Annual Report

2007 | 2008

Instituto de Tecnologia Química e Biológica
Universidade Nova de Lisboa

Foreword

My first words are to warmly welcome the new ITQB Director, Prof. José Artur Martinho Simões, who accepted this challenge, in a moment where large transformations at ITQB and in the Portuguese Academic and Scientific fields are occurring. I wish him the best success and strength to carry on his mandate.
Secondly, I want to express my gratitude to the President of the Foundation for Science and Technology, for his support to ITQB during this period.

This is my second and last Annual Report, as an *interin* Director of ITQB. An appointment initially meant to last for two months was extended for nineteen months (August 2006 – February 2008). And as soon as it became clear (already by mid-August 2006) that this mandate would be longer than anticipated, I decided to undertake a large series of long due changes to establish a scientific and administrative management at ITQB, compatible with the current challenges of any research institution. This was made always keeping in mind the vision for ITQB at its inception - a vision as valid today as it was in 1989 - of an interdisciplinary high-quality research institute at the interface of chemistry and life sciences, and related technologies; an institute whose core activity is research, a concept almost unique in the Portuguese Academia; an institute where advanced education at the PhD level comes naturally together with the research activity.

Some characteristics, such as transparency and a clear information flow, are essential for the success of any Organization. These are the basis to achieve efficiency, to avoid casuistic actions and favoritisms, and to create a spirit of *corpus*. In my view an institute such as ITQB must have:

• An efficient administration based in modern organization concepts where traditional *walls* and bureaucracy are reduced to a minimum, but where hierarchies and responsibilities are clear and respected by all.

• An efficient and proactive scientific management, only possible with serious and periodic in-depth evaluations, that contemplates scientific and strategic discussions and decisions, career development programs, and the development of coherent research programs. Quoting a sentence from the present Minister for Science, Technology and Higher education, written already many years ago, “the cornerstone of a scientific policy is a sound scientific evaluation” (free translation).

• A serious commitment of all ITQB members, only possible through a continuous increase of flow of information. *Information is power*, if kept closed.

It was clear to me that changes along these lines would face internal and external resistance, but not at the level that occurred. Effective changes, particularly behavioral ones, are difficult. But, I believe, they are also required if we want to continue to level with the top academic institutions worldwide. This is a time that changes such as those are clearly needed in view of the new ByLaws of the Portuguese system (occurring at this precise moment) which should have been clear to the University, and at a time when all Portuguese Research Centres are being evaluated. While it might be easier, in the short term, to act as an ostrich, or satisfy others interests external to ITQB - an attitude that always paralised the Portuguese Academia - the main goal of a renowned Research Centre, such as ITQB, should be to continue its aim of international excellence in competitive research, with the freedom (and the interconnected evaluation and accountability) that is associated with the scientific endeavor itself.

While some minor changes could be implemented without external advice, major ones required professional, impartial, and objective inputs. In this light, both researchers and staff were evaluated by independent teams: a specialized team from ISCTE made an exhaustive study of the administrative services at ITQB; a panel of renowned scientists from USA and Europe made a major scientific evaluation of all ITQB Laboratories, on the basis of a pre-analysis of written reports by each group leader, followed later by three days of interviews with each Pi and respective group members – this is much more than a simple bibliometric evaluation.

Foreword

For the sake of transparency, free information and accountability, the main part of the Scientific evaluation is presented, *ipsis verbis* in the following pages.
An evaluation performed in this way (by coincidence, essentially the same way in which the Portuguese research centres will be evaluated in 2008) allowed that the evaluations were not biased by fragmented panels for each division, was not based on "blind" bibliometric methods, and allowed an effective and private exchange of information between each Lab Coordinator and the panel members.

I finish this Foreword by thanking all administrative and scientific staff that understood and collaborated in this need for changes, and most particularly the Vice-Director Prof. Cláudio M. Soares, for his unconditional dedication to ITQB and with the certainty that many necessary changes were done - the road for more in depth changes has been opened, i.e., we paved the way for the next Direction.

I wish the best success for ITQB and his staff, and I want to remember all that you should be proud to work at one of the best Research Institutions in Portugal, as revealed by many types of evaluation. An Institution where the *leitmotif* should be *interdisciplinarity*, in the interface of Chemistry and Biology, *quality* and commitment of the research(ers), together with the continuous desire and dynamism to improve, and discuss the relevant issues in an open and truthfull manner, either scientific of managerial.
A major problem at ITQB remained to be solved – the relation between ITQB and IBET. The latter being a private Institute that uses human, scientific and financial resources of the Public and Academic Research Institution that is ITQB.

The main mission of ITQB should continue to be to develop high-quality research, taking advantage of the unique mutidisciplinarity of the Institute. Always following the essential principle of Science, based on individual freedom for research, on its intrinsic unpredictability, on the search for truth (even if never attained).

Miguel Sepúlveda Teixeira
Professor of Biological Chemistry
ITQB Director
August 2006- February 2008

Foreword

External Scientifc Evaluation of ITQB

The ITQB Direction, at the beginning of 2007, decided to undertake a thorough Scientific Evaluation of all research laboratories working at ITQB, by a renewed and larger External Advisory Board, entirely constituted by foreigner members. ITQB had almost since its start such a Board, which evaluated through the years the Scientific development at ITQB. The process was conducted in the following way : names were proposed by the five ITQB Divisions, and the final choice was made by the Direction, after consulting several other researchers, both from ITQB or foreigner. Several changes had to be made along the process, according to the availability of each panel member. The final panel was coordinated by Prof. Geoffrey Cole, and was constituted by the following Scientists:

Professor Jeff Cole (Birmingham, UK) Panel Coordinator; Microbiology and Biotechnology.

Professor Gerard Canters (Leiden, NL) Biological Chemistry
Professor Charles Cooney (MIT, USA) Biochemical Engineering
Professor Leslie Dutton (Philadelphia, USA) Biophysics
Professor Wolfgang Hillen (Erlangen, DE) Microbiology
Professor Mike Kearsey (Birmingham UK) Quantitative Genetics
Professor Joachim Klein (Braunschweig, DE) Biochemical Engineering.
Professor Werner Nau (Bremen, DE) Chemistry
Professor Robert Poole (Sheffield, UK) Microbiology.
Professor Pere Puigdomenech (Barcelona, ES) Plant Biology and Genetics
Professor Horst Vogel (Lausanne, Ch) Physical Chemistry

Terms of reference.

The Panel Coordinator, Jeff Cole, visited ITQB for a one day meeting with the Director and Deputy Director of ITQB and, later in the day, with the Director of IBET. They agreed the following terms of reference.

1. The panel was asked to review all aspects of ITQB, not only the scientific excellence of each of the PIs and their groups but also to review management and communication structures, finances, and strategic developments, some of which were extremely imminent. ITQB needed to redefine not only its scientific strategy for the next few years, but also its working relationship with IBET.

2. Three factors made this review timely. First, 14 new staff would be appointed in 2007, probably before October 2007, with a further 4 at the IBET. Secondly, there was currently little or no free space in which to house these 18 new research groups. Thirdly, a steady decline in block grant funding meant that there was no income stream from which to fund equipment and running expenses for the new groups.

3. In addition to the above three reasons for the review, many additional factors made this review timely. Not all groups contribute equally to the excellence of ITQB, and some revision of strategy is overdue.

4. Communications and esprit-de-corps within the Institute could be improved. The panel was invited to look closely at the management structures.

Procedures used for the review.

In preparation for the site visit, each Principal Investigator (PI) submitted a brief CV plus a proforma providing information under six headings: the composition of the research team; details of papers published in the last five years; the four most significant papers from the group published in the last five years, with a

Foreword

50-word summary explaining their importance; evidence of their international esteem (Editorships; major honours); a list of current and previous funding from competitive sources awarded in the last five years; and future research aspirations.

Each group was assessed numerically on the basis of three criteria:

• the international standing of the group;
• the quality of current projects and the funding available to support them; and
• the long-term sustainability of the research programme.

The evaluation was performed without involvement of the Direction, except in providing the information asked by the panel. As a first step, a Form with a set of questions was sent by the Panel coordinator to each Head of Laboratory, and the answers were provided directly to the Panel. In a second stage, in October 2007, an *in site* visit of three days was performed, with all panel members, organized as follows: On day one, there was a presentation by the ITQB and IBET Directors and Vice Directors of both Institutions to the panel. This meeting was then followed by a short presentation of the Panel to the Scientifc Council, and by direct interviews of at least two panel members to each Head of Laboratory, as well as to the Laboratories members, in the absence of the Head (ca one hour for each Laboratory). On the third day a preliminary overview of the report was presented to the ITQB and IBET Directions, which was then presented to the Scientifc Council of ITQB. A final report was latter prepared by the Panel, and sent Directly to the ITQB and IBET Directions, to the UNL rector and to the President of the Foundation for Science and Technology. To the ITQB members, the individual evaluations were sent only to each Laboratory Head; only the general report was sent to all Laboratories. Of course, this Report was delivered to the new ITQB Direction.

Major conclusions of the 2007 Review Panel

• The majority of the research at ITQB is competitive with other laboratories worldwide: some groups are amongst the leaders in their fields. Research excellence was found in all five Divisions of ITQB.

• ITQB remains the leading research centre for the chemical and life sciences in Portugal: it is a major national resource for fundamental and applied research.

• ITQB depends on a reliable and continued source of funding from the Portuguese government via FCT as well as on competitive funding for project grants. Its success in securing these funds is justified by the high quality of research at the ITQB.

• The addition of new groups in diverse areas to already existing groups makes a long-term strategic review imperative, because of potentially competing or complementary requirements for space and other resources.

• Although the overall impression of the ITQB performance was positive, there are groups that make an unacceptably low contribution to ITQB and its partner, IBET. There is an urgent need to introduce an effective staff development scheme that encourages all research staff in the Institute to set their own objectives, to balance their research efforts with other responsibilities such as teaching, and agree on targets. A mechanism needs to be established to monitor target accomplishment and rectify institutional deficiencies that result in under-performance. Within the staff development scheme, the procedures and conditions for promotion and the positions (auxiliary investigator - principal investigator - professor) accessible to each PI should be defined.

• The provision for the first time this year of substantial start-up financial support for the recently appointed PIs is welcomed, but was long overdue. In the absence of sufficient initial support, young investigators will

potentially waste the most productive years of their careers.

• ITQB management should immediately establish an effective mentoring system in which each new PI is allocated a senior member of ITQB, preferably not a previous supervisor, to offer regular advice on how to obtain funding, focus their research, develop and plan their career, and make their group more productive and internationally visible.

• Investment in major equipment is long overdue: this was identified as a priority in reviews completed in 2000 and 2004, but has only partially been addressed (for example, with the provision this year of the large NMR facility).

• Some facilities, for example fume hoods and cupboards, are in such a state of disrepair that there are serious safety issues requiring immediate action.

• There is an urgent need for an in-depth strategic review of the relationship between ITQB and IBET that must include a review of the mandate of IBET within ITQB, and how this mandate is implemented. Points requiring clearer definition include space allocation, finances, and staff affiliations.

Major observations and recommendations

Is ITQB fulfilling its mission?

ITQB was created to be a scientific research and advanced training centre from the Universidade Nova de Lisboa. Its mission is to develop scientifically recognised research in chemistry and the life sciences. Its mandate is very broad: to consider all levels of complexity and its applications, so as to contribute to the understanding of life's mechanisms and ultimately benefit society (ITQB Annual Report and Plan, 2006-2007).

The majority of the research at ITQB is competitive with other laboratories worldwide: some groups are amongst the leaders in their fields. Furthermore, research excellence was found in all five Divisions of ITQB. ITQB is clearly the leading research institute for the chemical and life sciences in Portugal, representing a major national resource for both fundamental and applied research. Excellent collaborations have been established between groups within the Institute, with the associated laboratories, between institutes in the Lisbon area, within Portugal, and with other leading laboratories across the world. There are some examples of successful technology transfer.

• The first conclusion is therefore that ITQB continues to fulfill its mission as the leading research institute for the chemical and life sciences in Portugal.

Some basic statistics can be offered to back this assertion.
When dividing the overall scores into bins of 1/2, 3/4, ..., 11/12 the distribution is:

1/2	*2 groups*
3/4	*1*
5/6	*6*
7/8	*11*
9/10	*17*
11/12	*11*

Foreword

This confirms our impression of the overall high standing of the ITQB. The average scores of each of the five divisions are:

Biological Chemistry	*12 groups: average score: 9.8*
Biology	*10 groups: average score: 9.4*
Technology	*8 groups: average score: 8.4*
Chemistry	*7 groups: average score: 7.8*
Plant sciences	*9 groups: average score: 6.9*

It confirms the quality of the biological chemistry and biology sections and underlines the need for reinforcement of the plant sciences and chemistry divisions.

Relevance of ITQB to science in Portugal.

ITQB receives 34% of its income from FCT in the form of an institutional block grant, and a further 41% in competitively awarded research contracts. It is twice as successful in winning grant support compared with the average across all applications considered. This results in the appointment of new post-doctoral researchers, as well as talented PhD and research students who apply to work with a specific PI. However, PhD training scholarships are awarded with only minimal and totally inadequate financial support for consumables and other research expenses, and they are decoupled from the FCT research grants, which provide funding for consumables and limited payment for research students. There appears to be no requirement that the laboratory in which the successful applicant works should be adequately funded from other sources.

The Portuguese Government aspires to create a significant number of new junior research positions in multiple scientific institutions. While the injection of new funding is welcome, these initiatives have consequences for the institutions in implementation. This is especially acute for a successful and productive organization such as ITQB. The acquisition of new groups adds vitality to the Institution, but in the absence of mechanisms to terminate unproductive groups, it inevitably results in increased pressure on space, central services, and the financial resources of the institute. A major concern is that the panel found several examples of young researchers who had clearly not been provided with adequate start-up funding to support their research. In specific cases, sufficient laboratory space had been granted to freshly appointed auxiliary investigators, but this welcome space had been used inefficiently for periods from several months up to two years due to the lack of accompanying consumables and equipment grants, which also delayed the hiring of coworkers.

• The panel applauds the long-overdue introduction by the current Acting Director of significant start-up funding for the new PIs appointed in 2007.

Major reasons why ITQB needs to review its strategy.

There is a need for a high-level strategic review and plan for ITQB. This review should assess where it is now relative to where it wants to be. It should review the viability of existing programmes and consider priority areas for focus. The plan should consider both scientific direction and the allocation of resources, including space. With the imminent arrival of a new ITQB Director, this is a particularly timely point to complete this review. Especially important issues to address are the following.

• Plant biology is critical to Portugal. More than half the groups in the Plant Sciences Division were work-

ing at a good international level with strong funding for both pure and applied topics. They had significant publication records in international journals and had attracted many good PhD students. These groups were leaders in Portuguese plant sciences. However, there were also groups that had existed for some time but were struggling to find funding and seemed unsure of their scientific ambitions or direction. The viability of these less successful groups should be considered with some urgency. There was general concern about the lack of temperature controlled greenhouse facilities and dispersal of the groups around the Institute that hampers collaboration.

• There has been inadequate support of the chemistry group that has led to no allocation of new positions to chemistry, absence of timely replacements for retirements, old facilities in a separate and distant building, and the separation of analytical chemistry capabilities presently affiliated to the Technology Division. We recommend the replacement of retiring positions with new people working in important and topical areas of chemistry.

• The panel notes that the open door policy results in the allocation of available space to some groups that are less relevant to the mission. It is timely to consider relocation of low priority areas of these less relevant groups, and adjust laboratory space according to need and productivity.

A parallel need to review the IBET mission and its relationship with ITQB.

The panel found some positive examples of successful technology transfer but there is concern that the impact of IBET on ITQB has not been optimized. The mission of IBET is "to act as an interface to the economic world, transferring knowledge to the economic sector and fostering collaboration with industry." However, there is tension between the managements of ITQB and IBET. This creates confusion and poor morale in some groups. There is a need to bring clarity into the respective missions and transparency to the relationship.

• There is an urgent need for an in-depth strategic review of the relationship between ITQB and IBET that must include a review of the mandate of IBET within ITQB, and how this mandate is implemented. Points requiring clearer definition include space allocation, finances, and staff affiliations.

The panel came across a number of cases where the PI seemed to have affiliations with both ITQB and IBET and commitments to both institutes in a non-transparent manner. Duties seemed to include work of a servicing nature for a large percentage of the time, the performance of application oriented research as well as a minimal amount of fundamental research aimed at preserving some scientific credibility for the group. A particularly striking example is one group that is assigned a 100% IBET affiliation by the IBET Director, yet the PI is 100% paid by the University of Lisbon (as assistant professor). The group reported that they spend 50% of their time for teaching, and that nevertheless they devote a substantial amount of time for fundamental research for the ITQB (in order to produce papers to allow them to compete for promotion). This division of affiliations and tasks appears not to be successful. The environment in which such cross-institutional collaborations are conducted should be synergetic and inspiring, and avoid possible conflicts of interest. The following is the view of about half of the panel members: other members felt it would be inappropriate for the ITQB panel to comment.

"The panel recognized that it had not been asked to review IBET as an institution: consequently, panel members had neither the necessary in-depth information nor the mandate to do so. However, in view of the serious implications of the link between ITQB and IBET and the current tensions between them, many members of the panel felt it would be helpful, and appropriate, to suggest the following possible way forward.

IBET's growth into an independent research activity within ITQB arose in the absence of technology ready for transfer from ITQB in its early days. While the growth was the result of laudable initiatives, it is timely that the technical and analytical research laboratories currently identified as IBET be returned to the direct responsibility of the ITQB directorate. If it is deemed advantageous for the institution, the IBET operation

could be put in the hands of a technology transfer professional and scientific/paralegal staff. This technology transfer professional would report to the ITQB Director. The presence of functioning "companies" occupying ITQB laboratories warrant constructive review in view of the increased space pressure in ITQB."

Introduction of effective mentoring for young PIs.

It is important for the ITQB to carefully manage its response to the initiative to create new positions. The 18 new groups require support in addition to financial resources, collaborators, and space. Mentoring will be especially important as these new people are integrated into the programs of ITQB, not least to provide advice about how best to focus their research, information on sources of funding, guidance on how to increase the chances of grant success, feedback on draft grant applications, advice on how to manage their groups, and how to plan and develop their careers efficiently.

Criteria for performance assessment should be clear and transparent, and regular performance reviews should be applied to all PIs and groups with an emphasis on Junior and mid-career faculty. Promotion procedures and schedules need to be defined and initiated.

• The panel urges ITQB Management as a matter of immediate priority to instigate a system of mentoring for all young researchers especially, but not only, those that are currently being recruited. The mentor should be a successful, senior member of ITQB, preferably not someone who has previously supervised the newly appointed PI. There should be annual performance reviews and contract renewal. Where applicable, promotion procedures need to be initiated at least 1.5 to 1 year before the end of a contract period. Provision should be made to allow exceptionally successful researchers to initiate such procedures earlier, either themselves or upon nomination by the mentor. In this context, the positions and track of positions (auxiliary investigator - principal investigator - professor) accessible to each PI should be defined in a transparent manner.

• It is important to provide effective start-up packages (space, consumables, access to technical and analytical staff and services) to enable new PIs to initiate their research.

Introduction of an effective staff development scheme across the institute for new and existing PIs.

One of the two issues that caused the panel greatest concern was the realisation that a minority of groups are struggling to achieve success, yet there are no effective staff development and support procedures in place to help them. Especially alarming was the realisation that a few of these less successful groups are led by young PIs who were given empty laboratory space but no start-up funding, no clear mandate of their roles in ITQB, and no advice about how to set, achieve or monitor the delivery of their strategic research objectives.

Each person should agree with their reviewer how the institutional management might help them achieve their objectives. Targets should be set, monitored and any reasons for failure to meet them need to be identified. The progress of each group should be monitored at regular intervals in a supportive, not in a threatening way. Such reviews might facilitate the relocation of some groups to more supportive or more appropriate environments, or even propose combining small groups into a more productive unit. The aim should be to ensure that every ITQB staff member is valued: successful implementation would ultimately result in more effective use of space and other resources.

• The panel strongly recommends that ITQB management immediately introduce more effective staff development reviews in which each individual – from PIs to post-doctoral staff and PhD students - set their personal objectives.

Infrastructure Issues

Many groups commented that there are infrastructure issues that need to be addressed. Clearly the main reason for these problems is the serious shortage of institutional funding to correct them. However, there are serious safety implications in some of the deficiencies, especially fume hoods and cupboards in the Chemistry Division, or the lack of venting in the autoclave room.

There is a particular problem with lack of trained technical support for key central facilities such as proteomics, mass spectrometry, microscopy and greenhouses.

Many groups were concerned about the lack of uniform and institution-wide access to scientific literature in journals, especially electronic access. Similarly, there is frustration about the absence of access to structural databases for chemistry. There is a general deficiency in IT support for both hardware and software, and also for advice on statistical analyses and bioinformatics support.

Specific concerns repeatedly mentioned were difficulties in accessing mass spectrometry and other spectroscopic facilities, and lack of clarity regarding what facilities were available to all members of ITQB (and if so, on what conditions), whether specialized equipment could be accessed by agreement with the relevant PI, or whether it is unavailable for use outside the research group that purchased or until now exclusively used it. Some technical and analytical services are being charged, others are available for free, while access to other techniques appears to come at the expense of co-authorship, if the equipment is available only in a particular research group.

There was considerable concern among the Plant Scientists over lack of microscopy facilities, particularly confocal equipment.

• Management is urged to develop a more effective strategy for replacing major items of equipment, and for raising funds to bring in new facilities.

• Safety issues demand immediate attention: management will be liable for prosecution, should an accident result in injury.

• Communications within ITQB need to be improved, not least to ensure that all researchers are aware of the communal facilities that are available to them.

• Greater transparency is required concerning the basis on which technical support is provided.

As a final comment, I just want to say that I agree with the report and its recommendations, much of which were coincident with the Plans established by the ITQB Direction. The only point the ITQB Direction did not understand was the appointed lack of sufficient literature access – ITQB has direct access on-line to over 4000 Journals, the large majority through the National facility b-on, and some from ITQB own resources, to be extended in 2008 by a joint venture within UNL.

M.S.T.

ITQB at a glance
in 2007

Research Institute of Universidade Nova de Lisboa

Director: Miguel Sepúlveda Teixeira

157 PhD holders

160 PhD students

Laboratório Associado ITQB in partnership with IGC and IBET

600 researchers

6 biotechnology companies

19 active patents

Multidisciplinary research

5 research divisions

53 research laboratories

105 ongoing research projects

194 papers published in 2007

Large Research Infrastructures

GLP Certified Analytical Services

Advanced Training

ITQB PhD program

Masters Course

35 PhDs awarded in 2007

Introduction

The Instituto de Tecnologia Química e Biológica (ITQB) is a scientific research and advanced training centre of Universidade Nova de Lisboa. Its mission is to develop 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, initially *Centro de Tecnologia Química e Biológica* (CTQB) 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 (1943-2006). Operating since 1989, CTQB started its activities with a few research groups setting their laboratories next to 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 Nacional*.

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 53 independent research groups, forming a scientific staff of more than 350 researchers. The researchers are assisted in their activities by the infrastructure support services that include around 60 people.

Over the years, the Institute has been supported 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 1200 papers published in international journals since 2000 and over 14,000 corresponding citations.

ITQB is headed by its director and one vice-director, 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.

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 M. Soares (Vice-Director) Rosina Gadit (Secretary to the SC)
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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)

ADVISOR, SCIENCE ADMINISTRATION & PLANNING

Margarida Senna-Martinez

Organization of the Institute

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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
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Kenneth R. Seddon, The Queen´s University of Belfast, UK	Ionic Liquids
M. Teresa Duarte, Instituto Superior Técnico, UTL , PT	Small Molecule X-Ray Cristallography
Peter F. Lindley, Birkbeck College London, UK	Structural Biology
Peter G. Hildebrandt, Technische Universität Berlin, DE	Raman Spectroscopy
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Teresa Baptista da Silva	Equipment Washrooms
Teresa Crespo	Analytical Services
Cláudia Almeida	Lab Manager

SAFETY AND FLOOR COORDINATION COMMITTEE

Abel Oliva	Chairman
Miguel Teixeira	Director
Mafalda Mateus	Secretary
Helena Matias	Safety and Hygiene at Work Advisor
Henrique C.Nunes	First Floor
(Nuno Monteiro)	First Floor
Fernando Tavares	Second Floor
(Nuno Lopes)	Second Floor
Inês Cardoso Pereira	Third Floor
(Cláudio Gomes)	Third Floor
Abel Oliva	Fourth Floor
(Luís Paulo Rebelo)	Fourth Floor
Teresa Crespo	Fifth Floor
Cândido Pinto Ricardo	Sixth Floor
(Margarida Oliveira)	Sixth Floor
Ana Luisa Simplicio	Seventh Floor
(António Lopes)	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
Beatriz Royo	Solvent Handling
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 & Stores
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

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 (until March)
Cláudia Lopes Pinheiro (until November)
Isabel Cristina Respício Valente Almeida Lopes
Maria de Fátima Costa Madeira
Rosina Faruk Gadit

EXTERNAL AFFAIRS & SCIENCE COMMUNICATION
Ana Maria Beirão Reis de la Fuente Sanchez
Cláudia Lopes Pinheiro
Luís Manuel Ramalho Morgado

Organization of the Institute

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

LIBRARY
Susana Lopes Ferreira

LAB MANAGER
Cláudia Almeida

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
João Carlos Zanão Simões
José Costa (until June)
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

WASHROOMS
Scientific Coordinator: Teresa Baptista da Silva [Manuela Regalla (until September)]
Ana Cristina Martins Barreiros
Carmen Popula Pereira de Jesus Fernandes
Helena Isabel Pinto Vilaranda
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
Hugo Gonçalo Metelo dos Santos Cordeiro (since April)
José Miguel de São Bento Figueiredo Loureiro
Maria Isabel da Costa Baía (until March)
Maria Manuel Isaías Paulo Rato
Miguel Paulo Vinhas Pires Bento Ribeiro (until January)
Rui Pinto Garcia Fernandes (since March)

Organization of the Institute

ANALYTICAL SERVICES

Ana Maria de Jesus Bispo Varela Coelho – Mass Spectrometry
Maria da Conceição Lucas Carvalho Pereira de Almeida – Elemental Analysis / Mass Spectrometry
Maria Manuela Sobral Martins Alberto Regalla – Protein Sequencing
Paula Maria Gonçalves de Oliveira Roldão Chicau – Amino Acid Analysis

CRYSTALLOGRAPHY

Isabel Bento – Small Molecule X-Ray Crystallography

CERMAX

Helena Matias – Nuclear Magnetic Resonance
João Pires – Nuclear Magnetic Resonance
Pedro Lamosa - Nuclear Magnetic Resonance

FERMENTATION

João Nuno Carichas Carita

TEACHING LABORATORY

Teresa Baptista da Silva

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 11.5 % 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. This situation calls for an urgent action to increase permanent research positions at ITQB, and to establish clear conditions for career development based on scientific merit (and not solely on erroneous bibliometric formulas, such as that of the Leiden study). There is no substitute for a peer-based evaluation, as performed in 2007.

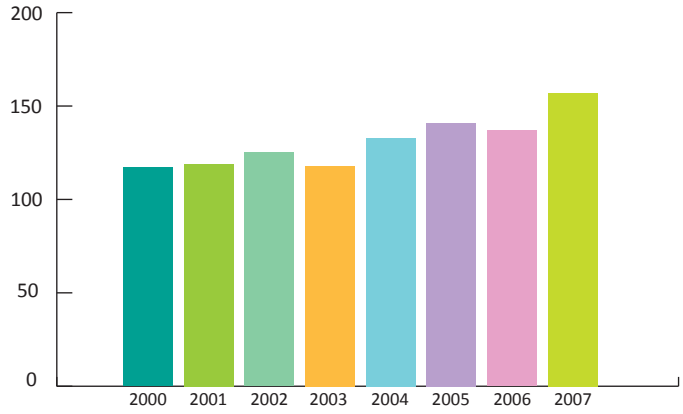
Currently, research at ITQB is supported by **350 researchers** excluding undergraduates and visiting scientists.

PhD holders	**157**
Permanent Staff	18
LA Contract	18
Other PhDs	7
Other institutions	32
Post Doctoral Fellows	82

PhD holder by gender: Female 93 / Male 64

Average age of a PhD holder: 39,3 years

PhD holders over the years:

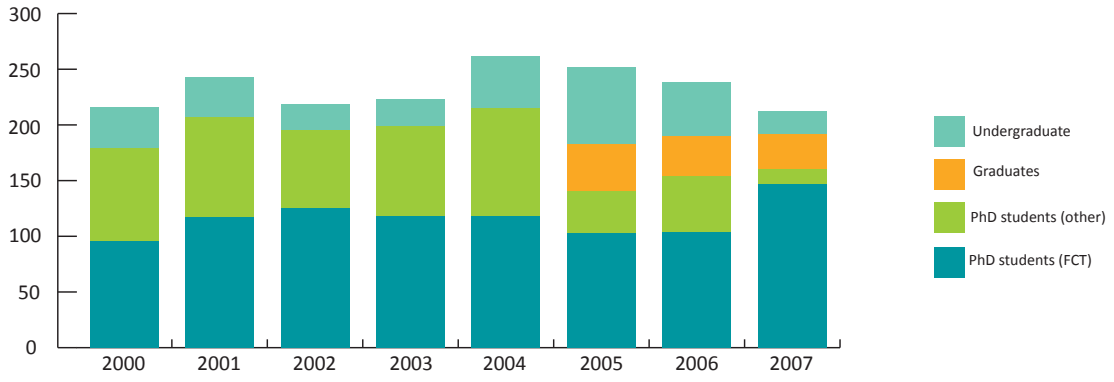


ITQB Relevant Statistics

Graduate students at ITQB, mostly PhD students, are integrated into ITQB's research groups where they undergo their training in scientific research (see also Education section). ITQB also welcomes students in the final year of their degrees for short periods of training in scientific research.

PhD students	**160**
FCT fellowships	147
Other sources of funding	13
Other Graduates (Science grantees)	**32**
Undergraduates	**20**

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.

Researchers Funding

The permanent research staff at ITQB is limited to a small number, currently 25. Additionally 18 researchers are supported through *Laboratório Associado* contracts. Most other researchers at ITQB are either staff members of other academic institutions (32 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 209 FCT grantees (147 PhD students and 62 post-docs).

RESEARCH

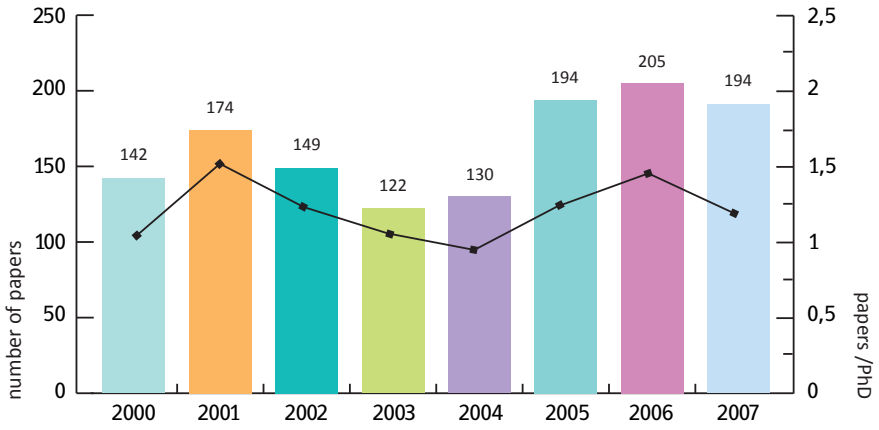
Each research laboratory at ITQB, variable both in size and structure, is managed independently by a senior researcher (Laboratory Coordinator). **Research Laboratories - 53 in 2007 -** are organized in Research Divisions - Chemistry, Biology, Biological Chemistry, Plant Sciences, and Technology – covering a wide range of scientific topics and methodologies. In many cases the allocation of a particular Laboratory to a Division is an organizational convenience and collaboration between Divisions is strongly encouraged.

Publications

During 2007, ITQB research scientists published **194 papers** in peer reviewed international journals. The multidisciplinary nature of ITQB research is visible in the number of papers (16 %) resulting from collaborations between different laboratories within or transversal to the research divisions. Scientific research is increasingly collaborative and international in nature. The number of publications involving other research institutions reflects ITQB's openness to external collaborations at national (60%) and international level (59%).
As of February 2008, some 84 papers had been published or accepted for publication.

Publications in peer reviewed journals over the years:

(absolute number and ratio per PhD holder)



ITQB Relevant Statistics

Projects

Research at ITQB is mainly supported by contracted projects with R&D funding agencies. Currently ITQB coordinates 75 research projects and further participates in 30 more. The total **105 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. This numbers do not include those that were approved on the 2006 call, whose starting date is in 2008.

Ongoing projects:

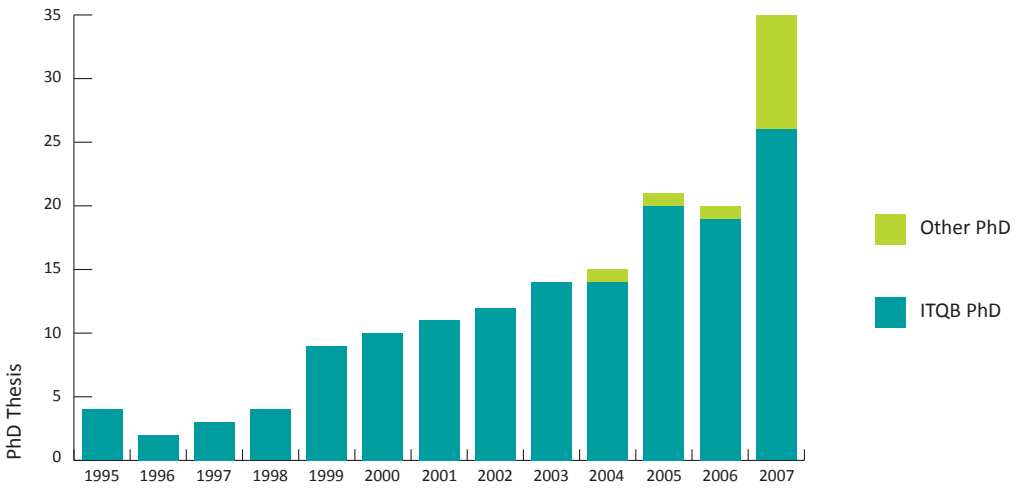
86 projects Fundação para a Ciência e a Tecnologia
1 project Re-equipment Program FCT
2 projects Fundação Calouste Gulbenkian
1 project Agência de Inovação
1 project Agência Portuguesa do Ambiente
1 project Sociedade Portuguesa de Gastrenterologia
8 projects European Commission
2 projects NIH
1 project European Free Trade Association
2 projects as Subcontracting Parties

ADVANCED EDUCATION

PhD Degrees

ITQB awards PhD degrees in Chemistry, Biology, Biochemistry and Chemical Engineering. Since 1995 ITQB has awarded **160 PhD degrees**.

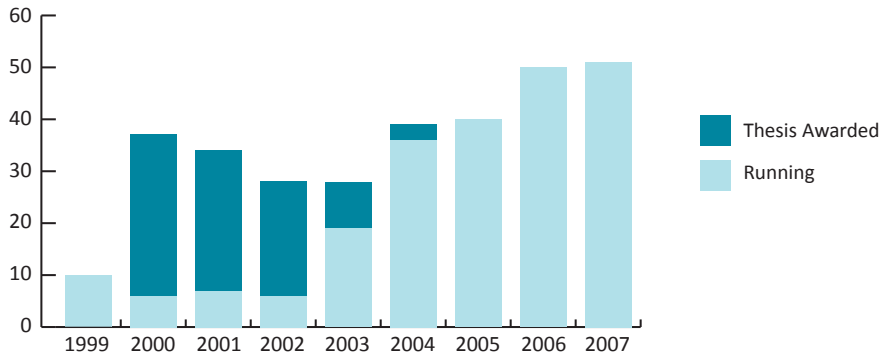
PhD thesis awarded at ITQB over the years:



In 2007, **35 PhD theses** were awarded at ITQB. This total includes 26 ITQB PhD students and 9 PhD students from other institutions and is distributed as follows: 16 in Biology, 12 in Biochemistry, 3 in Chemical Engineering, and 4 in Chemistry.

Throughout 2007, 51 new graduates registered at ITQB adding to a total of 225 registered PhD students by the end of the year. About 65 of these students do research at other institutions.

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.

ITQB is involved in the **Masters Degree in Medical Microbiology**, a collaborative Masters Course from Universidade Nova de Lisboa also envolving the Instituto de Higiene e Medicina Tropical, Faculdade de Ciências Médicas and Faculdade de Ciências e Tecnologia. During 2007, the **20 Master students** registered at ITQB have been preparing their dissertation theses.

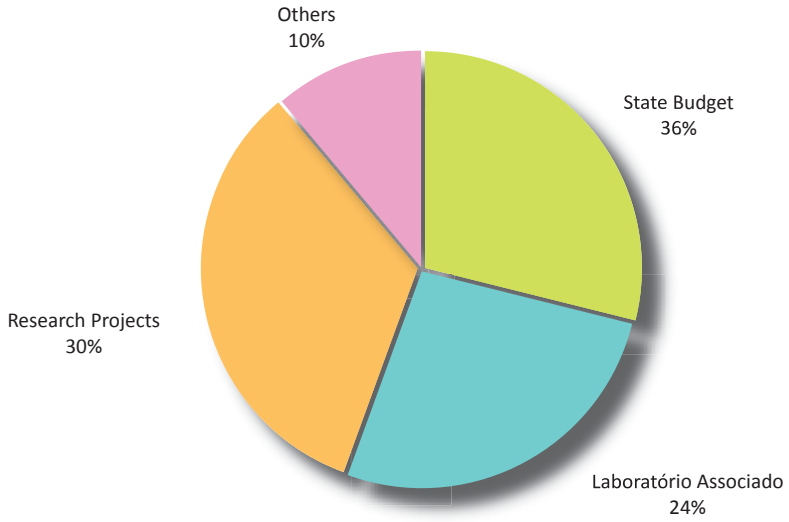
In 2007, 28 students have attended the **ITQB PhD program**, an intensive multidisciplinary course mandatory for first year PhD students at ITQB. Also, each PhD Student has to present two oral communications at during their doctorate period.

BUDGET

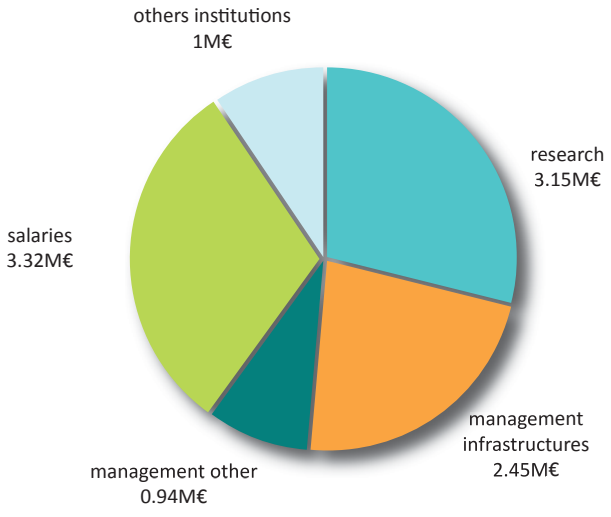
ITQB has two main sources of revenue; the State Budget (OE), attributed by the Ministry of Science, Technology and Higher Education, and the national science funding agency, *Fundação para a Ciência e a Tecnologia (FCT)*. The contribution from the State Budget, through Universidade Nova de Lisboa, represents less than half of the overall ITQB budget. FCT accounts for two sources of financial support, through the *Laboratório Associado* contract and through project funding, both of competitive nature. Additional sources for research projects include the European

ITQB Relevant Statistics

Commission, the *Fundação Calouste Gulbenkian*, the NIH and international cooperation projects. Other funding includes revenues from Masters Courses, the sale of analytical services, rental of rooms and facilities, etc.
The overall budget of ITQB for 2007 was around 10,8 M € originating from the following:



The corresponding expenses, summarized below, can be divided in: **research funds**, managed by the research groups (eg. research projects excluding overheads); **salaries**, a figure that comprises both administrative and research staff (including LA contracts); outflows to **other institutions**, through the LA contract or research projects; and **institute management**. Management comprises the **institute's infrastructures**, and **other management expenses**, such as the acquisition and maintenance of large common equipment, and support to research groups. For promoting a scientific strategy, the direction relies solely on this last parcel of the budget (0.94 M€).
In 2007, instead of distributing minor amounts of funds to all groups, the Direction deliberately decided to bet in the strategic development of the institute by allocating part of these rather short funds to support the installation of new ITQB researchers (Ciência 2007) and by investing in essential instrumentation. Groups facing financial difficulties, due to the continuous delay in payments from funding institutions, were also supported, as well as grantees, among other small expenses.



Institutional Relationships

Protocols established between ITQB and other institutions:

ITQB/IBET

ITQB's association with the Instituto de Biologia Experimental e Tecnológica (IBET), located in the same building, enables an interface between fundamental research and industrial applications.

Laboratório Associado

ITQB was one of the first research institutions to be awarded the status of *Laboratório Associado* (LA) by the Minister of Science and Technology, in 2001. Under the LA programme the Institute established a partnership with IGC and IBET to maximize its research and development potential.

ITQB/FCT/IBET

In 2007 was signed a protocol between the three institutions aiming at joint collaborations in undergraduate and graduate courses.

ITQB/FCUL

Protocol signed in 2005 between ITQB and Faculdade de Ciências da Universidade de Lisboa to regulate the cooperation of both institutions in research activities and advanced education.

ITQB/ISA

Protocol signed in 2004 between ITQB and Instituto Superior de Agronomia expressing a commitment to cooperate in scientific research activities and advanced education and to strengthen the research efforts in applied biology and biotechnology of both institutions.

Rede Nacional de Ressonância Magnética Nuclear

ITQB participates in Portuguese NMR network created with the support of the Portuguese Foundation for Science and Technology (FCT) in the frame of a national programme for acquisition and upgrading of scientific equipment. The network aims to stimulate the use of advanced facilities by Portuguese researchers and the sharing of the national scientific resources. The following laboratories are part of the Portuguese NMR Network: Universidade de Aveiro , Universidade de Coimbra through its Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa through Faculdade de Ciências e Tecnologia and the Instituto de Tecnologia Química e Biológica, Universidade Técnica de Lisboa through the Instituto Superior Técnico, Universidade da Madeira, Universidade do Minho and Universidade do Porto through its Faculdade de Ciências

Institutional Relationships

Rede Nacional de Espectrometria de Massa

Mass spectrometry is one of the main instrumental supports in scientific research, namely in life, chemical and environmental sciences. ITQB is one of the nine Portuguese Academic Institutions that integrates RNEM(Rede Nacional de Espectrometria de Massa), an infrastructural network launched in 2007, under the general National Program for Scientific Re-equipment.

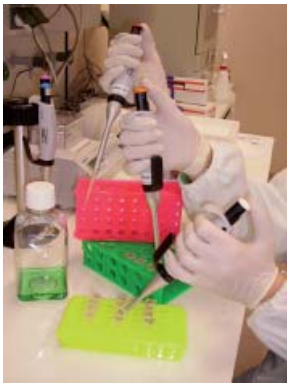
Massachusetts Institute of Technology - Portugal

The MIT - Portugal partnership is an international collaboration between the Portuguese state and the MIT, focused on the areas of engineering systems. This collaboration is centred on research, technology and higher education, and aims at promoting scientific and technological development. The Associated Laboratory ITQB/IGC/IBET is directly involved in the focus area of Bioengineering Systems.

ITQB- Unicat – Unifying Concepts in Catalysis, German Cluster of Excellence

ITQB is an International Partner of UniCat, the acronym for a new initiative on the area of catalysis research in the Berlin-Brandenburg area (Germany). UniCat is a Cluster of Excellence developed within the Excellence Initiative started by the German Federal and State Governments, under the supervision of the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG). The Cluster operation started in November 2007 and is a Consortium of the three Berlin Universities, two Max-Planck Institutes and the University of Postdam, being coordinated by the Technische Universität Berlin. As External Partners, it includes several large companies, and several Foreigner Academic Partners: besides ITQB, the Boreskov Institute of Catalysis, Novosibirsk, Russia, and Departments/ Research Centers from Cardiff University, UK, Hebrew University of Jerusalem, Israel, Northwestern University, Eindhoven, The Netherlands, Evanvston, USA, Pennsylvania Sate University, USA, University of Messina, Italy, University of Oxford, UK, Upsalla University, Sewden and Vrije Universiteit Amsterdam, The Netherlands.

The main scientific objective of this initiative is to unify concepts in catalysis by bridging the gaps between homogeneous and heterogeneous catalysis, between elementary gas-phase reactions and complex processes in highly organised biological systems, as well as between fundamental and applied catalysis research (http://unicat.tu-berlin.de).ITQB involvement will be mainly focused on biological catalysis, a key research topic at ITQB.
For 2008, are already planned one joint wokshop in Oeiras (ITQB/TU-Berlin), in September, and a Monographic Course on Vibrational Spectroscopy.

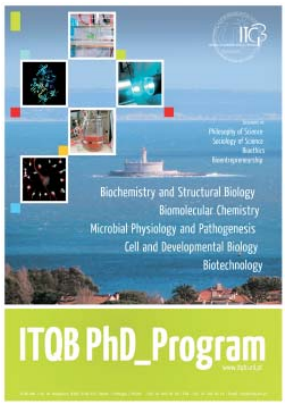


LABORATORY MANAGEMENT COURSE

A laboratory management course for independent scientists: "The art of leadership – fewer conflicts, more results" was organized within the framework of a mentoring program for younger group leaders at ITQB. Course coordination was assigned to Conor John Fitzsimons and Klaus Wagenhals, from MetisLeadership, the consultant team responsible for the EMBO Lab Management courses. The course took place in November, in Ericeira.

The aim of the Lab Management course was to help group leaders improve their skills in the areas of staff selection, leadership and team development, effective conflict and problem solving. The course consisted of brief presentations of management tools and several practical exercises in the form of role-play and discussion groups, providing hands-on experience.

Two editions of the course took place, with up to 16 participants each, from ITQB, IGC and IBET. Participants from the three institutions of the *Laboratório Associado* were intentionally mixed in both editions, with the purpose of promoting personal interactions that may evolve to scientific interactions in the future.
The course, assessed by means of a short questionnaire to the participants, was considered very positive: more than 95% of the participants said they liked the course very much and considered it useful to improve their performance as a group leader. The participants also expressed their interest in taking a follow up course focused on other relevant issues such as grant writing or communication skills.



A NEW ITQB PhD PROGRAM

Since 2002, research training leading to the degree of Ph.D. includes an element of formal teaching in addition to original research. The major goal of the ITQB PhD Program, totally restructured in 2007, is to provide further knowledge in the following areas: Biochemistry and Structural Biology, Biomolecular Chemistry, Microbial Physiology and Pathogenesis, Cell and Developmental Biology and Biotechnology. Advantage is taken of the multidisciplinary environment available at the campus.

During 5 weeks, in a full-time course, knowledge of the state-of-the-art science developing at the ITQB is transmitted to the students as well as the basics about advanced techniques in Biology, Chemistry, Biochemistry and Technology. A significant proportion of the course is devoted to developing critical thinking and interaction between the students. Additional sessions on the Sociology of Science, Philosophy of Science, Bioethics and Bioentrepreneurship take place. At the end, students are evaluated on the basis of a written critical appraisal of a scientific article.

The first edition of this ITQB PHD Program involved 28 students and was rated as very positive by both students and internal teaching staff.

ON THE COVER

One of the 2007 issues (volume 274) of the FEBS Journal displays on its cover a picture by Nuno Micaelo, PhD student at the Protein Modeling Laboratory/ITQB, featuring the article "Modeling hydration mechanisms of enzymes in non-polar and polar organic solvents" by Nuno Micaelo and Claudio Soares that can be found on pages 2424-2436.

Highlights 2007

CERMAX – CENTRO DE RESSONÂNCIA MAGNÉTICA ANTÓNIO XAVIER

Three new NMR spectrometers (400, 500, and 800 MHz - the highest field NMR spectrometer in Portugal) were installed at ITQB as part of the National Program for Scientific Re-equipment sponsored by FCT. This equipment is part of the National NMR Facility that was officially launched on July 9, 2007 with a Symposium that also honoured the memory of Prof. António Xavier, founder of ITQB and pioneer in the development of NMR in Portugal. Prof. Kurt Wüthrich, Nobel laureate in 2002 for his work on structural NMR of biological macromolecules, delivered a lecture at this Symposium. Pedro Lamosa, the Facility Manager of CERMAX, together with Patrick Groves and Manolis Matzapetakis (both holding Ciência 2007 positions), will promote collaborations with teams with important scientific questions that can be solved by NMR. CERMAX is coordinated by Helena Santos together with David Turner and Carlos Romão, and technical support is provided by Helena Matias and João Pires.

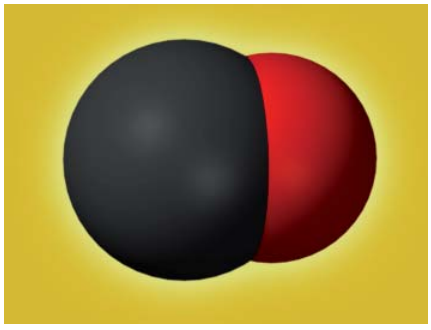
CIÊNCIA 2007

Under the Portuguese government initiative Ciência 2007, ITQB and IBET have appointed 18 research scientists, 15 and 3 respectively. The new researchers are expected to develop strategic areas of research as well as to conduct and support research associated with the National Facilities or critical technologies.

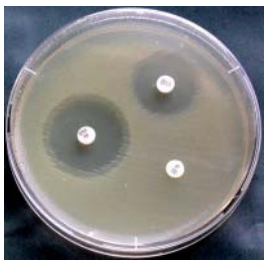
By recruiting additional high quality researchers, ITQB hopes to stay at the forefront of the Life Sciences and related fields, to strengthen some on-going research programs and to foster a closer association within the Laboratório Associado.

THE BRIGHT SIDE OF CARBON MONOXIDE

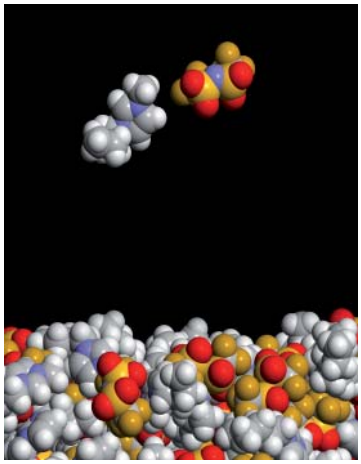
Research work led by the ITQB researcher, Lígia Saraiva, has demonstrated for the first time that carbon monoxide kills different kind of bacteria, including *Staphylococcus aureus*, a well known pathogen that has been acquiring a worrying resistance to currently used antibiotics. Also involved in this study were Carlos Romão, João Seixas and Lígia Nobre. Both commercially available and new CO-releasing compounds – produced and patented by the start-up company Alfama – have been tested and their ability to release CO inside the bacteria leading to their rapid death has been demonstrated. These findings could be the starting point for the development of novel types of therapeutic drugs designed to combat antibiotic resistant pathogens, which are widespread and presently a major public health concern. This discovery was patented by Alfama and the work was published in the journal Antimicrobial Agents and Chemotherapy.



BECOMING RESISTANT



A team led by Alexander Tomasz, invited full professor at ITQB and head of the Laboratory of Microbiology at Rockefeller University, with the participation of Herminia de Lencastre, head of the Molecular Genetics Laboratory at ITQB, studied samples of bacteria taken from the blood of a single patient over time and, by analyzing their genome, was able to track how bacteria modify their genes to become fully resistant to antibiotics in only three months. The research was reported in Proceedings of National Academy of Sciences.



THE MOST CITED PAPER IN CHEMISTRY

The paper on ionic liquids published last year in Nature by the team of Luis Paulo Rebelo, professor at ITQB, has now reached position #1 in the list of the most cited papers in chemistry.

This ranking from ISI Web of Knowledge considers all the papers published in the last two years and the corresponding citations in bimonthly periods. The citation rates are used by Science Watch, the editorial component of Thompson Scientific, to track trends and analyse performance in basic research.

The paper "The distillation and volatility of ionic liquids" was cited more than 40 times in June/July 2007 by papers from such diverse areas as chemistry, physics, biochemistry, biology and even astronomy, underpinning the growing importance of ionic liquids and its uses.

WASH YOUR HANDS

As part of their long-standing interest in bacterial epidemiology and infection control, the Laboratory of Molecular Genetics at ITQB developed a campaign to improve hand hygiene among children attending day care centres. From January to March 2007, and together with "Super Clean Hands", an attractive mascot created for this project, researchers visited eleven day care centres and talked with almost 1000 children about microbes and infections explaining that good hand hygiene is the first step to prevent transmission of common infectious diseases. This initiative met great success with important feedback from the schools. Parents and educators were also contacted to participate in the research project itself that enrolled over 800 children. Nasopharyngeal swabs were collected and analysed; bacteria were isolated, tested for antibiotic resistance, and further characterized by DNA fingerprinting techniques.

The whole research work is part of a European project (PREVIS) involving scientists, nurses, and pediatricians from different countries, with the aim of identifying bacterial genetic determinants and host factors associated with *Streptococcus pneumoniae* infections.

Highlights 2007

JOINING HEADS

The heads of laboratory of the *Laboratório Associado* ITQB got together for an informal meeting that took place in Vimeiro in February. During this symposium researchers from the three institutions that constitute the LA (ITQB, IBET and IGC) had the chance to introduce their research activities and expertises and look for new opportunities for collaboration. With this annual joint meeting the directors of the three institutes hope to further strengthen the scientific competences of the LA.

PROMOTING INTERNAL COLLABORATIONS

In order to strengthen the collaboration within the LA, the heads of the three institutions (ITQB, IBET and IGC) created a special fund for research projects involving researchers from at least two of these institutions. After a call for joint project proposals in May, six projects were selected and awarded with 10,000 Euros, distributed equally between each of the participant laboratory heads. The funded projects are listed below.

- Computational and experimental dissection of an ancient, robust protein shield.
  Adriano Henriques (ITQB) / José Leal (IGC)
- FlyingMould - Functional screening of anti-cancer activity of neoteric fungi metabolites.
  Cristina Silva Pereira (ITQB) / Rui Martinho (IGC) / Jörg Becker (IGC)
- How does a coccus divide in three ordered orthogonal planes over successive division cycles?
  Jorge Carneiro (IGC) /Mariana Pinho (ITQB)
- Studies on H_2S metabolism in Drosophila melanogaster.
  Manuela Pereira (ITQB) / Rui Martinho (IGC)
- Molecular and conformational analysis of Melanophilin domains and interacting partners.
  Miguel Seabra (IGC) / Cláudio Gomes (ITQB)
- Effect of HoxB4 on glycosylation patterns in differentiating mouse embryonic stem cells.
  Moises Mallo (IGC) / Júlia Costa (ITQB)

ALUMNI ITQB

During the last twelve years, ITQB has been playing a key role in the advancement of Portuguese Science. The training of specialized human resources, in particular PhD students, has been one of the major areas in which the institute has contributed to national scientific excellence. The current number of PhD holders trained at ITQB is now over 150. These former PhD alumni have followed the most diverse professional routes such as starting their own technology-based companies, leading international research groups in fundamental research areas, teaching, and have taken R&D positions in industry or in other research institutions.

Last year a few of us (Cláudio Gomes, Sergio Filipe and Karina Xavier) were challenged by the ITQB Director to start promoting a network between former ITQB PhD students – the ITQB alumni. With this aim we started by contacting all former ITQB alumni through a questionnaire in order to update their contact information, determine current professional activities, career paths and other aspects related to the fact of having received PhD training at ITQB. The feedback obtained from former students which have started their PhD formation at ITQB between 1996 and 2006, allowed some interesting observations. For example, we have learnt that 31% of former ITQB PhD students have obtained academic positions and are now doing research as independ-

ent group leaders, or integrated in existing research groups, in Portugal and abroad. On the other hand, around 13% have joined Biotech or IT companies, some of which abroad. The remaining former ITQB PhD students (47%) have had their PhD more recently and are still carrying out postdoctoral formation in different countries.

The ITQB alumni web site (www.itqb.unl.pt/alumni) was also implemented as a forum to disseminate the information gathered about previous PhD students, their research themes, professional trajectories and personal testimonials. Another initiative started in 2007 was the ITQB Alumni seminar series, in which previous alumni have the opportunity to present their career paths, talk about current interests and activities, and meet current students in an informal environment. After having launched this initiative in 2007 with two seminars, the program for 2008 is already prepared with a new talk every three months.

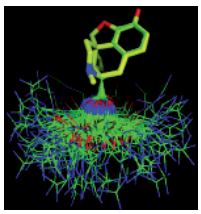
Overall, these are the initial steps towards a series of other initiatives that we aim to establish for setting up a stronger interaction between former ITQB Alumni, prospective students and the community. Such a network will be a powerful tool both to former ITQB students in shaping their current professional activities, and also to assist current and future PhD students, making them aware of the different career opportunities which are available to ITQB graduates. It is also the mirror of the scientific excellence of ITQB, and it is an important way to provide advise for scientific and teaching management at ITQB.

ANTÓNIO V. XAVIER SCIENTIFIC GRANTS

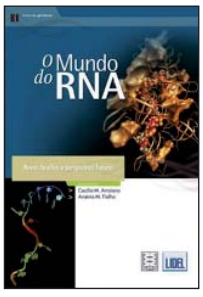
In 2006, the Oeiras City Council established a Scientific Grants Programme to honour the memory of Professor António V. Xavier, distinguished scientist and founder of ITQB, and to promote scientific development in Oeiras. This Grant Programme contemplates two types of grants: scientific excellence grants to be awarded to international renowned visiting scientists and "starting in Oeiras" grants to be awarded to investigators who are starting their own research group.

After a proposal of the Grants Scientific Commission, in 2007/8, the Oeiras City Council awarded two scientific excellence grants to Agílio Pádua and to Margarida Costa Gomes and two grants "Starting in Oeiras" to Cristina Silva Pereira and to Luis Jaime Mota.

ANALGESIC ON THE COVER



An article by Miguel Machuqueiro and António Baptista is highlighted on the cover of the Biophysical Journal (volume 92, issue 6) with the structure of the analgesic dipeptide kyotorphin. In their work "The pH-Dependent Conformational States of Kyotorphin: A Constant-pH Molecular Dynamics Study" the authors look at the effect of pH on the kyotorphin structure and infer on the nature of its receptor. Surprisingly, while known opioid receptors do not bind kyotorphin, some conformations of this endogenous neuropeptide are very similar to morphine, suggesting that the unknown kyotorphin receptor is probably more similar to the structural family of opioid receptors than anticipated.



RNA IN PORTUGUESE

The book "O mundo do RNA" coordinated by Cecília Arraiano, Senior Researcher at ITQB, and Arsénio Fialho, Associate Professor at Instituto Superior Técnico, and joining the contributions of Portuguese researchers with recognized work in the field of RNA, a crucial molecule for the control of gene expression, was published in March 2007.

This is the first book written in Portuguese about RNA biology, a topic of increased interest both for the understanding of genetics and for novel applications in Medicine. The list of book authors includes other ITQB researchers.

Awards and Nominations

Prémio Câmara Pestana 2007

Attributed to the research work "Biosynthetic pathways of inositol and glycerol phosphodiesters used by the hyperthermophile *Archaeoglobus fulgidus* in stress adaptation" published in the Journal of Bacteriology (2006).

This research project elucidated the biosynthetic pathways of three new solutes found in hyperthermophilic microbes, which are organisms effectively able to grow in extremely hot environments with temperatures close to 100ºC. The described compounds contribute to the stability of biological molecules at high temperatures and are therefore particularly important for biotechnological applications.

Christopher Maycock
Organic Synthesis Lab
(Head)

Filipa Siopa
Organic Synthesis Lab
(Graduate student)

Helena Santos
Cell Physiology and NMR Lab
(Head)

Luis Gafeira
Cell Physiology and NMR Lab
(Post-Doctoral researcher)

Marta Rodrigues
Cell Physiology and NMR Lab
(PhD student)

Nuno Borges
Cell Physiology and NMR Lab
(Post-Doctoral researcher)

Pedro Lamosa
Cell Physiology and NMR Lab
(Post-Doctoral researcher)

Rita Ventura
Bioorganic Chemistry Lab
(Head)

ISASF Best Thesis

Ana Rita Cruz Duarte (Former ITQB PhD Student - Nutraceuticals and Delivery Laboratory) "Exploring supercritical fluid technology for the development of controlled drug delivery systems", was distinguished by the International Society for advancement of Supercritical Fluids (ISASF) with the award for the Best Thesis in 2006, in this area.

Awards and Nominations

Technical University of Lisbon (UTL) / Santander Totta Awards

Manuela Chaves / ISA (Head of the Plant Molecular Ecophysiology Laboratory)
Distinguished by the Technical University of Lisbon with a first prize of the UTL/Santander-Totta award in Agronomy.

Ricardo Boavida Ferreira / ISA (Head of the Disease and Stress Biology Laboratory)
Distinguished by the Technical University of Lisbon (UTL) with an honourable mention of the UTL/Santander Totta award.

Lucélia Tavares (PhD - Disease and Stress Biology Laboratoy)
Distinguished by the Technical University of Lisbon (UTL) with a prize for best students of UTL/Santander Totta.

Awards at National and International Meetings

Cristina Escrevente (PhD Student - Glycobiology Laboratory)
Prize for Best Poster , Glupor 7, 7th International Meeting of the Portuguese Carbohydrate Group, ITQB, Oeiras

José Andrade (PhD - Control of Gene Expression Laboratory)
1st Prize for oral communication presented under the topic “Cell Physiology, Biochemistry and Molecular Biology” in the Congress of the Portuguese Societies of Microbiology, Biotechnology and Genetics (MICRO/BIOTEC/XXXIIIJPG2007).

Sandra Viegas (Post Doc - Control of Gene Expression Laboratory)
2nd Prize for oral communication presented under the topic “Genetics and Comparative and Evolutionary Genomics” in the Congress of the Portuguese Societies of Microbiology, Biotecnology and Genetics (MICRO/BIOTEC/XXXIIIJPG2007).

Tiago Vicente (PhD Student - Animal Cell Technology Laboratory)
1st prize for communication at the 14th International Conference on Biopartitioning and Purification: “Understanding anion exchange membrane chromatography: optimization of Rotavirus like-particles purification” Vicente T., Sosa M. Q., Peixoto C., Carrondo M. J. T., Alves P. M.

Science and Society at ITQB

ITQB's commitment to excellence in scientific research contemplates a program for raising public awareness of science. In the present scientific and technological society, only well informed citizens can attain full citizenship. While placing emphasis on children and young students, the outreach activities at ITQB aim to promote the appreciation and understanding of science across all sectors of society. In this view, ITQB has four main lines of action for bringing science closer to the public. To reach young people and teachers; to encourage researchers to engage with the public; to promote science by stimulating the interface with other disciplines; and to keep people informed and up-to-date on ITQB main achievements.

Reaching young people and teachers

Following a practice established long ago, ITQB receives visits from high school students throughout the year; each visit being organized according to the teacher's requests on specific research topics. In total, almost 500 students from all over the country visited ITQB in 2007. Overall, teachers rated the visits as "very interesting" and "very useful" and ITQB researchers enjoy interacting with both students and teachers.

ITQB laboratories also welcome University students group's visits. These tend to be more focused on specific scientific issues and represent valuable opportunities to discuss research career perspectives.

In April, ITQB hosted the Olímpiadas da Química Junior. In this event, organized by the Sociedade Portuguesa de Química, high school students are quizzed for their chemical knowledge.

To celebrate the National Science and Technology week in November, ITQB hosted a new series of debates on topics ranging from astrobiology to science policy, and including a session on careers in research. The debates were attended by students and teachers from neighbouring schools that engaged in discussions with ITQB researchers. Thanks to the ITQB / Oeiras City Council joint project, participating schools were awarded vouchers for educational material to support science teaching.

Summer time at ITQB was highlighted once more by the presence of high school students in the laboratories, as part of the Ciência Viva Program Ciência nas Férias. This time, ITQB also welcomed four students from Spain that, together with their Portuguese counterparts, got acquainted with life at the bench.

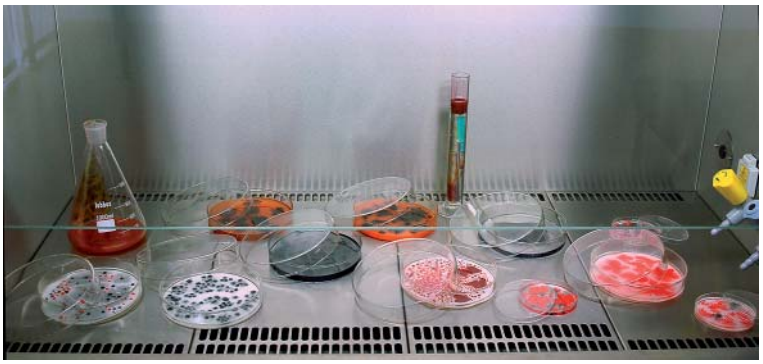
Encourage researchers to engage with the public

Besides interacting with students and teachers, researchers are encouraged to engage with the public at large in other initiatives; and the most illustrative event is the ITQB Open Day. On this day, the institute's research activities are displayed on the entrance floor of the main building in many attractive formats, such as demonstrations and hands-on experiments, and the visiting public is invited to interact with the researchers. The work and dedication of researchers and staff that, starting months ahead, plan and put together this day is an impressive example of the cooperative spirit present at ITQB, only matched by the visitor's enthusiasm. In 2007, ITQB welcomed over 1500 visitors who spent a Saturday discovering how science can be exciting and fun.

2007 was also a time to celebrate Portuguese Science under the Ciência 2007 initiative and all Associate

Laboratories were invited to organize a science day at Pavilhão do Conhecimento. At ITQB's stand, visitors were invited to appreciate the complexity of life by building their own models of cells and proteins. Starting from these models, ITQB researchers talked about their own research activities.

Promote science by stimulating the interface with other disciplines – Art & Science

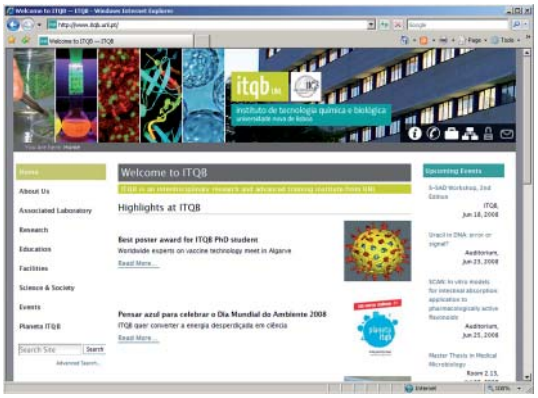


Art and artists have been amongst ITQB's science, namely through the work of Nuno Micaelo, Paisaigens moleculares (Molecular Landscapes), and more recently with the residence of Marta de Menezes at the Laboratory of Microbial Technology (project Decon).

In 2007, ITQB participated in the initiative for Artistic Residences in the Laboratories, promoted by Ciência Viva and Direcção-Geral das Artes. Many artists submitted projects to develop their work at ITQB. The project *Diferentes olhares sobre os objectos científicos* (Different looks on scientific objects), by Patricia Noronha, was selected. The artist worked at ITQB over a three-month period developing art pieces that join common microbiology materials with artist's pigments and resins. These pieces were already on display at ITQB's Open Day 2008.

The work of Patricia Noronha is now being extended, as a tool to stimulate children to expand their artistic skills, by being challenged to use non-conventional painting methods, and at the same time stimulate their scientific curiosity on microbial life.

Keep people informed and up-to-date on ITQB research achievements



Science as the pursuit of knowledge about the world is intrinsically interesting. But science and its outcomes are sometimes difficult to grasp by non specialists (and these include scientists from other fields). ITQB recognizes its mission of keeping society informed on its research activities.

In 2007, the webpage of the institute was fully restructured to facilitate updated information to all interested parties. To give just a few examples, all laboratory pages include a segment (in Portuguese) that explains in lay terms what the mains objectives of the laboratory are; main research achievements are highlighted on the homepage; a science and society section keeps the public informed on upcoming, and past, outreach activities; and there is a page dedicated to the press. All the information in the webpage is complemented with leaflets and brochures targeting different segments of the public.

Besides being available for all media contacts, ITQB regularly issues press releases, informing on major scientific breakthroughs or other relevant activities at the Institute.

The role of the Science Communication Office is essentially to establish bridges between the scientific endeauvour and other sectors of society. For further details please visit our webpage at www.itqb.unl.pt.

Scientific Services

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 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
Pedro Lamosa, lamosa@itqb.unl.pt

GLP 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. This unit provides services to researchers from within ITQB or elsewhere but also to private or public companies.

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 LAB

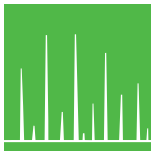
Analytical Laboratory (LA)

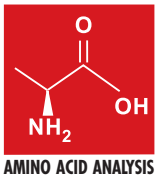
The laboratory has a long track record of providing services using chromatographic (HPLC and GC with several detectors) and electrophoretic methods for pharmaceutical, agro and chemical industry and academia.
António Ferreira, antoniof@itqb.unl.pt

MICROBIOLOGY LAB

Microbiology Laboratory (LM)

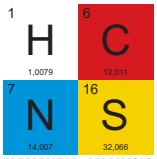
The laboratory has provided services for the pharmaceutical industry, for pharmaceutical formulas and API, agro-industry and academia. Services include in vitro potency assays, protein quantification, molecular biology analysis (GMOs in food and feed and other) and detection and quantification of impurities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


MASS SPECTROMETRY



AMINO ACID ANALYSIS


PROTEIN SEQUENCING



ELEMENTAL ANALYSIS

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 inorganic compounds to peptides, oligosaccharides, nucleotides and proteins. Part of this laboratory is associated with the Mass Spectrometry National Facility.

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

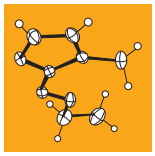
Free or proteic amino acids analysis. Some less common amino acids such as hydroxyproline, hydroxylisine, glucosamine and galactosamine can also be quantified on request.
Paula Chicau, chicau@itqb.unl.pt

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 in carbon, nitrogen, hydrogen and sulphur.
Conceição Almeida, salmeida@itqb.unl.pt


SMALL MOLECULE X-RAY CRYSTALLOGRAPHY

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
Coordinator: M. A. Carrondo

Other Facilities

Library

In 1999 the Library started with a few print and online subscriptions. Today we have access to the largest publishers and the most important journals in science (over 4000 titles).
In 2004 we took part in the beginning of the national initiative b-on (Biblioteca do conhecimento online). Since then we have online access to Elsevier, Springer, Wiley, American Chemical Society and more.
Apart from the specific resources that we subscribe on our own, 2007 was a year of collaborations. Together with the Universidade Nova de Lisboa we subscribed to: Blackwell, Science, Nature, JSTOR and Kluwer Law.

ITQB alone subscribes to:
- American Society for Microbiology (11 journals)
- Annual Reviews Back files
- Journal of Biological Chemistry
- Plant Cell and Plant Physiology
- Proceedings of the National Academy of Sciences (2008)
- Structure (Cell Press)

And the very important book series Methods in Enzymology (online).
Susana Lopes Ferreira, biblio@itqb.unl.pt
Coordinator: M. S. Teixeira

Fermentation Unit

This unit is only available for inhouse researchers, and is devoted to small or large scale cell growth of a multitude of diferent organisms. The unit is in charge of keeping the relevant collection of bacterial strains.
João Carita, carita@itqb.unl.pt
Coordinator: M. S. Teixeira

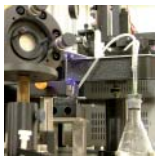
Teaching Laboratory

The Teaching Laboratory is designed and equipped to support the teaching activities of the Institute in areas ranging from Biochemistry to Genetics. These have included advanced courses (e.g., the MX course on protein crystallography), Master Degree courses, or science education and communication activities (e.g., “Olimpíadas da Química Junior”).
The staff of the teaching laboratory has provided support to two formal Courses at the Master degree level:
i) the Master Degree Course in Medical Microbiology, a joint initiative of four Institutions belonging to Universidade Nova de Lisboa. Instituto de Higiene e Medicina Tropical (IHMT), Instituto de Tecnologia Química e Biológica (ITQB), Faculdade de Ciências Médicas (FCM) and Faculdade de Ciências e Tecnologia (FCT);
ii) the Master Degree Course in Clinical Microbiology, which is organized by Faculdade de Medicina da Universidade de Lisboa (FM-UL).
Teresa Baptista da Silva, teresas@itqb.unl.pt
Coordinator: A. O. Henriques

Laboratory Manager

The ITQB Lab Manager is responsible for the management of all purchasing, maintenance and servicing activities of scientific equipment and consumables for the Institute. This new position, created in 2007 aims at having a efficient and professional way of purchasing, and supervise the common scientific equipment, supporting the researchers and leading to significant budget savings.
Cláudia Almeida, calmeida@itqb.unl.pt
Coordinator: L. M. Saraiva

Major equipment available at ITQB



- ATR-FT- Infra Red Spectroscopy
- Circular Dichroism Spectropolarimeter
- Computational Cluster for Structural Bioinformatics
- Dynamic Light Scattering Particle Sizer / Instrument for Zeta Potential and Molecular Mass Determination
- Electron Paramagnetic Resonance Spectroscopy
- Fluorescence Deconvolution Microscope
- Fluorescence Recovery After Photobleaching (FRAP)
- Greenhouse 300m2
- High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection
- ITQB/IBET Pilot Plant
- Mass Spectrometer (MALDI-TOF, API-3D ion trap, LC-API-3D ion trap, LC-nanoESI-linear ion trap)
- NMR spectrometers (300, 400, 2x500 and 800 MHz)
- Raman Spectrometer, including surface enhanced and time resolved Raman
- Room Temperature Time Resolved Phosphorescence
- Steady State Fluorescence and Steady State Fluorescence Anisotropy
- Surface Plasmon Resonance (Biacore)
- Walk-in Plant Growth Chambers 2x6m2 and Rooms 3x2m2
- X-ray Difractometer
- Several Stopped Flow Systems
- Oxygen and nitric oxide amperometers
- Differential Scanning Calorimeter

Ciência 2007

As a result of the LA proposal submission to the Ciência 2007 call for researcher positions, the three institutions were awarded 32 positions: 15 for ITQB, 3 for IBET and 14 for the IGC.

The ITQB/IBET positions were announced jointly and were widely advertised at the ITQB's website, the official FCT website, in Nature jobs, in national newspapers, through national and international societies such as the Society of Biological Inorganic Chemistry, and by direct contacts with European research institutions.

The jury for ITQB/IBET positions was formed by the Directors of the three LA institutions, the Vice-Director of ITQB, the Executive Director of IBET, representatives of the main research areas of ITQB, and two renowned scientists from other European institutions. For particular research areas and when found necessary, other scientists were asked for advice.

For the 18 positions announced, 196 applications were received. The whole juri had access to all applications, but sub-committees were formed to analyse each position. These sub-committees reported back to the whole juri where final decisions were taken.

Each appointed candidate discussed with the ITQB Direction the conditions needed for their research plan in terms of lab space, budget, and equipment availability.

The list of researchers appointed under Ciência 2007 is the following:

Andreas Bohn (ITQB)

PhD in Physics, 2003, Darmstadt University of Technology, Germany

Systems Biodynamics

The Systems Biodynamics Group applies quantitative computational tools to study dynamical processes in multicellular systems under experimental conditions close to real-world environments. The current focus is on stochastic growth processes in phototrophic biofilms and the synchronization of interacting circadian rhythms.

The large-scale objective in fundamental research is to contribute to the discovery of universal principles of robust functioning of living organisms in fluctuating environments. In applied research, the aim is to catalyze the rate of scientific knowledge discovery by pursuing a systems biology approach. The group aims to integrate data-driven analysis and information-management tools with hypothesis-driven methods for quantitative modeling and simulation. The transversal nature of this approach enables interdisciplinary interactions with a large spectrum of laboratories, in particular in the area of environmental biosciences and -technology.

Selected Publications

1) Bohn A, García-Ojalvo J. 2008. Synchronization of coupled biological oscillators under spatially heterogeneous environmental forcing. J Theor Biol 250: 37-47.

2) Bohn A, Zippel B, Almeida JS, Xavier JB. 2007. Stochastic modeling for characterization of biofilm development with discrete detachment events (sloughing). Water Sci Technol 55 (8-9): 257-264.

Cristina Silva Pereira (IBET)

PhD in Biochemistry, 2004, ITQB-UNL

Applied and Environmental Mycology

Filamentous fungi ensure the most important degradative event in the carbon cycle of earth - the decomposition of the highly recalcitrant plant composites. Their use in biodegradation/bioremediation processes is often sub-optimal because there are still critical knowledge gaps concerning the diversity, activity and dynamics of the microbial communities responsible for those processes. A multi-functional approach linking community composition to function, in specific environments, is a major challenge. For that critical fungal metabolic pathways are being initially pursuit (for e.g. biocomposites and POPs degradation).

Fungal degradation of water-insoluble residues to their forming elements, controlling biocatalysis efficiency trough the solvent, represents an ambitious scientific breakthrough. Ionic liquids are by definition salts liquid at room, or nearby, temperature. The high plasticity of their synthesis, the possibility of tailoring their functionality, and their tremendous solvating properties are being explored as to augment fungi biotechnological potential. Fundamental and applied questions are being pursuit: fungi transcriptional response in the salty-milieu, and IL-biocatalysis potential use for producing biological active molecules and as a technology for toxic waste management, respectively. This pioneering research line involves a scientific partnership with Prof. LPN Rebelo (ITQB-UNL) and Dr. J Becker (GE).

Selected Publications

1) C. Silva Pereira, GAM Soares, AC Oliveira, ME Rosa, H Pereira, N Moreno and MVS Romão. Effect of fungal colonization on mechanical performance of cork. 2006. Int Biodeter Biodegr. 57(4): 244-250 IBB: MS501208

2) I.S. McLellan, M. Carvalho, I. Martins, C. Silva Pereira, A.S. Hursthouse, C. Morrison, P. Tatner, M.C. Leitão and M.V. San Romão. 2007. Polychlorinated phenols and cork forest ecosystems: A review. J. Environ. Monit. DOI:10.1039/B701436H

Dirk-Jan Scheffers (ITQB)

Ph.D. in Molecular Microbiology, 2001, University of Groningen, the Netherlands

Bacterial Membrane Proteomics

Bacteria carefully regulate their shape by coordinating cell division and growth of the cell wall. These processes require the coordinated action of various proteins which are thought to be organised in large complexes. The molecular composition of these complexes however, is not known, and studies into the composition of these complexes are complicated by the fact that most of the proteins involved are embedded in the bacterial membrane.

The aim of the Bacterial Membrane Proteomics laboratory is to isolate complexes of membrane proteins involved in cell division and cell wall growth using native conditions. This will allow the identification of complex components, study of the protein-protein contacts involved in complex formation, and the analysis of factors involved in targeting of complex components to their site of action. Initially, the studies will be undertaken in the model organisms *Escherichia coli* and *Bacillus subtilis*. Once established, the methods will be applied to membrane protein complexes involved in other processes like respiration, signalling etc, and other micro-organisms.

Selected Publications

1) Scheffers D.-J., Robichon, C., Haan, G.J., Den Blaauwen, T., Koningstein, G. Van Bloois, D.W., Beckwith, J. and Luirink, J. (2007) Contribution of the FtsQ transmembrane segment to localization to the cell division site. Journal of Bacteriology, 189: 7273-7280

2) Scheffers, D.-J. and Pinho, M.G. (2005) Bacterial cell wall synthesis – new insights from localization studies. Microbiology and Molecular Biology Reviews, 69: 585-607

Luís Jaime Mota (ITQB)

PhD in Biology (2001) by Universidade Nova de Lisboa - Instituto de Tecnologia Química e Biológica.

Infection Biology

Our laboratory will study type III secretion (T3S), a virulence mechanism used by many pathogenic Gram-negative bacteria to deliver effector proteins into the cytoplasm of animal or plant cells. The effectors interfere with diverse functions of the host cell. We intend to focus on the molecular mechanisms which guarantee that effector secretion is specifically triggered upon contact between a bacterium and a host cell, and on the molecular and cellular function of effector proteins, which are priceless tools to study eukaryotic cell biology.

Our first model system will be Yersinia. In humans, pathogenic Yersinia cause diseases ranging from bubonic plague, caused by *Y. pestis*, to gastrointestinal syndromes, caused by *Y. enterocolitica* and *Y. pseudotuberculosis*. Yersinia virulence is dependent on the well characterised Ysc T3S system. In addition, high virulent Y. enterocolitica strains possess a second T3S system, named Ysa, whose role in virulence and mode of action are largely unknown. We will study the Ysa system, which secretes seven effector proteins of uncharacterised function, also aiming to contribute to a better understanding of *Yersinia virulence*.

Selected publications

1)Ramsden, A. E., Holden, D. W., Mota, L. J. (2007). Membrane dynamics and spatial distribution of Salmonella-containing vacuoles. Trends Microbiol 15: 516-524.

2)Mota, L. J., Journet, L, Sorg, I., Agrain, C. and Cornelis, G. R. (2005). Bacterial Injectisomes: Needle Length Does Matter. Science 307: 1278.

Manolis Matzapetakis (ITQB)

Ph.D. 2004 in Biological Chemistry, University of Michigan, Ann Arbor, USA

Biomolecular NMR

Antonio Xavier Magnetic Resonance Centre - CERMAX

Our focus is the application of NMR to various biomolecular problems. We are interested in protein structure determination - dynamics, protein-protein interactions including the study of metalloproteins and large proteins.

Topics of research include the:

• Study or ferrous transport mechanisms in pathogenic bacteria.

While significant amount of research has been done in the field of sequestering and transport system of iron we are still discovering new proteins that seem to function as transporters. We are interested in the structural and functional characterization of such proteins with an emphasis in pathogenic bacteria.

• Development of protocols for NMR based structural proteomics,

Develop protocols for high throughput use of high field NMR in proteomics. Application of fast NMR acquisition and automated structure determination. Optimization of NMR friendly, high throughput protocols for protein production.

• In cell protein NMR.

Use in-cell NMR as a quick way to evaluate protein folding inside the cell.

Selected Publications:

1)Bermel, W., I. Bertini, I.C. Felli, M. Matzapetakis, R. Pierattelli, E.C. Theil, P. Turano, A method for C<alpha> direct-detection in protonless NMR. J. Mag. Res., 2007. 188(2): p. 301-10.

2)Matzapetakis, M., P. Turano, E.C. Theil,I. Bertini, (13)C- (13)C NOESY spectra of a 480 kDa protein: solution NMR of ferritin. J Biomol NMR, 2007. 38(3): p. 237-42.

Patrick Groves (ITQB)

PhD in Chemistry, 1994, Cambridge University, UK

Molecular Interactions and NMR

Antonio Xavier Magnetic Resonance Centre - CERMAX

Transfer-NMR (TR-NMR) experiments can specifically provide the spectrum of a ligand that binds to a biomolecule (diffusion NMR – DOSY), even if it is present in a mixture. Saturation Transfer Difference (STD) NMR gives the interaction surface for the bound molecule. Transfer nuclear Overhauser effect spectroscopy (TR-NOESY) gives the bound conformation around flexible bonds. TR-NMR is used to screen small molecule libraries (pharmaceutical applications) and to investigate specific ligands (biochemical applications). TR-NMR methods have wide applicability at the ITQB.

Selected Publications:

1) P. Groves, K.E. Kövér, S. André, J. Bandorowicz-Pikula, G. Batta, M. Bruix, R. Buchet, A. Canales, F.J. Cañada, H-J. Gabius, D.V. Laurents, J.R. Naranjo, M. Palczewska, S. Pikula, E. Rial, A. Strzelecka-Kiliszek, and J. Jiménez-Barbero "Effect of temperature in Saturation Transfer Difference NMR experiments", Magn. Reson. Chem., 2007, 45, 745-8.

2) K.E. Kövér, P. Groves, J. Jiménez-Barbero and G. Batta (2007) "Molecular recognition and screening using STD NMR: 15N-group selective STD NMR experiment to study intermolecular interactions in heavily overlapped spectra" Journal of the American Chemical Society, 129, 11579-11582.

Olga Iranzo Casanova (ITQB)

PhD in Chemistry 2003, University at Buffalo - SUNY, NY

Bioinorganic Chemistry and Metallopeptide/protein Design

The research in this group is focused on the design and characterization of new metallopeptides with the final objective of obtaining functional molecules. This research hinges on the central theme of using small peptides as starting frameworks to introduce metal centers that either will mimic the active site of natural metalloenzymes or will be novel metal binding sites. These constructs are excellent starting points for tailoring known and novel functionalities that are difficult to engineer in any other molecular scaffold, in particular in small molecules. This is very appealing because relevant natural enzymes are not available for many industrial applications, mainly due to the high substrate selectivity that enzymes have and the reaction conditions they require. The fact that these peptides can be produced by chemical synthesis opens the door to the introduction of unnatural amino acids and useful chemical probes in specific positions, expanding in this way the scope of functionalities that one can evolve. This research will improve our understanding of how the peptidic framework can tailor the metal ion properties to achieve the target functionality, and lay the groundwork for designing therapeutic peptides; novel catalysts for chemical, biochemical and biotechnological applications; and biosensors for metal ions.

Selected Publications:

1) O. Iranzo, P.V. Thulstrup, S-B. Ryu, L. Hemmingsen and V. L.Pecoraro. "The application of 199Hg NMR and 199mHg PAC Spectroscopies to Define the Biological Chemistry of Hg(II): A Case Study Using Designed Two and Three Stranded Coiled Coils". Chem. Eur. J., 2007, 13, 9178-9190.

2) O. Iranzo, C. Cabello and V .L. Pecoraro. "Heterochromia in Designed Metallopeptides: Geometry-Selective Binding of Cd(II) in a De Novo Peptide". Angew. Chem. Int. Ed., 2007, 46, 6688-6691.

Pedro M. Domingos (ITQB)

PhD in Developmental Biology, 2001, University College London

Developmental Neurobiology

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

Selected Publications

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

2) Domingos PM, Mlodzik M, Mendes CS, Brown S, Steller H, Mollereau B. (2004) Spalt transcription factors are required for R3/R4 specification and establishment of planar cell polarity in the Drosophila eye. Development. Nov;131(22):5695-702.

Smilja Todorovic (ITQB)

PhD in Physical Chemistry, 2000, University of Belgrade / Mayo Clinic, Rochester, MN

Raman Spectroscopy of Metalloproteins

Our research is focused on understanding how different proteins fine-tune the reactivity of their metal cofactors and additionally, how the metal centres help to define the functional features, dynamics and stability of protein molecules. We use resonance Raman spectroscopy, in stationary and time resolved modes, on a wide variety of structurally and functionally different proteins in order to access the details of the interplay between the protein matrix and the metal.

Resonance Raman spectroscopy of the heme, blue copper, non-hemic iron and iron-sulfur proteins that we study, can reveal highly specific and sensitive information on discrete metal site(s): its ligation pattern, the thermodynamic parameters that control electron transfer in redox proteins, or short-lived intermediates of the catalytic cycle of an enzyme. We use surface enhanced resonance Raman spectroscopy for studying redox properties of respiratory chain complexes immobilized on metal electrodes. If coated with biocompatible monolayers, they can mimic some basic features of a biological membrane, allowing investigations of the proteins in a quasi-physiological environment.

Selected Publications

1) Todorovic, S., Pereira M., Bandeiras, T., Teixeira, M., Hildebrandt, P., and Murgida, D. H. `Midpoint potentials of Hemes a and a3 in the Quinol Oxidase from *Acidianus ambivalens* are Inverted` (2005) J. Am. Chem. Soc. 127 13561-13566

2) Todorovic, S., Leal, S. S., Salgueiro, C. A., Zebger, I., Hildebrandt, P., Murgida, D. H. and Gomes, C. M. ´A Spectroscopic Study of the Temperature Induced Modifications on Ferredoxin Folding and Iron-Sulfur Moieties´ (2007) Biochemistry 46 10733-10738

Yann Astier (ITQB)

PhD in Physical Chemistry 2001, Southampton University

Single Molecule Processes

This new group at ITQB is focussed on the study of chemical and biochemical processes at the single molecule level. Observing a single molecule and its stochastic interactions with another gives a bottom up understanding of bulk chemistry. While bulk chemistry relies on the notion of equilibrium, single molecule processes know no such thing.

We use protein nanopores such as alpha-hemolysin in a single channel electrophysiology set-up. The alpha-hemolysin, with a 1.5 nm diameter at the narrowest part of the pore, is used as an ionic magnifying lens to observe a single molecule trapped inside it.

Selected Publications

1) Yann Astier, Denis E. Kainov, Hagan Bayley, Roman Tuma, Stefan Howorka,.Stochastic Detection of Motor Protein-RNA Complexes by single channel current recording. Chem. Phys Chem. 2007, 8, 2189 . 2194.

2) Haichen Wu, Yann Astier, Giovanni Maglia, Ellina Mikhailova and Hagan Bayley. α-Hemolysin pores with covalently attached molecular adapters. J. Am. Chem. Soc.(2007), 129 (51), 16142 -16148.

Ciência 2007

Catarina Duarte (IBET)

PhD in Chemistry, 1997, Universidade Nova de Lisboa

Nutraceuticals and Controlled Delivery

Scientific Area: Particle Engineering using supercritical fluids

Developing clean processes for obtaining bioactive natural concentrates, new functional foods/nutraceuticals and improved delivery systems.

Carla Pinheiro (ITQB)

PhD in Biology, 2004, ITQB (UNL)

Laboratory: Plant Biochemistry

Scientific Area: Plant Sciences

Develop proteomics workflows for the study of plant responses to abiotic stress and to promote the use of proteomics techniques by ITQB researchers.

Célia Maria Valente Romão (ITQB)

PhD in Biochemistry, 2003,ITQB, Universidade Nova de Lisboa

Lab.: Metalloproteins and Bioenergetics/ Macromolecular Crystallography Unit - Structural Genomics

Scientific Area: Structure and biochemistry of metalloproteins/Metallomics

Study of metal containing proteins, namely those involved in iron metabolism in bacteria.

Isabel Maria Travassos de Almeida de Jesus Bento (ITQB)

Ph.D. in Biochemistry, 2003, Universidade Nova de Lisboa, ITQB

Laboratory: Structural Genomics - Macromolecular Crystallography Unit

Scientific Area: Biological Chemistry

Coordination of the small molecule X-ray crystallography facility at ITQB.

José Manuel da Silva Simões Esperança (ITQB)

PhD in Chemical Engineering 2004, Universidade Nova de Lisboa
Laboratory of Molecular Thermodynamics
Scientific Area: Ionic liquids for Chemical and Biological Processes.
Synthesis, characterization, and study of properties of new liquid salts and ionic liquid containing solutions aiming at exploiting new chemical and biological extractive and purification processes.

Nelson José Madeira Saibo (ITQB)

Ph.D. in Biotechnology 2003, Ghent University (Belgium)
Laboratory: Plant Genetic Engineering
Scientific Area: Functional analysis of transcription factors in plants
Functional analysis of transcription factors involved in plant responses to abiotic stress: an integrative approach.

Nuno Miguel Formiga Borges (ITQB)

PhD in Biochemistry, 2004, Universidade Nova de Lisboa, ITQB
Laboratory: Cell Physiology and NMR
Scientific Area: Physiology of extremophiles
Understanding how hyperthermophilic archaea sense and respond to heat stress.

Tiago Miguel Guerra Miranda Bandeiras (IBET)

PhD in Biochemistry, 2005, ITQB, Universidade Nova de Lisboa
Laboratory: Pilot Plant
Scientific Area: Structural Biology and Drug Discovery.
Protein Expression and Purification for academic and industrial partners.

Research Laboratories

"*Coomassie*" Ricardo Gouveia, PhD Student
ITQB from within - Internal photo competition held by the occasion of the Open Day 2007

Chemistry Division

The chemistry division consists of seven research groups and is the smallest at the ITQB. It will increase in size early 2008 to nine groups with the inclusion of a Bioinorganic chemistry group (Olga Iranzo) and a Nanotechnology group (Yann Astier) as a result of the Ciência 2007 program. This will increase the already wide range of activities of the division and present excellent opportunities for intra-divisional collaborations.

The activities within the division 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 and 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 contributs to the nations research status but also to the economic well-being of its means of production.

The Colloids, Polymers and Surfaces Laboratory, headed by António Lopes, has continued the several lines of action in the colloidal domain, namely, i) the modification of natural-occurring water soluble polysaccharides giving rise to biocompatible hydrogel systems applicable as pharmaceutical patches and the studies of the drug release from them (in collaboration with Hospital Garcia de Orta); ii) the studies of the self-aggregation of Room Temperature Ionic Liquids and the characterization of such aggregates (surface tension, NMR, fluorescence, light-scattering); iii) the extraction and characterization of the polyphenolic materials from cork origin.
The Laboratory of Microheterogeneous Systems (Eurico Melo) is involved in the study of molecular diffusion and percolation in lipid lamellar phases using fluorescence recovery after photobleaching, and singlet and triplet states emission quenching and emission anisotropy. Present work involves the structural characterization of lipid aggregates with high cholesterol content, such as those 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, probably related to what is known as “lipid rafts”.

The Homogeneous Catalysis group (Beatriz Royo) has been studying new uses for dioxo-molybdenum(VI) and oxo-rhenium(VII) and (V) complexes as catalysts in reduction reactions. The group has demonstrated the excellent efficiency of MoO_2Cl_2 as catalyst for the hydrosilylation of carbonyl groups, and has extended the use of high-valent oxo complexes to the hydrogenation of unsaturated organic molecules by demonstrating the capability of these compounds to activate H_2 under mild conditions.

Another area of interest in the group is the use of N-heterocyclic carbenes (NHC) as ligands for middle and late transition metals. This year was synthesized the first chiral Cp*-NHC ligand and was studied is coordination to different metal centers.

Reversing chemical tradition and lore has been the aim of the Organometallics group (Carlos Romão) turning oxidising metal oxides into catalysts for reducing reactions. Also the well known toxin carbon monoxide has been turnewd into bacteriocides and anti-inflammatory drugs by coordinating it to a metal so that it is released slowly and therefore dangerously high concentrations are avoided.
Several new aza and oxaaza macrocycles have been prepared by the Supramolecular Chemistry group (Rita Delgado) and show interesting complexation properties both for cationic species and anions derived from organic acids. The application of several ligands to Magnetic Resonance Imaging have also been a subject of study. These chelating agents have a large number of potential applications both for medicine and bioremediation.

The Bioorganic Chemistry group (Rita Ventura) has begun a new project related to the synthesis of hypersolutes and methods of high throughput screening for new candidates. Using resins as scaffold di and trisaccharides are being constructed which will be screened using an ELISA instrument. The use of

magic angle spinning NMR has been important for the characterisation of the immobilised compounds and this has been possible due to the installation of the CERMAX . The conditions which effect the selectivity of glycosidation reactions in non-immobilised systems is also a subject of ongoing general carbohydrate research.

Chemical resolution of cyclopentenone and cyclohexenone systems, which have wide ranging application in organic synthesis using a novel aziridination reaction has been the primary aim of the Organic Synthesis group (Christopher Maycock). The stereoselective reactions of these acyl aziridines is also of interest. A project involving the functionalisation of nanoparticles for use as probes for biological systems has also begun. Complex magnetic or fluorescent nanoparticles can be prepared having a wide range of molecular appendages which permit biocompatibilty and transport within biological entities.

Bioorganic Chemistry

Rita Ventura

Auxiliary Investigator

PhD 1999 in Chemistry, Universidade Nova de Lisboa

CDP-D-*myo*-inositol and CDP-L-*myo*-inositol **1** (Fig. 1) were synthesised for the Helena Santos research group (Cell Physiology and NMR). Enantiomerically pure D and L-*myo*-inositol 1-P **2** (Fig. 1) were obtained through a significant modification and improvement of the established procedures and then coupled to form CDP-D-*myo*-inositol and CDP-L-*myo*-inositol. In order to discover new compounds that inhibit protein aggregation and/or are able to rescue misfolding defects, we started to construct a library of carbohydrate derived solutes using solid support synthesis. The vast diversity of compounds will be used in the study of the mechanisms by which these solutes influence the aggregation, fibrillation and misfolding phenomena. The more active compounds could be employed as possible therapeutic drugs against amyloidal diseases or provide clues and serve as a basis for the future development of such drugs.

The useful separable galactose thioglycosides **3** and **4** (Fig. 2) were efficiently prepared based on the same reactions used previously for the glucose thioglycoside **5** (obtained as a mixture of anomers where the α-anomer was the major one). The easily differentiated C-6 hydroxyl group in these compounds was important for the constraction of suitable intermediaries linked at this position to a Tentagel resin (**6**, Fig. 2), and these polymer bound glycosyl donors are some of the scaffolds to be used to construct our library. Oxidation of the primary C-6 alcohol in **5** and its corresponding epimer mannose afforded the glucuronic and mannuronic acids **7** and **8** (Fig. 2), which are two other scaffolds. The outcome of this reaction was highly dependent on the quality of the oxidant reagent. Glycosylation reactions using the methyl esters of **7** and **8** as glycosyl donors did not afford high anomeric selectivity, but this problem could be easily overcome changing our synthetic strategy. With the uronic acid solute analogues another charged group is introduced in the final solute structure.Glucosyl donor **5** afforded high α-selectivities in the glycosylation reaction. However the degree of selectivity was dependent on the glycosyl acceptor. The highest selectivities (α:β >>9:1) were obtained when the glycosyl acceptor was another glucose derivative, thus being very useful for the synthesis of di- and higher saccharides. The selectivity obtained with the corresponding galacotsyl and mannosyl donors were not so good and further studies are under way.

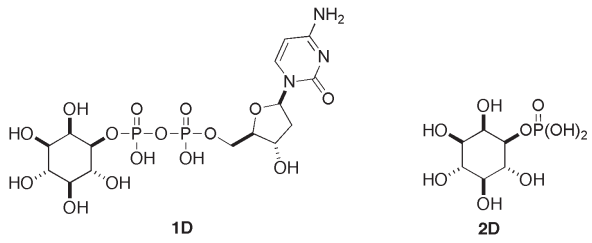


Figure 1

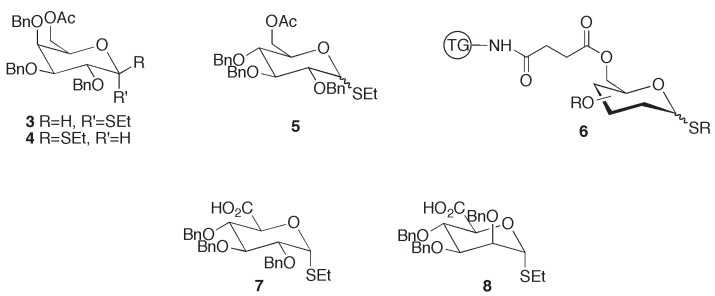


Figure 2

Group Members

Eva Lourenzo	Master Student
Matia Curci	Graduate
Daniela Canilhas	Undergraduate
Nuno Soares	Undergraduate

Selected Publications

Rodrigues M. V., Borges N., Henriques M., Lamosa P., Ventura R., Fernandes C., Empadinhas N., Maycock C., da Costa M. S. and Santos H. (2007)."*Bifunctional CTP: Inositol-1-phosphate cytidylyltransferase/CDP-inositol: Inositol-1-phosphate transferase, the key enzyme for di-myo-inositol-phosphate synthesis in several, (hyper)thermophiles*." Journal of Bacteriology **189**(15): 5405-5412.

Colloids Polymers and Surfaces (CoPoS)

António Lopes

Professor Associado - Universidade Lusófona de Humanidades e Tecnologias

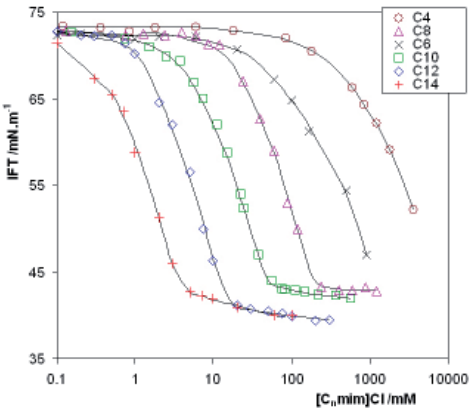
Phd 1997, Universidade Nova de Lisboa -ITQB

Group Members

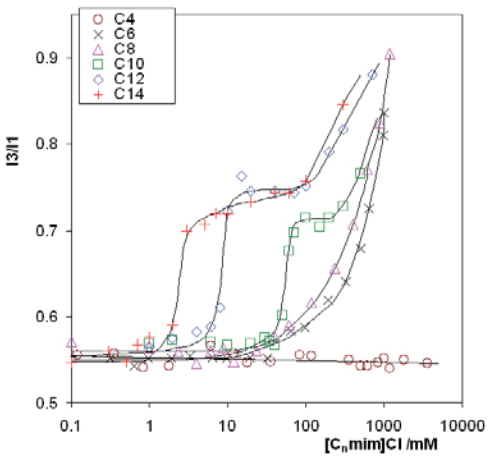
Rui Silva	Graduate
José Almeida	PhD Student
Carla Antunes	PhD Student
Marijana Blesic [1]	PhD Student

[1] co-supervision (Luis Paulo Rebelo)

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 into 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 focusing on 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.



Monitoring the self-aggregation of [C$_n$mim]Cl (RTIL of the 1-alkyl-3-methylimidazolium chloride family) in water using surface tension results for different chain lengths: *n*= 4-14



1-alkyl-3-methylimidazolium chloride family) in water using fluorescence (of pyrene) results for different chain lengths: *n* = 4-14

Selected Publications

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**(5): 481-490.

Coordination and Supramolecular Chemistry

Rita Delgado

Associated Professor with agregação at Instituto Superior Técnico (UTL)

PhD 1985 in Chemistry, Instituto Superior Técnico

The design of molecular architectures, as receptors for the detection and the selective removal of pollutants, such as pesticides and aromatic hydrocarbons, or dicarboxylate anions, was carried out. The receptors form homodinuclear copper(II) complexes and can recognize substrates that bridges the metal centres, forming cascade species, see an example in Figure 1. They can also selectively encapsulate substrates forming supermolecules, see Figure 2. Several families of receptors have been synthesized and the binding affinities between them and the substrates have been studied by several techniques. For instance, the values of the binding constants revealed that the affinity of the H_i[26]phen$_2$N$_4$O$_2^{i+}$ receptor increases with the number of coupled aromatic units and with the number of carboxylate groups of the substrate; consequently, very large association constants were found for pyrene-1-carboxylate and benzenetricarboxylate anions, revealing that this receptor can be used for the uptake of both anions from a solution containing other carboxylate anions at pH values of 3.0 and 5.5, respectively. Our studies also showed that the relative positions of the two carboxylate groups in the benzene ring are responsible for different binding affinities.

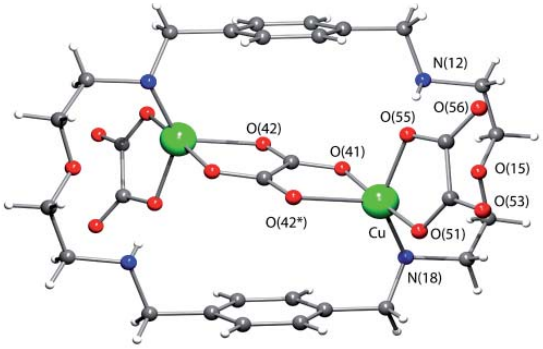


Figure 1. Overall structure of a cascade copper(II) complex containing oxalate anions.

The synthesis of three macrocyclic compounds useful for the selective uptake of toxic metal ions, such as Cd^{2+} and Pb^{2+} has been achieved. The compounds are a bis-*N*,*N*-(5-methylimidazol-4-ylmethyl) derivative of a 14-membered tetraaza macrocycle, and the dioxadiaza- and trioxadiaza-macrocycles containing one rigid dibenzofuran unit and *N*-(2-aminoethyl) pendant arms.

A 13-membered benzodioxotetraaza macrocycle (13bzN$_4$) exhibited an excellent affinity for copper(II), due to the good fit of copper(II) into its cavity. This chelator was labeled successfully with ^{67}Cu (yield > 98%) in mild conditions, indicating that this chelate is promising for future applications in nuclear medicine. The most common chelators used in MRI and as radiopharmaceuticals for medical diagnosis and tumour therapy, H$_4$dota, H$_4$teta, H$_8$dotp and H$_8$tetp, were examined from a chemical point of view.

The remarkable differences of chelation ability between the 12- and the 14-membered tetraazamacrocyclic derivatives with methylcarboxylate and methylphosphonate pendant arms were discussed on the basis of their thermodynamic stability constants, X-ray structures and theoretical studies.

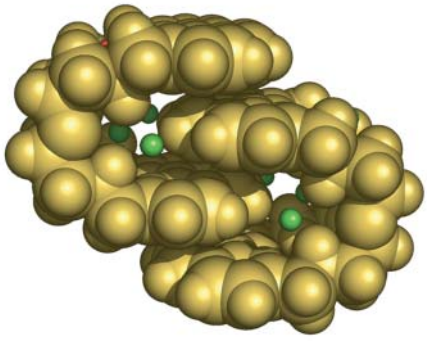


Figure 2. Structure of [26]phen$_2$N$_4$O$_2$ as bromide salt, side view of two interpenetrated supermolecules composed of two units. Oxygen atoms from water molecules are in red, bromide atoms are in green, and receptors are in yellow.

Group Members

Luís Lima	PhD Student
Carla Cruz	PhD Student
Pedro Mateus	PhD Student
Ana Piloto	PhD Student
Nicolas Bernier	Post Doc

Selected Publications

Carvalho S., Delgado R., Drew M. G. B., Félix V. (2007) " *Dicopper(II) complexes of a new di-p-xylyldioxatetraazamacrocycle and cascade species with dicarboxylate anions: thermodynamics and structural properties*" , Dalton Trans., 2431-2439.

Cruz C., Delgado R., Drew M. G. B. and Félix V. (2007) " *Evaluation of the binding ability of a novel dioxatetraazamacrocyclic receptor containing two phenanthroline units: selective uptake of carboxylate anions*" , J. Org. Chem., **72**, 4023-4034.

Delgado R., Félix V., Lima L. M. P. and Price D. W. (2007) " *Metal complexes of cyclen and cyclam derivatives useful for medical applications: a discussion based on thermodynamic stability constants and structural data*", Dalton Perspective, Dalton Trans., 2734-2745.

Homogeneous Catalysis

Beatriz Royo

Auxiliary Investigator

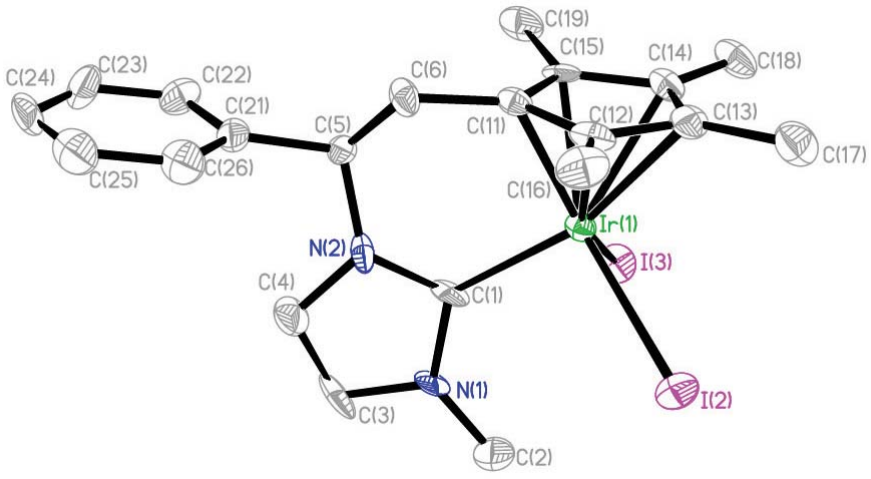
PhD in 1992, Sussex University

Group Members

Patrícia Reis	Post Doc
Kandepi Mohan	Post Doc
André Costa	PhD Student
José Brito	PhD Student

High-valent transition metal oxo complexes are commonly encountered in oxidation reactions such as olefin epoxidation and oxygen transfer. Recently, rhenium and molybdenum oxo complexes have been shown to catalyze reductions of organic compounds. This novel reactivity represents a complete reversal from the traditional role of these compounds as oxidation catalysts. During the last three years, we have been studying new uses for dioxo-molybdenum(VI) and oxo-rhenium(VII) and (V) complexes as catalysts in reduction reactions. We have demonstrated the excellent efficiency of MoO_2Cl_2 as catalyst for the hydrosilylation of carbonyl groups and we have proposed a new mechanism for the hydrosilylation reaction in which radical intermediates are involved. We have extended the use of high-valent oxo complexes to the hydrogenation of unsaturated organic molecules by demostrating the capability of these compounds to activate H_2 under mild conditions.

These species are efficient catalysts for the hydrogenation of alkynes to alkenes using H_2 and we have also proved their ability to perform the catalytic deoxygenation of substrates such as sulfoxides and epoxides using H_2 as a reducing agent and water as the final oxygen sink. Another area of interest in our group is the use of *N*-heterocyclic carbenes (NHC) as ligands for middle and late transition metals. This year we have synthesized the first chiral Cp*-NHC ligand shown in Figure 1, and we have studied its coordination to different metal centers. The novel chiral Ir complex (Cp*-NHC)IrI_2, Figure 1, has been prepared and characterized by X-ray diffraction studies. The new compound showed high catalytic activity toward hydrogenation transfer, b-alkylation of secondary alcohols with primary alcohols and amination with primary alcohols.



X-Ray diffraction molecular structure of (Cp*-NHC)IrI_2

Selected Publications

Reis P. M. and Royo B. (2007). "*Perrhenic acid as catalyst for the hydrosilylation of aldehydes and ketones and for the dehydrogenative silylation of alcohols*." Catalysis Communications **8** (7): 1057-1059.

Costa P. J., 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.

Laboratory of Micro-Heterogeneous Systems

Eurico Melo

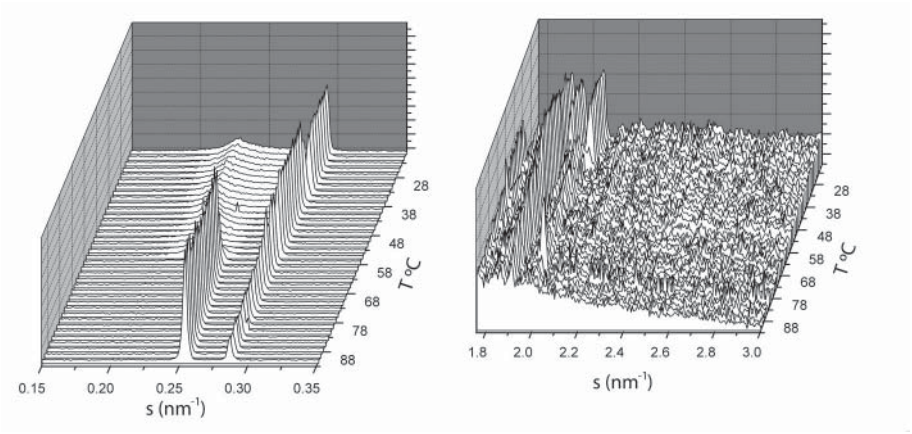
Assistant Professor at Instituto Superior Técnico, UTL

PhD 1986 in Chemical Engineering, Universidade Técnica de Lisboa (IST)

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 modeling 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 cholesterol content, such as those 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, probably related to what is known as “lipid rafts”.

Along 2007 we studied the mixtures of lipids with cholesterol, namely mixtures of ceramide C16 with cholesterol and those of palmitoyl oleoyl phosphatidylcholine and sphingomyelin with cholesterol. Both systems where characterized by small and wide-angle X-ray scattering and the results of this research are now submitted for publication. We have also initiated the comparative study of the diffusion of phospholipids with saturated and unsaturated carbon tails, in an attempt to prove the formation of 2-dimensional lipid protoaggregates due to caophilic-caophobic interactions.



Temperature dependence of the structure of a ceramide:cholesterol (40:60) mixture followed by small and wide-angle X-ray scattering. For this particular composition a well characterized complex is observed.

Group Members

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

Selected Publications

Polívka T, Pellnor M, Melo E, Pascher T, Sundström V, Osuka A, and Razi Naqvi K (2007) “*Polarity-Tuned Energy Transfer Efficiency in Artificial Light-Harvesting Antennae Containing Carbonyl Carotenoids Peridinin and Fucoxanthin*” J. Phys. Chem. C 111:467-476

Organic Synthesis

Christopher D. Maycock

Associate Professor, , Faculdade de Ciências, Universidade de Lisboa

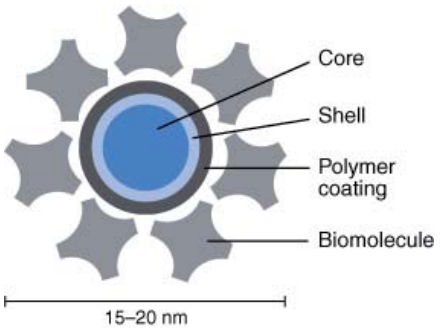
Phd 1978 in Organic Chemistry, University of Newcastle upon Tyne, UK

Group Members

Avedissian Hovsep	Post Doc
Paula Rodrigues	PhD Student
António Fernandez	Graduate
Vera Lopes	Technician

One of our key projects is the development of new strategies for the synthesis of natural products. Complex highly functionalised asymmetric compounds need to be synthesised estereoselectively and ultimately enantioselectively. The use of natural products as starting materials available from renewable biomass is a sustainable process which is environmentally friendly. Studies using natural chirality have continued.

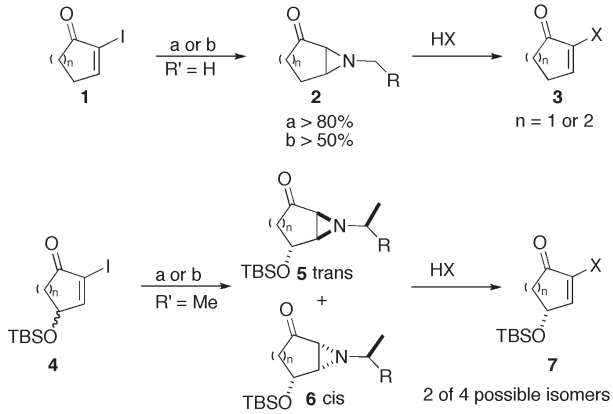
Aziridines fused to cyclohexanones or cyclopentanones have proven to be interesting intermediates for synthesis. 2-Oxo-7-azabicyclo[4.1.0]heptanes and 2-oxo-6-azabicyclo[3.1.0] hexanes can be prepared readily from the corresponding 2-iodocyclohexenones by reaction with primary amines. These compounds can be stereoselectively functionalised at other positions on the bicycle and the aziridine removed to form 2-heteroatom substituted cycloalkenones. Thus the aziridine group functions as a protecting group. Reaction of racemic 4-substituted 2-iodocyloalkenones with chiral amines produces diastereoisomers which can be separated. They can be reconverted into optically pure 4-substituted cycloalkenones or used for stereoselective reactions and then converted into further substituted cyclohexenones. A highly stereoslective aziridination method has been found. The aziridine group thus serves as a protecting group a method of resolution of enantiomers and as a stereodirecting group. We hope to apply all of these attributes into a synthesis of an optically active molecule.



Functionalised nanoparticles can have complex structures

Nanotechnology is a rapidly expanding field and the synthesis and manipulation of nanoparticles has become an important area of study. The functionalisation of nanoparticles with organic molecules for use as biological probes has made them into useful tools for the diagnosis and treatment of a wide range of life threatening ailments. Nanoparticles can have many useful physical properties, thay can be fluorescence or magnetic or just inert materials to which ligands can be readily attached. They can be functionalised to form quite complex structures with specific transport properties and specificities which are dependent upon the ligands which can be attached covalently or weakly via electroststic (acid/base) interactions.

In collaboration with the group of Dr. Abel Oliva we are preparing functionalised quantum dots and magnetic nanoparticles for probing parasite infected blood cells. This work will be expanded to other fields for which nanoparticles will be designed to target specific biological membranes and to assist in the discovery of the pathways for diseases development.



Aziridines can be used for the resolution of racemates

Selected Publications

Marta V. Rodrigues, Nuno Borges, Mafalda Henriques, Pedro Lamosa, Rita Ventura, Chantal Fernandes, Nuno Empadinhas, Christopher Maycock, Milton S. da Costa, and Helena Santos, *"Bifunctional CTP: Inositol-1-Phosphate Cytidylyltransferase/ CDP-Inositol:Inositol-1-Phosphate Transferase, the Key Enzyme for Di-myo-Inositol-Phosphate Synthesis in Several (Hyper)thermophiles"*,Journal of Bacteriology,2007, **189**, 5405-5412.

Organometallic Chemistry

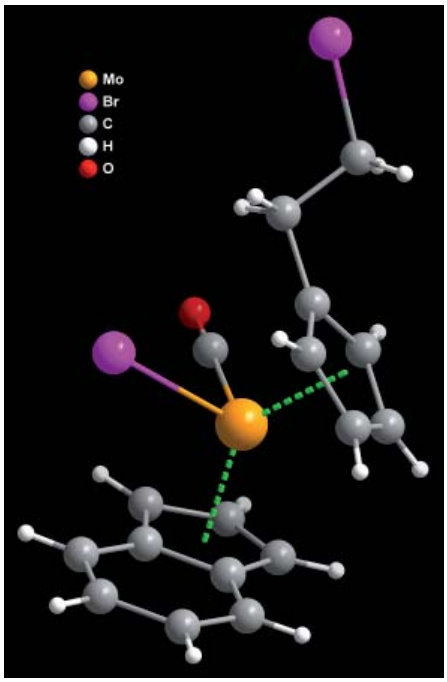
Carlos C. Romão

Full Professor

Phd 1979, Instituto Superior Técnico, Technical University of Lisbon, Portugal

Bridging Inorganic and Organic Chemistry, Organometallic Chemistry (OMC) strongly changed chemical synthesis by creating catalysts for many applications widely used in both largescale (refineries) and small scale (fine chemicals and pharmaceuticals) industries. Importantly, organometallic compounds have found applications in 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 (H_2O_2) 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 2007 new experimental systems were introduced both homogeneous (soluble) and heterogeneous (insoluble).In 2005 we had disclosed anew 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 like 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.

Throughout 2007 further results on this area include a thorough computational study of these reactions and experimental advances to other applications.The development of organometallic compounds for therapeutical applications is the other main focus of our research within the independent company, Alfama Inc., operating at 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. This topic was enriched with a new family of molybdenocene compounds. However, the highlight in this area comes from the discovery of the bactericidal capacity of CO releasing molecules. The potential for the development of a new type of bactericidal or antibiotic drugs remains to be explored.



The molecular structure of a derivative of Molybdenocene which has been functionalized to acquire better biocompatibility features for anti-tumoral applications

Group Members

Ana C. Fernandes	PhD
Carla Reis	PhD
Jan Honzicek	Post Doc
José Fernandes	Post Doc
João Seixas	PhD Student

Selected Publications

Nobre L. S., Seixas J. D., Romão C. C. and Saraiva L. M. (2007)."*Antimicrobial action of carbon monoxide-releasing compounds*."Antimicrobial Agents and Chemotherapy **51**(12): 4303-4307.

Costa P. J., 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.

Honzicek J., Paz F. A. A. and Romão C. C. (2007). "*Synthesis, characterization and stability of spirodiene complexes of Molybdenum(II): New route to ansa-molybdenocene and ring-functionalized molybdenocene compounds*" European Journal of Inorganic Chemistry(18): 2827-2838.

Biological Chemistry Division

The 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 defences, 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 and extracellular respiration of importance in bioremediation of contaminated environments. More applied studies target the structural characterisation of proteins with pharmacological or health importance, and the nanoengineering of enzymes through rational and directed evolution approaches for 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 O_2 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 2007 the Macromolecular Crystallography Laboratory was divided into three new Laboratories: Structural Genomics, Structural Biology and Industry and Medicine Applied Crystallography. Together with the Membrane Protein Crystallography, they associated to form the Macromolecular Crystallography Unit, sharing a common infrastructure while pursuing different and complementary research interests. The Membrane Protein Crystallography is one of the participants on a Marie Curie Initial Training Network (FP7), together with 12 other European academic research groups and 3 industrial companies partners to work on "Structural Biology of Membrane Proteins".

Recent discoveries in the Molecular Genetics of Microbial Resistance Laboratory within the Division demonstrated that CO-releasing compounds causes rapid death of phatogenic bacteria like *E. coli* and *S. aureus*. This constitutes the first evidence that CO can be used as an antimicrobial agent and is the starting point for the development of a novel type of therapeutic drugs. The mechanisms underlying the bactericidal action of CO are being investigated.

During 2007 the BC division was comprised of 14 laboratories, 35 PhD researchers, 41 PhD students, 8 graduate students and 3 undergraduates. 30 national projects were coordinated by members of the BC division, which also participated in 7 EU projects. 9 PhD students defended their thesis. In 2007 40 papers were published in international journals. The BC division is characterised by an extensive network of collaboration with other ITQB laboratories, which is reflected in the number of papers indicating internal collaborations (15). This characteristic, which has been a constant throughout the years, is a key point of its success.

In 2007 meetings were organised by members of the division: "2007 BIOXHIT Workshop on S-SAD diffraction data phasing of macromolecule single crystals from home and Synchrotron X-ray sources", October 3-6, at ITQB, Oeiras; 7th Short course of the Portuguese Biophysical Society "Biospectroscopy and Imaging". 19-21 of October 2007, Santarém; 7th Young Scientist Forum in Vienna as a satellite meeting of 32nd FEBS Congress. During the Forum a round table was organized on issues related with the Young Scientist career.

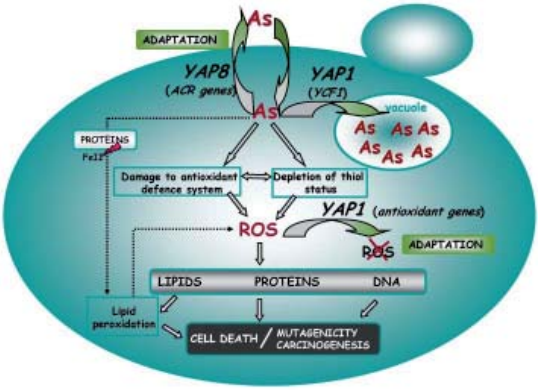
Genomics and Stress Laboratory

Cláudina Rodrigues-Pousada

Invited Full Professor

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

1. Deciphering the functional roles of Yap1 and Yap8 in yeast adaptation to arsenic compounds Yap1, a member of Yap family is the major regulator of oxidative stress response and plays a role in protecting cells against heavy metals and metalloids in *Saccharomyces cerevisiae*. Yap8, another Yap member, plays an essential and well-defined role in arsenic stress responses by regulating the expression of an arsenate reductase (*ACR2*) and an arsenite efflux-protein *(ACR3)*. These factors share a common activation mechanism through nuclear accumulation mediated by the disruption of the interaction of the exportin Crm1 to the nuclear export signal (NES) and recognize the same *cis*-element TGATTAATAATCA. Besides the contribution of Yap1 to arsenite detoxification through the regulation of the vacuolar-pump encoded *YCF1*, we showed that it is even more important to prevent the accumulation of reactive oxygen species (ROS) generated by inorganic arsenic exposure. Lipid peroxidation contributes to ROS formation in cells exposed to arsenic compounds being the antioxidant defences encoded by *GSH1, SOD1* and *TRX2* induced in a Yap1-dependent manner. The level of induction is well-correlated with the increased amounts of arsenite retained in the cytoplasm. Genome transcriptional profiling of the wild type strain stressed with arsenate reveals that genes of the functional categories related with sulphur and methionine metabolism and with the maintenance of the cell redox homeostasis are highly activated. To analyze whether both activators share similar signal transduction mechanisms we are investigating the effect of mutations in specific subunits of the tail module of the mediator complex, which is involved in the transduction of the signal from specific transcription factors to the basal transcriptional machinery.



Pathways of arsenic damage and contribution of Yap1 and Yap8 to the adaptation process

2. Decoding the anaerobe bacterium *Desulfovibrio gigas* **genome sequence** The negative impact of hydrogen sulphide in the oil industry and human health make the study of SRB metabolism very attractive. Deciphering the complete genomes of SRBs will contribute to unravel the energy transduction pathways of these bacteria. In collaboration with STAB Vida, we have recently launched a project to sequence the genome of *Desulfovibrio gigas*. Preliminary analysis of the genome sequence is summarized in Table1. The encoded predicted polypeptides involved in (i) cellular processes, (ii) environmental and genetic information and (iii) metabolism. One third of the genes have no function assigned. A whole genome survey of *D.gigas* revealed the presence of 16 NorR-like members. A NorR conserved molecular signature, in addition to the phylogenetic analysis of the 16 members, suggests that only one member should be a NoR transcription factor. The ongoing biochemical analysis with a strain mutated for the putative NorR of *D.gigas* will undoubtedly help to elucidate its role in NO detoxification.

Description	Number
Double stranded plasmid templates	14782
Average of edited read length (bp)	762
Number of bases assembled	18×10^6
Number of unique bases	3.88×10^6
Contig number	188
Average of gap length (bp)	1700
Actual sequence coverage	4.75x
Estimated genome size (Mb)	4.2
Percentage of G+C	63,66
Estimated number of genes	2620

Table1. General features of the genome of *Desulfovibrio gigas*

Group Members

Regina Menezes	Post Doc
Tracy Nevitt	Post Doc
Catarina Pimentel	Post Doc
Nedja Medeiros	Undergraduate
Teresa Barata	Master Student
Cristina Alves	Master Student
Liliana Nascimento	PhD
Jorge Pereira	PhD
Catarina Amaral	PhD
Iulia Bleoanca	PhD Student

Selected Publications

Azevedo D, Nascimento L, Labarre J, Toledano MB, Rodrigues-Pousada C. "*The S. cerevisiae Yap1 and Yap2 transcription factors share a common cadmium-sensing domain*." FEBS Lett. 2007 Jan **23**;581(2):187-95.

Broco M, Soares CM, Oliveira S, Mayhew SG, Rodrigues-Pousada C. "*Molecular determinants for FMN-binding in Desulfovibrio gigas flavoredoxin*" .FEBS Lett. 2007 Sep 18;**581**(23):4397-402.

Pimentel C, Van Der Straeten D, Pires E, Faro C, Rodrigues-Pousada C. "*Characterization and expression analysis of the aspartic protease gene family of Cynara cardunculus L.*" FEBS J. 2007 May;**274**(10):2523-39.

Inorganic Biochemistry and NMR

Ricardo O. Louro

Auxiliary Investigator

PhD 1998 in Biochemistry, Universidade Nova de Lisboa

Group Members

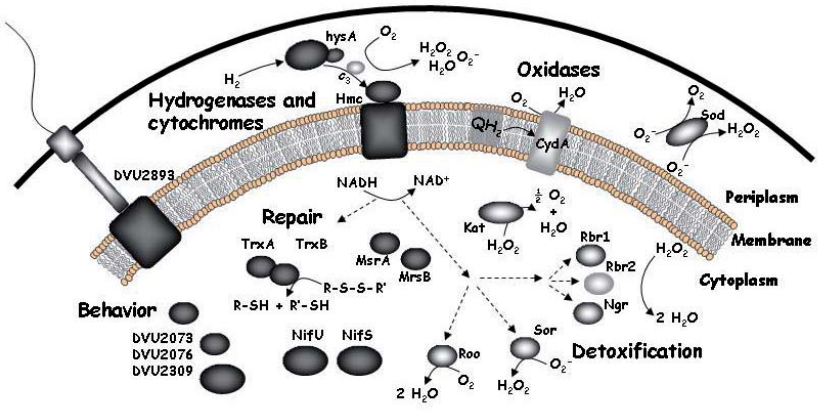
Catarina Paquete	Post Doc
Bruno Fonseca	Master Student
Ivo Saraiva	Graduate
Isabel Pacheco	Technician
Laura Portugal	Graduate
José Feliciano	Graduate

This research group studies the bioenergetic metabolism of sediment organisms that are capable of using metal compounds and ores as sources or sinks of electrons. In the case of respiration this has been named extracellular respiration and involves delivery of electrons to the outside of the cells. This task is accomplished by complex electron transfer networks involving novel proteins organised in novel arrangements. This is essential in order to maintain an effective coupling of the extracellular reduction of the solid terminal acceptor with the production of ATP associated to the inner membrane. It has been found that heme proteins play key roles in these novel respiratory chains.

The capability of these organisms to interact with solid substrates including metal ores and radionuclides makes them prime targets for application in bioremediation of contaminated environments and for the assembly of microbial fuel cells that use waste or sediments to produce electricity.

NMR is used for the structural and functional characterization of selected proteins with key roles in these novel respiratory chains complemented by other biophysical and biochemical techniques. The aim is to understand the mechanistic details that allow these organisms to live off insoluble substrates in order to improve their suitability for biotechnological applications.

During 2007 in addition to the three running projects funded by FCT a fourth project in collaboration with the Portuguese Environment Agency was initiated that allowed to reinforce the research group. This was further enhanced at the end of the year by the award of Master degree to one of the students working in this lab. Extramural collaborations with the Oak Ridge National Laboratory and the University of Tennessee in a work that also involved the Microbial Biochemistry group came to fruition and two papers have been accepted for publication. These papers deal with the genomicresponse of *Desulfovibrio vulgaris* Hildenborough to oxidative stress and a variety of electron donors and acceptors.



Cartoon of the transcriptional response of *D. vulgaris* genes to oxidative stress. Dark grey indicates genes that were up-regulated, medium grey indicates genes that show no significant changes, and light grey indicates genes that were found down regulated. Figure adapted from Dolla, A., D. M. Kurtz, Jr., M. Teixeira and G. Voordouw (2007) "Biochemical, proteomicand genetic characterization of oxygen survival mechanisms in sulphate-reducing bacteria of the genus *Desulfovibrio*". in Sulphate-Reducing Bacteria: Environmental and Engineered Systems, pp 185-213, Larry L. Barton and W. Allan Hamilton Eds., Cambridge University Press

Selected Publications

Paquete, C.M., Louro, R.O., Turner, D.L., Xavier, A.V., Catarino, T. "*Thermodynamic and kinetic characterisation of individual haems in multicentre cytochromes c_3*", Biochim Biophys Acta. 1767, 1169-79 (2007)

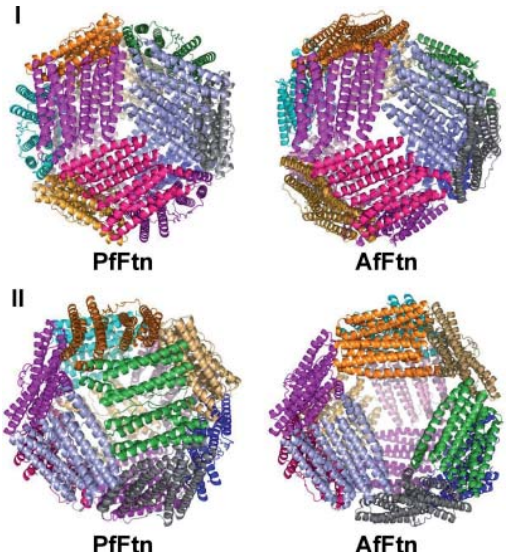
Paquete, C.M., Pereira, P.M., Catarino, T., Turner, D.L., Louro, R.O., Xavier, A.V. "*Functional properties of type I and type II cytochromes c_3 from Desulfovibrio africanus*", Biochim Biophys Acta 1767, 177-188 (2007)

Louro, R.O. "*Proton thrusters: overview of the structural and functional features of soluble tetrahaem cytochromes c_3*",J. Biol. Inorg. Chem. **12**, 1-10 (2007)

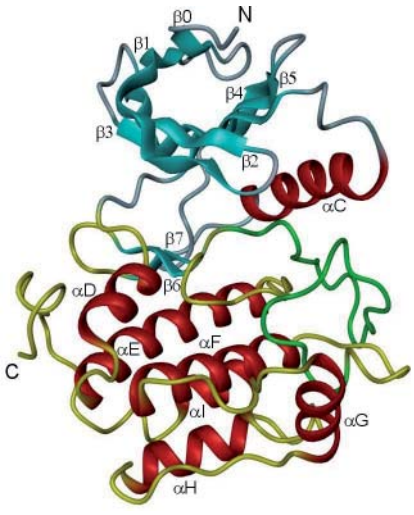
Industry and Medicine Applied Crystallography [Macromolecular Crystallography]

Pedro Matias
Auxiliary Investigator
PhD 1986 in Crystallography, University of Pittsburgh, USA

Our main activity is centred in structural studies by X-ray crystallography of single crystals of macromolecules with potential industrial and medical applications, in collaboration with other Laboratories at ITQB or elsewhere, using the Research Infrastructure of the Macromolecular Crystallography Unit, and resorting whenever necessary to European Synchrotron Radiation Facilities to carry out experimental measurements.Current activities are funded by two recently awarded FCT (Fundação para a Ciência e Tecnologia) grants. Additional funds are also available through collaborations with pharmaceutical companies, fostered via IBET. Highlights from the 2007 research activities: The three-dimensional structure of the kinase domain of human Plk-1 in complex with a selective Designed Ankyrin Repeat Protein (DARPin) was determined. This work underlined the power of selective DARpins as crystallization tools. The refined structure shows the active conformation of Plk-1 and broadens the basis for modeling and co-crystallization studies for drug design. A manuscript was accepted in Acta Crystallographica F for publication in 2008 (Work in collaboration with the Structural Genomics Laboratory [M. A. Carrondo] and the Global Drug Discovery Lab. of Bayer Schering Pharma [Berlin, Germany]). The three-dimensional structure of a ferritin from the archaeon, hyperthermophile and anaerobe *Pyrococcus furiosus* (PfFtn) was determined in three crystal forms: "as isolated", Fe- and Zn-soaked. The PfFtn monomers contain a ferroxidase centre with three possible iron binding sites. The "as-isolated", aerobically crystallized ferritin contains one iron at the site A; sites B and C only become occupied upon iron or zinc soaking. The extreme thermostability of PfFtn is proposed to originate mostly from the observed high number of intra-subunit hydrogen bonds, which leads to a preservation of the monomer fold, rather than the 24-mer assembly (Work in collaboration with Prof. W. R. Hagen and Dr. J. Tatur [T.U. Delft, The Netherlands]). An article was published in the Journal of Inorganic Biochemistry.Work is in progress aimed at the crystal structure determination of Mannosyl-3-phosphoglycerate synthase (MPGS) from *R.marinus* and *T.thermophilus* - collaboration with the Cell Physiology and NMR Laboratory (H. Santos), and [NiFeSe] hydrogenase from *D.vulgaris* Hildenborough - collaboration with the Microbial Biochemistry (I. A. C. Pereira) and Protein Modelling (Cláudio M. Soares) Laboratories.



Ribbon diagrams of the ferritin 24-mers from *P.furiosus* (PfFtn) and *A.fulgidus* (AfFtn, PDB 1S3Q). (I) View down a non-crystallographic 3-fold axis; (II) View rotated by ca. 90º about a vertical axis, showing the different monomer arrangement, which leads to the appearance of large pores in AfFtn.



Ribbon diagram of the Plk-1 molecule. The C-α ; chains of the N-terminal and C-terminal lobes are colored gray and yellow, respectively. The secondary structure elements (SSE) are highlighted (cyan for β-strands, red for α-helices). The activation segment, located in the C-terminal domain, is shown in green.

Group Members

Susana Gonçalves PhD Student

Selected Publications

Carrondo M. A., Bento I., Matias P. M. and Lindley P. F. (2007). "*Crystallographic evidence for dioxygen interactions with iron proteins*."Journal of Biological Inorganic Chemistry **12**(4): 429-442. Tatur J., Hagen W. R. and Matias P. M. (2007). "*Crystal structure of the ferritin from the hyperthermophilic archaeal anaerobe Pyrococcus furiosus*."Journal of Biological Inorganic Chemistry **12**(5): 615-630.

Membrane Protein Crystallography [Macromolecular Crystallography]

Margarida Archer

Auxiliary Investigator

PhD in 1999 in Biochemistry at Instituto de Tecnologia Química e Biológica - UNL

Group Members

Luísa Rodrigues[1]	Post Doc
Meike Stelter	Post Doc
Diana Plácido [2]	PhD Student
José Brito [3]	PhD Student
Tânia Oliveira [4]	PhD Student

[1] co-supervision (I.A.C. Pereira)

[2] co-supervision (M.A. Carrondo/ A. Henriques)

[3] co-supervision (P. Henderson)

[4] co-supervision (I.A.C. Pereira)

Our group is mainly interested on the structural characterization of membrane-bound proteins and complexes, namely those involved in respiration, metabolism and transport systems. We share common equipment and infrastructures with other laboratories within the Macromolecular Crystallography Unit. We also have regular access to european synchrotron sources.We have recently solved the X-rastructure of the membrane-bound cytochrome *c* nitrite reductase NrfHA in complex with an inhibitor (HQNO), which allowed the identification of the quinol binding site in the membrane anchored NfrH subunit and the electron transfer flow from quinol to the NrfA catalytic subunit. The manuscript describing the structural and functional characterization of NrfHA is on the final stage of preparation, a review on this subject was done (Work in collaboration with Inês Pereira - Microbial Biochemistry Laboratory). A joint FCT project has recently started.

Structural analysis of porcine pancreatic elastase in complex with novel inhibitors was done in collaboration with Rui Moreira (Faculdade Farmácia), for which two papers were published and a FCT project was approved.

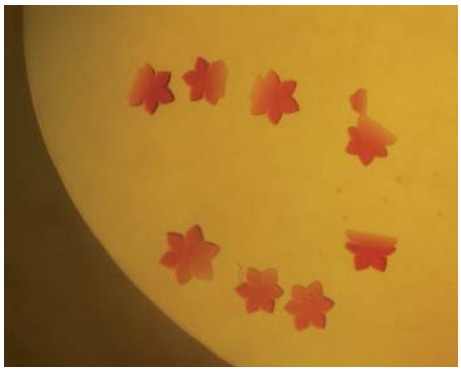


Fig 1 - Crystals of NrfHA nitrite reductase complex

The three-dimensional structure of transglutaminase from *Bacillus subtilis* was determined which provided important insights into the overall fold and catalytic site. The structural information together with mutagenesis studies and activity assays allow for a better understanding of the reaction mechanism (Work in collaboration with Adriano Henriques - Microbial Development Laboratory). The production and preliminary crystallographic studies of transglutaminase were published.

The crystal structure of two metalloproteins from extremophiles have been characterized (HiPIP and cytochrome c). Work in collaboration with Miguel Teixeira (Metalloproteins and Bioenergetics). One paper published, two other are in preparation.We have also 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 transporters which belong to the Major Facilitator Superfamily, usually comprising 12-14 transmembrane α-helices; which are responsible for uptake of sugars, nucleosides and amino acids; and those needed for efflux of antibiotics. A FCT project was awarded to this project.

Our Laboratory is an Associated Member of the European Membrane Protein Consortium (E-MeP) and is also one of the partners in the Structural Biology of Membrane Proteins Marie Curie Training Network (FP7) that comprises 13 academic research groups and 3 industrial companies. This network combines expertise of experimental and theoretical approaches, such as *in vivo* and *in vitro* expressions systems, functional/biochemical/biophysical characterisation, X-Ray diffraction, electron microscopy (EM), atomic force microscopy (AFM), single-molecule force spectroscopy (SMFS), liquid and solid state NMR, numerical simulations.

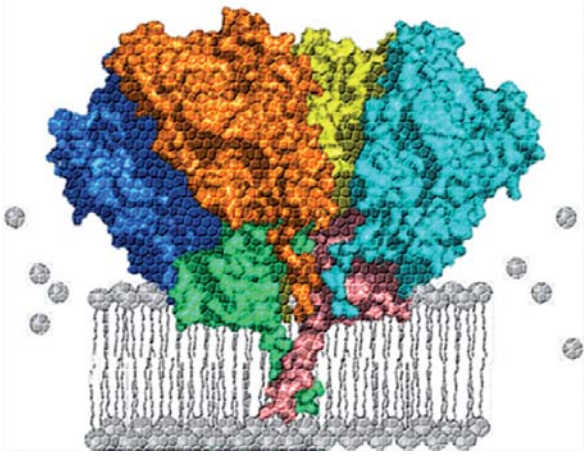


Fig 2 - Pictorial represention of NrfHA complex inserted in the membrane

Selected Publications

Mulchande J., Martins L., Moreira R., Archer M., Oliveira T. F. and Iley J. (2007). "*The efficiency of C-4 substituents in activating the beta-lactam scaffold towards serine proteases and hydroxide ion.*"Organic & Biomolecular Chemistry **5**(16): 2617-2626.3.

Stelter M.,Placido D., Fernandes C., Isidro A., Carrondo M. A., Henriques A. and Archer M. (in press 2008). "*Auto-induction and purification of a Bacillus subtilis transglutaminase (Tgl) and its preliminary crystallographic characterization*". Protein Expression and Purification

Rodrigues M.L., Pereira I. and Archer M. (in press 2008). "*Membrane-bound cytochrome c quinol dehydrogenase NrfH", Handbook of Metalloproteins*."

Metalloproteins and Bioenergetics

Miguel Sepúlveda Teixeira

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

The research in this laboratory has as its main goal to understand at a molecular level the structure and function of enzymes involved in aerobic respiration and detoxification of dioxygen, nitric oxide and superoxide. The main target organisms are aerobic or anaerobic bacteria and archaea, which includes, not only some model organisms, such as *E.coli* and *Synechocystis sp*, but also *Rhodothermus marinus*, *Acidianus ambivalens*, *Archeoglobus fulgidus*, and *Nanoarchaem equitans*, which, mainly due to their phylogenetic distance from the most studied prokaryotes, contain quite diverse and new proteins/enzymes. The studies are now being extended to higher organisms, i.e., protozoa and fungi. The research involves a variety of complementary approaches: cell growth, protein chemistry, enzymatic assays, fast kinetics, spectroscopies (UV-Visible, EPR, Raman, Fluorescence), potentiometry and amperometry, and reconstitution in artificial liposomes. These approaches are further complemented through several of collaborations at ITQB or international. The studies have been progressing at a continuously degree of a more detailed molecular level, but in parallel with a step forward in the use of closer *in vivo* situations, such as membrane vesicles.

In 2007, several achievements were: a clear identification of a novel membrane complex from *R. marinus*, which was shown to constitute a new type of quinol: acceptor oxidoreductase; molecular studies of the superoxide reductase from *N. equitans*, which further contributed to understand the functional mechanism and the role of key amino acids present in this enzyme family; as a first step for a thorough analysis of haem-copper oxygen reductases, a comprehensive redox study of members of the three different families of this superfamily, was undertaken. 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 behavior among all the studied enzymes, which on one hand points to subtle differences on proton-electron coupling, but on the second hand also enabled to start to reveal which elements are really important for the functional mechanism of these enzymes, which is still poorly understood.

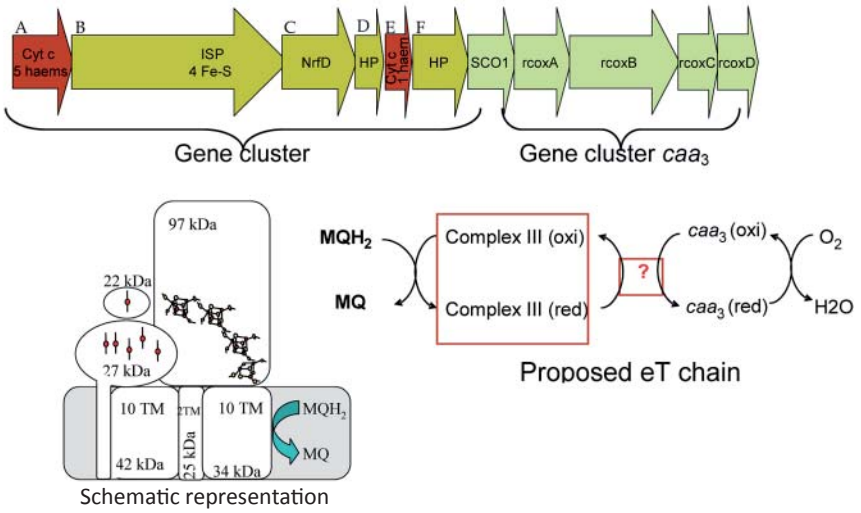


Figure: Novel quinol - acceptor oxide reductase, a prototype of an alternative complex III

Group Members

Manuela Pereira[1]	Auxiliary Inv.
Ana Melo	Assist. Prof. ULuso.
Andreia Fernandes	Assist. Prof. UAlg.
Célia Romão[2]	Post Doc
João Vicente[3]	Post Doc
Filipa Sousa	PhD student
Andreia Veríssimo	PhD student
Maxyme Cuypers	PhD student/ESRF
Vera Gonçalves[4]	PhD student
Ana Filipa Pinto	PhD student
Ana Refojo	PhD student
Ana Baptista	PhD student
Pedro Sousa	Undergraduate
Sara Silva	Undergraduate
Fay Mossel	Undergraduate

[1] coordinator of the Bioenergetics Program

[2] co-supervision (M.A. Carrondo)

[3] co-supervision (U.Singh, Stanford Univ. School of Medicine)

[4] co-supervision (Lígia M. Saraiva)

Selected Publications

Veríssimo A.F., Sousa F.L., Baptista A.M., Teixeira M., Pereira M.M. (2007) "*Thermodynamic redox behavior of the heme centers of cbb3 heme-copper oxygen reductase from Bradyrhizobium japonicum*". Biochemistry 46, 13245-53.

Pereira M.M., Refojo P.N., Hreggvidsson G.O., Hjorleifsdottir S., Teixeira M. (2007) "*The alternative complex III from Rhodothermus marinus - a prototype of a new family of quinol:electron acceptor oxidoreductases*". FEBS Lett. , 581, 4831-5.

Rodrigues J.V., Saraiva LM, Abreu IA, Teixeira M, Cabelli DE. (2007) "*Superoxide reduction by Archaeoglobus fulgidus desulfoferrodoxin: comparison with neelaredoxin*". J Biol Inorg Chem., 12, 248-56.

Microbial Biochemistry

Inês Cardoso Pereira

Auxiliary Investigator

PhD 1993, Oxford University, UK

Group Members

Filipa Valente	PhD Student
Luisa Rodrigues[1]	Post Doc
Sofia Silva	PhD Student
Sofia Venceslau[2]	PhD Student
Tânia Oliveira[1]	PhD Student
Isabel Pacheco	Technician

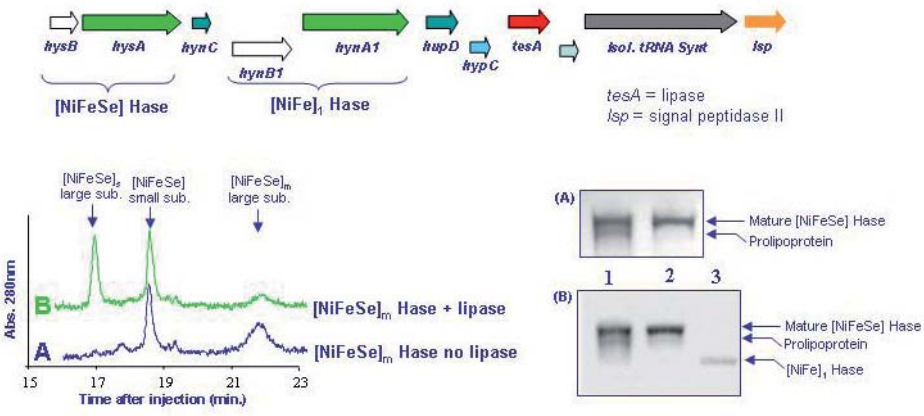
[1] co-supervision (Margarida Archer)

[2] co-supervision (Lígia M. Saraiva)

The Microbial Biochemistry Laboratory is interested in studying bacteria that grow by Anaerobic Respiration, a type of energy metabolism in which an organic or inorganic compound is used as terminal electron acceptor instead of oxygen. The majority of higher organisms and many microorganisms rely on the reduction of oxygen and have quite similar respiratory chains. In contrast, in anaerobic organisms a vast diversity of strategies for obtaining energy is observed. Many of the processes involved are of interest for biotechnological applications such as Waste Treatment, Bioremediation, Microbial Fuel cells or BioHydrogen production.

Currently we investigate the mechanisms and proteins used by a large group of ubiquitous bacteria that respire sulphur compounds (like sulfate and sulfite). 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 H_2 oxidation, as well as membrane-associated protein complexes that may transfer electrons to the cytoplasmic reduction of sulfate. In addition, we are also interested in the role played by these bacteria within the human intestinal flora.

In 2007, an important achievement was the discovery that the highly active and oxygen-resistant [NiFeSe] hydrogenase from *Desulfovibrio vulgaris* is a bacterial lipoprotein, which explains its association with the membrane. This protein is the first example of a lipoprotein lacking a standard lipoprotein signal peptide, and which is translocated by the Tat and not the Sec pathway. These results provided the first evidence that lipoprotein processing does not require the presence of the signal peptide, but only of a lipobox.Another important study revealed the importance of hydrogen metabolism for the bacterium *Bilophila wadsworthia*, an opportunistic pathogen in the human gut.



D. vulgaris Hildenborough gene locus containing the [NiFeSe] hydrogenase, a lipase and signal peptidase II; HPLC traces showing that treatment with a lipase converts the membrane-bound form of the [NiFeSe] hydrogenase large subunit into a soluble form; Western blot (A) and activity-stained native gel (B) showing that in cells treated with globomycin (1) a prolipoprotein form of the [NiFeSe] hydrogenase appears, which is not observed in the absence of globomycin (2).

Selected Publications

Valente F. M. A., Pereira P. M., Venceslau S. S., Regalla M., Coelho A. V. and Pereira I. A. C. (2007). "*The [NiFeSe] hydrogenase from Desulfovibrio vulgaris Hildenborough is a bacterial lipoprotein lacking a typical lipoprotein signal peptide.*" FEBS Letters **581**(18): 3341-3344.

Pereira I. A. C. (2007) "*Membrane complexes in Desulfovibrio.*" *in* Microbial Sulfur Metabolism. (C. Friedrich and C. Dahl)**:** 24-35.

Pereira I. A. C., Haveman S. A. and Voordouw G. (2007) "*Biochemical, genetic and genomic characterization of anaerobic electron transport pathways in sulphate-reducing delta-proteobacteria.*" *in* Sulphate-Reducing Bacteria: Environmental and Engineered Systems. (L. L. Barton and W. A. Hamilton)**:**215-240.

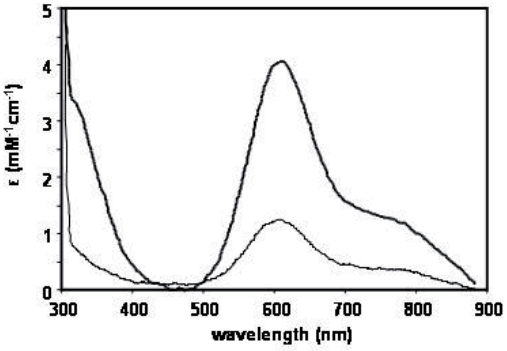
Microbial and Enzyme Technology

Lígia O. Martins

Invited Assistant Professor

PhD 1994 in Biotechnology , Universidade Técnica de Lisboa, IST

The research highlight of the year was the production of fully Cu-loaded recombinant CotA-laccase (from the multicopper oxidase family of enzymes) in the cytoplasm of *Escherichia coli*. Incomplete copper incorporation into the CotA-laccase had always been observed during the course of our work and was identified as a major limiting factor in our studies of structure-function relationships, involving the biochemical and structural analysis of site-directed mutants. One possibility was that overproduction of fully Cu-loaded CotA-laccase was impaired by the presence of a low concentration of this metal in the *E. coli* cytoplasm. In fact, due to the cytotoxicity of this metal its intracellular quota is maintained within a narrow range in the cytoplasm of *E. coli* (ca.10μM) through elaborated homeostasis mechanisms [1]. Also known was that copper physiology in *E. coli* is dependent on oxygen availability; higher copper content was measured in anaerobic as compared with aerobic-grown cells [2]. In our work we have shown that recombinant protein samples produced *E. colie* xpressing the *cotA* gene, after growth in microaerobic conditions, present a more intense blue colour and contain a 4 copper/protein stoichiometry, ensuring that all four copper ions required for enzyme activity were incorporated into the active sites, while purified enzyme from aerobic cultures exhibit an incomplete metal incorporation (0.5:1 Cu/protein) (Fig 1). The holoCotA produced under microaerobic conditions showed, as expected, higher catalytic rates (differences as large as 200-fold were measured) as well as higher kinetic and thermal stability. Taking into consideration that this holoenzyme was produced in conditions where cells accumulated increased copper content (80-fold higher than aerobic grown cells, as measured by atomic absorption) it is most likely that at least part remains biologically available at the cytoplasm, ready for incorporation into newly synthesized recombinant enzymes. Furthermore, our studies of copper incorporation into apo-CotA enzymes, as monitored by optical and EPR spectroscopies, point to a crucial role of copper in the folding of recombinant CotA-laccase in the cytoplasm of *E. coli*. Two types of apo-forms were prepared and studied. *In vitro* reconstitution of copper to CotA apoform produced by *E. coli* in copper deficient conditions, resulted in partial recovery of the enzyme biochemical properties. However,reconstitution of apoforms, produced in the presence of copper (holo) but further depleted *in vitro* with EDTA, results in the complete recovery of the native conformation as monitored by spectroscopic, kinetic and thermal stability analysis. [1] Finney LA, O'Halloran, TV (2003) Science 300, 931-936; [2] Outten, FW, Huffman, DL, Hale, JA, O'Halloran, TV (2001) J. Biol. Chem. 276: 30670-30677.



UV-Visible spectra of as-isolated CotA species produced in microaerobic (thickline) and in aerobic conditions (thin line). MCOs are characterized by having four Cu(II) ions that are classified into three distinct types of Cu sites, namely type 1 (T1), type 2 (T2) and type 3 (T3). The T1 copper site is characterized by an intense absorption band at around 600 nm, conferring an intense blue colour to these enzymes. T2 copper site is characterized by the lack of strong absorption bands and the T3 or coupled binuclear Cu site is characterized by an absorption band at 330 nm. The function of the T1 Cu site is to shuttle electrons from substrates to the trinuclear Cu centre (formed by the T2 and T3 Cu sites) where molecular oxygen is reduced to two molecules of water during the complete 4-electron catalytic cycle.

Group Members

Luciana Pereira	Post Doc
Vânia Brissos	Post Doc
Zhenjia Chen	Post Doc
André Fernandes	PhD Student
Paulo Durão	PhD Student

Selected Publications

Fernandes AT, Soares CM, Pereira MM, Huber R, Grass G, and Martins, LO. 2007. "*A robust metallo-oxidase from the hyperthermophilic bacterium Aquifex aeolicus*." FEBS J. 274: 2683-2694

Sanchez-Sutil MC, Gomez-Santos N, Moraleda-Munoz A, Martins LO, Perez J and Munoz-Dorado, J. 2007. "*Differential expression of the three multicopper oxidases from Myxococcus xanthus*." J. Bacteriol. 189: 4887-4898

Durão P, Chen Z, Fernandes AT, Hildebrandt P, Murgida DH, Todorovic S, Pereira MM, Melo EP and Martins, LO. 2008. "*Copper Incorporation Into Recombinant CotA-laccase from Bacillus subtilis - Characterization of Fully Cu-Loaded Enzymes*." J. Biol. Inorg. Chem. 13, 183-93

Molecular Genetics of Microbial Resistance

Lígia M. Saraiva

Auxiliary Investigator

PhD 1993 in Biochemistry, Universidade Nova de Lisboa

Group Members

Ana Tavares	PhD Student
Joana Baptista	PhD Student
Lígia Nobre	PhD Student
Marta Justino	PhD Student
Sofia Venceslau[1]	PhD Student
Susana Lobo	PhD Student
Vera Gonçalves[2]	PhD Student

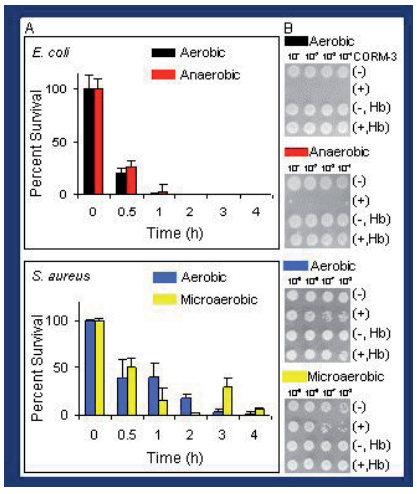
[1] co-supervision (Inês A. C. Pereira)

[2] co-supervision (Miguel S. Teixeira)

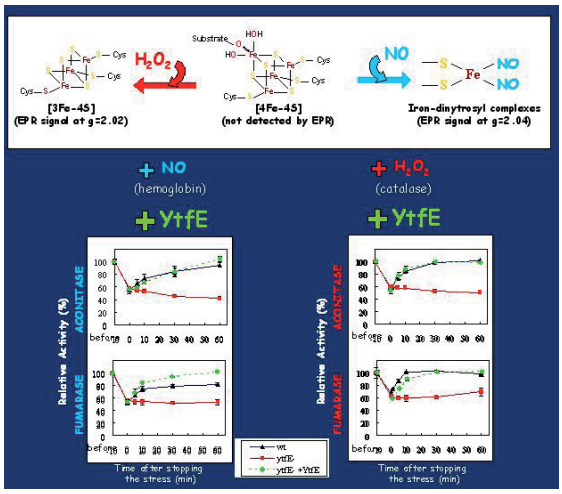
Our laboratory focuses on the study of the strategies used by pathogenic microbes to resist host defences, in particular, the antimicrobial actions of reactive oxygen and nitrogen species generated by the human immune system. Iron-sulfur [Fe-S] clusters are very simple, almost ubiquitous and evolutionary ancient prosthetic groups, that are essential for the function of proteins involved in a wide range of biological processes. Although in many cases *in vitro* assembly of [Fe-S] clusters and incorporation into apoproteins can occur spontaneously in other cases [Fe-S] cluster biogenesis requires specific accessory proteins. Furthermore, proteins that contain iron-sulfur clusters are one of the major targets of nitrosative and oxidative compounds that cause displacement of the iron atoms of the cluster and consequent malfunction of the protein/enzyme. We have shown that the di-iron protein *E. coli* YtfE represents a novel system involved in the repair of iron-sulfur clusters which is particularly important in cells submitted to oxidative and nitrosative stress conditions. This is also the first repair system reported for *E. coli* and homologs of this protein are found to be widely spread in pathogenic bacteria.

Carbon monoxide (CO) is endogenously produced in the human body where it plays important physiological roles in vasorelaxation, neurotransmission, and in the immune system. Besides these findings in the physiology and biology of mammals, nothing was until now known about the action of CO on bacteria. We have very recently discovered that carbon monoxide releasing compounds (CORMs) causes rapid death of pathogenic bacteria like *Escherichia coli* and *Staphylococcus aureus*. This result is the first evidence that CO can be utilized as an antimicrobial agent and may constitute the starting point for the development of a novel type of therapeutic drug.

We are also interested in analyzing the mechanisms of oxygen tolerance in sulphate reducing bacteria (SRB) which have environmental bioremediation importance and that have been associated with human pathogenicity. SRB of the *Desulfovibrio genus* are considered anaerobes, in spite of the fact that they are frequently isolated close to oxic habitats. We provided the first example of a SRB, *Desulfovibrio desulfuricans* ATCC 27774, that is able to grow in the presence of nearly atmospheric oxygen levels and we have characterized the oxygen reducing systems that enable this bacterium to cope with oxygen.



Escherichia coli and *Staphylococcus aureus* cells treated with 250 micromolar of CORM-2



Escherichia coli YtfE is necessary for the repair of NO and hydrogen peroxide-damaged [Fe-S] clusters

Selected Publications

Nobre LS, Seixas JD, Romão CC and Saraiva LM (2007) "*The Antimicrobial Action of Carbon Monoxide Releasing Compounds.*" Antimicrob Agents Chemother. **51**, 4303-7.

Justino M.C., Almeida, C.C., Teixeira, M., amd Saraiva, L.M. (2007) "*Escherichia coli di-iron YtfE protein is necessary for the repair of stress-damaged iron-sulfur clusters.*" J. Biol. Chem. **282**, 10352-9.

Lobo SA, Melo AM, Carita JN, Teixeira M, Saraiva LM (2007) "*The anaerobe Desulfovibrio desulfuricans ATCC 27774 grows at nearly atmospheric oxygen levels.*" FEBS Lett. **581**, 433-6.

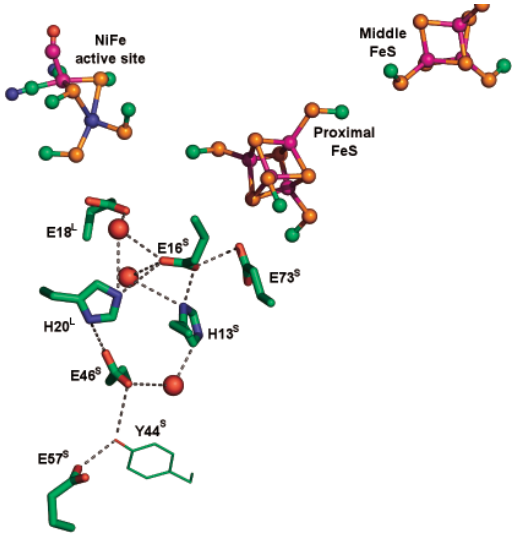
Molecular Simulation

António M. Baptista

Auxiliary Investigator

PhD 1998, Universidade Nova de Lisboa, Portugal

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. This made possible the detailed study of the structural changes induced by pH on several peptides and proteins, such as kyotorpin (an analgesic neuropeptide) and lysozyme (an enzyme that breaks the walls of Gram-positive bacteria). Another example is our recent study of hydrogenase, an enzyme which catalyzes the cleavage of molecular hydrogen into protons and electrons, in which case we could identify both access pathways for molecular hydrogen and exiting pathways for the resulting protons. Other investigated subjects include ligand-induced structural effects, protein folding/misfolding, characterization of energy landscapes, and modeling of electron-proton coupling in oxygen reductases. A recent line of work is the study of the interaction of peptides and proteins with biological membranes.



Proposed major proton-transfer pathway in the [NiFe]-hydrogenase of *Desulfovibrio gigas*, a sulfate-reducing bacteria. The figure shows the amino-acid residues (sticks) and water molecules (red spheres) involved in proton transfer, as well as the metal centers (ball-and-sticks) involved in catalysis and electron transfer.

Group Members

Miguel Machuqueiro	Post Doc
Sara Campos	PhD Student
Vitor Teixeira[1]	PhD Student

[1] co-supervision (Cláudio Soares)

Selected Publications

Machuqueiro M. and Baptista A. M. (2007). " *The pH-dependent conformational states of kyotorphin: a constant-pH molecular dynamics study.*" Biophysical Journal **92**:1836-1845.

Veríssimo A. F., Sousa F. L., Baptista A. M., Teixeira M. and Pereira. M. M. (2007). " *Thermodynamic redox behavior of the heme centers of cbb3 heme-copper oxygen reductase from Bradyrhizobium japonicum?*" Biochemistry **46**:13245-13253.

Gaspar P., Neves A. R., Shearman C. A., Gasson M. J., Baptista A. M., Turner D. L., Soares C. M. and Santos H. (2007). " *The lactate dehydrogenases encoded by the ldh and ldhB genes in Lactococcus lactis exhibit distinct regulation and catalytic properties: comparative modelling to probe the molecular basis.*" FEBS Journal **274**:5924-5936.

Protein Biochemistry, Folding and Stability

Cláudio M. Gomes

Auxiliary Investigator

PhD 1999 in Biochemistry, Universidade Nova de Lisboa

Group Members

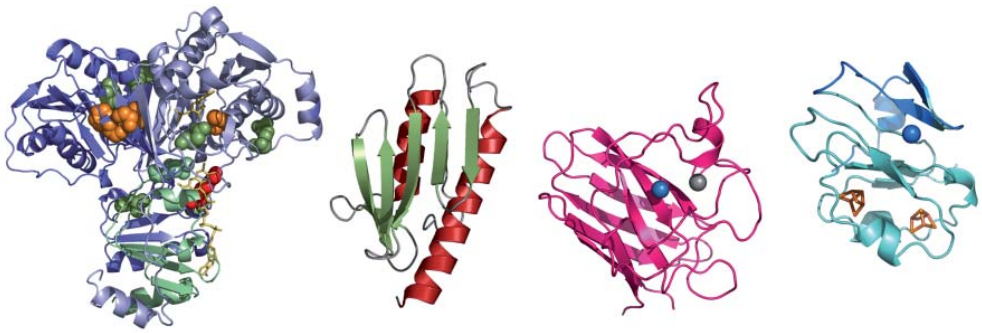
Sónia Leal	PhD Student
Vesna Prosinecki	PhD Student
Ana Correia	PhD Student
Bárbara Henriques	PhD Student
Hugo Botelho	PhD Student
João Rodrigues	Post Doc
Patrícia Faísca	Post Doc

The 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. Experimentally, we use a complementary set of tools, ranging from biochemical and molecular biology methodologies to biophysical, spectroscopic and proteomics techniques. A long lasting interest of the laboratory concerns the study of the interplay between a protein and its metal centres and cofactors.

In 2007 we have carried out a detailed spectroscopic study on the thermal unfolding of a ferredoxin model, combining different biophysical methods. This has allowed describing how the metalloprotein structural organization is intertwined with disassembly of the iron-sulfur centres, denoting the conformational interplay of the protein backbone with cofactors. Along these lines, we have characterized a molten globule state of the same protein, a conformation which may be involved in the protein folding pathway and iron-sulfur centre assembly. Studies on lactoperoxidase, a more complex metalloprotein, have shown that folding and catalysis relies on the stabilization of its α -helix rich core domain, in which the heme cofactor plays a key role.

In recent years we have been increasingly engaged in the investigation of human diseases which result from protein misfolding or destabilization. We are currently studying proteins involved in different types of disorders: frataxin and Cu/Zn superoxide dismutase (neurodegeneration), electron-transfer flavoprotein (lipid metabolism) and phenylalanine hydroxylase (amino acid metabolism). A common feature of these models is that the conformational states and phenotypes of some of the pathological mutations of interest, are dependent on cellular factors such as cofactor insertion and availability, temperature, molecular crowding, and oxidative and nitrosative stresses. In order to understand how disease-related genetic variability impacts on the cell, we are designing experiments aimed at characterizing specific conformational changes in the mutant proteins, but also broader cellular effects, such as intervention of molecular chaperone responses and oxidative modifications. For example, during 2007 we have started an investigation of how flavinylation improves the folding of human ETF, thus providing a structural and molecular framework to rationalize the therapeutic effects of riboflavin supplementation in multiple acyl-CoA dehydrogenation defective patients. Simultaneously, the *in vivo* folding and degradation processes are being investigated using molecular chaperones and proteases.

Specific national and international funding raised during this year will allow pursuing further research in these themes. More details and complete list of 2007 publications at www.itqb.unl.pt/pbfs



Some of the current target proteins (Left to right): Human ETF, frataxin, Cu/Zn-SOD and ferredoxin

Selected Publications

Leal S. S. and Gomes C. M. (2007). "*Studies of the molten globule state of ferredoxin: Structural characterization and implications on protein folding and iron-sulfur center assembly.*" Proteins-Structure Function and Bioinformatics **68**(3): 606-616.

Boscolo B., Leal S. S., Ghibaudi E. M. and Gomes C. M. (2007). "*Lactoperoxidase folding and catalysis relies on the stabilization of the alpha-helix rich core domain: A thermal unfolding study.*" Biochimica Et Biophysica Acta-Proteins and Proteomics 1774(9): 1164-1172.

Todorovic S., Leal S. S., Salgueiro C. A., Zebger I., Hildebrandt P., Murgida D. H. and Gomes C. M. (2007). "*A spectroscopic study of the temperature induced modifications on ferredoxin folding and iron-sulfur moieties.*" Biochemistry **46**(37): 10733-10738.

Protein Modelling

Cláudio M. Soares

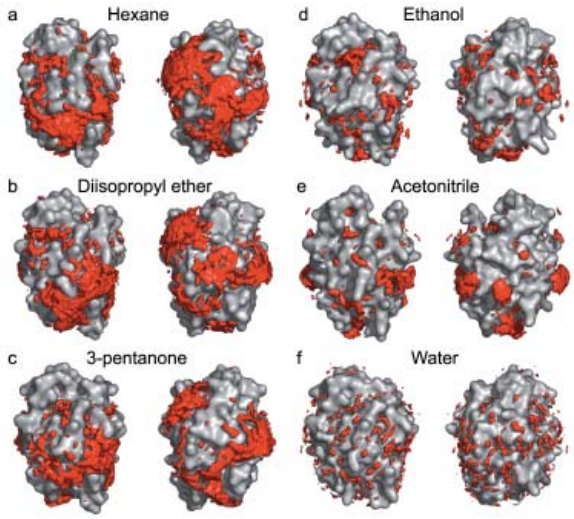
Associate Professor

PhD 1994 in Theoretical Biochemistry, Universidade de Lisboa / Uppsala University

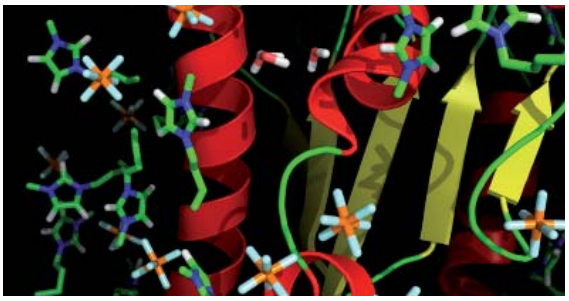
The Protein Modelling Laboratory works on molecular modelling of proteins using physical methods. Our areas of work range from basic research in modelling methodologies to applications with biotechnological and biomedical interest. Modelling redox proteins is one of our interests, and in 2007 we published several works, namely in predicting the structure of bacterial laccases and studying their molecular mechanisms (in collaboration with the Microbial and Enzyme Technology Lab and the Structural Genomics Lab), on the proton transfer mechanisms on [NiFe] hydrogenase (in collaboration with the Molecular Simulation Lab), on the molecular determinants of FMN binding in flavodoxin from *D.gigas*, and on structure prediction of neelaredoxins (in collaboration with the Metalloproteins and Bioenergetics Lab).

Studying enzymes aimed at biotechnological applications is another area of work. We published a paper on enzyme engineering aimed at textile applications (in collaboration with a Universidade do Minho Lab). We continued work on enzymes in non-aqueous solvents and we published a paper on enzyme hydration in organic solvents with different polarity, highlighting the common and different features of these hydration mechanisms. This year a paper got accepted, containing the first simulation study of an enzyme in two different ionic liquids with different protein stabilising features. Our results correlate with experimental knowledge and evidence, for the first time, the molecular reasons for this phenomenon.

This year a paper from our lab on the mechanisms of protein stabilisation by charged compatible solutes was accepted. Using MD simulations we studied the stabilization phenomenon and the molecular reasons for it.



Cutinase in organic solvents. Spatial distribution probability density of water in (a) hexane, (b) diisopropyl ether, (c) 3-pentanone, (d) ethanol, and (e) acetonitrile with 25% of water and (f) the fully hydrated system. For each organic solvent, two sides of the enzyme are shown in order to obtain a complete view of the surface. Micaelo e Soares (2007) *FEBS J.*, **274**, 2424-2436.



Molecular dynamics simulation of cutinase in the ionic liquid [BMIN][PF6]. Micaelo and Soares, (2008) *J.Phys.Chem.B*, accepted

ABC transporters are important membrane proteins involved in the translocation of diverse substrates. We are studying domains and complete ABC transporters inserted into membranes, with the aim at understanding their molecular translocation mechanisms. Comparative modelling techniques are used in our lab, to predict the structure of proteins and understand molecular mechanisms. Besides the works mentioned above, this year we published a study on the understanding the effect of mutations on the AraR repressor binding to DNA in *B. subtilis* (in collaboration with the Microbial Genetics Lab) and another study on the catalytic properties of different lactate dehydrogenases in *L. lactis* (in collaboration with the Cell Physiology and NMR Lab).

Group Members

Bruno Victor	PhD Student
Nuno Micaelo	PhD Student
Diana Lousa	PhD Student
João Damas	PhD Student
Vitor Teixeira[1]	PhD Student
Ana Oliveira	PhD Student
Zélia Ferreira	Graduate

[1] co-supervision (António Baptista)

Selected Publications

Micaelo, N, Soares, CM (2007) *" Hydration mechanisms of enzymes in nonpolar and polar organic solvents "*, FEBS J., **274**, 2424-2436.

Franco, I, Mota, LJ, Soares, CM, Sá-Nogueira, I (2007) *"Probing key DNA contacts in AraR-mediated transcriptional repression of the Bacillus subtilis arabinose regulon "*, Nucleic Acids Res., **35**, 4755-4766

Fernandes, AT, Soares, CM, Pereira, MM, Huber, R, Grass, G, Martins, LO (2007) *"A robust metallo-oxidase from the hyperthermophilic bacterium Aquifex aeolicus "*, FEBS J., **274**, 2683-2694.

Structural Biology

Carlos Frazão

Auxiliary Investigator

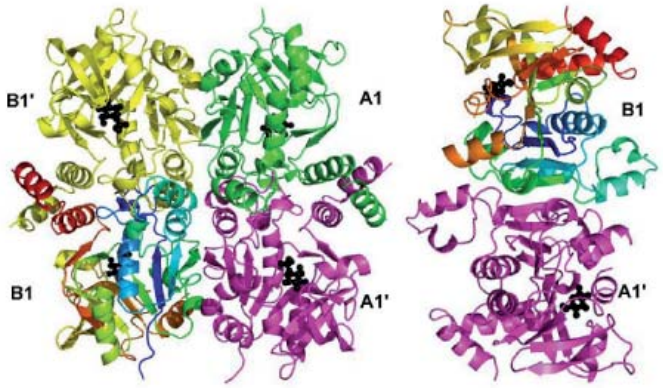
PhD 1988 in Chemistry, Technical University of Munich, Germany

Group Members

Joana Rocha	PhD Student
David Aragão	PhD Student

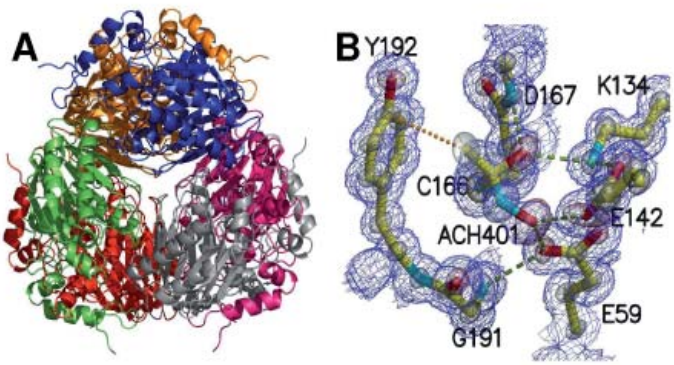
The visualization of biological molecules at atomic detail allows a deep understanding on the biological phenomena in which they are involved, because it makes possible the rationalization of the chemical mechanisms underlying those processes. We have been studying a variety of biological macromolecules using crystallographic X-rays diffraction analysis, both running in-house equipement at ITQB within the the Macromolecular Chrystallography Unit, as well as using several synchrontron facilites within Europe. In 2007 we examined in detail the structure of two industrially relevant enzymes. In collaboration with Profs. I. Sá-Correia and A. Fialho from IST-UTL, we studied the structure of one of the enzymes involved in the bacterial synthesis of gellan gum, approved in USA and EU as a food gelling, stabilizing and suspending agent (Figure 1). In collaboration with Prof. A. Karmali, ISEL, we determined a first structure of an aliphatic amidase, an enzyme with potential applications that span through chemical and pharmaceutical industries as well as in bioremediation (Figure 2).

Figure 1



Ribbons representations of two orthogonal views of the tetrameric *Sphingomonas eloedea* ATCC 31461 glucose-1-phosphate uridylyltransferase, responsible for the reversible conversion of glucose-1-phosphate and UTP into UDP-glucose and pyrophosphate, which is a key step in the biosynthesis pathway of gellan gums. Each monomer is represented with different colors, with monomer B1 colored from blue to red in a ramping scheme from the N-to C-termini. The complexed substrate glucose-1-phosphate is shown as back ball-and-stick. This enzyme revealed a novel quaternary structure, unique amongst NDP-sugar pyrophosphorylase members (Aragao et al. J Bact. 2007).

Figure 2



(A) Ribbon representation of hexameric *Pseudomonas aeruginosa* aliphatic amidase, showing each monomer in a different color. This enzyme hydrolyzes amides but also exhibits acyl transfer reactivity in the presence of hydroxylamine, thus originating hydroxamic acids. Some of these are potent inhibitors of metallo-proteases and have been investigated in therapeutic applications as anti-human immunodeficiency virus or anti-malarial agents. (B) View of the enzyme active site showing the atomic electron density as blue mesh, anisotropic atomic displacements at 0.5 probability as transparent ellipsoids, atoms as sticks (carbon in yellow, nitrogen in blue, oxygen red and sulfur green), hydrogen bonds in dashed green, and van der Waals interactions as pointed orange. The nucleophile in the covalent catalysis, Cys166 at the back, is bound to a trapped acyl transfer reaction intermediate state, ACH401, which is stabilized by several H-bonds to conserved residues at the bottom of the 13 A deep catalytic cavity. Such a detailed view of the active site enabled to understand the enzyme puzzling specificity and activity properties (Andrade et al. JBC 2007).

Selected Publications

Andrade J., Karmali A., Carrondo M. A. and Frazao C. (2007). "*Structure of amidase from Pseudomonas aeruginosa showing a trapped acyl transfer reaction intermediate state.*" Journal of Biological Chemistry **282**(27): 19598-19605.

Aragao D., Fialho A. M., Marques A. R., Mitchell E. P., Sa-Correia I. and Frazao C. (2007). "*The complex of Sphingomonas elodea ATCC 31461 glucose-1-phosphate uridylyltransferase with glucose-1-phosphate reveals a novel quaternary structure, unique among nucleoside diphosphate-sugar pyrophosphorylase members.*" Journal of Bacteriology **189**(12): 4520-4528.

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.

Structural Genomics [Macromolecular Crystallography]

Maria Arménia Carrondo

Full Professor

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

Our Laboratory is mainly interested in a structural genomics approach to the 3D-structural study of proteins related with human health and its biochemical applications. Within European Union projects the Laboratory is a partner in the Integrated Project SPINE2-Complexes "From Receptor to Gene: structures of complexes from signalling pathways linking immunology, neurobiology and cancer", in the Specific Support Action TEACH-SG, in the Infrastructure Cooperation Network and a TID centre of the Integrated project BIOXHIT. In SPINE2-Complexes our work primarily focuses on strategies to produce protein complexes involved in the innate immune response employing co-expression, refolding and truncation library methods.

Recently the structure of SorC, a transcriptional regulator, has been solved revealing a mechanism of DNA binding and identification of the ligand regulator-binding region. During 2007 the 3D-structure of 1,3-propanediol-dehydrogenase from the opportunistic human pathogen *Klebsiella pneumoniae* showed an unexpected quaternary structure, a decamer. This allowed a better understanding of the final step in the biological production of 1,3-propanediol, a compound which is of considerable industrial interest, used in the production of polymers, cosmetics, foods, lubricants and drugs.

The structure of two cotA laccase mutants from *B. subtilis*, M502L and M502F, reported in the previous annual report, were chosen as the annual cover for Journal of Biological Inorganic Chemistry in 2007. Additionally the structure of two other mutants, I494A and L386A, were solved. These mutations are also located in the vicinity of the T1 copper centre, and induced an increase in the solvent accessibility of the centre. This effect was more pronounced in the I494A mutant where a water molecule was observed within a coordination distance of the T1 copper ion. The changes observed in both mutants affected the redox potential of the centre and consequently the enzyme activity.

In the collaboration through IBET with Merck Serono the work focused on the design of new constructs for the target proteins, using limited proteolysis digestions followed by protein expression, purification. The collaboration with Bayer Schering Pharma was continued with the finalization of a joint PhD project and the negotiation of new projects. Crystallization trials in the nano-scale are optimized and scaled-up in order to produce good diffracting crystals which are finally used to solve the 3D-structure of protein:ligands complexes, for projects within both industrial IBET collaborations.

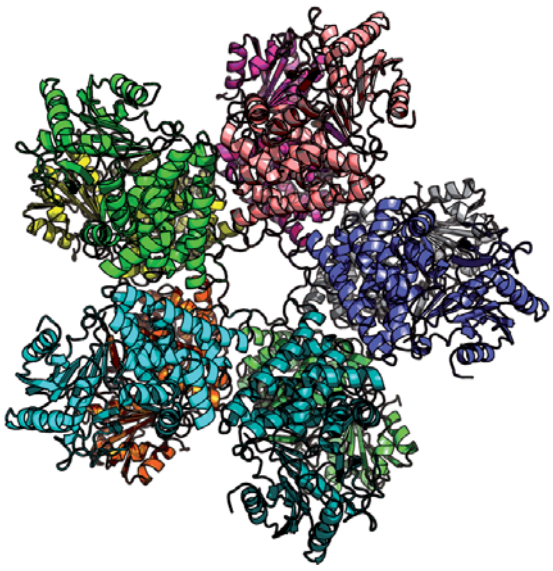


Figure 1. Structure of 1,3-propanediol-deydrogenase from *Klebsiella pneumoniae*. The x-ray structure showed a "star-like" shape decamer (with a diameter of approximately 150 Å), with a hydrophilic funnel-like structure in the middle that opens a central pore with a diameter of approximately 20 Å.

Group Members

Isabel Bento	Post Doc
Colin McVey	Post Doc
Daniele deSanctis	Post Doc
Tiago Bandeiras	Post Doc
Célia Romão[1]	Post Doc
Ricardo Coelho	Technician
Sabine Gorynia	PhD Student
Diana Plácido [2]	PhD Student
Ana Rêgo	PhD Student
David Marçal	PhD Student
Miguel Lopes	Graduate
Catarina Silva	Graduate
S. Palanivelo	Graduate
Ana Ferreira	Undergraduate

[1] co-supervision (Miguel Teixeira)

[2] co-supervision (M. Archer / A. Henriques)

Selected Publications

Carrondo M. A., Bento I., Matias P. M. and Lindley P. F. (2007). "*Crystallographic evidence for dioxygen interactions with iron proteins.*" Journal of Biological Inorganic Chemistry **12**(4): 429-442

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.

de Sanctis, D. Rego, A. T. Marcal, D. McVey, C. E. Carrondo, M. A. Enguita, F. J. (2008) "*Overexpression, purification and crystallization of the tetrameric form of SorC sorbitol operon regulator*" Acta Crystallogr Sect F Struct Biol Cryst Commun, **64**, pg 22-4

Raman Spectroscopy of Metalloproteins

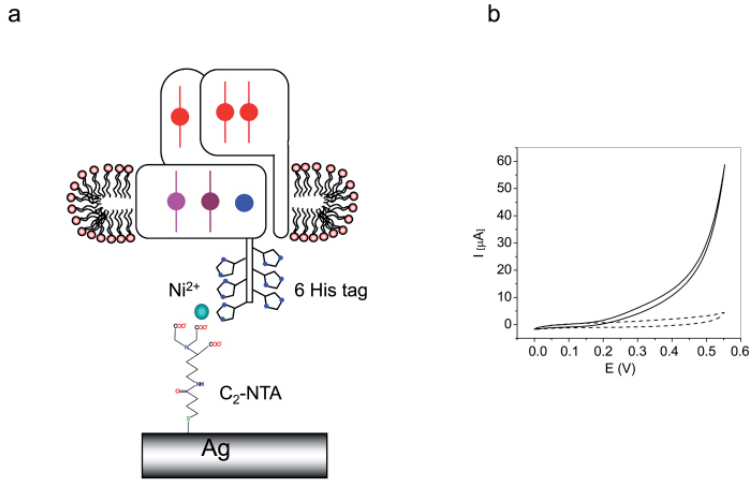
Smilja Todorovic
Auxiliary Investigator
PhD 2000, in Physical Chemistry, University of Belgrade / Mayo Clinic Rochester MN

Our research is focused on the biophysico-chemical aspects of the functioning of various metalloproteins. In particular, we are interested in understanding how different proteins fine-tune the reactivity of their metal cofactors and additionally, how the metal centers help to define the functional features, dynamics and stability of protein molecules. We use resonance Raman (RR) and Surface Enhanced RR (SERR) spectroscopy, in both stationary and time resolved modes, on a wide variety of structurally and functionally different proteins, sometimes with specifically designed point mutations, in order to access the details of the interplay between the protein matrix and the metal.

Resonance Raman spectroscopy of the heme, blue copper, non-hemic iron and iron-sulfur proteins that we study, can reveal highly specific and sensitive information on discrete metal site(s) within a protein. It is also capable of providing information on: the thermodynamic parameters that control electron transfer in redox proteins, the ligation pattern of the metal center, or short-lived intermediates of the catalytic cycle of an enzyme.

In the studies of temperature induced unfolding of a small electron carrier protein ferredoxine, RR provided a detailed description of the unfolding processes on the level of each Fe-S cluster, due to distinctive RR fingerprints of the 3Fe-4S and the 4Fe-4S centers. Also, RR spectroscopy is a powerful tool for investigating the coordination geometry of the T1 site of multicopper oxygen reductases. It can be used to address the role of the Cu-S bond strength in controlling the spectroscopic, electrochemical and catalytic features of the T1 copper site of CotA and specially designed mutants with tuned electrochemical features, which possess potentially interesting and diverse biotechnological applications.

Special emphasis in our research during this year was paid to heme copper oxygen reductases, terminal enzymes in the respiratory chains of various bacteria and archaea. In spite of their major role in the oxygen consumption by living organisms, the mechanism of their function, the coupling between the redox processes and proton translocation is not well understood yet. A prerequisite for understanding the functioning of these complex multi-center redox enzymes is determination of individual midpoint potentials and their possible coupling. SERR spectroelectrochemical titrations allow determination of the redox potentials of different heme groups for protein molecules immobilized on nanostructured silver electrodes, under conditions that mimic basic features of the physiological membrane, such as: hydrophobic environment, restricted motion and defined directionality of electron transfer. Also, RR and SERR can reveal differences in terms of catalytic cycle, regulation of electron flow and proton translocation between the oxidases of different types.



A pentahemic cbb3 oxygen reductase embedded into reconstituted lipid bilayer. The protein immobilized on nanostructured Ni-NTA coated silver electrode via His tag (a), shows large catalytic currents (solid line) in the presence of electron donor and only capacitive currents (dashed line) in its absence (b).

Selected Publications

Todorovic, S., Leal, S. S., Salgueiro, C. A.; Zebger, I.; Hildebrandt, P.; Murgida, D. H.; Gomes, C. M. ´A Spectroscopic Study of the Temperature Induced Modifications on Ferredoxin Folding and Iron-Sulfur Moieties‘ 2007, Biochemistry, 46(37); 10733-10738.

Durao, P., Chen, Z., Fernandes, A., Hildebrandt, P., Murgida, D., Todorovic, S. Pereira, M., Melo, E., Martins, L.`Copper Incorporation into Recombinant CotA- laccase from Bacillus subtilis- Characterization of Fully Copper Loaded Enzymes´ J. Biol. Inorg. Chem. 13 183-193 available on-line October 2007

Biology Division

Integrative Biology to Understand and Control Microbes

The Biology Division is presently formed by 11 Laboratories, which comprise 34 PhD researchers, 40 PhD students, and about 15 other research fellows, mostly undergraduate students. Most of the groups are engaged in research aiming at understanding basic fundamental processes that support life, its resilience, and evolution.

A common trait within the Division is a multidisciplinary approach to research. For some groups the start point is often genetics, and the emphasis is on the use of classic or molecular genetics, as well as on molecular and cell biology methodologies. For other groups on the entry point for some projects may be more on the biochemistry and structural biology sides. A trend can also be seen in the use of global or systems biology type of approaches to achieve integrated, multiple-level descriptions of complex phenomena such as metabolism, cell division, development, and the complex behavior of populations in pathogenesis or drug resistance. A strong network of collaborative work exists both within the Division, with other groups of the LAO (Laboratório Associado, Oeiras) and importantly, also at the national and international levels.

Several fundamental processes that support life are actively studied by researchers in the Biology Division. Bacterial cell growth and division is an example, as is the morphogenesis of cellular structures, cell differentiation, cell-cell signaling mechanisms used by bacteria for intra- and inter-species communication, the regulation of gene expression, RNA metabolism in different systems with particular emphasis on RNases, mechanisms of RNA degradation and the regulatory role of small RNAs, or the dissection of the networks and regulatory circuits involved in sugar metabolism in pathogens and model bacteria.

The contribution of the division to our understanding of the mechanisms underlying the resilience of life is illustrated by our commitment to the study of the biology of extremophiles, including the production and uses of novel compatible solutes, the biology of endospores, and the mechanisms by which bacteria are able to resist antibiotics or to evade the host immune system.

Evolutionary biology is also present, and exemplified by several projects: the study of the evolution of different populations of pathogens worldwide to identify isolates which are most proficient, for example, in resisting antibiotics, causing disease or evading vaccination programs; comparative genomics of domesticated versus undomesticated model organisms; origin and evolution of ancient developmental programs.

Although overall the main emphasis is on basic mechanisms, many projects within the Division have a clear applied angle, which has not been neglected. Examples are, on one hand, the use of compatibles solutes for biomaterials preservation, or the identification of metabolic products of interest, and nutraceuticals. Other examples include the identification of virulence factors or new cellular targets that, if impaired, will contribute to the killing of bacteria by new or old antibiotics, that may help the infected host immune system to successfully cope with infection.

Molecular Microbiology is a unifying theme within the Division, but our activities are not limited to prokaryotes. Other projects focus on glycobiology and fucosyltransferases from mammalian cells, on the analysis of glycopeptides in bacterial infections, and on the development of chemical chaperones from hyperthermophiles to prevent protein aggregation associated with neurodegenerative diseases such as Alzheimer´s disease or amyotrophic lateral sclerosis.

Lastly, two new Laboratory Heads – Jaime Mota (Infection Biology) and Pedro Domingos (Developmental Neurobiology) – have been hired through the “Ciência 2007” Program. The Division warmly welcomes the new colleagues, whishes their success among us, and the flourishing of new levels of integration of our activities.

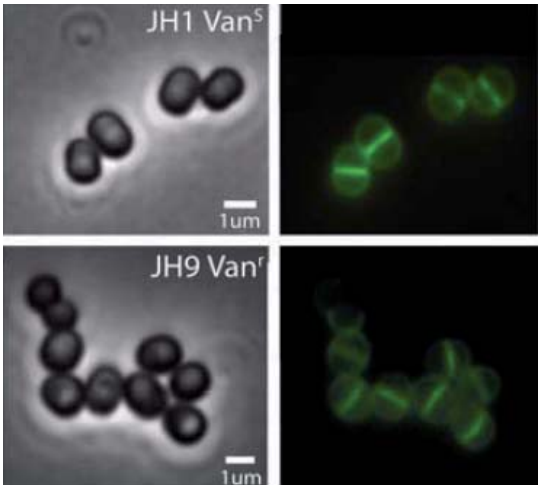
Bacterial Cell Biology

Mariana Gomes de Pinho

Auxiliary Investigator

PhD in 2001, Universidade Nova de Lisboa

In the Bacterial Cell Biology laboratory, we study cell division and antibiotic resistance in *Staphylococcus aureus*. We are currently interested in understanding at a molecular level, the organization and the temporal and spatial regulation of the cell wall synthetic machinery which is responsible for the synthesis of the division septum, as well as in understanding the bacterial mechanisms of resistance against antibiotics that target cell wall synthesis *S. aureus* is a Gram positive pathogen and the most common cause of antibiotic-resistant nosocomial infections. Over the last years, Vancomycin Intermediate resistant *S. aureus* (VISA) strains have emerged. Vancomycin is one of the few antibiotics that have remained effective against methicillin-resistant *S. aureus* (MRSA) and its multidrug resistant clones. Almost a decade has passed since the first reports of VISA strains and it is known that these strains usually have a thicker cell wall and are able to bind more vancomycin molecules, but their mechanism of resistance is still not completely understood. We have developed a new method of fluorescence ratio imaging microscopy and used it to compare the *in vivo* binding capacity and the access of a fluorescent derivative of vancomycin to the cell wall synthetic sites, in isogenic pairs of vancomycin susceptible and resistant *S. aureus* strains. Live cells of resistant strains were found to bind approximately 1.5 times more antibiotic but there was no correlation between the increased binding capacity and the vancomycin minimum inhibitory concentration (MIC) values of the strains, indicating that this increased binding capacity is certainly not the only factor responsible for resistance. We have also shown that in both susceptible and resistant bacteria, the subcellular sites of wall synthesis were localized at the division septa. However, the rate of diffusion of drug molecules to these sites, where the target of vancomycin (lipidII) is located, was reduced in resistant cells. These findings allowed a reinterpretation of the mechanism of vancomycin resistance, in which the slower diffusion of antibiotic molecules to its lethal target is an essential feature of this mechanism. Additionally, we have shown that the path of vancomycin to its target is through the division septum and therefore is dependent on the stage of the staphylococcal cell cycle, emphasizing the importance of cell cycle studies in pathogenic bacteria.



Diffusion of a fluorescent derivative of vancomycin (Van-FL) through the cell wall of *S. aureus* cells pre-labeled with non-fluorescent vancomycin. Fluorescent vancomycin was able to reach the division septum in both vancomycin susceptible strain JH1 and resistant strain JH9.

Group Members

Patricia Reed	Post Doc
Margarida Santos	Post Doc
Ana Jorge	PhD Student
Helena Veiga	PhD Student
Pedro Matos	PhD Student

Selected Publications

Pereira, P.M., S.R. Filipe, A. Tomasz and M. G. Pinho. 2007. "*Fluorescence imaging microscopy shows decreased access of vancomycin to cell wall synthetic sites in Vancomycin-Resistant Staphylococcus aureus*", Antimicrobial Agents and Chemotherapy, **51**:3627-3633

Bacterial Cell Surfaces and Pathogenesis

Sérgio Raposo Filipe

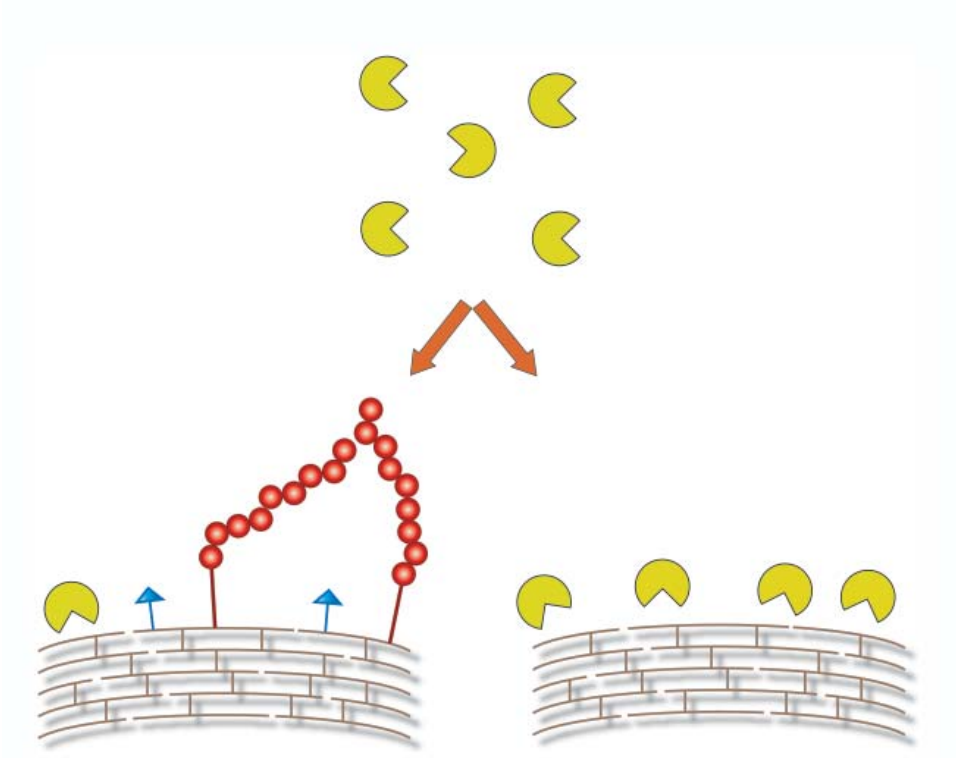
Auxiliary Investigator

PhD 2001 in Biology, Universidade Nova de Lisboa, ITQB

Group Members

James Yates	Post Doc
Magda Atilano	PhD Student
Mafalda Henriques	Graduate
Madalena Pereira	Graduate
Luis Ferreira	Master Student

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 cell wall 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. During this year we have created a small library of *Staphylococcus aureus* mutants with an altered cell wall metabolism. We are currently analysing the composition of the cell wall of these *S. aureus* mutants and determining the ability of these bacterial cell walls to induce an inflammatory response. On the other hand we have secured funding to start a complementary line of research that will allow us to better understand how the bacterial peptidoglycan is recognized by the host innate immune system. We have now produced novel genetic tools to analyse the cell wall synthesis in *Streptococcus pneumoniae*. We are particularly interested in how the pneumococcal capsular polysaccharide is synthesized and how is this process coordinated with the peptidoglycan synthesis. We look forward to determine the contribution of the capsular polysaccharides in the modulation of the inflammatory activity of the bacterial peptidoglycan.



Schematic representation of the bacterial cell wall (left) and peptidoglycan (right). Access of a peptidoglycan detector protein or a peptidoglycan hydrolytic enzyme (yellow symbol) can be impaired when peptidoglycan is covered with teichoic acids (red circles) or when it presents modifications (blue triangle). In live bacteria peptidoglycan is usually found attached to other bacterial polysaccharides or proteins.

Selected Publications

Pereira P. M., Filipe S. R., Tomasz A. and Pinho M. G. (2007). "*Fluorescence ratio imaging microscopy shows decreased access of vancomycin to cell wall synthetic sites in vancomycin-resistant Staphylococcus aureus.*" Antimicrobial Agents and Chemotherapy **51**(10): 3627-3633.

Bacterial Signalling

Karina B. Xavier

Auxiliary Investigator

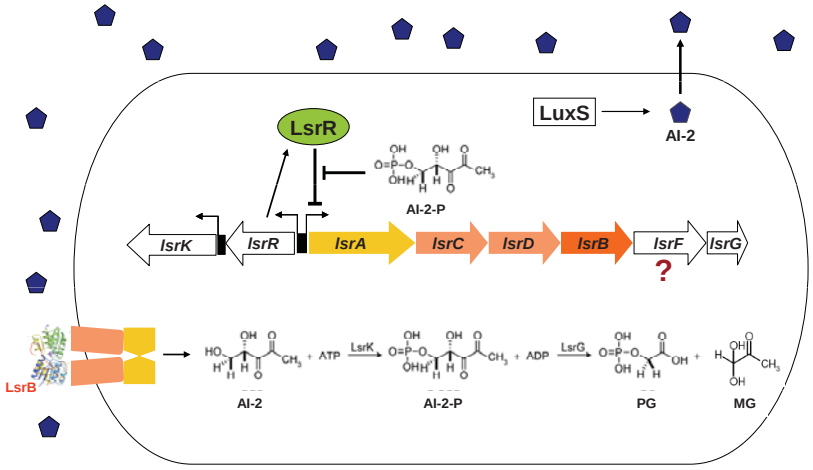
PhD in 1999, Universidade Nova de Lisboa ,ITQB

Quorum sensing is a process many bacteria use to assess cell-population density and synchronize behavior on a community-wide scale. Communication is mediated by the production, release, and detection of signal molecules called autoinducers. The LuxS synthase produces 4,5-dihydroxy-2,3-pentanedione (DPD), the precursor to a set of inter-converting molecules, used by many different bacteria as a quorum sensing autoinducer called autoinducer-2 (AI-2) that fosters inter-species communication.

In enteric bacteria, AI-2 production induces the assembly of a transport apparatus (called Lsr for LuxS Regulated) that internalizes endogenously produced AI-2 as well as AI-2 produced by other bacterial species, thus eliminating the signal from the environment. In previous work we have shown that using this system to internalize AI-2 enteric bacteria have the capacity of interfering with the signaling capabilities of neighboring species of bacteria like the human pathogen *Vibrio cholerae* and their ability to regulate group behaviours such as virulence and biofilm formation.

We are currently elucidating the molecular mechanisms involved in the system of interference with AI-2 mediated signaling. In *Salmonella typhimurium* and *Escherichia coli* the AI-2 receptor binds a specific cyclic derivative of DPD but following AI-2 internalization by the Lsr system, intra-cellular AI-2 is phosphorylated to AI-2-Phosphate (AI-2-P) by a kinase (LsrK) that phosphorylates the open form of DPD. AI-2-P is the inducer of the lsr operon and acts by binding to the repressor of this operon. We have shown that LsrG, another member of the *lsr* operon, catalyzes the retro-aldol cleavage of AI-2-P stopping its ability to induce the system. Therefore, LsrG is the first enzyme involved in terminating the signaling cycle of AI-2 interference.

We predict that this mechanism of interference with AI-2 signaling has important consequences in natural niches colonized by enteric bacteria such as the human gut where many different bacterial species co-exist and depend on quorum sensing for efficient colonization. To further understand the role of this mechanism in Nature we are also studying the impact of this system in the behavior of other bacterial species that colonize the human gut.



In *Escherichia coli* and *Salmonella typhimurium*, AI-2 production induces the assembly of a transport apparatus (called Lsr for LuxS Regulated) that internalizes endogenously produced AI-2 as well as AI-2 produced by other bacterial species eliminating the signal from the environment, and thus interfering with the signaling capabilities of neighboring species of bacteria.
This mechanism of AI-2 interference involves the production of the AI-2 precursor 4,5-dihydroxy-2,3-pentanedione (DPD) by the synthase LuxS, a periplasmic receptor protein (LsrB), an uptake transport system (Lsr transporter), the transcriptional repressor (LsrR) and at least two processing enzymes the AI-2 kinase LsrK, and the newly characterized aldolase, LsrG. We have shown that LsrK phosphorylates AI-2 and the phosphorylated compound, termed AI-2-P, binds to LsrR, an event that leads to induction of the *lsr* operon. This induction is ceased by the action of LsrG which catalysis a retro-aldol reaction cleaving AI-2-P to 2-phosphoglycolic acid (PG) and methylglyoxal (MG). Importantly, the products of the LsrG reaction do not bind to LsrR therefore when AI-2-P is cleaved lsr repression is restored terminating the AI-2 signaling cycle.

Group Members

Michal Bejerano-Sagie	Post-Doc
Catarina Pereira	PhD Student
João Marques	Master Student
António Santos	Short Term Apprentice

Selected Publications

Bejerano-Sagie M. and Xavier K. B. (2007). "*The role of small RNAs in quorum sensing.*" Current Opinion in Microbiology **10**(2): 189-198.

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.

Cell Physiology & NMR

Helena Santos

Full Professor

PhD 1984 in Biophysics (First Class), Universidade Nova de Lisboa

Group Members

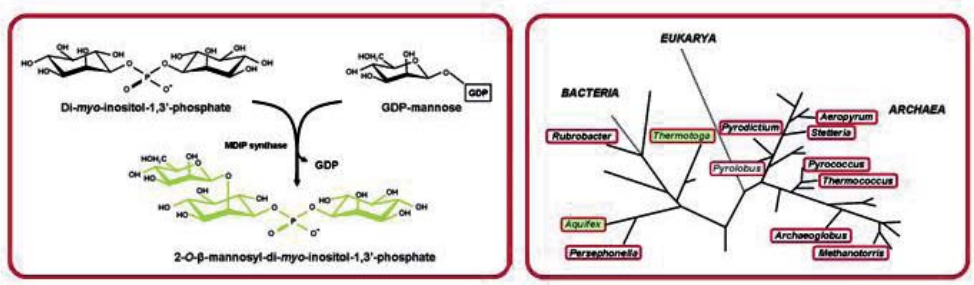
Pedro Lamosa	Post Doc
Nuno Borges	Post Doc
Tiago Faria	Post Doc
Luís Fonseca	Post Doc
Melinda Noronha	Post Doc
Tony Collins	Post Doc
Luís Gafeira	PhD Student
Filipa Cardoso	PhD Student
Carla Jorge	PhD Student
Paula Gaspar	PhD Student
Rute Castro	PhD Student
Marta Rodrigues	PhD Student
Tiago Pais	PhD Student
Ana Carvalho	PhD Student
Ana Mingote	Technician
Carla Almeida	Graduate
Sandra Carvalho	PhD Student
David Turner	Invited Full Professor
Teresa Catarino	Assist. Prof. FCT-UNL
Pedro Quintas	PhD Student

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 systems for the industrial production of hypersolutes; characterization of the molecular basis for protein stabilization by solutes: effects on protein structure and dynamics; new applications for compatible solutes as protein stabilizers.

Systems Biology 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 sugar 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.

Structure and Properties of Haem Proteins. In the end of 2007 a sub-group with Prof. D. L. Turner and Prof. T. Catarino as senior scientists joined my Team.

Scientific Highlight. A major achievement of our team in 2007 was the elucidation of the pathway for the synthesis of a novel solute: mannosyl-di-*myo*-inositol phosphate. The gene encoding the synthase was identified and the function confirmed in the recombinant protein. In addition, in collaboration with the group of Prof. Imanaka/ Dr. Atomi, Kyoto, a mutant of *Thermococcus kodakaraensis* deficient in the synthesis of di-myo-inositol phosphate was constructed. This opens the way to the elucidation of the physiological role of this canonical solute of hyperthermophiles.



Pathway for the synthesis of mannosyl-di-*myo*-inositol-phosphate (MDIP), a novel solute accumulating in the hyperthermophilic bacteria of the genera *Thermotoga* and *Aquifex*. The stereochemistry was established by 13C-labeling and NMR, using the recombinant synthase. The distribution of the precursor, DIP, is highlighted with red boxes in the Tree of Life.

Selected Publications

Jorge C. D., Lamosa P. and Santos H. (2007). "*alpha-D-Mannopyranosyl-(1 -> 2)-alpha-D-glucopyranosyl-(1 -> 2)-glycerate in the thermophilic bacterium Petrotoga miotherma - structure, cellular content and function.*" FEBS Journal **274**:3120-3127.

Rodrigues M. V., Borges N., Henriques M., Lamosa P., Ventura R., Fernandes C., Empadinhas N., Maycock C., da Costa M. S. and Santos H. (2007). "*Bifunctional CTP: Inositol-1-phosphate cytidylyltransferase/CDP-inositol: Inositol-1-phosphate transferase, the key enzyme for di-myo-inositol-phosphate synthesis in several, (hyper)thermophiles.*" Journal of Bacteriology **189**:5405-5412.

Gaspar P., Neves A. R., Shearman C. A., Gasson M. J., Baptista A. M., Turner D. L., Soares C. M. and Santos H. (2007). "*The lactate dehydrogenases encoded by the ldh and ldhB genes in Lactococcus lactis exhibit distinct regulation and catalytic properties - comparative modeling to probe the molecular basis.*" FEBS Journal **274**:5924-5936.

Control of Gene Expression

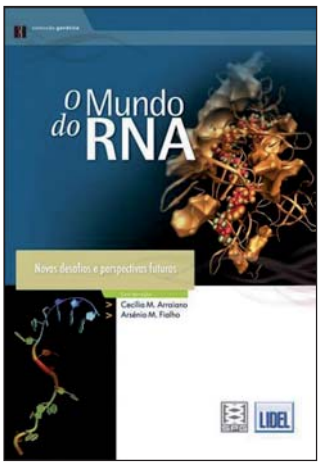
Cecília Arraiano

Principal Investigator with Agregação

PhD 1989 in Genetics, University of Georgia, Athens, USA

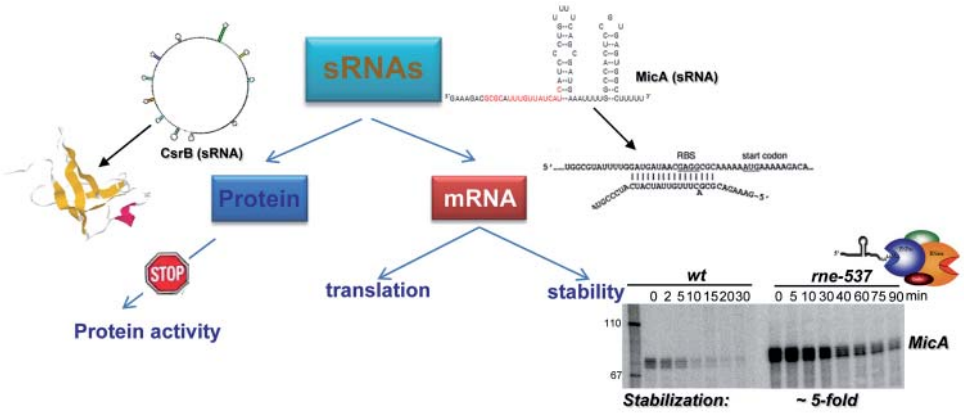
Many biological processes can not be fully understood without detailed knowledge of RNA metabolism. The analyses of RNA degradation have been difficult in all systems and, despite numerous studies, the process of RNA degradation is still poorly understood. Recent results appear to show that the similarities between mRNA decay in the pro- and eukaryotic systems are greater than were generally believed. This year we have edited the first Portuguese book on RNA metabolism **"O Mundo do RNA"**. This book will be very useful as an instrument of study for university Professors and students as well as for the general public interested in Science. This book explains in a simple way from basic concepts of RNA biology to the most recent discoveries. Research in this topic is more and more recognized as crucial in the control of gene expression and it was distinguished with 2 Nobel Prizes in 2006. Our research objectives are:

- **Post-Transcriptional Control of Gene Expression.**
- **Mechanism and Control of mRNA degradation.**
- **Characterization and Study of Ribonucleases.**
- **Metabolism of the Poly(A) tail in Bacterial mRNAs.**
- **Control of Cell Division under stress and stationary phase.**
- **Small RNAs and Control of Gene Expression.**



The cover of the Book: "O Mundo do RNA, Novos Desafios e Perspectivas Futuras". Coordination: CM. Arraiano and AM Fialho, Editora Lidel. Published Jan 2007

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. We have unravelled the dynamics of RNA degradation by RNase II and its RNA-bound complex (Frazão et al, Nature, 2006). This year, using RNase II hybrid proteins we were able to gain further insight into the role of the S1 domain in exoribonucleolytic activity (Amblar et al, RNA, 2007).In pathogenic bacteria, a large number of sRNAs coordinate adaptation to stress and expression of virulence genes. We have constructed several RNase mutants and characterized the role of ribonucleases in *Salmonella* small RNA decay (Viegas et al, NAR, 2007). Our study provided initial clues into the mechanisms of sRNA regulation in *Salmonella* and indicated specific contributions of the RNA decay machinery components to the turnover of individual sRNAs.



Modes of Action of Small RNAs. Small RNAs are divided in two main classes according to their mode of action. They can act on proteins changing their activity (e.g. CsrB) or directly bind to a target mRNA (e.g. MicA) affecting its translation and/or stability (results from Viegas et al, NAR, 2007).

Group Members

Patrick Freire	Post Doc
Sandra Viegas	Post Doc
Ana Barbas	Post Doc
Susana Domingos	Post Doc
José Andrade	PhD
Inês Guinote	PhD
Rute Matos	Master Student
Ana Matos	Master Student
Ricardo Moreira	Graduate
Inês Silva	Graduate
Vânia Pobre	Graduate
Ana Reis	Graduate
Mauro Conde	Graduate

Selected Publications

Viegas, S.C., Pfeiffer, V., Sittka, A., Silva, I.J., Vogel, J., and Arraiano, C.M. (2007) "*Characterization of the role of ribonucleases in Salmonella small RNA decay*". Nucleic Acids Research **35**:7651-64.

Amblar+, M., Barbas+, A., Gomez-Puertas P., Arraiano, CM. +Contributed equally (2007) "*The role of the S1 domain in Exoribonucleolytic Activity: Substrate Specificity and Multimerization*". RNA **13**:317-27.

Book: "*O Mundo do RNA, Novos Desafios e Perspectivas Futuras*". Coordination: CM. Arraiano and AM Fialho, Editora Lidel. Published Jan 2007

Glycobiology

Júlia Costa

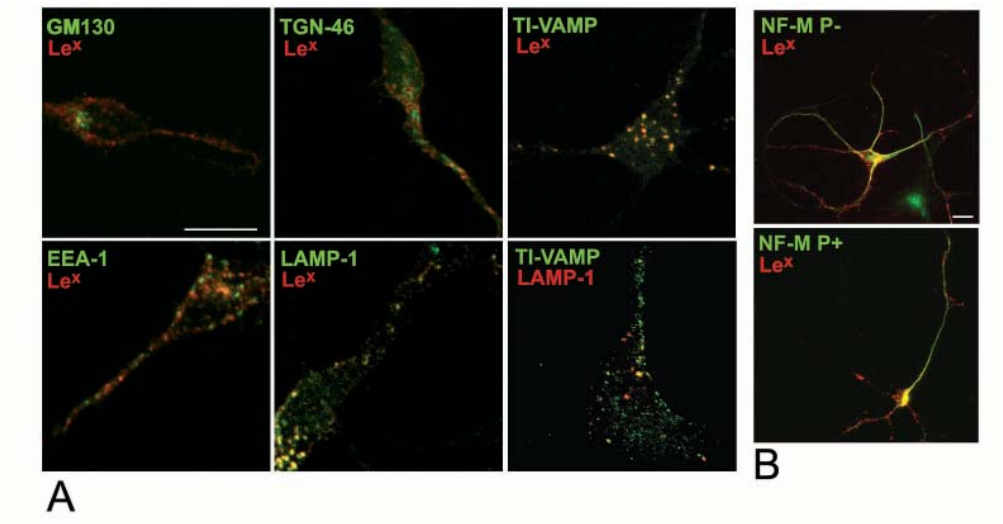
Principle Investigator

PhD 1994 in Biochemistry, University of Lisbon

Group Members

Catarina Gomes	PhD Student
Ricardo Gouveia	PhD Student
Eda Machado	PhD Student
Cristina Escrevente	PhD Student

Most mammalian proteins contain oligosaccharides covalently linked. The initial step in *N*-glycosylation occurs in the endoplasmic reticulum (ER) and consists of the transfer of the oligosaccharide $Glc_3Man_9GlcNAc_2$ onto the consensus site Asn-X-Ser/Thr of the nascent polypeptide chain. Processing of this precursor oligosaccharide by several glycosidases and glycosyltransferases in the ER and Golgi apparatus, results in the final *N*-linked oligosaccharide. Glycosyltransferases catalyze the transfer of a monosaccharide from a sugar-nucleotide donor onto the oligosaccharide acceptors. Protein glycosylation depends on several factors including the three-dimensional structure of the protein and the set of glycosidases and glycosyltransferases of the host system. Oligosaccharides have been shown to be important for several processes including protein folding, intracellular transport, cell-cell interactions and signalling.We have been investigating the importance of *N*-linked glycosylation in several glycoproteins through mutagenesis of the occupied sites. One example is nicastrin, a glycoprotein from the g -secretase complex, which produces A b , the pathological hallmark of Alzheimer's disease. Peripheral fucosyltransferases are late acting glycosyltransferases localized in the *trans*-Golgi and *trans*-Golgi network. They transfer Fuc from GDP-Fuc onto *N*- or *O*-linked oligosaccharides with the synthesis of the carbohydrate adhesion determinants of the Lewis type. Alterations of cell surface glycosylation are common to almost all types of tumors. In this context, we have modified the fucosylation of ovarian carcinoma cells to investigate consequences for cell phenotype. Fucosylation, Lewis^x detection and factors affecting neurite outgrowth have also been studied in human NT2N neurons differentiated in vitro from teratocarcinoma NT2$^-$ cells (Figure). Our results suggest that Lewis^x underlies cell differentiation, cell adhesion and neurite outgrowth in NT2N neurons.The Golgi apparatus is found disrupted in the neurodegenerative disease amyotrophic lateral sclerosis (ALS). We are investigating consequences for secretion and glycosylation of glycoproteins expressed in cellular models of the disease. Furthermore, we are analyzing glycoproteins from plasma and cerebrospinal fluid of ALS patients by proteomics, to find a possible biomarker.



Localization of Lewis^x in NT2N neurons by immunofluorescence confocal microscopy.(A) Co-localization analysis of Le^{x}with markers of the endocytic and exocytic pathways and of LAMP-1 with TI-VAMP. (B) Co-localization analysis of Le^x with dendrite (hypophosphorylated medium molecular weight neurofilament protein, NF-M P-) and axon (hyperphosphorylated medium molecular weight neurofilament protein, NF-M P+) markers. Bar 10 mm.

Selected Publications

Brito C., Escrevente C., Reis C. A., Lee V. M. Y., Trojanowski J. Q. and Costa J. (2007). "*Increased levels of fucosyltransferase IX and carbohydrate Lewis(x) adhesion determinant in human NT2N neurons.*" Journal of Neuroscience Research **85**(6): 1260-1270.

Gomes C., Keller S., Altevogt P. and Costa J. (2007). "*Evidence for secretion of Cu,Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis.*" Neuroscience Letters **428**(1): 43-46.

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.

Lactic Acid Bacteria and in vivo NMR

Ana Rute Neves
Auxiliary Investigator
Ph.D. 2001 in Biochemistry,
Instituto de Tecnologia Qumica e Biolgica (ITQB)/ Universidade Nova de Lisboa (UNL)

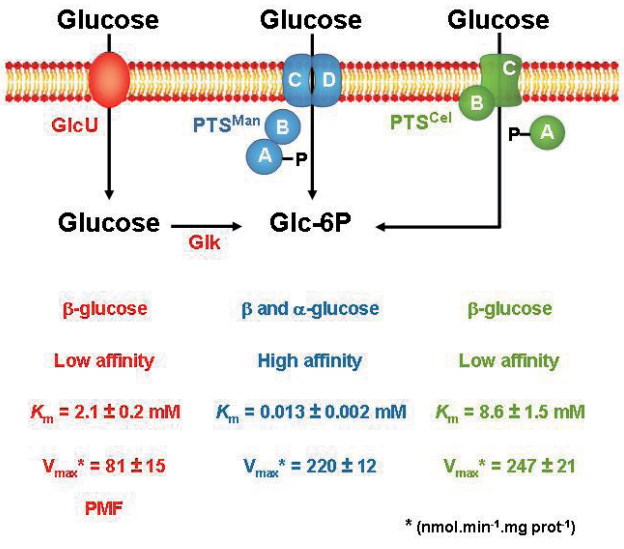
Lactic acid bacteria (LAB) comprise a group of Gram-positive bacteria with habitats ranging from milk to specific niches in the human body. Some members have been used for thousands of years in food fermentations (e.g. *Lactococcus*, *Lactobacillus* and Bifidobacteria), whereas others are well-known 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 members of the LAB, 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 dairy organism *Lactococcus lactis*. 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 the 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. peumoniae*, 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 past year we were able to establish a connection between nutrients in the growth medium (nitrogenous bases) and capsule production in *S. pneumoniae* (Carvalho *et al.*, unpublished results). This finding paves the way for new strategies envisaging the obstruction of capsule synthesis. Our long-term efforts to unravel the mechanisms involved in sugar transport in *L. lactis* have produced good results (collaboration with H. Santos). We have proven that the preferred sugar glucose can be transported by three different routes, the mannose-PTS (PTS, phospho*enol*pyruvate-phosphotransferase system), the cellobiose-PTS (Pool, Neves *et al.*, 2006) and the newly identified non-PTS permease, GlcU (Castro *et al.*, manuscript in preparation). This discovery has important implications in metabolic engineering of *L. lactis*, due to the key role of glucose in the regulation of sugar metabolism. Our work provides the first complete picture of glucose transport in a lactic acid bacterium (see figure).



Schematic representation of glucose transport systems and their properties in *L. lactis*.

Group Members

Rute Castro	PhD Student
Ana Carvalho	PhD Student
Sandra Carvalho	PhD Student

Selected Publications

Gaspar P, Neves, AR, Shearman CA, Gasson MJ, Baptista AM, Turner DL, Soares CM and Santos H . (2007) "*The lactate dehydrogenases encoded by the ldh and ldhB genes in Lactococcus lactis exhibit distinct regulation and catalytic properties - comparative modeling to probe the molecular basis.*" FEBS J. **274**: 5924-5936.

Sánchez C, Neves AR, Cavalheiro J, dos Santos MM, García-Quintáns N, López P and Santos H. (2007) "*The contribution of citrate metabolism to the growth of Lactococcus lactis CRL264 at low pH.*" Appl. Environ. Microbiol. [Epub ahead of print]. PMID:18156322.

Microbial Development

Adriano O. Henriques

Associate Professor

Ph.D 1993 in Cellular Biology, University of Coimbra, Coimbra, Portugal.

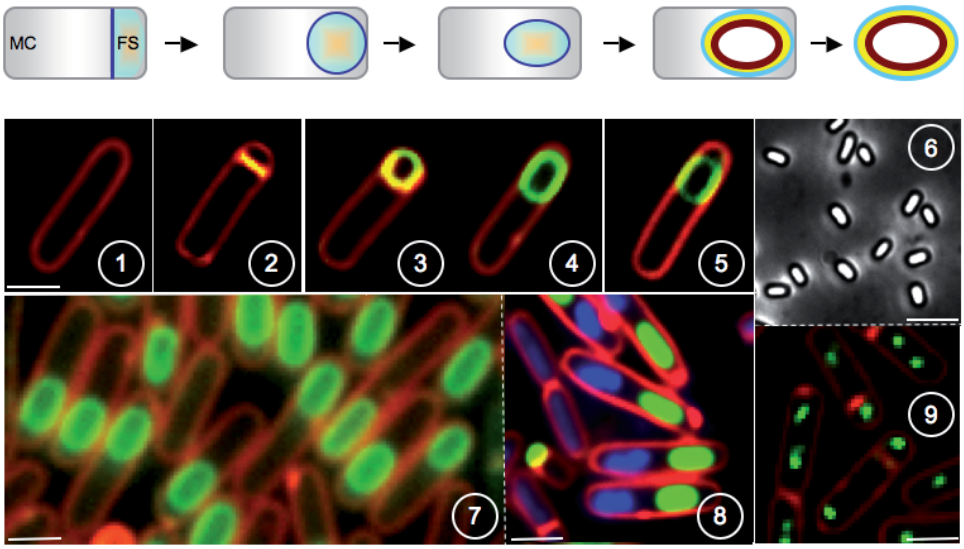
Group Members

Mónica Serrano	Post Doc
Gonçalo Real	Post Doc
Anabela Isidro	Post Doc
Filipa Valente	Post Doc
Luísa Côrte	PhD Student
Cláudia Serra	PhD Student
Catarina Fernandes	Graduate
Carla Esteves	Master Student
Pedro Rodrigues	Master Student
Diana Plácido [1]	PhD Student
Sandro Pereira [2]	PhD Student

[1] co-supervision (M. Archer and M.A. Carrondo)

[2] co-supervision (A. Tomasz and H. de Lencastre)

The Microbial Development group studies development in the model organism *Bacillus subtilis*, which involves metamorphosis of the rod-shaped vegetative cell into a dormant, highly resistant spore. At its onset, the cells abandon medial (symmetrical) cell division, and switch to a mode of polar (asymmetric) division, which gives rise to a dissimilar progeny, the mother cell and the smaller prespore (figure below). The prespore will differentiate into the mature spore, whereas the mother cell eventually lysis to release the spore (see figure). Polar division is served by a specialized mode of chromosome segregation that ensures that one of the replicated chromosomes is partitioned into the prespore, while the other stays in the mother cell (see the figure below), and one project in the laboratory deals with the coordination between asymmetric division and chromosome segregation. Gene expression in the prespore and the mother cell is then orchestrated by a cascade of RNA polymerase sigma subunits, which are activated in a cell type-specific manner, in response to the course of morphogenesis and as the result of cell-cell signaling pathways that link the two lines of gene expression. The group is also investigating a pathway by which the mother cell induces activation of the late prespore-specific sigma factor. It functions only after the complete engulfment of the prespore by the mother cell (see figure), and involves a novel type of synapse-like complex that connects the two cells, as well as a new mechanism for maintaining the polymerase in the prespore inactive until the appropriate signal is perceived. Keeping the programs of gene expression in the two cells in close register with the course of morphogenesis allows the temporal differentiation of classes of gene expression, whose proper deployment is important for the fidelity of morphogenesis. In a dramatic example, heterochronic mutants, in which an early morphogenetic gene (cotE) is transplanted to late classes of expression, show suppressed morphogenesis of a spore protective layer. In addition, and importantly, much of the differentiation process relies on the sub-cellular localization of proteins, and the group is also interested on the mechanisms by which proteins are targeted to their final addresses. An example is the localization of an integral membrane protein required for cortex biogenesis, a layer of peptidoglycan that confers heat resistance to the spore (see figure). Lastly, knowledge is also being translated into research with a more applied angle, from the use of spores as display systems for bioactive molecules, to the characterization of spore-formers with a potential use as probiotics.



The figure (top) shows the main morphological stages of sporulation in B. subtilis, from the polar division event that forms the larger mother cell (MC) and the smaller forespore (FS), engulfment of the FS by the MC, synthesis of the spore protective layers (brow, cortex peptidoglycan; yellow, inner coat; blue, outer coat), to spore release upon lysis of the mother cell. The figure (bottom) also depicts the engulfment sequence (1 through 5) and the sub-cellular locaization of a mambrane protein fused to GFP. Phase bright free spores are shown in panel 6. Panel 7 shows the localization of SpoVE-GFP, a membrane protein essential for synthesis of the spore cortex peptidoglycan; the forespore-specific expression of the late forespore regulator SigG fused to GFP is shown in 7, and the origin of chromosome replication (as marked by Spo0J-GFP) is shown in panel 9 (note the presence of one focus of Spo0J-GFP in them other cell and one in the forespore of the two specimens in the center). Cell and spore membranes are in red. Scale bar, ≈1μm.

Selected Publications

Costa, T. V., M. Serrano, C. P. Moran Jr., and A. O. Henriques. 2007. "*The timing of cotE expression affects Bacillus subtilis spore coat morphology but not resistance to lysozyme.*" J. Bacteriol., **189**:2401-2410.

Henriques, A. O., and C. P. Moran Jr. 2008. "*Structure, assembly and function of the spore surface layers.*" Ann. Rev. Microbiol., **61**:555-588.

Real, G., Fay, A., Eldar, A., Henriques, A. O., and J. Dworkin. 2008. "*Determinants for the sub-cellular localization and function of a non-essential SEDS protein.*" J. Bacteriol., **190**:363-376.

Microbial Genetics

Isabel M. G. de S Nogueira

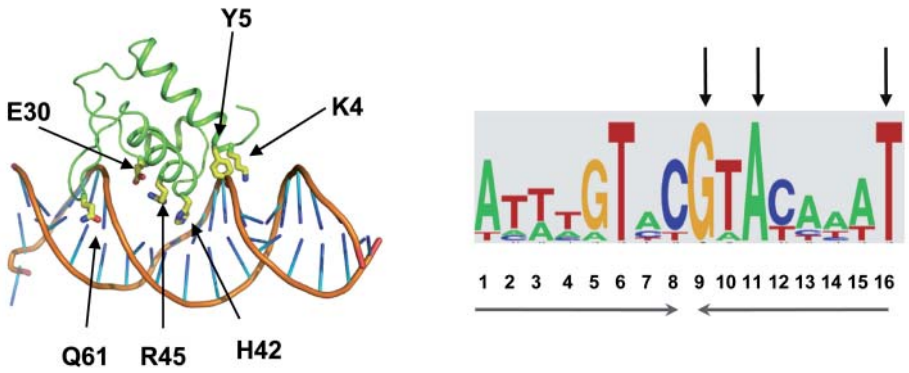
Associate Professor, Faculdade Ciências e Tecnologia, Universidade Nova Lisboa

PhD 1991 in Biology, Universidade Nova Lisboa

The main area of interest in the Microbial Genetics Laboratory 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*. In 2007, we carried on two major lines of research: the first centered on mechanisms of gene regulation that involve the AraR transcription factor, and the second addressing the genetic and biochemical characterization of a hemicellulolytic enzymatic system. AraR controls a set of genes required for the extracellular degradation of polysaccharides, transport of oligomers and simple sugars, and further intracellular catabolism.

Studies of the AraR repressor-operator system aimed at understanding protein-DNA interactions and allosteric mechanisms of gene regulation. The effect of residue substitutions in the AraR N-terminal DNA-binding domain and of base-pair exchanges into an AraR operator site were examined by assaying for AraR-mediated regulatory activity *in vivo* and DNA-binding activity in vitro. The results showed that particular residues, located in or near the winged-helix DNA-binding motif, were the most critical for AraR function (Figure). In addition, the analysis of the various mutations in a palindromic operator sequence highlighted specific bases crucial for AraR binding activity. These findings extend the knowledge on the nature of AraR nucleoprotein complexes and the mode of action of AraR and its orthologues.

The synthesis of hemicellulolytic enzymes involved in the degradation of arabinose-containing polysaccharides, is subjected to carbon catabolite repression (CCR). We identified *trans*-acting factors and *cis*-elements involved in glucose repression of arabinases genes. CcpA is the major regulator and acts via binding to cre elements located primarily in each promoter region of these genes. Contributions of co-effectors HPr and Crh to CCR differ according to growth phase. HPr dependency occurs both during exponential growth and transition phase, while Crh dependency is mainly detected at transition phase. Our results suggest that Crh synthesis may increase at the end of exponential growth and consequently contribute to this effect together with other factors (Inácio and Sá -Nogueira, 2007).



AraR-DNA key contacts. Structure of the modelled N-terminal domain of AraR (only one monomer is represented), highlighting the residues in which site-directed mutations lead to a constitutive phenotype (left). Consensus 16-bp palindromic operator sequence inferred from eight AraR boxes (right). Arrows point out the most critical bases for AraR-DNA interaction. Picture adapted from Franco et al., 2007.

Group Members

Irina Franco	PhD Student
José Inácio	PhD Student
Isabel Correia	PhD Student
Sónia Custódio	Graduate
Zélia Gouveia	Graduate
Margarida Augusto	Graduate
Samuel Costa	Undergraduate

Selected Publications

Franco I. S., Mota L. J., Soares C. M. and de Sá-Nogueira I. (2007) "Probing key DNA contacts in AraR-mediated transcriptional repression of the *Bacillus subtilis* arabinose regulon." Nucleic Acids Research **35**(14): 4755-4766.

Inácio J. M. and de Sá-Nogueira I. (2007) "*Trans-acting factors and cis elements involved in glucose repression of arabinan degradation in Bacillus subtilis.*" Journal of Bacteriology **189**(22): 8371-8376.

Laboratory of Microbial Epidemiology

Rosário Mato

Auxiliary Investigator

PhD 1992 in Medical Microbiology, Universidad Complutense de Madrid, Spain.

Group Members

Filipe Almeida	Graduate
Raquel Portela	Undergraduate

Epidemiology and evolution of enterococci

Epidemiological surveillance studies were carried out by our team involving two Lisbon hospitals. The collection of isolates under study included enterococci recovered from infection products from inpatients of all hospital services during a 4 year longitudinal study (2004-2007) and enterococci associated with colonization collected at a haematological malignancies ward and at a neonatal intensive care unit-NICU. The results obtained were of clinical relevance, as briefly illustrated. Data from the longitudinal study showed that: i) the number of infections caused by multidrug-resistant enterococci, and resistance to most of the antibiotics tested mainly *E. faecalis* increased ii) resistance to glycopeptides among *E. faecium* isolates was 14% in 2007; iii) most of the *E. faecium* isolates are members of an international and widely disseminated clonal complex; iv) one *E. faecalis* HLGR clone acquired virulence determinants over time and became dominant in 2007 (Fig. 1); v) new *E. faecium* clones of vancomycin-resistant strains with different genetic backgrounds, virulence profiles determinants emerged during 2007. Results from the colonization surveillance studies showed that newborns were mostly colonized by *E. faecalis* (92%), lower enterococcal rectal carriage rates (68%)and, yet resistance frequencies to almost all antimicrobial tested were higher it waere not detected enterococci resistant to vancomycin. The dominant *E. faecalis* HLGR clones associated with colonization of newborns and with clinical products of inpatients were unrelated. Figure 2. In contrast, colonization and infection isolates from haematological patients were clonal. The lymphocytis as underlying haematological malignancy diseases seem to be a risk factor for the colonization and infection with *E. faecium* vancomycin-resistant.

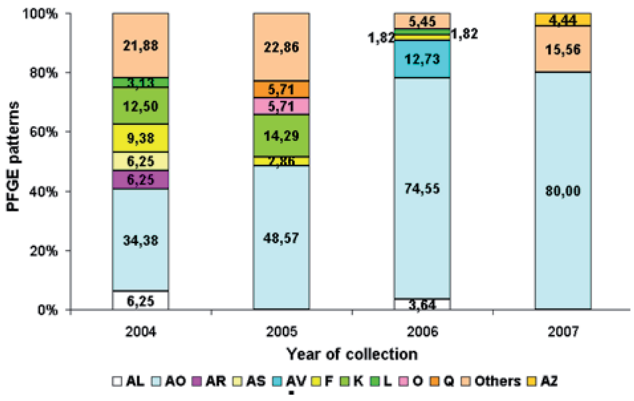
These results were included in two communications (17Th ECCMID, Munich, Germany, March 2007 and MICRO' 2007, BIOTEC'2007, Lisbon, Portugal) and in two theses to obtain Master's degree (MSc) in Molecular Biology and Genetic (FC/UL) and Graduation's degree in Molecular and Cellular Biology (FCT/UNL). Supervisor: Rosario Mato, PhD. ITQB/UNL.

Nasopharyngeal and oropharyngeal colonization of pre-school children by beta-hemolytic streptococci (BHS)

BHS recovered from nasopharyngeal and oropharyngeal samples collected in 2007 from healthy children attending Day Care Centers (DCC) located at the Oeiras area were study. The majority of BHS isolates from nasopharyngeal samples were susceptible to macrolides. Related with oropharyngeal samples, a group of bacitracin-resistant (BacR) Group A streptococci (GAS) was studied the results show that belong to the international BacR e*mm*28-ST52 lineage (resistant to bacitracin) .

These results were included in two communications at the national Congress MICRO'07 BIOTEC'07 Lisbon, Portugal, and in two graduation thesis to obtain a Graduation's degree in Cellular and Molecular Biology by FCT/UNL. Co-Supervisor: Rosario Mato, PhD. ITQB/UNL.

These projects have been carried out in collaboration with I. Sanches, PhD (CREM; FCT/UNL), A. Brito-Avô, MD and A. Morais and associated teams (Centro de Saúde de Oeiras). Nasopharyngeal swabs were provided by H. de Lencastre, PhD (Laboratory of Molecular Genetics. ITQB/UNL.



Legend. Picture 1. Clonal evolution of *Enterococcus faecalis* high-level gentamicin resistant and vancomycin-resistant recovered from infection products of inpatients, over four year study period.

Molecular Genetics

Hermínia de Lencastre

Full Professor

1981, in Molecular Genetics, Universidade Nova de Lisboa

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. *S. aureus* has a remarkable ability to rapidly adapt to antibiotic pressure. The characterization of different type of mutants and several DNA microarrays approaches has provided insights not only on mechanisms of resistance to methicillin and vancomycin, but also on cell physiology and cell wall biosynthesis regulation. Functional cooperativity and regulatory networks linking peptidoglycan biosynthetic genes and the methicillin and vancomycin resistance phenotype are being explored.Presently, one of the essential methods for characterization of MRSA is SCC*mec* typing. To this end we have recently developed a strategy, which consists in three independent assays: (i) *ccrB* typing; (ii) SCC*mec* multiplex PCR; and (iii) SCC*mec* type IV multiplex.We have performed recently a quantitative assessment of the congruency of the major typing methods for *S. aureus*, using a large collection previously characterized by PFGE, *spa* typing, MLST, and, for MRSA, SCC*mec* typing. The study clarified how the different typing methods perform in comparison to each other, which may guide the choice of the methodology to be used. Overall the most cost effective combination of techniques for a detailed characterization of *S. aureus* isolates was found to be the combination of PFGE with *spa* typing.In the light of the most recent findings on the evolution, population structure, and epidemiology of *S. epidermidis* we reviewed the usefulness and applicability of PFGE, MLST, and SCC*mec* typing to characterize *S. epidermidis* strains. The quantitative assessment of the correlation of the three methods showed that the combination of PFGE and SCC*mec* provides reliable information on both short and long-term epidemiology, which allowed us to define a *S. epidermidis* clone. Children at Portuguese day-care centers (DCC) frequently carry pneumococci. To understand the dynamics of colonization with pneumococci we did a 1-year longitudinal study on colonization in the DCC and showed that cross-transmission occurred with a very high frequency as 98% of all isolates belonged to clones shared by at least two children. Studies on the impact of the private use of the 7-valent pneumococcal conjugate vaccine showed that a high proportion (~60%) of children are being vaccinated and a dramatic shift on the serotypes carried has occurred among vaccinated and non-vaccinated children (indirect herd effect). An increase in the rarely carried serotype 1 was observed. Antimicrobial resistance rates have remained stable.

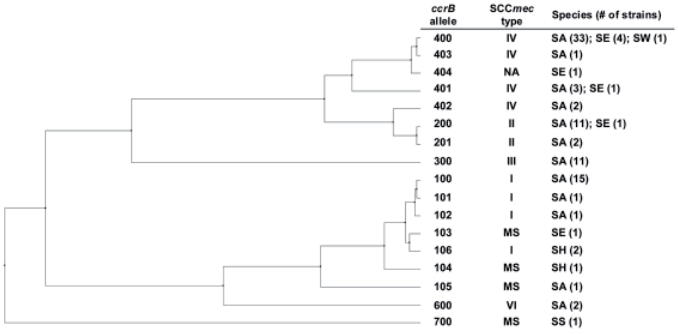


Figure 1. Average distance tree using identity percentage for all prototype sequences available on November 15, 2007 at the "*ccrB* Typing Tool" online resource. The tree was automatically drawn by the Java applet available through the "*ccrB* Typing Tool" using the default parameters. For each prototype sequence (*ccrB* allele) it is indicated the species where it was described and, between parenthesis, the number of strains of each species with that allele. Abbreviations: SA, *S. aureus*; SE, *S. epidermidis*; SH, *S. hominis*; SS, *S. saprophyticus*; SW, *S. warneri,* NA, not available; MS, methicillin-susceptible (SCC*mec* negative). (Oliveira DC, M Santos, C Milheiriço , JA CarriçoS Vinga, AL Oliveira, and H. de Lencastre. 2008. *ccrB* typing tool: an online resource for staphylococci *ccrB* sequence typing. J Antimicrob Chemother, in press).

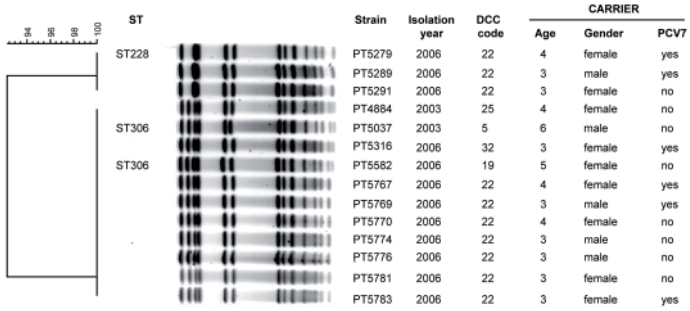


Figure 2. Genotypic relationship of serotype 1 pneumococcal isolates according to pulsed-field gel electrophoresis. ST, sequence type; DCC, day-care centre; PCV7, vaccinated with seven-valent pneumococcal conjugate vaccine (adapted from Nunes, S., R. Sá-Leão, L. Curto, and H. de Lencastre. 2008. Emergence of a serotype 1 *Streptococcus pneumoniae* lineage colonizing healthy children in Portugal in the 7-valent conjugate vaccination era. Clin. Microbiol. Infect. 14:82-84).

Group Members

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Alexandra Simões	PhD Student
Teresa Figueiredo	PhD Student
Sónia Nunes	PhD Student
Pedro Arede	PhD Student
Liliana Pereira	Master Student
Ana Tavares	Graduate
Joana Rolo	Undergraduate
Isilda Gueifão	Technician
Manuela Nogueira	Secretary

Selected Publications

1.Sobral, R. G., A. E. Jones, S. G. Des Etages, T. J. Dougherty, R. M. Peitzsch, T. Gaasterland, A. M. Ludovice, H. de Lencastre, and A. Tomasz. 2007. "*Extensive and genome-wide changes in the transcription profile of Staphylococcus aureus induced by modulating the transcription of the cell wall synthetic gene mur*" F. J. Bacteriol. **189**:2376-91.

2.Miragaia, M., J. C. Thomas, I. Couto, M. C. Enright, and H. de Lencastre. 2007. "*Inferring a population structure for Staphylococcus epidermidis from multilocus sequence typing (MLST) data.*" J. Bacteriol. **189**:2540-52.

3.Mwangi M. M, S. W. Wu, Y. Zhou, K. Sieradzki, H. de Lencastre, P. Richardson, D. Bruce, E. Rubin, E. Myers, E. D. Siggia, and A. Tomasz. 2007. "*Tracking the in vivo evolution of multidrug resistance in Staphylococcus aureus by whole-genome sequencing.*" Proc Natl Acad Sci U S A. **104**:9451-6.

Plant Sciences Division

Plants efficiently colonize highly diverse habitats due to a great genetic plasticity, but most plants utilized by man have been displaced from the original environments and have lost resistance genes during the extensive breeding programs. As a result, they are highly susceptible to stress (both abiotic and biotic), with consequent high economic losses that are predicted to increase in the near future due to the climatic changes. This is the concern of the 9 Laboratories of the Plant Sciences Division, which have a marked scientific interest in the plant responses to stress, performing research that extends from cell biology and developmental genetics, through biochemistry, genetic engineering and biotechnology, to ecophysiology. Economical relevant plants, such as, rice, maize, grain legumes, tomato, grapevine, olive, almond, coffee, pine, cork oak and eucalypt are being studied. The model-plants *Arabidopsis thaliana, Thellungiella halophila, Medicago truncatula, Oryza sativa (rice)*, tobacco, *Antirrhinum majus* and *Linaria* sp. are also being used, with the purpose of elucidating basic aspects of development, biochemistry and responses to stress, to support the studies with economical plants. Research with Portuguese endemic plants has also been initiated with the aim to elucidate the antimicrobial and pharmacological value of their secondary metabolites.

Proteins, metabolites and genes are currently being studied in association with the control of plant development and stress responses. The identification of upstream gene regulators is increasingly important, and an integrative approach to study the role of transcription factors (TFs) in plant stress-tolerance has been applied using rice as model plant. Novel TFs involved in cold, salt and drought stress adaptation have been isolated and identified. On a reduced scale, this work is being extended to a Rosaceae fruit tree (almond). A cytogenomic approach was initiated to study chromatin remodelling, the spatial distribution of TF genes and the redistribution of ribosomal genes and proteins, after imposed abiotic stresses.

The endogenous transposons of *Antirrhinum* continued to be used as tools for genetic, molecular and cellular analyses and equivalent tools for *Linaria* (a member of the *Antirrhinum* tribe) are sought. This would provide opportunities for studies on the evolution of developmental mechanisms that incorporate comparisons of mutants in a pair of close relatives. A somatically and germinally mobile copy of a CACTA transposon was already identified in *Linaria* and a number of *Linaria* homologues of *Antirrhinum* genes involved in flower development were characterized.

Arabidopsis and the related stress tolerant *Thellungiella* are being used in a comparative study to screen proteins associated with survival under severe drought conditions. The drought responses have also been investigated in *M. truncatula* and tobacco in relation to the participation in those mechanisms of trehalose-phosphate synthase, arginine carboxilase and DSP22 (an ELIP-like protein). Proteins and genes shown to be differentially expressed under boron deficiency continued to be studied in *Arabidopsis* and, conversely, studies were initiated of cell wall proteins that respond to boron

The successful production of recombinant proteins in plant-based systems continued to be studied with both whole plants and cell suspension cultures of the model species *M. truncatula*, *Arabidopsis* and tobacco. The advantages and disadvantages of these systems are being compared with the aim to identify the mechanisms involved in the correct sorting of recombinant proteins and to achieve high yields and an easy purification procedure.

Proteomic work has been conducted to evaluate the potential impact on human health of genetically modified foods (e.g., rice). Transcriptomic comparison of mutagenized versus transgenic plants produced interesting results. Concerning Portuguese maize landraces used for bread (“broa”) production, molecular diversity has been studied and seed proteins analysed in two landraces contrasting in “broa” quality. The Osborne solubility fractions were prepared and separated by 2-DE in order to identify the proteins by MS.

Grapevine has been actively investigated at the molecular level. Genes responsible for phenotypic variation of resistance to stress and berry quality and the events that control grape berry maturation, at the proteomic and transcriptomic level, are being studied.

In pine, the transcriptomics of wood formation and somatic embryogenesis continued to be investigated. Somatic embryogenesis is used both as a cloning technology and as a system for studying molecular regulation of embryo development. The recent success with the induction of somatic embryos from adult tissues has represented a major advance for the propagation of genotypes of proven value. Nitrogen metabolism and vascular differentiation are other aspects that are being studied as relevant mechanisms in tree growth and development.

In eucalypt, cell wall proteins related to wood formation were studied and of 46 new sequences contributed to the data base, four xyloglucan endotranglycoslyase/hydrolases (XHTs) are being expressed in *Pichea pastoris*. Another eucalypt protein of interest, associated with wood formation, is a KORRIGAN (KOR)-like endo-1,4,β-D-endoglucanase. *Arabidopsis* KOR1-1 mutants (with a severe dwarf phenotype and deficient cell wall formation) will be complemented with KOR and the KOR-like eucalypt sequence, in order to provide insight into the function of this protein in eucalypt.

In cork oak, the proteomics of cork forming tissues has been studied and the gene sequence of chalcone synthase was obtained. This sequence together with those of PAL (phenylalanine ammonia lyase) and CAD (cinnamyl alcohol dehydrogenase), already available in public databases, will be used to study the phenylpropanoid metabolism under environmental stress and in relation to cork formation.

In coffee, a study on the mechanisms underlying tolerance to adverse environment was initiated. Special attention has been given to the control of oxidative stress at the leaf level.

Regarding the Portuguese endemic species, their secondary metabolites are being screened during growth and under stress conditions, and the antioxidant and anti-proliferative bioactivities of selected compounds are being confirmed in vivo on several human cell lines; the effect of these substances on the ubiquitin/proteasome pathway will also be evaluated.

Disease and Stress Biology

Ricardo Boavida Ferreira

Full Professor at Instituto Superior de Agronomia, UTL

PhD 1987 in Biochemistry, University of East Anglia, U.K

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), 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.;

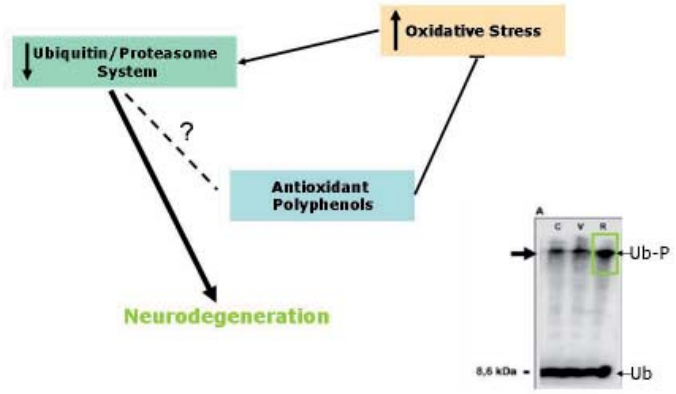
Biomedical applications of plant polyphenols

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

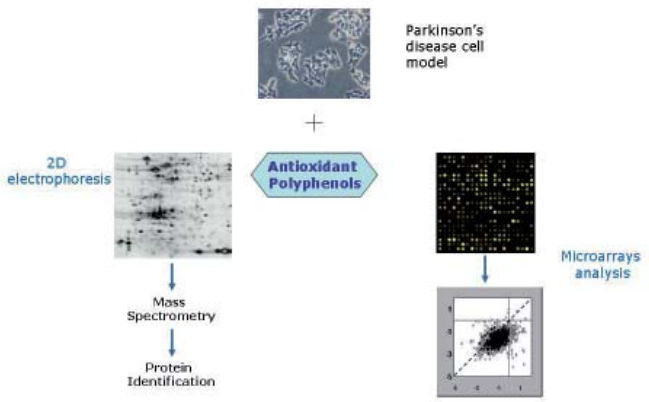
• Screening of the polyphenols for neuroprotective and anti-proliferative activities using cultured neuroblastoma cells and for anti-fungal activity towards human and plant pathogens;

• 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.



Effect of polyphenol treatment on the role played by the ubiquitin/proteasome pathway in Parkinson's disease



Transcriptomic and proteomic analyses of polyphenol treatment in Parkinson's disease

Group Members

Claudia Santos	Post Doc
Sara Monteiro	Post Doc
Damiano Vesentini	Post Doc
Dina Carrilho	Post Doc
Alexandra Carreira	Post Doc
Regina Freitas	PhD
Lucélia Tavares	PhD
Ana Ribeiro	PhD
Cristina Price	PhD
Vanessa Borrego	Graduate
David Barata	Undergraduate
Daniel Duarte	Undergraduate

Selected Publications

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.

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.

Ferreira, R. B., Monteiro, S., Freitas, R., Santos, C. N., Chen, Z., Batista, L. M., Duarte, J., Borges, A. and Teixeira, A. R. (2007) "*The role of plant defence proteins in fungal pathogenesis.*" Molecular Plant Pathology, **8** : 677-700.

Forest Biotech (ITQB/IBET)

Célia Miguel

Auxiliary Investigator

PhD 1999 in Plant Biotechnology, Universidade de Lisboa

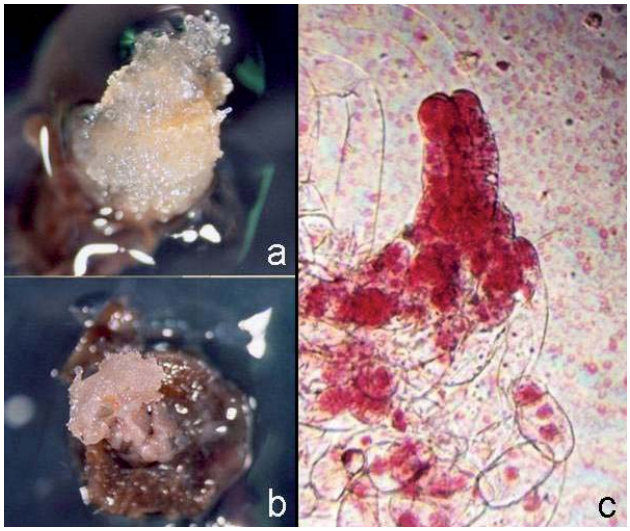
Group Members

Ravindra Malabadi	Post Doc
Sónia Gonçalves	Post Doc
Liliana Marum	PhD Student
Marta Simões	PhD Student
Ana Milhinhos	PhD Student
Lara Currais	Master Student
Andreia Miguel	Graduate

The Forest Biotechnology Lab is working on specific aspects of plant development and growth with a potential impact on the breeding and propagation of forest tree species that are relevant to the Portuguese economy, such as maritime pine. We had previously established a somatic embryogenesis system from juvenile tissues of maritime pine, and recently the induction of somatic embryos from adult tissues has also been achieved representing a major advance in terms of application potential. We are also continuing our work on the identification and characterization of genes that regulate specific stages of embryogenesis. Analysis of DNA methylation levels in the embryos under artificial (*in vitro*) conditions as compared to the zygotic embryos, and of genomic DNA sequence stability along the several steps of the somatic embryogenesis process has been going on as a way of assessing true-to-typeness.

Another aspect of plant development being studied is related to vascular development. In plants, long distance transport is accomplished by two vascular tissues: xylem and phloem. Wood formation is a process derived from secondary growth and anatomically, wood is secondary xylem, the basic component characterizing trees. We are interested in the molecular mechanisms involved in vascular cell specification as potential regulators of secondary growth. The study of a few genes known to be involved in these processes is being pursued using model species.

Another research line going on in the lab is focused on the functional characterization of nitrogen metabolism related genes by expression level manipulation in transgenic pine.



Somatic embryogenesis from adult tissues of maritime pine. (a, b) Embryogenic tissue induced from *in vitro* cultured explants. (c) Early somatic embryo visible in the induced embryogenic tissues

Selected Publications

Gonçalves S., Cairney J., Pérez Rodríguez M., Cánovas F., Oliveira M., Miguel C.M. (2007) "*PpRab1, a Rab GTPase from maritime pine is differentially expressed during embryogenesis.*" Mol Genet Genomics **278**: 273-282.

Rocheta M., Cordeiro J., Oliveira M., Miguel C.M. (2007) "*PpRT1 (Pinus pinaster retrotransposon 1): the first gypsy-like retrotransposon isolated in Pinus pinaster.*" Planta **225**: 551-562.

Tereso S., Miguel C.M., Zoglauer K., Milhinhos A., Oliveira M.M. (2007) "*Zygotic and somatic embryo morphogenesis in Pinus pinaster: histological and histochemical comparative study.*" Tree Physiol **27**: 661-669.

Plant Biochemistry

Cândido Pinto Ricardo

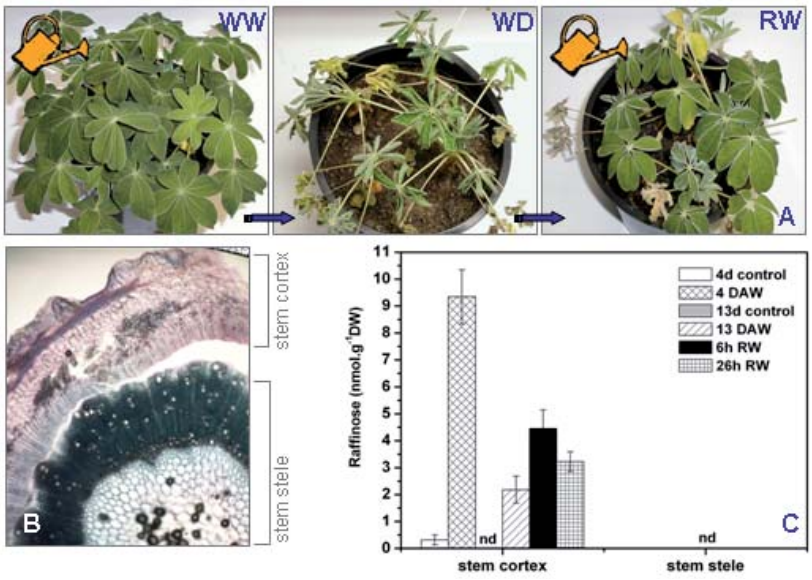
Emeritus Professor, ISA, Universidade Técnica de Lisboa

Phd 1968 in Plant Biochemistry Cambridge University, UK

The Plant Biochemistry Laboratory continued to study plant development and the responses to abiotic stress (mainly drought, temperature and mineral imbalance), through the analysis of proteins, metabolites and genes. A new project on "The wild relatives of *Beta* : genetic diversity assessment and biochemical studies" (coordination of Cristina Duarte from IICT) was started. It aims to study wild populations of beet plants (*Beta* sp.) from mainland Portugal and the islands of Madeira, Azores and Cape Verde in relation to tolerance to drought and salinity. These populations are very important in ecological and agronomical terms and at the same time enable to assess the basic aspects of plant survival under extreme adverse conditions. Field surveys and seed collection were undertaken and germination conditions are being tested.

Proteins (separated by 2-D electrophoresis, 2-DE, and identified by MS techniques) A comparative study between *Arabidopsis* and the related stress tolerant *Thellungiella* was performed to screen proteins associated with survival under severe drought (12-15% soil water content, comparing to 70-80% for control plants). The study of phospho-proteins of drought stressed *Lupinus* evidenced the need for LC-MS proteomic facilities. In grapevine subjected to drought (in collaboration with Manuela Chaves) the skin proteins of the developing berry continued to be studied. The proteins of the maize seed (Osborne fractions) from two Portuguese cultivars differing in technological traits for bread production were also studied. MS identification of selected spots is underway.

Metabolites (analyzed by general biochemical techniques, NMR or LC-MS) The metabolites of leaves of water stressed cork oak and of skin berry of water stressed grapevine continued to be studied by ^{1}H-NMR. Using a more sensitive technique, porous graphic carbon LC electrospray ionisation MS (in collaboration with Jane Thomas-Oates; University of York) it was possible to detect in water stressed *Lupinus* significant changes in sugar composition in the cortex and the stele components of the stem, that react differently to the stress. Genes In *Arabidopsis* , genes shown to be differentially expressed under boron deficiency started to be studied. In cork oak, the gene sequence of chalcone synthase was obtained (in collaboration with Pedro Fevereiro). This sequence together with those of PAL (phenylalanine ammonia lyase) and CAD (cinnamyl alcohol dehydrogenase), already available at databases, will be used to study the phenylpropanoid metabolism under environmental stress.



Water stress responses of *Lupinus albus*. (A) Three distinct physiological conditions - WW, well watered; WD, subjected to severe stress; RW, rewatered after severe stress. (B) Lupin stem cross section (partial view). (C) Water stress effect on stem stele and stem cortex raffinose levels (by LC/MS in collaboration with Jane Thomas-Oates).

Group Members

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Tiago Lourenço [3]	PhD Student
Carla Antunes [4]	PhD Student
Cátia Machado	Graduate

[1] co-supervision (Manuela Chaves)

[2] co-supervision (Phil Jackson)

[3] co-supervision (Margarida Oliveira)

[4] co-supervision (António Lopes)

Selected Publications

Soares , N.C. , Francisco R., Ricardo, C.P., Jackson, P.A. 2007 "*Proteomics of ionically bound and soluble extracellular proteins in Medicago truncatula leaves*". Proteomics **7**, 2070–2082.

Batista, R., Martins, I., Jenö, P., Ricardo, C.P., Oliveira, M.M. (2007) "*A proteomic study to identify soya allergens – the human response to transgenic versus non-transgenic soya samples*". International Archives Allergy and Immunology **144**, 29–38 .

Plant Cell Biology

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PhD 2000 in Cell Biology, University of East Anglia UK

Group Members

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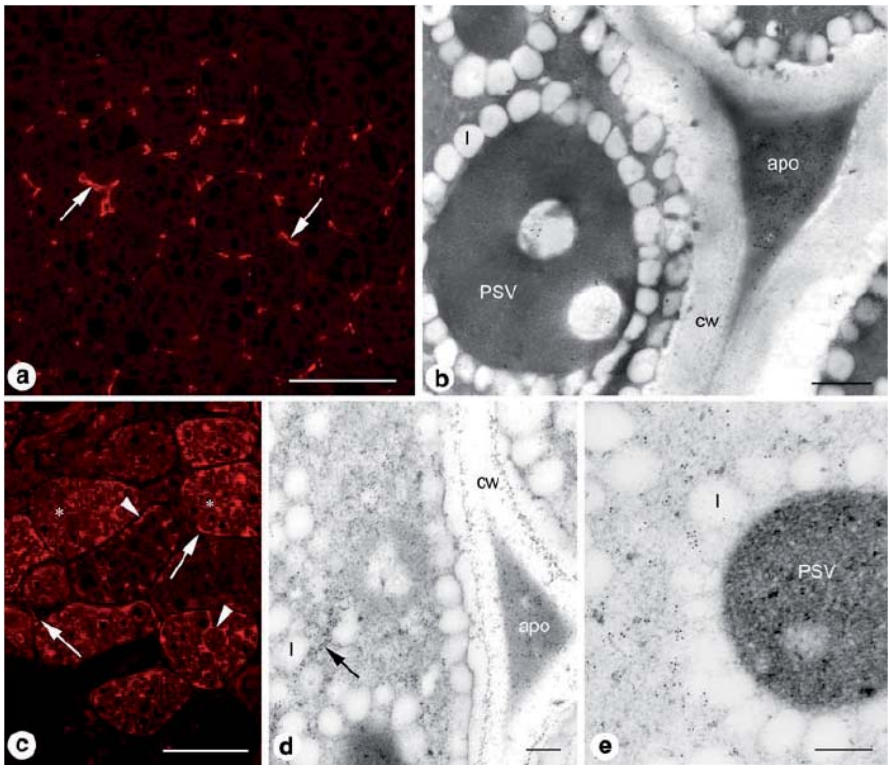
Margarida Figueiredo	Graduate

The Plant Cell Biology Lab is interested in Molecular Farming - the large scale production of recombinant proteins in plants. This is a challenging area of plant research which is developing rapidly, and several molecules have already been produced using plant based systems. However we are still far from fully understanding the factors that control the successful production of functional recombinant proteins.In the past year our lab has focused on the study of species- and tissue-specific factors that affect protein production. In plant cells, the endomembrane system differentiates in a tissue-specific manner, and is particularly complex in storage organs such as seeds, where it gives rise to a number of distinct storage organelles. Several recent reports suggest that the functional specialization of plant cells in storage organs can influence subcellular protein sorting, so that the fate of a recombinant protein tends to differ between seeds and leaves. In order to test the general applicability of this hypothesis, we investigated the fate of a model recombinant glycoprotein in the leaves and seeds of the model plant *Medicago truncatula*. We concluded that the previously observed aberrant deposition of recombinant proteins into storage organelles of seed tissue is not a general reflection of functional specialization, but also depends on the species of plant under investigation.Additionally, we investigated the potential of plant cell cultures derived from transgenic plants as platforms for production of recombinant proteins. We generated cell suspension cultures from transgenic *Medicago truncatula* plants, in which the recombinant protein was secreted and accumulated to remarkable levels in the culture medium. We showed that this suspension cell culture system offers a viable alternative that combines the advantages of protein production in whole plants with the additional benefits of cell cultures, resulting in a reliable and controllable expression system.



Immunolocalization of a recombinant protein in seed cotyledons of *Medicago truncatula*. (a, b) the protein is secreted into the extracellular space; (c, d, e) the protein is retained in the Endoplasmic Reticulum. (a, c) Fluorescence microscopy. (b, d, e) Electron microscopy. Bars: a, c 20 µm; b, d, e 0.5 µm.

Selected Publications

Abranches R, Arcalis E, Marcel S, Altmann F, Ribeiro-Pedro M, Rodriguez J, Stoger E. "*Functional specialization of Medicago truncatula leaves and seeds does not affect the subcellular localization of a recombinant protein.*" Planta **227**(3):649-658. Epub 2007 Oct 18.

Plant Cell Biotechnology

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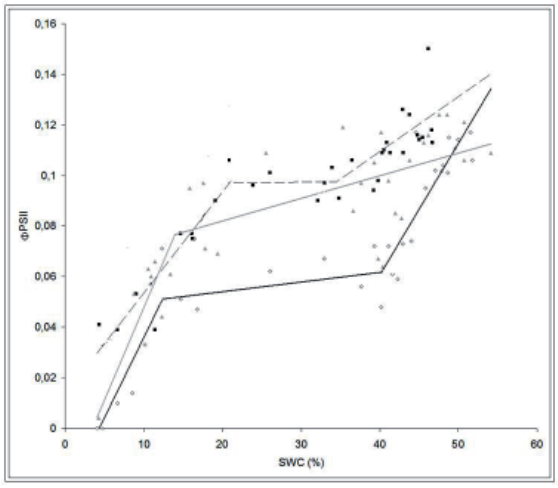
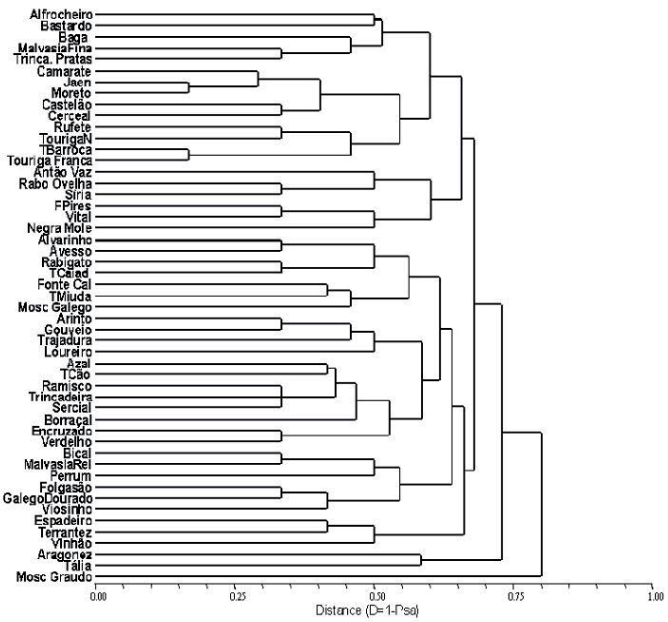


Variation of the Effective Quantum Yield of Photosystem II (FPSII) of Wild Type (WT), B5H and B1F transgenic tobacco plant lines harbouring the cDNA of the trehalose phosphate synthase gene from *Arabidopsis thaliana*, as a consequence of changes in soil water content (SWC) under an actinic light of 1850 m mol photons m–2 s–1. Polyphasic trend lines were established for WT (black line), B5H (dashed line) and B1F (gray line) plant lines. Transgenic lines display significantly better responses to water withdraw when compared to its wild type counterpart.

In 2007 the researchers of Plant Cell Biotechnology Laboratory published 12 papers in international refereed journals. Our Laboratory was involved in ten funded research projects, from which three are international (ERA-PG), six supported by Fundação para a Ciência e Tecnologia and one by Agência de Inovação. Two of the international and two of the national projects are developed in collaboration with private companies. Besides the strict research activities, our Laboratory was involved, mainly through the presidency of the Centre for Biotechnology Information, in various science communication events. The Plant Cell Biotechnology Laboratory has the following objectives: to develop molecular strategies to support plant selection and breeding programs including molecular biodiversity analysis, quantitative genetics and molecular marker-trait associations; to develop a model to introduce and to study the expression of genes of interest related with water deficit tolerance; to apply biotechnology to the development of company's strategies with incidence on forest trees; to apply and develop strategies to produce bio-products (metabolites, enzymes, recombinant proteins); to teach and train researchers and technicians in plant biotechnology and plant molecular biology; to develop public awareness and professional certification on Plant Biotechnology.

Some of the achievements of 2007 were: the description of the molecular diversity of maize landraces used for "broa" production and of the Portuguese grapevine varieties; the evaluation of the integration of trehalose phosphate synthase, of Arginine decarboxilase and of DSP22 (an ELIP like protein) on the response of tobacco and lucern to water deficit; the transcriptomic of wood production in maritime pine; the optimization of the production of Taxol using heat shock; and the optimization of bacteriocin production by *Lactobacillus plantarum* 17.2b.



Dendrogram of the 51 most important grapevine varieties used in Portugal for wine making, using the proportion of shared alleles to compute the genetic distances and UPGMA as the agglomeration method.

Group Members

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Susana Neves	Post Doc
Jingsi Liang	Post Doc
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Pedro Moreira	PhD Student
Inês Trindade	PhD Student
Jorge Cunha	PhD Student
Mara Alves	Master Student
Leonor Tomaz	Technician
Changhe Zangh	Post Doc
Nuno Almeida	Graduate
Filipa Silva	Master Student
José Salvado	Master Student
Marisa Martins	Master Student
Rita Morgado	Master Student
Rita Caré	Science Commun.

Selected Publications

Almadanim M. C., Baleiras-Couto M. M., Pereira H. S., Carneiro L. C., Fevereiro, P., Eiras-Dias J. E., Morais L., Viegas W., Veloso M.M. (2007) "*Genetic diversity of the grapevine (Vitis vinifera L.) cultivars most utilized for wine production in Portugal.*" VITIS - Journal of Grapevine Research **46**(3): 116-119.

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.

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.

Plant Cell Wall

Phil A. Jackson

Investigador Auxiliar

PhD 1997 in Biochemistry, Universidade Nova de Lisboa, ITQB

Group Members

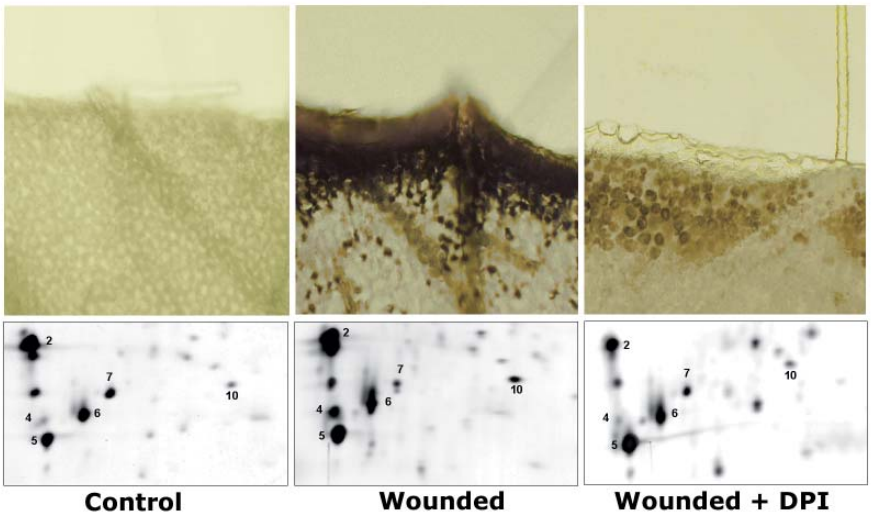
Luis Goulão	Post Doc
Nelson Soares	PhD Student
Ada Vatulescu	PhD Student
José Ribeiro	PhD Student

Proteomics of plant development and stress responses, with emphasis on apoplastic proteins.

One major area of activities is to characterise stress-related changes in the apoplast proteome using 2DE and MALDI-TOF/TOF analyses. The wounding response in Medicago has provided a useful system to probe the role of reactive oxygen species in coordinating stress-related changes in the legume apoplast (see figure). A further area of investigation concerns the effect of boron toxicity in tomato.

Plant cell wall nanostructure and properties.

The extracellular matrix of plants has been called a *nanostructure* in recognition of its complexity and the dificulty in relating the roles of its constituent polymers to its global properties. One of the major activities in the laboratory is to elucidate the role of extensin, a major structural protein in dicot cell walls. Our results indicate that the oxidative cross-linking of extensin within both development and stress responses directly effects primary wall properties, and is likely to play an essential role in forming cell-cell adhesion and the definition of cell morphology.



The accumulation of ROS species in wounded leaves plays a major coordinating role in adaptive modification to the leaf apoplast in the legume, *Medicago truncatula*.

Selected Publications

Nelson C. Soares, Rita Francisco, Cândido Pinto Ricardo and Phil A. Jackson (2007). "*Proteomics of ionically bound and soluble extracellular proteins in Medicago truncatula leaves.*" Proteomics **7**: 2070-2082.

Plant Genetics

Jorge Almeida

Associate Professor Instituto Superior de Agronomia, UTL

Phd 1989 in Plant Molecular Genetics, John Innes Institute, UEA, UK

Flower development. Flowers of several species have spurs, that is, petal outgrowths where nectar is stored. There have been many studies on how interactions between evolving floral spurs and pollinators might have influenced speciation events. However, mechanisms underlying spur development remain poorly understood, perhaps because most species with spurred flowers are not readily amenable to molecular genetic analysis. One aim of our research is to develop a transposon-based system for molecular genetic analysis in Linaria, a genus with spurred flowers (see figure) which is closely related to the unspurred model species *Antirrhinum majus*. As a first step, we have identified a mobile copy of a CACTA transposon in the gene encoding dihydroflavonol-4-reductase, an enzyme required for the synthesis of red floral pigment. In addition, we have isolated a number of Linaria genes which, by analogy to their Antirrhinum counterparts, may have roles in spur development. Amongst these is *DIVARICATA (DIV),* a gene that interacts with flower symmetry determinants to specify ventral petal identity in Antirrhinum. In Antirrhinum *div* mutants, the petal at the ventral position of the flower adopts lateral identity. Because the Linaria spur is an outgrowth of the ventral petal, it is possible that loss of *DIV* function leads to spur loss. This may be tested by using a reverse strategy to select for transposon insertions in the Linaria *DIV* gene.

Pollinator visiting spurred flower of Linaria

Group Members

Lisete Galego	Auxiliary Investigator
Hugo Tavares	Specify other category

Selected Publications

Galego L. and Almeida J. (2007). "*Molecular genetic basis of flower colour variegation in Linaria.*" Genetical Research **89**(3): 129-134.

Plant Genetic Engineering

M. Margarida Oliveira

Assistant Professor with Agregação, Faculdade de Ciências de Lisboa

Phd 1993 in Plant Biotechnology, Universidade de Lisboa

Group Members

Bárbara Emmerich	PhD Student
Nelson Saibo	Post Doc
Ana Santos	Post Doc
Isabel Abreu	Post Doc
Tiago Lourenço	PhD Student
Ana Santos	PhD Student
Ana Farinha	PhD Student
Sónia Negrão	PhD Student
Rita Batista	PhD Student
Subhash Chander	Post Doc
Milene Costa	PhD Student
Pedro Barros	PhD Student
Duarte Figueiredo	PhD Student
Tânia Serra	PhD Student
Pedro Babo	Master Student
Liliana Ferreira	Master Student
Sheilla Borges	Undergraduate
Mafalda Rodrigues	Undergraduate
Ana Sanchez	PhD

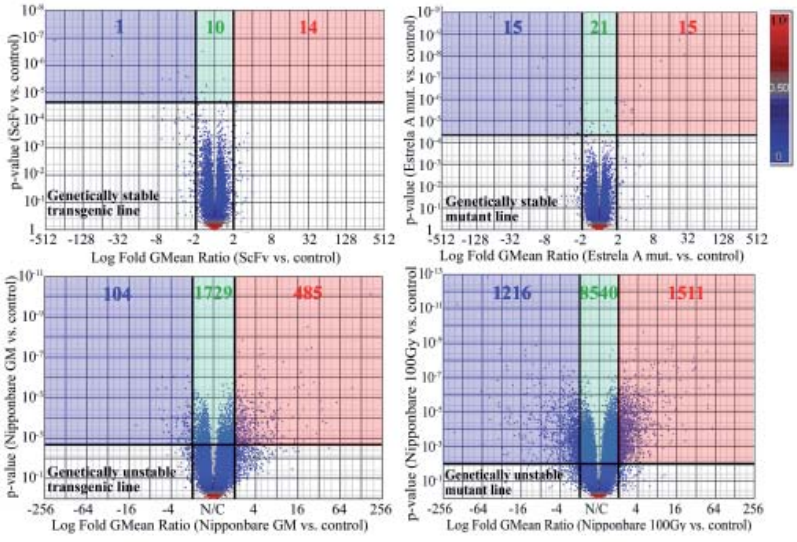
Our team focuses on transcriptional regulatory networks that control all aspects of plant development and response to biotic and abiotic stress. We use molecular biology techniques (including mutant analyses), tissue culture and genetic engineering (for over-expression and RNAi) as tools for gene functional studies. We also exploit such strategies for crop improvement and to study important plant agronomic traits. In collaboration with other labs, proteomic and transcriptomic work has also been conducted to evaluate the potential impact on human health of genetically modified foods and of fungi living in human environments and to compare mutagenized versus transgenic plants (see figure).

The identification of stress response regulators is increasingly important and we are applying an integrative approach to study the role of transcription factors (TFs) in plant stress tolerance, using rice as model plant. We are isolating and identifying novel TFs using protein-DNA (Y1H) and protein-protein (Y2H and Tap-tag) screens. Transgenic rice plants carrying various TFs involved in cold, salt and drought stress adaptation (homozygous lines carrying a single insertion) have been analysed at phenotypic, physiologic and molecular levels. We have prepared cold- and salt-induced cDNA expression libraries. Using these libraries, we identified several new TFs that are being characterized at molecular level, in homologous and heterologous systems. At a more reduced scale, this work is also being extended to almond, since little is known on fruit tree TFs putatively involved in abiotic stress response. In almond, we are also interested in TFs putatively involved in plant dormancy and started to clone them. From previous work, we have several almond TFs putatively assigned as key genes implicated in flowering or adventitious shoot induction. Some flowering genes were already transformed in Arabidopsis for phenotypic analyses.

We are also investigating the functional 3D organization of rice chromatin as part of the genomic responses to abiotic stresses. A cytogenomic approach was initiated to study chromatin remodelling putatively caused by abiotic stresses, the spatial distribution of TF genes known to be involved in the plant adaptation to abiotic stresses and the redistribution of ribosomal genes and proteins after imposed abiotic stresses.

Marker-assisted breeding has been used to introgress high production and disease resistance traits in rice varieties of national interest and the plants were grown in soil and analysed for agronomic and grain quality.

Besides almond and rice, other plants are also being studied in collaboration with other groups (i.e.: maritime pine, with Forest Biotech Lab). The lab counts at present with two international (EU) and three national (FCT) projects, collaborates in another two FCT projects and has two funded international collaborations (bilateral and COST).



Rice plants improved by conventional means (mutagenesis) were compared with others improved by genetic engineering regarding their transcription profiles. Also, recently improved plants (unstable) were compared with those that went through several generations of self-pollination after the event (stable). Differentially expressed genes appear above the thick horizontal line. Those induced over 2-fold (pink area and red number) are on the right of the right vertical thick line and those repressed over 2-fold (blue area and blue number) are on the left of the left vertical thick line. Genes differentially expressed below two-fold are in the green area. PNAS *(in press)*.

Selected Publications

Batista R., Martins I., Jenö P., Ricardo C. P., Oliveira M. M. (2007). " *A proteomic study to identify soya allergens- The human response to transgenic versus non-transgenic soya samples."* International Archives of Allergy and Immunology **144**: 29-38.

Jayamani P, Negrão S, Brites C, Oliveira MM (2007) "*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, **46**: 178-186.

Batista R., Saibo N., Lourenço T., Oliveira M. M. (2008). "*Mutagenesis vs. transgenesis: a transcriptomic approach*." Proceedings of the National Academy of Sciences (in press)

Plant Molecular Ecophysiology

Manuela Chaves

Full Professor Instituto Superior de Agronomia, UTL

PhD 1986, Universidade Técnica de Lisboa

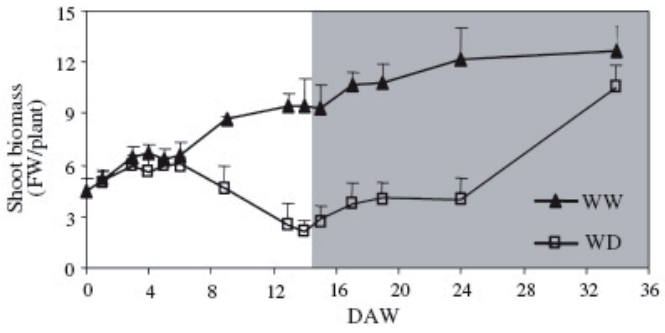
As part of our general research interests that concern the understanding of physiological and molecular mechanisms underlying plant responses to environmental stresses (drought, high light and temperature) we studied during 2007:

• grapevine photosynthetic carbon assimilation, growth and yield under water scarcity as well as the chemical and hydraulic regulations originated in the root system and affecting shoot metabolism.

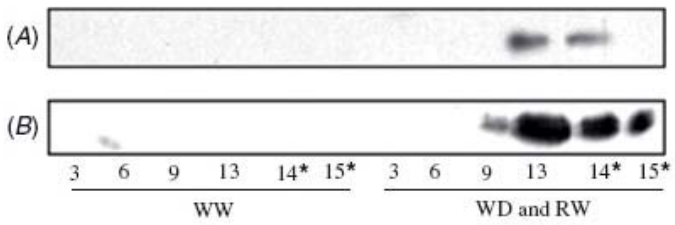
• molecular and biochemical events that control grape berry maturation under the influence of environmental stresses in particular by using a proteomic approach.

• organ specific response to drought in legumes (*L albus*); work in cooperation with Plant Biochemistry Lab.

• cold and water stress effects in eucalypt tree seedlings. We demonstrated in grapevine that deficit irrigation practices 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 combined hormonal and hydraulic signals originated in roots under dehydration. For the comparison of grape berry proteomes under different irrigation strategies 2-DE protein patterns were analyzed using adequate imaging software. Results obtained revealed quantitative/qualitative changes of several protein spots at 'veraison' under the drought conditions prevailing in non-irrigated treatment as compared with the irrigated treatments (Full irrigation and Regulated deficit irrigation). The expression of dehydrins studied in different tissues of lupin in response to drought revealed the presence of a 29-kDa polypeptide, the SK3 dehydrin LaDhn1, in roots, regardless of the water state, whereas in stem it depended on the water state. We found that dehydrins are always present in some tissues and are inducible by stress in other tissues. Antioxidative defence mechanisms do occur in both roots and shoots of eucalypt tree seedlings in response to cold and water deficits. Part of the defence mechanisms at the molecular level are shared by both stresses. This explains why the genotype most resistant to drought was also the most resistant to cold.



Total aboveground shoot biomass fresh weight (blade, petiole and stem) of *Lupinus albus* plants under well water conditions (WW) and when subjected to water deficit (WD) for 13 days after withholding water (13 DAW). Subsequent rewatering is represented by the shaded area. Data are the means ± SD of four independent assays.



Detection of RAB16-like proteins in *L. albus* stem components (cortex and stele). Samples of 15 mg extracted proteins were separated by SDS-PAGE and blotted onto PVDF membranes for western analysis. The ECL system was used for detection. A, stem cortex and B, stem stele. Approximate molecular weights of detected polypeptides are 29 kDa. Numbers indicate days of treatment. WW, plants grown in well-watered conditions; WD, subjected to water deficit; RW, rewatered (numbers with asterisk).

Group Members

Alla Shvaleva	Post Doc
Maria Galud	Post Doc
Miguel Costa	Post Doc
Tiago Santos	Post Doc
Carla Pinheiro	Post Doc
Inês Chaves	Post Doc
Ana Rodrigues	PhD Student
Rita Francisco	PhD Student
Ana Leandro	PhD Student
Raissa Santos	Master Student
Fernando Broetto	Specify other category
Ana Regalado	Post Doc
Olfa Zarrouk	Post Doc

Selected Publications

Grant OM, Tronina L, Jones HG, Chaves MM, 2007 "*Thermal imaging successfully detects stress-related differences between grapevine canopies receiving different irrigation.*" J exp Botany **58**, 815-825.

Costa e Silva F, Shvaleva A, Almeida MH, Chaves MM, Pereira JS 2007. "*Responses to chilling of two Eucalyptus globulus clones with contrasting drought resistance.*" Functional Plant Biology **34**, 793–802.

Chaves M.M., Santos, T., Souza, C.R., Ortuño, M.F., Rodrigues, M.L, lopes, C., Maroco, J., Pereira, J.S . 2007 "*Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality*" Ann Appl Biol **150**, 237-252.

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, and 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 Division integrates technology transfer of many core-areas of fundamental research developed at the institute, and, therefore, bridges distinct research fields ans disciplines; diversity of activities is thus, inherently, a major characteristic of the Division.

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.

In 2007 , the Division published 60 articles as spotted by ISI Thomson matching an average rate of publications of *ca.* 1.8 papers/Phd holder.

Brief accounts of 2007 highlights follow:

In the frame of the 2007 Ciência Program, five new positions at the auxiliary researcher level were filled in the areas of Computational Biology, Ionic Liquids for Chemical and Biological Processes, Applied and Environmental Mycology, Pilot Plant, and Particle Engineering using Supercritical Fluid Technology.

Two European projects amounting to a total close to 2,000,000 € were awarded through the EEA Financial Mechanism for the period 2007-2011:
Awake of Green Biotech in Portugal: Waste Elimination Using Genetically Manipulated Fungal Species in an Ionic Liquid Environment and Development and Validation of Integrated Drinking Water Treatment Processes in Portugal and Norway.

As for publications, (i) studies on new strategies for cell cryopreservation led to a *Biotechnology and Bioengineering* paper and awarded presententation to the Cell Centre Engineering Conference ; (ii) a *JACS* paper on the Cohesive Energy of Ionic Liquids was ranked in the top-3 most cited in 2007 of the Journal of the American Chemical Society; and (iii) a *Nature* paper published in 2006 on the Distillation and Volatility of Ionic Liquids, already with more than 150 citations, received the ISI award of rank #1 (world most-cited article) in all areas of Chemistry for June-July 2007.

Analytical Chemistry [ITQB/IBET]

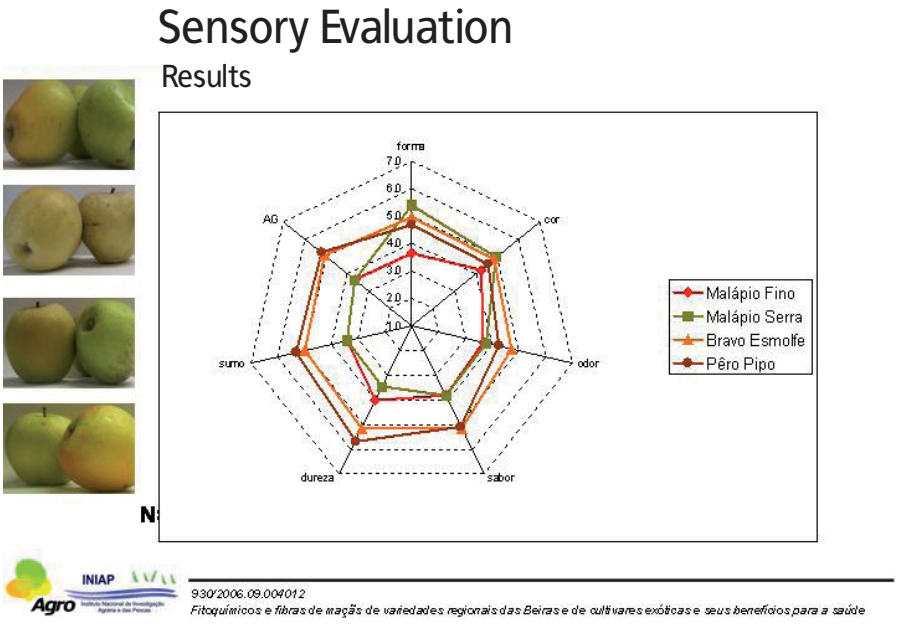
Luis Vilas Boas
Associate Professor at Universidade Técnica de Lisboa, IST
PhD 1974 in Chemistry, University of Kent

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

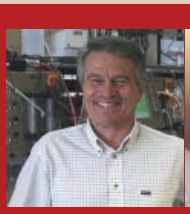
Food products are the source of nutrients necessary for survival, and play an important rule in human health and well-being, as some compounds may help to prevent some diseases (*e.g.* antioxidants), or may influence the consumers' preferences for foods (*e.g.* key aroma compounds). The main goals of 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. A collaboration was started with IICT (project: PTDC/AGR-AAM/71866/2006) to study coffee plant.

Group Members

Antero Ramos	Technician
José Dias	Master Student
Nubélia Bravo	PhD Student
Rodrigo Feliciano	Technician
Valentim Almeida	Post Doc
Ana Fulgêncio	Master Student
Cláudia Antunes	Technician



Apple varieties: Sensory evaluation by 70 consumers



Animal Cell Technology [ITQB/IBET]

Manuel J.T. Carrondo
Full Professor, FCT-UNL , IBET CEO
PhD in 1979, Environmental Engineering, Imperial College, London

Paula M. Alves
Auxiliary Investigator, ITQB/IBET
PhD 2001 in Biochemistry, Universidade Nova de Lisboa, ITQB

Group Members

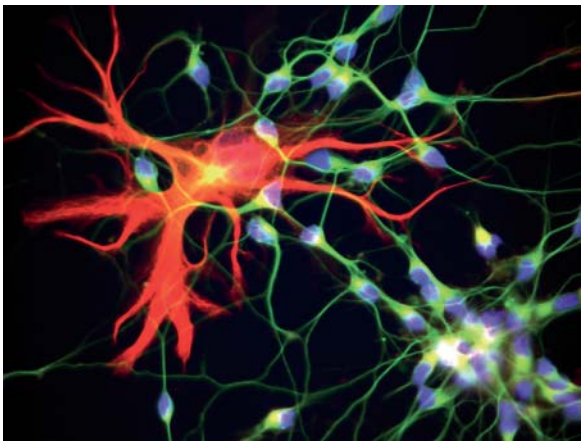
Pedro Cruz	Senior Researcher
Ana Coroadinha	Auxliliary Inv.
José Bragança	Auxliliary Inv.
Helena Vieira	Post Doc
Joana Miranda	Post Doc
Isabel Marcelino	Post Doc
Vicente Bernal	Post Doc
Teresa Rodrigues	PhD Student
Tiago Vicente	PhD Student
Carina Silva	PhD Student
Cândida Mellado	PhD Student
Marlene Carmo	PhD Student
Rita Malpique	PhD Student
Ana Amaral	PhD Student
Sónia Santos	PhD Student
António Roldão	PhD Student
Nuno Carinhas	PhD Student
Sofia Leite	PhD Student
Margarida Serra	PhD Student
Catarina Brito	Post Doc
Telma Lança	Graduate
Ana Mendes	Graduate
Ana Teixeira	PhD Student
Leonor Norton	PhD Student
Cristina Peixoto	Technician
Marcos Sousa	Technician
Rosário Dias	Technician
Cláudia Queiroga	Graduate
Daniela Ferreira	Graduate
Ana Rodrigues	Graduate
Ricardo Perdigão	PhD Student
Ana Tátá	Graduate

Selected Publications

Serra M., Leite S. B., Brito C., Costa J., Carrondo M. J. T. and Alves P. M. (2007). "*Novel culture strategy for human stem cell proliferation and neuronal differentiation.*" Journal of Neuroscience Research **85**(16): 3557-3566.

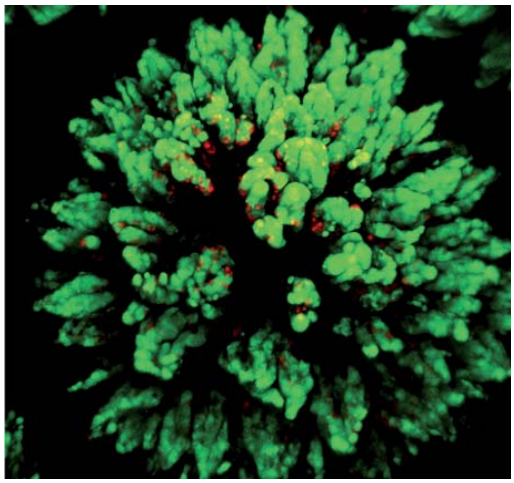
Roldão A., Vieira H.L.A., Alves, P.M., Oliveira R., Carrondo M.J.T. (2007) "*Intracellular dynamics in Rotavirus-like particles production: Evaluation of multigene and monocistronic infection strategies.*" Process Biochemistry, **41**, 2188-2199.

Malpique R., Katsen-Globa A., Carrondo M. J. T., Zimmermann H. and Alves P. M. (2007). "*Cryopreservation in micro-volumes: Impact upon Caco-2 colon adenocarcinoma cell proliferation and differentiation.*" Biotechnology and Bioengineering **98**(1): 155-166.



In vitro culture of Human neurons derived from Cancer Stem Cells: Precursors labeled with Nestin (red) and neurons with beta-III-tubulin (green). Nuclei stained with DAPI (blue).

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 for improved recombinant protein production (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 2007, a novel culture strategy for NT2 proliferation and differentiation was developed in order to control, expand and enhance the efficiency of neuronal production. NT2 cells were cultured as 3D cell aggregates (neurospheres) in the presence of retinoic acid, using stirred small scale bioreactors. Successful results showed that this novel culture strategy can greatly improve the neuronal differentiation efficiency of NT2 cells (a 10-fold increase was obtained) concomitantly reducing simultaneously the time needed for the differentiation process. This culture method is a promising approach for ES cell proliferation and differentiation by assuring an efficient and saleable system under a fully controlled environment, thus overcoming one of the challenges of stem cell bioengineering (this work was published at the *J Neurosc Research*).



Novel strategies for cryopreservation of adherent cells. The photo shows N2a cells encapsulated on alginate gel beads (0,4% alginate, 20 min gelling with BaCl2): more than 78% cells with intact plasma membrane integrity after thawing

Antibiotic Stress and Virulence of Enterococci [ITQB/IBET]

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

Opportunistic pathogens constitute a very interesting and challenging area of research. That is the case of bacteria from the genus *Enterococcus*. These gut commensals are also found in food products, water, sand and soil, therefore maintaining with humans and animals a very tight and happy coexistence. However, in the last decades, due to the increased use of antibiotics, both in animal breeding and in Hospitals and veterinary clinical practice, enterococci became a major concern in terms of nosocomial and community acquired infections. This urged us to find new ways of dealing and controlling enterococcal infections, thus directing our research into six main topics:

1- ANTIBIOTIC RESISTANCE. Studies aimed at understanding resistance/tolerance mechanisms, in particular to vancomycin and bacitracin, were carried out. Renata work revealed that bacitracin resistance is widely spread and is on the way to unravel new insights into the mechanism of resistance. Marta Ruivo is close to find the reasons why we have previously found VACISE strains. Tânia has been working with sub-inhibitory and clinically relevant vancomycin concentrations to find how this antibiotic influences cell functions by transcriptomic and proteomic analysis and is close to reveal new vancomycin cell "targets";

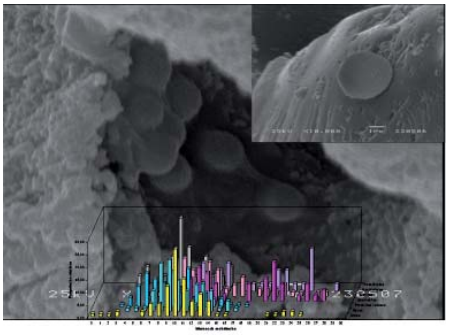
2- RESISTANCE IN THE ENVIRONMENT. Enterococcal isolates from different environments (food, hospital, pets, water, and sand) have been typed using molecular techniques and characterized concerning their antibiotic resistance and virulence potential. This work was done by Vera and Daniela;

3- STRESS RESPONSE. We are convinced that expression of many virulence traits is a consequence of stress response. Using trasnscriptiomics, Marta Abrantes is on the way to understand how enterococcal cells respond to metal ion stress. Cell-wall stress has also been the subject of Tania';s work, who made beautiful pictures of enterococcal cell-wall under stress using CEMOVIS;

4- VIRULENCE. We have focused our attention on three virulence factors (gelatinase, *fsr* and cytolysin). Frederic has put a final point in the debate around cytolysin genotype vs phenotype. Together with Neuza and Paulo, they are close to clarify the mystery around the same debate on gelatinase. Paulo, with the help of Tiago and Neuza, is working on the pos-transcriptional control of the *fsr* operon, which regulates several virulence genes in *E. faecalis*;

5- TRANSITION FROM COMMENSALISM TO VIRULENCE. We are involved with a European consortium searching for genes involved in the transition of enterococcal cells to the virulent behavior. The whole team of SAVE LAB is involved, although Renata and Daniela are the preponderant "hands"; in this project;

6- BIOCIDE. Mechanisms of resistance to biocides are poorly understood in enterococci, but urge clarification. Teresa is working hard to bring some light into this subject.



Electronic microscopy images of sand grains (from Sesimbra). Big picture- small group of bacteria inside a grain cavity (15000x); Small picture- bacterial cell attached to a sand grain (10000x); Graph- distribution of antibiotic resistance frequency among enterococcal isolates from different environments, where we can see a small group of multiresistant sand strains (yellow) showing a similar behavior to clinical and veterinary strains.

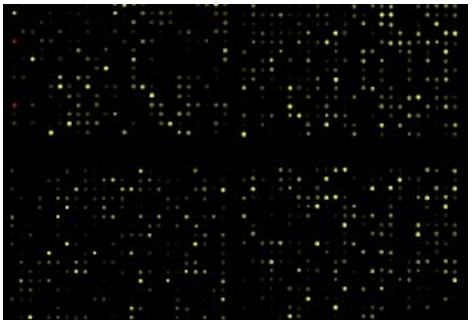


Image of a DNA microarray chip obtained after copper stress on *E. faecalis*.Microarray chips measure transcriptomes, which are the entire collection of RNA transcripts within a cell under the given conditions. Merged image showing green, red and yellow spots, indicating transcripts that are expressed under both sets of conditions.

Group Members

Paulo Marujo	Post Doc
Tânia Ribeiro	PhD Student
Marta Abrantes	PhD Student
Frederic Gaspar	PhD Student
Marta Ruivo	Master Student
Neuza Teixeira	Master Student
Tiago Amado	Master Student
Teresa Braga	PhD Student
Renata Matos	Graduate
Daniela Pinto	Graduate

Selected Publications

Corsetti, A, Settanni, L, Braga, MT, Lopes, MFS, Suzzi, G (2007)."*An investigation on the bacteriocinogenic potential of lactic acid bacteria associated with wheat (Tricum durum) kernels and non-conventional flours*." LWT-Food Science and Technology (2007), doi:10.1016/j.lwt.2007.07.022

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.

Biomathematics

Jonas S Almeida

Full Professor, University of Texas, USA

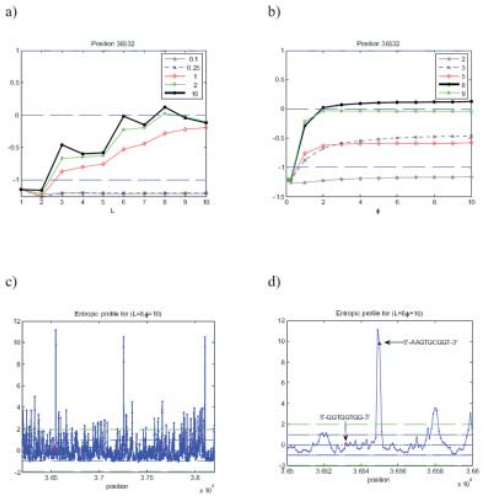
PhD 1995 in Biological Engineering, Fac. Ciências e Tecnologia, Universidade Nova de Lisboa

Group Members

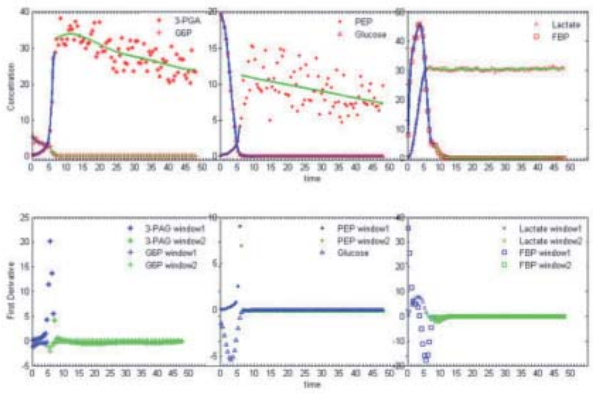
Helena Deus	PhD Student
Marco Vilela	PhD Student

Reflecting the increasing involvement in international projects proposing to integrate heterogeneous biomolecular datasets, the Biomathematics Laboratory has been widening its focus to integrate elements of variable selection and variable discovery. The reporting year of 2007-2008 has accentuated this tendency with the use of semantic web and other knowledge engineering tools alongside conventional computational statistical and machine learning procedures. In that regard, the collaboration with the molecular epidemiology group of Prof Herminia de Lencastre at ITQB / Univ Rockefeller and our European collaborators from the EURIS and PREVIS EU projects remains central to our integrative bioinformatics research. In Summary, the Biomathematics group is refocusing its efforts on data driven mathematical modeling.

If the previous paragraph describes a focus on integrated data analysis and data management, the reverse engineering of Biological networks from time series acquired in vivo offers a good illustration of the numerical computation aspects of our work (see for example manuscripts by Vilela et al, PMEDID). This work is performed in close collaboration with the groups of Helena Santos and Rute Neves at ITQB, Eberhard Voit at Georgia Institute of Technology in Atlanta, and Susana Vinga and Ana Freitas of INESC/IST where our joint FCT project (Dynamo) is centered.



Entropic profile (EP) for sequence Hi – complete genome of H. influenzae. a) and b) Analysis of position 36532 (from the beginning of replication). c) and d) Detail for the EP for positions 36200 to 38200 and 36500 to 36600. The highest peaks in the EP correspond to uptake signal sequences (USS+) 5'-AAGTGCCGGT-3', its reverse complement (USS-) 5'-ACCGCACTT-3' and related motifs, such as AGTGCGGT and AAGTGCGG. The Chi sites are not particularly well conserved neither overexpressed [24] and therefore are not easily detected with this approach.



Result in real data. Illustration of the smoothing procedure applied to in vivo Lactococcus lactis time series for Glucose, Glucose 6-phosphate (G6P), Fructose 1,6-bisphosphate (FBP), 3-Phosphoglycerate (3-PGA), Phosphoenolpyruvate (PEP), and Lactate [16]. The first derivative is shown below the corresponding metabolic time series. The window partitions are shown with distinct colors. It is noteworthy that the shift in noise structure, which segments the signal into smaller temporal windows with noise invariance, is approximately the same for all metabolites except FBP. Since the smoothing procedure is applied independently to each metabolite, this coupling suggests shared dependency on some molecular machinery, which changes when its main substrate, Glucose, is depleted at t ~ 6 min.

Selected Publications

Vinga S, Almeida JS. (2007) Local Renyi entropic profiles of DNA sequences. BMC Bioinformatics. 2007 Oct 16;8(1):393.

Sá-Leão R, Nunes S, Brito-Avô A, Alves CR, Carriço JA, Saldanha J, Almeida JS, Santos-Sanches I, de Lencastre H. (2008) High rates of transmission of and colonization by *Streptococcus pneumoniae* and Haemophilus influenzae within a day care center revealed in a longitudinal study. J Clin Microbiol. Jan;46(1):225-34.

Vilela M, Borges CC, Vinga S, Vanconcelos AT, Santos H, Voit EO, Almeida JS. (2007) Automated smoother for the numerical decoupling of dynamics models. BMC Bioinformatics 8(1):305.

Biomolecular Diagnostics

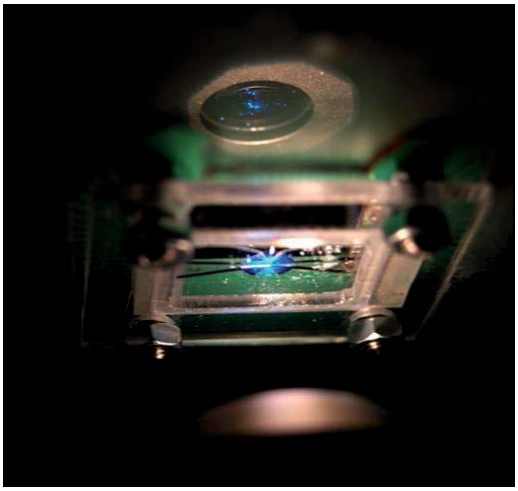
Abel González Oliva

Auxiliary Investigator

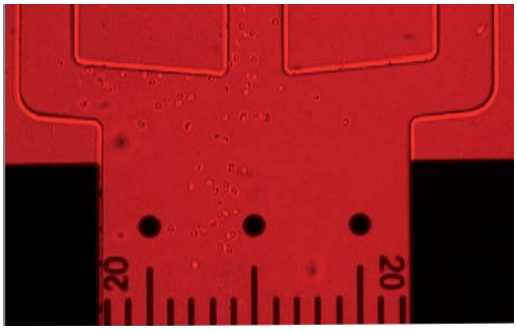
PhD in 1987 in Agriculture Technology , Hohenheim University/Germany

Our multidisciplinary team is committed to develop new instrumental tools for disease diagnostic and bioprocess operation.It has been working on developing innovative diagnostics of veterinary diseases based on optical immunodetection of new disease targets (e.g. African swine fever virus; tick and tick-born disease parasites, like *Theileria sp*., *Brucella sp*., *Babesia sp*.). Technologies like micro-fluidics and micro-fabrication, development of nanoparticles (Quantum dots) and functionalization with specific molecules allows the study of infected cells, as well as cell handling and characterization.

The laboratory main areas of expertise include immunological studies and characterization of antigens and antibodies for their use in biosensors; development of ELISA assays and rapid tests; development of immobilization protocols for antibodies/antigens on solid supports; development of flow-cells and microfabricated channels for cell handling and characterization and development of nanoparticles (Quantum dots) for use in imaging, sensing and in cell characterization studies. Developed target applications range from disease diagnosis (African Swine Fever Virus immunosensor; /Brucella sp/. immnunosensor), environmental monitoring (detection of nitrite in drinking water), bioprocess monitoring and control (optical density measurement in fermentors) or fibre optic sensor for monitoring dissolved oxygen in rivers.



Characterization of *Babesia sp* infected cells red blood cells by impedance spectroscopy (IS) in a microchannel chip.



Separation of *Babesia sp* infected red blood cells in a microfluidic channel by dielectrophoresis (DEP).

Group Members

Marta Gomes	PhD Student
Sofia Miguel [1]	PhD Student
Cláudia Sanchez	Post Doc
José Palmeira	Master Student
Elisa Campos	Graduate
Gisela Henriques	Graduate

[1] co-supervision (Chris Maycock)

Selected Publications

T. Braschler, N. Demierre, E. Nascimento, A. G. Oliva, and P. Renaud, (2008) "*Continuous separation of cells by balanced dielectrophoretic forces at multiple frequencies*" Lab on a Chip., DOI: 10.1039/b710303d

Claudia Kuttel ; Elisabete Nascimento; Nicolas Demierre; Tiago Silva.; Thomas Braschler; Philippe Renaud and Abel G Oliva. (2007) "*Label-free detection of Babesia bovis infected red blood cells using impedance spectroscopy on a microfabricated flow cytometer.*" Acta Tropica 102 (2007) 63-68

Marta G. Silva, Silvina Wilkowsky, Susana Echaide, Marisa Farber, Abel Oliva (2006) "Development of an immunosensor for the diagnosis of bovine Anaplasmosis." Ann N Y Acad Sci ;1081:379-81

Mass Spectrometry

Ana Maria Varela Coelho

Assistant Professor, Universidade de Évora

Phd 1998 in Chemistry (Biochemistry), Évora University, Portugal

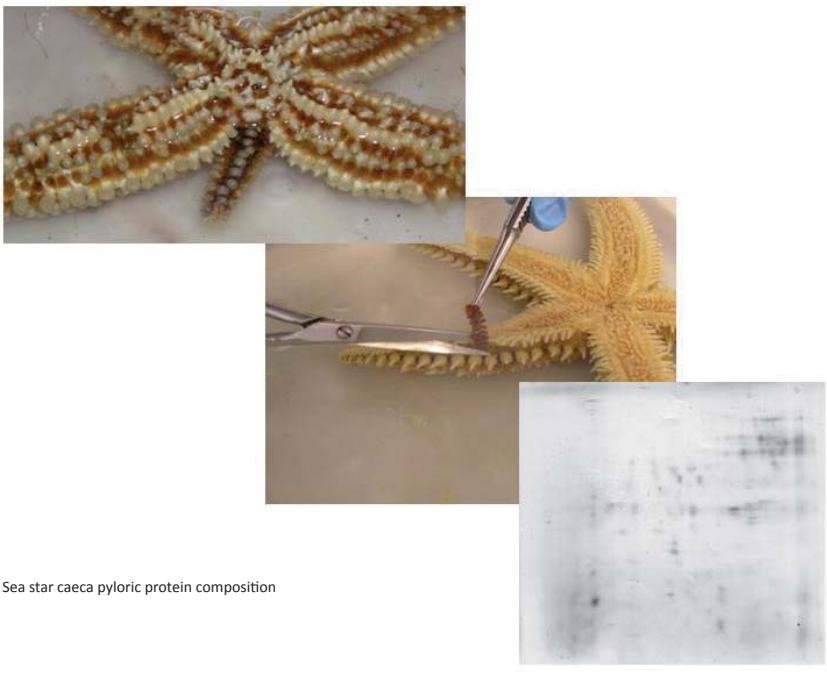
Group Members

Alexandre Campos	Post Doc
Romana Santos	Post Doc
André Almeida	Post Doc
Gonçalo Costa	PhD Student
Duarte Toubarro	PhD
Patrícia Alves	PhD Student
Sérgio Mota	PhD Student
Elsa Lamy	PhD Student
Catarina Franco	PhD Student
Elisabete Pires	Technician
Catarina Pereira	Technician
Liisa Arike	PhD Student

The information obtained with the powerful Mass Spectrometry (MS) techniques is fundamental for structural characterization of chemical and biochemical species. Besides precise mass determination it is possible to perform controlled fragmentation of the molecular ions, which allows obtaining detailed structural information, like comparative identification of organic compounds, peptide and oligosaccharide sequencing. We are involved in projects that allow the development and implementation of new MS applications, namely involving identification of proteins, their characterization and studies on their biological interactions.

One of the projects aims a deeper knowledge on the architecture of kinetochores (KC), large multiprotein complexes that assemble at the centromeric chromosomes during mitosis, which is the division and equal distribution of genetic material to daughter cells. This biological process requires dynamic attachment of chromosomes to spindle microtubules and this interaction is mediated largely by KC. It is important to improve the knowledge on this process due to the fact that aberrant cells, including carcinogenic, may result from incorrect mitosis. We have identified 26 new proteins from Drosophila mitotic KC and provided evidence that 7 of these proteins are essential for correct chromosome congression and segregation. We have shown that human homologues of two of the new kinetochore components, also functions to ensure precise chromosome segregation. Work in collaboration with the Mitosis Group at IGC (Coord: Ivaro Tavares).

Another project currently in progress concerns the identification of proteins and bioactive peptides that are implicated in the regeneration process of Marthasterias glacialis, a common sea star of the Portuguese coast. Regeneration is a usual trait in all echinoderm classes being used to replace organs, which are lost following traumatic injury, predation, autotomy or as a reproduction strategy. Preliminary experiments aiming the characterization of pyloric caeca (circulatory and digestive organ), radial nerve cord and coelomic fluid (fluid that is in contact with internal organs) proteome have been performed. The identification of differentially expressed proteins and peptides by a sea star under regeneration will allow the identification of potential regeneration factors.



Sea star caeca pyloric protein composition

Selected Publications

Zhenjia C, Franco CF, Baptista RP, Cabral JMS, Coelho AV, Rodrigues CJ, Melo EP, "*Purification and identification of cutinases from C kahawae and C gloesporioides*" Applied Microbiology and Biotechnology, (2007), **73**(6):1306-13

FM Valente, PM Pereira, SS Venceslau, M Regalla, AV Coelho and IA Pereira "*The [NiFeSe] hydrogenase from D vulgaris Hildenborough is a bacterial lipoprotein lacking a typical lipoprotein signal peptide*" FEBS Letters (2007); **581**(18):3341-4

Santos SC, Vala I, Miguel C, Barata JT, Garo P, Agostinho P, Mendes M, Coelho AV, Calado A, Oliveira CR, e Silva JM, Saldanha C "*Expression and subcellular localization of a novel nuclear acetylcholinesterase protein*" JBC (2007); **282**(35):25597-603

Microbioloy of Man-Made Environments [ITQB/IBET]

Maria Teresa Crespo
Senior Research Fellow at IBET
Phd 1992 in Biological Engineering, FCT, Universidade Nova de Lisboa

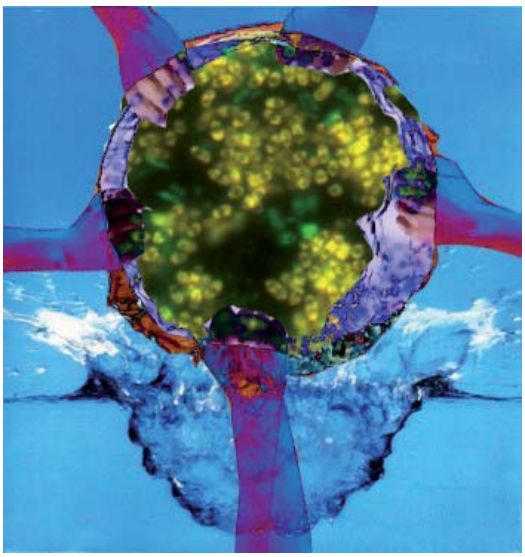
The activities of the group in the past year have been directed essentially to the microbial characterization of drinking water sources and microbial population characterization of wastewater treatment biological systems.

Taking into account that water supply can be considered as a potential carrier for contaminants to human beings and thus a direct threat to human health, production and distribution of drinking water with high chemical and microbiological quality has been object of growing interest by water providers and researchers. Our research involved initiating the development of integrated nanofiltration and disinfection techniques (ultraviolet radiation, ozonation, advanced oxidation processes and chlorination) for drinking water treatment in order to guarantee effective microbial inactivation in terms of bacteria (*Escherichia coli*, total coliforms, and *Enterococcus spp.*), viruses (bacteriophages), filamentous fungi, and yeasts.

Biological wastewater treatment is carried out by mixed microbial communities that continuously evolve and compete with each other. As a result, the microbial population profiles change in a dynamic adaptation to process operational conditions. Our research in this field focused on characterizing the mixed microbial populations enriched in systems aiming at removing specific pollutants, such xenobiotics, mercury and phosphorus, as well as those associated with good and bad performance of membrane bioreactors for domestic wastewater treatment.

Identification of the bacteria dominant in these systems was carried out by molecular techniques, such as Fluorescence *in situ* Hybridization and quantitative PCR, as well as by classical isolation methods. Additionally, fingerprinting techniques (DGGE) were used to follow the microbial profile of a sludge in order to evaluate the impact of changes in operation strategies on population diversity.

Ultimately, a correlation between the microbial populations and the bioreactor's operation and its performance can be established, which can provide the operator/designer with additional tools for plant optimization.



A look inside water microbial communities (Microphotograph of epifluorescent microscope image of sludge hybridized with FISH probes for polyphosphate accumulating organism and other bacteria).

Group Members

Gilda Carvalho	Post Doc
Vanessa Pereira	Post Doc
Ana Filipa Silva	PhD Student
Frédric Gaspar	PhD Student
Helena Santos	PhD Student
Diana Fernandes	Master Student
Bárbara Almeida	Graduate
Ana Dourado	Undergraduate
Paula Alves	Technician
Michel Domingues	Graduate
Sandra Sanches	Graduate

Selected Publications

Pimentel L. L., Semedo T., Tenreiro R., Teresa M., Crespo B., Manuela M., Pintado E. and Malcata F. X. (2007). " *Assessment of safety of enterococci isolated throughout traditional terrincho cheesernaking: Virulence factors and antibiotic susceptibility.*" Journal of Food Protection **70**(9): 2161-2167.

Carvalho G., Lemos P. C., Oehmen A. and Reis M. A. M. (2007). " *Denitrifying phosphorus removal: Linking the process performance with the microbial community structure.*" Water Research **41**(19): 4383-4396.

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.

Physiology of Environmentally Conditioned Microbiota [ITQB/IBET]

Maria Vitória San Romão

Principal Investigator, INRB

Phd 1987, in Biochemistry, Faculdade de Farmácia, Universidade do Porto

Group Members

Cristina Pereira	Post Doc
Ofélia Anjos [1]	Post Doc
Ana Marques [2]	PhD
M. Carmo Basílio [3]	PhD
Mariana Carvalho [4]	PhD Student
Isabel Martins [5]	PhD
Adélia Varela [6]	PhD Student
M. Cristina Leitão	Technician
Helga Garcia	Graduate
Cátia Rodrigues	Graduate

[1] co-supervision (Luís Villas-Boas)

[2] co-supervision (Rogério Tenreiro)

[3] co-supervision (Teresa Crespo)

[4] co-supervision (Cristina Pereira)

[5] co-supervision (Cristina Pereira and Luís P. Rebelo)

[6] co-supervision (Cristina Pereira)

Group activity merges IBET/ITQB-UNL priorities, covering fundamental and applied disciplines. It also integrates and highlights EVN-INIA mission, especially in the field of food-safety and environment sustainability.

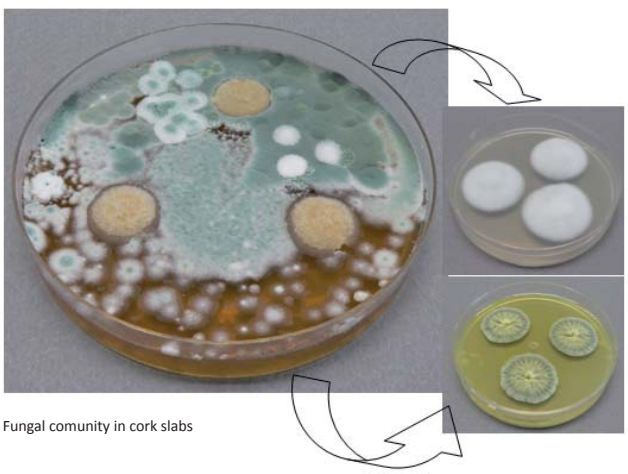
1- **Bacteria in conditioned environments - Quality/safety assessment** Biogenic amines (BA) are undesirable metabolic compounds in food due to their putative healthy hazards. BAs are possible wine sub-products, produced after malo-lactic fermentation. We aim to identify *Oenococcus oeni* adequate starters (coll. PROENOL and R Tenreiro, FCUL). The differential expression of genes codifying enzymes associated with BA synthesis is being analysed.

2- **Fungi communities** The cork stoppers manufacturing process involves one step where fungi actively colonise cork. This community can be involved in the formation of off-flavours, but present valuable potential (e.g. production of cellulases and lipases). On-going work (coll. AmorimandIrmão) aims at solving, by molecular methods (coll. R Samson, CBS) the species in this population. This includes identification/characterisation of novel taxonomic metabolic markers. Data will help in further understanding the dynamics of other complex fungal populations.

Fungi and their metabolites are yet to be considered in water quality assessment. However, amongst fungi metabolites there are several presenting significant eco-toxicity, which can endanger Human health. This work aims at evaluating the efficiency of drinking-water treatment processes, including elimination and control of contaminant fungi (EEA grant, coordinated M Reis, IBET).

3- **Applied and Environmental Mycology**, area coordinated by C Silva Pereira (recently appointed through Ciência2007).Filamentous fungi are key organisms in the carbon cycle of earth. Biodegradation, biodeterioration and bioremediation processes highlight the manifold relations of fungi and composites, thus fungi biotechnological significance. Adequate models were initially selected: • suberin for biodegradation• pentachlorophenol (PCP) for bioremediation (coll. A Hursthouse, U. West of Scotland)• Tunisia Oak forest (high probable PCP risk zone) for on-site studies (NATO sfp grant) • labdanoic acid for oriented biocatalysis (coll. C Afonso, IST)Fungal enzymes involved in these important processes are being pin-pointed by 2D and DIGE (coll. P. Spencer-Philips, U. West of England), the degradation intermediates formed analysed by chromatography and the substrate compositional alterations fingerprinted by spectroscopy. Data integration will solve these metabolic-pathways.

4- **Green biotechnology** (coll. LPN Rebelo, ITQB):• Producing ionic liquids-IL adapted fungi for biotechnological applications:o Novel whole-cell biocatalysis in ILo Novel biochemical pathways for drug productiono Task oriented bioremediation in IL o Waste management - biodegradability in IL (EEA grant).



Fungal comunity in cork slabs

Selected Publications

2007, Iain McLellan, Mariana Carvalho, Cristina Silva Pereira, Andrew Hursthouse, Calum Morrison, Paul Tatner, Isabel Martins, M. Vitória San Romão and Maria Leitão. J. Environ. Monit., 9, 1055 - 1063, DOI: 10.1039/b701436h. The environmental behaviour of polychlorinated phenols and its relevance to cork forest ecosystems: a review.

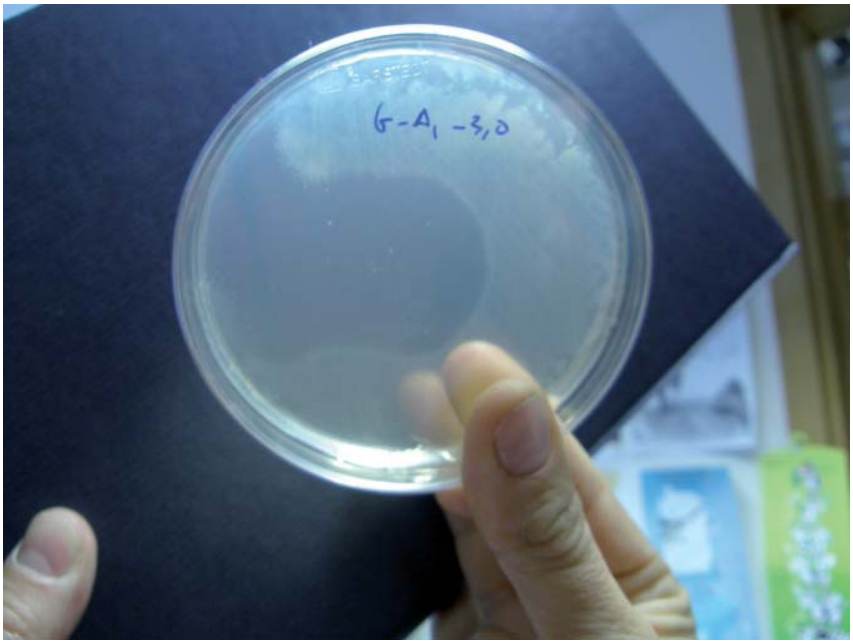
2007, Pires J M, Pereira H, San Romão MV. Study of humidity and water activity of cork slabs during cork stoppersmanufacturing process-Prelimonary results. Ciência Tec Vitiv, 22 (1) 15-20.

2007, Vitorino SI, Neves ESG, Gaspar F, Figeiredo Marques JJ, San Romão MV. Suberin utilization by *Chrysonilia sitophila*: evidence for lipolytic enzymes production. Ciência Tec Vitiv , 22 (1) 1-4.

Food Microbial Technology [ITQB/IBET]

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

Research achievements. Microbial biodiversity of traditional fermented foods . These foods are part of each country's heritage and are intrinsically connected to SMEs. The microbial metabolites that accumulate during fermentation determine the product' s flavor. Bacteriocins from Lactic Acid Bacteria (LAB) are believed to play a relevant role in these food ecosystems. In order to improve the quality of Portuguese table olives, a search for bacteriocin producers was conducted on the LAB strain collection from fermenting brines. The operon genes that codifies for plantaricin S, firstly isolated in Spain and widely found in Spanish olives, was found in many of the identified producer strains that produced the largest inhibition halos. We are currently confirming the transcription of the gene, trough the presence of the corresponding m-RNA, in alternative to protein purification. A paper covering these aspects is under preparation and will be submitted soon. The team are involved in a COST action that aims at setting up a network of research groups that study microbial biodiversity of traditionally fermented foods and the innovative potential of the associated microbial strains ; the project is currently in the second evaluation stage. Novel applications for bacteriocins. In recent years, public concern about the safety of foods of animal origin has heightened and there is a growing tendency to diminish or avoid the use of antibiotics as growing factors in feed production. A project is being developed under contract for a private company and IBET. It aims the valorization of an industrial waste trough the *in situ* bacteriocin production, resulting in a feed additive. Due to their properties, bacteriocins are expected to stand thermal treatments and other processing operations. Besides the optimization of the bacteriocin-producing-strains, the inhibition of some relevant pathogens of porcine' s gut is under evaluation, as well as tests in a simulated rumen. Human health applications cover alternatives to antibiotics. *Helicobacter pillory (HP)* is an example of a human pathogen in which multiple resistances to antibiotics are common. As a result of the project "Pesquisa de probióticos em azeitona de mesa Portuguesa com potencial actividade anti-*Helicobacter pylori*" we found anti-*Helicobacter pilory* activity caused by LAB strains from table-olives, possibly associated to bacteriocins. We are currently optimizing a culture medium to evaluate the HP inhibition by various compounds. It will be a powdered formulation with no complex ingredients, and favouring the agar-diffusion of large molecules.



Inhibition of the growth of a clinical isolate of Helicobacter pylori caused by diffusible metabolites (other than lactic acid) produced by a Lactobacillus strain, isolated from table-olives

Group Members

Dulce Brito	Post Doc
Amélia Delgado	Post Doc
Cristina Serrano	Graduate
Luís Catulo	Graduate
Luisa Reis	Technician
Alexandra Pereira	Graduate
Rúben Rocha	Master Student
Helena Pereira	Master Student

Selected Publications

Delgado, A., Arroyo-López, FN, Brito, D, Peres, C., Fevereiro, P., Garrido-Fernández, A. "*Optimum bacteriocin production by Lactobacillus plantarum 17.2b requires absence of NaCl and apparently follows a mixed metabolite kinetics*". J. Biotechnol. **130**: 193-201.

Molecular Thermodynamics Laboratory

Luis Paulo N. Rebelo

Associate Professor with Agregação

PhD in 1989, Universidade Nova de Lisboa

Group Members

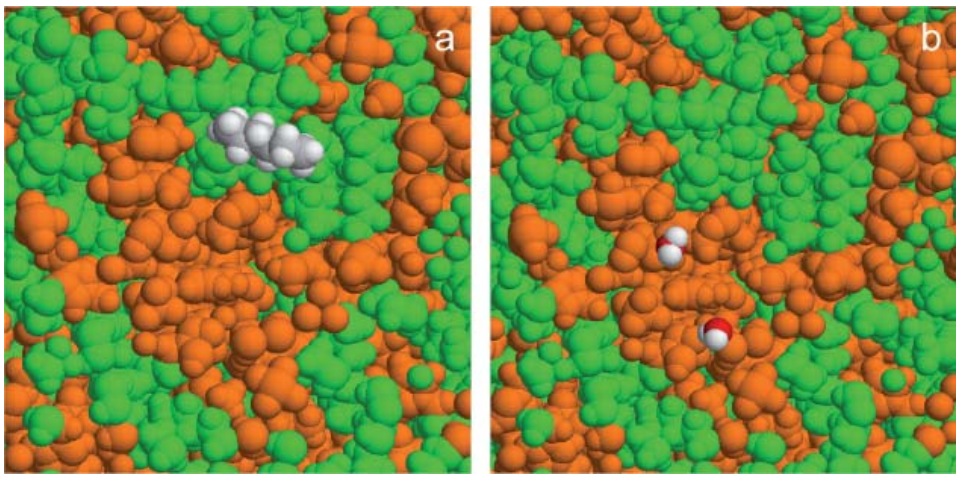
José Lopes	Invited Professor
Zoran Visak	Post Doc
José Esperança	Post Doc
Mohammad Tariq	Post Doc
Marijana Blesic	PhD Student
Marija Petkovic [1]	PhD Student
Isabel Martins [2]	PhD Student
Rui Ferreira	Graduate
Joana Trindade	Undergraduate

[1] co-supervision (Cristina Pereira)

[2] co-supervision (Cristina Pereira)

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.



Low-charge (green) and high-charge (orange) density domains in [C8mim][NTf2], showing schematically its preferential spatial interactions with (a) an apolar solute (n-hexane) and (b) a dipolar and/or associative solute (water).

Selected Publications

Rebelo L. P. N., Lopes J. N. C., Esperança J., Lachwa H., Najdanovic-Visak V. and Visak Z. P. (2007). "*Accounting for the unique, doubly dual nature of ionic liquids from a molecular thermodynamic,and modeling standpoint.*" Accounts of Chemical Research **40**(11): 1114-1121

Santos L., Lopes J. N. C., Coutinho J. A. P., Esperança J., 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

Leal J. P., Esperança J., da Piedade M. E. M., Lopes J. N. C., Rebelo L. P. N. and Seddon K. R. (2007). "*The nature of ionic liquids in the gas phase.*" Journal of Physical Chemistry A **111**(28): 6176-6182

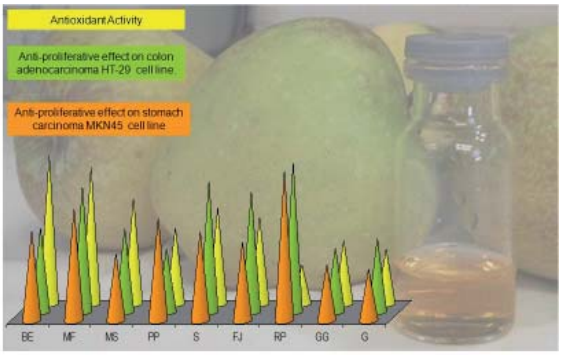
Nutraceuticals and Controlled Delivery [ITQB/IBET]

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

The main activities of Nutraceuticals and Controlled Delivery group are centred on i) the production of bioactive concentrates from several botanical sources, using clean technology; ii) the formulation of new functional products, with application in food, pharmaceutical and cosmetic industries, by incorporation of high-value natural bioactive ingredients; iii)the development of appropriate tests for bioactivity evaluation and iv) the development of efficient processes that uses alternative clean technologies (namely with supercritical fluids) for particle formation (micro and nano-scale), and incorporation of active substances in biocompatible and biodegradable matrixes, with application on the preparation of sustained delivery systems.

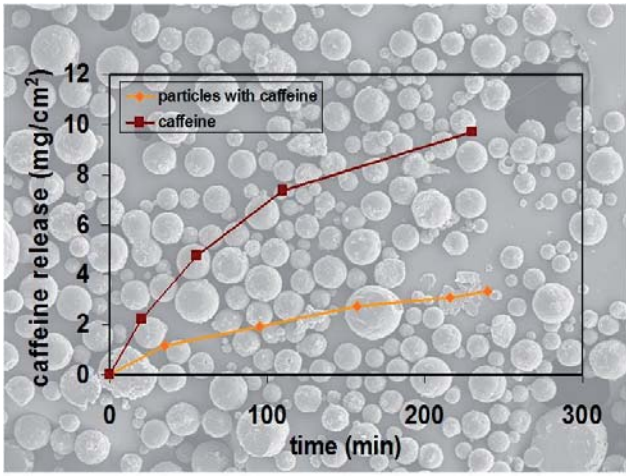
During 2007, we were involved in several projects:

• Isolation of bioactive ingredients from agro-food industrial wastes and by-products. Our attention was mainly focused on the application of supercritical fluid extraction for the isolation of high-value fractions with specific bioactivity answers, namely novel and powerful antioxidants and potent anti-inflammatory agents.



Bioactivity studies on portuguese and exotic varieties of apples. BE: bravo esmolfe; MF: malá pio fino, MS:malá pio serra, PP:pêro pipo, S:starking, F:fuji; RP: reineta parda, GG: gala galaxy, G:golden.

• Characterization of typical Portuguese apples. We evaluated the bioactivity, namely the antioxidant and antiproliferative activity, of 9 different apple cultivars. As a first approach the total of polyphenols content was measured and the global antioxidant activity was studied using complementary assays. Intracellular antioxidant and antiproliferative activities were assessed at cellular level in appropriate human cell lines searching for protection responses to environmental stresses and cell death signals.



Comparison of kinetic release of caffeine through a cellulose acetate membrane using a Franz cell.

• Development of bio-functional food-products. The activities under this theme were mainly supported by national agro-food industries. The formulation of new products by incorporation of natural extracts is nowadays widely in use, as the trend of the future is moving towards nutraceuticals and functional food with specific health effects. We consolidated scientific evidence supporting the existence of a synergetic positive effect between the compounds present on natural matrices.

• In the field of material science, this group conducted an intensive research work related with the the impregnation of bio-polymers with biological active drugs and the preparation of lipid-based particles, using supercritical fluid technology, that enable the production of low-micro systems as carriers or solubility enhancers for active substances.

Group Members

Raquel Frade	Post Doc
Ana Matias	PhD Student
Ana Serra	PhD Student
Mariana Costa	PhD Student
Mónica Faustino	Master Student
Ana Sousa	Post Doc
Rodrigo Silva	Technician

Selected Publications

Duarte A. R. C., Simplicio A. L., Vega-Gonzalez A., Subra-Paternault P., Coimbra P., Gil M. H., de Sousa H. C. and Duarte C. M. M. (2007). "*Supercritical fluid impregnation of a biocompatible polymer for ophthalmic drug delivery.*" Journal of Supercritical Fluids **42**(3): 373-377.

de Sousa A. R. S., Simplicio A. L., de Sousa H. C. and Duarte C. M. M. (2007). "*Preparation of glyceryl mono stearate-based particles by PGSS((R)) - Application to caffeine.*" Journal of Supercritical Fluids **43**(1): 120-125.

Raquel F.M. Frade, Ana Matias, Luís C. Branco, Carlos A.M. Afonso, Catarina M.M.Duarte(2007), "*Effect of ionic Liquids on human colon carcinoma HT-29 and CaCo-2 cell lines*" Green Chem, **9**, 873-877.

Pharmacokinetics and Biopharmaceutical Analysis [ITQB/IBET]

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Group Members

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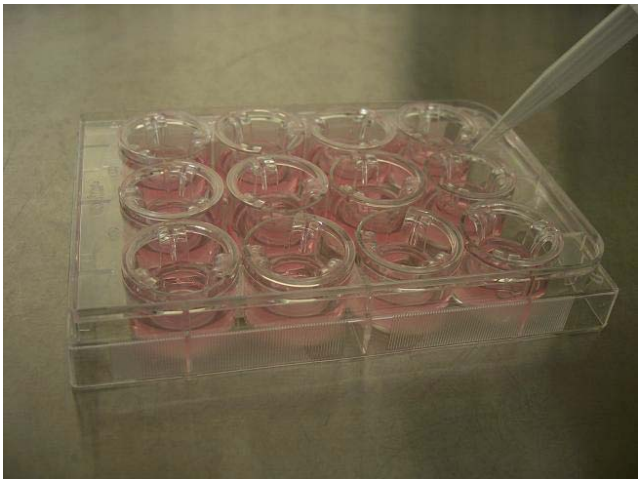
In the development of new drugs and new drug formulations for oral administration it is particularly important to investigate how the active principles are absorbed and metabolized before reaching the site of action.

In our laboratory we use well established *in vitro* models, such as the Caco-2 cell model, to evaluate the permeability of pharmacologically active compounds as well as the interaction between different compounds and nutrients. We are also interested in using those models for risk evaluation of persistent organic pollutants.

The Caco-2 and other cell models still lack some characteristics that limit their correlation with *in vivo* observations and therefore we also try to improve them by association with other *in vitro* methodologies in order to widen their applicability range. Genetic manipulation is the next step we are taken in consideration.

The absorption profile is highly dependent on the chemical characteristics of the drugs and therefore sometimes it is necessary to prepare a derivative that is more stable, more soluble or just more permeable through biological membranes, in order to increase its bioavailability. The derivative is than converted back to the original compound before or at the site of action and this is called the prodrug approach. We are also involved in the development and evaluation of prodrugs for low solubility compounds.

Pre-absorption metabolism may also have a significant effect on bioavailability and on inter-patient or inter-species variability in the response to drugs. We are currently developing capillary electrophoresis *in capillary* methods for the establishment of *in vitro-in vivo* correlations of inter-species metabolism.



Permeability model Caco-2

Selected Publications

Simplicio A. L., Clancy J. M. and Gilmer J. F. (2007). "*beta-aminoketones as prodrugs with pH-controlled activation.*" International Journal of Pharmaceutics **336**(2): 208-214.

de Sousa A. R. S., Simplicio A. L., de Sousa H. C. and Duarte C. M. M. (2007). "*Preparation of glyceryl mono stearate-based particles by PGSS((R)) - Application to caffeine.*" Journal of Supercritical Fluids **43**(1): 120-125.

Duarte A. R. C., Simplicio A. L., Vega-Gonzalez A., Subra-Paternault P., Coimbra P., Gil M. H., de Sousa H. C. and Duarte C. M. M. (2007). "*Supercritical fluid impregnation of a biocompatible polymer for ophthalmic drug delivery.*" Journal of Supercritical Fluids **42**(3): 373-377.

Research Output

"*Tríptico*" Sofia Miguel, PhD Student

Publications 2007

1. Abrantes M., Balula M. S., Valente A. A., Paz F. A. A., Pillinger M., Romão 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.

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89. Vicente J. B., Justino M. C., Goncalves V. L., Saraiva L. M. and Teixeira M. (2008) "Biochemical, spectroscopic, and thermodynamic properties of flavodiiron proteins." in Globins and Other Nitric Oxide-Reactive Proteins, Part B Methods in Enzymology 437: 21-45.

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Scientific events organized by ITQB members

Biosafe project meeting, IBET, Oeiras, March 2007.
Maria Teresa Crespo, organizer

Europneumo 2007 8th European Meeting on the Molecular Biology of Pneumococci.
Oeiras, Portugal. April 14-17, 2007.
Hermínia de Lencastre, organizer

International Meeting "Harmonisation and Distribution of Pathogen Detection and Differentiation Tools", 15-17th May 2007, Lisbon, Portugal.
Abel González Oliva, organizer

40th ESCMID Postgraduate Education Course:
Bacterial molecular typing a Practical and Theoretical Course organized under the auspices of ESGEM (ESCMID Study Group on Epidemiological Markers) and ESCMID (European Society of Clinical Microbiology and Infectious Diseases). Oeiras, Portugal. April 29-May 4, 2007.
Hermínia de Lencastre, organizer

IUFRO Tree Biotechnology 2007, Ponta Delgada, Azores (3-8 June, 2007).
Célia Miguel, M. Margarida Oliveira, organizers

Inauguração da Rede Nacional de Ressonância Magnética Nuclear
Recordar António V. Xavier, Oeiras, 9 de Julho 2007.
Helena Santos, organizer

1º Encontro da Plataforma de Genómica Funcional de Plantas, Faro, Portugal, 12-13 July, 2007.
M. Margarida Oliveira, organizer

2nd Congress on Ionic Liquids (COIL - 2),August 2007, Yokohama, Japan.
Luis Paulo N. Rebelo, Member International Scientific Advisory Committee and Organizing Committee

Glupor 7
7th International Meeting of the Portuguese Carbohydrate Group, ITQB, Oeiras.
Júlia Costa, Rita Abranches, Rita Ventura, Sérgio R. Filipe, organizers

Scientific events organized by ITQB members

"S-SAD diffraction data phasing of macromolecule single crystals from home and Synchrotron X-ray sources" 3-6 October 2007, International Course organized as TID center of BIOXHIT.
Maria Arménia Carrondo, **Pedro Matias** organizers

7th Short course of the Portuguese Biophysical Society " Biospectroscopy and Imaging"
19-21 of October 2007, Santarém, Portugal.
Cláudio M. Soares, Manuela M. Pereira, Bruno Victor, Miguel Teixeira, organizers

6th European Congress on Grain Legumes Integrating Legume Biology for Sustainable Agriculture
12-16 November 2007. Lisbon Congress Centre. Portugal.
Carlota Vaz Patto, Chair of the Local Organizing Committee

IV National Meeting on RNA (RNA07)
November 2007, Vimeiro, Portugal.
Cecilia Maria Arraiano, organizer

5º Encontro Nacional de Cromatografia,
Aveiro, 10-12 December 2007.
Luis Vilas Boas / Maria do Rosário Bronze, organizers

7th Conference of Inorganic Chemistry of the Sociedade Portuguesa de Química,
Fátima, 30 November-1 December 2007,Portugal.
Carlos C. Romão, Member of the Organizing Committee

Young Scientist Forum
in Vienna as chairman of the FEBS young scientists career (2007).
Claudina Rodrigues-Pousada, organizer

PhD Theses at ITQB in 2007
(by chronological order)

Clara Pinheiro Vieira Correia dos Reis (Biochemistry)
"Study of Trf4 and Trf5 in Sacharomyces cerevisae: Poly(A) Polymerases with a Function in Histone Homeostasis"
Supervisor: Judith Campbell/Sukalyan Chatterjee (IGC)

Beatriz Garcia Fernández (Biology)
"Analysis of the role of Dpp signalling during the morphogenetic process of Dorsal Closure in Drosophila melanogaster"
Supervisor: António Jacinto/Moises Mallo (IGC)

Marta Sofia Zambujo Carapuço (Biology)
"The role of Hox genes in the development of the branchial arches and axial skeleton"
Supervisor: Moisés Mallo (IGC)

João Filipe Bogalho Vicente (Biochemistry)
"The Role of Flavodiiron Proteins in Nitric Oxide Detoxification"
Supervisor: Miguel Teixeira

Vítor Hugo Oliveira Teixeira (Biochemistry)
"Molecular Modelling of Redox Proteins Involved in the Hydrogen Metabolism"
Supervisors: Cláudio M. Soares and António M. Baptista

Maria Angelina de Sá Palma (Biochemistry)
"Amyotrophic Lateral Sclerosis: Identification and Characterisation of Biochemical and Molecular Marker"
Supervisor: Júlia Costa

Rute Conceição do Nascimento Veríssimo Afonso (PGDBM) (Biology)
"A non-assigned herpesvirus gene inducing cell cycle arrest and apoptosis"
Supervisor: Michael Parkhouse (IGC)

Isabel Eloi Marcelino-Mista (Engª. Química)
"Development of a Vaccine Candidate Against Heartwater"
Supervisor: M.Carrondo/Paula Alves/D. Martinez

Tiago Bruno Pereira Soares Ferreira (Engª. Química)
"Adenovirus as a Biopharmaceutical: Optimisation of Vector Production"
Supervisor: Manuel Carrondo

Melinda Carmen Noronha (Chemistry)
"Time-Resolved Fluorescence of Tyrosine Residues: A New Tool to Characterize Protein Conformational Changes"
Supervisor: Antº Maçanita/Helena Santos

Ana Filipa de Matos Almeida Valente (Biochemistry)
"Hydrogen Metabolism in *Desulfovibrio* vulgaris Hildenborough"
Supervisor: Inês Cardoso Pereira

PhD Theses at ITQB in 2007
(by chronological order)

Claudia Istrate (Biology)
"Host and Immune Responses to Natural and Experimental Rotavirus Infection"
Supervisor: Manuel Carrondo/Paula Alves

Sara Newbery Raposo de Magalhães (Biology)
"To eat and not to be eaten. Do plan-inhabiting arthropods tune their behaviour to predation risk?"
Registo de tese/Universidade de Amsterdão

Irina Luísa Saraiva Franco (Biology)
"Regulation of arabinose metabolism in *Bacillus subtilis*: linking structure and function"
Supervisor: Isabel Sá Nogueira

Susana de Sousa Araújo (Biology)
"Expression of the ELIP-like protein DSP22 in *Medicago truncatula*: an approach to improve the response of a legume plant towards water deficit"
Supervisor: Manuel Pedro Fevereiro

Marta Alexandra Garcia Campos (Biology)
"The role of VEGF during a malaria infection"
Supervisor: Maria Mota (IGC)

Ana Sofia Gírio Veloso (Biology)
"Regulation of the pró-apoptotic protein Bim by the kinase Erk5 in mitosis"
Supervisor: Sukalyan Chatterjee (IGC)

Alexandre Alves Neves (Biology)
"Deciphering Notch transcriptional synergy in Caenorhabditis elegans embryos"
Supervisor: Adriano O. Henriques

Jorge Bruno Reis Wahnon (Chemistry)
"Asymmetric synthesis using chiral pool hydroxyacids"
Supervisor: Christopher Maycock

Maxime Guy Leonard Cuypers (Biochemistry)
"Structural studies of iron storage proteins: Dps and Bacterioferritin"
Supervisor: Miguel Teixeira, Robert Crichton, Sean McSweeney, Edward Mitchell, Célia Romão

João Vieira Rodrigues (Biochemistry)
"Superoxide defence strategies in anaerobic prokariotes: The mechanism of superoxide reductases"
Supervisor: Miguel Teixeira

David Guerra Aragão (Biochemistry)
"Structure to function studies in hybrid cluster proteins and glucose-1-phosphate urydylyltranferases"
Supervisor: Carlos Frazão, Edward Mitchell

Nuno Miguel da Silva Micaêlo (Biochemistry)
"Enzyme structure and function in nonaqueous solvents"
Supervisor: Cláudio Soares

PhD Theses at ITQB in 2007
(by chronological order)

Krassimira Assenova Passos Guerra (Chemistry)
"Heterodinuclear Metal Complexes as Models of Active Centres of Proteins"
Supervisor: Rita Delgado

Ana Catarina Maurício Brito Ataíde Montes (Biochemistry)
"Fucosyltransferase IX: characterization and biological role"
Supervisor: Júlia Costa

Larissa Consani Textor (Biochemistry)
"Structural insights into the new features of the transcription factor MafB"
Supervisor: Matthias Wilmanns, Maria Arménia Carrondo

Filipa Susana Caldas Pinto (Biology)
"p63 function in epithelial stem cells"
Supervisor: Frank McKeon, Sukalyan Chatterjee (IGC)

Sílvia Cristina Ferreira de Carvalho (Chemistry)
"New Molecular Architectures for the Encapsulation of Anions"
Supervisor: Rita Delgado

Sílvia Cristina de Paiva e Almeida (Biology)
"Manipulation of T and B cell biology through transgenic expression of a vírus gene inhibiting transcription via NF-kB and NFAT"
Supervisor: Michael Parkhouse (IGC)

Amélia Maria de Sousa Martins Muralha Delgado (Biology)
"Bacteriocins from Lactic Acid Bacteria of Portuguese Table-Olives"
Supervisor: Cidália Peres, Manuel Pedro Fevereiro

Ana Serbanovic (Engª. Química)
"Alternative Solvents: Phase Behaviour and Application in Catalysis"
Supervisor: Manuel Nunes da Ponte

Sandra Cristina de Oliveira Viegas (Biology)
"Microbial Ribonucleases: control of gene expression and regulation of small non-coding RNAs"
Supervisor: Cecília Arraiano

Bruno Lourenço da Silva Victor (Biochemistry)
"Molecular modeling of redox proteins involved in the oxygen and nitric oxide metabolism"
Supervisor: Cláudio Soares

Catarina Maria de Castro Dias Vieira Figueiredo (Biology)
"Role of neuron-microglia interactions in NMDA receptor-mediated cell death"
Supervisor: Sukalyan Chatterjee (IGC)

Ana Lúcia Freitas de Mesquita Barbas Sant'Ana de Miranda (Biology)
"Functional and Structural Studies on *Escherichia coli* RNase II: a crucial enzyme in RNA metabolism"
Supervisor: Cecília Arraiano

PhD Theses at ITQB in 2007
(by chronological order)

Ana Raquel Sampaio de Sousa (Biology)
"Development of Functional Particles using Supercritical Fluid Technology"
Supervisor: Catarina Duarte

Feng Li (Chemistry)
Coordination and Supramolecular Chemistry of New Macrocycles and Cryptands as Receptors for Cations and Anions.
Supervisor: Rita Delgado

Ricardo Hugo Jorge Pires (Biochemistry)
Membrane-bound metalloproteins involved in Sulfate Respiration.
Supervisor: Inês Cardoso Pereira (António Xavier)

Ongoing Research Projects
as in December 2007

Project title	Project nº	Leader/Participant [1]	Funding [2] in €	Duration
Projects funded by FCT:				
RNases and polyadenylation in the adjustment of bolA mRNA levels necessary for cells growth and survival	POCTI/BME/42377/01	Cecília Arraiano	93.480	2002-2007
Unravelling the acrobic respiratory chain of the "anaerobic" sulfate reducing bacteria	POCTI/BME/37406/01	Miguel Teixeira	74.500	2003-2007
Reduction of nitric oxide in prokaryotes: new metabolic routes	POCTI/BME/44597/02	Miguel Teixeira	102.456	2003-2007
Increasing realism in protein modelling: including pH and rdox effects into molecular dynamics simulations	POCTI/BME/45810/02	António Baptista	51.528	2003-2007
Anaerobic metabolism of the human pathogen bilophila wadsworthia	POCTI/ESP/44782/02	Inês Cardoso Pereira	86.808	2003-2007
Structure, tropisms and molecular dynamics of the stratum corneum lipid matrix. A study in model systems	POCTI/QUI/45090/02	Eurico de Melo	53.470	2003-2007
Thermodynamics of metalloprotein folding and stability	POCTI/QUI/45758/02	Cláudio Gomes	69.440	2003-2007
Energy transduction in a plant symbion from Sinerhizobium melitoli	POCTI/BME/45122/02	Manuela Pereira	74.000	2004-2007
Cell wall proteins with roles in xylogenic programmes in eucalyptus	POCTI/AGR/46671/02	Philip Jackson	76.854	2003-2007
Oxidative Phosphorylation in sulfate respiration	POCTI/QUI/47866/02	David Turner	58.440	2003-2007
Study of ribonucleases from lactid acid bacteria for the construction of strains important for food processing by the dairy industry	POCTI/AGR/49306/02	Cecília Arraiano	120.680	2003-2008
Global experimental approches to medel central metabolism in L. lactis: modulation of the elvels of key-enzymes	POCTI/BIO/48333/02	Helena Santos	98.738	2004-2007
Reforestation woth corl oak: genetic variability and seed storage biology	POCTI/AGG/41359/02	Cândido Pinto Ricardo	25.200	2003-2007
Macrocyclic ompounds selective for heavy metals poisoning: Cd(II), Hg(II), and Pb(II)	POCTI/QUI/49114/02	Rita Delgado	6.563	2003-2007
Searching for rhizobial strains with improved skills to thrive in arid lands	POCTI/AGG/46371/02	Helena Santos	17.000	2004-2007
Kinetic modeling of cellular chemoreception: An experimental study	POCTI/POCTI/BCI/46174/02	Eurico de Melo	3.000	2004-2008
The role of RNase R and its homologues in the control of gene expression: structural and functional studies	POCI/BIA-MIC/55106/04	Cecília Arraiano	90.000	2005-2009
Role of the interaction between AgfA and AgfB proteins from Salmonella enterica serovar Typhimurium in the surface polimerization of the amyloid-like thin aggregative fimbriae.	POCI/SAU-IMI/55520/04	Francisco Enguita	71.872	2006-2008
Rhenium, molybdenum and tungsten oxo complexes: new class of catalysts for reduction reactions	POCI/QUI/555862/04	Beatriz Royo Cantabrana	55.500	2005-2008
Structural characterization of membrane proteins of the respiratory chain of a thermoacidophilic organism	POCI/BIA-PRO/55621/04	Margarida Frazão	78.410	2005-2008

Ongoing Research Projects
as in December 2007

Project title	Project nº	Leader/Participant [1]	Funding [2] in €	Duration
Role of defense-related genes during the establishment of root-nodule symbioses between higher plants and nitrogen-fixing bacteria	POCI/AGR/55651/04	Ana Ribeiro	57.360	2005-2008
Characterisation of membrane bound cytochrome involved in the anaerobic respiration in sulphate reducing bacteria	POCI/QUI/55690/04	Ricardo Louro	54.500	2005-2008
Structure function studies of murine Toll-like receptors: activation of the innate immune response	POCI/SAU-IMI/55729/04	Maria Arménia Carrondo	50.420	2005-2008
Transgenic plants as models to study regulation of transgene expression and recombinant protein deposition	POCI/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	POCI/BIA-BCM/56063/04	Nelson Saibo	50.000	2005-2008
Nitrosative Stress Responses of Human Pathogens	POCI/SAU-IMI/56088/04	Lígia Saraiva Teixeira	99.648	2005-2008
Organizations of the staphylococcal cell wall synthetic machinery	POCI/BIA-BCM/56493/04	Mariana Pinho	90.000	2005-2008
Role of bacterial cell wall on the host innaste immune response	POCI/SAU-IMI/56501/04	Sérgio Filipe	99.999	2005-2008
Molecular ercognision of phthalate and phthalic acid esters pollutants by ditopic receptors by cascade dicopper systems	POCI/QUI/56569/04	Rita Delgado	43.640	2005-2008
Molecular markers for Portuguese pine wood quality	POCI/AGR/56658/04	Pedro Fevereiro	22.066	2005-2008
Improving tolerance to water stress in legumes using the model *Medicago truncatula*	POCI/BIO/56659/04	Pedro Fevereiro	16.144	2005-2008
The role of small non-coding RNAs and RNases on the pathogenicity of Salmonella	POCI/CVT/56811/04	Cecília Arraiano	87.879	2005-2009
Nano-Engineering of bacterial laccases	POCI/BIO/57083/04	Lígia Martins	90.459	2005-2008
Functional characterization of genes related to nitrogen metabolism in genetically modified maritime pine	POCI/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	POCI/BIA-PRO/57263/04	Helena Santos	63.102	2005-2008
Bioremediation of PCP by the co-metabolism of cork endogenous moulds	POCI/AMB/57374/04	Cristina Silva Pereira	82.187	2005-2008
Staphylococcus aureus and *Staphylococcus epidermidis*: links between hospital and community	POCI/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.	POCI/AGR/57994/04	Carlota Vaz Patto	37.060	2005-2008
Epidemiology of multidrug resistant enterococci in a Lisbon Hospital – Surveillance study in malignancy ward	POCI/SAU-ESP/58030/04	Rosario Mato Labajos	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	POCI/BIA-PRO/58374/04	Manuela Pereira	51.600	2005-2008

Ongoing Research Projects
as in December 2007

Project title	Project nº	Leader/Participant [1]	Funding [2] in €	Duration
Regulation of cell wall synthetic genes and enzymes in B-lactam resistant *Staphylococcus aureus*	POCI/BIA-MIC/58416/04	Hermínia de Lencastre	80.000	2005-2008
Screening hyperthermophilic proteomes for hyperstable proteins	POCI/BIO/58465/04	Cláudio Gomes	51.829	2005-2008
Heme-copper oxygen reductases – mechanisms of electron/proton transfer and oxygen reduction	POCI/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	POCI/BIO/58652/04	Ricardo Louro	80.500	2005-2008
Characterization of CymA: a focal protein in anaerobic respiration by Shewanella	POCI/BIA-PRO/58722/04	Ricardo Louro	39.600	2005-2008
Cytochrome c: A model protein to probe thermodynamic and choreographic constraints in electroprotonic energy transducers	POCI/QUI/58985/04	David Turner	69.000	2005-2009
Understanding defence responses of grapevine to drought stress-metabolic regulation at the leaf and berry levels	POCI/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*	POCI/BIA-MIC/59310/04	Helena Santos	90.000	2005-2008
Studies on quinine-protein interaction in complexes of respiratory chains	POCI/QUI/59824/04	Manuela Pereira	73.000	2005-2008
Molecular characterization of a microbial hemicellulolytic system	POCI/POCTI/AGR/60236/04	Isabel Sá Nogueira	74.500	2005-2008
Transcriptional control of the mecA gene, the central element of methicillin-resistance in staphylococci.	POCI/BIA-MIC/60320/04	Duarte Oliveira	72.233	2005-2008
Interactions between proteins in adjacent sister cells that signal the activation of RNA polymerase in response to cellular morphogenesis	POCI/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	POCI/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	POCI/EQU/61550/04	Rui Ruivo	31.728	2005-2008
Studies on the synthesis and applications of 2-Oxoaza [x.1.0] bicycles	POCI/QUI/62794/04	Christopher Maycock	44.500	2005-2009
Constraints to carbon gain by tree age in Eucalyptus globules (Labill.) stands.	POCI/AGR/61980/04	Manuela Chaves	22.194	2005-2008
Rationalization of cutinase enantioselectivity in non-aqueous media	POCI/BIO/57193/04	Cláudio Soares/Isabel Sá Nogueira	35.088	2005-2008
Mechanism and kinetics of protein stabilization by osmolytes.	POCI/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	POCI/AMB/56075/04	Helena Santos	10.080	2005-2008
Synthesis, structure and reactivity of transition metal complexes with potential application in oxidative catalysis	POCI/QUI/55985/04	Carlos Romão	3.600	2005-2008
Gene expression changes during hepatitis delta virus infection I. Analysis of the cellular proteome.	POCI/SAU-IMI/55112/04	Ana Coelho	13.200	2005-2008

Ongoing Research Projects
as in December 2007

Project title	Project nº	Leader/Participant [1]	Funding [2] in €	Duration
Optical fibre sensors for distributed monitoring of dissolved oxygen and temperature.	POCI/AMB/56132/04	Abel Oliva	30.028	2006-2008
Thermodynamical and structural characterization of ionic liquids and others associated fluids.	POCI/QUI/57716/04	Luís Paulo Rebelo	25.000	2005-2008
Ñature's shields to environmental stress. Biosynthesis of compatible solutes in extremely radiation-resistant Rubrobacter spp.	POCI/BIA-MIC/56511/04	Helena Santos	18.000	2005-2008
Proteomics of chronic lung diseases leading to biomarkers and therapeutic target dicovery	POCI/SAU-MMO/56163/04	Ana Varela Coelho	16.904	2005-2008
Tannin polymerase, a novel and previously unknown enzyme that is required by obligate pathogens to invade grapevine tissues	PTDC/AGR-AAM/65611/06	Ricardo Boavida Ferreira	126.456	2007-2010
Two-dimensional micelles and emulsions in lipid bilayers resulting from caophylic/caophobic amphiphilicity of some phospholipids. Biological consequences	PTDC/QUI/68242/06	Eurico de Melo	93.238	2007-2010
Understanding sulfate respiration at molecular level: studies of two conserved membrane complexes	PTDC/QUI/68368/06	Inês Cardoso Pereira	74.700	2007-2009
Z-DNA and Z-RNA: the long road to a biological function	PTDC/SAU-MII/69084/06	Maria Arménia Carrondo	159.643	2007-2010
Novel stroke model for neuroprotective research: bioreaction of primar brain cell aggregates	PTDC/BIO/69407/06	Paula Alves	169.000	2007-2010
Functional analysis and origins of the pathophysiology of mutations within genes of the fatty acid oxidation pathway: implications in the multiple acyl-CoA dehydrogenase deficiency disorder	PTDC/SAU-GMG/70033/06	Cláudio Gomes	70.801	2007-2010
In search of ideal protein stabilisers: compound libraries inspired by solute from hyperthermophiles	PTDC/BIO/70806/06	Pedro Lamosa	105.000	2007-2010
Analysis of the breeding potential of fasciation traits in Portuguese maize landraces	PTDC/AGR-AAM/70845/06	Carlota Vaz Patto	77.100	2007-2010
Molecular Adaptation to Extreme Environments: Structural Studies of Proteins Involved in the Synthesis of Osmolytes in (hyper)thermophylic micro-organisms	PTDC/QUI/71142/06	Pedro Matias	85.500	2007-2010
Contribution of the capsular polysaccharide to the inflammatory ability of the bacterial peptidoglycan	PTDC/SAU-MII/75696/06	Sérgio Filipe	159.686	2007-2010
Exploiting genetic variability of resistance genes in major European food legumes to improve varieties for sustainable agriculture	ERA-PG/0008/06	Carlota Vaz Patto	101.300	2007-2010
Genomic research-assisted breeding for sustainable production of quality GRAPEs and WINE	ERA-PG/005/06	Pedro Fevereiro	85.700	2007-2010
Genome-wide analysis of short RNAs as modulators in dehydration stress tolerance using yolerant and genetic model systems	ERA-PG/001/06	Pedro Fevereiro	126.588	2007-2010
Molecular Evaluation and Characterization of Tolerance Mechanisms to Adverse Environmental Conditions in Coffea sp. - Central Role of the Oxidative Stress Control at Leaf Level	PTDC/AGR-AAM/64078/06	Manuela Chaves	29.930	2007-2010
Vapour liquid equilibrium of pure ionic liquids and their mixtures with organic solvents	PTDC/EQU-FTT/65252/06	Luís Paulo Rebelo	16.920	2007-2010
Quantitative Modeling of Passive Trancytotic Diffusion of Amphiphilic Molecules Across the Blood-Brain Barrier	PTDC/SAU-FCF/69072/06	Eurico de Melo	15.000	2007-2010

Ongoing Research Projects
as in December 2007

Project title	Project nº	Leader/Participant [1]	Funding [2] in €	Duration
DynaMo - Dynamical modeling, control and optimization of metabolic networks	PTDC/EEA-ACR/69530/06	Helena Santos	81.621	2007.2010
Enzymatic degradation and synthesis of Azo and antraquionic dyes	PTDC/BIO/72108/06	Ana Coelho	16.800	2007-2010
The wild relatives of Beta: genetic diversity assessment and biochemical studies	PTDC/AGR-AAM/73144/06	Cândico Pinto Ricardo	46.740	2007-2010
Lessons from the physiology of hypertermophilic microorganisms: Potential of new molecules to inhibit protein misfolding abd aggregaton	010.6/A004/05	Helena Santos	99.996	2005-2008
Structural genomics and human pathogenic bacteria	010.6/A004/05	Maria Arménia Carrondo	100.000	2005-2008
Projects funded by FCT, under the re-equipment call:				
National Facility for High-Field Nuclear Magnetic Resonance	REDE/1517/RMN/05	Helena Santos	6.500.000	2005-2007
Projects funded by FCG:				
Creation of a reference collection of antimicrobial resistant gram-positive bacteria serving the national and international scientific and clinical communities	61052/03	Herminia de Lencastre	88.000	2004-2007
Infeccion and colonization by multidrug-resistant Enterococci recovered from neonatal intensive care units. Epidemiological surveillance and infection control	65882/04	Rosario Mato Labajos	45.124	2004-2007
Projects funded by Agência de Inovação:				
Gigasnome	ADI/2006/M.2.3/0031	Claudina Rodrigues-Pousada	114.153	2005-2007
Projects funded by Agência Portuguesa do Ambiente:				
Protocolo entre a Agência Portuguesa do Ambiente e o ITQB	200/2006	Ricardo Louro	88.068	2006-2008
Projects funded by Sociedade Portuguesa de Gastrenterologia:				
Pesquisa de probióticos em azeitona de mesa portuguesa, com potencial actividade anti-Helicobacter pylori	01/2006	Cidália Peres	14.850	2006-2008

Ongoing Research Projects
as in December 2007

Project title	Project nº	Leader/Participant [1]	Funding [2] in €	Duration
Projects funded by European Comission:				
From receptor to gene: structures of complexes from signalling pathways linking immunology, neurobiology and cancer (SPINE2-COMPLEXES)	LSHG-CT-2006-031220	Maria Arménia Carrondo	206.750	2006-2009
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
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	2004-2008
European macromolecular crystallography infrastructure network 2 (MAX-INFO 2)	RICA 505977	Maria Arménia Carrondo	36.700	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	Ligia 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 through Rockfeller University:				
Evolution and acquisition of drug resistance in MRS		Sérgio Filipe	51.289,29	2005-2009
Pathogen – specific drug targets for weaponized bacteria		Adriano O. Henriques	21.363,93	2005-2009
Projects funded by the European Free Trade Association:				
Awake of green Biotech in Portugal: waste elimination using genetically manipulated fungal species in an ionic liquid environment	Gree Bio-Awake	Cristina Silva Pereira	1.470.143	2007-2011
Subcontracting Parties:				
Biocrystallography (X) on a highly integrated Technology platform for European structural genomics	LSHG-CT-2003-503420	Maria Arménia Carrondo	49.200	2006-2008
Assessment of pathway design through multi-level modeling and experiments	NSF	Helena Santos	39.900	2006-2009

1. Projects are displayed in black when coordinated by ITQB researchers.
2. Funding refers solely to the budget allocated to ITQB and not to the project total funding.

ITQB PhD Programme

COMMITTE

Inês C. Pereira
Beatriz Royo
Rita Abranches
Sérgio Filipe
Júlia Costa

ADVISORS

Luis Paulo Rebelo
Cláudio Soares
Adriano Henriques

1st Module

Biochemistry and Structural Biology (BSB)

Opening - Miguel Teixeira
Biochemistry and Structural Biology presentation - Inês Pereira
Metals in Biology - Miguel Teixeira
Introduction to spectroscopy - Eurico Melo
Electron paramagnetic ressonance in Biology - Miguel Teixeira
Energy transduction in respiratory systems (group activities/paper discussion) - Manuela Pereira/Andreia Fernandes
Protein structure - Margarida Archer
Protein and small molecule crystallography - Isabel Bento
How to interpret X-ray Crystallographic Data (group activities/paper discussion) - Pedro Matias/Carlos Frazão
Raman spectroscopy: theoretical basis and applications - Smilja Todorovic
Protein folding and stability - Cláudio Gomes
Hot topics in Protein Folding research (group activities/guided paper discussion) - Cláudio Gomes/Patrícia Faísca
Nuclear Magnetic Resonance in Structural Biology - David Turner/Pedro Lamosa
Molecular mechanics/ dynamics of biomolecules - Cláudio M. Soares
Anaerobic microorganisms: from proteins to applications (group activities/presentation of papers) - Inês Cardoso Pereira/ Ricardo Louro/Catarina Paquete
Homology - detection and functional inference - José Leal, IGC
Protein electrostatics - António M. Baptista
Protein structure prediction - Cláudio M. Soares
Molecular visualisation - Cláudio M. Soares/António M. Batista

2nd Module

Biomolecular Chemistry (BC)

Biomolecular Chemistry Presentation - Beatriz Royo
Natural products isolation and asymmetric catalysis - Christopher Maycock
Organocatalysis: synthesis of novel catalysts - Rita Ventura
Mass spectrometry - Ana Coelho (inc. visit to the lab)
Supramolecular chemistry: an overview - Rita Delgado
Metal based drugs - Carlos C. Romão
Inorganic chemistry, biology and medicine (group activities/paper discussion) - Beatriz Royo/Patrícia Reis
UV-Vis Spectroscopy - Eurico Melo
Atomic Force Microscopy - Miguel Castanho
Equipment and methods in UV-Vis spectroscopy - Eurico Melo (Visit to the lab)
Radioisotopes and biomedical applications - Isabel Santos
Radioisotopes and biomedical applications - António Paulo
Potentiometry with ion-selective electrodes and cyclic voltametry - Margarida Santos (IST)
Hyperthermophiles as a source of new protein stabilizers - Helena Santos
Biological targets for prodrug strategy - Ana Luísa Simplício
Presentation of the PhD. projects by the students (4+1min) - Module Coordinators

3rd Module

Microbial Physiology and Pathogenesis (MPP)

Microbial Physiology and Pathogenesis presentation - Sérgio Filipe
Partitioning of the transcriptional space I - Adriano Henriques
Partitioning of the transcriptional space II - Adriano Henriques
Transcriptional regulation: protein-DNA interactions - Isabel Sá-Nogueira
Introduction to bacterial stress (group activities) - Sérgio Filipe/James Yates
Control of gene expression: the central role of RNA - Cecilia Arraiano
Metabolic networks and integration of metabolism - Ana Rute Neves
Bacterial stress (presentation of papers /group activities) - Sérgio Filipe/James Yates
Microbial Survival To Nitrosative And Oxidative Stress Imposed By The Immune System - Lígia Saraiva
Surviving antibiotics: extensive changes in transcription profile induced by antibiotics - Rita Sobral
Introduction to post-transcriptional regulation (group activities) - Karina Xavier/Rita Sobral
Bacterial cell surface – composition, metabolic pathways and role in microbial pathogenesis - Sérgio Filipe
Immune mechanisms of host resistance or susceptibility to bacterial pathogens - Manuel Vilanova
Post-transcriptional regulation (presentation of papers/group activities) - Karina Xavier/Rita Sobral
Quorum-sensing and bacterial host relationship - Karina Xavier
Evolution of the microbial physiology - from food to disease - Maria Fátima Lopes
Enterococci from commensal to pathogenic bacteria - Rosário Mato
Philosophy of Science

4th Module

Cell and Developmental Biology (CDB)

Cell and Developmental Biology Presentation - Rita Abranches
Cellular Organization of Genome Function - Rita Abranches
Light Microscopy in Cell Biology - Gabriel Martins
3D Live Cell Imaging - Gabriel Martins
Microscopy – Viewing samples under the fluorescence microscope. Overview of different techniques to visualize genes and genomes - Ana Paula Santos/Nuno Geraldo
Plant Secondary Metabolites - C. Pinto Ricardo
Growth and morphogenesis at the cellular level: ion dynamics and pollen tube growth - Jose Feijó
Plant Cell Wall - Phil Jackson
Sociology of Science - The sociology of science: theory and empirical research (a brief overview and some recent studies) - António Firmino da Costa
Science for the 21st Century - João Caraça
Bacterial Cell Division - Mariana Pinho
Cell Cycle and Cell Division Mechanisms - Alvaro Tavares
Telomeres and Cancer - Miguel Godinho
CBD Journal club - Nuno Geraldo/Rita Abranches
A View at specific processes essential for vertebrate Development - Moises Mallo
Prokaryotic Development - Adriano Henriques
Cell and Developmental Biology Paper Presentations – part I
Limits and Applications of Electron Microscopy in the age of live imaging and molecular constructs - Rui Malho, FCUL
The Secretory Pathway - Júlia Costa
Signaling in Yeast under stress response - Claudina R.Pousada
Cell and Developmental Biology Paper presentations – part II

ITQB PhD Programme

5th Module

Biotechnology

Biotech Presentation - Júlia Costa
Main strategies and achievements in plant genetic engineering - Margarida Oliveira
Molecular pharming - Rita Abranches
Integrating protein science and technology: the laccase-study - Lígia Martins
Biological issues, technologies and ethics in plant genetic engineering (demonstration/group discussion) - Célia Miguel/Ana Sanchez
Animal cell culture - Paula Alves
Molecular biology of animal cells - Ana Sofia Coroadinha
From genes to biopharmaceuticals - Manuel Carrondo
Bioethics – challenges and dilemmas for our times - Paula Martinho da Silva
Fermentation and large-scale production (class+visit pilot plant) - António Cunha
Downstream processing for complex biopharmaceuticals - Paula Alves
Protein glycosylation - Júlia Costa
Recombinant human protein therapeutics: quality, safety, structural and approval aspects - Harald Conradt
Cell and Gene Therapy Technologies and Clinical applications (group activities/short presentation and discussion of papers) - Ana Sofia Coroadinha/Pedro Cruz
Biosensors - Abel Oliva
Nutraceuticals - Catarina Duarte
Good Laboratory Practices - Teresa Crespo
Protein technologies from bacteria and yeast (group activities/presentation of papers) - Lígia Saraiva/Regina Menezes
Green solvents for separations - Luis Paulo Rebelo
Biocatalysis using green solvents - Cristina Pereira
A Technological mix of Microbiology and Chemistry (group activities/discussion of papers) - Luis Paulo Rebelo/José Nuno Lopes/Cristina Pereira

art and science at ITQB

"*nova espécie por identificar*"
Acrylic on resin inside Petri dish
by Patrícia Noronha
resident artist at ITQB in 2007

Photograph by Alexandra Ceregeiro