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Lecture

Prof. Dr. Joaquim Ferreira

- Sub-director faculty of Medicine of Lisbon (FMUL)
- Associate Professor at FMUL since 2012
- Director - Laboratory of Clinical Pharmacology and Therapeutics, FMUL (2011)
- MD (1992) and PhD (2009) in Neurology, FMUL

"Not Everything Is What It Seems":

Challenges in Treating Neurodegenerative Diseases

GDNF Signaling Dysfunction in Absence Seizures

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Abstract

Childhood absence epilepsy (CAE) is a prevalent paediatric epilepsy characterized by recurrent absence seizures (ASs) and generalized 2.5-4 Hz spike-and-wave discharges arising from thalamocortical circuit dysfunction. While alterations in GABAergic inhibition are central to CAE pathophysiology, increasing evidence suggests that neurotrophic factors may modulate seizure activity and long-term synaptic plasticity.

Glial cell line-derived neurotrophic factor (GDNF) exhibits neuroprotective and anticonvulsant properties in other epilepsy models; however, its impact in ASs remains poorly defined. This study aimed to characterize molecular and functional alterations in hippocampal GDNF signalling in CAE, focusing on astrocytic involvement and synaptic plasticity.

Experiments were performed using adult (90 day old) Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and non-epileptic control (NEC) rats. Acute hippocampal slices were prepared and incubated with GDNF (2 nM) for at least 1 hour. Long-term potentiation (LTP) was assessed by recording field excitatory postsynaptic potentials (fEPSP) at the hippocampus CA1-CA3 synapses following theta-burst stimulation. All procedures were conducted in accordance with institutional and national ethical guidelines for animal research.

In NEC rats, GDNF incubation resulted in a significant reduction in LTP magnitude, indicating an inhibitory modulation of synaptic plasticity under on-epileptic conditions. In contrast, this modulatory effect was markedly attenuated in GAERS. Suggesting a reduced responsiveness to GDNF-mediated regulation of synaptic efficacy.

Together, these findings indicate that GDNF modulates hippocampal synaptic plasticity in physiological conditions, while this effect is blunted in absence epilepsy. This altered sensitivity to GDNF may reflect disruptions in inhibitory control mechanisms and neurotrophic signalling associated with the epileptic state.

Keywords: **Childhood absence epilepsy, absence seizures, GDNF signalling, hippocampal synaptic plasticity, astrocytic modulation, thalamocortical dysfunction**

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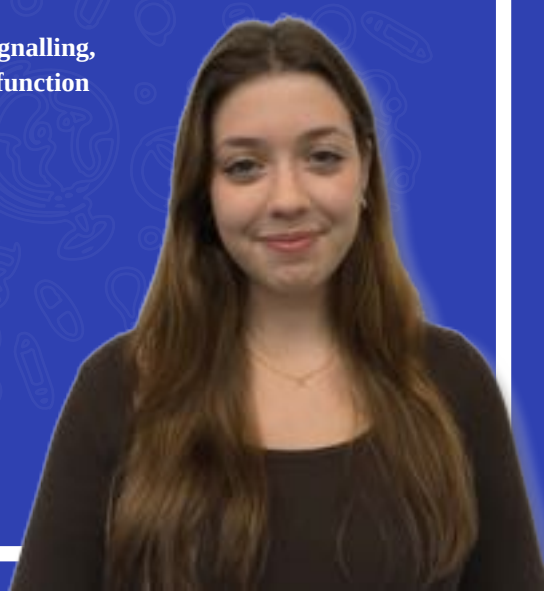

Glial Cell Populations in Absence Epilepsy: A Developmental Analysis of Microglia

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Abstract

Absence Seizures (ASs) are genetic generalized seizures characterized by brief lapses of consciousness with 2.5-4 Hz spike-and-wave discharges (SWDs) on electroencephalogram (EEG). ASs are the most prevalent seizure types in children and occur in many pediatric epilepsies, as well as in adults. Generalized ASs arise from neuronal hyperexcitation and hypersynchronization within cortico-thalamic circuits, resulting from an imbalance between gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the brain, and glutamate, the primary excitatory neurotransmitter. Recent studies suggest that besides neurons, glial cells, such as microglia, are crucial to ASs pathophysiology. Microglia, the innate immune cells of the central nervous system, are involved in neuroinflammation, glutamate excitotoxicity, and the balance between excitatory and inhibitory neurotransmission, underscoring their dual role in seizure promotion and protection. Despite these insights, a comprehensive study of microglia populations in ASs has not yet been conducted. This study addresses the existing gap by assessing the morphology and inflammatory protein expression in microglia across a developmental timeline in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) animal model. Our preliminary observations in the somatosensory cortex suggest an age-dependent association between microglial phenotype and absence seizures. Younger GAERS (P10) display a more amoeboid-like microglial phenotype, consistent with a pro-inflammatory profile, when compared with age-matched NEC rats. In contrast, older GAERS (P14-18) exhibit a more ramified phenotype relative to NEC animals, suggesting a shift toward a more anti-inflammatory state. This pattern is particularly relevant given that ASs emerge within a defined neurodevelopmental window, corroborating that maturational changes in glial function may influence disease onset, severity, and persistence. Characterizing these age-related microglial changes may therefore help identify correlations between seizure progression and neuroinflammatory state. In the future it may also provide novel therapeutic avenues, potentially improving seizure control and better clinical outcomes for patients with this form of epilepsy.

Keywords: CNS; Absence Seizures; Microglia; Cell Morphology; Development

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Engineering S100 Chaperones to Target Amyloid-β Conformers in Alzheimer's Disease

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Abstract

In Alzheimer's disease (AD), the pathological self-assembly of amyloid-β (Aβ) and tau into toxic oligomers and fibrils drives neurodegeneration. While fibrils form the hallmark plaques and tangles observed in AD, oligomeric conformers are increasingly recognised as the most neurotoxic. Given the profound societal burden of AD, the ongoing pursuit of effective disease-modifying therapies has highlighted molecular chaperones as promising suppressors of Aβ aggregation, given their ability to selectively modulate distinct steps of the aggregation pathway. Among chaperones, members of the S100 protein family, notably S100B, are found to co-localize with amyloid plaques and interact with Aβ in a conformation- and context-dependent manner. Upon calcium binding, S100B exposes hydrophobic interfaces that enable selective binding to Aβ42 oligomers and fibrils, thereby inhibiting surface-catalysed secondary nucleation and rescuing neuronal viability. Fine-tuning the binding specificity and calcium-dependent dynamics of S100B could offer a strategy to enhance therapeutic potential. Additionally, preliminary data from our group demonstrates that proteins from the S100 family display distinct inhibitory profiles and selectivities towards Aβ conformers, highlighting important functional differences between these chaperones that provide a rationale for optimization. With this project, we aim to produce optimized S100B mutant variants inspired by other S100 family proteins and identify the biochemical determinants of S100 chaperone activity and specificity, with the long-term goal of designing precision chaperones for therapeutic intervention in AD and related proteinopathies.

Keywords: Neurodegenaration, Protein Misfolding, Molecular Chaperones, Protein Engeenering

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Mechanistic Insights into S100A9-Driven Regulation of α–Synuclein Self-Assembly

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Abstract

"Alpha-Synuclein (α-Syn) is an intrinsically disordered neuronal protein whose misfolding and aggregation are central to the pathogenesis of synucleinopathies such as Parkinson's disease. Under molecular crowding, α-Syn can form dynamic, membrane-less liquid droplets that are thought to facilitate the nucleation of toxic oligomers and amyloid fibrils, but the cellular factors that regulate these early events remain poorly understood. S100A9 is an EF-hand Ca^{2+}-binding pro inflammatory protein of the S100 family, highly expressed in neutrophils and monocytes and involved in innate immune signalling. Notably, S100A9 has been shown to interact with α-Syn, co-localizing in Lewy bodies. However, the precise contribution of S100A9 to PD pathogenesis remains to be elucidated.
In this work, we present preliminary data exploring how S100A9 may modulate α-Syn self-assembly and phase behaviour using a droplet microfluidic platform (PhaseScan) with LLPS turbidity measurements and bulk aggregation assays. PhaseScan mapping indicates that S100A9 undergoes LLPS over a range of crowding and ionic conditions and that this phase separation occurs independently of Ca^{2+}, while increasing NaCl concentration promotes S100A9 condensate formation. Turbidity-based LLPS assays further show that mixtures of α-Syn and S100A9 display increased turbidity relative to the individual proteins, consistent with the formation of heterotypic condensates, although the impact of these droplets on downstream amyloid formation remains unresolved at this stage. Aggregation experiments confirm robust, concentration-dependent α-Syn fibril formation under our conditions, providing a platform to quantitatively assess the effect of S100A9 on aggregation kinetics in future work. Together, these preliminary results define experimental conditions under which S100A9 and α-Syn co-exist in condensed phases, providing a starting point to unravel the molecular basis of S100A9 mediated modulation of α-Syn self-assembly.
"

Keywords: **Protein aggregation; Parkinson's disease; Phase separation; Neurodegeneration; Liquid-liquid phase separation; Protein misfolding**

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Protonation-Dependent Stability of DJ-1 Dimers and its Implications for Parkinson's Disease

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Abstract

DJ-1 is a multifunctional, neuroprotective protein that acts both as an oxidative stress sensor and as a protein deglycase. Its stability and function are highly dependent on its oligomeric state, naturally occurring as a dimer. The dimerization process has been proposed to be strongly modulated by the protonation states of two carboxylic acids, Glu15 and Asp24, which form a unique hydrogen bond at the dimer interface. Mutations in DJ-1, as well as oxidation state modifications, such as those in Cys106, can compromise its structural integrity, impair dimerization, and ultimately lead to aggregation, which is intimately associated with neurodegenerative diseases, such as Parkinson's Disease (PD).

In this study, we use constant-pH molecular dynamics (CpHMD) simulations to understand the role of protonation and carboxylic acids' H-bonds in the regulation of DJ-1 dimer stability. We will also investigate the effect of a set of Parkinson's disease-associated mutations (such as L166P) and of Cys106 oxidation to determine their impact on dimerization and on the overall protein aggregation propensity. CpHMD is particularly important for this study because it enables titratable residues to change protonation state during the simulation, allowing simultaneous sampling of conformational and protonation states that influence DJ-1 dimer stability. This provides valuable insights into dimerization and the mechanisms underlying neurodegeneration in PD.

We have built and equilibrated wild-type monomeric and dimeric models of human DJ-1 and simulated them at five pH values (4.2, 5.2, 6.2, 7.2 and 8.2), each with five replicas, to obtain titration curves and evaluate protonation under physiological and stress-related conditions.

These simulations are complete, and preliminary data will be presented in this communication.

Keywords: **DJ-1 protein, constant-pH molecular dynamics (CpHMD), protein dimerization, protonation states, Parkinson's disease, protein aggregation**

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Deep Learning How to Inhibit Aβ-42 Aggregation

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Abstract

Alzheimer's Disease (AD) is the most prevalent neurodegenerative disease being driven, in part, by the misfolding and aggregation of the amyloid-β peptide (Aβ), particularly Aβ42. Its intrinsically disordered nature poses limitations for traditional small-molecule therapeutics due to the lack of stable binding pockets. Although the mechanisms underlying Aβ42 aggregation remain elusive, aggregation-prone regions have been identified, including the 16-22 residue region.
This project combines molecular dynamics (MD) simulations with AI-based peptide design to investigate the structural and conformational changes of Aβ42 in its monomeric and early oligomeric states. Using RFdiffusion, we designed de novo peptides targeting the 16-22 region of Aβ42. Peptide 1 is a seven residue β-sheet rich peptide and Peptide 2 is a 15 residue α-helix rich peptide. MD simulations were performed on Aβ42 monomeric systems using both the RFdiffusion proposed bound poses and unbiased, unbound configurations.
The peptides showed mixed binding behaviors. Peptide 1 demonstrated greater binding stability and more frequent association with Aβ42, whereas Peptide 2 struggled to sustain binding in the proposed pose and showed limited binding in unbound simulations. When bound in the ideal pose, both peptides induced significant structural rearrangements and remodeled the accessible conformational space of Aβ42, with distinct intramolecular contact patterns underlying these effects.
These findings highlight the potential of RFdiffusion for designing peptides targeting intrinsically disordered proteins, while also revealing challenges associated with stable binding to highly dynamic targets. Ongoing work in our group extends this approach to additional RFdiffusion designed peptides and early oligomeric Aβ42 systems, including dimers and tetramers. In parallel, we are investigating the role of the hydrophobic effect on IDPs conformational landscape and aim to characterize the energetic and structural evolution during the initial stages of oligomerization.

Keywords: Alzheimer's Disease, Molecular Dynamics, Deep Learning, Protein Aggregation, RFdifussion

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In vivo Dissection and Validation of Common Molecular Pathways Affected in pre–Symptomatic Drosophila Models for ALS and SMA

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Abstract

Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA) are two of the most prevalent forms of motor neuron diseases (MNDs), both leading to progressive neurodegeneration and ultimate loss of motor neurons. Despite their distinct genetic backgrounds and clinical presentations, growing evidence strongly suggests the existence of convergent molecular mechanisms underlying their pathogenesis, particularly through the disruption of RNA-binding proteins (RBPs) function, important regulators of the RNA metabolism.

Comparative transcriptomics analysis using presymptomatic Drosophila disease models revealed that the silencing of the RBPs associated with ALS (Caz, Tbph) and SMA (Smn) induces transcriptomic alterations that converge on functional protein modules with critical roles in neuronal health. Among these, the neuromuscular junction (NMJ) emerged as a particularly affected and disease-relevant module for further investigation, since it was enriched in proteins encoded by MND-linked gene orthologs.

Based on these recent findings, in this project we aim to validate in vivo the contribution of candidate pathways to NMJ dysfunction and disease initiation. By combining Drosophila genetics, high-resolution confocal and Ca^{2+} imaging, as well as larval crawling behavioral assays, we will examine how perturbations impact synaptic structure and function in disease context. Functional rescue assays will further test the reversibility of identified effects. Ultimately, through the integration of molecular genetics, imaging and functional analyses, this work seeks to unravel early cellular events driving ALS and SMA progression and to discover shared therapeutic targets, thereby guiding the development of innovative strategies to combat these disorders.

Keywords: ALS, SMA, Drosophila, Neuromuscular Junction, Motor neuron disease, Neurodegeneration, Neuroplasticity

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Characterizing the Functional Impact of SMA and fALS Disease Gene Loss-of-Function on Cellular Protein Complexes

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Abstract

Motor neuron diseases (MNDs), including amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), are neurodegenerative disorders caused by genetic mutations that impair motor neuron function. Although genetically and clinically distinct, ALS and SMA converge on common molecular pathways involving key RNA-binding proteins such as TDP-43, FUS and SMN1. Recent findings in Drosophila models revealed that the knockdown of the fly orthologues of the human genes TARDBP, FUS and SMN1 caused coordinated expression changes in over 500 fly genes and had a concerted effect on neuronal protein complexes involved in critical cellular pathways for motor neurons, including neuromuscular synaptic transmission. These findings highlight conserved molecular mechanisms across MNDs and provide a foundation for investigating similar alterations in human neuronal models. Building on this, the present project aims to bridge findings from fly to human systems by generating and characterizing inducible knockdown models for MND-associated genes and exploring the transcriptomic and proteomic alterations associated with gene loss-of-function. As such, predesigned, commercially available short hairpin RNA (shRNA) constructs targeting TARDBP, FUS, and SMN1 will be stably transfected into SH-SY5Y cells using the Flp-In T-REx system. This platform offers two major advantages: first, it ensures the generation of isogenic cell lines by inserting the shRNA cassette at a defined genomic location via Flp-mediated recombination; and second, it allows controlled, tetracycline-inducible expression of the integrated construct, thereby enabling precise control over the timing of expression. Once stable clones are established, functional assays will be performed to determine the impact of gene knockdown. Ten candidate genes will be selected for mRNA-level characterization, with transcript abundance (including isoform-specific expression) quantified by RT-qPCR in undifferentiated and differentiated cells. Based on these findings, five candidate genes will be prioritized for protein-level analyses in differentiated neurons, with protein abundance and subcellular localization assessed by Western blot and immunofluorescence microscopy.

Keywords: **Motor Neuron Diseases, Amyotrophic Lateral Sclerosis (ALS), Spinal Muscular Atrophy (SMA), RNA-binding Proteins, Human Knockdown Models, Short Hairpin RNA (shRNA)**

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Development of Metal-Based Complexes as Potential Theranostic Agents for Cancer Therapy

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Abstract

Cancer is still one of the main causes of death worldwide, and many current treatments are limited by severe side effects and incomplete tumour control. Metal-based complexes are emerging as versatile tools in oncology, because their structures can be tuned to combine imaging and therapy in a single molecule or material. In this project, a library of iron- and rhenium-based metallic complexes is developed to pair MRI contrast with redox-driven anticancer activity. The complexes are obtained using standard coordination-chemistry methods and characterised by NMR, UV-Vis and IR spectroscopy, cyclic voltammetry, powder x-ray diffraction and, when crystals are available, single-crystal X-ray diffraction, to clarify their structure, stability and redox properties. Their biological activity is explored in vitro using MTT and IC50 assays in triple negative breast cancer (MDA-MB-231), hormone dependent breast cancer (MCF-7), liver cancer (Hep G2) and colorectal cancer (CACO), using cisplatin and healthy cells as references for potency and selectivity. Mechanistic studies by flow cytometry focus on reactive oxygen species formation, changes in cell-cycle distribution and induction of apoptosis, complemented by confocal microscopy to visualise cellular uptake and localisation. Here we report the synthesis of five iron (III) and three rhenium(I) complexes characterised by NMR, UV-Vis, IR spectroscopy, powder x-ray diffraction and cyclic voltammetry. The magnetic moment of the iron (III) complexes was also determined to access their use as MRI agents. Finally, Nuclear Magnetic Relaxation Dispersion and relaxivity measurements under conditions that mimic the physiological environment are used to assess MRI performance. Bringing together the structural, electrochemical, biological and relaxometric data, this work aims to draw simple structure-activity-relaxivity links and to highlight a family of iron- and rhenium-based complexes with realistic dual diagnostic and therapeutic potential as theranostic candidates for cancer.

Keywords: **Metal-based anticancer complexes, iron and rhenium complexes, cancer theranostics, MRI contrast agents, redox-driven cytotoxicity, structure–activity relationships** Theranostic agents, Cancer therapy, metal-complexes, coordination chemistry, diagnostic imaging, metallodrugs

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Mode of Action and Metabolic/Plasma Stability of Emerging Metal-Based Anticancer Agents

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Abstract

According to the World Health Organization (WHO) cancer is the second leading cause of death worldwide, surpassed only by cardiovascular diseases. Chemotherapy is the cornerstone for cancer treatment, however the current approved drugs for such treatments present major limitations such as low selectivity, leading to serious side effects, and resistance to therapy, which limits the number of effective options.

Metallodrugs have been developed to overcome these limitations due to their unique properties and diverse mechanisms of action, which can improve selectivity, reduce systemic toxicity, and bypass platinum resistance. Among them, Fe(II) and Ru(II)-cyclopentadienyl compounds developed by our Group have shown promising results, being cytotoxic against a wide panel of cancer cell lines and, in particular, against cisplatin-resistant cell lines. Building on these findings, the present work explores the effect of substituting triphenylphosphine with a diphenyl-2-pyridylphosphine ligand in both Fe(II) and Ru(II)-cyclopentadienyl complexes, with the aim of evaluating their efficacy against cisplatin-resistant cancers such as non-small cell lung cancer (NSCLC).

To assess the cytotoxic potential against cancer cells, the MTT assay was performed in the A549 cisplatin-resistant NSCLC cell line. Results showed that these compounds exhibited great cytotoxic potential, with IC50 values significantly lower than that of cisplatin. Some of these compounds have exhibited IC50 < 1μM, while cisplatin commonly used in chemotherapy, has an IC50 >100 μM.

This work also aims to evaluate the cell death mechanism through Annexin V/PI staining (flow cytometry analysis), colony formation assay to verify the selected compounds to inhibit the formation of colonies, cellular uptake and intracellular distribution by ICP-MS (Inductively Coupled Plasma Mass Spectrometry), and metabolic and plasma stability by LC-HRMS (Liquid Chromatography-Tandem High Resolution Mass Spectrometry).

Keywords: **Anticancer agents; Metallodrugs; Ruthenium(II)-cyclopentadienyl compounds; Iron(II)-cyclopentadienyl compounds; Non-Small Cell Lung Cancer;**

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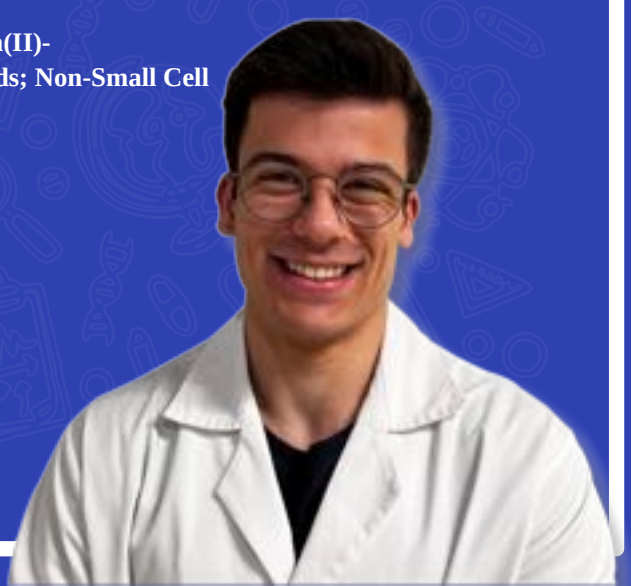

In vitro Evaluation of Innovative Peptides Towards Breast Cancer Metastization

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Abstract

Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype, associated with poor clinical outcomes. The lack of estrogen, progesterone, and HER2 receptors limits approved targeted therapies, underscoring the need for selective molecular strategies. In this context, aberrant activation of the Wnt/β-catenin pathway, driven by Frizzled-7 (FZD7) overexpression, is a critical driver of TNBC tumorigenesis, metastatic dissemination, and resistance to therapy. To selectively target FZD7, we engineered linear peptides derived from the complementary-determining regions (CDRs) of an anti-FZD7 antibody, preserving high-affinity receptor recognition while mitigating limitations associated with full-length antibodies. Given their strong preclinical performance, these peptides formed the basis for a subsequent optimization phase in which stapling strategies were applied to enhance chemical stability and pharmacological performance, yielding a more advanced generation of anti-FZD7 candidates. Stapling introduces intramolecular covalent crosslinks that constrain peptide conformation into bioactive α-helices. Several stapled variants and N-terminally labelled analogues (Quasar 670 or biotin) were synthesized for mechanistic and localization studies.
To evaluate safety, systemic membrane toxicity was assessed using a red blood cell hemolysis assay. The linear peptide showed the highest membrane-disruptive activity, while mono- and double-stapled peptides exhibited reduced hemolysis, with no peptide reaching HC_{50} values. In 2D TNBC cultures, the linear and mono-stapled peptides demonstrated comparable cytotoxicity with IC_{50} values around 50 micromolar, whereas the double-stapled peptide showed limited activity under short-term exposure. In contrast, in 3D TNBC spheroid models that better recapitulate tumor architecture, stapled peptides outperformed the linear sequence, producing stronger and more sustained inhibition of spheroid growth.Ongoing work includes peptide characterization, analysis of cell binding kinetics, biodistribution and pharmacokinetics in zebrafish models, and assessment of FZD7 signaling inhibition. Collectively, these efforts support stapled anti-FZD7 peptides as promising targeted agents for TNBC.

Keywords: **Triple-negative breast cancer, Frizzled-7 (FZD7), Wnt/β-catenin signaling, stapled peptides, targeted therapy, peptide therapeutics**

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Evaluating the Anticancer Potential of Peptide-Drug Conjugates in a Blood-Brain Barrier Integrated *In Vitro* Model of Brain Metastases

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Abstract

Breast cancer is the most commonly diagnosed cancer worldwide and one of the leading causes of cancer-related mortality in women, mainly due to metastases. Among its subtypes, triple-negative breast cancer (TNBC), which accounts for about 15-20% of cases, is characterized by high aggressiveness, a high propensity for brain metastases and poor clinical prognosis. The treatment of these metastases is severely limited by the blood-brain barrier (BBB), which restricts the entry of many drugs.
Poly(ADP-ribose) polymerase inhibitors (PARPi) are clinically effective in patients with BRCA1/2 mutations and TNBC shares several molecular and functional features with BRCA-mutated tumors, supporting the rationale for PARP inhibition in this subtype. Nevertheless, the efficacy of PARPi in brain metastases remains limited by insufficient BBB permeability.
This project aims to develop and validate advanced in vitro models integrating the BBB and TNBC spheroids to evaluate peptide-drug conjugates (PDCs), that combine PARP inhibitors with BBB peptide shuttles, enabling BBB crossing and anticancer activity in TNBC brain metastases.
As preliminary results, TNBC spheroids were optimized for application in transwell and organ-on-chip models, and cell viability assays in 2D TNBC cultures treated with the PARPi olaparib demonstrated a dose-dependent reduction in metabolic activity.
Future work will focus on evaluating BBB transport and anticancer efficacy of PDCs using transwell models as an initial predictor of permeability, followed by functional validation in an organ-on-chip model of brain metastases (BMoC), which integrates microfluidic BBB and TNBC spheroids. These models will be used to assess cellular toxicity, apoptosis, oxidative stress and permeability, with the goal of establishing a predictive in vitro screening system for the development of therapies targeting TNBC brain metastases.

Keywords: **Triple-Negative Breast Cancer, Brain metastases, Peptide drug conjugates, PARP inhibitors, Organ-on-chip**

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Design and Characterization of Trojan-Like Lipid Droplets for Targeted Drug Delivery and Metabolic Modulation in TNBC

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Abstract

Female breast cancer remains the most commonly diagnosed cancer worldwide, with an estimated 2.3 million new cases and 670,000 deaths in 2022, underscoring its persistent public health burden. Among its subtypes, triple-negative breast cancer (TNBC) is particularly lethal, owning to metabolic plasticity and frequent resistance to standard chemotherapies, including Paclitaxel (PTX). Although PTX is a first-line agent, its extreme hydrophobicity requires solubilizing excipients, which are often toxic, limiting therapeutic efficacy. Despite extensive efforts to develop biocompatible drug delivery systems (DDS), therapeutic responses remain limited and relapse is common. To sustain proliferation, TNBC cells exhibit heightened lipid demand and actively scavenge exogenous lipids, using lipid droplets (LDs) to buffer lipid toxicity and metabolic stress. Interestingly, LD lipid composition critically regulates LD-associated proteomes, intracellular organization, and stress-adaptive signaling, thereby supporting TNBC survival and therapy resistance. This project aims to exploit TNBC lipid dependency, by using artificial LD (aLD) with engineered lipid composition, designed to encapsulate PTX while actively engaging and modulating LD-associated metabolic pathways to sensitize TNBC cells to therapy.
aLD were systematically characterized across formulation parameters, including phospholipid-oil balance, mechanical mixing speed, and ultrasonic energy input, using DLS, NTA, and fluorescence spectroscopy. Parameter-mapping indicates that sonication-based methods optimally regulate aLD size and interfacial stability. Optimized aLDs exhibit time-dependent uptake in TNBC cell lines (MDA-MB-231 and 4T1), consistent with LD-mimetic internalization. Future work will focus on optimizing the lipid composition and ultrasonication parameters to enhance stability, PTX loading, and in vitro therapeutic responses.

Keywords: TNBC, Metabolic reprogramming, Paclitaxel, Lipid Droplets, Drug Delivery System

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Assessing How Sphingolipid Structure Regulates Membrane Compartmentalization by Advanced Fluorescence Techniques

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Abstract

The plasma membrane (PM) of eukaryotes shows an asymmetric lipid distribution essential for processes like apoptosis, vesiculation, and cell fusion. In yeast PM, sphingolipids are mainly in the outer leaflet and ergosterol in the inner, shaping membrane properties and forming several types of membrane domains, namely the unique sphingolipid-enriched domains (SLEDs), discovered in our lab, absent in mammalian PM. Unlike mammals, fungi feature stable, large membrane compartments with distinct proteins, where sphingolipids and ergosterol seem to associate with different domains: the Pma1 H+-ATPase compartment (MCP) for sphingolipids and the Can1p arginine/H+ symporter compartment (MCC) for ergosterol. Thus, in the yeast membrane there seems to be both transversal and lateral segregation of sphingolipids and sterols. This discovery together with the differences in fungal and mammalian (sphingo)lipidomes alongside the structural differences of the sphingolipids (chain length, functional groups, stereochemistry) led to the hypothesis that PM sphingolipid profile plays a crucial role in PM dynamics, and therefore, on the organization and interplay of protein membrane compartments. In this project we aim to reveal some of the mysteries behind PM organization, structure and composition. We also aim to reveal how sphingolipid profile impacts the two major membrane compartments in yeast PM, MCP and MCC and to disclose the intricate relation between membrane protein organization and lipid domains in fungi. So far, we tested the association of di-4-ANEPPS and di-12-ANEPPQ in intact and permeabilized wild-type Saccharomyces cerevisiae cells. Di-4-ANEPPS shows little fluorescence shift between intact and permeabilized cells, suggesting it can access most cellular membranes irrespective of permeabilization, whereas higher quencher concentrations induce a red shift in excitation spectra, a result that remains to be interpreted. The behavior of di-12-ANEPPQ is currently under investigation, and it is still too early to draw firm conclusions.

Keywords: **Yeast membranes, fluorescence spectroscopy, di-4-ANEPPS, Sphingolipid enriched Domains (SLEDs)**

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Characterization of Erythroblastic Islands and Their Dynamic Interactions with Patient-Derived Acute Myeloid Leukemia Cells using Live Imaging in ZAvatars

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Abstract

Acute Myeloid Leukemia (AML) profoundly remodels the bone marrow microenvironment, leading to ineffective erythropoiesis and severe anemia. Central to physiological red blood cell production are erythroblastic islands (EBIs), specialized macrophage-centered niches that coordinate erythroid maturation and regulate iron availability. Emerging evidence indicates that EBI macrophages can be recruited to malignant niches, redirecting iron away from erythroblasts to support tumor growth. In AML, this diversion compromises normal erythropoiesis while leukemic cells adapt to the hypoxic bone marrow niche and associate with erythroid-supportive, iron-rich microenvironments that promote survival. Here, we provide the first in vivo characterization of EBIs in zebrafish and leverage the zAvatar model to investigate their dynamic interactions with patient-derived AML cells. Hemolytic stress induced the rapid formation of structurally organized EBIs in 2 days post-fertilization zebrafish, predominantly exhibiting a domed architecture. High-resolution live imaging revealed that these niches are highly dynamic, supporting erythroblast recruitment, inter-island transitions, and coordinated migration along the tail, indicating an active spatial organization of the erythroid compartment. To enable transcriptomic analysis of EBIs, we established an optimized dissociation and isolation protocol compatible with downstream profiling. Ongoing work focuses on bulk RNA sequencing to define the transcriptional landscape of EBIs and on dissecting EBI–AML interactions through live imaging following leukemic cell injection. Together, this work establishes zebrafish as a powerful in vivo model to study erythroid niche biology and provides a platform to uncover how erythropoietic microenvironments contribute to tumor survival and anemia in AML.

Keywords: Acute Myeloid Leukemia (AML), Erythroblastic Islands (EBIs), Erythropoiesis, Anemia, Zebrafish, zAvatar model, Bone Marrow Microenvironment.

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Drug Repositioning to Restore Radioactive Iodine Sensitivity in Refractory Thyroid Cancer: Functional Validation of Gene Targets

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Abstract

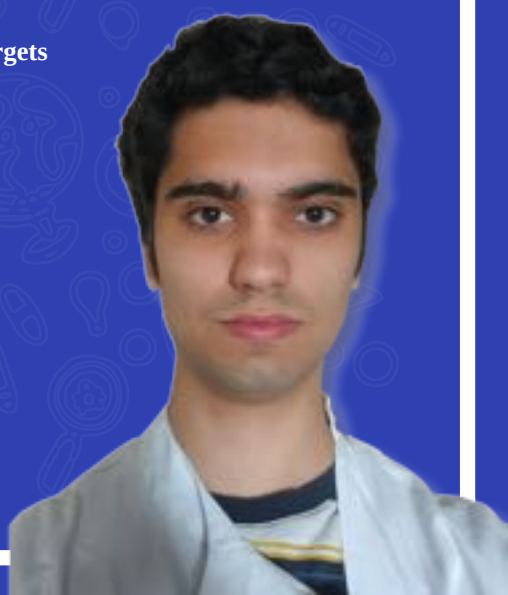
Differentiated thyroid carcinoma (DTC) is the most prevalent malignant endocrine neoplasm and usually has a good prognosis. The main therapeutic strategy in addition to surgery is the use of radioactive iodine (RAI), whose effectiveness depends on the functional expression of the sodium/iodide symporter (NIS), a protein located in the basolateral membrane of thyroid follicular cells and responsible for iodide uptake. However, in 5–15% of all DTC cases and in about 30% of metastatic cases, tumors become refractory to RAI treatment due to loss of expression or relocalization of NIS from the plasma membrane, impairing iodide incorporation and limiting the therapeutic options available

This project aims to identify and validate FDA approved drugs capable of restoring NIS expression and functionality in refractory cells, thereby resensitizing them to iodine treatment. The work will be carried out in transfected cell lines engineered to express HS-YFP, a more suitable and specific tool than normal YFP for functional assays that assess the efficiency of iodine uptake and utilization by follicular cells, due to its higher sensitivity for detecting functional halide ions such as intracellular iodide.

To assess cell viability, proliferation and functional iodide uptake, we will employ, for the first time, a multiplex assay in 96-well plates, using an in-house optimized protocol for the Infinite 200 PRO plate reader for cinetic YFP-HS Fluorescence decay reading and subsequent NIS activity quantification.

Subsequent molecular analyses such as qPCR, Western blot, immunofluorescence, and cell surface biotinylation will be performed to characterize changes in NIS expression and localization. The combination of functional data with transcriptomic analyses will help elucidate the molecular mechanisms underlying refractory cells and NIS recovery. This study may pave the way for new therapeutic approaches in the clinical management of RAI-refractory DTC, expanding possible treatment options and improving prognosis.

Keywords: Refractory Thyroid Cancer, Drug Repositioning, Gene Targets



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Construction of a Single-Chain Variable Fragment Library Against AQP9

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Abstract

Aquaporins (AQPs) are a family of transmembrane channel proteins essential for osmotic regulation and solute transport. Among them, Aquaporin-9 (AQP9) serves as a critical facilitator for water, glycerol, and other small solutes in leukocytes and hepatocytes. Recent evidence implicates AQP9 upregulation in the pathophysiology of inflammatory diseases, where it facilitates immune cell migration and proliferation, antigen uptake, cytokine secretion and metabolic adaptations. While AQP9 represents a promising therapeutic target, current pharmacological inhibitors are limited by high cytotoxicity and lack of specificity. This project aims to overcome these limitations by developing a single-chain variable fragment (scFv) library targeting AQP9. The methodology involves the extraction of RNA from the spleen and bone marrow of rabbits previously immunized with a specific peptide corresponding to an extracellular loop of the AQP9 pore. Following cDNA synthesis, Variable Heavy (VH) and Variable Light (VL) chain domains will be amplified and combined by PCR to reconstruct the antigen-binding site, preserving natural immunological diversity. The resulting scFv repertoire will be cloned into phagemid vectors to construct a phage display library, where the high-affinity binders capable of recognizing the native channel will be selected. The final phase involves the functional validation of selected scFvs to confirm their ability to block AQP9 pore-mediated transport and attenuate inflammatory responses in vitro. This study seeks to generate a novel, low-toxicity therapeutic tool to modulate AQP9 activity, offering a targeted approach to manage systemic inflammation and other AQP9 overexpression-associated pathological conditions.

Keywords: Immunology; Aquaporin 9 (AQP9); single-chain variable fragment (scFv); Inflammation

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Design and Evaluation of Novel DprE1-Targeting Agents Against Mycobacterium tuberculosis

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Abstract

Drug-resistant tuberculosis (TB) remains a critical global health challenge. The continued emergence of multidrug and extensively drug-resistant Mycobacterium tuberculosis (Mtb) underscores the need for novel antitubercular agents with new mechanisms of action. Previous work at iMed.ULisboa identified dinitrobenzamide (DNB) derivatives as a novel class of compounds displaying significant antibacterial activity against Mtb, and whose target is a critical enzyme, DprE1, located in the periplasm of the bacterium. Thus, this project aims to further optimize the DNB-derivative library of compounds towards the optimization of their antibacterial activity against Mtb using a combined in silico and experimental strategy.
To date, a total of six new DNB derivatives have been added to the already existing chemical library, in this first stage the development of a reliable synthetic pipeline is still being developed. The biological evaluation of selected compounds will include the determination of minimum inhibitory concentration (MIC) against Mtb, in collaboration with a specialized mycobacteriology laboratory. In parallel, molecular modeling studies performed at BioISI (FCUL) are being conducted in an attempt to develop predictive models of DprE1 inhibition. Specifically, post-processing of docking poses are bing used to identify regions of high chemical group propensity in the protein target, which hopefully can guide the rational design and selection of novel derivatives.

Keywords: Drug-resistant Tuberculosis, Dinitrobenzamide Derivatives, Mycobacterium tuberculosis, DprE1 Inhibition, Structure-based Drug Design, Molecular Docking

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FT-ICR-MS Analysis of Glycation Effects on TDP-43 Aggregation and Metabolic Changes in S. cerevisiae

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Abstract

Amyotrophic Lateral Sclerosis (ALS) remains a poorly understood disease, with several known risk factors but no specific root causes for its onset. Most importantly, this illness continues to be very difficult to diagnose in an early stage, due to the lack of confirmed and standardized disease biomarkers.
Fortunately, in recent years, FT-ICR-MS, due to its ultra-high resolution, mass accuracy, and dynamic range, has been increasingly used for characterizing neurodegenerative diseases through proteomics and metabolomics, employing a well-established cellular model, Saccharomyces cerevisiae. However, to date, no papers have been published on the metabolic alterations that occur in the TDP-43-expressing yeast model, leaving room for fascinating breakthroughs.
Methylglyoxal (MGO) associated glycation events inevitably occur under high-glucose conditions, leading to the non-enzymatic modification of proteins and nucleic acids. These have been reported to play a critical role in neurodegenerative complications, including neuroinflammation, mitochondrial dysfunction, and oxidative imbalance. There's evidence that this post-translational modification causes profound alterations in folding and aggregation dynamics and, consequently, may lead to worse disease outcomes.
Here, we will focus on understanding how glycation can affect human TDP-43 aggregation in vivo and how this results in metabolite level changes in different yeast mutants lacking important genes associated with MGO detoxification, such as GLO1 and GLO2, which code for enzymes of the glyoxalase system, and GRE3, responsible for MGO secondary metabolism.
Spotting assays revealed that the expression of the heterologous protein impaired cell viability, with greater effects on the mutant yeast cells, highlighting the relevance of the deleted genes in protecting cells against glycation stress. Metabolomics analysis confirmed interesting differences between protein expressing and non-expressing variants, reinforcing the need to understand which cellular processes are being affected.

Keywords: Neurodegeneration, Amyotrophic Lateral Sclerosis (ALS), TDP-43, FT-MS, Metabolomics, Yeast

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Recovering AlphaFold Inaccessible Pockets

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Abstract

Accurate identification of novel pockets is crucial for drug discovery. However, while current structure prediction models provide highly accurate protein structures, their reliability for characterizing ligand-induced, allosteric or cryptic pockets remains limited. This project aims to identify poorly predicted pockets in the AlphaFold Protein Structure Database and develop strategies for improving their identification and characterization.

Using a dataset of experimental proteins in complex with druglike ligands, AlphaFold (AF) predictions were analyzed through pocket mapping, structural alignment and residue-level accuracy assessment using local Distance Difference Test (lDDT) scores. Pockets were then categorized into good, medium and poor prediction classes, allowing targeted investigation of problematic cases.

Initial results have shown that most of the pockets were well characterized by AlphaFold, highlighting its strong performance in structure prediction. Pockets with AF confidence scores (plDDT) above 70 are generally considered reliable, however, a significant fraction of poorly predicted pockets also have high plDDT scores. In contrast, lower confidence scores strongly correlate with local structural inaccuracies, suggesting that AlphaFold can partially recognize its own failure modes. We have also found that the majority of poor pocket predictions are not associated with the presence of similar proteins in the AlphaFold training set, implying that other factors contribute to pocket mischaracterization.

Ongoing work is focused on further exploring contributing factors for poor predicted pockets, evaluating existing pocket identification methods and describing strategies aimed at improving pocket characterization. By combining the most promising strategies, we seek to establish a final protocol that maximizes pocket identification.

Keywords: AlphaFold; Druggable pockets; Pocket characterization; Drug design; Optimization strategies

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Decoding the Mechanisms Involved in Manganese Oxidation by Bacterial DyP-Type Peroxidase BsDyP

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Abstract

The enzymatic degradation of lignin, the largest natural reservoir of aromatic compounds, is largely driven by microbial peroxidases that catalyze, among other reactions, the oxidation of Mn(II) to diffusible Mn(III). This work investigates the catalytic properties and structural mechanisms underlying Mn(II) oxidation by the DyP-type peroxidase from the bacterium Bacillus subtilis (BsDyP) and its evolved variant, CFR-5G5. In contrast to fungal peroxidases, which can employ Direct Electron Transfer (DET) through heme-proximal binding sites, BsDyP contains clusters of acidic residues on the protein surface located far from the heme cofactor, suggesting a Long-Range Electron Transfer (LRET) mechanism. Using X-ray crystallography combined with biochemical and kinetic analyses, we identified and validated two distinct Mn(II) surface-binding sites: a proximal and a distal site. Kinetic characterization shows that the engineered CFR-5G5 variant outperforms the wild type, displaying twofold and fourfold increases in catalytic efficiency toward ABTS and Mn(II) oxidation, respectively. In ongoing work, site-directed mutagenesis coupled with kinetic analysis will be used to clarify the structural and functional basis of the electron transfer pathways involved in Mn(II) oxidation by BsDyP and to further demonstrate the effectiveness of directed evolution in enhancing peroxidase performance for lignin valorization and bio-waste reduction.

Keywords: Dye-decolorizing peroxidases; Bacillus subtilis; Mn(II) oxidation; Long-range electron transfer; Directed evolution; Lignin valorization

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Evaluation of the Toxic Potential of New Psychoactive Substances (NPS)

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Abstract

In recent decades, there has been a global increase in the consumption of New Psychoactive Substances (NPS), including in Portugal, however there is a lack of data on their toxicity and long-term effects. Some NPS, namely synthetic cathinones, have been associated with severe episodes of intoxication and fatalities, highlighting the need to characterize their mechanisms of action in human cell models. Although previous studies have determined the toxicity and bioavailability of several cathinones, the cellular mechanisms underlying these effects remain poorly understood. In this context, the present project aims to evaluate and understand the cellular mechanisms involved in the toxicity of selected synthetic cathinones, based on substances identified in illicit markets and in previous studies. The study focuses on primary human fibroblast cell lines, as a non-tumor model, to assess the toxic potential of these NPS.

In vitro assays were performed to evaluate the cytotoxicity of cathinones, with cell viability assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to determine the half-maximalinhibitory concentration (IC_{50}). Ongoing studies aim to investigate the cellular mechanisms of toxicity, including alterations in reactive oxygen species production and membrane integrity. Additionally, changes in the expression of proteins associated with stress responses and cell death will be analyzed, as well as alterations in the metabolomic profile, to identify affected metabolic pathways. The expected results will contribute to a better understanding of the cellular processes affected by exposure to synthetic cathinones and to a more informed assessment of the risks associated with the consumption of these NPS.

Keywords: New Psychoactive Substances (NPS), Synthetic Cathinones, Cellular toxicity, Metabolomic alterations

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Nanotechnology-Enhanced Delivery of Seaweed Bioactive Compounds for Health and Well-Being Applications

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Abstract

Seaweed represents an environmentally sustainable source of bioactive compounds with antioxidants, antimicrobial, and anti-inflammatory properties [1]. Despite this beneficial potential, their applications are limited by poor stability, low solubility and limited skin penetration of these compounds [2].
To address these challenges, nanotechnology emerged as an ideal delivery strategy. Silver nanoparticles (AgNPs) could provide a dual benefit, by acting both as carriers, ensuring bioactives loading and delivery systems. However, AgNPs potential cytotoxicity may restrict biological applications. This problem can be overcome by natural compounds, which may also contribute to additional bioactive effects[3][4]. The main objective of this work is to develop two formulations incorporating AgNPs of Gracilaria sp. red seaweed extract, with anti-inflammatory and antimicrobial properties for dermatological application.
An aqueous extract of seaweed biomass (12.48 % extraction yield) was prepared and subsequently, purified via ethanol precipitation to remove polysaccharides (9.36 % purification yield).The polysaccharide-free extract was characterized showing 4.9 ± 0.3 mg PGE/g of total phenolics content and 28% antioxidant activity via DPPH assay. AgNPs were synthesized using a green method, by reducing silver nitrate with sodium borohydride under magnetic stirring in an ice bath at several extracts concentrations.The average size of the AgNPs and the PdI were evaluated by dynamic laser scattering followed by zeta potential analysis using the electrophoretic light scattering. The nanoformulation containing 25 mg of purified extract was seen as the most effective for synthesis (221.95 ± 57.7 nm; PdI 0.46± 0.03 ;29.9 ± 1.2 mV). This formulation was selected for the ongoing investigation, through the development of a prototype gel and a spray for topic applications. Future work will focus on the physicochemical characterization of the formulations and validation of safety and efficacy, using Artemia salina and Human Dermal Fibroblasts, alongside with antimicrobial tests. Finally, skin permeation and release studies using Franz diffusion cells will be performed.

Keywords: **Seaweed bioactives, silver nanoparticles, green synthesis, antimicrobial activity, anti-inflammatory activity, dermatological nanocarriers**

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Evaluation of Human Messenger RNA Therapy for Restoring Ciliary Function and Motility in Zebrafish Models of PCD

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Abstract

Primary Ciliary Dyskinesia (PCD) is a rare and heterogeneous ciliopathy, most inherited in an autosomal recessive manner. This condition presents a set of symptoms associated with the respiratory system, including mucus accumulation or recurrent respiratory infections, due to impaired mucociliary clearance. Additional manifestations may involve other organs containing motile cilia, namely infertility and subfertility, hydrocephalus, and laterality defects, such as situs inversus, situs ambiguous, and heterotaxy, the latter often associated with congenital heart diseases. Worldwide, 1 in 7,500 individuals is affected, and in 50% of the cases, left-right (LR) axis laterality defects, established during embryogenesis, are observed. Given the rarity of PCD, both diagnosis and treatment remain challenging.

The zebrafish (Danio rerio) model is widely used in biomedical research, due to its amenability to genetic manipulation, embryo transparency, and high genetic similarity to humans (~70%). This organism has been used to study PCD because of its conserved features with humans. The LR organizer in zebrafish, also known as Kupffer's Vesicle, is analogous to the mammalian embryonic node, despite some structural differences, and the ciliated cells of the zebrafish olfactory pit resemble those in the human respiratory tract. For these reasons, and based on previous studies using this model, mutant lines have been generated for some of the approximately 50 identified PCD genes, such as dnah5.

This work aims to (i) characterize dnah5 homozygous mutants in terms of LR development and assess the olfactory pit as a model organ to study multiciliated cell function, and (ii) evaluate the restoration of ciliary motility and beating patterns after bath-administered human mRNA therapy, provided by ETHRIS through a non-commercial collaboration.

Keywords: **Primary Ciliary Dyskinesia, zebrafish model, dnah5 gene, left-right axis development, ciliary motility, mRNA therapy**

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Testing a Ciliary Origin for Cardiovascular Defects in Patients with Down Syndrome

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Abstract

Trisomy 21, also known as Down syndrome (DS), is the most common human chromosomal disorder and is associated with cardiovascular, musculoskeletal, and neurological abnormalities. Individuals with trisomy 21 frequently present with congenital heart defects (CHDs) that arise from errors in embryonic cardiac development, where cilia play an important role. Although the cellular mechanisms are poorly understood, current evidence suggests that disruption of cilia formation and function contribute to the pathogenesis of CHDs. With this knowledge, and the fact that ciliary genes in chromosome 21 are differently expressed in DS, we will test the hypothesis that defects in these genes are important contributors to heart defects found in the majority of DS individuals. Zebrafish (*Danio rerio*) provide a powerful model to investigate these mechanisms as their transparent embryos, rapid external development, fully sequenced genome and molecular accessibility for genetic manipulation allow for detailed examination of ciliary function and heart morphogenesis. This project aims to generate zebrafish models of trisomy 21 to investigate how ciliary gene dysregulation influences cardiac development at the whole-organ level and in the context of inter-organ communication.

Keywords: Down syndrome, trisomy 21, congenital heart defects, ciliary dysfunction, zebrafish model, cardiac development

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Uncovering How the Structure and Function of ETF Variants Shape the Metabolic Disorder MADD

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Abstract

Multiple Acyl-CoA Dehydrogenase Deficiency (MADD, OMIM #231680) is an autosomal recessive disorder leading to an inborn error of metabolism (IEM) that affects fatty acid, amino acid and choline metabolism. This disorder is caused by mutations on the electron transfer flavoprotein (ETF) or ETF:ubiquinone oxidoreductase (ETF:QO), proteins that are crucial to transfer electrons from several dehydrogenases to the mitochondrial respiratory chain. Thus, mutations on their genes lead to compromised mitochondrial β-oxidation and impaired energy production. Clinically, MADD can be presented in a severe neonatal-onset form, which is fatal, as well as in a mild later-onset form, for which molecular mechanisms underlying symptoms and age of onset still remain poorly understood, despite the advancements in genetic diagnosis. Currently, no effective therapy options are available, therefore the lack of knowledge complicates prognosis.

To improve the understanding of the genotype-phenotype relationship and the disease mechanisms on the milder forms of this metabolic disorder, we are assessing the structural and functional impact that the disease variants ETFα:p.L95V, ETFα:p.R122K, ETFα:p.G255V, and ETFα:p.R249C, which map on different structural features of ETF, have on the protein . These studies include spectroscopy analysis (UV-visible absorption, circular dichroism (CD), and fluorescence) to evaluate protein folding and conformational stability, as well as enzymatic assays using Medium-Chain Acyl-CoA Dehydrogenase (MCAD), an enzyme ETF partner.

So far, the data collected shows that the variants ETFα:p.L95V and ETFα:p.R122K are wild-type like, but the ETFα:p.G255V, which is located near the FAD binding site, presents significant changes in ETF conformation, cofactor binding and function. At the end the experimental results obtained within this project will be combined with computational studies already performed by the host lab, and with clinical information available in the literature aiming to improve knowledge on mild MADD.

Keywords: Metabolic Disorder; Metabolism; Protein Folding; Biophysical methods; FAD

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Impact of a Nutritional Approach on Obesity-Induced Hepatic Dysfunction

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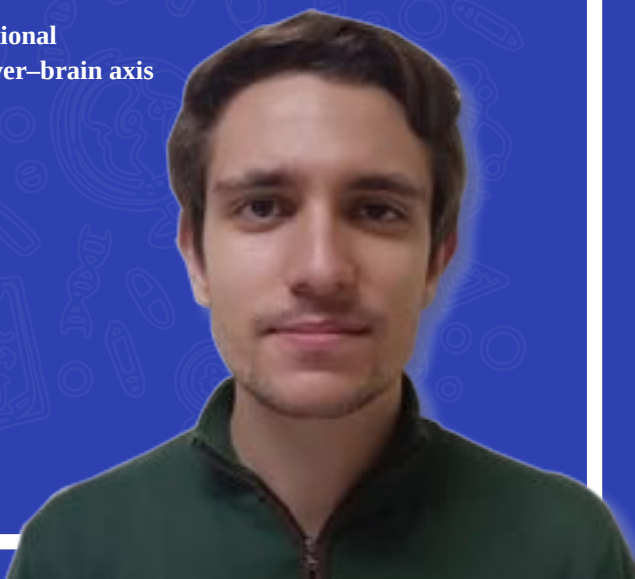
Abstract

Obesity has emerged as one of the most critical public health challenges worldwide, being strongly associated with metabolic dysfunctions and chronic liver diseases, particularly non-alcoholic fatty liver disease (NAFLD). Excessive hepatic lipid accumulation initiates a cascade of oxidative stress, inflammatory responses, and fibrotic remodeling, promoting the progression from steatosis to non-alcoholic steatohepatitis and, in advanced stages, cirrhosis. Although pharmacological therapies such as glucagon-like peptide-1 (GLP-1) receptor agonists have demonstrated efficacy in weight reduction and improvement of liver function, their widespread use is limited by high cost and relevant adverse effects, including gastrointestinal disturbances and potential associations with pancreatitis and endocrine deregulations. Therefore, safe and accessible alternatives are urgently needed.

Nutritional interventions represent a promising strategy to prevent and reverse metabolic and hepatic alterations induced by excessive caloric intake. Preclinical evidence indicates that specific dietary approaches can modulate hepatic lipid metabolism, attenuate oxidative stress, and suppress inflammatory signaling, thereby promoting liver recovery. In parallel, obesity-associated liver dysfunction is increasingly recognized to be interconnected with neurobiological alterations affecting feeding behavior, reward processing, and energy homeostasis. Understanding the bidirectional communication between the liver and the brain is thus essential for developing integrative therapeutic strategies.

The present project aims to analyze biochemical, histological, and molecular markers of hepatic function, as well as glucose and lipid metabolism, in obesity under a nutritional intervention, and to compare its effects with those of GLP-1 analog treatment. Hepatic lipid content and the degree of steatosis, inflammation, and fibrosis will be evaluated histologically. Key molecular pathways involved in lipid metabolism, oxidative stress, and inflammation will be assessed through western blot and immunofluorescence analyses. In addition, modulators of hepatic metabolism, including adenosine, will be quantified by HPLC. These hepatic and metabolic parameters will be correlated with neurobehavioral outcomes assessed through in vivo behavioral tests of memory, learning, anxiety, and stress.

Keywords: **Obesity, non-alcoholic fatty liver disease, nutritional interventions, GLP-1 receptor agonists, hepatic lipid metabolism, liver–brain axis**



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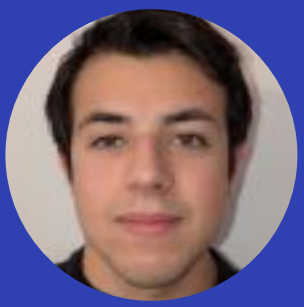
P15	Mariana Guerreiro Antunes
P16	Miriam Rodrigues Veloso
P17	Rúben Filipe Gavela da Silva
P18	Salomé Avelar Martins
P19	Tiago João Garcia Gregório
P20	Vítor Manuel Lapa Fernandes

Organizing Commitee

Cristiano Rocha

Ana Estrela

Diogo Velez



João Matias

Pedro Lourenço

Salomé Martins

Joana Ratado

Mariana Ventura

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Message from the Programme Coordination

The MSc in Biochemistry and Biomedicine (MBB) at the Faculty of Sciences of the University of Lisbon represents a consolidated and forward-looking training programme in the biomedical sciences. Built on a strong academic tradition in Biochemistry, the MBB has evolved to fully embrace the interdisciplinary and research-driven nature of modern biomedicine, offering students an intellectually demanding and highly stimulating educational environment.

A defining feature of the programme is its strong integration of original research developed in high-quality academic laboratories. Through their dissertation projects, students acquire advanced scientific and technical expertise while developing the broad set of transferable skills that are intrinsic to scientific practice and essential in any demanding professional context, including critical thinking, effective communication, problem-solving, teamwork, autonomy, adaptability and lifelong learning.

The Annual MSc Meeting, now in its 8th edition, is a flagship activity of the programme and a key component of this integrated training model. It provides students with hands-on experience in the organisation of a scientific event, opportunities to communicate their research to a broad audience, and a platform to expand their professional networks. The 2024–2026 cohort has demonstrated outstanding commitment and professionalism, playing an active role in shaping the scientific programme and in the preparation of this book of abstracts.

The abstracts presented in this volume showcase the scientific quality, diversity and ambition of the work developed within the MSc in Biochemistry and Biomedicine. They also reflect the profile of our graduates: well-trained, critically minded and prepared to excel in the next stage of their professional careers. We hope this book will serve as a compelling showcase of the excellence and impact of the training provided by the MBB for both prospective students and future employers.

Margarida Gama Carvalho (Coordinator)
Rodrigo Almeida
Bárbara Henriques