CNC
CENTER FOR NEUROSCIENCE AND CELL BIOLOGY
UNIVERSITY OF COIMBRA, PORTUGAL

Center for Neuroscience and Cell Biology

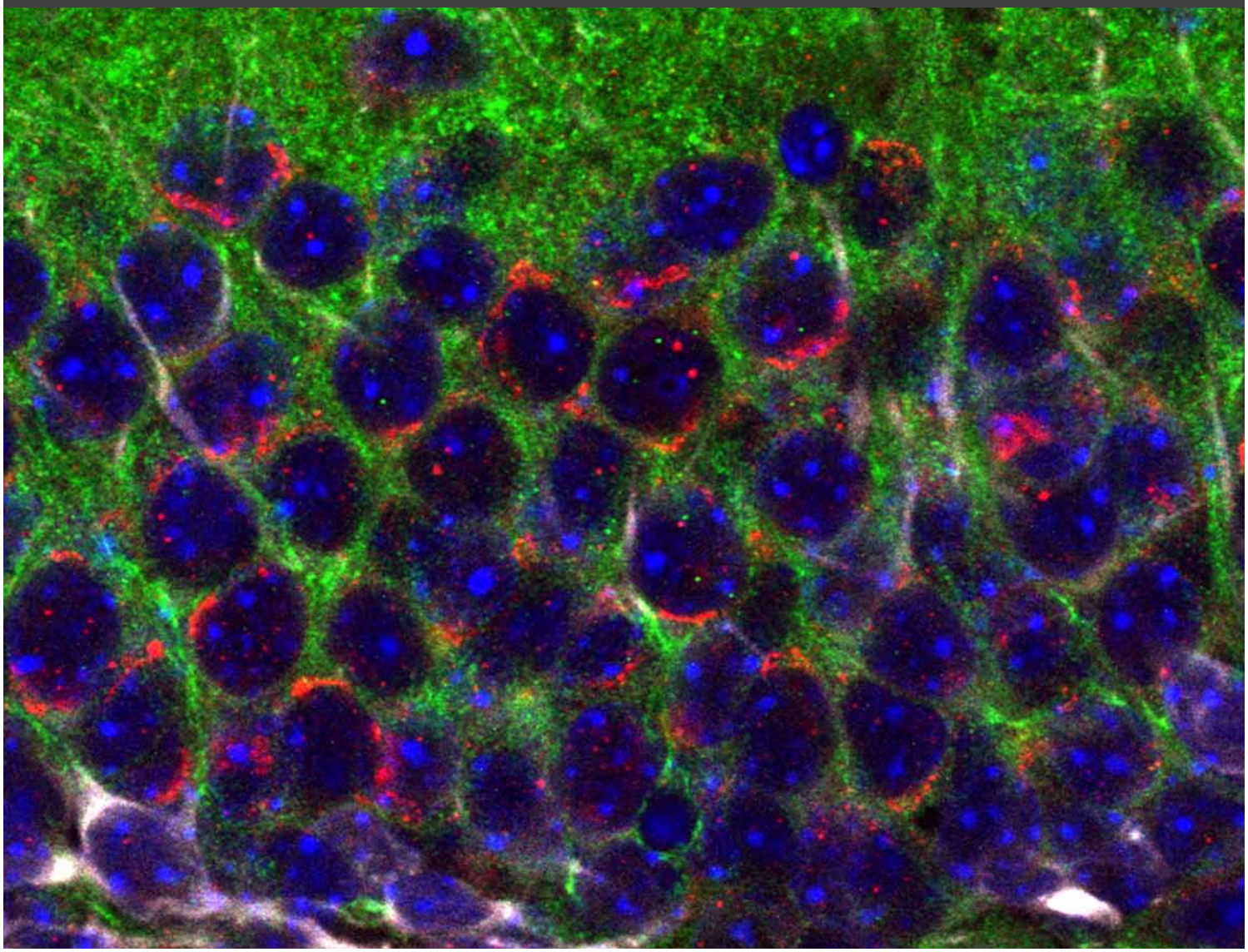
UNIVERSITY OF COIMBRA

A new culture through Scientific Research

2014 Annual Report

Biology | Neurosciences | Health and Disease | Biotechnology

Experimental Biology and Biomedicine | Research Programmes



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INTRODUCTION

CNC is a multidisciplinary research Institute, which brings together researchers from various Faculties and affiliated hospitals in the University of Coimbra. In 1990 CNC was recognized by FCT as a *Laboratório Associado* with the major mission of fostering fundamental and translational research and advanced training in biomedical sciences with a particular focus in neurosciences.

The current aims at CNC are: 1) Fundamental and Translational research in Neuroscience, Cell Biology and Molecular Biotechnology, 2) Advanced training; 3) Technology transfer and to provide specialized services to the community; 4) Outreach Programme (science and society).

In 2014, 208 scientific papers were published and 22 new research projects were financed (9 FCT projects, 3 national projects and 10 international projects).

The core scientific activity of CNC is the study of the molecular basis of degenerative processes common to aging and neurodegenerative disorders. In parallel, several groups explore mechanisms of neuroprotection and regeneration, which may be future candidates for the development of potential therapeutic strategies. 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.

Post-graduate education is a major goal at CNC. The Doctoral Programme in Experimental Biology and Biomedicine (PDBEB) and the participation in the MIT/Portugal Doctoral Programme provide Master and PhD students with a multi-faceted education in molecular life sciences related to disease and contribute to international scientific networking. Development of new technologies routed on solid fundamental research, and stimulated by the growing interest in translational research, led to reorganization of the services sector and to the creation of a research institute in the field of biotechnology, the CNC-Biotech Institute at BIOCANT. Research performed in this Institute is crucial to promote technology transfer and the creation of novel biomedical and biotechnology enterprises, which is one of the aims of CNC at BIOCANT Park.

The Outreach programme, the fourth current aim of CNC, aims at society scientific education and public perception of the importance of science for human health. To reach this goal, specific scientific programmes continued to be implemented in collaboration with schools and several social and cultural associations.

Future plans of CNC for the next two coming years include the reinforcement and expansion of the ongoing competitive basic research focused on the molecular mechanisms of neurodegeneration, neuroprotection, neurogenesis and brain repair, from the cellular level to in vivo animal models, as specified in each group research plan in this Annual Report. Perform high quality research, with international impact in fundamental cellular and molecular neuroscience and mechanisms of brain disease, is a common goal of most of the groups, some of which are currently working in the borderline between basic and applied research. Pushing forward some translational research approach to boost the development of high quality translational research in Neuroscience is one of the aims in a near future. Promoting internal collaborations between groups working in different areas at CNC will allow using biocompatible carriers for drug and gene delivery, such as viral vectors, molecular biology and proteomics approaches and the use of new sensors and electrodes to study brain function. Simultaneously, in the area of Biotechnology, the development of cutting-edge research projects, namely in the areas of stem cells and computational biology, allowing interdisciplinary approaches, will lead to innovation and to the increase of research projects of excellence. Post-graduate programmes will continue in the next coming years. Besides the CNC PhD Programme (PDBEB), CNC is a partner in the European Master Program (Neurasmus) and the European PhD Programme developed under the scope of ENC Network, as well as the MIT-Portugal Programme.

Technology transfer programme will strongly benefit with the “CNC Biotech – Investigação em Biotecnologia e capacitação do sector empresarial” project, which will be carried out in the Biotechnology unit at Biocant-Park, UC-Biotech.

Regarding the Outreach Programme, the strong collaboration that exists with “Ciência Viva”, “Instituto de Educação e Cidadania”(IEC) and several high schools will be maintained, and steady extended to other institutions.

CNC will pursue its involvement as a partner of MIT-Portugal and HMS-Portugal programs and a founder member of Health Cluster Portugal (HCP).

The 2014 Annual Report highlights the CNC accomplishments and the contribution of its dedicated researchers, students, support teams and administrative staff to achieve the main scientific goals of this research Center.

Facts & Figures (2014)

RESEARCH STAFF

Members holding Ph.D.	206 (80 Post Doctoral Fellows)
Ph.D.Students	156
MSc Students	64
Grant Technicians	34

PUBLICATIONS

Scientific papers published	210
Scientific papers *In Press*	27

THESIS CONCLUDED

Ph.D. thesis	27
MSc thesis	40

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%), centro Hospitalar da Universidade de Coimbra, Fundação para a Ciência e Tecnologia, AIBILI, Fundação Bissaya Barreto and two comercial firms – Reagente 5 and ILC.

GOVERNING BODY

President	*João Ramalho-Santos*
Vice Presidents	*Carlos Duarte*
	Carlos Faro
	Luís Pereira de Almeida
Honorary President	*Arsélio Pato de Carvalho*
Executive Council	Directors of the Departments
Research Council	CNC members holding PhD
"Conselho Fiscal"	A. Rodrigues, Leal e Carreira, A. Mourão
"Revisor Oficial de Contas"	Leal e Carreira, Sociedade Revisora de Contas

External Advisory Commitee: Fernando Lopes da Silva (NL); John Greenwood (UK); Rainer Goebel (NL); Marc Peschanski (FR); Xandra Breakefield (USA); Matthijs Vehage (NL)

SCIENTIFIC AREAS AND RESEARCH GROUPS

At present, research programmes and projects are organized in 3 research 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 2014, the research groups for each area can be identified, according to the following organization:

Neuroscience and Disease | *Carlos Duarte*

Neuromodulation Group (*Head: Rodrigo Cunha*)

Synapse Biology Group (*Head: Ana Luísa Carvalho*)

Growth Factor Signaling and Brain Ischemia Group *(Head: Carlos B. Duarte*)

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group (*Head: A. Cristina Rego*)

Neuroendocrinology and Aging Group (*Head: Claudia Cavadas*)

Redox Biology and Brain Sensing Group (*Head: João Laranjinha*)

Biotechnology | *Luis Pereira de Almeida*

Molecular Biotechnology Group (*Head: Carlos Faro)*

Computational and Systems Biology Group (*Head: Armindo Salvador)*

Vectors and Gene Therapy Group (*Head: M. Conceição Pedroso Lima*)

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

Pharmacometrics Group (*Head: Amílcar Falcão*)

Medicinal Chemistry & Drug Discovery Group (*Head: Maria Luísa Sá e Melo*)

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

Medical Microbiology Group (*Head: Teresa Gonçalves*)

Molecular Mycobacteriology Group (Head: Nuno Empadinhas) - *Emerging Group*

Metabolism Age, and Disease | *João Ramalho Santos*

Biology of Reproduction and Stem Cell Group (*Head: João Ramalho Santos)*

Cell Metabolis and Quality Control Group (*Head: Paula Moreira*)

Mitochondria, Metabolism and Disease Group (*Head: Paulo Oliveira*)

Obesity Diabetes and Complications Group (*Head: Eugénia Carvalho)*

ImmunoMetabolic Pharmacology Group (*Head: Mª Margarida Carneiro*)

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

Neuroscience and Disease Scientific Research Line

Coordinator: Carlos Duarte

The activity of the Neuroscience and Disease area in 2014 focused in i) understanding the molecular and cellular processes regulating synaptic activity, ii) how alterations in synaptic activity contribute to acute and chronic neurodegenerative processes, as well as to neuropsychiatric disorders, and iii) the development of neuroprotective strategies. Molecular genetics tools were also applied to embryonic stem (ES) cells to generate novel animal models of neuropsychiatric disorders.

The activity of the brain under normal conditions is controlled by several different regulatory molecules targeting specific receptors, and dysregulation of these mechanisms is associated with numerous diseases of the nervous system. The modulatory effects of neurotrophic factors were investigated from different perspectives: in the regulation of synaptogenesis, as modulators of synaptic plasticity, the changes in signaling mechanisms and neuroprotection in brain ischemia, which leads to acute neuronal damage, and the neuroprotective effects in Huntington's disease. The mechanisms underlying the cognition-enhancing effects of ghrelin, a peptide affecting energy balance and growth hormone release, were also investigated. The beneficial effects of caffeine and other antagonists of adenosine A_{2A} receptors were studied in the context of Huntington's disease and neuropsychiatric disorders. The role of neuropeptide Y and ghrelin in the induction of autophagy in the hypothalamus following caloric restriction was investigated as a strategy aiming at delaying aging and aging-related diseases. Finally, in vivo studies addressed the role of nitric oxide as a neuromodulator and mediator of neurovascular and neurometabolic coupling.

The molecular mechanisms of neurodegeneration and neuroprotection were also investigated with the aim of identifying novel therapeutic targets. These studies focused on the i) alterations in excitatory synapses in Alzheimer's disease, ii) changes in gene expression in brain ischemia and iii) mitochondrial dysfunction in Parkinson's disease.

Neuromodulation Group

Rodrigo A. Cunha	PhD – *head of group*
Paula G. Agostinho	PhD
Ângelo José Ribeiro Tomé	PhD
Attila Köfalvi	PhD
Ricardo Jorge A. Rodrigues	PhD
Henrique Bernardo Silva	PhD
Ana Patrícia Simões	Post-Doctoral Fellow
Catarina Alexandra Gomes	Post-Doctoral Fellow
Daniel Rial	Post-Doctoral Fellow
Joana Isabel Real	Post-Doctoral Fellow
João Pedro O. S P Lopes	Post-Doctoral Fellow
Joana Marques	Post-Doctoral Fellow
Nélio da Mota Gonçalves	Post-Doctoral Fellow
Paula M. Canas	Post-Doctoral Fellow
Samira Ferreira	Post-Doctoral Fellow
Amber Kherkoffs	PhD Student
Ana Cristina Lemos	PhD Student
Anna Pliassova	PhD Student
*António Manuel C. da Silva	PhD Student
Francisco M. Gonçalves	PhD Student
Jimmy George	PhD Student
Nuno Jesus Machado	PhD Student
Sofia Alexandra Ferreira	PhD Student
Patrícia Sofia Morais	PhD Student
Tiago Manuel P. Alfaro	PhD Student
Xinli Xu	PhD Student
Ana Carolina Xavier	MSc Student
João Filipe Amorim	MSc Student
Liliana Caetano	MSc Student
Paula Silva	MSc Student
Rui Oliveira Beleza	MSc Student
Caroline Delgado Veloso	Grant Technician

Synapse Biology Group Group

Ana Luísa Carvalho	PhD – *head of group*
João Miguel Peça Silvestre	PhD
Paulo Pinheiro	PhD
Joana Fernandes	Post-Doctoral Fellow
Susana Louros	Post-Doctoral Fellow
Tatiana Catarino	Post-Doctoral Fellow
Carlos Adriano A. Matos	PhD Student
Dominique Fernandes	PhD Student
Gladys Caldeira	PhD Student
Jeannette Schmidt	PhD Student
Lara Franco	PhD Student
Mariline Silva	PhD Student
Mohamed Hussien	PhD Student
Bruno Cruz	MSc Student
Mário Carvalho	MSc Student
João Calmeiro	MSc Student

Growth Factor Signaling and Brain Ischemia Group

Carlos B. Duarte	PhD – h*ead of group*
Armanda E. Santos	PhD
Emília P. Duarte	PhD
Michele Curcio	PhD
Ramiro Almeida	PhD
Graciano Leal	Post-Doctoral Fellow
Marta Dias M. Vieira	Post-Doctoral Fellow
Miranda Mele	Post-Doctoral Fellow
Rui Costa	Post-Doctoral Fellow
Ivan Salazar	PhD Student
Joana Pedro	PhD Student
Maria Joana Pinto	PhD Student
Pedro João Afonso	PhD Student
Sara Oliveira	PhD Student
Susana Sampaio	PhD Student
Eduardo Morais	MSc Student
Helena Martins	MSc Student
Ricardo Vieira	MSc Student
Luís Martins	Grant Technician
Marisa Marques	Grant Technician
Pedro Alves	Grant Technician

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Ana Cristina Rego	PhD – *head of group*
Ildete Luisa Ferreira	PhD
Elisabete Ferreiro	Post-Doctoral Fellow
Jorge Valero	Post-Doctoral Fellow
Mário Laço	Post-Doctoral Fellow
Sandra Mota	Post-Doctoral Fellow
*António M. Silva	PhD Student

Carla Maria Nunes Lopes	PhD Student
Luana Carvalho Naia	PhD Student
Marta Cerejo	PhD Student
Catarina Carmo	MSc Student
Inês Saragoça Dias	MSc Student
Irina Fonseca	MSc Student
Maura De Rosa	MSc Student
Vilte Sauliūnaitė	MSc Student
Ana Margarida Oliveira	Grant Technician

Neuroendocrinology and Aging Group

Cláudia Cavadas	PhD – *head of group*
Ana Rita Álvaro	PhD
Ana S. Carvalho	PhD
António Pedro Gomes	PhD
Armando Cristóvão	PhD
Caetana Carvalho	PhD
Célia Aveleira	PhD
Joana R. Salgado	PhD
Magda Santana	PhD
Mª Céu Sousa	PhD
Ligia Ferreira	Post-Doctoral Fellow
Ana Patricia Marques	PhD Student
Janete Cunha Santos	PhD Student
Joana Duarte Neves	PhD Student
Joana Vindeirinho	PhD Student
Mariana Botelho Rocha	PhD Student
Sara Matias Silva	PhD Student
Sonya Costa	PhD Student
Helena Leal	MSc Student
Liliana Santos	MSc Student

Redox Biology and Brain Sensing Group

João Laranjinha	PhD – *head of group*
Ana Ledo	PhD
Leonor Almeida	PhD
Rui Barbosa	PhD
Teresa Dinis Silva	PhD
Carla Nunes	Post-Doctoral Fellow
Cátia Lourenço Marques	Post-Doctoral Fellow
Ricardo dos Santos	Post-Doctoral Fellow
Bárbara Rocha	PhD student
Cassilda Pereira	PhD student
Diana Serra	PhD student
Nuno Ferreira	PhD student
Miguel António Azinheira	PhD student
Ana Sofia Miranda	Grant Technician
Cândida Dias	Grant Technician
Sónia Pereira	Grant Technician

Neuromodulation Group

Head: Rodrigo A. Cunha

Objectives

The general objective of the group is to identify modulation systems that can be targeted to interfere with the evolution of neurodegenerative diseases, with a central focus on purines (adenosine and ATP). We mostly focus on the initial stages of neurodegenerative disorders, under the working hypothesis that one of the key early features transversal to different such diseases is the dysfunction of synapses. This involves both neuronal and glial (astrocytes and microglia) maladaptive changes, with alterations of receptors, metabolic support and neuroinflammatory status, leading to abnormal synaptic plasticity and synaptic pruning that recapitulates features of neurodevelopment.

Our efforts over the years have identified a key role of adenosine A2A receptors (A2AR) in the control of neurodegenerative disorders; A2AR selectively control synaptic plasticity and they are up-regulated in afflicted areas upon brain diseases. We have shown that their blockade prophylactically prevents alterations in animal models of Alzheimer's disease, epilepsy or diabetic encephalopathy; this is in remarkable agreement with the prophylactic benefit afforded by the regular consumption of caffeine (an adenosine receptor antagonist) against diseases such Alzheimer's or Parkinson's. We are currently engaged in consolidating this concept that caffeine and selective A2AR antagonists can effectively control brain damage in different neuropsychiatric conditions. Additionally, we are exploring the mechanisms of action of A2AR in different brain areas (hippocampus, prefrontal cortex, amygdala and striatum) mingling the use of different A2AR-selective drugs, transgenic mice with tissue selective deletions of A2AR, virus designed to over-express or down-regulate A2AR and opto-genetic tools to selectively manipulate A2AR-containing cells combined with parallel behavioral, electrophysiological, morphological and neurochemical approaches exploiting subcellular fractionation techniques.

We now post that A2AR up-regulation may actually be a causative factor of aberrant synaptic plasticity underlying abnormal phenotypic changes, through a combination of direct neuronal control of synaptic plasticity, and glial control of synaptic function involving altered astrocyte-to-neuron communication and modified microglia-dependent neuro-inflammatory context.

In parallel, two emergent lines within the group are exploring the role of purines and of cannabinoids in the control of brain metabolism (Attila Kofalvi) and the role of purines, namely of extracellular ATP, in different processes characteristic of neurodevelopment (Ricardo Rodrigues).

Main Achievements

1-We have developed a novel chimeric molecule enabling a light-induced recruitment of A2AR transducing systems, which was applied to define that A2AR activation is sufficient to imbalance hippocampal synaptic plasticity and deteriorate cognitive performance.

2-Likewise, the over-expression of A2AR is also deleterious for mood-related performance of rodents.

3-In accordance with the beneficial effect of caffeine and A2AR antagonists to prevent memory deterioration in Alzheimer's disease, we concluded that A2AR inactivation also prevents memory impairment at early stages of Huntington's disease models.

4-We defined that the regular consumption of moderate doses of caffeine is safe in adolescent rodents.

5-We continued exploring the interaction of A2AR with different modulator systems. We reported interactions of A2AR with cannabinoid CB1 receptors in presynaptic terminals of the striatum, with D1 receptors in animal models of Huntington's disease and between A2BR and A1R in hippocampal terminals.

6-By exploring the role of A2AR in astrocytes, we unraveled an astrocyte-to neuron wave of communication, so that the selective elimination of astrocytic A2AR causes a synaptic imbalance and a schizophrenia-like phenotype. Additionally, we reviewed the therapeutic potential of A2AR to manage schizophrenia.

7-We continued to explore the role of A2AR in microglia and in the control of neuroinflammation, showing that ATP-derived adenosine control microglia proliferation and that microglia bolsters synapse formation during development.

8-We consolidated the role of ATP as a danger signal in the brain by showing that the blockade of P2X7R or of P2Y1R to control the deleterious impact associated with Parkinson's disease and brain ischemia.

9-We unraveled the role of proteins associated with Parkinson's disease, namely Parkin and GPR37 in the functioning of cortical networks and their interaction with A2AR.

Glutamatergic Synapses Group

Head: Ana L. Carvalho

Objectives

Synapses are neuronal specializations that transduce information between cells and mediate the precise flow of information between neuronal circuits. Memories and behaviors are encoded and shaped by changes in the structure and efficacy of synapses. As such, a current hypothesis is that the etiology of brain disorders either stems from, or gives rise to, synaptic malfunction. The Synapse Biology group focuses on understanding the molecular and cellular processes regulating synaptic biology, in identifying and mapping synaptic circuits underlying behavioral programs and in better understanding their dysfunction in neuropsychiatric disorders.

The **Synapse Biology** group is composed of three research sub-groups which share interest in the regulation of different aspects of synapse function.

Molecular and Cellular Mechanisms of Synaptic Plasticity (PI: Ana Luisa Carvalho)

Synaptic plasticity refers to neuronal-activity changes in synaptic strength. Depending on the pattern of activity, persistent increases or decreases in synaptic strength are induced, which changes the contribution of a set of synapses to information processing. Synaptic plasticity can persist for long periods of time, and it is considered the cellular correlate of learning and memory. In addition to this form of rapidly induced input-specific plasticity, forms of global homeostatic plasticity function to maintain neuronal activity within functional ranges. Homeostatic plasticity scales synaptic strength up or down while preserving relative synaptic weights. Together these forms of plasticity are necessary for activity-dependent changes in synapses and circuits and for adaptive cognitive processes in response to an environment that is constantly changing. We are interested in understanding at the molecular and cellular levels how these forms of plasticity are produced. Importantly, mutations in synaptic proteins implicated in synaptic plasticity have been associated with several neuropsychiatric disorders, such as intellectual disability, schizophrenia and autism.

The objectives for 2014 were 1) to establish the role of the transmembrane AMPA-type glutamate receptor associated protein (TARP) stargazin in homeostatic and experience-dependent plasticity; 2) to understand how the appetite-stimulating hormone ghrelin affects synaptic transmission and plasticity in the hippocampus.

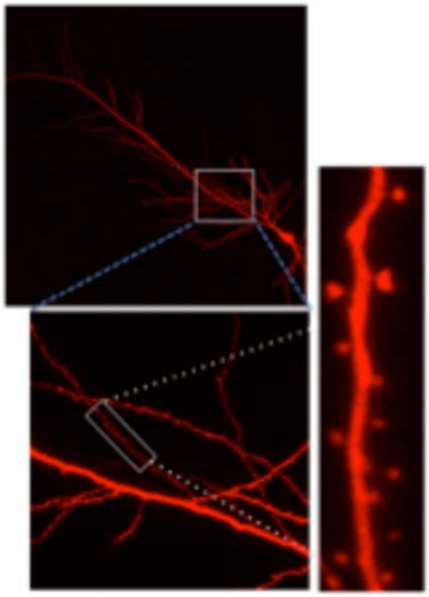
Neuronal Circuits and Behavior (PI: João Peça)

During 2014 our main goal was to consolidate research lines and implement critical experimental techniques: a) molecular genetics applied to murine ES cells in order to generate novel animal models of neuropsychiatric disorders; b) to setup and optimize electrophysiology recordings in awake behaving mice.

Neurosecretion and Neurotransmission (PI: Paulo Pinheiro)

Understanding the exocytotic process is a major step towards deciphering the immense complexity of brain function. We have been interested in how different proteins may be implicated in the exocytosis of neurotransmitter-containing vesicles, both directly and indirectly. One protein that received much focus in recent year is Doc2b, initially claimed as the calcium sensor for spontaneous release, and answering a long standing question. This view was later confounded with reports of it being a calcium sensor for asynchronous release and a promoter of spontaneous release in a calcium-independent manner. Therefore, one of our objectives in 2014 was to more finely dissect the functions of Doc2b after publishing, in 2013, a new view on its function in exocytosis.

We were also interested in the possible function of proteins of the BAR domain family in exocytosis, specifically in PICK1 (protein interacting with C-kinase 1). BAR domain proteins have the ability to sense/induce membrane curvature, and PICK1, in particular, has been implicated in the traffic of several membrane proteins, including AMPA receptor endocytosis during synaptic plasticity. However, a possible function for this class of proteins in exocytosis had not been demonstrated. Our aim was to dissect a possible function of PICK1 in the exocytosis of large dense core vesicles from chromaffin cells.



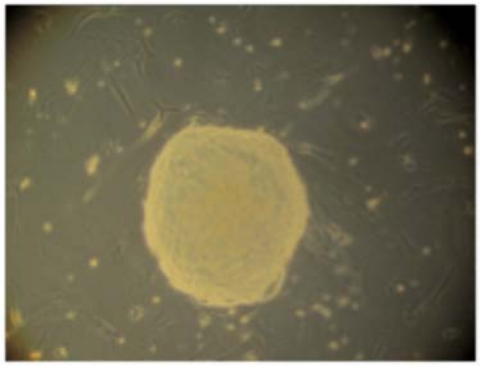
CA1 pyramidal neuron in hippocampal organotypic slices expressing mCherry. This system is well suited to study the morphology and dynamic behavior of dendritic spines.

Main Achievements

Molecular and Cellular Mechanisms of Synaptic Plasticity (PI: Ana Luisa Carvalho)

During development, neurons are constantly refining their connections in response to changes in activity. Experience-dependent plasticity is a key form of synaptic plasticity, involving changes in AMPAR accumulation at synapses. We found a critical role for the AMPAR auxiliary subunit stargazin in this plasticity. In the absence of stargazin, the refinement of the retinogeniculate synapse is specifically disrupted during the experience-dependent phase. Importantly, we found that stargazin expression and phosphorylation increased with visual deprivation. To test whether stargazin plays a role in homeostatic plasticity, we turned to cultured neurons and found that stargazin phosphorylation is essential for synaptic scaling. Overall, our data reveal an important role for stargazin in regulating AMPAR abundance and composition at glutamatergic synapses during homeostatic and experience-dependent plasticity (Louros et al. *Cell Reports* 2014, **7**: 1-12).

Ghrelin is a peptide mainly produced by the stomach and released into circulation, affecting energy balance and growth hormone release. These effects are guided largely by the expression of the ghrelin receptor growth hormone secretagogue type 1a (GHS-R1a) in the hypothalamus and pituitary. However, GHS-R1a is expressed in other brain regions, including the hippocampus, where its activation enhances memory retention. We explored the molecular mechanism underlying the action of ghrelin on hippocampal-dependent memory. Our data show that GHS-R1a is localized in the vicinity of hippocampal excitatory synapses, and that its activation increases delivery of AMPARs to synapses, producing functional modifications at excitatory synapses. Moreover, GHS-R1a activation enhances two different paradigms of long-term potentiation in the hippocampus, activates the phosphatidylinositol 3-kinase, and increases GluA1 AMPAR subunit and stargazin phosphorylation. We propose that GHS-R1a activation in the hippocampus enhances excitatory synaptic transmission and synaptic plasticity by regulating AMPAR trafficking. Our study provides insights into mechanisms that may mediate the cognition-enhancing effect of ghrelin, and suggests a possible link between the regulation of energy metabolism and learning. (Ribeiro et al. PNAS 2014, **111**:E149-58 [2014]).



Recombinant ES cell colonies for the generation of genetically-modified mice

Neurosecretion and Neurotransmission (PI: Paulo Pinheiro)

Following up on the work where we demonstrated that Doc2b had a dual function in exocytosis – promoting priming of vesicles at resting calcium, while putting a break on priming at high calcium - we were now able to demonstrate the functional consequences of interactions of Doc2b with other exocytotic proteins that could explain its functions. We showed that binding to SNAREs and calcium affects priming at rest, while binding to Munc13 affects priming at high calcium. Binding to other putative partners seems to play no role. Furthermore, previously published mutations in calcium binding were now better characterized and shown to cause partial gain-of-function. The work is now reaching publication phase.

We were also able to demonstrate that PICK1 does not directly participate in the process of exocytosis but that it plays a crucial role in the biogenesis of secretory vesicles: In the absence of PICK1 catecholamine secretion is severely impaired due to the formation of fewer and smaller vesicles. However, single vesicle or vesicle pool fusion kinetics were unaltered, as was the calcium dependence of release.

Neuronal Circuits and Behavior (PI: João Peça)

We have setup the pipeline for the generation of genetically engineered mouse models and we have established collaborations to assist in the creation and characterization of these mice. We have also successfully optimized the in vivo recording in awake-behaving mice during instances of social behaviors.

Neuronal Cell Death and Neuroprotection Group

Head: Carlos B. Duarte

Objectives

Neurotrophic factors play numerous roles in the nervous system, including the regulation of neuronal development, long-term modulation of synaptic transmission and in neuronal survival and neuroprotection under several different injury conditions. These effects are mediated by activation of specific receptors with tyrosine kinase activity, thereby inducing several parallel intracellular signaling cascades. Alterations in these signaling mechanisms have been associated with various disorders of the central and peripheral nervous systems. This group focuses on i) understanding the molecular mechanisms induced locally by neurotrophic factors to regulate neuronal development and synaptic plasticity, and ii) on the alterations in neurotrophic factor signaling in brain ischemia. Another major interest of the group is the understanding of the neurotoxic signaling mechanisms activated in brain ischemia.

Four core questions related to neurotrophic factor function/dysfunction and neurotoxic signaling mechanisms are currently pursued:

i) Local protein regulation in neuronal development (PI: Ramiro Almeida)

It has been known for many years that axons are capable of "locally responding" to guidance cues but only now are the mechanisms responsible for these phenomena starting to be understood. Recent data has shown that local translation is required for other neurodevelopmental mechanisms like neuronal survival and axonal pathfinding. In fact, a significant number of mRNAs has been found in pure preparations of distal axons and growth cones and its composition is far more complex than initially thought. This observation leads us to ask if local mRNA translation may play an important role in other neurodevelopmental processes like presynaptic differentiation. One goal of our research is to identify which mRNA(s) are required for presynaptic differentiation in response to neurotrophic factor stimulation. For that purpose we developed reporter assays to address this objective and monitor local translation in live cells.

ii) Local protein regulation in long-term synaptic potentiation (PI: Carlos B. Duarte)

The neurotrophin brain-derived neurotrophic factor (BDNF) is known to contribute to long-term synaptic potentiation

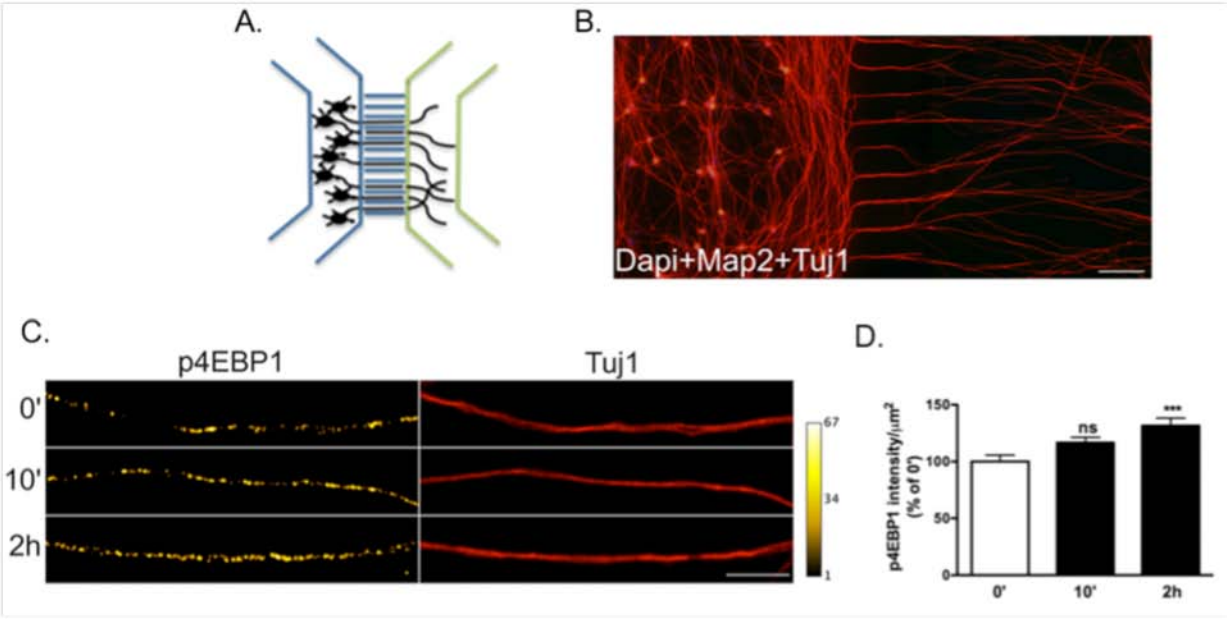


*Fig. 1 - FGF22 induces intra-axonal translation. (A, B) Axonal isolation in microfluidic chambers. Schematic illustration (A) and representative images (B) of ciliary ganglia neurons cultured in microfluidic devices. At DIV3, primary cultures of ciliary ganglia neurons were immunostained for Tuj1 (red), an axonal marker, and MAP2 (green), a dendritic marker (B). The image shows that axons crossed into the axonal side but cell bodies (blue) and dendrites (green) are restricted to the somal side. Scale bar is 100 µm. (C) Effect of FGF22 in axonal translation initiation. Ciliary ganglia neurons were stimulated with FGF22 nM, for the indicated time points. 4EBP1 phosphorylation (p4EBP1) was evaluated by immunocytochemistry using anti-p4EBP1 antibody and axons were immunostained using an anti-Tuj1 antibody (red). Scale bar is 5 µm. (D) Quantification of integrated intensity of p4EBP1/axonal area (μm^2). Results show that p4EBP1 levels increase after 2h of FGF22 stimulation, demonstrating that intra-axonal translation occurs after axonal-specifi application of FGF22. Fluorescence intensity was measured using Image J 1.45e software. Bars represent the mean ±SEM of 20 images from randomly selected areas of 4 independent experiments. *** Represents p<0.001 by one-way analysis of variance using Dunnett's multicomparison test when compared to 0'.*

(LTP) in the hippocampus, but the mechanisms involved are not fully elucidated. These effects of BDNF have been largely attributed to local protein synthesis, which requires the delivery of transcripts to the synapse, and one of our goals is to elucidate the role of this neurotrophin in the regulation of the traffic of RNA-binding proteins along dendrites. Since the ubiquitin-proteasome system (UPS) for protein degradation is also present at the synapse, we have investigated whether BDNF also regulates the synaptic proteome by modulating protein degradation in synaptic plasticity events.

iii) Synaptic dysregulation in brain ischemia (PIs: Carlos Duarte and Emília Duarte)

The increase in the $[Ca^{2+}]_i$ in brain ischemia leads to an abnormal stimulation of calpains (Ca^{2+}-dependent proteases), with consequent cleavage and downregulation of different proteins, including trophic factors. We are currently investigating whether a downregulation of trophic factor signaling contributes to neuronal damage in the ischemic brain.

iv) Alterations in gene expression in brain ischemia and neuronal death (PI: Armanda Santos)

Brain ischemia and excitotoxic phenomena lead to changes in the pattern of gene expression. At present we are addressing changes in the neuronal transcriptome in order to identify new genes involved in neuronal death or survival, thus broadening the set of possible therapeutic targets in brain ischemia

Main Achievements

i) Local protein regulation in neuronal development (PI: Ramiro Almeida)

Our goal is to detect if local mRNA translation is required upon induction of presynaptogenesis. We observed that presynaptic assembly requires axonal translation, indicating that local protein translation can regulate the formation of new synapses. Moreover, FGF22 stimulation induces a significant increase in the levels of the β-actin reporter, and in the number of F-actin rich puncta suggesting that local translation of β-actin mRNA regulates presynaptic differentiation. To assess the role of β-actin in presynaptic differentiation we developed a new nerve-muscle co-culture system. We observed that overexpression of a construct that mimics endogenous β-actin mRNA blocks NMJ formation *in vitro*. These results demonstrate that β-actin mRNA is trafficked to axons, where is locally translated in response to a synaptogenic cue, giving rise to a new presynaptic terminal.

ii) Local protein regulation in long-term synaptic potentiation (PI: Carlos B. Duarte)

Dendritic protein synthesis plays a critical role in several forms of synaptic plasticity, including BDNF (brain-derived neurotrophic factor)-mediated long-term synaptic potentiation (LTP). Dendritic transcripts are typically transported in a repressed state as components of large ribonucleoprotein complexes, and then translated upon stimulation at, or in the vicinity, of activated synapses. We found that neuronal activity and BDNF induce the synaptic delivery of Heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNP A2/B1), a trans-acting factor involved in dendritic mRNA trafficking (PLoS One 9, e108175 [2014]). Furthermore, we showed that inhibition of the proteasome by BDNF contributes to the effect of the neurotrophin in synaptic potentiation (J Neurosci 35, 3319-3329 [2015]). The effects of BDNF on protein synthesis, together with the regulation of protein degradation by the UPS, are expected to contribute to a tight regulation of the synaptic proteome thereby contributing to synaptic potentiation.

iii) Synaptic dysregulation in brain ischemia (PIs: Carlos Duarte and Emília Duarte)

Glial cell line-derived neurotrophic factor (GDNF) plays an important role in neuronal survival through binding to the GFRα1 receptor and activation of the receptor tyrosine kinase Ret. Brain ischemia alters the expression of the GDNF signaling machinery but the molecular mechanisms involved and the functional implications are not yet elucidated. We found that excitotoxic stimulation with glutamate as well as in vivo and in vitro (oxygen-glucose deprivation [OGD] in cultured hippocampal neurons) ischemia downregulate Ret protein levels via a calpain-dependent mechanism. Although calpain inhibitors prevented the downregulation of Ret receptors following excitotoxic stimulation, they did not fully prevent the downregulation of GDNF-induced intracellular signaling activity, suggesting that additional mechanisms may be involved. This alteration of the neuroprotective GDNF support to neurons may contribute to neuronal death in brain ischemia (Cell Death Dis 6, e1645 [2015]). These findings together our previous results showing a downregulation of BDNF-TrkB signaling under the same conditions (J Neurosci 32, 4610-4622 [2012]), suggest that a general failure of the trophic factor signaling mechanisms may play an important role in neuronal demise in the ischemic brain.

iv) Alterations in gene expression in brain ischemia and neuronal death (PI: Armanda Santos)

Brain ischemia induces a transcriptional response that has an important role both in neuronal survival and in neuronal death. By means of a whole genome DNA microarray we are investigating the transcriptome of rat hippocampal neurons challenged by OGD, an in vitro ischemia model (PLoS One 9, e99958 [2014]). This approach allowed to identify an up-regulation of RIP3, a crucial kinase mediating the regulated necrosis cell death program, upon ischemic stimuli (Neurobiol Dis. 68, 26-36 [2014]). Other interesting genes that came up on our analysis code for synaptic scaffolds, proteins involved in synaptic translation, and ionic channels (PLoS One 9, e99958 [2014]). Further studies will be pursued to understand the role of these genes on cell fate as this will pave the way to identify new therapeutic targets in cerebral ischemia.

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Head: A. Cristina Rego

Objectives

Neurodegenerative diseases are chronic and debilitating disorders of the central nervous system, characterized by cognitive decline and selective brain neurodegeneration. The latter has been attributed to mitochondrial dysfunction and protein misfolding. However, how modified or mutant proteins interfere with neuronal and mitochondrial function is not completely clear. Our research characterizes molecular targets for therapeutic intervention by evaluating glutamate postsynaptic dysfunction and mitochondrial dysfunction and interrelated signaling pathways in distinct neurodegenerative disorders, namely Alzheimer's disease (AD), Huntington's disease (HD), a polyglutamine expansion disorder, and Parkinson's disease (PD).

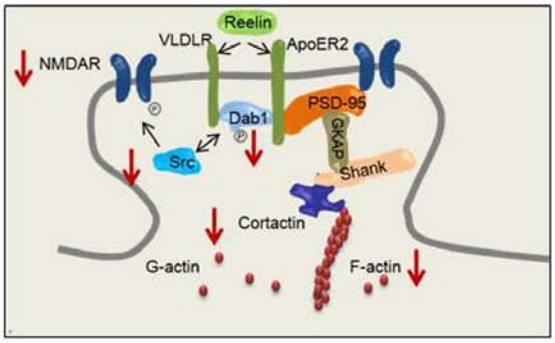
Early cognitive deficits in AD have been related to deregulation of N-methyl-D-aspartate receptors (NMDARs) and synaptic dysfunction in response to amyloid-beta peptide (Abeta). NMDAR anchorage to postsynaptic membrane depends in part on Src kinase, also implicated in NMDAR activation, and actin cytoskeleton stabilization. Thus, we analyzed P-GluN2B (NMDAR subunit) and the levels of proteins involved in Src signaling linking the Tyr kinase to actin cytoskeleton polymerization, namely reelin, disabled-1 (Dab1) and cortactin, in hippocampal samples obtained from the triple transgenic AD mice (3xTg-AD) *versus* age-matched wild-type mice. Moreover, we evaluated regional post-synaptic actin polymerization using phalloidin labeling in hippocampal slices (Mota et al., *Exp. Neurol*., 2014). Considering that Abeta interferes with NMDAR activity and that this may be a relevant factor for Abeta1-42-induced mitochondrial toxicity and neuronal dysfunction, we followed *in situ* single cell simultaneous measurement of cytosolic free Ca2+ (Ca2+i) and mitochondrial membrane potential (mmp) in primary cortical neurons (Ferreira and Ferreiro, *Neurobiol. Aging*, 2015).

Changes in α-synuclein (α-syn) post-translational modifications, including α-syn phosphorylation (P), mitochondrial dysfunction and oxidative stress constitute key pathogenic events of PD, the most common age-related neurodegenerative movement disorder. Using neuroblastoma SH-SY5Y cells expressing WT or mutant A53T α-syn, we analyzed the effects of prolonged exposure to oxidative stress and mitochondrial dysfunction, induced by iron and rotenone (complex I inhibitor), respectively, and determined the correlation between P-α-syn at Ser129, the formation of reactive oxygen species (ROS) and mitochondrial dysfunction.

HD is an autosomal dominant disease caused by an expansion of CAG repeats in the *HTT* gene. Brain metabolic dysfunction and altered Akt signaling pathways have been associated with disease progression. Nevertheless, conflicting results persist regarding the role of insulin-like growth factor-1 (IGF-1)/Akt pathway in HD. Thus, we investigated motor phenotype, peripheral and central metabolic profile, and striatal and cortical signaling pathways in YAC128 mice subjected to intranasal

Alzheimer's disease

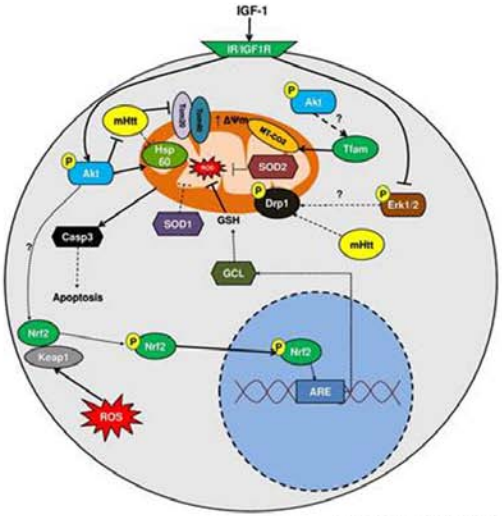
Impaired Src signaling and postsynaptic actin polymerization in AD mice hippocampus — linking NMDAR and the reelin pathway



3xTg-AD male hippocampus

Huntington's disease

IGF-1 improves mitochondrial function in a PI-3K/Akt-dependent manner and reduces mitochondrial generation of ROS in HD knock-in striatal cells



administration of recombinant human IGF-1 (rhIGF-1) for 2 weeks, in order to promote IGF-1 delivery to the brain (Lopes et al., *Mol. Neurobiol*., 2014). IGF-1 was previously shown to protect HD cells, whereas insulin prevented neuronal oxidative stress. Thus, we analyzed the role of insulin and IGF-1 in striatal cells derived from HD knock-in mice on mitochondrial ROS and related antioxidant and signaling pathways influencing mitochondrial function (Ribeiro et al., *Free Radic. Biol. Med*., 2014). We also used lymphoblasts obtained from HD patients or unaffected parentally related individuals to study the protective role of IGF-1 *versus* insulin on signaling and metabolic and mitochondrial functions (Naia and Ferreira et al., *Mol. Neurobiol*., 2015).

Main Achievements

In the context of AD we showed that young/pre-symptomatic 3xTg-AD male mice hippocampus exhibited decreased P-GluN2B at Tyr1472 and reduced Src activity. We also observed diminished P-Dab1 and cortactin protein levels in the hippocampus of young 3xTg-AD mice. A significant decrease in postsynaptic F-actin was also observed in 3xTg-AD CA1 and CA3 hippocampal regions, evidencing deregulated Src-dependent signaling pathways involving GluN2B-composed NMDARs and postsynaptic actin cytoskeleton depolymerization in the hippocampus in early stages of AD (Mota et al., *Exp. Neurol*., 2014). In single neuronal experiments, direct exposure to Abeta1-42 oligomers plus NMDA increased Ca2+i and induced immediate mitochondrial depolarization, compared with Abeta or NMDA alone. Exposure to Abeta+NMDA also evoked higher mitCa2+ retention, which was ameliorated in GluN2B-/- cortical neurons, involving this NMDAR subunit. Moreover, pharmacologic inhibition of endoplasmic reticulum (ER) inositol-1,4,5-triphosphate receptor (IP3R) and mitCa2+ uniporter (MCU) evidenced that Abeta+NMDA-induced mitCa2+ rise involved ER Ca2+ release through IP3R and mitochondrial entry by the MCU. Data highlight mitCa2+ dyshomeostasis and subsequent dysfunction as mechanisms relevant for early neuronal dysfunction in AD linked to Abeta-mediated GluN2B-composed NMDARs activation (Ferreira and Ferreiro et al., *Neurobiol. Aging*, 2015). These data emphasize mitochondria and GluN2B subunits as therapeutic targets in early stages of AD pathogenesis.

In the context of PD, prolonged expression of mutant A53T α-syn altered mitochondria morphology, causing mitochondrial dysfunction and increased superoxide formation. Increased susceptibility of mutant A53T α-syn cells to ROS production correlated with enhanced P-α-syn at Ser129, along with decreased activity of protein phosphatase 2A. Exposure to iron or rotenone further enhanced intracellular ROS levels, P-α-syn Ser129 and mitochondrial depolarization, particularly in A53T mutant α-syn expressing cells. Data suggested that stimuli that promote ROS formation and mitochondrial alterations highly correlate with mutant P-α-syn at Ser129, which may precede cell degeneration in PD (Perfeito et al., *Mol. Cell. Neurosci*., 2014).

In the context of HD, IGF-1 supplementation through intranasal administration enhanced IGF-1 cortical levels and improved motor activity, and both peripheral and central metabolic abnormalities in YAC128 mice. Upregulation of Akt following rhIGF-1 treatment occurred concomitantly with increased P-mHtt at Ser421. Data suggest that intranasal rhIGF-1 ameliorates HD-associated glucose metabolic brain abnormalities and mice phenotype (Lopes et al., *Mol. Neurobiol*., 2014). This provides an experimental basis for establishing intranasal administration as an important therapeutic route for the administration of neurotrophic factors in neurodegenerative diseases. In striatal cells derived from HD knock-in mice, insulin and IGF-1 improved mitochondrial function and reduced mitochondrial ROS caused by activating the PI-3K/Akt signaling pathway, in a process independent of Nrf2 transcriptional activity, but involving enhanced mitochondrial levels of Akt and mitochondrial-encoded complex IV subunit (Ribeiro et al., *Free Radic. Biol. Med.,* 2014). In HD human lymphoblasts deregulation of insulin/IGF-1 receptor (IR,IGF-1R) signaling pathways were largely restored by IGF-1. Both IGF-1 and insulin stimulated P-HTT at Ser421 and rescued energy levels in HD cells. IGF-1 also ameliorated O2 consumption and mmp in HD lymphoblasts. Indeed, constitutive P-HTT was able to restore the mmp in HD lymphoblasts. We demonstrate that IGF-1-mediated activation of IR/IGF-1R enhances mitochondrial function in human HD cells through the activation of intracellular signaling pathways and P-HTT at Ser421 (Naia and Ferreira et al., *Mol. Neurobiol.,* 2015).

Neuroendocrinology and Neurogenesis Group

Head: Cláudia Cavadas

Objectives

In our group we investigate the hypothalamus as an underlying mediator and a target for interventional strategies in counteracting aging and related diseases. In this context the group focuses the research on the following scientific questions:

i) Does caloric restriction (CR) delay aging and aging-related diseases through hypothalamus-related mechanisms?
ii) How aging and aging related disease change hypothalamus?
iii) Can we delay premature aging of Hutchinson Gilford progeria syndrome (HGPS) rodent models, normal aging or aging related diseases, by targeting the hypothalamus or using hypothalamic related mechanisms?

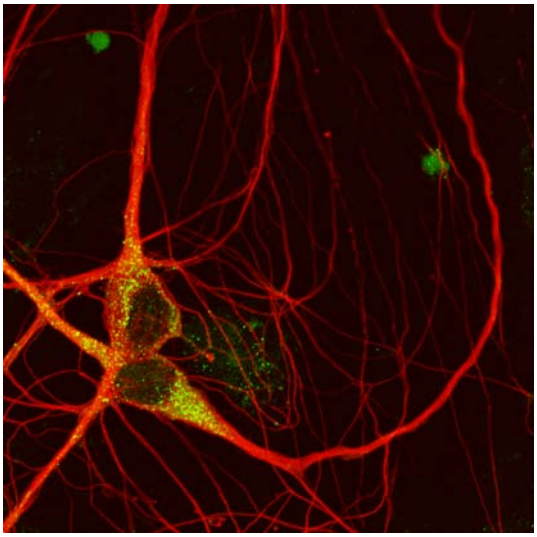


Fig. 1. Neuropeptide Y (NPY) enhances autophagy in rodent hypothalamic neurons; microscopy confocal image of rat hypothalamic neurons co-labeled with the neuronal marker beta III Tubulin (red) and autophagy marker (LC3B, green)

Main Achievements

1. We demonstrate that CR induces autophagy in hypothalamic neurons, and this effect is mediated, in part, by NPY receptors activation. In addition, evidence from both hypothalamic neuronal *in vitro* models and mice overexpressing NPY in the hypothalamus, show that NPY *per se*, stimulates autophagy in the hypothalamus (Figure 1). Mechanistically, the activation of NPY Y_1 and Y_5 receptors increases autophagy in hypothalamic neurons and this effect is tightly associated with the concerted activation of PI3K, MEK/ERK and PKA signaling pathways. Since both hypothalamic autophagy and NPY levels decrease with age, the rescue of hypothalamic NPY levels provides a new putative strategy to delay aging (Aveleira and Botelho et al., in revision).
2. The microRNA pathway is impaired in the hypothalamus of obese rodents as shown by alterations in the expression levels of miRISC genes and specific microRNAs.
3. The hypothalamus of premature aging mouse (Zmpste24-/- mouse) has lower levels of NPY, compared to wild type. Therefore, we hypothesized that increasing NPY levels in the hypothalamus could rescue the premature aging of these mice. In fact, our preliminary studies show that hypothalamic NPY increase, achieved by stereotaxic injection of gene delivery approach in the hypothalamus, delays or reverts aging markers in Zmpste24-/- mouse: alopecia, memory impairment, lipodistrophy, skin and hypothalamic aging markers (Botelho and Aveleira et al., in preparation).
4. Caloric restriction (CR) mimetic medium induces autophagy in rat cortical neurons in culture and blocking NPY or Ghrelin receptors inhibits this effect. Moreover, NPY and ghrelin, *per se*, stimulate autophagy and NPY mediates, in part, ghrelin-induced autophagy in rat cortical neurons. Since autophagy impairment occurs in aging and age-related neurodegenerative diseases, this NPY and ghrelin synergistic effect on autophagy stimulation may suggest a new strategy to delay aging process (Marques and Aveleira et al, in revision).
5. CR and NPY modulate the proliferation and differentiation of rodent hypothalamic neuroprogenitor cells.
6. Modulation of ataxin-2 in mice hypothalamus regulates energy balance and metabolism: including changes in body weight, white and brown adipose tissue, hypothalamic NPY levels, and response to insulin (Matias et al., in preparation).
7. SIRT2 is abundantly expressed in major mouse hypothalamic nuclei and hypothalamic SIRT2 expression changes upon CR or consumption of a high fat diet (HFD), which triggers insulin resistance, suggesting that hypothalamic SIRT2 levels are modulated by nutrient availability (Santos et al., in preparation).
8. Machado-Joseph disease (MJD) is a fatal dominantly inherited neurodegenerative disorder associated with an expanded polyglutamine tract within the ataxin-3 protein, and characterized by progressive impairment of motor coordination, associated to neurodegeneration of specific brain regions including cerebellum and striatum. We observed that NPY levels are decreased in two MJD patients' *cerebella* and in *striata* and *cerebella* of MJD mouse models. Furthermore, CR or overexpression of NPY in specific brain areas alleviate the motor coordination impairments and attenuated the related MJD neuropathological parameters (Duarte-Neves et al., in revision; Cunha-Santos et al., in preparation).

Redox Biology and Brain Sensing Group

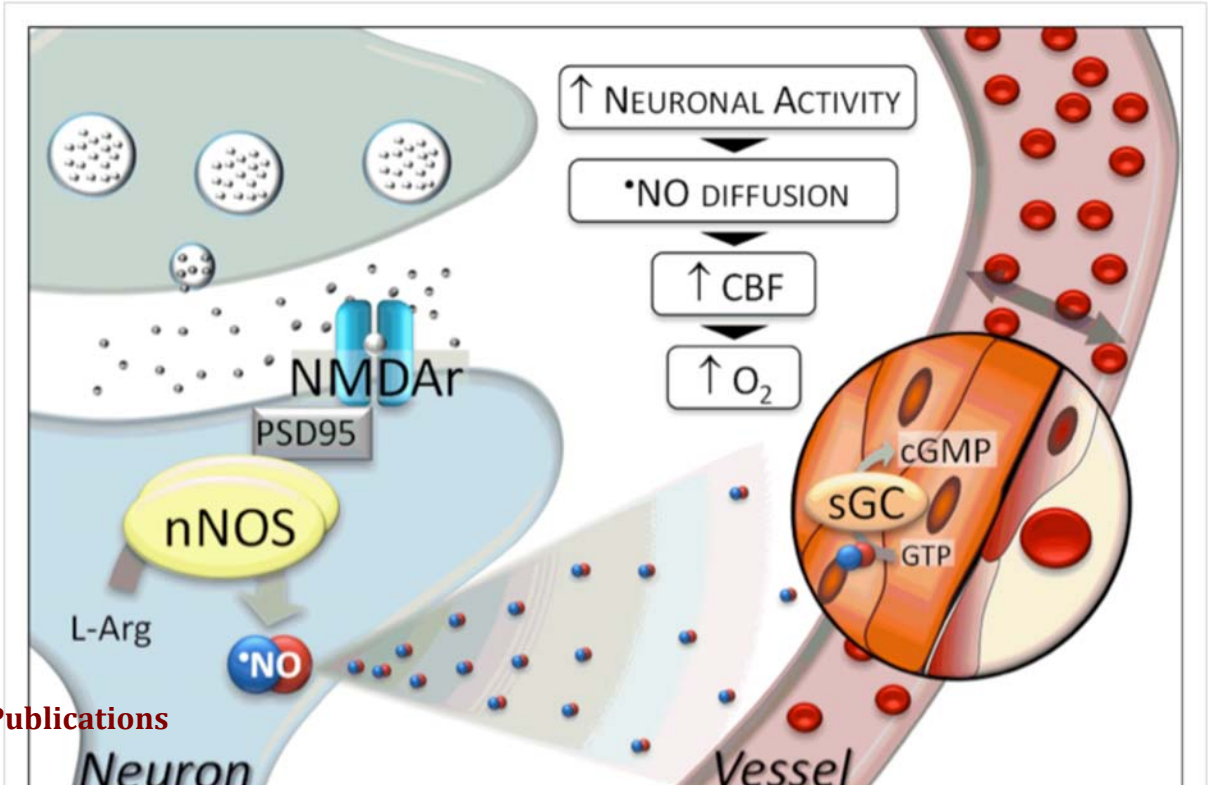
Head: João Laranjinha

Objectives

We are interested in: (a) the study in vivo in anesthetized and in freely moving animals of the molecular mechanisms inherent in neuromodulation and aging that critically involve nitric oxide (NO) in the brain, deciphering the mechanisms that support its role as a neuromodulator and as the mediator of neurovascular and neurometabolic coupling; (b) the analysis of the mechanisms of action of plant-derived dietary phenolic compounds, particularly those present in wine, in terms of protection against vascular endothelial dysfunction, anti-inflammatory properties, as well as their impact on nitrite-driven regulatory processes.

Main Achievements

1. We have mapped neuronal isoform of nitric oxide synthase (nNOS) and correlated it with the profile of NO changes in time and space in different brain areas (hippocampus, cortex, striatum). We observed that the distribution of nNOS does not necessarily correlate with NO concentration dynamics in different brain areas upon neuronal stimulation. This has profound consequences for NO studies in the brain. Conversely to the vast majority of data available regarding bioactivity of NO, only the concentration dynamics of NO provides a refined piece of information for the understanding of its actions as it reflects the fine regulatory mechanisms involving NO production and inactivation in vivo.
2. We have demonstrated in vivo and in a dynamic and real-time fashion that neuronal-derived nitric oxide (NO) is the direct mediator of neurovascular coupling in hippocampus. The mechanistic link to match blood supply with metabolic demands imposed by increased neuronal activity has been a matter of controversy. We showed that this process relies on volume signaling conveyed by NO that, upon neuronal stimulation, diffuses from neurons towards blood vessels, inducing restricted vasodilation via interaction with soluble guanylate cyclase.
3. We have described a novel pathway for systemic biological effects of dietary polyphenols in connection with NO biology that short-cuts the standard pathways that involve their absorption from the intestine. This hypothesis posits that inclusive biological impact of polyphenols is conveyed by NO and related oxides via modulation of the nitrate.nitrite:NO pathway in the gastric compartment. The nitrate-nitrite-NO pathway may therefore provide a short cut to the signaling effects of polyphenols (including the brain), linking directly diet composition and the biological outcome.
4. We have revealed the mechanisms for antiinflammatory activity of resveratrol in terms of downregulating the production of pro-inflammatory mediators (NO, PGE2), pro-inflammatory enzymes (iNOS, COX-2 mRNAs and proteins) and ROS formation induced by cytokines. Moreover, our work gave a step forward unravelling JAK-STAT as well as MAPK signaling as key cascades involved in rescveratrol anti-inflammatory protection and in its potential anticancer action. *In vivo* experiences, in a rat model of inflammation, treated with an anthocyanin rich extract obtained from blueberries (*Vaccinium corymbosum L.*), confirmed the higher anti-inflammatory action of anthocyanins and their benefits in the inflammed intestinal lumen. Taking into account the high concentrations of dietary anthocyanins and other Thus, polyphenols may be envisaged as promising nutraceuticals, giving complementary benefits in the context of inflammatory cascades.



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Biotechnology Scientific Research Line

Coordinator: Luis Pereira de Almeida

The Stem Cell-Based and Molecular Therapies Thematic Strand involves 8 groups performing **specific** fundamental and translational science in diagnostics and therapeutics in the areas of cardiovascular, oncological neurodegenerative and microbial diseases. In addition, the 8 groups have a joint scientific program that combines the different expertises into a collaborative project. In this case, all the 8 groups are committed to develop diagnostics and therapeutics to re-establish the homeostasis of blood-brain barrier (BBB) in neurodegenerative diseases and to develop vectors able to cross efficiently the BBB.

The main goals of the Stem Cell-Based and Molecular Therapies Thematic Strand are:

a) To use stem cell-based therapies for the treatment of ischemic diseases, to develop innovative strategies for cell reprogramming and stem cell modulation/differentiation, and to implement stem cell–based assays and *in silico* approaches for drug screening.

b) To use molecular therapy approaches to accelerate understanding and treatment of disease. For this we take advantage of viral and non-viral vectors, for nucleic acid and drug delivery aiming at i) establishment of disease models and study of molecular mechanisms and ii) development of new molecular therapeutic approaches. Translational molecular therapy approaches will be investigated for cancer, neurodegenerative (e.g. Machado-Joseph and retinal disease*)* and infectious diseases using nucleic acids, which will in some cases be translated into therapies with compounds.

c) To identify novel therapeutic compounds, natural and synthetic, by both in silico and high throughput screening of chemical libraries. These candidates will be delivered using nanotechnology-based non-viral vectors. Imaging and pharmacometric studies coupled with biomarker, animal behavior and pathology will allow extensive multilevel evaluation of disease alleviation.

Collaborative project aims:

a) to develop *in vitro* models of healthy and disease BBB.

b) to understand the disease state of BBB in neurodegenerative diseases and to develop neurotherapeutics to re-establish its homeostasis.

c) to develop nanomedicine platforms able to cross BBB and to regenerate brain after disease.

The Stem Cell-Based and Molecular Therapies Thematic Strand has around 70 PhD holding integrated members distributed over 8 groups, the first 2 of these of large dimension (Vectors and Gene Therapy; Biomaterials and Stem Cell-Based Therapeutics) and 5 other smaller groups. Recently hired young investigators: Filipe Pereira; Miguel Mano; Ricardo Pires will further expand this strand.

During 2014 the groups of the Stem Cell-Based and Molecular Therapies Thematic Strand have:

Cardiovascular Disease

a) differentiated human pluripotent stem cells into embryonic arterial endothelial cells and screened compounds that affect specifically embryonic vasculature

b) generated a human in vitro model of ageing based on induced pluripotent stem cells (iPSCs) derived from patients with Progeria, and studyied the reasons of Progeria-SMCs vulnerability providing new opportunities for the treatment of progeria and diseases related to vascular ageing.

Oncological disease

a) Developed light-activatable nanoparticles to improve the intracellular delivery of retinoic acid in leukemic cells with potential for activating the differentiation of the cells at the bone marrow niche and interfering with the leukemic stem cell niche.

b) Identified new molecules to produce efficient nucleic acid delivery systems and established structure-activity relationships - gemini surfactants, copolymers and cell penetrating peptides.

c) developed a new antitumoral strategy involving silencing of the oncomir miR-21, overexpressed in glioblastoma (GBM), through delivery of LNA oligonucleotides via tumor-targeted stabilized nucleic acid lipid particles (SNALPs) followed by cell exposure to sunitinib.

d)

e) Showed that regulation of microRNA expression levels combined with low amounts of chemotherapeutic agents results in a significant and synergistic cell death effect in pancreatic cancer models.

Brain Disease

a) generated lentiviral and adeno-associated viral vectors to study the pathogenesis of Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD) and demonstrated that gene silencing, autophagy activation, proteolysis inhibition and neural stem cell replacement are promising approaches to alleviate this disorder.

b) identified specific miRNAs whose levels are deregulated in AD patients.

c) Identified a compound, monoterpene necrodane ketone, from the oil of *L. luisieri* that displayed a dose-dependent inhibition of BACE-1 in cellular/mouse models of Alzheimer's disease and was capable of passing through cellular membranes and the BBB.

d) **modeled the permeation** of a homologous series of amphipatic molecules, 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-labeled alkyl chain amphiphiles (NBD-Cn, n=2-16) **across the BBB**, to obtain rules that relate permeant structure to permeability.

e) Clarified and modelled the **biodistribution and pharmacokinetics** of the antiepileptic drug carbamazepine, **after intranasal and intravenous administrations**. Higher concentrations in the olfactory bulb and frontal cortex following intranasal instillation, suggest the involvement of a **direct transport from nose to brain**.

Microbial disease

a) Reported the modulation of *Alternaria infectoria* cell wall chitin and glucan synthesis by cell wall synthase inhibitors.

b) identified genes and characterized the enzymes involved in the mycobacterial methylglucose lipopolysaccharides byosynthesis and, deciphered the three-dimensional structures of essential enzymes, establishing experimental scaffolds for drug screening and design.

c) Developed new synthetic methodologies to produce new oxysterol tetraoxane chemotypes, for antimalarial drug discovery, with the ability to circumvent drug resistance.

d) Demonstrated the transfer of resistance genes among bacteria of clinical and animal origin showing the resistance gene transfer contributing to the clarification of the mechanism of antimicrobial susceptibility and mechanisms of resistance.

e) provided the the first report on a retropepsin-like protease in the gram-negative intracellular bacteria Rickettsia, which may be a new target for therapeutic intervention against fatal rickettsioses.

f) clarified the adjuvanticity mechanisms of chitosan nanoparticles, which increased antigen nasal residence, induced the production of IL-1β by DC cells, via a NLRP3 inflammasome-dependent pathway and promoted mast cell activation

Substantial funding is available for 2015 research activity and the groups have already secured funding for the following years namely under European programs E-Rare and JPND Transnational calls, MIT-Portugal program, ERC funding and research contracts with industry until 2018. Applications for funding in all the areas of the strand are pending including H2020 proposals under evaluation.

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Computational and Systems Biology Group

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Vectors and Gene Therapy Group

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Liliana Mendonça	Post-Doctoral Fellow
Pedro Costa	Post-Doctoral Fellow
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Raquel Costa	MSc Student
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Medicinal Chemistry & Drug Discovery Group

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Microbiology of Extreme Environments Group

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Luís André A. França	PhD Student
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Medical Microbiology Group

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Molecular Mycobacteriology Group (*Emerging Group*)

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Molecular Biotechnology Group

Head: Carlos Faro

Objectives

Our group has a main interest on proteolytic enzymes and their role in regulating complex and highly dynamic protein cascades, networks and signaling pathways, in addition to their degradative function and biotechnological potential. Furthermore, we have been interested on the structural/biophysical characterization of neuronal proteins involved in human brain diseases. Also, activities have been developed on characterization of pollen proteases and their role on inflammatory and immunological response. Our research activities are subdivided into 4 focus areas:

Biochemistry, biology and biotechnology potential of plant aspartic proteases (APs)

Proteases exert critical roles in different plant developmental processes as well as stress responses. However, our understanding of this full protease web is still in its infancy for plant proteases. Identification of native substrates (degradomes), correlation of processing events with biological processes and a better understanding of structure-function relationships are, therefore, crucial tasks to understand the role of proteases in plant biology. Our work focuses on APs, the second largest class of plant proteases. Recent studies implicate APs as important players in developmental processes/stress responses. Based on the huge potential of system-wide proteomic approaches, our goal is to generate an integrated platform on proteases, their substrates, and their function - thereby enabling the elucidation of the biological roles of APs in plants. We aim to contribute for the development of a holistic system on plant AP networks.

Biochemistry and biology of prokaryotic aspartic proteases (APs) and their role as potential therapeutic targets in pathogenic Bacteria

The relevance of proteolytic events for bacterial pathogenicity and the progressive increase in antibiotic resistance among pathogenic bacteria contribute to positioning proteases as potential candidate targets for the development of alternative antibacterial strategies. The presence of APs of both pepsin and retropepsin-type in prokaryotes has always been a matter of debate and our work has provided the first unequivocal documentation of these types of activities in prokaryotes. Our goal is to generate an integrated platform for the discovery, characterization (biochemical/structural/functional) and evaluation of "targetability" of APs from different (pathogenic) bacteria. In this way, we aim to contribute for the development of a holistic system on prokaryotic (aspartic) protease networks.

Structural and biophysical characterization of neuronal proteins involved in human brain diseases

Through the study of the structure and the dynamics of interaction of neuronal proteins with either protein- (PPI) or carbohydrate-interactors (PCI), we aim at unravelling the role of these PPIs and PCIs on the molecular mechanisms underlying different neuronal diseases and further explore if/how these interactions can be eventually modulated to ameliorate disease states. Our focus is on the structural /biophysical characterization of the interaction of laforin (a human phosphatase) and carbohydrates, as this protein is involved in Lafora disease, a hereditary form of epilepsy; as well as on the detailed structural/interactomics' characterization of SAPAP3, a postsynaptic scaffolding protein, suggested to be involved in obsessive-compulsive disorder. Moreover, we pursued studies on natural inhibitors of BACE-1, a protease implicated in Alzheimer's disease.

The role of pollen proteases in allergic respiratory disorders.

Pollens are important triggers for allergic disorders. In the past we have established that pollen grains, with distinct allergenic abilities, release proteases that are able to compromise epithelium barrier integrity by disruption of transmembrane adhesion protein. On-going activities include purification and functional characterization of proteases to evaluated their contribution on immunologic and inflammatory response.

Main Achievements

1) **Biochemistry, biology and biotechnology potential of plant APs**

- We developed & optimized a eukaryotic-based expression platform for the PSI domain of cirsin in the GRAS yeast *K. lactis* and confirmed its antifungal activity (Curto *et al*, AEM, 2014; Q1 Biotechnology & Applied Microbiology).
- We developed a new cardosin B-derived rennet produced in the GRAS yeast *K. lactis* (named VRen). VRen's effectiveness in cheese production was demonstrated by manufacturing sheep, goat, and cow cheeses (Almeida *et al*, AMB, 2014;Q1 Biotechnology & Applied Microbiology).
- We pursued with the functional characterization of 2 atypical APs from Arabidopsis suggested to be involved in PCD. The work developed included: analysis of expression patterns, confirmation of homozygous T-DNA knockout lines for both genes; and phenotypic analyses of KO lines and comparison with WT plants. The results obtained thus far reveal a reduction in root growth for the KO lines.

2) **Biochemistry & biology of prokaryotic APs and their role as potential therapeutic targets in pathogenic Bacteria**

- We reported the characterization of a novel membrane embedded AP of the retropepsin-type (APRc), conserved in 51 Rickettsia genomes. APRc shares several enzymatic properties with retropepsins, and is expressed, at least, in 2 pathogenic species of *Rickettsia.* APRc's inhibition by specific HIV-protease inhibitors in vitro suggests that this protease may be a candidate for the development of new protease-targeted therapies (Cruz *at al*, Plos Pathogens, 2014;Q1 Microbiology, Parasitology and Virology). Reinforcing this, we demonstrated that addition of a specific HIV-1 protease inhibitor to R. conorii-infected cell cultures, results in a dramatic decrease in the ability of R.

conorii to bind to these cells and subsequently proliferate within them. Also, 3 different crystal forms of APRc's soluble domain were obtained by X-ray diffraction (at 2-Å, 2.45-Å and 2.59-Å resolutions), and, as expected, the fold of the APRc monomer resembles the canonical fold of retropepsins (manuscript in preparation).

3) Structural &biophysical characterization of neuronal proteins involved in human brain diseases

- We characterized BACE-1 inhibition by a monoterpene necrodane ketone, from the oil of *L. luisieri*. The compound displayed a dose-dependent inhibition of BACE-1 in cellular/mouse models of Alzheimer's disease and was capable of passing through cellular membranes and the BBB (Videira *et al*, JNP, 2014; Q1 Plant Sciences; Chemistry, Medicinal; Pharmacology & Pharmacy).
- We pursued with the biophysical characterization of laforin-carbohydrate interaction using soluble glycans. We demonstrate an increased preference of laforin for the interaction with glycans with higher order of polymerization. We confirmed the importance of Trp residues for glycan interaction, which occurs with a positive enthalpic contribution counterbalanced by a negative entropic contribution (manuscript in preparation).
- We used ESPRIT technology to identify SAPAP3 soluble domains in *E. coli*. 28 000 clones were initially screened. From here, 4 domains with higher yields of soluble protein were produced in larger scale. SAPAP3 domains are now being screened for crystallization conditions and also used in interactomics studies using isolated synaptoneurosomes.

4) The role of pollen proteases in allergic respiratory disorders.

- Serine and metalloproteases isolated from *C. album*, *P.judaica* and *P.sylvestris* were tested on Calu-3 cells grown in an air-liquid interface system. The disruption of intercellular complexes was identified using immunoblotting and immunofluorescence assays. PAR- 2 activation and subsequent interleukin release were monitored using single-cell imaging and flow cytometry, respectively. These proteases disrupted the several transmembrane adhesion proteins. Pollen proteases from *C. album* and *P.sylvestris* were capable of activating PAR-2. Additionally, all proteases increased the release of IL-6 and IL-8.

Computational and Systems Biology Group

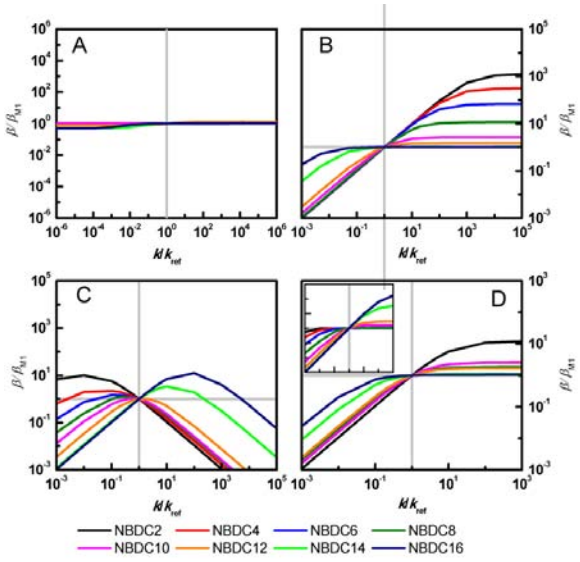
Head: Armindo Salvador

Objectives

Research at the Computational & Systems Biology Group is distributed by the following three research lines:

1. Organization principles of biochemical systems. The main goal of this research line is to discover, understand and exploit generic rules (organization principles) that hold across processes, cell types and organisms. We are focusing on (a) rules relating the design (i.e. naturally evolved molecular mechanisms) of biochemical networks to their function, and (b) explaining generic phenomena of cell physiology (e.g. growth laws, stress responses, hormesis) from fundamental principles of physical chemistry and evolution. We envisage that the network-structure / function relationships in (a) will play in biomedicine and bioengineering a role analogous to that of QSAR in pharmacology. With regards to (b) we are finding that some apparently complex phenomena represent optimal cellular responses under physical-chemical constraints that apply universally. Importantly, these phenomena can be predicted without a detailed knowledge of mechanisms, supporting the application of coarse-grained constraint-based models to help understand the considerations and trade-offs that shape cell fates.

Objects of interest in this research line are metabolic networks, stress responses and redox signaling. We are working towards exploring translational implications of these design/function relationships in degenerative diseases. In parallel, we are developing novel experimental (fluxomics and synthetic biology) methodologies to determine critical parameters in these applications.



2. Modeling the permeation through physiological barriers. The long-term goal of this research line is to develop quantitative structure-activity relationships (QSAR) for the permeation of physiological barriers by drugs, namely tight endothelia such as the blood-brain barrier (BBB). Failure to cross the BBB is the main factor of attrition in the development of psycho-active drugs, and is causing some of the main pharmaceutical corporations to abandon the development of such drugs altogether. The bioavailability of xenobiotics at the brain is strongly affected by their interaction with lipid bilayers and blood components (albumin, lipoproteins, erythrocytes and membranes of endothelial cells). Our work shows that the partition of drugs among the compartments strongly affects the timing and effectiveness of their permeation across the BBB, and that bioaccumulation may be limited by several distinct steps in the permeation pathway. We are working towards modeling how molecular features of the xenobiotics impact on the kinetics of these critical steps and to achieve better predictions of overall permeability.

3. Computational tools for biomolecular systems. The main goal of this research line is to develop effective computational tools to simulate and analyze complex biomolecular systems and reaction networks. Namely, in support of the activities of the research lines described above. Developments range from very fundamental computer-science methods that vastly speed-up numerical computation in a broad range of computational biology applications, to tools for characterizing the relationship between design and performance of biomolecular reaction networks.

Main Achievements

Fast-growing microorganisms are more susceptible to acute environmental stresses than slow-growing ones, but the reasons for this phenomenon are unclear. Using idealized models of self-replicating cells we found that it can be explained by the interplay among three fundamental principles: (a) maximization of growth rate, (b) unavoidability of damage to cellular components, and (c) growth-related damage dilution. Thus, under permissive condition high growth rates can effectively dilute damaged components, and the resource expenditure in defenses would decrease growth. In contrast, under conditions forbidding fast growth damaged components cannot be readily diluted, and growth is then maximized by a higher expression of defenses. As result, slow-growing cells become pre-adapted to acute environmental stresses. Over 2014 we parameterized the models based on experimental data for the bacterium *Escherichia coli* and demonstrated that the hypothesis and models can quantitatively describe multiple aspects of the growth and stress responses on this organism [Bolli & Salvador (2015), manuscript in preparation].

One great challenge in pharmacokinetics is to optimize the transport across cell barriers. We modeled the permeation of a homologous series of amphipatic molecules, 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD)-labeled alkyl chain amphiphiles (NBD-Cn, n=2-16) across of the BBB, to obtain rules that relate permeant structure to permeability. The amphiphile enters the system from the serum equilibrated with serum albumin and lipoproteins, and its sequestration by serum components, interaction with the endothelium, and accumulation in the tissue is followed over time. The

dependence of the permeability coefficient on the number of carbons of the amphiphile's alkyl chain has a parabolic-like shape. After a threshold value, an increase in the hydrophobicity of the amphiphile along the homologous series results in a decrease in the characteristic rate of permeation to the tissue. Sequestration in the serum and rate of desorption from the endothelium were the determinant processes for some amphiphiles, while for others translocation was the rate limiting step. For some amphiphiles several steps contributed significantly to the overall permeation. [Filipe *et al.* (2014). *Molec. Pharmacol.* 11:3696-3706]

In molecular dynamics (MD) simulations the calculation of nonbonded interactions is the major performance bottleneck. Processor architecture and how finely the simulation can be distributed across multiple processors/cores limit the speed of simulation. Simulations in the range of 10s of thousands of atoms will not run faster on a supercomputer than on a 64 core server. An algorithm was previously developed to provide a 'non-computational' approach followed by incremental computation to achieve significant improvements in performance. This algorithm was shown to determine nonbonded interaction results using Lennard-Jones with reaction field on the Intel Core i7 'Sandy Bridge' up to 14-15 times faster than the GROMACS 4.5.4. In 2014, the study performed over 170 μs of simulations on five amino acid side chain analogues to provide the basis for the analysis of statistical equivalence and superiority of the developed algorithm compared with a zone of equivalence and superiority defined using the three widely used builds of GROMACS 4.5.4. The study showed that the results from the developed algorithm were statistically equivalent and indistinguishable to GROMACS 4.5.4. Prior studies of these amino acid side chain analogues by others showed that the free energy results for these simple systems calculated using GROMACS were comparable to the experimentally determined values. This study provides strong evidence that the algorithm may be used in general purpose molecular dynamics simulations and for other computational purposes without causing side effects. The algorithm can also be applied to other general computational problems.

Vectors and Gene Therapy Group

Head: Mª Conceição P. Lima

Objectives

The research in the Group of Vectors and Gene Therapy has been devoted to the design and development of carriers, including viral and nonviral vectors, for nucleic acid and drug delivery aiming at their application as technological platforms for 1) establishment of disease models, 2) study of disease mechanisms and 3) development of new molecular therapeutic approaches for cancer and neurodegenerative disorders and of prophylactic strategies. Our studies on non-viral vectors have been mainly focused on the evaluation of the potential of novel lipid-based nanosystems and polymeric nanoparticles in gene therapy strategies for the treatment of both cancer and neurodegenerative disorders, and for the development of vaccines.

Non-viral vectors, such as cationic liposomes, stable nucleic acid lipid particles and cell-penetrating peptides have been explored as carrier systems to deliver nucleic acids, including plasmid DNA encoding therapeutic proteins, as well as antisense oligonucleotides, siRNAs and anti-miRNA locked nucleic acids, aiming at promoting silencing of known oncogene proteins and both cancer-related and pro-inflammatory miRNAs. The group is interested in investigating the anti-tumoral effect of gene therapy strategies, either *per se* or in combination with chemotherapeutic agents, both *in vitro* and in animal models for different types of cancer. A lipidomic approach to cancer has been developed using RNA interference to unravel the role of membrane lipids in cancer cell signaling and chemoresistance. In addition, non-viral vectors are currently being developed to study the role of miRNAs in neuroinflammation, aiming at promoting neuronal survival by targeting the inflammatory pathways associated with neurodegenerative diseases.

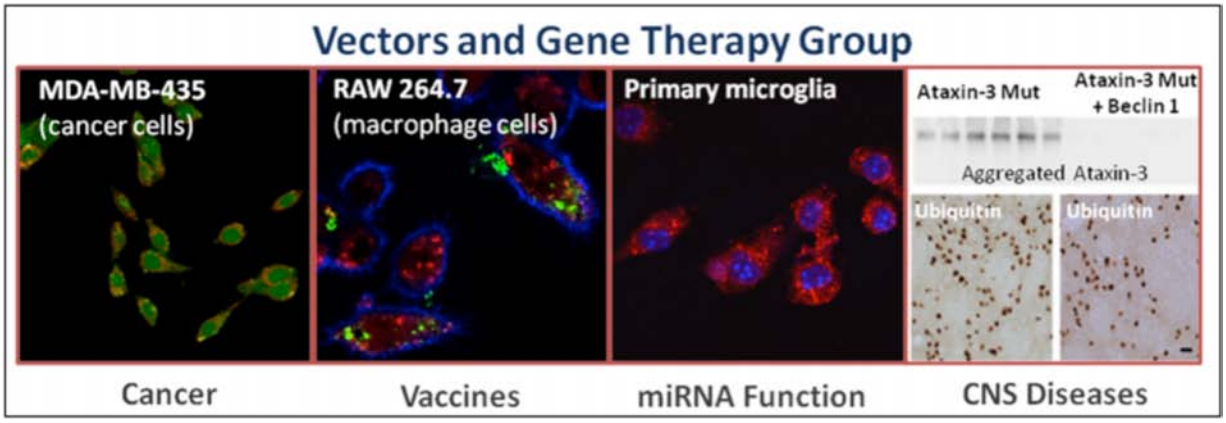
Fundamental research work addressing the development and physicochemical characterization of new nucleic acid delivery systems has also deserved the attention of our group. Research efforts have been developed to define through a biophysical approach the architecture parameters that endow vectors with the ability to transpose membranes and efficiently deliver their cargo into the cell.

In addition, the fact that tumor survival and proliferation are largely dependent on the microenvironment, represents an opportunity to engineer novel therapeutic strategies to address unmet medical needs, upon choosing more than one target from the pool of tumor–stroma interactions. Therefore, the study of the functional contribution of tumor microenvironment on cancer progression and metastasis, aiming at identifying novel therapeutic targets is becoming an emergent area of research in our group. This is aligned with the design and understanding of the mechanistic basis of non-viral carriers aiming at targeting drugs and nucleic acids to the tumor microenvironment, in orthotopic murine models of cancer. These lines of research have included a component of translational research, following the collaboration with the Portuguese Institute of Oncology from Coimbra and the Faculty of Medicine and the Hospital of the University of Coimbra. Viral vectors, particularly lentiviral and adeno-associated viruses are powerful technological platforms for gene delivery to the CNS, which we have been using for investigating the pathogenesis and modeling of neurodegenerative diseases, with a focus on Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD). This knowledge is expected to allow the generation of disease-modifying approaches for MJD therapy.

The group also addresses mucosal vaccination (oral and nasal) using antigens (protein or DNA) encapsulated in polymeric nanovectors, to target the lymphoid structures of the mucosal immune system. In this regard, new chitosan-based delivery systems able to simultaneously encapsulate antigens and an immunopotentiator (mast cell activator c48/80, aluminum compounds and exosomes) have been developed and tested (*in vitro* and *in vivo*) with the purpose of improving immune response modulation.

Main Achievements

Regarding non-viral-mediated gene delivery, an extensive screening of a variety of molecules (gemini surfactants, copolymers and cell penetrating peptides) for their capacity to produce efficient nucleic acid delivery systems has been carried out and structure-activity relationships, established. Several characteristics susceptible of modulation emerged as critical to improve vector performance, e.g. hydrocarbon chain length and spacer chemical nature in gemini surfactants; combinatory proportions of copolymer



components and the corresponding cloud point; amino-acid sequence, presence of specific amino acid residues (e.g. histidine) and acylation in cell penetrating peptides.

Regarding targeted cancer gene therapy, we have generated novel lipid-based systems exhibiting the ability to specifically and efficiently deliver DNA into hepatocellular carcinoma cells through its specific binding to the asialoglycoprotein receptor. A new antitumoral strategy was also developed involving silencing of the oncomir miR-21, overexpressed in glioblastoma (GBM), through delivery of anti-miRNA LNA oligonucleotides via tumor-targeted stabilized nucleic acid lipid particles (SNALPs) followed by cell exposure to sunitinib. We have shown that SNALP-mediated miR-21 silencing enhances the cytotoxic effect of sunitinib in different glioma cell lines and significant tumor accumulation of targeted SNALP-formulated anti-miR-21 oligonucleotides occurs upon systemic injection in an orthotopic mouse model of GBM, with minimal delivery to normal tissues. An enhancement of GBM cell susceptibility to chemotherapeutics was also obtained by modulating membrane lipid composition through the delivery of siRNAs addressing the activity of key-enzymes of lipid metabolism. Moreover, we have demonstrated that regulation of microRNA expression levels combined with low amounts of chemotherapeutic agents results in a significant and synergistic cell death effect in pancreatic cancer cell lines and primary culture models. We have also developed a novel ligand-mediated targeted lipid-based nanoplatform for the delivery of rationally designed drug combinations. It was demonstrated that the drug combination tested developed a synergistic interaction at a specific drug molar ratio. Additionally, our study suggested that the strategy of delivery may alter the nature of drug interaction, since liposomal targeted formulation, encapsulating an additive/mildly antagonistic drug combination, demonstrated singularly a cytotoxic effect above 90%, for an incubation period as short as 4 h. Overall, these results demonstrated that targeted intracellular delivery of different molar ratios of drug combinations, with diverse drug interactions, enabled a relevant increased efficacy against chemotherapy resistant cancer cells.

Regarding neurodegenerative diseases, we have generated lentiviral and adeno-associated viral vectors to study their pathogenesis focusing on Machado-Joseph disease/spinocerebellar ataxia type 3 (MJD). Development of lentiviral-based in vivo models of MJD, in which we are experts, allowed fruitful investigation of disease-modifying strategies involving gene silencing, autophagy activation, proteolysis inhibition and neural stem cell replacement. We have also investigated the contribution of immune-related miRNAs to innate immune response in the context of Alzheimer's disease (AD) and have identified specific miRNAs whose levels are deregulated in AD patients with respect to healthy controls. It is expected that these studies contribute to the finding of new therapies for these devastating disorders for which no effective therapy is available.

Regarding DNA-based vaccination, we clarified the adjuvanticity mechanisms of chitosan nanoparticles, which increased antigen nasal residence, induced the production of IL-1β by DC cells, via a NLRP3 inflammasome-dependent pathway and promoted mast cell activation. The *in vivo* immunogenicity of antigens was considerably increased.

Biomaterials and Stem Cell-Based Therapeutics Group

Head: Lino Ferreira

Objectives

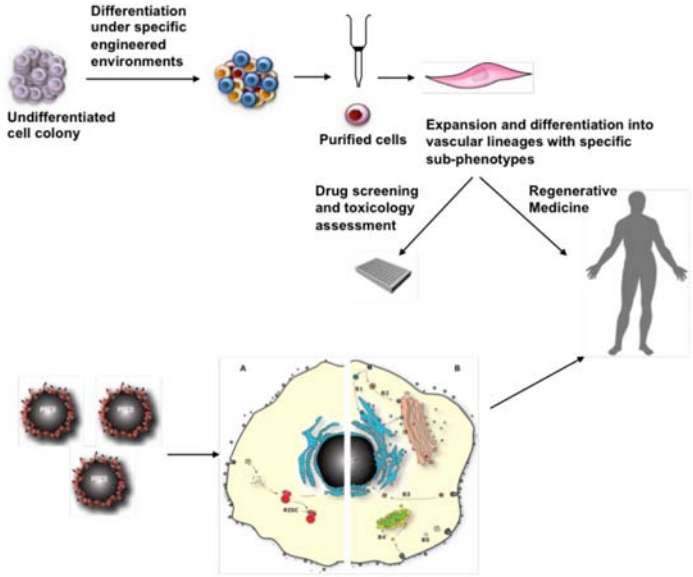
The research group has two main avenues of research: (i) development of bioengineering platforms to modulate the differentiation and maturation of stem cells, (ii) development of nanomedicine platforms to modulate the activity of stem cells and their progenies.

1- Bioengineering platforms to modulate the differentiation and maturation of stem cells. One of the main objectives of the research group is to develop biomaterials and bioengineering platforms for the efficient differentiation, maturation and engraftment of stem cells and their progenies (focus: cardiovascular lineages). We are primarily working with human pluripotent stem cells (induced pluripotent stem cells and human embryonic stem cells) and fetal hematopoietic stem cells (human cord blood). The group is developing scaffolds capable of retaining the cells at the desired location, while serving as a template for cell assembly, survival, differentiation and engraftment. The group is also designing biomaterials that provide several different types of information to stem cells, with the purpose of controlling their differentiation. New strategies based on topography and fluid shear stress to modulate the differentiation of mesoderm cells such as vascular cells and cardiomyocytes derived from human pluripotent stem cells are under development.

2- Nanomedicine platforms to modulate the activity of stem cells and their progenies. The development of a wide spectrum of nanotechnologies (referred as Nanomedicine by National Institutes of Health for applications in the biomedical area) during the last years are very promising for the study of stem cell biology and to control exogenous and endogenous stem cells for regenerative medicine. Our group is particularly interested to use these tools to induce *in vivo* stem cell differentiation and to mobilize stem cells from their niches to treat cardiovascular diseases. For this purpose, we are developing nanomaterials that release efficiently small molecules or non-coding RNA (miRNAs) to manipulate stem cells or their progenies.

The 2 avenues of research of the group target cardiovascular diseases. Cardiovascular diseases (CVDs), a group of disorders of the heart and blood vessels, are the number one cause of death globally. More people die annually from CVDs than from any other cause. Stem cells are an important source of cells for regenerative medicine applications. Several clinical trials are underway to investigate their therapeutic effects. Yet, it is of utmost importance to understand the bioactivity of stem cells and eventually to control it. The paracrine effect of stem cells remains to be elucidated as well as new platforms to improve stem cell survival after transplantation. Stem cells are also an important source of cardiac and vascular cells for drug screening and toxicological assessment. They can be an useful *in vitro* model to study specific diseases and to find new therapeutic targets.

The research group uses many approaches to answer research questions, including the design of new biomaterials with relevant biological information, molecular and cell biology, microfluidic systems, high content analysis, and animal experimentation. The group uses molecular analysis to identify signaling pathways that are activated when cells are exposed to specific signals or extracellular matrixes. The group has strong experience in the differentiation of pluripotent stem cells into vascular lineages.



Main Achievements

During the last year, the group has done progresses to address the following scientific questions: **(i) can we use stem cells to generate in vitro models of ageing and drug screening? (ii) can we develop nanomaterials to modulate stem cell niche?** To tackle the first question we have developed a novel blood vessel on a chip, combining vascular cells differentiated from induced pluripotent stem cells and microfluidic systems (manuscript submitted). We have differentiated human pluripotent stem cells into embryonic arterial endothelial cells (ECs), which were then cultivated under static or flow conditions to screen compounds that affect specifically embryonic vasculature. Using this platform, we have identified two compounds from a library of 1,200 chemical compounds that are toxic for embryonic ECs. The vascular toxicity of the compound was further validated in prenatal mouse ECs and in mice embryos. In a separate work, we have generated a human in vitro model of ageing based on induced pluripotent stem cells (iPSCs) derived from patients with Progeria. Progeria is a rare, progressive aging disease in children that leads to premature death. SMCs are the most affected cells in Progeria patients, although the reason for such sensitivity remains poorly understood. Therefore we have studied the reasons of Progeria-SMCs vulnerability using iPSCs obtained from Progeria

fibroblast patients (manuscript in preparation). SMCs differentiated from HGPS-iPSCs showed impaired maturation as confirmed by a low expression of calponin and SMMHC genes and individualized calponin fibers. HGPS-iPSC SMCs shared similar features observed on progerin-expressing cells such as activation of several effectors of NOTCH signaling pathway and response to farnesyltransferase inhibitors. When HGPS-iPSC SMCs are cultured under arterial flow conditions they show an up-regulation of progeria and osteogenic markers followed by their detachment from the culture substrate. This finding opens new opportunities for the treatment of HGPS disease and diseases related to vascular ageing.

To tackle the second question we have synthesized new advanced nanomaterials. Acute myeloid leukemia (AML) is a group of heterogeneous diseases defined morphologically by an abnormal increase in myeloblasts in the bone marrow (BM). Current therapies include the use of all trans retinoic acid (RA) to induce the differentiation of leukemic cells. Unfortunately, the efficiency of the process is limited, even if coupled with chemotherapy, since patients can relapse after remission (25% of the patients actually die). This relapse is likely related to an induction of RA-binding protein and increased RA metabolism by cytochrome P-450-mediated reactions. In addition, one-third of patients develop the so-called “RA syndrome” characterized by dyspnea, fever, weight gain and hypotension. In the last years, RA delivery formulations have been proposed to overcome these unwanted effects and some of them evaluated in human clinical trials. A lipossomic RA delivery system has been shown to improve the pharmacokinetic profile of RA as compared to a non lipossomal formulation and maintained higher and sustained plasma drug concentrations. Further, the liposomal formulation decreased the probability of relapse after remission but not eliminate it. This might due to the survival of leukemic stem cells in the bone marrow niche. Recently (manuscript in preparation), we have developed light-activatable nanoparticles to improve the intracellular delivery of RA in leukemic cells. The possibility of remotely activating the drug delivery system opens new therapeutic opportunities due to the possibility of activating the differentiation of the cells at the bone marrow niche and potentially interfering with the leukemic stem cell niche.

During 2014, the group has filled 1 patent, published 6 publications in international journals (being 2 publications in journals with impact factor above 7) and submitted 12 publications. The group has attracted additional funding from FCT (“CARDIOSTEM- Engineered cardiac tissues and stem cell-based therapies for cardiovascular applications”, Project reference: MITP-TB/ECE/0013/2013). In addition, one PhD student has defended his PhD thesis during 2014.

Pharmacometrics Group

Head: Amílcar Celta Falcão

Objectives

The principal aim of the Pharmacometrics Group is to early predict the kinetics of drug candidates since this area is regarded as one of the major reasons for the failure of new drug candidates *in vivo*. Thus, in silico, in vitro and in vivo techniques are carried out in order to characterize the bioavailability and biodisposition of new therapeutic drugs, evaluating the concentration of parent compounds and active metabolites in plasma and tissues (including liver, kidney, brain, etc). Specifically considering the central nervous system (CNS) drug discovery programs, the limited permeability of the blood-brain barrier, which mainly restricts the entry of various substances into the brain, remains the bottleneck of a fast and successful development of CNS-acting drugs. Drugs and drug candidates that act at the Central Nervous System, including antiepileptic drugs and antiparkinsonian drugs, remain particularly under investigation within our group.

Thus, similarly to the *in vitro* methodologies developed within our research group in 2012 to estimate drug human fraction absorption, the plasma protein binding and identify substrates of P-glycoprotein, we are presently trying to develop in silico / in vitro models to foresee the ability of new therapeutic compounds to reach the brain. Moreover, a new strategy involving the intranasal administration of drugs is being employed attempting to directly deliver therapeutic agents into the brain. Indeed, the olfactory region is the only site in the human body where the nervous system is in direct contact with the surrounding environment, providing a great opportunity for drugs administered by the intranasal route to be directly transported into the CSF and brain, circumventing the BBB. Thus, one of the main objectives of the Pharmacometrics Group during the year of 2014 consisted on assessing the pharmacokinetics of the typical antiepileptic drug under investigation within our research group, carbamazepine, after intranasal and intravenous administrations in order to investigate whether a direct transport of the drug from nose to brain may be involved.

Moreover, the *in vitro* and non-clinical *in vivo* pharmacokinetic/pharmacodynamic studies are being performed to evaluate the new candidate drug for Parkinson's disease, Opicapone.

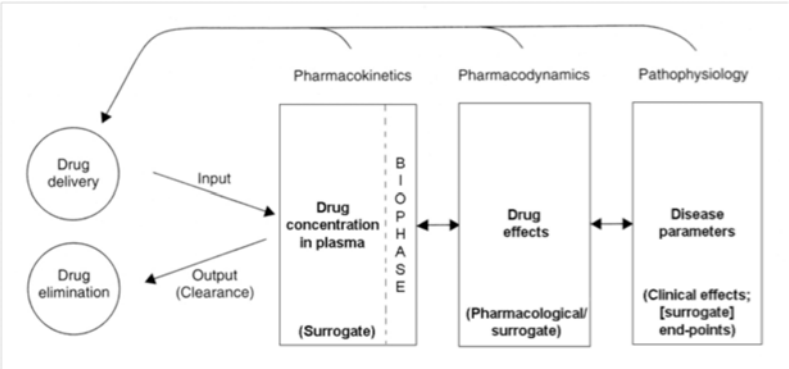
Main Achievements

In vivo intranasal and intravenous administrations of carbamazepine revealed similar pharmacokinetic profiles in plasma, brain (biophase) and liver matrices, indicating that, after intranasal delivery, carbamazepine reaches quickly and extensively the bloodstream, achieving the brain predominantly via systemic circulation. However, the brain biodisposition studies revealed higher concentrations in the olfactory bulb and frontal cortex following intranasal instillation, and a homogenous brain distribution pattern after intravenous injection, suggesting the involvement of a direct transport of carbamazepine from nose to brain. This proof-of-concept is currently encouraging us employ this novel strategy to other CNS-acting drugs.

Since pharmacometric analysis of other CNS-acting drugs is predicted to be performed in a near future, the development and full validation of bioanalytical methodologies are being tested in order to quantify the compounds in relevant biological samples by HPLC. Indeed, the increasing know-how of Pharmacometric Group in the field of bioanalysis of biological samples during pharmacokinetic/pharmacodynamic *in vivo* studies is internationally well-recognized and it is supporting other investigations.

It is also important to highlight that our expertise in *in vivo* studies and pharmacokinetic analysis remain allowing us to find relevant *in vivo* drug-drug interactions between herbal extracts and narrow therapeutic index drugs.

The contribution of the Pharmacometric group is also evidenced by the pharmacokinetic analysis carried out during the clinical studies performed for those compounds. This information is extremely important as Pharmacometrics aims to assess quantitatively the pharmacokinetics and pharmacodynamics of drugs, using data from various phases of drug development which are then linked together and quantitatively related to each other.



Medicinal Chemistry & Drug Discovery Group

Head: Maria Luísa Sá e Melo

Objectives

1.Steroids comprise a wide range of structurally related compounds with important functions *in vivo* and have shown a great therapeutic value due to anticancer, antiviral and antimicrobial activities. Our group has generated a library of oxysterols with a vast array of structural variations and diverse biological activities, including the ability to increase the sensitivity of tumor cells to other chemotherapeutic agents. More recently, these oxysterols have been screened for antiplasmodial activity against *P. falciparum* W2 (chloroquine resistance) and some of them presented low micromolar IC_{50} values. The found antimalarial activity is very representative of their rich structural molecular diversity (unpublished results *in coll* with Malaria Group, iMed). Knowing that drug resistance requires new drugs, the design of hybrid antimalarials, based on the most potent oxysterol scaffolds and on stable tetraoxanes, synthetic analogues of artemisin, has been studied. With this in mind and aware of the importance of multitarget therapies in malaria as a promising approach to circumvent drug resistance, their syntheses have been planned.

2.Pentacyclic triterpenoids are a class of pharmacologically active and structurally rich natural products with privileged motifs for further modifications and SAR analyses. We focused on the the anti-Leishmania activity of semisynthetic lupane triterpenoids derivatives of betulin and betulinic acid. Effects on the cell cycle, apoptosis/necrosis events, morphology and DNA integrity were also planned.

3.The understanding of the GPR30 receptor, concerning specific ligands, their structure and type of action, *in vitro* and *in vivo*, is another aim. Through SAR studies we will search for more effective ligands and will explore the selective modifications on the estradiol scaffold and relative binding affinity of each compound towards the nuclear and membrane-associated ERs. *In vitro* pharmacologic approaches and selective assays in cell lines differentially expressing those receptors will be done. From SAR studies, information about the receptor will be incorporated into a 3D model of GPR30 to direct future syntheses.

4.Antimicrobial resistance is an important problem of public health. The development of new antibiotics has slowed down in recent years and new strategies and potential antimicrobial compounds are needed to fight resistant infections. We aim to characterize the molecular antimicrobial resistance mechanisms, to unravel the mechanisms that facilitate the horizontal movement of antimicrobial resistance genes that lead to adaptation to pharmaceuticals and to understand the molecular epidemiology of bacterial strains. This is an important step to control antibiotic resistance and to prevent further spread of antimicrobial resistance. Furthermore, we aim to discover potential antimicrobials compounds and to design new alternative drugs. New semi-synthetic triterpenoids molecules were developed showing promising antibacterial activity. Additional in vitro and in vivo tests will be performed to unravel the mechanism of action and its cell toxicity.

The research activities of the group are supported by the following expertise:

a) Computational approaches in drug discovery: 4D (pocket ensemble) molecular docking; pharmacophore- and structure-based drug design; virtual screening; focused library design based on hit and target.

b) Synthesis in drug discovery: asymmetric synthesis for chiral drugs; biocatalysis; chemo-enzymatic methods; clean processes.

c) Biological evaluation *in vitro*.

d) Analysis of structure-activity relationships (SAR) to predict potency and improve “hits” to “lead candidates” by optimizing their selectivity against the target and pharmacokinetics.

e) To test in vitro antimicrobial activity

f) Molecular biology: molecular characterization of resistance genes and genetic support; evaluation of horizontal gene transfer (conjugation and natural tansformation) and molecular bacterial epidemiology

g) Biologic evaluation of new compounds

Main Achievements

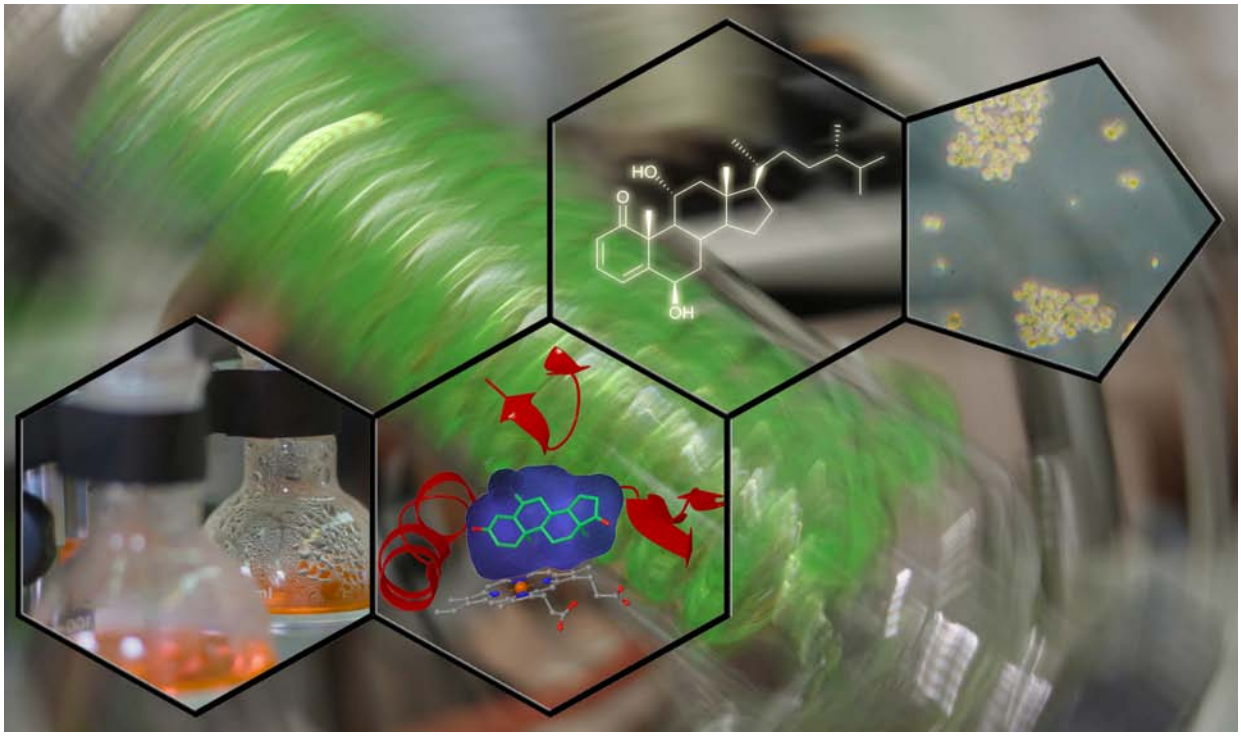
1.To pursue the aim to prepare new hybrid antimalarials based on the most potent oxysterol scaffolds extracted from our study on antiplasmodial activity against *P. falciparum* W2 (chloroquine resistance) and stable tetraoxanes, synthetic analogues of artemisin, have been performed. Different synthetic methodologies and chemical processes were studied to obtain four new chemical hybrid entities. One of our synthetic methods, still under way, is the development of a green chemistry approach, which makes use of a cheap and non-toxic metal catalyst to produce new oxysterol tetraoxane chemotypes, for antimalarial drug discovery, with the ability to circumvent drug resistance.

2.The anti-Leishmania activity of sixteen semisynthetic lupane triterpenoids derivatives of betulin and betulinic acid were evaluated. Drug interactions between the active compounds and one current antileishmanial drug, miltefosine, were assessed using the fixed ratio isobologram method. In addition, effects on the cell cycle, apoptosis/necrosis events, morphology and DNA integrity were studied. The derivatives (3b-Hydroxy-(20R)-lupan-29-oxo-28-yl-1H-imidazole-1-carboxylate) and (28-(1H-imidazole-1-yl)-3,28-dioxo-lup-1,20(29)-dien-2-yl-1H-imidazole-1-carboxylate) were found to be the most active,

with IC50 values of 50.8 mM and 25.8 mM, respectively (PLOS ONE, 2014, 9 (3), e89939).

3.The work developed has been focused on the evaluation of the role of the GPR30 as mediator of non-genomic answers, induced by estrogens, as 17-beta-estradiol, and by the specific agonist G-1, in diferente types of cells. The expression of this receptor in the human cancer cell lines, T47-D and HepG2, and the endotelial cell line, BAEC, has been evaluated. The same type of study has been performed in previously synthesised compounds (unpublished results). Furthermore, the evaluation of the potential activity imunomodulator and antiproliferative of steroids and non-steroids has been started, with the aim to establish structure - activity relationships and to clarify the role of the GPR30 in these effects.

4.The antimicrobial susceptibility and the mechanisms of resistance were characterized in bacteria of clinical and animal origin showing the resistance gene transfer among different strains. Moreover, the finding of virulence traits of *Salmonella enterica* serovar Typhi were found in a S. Typhimurium isolated from poultry, demonstrating the transfer of the cdt Typhi toxin. Preliminary work on natural transformation with clinical *Acinetobacter baumannii* isolates as recipient, revealed the ability of some isolates to develop competence and produce transformants showing a new way of acquisition of resistance genes. Preliminary work showed the antimicrobial activity of semi-synthetics derivatives of ursolic acid against Gram-positive bacteria.



Microbiology of Extreme Environments Group

Head: Milton Costa

Objectives

The objectives for 2014 were:

1. Continued studies on the mechanisms involved in stress adaptation of thermophilic, halophilic and desiccation-resistant bacteria and also in members of the *Planctomycetes*, an unusual deep-rooted lineage of bacteria.

2. To identify new compatible solutes and elucidate their biosynthetic pathways and their role in stress tolerance.

3. To isolate and characterize novel organisms from extreme environments for basic studies and for their biotechnological potential, namely the Deep Mediterranean Sea (about 5.000 meters) and the Deep Atlantic Ocean (3.000 to 4.000 meters(.

4. The study the biodiversity of the brine and brine-seawater interface of Deep Mediterranean Brine Lake Medee, with high sodium and chloride levels to obtain enzymes of biotechnology value.

5. To determine the genetic structure and allelic diversity of *L. pneumophila* populations inferred from virulence-related genes and to identify the molecular mechanisms operating in the evolution of these genes.

6.-To construct a metagenomic clone library derived from the microbial populations associated with the digestive system (comprising the stomach, hindgut and hepatopancreas) of Porcellio dilatatus (Crustacea:Isopoda). The library will be screened to identify clones with xylanase, chitinase, cellulase and amylase activity.

7. To compare the microbial diversity of several continental serpentinization-driven deep aquifers.

Main Achievements

During 2014:

1. We have completed the genome sequence of *Dehalogenimonas lykanthroporepellens* type strain (BL-DC-9) and the complete genome sequence of *Halorhabdus tiamateia* from a Deep Mediterranean brine

2. We completed the genome sequence *Rubrobacter radiotolerans*.

3. We have isolated and characterized several novel bacterial species such as, *Natrinema salacieae*, *Heliimonas saccharivorans, Rhodopirellula lusitana* and *Rhodopirellula rubra*, *Palleronia abyssalis*, *Rubrobacter calidifluminis* and *Rubrobacter naiadicus*.

4. We completed a complex but not stable autochthonous community structure of groundwater samples between different replicas. We observed that the bottling procedures and storage time induced profound modifications on groundwater diversity. A high diverse bacterial composition and low archaeal diversity were detected in groundwater and in bottled water samples. The majority of the sequences collected from groundwater were from autotrophic populations, mainly Gram-negative organisms. On the other hand, bottle environments were dominated by Gram-negative heterotrophic organisms.

5. We have described a new bacterial hydrolase specific for the compatible solutes α-D-mannopyranosyl-(1→2)-D-glycerate and α-D-glucopyranosyl-(1→2)-D-glycerate.

6. We construct a 17 000 clone library based on the microbiome of Porcellio dilatatus (Crustacea:Isopoda) digestive system. Partial screening of the library was alredy performed and several clones with xylanase, chitinase and cellulase activity were identified.

7. We determined the digestive system microbiome of several distinct populations of Porcellio dilatatus (Crustacea:Isopoda).

8. We produced metagenomic data to determine the structural and functional microbial diversity of diverse continental serpentinization-driven deep environments.

Medical Mycology – Yeast Research Group

Head: Teresa Gonçalves

Objectives

A. *Alternaria infectoria* an opportunistic agent of human infection and of severe allergies"

Objectives 2014:

1. Characterization of the macrophage response to *in vitro* infection by *A. infectoria* spores

2. Hyphal cell wall nanoparticles

3. the evaluation of the morphological and molecular alterations in *Alternaria infectoria* during exposure to caspofungin and nikkomycin, a β-glucan and a chitin synthesis inhibitor, respectively. Moreover, we aimed at the identification of a potential synergistic effect between chitin synthase, β-glucan synthetase and DHN-melanin synthesis inhibitors.

B. Role of adenosine and adenosine receptors in *Candida albicans* infection

Objectives 2014:

1. Involvement of adenosine and adenosine A_{2A} receptor in *C albicans* infection

2. *C. albicans* infection of A_{2A} knockout mice peritoneal macrophages

3. Differential gut infection of C. albicans in aged mice. Involvement of A_{2A} receptors

4. Characterisation of ectophosphatases and ectonucleotidase activity of *C. albicans*.

C. Validation of Chromogenic media for the identification of pathogenic yeasts of parapailosis group

D. HIV-1 Vpr variants impact in AIDS progression and in variation of genes involved in fungal infection recognition (Interinstitutional)

1. Patients blood sample colection and HIV-VPR sequencing

E. National Natural Resources antimicrobial potential

Initial screnning of the antifungal and antiviral potential of national natural resources, such as from extracts obtained from the algae and olives

Main Achievements

A. *Alternaria infectoria* an opportunistic agent of human infection and of severe allergies

1. Susceptibility to caspofungin and nikkomycin Z. Collaboration with Professor Neil Gow of the Institute of Medical Sciences of Aberdeen, UK.

Papers

C Fernandes, J Anjos, LA Walker, BMA Silva, L Cortes, M Mota, CA Munro, NAR Gow, T Gonçalves (2014). Modulation of *Alternaria infectoria* cell wall chitin and glucan synthesis by cell wall synthase inhibitors. Submitted to Antimicrobial Agents and Chemotherapy.

Gene sequences deposited in the NCBI database:

Accession numbers JX436211 to JX436224, JX443517, and JX443518

2. DHN-melanin production and antifungal efficency in *A. infectoria*

Paper under submission process:

Fernandes C, Prados-Rosales R, Silva BMA, Nakouzi-Naranjo A, Chatterjee S, Stark RE, Eusébio E, Casadevall A, Gonçalves T. DHN-melanin characterization and biosynthesis in *Alternaria infectoria*.

3. We also evaluated how macrophages respond to *A. infectoria* conidia and observed the absence of inflammation hallmarks.

Cruz-Almeida M, Silva B, Rodrigues L, Abrunheiro A, Mota M, Cortes L and Gonçalves T. Characterization of the macrophage response to Alternaria infectoria conidia infection. Submitted Medical Mycology

B. Role of adenosine and adenosine receptors in *C. albicans* infection

1. During 2013 we continued tackling the involvement of purines and of the adenosine A2A receptor in *C. albicans* infection of macrophages.

MS under submission process to mBio

L Rodrigues; F Curado; C Coelho; V Cabral; L Cortes, RA. Cunha; T Gonçalves. INVOLVMENT OF ADENOSINE A2A RECEPTORS IN MACROPHAGE INFECTION BY *CANDIDA ALBICANS*.

Characterisation of ectophosphatases and ectonucleotidase activity of *C. albicans*.

Rodrigues L, Russo-Abrahão T, Cunha RA, Gonçalves T, and Meyer-Fernandes JR (2015b). Characterisation of extracellular nucleotide metabolism in Candida albicans. Submitted to *Journal of Biological Chemistry*

Prototype of kit for the identification of *Candida parapsilosis* group

Molecular Mycobacteriology - *Emerging Group*

Head: Nuno Empadinhas

Objectives

Antimicrobial resistance is widespread worldwide and it is estimated that, in a near future, most of the currently available antibiotics will no longer be effective. The numbers of multidrug-resistant pathogens circulating in hospitals worsen this scenario. Efforts between universities and the pharmaceutical industry are urgent to tackle this serious health threat.

Our group is focused on mycobacterial pathogens such as M. tuberculosis and M. leprae, the agents of tuberculosis (TB) and leprosy, diseases known to man for millennia. In addition to these, there are over 160 species of nontuberculous mycobacteria (NTM), which include multidrug-resistant pathogens established in our water distribution systems that cause life-threatening infections in patients with chronic diseases, with immune fragilities, and in the elderly. Unlike TB, NTM are already a growing epidemic in developed countries.

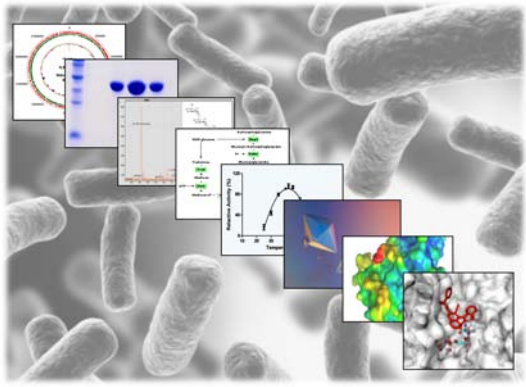
Despite decades of research, many metabolic pathways in mycobacterial pathogens remain enigmatic, which protracts the path toward new therapies. We have identified the genes for the early steps for biosynthesis of methylglucose lipopolysaccharides (MGLP), intracellular structures regulating fatty acids metabolism. We aim at the comprehensive elucidation of this intricate pathway. We are also interested in identifying the host targets of a secreted M. tuberculosis protease, which we propose to be critical for translocation of this pathogen across the blood-brain-barrier leading to central nervous system tuberculosis (CNS-TB).

Our funding perspectives include partnership with the pharmaceutical industry (drug screening) and with chemical companies (distribution of rare mycobacterial metabolites), which we have made available for research needs. We have an agreement with a one such company to screen an antitubercular compounds library for inhibitors of 2 target enzymes.

As a longer-term strategy we will explore microbe-gut-metabolism and microbe-gut-brain links in different pathologies. We have assembled a multidisciplinary team to probe microbiome-brain links in Parkinson's disease (PD) and will look for specific microbial taxa and also establish cellular/animal models to validate our microbe-PD hypothesis.

In 2014 we have joined a 15-partner consortium in the scope of a H2020-PHC2015 grant application "Find2Care - Fluorescence Imaging Assisted Infection Diagnostics in Diabetic Foot Care", which reached the 2nd stage with final decision expected for 2015. Our group will coordinate WP1 "Diabetic ulcer microbiome profiling". Diabetic patients selected by healthcare providers at APDP (Diabetics Association) and at ESEnfC (Nursing School of Coimbra) will be sampled for microbiome and metatranscriptome profiling to understand their dynamics towards development of highly sensitive probes for early detection of infection by innovative fluorescence-based technology to be engineered within the project. The ultimate goal is to prevent chronic wound progression and amputations.

Threats: The difficulty to attract and fix key students/researchers to longer-term projects and poor predictability in national funds and imprudent government scientific policy are major obstacles for the effective implementation of the plans described above. Our Group's objectives have been strategically aligned with some major priorities of "Horizon 2020": Antimicrobial resistance, infectious diseases, chronic and ageing-related disorders.



Main Achievements

Our ability to explore a wide range of microbiology research topics (microbial metabolism, genomics, antimicrobial susceptibility, microbiome dynamics) is the result of an expanding network of collaborations (medical microbiologists, bioinformaticians, neurologists, cell biologists, chemists and crystallographers) within and outside CNC. It reflects an ongoing effort to combine our know-how with priority H2020 research areas. In a recent past we have been able to attract funding from public (FCT) and private (Mizutani Foundation for Glycoscience, Institute Piaget) agencies or from Entrepreneurship programs (InovC 2014).

In 2014 we have identified additional genes and characterized the enzymes involved in the mycobacterial MGLP pathway and, in collaboration with crystallographers, we deciphered the three-dimensional structures of essential enzymes, establishing experimental scaffolds for drug screening and design. The planned enzyme inhibition trials, microbial viability assays, and pharma/toxicological profiling in cell/animal models will benefit from the know-how assembled at the "New Preventive and Therapeutic Strategies" area at CNC.

We have filed 2 provisional patent applications for exploitation of a new enzyme target in drug discovery trials and for a new method for the synthesis of a rare phosphorylated MGLP intermediate from M. tuberculosis. In collaboration with chemists we developed a new chemical synthesis method for MGLP acylated intermediates, also to be patented in 2015.

Agreements with pharmaceutical companies for drug screening with TB targets, a recently established collaboration with a group with access to a large cohort of PD patients' gut microbiomes, a new collaboration with Fiocruz on emerging NTM infections, and the participation in a European consortium as the leading partner of the microbiome profiling workpackage of a H2020-PHC2015 action, are among our recent strategic achievements to seek new important research developments in the scope of our present and future projects.

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Metabolism, age and Disease thematic line

Coordinator: João Ramalho Santos

The Metabolism, Aging and Disease (MAD) strand includes Research Groups or varying size and structure, from relatively small groups with only one PI and few research lines (Biology of Reproduction and Stem Cells, Intermediary Metabolism, Obesity Diabetes and complications), to larger groups with several PIs and research lines that are clustered along common goals (ImmunoMetabolic Pharmacology, Mitochondria, Metabolism and Disease; Cell Metabolism and Quality Control).

The main goal of all groups is to carry out research on the metabolic aspects of human disorders, and notably on those disorders that have clear metabolic origins, and how they may be interlinked. Researchers in this Strand carry out basic research on metabolic pathways and mitochondrial function, but also aim to perform translational research with relevant models (cell cultures, animal models, human samples) in order to address distinct issues, from novel diagnostic tools to possible therapeutic interventions.

One crucial main achievement is that the Groups have retained a reasonable amount of funding in the past years, and have been active in tapping wide variety of funding sources, both National and International, Public and Private, Academic and Enterprises (including several service contracts).

In terms of research there are three main focuses that are worthy of attention: 1- Using mitochondrial activity to characterize and possibly treat several disorders; 2- Developing novel metabolic-based diagnostic tools; 3- Studying the role of metabolism in defining immunological and inflammation responses in several conditions.

1- Researchers in this strand have provided clear links between several aspects of mitochondrial (dys)function and the possibility of predicting/controlling cancer and stem cell fate, or in terms the development and progression of several Neurodegenerative disorders (Alzheimers, Parkinsons), as well as diabetes, cancer and infertility.

2- The development of several diagnostic tools that can allow for the characterization of metabolic fluxes and metabolic status in several types of disorders (Diabetes, fatty liver disease, male and female infertility immunology-based disorders) has also been a staple of this Strand. In some cases using non-invasive NMR-based methodologies, which are currently being moved into more extensive patient studies.

3- The metabolic basis of the immune response and inflammation and how it can contribute to the management of rheumatoid arthritis, ostheoarthritis, allergies, diabetic wound healing or transplantation have also been studied, in all cases with close clinical collaborations that we hope will accelerate translational impact.

Biology of Reproduction, and Stem Cells Group

João Ramalho de S. Santos	PhD – *head of group*
Ana Paula Sousa	PhD
Mª Alexandra Amaral	Post-Doctoral Fellow
Sandra Amaral	Post-Doctoral Fellow
Paula Mota	Post-Doctoral Fellow
Sandro Pereira	Post-Doctoral Fellow
Ana Sofia Rodrigues	PhD Student
Ângela Crespo	PhD Student
Beatriz Sousa	PhD Student
Carla Paiva	PhD Student
Marcelo Correia	PhD Student
Mª Inês Almeida Sousa	PhD Student
Marília Cordeiro	PhD Student
Rodrigo Santos	PhD Student
Tânia Perestrelo	PhD Student
Alexandra Carvalho	MSc Student
Joana Portela	MSc Student
Marcelo Ribeiro	MSc Student
Sara Rebelo	MSc Student
Solange Machado	MSc Student
Marta Baptista	Grant Technician
Renata Tavares	Grant Technician

Cell Metabolism and Quality Control Group

Paula Moreira	PhD – *head of group*
Alexandrina Mendes	PhD
Cláudia Mª F. Pereira	PhD
Sandra Cardoso	PhD
Teresa Cruz Rosete	PhD
Ana Isabel Duarte	Post-Doctoral Fellow
Ana Raquel Esteves	Post-Doctoral Fellow
Ana Silva	Post-Doctoral Fellow
Diana F.F. Silva	Post-Doctoral Fellow
Rosa M. Matos Resende	Post-Doctoral Fellow
Sofia Guedes	Post-Doctoral Fellow
Sónia Correia	Post-Doctoral Fellow
Susana Cardoso	Post-Doctoral Fellow
Ana Catarina Fonseca	PhD Student
Ana Teresa Rufino	PhD Student
Ana Plácido	PhD Student
Cátia Sousa	PhD Student
Emanuel Candeias	PhD Student
Joana Liberal	PhD Student
João Martins	PhD Student
João Pedro Oliveira	PhD Student
Júlia Valente	PhD Student
Maria Fernandes	PhD Student
Renato Xavier Santos	PhD Student
Catarina Xavier	MSc Student
Guilherme Loureiro	MSc Student
Inês Sebastião	MSc Student
Leisa Évora	MSc Student
Marcelo Catarino	MSc Student
Rafael Paiva	MSc Student
Rui Gomes	MSc Student
Rui Simões	MSc Student
Vanessa Rodrigues	MSc Student
Cristina Carvalho	Grant Technician
Isabel Ferreira	Grant Technician
Joana Sousa	Grant Technician
João Ferreira	Grant Technician

Mitochondria, Metabolism and Disease Group

Paulo Jorge Oliveira	PhD – h*ead of group*
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Carlos Palmeira	PhD
José Custódio	PhD
Maria Carmen Alpoim	PhD
Maria Sancha Santos	PhD
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Carlos Rodrigues	Post-Doctoral Fellow
Filipe Valente Duarte	Post-Doctoral Fellow
João Paulo Teodoro	Post-Doctoral Fellow
Mª Teresa Cunha Oliveira	Post-Doctoral Fellow
Mariana Ponte Ribeiro	Post-Doctoral Fellow
Teresa Serafim	Post-Doctoral Fellow
Vilma Sardão Oliveira	Post-Doctoral Fellow
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Inês Barbosa	PhD student
Filipa Carvalho	PhD student
Katia Mesquita	PhD student

Nuno Gabriel Machado	PhD student
Susana Pereira	PhD student
Ana Marta Silva	MSc Student
Ana Raquel Coelho	MSc Student
João Filipe Amorim	MSc Student
Renata Couto	MSc Student
Silvia Magalhães	MSc Student
Claudia Deus	Grant Technician
Mariana Val	Grant Technician
Tatiana Martins	Grant Technician

Obesity Diabetes and Complications Group

Eugénia Carvalho	PhD – *head of group*
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Ermelindo Leal	Post-Doctoral Fellow
Susana Guerreiro	Post-Doctoral Fellow
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Ana Tellechea	PhD Student
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Roksana Pirzgalska	PhD Student
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Ana Rita Rocha	Msc Student
Fabio Carvalho	Msc Student
Manuela Cerqueira	Msc Student
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ImmunoMetabolic Pharmacology Group

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Fernando Nogueira	PhD
Rui Carvalho	PhD
Ana Filipa Henriques	Post-Doctoral Fellow
Helena Mª Carvalheiro	Post-Doctoral Fellow
Ana Romero	PhD Student
Ludgero Tavares	PhD Student
Mónica Teresa P. Abreu	PhD Student
Tiago R. Sousa	PhD Student
Ana Xavier	PhD Student
Fábio Paiva	Grant Technician

Intermediary Metabolism Group

John Jones	PhD – *head of group*
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João Duarte	PhD student
João Rito	PhD student
João Silva	PhD student
Catia Marques	MSc student
Paula da Silva	MSc student
Cristina Barosa, PhD	Grant Technician
Filipa Simões	Grant Technician
Margarida Coelho	Grant Technician

Biology of Reproduction, and Stem Cells Group

Head: João Ramalho-Santos

Objectives

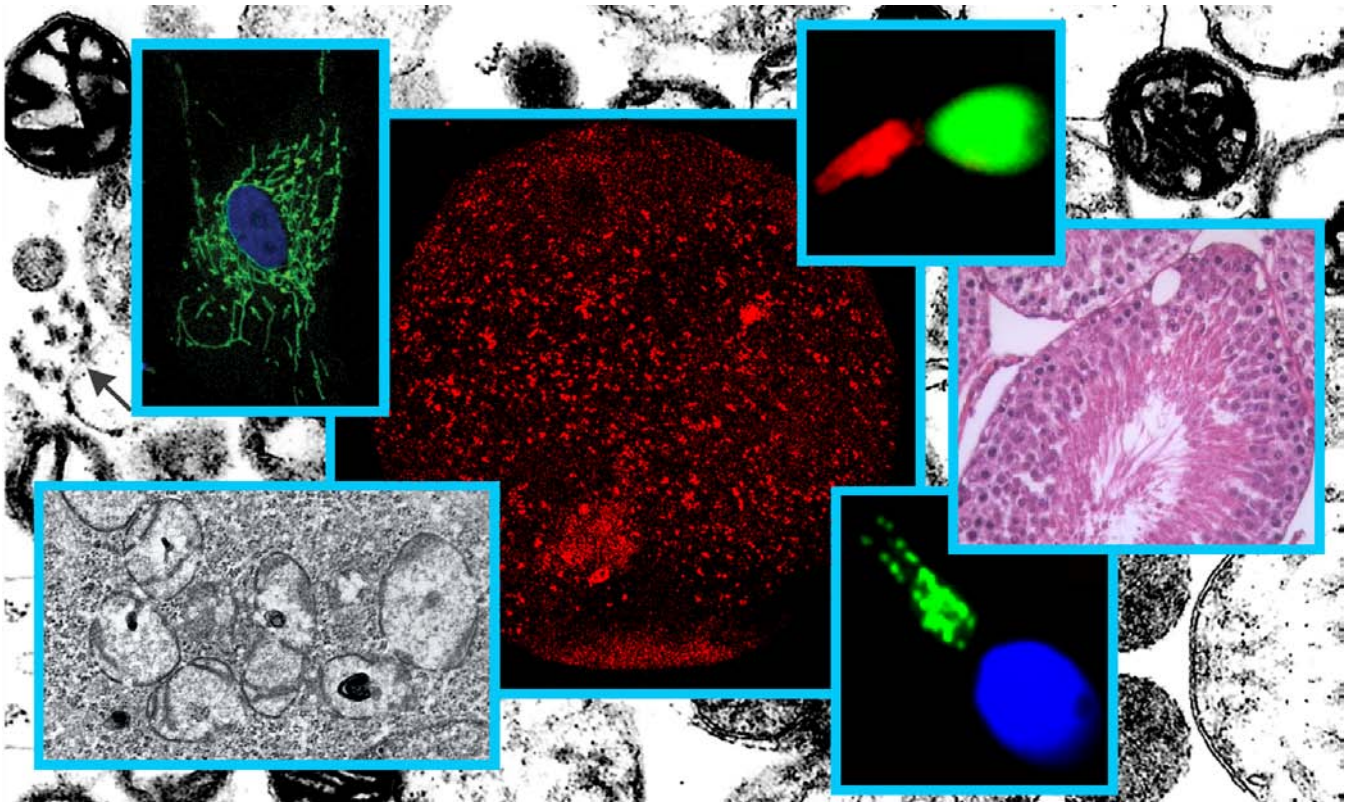
The main Objectives of the group are the characterization of metabolic pathways focusing on mitochondrial activity, and how they can be used to determine and modify human gamete functionality; and as cues to modulate pluripotent stem cell fate.

In terms of research in reproduction the goals are always two-fold: to decipher basic molecular mechanisms, and to translate those finding into clinically relevant deliverables and novel methodologies for Assisted Reproduction, both as diagnostic and as interventions. In the past year basic research focused on novel projects on sperm proteomics and metabolomics. The former study has suggested novel metabolic pathways relevant for human sperm function that might be actively used to increase fertilization rates. Research has also focused on how a heterogeneous population of sperm may be separated into subpopulations, thus allowing the use of only the more functional gametes in Assisted Reproduction.

In terms of applied research the group has pioneered a simple and cost-effective assay to analyze sperm chromatin status, and this assay has been further validated in a large study. We also were able to pinpoint novel ways in which environmentally relevant contaminants, pharmaceutical drugs and diabetic simulated conditions may affect human sperm function at a non-genomic level, by interfering with sperm metabolism and mitochondrial function. Additionally, the group has collaborated with the Coimbra University Health System (CHUC) in order to preserve germinal tissues from oncological patients that may have their fertility potential compromised following chemotherapy and radiotherapy. This project, named Oncofertility, is ongoing. In terms of preserving the germline of rare individuals, similar strategies are being implemented to conserve gonadal tissue of animals from at-risk species, namely wild felids, using the domestic cat as a model.

During this year our group initiated the development of several strategies to promote and study spermatogenesis in vitro, such as in vitro spermatogonial stem cell culture and in vitro testicular tissue organ culture, using several animal models. These techniques aim to evaluate the effects of several media and supplements on spermatogenesis development, and study the effects of metabolic conditions such as those related to diabetes.

Another research venue focuses on the study of pluripotent stem cells and how metabolism can dictate their final fate. The Group continues to use metabolic cues in order to control the pluripotency or differentiation ability of stem cells, with implications for tissue engineering. Additionally, in the course of these experiments unexpected parallels between pluripotent stem cells and cancer cells were discovered, and further explored, at the metabolic level, showing that both cell types control mitochondrial activity and metabolic pathways in a similar manner. Moreover, the group is focusing on cytoskeleton dynamics as well as on the putative role of sirtuins as central players on the interplay of metabolism and epigenetics in embryonic stem cells. This novel potential will be explored in the near future.



Main Achievements

1- We have characterized human sperm proteomics and metabolomics as related to sperm motility, and identified possible targets that may be useful in diagnosis-treatment (publications 1 and 9). In the case of human sperm metabolomics this is the first such study, using both NMR and Mass-Spectometry (accepted Manuscript). We have also established that ROS levels identify human sperm with distinct fertilizing ability (publication 4).

2- In order to explore possible parallels between pluripotent stem cells and cancer cells we have researched the possible use of metabolic cues in embryonic stem cells using defined chemical compounds previously described in clinical trials for cancer treatment (3-Bromopyruvate and Dichloroacetic acid). We have discovered that both cell types control mitochondrial activity and metabolic pathways in a similar manner, and that the metabolic alteration caused by these compounds leads to differentiation of embryonic stem cells (two submitted papers and publication 5).

Additionally we have started to apply molecular approaches, namely gene silencing, to address specific protein functions within embryonic stem cells, in order to avoid indirect effects of pharmacological approaches. Furthermore, the Group has also focused on the effect of antioxidant compounds on the pluripotency of embryonic stem cells, and on cytoskeleton dynamics in these cells.

3- The group established that the endocrine disruptor DDE (the main metabolite of the pesticide DDT) can act on human sperm in a non-genomic manner and at environmentally relevant concentrations, ultimately affecting sperm fertilizing ability. These results suggest a new possible mechanism to explain the negative role of these compounds on male infertility (Publication 8).

4- Characterization of human sperm function in diabetic-simulated conditions in vitro. Among several non-conventional parameters evaluated we found that high glucose levels only affect the acrossome integrity of the spermatozoa, suggesting that alterations might exist at other levels, such as spermatogenesis. In order to test this hypothesis, organ culture experiments with testis fragments exposed to high glucose conditions demonstrated an increase in the luminal area and in the Sertoli cell number. New experiments in Sertoli cells are being performed, also in high glucose conditions, in order to clarify how this important cell is affected and how this might influence spermatogenesis (two submitted manuscripts).

5- We demonstrated that Sildenafil citrate (Viagra) affects sperm motility and mitochondrial function. The energetic status of the cell was also affected and we suggested that alterations at this level might be responsible for the observed changes in sperm functionality (Publication 2).

6-Spermatogenesis development and possible determining factors have been accessed using the domestic cat as animal model. We were able to establish a specific marker for felid spermatogonial stem/progenitor cells, the lectin Dolichos bifluros that as allowed further observation of species' specificities of spermatogenesis organization which are now being explored. The use of a gas-liquid interphase testicular tissue organ culture, proven to promote progression of spermatogenesis from spermatogonial stem cell to sperm in mice, was used to test survival and progression of spermatogenesis of higher mammals testicular tissue. Manuscripts congregating the information obtained in these experiments are being prepared.

Cell Metabolism and Quality Control Group

Head: Paula Moreira

Objectives

Our research aims to unveil the involvement of mitochondria, inflammasome and quality control systems in aging and age-related neurodegenerative pathologies, namely Alzheimer´s disease (AD) and Parkinson´s disease (PD), as well as in other age-related diseases such as osteoarthritis. The mechanisms underlying diabetes-associated central and peripheral damage as well as their role as risk factors for several diseases are also studied. We intend to clarify the mechanisms involved in mitochondrial trafficking and signaling pathways and the crosstalk with other organelles such as the endoplasmic reticulum in the aforesaid diseases. The mechanisms of protein quality control present in these organelles and in the cytosol, and their role in inflammasome activation, are another focus of our research. Ultimately, our goal is to identify novel strategies for therapeutic intervention.

Specific objectives of the Research Lines:

Mitochondrial metabolism and insulin signaling in neurodegenerative and metabolic disorders (PI – Paula Moreira)

1) to clarify how mitochondrial homeostasis and trafficking are affected in AD and diabetic brains;
2) to understand the role of mitochondrial anomalies in the increased susceptibility of the diabetic brain to cognitive deficits and AD-like neurodegeneration;
3) to test the efficacy of antidiabetic agents in AD and diabetes-related brain damage.

Mitochondrial-regulation of molecular mechanisms involved in cellular degeneration (PI – Sandra Cardoso)

1) to elucidate the role of mitochondrial metabolism signaling in AD and PD neurodegenerative pathways;
2) to clarify how mitochondrial-driven defects in quality control systems, namely macroautophagy, and in intracellular traffic trigger AD and PD neuropathological hallmarks such as protein aggregation and axonal and synaptic loss;
3) to identify potential molecular targets that could be manipulated in order to arrest the degenerative pathways occurring in these brain pathologies.

Endoplasmic reticulum (ER) stress response and cellular quality control in aging and brain disorders (PI – Cláudia Pereira)

1) to investigate ER stress as a crucial molecular mechanism implicated in neuronal and endothelial dysfunction through the deregulation of calcium and redox homeostasis, mitochondrial dysfunction and impairment of protein homeostasis during aging and in brain pathologies, in particular in age-related neurodegenerative disorders such as AD.

Inflammation and cellular quality control in cartilage aging and osteoarthritis (PI: Alexandrina Mendes)

1) to elucidate the mechanisms by which diabetes favours the development and progression of osteoarthritis;
2) to identify new compounds in plant extracts with potential anti-osteoarthritic activity, as well as with potential activity against other diseases with a chronic inflammatory component.

Innate immunity in inflammation-related diseases (PI – Teresa Cruz Rosete)

1) *in silico* and *in vitro* development of non-animal cell-based approaches to detect skin and respiratory allergens;
2) to screen lead molecules with anti-inflammatory and anti-tumoral properties obtained from medicinal plants;
3) to evaluate the cross-talk between autophagy and inflammasome in antigen presenting cells.

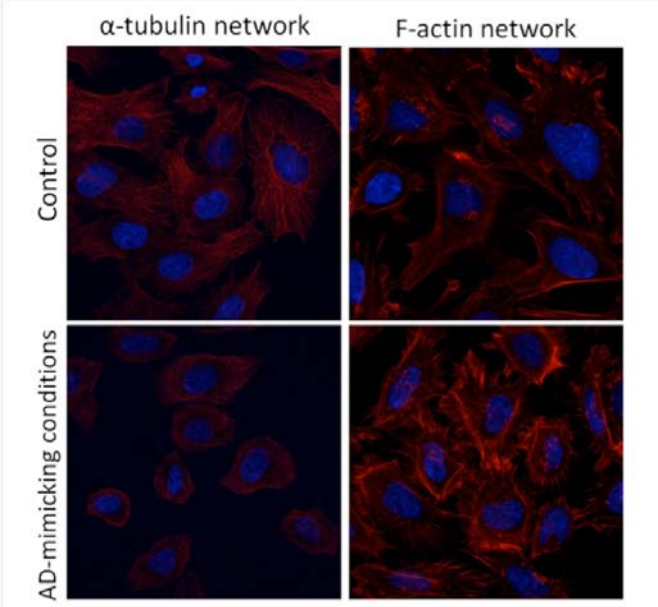


Figure 1: Rat brain endothelial cells exposed to AD-mimicking conditions show pronounced cytoskeletal alterations that are associated with mitochondrial dysfunction, ER stress and redox imbalance. Cells were stained for α-tubulin or F-actin (red) and DNA (blue), and visualized using a Zeiss LSM 510 Meta confocal microscope with a plan apochromat objective (63Å~, 1.4 NA)

Main Achievements

Mitochondrial metabolism and insulin signaling in neurodegenerative and metabolic disorders (PI – Paula Moreira)

Our lab has demonstrated that brain aging and type 2 diabetes (T2D) cause vascular, oxidative, and synaptosomal abnormalities. Although aging and T2D differently affect brain vessels and synaptosomes, both conditions increase the vulnerability of brain structures to degenerative events. In fact, in vitro studies showed that high glucose levels increase the susceptibility of brain microvascular

endothelial cells to amyloid β (Aβ) toxicity supporting the idea that hyperglycemia is a major risk factor for vascular injury associated with AD. It was also observed that during the early stages of T2D, brain mitochondrial function is maintained in part due to a delicate balance between mitochondrial fusion-fission and biogenesis and autophagy. Interestingly, type 1 diabetes (T1D) also seems to induce compensatory changes in mitochondrial dynamics and biogenesis in order to maintain mitochondrial function and synaptic integrity. In addition, insulin treatment was effective in normalizing brain alterations promoted by T1D, including the increase in tau protein phosphorylation, which supports the importance of insulin signaling in the brain. Rats chronically treated with methylglyoxal (MG), an important intermediate in advanced glycation end products (AGEs) synthesis, also showed brain mitochondrial impairments supporting the idea that AGEs accumulation predispose to (pathological) brain aging.

Mitochondrial-regulation of molecular mechanisms involved in cellular degeneration (PI – Sandra Cardoso)
We proved that autophagy is dependent on a functional mitochondrial network, since the stimulation of macroautophagy did not rescue the α-synuclein oligomers burden, neither prevented apoptosis in cells containing mitochondria from PD patients. We demonstrated that mitochondrial impairments cause the loss of microtubule network, culminating in intracellular trafficking deficits, which enhanced protein aggregation, due to disturbances in the autophagic-lysosomal pathway.

Endoplasmic reticulum (ER) stress response and cellular quality control in aging and brain disorders (PI – Cláudia Pereira)
We found that in addition to neurons and glia cells, other cell types are affected by the AD-associated oligomeric Aβ, namely hypothalamic and endothelial cells. We demonstrated for the first time that Aβ oligomers are toxic to hypothalamic cells, leading to superoxide radical production, calcium rise and mitochondrial dysfunction, which culminates in apoptotic cell death. Furthermore, the metabolic peptides ghrelin and leptin were shown to ameliorate in a receptor-dependent manner the hypothalamic cell dysfunction triggered by oligomeric Aβ. Data obtained in Aβ-treated endothelial cells from cerebral microvasculature supported that the loss of proteostasis mechanisms, namely ER stress-induced unfolded protein response (UPR), the ubiquitin-proteasome system (UPS) and macroautophagy, play a major role in the vascular alterations occurring in the AD brain.

Inflammation and cellular quality control in cartilage aging and osteoarthritis (PI: Alexandrina Mendes)
We showed that hyperglycemia and hyperinsulinemia independently induce the inflammatory response in human chondrocytes. It was also demonstrated that myrcene has anti-inflammatory and anti-catabolic activities in human chondrocytes suggesting that myrcene is a potential anti-osteoarthritic compound.

Innate immunity in inflammation-related diseases (PI – Teresa Cruz Rosete)
We developed *in vitro/in silico* methods for quantifying the potency of skin allergens and the identification of respiratory allergens (Provisional Patent Applications nº 20141000087015 and nº 20141000021388).

Mitochondria, Metabolism and Disease Group

Head: Paulo Oliveira

Objectives

Mitochondria are critical organelles for cell physiology. Mitochondria are the cell energy powerplants, producing the majority of chemical energy for cell metabolism, and playing an important role in cell death and quality control processes. Since mitochondria are also active players in cellular redox and calcium homeostasis, as well as in intermediate metabolism, the general objective of our research group is to provide insights into the role of mitochondria in cellular metabolism, redox signaling and stress responses associated with chemical toxicology, cancer, cardiovascular and hepatic diseases, aging, and stem cell differentiation. The group has a multiple prong approach to the scientific question, focusing in various specific aims:

1) Investigate whether intrinsic, pharmacological or non-pharmacological (exercise or diet) regulation of mitochondrial biogenesis/metabolism and quality control reduces organ injury during disease or chemical toxicity.

2) Decipher mitochondrial signals triggered by the axis sestrin -sirtuin and how its unbalance disrupts mitochondrial dynamics/energetic metabolism, resulting in increased susceptibility to physiologic/pathologic stress.

3) Identify mitochondrial remodeling steps and mechanisms during cancer stem cell differentiation and carcinogenesis.

4) Investigate mechanisms of Cr(VI)-induced lung carcinogenesis and the role of cancer stem cells, microenvironment and mitochondrial metabolism in tumor physiology.

5) Unravel mechanism of mitochondrial dysfunction caused by different xenobiotics, including drug-induced injury (e.g. retinoids, anthracyclines).

6) Study bone cell mitochondrial bioenergetics impairment and mitochondrial/peroxisomal fatty acid beta-oxidation unbalance on estrogen-deprivation-induced menopause.

7) Design and testing novel mitochondrial-directed antioxidants based on dietary components in models for human diseases (cardiovascular/hepatic) as well as the development of new pharmacological conditioning strategies, resulting in the reduction of morbidity and mortality of liver resection surgery.

8) Develop high-throughput methods to investigate mitochondrial function.

9) Identify molecular mechanisms responsible for miRNA regulation in several biological processes, particularly the miRNAs acting in mitochondria or in mitochondria-related mechanisms.

Main Achievements

1) In collaboration with the University of Porto, we demonstrated that endurance treadmill training and voluntary free-wheel exercise prevented doxorubicin (DOX)-induced cardiac mitochondrial dysfunction and oxidative stress in a rodent model and preserved the hepatic mitochondrial phospholipid profile, tissue architecture and overall mitochondrial function in a rodent model for non-alcoholic fatty liver disease. Diclofenac-induced hepatic mitochondrial alterations and apoptotic markers were also decreased by both exercise protocols.

2) We compared mitochondrial physiology and metabolism between P19SC embryonal carcinoma stem cells before/after differentiation and observed a unique fingerprint of the association between mitochondrial activity, cell differentiation and stemness. In comparison with their differentiated counterparts, pluripotency of P19SCs was correlated with a strong glycolytic profile and decreased mitochondrial biogenesis and complexity with a closed permeability transition pore. This decreased mitochondrial capacity increased their resistance against dichloroacetate. Stimulation of mitochondrial function by growing P19SCs in glutamine/pyruvate-containing medium reduced their glycolytic phenotype, induced loss of pluripotent potential, compromised differentiation and converted P19SCs sensitive to dichloroacetate.

3) By exploring differences in cancer mitochondria, including hyperpolarization, we developed novel cationic dimethylaminopyridine derivatives of pentacyclic triterpenes which target mitochondria in human breast cancer cell lines and trigger the mitochondrial permeability transition pore, followed by apoptotic cell death.

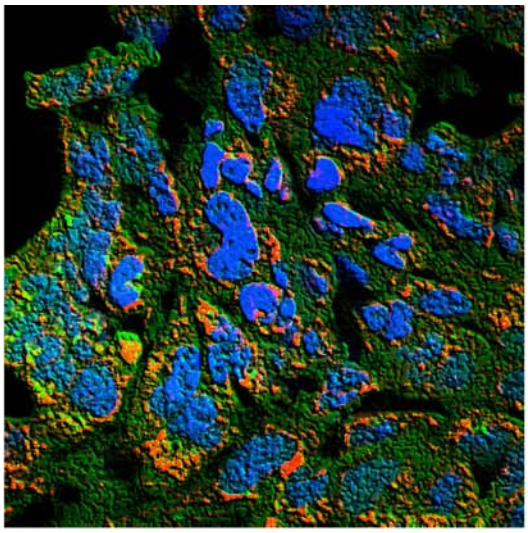
4) We demonstrated that the apoptosis-inducing factor (AIF) is released from mitochondrial during DOX treatment of H9c2 cardiomyoblasts, being translocated to the nucleus and causing caspase-independent DNA damage. AIF knockdown using a small-interfering RNA approach resulted in a reduction of doxorubicin toxicity, including decreased p53 activation and poly-ADP-ribose-polymerase cleavage. Among the proteases that could be responsible for AIF cleavage, DOX decreased calpain activity but increased cathepsin B activation, with inhibition of the latter partly decreasing DOX toxicity.

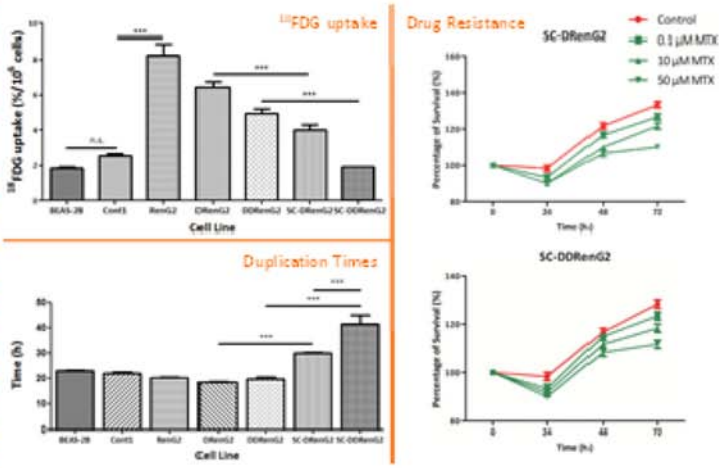
5) We tested estradiol (E2) and a phytochemical substitute, coumestrol (COU) in ovariectomized rats in order to compare bone loss with sham-operated animals. For the first time, we assessed the metabolic and lipid profiles of osteocytes ex vivo. Higher lactate/alanine and acetate/alanine ratios in the OVX group, and especially in the E2 group, were observed. Increased content in palmitic acid, alfa/gama-linolenic acids and arachidonic acid was found in the E2 group, while a 62% decrease in tetradecenoylcarnitine and a 2.5-fold increase in indocosanoic acid was identified in the OVX group, when compared with the control group. Ovariectomy clearly

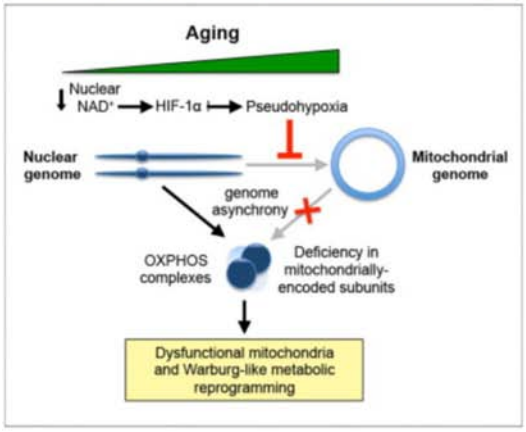
changed lipid profile inducing significant changes in both diacyl- and choline-plasmalogens content (comparatively with SHAM and OVX+E2 groups).

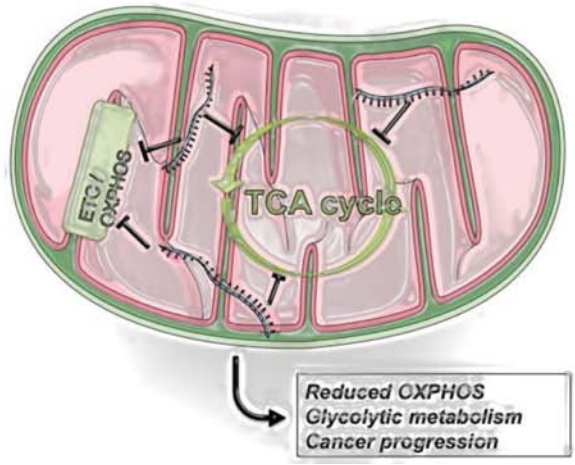
6) In the context of obesity and metabolic dysfunction, we have observed that acute impaired OXPHOS capacity lead to an unbalance in mitochondrial biogenesis and that OXPHOS stimulation prevents the perpetuation of metabolic dysfunction.

7) We demonstrated that the tumor microenvironment modulates malignant cells phenotype and, consequently, we concluded that there is not a unique source of CSCs. We also identified three cytokines that are possible important players in CSCs reprogramming process. Seven cellular systems (BEAS-2B, Cont1, RenG2, DRenG2, DDRenG2, SC-DRenG2 and SC-DDRenG2) were generated from parental tumorigenic cells, showing progressively malignancy and resistance to chemotherapy.









Obesity, Diabetes and Complications Group

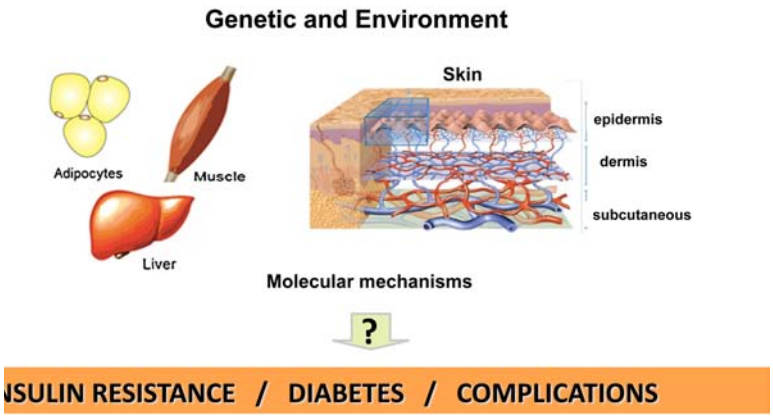
Head: Eugénia Carvalho

Objectives

a) Immunosuppressive agents, such as cyclosporine and rapamycin cause dyslipidemia and diabetes in solid organ-transplantation. Development of these metabolic complications increases the risk for graft failure and patient death. However, the molecular mechanisms underlying these metabolic effects are not fully elucidated. We aimed to understand the effects of therapeutic doses of CsA (5 mg/kg/day) and SRL (1 mg/Kg/day) in glucose and lipid metabolism in peripheral insulin sensitive tissues.

b) Diabetes is one of the most widespread and costly diseases in the world. It may cause diabetic foot ulcers, decreasing the welfare of patients. Peripheral neuropathy impairs wound healing. We have used different cellular and animal models to unveil the molecular mechanisms of wound healing. Recent studies suggest that neuropeptides such as substance P and neurotensine participate in wound healing but the mechanisms of their action are not clear. Our main objective here was to understand the role of substance P and neurotensin in wound healing impairment and how much they contribute to wound healing amelioration if applied topically to the wound site. Furthermore, natural biopolymers like chitosan, collagen are presently receiving great attention as wound dressing materials for wound healing applications. Employing these chitosan or collagen derivatives as dressings and as platforms for the delivery of a neuropeptide, neurotensin (NT) has not yet been evaluated and it is being addressed in our work.

c) Congestive heart failure (HF) is a major health care burden and life-threatening condition. Insulin resistance, impaired glucose tolerance and overt diabetes are associated with the disease, which is accompanied by inflammation and oxidative stress. Epicardial adipose tissue (EAT) has been related to HF and myocardial dysfunction through unidentified mechanisms. We aim at understanding the role of EAT in HF conditions. Our objective is to study the role of EAT on the heart muscle, not only at the metabolic and inflammatory levels, but also to assess oxidative and ER stress, autophagy, apoptosis and mitochondrial dysfunction in these tissues derived from patients with diabetes and the association of these factors with the presence of CVD.



d) One of our objectives has been to understand the role of microRNAs in the complications of diabetes namely those particularly involved in impaired wound healing in diabetes.

e) More recently we have been interested in evaluating the role of T-cells in wound healing in patients with and without diabetic foot ulcers.

Main Achievements

A) Cyclosporine A (CsA) and sirolimus (SRL) are immunosuppressive agents (IA) associated with new-onset diabetes after transplantation (NODAT). This study aimed to evaluate the effects of 3-weeks of treatment with either CsA (5 mg/kg BW/day) or SRL (1 mg/kg BW/day) on insulin signaling and expression of markers involved in glucose metabolism in insulin-sensitive tissues, in Wistar rats. An increase in glucose-6-phosphatase protein levels (68%, $p < 0.05$) and in protein-tyrosine phosphatase 1B (163%, $p < 0.05$), a negative regulator of insulin was observed in the CsA-treated group in the liver, indicating enhanced gluconeogenesis and increased insulin resistance. On the other hand, glucokinase protein levels were decreased in the SRL group (35%, $p < 0.05$) compared to vehicle, suggesting a decrease in glucose disposal. SRL treatment also reduced peroxisome proliferator-activated receptor γ coactivator 1 alpha protein expression in muscle (~50%, $p < 0.05$). Moreover, the phosphorylation of key proteins of the insulin signaling cascade was suppressed in the SRL group, but was unchanged by the CsA treatment.

Moreover, lipolysis was evaluated in isolated adipocytes, while triglycerides (TG) and non-esterified fatty acid (NEFA) were measured in serum. CsA and SRL treatments of rats for 3 and 9weeks increased isoproterenol-stimulated lipolysis by 5-9 fold and 4-6 fold in isolated adipocytes, respectively. While CsA increased adipocyte weight and diameter, as well as NEFA and TG levels in circulation after 9weeks, SRL treatment caused ectopic deposition of TG in the liver after 3weeks. Moreover, ACC1 and FAS protein expression was increased after 3weeks (>100%, $p<0.01$), while HSL was increased after 9weeks of CsA treatment. On the other hand, SRL decreased the expression of lipogenic genes, including ACC1 (50%, $p<0.05$), lipin1 (25%, $p<0.05$), PPAR-γ (42%, $p<0.05$) and SCD1 (80%, $p<0.001$) in adipose tissue, after 3weeks of treatment. The effects of both IAs on expression of lipolytic and lipogenic genes suggest that these agents influence lipid metabolism, thus contributing to the dyslipidemia observed during immunosuppressive therapy. Moreover, these data suggest that CsA treatment enhances gluconeogenic factors in liver, while SRL treatment impairs insulin signaling in peripheral tissues.

b) Diabetic foot ulceration (DFU) occurs almost exclusively in the presence of diabetic neuropathy. Substance P (SP) is involved in wound healing, but its

effect in diabetic skin wounds is unclear. We examined the effect of exogenous SP delivery on diabetic mouse and rabbit wounds. SP treatment improved wound healing in mice and rabbits, whereas the absence of SP or its receptor impaired wound progression in mice. Moreover, SP bioavailability in diabetic skin was reduced as SP gene expression was decreased, whereas the gene expression and protein levels of the enzyme that degrades SP, neutral endopeptidase, were increased. Diabetes and SP deficiency were associated with absence of an acute inflammatory response important for wound healing progression and instead revealed a persistent inflammation throughout the healing process. SP treatment induced an acute inflammatory response, which enabled the progression to the proliferative phase and modulated macrophage activation toward the M2 phenotype that promotes wound healing. In conclusion, SP treatment reverses the chronic proinflammatory state in diabetic skin and promotes healing of diabetic wounds.

Moreover, collagen based dressings were prepared to be applied as support for the delivery of neurotensin (NT), a neuropeptide that acts as an inflammatory modulator in wound healing. The performance of NT alone and NT-loaded collagen matrices to treat wounds in streptozotocin (STZ) diabetic induced mice was evaluated. NT-loaded collagen dressings induced faster healing (17% wound area reduction) in the early phases of wound healing in diabetic wounded mice. In addition, they also significantly reduced inflammatory cytokine expression namely, TNF-α ($p<0.01$) and IL-1β ($p<0.01$) and decreased the inflammatory infiltrate at day 3 post-wounding (inflammatory phase). After complete healing, metalloproteinase 9 (MMP-9) is reduced in diabetic skin ($p<0.05$) which significantly increased fibroblast migration and collagen (collagen type I, alpha 2 (COL1A2) and collagen type III, alpha 1 (COL3A1)) expression and deposition. These results suggest that collagen-based dressings can be an effective support for NT release into diabetic wound enhancing the healing process.
Furthermore, N-carboxymethyl chitosan, 5-methyl pyrrolidinone chitosan (MPC) and N-succinyl chitosan, were presented as potential biomaterials for wound healing applications. NT-loaded and non-loaded MPC dressings were applied to control/diabetic wounds to evaluate their in vitro/in vivo performance. The results show that the former induced more rapid healing (50% wound area reduction) in the early phases of wound healing in diabetic mice. A NT-loaded MPC foam also reduced expression of the inflammatory cytokine TNF-α ($P<0.001$) and decreased the amount of inflammatory infiltrate on day 3. On day 10 MMP-9 was reduced in diabetic skin ($P<0.001$), significantly increasing fibroblast migration and collagen (COL1A1, COL1A2 and COL3A1) expression and deposition. These results suggest that MPC-based dressings may work as an effective support for sustained NT release to reduce DFUs.

C) Epicardial Adipose Tissue (EAT) is an active endocrine and paracrine organ located on the surface of the heart surrounding the large coronary arteries that may influence the development of CVD and has been implicated in the pathogenesis of coronary artery disease. Our findings are that in non-diabetic patients, insulin-stimulated glucose transport is significantly lower in EAT cells, compared to subcutaneous adipose (SAT) cells of the same patients, highlighting the possible physiologic, metabolic, endocrine and inflammatory differences present between both types of adipose tissue. In diabetic patients with congestive heart failure, the insulin-stimulated glucose uptake was impaired in either SAT or EAT. This impairment in activation of glucose transport by insulin could possibly be due to reduced GLUT4 protein expression. In fact, at the mRNA level, GLUT4 gene expression was significantly decreased in EAT of diabetic patients. In addition, IL-α gene was significantly increased in EAT of diabetic patients. Dysfunctional EAT maybe a marker of CVD development.

D) Overweight and obesity are major problems in today's society, driving the prevalence of diabetes and its related complications. The highly conserved endogenous small non-coding RNA molecules, the micro RNAs (miRNAs) have in recent years been found to be involved in a number of biological processes, including the pathogenesis of disease. Their main function is to regulate post-transcriptional gene expression by binding to their target messenger RNAs (mRNAs), leading to mRNA degradation, suppression of translation or even gene activation. MiRNAs are promising therapeutic targets and demonstrate great potential as diagnostic biomarkers for disease. We have reviewed the most recent findings regarding the important roles of miRNAs in diabetes and its complications, with special attention given to the different phases of diabetic wound healing. In collaboration with a colleague in Denmark we have ongoing studies trying to screen and identify microRNAs that might be altered in skin wound healing in animal models we have used in the previous studies.

E) Early studies involving murine models have unraveled a dual role of T-cells in both the inflammatory and proliferation phases of wound healing, but their *in vivo* impact on diabetic foot ulceration (DFU) has remained elusive. we analyzed how diabetes in general and DFU in particular affect TCR diversity, by PCR based studies, and the relative distribution of the most representative T-cell populations (naive, activated/memory and effector), by flow cytometry. Our results show that diabetes has a profound impact on the circulating T-cell pool of diabetic patients, lowering naïve T-cell numbers to a point where TCR diversity may become an issue to the immune response and some pathogen epitopes may no longer be recognized. We have also shown that this reduced TCR repertoire diversity is mainly due to the accumulation of effector T-cells, which are major TNF-α producers. In conclusion, our results may explain the self-sustaining systemic inflammatory environment that is driven by the accumulation of effector cells.

ImmunoMetabolic Pharmacology Group

Head: Margarida Carneiro

Objectives

a) Identify alterations in the different B cell subpopulations in peripheral blood samples of HIV-negative pulmonary Multidrug-Resistant Tuberculosis patients.

b) Reveal the molecular pathways underlying chronic inflammation in NADPH-Oxidase 2 complex deficient human donors and corresponding mouse models.

c) Test whether genetically controlled mice with a point-mutation in the Ncf1 gene present increased predisposition to develop chronic colitis.

d) Evaluate and characterize metabolic alterations in differentiated cancer cells and stem cells by ^{13}C NMR isotopomer analysis.

Main Achievements

A: Identify alterations in the different B cell subpopulations in peripheral blood samples of HIV-negative pulmonary Multidrug-Resistant Tuberculosis patients when compared to healthy donors.
Multidrug-Resistant Tuberculosis (MDR-TB) patients, similarly to what has been observed in other chronic inflammatory diseases, have a much lower frequency of peripheral blood unswitched IgD+CD27+ memory B cells. Equally novel are the findings that in MDR-TB patients there is a reduction in the circulating plasma cell pool and that in MDR-TB there is an increased frequency of circulating type 1 transitional IgD+CD38++, activated CD69+ and TLR9-expressing B cells. These results document disease-related shifts in peripheral blood B cell subsets in MDR-TB and suggest that such changes should be taken into account when designing new strategies to boost the cellular and humoral immune response against Mycobacterium tuberculosis.

B: Reveal the molecular pathways underlying chronic inflammation in NADPH-Oxidase 2 complex deficient human donors and corresponding mouse models.
A prominent type I interferon (IFN) response signature that was accompanied by elevated autoantibody levels was identified in both mice and humans lacking functional NADPH-Oxidase 2 (NOX2) complex. To further underline the systemic lupus erythematosus (SLE)-related autoimmune process, we show that naïve Ncf1(m1J) mutated mice, similar to SLE patients, suffer from inflammatory kidney disease with IgG and C3 deposits in the glomeruli. Expression analysis of germ-free Ncf1(m1J) mutated mice reproduced the type I IFN signature, enabling us to conclude that the upregulated signaling pathway is of endogenous origin. Our findings link the previously unexplained connection between ROS deficiency and increased susceptibility to autoimmunity by the discovery that activation of IFN signaling is a major pathway downstream of a deficient NOX2 complex in both mice and humans.

C: Test whether genetically controlled mice with a point-mutation in the Ncf1 gene present increased predisposition to develop chronic colitis.
Clinical scores demonstrated a more severe colitis in Ncf1-mutant mice than controls, with no recovery during the resting period and a severe chronic colitis after the 2nd cycle, confirmed by histopathology and presence of infiltrating neutrophils, macrophages, plasmocytes and lymphocytes in the colon. Severe colitis was mediated by increased local expression of cytokines (IL-6, IL-10, TNF-α, IFN-γ and IL-17A) and phosphorylation of Leucine-rich repeat kinase 2 (LRRK2). Serological cytokine titers of those inflammatory cytokines were more elevated in Ncf1-mutant than control mice, and were accompanied by systemic changes in functional subsets of monocytes, CD4+ T and B cells. Overall, our data suggest that an ineffective oxidative burst leads to severe chronic colitis through local accumulation of peroxynitrites, pro-inflammatory cytokines and lymphocytes and systemic immune deregulation similar to what is observed in patients with Chronic Granulomatous Disease.

D: Evaluate and characterize metabolic alterations in cancer cells and stem cells by ^{13}C NMR isotopomer analysis.
Pluripotency of undifferentiated P19SC cells was correlated with a strong glycolytic profile and decreased mitochondrial biogenesis and complexity. This decreased mitochondrial capacity increased their resistance against dichloroacetate. Stimulation of mitochondrial function by growing P19SCs in glutamine/pyruvate-containing medium reduced their glycolytic phenotype, induced loss of pluripotent potential, compromised differentiation and became P19SCs sensitive to dichloroacetate. Because of the central role of this type of SCs in teratocarcinoma development, our findings highlight the importance of mitochondrial metabolism in stemness, proliferation, differentiation and chemoresistance.
When comparing the intermediary metabolism of several colon carcinoma cell lines under normoxia there was a significantly higher lactate production –reminiscent of the Warburg effect typically observed in tumors- was observed for all cell lines. However, the adaptation to hypoxia conditions was different between the studied cell lines. Since the different cell lines correspond to distinct characteristics and regions of the colon and colonic adenocarcinoma types, these differential metabolic behaviors stress the importance to gain an adequate knowledge of the “metabolic remodeling” that follows a given cancer treatment. Thus influencing the correct (re)design of therapeutic strategies against cancer.

Intermediary Metabolism Group

Head: John G. Jones

Objectives

a) The effects of high fructose feeding on hepatic lipid and carbohydrate fluxes: The Western diet is characterized by high intake of refined sugar and high-fructose corn syrup and is implicated in the soaring rates of diabetes and non-alcoholic fatty liver disease. Fructose is a carbohydrate that is solely metabolized by liver, hence diets high in fructose present the liver with a substantial nutritional challenge. The immediate fate of fructose is phosphorylation and conversion to triose phosphates. Triose phosphates may in turn be metabolized to pyruvate and acetyl-CoA via glycolysis and pyruvate dehydrogenase activities. This acetyl-CoA in turn can be recruited for *de novo* lipogenesis. Triose phosphates can be also converted to glucose and glycogen *via* gluconeogenic pathways resulting in elevated hepatic glucose production and glycogen synthesis. Since high fructose feeding is associated with both excessive hepatic lipid levels (possibly related to increased rates of *de novo* lipogenesis) and impaired control of hepatic glucose production (possibly related to increased rates of gluconeogenesis), determining the flux of fructose carbons into glucose/glycogen and into hepatic triglyceride is a key objective. To this end, we have been developing novel noninvasive stable isotope tracer methods to determine the contribution of dietary fructose to the synthesis of hepatic glucose, glycogen and triglyceride. This approach will allow us to determine if fructose is directly contributing carbons for *de novo* lipogenesis and/or facilitating *de novo* lipogenesis from all acetyl-CoA sources, possibly by upregulation of *de novo* lipogenesis enzymes. These methodologies are being currently applied to animal models but we are also translating to human studies where they will be applied to characterize hepatic metabolic fluxes during high sugar feeding.

b) Effect of oral medium-chain triglyceride on cerebral substrate utilization in rodent disease models: Diseases such as Alzheimers and epilepsy are characterized by a decrease in cerebral glucose oxidation. In the initial stages, restricted glucose conversion to acetyl-CoA is hypothesized to be an important contributory factor. In this setting, the neurons are believed to intact but in a hypometabolic state, which may compromise their energetic and functional capacities. If this is the case, provision of alternative oxidizable substrates to generate acetyl-CoA may restore cellular Krebs cycle flux and energetic state. While glucose is the principal oxidizable substrate for brain metabolism, ketone bodies can also be efficiently utilized as a source of acetyl-CoA. Therefore, the initial objectives are to quantify competition of glucose and ketone bodies to cerebral acetyl-CoA synthesis in isolated brain slices. This will be initially applied to healthy rodents in order to optimize experimental protocols and methodologies. When this is accomplished, the protocol may then be applied to appropriate disease models.

c) Characterizing dietary glycerol utilization by seabass: The European seabass is an important farmed marine fish species. As carnivorous fish, their metabolism is adapted to high levels of dietary protein, thus their efficiency in utilizing dietary carbohydrates is poor. Increased carbohydrate utilization would be both economically and environmentally beneficial, since high-cost fish meal could be substituted in part by lower cost substrates while at the same time the conversion of dietary amino acids to glucose and generation of waste ammonia would be spared. Glycerol is a by-product of biodiesel synthesis and it has been evaluated as a feed supplement in rainbow trout and channel catfish. In mammals, it is efficiently converted to glucose via gluconeogenesis, but its metabolism by fish is not known. We hypothesize that glycerol effectively competes with dietary amino acids for gluconeogenic carbons thereby sparing their conversion to glucose.

Main Achievements

1. ^{2}H-enrichment distribution of hepatic glycogen from $^{2}H_2O$ reveals the contribution of dietary fructose to glycogen synthesis: ^{2}H-enrichment of glycogen positions 5 and 2 from $^{2}H_2O$ informs direct and indirect pathway contributions to glycogenesis. Inclusion of position 6S ^{2}H-enrichment data allows indirect pathway sources to be resolved into triose-phosphate and Krebs cycle precursors. This analysis was applied to 6 rats that had fed on standard chow (SC), and 6 fed on SC plus 35% sucrose in the drinking water, all of which were also given $^{2}H_2O$. Overnight net hepatic glycogen synthesis was similar between HS and SC rodents. Direct pathway contributions were also similar (403 ± 71 vs. 578 ± 76 mmol/gdw), but triose-phosphate contributions were significantly higher for HS (382 ± 61 vs. 87 ± 24 mmol/gdw, $p<0.01$) while Krebs cycle inputs were lower for HS (110 ± 9 mmol/gdw vs. 197 ± 32 mmol/gdw, $p<0.05$). Hence, the ^{2}H-enrichment distributions of hepatic glycogen and glucose from $^{2}H_2O$ informs the contribution of dietary fructose to hepatic glycogen and glucose synthesis.

2. Effects of transaldolase exchange on estimates of gluconeogenesis in type 2 diabetes: Transaldolase exchange (TA) overestimates gluconeogenesis measured with $^{2}H_2O$. However, it is unknown if TA differs in people with type 2 diabetes (T2DM). $^{2}H_2O$ was ingested and [1-^{13}C]acetate and [3-^{3}H]glucose infused in T2DM (n=10) and healthy nondiabetic (ND, n=8) subjects. TA was assessed from the ratio of ^{13}C3 to ^{13}C4 glucose enrichment (^{13}C3/^{13}C4) measured by ^{13}C NMR. Glucose turnover was measured before (~16hr fast) and during hyperglycemic (~10mM) moderate dose insulin (~0.35 mU/kg/min) clamp.

^{13}C3/^{13}C4 in T2DM vs. ND was no different at baseline and clamp indicating equivalent TA. To determine if incomplete triose-phosphate isomerase exchange (TPI) contributed to asymmetric ^{13}C3/^{13}C4, [U-^{13}C]glycerol was infused in lieu of [1-^{13}C]acetate at a separate visit in a subset of ND (n=7)

subjects. Both tracers yielded $^{13}C3/^{13}C4 < 1.0$ at baseline and at clamp conditions indicating that TPI exchange was essentially complete and did not contribute to asymmetric glucose enrichment. Uncorrected and corrected rates of gluconeogenesis were no different in T2DM vs. ND both at baseline and during clamp. TA correction resulted in equivalent estimates of corrected gluconeogenesis in T2DM and ND that were ~25-35% lower than uncorrected gluconeogenesis both at baseline and during the clamp. In conclusion, TA exchange does not differ between T2DM and ND under these conditions and the ^2H_2O method provides an accurate comparison of gluconeogenic fluxes in subjects with and without diabetes.

3. Noninvasive measurement of murine hepatic acetyl-CoA ^{13}C-enrichment following overnight feeding with ^{13}C-enriched fructose and glucose. The ^{13}C-isotopomer enrichment of hepatic cytosolic acetyl-CoA of overnight-fed mice whose drinking water was supplemented with [U-^{13}C]fructose, and [1-^{13}C]glucose and *p*-amino benzoic acid (PABA) was quantified by ^{13}C NMR analysis of urinary *N*-acetyl- PABA. Four mice were given normal chow plus drinking water supplemented with 5% [1-^{13}C]glucose, 2.5% [U-^{13}C]fructose, and 2.5% fructose (Solution 1) overnight. Four were given chow and water containing 17.5% [1-^{13}C]glucose, 8.75% [U-^{13}C]fructose and 8.75% fructose (Solution 2). PABA (0.25%) was present in both studies. Urinary *N*-acetyl-PABA was analyzed by ^{13}C NMR. In addition to [2-^{13}C]- and [1,2-$^{13}C_2$]acetyl isotopomers from catabolism of [U-^{13}C]fructose and [1-^{13}C]glucose to acetyl-CoA, [1-^{13}C]acetyl was also found indicating pyruvate recycling activity. This precluded precise estimates of [1-^{13}C]glucose contribution to acetyl-CoA while that of [U-^{13}C]fructose was unaffected. The fructose contribution to acetyl-CoA from Solutions 1 and 2 was 4.0 ± 0.4% and 10.6 ± 0.6%, respectively, indicating that it contributed to a minor fraction of lipogenic acetyl-CoA under these conditions.

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BIOMEDICAL INTER-INSTITUTIONAL RESEARCH PROGRAMME

Psychiatry Research

António Ferreira de Macedo, Ana Telma Pereira (FMUC, CNC)

Molecular genetics studies of complex disorders

The Center for Genomic Psychiatry at USC, led by Professor Carlos Pato and Michele Pato at Department of Psychiatry and the Behavioral Sciences, Keck School of Medicine, at University of South California (USC) remains one of our principal collaborations focused on genomic psychiatry with an emphasis on population based genetic studies. This collaboration was establish over 25 years and have been followed the Portuguese Cohort studies. These studies have been highly productive family based and population based studies. In 2014, we have contributed 450 additional participants as 150 trios.

In the last 10 years, this collaborative has expanded through a number of extensive expansion throughout the world. Culminating in creating a large new cohort, the Genomic Psychiatry Cohort (GPC). To date the GPC based and directed by Professors Carlos Pato and Michele Pato at USC has brought in close to 39,000 participants, including 10,000 patients suffering from schizophrenia, 5,000 patients suffering from bipolar disorder, and 15,000 control participants, and other disorders. Of this total, 9,000 are drawn from long-term studies of specific populations, and over 24,000 have joined as new partner participants. These participants have all contributed DNA, and cells, that are sharable through the NIMH repository. All have agreed to prospective follow-up. Further, over 88% allow us to re-contact them for future studies, over 20 years or more, including the evolving electronic health record. This captures overall health history for all medical disorders among our patients suffering from psychiatric disorders and the over 15,000 control participants. This is a tremendous demonstration of the approach to patient engagement pioneered by Michele Pato.

The GPC includes 6,000 African-Ancestry, close to 8,000 Latino, and over 19,000 Euro-Caucasian participants. Over 5,000 African Americans have been completed as wave 1. Wave 2 has completed over 6,000 Latino subjects. Wave 3 focused on Caucasian participants have now 3,700 completed and may ultimately include over 12,000 subjects.

Our studies have utilized the more recent DNA and RNA microarray technology to identify chromosomal regions of linkage to each disorder, genetic association information, as well as areas of differential gene expression in the presence of illness. This convergent genetic-genomic approach has led to the identification of several areas in the human genome that may harbour susceptibility genes for Sz or BP. In Sz, our group identified a region on 5q31–5q35 with a NPL score of 3.28 which was replicated in the BP sample with psychosis. Further study of this region showed positive SNP associations with several GABA receptor subunit genes in patients with SZ. In BP, the identification of a region on 6q22 (NPL-Z=4.2), was also an important finding. In our case-control studies a number of significant associations were reported for several genes: syntaxin 1A; NRG1, GABA receptor subunit genes; Neurogranin; CHRNA7, and DRD2. More recently, as published in *Nature*, our studies with copy number variants (CNVs) led to the identification of 22q11.2, 15q13.2 and 1q21.1 as regions with excess CNVs in Sz. An exploratory WGA study in the Portuguese Sz probands was carried out identiying a total of 55 SNPs that showed nominally significant associations with schizophrenia at a threshold of $P < 1 \times 10^{-4}$. Two of these SNPs survived FDR correction (rs6638512 on chromosome X, and rs4907606 on chromosome 13). However, in this study, when considering the region of maximal linkage on Chromosome 5q31-35, only one of the 22 candidate genes, glutamate receptor, ionotropic, AMPA 1 (GRIA1) was found to have multiple SNPS showing significant association at $p<10^{-4}$ (Middleton et al, 2012).

More recently, we are contributing to a genome-wide analysis of rare and common SNPs, common haplotypes, and CNVs using the Illumina 2.5 million SNP Platform. This is a unique opportunity to study populations that trace ancestry to continents other than Europe. We believe this has the potential to lead us to novel risk factors and to alleles for which discovery power is different in different populations. As well as, increase our understanding of the genetics of human populations and population admixture.

Nonetheless, the problem of the phenotypic heterogeneity in the area of psychosis still remains to be solved and we have to face the possibility that it could even be increased in samples of the magnitude used in GWAS. It is necessary, in parallel with these large GWAS, to implement nested studies, using clinical covariates that shows high familiality and are potentially under the control of a smaller set of genes, defining more homogeneous sub-samples. One of the areas of expertise of our team is phenotypic definition,

and in this context, we have been contributing by developing phenotypic measures potentially more adequate to dissect the underlying pathologic mechanisms.

Some of the phenotypes that have received greatest attention to date are those relating to psychosis because both population-based studies and molecular genetic studies, either linkage or association studies, show evidence that SZ and BP partly share a common genetic cause. Thus, based on the assumption that we can expect substantial overlaps of genetic susceptibility across diagnostic categories and substantial heterogeneity within diagnostic categories we are now also interested in investigating some key phenotypic measures/symptom dimensions.

In May 2013 we have obtained limited funding from the "Gabinete de Apoio à Investigação" (Office of Research Support) from Faculty of Medicine-University of Coimbra, to continue developing the research project entitled "*Phenotypic Dimensions in Psychosis*" (Pereira04.01.13). The project duration is 12 months. Our aims include: 1. Assess 200 SZ/BD/SzA probands (from multiplex families and unrelated cases) – diagnostic classification and lifetime-ever occurrence of symptoms using all available clinical information; 2. Deposit the 200 Blood/DNA samples in the FMUC (Laboratório de Citogenética) repository for future studies; 3. Contribute to phenotypic refinement and formulation of alternative phenotypes: symptom dimensions and subphenotypes.

During 2014 we have collected data and inputed sufficient data to achive these aims in 2015, including aim 3, that had not yet begun in 2013.

At the moment, our sample, including data collected with EP-GENE (aproximatelly 120 psychotic patients) and data collected within other projects but adapted and inputed to be in accordance with the EP-GENE, is composed of 684 psychotic patients: Schizophrenia/Sz (73.8%), Other non-organic psychotic disorders/ONPD (6.0%), Schizoaffective disorders/SzA (1.4%), Bipolar disorders/BP (18.1%) and Severe depression with psychosis/SDP (.8%).

Three doctoral thesis, which we are supervising, are in progress within this line of research "Phenotypic Dimensions in Psychosis": 1-Schizophrenia - Subphenotypes and dimensions (Dra. Raquel Alexandra da Silva Correia, FMUP); 2-Subphenotypes in Bipolar Disorders (Dr. José Valente, FMUC); 3-Social cognition in bipolar disorder and schizophrenia: Clinical phenotypes and neural basis (Dr. Nuno Madeira, FMUC).

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 psychopathology. Our correlational studies have established an association between the maladaptive aspects of perfectionism and a broad range of psychopathological conditions and health problems (e.g. sleep problems). However, as the cognitive mechanisms that mediate this association are not fully understood, we are now developing a project to investigate the role of multilevel cognitive processes in the relationship between psychological distress (PD) and perfectionism in a non-clinical sample of undergraduate students and a clinical sample of depressive and anxiety disorders. The second data wave (prospective study) was completelly collected in 2014; the data input have also started in 2014 and is in progress. It was also in 2014 relevant results were presented/published.

Also in 2014 we have started a new research project within this line, but focusing on a transgenerational perspective. As, in 2014, we have confirmed our hypothesis that Repetitive Negative Thinking (RNT) is a significant mediator of the relationship between perfectionism and psychological distress, as well as, with disordered eating behaviours and OC symptoms, we are now also interested in the role of parental perfectionism and RNT in the development of children perfectionism and psychopathology levels, which warrants further study. Our aim is to analyse the role of parental perfectionism and RNT in the children emotion regulation and psychological distress/psychopathology symptoms (anxiety, depression, OC and eating disorders symptoms). In 2014, we have already started the first wave (transversal) of this prospective study with a sample composed of college and high school students (aged≥ 15 years old) and their parents. During 2015 we plan to have data (personality, cognitive and the psychopathological variables) from approximately 500 families.

Another important area of interest in which we have developed a line of research is the study of affective disorders in the perinatal period, a topic which have been relatively neglected.

Our team have also acquired an extensive expertise in the field of psychometrics and diagnostic methodologies, developing and adapting diagnostic tools, and several scales which have been validated to be used in the above mentioned studies.

Publications

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Neurology Research

Studies on neurodegenerative disorders

Luis Cunha, Isabel Santana (FMUC, CHUC); Inês Baldeiras, Catarina Oliveira (FMUC, CNC)

Biomarkers for the early differential diagnosis of Dementia is one of our main areas of interest. Core cerebrospinal fluid (CSF) biomarkers - Aβ42, Tau and pTau – have been recently incorporated in the new proposed revised criteria for Alzheimer's Disease (AD). However their widespread clinical application is still hampered by lack of standardization. Pre-analytical sample handling and storage procedures play an important role in the reliable measurement of these biomarkers. In the context of an EU Joint Programme - Neurodegenerative Disease Research (JPND) project (BIOMARKAPD), supported by FCT through JPND/0005/2011, we have lead a task on *in vitro* pre-analytical confounders regarding CSF manipulation and storage, that involved four different centers (Coimbra, Portugal; Kuopio, Finland; Copenhagen, Denmark and Lyon, France). We have focused on the influence of spinning conditions of the CSF samples (temperature: RT vs. 4^{o}C; speed: 500g vs. 2000g vs. 3000g), storage volume variation (25%, 50% and 75% of tube total volume) as well as freezing-thaw cycles (up to 5 cycles) in AD-biomarkers quantification. Centrifugation conditions did not influence biomarkers levels, except for samples with a high CSF total protein content, where non centrifugation or centrifugation at RT, compared to 4ºC, led to higher Aβ42 levels. Reducing CSF storage volume from 75% to 50% of total tube capacity, decreased Aβ42 concentration, this reduction being in the analytical CV of the assay, whereas no change in Tau or pTau was observed. Moreover, the concentration of Tau and pTau appeared to be stable up to 5 freeze-thaw cycles, whereas Aβ42 levels decreased if CSF was freeze-thawed more than three times. This systematic study contributes to the establishment of uniformized standard operating procedures that will help reducing inter-lab variability of CSF-AD biomarkers evaluation.

Protein 14-3-3 is a reliable marker of rapid neuronal damage and has been detected in the cerebrospinal fluid (CSF) of several progressive neurological disorders. It is specifically increased in sporadic Creutzfeldt-Jakob disease (CJD) patients, currently being used as a supportive marker for clinical diagnosis. However, detection of 14-3-3 protein is usually performed by Western Blot (WB), which is an expensive and time consuming technique, prone to methodological and inter-rater reliability problems. Moreover, this qualitative assay can lead to inconclusive or borderline results, hampering a final clinical diagnosis. In order to improve the laboratorial diagnosis of sCJD, efforts have been done to develop a quantitative assay. Under the aegis of an EU Joint Programme - Neurodegenerative Disease Research (JPND) project (DEMTEST), supported by FCT through JPND/0001/2011, we have evaluated the diagnostic performance of a recently developed quantitative enzyme linked immunosorbent (ELISA) assay for 14-3-3γ and compared it with the previously established WB technique, as well as with other neurodegeneration related biomarkers (Tau and p-Tau). CSF samples from a total of 145 patients with an initial clinical suspicion of prion disease, further classified as definite sCJD (n=72) or with a final alternative diagnosis of non-prion disease (Non-CJD; n=73) were included in this study. 14-3-3γ levels were significantly increased (p<0,001) in sCJD compared to non-CJD patients, showing a very good accuracy to differentiate between the two groups (AUC = 0.982; sensitivity =97%; specificity =94%). 14-3-3γ ELISA matched the 14-3-3 WB results in 82% of cases and strongly correlated with both Tau and pTau levels (p<0,0001), but showed higher specificity than any other test (14-3-3 WB – 68%; Tau - 90% and pTau/Tau ratio - 88%). In cases with a 14-3-3 WB borderline result (n=44), the 14-3-3γ ELISA correctly classified 41 patients (93%). Additionally, logistic regression analysis identified 14-3-3γ ELISA as the best single predictive marker for sCJD, with an overall accuracy of 95%. In routine sCJD diagnosis, this commercial quantitative assay can be employed as a first test, well standardized and easy to apply, allowing a higher sample throughput and an unequivocal result.

Publications

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Research in neurodegenerative diseases:

Frequency of SQSTM1 mutations in Lewy Body Disease

Miguel Pereira, Isabel Santana, Beatriz Santiago, Ana Gouveia, Maria Helena Pinto, Maria Rosário Almeida

In Lewy body disease (LBD), alpha-synuclein, the main component of the Lewy bodies, is degraded via the ubiquitin-proteosome system and the autophagy-lysosomal system. Several lines of evidence show that in LBD, disrupted proteolysis occurs in which p62 protein might be involved, due to its known role in the clearance of misfolded proteins and aggregation-prone proteins. p62 is present in neuronal and glial ubiquitin-positive inclusions in various taupathies and synucleinopathies and recently using mouse models of LBD that lacks p62, an enhanced α-synuclein pathology has been observed. Therefore, p62 deficiency seems to affect inclusion formation and abnormal protein accumulation. Rare mutations in the sequestosome 1 (*SQSTM1*) gene, which encodes p62 protein, have been recently reported in patients with ALS and FTLD but no studies have been performed yet to test whether mutations in this gene are also a cause of LBD.

In the present work we aimed to investigate the genetic contribution of the *SQSTM1* gene mutations to the LBD etiopathology. Fifty patients with clinical diagnosis of sporadic LBD assisted in the Dementia outpatient clinic of CHUC have been enrolled in the study. The entire coding region of *SQSTM1* gene has been screened for mutations by direct sequencing. Although rare, *SQSTM1* mutations have been identified in our cohort of patients clinically diagnosed with LBD. *SQSTM1* mutations were present in our LBD patients cohort. However, due to the fact that p62 is a multifunction protein mainly involved in clearance of ubiquitinated proteins via autophagy and/or proteosomal degradation, it is predictable its involvement in various neurodegenerative diseases. The previous observation of rare mutations in both FTLD and ALS patiens suggests an involvement of p62 possibly through a common disease pathomechanism. With this study, we wide the spectrum of neurodegenerative diseases caused by p62 mutations and provide further evidence for a putative role of rare mutations in SQSTM1 in the genetic etiology of LBD.

Reverse Translational Biomedical Research in Bigenomic Disorders and Personalized Medicine

Manuela Grazina

Biochemical genetics study in Metabolic and proliferation disorders

Manuela Grazina (FMUC, CNC), Luisa Diogo (CHUC, CNC), Catarina R. Oliveira (FMUC, CNC)

Collaborators: *Carmo Macário, Paula Garcia, Beatriz Costa, Ana Patrícia Domingues, Paulo Moura, Pedro Fonseca, Eduardo Silva (CHUC); Filomena Botelho, Margarida Abrantes (FMUC, IBILI); Filipe Silva, Miguel Castelo Branco (IBILI); Paulo Oliveira (CNC).*

The pathogenic mechanisms underlying mitochondrial respiratory chain (MRC) diseases are still unknown in the majority of cases. The genetic causes of these complex disorders are located either in mtDNA or nuclear DNA, affecting the subunits of MRC system and all factors involved in mitochondrial biogenesis or mtDNA replication, transcription or stability. However, the functional genomics demonstration of pathogenicity has also many gaps to be explained.

Project PTDC/DTP/EPI/0929/2012: Leber´s hereditary optic neuropathy (LHON, ORPHA104, MIM#535000) is a maternally inherited mitochondrial disorder characterized by retinal ganglion cell degeneration leading to optic atrophy, acute onset central bilateral visual loss and blindness, particularly affecting young adult males. It affects both eyes simultaneously or sequentially, with vision loss in the second eye occurring weeks to months after the first. Through the investigation of the "mitochondriome" (interaction of the mitochondrial and nuclear genomes, transcripts and proteins related to

OXPHOS function), we aim to clarify the disease etiology. So far, the mitochondrial DNA investigation was performed in 18 samples, on Baylor College of Medicine - Houston Texas, by Next Generation Sequencing. The results show that 5 patients have one of the three primary mtDNA point mutations: m.11778G>A, m.3460G>A and m.14484T>C. The m.11778G>A mutation, as expected from the literature, is the most frequent, found in 3 patients. The mutations m.3460G>A and m.14484T>C were found each in only one patient. The others 13 patients did not have any primary mutation associated with LHON. The exome analysis is ongoing and the first results are being analysed. The results from the two genomes will be correlated. To date, the study of complexes of MRC was performed on 15 samples, with 9 presenting a deficiency. So far, the technology necessary to evaluate anatomical changes, by multimodal techniques to correlate with function. A patient (homoplasmic mutante for m.11778G>A) and 15 healthy relatives of the same pedigree were analysed. The results revealed that the changes occur in cortical thickness due to the presence of the mutation, suggesting asymptomatic degeneration of ganglion cells associated with regional characteristics of the human visual cortex. These results were described in d'Almeida et al., 2013 and were an important basis for implementation of analysis of brain metabolism by functional imaging. CoQ10 content was analyzed in 8 samples of LHON patients and results suggest decreased content in patients.

In collaboration with Dr. Beatriz Costa (Surgery Department, CHUC), we have analyzed 70 plasma samples of critical surgical/ trauma patients, in order to determine the glutaminemia, argininemia and the plasma arginine bioavailability (PAB) and to analyze its correlation with the prognosis. Our preliminary results indicate the importance of glutamine and arginine determination in the in the context of these patients. Two abstracts were submitted for ESPEN (The European Society for Clinical Nutrition and Metabolism) and an article is in preparation for submission.

A preliminary MRC study for evaluating the effect of some chemical compounds in control and mtDNA-mutated skin derived fibroblasts, in collaboration with Paulo Oliveira (CNC) was initiated, So far 10 samples were analysed.

A collaboration with Filomena Botelho's group was completed and results were published. Our contribution to the work of metabolic effects of hypoxia in colorectal cancer was the evaluation of oxygen consumption (42 samples), complex IV, lactate dehydrogenase and citrate synthase activities (189 samples) in the different cell lines in study. Abrantes et al. (2014) demonstrated that three colorectal cell lines showed differential metabolic behaviors to hypoxia. This distinct responsiveness was determined to be a crucial parameter to be evaluated aiming at the implementation of more efficient anticancer therapeutic strategies.

Publications

Abrantes AM, Tavares LC, Pires S, Casalta-Lopes J, Mendes C, Simões M, Grazina M, Carvalho RA, Botelho MF, "Metabolic Effects of Hypoxia in Colorectal Cancer by 13C NMR Isotopomer Analysis", Biomed Res Int. 2014;2014:759791-759801. doi: 10.1155/2014/759791. Epub 2014 Jul 1.

Bigenomic investigation in Neurodegenerative disorders

Manuela Grazina (FMUC, CNC), Isabel Santana (FMUC, CHUC, CNC), Catarina R. Oliveira FMUC, CNC)

Collaborators: *Beatriz Santiago, Diana Duro (CHUC), Filipe Silva (IBILI)*

Neurodegenerative disorders, particularly dementias, are complex and the mechanisms underlying its phenotypic expression are not clarified. Finding genetic risk factors, from bigenomic origin, will contribute to identify new tools for early diagnosis and contribute for a better knowledge of the underlying causes.

Project PTDC/SAU/EPI/121811/2010: Frontotemporal Lobar Degeneration (FTLD) is the second most common early-onset dementia, clinically heterogeneous and characterized by a progressive decline in behaviour and/or language difficulties. A growing number of evidence implicating mitochondrial DNA (mtDNA) in neurodegenerative diseases, led to the hypothesis about its involvement in FTLD. Mitochondrial dysfunction and oxidative damage have been suggested to have an important role in ageing-related neurodegenerative disorders. One of the possible mechanisms is related to mtDNA alterations that may contribute to MRC impairment. The sequencing of mtDNA genes was performed in 91 FTLD patients. A total of 501 different alterations were found in genes *MT-ND1,2,3,4,4L,5,6, MT-ATP6,8, MT-CO1,2,3, MT-CYB, 22 MT-tRNAs and 2 MT-RNAs*. The majority of the alterations identified have been described as polymorphisms, some are also mutations that have been already associated to other diseases and 7 variations are unpublished variants. Although the majority of these alterations are not pathogenic, an interaction with other mutations may occur, leading to the disease, worsening its expression or influencing age of onset. Complex I activity was also decreased. To our knowledge, this is the first report of complete sequence of the mtDNA genes in FTLD patients and the high number of mtDNA variations in the FTLD population studied, suggests the involvement of mtDNA alterations in this disease.

Personalized Medicine and Pharmacogenomics

Manuela Grazina (FMUC, CNC), Carolina Ribeiro (CNC)

Collaborators: *Ana Valentim, Ana Eufrásio, Teresa Lapa (CHUC); Adrián Llerena and Eva Peñas-Lledó (Univ. Extremadura); João Curto (ARS-C)*

The pharmacogenomics studies have been imple,mented in 2007 in this group and Manuela Grazina is a member of the CEIBA.FP Consortium of the Ibero-American Network of Pharmacogenetics and Pharmacogenomics (RIBEF), by invitation, since February 2012.

The main aim of the research performed is mainly to identify genetic alterations and copy number variation that will determine the metabolic profile or targeting depending on genetics, for providing tools for more rational treatments, managing risks and preventing drug adverse reactions.

Investigation of CYP2D6 copy number and sequence variants (*2, *3, *4 and *10 alleles) to infer susceptibility to Alzheimer's disease related to these gene alterations and metabolic profiles. The results reveal a positive association with the age, age of onset and depression features with alleles *4 and *10, and a positive association between APOE ε4/ ε4 and ultra-rapid metabolizers. These data suggest that genetic variations previously associated to decreased CYP2D6 activity may be a protective factor on the manifestation and progression of Alzheimer's disease.

On the other hand, pharmacogenetic characterization of CYP2D6 and correlation with post-caesarean pain treated with morphine, in Portuguese Caucasian adult women revealed a positive association between CYP2D6 reduced activity and pain.

In 2013 the group published the genetic screening of the Portuguese population and determined the metabolic profiles for CYP2D6 (Albuquerque et al., 2013), aiming to identify profiles for treatment optimization and predicting the risk heterogeneity. This work is an important basis for further comparisons with patients' populations.

Presently, a pharmacogenomic and functional genomics study is ongoing, focused on drug addicts undergoing drug withdrawal with methadone therapy, aiming to understand the genetic factors underlying heterogeneity in detoxification fulfillment. This study will contribute for the development of new therapeutic strategies helping the reintegration of these individuals in the society, with direct impact in public health. So far, the genes HTR2A, COMT and OPRM gene and predicted CYP2D6 metabolic profile have been studied in 95 patients for further analysis and correlation with clinical data. Furthermore, analysis of mitochondrial respiratory chain activity in 24 patients showed a significant reduction of energy production capacity.

These approaches are a step forward in the clinical practice, taking advantage of the most recent techniques in Biochemistry and Molecular Genetics.

Publications

Apellániz-Ruiz M, Inglada-Pérez L, Naranjo MEG, Sánchez L, Mancikova V, Currás-Freixes M, de Cubas AA, Comino-Méndez I, Triki S, Rebai A, Rasool M, Moya G, Grazina M, Opocher G, Cascón A, Ingelman-Sundberg M, Carracedo A, Robledo M, Llerena A, Rodríguez-Antona C. High frequency and founder effect of the CYP3A4*20 loss-of-function allele in the Spanish population classifies CYP3A4 as a polymorphic enzyme. The Pharmacogenomics Journal advance online publication, 4 November 2014; doi:10.1038/tpj.2014.67.

Dermatology Research

Margarida Gonçalo (FMUC,CHUC), Américo Figueiredo (FMUC, CHUC), Teresa Cruz (FFUC, CNC), Bruno Neves (UA), Celeste Lopes (FFUC, CNC)

Like contact sensitizers, we have shown that systemic drugs that cause T-cell mediated cutaneous adverse drug reactions (CARD) also induce an innate immune response in dendritic cells (DC) that very probably enhances their antigen presentation and T cell response involved in CADR.
We have shown that, *in vitro,* some drugs for systemic use, particularly allopurinol/oxypurinol and carbamazepine, exert cytotoxicity on THP-1 cells, with an intensity that seems to correlate with the severity of the CADR they cause. Also, they induce p38 MAPK activation, evaluated by Western Blot, and upregulate the expression of genes coding for DC maturation markers (CD40/CD83), pro-inflammatory cytokine/chemokines (IL-8; IP-10) and the detoxifying intracellular enzyme, hemeoxygenase 1 (HMOX-1), evaluated by real-time RT-PCR. Similarly to contact sensitizers that induce allergic contact dermatitis, a direct activation of monocytic or dendritic cells that participate in antigen presentation may be an important step in the pathophysiology of delayed immune mediated CADR.
At present we are evaluating the *in vitro* effect of concomitant factors (exposure to ROS, LPS and other microbial or viral products or increased temperature) during THP-1 stimulation by drugs. We intend to mimic the *in vivo* setting where a concomitant effect of viral/bacterial infection or chronic disease with high cellular damage have shown to enhance sensitization to systemic drugs and the elicitation of CADR.

Publications

Alergia de contacto a metais num period de 20 anos no centro de Portugal: implicações das directivas europeias.
Vera Teixeira, I Coutinho, M Gonçalo
Acta Médica Portuguesa 2014: 27 (3): 295-303

Allergic contact dermatitis caused by acrylates and methacrylates - a 7-year study.
Ramos L, Cabral R, Gonçalo M.
Contact Dermatitis. 2014: 71: 102-7 May 27. doi: 10.1111/cod.12266.

Patch testing with a textile dye mix - a multicentre study.
Ryberg K, Agner T, Andersen KE, Bircher A, Diepgen T, Foti C, Giménez-Arnau A, Gonçalo M, Goossens A, Johansen JD, Le Coz C, Maibach HI, Bruze M.
Contact Dermatitis. 2014 May 12. doi: 10.1111/cod.12244. [Epub ahead of print]

The EAACI/GA(2) LEN/EDF/WAO Guideline for the definition, classification, diagnosis, and management of urticaria: the 2013 revision and update.
Zuberbier T, Aberer W, Asero R, Bindslev-Jensen C, Brzoza Z, Canonica GW, Church MK, Ensina LF, Giménez-Arnau A, Godse K, Gonçalo M, Grattan C, Hebert J, Hide M, Kaplan A, Kapp A, Abdul Latiff AH, Mathelier-Fusade P, Metz M, Nast A, Saini SS, Sánchez-Borges M, Schmid-Grendelmeier P, Simons FE, Staubach P, Sussman G, Toubi E, Vena GA, Wedi B, Zhu XJ, Maurer M.
Allergy. 2014 Jul;69(7):868-87. doi: 10.1111/all.12313. Epub 2014 Apr 30

Coupled exposure to ingredients of cosmetic products: III. UV filters. Wolfgang Uter, Margarida Gonçalo, Kerem Yazar, Eva-Maria Kratz, Gerd Mildau, Carola Lidén. Contact Dermatitis 2014: 71(3):162-9. doi: 10.1111/cod.12245.

Multicentre patch testing with methylisothiazolinone by the European Environmental and Contact Dermatitis Research Group.
Isaksson M, Andersen KE, Gonçalo M, Goossens A, Gruvberger B, Johansen JD, Maibach HI, Rustemeyer T, Le Coz CJ, White IR, Bruze M.
Contact Dermatitis. 2014 May;70(5):317-20. doi: 10.1111/cod.12220.

Methylisotiazolinoe: second “epidemic” of isothiazolinone sensitization.
Ana Gameiro, I Coutinho, L Ramos, M Gonçalo.
Contact Dermatitis 2014: 70 (4): 242-3. DOI: 10.1111/cod.12200

Repeated open application test with methylisothiazolinone in individuals sensitive to methylchloroisothiazolinone/ methylisothiazolinone.
Marleène Isaksson, Birgitta Gruvberger, Margarida Gonçalo, An Goossens, Christophe-J. Le Coz, Magnus Bruze.
Contact Dermatitis. 2014: 70: 244-46 - DOI: 10.1111/cod.12215

DR(E)SS ao abacavir confirmado por testes epicutâneos.
Leonor Ramos, MM Brites, J Oliveira, M Gonçalo
Revista da Soc Port Dermatol Venereol 2014: 72 (3), 411-6

Systemic drugs inducing non-immediate cutaneous adverse reactions and contact sensitizers evoke similar responses in THP-1 cells.
Gonçalo M, Martins J, Silva A, Neves B, Figueiredo A, Cruz T, Lopes C.
J Appl Toxicol. 2015: 35 (4):398-406. doi: 10.1002/jat.3033.

Sensitization to palladium in Europe
Muris J, Goossens A, Gonçalo M, Bircher AJ, Gimenez-Arnau A, Foti C, Bruze M, Andersen KE, Rustemeyer T, Feilzer AJ, Kleverlaan CJ
Contact Dermatitis 2015:72(1):11-9. doi: 10.1111/cod.12295

Arthritis Research

Fernando Judas (HUC, FMUC), Alexandrina Mdeens (FFUC, CNC) Carlos Cavaleiro (FFUC, CEF), Ali Mobasheri (U. Nottingham,U.K.), Celeste Lopes (FFUC, CNC)

Inflammation and osteoarthritis

In collaboration with the Orthopedic and Bone Bank Departments of CHUC, we are using normal and osteoarthritic (OA) human articular cartilage and chondrocytes to identify molecular mechanisms relevant for the development of target-and pathway-specific drugs to halt the development and/or progression of distinct osteoarthritis (OA) phenotypes. The studies concluded during 2014 showed that i) hyperglycemia and hyperinsulinemia independently induce inflammatory responses in human chondrocytes that can contribute to diabetes-induced OA development and progression; ii) myrcene, a small compound of natural origin, has anti-inflammatory and anti-catabolic activities in human chondrocytes thus showing potential as an anti-osteoarthritic drug.

Publications

Rufino AT, Ribeiro M, Judas F, Salgueiro L, Lopes MC, Cavaleiro C, Mendes AF. 2014. Anti-inflammatory and Chondroprotective Activity of (+)-α-Pinene: Structural and Enantiomeric Selectivity. *J Nat Prod.*, *77* (2): 264–269; doi: 10.1021/np400828x.

Serra D, Rufino AT, Mendes AF, Almeida LM, Dinis TC. 2014. Resveratrol modulates cytokine-induced Jak/STAT activation more efficiently than 5-aminosalicylic acid: an in vitro approach. PLoS One 9(10):e109048.

Rufino AT, Ribeiro M, Judas F, Lopes MC, Mendes AF. 2014. Hyperglycemia-like culture conditions induce IL-1β and TNF-α expression and impair autophagy in human chondrocytes. Osteoarthritis Cartilage, 22 (Suppl.): S165-S166 (P274).

Rufino AT, Ribeiro M, Judas F, Lopes MC, Mendes AF. 2014. Culture of human chondrocytes in high glucose induces inflammatory markers and impairs autophagy. A*nn Rheum Dis* 2014;73:A63, doi:10.1136/annrheumdis-2013-205124.143.

Arthritis Research

Margarida Carneiro (CNC), Pereira da Silva (FMUC; Rheumatology CHUC)

The main objective of the cooperation work between the ImmunoMetabolic Pharmacology Group and the Department of Rheumatology is to clarify the role of CD8+ T lymphocytes in the pathogenesis of rheumatoid arthritis (RA). In particular, we have been focusing on how alterations in functional subsets of CD8+ T lymphocytes and in their capacity to produce cytokines and cytolytic molecules relate to disease activity and severity in rheumatoid arthritis patients. To address this questions, we have collected and analyzed, during the past 5 years, blood and synovial fluid samples from around 100 patients. The results have been recently published as a full report in Arthritis & Rheumatology (doi: 10.1002/art.38941; Accepted manuscript online: 4 NOV 2014 11:07AM EST). We observed that blood CD8+ T lymphocytes from RA patients with active disease exhibited a predominantly effector phenotype, with elevated expression of proinflammatory cytokines when compared to healthy controls. In a state of remission, the same phenotype observed in patients with active disease persisted, including a significant increase in the frequency of activated cells, but with lower cytokine production. Synovial fluid CD8+ T lymphocytes from RA patients presented more robust effector memory and activated profiles compared to the CD8+ T lymphocyte subsets in paired blood samples. Production of cytokines by CD8+ T cells from RA blood was positively correlated to synovial fluid within individual donors. The production of TNFα, IFNγ, and IL-17A by CD8+ T lymphocytes from RA blood positively correlated with the Disease Activity Score in 28 joints. This study has been supported by an unrestricted grant from Abbvie Ltd.

This study on patient samples has proven the essential role of CD8+ T lymphocytes in the pathogenesis of rheumatoid arthritis, which had already been suggested by our previous observations in experimental mouse models of arthritis (K/BxN and collagen-induced arthritis) (doi: 10.1002/art.27729). Hence, paving the way to new research endeavors exploring the manipulation of CD8+ T lymphocytes for diagnostic and/or therapeutic purposes.

Research in brain tumors

Alberto Orfão (CSIC, University Salamanca), Maria Dolores Tabernero (University Hospital, Salamanca), Hermínio Tão (HUC), Olinda Rebelo (HUC), Marcos Barbosa (FMUC, HUC), Anália do Carmo (CNC), M. Celeste Lopes (FFUC, CNC)

In this project, we analysed the incidence of numerical/structural abnormalities of chromosomes in samples of human gliomas by using interphase fluorescence *in situ* hybridization (iFISH). The results revealed complex and heterogeneous cytogenetic profiles in this type of tumors with distinct pathways of clonal evolution being detected, which were associated with both the histopathological subtype and the grade of the tumor.
Gene expression profiles (GEP) of tumor cells were analysed in these samples using cDNA oligonucleotide microarrays, in order to assess the potential impact of individual chromosomal changes and cytogenetic profiles in the tumors-associated patterns of gene expression. The results of this study demonstrated a clear association between the GEP of gliomas and tumor histopathology, and the most discriminating genes between low- and high-grade being genes involved in the regulation of cell proliferation, apoptosis, DNA repair and signal transduction. Regarding the cell signalling transduction pathways, our results performed in glioma cell lines indicate that the activation of PI3K/Akt and MAP kinase signaling pathways contribute to the chemoresistance that characterizes glioma cells.
High-density single-nucleotide polymorphism array (SNP-array) was performed to investigate genome-wide copy number (CN) alterations in glioblastoma multiforme (GBM) samples. We have shown that combining both genomic and transcriptional data to differentiate genes with concordant CN alterations and expression patterns is crucial to disclose which of those genes may have functional relevance in GBM pathogenesis.

Studies of multiparametric flow cytometry were performed to identify and characterize the different cell population coexisting in meningiomas, and their patterns of protein expression. The results suggest the involvement of different signalling pathways in the distinct cytogenetic subgroups of meningiomas, at the same time they would contribute to explain the close association between tumor cytogenetic and patient outcome.

Publications

Balça-Silva J, Matias D, Carmo A, Girão H, Moura-Neto V, Sarmento-Ribeiro A, Lopes MC (2014). Tamoxifen in combination with Temozolomide induce a synergistic inhibition of PKC-pan in GBM cell lines. **Bioch. Biophys. Acta**, 1850: 722-732.

Melo-Lima S, Lopes MC, and Mollinedo F (2014). Necroptosis is associated with low procaspase-8 and active RIPK1 and -3 in human glioma cells. **Oncoscience**, 1(10): 649-664.

Domingues PH, Sousa P, Otero A, Gonçalves JM, Oliveira C, Lopes MC, Orfao A, Tabernero MD (2014). Proposal for a new risk stratification classification for meningioma based on patient age, WHO tumor grade, size, localization, and karyotype. **Neuro Oncol.**;16(5):735-47. doi: 10.1093/neuonc/not325.

Melo CA and Melo CA (2014). RNA mapping: microrna biogenese, Spring New York, vol. 1182, p. 219-226

Carvalho D, Mackay A, Bjerke L, Grundy R, Lopes MC, Reis RM and Jones C (2014). The prognostic role of intragenic copy number breakpoints and identification of novel fusion genes in paediatric high grade glioma. ***Acta Neuropathol. Comm.***, v. 2, n. 1, p. 23-23, doi:10.1186/2051-5960-2-23

Domingues P, Tablas MG, Otero A, Pascual D, Ruiz L, Miranda D, Sousa P, Gonçalves JM, Lopes MC, Orfao A and Tabernero MD (2015). Genetic/molecular alterations of meningiomas and the signalling pathways targeted. **Oncotarget;** review Meningiomas. Ms oncotarget 003725, *(in press)*

Rebelo I, Vital AL, Gonzalez T M, Patino MC, Otero A, Lopes MC, Oliveira C, Domingues PH, Orfao A, Tabernero MD (2015). Molecular and genomic alterations in GBM. **Am. J. Path**. Review gliomas. ref, MS AJP14-0661, *(in press)*

Domingues PH, Teodósio C, Otero A, Sousa P, Gonçalves JM, Nieto AB, Lopes MC, Oliveira C, Orfao A, Tabernero MD (2015). The protein expression profile of meningioma cells is associated with distinct cytogenetic tumour subgroups. **Neuropathol Appl Neurobiol.** 41(3):319-32, doi: 10.1111/nan.12127. PMID:24612434.

Melo-Lima S, Lopes MC and Mollinedo F (2015). ERK1/2 acts as a switch between necrotic and apoptotic cell death in ether phospholipid edelfosine-treated glioblastoma cells. **Pharmacol. Research:** 95, 2-11, DOI: 10.1016/j.phrs.2015.02.007

HIV-1 Vpr variants and disease progression

Using a yeast model to predict AIDS progression

Rui Soares (CNC), Graça Rocha (CHUC, FMUC), Cristina Valente (CHUC), A. Meliço-Silvestre (CHUC, FMUC), António Vieira (CHUC), Andrea Spiegel (CHUC) Teresa Gonçalves (CNC)

The biological functions of HIV-1 Vpr have been involved in the replication and pathogenesis of the virus. Part of this collaboration is an ongoing work aimed to study the correlation, in a population of infected subjects, between the Vpr variant present and disease progression.
During the period considered we worked with the previous gathered samples and clinical data of 167 patients belonging to the following groups: HIV infected, assyntomatic, no therapy needed; HIV infected, assyntomatic, that initiated therapy; HIV infected, under different therapeutic programs. The analysis of Vpr sequences in 80 patients is completed and characterised in terms of the mutation R77Q. We initiated a study aimed to characterise the occurrence of polymorphisms in genes associated with fungal diseases as a marker of susceptibility to fungal infections.

During 2013 the collaborative protocol CHC and FMUC/CNC was transferred to CHUC due to the hospital fusion.

Publication

Soares R, Rocha G, Nogueira C, Meliço-Silvestre A, **Gonçalves T** (2014). R77Q and Q3R HIV1-VPR mutations in an otherwise asymptomatic 5-year-old child with repeated ear infections. JMM Case Reports. doi 10.1099/jmmcr.0.002709

Novel techniques for the diagnosis and treatment of human Infertility

Teresa Almeida Santos (HUC, FMUC), Ana Paula Sousa (HUC, CNC), Marta Baptista (CNC), Raquel Brito (HUC), João Ramalho-Santos (CNC, FCTUC)

Infertility is a growing problem, affection about 15% of couples worldwide. A partnership has been established between CNC and the Assisted Reproduction Laboratory of the University Hospitals of Coimbra (HUC) to develop novel assays to monitor human sperm and oocyte quality with the ultimate goal of improving Assisted Reproduction.

For sperm analysis the focus has been on complementing traditional analysis by including new parameters with a higher predictive value in terms of defining proper sperm function. These parameters include sperm viability, sperm mitochondrial activity, and sperm chromatin status, monitored using simple, easy and quick assays that can be implemented clinically with minimal effort. The collaboration was extended to two other Portuguese labs (University of Oporto and Gaia Hospital) and one in France (Clinique Pasteur, Brest) for a multi-center evaluation and validation of procedures. Papers describing a novel methodology to assess sperm chromatin routinely, and how to correctly determine sperm mitochondrial function have been published, and collaboration with the Computer Science Department (FCTUC) is underway to establish an automated analysis.

In terms of oocyte evaluation novel non-invasive techniques are being pioneered to select the best oocytes (and, ultimately, the best embryos) to be used in Assisted Reproduction.

Additionally results on evaluation of sperm quality have led to a contract with the Pharmaceutical Company INNOTECH to test the effectiveness of contraceptives using techniques developed by the group.

The collaboration also involves improving the cryo-banking and subsequent use of ovarian tissue for patients undergoing chemotherapy, as this type of treatment often leads to female infertility. This approach is labeled “Oncofertility” and the Minister of Health established a National Reference Facility for this purpose in 2014. This facility is located in CHUC (Pediatric Hospital) and involves the scientific consultancy of CNC.

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 in collaboration

Neuroscience and Disease Research Line

Neuromodulation Group

Networks:

Member of the Steering Committee of the European Neuroscience Campus (with Univ. Amsterdan, The Netherlands; Univ. Bordeaux, France; Univ. Zurich, Switzerland; Univ. Gottingen, Germany)

Member of the European Network of Neurosciences Institutes (ENI-Net)

EU Joint Programme – Neurodegenerative Disease Research (JPND, BIOMARKAPD) with Alexandre de Mendonça (Inst. Molecular Medicine, Univ. Lisbon), Magda Tsolaki (Univ. Thessaloniki, Greece), Sermin Genc (Univ. Izmir, Turkey), Anja Simonsen (Univ.Copenhagen, Denmark), Elisabeth Kapaki (Univ. Athens, Greece)

Member of the Coffee and Health Forum managed by the Institute for Scientific Information of Coffee

Research grants:

Joint research project with Ki Ann Goosens and Ann Graybiel (McGovern Institute, MIT, USA)

Ciência sem Fronteiras program with Lisiane Porciúncula (Univ. Federal Rio Grande do Sul, Brazil)

Graduate training:

Co-supervision of a post-doctoral student (Samira Ferreira) with Nuno Sousa (Univ. Minho)

Co-supervision of a PhD student (Silvia Sousa) with Christophe Mulle (Univ. Bordeaux, France)

Co-supervision of a PhD student (Marta Carmo) with Geanne Matos (Univ. Federal Ceará, Brazil)

Co-supervison of a PhD student (Filipe Matheus) with Rui Prediger (Univ. Federal Santa Catarina, Brazil)

Co-supervision of a PhD student (Jimmy George) with Thierry Amédée (Univ. Bordeaux, France)

Co-supervision of a PhD student (Amber Kerkhofs) with Huibert Manvelder (Univ.Amsterdam, The Netherlands)

Co-supervision of a PhD student (Xu Xinliu) with Nelson Rebola (Univ. Bordeaux, France)

Graduate teaching:

Course entitled 'Fronteiras da Ciência', Univ. Federal Santa Maria, Brazil

Synapses Biology Group

Ann Marie Craig, University of British Columbia, Vancouver, Canada

Carlos Pato, University of Southern California, Los Angeles, USA

Chinfei Chen, Harvard Medical School, Boston, USA

Daniel Choquet, Bordeaux Neuroscience Institute, France

Hey-Kyoung Lee, Johns Hopkins University, Baltimore, USA

José Esteban, Centro de Biologia Molecular Severo Ochoa, Madrid, Spain

Laurent Groc, Bordeaux Neuroscience Institute, France

Joint PhD student (Blanka Kellermayer) with Laurent Groc (IINS, Bordeaux), within the European Neuroscience Campus (ENC) PhD program.

Growth Factor Signaling and Brain Ischemia Group

Collaborative publications:

Neto E, Alves C, Sousa D, Alencastre I, Lourenço A, Leitão L, Ryu R, Jeon NL, Fernandes R, Aguiar P, Almeida RD, Lamghari M. Sensory neurons and osteoblasts: close partners in a microfluidic platform. Integr Biol (Camb). 2014 Jun;6(6):586-95.

Pimenta AC, Martins JM, Dourado DFAR, Melo A, Cordeiro MNDS, Almeida RD, Morra G, Moreira IS. Dynamic structure of NGF and proNGF complexed with p75NTR: pro-peptide effect. J Chem Inf Model. 2014 Jul 28;54(7):2051-67.

M Vieira, J Fernandes, L Carreto, B Anuncibay-Soto, M Santos, J Han, A Fernández-López, CB Duarte, AL Carvalho, AE Santos (2014) Ischemic insults induce necroptotic cell death in hippocampal neurons through the up-regulation of endogenous RIP3. Neurobiol Dis. 68, 26-36.

M Mele, L Ribeiro, AR Inácio, T Wieloch, CB Duarte (2014) $GABA_A$ receptor dephosphorylation followed by internalization is coupled to neuronal death in in vitro ischemia. Neurobiol Dis 65, 220–232.

MV Caldeira, IL Salazar, M Curcio, LMT Canzoniero, CB Duarte (2014) Role of the ubiquitin-proteasome system in brain ischemia: Friend or foe? Prog Neurobiol 112, 50-69.

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

Organization of an international PhD course:

"Neuroscience and Mental Health" course, The Doctoral Programme in Health Sciences, PhDHS (http://www.phdhs.org/), Faculty of Medicine, University of Coimbra (5-9th May, 2014).

Participation in international meetings:

Society for Neuroscience Meeting 2014, November 15-19, Washington, DC, USA (1 abstract).

Invited speaker in international meeting:

18th European Bioenergetics Conference (EBEC), 12-17th July, 2014; Faculty of Sciences, University of Lisbon, Lisbon, Portugal (AC Rego invited speaker).

Research collaboration with:

Sandrine Humbert (PhD), Institut Curie, Orsay, France _ study of phosphorylated huntingtin; doctoral work of Carla Lopes.

Frederic Saudou (PhD), Institut Curie, Orsay, France _ study of phosphorylated huntingtin.

Michael Hayden (MD, PhD), The University of British Columbia, Vancouver, Canada _ studies in the YAC128 mice.

Tiago Fleming Outeiro (PhD), University Medizin Goettingen, Goettingen, Germany _ study of phosphorylated alpha synuclein (undergoing); doctoral work of Raquel Pinho.

Collaborative publications:

Silva A., Naia L., Dominguez A., Ribeiro M., Rodrigues J., Vieira O. V., Lessmann V., Rego A. C. (2015) Neurodeg. Dis. (in press).

Naia L., Ferreira I. L., Cunha-Oliveira T., Duarte A. I., Ribeiro M., Rosenstock T. R., Laço M. N., Ribeiro M. J., Oliveira C. R., Saudou F., Humbert S., Rego A. C. (2015) Mol. Neurobiol. 51, 331-348.

Perfeito R., Lázaro D. F., Outeiro T. F., Rego A. C. (2014) Mol. Cell. Neurosci. 62, 51-59.

Lopes C., Ribeiro M., Duarte A. I., Humbert S., Saudou F., Pereira de Almeida L., Hayden M., Rego A. C. (2014) Mol. Neurobiol. 49, 1126-1142.

Neuroendocrinology and Aging Group

On going collaborators:

Carlos Lopez Otin - Departamento de Bioquímica y Biología Molecular Facultad de Medicina, Universidad de Oviedo, Oviedo, Spain. (Project collaborator).

Leonard Guarente - Glenn Laboratory for the Science of Aging at MIT; USA - (Co-supervisor of PhD student)

Licio Velloso - University of Campinas, Brasil (FCT-Capes Project)

Tamas Horvath - Section of Comparative Medicine; Yale School of Medicine PO Box 208016, New Haven, USA (Co-supervisor of PhD student)

Redox Biology and Brain Sensing

Lourenço CF, Ferreira NR, Lukacova N, Barbosa RM and Laranjinha J. (2014) The pattern of glutamate-induced nitric oxide dynamics in vivo and its correlation with nNOS expression in rat hippocampus, cerebral cortex and striatum. Brain Research 1554, 1-11.

In collaboration with Nadia Lukacova from the Institute of Neurobiology, Slovak Academy of Sciences, Soltésovej 4, 040 01 Kosice, Slovak Republic.

Lourenço CF, Santos RM, Barbosa RM, Cadenas E, Radi R and Laranjinha J. (2014) Neurovascular coupling in hippocampus is mediated via diffusion by neuronal-derived nitric oxide. Free Radic Biol Med 73C, 421-429.

In collaboration with Enrique Cadenas from the Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA.

In collaboration with Rafael Radi from the Department of Biochemistry and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la Republica, Montevideo, Uruguay.

List of collaborators and of respective research programmes:

Enrique Cadenas - Dept. Pharmaceutical Sciences, University of Southern California, USA. Nitric oxide in neurodegeneration and aging.

Greg Gerhardt - Dept. Anatomy and Neurobiology, and Center for Microlelectrode Technology (CenMet) University of Kentucky, Lexington, Kentucky, USA. Development of microsensors for nitric oxide measurement in tissues.

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

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

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

Nadezda Lukacova - Institute of Neurobiology, Centrum of Excellence, Slovak Academy of Sciences, Košice, Slovak Republic. Immunolocalization of nNOS in the barin and the correlation with nitric oxide dynamics.

Juan Sastre – Faculty of Pharmacy, University of Valencia, Spain. Prevention of inflammatory processes in the gastrointestinal epithelia by dietary flavonoids.

Biotechnology Research Line

Molecular Biotechnology Group

Collaborative publication:
Cruz R., Huesgen P., Riley S.P., Wlodawer A., Faro, C., Overall C. M., Martinez, J. J. and Simões I.**(2014)** RC1339/APRc from Rickettsia conorii is a Novel Aspartic Protease with Properties of Retropepsin-Like Enzymes. **PLoSPathog** 10(8): e1004324. doi:10.1371/journal.ppat.1004324. (Impact factor 2013: 8.057; Quartile in categories Microbiology, Parasitology and Virology: Q1).

Curto P., Lufrano D., Pinto C., Custódio V., Gomes A.C., Trejo S., Bakás L., Vairo-Cavalli S., Faro C., and Simões I. **(2014)**"Establishing the yeast *Kluyveromyceslactis* as an expression host for production of the saposin-like domain (plant-specific insert) from the aspartic protease cirsin". **Applied and Environmental Microbiology**, 80:86-96. doi: AEM.03151-13. (Impact factor 2013: 3.952; Quartile in category Biotechnology & Applied Microbiology: Q1; Microbiology: Q2).

Collaborative research:
Dr. Alexander Wlodawer, Macromolecular Crystallography Laboratory, NCI-Frederick, USA,
Dr. Alice Y. Cheung, University of Massachusetts at Amherst, Amherst, USA.
Dr. HertaSteinkellner, Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Vienna, Austria
Dr. Juan J. Martinez, Department of Pathobiological Sciences, LSU School of Veterinary Medicine, Baton Rouge, USA
Dr. Pitter Huesgen, Central Institute for Engineering, Electronics and Analytics (ZEA-3), ForschungszentrumJülich, Germany

Computational and Systems Biology Group

Massachusetts Institute of Technology (U.S.A.):

Researchers: Timothy Lu

Project: Developing a synthetic biology *E. coli*-based H_2O_2 sensor with memory

University of Otago (New Zealand):

Researchers: Christine Winterbourn

Project: Characterizing the operation of the Prx2/Trx1/TrxR system in human erythrocytes.

University of Saarland (Germany):

Researchers: Elmar Heinzle

Project: Development and application of a method for profiling mitotic-cycle-dependent metabolism without having to synchronize cells

University of Lleida (Spain):

Researchers: Rui Alves

Project: Uncovering the evolutionary adaptations of protein aminoacid sequence and structure to O_2-rich environments

VIT University (India):

Cooperation in research training of B. Tech. and M. Sc. students

Vectors and Gene Therapy Group

Collaborative publication:

1. A.M. Cardoso, T. Calejo, C. Morais, A.L. Cardoso, R. Cruz, K. Zhu, M.C. Pedroso de Lima, Maria, A. Jurado, B. Nystrøm, Bo, "Application of Thermoresponsive PNIPAAM- PAMPTMA Diblock Copolymers in siRNA Delivery", Molecular Pharmaceutics, 2014, 11 (3), 819-827 (IF 4.8, Q1)
2. Anna-Lena Kjoniksen, Maria Teresa Calejo, Kaizheng Zhu, Ana Maria S. Cardoso, Maria C. Pedroso de Lima, Amalia S. Jurado, Bo Nystrom, Sverre Arne Sande. "Sustained Release of Naltrexone from PNIPAAM microgels", Journal of Pharmaceutical Sciences 103 (2014) 227–234 (IF 3.007, Q2)
3. Gonçalves FA, Costa CS, Fabela IG, Farinha D, Faneca H, Simões PN, Serra AC, Bártolo PJ, Coelho JF. 3D printing of new biobased unsaturated polyesters by microstereo-thermal-lithography. *Biofabrication* 2014;6:035024 (IF 4.302, Q1)
4. Dubinsky AN, Dastidar SG, Hsu CL, Zahra R, Djakovic SN, Duarte S, Esau CC, Spencer B, Ashe TD, Fischer KM, MacKenna DA, Sopher BL, Masliah E, Gaasterland T, Chau BN, Pereira de Almeida L, Morrison BE, La Spada AR. Let-7 Coordinately Suppresses Components of the Amino Acid Sensing Pathway to Repress mTORC1 and Induce Autophagy. Cell Metab. 2014 Oct 7;20(4):626-38. doi: 10.1016/j.cmet.2014.09.001. PMID: 25295787. (IF: 16.7; Q1).
5. Clévio Nóbrega, *Isabel Nascimento-Ferreira, Isabel Onofre, David Albuquerque; Nicole Déglon, Luís Pereira de Almeida. RNA interference mitigates motor and neuropathological deficits in a cerebellar mouse model of Machado-Joseph disease. PlosOne. August 2014 | Volume 9 | Issue 8 | e100086. DOI: 10.1371/journal.pone.0100086.(IF: 3.53; Q1).

Research:
1. SynSpread: 2013 JPND Transnational call: €150.000; Mar 2015-Mar 2018. European network with groups from Luxembourg, and France.
2. Eranet E-Rare4/0003/2012, €141581; Mar 2013 – Feb 2016. European network with german, dutch and israeli groups.
3. FP7-PEOPLE2012-ITN, €209781, 2013 – 2016. (CAFFEIN - Cancer associated fibroblasts function in tumor expansion and invasion).
4. FP7-PEOPLE2012-ITN, 264508 SEVENTH FRAMEWORK PROGRAMME; €211441; Mar 2011 - Mar 2015. Treat PolyQ

Graduate Training:
Advanced course on *Principles and Practice in Drug Development* - MIT-Portugal program and CNC PhD program on *Biomedicine and Experimental Biology* - João Nuno Moreira, Luís Pereira de Almeida, Sérgio Simões and Stan Finkelstein; CNC, March 31st - April 10th.

Biomaterials and Stem Cell-Based Therapeutics

Participation at the international program MIT-Portugal, focus area of bioengineering. Lino Ferreira is contributing for the "Cell and Tissue Engineering" module with Robert Langer (MIT) and Joaquim Cabral/Cláudia Lobato (IST).

During 2014, several networks involving international researchers have been established or continued:

1- Three-dimensional matrices for cell culture and transplantation. 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 (CNC, Portugal), Sezin Aday (CNC, Portugal), Lino Ferreira (CNC, Portugal).

2- Nanomaterials for wound healing. Josephine Blersh (CNC, Portugal), Michela Comune (CNC, Portugal), Veronique Preat (University of Louvain, Belgique), Klaus Liedl (University of Insbruck, Austria), Lino Ferreira (CNC, Portugal).

3- Nanomaterials to modulate cardiac cells. Thomas Braun (Max Planck Institute), Catarina Rebelo (CNC, Portugal), Sónia Pinho (CNC, Portugal), Carolyn Carr (University of Oxford), Lino Ferreira (CNC, Portugal).

4- Cell reprogramming/stem cell modulation. Tariq Enver (University College of London, UK), Carlos Boto (CNC, Portugal), Emanuel Quartin (CNC, Portugal), Ana Lima (CNC, Portugal), Ricardo Neves (CNC, Portugal), DengLi (University of Shanghai), Lino Ferreira (CNC, Portugal).

5- Unraveling the effect of arterial flow in smooth muscle cells derived from induced pluripotent stem cells containing Hutchinson-Gilford Progeria Syndrome (HGPS). Xavier Nissam/ Marc Peschanski (i-Stem, France), Patrícia Pereira (CNC, Portugal), Helena Vazão (CNC, Portugal), Luis Estronca, Lino Ferreira (CNC, Portugal).

6- Cardiac kit. Christine Mummery/Robert Passier (University of Leiden, Netherlands), Leonardo Ricotti/Ariana Menciassi (University of Pisa, Italy), Paula Alves (ITQB, Lisbon), Bernardo Abecassis (ITQB), Pedro Gouveia (CNC, Portugal), Ricardo Neves (CNC, Portugal), Susana Rosa (CNC, Portugal), Lino Ferreira (CNC, Portugal).

7- Cardiac regeneration. Jeffrey Karp (Harvard-MIT Division of Health Science and Technology, USA), Ivana Kostic (CNC, Portugal), Lino Ferreira (CNC, Portugal).

8- In vitro blood-brain barrier models. Romeo Cechelli (University of Lille, France), Sezin Aday (CNC, Portugal), Catarina Almeida (CNC, Portugal), Susana Rosa (CNC, Portugal), Lino Ferreira (CNC, Portugal).

Medicinal Chemistry & Drug Discovery

Collaborative Publications:

Anastácio S; Carolino N; Sidi-Boumedine K; da Silva GJ "Q fever dairy herd status determination based on serological and molecular analysis of bulk tank milk" Transboundary and emerging diseases,2014, doi:10.1111/tbed.12275.

Da Silva G J; Van Der Reijden T; Domingues S; Mendonça N; Petersen P; Dijkshoorn L "Characterization of a novel international clonal complex (CC32) of Acinetobacter baumannii with epidemic potential" Epidemiol. Infect. 2014, 142, 1554–1558. doi:10.1017/S0950268813002288

Inwezerua C; Mendonça N; Calhau V; Domingues S; Adeleke OE; da Silva GJ "Occurrence of extended-spectrum beta-lactamases in human and bovine isolates of Escherichia coli from Oyo state, Nigeria" J Infect Dev Ctries, 2014, 8(6):774-779. doi:10.3855/jidc.3430

Salvador JAR; Santos,Rita C; Figueiredo SA C; Jing YK "Antitumor Effects of Celastrol and Semi-Synthetic Derivatives" Mini-Reviews in Organic Chemistry, 2014, 11, 400-407.

Research, Graduate Training Networks:

FCT: SFRH/BD/77823/2011, Coxiella burnetii and Q Fever: an emergent zoonosis in Portugal - Co-supervisor: Dr. Karim Sidi-Boumedine, DVM, PhD, Co-Head of the National Reference Laboratory on Q fever, French Agency for Food, Environmental and Occupational Health Safety (ANSES), Sophia-Antipolis, France

FCT: SFRH/BD/78833/2011, Microarray-based detection of antibiotic resistance and virulence factors genes of Salmonella spp. isolated from food-producing animals and processed food - Co-supervisor: Dr. Muna Anjum, Honorary Associate Professor, Molecular Lead: Antimicrobial resistance and enteric pathogens, Dept. of Bacteriology, Animal and Plant Health Agency, Woodham Lane, London, United Kingdom

Microbiology of Extreme Environments

The Principal Investigator is the Chair of the International Committee on the Systematics of Prokaryotes (ICSP) since July 2014 that is part of the International Union of Microbiological Societies (IUMS).

As can be noted from the publications almost all are in collaboration with microbiologists or chemists from other countries, which indicate that there is much interest and internationalization.

At this time and since the middle of 2014 one Ph. D. students (Tânia Leandro) working with Professor Ricardo Amils of the Severo Ochoa Institute of Universidad Autonoma de Madrid on the biodiversity of deep borehole aquifers (about 600 meters) in the Iberian Pyrite Belt. This work involves isolation of organism and in situ metagenome analysis to be followed by physiological analysis of autotrophy in this lab.

Medical Microbiology

Professor Neil AR Gow, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK

Fernandes C, Anjos A, Walker LA, Silva BMA, Cortes L, Mota M, Munro CA, Gow NAR, **Gonçalves T** (2014). Modulation of *Alternaria infectoria* cell wall chitin and glucan synthesis by cell wall synthase inhibitors. Antimicrob Agents Chemother 58(5):2894. doi: 10.1128/AAC.02647-13

Professor JR Meyer-Fernandes (Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazi)

Rodrigues L, Russo-Abrahão T, Cunha RA, Gonçalves T, and Meyer-Fernandes JR (2015b). Characterisation of extracellular nucleotide metabolism in Candida albicans. Submitted to *Journal of Biological Chemistry*

Professor Arturo Casadevall, Albert Einstein School of Medicine, NY, USA

Paper under submission process:

Fernandes C, Prados-Rosales R, Silva BMA, Nakouzi-Naranjo A, Chatterjee S, Stark RE, Eusébio E, Casadevall A, Gonçalves T. DHN-melanin characterization and biosynthesis in *Alternaria infectoria*.

Molecular Mycobacteriology

The Group was formed in 2014 and students received training in laboratories abroad in the scope of new collaborations relevant to our research goals:

Ana Maranha worked at the University of Guelph, Canada, for 6 months (collaboration with Prof. Anthony Clarke). The results were submitted for publication: Maranha A, Moynihan PJ, Miranda V, Correia E, Nunes-Costa D, Fraga J, Pereira PJ, Macedo-Ribeiro S, Ventura R, Clarke AJ, Empadinhas N. Octanoylation of early intermediates of mycobacterial MGLP. Submitted to PNAS.

Vitor Mendes develops post-doctoral work at the University of Cambridge, UK, under a collaboration established with Prof. Tom Blundell, which pioneered structure-guided drug discovery and is focused on new anti-TB drugs with the Bill & Melinda Gates Foundation.

New approaches to fight TB were explored in a joint publication (review) with Dr. Carla Bento at Department of Medical Genetics, University of Cambridge: Bento CF, Empadinhas N, Mendes V. Autophagy in the fight against tuberculosis. DNA & Cell Biology. doi: 10.1089/dna.2014.2745.

We isolated multidrug-resistant mycobacteria from a hospital in Coimbra and initiated collaboration with Prof. Margareth Dalcolmo (Fiocruz, Brasil). A manuscript was submitted: Nunes-Costa D, Alarico S, Dalcolmo M, Correia-Neves M, Empadinhas N. The rising tide of nontuberculous mycobacterial infections in Portugal and Brazil. (Submitted to Emerging Infectious Diseases).

Sónia Pereira was at BIDMC, Harvard University for 6 months to investigate microbial biofilms (with Prof. Janice Zabolotny). The manuscript “Microbiome dynamics and immune response in diabetic foot ulcers: new opportunities for wound healing” will be submitted soon for publication.

In 2014 we joined an H2020-PHC2015 consortium and submitted the application “Find2Care - Fluorescence Imaging Assisted Infection Diagnostics in Diabetic Foot Care”, which recently reached the 2nd stage. We coordinate WP1 “Diabetic ulcer microbiome profiling”.

Metabolism, aging and Disease Research Line

Biology of Reproduction, and Stem Cells

Ongoing International collaborations include:

Sperm Proteomics and Metabolomics (University of Barcelona, Spain). Collaboration with Rafael Oliva. Group Members involved: Alexandra Amaral, Carla Paiva.

Novel methods for sperm quality assessment (University of Muenster, Germany). Collaboration with Stefan Schlatt/Con Mallidis. Group Member involved: Sandra Amaral.

Xenografting and Male fertility preservation in humans and endangered species (University of Muenster, Germany). Collaboration with Stefan Schlatt. Group Member involved: Paula Mota.

Internationalization also involves PhD students doing collaborative work and/or being co-supervised with other Researchers, that do a large part of their work abroad:

Beatriz Lacerda: Regulation of stem cell pluripotency by NRF-1 (University of California-San Francisco, USA). Collaboration with Miguel Ramalho-Santos.

Marília Cordeiro: Ovarian follicle dynamics (Northwestern University, USA). Collaboration with Teresa Woodruff.

Carla Paiva: Comparative sperm proteomics and relation to metabolism and movement (University of Barcelona, Spain). Collaboration with Rafael Oliva.

Tânia Perestrelo: Physical properties and their role in stem cell pluripotency (John Hopkins University, USA). Collaboration with Denis Wirtz.

Ângela Crespo: NK cells and maternal-fetal immunity (Harvard University, USA). Co-supervision with Jack Strominger.

Cell Metabolism and Quality Control

Russell H Swerdlow (Kansas University, USA);

Marcia Haigis (Harvard Medical School, USA)

Merari Ferrari (Instituto de Biociências – USP, Brasil);

Laszlo Otvos (Temple University, Philadelphia, USA);

Catherine Lawrence (University of Manchester, UK);

William L. Klein (Northwestern University, Evanston, USA);

Jeroen Hoozemans (VU University Medical Center, Amsterdam, Netherlands);

Carmen García-Rodriguez (CSIC-University of Valladolid, Spain);

Maurício Sforcin (Instituto de Biociências, UNESP, Brasil);

Ali Mobasheri (University of Nottingham, UK);

Francisco Blanco (Centro Hospitalario Universitario A Coruña, Spain);

George Perry (University of Texas at San Antonio, USA);

Xiongwei Zhu (Case Western Reserve University, USA);

David Busija (Tulane University School of Medicine, USA);

James Bennett (Virginia Commonwealth University, USA);

Afshan Malik (School of Medicine, King's College London, UK);

Frederick Bellinger (John A. Burns School of Medicine, University of Hawaii, USA);

Maria Björkqvist (Lund Medical School, Sweden)

Mitochondria Metabolism and Disease Group

Visiting researchers:

Alecsandra Souza (University of São Paulo, Brazil)
Lílian Pereira (University of São Paulo, Brazil)
Irina Starostina (Kazan Federal University, Russia)
Vilena Ivanova (Kazan Federal University, Russia)

Collaborations

Albert Rizvanov, Kazan Federal University, Russia (P. Oliveira)
Anatoly Zhitkovich, Brown University, USA (C. Alpoim)
Anika Hartz, Bjorn Bauer, University of Kentucky, USA (V. Sardão)
Clemens Steegborn, University of Bayreuth, Germany (C. Palmeira, A. Rolo)
Daniel Dorta, University of São Paulo, Brazil
David Sinclair, Harvard Medical School, USA (C. Palmeira/A. Rolo)
Edward Perkins, Mercer University, USA (P. Oliveira)
Faustino Mollinedo, CSIC, Spain (P. Oliveira)
Ignacio Vega-Naredo, University of Oviedo, Spain (P. Oliveira)
Kendall Wallace, University of Minnesota, USA (A. Rolo, C. Palmeira, P. Oliveira)
Jan Kopecky, Academy of Sciences, Czech Republic (C. Palmeira, A. Rolo)
Jiiri Neuzil, Griffith University, Australia (P. Oliveira)
Joan Rosselo, CSIC, Spain (C. Palmeira, A. Rolo)
John Wise, University of Maine, Portland (C. Alpoim)
Maria Almeida, University of Arkansas, USA (V. Sardão)
Mariusz Wieckowski, Nenki Institute, Poland (P. Oliveira)
Mark Nijland, Laura Cox, Peter Nathanielsz, University of Texas Health Science Center, USA (P. Oliveira)
Michael Sack, NHLBI, National Institutes of Health, USA (P. Oliveira)

Nika Danial, Dana-Farber Cancer Institute, USA (C. Palmeira)
Patricia Scott, Jon Holy, Pavel Krasutsky, University of Minnesota, USA (P. Oliveira)
Piero Portincasa, University of Bari, Italy (P. Oliveira)
Saber Hussain, Wright State University, USA (C. Palmeira)

Obesity Diabetes and Complications

The group has a broad range of international active collaborations in the different fields, we collaborate with Dr. A. Veves & Dr J. Zabolotny, at Harvard Medical School, USA, for the study of inflammation and wound healing and to gain experience working with transgenic animal models. Dr Veves is Research Director at the Beth Israel Deaconess Medical Center Foot Center and Microcirculation Lab Harvard Medical School, his particular interest is in wound healing in diabetes and is involved in both basic research in animal models and particularly in translational research that involves human subjects. With him we learn techniques in the field of wound healing in human subjects, particularly, the Doppler and laser Doppler imaging technique to evaluate the microvascular function of diabetic patients and the Medical Hyperspectral Imaging technique to evaluate the skin oxigenation in patients. Dr. Zabolotny's laboratory is in the Division of Endocrinology, Diabetes, and Metabolism at Beth Israel Deaconess Medical Center and Harvard Medical School. Dr. Zabolotny's group is focused on understanding the molecular mediators of insulin and leptin resistance in obesity, and impaired wound healing in diabetes and inflammatory bowel disease, with a particular focus on the role of inflammation in the pathogenesis of these disorders. Her group has significant experience in generating and studying transgenic and knockout mouse models. We have several students perform part of their studies in their laboratories, and some of their travel expenses have been paid by fellowships from the European Foundation for the Study of Diabetes.

In addition we also collaborate with Prof. J. Eriksson, Global Medical Science Director (executive level) Global Medicines Development, Cardiovascular/Gastrointestinal, Clinical Discovery, AstraZeneca R&D in Sweden, a specialist in Internal medicine and in Endocrinology (including diabetology). With him we have been investigating the role of the immunosuppressive agents, rapamycin, cyclosporin A and tacrolimus in lipolysis and their effects in altering the expression of genes involved in lipid metabolism in human adipose tissue. In his laboratory we have had a PhD student, Maria Joao Pereira, who has just defended her thesis.

Moreover, our collaboration with Prof A. Valverde, at the Instituto de Investigaciones Biomedicas Alberto Sols, Spain, is related to insulin action, insulin resistance and brown adipocytes. We presently have a Master student at her lab to perform part of his studies on brown adipocytes regarding their modulation by immunosuppressive agents. Finally with Prof G. Lopaschuk, at the University of Alberta, Canada, who is an expert on the heart, we are performing heart studies on human epicardial fat tissue. We have recently published a review together "Cherian S, Lupaschuk DG and **Carvalho E**. Cellular cross-talk between epicardial adipose tissue and myocardium in relation to the pathogenesis of cardiovascular disease. *Am J Physiol Endocrinol Metab. 2012 Oct;303(8):E937-49: IF: 4.7*".

More recently we have initiated a collaboration with the research group of Dr Louise Torp Dalgaard at Roskilde University, Roskilde, Denmark, who's specialties are in depth knowledge of metabolism, type 2 diabetes, obesity, beta-cell dysfunction, gene-expression, microRNAs and uncoupling proteins. With her laboratory we are studying the role of microRNAs in wound healing in our models.

Actively involved in the organization of 2014-2015: H2020 consortia:

1. PHC 11 -2015: The development of innovative *in vivo* imaging tools and technologies.

2. PHC-03-2015: Understanding common mechanisms of diseases and their relevance in co-morbidities.

Intermediary Metabolism Group

Contract and collaboration with Prof Michael Roden of the German Diabetes Foundation (DDZ) for ^{2}H NMR analysis of plasma and urine samples to quantify hepatic gluconeogenesis from a study of healthy subjects infused with different lipid mixtures and administered with deuterated water.

Collaboration with Radboud University Medical Center, Nijmegen on *in vivo* measurement of hepatic lipid fluxes during high fructose feeding resulted in one publication during 2013 a newly accepted paper: Nunes. P.M., Wright, A.J., Veltien, A., van Asten, J.J.A., Tack, C.J., Jones, J.G. and Heerschap, A. 2014. Dietary lipids do not contribute to the higher hepatic triglyceride levels of fructose compared to glucose fed mice. FASEB J. (*in press*).

Participation in the organization of scientific meetings

March 2014

Seminar "Tales from the crypt, characterization of candidate colorectal cancer genes"
Date: March 12, 2014
CNC members involved in the organization: Paulo Oliveira

April 2014

Member of the Organizing Committee of the Bergey's International Society for Microbial Systematics (BISMiS)
Date: April 7 -10, 2014
CNC members involved in the organization: Milton Costa

Integrative genomics of ageing: New approaches for an "old" problem
Date: April 24, 2014
CNC members involved in the organization: Claudia Cavadas

Workshop "Mitochondrial Translational Medicine", part of the Annual Meeting of the European Society of Clinical Investigation
Date: April 28-May 2, 2014
CNC members involved in the organization: (P Oliveira, C Palmeira)

May 2014

Organization of the Seminar "Brain and language"
Date: May 9, 2014
CNC members involved in the organization: Cristina Rego

June 2014

Symposium Showcasing Portuguese Pharma Science: Advances in Discovery, Development and Manufacturing. Hovione and MIT-Portugal
Date: June 30th, 2014
CNC members involved in the organization: João Nuno Moreira

July 2014

Organization of a symposium entitled 'Purine Receptors in Neuroinflammation and neuro-degeneration' at Nucleotides, Nucleosides and Nucleobases International Conference on Signalling, Drugs and Targets
Date: July 2014
CNC members involved in the organization: Rodrigo Cunha

SIRTUINS, NAD, AND HEALTH
Date: July 1, 2014
CNC members involved in the organization: Claudia Cavadas

Organization of the Seminar "Familial Amyloidotic Polyneuropathy: from bench to bedside" (IBMC), Universidade do Porto, Portugal)
Date: July 11, 2014.
CNC members involved in the organization: Cristina Rego

Member of the Scientific Committee of the Congress of the International Union of Microbiological Societies (IUMS), Montreal, Canada
Date: July 27 to August 1, 2014
CNC members involved in the organization: Milton Costa

Chair of the Symposium on Environmental and Applied Metagenomics. International Congress of Bacteriology and Applied Microbiology (BAM), International Union of Microbiological Societies (IUMS), Montreal, Canada
Date: July 27 to August 1, 2014
CNC members involved in the organization: Milton Costa

September 2014

Organization of seminar "Transplantation of bone marrow mesenchymal stem cells decreases oxidative stress, apoptosis, and hippocampal damage in brain of a spontaneous stroke model to healthy levels"
Date: September 9, 2014.
CNC members involved in the organization: Cristina Rego

Member of the Organizing Comittee of the International Symposium on Medicinal Chemistry, ISMC, of the European Federation of Medicinal Chemistry, EFMC, Lisbon, Portugal
Date: September 9-11, 2014
CNC members involved in the organization: M Luisa Sá e Melo

President of the Organizing Comittee of the 4th ENOR Symposium, on behalf of the European Network for Oxysterol Research, on "Translational Research on Oxysterols", Coimbra, Portugal
Date: September 18-19, 2014
CNC members involved in the organization: M Luisa Sá e Melo

October 2014

Seminar "Acquisition of mtDNA by cancer cells with damaged mitochondrial genome causes restoration of mitochondrial function and tumour-initiating activity"
Date: October 20, 2014
CNC members involved in the organization: Paulo Oliveira

November 2014

Workshop "Integrating Biomarkers and Cell Physiology for Metabolic Profiling: Next Generation Approaches and Technologies"
Date: November 21, 2014
CNC members involved in the organization: Paulo Oliveira

Biocant Workshop Co-organizer: "Integrating Biomarkers and Cell Physiology for Metabolic Profiling: Next Generation Approaches and Technologies"
Date: November 21, 2014
CNC members involved in the organization: John Jones

Heart without Borders"- International conference in cardiovascular development, disease and repair
Date: November 28-29, 2014
CNC members involved in the organization: Lino Ferreira

December 2014

Organization and host of Seminar Neuro-skeletal network and bone repair
Date: December 12, 2014
CNC members involved in the organization: Claudia Cavadas

Organization of the XVIII Congress of the Portuguese Biochemical Society
Date: December 17-20, 2014
CNC members involved in the organization: P Oliveira

GRADUATE STUDIES PROGRAMME

During 2014 CNC organized 6 Advanced Courses and hosted 62 seminars. Local graduate students and researchers attended the seminars, whereas the advanced courses also met the interest of people from other Portuguese Universities. Besides the organization of courses and seminars, CNC also supported ongoing research work for Ph.D. and M.Sc. theses. Throughout this year 27Ph.D. and 40 M.Sc. theses 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 foreign scientists in collaboration with local investigators, laboratory rotations and research work to be carried out within international networks organized by CNC. Students from the European Neuroscience Campus (ENC) Erasmus Mundus PhD and PhD students from several Marie Curie International Training Networks (ITNs) in which CNC is a partner, and who perform part of their work at the Institute, are also enrolled in PDBEB.

Advanced Courses 2014

Neuronal Circuits and Behavior
January 20 - 31
João Peça

Functional Neuroanatomy
February 10 - 20
Emília Duarte

Speed Dating with Cancer: basic bioloy and beyond
February 21 - 26
Giuseppe Viglietto, Ana Sofia Ribeiro, Maria José Oliveira, Carmen Jerónimo
Sérgio Dias, João Nuno Moreira, Raquel Seruca

Hands-on course on Microscopy Imaging Analysis
March 12 - 14
Luísa Cortes

Neuroscience Course 2014 [MIT-Portugal PhD Program in Bio-Engineering]
March 17 - 28
Ana Luísa Carvalho

Principles and Practice in Drug Development
March 31 - April 10
João Nuno Moreira, Luís Pereira de Almeida, Sérgio Simões

Seminars

January

Excellent Science in Horizon 2020: ERC and Marie Curie Actions
2014.1.10
Elsa Henriques
Fundraising & Project Management
Center for Neuroscience and Cell Biology
University of Coimbra
Coimbra, Portugal

Role of adenosine A2A receptor in the control of synaptic plasticity in the striatum
2014.1.17
Daniel Rial
Neuromodulation Research Group
Center for Neuroscience and Cell Biology
University of Coimbra
Coimbra, Portugal

Alzheimer´s disease: the brain diabetes
2014.1.24
Sónia Correia
Molecular Mechanisms of Disease Research Group
Center for Neuroscience and Cell Biology
University of Coimbra
Coimbra, Portugal

Confocal Spectral Microscopy Leica : the next dimension
2014.1.27
Leica Microsystems

T-cell response in normal and pathological wound healing
2014.1.31
João Moura
Center for Neuroscience and Cell Biology
University of Coimbra
and Instituto Politécnico de Viana do Castelo (IPVC)

February

IEC: a new strategy to engage citizens in Science
2014.2.7
Ana Carvalho and Sónia Ferreira
Instituto de Educação e Cidadania (IEC)
Mamarrosa, Portugal

Methamphetamine: speeding into neurochemical and behavioral crash
2014.2.14
Frederico Pereira
Pharmacology and Experimental Therapeutics Research Group
IBILI, Faculty of Medicine
University of Coimbra
Coimbra, Portugal

Towards the Aβ aggregation mechanism and the role of inhibitors
2014.2.18
Sara Linse
Department of Biochemistry and Structural Biology, Lund Univesity
Lund, Sweden

March

ERC Grant Funding Schemes – further insights (what's new; how to apply; evaluation criteria & procedures; lessons from previous calls)
2014.3.7
Elsa Henriques
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Spatial and temporal regulation of axon guidance
2014.3.14
Alyson Fournier
Montreal Neurological Institute
McGill University
Montréal, Canada

Isotopic tracing of nucleic acids to probe metabolic heterogeneity over cell division cycle
2014.3.14
Inês Miranda
Molecular Systems Biology Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Immunologic aspects of sport
2014.3.21
Ana Teixeira
Faculty of Sport Sciences and Physical Education
University of Coimbra
Coimbra, Portugal

Disruption of hippocampal function using pharmacogenetics
2014.3.26
Carmen Varela
Picower Institute for Learning and Memory, MIT
Cambridge, USA

Involvement of adenosine A2A receptors in the Aβ1-42-induced impairment of mouse hippocampal LTP
2014.3.28
João Pedro Lopes
Neuromodulation Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

April

Progenitor endothelial cells (EPCs) and Physical Exercise
2014.4.4
Paula Tavares
Faculty of Sport Sciences and Physical Education
University of Coimbra
Coimbra, Portugal

Neuropeptide Y, a caloric restriction mimetic, induces autophagy in hypothalamic neurons through a MTOR-independent pathway
2014.4.11
Célia Aveleira
Neuroendocrinology and Aging Research Group
Center for Neuroscience and Cell Biology (CNC)

Integrative genomics of ageing: New approaches for an "old" problem
2014.4.24
João Pedro Magalhães
Integrative Genomics of Ageing Group
University of Liverpool
United Kingdom

Building predictive oncology models
2014.3.31
Darrin Stuart
Novartis Institutes for Biomedical Research
USA

May

Positioning the cell nucleus for cell migration and muscle function
2014.5.9
Edgar R. Gomes
Instituto de Medicina Molecular (IMM)
Faculty of Medicine, University of Lisbon
Lisbon, Portugal

The nitrate-nitrite-NO pathway and gut microbiota on gastric protein nitration: implications for peptic ulcer disease
2014.5.16
Bárbara Rocha
Redox Biology and Brain Sensing Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Synapse specific modulation of inhibitory synaptic transmission by adenosine at the hippocampus
2014.5.23
Ana M. Sebastião
Instituto de Medicina Molecular (IMM)
Faculty of Medicine, University of Lisbon
Lisbon, Portugal

The role of stargazin in experience-dependent plasticity
2014.5.30
Susana Louros
Synapse Biology Research Group
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

June

Manipulating interspecies quorum sensing in bacterial consortia
2014.6.6
Karina Xavier
Instituto Gulbenkian de Ciência (IGC)
Oeiras, Portugal

The Crosstalk between reprodutive senescence and Alzheimerr disease hallmarks in type 2 diabetic brain: a role for peripheral exendin-4 therapy?
2014.6.13
Ana Duarte
CNC, University of Coimbra

Bending membranes to make vesicles: a role for the BAR domain protein PICK1 in vesicle biogenesis
2014.6.20
Paulo Pinheiro
CNC, University of Coimbra

Modulation of GABAA receptors and neuronal death in brain ischemia
2014.6.27
Miranda Mele
CNC, University of Coimbra

July

SIRTUINS, NAD, AND HEALTH
2014.7.1
Leonard Guarente
Novartis Professor of Biology and
Director of the Glenn Labs for the Science of Aging
MIT

Familial amyloidotic polyneuropathy: from bench to bedside
2014.7.11
Maria João Saraiva
IBMC and ICNAS
University of Porto

September

Transplantation of bone marrow mesenchymal stem cells decreases oxidative stress, apoptosis, and hippocampal damage in brain of a spontaneous stroke model to healthy levels
2014.9.9
Clélia Bertoncini
CEDEME, Federal University

Say Cheese
2014.9.10
Isaura Simões
CNC, UC-Biotech

DNA methylation in cognitive functions
2014.9.12
Ana M. M. Oliveira
Neurobiology Department,
University of Heidelberg, Germany

Carrier Devices for Target Delivery
2014.9.17
Eduardo Silva
Department. of Biomedical Engineering
University of California, Davis, USA

Uncovering and Characterizing the Phenotypic Repertoire of Complex Biochemical Systems
2014.9.19
Michael A. Savageau
Biomedical Engineering Department and Microbiology Graduate Group, University of California – Davis, USA

The stressed brain
2014.9.19
Nuno Sousa
ICVS, School of Health
Sciences, University of Minho

Noninvasive Studies of Metabolism Using Deuterated Water and Deuterium NMR
2014.9.24
John Jones
CNC, UC-Biotech

The Potential Long-term Health Impacts of the Gulf of Mexico Oil Crisis: Insights from Whale Cells and Tissues
2014.9.25
John Wise
Director, Maine Center for Toxicology and Environmental Health
Professor of Toxicology and Molecular Epidemiology
Department of Applied Medical Sciences
University of Southern Maine, Portland, USA

Life factors and hippocampal adult neurogenesis in animal models of Alzheimer´s disease
2014.9.26
Jorge Valero
CNC, University of Coimbra

Insights into the Mechanism of Chromium Carcinogenesis: Are Increased Chromosome Instability and DNA Repair Deficiency Permanent, Heritable Cellular Changes?
2014.9.26
Sandra Wise
Program Director, Wise Environmental & Genetic Laboratory
Department of Applied Medical Sciences
University of Southern Maine, Portland, USA

October

Microsystems for biomedical applications: from advanced in vitro diagnostics to in vivo neuronal signal detection
2014.10.3
Luís Ferreira Moita
Instituto Gulbenkian Ciência

Anthracyclines Induce DNA Damage Response-Mediated Protection against Severe Sepsis
2014.10.8
Luís Ferreira Moita
Instituto Gulbenkian Ciência

Alteration in trophic support and neuronal death in brain ischemia
2014.10.10
Michele Curcio
CNC, University of Coimbra

Disease-mediated GPCR oligomer dynamics in the brain
2014.10.10
Francisco Ciruela
University of Barcelona

Modulation of neuronal mitochondrial dynamics, autophagy and huntingtin proteostasis by selective HDAC inhibitors
2014.10.17
Jorge A. Oliveira
REQUIMTE, Faculty of Pharmacy,
University of Porto

Strength in numbers: Understanding microbial communities in health and disease
2014.10.17
João Xavier
Memorial Sloan Kettering Cancer Center,
New York, USA

Acquisition of mtDNA by cancer cells with damaged mitochondrial genome causes restoration of mitochondrial function and tumour-initiating activity
2014.10.17
Jiri Neuzil
Griffith University, Southport, Qld, Australia
& Academy of Sciences of the Czech Republic, Prague, Czech Republic

From Bench to Bedside: the example of LUZ11
2014.10.22
Luís Arnaut
Chemistry Department, University of Coimbra

Effect of the cigarette smoke exposure on the metabolic profile of Wistar rats
2014.10.22
Prof. Dr. Patricia Monteiro Seraphim
Faculty of Sciences and Technology
Paulista State University – Presidente Prudente Campus
Presidente Prudente, São Paulo, Brasil

Ataxin-2 as a molecular target in Machado-Joseph disease: from translation modulation to disease alleviation
2014.10.24
Clévio Nóbrega
CNC, University of Coimbra

Insulin Clearance (Dys)Regulation: clarifying the mist
2014.10.31
Paula Macedo
CEDOC, NOVA Medical School, Lisbon

November

Modulation of cell stemness and differentiation by biochemical and mechanical factors
2014.11.5
Mário Grãos
Cell Biology
Biocant, Cantanhede, Portugal

Next Wondrous Wednesdays
2014.11.5
Prof. João Nuno Moreira
On the evaluator's perpective.

Next Wondrous Wednesdays
2014.11.5
Dr. Rui Travasso
On the applicant's perspective

Design of antimicrobial and biocompatible nanoparticles with in-vivo antimicrobial efficacy on a systemic infection model
2014.11.7
Akhilesh Rai
Center for Neuroscience and Cell Biology (CNC), UC-Biotech
University of Coimbra
Biocant-Center of Innovation and Biotechnology
Cantanhede, Portugal

Fighting disease at a molecular level: Snapshots of targets provide the framework for rational drug design
2014.11.14
Pedro J. B. Pereira
Institute for Molecular and Cell Biology (IBMC)
University of Porto
Porto, Portugal

Bio-inspired Optimization
2014.11.20
Francisco Pereira
Coimbra Institute of Engineering, (ISEC-IPC) & Evolutionary and
Centre for Informatics and Systems of the University of Coimbra (ECOS-CISUC)
Coimbra, Portuga

Carotenoids and Cardiovascular Disease
2014.11.21
Tatiana Emanuelli
Federal University of Santa Maria, Santa Maria, Brasil
and
CNC, University of Coimbra, Portugal

Dissecting the pancreas genetic networks in zebrafish
2014.11.28
José Bessa
Institute for Molecular and Cell Biology (IBMC)
University of Porto
Porto, Portugal

December

From the idea to entrepreneurship: the role of science
2014.12.3
André Gomes
Crioestaminal
Cantanhede, Portugal

MICC-CNC has new "toys
2014.12.5
Luísa Cortes
Microscopy Imaging Center of Coimbra (MICC)
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

Neuro-skeletal network and bone repair
2014.12.12
Meriem Lamghari
New Therapies Group
Institute for Molecular and Cell Biology (IBMC)
Institute of Biomedical Engineering (INEB)
University of Porto
Porto, Portugal

Title not available
2014.12.17
Raquel Seruca
IPATIMUP
Porto, Portugal

Mitochondrial dysfunction in metabolic disease: the role of miRNA
2014.12.19
Filipe Duarte
Center for Neuroscience and Cell Biology (CNC)
University of Coimbra
Coimbra, Portugal

PhD thesis concluded in 2014

Ana Cardoso
Nucleic Acid Delivery Systems: From Biophysics to Biological Activity
Mª Amália Jurado

Ana C Fonseca
Amyloid-beta peptide as a trigger of ER stress-mediated autophagy in endothelial cells: relevance to Alzheimer's disease etiopathogenesis
Doutoramento em Biologia Celular, Faculdade de Ciências e Tecnologia, Universidade de Coimbra.
Cláudia Pereira

Ana Sofia Rodrigues (Cell Biology)
João Ramalho

Ana Tellechea
Cellular and Molecular Mechanisms of Impaired Wound Healing in Diabetes: understanding the role of Substance P and Mast Cells
Dec 2014
Eugenia Cavalho

Carlos Manuel Almeida Guedes de Melo
Long noncoding RNAs at the service of p53
Dezember 2014
Celeste Lopes

Carlos Rodrigues
Hexavalent Chromium and Lung Cancer: A View to a Kill!
Ph.D. in Biosciences, FCTUC, Portugal, 2014
supervisor: C. Alpoim

Carlos Samuel Marques Boto
Leukemia cells modulation by light-activatable nanoparticles
Universidade de Coimbra (Chemical Engineering Department). Area: Chemical Engineering; speciality: Biotechnology.
Lino Ferreira

Diana FF Silva
Mitochondrial signaling pathway in Alzheimer´s disease: disclosure of new therapeutic targets
Doutoramento em Biociências, Faculdade de Ciências e Tecnologia, Universidade de Coimbra
Sandra Cardoso

Elisabete de Oliveira Augusto
Characterization of ecto-5'-nucleotidase (CD73) in the brain – role in adenosine A2A receptor activation
Rodrigo Cunha

Fatima Martins
Brain-Liver Nitric Oxide Axis – A Major Regulatory Player in Insulin Clearance and Action
December 2 2014
John Jones

Filipa Sofia Libório Carvalho
Clarification of the mitochondrial role in the cardiotoxicity of doxorubicin using a whole heart perfusion system - impact of different doxorubicin treatment regimens
Dr. Paulo Oliveira, Margarida Carneiro

Graciano Silva Leal
Regulation of hnRNP A2/B1 and hnRNP K by synaptic activity and BDNF in the hippocampus
University of Coimbra
Carlos Duarte

Helena Carvalheiro

(2009-2014) Universidade de Coimbra, Faculdade de Farmácia.
Further Co-supervisors: José António Pereira da Silva (FMUC); Celeste Maria Lopes (FFUC) (Margarida Carneiro)

Ines Barbosa
TRAP1 regulation of mitochondrial homeostasis and cellular quality control
PhD in Biology, FCTUC, Portugal, 2014
supervisor: P Oliveira

Joana Filipa Coelho Fernandes
In vitro ischemia-induced changes in the transcriptome of hippocampal neurons: Neuroprotective pathways in brain ischemia
July 2014
Ana Luisa Carvalho, Carlos Duarte

Margarida Gonçalo
Cutaneous adverse drug reactions. Contributions to understand pathophysiologic mechanisms underlying delayed reactions
Dezember 2014
Celeste Lopes

Marta Baptista (Cell Biology)
João RAmalho

Marta Isabel Dias da Mota Vieira
Molecular Mechanisms underlying in vitro cerebral ischemia: multiple neuronal death pathways
July 2014
Ana Luisa Carvalho, Carlos Duarte

Marta Passadouro
MicroRNA Modulation in Combination with Chemotherapeutic Drugs as a Novel Therapeutic Strategy for Pancreatic Cancer
Luis Pereira de Almeida

Márcio José do Coito Ribeiro
Oxidative stress in Huntington's disease knock-in striatal cells
9th July, 2014
AC Rego supervised

Patricia Lopes
Molecular mechanisms involved in glucose and lipid metabolism after immunosuppressive therapy
Defended Jul 2014,
Eugenia Carvalho

Paulo Alexandre da Costa Gameiro Guerreiro
On the reprogramming of the Krebs cycle in hypoxic and VHL-deficient cancer cells
Margarida Carneiro

Renata Tavares (Cell Biology)
João Ramalho

Renato X Santos
The involvement of mitochondrial fission, fusion and biogenesis and autophagy in the diabetic brain
Doutoramento em Biologia Celular, Faculdade de Ciências e Tecnologia, Universidade de Coimbra
Paula Moreira

Rodrigo Santos (Experimental Biology and Biomedicine)
João Ramalho

Rui Gonçalo Batista Mamede da Cruz
New retroviral-like membrane-associated aspartic proteinases from Rickettsiae: Biochemical characterization and specificity profiling
November 26th 2014
Co-supervisors: Isaura Simões, Carlos Faro

Silvia Margarida Viana da Silva
Pathophysiology of hippocampal CA3 neurons in the APP/PS1 mouse model of Alzheimer's disease
Rodrigo Cunha

Master Thesis

ABDULLAH NAWABJAN SHAIK
Effect of Insulin on impaired diabetic wound healing
Eugenia Carvalho

Ana Carolina Gonçalves de Almeida Xavier
Role of adenosine receptors in suicide
Rodrigo Cunha

Ana Coelho
Role of Sirtuin 3 on Doxorubicin-induced Toxicity on H9c2 Cardiomyoblasts
supervisor: P Oliveira

Ana Raquel M Nunes
Effect of the Daily Consumption of White Tea in the Cerebral Cortex of Prediabetic Rats
Paula Moreira

Ana Ricardo Xavier
Universidade de Lisboa, Faculdade de Ciências.
Margarida Carneiro

Bruno Cruz
Intelligent design of color-tuned ChR2 variantes for optogenetics applications
Ana Luisa Carvalho

Cátia Filipa Oliveira Correia
Preparação de novos derivados de esteróides através de cicloadição [8p+2p] de aniões 1,2-diazafulvénio
September 30, 2014
Jorge António Ribeiro Salvador

Diana Cristina Simões Maurício
Purification and Partial Characterization of Metalloproteinases and Chitinase from Laetiporus sulphureus
Carlos Faro?

Diogo Reis
Perfis de resistência a agentes antimicrobianos de 5 estirpes do género Mycobacterium isoladas de ambiente Hospitalar
Supervisor: Nuno Empadinhas

Eduardo Manuel Firmo Morais
Activity-dependent changes in the dendritic distribution of hnRNP K: functional implications
Carlos Duarte

Fabio Carvalho
The role of immunosuppressors in metabolism
Eugenia Carvalho

Filipa Raquel Grilo Tavares
Prospecção e obtenção de compostos com potencial terapêutico, a partir de subprodutos industriais
September 30, 2014
Jorge António Ribeiro Salvador

Giorgia Mastrella
Characterization of neurogenesis in a transgenic model of Alzheimer's disease
June, 2014
E Ferreiro supervised and AC Rego co-supervised

Helena Sofia Caria Martins
Ribosomal regulation in axonal development
Carlos Duarte

Inês Carolina Sebastião
Brain dysfunction and cell death in type 2 diabetes: a neuroprotective role for the peripheral treatment with Exendin-4

Paula Moreira

Ines Simões
Remodeling of white adipose tissue and metabolic disease
supervisor: A. Rolo

Joana Gomes
Universidade de Coimbra, Faculdade de Ciências e Tecnologia.
Margarida Carneiro

Joana Portela (Cell & Molecular Biology)
João Ramalho

João Filipe Alves Amorim
Mitochondria in glutamatergic nerve terminals are selectively affected by exposure to Aβ1-42 modeling early Alzheimer's disease
Rodrigo Cunha, Anabela P. Rolo

Liliana Ricardina Oliveira Caetano
Purinergic involvement in microglial responses to immunomodulation during neurodevelopment
Rodrigo Cunha

Maria Braz da Silva
Modulação do ciclo TG/(GL+ FFA) como alvo terapêutico na obesidade
supervisor A. Rolo

Maria Cristina Aspromonte
Alteration of $GABA_AR$ trafficking during cerebral ischemia: the role of Huntingtin-associated protein 1
University of Coimbra.
Carlos Duarte

Mário Carvalho
Molecular mechanisms of the synaptic and cognitive effects of ghrelin
Ana Luisa Carvalho

Mirco Martino
Effect of Bexarotene and Aβ in microglia and macrophage TREM2 expression and M1/M2 polarization
Conceição P Lima

Paula Sofia Valente da Silva
Ketone bodies as brain substrates
Rodrigo Cunha

Pedro Rafael Mendes Reis
Comparação de métodos de fingerprints para o rastreio virtual de inibidores da 5a-redutase
Jorge António Ribeiro Salvador
September 29, 2014

Renata Couto
Protection Provided by Phytoestrogens Against Cardiac Lipotoxicity Induced by Palmitic Acid
supervisor: P Oliveira

Ricardo Jorge Cruz Oliveira
Estudo comparativo do potencial anti-inflamatório e antioxidante da cianidina-3-glucósido e do ácido protocatecuico em células intestinais
Setembro 2014
João Laranjinha

Rossella Vetrone
Redistribution of the Proteasome in Brain Ischemia
University of Sannio (Benevento, Italy)
Carlos Duarte

Rúben Branco
MiRNA Profilling and Modulation in Cancer Stem Cells isolated from Glioblastoma

Conceição P Lima

Rui F Simões
Brain endothelial cells protection by pinacidil preconditioning in an okadaic acid-induced Alzheimer's disease model: role of mitoKATP channels, ROS and HIF-1α
Paula Moreira

Sara Rebelo (Cell & Molecular Biology)
João Ramalho

Sílvia Magalhães
Cardiolipin Content in P19 Embryonal Carcinoma Cells" Master Degree in Biochemistry
supervisor: P Oliveira

Solange Machado (Biochemistry)
João Ramalho

Susana Cecílio
Physico-chemical and biological characterization of nanoparticles targeting human leukemia
Conceição P Lima

Tatiana Martins
Berberine Modulation on Doxorubicin-induced Cell Death and Autophagy
supervisor: P Oliveira

Teresa Silva
Células estaminais mesenquimatosas para terapia da Doença Machado-Joseph

Susana Cecílio
Physico-chemical and biological characterization of nanoparticles targeting human leukemia
Conceição P Lima

Vanessa Nobre Corceiro
Study of the impact of pollutant compounds and oxidative stress in Human Health
October 14, 2014
Supervisor: Jorge António Ribeiro Salvador

Vânia Filipa Moreira
Caracterização da infecção conjunta de Escherichia coli e de Candida albicans
Julho de 2014
Teresa Gonçalves

Vanessa RF Rodrigues
Avaliação da atividade anti-inflamatória do óleo essencial de Thymus zygis subsp. sylvestris e dos seus compostos principais
Paula Moreira

TECHNOLOGY TRANSFER

Translational research and technology transfer have been progressively developed in CNC leading to a promising interaction with Industry and local authorities.

The main contribution of CNC for that goal was the creation of a technology transfer unit, Biocant, in collaboration with Cantanhede Municipal Council. This unit became the anchor of Biocant Park, a Biotechnology Park that is rapidly growing by atracting new Biotechnology companies.

BIOCANT

Biocant is a private, non-profit, innovation centre created by CNCB together with the municipality of Cantanhede for technology transfer in biotechnology. Founded 8 years ago, 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 involving research institutions, hospitals and companies.

Companies operating in Biocant Park

At the present 20 companies operate in Biocant Park: AP-Bio, Biocant Ventures, Biotrend, Converde/CEV, Crioestaminal, Equigerminal, Hittag Biotecnology, Interactome, GenePrediT, Genebox, GeneLab, Matera, Vetdiagnos, 4Health, Cell2B, Klon, NutriAdd, Treat U, Reg4Life and Coimbra Genomics. Along with Biocant they form a biotech cluster of excellence that attracted altogether over 70M€ euros investment (50% is private) and generated 400 highly qualified jobs.

OUTREACH PROGRAMME

Outreach / Science and Society

Teresa Girão, PhD (Jan-July 2014)
& CláudiaCavadas, PhD (Sept-Dec 2014)

The Science Communication Office is responsible for the cultural, textual, discursive and visual mediation of CNC scientific work, fostering a social appropriation of the scientific world, contextualized in the different perspectives of our extra/intra/interdisciplinary publics. The mediation is conducted:

- in a macro-level through a process of public relations with regional, national and international media, in coordination with the University of Coimbra Press Office;

- in a meso-level through the internet (social media, CNC website, e-mail) schools, associations, science centers, university institutions and local science communication events inserted in national strategies (of Ciência Viva - National Agency for Scientific and Technological Culture, and Portuguese Society of Neurosciences), or international strategies (Dana Foundation and Federation of European Neuroscience Societies);

-in a micro-level within CNC research community

CNC Science Communication Office objectives:

- Provide public accountability, ethically justified by the public nature of CNC funding;

- Anticipate and resolve (media and institutional) communication crises that compromise the fundamental relationship between science and society;

- Consolidate CNC institutional image for the national and international scientific system, national and regional political decision-makers, public and private funders, and different types of publics;

- Mediate contextualized scientific knowledge as an investment in a society with a critical thinking about new scientific challenges;

- Create bridges and institutional belonging between CNC research groups

CNC yearly participates in various activities exclusively planned to different publics, namely during the Brain Awareness Week, Science and Technology Week, and European Researchers Night. Elementary to high school students are also a committed public of the CNC's outreach actions. CNC intensively collaborates with the Ciência Viva Agency, the Portuguese Society for Neuroscience, the University of Coimbra Science Museum, and Exploratório Infante D. Henrique Science Center for the organization of science communication actions.

Some of our activities are also carried out through the "Instituto de Educação e Cidadania" (IEC, Mamarrosa), a non-profit institution, dedicated to education and to promoting science and knowledge in schools, and among the rural populations in underprivileged areas. The IEC is housed in a modern building, provided with modern equipment, and includes classrooms and laboratories for students and teachers. The IEC has established protocols with several schools, and the CNC channels some of its outreach activities through IEC and the schools it is linked to.

The Science Communication Office is in charge of the public relations process with the media, elaborating press releases with news-values in the context of different agenda-settings, and successfully liaising CNC researchers with journalists. CNC research and science communication activities have been recognized through numerous media articles and broadcasts.

Year of The Brain 2014 – How to intervene in the brain ? Repair or enhance? - February

A contest for 9th grade students (Natural Sciences) and secondary students (Physics, Chemistry, Philosophy, Biology), across the country, to debate neuroscience themes. Themes: 1) "When the brain betrays us, is Alzheimer's?" (Catarina Resende de Oliveira and Claudia Pereira Fragão); 2) "Stem Cells of the Brain - Memory and Aging (João Malva)

Brain Awareness Week (BAW), March 10 – April 03

In 2014, Center for Neuroscience and Cell Biology's activities included several events for the general public:

1) "Brain Myths and Facts" – a public session about some common myths and facts about the brain, preceded by hands-on activities; both the hands-on activities and the conference organization had the involvement of local high school students.

2) “Brain Buskers” – CNC neuroscientists performed hands-on activities, games, and brain teasers at public spaces (e.g. a public garden) with the support of the local Science Center.

3) “Neuroquiz” – the neuroscientists organized a public quiz at local cafés to challenge the participants to explore brain-related issues. Our programme also included a variety of activities for school-age children:

4) Neuroscientists go to Schools; during this event neuroscientists visited schools and gave lectures on brain related subjects to middle and high school students; elementary school students had the opportunity to perform several hands-on activities, games, and brain teasers.

5) Open Laboratories; during this event students visited CNC’s laboratories and listened to talks about neuroscience research. The total audience was 1215 students from 16 schools. Overall, 62 CNC researchers took part in the activities dedicated to the school and different publics.

Ask me Science (2º Edition) – March – April

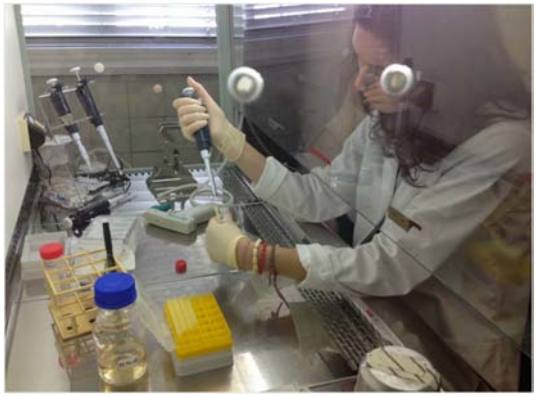
“Ask me Science…” is a scientific enrichment and engagement project that promotes the approach between high school students (and teachers) and the reality of universities and research centers. Following a competition model between researchers, we expect that this project develops skills among students and researchers, arousing curiosity and contributing for the understanding of the scientific process. We intend it to be an encouragement for scientific careers. Scientists of several research areas of CNC will have the opportunity of interacting with students in different contexts. Students could vote for the scientists that they think should win the competition. In the second edition three researchers, with different backgrounds and expertise, were enrolled in "Ask me Science": Anabela Rolo – “What happens when the stress installs?”; Jorge Valero - “New neurons for new memories: the effect of a healthy life”; Cláudia Cavadas - “In search of new drugs for brain diseases and ageing”. The researchers and their teams developed different activities: video-conference classes; laboratorial activities, debates and on-line student’s questions. Cláudia Cavadas was the winner of the project and received a prize of five hundred euros, converted into laboratory materials.
More information: http://perguntameciencia.cnc.uc.pt/

C3 – Children with Sciences, March-May

C3 is a Associação de Pais do Centro Educativo de Condeixa-a-Nova project for pre-school children with the following objectives: meet the intrinsic curiosity of students, create habits and attitudes that encourage critical thinking, meet educational needs in the classroom. The project encourages interaction between parents, students and scientific organizations, providing opportunities for the students to perform theoretical and practical actions at school and at the partner institutions. CNC was visited by 143 children.

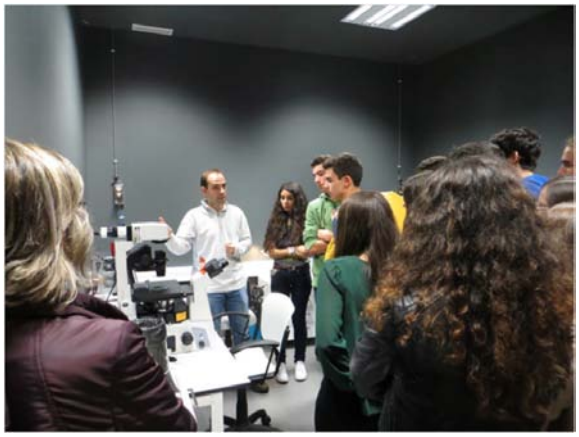
“Science in the Holidays” Programme (Ocupação Científica de Jovens nas Férias), July15-25

Seven Portuguese high-school students participated in a 10 day program during Summer Holidays, promoted by Ciência Viva Agency. Students were tutored by CNC researchers and included in different research groups (João Ramalho-Santos, Ramiro de Almeida and Paula Moreira). The students had the opportunity to run several molecular/cell biology techniques as part of short projects, adding to visits to facilities and laboratories.



European Researchers' Night, September 26

Together with the Science Museum of the University of Coimbra, CNC took part for the seventh time in the organization of the activities of the European Researchers' Night. This initiative is promoted by the European Commission in order to bring the different publics closer to the researchers. The theme of 2014 was "Citizen Science". CNC researchers developed "hands-on" activities for different publics, participated in a theatre play, built a "scientific photo boot" and participated in "speed dating" activity. The event engaged 56 CNC researchers and reached about 1000 people.



Science and Technology Week, November November 22-30

During the Science and Technology week, and the National Day for Scientific Culture, CNC organized visits to the schools, received 70 students in the UC-Biotech and offered two 'Café Scientifiques'. The 'Café Scientifiques' took place at Vila Nova de Poiares (with Manuela Grazina) and Lousã (with João Laranjinha). The total audience of the Science and Technology Week was 300 (240 were students).

Social media

The growing importance of social media for strong relationships between scientists and society is evident in the CNC Facebook strategy results: 2462 page 'likes' in 2014 (growth of 52% since 2013). The Science Communication Office invests in this networking tool for the mediation of CNC news (along with CNC Youtube Channel), seminars, communication activities, awards and science culture.

CORE FACILITIES

ANIMAL HOUSE

Head of Unit: Prof. João Laranjinha

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.

Staff: Carmen Semião (caretaker)
Fátima Graça (assistant technician)
Maria Eugénia Campos (assistant technician)
Patrícia Ribeiro (Veterinary Doctor)

Animal room – IVC cages (type I)

Laminar flow chamber

FLOW CYTOMETRY UNIT

Head of Unit: Isabel Nunes Correia

The flow cytometry unit provides scientific and technical support both to CNC and external researchers. Currently, it is equipped with a Becton Dickinson FACSCalibur cell analyser and a Partec CyFlow® Space cell sorter. For researchers wishing to use flow cytometry, the unit offer 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.

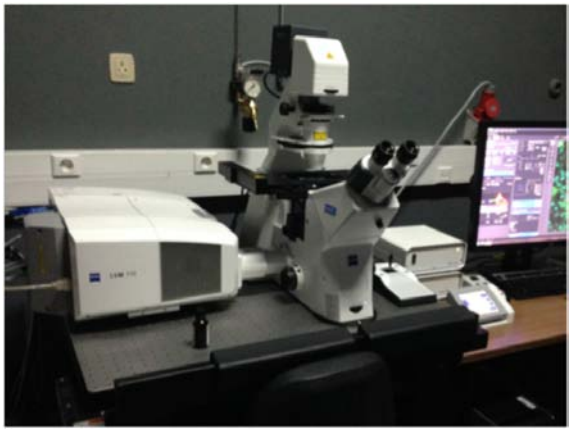
Since 2007, when the unit was created, the number of users is increasing every year, and presently flow cytometry is an important and central technique for the fulfilment of many CNC investigation projects.

FACSCalibur cell analyzer

MICROSCOPY IMAGING CENTER OF COIMBRA - CNC

Head of Unit: Luísa Cortes

The Microscopy Imaging Center of Coimbra, at the Center for Neuroscience and Cell Biology (MICC-CNC), is a open infrastructure where users receive the support needed to carry out conventional and advanced imaging techniques, based on Light Microscopy. MICC-CNC is a reference partner of Carl Zeiss initiative Microscopy Labs@location. Moreover, MICC-CNC is part of the Portuguese Platform for BioImaging (PPBI), and it is the coordinating node of this platform for the Center pole of Portugal. MICC-CNC participates in the EuroBioImaging network, which is an ESFRI initiative.



Confocal LSM 710 (34 channels)

The MICC has highly skilled and multidisciplinary scientific staff, which is involved in several activities present on the imaging platform, namely:

• Training users to operate confocal microscopes, and fluorescence microscope and implement advanced techniques. First time users receive training by the technician in charge for the equipment.

• Designing robust image analysis and data presentation regimes.

• Organizing advanced courses that provide the PhD and Master students with the fundamentals of light microscopy, fluorescence microscopy, live cell imaging applied to Biomedicine, in the scope of Masters and PhD training courses.

• Testing and specifying new equipment and software.

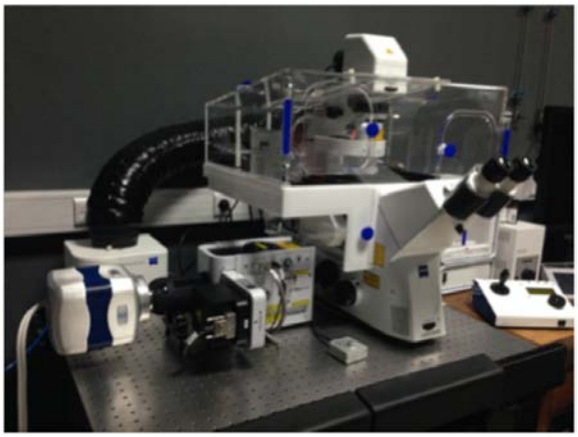
• Maintaining strong relationships with the microscope manufacturers and service teams.

• Troubleshooting, repairing and overseeing maintenance of the microscopes.

• Responsibility for the safety issues pertaining to microscopes.

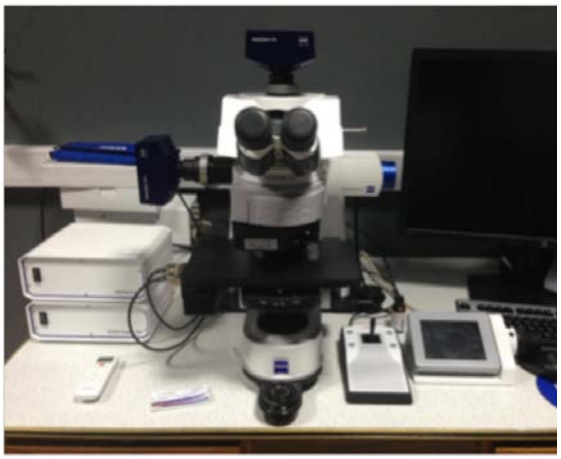
• Keeping the information about MICC-CNC updated on the CNC webpage.

• Disseminate the available resources and services on other national technologic platforms, such as SciPort and PPBI webpages.



Confocal Cell Observer Spinning-Disk

Presently, the unit manages two laser scanning confocal microscopes, a spinning disk confocal microscope, a live cell imaging station, a epifluorescence microscope with structure illumination (Apotome2) and stereology analysis



(StereoInvestigator), a P.A.L.M. laser microdissection microscope, a single cell calcium imaging system, and other brightfield microscopes. The systems are prepared for advanced applications, including live cell imaging and single cell calcium measurements, enabling the researchers to image dynamic events and molecular interactions. The P.A.L.M. laser microdissection microscope is a perfect tool for the isolation of different cell populations within a sample, allowing it full characterization. The MICC-CNC has dedicated workstations for imaging analysis, with the following software: Neurolucida, Huygens, Matlab, Metafluor, FIJI/ImageJ, and CellProfiler.

Widefield AxioImager Z2 with ApoTome2

MASS SPECTROSCOPY UNIT

Head of Unit: Bruno Manadas

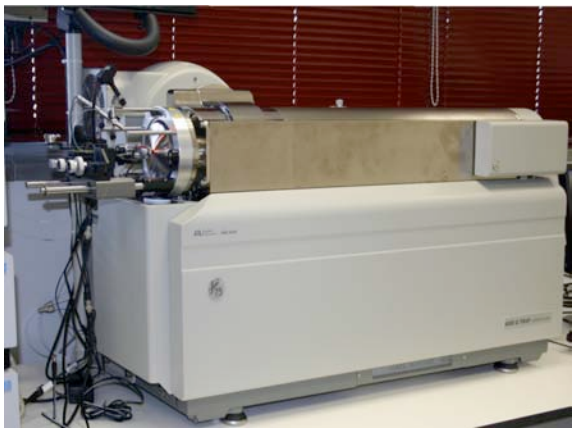
The Mass Spectrometry Unit is specialized in identification and quantification of proteins from simple and complex samples; identification and quantification of post-translational modifications, and identification and quantification of metabolites. The Unit is also involved in the identification of biomarkers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

Presently, the Mass Spectrometry Unit is equipped with state of the art technology, namely: a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex), hybrid triple quadrupole/ion-trap mass spectrometer with capacity of MS3, and a two-dimensional liquid chromatography system Ultimate 3000 (Dionex/LCPackings). The unit also contains several software packages for data processing, including Protein Pilot and PEAKS for protein identification, post-translational modifications and de novo sequencing.

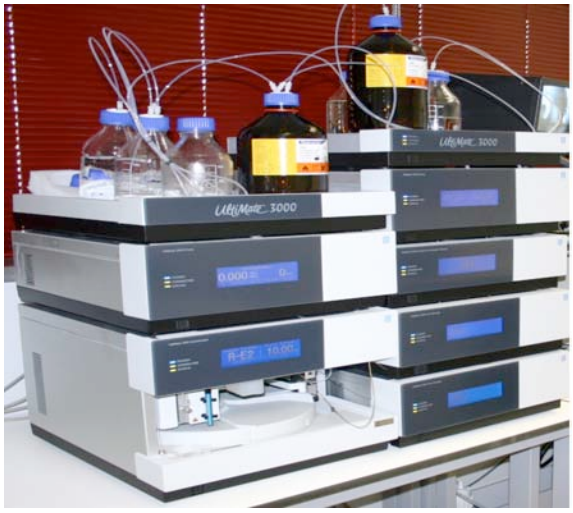
By combining the high resolving power of the LC system with the structure elucidation from the mass spectrometer, the Mass Spectrometry Unit is able to identify peptides, metabolites, drugs, pesticides, among others, from complex mixtures.

The Unit integrates the National Mass Spectrometry Network (RNEM).

Staff: Vera Mendes (technician)



4000 QTRAP mass spectrometer



Bidimensional chromatography modular system coupled to the 4000 QTRAP spectrometer

Services

LABORATORY OF BIOCHEMICAL GENETICS

Coordinator: Manuela Grazina

Certification NP EN ISO 9001:2008

The coordinator of LBG (Manuela Grazina) maintains international collaborations, allowing significant developments in the assays performed, namely with Prof. Lee-Jun Wong and Doctor Fernando Scaglia (Baylor College of Medicine, Houston – Texas, USA), Prof. Massimo Zeviani (MRC Mitochondrial Biology Unit, Cambridge, UK), Prof. Robert Taylor (Mitochondrial Pathology, University of Newcastle upon Tyne, UK) and Dr. Rafael Artuch (Hospital San Juan de Dios-Barcelona, Spain).

Biochemical analysis

Mitochondrial Respiratory Chain (MRC) and Krebs cycle enzymes

Biochemical assays related to mitochondrial respiratory chain biogenesis, functioning and maintenance are essential for achieving the probable diagnosis of Mitochondrial Respiratory Chain and Krebs Cycle Diseases.

There were studied 53 subjects suspected of Mitochondrial Cytopathy, corresponding to the analysis of 53 samples, in 530 assays, including lymphocytes isolated of peripheral blood (19), muscular biopsies (28), liver (5) and fibroblasts (1). A MRC deficiency was detected in 20 patients (38%).

Krebs cycle enzymes (fumarase, alfa-ketoglutarate dehydrogenase, malate dehydrogenase, aconitase, isocytrate dehydrogenase) analysis was performed in 1 patient, corresponding to 7 assays. These tests represent an important set up for improving diagnostic of mitochondrial bioenergetic defects. Control values have been set up to offer theses analyses as a service available at LBG.

Analysis of Coenzyme Q10

The equipment available is out of order and the samples were analysed in collaboration with Dr. Rafael Artuch, at Hospital San Juan de Dios- Barcelona, Spain.

There were studied 2 samples (plasma, muscle, liver), in 14 assays. A deficiency of CoQ10 content was found in two patients' samples.

Detection of Coenzyme Q10 deficiency represents a key tool in diagnosis of MRC diseases (MRCD), since this is the only treatable deficiency in this group of inherited errors of metabolism.

Amino Acid Analysis

The patients investigated 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.

We have received 234 samples (185 plasma, 35 urine and 14 cerebrospinal fluid) of physiological fluids for amino acid analysis, corresponding to 702 assays. The majority of samples are from children, although less frequently, adults and adolescents are also monitored.

Amino acids analysis is a very important approach in early metabolic disorder diagnosis, and frequently helps to prevent mental retardation or even death.

Genetic analysis

Genetic screening is the only available tool for reaching a definitive diagnosis in many diseases. Concerning OXPHOS disorders and given its dual genetic origin, the study of nuclear genome, mitochondrial DNA and bigenomic crosstalk factors, the genetic integrative approach is mandatory.

***Mitochondrial DNA (mtDNA) and nuclear (nDNA) genomes studies*:** 91 samples (blood - 45, muscle -39, liver – 6 and other tissues - 1) were received for DNA extraction.

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. Total mtDNA sequencing or gene panel analysis is also performed in selected samples, according to clinic manifestations and results from previous biochemical and/or genetic screening.

There were studied 108 patients suspected of Mitochondrial Cytopathy, corresponding to the analysis of 111 samples (some patients had 2 or more tissues analysed), in 5808 assays, allowing detection of 762 mtDNA alterations. A pathogenic mutation was found in 31 patients. There were also analysed 4 cases (family relatives) for confirmation of mutations detected in index cases.

Mitochondrial **DNA depletion syndrome** (MDS) is caused by defects in intergenomic communication and comprises a heterogeneous group of diseases, namely due to nuclear genes mutations leading to severe reduction of mtDNA content, with energy failure. Copy number (mtDNA) assays are now part of the genetic mitochondrial genome screening. Nuclear genes screening includes 9 genes related to MRC function and or mtDNA biogenesis.

Concerning **mtDNA copy** number assays for depletion screening, we investigated 16 samples of 15 patients, comprising a total of 448 real time PCR assays.

Implementation of analysis for other genes, such as ANT, TK, MPV17 and twinkle has continued, in the attempt of finding the cause for mtDNA depletion or multiple deletions, but limitations in personnel available did not allow the completion of this objective.

Concerning the **screening of *nDNA defects causative of MRCD***, we have screened 49 samples, comprising a total of 4,778 assays.

POLG1 and POLG2 genes were screened in 35 samples of 35 patients. We have identified 240 sequence variations and 3 pathogenic mutations in 29 patients, comprising a total of 4,278 assays.

DGUOK gene screening was performed in 2 samples of 2 patients (110 assays) allowing identification of 6 sequence variations, 5 of which are probable pathogenic related to mtDNA depletion, relevant for genetic diagnosis and genetic counselling.

Screening of SURF1 gene (2 samples of 2 patients, 140 assays) allowed detection of 8 sequence variations.

TP gene screening was performed in 1 sample of 1 patient (48 assays) and identified 6 sequence variations, 1 of which is probable pathogenic related to mtDNA depletion, relevant for

genetic diagnosis and genetic counselling.
Screening of OPA1 gene (1 sample of 1 patient, 178 assays) didn't show any alteration.
There were also analysed 3 cases (family relatives) for confirmation of mutations detected in index cases.

Staff: *Staff: Cândida Mendes; Carla Veríssimo; João Pratas; Marta Simões; Maria João Santos. Carolina Ribeiro has participated as voluntary*

LABORATORY OF NEUROCHEMISTRY

Coordinators: Catarina Resende Oliveira, Inês Baldeiras

The Neurochemistry Unit is integrated in the Neurology Department of the University Hospitals of Coimbra (CHUC) and develops its activity in essentially two areas: laboratorial support of diagnosis and follow-up of neurological and metabolic diseases and clinical research of neurodegenerative disorders.

In what concerns the immediate support to the patient, the Neurochemistry Unit provides several test that help in the diagnosis and control of progression of neurodegenerative, demielinizing, neuromuscular and metabolic disorders:

- Cerebrospinal Fluid (CSF) cell count and chemical analysis

- Electrophoresis of CSF/serum proteins

- Detection of Immunoglobulin G Oligoclonal Bands in CSF/serum by Isoelectrical Focusing

- Determination of plasma Vitamin A and E levels by high-performance-liquid chromatography (HPLC)

- Evaluation of plasma and CSF redox status

- Quantification of urinary levels of purines and pyrimidines by HPLC

- Evaluation of the urinary activity of Arylsulfatase A

- Seric evaluation of anti-neuronal antibodies in patients with polineuropathies

- Quantification of serum levels of antiepileptic drugs in patients under therapy

- Determination of serum neutralyzing antibodies (NABs) against Interferon-β (IFN-β) in multiple sclerosis patients undergoing treatment with IFN-β.

Early and differential diagnosis of dementias is a particular important area of work of this laboratory. The Neurochemistry unit is, in the framework of the Portuguese Epidemiological Surveillance Program for Human Prion Diseases, the national reference laboratory for Cerebrospinal Fluid (CSF) analysis, and it performs:

- Quantification of CSF levels of total-Tau protein, phosphorylated-Tau protein and β-amyloid1-42 peptide for dementia diagnosis

- Detection of 14-3-3 protein in CSF in suspected cases of Creutzfeldt-Jakob Disease (CJD)

- Immunodetection of Prion protein isoforms in brain extracts of CJD patients

Characterization of oxidative status in neurodegenerative disorders is also a specific interest of this unit. In this context, we perform, either in patient's blood or in several cellular extracts, the:

- Evaluation of plasma and cellular oxidative stress

This includes the determination of a broad spectrum of non-enzymatic (uric acid, vitamin E, oxidized and reduced glutathione) and enzymatic antioxidants (glutathione reductase and peroxidase), nitrogen oxidative species and lipid (malondialdehyde) and protein (carbonyls) oxidation markers.

During the year of 2014, the Neurochemistry Unit has received around 700 blood and 550 CSF samples and has performed the following analysis:

	Blood (Serum/Plasma)	CSF	Urine	Brain extracts	Other extracts
Cytochemistry and electrophoresis	383	383			
IgG Oligoclonal bands	215	215			
Vitamin A/E	162				
Redox Satus	31				138
Purines & Pyrimidines			5		
Arylsulfatase A			0		
Anti-neuronal antibodies	64				
Antiepileptic drugs	5				
NABs against INFβ	2				
CSF Tau, p-Tau and Aβ42		264			
CSF 14-3-3 protein		90			
Prion protein isoforms				27	
Oxidative Stress	2				60

LABORATORY OF MOLECULAR GENETICS CARDIOPATHIES

Coordinator: Isabel Marques Carreira

Screening of mutations in 53 genes associated with cardiopathies

In the laboratory of Molecular Genetics of Cardiopathies (LGMC) the main study area is the Hypertrophic Cardiomyopathy(HCM) and Sudden Death (SD)

HCM can present at any age and is highly variable. Patients can remain asymptomatic throughout their life, but is also associated with adverse clinical events, like heart failure, stroke and sudden cardiac death.

In about half of the HCM patients a disease causing mutation can be detected in one of the genes encoding for sarcomeric proteins. More than 1000 distinct sarcomere protein gene mutations have been identified to cause HCM. Identification of a disease causing mutation in a HCM patient (the proband) implies the opportunity of screening by means of predictive DNA testing in relatives, and can thus better identify the relatives at risk for HCM and associated death.

In our lab (LGMC), genotyping is achieved through a high-throughput and high accurate DNA Microchip platform optimized for genetic analysis using an iPlex MassArray system, which analyzes mutations in 53 genes associated with the development of cardiopathies. The procedure involves collaboration with a laboratory in Lisbon. Validation and interpretation of the results as well as the familial studies are done in the LGMC.

In 2014, the LGMC was revalidated the quality certificate (APCER), continuing to be a certified laboratory for the "Research of mutations in genes associated with cardiopathies".

Team: Ana Cristina Santos

LABORATORY OF NEUROGENETICS

Coordinator: Maria do Rosário Almeida

Molecular testing of Neurodegenerative diseases

The Neurogenetics Laboratory continues to provide the molecular diagnostic tests for several Neurodegenerative diseases such as: Frontotemporal Lobar degeneration (FTLD), Familial Alzheimer Disease (AD) and Parkinson´s Disease (PD). As has been the case in the previous years, a continuous effort has been made to ensure that the methodologies and diagnostic strategies used in the laboratory are in accordance with the established guidelines for the different diseases and are compatible with current scientific knowledge. Therefore, during 2014, the determination of the serum progranulin level was introduced as a pre-screen procedure to identify patients harboring null PGRN mutations prior to direct sequencing to be performed. This additional approach significantly decreased the price of the test and the required PGRN mutation analysis workload in FTLD diagnosis. Moreover, this year there was an increase in the number of referrals to detect the hexanucleotide repeat expansion in C9orf72 gene and the mutations in the *SQSTM1* gene, both genes associated with FTLD and Amyotrophic lateral sclerosis (ALS). Regarding the genetic tests available for PD, several requests were also arrived in the laboratory, not only to study the most common causative-genes, LRRK2 and Parkin but also to the susceptibility factor, glucocerebrosidase, special for PD patients with cognitive impairment. The group was aware that the next step to improve the molecular diagnosis of these neurodegenerative diseases requires the acquisition of next generation sequencing technology, already set up in different international reference laboratories, therefore, the team members were also involved in exploring this opportunity.

Finally, the Neurogenetics Laboratory which was certified according to NP EN ISO 9001 : 2008 by APCER , Record No. PT- 2011 / CEP.3971, was audited on 15th May and obtained a renewal of the certificate ensuring the required quality in all the procedures.

***Team**: Mª Rosário Almeida and Ana Cristina Santos*

LABORATORY OF CELL BIOLOGY

Coordinator: Mário Grãos

The Laboratory of Cell Biology develops its activity between research projects and service providing.

In terms of research, the year of 2014 resulted in the ongoing participation in 4 FCT-funded projects, mostly in the area of stem cells, neural differentiation and neurodegenerative disorders. Two international peer-reviewed papers were published and 1 MSc thesis was produced.

Concerning service providing, the laboratory has continued its 2 services. One service provides the determination of bio-molecules using the multiplex xMAP technology, and during 2014, 13 kits were processed (each kit allows the determination of multiple analytes per sample depending on the kit, which considering its 96-well plate format, represents a multitude of data points). Another service consists on testing the viability of human cryopreserved samples. During the year of 2014, a total of 2,380 cryopreserved samples were tested.

LABORATORY OF IMMUNOLOGY AND ONCOLOGY

Coordinator: Paulo Rodrigues Santos

Scope

Our laboratory provides complementary scientific or technological services to external entities, public or private, developing new tests for diagnostics and therapy monitoring of malignant diseases. The achievement of this goal results from the effective cooperation with other national and international institutions.

Available Tests

The laboratory provides combined molecular and cellular tests involving immunology and oncology knowledge.

Currently, the available tests include:

- BCR-ABL1, qualitative, RT-PCR
- BCR-ABL1, quantification, real-time quantitative PCR
- ABL KD, mutation screening, High-resolution melting (HRM) real-time PCR
- ABL KD, mutation identification, Next-generation sequencing (NGS)
- BCR-ABL1^{+} leukemic stem cells, Fluorescence-activated cell sorting (FACS)/RT-qPCR
- Gene expression profile, RT-qPCR

Service activity

The laboratory established during the last five years a robust and sustainable service, increasing its capacity to provide specialized tests to the community.

Development and Innovation

During 2014, our laboratory was developing new tests for the identification and culture of circulating tumour cells in order to monitor solid tumour therapies. Those news tests will be available during the last quarter of 2015.

High-resolution melting for ABL KD mutation screening

higher sensitive method has been used in our laboratory for screening of ABL KD mutations in CML patients.

ABL1 kinase domain mutation identification using next-generation sequencing

sequencing option for ABL1 KD mutation identification for our services.

Collaborations

- Paulo Freitas-Tavares and Lenka Růžičková, Hematology Service, Coimbra Hospital and Universitary Centre, Portugal.
- Célia Gomes, Flávio Reis, Belmiro Parada and Frederico Costa Pereira, Laboratory of Pharmacology & Experimental Therapeutics, Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine University of Coimbra, Portugal.
- Simona Soverini, Institute of Hematology and Medical Oncology, University of Bologna, Italy.
- Anahid Jewett, Tumor Immunology Laboratory, Division of Oral Biology and Medicine, and Wintraub Center for Reconstructive Biotechnology, UCLA School of Medicine and Dentistry, Los Angeles, USA.
- Torsten Haferlach, MLL Munich Leukaemia Lab, Munich, Germany.

International Networking

IRON III (Interlaboratory Robustness of Next-Generation Sequencing)

Collaboration within the framework of the international consortium for the investigation of the robustness, precision and reproducibility of 454 amplicon next-generation sequencing for the study of hematologic malignant diseases across 10 laboratories from 8 countries (Soverini *et al.* Blood. 2013 Aug 29;122(9):1634-48).

Conference Proceedings abstracts

1. Couceiro P, Soverini S, Gomes-Silva D, Freitas-Tavares P, **Rodrigues-Santos P**. Screening of ABL kinase domain mutations in Chronic Myeloid Leukemia using Next-Generation Sequencing: impact in monitoring response to tyrosine kinase inhibitor therapy. Rev Port Pneumol 2014, 20:8-32.

2. Coirada F, **Couceiro P**, Mazini P, Gomes-Silva D, Alves V, Visentainer JEL, Santos-Rosa M, Freitas-Tavares P, **Rodrigues-Santos P**. CD137 (4-1BB) as therapeutic target in Chronic Myeloid Leukemia: immunophenotyping, gene expression profile and functional studies in lymphocyte subsets on patients treated with imatinib mesylate. Rev Port Pneumol 2014, 20:1-7.

Publications

1. Sereno J, **Rodrigues-Santos P**, Vala H, Rocha-Pereira P, Alves R, Fernandes J, Santos-Silva A, Carvalho E, Teixeira F, Reis F. Transition from cyclosporine-induced renal dysfunction to nephrotoxicity in an *in vivo* rat model. Int J Mol Sci. 2014 May 20;15(5):8979-97.

2. Sereno J, Nunes S, **Rodrigues-Santos P**, Vala H, Rocha-Pereira P, Fernandes J, Santos-Silva A, Teixeira F, Reis F. Conversion to sirolimus ameliorates cyclosporine-induced nephropathy in the rat – focus on serum, urine, gene and protein renal expression biomarkers. BioMed Res Int 2014; 576929.

3. Marques C, Mega AC, Gonçalves A, **Rodrigues-Santos P**, Teixeira-Lemos E, Teixeira F, Fontes-Ribeiro C, Reis F, Fernandes R. Sitagliptin prevents inflammation and apoptotic cell death in the kidney of type 2 diabetic animals Mediators Inflamm 2014, 538737.

4. Mega C, Vala H, **Rodrigues-Santos P**, Oliveira J, Teixeira F, Rosa Fernandes, Reis F, Teixeira-Lemos E. Sitagliptin prevents aggravation of endocrine and exocrine pancreatic damage in the Zucker Diabetic Fatty rat - focus on amelioration of metabolic profile and tissue cytoprotective properties. Diabetology & Metabolic Syndrome 2014, 6:42.

Research Funding

NK cell-based adoptive therapy targeting human bladder cancer stem cells: impact on tumor

progression using a humanized orthotopic animal model

Célia Gomes, Flávio Reis, Belmiro Parada, Margarida Teixeira, ***Paulo Rodrigues Santos***

The Astellas European Foundation Uro-Oncology Grant 2013.

150.000,00 USD

Stepping on the RAGE network: physical exercise In the management of Parkinson

Frederico Costa Pereira (PI), Carlos Fontes Ribeiro, ***Paulo Rodrigues Santos****, Rui Daniel Prediger, Sofia Viana, Syed Ali.*

Fundação para a Ciência e Tecnologia 2014: EXPL/DTP-DES/0104/2013.

47.519,00 EUR

Team*: Patricia Couceiro*

Genome Sequencing Biology

Coordinator: Conceição Egas

Sequencing services:
Type of clients: R&D groups and companies
Type of services: Large scale genotyping, metagenomics, RT-PCR and transcriptomics.

Group Publications:

1. Simões MJ, Lobo C., Egas C, Nunes S, Carmona S, Costa MA, Duarte T, Ribeiro L, Faro C, Cunha-Vaz J. 2014. Genetic variants in ICAM1, PPARGC1A and MTHFR are potentially associated with different phenotypes of diabetic retinopathy. Ophthalmologica 2014; 232:156-162. doi:10.1159/000365229.
2. Rocheta M, Sobral R, Magalhães J, Amorim MI, Ribeiro T, Pinheiro M, Egas C, Morais-Cecílio L, Costa MMR. 2014. Comparative transcriptomic analysis of male and female flowers of monoecious *Quercus suber*. Frontiers in Plant Science. 5. Doi:10.3389/fpls.2014.00599.

Clients publications and/or citations
(Results obtained with the sequencing and data analysis from the Genome Sequencing Unit)

1. Cristiane C. P. Hardoim, Massimiliano Cardinale, Ana C. B. Cúcio, Ana I. S. Esteves, Gabriele Berg, Joana R. Xavier, Cymon J. Cox, and Rodrigo Costa. Effects of sample handling and cultivation bias on the specificity of bacterial communities in keratose marine sponges. Front Microbiol. 2014; 5: 611.
2. Ana Catarina Alves, Aitor Etxebarria, Anne Katherine Soutar, Cesar Martin, Mafalda Bourbon.2014. Novel functional APOB mutations outside LDL-binding region causing familial hypercholesterolaemia. Mol. Genet. (2014) 23 (7):1817-1828. doi: 10.1093/hmg/ddt573.
3. V. Oliveira, Newton C.M. Gomes, Daniel F.R. Cleary, A. Almeida, Artur M.S. Silva, Mário M.Q. Simões, H. Silva, A. Cunha. Halophyte plant colonization as a driver of the composition of bacterial communities in salt marshes chronically exposed to oil hydrocarbons. FEMS Microbiology Ecology. 2014, 90 (3) 647-662; DOI: 10.1111/1574-6941.12425.

4. S. Schreiter, G-C. Ding, R. Grosch, S. Kropf, K. Antweiler, K. Smalla. Soil type-dependent effects of a potential biocontrol inoculant on indigenous bacterial communities in the rhizosphere of field-grown lettuce. FEMS Microbiology Ecology. 2014, 90 (3) 718-730; DOI: 10.1111/1574-6941.12430.
5. Ding G-C, Radl V, Schloter-Hai B, Jechalke S, Heuer H, Smalla K, Schloter M. (2014) Dynamics of Soil Bacterial Communities in Response to Repeated Application of Manure Containing Sulfadiazine. PLoS ONE 9(3): e92958. doi: 10.1371/journal.pone.0092958.

Undergoing Projects

1. Project DoIT - Development and Operation of Translational Research. This Project involved a consortium of Portuguese R&D institutions and companies: AIBILI, BIAL, BIOCANT, Center for Neuroscience and Cell Biology, CRITICAL HEALTH, S.A., EUROTRIALS - FRULACT, GENETEST, Hospitals of the University of Coimbra, S. João Hospital- Porto, IMM – Institute for Molecular Medicine, Portuguese Institute of Oncology, IPATIMUP, PLUX – Biosensor Engeneering, SIEMENS, Têxtil Manuel Gonçalves, University of Aveiro, University of Coimbra, University of Minho. Financed by QREN, 2012-2015. Our group (CNC) was involved in the task "Diamarker: Genetic susceptibility of multisystemic complications of diabetes type 2: novel biomarkers for diagnosis and monitoring of therapy".
   - Project duration: 2012-2015, finnanced by the Portuguese Innovation Agency and QREN - the Portuguese Strategic Reference Framework (Projecto Mobilizador n.º 13853)
   - The project involves the sequencing and analysis of 100 exomes of diabetic patients, the organization of the variants found in a database and the development of variant annotation and prioritization tools for data analysis.
   - Main results are the identification of candidate genes with rare variants for T2D complications (diabetic retinopathy, diabetic nephropathy).

Laboratory of Brettanomyces by FCM

Coordinator: Margarida Carneiro

The main activity of the Laboratory of Brettanomyces by FCM is to provide accurate and rapid determination of the presence of contaminating Brettanomyces/ Dekkera yeast in wines during the different stages of wine maturation. This service is offered to wine producers in Portugal. During the year 2014 the laboratory has carried out over 400 analyses. The bulk (90%) of these analyses has been done under long-term fidelity contracts celebrated with 4 wine producers, but sporadic analysis were done on fee-for-service basis. In the future we plan on expanding both the number of long-term contracts as well as the pallet of analytic services offered to the wine producers. A mid-term goal is to get an official quality certification for the brettanomyces analysis.

FUNDING

In 2014 funding of "Laboratório Associado – Centro de Neurociências e Biologia Celular" ascended the amount of 9.597.780,70€.

The main financing contribution was made by "Fundação para a Ciência e Tecnologia (FCT)", concerning global institution programs and national projects, in the amount of 4.490.807,76 € distribuited as follows:

Strategical Project_ PEst-C/SAU/LA0001/2013	1.725.877,73 €
Incentivo/SAU/LA0001/2014:	40.950,09€
Projects:	2.243.996,29€
Science Program:	479.983,65€

The related items supported the main part of Center for Neuroscience and Cell Biology expenses during 2014.

Besides the above-mentioned, the Center for Neuroscience is financed by other national and international agencies. In 2014 Center for Neuroscience received the amount of 2.673.048,30€ concerning other national projects and 1.417.663,23€ concerning international projects.

The provision of services is another important vector in the CNC, which amounts the 642,783.98€

The amount of other resting funds, which are not listed, ascends a value of 373.477,43€.

On the following pages a description of the projects is made, which includes FCT projects, other National Projects and International projects

Note: Financing values are based on expenditure values 2014

ONGOING PROJECTS

Title	Financing Agency	Duration	Budget (CNC)	Expenditure 2014
National Projects:				
"Rede Nacional de Espectrometria de Massa" Coordinator: Euclides Pires	FCT Refª: REDE/1506/REM/2005	01/01/2009 to 30/12/2015	25.400,00	25.374,24€
"Caracterização dos princípios de design de circuitos metabólicos prevalentes." Coordinator: Armindo Salvador Participants: Universidade de Coimbra; Universidade do Minho	FCT Refª: PTDC/QUI-BIQ/119657/2010	01/04/2012 to 30/09/2015	117.226,00	44.308,09€
"Terapia génica Não invasiva e Não viral da doença de Machado-Joseph" Coordinator: Luis Almeida	FCT Refª: PTDC/SAU-FAR/116535/2010	01/04/2012 to 31/08/2015	108.280,00	18.168,78€
"Estudo do mecanismo patogénico da Doença de Machado-Joseph num novo modelo de células estaminais pluripotentes induzidas." Coordinator: Luis Almeida	FCT Refª: PTDC/SAU-NMC/116512/2010	24/01/2012 to 30/07/2015	145.360,00	41.439,65€
"Avaliação Neuropsicológica e Investigação Bigenómica nas Demência Frontotemporal." Coordinator: Maria Manuela Grazina	FCT Refª: PTDC/SAU-EPI/121811/2010	01/01/2012 to 30/06/2015	199.699,00	33.127,60€
"Impacto da terapia com exendina-4 nos mecanismos moleculares subjacentes à disfunção cerebral associada à diabetes tipo 2 a longo prazo." Coordinator: Ana Isabel Duarte	FCT Refª: PTDC/SAU-TOX/117481/2010	01/05/2012 to 31/10/2014	144.305,00	66.088,75€
"Papel da proteína p66Shc na Persistência de Danos Mitocondriais Induzidos por Fármacos." Coordinator: Ignacio Vega Naredo	FCT Refª: PTDC/SAU-TOX/117912/2010	01/03/2012 to 31/08/2014	79.291,00	20.277,83€
"TranstirRetina é uma metaloprotease: possíveis implicações em doenças do sistem nervoso." Coordinator: Sukalian Chaterjee Proponent: Instituto de Biologia Molecular e Celular (IBMC)	FCT Refª: PTDC/SAU-ORG/118863/2010	01/05/2012 to 30/09/2015	56.152,00	32.112,08€
"Alterações na transmissão sináptica GABAérgica na isquemia cerebral - mecanismos moleculares responsáveis pela internalização dos receptores GABAA." Coordinator: Carlos Duarte	FCT Refª: PTDC/SAU-NMC/0198/2012	01/07/2013 to 30/06/2015	141.136,00	69.695,35€

“DEMTEST: Diagnóstico de demencias rapidamente progressivas baseado em biomarcadores - optimização de protocolos de diagnóstico.” Coordinator: Catarina Resende de Oliveira	FCT Refª: JPND/0001/2011	01/06/2012 to 31/05/2015	35.000,00	6.540,82€
“Regulação do metabolismo energético no cérebro pelo óxido nítrico: solução para a glicólise aeróbia” Coordinator: João Laranjinha	FCT Refª: PTDC/BBB-BQB/3217/2012	03/07/2013 to 02/07/2015	134.938,00	45.498,64€
“Previsão da diabetes e feridas em familiares em primeiro grau de diabéticos tipo 2” Coordinator: John Jones Proponent: Associação Protectora dos Diabéticos de Portugal (APDP)	FCT Refª: EXCL/DTP-PIC/0069/2012	01/06/2013 to 31/05/2016	173.264,00	62.314,73€
“Estudo da contribuição dos miRNAs para o metabolismo do peptídeo b-amilóide: desenvolvimento de uma plataforma lentiviral para expressão de múltiplos miRNAs no contexto da doença de Alzheimer” Coordinator: Ana Luísa Colaço Cardoso	FCT Refª: PTDC/BIM-MEC/0651/2012	01/03/2013 to 31/08/2015	100.800,00	56.727,43€
“Silenciamento da Doença de Machado-Joseph pela via sistémica” Coordinator: Rui Jorge Gonçalves Pereira Nobre	FCT Refª: EXPL/NEU-NMC/0331/2012	01/03/2013 to 30/04/2014	48.900,00	4.619,14€
“Do controlo da neuroinflamação à neuroproteção: bloqueio dos reptores A2A para o tratamento do glaucoma” Coordinator: Ana Raquel Sarabando Santiago	FCT Refª: PTDC/BIM-MEC/0913/2012	01/06/2013 to 30/09/2015	32.401,00	19.067,50€
“Tecido cardíaco humano para a avaliação de toxicidade – CARDIOTOX” Coordinator: Susana Carvalho Rosa	FCT Refª: EXPL/DTP-FTO/0570/2012	01/07/2013 to 30/09/2014	36.800,00	27.381,23€
“Efeitos do peptídeo orexigénico grelina na transmissão sináptica glutamatérgica” Coordinator: Sandra Manuela Domingues dos Santos	FCT Refª: PTDC/NEU-NMC/1098/2012	01/07/2013 To 30/06/2015	199.975,00	65.688,99€
Porque é a mitoxantrona um veneno para o coração? Em destaque os mecanismos mitocondriais, sobre o citoesqueleto e de bioactivação metabólica Coordinator: Vera Costa	FCT Refª: EXPL/DTP-FTO/0290/2012	01/05/2013 to 31/10/2014	1.320,00	1.100,24€
A Sabedoria do faminto: modulação por ghrelina da neurogénese e da sua relação com a memória Coordinator: Jorge Gómez	FCT Refª: EXPL/NEU-SCC/1193/2012	01/04/2014 to 30/09/2015	49.980,00	29.244,40€
A natureza das ligações de carbono e azoto como fator discriminante da origem da matéria orgânica solúvel em água de aerossóis atmosféricos Coordinator: Luisa Ramos	FCT Refª: PTDC/AAG-MAA/2584/2012	01/07/2013 to 30/06/2015	3.720,00	1.049,46€

CARDIOSTEM: Tecidos cardíacos e terapias baseadas em células estaminais para aplicações cardiovasculares Coordinator: Lino Ferreira Participants: Associação do Instituto Superior Técnico para a Investigação e o Desenvolvimento (IST-ID); Faculdade de Medicina Veterinária (FMV/UTL); Instituto de Biologia Experimental e Tecnológica (IBET); Instituto Nacional de Engenharia Biomédica (INEB Porto)	FCT Refª: MITP-TB/ECE/0013/2013	01/12/2014 to 30/11/2017	405.316,00	0,00€
"Análise sistemática de proteínas Rab na fagocitose e na maturação do fagossoma do Mycobacterium tuberculosis." Coordinator: Maria Otilia Vieira Participants: Instituto de Biologia Molecular e Celular - IBMC/UP	FCT Refª: PTDC/BIA-BCM/112138/2009	01/01/2011 to 30/06/2014	171.993,00	22.595,77€
"Actividade Protectora da SIRT3 na Disfunção Mitocondrial Induzida por Fármacos." Coordinator: Paulo Oliveira	FCT Refª: PTDC/SAU-TOX/110952/2009	01/03/2011 to 30/08/2014	128.800,00	16.864,30€
"Transporte entre células da alfa-sinucleina na doença de Parkinson. O factor de progressão?" Coordinator: Manuel Garrido	FCT Refª: PTDC/SAU-NMC/109955/2009	01/04/2011 to 30/09/2014	144.738,00	44.350,48€
"Uma nova formulação de nanopartículas para aplicação de terapia génica em tumores sólidos." Coordinator: Henrique Faneca	FCT Refª: PTDC/QUI-BIQ/116080/2009	01/04/2011 to 30/09/2014	94.000,00	39.022,23€
"Simugrowth-Desenvolvimento de um modelo computacional para a simulação das propriedades biomecânicas de cartilagem desenvolvida in-vitro em função do estimulo mecânico em bioreactor." Coordinator: Alexandrina Mendes Proponent: Universidade de Aveiro Participants: Universidade do Minho (UM)	FCT Refª: PTDC/EME-TME/113039/2009	03/04/2011 to 31/03/2014	28.830,00	2.668,41€
"O papel do intestino no desenvolvimento da esteatose hepatica induzida pela frutose." Coordinator: John Jones	FCT Refª: PTDC/SAU-MET/111398/2009	01/07/2011 to 30/12/2014	139.476,00	43.117,50€
"Nitrato:nitrito:óxido nítrico: uma via crítica que suporta o impacto benéfico do vinho e do azeite na fisiologia gastrointestinal e cardiovascular." Coordinator: João Laranjinha	FCT Refª: PTDC/AGR-ALI/115744/2009	01/03/2011 to 31/12/2014	142.474,00	38.382,87€
"Indução de células estaminais pluripotentes a partir de células do sangue do cordão umbilical através de metodologia não-viral e a sua diferenciação em cardiomiócitos – iPSCardio."	FCT Refª: PTDC/SAU-ENB/113696/2009	01/04/2011 to 31/12/2014	135.649,00	31.582,81€

Coordinator: Ricardo Das Neves				
“Targets - TARgeted GEne Therapy Strategies to treat nerve injury.” Coordinator: Sérgio Paulo de Magalhães Simões Proponent: INEB Participants: Instituto de Biologia Molecular e Celular - IBMC/UP; ADFC/FC/UP	FCT Refª: PTDC/CPM-NAN/115124/2009	01/04/2011 to 30/09/2014	3.060,00	0,00€
“Regulação do sistema ubiquitina-proteassoma pelo BDNF nas sinapses do hipocampo: importância na plasticidade sináptica.” Coordinator: Carlos Duarte	FCT Refª: PTDC/SAU-NMC/120144/2010	10/02/2012 to 31/08/2015	154.678,00	18.967,71
“Fibrilas Interrompidas: Inibição de interacções aberrantes proteína-proteína em Amilóides.” Coordinator: Rui Brito	FCT Refª: PTDC/QUI-QUI/122900/2010	01/03/2012 to 31/08/2015	113.768,00	28.295,92€
“Nova Abordagem na Luta Contra a Tuberculose.” Coordinator: Maria Otília Vieira	FCT Refª: HMSP-ICT/0024/2010	01/01/2012 to 30/06/2015	206.610,00	48.625,26€
“Libertação de neuropeptídeos em feridas: uma nova terapêutica para o tratamento do pé diabético.” Coordinator: Ermelindo Leal	FCT Refª: PTDC/SAU-FAR/121109/2010	01/04/2012 to 30/09/2014	106.872,00	55.433,75€
“Contribuição para a erradicação da malária. Uma nova abordagem para atingir multi-alvos no ciclo de vida do parasita.” Coordinator: Luísa Melo Proponent: Faculade de Farmácia da Universidade de Coimbra; Participants: Instituto de Medicina Molecular (IMM/FM/UL)	FCT Refª: PTDC/SAU-FAR/118459/2010	01/03/2013 to 31/08/2015	5.500,00	1.887,38€
“O Óxido Nítrico na Doença de Alzheimer - Molécula Sinalizadora e Mediador de Patogénese.” Coordinator: Ana Ledo	FCT Refª: PTDC/BIA-BCM/116576/2010	01/04/2012 to 31/03/2015	81.698,00	28.182,04€
“Desenvolvimento de nanoparticulas multifuncionais inovadoras para o tratamento do cancro de mama.” Coordinator: João Nuno Moreira Proponent: Universidade do Minho	FCT Refª: PTDC/SAU-DMA/121028/2010	20/04/2012 to 30/09/2015	76.857,00	8.909,80€
“O sistema neuropeptídeo Y: potencial novo alvo terapêutico na retinopatia diabética” Coordinator: Francisco Ambrósio Proponent: Universidade de Coimbra	FCT Refª: PTDC/NEU-OSD/1113/2012	01/05/2013 to 31/08/2015	36.000,00	18.944,33
“Estratégia terapêutica combinada baseada na modulação de miRNAs direcionada para glioblastoma multiforme: um novo nanosistema de base lipídica para entrega sistémica.”	FCT Refª: PTDC/DTP-FTO/0265/2012	02/03/2013 to 01/06/2015	99.768,00	54.241,75€

Coordinator: Maria Conceição Pedroso Lima				
"Um Novo Modelo para a Esquizofrenia: Defeitos na Plasticidade Homeostática Mediada por Stargazina." Coordinator: Ana Luísa Carvalho	FCT Refª: PTDC/NEU-NMC/0750/2012	01/07/2013 to 30/09/2015	117.262,00	54.591,37€
"Doença de Machado-Joseph, agregação e degradação proteicas, biologia de células estaminais, proteostase, neurodegeneração." Coordinator: Luís Almeida	E-RARE4/0003/2012	01/03/2013 to 29/02/2016	141.581,00	42.781,11€
"Ambiguidade e virulência em patogénios humanos." Coordinator: Nuno Empadinhas Proponent: IBMC Instituto de Biologia Molecular e Celular - IBMC/UP	FCT Refª: PTDC/BBB-BEP/0695/2012	01/07/2013 to 30/09/2015	69.840,00	27.566,57€
"Papel dos receptores P2Y1 na polaridade neuronial e no crescimento axonial: implicações na proliferação das fibras musgosas na epilepsia." Coordinator: Ricardo Rodrigues	FCT Refª: EXPL/NEU-NMC/0671/2012	10/03/2013 to 09/04/2014	48.240,00	6.075,14€
"Tratamento da doença de Alzheimer com um novo peptídeo inibidor da BACE1." Coordinator: Armanda Santos	FCT Refª: PTDC/SAU-SCC/1351/2012	15/06/2013 to 30/09/2015	177.611,00	38.124,93€
"Plataformas combinatoriais para promover a sobrevivência celular- PROSURVIVAL." Coordinator: Hugo Fernandes	FCT Refª: PTDC/BIM-MED/1118/2012	01/07/2013 to 30/09/2015	130.000,00	43.475,95€
"Mecanismos associados à regulação ribosomal durante o desenvolvimento axonal." Coordinator: Rui da Costa	FCT Refª: EXPL/NEU-NMC/0541/2012	01/07/2013 to 31/12/2014	49.998,00	21.086,40€
"Papel da sirtuina 3 na função mitochondrial e desacetilação de alvos mitocondriais: relevância para a doença de Huntington Coordinator: Tatiana Rosado Rosentstock	FCT Refª EXPL/BIM-MEC/2220/2013	01/04/2014 to 30/09/2015	37750,89€	20.302,05€
''Projeto de investigação Exploratória'' Coordinator: João Peça	FCT Refª IF/00812/2012/CPO151/CT0001	19/06/2014 To 18/06/2018	50.000,00€	3.999,90€
''Projeto de investigação Exploratória" Coordinator: Ricardo Pires	FCT Refª IF/00123/2013	01/08/2014 to 31/07/2018	50.000,00€	18.618,43€
''Desvendar a vulnerabilidade das células do musculo liso de pacientes com Progeria - Smooth_Progeria'' Coordinador: Lino Ferreira	FCT Refª: EXPL/BIM-MED/2267/2013	01-03-2014 to 31-05-2015	49.800,00	22.129,92
"Regulação das proteínas hnRNP pela neurotrofina BDNF: importância da plasticidade sináptica." Coordinador: Carlos Duarte	FCT Refª: PTDC/SAU-NEU/104297/2008	15/09/2010 to 14/03/2014	120.000,00	745,00€

"Parametrização do metabolismo e crescimento tumorais através da análise de fluxos metabólicos e engenharia metabólica." Coordinador: Rui Carvalho	FCT Refª: PTDC/EBB-EBI/115810/2009	01/01/2011 to 30/06/2014	169.578,00	28.738,33€
"Caracterização da interacção Proteína - Carbohidrato da Laforina - Poteína humana envolvida na Doença de Lafora." Coordinador: Carlos Geraldes Proponent: Biocant	FCT Refª: PTDC/BIA-PRO/111141/2009	01/03/2011 to 30/08/2014	31.140,00	13.435,10€
"Regulação por fosforilação da ataxina-3, a proteína mutada na Doença de Machado Joseph." Coordinador: Ana Luísa Carvalho Participants: UM; IBMC	FCT Refª: PTDC/SAU-NMC/110602/2009	01/01/2011 to 30/06/2014	123.777,00	37.434,85€
"Via para a síntese do MGLP de micobactérias. Caracterização bioquímica e estrutural das enzimas envolvidas." Coordinador: Nuno Empadinhas Participants: IBMC	FCT Refª: PTDC/BIA-PRO/110523/2009	01/01/2011 to 30/06/2014	130.624,00	17.456,10€
"Desenvolvimento de uma vacina contra a hepatite B para ser administrada através das mucosas: Desenho e estudos mecanísticos de um protótipo de um sistema de libertação multicomponente nanoparticular." Coordinador: Olga Ribeiro	FCT Refª: PTDC/SAU-FAR/115044/2009	01/01/2011 to 31/03/2014	122.060,00	16.796,97€
"Desenvolvimento de uma nova estratégia terapêutica para o cancro do pâncreas envolvendo uma acção concertada de terapia génica e quimioterapia." Coordinador: Henrique Faneca	FCT Refª: PTDC/SAU-BMA/114482/2009	01/01/2011 to 30/06/2014	100.000,00	21.498,15€
"iCALP - Identificação das funções fisiológicas das calpaínas no controlo da proliferação e migração celulares no sistema nervoso central." Coordinador: Inês Araujo	FCT Refª: PTDC/SAU-NMC/112183/2009	01/03/2011 to 31/08/2014	142.560,00	61.835,14€
Programa MIT Coordinator: Catarina Oliveira, Lino Ferreira	FCT Refª: MIT-Portugal 2014	01/01/2014 to 31/12/2014	13 674,00	13.243,37€
"Novas estratégias para a recuperação da fertilidade e potencial genético de felídeos selvagens: desenvolvimento do xenotransplante e da transplantação de células espermatogoniais estaminais em gato doméstico como modelo para felídeos selvagens." Coordinator: Paula Mota	FCT Refª: PTDC/CVT/119477/2010	01/05/2012 to 30/09/2015	62.813,00	11.501,67€
"Modulação da actividade de células estaminais hematopoiéticas por acção de nanopartículas capazes de libertar factores de transcrição – STEMCELLMODULATORS." Coordinator: Ricardo Pires das Neves	FCT Refª: PTDC/CTM-NAN/120552/2010	01/05/2012 to 30/09/2015	115.884,00	31.055,37€

“Modulação da piruvato desidrogenase cinase e pluripotência: Implicações para cancro e biologia de células estaminais.” Coordinator: João Ramalho	FCT Refª: PTDC/QUI-BIQ/120652/2010	06/05/2012 to 31/08/2015	130.000,00	34.933,94€
“Produção e propagação de linhas de células estaminais pluripotentes usando modulação metabólica.” Coordinator: João Ramalho	FCT Refª: PTDC/EBB-EBI/120634/2010	06/05/2012 to 05/09/2015	94.000,00	18.040,28€
“Papel fisio-patológico da ecto-5`-nucleotidase - um novo alvo para neuroprotecção.” Coordinator: Rodrigo Cunha	FCT Refª: PTDC/SAU-TOX/122005/2010	01/05/2012 to 31/08/2014	147.605,00	44186,06€
“Papel do accumbens e amigdala no controlo da neuropatologia causada por stress crónico.” Coordinator:Rodrigo Cunha	FCT Refª: PTDC/SAU-NSC/122254/2010	01/04/2012 to 30/09/2014	148.080,00	55.383,03€
“BIOMARKAPD: Biomarcadores para Doença de Alzheimer e Doença de Parkinson.” Coordinator:Catarina Oliveira	FCT Refª: JPND/0005/2011	01/06/2012 to 31/05/2015	48.500,00	17.246,39€
“Bioprospecção de enzimas com capacidade de degradar biomassa vegetal no metagenoma do sistema digestivo de Porcellio dilatatus (Crustacea,Isopoda).” Coordinator: António Veríssimo	FCT Refª: PTDC/AGR-TEC/3789/2012	01/05/2013 to 30/09/2015	90.000,00	47.194,00€
“Patofisiologia da Toxicidade Cardíaca Persistente da Doxorubicina: Uma ligação entre Mitocôndria e Epigenetica” Coordinator: Paulo Oliveira	FCT Refª: PTDC/DTP-FTO/1180/2012	01/05/2013 to 31/08/2015	175.000,00	79.296,65€
“O metilfenidato e as alterações na barreira hemato-encefálica numa situação fisiológica e na perturbação de hiperatividade com défice de atenção” Coordinator: Ana Paula Silva Proponent: Universidade de Coimbra	FCT Refª: PTDC/NEU-OSD/0312/2012	01/06/2013 to 30/09/2015	60.336,00	14.269,63€
“Mecanismos de protecção neuronal contra stress oxidativo mediados pela DJ-1: implicações na doença de Parkinson” Coordinator:Bruno Manadas Participant:Biocant, Univ.Minho, U.Beira Interior	FCT Refª: PTDC/NEU-NMC/0205/2012	01/05/2013 to 30/09/2015	113.870,00	64.018,08€
“Biossíntese de polissacáridos raros de metilmanose em micobactérias não tuberculosas” Coordinator:Nuno Empadinhas Participant:IBMC,ITQB	FCT Refª PTDC/BIA-MIC/2779/2012	01/07/2013 to 30/09/2015	100.360,00	47.057,06€
“Investigação bigenómica translacional na Neuropatia Ótica Hereditária de Leber: Correlação Genótipo-Fenótipo” Coordinator:Manuela Grazina Participant:CCMAR-Alg	FCT Refª: PTDC/DTP-EPI/0929/2012	01/04/2013 to 31/08/2015	192.780,00	36.913,01€

"Células estaminais tumorais e progressão tumoral: dos mecanismos moleculares às consequencias clínicas" Coordinator:Maria Carmen Alpoim	FCT Refª: PTDC/BBB-BQB/2450/2012	01/05/2013 to 30/09/2015	132.248,00	24.800,13€
"Mecanismos e estrategias de tratamento da deficiência da cicatrização cutânea na diabetes" Coordinator: Susana Guerreiro	FCT Refª: PTDC/BIM-MED/0492/2012	01/07/2013 to 30/06/2014	50.000,00	37.961.00€
''Projeto de investigação Exploratória'' Coordinator: Miguel Mano	FCT Refª: IF/00694/2013	10/07/2014 to 30/06/2018	50.000,00	2.463,06€
''Projeto de investigação Exploratória'' Coordinator: Paulo Pinheiro	FCT Refª: IF/01302/2012	01-01-2014 to 30-09-2018	50.000,00	0,00€
"Nova abordagem da disfunção reprodutora na diabetes: análise 3D da espermatogénese e microscopia confocal Raman para análise da função mitocondrial" Coordinator:Sandra Amaral	FCT Refª: PTDC/BEX-BCM/0224/2012	03/07/2013 to 02/07/2014	48132,00	33.960,36€
Sub – Total FCT				**2.243.996,29€**
Other National Projects				
"CNC Biotech – Investigação em Biotecnologia e capacitação do sector empresarial Coordinator: Carlos José Fialho da Costa Faro	Mais Centro-Programa Operacional Regional do Centro Refª: ICT_2009_02_041_1769	18/06/2009 to 30/06/2015	10.834.801,05	1.296.046,47
"Ibercivis.pt - Uma plataforma de computação voluntária para a Península Ibérica." Coordinator: Rui Manuel Pontes M. F. Brito	UMIC - Agência para a Sociedade do Conhecimento	16/06/2010 to 30/06/2015	87.380,00	2.117,38€
''DoIT – projeto nº 013853'' Coordinator: Catarina Oliveira	Agência da Inovação, S.A.	01/07/2010 to 28/02/2015	378.154,38	92.866,97€
"Aging,Stress and Chronic Diseases:From mecanisms to therapeutics" Coordinator:Luis Almeida Proponent:Universidade de Coimbra	Mais Centro-Programa Operacional Regional do Centro Refª: SCT_2011_02_006_4819	01/06/2013 to 30/06/2015	128.093.92	63.965,28€
"New Strategies do manage Brain Diseases." Coordinator: Luís Almeida Proponent: Universidade de Coimbra	Mais Centro-Programa Operacional Regional do Centro Refª:SCT_2011_02_002_4756	01/06/2013 To 30/06/2015	305.220,15	175.112,08€
QREN-Amilotera: 021622 Coordinator: Rui Brito	Agência da Inovação, S.A	01/09/2011 to 31/08/2014	85.804.45	24.508,37€
"Engenharia Epigenética para reverter o Fonótipo Celular da Doença de Parkinson" Coordinator: André Valente/Paulo Olieira	Fundação Montepio	01/06/2014 To 31/05/2016	105.950,00€	13.731,24€
"Stemcell based platforms for Regenerative and Therapeutic Medicine" Coordinator: Carlos José Fialho da Costa Faro Proponent: Universidade de Coimbra	Mais Centro-Programa Operacional Regional do Centro Refª: SCT_2011_02_008_4832	01/02/2013 To 28/02/2015	682.875,01	325.763,42€

“Plataformas de Bioimagem, Comportamento e Electrofisiologia@CNC Coordinator: Catarina Isabel Neno Resende de Oliveira	Mais Centro-Programa Operacional Regional do Centro Refª: ICT-2013-05-030-5377	01/01/2014 to 31/03/2015	987.923,50€	663.474,60€
“Evaluation of oxidative stress and mitochondrial dysfunction in animal models and patients of Huntington`s disease using Cu(II)-ATSM PET Coordinator: Ana Cristina Carvalho Rego	Santa Casa da Misericórdia de Lisboa: “Prémio Mantero Belard´2013"	01/01/2014 to 31/12/2016	99.072,00€	15.462,49€
Sub – Total Other				**2.673.048,30€**
Total National Projects				**4.917.044,59€**
International Projects:				
“Cellular and Synaptic Dissection of the Neuronal Circuits of Social and Autistic Behavior” Coordinator: João Peça Silvestre	Brain & Behavior Research Foundation: “2013 Narsad Young Investigator Grant”	15/01/2014 to 14/01/2016	45.000€	25.832,48€
“Silencing Machado-Joseph Disease/Spinocerebellar ataxia type 3 through the systemic route” Coordinator: Rui Nobre Jorge	National Ataxia Foundation	01/01/2014 to 31/12/2014	10.823,71€	1.785,76€
“Promoting endothelial progenitor cell function in diabetes would healing” Coordinator: Ermelindo Carreira Leal	European Foundation for the Study of Diabetes/JDRF/Novo Nordisk European Programme in Type 1 Diabetes Research	01/01/2013 to 31/12/2014	50.000,00€	11.240,53€
"Industrial Academic Initial Network towards treatment of Polyglutamine diseases" Coordinator: Luís Almeida	Marie-Curie-264508 Ref.ª FP7-PEOPLE-ITN-2010	01/03/2011 to 28/02/2015	202.332,86	62.717,84€
Novel nanoparticles for drug delivery to the skin Coordinator: Lino Ferreira	Queen Mary - 289454 Ref.ª: FP7-PEOPLE-2011-ITN	01/11/2011 to 31/10/2015	471.627,60	119.023.22€
"Docotral Candidate Agreement 159302-1-2009-NL-Era Mundus-EMJD" Coordinator: Rodrigo Cunha	Marie-Curie-Cycle 2-2011-PT	21/06/2011 to 30/08/2014	89.680,00	67.625,09€
“The role of local mRNA translation in synapse formation” Coordinator: Ramiro Daniel Carvalho de Almeida	Marie Curie Actions Refª: PIRG-GA-2009-249288	01/04/2010 to 31/03/2014	100.000,00	22.799,01€
"The effect of TCF7L2 on Glucose Metabolism" Coordinator: John Jones	Mayo Clinic 5Ro1DK078646-07	01/08/2013 to 31/07/2014	15.701,04	8359,25€
"The effect of TCF7L2 on Glucose Metabolism" Coordinator: John Jones	Mayo Clinic 5Ro1DK078646-08	01/08/2014 To 31/07/2015	14.223,58€	7.503,00€

“Activating autophagy to block Machado-Joseph disease progression Coordinator: Luís Pereira de Almeida	Association Française contre les Myopathies Ref.ª: 180151	01/08/2014 to 31/07/2015	60.000,00	0€
“New Treatments for Stress-induced Dysregulation of Circuits Regulating Reward, Fear, and Habit Learning”. Coordinator: Rodrigo Cunha	Massachusetts Institute of Technology Ref.ª: DARPA-BAA-009-68	01/04/2010 to 30/11/2015	944.680,00	276.978,58€
“DFRH/WIIA/51/2011 - Welcome II” Coordinator: Catarina Oliveira/Otília Vieira	Marie Curie Actions DFRH/WIIA/51/2011 - Welcome II	01/02/2012 to 31/01/2014	119.740,50	65.251,92€
“Unravelling the early steps in the biosynthesis of the mycobacterial MGLP.” Coordinator: Nuno Empadinhas	Mycobacterial MGLP	01/04/2012 to 31/03/2014	19.758,57	1.671,70€
“CAFFEIN-Cancer Associated Fibroblasts (CAF) Function in Tumor Expansion and Invasion”. Coordinator: João Nuno Moreira	Marie Curie grant 316610 Refª FP7-People-2012-ITN	01/10/2012 to 30/09/2014	209.781,00	39.387,39€
"Trigerralde nanomaterials to modulate cell activity" Coordinator:Lino Ferreira	European Research council executive agency" Ref.ª ERC-2012-StG 307384-NanoTrigger	01/11/2012 to 30/10/2017	1.699.320,00	440.065,12€
“Caffeine alleviation of MJD/SCA3” Coordinator: Luís Almeida	National Ataxia Foundation	01/01/2013 to 31/12/2014	11.186,27€	0€
“Transplantation of neural stem cells (NSC) as a new therapeutic strategy for Machado-Joseph disease (MJD). Coordinator: Liliana Mendonça	National Ataxia Foundation	01/01/2014 To 31/12/2014	10.823,71€	3.678,90
“LRRK2 role on auto-antibody production by human B cells.” Coordinator: Margarida Carneiro	The Michael J. Fox Foundation for Parkinson’s Research	16/05/2013 to 16/03/2014	81.325,76€	27.389,23€
“Mitochondrial Trafficking In Alzheimer Disease: Revealing the Role of Hummr.” Coordinator: Paula Moreira	Alzheimer Association NIRG-13-282387	01/11/2013 to 31/10/2014	71.495,56€	15.704,89€
“In chemico, in silico and in vitro modelling to predict human respiratory allergens” Coordinator: Maria Teresa Cruz Rosete	John Hopkins Bloomberg Ref.ª 2014-07	01/02/2014 To 31/01/2015	11547,12€	10.764,74€
‘‘Pharmacological activation of autophagy to alleviate Machado-Joseph disease’’ Coordinator: Luís Almeida	National Ataxia Foundation	01-01-2014 to 31-12-2014	72.249,25	71.443,87
‘‘The role of ataxin-2 in in Machado-joseph disease:a molecular therapy approach with viral vectors’’ Coordinator: Clévio Nobrega	National Ataxia Foundation	01-01-2014 to 31-12-2014	10.823,71	5.761,25
‘‘ENC Network Cycle 4-2013 - PT - 04 -Amber Kerkhofs’’ Coordinator: Rodrigo Cunha	European Neuroscience Campus Network Cycle	01/10/2013 to 30/09/2015	121.900,00	35.097,58€
‘‘159302-1-2009-1-NL-ERA MUNDUS-EMJD’’ Coordinator: Ana Luísa Carvalho	European Neuroscience Campus Network	15-09-2014 to 14-09-2016	121.900,00	10.736,56€

‘‘Role of Adenosine A2A Receptors in the Accumbens and mygdala in the control of Chronic Stress Neuropathology’’ Coordinator: Rodrigo Cunha	Brain & Behavior Research Foundation: “2014 Narsad Independent Investigator Grant”	15-09-2014 to 14-09-2016	78.204,00	13.889,24€
‘‘ENC Network Cycle 4-2013 - PT - 07 - Xin-Li Xu’’ Coordinator: Rodrigo Cunha	European Neuroscience Campus Network	01/10/2013 to 30/09/2015	126.400,00	44.034,84€
"Cellular and synaptic dissection of the neuronal circuits of social and autistic behavior" Coordinator: João Peça	Marie Curie FP7-People-20123-CIG PCIG13-GA-2013-618525	01/08/2013 To 31/07/2017	100.000,00	15.501,04€
"Chronic effects of silver nanoparticles (AgNPs) on rat liver, kidney and heart mitochondrial function" Coordinator: Carlos Manuel M. Palmeira	DFAS_Indianopolis Center EOARD FA8655-13-1-3036	25/02/2013 To 28/02/2014	18.461,44	7.559,77€
Total International Projects				**1.417.663,23€**
TOTAL				**6.334.707,82€**

LIST OF STAFF AND RESEARCH STUDENTS

GENERAL LIST

Members holding PhD		**Time % at CNC**
Akhilesh Rai	(Assistant Inv., CNC)	100
Alcino Jorge Lopes Leitão	(Assistant Prof., FFUC)	60
Alexandrina F. Mendes	(Assistant Prof., FFUC)	80
Amílcar Falcão	(Full Prof., FFUC)	50
Ana Bela Sarmento Ribeiro	(Assistant Prof., FMUC)	40
Ana Cristina Fortuna	(Inv. Assistant Prof., FFUC)	50
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Ledo	(Assistant Inv., CNC)	100
Ana Luísa Cardoso	(Assistant Inv., CNC)	100
Ana Luísa Monteiro de Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula M. Sousa	(Investigator, CHUC)	50
Ana Rita Costa Álvaro	(Assistant Inv., CNC)	100
Ana Santos Carvalho	(Assistant Inv., IEC)	20
Anabela P. Rolo	(Assistant Prof., FCTUC)	80
Anália do Carmo	(Assistant Prof., Univ. Vasco da Gama)	80
André Xavier C. Negrão Valente	(Assistant Inv., CNC)	100
Ângelo R. Tomé	(Assistant Prof., FCTUC)	70
António Macedo Santos	(Assistant Prof., FMUC)	30
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	60
António Pedro Gomes	(Assistant Inv., CNC)	100
Armanda E. Santos	(Assistant Prof., FFUC)	80
Armando Cristóvão	(Assistant Prof., FCTUC)	20
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Arsélio P. Carvalho	(Full Prof., FCTUC)	100
Attila Köfalvi	(Assistant Inv., CNC)	100
Bruno José F. Manadas	(Assistant Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	100
Carla Vitorino	(Assistant Prof., FFUC)	50
Carlos B. Duarte	(Associate Prof., FCTUC)	80
Carlos Cavaleiro	(Assistant Prof., FFUC)	50
Carlos Faro	(Associate Prof., FCTUC)	50
Carlos Matias	(Investigator, UTAD)	50
Carlos M. Palmeira	(Full Professor, FCTUC)	80
Catarina R. Oliveira	(Full Prof., FMUC)	60
Célia Aveleira	Assistant Inv., CNC)	100
Célia Laurinda Nogueira	(Assistant Prof., FMUC)	60
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	60
Cristiana Paulo		Collaborator
Emília P. Duarte	(Assistant Prof., FCTUC)	80

Euclides Pires	(Associate Prof., FCTUC)	50
Eugénia Carvalho	(Assistant Inv., CNC)	100
Faraj Barah	(Investigator, CNC)	100
Fernando Monteiro Judas	(Assistant Prof., FMUC)	Collaborator
Fernando Nogueira	(Associate Prof., FFUC)	Collaborator
Fernando Ramos	(Associate Prof., FFUC)	50
Gabriela Silva	(Assistant Prof., FFUC)	60
Henrique Faneca	(Assistant Inv., CNC)	100
Henrique Bernardo Silva	(Assistant Inv., CNC)	100
Hugo Fernandes	(Assistant Inv., CNC)	100
Ildete Luísa Ferreira	(Assistant Inv., CNC)	100
Inês Esteves Baldeiras	(Investigator, FMUC)	30
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	30
Isaura Simões	(Assistant Inv., CNC)	100
Joana Cardoso Costa	(Assistant Professor, FCTUC)	60
Joana Simões Correia	(Assistant Inv., CNC)	100
Joana Rosmaninho-Salgado	(MD, CHUC)	20
João José Sousa	(Associate Prof., FCTUC)	50
João Laranjinha	(Full Prof., FFUC)	60
João Moura	(Assistant Prof., Inst. Pol. Viana Castelo)	50
João Nuno Moreira	(Assistant Prof., FFUC)	80
João Peça-Silvestre	(Assistant Inv., CNC)	100
João Ramalho Santos	(Associate Prof., FCTUC)	80
John Griffith Jones	(Principal Inv., CNC)	100
Jorge António R. Salvador	(Full Prof., FFUC)	60
Jorge Valero Gomez-Lobo	(Assistant Inv., CNC)	100
José Alberto Correia e Vale	(MD, Univ. Salamanca)	Collaborator
José Custódio	(Associate Prof., FFUC)	80
José Dionisio	(Assistant Prof., FFUC)	75
Leonor Almeida	(Full Prof., FFUC)	40
Lígia Salgueiro	(Full Prof., FFUC)	50
Lino Ferreira	(Investigator, CNC)	100
Luís Loura	(Associate Prof., FCTUC)	Collaborator
Luís M. Rosário	(Associate Prof., FCTUC)	60
Luís Pereira Almeida	(Assistant Prof., FFUC)	80
Magda Santana	(Assistant Inv., CNC)	100
Manuel Garrido	(Investigator, Genibet)	30
Mª Amália Jurado	(Assistant Prof., FCTUC)	80
Mª Carmen Alpoim	(Associate Prof., FCTUC)	60
Mª Celeste Lopes	(Full Prof., FFUC)	80
Mª Ceu Sousa	(Assistant Prof., FFUC)	40
Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Dolores T. Redondo	(Investigator, Univ. Salamanca)	Collaborator
Mª do Rosário Almeida	(Assistant Inv., CNC)	100

Mª Emilia O. Quinta Ferreira	(Associate Prof., FCTUC)	60
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	80
Mª Isabel J. Santana	(Associate Prof., FMUC)	30
Mª João Silvestre	(Assistant Prof., FCTUC)	Collaborator
Mª José Gonçalves	(Assistant Prof., FFUC)	Collaborator
Mª Luisa Sá e Melo	(Full Prof., FFUC)	60
Mª Manuel da Cruz Silva	(Assistant Prof., FFUC)	60
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
Mª Margarida Catalão Castro	(Assistant Prof., FCTUC)	Collaborator
Mª Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Mª Sancha Santos	(Principal Inv., FCTUC)	100
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Miguel Silva Empadinhas	(Assistant Inv., CNC)	100
Olga Maria F. Borges Ribeiro	(Assistant Prof., FFUC)	60
Paula G. Agostinho	(Investigator, FMUC)	60
Paula Isabel Moreira	(Assistant Prof., FMUC)	60
Paula Veríssimo Pires	(Assistant Prof., FCTUC)	60
Paulo J. Oliveira	(Assistant Inv., CNC)	100
Paulo Pinheiro	(Assistant Inv., CNC)	100
Pedro Castanheira	(Investigator, Biocant)	Collaborator
Raghu Kalluri	(Investigator, HMS)	35
Ramiro Almeida	(Assistant Inv., CNC)	100
Ricardo Neves	(Assistant Inv., CNC)	100
Ricardo Pires	(Assistant Inv., CNC)	100
Ricardo Rodrigues	(Assistant Inv., CNC)	100
Renata Silva	(Assistant Inv., CNC)	100
Rodrigo A. Cunha	(Associate Prof., FMUC)	75
Rosa M. Santos	(Assistant Prof., FCTUC)	60
Rui A. Carvalho	(Assistant Prof., FCTUC)	50
Rui Barbosa	(Assistant Prof., FFUC)	60
Rui M. M. Brito	(Associate Prof., FCTUC)	Collaborator
Rui Pinto	(Assistant Prof., EUVG)	30
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Assistant Inv., FMUC)	60
Sara Domingues	(Assistant Prof., FFUC)	60
Sérgio Simões	(Associate Prof., FFUC)	80
Teresa Dinis Silva	(Associate Prof., FFUC)	40
Teresa Gonçalves	(Assistant Prof., FMUC)	60
Teresa Cruz Rosete	(Assistant Prof., FFUC)	80
Teresa Maria C. Martins	(Assistant Investigator, IPO)	80
Vera Lúcia Dantas Moura	(Manager Science & Tech., UC)	50
Vitor Manuel C. Madeira	(Full Prof., FCTUC)	80

Post-Doc Members	Time % at CNC
Adrian Balsa	100
Alessandro Boli	100
Ana Burgeiro	100
Ana Filipa Henriques	30
Ana Isabel Duarte	100
Ana Maria Cardoso	100
Ana Oliveira	100
Ana Patricia Simões	100
Ana Raquel Esteves	100
Ana Silva	Collaborator
Ana Teresa Simões	100
Ana Teresa Varela	100
Cândida Gonçalves da Silva	35
Carla Nunes	100
Carlos Rodrigues	100
Catarina Alexandra Gomes	100
Catarina Miranda	100
Cátia Marques	100
Chantal Fernandes	100
Clévio Nóbrega	100
Daniel Rial	100
Diana Silva	100
Elsa Henriques	100
Elisabete Baptista Ferreiro	100
Ermelindo Leal	100
Filipe Valente Duarte	100
Graciano Leal	100
Helena Vazão	100
Igor Tiago	100
Ivan Viegas	100
Joana Isabel Real	100
Joana Fernandes	100
Joana Marques	100
João Fernando S. Carvalho	Collaborator
João Paulo Teodoro	100
João Pedro Lopes	100
*Lígia Maria S. Ferreira	100
Liliana Mendonça	100
Luis Miguel Estronca	100
Margarida Caldeira	100
Mariana Ponte Ribeiro	100

Mª Alexandra B. Amaral	100
Mª Teresa Cunha Oliveira	100
Anabela Marisa Azul	100
Mário Laço	100
Marta Mota Vieira	100
Michele Curcio	100
Miranda Mele	100
Nelio Gonçalves	100
Nuno Mendonça	100
Patricia Ribeiro	100
Paula M. Canas	100
Paula Mota	100
Pedro Costa	100
Pedro Miguel Coelho	100
Renato Cardoso	100
Ricardo Santos	100
Rita Perfeito	100
Rosa M. B. Matos Resende	100
Rui Lopes	100
Rui Nobre	100
Rui Oliveira Costa	100
Samira Ferreira	100
Sandra Catarina G. Amaral	100
Sandra Isabel F. Mota	100
Sandro Pereira	100
Sezin Aday	100
Sofia Guedes	100
Sónia Correia	100
Sónia Duarte	100
Sonia Luzia Pinho	100
Susana Cardoso	100
Susana Guerreiro	50
Susana Isabel E. Alarico	100
Susana Ribeiro Louros	100
Susana Rosa	100
Tatiana Catarino	100
Tatiana Emanuelli	50
Teresa Serafim	100
Vilma Sardão Oliveira	100
Vera Francisco	50
Vitor Mendes	100

PhD Students	Time % at CNC
Amber Kherkoffs	100
Ana Carolina Romero	100
Ana Maranha	100
Ana Cristina F. Lemos	100
Ana Cristina Gonçalves	100
Ana Cristina Gregório	100
Ana Catarina Ferreira	100
Ana Catarina R. Graça Fonseca	100
Ana Cristina Ferreira	100
Ana Filipa Cruz	100
Ana Francisca Lima	100
Ana Isabel Serralheiro	100
Ana Luísa Nobre	100
Ana Mª Silva	100
Ana Patricia Marques	100
Ana Plácido	100
Ana Sofia Lourenço	100
Ana Sofia C. Valdeira	100
Ana Sofia V. Cunha	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa Rufino	100
Ana Teresa Viegas	100
Ana Xavier	100
André Filipe M. Soares	100
Andreia Freitas	100
Andreia Gomes	100
Ângela Valério-Fernandes	100
Ângela Pascoal Crespo	100
Anna Vladimirovna Pliassova	100
*António Silva	100
Bárbara Rocha	100
Beatriz Lacerda de Sousa	100
Bruno Miguel F. Gonçalves	100
Carla Mª Nunes Lopes	100
Carla Patrícia R. Paiva	100
Carlos Adriano Matos	100
Carlos Samuel M. Boto	100
Cassilda Pereira	100
Catarina Mendes Morais	100
Catarina Praça de Almeida	100
*Cátia Moreira de Sousa	100

Daniela Gonçalves	100
Daniela Pereira S. Alho	100
David Bowman	25
Diana Jurado S. Serra	100
Dina Pereira	100
Dominique Fernandes	100
Dulce Bento	100
Edna Filipa Soares	100
Elsa Rodrigues	100
Emanuel Candeias	100
Emanuel Costa	100
Filipa L. Carvalho	100
Filipa Lebre	100
Francisco Manuel Queiroz	100
Geetha Vijayakumar	100
Gianluca Selvaggio	100
Gladys Caldeira	100
Graça Rocha	40
Graciana Tribuna	50
Gustavo Costa	100
Inês Biscaia Barbosa	100
Inês Honório	100
Inês Vasconcelos M. Santos	75
Isabel Maria Santos Onofre	100
Isabel Maria Ramalho	100
Ivan Salazar	100
Ivana Kostic	100
Janete Cunha Santos	100
Jeannette Schmidt	100
Jimmy George	100
Joana Bicker	100
*Joana Liberal	100
Joana Domingues Vindeirinho	100
*Joana Duarte Neves	100
Joana Pedro	100
Joana Ribeiro Guedes	100
Joana Sousa	100
João André Freitas	50
João Ribas Almeida	100
João Demétrio B. Martins	100
João Pedro Oliveira	20
Josephine Blerch	100
*Júlia Valente	100
Kátia Mesquita	100

Lara Franco	100
Lisa Rodrigues	100
Luana Naia	100
Ludgero C. Tavares	100
Luís André A. França	100
Mafalda Costa	100
Marcelo Correia	100
Maria Fernandes	100
Mª Inês Almeida Sousa	100
Mª Joana G. Pinto	100
Mª la Salete J. Baptista	100
Mª Madalena Ribeiro	100
Mariana Botelho da Rocha	100
Mariana Oliveira Conceição	100
Mariangela Natale	100
Marília Henriques Cordeiro	100
Mariline Silva	100
Marisa Gaspar	100
Marta Cerejo	100
Marta Daniela Passadouro Caetano	100
Marta Pereira	30
Michela Comune	100
Miguel António azinheira	100
Miguel Maria Lino	100
Mohamed Hussien	100
Mónica Abreu	80
Nuno Ferreira	100
Nuno André Fonseca	100
Nuno Gabriel Machado	100
Nuno Miguel Jesus Machado	100
Nuno Mendonça	25
Nuno Silva	100
Patrícia Lopes	100
Patrícia Raquel Pereira	100
Patrícia Rosado	100
Patrícia Sofia Morais	100
Paulo Magalhães	100
Pedro Curto	100
Pedro João Madeira Afonso	100
Pedro José Gouveia	100
Pedro Manuel Batista Branco	100
Raquel Costa	100
Ravi Adusumalli	100
Renato Xavier C. Santos	100

Ricardo Romão Leão	25
Rodrigo Luiz Santos	100
Roksana Pirzgalska	100
Rui Benfeitas Vicente	100
Rui Soares	60
Rui Cruz	100
Rui Figueiredo	100
Sandra Figueiredo	100
Sandra Cristina Jesus	100
Sandra Marina A. Santos	100
Sara Amaral	100
Sara Lopes	100
Sara Matias Silva	100
Sara Raquel Oliveira	100
Sofia Anastácio	100
Sofia Alexandra Ferreira	100
Sofia Romano	100
Sonya Costa	100
Susana Patrícia S. Pereira	100
Susana Sampaio	100
Tânia Leandro	100
Tânia Perestrelo	100
Tiago Rodrigues Sousa	70
Tiago Alfaro	75
Vanessa Isabel S. Mendes	100
Vera Calhau	100
Vitor Manuel Carmona	100
Xinli Xu	100

MSc Students	**Time % at CNC**
Abdullah Nawabjan Shaik	100
Alexandra Carvalho	100
Ana Alves	100
Ana Rita Rocha	100
Ana Catarina Cardoso	100
Ana Marta Silva	100
Ana Pica-Milho	100
Ana Raquel Coelho	100
Ana Rita Cruz	100
Ana Xavier	100
Ângelo Serani	100

Bruno Cruz	100
Catarina Carmo	100
Catarina Xavier	30
Daniela Costa	100
Diana Maurício	100
Diogo Reis	100
Edmilson Semedo	100
Eduardo Morais	100
Fabiana Soares	20
Fábio Carvalho	100
Gabriela Leão	100
Guilherme Loureiro	100
Helena Leal	100
Helena Martins	100
Inês Saragoça Dias	100
Inês Sebastião	100
Irina Fonseca	100
Joana Portela	100
João Filipe Amorim	100
José Miguel Codesso	100
Liliana Caetano	100
Liliana Santos	100
João Calmeiro	100
Leisa Évora	100
João Filipe Amorim	100
Manuela Cerqueira	50
Marcelo Catarino	50
Marcelo Ribeiro	100
Mariana Magalhães	100
Mário Carvalho	100
Maura De Rosa	100
Nanci Ferreira	100
Paula Silva	90
Paulo Silva	40
Pedro Cunha	100
Rafael Paiva	50
Renata Couto	100
Ricardo Silva	100
Ricardo Vieira	100
Ruben Branco	100
Rui Beleza	100
Rui Gomes	100
Rui Simões	100
Rute Araújo	100

Sara Dias	100
Sara Rebelo	100
Sílvia Magalhães	100
Susana Cecílio	100
Susana Paixão	100
Solange Machado	100
Teresa Silva	100
Vanessa Rodrigues	100
Vânia Moreira	100
Vilte Sauliūnaitė	25

Grant Technicians	**Time % at CNC**
Alexandra Isabel Abrunheiro	100
Ana Margarida Oliveira	100
Ana Sofia L. Coelho	100
Ana Sofia Miranda	100
Andreia Madeira	50
Cândida Dias	100
Caroline Delgado Veloso	100
Cláudia Maria C. Deus	100
Cristina Barosa, PhD	100
Cristina Carvalho, PhD	100
Dina Farinha	100
Fabio Paiva	100
Fatima Nunes	25
Hugo Filipe, PhD	50
Isabel Ferreira	100
Joana Sousa	100
João Ferreira	100
José Miguel J. Paiva	100
Luís Martins	100
Marisa Marques	100
Marta Baptista	100
Marta Mota	40
Mónica Zuzarte	50
Paulo Dias	25
Pedro Alves	50
Renata Tavares	100
Rita Pereira	100
Sandra Pinto	100
Sónia Neto R. Pereira	100
Sónia Pereira	30
Tatiana Martins	100

Vanessa Anjos	100
Vanessa Monteiro	100
Vinicio Oliveira	100

MD	**Time % at CNC**
Fernando Judas	20
Hermínio José T. Espírito Santo	30
Maria Isabel Santana	30
Luís Cunha	Collaborator
Luísa Diogo	Collaborator
Mª Margarida Martins Gonçalo	40
Maria Olinda R. Rebelo	Collaborator

SERVICE STAFF

		Time % at CNC
Ana Cristina F. Barbosa Soares	(Graduate Technician, CNC)	100
Ana Cristina Franco dos Santos	(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
Mª Helena Garrucho Ribeiro	(Graduate Technician, HUC	20
Maria João Ferreira Canas dos Santos	(Graduate Technician, CNC)	100
Marta Sofia Marques Simões	(Graduate Technician, CNC)	100
Mónica Alexandra V. Serrano	(Graduate Technician, CNC)	100
Paulo Rodrigues-Santos	(Graduate Technician)	20

TECHNICAL STAFF

		Time % at CNC
Cármen Lídia Graça Semião	(Graduate Technician, CNC)	100
Diana dos Santos Simões Graça	(Technician, CNC)	100
Fátima Cristina dos S. Carvalho Graça	(Technician, CNC)	100
Isabel Conceição Calado Esteves Costa	(Technician, CNC)	100
Isabel Nunes Correia	(PhD, Graduate Technician, CNC)	100
Isabel Dantas Fernandes	(Graduate Technician, CNC)	100
Luisa Leitão Cortes	(PhD, 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
Vera Mónica M. Mendes	(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
Sandra Manuela Domingues dos Santos	(PhD, Graduate Technician, CNC)	100
Sara da Costa Jordão A. Lopes	(Technician, CNC)	100
Sandra Freire	(Technician, CNC)	100
Vera Oliveira	(Graduate Technician, CNC)	100

ADMINISTRATIVE STAFF

		Time % at CNC
Carla Lopes	(Administrative Assistant, CNC)	100
Catarina Alexandra Ferreira Gomes	(Graduate Administrative, CNC)	100
Heidi Maria da Silva Lopes Gonçalves	(Graduate Administrative, CNC)	100
Lia da Costa Jordão Aparício Lopes	(Graduate Administrative, CNC)	100
Mª Luísa R. Caldeira Bonito	(Graduate Administrative, CNC)	100
Mónica Alexandra Rodrigues Morais	(Graduate Administrative, CNC)	100
Nilza Clara F. Marques Manadas	(Administrative Assistant, CNC)	100
Rosa Alexandra Folhas Fernandes	(Graduate Administrative, CNC)	100
Sandra Cristina Santos Luís	(Graduate Administrative, CNC)	100
Sílvia Marisa Esteves de Sousa	(Graduate Administrative, CNC)	100
Susana Adelaide Rocha da Silva	(Administrative Assistant, CNC)	100
Tatiana de Azevedo Paula	(Graduate Administrative, CNC)	100

RESEARCH STAFF AND STUDENTS / SCIENTIFIC RESEARCH LINE

Neuroscience and Disease

Carlos Bandeira Duarte, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Ledo	(Assistant Inv., CNC)	100
Ana Luísa Carvalho	(Assistant Prof., FCTUC)	80
Ana Rita Costa Álvaro	(Assistant Inv., CNC)	60
Ana Santos Carvalho	(Assistant Inv., IEC)	20
Ângelo Tomé	(Assistant Prof., FCTUC)	70
António Macedo Santos	(Assistant Prof., FMUC)	30
António Pedro Gomes	(Assistant Inv., CNC)	100
Armanda E. Santos	(Assistant Prof., FFUC)	80
Armando Cristóvão	(Assistant Prof., FCTUC)	20
Arsélio P. Carvalho	(Full Prof., FCTUC)	100
Attila Köfalvi	(Assistant Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	100
Carlos B. Duarte	(Associate Prof., FCTUC)	80
Catarina R. Oliveira	(Full Prof., FMUC)	60
Célia Aveleira	(Assistant Inv., CNC)	100
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Emília P. Duarte	(Assistant Prof., FCTUC)	80
Henrique Bernardo Silva	(Assistant Inv., CNC)	100
Ildete Luísa Ferreira	(Assistant Inv., CNC)	100
Inês Esteves Baldeiras	(Investigator, FMUC)	35
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	30
Joana Rosmaninho-Salgado	(MD, CHUC)	20
João Laranjinha	(Full Prof., FFUC)	60
João Peça-Silvestre	(Assistant Inv., CNC)	100
Jorge Valero Gomez-Lobo	(Assistant Inv., CNC)	100
Leonor Almeida	(Full Prof., FFUC)	40
Magda Santana	(Assistant Inv., CNC)	100
Mª Céu Sousa	(Assistant Prof., FFUC)	40
Mª do Rosário Almeida	(Assistant Inv., CNC)	100
Mª Isabel J. Santana	(Associate Prof., FMUC)	30
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
Paula G. Agostinho	(Investigator, FMUC)	60
Paulo Pinheiro	(Assistant Inv., CNC)	100
Ramiro Almeida	(Assistant Inv., CNC)	100
Ricardo Rodrigues	(Assistant Inv., CNC)	100

Rodrigo A. Cunha	(Associate Prof., FMUC)	75
Rui Barbosa	(Assistant Prof., FFUC)	60
Sandra Maria R. Carvalho Bós	(Investigator, FMUC)	60
Teresa Dinis Silva	(Associate Prof., FFUC)	40

Post-Doc Members	**Time % at CNC**
Ana Patricia Simões	100
Catarina Alexandra Gomes	100
Catia Marques	100
Carla Nunes	100
Daniel Rial	100
Elisabete Baptista Ferreiro	100
Joana Isabel Real	100
Joana Fernandes	100
Joana Marques	100
João Pedro Lopes	100
Lígia Maria Ferreira	100
Mário Laço	100
Marta Mota Vieira	100
Michele Curcio	100
Miranda Mele	100
Nélio Gonçalves	100
Paula M. Canas	100
Ricardo Santos	100
Rui Oliveira Costa	100
Samira Ferreira	100
Sandra Mota	100
Susana Ribeiro Louros	100
Tatiana Catarino	100

PhD Students	**Time % at CNC**
Amber Kherkoffs	100
Ana Cristina F Lemos	100
Ana Patricia Marques	100
Anna Vladimirovna Pliassova	100
António Manuel C da Silva	100
Barbara Rocha	100
Carla Maria Nunes Lopes	100
Carlos Adriano A. Matos	100
Cassilda Pereira	100
Diana Serra	100
Dominique Fernandes	100

Francisco Manuel Q Gonçalves	100
Gladys Caldeira	100
Ivan Salazar	100
Janete Cunha Santos	100
Jeannette Schmidt	100
Jimmy George	100
Joana Duarte Neves	100
Joana Pedro	100
Joana Vindeirinho	100
Lara Franco	100
Luana Carvalho Naia	100
Mª Joana Pinto	100
Mariana Botelho Rocha	100
Mariline Silva	100
Miguel António Azinheira	100
Mohamed Hussien	100
Nuno Ferreira	100
Nuno Jesus Machado	100
Patrícia Sofia Morais	100
Pedro João Afonso	100
Sara Matias Silva	100
Sara Oliveira	100
Sonya Costa	100
Sofia Alexandra Ferreira	100
Susana Sampaio	100
Tiago Manuel P. Alfaro	75
Xinli Xu	100

MSc Students	**Time % at CNC**
Ana Xavier	100
Bruno Cruz	100
Catarina Carmo	100
Eduardo Morais	100
Helena Leal	100
Helena Martins	100
Inês Saragoça Dias	100
Irina Fonseca	100
João Calmeiro	100
João Filipe Amorim	100
Liliana Caetano	100
Liliana Santos	100
Mário Carvalho	100
Maura De Rosa	100
Paula Silva	90
Ricardo Vieira	100
Rui Beleza	100
Vilte Sauliūnaitė	25

Grant Technicians	Time % at CNC
Ana Margarida Oliveira	100
Ana Sofia Miranda	100
Candida Dias	100
Caroline Veloso	100
Luis Martins	100
Marisa Marques	100
Pedro Alves	50
Sónia Pereira	100

Biotechnology

Luis Pereira de ALmeida, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Akhilesh Rai	(Assistant Inv., CNC)	100
Alcino Jorge Lopes Leitão	(Assistant Prof., FFUC)	60
Amílcar Falcão	(Full Prof., FFUC)	50
Ana Cristina Fortuna	(Assistant Prof., FFUC)	50
Ana Luísa Cardoso	(Assistant Inv., CNC)	100
Ana Bela Sarmento Ribeiro	(Assistant Prof., FMUC)	40
Anália do Carmo	(Assistant Prof., Univ. Vasco Gama)	80
André Xavier C. Negrão Valente	(Assistant Inv., CNC)	100
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	60
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Bruno Manadas	(Investigator, CNC)	100
Carla Vitorino	(Assistant Prof, FFUC)	50
Carlos Cavaleiro	(Assistant Prof, FFUC)	50
Carlos Faro	(Associate Prof., FCTUC)	50
Célia Nogueira	(Assistant Prof, FMUC)	60
Cristiana Paulo		Collaborator
Euclides Pires	(Associate Prof., FCTUC)	50
Fernando Ramos	(Associate Prof, FFUC)	50
Gabriela Silva	(Assistant Prof., FFUC)	60
Henrique Faneca	(Assistant Inv., CNC)	100
Hugo Fernandes	(Assistant Inv., CNC)	100
Isaura Simões	(Assistant Inv., CNC)	100
Joana Cardoso Costa	(Inv. Assistant Prof., FCTUC)	60
Joana Simões Correia	(Assistant Inv., CNC)	100
João José Sousa	(Associate Prof, FFUC)	50
João Nuno Moreira	(Assistant Prof., FFUC)	80
Jorge António R. Salvador	(Full Prof, FFUC)	60
Lígia Salgueiro	(Full Professor, FFUC)	50
Lino Ferreira	(Assistant Inv., CNC)	100
Luís Loura	(Associate Prof., FCTUC)	Collaborator
Luís Pereira Almeida	(Assistant Prof., FFUC)	80
Manuel Garrido	(Investigator, Genibet)	30
Mª Amália Jurado	(Assistant Prof., FCTUC)	80
Mª Celeste Lopes	(Full Prof., FFUC)	80
Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	60
Mª João Silvestre	(Assistant Prof., FCTUC)	Collaborator
Mª José Gonçalves	(Assistant Prof., FFUC)	Collaborator

Mª Luísa Sá e Melo	(Full Prof., FFUC)	60
Mª Manuel da Cruz Silva	(Assistant Prof., FFUC)	60
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Empadinhas	(Assistant Inv., CNC)	100
Olga Maria F. Borges Ribeiro	(Assistant Prof., FFUC)	60
Paula Veríssimo Pires	(Assistant Prof., FCTUC)	60
Pedro Castanheira	(Investigator, Biocant)	Collaborator
Raghu Kalluri	(Investigator, HMS)	35
Renata Dias da Silva	(Assistant Inv., CNC)	100
Ricardo Neves	(Assistant Inv., CNC)	100
Ricardo Pires	(Assistant Inv., CNC)	100
Rui M. M. Brito	(Associate Prof., FCTUC)	Collaborator
Rui Miguel Pinto	(Assistant Prof., EUVG)	30
Sara Domigues	(Assistant Prof., FFUC)	60
Sérgio Simões	(Associate Prof., FFUC)	80
Teresa Gonçalves	(Assistant Prof., FMUC)	40
Teresa Maria C. Martins	(Assistant Inv., IPO)	80
Vera Lúcia Dantas Moura	(Manager Science & Tech., UC)	50

Post-Doc Members	**Time % at CNC**
Adrian Balsa	100
Alessandro Boli	100
Ana Maria Cardoso	100
Ana Teresa Simões	100
Cândida Gonçalves da Silva	35
Catarina Miranda	100
Chantal Fernandes	100
Clévio Nóbrega	100
Elsa Henriques	100
Helena Vazão	100
Igor Tiago	100
João Fernando S. Carvalho	Collaborator
Lígia Maria S. Ferreira	100
Liliana Mendonça	100
Luís Estronca	100
Nuno Mendonça	100
Patrícia Ribeiro	100
Pedro Miguel Coelho	100
Pedro Miguel Costa	100
Renato Cardoso	100
Rita Perfeito	100
Rui Lopes	100
Rui Nobre	100
Sezin Aday	100

Sónia Luzia Pinho	100
Sónia Patricia Duarte	100
Susana Alarico	100
Susana Rosa	100
Vítor Mendes	100

PhD Students	**Time % at CNC**
Ana Catarina Ferreira	100
Ana Cristina Gonçalves	100
Ana Cristina Gregório	100
Ana Cristina Ferreira	100
Ana Filipa Cruz	100
Ana Francisca Lima	100
Ana Isabel Serralheiro	100
Ana Maranha	100
Ana Nobre	100
Ana Sofia Cunha	100
Ana Sofia Lourenço	100
Ana Sofia C. Valdeira	100
Ana Teresa Viegas	100
André Filipe M. Soares	100
Andreia Freitas	100
Ângela Valério-Fernandes	100
Bruno Miguel F. Gonçalves	100
Carlos Samuel M. Boto	100
Catarina Mendes Morais	100
Catarina Oliveira Almeida	100
Cátia Moreira de Sousa	100
Daniela Gonçalves	100
Daniela Pereira S. Alho	100
Dina Pereira	100
Dulce Marisa Bento	100
Emanuel Costa	100
Edna Filipa Soares	100
Elsa Rodrigues	100
Filipa Lebre	100
Geetha Vijayakumar	100
Graça Rocha	100
David Bowman	25
Gianluca Selvaggio	100
Graciana Tribuna	50
Gustavo Costa	100
Inês Honório	100

Inês Vasconcelos Miranda Santos	75
Isabel Maria Santos Onofre	100
Isabel Mª Ramalho	100
Ivana Kostic	100
João Ribas Almeida	100
João Freitas	50
Joana Bicker	100
Joana Filipa Neves	100
Joana Liberal	100
Joana Ribeiro Guedes	100
Joana Sousa	100
Josephine Blersch	100
Júlia Valente	100
Lisa Rodrigues	100
Luís França	100
Mafalda Costa	100
Mª de la Salete J. Baptista	100
Mariana Conceição	100
Mariangela Natale	100
Marisa Gaspar	100
Marta Daniela Passadouro Caetano	100
Marta Pereira	30
Michela Comune	100
Miguel Maria Lino	100
Nuno Fonseca	100
Nuno Mendonça	25
Nuno Silva	100
Patrícia Raquel Pereira	100
Patrícia Rosado	100
Paulo Magalhães	100
Pedro Curto	100
Pedro José Gouveia	100
Pedro Manuel Batista Branco	100
Ravi Adusumalli	100
Ricardo Romão Leão	25
Rui Cruz	100
Rui Figueiredo	100
Rui Benfeitas Vicente	100
Rui Soares	60
Sandra Cristina Jesus	100
Sandra Figueiredo	100
Sandra Marina A. Santos	100
Sara Lopes	100
Sofia Anastácio	100

Sofia Pereira Romano	100
Tânia Leandro	100
Vanessa Mendes	100
Vera Calhau	100
Vitor Carmona	100

MSc Students	**Time % at CNC**
Ana Alves	100
Ana Catarina Cardoso	100
Ana Pica-Milho	100
Ana Rita Cruz	100
Angelo Serani	100
Daniela Costa	100
Diana Maurício	100
Diogo Reis	100
Edmilson Semedo	100
Fabiana Soares	20
Gabriela Leão	100
José Miguel Codeso	100
Mariana Magalhães	100
Nanci Ferreira	100
Paulo Silva	40
Pedro Cunha	100
Raquel Costa	100
Ricardo Silva	100
Ruben Branco	100
Rute Araújo	100
Sara Dias	100
Susana Cecílio	100
Susana Paixão	100
Teresa Silva	100
Vânia Moreira	100

Grant Technicians	**Time % at CNC**
Alexandra Abrunheiro	100
Ana Sofia L. Coelho	100
Dina Farinha	100
Fátima Nunes	25
Hugo Filipe (PhD)	50
José Paiva	100
Marta Mota	40
Paulo Dias	25
Rita Pereira	100

Sandra Pinto	100
Sónia Pereira	30
Vanessa Rebelo Anjos	100
Vanessa Monteiro	100
Vinicio Oliveira	100

Metabolism, Age and Disease

João Ramalho Santos, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Alexandrina F. Mendes	(Assistant Prof., FFUC)	80
Ana Paula Marques de Sousa	(Investigator, CHUC)	50
Anabela P. Rolo	(Assistant Prof., FCTUC)	80
Carlos M. Palmeira	(Full Professor., FCTUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	60
Eugénia Carvalho	(Assistant Inv., CNC)	100
Fernando Nogueira	(Associate Prof., Brasil)	Collaborator
João Moura	(Assistant Prof., Inst Pol. Viana Castelo)	50
João Ramalho Santos	(Associate Prof., FCTUC)	80
José Custódio	(Associate Prof., FFUC)	80
Mª Carmen Alpoim	(Associate Prof., FCTUC)	60
Mª Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Maria S. Santos	(Principal Inv., FCTUC)	100
Paula Isabel Moreira	(Assistant Prof., FMUC)	60
Paulo J. Oliveira	(Assistant Inv., CNC)	100
Rui A. Carvalho	(Assistant Prof., FCTUC)	50
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	60
Teresa Cruz Rosete	(Assistant Prof., FFUC)	80

Post-Doc Members	**Time % at CNC**
Ana Burgeiro	100
Ana Duarte	100
Ana Filipa Henriques	30
Ana Raquel Esteves	100
Ana Silva	20
Ana Teresa Varela	100
Carlos Rodrigues	100
Denisa Daud Mateus	100
Diana Silva	100
Ermelindo Leal	100
Filipe Valente Duarte	100
Helena Carvalheiro	100
João Paulo Teodoro	100
Mª Alexandra B. Amaral	100
Mª Teresa Cunha-Oliveira	100
Mariana Ponte Ribeiro	100
Paula Mota	100
Rosa Resende	100
Sandra Catarina G. Amaral	100
Sandro Pereira	100

Sofia Guedes	100
Sonia Correia	100
Susana Cardoso	100
Susana Guerreiro	50
Tatiana Emanuelli	50
Teresa Serafim	100
Vera Francisco	50
Vilma Sardão Oliveira	100

PhD Students	**Time % at CNC**
Ana Carolina Romero	100
Ana Catarina Fonseca	100
Ana Mª Silva	100
Ana Plácido	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa Rufino	100
Ana Xavier	100
Ângela Pascoal Crespo	100
Beatriz Lacerda de Sousa	100
Carla Patrícia R. Paiva	100
Emanuel Candeias	100
Filipa Carvalho	100
Inês Biscaia Barbosa	100
Joana Liberal	100
João Demétrio B. Martins	100
João Pedro Oliveira	20
Júlia Valente	100
Katia Mesquita	100
Ludgero Tavares	100
Marcelo Correia	100
Maria Fernandes	100
Mª Inês Almeida Sousa	100
Mª Madalena Ribeiro	100
Marília Cordeiro	100
Mónica Abreu	80
Nuno Gabriel Machado	100
Patrícia Lopes	100
Renato Santos	100
Rodrigo Santos	100
Roksana Pirzgalska	100
Susana Pereira	100
Tânia Perestrelo	100
Tiago R. Santos	70

MSc Students	**Time % at CNC**
Abdullah Nawabjan Shaik	100
Alexandra Carvalho	100

Ana Marta Silva	100
Ana Raquel Coelho	100
Ana Rita Rocha	100
Catarina Xavier	30
Fabio Carvalho	100
Guilherme Loureiro	100
Joana Portela	100
João Filipe Amorim	100
Leisa Évora	100
Manuela Cerqueira	50
Marcelo Catarino	50
Marcelo Ribeiro	100
Rafael Paiva	50
Renata Couto	100
Rui Gomes	100
Rui Simões	100
Sara Rebelo	100
Silvia Magalhães	100
Solange Machado	100
Vanessa Rodrigues	100

Grant Technicians	**Time % at CNC**
Andreia Madeira	50
Cláudia Mª Deus	100
Cristina Carvalho	100
Fábio Paiva	50
Isabel Ferreira	100
Joana Sousa	50
João Ferreira	100
Mariana Val	100
Marta Baptista	100
Mónica Zuzarte	50
Renata Tavares	100
Tatiana Martins	100

MD Members

Herminio Espirito Santo	Collaborator
Mª Margarida Gonçalo	40

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