M. and Svenssons L. (2006) “Role of nitric oxide during rotavirus infection.” Journal of Medical Virology 78(7): 979-985.

164. Roldao A., Vieira H. L. A., Alves P. M., Oliveira R. and Carrondo M. J. T. (2006) “Intracellular dynamics in rotavirus-like particles production: evaluation of multigene and monocistronic infection strategies.” Process Biochemistry 41(10): 2188-2199.

165. Roxo-Rosa M., da Costa G., Luider T. M., Scholte B. J., Coelho A. V., Amaral M. D. and Penque D. (2006) “Proteomic analysis of nasal cells from cystic fibrosis patients and non-cystic fibrosis control individuals: Search for novel biomarkers of cystic fibrosis lung disease.” Proteomics 6(7): 2314-2325.

166. Roxo-Rosa M., Xu Z., Schmidt A., Neto M., Cai Z. W., Soares C. M., Sheppard D. N. and Amaral M. D. (2006) “Revertant mutants G550E and 4RK rescue cystic fibrosis mutants in the first nucleotide-binding domain of CFTR by different mechanisms.” Proceedings of the National Academy of Sciences of the United States of America 103(47): 17891-17896.

167. Sá Santos S., Gibson G. E., Cooper A. J. L., Denton T. T., Thompson C. M., Bunik V. I., Alves P. M. and Sonnewald U. (2006) “Inhibitors of the a-ketoglutarate dehydrogenase complex alter [1-13C]glucose and [U-13C]glutamate metabolism in cerebellar granule neurons.” Journal of Neuroscience Research 83(3): 450-458.

168. Sá-Leao R., Simoes A. S., Nunes S., Sousa N. G., Frazao N. and de Lencastre H. (2006) “Identification, prevalence and population structure of non-typable *Streptococcus pneumoniae* in carriage samples isolated from preschoolers attending day-care centres.” Microbiology-SGM 152(2): 367-376.

169. Sampaio de Sousa A. R., Calderone M., Rodier E., Fages J. and Duarte C. M. M. (2006) “Solubility of carbon dioxide in three lipid-based biocarriers.” Journal of Supercritical Fluids 39(1): 13-19.

170. Santos A. P., Wegel E., Allen G. C., Thompson W. F., Stoger E., Shaw P. and Abranches R. (2006) “*In situ* methods to localize transgenes and transcripts in interphase nuclei: a tool for transgenic plant research.” Plant Methods 2(18): 18-.

171. Santos C., Gaspar M., Caeiro A., Branco-Price C., Teixeira A. and Ferreira R. B. (2006) “Exposure of *Lemna minor* to arsenite: Expression levels of the components and intermediates of the ubiquitin/proteasome pathway.” Plant and Cell Physiology 47(9): 1262-1273.

172. Santos H., Lamosa P. and Borges N. (2006) “Characterization and quantification of compatible solutes in (hyper)thermophilic microorganisms.” in Extremophiles Methods in Microbiology 35: 173-199.

173. Santos J. M., Freire P., Mesquita F. S., Mika F., Hengge R. and Arraiano C. M. (2006) “Poly(A)-polymerase I links transcription with mRNA degradation via 3C3S proteolysis.” Molecular Microbiology 60(1): 177-188.

174. Schucht R., Coroadinha A. S., Zanta-Boussif M. A., Verhoeyen E., Carrondo M. J. T., Hauser H. and Wirth D. (2006) “A new generation of retroviral producer cells: Predictable and stable virus production by Flp-mediated site-specific integration of retroviral vectors.” Molecular Therapy 14(2): 285-292.

175. Schweitzer B., Felippe A. C., Dal Bo A., Minatti E., Zanette D. and Lopes A. (2006) “Sodium dodecyl sulfate promoting a cooperative association process of sodium cholate with bovine serum albumin.” Journal of Colloid and Interface Science 298(1): 457-466.

Publications 2006

176. Serafim L. S., Lemos P. C., Rossetti S., Levantesi C., Tandoi V. and Reis M. A. M. (2006) “Microbial community analysis with a high PHA storage capacity.” Water Science and Technology 54(1): 183-188.

177. Shvaleva A. L., Silva F. C. E., Breia E., Jouve L., Hausman J. F., Almeida M. H., Maroco J. P., Rodrigues M. L., Pereira J. S. and Chaves M. M. (2006) “Metabolic responses to water deficit in two Eucalyptus globulus clones with contrasting drought sensitivity.” Tree Physiology 26(2): 239-248.

178. Sillero J. C., Fondevilla S., Davidson J., Vaz Patto M. C., Warkentin T. D., Thomas J. and Rubiales D. (2006) “Screening techniques and sources of resistance to rusts and mildews in grain legumes.” Euphytica 147(1-2): 255-272.

179. Silva S., Gomes L., Leitao F., Coelho A. V. and Boas L. V. (2006) “Phenolic compounds and antioxidant activity of *Olea europaea* L. fruits and leaves.” Food Science and Technology International 12(5): 385-395.

180. Silvestre O. F., Silva M. G., Oliva A. G. and Cruz H. J. (2006) “Spherical vs. granular immobilization support selection and performance on an optical flow cell immunosensor based on the fluorescence of cyanine-5.” Preparative Biochemistry & Biotechnology 36(4): 333-353.

181. Simplicio A. L., Matias P., Gilmer J. F. and Clancy J. M. (2006) “Chiral separation and identification of beta-aminoketones of pharmacological interest by high performance liquid chromatography and capillary electrophoresis.” Journal of Chromatography A 1120(1-2): 89-93.

182. Sobral R. G., Ludovice A. M., de Lencastre H. and Tomasz A. (2006) “Role of murF in cell wall biosynthesis: Isolation and characterization of a murF conditional mutant of *Staphylococcus aureus*.” Journal of Bacteriology 188(7): 2543-2553.

183. Sousa A. R. S. d., Calderone M., Rodier E., Fages J. and Duarte C. M. M. (2006) “Solubility of carbon dioxide in three lipid-based biocarriers.” Journal of Supercritical Fluids 39(1): 13-19.

184. Tam N. M. K., Uyen N. Q., Hong H. A., Duc L. H., Hoa T. T., Serra C., Henriques A. O. and Cutting S. M. (2006) “The intestinal life cycle of *Bacillus subtilis* and close relatives.” Journal of Bacteriology 188(7): 2692-2700.

185. Teixeira A., Clemente J. J., Cunha A. E., Carrondo M. J. T. and Oliveira R. (2006) “Bioprocess interative batch-to-batch optimization based on hybrid parametric/nonparametric models.” Biotechnology Progress 22(1): 247-258.

186. Teixeira D. M., Patao R. F., Coelho A. V. and da Costa C. T. (2006) “Comparison between sample disruption methods and solid-liquid extraction (SLE) to extract phenolic compounds from *Ficus carica* leaves.” Journal of Chromatography A 1103(1): 22-28.

187. Teixeira F. C., Ramos H., Antunes I. F., Curto M. J. M., Duarte M. T. and Bento I. (2006) “Synthesis and structural characterization of 1-and 2-substituted indazoles: Ester and carboxylic acid derivatives.” Molecules 11(11): 867-889.

188. Teixeira V. H., Baptista A. M. and Soares C. M. (2006) “Pathways of H-2 toward the active site of [NiFe]-hydrogenase.” Biophysical Journal 91(6): 2035-2045.

189. Tereso S., Goncalves S., Marum L., Oliveira M., Maroco J. and Miguel C. (2006) “Improved axillary and adventitious bud regeneration from Portuguese genotypes of *Pinus pinaster* Ait.” Propagation of Ornamental Plants 6(1): 24-33.

190. Tereso S., Miguel C., Maroco J. and Oliveira M. M. (2006) “Susceptibility of embryogenic and organogenic tissues of maritime pine (*Pinus pinaster*) to antibiotics used in Agrobacterium-mediated genetic transformation.” Plant Cell Tissue and Organ Culture 87(1): 33-40.

191. Tereso S., Miguel C., Zoglauer K., Valle-Piquera C. and Oliveira M. M. (2006) “Stable Agrobacterium-mediated transformation of embryogenic tissues from *Pinus pinaster* Portuguese genotypes.” Plant Growth Regulation 50(1): 57-68.

192. Thompson L. S., Beech P. L., Real G., Henriques A. O. and Harry E. J. (2006) “Requirement for the cell division protein DivIB in polar cell division and engulfment during sporulation in *Bacillus subtilis*.” Journal of Bacteriology 188(21): 7677-7685.

193. Todorovic S., Jung C., Hildebrandt P. and Murgida D. H. (2006) “Conformational transitions and redox potential shifts of cytochrome P450 induced by immobilization.” Journal of Biological Inorganic Chemistry 11(1): 119-127.

194. Troncoso J., Cerdeirina C. A., Sanmamed Y. A., Romani L. and Rebelo L. P. N. (2006) “Thermodynamic properties of imidazolium-based ionic liquids: Densities, heat capacities, and enthalpies of fusion of [bmim][PF6] and [bmim][NTf2].” Journal of Chemical and Engineering Data 51(5): 1856-1859.

195. Urich T., Gomes C. M., Kletzin A. and Frazao C. (2006) “X-ray structure of a self-compartmentalizing sulfur cycle metalloenzyme.” Science 311(5763): 996-1000.

196. Valente F. A. A., Almeida C. C., Pacheco I., Carita J., Saraiva L. M. and Pereira I. A. C. (2006) “Selenium is involved in regulation of periplasmic hydrogenase gene expression in *Desulfovibrio vulgaris* Hildenborough.” Journal of Bacteriology 188(9): 3228-3235.

197. Vaz Patto M. C., Fernandez-Aparicio M., Moral A. and Rubiales D. (2006) “Characterization of resistance to powdery mildew (Erysiphe pisi) in a germplasm collection of *Lathyrus sativus*.” Plant Breeding 125(3): 308-310.

198. Vaz Patto M. C., Skiba B., Pang E. C. K., Ochatt S. J., Lambein F. and Rubiales D. (2006) “*Lathyrus* improvement for resistance against biotic and abiotic stresses: From classical breeding to marker assisted selection.” Euphytica 147(1-2): 133-147.

199. Veiros L. F., Prazeres A., Costa P. J., Romao C. C., Kuhn F. E. and Calhorda M. J. (2006) “Olefin epoxidation with tert-butyl hydroperoxide catalyzed by MoO2X2L complexes: a DFT mechanistic study.” Dalton Transactions 11: 1383-1389.

200. Vieira H. L. A., Pereira A. C. P., Carrondo M. J. T. and Alves P. M. (2006) “Catalase effect on cell death for the improvement of recombinant protein production in baculovirus-insect cell system.” Bioprocess and Biosystems Engineering 29(5-6): 409-414.

201. Visak Z. P., Szydlowski J. and Rebelo L. P. N. (2006) “A simple quantum statistical thermodynamics interpretation of an impressive phase diagram pressure shift upon (H/D) isotopic substitution in water+3-methylpyridine.” Journal of Physical Chemistry B 110(3): 1377-1387.

202. Voit E., Neves A. R. and Santos H. (2006) “The intricate side of systems biology.” Proceedings of the National Academy of Sciences of the United States of America 103(25): 9452-9457.

203. Voit E. O., Almeida J., Marino S., Lall R., Goel G., Neves A. R. and Santos H. (2006) “Regulation of glycolysis in *Lactococcus lactis*: an unfinished systems biological case study.” Iee Proceedings Systems Biology 153(4): 286-298.

204. Wang L. H., Weber A. N., Atilano M. L., Filipe S. R., Gay N. J. and Ligoxygakis P. (2006) “Sensing of Gram-positive bacteria in *Drosophila*: GNBP1 is needed to process and present peptidoglycan to PGRP-SA.” EMBO Journal 25(20): 5005-5014.

205. Zanette D., Felippe A. C., Schweitzer B., Dal Bo A. and Lopes A. (2006) “The absence of cooperative binding in mixtures of sodium cholate and poly(ethylene oxide) as indicated by surface tension, steady-state fluorescence and electrical conductivity measurements.” Colloids and Surfaces a-Physicochemical and Engineering Aspects 279(1-3): 87-95.

206. Zhang C. H., Ferreira T. B., Cruz P. E., Alves P. M., Haury M. and Carrondo M. J. T. (2006) “The importance of 293 cell cycle phase on adenovirus vector production.” Enzyme and Microbial Technology 39(6): 1328-1332.

207. Zuber B., Haenni M., Ribeiro T., Minnig K., Lopes F., Moreillon P. and Dubochet J. (2006) “Granular layer in the periplasmic space of gram-positive bacteria and fine structures of *Enterococcus gallinarum* and *Streptococcus gordonii* septa revealed by cryo-electron microscopy of vitreous sections.” Journal of Bacteriology 188(18): 6652-6660.

Patents 2006

Method for treating a mammal by administration of a compound having the ability to release CO and pharmaceutical compositions thereof.
Haas W., Romão C., Royo B., Fernandes A. C., Gonçalves I. (2006) US 7,011,854 (property of Alfama)

Method for treating a mammal by administration of a compound having the ability to release CO and pharmaceutical compositions thereof.
Haas W., Romão C., Royo B., Fernandes A. C., Gonçalves I. (2006) "US 2006/0148900 A1 (property of Alfama)

Method for treating a mammal by administration of a compound having the ability to release CO.
Haas W., Romão C., Royo B., Fernandes A. C., Gonçalves I. (2006) US 2006/0233890 A1 (property of Alfama)

Derivados sintéticos de manosilglicerato para a estabilização e/ou preservação de biomateriais.
P. Lamosa, T. Q. Faria, M. R. Ventura, C. D. Maycock & H. Santos. Patente PAT 103442 P; 2006/02/24. (Submitted by: STAB VIDA, Lda.)

Utilização de uma proteína de plantas do género Lupinus como bioestimulante do crescimento e desenvolvimento de plantas e na formulação de preparados proteicos para alimentação humana e/ ou animal. R.B. Ferreira, S. Monteiro,V. Loureiro, and A. Teixeira (2006).N.P.I. application nº PT103511

Protein extracted from plants of the genus Lupinus or produced in recombinant form, nucleotide sequence encoding it and its use in animal nutrition, as a plant growth promoter and in the fight against pathogenic fungi.
R.B. Ferreira, S. Monteiro,V. Loureiro, and A. Teixeira (2006) International Bureau of the World Intellectual Property Organization, publication nº WO2007010459.

Methods for preparing sustained-release therapeutic ophthalmic articles using compressed fluids for impregnation of drugsH.
C. de Sousa, M.H.M. Gil, E.O.B. Leite, C.M.M. Duarte, A.R.C. Duarte, application 05398006 registered at 27/06/2005, publication at European Patent Bulletin on January 4, 2006. EP Patent EP1611877A1.

Method for preparing therapeutic ophthalmic articles using compressed fluids.
H.C. de Sousa, M.H.M. Gil, E.O.B. Leite, C.M.M. Duarte, A.R.C. Duarte, application 11/167083 registered at 28/06/2005, publication on January 12, 2006. US Patent US20060008506A1

Method for preparing therapeutic ophthalmic articles using compressed fluids.
H.C. de Sousa, M.H.M. Gil, E.O.B. Leite, C.M.M. Duarte, A.R.C. Duarte, application 05002937 registered at 28/06/2005, publication on December 28, 2006. CA Patent CA2510960A1

Submitted:

Method of obtaining a natural Hydrioxityrosol-rich concentrate from olive tree residues and sub-products using clean technologies.
International patent request, July 2006, PCT/IB/2006/052552. (Catarina Duarte)

Pastas de Azeitona-Fórmulas INIAP
Industrial Rights Registration (in course) (Cidália Peres)

Publications 2007

Published or in press as of Feb. 2007

1. Abrantes M., Balula M. S., Valente A. A., Paz F. A. A., Pillinger M., Romao C. C., Rocha J. and Goncalves I. S. (2007). "Structural and catalytic studies of a trimethyltin vanadate coordination polymer." Journal of Inorganic and Organometallic Polymers and Materials 17(1): 215-222.
2. Almeida A. M., Cardoso L. A., Santos D., Torné J. M. and Fevereiro P. S. (2007 in press). "Trehalose and its applications in plant biotechnology." In Vitro Cellular and Developmental Biology - Plant
3. Almeida A. M., Santos M., Villalobos E., Araujo S. S., van Dijck P., Leyman B., Cardoso L. A., Santos D., Fevereiro P. S. and Torne J. M. (2007). "Immunogold localization of trehalose-6-phosphate synthase in leaf segments of wild-type and transgenic tobacco plants expressing the AtTPS1 gene from Arabidopsis thaliana." Protoplasma 230(1-2): 41-49.
4. Almeida A. M., Silva A. B., Araujo S. S., Cardoso L. A., Santos D. M., Torne J. M., Silva J. M., Paul M. J. and Fevereiro P. S. (2007). "Responses to water withdrawal of tobacco plants genetically engineered with the AtTPS1 gene: a special reference to photosynthetic parameters." Euphytica 154(1-2): 113-126.
5. Amblar M., Barbas A., Gomez-Puertas P. and Arraiano C. M. (2007). "The role of the S1 domain in exoribonucleolytic activity: Substrate specificity and multimerization." Rna-a Publication of the Rna Society 13(3): 317-327.
6. Andrade J., Karmali A., Carrondo M. A. and Frazao C. (2007). "Crystallization, diffraction data collection and preliminary crystallographic analysis of hexagonal crystals of Pseudomonas aeruginosa amidase." Acta Crystallographica Section F-Structural Biology and Crystallization Communications 63: 214-216.
7. Antunes P., Delgado R., Drew M. G. B., Felix V. and Maecke H. (2007). "Copper complexes of new benzodioxotetraaza macrocycles with potential applications in nuclear medicine." Inorganic Chemistry 46(8): 3144-3153.
8. Araújo R., Silva C., O'Neill A., Micaelo N., Guebitz G., Soares C. M., Casal M. and Cavaco-Paulo A. (2007 in press). "Tailoring cutinase activity towards polyethylene terephthalate and polyamide 6.6 fibers." Journal of Biotechnology.
9. Azevedo D., Nascimento L., Labarre J., Toledano M. B. and Rodrigues-Pousada C. (2007). "The S-cerevisiae Yap1 and Yap2 transcription factors share a common cadmium-sensing domain." FEBS Letters 581(2): 187-195.
10. Batista R., Martins I., Jenö P., Ricardo C. P. and Oliveira M. M. (2007 in press). "A proteomic study to identify soya allergens- The human response to transgenic vs. non-transgenic soya samples." International Archives of Allergy and Immunology.
11. Bejerano-Sagie M. and Xavier K. B. (2007). "The role of small RNAs in quorum sensing." Current Opinion in Microbiology 10(2): 189-198.
12. Bento I., Peixoto C., Zaitsev V. N. and Lindley P. F. (2007). "Ceruloplasmin revisited: structural and functional roles of various metal cation-binding sites." Acta Crystallographica Section D-Biological Crystallography 63: 240-248.
13. Blesic M., Marques M. H., Plechkova N. V., Seddon K. R., Rebelo L. P. N. and Lopes A. (2007). "Self-aggregation of ionic liquids: micelle formation in aqueous solution." Green Chemistry 9: 1-11.
14. Brito C., Barata B., Escrevente C., Reis C., Lee V. M.-Y., Trojanowski J. Q. and Costa J. (2007 in press). "Increased levels of fucosyltransferase IX and carbohydrate Lewisx adhesion determinant in human NT2N neurons." Journal of Neuroscience Research.
15. Cardoso F. S., Castro R. F., Borges N. and Santos H. (2007). "Biochemical and genetic characterization of the pathways for trehalose metabolism in Propionibacterium freudenreichii, and their role in stress response." Microbiology-SGM 153: 270-280.
16. Chaves M. M., Santos T. P., Souza C. R., Ortuno M. F., Rodrigues M. L., Lopes C. M., Maroco J. P. and Pereira J. S. (2007). "Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality." Annals of Applied Biology 150(2): 237-252.
17. Chen Z. J., Franco C. F., Baptista R. P., Cabral J. M. S., Coelho A. V., Rodrigues C. J. and Melo E. P. (2007). "Purification and identification of cutinases from Colletotrichum kahawae and Colletotrichum gloeosporioides." Applied Microbiology and Biotechnology 73(6): 1306-1313.
18. Costa M. S., Duarte A. R. C., Cardoso M. M. and Duarte C. M. M. (2007). "Supercritical antisolvent precipitation of PHBV microparticles." International Journal of Pharmaceutics 328(1): 72-77.
19. Costa P. M., Romão C. C., Fernandes A. C., Royo B., Reis P. M. and Calhorda M. J. (2007). "Catalyzing Aldehyde Hydrosilylation with a Molybdenum(VI) Complex: A Density Functional Theory Study." Chemistry - A European Journal 13(14): 3934-3941.
20. Costa T., Serrano M., Steil L., Volker U., Moran C. P. and Henriques A. O. (2007). "The timing of cotE expression affects Bacillus subtilis spore coat morphology but not lysozyme resistance." Journal of Bacteriology 189(6): 2401-2410.
21. Cravo C., Duarte A. R. C. and Duarte C. M. M. (2007). "Solubility of carbon dioxide in a natural biodegradable polymer: Determination of diffusion coefficients." Journal of Supercritical Fluids 40(2): 194-199.
22. Cruz C., Delgado R., Drew M. G. B. and Felix V. (2007 in press). "Evaluation of the Binding Ability of a Novel Dioxatetraazamacrocyclic Receptor that Contains Two Phenanthroline Units: Selective Uptake of Carboxylate Anions." Journal of Organic Chemistry.
23. David T. S., Henriques M. O., Kurz-Besson C., Nunes J., Valente F., Vaz M., S. P. J., Siegwolf R., Chaves M. M., Gazarin L. C. and David J. S. (2007). "Water use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought." Tree Physiology 27(5): 793-803.

Publications 2007

24. Delgado A., Arroyo Lopez F. N., Brito D., Peres C., Fevereiro P. and Garrido-Fernandez A. (2007 in press). “Optimum bacteriocin production by Lactobacillus plantarum 17.2b requires absence of NaCl and apparently follows a mixed metabolite kinetics.” Journal of Biotechnology.

25. Duarte A. R. C., Roy C., Vega-Gonzalez A., Duarte C. M. M. and Subra-Patemault P. (2007). “Preparation of acetazolamide composite microparticles by supercritical anti-solvent techniques.” International Journal of Pharmaceutics 332(1-2): 132-139.

26. Duarte A. R. C., Simplicio A. L., Vega-Gonzalez A., Subra-Patemault P., Coimbra P., Gil M. H., Sousa H. C. and Duarte C. M. M. (2007 in press). “Impregnation of intraocular lens for opthalmic drug delivery.” Journal of Supercritical Fluids.

27. Duarte A. R. C., Simplicio A. L., Vega-Gonzalez A., Subra-Paternault P., Coimbra P., Gil M. H., Sousa H. C. and Duarte C. M. M. (2007 in press). “Supercritical fluid impregnation of a biocompatible polymer for ophthalmic drug delivery.” Journal of Supercritical Fluids.

28. Duque A. S., Araújo S. S., Cordeiro M. A., Santos D. M. and Fevereiro P. S. (2007 in press). “Use of fused gfp and gus reporters for the recovery of transformed Medicago truncatula somatic embryos without selective pressure.” Plant Cell Tissue and Organ Culture.

29. Egorova K., Grudieva T., Morinez C., Kube J., Santos H., Costa M. and Antranikian G. (2007 in press). “High yield of mannosylglycerate production by upshock fermentation and bacterial milking of trehalose-deficient mutant Thermus thermophilus RQ-1.” Applied Microbiology and Biotechnology.

30. Fernandes A. C. and Romao C. C. (2007 in press). “Reduction of amides with silanes catalyzed by MoO2Cl2.” Journal of Molecular Catalysis A: Chemical.

31. Fernandes A. T., Soares C. M., Pereira M. M., Huber R., Grass G. and Martins L. O. (2007 in press). “A robust metallo-oxidase from the hyperthermophilic bacterium Aquifex aeolicus.” FEBS Journal.

32. Fernandes J., Ruivo R. and Simoes P. (2007). “Dynamic model of a supercritical fluid extraction plant.” Aiche Journal 53(4): 825-837.

33. Ferreira T. B., Carrondo M. J. T. and Alves P. M. (2007). “Effect of ammonia production on intracellular pH: consequent effect on adenovirus vector production.” Journal of Biotechnology 129(3): 433-438.

34. Franklin G., Oliveira M. and Dias A. C. P. (2007 in press). “Production of transgenic Hypericum perforatum plants via particle bombardment-mediated transformation of novel organogenic cell suspension cultures.” Plant Science.

35. Freitas R. L., Teixeira A. R. and Ferreira R. B. (2007). “Vicilin-type globulins follow distinct patterns of degradation in different species of germinating legume seeds.” Food Chemistry 102(1): 323-329.

36. Gouveia R. M., Morais V. A., Peixoto C., Sousa M., Regalla M., Alves P. M. and Costa J. (2007). “Production and purification of functional truncated soluble forms of human recombinant L1 cell adhesion glycoprotein from Spodoptera frugiperda Sf9 cells.” Protein Expression and Purification 52(1): 182-193.

37. Grant O. M., Tronina L., Jones H. G. and Chaves M. M. (2007). “Exploring thermal imaging variables for the detection of stress responses in grapevine under different irrigation regimes.” Journal of Experimental Botany 58(4): 815-825.

38. Honzícek J., Paz F. A. A. and Romão C. C. (2007 in press). “Synthesis, characterization and stability of spirodiene complexes of molybdenum(II): New route to ansa-molybdenocene and ring functionalized molybdenocene compounds.” Journal of Molecular Catalysis A: Chemical.

39. Jayamani P., Negrao S., Brites C. and Oliveira M. M. (2007 in press). “Potential of Waxy gene microsatellite and single-nucleotide polymorphisms to develop japonica varieties with desired amylose levels in rice (Oryza sativa L.).” Journal of Cereal Science.

40. Jorge C. D., Sampaio M. M., Hreggvidsson G. O., Kristjanson J. K. and Santos H. (2007). “A highly thermostable trehalase from the thermophilic bacterium Rhodothermus marinus.” Extremophiles 11(1): 115-122.

41. Justino M. C., Almeida C. C., Teixeira M. and Saraiva L. M. (2007). “Escherichia coli Di-iron YtfE Protein Is Necessary for the Repair of Stress-damaged Iron-Sulfur Clusters.” Journal of Biological Chemistry 282(14): 10352-10359.

42. Ladero V., Ramos A., Wiersma A., Goffin P., Schanck A., Kleerebezem M., Hugenholtz J., Smid E. J. and Hols P. (2007). “High-level production of the low-calorie sugar sorbitol by Lactobacillus plantarum through metabolic engineering.” Applied and Environmental Microbiology 73(6): 1864-1872.

43. Leal S. and Gomes C. M. (2007 in press). “Studies of the molten globule state of ferredoxin: structural characterisation and implications on protein folding and iron-sulfur centre assembly.” Proteins.

44. Li F., Li L., Delgado R., Drew M. G. B. and Felix V. (2007). “New dioxadiaza- and trioxadiaza-macrocycles containing one dibenzofuran unit with two amino pendant arms: synthesis, protonation and complexation studies.” Dalton Transactions(13): 1316-1324.

45. Lobo S. A. L., Melo A. M. P., Carita J. N., Teixeira M. and Saraiva L. M. (2007). “The anaerobe Desulfovibrio desulfuricans ATCC 27774 grows at nearly atmospheric oxygen levels.” FEBS Letters 581(3): 433-436.

46. Machuqueiro M. and Baptista A. M. (2007). “The pH-dependent conformational states of kyotorphin: A constant-pH molecular dynamics study.” Biophysical Journal 92(6): 1836-1845.

Publications 2007

47. Malpique R., Katsen-Globa A., Carrondo M. J. T., Zimmermann H. and Alves P. M. (2007 in press). “Cryopreservation in micro-volumes: impact upon Caco-2 Colon Adenocarcinoma cell proliferation and differentiation.” Biotechnology and Bioengineering.

48. Marcal D., Rego A. T., Fogg M. J., Wilson K. S., Carrondo M. A. and Enguita F. J. (2007). “Crystallization and preliminary X-ray characterization of 1,3-propanediol dehydrogenase from the human pathogen Klebsiella pneumoniae.” Acta Crystallographica Section F-Structural Biology and Crystallization Communications 63: 249-251.

49. Miragaia M., Thomas J. C., Couto I., Enright M. C. and de Lencastre H. (2007). “Inferring a population structure for Staphylococcus epidermidis from multilocus sequence typing data.” Journal of Bacteriology 189(6): 2540-2552.

50. Monteiro S., Picarra-Pereira M. A., Loureiro V. B., Teixeira A. R. and Ferreira R. B. (2007). “The diversity of pathogenesis-related proteins decreases during grape maturation.” Phytochemistry 68(4): 416-425.

51. Morgado P., Zhao H., Blas F. J., McCabe C., Rebelo L. P. N. and Filipe E. J. M. (2007). “Liquid Phase Behavior of Perfluoroalkylalkane Surfactants.” Journal of Physical Chemistry B 111(11): 2856-2863.

52. Najdanovic-Visak V., Rodriguez A., Visak Z. P., Rosa J. N., Afonso C. A. M., da Ponte M. N. and Rebelo L. P. N. (2007 in press). “Co-solvent effects in LLE of 1-hydroxyethyl-3-methylimidazolium based ionic liquids + 2-propanol + dichloromethane or 1,2-dichloroethane.” Fluid Phase Equilibria.

53. Noronha M. C., Lima J. C., Paci E., Santos H. and Macanita A. L. (2007). “Tracking Local Conformational Changes of Ribonuclease A using Picosecond Time-Resolved Fluorescence of the Six Tyrosine Residues.” Biophysical Journal: biophysj.106.093625.

54. Nunes A. V. M., Matias A. A., da Ponte M. N. and Duarte C. M. M. (2007). “Quaternary phase equilibria for scCO(2) plus biophenolic compound plus water plus ethanol.” Journal of Chemical and Engineering Data 52(1): 244-247.

55. Nunes C. D., Vaz P. D., Fernandes A. C., Ferreira P., Romao C. C. and Calhorda M. J. (2007 in press). “Loading and delivery of sertraline using inorganic micro and mesoporous materials.” European Journal of Pharmaceutics and Biopharmaceutics.

56. Oliveira P. B., Silva M. J., Ferreira R. B., Oliveira C. M. and Monteiro A. A. (2007). “Dry matter partitioning, carbohydrate composition, protein reserves, and fruiting in ‘Autumn bliss’ red raspberry vary in response to pruning date and cane density.” Hortscience 42(1): 77-82.

57. Oliveira T. F., J. M., Moreira R., Iley J. and Archer M. (2007). “Crystallization and preliminary diffraction studies of porcine pancreatic elastase in complex with a novel inhibitor.” Protein & Peptide Letters 14: 93-95.

58. Peixoto C., Sousa M. F. Q., Silva A. C., Carrondo M. J. T. and Alves P. M. (2007). “Downstream processing of triple layered rotavirus like particles.” Journal of Biotechnology 127(3): 452-461.

59. Pereira S. F. F., Henriques A. O., Pinho M. G., de Lencastre H. and Tomasz A. (2007). “Role of PBP1 in Cell Division of Staphylococcus aureus.” Journal of Bacteriology 189(9): 3525-3531.

60. Pimentel C., Van Der Straeten D., Pires E., Faro C. and Rodrigues-Pousada C. (2007 in press). “Characterization and expression analysis of the aspartic protease gene family of Cynara cardunculus L.” FEBS Journal.

61. Pinto F. R., Carrico J. A., Ramirez M. and Almeida J. S. (2007). “Ranked Adjusted Rand: integrating distance and partition information in a measure of clustering agreement.” Bmc Bioinformatics 8: 13.

62. Polivka T., Pellnor M., Melo E., Pascher T., Sundstrom V., Osuka A. and Naqvi K. R. (2007). “Polarity-tuned energy transfer efficiency in artificial light-harvesting antennae containing carbonyl carotenoids peridinin and fucoxanthin.” Journal of Physical Chemistry C 111(1): 467-476.

63. Reis P. M. and Royo B. (2007 in press). “Perrhenic acid as catalyst for hydrosilylation of aldehydes and ketones and dehydrogenative silylation of alcohols.” Catalysis Communications.

64. Ribeiro T., Abrantes M., Lopes M. D. S. and Crespo M. T. B. (2007). “Vancomycin-susceptible dairy and clinical enterococcal isolates carry vanA and vanB genes.” International Journal of Food Microbiology 113(3): 289-295.

65. Rocheta M., Cordeiro J., Oliveira M. and Miguel C. (2007). “PpRT1: the first complete gypsy-like retrotransposon isolated in Pinus pinaster.” Planta 225(3): 551-562.

66. Rodrigues J. V., Saraiva L. M., Abreu I. A., Teixeira M. and Cabelli D. E. (2007). “Superoxide reduction by Archaeoglobus fulgidus desulfoferrodoxin: comparison with neelaredoxin.” Journal of Biological Inorganic Chemistry 12(2): 248-256.

67. Rodrigues T., Carrondo M. J. T., Alves P. M. and Cruz P. E. (2007). “Purification of retroviral vectors for clinical application: Biological implications and technological challenges.” Journal of Biotechnology 127(3): 520-541.

68. Rodrigues T., Carvalho A., Carmo M., Carrondo M. J. T., Alves P. M. and Cruz P. E. (2007). “Scaleable purification process for gene therapy retroviral vectors.” Journal of Gene Medicine 9(4): 233-243.

69. Roldao A., Vieira H. L. A., Charpilienne A., Poncet D., Roy P., Carrondo M. J. T., Alves P. M. and Oliveira R. (2007). “Modeling rotavirus-like particles production in a baculovirus expression vector system: Infection kinetics, baculovirus DNA replication, mRNA synthesis and protein production.” Journal of Biotechnology 128(4): 875-894.

70. Romao C. C. and Veiros L. F. (2007). “Haptotropic Shifts and Fluxionality of Cyclopentadienyl in Mixed-Hapticity Complexes: A DFT Mechanistic Study.” Organometallics 26(7): 1777-1781.

Publications 2007

71. Ruivo R. M., Couto R. M. and Simoez P. C. (2007). “High-pressure phase equilibria of the ternary system oleic acid plus squalene plus carbon dioxide.” Journal of Chemical and Engineering Data 52(2): 566-570.

72. Santos L. M. N. B. F., Canongia Lopes J. N., Coutinho J. A. P., Esperanca J. M. S. S., Gomes L. R., Marrucho I. M. and Rebelo L. P. N. (2007). “Ionic Liquids: First Direct Determination of their Cohesive Energy.” Journal of the American Chemical Society 129(2): 284-285.

73. Santos T. P., Lopes C. M., Rodrigues M. L., Souza C. R., Silva J. R., Maroco J. P., Pereira J. S. and Chaves M. M. (2007). “Effects of deficit irrigation strategies on cluster microclimate for improving fruit composition of Moscatel field-grown grapevines.” Scientia Horticulturae 112(3): 321-330.

74. Simplicio A. L., Clancy J. M. and Gilmer J. F. (2007 in press). “[beta]-Aminoketones as prodrugs with pH-controlled activation.” International Journal of Pharmaceutics.

75. Soares N. C., Francisco R. and Ricardo C. P. (2007 in press). “Proteomics of ionically bound and soluble extracellular proteins in Medicago truncatula leaves.” Proteomics.

76. Sobral R. G., Jones A. E., Des Etages S. G., Dougherty T. J., Peitzsch R. M., Gaasterland T., Ludovice A. M., de Lencastre H. and Tomasz A. (2007). “Extensive and genome-wide changes in the transcription profile of Staphylococcus aureus induced by modulating the transcription of the cell wall synthesis gene murF.” Journal of Bacteriology 189(6): 2376-2391.

77. Teixeira A. P., Alves C., Alves P. M., Carrondo M. J. T. and Oliveira R. (2007). “Hybrid elementary flux analysis/nonparametric modeling: application for bioprocess control.” Bmc Bioinformatics 8: 15.

78. Tereso S., Miguel C., Zoglauer K., Milhinhos A. and Oliveira M. M. (2007). “Zygotic and somatic embryo morphogenesis in Pinus pinaster: comparative histological and histochemical study “ Tree Physiology 27: 661-669.

79. Thomas J. C., Vargas M. R., Miragaia M., Peacock S. J., Archer G. L. and Enright M. C. (2007). “Improved multilocus sequence typing scheme for Staphylococcus epidermidis.” Journal of Clinical Microbiology 45(2): 616-619.

80. Trindade J. R., Dias A. M. A., Blesic M., Pedrosa N., Rebelo L. P. N., Vega L. F., Coutinho J. A. P. and Marrucho I. M. (2007). “Liquid-liquid equilibrium of (1H,1H,7H-perfluoroheptan-1-ol plus perfluoroalkane) binary mixtures.” Fluid Phase Equilibria 251(1): 33-40.

81. Trindade J. R., Visak Z. P., Blesic M., Marrucho I. M., Coutinho J. A. P., CanongiaLopes J. N. and Rebelo L. P. N. (2007 in press). “Salting-Out Effects in Aqueous Ionic Liquid Solutions: Cloud-Point Temperature Shifts.” Journal of Physical Chemistry B.

82. Vaz Patto M. C., Fernández-Aparicio M., Moral A. and Rubiales D. (2007 in press). “Resistance reaction to powdery mildew (Erysiphe pisi) in a germplasm collection of Lathyrus cicera from Iberian origin.” Genetic Resources and Crop Evolution.

83. Vaz Patto M. C., Moreira P. M., Carvalho V. and Pêgo S. (2007 in press). “Collecting maize with technological ability for bread production in Portugal.” Genetic Resources and Crop Evolution.

84. Verissimo A. F., Pereira M. M., Melo A. M. P., Hreggvidsson G. O., Kristjansson J. K. and Teixeira M. (2007). “A ba(3) oxygen reductase from the thermohalophilic bacterium Rhodothermus marinus.” FEMS Microbiology Letters 269(1): 41-47.

85. Vicente J. B., Scandurra F. M., Rodrigues J. V., Brunori M., Sarti P., Teixeira M. and Giuffre A. (2007). “Kinetics of electron transfer from NADH to the Escherichia coli nitric oxide reductase flavorubredoxin.” FEBS Journal 274(3): 677-686.

86. Wolf G., Almeida J. S., Crespo J. G. and Reis M. A. M. (2007). “An improved method for two-dimensional fluorescence monitoring of complex bioreactors.” Journal of Biotechnology 128(4): 801-812.

87. Xavier K. B., Miller S. T., Lu W. Y., Kim J. H., Rabinowitz J., Pelczer I., Semmelhack M. F. and Bassler B. L. (2007). “Phosphorylation and processing of the quorum-sensing molecule autoinducer-2 in enteric bacteria.” Acs Chemical Biology 2(2): 128-136.

88. Zhang C. H. and Fevereiro P. S. (2007). “The effect of heat shock on paclitaxel production in Taxus yunnanensis cell suspension cultures: Role of abscisic acid pretreatment.” Biotechnology and Bioengineering 96(3): 506-514.

89. Zhang C. H., Fevereiro P. S., He G. and Chen Z. (2007). “Enhanced paclitaxel productivity and release capacity of Taxus chinensis cell suspension cultures adapted to chitosan.” Plant Science 172(1): 158-163.

Scientific events organized by ITQB members

Meeting on Microbial Respiratory Chains
Tomar, Portugal, 19-23 March 2006. Lígia M. Saraiva, organizer

Last meeting of the "HotSolutes" Project
Carcavelos, Portugal, May 2006, Helena Santos, organizer

8th European Biological Chemistry Conference, EUROBIC 8
Aveiro, Portugal 2-6 July, 2006. Miguel Teixeira (Chair), M. Arménia Carrondo, Cláudio Soares, Manuela Pereira, Pedro Matias, Ricardo Louro, organizers

EUROBIC Young Investigator's Forum
Aveiro, Portugal, 1-2 July 2006. Manuela M. Pereira, Ricardo O. Louro, Smilja Todorovic
Julia Steuber (Zürich), organizers

Peroxidase 2006
Aveiro, Portugal, 6-9 July 2006. Phil Jackson, organizer

3rd European Meeting of Oxizymes -"Oxizymes in Oeiras 2006
ITQB, Oeiras, Portugal, 7-9 September 2006. Lígia O. Martins, organizer

VI Ibero-American Congress of Biophysics
Madrid, Spain, 24-27 September 2006. Cláudio Soares, organizer

BIOCRYS 2006
ITQB, Oeiras, Portugal, 6-13 October 2006. M. Arménia Carrondo and Thomas Schneider (Italy), organizers

Workshop "Nutrigenomics"
ITQB, Oeiras, Portugal, November 2006, under the scope of the Specific Support Action, Thematic Priority Food Quality and Safety. Teresa Crespo, organizer

FEBS Young Scientist Forum 2006 (satellite meeting of the 31th FEBS Congress)
Istanbul, 22 - 24 June 2006. C. Rodrigues-Pousada, organizer

Jornada de Reflexão sobre a Investigação em Olivicultura em Portugal
2006. Cidália Peres, President of the Organizing Committee

IV Simpósio Nacional de Olivicultura
ENMP, Elvas, Portugal, November 2006. Cidália Peres, Convener

Workshop Parasitic Plant Management in Sustainable Agriculture
ITQB, Oeiras, Portugal, 23-24 November 2006. Carlota Vaz Patto, organizer

PhD Theses at ITQB in 2006 (by chronological order)

Joana Paiva Gomes Miranda (Biochemistry)
Identification and characterization of immunodominant proteins of *Theileria uilenbergi* to be used in the diagnosis of ovine theileriosis in China. **Supervisor**: Abel Oliva

Rute Isabel Alves Rodrigues (Biology)
Functional Analysis of *Desulfovibrio gigas* ROO and Ech hydrogenase. **Supervisor:** Claudina Rodrigues-Pousada

Vanessa Alexandra dos Santos Morais (Biochemistry)
Role of Nicastrin in Alzheimer's Disease: Importance of the Transmembrane Domain and Ectodomain. **Supervisor:** Júlia Costa

Jorge Almiro Barceló Caldeira Pinto Paiva (Biology)
Phenotypic and Molecular Plasticity of Wood Forming Tissues in Maritime Pine (*Pinus pinaster* Ait.). **Supervisor:** Pedro Fevereiro / Christophe Plomion

João André Nogueira Custódio Carriço (Biology)
New Perspectives on Microbial Typing. **Supervisor:** Jonas Almeida

Susana Maria Lavado de Oliveira Gardete (Biology)
Towards the understanding of the mechanism of methicillin resistance in *Staphylococcus aureus*. **Supervisor:** Ana Madalena Ludovice/Hermínia de Lencastre

Ana Sofia Correia Fortunato (Biology)
Involvement of defence-related genes during nodulation of *Casuarina glauca*. **Supervisor:** Ana Isabel Ribeiro/Cândido Pinto Ricardo

Ana Rita Cruz Duarte (Chemical Engineering)
Exploring supercritical fluid technology for the development of controlled drug delivery systems. **Supervisor:** Catarina Duarte

Jingsi Liang (Biology)
Heterologous gene expression in legume nodules of *Medicago truncatula*. **Supervisor:** Ana Isabel Ribeiro/Pedro Fevereiro

Inês Maria da Silva de Almeida Chaves (Biochemistry)
Protein expression in plant cells during programmed cell death induced by stress. **Supervisor:** Cândido Pinto Ricardo/Ana Paula Regalado

Luís Maria de Figueiredo Mascarenhas Lopes da Fonseca (Biochemistry)
Metabolism of primary astrocytes studied by NMR: metabolic trafficking and neuroprotection. **Supervisor:** Helena Santos

Patrícia dos Santos Antunes (Chemistry)
Evaluation of macrocyclic complexes and somatostatin-based radiopharmaceuticals for nuclear medicine. **Supervisor:** Rita Delgado

Feng Li (Chemistry)
Coordination and Supramolecular Chemistry of New Macrocycles and Cryptands as Receptors for Cations and Anions. **Supervisor:** Rita Delgado

PhD Theses at ITQB in 2006 (by chronological order)

Maria Leopoldina C.C.A. Miragaia Ryder (Biology)
The evolution of methicillin-resistant *Staphylococcus epidermidis*: diversity in genetic backgrounds and in the structure of the resistant determinant SCCmec. **Supervisor:** Hermínia de Lencastre/Isabel Couto

Maria Inês Crisóstomo Ramos (Biology)
Contrasts and similarities in the β-lactam resistance of pneumococci and *Staphylococcus aureus*: mechanisms, evolution and epidemiology. **Supervisor:** Hermínia de Lencastre/Alexander Tomasz

César Miguel Pereira Soares Mendes (Biology)
Cell Death Mechanisms in the Retina of *Drosophila melanogaster*. **Supervisor**: Álvaro Tavares/Hermann Steller/Bertrand Mollereau. IGC PhD Student

Rita Gonçalves Sobral de Almeida (Biology)
Modulating *Staphylococcus aureus* cell physiology through a murein ligase. **Supervisor:** Hermínia de Lencastre/Alexander Tomasz/Ana Madalena Ludovice

Patrícia Matos Cruz da Silva Pereira (Biochemistry)
Biochemical and genomics studies of proteins involved in the bioenergetic metabolismo of Sulfate-Reducing Bacteria. **Supervisor:** Ricardo Louro/Inês Cardoso Pereira (António Xavier).

Catarina Morais Vaz Paquete (Biochemistry)
Thermodynamic and kinetic characterisation of multiheme cytochromes. **Supervisor:** Teresa Catarino (António Xavier)

Ricardo Hugo Jorge Pires (Biochemistry)
Membrane-bound metalloproteins involved in Sulfate Respiration. **Supervisor:** Inês Cardoso Pereira (António Xavier)

Ongoing Research Projects

Project title	Project n'	Leader/Participant	Funding €	Duration
Projects funded by FCT:				
Approaches to the synthesis of optically pure natural compounds having a dehydrodecalin nucleus	QUI/43313/01	Christopher Maycock	60.200	2002-2006
RNases and polyadenylation in the adjustment of bolA mRNA levels necessary for cells growth and survival	BME/42377/01	Cecília Arraiano	93.480	2002-2007
Adventitious organogenesis in almond: an histological and molecular approach	AGR/38507/01	Ana Sanchez	60.000	2003-2006
Molecular basis of the functioning of immobilised redox enzymes in bioelectronic devices	BIO/43105/01	Daniel Murgida	120.000	2003-2006
Unravelling the aerobic respiratory chain of the "anaerobic" sulfate reducing bacteria	BME/37406/01	Miguel Teixeira	74.500	2003-2007
Genes involved in bioenergetic mechanisms in the sulphate reducing bacterium Desulfovibrio gigas	BME/37480/01	Claudina R. Pousada	100.584	2003-2006
Structure and function of the centrosomal proteins HsMob in cell division	CBO/39099/01	Mª Arménia Carrondo	20.000	2003-2006
Green processing with ionic liquids coupled to supercritical CO2 extraction or membrane pervaporation	EQU/35437/99	Luis Paulo Rebelo	28.000	2003-2006
Reduction of nitric oxide in prokaryotes: new metabolic routes	BME/44597/02	Miguel Teixeira	102.456	2003-2006
Increasing realism in protein modelling: including pH and redox effects into molecular dynamics simulations	BME/45810/02	António Baptista	51.528	2003-2006
Anaerobic metabolism of the human pathogen bilophila wadsworthia	ESP/44782/02	Inês Cardoso Pereira	86.808	2003-2006
Structure, tropisms and molecular dynamics of the stratum corneum lipid matrix. A study in model systems	QUI/45090/02	Eurico de Melo	53.470	2003-2006
Thermodynamics of metalloprotein folding and stability	QUI/45758/02	Claudio Gomes	69.440	2003-2006
Structural characterization of MrkD protein from *Klebsiella pneumoniae*: implications in the epithelial adhesion properties of the microorganism	ESP/46428/02	Francisco Enguita	61.037	2003-2006
Energy transduction in a plant symbion from *Sinerhizobium melitoli*	BME/45122/02	Manuela Pereira	74.000	2004-2006
Cell wall proteins with roles in xylogenic programmes in eucalyptus	AGR/46671/02	Philip Jackson	76.854	2003-2006
Oxidative Phosphorylation in sulfate respiration	QUI/47866/02	António V. Xavier	58.440	2003-2006
Dissection of a checkpoint linking chromosome segregation to asymmetric cell division at the onset of endospore development in *Bacillus subtilis*	BCI/48647/02	Adriano O. Henriques	63.724	2003-2005
Study of ribonucleases from lactid acid bacteria for the construction of strains important for food processing by the dairy industry	AGR/49306/02	Cecília Arraiano	120.680	2003-2006

Ongoing Research Projects

Project title	Project n'	Leader/Participant	Funding €	Duration
Global experimental approches to model central metabolism in *L. lactis*: modulation of the levels of key-enzymes	BIO/48333/02	Helena Santos	98.738	2004-2007
Clinical, neurophysiological and neuro-chemical studies in amyotrophic lateral sclerosis	CBO/43952/02	Júlia Costa	34.174	2003-2006
Reflorestation with corl oak: genetic vari-ability and seed storage biology	AGG/41359/02	C.Pinto Ricardo	25.200	2003-2006
Macrocyclic compounds selective for heavy metals poisoning: Cd(II), Hg(II), and Pb(II)	QUI/49114/02	Rita Delgado	6.563	2003-2006
New compatibles solutes from thermophil-es and hyperthermophiles: screening, identification and physiological role	BIO/42331/02	Helena Santos	49.000	2003-2006
Searching for rhizobial strains with im-proved skills to thrive in arid lands	AGG/46371/02	Helena Santos	17.000	2004-2007
Folding, processing and function of nor-mal and mutant cystic fibrosis transmem-branar conductance regulator: structural implications	MGI/47382/02	Cláudio Soares	9.969	2003-2006
The role of RNase and its homologues in the control of gene expression: structural and functional studies	BIA-MIC/55106/04	Cecília Arraiano	90.000	2005-2008
Role of the interaction between AgfA and AgfB proteins from *Salmonella enterica* serovar Typhimurium in the surface polimerization of the amyloid-like thin ag-gregative fimbriae	SAU-IMI/55520/04	Francisco Enguita	71.872	2006-2008
Rhenium, molybdenum and tungsten oxo complexes: new class of catalysts for reduction reactions	QUI/555862/04	Beatriz Royo	55.500	2005-2008
Structural characterization of membrane proteins of the respiratory chain of a thermoacidophilic organism	BIA-PRO/55621/04	Margarida Frazão	78.410	2005-2008
Role of defense-related genes during the establishment of root-nodule symbioses between higher plants and nitrogen-fixing bacteria	AGR/55651/04	Ana Ribeiro	57.360	2005-2008
Characterisation of membrane bound cytochrome involved in the anaerobic respiration in sulphate reducing bacteria	QUI/55690/04	Ricardo Louro	54.500	2005-2008
Structure function studies of murine Toll-like receptors: activation of the innate immune response	SAU-IMI/55729/04	Mª Arménia Carrondo	50.420	2005-2008
Transgenic plants as models to study regulation of transgene expression and recombinant protein deposition	BIA-BCM/55792/04	Rita Abranches	69.621	2005-2008
Transcription factors regulating abiotic stress response in rice (Orysa sativa): a transgenic approach to improve tolerance, and search for novel players	BIA-BCM/56063/04	Nelson Saibo	50.000	2005-2008
Nitrosative Stress Responses of Human Pathogens	SAU-IMI/56088/04	Lígia Saraiva Teixeira	99.648	2005-2008

Ongoing Research Projects

Project title	Project n'	Leader/Participant	Funding €	Duration
Organizations of the staphylococcal cell wall synthetic machinery	BIA-BCM/56493/04	Mariana Pinho	90.000	2005-2008
Role of bacterial cell wall on the host inate immune response	SAU-IMI/56501/04	Sérgio Filipe	99.999	2005-2008
Molecular recognision of phthalate and phthalic acid esters pollutants by ditopic receptors by cascade dicopper systems	QUI/56569/04	Rita Delgado	43.640	2005-2008
Molecular markers for Portuguese pine wood quality	AGR/56658/04	Pedro Fevereiro	22.066	2005-2008
Improving tolerance to water stress in legumes using the model *Medicago truncatula*	BIO/56659/04	Pedro Fevereiro	16.144	2005-2008
The role of small non-coding RNAs and RNases on the pathogenicity de Salmonella	CVT/56811/04	Cecília Arraiano	87.879	2005-2008
Nano-Engineering of bacterial laccases	BIO/57083/04	Lígia Martins	90.459	2005-2008
Functional characterization of genes related to nitrogen metabolism in genetically modified maritime pine	AGR/57157/04	Susana Tereso	77.700	2005-2008
Structural determinants of protein stabilization by compatible solutes from hyperthermophiles: in search of guidelines solute improvement	BIA-PRO/57263/04	Helena Santos/ Christopher Maycock	79.902	2005-2008
Bioremediation of PCP by the co-metabolism of cork endogenous moulds	AMB/57374/04	Cristina Silva Pereira	82.187	2005-2008
Staphylococcus aureus and *Staphylococcus epidermidis*: links between hospital and community	SAU-ESP/57841/04	Hermínia de Lencastre	63.245	2005-2008
Maize "broa" quality attributes: Identifying genes that affect the technological ability for bread production	AGR/57994/04	Carlota Vaz Pato	37.060	2005-2008
Epidemiology of multidrug resistant enterococci in a Lisbon Hospital – Surveillance study in malignancy ward	SAU-ESP/58030/04	Rosário Mato	63.951	2005-2008
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	BIA-PRO/58374/04	Manuela Pereira	51.600	2005-2008
Regulation of cell wall synthetic genes and enzymes in B-lactam resistant *Staphylococcus aureus*	BIA-MIC/58416/04	Hermínia de Lencastre	80.000	2005-2008
Screening hyperthermophilic proteomes for hyperstable proteins	BIO/58465/04	Cláudio Gomes	51.829	2005-2008
Heme-copper oxygen reductases – mechanisms of electron/proton transfer and oxygen reduction	BIA-PRO/58608/04	Miguel Teixeira	55.200	2005-2008
Characterization of metal and sulphur respiratory chains in a marine organism targeted for bioremediation applications	BIO/58652/04	Ricardo Louro	80.500	2005-2008
Characterization of CymA: a focal protein in anaerobic respiration by Shewanella	BIA-PRO/58722/04	Ricardo Louro	39.600	2005-2008

Ongoing Research Projects

Project title	Project n'	Leader/Participant	Funding €	Duration
Cytochrome c: a model protein to probe thermodynamic and choreographic constraints in electroprotonic energy transducers	QUI/58985/04	David Turner	69.000	2005-2008
Understanding defence responses of grapevine to drought stress-metabolic regulation at the leaf and berry levels	AGR/59079/04	Manuela Chaves	71.575	2005-2008
Strategies of life adaptation to hot environments: heat and osmotic stress responses in the extremely thermophilic bacterium *Rhodothermus marinus*	BIA-MIC/59310/04	Helena Santos	90.000	2005-2008
Studies on quinine-protein interaction in complexes of respiratory chains	QUI/59824/04	Manuela Pereira	73.000	2005-2008
Molecular characterization of a microbial hemicellulolytic system	AGR/60236/04	Isabel Sá Nogueira	74.500	2005-2008
Transcriptional control of the mecA gene, the central element of methicillin-resistance in staphylococci.	BIA-MIC/60320/04	Duarte de Oliveira	72.233	2005-2008
Interactions between proteins in adjacent sister cells that signal the activation of RNA polymerase in response to cellular morphogenesis	BIA-BCM/60855/04	Adriano O. Henriques	84.000	2005-2008
Mechanisms of repression by AraR, a key regulator of carbohydrates utilization in *Bacillus subtillis*	BIA-MIC/61140/04	Isabel Sá Nogueira	80.500	2005-2008
Process integration of supercritical fluid extraction and membrane separation to recover "Vegetal" squalene from olive oil residues	EQU/61550/04	Rui Ruivo	31.728	2005-2008
Studies on the synthesis and applications of 2-Oxoaza [x.1.0] bicycles	QUI/62794/04	Christopher Maycock	44.500	2005-2008
Constraints to carbon gain by tree age in Eucalyptus globules (Labill.) stands.	AGR/61980/04	Manuela Chaves	22.194	2005-2008
Rationalization of cutinase enantioselectivity in nonaqueous media	BIO/57193/04	Claudio Soares/Isabel Sá Nogueira	35.088	2005-2008
Mechanism and kinetics of protein stabilization by osmolytes.	QUI/56585/04	Helena Santos	7.560	2005-2008
Metabolism and characterization of mixed cultures in wastewater processes for simultaneous removal of nitrogen and phosphorus	AMB/56075/04	Helena Santos	7.440	2005-2008
Synthesis, structure and reactivity of transition metal complexes with potential application in oxidative catalysis	QUI/55985/04	Carlos Romão	3.600	2005-2008
Gene expression changes during hepatitis delta virus infection I. Analysis of the cellular proteome	SAU-IMI/55112/04	Ana Varela Coelho	13.200	2005-2008
Optical fibre sensors for distributed monitoring of dissolved oxygen and temperature	AMB/56132/04	Abel Oliva	30.028	2006-2008
Thermodynamical and structural characterization of ionic liquids and others associated fluids	QUI/57716/04	Luis Paulo Rebelo	25.000	2005-2008

Ongoing Research Projects

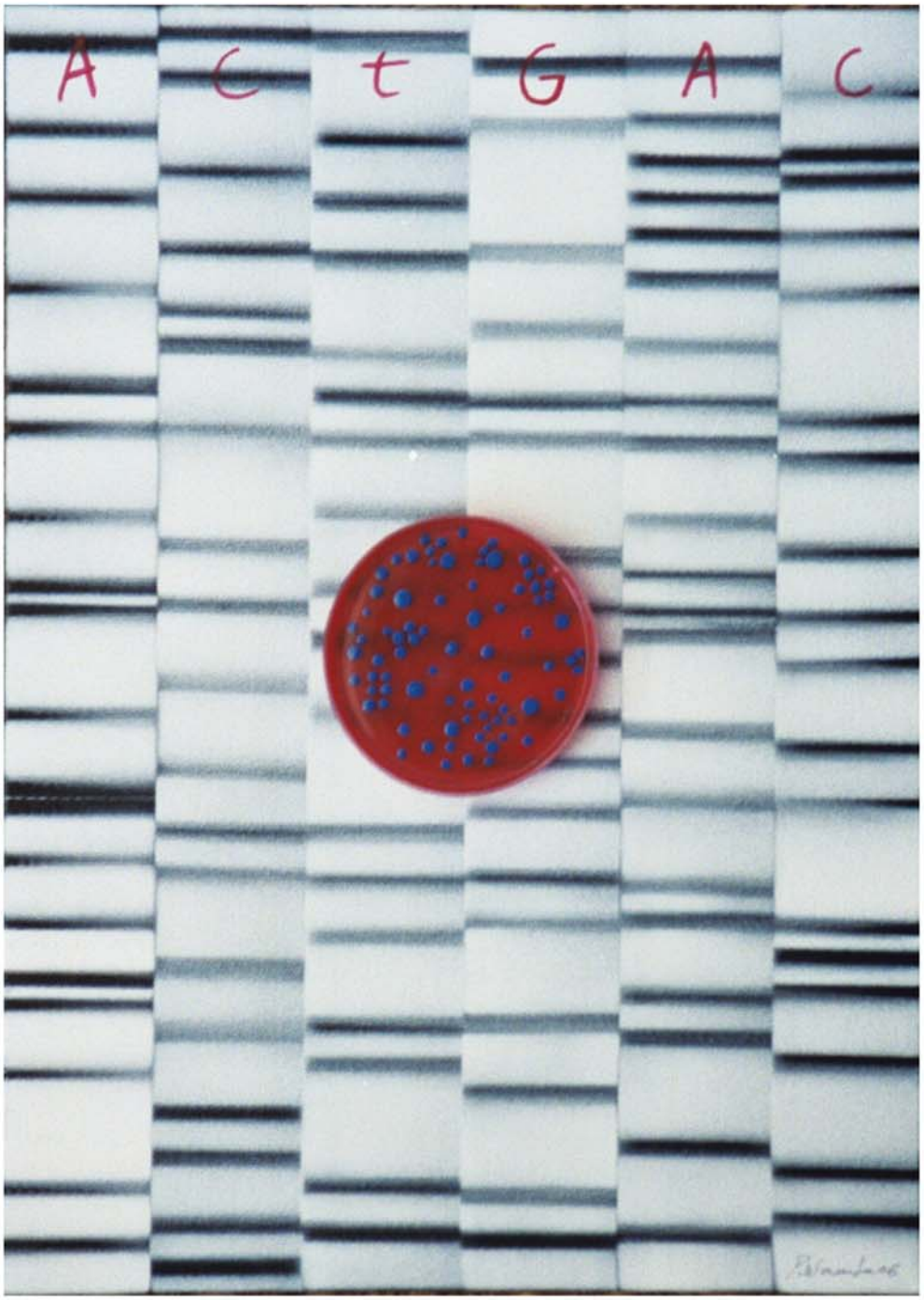
Project title	Project n'	Leader/Participant	Funding €	Duration
Nature's shields to environmental stress. Biosynthesis of compatible solutes in extremely radiation-resistant Rubrobacter spp	BIA-MIC/56511/04	Helena Santos	18.000	2005-2008
Proteomics of chronic lung diseases leading to biomarkers and therapeutic target discovery	SAU-MMO/56163/04	Ana Varela Coelho	16.904	2005-2007
Projects funded by FCT, under the Re-Equipment call:				
Interaction at the molecular level between vines and fungi	REEQ/122/AGR/2005	Ricardo Ferreira	51.763	2005-2006
Structure, dynamics and functions of proteins	REEQ/336/BIO/2005	Miguel Teixeira	399.758	2005-2006
Plant development under environmental controlled conditions to study the response to biotic and abiotic stresses, at the genomic, physiological, biochemical and structural levels	REEQ/374/BIO/2005	Cândido Pinto Ricardo	76.667	2005-2006
A platform for protein expression profiling, cell mapping, and the analysis of protein-protein interactions	REEQ/392/BIO/2005	Adriano O. Henriques	240.000	2005-2006
Study of plant response to stress using Thermography and Fluorescence Imaging	REEQ/834/BIO/2005	Manuela Chaves	33.597	2005-2006
National Facility for Mass Spectrometry	REDE/1504/REM/2005	Ana Varela Coelho	326.571	2005-2006
National Facility for High-Field Nuclear Magnetic Resonance	REDE/1517/RMN/2005	Helena Santos	2.492.600	2005-2006
Projects funded by FCG:				
Creation of a reference collection of anti-microbial resistant gram-positive bacteria serving the national and international scientific and clinical communities	61052/03	Hermínia Lencastre	88.000	2004-2006
Infeccion and colonization by multidrug-resistant Enterococci recovered from neonatal intensive care units. Epidemiological surveillance and infection control	65882/04	Rosario Mato	45.124	2004-2007
Projects funded by Ag|encia de Inovação:				
Gigasnome	ADI/2006/M.2.3/0031	Claudina R. Pousada	114.153	2005-2007
Projects funded by European Comission:				
Structural proteomics in Europe (SPINE)	QLG2-CT-2002-00988	Mª. Arménia Carrondo	190.475	2002-2006
From receptor to gene: structures of complexes from signalling pathways linking immunology, neurobiology and cancer (SPINE2-COMPLEXES)	LSHG-CT-2006-031220	Mª. Arménia Carrondo	206.750	2006-2009

Ongoing Research Projects

Project title	Project n'	Leader/Participant	Funding €	Duration
Genomics to combat resistance against antibiotics in community – acquired LRTI in Europe (GRACE)	LSHM-CT-2005-518226	Hermínia de Lencastre	129.216	2006-2011
Molecular mechanisms of resistance, virulence and epidemicity in Streptococcus pneumoniae (PREVIS)	LSHM-CT-2003-503413	Hermínia de Lencastre	259.810	2004-2006
New applications for compatible solutes from extremophiles (HOTSOLUTES)	COOP-CT-2003-508644	Helena Santos	242.520	2004-2007
Signalling and membrane trafficking in transformation and differentiation (SIGNALLING AND TRAFFIC)	LSHG-CT-2004-503228	Júlia Costa	162.700	2004-2007
Water resources strategies and drought alleviation in western Balkan agriculture (WATERWEB)	INCO-CT-2004-509163	Manuela Chaves	113.720	2004-2007
European macromolecular crystallography infrastructure network 2 (MAX-INFO 2)	RICA 505977	Mª Arménia Carrondo	41.700	2004-2009
White biotechnology for added value products from renewable plant polymers: design of tailor-made biocatalysts and new industrial bioprocesses (BIORENEW)	NMPT2-CT2-2006-026456	Lígia O. Martins	417.000	2006-2010
A doctoral training network in integrative studies of plant stress biology (ADONIS)	MEST-CT-2005-020232	Margarida Oliveira	151.122	2006-2010
Projects funded by NIH (Rockefeller University):				
Evolution and acquisition of drug resistance in MRS		Sérgio Filipe	200.500	2005-2009
Pathogen – specific drug targets for weaponized bacteria		Adriano O. Henriques	91.000	2005-2009

Projects coordinated by ITQB researchers are displayed in black; funding refers solely to the budget allocated to ITQB and not to the project total funding.

Instituto de Tecnologia Química e Biológica
Universidade Nova de Lisboa
tel. +351 21 446 93 18 | info@itqb.unl.pt | www.itqb.unl.pt
ITQB | Av. da República | EAN | 2780-157 Oeiras | Portugal



"*cultura pura*"
Acrylic on canvas with applied painted Petri dish, 40x30 cm
by Patrícia Noronha
former ITQB PhD student