Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Screening of compounds against malaria parasites

Synopsis: Malaria is caused by protozoan parasites of the genus *Plasmodium* and it is still one of the most prevalent infectious diseases in the world, affecting primarily children under the age of 5 years old. Despite the achievements in the treatment of malaria, there is still an urgent need for the discovery of new drugs that tackle infection by *Plasmodium*. The obligatory and clinically silent nature of the liver stage of the *Plasmodium* life cycle in the mammalian host, which precedes disease onset, makes it an appealing target for drug development.

This Master's Proposal will focus on the *in vitro* screening of compounds for their potential activity against the hepatic stage of infection by the rodent *P. berghei* parasite. Given the cost and time constraints associated with *de novo* drug development, the library of compounds to be tested may include drugs that are already approved for other purposes, an approach known as drug repositioning or repurposing, or compound libraries including novel chemotypes. Initially, compounds will be screened for their overall activity against *Plasmodium* liver stage by a well-established, bioluminescence-based, assay. Next, their activity will be further assessed by flow cytometry and fluorescence microscopy techniques to identify the specific steps of hepatic infection that are affected by the compound. Subsequently, the ability of selected compounds to block invasion of hepatocytes by the human malaria-causing parasite *P. falciparum* will also be assessed. Results obtained will inform the selection of hit compounds that may subsequently be tested *in vivo* in a rodent model of *Plasmodium* infection.

Overall, this project has the potential to identify new compounds that act against the liver stage of *Plasmodium* infection, which can contribute to the global effort of malaria eradication.

Supervisor: *Diana Fontinha, Post-doctoral Researcher, Miguel Prudêncio Lab; dfontinha@medicina.ulisboa.pt*
Co-Supervisor: *Miguel Prudêncio, Group Leader, Miguel Prudêncio Lab; mprudencio@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

Fontinha, D., Moules, I., & Prudêncio, M. (2020), "Repurposing Drugs to Fight Hepatic Malaria Parasites", *Molecules* (Basel, Switzerland), 25(15), 3409.

Prudencio, M.; Mota, M. M.; Mendes, A. M. (2011), "A toolbox to study liver stage malaria", *Trends in Parasitology*, *27* (12), 565-74.

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title:

Deciphering the bioenergetics signature of brain mitochondria

Synopsis:

Neurons are morphologically polarized cells and mitochondria have been observed in all neuronal sub-compartments. However, this distribution has been shown to be heterogeneous, with presynaptic and postsynaptic terminals containing more mitochondria than other neuronal domains. Additionally, these neuronal compartment-specific mitochondria appear to have different dynamics and morphological features, raising the question of whether these are functionally similar or actually have specialized functions adapted to the environment where they reside. Therefore, defining the intrinsic properties preferentially used by synaptic mitochondria to maintain their overall health is of particular relevance in the context of neuron function.

Mitochondria homeostasis is a process involving an intimate crosstalk between energy production, quality control and mitophagy. Perturbances of this intricate system are widely speculated to contribute to neurodegeneration. Our work focuses on elucidating these mitochondrial mechanisms crucial for brain function, and how a dysregulation in these processes can be fatal for the mitochondria itself or for the neuron.

To achieve this and in order to decipher the molecular mechanisms that regulate synaptic mitochondrial, this project will build on a previously obtained proteomic dataset from the host laboratory where protein abundance from synaptic mitochondria was directly compared to non-synaptic mitochondria. Bioinformatics analysis revealed several top candidate proteins significantly upregulated in the synaptic pool that are connected to mitochondrial bioenergetics, dynamics and turnover pathways.

Therefore, this research project aims to validate these top candidate proteins and to further understand their role within regulating synaptic mitochondria's unique fingerprint. This project will use mouse models and their derived primary neuronal cultures to perform cell biology, imaging, biochemical and bioenergetics approaches.

Supervisor:

Vanessa A. Morais
VMorais Lab
vmorais@medicina.ulisboa.pt

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Exploring the contribution of transposable elements for the pathogenesis of Rett syndrome

Synopsis:

This project aims at exploring an understudied mechanism that could contribute to the etiology of Rett syndrome. Rett syndrome (RTT) is a rare neurodevelopmental disorder caused by mutations of the MECP2 gene, located on the X chromosome. The lack of a fully functional MECP2 protein causes dysfunction of several parts of the central nervous system, affecting patients physically and intellectually. The MECP2 protein is able to bind methylated cytosines, which are usually found within the regulatory regions of genes and transposable elements. As such, MECP2 is believed to act as a transcriptional repressor in neuronal lineages. One known target of MECP2 are LINE-1 retrotransposons, the largest family of transposable elements (TEs) in mammalian genomes. Indeed, previous studies reported a higher activity of LINE-1 elements in animal models, in neurons derived from RTT human induced pluripotent stem cells (iPSCs) and in post-mortem brain samples from RTT patients. Given the importance of DNA methylation on the global silencing of TEs, it is reasonable to speculate that the influence of MECP2 on TEs might extend beyond the control of LINE-1 elements in RTT neuronal lineages; this however remains to be investigated. Moreover, whether the aberrant activity of LINE-1 is a driver or a bystander of the disease phenotype remains to be formally tested.

In this project, we will take advantage of iPSC cellular models for Rett syndrome, advanced genomic engineering and 2D neuronal differentiation to test the contribution of TEs for the pathogenesis of Rett syndrome. To address this question, we will use CRISPR-interference in RTT iPSCs to silence the expression of LINE-1 and other TEs found to be aberrantly expressed (through an ongoing bioinformatics analysis) in RTT samples. After differentiation in mature neurons, we will determine whether defects in the transcriptome and in neuronal morphology, maturation, activity and synaptic function can be rescued upon silencing of specific TE families.

Most current therapeutic approaches for RTT are centered on the re-establishment of adequate levels of functional MECP2 protein. In this project, we propose to focus on studying and correcting pathways that are downstream from MECP2. Given the growing number of studies focusing on the role of TEs in the brain, both in physiological and pathological contexts, it is likely that the misregulation of TEs impacts human pathogenesis more than previously thought. The set of approaches proposed here will allow to determine whether and how misregulated TEs impair neuronal function in RTT, which could lead to the identification of new therapeutic targets.

Supervisor: *Anne-Valerie Gendrel, Sergio de Almeida Lab, anne.gendrel@medicina.ulisboa.pt*
Webpage of the group

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Determine the impact of nuclear envelope rupture on cell homeostasis

Synopsis: The nuclear envelope (NE) protects the eukaryotic genome by providing a physical barrier between the chromatin and cytosol. The major components of the NE are the nuclear membranes, which are contiguous with the ER and each other, nuclear pore complexes, which regulate large molecule transport, and the nuclear lamina (NL). The genome contacts the NL in broad lamina-associated domains (LADs), which are believed to aid the spatial organization of chromosomes and contribute to transcription regulation[1].The maintenance of the nucleo-cytoplasmic barrier is essential to protect the chromatin from cytoplasmic enzymes such as nucleases and to prevent unprocessed pre-mRNAs to enter the cytoplasm for translation.
However, the movement of cells or the cell nucleus through confined spaces can cause mechanical stress that results in damage to the nuclear lamina and membrane which may lead to nuclear envelope rupture[2,3]. Our initial observations show in addition, that DNA damage and specifically exacerbated DNA double strand breaks can as well lead to disruption of the balance between the Lamin network and the attached chromatin and results in nuclear membrane blebbing and even rupture. A breach of the nuclear membrane exposes the DNA to cytoplasmatic proteins including DNases such as the three-prime repair exonuclease 1 (TREX1; also known as DNase III). A recent study demonstrated that TREX causes DNA damage, probably by entering the nucleus after nuclear membrane rupture events. The increased DNA damage then triggers downstream pathways which can increase tumor cell invasiveness[4].
Despite the recent progress in understanding the consequences of nuclear envelope rupture events, it is largely unclear how the exchange of molecules between the formerly separated nucleus and cytoplasm takes place and if an immediate damage response mechanism exists. We hypothesize that cells possess acute response mechanisms that act upon nuclear membrane rupture, to i.e., prevent excessive genomic DNA damage by nucleases.
In this project we aim to investigate the events that follow a rupture of the nuclear membrane and measure how the resulting exchange of molecules between the nucleus and cytoplasm takes place. We will use induced DNA damage in cultured cells to effect nuclear envelope rupture and use live cell fluorescence microscopy to determine the distribution and dynamics of molecules in the nucleus and cytoplasm following nuclear membrane breaches.

Supervisor: *Robert M. Martin, Sérgio de Almeida Lab, robertmartin@medicina.ulisboa.pt*
Co-Supervisor: *Sérgio de Almeida, Sérgio de Almeida Lab, sergioalmeida@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

1. Guerreiro, I. & Kind, J. Spatial chromatin organization and gene regulation at the nuclear lamina. *Curr. Opin. Genet. Dev.* **55**, 19 (2019).
2. Nader, G. P. de F. *et al.* Compromised nuclear envelope integrity drives TREX1-dependent DNA damage and tumor cell invasion. *Cell* **184**, 5230-5246.e22 (2021).
3. Denais, C. M. *et al.* Nuclear envelope rupture and repair during cancer cell migration. *Science* **352**, 353–358 (2016).
4. Gomes, E. R. & de Almeida, S. F. TREX1-dependent DNA damage links nuclear rupture to tumor cell invasion. *Dev. Cell* **56**, 3040–3041 (2021).

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Investigating R-loops as regulators of splicing

Synopsis:

R-loops are three stranded nucleic acid structures that form co-transcriptionally, when an RNA hybridizes with the complementary template DNA strand, forming a DNA/RNA hybrid and a displaced single stranded DNA (ssDNA)[1]. Although these structures are often seen as sources of genomic instability, R-loops are also emerging as powerful regulators of several processes, such as gene expression, class-switch recombination, and telomere stability. R-loops have both beneficial and deleterious roles. They serve as binding site for factors regulating chromatin states, epigenetic modifications, transcription initiation and termination, antibody diversification and DNA repair. However, conflicts between R-loop-stalled transcription complexes and replication forks are a potent source of DNA damage and threaten genome stability[2].

Splicing is the co-transcriptional removal of introns from the pre-messenger RNAs (pre-mRNAs) and is a versatile means of genetic regulation that is often disrupted in human diseases[3]. Surprisingly, R-loops have never been shown to directly impact or regulate splicing, even though most human genes are alternatively spliced, and R-loops may form genome wide in virtually any gene. Nevertheless, previous data raise the hypothesis that such a role may indeed exist. For instance, several splicing factors are known to interfere with R-loop formation, R-loops form frequently in introns and G/C-rich DNA sequences, known to favour R-loop formation are enriched upstream of weak 3' splice sites (3'SS) in non-canonical introns[4,5]. Our data indicate that R-loop formation juxtaposed upstream a weak 3' splice site increases the splicing of that intron. Altogether, these findings support our hypothesis that R-loops can directly interfere with splicing.

In this project we aim to investigate how R-loop formation affects splicing. To achieve this, we generated splicing reporter systems together with tools to efficiently remove R-loops which allows us to directly address the impact of R-loops on splicing. We will use molecular biology techniques to determine splicing efficiencies and fluorescence microscopy to observe our tools to remove R-loops in live cells.

Supervisor: *Robert M. Martin, Sérgio de Almeida Lab, robertmartin@medicina.ulisboa.pt*
Co-Supervisor: *Sérgio de Almeida, Sérgio de Almeida Lab, sergioalmeida@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

1. Santos-Pereira, J. M. & Aguilera, A. R loops: new modulators of genome dynamics and function. *Nat. Rev. Genet.* **16**, 583–597 (2015).
2. Crossley, M. P., Bocek, M. & Cimprich, K. A. R-Loops as Cellular Regulators and Genomic Threats. *Mol. Cell* **73**, 398–411 (2019).
3. Wang, E. & Aifantis, I. RNA Splicing and Cancer. *Trends in Cancer* vol. 6 631–644 (2020).
4. Sridhara, S. C. *et al.* Transcription Dynamics Prevent RNA-Mediated Genomic Instability through SRPK2-Dependent DDX23 Phosphorylation. *Cell Rep.* **18**, 334–343 (2017).
5. Murray, J. I., Voelker, R. B., Henscheid, K. L., Warf, M. B. & Berglund, J. A. Identification of motifs that function in the splicing of non-canonical introns. *Genome Biol.* **9**, R97 (2008).

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: High-throughput sequencing technologies to decipher co-transcriptional mRNA processing kinetics

Synopsis:

Long DNA molecules inside the nucleus of eukaryotic cells work as handbooks for all cellular behaviours, but they never act directly. Thus, DNA chemical information is transcribed into pre-mRNA molecules, which are then exported to cytoplasmatic ribosomes. However, it requires a preview intense maturation journey of pre-mRNA to mRNA, which comprises such mechanisms as splicing, capping or 3'end processing. The systematic failure of any RNA maturation processing mechanism leads to aberrant transcripts production, which is the reason for several diseases and birth deaths. Surprisingly, RNA processing occurs largely at the same time as transcription, laying on a complex regulatory system that allows a perfect interplay between RNA production and its maturation.

To dissect such a hot topic in the transcriptomic field, our laboratory jointly with Nicholas Proudfoot laboratory from the University of Oxford have recently developed a novel approach to successfully immunoprecipitate elongating RNA Polymerase II (Pol II), followed by the isolation of the intact nascent RNA bound to Pol II active site - POlymerase Intact Nascent Technology (POINT). During this master project, we propose to employ the POINT high-throughput sequencing technology to better understand the intercommunication between distinct RNA processing mechanisms, as well as their coordination with transcription.

We are seeking highly motivated students, with strong bioinformatic skills, biological background, critical thinking and open-minded.

Supervisor: *Maria Carmo-Fonseca, Maria Carmo-Fonseca Lab, carmo.fonseca@medicina.ulisboa.pt*
Co-Supervisor: *Rui Sousa-Luís, Maria Carmo-Fonseca Lab, rluis@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

Sousa-Luís, R., Dujardin, G., Zukher, I., Kimura, H., Carmo-Fonseca, M., Proudfoot, N. J., & Nojima, T. (2021). *POINT Technology illuminates the processing of polymerase-associated intact nascent transcripts.* Molecular Cell, 1935–1950. https://doi.org/10.1016/j.molcel.2021.02.034

Prudêncio P. Savisaar R. Rebelo K. Gonçalo Martinho R. and Carmo-Fonseca M. (2022) *Transcription and splicing dynamics during early Drosophila development* RNA http://www.rnajournal.org/cgi/doi/10.1261/rna.078933.121

Nojima, T., Gomes, T., Carmo-Fonseca, M., & Proudfoot, N. J. (2016). *Mammalian NET-seq analysis defines nascent RNA profiles and associated RNA processing genome-wide.* Nature Protocols, 11(3), 413–428. https://doi.org/10.1038/nprot.2016.012

Tammer L, Hameiri O, Keydar I, Roy VR, Ashkenazy-Titelman A, Custódio N, Sason I, Shayevitch R, Rodríguez-Vaello V, Rino J, Lev Maor G, Leader Y, Khair D, Aiden EL, Elkon R, Irimia M, Sharan R, Shav-Tal Y, Carmo-Fonseca M, Ast G. (2022). *Gene architecture directs splicing outcome in separate nuclear spatial regions.* Molecular Cell. https://doi.org/10.1016/j.molcel.2022.02.001

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Spatial organization of co-transcriptional pre-mRNA splicing

Synopsis:

Splicing of pre-mRNAs is a fundamental process for gene expression in eukaryotic cells and its mis-regulation is a hallmark of many human diseases. Understanding the processes involved in splicing regulation is essential to deciphering disease mechanisms and to developing new targeted treatment strategies.

The molecular mechanisms of the splicing reactions have been extensively characterized, and proteins that bind to the pre-mRNA (RNA-binding proteins, RBPs) are known to influence splicing decisions. However, the principles governing splicing regulation remain elusive. It has been proposed that Pol II assembles with splicing factors into local higher-order complexes that control the efficiency of the splicing process, but currently very little is known about the higher-order organization of pre-mRNA in the nucleus.

Recently, methods were developed to measure higher-order RNA and protein contacts within 3D structures. This project aims to explore the potential application of these methods to test the hypothesis that nascent transcripts are organized in 3D territories where splicing-related RBPs and their binding sites are highly enriched.

We are seeking highly motivated students, with strong background on biochemistry and molecular biology.

Supervisor: *Maria Carmo-Fonseca, Maria Carmo-Fonseca Lab, carmo.fonseca@medicina.ulisboa.pt*
Co-Supervisor: *Pedro Prudêncio, Maria Carmo-Fonseca Lab, pprudencio@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

Sousa-Luís, R., Dujardin, G., Zukher, I., Kimura, H., Carmo-Fonseca, M., Proudfoot, N. J., & Nojima, T. (2021). *POINT Technology illuminates the processing of polymerase-associated intact nascent transcripts.* Molecular Cell, 1935–1950. https://doi.org/10.1016/j.molcel.2021.02.034

Prudêncio P. Savisaar R. Rebelo K. Gonçalo Martinho R. and Carmo-Fonseca M. (2022) *Transcription and splicing dynamics during early Drosophila development* RNA http://www.rnajournal.org/cgi/doi/10.1261/rna.078933.121

Nojima, T., Gomes, T., Carmo-Fonseca, M., & Proudfoot, N. J. (2016). *Mammalian NET-seq analysis defines nascent RNA profiles and associated RNA processing genome-wide.* Nature Protocols, 11(3), 413–428. https://doi.org/10.1038/nprot.2016.012

Tammer L, Hameiri O, Keydar I, Roy VR, Ashkenazy-Titelman A, Custódio N, Sason I, Shayevitch R, Rodríguez-Vaello V, Rino J, Lev Maor G, Leader Y, Khair D, Aiden EL, Elkon R, Irimia M, Sharan R, Shav-Tal Y, Carmo-Fonseca M, Ast G. (2022). *Gene architecture directs splicing outcome in separate nuclear spatial regions.* Molecular Cell. https://doi.org/10.1016/j.molcel.2022.02.001

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Harnessing the potential of *Trypanosoma brucei*-derived compounds to tackle obesity

Synopsis: *Trypanosoma brucei* is an unicellular and extracellular protozoan parasite that causes sleeping sickness in humans and nagana in cattle. In the mouse model of infection, *T. brucei* colonizes the adipose tissue (AT) in disproportionately high numbers when compared to other organs[1,2]. During this colonization, the host experiences a marked loss of AT mass through a process dependent on adipocyte neutral lipolysis[3]. Interestingly, induction of adipocyte lipolysis is recapitulated *in vitro* using adipocyte-*T. brucei* co-cultures or following stimulating adipocytes with molecules secreted by the parasite. In this project, we aim to establish the feasibility of using *T. brucei*-derived compounds for the treatment of obesity, a major public health problem. To achieve this, the selected candidate will use a combination of biochemical and genetic tools to identify and isolate lipolysis-inducing compounds. Afterwards, the most promising compounds will be tested in preliminary pre-clinical safety and subsequent preliminary pre-clinical efficacy studies using appropriate rodent models.

Supervisor: *Luísa M. Figueiredo, Luisa Figueiredo Lab, lmf@medicina.ulisboa.pt*
Co-Supervisor: *Henrique Machado, Luisa Figueiredo Lab, henriquemachado@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

Trindade, S. et al. *Trypanosoma brucei* parasites occupy and functionally adapt to the adipose tissue in mice. Cell Host Microbe (2016). https://doi.org/10.1016/j.chom.2016.05.002

Silva Pereira, S. et al. Tissue tropism in parasitic diseases. Open Biology (2019). http://dx.doi.org/10.1098/rsob.190036.

Grabner, G.F. et al. Lipolysis: cellular mechanisms for lipid mobilization from fat stores. Nature Metabolism (2021). https://doi.org/10.1038/s42255-021-00493-6

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal 2022-2023

Title: The role of JAK-STAT signaling pathway in early arthritis

Synopsis:
Rheumatoid arthritis (RA) is a chronic, systemic immune-mediated inflammatory disease that can lead to joint destruction, functional disability and substantial comorbidity due to the involvement of multiple organs and systems. Despite the efficacy of synthetic and biologic disease modifying anti-rheumatic drugs (DMARDs) in the treatment of RA, few patients reach sustained remission and refractory disease is a concern that needs critical evaluation and close monitoring. Janus kinase (JAK) inhibitors or JAKi are a new class of oral medications recently approved for the treatment of RA. JAK inhibitors suppress the activity of one or more of the JAK family of tyrosine kinases [JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2)], thus interfering with the JAK-Signal Transducer and Activator of Transcription (STAT) signaling pathway. JAK-STAT signaling pathway is critical for immune cell proliferation, survival and differentiation and its inhibition leads to multi-cytokine blockade and abrogated inflammation. Tofacitinib is a JAK1 and JAK3 inhibitor, approved for the treatment of established refractory RA patients. However, preliminary clinical evidence indicate that patients treated in an earlier phase of the disease have a better response. Furthermore, our group has previously demonstrated that early treatment with tofacitinib in animal models of arthritis can abrogate disease and completely prevent bone and cartilage damage. Therefore, we hypothesize that JAK-STAT signaling pathway is key to chronic arthritis onset and its early inhibition might have a major effect on the immune cascade, allowing lasting disease control. The main goal of this study is to characterize the JAK-STAT signaling pathway activation in untreated early arthritis patients and to analyze the impact of conventional treatment on its activity. For that, blood samples will be collected from untreated early arthritis patients (<1 year of disease duration) followed up at the Rheumatology Department, Hospital de Santa Maria, Lisbon Academic Medical Centre, Portugal at baseline and after treatment with conventional DMARDs. At each time point, peripheral blood mononuclear cells (PBMC) will be isolated by density-gradient centrifugation, cell viability will be estimated with Trypan Blue dye exclusion and cells will be cryopreserved at -80ºC until use. After thawing frozen cells, the frequency, phenotype and JAK-STAT signaling pathway activation will be evaluated on peripheral blood leukocytes (B cells, T cells, monocytes and dendritic cells) by flow cytometry. In addition, a group of age and gender-matched healthy volunteers will be included as controls. All samples will be used for research purposes only. All the experimental work will be developed at Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Portugal.

Supervisor:
Rita Moura, PhD
João Eurico Fonseca Lab, Instituto de Medicina Molecular João Lobo Antunes
Faculdade de Medicina da Universidade de Lisboa
Edifício Egas Moniz, Av. Professor Egas Moniz, 1649-028 Lisboa
Email: ritaaguiarmoura@gmail.com; rmoura@medicina.ulisboa.pt

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: The effect of tofacitinib on peripheral blood leukocytes from early arthritis patients

Synopsis:

Rheumatoid arthritis (RA) is a chronic, systemic immune mediated disease that mainly affects joints, characterized by synovial inflammation, bone erosion and cartilage destruction. Disturbances in both innate and adaptive immune systems have been described in RA patients. Janus kinase (JAK) inhibitors or JAKi are a new class of oral medications recently approved for the treatment of RA. JAK inhibitors suppress the activity of one or more of the JAK family of tyrosine kinases [JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2)], thus interfering with the JAK-Signal Transducer and Activator of Transcription (STAT) signaling pathway. JAK-STAT signaling pathway is critical for immune cell proliferation, survival and differentiation and its inhibition leads to multi-cytokine blockade and abrogated inflammation. Tofacitinib, an oral JAK1 and JAK3 inhibitor, was recently approved for the treatment of RA patients who have had an inadequate response or intolerance to conventional synthetic or biologic disease modifying anti-rheumatic drugs (DMARDs). However, preliminary clinical evidence indicate that patients treated in an earlier phase of the disease have a better response. Furthermore, our group has previously demonstrated that early treatment with tofacitinib in animal models of arthritis can abrogate disease and completely prevent bone and cartilage damage. Therefore, we hypothesize that JAK-STAT signaling pathway is key to chronic arthritis onset and its early inhibition with tofacitinib might have a major effect on the immune cascade, allowing lasting disease control. The main goal of this study is to analyze the in vitro effect of tofacitinib on peripheral blood leukocytes (B cells, T cells, monocytes and dendritic cells) from untreated early arthritis patients. For that, blood samples will be collected from untreated early arthritis patients (<1 year of disease duration) followed up at the Rheumatology Department, Hospital de Santa Maria, Lisbon Academic Medical Centre, Portugal. A group of age and gender-matched healthy volunteers will be also included as controls. Peripheral blood mononuclear cells (PBMC) will be isolated by density-gradient centrifugation and cell viability will be estimated with Trypan Blue dye exclusion. Cells will be cultured during 24h-72 hours at 37ºC, 5% CO2 in complete medium, in the presence or in the absence of tofacitinib and appropriate cell stimuli. After culture, cells will be collected and JAK-STAT signaling pathway activation will be evaluated on peripheral blood leukocytes (B cells, T cells, monocytes and dendritic cells) by flow cytometry. In addition, supernatants will be harvested and stored at -80ºC to analyze antibody and cytokine production by enzyme-linked immunosorbent assay (ELISA) and/ or multiplex assay. All samples will be used for research purposes only. All the experimental work will be developed at Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Portugal.

Supervisor:

Rita Moura, PhD
João Eurico Fonseca Lab, Instituto de Medicina Molecular João Lobo Antunes
Faculdade de Medicina da Universidade de Lisboa
Edifício Egas Moniz, Av. Professor Egas Moniz, 1649-028 Lisboa
Email: ritaaguiarmoura@gmail.com; rmoura@medicina.ulisboa.pt

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Immune regulation of Stem Cell Aging

Synopsis:

The consequences of aging on human health are broadly apparent, yet the causes and drivers of the aging process are just beginning to be understood. Adult stem cells are major regulators of organismal homeostasis, sustaining tissue renewal and repair throughout life. However, knowledge regarding what drives stem cell aging and how this contributes to tissue deterioration is just starting to emerge. Our lab aims to understand the process of stem cell aging, with the goal of developing new therapeutic solutions to slow degenerative changes in organs and design stem cell-based therapies for age-related diseases.

The skeletal muscle is a paradigmatic model to study age-related loss of repair capacity. Skeletal muscle regeneration is sustained by a population of adult resident stem cells, termed muscle stem cells (MuSCs). During ageing, muscle regenerative capacity is lost due to MuSC impaired activity. Aged MuSCs present multiple functional defects, including an increased propensity to convert into alternative cellular lineages. Some environmental drivers of impaired MuSC function have been defined in recent years. However, what drives MuSC alternative lineage commitment with age is still unknown. Yet, such divergence in cellular identity is likely to have a major impact on muscle functional decline with age.

The immune system, a central player in the regulation of skeletal muscle repair, is also affected by aging, with consequences for MuSC function. Ongoing studies in our lab uncovered defects in immune signaling in the aged regenerating muscle. We were able to recapitulate these age-related immune defects in mouse models of immune dysfunction. Importantly, we found that changes in immune signaling during the regenerative process were sufficient to cause impairments in MuSCs function and lineage commitment. This work led to the novel hypothesis that dysregulated immune signaling plays a central role in the loss of MuSC function and lineage identity in ageing. We propose to understand how immune dysfunction drives MuSC functional decline through a detailed characterization of age-related changes in immune populations and signaling events associated with muscle repair, and evaluation of the causal nature of those changes over MuSC intrinsic defects.

Supervisor: *Pedro Sousa-Victor, NevesVictor Lab, psvictor@medicina.ulisboa.pt*
Co-Supervisor: *Joana Neves, NevesVictor Lab, joana.neves@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

P. Sousa-Victor, L. Garcia-Prat, P. Munoz-Canoves, Control of satellite cell function in muscle regeneration and its disruption in ageing. *Nature Reviews Molecular Cell Biology* 23, 204 (Mar, 2022).

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Mechanisms of MANF-mediated immune modulation in tissue repair

Synopsis:

Regenerative medicine is emerging as a strategy for tissue rejuvenation, holding the potential to improve human health by delaying age-related disease. Nevertheless, the efficacy of such therapeutic approaches is compromised by the inefficient repair capacity of old, degenerating tissues. Age-associated dysregulation of inflammatory signaling is an important roadblock for the success of regenerative therapies, and immune modulatory interventions that reestablish a regulated inflammatory response can be effective strategies to promote regenerative success in aging. Macrophages, as important integrators of inflammatory signaling and regulators of tissue homeostasis, are key candidates to mediate the effects of immune modulatory mechanisms during tissue repair.

The skeletal muscle has been extensively used as a model system to understand the age-related decline in tissue repair capacity, providing important insight into the role of macrophages in coordinating the inflammatory response during regeneration, and on the limitations imposed by chronic inflammation and the aged immune system to efficient muscle repair. However, we have an incomplete understanding of the cellular processes and molecular effectors that ensure the maintenance of a regulated inflammatory response during a repair event, and how their dysregulation contributes to the observed chronic activation of inflammatory signaling during aging. Highlighting the utility of macrophage-based strategies in regenerative medicine, recent studies have shown that interventions that harness the repair potential of macrophages can improve muscle regenerative capacity.

Our recent work uncovered an essential role for the immune modulator mesencephalic astrocyte-derived neurotrophic factor (MANF) in the skeletal muscle regenerative process. We found that MANF-deficiency leads to a dysregulated immune response during muscle repair and structural alterations in the macrophage population. We observed similar alterations in aged animals. We now aim to understand how MANF-mediated immune modulation controls macrophage function during tissue repair and how this mechanism is disrupted during aging.

Supervisor: *Joana Neves, NevesVictor Lab, joana.neves@medicina.ulisboa.pt*
Co-Supervisor: *Pedro Sousa-Victor, NevesVictor Lab, psvictor@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

Neves, J., Zhu, J., Sousa-Victor, P., Konjikusic, M., Riley, R., Chew, S., Qi, Y., Jasper, H., and Lamba, D.A. (2016). Immune modulation by MANF promotes tissue repair and regenerative success in the retina. Science 353, aaf3646.

Sousa-Victor, P., Neves, J., Cedron-Craft, W., Ventura, P.B., Liao, C.Y., Riley, R.R., Soifer, I., van Bruggen, N., Kolumam, G.A., Villeda, S.A., et al. (2019). MANF regulates metabolic and immune homeostasis in ageing and protects against liver damage. Nature metabolism 1, 276-290.

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Onco-immuno-microbial profiling of the breast tumour microenvironment for therapeutic targeting

Synopsis:

Breast cancer (BC) is the most commonly diagnosed cancer type and the leading cause of cancer-related death in women worldwide. The tumour microenvironment (TME) is now recognized as an active player in cancer progression and response to treatments. A multi-dimensional network of non-malignant cells populate the TME, including fibroblasts, adipose and immune cells, namely T cells and macrophages. Evidence suggests that immune cells (ICs) play an ambiguous role in regulating both protumour and antitumor immune responses in BC. Strikingly, a pan-cancer study found that BC has a particularly rich and diverse microbiome compared to other cancer types [1]. This suggests that intra-tumoural bacteria exist, are active, and more provocatively that they may play key roles in cancer pathogenesis and response to therapy. Indeed, some of these tumour-associated bacteria can activate immune responses against tumours, while others produce enzymes able to hamper chemotherapy, helping cancer cells escape from the immune system [2]. Nevertheless, the complex interaction between microbiota, the immune system and malignant cells is still poorly described, as are the mechanisms by which they modulate immunity and therapy response. Thus, a more comprehensive understanding of these mechanisms will be a step forward to discover novel targets in BC treatment and prevention. We hypothesize that the microbiome interacting with tumour cells as well as the host's immune system may be a key determinant *in situ*, constituting an independent component of the TME.

We therefore propose a computational biology MSc project aimed at deciphering new rules illustrating the interaction between BC cells and their TME components, namely ICs and tumour-associated microbiota, as they have been found to independently act on tumour progression, response to therapy, and patient survival. The student will, for instance, mine and analyse publicly available and clinically annotated BC whole-genome and RNA sequencing datasets and participate in the development of a computational framework to characterise the tumour-associated microbiota. Using dimensionality reduction combined with machine learning methods, novel associations between specific bacteria species, immune silent or active TME, and unfavourable or favourable clinical outcome in the various BC subtypes will be uncovered. By dissecting the crosstalk between those different layers, this project will unravel novel molecular signatures of BC progression and provide novel insights with implications for BC prognosis and personalized therapy.

This project will be integrated in the iMM-Laço Hub initiatives and benefit from the collaborative and interdisciplinary environment of iMM and host teams. Observations from computational analyses of omics data will be subsequently validated in diagnostic biopsies and surgical samples from an independent local cohort of BC patients. Moreover, this project will trigger hypotheses about the tripartite onco-immuno-microbial crosstalk in BC that will be functionally tested *in vitro* and *in vivo*.

Supervisor: *Paulo Caldas, Nuno Morais Lab and iMM-Laço Hub, paulocaldas90@gmail.com*
Co-Supervisors: *Nuno Morais, Nuno Morais Lab and iMM-Laço Hub, nmorais@medicina.ulisboa.pt*
Karine Serre, Bruno Silva-Santos Lab and iMM-Laço Hub, karineserre@medicina.ulisboa.pt
Webpage of the group

Instituto de Medicina Molecular João Lobo Antunes

http://imm.medicina.ulisboa.pt/group/distrans/
https://laco.imm.medicina.ulisboa.pt/

Bibliography:

[1] Nejman, D. *et al.* The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science* **368,** 973–980 (2020).

[2] Geller, L. T. *et al.* Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science* **1160,** 1156–1160 (2017).

Proposal for Masters Project 2022-2023
Supervisor: Ângelo Ferreira Chora, Maria Mota lab, iMM-JLA

Mechanisms of γδ T cell activation during the hepatic stage of *Plasmodium* infection

The co-evolution of *Plasmodium spp*., the etiological agents of malaria, and humans over thousands of years has established an intricate equilibrium between the immunity of the host and parasitic virulence factors, guaranteeing maximal parasite transmission with limited host damage. A major factor contributing for this "quid pro quo" is the development of naturally acquired immunity (NAI).

The nature of NAI is, most certainly, multifactorial. Although cumulative exposure to natural *Plasmodium* infections results in decreased levels of circulating parasites, the major readout of acquired immunity to malaria is protection from severe disease and death. While the establishment of such protective responses has been extensively explored, no single molecular signature, key cellular determinant, or immune mechanism has been causally associated with clinical protection from severe malarial disease[1].

We have developed a rodent model of infection that recapitulates the main features of NAI, i.e., decreased parasite burden and protection from severe pathology, echoing what is observed in clinically-asymptomatic humans living in high- to holoendemic malaria regions[2]. This experimental infection system allows for the uncoupling of the two distinct development stages of *Plasmodium* within the mammalian host, while controlling for the initial blood-stage (BS) parasite inoculum. It also bypasses the mosquito stage of *Plasmodium* development, which has been suggested to alter parasite virulence[3]. In accordance, C57BL/6 mice pre-exposure to a single bolus of non-productive, irradiated hepatotropic parasites (sporozoites; spz), followed 2 days later, by a BS infection initiated by the transfusion of PbANKA-infected red blood cells (iRBCs) are fully protected from severe malarial disease. This is in sharp contrast to control mice receiving the same inoculum of iRBCs 2 days after a hepatic mock infection (equivalent amount of uninfected mosquitoes' salivary gland material) that succumbed all from experimental cerebral malaria (ECM). This rodent neurological syndrome largely recapitulates the main features of severe neuropathology following *Plasmodium* infection in humans, and is typically associated with significant intravascular accumulation of mononuclear cells, intracerebral haemorrhage, enhanced blood-brain barrier permeability and oedema[4,5].

Until now, we were able to establish that the cellular basis for the protection against ECM afforded by exposure to spz prior to a BS infection relies on the activation of a subset of T lymphocytes harbouring the gamma (γ) and delta (δ) chains of the T cell receptor, i.e., γδ T cells. In fact, while wild-type (WT) C57BL/6 mice pre-exposed to irradiated spz, followed 2 days later, by a BS infection are fully protected from ECM establishment, C57BL/6 mice with genetic depletion of γδ

T cells (TCR$\delta^{-/-}$ mice) succumb to ECM in the same experimental setting. Production of IL-17 by $\gamma\delta$ T cells associates with increased extra-medullary erythropoiesis and concomitant reticulocytosis throughout infection, thus impacting on parasite virulence and the concomitant establishment of ECM.

We now aim at identifying (and characterizing) the signals generated by spz during the hepatic stage of infection leading to the activation of hepatic (and potentially, of non-hepatic) $\gamma\delta$ T cells. We will do so by using a library of genetically-modified parasite strains to functionally identify parasite-derived processes (and molecules) relevant for protection from ECM upon spz exposure followed by a BS infection. For this, C57BL/6 mice exposed to spz from each parasite line will receive a BS infection and infection progression and ECM establishment will be assessed. In parallel, analysis of hepatic $\gamma\delta$ T cell activation (CD44 expression) and functional specification (cytokine production) will be assessed by flow cytometry at relevant time points.

We then intend to disclose the molecular signature(s) of $\gamma\delta$ T cell activation following exposure to *Plasmodium* spz. For this, hepatic $\gamma\delta$ T cells (and other liver-resident and infiltrating leukocytes) will be isolated from the liver of C57BL/6 mice receiving spz, or from non-infected mice, at defined time points, at a single cell level. Single cells will be sorted into separate wells of a 384-well plate, with 5 plates per cell population per mouse. We will apply the MARS-seq protocol[6] to obtain the single cell transcriptomes of thousands of single cells of each type and the data obtained will be analyzed using the Seurat software[7]. Cells will be clustered in an unbiased manner and differential gene expression will be performed between the transcriptomes of the different cell types isolated from spz-exposed, or non-exposed, hosts. Time allowing, we will proceed by functionally validating candidate molecules and/or molecular pathways disclosed by our unbiased approach.

Trainee Goals:

During the development of the experimental work in the laboratory, the student is expected to:

- take part in all common activities in the laboratory, engaging and interacting with other team members and understand, present and scientifically discuss the data obtained in periodical presentations during laboratory meetings;
- acquire the basic knowledge on the techniques required for malarial infection establishment and follow-up in the rodent model, including isolation of both parasitized RBC and hepatocyte-infectious parasites (spz) from mosquitoes salivary glands; isolation of and determination of parasite load both during the liver and blood stages of infection;
- learn basic techniques of animal experimentation including handling and restraining; maintenance of mouse colonies necessary for the development of the project by genotyping of mice offspring and layout of husbandry schemes; administration of substances by intraperitoneal and/or intravenous routes and in vivo or post-mortem collection of biological samples;

- learn laboratory techniques including: isolation of tissue immune and non-immune cell subsets; flow-cytometric acquisition and analysis of isolated cells; fluorescence-activated single cell sorting; basic analysis of transcriptomic large data sets; cell culture techniques and; gene expression quantification by real time quantitative PCR.

Bibliography

1. Langhorne, J., Ndungu, F. M., Sponaas, A. M. & Marsh, K. Immunity to malaria: More questions than answers. *Nature Immunology* **9,** 725–732 (2008).
2. Lindblade, K. A., Steinhardt, L., Samuels, A., Kachur, S. P. & Slutsker, L. The silent threat: Asymptomatic parasitemia and malaria transmission. *Expert Review of Anti-Infective Therapy* **11,** 623–639 (2013).
3. Spence, P. J. *et al.* Vector transmission regulates immune control of Plasmodium virulence. *Nature* **498,** 228–231 (2013).
4. Langhorne, J. *et al.* The relevance of non-human primate and rodent malaria models for humans. *Malaria Journal* **10,** (2011).
5. Dorovini-Zis, K. *et al.* The neuropathology of fatal cerebral malaria in Malawian children. *Am. J. Pathol.* **178,** 2146–2158 (2011).
6. Brette, F. *et al.* Massively Parallel Single-Cell RNA-seq for marker-free decomposition of tissue into cell types. *Science (80-.).* (2014). doi:10.1126/science.1242747
7. Butler, A., Hoffman, P., Smibert, P., Papalexi, E. & Satija, R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat. Biotechnol.* **36,** 411–420 (2018).

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Studying the nuclear envelope during human healthy aging – a biophysics approach

Synopsis: Aging acts as a major risk factor for many human pathologies, including cancer, neurodegenerative disorders, diabetes and cardiovascular diseases. Nonetheless, information on the determinants of biological deterioration is still required if we aim at providing a better quality of life for aging individuals. In our Lab, we are focused on understanding why human cell nuclei tend to lose structural integrity as age progresses, ultimately leading to functional impairment. In other words, we aim at explaining why aged individuals are more prone to certain diseases and how does it relate with the loss of nuclear structural and mechanical properties.

In mammalian cells, the nuclear structural integrity is thought to be safeguarded by the nuclear envelope (NE), in particular the nuclear lamina (NL). The NL is mainly composed of lamin proteins that assemble into filaments and bind both heterochromatin and the inner nuclear membrane. However, the role of specific NE lipids and lipid-lamin interactions in the nuclear compartment has been mainly disregarded, mostly due to the complexity of the double lipid bilayer. In this project, we aim at directly circumventing these issues by carefully designing simplified model systems with lipid features characteristic of the NE at different stages of healthy age progression. We have started by identifying the age-related lipid composition of the nuclear envelope by mass spectrometry (lipidomics). Now, the next step is to **design and characterize membrane models mimicking this age-tuned lipid composition** (with the possibility of adding lamin proteins for a more physiological approach).

Briefly, small, large and giant unilamellar vesicles will be prepared with age-specific lipid compositions matching the lipidomics results. The biophysical characterization of the models will then be accomplished by a multitude of techniques, including fluorescence spectroscopy and microscopy, dynamic light scattering (DLS) and atomic force microscopy (AFM)-based approaches. Upon characterization, the preparation of more complex systems including lamin proteins can be attempted.

Ultimately, this project will allow for the first time the implementation of a free-standing nucleus-sized vesicle system with age-tuned lipid composition (and possibly lamin proteins), **creating a novel platform to study nuclear aging *in vitro***.

Supervisor: *Maria João Sarmento, Nuno Santos Lab, maria.sarmento@medicina.ulisboa.pt*
Co-Supervisor: *Nuno Santos, Nuno Santos Lab, nsantos@medicina.ulisboa.pt*
Webpage of the group

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Harnessing the biotechnological potential of microbial pigments to develop antimicrobial solutions against multidrug resistant human pathogens

Synopsis:

Microbial pigments are colored secondary metabolites of diverse chemical classes (e.g., carotenoids, pyrroles, phenazines, flavonoids, melanins), which play important roles in the physiology and competitiveness of microbial cells and populations, by being involved in iron uptake and growth inhibition of other microbial species, protection against UV radiation and oxidative damage, signaling cascades modulating gene expression, and in microbial pathogenesis [1]. In particular, the pigments violacein and prodigiosin have been extensively studied by their versatile biomedical potential, including antimicrobial activity against a broad range of Gram-negative and Gram-positive bacteria, yeasts, fungal and viral human pathogens [2].

The urgent global need to develop groundbreaking antimicrobial solutions has also prompted the biotechnological prospection of the microbial biomolecular catalogue as a source of novel bioactive compounds, against pathogens associated to the multidrug resistant trait (e.g., strains of *Pseudomonas aeruginosa, Enterobacter* spp., *Klebsiella* spp*., Staphylococcus aureus, Streptococcus* spp., *Candida* spp.) [3]. The combination of microbial pigments with drug delivery systems, such as metal nanoparticles (MNPs), may be fundamental for their clinical implementation [4,5], although their development and characterization has not been systematically explored. Furthermore, the functionalization of MNPs (e.g., silver nanoparticles) can be modulated to improve the stability of the conjugant-MNP complex, low toxicity/immunogenicity and affinity for the target, resulting in efficient antimicrobial agents, which implicates reduced health-care costs [6].

Our group at iMM has a solid record studying the molecular mechanism of action of antimicrobial compounds (e.g., peptides and peptide-based nanosystems), against bacterial, fungal and viral pathogens, and in the development of bio- and nano-based antimicrobial solutions for biomedical application. Moreover, we hold a library of pigment-producing bacterial and fungal strains, which are being characterized regarding the pigment production process and the respective biotechnological potential.

The work plan will be carried out at iMM, in the scope of ongoing projects of the group, mainly focused on the biomedical properties of violacein and prodigiosin, envisaging:

(i) the production of the bacterial pigments in liquid cultures and evaluation of the effect of abiotic factors in the production yield (e.g., type of carbon and nitrogen sources, temperature, pH, micronutrients);

(ii) the extraction, purification and quantification of the bacterial pigments produced in the optimized culture conditions;

(iii) characterization of the purified pigments by spectroscopic methodologies and relevant biochemical methods;

(iv) evaluation of the antimicrobial properties of the pigments, as purified fractions and in MNP-pigment conjugants, against major human pathogens;

(v) revision of the state-of-art regarding the application of the studied bacterial pigments and thesis writing.

Students who are interested in the proposal must meet with both supervisors before selecting this Masters project. The work plan may be further shaped (as much as possible) to the applicant's profile.

Supervisor: *Pedro Castro, Nuno Santos Lab, pedrocastro@medicina.ulisboa.pt*
Co-Supervisor: *Sónia Gonçalves Abreu, Nuno Santos Lab, sabreu@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

[1] Venil, C. K., Zakaria, Z. A., & Ahmad, W. A. (2013). Bacterial pigments and their applications. Process Biochemistry, 48(7), 1065-1079. doi: 10.1016/j.procbio.2013.06.006;
[2] Choi, S. Y., Lim, S., Yoon, K. H., Lee, J. I., & Mitchell, R. J. (2021). Biotechnological activities and applications of bacterial pigments violacein and prodigiosin. Journal of Biological Engineering, 15(1), 1-16. doi: 10.1186/s13036-021-00262-9;
[3] Miethke, M., Pieroni, M., Weber, T., Brönstrup, M., Hammann, P., Halby, L., et al. (2021). Towards the sustainable discovery and development of new antibiotics. Nat. Rev. Chem. 5(10), 726-749. doi:10.1038/s41570-021-00313-1;
[4] Arif, S., Batool, A., Khalid, N., Ahmed, I., & Janjua, H. A. (2017). Comparative analysis of stability and biological activities of violacein and starch capped silver nanoparticles. *RSC advances*, *7*(8), 4468-4478. doi: 10.1039/C6RA25806A;
[5] El-Batal, A. I., El-Hendawy, H. H., & Faraag, A. H. (2017). In silico and in vitro cytotoxic effect of prodigiosin-conjugated silver nanoparticles on liver cancer cells (HepG2). Journal of Biotechnology Computational Biology and Bionanotechnology, 98(3). doi: 10.5114/bta.2017.70801;
[6] Makowski, M., Silva, Í. C., Pais do Amaral, C., Gonçalves, S., & Santos, N. C. (2019). Advances in lipid and metal nanoparticles for antimicrobial peptide delivery. Pharmaceutics, 11(11), 588.

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Optimizing the production of natural microbial pigments for the sustainable dyeing of textile materials

Synopsis:

Microbial pigments are colored secondary metabolites of diverse chemical classes (e.g., carotenoids, pyrroles, phenazines, flavonoids, melanins), which play important roles in the physiology and competitiveness of microbial cells and populations, by being involved in iron uptake and growth inhibition of other microbial species, protection against UV radiation and oxidative damage, signaling cascades modulating gene expression, and in microbial pathogenesis [1]. Having in mind these diverse roles, several microbial pigments have been extensively studied as novel bioactive molecules, towards biomedical (e.g., with antimicrobial, antioxidant and anticancer activities) as well as industrial applications (e.g., natural food additives and textile colorants) [2]. Pigment-producing microorganisms are mostly preferred over plant-based pigment sources, due to their fast and cheap cultivation processes, simple pigment extraction protocols associated to higher yields, easier genetic manipulation, among other advantages [1].

The textile industry is the 2nd most polluting industry worldwide, and accounts for 20% of the chemical pollution of water resources, mainly derived from the textile dyeing processes. The study of microbial pigments as ecological substitutes of the conventional synthetic dyes is thus an emerging and promising field of research [3,4]. Our group is a member of the BIOCOLOUR consortium, which aims at the sustainable dyeing of textiles based on microbial pigments. In the scope of this project, we hold a library of bacterial and fungal strains producing different pigments, which are being characterized regarding the production process and their respective biotechnological potential.

The work plan will be carried out at iMM, mainly envisaging:

(i) the systematic characterization of the pigment production process in liquid cultures and evaluation of the effect of abiotic factors in the production yield (e.g., type of carbon and nitrogen sources, temperature, pH, micronutrients), by using molecular, biochemical and phenotypic approaches;

(ii) the extraction, purification and quantification of the bacterial pigments produced in the optimized culture conditions;

(iii) the characterization of the purified pigments by spectroscopic methodologies and relevant biochemical methods, towards their application for dyeing textile fibers.

Students who are interested in the proposal must meet with both supervisors before selecting this Masters project. The work plan may be further shaped (as much as possible) to the applicant's profile.

Supervisor: *Pedro Castro, Nuno Santos Lab, pedrocastro@medicina.ulisboa.pt*
Co-Supervisor: *Sónia Gonçalves Abreu, Nuno Santos Lab, sabreu@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

[1] Venil et al. 2014. Current perspective on bacterial pigments: emerging sustainable compounds with coloring and biological properties for the industry–an incisive evaluation. RSC Advances, 4(74), 39523-39529. doi: 10.1039/C4RA06162D

[2] Narsing Rao et al. 2017. Fungal and bacterial pigments: secondary metabolites with wide applications. Frontiers in microbiology, 8, 1113. doi: 10.3389/fmicb.2017.01113

[3] Wang, F., Gong, J., Ren, Y., & Zhang, J. (2018). Eco-dyeing with biocolourant based on natural compounds. Royal Society Open Science, 5(1), 171134. doi: 10.1098/rsos.171134;

[4] Panesar, R., Kaur, S., & Panesar, P. S. (2015). Production of microbial pigments utilizing agro-industrial waste: a review. Current Opinion in Food Science, 1, 70-76. doi: 10.1016/j.cofs.2014.12.002

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: The interaction between pollution and other environmental measures on systemic lupus erythematosus

Synopsis:

Systemic Lupus Erythematosus is a chronic immune-mediated disease, associated with high morbidity and mortality. In women 15-24 YO lupus is the 7th cause of non-traumatic death, even more than sepsis, HIV or diabetes (1). It is well known that our genetic background determines the risk of developing this disease and shapes its manifestations, but other factors certainly contribute, including epigenetics. Interestingly, geographic factors are clearly important, since lupus is more frequent in urban than in rural areas (2,3). Another factor that affects disease activity is sun exposure. Patients are more frequently diagnosed in the summer and have more flares in this season. UV light exposure increases apoptosis and facilitates exposure of self-antigens, which may be recognized by stochastically generated auto-reactive immune cells. Air pollution contributes to the depletion of the ozone layer, which is crucial to absorb UVC radiation and a large amount of UVB radiation. We definitely need to know more between the connection between the environment and lupus.

We gathered a team of rheumatologists, immunologists, neuroscientists, bioinformatics and geographers to explore why the place where we live determines lupus activity.

We will use the database Reuma.pt to select patients with Systemic Lupus Erythematosus and we will analyze where they live, work and spend their leisure time. We will study the environment characteristics, namely the exposure to the air pollution – more specifically ambient particulate matter (PM) with several aerodynamic diameters (≤2.5μm, $PM_{2.5}$; ≤10μm, PM_{10}), as well as some outdoor comfort measures (temperature, solar radiation and humidity). Disease activity will be evaluated using a validated clinical scale (SLEDAI-2k). The number of relapses and the treatment strategies used will be studied. The clinicians will collect all the demographic and clinical variables.

The specific tasks that the master student will develop include statistical analysis attempting to establishing links between the environmental data with the clinical data. It will also involve organization of the database to accommodate both types of data (clinical and nonclinical). For the environmental data, we will take advantage of the collaboration between the team members with the "Instituto de Geografia e Ordenamento do Território – Universidade de Lisboa".

Our goal is to better understand how the environment shapes lupus activity in order to create strong foundations to better control this disease.

Supervisor: *Bruno Miranda M.D. Ph.D.*, *bruno.miranda@campus.ul.pt*
Co-Supervisor: *Patrícia Costa Reis M.D. Ph.D., João Eurico Fonseca Lab, pcr.patricia@gmail.com*
Webpage of the group

Instituto de Medicina Molecular João Lobo Antunes

Bibliography:

1. Yen EY, Singh RR. Brief Report: Lupus-An Unrecognized Leading Cause of Death in Young Females: A Population-Based Study Using Nationwide Death Certificates, 2000-2015. Arthritis Rheumatol. 2018 Aug;70(8):1251-1255. doi: 10.1002/art.40512. Epub 2018 Jun 27. PMID: 29671279; PMCID: PMC6105528.
2. Chakravarty EF, Bush TM, Manzi S, Clarke AE, Ward MM. Prevalence of adult systemic lupus erythematosus in California and Pennsylvania in 2000: estimates obtained using hospitalization data. Arthritis Rheum. 2007 Jun;56(6):2092–4.
3. Barnabe C, Joseph L, Belisle P, Labrecque J, Edworthy S, Barr SG, et al. Prevalence of systemic lupus erythematosus and systemic sclerosis in the First Nations population of Alberta, Canada. Arthritis Care Res. 2012 Jan;64(1):138–43.

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Título: Combater vírus e/ou bactérias usando fármacos seletivos para membranas

Resumo:

O estágio pode incidir sobre uma das seguintes duas temáticas, a combinar com o(s) futuro(s) estagiário(s):
1- Vários vírus estão envoltos por uma membrana de lípidos, formada a partir da membrana da célula hospedeira. Vírus como o SARS-CoV-2, HIV, Dengue, Ébola ou Zika são deste tipo. Apesar dos esforços, ainda não foi possível desenvolver uma cura ou vacina para nenhum deles. No entanto, alguns tratamentos disponíveis têm sido bem-sucedidos em reduzir tanto a mortalidade como a morbilidade associados à infeção por alguns destes vírus. Os inibidores de entrada do HIV-1 são uma nova classe de compostos antivirais com provas dadas e grande potencial de desenvolvimento, por exemplo. Estes inibidores atuam no meio extracelular, em diferentes passos do processo de entrada do vírus, impedindo a sua entrada nas células alvo. É possível aplicar estratégias já testadas em alguns destes vírus, nomeadamente com péptidos, para desenvolver moléculas antivirais contra vírus congéneres.
2- Alguns péptidos têm propriedades antibacterianas, atacando seletivamente a membrana de bactérias. Uma vez que o alvo destes antibióticos é a estrutura da membrana, o desenvolvimento de resistência por parte das bactérias é extremamente difícil. Por esta razão, o desenvolvimento de novos antibióticos deste tipo é considerado uma das poucas esperanças de vencer bactérias multirresistentes. As bactérias multirresistentes são atualmente uma das maiores causas de vítimas mortais em infeções hospitalares e um dos maiores desafios da medicina moderna.

O(s) estágio(s) terá(ão) como objetivo estudar formas eficientes de:
1) bloquear a entrada de vírus em células (estágio 1), sobretudo ao nível do Sistema Nervoso Central, ou
2) perturbar as membranas de bactérias (estágio 2), sobretudo quando se formam biofilmes,
através da interação de fármacos inovadores com sistemas modelo de membranas

Serão usadas técnicas de microscopia de força atómica (AFM), citometria de fluxo, e técnicas espectroscópicas como fluorescência, dispersão dinâmica de luz e potencial zeta em sistemas modelo de membranas, culturas celulares e bactérias.

Orientador: *Miguel Castanho, Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa (https://imm.medicina.ulisboa.pt/investigation/laboratories/miguel-castanho-lab/#intro); macastanho@medicina.ulisboa.pt*

Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Crosstalk between PLCγ1 and Estrogen Receptor Modulates the Prognosis and Therapy Resistance of Luminal Breast Cancer

Synopsis: PLCγ1 is a direct downstream effector of receptor and non-receptor tyrosine kinases which catalyses the hydrolysis of the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) (1). PLCγ1 is involved in the tumorigenesis and progression of different types of cancer, including breast cancer (BC) (2). Previous studies showed that PLCγ1 downregulation in BC cell lines resulted in a strong inhibition of metastasis formation in nude mice (2). Additionally, overexpression of PLCγ1 is a risk factor for distant relapse in early luminal A and B breast cancer patients (3). Our previous work showed that PLCγ1 induces cetuximab resistance in metastatic colorectal cancer by activating ERK and AKT pathways (4). In this project, we aim to understand the link between PLCγ1 and Estrogen Receptor signalling and how it impacts the prognosis and therapy of luminal breast cancer. To answer that question, we will use both human samples available in our biobank and *in vitro* models of breast cancer cell lines depleted (knockout) or overexpressing PLCγ1 in several cellular assays such as: cell viability, drug resistance, apoptosis, invasion and signalling.

Supervisor: *Marta Martins, Luís Costa Lab, marta.martins@medicina.ulisboa.pt*
Webpage of the group

Bibliography:

1 - Bunney TD, Esposito D, Mas-Droux C, Lamber E, Baxendale RW, Martins M, Cole A, Svergun D, Driscoll PC, Katan M. Structural and functional integration of the PLCγ interaction domains critical for regulatory mechanisms and signaling deregulation. Structure. 2012 Dec 5;20(12):2062-75.

2 - Sala G, Dituri F, Raimondi C, Previdi S, Maffucci T, Mazzoletti M, Rossi C, Iezzi M, Lattanzio R, Piantelli M, Iacobelli S, Broggini M, Falasca M. Phospholipase Cgamma1 is required for metastasis development and progression. Cancer Res. 2008 Dec 15;68(24):10187-96.

3 - Lattanzio R, Marchisio M, La Sorda R, Tinari N, Falasca M, Alberti S, Miscia S, Ercolani C, Di Benedetto A, Perracchio L, Melucci E, Iacobelli S, Mottolese M, Natali PG, Piantelli M; CINBO (Consorzio Interuniversitario Nazionale per Bio-Oncologia). Overexpression of activated phospholipase Cγ1 is a risk factor for distant metastases in T1-T2, N0 breast cancer patients undergoing adjuvant chemotherapy. Int J Cancer. 2013 Mar 1;132(5):1022-31.

4 - Cruz-Duarte R, Rebelo de Almeida C, Negrão M, Fernandes A, Borralho P, Sobral D, Gallego-Paez LM, Machado D, Gramaça J, Vílchez J, Xavier AT, Ferreira MG, Miranda AR, Mansinho H, Brito MJ, Pacheco TR, Abreu C, Lucia-Costa A, Mansinho A, Fior R, Costa L, Martins M. Predictive and Therapeutic Implications of a Novel PLCγ1/SHP2-Driven Mechanism of Cetuximab Resistance in Metastatic Colorectal Cancer. Clin Cancer Res. 2022 Mar 15;28(6):1203-1216.

Instituto de Medicina Molecular João Lobo Antunes

Master's Project Proposal

Title: Targeting cancer with malaria parasites

Synopsis: This Master's Proposal will focus on the combined use of transgenic malaria parasites and rodent models of cancer to explore the potential of the former to combat the latter. The project will involve multiple experimental methodologies, including animal experimentation, molecular biology, immunofluorescence microscopy, quantitative reverse transcriptase PCR and histopathology, and will be carried out at Instituto de Medicina Molecular (iMM) under the supervision of Maria M. Mota and the co-supervision of Miguel Prudêncio. The student will operate at the interface between these laboratories and other iMM research groups with expertise in the biology of cancer. Applicants are expected to display proactivity, a capacity to operate at a multidisciplinary level, and a strong ability to gain autonomy. Besides her/his supervisors, the selected candidate will interact with and have the opportunity to learn from the members of different research teams at iMM.

Submit applications by email to Sofia Marques, Mota Lab**:** smarques@medicina.ulisboa.pt including your CV, a Motivation Letter and the name(s) and contact details of at least 1 reference.

Supervisor: *Maria M. Mota, Maria Mota Lab, mmota@medicina.ulisboa.pt*
Co-Supervisor: *Miguel Prudêncio, Miguel Prudêncio Lab, mprudencio@medicina.ulisboa.pt*
Webpage of the group

Remunerated or volunteer training: This is a VOLUNTEER training offer.