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Biomedicine Projects

Mechanisms of protein dysfunction in mitochondrial leukodystrophies – studies on glutamyl-tRNA synthetase (EARS2)

Place of work: Protein Misfolding and Amyloids in Biomedicine laboratory (Lab 8.5.56),
BioISI,Faculdade Ciências Universidade de Lisboa

Supervisor: Dr. Bárbara J. Henriques; ORCID
Assistant Researcher at the Protein Misfolding and Amyloids in Biomedicine laboratory (BioISI, FCUL)
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Co-supervisor: Dr. Cláudio M. Gomes; ORCID
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Group leader of the Protein Misfolding and Amyloids in Biomedicine laboratory (BioISI,FCUL)
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MSc Research Plan

Mitochondrial aminoacyl-tRNA synthetase-related neurological diseases are a heterogeneous group of rare mitochondrial disorders (MDs), that lead to a wide diversity of phenotypes commonly affecting mitochondrial morphology and bioenergetics. Among them are the rare leukodystrophies, causing abnormal development or destruction of the white matter of the brain, such as LBTL (leukoencephalopathy with thalamus and brain stem involvement and high lactate) caused by mutations on glutamyl-tRNA synthetase (EARS2).

In the recent years over 30 mutations on EARS2 gene have been identified, however the molecular basis for leukodystrophies associated to EARS2 mutations remains to be established. It has been suggested that the location of missense mutations in certain domains might influence disease phenotype, but the structure of these enzyme remains poorly characterized which is a major gap towards effective therapies. Here, we propose to investigate the molecular pathophysiology associated to defects on glutamyl-tRNA synthetase by comprehensively characterize EARS2, wild-type and variants associated to LBTL, using biochemical and biophysical methodologies.

Specifically, this work will involve the following tasks:

- Optimization of recombinant protein expression (EARS2 disease-related variants, EARS2-p. Gly110Ser, EARS2-p. Asp349Asn and EARS2-p. Arg489Gln);
- Purification EARS2 variants using a combination of chromatographic methodologies (his-tag affinity or ion exchange columns and gel filtration columns);
- Characterize the variants regarding structure and conformation stability using circular dichroism (CD), Fourier Transform Infrared Spectroscopy (FTIR), fluorescence spectroscopy;
- Establish protocols for EARS2 enzymatic activity determination.

Research at the Protein Misfolding and Amyloids in Biomedicine laboratory takes place in a highly multidisciplinary and collaborative environment, at the national and international levels. We seek candidates which are highly motivated to tackle a challenging research activity, ability to work independently, and to undertake intensive learning and training in multiple methodologies, with an excellent academic track record and communication skills.

Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship (BII), being the selection criterium the academic merit of the candidates).

Rescue of Rare CFTR Mutations by Novel CFTR Correctors

Place of work: BioISI/DQB-FCUL, C8 building (labs 8.1.74, 8.3.68 and 8.4.62), (http://bioisi.pt/fungp)

Supervisor: Dr. Miquéias Lopes-Pacheco (mlpacheco@fc.ul.pt), BioISI/FCUL (ORCID ID: 0000-0002-7444-9359)

Co-Supervisor: Professor Margarida D. Amaral (mdamaral@fc.ul.pt), BioISI/FCUL (ORCID ID: 0000-0002-0828-8630)

Background: Cystic fibrosis (CF) is a monogenic disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) protein, an anion channel expressed at the plasma membrane (PM) of secretory epithelia. The p.Phe508del is the most prevalent CF-causing mutation and leads to CFTR protein misfolding (*i.e.,* class II CFTR mutation) that is retained by the endoplasmic reticulum quality control (ERQC), thereby precluding its processing and trafficking to the PM, being instead targeted for proteasomal degradation. Although recent therapeutic progress has been achieved in developing CFTR corrector drugs for p.Phe508del-CFTR, a considerable number of rare CFTR mutations (so-called 'orphan' mutations) remains without any effective causal therapy. Various CFTR mutations exhibit a primary defect similar to that of p.Phe508del-CFTR. However, despite mutations in the same class are expected to be treated by the same approach (*i.e.,* correctors for class II CFTR mutations), they might not be efficiently rescued by the same chemical compound. We have recently investigated the efficacy and the mechanism of action of novel correctors (PTI-801, ABBV-2222, FDL-169) in rescuing p.Phe508del-CFTR, but their effects on other class II CFTR mutations remains to be exploited.

Objectives: To investigate the potential ability of novel correctors (PTI-801, ABBV-2222, FDL-169) in rescuing processing, trafficking and function of rare class II CFTR mutations, namely p.Glu92Lys, p.Val232Asp, p.Ala455Glu, p.Ser492Phe, p.Leu1077Pro and p.Gly1249Arg.

Methodology: The techniques/assays used in this project include:

1) Site-directed mutagenesis and cloning to generate cell lines expressing the CFTR mutation under study;
2) Halide sensitive-yellow fluorescence protein (HS-YFP) assay on a plate reader to determine CFTR function;
3) Western blotting to determine CFTR protein level and pattern;
4) Immunofluorescence microscopy to determine CFTR localization in the cell;
5) Ion transport measurements in micro-Ussing chamber.

Bibliography:

1. Bacalhau M *et al* (2023) Identification of novel F508del-CFTR traffic correctors among triazole derivatives. *Eur J Pharmacol* 938: 175396.
2. Lopes-Pacheco M *et al* (2022) Rescue of Mutant CFTR Trafficking Defect by the Investigational Compound MCG1516A. *Cells* 11 (1): 136.
3. Silva IAL *et al* (2022) Advances in Preclinical In Vitro Models for the Translation of Precision Medicine for Cystic Fibrosis. *J Pers Med* 12 (8): 1321.
4. Lopes-Pacheco M *et al* (2021) Discovery of CFTR Modulator for the Treatment of Cystic Fibrosis. *Expert Opin Drug Discov* 16 (8): 897-913.
5. Lopes-Pacheco M (2020) CFTR Modulators: The Changing Face of Cystic Fibrosis in the Era of Precision Medicine. *Front Pharmacol* 10: 1662.
6. Botelho HM (2015) Protein Traffic Disorders: an Effective High-throughput Fluorescence Microscopy Pipeline for Drug Discovery. *Sci Rep* 5: 9038.
7. Pedemonte N, *et al* (2011) High-throughput Screening of Libraries of Compounds to Identify CFTR Modulators. *Methods Mol Biol* 741: 13-21.

Title: The role of proteoform stoichiometry on the behavior and function of STAT3 dimers

MSc em Biologia Molecular e Genética

Place of work: Departamento de Química e Bioquímica, FCUL

Supervisor: Federico Herrera (fherrera@fc.ul.pt)

Abstract

Protein self-association in homodimers and oligomers is very common in nature, playing key roles in both physiological and pathological conditions. Protein homodimers frequently display some structural symmetry and are generally assumed to be formed by identical molecules, not only in terms of amino acid composition, but also in terms of post-translational modifications (PTMs). However, a perfect symmetry is very unlikely considering the high number and dynamic nature of the different proteoforms that can co-exist at any given time and for the same protein (i.e. the proteoform stoichiometry). We have recently reported that asymmetrically phosphorylated huntingtin homodimers/oligomers showed a distinct aggregation pattern, with implications for Huntington´s disease; and that the intracellular distribution of STAT3 homodimers changed strikingly when specific PTMs could not occur on only one of the monomers. Based on these results, we launched the hypothesis that PTM asymmetry could constitute a new level of functional regulation for self-associating proteins. To challenge this hypothesis, the student will study the putative role of asymmetric PTMs and alternative splicing isoforms on the behavior and function of STAT3 homodimers by means of a multidisciplinary combination of advanced biochemistry and bioimaging methods. During this thesis, the student will learn mammalian cell culture, advanced microscopy methods, flow cytometry, protein biochemistry methods, cloning and site-directed mutagenesis, as well as improve his soft skills (writing, presenting, producing professional graphs and statistics). The ideal candidate must be an organized, hard-working and team player individual, and have good English level (the language we use in lab meetings). Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.

Title: The effect of melatonin on prokaryotic and eukaryotic respiratory proteins: a possible explanation for its antimicrobial, neuroprotective and antitumoral properties

MSc em Biologia Molecular e Genética

Supervisors: Federico Herrera (fherrera@fc.ul.pt), Manuela Pereira (mmpereira@ciencias.ulisboa.pt)

Abstract

Melatonin is a highly pleiotropic hormone present in all natural kingdoms, from archaeobacteria to humans. Usually associated with regulation of biological responses to light/darkness cycles (e.g. circadian and circannual rhythms or skin pigmentation), melatonin has shown notable and extremely diverse therapeutic potential. Melatonin is generally antioxidant and cytoprotective for normal cells, preventing neuronal death in models of Parkinson or Alzheimer´s disease, or preserving frozen sperm quality for longer periods of time. However, it is also antiproliferative and toxic for cancer cells which metabolism is based on aerobic glycolysis (see Warburg effect) and, interestingly, for pathogenic bacteria. The origin of such pleiotropic and apparently opposite effects remains unknown. Herrera and Pereira labs @ FCUL recently joined their expertise in melatonin and bioenergetics to test the hypothesis that melatonin´s pleiotropic effects are related to a fundamental effect of the hormone on both eukaryotic and prokaryotic respiratory complexes. To challenge this hypothesis, the student will study the effect of melatonin and other indoles on the activity of recombinant respiratory proteins from pathogenic bacteria (e.g. Staphylococcus aureus or Pseudomonas aeruginosa) and mammalian cells (e.g. AMID) in vitro. Results will be confirmed in living bacteria and mitochondria isolated form human cells, and the effect of selected concentrations of melatonin in the survival and growth of bacteria and both tumoral and healthy human cells will be analyzed. During this multidisciplinary project, the student will learn bacterial and mammalian cell culture; cloning; production, purification and activity assays of recombinant proteins in bacteria, and other advanced cell and molecular biology methods, as well as improve their soft skills (writing, presenting, producing professional graphs and statistics). The ideal candidate must be an organized, hard-working and team player individual, and have good English level (the language we use in lab meetings). Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.

Title: Asymmetric post-translational modifications (PTMs) as a new regulatory mechanism in self-associating signaling proteins

MSc em Biologia Evolutiva e do Desenvolvimento

Place of work: Departamento de Química e Bioquímica, FCUL

Supervisor: Federico Herrera (fherrera@fc.ul.pt)

Abstract

Protein self-association in homodimers and oligomers is very common in nature, playing key roles in both physiological and pathological conditions. Protein homodimers frequently display some structural symmetry and are generally assumed to be formed by identical molecules, not only in terms of amino acid composition, but also in terms of post-translational modifications (PTMs). However, a perfect symmetry is very unlikely considering the high number and dynamic nature of the different PTM proteoforms that can co-exist at any given time and for the same protein (i.e. the proteoform stoichiometry). We have recently reported that asymmetrically phosphorylated huntingtin homodimers/oligomers showed a distinct aggregation pattern, with implications for Huntington´s disease; and that the intracellular distribution of STAT3 homodimers changed strikingly when specific PTMs could not occur on only one of the monomers. Based on these results, we launched the hypothesis that PTM asymmetry could constitute a new level of functional regulation for self-associating proteins. To challenge this hypothesis, the student will study the putative role of asymmetric PTMs on the behaviour and function of STAT3 homodimers by means of a multidisciplinary combination of advanced bioimaging methods in living cells and proteomics. This project is the basis of my last applications to FCT grants, two current projects within FCUL MSc programmes and 4 MSc theses and 1 PhD thesis in my laboratory. During this thesis, the student will learn mammalian cell culture, advanced microscopy methods, flow cytometry, protein biochemistry and proteomics methods, cloning and site-directed mutagenesis, as well as improve his soft skills (writing, presenting, producing professional graphs and statistics). The ideal candidate must be an organized, hard-working and team player individual, and have good English level (the language we use in lab meetings). Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.

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Title: Radiation as a therapeutic tool against neurodegeneration: pilot studies in cellular and animal models of Huntington´s disease

MSc em Bioquímica e Biomedicina

Place of work: Departamento de Química e Bioquímica, FCUL; ITQB-NOVA

Supervisors: Federico Herrera (fherrera@fc.ul.pt), Gonçalo Poças (goncampocas@itqb.unl.pt)

Abstract

Radiotherapy (RT) is a relatively safe and established treatment for cancer, where the goal is to kill tumoral cells with the lowest toxicity to healthy tissues. Using it for neurodegenerative disorders involving cell loss is counterintuitive. However, ionizing radiation has a hormetic nature: it can have deleterious or beneficial effects depending on how it is applied. For example, low-dose RT can trigger antioxidant, anti-inflammatory and tissue regeneration responses. RT has been used to treat peripheral amyloidosis, which is very similar to neurodegenerative disorders from a molecular perspective. Although some hypotheses have been formulated, the mechanism of action of RT on systemic amyloid deposits is still unclear, and its impact in the central nervous system remains uncertain. We want to explore the potential of RT to treat neurodegenerative disorders using cellular, fly and worm models of Huntington´s disease, where mutated huntingtin aggregates and produces pathological phenotypes. The student will be trained in methods to work with Drosophila flies and mammalian cell cultures, as well as classic cell and molecular biology methods (western blotting, cloning,…) and advanced bioimaging, especially widefield and confocal microscopy. The student should have her/his own means of transportation, as the experiments will require driving between three locations: Lisbon (FCUL, Campo Grande, cell culture experiments), Oeiras (ITQB NOVA, fly experiments) and Sacavém (CTN, for irradiation). Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.

Title: Radiotherapy beyond cancer: simulating the effect of radiation on pathological protein amyloids associated with neurodegenerative disorders

MSc em Biologia Computacional

Place of work: Departamento de Química e Bioquímica, FCUL

Supervisors: Federico Herrera (fherrera@fc.ul.pt), Daniel Galaviz (galaviz@fc.ul.pt)

Abstract

Radiotherapy (RT) is a relatively safe and established treatment for cancer, where the goal is to kill tumoral cells with the lowest toxicity to healthy tissues. Using it for neurodegenerative disorders involving cell loss is counterintuitive. However, ionizing radiation has a hormetic nature: it can have deleterious or beneficial effects depending on how it is applied. For example, low-dose RT can trigger antioxidant, anti-inflammatory and tissue regeneration responses. RT has been used to treat peripheral amyloidosis, which is very similar to neurodegenerative disorders such as Alzheimer or Parkinson from a molecular perspective. Both types of disorders are associated to the toxic accumulation of proteins in structures known as amyloids. Although some hypotheses have been formulated, the mechanism of action of RT on systemic amyloid deposits is still unclear, and its impact in the central nervous system remains uncertain. We want to explore the potential of RT to destroy or modify pathological amyloids. The student will be trained in Monte Carlo simulations and simulation engines, such as GEANT4-DNA and TOPAS. The student will develop and optimize a simulation tool based on the GEANT4-DNA and TOPAS engines to study the intrinsic characteristics of the effect of traditional and proton RT on toxic protein amyloid structures. If the student is interested, he/she can learn basic cell and molecular biology methods at Herrera´s lab, to confirm simulation results experimentally. Both supervisors have experience in creating multidisciplinary scientific profiles in the edge between computation, physics and biology. Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.

Title: Stress Out: Promoting Anticancer Activity by Fostering Reductive Stress with Metal Complexes

MSc em Química

Place of work: Departamento de Química e Bioquímica, FCUL; ITQB-NOVA

Supervisors: Federico Herrera (fherrera@fc.ul.pt), Ana Petronilho (ana.petronilho@itqb.unl.pt)

Abstract:
In this project, we will develop a new class of anticancer drugs able to disrupt redox homeostasis by promoting reductive stress. We will do so by developing metal complexes able to act as reduction catalysts inside cells. Previous work from our labs has shown that metal complexes can induce reductive stress in cancer cells that ultimately leads to cell death. The main objective is to develop compounds that are able to operate with complementary modes of action to that of existing drugs, to be able to overcome resistance and associated side effects.
To achieve our goal, we plan to fulfil the following tasks:
Task 1. Synthesis of metal complexes based on iridium bearing triazoles ligands (ITQB NOVA). Triazole ligands will be synthesized via click chemistry, using copper and ruthenium catalysts. Synthesized compounds will be characterized by NMR spectroscopy and mass spectrometry, and the stability of the complexes in physiological media will be monitored by NMR.
Task 2. Evaluation of the Metal complexes to promote redox reactions in vitro (FCUL/ITQB NOVA). In this task, we will evaluate the catalytic activity of the metal complexes synthesized in Task 1 in two reactions pivotal to achieve reductive stress: 1) reduction of NAD+ to NADH and 2) reduction of GSSH to GSH.
Task 3. Evaluation of the anticancer activity of iridium complexes (FCUL). The antiproliferative activity or cytotoxicity of our metal complexes will be tested in cancer cell lines from different origins, and determine their IC50 values and safety indexes. The mechanisms involved in their antitumoral activity will be analyzed.
Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.

Impact of BCL-6 downregulation in the oncogenic properties of breast cancer cells

Place of work/: Laboratório de oncobiologia e transdução de sinal, Departamento de Genética
Humana, Instituto Nacional de Saúde Doutor Ricardo Jorge

Supervisors: Patrícia Barros (patricia.barros@insa.min-saude.pt); Peter Jordan (peter.jordan@insa.min-saude.pt)

Breast cancer (BC) is the most frequent type of cancer and the leading cause of cancer-related deaths in women. In recent years, the improvement in diagnosis techniques led to an earlier detection of the disease and a consequent decline in the mortality from BC in developed countries. Moreover, the development of targeted therapies contributed to increased treatment efficacy, reducing the number of fatalities from this type of cancer. However, BC is still a major health issue, in which the acquisition of therapy resistance, disease recurrence, and the formation of metastasis accounts for most BC-related deaths. Therefore, the understanding of the mechanisms behind BC resistance and recurrence is crucial for developing new therapeutic strategies to reduce BC mortality and morbidity. The host lab has identified a signaling pathway through which the downregulation of the transcriptional repressor BCL-6 contributes to colorectal cancer cell survival and chemoresistance [1,2]. Meanwhile, the lab discovered that BCL-6 is also downregulated in BC and, using a transcriptomic approach, identified a cluster of candidate genes that become upregulated in BCL-6-depleted BC

cells. The proposed Master's project, envisions the validation of BCL-6 as a regulator of these candidate genes in BC cells and the evaluation of the impact of downregulating BCL-6 or its identified target genes in the viability, migration and invasive properties of BC cells.

Methodologies: Culture and transfection of breast cancer cell lines; RNA interference, isolation and purification of nucleic acids; RT-qPCR; MTT assays (cell proliferation and viability); Boyden chamber assays (chemotactic cell migration); 3D-matrix invasion assays (cell invasive behavior).

References:

1. Barros P, Lam EW, Jordan P, Matos P (2012). Rac1 signaling modulates a STAT5/BCL-6 transcriptional switch on cell-cycle-associated target gene promoters. Nucleic Acid. Res. 40, 7776-7787 (doi: 10.1093/nar/gks571).

2. Barros P, Jordan P and Matos P (2009). Rac1 signaling modulates BCL-6-mediated repression of gene transcription. Mol. Cell. Biol. 29, 4156-4166 (doi: 10.1128/MCB.01813-08).

Targeted CFTR Expression in Specific Airway Cell Types

Place of work/: BioISI, DQB-FCUL (C8 building)

Supervisors: Supervisor 1 Dr. Ines Pankonien (ipankonien@fc.ul.pt) BioISI/FCUL; ORCID 0000-0002-4622-7521;
Supervisor 2 Prof. Margarida D. Amaral (mdamaral@fc.ul.pt) BioISI/FCUL; ORCID 0000-0002-0828-8630

Background: *Cystic Fibrosis (CF) is caused by variants in the CF transmembrane conductance regulator (CFTR) gene encoding a Cl^-/HCO_3^- channel expressed at the apical plasma membrane (PM) of epithelial cells. The variant p.Phe508del is the most common CF-causing variant which leads to defective PM traffic of CFTR. While CF affects multiple organs, lung disease represents the primary cause of morbidity and mortality [1,2]. The airway epithelium is mainly composed of basal, secretory club and goblet, ciliated, neuroendocrine and tuft cells which all play specific roles. The cell types expressing CFTR are still being discussed. For a long time it was believed that ciliated cells are the predominant CFTR-expressing cells. However, pulmonary ionocytes were recently discovered as a novel rare cell type with unexpected high CFTR expression levels. Other reports also describe a CFTR expression in secretory club and basal cells. However, it is necessary to understand which cell types express CFTR under physiological conditions in order to directly target CFTR by possible novel therapeutic approaches. In our previous studies we used a recently described human respiratory multipotent basal cell line from large airways (BCi-NS cells) [3,4] and generated a basal CF cell line by introducing p.Phe508del /p.Phe508del (BCi-CF cells) using CRISPR/Cas9. Both, BCi-NS and BCi-CF cell lines differentiate into the various airway epithelial cell types when cultured at air-liquid interface (ALI). These two novel cell lines are unique models which allow us to investigate CFTR expression in different cell types.*

Objective: *The aim of the MSc work is to identify in which specific cell type(s) CFTR expression contribute(s) to physiological functioning of the airway epithelium.*

Methodology: *The MSc project proposal comprises the following tasks:*

1) *Cloning of cell type-specific promoters into lentiviral CFTR expression vector;*

2) *To generate stable BCi-CF cell lines expressing wildtype-CFTR under cell type-specific promoters.*

The techniques applied within this project include: Mammalian cell culture, PCR based cloning, Western blot, Immunofluorescence microscopy and Ussing Chamber.

References

1. De Boeck K, Amaral MD (2016) Lancet Respir Med 4: 662–674 [PMID:27053340]
2. Riordan et al. (1989) Science, 245(4922), 1066–1073. [PMID: 2475911]
3. Walters et al. (2013) Respir Res 14:135 [PMID: 24298994]
4. Simoes et al. (2019) Life Sci Alliance 2(6):e201900462. [PMID: 31732694]

Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship (BII)), being the selection criterium the academic merit of the candidates.

Deciphering the transcriptional regulation underlying miR-34c-5p TCR-dependent induction in CD4+ T cells

Place of work/: RNA Systems Biology Lab – BioISI, FCUL

Supervisors: Margarida Gama-Carvalho (mhcarvalho@ciencias.ulisboa.pt)

microRNAs (miRs) are small non-coding RNAs of 21-23 nucleotides in length that regulate gene expression post-transcriptionally that play a key role in cell fate determination processes including the regulation of T cell activation, proliferation, and differentiation, a central process for mounting an effective immune response. miR-155 is a well characterized regulator of the innate and adaptive immune responses, being induced in response to CD4+ T cell activation and required for the polarization of different Th subtypes. In addition to miR-155, miR-34c-5p has been identified by the host lab as a novel regulator of T cell activation, displaying an activation dynamic significantly distinct from miR-155. The transcriptional mechanisms, as well as key regulators involved in the TCR-dependent induction of miR-34c-5p remain poorly characterized, thus defining the core aims of this project. Prior work from the lab has led to the identification of two genomic regions with the potential to enhance or inhibit miR-34c-5p expression, and some putative regulatory transcription factors (TFs) acting on them. In this project, we aim to expand this work to generate a highly detailed mapping of the transcriptional control of miR-34c expression.
Luciferase reporter platforms will be used to investigate additional genomic segments and address the combinatorial effects of key TFs that act on these regions. Validation of the sequence-dependent nature of the observed effects will be demonstrated by analyzing the direct binding of TFs to the genomic regions and introducing point mutations to abrogate the observed interactions.
The identified transcriptional regulatory elements will be further validated in primary CD4+ T cells by a combination of approaches: 1) transfection of the luciferase vectors coupled to TCR stimulation to assess their ability to simulate the miR-34c transcriptional response; 2) time-course analysis of the expression level, subcellular localization, and post-transcriptional modification of the candidate regulatory factors in response to TCR stimulation; and (if time permits) 3) chromatin-immunoprecipitation (ChIP) in unstimulated and stimulated cells to validate direct association to the target genomic region in a physiological context.
This project will allow the student to acquire a deep understanding of the transcriptional regulation in T cell activation, develop significant expertise in genetic engineering methods, as well as in the growth, manipulation and analysis of human cells.

Computational approaches to understand the selective basis of motor-neuron degeneration

Place of work/: RNA Systems Biology Lab – BioISI, FCUL

Supervisors: Margarida Gama-Carvalho (mhcarvalho@ciencias.ulisboa.pt)

Hereditary mutations in essential proteins involved in RNA metabolism that also present ubiquitous expression are linked to many human genetic diseases, but show striking association to neurodegenerative and, in particular, motor neuron disorders. Several models have been put forth to explain the unexpected cell-type specific manifestation of the disease phenotype, including the concept that motor-neurons may be more sensitive to changes in splicing or our lab's proposal that these proteins tend to coordinate the expression of proteins that integrate the same tissue-specific functional consortia. This project aims to expand on our previous work and available RNA-seq data by exploring different models that justify the predominant neuronal impact of these proteins. The workplan will explore the impact of disease-associated mutations on the neuronal transcriptome with an emphasis on the analysis of transcript structure based both on our available data and public data-sets though the implementation of different RNA-seq data analysis pipelines. These results will contribute to the second aim of the project, om which the concept of BioInt libraries, developed by our group to explore tissue-specific protein consortia and how they are altered by disease causing mutations, will be expanded to include information on transcript and protein isoform expression. Finally, a pipeline for cross-species mapping and comparison of these functional proteins networks will be developed.

The outputs of this project will contribute to build a better understanding of the molecular functional diversity that is found across tissues and how it connects to the manifestation of disease phenotypes. The student will gain an in-depth know-how on RNA-seq data analysis and protein-network analysis working mostly in R.

References:

Garcia-Vaquero, M., Heim, M., Flix, B., Pereira, M., Palin, L., Marques, T.M., Pinto, F.R., de Las Rivas, J., Voigt, A., Besse, F., Gama-Carvalho, M. (2022). Analysis of pre-symptomatic Drosophila models for ALS and SMA reveals convergent impact on functional protein complexes linked to neuro-muscular degeneration bioRxiv 2022.06.20.496821; doi: 10.1101/2022.06.20.496821

Garcia-Vaquero M, Gama-Carvalho M, Pinto FR, De Las Rivas J. 2022. Biological Interacting Units identified in human protein networks reveal tissue functional diversification and its impact on disease. Computational and Structural Biotechnology Journal 20: 3764-3778. doi: 10.1016/j.csbj.2022.07.006

A role for astrocytes in modulation of GABAergic transmission and synaptic plasticity by VIP?

Place of work/: Epilepsy and Aging Team, BioISI – GER, FCUL.

Supervisor(s): Diana Cunha Reis, PhD (dcreis@fc.ul.pt). ORCID: 0000-0002-0900-9306;

GABA is the main inhibitory neurotransmitter in the mammalian brain where it plays an important role in regulating brain activity states and preventing excessive neuronal excitation. Synaptic plasticity events are cellular mechanisms that play a crucial role in learning and memory formation. Vasoactive intestinal peptide (VIP), acting on $VPAC_1$ receptors, is released during synaptic plasticity induction and influences hippocampal synaptic plasticity through modulation of GABAergic transmission(*1*, *2*). VIP is present only in a few populations of GABAergic interneurons and regulates GABA release through activation of $VPAC_1$ and $VPAC_2$ receptors(*3*). Astrocytes are another important cellular component of GABAergic synapses and can greatly influence synaptic GABA availability. In addition, astrocytes can be activated by synaptic GABA, that enhances intracellular Ca^{2+} through several signaling pathways. This may in turn trigger the release of gliotransmitters that can influence LTP(*4*). Gliotransmitters released from astrocytes, including neuropeptides, have been described to modulate LTP induction and expression(*5*). Astrocytes express both $VPAC_1$ and $VPAC_2$ VIP-selective receptors(*6*) but their role in VIP modulation of GABAergic transmission and synaptic GABA availability was never investigated. Likewise, the influence of these receptors in astroglial responses to GABA stimuli was never studied.

This project aims to use hippocampal astrocyte cultures and hippocampal slices to investigate: 1) The influence of $VPAC_1$ and $VPAC_2$ receptors on astroglial GABA uptake; 2) The astrocytic Ca^{2+} responses to transient GABA stimuli, as would occur at GABAergic synapses during synaptic plasticity; 3) the presence of VIP, $VPAC_1$ and $VPAC_2$ receptors in astrocyte cultures as accessed by immunocytochemistry.

The influence of $VPAC_1$ and $VPAC_2$ receptors on astroglial GABA uptake kinetics mediated by GAT-1 and GAT-3 receptors will be studied as previously described(*7*), using selective agonists and antagonists for these receptors. Astrocytic Ca^{2+} responses to transient GABA stimuli will be monitored using the Ca^{2+}-sensitive fluorescent dye fura-2 acetoxymethyl ester (fura-2 AM)(*8*). VIP, $VPAC_1$ and $VPAC_2$ receptors will be detected in hippocampal astrocyte cultures by immunocytochemistry with specific antibodies. Experimental work will be conducted with the support of the BioISI cell culture and FCUL microscopy facilities.

References:

1. D. Cunha-Reis, A. Caulino-Rocha, *Front. Cell. Neurosci.* **14**, 153 (2020).
2. A. Caulino-Rocha, N. C. Rodrigues, J. A. Ribeiro, D. Cunha-Reis, *Biol. 2022, Vol. 11, Page 627*. **11**, 627 (2022).
3. D. Cunha-Reis, J. A. Ribeiro, R. F. M. de Almeida, A. M. Sebastião, *Br. J. Pharmacol.* **174**, 4725–4737 (2017).
4. A. Araque *et al.*, *Neuron*. **81**, 728–739 (2014).
5. C. Henneberger *et al.*, *Neuron*. **108**, 919-936.e11 (2020).
6. O. Masmoudi-Kouki *et al.*, *J Neurochem*. **117**, 403–411 (2011).
7. J. Y. Chatton, L. Pellerin, P. J. Magistretti, *Proc. Natl. Acad. Sci. U. S. A.* **100**, 12456–12461 (2003).
8. P. F. Jacob, S. H. Vaz, J. A. Ribeiro, A. M. Sebastião, *Glia*. **62**, 1211–1226 (2014).

Alternative topics: (please contact the supervisor)**:**

- Investigating the alterations in synaptic lipid rafts using atomic force microscopy and immunogold staining.
- Synaptic plasticity in the hippocampus during post weaning development and aging: influence of phosphorylation of synaptic enzymes and channels.

Investigating altered lipid raft dynamics following seizures at hippocampal synapses with AFM

Place of work/: Epilepsy and Aging Team, BioISI – GER and BioISI – BiopHysNano, FCUL.

Supervisor(s): Diana Cunha Reis, PhD (dcreis@fc.ul.pt) ORCID: 0000-0002-0900-9306; Ana Carapeto (apcarapeto@fc.ul.pt) PhD ORCID: 0000-0003-2654-6848.

Lipid rafts are membrane nanodomains (100–300 nm), not detectable by light microscopy, involved in synaptic receptor (R) clustering, synaptic signaling, synaptic vesicle recycling, neurotransmitter release and synaptic plasticity, a cellular process involved in learning and memory (*1*). Recently we showed that classic raft-associated proteins like caveolin-1 and flotillin-1 are dramatically reduced at synapses after seizure-like events (*2*). Likewise, postsynaptic proteins like PSD-95, an NMDA/AMPA R anchoring protein present at glutamatergic synapses, and gephyrin, a $GABA_A$ R anchoring protein present at GABAergic synapses, are markedly altered following *in vitro* seizure-like activity (*2*), suggesting altered lipid raft stability may be crucial to deficits in synaptic plasticity in epilepsy.

This work will use atomic force microscopy (*3*, *4*)(AFM) to unravel changes in synaptic and neurite membrane structure and lipid raft size and distribution in 1) synaptosomes obtained from hippocampal slices subjected to seizure-like activity and 2) hippocampal neuron neurites in culture exposed to similar seizure-like activity.

To unravel the subsynaptic location of the main lipid domains affected by seizures, we will use immunogold electron microscopy detection of caveolin-1 and flotillin-1 in the synaptosome preparations and neuronal cultures used in AFM studies.

Experimental work will be conducted with the support of the BioISI cell culture and FCUL microscopy facilities.

References:

1. D. Cunha-Reis, A. Caulino-Rocha, *Front. Cell. Neurosci.* **14**, 153 (2020).
2. J. Carvalho-Rosa, N. Rodrigues, A. Silva-Cruz, S. Vaz, D. Cunha-Reis, *bioRxiv*, in press, doi:10.1101/2022.12.06.519267.
3. A. P. Carapeto *et al.*, *Int. J. Mol. Sci.* **21**, 2916 (2020).
4. J. S. Cristóvão *et al.*, *ACS Chem. Neurosci.* **11**, 2753–2760 (2020).

Alternative topics: (please contact the supervisor)**:**

- A role for astrocytes in modulation of GABAergic transmission and synaptic plasticity by VIP?.
- Synaptic plasticity in the hippocampus during post weaning development and aging: influence of phosphorylation of synaptic enzymes and channels.

Synaptic plasticity in the hippocampus during post weaning development and aging: influence of phosphorylation of synaptic enzymes and channels

Place of work/: Epilepsy and Aging Team, BioISI – GER, FCUL.

Supervisor(s): Diana Cunha Reis, PhD (dcreis@fc.ul.pt) ORCID: 0000-0002-0900-9306.

Synaptic remodelling is believed to contribute to altered cognition and synaptic plasticity during postnatal development and upon ageing. Recently, we have shown that hippocampal LTP, a crucial cellular mechanism for learning and memory, undergoes postweaning developmental maturation (*1*). LTP also changes substantially with ageing (*2*). Hippocampal CA1 LTP depends on rapid events like phosphorylation of AMPA receptors, the autophosphorylation of CaMKII and other protein kinases like PKM (or PKCzeta). As such, the basal phosphorylation status of these proteins may be a strong conditioning for LTP expression. It has not been determined how the basal levels and phosphorylation status of these proteins change during postweaning development or during aging. Modulation of hippocampal LTP by the GABAergic-associated neuropeptide vasoactive intestinal peptide (VIP) also undergoes postweaning developmental changes (*3*, *4*).

This project aims to study the changes in enzymes and channels involved in synaptic plasticity as well as VIP and VIP receptors that are associated with postweaning development and ageing. This will be evaluated by western blot analysis in total hippocampal membranes and/or Percoll-purified hippocampal synaptosomes(*1*, *5*) obtained from:

a) 3-12-week-old rats (postweaning development).

b) 4-21-month-old rats (throughout aging).

to evaluate phosphorylation levels of AMPA receptor subunit GluA1 (associated with enhanced LTP), CaMKII and PKM. In addition, membrane levels of NMDA receptor subunits GRIN1, GRIN2 and AMPA GluA2 subunit (associated with pathological synaptic mechanisms) will also be evaluated. The expression of VIP and VIP $VPAC_1$ and $VPAC_2$ receptors and synaptic GABAergic markers may also be investigated.

For targets showing enhanced membrane levels, PCR will be performed to determine changes in gene expression (as opposed to membrane recruitment from intracellular stores).

Functional studies in hippocampal synaptosomes may be additionally performed using FM1-43 imaging *in vitro*.

References:

1. N. C. Rodrigues *et al.*, *Eur. J. Neurosci.* **54**, 5272–5292 (2021).
2. E. S. Rosenzweig, C. A. Barnes, *Impact of aging on hippocampal function: Plasticity, network dynamics, and cognition* (Elsevier Ltd, 2003), vol. 69.
3. D. Cunha-Reis, A. Caulino-Rocha, *Front. Cell. Neurosci.* **14**, 153 (2020).
4. A. Caulino-Rocha, N. C. Rodrigues, J. A. Ribeiro, D. Cunha-Reis, *Biol. 2022, Vol. 11, Page 627*. **11**, 627 (2022).
5. D. Cunha-Reis, J. A. Ribeiro, R. F. M. de Almeida, A. M. Sebastião, *Br. J. Pharmacol.* **174**, 4725–4737 (2017).

Alternative topics: (please contact the supervisor)**:**

- A role for astrocytes in modulation of GABAergic transmission and synaptic plasticity by VIP?
- Investigating altered lipid raft dynamics following seizures at hippocampal synapses with AFM.

Blood-brain barrier transposition by VIP $VPAC_1$ selective ligands

Place of work/: Epilepsy and Aging Team, BioISI – GER, FCUL & FunGP-Functional genomics and proteomics at BioISI.

Supervisor(s): Diana Cunha Reis, PhD (dcreis@fc.ul.pt, BioISI) ORCID: 0000-0002-0900-9306, Teresa Faria Pais, PhD (IGC and FCUL) & Hugo Botelho, PhD (BioISI).

Vasoactive intestinal peptide (VIP) $VPAC_1$ receptors (Rs) regulate synaptic plasticity in both physiological and pathological conditions, such as pro-epileptogenic events(*1–3*). This has implications to brain neuroprotection. Exogenous VIP was reported to cross the rat blood-brain barrier (BBB) *in vivo* through a non-saturable mechanism (likely transmembrane diffusion)(*4*). Other peptides in the VIP-PACAP-secretin family cross the BBB by peptide transporter 6 (PepT6)(*5*). Selective $VPAC_1$ R ligands (agonists and antagonists) are chimeric peptides based on the sequence of peptides in this family and may well benefit from the same ability to cross the BBB(*2*). This project will use cultures of rat brain microvascular endothelial cells (RBMVECs) to investigate the ability of $VPAC_1$ R ligands to cross the BBB. BBB spheroids (3D culture) may be used to further investigate the mechanisms of VIP BBB transposition.

RBMVECs will be obtained from the rat brain as described(*6*) and seeded on transwell inserts (0.4 µm; 8×10^4 cells/insert) for BBB transposition studies and in 96-well plates over an extracellular matrix coat to study peptide cell accumulation. Establishment of the BBB will be assessed by trans endothelial electrical resistance (TEER) measurements (~10-15 days). At this stage, the ability of VIP (positive control) or two $VPAC_1$ selective ligands (PG 97-269, a $VPAC_1$ R antagonist, or [K^{15}, R^{16}, L^{27}] VIP (1-7)/GRF (8-27), a $VPAC_1$ R agonist) to cross the BBB or to enter the endothelial cells will be evaluated by quantifying the presence of peptides in the lower culture media compartment by mass spectrometry (MS) or with a fluorescent plate reader (upon sample concentration) by using FAM/FITC-labelled peptides. These will also be used to monitor accumulation of $VPAC_1$ ligands inside the cells using confocal microscopy. The presence of VIP $VPAC_1$ receptors, PepT6 transporter, vascular cell adhesion molecule 1 (VCAM), zonula occludens-1 (ZO-1) or occludin-5 and -1 may also be evaluated by immunocytochemistry. A BBB spheroid 3D culture may be used to confirm and refine the results obtained.

References:

1. A. Caulino-Rocha, N. C. Rodrigues, J. A. Ribeiro, D. Cunha-Reis, *Biol. 2022, Vol. 11, Page 627*. **11**, 627 (2022).
2. D. Cunha-Reis, A. Caulino-Rocha, P. Correia-de-Sá, *Pharmacol. Res.* **164**, 105356 (2021).
3. J. D. Carvalho-Rosa, D. Cunha-Reis, Endogenous VIP VPAC1 receptor activation during ictal and interictal-like activity induced in vitro by biccuculine and 0-Mg2+ modulates subsequent LTP expression in the rat hippocampus. *Front. Cell. Neurosci.* (2019), , doi:10.3389/conf.fncel.2019.01.00028.
4. D. Dogrukol-Ak, W. A. Banks, N. Tuncel, M. Tuncel, *Peptides*. **24**, 437–444 (2003).
5. D. Dogrukol-Ak *et al.*, *J. Cereb. Blood Flow Metab.* **29**, 411–422 (2009).
6. Y. Liu *et al.*, *Microvasc. Res.* **90**, 199–205 (2013).

Alternative topics: (please contact the supervisor)**:**

- A role for astrocytes in modulation of GABAergic transmission and synaptic plasticity by VIP?
- Investigating altered lipid raft dynamics following seizures at hippocampal synapses with AFM.
- Synaptic plasticity in the hippocampus during post weaning development and aging: influence of phosphorylation of synaptic enzymes and channels

Characterizing the regulation of the CFTR protein by the kinase GRK5 in Cystic Fibrosis models

Place of work: Functional Genomics and Proteostasis Lab, BioISI (FCUL, C8)

Supervisors: Hugo M. Botelho (hmbotelho@ciencias.ulisboa.pt, http://webpages.fc.ul.pt/~hmbotelho)

Bacground: Cystic Fibrosis (CF) is the most common life limiting rare disease in the Caucasian population (~90,000 individuals worldwide). CF is caused by mutations in the CFTR gene, which encodes an anioinc channel located at the apical plasma membrane (PM) of several epithelial cell types. CFTR mutations impair transepithelial chloride transport and lead to a progressive lung disease, the foremost cause of morbidity and mortality in CF. One single mutation– p.Phe508del – accounts for 85% of all CF cases. In the cell, p.Phe508del-CFTR is retained in the endoplasmic reticulum (ER), thereby failing to reach its physiological location at the PM and leading to defective transepithelial anionic transport, which negatively affects the epithelium in the lungs and other organs. Drugs which correct, to some degree, the molecular defects of selected CFTR mutants – such as the Kaftrio cocktail – are already available to most people with CF. Nevertheless, this pharmacotherapy is not available to many individuals and healthcare systems due to its high cost and lifelong need. The supervisor laboratory has recently discovered that the efficacy of the drug cocktail is maximized in CF cellular models when the kinase GRK5 is inhibited with a specific inhibitor known as 9g. The mechanism whereby GRK5 participates in recovering the activity of mutant CFTR is not known and may hold important implications towards improving CF therapy, which this proposal will aim at leveraging.

Objective: Identify the composition of the signaling pathway responsible for regulating p.Phe508del-CFTR traffic and activity through GRK5.

Work plan: This proposal includes the following laboratorial work:

1) Culturing human lung epithelial cell lines expressing the wild type or p.Phe508del CFTR variants, with and without tags for fluorescence microscopy (mCherry-Flag);
2) Performing a genetic screen through automated fluorescence microscopy to identify genes whose expression is essential for the rescue of p.Phe508del-CFTR by the inhibitor 9g. This task will make use of siRNA libraries to silence individual genes;
3) Validation of screen hits through Western blot (biochemical detection of mutant CFTR release from the ER) and HS-YFP (quantification of anionic transport through CFTR);
4) Proposal of a model for the signaling pathway coupling GRK5 inhibition to p.Phe508del-CFTR ER release and activation.

References:

1) Amaral MD et al (2020) CFTR processing, trafficking and interactions. J Cyst Fibros (2020) 19(Suppl 1): S33-S36
2) Botelho HM et al (2015) Protein Traffic Disorders: an Effective High-Throughput Fluorescence Microscopy Pipeline for Drug Discovery. Sci Rep. 5: 9038

BioISI Junior

Computational Biology Projects

A unifying network regulating CFTR trafficking and function

Place of work/: RNA Systems Biology Lab & Cystic Fibrosis Research Lab, BioISI, FCUL

Supervisors: Francisco Rodrigues Pinto (frpinto@fc.ul.pt), Carlos Miguel Farinha (cmfarinha@fc.ul.pt)

Background and objective

Most Cystic Fibrosis is caused by the deletion of F508, that results in retention of the mutant protein within the ER. This is due to the interaction of immature forms of CFTR with ERQC proteins, which target misfolded conformations for proteasomal degradation. Although many (large scale) studies have addressed CFTR protein-protein interactions, a unifying approach to reconcile such information is lacking. Here, we propose to use network analysis combining the available omics studies in the field to determine the most relevant pathways in the regulation of CFTR trafficking and function. This work will explore the strong expertise of the supervising team and host labs in network biology and CFTR trafficking (as shown in previous publications – see below).

Workplan

To fulfil this objective, the work proposed involves:

1. Data collection – Identification of the relevant studies to be included and collect the data previously published that is relevant to the identification of the proteins expressed, interacting or regulating CFTR trafficking.
2. Network construction - An integrated network will be assembled including: CFTR; CFTR interactors (retrieved from the studies described above); Proteins associated with other rare conformational diseases (retrieved from DisGeNet and Open Targets); Proteins involved in protein trafficking pathways; Minimum number of proteins needed to link all the previous proteins in a connected network, using protein interactions retrieved from public databases (APID, HuRI, StringDB).
3. Network mining – Development of protein metrics that integrate 1) gene or protein expression measurements, 2) type of experiment or cellular model where interactions were detected, 3) connectivity to other conformational disease associated proteins, 4) network centralities and 5) functional annotation. These metrics will be used to prioritize the most relevant network nodes which may have a higher impact in the regulation of CFTR trafficking.
4. Validation of the network and protein candidates obtained – If time allows, and to validate the obtained network, we will select the most relevant nodes and pathways identified and pick 10-20 proteins whose down-regulation will then be assessed in terms of F508del-CFTR exit from the ER (assessed by Western blot, fluorescence microscopy and iodide efflux assay).

Identification of tissue specific cancer driver gene interactors

Place of work/: RNA Systems Biology Lab, BioISI, FCUL

Supervisors: Francisco Rodrigues Pinto (frpinto@fc.ul.pt)

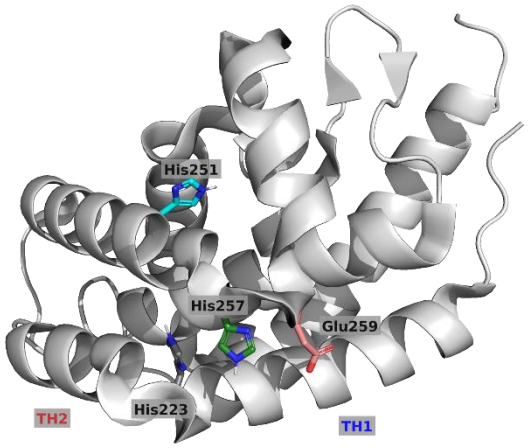
Cancer driver genes, when mutated, contribute to cancer development. Driver genes are usually identified by being frequently mutated in cancer samples. Some drivers are involved in many cancer types (generalist drivers), but most of them are associated with a restricted number of cancers (specific drivers). Recently we observed that specific drivers tend to have different interactions (protein physical interactions, signaling and regulatory interactions) in the tissues where they contribute to cancer development in comparison with other tissues. This observation suggests that these differential tissue specific interactors may play relevant roles in cancer development. This project aims to identify and characterize such tissue specific cancer driver interactors for multiple cancers using a comparative interactome analysis across tissues. The project consists in: 1 – collecting molecular interaction data to build a global interactome, 2- construction of tissue specific interactions through the analysis of tissue gene expression datasets (GTEX, Human Protein Atlas, TCGA), 3- development of quantitative scores to rank driver gene interactors according to their ability to preferentially interact with driver genes in the tissues where these drivers are (or are not) associated with cancer development, 4- identification, in each tissue, of tissue specific cancer driver gene interactors, 5- functional characterization of these driver interactors. We expect to find potential drug targets or biomarkers among these tissue specific driver interactors, contributing for the improvement of patient stratification and personalized medicine goals.

Unraveling the molecular details of the pH-dependent trigger in diphtheria toxin T domain

Place of work: BioISI-FCUL (C8, 8.5.50D)

Supervisors: Miguel Machuqueiro (machuque@ciencias.ulisboa.pt)

Diphtheria is a severe illness, which can be fatal if left untreated. The diphtheria toxin is produced by the *Corynebacterium diphtheriae* bacterium and is spread through respiratory droplets. The diphtheria toxin T (DTT) domain (see Figure) [1] translocates the toxin's catalytic domain across the endosomal membrane into the host cell cytoplasm, leading to cell death. The translocation process is triggered by endosome acidification and DTT undergoes a conformational change exposing a hydrophobic region (TH1-TH2) that enables it to insert into the endosomal membrane. The DTT domain is a potential target for therapeutics aimed at disrupting the translocation process and preventing the spread of the disease. The conformational destabilization of the DTT domain in the endosome is likely triggered by the protonation of key residues, although the molecular details of this process are still unknown. Several histidine residues have been proposed to be the pH sensors (see Figure), yet other typical protonatable residues, like aspartic and glutamic acids, cannot be excluded.



In this proposal, we aim to study the impact of endosomal acidity on the structural stability of DTT. We will use our state-of-the-art constant-pH molecular dynamics (CpHMD) method [2] to perform computational MD simulations under an acidic environment and characterize the conformational transitions triggered by the protonation of key residues, including the histidine residues that have been proposed by our collaborators (Prof. Alexey Ladokhin, Univ. Kansas, USA) [3]. The following tasks will be performed:

1. CpHMD simulations of the wild-type DTT protein at different pH values (3.0–7.0) to evaluate the impact of acidity on the protein.
2. Extend the previous simulations to several DTT mutants, including H223Q and H257Q, to help the experimentalists rationalize their experimental data and propose a detailed mechanism for the acid-induced conformational transition.
3. Perform CpHMD simulations in the presence of a membrane model at low pH to assess the role of the lipids in facilitating the exposure of the hydrophobic regions of DTT.
4. Compile all results in an MSc thesis and in a paper for an international scientific journal.

[1] Steere B, Eisenberg D. Worldwide Protein Data Bank; 2000. doi:10.2210/pdb1f0l/pdb
[2] Oliveira NFB, Pires IDS, Machuqueiro M. J Chem Theory Comput. 2020;16: 6368–6376.
[3] Rodnin, M. V., Li, J., Gross, M. L., Ladokhin, A. S. Biophys. J., 2016, 111, 1946–1953.

Using polygenic risk scores to address the heterogeneity of Autism Spectrum Disorder

Place of work/: Instituto Nacional de Saúde Doutor RIcardo Jorge

Supervisors: Hugo Martiniano (hugo.martiniano@insa.min-saude.pt); Astrid Vicente (astrid.vicente@insa.min-saude.pt);

Non-coding RNA ((ncRNA) are RNAs that do not encode proteins. These are the majority of human genes and several ncRNAs play a crucial role in regulating gene expression and other biological processes.

However, when compared with protein-coding genes, little is known of the function of most ncRNAs. The state-of-the-art approach to systematization of biological knowledge of genes and gene products is the Gene Ontology (GO). While annotation of proteins is well-developed (albeit not finished), this is not true for ncRNAs. Closing this knowledge gap is an essential step in understanding living systems. In particular when related to health and disease states.

In this project we propose the development of machine learning methods to predict GO annotations for ncRNA molecules. Using a dataset composed of a network of ncRNAs and their associations to each other and to protein-coding genes, the problem of predicting GO annotations can be framed as a link prediction task. For this purpose we propose the use of recently-developed graph neural networks, which have been shown to have excellent performance for link-prediction in complex networks.

The methods developed in the context of this project will be applied to the identification of pathogenic genetic variants in a cohort of children with Autism Spectrum DIsorder.

The candidate is expected to have knowledge of the Python programming language and an interest in machine learning methods.

Prediction of Genes associated With Autism Spectrum Disorder Using Graph Embedding methods

Place of work: Instituto Nacional de Saúde Doutor RIcardo Jorge

Supervisors: Hugo Martiniano (hugo.martiniano@insa.min-saude.pt); Astrid Vicente (astrid.vicente@insa.min-saude.pt);

In recent years machine learning methods designed to work with graph data have emerged. Many biological datasets are naturally structured in this way, such as protein-protein interaction (PPI) networks or Biological Pathways so the application of these algorithms to data in this domain is often straightforward.

In this project we propose the application of these methods to networks of interactions between genes, based on protein-protein interactions, as well as to other types of biological networks, obtained from publicly-accessible databases, such as gene-gene similarity networks derived from the Gene Ontology.

The ultimate aim is the identification of risk genes associated with Autism Spectrum DIsorder (ASD), a prototypical complex disorder with a strong genetic component. More specific aims are the comparison of the performance of several graph embedding methods and different data sources on the task of predicting the association of genes to ASD.

The main task are:

1 - Apply in-house Machine learning pipelines to perform binary classification of genes
2 - Extend and adapt existing pipelines for new networks

Knowledge of Python and common Python Machine Learning and processing frameworks (scikit-learn, pandas), Bash and the linux command line utilities would be useful.

pKaMD: Development of a fast AI-based method that includes conformational sampling in the predictions of protein pK_a values

Place of work: BioISI-FCUL (C8, 8.5.50D)

Supervisors: Miguel Machuqueiro (machuque@ciencias.ulisboa.pt);
Pedro Reis, Bayer AG, Berlin, DE (pedro.reis@bayer.com)

The importance of pH in the structure and function of proteins is illustrated by the fact that about a quarter of amino acid residues contain ionizable side chains. The pH-dependent ionizations that arise can generate strong electrostatic interactions that inevitably influence the stability of proteins and their ability to bind to other molecules, for example to substrates. Many research groups have invested in the development and improvement of computational methods capable of modeling the protonation equilibrium and predicting the pK_a values of proteins. One of the greatest difficulties encountered is related to the great conformational variability of proteins, leading to a very strong coupling between protonation and conformation. To deal with this difficulty, constant-pH molecular dynamics (CpHMD) methods were developed [1-2]. Recently, with advances in computational power, this type of methodology has become relatively accessible and promises to become state-of-the-art in terms of pK_a predictions in proteins. Furthermore, machine learning methods have been shown to accelerate pK_a calculations by a factor of up to three orders of magnitude [3]. Nonetheless, the introduction of conformational sampling produces a significant computational penalty. The aim of the MSc. thesis work is the development of a new pK_a calculation method based on short simulations of the current AI-accelerated CpHMD method. Several tasks will be carried out during the period of the work plan:

1. Adapt the CpHMD method for short runs (optimizing its parameters) aiming at both efficiency and accuracy of the pK_a predictions;
2. Apply the method and benchmark with a set of proteins for which there is abundant experimental pK_a data [4].
3. Implement an online UI and merge it with the current infrastructure (pypka.org) and its backend servers to make this service available to the scientific community.
4. Compile the results into an MSc. thesis and write a scientific article to be published in an international journal.

[1] Machuqueiro, M., Baptista, A. M. (**2006**) *J. Phys. Chem. B*, 110, 2927.
[2] Vila-Viçosa, D., Reis, P.B.P.S., Baptista, A.M., Oostenbrink, C., Machuqueiro, M. (**2019**) *J. Chem. Theory Comput.*, 15, 3108-3116.
[3] Reis, P.B.P.S, Bertolini, M., Montanari, F., Rocchia, W., Machuqueiro, M., Clevert, DA. (**2022**) J. Chem. Theory Comput., 18, 8, 5068–5078
[4] Pahari, S., Sun, L., Alexov, E. (**2019**) *Database*, baz024.

Predicting non-coding RNA function using graph neural networks

Place of work/: Instituto Nacional de Saúde Doutor RIcardo Jorge

Supervisors: Hugo Martiniano (hugo.martiniano@insa.min-saude.pt); Francisco Couto (fjcouto@edu.ulisboa.pt);

Non-coding RNA ((ncRNA) are RNAs that do not encode proteins. These are the majority of human genes and several ncRNAs play a crucial role in regulating gene expression and other biological processes.

However, when compared with protein-coding genes, little is known of the function of most ncRNAs. The state-of-the-art approach to systematization of biological knowledge of genes and gene products is the Gene Ontology (GO). While annotation of proteins is well-developed (albeit not finished), this is not true for ncRNAs. Closing this knowledge gap is an essential step in understanding living systems. In particular when related to health and disease states.

In this project we propose the development of machine learning methods to predict GO annotations for ncRNA molecules. Using a dataset composed of a network of ncRNAs and their associations to each other and to protein-coding genes, the problem of predicting GO annotations can be framed as a link prediction task. For this purpose we propose the use of recently-developed graph neural networks, which have been shown to have excellent performance for link-prediction in complex networks.

The methods developed in the context of this project will be applied to the identification of pathogenic genetic variants in a cohort of children with Autism Spectrum DIsorder.

The candidate is expected to have knowledge of the Python programming language and an interest in machine learning methods.

BioISI
Junior

Biological Chemistry Projects

Antimicrobial nano-agents for bio-threats prevention

Place of work/: The work will be mainly performed at the Laboratory 8.6.42 (BioISI) and - with some training stages at the Laboratório de análises do IST - LAIST (https://la.tecnico.ulisboa.pt/)

Supervisors: BioISI: Elisabete R. Silva (ersilva@ciencias.ulisboa.pt); LAIST: Ricardo Santos (ricardosantos@tecnico.ulisboa.pt).

Pathogenic microorganisms accommodated in biofilms on surfaces are a global concern for all societal infrastructures, for example, water utility management systems (e.g., water distribution and treatment). Biofilms lead to serious consequences, including premature biocorrosion and waterborne biothreats, posing a major risk to industrial systems sustainability and public health, and its disinfection is recognized as a critical and challenging process. The most effective bio-decontamination strategies include the controlled release of toxic and cumulative bioactive agents into the aquatic environment, which has a limited life cycle and brings ecological issues. This work aims at contributing to overcoming these decontamination strategies challenges, by providing novel approaches for the immobilization of commercial antimicrobial agents (synthetic or natural-based) on the surface of metal oxide nanoparticles, allowing their bioactivity to be amplified, particularly for major pathogenic microorganisms and generated without leaching agents into aquatic media, extending their potential application.

The key workplan activities comprises:

(i) Surface design through the modification of nanoparticles with bioactive agents; **(ii)** Physicochemical characterization of the NPs (e.g., XRD, DRIFT, SEM, elementary analysis, BET); **(iii)** Antimicrobial susceptibility and ecotoxicity evaluation of (un)modified nano-agents; **(iv)** Dissemination of the work, which may include contributions and participation in events/training actions and writing the thesis.

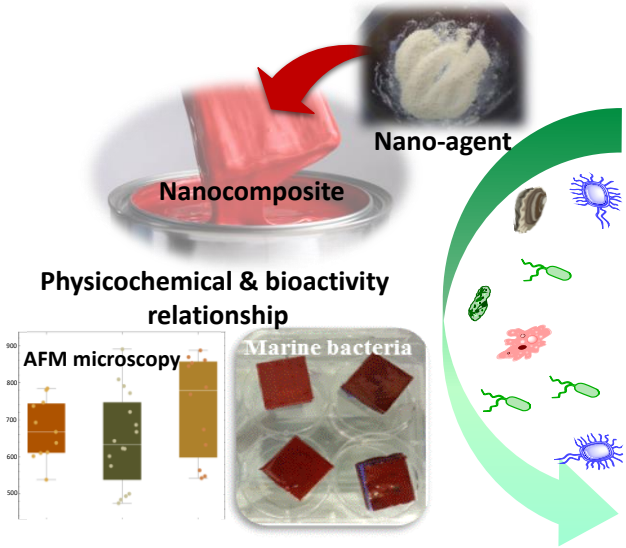
Ultimately, the most promising nano-agents will be immobilized in commercial coatings to coat small prototypes for proof-of-concept in simulated conditions as part of an ongoing national collaborative project (4 partners). During his thesis, the student will be a member of this collaborative project team, with the possibility of continuing his work depending on his performance and goals.

Anti-biofouling nanocomposite coatings for bio-threats prevention

Place of work/: The work will be mainly performed at the Laboratory 8.6.42 and 8.4.23.

Supervisors: BioISI: Elisabete R. Silva (ersilva@ciencias.ulisboa.pt); Ana Carapeto (apcarapeto@ciencias.ulisboa.pt).

Global efforts have been promoted to face pathogenic bio-threats, since microorganism are prone to colonize and form biofilms on surfaces. These threats are particularly relevant on surfaces in contact with water, such as wastewaters circuits and those highly exposed to pathogens (e.g., water circuits and medical devices in hospitals). Most effective antimicrobial protection strategies on surfaces rely on chemical-based disinfection, which release toxic and persistent agents into the environment, remaining ineffective in preventing biofilm formation and progressive biofouling on surfaces under the current environmental demand and guidelines. In a previous work, newly synthesised bioactive nano-agents demonstrated auspicious antimicrobial effects. This project aims to foster these findings to achieve application validation of the most promising nano-agents as anti-biofouling coatings suitable for various industrial applications, including those involving an aquatic environment (e.g., water treatment, marine infrastructures).
Three interrelated R&D objectives can be outlined:



- Formulate and optimise nanocomposite coatings containing immobilised nano-agents suitable for different applications.
- Investigate the relationship between nanocomposite coatings´ physicochemical properties. The supervisor team provides expertise and specific resources for antifouling coatings development and several AFM facilities essential for the biophysical properties evaluation of the nano-systems. For example, morphological characteristics of surfaces (e.g., roughness, dispersion), and adhesion of organic matter and bacteria on coating films using AFM tip functionalization.
- Evaluate the anti-biofouling potential of nanocomposite coatings under laboratory and simulated conditions. *This part of the work will be performed in collaboration with a partner of an ongoing national collaborative project. During his thesis, the student will be a member of this collaborative project team, with the possibility of continuing his work depending on his performance and goals.*

Design of Cyclic Peptide Drugs for Parkinson's Disease through Molecular Simulations

Place of work/: BioISI Ed. C8 – piso 5 Workspace

Supervisors: Nuno Galamba (njgalamba@fc.ul.pt); Gabriel Martins (gfmartins@fc.ul.pt)

Protein aggregation is implicated in several human pathologies, ranging from sickle cell disease, a red blood cell disorder, to type 2 diabetes mellitus, and various neurodegenerative diseases, such as Alzheimer's, Huntington's, or Parkinson's disease (PD), commonly known as proteinopathies. A major challenge regarding drug design aiming at inhibiting or delaying protein aggregation for some of these diseases concerns the lack of specific protein binding regions. Over the past years several seemingly key regions governing the aggregation of α–synuclein, the most important protein involved in the formation of cytotoxic oligomers in PD, have been identified. Herein, we propose to design and probe, through computational chemistry methods, the binding affinity of cyclic peptides toward some of these regions. The project will involve the use of coarse-grained and all-atom molecular dynamics simulations of α–synuclein and several tailor-made cyclic peptides. Their affinity toward the aforementioned regions and potential implications to the structure of the monomer will be assessed and putative structural descriptors allowing to predict their influence on the protein's aggregation tendency will be investigated.

Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship BII), being the selection criterium the academic merit of the candidates.

Drug Design Pipeline Development for Proteinopathies

Place of work/: BioISI Ed. C8 – piso 5 Workspace

Supervisors: Nuno Galamba (njgalamba@fc.ul.pt); Hugo Martiniano (hugo.martiniano@insa.min-saude.pt)

Protein aggregation is implicated in several human pathologies, ranging from sickle cell disease (SCD), a red blood cell disorder, to type 2 diabetes mellitus, and various neurodegenerative diseases (NDs), such as Alzheimer's (AD), Huntington's (HD), or Parkinson's disease (PD), commonly known as proteinopathies. A major challenge regarding *in silico* drug design studies concerns the assessment of the aggregation inhibitory activity of drugs found to bind to the proteins involved in these diseases. Thus, the fact that a drug binds to a specific pocket or region of the protein of interest does not necessarily imply its ability to interfere with the aggregation process. The reason for this difficulty is associated with the large size of these systems and the fact that protein aggregation and dissociation may occur on time scales not available through typical all-atom molecular simulation methods. To address this problem we propose to develop a pipeline which integrates a set of coarse grained and all-atom molecular simulations and develop a set of structural descriptors that can predict the aggregation inhibitory activity of drugs already studied *in vitro* for simple model proteins and peptides. These will then be used to evaluate the performance of a series of potential drug candidates for one or more proteins involved in proteinopathies such as sickle cell anemia, Parkinson's disease or Alzheimer's disease.

The candidate is expected to have an interest in learning about molecular simulations as well as in the development of python-based molecular and statistical analysis tools. A simple pipeline encompassing molecular simulations, performed with the program GROMACS, and analysis tools, that allows probing the effect of any drug on protein aggregation,is expected to be developed.

The project will be developed at BioISI-FCUL under the supervision of the researchers Nuno Galamba and Hugo Martiniano. Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship BII), being the selection criterium the academic merit of the candidates.

BioISI Junior

Folding equilibrium of cyclic decapeptides mediated by odd noncovalent interactions

Place of work/: Computational Chemistry & Molecular Interactions Lab (https://ccmi.rd.ciencias.ulisboa.pt/), BioISI

Supervisors: Paulo J. Costa (pjcosta@ciencias.ulisboa.pt)

The mechanism of protein folding/unfolding is one of the most fundamental questions in structural biology, as this process is paramount for regulating biological activity and targeting proteins in different cellular locations [1]. When folded, the main secondary structure motifs in proteins, most commonly α–helices and β–sheets, are characterized by the existence of noncovalent interactions, namely, hydrogen bonds (HBs), between the N–H and C=O groups of the main chain, thus, the understanding of these interactions is paramount. Cyclic peptides (CPs) are a class of potent and selective target-binding molecules with attractive features in drug design such as the ability to bind to protein targets, tunable permeability, resistance to proteolytic enzymes, and fewer conformational degrees of freedom than linear peptides. CP-based drugs have been developed in the past two decades [2], and fine-tuning their folding/unfolding equilibrium is required to modulate their target-binding properties and also for tunning their remarkable membrane permeability. Indeed, the stabilization and modulation CP conformations can be achieved not only by the usage of the ubiquitous hydrogen bond but also by using odd noncovalent interactions such as halogen bonds [3]. Our Lab has been focused on the study of halogen bonding with biomolecular applications [4] using molecular modeling techniques such as molecular dynamics simulations. In this project, we will study the conformational space and folding/unfolding equilibria of a series of CPs [3] whose stabilization of a β–hairpin structure can be achieved by both hydrogen or halogen bonds. This study aims at testing the limits of force field parametrization but also to provide insights into the potential membrane permeability and drug potential of such entities. The usage of the extensive experimental NMR structural data available allows for the validation and tuning of the parameters that describe the noncovalent interactions responsible for the stability of the β–hairpin structures, thus allowing an experimental validation of our results. This project requires someone motivated to study biochemical systems using computational methods. The results will be used not only for the Master Thesis but also to be published in a peer-review journal. Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium, the academic merit of the candidates.

[1] C. M. Dobson, Nature, 2003, 426, 884–890.
[2] H. Zhang, S. Chen, RSC Chem. Biol., 2022, 3, 18-31.
[3] E. Danelius, H. Andersson, P. Jarvoll, K. Lood, J. Gräfenstein, M. Erdélyi, Biochemistry 2017, 56, 3265–3272
[4] R. S. Nunes, D. Vila-Viçosa, P. J. Costa, J. Am. Chem. Soc. 2021, 143, 4253−4267

BioISI
Junior

Biological Physics Projects

The effect of intermediate filament, microtubule and microfilament polymerization on the mechanical properties of cells

Place of work/: Atomic Force Microscopy and Related Techniques Lab & Cell Structure and Dynamics Lab, FCUL, Lisbon

Supervisors: Mário Rodrigues (mmrodrigues@ciencias.ulisboa.pt); Federico Herrera (fherrera@fc.ul.pt)

WORKPLAN

The effects of mechanical interactions are determinant in a number of cell aspects, like mechanotransduction, morphogenesis, disease progression, metastasis, drug cell interactions, etc. However, establishing the link between said properties tends to be highly complex and despite some success, the links are still poorly understood. With a clear strategy for measuring cell viscoelasticity, we plan to pharmacologically inhibit intermediate filament, microtubule and microfilament polymerization in glioma cells from the nervous tissue. This can be achieved by using chemical inhibitors such as withaferin, nocodazole or latrunculin, respectively or by genetically removing specific intermediate filaments or their organizers (ex: sacsin or plectin). The respective cell culture and modification will be carried out at F. Herrera' biochemistry laboratory under the supervision of F. Herrera's team. The alterations of the cytoskeleton microtubules and actin filaments will be characterized by confocal fluorescence imaging. This will permit the estimation of the density of each type of fibre, arrangement and shape, cell nucleus size, presence/absence of cytoskeleton-binding proteins, and, through combination with membrane markers, the link between the cytoskeleton and the membrane. The mechanical properties of the cells will then be measured with atomic force microscopy. The main goal is to establish correlations between cytoskeleton morphology and the viscoelastic properties of the cell by using fluorescence microscopy and both conventional and nonconventional AFM strategies to measure the differences in the mechanical properties as a consequence of aforementioned cell alterations.

This work is in line and integrated with the FCT project: Viscoelastic Cells - new experimental approaches based on atomic force microscopy PTDC/FIS-MAC/2741/2021

BioISI
Junior

Biotechnology Projects

Soil Microbiome analysis to improve drought resilience in maize

Place of work/: BioISI (Plant Functional Genomics Group) and Department of Plant Biology / Centro Nacional de Competências das Culturas do Milho e Sorgo (INOVMILHO), Coruche

Supervisors: Supervisor 1: Ana Margarida Fortes (amfortes@fc.ul.pt); Supervisor 2 Jorge Marques da Silva (jmlsilva@fc.ul.pt)

Water availability may be the most important environmental factor limiting crop growth and productivity. Climate change will likely make drought events more frequent in many regions, including Portugal, and increasing the demand on freshwater resources and creating major challenges for sustainable agriculture. Maize (*Zea mays*) is an extremely important crop cultivated worldwide, being used for food for both humans and livestock, as well as biofuel, and as a crude material in industry. However, it demands high amounts of water, and is even considered a "thirsty" crop.

Microbiomes support plant health and adaptation to environmental transitions, exhibiting increased phenotypic plasticity comparing to plants with lesser dynamic genomes. This Master thesis aims to contribute to sustainable production of maize by identifying the putative functional microbiome that can improve resilience of *Zea mays* to drought stress.

The work plan is proposed as follows:

1- Extraction of Metagenomic DNA from soil associated with two cultivars, one resilient and one susceptible to drought, and growing under different water regimes (full irrigation, deficit irrigation).

2- Identification of microbiome composition by bioinformatic analysis of 16S rRNA gene metabarcoding.

3- Measurements of plants' optical signals to infer physiological responses to drought in both resilient and susceptible genotypes.

Analysis of periderm development in potato microtubers

Place of work: *ForGen Lab – Forest Genomics and Molecular Genetics Laboratory* - Faculdade de Ciências da Universidade de Lisboa (FCUL)
https://forgen.rd.ciencias.ulisboa.pt/

Supervisors: Vera Inácio (vlinacio@fc.ul.pt), Célia Miguel (cmmiguel@fc.ul.pt FCUL)

Abstract / MSc thesis project proposal

The periderm envelops the roots and shoots of species with secondary growth functioning as an outer protective shield against biotic and abiotic stresses. The periderm is not only crucial for plant survival but is also explored in a sustainable and profitable cork industry, where cork oak is the exclusive commercial source. The periderm is mainly composed of phellem or cork cells resulting from the meristematic activity of the phellogen. After phellogen initiation, cork cells undergo a differentiation process that includes cell wall suberization and programmed cell death. Potato tubers also form a periderm and when potatoes are harvested, the suberization process continues for a few days, resulting in a “mature” potato skin.
In this work, we will use potato microtubers to characterize the phellogen activity and periderm formation at different developmental stages. Microtubers can be induced very quickly in *in vitro* potato cultures using a synthetic cytokinin and it is a valuable tool to study periderm development in a prompt way.

The plan includes:

(1) Induction of microtuber formation in *in vitro* potato cultures;
(2) Time-series histological study of microtuber skin to determine periderm developmental/maturation stages using fluorescence, confocal microscopy, and transmission electron microscopy with established methods;
(3) gene expression analysis by RT-qPCR of genes involved in periderm development to validate phellogen activity/inactivity.

This work will serve as a framework for future studies on the regulation of periderm development at the molecular and chromatin levels.

The student will be integrated into a multidisciplinary team of researchers and will develop his/her knowledge of experimental tools and techniques and teamwork ability, autonomy, organization, and critical thinking, essential in any professional area. Specifically, the student will acquire skills in histology and microscopy techniques, and molecular biology and bioinformatics, transversal to research areas. The student selected for this project, after thesis registration, is eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.

Analysis of histone modifications in potato periderm development

Place of work: *ForGen Lab – Forest Genomics and Molecular Genetics Laboratory* - Faculdade de Ciências da Universidade de Lisboa (FCUL)
https://forgen.rd.ciencias.ulisboa.pt/

Supervisors: Vera Inácio (vlinacio@fc.ul.pt), Célia Miguel (cmmiguel@fc.ul.pt FCUL)

Abstract / MSc thesis project proposal

The periderm is the outermost line of defense against external stresses in plants with secondary growth and it is formed by three tissues: a secondary meristem, the phellogen; suberized phellem or cork produced by the phellogen; and the phelloderm also produced by the phellogen. The potato tuber periderm is widely used as a model to study periderm development. Early in the development of potato tuber periderm, the phellogen is activated and produces the immature skin, while during tuber maturation, the phellogen becomes inactive and the skin adheres to the tuber flesh.
Previous findings established that key regulatory networks of secondary meristems involve histone post-translational modifications (HPTMs). These modifications are critical for plant development being involved in gene activity control at the chromatin level, cell-cycle regulation, cell differentiation, and tissue specification.
Our main goal is to investigate the role and dynamics of antagonist H3K27me3 (chromatin mark of developmentally repressed genes) and H3K27ac (a chromatin mark associated with actively transcribed genes) chromatin marks in potato periderm formation and phellogen activity.

The plan includes:

1) Isolation of phellogen nuclei at distinct periderm developmental stages (active vs inactive phellogen) by fluorescence-activated nuclei sorting (FANS);
2) Chromatin extraction of sorted phellogen nuclei and immunoprecipitation with antibodies against antagonist HPTMs (H3K27me3/H3K27ac);
3) Analysis of regions of interest of candidate genes (gene regulatory regions of genes known/likely to be associated with H3K27me3/H3K27ac marks) in the immunoprecipitated samples by qPCR.

This innovative project will contribute to elucidating the role of these two antagonist chromatin marks in potato periderm development and contribute to advancing the knowledge of chromatin regulatory mechanisms underlying periderm formation and phellogen activity.

The student will be integrated into a multidisciplinary team of researchers and will develop his/her knowledge of experimental tools and techniques and teamwork ability, autonomy, organization, and critical thinking, essential in any professional area. Specifically, the student will acquire skills in molecular biology (DNA/RNA/chromatin extraction and immunoprecipitation, qPCR), flow cytometry, and bioinformatics, transversal to research areas. The student selected for this project, after thesis registration, is eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.

SNP genotyping in the identification of *Pinus pinaster* plants more resistant to the pine wilt disease

Place of work: *ForGen Lab – Forest Genomics and Molecular Genetics Laboratory* - Faculdade de Ciências da Universidade de Lisboa (FCUL) https://forgen.rd.ciencias.ulisboa.pt/; **Supervisors:** Célia Miguel (cmmiguel@fc.ul.pt), Vera Inácio (vlinacio@fc.ul.pt)

The student will be part of a project that aims at confirming the association of previously identified single nucleotide polymorphisms (SNPs) in maritime pine (*Pinus pinaster*) with the species ability to survive inoculation by the pinewood nematode, the causal agent of the pine wilt disease (PWD). PWD is one of the most serious diseases currently affecting coniferous forests in East Asia and the Iberian Peninsula. It has caused high economic losses and threatened forest ecosystems in affected areas. Maritime pine showed extreme susceptibility to the disease. In recently performed work in our lab it was possible to identify candidate genes and microRNAs involved in resistance (Modesto et al. 2021, https://doi.org/10.3389/fpls.2021.690857; Modesto et al. 2022, https://doi.org/10.1038/s41598-022-09163-3), as well as genetic variants or SNPs (Single Nucleotide Polymorphisms) possibly associated to the plant response (Modesto et al. 2022, https://doi.org/10.3390/f13060946).

The main objective of this project is to validate the usefulness of previously identified SNPs in the discrimination of PWD resistant and susceptible plants of maritime pine at an early stage of the plant life cycle. The work plan includes:

(I) PWN inoculation trial and symptom recording;

(II) DNA extraction from tissue samples of resistant and susceptible plants;

(III) Design of primers for amplifying regions containing the selected SNPs

(IV) SNP genotyping of DNA samples from resistant and susceptible plants by PCR and Sanger sequencing;

(IV) Analysis of the suitability of the tested SNP in plant screening

This work will be integrated in a large national project involving public and private entities aiming at the conservation and improvement of forest genetic resources in Portugal. The student selected for this project, after thesis registration, is eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship (BII), being the selection criterium the academic merit of the candidates.

Functional characterization of genes associated to cork formation by genetic complementation approaches

Place of work: *ForGen Lab – Forest Genomics and Molecular Genetics Laboratory* - Faculdade de Ciências da Universidade de Lisboa (FCUL) https://forgen.rd.ciencias.ulisboa.pt/; **Supervisors:** Célia Miguel (cmmiguel@fc.ul.pt), Ana Milhinhos (afmilhinhos@fc.ul.pt ITQB-NOVA)

The student will be part of a larger project that aims at understanding the role of previously identified cork oak genes associated with cork formation (Lopes et al. 2020, https://doi.org/10.1093/treephys/tpz118). Cork is part of the bark that protects plants from environmental stresses, being at the same time a relevant biomaterial for the industry. Therefore, understanding the molecular mechanisms underlying its formation will be important to devise strategies for increased plant resilience and cork production for industrial purposes. The integration in this project will allow the student to have contact with different methodologies used in a molecular genetics lab, including primer design, molecular cloning, plant genetic transformation and phenotyping.

In previous work, the putative orthologs of the cork oak genes of interest have been identified in Arabidopsis and loss-of-function mutants with characteristic phenotypes are currently available in the lab. The main objective of this project is to generate genetic complementation lines to check whether the expression of the cork oak genes in Arabidopsis loss-of-function mutants can rescue mutant phenotypes. The work plan includes:

(I) Primer design for amplifying the sequences of the genes of interest in cork oak and the promoter regions of the putative orthologs in Arabidopsis;

(II) Preparation of transformation vectors with Arabidopsis promoters driving the expression of cork oak genes;

(III) Genetic transformation and selection of transformant seedlings of *Arabidopsis*

(IV) Phenotyping of transformed plants using confocal microscopy

The student selected for this project, after thesis registration, is eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship (BII), being the selection criterium the academic merit of the candidates.

Developing molecular tools to improve grapevine resilience against pathogens: new insights on the role of chloroplasts lipids

Place of work/: GPS lab and Plant Physiology Lab, C2 building, 4th floor

Supervisors: Andreia Figueiredo/Ana Rita Matos

Contact: aafigueiredo@fc.ul.pt / armatos@fc.ul.pt

Grapevine (Vitis vinifera L.) represents a great agricultural and economic value worldwide, with deep ties to human culture for more than 5000 years. In 2019, grapevine plantation areas reached 7.4 Mha, with Spain, Italy, and France leading these plantation areas in Europe (OIV data, 2020). The European elite grapevine cultivars are highly susceptible to various pathogens and several phytochemical applications each growing season are made to control the main grapevine pathogens. In the last years, there is an increasing demand for more sustainable agricultural practices, with several guidelines being established within the European Union (Directive 2009/128/EC) which fosters the need to develop new approaches to reduce the use of these phytochemicals. On the past years, studies by our group focusing on the comparision between tolerant and susceptible grapevine genotypes, has highlighted several key components of the resistance mechanisms to pathogen challenge, namely associated to lipid metabolism and signaling events . With this Master thesis project, we aim at deepening our knowledge regarding the role of chloroplasts in the the defence mechanisms to downy mildew pathogen. Both molecular biology and biochemical approaches will be conducted namely fatty acid and lipid profiling (GC and TLC), expression analysis of known defense marker genes (qPCR). In parallel, gene families coding enzymes involved in plastidial lipid metabolism will be characterized in *Vitis vinifera* and the expression of some selected genes will also be assessed.

Techniques: Bioinformatic tools, Quantitative real time PCR, chloroplast isolation, Chloroplast isolation Gas and Thin layer Chromatography, Chloroplasts isolation,

Project Integration in BioISI strategic program: BioISI is a multidisciplinary research centre that selected grapevine research as a flagship project. This thesis project is fully integrated in the BioTech – Biotechnology line and will contribute for strengthening the BioISI's position in the agricultural and wine sector.

Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates.

Exploring the Hidden World of Microbes: Unravelling Microbial Communities in Complex Multi-dimensional Systems

Place of work: TecLabs – Ulisboa

Supervisors: Monica Nunes (msnunes@fc.ul.pt); Ricardo Dias (rpdias@fc.ul.pt)

The study of microbial communities in complex environmental samples has become an increasingly important field in microbiology and environmental science. These communities play critical roles in maintaining ecosystem functions and have significant impacts on human health and disease. However, characterizing these communities is challenging due to their vast diversity and complexity.

One of the most important steps in these studies is proper sample collection, concentration, and processing, to accurately characterize the microbial communities.

This study will focus on exploring the different techniques available for characterizing microbial communities in complex environmental samples. Different methods for collecting and preserving environmental samples, techniques for extracting and purifying microbial DNA, and various molecular methods for profiling microbial communities, including amplicon sequencing and metagenomics, will be explored. Bioinformatics tools and pipelines used for analysing and interpreting large-scale microbial community data, including different software packages for quality control, taxonomy assignment, and statistical analysis will also be addressed.

These techniques will be applied to analyse complex environmental samples, such as water, and air samples, and to characterize the diversity of microbial communities in different ecosystems. The project can be tailored to the student's specific interests and may involve collaborations with other researchers or institutions in the field if needed.

The development of this study will provide a systematic framework for understanding the functional relationships between microbial communities in complex environmental systems, advancing our knowledge of how these communities interact and contribute to ecosystem functioning.

ULisboa Master Programmes of interest:

- Master Programme in Microbiology
  (Coordinator Prof. Jorge Leitão - jorgeleitao@tecnico.ulisboa.pt)
- Master Programme in Molecular Biology and Genetics
  (Coordinator Prof. Francisco Dionísio -fadionisio@fc.ul.pt)
- Master Programme in Applied Microbiology
  (Coordinator Prof. Mónica Cunha - mscunha@ciencias.ulisboa.pt)

Information: Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship (BII), being the selection criterium the academic merit of the candidates.

Conversion of a tropical forest to agriculture – Effects on the diversity of soil microbial communities as revealed by a DNA metabarcoding approach

Place of work/: [Plant Functional Genomics Unit, FCUL]

Supervisors: Supervisor 1: Mónica Sebastiana (mgsebastiana@fc.ul.pt); Supervisor 2: Francisco Pina-Martins (f.pinamartins@gmail.com)

It is estimated that 25% of all biodiversity lives in the soil. Soil microorganisms contribute to a number of life-support functions, including maintaining of soil fertility essential for the production of food for human consumption. However, together with climate change, the increasing intensification of agriculture jeopardizes the functionality of soils, contributing to their degradation. Several studies point to a generalized loss of soil biodiversity with the intensification of agriculture, due to the use of methods such as tilling, use of chemical fertilizers and pesticides. Land-use change, specifically deforestation, is one of the most important drivers of change in soil biodiversity. However, its impact on soil microbial communities is still largely unknown. Increasing the knowledge on how microbial soil communities change following land conversion to agriculture can provide valuable information for soil management and assessment of the impact of deforestation of vulnerable ecosystems.

This study aims to evaluate how the conversion of a tropical native forest into a field for agricultural production impacts the taxonomic, phylogenetic, and functional diversity of the soil microbial community. The soils being analyzed were collected in Guiné-Bissau, at 3 locations with different land uses: a primary forest, an annual crop field (peanut) and a perennial crop field (cashew), during the wet and dry season. Specifically, we want to test how the soil microbiome responds to changes in land use, as well as to seasonal changes, on each soil type. To achieve this, we will use a DNA metabarcoding analyses targeting bacterial 16S rRNA and fungal ITS regions. Datasets from Illumina sequencing will be processed, clustered and annotated using software tools, such as QIIME [1], MicrobiomeAnalyst [2] or mothur [3]. Taxonomic identity will be performed searching against the SILVA database for prokaryotic operational taxonomic units (OTUs) and the

UNITE database for fungal OTUs. The FUNguild tool [4] will be used for functional assignment (e.g., saprotrophs, pathogens, and symbionts) of the fungal OTUs. Diversity estimates (OTU richness, Michaelis-Menten fit, Chao1, Shannon, Simpson) and rarefaction curves will be calculated, and statistical methods will be used to support any findings. The work benefits from an ongoing project with the Guinean Institutions financed by the FAO (Food and Agriculture Organization) of the United Nations and will increase soil biodiversity knowledge in this understudied region of the world.

[1] Caporaso J.G. et al. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336 (2010).

[2] Dhariwal A. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Research, 45, (2017).

[3] Schloss P.D. et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol., 75, 7537–7541 (2009).

[4] Nguyen, N. H. et al. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248 (2016)

"Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates."

From sea to vineyard: use of marine bacteria and seaweed extracts as biotools against Botrytis cinerea infection in grapevines.

Place of work/: GPS Lab (https://grapevinesyslab.rd.ciencias.ulisboa.pt) and BIOTOX Lab (https://biotoxlab.wixsite.com/home) (C2 building, 4th floor)

Supervisors: Doutor Bernardo Duarte (baduarte@fc.ul.pt), Professora Andreia Figueiredo (aafigueiredo@fc.ul.pt)

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops worldwide with over 7.4 million hectares of cultivated area. During the past decades, climate change brought novel challenges for grapevine production, not only through abiotic factors as extreme weather events (eg heatwaves) but also through biotic factors, namely the appearance of new diseases or a higher incidence of well-known diseases. In the last years we have been focused on understanding how grapevine cope with oomycete and fungi-associated diseases through multi-OMIC approaches. More recently we have been looking also to molecules that have either priming or biocide capabilities. On that sense, a novel approach is being exploited, based on marine-driven resources and their application in regenerative agriculture practices – a sea-to-vineyard approach.
With this project, we intend to test the application of either marine bacteria consortia (as priming agents) or invasive seaweed species extracts (as a source of secondary metabolites and biocide activity) in the control of grapevine grey mold disease caused by the fungus *Botrytis cinerea*. Priming or biocide capabilities will be evaluated through a combination of gene expression, biochemical and phenotyping approaches.

This project will be developed under the frame of the REVINE: *Regenerative agricultural approaches to improve ecosystem services in Mediterranean vineyards* project (https://www.revine-prima2020.org)

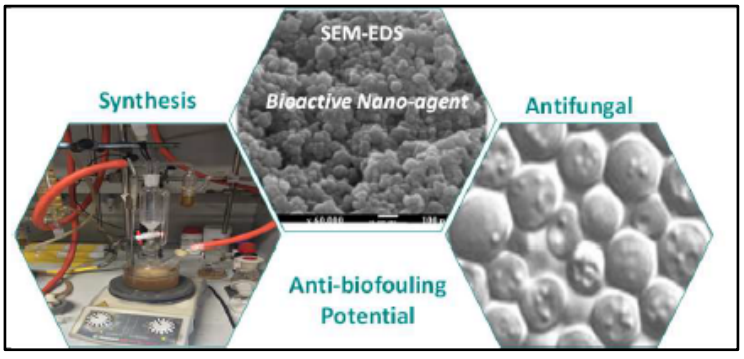
"Students selected for this project, after thesis registration, are eligible to apply to the BioISI Junior Programme (supporting 8 students with a 6-month Scholarship(BII), being the selection criterium the academic merit of the candidates."

Evaluation of the anti-biofouling potential of novel biocidal nano-agents in a biological model system

Place of work: BioISI, Lab Microbiology & Biotechnology Edificio Teclabs

Supervisors: Lisete Fernandes (lcfernandes@ciencias.ulisboa.pt); Elisabete Silva (ersilva@ciencias.ulisboa.pt)

The spontaneous colonization of microorganisms on surfaces, which promotes biofouling formation, is a global concern for all societal infrastructures, ranging from water service management systems (e.g., water distribution and treatment) to marine operations (e.g. transportation, offshore renewable energy systems).
Biofouling has serious consequences, including premature biocorrosion and waterborne biocontamination, which endangers industrial sustainability and public health.
Controlling biofouling through disinfection or prevention strategies is a critical process.
The most effective bio-decontamination strategies include the controlled release of bioactive compounds. However, these are frequently toxic and cumulative in the environment, resulting in a limited life cycle and ecological issues that entail new strategies, as well as a better understanding on the agent's bioactivity effects towards bio-foulants.
E. R. Silva's work develops new nanocomposite agents containing immobilized biocidal compounds, aiming to amplify their original bioactivity, and minimize the release of chemical compounds into aquatic systems when employed.
Exposure to environmental aggressions, stresses, is a universal phenomenon constantly affecting all living cells within a multicellular or unicellular organism context. Due to the knowledge built from extensive studies which includes 'omics as well as genetics manipulations, yeast *Sacharomyces cerevisiae* remains a reliable biological system to easy assess cellular impact of biocidal nano-agents.
Assess if nano-agents have biological impact on yeast will allow to ascertain nonspecific or side effects of the agents, as well as will better define/characterize its biological target activity.



Find out more!