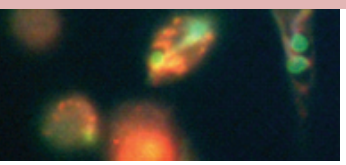
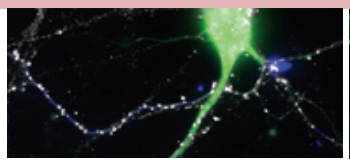
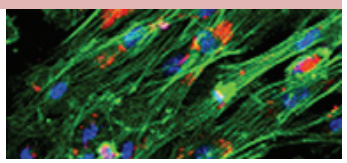
CENTER FOR NEUROSCIENCE AND CELL BIOLOGY

ASSOCIATE LABORATORY

Annual Report

Biology | Neuroscience | Health and Disease | Biotechnology

A new culture through Scientific Research

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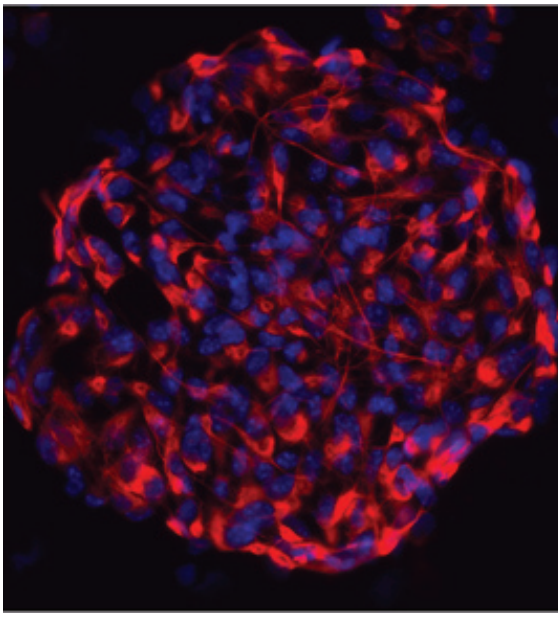

EXPERIMENTAL BIOLOGY AND BIOMEDICINE | RESEARCH PROGRAMMES

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Introduction

The Center for Neuroscience and Cell Biology (CNC) is a non-profit research Center of public utility at the University of Coimbra, recognized as an Associate Laboratory of the Portuguese Foundation for Science and Technology in November 2000, 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: Neuroscience and Disease, Molecular Biotechnology and Health, Cell and Molecular Toxicology, Microbiology, Biophysics and Biomedical NMR, Cell and Development Biology.

The CNC gathers researchers in several groups from the Faculties of Medicine, Pharmacy and Science and Technology, at the University of Coimbra, from the Coimbra University Hospital (HUC) and Institute of Oncology (IPO) in a total of 87 permanent researchers, 27 Post-Doc Members, 27 technicians and 9 administrative officers.

The scientific productivity of CNC researchers in 2008 is demonstrated by 163 papers, published in international journals, a effort support by 86 grant projects

Fundamental research is complemented by an effort on translational research, documented by posting 12 patents in 2008, by providing specialised services to Hospitals and Pharmaceutical Industries, and by the participation in the creation of the first biotechnology park in the centre of Portugal (Biocant).

CNC also supported the training of 117 PhD students in 2008, (21 PhD thesis concluded) and 27 Master students (24 Master thesis concluded) and post-docs, hosts a PhD program in Experimental Biology and Biomedicine, participates in the MIT/Portugal Protocol Doctoral Programme and has organised several European courses (FENS, PENS, FEBS). CNC integrates international networks such as the MIT-Portugal program and the European Excellence Neuroscience Institutes network (ENI-net) and is a founder of Health Cluster Portugal (HCP).

Under the scope of its Outreach Programme, 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.

The CNC management structure includes:

1.The Board of Trustees (General Assembly) is composed by representatives of the University of Coimbra (the Rector), of associate members, and the directors of departments. It meets once per year to analyse the scientific and finance report and to approve the plan of activities and budget for the next year;

2.The Board of Directors, which includes the President and Vice-Presidents and the Directors of Departments, takes into account the proposals of the Research Council (composed by all CNC PhD members) and the External Advisory Committee. It is responsible for the proposal of: 1. Research Lines; 2.Training Programs; 3.Science and Society Programs; 4. Specialized Services to the community; 5. New Programs.

3. The Executive Board, composed by the President and 3 Vice-Presidents is responsible by the implementation of decisions.

4.Financial performance is scrutinized by the "Conselho Fiscal" and "Revisor Oficial de Contas". Both the Board of Directors and the finance scrutinizing council, report to the Board of Trustees.

General Objectives

The CNC major mission is to foster 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 core scientific activity of CNC is the study of the molecular basis of neurodegenerative processes common to aging, neurodegenerative disorders, cerebral ischemia and epilepsy. In parallel, several groups explore mechanisms of neuroprotection and regeneration, which may be future candidates for the development of potential therapeutic strategies to manage these disorders. This core activity is complemented by supporting areas which also develop their own research activity, opening the scope of intervention of CNC in the biomedical field, while providing novel lines of research applicable to Neuroscience, namely: A) molecular biotechnology, with expertise in genetic screening of diseases, structure-function relation of proteins with biomedical or biotechnological interest and development of new vectors for delivery of drugs and genetic material and biomaterials for stem cell- based therapeutics; B) molecular and cellular toxicology, focused on the study of drug and disease-induced cell dysfunction, aiming to understanding the molecular basis for clinical drug toxicity, with particular expertise in processes involving mitochondrial dysfunction and free radicals; C) biomedical NMR and metabolomics with a strong focus on the development of inorganic compounds for medical diagnosis (eg MRI contrast agents), intermediate metabolism and diabetes; D) cellular and developmental biology, whose programs focused on human infertility, disruption of human cell function in cancer, contact dermatitis, osteoarthritis, auto-immune disease, obesity and pathogens biology, involve close partnerships with clinicians at HUC and IPO; E) microbiology with emphasis on the strategies for adaptation of micro-organisms to extreme environments, the screening and development of new anti-mycobacterial drugs and the susceptibility to legionella and fungal infection.

The CNC aims to focus on : 1. solid basic research, reinforcing the responsibility of a few highly qualified researchers to carry out first rate research according to international standards; 2.translational research, promoting the interaction with Hospitals and Pharmaceutical Companies; 3. the development of new technologies, promoting the creation of novel Biomedical and Biotechonology enterprises on a biotechnology hub, at Biocant.

These aspects lead to the expansion of CNC outreach program and recent hires have taken this into account, focusing on novel tools and translational research, bringing new competences and strengthening some of the research lines.

Post-graduate education is a major goal at CNC. Its Doctoral Programme in Experimental Biology and Biomedicine and the participation in the MIT/Portugal Protocol Doctoral Programme provides Master and PhD students with a multi-faceted education in molecular life sciences related to disease.

In 2008, the expansion of the network of national and international collaborations and stronger interactions between research groups at CNC were accomplished. The core facility units which are run by an expert to guarantee efficiency and correct maintenance are being set up and have contributed for the reinforcing of these collaborations.

Main Achievements during the year of 2008

In the previous years, CNC gave a significant contribution in the elucidation of cellular and molecular mechanisms of brain diseases. In 2008 we pursued the study of the molecular and cellular basis of neurodegeneration (glutamate receptors overactivation, synaptic dysfunction with the deregulation of intracellular calcium homeostasis, amyloidogenic peptides, mitochondrial dysfunction and alteration of inter-organelle crosstalk, free radical production, including NO) and neuroprotection induced by modulators of synaptic function, including classic neuromodulators (purines, NPY), cannabinoids, neurotrophins, antioxidant and mitoprotective mechanisms.

The study of mitochondria as signalling organelles, led to the demonstration that they are a primary target in drug-induced and disease-related cell dysfunction, as occurring in heart and hepatic disorders, cancer and metabolic syndrome. Curiously, diabetes-induced mitochondrial dysfunction was shown to be prevented by insulin. Endocytosis disturbance of negatively charged LDL, due to lipid peroxidation, and

the evidence that polyphenolic antioxidants interfere with NO metabolism and endothelial function points to new potential therapeutical targets in atherosclerosis prevention. Characterization of mitochondrial bioenergetics and immunological profiles in heart ischemia and ischemia/reperfusion, evaluation of metabolic fluxes in hippocampus and protection of cognition by caffeine were demonstrated by NMR. The development of new potential MRI contrast agents and the first evidence for P2X receptors in pancreatic β-cells which modulate insulin release, together with the identification of Substance P as an orexigenic neuropeptide, crucial in wound healing in diabetes were important achievements. The identification of the molecular mechanisms of phagocytosis involving Rab proteins, the discovery of the function of the gene product of an essential gene in Mycobacterium spp, the understanding of yeast metabolic response to bacterial endotoxin, the identification of novel species of extreme environment bacteria and analysis of their adaptive response, open potential new therapeutic pathways, which can benefit of pharmacosatistical methods to study drug efficacy and toxicity. New exciting developments of neuroprotection and tissue repair, using gene delivery, created the basic conditions to develop strong research lines devoted to the use of viral vectors and gene delivery as tools to stop the progression of diseases, particularly cancer and brain diseases. A transversal interest, involving different research areas, in stem cells biology and tissue engineering, namely on the use of stem cells in tissue repair, led to the clarification of the mechanisms of stem/progenitor cell proliferation and differentiation, involving not only neurons and oligodendrocytes, but also cardiac cells. Under this scope two patents were produced and several collaborations established. Translational research of neurodegenerative disorders genetics, in collaboration with Neurogenetics Lab, NIH, led to the description of novel mutations in FTD and AD. An algoritm to unveal clusters of genes associated with human diseases was established. In 2008 14 prizes were awarded to young talented investigators at CNC. The following five are representative: “Medal of Honor L’Oreal for Women in Science”, L’Oreal Portugal/Unesco/FCT; Crioestaminal Prize; GSK IdeaSpring Prize BioTecnologia; Programa Estímulo à Investigação 2008, FCG; prize “ Nunes Correa Verdades de Faria”

Facts and Figures (2008)

RESEARCH STAFF

Members holding Ph.D.	87
Post-Doc Members	27
Ph.D.Students	117
MSc Students	27
Grant Technicians	11

PUBLICATIONS IN 2008 **163**

THESIS CONCLUDED – 2008

Ph.D. thesis	21
MSc thesis	24

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 2008, the research groups for each area can be identified, according to the following organization:

Neuroscience and Disease | *Catarina Oliveira*

Neuromodulation Group (*Head: Rodrigo Cunha*)

Glutamatergic Synapses 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: Claudia Cavadas*)

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*)

Emerging Group

Biomaterials and Stem Cell-Based Therapeutics Group (*Head: Lino Ferreira*)

Cell and Molecular Toxicology | *Leonor Almeida*

Mitochondrial Toxicology and Pharmacology Group (*Head: Paulo Oliveira*)

Free Radicals and Antioxidants Group (*Head: João Laranjinha*)

Membrane Toxicity Group (*Head: Amália Jurado*)

Pharmacometrics Group (*Head: Amilcar Falcão*)

Microbiology | *Milton Costa*

Microbiology of Extreme Environments Group (*Head: Milton Costa*)

Medical Mycology - Yeast Research Group (*Head: Teresa Gonçalves*)

Biophysics and Biomedical NMR | *Carlos Geraldes*

Inorganic Biochemistry and Molecular Imaging Group *(Head: Carlos Geraldes)*

Intermediate Metabolism Group *(Head: John Grifith Jones)*

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)*

Insulin Resistance and Adipocyte Group (*Head: Eugénia Carvalho)*

Area A | Neuroscience and Diesease

Coordinator | Catarina Resende Oliveira

General Objectives

Research is focused on understanding the modulation of synaptic function and the molecular mechanisms leading to synaptic dysfunction and neuronal death, with the goal of devising new targets to prevent or cure neurodegenerative disorders.
Special attention is devoted to: 1- study synaptic fine-tuning by adenosine, ATP, cannabinoid, nicotinic receptors; 2- demonstrate cellular and molecular mechanisms of glutamatergic synapses regulation; 3- study excitotoxic cell damage, in hippocampus, a brain region important in memory but particularly vulnerable to glutamate; 4- identify molecular insults and signalling pathways involved in brain and retina cell injury; 5- investigate primary cellular and molecular events induced by amyloidogenic peptides, α-synuclein, hyperglycemia and toxins with a main focus on mitochondria and inter-organelle cross-talk; 6- evaluate neuroprotective strategies, involving growth factors, pharmacological or cell replacement therapies; 7- develop new competences and tools to study neuroprotective and neuroregenerative pathways.

Main Achievements

The seven groups in this Line of research, using cell and animal models of disease, found that: 1- A1 and A2A Adenosine receptors work together in the control of glutamatergic synapsis, involved in the control of motor activity (forebrain) and memory (hippocampus). Receptors blockade and chronic caffeine consumption abrogate memory impairment and synaptotoxicity; 2- novel cytoskeleton and motor proteins, RNA processing proteins and kinases interact with glutamate AMPAR ; adhesion proteins, affect cell surface and synaptic expression of AMPAR subunits. NR2B subunit of NMDA receptors is necessary to NMDARs synaptic scaffold; 3- excitotoxic cell death involves: JNK- induced AMPARs phosphorylation, deregulation of ubiquitin-proteasome system through extra-synaptic NMDARs activation and down regulation of full-length BDNF TrkB receptors in hippocampal and striatal neurons; 4- diabetes changes the expression of glutamate receptors in human retina and high glucose alters the purinergic system. Ecstasy induces retinal cell death. NPY and NO stimulate endogenous progenitor cells proliferation; 5- ER/mitochondria interplay is a primary mechanism of Aβ, PrP or MPP+ induced neuronal loss due to Ca2+ homeostasis deregulation and oxidative stress. ER stress increases GSK3β tau phosphorylation, which is related with Cdk5 activation induced by the peptides. Mitochondria impairment leads to oxidative stress, proteasome deregulation, microtubule depolymerization and α-synuclein aggregation in a PD cellular model; 6- Aβ oligomers increase membrane surface NR2B NMDAR subunits in mature hippocampal neurons. In HD, BDNF signalling pathway activation prevents 3NP toxicity and alterations in mitochondrial proteome and complex I activity in the mice brain. Insulin increases expression of antioxidant proteins and prevents apoptosis. In MJD, an increase in ataxin-3 de-ubiquitinating activity was detected. Heroin/cocaine adducts neurotoxicity is higher than that of each drug per se; 7- a novel method based on single cell imaging technology allows the functional evaluation of differentiated stem cell cultures. NPY and TNFα have a concentration- dependent proneurogenic effect in SVZ stem cell niche.

Future Research

Research groups in this Line (Neuromodulation and Retinal Dysfunction and Neurogenesis) continue to develop collaborative research projects with pharmaceutical companies (BIAL, Portugal; Sanofi-Aventis, France) with which confidential contract agreements have been established.
In the frame of PDBEB at CNC (http://beb.cnbc.pt/) and the Doctoral Program at Faculty of Medicine, University of Coimbra (www.uc.pt/en/fmuc/phdhs), international courses on Neuroscience (at least 1-2

per year) will be proposed by the groups of the Neuroscience and Disease Line. Members of the Neuroscience and Disease area also participated as Faculty members at MIT-Portugal PhD Programme involving other Portuguese research Units (ITQB/IGC, IST, University of Minho, BIAL).
Mitochondria and Cell Death group will be involved in organization and teaching in the "2009 PENS Summer School- Metabolic Aspects of Chronic Brain Disorders", that will be held in Gunzburg, Germany (organizing committee: Patrick Weydt, Germany, Asa Petersen, Sweden, Ana Cristina Rego, Portugal, Maja Bresjanac, Slovenia).
An international Symposium on "Crosstalk Between Basic and Clinical Research", involving CNC groups and the Neurological Clinic, Coimbra University Hospital, is being proposed for 2010.
A patent resulting from the collaboration between different groups at CNC and the University of Minho - Silva B., Oliveira PJ, Dias ACP, Malva JO: Neuroprotection by phenolic compounds present in hypericum perforatum extracts. US Provisional PAT-US 61/020,226.

Future Plans

The wealth of expertise in studying glutamatergic synapses, mainly focused on glutamate receptors trafficking and synaptic stabilization, regulation of their functional properties, either by purines, neuropeptides or phosphorylation, the role of mitochondria dysfunction and changes in interorganelle crosstalk will be further explored, to look at synaptic dysfunction and excitotoxic cell death that have been observed in several neurodegenerative diseases, stroke and epilepsy. We plan to expand the competitive ongoing research on the molecular determinants of neurodegeneration, neuroprotection and neurogenesis, from the molecular and cellular level to in vivo animal models of disease with impact on human care strategies and offer competitive research service facilities to pharmaceutical industry.Future strategic development of research activity will be based on three main pillars: Neuroprotective strategies; Novel approaches of brain repair based in gene therapy; The use of stem and progenitor cells for brain repair.
Accordingly, we will pursue the study of the molecular and cellular basis of neuroprotection induced by modulators of synaptic function, including classic neuromodulators, neurotrophins and downstream antioxidant and mitoprotective mechanisms. New exciting developments of neuroprotection and neural tissue repair using gene delivery created the basic conditions to develop strong research groups devoted to the use of viral vectors and gene delivery as tools to stop the progression of brain diseases. Moreover, the use of stem cells and particularly neural stem/progenitor cells will contribute to develop novel approaches able to promote the replacement of death neurons and oligodendrocytes, with new functional cells, in models of brain diseases.
The close collaboration with the Neurology and Ophtalmology departments at the University Hospital will support the research using human tissues. This integrated approach will require the creation or updating of research units such as neuroproteomics, electrophysiology, viral vectors and gene therapy, microdyalisis experimental surgery and behaviour studies. To meet the needs of the use of genetically modified animals as models of disease, the expansion and re-organization of the animal facility is a priority

Neuromodulation Group

Rodrigo A. Cunha	(PhD – *Head of group*)
Paula G. Agostinho	(PhD)
Attila Köfalvi	(PhD)
Lisiane O. Porciúncula	(PhD)
Geanne M. Cunha	(PhD)
Manuella P. Kaster	(Post-Doctoral Fellow)
Paula M. Canas	(PhD Student)
Ana Patrícia Simões	(PhD Student)
Carla Sofia G. Silva	(PhD Student)
Rui Sanches	(PhD Student)
Pedro Manuel V. Garção	(PhD Student)
Marco António P. Matos	(PhD Student)
Elisabete O. Augusto	(PhD Student)
Pablo Pandolfo	(PhD Student)
Alexandre S. Rodrigues	(PhD Student)
Samira C. Ferreira	(PhD Student)

Glutamatergic Synapses Group

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Neuroprotection and Neurogenesis in Brain Repair Group

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Liliana Bernardino	(Post-Doctoral Fellow)
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Raquel Ferreira	(PhD Student)
Sofia Grade	(PhD Student)
Alexandra Rosa	(PhD Student)
Joana Gonçalves	(PhD Student)
Clarissa S. Schitine	(PhD Student)
Sofia Baptista	(PhD Student)

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Ramiro Almeida	(PhD)
Bruno O. Manadas	(Post-Doctoral Fellow)
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Marta Dias M. Vieira	(PhD Student)
Márcio S. Baptista	(PhD Student)
João T. Costa	(PhD Student)
Joana F. C. Fernandes	(MSc Student)
Patrícia Rebelo	(MSc Student)
Graciano Leal	(Undergraduate Student)

Mitochondrial Dysfunction and Cell Death Group

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Ana Isabel Duarte	(Post-Doctoral Fellow)
Tatiana R. Rosenstock	(Post-Doctoral Fellow)
Mª Teresa Cunha Oliveira	(Post-Doctoral Fellow)
Mário Laço	(PhD Student)
Ana Catarina H. Oliveira	(PhD Student)
Rita Perfeito	(PhD Student)
Ana Cristina Silva	(PhD Student)
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Molecular Mechanisms of Disease Group

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Daniela M. Arduíno	(PhD Student)
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Retinal Dysfunction and Neurogenesis Group

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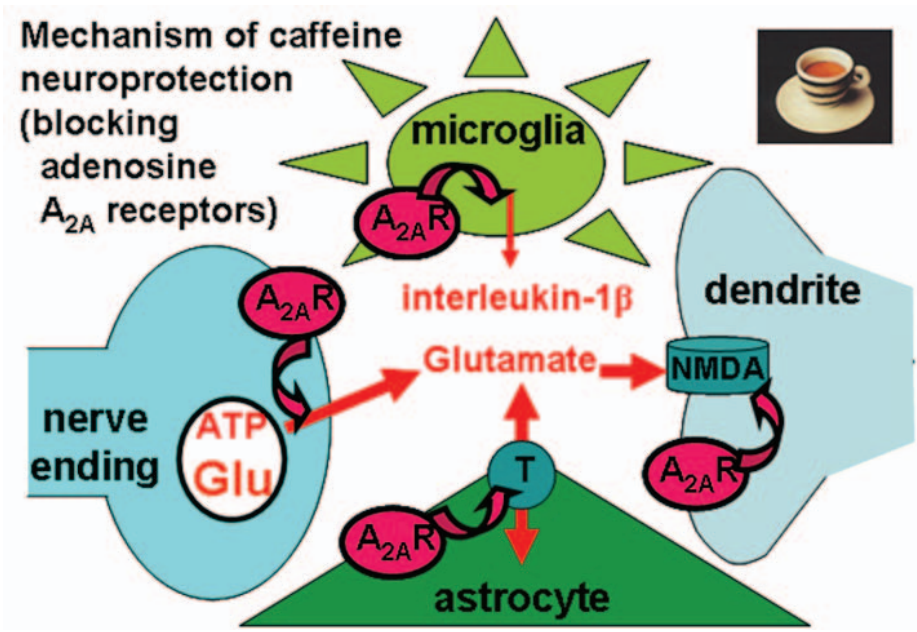
Inês Araújo	(PhD)
Joana Salgado	(Post-Doctoral Fellow)
Ana Rita Álvaro	(Post-Doctoral Fellow)
Bruno Carreira	(PhD Student)
Gabriel Costa	(PhD Student)
Maria Inês Morte	(PhD Student)
Ana S. Carvalho	(PhD Student)
Magda Santana	(PhD Student)
Mª João Catarino	(PhD Student)
Vera Raquel Cortez	(MSc Student)
Ana Sofia S. B. Baptista	(Grant Technician)

Neuromodulation | *Head: Rodrigo Cunha*

Objectives

Purines are prototypical neuromodulators in the brain, i.e. they do not directly transmit information between neurons, but instead they fine-tune the efficiency of information transfer. Adenosine is a metabolic product of ATP and is released from cells to act as a paracrine signal to prompt tissue adaptation to differences in workload or noxious conditions. This is achieved by activation of adenosine receptors (inhibitory A1 and facilitatory A2A receptors), which are the only known molecular targets of non-toxic doses of caffeine. Our aim is to investigate synaptic modulation systems preventing the earlier events in neurodegeneration. Our primary focus is adenosine A2A receptors (A2ARs) and we also explore other modulation system such as ATP P2Rs cannabinoid CB1Rs and nicotinic receptors. 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.



Main Achievements

We found that A1 and A2A receptors work together to define the salience of information encoding in brain circuits through control of excitatory (glutamatergic) synapses. The excessive firing of excitatory synapses can lead to glutamate toxicity and brain damage. Thus, neurodegenerative diseases begin with an early loss of synapses that precedes neuronal death, which makes synaptic modulation systems controlling glutamatergic synapses and synaptic degeneration attractive therapeutic candidates to prevent the early stages of degenerative diseases.

1. In cell type-selective A2ARs knockout mice it was demonstrated that A2ARs in extrastriatal neurons provides a prominent excitatory effect on psychomotor activity.

2. A2ARs activity in forebrain neurons is critical to control motor activity, whereas in brain cells other than forebrain neurons (likely glial cells) are important components for protection in a model of Parkinson's disease.

3. A2ARs act in concert with GDNF in the control of cortico-striatal glutamatergic pathways .

4. Postsynaptic A2ARs affect information processing in hippocampus (CA3 region) neuronal networks and memory performance

5. Although A2ARs do not affect general processes of memory impairment, they play a crucial role in memory dysfunction associated with Alzheimer's conditions involving an insidious synaptic deterioration.

6. The blockage of A2ARS and chronic caffeine consumption abrogate memory impairment and synaptotoxicity in adults which suffered from a convulsive period early in life.

7. The density of A2ARs tends to increase with aging, while the levels of inhibitory A1Rs and CB1Rs augment.

Glutamatergic synapses | *Head: Ana Luisa Carvalho*

Objectives

We are interested in understanding the connections between nerve cells in the brain, and how they are modified with experience. The ability of synapses to change their strength is thought to be the cellular correlate of learning and memory, and synaptic dysfunction is correlated with several neurodegenerative diseases. We focus on excitatory glutamatergic synapses, and study their regulation from a cellular and molecular biology viewpoint. We use a combination of primary neuronal cultures, molecular cell biology and biochemistry to address these questions.

1. Proteomic analysis of the interactome of AMPA-type glutamate receptors (AMPAR). AMPAR binding partners regulate their synaptic targeting. We identified several novel interactors for AMPAR subunits in a proteomic screening, and are currently addressing their function in regulating AMPARs.

2. Regulation of the mRNA stability of the mRNA for GluR1 AMPAR subunit. We found evidence for post-transcriptional regulation of the mRNA levels for GluR1, and are addressing its mechanisms.

3. Mechanisms of synaptic traffic of NMDA receptors (NMDAR). NMDARs play a role in the induction of synaptic plasticity. We use cultured neurons from knock-out mice for NMDAR subunits to understand subunit-specific rules that govern synaptic targeting of NMDARs.

4. Regulation of glutamatergic transmission by ghrelin in the hippocampus. Ghrelin is an appetite-stimulating hormone which was shown to enhance memory processes and synaptic plasticity in the hippocampus. We are characterizing the subcellular localization of ghrelin receptors in the hippocampus, and evaluating how ghrelin receptors affect glutamatergic transmission.

5. Synaptic dysfunction in a mouse model of Machado-Joseph disease (MJD). MJD is a spinocerebellar ataxia caused by a glutamine expansion in ataxin-3, an ubiquitin protease. We are using a transgenic mouse model of MJD to address the role of excitotoxicity and synaptic dysfunction in the pathogenesis of this disease.

Main Achievements

Proteins that interact with AMPA-type glutamate receptors (AMPAR) are crucial for appropriate targeting of receptors to synapses (*Santos et al. 2009*). We have performed a proteomic screening for binding partners of Ca^{2+}-permeable AMPARs. After re-isolating known AMPAR interactors, we identified novel interactors, such as motor proteins and proteins of the neuronal RNA granules. Moreover, we isolated the cell adhesion molecule Contactin associated protein 1 (Caspr1). Functional analyses revealed that Caspr1 affects the cell surface and synaptic expression of AMPAR subunits in cultured hippocampal neurons (*Manuscripts in preparation*).

NMDA receptors (NMDAR) are the coincidence detector in the induction of synaptic plasticity. We are addressing the mechanism of synaptic accumulation of NMDARs, and found that the NR2B subunit of NMDARs is necessary to establish the synaptic scaffold for NMDARs, since synaptic clusters of NMDARs are dramatically reduced in primary hippocampal neuronal cultures from NR2B knock-out mice. Engineered subunits of the NMDAR complex are being reintroduced into neuron cultures from knockout mice, to dissect molecular signals involved in NMDAR trafficking.

The number of dendritic spines, where excitatory synapses are located, is regulated by molecules that organize their actin cytoskeleton, e.g. cortactin. We are studying how cortactin acetylation influences its role in the morphogenesis of spines, and found that overexpression of the acetylation and deacetylation mimetics of cortactin changes the density of synapses in hippocampal neurons in culture (*Fig.1*).

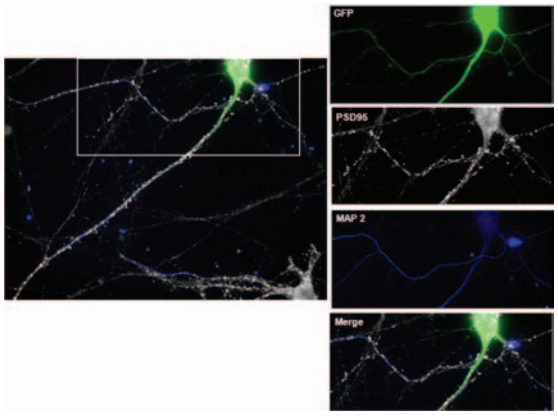


Fig.1. Hippocampal neurons in culture transfected at 7 DIV with GFP and cortactin, and labeled at 15 DIV for a marker of excitatory synapses (PSD95), and a somatodendritic marker (MAP2). Expression of cortactin changes the density of synapses in cultured hippocampal neurons.

Another interest in the group is to study dysfunction of glutamatergic transmission in pathology. We are interested in ataxin-3, the polyQ-containing protease involved in Machado-Joseph disease (MJD). We have characterized its nucleocytoplasmic shuttling activity (Macedo-Ribeiro et al. 2009), and will now test the hypothesis that there are alterations in glutamatergic synapses in MJD.

Neuroprotection and Neurogenesis in Brain Repair | *Head: João Malva*

Objectives

The research activity of the "Neuroprotection and Neurogenesis in Brain Repair" group is founded on four main research pillars: 1- Neuromodulation; 2- Neuroprotection; 3- Neuroinflammation; 4- Neural stem cells and brain repair. This research effort aims at finding novel molecular and cellular targets to prevent the progression of brain damage and to treat brain diseases.

In 2008 we focused particularly on the impact of neuroinflammation in neural stem cell fate. The main objective of the research group has been the development of new competences and new tools to study neuroprotective and neuroregenerative pathways for brain repair purposes.

Specific objectives included:

1) To establish a novel single cell calcium imaging-based tool to functionally identify different neural phenotypes differentiating from neural stem cell cultures

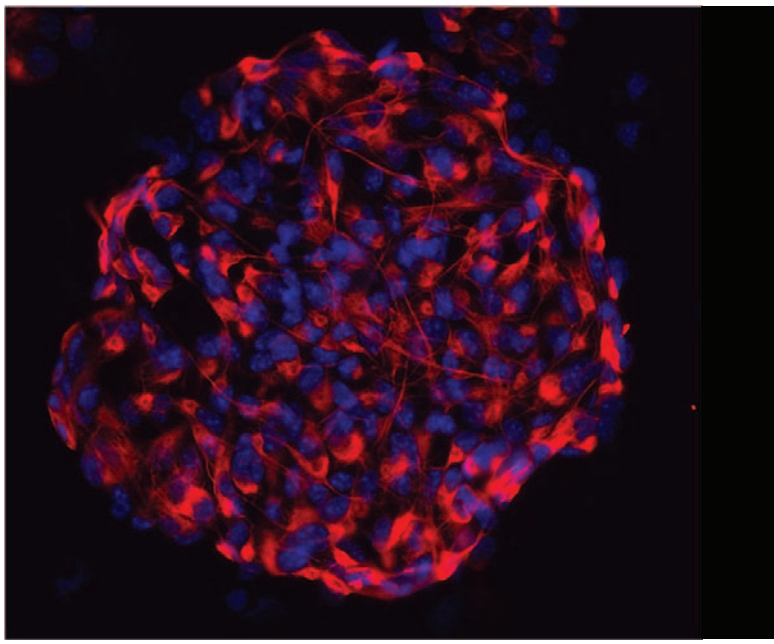
2) To identify novel proneurogenic and pro-oligodendrogenic factors as well as new drugs affecting neural stem cell physiology/differentiation useful for stem cell-based brain repair strategies

3) To develop new pharmacological procedures to treat neural stem cell cultures in order to boost differentiation of specific neural stem cell phenotypes in central nervous system disease models. These models included: temporal lobe epilepsy; methamphetamine-induced neural damage; demyelinating diseases; retinal excitotoxic injury.

At the long-term, we aim to contribute with unique tools to discover new molecular and cellular targets to better understand neural stem cell biology. Ultimately, these tools will allow the identification of new targets for drug discovery and new pre-transplantation procedures of neural stem cells into to the diseased brain.

Main Achievements

We developed a novel robust method useful to identify the phenotype of living cells differentiating from neural stem cell cultures. This method is based in single cell calcium imaging technology and allows the simultaneous identification of more than 100 cells in neural stem cell populations, matching unique functional profile of responses with the phenotypic markers of neural cell lineages. This method has proven to be a valuable tool for drug discovery and to functionally evaluate the quality of differentiated stem cell cultures for pre-transplantation procedures. With this method we are able to functionally characterize a diversity of cells in the same cultures including: immature cell, astrocytes, oligodendrocytes, progenitors and neurons. Two patents were produced (WO2008100168-A1, PAT2008100089504/0198).



We could identify the proneurogenic effect of neuropeptide Y (NPY) and tumor necrosis factor alpha (TNFalpha) in neural stem cell cultures derived from the subventricular zone of the mice (SVZ). Interestingly, according to previous findings of the group in organotypic brain slice cultures, we found that TNFalpha exerts ambiguous effects in SVZ cultures. At low concentrations TNFalpha induces proliferation and differentiation of neurons (involving JNK activation and axonogenesis) whereas at high concentrations this cytokine is mainly toxic. These data reinforces the double effect of neuroinflammation in brain repair, depending on the magnitude of the inflammatory response to brain injury. Moreover, we also determined that NPY is a potent proliferative and proneurogenic peptide in the SVZ stem cell niche. NPY Y1 receptor is expressed and functionally active in the adult mice SVZ and its activation causes phenotypic and functional neuronal differentiation. These projects resulted in the publication of three major manuscripts (one in Rejuvenation Research; two in Stem Cells).

Neuronal Cell Death and Neuroprotection | *Head: Carlos Duarte*

Objectives

Numerous disorders of the CNS are characterized by neuronal cell death, which may arise from the deregulation of the activity of neurotransmitter systems or insufficient neurotrophic support. 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.

Main Achievements

The contribution of Ca2+-permeable AMPA receptors overactivation to dell death was investigated in HEK293 cells constitutively expressing GluR4-containing AMPARs. Excitotoxicity mediated by these receptors was found to involve the calcium-dependent activation of the JNK pathway. The GluR4 subunit was shown to bind the JIP-1 protein, a scaffold that participates in the JNK cascade induced by death stimuli in neurons, and current studies will allow mapping the JIP-1 domain interacting with GluR4. Moreover, JNK-specific GluR4 phosphorylation was increased upon excitotoxic stimulation. Studies with the GluR4 subunit mutated at the phosphorylation site will allow understanding the functional role of this phosphorylation.

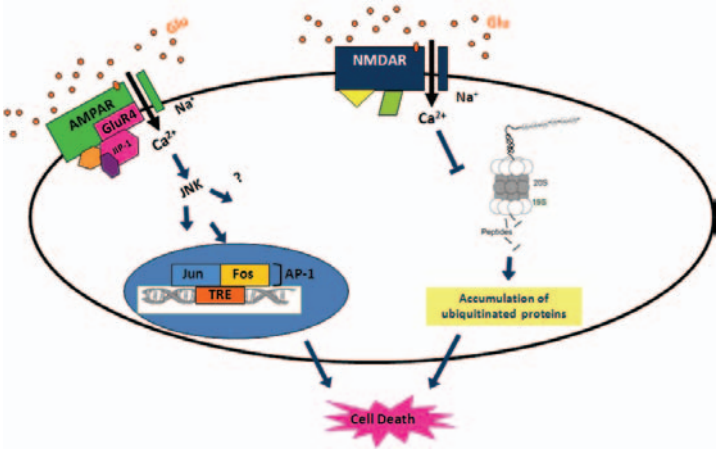


Fig. 1. Excitotoxic stimulation of Ca^{2+}-permeable AMPA and NMDA receptors for the neurotransmitter glutamate leads to cell death due to activation of the JNK signaling pathway and inhibition of the ubiquitin-proteasome system, respectively.

Cell death under excitotoxic conditions is mediated by deregulation of the major proteolytic systems in neurons. Excitotoxic stimulation of hippocampal neurons down-regulated proteasome activity by a mechanism sensitive to cathepsin-L inhibitors. The decrease in proteasome activity was more significant in the nuclear fraction than in the cytoplasmic fraction, and was mediated by activation of extrasynaptic NMDA receptors. Excitotoxic stimulation with glutamate also decreased the deubiquitinase activity, pointing to a general deregulation of the ubiquitin-proteasome system.

Excitotoxic stimulation was found to down-regulate the full-length TrkB (TrkB-FL) receptors for the neurotrophin BDNF in hippocampal and striatal neurons, by a calpain-dependent mechanism, and upregulated the truncated form of the receptors (TrkB-T). Under the same conditions there was a decrease in the signaling activity of TrkB-FL, and current studies will allow determining the role of a putative dominant negative effect of TrkB-T. The effect on TrkB-T occurred at the transcription level, and was coupled to the down-regulation of RhoA signaling activity. Future studies will address how the change in TrkB protein levels affect neuroprotection under excitotoxic conditions.

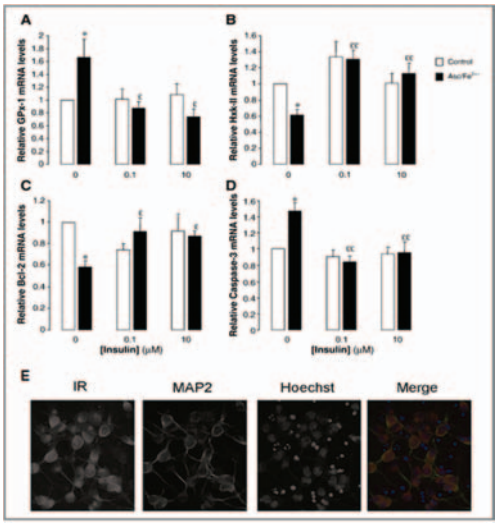
Mitochondrial Dysfuntion and Cell Death | *Head: Ana Cristina Rego*

Objectives

Selective neurodegeneration in irreversible disorders of the CNS has been largely attributed to protein misfolding, excitotoxicity and mitochondrial impairment. However, how modified or mutant proteins interfere with mitochondrial and neuronal function is largely unclear. Furthermore, several neurotoxic substances cause neuronal death through changes in mitochondrial activity, deregulation of intracellular calcium homeostasis and oxidative stress. Thus, the main objective of our group is to study defective intracellular signaling mechanisms and identify molecular targets for therapeutic intervention underlying excitotoxicity, mitochondrial dysfunction, oxidative stress and neuronal death in neurodegenerative diseases, including 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 cell function and thus cell survival in animal and cellular models of neurodegenerative disorders. The protective effect of growth factors that act as trophic signalling molecules assumes a high importance in neurodegeneration. Therefore, we will examine the influence of brain-derived neurotrophic (BDNF) and glial cell-derived neurotrophic factor (GDNF) either directly or after transduction into stem cells acquiring a neural phenotype, and the neuroprotective effect of insulin-growth factor (IGF-1), insulin and histone deacetylase modulators in PD and HD models.

Main Achievements

In the context of HD, our group showed that activation of BDNF signaling pathways prevent 3-NP toxicity and transcriptional deregulation in neurons. We also observed alterations in the mitochondrial proteome of HD mice brain and in complex I activity, which were prevented by BDNF. These data implicate expression of mitochondrial proteins and molecular activity in this organelle as HD therapeutic targets. Moreover, we demonstrated insulin neuroprotection against oxidative stress through increased expression of proteins involved in antioxidant defense, glucose metabolism and prevention against apoptosis (*Duarte et al, 2008*). We also observed insulin protection against oxidative stress in striatal cells derived from HD knock-in mice. Mutant cells revealed increased ROS formation, decreased GSH synthesis and in the activity of antioxidant enzymes. We further studied markers of apoptosis and mitochondrial dysfunction in peripheral blood cells of HD patients (Almeida et al, 2008). We found increased Bax expression in B and T lymphocytes, and monocytes from HD patients. B lymphocytes also showed decreased mitochondrial potential, suggesting that these cells may reflect changes observed in HD brain. In the context of MJD, we observed an increase in ataxin-3 deubiquitinating activity in vitro after long periods of incubation with valosin-containing protein (VCP/p97). Using human SH-SY5Y cells transfected with WT and mutant A53T alpha-synuclein (a-syn) subjected to iron and rotenone as models of PD, we detected increased P-Ser129-a-syn, decreased mitochondrial potential and ROS production, in the absence of a-syn aggregates. Within the scope of AD pathogenesis, we observed that amyloid-beta peptide (Abeta) oligomers increase NR2A de novo protein synthesis, but enhance membrane surface of NR2B NMDAR subunits in mature hippocampal neurons. Abeta evoked calcium rise sensitive to NMDAR antagonists and decreased tubulin-beta III levels, suggesting the involvement of NMDAR subunits on neuronal dysfunction caused by Abeta oligomers. Regarding research on drugs of abuse we showed that combination of cocaine and heroin enhances the neurotoxicity of the drugs alone and that cocaine-morphine adducts shift cell death pathways towards necrosis.



*Insulin-mediated mRNA expression of glutathione peroxidase-1 (**A**), hexokinase-II (**B**), Bcl-2 (**C**) and caspase-3 (**D**) upon oxidative stress in cultured cortical neurons labeling the insulin receptor (IR) (**E**) [Duarte et al., 2008].*

Molecular Mechanisms of Disease | *Head: Cláudia Pereira*

Objectives

Alzheimer's disease (AD), Parkinson's disease (PD) and prion-related encephalopathies (PRE) are progressive neurodegenerative disorders characterized by the extracellular deposition of amyloid-beta peptide (Aβ), alpha-synuclein and the scrapie isoform of prion protein (PrPSc), respectively. 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. The aim of the Molecular Mechanisms of Disease group during 2008 was to investigate the primary molecular and cellular events induced by these disease-related peptides and their causal relationships in order to identify potential targets for therapeutic intervention.

The involvement of mitochondria dysfunction, which has emerged as a potential 'lowest common denominator' linking several neurodegenerative disorders, was investigated in studies conducted in mitochondrial DNA-depleted rho0 cells and in cultured neurons isolated from rat brain cortex exposed to Aβ or PrP peptides or to MPP+ (which causes parkinsonism by killing dopamine-producing neurons in the substantia nigra). Using these AD, PD and PRE cell models, we further explored the mitochondria/endoplasmic reticulum (ER) cross-talk as a primary molecular mechanism leading to neuronal loss. To get a better insight into the role of mitochondrial dysfunction in AD and PD we have used cybrid cells obtained from the fusion of rho0 cells with platelets isolated from AD or PD patients (or age-matched controls) that recapitulate the focal mitochondrial respiratory chain defects, and also human brain tissue (cortex and hippocampus) and fibroblasts from AD patients and control subjects. Using the triple transgenic mouse model of AD (3xTg-AD), we analysed pathogenic mechanisms, in particular, oxidative stress and cell cycle reactivation, during the progression of AD-like neuropathology.

Another focus of research of our group during 2008 was the role of neuroinflammation in AD and PRE. Using co-cultures of microglia/cortical neurons challenged with Aβ or PrP or Aβ-treated cultured astrocytes, we investigated the involvement of glia activation in neuronal injury.

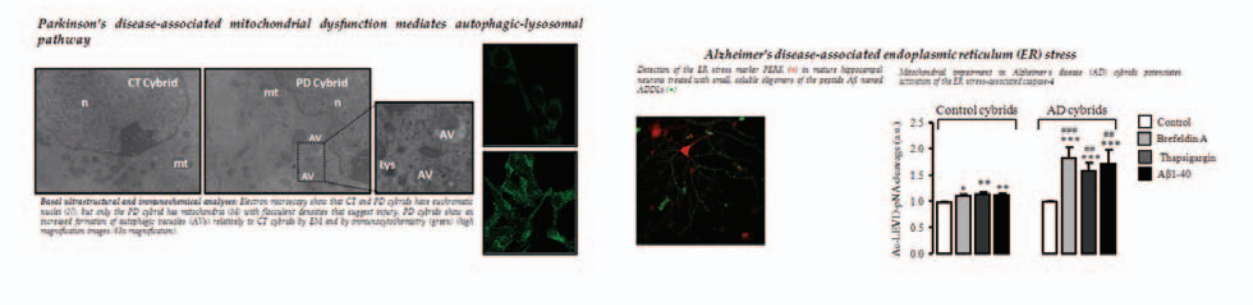
Main Achievements

One of the group's achievements was that the interplay between ER and mitochondria represents a primary mechanism leading to neuronal loss triggered by Aβ, PrP or MPP+. Data obtained in cortical neurons, corroborated by studies in rho0 cells, demonstrate that Aβ and PrP activate the ER stress-mediated apoptotic pathway by a mitochondrial-dependent process. ER stress was also shown to be involved in GSK3β-mediated tau phosphorylation induced by Aβ. Additionally, the Aβ-induced abnormal mitochondrial dynamics via differential modulation of fission/fusion proteins was revealed.

In Aβ- or PrP-treated neurons, Cdk5 is activated leading to tau phosphorylation and also apoptosis due to abortive cell cycle reactivation. In 3xTg-AD mice, neuronal cell cycle reactivation is not a direct consequence of Aβ and tau pathologies. In this transgenic model, oxidative stress was shown to be an early event during the neuropathological process. 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.

Our data supports the role of neuroglia dysregulation in AD and PRE. In co-cultures of microglia/cortical neurons challenged with Aβ or PrP, IL-6 released by activated microglia contributes to neuronal injury. Aβ also regulates the activity/levels of glutamate transporters in cultured astrocytes decreasing glutamate clearance.

We also contributed to elucidate the mechanisms underlying cell degeneration in PD. Using rho0 cells and PD cybrids, we demonstrated that mitochondria impairment leads to oxidative stress, inducing proteasomal deregulation. Moreover, we showed the impact of mitochondrial dysfunction in microtubule depolymerization and its relevance to alpha-synuclein aggregation.



Retinal Dysfunction and Neurogenesis | *Head: Claudia Cavadas*

Objectives

The retina is a neuronal structure highly susceptible to several insults, such as hyperglycemia, excitotoxicity, inflammation and exposure to drugs. Our group is actively committed to identify important players and mechanisms mediating retinal and neuronal damage, with the main goal of devising new therapeutic targets and strategies to treat retinal or brain degenerative diseases

We will clarify the role(s) of NPY in retinal physiology, and further investigate the potential neuroprotective and regulatory effects of NPY on retinal progenitor cell proliferation and differentiation.

We will continue to study the impact of hyperglycemia in the retina, namely on exocytosis events, on the molecular mechanisms underlying changes in AMPA receptor subunits expression in retinal cells, and on the regulation of retinal microglia.

The evaluation of the potential harmful effect of ecstasy in rat retinal physiology and morphology is another goal of our group.

The identification of the signaling pathways and molecular mechanisms responsible for the proliferative/antiproliferative effects of nitric oxide on neural stem cells is another main research interest of our group.

In a project of close cooperation with the pharmaceutical industry, we are evaluating the neurotoxicity/safety profile of eslicarbazepine acetate (developed by BIAL, Portugal) and its metabolites, as compared to other antiepileptic drugs (AEDs). We are also investigating the effects of AEDs on the proliferation and fate of neural stem cells.

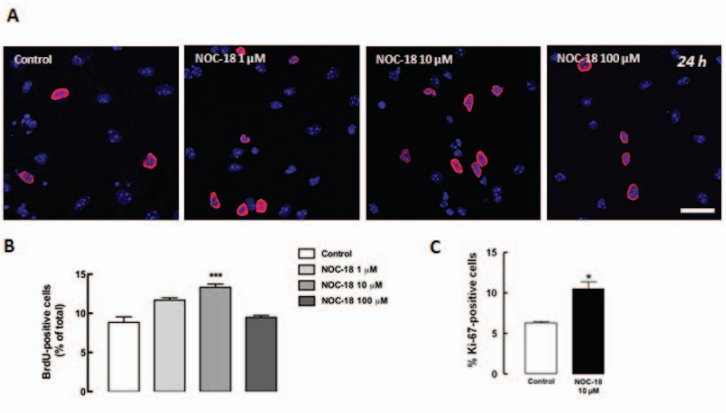


Fig. 1. Stimulation of the proliferation of neural stem cells from the mouse subventricular zone by nitric oxide (NOC-18 = NO donor). Cell proliferation was quantified by assessing the number of cells that incorporate 5-bromo-2-deoxyuridine (A,B) or express Ki-67 (C). Scale bar =30 µm.

Main Achievements

Starting by the hypothesis that glutamate, the main excitatory neurotransmitter in the retina, might be involved in the pathogenesis of diabetic retinopathy, we found that diabetes or elevated glucose levels can impair the uptake and the release of excitatory neurotransmitters in the retina and alter the expression of ionotropic glutamate receptor subunits. In fact, we observed that diabetes changes the expression of ionotropic glutamate receptor subunits in the human retina, suggesting that glutamatergic transmission in the retina might be compromised early in the course of diabetes. Moreover, inflammation, and activation of microglial cells, has been shown to have an important role in diabetic retinopathy. We have shown that high glucose alters the purinergic signaling system in the retina, resulting high levels of extracellular ATP that may lead to inflammation involved in the pathogenesis of diabetic retinopathy. Moreover, we observed that Muller cells do not influence the adhesion of leukocytes to retinal endothelial cells

We have also described that ecstasy (MDMA) induces retinal cell death, and we identified neuropeptide Y (NPY) as neuroprotective agent against this insult. Moreover, NPY stimulates the proliferation of retinal progenitor cells mediated by the activation of NPY Y1, Y2, and Y5 receptors as well as the by the nitric oxide (NO)-guanylyl cyclase pathway. This pathway is also activated by NPY when it stimulates catecholamine release from mouse neuronal-like cells (chromaffin cells).

The study on proliferation of endogenous neural progenitor cells, as a strategy to promote neuronal repair, showed that NO stimulates the proliferation of neural stem cells by passing the epidermal growth factor receptor.

Moreover, in a parallel research line, our results show that eslicarbazepine acetate (BIA 2-093) and its metabolites are not toxic to hippocampal neurons compared to carbamazepine or oxcarbazepine.

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Area B | Molecular Biotechnology and Health

Coordinator | Euclides Pires

General Objectives

Living organisms are built of interacting components whose correct function or dysfunction leads respectively to health or disease conditions. Understanding interactions, from the molecular level to the system level, is a tremendous task crucial to the design of strategies capable of correcting dysfunction.

The Biotechnology and Health Line of CNC has been "shapped" steadly by hiring young scientists with the competence to investigate interactions at different levels and apply this know - how to a selected set of pathologies. At present this Line comprises five research groups whose general objectives are: 1) unveal clusters of genes associated with some diseases; 2) exploit design principles in metabolic networks; 3) investigate molecular interactions as well protein folding and unfolding using computational methodologies; 3) search, structural characterization and kinetics of proteases with biomedical or biotechnological interest; 5) development of viral and non-viral drug carriers; 6) development of biomaterials for stem cell-based therapeutics; 7) development of a technological platform to generate new models of human CNS diseases.

Main Achievements

• A generic approach for characterizing and quantifying global tolerance of Biochemical systems was proposed.

• A computational tool for integrated visualization of protein interaction networks and RNA expression was devised.

• A prototype for a public open platform to share protein folding and unfolding was developed.

• New data mining tools for analysis of multiple protein folding and unfolding simulations started to be developed.

• The most common and abundant allergenic pollens were shown to contain serine and/or aminopeptidase activity which is instrumental in degrading intercellular adhesion proteins.

• A novel lipid – based nanosystem that has the potential to target the microenvironment of human breast tumor was developed.

• A new approach for delivery of vascular growth factors into embryonic stem cells was established.

• New nanomaterials for drug release and cell tracking were developed.

Future Research

Patents/prototypes

"Human embryoid bodies containing nano- and micro-particulate delivery vehicles" Ferreira, L., Kohane, D., Langer, R. Patent pending, 2008.

"A biodegradable and biocompatible Gecko-inspired tissue adhesive".Lino Ferreira, Alborz Mahdavi, Cathryn Sundback, David J. D. Carter, Chris Bettinger, Andreas Zumbuehl, Jeff Borenstein, Joseph Vacanti, Robert Langer, Jeffrey M. Karp. Patent pending, 2008.

"Nano-transportadores de base lipídica para entrega direccionada de vectores virais e processo para a sua produção"Ana Filipe, M. C. Pedroso de Lima, Mauro Giacca and Sérgio Simões. Request number: PCT/IB2008/054399.

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"Human papilloma virus detection kit" Nobre, R., Pereira de Almeida L., Martins T., US Provisional Patent Application: Ref. PAT-US 38267/08.

"Stable and readily dissolved compositions of Candesartan cilexetil prepared with wet granulation"Gabriel Silva, Sérgio Simões and Frank Gindulis.; 91881 EP (BE/BS).

"Synthesis of Compounds Relating to Photodynamic Therapy of Cancer". L. G. Arnaut, M. M. Pereira, S. J. Formosinho, S. Simões, G. Stochel and K. Urbanska. Universidade de Coimbra. UK Patent Application no. 0819594.3, 24th of October, 2008.

Organization of conferences

Program Committee, International Conference on Molecular Systems Biology, Diliman (Philippines)

Industry contract research

"Design of a biomimetic injectable scaffold for the transplantation and differentiation of umbilical cord stem cells- InjectCord". Funding: 70,000 euros. Source: Crioestaminal.

"Antifungal nanocoatings". Funding: 150,000 euros. Source: Biocant Ventures. BIOCANT VENTURES

"Production of recombinant growth factors for cell culture", Source: Criostaminal

"Production of a new milk coagulant based on cardosins", Source: BIOCANT VENTURES

"Nanomedicine and new therapeutic strategies: development of supramolecular strategies for therapy of ophthalmologic diseases",Partnership with the industry Bluepharma. Source: CCRDC – Coordinator committee for regional development of the Center.

Future Plans

The research activity developed by the investigators of this line aims at: 1) understanding interactions of living organisms components from a molecular level to system level; 2) design strategies with the potential to modulate these interactions. The work performed by the Molecular Systems Biology, the Structural and Computational Biology and by the Molecular Biotechnology groups is concerned with the first aim whereas the work performed by the groups Vectors and Gene Therapy and Biomaterials and Stem Cell – based therapy is concerned with the second aim.

In brief the future working plans of the groups concerned with the first aim are: to pursue with the analysis of the design principles of metabolism and to proceed to experimental validation of models purposed; to carry on the search for clusters of genes associated with diseases; to characterize mechanisms of protein aggregation that lead to diseases; rational design and testing of lead compounds for inhibition of amyloid formation; identification of the natural substrates of the recent unvealed proteases CDR 1 (constitutive disease resistant protease) and RCS1 (promoter of cell survival protease); proceed with the molecular characterization of the pollen proteases that show high ability to cause airway damage. In what it concern the second aim the future working plans are: development of the use of viral and non viral technological platforms to generate new models of CNS diseases; do develop new approachs for transplantation of stem cells, in particular vascular progenitor cells; to develop new tools to image, non – invasively, transplanted stem cells; to develop antimicrobial coating for medical device surfaces.

Molecular Biotechnology Group

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Paula Veríssimo Pires	(PhD)
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Rui Cruz	(MSc Student)
Raquel Vinhas	(MSc Student)
Nair Monteiro	(Undergraduate Student)
Maria Inês Coelho	(Undergraduate Student)

Molecular Systems Biology Group

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Pedro Miguel Brás M. Coelho	(PhD Student)
Inês Vasconcelos Miranda Santos	(PhD Student)
Pedro Manuel Batista Branco	(PhD Student)
Pedro Alexandre Martins	(MSc Student)
Flávio Fortes R. Sousa	(MSc Student)

Structural and Computational Biology

Rui M. M. Brito	(PhD – *Head of group*)
Elsa Henriques	(PhD)
Daniela Cipreste Vaz	(PhD Student)
Cândida S. Gonçalves da Silva	(PhD Student)
Carlos José Vieira Simões	(PhD Student)
Catarina Sofia H. Jesus	(PhD Student)
Pedro Cruz	(Grant Technician)

Vectors and Gene Therapy Group

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Sérgio Simões	(PhD)
João Nuno Moreira	(PhD)
Luís Almeida	(PhD)
Henrique Faneca	(PhD)
Clévio Nóbrega	(Post-Doctoral Fellow)
Ana Luísa Cardoso	(Post-Doctoral Fellow)
Adriana Santos	(PhD Student)
Ana Filipe	(PhD Student)
Mª Isabel Nascimento Ferreira	(PhD Student)
Lígia Mª Sousa Ferreira	(PhD Student)
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Ligia Gomes da Silva	(PhD Student)
Nélio Gonçalves	(PhD Student)
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Sara Trabulo	(PhD Student)
Sónia Duarte	(PhD Student)
Ana Teresa Simões	(PhD Student)
Luís Bimbo	(MSc Student)
Tiago Francisco S. Ferreira	(Grant Technician)

Emerging Group

Biomaterials and Stem Cell-Based Therapeutics

Lino Ferreira	(PhD - *Head of group*)
Dora Pedroso	(Post-Doctoral Fellow)
Helena Vazão	(PhD Student)
Cristiana Paulo	(PhD Student)
Maria Nunes Pereira	(PhD Student)
Renata S. M. Rodrigues	(PhD Student)
Paula Sofia S. Lacerda	(PhD Student)

Molecular Biotechnology | *Head: Carlos Faro*

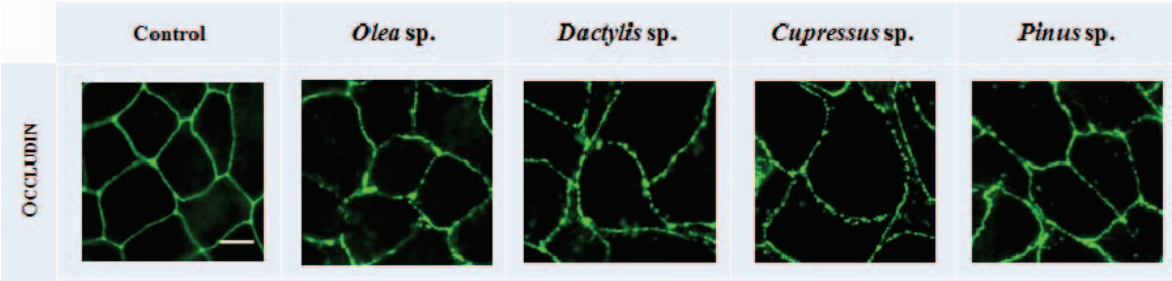
Objectives

The Molecular Biotechnology group has a long-time interest in studying biotechnologically and/or biomedically relevant plant proteases. Understanding the structure-function relationship of plant aspartic proteases has been the main research objective. Initial studies used cardosins, the milk-clotting enzymes from the flowers of cardoon, as working models. Since the sequencing of Arabidopsis genome our interest shifted towards the study of aspartic proteases from this model organism, involved in disease resistance and stress responses. The systematic biochemical analysis of the plant aspartic proteases has given important information on the mechanistic aspects of enzyme activity and inhibition, a valuable mean to understand the possible biological functions of this family of enzymes.

Another line of research is devoted to study serine proteases from allergenic pollens. The enzymes have been purified and caracterized in our laboratory and seem to play an important role in allergy. The overall goal is to understand the molecular mechanism underlying the possible involvement of these proteases in eliciting the allergenic response as well as to assess whether or not they can be good therapeutic targets.

Main Achievements

Allergy is a major health issue that is increasing worldwide and affects one third of the European population. Pollens are important triggers for allergic rhinitis, conjunctivitis and asthma. Over the past year we evaluated the presence of protease activity in several allergenic pollens. All pollen diffusates were shown to have high molecular proteases with low pI and predominant serine and/or aminopeptidase activity. Moreover all pollen extracts, with distinct allergic potential, were able of increasing transepithelial permeability and cell detachment in vitro by degrading intercellular adhesion proteins. These results suggested that the proteases normally presented in the pollen grains, even those with less allergenic capacities, might be involved in the sensitization to a range of airborne allergens by facilitating their contact to subepithelial immune cells.



Diffusates from different allergenic pollen types cause loss of epithelial integrity through disruption of intercellular adhesion proteins. MDCK monolayers were incubated with four pollen diffusates. Immunofluorescence results show that occludin, a transmembrane protein from tight junctions, is degraded by each pollen extract at the same extent after only 1h of exposure. Cell shape is also altered. Representative images are shown for each stimulus. Bar = 20 μm

Molecular Systems Biology | *Head: Armindo Salvador*

Objectives

Main objectives of our group are:

1. Discovering and understanding the naturally evolved principles of quantitative design of the most prevalent elementary circuits in metabolic networks. These design principles are rules associating quantitative design (e. g., relationships among enzyme kinetic parameters or among these and effector concentrations) to function.

2. Kinetic modeling and systems analysis of the biogenesis, fate and regulation of reactive metabolic intermediates. Reactive intermediates are involved in many pathologies and, much for the same reasons that make them toxic, also participate in important regulatory mechanisms. We seek a better grasp of the trade-offs involved in the metabolic processes that generate these species and to understand how these trade-offs inpend on the regulatory design of these processes.

3. Modeling the permeation of the blood-brain barrier by amphiphiles. The aim is to understand how the interactions of amphiphilic drugs with the various relevant aqueous, lipid, and glycocalyx compartments to which they partition in the blood stream and while crossing the blood-brain barrier affect the efficacy of their delivery to brain tissues.

4. Developing a method for profiling mitotic-cycle-dependent metabolism without having to synchronize cells.

5. Developing an Internet-based platform for distributed colaboration in kinetic modeling of biochemical processes. Current solutions for archival and communication of kinetic models just store "frozen" versions of the models and do not promote discussion and further development. This is a major limitation beacuse model development should be viewed as a dynamic process reflecting the evolving knowledge about biochemical processes. We seek to develop a platform — WikiModels — for developing models as a comunity activity through constant open peer-review of modeling decisions, recording successive states of a model and tracking credit for contributions.

Main Achievements

We tested if the design principles we derived for moiety-transfer cycles [Coelho PMBM, Salvador A, Savageau MA (2009). PLoS Comput. Biol. 5:e1000319, and paper in preparation] apply extensively. Tests so far focused mainly redox cycles and were based on both detailed analyses of well-characterized instances and broad surveys of metabolite concentrations and enzyme-kinetic parameters. We examined in detail glutathione, NADPH, cyt b5 and FAD cycles in human erythrocytes and found them to adhere to the predicted design principles. A set of KMs for >50 enzymes involved in NADPH redox cycles in E. coli and S. cerevisiae indicates that the predicted design principles hold extensively.

We developped a mathematical approach for systematically constructing a "dimensionally-compressed" design space and partitioning this into discrete regions of qualitatively different performance [*Savageau MA, Coelho PMBM, Fasani RA, Tolla DA, Salvador A (2009). Proc. Natl. Acad. Sci. USA 106:6435*].

Activation of uncoupling protein 2 (UCP2) by 4-hydroxynonenal (HNE) decreases the protonmotive potential across the mitochondrial inner membrane, with ensuing decrease in superoxide (SO) formation by Complex I. Because SO is one of the main initiators of lipid peroxidation, of which HNE is an end-product, the overall process is a negative feedback loop on SO production. We have investigated possible functional advantages of this indirect feedback loop versus direct UCP2 activation by SO, which would in principle permit faster regulation. The analysis indicates that the HNE-mediated design is better at managing the trade-off between efficiency of energy production and minimization of the generation of reactive intermediates.

We developped a mathematical model for the redistribution of amphiphiles among lipid compartments in human blood and crossing of the blood-brain barrier. The model permitted identifying limiting steps in the transport of amphiphiles from bloodstream to the brain tissue.

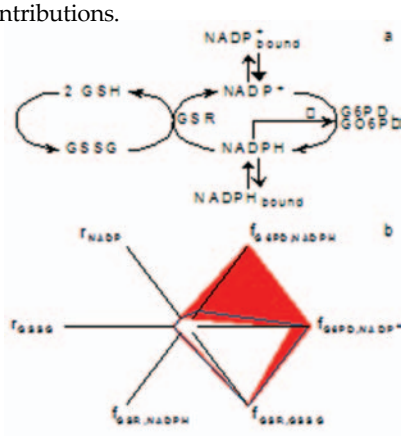


Fig.1. Design of the coupled NADPH and GSH redox cycles (a) in human erythrocytes. (b) Good performance requires orchestration among parameters. Each parameter is represented in a radial axis extending from 0 (inner tip) to 1 (outer tip). A polygon joining values in each axis indi-cates the parameter set for a given realization of the circuit. The red polygons indicate the realizations that warrant a level of performance matching or exceeding that in vivo. The dashed blue polygon indicates the parameter set that holds in normal human erythrocytes.

Structural and Computational Biology | *Head: Rui Brito*

Objectives

The group is strategically focused on the use of experimental and computational methodologies to study the molecular basis of human and animal disorders, in particular amyloid diseases. 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 (TTR), the causative agent of Familial Amyloid Polyneuropathy (FAP). 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. Significant efforts in Grid computing and volunteer computing are being made by the group and in the near future will be publicized.

Additionally, using time-dependent density functional theory (TDDFT) we are currently studying the process of light emission in the luciferin/luciferase system, present in fireflies and some beetles.

Main Achievements

a. Development of the first prototype for a public open platform to share protein folding and unfolding simulations, the P-found system (www.p-found.org).

b. Development of new data mining tools for the analysis of multiple protein unfolding simulations in order to infer rules that may discriminate between amyloidogenic and non-amyloidogenic protein unfolding behavior.

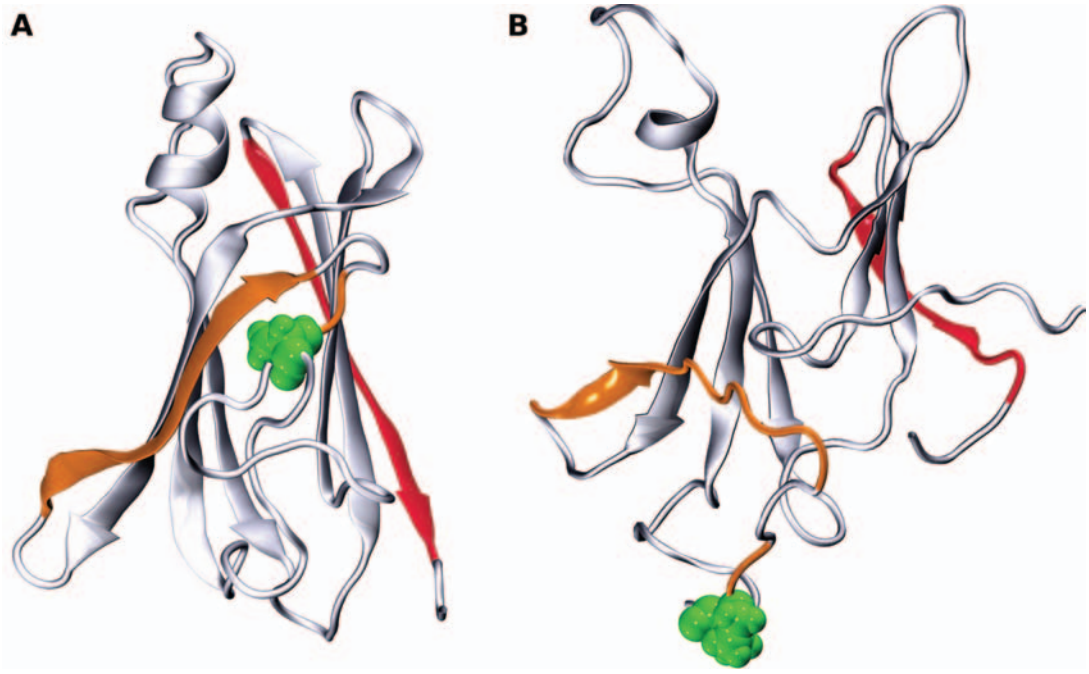


Fig. 1. Structural model of a potentially amyloidogenic intermediate of the protein transthyretin (TTR). (A) Ribbon representation of the subunit native structure of the amy-loidogenic L55P-TTR variant; (B) Partially unfolded monomer of L55P-TTR with fully disrupted α-helix and fully displaced β-strands C and D (in orange). The residue at the mutation site is represented with green spheres, and the unstable β-strand H is shown in red. Partially unfolded TTR monomers have been produced by Molecular Dynamics simulations in unfolding conditions, and potentially amyloidogenic intermediates have been identified by cluster analysis of thousands of structures.

Vectors and Gene Therapy | *Head: Maria da Conceição Pedroso de Lima*

Objectives

The Group 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.

Regarding the development of non-viral vectors, the specific aims include the generation of novel lipid-based nanosystems for efficient intracellular delivery of drugs and nucleic acids (plasmid DNA, antisense oligonucleotides or siRNAs) and evaluation of their potential in therapeutic approaches for two major diseases: cancer and neurodegenerative disorders. For this purpose, our research has been focused on the selection of appropriate lipids, ligands and cell-penetrating peptides, and on the development of technology to generate nanosystems with adequate features for in vivo use, allowing targeting to specific cells or tissues and enhanced intracellular nucleic acid delivery. In parallel, 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. Evaluation of the therapeutic activity mediated by the developed systems has been performed in several in vitro and in vivo models for cancer and neurodegenerative disorders, and also constitutes an important goal of this Group. Regarding viral vectors, the group aims at developing and using viral vectors as a technological platform to generate genetic models of neurodegenerative diseases, such as Machado-Joseph disease, and to develop new molecular therapeutic strategies involving gene transfer or silencing of mutant genes by expression of short hairpin RNAs.

Main Achievements

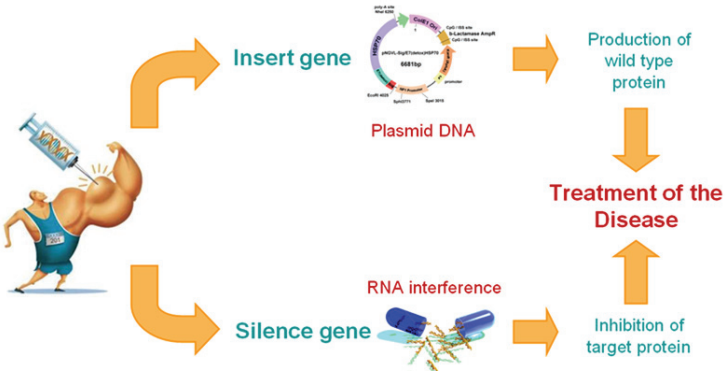
Regarding the development of improved non-viral gene delivery vectors, we demonstrated the capacity of the S413-PV cell-penetrating peptide, either per se or in association with cationic liposomes, to very efficiently mediate the intracellular delivery of plasmid DNA.

Regarding the evaluation of the developed nanosystems in anticancer strategies, we have shown that combination of antineoplastic agents with suicide gene therapy mediated by albumin-associated lipoplexes results in a remarkable synergistic antitumor effect, highlighting the potential of this approach for future applications in antitumoral therapy.

We have also developed a novel lipid-based nanosystem that has the potential to target the microenvironment of human breast tumors at two different levels: tumor cells and endothelial cells of tumor blood vessels. Such features led to a dramatic improvement of cytotoxicity of encapsulated small molecular weight drug, as compared to the non-targeted formulation. The targeting ability of the developed nanosystem was confirmed in tumor cells harvested from tumors of patients submitted to mastectomy.

Concerning the potential of the developed nanosystems in gene silencing approaches for neurodegenerative disorders, we achieved significant downregulation of gene expression upon neuronal siRNA delivery mediated by transferrin-associated lipoplexes, both in vitro and in vivo. Moreover, promising results were obtained regarding the application of these systems to mediate downregulation of specific pro-apoptotic targets, which may prove useful in therapeutic approaches to neuronal protection and repair.

Using lentiviral vectors (LV) to transduce the rat brain we overexpressed polyglutamine-expanded ataxin-3 in this way replicating Machado-Joseph disease neuropathology. LVs also allowed the first demonstration of in vivo allele-specific gene silencing. More recently we did not observe toxicity upon endogenous wild-type ataxin-3 silencing and showed that LV-mediated non-allele-specific silencing of ataxin-3 is effective and well tolerated in vivo.

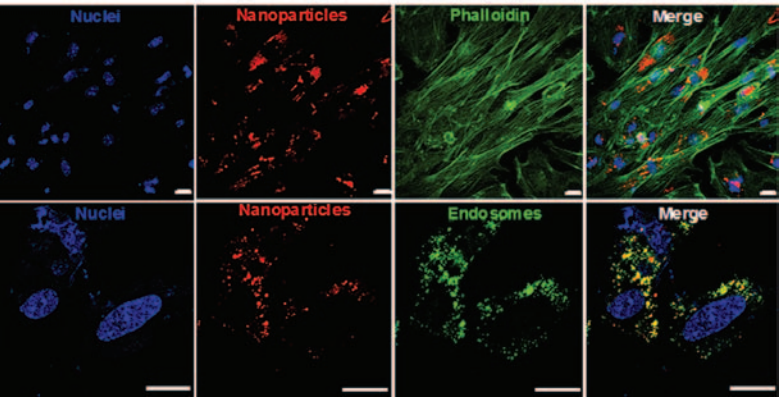


Biomaterials and Stem Cell-Based Therapeutics | *Head: Lino Ferreira*

Objectives

The group of biomaterials and stem cell-based therapeutics created in September 2006 is an emerging group at CNC. The group has two major avenues of research: i) to develop new biomaterials for stem cell differentiation, tracking and transplantation, and ii) to develop biomaterials with antimicrobial properties. 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 vascular or cardiomyocyte lineages and to obtain fundamental knowledge regarding the effect of chemistry, mechanics and three-dimensional organization of the scaffold in terms of stem cell differentiation.

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.



Confocal microscopy of cellular uptake of nanoparticles. Blue Topro-3 stains the nucleus, green lysosensor indicates endosomes, green phalloidin indicates cytoplasm, and TRITC-labelled nanoparticles are displayed in red. Nanoparticles can be seen co-localized with endosomes as a yellow colour and distributed mainly in the perinuclear region. For all pictures, bar corresponds to 20 μm. Taken from Ferreira et al. Advanced Materials 2008.

Main Achievements

Recently we reported a new approach for the delivery of vascular growth factors into human embryonic stem cells (hESCs), by incorporating growth factor-release particles into embryoid bodies (EBs) (Ferreira et al., Advanced Materials 2008). We demonstrated that the incorporation of these polymeric biodegradable particles had a minimal effect on cell viability and proliferation but a great impact on differentiation. In some cases, the effect on vascular differentiation of incorporation of particles containing growth factors was superior to that produced by exposing EBs to large extrinsic doses of the same growth factors. Recently we have also contributed for the development of new nanomaterials for drug release and cell tracking (Fuller et al., Biomaterials 2008). Highly fluorescent core-shell silica nanoparticles made by the modified Stober process (C dots) are promising as tools for sensing and imaging subcellular agents and structures. We reported that C dots can be electrostatically coated with cationic polymers, changing their surface charge and enabling them to escape from endosomes and enter the cytoplasm and nucleus. As an example of cellular delivery, we demonstrated that these particles can also be complexed with DNA and mediate and trace DNA delivery and gene expression. During 2008, our research group was also involved in the development of new bio-adhesives (Mahdavi et al., PNAS 2008). We approached this objective by utilizing a novel polymer poly(glycerol sebacic acid acrylate) (PGSA) and modifying the surface to mimic the nanotopography of gecko feet that allows attachment to vertical surfaces. Coating these nano-molded pillars of biodegradable elastomers with a thin layer of oxidized dextran significantly increased the interfacial adhesion strength on tissue either in vitro or in vivo environments. This gecko-inspired medical adhesive has potential applications for sealing wounds and for replacement or augmentation of sutures or staples.

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Area C | Cell and Molecular Toxicology

Coordinator | Leonor Almeida

General Objectives

This Area is maintaining a focus on the study of drug and disease-induced cell dysfunction, in which mitochondria lipid membranes or free radicals could be involved, aiming to translate this knowledge into disease treatment and prevention. Different groups accomplish such goals: Mitochondrial Toxicology and Pharmacology, focused on the role of mitochondria as a primary target in the initiation of drug- and disease-related cell dysfunction and on its potential usefulness as a target in drug therapeutics; Membrane Toxicity, centred on changes of physical properties of lipid membranes related to a wide range of chemical compounds and cell malfunctioning; Free Radicals and Antioxidants in Biomedical Research focused on i) molecular mechanisms inherent to neuromodulation and aging that involve free radicals, particularly •NO and ii) molecular mechanisms underlying the health-promoting role of polyphenols in connection to protection against endothelial dysfunction; the more recent group, Pharmacometrics, brings a greater insight into the optimization of drug efficacy and safety, in order to prevent costly and life-threatening drug-induced toxicity.

Main Achievements

According to our objectives and using different experimental strategies, the groups obtained a vast range of results, as indicated in their individual reports.

The relevance of mitochondrial dysfunction to the pathogenesis of some diseases, such as non-alcoholic fatty liver disease has been shown using animal models. Also, the role of mitochondria as a primary target in xenobiotic-induced cell dysfunction has been demonstrated for a wide range of compounds with clinical relevance, in particular, for some anticancer agents. The selective cytotoxicity of two natural alkaloids, berberine and sanguinarine, on human and mouse metastatic melanoma cells, by targeting mitochondria, are very promising results for these potential anticancer agents. Also, alterations of the structural order and organisation of membrane lipids have been identified as common strategies for a variety of drugs and environmental pollutants to alter the homeostatic equilibrium of biological systems.

In the scope of free radicals and antioxidants research, worthy of notice is the development of selective electrochemical micro sensors for in vivo insertion into the rat brain to measure •NO in a real-time fashion, which permitted to show, for the 1st time, the •NO concentration dynamics, in vivo, in rat hippocampus, upon stimulation of glutamate NMDA receptor, a highlight in the context of molecular mechanisms inherent to neuromodulation and aging. Regarding the antioxidants research line, the mechanistic studies of polyphenols as nitrite reductants in the stomach, and as modulators of vascular signalling pathways, beyond their antioxidant activity, support new potential beneficial effects of such compounds on •NO metabolism and endothelial function.

A new research line has been implemented related to the optimization of drug efficacy and safety. The pharmacokinetics and the oral biodisposition of eslicarbazepine acetate (ESL), a promising antiepileptic agent, and its metabolites in CD-1 mice, in the scope of the preclinical research studies to this new drug, have been characterized.

Future Research

This Area carried out the 4th edition of the "International Courses on Toxicology at the CNC", organized on a yearly basis, entitled "Metabolic Toxicology, From Pathway to Organism", which had the participation of highly recognized scientists: Vitor M. C. Madeira (Portugal), Yvonne Will (USA), Elaine Holmes (UK), Piero Portincasa (Italy), Maria de Lourdes Bastos (Portugal), Rui A. Carvalho (Portugal), Carlos M. Palmeira (Portugal) (April 9-11, 2008).

Future Plans

This area is maintaining a focus on the study of cellular and molecular basis of disease as well as drug-induced cell toxicity, in which mitochondria, lipid membrane or free radicals could be involved, for the purpose of translating this knowledge into disease treatment and prevention. Future research plans encompass mainly the continuation of ongoing research and new objectives, including the use of novel techniques and biological and non-biological models, as specified by each group. Also, the recently created group, Pharmacometrics, is bringing into the CNC the expertise on developing and applying mathematical and statistical methods to a better understanding and prediction of drug pharmacokinetic and pharmacodynamic behaviour, which are critical for optimizing drug efficacy and minimizing its toxicity. In silico techniques are becoming now popular in drug discovery and drug toxicolgy. Optimization of such approaches and the potential synergism with other groups within CNC constitute a great challenge in near future. It is worthy of note that besides the ongoing basic research from the molecular and cellular level to in vivo animal models, as specified by each group, future research plans encompass also some translational research. In fact, a few lines of research are currently working in the borderline between the bench and the bedside and all the groups are pushing forward some translational research projects in a near future.

On the other hand, aware of the relevance of the cohesion and synergy within the area, a trend is being implemented to conjugate the expertise of groups. Also, we are engaged in expanding the network of collaborations within the CNC and abroad. A great effort will be done to maintain the organization of the annual International Courses on Toxicology at CNC with the participation of highly recognized scientists. The organization of conferences and advanced courses, mainly in the scope of the CNC Doctoral Programme, by joining the efforts of the research groups will be stimulated.

Mitochondrial Toxicology and Pharmacology Group

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Maria S. Santos	(PhD)
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Ana Filipa Branco	(PhD Student)
Gonçalo Pereira	(PhD Student)
Inês Barbosa	(PhD Student)
Teresa Serafim	(PhD Student)
Ana Patricia S. Gomes	(PhD Student)
Ana Teresa I. Varela	(PhD Student)
Sandro Pereira	(PhD Student)
João Monteiro	(PhD Student)
Cláudia Pereira	(PhD Student)
Cátia Diogo	(PhD Student)
Filipe Duarte	(PhD Student)
João Teodoro	(PhD Student)
Susana Cardoso	(PhD Student)
Cristina Carvalho	(PhD Student)
Susana Pereira	(Grant Technician)
Anabela Simões	(Grant Technician)
Nuno Gabriel Machado	(MSc Student)
Ana Carolina Moreira	(MSc Student)
Filipa Carvalho	(MSc Student)
Ricardo Marques	(MSc Student)
Tiago Capote	(MSc Student)

Free Radicals and Antioxidants Group

João Laranjinha	(PhD – *Head of group*)
Leonor Almeida	(PhD)
Teresa Dinis Silva	(PhD)
Rui Barbosa	(PhD)
Ana Ledo	(Post-Doctoral Fellow)
João Frade	(PhD Student)
Carla Nunes	(PhD Student)
Bruno Gago	(PhD Student)
Cátia Marques	(PhD Student)
Ricardo Santos	(PhD Student)
Joana Paixão	(PhD Student)
Bárbara Rocha	(PhD Student)
Cassilda Pereira	(Grant Technician)
Jaime Louro	(Grant Technician)

Membrane Toxicity Group

Mª Amália Jurado	(PhD – *Head of group*)
Mª Fátima V. Lopes Pinto	(PhD Student)
Sandra Marina A. Santos	(PhD Student)
João Demétrio Martins	(MSc Student)
Ana Cardoso	(MSc Student)
Catarina Morais	(MSc Student)

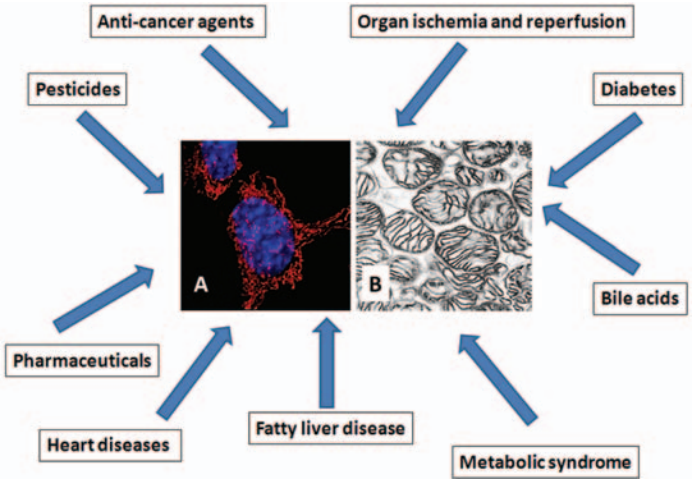
Pharmacometrics Group

Amílcar Falcão	(PhD – *Head of group*)
Marília Rocha	(PhD)
Anabela A. Francisco	(PhD)
Gilberto Alves	(PhD)
António Sales Mano	(PhD Student)
Ana C. Fortuna	(PhD Student)
Anaísa Pires	(PhD Student)
Graciana Tribuno	(PhD Student)
Bruno Lopes	(PhD Student)

Mitochondrial Toxicology and Pharmacology | *Head: Paulo Oliveira*

Objectives

Mitochondria are the energy powerplants of cells by producing the majority of the chemical energy cell use for their processes. The major breakthrough happened with the discovery that mitochondria play an important role in cell death processes. Together with the fact that mitochondria also are active players in cytosolic calcium homeostasis and in intermediate metabolism, it is pertinent to question if different molecules, which can interact with living systems, or even disease conditions, promote their biological effects through mitochondrial-mediated effects. In fact, numerous examples of mitochondria-mediated cell injury can be found in the literature; not only chemicals can negatively affect mitocondrial function but also the origin and progression of several pathologies is closely related with disruption of mitochondrial homeostasis. The main and general objective of the "Mitochondrial Toxicology and Pharmacology 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. The main particular objective is to understand how mitochondria are involved in the pathophysiology of several diseases, including diabetes and cholestasis and how mitochondrial function can be altered by chemotherapy, not only to decrease the side effects of agents commonly used in oncology, but also to specifically identify new mitochondrial targets in tumor cells. We use different in vitro (isolated mitochondrial fractions, cultured cell lines) and in vivo models (animal models of stress or disease-induced mitochondrial alterations) in order to gather information on how mitochondria are affected by xenobiotics or stressful situations (e.g. exercise, different pathologies) and the relevance for the tissue homeostasis.



Main Achievements

Our research group has provided several seminal works in different lines of research, in which the following:

1) Mitochondrial toxicity of anticancer agents: We have investigated the role of mitochondria, not only as a plausible target of novel chemotherapeutics in cancer cells, but also as a mediator of toxicity of clinically used anti-neoplastic agents. We have demonstrated that two plant alkaloids, berberine and sanguinarine, present a selective cytotoxic effect on human and mouse metastatic melanoma cells by targeting mitochondria, which can be a breakthrough in the treatment of advanced melanoma. We have identified the adenine nucleotide translocator as a critical target of berberine. We also concluded that the activation of the p53-Bax axis occurs upstream of mitochondrial dysfunction induced by the cardiotoxicant doxorubicin in H9c2 myoblasts.

2) Mitochondrial alterations during hepatic diseases: Non-alcoholic fatty liver disease (NAFLD) is an increasingly reported pathology, characterized by fat accumulation within the hepatocyte. We have demonstrated that choline-deficient animals have disturbed mitochondrial calcium accumulation and bioenergetics, as well as increased oxidative stress, which suggests a mechanism for the development of NALFD associated with altered mitochondrial function.

3) Xenobiotic-induced mitochondrial alterations: We have screened the toxicity of several compounds with clinical relevance in both heart and liver mitochondrial fractions. Of special relevance, we tested Indirubin-3'-oxime, which impairs mitochondrial oxidative phosphorylation and prevents mitochondrial permeability transition induction, the insecticide methoprene, which showed a lower toxicity when compared with other compounds of the same family, mildronate, which was shown to prevent AZT-induced mitochondrial toxicity and Sildenafil citrate, which was shown to decrease mitochondrial oxidative stress at concentrations that did not affect mitochondrial bioenergetics.

Free Radicals and Antioxidants in Biomedical Res. | *Head: João Laranjinha*

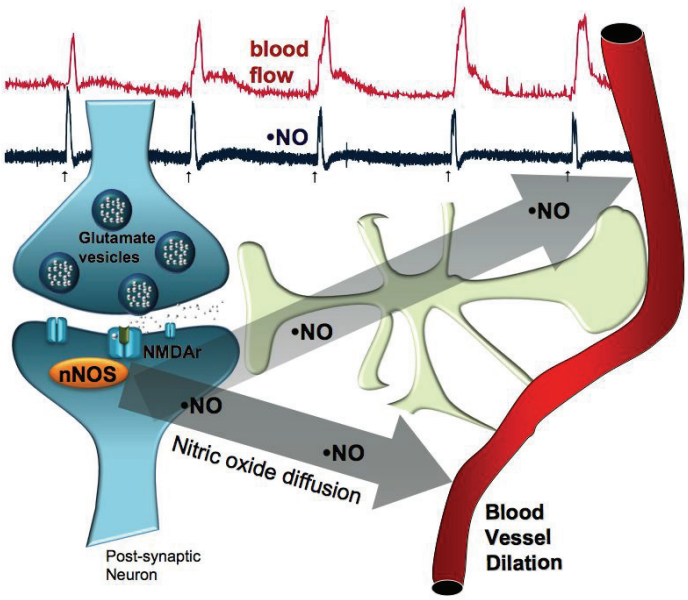
Objectives

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 are:

1) To study molecular mechanisms inherent to neuromodulation, and aging that critically involve free radicals and oxidants, particularly nitric oxide (•NO). Emphasis is put on the dynamic profiles of NO in hippocampus in connection with its neuromodulatory role and as the mediator of neurovascular coupling.

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, anti-inflammatory properties as well as the non-enzymatic production of nitric oxide from dietary nitrite in the gastric compartment. Nitrite-driven regulatory process with impact in physiology and in pathology.



Main Achievements

1. We have discovered new molecules (ethyl nitrite) formed in vivo in the human stomach from the interaction of wine ethanol and dietary nitrite that act as nitric oxide-donors, inducing muscle relaxation and have proposed a new pathway for the biological impact of dietary nitrite and dietary polyphenols, beyond their well-known antioxidant activity.

2. We have established that wine polyphenols may exert cardioprotective effects by interfering with cell signaling pathways. In particular, resveratrol protects vascular smooth muscle cells proliferation, promoted by oxidized LDL, by disrupting the mTOR signaling pathway, pointing to a new potential pharmacologic target in atherogenesis. Also, malvidin-3 glucoside, a typical wine anthocyanin, was shown to protect peroxynitrite-triggered endothelial cells toxicity by up-regulating cellular NO and down-regulating NF-kB.

3. We have published for the first time since nitric oxide hás been discovered, its the concentration dynamics of nitric oxide in vivo in the rat hippocampus upon stimulation of glutamate NMDA receptor.

4. We have propsosed a new mechanism for neuronal protection involving glutamate-dependent astrocyte gluthathione release.

5. We have proposed a new pathway for cell death associated with parkinson's disease envolving nitric oxide and dopamine metabolism

6. We have developed selective electrochemical micro sensors for in vivo insertion into the rat brain to measure nitric oxide in a real-time fashion.

Membrane Toxicity | *Head: Maria Amália Jurado*

Objectives

The main purpose of our research has been to find out more about the particular role played by lipids and the lipid-bilayer component of cell membranes in health and disease conditions. The emphasis is on biophysical properties of the lipid-bilayer and on the way they affect membrane functions, that is a lipidomics approach. Advances in the elucidation of the aspects of lipid-bilayer structure and dynamics potentially involved in abnormal membrane functioning and disease have been built upon experimental approaches considering a serial stepwise increase in biological complexity, from model membranes prepared with synthetic and native membrane lipids, to subcellular fractions (biological membranes, mitochondria, protoplasts) and prokaryotic and eukaryotic cell cultures. The area of research has included the study of a wide range of biological and chemical compounds, such as DNA, sterols, surfactants, drugs, environmental pollutants and nanomaterials.

To investigate how membrane composition, structure and dynamics are involved in cell functioning or dysfunction, the group has been developing different experimental strategies, namely: a) To elucidate how cell functioning and pharmacological/toxicological effects of membrane-active drugs are influenced by diet-induced membrane lipid composition changes, in rats, and by alterations of membrane lipids as a response to environmental stress, in bacteria; b) To identify alterations of the physical properties of the lipid bilayer related with cell malfunctioning and disease. Additionally, the group has been also interested on the characterization of DNA physical interactions with lipid membranes, envisaging to contribute to the amelioration of liposomal gene delivery systems and to further clarify the biophysical principles, which govern efficient liposome-mediated transfection.

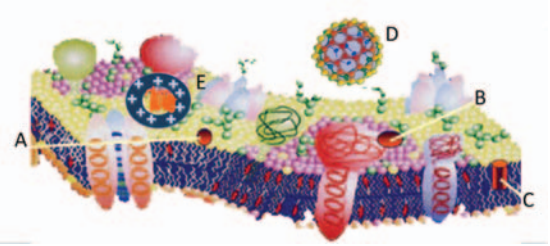


Fig.1. Interaction of chemical agents with membranes. Small molecules interact with the membrane surface, in fluid (A) and lipid raft (B) domains, or penetrate in the membrane core (C). Nanostructures such as fullerenes (D) or lipid-based DNA vectors (E) establish different interactions with the membrane, depending on their size, surface chemistry and charge.

Main Achievements

A large experience has been accumulated in our lab concerning pesticides effects on membrane physical properties using different model systems. A particularly important aspect of this work is the estimation of the partition coefficients of the compounds in model and native membranes. These studies are instrumental to evaluate their potential for uptake and accumulation in living cells. Thereafter, biophysical techniques, fluorescence spectroscopy, differential scanning calorimetry and magnetic resonance spectroscopy (^{31}P-NMR), have helped to characterise the perturbations promoted by the compounds across the bilayer thickness and to identify their potential accumulation in differentiated regions of the heterogeneous membrane structure, allowing to predict a preferential interaction on specific lipid-protein environments.

On the basis of collected data and knowledge, these studies have been extended to a variety of compounds whose physical-chemical characteristics make them presumable disturbers of membrane properties. Thus, the cellular effects of different chemical compounds with pharmacological or toxicological interest have been correlated to their ability to affect and modulate lipid-membrane organisation. Alterations induced in the structural order and organisation of lipid membranes have shown to be strictly correlated with adverse effects on bioenergetics, cell growth and viability, suggesting to be involved in xenobiotic mechanisms of action focused on the target cells and/or on xenobiotic non-selective side-effects.

We emphasise the following conclusive aspects:

Bacterial models can be used as a suitable research approach to assess unspecific membrane cytotoxic effects mediated by pesticides or drugs;

Lipid composition changes induced by physical or chemical stress in bacteria have indicated that rather than fluidity (the lipid membrane micro-structure), other membrane properties, such as structural heterogeneity and curvature stress, directly account for cell function impairment.

Alterations of the structural order and organisation of membrane lipids, disturbance of the bilayer lateral pressure profile and induction or remodelling of a membrane microphase pattern have been identified as common strategies for a variety of drugs and environmental pollutants to alter the homeostatic equilibrium of biological systems.

Pharmacometrics | *Head: Amílcar Falcão Ferreira*

Objectives

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. In effect, pharmacometrics is the science of interpreting and describing pharmacology in a quantitative fashion.

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). 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.

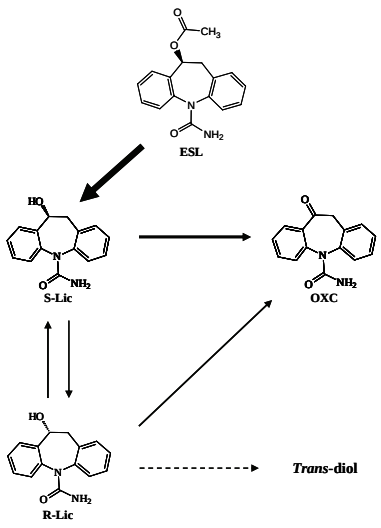
Main Achievements

The Pharmacometrics Group as a new group (started in 2007), has a small experience to share at the present time. Therefore, some individual ongoing projects were incorporated in the group and some of our achievements can be easily found analysing the productivity section. Nevertheless, our preclinical research studies in new drug development process focused on eslicarbazepine acetate could be the considered the main structured achievement in 2008.

Eslicarbazepine acetate (ESL) is a promising central nervous system-active compound recently accepted by the authorities to be put on the European market for the treatment of epilepsy (submission to FDA are going on). In the last years, we have been involved in the ESL development program to complete the pharmacokinetic data obtained from preceding and ongoing ESL clinical trials. Since pharmacokinetic studies imply the evaluation of drug concentrations over the time in the biological matrices of interest, we developed and validated the first chiral liquid chromatographic method with ultraviolet detection (LC-UV) to determine ESL and its metabolites [S-licarbazepine (S-Lic), R-licarbazepine (R-Lic) and oxcarbazepine (OXC)] in mouse plasma and brain, liver and kidney tissue homogenates. Additionally, a similar LC-UV method was also developed in human plasma, making available a useful tool not only to support ESL clinical research but also to the routine therapeutic drug monitoring assays.

Recently we characterized the pharmacokinetics and the oral biodisposition of ESL in adult male CD-1 mice, and then we studied the pharmacokinetics and the enantioselective disposition of S-Lic and R-Lic (pharmacologically active metabolites of ESL).

ESL is rapidly and extensively metabolised in mice to S-Lic (major metabolite), which is then oxidised to OXC to a small extent. R-Lic was not found in measurable amounts in all matrices. On the other hand, following the oral administration to mice of each licarbazepine enantiomer separately, it was noted that S-Lic and R-Lic were rapidly absorbed and immediately distributed, at least, to the highly perfused tissues. Both licarbazepine enantiomers were metabolized to a small extent but, even so, the bi-directional chiral inversion was observed and it occurred preferentially in the R→S direction.



Proposed metabolism of ESL, S-Lic and R-Lic in CD-1 mice. The thickness of the arrows indicates the relative extent of the metabolic pathways.

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Area D | Microbiology

Coordinator | Milton Costa

General Objectives

The Extreme Environment Group will gain a better understanding of the microbial diversity in geothermal areas, hypersaline environments and extremely alkaline springs. One of the primary objectives involves the study of mechanisms that confer radiation and desiccation resistance in species of the genus Deinococcus and Rubrobacter. We are also studying the population structure of Legionella species in natural environments to define clones that will cause colonization of man-made fixtures and cause disease.

Another line of research focuses on the characterization of the pathways for the synthesis of compatible solutes in hyperthermophiles. This lead us to examine the synthesis of the essential lipopolysaccharides in Mycobacterium species.

The Yeast Research Group is unraveling the resistance of Candida albicans to macrophages as well as the epidemiology of yeast infections in a local hospital.

Main Achievements

The Extreme Environments Group has described a large number of novel species, one of which we named Haloplasma contractile isolated from a deep sea brine represents a completely new bacterial phylum. Among other organism described was the first anaerobic member of the archaeal family Halobacteriaceae. It is also the first non-pigmented strain know.

Our research has lead to the discovery a three new pathways for the synthesis of compatible solutes in thermophiles.

We have also discovered the function of the gene product of an essential gene in Mycobacterium spp. that could lead to the development of a very specific antibiotic. The structure of this enzyme was elucidated and the structure of the active site described.

He found that Legionella clones in natural environments are different from those in man-made fixtures that cause disease.

The genome of extremely radiation resistant strain of Rubrobacter radiotolerans and a radiation-sensitive strain of Deinococcus radiomllies were completely sequenced and genes compared with other radiation resistant strains of Deinococcus and Rubrobacter. The genes and replicons of genes involved in DNA repair of the radiation-sensitive strain are the same as in the radiation-resistant Deinococcus strains, leading to the hypothesis that additional factors are involved in the resistance to radiation.

The Yeast Research group has achieved the following: Yeast metabolic response to the presence of bacterial endotoxin (one paper submitted); Combined effect of anti-fungal cell wall inhibitors in A. infectoria: identification and cloning of the AiFKS gene and its regulator AiRHO; caspofungin susceptibility (2 papers under preparation)

Future Research

Organization of conferences:

Chair Programme Committee and Co-chair Organizing Committee 3rd FEMS Congress of Microbiology, Goteborg, 28th June-2nd July, 2009.

Industry contract research:

Ongoing contracts with the Sociedade das Águas de Luso, S.A. (Luso Mineral Water Company).

Future Plans

Microbiology of Extreme Environments Group Our laboratory will participate in the first Portuguese exploration of the Atlantic sea-floor at depths of 6000. The samples retrieved and others from the Red-sea deeps and hot springs from the Azores will be used for isolation of organisms and metagenomic studies. We will evaluate the functional diversity of an alkaline groundwater environment by screening genomic libraries of conserved genes involved in central metabolic processes. We aim to study the homeostasis of compatible solute (CS) pools in extremophiles through regulation of biosynthesis and catabolism since the regulation of catabolism/export is scarce. We will continue to study the pathways for recently identified CSs. Glucosyl-glucosylglycerate for example, found in a thermophilic bacterium, was detected in mycobacteria and proposed to be a precursor of methylglucose polysaccharides. We will elucidate the biosynthesis of the methylglucose polysaccharides from mycobacteria. After the identification of the genes involved we will obtain the structure of the corresponding enzymes, essential for probing the catalytic mechanism and design/development of specific inhibitors to act as anti-mycobacterial drugs. We will probe the importance of recombination events on speciation mechanisms within Legionella and the distribution of virulence-related genes as a driving force on the evolution of the pathogen L. pneumophila. We will additionally design of a universal, portable and unambiguous epidemiological tool capable of correlating L. pneumophila population structure and virulence.

Medical Mycology – Yeast Research Group We will characterize the sensing mechanism by which yeasts are able to detect and respond to the presence of LPS, to study in vivo models of mixed infections and to assess yeast gene modulation by LPS. The in vivo and in vitro effect of purines in the interaction of C. albicans- macrophages will be studied, together with the molecular and pharmacological characterization of purine receptor and transporters in C. albicans. The inefficiency of single therapeutic strategies to eradicate dematiaceous infections prompts us to study the sinergism between caspofungin and chitin synthetase inhibitors and how this affects the A. infectoria-host interaction.

Microbiology of Extreme Environments Group

Milton Simões da Costa	(PhD – *Head of group*)
António Manuel Veríssimo Pires	(PhD)
Mª Fernanda P. N. Gomes Nobre	(PhD)
Nuno Miguel Silva Empadinhas	(PhD)
Joana Cardoso da Costa	(Post-Doctoral Fellow)
Susana Isabel Elias Alarico	(PhD Student)
Igor Clemente Tiago	(PhD Student)
Chantal Ana Vicência Fernandes	(PhD student)
Ana Luísa N. Gomes Nobre	(PhD Student)
Vitor Gonçalo Silva C. Mendes	(PhD Student)
Luis França	(PhD Student)
Ana Sofia V. Cunha	(PhD Student)

Medical Mycology – Yeast Research Group

Teresa Gonçalves	(Ph.D. – *Head of group*)
Carolina Coelho	(PhD Student)
Filipa Curado	(Grant Technician)
Vitor Hugo Rodrigues Cabral	(Grant Technician)
Nelson Cunha	(Undergraduate Student)
Branca Silva	(Undergraduate Student)

Microbiology of Extreme Environments | *Head: Milton Costa*

Objectives

The objectives for 2008 were:

1) To isolate and characterize organisms from extreme environments for basic studies and for their biotechnological potential.

2) To continue our studies on the osmotic adaptation of thermophilic bacteria.

3) To identify new compatible solutes and elucidate the pathways by which they are synthesized and their role in stress tolerance.

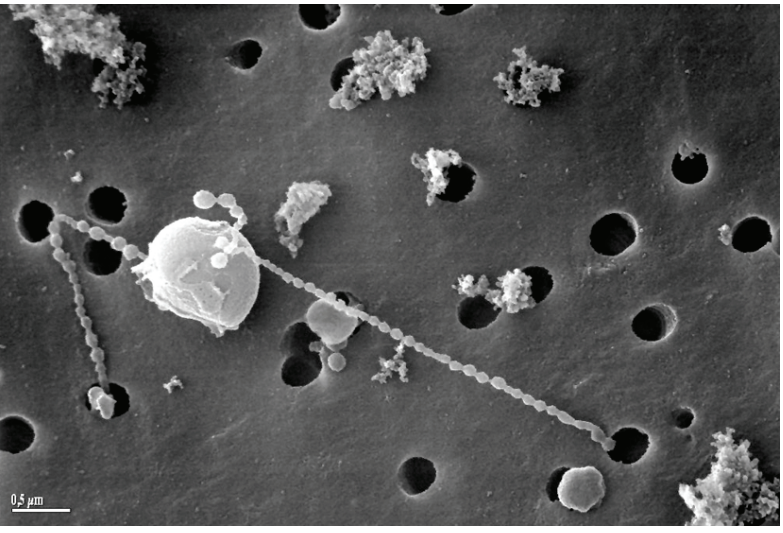
4) To determine the mycobacterial pathway leading to the synthesis of a methylglucose polysaccharide (MGLP) exclusively found in these organisms.

5) To determine the usefulness of ultraviolet light in the control of hydrothermal spa water contaminated with Legionella spp.

6) To determine the contribution of natural environmental Legionella pneumophila strains into the molecular evolution of the virulent-related dotA gene.

7) To determine the presence of viable but non-culturable Legionella spp. in treated waters and their persistence for long periods of time.

8) To determine the microbial diversity related to stalactite/stalagmite system in a subterranean karstic environment by culture- independent community analysis.



Main Achievements

During 2008:

Isolation and description of 1 new bacterial Order (Haloplasmatale), 3 new Genera and 9 new Species of bacteria and archaea, from distinct environments, namely thermal springs, alpine and saline ecosystems.

Identification of a unique combination of genetic systems for the synthesis of trehalose in Rubrobacter xylanophilus. We detected genes of four different pathways for trehalose synthesis in the genome of this organism. Both the Tps/Tpp and the TreT pathways were active in vivo.

Trehalose supports the growth of Thermus thermophilus but the absence of obvious genes for its hydrolysis led us to search for new enzymes. We expressed a putative α-glucosidase gene, characterized the recombinant enzyme, and found that the preferred substrate was α,α-1,1-trehalose, a new feature among α-glucosidases.

We have studied a mycobacterial pathway leading to the synthesis of a methylglucose polysaccharide (MGLP) exclusively found in these organisms. We have biochemically characterized the enzyme catalyzing the first glucoyl transfer in this pathway, both from Mycobacterium bovis BCG and from M. smegmatis. Since the corresponding gene had been considered essential for the growth of M. tuberculosis, we have determined the three-dimensional structure of this pathogen's enzyme laying the foundation for the design of specific inhibitors and novel anti-tuberculosis therapies.

We evaluated the usefulness of UV disinfection against Legionella spp. present on groundwater used as water supply in a therapeutic spa. We have demonstrated that the UV disinfection provides effective control with the advantage of being a method that, alike chemical disinfectants, did not altered the physicochemical composition of the water.

Culture-independent community analysis performed on stalactite/stalagmite system revelled that the majority of the populations detected were very close related to the populations previously isolated. Archaea and micro-Eukaryotes SSU rRNA genes were not detected.

Medical Mycology – Yeast Research | *Head: Teresa Gonçalves*

Objectives

1. With the final purpose of unravel the resistance of Candida albicans to macrophage attack a study was initiated with the aim to study the role of extracellular purines since ATP and its metabolite, adenosine, have been implicated in the immune-inflammatory response as STOP signals.

2. The increased number of yeast infections prompted us to study the epidemiology of yeast infections in the Portuguese population attending a Central Hospital.

3. Our recent finding that the yeast Saccharomyces cerevisiae responds to bacterial endotoxin activating the HOG Signalling Pathway [J Biol Chem 281: 24687-24694(2006)] prompted us to study the impact of such activation in yeast metabolism.

4. With the identification in the dematiaceous fungus Alternaria infectoria of AiFKS and AiRHO, together with caspofungin susceptibility, took us to study the combined effect of anti-fungal cell wall inhibitors in collaboration with Prof Neil Gow of the Institute of Medical Sciences, University of Aberdeeen, UK (manuscripts under preparation).

Main Achievements

1. Yeast metabolic response to the presence of bacterial endotoxin (one paper submitted)

2. Combined effect of anti-fungal cell wall inhibitors in A. infectoria: identification and cloning of the AiFKS gene and its regulator AiRHO; caspofungin susceptibility (2 papers under preparation)

3. Clinical mycology: a pathogenic yeast collection with 1000 isolates identified and characterised using molecular biology; an epidemiological study; a new diagnostic technology is being patented

4. An in infection model using macrophage cells infected with Candida albicans is currently being used to study:

a. the effect of purines and purinergic receptors in C. albicans clearance, indicating that adenosine might affect this process trough specific adenosine receptors (ongoing work)

b. together with the Faculty of Medicine of Porto a study was finished indicating that CUG ambiguity in C. albicans affects the phagocytic efficiency (one paper under preparation)

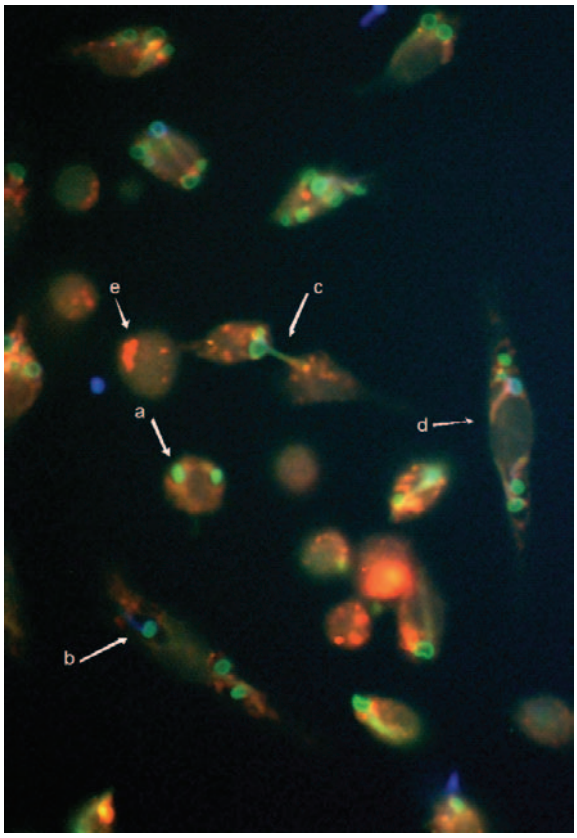


Fig.1. a. C. albicans cell ingested by RAW264.7 macrophage
b.,c. Yeast cells evading macrophage cell
d. Yeast cells inside the macrophage, forming germ tube
e. Yeast cell destroyed by phagolysosome

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Area E | Biophysics and Biomedical NMR

Coordinator | Carlos F. Geraldes

General Objectives

Cell Biophysics studies stimulus-secretion coupling mechanisms contributing to pancreatic β-cell and islet oscillations. In particular, the presence and function of ionotropic purinergic receptors were addressed, as well as the contribution of other ion channels besides the ATP-sensitive K+ channel to bursting electrical activity.

Inorganic Biochemistry and Molecular Imaging evaluates diagnostic imaging tools -metal based nanoparticles and chelates as multimodal targeted agents–in vitro and in animal models using MRI and nuclear techniques; Inorganic drugs for therapy–Li+ and bipolar disorder and V(IV/V) complexes; Environmental and toxicological effects of Cr(VI) species using cell studies; NMR and DFT studies of ion-polymer complexes.

Biomedical NMR involves studies of intermediary metabolism using 2H, 13C and 15N stable isotope tracers. Glucose, protein and lipid homeostasis are studied in humans, animal models of human diseases, and isolated perfused animal organs.

Main Achievements

Cell Biophysics

First evidence for the presence of P2X receptors in β-cells cells (rapidly desensitizing P2X1 and P2X3 receptors in mouse), which may contribute to the initial outburst of glucose- or acetylcholine-evoked insulin release. Glucose enhancement of bursting electrical activity, [Ca2+]i and 5-HT/insulin oscillatory activity in absence of functional KATP channels, indicating that early glucose sensing involves other channels besides the KATP channel (e.g. voltage-sensitive Cav1 channels).

IB and MI

Development of new classes of Gd+-based chelates and nanoparticles with optimized relaxivities as potential MRI CAs. Evaluation in cells and animals of agents optimized for MRI at high magnetic fields and targeted agents to liver Kupfer cells using DCE-MRI and nuclear imaging.

Show by 13C NMR that Li+ effects on metabolism of 13C-labeled substrates in adult rat brain and cortical neurons and astrocytes result from a reduction of neuronal glucose uptake resulting in a decrease of glutamatergic and GABAergic neurotransmission with no effects on astrocytic metabolism.

Chemical and cell toxicity studies of new V(IV/V) complexes as oral insulin-enhancing agents for Type 2 diabetes

Implementation of an in vitro model of Cr(VI)-induced malignant transformation of human bronchial epithelial cells

NMR and DFT studies of polymers and their ion complexes.

Defining the effects of transaldolase exchange activity on tracer assays of hepatic gluconeogenesis and indirect pathway fluxes.

Automated Bayesian analysis for 2H NMR spectra of urinary glucuronide.

Correlating tracer measurements of hepatic glycogenolytic fluxes in Type 1 diabetes patients with in vivo measurements by localized 13C MRS.

Preservation of metabolic fluxes in hearts using cardioplegic solutions. Characterization of mitochondrial bioenergetics and immunological profiles in ischemia and ischemia/reperfusion.

Evaluation of metabolic fluxes in hippocampus and protection of cognitive performance by caffeine. Correlation of energy metabolism with adenosine receptor-mediated neuroprotection.

Future Plans

The Cell Biophysics group will investigate the role played by voltage-sensitive Ca2+ (Cav1) channels as early sensors of glucose metabolism in pancreatic β-cells, acting in concert with KATP channels to support bursting electrical activity and pulsatile insulin release. The working model assumes that Cav1 channels undergo slow and voltage-independent inactivation, modulated by either glucose metabolites or products arising from glucose metabolism. Further work will be carried out to assess whether Cav1 channels might be involved in β-cell dysfunction, using an animal model of type 2 diabetes (Goto-Kakizaki rats).

The IB and MI group will develop new metal containing multimodal targeted MI diagnostic tools, eg. MRI CAs, optimizing efficacy, safety and sensitivity of reporter groups, based on chelates or nanoparticles, and the specificity of targeting vectors. Best agents will be studied with cell and animal models.

Study non-covalent binding of Ln-chelates to proteins using STD NMR and develop and study new Ln binding tags rigidly attached to multidomain proteins for NMR structural and dynamic studies.

Study binding of Li+ to cells using 7/6Li and MQ 23Na NMR techniques and effects of new V complexes on glucose uptake, metabolism and toxicity in adipocytes and a rat model of Type 2 diabetes, using biochemical and NMR tracer techniques. Study toxicological effects of Cr(VI) compounds and conjugated polymers as biosensors.

The Biomedical NMR group will quantify de novo lipogenesis (DNL) fluxes by D2O and 2H NMR and determine the contribution of DNL and [U-13C]enriched dietary triglycerides to hepatic lipid levels in normal and pathophysiological states.; further develop LC-MS based assays of 2H and 13C-enrichment of glucose and glucuronide and apply these to animal and human glucose clamp studies; metabolic characterization of neurodegenerative disorders, correlating cognitive impairment with alterations in intermediary metabolism.

Inorganic Biochemistry and Molecular Imaging Group

Carlos G. Geraldes	(PhD – *Head of group*)
Mª Margarida Catalão Castro	(PhD)
Mª Carmen Alpoim	(PhD)
Mª Luisa D. Ramos	(PhD)
Licinia J. Simões	(Post-Doctoral Fellow)
Giovannia Araujo de Lima Pereira	(Post-Doctoral Fellow)
Carlos Rodrigues	(PhD Student)
Sara Figueiredo	(PhD Student)
Maria João Rodrigues Pereira	(PhD Student)
André Martins	(PhD Student)
João André Duarte	(PhD Student)
João Teixeira	(PhD Student)
Filipe Coreta Gomes	(PhD Student)
Ines Ribeiro Violante	(Undergraduate Student)
Marta Daniela P. Caetano	(Grant Technician)

Intermediary Metabolism Group

John Griffith Jones	(Ph.D. – *Head of group*)
Rui A. Carvalho	(PhD)
Mª Madalena Caldeira Santos	(PhD)
Ivana Jarak	(PhD)
Tiago Alves	(PhD Student)
Ana Francisca L. Silva Soares	(PhD Student)
Sara Gonçalves	(PhD Student)
Ivan Viegas	(PhD Student)
Pedro Coxito	(PhD Student)
Marco Alves	(PhD Student)
Cristina Barosa	(PhD Student)
Ilídio Martins	(PhD Student)
Paulo Gameiro Guerreiro	(PhD Student)
Daniela Pinheiro	(Grant Technician)

Patrícia Nunes	(Grant Technician)
Ludgero Tavares	(MSc Student)
Fátima Martins	(MSc Student)
Nuno Machado	(MSc Student)

Cell Biophysics Group

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Ângelo R. Tomé	(PhD)
Célia M. Antunes	(PhD)
Rosa M. Santos	(PhD)
Joana I. Real	(PhD Student)
Hugo Figueiredo	(PhD Student)

Inorganic Biochemistry and Molecular Imaging | *Head: Carlos Geraldes*

Objectives

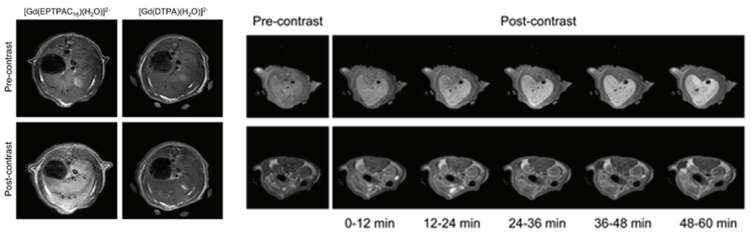
Our general objective is the study of inorganic compounds for medical diagnostic imaging (in particular MRI contrast agents), inorganic drugs for medical therapy, and the study of environmental and toxicological effects of inorganic species. The design and development of metal based agents for multimodal targeted molecular imaging agents, is followed by in vitro cell studies and animal model evaluation using MRI and nuclear imaging techniques. These agents include Ln3+-based paramagnetic nanoparticles with interesting photoluminescence properties for optical imaging (OI), and high r2 relaxivities, especially at high fields, yielding negative contrast in T2-weighted MRI images. The r1 relaxivity of new lanthanide chelates will be increased by designing new chelating agents which increase the number of inner sphere water molecules and optimize the water exchange rates. Second-sphere water relaxation contributions should also be optimized.

We also study the molecular and cellular mechanisms of action of lithium salts in the therapy of the bipolar disorder, using cell and animal systems, in particular by studying the Li+ effects on the adult rat brain and in primary cultures of cortical neurons and astrocytes during the metabolism of (1-13C) glucose or (2-13C) using 13C NMR of organ and cell extracts, in order to clarify the effects of Li+ on glutamatergic and GABAergic neurotransmission.

Several types of new inorganic vanadium(IV/V) complexes are synthesised, chemically characterized in aqueous solution and their potential use as efficient oral insulin-enhancing agents for type II diabetes and toxicity effects is investigated in different cell systems.

Environmental and toxicological effects of inorganic species of Cr(VI) are investigated by implementation of an in vitro model to study the multistage bronchial epithelial carcinogenesis induced by Cr(VI).

Other projects in Inorganic Chemistry include a) NMR structural and DFT theoretical studies of end-capped conjugated oligomers and polymers for molecular electronic device applications, of poly(9,9-dialkylfluorene)s, of luminescent gold(I) compounds and of organic compounds relevant in the production of zeolite-type materials for gas storage and molecular selection; b) studies of metal ion interactions with polyelectrolytes.



Main Achievements

1)Ln3+ paramagnetic nanoparticles (NPs) are useful for molecular imaging (MI) applications: Ln silicates containing Eu;Tb/Gd, with tuneable photoluminescence properties for OI; aqueous Ln-zeolite-type silicate (Ln-AV-9) have high r2* and r2 relaxivities, useful reporter groups for high fieldT2w or T2w* MRI; 2) A Gd3+-substituted DTTA complex has improved properties relative to the parent DTTA; the Gd3+ complexes of 5 phospho(i)nate ligands lacking an inner-sphere water molecule, have a significant contribution of second-sphere water to r1 relaxivity; 3)In vivo properties of potential MRI CAs: Ln3+ complexes of 6 DOTA-tetraamide ligands (ParaCEST agents), labeled with 153Gd or 177Lu, with renal excretion and no dissociation in vivo in Wistar rats, but all three tri-cationic complexes studied are acutely toxic to the heart at MRI CA doses; Gd3+ chelates were evaluated by DCE MRI in vivo: i) Gd3L (a trimeric DTTA chelate) has high r1 at high fields and MRI experiments at B0 = 9.4 T in mice showed much higher signal enhancement in the kidney medulla and cortex than the commercial GdDOTA at an identical dose; ii) micellar Gd3+ chelate of EPTPAC16 targeted to the RES, give strong positive liver contrast in T1w images (results supported by studies with the 153Sm3+-labeled compounds; 4)Li+ effects on metabolism of (1-13C) glucose or (2-13C) acetate in rat brain and primary cultures of neurons and astrocytes were investigated by 13C NMR, indicating that Li+ effects are mediated through a reduction of neuronal glucose uptake resulting in a decrease of glutamatergic and GABAergic neurotransmission without effects on astrocytic metabolism; 5)Aqueous speciation of the pyrimidinone complex (VV-MHCPE) gives (VVO2)L2 and (VVO2)LH–1 main species. Cytotoxicity study of this, a pyridinone and a salicylaldehyde VV-complexes showed potential antitumor activity for VV-MHCPE; 6) An in vitro model of Cr(VI)-induced malignant transformation of human bronchial epithelial cells was implemented.

Intermediary Metabolism | *Head: John Jones*

Objectives

In settings of insulin resistance and non-insulin-dependent (Type 2) diabetes, the loss of glucose and lipid homeostasis results in secondary complications such as heart disease and blindness. To understand the precise effects of substrate imbalances and secondary complications on the metabolic function of liver, heart and brain, we are developing detailed yet practical stable-isotope tracer measurements of intermediary metabolism in both humans and in animal models of diabetes. These assays are designed to quantify fluxes though the principal mammalian pathways of intermediary metabolism including glycolysis, gluconeogenesis, glycogen synthesis, pentose cycle, lipogenesis and the Krebs cycle. These measurements are providing new insights about how intermediary metabolism is modified in the liver, heart and brain in the setting of insulin resistance, hyperglycemia and diabetes.

Main Achievements

1. Defining the effects of transaldolase exchange activity on tracer measurements of hepatic gluconeogenesis and indirect pathway fluxes.

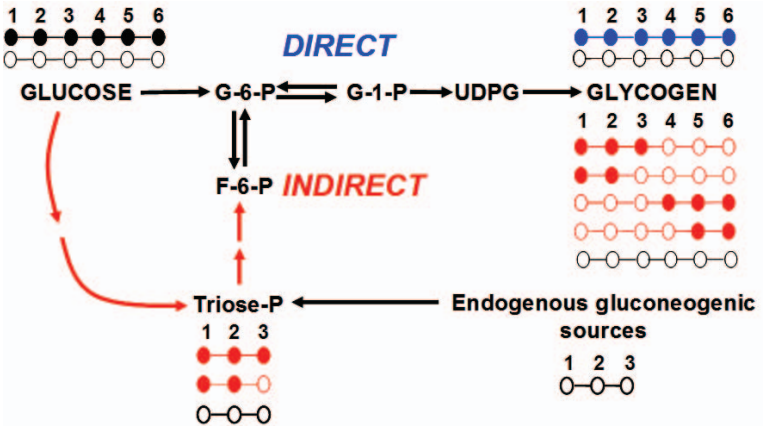
2. Development of a user-independent Bayesian analysis for 2H NMR spectra derived from urinary glucuronide.

3. Correlating noninvasive tracer measurements of hepatic glycogenolytic fluxes in Type 1 diabetes patients with direct in vivo measurement of hepatic glycogen levels by localized 13C magnetic resonance spectroscopy.

4. Quantifying plasma glucose 2H-enrichment from microliter blood samples via LC-MS.

5. Preservation of metabolic fluxes in hearts using cardioplegic preservation solutions. Characterization of mitochondrial bioenergetics and immunological profiles in ischemia and ischemia/reperfusion.

6. Evaluation of metabolic fluxes in hippocampus and protection of cognitive performance by caffeine. Correlation of energy metabolism with adenosine receptor-mediated neuroprotection.



Cell Biophysics | *Head: Luís Martinho do Rosário*

Objectives

Pulsatile insulin release from pancreatic islets is critical for glucose homeostasis, and its loss represents an early event in type 2 diabetes. Bursting electrical activity of beta-cells is thought to play an important role in islet pulsatility. Our general aim was to investigate stimulus-secretion coupling mechanisms contributing to beta-cell and islet oscillations, with a particular emphasis in ion channel function and membrane receptors.

Main Achievements

Autocrine and paracrine interactions involving extracellular ATP: Glucose-induced insulin secretion from pancreatic beta-cells is modulated by several hormones and transmitters, namely

adenosine triphosphate (ATP) via purinergic receptors. Although metabotropic (P2Y) receptors are well documented in beta-cells cells, the presence of ionotropic (P2X) receptors remained elusive. We presented the first electrophysiological evidence for the presence of P2X receptors in single beta-cells cells of different species. Specifically, mouse beta-cells express rapidly desensitizing P2X1 and P2X3 receptors. Paracrine or neural activation of these receptors may contribute to the initial outburst of glucose- or acetylcholine-evoked insulin release, thus enhancing the islet secretory response. It is also possible that granule-stored ATP might be released in a pulsatile fashion to coordinate the islet activity as a syncythium.

Oscillatory electrical activity: The glucose sensitivity of bursting electrical activity and pulsatile insulin release from single islets was determined in absence of functional KATP channels. Raising glucose or alpha-ketoisocaproic acid concentration increased spiking activity and burst plateau duration in islets exposed to KATP channel blockers (tolbutamide or glibenclamide) and high extracellular Ca2+. Glucose enhanced both [Ca2+]i and 5-HT/insulin oscillatory activity. It is concluded that beta-cells exhibit graded electrical and secretory responses to glucose in absence of functional KATP channels. This suggests that, under physiological conditions, early glucose sensing may involve other channels besides the KATP channel (e.g. voltage-sensitive Cav1 channels).

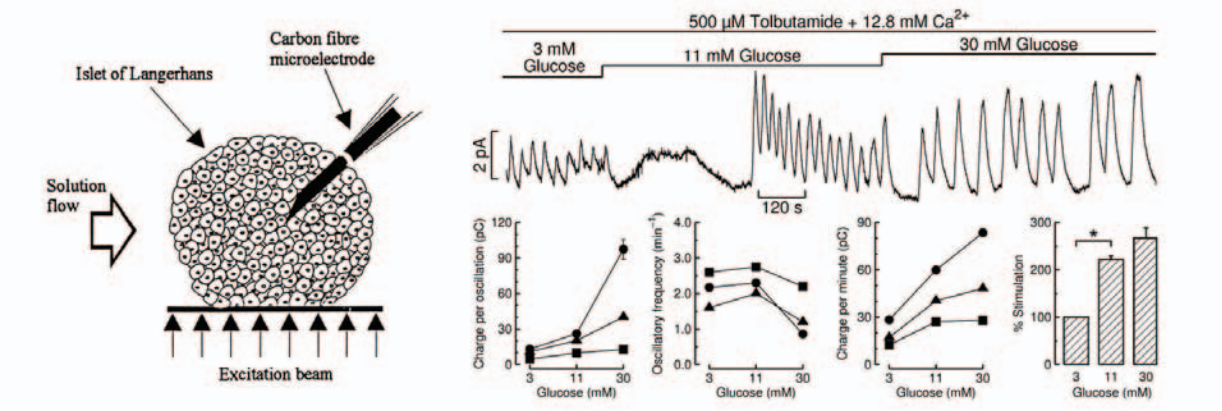


Fig.1. Pulsatile 5-HT / insulin release from a single islet, as detected by 5-HT carbon fibre microamperometry. Effect of different glucose concentrations in absence of functional ATP-sensitive K+ channels.

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Area F | Cell and Development Biology

Coordinator | Maria Celeste Lopes and João Ramalho Santos

General Objectives

The key identifying feature of the "Cell and Development Biology" area is CNC Researchers whose programs involve close partnerships with clinicians at FMUC/HUC, both in terms of basic research with human samples, setting up novel clinically-relevant services and trials, and hopefully furthering translational research. Partnerships already in place include Immunology, Oncobiology, Genetics, Neurology, Dermatology, Reproduction, Endocrionology (Obesity, Diabetes), and likely others.

One of the major strengths of the groups included in the "Cell and Development Biology" area is the strong collaboration with clinical departments, allowing the collection of human tissues and samples for the development of translational investigation in several distinct, yet interconnected research lines. In line with this, the major goal in 2008 was the consolidation of the research projects being carried out, which was achieved as the publication record for the various groups in this area demonstrates.

Main Achievements

As mentioned in the previous report, the main purpose for this area was to continue the consolidation of the research carried out, as well as the recruitment of new researchers to address specific needs. In this regard, in 2008, the Reproduction group has now established solid grounds in the fields of stem cell biology and tissue engineering. The Cellular Immunology and Oncobiology group was able to strengthen national and international collaborations established in previous years, which will become more apparent in the near future when collaboration manuscripts already submitted become published. The Phagocytosis and Pathogens group reached a significant dimension in line with the process of new recruitments initiated in the previous year. The Metabolism, Insulin Resistance and Complications group is now more firmly established within CNBC, especially due to collaborations with HUC services and CNBC's groups.

Future Research

Nobre R, Almeida LP, Martins TC: A new methodology for the complete genotyping of mucosal human papillomavirus using a restriction fragment length polymorphism analysis and an original typing algorithm. US Provisional PAT-US 38267/08.

"Oncobiology Course" - Doctoral Program of CNC, Coimbra University, 2007/2008.

http://cnc.cj.uc.pt/BEB/course0708.php

"Neuroimmunology Course" - Doctoral Program of CNC, Coimbra University, 2007/2008.

http://cnc.cj.uc.pt/BEB/course0708.php

"Immunology Course" - Doctoral Program of CNC, Coimbra University, 2007/2008.

http://beb.cnbc.pt/det_courses.asp?id=275

Future Plans

There is an enormous wealth of expertise in terms of healthcare, medical know-how; sample collections and patient groups at HUC/FMUC, which could be explored further, provided there are common interests and the partnerships are mutually potentiating. However the CNC should conduct organized prospecting in terms of novel possibilities for clinical research.

The "pitfalls" of the approach include encroachment of both clinical and research perspectives (i.e. "territorial" issues), which must be made to dialogue with vocabularies that are not exactly the same, although they may sound similar. An important point is that value-frames and time-frames also are different, from day-to-day clinical care, to long-term research approaches. It is thus crucial to identify willing partners on both sides, and nurture the dialogue continuously. It can be done.

Some examples of possible joint approaches are:

- Using Induced Pluripotency to create Stem-cell-like cells from patients with different pathologies, thus enabling the creation of human cell line models on which the disease can be modeled, for drug and gene expression screens, etc.

- Tissue engineering for tissue repair in cardiology or other specialties.

- The development of new animal models for specific diseases of interest (e.g. transgenic rats or mice).

- Development of trans-services core facilities (microscopy, flow cytometry, sequencing, gene expression, etc) that are not involved in day-to-day operations and are therefore available for research purposes.

The groups in the "Cell and Development Biology" area will continue to develop the research lines in which they are engaged, further strengthening existing collaborations and seeking new ones, both national and international. In terms of funding, all groups will continue to apply for grants from FCT and other national and international institutions.

Cellular Immunology and Oncobiology Group

Maria Celeste Lopes	(PhD – *Head of group*)
Alexandrina F. Mendes	(PhD)
Ana Bela Sarmento Ribeiro	(MD, PhD)
Maria Teresa Cruz Rosete	(PhD)
Sukalyan Chaterjee	(PhD)
Teresa Maria C. Martins	(PhD)
Artur Augusto Paiva	(PhD)
Anália do Carmo	(Post-Doctoral Fellow)
Ana Luisa Vital	(PhD Student)
Ana Raquel M. Soares	(PhD Student)
Ana Teresa Rufino	(PhD Student)
Bruno Miguel das Neves	(PhD Student)
Hugo Prezeres	(PhD Student)
Inês Crespo	(PhD Student)
José Mário Tenera Morgado	(PhD Student)
Mariana Freitas	(PhD Student)
Marta Viegas da Silva	(PhD Student)
Rui Nobre	(PhD Student)
Sara Tavares M. Lima	(PhD Student)
Susana Carvalho Rosa	(PhD Student)
Vera Lúcia G. Francisco	(PhD Student)
Vera P. Gonçalves	(PhD Student)
Ana Catarina Oliveira	(MSc Student)
Diana Moreira	(MSc Student)
Helena Carvalheiro	(MSc Student)
Patricia Henriques Domingues	(MSc Student)

Biology of Reproduction and Human Fertility Group

João Ramalho Santos	(PhD – *Head of group*)
Mª Alexandra Amaral	(PhD Student)
Sandra Amaral	(PhD Student)
Ana Paula Marques de Sousa	(PhD Student)

Paula Mota	(PhD Student)
Sara M. Diniz Martins Lopes	(PhD Student)
Ana Sofia Rodrigues	(PhD Student)
Marta Isabel Rodrigues Baptista	(PhD Student)
Beatriz Lacerda de Sousa	(PhD Student)
Renata Santos Tavares	(PhD Student)
Marília Cordeiro	(MSc Student)
Raquel Brito	(MSc Student)
Rita Silva	(Undergraduate Student)
Ana Carolina Borralho	(Undergraduate Student)

Emerging Groups

Infection, Phagocytosis and Pathogens Group

Otilia Vieira	(PhD – *Head of group*)
Carla Margarida Cardoso	(Post-Doctoral Fellow)
Luis Miguel Estronca	(Post-Doctoral Fellow)
Daniel Oberdorefer	(Ph.D. Student)
Diego Hartmann	(Ph.D. Student)
Michelle Stumpf Viegas	(Grant Technician)

Insulin Resistance and Adipocyte group

Eugénia Carvalho	(PhD – *Head of group*)
Maria João R. Pereira	(PhD Student)
Ana Tellechea	(PhD Student)

Cellular Immunology and Oncobiology | *Head: Maria Celeste Lopes*

Objectives

The researchers of the cellular immunology and oncobiology group 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, namely to allergic contact dermatitis, osteoarthritis, autoimmunity and cancer.

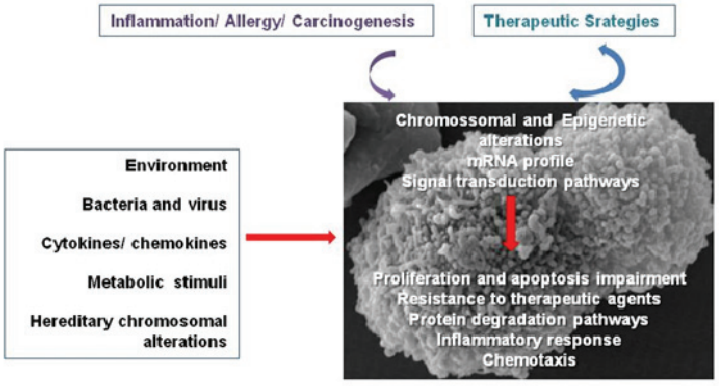
One of the strengths of this group is the variety of approaches, ranging from in vitro studies in human primary cell cultures and established cell lines, to in vivo experiments with animal models and analysis of clinical samples made in close collaboration with hospital clinical units, namely with the: i) Dermatology Department of the University Hospital of Coimbra (HUC); ii) Orthopaedic and Bone Bank Departments of HUC; iii) Clinical Hematology Department of HUC; iv) Portuguese Oncology Institute of Coimbra; v) Neuropathology Laboratory and Neurosurgery Service of HUC and vi) Center for Cancer Research of the Salamanca University, Spain.

Research on cellular immunology focused in:

i) how different maturation stimuli, namely contact sensitizers, irritants, an endotoxin and the parasite Leismania infantum, modulate the expression of dendritic cell (DC) surface molecules (chemokine and cytokine receptors), cytokine production and transcription factors activation; ii) identifying defective processes involved in chondrocyte susceptibility to high glucose-induced cell damage, thus contributing to the development and/or progression of OA; iii) the role of the CD38 on the regulation of immune responses, namely infection and autoimmunity.

Research on oncobiology focused in:

i) molecular changes relevant to the thyroid, breast and cervical carcinogenesis; ii) the involvement of oxidative stress and cell signalling in haematological neoplasias and chemoresistance and its implications on the therapeutic approach; iii) chromosomal and genetic abnormalities of human gliomas and cell signalling pathways involved in tumour progression and migration.



Main Achievements

Cellular Immunology:

Effects of maturation stimuli on dendritic cell (DC) protein expression: LPS increases the expression of co-stimulatory proteins and cytokines/chemokines in DC. Th2 cytokine production induced by LPS is dependent on NF-kB and ERK activation, being negatively modulated by p38 MAPK. The contact of virulent L. infantum parasites with DC activates AKT and ERK1/2, but was unable to fully activate DCs, as demonstrated by low expression of cell surface co-stimulatory markers and MHC molecules.

Modulation of chondrocyte functions by metabolic stimuli: unlike normal counterparts, OA human chondrocytes under hyperglycemia are unable to adjust glucose transport and undergo oxidative stress. OA chondrocytes express functional insulin receptors, while showing IGFR resistance.

Role of CD38 in immune function: using CD38KO mice, we found that CD38 is required for effective macrophage activation by T cells, NO production, chemotaxis and chemokine secretion during immune responses against mycobacteria; and for the control of systemic autoimmunity.

Oncobiology:

Pathways involved in thyroid and cervical cancer: we unravelled a new pathway involved in non-medullary thyroid cancer involving LRP1B and Wnt; identified and characterized a new probable-high risk HPV (HPV108); and identified changes in defensins associated with increased susceptibility to HPV infection and cervical cancer.

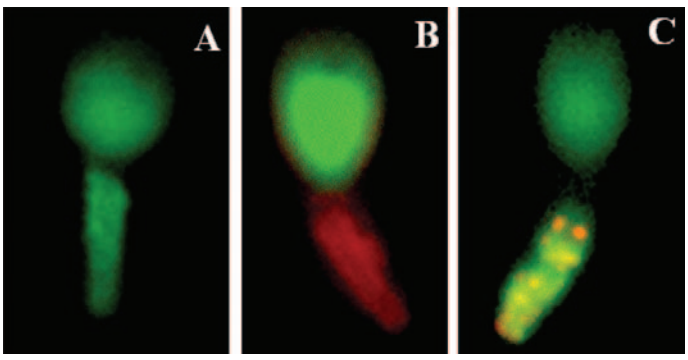
Cell signalling pathways involved in cancer and chemoresistance: we found a decrease in antioxidant defences and an increase in peroxides formation, which decreases at relapse. In cells resistant to azaguanine, occurs a decrease in the expression of pro-apoptotic proteins.

Genomic and phenotypic abnormalities of human gliomas: The results show genetic heterogeneity among human gliomas and support the existence of different cytogenetic pathways of intratumoral evolution in high versus low grade tumours, which could explain their different histopathological behaviour.

Biology of Reproduction and Human Fertility | *Head: João Ramalho*

Objectives

The main goal consists in determining what makes a good sperm, from a cellular, biochemical and molecular standpoints, with focus on the mitochondria and bioenergetics as it relates to cell function and homeostasis. Several animal models are used (horse, rat, cat, human), for different purposes. The horse has been used to both characterize native Portuguese breeds, and as a tool to improve animal management (reproduction, semen banking, artificial insemination) in collaboration with the Agricultural School of Coimbra. Human work is carried out at the University Hospitals of Coimbra where the group is involved in quality control, gamete and embryo evaluations, gamete and tissue banking for oncology patients, and research aimed at directly improving the quality of service in the Human Reproduction Clinic. The rat has been used as a model to characterize gametogenesis from a bioenergetics standpoint, and to assess the effect of diabetics on reproductive parameters. The cat is used as a model for endangered felids, in terms of preservation of the germline and xenotransplantation. 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 and horse (in)fertility. Another goal is to develop simple tests to monitor sperm quality in an ejaculate in terms of nuclear DNA status which can be applied both in field studies concerning the management of endangered species, and in the clinic. Furthermore, the group's expertise has recently led to collaborations regarding the effect of mitochondrial bioenergetics on human embryonic stem cell pluripotency, and differentiation.



Main Achievements

1- Detailed analysis of ATP production, mitochondrial function, membrane stability, apoptosis, oxidative stress and antioxidant defenses in equine sperm, and impact on stallion fertility in order to determine the best possible indicators for stallion fertility, with relevance for animal breeding and semen banking (part of this work has been published). Despite their economic importance for decades this work also provided the first characterization of native breeds (Lusitano and Sorraia) in terms of breed-specific semen parameters (published).

2-Simultaneous use of several fluorescence-based assays to monitor human sperm quality and provide a more accurate diagnosis of patient sperm quality and male infertility (previously published). However, this work also clearly showed that routine clinical applications would have to be both cheaper and easier, which led to the development of a novel simple assay to monitor human sperm DNA status, an important parameter that is not usually quantified. This assay was derived from previous work carried out in the cat, and its usefulness in predicting treatment outcomes has been validated 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). (*This work is in press*).

3- Successful proof of principle that primate sperm retains reproductive potential after freeze-drying (*paper published and on journal cover*).

4- Characterization of testicular bioenergetics as distinct for that of other organs, and implication of testicular bioenergetics and uncoupling proteins in aging of the male reproductive tract (*three papers in press*)

5- Discovery of a role for mitochondria in maintaining human embryonic stem cell pluripotency. Mitochondrial inhibition using antimycin A results in an up-regulation of pluripotency markers such as Nanog, in stem cells, while maintaing essential cellular characteristics. In fact, antimycin A in culture media can actually replace the role of some growth factors, namely bFGF (*work submitted*).

Infection, Phagocytosis and Pathogens | *Head: Maria Otilia Vieira*

Objectives

Project 1: Use of Surfactants in the Prevention of Sexually Transmitted Infections and Unwanted Pregnancies

We aim at a comprehensive research program that includes the development and screening in vitro of surfactants that have potential for use as microbicidal and spermicidal agents. Initially, we shall design and test new surfactants with the intuition that they should inhibit membrane fusion. To the best of our knowledge this rationale has not been used in the scientific literature so far and has certainly not been considered in all the (unsuccessful) Phase III trials that have been conducted to date. We will test the effect of these compounds, in a systematic manner, on the growth of bacteria, fungi, and viruses that are clinically relevant pathogens in sexually transmitted infections, urino-genital tract infections, and neonatal infections.

Project 2: Identification of the molecular machinery involved in phagosomal maturation

Identification and elucidation of the function of host molecular determinants such as small G proteins (Rab proteins) in the entry of two different particles: IgG-opsonized inert particles and Mycobacterium tuberculosis.

To identify host molecules that can affect maturation of phagosomes containing IgG-opsonized particles and Mycobacterium tuberculosi using RNAi and confocal imaging.

Project 3: Role and molecular mechanisms underlying CD36-mediated phagocytosis of apoptotic cells: implications for atherosclerosis.

To obtain a better understanding of the molecular processes underlying phagocytosis of apoptotic cells by macrophages and defective phagocytosis in atherosclerotic lesions. Initially, we want to study the contribution of CD36 and PSR to phagocytosis, individually and in combination, and then the downstream signaling events leading to engulfment of apoptotic cells. We hope to elucidate some of the fundamental principles involved in clearance of apoptotic cells.

Main Achievements

Project 1: Commercially available quaternary ammonium surfactants were shown to exhibit bactericidal and fungicidal properties at concentrations that were not toxic towards mammalian cells. However, neither this class of surfactants, nor commercially available surfactants of the non-ionic, zwitterionic, or anionic families were found to be spermicidal or anti-viral at concentrations that were sub-toxic to mammalian epithelia. Conversely, the newly synthesized surfactants were able to prevent viral infection of non-encapsulated virus. These promising compounds are at the moment being tested for bactericidal and fungicidal properties.

Project 2: We showed that Rab10 association with phagosomes is transient and live microscopy revealed detectible levels of Rab10 on phagosomal membranes at very early time points. The recruitment of Rab10 had strong functional consequence, as the depletion of Rab10 by RNAi or overexpression of Rab10 dominant negative mutants delayed maturation of phagosomes of IgG opsonized latex beads or dead mycobacteria. Of note, overexpression of the constitutively active mutant Rab10 rescued, at least partially, live Mycobacterium containing phagosomes' maturation. Altogether these results indicate that Rab10 plays a prominent role in phagolysosome formation and can modulate Mycobacterium containing phagosomes' maturation.

Project 3: We started this project by assessing the effect of LDL charge, resulting mainly from products from their lipid oxidation, in the internalization of these particles by macrophages. For this purpose, we successful generate LDL in which only the lipidic fraction is negatively charged by the incorporation of cholesteryl hemi-ester (a molecule that mimics one of main oxidation products of LDL). We found that we need at least the incorporation of around 600 molecules of negatively charged cholesteryl ester per LDL particle to have foam cell formation, an early event in the atherogenesis. These negatively charged particles are also able to induce massive apoptosis. Furthermore, when we follow their internalization we found that our LDL model is internalized faster than native but at slower rates than acetylated LDL.

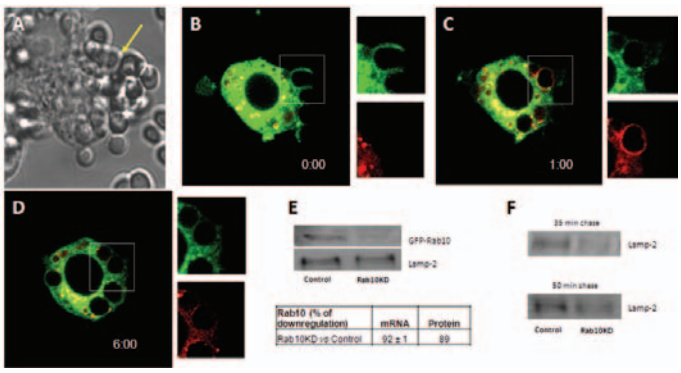


Fig.1. Distribution of Rab10 and Rab5 during phagocytosis and the functional relevance of Rab10 in phagolysosome formation

Insulin Resistance and Adipocyte | *Head: Eugenia Carvalho*

Objectives

• Study the possible environmental and molecular mechanisms responsible for insulin resistance, type 2 diabetes and obesity.

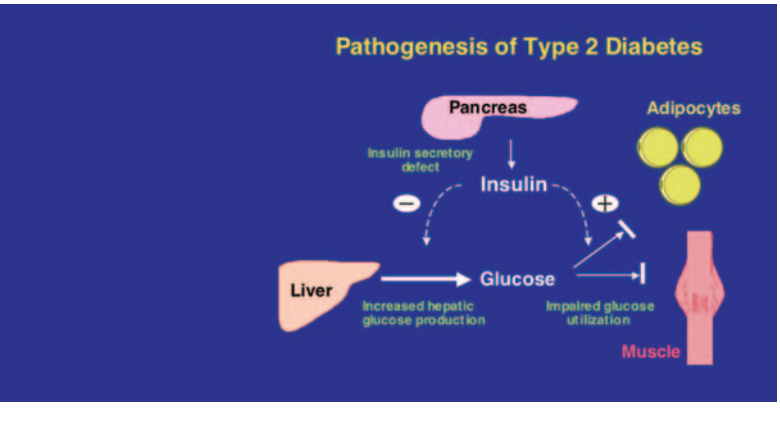
• To examine whether several biomarkers, including circulating EPCs, that are associated with the development of vascular disease, are able to predict the development of DFU. In addition, we intend to study different therapeutic strategies, neuropeptide treatment and cell-based treatments including transplantation of bone marrow derived vascular precursor cells (BM-PC) and vascular progenitor cells derived from human embryonic stem cells (hESCs), in a diabetic animal model with impaired wound healing.

• To evaluate the wound healing progress, cytokine expression, angiogenesis and inflammatory signaling downstream of neuropeptide receptors, in skin of wild type diabetic and non-diabetic mice after peripheral administration of Neurokinin receptor (NK-1R), and Neurotensin (NT) receptor 1 antagonists. In addition, we want to delineate the effects of NK-1R, and NT receptor 1 signaling in skin cells, such as, keratinocytes, Langerhans cells, dermal fibroblasts, and endothelial cells under normoglycemic and hyperglycemic conditions, both in vitro cell cultures and in human skin explants.

• Evaluate the cross-talk dinamics between epicardial fat and the heart – study glucose metabolism, insulin signalling and inflammatory adipokines in explanted epicardial adipose tissue and the myocyte in pre-transplant patients.

• To study the effect of both glucocorticoids and immunosuppressive agents in insulin action and glucose homeostasis in vivo and ex vivo. These drugs have been implicated in the development of the metabolic syndrome, visceral obesity and PTD. Our studies are of substantial clinical relevance because of the increasing use of GC and IA in transplant recipients and also for the treatment of the broad spectrum of autoimmune diseases.

• We plan to further investigate the molecular mechanisms of action of different neuropeptides in wound healing at the skin level both in animal models and in humans with diabetes. In addition, we plan to explore further the link between insulin, glucocorticoids and immunosuppresors in visceral and subcutaneous adipose tissue in the development of insulin resistance and post-transplant diabetes.



Main Achievements

1. 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. 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.

2. 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.

3. The induction of insulin resistance by GCs and IA is a process that is still poorly understood. The main hypothesis is that GCs and IA are associated with insulin resistance, causing major metabolic chages in adipocytes leading to impaired insulin sensitivity. Our preliminary results indicate that the treatment of isolated rat fat cells with IA (*cyclosporin A, tacrolimus, Prednisolone and Dexamethasone*) causes a significant decrease in the insulin stimulated glucose uptake. These results demonstrate that both CsA, FK, P and D can inhibit insulin stimulated glucose uptake ex-vivo, promoting insulin resistance and causing major metabolic chages in adipocytes.

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Neves BM, Cruz MT, Francisco V, Gonçalo M, Figueiredo A, Duarte CB, Lopes MC (2008) Differential modulation of CXCR4 and CD40 protein levels by skin sensitizers and irritants in the FSDC cell line. *Toxicol. Lett*. 177: 74-82.

Neves BM, Francisco V, Cruz MT, Gonçalo M, Figueiredo A, Duarte CB, Lopes MC (2008) Modulation of CXCR4 by sensitizers and irritants in dendritic cells. *Contact Dermatitis* 58: 10-11.

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Vieira O, Hartmann D, Cardoso C, Oberdoerfer D, Baptista M, Santos M, Almeida L, Ramalho-Santos J and Vaz WL (2008) Surfactants as microbicides and contraceptive agents: A systematic in vitro study. *PLoS ONE* 6; 3(8):e2913. *Corresponding author.

1. Psychiatry Research

Molecular Genetics Studies of Complex Disorders

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

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.

1.1 Clinical Research – Phenotypic Studies of Complex Disorders

In parallel with the 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* has been completed. 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.

Publications

Carvalho Bos S, Pereira AT, Marques M, Maia B, Soares MJ, Valente J, Gomes A, Macedo A, Azevedo MH (2008) The BDI-II factor structure in pregnancy and postpartum: two or three factors? *European Psychiatry* doi:10.1016/j.eurpsy.2008.10.003.

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Pereira AT, Maia B, Bos S, Soares MJ, Cabral AS, Macedo A, Azevedo MH (2008) The Portuguese short form of the Eating Attitudes Test -40. *European Eating Disorders Review;* 16: 319– 325.

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Ruano D, Aulchenko Y, Macedo A, Soares MJ, Valente J, Azevedo MH, Hutz MH, Gama CS, Lobato MI, Belmonte-de-Abreu P, Goodman AB, Pato C, Heutink P, Palha JA (2008) Association of the gene encoding neurogranin with schizophrenia in males. *Journal of Psychiatric Research*; 42: 125-133.

2. Neurology Research: studies on neurodegenerative disorders

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

Oxidative stress has been shown to be a triggering event in the pathogenesis of Alzheimer's disease (AD). However, few evidences exist on the role of oxidative imbalance in Mild Cognitive Impairment (MCI), a group with a high risk of progression to AD.

In this context, a study was conducted to evaluate peripheral levels of a broad spectrum of non-enzymatic and enzymatic antioxidants, of nitrogen oxidative species and lipid and protein oxidation markers in a homogenous and clinically well characterized group of MCI and early-stage AD patients (mild AD), compared with age-matched healthy subjects. In the same groups of patients, the presence of the ApoE ε4 allele, a major risk factor for sporadic AD, was also analysed in order to verify the relationship between the oxidative parameters and ApoE genotype. We also searched for possible correlations between oxidative and clinical variables, including age, gender and cognitive evaluation. The study showed that most of the oxidative changes found in mild AD patients are already present in the MCI group, and that progression to AD might be accompanied by antioxidant depletion.

Baldeiras I, Santana I, Proença MT, Garrucho MH, Pascoal R, Rodrigues A, Duro D, Oliveira CR (2008) Peripheral oxidative damage in mild cognitive impairment and mild Alzheimer's disease. *JAD* Sep 15(1):117-28.

The clinical diagnosis of sporadic Creutzfeldt-Jakob disease (sCJD) is difficult and reliable markers are highly desired. In a clinical setting, putative cerebrospinal fluid (CSF) markers would be most useful in identifying sCJD cases in a cohort of mixed pathologies with a similar presentation of rapidly progressive dementia and therefore suspected to have sCJD.

In this study, conducted in the framework of the Portuguese Epidemiological Surveillance Program for Human Prion Diseases, we evaluated the utility of several CSF protein markers (14-3-3 protein, t-tau, p-tau, Aβ42 and S-100b) in a population of patients with suspected sCJD. We also analysed the influence of patients clinical and genetical characteristics on the sensitivity/specificity of the CSF markers. Both 14-3-3, t-tau and S-100b were sensitive markers for sCJD, but 14-3-3 specificity seemed to be lower in this special clinical setting of rapidly progressing dementias. The sensitivity of 14-3-3, as well as of p-tau181/t-tau ratio, was decreased in younger patients with long disease duration, with the prion protein 2A isotype and MV genotype. We propose that in cases with a 14-3-3 weak positive result, or in young patients with long disease duration, a second CSF marker would be valuable for the diagnosis of sCJD.

Baldeiras IE, Ribeiro MH, Pacheco P, Machado A, Santana I, Cunha L, Oliveira CR. Diagnostic value of CSF protein profile in a Portuguese population of sCJD patients. *J. Neurol.* (*In press*)

3. Pediatric Research: metabolic disorders

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

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.

The mtDNA copy number/mutation quantification by real time PCR was initiated. One patient was investigated, with collaboration of University of Newcastle upon Tyne, and a copy number variation was detected. This approach will be implemented for diagnosis and research purposes, and represents a major advance for our centre in this area.

We have continued the set up of the evaluation of Pyruvate dehydrogenase and Krebs cycle enzyme activities for diagnostic and research purposes. The analysis of fumarase activity in lymphocytes isolated from blood was performed in 4 control samples.

Publications

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4. DNA investigations in Neurodegenerative disorders

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

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, 4329-4400 e 12850-13700, corresponding to genes coding for 16s rRNA, ND1, tRNA Gln and ND5, have been sequenced and analysed. The MRC complexes activity was also evaluated in more 15 FTD patients. We have found 69 sequence variations in 31 (out of 39) patients, corresponding to 30 different alterations that include 1 pathogenic mutation and 8 novel variants. We have accomplished a phenotype-genotype correlation concerning MRC activity and *MTND1* gene in 27 patients. The results shows heterogeneous patterns but normal MRC is preferentially associated to the absence of mtDNA variations (7% higher). The results suggest the involvement of mtDNA and MRC in FTD. The role of mtDNA needs further examination, but our results support mitochondrial cascade hypothesis in FTD etiopathogeny.

Additionally we have continued the genetic characterization of dementias related to 5HTR2A, BDNF genes, aiming to perform a pharmacogenomic characterization of the patients. We have investigated 101 AD patients, 37 FTD subjects and 212 controls. No statistical differences were observed concerning the polymorphisms studied.

We have continued the genetic studies in eye disorders, in collaboration with IBILI and Serviço de Oftalmologia dos HUC.

The MD student Sofia Cleto was involved in the mtDNA investigation in FTD patients and presented the preliminary results at the "X Jornadas JOCEM". The work won the 2nd prize for clinical research.

In 2008 we continued to develop the research programme in the genetic basis of neurodegenerative disorders, focused on dementia, initiated previously in collaboration with the Neurology Department of University Hospital and the Neurogenetics Laboratory at NIH. Under the scope of this project 2 Ph.D. students have been developing their Ph.D. research work in co-supervision. The description of *LRRK2* and *PRKN* mutations in Parkinson disease patients, of a novel pathogenic insertion in the PGRN gene in a family with Frontotemporal dementia and the systematic reassessment of pathogenicity of the genes involved in AD leading to propose and use of a systematic algorithm to classify the putative pathology of AD mutations were the most relevant achievments.

Publications

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Bras J, Guerreiro R, Ribeiro M, Morgadinho A, Januario C, Dias M, Calado A, Semedo C, Oliveira C, Hardy J and Singleton A (2008)Analysis of Parkinson disease patients from Portugal for mutations in SNCA, PRKN, PINK1 and LRRK2. *BMC Neurology* 8:1.

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5. Dermatology research: contact dermatitis

Margarida Gonçalo (HUC), Américo Figueiredo (FMUC, HUC), Teresa Cruz (FFUC, CNC), Rosário Domingues (UA), Pedro Domingues (UA), Celeste Lopes (FFUC, CNC)

In collaboration with the Dermatology Department of the University Hospital of Coimbra and the Chemistry Department of the University of Aveiro, we are investigating the effect of skin sensitizers and irritants on the chemokine/cytokine release and on co-stimulatory molecules profile of skin dendritic cells. We observed that contact sensitizers selectively modulated CXCR4 and CD40 receptors and the chemokines IP-10 and CXCL2. Moreover, and by proteomic analysis, we observed that contact sensitizers selectively modulate antioxidant and detoxifying proteins.

Publications

Neves BM, Cruz MT, Francisco V, Gonçalo M, Figueiredo A, Duarte CB, Lopes MC (2008) Differential modulation of CXCR4 and CD40 protein levels by skin sensitizers and irritants in the FSDC cell line. *Toxicol. Lett.* 177: 74-82.

Neves BM, Francisco V, Cruz MT, Gonçalo M, Figueiredo A, Duarte CB, Lopes MC (2008) Modulation of CXCR4 by sensitizers and irritants in dendritic cells. *Contact Dermatitis* 58: 10-11.

Francisco V, Neves BM, Cruz MT, Gonçalo M, Figueiredo A, Duarte CB, Lopes MC (2008) The sensitizer nickel increases protein thioredoxin in dendritic cells. *Contact Dermatitis* 58: 48.

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6. Arthritis research: inflammation

José António Pereira da Silva (HUC, FMUC), Fernando Judas (HUC), Alexandrina Mendes (FFUC, CNC) Carlos Cavaleiro (CEF/FFUC), Ali Mobasheri (U. Nottingham,U.K.), Margarida Carneiro (CNC), Celeste Lopes (FFUC, CNC)

In collaboration with the Orthopedic and Bone Bank Departments of HUC, we are developing three projects, using normal and osteoarthritic (OA) human articular cartilage and chondrocytes, that aim at 1) improving the survival rate of implanted osteochondral allografts, thus direct and positively affecting the clinical outcome; 2) identifying cellular and molecular mechanisms relevant in OA pathogenesis that can be translated into new therapeutic strategies; and 3) identifying compounds with potential anti-osteoarthritic activity. The first project identified arbutin as a new cryoprotective agent more effective than traditional agents. In the second project, developed in collaboration with the School of Veterinary Science and Medicine, University of Nottingham, we found that OA chondrocytes under hyperglycemia are unable to adjust glucose transport rate and undergo oxidative stress. We are currently investigating the mechanisms that regulate glucose transport in normal chondrocytes under hyperglycemia and whether the defective mechanism in OA chondrocytes is a pre-existing condition related or not to chondrocyte aging. The third project, developed in collaboration with CEF/FFUC, identified ⊚-pinene as a NF-⊚B and NO production inhibitor. Current work is underway to identify active compounds present in other fractions of the same essential oil.

CD8+ T cells represent 40% of the total T cells infiltrating the rheumatoid synovial membrane, and around 50% of the T cells found in the synovial fluid of rheumatoid arthritis (RA) patients. However, their role in RA is still ill defined. Our recent data in a mouse model of chronic polyarthritis, show that CD8+ T cells might have a dual role in the disease, with both cytotoxic and regulatory functions. Our present project aims at understanding how CD8+ T cells participate in the recruitment and/or regulation of other immune cells and in the maintenance of the chronic inflammation in RA.

Publications

Rosa SC, Judas F, Lopes MC, Mendes AF (2008) Nitric oxide synthase isoforms and NF-⊚B activity in normal and osteoarthritic human chondrocytes: Regulation by inducible nitric oxide. *Nitric Oxide* 19**:** 276-283.

Mobasheri A, Bondy CA, Moley K, Mendes AF, Rosa SC, Richardson S, Hoyland JA, Barrett-Jolley R, Shakibaei M (2008) Articular Chondrocytes: Expression, Distribution and Functional Regulation of GLUT Isoforms by Hypoxia, Hypoxia Mimetics, Growth Factors and Pro-Inflammatory Cytokines. *Adv. Anat. Embryol. Cell Biol.* 200**:**1-84.

Lopes MC, Rosa SC, Gonçalves J, Judas F, Mendes AF. (2008) Cryoprotection of human articular chondrocytes by the glycosylated hidroquinone, arbutin. *Rev. Farmacol. Chile* 1: 199.

Rosa SC, Gonçalves J, Judas F, Lopes MC, Mendes AF (2008) Evaluation of the cryoprotective efficacy of combinations of dimethylsulfoxide, glycerol and arbutin in human tibial plateaus. *Osteoarthritis Cartilage* 16: 489.

Rosa SC, Judas F, Mobasheri A, Lopes MC, Mendes AF (2008) Dysregulation of glucose transport and GLUT-1 protein in osteoarthritic chondrocytes in response to high extracellular glucose. *Osteoarthritis Cartilage* 16:202.

Gonçalves J, Rosa SC, Judas F, Salgueiro L, Cavaleiro C, Lopes MC, Mendes AF (2008) Dual inhibition of IL-1-induced NF-⊚B activation and iNOS enzyme activity, in human chondrocytes, by natural and commercial ⊚-pinene. *Osteoarthritis Cartilage* 16: 546.

7. Research in brain cancer: genetic heterogeneity of gliomas

Alberto Orfão (CSIC, Univ. Salamanca), Fernando Gomes (HUC), Olinda Rebelo (HUC), Celeste Lopes (FFUC, CNC)

The project entitled "Whole human genome analysis of genetic imbalance and numerical abnormalities by single-nucleotide polymorphism (SNP)-arrays in gliomas: correlation with clinical and biological features of the disease" is being developed in collaboration with the Neuropathology Laboratory and Neurosurgery Service of the University Hospital of Coimbra and with the 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). The gene expression profiling is performed by cDNA micro-arrays, and a full screening of the tumoral cell genome is being done by single-nucleotide polymorphism (SNP)-array analysis. The tissue samples are obtained from patients diagnosed with gliomas undergoing surgery at the University Hospital of Coimbra. Our data, of iFISH evaluation, revealed a complex cytogenetic heterogeneity in these type of tumours and distinct gene expression profiles were found between tumours of different histological grades. Now, genome-wide allelotyping, for detection of new genetic lesions, are being performed in gliomas and this analysis will facilitate the identification of new genetic/chromosomal changes, relevant for the understanding of the pathogenesis of the disease.

Publications

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Carmo A, Patricio M, Amaro P, Cruz MT, Lopes MC (2008) CXCR4 expression mediates the survival and proliferation of glioma cells. *Europ. J. Cancer* 6*:* 20.

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.

Projects jointly with laboratories abroad

Neuroscience and Disease

Adenosine A2A and dopamine D4-7 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).

Axonal transport of mitochondria in the triple transgenic mouse model of Alzheimer disease. J. Busciglio (Univ. California, USA), Claudia Pereira (CNC, Portugal).

Cell cycle reactivation in the triple transgenic mouse model of Alzheimer disease. S. Oddo / F. LaFerla (Univ. California, USA), Rui Costa , 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 B. Duarte (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 β-syn to shut them down. R. Swerdlow (Univ. Virginia Health System, USA), Claudia Pereira (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).

Endocannabinoids and neurogenesis in the subventricular zone. Prof. Giovanni Marsicano (Institute François Magendie, Bordeaux, France), Ana P. Silva, Sara Xapelli, João O. Malva (CNC, Portugal).

Endothelial - neural stem cells crosstalk in stem cell niches: implications in brain tumor development. Prof. Florence Hoffmann (University of South California, Los Angeles), Fabienne Agasse, Sofia Grade, Alexandra Rosa (CNC, Portugal).

Grafting SVZ neural stem cell cultures in mice models of temporal lobe epilepsy. Prof. Mohamed Jaber (Univesity of Poitiers, France), Fabienne Agasse, Raquel Ferreira (CNC, Portugal).

Inflammation and neurogenesis in models of epilepsy. Prof. Annamaria Vezzani (Institute Mario Negri, Milan), Liliana Bernardino, João O. Malva (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).

Muller cells as a source of repairing cells in retinal dysfunction. Prof. Fernando Mello (Universidade Federal do Rio de Janeiro, Brasil), João O. Malva, Ricardo Reis, Clarissa Schitine (CNC, Portugal).

Neuroprotection by adenosine A2A 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. Prof. Patrik Brundin (Wallenberg Neuroscience Center, Lund, Sweden) Ana Cristina Rego (CNC, Portugal).

NPY and inflammation in hippocampal neurogenesis. Prof. William Gray (University of Southampton, UK), João O. Malva (CNC, Portugal).

NPY and modulation of backpropagation activity in epilepsy. Prof. William Colmers (University of Alberta, Canada), Sara Xapelli, João O. Malva (CNC, Portugal).

NPY and their receptors in cell fate decision in the subventricular zone of the mice Impact of methamphetamine administration in NPY and NPY receptor levels. Prof. David Woldbye (University of Copenhagen, Denmark), Ana Paula Silva, Joana Gonçalves; Fabienne Agasse, Raquel Ferreira, João O. Malva (CNC, Portugal).

Nurr1 and GDNF genetic modification of mice adult neural stem cells and human cells derived from the umbilical cord – replacement cell therapy in a Parkinson's disease mouse model. Prof. Ernest Arenas (Stem Cell Neurobiology Unit, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden) Ana Catarina Henriques Oliveira, Ana Cristina Rego (CNC, Portugal).

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

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

Regulation of glutamatergic transmission by ghrelin in the hippocampus.José Esteban (Centro de Biologia Molecular Severo Ochoa, Universidad Autonoma de Madrid/CSIC, Madrid, Spain), Ki Ann Goosens McGovern (Institute for Brain Research, MIT, Cambridge, MA, USA), Ana Luisa Carvalho (CNC, Portugal).

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

Retinal Dysfunction and Neurogenesis Group in collaboration with Alistair Barber and David Antonetti (Penn State Retina Research Group, Penn State College of Medicine, Hershey, Pennsylvania, USA) Claudia Cavadas (CNC, Portugal).

Retinal Dysfunction and Neurogenesis Group in collaboration with Ben Bahr (Department of Pharmaceutical Sciences and The Neurosciences Program, University of Cunnecticut, Storrs, CT, USA) Claudia Cavadas (CNC, Portugal).

Retinal Dysfunction and Neurogenesis Group in collaboration with Eric Grouzmann (Division of Clinical Pharmacology and Toxicology, Lausanne University Medical School, 1011, Lausanne, Switzerland) Claudia Cavadas (CNC, Portugal).

Retinal Dysfunction and Neurogenesis Group in collaboration with John Forrester (Department of Ophthalmology, Institute of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, UK) Claudia Cavadas (CNC, Portugal).

Retinal Dysfunction and Neurogenesis Group in collaboration with Michael J. Young (The Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts, USA) Claudia Cavadas (CNC, Portugal).

Retinal Dysfunction and Neurogenesis Group in collaboration with Patrik Brundin (Section for Neuronal Survival, Wallenberg Neuroscience Center, Lund University, Lund, Sweden) Claudia Cavadas (CNC, Portugal).

Retinal Dysfunction and Neurogenesis Group in collaboration with Tamas Horvath (Section of Comparative Medicine; Yale University School of Medicine, USA) Claudia Cavadas (CNC, Portugal).

Retinal Dysfunction and Neurogenesis Group in collaboration with Willem Kamphuis (Netherlands Institute for Neuroscience (NIN)-KNAW, Department of Astrocyte Biology and Neurodegeneration, Amsterdam, The Netherlands) Claudia Cavadas (CNC, Portugal).

Role of presynaptic and microglial adenosine A2A receptors in neuroprotection. Jiang-Fan Chen (Boston University, USA), Rodrigo Cunha (CNC, Portugal).

Role of adenosine A2A 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 B. 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 NPY in proliferation and cell fate decision in rat cultures of SVZ neural stem cells. Dr. Valerie Coronas and Dr. Nathalie Thiriet, (University of Poitiers, France), Fabienne Agasse (CNC, Portugal).

Role of NMDAR subunits in endoplasmic reticulum stress induced by ADDLs. William L Klein (Northwestern University, Chicago, USA), Claudia Pereira (CNC, Portugal).

Role of nucleus ataxin-3 on mitochondrial function – implication for neurodegeneration in Machado-Joseph disease. Prof. Henry L. Paulson (Department of Neurology, University of Michigan Health System, Michigan, USA), Mário Luís Nôro Laço, Ana Cristina Rego (CNC, Portugal).

Role of presynaptic A2A 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 the JNK/C-Jun pathway on excitotoxic cell death. Lloyd Greene (Medical School, Columbia University, NY), Jonathan Ham (Institute of Child Health, University College London, UK), Michael Courtney (Virtainen Institute, University of Kuopio, Finland), Carlos B. Duarte (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 mitochondrial dysfunction and oxidative stress in Alzheimer disease. G. Perry (Univ. Texas at San Antonio, USA), Xiongwei Zhu (Case Western Reserve Univ., Cleveland, USA), Mark Smith (Case Western Reserve Univ., Cleveland, USA), Claudia Pereira (CNC, Portugal).

Toxic pathways triggered by activation of Ca2+-permeable AMPA receptors. Edward Barsoumian, Masaki Iizuka (Nippon Boehringer Ingelheim Co., Ltd, Kawanishi Pharma Research Institute, Kawanishi, Japan), Carlos B. Duarte (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 structure of metabolic networks. George Stephanopoulos (M.I.T., U.S.A.), ArmindoSalvador (CNC, Portugal).

Antimicrobial coatings. Robert Langer (Department of Chemical Engineering, Massachusetts Institute of Technology, MIT, EUA), Andreas Zumbuehl (Department of organic Chemistry, University of Geneva, Switzerland), Cristiana Paulo, Lino Ferreira (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).

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

Design principles of biochemical circuits, mathematical methods for systems analysis of biochemical networks. Mchael Savageau (U.C. Davis, U.S.A.), Armindo Salvador (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).

Development of three-dimensional matrices for differentiation and transplantation of human stem cells for regenerative medicine. Robert Langer (Department of Chemical Engineering, Massachusetts Institute of Technology, MIT, EUA), Ali Khademhosseini (Harvard-MIT Division of Health Science and Technology, USA), Helena Vazão, Lino Ferreira (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).

Energetic constraints on gene expression in S. cerevisiae, methods and software for kinetic modeling, factors shaping proteins' aminoacid usage. Rui Alves, Albert Sorribas, Ester Villaprinyó (University of Lleida, Spain), Armindo Salvador (CNC, Portugal).

Gecko-inspired tissue adhesive. Robert Langer (Department of Chemical Engineering, Massachusetts Institute of Technology, MIT, EUA), Jeffrey Karp (Harvard-MIT Division of Health Science and Technology, USA), Maria Pereira, Lino Ferreira (CNC, Portugal).

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

Lentiviral vectors-mediated ataxin-3 gene silencing. Nicole Déglon, Philipe Hantraye (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).

Nanomaterials for cell tracking. John Martin (Centre for Cardiovascular Biology and Medicine, University College of London, UK), Renata Gomes (CNC, Portugal), Lino Ferreira (CNC, Portugal).

Plant proteases for food industry. Dra. Nora Priolo, Sandra Vairo (LIPROVE, Universidade de La Plata, Argentina), Carlos Faro (CNC, Portugal).

Pollen Proteases and Allergy. Dr. Sónia Barbensis, Cristina Barcia (Universidade San Luís, Argentina), Carlos Faro (CNC, Portugal).

Profiling of the metabolism of proliferating cells. Craig Thompson (University of Pennsylvania, U.S.A.), Armindo Salvador (CNC, Portugal).

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

Protein structure and dynamics using high field, multidimensional solution NMR. Doctor Christina Redfield (Oxford University, UK), Rui Brito (CNC, Portugal).

Protein structure using high field solid state NMR. Professor Hartmut Oschkinat (Leibniz-Institut für Molekulare Pharmakologie, Berlin, Germany), Rui Brito (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), Teresa Pinheiro (Department of Biological Sciences, University of Warwick, UK), M.ª Amália Jurado, Paulo J. Oliveira (CNC, Portugal).

Anticancer Effects of of Phytochemicals. Jon Holy (University of Minnesota, Duluth, USA), Paulo J. Oliveira (CNC, Portugal).

Cancer Stem Cell Responses to DNA Damage. Edward Perkins (Mercer University School of Medicine, Savannah, USA), 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), João Laranjinha (CNC, Portugal).

DNA in lipoplexes: bilayer properties and adsorption factors. Rita Dias, Tommy Nylander (Department of Physical Chemistry 1, Lund University, Sweden), M.ª Amália Jurado (CNC, Portugal).

Doxorubicin-induced Mitochondrionopathy. Kendall B. Wallace (University of Minnesota, Duluth, USA), Paulo J. Oliveira (CNC, Portugal).

Hyperglycaemia-mediated Mitochondrial Dysfunction. Kendall B. Wallace (University of Minnesota, Duluth, USA), Carlos M. Palmeira, Anabela P. Rolo (CNC, Portugal).

New biological functions for wine polyphenols: Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite. Rafael Radi, Homero Rubbo (Facultad de Medicina, Universidad de la República, Montevideo, Uruguay), Jon O. Lundberg (Department of Physiology and Pharmacology, Karolinska Institutet, Sweden), João Laranjinha (CNC, Portugal).

Mesenchimal Stem Cells as Anti-Cancer Weapons. Teresa Rose-Hellekant (University of Minnesota, Duluth, USA), Vilma A. Sardao (CNC, Portugal).

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

Mitochondrial Tolerance and Liver Ischemic Preconditioning: Pathophysiological Mechanisms. Joan Rosseló (CSIC, Barcelona, Spain), Anabela P. Rolo, Carlos M. Palmeira (CNC, Portugal).

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

Role of Sirt 1 and Sirt 3 as Modulators of Mitochondrial Biogenesis. David A. Sinclair (Harvard Medical School, USA), Carlos M. Palmeira, Anabela P. Rolo (CNC, Portugal).

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

Microbiology

Microbiology of Extreme Environments Group in collaboration with Fred A. Rainey (Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 7080, USA), Milton Costa (CNC, Portugal).

Microbiology of Extreme Environments Group in collaboration with José Berenguer (Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid-C.S.I.C., 28049 Madrid, Spain), Milton Costa (CNC, Portugal).

Microbiology of Extreme Environments Group in collaboration with Garo Antranikian (Institute of Technical Microbiology, Hamburg University of Technology, Hamburg, Germany), Milton Costa (CNC, Portugal).

Biophysics and Biomedical NMR

Automated metabolic flux analysis of 2H NMR data from the 2H2O ingestion measurement of gluconeogenesis in humans. Matthew Merritt, Shawn Burgess (U.T. Southwestern Medical School Advanced Imaging Center, Dallas, TX), John Jones (CNC, Portugal).

Cell labeling for Molecular Imagin applications. Milena Salerno (CNRS, Faculty of Medicine, University of Paris 13, France), Carlos F. G. C. Geraldes (CNC, Portugal).

Cellular and molecular targets of the therapeutic action of Li+ in bipolar disease. Virtudes Moreno Martinez (Inorganic Chemistry Department, University of Barcelona, Spain), Fernando Avecilla Porto (Fundamental Chemistry Department, Universidade da Coruna, Spain), Duarte Mota de Freitas (Loyola University of Chicago, USA), Sebastian Cerdán (Laboratorio de RMN, Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC, Universidade Autónoma de Madrid, Spain), M.M. Castro (CNC, Portugal).

Characterization of cardiac intermediary metabolism during cardioplegic preservation. Gary Lopaschuk (Mazankowski Alberta Heart Institute, Edmonton, Canada), John Jones (CNC, Portugal).

Collaboration with Coral A. Lamartiniere (University of Alabama at Birmingham, USA), Thomas Liehr (Institute of Human Genetics and Anthropology), Friedrich-Schiller (University Jena, Jena, Germany), Virtudes Moreno Martinez (Universidade de Barcelona, Spain), M.C. Alpoim (CNC, Portugal).

Comparison of human hepatic glycogenolysis by the 2H2O assay with direct in vivo 13C NMR measurement of hepatic glycogen levels. Michael Roden (Department of Medicine/Metabolic Diseases, Heinrich-Heine University Düsseldorf, Germany), John Jones (CNC, Portugal).

Correlating hepatic expression of glucose and lipid metabolic pathway enzymes with fluxes through these pathways. Robert O´Doherty, Donald Scott (University of Pittsburgh Medical Center), John Jones (CNC, Portugal).

Defining insulin resistance in Type 1 diabetic patients. Michael Roden (Department of Medicine/Metabolic Diseases, Heinrich-Heine University Düsseldorf, Germany), John Jones (CNC, Portugal).

Design of new vanadium compounds: towards their application as anti-diabetic and anticancer agents. Virtudes Moreno Martinez (Inorganic Chemistry Department, University of Barcelona, Spain), Fernando Avecilla Porto (Fundamental Chemistry Department, Universidade da Coruna, Spain), Duarte Mota de Freitas (Loyola University of Chicago, USA), Sebastian Cerdán (Laboratorio de RMN, Instituto de

Investigaciones Biomédicas "Alberto Sols", CSIC, Universidade Autónoma de Madrid, Spain), M.M. Castro (CNC, Portugal).

Disruption of hepatic glucose and lipid metabolism by immunosuppressive agents: implications for diagnosis and treatment of posttransplant diabetes. Virtudes Moreno Martinez (Inorganic Chemistry Department, University of Barcelona, Spain), Fernando Avecilla Porto (Fundamental Chemistry Department, Universidade da Coruna, Spain), Duarte Mota de Freitas (Loyola University of Chicago, USA), Sebastian Cerdán (Laboratorio de RMN, Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC, Universidade Autónoma de Madrid, Spain), M.M. Castro (CNC, Portugal).

Electrophysiology of purinergic P2X receptors. Prof. Stanley Misler (Departments of Medicine and Cell Biology/Physiology, Washington University Medical School, St Louis, MO, USA), Luis M. Rosário (CNC, Portugal).

European Molecular Imaging Laboratory (EMIL) Network of Excellence (NoE); European Union FP6, involving a netwpork of about 56 European Laboratories working on Molecular Imaging, Carlos F. G. C. Geraldes (CNC, Portugal).

In vivo NMR studies of hippocampal metabolism. Rolf Gruetter (EPFL, Switzerland), John Jones (CNC, Portugal).

Metabolic modeling of 13C and 2H-tracer enrichments of glucose and other metabolites to hepatic intermediary metabolic fluxes. Matthew Merritt, Shawn Burgess (U.T. Southwestern Medical School Advanced Imaging Center, Dallas, TX), John Jones (CNC, Portugal).

Metal-based systems for Molecular Imaging applications. Involving about 45 European Laboratories working on Molecular Imaging, Carlos F. G. C. Geraldes (CNC, Portugal).

Noninvasive Analysis of hepatic lipid kinetics in rodent diabetes models by 1H MRS. Sebastian Cerdan (CSIC, Madrid), John Jones (CNC, Portugal).

Quantifying transaldolase exchange activity and its effects on gluconeogenic flux measurements using the deuterated water tracer method. Robert Rizza, Rita Basu (Mayo Clinic, Minnesota, USA), John Jones (CNC, Portugal).

SAXS study of aggregation behaviour of fluorene based conjugated polyelectrolytes. Dr. Matti Knaapila (MAX-lab, Lund University, Sweden), L. Ramos (CNC, Portugal).

Cell and Development Biology

Assessment of genetic heterogeneity in gliomas: impact on the clinical and biological behaviour of the disease. Alberto Orfão (Center for Cancer Investigation, University of Salamanca, Spain), M.ª Celeste Lopes (CNC, Portugal).

CD38 and immune regulation. Fran Lund (Rochester University, USA), M.ª Celeste Lopes (CNC, Portugal).

CD38 and immune responses against Mycobacterium tuberculosis. Andrea Cooper (Trudeau Institute, USA), M.ª Celeste Lopes (CNC, Portugal).

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

Freeze-dried primate sperm. Gabriel Sanchez-Partida (Monash University, Australia), João Ramalho Santos (CNC, Portugal).

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

Insulin resistance and fat cell biology. Dr. Jan Eriksson, University of Gothenburg, Sweden, Eugénia Carvalho, Maria Pereira (CNC, Portugal).

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

Mitochondria and embryonic stem cell pluripotency. Christopher Navara, Gerald Schatten (University of Pittsburgh, USA/University of Texas, San Antonio, USA), Sandra Varum (CNC, Portugal).

Model of neuropeptide receptor KO mice in response to skin injury.Dr. Aris Veve, (Harvard Medical School, USA), Eugénia Carvalho (CNC, Portugal).

New methods to evaluate human sperm quality. Juan Vellez de la Calle (Clinique Pasteur, Brest, France), Vasco Almeida (Univ. Porto, Portugal), Helena Figueiredo (Hospital de Gaia, Portugal), Ana Paula Sousa, Renata Tavares (CNC, Portugal).

Role and molecular mechanisms underlying CD36-mediated phagocytosis of apoptotic cells: implications for atherosclerosis. Dr. Paul Verkade (Departments of Biochemistry and Physiology & Pharmacology, School of Medical Sciences, University of Bristol, Bristol, United Kingdom), Otília Vieira (CNC, Portugal).

Role of RabGTPases (Rab8 and Rab35) on Phagocytosis and Phagosomal Maturation of IgG-Opsonized Heat-killed and Virulent *Mycobacterium tuberculosi.* Prof. Marino Zerial (Max-Planck Institute for Molecular Cell Biology and Genetics. Dresden, Germany), Otília Vieira (CNC, Portugal).

Studies on the regulatory pathways involved in energy metabolism in the heart, particularly the integrated regulation of fatty acid oxidation and carbohydrate metabolism in both the normal heart and the reperfused ischemic heart. Dr. Gary Lopaschuk, (Dept. Pediatrics and Pharmacology, University of Alberta, Canada), Sara Goncalves, Eugénia Carvalho (CNC, Portugal).

Study of the cytokine release profile, by protein arrays, of dendritic cells. Carmen García-Rodriguez (Institute of Biology and Molecular Genetic. CSIC-University of Valladolid, Spain), M.ª Celeste Lopes (CNC,Portugal).

Surfactants in the Prophylaxis of Sexually Transmitted Infections and in Oral Hygiene. Dr. Alfin Vaz (Pfizer Laboratories, Groton, USA), Otília Vieira (CNC, Portugal).

Testicular organization and xenotransplanting of testicular tissue in cats. Stefan Schlatt (University of Pittsburgh, USA/ University of Muenster, Germany), Paula Mota (CNC, Portugal).

The role of PTP1b in inflammation. Dr. Janice Zabolotny (Harvard Medical School, USA), Eugénia Carvalho (CNC, Portugal).

Participation in the organization of scientific meetings

January 2008

"The role of the AP-1 transcription factors and their target genes in developmental cell death." Doctoral Programe in Experimental Biology and Biomedicine, organized by the Center for Neuroscience and Cell Biology
Date: 25th January, Coimbra
CNC members involved in the organization: Armanda Santos

"Neurodegenerative disorders" Doctoral Programe in Experimental Biology and Biomedicine, organized by the Center for Neuroscience and Cell Biology, University of Coimbra, Portugal
Date: Jan 28 – Feb 1, Coimbra
CNC members involved in the organization: Ana C. Rego, Cláudia Pereira, Luís P. Almeida, Paula Agostinho, Paula Moreira, Sandra M. Cardoso

February 2008

"Retinal Physiology and Disease: from Bench to Clinics." Doctoral Programe in Experimental Biology and Biomedicine, organized by the Center for Neuroscience and Cell Biology
Date: 4 - 8 February, Coimbra
CNC members involved in the organization: António Francisco Ambrósio

International Conference on Molecular Systems Biology, Diliman.
Date: February 25 to 28 (Philippines)
CNC members involved in the organization: Armindo Salvador

"Mitochondrial Permeabilization in Necrosis, Apoptotis and Autophagy"
Date: February 9
CNC members involved in the organization: Paulo Oliveira

"Immunology Course." Doctoral Programe in Experimental Biology and Biomedicine, organized by the Center for Neuroscience and Cell Biology
Date: February 11 to 15
CNC members involved in the organization: Anabela C. Silva, M. Celeste Lopes, Sukalyan Chatterjee

"The commodification of life, health and environment: challenges and responses." Science & Society: Challenges of the Post-Genome Era (CNC/CES/Gulbenkian Foundation)
Date: 12 February 2008
CNC members involved in the organization: João Ramalho Santos

March 2008

"O Cérebro no Mundo das Drogas." Participation on the activities of "Brain Awareness Week" 2008
Museu da Ciência da Universidade de Coimbra
Date: March 13, Coimbra
CNC members involved in the organization: Cláudia Cavadas

"Cérebros à solta." Participation on the activities of "Brain Awareness Week" 2008 organized by CNC at the Science Museum of the University of Coimbra
Date: 10-16 March, Coimbra
CNC members involved in the organization: Cláudia Cavadas and Ana Rita Álvaro

"Oncobiology Course" - Doctoral Programe in Experimental Biology and Biomedicine, organized by the Center for Neuroscience and Cell Biology
Date: March 10 to 14
CNC members involved in the organization: Anália do Carmo, Maria Celeste Lopes, Sukalyan Chaterjee

"Neuroimunology Course" - Doctoral Programe in Experimental Biology and Biomedicine, organized by the Center for Neuroscience and Cell Biology
Date: March 31 to April 3
CNC members involved in the organization: Anália Carmo, Teresa Cruz, Emília Duarte

"Fuelling of Obesity and Type 2 Diabetes"
Date: 9-12th March
CNC members involved in the organization: Eugenia Carvalho and John G. Jones

April 2008

"Investigação em Neurociências: Que Futuro" FNAC Café, Coimbra
Date: 16 th April. Coimbra
CNC members involved in the organization: Catarina Resende Oliveira, Carlos Lima, Inês Araújo

"Drogas de Abuso: a rua para a bancada do laboratório"
Speaker: Departamento de Biologia da Universidade de Aveiro
Date: April 15, Aveiro
CNC members involved in the organization: Cláudia Cavadas

"Metabolic Toxicology, From Pathway to Organism." International Courses on Toxicology 2008 at the Center for Neurosciences and Cell Biology, University of Coimbra
Date: April 9-11, Coimbra
CNC members involved in the organization: . Rui A. Carvalho, Carlos M. Palmeira, Paulo Oliveira

"Risk, Uncertainty and Decision Analysis for Nanomaterials: Environmental Risks and Benefits and Emerging Consumer Products" Nato Meeting.
Date: April 27-30, 2008
CNC members involved in the organization: Paulo Oliveira

"Metabolic Toxicology: from pathway to organism." 3rd Edition of the International courses of Toxicology at Center for Neurosciences and Cell Biology. Co-organizer. Coimbra
Date: 9-11 April, Coimbra
CNC members involved in the organization: João Laranjinha

May 2008

Member of the organizing committee of 10th International Symposium on Metal Ions in Biology and Medicine, Bastia, Corsica, France
Date: 19 - 22 May, France
CNC members involved in the organization: M.C. Alpoim

"The reshaping of human life: assisted reproduction, stem cells and genetics." Science & Society: Challenges of the Post-Genome Era (CNC/CES/Gulbenkian Foundation)
Date: 13 May 2008
CNC members involved in the organization: João Ramalho Santos

July 2008

"Neurogenesis and Gliogenesis in Brain Repair." 6th Forum of European Neuroscience Societies
Date: July 16, Geneva, Switzerland
CNC members involved in the organization: João O. Malva

International Symposium on Drug Abuse sponsored by IDARS, The International Drug Abuse Research Society
Date: July 28-29, Ponta Delgada, S. Miguel island, Açores
CNC members involved in the organization: Ana Cristina Rego

6th International Vanadium Symposium, organized jointly by Researchers from IST-TU, FC-UL, ITN,Sacavém, FCT-Algarve, and CNC, U Coimbra
Date: 17-19 July, Lisbon
CNC members involved in the organization: Carlos F. G. C. Geraldes, Maria Margarida C. A. Castro

September 2008

"Peptides, Neurogenesis and Neuronal Phenotype" I IBRO/LARC Neuroscience Meeting. Latin-America, Iberia and Caribbean regions.
Date: September 1-4, Búzios, Brazil,
CNC members involved in the organization: João O. Malva

"2nd Portuguese-Brasilian symposium on Neurochemistry"
Date: September 1, Búzios, Brazil
CNC members involved in the organization: João O. Malva

"I Reunião Ibérica de RMN", organized jointly by the GERM-Grupo Espanhol de Ressonância Magnética of the Real Socidade Espanhola de Química and the GPRM- Grupo de RMN da Sociedade Portuguesa de Química
Date: 21-24 September, Sevilha, Espanha
CNC members involved in the organization: Carlos F. G. C. Geraldes

October 2008

7th ESH Interdisciplinary EuroConference on Mechanisms of Cell Death and Disease: Advances in Therapeutic Intervention and Drug Develoment, organized by the ESH, European School of Haematology
Date: Oct 30 – Nov 3, Cascais
CNC members involved in the organization: Ana Cristina. Rego

"Diabetes tipos I e II. Resistência à insulina na diabetes tipo II" In collaboration with the Instituto de Educação e Cidadania (IEC) organized a cycle of seminars to general public.
Date: 25 th October, Mamarrosa
CNC members involved in the organization: Caetana Carvalho

"Consequências da Diabetes" In collaboration with the Instituto de Educação e Cidadania (IEC) organized a cycle of seminars to general public.
Date: 25 th October, Mamarrosa
CNC members involved in the organization: Paulo F. Santos

December 2008

"Cell Biology of Infection". Doctoral Programe in Experimental Biology and Biomedicine, organized by the Center for Neuroscience and Cell Biology
Date: December, Coimbra
CNC members involved in the organization: Otilia Vieira

Graduate Studies Programme

During 2008, CNC organized 19 Advanced Courses and hosted 42 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, 21 Ph.D. and 24 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.

Advanced Courses 2008

January 2008

Proteolysis
January 7 - 11
Paulo Pereira

Molecular Neuroscience
January 14 - 18
Ana Luisa Carvalho

Cell Death
January 21 - 25
Armanda Santos

Neurodegenerative Disorders
January 28 - February 1
Ana C. Rego, Cláudia Pereira, Luís P. Almeida, Paula Agostinho, Paula Moreira, Sandra M. Cardoso

February 2008

Retinal Physiology and Disease: from Bench to Clinics
February 4 - 8
Miguel Castelo-Branco, Francisco Ambrósio

Immunology
February 11 - 15
Anabela C. Silva, M. Celeste Lopes, Sukalyan Chatterjee

Microbiology
February 18 - 22
Milton Costa

Genome Biology
February 25 - 27
Isabel Carreira

March 2008

Synapse, Circuitry and Behavior
March 3 - 7
Chinfei Chen, Guoping Feng

Oncobiology
March 10 - 14
Anália do Carmo, Maria Celeste Lopes, Sukalyan Chaterjee

Neuroimunology
March 31 - April 3
Anália Carmo, Teresa Cruz, Emília Duarte

April 2008

Extra-activities
April 7 - 7

Toxicology
April 9 - 11
João Laranjinha, Leonor Almeida, Paulo Oliveira, Carlos Palmeira

September 2008

Biostatistics
September 30 - October 3
Chris Palmer

October 2008

Molecular Biotechnology
October 6 - 17
Paula Veríssimo, Bruno Manadas, Sandra Ribeiro, Rui Cruz

Lab Rotations
October 20 - November 14

November 2008

Molecular Cell Biology
November 17 - 21
Edgar Gomes, João Ramalho-Santos

Biology of Proteolysis
November 24 - 28
Sandra M. Cardoso, Cláudia Pereira, Paulo Pereira

December 2008

Cell Biology of Infection
December 3 - 5
Otília Vieira

Biology of Reproduction and Embryonic Stem Cells
December 8 - 12
João Ramalho

Science Writing
December 15 - 16
Sofia Araújo

CNC Annual Meeting
December 16 - 17

Seminars

2008 Series | CNC Audithorium 16:00 h

January

11.1.2008
Regulation of Proteasome Activity
Daniel Finley | *Department of Cell Biology, Harvard Medical School, Boston, USA*

17.1.2008
Assembly and plasticity of hippocampal synapses
Ann Marie Craig | *Brain Research Centre - Department of Psychiatry - University of British Columbia - Vancouver, BC, Canada*

25.1.2008
The role of AP-1 transcription factors and their target genes in developmental neuronal death
Jonathan Ham | *Molecular Haematology and Cancer Biology Unit, - Camelia Botnar Laboratories - Institute of Child Health - University College London - London WC1N 1EH, UK*

February

1.2.2008
Protein misfolding disorders – mechanisms of protein aggregation and toxicity
David Borchelt | *Department of Neuroscience, McKnight Brain Institute, University of Florida, Gainesville, FL, USA*

8.2.2008
Regulation of synaptic transmission through presynaptic glutamate transporters in the retina
Espen Hartveit | *Department of Biomedicine, University of Bergen, Jonas Lies vei 91, 5009 Bergen Norway*

15.2.2008
Role of invariant Natural Killer T cells in airway inflammations: foccus on allergic asthma
Maria do Carmo Leite-de-Moraes | *CNRS, Faculté de Médecine, René Descartes, Paris V, Hôpital Necker, Paris*

15.2.2008
Analysis of DNA binding site motifs in Saccharomyces cerevisiae using the YEASTRACT-DISCOVERER tools
Ana Teresa Freitas | *IST and INESC*

19.2.2008
Mitochondrial Permeabilization in Necrosis, Apoptotis and Autophagy
John J. Lemasters | *Medical University of South Carolina, USA*

22.2.2008
The Microbe Hunters: The Search for new Taxa: From Species to Phyla
Fred Rainey | *Louisiana State University, USA*

29.2.2008
Poised RNA polymerase II complexes in epigenetics and genome architecture
Ana Pombo | *MRC, Imperial College, London*

March

7.3.2008
Synaptic and Circuitry Mechanisms of Obsessive-Compulsive Disorder-like Behavior in Mice
Guoping Feng | *Department of Neurobiology, Duke University Medical Center, USA*

12.3.2008
Bone marrow-derived stem/progenitor cells control primary tumor growth, and initiation and progression of metastasis
Vivek Mittal | *Cold Spring Harbor Laboratory - New York, USA*

April

3.4.2008
Glutamate and glutamate receptors at the Immunosynapse
Denis Soulet | *Dept. of Experimental Medical Science, Lund University, Sweden*

4.4.2008
Transcriptional activation of redox sensitive genes in vascular endothelial and smooth muscle cells
Giovanni Mann | *Cardiovascular Division, School of Medicine King`s College London, U.K*

4.4.2008
Reactive oxygen species signaling in regulation of the blood-brain barrier
Paul Frazer | *Cardiovascular Division, School of Medicine King`s College London, U.K*

11.4.2008
Drug development and safety- past, present and future
Yvonne Will | *Early Safety Differentiation Group, Pfizer Groton, CT, USA*

May

9.5.2008
Embryonic stem cells-derived cardiomyocytes: functionality and tissue-engineered strategies for heart regeneration
Marisa Jaconi | *Dept. Pathology and Immunology, Faculty of Medicine, Geneva University*

16.5.2008
Prions : folding, aggregation and toxicity
Teresa Pinheiro | *Dept. of Biological Sciences, University of Warwick Coventry United Kingdom*

June

6.6.2008
Genetic dissection of the physiological functions of the endocannabinoid system
Giovanni Marsicano | *AVENIR INSERM U862 - NeuroCentre Magendie, Bordeux, France*

13.6.2008
Cells caught in the act: Morphodynamic imaging and modelling of living cells
Jorge Carneiro | *Inst. Gulbenkian de Ciência, Oeiras, Portugal*

16.6.2008
Why Alzheimer is a disease of memory: the attack on synapses by Abeta oligomers
William Klein | *Cognitine Neurology and Alzheimer´s Disease Center, Northwestern University, USA*

20.6.2008
Different flavors of pluripotency
Niels Geijsen | *Harvard Medical School, USA*

24.6.2008
Peroxynitrite decomposition catalysts and mitochondrial-targeted antioxidants: its promising pharmacological action in neurodegenerative disorders
Rafael Radi

25.6.2008
In search of the in vivo identity of mesenchymal stem cells
Nance Beyer, Nardi | *Department of Genetics, Universidade Federal do Rio Grande do Sul, Brazil*

27.6.2008
Biomarkers of Brain Tumor´s Development, Progression and Therapeutic Response
Rui Reis | *Life and Health Science Research Institute, University of Minho, Portugal*

July

10.7.2008
Gene and cell therapy for tissue angiogenesis and myocardial regeneration
Mauro Giacca

September

4.9.2008
Targeting metabolic dysfunction in Huntington´s disease
Etienne Regulier | *Novartis Pharma AG, NIBR Basel, Switzerland*

4.9.2008
Targeting metabolic dysfunction in Huntington s disease

18.9.2008
Gene loss in intracellular endosymbionts and parasites: sacrificing redundancy for diversity
Luís Pereira Leal | *IGC Lisbon*

18.9.2008
Treating Experimental Arthritis by CD8 T cell Depletion
Margarida Carneiro | *Instítuto GulbenKian de Ciência*

October

17.10.2008

Catalytic RNAs in Biomedicine

Benjamin Haibe-Kains | *Computer Science Dept. Univ. Libre Bruxelles and Functional Genomics Unit, Jules Bordet Institute*

30.10.2008

BDNF mRNA splice variants: A spatial code to regulate local Plasticity of Dendrites and spines

Enrico Tongiorgi | *Brain Centre for Neuroscience. Department of Biology at University Trieste, triest, Italy*

November

7.11.20087

Molecular mechanisms of wound healing

Eugénia Carvalho | *CNC- Centro de Neurociências e Biologia Celular*

11.11.2008

Jan Motlik | *Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Czech Republic*

20.11.2008

Effect of Acetaminophen (Paracetamol) Hepatotoxicity on Expression and Regulation of Hepatobiliary Drug Transporters: Role of Oxidative Stress and Kupffer Cell Function

José Manautou | *Dept. Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, USA*

21.11.2008

Integrating actin dynamics with adhesion in cell migration

Clare M. Waterman | *Laboratory of Cell and Tissue Morphodynamics ?National, Heart, Lung and Blood Institute ?National Institutes of Health, Bethesda Maryland, USA*

28.11.2008

Pathophysiology of chaperone-mediated autophagy

Ana Maria Cuervo | *Albert Einstein College of Medicine, New York, USA*

28.11.2008

Dissecting neurological functions through gene transfer

Nicole Déglon | *Atomic Energy Commission (CEA) - Deputy Director Molecular Imaging Research Center (MIRCen)*

December

4.12.2008

Molecular dynamics and docking studies of ligands to Cytochrome P450 3A4

Paulo Martel | *Centro de Biomedicina Molecular e Estrutural - IBB-Instituto Biotecnologia Bioengenharia,Universidade do Algarve*

5.12.2008

Unexpected fate and function of a single glycolipid in pigmentation

Gerrit Van Meer | *Membrane Enzymology Bijvoet Center/Institute of Biomembranes Utrecht University, The Netherlands*

12.12.2008

Pluripotency Strategies and Sophisticated Non-Invasive Imaging to Realize the Promises of Regenerative Medicin

Gerald Schatten | *University of Pittsburgh, USA*

15.12.2008

Cationic lipid-mediated delivery of genes and oligonucleotides into eukaryotic cells: mechanism and cellular barriers

Dick Hoekstra | *Faculty Medical Sciences, University of Groningen, The Netherlands*

Thesis concluded in 2008

Artur Augusto Paiva

“Estudo da aloreactividade das células de Sangue do Cordão Umbilical”.

December 12, 2008

Supervisor: Arsélio P. Carvalho

Ana Cristina Silva Filipe

"Terapia génica para doenças vasculares: Desenvolvimento de vectores para administração intracelular de genes de factores angiogénicos".

July 10th 2008

Supervisor: Mº Conceição Pedroso Lima

Ana Luísa Colaço Cardoso

“Gene silencing as a promising strategy for neuroprotection: On the development of a lipid-based system for the delivery of siRNAs to the central nervous system”.

December 15, 2008

Supervisor: Mº Conceição Pedroso Lima

Ana Raquel Sarabando Santiago

"Mecanismos de neurodegeneração na retina nas fases iniciais da retinopatia diabética: papel do glutamato".

June 30, 2008

Supervisor: António Francisco Ambrósio

Ana Rita Álvaro

"O Neuropeptídeo Y e os seus receptores na retina: da proliferação celular à neuroprotecção".

May 15, 2008

Supervisor: Cláudia Cavadas; Co-supervisor: António Francisco Ambrósio

Anabela Maduro de Almeida

“Estudo da lamotrigina em doentes epilépticos submetidos a monitorização vídeo-electroencefalográfica.”

July 23, 2008

Supervisor: Amilca Falcão Gilberto Lourenço Alves

Bruno Alexandre Correia da Silva

“Neuroprotective potential of extracts and compounds isolated from Hypericum perforatum”.

January 11, 2008.

Supervisor: Alberto Dias; Co-supervisor: João O. Malva

Carla Maria dos Santos Nunes

“New pathways for cell death in Parkinson´s disease involving nitric oxide and dopamin metabolism.”
December 19, 2008

Supervisor: João Laranjinha

Elisabete Ferreiro

"Cross-talk between endoplasmic reticulum and mitochondria in the neurotoxic effect of amyloid-beta and prion peptides. Implications for the etiopathogenesis of Alzheimer's diasease and spongiform encephalophaties."

July 3, 2008

Supervisor: Claudia Pereira

Ema Luís Pereira Gomes Alves

"Neurotoxicity of methylenedioxymethamphetamine (MDA; Ecstasy) and its main metabolites, on rat brain mitochondria in vitro and in vivo – Behavioral consequences".

Supervisor: Paulo Oliveira

Ermelindo Carreira Leal

"Mecanismos de degeneração celular na Retinopatia Diabética: o envolvimento de mediadores inflamatórios".

December 9, 2008

Supervisor: António Francisco Ambrósio

Gilberto Lourenço Alves

"Caracterização Farmacocinética do Acetato de Eslicarbazepina e dos seus metabolitos S-Licarbazepina e R-Licarbazepine em murganhos."

October 01, 2008

Supervisor: Amilca Falcão

Giovannia Araujo de Lima Pereira

"Paramagnetic Systems as Potential MRI Contrast Agents: Evaluation of Physico-chemical Properties" January 24, 2008

Supervisor: Carlos F.G.C. Geraldes; Co-supervisor: Dr. J. A. Peters

Joana Cardoso Costa.

"Pathways for the synthesis of glucosylglycerate. From Antarctica to deep sea hydrothermal vents. Genes, enzymes and evolutionary implications".

January 21, 2008

Supervisor: Milton Costa

João Gonçalo Leal O. S. Frade

"Real-time change of nitric oxide in rat hippocampal slices and astrocytic gluthathione release via glutamate-dependent pathways."

September 15, 2008.

Supervisor: João Laranjinha

Liliana Inácio Bernardino

"Inflammation in excitotoxic neuronal death and survival: involvement of tumor necrosis factor-a and interleukin -1b".

February 11, 2008.

Supervisor João O. Malva

Nelson Alexandre Marchante Rebola

"Physiological and pathophysiological role of adenosine A2A receptors in the hippocampus"

January 10, 2008

Supervisor: Rodrigo A. Cunha

Rosa Resende

"Neurotoxicity induced by amyloid-beta peptide. The role of different peptide assembies".

December 18, 2008

Supervisor:: Catarina Resende Oliveira

Sandra Cristina Vicente e Almeida

"Mitochondrial dysfunction in cellular and animal models of Huntington's disease – Effects of FK506 and neurotrophins".

March 13, 2008

Supervisor: Ana Cristina Rego

Sara Alves Xapelli

"Neuroprotective and antiepileptic role of neuropeptide Y".

February 28, 2008

Supervisor: João O. Malva

Vilma Marisa Arrojado Soares Sardão

"H9c2 myoblasts as a tool to study doxorubicin-induced cardiomyopathy: mechanisms of cell death and relevance of differentiation state."

February 18, 2008

Supervisor: Catarina Resende Oliveira

Master Thesis

Ana Catarina Rodrigues Fonseca

"Ectoenzimas multifuncionais CD38 e CD157: funções em linfócitos T, linfócitos B e monócitos."

June 27, 2008

Supervisor: Teresa C. Martins

Ana Catarina Rodrigues Oliveira

"Sinalização celular mediada pelo receptor Flt3 e proteínas Ras como alvos terapêuticos no tratamento da síndrome mielodisplásica".

July 15, 2008

Supervisor: Ana Bela Sarmento

Ana Filipa Roque Branco

"Cell alterations involved in the different susceptibility of differentiated and non-differentiated H9c2 myoblasts to doxorubicin".

Supervisor: Paulo Oliveira

Ana Isabel Vicente Rafael

"Estudos dos efeitos tóxicos do Cr(VI) em rim e fígado de rato."

January 15, 2008

Supervisor: M.C. Alpoim; Co-supervisor: AMS Cabrita

Ana Sofia de Jesus Rodrigues

Cell Biology

July, 2008

Supervisor: João Ramalho Santos

Ana Sofia Ventura Cunha

"Dokdonella fugitiva sp. nov., uma nova espécie de classe Gammaproteobacteria isolada de solo de compostagem".

Supervisor: António Veríssimo

Andrea Silva

"Factores virais e do hospedeiro envolvidos na infecção por HPV".

February 26, 2008

Supervisor: Teresa C. Martins.

António Domingues

"Mitochondrial dysfunction and proteasomal alteration are key events in Parkinson's disease."

Supervisor: Claudia Pereira

Áurea Filipa de Aguiar Castilho

"O papel protector da hemo-oxigenase-1 na retinopatia diabética"

March 6, 2008

Supervisor: Claudia Cavadas

Cristiana da Silva Oliveira Paulo

"Yeast infections in Portugal. Characterisation of risk groups and identificatication of oplace of infection and aetiological agents using molecular biology tools."

Supervisor: Teresa Gonçalves

Cristina Isabel Marques Maurício de Carvalho

"Effects of metformin on mitochondrial bioenergetics and oxidative stress."

Supervisor: Paulo Oliveira

Diana Moreira

"Novas abordagens terapêuticas no cancro oral - Avaliação de um inibidor de cinases de tirosina e de um inibidor do proteasoma".

July 16, 2008

Supervisor: Ana Bela Sarmento

Evanilda Tavares da Silva

"Susceptibilidade genética para carcinoma não medular da tiróide familiar: análise mutacional do gene TIMM44."

September 18, 2008

Supervisor: Teresa C. Martins.

José Afonso Rodrigues Moreira

"Avaliação da capacidade antiagregante plaquetar do ácido acetilsalicílico".

December 17, 2008

Supervisor: Amilca Falcão

Luís André Antunes Baptista França

"Descrição de duas novas espécies halofílicas moderadas".

Supervisor: Milton Costa

Marco Matos

"The effect of Amyloid-beta peptide on the glutamate uptake by astrocytes."

Supervisor: Paula Aostinho

Marta Isabel Baptista

"O efeito de Surfactantes na Viabilidade de Gâmetas Masculinos Humanos."

July 24, 2008

Supervisor: João Ramalho Santos

Marta Isabel Dias da Mota Vieira

"Excitotoxic mechanisms associated to Ca2+-permeable AMPA receptors: the role of the JNK signalling pathway."

September 16, 2008

Supervisor: Carlos Duarte

Raquel Patrícia Gomes Silvestre Vinhas

"Proteases de Pólenes: Relevância em Doenças Alérgicas. "

Supervisor: Paula Veríssimo

Renata Santos Tavares

"Novos métodos de avaliação da morfologia e integridade do ADN Nuclear em espermatozóides Humanos."

July 24, 2008

Supervisor: João Ramalho Santos

Ricardo Jorge Fernandes Marques

"Study of pharmaceuticals with cardioprotective action in ischemia and reperfusion: Molecular mechanisms of pharmacological and toxicological effects".

Supervisor: Paulo Oliveira

Rosete Pais

"Papilomavirus humano e cancro do colo do útero: estudo de factores imunológicos do hospedeiro que podem condicionar o desenvolvimento de doença".

September 30, 2008

Supervisor: Teresa C. Martins

Rui Gonçalo Batista Mamede da Cruz

"Aminopeptidase N de Arabidopsis thaliana: Expressão e Caracterização."

Supervisor: Paula Veríssimo

Sara Diniz

Animal Biology

May, 2008

Supervisor: João Ramalho Santos

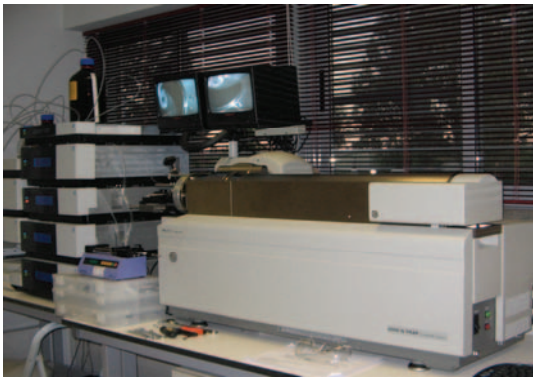
Technology Transfer

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

CNC as promoter of Science Divulgation

Under the scope of *Laboratório Associado*, CNC has launched several activities aimed to promote Science Divulgation near the non-scientific community:

1. "Ciência Viva" programme.

Six portuguese students and four spanish students from secondary schools participated in this programme during Summer Holidays which was run for 10 days. Adding to visits to facilities and laboratories, students had the opportunity to run themselves several molecular/cell biology techniques as part of short projects (e.g. *"Neurotoxic effects of drugs of abuse"*).

2. Brain Awareness Week (BAW)

In order to promote contact between students and neuroscientists at school, several activities entitled "Journey through the center of the Brain" and "Crash of the Brain" were planned for 5-17 years old students, including modelling by using playdough, puzzles, painting, brief PowerPoint presentations (e.g. brain anatomy 3-D film, five senses, optical illusions and neuronal damage by alcohol and drugs of abuse). More than 500 students participated in these activities.

Students from secondary schools visited several laboratory facilities and were allowed to perform techniques currently used in a neuroscience laboratory ("Open Laboratories").

Public Sessions at book stores, dealing with themes such as "Neuroscience Research in Portugal" were organized.

In collaboration with the new Museum of Science of the University of Coimbra, CNC organized Multidisciplinary Conferences ("Brain and Society"), with speakers from different areas, open for general public but the target audience was students (6-18 years old), In the entrance hall of the Museum of Science an interactive exposition called *"Brain: from anatomy to behavior"* took place that was visited by around 900 persons.

3. European Science and Technology week

General Public Sessions and "Open Laboratories" activities were developed similarly to what was described above for BAW.

The activities in which CNC has been enrolled in 2008 related with Science Divulgation were partially funded by Ciência Viva (the national council for the scientific and technological culture), the Federation of European Neuroscience Societies (FENS) and the Portuguese Society for Neurosciences (SPN) and also by several private entities, such as Calouste Gulbenkian Foundation. These activities were promoted in several web pages including "Ciencia Viva" (www.cienciaviva.pt/home/).

In addition to the above mentioned activities, CNC enthusiastically participated in the exposition *Forum Ciência* at the "Pavilhão do Conhecimento", Lisbon, where research and outreach activities were exhibited.

In the scope of the collaboration between CNC and *Instituto de Educação e Cidadania* (IEC), Mamarrosa, several activities took place during BAW (primary and secondary schools near IEC) and CNC's researchers participated in public sessions at IEC, actively promoting the interaction between neuroscientists and society, at a regional level.

In 2008, CNC established collaboration with Exploratorium (Center Ciência Viva in Coimbra), that will be launched in 2009/2010 to promote organized visits of students from secondary schools to laboratories ("Open Laboratories") as part of their scholar programme.

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)

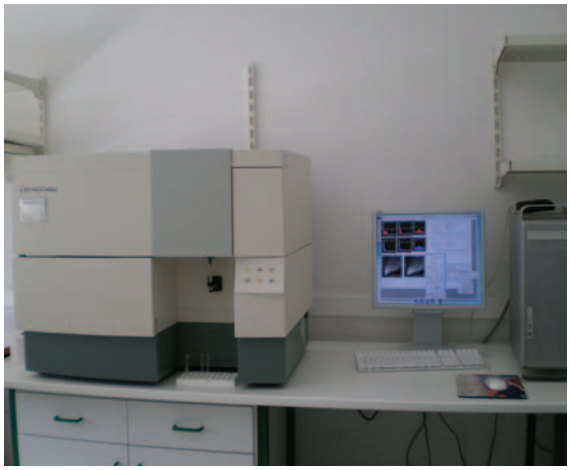
Laminar flow chamber

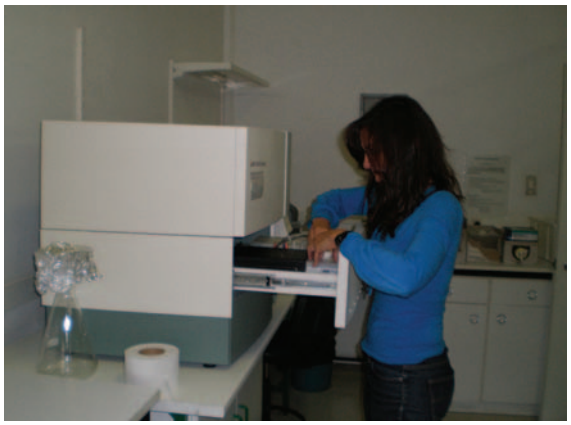
FLOW CYTOMETRY UNIT

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.

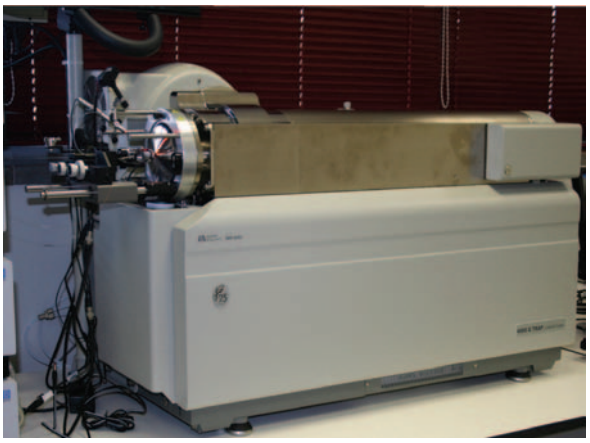

Laser scanning confocal microscope

MASS SPECTROMETRY UNIT

Head of Unit: Bruno Manadas | *Post-Doc, PhD in Cellular Biology (2008) at University of Coimbra*
Head of Facility since 2008

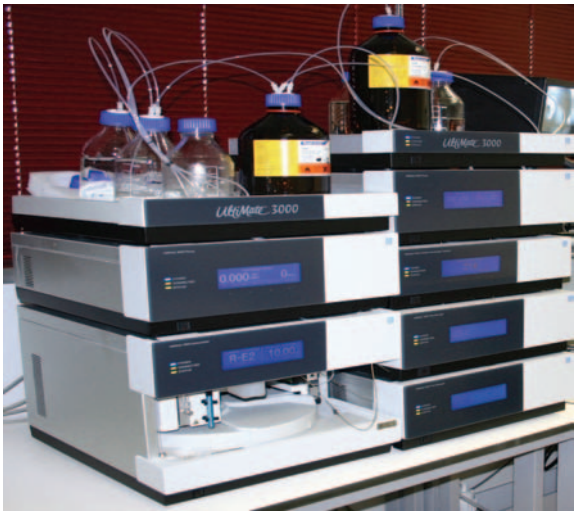
Staff: Vera Mendes (technician)

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.



4000 QTRAP mass spectrometer

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.



Bidimensional chromatography modular system coupled to the 4000 QTRAP spectrometer

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).

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

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

Biochemical and Molecular Biology Analysis:

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

Team:

Cândida Mendes

Carla Veríssimo

João Pratas

Maria João Santos

Marta Simões

Teresa Proença

Mª Helena Garruncho

Mitochondrial Respiratory Chain (MRC)

Oxygen consumption and double wavelength spectrophotometry evaluation

There were studied 61 subjects suspected of Mitochondrial Cytopathy, corresponding to the analysis of 74 samples (some patients had 2 or more tissues analysed), including 44 lymphocytes isolated of peripheral blood, 23 muscular biopsies, 6 liver and 1 heart samples. A MRC deficiency was detected in 20 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 that include 31 confirmed pathogenic mutations associated to MRC associated diseases. We have continued to screen deletions by flanking PCR of 6 hot-spot regions.

We have received 205 samples from 187 patients suspected of Mitochondrial Cytopathy, that **represent an increase of 73%, compared to year 2007**, for DNA extraction, including blood (172), muscle (22), liver (7), heart (3) and skin (1) tissues, comprising a total of 1115 and 1386 PCR reactions for point mutations and deletions analysis, respectively. Deletions have been detected in 8 samples and a total of 481 (150 different) known mtDNA sequence variations, 4 rearrangements and 50 novel variants have been detected in 84/99 samples analysed of 71/85 patients investigated. Further 97 PCR-RFLP analyses were performed to validate point mutations in 52 samples of 43 patients.

Amino Acid Analysis

Our laboratory received 480 samples (427 - plasma, 41 - urine and 12 - cerebrospinal fluid) of physiological fluids for amino acid analysis, representing a 12.9% increase, compared to 2007. The patients investigated (children, adolescents adults) were categorized 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.

Genetic testing of Neurodegenerative Disorders

In 2008 our laboratory has focused on several genetic factors known to underline the process of neurodegeneration. Our main interests are centered in the most common neurodegenerative diseases, including Alzheimer's (AD) and Parkinson's (PD) diseases.

A total number of 59 samples (22 Frontotemporal and 37 Alzeimer's disease patients), 33 Mild Cognitive Impairment and 20 Parkinson's disease patients were studied. Genes associated with these diseases were screened, and mutations were found in *LRRK2* and *PARK2*. Furthermore, 9 control samples from healthy individuals with ages matching those of the patients, were also collected.

On the dementia series Apo-E and COMT genotypes were assessed.

AIBILI Services

1. Introduction

AIBILI - Association for Innovation and Biomedical Research on Light and Image is a private non-profit organisation, founded in 1989, established to support technology transfer to industry.

AIBILI is a Research Technology Organisation (RTO) in the Health Market dedicated to help the development of new products for health imaging, 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) including

Quality Management Unit (UGQ)
Technology Transfer Unit (UTT)
Projects Unit (PU)

AIBILI is located at the Health Campus of Coimbra University since 1994 and has 15.296 sq. feet with state-of-the-art equipment. Regarding human resources we have 7 investigators, 12 technicians, 5 study coordinators and 3 administrative staff full time. Also collaborate regularly with AIBILI 49 investigators, 5 technicians for diagnostic procedures and 7 nurses in different variable percentages of time. We have, therefore, a total of active 56 researchers.

AIBILI is partner of the CNC – Centre of Neuroscience and Cell Biology of the University of Coimbra, as an Associate Laboratory of the Portuguese Foundation for Science and Technology (FCT).
As a complement of CNC laboratory research activities and taking into consideration pharmaceutical industry needs, AIBILI provides clinical trials and physical-chemical testing services to industry.

2. Areas of Expertise / Research / Staff

2.1. Centre for Clinical Trials

The Centre for Clinical Trials (CEC) performs randomized clinical trials with special emphasis on Ophthalmology and, more specifically, in diabetic retinopathy, age-related macular degeneration, glaucoma, cataract surgery and ocular inflammation.

It is the purpose of the Centre for Clinical Trials to work with the Industry and to function as liaison between the Drug and Medical Device Industry and the Health Services.

CEC has dedicated facilities and the most modern ophthalmological equipment. Its permanent staff includes two Ophthalmologists full time, one Pharmacist, five experienced Study Coordinators, five Technicians for Diagnostic Procedures, four Nurses, one Laboratory Technician and two Administrative Secretaries. Eighteen Ophthalmological Consultants and one Endocrinologist, collaborate regularly with the Centre.

The professional organisation of the Centre for Clinical Trials with a Manual of SOP (Standardized Operating Procedures) and its convenient location, next to the University Hospital of Coimbra and its Department of Ophthalmology are a guarantee that the deadlines are successfully met and in compliance with the ICH Good Clinical Practice Guidelines. The Centre for Clinical Trials renewed its Certificate ISO 9001:2000 to perform clinical trials, thus guaranteeing the continual improvement of its work in Ophthalmological Research.

CEC is also certified as an "EVI.CT.SE Site of Excellence", that is a clinical trial in ophthalmology that complies with ICH GCP Guidelines with written SOPs, has the necessary equipment and personnel to perform clinical trials and has proven expertise and scientific publications in this area.

Areas of Expertise

Characterisation and evaluation of the most recent methods to study the initial stages of diabetic retinopathy. Evaluation of new methodologies for multimodal mapping of the macula.

Studies of the diseases of the choroid and retina and especially of their blood circulation, particularly in age-related macular degeneration.

Correlation between structure-function with psychophysics tests and study the early signs of the disease.

Testing new methods of early diagnosis and characterisation of macular edema and retinal vascular pathology.

Evaluation of new drugs to treat glaucoma. Development of methods to correlate clinical indicators of disease progression, particularly regarding optic nerve degeneration and the mechanisms of the actions of drugs being tested.

To test the pos-operative inflammation after cataract surgery and evaluation of drugs for the prevention and treatment of ocular inflammation after cataract surgery.

Research

Ongoing Clinical Trials

Clinical Trials in Ophthalmology

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

Protocol B7A-MC-MBDL. Reduction in the Occurrence 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

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 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 the Center 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

A phase 1, Open-Label study of Latanoprost Acid Plasma Concentrations in Pediatric and Adult Glaucoma Patients Treated with Latanoprost 0,005%

A phase 3 Prospective, Randomized, Double-Masked, 12-week, parallel group Study evaluating the efficacy and safety of Latanoprost and Timolol in Pediatric subjects with Glaucoma

Age-Related Macular Degeneration

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 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, long-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)

Early Markers of choroidal neovascularization (CNV) in fellow eyes of patients with Age-Related Macular Degeneration (AMD) and CNV in one eye

A 6-Month, Single-Masked, Multicenter, Randomized, Controlled Study to Assess the Safety and Efficacy of 700μg Dexamethasone Posterior Segment Drug Delivery System Applicator System as Adjunctive Therapy to Lucentis® Compared with Lucentis® Alone in the Treatment of Patients with Choroidal Neovascularization Secondary to Age-Related Macular Degeneration

A randomized, double-masked, active controlled, phase 3 study of the efficacy, safety, and tolerability of repeated doses of intravitreal VEGF Trop-Eye in subjects with neovascular age-related

Cataract

A Multicenter, Investigator-Masked, Parallel-Group, Randomized, Study of the Efficacy and Safety of Indomethacin 0,1% Eyedrops Compared with Kerorolac 0,5% Eyedrops in Ocular Inflammation After Cataract Surgery

Multiple Sclerosis

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 12-month double-blind, randomized, multicenter, active-controlled, parallel-group study comparing the efficacy and safety 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

Retinal Toxicity

Long term (3 years) ophthalmic safety and cardiac efficacy and safety of ivabradine administered at the therapeutic recommended doses (2.5/5/7.5 mg b.i.d.) on top of anti anginal background therapy, to patients with chronic stable angina pectoris. An international, double-blind placebo controlled study

Retinitis Pigmentosa

An Exploratory, Multicenter, Patient-masked, Dose-escalation, Paired-eye Comparison, Sham-controlled, 6-Month (Plus 6-Month Extension) Study to Evaluate the Safety and Effects on Visual Function of 100ug, 200 ug, and 400 ug Brimonidine Tartrate Posterior Segment Drug Delivery System (Brimonidine Tartrate PS DDS) Applicator System in Patients with Retinitis Pigmentosa

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

Observational Studies

Functional Genomics of the Retina in Health and Disease (EVI-GENORET)

The phenotyping component of EVI-GENORET Project is collecting data on well-defined phenotypes of patients with Age-Related Macular Degeneration and Monogenic Retinal Dystrophies. A case report form (e-CRF) has been developed and is fully implemented as an internet based Database.

Medical doctors from AIBILI are also including data according to this specific protocol to the consortium database

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2.2. Centre for Bioavailability Studies

The Centre for Bioavailability Studies (CEB) was structured to be a qualified resource, skilled to collaborate with Pharmaceutical Industry in all the different phases of drug development.

The main area of activity has been the performance of Bioavailability/Bioequivalence Studies in human healthy volunteers to assess the efficacy and security of drugs. The elaboration of protocols and other documents needed to the studies execution, the organization of the processes to the regulatory authorities approval and the development of analytical methods for the drugs quantification have been of CEB's responsibility.

Recently, CEB improved competences and facilities for clinical trials performance (Phases I to III), one of the most relevant areas of activity at the moment. The proximity of the University Hospital of Coimbra and an important relation with a pro-active national pharmaceutical industry are key-points of this development.

Regarding human resources, the team includes a coordinator, a study director and four laboratory technicians also performing study-coordination functions in clinical trials. Multidisciplinary medical doctors, pharmacists and nurses also collaborate in the clinical trials executed at CEB.

The technical resources correspond to the most up-to-date and suitably calibrated equipment, in order to ensure precision and quality of the results presented.

All the activities performed at CEB follow quality standards such Good Laboratory Practices (certification since 1999 by INFARMED for the performance of Bioavailability/Bioequivalence and Pharmacokinetic Studies), Good Clinical Practices and ISO 9001 Guidelines (certification since 2004 for the performance of Clinical Trials, Bioavailability/Bioequivalence Studies and Drug Dosages at CEB).

Areas of Expertise

Studies of absolute bioavailability of a drug.

Elaboration of documentation to submit for the regulatory authorities, associated with bioequivalence studies.

Bioequivalence studies of pharmaceutical products having the same drug in the same formulation or different formulations.

Elaboration of documents associated with clinical trials performance.

Clinical trials performance.

Development and validation of analytical methods.

Dosage of drugs in the finished product or during the manufacturing process and in biological matrixes.

Clinical studies on the variability of different batches of preparation from a single manufacturer.

Chemical control of raw materials and manufactured products.

Organisation and scientific coordination of reviews or reports for the introduction of drugs in Portugal and the European Union.

Research

Ongoing Studies

Bioavailability/Bioequivalence Studies

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

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

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

A 52-week open-label extension study of the long-term safety and efficacy of rosiglitazone extended-release (RSG XR) as adjunctive therapy to acetylcholinesterase inhibitors in subjects with mild-to-moderate alzheimer´s disease (REFLECT-4)

A randomized, double-blind, parallel group, multi-center, comparative, flexible dose trial of pregabalin versus gabapentin as adjunctive therapy in subjects with partial seizures.

A Multi-Center, Placebo-Controlled, Double-Blind Trial To Examine the Safety and Efficacy of ACP-103 in the Treatment of Phychosis in Parkinson's Disease

A Multi-Center, Open-Label Extension Study to Examine the Safety and Tolerability of ACP-103 in the Treatment of Psychosis in Parkinson's Disease

A Historical-controlled, Multicenter, Double-blind, Randomized Trial to Assess the Efficacy and Safety of Conversion to Lacosamide 400mg/day Monotherapy in Subjects with Partial-onset Seizures

A Multicenter, Open-label Extension Trial to Assess the Long-term Use of Lacosamide Monotherapy and Safety of Lacosamide Monotherapy and Adjunctive Therapy in Subjects with Partial-onset Seizures

Drug Dosages Studies

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

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Funding

Introduction

The funding of "Laboratório Associado – Centro de Neurociências e Biologia Celular" comprises the following financing items: Global funding by FCT, Project FCT funding, national and international project funding by other financing agencies as well as other funding.

During 2008 global funding by "Fundação para a Ciência e Tecnologia (FCT)", concerning global institution programs ascended to 2.464.977,48€, namely:

Extension of Plurianual 2006:	146.268,32€
Plurianual 2008	1.882.328,78€
"Infrastructures"	239.396,45€
Science Program 2008	139.492,44€
Doctoral Program	57.491,49€

Funding of projects, namely FCT, national and international projects supports the main part of the running costs of Center for Neuroscience and Cell Biology.

FCT ongoing projects, as well as other national and international projects, which are discriminated below, comprised the total value of: 1.689.991,55€.

Projects financed by FCT ascended to 1.504.761,52€. National projects amounted to 135.042,36€**,** wheras international projects reached the financing value of 50.187,67 €.

The amount of other funding represented a financing value of 85.957,53€.

Note: Referred financing values are based on expenditure values 2008

ONGOING PROJECTS

Title	Financing Agency	Duration	Total Financing (CNC)	Financing 2008
National Projects:				
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	40.891,17
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	3.749,74
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	18.607,28
"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	2.898,21
Targetin of genes (…). Entidade Proponente INEB Porto Coordinator CNC: Sérgio Magalhães Simões	FCT Ref.ª: POCI/SAU-BMA/58170/2004	01/01/2005 to 31/08/2008	8.700,00	9.130,64

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	1.147,59
"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	14.880,23
"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 30/06/2008	65.000,00	5.404,80
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	9.953,70
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: PPCDT/AGR/59919/2004	02/05/2005 to 30/06/2009	64.280,00	4.195,98
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 30/09/2008	89.985,00	22.642,84
"Manipulação de DNA em solução e interfaces" Coordinator: Sérgio Paulo de Magalhães Simões Participants: Faculdade de Ciências e Tecnologia	FCT Refª: PPCDT/QUI/58689/2004	01/07/2005 to 30/06/2009	4.440,00	2.434,77

"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	5.473,95
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	20.162,10
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 Refª: POCTI/SAU-NEU/57310/2004	01/09/2005 to 31/08/2008	98.103,00	13.166,70
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	FCT Refª: POCI/SAU-MMO/56055/2004	01/09/2005 to 31/08/2008	30.000,00	445,61
Vectorização de fármacos para os vasos sanguíneos tumorais: uma nova terapia para o cancro da mama humano. Coordinator: João Nuno Moreira	FCT Refª: POCI/SAU-OBS/57831/2004	01/09/2005 to 29/02/2008	45.000,00	2.336,99
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	27.203,18

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	96,74
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	37.485,48
"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: PPCDT/QUI/56949/2004	01/10/2005 to 30/08/2009	29.500,00	6.428,88
"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	36.980,15
"Effecto das purinas no desenvolvimento do hipocampo: Consequências para o establecimento de circuitos relacionados com aprendizagem e memória" Coordinator: Rodrigo Pinto Cunha	FCT Refª: PTDC/SAU-NEU/74318/2006	01/07/2007 to 30/09/2010	94.439,00	52.891,98
"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	10.733,68

"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	28.488,58
"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	2.790,76
"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	39.876,17
"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	41.260,53
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	70.134,74
"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; Faculdade de Farmácia; IBILI;	FCT Refª: PTDC/SAU-FCF/67053/2006	01/05/2007 to 30/04/2010	88.000,00	20.286,90
Interacção entre a nicotina e a cafeína no núcleo estriado. Relevância na doença de Parkinson Coordinator: Rodrigo A. Cunha	FCT Refª: PTDC/SAU-NEU/81064/2006	01/05/2007 to 30/04/2010	94.378,00	39.025,66

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	55.613,10
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	58.493,06
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 31/08/2010	78.936,00	31.894,24
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 13/03/2010	115.800,00	43.306,63
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 31/04/2010	85.000,00	37.624,96
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	4.187,80

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 31/12/2009	25.059,00	16.220,34
Função da cortactina no tráfego celular dos receptores do glutamato do tipo do tipo AMPA Coordinator: Ana Luísa Monteiro de Carvalho	FCT Refª: PTDC/BIA-BCM/71789/2006	01/04/2008 to 31/03/2011	89.000,00	29.299,76
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	3.879,34
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	39.060,12
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	4.155,85
Estabilidade conformacional de proteínases aspárticas com importância biotecnológica e médica - O unfolding/refolding de proteínas diméricas e monoméricas. Coordinator: Marlene Barros	FCT Refª: PTDC/QUI/60791/2004	01/01/2007 to 04/03/2009	9.480,00	6.991,18

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 Refª: PTDC/SAU-NEU/65846/2006	01/05/2007 to 30/04/2010	115.256,00	41.033,59
Neuropeptídeo Y na retina: porquê? E para quê? Coordinator: Cláudia Cavadas	FCT Refª: PTDC/SAU-NEU/73119/2006	01/05/2007 to 30/04/2010	123.668,00	39.069,68
"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 Refª: GRID/GRI/81809/2006	01/06/2007 to 31/05/2010	27.545,00	12.923,41
Estudo de processos de bioluminescência. Coordinator: Rui Brito Participants: ADDF	FCT Refª: PTDC/FIS/73578/2006	01/07/2007 to 30/06/2010	50.928,00	22.959,25
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 Refª: PTDC/AGR-ALI/71262/2006	15/05/2007 to 14/05/2010	123.478,00	16.373,52
Papel e mecanismos moleculares do receptor CD36 na fagocitose de células apoptóticas: implicações para a aterosclerose Coordinator: Otília Vieira	FCT Refª: PTDC/SAU-MII/66285/2006	01/09/2007 to 31/08/2010	159.936,00	52.088,94
Actividade metabólica e viabilidade do condrócito em enxertos osteocartilagíneos humanos criopreservados. Coordinator: Celeste Lopes	FCT Refª: PTDC/SAU-OSM/67936/2006	01/09/2007 to 31/08/2010	32.648,83	28.946,60

Design, synthesis and biological assessment of multifunctional compounds as anti-Alzheimer drugs Coordinator: Paula Agostinho Participantes: Faculdade Farmácia Univ. Lisboa	FCT Refª: PTDC/SAU-NEU/64151/2006	01/08/2007 to 31/07/2010	12.740,00	0,00
"Modulação das vias metabólicas envolvidas no stress oxidativo mitocondrial em condições de hiperglicémia: sua relevância na prevenção da diabetes." Coordinator: Carlos Palmeira	FCT Refª: PTDC/QUI/72826/2006	01/01/2008 to 31/12/2009	36.000,00	16.679,23
"Searching for high level rules in protein folding and unfolding: from amyloid diseases to protein structure prediction", Coordinator: Rui Brito Participants: Universidade do Minho	FCT Refª: PTDC/BIA-PRO/72838/2006	01/01/2008 to 31/12/2010	39.556,00	12.727,70
"Nanoestruturas endereçadas para imagem molecular médica multimodal." Coordinator: Carlos Geraldes Participants:Universidade do Minho, Faculdade de Medicina Universidade de Coimbra	FCT Refª: PTDC/QUI/70063/2006	01/01/2008 to 31/12/2010	32.352,00	825,13
"Nanoquimica de compósitos magnéticos/luminescentes para aplicações de diagnóstico médico in vitro" Coordinator: António Guiomar Participants: Universidade de Aveiro	FCT Refª: PTDC/QUI/67712/2006	01/01/2008 to 31/12/2010	15.300,00	606,00
"Reconstrução e análise sistémica da rede reaccional de espécies reactivas de oxigénio, azoto e enxofre em sistemas fisiológicos representativos." Coordinator: Armindo Salvador Participants: Fundação da Faculdade de Ciências, Universitat de Lleida.	FCT Refª: PTDC/QUI/70523/2006	01/01/2008 to 31/12/2010	162.752,00	17.918,39

"BIOINK - Aprendizagem incremental de Kernel Machines para análise de dados em bioinformática" Coordinator: Paula Verissimo Participants: Faculdade Ciências Tecnologia da Universidade de Coimbra, Instituto Superior de Engenharia de Coimbra.	FCT Refª: PTDC/EIA/71770/2006	01/01/2008 to 31/12/2010	4.200,00	174,21
"Proteases de Polens, relevância nas doenças alérgicas." Coordinator: Paula Verissimo	FCT Refª: PTDC/SAU-ESA/72571/2006	01/05/2008 to 30/04/2011	199.850,00	25.906,45
"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 Lopes	Fundação Calouste Gulbenkian Refª: 68708	01/02/2005 to 31/07/2008	30.000,00	11.528,90
"Histamine in the neural and cancerstem cell niche: a role inglioblastoma ontogeny". Coordinator: Liliana Bernardino e Fabienne Agasse	Fundação Calouste Gulbenkian Refª: 96542	10/11/2008 to 09/11/2011	50.000,00	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	82.307,40
Programa MIT Coordinator: Catarina Oliveira, Lino Ferreira	FCT Refª: MIT-Portugal	01/09/2006 to 31/08/2011	854.145,02	294.597,31

"Studies of the molecular and cellular actions of Eslicarbazepine Acetate (BIA 2-093) as compared to other anti-epileptic drugs" Coordinator: Caetana Carvalho, Inês Araújo	BIAL	01/04/2008 to 31/03/2010	145.000,00	41.206,06
Sub - Total				**1.639.803,88**
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 30/04/2008	217.313,00	1.133,48
EMIL Coordinator: Carlos Geraldes	EMIL Ref.ª EMIL: LSHC-CT-2004-503569	01/07/2005 to 30/06/2009	43.646,87	6.684,39
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	42.369,80
Sub - Total				**50.187,67**
TOTAL				**1.689.991,55**

Members holding PhD		**Time % at CNC**
Alexandrina F. Mendes	(Auxiliar Prof., FFUC)	80
Amílcar Falcão	(Full Prof., FFUC)	80
Ana Bela Sarmento Ribeiro	(Auxiliar Prof., FMUC)	40
Ana Cristina Rego	(Assistant Prof., FMUC)	80
Ana Luísa Carvalho	(Auxiliar Prof., FCTUC)	80
Ana Paula Silva Martins	(Auxiliar Inv., FMUC)	Collaborator
Anabela A. Francisco	(Auxiliar Prof., Univ. Vasco Gama)	80
André Xavier C. Negrão Valente	(Auxiliar Inv., CNC)	100
Ângelo R. Tomé	(Auxiliar Prof., FCTUC)	70
António F. Ambrósio	(Auxiliar Inv., FMUC)	Collaborator
António Manuel Veríssimo Pires	(Auxiliar Prof., FCTUC)	80
Armanda E. Santos	(Auxiliar Prof., FFUC)	60
Armando Cristóvão	(Auxiliar Prof., FCTUC)	40
Armindo J. Alves S. Salvador	(Auxiliar Inv., CNC)	100
Arsélio P. Carvalho	(Full Prof., FCTUC)	80
Artur Augusto Paiva	(Graduate Technician, HUC)	50
Attila Köfalvi	(Auxiliar Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	80
Carlos B. Duarte	(Associated Prof., FCTUC)	80
Carlos G. Geraldes	(Full Prof., FCTUC)	60
Carlos Manuel Matias	(Auxiliar Inv., FCTUC)	60
Carlos Faro	(Associated Prof., FCTUC)	80
Carlos M. Palmeira	(Associated Prof., FCTUC)	60
Catarina R. Oliveira	(Full Prof., FMUC)	80
Célia M. Antunes	(Auxiliar Prof., FCTUC)	80
Cláudia Cavadas	(Auxiliar Prof., FFUC)	60
Cláudia M. F. Pereira	(Auxiliar Inv., FMUC)	60
Elsa Henriques	(Auxiliar Inv., FCTUC)	100
Emília P. Duarte	(Auxiliar Prof., FCTUC)	60
Euclides Pires	(Associated Prof., FCTUC)	60
Eugénia Carvalho	(Auxiliar Inv., CNC)	100
Geanne M. Cunha	(Auxiliar Prof., Brasil)	25
Gilberto Alves	(Auxiliar Prof., Univ Beira Int.)	Collaborator

Henrique Faneca	(Auxiliar Inv., CNC)	100
Inês Araújo	(Auxiliar Inv., CNC)	100
Isabel Maria Marques Carreira	(Auxiliar Prof., FMUC)	60
Ivana Jarak	(Auxiliar Inv., CNC)	100
João Laranjinha	(Associated Prof., FFUC)	60
João Nuno Moreira	(Auxiliar Prof., FFUC)	60
João O. Malva	(Principal Inv., FMUC)	100
João Ramalho Santos	(Associated Prof., FCTUC)	80
John Griffith Jones	(Principal Inv., CNC)	100
José Custódio	(Associated Prof., FCTUC)	80
Leonor Almeida	(Full Prof., FFUC)	70
Lino Ferreira	(Auxiliar Inv., CNC)	100
Lisiane O. Porciúncula	(Auxiliar Prof., Brasil)	30
Luís M. Rosário	(Associated Prof., FCTUC)	80
Luís Pereira Almeida	(Auxiliar Prof., FFUC)	60
Mª Amália Jurado	(Auxiliar Prof., FCTUC)	60
Mª Carmen Alpoim	(Associated Prof., FCTUC)	60
Mª Celeste Lopes	(Full Prof., FFUC)	80
Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Conceição Venâncio Egas	(Auxiliar Inv., FCTUC)	100
Mª Emilia O. Quinta Ferreira	(Associated Prof., FCTUC)	80
Mª Fernanda P. N. Gomes Nobre	(Principal Inv., FCTUC)	60
Mª Isabel J. Santana	(Associated Prof., FMUC)	80
Mª Luisa D. Ramos	(Auxiliar Inv., FCTUC)	80
Mª Madalena Caldeira Santos	(Associated Prof., FCTUC)	100
Mª Manuela Monteiro Grazina	(Auxiliar Prof., FMUC)	60
Mª Margarida Catalão Castro	(Auxiliar Prof., FCTUC)	60
Mª Otilia Vieira	(Auxiliar Inv., CNC)	100
Mª Sancha Santos	(Principal Inv., FCTUC)	60
Mª Teresa Cruz Rosete	(Auxiliar Prof., FFUC)	60
Marília Rocha	(Investigator, HUC)	60
Marlene Maria Tourais Barros	(Auxiliar Prof., FCTUC)	60
Milton Simões da Costa	(Full Prof, FCTUC)	80
Nuno Miguel Silva Empadinhas	(Auxiliar Inv., CNC)	100
Paula G. Agostinho	(Auxiliar Inv., FMUC)	60
Paula Isabel Moreira	(Auxiliar Prof., FMUC)	60
Paula Veríssimo Pires	(Auxilar Prof., FCTUC)	60
Paulo J. Oliveira	(Auxiliar Inv., CNC)	100

Paulo Santos	(Auxiliar Prof., FCTUC)	60
Ramiro Almeida	(Auxiliar Inv., CNC)	50
Ricardo Reis	(Associated Prof., Brasil)	100
Rodrigo A. Cunha	(Associated Prof., FMUC)	80
Rosa M. Santos	(Auxiliar Prof., FCTUC)	60
Rui A. Carvalho	(Auxiliar Prof., FCTUC)	60
Rui Barbosa	(Auxiliar Prof., FFUC)	50
Rui M. M. Brito	(Associated Prof., FCTUC)	50
Sandra Isabel M. Cardoso	(Auxiliar Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Auxilar Inv., FMUC)	60
Sérgio Simões	(Auxiliar Prof., FFUC)	60
Sukalyan Chaterjee	(Principal Inv., CNC)	100
Teresa Dinis Silva	(Associated Prof., FFUC)	70
Teresa Gonçalves	(Auxiliar Prof., FMUC)	60
Teresa Maria C. Martins	(Associated Prof., FFUC)	100
Vitor Manuel C. Madeira	(Full Prof., FCTUC)	80

Post-Doc Members

Ana Isabel Duarte	100
Ana Ledo	100
Ana Luísa Cardoso	100
Ana Rita Álvaro	100
Anabela P. Rolo	100
Anália do Carmo	100
Bharathi Pandurangan	100
Bruno O. Manadas	50
Carla Margarida Cardoso	100
Clévio Nóbrega	100
Dora Pedroso	100
Elisabete Baptista Ferreiro	100
Fabienne Agasse	100
Giovannia Araujo de Lima Pereira	50
Ildete Luisa Ferreira	100
Joana Cardoso da Costa	100
Joana Salgado	100
Licinia J. Simões	100

Liliana Bernardino	100
Luis Miguel Estronca	100
Luis França	100
Manuella P. Kaster	100
Mª Teresa Cunha Oliveira	100
Rosa M. B. Matos Resende	100
Sara Xapelli	100
Tatiana R. Rosenstock	100
Vilma A. Oliveira	100

PhD Students

Adriana Santos	100
Alexandra Rosa	100
Alexandre S. Rodrigues	100
Ana Burgeiro	100
Ana C. Fortuna	100
Ana Catarina H. Oliveira	100
Ana Cristina Silva	100
Ana Filipa Branco	100
Ana Francisca L. Silva Soares	100
Ana Luísa N. Gomes Nobre	100
Ana Paula Marques de Sousa	100
Ana Patricia S. Gomes	100
Ana Patrícia Simões	100
Ana Raquel Esteves	100
Ana Raquel M. Soares	100
Ana Rita A. Santos	100
Ana S. Carvalho	100
Ana Sofia V. Cunha	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa I. Varela	100
Ana Teresa Simões	100
André Martins	100
Andrea Lobo	100
Anaísa Pires	100

Bárbara Rocha	100
Beatriz Lacerda de Sousa	100
Bruno Carreira	100
Bruno Gago	100
Bruno Lopes	100
Bruno Miguel das Neves	100
Cândida S. Gonçalves da Silva	100
Carla Sofia G. Silva	100
Carlos José Vieira Simões	80
Carolina Coelho	100
Catarina Sofia H. Jesus	100
Cátia Diogo	100
Cátia Marques	100
Chantal Ana Vicência Fernandes	100
Clarissa S. Schitine	100
Cláudia Pereira	100
Cristiana Paulo	100
Cristina Barosa	100
Daniel Oberdorefer	100
Daniela Cipreste Vaz	100
Daniela M. Arduíno	100
Diego Hartmann	100
Filipe Coreta Gomes	30
Filipe Duarte	100
Gabriel Costa	100
Gonçalo Pereira	100
Helena Vazão	100
Hugo Prazeres	100
Igor Clemente Tiago	100
Ilídio Martins	100
Inês Vasconcelos Miranda Santos	100
Ivan Viegas	100
Joana Ferreira	100
Joana I. Real	100
Joana Paixão	100
João André Duarte	100
João Monteiro	100
João R. Gomes	100

João Teixeira	100
João Teodoro	100
João T. Costa	100
José Mário Tenera Morgado	100
Ligia Gomes da Silva	100
Liliana Mendonça	100
Luís Ribeiro	100
Magda Santana	100
Márcio S. Baptista	100
Márcio Ribeiro	100
Marco António P. Matos	100
Mª Fátima V. Lopes Pinto	100
Mª Inês Morte	100
Mª Isabel Nascimento Ferreira	100
Mª João Rodrigues Pereira	100
Marco Alves	100
Mariana Freitas	100
Mário Laço	100
Marta Isabel Rodrigues Baptista	100
Marta Viegas da Silva	100
Nélio Gonçalves	100
Pablo Pandolfo	100
Paula M. Canas	100
Paula Mota	100
Paulo Gameiro Guerreiro	100
Pedro Manuel Batista Branco	100
Pedro Coxito	100
Pedro Manuel V. Garção	100
Pedro Miguel Brás M. Coelho	100
Raquel Ferreira	100
Renata Santos Tavares	100
Ricardo Santos	100
Rita Perfeito	100
Rui Oliveira Costa	100
Rui Sanches	100
Sandra Marina A. Santos	100
Sandro Pereira	100
Samira C. Ferreira	100

Sara M. Diniz Martins Lopes	100
Sara Tavares M. Lima	100
Sara Trabulo	100
Sofia Baptista	100
Sofia Grade	100
Sónia Correia	100
Sónia Duarte	100
Sueli Cristina Marques	100
Susana Carvalho Rosa	100
Susana Isabel Elias Alarico	100
Susana Cardoso	100
Tatiana Catarino	100
Teresa Serafim	100
Vera Lúcia G. Francisco	100
Vera Moura	100
Vitor Gonçalo Silva C. Mendes	100

MSc Students

Ana Cardoso	100
Ana Carolina Moreira	100
Ana Catarina Fonseca	100
Ana Catarina Oliveira	100
Carlos Adriano A. Matos	100
Catarina Morais	100
Diana Moreira	100
Fátima Martins	100
Filipa Carvalho	100
Flávio Fortes R. Sousa	100
Helena Carvalheiro	100
Joana F. C. Fernandes	100
João Demétrio Martins	100
Ludgero Tavares	100
Luís Bimbo	100
Luis M. Bajouco	100
Marília Cordeiro	100
Nuno Gabriel Machado	100

Patricia Henriques Domingues	100
Patrícia Rebelo	100
Pedro Alexandre Martins	100
Raquel Brito	100
Raquel Vinhas	100
Ricardo Marques	100
Rui Cruz	100
Tiago Capote	100
Vera Raquel Cortez	100

Grant Technician

Ana Sofia Simões Bento Baptista	100
Anabela Simões	100
Cassilda Pereira	100
Daniela Pinheiro	100
Jaime Louro	100
Michelle Stumpf Viegas	100
Patrícia Nunes	100
Pedro Cruz	100
Susana Pereira	100
Tiago Francisco Santos Ferreira	100
Vitor Hugo R. Cabral	100

Undergraduate Students

Ana Carolina Borralho	100
Branca Silva	100
Graciano Leal	100
Maria Inês Coelho	100
Nair Monteiro	100
Nelson Cunha	100
Rita Silva	100

SERVICE STAFF

		Time % at CNC
Sandra Manuela Domingues 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
Filomena Maria F. Pereira dos Santos	(Technician, CNC)	100
Isabel Nunes Correia	(Ph.D., Graduate Technician, CNC)	100
Isabel Dantas Fernandes	(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 Eugénia A. Silva Lopes Campos	(Technician, CNC)	100
Virginia Maria R. Ferreira Fonseca	(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
Catarina Alexandra Ferreira Gomes	(Graduate Administrative, CNC)	100

Neuroscience and Disease

Catarina Resende Oliveira, MD, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Ana Cristina Rego	(Assistant Prof., FMUC)	80
Ana Luísa Carvalho	(Auxiliar Prof., FCTUC)	80
Ana Paula Silva Martins	(Auxiliar Inv., FMUC)	Collaborator
António F. Ambrósio	(Auxiliar Inv., FMUC)	Collaborator
Armanda E. Santos	(Auxiliar Prof., FFUC)	60
Armando Cristóvão	(Auxiliar Prof., FCTUC)	40
Arsélio P. Carvalho	(Full Prof., FCTUC)	80
Attila Köfalvi	(Auxiliar Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	80
Carlos B. Duarte	(Associated Prof., FCTUC)	80
Carlos Manuel Matias	(Auxiliar Inv., FCTUC)	60
Catarina R. Oliveira	(Full Prof., FMUC)	80
Cláudia Cavadas	(Auxiliar Prof., FFUC)	60
Cláudia M. F. Pereira	(Auxiliar Inv., FMUC)	60
Emília P. Duarte	(Auxiliar Prof., FCTUC)	60
Geanne M. Cunha	(Auxiliar Prof., Brasil)	25
Inês Araújo	(Auxiliar Inv., CNC)	100
Isabel Maria Marques Carreira	(Auxiliar Prof., FMUC)	60
João O. Malva	(Principal Inv., FMUC)	100
Lisiane O. Porciúncula	(Auxiliar Prof., Brasil)	30
Mª Graça Santos P. Vale	(Full Prof., FCTUC)	80
Mª Emilia O. Quinta Ferreira	(Associated Prof., FCTUC)	80
Mª Isabel J. Santana	(Associated Prof., FMUC)	80
Mª Manuela Monteiro Grazina	(Auxiliar Prof., FMUC)	60
Paula G. Agostinho	(Auxiliar Inv., FMUC)	60
Paula Isabel Moreira	(Auxiliar Prof., FMUC)	60
Paulo Santos	(Auxiliar Prof., FCTUC)	60
Ramiro Almeida	(Auxiliar Inv., CNC)	50
Ricardo Reis	(Associated Prof., Brasil)	100
Rodrigo A. Cunha	(Associated Prof., FMUC)	80

Sandra Isabel M. Cardoso	(Auxiliar Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Auxilar Inv., FMUC)	60

Post-Doc Members

Ana Isabel Duarte	100
Ana Rita Álvaro	100
Bruno O. Manadas	50
Elisabete Baptista Ferreiro	100
Fabienne Agasse	100
Ildete Luisa Ferreira	100
Joana Salgado	100
Liliana Bernardino	100
Manuella P. Kaster	100
Mª Teresa Cunha Oliveira	100
Rosa M. B. Matos Resende	100
Sara Xapelli	100
Tatiana R. Rosenstock	100

PhD Students

Alexandra Rosa	100
Alexandre S. Rodrigues	100
Ana Catarina H. Oliveira	100
Ana Cristina Silva	100
Ana Patrícia Simões	100
Ana Raquel Esteves	100
Ana Rita A. Santos	100
Ana S. Carvalho	100
Andrea Lobo	100
Bruno Carreira	100
Carla Sofia G. Silva	100
Clarissa S. Schitine	100
Daniela M. Arduíno	100
Gabriel Costa	100
Joana Ferreira	100
João R. Gomes	100
João T. Costa	100

Luís Ribeiro	100
Magda Santana	100
Márcio S. Baptista	100
Márcio Ribeiro	100
Marco António P. Matos	100
Maria Inês Morte	100
Mário Laço	100
Pablo Pandolfo	100
Paula M. Canas	100
Pedro Manuel V. Garção	100
Raquel Ferreira	100
Rita Perfeito	100
Rui Oliveira Costa	100
Rui Sanches	100
Samira C. Ferreira	100
Sofia Baptista	100
Sofia Grade	100
Sónia Correia	100
Sueli Cristina Marques	100
Tatiana Catarino	100

MSc Students

Ana Catarina Fonseca	100
Carlos Adriano A. Matos	100
Joana F. C. Fernandes	100
Luis M. Bajouco	100
Patrícia Rebelo	100
Vera Raquel Cortez	100

Undergraduate Students

Graciano Leal	100

Grant Technician

Ana Sofia Simões Bento Baptista	100

Molecular Biotechnology and Health

Euclides Pires, PhD, Coordinator

Members holding PhD		**Time % at CNC**
André Xavier C. Negrão Valente	(Auxiliar Inv., CNC)	100
Armindo J. Alves S. Salvador	(Auxiliar Inv., CNC)	100
Carlos Faro	(Associated Prof., FCTUC)	80
Elsa Henriques	(Auxiliar Inv., FCTUC)	100
Euclides Pires	(Associated Prof., FCTUC)	60
Henrique Faneca	(Auxiliar Inv., CNC)	100
João Nuno Moreira	(Auxiliar Prof., FFUC)	60
Lino Ferreira	(Auxiliar Inv., CNC)	100
Luís Pereira Almeida	(Auxiliar Prof., FFUC)	60
Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Conceição Venâncio Egas	(Auxiliar Inv., FCTUC)	100
Marlene Maria Tourais Barros	(Auxiliar Prof., FCTUC)	60
Paula Veríssimo Pires	(Auxilar Prof., FCTUC)	60
Rui M. M. Brito	(Associated Prof., FCTUC)	50
Sérgio Simões	(Auxiliar Prof., FFUC)	60

Post-Doc Members	
Ana Luísa Cardoso	100
Bharathi Pandurangan	100
Clévio Nóbrega	100
Dora Pedroso	100

PhD Students	
Adriana Santos	100
Ana Teresa Simões	100
Cândida S. Gonçalves da Silva	100
Carlos José Vieira Simões	80
Catarina Sofia H. Jesus	100
Cristiana Paulo	100

Daniela Cipreste Vaz	100
Helena Vazão	100
Inês Vasconcelos Miranda Santos	100
Ligia Gomes da Silva	100
Liliana Mendonça	100
Mª Isabel Nascimento Ferreira	100
Nélio Gonçalves	100
Pedro Manuel Batista Branco	100
Pedro Miguel Brás M. Coelho	100
Sara Trabulo	100
Sónia Duarte	100
Vera Moura	100

MSc Students

Flávio Fortes R. Sousa	100
Luís Bimbo	100
Pedro Alexandre Martins	100
Raquel Vinhas	100
Rui Cruz	100

Undergraduate Students

Maria Inês Coelho	100
Nair Monteiro	100

Grant Technician

Pedro Cruz	100
Tiago Francisco Santos Ferreira	100

Cell and Molecular Toxicology

Leonor Almeida, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Amílcar Falcão	(Full Prof., FFUC)	80
Anabela A. Francisco	(Auxiliar Prof., Univ. Vasco Gama)	80
Carlos M. Palmeira	(Associated Prof., FCTUC)	60
Gilberto Alves	(Auxiliar Prof., Univ Beira Int.)	Collaborator
João Laranjinha	(Associated Prof., FFUC)	60
José Custódio	(Associated Prof., FCTUC)	80
Leonor Almeida	(Full Prof., FFUC)	70
Mª Amália Jurado	(Auxiliar Prof., FCTUC)	60
Maria S. Santos	(Principal Inv., FCTUC)	60
Marília Rocha	(Investigator, HUC)	60
Paulo J. Oliveira	(Auxiliar Inv., CNC)	100
Rui Barbosa	(Auxiliar Prof., FFUC)	50
Teresa Dinis Silva	(Associated Prof., FFUC)	70
Vitor Manuel C. Madeira	(Full Prof., FCTUC)	80

Post-Doc Members	
Ana Ledo	100
Anabela P. Rolo	100
Vilma A. Oliveira	100

PhD Students	
Ana Burgeiro	100
Ana C. Fortuna	100
Ana Filipa Branco	100
Ana Patricia S. Gomes	100
Ana Teresa I. Varela	100
Anaísa Pires	100
Bárbara Rocha	100
Bruno Gago	100
Bruno Lopes	100

Cátia Diogo	100
Cátia Marques	100
Cláudia Pereira	100
Filipe Duarte	100
Gonçalo Pereira	100
Joana Paixão	100
João Monteiro	100
João Teodoro	100
Mª Fátima V. Lopes Pinto	100
Ricardo Santos	100
Sandra Marina A. Santos	100
Sandro Pereira	100
Susana Cardoso	100
Teresa Serafim	100

MSc Students

Ana Cardoso	100
Ana Carolina Moreira	100
Catarina Morais	100
Filipa Carvalho	100
João Demétrio Martins	100
Nuno Gabriel Machado	100
Ricardo Marques	100
Tiago Capote	100

Grant Technician

Anabela Simões	100
Cassilda Pereira	100
Jaime Louro	100
Susana Pereira	100

Microbiology

Milton Costa, PhD, Coordinator

Members holding PhD		**Time % at CNC**
António Manuel Veríssimo Pires	(Auxiliar Prof., FCTUC)	80
Mª Fernanda P. N. Gomes Nobre	(Principal Inv., FCTUC)	60
Milton Simões da Costa	(Full Prof, FCTUC)	80
Nuno Miguel Silva Empadinhas	(Auxiliar Inv., CNC)	100
Teresa Gonçalves	(Auxiliar Prof., FMUC)	60

Post-Doc Members

Joana Cardoso da Costa	100

PhD Students

Ana Luísa N. Gomes Nobre	100
Ana Sofia V. Cunha	100
Carolina Coelho	100
Chantal Ana Vicência Fernandes	100
Igor Clemente Tiago	100
Luis França	100
Susana Isabel Elias Alarico	100
Vitor Gonçalo Silva C. Mendes	100

Grant Technician

Vitor Hugo R. Cabral	100

Undergraduate Students

Branca Silva	100
Nelson Cunha	100

Biophysics and Biomedical NMR

Carlos Geraldes, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Ângelo R. Tomé	(Auxiliar Prof., FCTUC)	70
Carlos G. Geraldes	(Full Prof., FCTUC)	60
Célia M. Antunes	(Auxiliar Prof., FCTUC)	80
Ivana Jarak	(Auxiliar Inv., CNC)	100
John Griffith Jones	(Principal Inv., CNC)	100
Luís M. Rosário	(Associated Prof., FCTUC)	80
Mª Carmen Alpoim	(Associated Prof., FCTUC)	60
Mª Luisa D. Ramos	(Auxiliar Inv., FCTUC)	80
Mª Madalena Caldeira Santos	(Associated Prof., FCTUC)	100
Mª Margarida Catalão Castro	(Auxiliar Prof., FCTUC)	60
Rosa M. Santos	(Auxiliar Prof., FCTUC)	60
Rui A. Carvalho	(Auxiliar Prof., FCTUC)	60

Post-Doc Members	
Giovannia Araujo de Lima Pereira	50
Licinia J. Simões	100

PhD Students	
Ana Francisca L. Silva Soares	100
André Martins	100
Cristina Barosa	100
Filipe Coreta Gomes	30
Ilídio Martins	100
Ivan Viegas	100
Joana I. Real	100
João André Duarte	100
João Teixeira	100
Maria João Rodrigues Pereira	100
Marco Alves	100

Paulo Gameiro Guerreiro	100
Pedro Coxito	100

MSc Students

Fátima Martins	100
Ludgero Tavares	100
Nuno Machado	100

Grant Technician

Daniela Pinheiro	100
Patrícia Nunes	100

Cell and Development Biology

Mª Celeste Lopes, PhD, João Ramalho Santos, PhD, Coordinators

Members holding PhD		**Time % at CNC**
Alexandrina F. Mendes	(Auxiliar Prof., FFUC)	80
Ana Bela Sarmento Ribeiro	(Auxiliar Prof., FMUC)	40
Artur Augusto Paiva	(Graduate Technician, HUC)	50
Eugénia Carvalho	(Auxiliar Inv., CNC)	100
João Ramalho Santos	(Associated Prof., FCTUC)	80
Mª Celeste Lopes	(Full Prof., FFUC)	80
Mª Graça Santos P. Vale	(Full Prof., FFUC)	80
Mª Otilia Vieira	(Auxiliar Inv., CNC)	100
Mª Teresa Cruz Rosete	(Auxiliar Prof., FFUC)	60
Sukalyan Chaterjee	(Principal Inv., CNC)	100
Teresa Maria C. Martins	(Associated Prof., FFUC)	100

Post-Doc Members	
Anália do Carmo	100
Carla Margarida Cardoso	100
Luis Miguel Estronca	100

PhD Students	
Ana Paula Marques de Sousa	100
Ana Raquel M. Soares	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Beatriz Lacerda de Sousa	100
Bruno Miguel das Neves	100
Daniel Oberdorefer	100
Diego Hartmann	100
Hugo Prazeres	100
José Mário Tenera Morgado	100
Mª João R. Pereira	100
Mariana Freitas	100

Marta Isabel Rodrigues Baptista	100
Marta Viegas da Silva	100
Paula Mota	100
Sara M. Diniz Martins Lopes	100
Sara Tavares M. Lima	100
Susana Carvalho Rosa	100
Renata Santos Tavares	100
Vera Lúcia G. Francisco	100

MSc Students

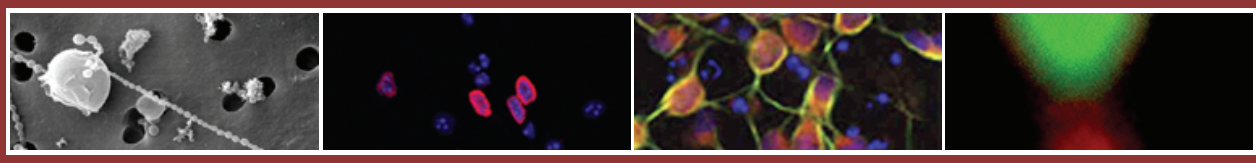
Ana Catarina Oliveira	100
Diana Moreira	100
Helena Carvalheiro	100
Marília Cordeiro	100
Patricia Henriques Domingues	100
Raquel Brito	100

Grant Technician

Michelle Stumpf Viegas	100

Undergraduate Students

Ana Carolina Borralho	100
Rita Silva	100



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CENTER FOR NEUROSCIENCE AND CELL BIOLOGY
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