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Introduction

INTRODUCTION

The 2007 annual report of activities, gives an overview of the achievements of the Center for Neuroscience and Cell Biology (CNC), in both science and education and confirms the CNC previous commitment to excellence in research in biomedicine.

The CNC is a non-profit research Center of public utility at the University of Coimbra, which is committed to high quality, internationally competitive research in Neuroscience, Cell and Molecular Biology and Biotechnology and allied sciences relevant to Biomedicine. Research at CNC is organized in six thematic areas, which are further divided into sub-themes, under the leadership of usually a young accomplished scientist : Neuroscience and Disease, Molecular Biotechnology and Health, Cell and Molecular Toxicology, Microbiology, Biophysics and Biomedical NMR, Cell and Development Biology.

In 2007, through the " Contrato Programa para Contratação de Doutorados" launched by FCT , novel group leaders or potential group leaders were recruited, bringing new competences and strengthening some of the research areas at CNC .

The main goal of the CNC has been to sustain and amplify the research in the biomedical area. Although the core activity of the Center is still the research in the molecular determinants of neurodegeneration and neuroprotection from the molecular level to in vivo animal models of disease and human patients, research groups with interests in inflammatory and reproductive systems and microbiology open the scope of intervention of CNC to different areas of Biomedicine while simultaneously providing novel lines of research applicable to Neurosciences.

The CNC brings together researchers in several groups from the Faculties of Medicine, Pharmacy and Science and Technology, at the University of Coimbra, as well as from the Coimbra University Hospital. The diverse scientific background of the CNC staff and the recruitment of new group leaders has been crucial to advance innovation in fundamental and translational research based on the integration of diverse scientific expertise in molecular and medical sciences. The multi-disciplinary nature of the CNC is one of its greatest assets to address future scientific and technological challenges.

The excellence of the core activity of Neurosciences at CNC led to the integration of the Center in the Network of European Neuroscience Institutes (ENI), which encourages the interaction with similar Neuroscience Centers in Europe and the development of research projects in an European context. CNC was also included as a partner in the protocol of collaboration between the Portuguese government and the Massachusetts Institute of Technology (MIT) in the focus area of Bio-Engineering Systems. This partnership has promoted the research capacity existing in the Center and developed emerging aspects of cell and tissue engineering and computational biology.

Education at CNC focuses on the domain of molecular life sciences related to disease, in the fields of Cellular and Molecular Biology, Neuroscience, Biotechnology and translational research. The aim of the CNC graduate studies programme is to provide Master and PhD students with a multi-faceted education in those scientific fields, through a Doctoral Programme in Experimental Biology and Biomedicine and the participation in the MIT/Portugal Protocol Doctoral Programme.

Under the scope of its Outreach Programme, the CNC continued involved in the promotion of science outside the scientific community through a strong participation in the "Brain Awareness Week", in the organization of high school students visits to CNC Laboratories and other "Ciência Viva" Programme initiatives.

There is also a particular emphasis on translational research involving Hospitals and Pharmaceutical Industries. As a founding partner of the biotechnology association Biocant, the CNC seeks to promote the transfer technology and the creation of novel Biomedical and Biotechonology enterprises.

Networking is a pillar of CNC, and in 2008, we intend not only to expand the network of national and international collaborations but also to foster stronger interactions between research groups at CNC. By approving the plans to reallocate groups and by appointing novel researchers as belonging jointly to more than one group will bring the research groups closer and it may promote collaboration.

For 2008, the CNC will pursue on its major mission, the understanding of the cellular and molecular basis of disease, trying to identify new therapeutical targets.

The annual report for 2007 highlights the researchers within the various research themes being developed at CNC and their contribution to achieve the main scientific goals of the Center.

Facts & Figures

II. FACTS AND FIGURES (2007)

RESEARCH STAFF

Members holding Ph.D. (92 and 11 collaborators)	103
Post-Doc Members	16
Ph.D.Students	152
MSc Students	36

PUBLICATIONS IN 2007

Publications in press	141
	69

THESIS CONCLUDED – 2007

Ph.D. thesis	13
MSc thesis	10

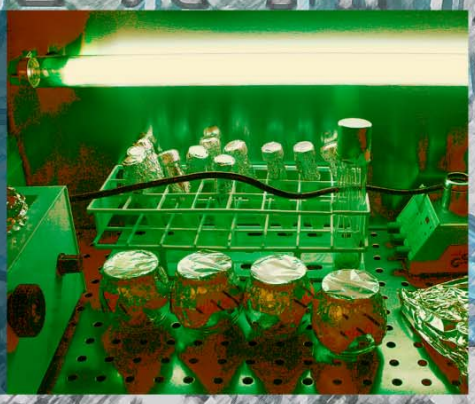
FUNDING - 2007

Pluriannual (includes salaries*)	2.100.967,32 €
International Projects	145.726,00 €
National Projects	1.222.994,00 €
Others	73.997,97 €
University of Coimbra (salaries*)	1.592.975,58€

*Salaries – Staff salaries are supported by:

1.	CNC Pluriannual	743.159,75€
2.	Projects (general expenses)	237.675,91€
3.	University of Coimbra (estimated as 60% of time dedicated to research)	1.592.975,58

Organization



ORGANIZATION

The Center for Neuroscience and Cell Biology (CNC) is a non-profit biomedical research center of public utility at the University of Coimbra. CNC brings together scientists from the Faculties of Science and Technology, Medicine and Pharmacy and from the University Hospital. The CNC is a "Laboratório Associado".

Associate Members of CNC are: Universidade de Coimbra (principal associate – 50%), Hospitais da Universidade de Coimbra, Fundação para a Ciência e Tecnologia, AIBILI, Fundação Bissaya Barreto and two comercial firms – Reagente 5 and ILC.

1- Governing Body

President: …….. *Catarina Resende de Oliveira*

Vice Presidents: …….. *Euclides Pires*
…….. *Carlos Faro*
…….. *Leonor Almeida*

Honorary President: ….. *Arsélio Pato de Carvalho*

Executive Council …….. Directors of the Departments
Research Council …….. CNC members holding PhD
"Conselho Fiscal" …….. T. Macedo, A. Rodrigues, Leal e Carreira
"Revisor Oficial de Contas" …….. Leal e Carreira, Sociedade Revisora de Contas

External Advisory Commitee ……. Bertil Fredholm (Sweden); Enrique Cadenas (USA); Roberta Brinton (USA); George Perry (USA); Helmut Sies (Germany); Stephen Zinder (USA).

2- Scientific Areas and Research Groups

At present, research programmes and projects are organized in 6 scientific areas, each coordinated by a senior scientist. The programme for each area is implemented by small research groups each headed by a research leader in his field of study. In 2007, the research groups for each area can be identified, according to the following organization:

Neuroscience and Disease | *Catarina Oliveira*
Neuromodulation Group (*Head: Rodrigo Cunha*)
Molecular Biology of Glutamate Receptors Group (*Head: Ana Luísa Carvalho*)
Neuroprotection and Neurogenesis in Brain Repair Group (*Head: João Malva*)
Neuronal Cell Death and Neuroprotection Group *(Head: Carlos B. Duarte*)
Mitochondrial Dysfunction and Cell Death Group (*Head: A. Cristina Rego*)
Molecular Mechanisms of Disease Group (*Head: Claudia Pereira*)
Retinal Dysfunction and Neurogenesis Group (*Head: Francisco Ambrosio*)

Molecular Biotechnology and Health | *Euclides Pires*
Molecular Biotechnology Group (*Head: Carlos Faro)*
Molecular Systems Biology Group (*Head: Armindo Salvador)*
Structural and Computational Biology Group (*Head: Rui Brito*)
Vectors and Gene Therapy Group (*Head: M. Conceição Pedroso Lima*)

Cell and Molecular Toxicology | *Leonor Almeida*
Mitochondrial Toxicology Group (*Head: Paulo Oliveira*)
Free Radicals and Antioxidants Group (*Head: João Laranjinha*)
Membrane Toxicity Group (*Head: Amália Jurado*)

Microbiology | *Milton Costa*

Microbiology of Extreme Environments Group (*Head: Milton Costa*)
Yeast Research Group (*Head: Teresa Gonçalves*)

Biophysics and Biomedical NMR | *Carlos Geraldes*

Intermediate Metabolism Group *(Head: John Grifith Jones)*
Inorganic Biochemistry and Molecular Imaging Group *(Head: Carlos Geraldes)*
Cell Biophysics Group *(Head: Luis Martinho do Rosário)*

Cell and Development Biology | *Celeste Lopes and João Ramalho Santos*

Cellular Immunology and Oncobiology Group (*Head: Celeste Lopes*)
Biology of Reproduction and Human Fertility Group (*Head: João Ramalho Santos)*

Emerging Groups

Infection, Phagocytosis and Pathogens Group (*Head: Otilia Vieira)*
Mechanisms of Insulin Resistance – The Role of the Adipocite (*Head: Eugénia Carvalho)*

Research Activities

AREA A: NEUROSCIENCE AND DISEASE

Coordinator-Catarina Oliveira

Introduction

The core subject of research activity in the area of Neuroscience and Disease is devoted to address some of the key issues that lead to a better knowledge of brain functioning and to the understanding of the mechanisms of brain disorders, ultimately fostering the development of novel neuroprotective strategies.

Research in this active area involves the development and use of cell and animal models of neurodegenerative disorders and the study of synaptic modulation and dysfunction that precedes neuronal death.
The modulation of glutamatergic synapses, the role of inflammation in excitotoxic injury and of mitochondria and inter-organelle cross-talk, namely endoplasmic reticulum/mitochondria, as a primary molecular mechanism of neuronal loss have been investigated.
The evaluation of neuroprotective strategies going from the mechanisms controlling the expression of neuroprotective factors to the manipulation of neurogenesis are promising fields of research being carried out in the Neuroscience and Disease area.

In 2007, under the supervision of investigators in this area, 5 Ph.D. thesis and 2 Master thesis were concluded.

The scientific collaborations established by the research groups included in the area, not only with national and international laboratories, but also with other areas at CNC, namely the Cell and Molecular Toxicology, the Cell and Development Biology, and the Biophysics and Biomedical NMR areas, can be stated by the co-authorship of published scientific papers, the organization of advanced courses integrated in the CNC Graduated Studies Programme, International Courses, such as the Ofir PENS Summer School and other scientific events, as reported in the Annual Report Internationalization section.

Several investigators of this area are strongly committed in translational research in the frame of the Biomedical Inter-Institutional research Programme at CNC, namely the research of complex brain disorders and metabolic disorders.

The commitment of the Neuroscience and Disease researchers and post-graduated students to the activities organized during the Brain Awareness Week, and to public sessions aiming to promote general public information about Neuroscience, has been highly relevant to the accomplishment of the society education programme at CNC.

The specific research highlights and ongoing and future work are described in the individual reports of the seven research groups encompassed in the Neuroscience and Disease area.

Neuromodulation Group

Rodrigo A. Cunha	(Ph.D. – *Head of group*)
Attila Kofälvi	(Post-Doctoral Fellow)
Lisiane O. Porciúncula	(Ph.D.)
Teresa Almeida	(Ph.D.)
Gianne M. Cunha	(Ph.D. Student)
Gabriele Ghisleni	(Ph.D. Student)
Nelson Rebola	(Ph.D. Student)
Ricardo J. Rodrigues	(Ph.D.Student)
Paula M. Canas	(Ph.D.Student)
João M.N. Duarte	(Ph.D.Student)
Manuella P. Kaster	(Ph.D.Student)
Gianna Cognato	(Ph.D.Student)
Ana Patrícia Simões	(Ph.D.Student)
Carla Silva	(Ph.D.Student)
Jean Oses	(Ph.D.Student)
Rui Sanches	(MSc Student)
Tiago M. Alfaro	(Junior Researcher)
Samira C. Ferreira	(Junior Researcher)

Glutamatergic Synapses Group

Ana Luísa Carvalho	(Ph.D. – *Head of group*)
Sandra D. Santos	(Ph.D. Student)
Tatiana Catarino	(Ph.D. Student)
Joana Ferreira	(Ph.D. Student)
Luís Ribeiro	(Undergraduate Student)

Neuroprotection and Neurogenesis in Brain Repair Group

João O. Malva	(Ph.D. – *Head of group*)
Fabienne Agasse	(Postdoctoral fellow)
Paulo S. Pinheiro	(Postdoctoral fellow)
Ana Paula Silva	(Ph.D.)
Joana Lourenço	(Ph.D.)
Liliana Bernardino	(Ph.D. Student)
Bruno Silva	(Ph.D. Student)
Sara Xapelli	(Ph.D. Student)
Raquel Ferreira	(Ph.D. Student)
Sofia Domingues	(Ph.D. Student)
Alexandra Rosa	(Ph.D. Student)
Sofia Grade	(Ph.D. Student)

Neuronal Cell Death and Neuroprotection Group

Carlos B. Duarte	(Ph.D. – *Head of group*)
Emília P. Duarte	(Ph.D.)
Armanda E. Santos	(Ph.D.)
Margarida V. Caldeira	(Ph.D.)
Bruno O. Manadas	(Ph.D. Student)
João R. Gomes	(Ph.D. Student)
Andrea Lobo	(Ph.D. Student)
Ana Rita A. Santos	(Ph.D. Student)
Marta Vieira	(MSc Student)
Márcio S. Baptista	(Research Grantee)
João T. Costa	(Undergraduate Student)
Graciano Leal	(Undergraduate Student)
Pedro Réu Carvalho	(Undergraduate Student)
Patrícia Rebelo	(Undergraduate Student)

Mitochondrial Dysfunction and Cell Death Group

Ana Cristina Rego	(Ph.D. – *Head of group*)
Ildete Luisa Ferreira	(Post-doctoral fellow)
Ana Isabel Duarte	(Post-doctoral fellow)
Teresa Cunha Oliveira	(Post-doctoral fellow)
Sandra Almeida	(Ph.D. Student)
Mário Laço	(Ph.D. Student)
Ana Catarina H. Oliveira	(Ph.D. Student)
Rita Perfeito	(Ph.D. Student)
Ana Cristina Silva	(Ph.D. Student)
Márcio Ribeiro	(MSc Student)
Maria Viegas Nascimento	(MSc Student)
Rui Costa	(Undergraduate student)

Molecular Mechanisms of Disease Group

Cláudia M.Fragão Pereira	(Ph.D. – *Head of group*)
Catarina R. Oliveira	(M.D., Ph.D.)
Paula Agostinho	(Ph.D.)
Paula Isabel Moreira	(Ph.D.)
Sandra Isabel M. Cardoso	(Ph.D.)
Elisabete Baptista Ferreiro	(Ph.D. Student)
Rosa M. B. Matos Resende	(Ph.D. Student)
Rui Oliveira Costa	(Ph.D. Student)
Sónia Correia	(Ph.D. Student)
Raquel esteves	(Ph.D. Student)
João Pedro Lopes	(Ph.D. Student)
Pedro Garção	(Ph.D. Student)
Marco Matos	(MSc Student)
Ana Catarina Fonseca	(MSc Student)
Cristina Carvalho	(MSc Student)
Filipa Domingues	(MSc Student)
Susana Cardoso	(Undergraduated Student)
Renato Xavier Santos	(Undergraduated Student)
Sandrina Silva	(Undergraduated Student)

Sueli Cristina Marques (Undergraduated Student)

Retinal Dysfunction and Neurogenesis Group

Cláudia Cavadas (Ph.D. – *Head of group*)

Francisco Ambrósio (Ph.D.)
Paulo Santos (Ph.D.)
Armando Cristóvão (Ph.D.)
Caetana Carvalho (Ph.D.)
Inês Araújo (Ph.D.)
Joana Salgado (Ph.D.)
Raquel Santiago (Ph.D.Student)
Ermelindo Leal (Ph.D. Student)
Ana Rita Álvaro (Ph.D. Student)
Bruno Carreira (PhD. Student)
Gabriel Costa (PhD Student)
Joana Gaspar (PhD Student)
João Martins (MSc Student)
Vera Cortez (MSc Student)

Evidence is accumulating indicating that some neurodegenerative diseases start with an early synaptic dysfunction and elimination that precedes neuronal death. Our aim is to investigate synaptic modulation systems preventing the earlier events in neurodegeneration. Our primary focus is adenosine A_{2A} receptors (A2ARs) and we also explore other modulation system such as ATP P2Rs and cannabinoid CB1Rs. We grasp their roles in controlling synaptic transmission and plasticity and their neuroprotective role in models of chronic brain diseases such as Alzheimer's or Parkinson's disease, epilepsy, diabetes or stress.

Research Highlights

In 2007, the following key conclusions were reached:

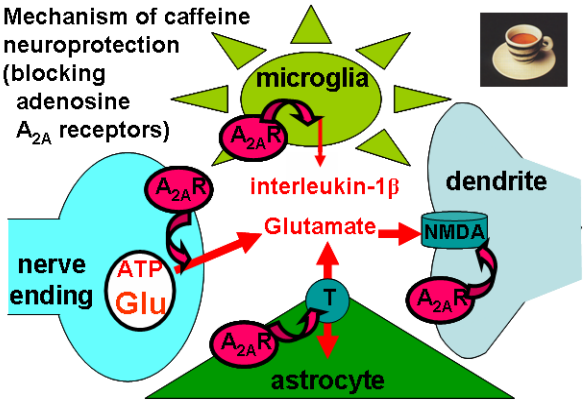
1-Caffeine and A2AR antagonists prevent memory dysfunction caused by exposure to β-amyloid peptides, a putative trigger of Alzheimer's disease.

2-Synaptic A2ARs control synaptic plasticity through a facilitation of NMDA receptors; this may help understanding how A2A receptors control neurodegeneration and memory dysfunction.

3-Synaptic A2ARs control neuronal apoptosis through control of mitochondrial dysfunction.

4-A2ARs are also located in microglia and control neuroinflammation.

5-Cell type-selective A2AR knockout mice revealed that presynaptic A2ARs play a crucial role in psychostimulant sensitization.



Ongoing and future work

Caffeine and A2AR antagonists control memory impairment caused by stress and by type II diabetes: We are exploring if and how prolonged caffeine consumption can prevent memory dysfunction and hippocampal damage (synaptic dysfunction/loss and setting of a pro-inflammatory status) in mice model of chronic unpredictable stress and both in mice and rat models of type II diabetes.

Role of presynaptic and microglia-located A2ARs in the control of neuroprotection: We are currently generating transgenic mouse lines with selective deletion of A2ARs in glutamatergic terminals and in microglia to probe their role in the control of neuronal damage in animal models of neurodegenerative diseases.

Additive neuroprotective effect of caffeine and A2AR antagonists to these of nicotine and cannabinoid receptor antagonists: Nicotinic and cannabinoid receptors are also synaptic receptors which manipulation also alleviates neuronal damage. Following our previous demonstration of the existence of receptor dimers, we are now seeking their putative role and potential beneficial effects as novel drug targets in neurodegenerative diseases.

Explore if adenosine receptor mediate stress-induced preconditioning: A near threshold noxious stimulus (e.g. restraint stress for 2 hours) decreases brain damage caused by subsequent stimuli (either kainate-induced convulsion or carotid occlusion), i.e. preconditioning. We found that the first stimulus up-regulates A1Rs and A1R blockade abrogates preconditioning.

Explore if ATP and P2Y1Rs are key elements in different models of brain damage: We found that different noxious stimuli trigger enhanced levels of extracellular ATP and we are now detailing the ability of antagonists of the ATP-P2Y1Rs to prevent neuronal damage and dysfunction in different animal models of disease (epilepsy, excitotoxicity, Alzheimer's disease, stress and diabetes).

Key References

Dall'Igna OP, Fett P, Gomes MW, Souza DO, Cunha RA, Lara DR. (2007) Caffeine and adenosine A2A receptor antagonists prevent β-amyloid (25-35)-induced cognitive deficits in mice. *Exp. Neurol.* 203:241-245.

Rebola N, Lujan R, Cunha RA, Mulle C. (2008) Long-term potentiation of NMDA-EPSCs at hippocampal mossy fiber synapses: an essential role for adenosine A2A receptors. *Neuron* 57:121-134.

GLUTAMATERGIC SYNAPSES (*Head: Ana Luísa Carvalho*)

Neurons have a complex morphology, with branched dendrites exhibiting thousands of synapses, the contacts where communication between neurons occurs. The ability of synapses to change their strength is thought to be the cellular correlate of learning and memory. Delivering and maintaining proteins at synapses requires fine-tuned mechanisms that are crucial both for synapse maintenance and for changes in synapse efficacy. Glutamate receptors mediate the majority of excitatory neurotransmission in the central nervous system, and changes in the characteristics and cellular localization of these receptors are thought to underlie the mechanisms for synaptic plasticity. Our group focuses on investigating the mechanisms that regulate ionotropic glutamate receptor activity and cellular traffic.

Research Highlights

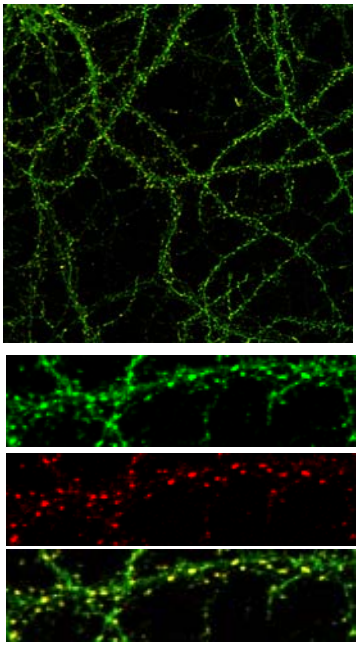
We have studied the regulation of AMPA-type glutamate receptors by phosphorylation, interaction partners and neurotrophins. We have shown that AMPA receptors containing GluR4 are phosphorylated and directly interact with PKCγ. This interaction is necessary for efficient receptor phosphorylation and cell surface expression (Gomes et al., Traffic 2007). Moreover, we have recently characterized novel splice variants of AMPA receptor subunits that give rise to truncated receptors, which play a dominant negative role. The truncated subunits are neuroprotective, are their transcripts are upregulated in cultured neurons after an excitotoxic stimulus, and are also detected in the hippocampus and cortex of epileptic rats (Gomes et al., Mol Cell Neurosci 2007). Another question that we have addressed concerns the regulation of the expression and synaptic targeting of AMPA and NMDA receptors by the neurotrophin BDNF. We have found that BDNF promotes the expression of AMPA and NMDA receptor subunits in hippocampal neurons in culture, and that it increases the synaptic expression of AMPA receptors in CA1 hippocampal neurons (Caldeira et al., J Biol Chem, Mol Cell Neurosci, 2007; reviewed in Carvalho et al., British J Pharmacol., 2007).

Ongoing and future work

1) Proteins that interact with AMPA-type glutamate receptors have been shown to be crucial for the appropriate targeting of receptor to synapses, their synaptic stabilization and also their removal from the synapse. We have performed a proteomic screening to identify novel binding partners for GluR4 AMPA receptor subunit, which is expressed in the hippocampus in the first postnatal weeks. After re-isolating α-actinin, a known GluR4 interactor, we have isolated several other molecules which may play a role in the traffic of AMPA receptors. Current studies are underway to assess the function of these new molecules in regulating AMPA receptor functional properties and traffic. (Electrophysiology studies for this project in collaboration with Christophe Mulle, University of Bordeaux, Bordeaux, France).

2) Changes in the surface and synaptic expression of AMPA receptors require endocytic and exocytic processes, coupled to cytoskeleton remodelling. We have been studying the role of the protein cortactin, an F-actin-binding protein implicated in endocytosis in other systems, in maintaining the synaptic expression of AMPA receptors in cultured hippocampal neurons. (In collaboration with Andras Kapus, The St. Michael's Hospital Research Institute, University of Toronto, Toronto, Canada).

3) Another major question that we are addressing concerns the mechanisms of subunit-specific synaptic delivery of NMDA-type glutamate receptors, under basal conditions and during synaptic plasticity. We use neuron cultures from knock-out mice for the NMDA receptor subunits, where we introduce engineered subunits of the NMDA receptor complex using a lentiviral system. This approach allows us to dissect the molecular domains required for NMDA receptor trafficking. (In collaboration with Ann Marie Craig, University of British Columbia, Vancouver, BC, Canada).



Labelling hippocampal neurons in culture (21 DIV) for GluR1 AMPA receptor subunit (in green) and an excitatory synaptic marker (PSD-95, in red), allows detection of receptor expression at the synapse.

Key references

Gomes AR, Ferreira JS, Paternain AV, Lerma J, Duarte CB, Carvalho AL. Characterization of alternatively spliced isoforms of AMPA receptor subunits encoding truncated receptors. *Mol Cell Neurosci.* 2007 [Epub ahead of print]

Carvalho AL, Caldeira MV, Santos SD, Duarte CB. Role of the brain-derived neurotrophic factor at glutamatergic synapses. *Br J Pharmacol.* 2007 [Epub ahead of print]

Gomes AR, Correia SS, Esteban JA, Duarte CB, Carvalho AL. (2007) PKC anchoring to GluR4 AMPA receptor subunit modulates PKC-driven receptor phosphorylation and surface expression. *Traffic* 8, 259-69.

NEUROPROTECTION AND NEUROGENESIS IN BRAIN REPAIR (*Head: João O. Malva*)

The search for new antiepileptogenic, neuroprotective and proneurogenic compounds is of critical relevance for the treatment of the pharmaco-resistant forms of brain diseases, and, at the long-term, will open new possibilities for brain repair.

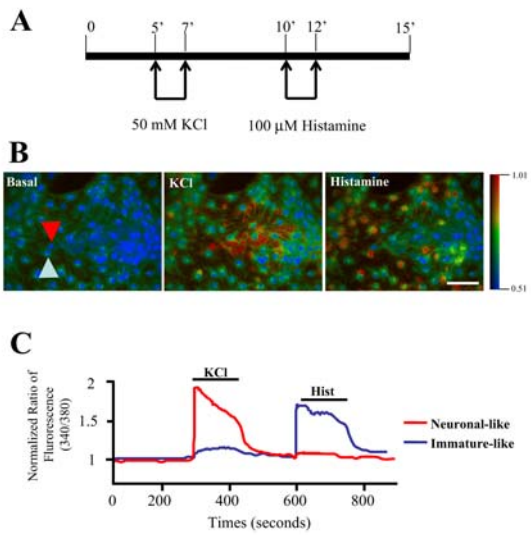
The major research pilars of the "Neuroprotection and Neurogenesis in Brain Repair" group are: 1) *Synaptic modulation* - Modulation of glutamatergic synapses by kainate and NPY receptors; 2) *Neuroprotection* – Neuroprotective properties of NPY and cytokines; 3) *Inflammation* – The role of inflammation in excitotoxic injury; 4) *Brain Repair* – Manipulation of neurogenesis and development of new strategies for brain repair.

Research Highlights

Mossy fiber-CA3 pyramidal cell synapses are endowed with presynaptic kainate receptors with unique characteristics. These receptors are involved in presynaptic forms of synaptic plasticity, are composed by GluR6/GluR7 subunits and seem to be calcium permeable. Our data favours the presence of these receptors at active zones nearby glutamate releasing sites where they facilitate glutamate release (Proc. Natl. Acad. Sci. USA 104: 12181-12186).

In status epilepticus a functional interaction between NPY Y2 receptors and PKC was found. This functional interaction favours excitability since it potentiates the release of glutamate and inhibits the functional effect of Y2 receptor activation. The common target was determined to be located downstream Y2 receptors or its coupled G-protein and it includes N-P/Q-type calcium channels (FASEB J 21: 671-681).

We developed a unique technology useful for the functional evaluation of neurogenesis from subventricular zone cell cultures (Rej. Res., In Press). This method allows us to perform pharmacological studies directed to cells differentiating from neural stem cells, and to screen for new proneurogenic factors.



Ongoing and future work

Currently we are investigating the impact of neuropeptide Y, inflammation and proinflammatory cytokines in neurogenesis in subventricular zone and hippocampal dentate gyrus cell cultures. The identification of new proneurogenic factors based in these important peptides will open new avenues for the search of new strategies to promote neurogenesis from grafted neural stem cells in the brain with neuronal damage.

The molecular signalling cascade between receptor activation, axonal outgrowth and neuronal differentiation is under investigation.

We are searching new strategies to promote and evaluate differentiation of new oligodendrocytes from subventricular zone cell cultures useful for new strategies of multiple sclerosis brain repair.

Key References

Pinheiro, P., Perrais, D., Coussen, F., Barhanin, J., Bettler, B., Mann, J.R., Malva, J.O., Heinemann, S.F. and Mulle, C. (2007) GluR7 is an essential subunit of presynaptic kainate autoreceptors at hippocampal mossy fiber synapses. *Proc. Natl. Acad. Sci. USA* 104: 12181-12186.

Agasse, F., Bernardino, L., Silva, B., Ferreira, R., Grade, S. and Malva J.O. (2008) Response to histamine allows the functional identification of neuronal progenitors, neurons, astrocytes and immature cells in subventricular zone cell cultures. *Rejuv. Res.* (*in press*).

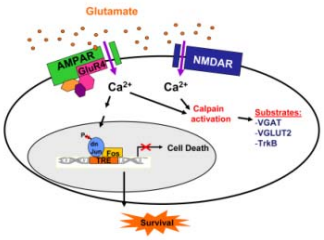
NEURONAL CELL DEATH AND NEUROPROTECTION (*Head: Carlos Duarte*)

Numerous disorders of the CNS are characterized by neuronal cell death, which may arise from the deregulation of the activity of neurotransmitter systems. In *brain ischemia* there is an excessive accumulation of the neurotransmitter glutamate, and the resulting overactivation of glutamate receptors causes neuronal death (excitotoxicity). The activity of glutamatergic synapses in the hippocampus is normally regulated by the neurotrophin BDNF (e.g. *J Biol Chem 282*: 12619-12628 [2007]), which is also an endogenous neuroprotectant, counteracting to some extent the effects of glutamate as a toxin. This group studies molecular mechanisms contributing to excitotoxic cell damage, particularly in the hippocampus, a brain region particularly vulnerable to glutamate toxicity, and neuroprotection by BDNF (brain-derived neurotrophic factor). Furthermore, this group investigates the mechanisms controlling the expression of neuroprotective factors upon neuronal injury, both in cell cultures and in a rat model of Parkinson's disease.

Research Highlights

In transient cerebral global ischemia, the selective death of hippocampal CA1 pyramidal neurons is mainly due to changes in the molecular composition of α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptors (AMPARs) leading to an enhancement of Ca^{2+} permeability. We found that excitotoxic cell death mediated by Ca^{2+}-permeable AMPARs requires the activation of the AP-1 transcription factor, which can be regulated by the c-Jun N-terminal kinase (JNK). The excessive activation of glutamate receptors in brain ischemia also cause a $[Ca^{2+}]_i$ overload and excessive activation of calpains. We found that the vesicular glutamate transporter, VGLUT2, and the vesicular GABA transporter, VGAT, are cleaved by calpain upon excitotoxic stimulation of cultured hippocampal neurons and in the striatum and cerebral cortex of mice subjected to transient middle cerebral artery occlusion (MCAO). Although VGLUT1 is also cleaved under excitotoxic conditions, the truncated VGAT is not targeted to the synapse, suggesting that the activity of the GABAergic synapses is impaired. The TrkB receptors for BDNF are also cleaved by calpain, giving rise to a stable product lacking most of the intracellular region of the protein, which may have a dominant negative effect, thereby precluding the neurotrophic effects of BDNF. In contrast to the effect on calpain, a toxic insult with glutamate decreases proteasome activity and leads to an accumulation of ubiquitinated proteins.

Giving the role played by protein synthesis in neuroprotection by BDNF, we carried out a large scale proteomics study aiming at characterizing the effect of the neurotrophin on the proteome of cultured hippocampal neurons. BDNF upregulates the protein synthesis machinery and some of the effects appear to be transcription-independent. Furthermore, BDNF changes the abundance of proteins of the ubiquitin-proteasome system (UPS) and displays rapid effects on the activity of the proteasome in hippocampal neurons. We have shown that, unlike in other systems, interleukin-1β (IL-1β) plays a neuroprotective role against dopaminergic injury in substantia nigra cell cultures. This cytokine is up-regulated and released upon selective injury to dopaminergic neurons, and mediates GDNF up-regulation by astrocytes. We found that both IL-1β and its receptor are expressed by neurons and astrocytes, suggesting autocrine and paracrine effects (Saavedra et al, Neurobiol Dis 25, 92-104, 2007).



Ongoing and Future Work

1) The molecular mechanisms linking Ca^{2+}-permeable AMPA receptors to the JNK cytotoxic pathway activation will be investigated in hippocampal neurons submitted to oxygen-glucose deprivation (OGD), which induces the targeting of Ca^{2+}-permeable AMPARs to the synapses at early times after the insult. We will identify molecular targets for a selective therapeutic approach aiming at disrupting the molecular links responsible for activation of a receptor-mediated toxic pathway while sparing the physiological activity of the receptors.

2) The VGLUT cleavage sites under excitotoxic conditions and the functional consequences of this cleavage will be investigated, focusing on the effects on the activity of the transporters and on their intracellular trafficking. The changes in the activity of the UPS under excitotoxic conditions will be further investigated, as well as the modulatory role played by BDNF.

3) In the 6-OHDA rat model of Parkinson's disease, we are looking at the expression of GDNF in the substantia nigra and the striatum, at different degrees of injury as assessed by tyrosine hydroxylase staining and the levels of dopamine and DOPAC. The glial response to injury is assessed by immunostainig for astrocyte and microglial markers. Our goal is to find whether damage to the nigrostriatal pathway induces GDNF expression in vivo, which cells express GDNF, and to identify the intercellular mediators involved in the protective neuron-glia crosstalk upon injury, both at the level of dopaminergic terminals in the striatum, and at the level of cell bodies in the substantia nigra. Cell cultures and nigrostriatal slices will be used to examine the relationship between activation/inhibition of adenosine receptors and GDNF expression and neuroprotective action, since antagonists of A2A receptors are emerging as promising therapeutic agents for Pakinson's disease.

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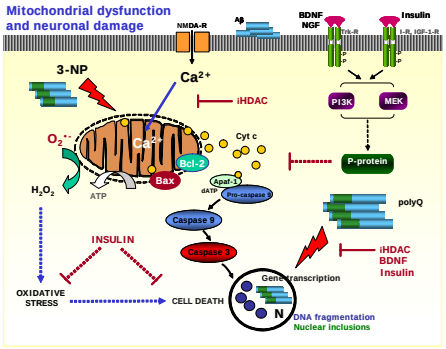
MITOCHONDRIAL DYSFUNCTION AND CELL DEATH *(Head: A. Cristina Rego)*

Selective neurodegeneration in irreversible disorders of the CNS has been largely attributed to mitochondrial impairment and protein misfolding. However, how modified or mutant proteins interfere with mitochondria is not clear. The main objective of our group is to determine defective intracellular mechanisms and identify molecular targets for therapeutic intervention underlying mitochondrial dysfunction and cell death in distinct neurodegenerative conditions, namely polyglutamine expansion (Huntington's (HD) and Machado-Joseph's (MJD) disorders), Parkinson's (PD) and Alzheimer's (AD) diseases, and in the neuropathology caused by drug addiction. We also aim to evaluate the efficacy of neuroprotective strategies (pharmacological and cell replacement therapies) that help recovering mitochondrial function and thus neuronal survival.

Research Highlights

During the past year our group studied: i) mitochondrial dysfunction in HD, MJD and in street heroin cytotoxicity; ii) neuroprotection induced by neurotrophins (in particular BDNF) in HD; iii) insulin neuroprotection in oxidative stress; and iv) genetically modified mouse neural stem cells (mNSC) for PD cell therapy. In collaboration with Prof. L Ellerby and Dr. B Schilling (Buck Institute for Age Research, CA, USA), we analysed the mitochondrial proteome of HD YAC128 transgenic mice. In addition, we investigated the effect of BDNF brain supplementation of YAC128 mice on the expression of mitochondrial proteins. Most alterations occurred in proteins belonging to the mitochondrial respiratory chain. Moreover, complex I activity was increased in the YAC128 mice and this alteration was prevented by BDNF. Also, we showed that complex II inhibition with 3-nitropropionic acid (3-NP) led to transcription dysregulation and mitochondrial-dependent neuronal death. BDNF prevented 3-NP-induced neurotoxicity, activated pro-survival signalling pathways and prevented transcriptional dysregulation. In collaboration with Profs. DG. Nicholls and L Ellerby (Buck Institute for Age Research), we compared the bioenergetic behavior of mitochondria isolated from different transgenic HD with *in-situ* respiratory parameters in intact HD striatal neurons, stressing the importance of assessing HD mitochondrial function in the cellular context (Oliveira et al, *J Neurochem*, 2007). In collaboration with Dr. C Januário (HUC) and Dr. S Cardoso (CNC) we examined the bioenergetic changes and mitochondrial-dependent apoptosis in HD and control cybrids. Glycolysis hyperstimulation occurred as a compensatory mechanism in response to bioenergetic dysfunction in HD *versus* control cybrids. HD cybrids showed increased susceptibility to 3-NP-induced apoptosis and oxidative stress. We also determined whether mitochondrial function was compromised in MJD. Our data suggest that mitochondria do not have a major role on neuronal death occurring in this polyglutamine disorder. Moreover, we studied mitochondrial dysfunction caused by street heroin (Cunha-Oliveira et al, *J Neurochem*, 2007). Street heroin-treated neurons showed cyt c release, decreased mitochondrial potential and Bcl-2/Bax. Early caspases activation was observed, culminating in PARP cleavage/DNA fragmentation. Nevertheless, street heroin cytotoxicity was independent of functional mitochondria.



By studying neuronal changes caused by oxidative stress and insulin neuroprotection, we observed that insulin stimulated PI-3K/Akt and inhibited GSK-3□ signaling pathways, modifying the expression of proteins involved in neuronal antioxidant defense, glucose metabolism and prevention against apoptosis (Duarte et al, *BBA-Mol Cell Res, in press*). In addition, we developed retroviral vectors (in collaboration with Dr. O Vieira, CNC) for the expression of genes involved in dopaminergic neuron development in mNSC: Nurr1 and glial cell line-derived neurotrophic factor (GDNF) for cell therapy in PD. Subventricular zone (SVZ) mNSC were successfully transduced with a GDNF-GFP retroviral vector.

Ongoing and future work

Currently we are examining oxidative stress in HD striatal cell lines. Taking into account that ~15% of HD patients develop diabetes and insulin neuroprotection, we will analyse insulin effects against alterations in glucose metabolism and cell viability in diabetic HD models. Furthermore, we will examine insulin antioxidant effect and its possible synergy with compounds previously tested in HD clinical trials. With the objective of clarifying ataxin-3 function in the cell and understand the pathways involved in mutant ataxin-3-derived neurodegeneration in MJD, we are investigating ataxin-3 activity and biology, especially focusing on ataxin-3 crosstalk with known interactors, in collaboration with Prof. Henry Paulson (Univ Michigan Med Sch, USA). We also aim to examine the link between mitochondrial dysfunction in PD, and the post-translational modifications of alpha-synuclein. We will further explore the contribution of NMDA receptor subtypes (NR2A/B) on mitochondrial function and the interplay with endoplasmic reticulum in the hippocampus of AD animal models. For future work, we will continue characterizing genetically modified mNSC regarding its use in PD and HD mouse models.

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MOLECULAR MECHANISMS OF DISEASE (*Head: Cláudia Pereira*)

The prion (PrPSc isoform), amyloid-β (Aβ) and α-synuclein (α-syn) peptides are crucial in the pathogenesis, respectively, of prion-related encephalopathies (PRE), Alzheimer disease (AD) and Parkinson disease (PD). Although the aberrant peptide accumulation is recognized as an important common feature in these neurodegenerative diseases, the mechanisms of pathogenesis remain an important subject of competing hypothesis and debate. Our general aims are: i) to investigate the primary molecular and cellular events induced by these disease-related peptides and their causal relationships; ii) to identify and test potential therapeutic agents that target the underlying disease mechanisms.

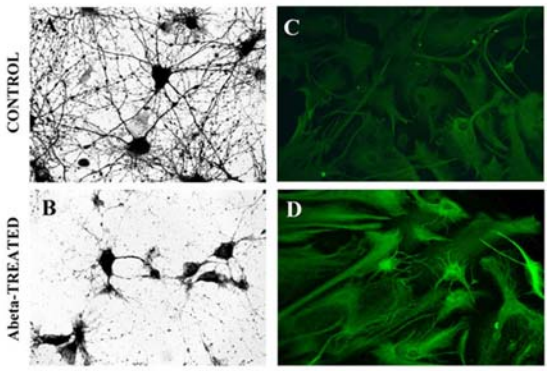
Research Highlights

One of the major interests was the investigation of endoplasmic reticulum (ER)/mitochondria cross-talk as a primary molecular mechanism leading to neuronal loss triggered by Aβ or PrP. In cortical neurons, we found that these peptides induce ER Ca^{2+} release activating the ER stress-mediated apoptotic pathway by a mitochondrial-dependent process. Ca^{2+} released from ER was shown to be involved in GSK3β-mediated tau phosphorylation and apoptosis induced by Aβ. Regulation of Ca^{2+} homeostasis was identified as one of the mechanisms by which Aβ-induced neuronal damage is rescued by statins that were predicted to interact with Aβ (collaboration with Structural and Computational Biology group). Neuronal Aβresponse was found to be changed due to the interaction with other proteins, namely with α-syn.

In neurons exposed to Aβ or PrP, levels of p25, a Cdk5 activator, are increased and lead to the formation of a p25/Cdk5 complex, which triggers tau hyperphosphorylation and apoptosis. Moreover, Cdk5 deregulation and redistribution to perinuclear regions underlie an abortive cell cycle reactivation. Data obtained in 3xTg-AD mice suggest that cell cycle reactivation in neurons is not a direct consequence of Aβ and tau pathologies. In this transgenic mouse model, oxidative stress was shown to be an early event occurring before the appearance of Aβ plaques and neurofibrillary tangles. Data obtained with human AD brain tissue co-substantiate the existence of prominent mitochondrial-related oxidative stress and showed that mitochondria are key targets of increased autophagic degradation in this neurodegenerative disease.

Another focus of research of our group was the role of neuroinflammation in AD and PRE. Using co-cultures of microglia/cortical neurons challenged with Aβ or PrP, we provided evidence that IL-6 released by activated microglia plays an active role in neuronal injury. Aβ was also shown to regulate the activity/levels of the glutamate transporters GLAST and GLT1 in cultured astrocytes, in a MAPKs-dependent manner, significantly decreasing glutamate clearance.

Mitochondria have also a major role in PD etiopathogenesis. Using an *in vitro* model of sporadic PD (cybrids that recapitulate mitochondrial deficits of PD patients) we proved that mitochondrial dysfunction induces aggregation of α-syn, one of the major constituents of Lewy bodies, increasing apoptotic features.



Abeta peptide induces neuronal dystrophy and astrocytes activation
A,B – Co2+ staining of cultured hippocampal neurons
C,D – anti-GFAP immunoreactivity (green) of cultured astrocytes

Ongoing and future work

Continuing our efforts to clarify the role of ER stress in Aβ-induced toxicity, we are exploring the role of different subunits of NMDARs in Aβ-induced ER stress (collaboration with Mitochondrial Dysfunction and Cell Death group). Alterations in the different subtypes of hippocampal nAChRs, induced by Aβ, are also under investigation (collaboration with Neuromodulation group). In a near future we will study whether glial nAChRs regulate the AD-associated neuroinflammatory process.

At the moment we are lauching a new project that concerns the role of endothelium dysfunction in AD. Our goal at this point is to look at the effect of Aβ on brain endothelial cells function and subsequent neuronal outcome, focusing on the involvement of mitochondria and angiogenesis growth factors signaling. Moreover, the impact of aging, hypoxia and diabetes in brain vascular endothelium will also be evaluated as (potential) risk factors for the development of AD.

Currently, using PD cellular models, we are addressing the role of mitochondria on microtubular alterations and proteasomal impairment. Moreover, the involvement of autophagic pathway in cell death occurring in PD is being studied.

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RETINAL DYSFUNCTION AND NEUROGENESIS (*Head: Claudia Cavadas*)

Age-related retinal diseases, such as diabetic retinopathy and age-related macular degeneration, are leading causes of blindness in developed countries. Their treatment has been hampered because the pathogenic processes are not clearly elucidated yet. Our main goal is to give insight into the molecular and cellular mechanisms underlying cell dysfunction in these pathologies. We are mainly focused on the role played by glutamate and glutamate receptors, ATP and by some inflammatory mediators, namely nitric oxide, interleukin 1-beta and TNF-alpha. We are also interested in identifying new molecular targets and developing new therapies, based on nanostructures that target VEGF and VEGF receptors, which are responsible for increased retinal vascular permeability and neovascularization.

Neuropeptide Y (NPY) is expressed in the retina, but its physiological role is unknown. We are investigating the potential physiological roles of NPY in the retina, as well as its potential neuroprotective properties, that might be useful to treat retinal degenerative disorders.

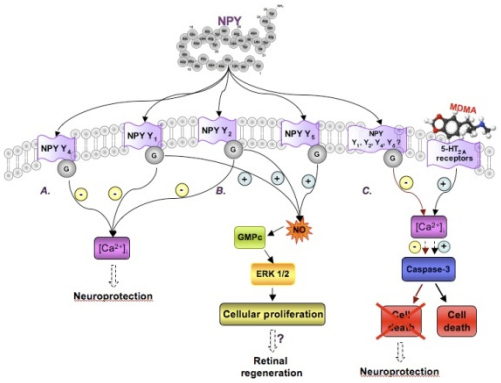
Drugs of abuse have harmful effects in the brain, but the effects of drugs of abuse in the retina are not elucidated, and thus we have been interested in understanding the impact of ecstasy in the retina.

Finally, we are also interested in the role played by calpains and nitric oxide in neurodegeneration, as well as the role of inflammation in neurogenesis, in models comprising injury/inflammation in CNS.

Research Highlights

We demonstrated that:

1. High glucose-induced apoptosis in retinal cells is independent of caspase activation and is mediated by the apoptosis-inducing factor (AIF); 2. Diabetes changes ionotropic glutamate receptor subunit expression in the human retina; 3. Inducible nitric oxide synthase isoform is a key mediator of leukostasis and blood-retinal barrier breakdown in diabetic retinopathy; 4. NPY is expressed by different cell types in the retina, including neurons, macroglial and microglial cells, and endothelial cells. Also, NPY exerts potent neuroprotective effects in retinal cells; 5. Activation of calpains is an early event in the neurodegeneration following *status epilepticus* in the rat hippocampus. Furthermore, in cultured hippocampal neurons, calpains are specifically activated by Ca^{2+} entering the cells via reversal of the sodium-calcium exchanger.



NPY effects on retinal neural cells

Ongoing and Future Work

1. Since AMPA receptors have a major role in retinal physiology, we are investigating the impact of hyperglycemia in the physiology of AMPA receptors, and the molecular mechanisms underlying changes in AMPA receptor subunits expression in retinal cells; 2. We will continue to investigate the mechanisms underlying the breakdown of blood-retinal barrier induced by inflammatory mediators, and the potential use of anti-inflammatory drugs to treat diabetic retinopathy; 3. We intend to elucidate the role played by ATP in the inflammatory events characteristic of diabetic retinopathy; 4. We are studying the impact of hyperglycemia on exocytosis in the retina, and in the hippocampus; 5. We will keep trying to develop new therapies, based on viral vectors and liposomes, to treat age-related macular degeneration; 6. We will proceed investigating the role of NPY in retinal physiology and in neurogenesis and cell differentiation; 7. We will evaluate the potential harmful effect of ecstasy in retinal physiology and morphology; 8. We are currently investigating the role of nitric oxide, under pathophysiological conditions, in the proliferation of neural stem cells. Its effect on cell differentiation will also be addressed.

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AREA B: MOLECULAR BIOTECHNOLOGY AND HEALTH

Coordinator - Euclides Pires

Introduction

In the last years Biological research has evolved from a reductionist paradigm of isolating and studying the structure and behavior of individual molecules, up to cluster interaction and back to the whole system studies, but now using mathematic and computational based approaches. The Molecular Biotechnology and Health area of CNC, whose programme covers basic and translational research themes, has accompanied that trend and comprises, at present, five research groups: 1) Molecular Biotechnology; 2) Structural and Computational Biology; 3) Molecular Systems Biology; 4) Vectors and Gene Therapy, and 5) Biomaterials and Stem Cell-Based Therapeutics.

Search for biotechnological or biomedical relevant proteins, in particular proteases has been a recurrent theme in this area of CNC. Substancial work has been carried out on the structure and function of plant aspartic proteases from Cynara cardunculus and Arabidopsis thaliana and serine proteases from allergenic pollens. Interest in Arabidopsis has risen recently owing to the fact that the majority of human genes, that were suspected to play a role in disease, were shown to have orthologs in Arabidopsis. Indeed, in what it concern oncogenes, the degree of similarity with human genes is 70%, thus in the range of models like Drosophila malanogaster and Caenorhabdites elegans, and far higher than in yeast ~ 41%. In addiction to plant models, animal models are also under the scope of this research programme and recently a system to develop a new model for Machado-Joseph disease was devised by CNC researchers.

Computational methodologies in combination with spectroscopic methodologies are being used by CNC researchers to study molecular basis of amyloid diseases, namely the characterization of molecular species involved in the initial stages of amyloid formation of transthyretin, aiming at the design of inhibitors. This experience is now being extended to model inhibitors of the amyloid formation by Aβ-peptide in Alzheimer's Disease. Computational methodologies, in particular data mining, were also successfully used, by CNC researchers in collaboration with Biocant Systems Biology unit, to unveal clusters of genes associated with some human diseases (patent submitted). A third theme where these methodologies are being extensively used is the exploitation of design principles of metabolic networks and the development of approaches for quantifying the tolerance of biological systems to potentially large perturbations.

Translational research in the frame of the Molecular Biotechnology and Health programme of CNC encompasses the design and development of carriers for drug and nucleic acid delivery, the development of biomaterials for stem cell differentiation and transplantation and the development of biomaterials with antibiotic properties. In the last years viral and non-viral carriers have been developed and recently a considerably effort has been made to increase efficiency and specificity. So, interaction with membranes, internalization routes and intracellular trafficking, as well as surface markers, in the case of tumor cells, have been throrougly investigated. In what it concern Biomaterials and Stem Cell-based therapeutics the goal is the identification of biomaterials capable of improving the differentiation of stem cells in a specific cell lineage, and eventually enhance their grafting after in vivo transplantation. Recently CNC researchers, in collaboration with MIT researchers isolated a vascular progenitor from embryonic cells and are at present actively working on the development of biomaterials to transplant these cells into animal model diseases.

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Ana Luísa Cardoso	(Ph.D. Student)
Maria Isabel Nascimento Ferreira	(Ph.D. Student)
Lígia Ferreira	(Ph.D. Student)
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Sandro Alves	(Ph.D. Student)
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Emerging Group

Biomaterials and Stem Cell-Based Therapeutics

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MOLECULAR BIOTECHNOLOGY (*Head: Carlos Faro*)

The Molecular Biotechnology group has a long-time research interest in biotechnology and/or biomedically relevant plant proteases such as aspartic proteases from *Arabidopsis thaliana* and serine proteases from allergenic pollens.

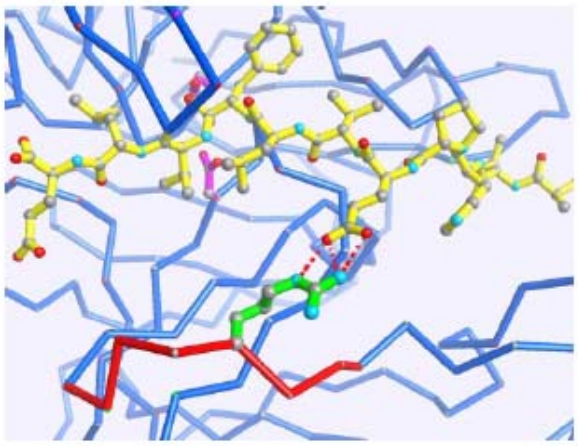
Research Highlights

The *Arabidopsis thaliana* Constitutive Disease Resistance 1 (CDR1) gene product is an aspartic proteinase that has been implicated in disease resistance signalling. Following optimization of a suitable heterologous expression system for production of recombinant CDR1, its enzymatic properties were studied in detail. The findings unveil a novel mechanism of regulation of proteolytic activity based on redox potencial, which emerged as a fundamental aspect in disease resistance signalling.

The Promoting Cell Survivor (PCS1) gene product is another aspartic protease from Arabidopsis whose expression and activity were associated with male sterility through an anti–cell death pathway. Over the past year, the recombinant form was produced in E.coli and its structural and enzymatic properties were also thoroughly characterised. Recombinant PCS displays rather unusual properties that make it unique among aspartic proteinases. However, the underlying mechanism involved in inhibition of plant developmental cell death still remains to be elucidated.

Pollen allergy has a remarkable clinical impact all over Europe. Over the past year we started the assessment and the characterization of the proteases present in pollens with distinct allergic potential: *Pinus sylvestris, Olea europaea*, *Dactylis glomerata* and *Cupressus sempervirens*. Moreover we evaluated the ability of the pollen proteases to damage airway epithelial cells and we started to establish a correlation between proteolytic and allergenic activity.

These results will increased our knowledge on the allergen delivery mechanism and will launch the basis for a new treatment approach of allergic disorders induced by pollens.



A C-alpha representation of CDR1 model is shown in blue with the two catalytically important aspartates marked pink

Ongoing and future work

Work currently in progress aims to further understand the structure-function relationship of plant aspartic proteinases, namely the identification of their natural substrates and inhibitors.

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MOLECULAR SYSTEMS BIOLOGY (*Head: Armindo Salvador*)

Our main interest is the discovery, explanation and exploitation of design principles in metabolic networks: *i. e.* of biologically widespread rules relating reaction/interaction structure of metabolic circuits to the function they perform. These principles emerge from the interplay between physical-chemical constraints, functional constraints (*i. e.* requirements for good performance) and evolutionary inherence (*i. e.* gradualness of biological innovations). Their discovery and understanding contributes for a much needed theoretical foundation for molecular biology and provides guidance for biochemical engineering.

Research Highlights

Pairs of consecutive reactions are the smallest and most prevalent non-trivial elementary circuits in metabolic networks, and therefore any principles that apply to their design and operation should be very relevant. We performed a statistical analysis of the modes of coupling of such pairs in the reconstructed metabolic networks of *Escherichia coli* and *Saccharomyces cerevisiae*. Our analysis showed that ~65% of the consecutive multi-substrate, multi-product reactions, comprising ~70% of all documented enzyme-catalyzed processes in these organisms form cycles. Most of these cycles occur at the interface between catabolism and anabolism, and play a role analogous to that of power-supply units in electronic circuits: they couple supply of molecular parts (moieties) to demand, ensuring that moieties are transferred to metabolic acceptors at a rate that is proportional to demand and insensitive to fluctuations in the outside supply.

A "central dogma" emerging in systems biology is that biological systems are selected for robustness to perturbations. However, the lack of effective approaches for quantifying the tolerance of biological systems to potentially large perturbations has hitherto hampered understanding of the molecular underpinnings of biological robustness.

We have addressed this problem by introducing a generic approach to identifying and characterizing the boundaries where the performance of a biological system deteriorates abruptly (Coelho *et al.*, submitted). This framework allows us to precisely define and quantify "global tolerance" as the minimum ratio between the normal value of a parameter and the value at such a boundary. The results from applying this approach to metabolic moiety-supply units show that selection for effective system design leads to large safety factors with respect to abrupt performance breakdown and yield insight regarding the design features that permit these large tolerances.

The level of a protein in a cell likely represents an optimal balance between the fitness advantages of providing the cell with more of the protein's function and the fitness cost of spending additional resources in maintaining higher levels of the protein.

Because growth and survival of *S. cerevisiae* are energy limited under many conditions and a very large fraction of the ATP generated in this yeast is spent in protein synthesis, the above mentioned fitness costs should strongly reflect the *energetic* cost of maintaining the level of the protein. Hence, protein levels should strongly reflect proteins' per-molecule cost rate (PMCR, ATP spent per molecule per time). Data for estimating PMCR in the yeast on a proteomic scale have recently become available. Our analysis of these data showed that: (a) there is indeed a very significant negative correlation between protein abundances and PMCR in cells growing in log-phase in rich media (Figure 1); (b) when cells shift to a nutrient-depleted environment and reach stationary phase, transcripts coding for costly proteins are more down-regulated than transcripts coding for less costly proteins. Altogether, these results point to energetic costs as an important consideration in interpreting gene expression profiles.

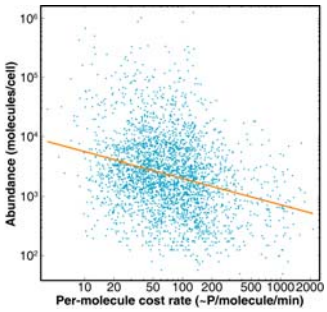

Fig. 1: Protein abundances (A) in yeast correlate negatively with per-molecule cost rates. Spearman rank correlation: -0.26 ($p<10^{-47}$). Red line, regression of Log(A) vs. Log(PMCR): $A \propto PMCR^{-0.44}$.

Ongoing and future work

We are currently investigating design principles that may apply to "moiety-supply units" as a general class of metabolic circuits and addressing the following questions. Do the "canonical" rules of design apply to concrete well-characterized biological realizations, or are many design features context-dependent? What rules apply where there are several equally important moiety-supplying processes and/or moiety-consuming processes? Are there functional reasons for some bi-bi enzyme mechanisms being selected over others in the context of these circuits? Is the evolution of large global tolerances a very frequent outcome in these circuits?

The intrinsic non-enzymatic reactivity of many metabolites is a potentially very important consideration in the design of biochemical networks. We are reconstructing and analyzing the network of reactive chemical species (RS) in representative physiological systems and profiling the covalent modifications of hemoglobin by RS. We seek to investigate: (a) what general strategies evolved to manage the trade-off between deleterious and useful actions of reactive species, (b) the extent to which different reactive species modify hemoglobin *in vivo*, (c) the potential use of profiles of covalent modifications of hemoglobin for the diagnosis of metabolic conditions.

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STRUCTURAL AND COMPUTATIONAL BIOLOGY (*Head: Rui M. M. Brito*)

Following up the reorganization of the structural biology area at the CNBC in 2006, with the creation of the Structural and Computational Biology Group, in 2007 two *Ciencia2007* PhD researchers joined the group: Elsa Henriques, as an integrated member of CNBC, and Rui Travasso, as a collaborator of the Centre. The group is now strategically focused on the use of experimental and computational methodologies to study the molecular basis of human and animal disorders, in particular amyloid diseases. Building on the previous experience on the use of spectroscopic and computational methodologies to study protein folding and stability, the group is now setting its goals on a wider approach to the study of human disorders, going from the detailed modelling of protein-protein and protein-ligand interactions using mixed molecular dynamics and quantum mechanics approaches, to virtual screening and rational drug design, and computer modelling of protein interaction networks.

Research Highlights

During 2007, the group concentrated its research efforts on the following topics: *i)* computational modelling of the initial stages of amyloid formation by Transthyretin (TTR), the protein responsible for the development of FAP (Familial Amyloid Polyneuropathy), a neurodegenerative disease of very poor outcome in most patients; *ii)* virtual screening and molecular modelling of inhibitors of amyloid formation by TTR and Aβ-peptide; *iii)* unfolding simulations of amyloidogenic and non-amyloidogenic proteins; *iv)* quantum mechanics simulations of light emission processes in bioluminescent organisms. Several research grants in these areas were awarded to members of the group in 2007. In order to increase the visibility of Computational Biology in general, and to show the work being developed at the University of Coimbra, the group also started a series of conferences titled "Computational Biology @ UC" given by invited scientists and members of the group, opened to the community in general and targeting in particular undergraduate and graduate students in Chemistry, Physics, Biology/Biochemistry and Computer Science.

Ongoing and future work

Combining the reach of experimental and computational methodologies, we are working on the characterization of the molecular species involved on the initial stages of amyloid formation by the protein Transthyretin.
Additionally, a significant effort is being made in the area of virtual screening and rational design of inhibitors of TTR amyloidosis. The experience gained with TTR is also now being used to model inhibitors of amyloid formation by the Aβ-peptide of Alzheimer´s, a project in collaboration with Doctor Claudia Pereira of CNBC.
Docking and Molecular Dynamics simulations in a massive parallel computer (*Milipeia*, UC) are being routinely used. Ongoing collaborations with computer scientists are allowing us to develop tools for data mining of large data sets produced in protein folding and unfolding computer simulations.
Additionally, using time-dependent density functional theory (TDDFT) we are currently studying the process of light emission in two systems present in bioluminescent organisms: *i)* the luciferin/luciferase present in fireflies and some beetles, and *ii)* mutants of the green fluorescent protein (GFP), namely the variant Y66W. These systems are of great importance, both from a fundamental and from a technological point of view.

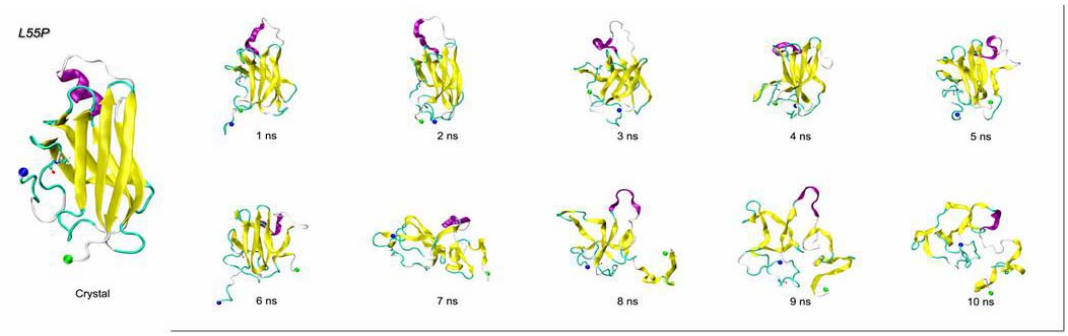

Fig. 1: Molecular Dynamics unfolding simulation of L55P-TTR, a highly amyloidogenic variant of Transthyretin.

Key References

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VECTORS AND GENE THERAPY (*Head: Mª Conceição Pedroso de Lima*)

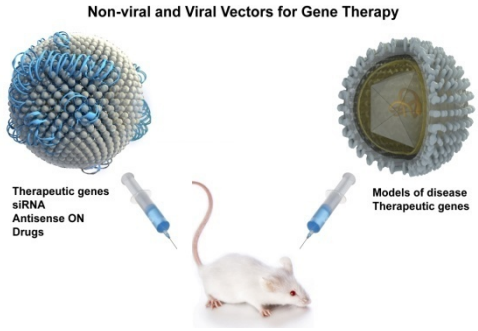
The CNC laboratory of vectors and gene therapy is devoted to the design and development of carriers, including viral and non-viral vectors, for nucleic acid and drug delivery aiming at their application in gene therapy and gene silencing approaches.

Research Highlights

Regarding the development of non-viral vectors our studies have been focused on the evaluation of the potential of novel nanosystems for the treatment of two major diseases: cancer and neurodegenerative disorders. Cancer has been the main target disease in which approaches dealing with delivery of both gene silencing agents (antisense oligonucleotides or siRNAs) and plasmids encoding therapeutic genes have been evaluated. Mechanistic studies on the interaction of the developed systems with target cells, including cell internalization and intracellular trafficking, have also been addressed aiming at their optimization for specific therapeutic applications. We were able to select siRNA sequences that, in vitro, downregulated the expression of Bcl-2 in small cell lung cancer cells but neither reduced their viability nor increased their sensitivity to cisplatin. Such results emphasize that target validation plays a key role in the success of any molecular targeted strategy. Several cancer animal models have been generated, for exploring the potential of the generated nanosystems. Important findings were achieved regarding the therapeutic effects observed upon application of protein-associated lipoplexes in suicide gene therapy and immuno gene therapy approaches in murine models (both for oral squamous cell carcinoma and mammary adenocarcinoma). Moreover, we have shown that vinblastine, even at low concentrations, significantly enhances cationic liposome-mediated transgene expression, showing the potential of combining gene therapy and chemotherapy in cancer treatment.

Our studies on the potential of the developed nanosystems in gene silencing approaches targeting neurodegenerative disorders and excitotoxic brain injury demonstrated that protein-associated lipoplexes can be successfully applied to mediate downregulation of both reporter and therapeutic genes in neuronal cell lines and primary neuronal cultures, as well as in animal models, such as Nf-kB reporter mice.

Moreover, lentiviral vectors-mediated expression of polyglutamine-expanded ataxin-3 in the rat brain was shown to induce motor and neuropathological abnormalities which replicate Machado-Joseph disease/ Spinocerebellar ataxia type 3, demonstrating that this strategy can be employed to produce a new genetic animal model of this disorder. Using this model, we applied lentiviral vectors to achieve efficient and allele-specific silencing of mutant ataxin-3 in the rat brain, which prevented neuropathological changes associated with Machado/Joseph disease.



Ongoing and future work

Our current and future work aims at further improving the different nucleic acid delivery systems, namely by enhancing their efficiency and targeting specificity. To this end, mechanistic studies on the process of nucleic acid delivery mediated by the developed lipid based-nanosystems, including their interaction with the cell surface, route of internalization and intracellular trafficking, are being addressed. Regarding cancer, significant efforts are currently devoted to promote specific delivery to different cells or tissues through functionalization of the developed systems with targeting agents, which selection will be made based on the tumor cell surface marker specificity. We aim at developing a novel antitumoral strategy, based on the combination of gene therapy and chemotherapy, exhibiting a synergistic therapeutic activity without causing significant side effects. Regarding our studies on neuronal disorders, we are currently focusing our research on the application of protein-associated lipoplexes to mediate downregulation of specific pro-apoptotic targets, aiming at evaluating their potential as research tools for elucidating death and survival pathways and as new therapeutic approaches to modulate neuronal damage.

Concerning Machado-Joseph disease two new lines of research are presently being pursued: The role of autophagy in the clearance of mutant ataxin-3 inclusions and gene transfer approaches for activating this protective mechanism; Involvement of calpains in ataxin-3 cleavage and generation of toxic fragments. Finally, AAV vectors are presently being used as a new strategy to study central and peripheral mechanisms involved in appetite and obesity (collaboration with Cláudia Cavadas).

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Emerging Group

BIOMATERIALS AND STEM CELL-BASED THERAPEUTICS (*Head: Lino Ferreira*)

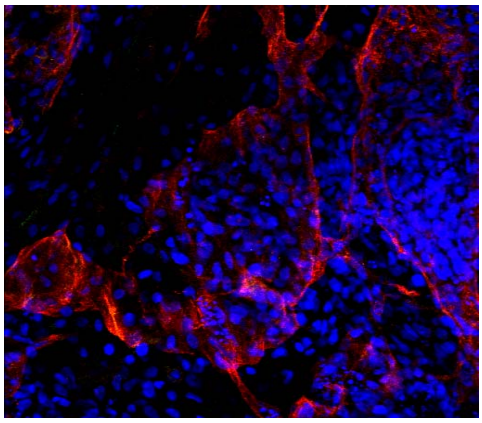
The group of biomaterials and stem cell-based therapeutics has two major avenues of research: i) to develop new biomaterials for stem cell differentiation and transplantation, and ii) to develop biomaterials with antimicrobial properties.

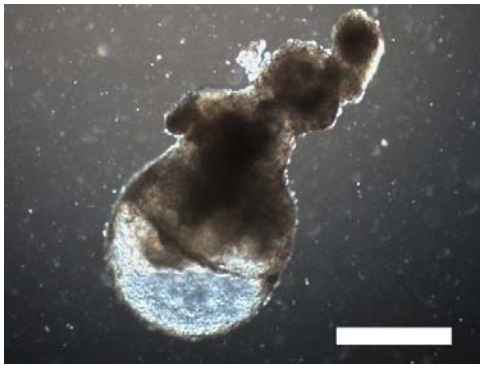
Research highlights

We are designing biomaterials which provide different types of information to stem cells, with the purpose of controlling their differentiation and enhancing their grafting after *in vivo* transplantation. In this context we are developing or modifying natural or synthetic polymers and to characterize their physico-chemical and biological properties. One of the major interests in our group is to identify biomaterials that will improve the differentiation of stem cells in a specific cell lineage and to obtain fundamental knowledge regarding the effect of chemistry, mechanics and three-dimensional organization of the scaffold in terms of stem cell differentiation. Recently we developed a synthetic stem cell niche for the vascular differentiation of human embryonic stem cells (hESCs) (1).

Three-dimensional hydrogels incorporating biomolecules (vascular endothelial growth factor and the cell adhesion epitope RGD) in their backbone increased the fraction of cells expressing VEGF receptor KDR/Flk-1, a vascular marker, up to 20-fold, as compared to spontaneously differentiated embryoid bodies. In another study, we reported that hyaluronic gel matrix could i) support long term self-renewal of hESCs in the presence of conditioned medium from mouse embryonic fibroblast feeder layer, and ii) direct cell differentiation in the presence of differentiation medium (Gerecht-Nir *et al.*, *PNAS* 2007, 104(27), 11298-11303).

Another focus of our group is the design of biomaterials with antimicrobial properties. A major problem associated with the implantation of biomedical devices in the human body is the inherent risk of microbial infections. We are developing effective strategies to control antimicrobial infections by developing coating technologies to immobilize antimicrobial agents. Recently, we reported an antifungal material, formed by a hydrogel and an antifungal agent-amphotericin B- that was able to kill fungi within 2 h of contact and could be reused for at least 53 days without losing its effectiveness against *Candida albicans* (2).





Human embryoid bodies containing vascular progenitor cells

Ongoing and future work

Recently, we isolated vascular progenitor cells from hESCs that might be an important source of vascular cells for the vascularization of tissues (3). At the present, we are developing biomaterials to transplant these cells in animal models of diseases. In parallel, we are developing durable antimicrobial coatings to modify material surfaces in a uniform fashion.

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AREA C: CELL AND MOLECULAR TOXICOLOGY

Coordinator – Leonor Almeida

Introduction

This area is mainly concerned to the study of cellular and molecular basis of disease as well as drug-induced cell toxicity for the purpose of translating this knowledge into disease treatment and prevention.

The area encompasses four research groups:

The *Mitochondrial Toxicology and Pharmacology Group,* particularly focused on the role of mitochondria as a primary intracellular target in initiation of drug- and disease-related cell dysfunction and on its potential usefulness as a target in drug therapeutics, in particular, anti-cancer therapy. Tamoxifen and its metabolite hydroxytamoxifen, doxorubicin and newly synthesized phenolic compounds, have been the main drugs under study by using *in vitro* and *in vivo* approaches. On the other hand, the relevance of mitochondrial dysfunction to the pathogenesis of some diseases, such as cholestasis, steatosis and metabolic syndrome in experimental rat models has been also demonstrated and underlying mechanisms have been indicated.

The *Free Radicals and Antioxidants Group,* centred on free radicals and oxidants, either as messenger molecules relevant to physiological functions, or as promoters of toxic pathways in the pathogenesis of chronic degenerative processes. This group has been particularly active in exploring the concentration dynamics of nitric oxide in rat hippocampal slices and, more recently, *in vivo*, in anesthetized rat brain, in the context of molecular mechanisms inherent to neuromodulation and aging. Also, it has been involved in the molecular mechanisms underlying the health-promoting role of dietary polyphenol antioxidants, in particular those present in red wine, mainly in connection with the protection against vascular endothelial dysfunction and the non-enzymatic production of nitric oxide in the gastric compartment.

The *Membrane Toxicity Group* has been focused on the study of membrane changes (biophysical approach) upon the interaction with xenobiotic molecules, in particular, environmental pollutants, such as insecticides and herbicides, by using bacterial and membrane models, aimed to elucidate the molecular mechanisms underlying their toxicological effects upon biological systems.

The *Pharmacometrics Group*, a novel research group that brings into CNC the expertise on developing and applying mathematical and statistical methods to collected experimental data, mainly in pre-clinical and clinical trials, in order to a better understanding and prediction of a drugs's pharmacokinetic and pharmacodynamic behaviour, which is of crucial importance for optimizing drug efficacy and minimizing its toxicity.

The specific research highlights and ongoing and future work are indicated in the group's individual reports.

Worthy of notice is the prize won in 2007 by a young researcher (Anabela Rolo), "Prize Medal L'Oréal Portugal for Women in Science", awarded by L'Oréal Portugal/Unesco/FCT, which together with others won by other researchers before, point that the quality and originality of the research carried out in this Area have been recognized by the scientific community.

Within the scientific activities of this area, 2 Ph.D. and 5 MSc thesis were concluded, and more 22 Ph.D. and 5 MSc thesis are ongoing. The interaction among the groups has been reinforced and interfaces established with other areas within CNC, namely Neuroscience and Disease and Cell and Development Biology, as indicated by joint publications and supervision of PhD and Master Thesis. Moreover, the groups have promoted fruitful collaborations with external national and foreign researchers, as evidenced mainly by co-authorship of published papers, jointed funded research projects and organization of scientific events.

Also of note, in 2007 this Area carried out the 3d edition of the "International Courses on Toxicology at the CNC", organized on a yearly basis, entitled "Stem Cells as a Tool in Toxicology", which had the participation of highly recognized scientists (see Graduate Studies Programme).

Mitochondrial Toxicology and Pharmacology Group

Paulo J. Oliveira	(Ph.D. – *Head of group*)
José Custódio	(Ph.D.)
Maria S. Santos	(Ph.D.)
Anabela P. Rolo	(Ph.D.)
Carlos M. Palmeira	(Ph.D.)
Ana Burgeiro	(Ph.D. Student)
Vilma A. Oliveira	(Ph.D. Student)
Filomena Silva	(Ph.D. Student)
Marco Alves	(Ph.D. Student)
Teresa Serafim	(Ph.D. Student)
João Teodoro	(Ph.D. Student)
Sandro Pereira	(Ph.D. Student)
Filipe Duarte	(Ph.D. Student)
Sónia Correia	(Ph.D. Student)
Gonçalo Pereira	(Ph.D. Student)
João Monteiro	(Ph.D. Student)
Anabela Simões	(M.Sc Student)
Ana Filipa Branco	(M.Sc Student)
Cristina Carvalho	(M.Sc Student)
Ricardo Marques	(M.Sc Student)
Susana Pereira	(Laboratory Technician)
Cláudia Pereira	(Undergraduate Student)
Cátia Diogo	(Undergraduate Student)
Nuno Machado	(Undergraduate Student)
Filipa Carvalho	(Undergraduate Student)
Ana Gomes	(Undergraduate Student)
Ana Varela	(Undergraduate Student)
Renato Xavier Santos	(Undergraduate Student)
Susana Cardoso	(Undergraduate Student)
Sandrina Silva	(Undergraduate Student)
Pedro Marques	(Undergraduate Student)
Tiago Capote	(Undergraduate Student)

Free Radicals and Antioxidants Group

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Leonor Almeida	(Ph.D.)
Teresa Dinis	(Ph.D.)
Rui Barbosa	(Ph.D.)
João Frade	(Ph.D. Student)
Carla Nunes	(Ph.D. Student)
Ana Ledo	(Ph.D. Student)
Paula Brito	(Ph.D. Student)
Bruno Gago	(Ph.D. Student)
Cátia Marques	(Ph.D. Student)
Ricardo Santos	(Ph.D. Student)
Joana Paixão	(Ph.D. Student)
Núria Simões	(Ph.D. Student)
Bárbara Rocha	(Ph.D. Student)

Membrane Toxicity Group

Mª Amália Jurado	(Ph.D. – *Head of group*)
Manuel J. Matos	
João Pedro P. Monteiro	(Ph.D. Student)
Mª de Fátima V. Lopes Pinto	(Ph.D. Student)
Sandra Marina A. Santos	(Ph.D. Student)
João Demétrio Martins	(M.Sc Student)
Ana Cardoso	(M.Sc Student)

Pharmacometrics Group

Amílcar Falcão	(Ph.D. – *Head of group*)
Marília Rocha	(Ph.D.)
Anabela Almeida	(Ph.D. Student)
António Sales Mano	(Ph.D. Student)
Gilberto Alves	(Ph.D. Student)
Ana Fortuna	(Ph.D. Student)
Bruno Lopes	(Ph.D. Student)

MITOCHONDRIAL TOXICOLOGY AND PHARMACOLOGY (*Head: Paulo Oliveira*)

Mitochondria play a pivotal role in cellular metabolism and in energy production. Research over the years has demonstrated that mitochondria, besides being the cell energy suppliers, have also important roles on cell calcium homeostasis and cell death phenomena.

The main and general objective of the group is to provide an insight into the role of mitochondria as a primary intracellular target in the initiation of drug- and disease-induced cell dysfunction and also investigate whether mitochondria can be a useful target in pharmacological anti-cancer therapy.

Research Highlights:

1) Cholestasis, steatosis and hepatic ischemia: bile acids therapy. Cellular mechanisms with relevance to mitochondrial dysfunction: Our present understanding of the pathogenesis of cholestasis and fatty liver disease (FLD) is based on studies performed with experimental animal models. Both pathologies result in more susceptible organs to hepatic ischemia/ reperfusion injury and in severe mitochondrial damage. We have performed studies showing mitochondrial alterations present in the livers of animal models and the toxicity/protection of bile acids at the hepatocytes level.

2) Mitochondria as Targets for Anti-Cancer Molecules: The active role of mitochondria in cell death pathways makes this organelle a very attractive target for anti-cancer therapy. Phytochemicals such as the alkaloids berberine and sanguinarine have yielded promising compounds as molecules capable of inducing cytotoxicity on tumor cell lines through a mitochondrial mechanism. Phenolic acid derivatives and polyamines complexed with platinum or palladium are also being tested with very promising results in triggering tumor cell death. One seminal study regards the *in vivo* mitochondrial toxicity of estradiol when associated with the anti-neoplastic agents tamoxifen and hydroxitamoxifen.

3) Mechanisms of Doxorubicin-induced Cardiotoxicity: We are also investigating the still unidentified mitochondrial mechanisms by which doxorubicin (DOX), a potent anti-neoplastic agent, is toxic to the heart. In studies conducted in H9c2 myoblasts, we correlated DOX toxicity with activation of the p53-Bax pathway, resulting in mitochondrial dysfunction. We also identified new morphological end-points for DOX-induced toxicity including the degradation of the nuclear cytoskeleton.

4) Hyperglycaemia-induced Mitochondrial Dysfunction*:* The group has demonstrated that hyperglycaemia regulates mtDNA copy number in response to hyperglycemia-induced ROS production. We concluded that the decrease of mtDNA content and inhibition of mitochondrial function may be pathogenic hallmarks in the altered metabolic status associated with diabetes. In a different context, the toxicity of metformin on liver mitochondria was also studied on isolated mitochondrial fractions.

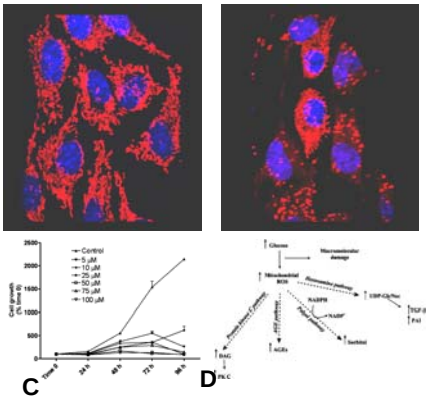

Fig. A) Control and B) Berberine-treated mouse melanoma K1735-M2 cells. Berberine causes mitochondrial fragmentation and depolarization, ultimately inducing inhibition of cell proliferation (C).
D) Hyperglycaemia-induced mitochondrial-mediated oxidative stress.

Ongoing and future work

We are currently studying mitochondrial mechanisms involved in the cytoprotection afforded by cardioplegic solutions against cardiac damage by ischemia and reperfusion. We intend to identify the components involved in mitochondrial protection, as well as to design alterations that ameliorate the protective effect. Besides, by using the myoblast cell line H9c2, we are investigating if the degree of cell differentiation (myoblast/ adult muscle cell) affects the response to cardiotoxic drugs, including isoproterenol and DOX. Tumour and non-tumour cell lines as well as isolated mitochondrial fractions are also being used to pinpoint mitochondrial targets of possible anti-cancer molecules and to help design new derivatives through quantitative structure-activity relationships. We are in the process of identifying promising and novel natural and synthetic anti-cancer agents against a variety of tumour cell lines, with relevance for melanoma and breast cancer-derived cell lines. Also of interest, we are interested in identifying key signalling pathways involving mitochondria which are altered in melanoma. We are also currently treating animal models with DOX, tamoxifen and hydrotamoxifen in order to investigate organ-specific mitochondrial alterations caused by the molecules. In the context of hyperglycaemia-induced mitochondrial dysfunction, we are exploring the crosstalk between mitochondrial reactive oxygen species and the transcriptional factor hypoxia factor-1 in brain and retinal endothelium. Lung mitochondrial toxicity of environmental pollutants with regional relevance and the hepatic mitochondrial toxicity of clinical relevant molecules (*viz. p*-cimene, acitretin or 13-*cis*-retinoid acid) are two other hot and developing topics of research in the group. Mitochondrial targeting of anti-cancer molecules or the mechanisms of mitochondrial damage during the course of hepatic diseases are also under continuous research.

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FREE RADICALS AND ANTIOXIDANTS (*Head: João Laranjinha*)

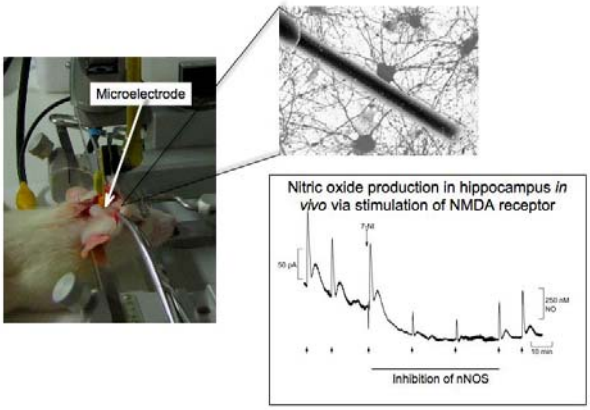
Reactive oxygen and nitrogen species play a pivotal in the regulation of critical cellular functions but extensive oxidative damage to biomolecules (oxidative stress) can lead to cell death by a variety of different mechanisms, either by turning off vital processes or by upregulating toxic cascades. Long term objectives of this group involve: 1) the study of molecular mechanisms inherent to neuromodulation, and aging that critically involve free radicals and oxidants, particularly nitric oxide (•NO); 2) To establish molecular mechanisms underlying the health-promoting role of plant-derived dietary phenolic compounds, particularly those present in wine, in connection with the protection against vascular endothelial dysfunction and the non-enzymatic production of nitric oxide from nitrite in the gastric compartment.

Research Highlights

It was shown for the first time the concentration dynamics of nitric oxide, •NO, (rate and pattern of change) in the extracellular space of hippocampus upon stimulation of *N*-methyl-D-aspartate (NMDA)-subtype glutamate receptors *in vivo*, in the anesthetized rat brain. This was achieved by using a microelectrode/micropipette array. The diffusion and the kinetics of •NO disappearance *in vivo* following pressure ejection of a •NO solution were experimentally substantiated and the half-life of •NO calculated.

Mechanistic studies of polyphenolic compounds as nitrite reductants in the stomach revealed a previously unrecognized pathway supporting potential beneficial effects of red wine on nitric oxide metabolism and endothelial function and in general on human health.

The activity of wine polyphenols as modulators of vascular signalling pathways was developed in connection with their use as potential anti-atherogenic agents. In particular, resveratrol was shown 1) to inhibit peroxynitrite-triggered endothelial cell apoptosis, by disrupting the mitochondrial pathway through modulation of Bcl-2 intracellular levels and 2) to prevent the vascular smooth muscle cells proliferation, promoted by oxidized LDL, by disrupting the mTOR signaling pathway, pointing to a new potential pharmacologic target in atherogenesis.



Ongoing and future work

To measure the patterns of •NO change in rat brain *in vivo* in connection with brain activities in which •NO plays a significant role.

To study the non-enzymatic production of •NO in the gastric compartment derived from dietary nitrate/nitrite and its pathophysiological impact.

To elucidate the cardioprotective mechanisms of phenolic compounds from red wines beyond their antioxidant properties, particularly those related with anti-inflammatory properties and protection of vascular dysfunction, by using cell models and apolipoprotein-E deficient mice submitted to a high cholesterol diet.

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MEMBRANE TOXICITY *(Head: Mª Amália Jurado)*

The main purpose of our research is to find out more about the particular role played by lipids and the lipid-bilayer component of cell membranes in cell functioning, in health and disease conditions, that is a lipidomics approach.

The research within the Membrane Toxicity Group focuses on developing strategies to approach two basic questions: a) Why are there so many different lipids into biological membranes? b) How membrane mediated cell functioning may be regulated (or impaired), both in space and time, by lipid membrane composition, structure and dynamics?

To investigate these central problems in lipid membrane research, two strategies have been and will be developed: a) to qualitatively and quantitatively analyse membrane lipid composition changes induced by physical and chemical agents, using bacterial cells as models, or promoted by diet fat manipulation and drugs administration in rats; b) to identify alterations of the physical properties of the lipid bilayer related with cell malfunctioning and disease.

With its focus on lipids and lipid biological relevance, the area of research covers lipid bilayers, biological membranes, bacterial cells/protoplasts and eukaryotic cells or subcellular fractions (mitochondria), as well as interactions of these systems with DNA, sterols, surfactants, drugs, environmental pollutants and nanomaterials. The experimental methodologies involve techniques of lipid analysis, thermodynamic measurements (calorimetry) and fluorescence techniques. The group also counts on the physical chemistry expertise from other groups of the Centre or from outside the Centre via collaborative efforts. On the other hand, the group, having adequate experience and background in lipid biochemistry and biophysics and membrane modelling, co-operates with other groups carrying out protein-centred research on membrane mediated cellular processes, contributing with an experimental approach in a lipid context.

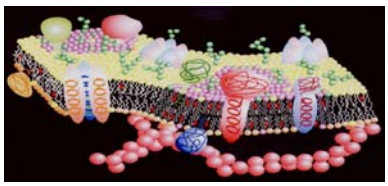
Research highlights

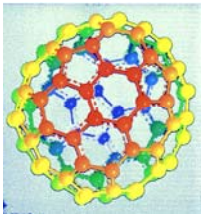
From previous work, we emphasise some conclusive aspects: a) Bacterial models have been shown as a suitable research approach to assess pesticides or drugs unspecific membrane mediated cytotoxic effects; b) A toxic action targeted on the structural order and organisation of membrane lipids has been identified as a common strategy for a variety of pesticides and environmental pollutants to induce adverse effects on biological systems; c) Lipid composition changes induced by physical or chemical stress in bacteria show that rather than fluidity, other, not so easily measured, membrane physical properties directly accounts for cell function impairment.

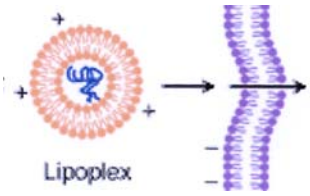
Ongoing and future work

The group, in collaboration with other research groups, is currently engaged with three new projects to be developed by Ph.D students: 1. A systematic study to assess the potential risks of the exposure to carbon nanomaterials, for human health and the environment. 2. Biophysical characterisation of DNA interactions with lipid membranes. 3. A lipidomics approach to mitochondria functioning with emphasis on the role of cardiolipin.

Studies centred on membranes







The membrane as a mediator for nanomaterials cell delivery

The biophysical principles which govern efficient liposome mediated transfection.

A lipidomics approach to the molecular mechanisms of mitochondrial toxicity.

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PHARMACOMETRICS (*Head: Amílcar Falcão*)

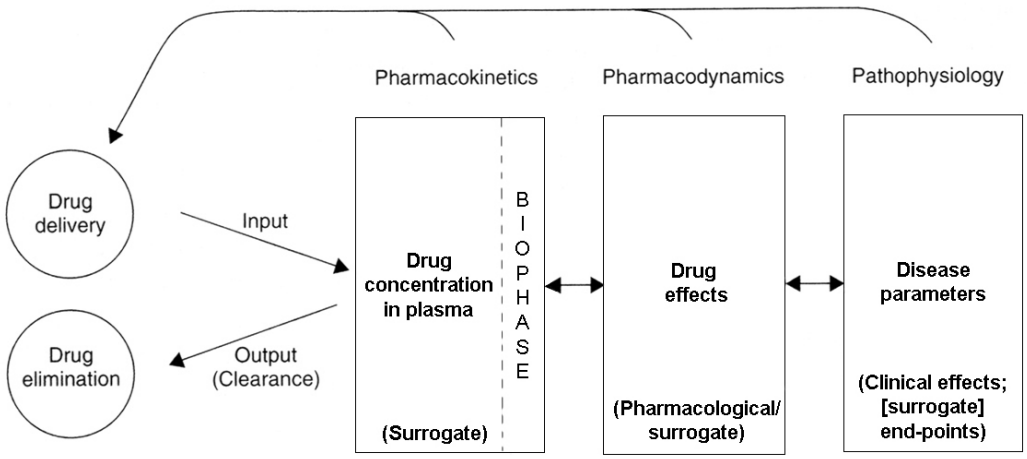
Pharmacometrics is the science of developing and applying mathematical and statistical methods to characterize, understand, and predict a drugs's pharmacokinetic, pharmacodynamic, and biomarker-outcomes behaviour (see scheme). In fact, pharmacometrics is the science of interpreting and describing pharmacology in a quantitative fashion.

Research Highlights

We explored methods to predict early in the drug development the ADME (Absorption, Distribution, Metabolism and Excretion) as well as drug-drug interactions of new chemical entities (NCEs). In the recent years drug development strategies undergoes several changes due to different reasons. Although we can easily identify some of these reasons (e.g. ethical, economic and legal reasons), the main point is that the pharmaceutical industry challenge concerning drug development is to do it better, faster and cheaper (keeping or even improving the quality of the final result). Therefore, *in silico* techniques becomes more and more popular and a lot of efforts are being done to optimise.

Ongoing and future work

Model-based drug development is characterised by the development and application of pharmacostatistical models of drug efficacy and safety from non-clinical and clinical data to improve drug development knowledge management and decision making.



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AREA D: MICROBIOLOGY

Coordinator – Milton Costa

Introduction

Microbiology research at CNBC has been devoted to microbial diversity and ecology as well as to the metabolic versatility found in extreme environments like deep-sea brines, boiling hydrothermal vents, alkaline groundwater, salt evaporation ponds and deserts. Classical microbiology and culture independent molecular methods allowed the description of several new microbial taxa from species to phylum as exemplified by *Haloplasma contractile*, a unique microorganism from the deeps of the Red Sea, and the phylogenetic characterization of a nonsaline alkaline groundwater environment (pH 11.4), apparently dominated by organisms that likely oxidize molecular hydrogen H_2 for primary productivity.

We have also probed one of the microbial strategies used in adaptation to thermal and osmotic stresses as well as the genetic grounds for such characteristics. Accordingly, we have identified the genes for synthesis of the compatible solutes mannosylglycerate, glucosylglycerate and di-myo-inositol-phosphate found in extremophiles and often designated chemical chaperones. Our contribution to the knowledge of compatible solutes biosynthesis has allowed a deeper understanding of the hyper/thermophilic and psychrophilic lifestyles.

A new line of research emerged from compatible solutes biosynthetic pathways, as we have characterized functionally related genes involved in the synthesis of unique polysaccharides strictly found in mycobacteria, including the human pathogens *M. leprae* and *M. tuberculosis*. The structural characterization of two of such highly conserved genes has also been accomplished considering that they might represent attractive targets for the development of new drugs, particularly urgent on the fight against neglected diseases like tuberculosis.

Another line of research deals with the capacity of yeast to sense and respond to bacterial LPS. Particularly in situations of mixed infections where yeasts are both exposed to live and lysed bacteria (due to macrophage attack), such a LPS-induced/sensing mechanism would be an advantage for the survival of yeast, and can be viewed as an evolutionary aspect of the mammalian inflammatory response. The understanding of the molecular responses of yeasts and molds to other organisms or to antifungals is also of medical relevance.

Microbiology of Extreme Environments Group

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Nuno Miguel Silva Empadinhas	(Ph.D.)
Ana Paula Kuan Yon Chung	(Ph.D.)
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Susana Isabel Elias Alarico	(Ph.D. Student)
Igor Clemente Tiago	(Ph.D. Student)
Chantal Ana Vicência Fernandes	(Ph.D. student)
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Sofia Cunha	(M.Sc Student)
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Medical Mycology – Yeast Research Group

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Cristiana Paulo	(M.Sc Student)
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Filipa Curado	(Undergraduate Student)

MICROBIOLOGY OF EXTREME ENVIRONEMENTS (*Head: Milton S. da Costa*)

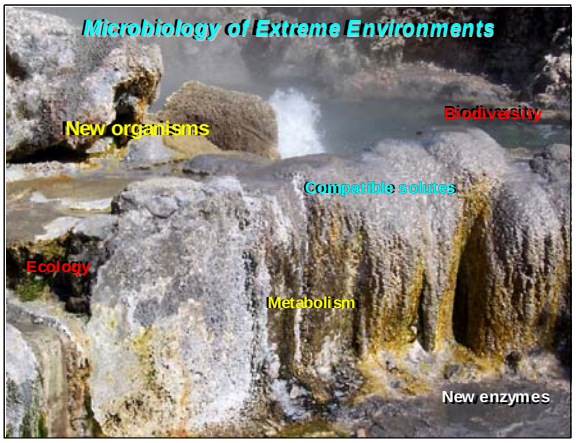
The discovery of microbial life in extreme environments has restructured all fields in Biology. Biodiversity and metabolic versatility continue to expand as new species are isolated from environments ranging from deep-sea brines to boiling hydrothermal vents and alkaline springs or from deserts to perpetually frozen habitats. The discovery of new microorganisms and their impact in their biotopes as well as the understanding of the strategies used to endure and adapt to extreme conditions are the general goals of our research. In particular, we pursue the answers to how microorganisms cope with osmotic and temperature stresses, and the genetic grounds for their adaptive success.

Research Highlights

Several new microbial species were isolated from a variety of environments with extreme conditions like deserts, thermal springs, deep-sea brines and alkaline environments. Many protect themselves from stress by accumulating compatible solutes, which are believed to act as chemical chaperones. Mannosylglycerate (MG), a compatible solute detected in many hyper/thermophilic bacteria and archaea was considered an archetypal compatible solute of these organisms. We identified the genes that govern MG synthesis and elucidated two distinctive biosynthetic pathways.

We identified the key-genes and pathways for the related solute glucosylglycerate (GG), initially detected in a few microorganisms in minute amounts. The increasing number of genomes available show that the GG genes are more prevalent than suspected, found in organisms from very cold to extremely hot environments, and that GG biosynthesis is likely to be fine-tuned by combined nutritional and abiotic stresses. Furthermore, GG is a versatile molecule found in unique methylglucose lipopolysaccharides (MGLP) from mycobacteria, including those causing leprosy and tuberculosis. Curiously, the key-gene for MG synthesis (*mpgS*) identified in the radiation-resistant bacterium *Rubrobacter xylanophilus*, represents a new class of glycosyltransferases and is homologous to the mycobacterial gene (*gpgS*) for the synthesis of GG found in the MGLP. We functionally characterized *gpgS*, which in *Mycobacterium tuberculosis* has been considered essential, hence a suitable target for new drugs against tuberculosis.

We studied the phylogenetic diversity in a nonsaline alkaline groundwater. This groundwater, generated by serpentinization process, has a high alkalinity (pH 11.4) with an extremely low ionic concentration. Results of extensive molecular analyses show that most microbial biomass, as reflected by rRNA gene abundance, is comprised of organisms of the kinds that derive energy for primary productivity from the oxidation of molecular hydrogen, H_2.



Ongoing and future work

Structural characterization of the mycobacterial GpgS: This project will be essential for design/development of specific enzyme inhibitors, which can be used as anti-mycobacterial drugs.

Identification of genes for MGLP synthesis: These MGLP lipopolysaccharides are crucial for the assembly of the mycobacterial cell wall. In addition to *gpgS*, five other genes are likely involved in their synthesis. It is important to elucidate each step to gather information to fight mycobacterial diseases. Specific *M. smegmatis* mutants will be constructed to evaluate the importance of these genes in mycobacterial physiology.

Biochemical and structural characterization of the ancient MpgS/GpgS from Rubrobacter xylanophilus: The catalytic properties and the structure of this unique bifunctional enzyme will shed additional light on the evolution of MG and GG biosynthesis.

Determination of genetic functional diversity of an alkaline groundwater: by screening genomic libraries composed by conserved protein coding genes involved in central metabolic processes, which may be used as functional markers.

Studies on the extreme desiccation tolerance of desert bacteria: bacteria from desert soils survive for decades in the absence of water and grow during sporadic rainfalls. We have evidence to support a contribution of specific compatible solutes for this extreme phenotype.

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MEDICAL MYCOLOGY – YEAST RESEARCH (*Head: Teresa Gonçalves*)

Research Highlights

The outbreak of patients bearing immunitary deficiencies has led to an increased incidence of opportunistic fungal infections, difficult to diagnose, treat and with poor outcome. Our main goal is to understand how yeasts and molds respond to the presence of other organisms (the host or bacteria) and to antifungals.

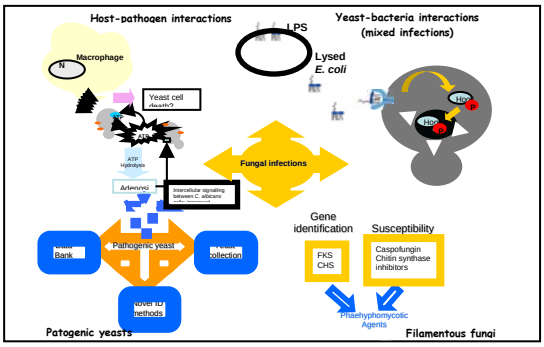
a) Clinical isolates, obtained from several hospitals and health centers, allowed us to construct a collection (1,000 different strains) of pathogenic yeasts, used in the validation of a novel chromogenic kit for the automatic identification of yeasts. This surveillance action allowed the elaboration of a epidemiological study in the portuguese population.

b) A case of cerebral phaeohyphomycosis in a child, caused by *Alternaria infectoria,* a dematiaceous fungus, prompt us to project, developed during 2006, allowing the complete identification, isolation and cloning of the AiFKS gene, and of caspofungin susceptibility of this mold. Preliminary results with the combination of two antifungals opens new perspectives in what regards the antifungal therapy of mold infections, difficult to treat and with high mortality.

c) The recognition that the ability of yeasts in sensing the presence of bacteria (through endotoxin detection) supports the idea of more robust yeast cells. We now finished a study showing that in the yeast *S. cerevisiae* the presence of LPS results in metabolic alterations. The Hog1 phosphorylation (sustained in cells grown under hyper-osmotic conditions – sorbitol 1 M) increases with the exposure to LPS. This increased phosphorylation corresponds to an activation of the pathway, or part of it, since at 30 min we observed an increased expression of the GPD1 gene. In the sequence of this events we observed: decreased activity of the glycolytic enzymes hexokinase and phosphofructokinase; a lower ATP pool; a decreased consumption of glucose and higher K_M for glucose initial transport; an increased expression of HXT1, and that yeast cells, when exposed to LPS are more prepared to deal with hyper-osmotic media, i. e., that this pre-activation increases the robustness of the yeast cells in what regards hyper-osmotic response.

d) A projectwas initiated with the aim of testing the novel hypothesis that purines (ATP and adenosine) and their sensing devices may constitute a key system exploited by *C.albicans* to evade macrophage attack, thus explaining its success as a pathogen.

e) Vpr1 is an HIV protein involved in the progression of AIDS. A study was initiated, together with clinical partners, with the final goal of constructing an yeast expression model that allows the prediction of mitochondrial disfunction as a marker of fast progressors related to different Vpr1 variants. In order to avoid HAART side effects, the ideal diagnosis test would include not only the HIV screening but also a tool to recognize the virus variant in what regards the potential disease progression. The development of such new identification test would also be expected to decrease the health costs with HIV patients, especially on low resource settings.



Ongoing and future work

Yeast response to bacterial endotoxin. In *S. cerevisiae* was initiated a study aimed to characterise the involvment of elements of the HOG pathway in the LPS response and the identification of putative binding sites for LPS.

Combined effect of anti-fungal cell wall inhibitors in Alternaria infectoria. Investigation of a possible synergistic effect between two different types of cell wall inhibitors (β-1,3-D-glucan synthase and chitin synthase inhibitors) and characterization of the effect of this inhibitors in the cell wall.

Role of extracellular ATP and characterization of purinergic receptors in the resistance of C. albicans to macrophage attack Release of ATP and adenosine in co-cultures of *C. albicans* and macrophages. Characterization of the extracellular enzymatic activities present in yeasts to degrade extracellular ATP.

Vpr1 expression in S. cerevisiae. With HIV Vpr sequences from fast progressor and from long-term survival patients a model will be constructed, based on the degree of mitochondrial dysfunction.

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AREA E: BIOPHYSICS AND BIOMEDICAL NMR

Coordinator –Carlos F.Geraldes

Introduction

In the last years the activity of this area has been organized in three sub-areas:

Cell Biophysics whose research is centered both on fundamental mechanisms of cellular excitability and on long–term potentiation (LTP). Cellular excitability work has been devoted to the study of mechanisms underlying stimulus secretion coupling in endocrine and neuroendocrine models of hormone secretion (pancreatic β–cells and adrenal chromaffin cells). In what it concerns LTP research, the aim is the involvement of the different Ca^{2+} change mechanisms in LTP in hippocampal CA1 area.

Biomedical NMR, involving studies of intermediary metabolism using stable isotope traces and NMR, from the cellular level to perfused organs, animal models of disease, and humans.

Inorganic Biochemistry and Molecular Imaging, whose goal is the study of inorganic compounds for medical diagnostic imaging (in particular MRI contrast agents), inorganic drugs for medical therapy, and the study of environmental and toxicological effects of inorganic species.

The last two sub areas are a result of several years of collaboration between researchers from the CNC and from the NMR Center of the University of Coimbra. This collaboration, and the sharing of many facilities at the University of Coimbra in these subareas has enriched both research centers and the results have appeared in previous annual reports of CNC. The present report includes only the work that is being performed in close collaborations and that is expected to lead to joint publications, leaving out many other fruitful interactions between the two research centers.

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Sara Figueiredo	(Ph.D. Student)
Maria João Rodrigues Pereira	(MSc Student)
Ines Ribeiro Violante	(Undergraduate Student)
André Martins	(Undergraduate Student)
João André Duarte	(Undergraduate Student)
João Teixeira	(Undergraduate Student)

Intermediary Metabolism Group

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Madalena Caldeira	(Ph.D.)
Ivana Jarak	(Ph.D.)
Tiago Alves	(Ph.D. Student)
Ana Francisca Leal Silva Soares	(Ph.D. Student)
Sara Gonçalves	(Ph.D. Student)
Ivan Viegas	(Ph.D. Student)
Marco Alves	(Ph.D. Student)
Cristina Barosa	(Ph.D. Student)
Ana Maria Silva	(MSc Student)
Patrícia Nunes	(MSc Student)
Daniela Pinheiro	(MSc Student)
Claudia Silva	(Postgraduate Research Fellow)
Manuela Almeida	(Undergraduate Student)
Sara Gomez	(Undergraduate Student)
Paulo Gameiro	(Undergraduate Student)
Isabel Fernandes	(Undergraduate Student)

Cell Biophysics Group

Luís M. Rosário	(Ph.D. – *Head of group*)
Ângelo R. Tomé	(Ph.D.)
Célia M. Antunes	(Ph.D.)
Rosa M. Santos	(Ph.D.)
Joana I. Real	(Ph.D. Student)

INORGANIC BIOCHEMISTRY AND MOLECULAR IMAGING *(Head: Carlos Geraldes)*

This group develops three main lines of research. Part of the work involving biological material is carried out in collaboration with CNC researchers.

Research Highlights

Aqueous suspensions of paramagnetic lanthanide oxide nanoparticles were studied by NMR relaxometry. The observed very strong R_2^* relaxivities were explained by the Static Dephasing Regime (SDR) theory. The corresponding R_2 relaxivities are considerably smaller and are strongly dependent on the interval between refocusing pulses in the spin echo sequence and increase with the magnetic moment of the lanthanide ion. They are thus very efficient reporter groups to be used in targeted nanodiagnostic agents in molecular imaging in T_2 or T_2*-weighted MRI images.

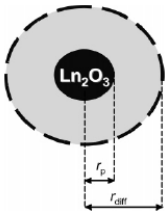
Data in cultured cortical neurons showed that the treatment with the mood stabilizing drugs had no effect on basal cAMP levels *in vitro*, but had differential effects *in vivo*. Direct stimulation of adenylate cyclase (AC) with forskolin increased cAMP levels both *in vitro* and *in vivo*, and this effect was significantly inhibited by all three mood stabilizers. Activation of dopamine D2-like receptors with quinpirole partially inhibited forskolin-induced increase in cAMP in untreated cultures, butno effect was observed in cortical neuron cultures treated with the mood stabilizing drugs.

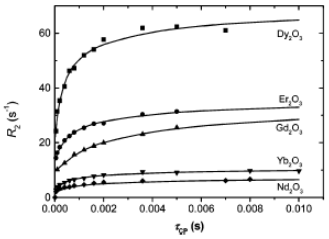
Ongoing and future work

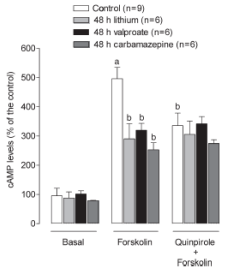
Inorganic agents for Molecular Imaging: Synthesis *in vitro* and *in vivo* characterization of metal-based agents for multimodal Molecular Imaging: (MI) by MRI and gamma imaging; optimization of relaxivity of Gd^{3+} chelates useful as contrast agents for Magnetic Resonance Imaging (MRI) (Gd-EPTPA-CH_2OH); Gd^{3+} complexes with bifunctional ligands derived from DOTA or DTPA targeted to liver cells: Gd^{3+}-glycoconjugates targeted to the internalizable liver asyaloglycoprotein membrane receptor (ASGPR): *in vitro* and *in vivo* characterization by MRI and gamma scintigraphy and biodistribution studies in animal models; Micellar Gd^{3+}-amphyphilic chelates targeted to by the liver reticuloendotelial system (Gd/^{153}Sm-EPTPA-C16): *in vitro* and *in vivo* characterization by MRI and gamma scintigraphy and biodistribution studies in animal models; relaxometric characterization of Gd^{3+}-based paramagnetic nanoparticles (Gd-zeolites and lanthanide oxides) as efficient T_2 contrast agents for Molecular Imaging

Metal Compounds for Therapy: Studies of the molecular and cellular mechanisms of the therapeutic action of Li^+ in bipolar disease; effects of Li^+ and other mood stabilizing agents (carbamazepine and valproate), on dopamine D2 receptor mediated inhibition of adenylate cyclase in cell models and the interaction of dopamine D2 and beta-adrenergic receptors in the prefrontal cortex of rats studied using microdialysis; metal Compounds for Therapy: characterization of new Schiff-base vanadium compounds as efficient oral insulin mimetic agents for type II diabetes.

Toxicology of metal ions: Study the signalling mechanisms of Cr(VI)-induced genotoxic stress; establishment of a cellular model for the study of Cr(VI)-induced lung carcinogenesis; studies with a human bronchial epithelial cell line (BEAS-2B); evaluation of the relation of possible genomic mutations with changes in growth cell characteristics associated with cancer cell-like characteristics; evaluation of the expression changes on genes and proteins involved in loss of contact inhibition, cell cycle control, metabolic alterations associated with cancer development and cell proliferation; toxicity of orally ingested Cr(VI) in using Wistar rats; involvement of the TGF-β pathway on Cr(VI)-induced hepatotoxicity; effects of orally ingested Cr(VI) on glucose metabolism.







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Torres S, Martins JA, André JP, Neves M, Santos AC, Prata MIM, Geraldes CFGC. (2007) Radiolabelled Sm-153 Chelates of Glycoconjugates: Multivalence and Topology Effects on the Targeting of the Asialoglycoprotein Receptor. *Radiochimica Acta*, 95: 343-349.

Montezinho LP, Mørk A, Duarte CB, Penschuck S, Geraldes CFGC, Castro MMCA (2007). Effect of mood stabilizers on dopamine D_2-like receptors-mediated inhibition of adenylate cyclase. *Bipolar Disorders*, 9: 290-297.

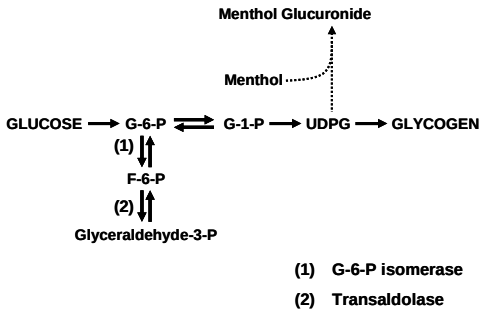
INTERMEDIARY METABOLISM (*Head: John G. Jones*)

In diabetes, the loss of glucose homeostasis results in secondary complications such as heart disease and blindness. To understand the precise effects of this on the function of liver, heart and brain, we are developing safe and practical stable-isotope tracer measurements of glucose metabolism in humans and in animal models of diabetes. These measurements are providing new insights about how glucose metabolism is modified in the liver, heart and brain in the setting of hyperglycemia and diabetes.

Research highlights

Clinical Research Studies: The measurement of hepatic glycogen synthesis by direct and indirect pathways with any tracer method may be significantly skewed by transaldolase (TA) activity, but there are currently no methods for determining the extent of TA flux and correcting its effects on estimates of indirect pathway contributions. We developed a metabolic model that allows these parameters to be derived with any gluconeogenic ^{13}C-tracer and applied this to human studies. We found that under fed conditions, TA activity results in substantial underestimate of the direct pathway contribution to hepatic glycogen synthesis (see Key Reference). Since indirect pathway flux has been reported to be altered in both Type 1 and Type 2 diabetes, this observation has important implications for interpretation of these data. To further utilize the central role of deuterated water (^2H_2O) tracer in our human studies, we developed new methodologies that will provide metabolic information on amino acid and lipid metabolism. We successfully quantified the enrichment of human hepatic glutamine from ^2H_2O by noninvasive chemical biopsy using phenylbutyric acid. This information allows us to determine the contribution of metabolic and proteolytic sources to the hepatic glutamine pool. This measurement may be a key parameter to link the overproduction of hepatic glucose with alterations in whole body protein and amino acid kinetics in Type 2 diabetes and also in chronic wasting conditions such as cardiac cachexia. In the rat, we applied NMR to quantify lipid enrichment from ^2H_2O and developed a novel model for quantifying the contribution of de-novo lipogenesis to the hepatic lipid pool. This method is directly translatable to humans.

Basic Research Studies: The neurochemical profile and the kinetics of glucose transport were evaluated in the hippocampus of streptozotocin (STZ)-induced diabetic rats, using non-invasive ^1H NMR spectroscopy. One month after STZ treatment, diabetic rats displayed significantly altered neurochemical profile in the hippocampus, such as increased glutamate, myo-inositol, glycerophosphocholine, phosphocholine, taurine, β-hidroxybutyrate, scyllo-inositol and total creatine and decreased glutathione and N-acetylaspartylglutamate concentrations. Glycaemic normalization restored all but the concentrations of myo-Inositol and phosphocholine, suggesting that the main effect of diabetes on brain metabolites is due to osmolarity dysregulation. We observed unaltered hippocampal glucose transport when compared to controls. The initial results obtained from STZ-treated rats consuming caffeine (1g/L caffeine in drinking water) showed that caffeine is able to prevent metabolic alterations in the hippocampus caused by diabetes. In another study, profiles of gene expression, mitochondrial bioenergetics and apoptotic effects were evaluated in hearts made ischemic for various periods in the presence of two distinct cardioplegic solutions, Celsior and HBS (histidine buffered solution). The two solutions seem to have similar cardioprotective effects. However, the HBS solution seems to be more effective in relation to the inhibition of the apoptotic process and to the maintenance of cardiac metabolism while the Celsior seems to be more effective in protecting from the inflammatory process. Finally we made an evaluation of the possible contribution of galactose to glycogen synthesis and detected a preferential use of this hexose when compared to glucose. This could prove of relevance in the scenario of improper glycogen storage by individuals like it happens in type I diabetes.



Transaldolase exchange of hepatic glucose metabolites.

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CELL BIOPHYSICS *(Head: Luís M. Rosário)*

Adrenaline release from adrenal chromaffin cells plays a key role in the body reactions to stress. Pulsatile insulin release from pancreatic islets is critical for glucose homeostasis, and its loss represents an early event in type 2 diabetes. Our general aim was to investigate stimulus-secretion coupling mechanisms in catecholamine- and insulin-secreting cells, with a particular emphasis in ion channel function and Ca^{2+}-coupled membrane receptors.

Research highlights

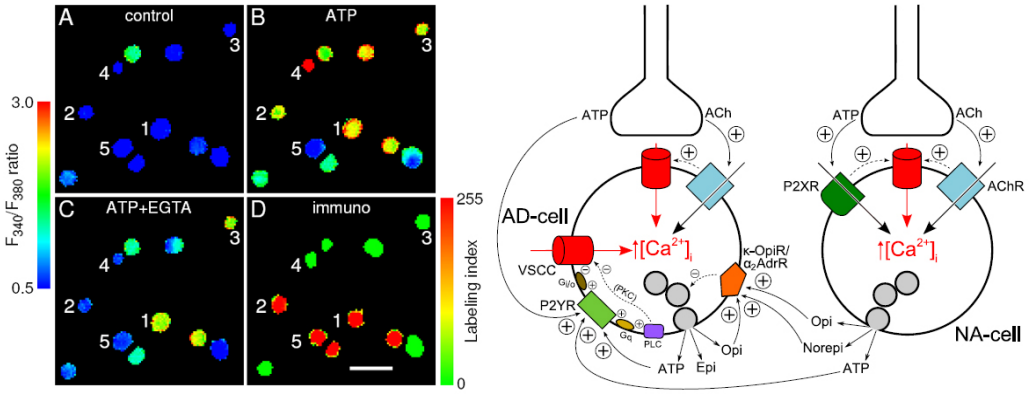
Several research projects were developed in 2007, focusing in: i) the differential role played by metabotropic (P2Y) and ionotropic (P2X) purinergic receptors in catecholamine release from adrenergic and noradrenergic chromaffin cells, aiming at identifying paracrine interactions involving extracellular ATP; ii) the electrophysiological assessment of ATP-gated channels in pancreatic β-cells, aiming at establishing its role in insulin release; iii) the role played by specific protein kinase C (PKC) isoforms in the cholinergic potentiation of glucose-induced insulin release; iv) the role played by ion channels distinct from the ATP-sensitive K^+ (K_{ATP}) channel in bursting electrical activity and pulsatile insulin release from β-cells. The cellular models used included isolated bovine chromaffin cells, isolated β-cells from different species and isolated mouse pancreatic islets.

Ongoing and future work

Autocrine and paracrine interactions involving extracellular ATP. We are presently investigating the role played by ATP released from nerve terminals, chromaffin cells, catecholaminergic neurons and pancreatic β-cells in catecholamine and insulin release. The working model for catecholamine-releasing cells (see figure) implies that ATP has both excitatory actions (via P2X receptors) and delayed inhibitory actions (via P2Y receptors), the latter serving as a refraining signal for excessive hormone release via inhibition of voltage-sensitive Ca^{2+} channels. The working model for insulin-releasing cells assumes that granule-stored ATP might be released in a pulsatile fashion to coordinate the islet activity as a syncythium.

Differential cellular actions of specific PKC isoforms. We are presently investigating the role played by cPKC and nPKC isoforms in acetylcholine-induced catecholamine release and pulsatile insulin release. The working models assume that these isoforms have differential actions on the reserve and immediately releasable hormone pools of chromaffin cells, catecholaminergic neurons and pancreatic β-cells.

Oscillatory electrical activity and pulsatile insulin release. We are presently investigating the role played by voltage-sensitive Ca^{2+} (Ca_V1) channels as early sensors of glucose metabolism in pancreatic β-cells, acting in concert with K_{ATP} channels and small-conductance KCa (SK) channels to support bursting electrical activity and pulsatile insulin release. The working model assumes that Ca_V1 channels undergo slow and voltage-independent inactivation, modulated by either glucose metabolites or products arising from glucose metabolism (e.g. MgATP, Ca^{2+} or phosphorylation mediated by non-serine/threonine kinases). Further work is being carried out to assess whether Ca_V1 channels might be involved in β-cell dysfunction, using an animal model of type 2 diabetes (Goto-Kakizaki rats).



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AREA F: CELL AND DEVELOPMENT BIOLOGY

Coordinator – Maria Celeste Lopes and João Ramalho Santos

Introduction

In this Area two main focal changing points were evident in 2007. On one hand the group involved in Diabetes and Obesity research has left the Center, but its departure was offset by increased collaborations and new researchers in the Cellular Immunology and Oncobiology Group, as well as by the continuing recruitment of collaborators and researchers (in the form of graduate students, or Post-Doctoral Fellows) by the recently established Phagocytosis and Pathogens Group, as predicted in the previous report. Furthermore, the Reproduction group has been involved in the emerging field on stem cell biology and tissue engineering, in collaboration with other CNC groups.

As stated in previous reports, the groups in this Area have a common strong medical component in addition to their basic science interests, and research projects have involved clinicians and clinical samples (both nationally and internationally), as can be quickly ascertained from their Research Highlights and Publication records, which rely heavily on translational research synergies. Furthermore all the groups listed have active collaborations with groups in other areas of the CNC (Neuroscience and disease, Molecular Biotechnology and Health, Cellular and Molecular Toxicology, Biophysics and Biomedical NMR) in addition to other national and international collaborations.

The general objectives of this Area accomplished in 2007 were: i) the involvement of the Reproduction Group in the emerging field on stem cell biology and tissue engineering, in collaboration with other groups, ii) the increased collaborations and integration of new researchers in the Cellular Immunology and Oncobiology Group and iii) the continuing recruitment of collaborators and researchers (in the form of graduate students, or Post-Doctoral Fellows) by the recently established Phagocytosis and Pathogens Group.

The main goal for this Area in 2008 will continue to be the consolidation of the research carried out, as well as the recruitment of new researchers to address specific needs. In this regard, it should be noted that both the Cellular Immunology and Oncobiology and the Biology of Reproduction and Human Fertility groups rely heavily on the use of University, Hospital and other non-CNC facilities.

Cellular Immunology and Oncobiology Group

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Alexandrina F. Mendes	(Ph.D.)
Ana Bela Sarmento Ribeiro	(M.D., Ph.D.)
Anália do Carmo	(Ph.D.)
Maria Teresa Cruz Rosete	(Ph.D.)
Sukalyan Chaterjee	(Ph.D.)
Teresa Maria C. Martins	(Ph.D.)
Ana Catarina Simões P. Oliveira	(Ph.D. Student)
Ana Luisa Vital	(Ph.D. Student)
Bruno Miguel das Neves	(Ph.D. Student)
Inês Crespo	(Ph.D. Student)
José Mário Tenera Morgado	(Ph.D. Student)
Mariana Freitas	(Ph.D. Student)
Marta Viegas da Silva	(Ph.D. Student)
Rui Nobre	(Ph.D. Student)
Susana Carvalho Rosa	(Ph.D. Student)
Diana Moreira	(MSc Student)
Vera Gonçalves	(Undergraduate Student)
Inês Patricio	(Undergraduate Student)
Vera Lúcia G. Francisco	(Undergraduate Student)
Andreia Madeira	(Technician fellowship)
Patricia Amaro	(Technician fellowship)
Patricia Silva	(Technician fellowship)

Biology of Reproduction and Human Fertility Group

João Ramalho Santos	(Ph.D. – *Head of group*)
Teresa Almeida Santos	(M.D., Ph.D.)
Alexandra Amaral	(Ph.D. Student)
Sandra Amaral	(Ph.D. Student)
Sandra Varum	(Ph.D. Student)
Ana Paula Marques de Sousa	(Ph.D. Student)
Paula Mota	(Ph.D. Student)
Sandra Gamboa	(Researcher, Ph.D. Student)
Sara M. Diniz Martins Lopes	(MSc Student)
Ana Sofia Rodrigues	(MSc Student)
Raquel Brito	(MSc Student)
Renata Santos Tavares	(MSc Student)
Marta Isabel Rodrigues Baptista	(MSc Student)
Rita Silva	(Undergraduate Student)
Ana Carolina Borralho	(Undergraduate Student)
Beatriz Lacerda de Sousa	(Undergraduate Student)

Emerging Groups

Infection, Phagocytosis and Pathogens Group

Otilia Vieira	(Ph.D. – *Head of group*)
Carla Cardoso	(Post-doctoral fellow)
Daniel Oberdoerfer	(Ph.D. Student)
Shyam P. Mohan	(Ph.D. Student)

Insulin Resistance and Adipocyte group

Eugenia Carvalho	(Ph.D. – *Head of group*)
Manuel Aureliano Alves	(Ph.D.)
Maria João Pereira	(Ph.D. Student)
Patricia Nunes	(Technician)
Daniela Pinheiro	(Technician)

CELLULAR IMMUNOLOGY AND ONCOBIOLOGY *(Head: Maria Celeste Lopes)*

The cellular immunology and the oncobiology sub-groups share common interests in identifying the cellular mechanisms that regulate the function of normal human cells and in understanding how disruption of these processes leads to disease.

The main focus of research of this group is the study of intra and inter cellular cross-talks in health and disease and to identify: 1) cellular mechanisms involved in the development and progression of inflammatory and allergic diseases; 2) genetic abnormalities, gene expression profiles and new prognostic markers in tumours; and 3) molecular targets for new therapeutic strategies for the prevention and/or treatment of chronic inflammation, allergy and cancer.

Research Highlights

In 2007, the research projects of the cellular immunology sub-group focused in studying: ; i) how skin sensitizers and irritants modulate the expression of dendritic cell surface molecules (chemokine and cytokine receptors), cytokine production and transcription factors activation; ii) the efficacy of cryoprotective agents to preserve the viability and metabolism of articular chondrocytes in the cartilage for allotransplantation, and the modulation of chondrocyte functions by different glucose concentrations; and iii) the role of the CD38 on the regulation of immune responses.

The research projects of the oncobiology sub-group focused on evaluating: i) chromosomal and genetic abnormalities of human glioma tumors; ii) the role of CD26/DPPIV expression and 8-azaguanine response in acute leukaemia, and proteasome inhibitors in chronic lymphocytic leukemia; and iii) the genetic risk factors in HPV-mediated cervical cancer.

Ongoing and future work

We are developing a research program that involves several clinical units:

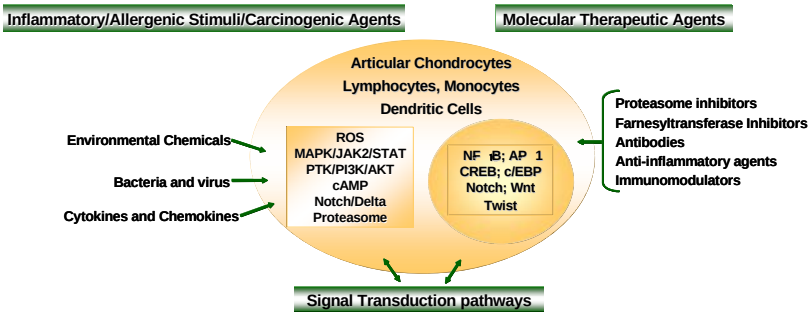
In collaboration with the Dermatology Department of the University Hospital of Coimbra, we are using skin dendritic cells in culture to investigate the effect of skin sensitizers and irritants on the: 1) activation of intracellular signalling pathways and 2) changes in the proteomic profile. We are looking for the cellular events suitable for the development of *in vitro* tests to predict the sensitizing potential of chemicals.

In collaboration with the Orthopaedic and Bone Bank Departments of the University Hospital of Coimbra, we are using 1) normal and osteoarthritic human articular cartilage and chondrocytes, from diabetic and non-diabetic patients, and a human chondrocytic cell line, to compare chondrocyte viability, dynamics of glucose transport and expression of glucose transporters; and 2) normal articular cartilage from cadaveric tissue donors to identify new effective cryoprotective agents that may be used with large human osteochondral pieces at tissue banks.

In collaboration with the Neuropathology Laboratory and Neurosurgery Service of the University Hospital, Coimbra, and with the Center for Cancer Research of Salamanca, we are evaluating the chromosomal abnormalities, using iFISH, and the gene expression profile, using cDNA micro-arrays and SNP-array analysis, in samples of human brain tumors, previously diagnosed as gliomas.

In collaboration with the Portuguese Oncology Institute of Coimbra, we are studying: 1) the role of multifunctional ectoenzymes CD38 and CD157 in infection and autoimmunity, and 2) the genetic risk factors of HPV-mediated cervical cancer.

In collaboration with the Clinical Hematology Department of the University Hospital of Coimbra, we are studying the role of immune system and signal transduction pathways, namely those involving Ras gene, in the prognostic and therapeutic of haematological neoplasias.



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Cruz MT, Gonçalo M, Figueiredo A, Duarte CB, and Lopes MC. (2007) Effect of skin sensitizers on inducible nitric oxide synthase expression and nitric oxide production in skin dendritic cells: role of different immunosuppressive drugs. *Immunopharmacol. and Immunotoxicol.* 29: 225-241.

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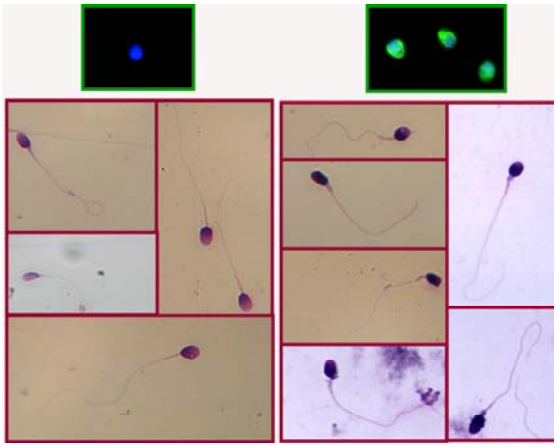
The main goal consists in determining what makes a good sperm, from a cellular, biochemical and molecular standpoints. Several animal models are used (horse, rat, cat, human). We are currently researching changes in sperm that may correlate with fertility (abnormal mitochondrial DNA replication, mitochondrial function, apoptosis, sperm chromatin status, ATP production, antioxidant defenses), as well as the effect of diabetes and age on testicular homeostasis, sperm production, metabolism and physiology. These studies are being carried out both in bulk populations of sperm from males with different semen characteristics, as well as in populations that have been sorted by either classical methods or flow cytometry, and have relevance for the diagnosis and management of human (in)fertility. Another goal is to develop simple tests to monitor sperm quality and we have recently developed an assay to determine the percentage of normal (below, left) and abnormal (below, right) sperm in an ejaculate in terms of nuclear DNA status. Furthermore, we are also studying the effect of mitochondrial bioenergetics on human embryonic stem cell pluripotency. In fact mitochondrial inhibition using antimycin A results in an up-regulation of pluripotency markers such as Nanog, in stem cells, while maintaing essential cellular characteristics. One recent highlight of this research is the finding that antimycin A in culture media can actually replace the role of some growth factors, namely bFGF.

Research Highlights

1- Detailed analysis of ATP production, mitochondrial function, membrane stability, apoptosis, oxidative stress and antioxidant defenses in equine sperm, and impact on stallion fertility. The idea is to come up with the best possible indicators for stallion fertility, with relevance for animal breeding (part of this work has been published). 2- Discovery of proteins related to mitochondrial DNA replication in sperm, the presence of which varies with semen characteristics, determines mitochondrial DNA copy number in mature sperm, and may thus have implications for human fertility (this work has been published).

Ongoing and Future Work

1- Development of a novel simple assay to monitor human sperm DNA status, an important parameter that is not usually quantified, with direct application for clinical practice. This assay was derived from previous work carried out in the cat (Mota & Ramalho-Santos, 2006), and is currently being evaluated in a multi-center collaboration involving samples from the University Hospitals of Coimbra, two labs affiliated with the University of Porto, and a lab in Brest (France).



2- Employment of different strategies to create and analyze homogeneous subpopulations of sperm from the heterogeneous initial ejaculate using flow cytometry with a variety of functional markers, as well as more classical methodologies. The goal is to determine if the differences we have found between individuals in terms of mitochondrial DNA, functional markers etc, also apply to distinct subpopulations within the ejaculate of the same individual.

3- Analysis of mtDNA replication in long-term culture of human sperm.

4- Development and testing of novel spermicides with antiviral activity. 5-Analysis of the energetic competency of discarded human oocytes and failed fertilizations using the vital metabolic dyes Brilliant Cresyl Blue and Alamar Blue on both oocytes and companion cumulus cells. This will allow further analysis of the same samples by immunocytochemistry in search of changes that may be related to successful human fertilization. 6- Implementation and optimization of protocols to cryopreserve human ovarian tissue for future transplantation following oncological interventions. We will also study the influence of cryopreservation on ovarian cell apoptosis, and attempts on culturing human samples following cryopreservation are already underway. 7- Characterization of testicular apoptosis and mitochondrial bioenergetics, how it compares with other tissues, and how it is affected by age and diabetes. 8- Dissecting the role of mitochondria and reactive oxygen species on embryonic stem cell pluripotency.

Key References

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Emerging Group

INFECTION, PHAGOCYTOSIS AND PATHOGENS (*Head: Otilia Vieira*)

Phagocytosis, a complex cellular event by which particles are recognized, engulfed and eliminated, plays an essential role in innate immunity (host defence) and also in the removal of apoptotic cells. After internalization, the organelle formed, the phagosome, starts to interact with the components of the endocytic pathway, a process referred to as phagosome maturation. In the vast majority of cases, the microbe inside the phagosome is killed and digested, but a number of important pathogens, including Mycobacterium tuberculosis, which kills around two million people each year, have acquired the ability to survive. In this context, one of our research interests is the identification of the molecular machinery involved in the engulfment and phagosomal maturation. We are also interested in the identification of the reasons accounting for defective phagocytosis in atherosclerotic lesions, given that macrophages, which are very effective at removing modified LDL and apoptotic cells, fail to prevent or resolve the atherosclerotic lesion. In fact, they appear to aggravate the lesion by releasing pro-inflammatory mediators, proteases and oxidants that damage the arteries.

Finally, since we are in the midst of global epidemics of both unwanted pregnancies and sexually transmitted infections options that provide protection are ideal. We are interested in the evaluation of surfactants as microbicides and contraceptive agents.

Research Highlights

1. Rab10 and Rab14 are required for phago-lysosome formation: Pulse-chase experiments showed the Rab10 and Rab14 associate transiently with phagosomes. Albeit, the expression of the DN or CA versions of these Rabs do not affect the engulfment capacity of phagocytes both seem to be required for phago-lysosome formation (1).

2. Quaternary ammonium compounds have anti-fungal and antimicrobial properties: At sub-toxic concentrations towards mammalian epithelial cells these surfactants neither inhibit viral infection nor are they spermicidal. However, we found that quaternary ammonium compounds, in a CMC-dependent way, exhibit bacteriostatic, bactericide and antifungal properties at concentrations substantially below their CMC (2).

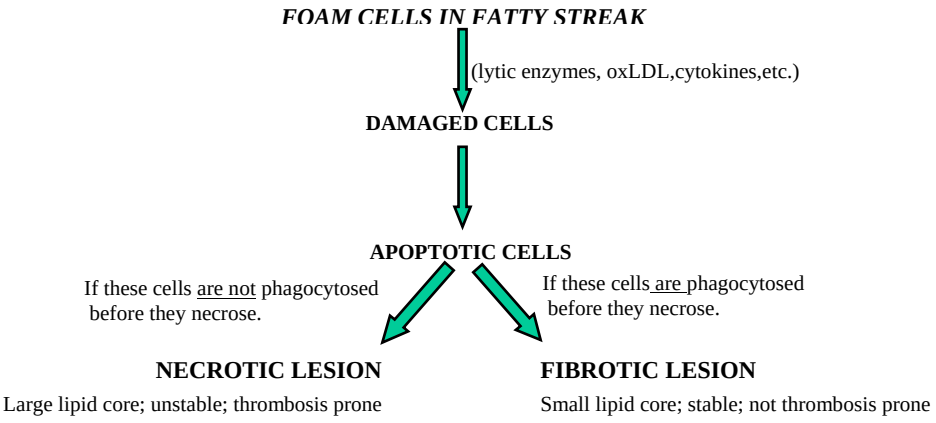
3. Charge changes in the lipid part of lowdensity lipoproteins (LDL) are sufficient to cause foam cell formation: We found that the incorporation of 500 molecules of negatively charged cholesterol per LDL particle leads to foam cell formation, an early event in the atherogenesis (scheme).

Ongoing and Future Work

1. Confocal live imaging to look at kinetics and interplays of Rab10 and 14 with other Rabs that play important roles in phagosomal maturatios such as: Rab5, 7, 4 and 11.

2. Test newly synthesized surfactants, not commercially available, and designed according to the results obtained in our previous studies, for antiviral and spermicidal activities.

3. Characterizing the entry process of our LDL model and their interference in the clearance of apoptotic cells.



The failure to clear apoptotic cells will favour the formation of vulnerable plaques

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[1]Corresponding author

Emerging Group

INSULIN RESISTANCE AND ADIPOCYTE (*Head: Eugenia Carvalho*)

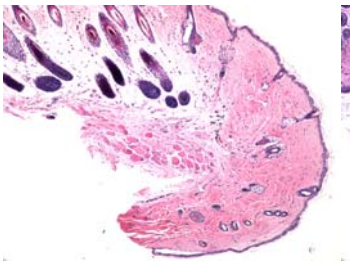
Mechanisms of insulin resistance. The homeostasis model assessment (HOMA) and the quantitative insulin sensitivity check index (QUICKI) as markers of insulin resistance in a population from the south of Portugal. Serum samples from 5711 subjects were collected for measurements of glucose, cholesterol, and triglycerides to assess the prevalence of impaired glucose tolerance and diabetes. We found that 11% of the population is hyperglycemic, and that these models can be used to assess insulin sensitivity in the population at large. In addition, we studied glycogen metabolism in primary adipocytes. Our results demonstrate that the standard method of glycogen extraction and isolation does not completely exclude glucose. With tissues that contain high glycogen levels such as liver and muscle, a residual amount of glucose may not significantly alter the estimates of glycogen concentrations. However, with adipocytes and other cells with low glycogen levels, the presence of a residual amount of glucose in the isolated glycogen preparation can inflate the estimate of glycogen concentration. Glucose oxidase treatment provides a convenient and quantitative means for scavenging residual glucose before glycogen is hydrolyzed.

The role of neuropeptides in the regulation of food intake. We demonstrated that Substance P is a novel anti-obesity target. SP acts in the brain as well as in the periphery as a neuropeptide, neurotransmitter and hormone affecting diverse physiological pathways, via its neurokinin-1 receptor (NK-1R). CJ 012,255, a SP antagonist which binds to NK-1R, administration prevented weight gain and accumulation of fat after two weeks of high fat feeding in mice, while similar CJ treatment in obese mice resulted in weight loss, reduction in adiposity and improvement of insulin sensitivity, in part due to inhibition of food intake. The effects of SP in the control of energy balance are, at least in part, leptin independent, since CJ treatment was also effective in leptin deficient mice. SP per se acts as an orexigenic neuropeptide and promotes weight gain in mice via NK-1R coupling. We speculate that NK-1R antagonists, already tested in clinical trials for various diseases, may represent a potential target against obesity.

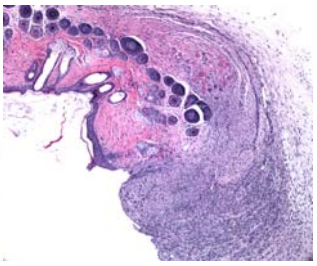
The role of neuropeptides in wound healing in diabetes. Impaired wound healing is a major clinical problem in diabetes. Peripheral neuropathy is a major contributing factor to tissue ischemia. We studied wound healing in a model that mimics the human condition by using NK-1R deficient mice and CJ, the NK-1R antagonist. The NK-1R deficiency was associated with 17% reduction in skin oxygenation at baseline and 24% ten days after wound induction. These mice showed a significant reduction of the wound area. Wound area reduction was impaired by 25% in the CJ treated wild-type mice when compared to the saline-treated mice. These results indicate that SP plays a crucial role in wound healing and that a major pathway is the reduction of tissue oxygenation. Manipulation of the SP pathway may prove a potential new therapeutic approach in treating diabetic foot ulceration.

Ongoing and Future Work

We plan to further study glycogen metabolism in healthy and insulin resistant human primary adipocytes, as well as, evaluate interactions between insulin, glucocorticoids and immunosuppresors in visceral and subcutaneous adipose tissue in the development of insulin resistance. In addition, we plan to explore the link of Melanin-Concentrating Hormone (MCH), an appetite regulating neuropeptide, with obesity to inflammation, as well as, investigate the role of diabetes and peripheral neuropathy on mast cell function.



WT mice pre-wound day 1



WT mice post-wound day 10

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Biomedical Inter-Institutional Research Programme

Biomedical Inter-Institutional Research Programme (CNC, HUC, CHC, IPO)

A strong and proficuos interaction has been developed between CNC and Clinical Faculties. The outcome of this interaction was the establishment of a Biomedical Inter-Institutional Research Programme envolving CNC (Centro de Neurociências e Biologia Celular), HUC (Hospitais da Universidade de Coimbra), CHC (Centro Hospitalar de Coimbra) and IPO (Instituto Português de Oncologia). The main ongoing joint research projects on this programme are described bellow.

1. Psychiatry Research: Molecular Genetics Studies of Complex Disorders

(Carlos Pato, Michele Pato (University of Southern California.), M.H. Azevedo (HUC, FMUC), C.R. Oliveira (CNC))

These studies are focused on the identification of candidate genes for Schizophrenia and Bipolar Disorder through the use of linkage and association analysis. For this purpose two populations have been analyzed: a relatively homogenous population from Azores, augmented by a similarly homogenous subsample from Madeira, and a mainland Portuguese population. To date we have collected 3000 DNA samples, including 700 schizophrenic patients, 500 bipolar patients, and 1400 unaffected family members. Additionally, 350 unaffected (i.e. no history of psychiatric disorder) subjects of Azorean descent have been collected as a control group. The schizophrenic sample includes 100 multiplex (2 or more affected members) families, and the bipolar sample includes 120 multiplex families.

Last year, these studies have utilized "state of the art" DNA and RNA microarray technology to identify chromosomal regions of linkage to each disorder, as well as areas of increased expression in the presence of illness. This convergent genetic-genomic approach has led to the identification of several areas in the human genome to target for follow-up study, most notably on Chromosome 5 for schizophrenia and psychosis, and on Chromosome 6 for Bipolar Disorder. Additionally, collaborators from the Broad Institute in the United States and from the Centre on Addiction and Mental Health (CAMH) in Toronto, Canada have been using the sample, collectively known as the "Portuguese Island Collection," to investigate specific candidate genes for schizophrenia, including neuregulin 1, syntaxin 1A, and genes from the dopaminergic and serotonergic systems. We have also formed the International Shizophrenia collaborative to use whole genome approaches to define the genomics of this disorder.

Clinical Research – Phenotypic Studies of Complex Disorders

In parallel with the molecular genetic studies of schizophrenia and bipolar disorder, we have developed a range of clinical investigations in areas in which a more clear understanding of the phenotypic definitions and boundaries were needed. These studies have focused in the area of personality, namely studying the perfectionism and the relationship between this trait and some disorders of the obsessive-compulsive spectrum (eating disorders and OCD) and sleep problems. Another important area under investigation is the postpartum depression, and for this purpose a funded project from the *Fundação para a Ciência e Tecnologia* is under way. One of the areas of expertise of our team is in the field of diagnostic methodologies and tools, and in this context several scales have been validated to be used in the above mentioned studies.

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2. Neurology Research: studies on neurodegenerative disorders

(*Luis Cunha (H.U.C.), Catarina Oliveira (CNC)*)

Cerebrospinal fluid (CSF) biomarker identification in neurodegenerative disorders, mainly in dementias, has been one of our areas of interest. Sporadic Creutzfeldt-Jakob Disease (sCJD) is a fatal and rapidly progressive disease, that can only be diagnosed *post-mortem*. CSF biomarkers would be of great value in the early diagnosis of this disease, and in fact the immunodetection of 14-3-3 protein in CSF has shown high specificity and sensitivity for sCJD. However, the detection of 14-3-3 protein is a qualitative test, that is strongly related to the duration of the disease. Therefore we have evaluated the utility of other CSF markers in the differential diagnosis of sCJD.

We studied a group of 25 patients with sCJD, 58 with Alzheimer's disease (AD), 17 with Fronto-Temporal Dementia (FTD) and 2 individuals with variant CJD (vCJD), evaluating CSF levels of total tau protein (t-tau), tau protein phosphorylated at threonine-181 (p-tau181), amyloid $\beta_{(1-42)}$ protein (Aβ42) and S-100b. We have found extremely increased levels of both t-tau and S-100b in sCJD patients relative to AD and FTD patients, while p-tau 181 in sCJD was significantly decreased in relation to AD, but similar to the levels found in FTD patients. The levels of Aβ42 were similar in sCJD and AD patients, but slightly decreased in relation to FTD patients. In the vCJD patients, although t-tau levels were similar to those found in sCJD patients, p-tau 181 was elevated. Overall, t-tau and the ratio p-tau 181 / t-tau presented with higher diagnostic value (sensibility, specificity and accuracy) and therefore should by useful in the early diagnosis of sCJD. The results also suggest that the ratio p-tau 181 / t-tau might help distinguish between sCJD and vCJD.

Biochemical characterization of Mild Cognitive Impairment (MCI) and early AD is also a specific research interest of our laboratory. A defect in the cholinergic system has been well established in AD, and several CSF markers of this system have been studied as putative indicators of diagnosis and severity. However the information regarding such markers in MCI is scarce. Therefore we have studied the activity of the acetylcholinesterase (AchE) and butirylcholinesterase (BuchE) in CSF in three diagnostic groups: 32 cognitively healthy age-matched controls, 13 patients with MCI and 50 with AD at different stages of the disease, without previous medication with cholinesterases inhibitors. We have found significantly decreased activity of both enzymes in AD patients, while MCI patients were similar to controls. In the AD group, we also found a negative relation between the levels of AchE activity or the ratio AchE/BuchE and clinical indicators of severity, while there was no clear relation between the activity of BuchE and the progression of disease. This study shows that, regarding the activity of CSF cholinesterases, MCI patients do not present with a profile similar to AD patients, suggesting that the cholinergic system might yet be preserved in non-symptomatic stages of dementia.

We have an ongoing collaboration with the Pediatric Hospital of Coimbra for the diagnosis of congenital errors of purines and pirimidines metabolism, and during this last year we have assessed the urinary levels of purines and pirimidines metabolites (uric acid, xanthine, hipoxanthine, pseudouridine, uracil and 7-methylguanine) in 22 patients by high performance liquid chromatography (HPLC) with diode array spectrophotometric detection. Also in collaboration with the Pediatric Hospital of Coimbra we have evaluated the plasmatic levels of vitamin E in 23 patients and the plasmatic redox state (simultaneous determination of the levels of piruvate, lactate, acetoacetate, β-hidroxybutirate and free fatty acids) in 41 patients, useful in the diagnosis of hereditary metabolic diseases with energetic failure.

PUBLICATIONS

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3. Pediatric Research: metabolic disorders

(Luísa Diogo (CHC); Catarina Oliveira (CNC); Manuela Grazina (CNC))

Mitochondrial respiratory chain diseases (MRCD) are a diverse group of disorders with a broad spectrum of clinical manifestations, characterised by defects in mitochondrial energetic function. Inherited defects causing mitochondrial dysfunction can be due to mutations either in nuclear DNA (nDNA) or mitochondrial DNA (mtDNA). Each mitochondrion contains its own DNA that codes for 13 peptides of the mitochondrial respiratory chain (MRC) system, where the oxidative phosphorylation (OXPHOS) occurs, plus the two structural rRNAs and 22 tRNAs necessary for mtDNA genes expression. Novel concepts of mitochondrial inheritance, such as mtDNA heteroplasmy, tissue distribution and threshold effect, have explained many of the clinical characteristics. Different gene mutations of mtDNA origin that produce MRC defects have been identified and have been classified as point mutations, large-scale mtDNA deletions, duplications or insertions. Additionally, other mutations affecting nDNA genes (either coding for MRC subunits or assembly/mtDNA stability factors) have also been recently identified; in particular, autosomically inherited disorders have been identified in cases with multiple mtDNA deletions. The major laboratory criteria for the diagnosis of MRCD include: ragged red fibbers (RRF's) on muscle biopsy, lactic acidosis, a specific deficiency in a mitochondrial respiratory enzyme complex and nDNA/mtDNA abnormalities. However, not all MRCD cases display RRF's, biochemical analyses of muscle tissue may show no apparent defects and, in a large proportion of patients with MRC enzyme deficiencies, no mutations have been found. Taking into account these facts, our main objective is to provide tools for the diagnosis of MCRD and a better understanding of the pathogenic mechanisms leading to the clinical phenotypes. This will provide new insight into mitochondrial dysfunctions and will be the basis for more rational therapies for the patients. The precise pathogenic mechanisms by which these biochemical abnormalities induce tissue dysfunction are not clearly understood and diagnosis of these disorders is complex, requiring specialised techniques and correlation between clinical and biochemical/ genetic data.

It was initiated the mtDNA mutation quantification, by real time PCR, with the collaboration of University of Newcastle upon Tyne, with particular interest on copy number variation for diagnosis of depletion syndrome.

We have continued the set up of the evaluation of Pyruvate dehydrogenase activity for diagnostic purposes, in a Spectramax PLUS 384 Microplat Reader - Molecular Devices, recently acquired, following the publication by the Reference Laboratory in Holland in *Clin Chem. 2005 Jan;51(1):151-60* (Optimized Spectrophotometric Assay for the Completely Activated Pyruvate Dehydrogenase Complex in Fibroblasts, by Schwab et al.).

A MSc Thesis (*Luis Miguel Oliveira*) has been presented in July 2007, concerning the "Determination of the frequency of mtDNA haplogroups in patients suspected of Mitochondrial Cytopathy".

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4. DNA investigation in Neurodegenerative disorders

(Catarina Oliveira (CNC); Manuela Grazina (CNC))

Neurodegenerative disorders are complex and the mechanisms underlying the phenotypic expression of this group of diseases are not clearly understood. Finding genetic risk factors, either from nuclear or mitochondrial genome origin, will contribute to identify new tools for early diagnosis, as well as to support the development of more rationale therapies, including the implementation of pharmacogenetic approach.

Our aim is to search for genetic risk factors in our population and identify disease risk groups.

We have continued, in collaboration with Neurology Department of University Hospitals, a Research Project for Medical Students, concerning the evaluation of mtDNA *ND1* sequence variations in a larger sample of FTD patients, following the evidences of the involvement of MRC complex I in FTD, reported in 2004 (Grazina M, Silva F, Santana I, Santiago B, Oliveira M, Cunha L, Oliveira C. Frontotemporal dementia and mitochondrial DNA transitions. *Neurobiol. Dis.* 2004; 15-2: 306-311). So far, the nucleotide regions 2300-3100, 3900-4500 e 12850-13700, corresponding to genes coding for 16s rRNA, ND1 and ND5, have been sequenced and analysed. We have found 21 different sequence variations (total 53) in 29 patients (out of 35), including 12 described polymorphisms; 2 pathogenic mutations, 1 secondary LHON mutation and 6 novel alterations. These results suggest a contribution of mtDNA variations to FTD ethiopathogenesis.

Additionally we have started the genetic characterization of dementias related to 5HTR2A, BDNF genes, aiming to perform a pharmacogenomic characterization of the patients. The correlation with levels of BDNF and serotonin are also included

We have started the genetic studies in eye disorders, in collaboration with IBILI and Serviço de Oftalmologia dos HUC. A project has been submitted to FCT in November 2007.

Over the past year the main goal of the Neurogenetics Laboratory at the CNC has continued to be the identification of genetic mutations leading to neurological diseases, mainly Alzheimer's (AD) and Parkinson's (PD) diseases. Additionally, we have also been interested in the genetic basis of a particular form of dementia, frontotemporal dementia (FTD), a disorder in which a new gene (*PGRN*) was recently discovered.

At present, the focus of this laboratory work is based in two major perspectives: 1) the identification of common genetic variability that confers risk for disease – in order to accomplish this we continue to collect samples from idiopathic forms of dementia and PD cases; 2) the study of rare familial forms of disease, where the common associated genes present no mutations, and then extrapolating the function of genes actually involved to related conditions – for this purpose, we are continuing to collect samples from individuals with positive familial history.

Over the past year we have published a number of papers, but perhaps the two most striking results are the association of mutations in the gene *GBA*, involved in Gaucher disease, with PD; and the description of cytokines as potential biomarkers for Mild Cognitive Impairment and AD.

PUBLICATIONS

Guerreiro, R.J., Santana, I., Bras, J.M., Santiago, B., Paiva, A., and Oliveira, C. Peripheral inflammatory cytokines as biomarkers in Alzheimer's disease and mild cognitive impairment. *Neurodegener. Dis.* 2007. 4(6): p. 406-12.

Bras J, Paisan-Ruiz C, Guerreiro R, Ribeiro MH, Morgadinho A, Januario C, Sidransky E, Oliveira C, Singleton A. Complete screening for glucocerebrosidase mutations in Parkinson disease patients from Portugal. *Neurobiol Aging*. 2007 Dec 19. (*in press*)

Bras, J., Guerreiro, R., Ribeiro, M., Morgadinho, A., Januario, C., Dias, M., Calado, A., Semedo, C., Oliveira, C., Hardy, J., Singleton, A. Analysis of Parkinson disease patients from Portugal for mutations in SNCA, PRKN, PINK1 and LRRK2. *BMC Neurol.* (*in press*)

Camargos S, Scholz S, Simon-Sanchez J, Paisan-Ruiz C, Lewis P, Hernandez D, Ding J, Gibbs JR, Cookson MR, Bras J, Guerreiro R, Oliveira CR, Lees A, Hardy J, Cardoso F, Singleton AB. (2008) DYT16, a novel young-onset dystonia-parkinsonism disorder: identification of a segregating mutation in the stress response protein prkra. *Lancet Neurology*. (*in press*)

5. Cardiovascular Research – Basic Research Unit in Cardiology

(Lino Gonçalves (HUC/IBILI), Pedro Monteiro (HUC/CNC)

Our aim was to determine the effects of pharmacological preconditioning induced by nicorandil, an agonist of the mitochondrial ATP sensitive potassium channel (mitoKATP) on the cardiac mitochondrial function during ischemia-reperfusion injury. Nicorandil abolished the deleterious effects of ischemia-reperfusion on the oxidative-phosphorylating system thus preserving the mitochondrial function, in particular its oxidative capacity and phosphorylating efficiency. In an *ex vivo* isolated heart model of global ischemia, nicorandil abolished not only the development of oxidative stress but also the opening of the mitochondrial permeability transition pore. Furthermore, in a cellular model of simulated anoxia-reoygenation using HL-1 cells, nicorandil was shown to decrease the oxidative stress levels and preserve the cellular viability. Our data suggests that nicorandil protects cardiac mitochondria from ischemia-reperfusion injury via reduction of the mitochondrial oxidative stress and calcium overloading, therefore inhibiting the opening of the mitochondrial permeability transition pore and cardiac cell death. Our work could, therefore, help to clarify the mechanisms involved in the positive effects of nicorandil in the treatment of coronary disease and ischemic cardiomyopathy.
In a project related to diabetes and cardiovascular disease, we sought out to clarify the cardioprotective effects of metformin, an oral antidiabetic drug and its interaction with AMPK (5'-AMP-activated kinase). Our hypothesis was that metformin reduces myocardial infarct size, after an ischemia-reperfusion injury, through the activation of AMPK.
Metformin was able to reduce the extension of myocardial necrosis through the prolonged activation of AMPK during reperfusion. This cardioprotective effect was abrogated in the presence of a specific chemical inhibitor of AMPK. In addition, the beneficial effects of metformin in an acute cardiac setting are also mediated through the activation of cardiac adenosine receptors. Finally, it was demonstrated that the metformin's cardioprotective effects are dependent of the production of nitric oxide (NO) (myocardial infarct size reduction is abolished in the presence of L- NAME, a chemical inhibitor of the NO production). Moreover, metformin is able to activate AMPK and inhibit PTEN (Phosphate and Tensin homologue deleted in chromosome 10) tumor suppressor, thus lowering the threshold for the activation of the RISK (Reperfusion Injury Salvage Kinase) pathway.

PUBLICATIONS

Carreira R, Monteiro P, Gonçalves LM; Providência LA. Nicorandil Preserves The Function of The Mitochondrial Phosphoraltyve and Oxidative System in an Animal Model OG Global Ischemia-Reperfusion. *Rev Port Cardiol* 2007; 28:521-8.

Carreira RS, Miyamoto S, Di Mascio P, Gonçalves LM, Monteiro P, Providência LA, Kowaltowski AJ. Ischemic Preconditioning Enhances Fatty Acid-Dependent Mitochondrial Uncoupling. (2007) *J Bioenerg Biomembr.* 39(4):313-20.

Paiva M, Gonçalves L, Providência L, Mocanu M M and Yellon D M. AMPK Activation at Reperfusion: A Novel Cardioprotective Target? *J of Mol and Cell Cardio*, 2007; 42 (6) (Suppl 1): S207.

6. Dermatology research: contact dermatitis

(Margarida Gonçalo (HUC), Américo Figueiredo (HUC), Teresa Cruz (CNC), Celeste Lopes (CNC))

In collaboration with the Department of Dermatology of the University Hospital, we have continued a research project with two main objectives: 1) the identification of new therapeutic targets for allergic contact dermatitis and 2) the identification of cellular markers that allow the in vitro recognition of the skin sensitization potential of environmental chemicals. We are using a mouse skin dendritic cell (DC) line and human monocyte-derived DC to study 1) intracellular signalling pathways activated by skin sensitizers; 2) changes in the expression of proteins (CD40, CXCR4, thioredoxin) or their mRNA, and in the release of soluble mediators after exposure to skin sensitizers; 3) to correlate the signalling pathways activated by skin sensitizers with the modifications in DC phenotype. This knowledge provides further information about the sensitizing process and thus the sensitizing potential of chemicals.

PUBLICATIONS

Cruz M.T, Gonçalo M., Figueiredo A., Duarte C.B. and Lopes M.C. (2007) Effect of skin sensitizers on inducible nitric oxide synthase expression and nitric oxide production in skin dendritic cells: role of different immunosuppressive drugs. *Immunopharmacol. and Immunotoxicol.,* , 29: 225-241.

Neves B., Cruz M.T., Gonçalo M., Figueiredo A., Duarte C.B., Lopes M.C. (2007) Differential CXCR4 expression induced by skin sensitizersor irritants in dendritic cell models. *J. Investigative Dermatol.*, 127, S2.

Neves B.M., Cruz M.T., Gonçalo M., Figueiredo A., Duarte C.B. and Lopes M.C. Differential modulation of CXCR4 and CD40 protein levels by skin sensitizers and irritants in the FSDC cell line. *Toxicology Letters* (in press)

7. Arthritis research: inflammation

(Fernando Judas (HUC), Alexandrina Mendes (CNC), Celeste Lopes (CNC))

In collaboration with the Orthopaedic and Bone Bank Departments of the University Hospital, we are currently developing the projects entitled "Impact of diabetes on articular chondrocyte functions: identification of pharmacological targets" and "Evaluation of chondrocyte viability and metabolic activity in different conditions of cryopreservation of osteochondral allografts" using normal and osteoarthritic human articular cartilage and chondrocytes. The first project aims at elucidating the role of high and low glucose concentrations as effector mechanisms modulating the chondrocyte functions, in order to identify cellular and molecular links between diabetes and OA. The major objective of the second project is to develop a method for the cryopreservation of osteochondral allografts that maintains chondrocyte viability and metabolic activity and that is suitable for use at the Bone Bank Department of the University Hospital. The results of these two projects are expected, respectively, 1) to identify molecular mechanisms that can be translated into new therapeutic strategies capable of minimizing diabetes-induced cartilage damage and the development of OA; and 2) to improve the survival rate of implanted osteochondral allografts, thus direct and positively affecting the clinical outcome.

PUBLICATIONS

Judas F, Rosa S, Teixeira L, Lopes MC, Mendes AF (2007). Chondrocyte viability in fresh and frozen large human osteochondral allografts: effect of cryoprotective agents. *Transplant. Proc.* 39: 2531-2534.

Rosa SC, Judas F, Lopes MC, Mendes AF. Glucose Uptake and Glucose Transporter-1 expression in human chondrocytes are differentially regulated by high and low glucose concentrations. *Osteoarthritis Cartilage.* 2007; 15 (Sup.C): P211.

Neves A, Rosa SC, Judas F, Salgueiro L, Cavaleiro C, Lopes MC, Mendes AF. α-Pinene prevents Interleukin-1ß-induced NF-kB activation and nitric oxide production in human chondrocytes. *Osteoarthritis Cartilage.* 2007; 15 (Sup.C): P417.

8. **Research in brain cancer: genetic heterogeneity of gliomas**

(Alberto Orfao (Centro de Investigación del Cancer, Salamanca), Fernando Gomes (HUC), Celeste Lopes (CNC))

The project 'Assessment of intratumoral genetic heterogeneity in gliomas: impact on the clinical and biological behaviour of the disease' is being developed in collaboration with Neuropathology Laboratory and Neurosurgery Service of University Hospital of Coimbra and with Research Unit of the University Hospital and Center for Cancer Research of Salamanca. In this project, allelic imbalances in chromosome regions of human gliomas are evaluated using interphase fluorescence *in situ* hybridization (iFISH) and the gene expression profiling is performed by cDNA micro-arrays and single-nucleotide polymorphism (SNP)-array analysis. Tissue samples are obtained from patients diagnosed with gliomas, undergoing surgery at the Neurosurgical Unit of the University Hospital of Coimbra. The iFISH evaluation of allelic imbalances, detected numerical abnormalities in the following chromosome regions: 1p36, 19q13, 7q11, 9p21, 9q34, 10q23, 13q14, 17p13 and 22q11, confirming the existence of complex cytogenetic abnormalities in these tumours. The analysis of larger groups of patients will help to establish the potential clinical relevance of these findings.

PUBLICATIONS

Vital L, Tabernero M D, Crespo I, Rebelo O, Tão H, Gomes F, Castrillo A, Lopes M C, Orfão A. (2007) Analysis of chromosome abnormalities by interphase fluorescence in situ hybridization (FISH) and gene expression profiles in human gliomas. *Chromosome Res.* 15, 1, 212-213.

Vital L, Tabernero M D, Crespo I, Rebelo O, Tão H, Gomes F, Lopes M C, Orfão A. (2007). Study of genetic profile and intratumoral patterns of clonal evolution in gliomas. *Eur. J. of Human Genetics.*, **15**, S 1, 160.

Maillo A, Orfao A, Espinosa AB, Sayagués JM, Merino M, Sousa P, Lara M, Tabernero MD. (2007).Early recurrences in histologically benign/grade I meningiomas are associated with large tumors and coexistence of monosomy 14 and del(1p36) in the ancestral tumor cell clone. *Neuro Onco* l9(4):438-46.

Internationalization

Internationalization

Internationalization has been a permanent concern of the CNC strategy. To attain this goal the researchers have been encouraged to establish colaborations and joint projects with laboratories abroad, and to colaborate in the organizaton of international scientific meetings. A third action line of the Internationalization strategy is the Graduate Studies Programme which is described in the next section of this report.

1. Projects jointly with laboratories abroad

Neuroscience and Disease

Adenosine A_{2A} and dopamine D_{4-7} receptor heteromers: molecular target for caffeine in attention-deficit hyperactivity disorder. Francisco Ciruela (University of Barcelona, Spain), Cristina Oliveira (Hospitais da Universidade de Coimbra, Portugal), Francisco Corte-Real (Instituto de Medicina Legal, Portugal), Rodrigo Cunha (CNC, Portugal).

Anti-NPY antibodies as a tool for the detection of amidated NPY. Eric Grouzmann (Centre Hospitalier Universitaire Vaudois, Division de Pharmacologie et Toxicologie Cliniques, Hôpital Beaumont, Lausanne, Switzerland), Cláudia Cavadas and João O. Malva (CNC, Portugal).

Ataxin-3 activity and its interactors – implication for neurodegeneration in Machado-Joseph disease. Henry Paulson (Department of Neurology, University of Michigan Medical School, MI, USA), Ana Cristina Rego (CNC, Portugal).

Axonal transport of mitochondria in the triple transgenic mouse model of Alzheimer disease. Jorge Busciglio (Department of Neurobiology and Behavior, University of California, Irvine, USA) , Claudia Pereira (CNC, Portugal).

Cell cycle reactivation in the triple transgenic mouse model of Alzheimer disease. Salvatore Oddo / Frank LaFerla (Department of Neurobiology and Behavior, University of California, Irvine, USA) , Claudia Pereira (CNC, Portugal).

Characterization of the BDNF-induced changes in the proteome of cultured hippocampal neurons. Michael Fountoulakis (Foundation for Biomedical Research of the Academy of Athens, Greece), Carlos Duarte (CNC, Portugal).

Cytotoxicity of Th1 and Th17 cells in experimental models of multiple sclerosis. Hartmut Wekerle (Max Planck Institute of Neurobiology, Department of Neuroimmunology, Planegg-Martinsried, Germany), Sofia Domingues and João O. Malva (CNC, Portugal).

Differentiation of oligodendrocytes from neural stem cells. João Relvas (Institute of Cell Biology, Zuerich, Switzerland), Sofia Grade, Fabienne Agasse and João O. Malva (CNC, Portugal).

Does enhanced adenosine formation prevent the early memory dysfunction and synaptotoxicity characteristic of Alzheimer's disease? Detlev Boison (R.S. Dow Neurobiology Laboratories, USA), Rodrigo Cunha (CNC, Portugal).

Dysfunctional mitochondria recruits oligomeric and fibrillar α-syn to shut them down. Russell Swerdlow (Department of Neurology, University of Virginia Health System, Charlottesville, USA), Claudia Pereira (CNC, Portugal).

Electrophysiology of epilepsy in cortical layer 5: role of NPY. William Colmers (Department of Pharmacology, University of Alberta, Edmonton, Canada), Sara Xapelli and João O. Malva (CNC, Portugal).

Electrophysiology of presynaptic kainate receptors. Christophe Mulle (Physiologie Cellulaire de la Synapse, UMR CNRS 5091, Institut François Magendie Bordeaux, France), Paulo Pinheiro and João O. Malva (CNC, Portugal).

Effect of purines in the developing hippocampus: consequences for the establishment of circuits related to learning and memory. Christophe Bérnard (Institut de Neurobiologie de la Méditerranée, France), Scott Rivkees (Yale University, USA), Rodrigo Cunha (CNC, Portugal).

Effect of the Contactin/Caspr complex on AMPA receptor-mediated excitatory postsynaptic currents in hippocampal neurons in culture. Christophe Mulle (University of Bordeaux, Bordeaux, France), Ana Luisa Carvalho (CNC, Portugal).

Functional binding of NPY receptors. David Woldbye (Laboratory of Neuropsychiatry, Rigshospitalet University Hospital O-6102 & Department of Neuroscience and Pharmacology, University of Copenhagen, Denmark), Ana Paula Silva, Fabienne Agasse, Raquel Ferreira and João O. Malva (CNC, Portugal).

Inflammation and epilepsy. Annamaria Vezzani (Institute Mario Negri for Pharmacological Research, Milan, Italy), Liliana Bernardino and João O. Malva (CNC, Portugal).

Interaction between nicotine and caffeine in the basal ganglia: relevance for Parkinson's disease. Krystyna Golembiowska (Polish Academy of Sciences, Poland), Rodrigo Cunha (CNC, Portugal).

In vivo grafting of neural stem cells for brain repair in mouse models of epilepsy. Mohamed Jaber (CNRS UMR 6187 & Université de Poitiers, Poitiers, France), Fabienne Agasse, Raquel Ferreira and João O. Malva (CNC, Portugal).

Mechanisms of cell degeneration in diabetic retinopathy. Alistair Barber and David Antonetti (Penn State Retina Research Group, Penn State College of Medicine, Hershey, Pennsylvania, USA), Francisco Ambrósio (CNC, Portugal).

Mechanisms of cell degeneration in diabetic retinopathy. John Forrester (Department of Ophthalmology, Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK), Francisco Ambrósio (CNC, Portugal).

Microglial nicotinic acetylcholine receptors: role in neuroinflammation and synaptic dysfunction in Alzheimer's disease. Marina Lynch (Trinity College, Ireland), Paula Agostinho (CNC, Portugal), Rodrigo Cunha (CNC, Portugal).

Neuropeptide Y and somastotatin as proneurogenic peptides. William Gray (Division of Clinical Neurosciences, University of Southampton, UK), Fabienne Agasse and João O. Malva (CNC, Portugal).

Neuroprotection by adenosine A_{2A} receptor antagonists: novel mechanisms for new antiparkinsonian drugs. Micaella Morelli (University of Cagliari, Italy), Michael Schwarzschild (Harvard Medical School, USA), Rodrigo Cunha (CNC, Portugal).

Neuroprotective role of insulin and IGF-1 against Huntington's disease-associated diabetes in vitro and in vivo. Patrik Brundin (Wallenberg Neuroscience Center, University of Lund, Sweden), Ana Cristina Rego (CNC, Portugal).

Organization of the Brazilian-Portuguese Symposium on Neurochemistry. Roberto Pais de Carvalho (Programa de Neuroimunologia; Instituto de Biologia, Universidade Federal Fluminense, Niterói, Brasil), João O. Malva (CNC, Portugal).

Organotypic hippocampal slice cultures as a model system in excitotoxicity research - Jens Zimmer (Anatomy & Neurobiology, Institute of Medical Biology, University of Southern Denmark, Odense, Denmark), Sara Xapelli and João O. Malva (CNC, Portugal).

PACAP as a modulator of neuronal phenotype in the retina. Fernando Mello (Instituto de Biofísica Carlos Chagas Filho, Programa de Biofísica, Universidade Federal do Rio de Janeiro, Brasil), João O. Malva (CNC, Portugal).

Prevention by caffeine of memory deficits caused by diabetes. Rolph Grutter (École Polytechnique Fédérale de Lausane, Switzerland), Rui A. Carvalho (CNC, Portugal), Rodrigo Cunha (CNC, Portugal).

Protein cleavage in the ischemic rat brain. Takaomi C. Saido (Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute, Wako, Saitama), Carlos Duarte (CNC, Portugal).

Protein cleavage in the ischemic rat brain. Tadeusz Wieloch (Wallenberg Neuroscience Center, Lund Sweden), Carlos Duarte (CNC, Portugal).

Regulation of AMPA receptors by hyperglycemia in the retina. Espen Hartveit (Department of Anatomy and Cell Biology, University of Bergen, Norway), Francisco Ambrósio (CNC, Portugal).

Relation between cannabinoid- and caffeine-mediated effects in the hippocampus: Relevance for learning & memory. Reinaldo Takahashi, Rui Prediger (Federal University of Santa Catarina, Brazil), Rodrigo Cunha (CNC, Portugal).

Retinal gene expression profiles in an experimental model for diabetic retinopathy. Willem Kamphuis (Netherlands Ophthalmic Research Institute, Amsterdam, The Netherlands) Reinier Schlingemann (Department of Ophthalmology, Academic Medical Center, Amsterdam, The Netherlands), Francisco Ambrósio (CNC, Portugal).

Role of adenosine A_{2A} receptors in the control of the early memory dysfunction caused by unpredictable stress. Jean-Marie Vaugeois (University of Rouen, France), Rodrigo Cunha (CNC, Portugal).

Role of calpains in excitotoxic neuronal damage. Ben A. Bahr (University of Connecticut, Storrs, USA), Carlos Duarte (CNC, Portugal).

Role of cortactin in AMPA receptor traffic. Andras Kapus (The St. Michael's Hospital Research Institute, University of Toronto, Toronto, Ontario, Canada), Ana Luisa Carvalho (CNC, Portugal).

Role of nitric oxide in neural stem cell proliferation. Patrik Brundin and Denis Soulet (Wallenberg Neuroscience Center, Department of Experimental Medicine, Neuronal Survival Unit, Lund University, Lund, Sweden), Francisco Ambrósio (CNC, Portugal).

Role of presynaptic A_{2A} receptors in the control of GDNF-mediated modulation of glutamatergic transmission in cortico-striatal pathways. Sergi Ferré (National Institute of Drug Abuse, USA), J. Alexandre Ribeiro (Instituto de Medicina Molecular, Portugal), Rodrigo Cunha (CNC, Portugal).

Role of presynaptic and microglial adenosine A_{2A} receptors in neuroprotection. Jiang-Fan Chen (Boston University, USA), Rodrigo Cunha (CNC, Portugal).

Structure-function analysis of the NMDA receptor domains involved in synaptic delivery under basal conditions and during synaptic plasticity. Ann Marie Craig (Brain Research Centre, University of British Columbia, Vancouver, BC, Canada), Ana Luisa Carvalho (CNC, Portugal).

The involvement of oxidative stress in Alzheimer disease. George Perry (College of Sciences, University of Texas at San Antonio, Texas, USA), Mark Smith (Institute of Pathology, Case Western Reserve University, Cleveland, USA), Claudia Pereira (CNC, Portugal).

Toxic pathways triggered by activation of Ca^{2+}-permeable AMPA receptors. Edward Barsoumian & Masaki Iizuka (Nippon Boehringer Ingelheim Co., Ltd, Kawanishi Pharma Research Institute, Kawanishi, Japan), Carlos Duarte (CNC, Portugal).

Toxic pathways triggered by activation of Ca^{2+}-permeable AMPA receptors. Jonhatan Ham (Institute of Child Health, University College of London, London, UK), Carlos Duarte (CNC, Portugal).

Transcription regulation by FK506 and BDNF control mitochondrial dependent cell death: a protective role in Huntington's disease. Lisa Ellerby and Birgit Schilling (Buck Institute for Age Research, Novato, CA, USA), Ana Cristina Rego (CNC, Portugal).

Two-photon imaging of neurogenesis in dentate gyris subgranular layer. Rosa Cossart (INSERM U29 – INMED, Parc Scientifique de Luminy, Marseille, France), Liliana Bernardino and João O. Malva (CNC, Portugal).

Use of caffeine to prevent cognitive decline in aging and in experimental models of Alzheimer's disease: influence of gender and of trophic factors. Diogo O. Souza (Federal University of Rio Grande do Sul, Brazil), Rodrigo Cunha (CNC, Portugal).

Molecular Biotechnology and Health

AAV vectors-mediated gene therapy. Sebastian Kugler (Department of Neurology, Faculty of Medicine, S2-Laboratory, University of Göttingen, University of Gottingen, Waldweg 33, 37073 Gottingen, Germany), Luís Pereira de Almeida (CNC, Portugal).

Analysis of the quantitative evolutionary design of metabolic circuits. Michael A. Savageau (University of California, Davis), Armindo Salvador (CNC, Portugal).

Application of non-viral suicide gene therapy approaches in animal models for cancer: molecular and cellular events associated with the antitumor response. Valérie Pierrefite-Carle (Unity INSERM, Faculty of Medicine, Nice, France), M. Conceição Pedroso de Lima (CNC, Portugal).

Application of Time-dependent Density Functional Theory (TDDFT) to study light emission in bioluminescent organisms. Angel Rubio, (Universidade do País Basco, Spain), Fernado Nogueira (CNC, Portugal).

Cell internalization mechanisms of anti-HIV peptides. Abraham Loyter (Department of Biological Chemistry, Institute of Life Sciences, Hebrew University of Jerusalem, Israel), M. Conceição Pedroso de Lima (CNC, Portugal).

Characterization of the metabolism of proliferating cells. Craig B. Thompson (Abramson Family Cancer Center, University of Pennsylvania), Armindo Salvador (CNC, Portugal).

Data Mining of Protein Unfolding Simulations. Paulo Azevedo, (Universidade do Minho, Portugal), Rui Brito e Cândida Silva (CNC, Portugal).

Development of lipid-based gene delivery systems for application in gene therapy. Nejat Duzgunes (University of the Pacific, San Francisco, USA), M. Conceição Pedroso de Lima (CNC, Portugal).

Development of non-viral vectors for siRNA delivery to the central nervous system. Ernst Wagner (Department of Pharmacy, University of Munich, Germany), M. Conceição Pedroso de Lima (CNC, Portugal).
Encapsulation of viral vectors into targeted nanolipid-based carriers: evaluation of therapeutic activity in animal models of ischemia. Mauro Giacca (Laboratory of Molecular Medicine, ICGEB - International Centre for Genetic Engineering and Biotechnology, Trieste, Italy) Sérgio Simões (CNC, Portugal).

Grid Warehousing and Data Mining of Protein Unfolding Simulations. Werner Dubitzky, (University of Ulster, UK), Rui Brito e Cândida Silva (CNC, Portugal).

Investigation of operating principles in systems biology: characterization in the case of the thermal stress response of *Saccharomyces cerevisiae.* Albert Sorribas (University of Lleida, Spain), Armindo Salvador (CNC, Portugal).

Lentiviral vectors-mediated ataxin-3 gene silencing. Nicole Déglon & Philipe Hentraye (URA CEA-CNRS 2210, Service Hospitalier Frederic Joliot, MIRCen Program, Departement de Recherches Medicales, Direction des Sciences du Vivant, Commissariat a l'Energie Atomique (CEA), 91401 Orsay Cedex, France), Luís Almeida (CNC, Portugal).

Lipoplex and peptide-based delivery of steric-block oligonucleotides and application in splice correction. Bernard Lebleu (University of Montpellier 2, UMR 5124 CNRS, Montpellier, France), M. Conceição Pedroso de Lima (CNC, Portugal).

Models of Machado-Joseph disease. Veronica Colomer (John Hopkins, School of Medicine, Baltimore, USA), Luís Pereira de Almeida (CNC, Portugal).

Novel aspartic proteases. John Kay (University of Cardiff, UK), Maria do Rosário F.C. Faro, Isaura Simões, Carlos Faro e Euclides Pires (CNC, Portugal).

Protein docking and drug design. Richard Michael Jackson, (Leeds University, UK), Rui Brito e Carlos Simões (CNC, Portugal).

Protein satability and amyloidogenesis in Ataxin-3. Sandra Macedo Ribeiro, (Universidade do Porto), Rui Brito e Daniela Vaz (CNC, Portugal).

Protein structure and dynamics using high field, multidimensionalsolution NMR. Christina Redfield, (Oxford University, UK) Rui Brito e Daniela Vaz (CNC, Portugal).

Protein structure using high field solid state NMR. Hartmut Oschkinat, (Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany), Rui Brito (CNC, Portugal).

Serine protease inhibitors. Maria Luiza Oliva (Escola Paulista de Medicina, S. Paulo, Brasil), Paula Verissimo, Carlos Faro e Euclides Pires (CNC, Portugal).

Structure-function relationship of aspartic proteases. Daniel Bur (Actelion, Switzerland), Isaura Simões, Carlos Faro e Euclides Pires (CNC, Portugal).

Substrate specificy and inhibition of aspartic proteases. Ben Dunn (University of Florida, Gainesville, USA), Isaura Simões, Paula Veríssimo Carlos Faro e Euclides Pires (CNC, Portugal).

Cell and Molecular Toxicology

A Biophysical Approach to the Role of Lipids in Hepatic Mitochondrial Toxicity. Catherine Brenner (University of Versailles/St Quentin, France), Paulo J. Oliveira (CNC, Portugal).

A biophysical approach to the role of lipids in hepatic mitochondrial toxicity. Teresa Pinheiro (Department of Biological Sciences, University of Warwick, UK), Mª Amália Jurado (CNC, Portugal).

Apoptosis Signaling as a Therapeutic Target in Melanoma. Faustino Mollinedo (Universidad de Salamanca-CSIC, Spain), Paulo J. Oliveira (CNC, Portugal).

Development of microsensors for nitric oxide measurement in tissues. Greg Gerhardt (Dept. Anatomy and Neurobiology, and Center for Microlelectrode Technology (CenMet) University of Kentucky, Lexington, Kentucky, USA), Rui Barbosa and João Laranjinha (CNC, Portugal).

DNA in lipoplexes: bilayer properties and adsorption factors. Rita Dias and Tommy Nylander (Department of Physical Chemistry 1, Lund University, Sweden), Mª Amália Jurado (CNC, Portugal).
Hyperglycaemia-mediated Mitochondrial Dysfunction and Doxorubicin-induced Mitochondrionopathy. Kendall B. Wallace (Medical School, University of Minnesota, Duluth), Paulo J. Oliveira, Carlos M. Palmeira and Anabela P. Rolo (CNC, Portugal).

Mitochondrial and Nuclear Effects of Phytochemicals With relevant Anti-neoplastic Activity. Jon Holy (Medical School, University of Minnesota, Duluth), Paulo J. Oliveira (CNC, Portugal).

Mitochondrial Genetics and Biochemistry. Mitochondrial Genetic Diseases. Gino Cortopassi (University of California, Davies, USA), Carlos M. Palmeira and Anabela P. Rolo (CNC, Portugal).

Mitochondrial Involvement in Neural Stem Cell Differentiation: Role of Morpho-functional Alterations and Relevance for Pos-Transplant Neuronal Death. Ernest Arenas (Karolinska Institute, Sweden), Paulo J. Oliveira (CNC, Portugal).

Molecular Biology Approaches to Investigate Anti-neoplastic Effect of Phytochemicals and Derivatives in Tumour Cells Edward Perkins (Medical School, University of Minnesota, Duluth), Paulo J. Oliveira (CNC, Portugal).

Mitochondrial Tolerance and Liver Ischemic Preconditioning: Pathophysiological Mechanisms. Joan Rosseló (CSIC, Barcelona, Spain), Paulo J. Oliveira (CNC, Portugal).

New biological functions for wine polyphenols: Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite. Rafael Radi (Facultad de Medicina, Universidad de la República, Montevideo, Uruguay), Rui Barbosa and João Laranjinha (CNC, Portugal).

New biological functions for wine polyphenols: Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite. Homero Rubbo (Facultad de Medicina, Universidad de la República, Montevideo, Uruguay), Rui Barbosa and João Laranjinha (CNC, Portugal).

New biological functions for wine polyphenols: Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite. Jon O. Lundberg (Department of Physiology and Pharmacology, Karolinska Institutet, Sweden), Rui Barbosa and João Laranjinha (CNC, Portugal).

Nitric oxide and excitotoxicity. The role of astrocytes. Simon Heales (Institute of Neurology, University College London), Rui Barbosa and João Laranjinha (CNC, Portugal).

Nitric oxide in neurodegeneration and aging. Enrique Cadenas (Dept. Molecular Pharmacology & Toxicology, University of Southern California, USA), João Laranjinha (CNC, Portugal).

Polyphenols and vascular cells redox signaling. Anne Nègre-Salvayre (INSERM-U, Institut Louis Bugnard CHU Rangueil, Toulouse, France), Teresa Dinis and Leonor Almeida (CNC, Portugal).

Microbiology

Cloning, Expression and Regulation of Genes for the Synthesis of Compatible Solutes in Thermus thermophilus. José Berenguer (Universidad Autónoma de Madrid, Spain), Milton Costa (CNC, Portugal).

Combined effect of anti-fungal cell wall inhibitors in *Alternaria infectoria.* Merck, Sharp & Dohme, Neil Gow (Institute of Medical Sciences, University of Aberdeen, UK), Teresa Gonçalves (CNC, Portugal).

Extremely Gamma Radiation-Resistant Bacteria: Taxonomy, Diversity and Physiology. Fred Rainey (Louisiana State University, Baton Rouge LA, USA), Milton Costa (CNC, Portugal).

Microbial Ecology and Diversity of the Hypersaline Deeps of the Red Sea. Robert Huber (University of Regensburg, Germany), Milton Costa (CNC, Portugal).

Molecular identification of pathogenic yeasts. Amparo Querol (IATA, Valencia, España)**,** Aristea Velegraki (Department of Microbiology, Medical School, University of Athens, Greece), Teresa Gonçalves (CNC, Portugal).

New Extremophiles and New Compatible Solutes. Garo Antranikian (Hamburg University of Technology, Germany), Milton Costa (CNC, Portugal).

Role of extracellular ATP and characterization of purinergic receptors in the resistance of C. albicans to macrophage attack. Concha Gil (Departamento de Microbiologia, Facultat de Farmacia, Universidade Complutense, Madrid, España), Neil Gow (Institute of Medical Sciences, University of Aberdeen, UK), Teresa Gonçalves (CNC, Portugal).

Biophysics and Biomedical NMR

Automated metabolic flux analysis of ^{2}H NMR data from the $^{2}H_2O$ ingestion measurement of gluconeogenesis in humans; Metabolic modelling of ^{13}C isotopomer data to hepatic Krebs cycle and gluconeogenic fluxes. Craig Malloy, Dean Sherry, Matthew Merritt, and Shawn Burgess (U.T. Southwestern Medical Center, Advanced Imaging Center, Dallas, TX), John Jones (CNC, Portugal).

Cardiac intermediary metabolism in obesity and diabetes. Gary Lopaschuk (University of Alberta, Mazankowski Alberta Heart Institute, Canada), John Jones (CNC, Portugal).

Cell uptake of Gd-based MRI contrast agents. Milena Salerno (CNRS, Faculty of Medicine, University of Paris 13), Carlos Geraldes (CNC, Portugal).

Characterization of Ga-based chelates as tracers for gamma and PET imaging. Frank Roesch **(**Institute of Nuclear Chemistry, Johannes Gutenberg Universitaet, Mainz, Germany), Carlos Geraldes (CNC, Portugal).

Characterization of Gd-based MRI Contrast Agents. A.D. Sherry (U.T. Southwestern Medical Center, Advanced Imaging Center, Dallas, TX**),** Carlos Geraldes (CNC, Portugal).

Chemical and in vivo animal characterization of MRI contrast agents. Eva Tóth (Institut de Biophysique, CNRS, University of Orleans, France), Carlos Geraldes (CNC, Portugal).

Cytotoxic and Therapeutic Effects of Transition Metal Ions. Virtudes Moreno Martinez (Universidade de Barcelona, Espanha), Carlos Geraldes (CNC, Portugal).

Development of mass spectrometry measurements of glucose deuteration from $^{2}H_2O$. Viswanathan Chandramouli (Case Western Reserve University, Cleveland, USA), John Jones (CNC, Portugal).

Effect of transaldolase activity on tracer measurements of human gluconeogenic flux. Robert Rizza (Mayo Clinic, Rochester Minnesota, USA), John Jones (CNC, Portugal).

European Molecular Imaging Laboratory. Bernard Tavitian (CEA, Orsay, Paris), Network of 56 European Universities, Carlos Geraldes (CNC, Portugal).

In vivo metabolic studies of the diabetic brain. Rolf Gruetter (EPFL, Lausanne, Switzerland), John Jones (CNC, Portugal).

Metabolic modelling of plasma and hepatic glucose kinetics during an oral glucose tolerance test. Karl Thomaseth (ISIB CNR, Padova, Italy), John Jones (CNC, Portugal).

Molecular and celullar mechanisms of lithium action in bipolar disease. Duarte Mota de Freitas (Loyola University of Chicago, USA), Carlos Geraldes (CNC, Portugal).

Modifying hepatic metabolic fluxes by genetic and endocrine manipulation. Robert O´Doherty and Don Scott (University of Pittsburgh Medical Center), John Jones (CNC, Portugal).

NMR and relaxometry of Gd-based complexes and nanoparticles as MRI contrast agents. Joop Peters (Technical University Delft, NL), Carlos Geraldes (CNC, Portugal).

NMR and relaxometric characterization Gd-based MRI Contrast Agents. Ivan Lukes (Charles University of Prague, Chech Republic), Carlos Geraldes (CNC, Portugal).

Non-invasive NMR studies of organ function with stable isotope tracers and contrast agents. Sebastian Cerdan (Laboratorio de RMN, Instituto de Investigaciones Biomédicas, Alberto Sols (CSIC, Universidade Autónoma de Madrid, Espanha), Carlos Geraldes and John Jones (CNC, Portugal).

PARACEST and LIPOCEST based targeted MRI contrast agents. Silvio Aime and Enzo Terreno (Center of Molecular Imaging, University of Torino, Italy), Carlos Geraldes (CNC, Portugal).

Regulation of PDH flux assessed by ^{1}H-[^{13}C]-NMR. Gerald Shulman (University of Yale, Yale School of Medicine), John Jones (CNC, Portugal).

Relaxometric characterization of potential MRI contrast agents. Lothar Helm (EPFL, Lausanne, Switzerland), Carlos Geraldes (CNC, Portugal).

Vanadium Complexes with Ligands derived from Pyrimidinones with Potential Insulin Mimetic Properties. Fernando Avecilla Porto (Universidade da Coruna, Espanha), João Pessoa (Universidade Técnica de Lisboa), Carlos Geraldes (CNC, Portugal).

Cell and Development Biology

Assessment of genetic heterogeneity in gliomas: impact on the clinical and biological behaviour of the disease. Alberto Orfão (Centro de Investigación del Câncer, Universidad de Salamanca, Espanha), Mª Celeste Lopes (CNC, Portugal).

Characterization of a new mucosatropic HPV type: HPV 108. Ethel de Villiers (DKFZ, Heidelberg, Germany), Mª Celeste Lopes (CNC, Portugal).

Implications of Claspin mutations in DNA replication, cell cycle checkpoints and oncogenesis. Raimundo Freire (University Hospital of Canarias, Tenerife, Spain), Mª Celeste Lopes (CNC, Portugal).

Metabolic activity and viability of chondrocytes in cryopreserved human osteochondral allografts. Ali Mobasheri (School of Veterinary Science and Medicine, University of Nottingham, UK), Mª Celeste Lopes (CNC, Portugal).

Mitochondria and embryonic stem cell pluripotency. Christopher Navara and Gerald Schatten (University of Pittsburgh, USA), João Ramalho Santos (CNC, Portugal).

Mitochondrial DNA alterations in sperm that may relate to human fertility. Justin St John (University of Warwick, UK), João Ramalho Santos (CNC, Portugal).

Multifunctional ectoenzymes CD38 and CD157: role in solid tumors and lymphoid malignancies. Fran Lund (Trudeau Institute, Saranac Lake, USA), Mª Celeste Lopes (CNC, Portugal).

New methods to evaluate human sperm quality. Juan Vellez de la Calle (Clinique Pasteur, Brest, France), João Ramalho Santos (CNC, Portugal).

Role of dendritic cells in leishmaniasis: intracellular signaling pathways activated by virulent and attenuated parasite Leishmania infantum. Carmen García-Rodriguez (Spanish Research Council (CSIC) and University of Valladolid, Spain), Mª Celeste Lopes (CNC, Portugal).

Testicular organization and xenotransplanting of testicular tissue in cats. Stefan Schlatt (University of Pittsburgh, USA), João Ramalho Santos (CNC, Portugal).

The role of leptin in energy metabolism. C. Mantzoros (Division of Medicine at the Department of Endocrinology at the Beth Israel Deaconess Medical Center and Harvard Medical School), Eugénia Carvalho (CNC, Portugal).

The role of neuropeptides in wound healing in diabetes. A. Veves (Department of Vascular Surgery at Beth Israel Deaconess Medical Center and Harvard Medical School), Eugénia Carvalho (CNC, Portugal).

The role of the neuropeptides as anti-obesity targets. E. Kokkotou (Division of Medicine at the Department of Gastroenterology at the Beth Israel Deaconess Medical Center and Harvard Medical School), Eugénia Carvalho (CNC, Portugal).

2. Participation in the organization of scientific meetings

January 2007

"Neurogenesis and Neural Stem Cells: potentials and limitations for brain repair" - Advanced Course of the PhD Programme in Experimental Biology and Biomedicine (PDBEB), hosted by the Center for Neuroscience and Cell Biology.
Date: January 29 - February 2, Coimbra
CNC members involved in the organization: Inês Araújo

March 2007

"Purines and related substances in brain research"' - 27th European Winter Conference of Brain Research
Date: March, Villars sur Ollon, Switzerland
CNC members involved in the organization: Rodrigo Cunha

"Stem cells as a Tool in Toxicology" - International Courses on Toxicology 2007 at the Center for Neurosciences and Cell Biology
Date: March 21-23, University of Coimbra
CNC members involved in the organization: Paulo J. Oliveira, Leonor Almeida, Joao Laranjinha, Carlos Palmeira

April 2007

"2nd International Meeting of the Portuguese Society for Stem Cells and Cellular Therapy (SPCE-TC)"
Date: April 27-28, Fundação Bissaya Barreto, Coimbra, Portugal
CNC members involved in organization: Ana Cristina Rego, Ana Catarina Oliveira, Ana Cristina Silva, António Francisco Ambrósio (Chair), Cláudia Cavadas, Inês Araújo, Ana Rita Álvaro, Bruno Carreira

July 2007

"Novel Molecular Strategies to treat Neurodegenerative Diseases" - PENS Summer School 2007
Date: July 8-15, Hotel Ofir, Esposende.
CNC members involved in organization: Ana Cristina Rego

August 2007

"11th Biennial Meeting of the International Society for Neurochemistry"
Date: August, Cancum, México,
CNC members involved in the organization: Rodrigo Cunha

September 2007

"Comunicar Ciência 2007", Workshop promoved by Associação Comunicar Ciência and CNC
Date: September 19-22, CNC, University of Coimbra
CNC members involved in the organization: Cláudia Pereira

October 2007

"SFRR Europe 2007 Meeting" - Meeting of the 'Society for Free Free Radical Research – Europe', organized by the portuguese and spanish groups of Free Radicals.
Date: October 10-13, Vilamoura, Portugal
CNC members involved in organization: Ana Cristina Rego, Catarina Resende Oliveira, João Laranjinha

"Science & Society: Challenges in a Post-Genome Era" - Ciclo de Colóquios
Date: October
CNC members involved in organization: João Ramalho,(co-organization CNC-CES)

December 2007

"XXXVIII Reunião Anual da Sociedade Portuguesa de Farmacologia"
Date: December 5-7, Coimbra
CNC members involved in organization: Cláudia Cavadas, Joana Rosmaninho Salgado, Ana Rita Álvaro

Graduate Studies Programme

Graduate Studies Programme

During 2007, CNC organized 22 Advanced Courses and hosted 37 seminars. The seminars were attended by local graduate students and researchers, whereas the advanced courses also met the interest of people from other Portuguese Universities. Besides the organization of courses and seminars, CNC also supported the ongoing research work for Ph.D. and M.Sc. thesis. Throughout this year, 13 Ph.D. and 10 M.Sc. thesis were concluded.

In October 2002 CNC, with the financial support of FCT, launched an International Doctoral Programme in Experimental Biology and Biomedicine to provide advanced, multidisciplinary, research-oriented training in emerging areas of modern Biology and Biomedicine. The programme included advanced courses in top research areas, taught by forein scientists in collaboration with local investigators, laboratory rotations and research work o be carried out within international networks organized by CNC. The programme provided fellowships to 12 students.

1. Advanced Courses 2007

January 2007

Biostatistics
January 10-12
Pedro Anastácio (Portugal)

Death and Survival Signaling
January 15-19
Armanda Santo(Portugal)s, Emília Duarte (Portugal), Paulo Santos (Portugal), Lloyd Greene (USA), Alun Davies (UK), Leonidas Stefanis (Grece)

Neurodegenerative diseases
January 22-26
Paula Agostinho (Portugal), Claudia Pereira (Portugal), Cristina Rego (Portugal), Luis Almeida (Portugal), Olga Corti (France), Ruth Luthi-Carter (Suisse), Salvatore Oddo (USA)

Neurogenesis and Neural Stem Cells
January 29 - February 02
Inês Araújo e Cristina Rego, Allison Ebert (USA), Mónica Sousa (Portugal), Jia-Yi Li (Sweden), João Bettencourt Relvas (Switzerland)

February 2007

Systems Neuroscience
February 05-09
Miguel Castelo-Branco (Portugal), Rainer Goebel (Netherlands), Rui Costa (USA), Marta Moita (Portugal), Serge Picaud (France)

Immunology
February 19-23
Alexandrina Mendes (Portugal), Teresa Cruz (Portugal), Celeste Lopes (Portugal), *António Bandeiras(France), Kai Zacharowski (UK), Anabela Cordeiro da Silva (Portugal), Carmen Garcia-Rodriguez (Spain)*

NMR
February 26 - March 02
Carlos Geraldes (Portugal), Margarida Castro (Portugal), Rui Carvalho (Portugal), John Jones (Portugal), Bernardo Celda Muñoz (Spain), Shawn Burgess (USA), Silvio Aime (Italy)

March 2007

Microbiology
March 05-09
Milton Costa (Portugal) Nuno Empadinhas (Portugal), André Antunes (Portugal), Paula Morais (Portugal), Gregor Grass (Germany), Teresa Gonçalves (Portugal), António Veríssimo (Portugal), Fred Rainey (USA)

Toxicology
March 21-23
Paulo Oliveira (Portugal), João Laranjinha (Portugal), Leonor Almeida (Portugal), Ernest Arenas (Sweden), Sérgio Dias (Portugal), Horst Spielmann (Germany), Edward Perkins (USA)

Molecular Biotechnology
March 26-30
Paula Veríssimo (Portugal), Luísa Cortes (Portugal)

April 2007

Oncobiology
April 02-06
Celeste Lopes (Portugal), Teresa Martins (Portugal), Alexandra Brás (Spain), Paula Soares (Portugal), Raquel Seruca (Portugal), Alberto Orfão (Spain)

Bioinformatics
April 11-13
Armindo Salvador (Portugal), José Leal (Portugal), Rui Alves (Spain), Ralf Hofestadt (Germany), Thoralf Töpel (Germany)

Modelling of Biological Systems
April 16-20
Armindo Salvador (Portugal), Rui Alves (Spain), Michael Savageau (USA), Jorge Carneiro (Portugal)

October 2007

Molecular Biotechnology
October 1-12
Paula Veríssimo (Portugal), Luísa Cortes (Portugal), Bruno Manadas (Portugal), Mónica Sousa (Portugal)

Biostatistics
October 3-5
Pedro Anastácio (Portugal), Isabel Gordo (Portugal)

November 2007

Molecular Cell Biology
November 12 to 16
Carlos Duarte (Portugal), Edgar R. Gomes (France), Phong Tran (France), Ewa Paluch (Poland), Helder Maiato (Portugal)

Transcription & RNA Biology
November 19 to 20
Sukalyan Chatterjee (Portugal)

Biology of RNA
November 21 to 23
Manuel Santos (Portugal)

December 2007

Reproductive Biology
December 3 to 7
João Ramalho-Santos (Portugal), Maria de Miguel (Spain), Eduardo Ruiz-Pesini (Spain), Miguel Ramalho-Santos (USA)

Development Biology
December 10 to 14
Sofia Araújo (Spain), Rui Martinho (Portugal), Leonor Saúde (Portugal), António Jacinto (Portugal), Isabel Palmeirim (Portugal), Ginés Morata (Spain), Richard Hampson (Portugal), Dan Shaye (Spain), Corinne Houart (UK), Hector Herranz (Spain), Enrique Martin-Blanco (Spain), Pedro Coutinho (UK), Diogo Castro (UK)

2. Seminars

2007 Series | IBILI 16:00 h

January

19 How do neurons die in neurodegenerative disorders and how can we prevent this? Insights from cellular models.
Lloyd Greene | *Columbia University Medical Center, New York, USA*

26 From Parkin gene mutations to Parkinson's disease: Pieces of the puzzle
Olga Corti | *INSERM U679 - Thérapeutique et Neurologie Expérimentale Hôpital de la Pitié-Salpêtriére, Paris, France*

February

2 Application of neural stem cells for brain repair in neurodegenerative disorders – the case of Parkinson's and Huntington's disease
Jia-Yi Li | *Neuronal Survival Unit, Department of Experimental Medical Science, Wallenberg Neuroscience Center, Lund University, Lund, Sweden*

9 Neural mechanisms underlying associative plasticity during auditory fear conditioning
Marta Moita | *Gulbenkian Institute for Science, Oeiras, Portugal*

16 Revisiting excitotoxicity: new insights into neuronal Ca2+ dynamics
Inês Araújo | *Centro de Neurociências e Biologia Celular, Coimbra*

23 Inflammation in the pathogenesis of cardiovascular diseases: new pharmacological approaches
Kai Zacharowski | *Dept. of Anaesthesia, BHI Bristol Royal Infirmary, Bristol, UK*

27 Core metabolism during cell proliferation: how do cells make more cells?
Ralph DeBerardinis | *Abramson Family Cancer Research Institute, The University of Pennsylvania, USA*

March

2 Magnetic Resonance Contrast Agents for Molecular Imaging Applications
Silvio Aime | *Chemistry Department and Center for Molecular Imaging, University of Torino, Torino, Italy*

9 Desiccation and ionizing radiation resistance: Adaptations to life in arid environments
Fred Rainey | *Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana, USA*

16 The role of the endocannabinoid system in neuronal communication and metabolism
Attila Köfalvi | *Center for Neurosciences of Coimbra, and Instituto de Investigação Interdisciplinar, University of Coimbra*

23 Stem cells and Parkinson's disease
Ernest Arenas | *Karolinska Institute, Sweden*

30 Functional genomics studies of endocytosis and signaling
Marino Zerial | *Max-Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*

April

3 Peptide-based delivery of nucleic acids
Bernard Lebleu | *UMR 5235 CNRS, Université Montpellier 2*

4 Diagnosis and follow-up of B-chronic lymphoproliferative disorders by flow cytometry immunophenotyping
Alberto Orfão | *Department of Medicine, Cytometry Service and Cancer Research Centre, University of Salamanca, Salamanca, Spain*

13 Metabolic network analysis and the detection of inborn errors
Ralf Hofestadt | *Institute of Bioinformatics and Medical Informatics, University Bielefeld (Germany)*

17 Mechanisms of protein tyrosine nitration in hydrophilic and hydrophobic biocompartments
Rafael Radi | *Facultad de Medicina Universidad de la República Montevideo, Uruguay*

20 Function design in the evolution of gene circuitry
Michael A Savageau | *Department of Biomedical Engineering, University of California, Davis (USA)*

27 El valor diagnstico de la Microscopia Electronica en Patologia
Juan Cuevas | *Hospital Universitário, Santiago de Compostela, Espana*

May

4 Communication within and between cells to decide life and death
Sukalyan | *Center for Neurosciences and Cell Biology, Coimbra*

18 Aplicações da espectrometria de massa em Biologia: uma introdução à proteómica
Pedro Domingues | *Departamento de Química, Universidade de Aveiro – Aveiro*

25 Interleukin-10 Underlies Distinct Susceptibilities to Mycobacterial Infections
Margarida Correia Neves | *Escola de Ciências da Saúde - Instituto de Investigação em Ciências da Vida e Saúde Universidade do Minho*

28 Dissection of the components for PIP2 activation and thermosensation in TRP channels
Ramon Latorre | *Department of Biophysics, Centro de Estudios Cientificos, Valdivia, Chile (Director)*

31 Dipeptidylpeptidase IV inhibition: the reverse side of the coin
Eric Grouzmann | *Division de Pharmacologie et Toxicology Centre Hospitalaire Universitaire Vaudois Lausanne, Switzerland*

June

1 Shared mechanisms during neural and tracheal morphogenesis in Drosophila melanogaster.
Sofia Araújo | *Institut de Recerca Biomedica de Barcelona (IRBB) IBMB-CSIC, Barcelona*

15 Targeting angiogenesis by polyphenols. From Mechanisms to therapeutics
Raquel Soares e Rita Araújo | *Serviço de Bioquímica, Faculdade de Medicina da Universidade do Porto*

22 LRT3 modulates FGF signalling during regeneration of the adult zebrafish caudal fin
Nuno Afonso | *Instituto Gulbenkian de Ciência, Oeiras, Portugal*

29 Unravelling the Molecular Basis of Parkinson's Disease
Tiago Fleming Outeiro | *Instituto de Medicina Molecular, Lisboa e MGH - Harvard Medical School, USAl*

July

6 The role of superoxide in age-related loss of skeletal muscle mass and function
Malcolm Jackson | *Head, Division of Metabolic and Cellular Medicine School of Clinical Sciences University of Liverpool*

16 Biology of Huntington's disease and Parkinson's disease
Christopher A. Ross | *Neuroscience Director, Division of Neurobiology, Baltimore Huntington's Disease, Center Johns Hopkins University School of Medicine, Baltimore, MD, USA*

17 Oxidative Stress in Alzheimer Disease
George Perry | *Dean and Professor, College of Sciences, The University of Texas at San Antonio*

26 Neurotoxicity of methamphetamine
Syed F. Ali | *Head, Neurochemistry Laboratory, Division of Neurotoxicology, National Center for Toxicological Research/FDA Jefferson, AR, USA*

November

16 *Seminar 1* – "Microfluidics, microtubules, and cellular pattern formation"
Phong Tran | *U. of Pennsylvania, Philadelphia USA & Institut Curie, Paris, France http://www.med.upenn.edu/cellbio/faculty/tran/*

16 *Seminar 2* – "Cell cortex mechanics and cell deformations"
Ewa Paluch | *MPI-CBG, Dresden, Germany & IIMCB, Warsaw, Poland; http://www.imprs-mcbb.de/groupleader/paluch.html*

23 mRNA Mistranslation in Biological Systems
Manuel Santos | *Universidade de Aveiro, Departamento de Biologia*

December

6 What can NMRS do for the study of cerebral metabolism; special emphasis on epilepsy and schizophrenia
Ursula Sonnewald | *Instituttleder INM | NTNU - Institutt for nevromedisin | MTFS, NO - 7489 Trondheim, Department of Neuroscience | NTNU - Faculty of Medicine, Trondheim, Norway*

7 Transcriptional Regulation of Embryonic Stem Cell Pluripotency
Miguel Ramalho-Santos | *University of California at San Francisco, USA*

14 Mechanisms directing epithelial cell replacement during morphogenesis
Enrique Martin-Blanco | *IBMB, CSIC, Barcelona, Spain*

3. Thesis concluded in 2007

Ph.D. Thesis

Ana Isabel Marques Duarte
"Influence of oxidative stress and type 2 diabetes mellitus on neuronal function and metabolism – the neuroprotective role of insulin".
12 de Novembro, 2007
Orientador: Ana Cristina Rego

Ana Margarida da Cruz Ledo
"Dinâmica de Concentração do Óxido Nítrico Produzido no Hipocampo de Rato por Activação de Receptores de Glutamato".
15 de Novembro, 2007
Orientadores: Rui M. Gomes Barbosa e João Laranjinha

Ângelo José Ribeiro Tomé
"Distribuição e função de receptores de ATP extracelular em células cromafins adrenais"
2007
Orientador: Luís Martinho do Rosário

Isabel Maria Nunes Correia
"Interacção de vírus providos de envelope lipídico com células cultura: das fases iniciais de internalização à importação nuclear"
22 de Março, 2007
Orientador: M. Conceição Pedroso de Lima
Co-orientador: Carlos José Faro

Joana Margarida Rosmaninho Salgado
"Mecanismos de regulação de catecolaminas nas células cromafins: papel da interleucina 1-beta e do neuropeptídeo Y"
1 de Junho, 2007
Orientadores: Cláudia Cavadas e Emília Duarte

Joana Maria do Amaral Campos Gil
"Mechanisms of neurodegeneration in transgenic models of Huntington's disease"
13 de Abril, 2007
Orientador: Ana Cristina Rego

Maria Teresa de Jesus Matos
"Alterações induzidas pelo sensibilizador químico DNFB em células dendríticas da pele: regulação das MAPKs, da libertação de IL-1B e de receptores membranares"
Novembro, 2007
Orientadores: Maria Celeste F. Lopes e Carlos Jorge B. Duarte

Maria Teresa Martins da Cunha Oliveira
"Neuronal dysfunction induced by drugs of abuse".
7 de Novembro, 2007
Orientador: Catarina Resende de Oliveira

Margarida V. Caldeira.
"Regulation of the expression of ionotropic glutamate receptors by BDNF in hippocampal neurons".
4 de Maio, 2007
Orientador: Carlos Duarte

Paula Isabel da Silva Moreira
"Disfunção mitocondrial na Doença de Alzheimer e na diabetes: efeito de compostos com propriedades antioxidantes."
16 de Julho, 2007
Orientadores: Maria S. Santos e Catarina R. Oliveira

Paula Matos de Brito
"Papel do resveratrol no contexto da prevenção da aterosclerose: mecanismos moleculares envolvidos na apoptose e proliferação celular".
17 de December, 2007
Orientadores: Teresa do Carmo Pimenta Dinis e Leonor Almeida

Ricardo Jorge Alves Rodrigues
"Neuromodulatory and Neuroprotective Roles of Synaptic P2 Receptors"
19 de Setembro, 2007
Orientador: Rodrigo A. Cunha

Tiago Brandão Rodrigues
"Neuroglial Coupling and the Cerebral Metabolism of Monocarboxylates as Detected by ^{13}C Nuclear Magnetic Resonance"
7 de Dezembro, 2007
Orientador: Carlos F. Geraldes e Sebastián Cerdán Garcia-Esteller

Master Thesis

Ana Carina Fernandes Pais
"Contributo das antocianinas para as actividades antioxidante e vaso-relaxante de vinhos tintos varietais Portugueses"
27 de Setembro, 2007
Orientadores: Leonor Almeida e Teresa do Carmo Pimenta Dinis

Ana Cristina Rosa da Silva
"Papel dos factores de crescimento e da matriz extracelular na diferenciação de células estaminais/precursoras neuronais de ratinho"
5 de Julho, 2007
Orientador: Ana Cristina Rego

Ana Luísa Nabais Gomes Nobre
"Biossíntese de trehalose em Rubrobacter xylanophilus: caracterização das enzimas TPS e TPP".
Maio, 2007
Orientador: Milton S. da Costa

Cátia Filipa Lourenço Marques
"Potenciais efeitos antiaterogénicos de componentes da dieta mediterrânea"
Julho, 2007
Orientador: João Laranjinha

Cristina Mendes de Azevedo
"Efeito protector das antocianinas na oxidação de LDL humanas; relação estrutura-actividade"
24 de Outubro, 2007
Orientadores: Teresa do Carmo Pimenta Dinis e Leonor Almeida

Esther Escribano Aranda
"Compostos de Vanádio com Potencial Actividade Terapêutica. Síntese e caracterização estrutural e alguns estudos dos seus efeitos biológicos num sistema celular modelo"
7 de Outubro 2007
Orientador: Virtudes Moreno e Maria Margarida C. A. Castro

João Paulo Soeiro Terra Teodoro
"Esteatose hepática: mecanismos celulares com relevância na função mitocondrial"
10 de Maio, 2007
Orientadores: Carlos M. Palmeira e Anabela P. Rolo

João Pedro Santos Prata Monteiro
"Avaliação Toxicológica do Metopreno por Dois Modelos Experimentais"
Maio de 2007
Orientadores: Maria Amália da Silva Jurado e António Joaquim Matos Moreno

Maria Inês Frade Marquez Varela Morte
"O papel fisiológico da oxidação lipídica e proteica no sémen de garanhões"
28 de Julho, 2007
Orientador: João Ramalho Santos

Rui Pedro Cunha Sanches
"Transducing systems operated by adenosine A_{2A} receptors in HEK 293 cells"
9 de Outubro, 2007
Orientador: Rodrigo A. Cunha

Technology Transfer

Technology Transfer

Translational research and technology transfer have been progressivly developed in CNC leading to a promising interaction with Industry and local authorities. The outcome of this interaction was the participation of CNC as a founding member of ABAP (Association envolving seven Municipal Councils of the Center Region of Portugal) aiming at knowledge based development). The main contribution of CNC for that goal was the creation of technology transfer unit (Biocant) in colaboration with Cantanhede Municipal Council.
This unit became the anchor of Biocant Park a Biotechnology Park that is rapidly growing by atracting new Biotechnology companies.

1. BIOCANT

Biocant is a private, non-profit, innovation centre created by CNCB together with the municipality of Cantanhede for technology transfer in biotechnology. Founded 3 years ago, Biocant has grown to become a reference in the field and the catalyst of Biocant Park, the first Portuguese biotechnology park.

Biocant is organized into seven main functional units with highly qualified teams and state of art equipment: Genomics, Cellular Biology, Molecular Biotechnology, Microbiology, Bioinformatics, System Biology, Tissue Engineering, and Advanced Services. Biocant provides services and R&D activities based on post-genomic platforms such as whole-genome sequencing, DNA chips, proteomics, interactomics and metabolomics.

Several research projects are currently in progress, some in collaboration with national or international research institutions, hospitals and companies. Throughout the past year Biocant has filed four patent applications and its researchers published papers in journals such as PNAS and JBC. Biocant expects to spin-out its first company by the end of 2008.

2. Companies operating in Biocant Park

At the present 8 companies operate in Biocant Park: Crioestaminal, GeneBox, GenePrediT, GeneLab, Novexem, Hematos, 4Health and Biocant Ventures. Along with Biocant they form a biotech cluster of excellence, bringing together over 100 researchers, in a unique enabling environment. Linking basic and applied research more closely to successful innovation, Biocant paved the way for a new paradigm of economic development in the Center Region of Portugal.

Outreach Programme

Outreach Programme

Outreach Programme aims society scientific education. To attain this goal specific scientific programes are implemented in colaboration with schools and several social and cultural associations.

Society Education Programme

1. **Interaction with high school students: students came to CNC**

"Ciência Viva" programme at CNC - 2007

In July, nine Portuguese and five Spanish students from different secondary schools students participated in the "Ciencia Viva" programme at CNC which was run for 15 solid days. Students were introduced to the organization and research activity of CNC, visited the CNC animal facility and several laboratories located at the Faculty of Medicine, and had also the opportunity to attend to several lecturers by CNC investigators and a seminar given by an international scientist. Students attending the programme were organized in groups in order to follow ongoing projects and the experiments that actually were going to be performed. Each group had also the opportunity to plan and to run themselves several molecular and cell biology techniques as part of brief projects, specially prepared for this event, untitled *"Neurotoxic effects of drugs of abuse"* and *"Hi, DNA!"* (collaboration with the Forensic Medical Institute from the Faculty of Medicine). At the end, each group had to prepare a Power Point document to be presented and discussed with the researchers involved in the scientific activity. The closing session took place at the Science Museum, University of Coimbra.


2. Brain Awareness Week 2007 activities

2.1. Neuroscience Activities at schools

Several activities were organized for 5-17 years old students in 9 schools of the Center region of Portugal. The major goal of the proposed activities (designed accordingly to the grade of education) was to promote contact between students and neuroscientists at school and to show the brain structure and function, what's the role of the brain in connection to the world (five senses), how brain is affected by several conditions, how can we contribute for a healthy brain. Activities included making a neuron model by using playdough, puzzles, painting, a 3-D film about brain areas, short PowerPoint presentation about brain structure and function, notion of a neuron and neuronal communication, five senses, brain curiosities, optical illusions and a healthy brain (role of nutrients, sleep, physical and mental exercise, neuronal damage by alcohol and drugs of abuse). Approximately 600 students participated in these activities.

2.2. "Open laboratories"

Students from a secondary school (about 50 students) had the opportunity to visit a cell culture room in the Institute of Biochemistry, Faculty of Medicine, and the microscopy unit of CNC and to follow a computer programme about some techniques currently performed in the laboratory.

2.3. Public Conferences

Two Public Conferences, attended by approximately 80 people, were organized and well-known researchers were invited. *"Brain and Mathematics"* and *"Stem cells therapy: a future for neurodegenerative disorders?"*


2.4. Interdisciplinary Conferences

During five days, interdisciplinary conferences entitled "*Brain and Mathematics"* were organized with the collaboration of the Department of Mathematics. These conferences, wherein researchers of different areas such as mathematics, informatics, electronics, architecture and neurosciences talked about brain in using different point of views, were open to general public but the target audience was students of different levels (5-18 years). These events took place at the amphitheatre "Chimico" of the new Museum of Science of the University of Coimbra.

2.5. Interactive exposition

The Science Museum has a big hall where the interactive exposition *"Brain: from anatomy to behavior"* took place. This exposition was visited by around 900 persons and was divided in three different stations:

Station 1 – "Brain as a game"

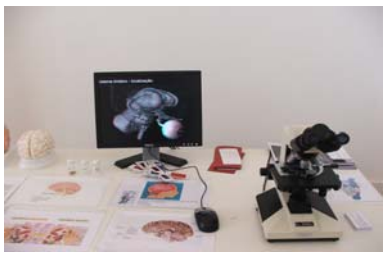
In this station the children and young students had the opportunity to perform simple activities about the brain: activities crosswords, puzzles, create a model of a neuron or a brain using playdough, painting diagrams of brains and neurons.

Station 2 – "Brain and neurons: how they look like?"

This station explained the anatomy of the brain and the structure of the neurons using different strategies: 1) plastic models of the brain; 2) Fixed rat brains of different ages in small vials; 3) poster explaining the anatomy of the brain; 4) Computer with brain images in 3D (the use of special glasses is needed) 5) Labeled neurons by cytochemistry (observation using a microscope).

Station 3 – "Brain, drugs and behavior"

In this station it was explained how neurons communicate and how drugs affect neurons, especially drugs of abuse such as alcohol, nicotine and ecstasy. To achieve this aim three strategies were used: 1) computer with a presentation explaining neurotransmitter release, receptors and effect of the drugs; 2) computer with an interactive presentation explaining the mechanism of action of drugs of abuse (alcohol, nicotine, heroin and ecstasy); 3) to explain the effect of the drugs on behavior it was demonstrated and explained short tests that we use in the lab to study memory, anxiety, motor coordination: hole board test, wire test.

2.6. Funding

The BAW activities were supported by: Federation of European Neuroscience Societies (FENS); Portuguese Society for Neurosciences (SPN); Crioestaminal (BioCant Park, Cantanhede); Reagente 5 (Porto); Ideias Concertadas (Coimbra).

Core Facilities

ANIMAL HOUSE

Head of Unit: Alexandre Pires

Graduate in Agricultural Engineering and Animal Production

Head of Facility since 2006

Staff: Carmen Semião (caretaker), Fátima Graça (assistant technician); Maria Eugénia Campos (assistant technician)

The Animal House is a shared resource that provides services in laboratory animal experimentation and husbrandy, for all CNC and FMUC scientists using animals in their research.
The present facility has a capacity to house about 3000 animals (rats/mice). This facility offers the following services: complete husbandry, including feeding, watering, daily cage changing, as well as routine procurement, inventory and care. In 2007, the facility started to provide specialized animal services, namely: breeding and housing of transgenic/knockout strains of mice as well as wild type colonies, production of rats/mice embryos and litters and maintenance of athymic nude mice.

The Animal House contains a barrier maintained facility, with 8 positive pressurised rooms, which are kept at 22ºC with a relative humidity of 55%. The rodents are breed in individually ventilated cages and a 12-hour light-dark cycle is maintained with an automatic timer. The facility has an animal identification system and software to monitor animal records.

Animal Room – IVC cages (type II)

Laminar flow chamber

FLOW CYTOMETRY UNIT

Head of Unit: Isabel Nunes Correia

PhD in Biochemistry Technology (2007) at University of Coimbra

Head of Facility since 2007

The Flow cytometry Unit provides technical support on flow cytometry both to CNC and external researchers. Currently, it is equipped with a FACSCalibur cell analyser and a separate computer and software to enable researchers to fully analyse their flow cytometry data. For researchers wishing to use flow cytometry in their studies, the unit provides assistance in planning projects, choosing fluorochromes, analyzing experimental results and presenting data.

The Unit organizes annual flow cytometry seminars with the purpose to initiate new users and make this powerful technology known to all researchers, endeavouring to deepen CNC research. Even though the unit has started to operate recently, several CNC research groups are already taking advantage of this facility, performing apoptose, receptor expression and siRNAs intracellular delivery studies, among others.

FACSCalibur cell analyzer

MICROSCOPY UNIT

Head of Unit: Luísa Cortes

PhD in Enzymology (2006) at University of Coimbra

Head of Facility since 2007

The Microscopy Unit provides technical support on the investigation made using Light Microscopy. Besides managing the resources, the unit assists in planning microscopy oriented projects, analysing experimental results, processing acquired images and presenting data. Presently, the unit manages a laser scanning confocal microscope (Zeiss LSM 510 Meta), a P.A.L.M. laser microdissecting microscope, a single cell calcium imaging system, 2 widefield systems and other brightfield microscopes. The systems are prepared for advanced applications which include live cell imaging and single cell calcium measurements, enabling the researchers of imaging dynamic events and molecular interactions.

P.A.L.M. laser microdissecting microscope

The P.A.L.M. laser dissecting microscope is a perfect tool for the isolation of different cell populations within a sample, allowing its full characterization. Using this technology, collaboration has been established, with the service of Anatomical Pathology from the FMUC, with the aim of studying the differences of gene expression between tumour cells at diverse stages.

Laser scanning confocal microscope

MASS SPECTROMETRY UNIT

Head of Unit: Bruno Manadas

Post-Doc, PhD in Cellular Biology (2008) at University of Coimbra

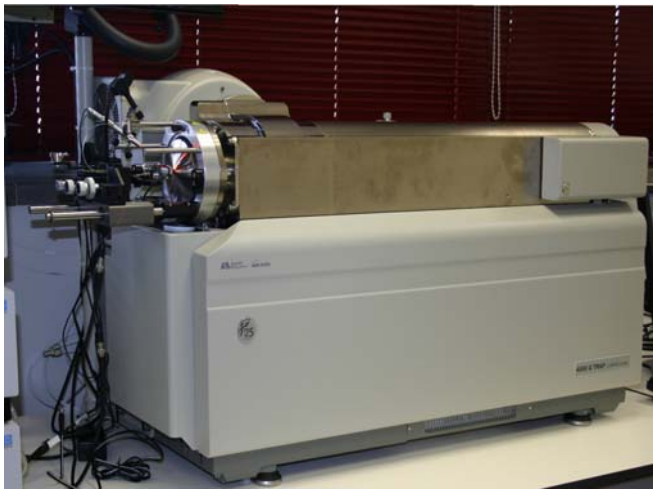
Head of Facility since 2008

Staff: Vera Mendes (technician)

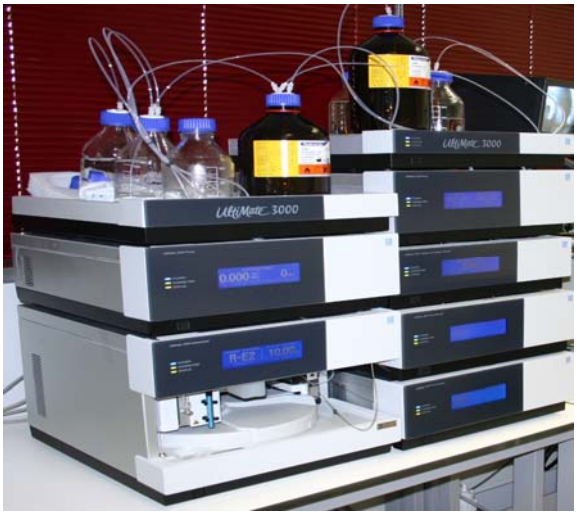
The Mass Spectrometry Unit is specialized in identification and quantification of proteins from simple and complex samples; identification and quantification of post-translational modifications, and identification and quantification of metabolites. The Unit is also involved in the identification of biomarkers through proteomics and metabolomics techniques with the purpose of developing new prognosis and diagnosis methods, in collaboration with other R&D units at CNC, Biocant, and external partners.

Presently, the Mass Spectrometry Unit is equipped with state of the art technology, namely: a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex), hybrid triple quadrupole/ion-trap mass spectrometer with capacity of MS^3, a two-dimensional liquid chromatography system Ultimate 3000 (Dionex/LCPackings), a ExQuest (Bio-Rad) – image acquisition and spot picking robot and a data processing station (connected to two data acquisition stations). The unit also contains several software packages for data processing, including PDQuest and ProteomeWeaver for 2D gel analysis, Protein Pilot and PEAKS for protein identification, post-translational modifications and de novo sequencing.

By combining the high resolving power of the LC system with the structure elucidation from the mass spectrometer, the Mass Spectrometry Unit is able to identify peptides, metabolites, drugs, pesticides, among others, from complex mixtures.
The Unit integrates the National Mass Spectrometry Network (RNEM).



4000 QTRAP mass spectrometer



Bidimensional chromatography modular system coupled to the 4000 QTRAP spectrometer

NMR SPECTROSCOPY UNIT

Head of Unit: Prof. Carlos Geraldes

PhD in Inorganic Chemistry (1976) at Oxford University, UK

Head of Facility since 2008

Staff: John Jones (Assistant Professor)

The Nuclear Magnetic Resonance Spectroscopy Laboratory provides technical support on analysis of liquid and semi-solid samples by Nuclear Magnetic Resonance (NMR) Spectroscopy and Electron Spin Resonance (EPR) Spectroscopy.

The Unit currently stands with a 600 MHz NMR Spectrometer (Varian VNMR 600), a narrow bore 500 MHz NMR Spectrometer (Varian Unity 500), a 20 MHz NMR relaxometer (Bruker mq20) and an X-band EPR Spectrometer (Bruker ESP 300 E).

The state-of-the art equipment comprise unique package of features that can provide information for NMR structural studies, metabolic studies in ex-vivo biosamples and biopsies. The unit also performs 1D, most 2D and some 3D NMR experiments on small-to-medium sized molecules and characterizes aqueous or non-aqueous samples, like paramagnetic and diamagnetic solutions, and biological tissues. Determine the quality control of various samples of industrial interest, such as water contents in oils, study small paramagnetic complexes and paramagnetic metalloproteins, and execute spin label and spin trap research, are also main areas of significance in our Unit.

This Unit integrates the Portuguese Nuclear Magnetic Resonance Network (PTNMR).

Varian 600 NMR Spectrometer

Services

SERVICES

Under the present contract, CNC Laboratório Associado in colaboration with AIBILI has the obligation to provide specialized services to community.

CNC SERVICES

Biochemical and Molecular Biology Analysis:

Coordinators: *Catarina Oliveira, Isabel Carreira, Manuela Grazina.*

Ana Cristina Franco dos Santos
Cândida Elsa Frias Mendes
Carla Margarida dos Santos Veríssimo
João Miguel Pratas
José Miguel Brás
Luís Miguel M. Vidal Oliveira
Marta Sofia Marques Simões
Maria do Rosário F. da Costa Faro
Maria Helena Garruncho
Manuela Grazina
Rita Guerreiro

1- Biochemical and Molecular Biology analysis

Mitochondrial Respiratory Chain (MRC)

Oxygen consumption and double wavelength spectrophotometry evaluation
There were studied 56 subjects suspected of Mitochondrial Cytopathy, corresponding to the analysis of 68 samples (some patients had 2 or more tissues analysed), including 47 lymphocytes isolated of peripheral blood, 17 muscular biopsies and 4 liver biopsies. A MRC deficiency was detected in 21 patients.

Mitochondrial DNA (mtDNA) studies

Molecular differential analysis of mitochondrial cytopathies, as a highthrouput screening, has been performed by sequencing analysis of 11 mtDNA regions, covering a total of 424 mtDNA sequence variations (point mutations/polymorphisms). We have continued to screen deletions by flanking PCR of 6 hot-spot regions.

We have received 118 samples from 108 patients suspected of Mitochondrial Cytopathy, for DNA extraction including blood (99), fibroblasts of skin biopsy (2), muscle (15) and liver biopsies (2), comprising a total of 1317 and 983 PCR reactions for point mutations and deletions analysis, respectively. Deletions have been detected in 36 samples (2 confirmed by sequencing analysis; 34 under study) and point mutations/polymorphisms have been detected in 117 samples of 101 patients. Further PCR-RFLP analysis was performed to validate point mutations in 76 samples of 33 patients.

Amino Acid Analysis

Our laboratory received 425 samples (362 - plasma, 54 - urine and 9 - cerebrospinal fluid) of physiological fluids for amino acid analysis. The subjects evaluated included children, adolescents and adults, in three clinical conditions: (1) selective screening of metabolic disorder, characterized by either primary or secondary abnormalities in the amino acid profile (2) amino acid profile changes secondary to proximal renal tubular or hepatic dysfunction of any origin; (3) nutritional evaluation of patients with protein restrictive diets.

The evaluation of homocysteine has been implemented for diagnosis and follow-up of metabolic disorders involving this amino acid, such as homocystinuria.

2- Genetic testing in neurodegenerative disorders

As last year we have continued to focus on the genetic factors known to underlie the process of neurodegeneration, mainly in Alzheimer's (AD) and Parkinson's (PD) diseases.

A total of 158 samples have been processed in the Neurogenetics Laboratory in 2007, including: 124 from dementia patients (17 from Frontotemporal dementia patients, 25 from Mild Cognitive Impairment patients, 39 from Alzheimer's disease and 43 from other types of dementia), 23 from Parkinson's disease patients, 5 from other neurological diseases and 6 control subjects. Genes associated with these diseases were screened in most samples, and mutations were found in *LRRK2*, *PS-2 PARK2, GBA, PGNR* and *HFE* . We have also found that common variability in a region of chromosome 6 may be associated with PD in our series of patients, and we are currently following up on these results. Apolipoprotein-E genotype, the common risk factor for Alzheimer's disease, was determined in 158 individuals and the COMT functional SNP in exon 4 (rs165688) was assessed in 29 subjects.

As of 2007, the Neurogenetics Laboratory at CNC offers genetic testing for AD and PD patients, in order to aid in the clinical diagnosis of these disorders.

AIBILI SERVICES

Ophthalmological clinical trials and studies on bioavailability are provided by AIBILI on the basis of protocol established between CNC and AIBILI, in the frame of the "Acordo de Contrato"signed between CNC and FCT.

AIBILI is a Research Technology Organisation (RTO) in the Health Market dedicated to help the development of new products for pharmaceutical and biotechnology companies.

AIBILI is certified by ISO 9001:2000 since 2004. Clinical Trials are performed in accordance with ICH Good Clinical Practice Guidelines and the Bioavailability and Pharmacokinetic Studies are also developed in compliance with the OCDE Principles of Good Laboratory Practice.

AIBILI has the following Units:

- *Centre for Clinical Trials (CEC)*
- *Centre for Bioavailability Studies (CEB)*
- Centre of New Technologies for Medicine (CNTM)
- Administrative Services (SA)
- Quality Management Unit (UGQ)
- Technology Transfer Unit (UTT)

1- Ongoing Clinical Trials in Ophthalmology

Director

Maria Luísa Ribeiro, MD, MSc

Principal Investigators

Maria Luísa Ribeiro, MD, MSc
Lilianne Duarte, MD
Conceição Lobo, MD PhD
João Figueira, MD MSc
Joaquim Murta, MD PhD
José Cunha-Vaz, MD PhD
Rufino Silva, MD MSc
Rui Daniel Proença, MD PhD

Investigators

Ana Esmeralda Costa, MD
Andreia Rosa, MD
Filipa Ponces, MD
Helena Azevedo, MD MSc
Isabel Pires, MD
Jorge Henriques, MD
Luís Cristóvão, MD
M.ª João Quadrado, MD MSc
M.ª Júlia Veríssimo, MD
M.ª Luz Cachulo, MD
Marília Rocha, PharmD
Mário Alfaiate, MD MSc
Patrícia Leitão Carvalho, MD
Paula Magro, MD
Pedro Faria, MD
Pedro Fonseca, MD

Study Coordinators

Adozinda Simão
Carla Duarte
Catarina Neves
Renata Castanheira

Technicians for Diagnostic Procedures

Aldina Reis, MSc
Ana Rita Santos
António Pedro Melo
Graciete Abreu
Mário Soares
Maria Pedro Silva

Nurses

Alexandra Tavares
Carla Duarte, BSc
Eugénia Cardoso
Maria do Céu Simões

Macular Edema after CRVO

- A Six- Month, Phase 3, Multicenter, Masked, Randomized, Sham-Controlled Trial (with Six-Month Open-Label Extension) to Assess the safety and Efficacy of 700µg and 350µg Dexamethasone Posterior Segment Drug Delivery System (DEX PS DDS) Applicator System in the Treatment of Patients with Macular Edema Following Central Retinal Vein Occlusion or Branch Retinal Vein Occlusion

Diabetic Macular Edema

- Reduction in the Occurence of center-threatening Diabetic Macular Edema
- The effect of Ruboxistaurin on clinically significant Macular Edema in patients with diabetes Mellitus, as assessed by optical coherence tomography
- An extension of the double-blind, randomized, placebo-controlled, parrallel-group, multicenter study evaluating safety, tolerability and effect on MRI lesion parameters of FTY720 vs placebo in patients with relapsing multiple sclerosis
- A randomized, double-masked, multicenter, phase II study assessing the safety and efficacy of two concentrations of ranibizumab (intravitreal injections) compared with non-treatment control for the treatment of diabetic macular edema with center involvement
- A randomized, double-masked, parallel group, multi-center, dose-finding comparison of the safety and efficacy of ASI-001A 0.5 µg/day and ASI-001B 0.2 µg/day fluocinolone acetonide intravitreal inserts to sham injection in subjects with diabetic macular edema
- A 12-month double-blind, randomized, multicenter, active-controlled, parallel-group study comparing the efficacy and afety of 0.5mg and 1.25mg fingolimod (FTY720) administered orally once daily versus interferon β-1a (Avonex®) administered i.m. once weekly in patients with relapsing-remitting multiple sclerosis
- A 3-Year, Phase 3, Multicenter, Masked, Randomized, Sham-Controlled Trial to Assess the Safety and Efficacy of 700 µg and 350µg Dexamethasone Posterior Segment Drug Delivery System (DEX PS DDS) Applicator System in the Treatment of Patients with Diabetic Macular Edema
- A Phase 2/3 Randomized, Controlled, Double-Masked, Multi-Center, Comparative Dose-Finding Trial, in Parallel Groups, to Compare the Safety and Efficacy of Intravitreous Injections of 0.3, 0.03 or 0.003mg Pagaptanib Sodium (Macugen®), Given as Often as Every 6 Weeks for 3 years, to Sham Injections, in Subjects with Diabetic Macular Edema (DME) Involving theCenter of the Macula

Glaucoma

- A five-year, multicenter, open-label study to evaluate the safety of once-daily evening instillation of travoprost 0,004% eyedrops (Travatan®) in subjects with open-angle glaucoma or ocular hypertension
- Study of the Efficacy and Safety of Travatan® Therapy compared with Cosopt® Therapy in Patients with Open-Angle Glaucoma or Ocular Hypertension

Age-Related Macular Degeneration

- A phase II/III randomized, double-masked, controlled, dose-ranging, multi-center comparative trial, in parallel groups, to establish the safety and efficacy of intravitreal injections of Eye001 (Anti-VEGF Pegylated Aptamer) given every 6 weeks for 54 weeks, in patients with exudative age-related macular degeneration (AMD)

- An evaluation of efficacy and safety of posterior juxtascleral injections of anecortave acetate 15mg (0.5ml of 30 mg/ml anecortave acetate sterile suspension) versus vehicle in patients with subfoveal choroidal neovascularization (CNV) due to exudative age-related macular degeneration (AMD)

- An evaluation of efficacy of posterior juxtascleral administration of Anecortave Acetate for depot suspension (15mg or 30mg) versus sham administrations in patients (enrolled in study "A" or study "B") at risk for developing sight-threatening choroidal neovascularization (CNV) due to exudative age-related macular degeneration (AMD)

- A phase 3B, randomized, active controlled, double-masked, single dummy, multi-center comparative trial, in parallel groups, to compare the safety and efficacy of intravitreous injections of Macugen™ given every 6 weeks for 54 weeks (to be extended to 102 weeks as indicated), to Macugen™ plus PDT with Visudyne®, in subjects with predominantly classic subfoveal chorodial neovascularization (CMV) secondary to age-related macular degeneration (AMD)

- An open label evaluation of long term efficacy and safety of posterior juxtascleral injections of anecortave acetate 15mg in patients with subfoveal exudative age-related macular degeneration (AMD)

- A randomized, double-masked, active-controlled, multicenter study comparing the efficacy and safety of ranibizumab (0.3mg and 0.5mg) administered as two dosing regimens in patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration

- An open label, non-comparative protocol for the use of pegaptanib sodium injection every 6 weeks in patients with exudative age-related macular degeneration (AMD)

- A 102-week, open label, multicenter trial to investigate the efficacy of macugen for the preservation of visual function in subjects with neovascular age-related macular degeneration (AMD) and to assess the benefit of treating early choroidal neovascularization (CNV)

- A phase IIIb, open-label, multi-center 12 month study to evaluate the safety, tolerability and efficacy and of ranibizumab (0.3mg) in patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration

- A phase 3, randomized, double-masked, parallel-assignment study of intravitreal bevasiranib sodium, administered every 8 or 12 weeks as maintenance therapy following three injections of Lucentis compared with Lucentis monotherapy every 4 weeks in patients with Exsudative Age-Related Macular Degeneration (AMD)

- A phase IV, lon-term, open-label, multicenter extension study to evaluate the safety and tolerability of ranibizumab in patients with subfoveal choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD)

Uveitis

- An 8-week, multicenter, masked, randomized trial (with an 18-week masked extension) to assess the safety and efficacy of 700 µg and 350 µg dexamethasone posterior segment drug delivery system (DEX PS DDS) applicator system compared with sham DEX PS DDS applicator system in the treatment of non-infectious ocular inflammation of the posterior segment in patients with intermediate uveitis

Ongoing Studies on Bioavailability

Coordinator

Tice Macedo, MD, PhD

Study Director

Carlos Fontes Ribeiro, MD, PhD

Director

Carla Neta, BSc

Principal Investigators

Carlos Fontes Ribeiro, MD, PhD
Luís Cunha, MD, PhD
Manuela Carvalheiro, MD, PhD
Tice Macedo, MD, PhD

Investigators

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Ana Isabel Rodrigues, BSc
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Cristina Machado, MD
Dírcea Rodrigues, MD
Fernando Silva, MD
Francisco José Inácio, MD
Júlia Figueiredo, BSc
Luís Jorge Negrão, MD
Maria Isabel Santana, MD, PhD
Marília Rocha, PharmD
Maria Vitor Campos, MD
Paula Pires, MD

Technical

Carla Neta, BSc
Ana Pedroso, BSc
Filipe Martins, BSc
João Silva

Nurses

Carla Duarte, BSc
Célia Marques
Maria Céu Simões
Olga Queirós dos Anjos, BSc
Paulo José Marques, BSc

- ***Bioavailability/Bioequivalence Studies***
  - Execution of an open, randomized and crossover study on the bioequivalence between suspensions containing 400 mg/5 ml of acyclovir from two different pharmaceutical laboratories
  - Execution of an open, randomized and crossed-over study on the bioequivalence between coated tablets containing 30 mg of deflazacort from two different pharmaceutical laboratories
  - Execution of an open, randomized and crossed-over study on the bioequivalence between coated tablets containing 6 mg of deflazacort from two different pharmaceutical laboratories
- ***Clinical Trials***
  - Real-Life Effectiveness and Care Patterns (in Portugal) of Diabetes Management – The RECAPP-DM study

- A 54-week, double-blind, randomized, placebo-controlled, parallel-group study to investigate the effects of rosiglitazone as adjunctive therapy to donepezil on cognition and overall clinical response in APOE e-stratified subjects with mild to moderate Alzeheimer's disease (REFLECT-2)

- Food-effect and dosage form proportionality study of esclicarbazepine acetate market formulation in healthy subjects

- Multicentre, double-blind, randomized, active and placebo controleed trial to investigate the efficacy and tolerability of nebicapone in parkinson's disease patients with "wearing-off" phenomenon treated with levodopa/carbidopa or levodopa/benserazide

- A randomized, comparative, double-blind, parallel-Group, Multicenter, Monotherapy, study of Pregabalin (Lyrica) and Lamotrigine (Lamictal) in patients with newly diagnosed partial seizures

- A Pan-European randomized, parallel group, two-arm placebo-controlled, double-blind multicenter study of Rimonabant 20mg once daily in the treatment of abdominally obese patients with impaired fasting blood glucose with or without other comorbidities

- A multicenter, randomized, double blind, parallel-group placebo and pramipexole controlled study to assess efficacy and safety of SLV308 monotherapy in the treatment of patients with early stage Parkinson's disease

- Clinical Trial to asses the efficacy of probiotic milk

- An open label SLV308 safety extension to study S308.3.003 in early PD patients

- ***Drug Dosages Studies***
  - Identification and quantification of azithromycin and related known and unknown substances in different batches of raw material

  - Dosage of S- and R-warfarin in plasma samples from a clinical trial

  - Dosage of metformin in plasma samples from the BIA-2093-125 clinical trial

  - Dosage of gliclazide in plasma samples from the BIA-2093-126 clinical trial

  - Dosage of antidiabetics in plasma samples from the BIA-2093-206 clinical trial

3. Financial Expenses

The financial expenses of AIBILI with respect to the Associate Laboratory in 2007 were 101.631,62€ and concern the payment of the salary of 3 graduate technician.

Funding

Funded Ongoing Projects

Title	Financing Agency	Duration	Total Financing (CNC)	Financing 2007
National Projects:				
Regulation of the expression of ionotropic glutamate receptors by BDNF in hippocampal neurons Coordinator: Carlos Jorge Alves Miranda Bandeira Duarte	FCT Refª: POCTI/BCI/46466/2002	01/03/2003 to 28/02/2007	97.964,00	0,00
Neuroprotective changes in the proteome induced by brain-derived neurotrophic factor (BDNF): proteomic studies in cultured hippocampal neurons Coordinator: Carlos Jorge Alves Miranda Bandeira Duarte	FCT Refª: POCTI/NSE/46441/2002	01/03/2003 to 28/02/2007	116.000,00	0,00
Neuroprotective effect of neuropeptide y in excitotoxicity and in temporal lobe epilepsy Coordinator: Catarina Isabel Neno Resende de Oliveira Participants: Faculdade de Medicina da Universidade de Coimbra (FMUC)	FCT Refª: POCTI/NSE/46848/2002	01/09/2003 to 31/08/2007	45.000,00	0,00
Targeted antisense therapy: a novel approach for the treatment of human small cell lung cancer Coordinator: João Nuno Sereno de Almeida Moreira Participants: Instituto Nacional de Engenharia, Tecnologia e Inovação (INETI)	FCT Refª: POCTI/FCB/48487/2002	01/09/2003 to 31/08/2007	33.700,00	0,00
Targeted Contrast Agents for MRI and Nuclear Scintigraphy (TARCAS) Project Coordinator: Carlos Frederico de Gusmão Campos Geraldes Departamento de Bioquímica, FCT, Universidade de Coimbra Participants: IBILI-Universidade de Coimbra; Universidade de Aveiro; Universidade de Minho	FCT Refª: POCTI/QUI/47005/2002	01/10/2003 to 30/09/2007	19.230,00	0,00
Biochemical and molecular analysis of hepsin, protease implicated in prostate cancer Coordinator: Carlos José Fialho da Costa Faro Participants: Instituto Português Oncologia (IPO)	FCT Refª: POCTI/CBO/49334/2002	01/10/2003 to 31/01/2007	68.400,00	0,00
Amyloid Fibril Formation by Transthyretin: searching for the amyloidogenic intermediates Coordinator: Rui Manuel Pontes Meireles Ferreira de Brito	FCT Refª: POCTI/BME/49583/2002	02/01/2004 to 01/01/2007	123.528,00	0,00

Aspartic proteinase content of Arabidopsis genome: identification, expression and characterization Coordinator: Carlos José Fialho da Costa Faro	FCT Refª: POCTI/BCI/48400/2002	02/01/2004 to 01/01/2007	60.000,00	0,00
Development of novel synthetic viral vectors for gene transfer: expression of angiogenic growth factors vascular diseases therapy Coordinator: Sérgio Paulo de Magalhães Simões	FCT Refª: POCTI/BIO/48735/2002	01/03/2004 to 28/02/2008	115.000,00	0,00
Mechanisms of nucleo-cytoplasmic trafficking of ASFV Egenome and design of anti-NLS/NS backbone cyclic peptides to block viral infection Coordinator: Mª da Conceição Monteiro Pedroso de Lima	FCT Refª: POCTI/CVT/44854/2002	01/12/2004 to 30/11/2007	80.000,00	20.067,67
Relação entre alterações metabólicas no hipocampo e défices de memória induzidos por diabetes. Coordinator: Rui Albuquerque Carvalho	FCT Ref.ª : POCI/SAU-NEU/56098/2004	01/01/2005 to 31/01/2008	89.663,00	36.329,63
Neuroprotecção por receptores de adenosina: acoplar o aumento da formação de adenosina com o bloqueio de receptores A2A Coordinator: Catarina Resende de Oliveira	POCI/SAU-FCF/59215/2004	01/01/2005 to 29/02/2007	85.000,00	12.923,32
Caracterização dos receptores purinérgicos nas fibras musgosas do hipocampo – papel na epilépsia Coordinator: Rodrigo Pinto dos Santos Antunes da Cunha	FCT Refª: POCI/SAU-NEU/59135/2004	01/01/2005 to 28/02/2007	86.500,00	0,00
Controlo pela adenosina da neuro-inflamação Coordinator: Rodrigo Pinto dos Santos Antunes da Cunha	FCT Refª: POCI/BIA-BCM/59980/2004	01/01/2005 to 28/02/2007	85.000,00	0,00
Endereçamento dos receptores AMPA para a sinapse: papel dos parceiros proteicos no endereçamento sináptico das subunidades GluR4 e GluR2L Coordinator: Ana Luísa Monteiro de Carvalho	FCT Refª: POCI/SAU-NEU/58955/2004	01/01/2005 to 31/12/2007	93.000,00	33.072,00
Ecologia e diversidade microbiana em ambientes abissais hipersalinos do Mar Vermelho Coordinator: Milton Simões da Costa	FCT Refª: POCI/BIA-BDE/56014/2004	01/01/2005 to 31/03/2008	90.000,00	30.720,00
Defesas contra agressão ambiental. Biossíntese de solutos compatíveis em bactérias extremamente resistentes a radiaçõesdo género Rubrobacter" Coordinator: Milton Simões da Costa Participants: Instituto Tecnologia Quimica e Biológica (ITQB)	FCT Refª: POCI/BIA-MIC/56511/2004	01/01/2005 to 30/04/2008	71.500,00	23.928,00

Veiculos baseados em biomateriais para medir o trnsporte de genes para o sistema nervoso periférico Entidade Proponente INEB Porto Coordinator CNC: Sérgio Magalhães Simões	FCT Ref.º POCI/SAU-BMA/58170/2004	01/01/2005 to 31/08/2008	8.700,00	1.041,99
New nanocrystalline ductile coatings for biomedical applications Entidade Proponente FCT Coordinator CNC: Carlos Duarte	FCT Ref.ª POCI/CTM/55967/2004	01/01/2005 to 31/08/2007	6.000,00	5.000,00
Compatible solutes from extremophiles: screening, biosynthesis and heterologous production Coordinator: Milton Simões da Costa	FCT Ref.ª : 010.6/A005/2005	01/03/2005 to 29/02/2008	99.168,00	37.342,08
O ATP e a retinopatia diabética: papel na regulação da microglia e na morte neuronal Coordinator: Paulo Fernando Martins dos Santos	FCT Refª: POCI/SAU-NEU/59003/2004	01/03/2005 to 31/08/2008	87.016,00	21.400,00
Caracterização funcional de espermatozóides equinos. Avaliação molecular da fertilidade de garanhões. Coordinator: João Ramalho de Sousa Santos	FCT Refª: POCTI/CVT/49102/2002	01/04/2005 to 31/03/2008	65.000,00	17.600,00
Interacção entre catecolaminas e neuropeptídeo Y nas células cromafins humanas Coordinator: Claúdia Margarida Gonçalves Cavadas	FCT Refª: POCI/SAU-FCF/60399/2004	01/04/2005 to 31/03/2008	55.000,00	18.500,00
Determinantes moleculares de neurotoxicidade e agregação da ataxina-3 na doença Machado-Joseph Coordinator: Sandra de Macedo Ribeiro	FCT Refª: POCI/SAU-MO/60156/2004	01/06/2005 to 31/12/2007	94.996,00	0,00
Acção protectora de polifenóis do vinho tinto na inflamação e disfunção do endotélio vascular: Implicações na prevenção da aterosclerose Coordinator: Leonor Martins de Almeida	FCT Refª: POCI/AGR/59919/2004	02/05/2005 to 30/06/2009	64.280,00	11.411,50
Influência das Soluções de Preservação para Transplante nos Perfis Metabólico e Imunológico Cardíacos Coordinator: Rui de Albuquerque Carvalho	FCT Refª: POCI/SAU-OBS/55802/2004	01/07/2005 to 30/06/2008	89.985,00	22.005,00
Formulações de inibidores de microtubulinas no tratamento de leishmaniose visceral zoonótica Coordinator: Sérgio Paulo de Magalhães Simões Participants: Instituto Nacional de Engenharia, Tecnologia e Inovação (INETI); Instituto de Higiene e Medicina Tropical	FCT Refª: POCI/CVT/56995/2004	01/07/2005 to 30/06/2008	5.644,00	0,00

Manipulação de DNA em solução e interfaces Coordinator: Sérgio Paulo de Magalhães Simões Participants: Faculdade de Ciências e Tecnologia	FCT Refª: POCI/QUI/58689/2004	01/07/2005 to 30/06/2008	4.440,00	2.040,00
Depressão Pós-parto e Sono Coordinator: Sandra Maria Rodrigues de Carvalho Bos Participants: Instituto de Psicologia Médica	FCT Refª: POCI/SAU-ESP/57068/2004	01/08/2005 to 31/07/2008	38.250,00	5.250,00
Estratégias de melhoramento do efeito terapêutico de fármacos antimicobacterianos Coordinator: Sérgio Paulo de Magalhães Simões Participants: Instituto Nacional de Engenharia, Tecnologia e Inovação (INETI), Universidade do Minho	FCT Refª: POCI/SAU-FCF/58355/2004	01/08/2005 to 31/07/2008	4.330,00	0,00
Algumas gotas de água; a diversidade microbiana na água de estalactites e estalagmites Coordinator: António Manuel Veríssimo Pires	FCT Refª: POCI/BIA-BDE/60704/2004	15/08/2005 to 31/12/2008	84.000,00	25.008,00
Controlo da morte celular dependente da mitocôndria por regulação da transcrição na presença de FK506 e BDNF - papel protector na doença de Huntington. Coordinator: Ana Cristina Rego	FCT POCTI/SAU-NEU/57310/2004	01/09/2005 to 31/08/2008	98.103,00	33.602,62
Vectores lentivirais e silenciamento de genes em doenças de poliglutaminas: Expressão de RNAs de cadeia dupla mediada por vectores lentivirais para silenciamento do gene da ataxina-3. Coordinator: Luis de Almeida	POCI/SAU-MMO/56055/2004	01/09/2005 to 31/08/2008	30.000,00	8286,00
Vectorização de fármacos para os vasos sanguíneos tumorais: uma nova terapia para o cancro da mama humano. Coordinator: João Nuno Moreira	FCT POCI/SAU-OBS/57831/2004	01/09/2005 to 29/02/2008	45.000,00	18. 912,00
A Microglia na Neo-neurogénese Associada à Epilepsia do Lobo Temporal: Papel das Citocinas Pró-inflamatórias e do Neuropeptídeo Y na Neuroprotecção dos Novos Neurónios Coordinator: João José Oliveira Malva	FCT Refª: POCI/SAU-NEU/58492/2004	01/09/2005 to 31/08/2008	76.000,00	25.333,00
Metabolismo hepático intermediário da glucose em crianças com e sem actividade da glucose-6 fosfatase hepática Coordinator: John Jones	FCT POCI/QUI/55603/2004	01/10/2005 to 30/09/2007	65.500,00	10.790,00
Efeito do consumo crónico de cafeína da neuromodulação exercida pela adenosina - possível relevância em processos de aprendizagem e memória. Coordinator: Rodrigo Cunha	FCT Refª: POCI/SAU-FCF/59601/2004	01/10/2005 to 31/12/2007o	56.400,00	24.000,00

Estudos estruturais da biogénese do proteassoma: determinação da estrutura do Ump1 e do seu complexo com precursores do proteassoma Coordinator: Sandra de Macedo Ribeiro	FCT Refª: POCI/BIA-PRO/58638/2004	01/03/2006 to 31/12/2007	26.094,00	6.881,25
Proteases aspárticas secretadas em Candida albicans: potencial uso como antigénicos alvo para vacinação contra candidíases sistémicas Participants: Universidade do Minho Coordinator: Carlos José Fialho da Costa Faro	FCT Refª: POCI/SAU-IMI/58014/2004	01/06/2005 to 31/05/2008	9.600,00	0,00
Estudo dos possíveis factores ambientais e moleculares que levam ao desenvolvimento de diabetes tipo 2 e obesidade em Portugal Coordinator: Eugénia Maria Lourenço de Carvalho	FCT Refª: POCI/SAU-MO/57598/2004	15/10/2005 to 31/10/2009	90.250,00	49.913,00
Desenvolvimento de novos compostos de Vanádio. Sua aplicação como agentes antidiabéticos e anticancerígenos. Coordinator: Maria Margarida Catalão Almiro e Castro Participants: Instituto Superior Técnico; Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA)	FCT Refª: POCI/QUI/56949/2004	01/10/2005 to 30/09/2008	29.500,00	16.440,00
Stress oxidativo na Doença Cardíaca: acção das catecolaminas Coordinator: Rui Albuquerque Carvalho Participants: Instituto de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA); Universidade de Aveiro	FCT Refª: POCI/SAU-OBS/55849/2004	01/10/2005 to 30/09/2008	8.644,00	2.394,00
Alterações nas vias fisiológicas e mecanismos moleculares reguladores da homeostase energética na obesidade e síndrome metabólico: identificação de novas estratégias e alvos terapeuticos Coordinator: Carlos Manuel Marques Palmeira	FCT Refª: PTDC/SAU-OSM/72443/2006	01/09/2007 to 31/08/2010	156.000,00	35.208,00
Effecto das purinas no desenvolvimento do hipocampo: Consequências para o establecimento de circuitos relacionados com aprendizagem e menória Coordinator: Rodrigo Pinto dos Santos Antunes da Cunha	FCT Refª: PTDC/SAU-NEU/74318/2006	01/07/2007 to 30/09/2010	94.439,00	6.000,00
Regulação dos receptores AMPA pela hiperglicémia na retina Coordinator: Francisco Ambrósio Participants: Faculdade de Medicina da Universidade de Coimbra (FMUC)	FCT Refª: PTDC/SAU-NEU/71228/2006	01/06/2007 to 31/05/2010	40.064,00	12.600,00

Desenvolvimento de novas estratégias para terapia anti-tumoral baseadas na utilização do peptídeo permeante S4(13)-PV com o objectivo de potenciar a entrega intracelular de ácidos nucleicos e proteínas com actividade terapêutica Coordinator: : Mª da Conceição Monteiro Pedroso de Lima	FCT Refª: PTDC/BIO/65627/2006	01/05/2007 to 30/04/2010	136.000,00	40.140,00
Nanostructured photoluminescent rare-earth nonotubes and microporous silicates: Coordinator: Carlos Frederico de Gusmão Campos Geraldes Participants: Universidade de Aveiro	FCT Refª: PTDC/CTM/73243/2006	01/12/2007 to 30/11/2010	14.544,00	744,00
Contribuição de subunidades dos receptores N-metil-D-aspartato na disfunção neuronal na doença de Alzheimer Coordinator: Ana Cristina Rego	FCT Refª PTDC/SAU-NEU/71675/2006:	01/09/2007 to 31/08/2010	99.944,00	6.000,00
Silenciamento da doença de Machado-Joseph: interferencia de RNA para a ataxina-3 mediada por vectores lentivirais Coordinator: Luis de Almeida	FCT Refª: PTDC/SAU-FCF/70384/2006	01/07/2007 to 30/06/2010	170.000,00	37.882,00
Alterações do metabolismo da glicose e lipído por agentes imunossupressores: implicações no diagnóstico e tratamento da diabetes pós-transplante Coordinator: John Jones	FCT Refª: PTDC/SAU-OSM/65140/2006	01/10/2007 to 30/09/2010	152.223,00	46.541,00
Alterações na Microglia e Neurónios do Hipocampo Induzidas por Metanfetamina: Papel das Citocinas Pró-inflamatórias e do Neuropeptídeo y Coordinator: Ana Paula Silva Martins Participants: AIBILI; Faculadade de Farmácia; IBILI;	FCT Refª: PTDC/SAU-FCF/67053/2006	01/05/2007 to 30/04/2010	88.000,00	7.800,00
Interacção entre a nicotina e a cafeína no núcleo estriado. Relevância na doença de Parkinson Coordinator: Rodrigo Pinto dos Santos Antunes da Cunha	FCT Refª: PTDC/SAU-NEU/81064/2006	01/05/2007 to 30/04/2010	94.378,00	40.892,00
Mecanismos de plasticidade sináptica e de neuroprotecção pelo BDNF no hipocampo: inibição da neurodegemeração vs. regeneração. Coordinator: Carlos Jorge Alves Miranda Bandeira Duarte	FCT Refª: PTDC/SAU-FCF/72283/2006	01/05/2007 to 30/04/2010	136.000,00	50.842,00
Células estaminais da região subventricular na reparação cerebral em epilepsia do lobo temporal. Coordinator: João José Oliveira Malva	FCT Refª: PTDC/SAU-NEU/68465/2006	01/05/2007 to 30/04/2010	148.828,00	22.000,00

Papel do ATP extracelular e caracterização dos receptores purinérgicos envolvidos na resitência da Candida albicans à resposta immune de macrógafos Coordinator: Teresa Maria Fonseca de Oliveira Gonçalves	FCT Refª: PTDC/SAU-FCF/81436/2006	01/06/2007 to 31/05/2010	78.936,00	36.156,00
Novos Mecanismos Mitocondriais Para a Toxicidade Cardioselectiva da Doxorubicina Coordinator: Paulo Jorge Gouveia Simões da Silva Oliveira	FCT Refª: PTDC/SAU-OSM/64084/2006	15/09/2007 to 14/09/2009	115.800,00	33.090,00
Influência do Estado de Diferenciação Celular na Apoptose Induzida por Isoproterenol em Células Ventriculares Embrionárias H9c2-Vias de Sinalização Envolvidas Coordinator: Paulo Jorge Gouveia Simões da Silva Oliveira	FCT Refª: PTDC/QUI/64358/2006	01/11/2007 to 31/10/2009	85.000,00	19.980,00
Modelação Quantitativa da Difusão Passiva Trans-Citótica de Moléculas Anfifílicas através da Barreira Hemato-Encefálica Coordinator: Armindo José Alves da Silva Salvador Participants: Faculdade de Ciências e Tecnologia da Universidade de Coimbra; Instituto de Tecnologia Química e Biológica	FCT Refª: PTDC/SAU-FCF/69072/2006	01/07/2007 to 30/06/2010	18.720,00	3.600,00
Papel da Células Dendriticas na Leishmaniose: estudos de sinalização intracelular na infecção pelo parasita Leismania infantum virulento ou atenuado Coordinator: Maria Teresa de Teixeira Cruz Participants: Instituto de Biologia Molecular e Celular	FCT Refª: PTDC/SAU-FCF/67351/2006	16/08/2007 to 15/08/2009	25.059,00	5.619,00
Acções troficas dos factores neurotróficos: dependência da coactivação de receptores A2A da adenosina. Coordinator: Emilia Conceição Pedrosa Duarte Participants: Instituto de Medicina Molecular; Faculdade de Farmácia da Universidade de Lisboa	FCT Refª: PTDC/SAU-NEU/64126/2006	01/07/2007 to 30/06/2010	29.907,00	10.500,00
Neuroprotecção pela insulina e IGF-1 na diabetes associada à doença de Huntington Coordinator: Ana Cristina Carvalho Rego	FCT Refª: PTDC/SAU-FCF/66421/2006	22/08/2007 to 21/08/2010	124.000,00	18.001,00
Elucidação de Mecanismos patológicos associados a forma juvenil da lipofuscinose ceróide neuronal: do modelo de levedura para sistemas mais complexos. Coordinator: João António Nave Laranjinha Participants: Universidade do Minho	FCT Refª: PTDC/SAU-NEU/70161/2006	01/07/2007 to 30/06/2010	25.000,00	3.889,00

Estabilidade conformacional de proteínases aspárticas com importância biotecnológica e médica - O unfolding/refolding de proteínas diméricas e monoméricas. Coordinator: Marlene Barros	FCT PTDC/QUI/60791/2004	01/01/2007 to 04.03.2009	9.480,00	8480,00
Clivagem dos transportadores vesiculares do glutamato (VGLUT) e do GABA (VGAT) em condições de excitotoxicidade: identificação dos locais de clivagem e implicações funcionais Coordinator: Carlos Duarte	FCT PTDC/SAU-NEU/65846/2006	01/05/2007 to 30/04/2010	115.256,00	42.900,00
Neuropeptídeo Y na retina: porquê? E para quê? Coordinator: Cláudia Cavadas	FCT PTDC/SAU-NEU/73119/2006	01/05/2007 to 30/04/2010	123.668,00	7.400,00
"P-found: computação GRID e armazenamento distribuido de dados de simulações de dobragem de proteínas." Coordinator: Rui Brito Participants: Univ. Minho, Faculdade Ciências Coimbra, Faculdade Ciências Tecnologia Univ. Coimbra, Critical Software	FCT GRID/GRI/81809/2006	01/06/2007 to 31/05/2010	27.545,00	4.822,00
Estudo de processos de bioluminescência. Coordinator: Rui Brito Participants: ADDF	FCT PTDC/FIS/73578/2006	01/07/2007 to 30/06/2010	50.928,00	3.158,00
Novas funções biológicas de compostos fenólicos do vinho: regulação celular e acção anti-inflamatória via formação de óxido nítrico a partir de nitrito contido na dieta. Coordinator: João Laranjinha	FCT PTDC/AGR-ALI/71262/2006	15/05/2007 to 14/05/2010	123.478,00	44.635,00
Papel e mecanismos moleculares do receptor CD36 na fagocitose de células apoptóticas: implicações para a aterosclerose Coordinator: Otília Vieira	FCT PTDC/SAU-MII/66285/2006	01/09/2007 to 31/08/2010	159.936,00	8.022,00
Actividade metabólica e viabilidade do condrócito em enxertos osteocartilagíneos humanos criopreservados.Coordinator: Celeste Lopes	FCT PTDC/SAU-OSM/67936/2006	01/09/2007 to 31/08/2010	32.648,83	8.065,00
Design, synthesis and biological assessment of multifunctional compounds as anti-Alzheimer drugs Coordinator: Paula Agostinho Participantes: Faculdade de Farmácia Univ. Lisboa	FCT PTDC/SAU-NEU/64151/2006	01/08/2007 to 31/07/2010	12.740,00	0,00
Neurociências e Doença Coordinator: Catarina Isabel Resende de Oliveira	FCT Refª: REEQ/651/SAU/2005	15/02/2005 to 31/12/2007	1.600.000,00	0,00

Estrutura e Função de Proteínas Coordinator: Euclides Manuel Vieira Pires	FCT Refª: REEQ/1028/BIO/2005	01/04/2005 to 31/12/2007	200.000,00	0,00
O Mundo dos Micróbios – Colecção Portuguesa de Bactérias (CPB) e Laboratório Acreditado de Controlo Microbiológico Coordinator: Milton Simões da Costa	FCT Refª: REEQ/851/BIO/2005	01/04/2005 to 31/12/2007	129.936,00	0,00
Estudos populacionais e clínicos para a quantificação da sobrevivênvia e identificação de factores de prognóstico – "Estudo da heterogeneidade genética intratumoral de gliomas: impacto no seu comportamento clínico e biológico Coordinator: Maria Celeste Fernandes Lopes	FCG Refª: 68708	01/02/2005 to 31/07/2008	30.000,00	0,00
A nanomedicina e as novas estratégias terapêuticas: desenvolvimento de estruturas supramoleculares para o tratamento de doenças oftalmológicas Coordinator: Carlos Faro, João Nuno Moreira; Luís Almeida Participants: AIBILI, IBILI, PRODEQ, BLUEPHARMA	iCentro Refª: ic-01-03-FDR-0035	01/01/2007 to 31/12/2008	85.051,00	43.875,00
Programa MIT Coordinator: Catarina Oliveira	FCT MIT-Portugal	01.09.2006 to 31/08/2011	854.145,02	96.962,52
Sub - Total			**7.989.038,00**	**1.225.994,00**
International Projects:				
Noninvasive measurement of hepatic glycogen kinetics in Type 1 diabetics. Coordinator: John Griffth Jones	JDRF Refª: 1-2006-74	01/05/2006 to 31/04/2007	217.313,00	145.726,00
EMIL Coordinator: Carlos Geraldes	EMIL Ref.ª EMIL: LSHC-CT-2004-503569	01/07/2005 to 30/06/2007	13.594,68	0,00
Sub - Total			**230.907,68**	**145.726,00**

Other				
Characterisation of FKS gene(s) in Alternaria infectoria Coordinator: Teresa Gonçalves	Merck Sharp Dohme MSG – P 1599	01/01/2006 to 31/03/2007	30.640,00	5597,97,00
Prevenção pela cafeína do deficit de memória causado pela diabete Coordinator: Rodrigo Cunha	Fundo Fundação Oriente/Johnson Johnson para a Saúde	01/01/2007 to 31/12/2007	20.000,00	20.000,00
Functional and pharmacological properties of CB1 receptor heteromers SANOFI Aventis Coordinator: Rodrigo Cunha	Sanofi Aventis	01.09.2007 to 31.08.2008	121.000,00	48.400,00
Sub - Total			**171.640,00**	**73.997,97**
TOTAL			**8.391.585,68**	**1.445.717,97**

Staff List

LIST OF STAFF AND RESEARCH STUDENTS | GENERAL LIST

Members holding Ph.D.		**Time % at CNC**
Alexandrina M. Ferreira S. Pinto Mendes	(Assistant Prof., FFUC)	60
Amílcar Celta Falcão Ramos Ferreira	(Full Prof., FFUC)	50
Ana Bela Sarmento A. Cruz Ribeiro	(M.D., Assistant Prof., FMUC)	40
Ana Cristina Carvalho Rego	(Assistant Prof., FMUC)	80
Ana Luisa Monteiro de Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula Pereira da Silva	(Assistant Investigator, FMUC)	Collaborator
Ana Fagulha	(M.D., HUC)	Collaborator
André Negrão Valente	(Auxiliar Investigator, CNC)	100
Ângelo Tomé José Ribeiro	(Aux. Prof., FCTUC)	60
António Francisco Gomes Ambrósio	(Investigator, FMUC)	Collaborator
António Freire Gonçalves	(M.D., Associate Prof., FMUC)	15
António Joaquim Matos Moreno	(Associate Prof., FCTUC)	15
António João F. Macedo Santos	(M.D., Assistant Prof., FMUC)	Collaborator
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	80
Armanda Emanuela Castro e Santos	(Assistant Prof., FFUC)	80
Armando Jorge Amaral Matias Cristóvão	(Assistant Prof., FCTUC)	80
Armindo Salvador	(Auxiliar Investigator, CNC)	100
Arsélio Pato de Carvalho	(Full Prof., FCTUC)	80
Attila Kofälvi	(Auxiliar Investigator, CNC)	100
Caetana Angélica E. Monteiro de Carvalho	(Full Prof., FCTUC)	80
Carla Baptista	(M.D., HUC)	Collaborator
Carlos Eduardo Paz Ferreira	(M.D., Ph.D, Assistant Prof., FMUC)	Collaborator
Carlos Frederico G. Campos Geraldes	(Full Prof., FCTUC)	60
Carlos Jorge Alves M. Bandeira Duarte	(Associate Prof., FCTUC)	80
Carlos José Fialho da Costa Faro	(Associate Prof., FCTUC)	80
Carlos Manuel Matias	(Inv. Auxiliar, UTAD)	20
Carlos Manuel Marques Palmeira	(Associate Prof., FCTUC)	20
Carlos Pato	(M.D., Prof., Univ. South California USA)	Collaborator
Catarina Isabel N. Resende de Oliveira	(M.D., Full Prof., FMUC)	80
Cláudia Margarida Gonçalves Cavadas	(Assistant Prof., FFUC)	80
Cláudia Maria Fragão Pereira	(Investigator, FMUC)	80
Célia Antunes	(Aux. Prof., Univ. Évora)	60
Cristina Januário Santos	(M.D., Assistant Prof. FMUC)	Collaborator
Emília da Conceição Pedrosa Duarte	(Assistant Prof., FCTUC)	80
Euclides Manuel Vieira Pires	(Associate Prof., FCTUC)	80
Eugénia Carvalho	(Investigator, CNC)	100
Henrique Faneca Manuel dos Santos	(Investigator, CNC)	100
Inês Baldeiras	(Aux. Prof., FMUC)	Collaborator
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	60
Ivana Jarak	(Principal Investigator, CNC)	100
João António Nave Laranjinha	(Associate Prof., FFUC)	80
João José Oliveira Malva	(Principal Investigator, FMUC)	100
João Nuno Sereno Almeida Moreira	(Assistant Prof., FFUC)	80
João Ramalho Santos	(Associate Prof., FCTUC)	80
John Griffith Jones	(Principal Investigator, CNC)	100
José António Pereira da Silva	(M.D., Associate Prof., FMUC)	20
José Antunes Barata Custódio	(Associate Prof., FFUC)	80
Leonor Martins de Almeida	(Full Prof., FFUC)	80
Lino da Silva Ferreira	(Auxiliar Investigator, CNC)	100
Lisiane O. Porciúncula	(Prof., Brasil)	10
Luís Augusto Salgueiro Cunha	(M.D., Full Prof., FMUC)	15

Luísa Maria Abreu Freire Diogo Matos	(M.D., Consultant of Pediatry CHC)	20
Luísa Barros	(M.D., HUC)	Collaborator
Luis Martinho do Rosário	(Associated Prof., FCTUC)	80
Luís Pereira Almeida	(Assistant Prof., FFUC)	60
Luís Pedro B. Sousa Inês	(M.D., HUC)	Collaborator
Manuel Aureliano Alves	(Prof. Univ. Algarve)	Collaborator
Manuela Carvalheiro	(M.D., HUC)	Collaborator
Maria Amália Silva Jurado	(Assistant Prof., FCTUC)	80
Maria Augusta de S. Fernandes dos Santos	(Investigator, FCTUC)	Collaborator
Maria Carmen M. de Carvalho Alpoim	(Associate Prof., FCTUC)	60
Maria Celeste Fernandes Lopes	(Full Prof., FFUC)	60
Maria Conceição Egas	(Auxiliar Investigator, CNC)	100
Maria da Conceição M. Pedroso de Lima	(Full Prof., FCTUC)	80
Maria Emília de Oliveira Quinta Ferreira	(Assistant Prof., FCTUC)	80
Maria Fernanda P. N. Gomes Nobre	(Principal Investigator, FCTUC)	100
Maria Graça Santos Pratas Vale	(Full Prof., FCTUC)	80
Maria Helena Pinto de Azevedo	(M.D., Full Prof., FMUC)	Collaborator
Maria Isabel Jacinto Santana	(M.D., Assistant Prof., FMUC)	80
Maria Madalena Mendes Caldeira Santos	(Aux. Prof., FCTUC)	60
Maria Manuela Monteiro Grazina	(Assistant Prof., FMUC)	80
Maria Margarida Catalão Almiro e Castro	(Aux. Prof., FCTUC)	60
Maria Otilia Vitoriana Vieira	(Aux. Investigator, CNC)	100
Maria Sancha Vieira Santos	(Investigator, FCTUC)	100
Maria Teresa de Teixeira Cruz Rosete	(Assistant Prof. FFUC)	80
Margarida Bastos	(M.D., HUC)	Collaborator
Michelle Pato	(M.D., Prof., Univ. South California USA)	Collaborator
Miguel Castelo Branco	(Assistant Prof., FMUC)	Collaborator
Milton Simões da Costa	(Full Prof., FCTUC)	80
Paula Cristina Veríssimo Pires	(Assistant Prof., FCTUC)	80
Paula Maria Garcia Agostinho	(Investigator, FMUC)	80
Paula Garcia	(M.D., HPC)	Collaborator
Paula Isabel da Silva Moreira	(Assistant Prof., FMUC)	60
Paulo Fernando Martins dos Santos	(Assistant Prof., FCTUC)	80
Paulo J. Oliveira	(Auxiliar Investigator, CNC)	100
Pedro Monteiro	(M.D., HUC)	Collaborator
Rodrigo Pinto dos Santos A. da Cunha	(Associate Prof., FMUC)	80
Rosa Maria Moreira Alves dos Santos	(Aux. Prof., FCTUC)	80
Rui de Albuquerque Carvalho	(Aux. Prof., FCTUC)	60
Rui M. Pontes Meireles F. de Brito	(Assistant Prof., FCTUC)	40
Rui Manuel Silva Gomes Barbosa	(Assistant Prof., FFUC)	80
Sandra Carvalho Bos	(Assistant Prof., FMUC)	80
Sandra Morais Cardoso	(Assistant Prof., FMUC)	80
Sérgio Paulo Magalhães Simões	(Assistant Prof., FFUC)	80
Sukalian Chatterjee	(Principal Investigator, CNC)	100
Teresa Carmo Pimenta Dinis	(Associate Prof., FFUC)	50
Teresa Maria Caldeira Martins	(Investigator, IPO)	20
Teresa Maria Fonseca de Oliveira Gonçalves	(Assistant Prof., FMUC)	80
Tiago Alfaro	(M.D., HUC)	20
Vítor Manuel Calado Madeira	(Full Prof., FCTUC)	60

Post-Doc Members

Ana Isabel Duarte	100
Ana Paula Kuan Yon Chung	Collaborator
Anália do Carmo	100
Anabela Pinto Rolo	100
Carla Margarida Pereira Cardoso	100
Clévio David Rodrigues Nóbrega	100

Dora Cristina dos Santos Pedroso	100
Fabienne Agasse	100
Ildete Luísa Ferreira	100
Inês Maria Pombinho de Araújo	100
Isabel Conceição Moreira Pereira Alonso	80
Jorge Fernandes dos Anjos	100
Margarida Alexandra Vaz Caldeira	Collaborator
Maria Teresa Martins da Cunha Oliveira	100
Nuno Miguel Empadinhas	100
Paulo César da Silva Pinheiro	5
Teresa Almeida	20

Ph.D. Students

Adriana Oliveira dos Santos	100
Alexandra Rosa	100
Ana Burgeiro	100
Ana Catarina Henriques Oliveira	100
Ana Catarina Simões Pinto Oliveira	100
Ana Cristina Rosa da Silva	100
Ana Cristina da Silva Filipe	100
Ana Fortuna	100
Ana Francisca Leal Silva Soares	100
Ana Luísa Colaço Cardoso	100
Ana Luisa N. Gomes Nobre	100
Ana Luisa Vital Carvalho	100
Ana Margarida Cruz Ledo	100
Ana Patricia Figueiredo Rocha Simões	100
Ana Paula Marques de Sousa	100
Ana Raquel Esteves	100
Ana Rita Costa Silva Álvaro	100
Ana Rita Araújo Santos	100
Ana Sofia Fraga de Almeida	100
Ana Teresa Simões	100
Anabela Almeida	100
André Rodrigues de Abreu Gomes	100
Andrea Catarina Lobo	100
António Sales Mano	100
Áurea Filipa de Aguiar Castilho	50
Bruno Pereira Carreira	100
Bruno Miguel Alves Fernandes Gago	100
Bruno José Fernandes Oliveira Manadas	100
Bruno Lopes	100
Bruno Miguel Rodrigues das Neves	100
Bárbara Rocha	100
Bruno Alexandre Cordeiro Silva	100
Cândida Susana Gonçalves da Silva	100
Carla Maria dos Santos Nunes	100
Carla Sofia Gomes Silva	100
Cátia Filipe Lourenço Marques	100
Célia Alexandra Ferreira de Oliveira Aveleira	Collaborator
Chantal Ana Vicência Fernandes	100
Claudia Silva	100
Cristina Barosa	100
Cristiana Paulo	100
Daniel Oberdoerfer	100
Daniela Arduíno	100
Daniela Maria Barroso de Moura Cipreste Vaz	100

Duarte A. Marques	100
Elisabete Batista Ferreiro	100
Ermelindo Leal	20
Filipe Duarte	100
Filomena José Pereira Silva	100
Gabriel Nascimento Ferreira da Costa	100
Gabriele Ghisleni	100
Gianna Cognato	100
Gilberto Alves	100
Geanne Cunha	10
Giovannia Araujo de Lima Pereira	10
Gonçalo de Castro Pereira	100
Helena Vazão	100
Hugo Prazeres	100
Igor Clemente Tiago	100
Inês Crespo	100
Inês Vasconcelos Miranda Santos	100
Isabel Dinis Ferreira	100
Ivan Viegas	100
Jean Oses	30
Joana Cardoso da Costa	100
Joana Ferreira	100
Joana Margarida Novalho Gaspar	30
Joana I. Real	100
Joana Catarina Cravo Lourenço	100
Joana Paixão	100
Joana Rosmaninho Salgado	
João Carlos Rodrigues Gomes	100
João Miguel das Neves Duarte	100
João Gonçalo Leal Frade	100
João Pedro Lopes	100
João Pedro P. Monteiro	100
Jorge Miguel de Ascenção Oliveira	100
José Mário Tenera Morgado	100
José Miguel Brás	100
Lígia Ferreira	100
Liliana Inácio Bernardino	100
Liliana Mendonça	100
Manuel Joaquim M. Gonçalves Matos	50
Manuella P. Kaster	100
Marco Aurélio Gouveia Alves	100
Maria Alexandra Barreto Amaral	100
Maria de Fátima Valente Lopes Pinto	100
Maria Isabel Nascimento Ferreira	100
Maria João Pereira	30
Maria José Simões	100
Maria Joana Lima Barbosa de Melo	80
Mariana de Oliveira Freitas	50
Mário Luis Noro Laço	100
Marta Viegas da Silva	100
Nelson Rebola	100
Nuno Ricardo Esteves Ferreira	100
Núria Simões	100
Paula Canas	100
Paula Matos de Brito	100
Paula Cristina Cardoso Ramos Mota	100
Pedro Manuel Batista Branco	100

Pedro Manuel Venâncio Garção	100
Pedro Miguel Brás Macedo Coelho	10
Raquel Margarida da Silva Ferreira	100
Raquel Santiago	100
Ricardo Jorge de Alves Rodrigues	75
Ricardo Miguel Oliveira dos Santos	100
Ricardo Jorge Ladeiro Tomé	100
Rita João Louro Guerreiro	100
Rita Catarina Gonçalves Perfeito	100
Rita Mafalda dos Santos Rocha	100
Rita Videira	100
Rosa Maria Matos Branco Resende	100
Rui Miguel Oliveira da Costa	100
Rui Jorge Gonçalves Pereira Nobre	100
Sara Alves Xapelli	100
Sandra Amaral	100
Sandra Cristina Vicente e Almeida	100
Sandra Manuela Domingues Santos	100
Sandra Gamboa	100
Sandra Marina de Almeida Santos	100
Sandra Filipa Tavares Varum	100
Sandro José Paiva Fernandes Alves	100
Sandro Lino Cardoso Pereira	100
Sara Figueiredo	10
Sara Gonçalves	100
Sara Trabulo	100
Shyam P. Mohan	100
Sofia Domingues	100
Sofia Cristina Soares de Morais Grade	100
Sónia Correia	100
Sónia Patricia Dias Duarte	100
Sílvia Sousa Neves	100
Susana Carvalho Rosa	100
Susana Isabel Elias Alarico	100
Tatiana Catarino	100
Tiago Alves	100
Tiago Brandão Rodrigues	10
Teresa Jesus Delgado	10
Teresa Louro Mandes Serafim	100
Vera Moura	100
Vilma Marisa Arrojado Soares Sardão Oliveira	100
Vitor Gonçalo Silva C. Mendes	100

MSc Students

Ana Cardoso	100
Ana Catarina Fonseca	100
Ana Filipa Branco	100
Filipa Domingues	100
Ana Isabel Rafael	100
Ana Maria Pereira da Silva	100
Ana Sofia Rodrigues	100
Anabela Simões	100
Cristina Carvalho	100
Cristina da Silva Oliveira Paulo	100
Daniela Pinheiro	100
Diana Moreira	50
Joana Medeiros Vieira Marques	100
João Demétrio Gonçalves Boto Martins	100

João Paulo Pereira	100
Luís Bimbo	100
Luis Gabriel Borges Rocha	100
Márcio Ribeiro	100
Marco Matos	100
Maria João Gonçalves	100
Maria João Rodrigues Pereira	10
Maria Viegas Nascimento	80
Marta Vieira	80
Marta Isabel Rodrigues Baptista	100
Patricia Maria Figueiredo Nunes	100
Paulo Nuno Centeio Matafome	100
Raquel Brito	100
Renata Santos Tavares	100
Ricardo Marques	100
Rui Abreu	100
Rui Sanches	100
Sara Margarida Diniz Martins Lopes	100
Sofia Cunha	100
Teresa Margarida Monteiro Garcia Louro	100

Undergraduate Students

Alexandra Faustino	100
Ana Carolina Borralho	100
Ana Gomes	100
Ana Varela	100
André Martins	10
Beatriz Lacerda de Sousa	100
Cátia Diogo	100
Cláudia Pereira	100
Filipa Carvalho	100
Filipa Curado	100
Graciano Leal	100
Inês Patricio	100
Inês Ribeiro Violante	10
Isabel Fernandes	100
João André Duarte	10
João Pedro Pereira	100
João T. Costa	100
João Teixeira	10
Luis Ribeiro	100
Manuela Almeida	100
Nuno Machado	100
Patrícia Rebelo	50
Paulo Gameiro	100
Pedro Costa	100
Pedro Marques	100
Pedro Réu Carvalho	100
Renato Xavier Santos	100
Rita Silva	100
Rui Benfeitas	10
Rui Costa	100
Sandrina Silva	100
Sara Gomez	100
Sueli Cristina Ferreira Marques	100
Susana Cardoso	100
Tiago Capote	100

Tiago Ferreira	100
Vera Mónica Gonçalves	100
Vera Lucia Francisco	100
Vitor Cabral	100

SERVICE STAFF

		Time % at CNC
Ana Cristina Franco dos Santos	(Graduate Technician, CNC)	100
Ana Margarida Ferreira	(Graduate Technician, CNC)	100
António José Azinhaga Teles Grilo	(Graduate Technician, CNC)	100
Cândida Elsa Frias Mendes	(Graduate Technician, CNC)	100
Carla Margarida dos Santos Veríssimo	(Graduate Technician, CNC)	100
João Miguel Pratas	(Graduate Technician, CNC)	100
Luciana C. Albuquerque Pinto	(Graduate Technician, CNC)	100
Luís Miguel M. Vidal Oliveira	(Graduate Technician, CNC)	100
Maria Helena Garruncho	(Graduate Technician, CNC)	Collaborator
Maria João Ferreira Canas dos Santos	(Graduate Technician, CNC)	100
Marta Sofia Marques Simões	(Graduate Technician, CNC)	100
Teresa Proença	(Graduate Technician, CNC)	Collaborator

TECHNICAL STAFF

		Time % at CNC
Alexandre Simão Vieira Pires	(Graduate Technician, CNC)	100
Cármen Lídia Graça Semeão	(Graduate Technician, CNC)	100
Diana dos Santos Simões Graça	(Technician, CNC)	100
Fátima Cristina dos S. Carvalho Graça	(Technician, CNC)	100
Isabel Conceição Calado Esteves Costa	(Technician, CNC)	100
Isabel Nunes Correia	(Ph.D., Graduate Technician, CNC)	100
Luisa Leitão Cortes	(Ph.D., Graduate Technician, CNC)	100
Maria do Céu Mendes Gomes	(Technician, CNC)	100
Maria Isabel Gonçalves	(Technician, CNC)	100
Maria da Rosário da Costa Faro	(Graduate Technician, CNC)	100
Odete Pereira Cabanelas	(Graduate Technician, CNC)	100

ADMINISTRATIVE STAFF

		Time % at CNC
Arménio Pereira Ferreira	(Administrative Assistant, FCTUC)	Collaborator
Benilde Maria Jesus Correia	(Administrative Assistant, FCTUC)	Collaborator
Carla Lopes Rodrigues	(Administrative Assistant, CNC)	100
Elisabete Cosmos dos Santos Machado	(Graduate Administrative, CNC)	100
Heidi Maria da Silva Lopes Gonçalves	(Graduate Administrative, CNC)	100
Lia da Costa Jordão Aparício Lopes	(Graduate Administrative, CNC)	100
Sandra Cristina Santos Luís	(Graduate Administrative, CNC)	100
Sílvia Marisa Esteves de Sousa	(Graduate Administrative, CNC)	100
Susana Adelaide Rocha da Silva	(Administrative Assistant, CNC)	100
Tatiana de Azevedo Paula	(Graduate Administrative, CNC)	100

RESEARCH STAFF AND STUDENTS | RESEARCH AREA

Neuroscience and Disease

Catarina Resende de Oliveira, M.D., Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
António Francisco Gomes Ambrósio	(Investigator, FMUC)	Collaborator
António Freire Gonçalves	(M.D., Associate Prof., FMUC)	15
Ana Cristina Carvalho Rego	(Assistant Prof., FMUC)	80
Ana Luísa Monteiro de Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula Pereira da Silva	(Assistant Investigator, FMUC)	Collaborator
Armanda Emanuela Castro e Santos	(Assistant Prof., FFUC)	80
Armando Jorge Amaral Matias Cristóvão	(Assistant Prof., FCTUC)	80
Arsélio Pato de Carvalho	(Full Prof., FCTUC)	80
Attila Kofâlvi	(Auxiliar Investigator, CNC)	100
Caetana Angélica E. Monteiro de Carvalho	(Full Prof., FCTUC)	50
Carlos Jorge Alves M. Bandeira Duarte	(Associate Prof., FCTUC)	80
Carlos Pato	(M.D., Prof., Univ. South California)	Collaborator
Catarina Isabel N. Resende de Oliveira	(M.D., Full Prof. FMUC),	80
Cláudia Margarida Gonçalves Cavadas	(Assistant Prof., FFUC)	80
Cláudia Maria Fragão Pereira	(Investigator, FMUC)	80
Emília da Conceição Pedrosa Duarte	(Assistant Prof., FCTUC)	80
João José Oliveira Malva	(Principal Investigator, FMUC)	100
Lisiane O. Porciúncula	(Prof., Brasil)	10
Luís Augusto Salgueiro Cunha	(M.D., Full Prof., FMUC)	15
Maria Helena Pinto de Azevedo	(M.D., Full Prof., FMUC)	Collaborator
Maria Isabel Jacinto Santana	(M.D., Assistant Prof., FMUC)	80
Maria Manuela Monteiro Grazina	(Assistant Prof., FMUC)	80
Maria Sancha Vieira Santos	(Assistant Prof., FCTUC)	100
Michelle Pato	(M.D., Prof., Univ. South California)	Collaborator
Miguel Castelo-Branco	(Assistant Prof., IBILI)	Collaborator
Paula Isabel da Silva Moreira	(Assistant Prof., FMUC)	80
Paula Maria Garcia Agostinho	(Investigator, FMUC)	80
Paulo Fernando Martins dos Santos	(Assistant Prof., FCTUC)	60
Rodrigo Pinto dos Santos A. da Cunha	(Associate Prof., FMUC)	80
Sandra Carvalho Bos	(Assistant Prof., FMUC)	80
Sandra Morais Cardoso	(Assistant Prof., FMUC)	80

Post-Doc Members

Ana Isabel Duarte	100
Fabienne Agasse	100
Ildete Luísa Ferreira	100
Inês Maria Pombinho de Araújo	100
Margarida Caldeira	100
Maria Teresa Martins da Cunha Oliveira	
Paulo César da Silva Pinheiro	5
Teresa Almeida	20

M.D. Members

António João F. Macedo Santos	(M.D., Assistant Prof., FMUC)	Collaborator
Carlos Eduardo Paz Ferreira	(M.D., HDE, Açores)	Collaborator
Cristina Januário Santos	(M.D., Assistant Prof. FMUC)	Collaborator
Luísa Maria Abreu Freire Diogo Matos	(M.D., Consultant of Pedriatry, CHC)	20
Tiago Alfaro	(M.D., HUC)	20

Ph.D. Students

Alexandra Rosa	100
Ana Catarina Henriques Oliveira	100
Ana Cristina Rosa da Silva	100
Ana Patricia Figueiredo Rocha Simões	100
Ana Raquel Esteves	100
Ana Rita Costa Silva Álvaro	100
Ana Rita Araújo Santos	100
André Rodrigues de Abreu Gomes	100
Andrea Catarina Lobo	100
Bruno José Fernandes Oliveira Manadas	100
Bruno Paulo Carreira	100
Bruno Alexandre Cordeiro Silva	100
Carla Sofia Gomes Silva	100
Daniela Arduíno	100
Elisabete Batista Ferreiro	100
Ermelindo Leal	20
Gabriel Nascimento Ferreira Costa	100
Gabriele Ghisleni	100
Geanne Cunha	10
Gianna Cognato	100
Jean Oses	30
Joana Ferreira	100
Joana margarida Novalho Gaspar	30
Joana Gil	100
Joana Catarina Cravo Lourenço	100
Joana Rosmaninho Salgado	100
João Miguel da Neves Duarte	100
João Carlos Rodrigues Gomes	100
José Miguel Brás	100
João Pedro Lopes	100
Jorge Miguel de Ascenção Oliveira	100
Lígia Ferreira	100
Liliana Inácio Bernardino	100
Manuella P. Kaster	100
Maria Joana Lima Barbosa de Melo	80
Mário Luis Noro Laço	100
Nelson Rebola	100
Paula Canas	100
Pedro Manuel Venâncio Garção	100
Raquel Ferreira	100
Raquel Santiago	100
Ricardo Jorge de Alves Rodrigues	75
Rita João Louro Guerreiro	100
Rita Catarina Gonçalves Perfeito	100
Rosa Maria Matos Branco Resende	100
Rui Miguel Oliveira da Costa	100
Sandra Cristina Vicente e Almeida	100
Sandra Manuela Domingues Santos	100
Sara Alves Xapelli	100
Sofia Domingues	100
Sofia Cristina Soares de Morais Grade	100
Sónia Correia	100
Tatiana Catarino	100

MSc Students

Ana Catarina Fonseca	100
Ana Rita Costa Silva Álvaro	100
Cristina Carvalho	100
Filipa Domingues	100
João Demetério Gonçalves Boto Martins	50
Marco Matos	100
Márcio Ribeiro	100
Maria Viegas Nascimento	80
Marta Vieira	80
Rui Sanches	100
Vera Cortez	100

Undergraduate Students

Graciano Leal	100
João T. Costa	100
Luis Ribeiro	100
Patrícia Rebelo	50
Pedro Réu Carvalho	100
Renato Xavier Santos	100
Rui Costa	100
Sandrina Silva	100
Sueli Cristina Ferreira Marques	100
Susana Cardoso	100

Molecular Biotechnology and Health

Euclides Pires, Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
Ana Margarida Vieira da Silva	(Assistant Prof., UVG)	50
André Negrão Valente	(Auxiliar Investigator, CNC)	100
Armindo Salvador	(Auxiliar Investigator, CNC)	100
Carlos José Fialho da Costa Faro	(Associate Prof., FCTUC)	80
Euclides Manuel Vieira Pires	(Associate Prof., FCTUC)	80
Henrique Faneca	(Investigator, CNC)	100
João Nuno Sereno Almeida Moreira	(Assistant Prof., FFUC)	60
Luís Pereira de Almeida	(Assistant Prof., FFUC)	60
Maria Conceição Egas	(Auxiliar Investigator, CNC)	100
Maria da Conceição M. Pedroso de Lima	(Full Prof., FCTUC)	60
Paula Cristina Veríssimo Pires	(Assistant Prof., FCTUC)	80
Rui M. Pontes Meireles F. de Brito	(Assistant Prof., FCTUC)	35
Sérgio Paulo Magalhães Simões	(Assistant Prof., FFUC)	60

Post-Doc Members	
Clévio Nóbrega	100

Ph.D. Students	
Adriana Oliveira dos Santos	100
Ana Catarina Simões Pinto	100
Ana Cristina da Silva Filipe	100
Ana Luísa Colaço Cardoso	100
Ana Sofia Fraga de Almeida	100
Ana Teresa Simões	100
Áurea Filipa de Aguiar Castilho	100
Cândida Susana Gonçalves da Silva	100
Cristiana Paulo	100
Daniela Maria Barroso de Moura Cipreste Vaz	100
Helena Vazão	100
Inês Vasconcelos Miranda Santos	100
Isabel Diniz Ferreira	100
Maria Isabel Nascimento Ferreira	100
Ligia Ferreira	100
Liliana Mendonça	100
Maria José Simões	100
Nuno Ricardo Esteves Ferreira	100
Pedro Manuel Batista Branco	100
Pedro Miguel Brás Macedo Coelho	10
Rita Rocha	100
Rita Videira	100
Sandro José Paiva Fernandes Alves	100
Sara Trabulo	100
Sílvia Sousa Neves	100
Sónia Patricia Dias Duarte	100
Ricardo Jorge Ladeiro Tomé	100
Vera Moura	100

MSc Students	
João Paulo Pereira	100
Luís Bimbo	100
Rui Abreu	100

Undergraduate Students

Alexandra Faustino	100
João Pedro Pereira	100
Pedro Costa	100
Rui Benfeitas	10
Tiago Ferreira	100
Vera Mónica Gonçalves	100

Cell and Molecular Toxicology

Leonor Almeida, Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
Amílcar Celta Falcão Ramos Ferreira	(Full Prof., FFUC)	50
Carlos Manuel Marques Palmeira	(Associate Prof., FCTUC)	20
João António Nave Laranjinha	(Associate Prof., FFUC)	80
José Antunes Barata Custódio	(Associate Prof., FFUC)	80
Leonor Martins de Almeida	(Full Prof., FFUC)	80
Maria Amália Silva Jurado	(Assistant Prof., FCTUC)	80
Maria Augusta de S. Fernandes dos Santos	(Investigator, FCTUC)	Collaborator
Maria Sancha Santos	(Princ. Investigator, FCTUC)	100
Paulo J. Oliveira	(Auxiliar Investigator, CNC)	100
Rui Manuel Silva Gomes Barbosa	(Assistant Prof., FFUC)	80
Teresa Carmo Pimenta Dinis	(Associate Prof., FFUC)	50
Vítor Manuel Calado Madeira	(Full Prof., FCTUC)	80
Post-Doc Members		
Anabela Pinto Rolo		100
Carla Margarida Pereira Cardoso		100
Fernanda Maria Lopes Ferreira		100
M.D. Members		
Luis Pedro B. Sousa Inês	(M.D., HUC)	Collaborator
Pedro Monteiro	(M.D., HUC)	Collaborator
Ph.D. Students		
Ana Burgeiro		100
Ana Margarida Cruz Ledo		100
Ana Fortuna		100
Anabela Almeida		100
António Sales Mano		100
Bárbara Rocha		100
Bruno Miguel Alves Fernandes Gago		100
Bruno Lopes		100
Carla Maria dos Santos Nunes		100
Cátia Filipa Lourenço Marques		100
Filipe Duarte		100
Filomena José Pereira Silva		100
Gilberto Alves		100
Gonçalo de Castro Pereira Pereira		100
Joana Paixão		100
João Gonçalo Leal Frade		100
João Pedro P. Monteiro		100
Manuel Joaquim M. Gonçalves Matos		50
Marco Aurélio Gouveia Marques Alves		100
Maria de Fátima Valente Lopes Pinto		100
Núria Simões		100
Paula Matos de Brito		100
Ricardo Miguel Oliveira Santos		100
Teresa Louro Mandes Serafim		100
Sandra Marina de Almeida Santos		100

Sandro Lino Cardoso Pereira	100
Sónia Correia	100
Vilma Marisa Arrojado Soares Sardão Oliveira	100

MSc Student

Ana Cardoso	100
Anabela Simões	100
Ana Filipa Branco	100
Cristina Carvalho	100
João Demétrio Gonçalves Boto Martins	100
Luis Miguel Borges Rocha	100
Ricardo Marques	100

Undergraduate Student

Ana Gomes	100
Ana Varela	100
Cátia Diogo	100
Filipa Carvalho	100
Cláudia Pereira	100
Nuno Machado	100
Pedro Marques	100
Renato Xavier Santos	100
Sandrina Silva	100
Susana Cardoso	100
Tiago Capote	100

Microbiology

Milton Costa, Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	80
Maria Fernanda P. N. Gomes Nobre	(Principal Investigator, FCTUC)	100
Milton Simões da Costa	(Full Prof., FCTUC)	80
Teresa Maria Fonseca de Oliveira Gonçalves	(Aux. Prof., FMUC)	25

Post-Doc Members

Ana Paula Kuan Yon Chung	Collaborator
Nuno Miguel Empadinhas	100
Jorge Fernandes dos Anjos	100

Ph.D. Students

Ana Luisa N. Gomes Nobre	100
Chantal Ana Vicência Fernandes	100
Igor Clemente Tiago	100
Joana Cardoso da Costa	100
Susana Isabel Elias Alarico	100
Vitor Gonçalo Silva C. Mendes	100

MSc Students

Cristina da Silva Oliveira Paulo	100
Joana Medeiros Vieira Marques	100
Sofia Cunha	100

Undergraduate Students

Filipa Curado	100
Vitor Cabral	100

Biophysics and Biomedical NMR

Carlos Geraldes, Ph.D., Coordinator

Members holding Ph.D.		**Time % at CNC**
Ângelo José Ribeiro Tomé	(Aux. Prof., FCTUC)	60
Carlos Frederico G. Campos Geraldes	(Full Prof., FCTUC)	60
Célia Antunes	(Aux. Prof., Univ. Évora)	60
Ivana Jarak	(Aux. Investigator, CNC)	100
John Griffith Jones	(Principal Investigator, CNC)	100
Luis Martinho do Rosário	(Associated Prof., FCTUC)	80
Madalena Caldeira	(Aux. Prof., FCTUC)	60
Maria Carmen M. de Carvalho Alpoim	(Associate Prof., FCTUC)	60
Maria Margarida Catalão Almiro e Castro	(Aux. Prof., FCTUC)	60
Rosa Maria Moreira Alves dos Santos	(Aux. Prof., FCTUC)	80
Rui de Albuquerque Carvalho	(Aux. Prof., FCTUC)	60

M.D. Members

Ana Fagulha	(M.D. HUC)	Collaborator
Carla Baptista	(M.D. HUC)	Collaborator
Luísa Barros	(M.D. HUC)	Collaborator
Manuela Carvalheiro	(M.D. HUC)	Collaborator
Margarida Bastos	(M.D. HUC)	Collaborator
Paula Garcia	(M.D. HPC)	Collaborator

Ph.D. Students

Ana Francisca Leal Silva Soares	100
Claudia Silva	100
Cristina Barosa	100
Duarte A. Marques	100
Giovannia Araujo de Lima Pereira	10
Ivan Viegas	100
Joana I. Real	100
Marco Aurélio Gouveia Alves	100
Sara Figueiredo	10
Sara Gonçalves	100
Teresa Jesus Delgado	10
Tiago Alves	100
Tiago Brandão Rodrigues	10

MSc Students

Ana Isabel Rafael	100
Ana Maria Pereira da Silva	100
Daniela Pinheiro	100
Maria João Gonçalves	100
Maria João Rodrigues Pereira	10
Patricia Maria Figueiredo Nunes	100

Undergraduate Students

André Martins	10
Inês Ribeiro Violante	10
Isabel Fernandes	100
João André Duarte	10

João Teixeira	10
Manuela Almeida	100
Paulo Gameiro	100
Sara Gomez	100

Cell and Development Biology

Celeste Lopes, Ph.D., João Ramalho Santos Ph.D., Coordinators

Members holding Ph.D.		**Time % at CNC**
Alexandrina M. Ferreira S. Pinto Mendes	(Assistant Prof., FFUC)	80
António Joaquim Matos Moreno	(Assistant Prof., FCTUC)	Collaborator
Ana Bela Sarmento A. Cruz Ribeiro	(M.D., Assistant Prof., FMUC)	40
Eugénia Carvalho	(Aux. Investigator, CNC)	100
João Ramalho Santos	(Associate Prof., FCTUC)	80
José António Pereira da Silva	(M.D., Associate Prof., FMUC)	20
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	60
Manuel Aureliano Alves		Collaborator
Maria Celeste Fernandes Lopes	(Full Prof., FFUC)	80
Maria Graça Santos Pratas Vale	(Full Prof., FCTUC)	80
Maria Otilia Vieira	(Investigator, CNC)	100
Maria Teresa de Teixeira Cruz Rosete	(Assistant Prof. FFUC)	80
Sukalian Chatterjee	(Principal Investigator, CNC)	100
Teresa Maria Caldeira Martins	(Investigator IPO)	20

M.D. Members

Adriana Teixeira	(M.D, HUC)	Collaborator
Américo Costa Figueiredo	(M.D., Ph.D., HUC)	Collaborator
Alberto Orfão	(M.D., Ph.D., CIC Salamanca***)	
Emília Cortesão	(M.D, MSc Student, FMUC)	Collaborator
Fernando Judas	(M.D., Ph.D., HUC)	Collaborator
Fernando Gomes	(M.D., HUC)	Collaborator
José António Pereira da Silva	(M.D., Ph.D.)	Collaborator
Hermínio José Tão Espírito Santo	(M.D., HUC)	Collaborator
Isabel Sousa	(M.D, HUC)	Collaborator
Maria Dolores Tabernero	(M.D., Ph.D., CIC Salamanca***)	Collaborator
Marília Dourado	(M.D., Ph.D., FMUC**** CIMAGO*****)	Collaborator
Maria Margarida Gonçalo	(M.D., HUC)	Collaborator
Olinda Rebelo	(M.D., HUC)	Collaborator
Teresa Almeida Santos	(M.D., Ph.D.)	Collaborator

Post-Doc Members

Anália do Carmo	100
Carla Margarida Prreira Cardoso	100

Ph.D. Students

Alexandra Amaral	100
Ana Paula Marques de Sousa	100
Ana Luisa Vital Carvalho	100
Ana Catarina Simões Pinto Oliveira	100
Bruno Miguel Rodrigues das Neves	100
Daniel Oberdoerfer	100
Inês Crespo	100
José Mário Tenera Morgado	100
Maria João Pereira	30
Mariana de Oliveira Freitas	50
Marta Viegas da Silva	100
Paula Cristina Cardoso Ramos Mota	100

Rui Jorge Gonçalves Pereira Nobre	100
Sandra Amaral	100
Sandra Gamboa	100
Sandra Filipa Tavares Varum	100
Shyam P. Mohan	100
Susana Carvalho Rosa	100

MSc Student

Ana Sofia Rodrigues	100
Diana Moreira	50
Marta Isabel Rodrigues Baptista	100
Paulo Nuno Centeio Matafome	100
Raquel Brito	100
Renata Santos Tavares	100
Sara Margarida Diniz Martins Lopes	100
Teresa Margarida Monteiro Garcia Louro	100
Vera Gonçalves	100

Undergraduate Student

Ana Carolina Borralho	100
Beatriz Lacerda de Sousa	100
Inês Patricio	100
Rita Silva	100
Vera Lucia Francisco	100

***IPO – Portuguese Institute for Oncology, Coimbra*

****CIC – Center for Cancer Research, University Salamanca*

*****FMUC - Faculty of Medicine, University of Coimbra*

******CIMAGO – Center of Investigation in Environment, Genetics and Oncobiology*