Università
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Luigi Vanvitelli

PhD Days 2024

PhD Programme in Biomolecular Sciences

Structural biology and protein function

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Tutor: Annamaria Sandomenico

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Martina Slapakova
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Vincenzo Massimiliano Vivenzio
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Awet Ghebretinsae Tewelde
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Tutor: Roberto Fattorusso

Domenico Sgambati
Zinc Finger Proteins: Roles and Functions in Eukaryotic and Prokaryotic Systems
Tutor: Paolo Vincenzo Pedone

Eunice Wairimu Maina
Effects of Plastic on Human Gut Bacteria
Tutor:Gaetano Malgieri

Ivan Mercurio
Disrupting protein-protein interactions involving PED/PEA15
Tutor: Roberto Fattorusso

Maryam Kamarehei
Design neuroprotective peptides against prion diseases
Tutor: Luigi Russo

Nataliia Ventserova
Insights into the formation of transient oligomeric species involved at the initial stages of Prion protein fibrillation
Tutor: Roberto Fattorusso

Bartosz Malinski
Use of Nuclear Extracts to study Cohesin at Single Molecule level
Tutor: Gijs Wuite

Linsheng Zhang
Role of the DDX11 DNA helicase at the interface between DNA replication and chromosomal cohesion
Tutor: Francesca Pisani

Maria Giuseppina Campanile
Purification of hortensins, type 1 ribosome inactivating proteins from the seeds of *Atriplex hortensis* L. *var. rubra*
Tutor: Antimo Di Maro

Mario Privitera
Alternative strategies against *Klebsiella pneumoniae* infection through structural studies

Tutor: Rita Berisio

Martina Filocaso
Advances in Galectin Research: new insights into Gal-3 interactors and Gal-7 characterization
Tutor: Michele Saviano

MohammadHossein Mosalaeizadeh
Bioinformatics analysis outlines the diffusion of a novel H-NS-like protein family largely present in Proteobacteria
Tutor: Roberto Fattorusso

Polina Selkova
"*In vitro* reconstitution of the *de novo* human cohesin loading pathway operating at the DNA replication fork"
Tutor: Francesca Pisani

Salvatore Mottola
Sustainable Ultrasound-Assisted Solid Phase Peptide Synthesis (SUS-SPPS)
Tutor: Anna Messere

Cancer Biology, Drug design, Immunology and Microbiology

Beatrice Cavalluzzo
Novel Immunological targets for Hepatocellular Carcinoma based on molecular mimicry
Tutor: Luigi Buonaguro

Carmela Casale
GADD45β interferes with the dual RIPK3-driven activation of RHIM-dependent NF-kB signaling and necroptosis
Tutor: Alessandra Pescatore

Ida De Chiara
Lactococcus lactis cell-free supernatant inhibits GBM cell line proliferation and maintains blood-brain barrier integrity
Tutor: Lidia Muscariello

Ilaria Mottola
Interleukin-4 inhibits the inflammatory cascade leading to villous atrophy in potential celiac disease
Tutor: Carmen Gianfrani

Pouria Savadi Someeh
Unraveling the role of poly (vinyl alcohol) in developing mucus- and biofilm-penetrating PLGA nanoparticles for pulmonary delivery of antimicrobial peptides
Tutor: Ivana D'Angelo

Saba Sadiq
Riboflavin production by a mutant *Limosilactobacillus fermentum* in vegetable beverages
Tutor: Donatella Cimini

THE PHUC NGUYEN
Human cell-based assays and advances in endotoxin analysis
Tutor: Paola Italiani

Davida Mirra
You are what you breathe: the impact of cigarette smoke and nanoplastics on airways disease
Tutor: Bruno D'Agostino

Giuseppe Ciccone
Selection of a novel RNA aptamer selectively targeting NSCLC-derived CAFs
Tutor: Silvia Catuogno

Iolanda Camerino
Unlike cisplatin, temozolomide inhibits migration and vasculogenic mimicry of glioblastoma cells
Tutor: Generoso Luca Colucci D'Amato

Marco Barretta
Nanomaterials-based strategies for the treatment of hepatocellular and colorectal cancer

Tutor: Assunta Borzacchiello

Milena Della Gala
Investigating the *Mycobacterium smegmatis TetR_3765* regulon
Tutor: Lidia Muscariello

Muhammad Waqas
AI-Driven Tool for Predicting Thermostability-Enhancing Mutations in GPCRs through Integrated Machine Learning Models
Tutor: Sandro Cosconati

Tinghao Liu
The innate immune memory of mast cells
Tutor: Diana Boraschi

Vincenzo Mazzarella
CXCR4 and FAP-1 as promising molecular targets for early-stage cancer diagnosis and treatment
Tutor: Salvatore Di Maro

Wenjie Yang
The innate memory molecular mechanism of monocytes and macrophages
Tutor: Diana Boraschi

Wenli Shi
Anti-tumor mechanism of natural mushroom polypeptide Gymnopeptide A
Tutor: Paola Italiani

Andrea Casale
Inhalable nanoparticles delivering peptidomimetic/antibiotic combinations for local treatment of CF lung infections
Tutor: Ivana d'Angelo

Fareeha Amjad
Targeting of the CHCHD4 import pathway for Neuroprotection
Tutor: Nunzianna Doti

Gaetano Caputo
Design and characterization of cyclodextrin complexed drugs and natural compounds against bovine coronavirus
Tutor: Carla Isernia

Michele Roggia
From algorithms to molecules: the identification of riboswitch-targeting compounds
Tutor: Sandro Cosconati

Mohammad Shaukat Ali
Targeting pharmacologically relevant anti-cancer agents by employing AI based methods PyRMD and PyRMD2Dock
Tutor: Sandro Cosconati

Roberto Cutolo

Development of amphiphilic dendrimers for targeting CXCR4/αvβ6/αvβ8 overexpressing cancers
Tutor: Salvatore Di Maro

Sohaib Bin Shabbir
Optimization of Microbial Melanin Production from *Streptomyces* strains
Tutor: Donatella Cimini

Suyin GE
Assessing systemic and mucosal immunity and predicting modulation of responses in the elderly and diseased population against future infections
Tutor: Diana Boraschi

Innovative omics technologies in biomolecular sciences

Chidoh Kootlole
NMR-based metabolomics and anti-leukemic activity of plants used in traditional medicine in Botswana
Tutor: Monica Scognamiglio

Domenico Romano
Specialized metabolites from natural sources as lead compounds to fight against emerging diseases
Tutor: Antonio Fiorentino

Mercy Ebunoluwa, Ayinde
Antibacterial Activities of Selected Nigerian Plants Against Clinically Important Human Pathogens
Tutor: Brigida D'Abrosca

Alessandro Giaquinto
Secretomic signatures in diagnostic stewardship for sepsis
Tutor: Prof. Angela Chambery

Angela Sorice
NMR-based metabolomics to discover bioactive cycloartane glycosides from *Astragalus* species
Tutor: Monica Scognamiglio

Carlo Raucci
Characterization and biological evaluation of *Trichoderma spp.* Metabolites
Tutor: Brigida D'Abrosca

Enza Canonico
Unravelling the TR3-56 cell proteome by Tandem Mass Tag-Based High-Resolution LC-MS/MS
Tutor: Angela Chambery

Erika Truppo
Bioactive small molecules from plants as ligands of DNA secondary structures
Tutor: Antonio Fiorentino

Federica Farinella
Machine Learning and Network Analysis for Biomarker Discovery in Neurodegenerative Diseases
Tutor: Prof. Bruna De Felice

Cellular and molecular bases of human diseases

Antonietta Esposito
Identify molecular pathway regulating cell proliferation through glycosphingolipids biosynthesis
Tutor: Seetharaman Parashuraman

Concetta Montanino
Non-coding RNAs as versatile regulators in Neurodegenerative Diseases (NDDs). A focus on MS (Multiple Sclerosis)
Tutor: Bruna De Felice

Debora Latino
Investigation of the molecular mechanisms activated in Leydig and Sertoli cells by D-Asp treatment to sustain steroidogenesis and spermatogenesis
Tutor: Maria Maddalena Di Fiore

Ezia Spinosa
Identification of Candidate Markers linked to Autoimmunity in Incontinentia pigmenti
Tutor: Francesca Fusco

Ilenia De Leo
Transcriptomic approach for studying miRNAs roles
Tutor: Nicoletta Potenza

Lucia Argenziano
Exploring the impact of the maternal-effect gene *Padi6* on female fertility, embryogenesis, and epigenetic reprogramming in mice
Tutor: Andrea Riccio

Maria Carannante
Reproductive cytotoxic and genotoxic impact of polystyrene microplastic on *Paracentrotus lividus* spermatozoa
Tutor: Lucia Rocco

Nagendra Sai Kumar Achanta
Regulatory mechanism and functional impact of Post-Translational Modifications on Human Paraoxonase2
Tutor: Manco Giuseppe

Nunzia Magnacca
miR-18a-5p reduces lipid accumulation by regulating SREBP1c expression *in vitro* and *in vivo NAFLD* models: link with ER stress response
Tutor: Antonia Lanni

Angela Pagano
Multiomic analysis of maternal *Padi6*-deficient blastocysts
Tutor: Flavia Cerrato

Anna Truda

Identification of genomic and epigenomic biomarkers in liquid biopsy by Next-Generation Sequencing in prostate cancer
Tutor: Nicoletta Potenza, Giovanna Marchese

Arif Mahmood
De novo variants in a gene encoding a *histone* underlie a novel Neurodevelopmental Disorder
Tutor: Manuela Morleo

Brunella Mongiardi
Identification of sex-specific therapeutic strategies in aging and dementia
Tutor: Elvira De Leonibus

Cristina Somma
Pharmacological stimulation of autophagy to rescue proteinopathy and cognitive decline in lysosomal storage disorders
Tutor: Elvira de Leonibus

Emilia D'Angelo
Omics analyses to molecularly characterise a large cohort of Silver-Russell Syndrome patients
Tutor: Flavia Cerrato

Giulia Grillo
Polystyrene microplastics impair steroidogenesis by inducing mitochondrion-endoplasmic reticulum dysregulation
Tutor: Alessandra Santillo

Isar Yahyavi
Serum D-serine Correlates with Age and Treatment in Parkinson's Disease
Tutor:Alessandro Usiello

Mariagrazia Di Gennaro
Unravelling the role of the TGN export machinery for basolateral proteins in Amyloid Precursor Protein (APP) transport and processing
Tutor: Alberto Luini

Pasquale Di Letto
Long read sequencing for elusive pathogenic variants
Tutor: Vincenzo Nigro

Raffaella di Vito
Untangling the intricate networks shaped by D-Asp in the developing brain
Tutor: Alessandro Usiello

Sarah Iffat Rahman
Optimizing Long-Read Sequencing Pipeline for Rare Disease Diagnostics
Tutor: Prof. Vincenzo Nigro

Giovanni Vicidomini
LANCELOT: A Molecular Newborn Screening of Treatable Conditions
Tutor: Vincenzo Nigro

Hira Khan
The effect of palmitoleic acid, an exerkine during fasting, in muscle cells
Tutor: Pieter de Lange

Ilaria Palmieri
Combined effect *in vitro* of TiO_2 nanoparticles and polystyrene microplastics on human spermatozoa
Tutor: Lucia Rocco

Ivana Milas
Development of stem cell-based models of cohesinopathies in vitro

Tutor: Marina Tarunina

Maria Ventriglia
Mitochondrial Unfolded Protein Response (UPR^{mt}) is involved in maintaining BAT mitochondria functionality of obese mice treated with 3,5-diiodothyronine (T2)
Tutor: Rosalba Senese

Maria Zawadzka
Gaining insight into the role of long noncoding RNA in inherited retinal disorders
Tutor: Sandro Banfi

Mariaceleste Pezzullo
A novel competing endogenous RNA network involving the lncRNA JPX, miR-378a-3p and its mRNA targets in lung adenocarcinoma
Tutor: Aniello Russo

Maria Elena Truppa
Morphological and functional characterization by preclinical imaging techniques of genetically modified mouse models for D-aspartate oxidase
Tutor: Prof. Alessandro Usiello

Nicole Lara
Role of Thyroid hormone (T3) in muscle metabolic signaling and differentiation
Tutor: Pieter de Lange

Sabrina Gargiulo
Investigating the role of the phosphatase SHP-1 in regulating cellular senescence
Tutor: Alessia Varone

Subham Saha
Methylation profile of the imprinting control regions and their variability in the normal population
Tutor: Andrea Riccio

Session 1:
Structural biology and protein function

Structural and functional characterization of the recombinant FVII of coagulation

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Session: Structural biology and protein function

Administration of recombinant Factor VII (rFVII) is a first-line therapy for treating patients with haemophilia and inhibitors. rFVII is a post-translationally modified (PTM) protein currently produced in mammalian cell lines (CHO or BHK). During purification, rFVII self-converts into its active form rFVIIa, the product administered to patients not without safety concerns.

We successfully expressed full-length FVII in human cells (EXPI 293). With the aim of obtaining an alternative to rFVIIa, we explored the possibility of obtaining a full-length product with the PTMs necessary for the protein to trigger the coagulative cascade initiated by its binding to Tissue Factor (TF). The protein structure and biological activity have been assessed via spectroscopic studies and functional assays.

To explore alternative, cost-effective purification methods that prevent rFVII self-activation, we generated non-inhibitory small synthetic ligand (F7-SPL) for use in affinity chromatography settings. F7-SPL immobilized on Sepharose 4B is able to capture rFVII from crude cell supernatants with promising enrichment yields.

To study the interaction between TF and FVII, several cyclic and bicyclic peptides were designed to mimic specific segments of TF extracellular domain. Peptides composed by spatially close TF segments at the interface with FVII, but distant in the primary sequence were joined to form single polypeptides which were cyclized or bicyclized following different folding strategies. Preliminary experiments performed with the synthetic molecules suggest that these peptides bind rFVII and inhibit its TF-mediated activation.

Development and application of new active packaging to preserve beneficial biomolecules to reduce fruit losses

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Session: Structural biology and protein function

Fruit is an important source of nutrients such as vitamins, fiber, and biologically active compounds, which levels can be influenced by abiotic and biotic factors. However, after harvest, fruits incline to deteriorate rapidly due to physiological and biochemical processes, leading to significant food losses. This project focuses on the development and application of eco-friendly and edible formulations, as coatings or films, to extend the shelf life of both whole and minimally processed fruits, thereby reducing food waste and preserving their valuable nutritional and health benefits. The project targets apples and pears proposed for the fresh-cut industry, while cherries have been selected as whole fruits, which are particularly perishable. In this research, two types of coatings and an active film have been developed. The first bi-layer coating, made of sodium alginate, carboxymethylcellulose, oxalic acid, and citric acid, was applied to 'Williams' pears and 'Annurca Rossa del Sud' apples minimally processed, 'Della Recca' cherries. The results showed that the coating effectively preserved fruit quality by enhancing antioxidant system, both enzymatic and non-enzymatic, and by preventing enzymatic browning, lipid peroxidation, and cellular membrane damage. Additionally, a slowdown in the senescence processes was observed in fresh fruits. Similar results were obtained with the other coating made of xanthan gum and chitosan applied to minimally processed 'Coscia' pears.

Moreover, in response to the growing demand for biodegradable packaging to replace non-eco-friendly ones, a biodegradable film made of polylactic acid and encapsulated lemongrass essential oil was developed. Encapsulation proved to be an effective method for reducing the direct impact of the essential oil on the fruit and protecting it from light and high temperatures, which could degrade it. The results indicated that the film extended the shelf life of minimally processed 'Fuji' apples, preserved the content of active biomolecules, reduced enzymatic browning and lipid peroxidation during cold storage, and provided both antioxidant and antimicrobial effects.

Strategies for fluorescent labelling of DNA-binding proteins in single-molecule biophysical studies

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Session: Structural biology and protein function

Correlative optical tweezers fluorescence microscopy (CTFM) is a powerful tool to visualise fluorescent proteins while they bind to and translocate onto DNA/RNA, providing high-resolution data about the forces involved during their interaction with the nucleic acid molecules. My objective has been to develop a strategy for the fluorescent labelling of DNA-binding proteins under investigation in my laboratory: the DNA helicases DDX11 and FANCJ and the proliferating cell nuclear antigen (PCNA). I adopted an enzymatic labelling protocol, covalently attaching small organic fluorophores to a short ybbR tag fused to the protein of interest. Insertion of the ybbR tag alone was enough to abolish the DNA helicase activity of FANCJ, while fluorescent ybbR-tagged DDX11 fully retained its activity. However, the DDX11 labelling efficiency was found to be extremely low. The same strategy was applied to fluorescent label PCNA. This protein can be easily produced in *Escherichia coli* with higher yield and purity compared to DDX11 and FANCJ, purified from transiently transfected mammalian cells. This allowed me to refine the labelling protocol, resulting in higher labelling efficiency. The labelled PCNA was then loaded by the replication factor C onto a single/double stranded DNA junction and visualized with the C-Trap system. In conclusion, the SFP-mediated protein labelling system is a versatile and powerful tool, but not suited for proteins produced with low yield, requiring extensive post-labelling purification steps.

Unmasking Viral RNA: targeting viral RNA capping machinery to tackle COVID-19 and future CoV emergencies

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Session: Structural biology and protein function

SARS-CoV-2 is a member of the Betacoronavirus family along with SARS-CoV-1 and MERS-CoV. These viruses have similar genome and replication strategies but differ in their pathogenicity for humans. The Replication-transcription complex (RTCs) is one of the vital machinery that enables the virus to recruit diverse proteins to maintain its life cycle. The nonstructural protein 10 is the central player in the RTCs, and represent an attractive therapeutic target for development of peptide and peptidomimetic therapeutics (PPTs) capable of inhibiting the viral RNA capping. By means of computational alanine scanning method, we have evaluated the binding mutation energy of NSP10/NSP16 and NSP10/NSP14 complexes, and unveiled the hotspots for the formation of such complexes. Along with identification of the conserved protein domain features on NSP10 across corona viruses, a cluster analysis of all the available PDB structures allowed us to highlight their structural and conformational changes. The design of PPTs and the biophysical characterization of NSP10 is in progress. Additional projects: we studied cytotoxicity and insulin sensitizing effects of Cuccumis prophetarum extracts in HepG2 and insulin resistance-L6C5 cells. Moreover, by integrating experimental and in silico data we also reported the molecular determinants of the interaction of β-CD with different pentapeptides bearing a tyrosine residue in one of the five different positions of the chain.

Biocompatible polymers for human health

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Session: Structural biology and protein function

My PhD project has focused on the production and biophysical characterisation of biocompatible polymers, including cellulose and important proteins for human health. In collaboration with the company K4B, we set up new protocols for the production of biocellulose of bacterial origin, with important for human health care applications. A strong focus of the second and third years was the production and biophysical characterisation of proteins involved in human pathogeneses. I studied the biochemical and biophysical features of the protein HtpG from M. tuberculosis. HtpG is an important protein involved in protein folding and, beside its biological role for M. tuberculosis pathogenesis,it is a promising vaccine against tuberculosis infection. Starting from our previous work, I managed to grow well diffracting crystals, to be used for structure determination using x-ray crystallography. Diffracting crystals of HtpG were obtained in complex with AMP-PNP, a non-hydrolysable form of ATP. Diffraction data were collected at ESRF synchrotron in Grenoble and the structure was solved using molecular replacement techniques. Using the newly acquired structural information and the data available from the literature, we also proposed a model for the orchestrated chaperone machinery of M. tuberculosis. Using the knowhow I acquired during my PhD I could also contribute to the understanding of key host-pathogen interaction processes involved in the infection by SARS-CoV-2.

Melleatin, a multitasking enzyme with rRNA N-glycosylase and nuclease activity from *Armillaria mellea* fruiting bodies

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Session: Structural biology and protein function

Armillaria mellea (Vahl) P. Kumm. is a basidiomycete fungus of the order Agaricales (1). It is widely distributed in the Northern Hemisphere temperate regions and can cause root-rot in different plant species (2-3). It is edible after proper cooking and rich of therapeutic compounds, enzymes/proteins and bioactive peptides with health-promoting effects and beneficial biological activities (4).

During the last year of my PhD, I performed a plethora of approaches to obtain the structural and enzymatic characterization of melleatin, a novel multitasking enzyme from *A. mellea* fruiting bodies. Melleatin is a basic, glycosylated and monomeric protein (17.5 kDa) isolated as an inhibitor of protein synthesis. This enzyme damages ribosomes like ribosome inactivating proteins (RIPs), being endowed with rRNA N-glycosylase activity.

Moreover, it exhibits nuclease activity and belongs to the His-Me finger endonucleases superfamily, having a fold like the biofilm-dispersing nuclease NucB isolated from the marine strain *Bacillus licheniformis* (5). Furthermore, the antibiofilm activity of melleatin confirms its structural and enzymatic similarity with NucB.

Additionally, melleatin possesses antifungal activity and is not cytotoxic, being unable to cross the cell membrane.

Overall, melleatin represents a novel biotechnological tool for its antibiofilm and antifungal activities or as a toxic component of biomedical bio-constructs.

Microbial and human lactonases for the control of virulence factors in pathogenic bacteria

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Session: Structural biology and protein function

The rise of multi-drug resistant (MDR) pathogenic bacteria has become a major public health concern in recent decades. Biofilm communities may serve as reservoirs for MDR bacteria. One strategy to combat these infections is degrading quorum sensing (QS) signals using lactonases. Our focus is on Phosphotriesterase-Like Lactonases (PLLs), initially studied as phosphotriesterases but later found to be primarily lactonases. We concentrated on AhlA, a PLL from *Rhodococcus erythropolis*. The AhlA enzyme was obtained from a synthetic his-tagged gene, expressed in *E.coli*, purified, and characterized. AhlA demonstrates high thermophilicity, thermostability, a long shelf life at 4°C, and stability under oxidizing conditions. The enzyme effectively quenches quorum sensing by hydrolysis of acyl-homoserine lactones, such as 3oxo-C12-HSL and C4-HSL, thereby inhibiting *Pseudomonas aeruginosa* (PAO1) biofilm formation. To enhance the impact on PAO1 biofilm, a formulation combining three enzymes: human rPON2, microbial his-AhlA, and SsoPox 4Mut was developed. This formulation was tested in wound healing assays on immortalized HeLa cells in vitro and on infected wound skin in vivo using CD1 mice. The results were encouraging, showing improved wound closure, a significant reduction in biofilm formation, and a decrease in bacterial cell numbers within the wound, suggesting potential for an effective treatment.

Experimental and computational methods to explore non covalent interactions in metal coordinating proteins and host-guest β-CD complexes

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Session: Structural biology and protein function

Understanding the three-dimensional structures of biomolecules, such as proteins, is essential in structural biology for elucidating their mechanisms of action and interactions. This field combines experimental techniques and theoretical methods, which are interdependent; experimental data inform theoretical models, and these models are refined based on experimental findings.
My Ph.D. research activity focused on two models of complexes stabilized by non-covalent interactions: metal ion-protein coordination compounds and host-guest complexes. First, we examined Ros, a zinc finger protein involved in horizontal gene transfer from *Agrobacterium tumefaciens* to its host plant. Ros contains a single Cys_2-His_2 zinc finger domain and adopts a βββαα fold stabilized by a metal cofactor and a hydrophobic core of 15 amino acids. The Ros/MucR gene, conserved across proteobacteria, is key to host-bacterium interactions. Sequence alignment revealed that in some homologs, the second cysteine is replaced by aspartate, leading us to study the Ros87C27D mutant to understand this substitution's impact on metal-binding and structural stability. For the second system, we investigated β-cyclodextrin (β-CD) inclusion complexes with model pentapeptides, analysing their structures using UV-Vis and NMR spectroscopy, as well as molecular docking. Insights into residue positioning in the sequence drive the design of effective peptide-CD complexes, valuable in drug delivery systems.

Purification, structural and functional characterization of FV zymogen from plasma fraction

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Session: Structural biology and protein function

Factor V (FV) plays a crucial role in the coagulation process by promoting the conversion of fibrinogen to fibrin with FXa and its deficiency can cause severe coagulopathies. To date, the only available treatment for patients with FV deficiency is fresh frozen plasma due to the complete absence of specific products. The objective of this project is to develop and characterize a stable FV zymogen concentrate potentially suitable for clinical trials. We focused on the development of a procedure for the isolation and/or enrichment of FV zymogen from human plasma fractions at laboratory scale and on its analytical, structural and functional characterization. A first purification process developed included several steps including delipidation, immunoglobulin depletion and size exclusion chromatography (SEC). However, the tests performed did not lead to the isolation of sufficient quantities of FV for subsequent analyses. Therefore, we improved the process by minimizing the purification steps. The new process includes only two purification steps: affinity chromatography on heparin columns and SEC. This allowed us to obtain sufficient quantities of FV for structural and functional studies. The resulting samples were characterized by SDS-PAGE, western blotting and mass spectrometry. In addition, to develop antibodies specific for the FV zymogen, which can be used as baits for the capture of the protein directly from plasma, we developed peptides that mimic the FV regions for the screening of phage display antibody libraries.

Galectin-3: an ever fashionable protein

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Session: Structural biology and protein function

Galectin-3 (Gal3), a member of the lectin protein family, binds glycans with β-galactoside bonds and is often elevated in cancers, making it a therapeutic target. With this in mind, the project explores LactoDHI, a derivative of 5,6-dihydroxyindole (DHI), the key building block of eumelanin, as a potential Gal3 inhibitor. LactoDHI, modified with a lactose unit to enhance the solubility, exhibits self-assembly properties that may sequester exceeded Gal3. The interaction between recombinant Gal3 ($Gal3^{CRD}$), produced in house, and LactoDHI, in monomeric and polymeric forms, was studied using DLS, ITC, and BLI. Results suggest LactoDHI's dynamic nature offers promise for designing novel Gal3 inhibitors.

During the period abroad, the research focused on using the host laboratory's expertise in human cerebral organoids to study neurodevelopment. The aim was to analyse the impact of Gal3 on the proliferation and differentiation of neuronal progenitors. It was done by overexpressing and inhibiting Gal3. The study provided insights into how modulation of Gal3 levels affects the behaviour of neuronal progenitors and pave the way for future research in this area.

Defining the Roles of MucR and MucR2 in Symbiotic Regulation and DNA Structuring in *Sinorhizobium meliloti*

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Session: Structural biology and protein function

Proteins of the MucR/Ros family are crucial in bacterial infection or symbiosis with eukaryotic hosts. In *Sinorhizobium meliloti*, MucR regulates symbiosis with the host plant *Medicago sativa*. Our study present the first mass spectrometry-based characterization of MucR purified from *S. meliloti* and demonstrate that, under native expression conditions, this protein forms higher-order oligomers as shown by SEC-MALS analysis. Our EMSA assays provide an in-depth characterization of the DNA targets recognized by MucR in *S. meliloti* and demonstrate that MucR binds AT-rich sequences without a specific consensus. We identified the intergenic region upstream the *ndvA* gene, coding for cyclin beta-1,2-glucan transporter, as a new MucR target. Additionally, an *in vitro* DNA-bridging assay confirmed the ability of MucR to form molecular bridges between DNA strands, crucial for genomic loop formation which aids DNA compaction, similarly to the well-known Histone-like Nucleoid Structuring (H-NS) protein involved in nucleoid structuring in *E. coli*. These findings establish MucR from *S. meliloti* as a member of a new family of H-NS proteins, thus explaining the multifaceted role of this protein in many species of alpha-proteobacteria.

Using a proteomic approach, we identified a second MucR/Ros family regulator, MucR2, in the *S. meliloti* proteome. Despite possessing structural elements for oligomerization, MucR2 remains monomeric in solution, precluding molecular bridge formation—a key feature of H-NS proteins. This suggests that MucR2 may have a different role in nucleoid structuring, possibly functioning as a DNA-bender. Overall, our findings deepen the understanding of transcriptional regulation related to symbiosis in *S. meliloti*.

Targeting Bacterial Carbonic Anhydrases: Identification, Structural Characterization, and Inhibition Studies in *Acinetobacter baumannii*

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Session: Structural biology and protein function

Given the pressing need to discover new antimicrobial targets, carbonic anhydrases (CAs) have emerged as a highly promising area of research. Targeting bacterial CAs presents a novel and innovative approach to antimicrobial drug development. In fact, the inhibition of these enzymes can disrupt bacterial homeostasis and metabolic functions, ultimately reducing virulence and inhibiting bacterial growth.

In this study, we describe the identification and characterization of three γ- and one β- recombinant CAs from the pathogenic bacterium *Acinetobacter baumannii* strain AYE. Upon the cloning of synthetic genes in proper plasmids, we heterologously expressed in *E. coli* systems the recombinant proteins and purified them using chromatographic techniques. Once sufficiently pure samples were obtained, proteins underwent biochemical and structural analysis through spectroscopic and crystallographic methods. Additionally, in collaboration with University of Florence, we performed kinetic assays evaluating kinetic parameters and inhibition profiles against a series of anionic and sulfonamide inhibitors.

In conclusion, we have characterized by structural and biochemical points of view, for the first time, several *A. baumannii* CAs, providing detailed insights into their properties. Results enhance our understanding on their functional mechanisms, inform future research directions in designing specific inhibitors that could regulate their activity, and offer new approaches for combating bacterial resistance.

The evaluation of in vitro genotoxicity and cytotoxicity of polystyrene nanoparticles on HeLa cells and of molecular basis of the processes involved

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Session: Structural biology and protein function

Humans are continually exposed to nanoplastics through ingestion, inhalation, and dermal contact. Our research aims to investigate how polystyrene nanoplastics (PNPs) interact with HeLa cells to understand the health risks associated with PNPs at cellular and molecular levels. We utilized the CBMN (cytokinesis block micro-nuclei) assay to evaluate the genetic damage caused by PNPs on HeLa cell lines. Cell viability and PNPs impact on cell cycle were assessed using flow cytometric analysis. Furthermore, we identified double-strand breaks in DNA by analyzing the phosphorylation of the γH2AX protein. Our findings showed that PNPs can penetrate HeLa cell nuclei at low doses, causing immediate DNA damage. We consistently observed genotoxic effects at all concentrations and time intervals, including the formation of multi-nuclei and micronuclei. Higher PNP concentrations also resulted in substantial cellular damage, cell death, and the presence of apoptotic cells, indicating acute cytotoxic impact. Overall, PNPs possess a remarkable ability to overcome cellular barriers, including the nucleus, and make direct contact with DNA, ultimately leading to genotoxic and cytotoxic effects. In our upcoming experiments, we will delve into the mechanistic insights of genotoxicity and study proteins involved in the process, shedding light on the carcinogenic impact of polystyrene nanoplastics.

Zinc Finger Proteins: Roles and Functions in Eukaryotic and Prokaryotic Systems

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Session: Structural biology and protein function

ZNF639, eukaryotic transcription factor, contains nine Cys_2His_2 zinc-fingers in the C-terminal region and a nuclear localization sequence at the N-terminus. ZNF639 plays a role in cancer. We demonstrated that ZNF639 interacts with the POZ-ZF protein ZBTB2. Analysing the interactome of both ZNF639 and ZBTB2, our attention has been drawn to two common interactors: Sm-D3 and Sm-F. These molecules are part of the Sm complex, a core component of snRNPs essential for splicing. This data suggests a potential role for ZNF639 and ZBTB2 in this cellular process. We plan to validate these interactions by immunoprecipitating Sm-D3 and Sm-F.

Zinc-finger proteins are also present in prokaryotes. Ros/MucR family comprises proteins involved in the regulation of the expression of genes involved in infection of and symbiosis with eukaryotic hosts. These proteins preferentially bind AT-rich DNA sequences by the C-terminal domain folding around zinc. The N-terminal domain mediates the formation of high-order oligomers allowing to bridge DNA forming DNA:protein:DNA complexes. We have shown that members of this family work as H-NS-like proteins. We recently identified a new Ros/MucR family member, MucR2 from *S. meliloti* by mass spectrometry. MucR2 contains a prolonged random coiled N-terminus and lacks a crucial residue for oligomerization at position 64. Light scattering analysis indicates MucR2 cannot form high-order oligomers and binds less efficiently to AT-rich DNA sequences. These findings have led us to classify MucR2 as an atypical member of the Ros/MucR family.

Effects of Plastic on Human Gut Bacteria

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Session: Structural biology and protein function

The intestinal microbiota is the complex microbial community that plays indispensable roles in organism development and human wellness. The balance of these bacteria is crucial for various physiological functions. Among the commonly known probiotics, Lactobacilli and Bifidobacteria are the most studied. Plastics in various forms have become a key threat to the environment as well as to human health. One of the many routes the plastics find their way to the human body is through ingestion which might impact gut microbial community negatively. Despite the many studies on their effects on bacteria, there is scarce information regarding the effects of plastics on human gut microflora. In this context, we used teflon (TEF) and nanopolystyrene (NP) to study the influence of plastic nanoparticles on probiotics (*Lactobacillus plantarum, Lactobacillus rhamnosus and Bifidobacterium breve* were used as representative of probiotics). We observe that the presence of TEF and NP affects surface properties (autoaggregation, hydrophobicity) and planktonic growth in a strain-dependent manner. Indeed, all the tested strains show increased biofilm formation with the exception of Bifidobacterium and variations in their surface properties. The impact of TEF and NP on the bacteria surface properties and adhesive ability are likely to cause alteration in the composition of gut microbiota. Currently more studies on plastic effects on both, eukaryotic and prokaryotic cells, are on-going.

Disrupting protein-protein interactions involving PED/PEA15

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Session: Structural biology and protein function

PED/PEA15 (*Phosphoprotein Enriched in Diabetes/Phosphoprotein Enriched in Astrocytic*) is 130 residues in length protein which is expressed ubiquitously in several human tissues. We employ computational methods to provide a detailed description of BPH03 interaction with PED, evidencing the presence of a hidden druggable pocket within its PLD1 binding surface. We also elucidate the conformational changes that occur during PED interaction with BPH03. Moreover, we report new NMR data supporting the in-silico findings and indicating that BPH03 disrupts the PED/PLD1 interface displacing PLD1 from its interaction with PED. PED/PEA15 is also overexpressed in cancer cells and involved in a protein-protein interaction with FADD protein, inhibiting the formation of the DISC complex and then inhibiting cell apoptosis. Disrupting PED/PEA15-FADD interaction could be helpful to develop new cancer treatments. In IRBM we employed NMR methods to screen a library of 180 fluorinated fragments to find putative compounds interacting in the PED/PEA15-FADD binding region.

Design neuroprotective peptides against prion diseases

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Session: Structural biology and protein function

Prions are fatal neurodegenerative disorders caused by misfolding of the cellular prion protein (PrP^{C}) into its pathological form, scrapie prion (PrP^{Sc}). This misfolding triggers a cascade of neurodegenerative processes, leading to severe symptoms and fatal outcomes associated with prion diseases. Due to the lack of effective treatments, prion diseases pose a significant public health threat, making the development of therapeutic interventions a critical research priority. Recently, we explored the structural and dynamical determinants controlling the prion misfolding process by which PrP^{C} HuPrP(90-231) is transformed to an amyloid fibril through the formation of a β-sheet-enriched intermediate state (β-PrPI). Moreover, our research highlighted the importance of transient electrostatic interactions between the N- and C-terminal domains in regulating the folding process of PrP^{C}. Building on our previous findings, the aim of this project is to develop peptide-based strategies able to interfere with the initial stages of prion misfolding avoiding the formation of stable intermediate states and/or oligomeric species involved in the amyloid aggregation. We designed a first 21-amino acid peptide encompassing the region from Lys23 to Ser43 of the N-terminal domain of HuPrP, called MANTRAP 1. NMR structural data revealed that this peptide has a high degree of conformational flexibility without adopting any preferential secondary structure and it is able to transiently interact with HuPrP(90-231) offering a promising way to design a novel class of peptides blocking prion misfolding and aggregation.

Insights into the formation of transient oligomeric species involved at the initial stages of Prion protein fibrillation

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Session: Structural biology and protein function

Prion disease (PD) are devastating neurodegenerative disorders caused by irreversible conversion of the native monomeric α-helix rich cellular prion protein (PrP^{C}) into a disease-related and predominately β-sheet-rich prion protein scrapie isoform (PrP^{Sc}). This misfolded PrP^{Sc} form incorporates into growing polymers, ultimately resulting in insoluble amyloid plaques, depositing in the central nervous system. Recently we identified that the amyloidgenic b-sheet-enriched intermediate state (b-PrP(I)) is the triggering event involved in the initial stages of PrP fibrillation, generating aggregation-prone species: 'β-PrPI-oligomers'. These oligomers are likely responsible for cytotoxicity, ultimately leading to cell death. However, developing therapeutic strategies targeting prion diseases by inhibiting PrP aggregation requires a precise understanding of the molecular mechanisms governing cytotoxic oligomer formation during amyloid fibrillogenesis, which is challenging due to their low abundance, transient nature, and dynamic properties. To address this purpose we applied Chemical Exchange Saturation Transfer (CEST) NMR experiments to obtain a detailed description of kinetic and thermodynamic parameters related to human prion conformational equilibria involving transient oligomeric species that drive amyloid fibril assembly mechanism. In the absence of oligomeric species, ^{15}N CEST data acquired at low temperature (15°C) did not show any detectable conformational exchange. On the contrary, in the presence of β-PrPI-oligomers, ^{15}N CEST experiments revealed that the HuPrp(90-231) monomeric state is in slow conformational exchange with "NMR-invisible" oligomers, having a pronounced β-strand character. Interestingly, ^{15}N chemical shift values of β-PrPI-oligomers are aligned with the Cryo-EM structure of prion fibrils, providing insights about structural rearrangement involving HuPrP(90-231) N-term tail.

Use of Nuclear Extracts to study Cohesin at Single Molecule level

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Session: Structural biology and protein function

Cohesin, a protein complex comprising SMC1, SMC3, and RAD21, is crucial for sister chromatid cohesion and gene regulation via loop extrusion, particularly when associated with the NIPBL/SCC2 loader complex. Better understanding of this mechanism could advance gene regulation research, potentially aiding in targeted therapies.

Although many imaging techniques exist to study fluorescent proteins at the single-molecule level, they typically rely on purified proteins, which may lack essential post-translational modifications or cofactors. To address these limitations, we developed methods using nuclear extracts and protein pull-downs to examine protein functionality in single-molecule experiments.

Our findings reveal that protein behaviour varies depending on extraction methods. Cohesin displayed distinct behaviours when comparing nuclear extracts to pulled-down proteins. In nuclear extracts, we observed that proteins remained bound to the same DNA location, while pulled-down proteins exhibited higher dissociation rates. Notably, pulled-down cohesin lost its ability to dissociate from DNA in the presence of nuclear extracts.

Role of the DDX11 DNA helicase at the interface between DNA replication and chromosomal cohesion

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Session: Structural biology and protein function

The molecular mechanism by which DNA replication and sister chromatid cohesion establish functional coupling is not well understood, nor is the specific role of the cohesion building factors, including DDX11 and TIMELESS, in this process. The inactivation of DDX11, a super-family 2 DNA helicase, leads to Warsaw breakage syndrome, a rare genetic disease characterised at cellular level by genomic instability and cohesion defects. It was demonstrated that DDX11 directly interacts with the cohesin complex and TIMELESS, a subunit of the replication fork protection complex. Since the DDX11 mutant allele deficient in TIMELESS-binding cannot correct the cohesion defect of DDX11-deficient HeLa cells, the interaction between DDX11 and TIMELESS is essential for recruiting the cohesin complex at the DNA replication fork and for establishing sister chromatid cohesion. In addition, DDX11/TIMELESS interaction has also been found to be critical in the chicken DT40 cell system for suppressing epigenetic instability at G-quadruplex-forming genomic loci and counteracting replication stress in mammalian cells.

In this project, I will analyse the interplay of DDX11, TIMELESS, and cohesin in coordinating chromosomal cohesion and DNA replication. So far, protocols related to the biochemistry part of the project have been standardized, including production of cohesin in insect cells, TIMELESS and DDX11 in mammalian cells; affinity purification of cohesin, TIMELESS, and DDX11; in vitro co-pull down assay of DDX11 and cohesin; prediction of the molecular interactions among cohesin, TIMELESS, and DDX11 by AlphaFold 3.

Purification of hortensins, type 1 ribosome inactivating proteins from the seeds of *Atriplex hortensis* L. *var. rubra*

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Session: Structural biology and protein function

Ribosome Inactivating Proteins (RIPs) are enzymes (EC: 3.2.2.22) mainly isolated from angiosperms, endowed with rRNA N-glycosylase activity (1). These enzymes remove a specific adenine of the Sarcin-Ricin Loop (SRL) located in the 28S ribosomal RNA, causing the inhibition of protein synthesis and cell death by apoptosis (2-3). Many RIPs have been isolated from edible plants belonging to Amaranthaceae family (Caryophyllales order), such as quinoin from *Chenopodium quinoa* Willd. and sodins from *Salsola soda* L.

In this framework, during the first year of my PhD, I have investigated the presence of type 1 RIPs in *Atriplex hortensis* L. *var. rubra*, edible plant belonging to Amaranthaceae family. To this aim, I purified four novel type 1 RIPs, from the seeds of *A. hortensins*, by applying a protocol for the obtainment of basic proteins. These enzymes, named hortensins 1, 2, 4, and 5, release the β-fragment and deadenylate salmon sperm DNA, thus, acting as polynucleotide:adenosine glycosilases. Structurally, hortensins have a different molecular weight, while only hortensins 2 and 4 are glycosylated. Finally, considering the higher homogeneity of hortensin 4 (~28.5 kDa, 0.71 mg /100 g), further studies will be performed to get information on its primary structure and the potential cytotoxicity.

Alternative strategies against *Klebsiella pneumoniae* infection through structural studies

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Session: Structural biology and protein function

Antimicrobial resistance (AMR) is currently a critical public health issue. In this context, a group of six pathogens, denoted as ESKAPE, have developed exceptional ability to survive to traditional antibiotics. One of the most harmful bacterium of this group – *Klebsiella pneumoniae* – uses various mechanisms to resist to antibiotic attacks. New strategies, successful on antibiotic resistant strains, need to be urgently identified.

A key role in resistance of *K. pneumoniae* is played by its cell envelope consisting of two main barriers: capsular polysaccharides (CPS) or lipopolysaccharides (LPS). Bacteriophages evolved to overcome these bacterial defences. Thanks to highly specific depolymerases, enzymes incorporated into virions' tail spike, they can hydrolyse CPS structures and make them susceptible to the antibiotic action. Understanding the structural factors involved in overcoming of antibiotic resistance is crucial for developing effective new therapeutic tools. My studies will start with the structural and functional characterisation of several capsular depolymerases, by exploiting both structural and biophysical techniques. Deep knowledge of these enzymes' structures and details of mechanisms of action is an essential step to engineer recombinant enzymes with a smaller size and a broader spectrum of action and achieve the goal to adjuvate antibiotics in the eradication of the infectious pathogen.

Advances in Galectin Research: new insights into Gal-3 interactors and Gal-7 characterization

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Session: Structural biology and protein function

Galectins are lectin proteins that specifically bind to β-D-galactoside sugars, commonly found on cell surfaces. Through their interactions with glycoproteins and glycolipids, galectins regulate cell signaling, cell adhesion, and immune responses, playing a critical role in processes such as inflammation and host-pathogen recognition, as well as in pathological conditions like cystic fibrosis and cancer progression. Given their relevance, galectins have emerged as targets for diagnostic and therapeutic approaches, with several inhibitors expected to be identified and characterized in the future. The most notable galectins are Galectin-1 (Gal-1), Galectin-3 (Gal-3), and Galectin-7 (Gal-7).

Since the interaction between Gal-3 and lipopolysaccharides (LPS) in pathogen recognition process is already known, a detailed characterization of this interaction has been performed. This research aims to gather valuable information for developing LPS receptor-targeted agonists and/or antagonists, such as galectins, as adjunctive therapies for pathogens infections and inflammatory disorders.

Moreover, while protocols for expression, purification, and biophysical characterization have been established for Gal-1 and Gal-3, this was not yet the case for Gal-7. Therefore, a set protocol was defined, involving the Gal-7 gene cloning, followed by heterologous protein expression, purification, and characterization by circular dichroism (CD) spectroscopy.

Bioinformatics analysis outlines the diffusion of a novel H-NS-like protein family largely present in Proteobacteria

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Session: Structural biology and protein function

The MucR family transcriptional regulator Ros, initially identified in *Agrobacterium tumefaciens* -a member of the alpha proteobacteria family- is demonstrated to be a zinc finger protein. Ros/MucR proteins are crucial in regulating the expression of genes involved in various cellular processes, including virulence, biofilm formation, and stress response. These regulators typically function by binding to DNA sequences through their zinc finger motifs, which contain conserved cysteine and histidine residues- although aspartate is more frequent than cysteine in the second coordinating position- that coordinate zinc ions for structural stability.

This study aims to determine the homology of Ros/MucR protein across diverse bacterial families, including both Gram-negative and Gram-positive bacteria, as well as eukaryotes. Special attention is given to the conserved position of the four amino acids containing the putative metal binding site. Our comprehensive bioinformatics analysis revealed significant homology of Ros/MucR protein among various bacterial families. Remarkably, our results also highlighted an unexpected similarity between Ros/MucR protein, and six proteins found in eukaryotic organisms: *Cyprideis torosa, Ptychographa xylographoides, Ricinus communis*, *Friedmanniomyces endolithicus, Drosophila suzukii,* and *Aspergillus fumigatus*.

Our bioinformatics results have allowed the choice of a Ros/MucR homologue to be structurally characterized through NMR and Cryo-EM techniques.

"*In vitro* reconstitution of the *de novo* human cohesin loading pathway operating at the DNA replication fork"

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Session: Structural biology and protein function

Cohesin, essential for linking sister chromatids and ensuring accurate chromosome segregation during mitosis, also plays a critical role in organizing higher-order DNA structures in interphase cells. These structures include DNA loops and topologically associating domains (TADs), which are vital for gene regulation, DNA repair, and recombination. Recent biochemical studies have revealed a conserved mechanism of *de novo* cohesin loading behind the DNA replication fork. This process involves the recruitment of the cohesin loader subunit Scc2/NIPBL through direct binding to the DNA polymerase sliding clamp PCNA. This mechanism highlights the dynamic regulation of cohesin during the cell cycle and its integration with the replication machinery to maintain timely sister chromatid cohesion. Understanding this regulation offers new avenues for exploring cohesin's role in various diseases, including cancer and cohesinopathies, where cohesin function is disrupted.

In my work, I have produced recombinant protein complexes, including the SMC1-SMC3^{FLAG}-RAD21-^{10XHIS}STAG1 cohesin complex and the $^{FLAG\text{-}Halo}$NIPBL^{10XHIS}/MAU2 cohesin-loader complex, using baculovirus-infected insect cells. Both complexes were labelled with fluorescent ligands for use with optical tweezers fluorescence microscopy (C-trap G2), facilitating detailed studies of cohesin dynamics and interactions at the single molecule level.

Sustainable Ultrasound-Assisted Solid Phase Peptide Synthesis (SUS-SPPS)

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Session: Structural biology and protein function

Peptides are increasingly represented among FDA-approved drugs, largely due to advancements in solid-phase peptide synthesis (SPPS) pioneered by Merrifield[1,2]. However, SPPS poses sustainability challenges, primarily due to its extensive use of hazardous solvents like DMF, NMP, and DCM, which are regulated under REACH[3]. Although SPPS incorporates some green chemistry principles, its reliance on protecting groups and substantial solvent use remains a concern. Addressing these issues may involve adopting green solvents compatible with current SPPS protocols. While it's difficult to find a single green solvent that optimizes all synthesis stages, reducing solvent use by selecting the most effective options can be environmentally beneficial. Evidence suggests green technologies, such as ultrasonication (US), can improve SPPS by reducing solvents, reagents, costs, and reaction times[4,5]. We propose a sustainable ultrasonic-assisted SPPS (SUS-SPPS) method that significantly reduces solvent use, washing steps, time, and reagents compared to standard SPPS. SUS-SPPS uses ultrasonication at each synthesis step with the COMU/Oxyma coupling strategy, requiring minimal reaction volumes and only one washing step. This method enables the synthesis of peptides up to 20-mer, including challenging sequences, with high yields and purity, achieving 75-87% solvent savings and meeting modern drug development sustainability goals.

Session 2:

Cancer Biology, Drug design, Immunology and Microbiology

Novel Immunological targets for Hepatocellular Carcinoma based on molecular mimicry

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality worldwide and about 800k cases are reported each year. Compared to other cancer types, a limited number of therapeutic options are available for this malignancy. Regarding the immunotherapy strategies, only a handful of effective antigenic targets are available. The present project aims to identify novel HCC-specific targets for the development of both active (vaccine-based) and passive (adoptive T-cell therapy) immunotherapeutic strategies, exploiting the concept of molecular mimicry. After an extensive analysis from public datasets, four HLA-A02:01 epitopes were identified from overexpressed cellular proteins in HCC (TAAs) showing significant sequence and structural homology with viral antigens (VirAs). A pMHC-tetramer staining analysis using PBMCs, from cancer patients and control subjects, revealed $CD8^+$ T cells cross-reacting with paired TAAs and VirAs. In order to verify a cross-reactive cytotoxic activity, PMBCs were stimulated *ex-vivo* with VirAs and challenged against HepG2 cells transfected for expressing TAAs (HepG2-TAAs). Results documented such a cross-reactive cytotoxicity. Further experimental validation is ongoing to estimate the immunogenicity of all identified coupled TAAs and VirAs and to investigate the cross-reactive $CD8^+$ T cell populations.

GADD45β interferes with the dual RIPK3-driven activation of RHIM-dependent NF-kB signaling and necroptosis

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Receptor-interacting protein kinase 3 (RIPK3) is a serine/threonine kinase that plays a critical role in both necroptosis and inflammation. Upon activation of necroptosis, receptor-interacting protein kinase 1 (RIPK1) interacts with RIPK3 through their RIP homotypic interaction motif (RHIM) domains, leading to the formation of the necrosome complex. In certain cellular contexts, RIPK3 also regulates NF-κB activity through its scaffolding functions, thereby influencing cytokine production and other immune processes. Here, we report a direct interaction between RIPK3 and GADD45β, confirmed through both Biolayer Interferometry (BLI) and immunoprecipitation experiments. Notably, our findings reveal that this interaction occurs independently of the RHIM domain of RIPK3. Furthermore, through co-immunoprecipitation, we demonstrate that the presence of GADD45β destabilizes RIPK3-RIPK3 homodimers and RIPK1-RIPK3 heterodimers. Interestingly, NF-κB activity assays revealed that GADD45β exerts an inhibitory effect on the canonical NF-κB pathway, with a dose-dependent reduction in NF-κB activation. Additionally, we investigate the expression levels of key necroptotic proteins across three different cholangiocarcinoma cell lines, assessing the impact of inducible GADD45β expression on necroptotic signaling. These findings suggest that GADD45β may serve as a negative regulator of RHIM-mediated activities of RIPK3, with potential implications for therapeutic strategies targeting necroptosis-related diseases and cancers.

Lactococcus lactis cell-free supernatant inhibits GBM cell line proliferation and maintains blood-brain barrier integrity

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Over the last decade, probiotics gained much attention within the medical, pharmaceutical, and food fields, given the health benefits of consuming these microorganisms. Postbiotics are functional compounds, such as organic acids, short-chain fatty acids, enzymes, and neurotransmitters, produced by probiotic bacteria during fermentation. As health-promoting microorganisms, probiotics show different therapeutic properties such as anti-pathogenic and cholesterol-lowering activities. Recently, anti-proliferative activity is one of the most interesting properties linked to probiotics. Here we studied the antiproliferative properties of cell-free supernatant (CFS) of three different *Lactococcus lactis* strains on human glioblastoma (GBM) cell lines. Cell vitality, migration, invasion, wound healing rate closure, and tumor spheroid formation of GBM cell lines were markedly inhibited by CFSs but no effects were observed on the viability of healthy primary astrocytes. Moreover, flow cytometry analysis revealed treated cells accumulated at the G0/G1 phase, but no apoptotic cells were detected. Furthermore, to investigate the effects of these metabolites on the blood-brain barrier (BBB), an *in vitro* co-culture model was established and revealed that the integrity of this latter was preserved. This study highlights, for the first time, the potential anticancer properties of probiotic *L. lactis* strains on human glioblastoma cell lines.

Interleukin-4 inhibits the inflammatory cascade leading to villous atrophy in potential celiac disease

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Session: Cancer Biology, Drug design, Immunology and Microbiology

The mechanisms preventing the intestinal villous atrophy in subjects with potential celiac disease-(CeD) are not completely elucidated. Recently, we have demonstrated a marked infiltration of IL4-secreting T cells in gut mucosa of potential-CeD children that correlated with clinical outcomes. Here, we further investigated the protective role of IL-4 in the evolution to acute-CeD. IL4 was used as growth factor of gluten-reactive, T-cell lines (TCLs) established from gut biopsies of either potential- and acute-CeD children (N=10 for each group). The effect of IL4 treatment on cytokine production, T-cell subset expansion and gluten specificity was evaluated by ELISA and multiparametric flow-cytometry. IL4 treatment on potential-CeD TCLs induced a statistically significant reduction of IFN-γ release in culture supernatants, inhibit the expansion of both CD8+ T cells and TCR γ/δ+ T cells compared to control (no IL4) TCLs. A slight reduction of IFN-γ production by gliadin-reactive T cells was also observed. In contrast, IL-4 induced no significant changes in TCLs from acute-CeD patients. Our study demonstrated an immunoregulatory function of IL4 on gluten-induced inflammation in gut mucosa of potential-CeD patients, suggesting an important role of this cytokine in counteracting the transition mechanisms to villous atrophy.

Unraveling the role of poly (vinyl alcohol) in developing mucus- and biofilm-penetrating PLGA nanoparticles for pulmonary delivery of antimicrobial peptides

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Inhaled antimicrobial peptides (AMPs) show potential for treating lung infections; however, infected lung barriers limit their effectiveness. To improve AMP delivery, this study examines the optimal polyvinyl alcohol (PVA) grade, considering molecular weight (Mw) and hydrolysis degree (HD), as a surface modifier for PLGA nanoparticles (NPs) to enhance mucus and biofilm penetration for efficient delivery of the model AMP, colistin (COL). This study analyzed the PVA shell obtained from four different grades (Mowiol® 4-88, 40-88, 10-98, and 56-98) and its influence on the diffusion of fluorescently-labeled NPs in porcine tracheal mucus (PTM) and E. coli biofilm using multiple particle tracking (MPT). The most promising formulations were then loaded with COL. The findings showed that low HD PVAs, 4-88 (low Mw) and 40-88 (high Mw), provided superior coating on PLGA NPs compared to high HD PVAs (10-98 and 56-98). MPT analysis in PTM revealed that PLGA-PVA 4-88, 40-88, 10-98, and 56-98 NPs diffused 395-, 300-, 90-, and 2-fold faster, respectively, than non-modified PLGA NPs. In E. coli biofilm, PLGA-PVA 4-88 and 40-88 NPs had only 9- and 26-fold reduced mobility compared to water, whereas non-modified PLGA NPs experienced a 3000-fold reduction. COL-loaded NPs using low HD PVAs exhibited similar diffusion behavior as bare NPs. In conclusion, Surface engineering of PLGA-NPs with low HD PVA led to the production of mucus- and biofilm-penetrating NPs suitable for the pulmonary delivery of COL.

Riboflavin production by a mutant *Limosilactobacillus fermentum* in vegetable beverages

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Riboflavin (vitamin B2) is vital for human health, aiding in metabolism and biological functions. Since humans cannot produce it, dietary intake is essential. Certain lactic acid bacteria, including *Limosilactobacillus fermentum*, possess the ability to synthesize riboflavin, a trait linked to the presence of the *rib* operon. This strain not only offers probiotic benefits but also enhances food preservation and health properties, sparking interest in riboflavin-fortified foods. So, the aim of the study was to select a riboflavin over producing food grade LAB mutant for the vitamin biofortification of fermented foods. The presence of riboflavin biosynthesis genes, namely *ribG*, *ribB*, *ribA* and *ribH* was investigated and verified in an *L. fermentum* strain isolated from buffalo milk (D'ambrosio et al., 2022). Enhanced exposure to roseoflavin has effectively demonstrated the potential to select a mutant strain of *L. fermentum* that overproduces riboflavin. These spontaneous mutants, overproducing riboflavin were used to ferment oat beverage in small scale bottle experiments. The scale up of the process in controlled conditions in 0.5 L and 3 L Biostat CT plus reactors (Sartorius) was conducted to further optimize riboflavin production. Results successfully demonstrated the enrichment of oat beverage with riboflavin up to 0.83 mg/L at 24h and 1.23 mg/L, paving the way for vitamin-enriched dietary options.

Human cell-based assays and advances in endotoxin analysis

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Although endotoxins or lipopolysaccharide (LPS) is responsible for cell activation in the innate immunity, the excessive cytokine induction can lead to cell death. The current cell-based tests for LPS detection still have limits. While the traditional cell-based tests rely on cytokine measurement, we propose in comparison with these tests, new approaches based on imaging analysis of NFκB GFP U937 or whole blood or PBMCs stimulated with different LPSs. The measurement of cytokines produced by monocytes, macrophages, U937-PMA, NFκB GFP U937-PMA were sensitive at 0.1 ng/mL LPS from *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica* (THP1 even less) and at 10 ng/mL *Pseudomonas aeruginosa* LPS. In terms of counting number fluorescent spot from images of NFκB GFP U937, the difference started from 0.02 ng/mL *E. coli* and *K. pneumoniae* LPS. Imaging analysis of dead cells showed the difference starting from 10^{-7} and 0.1 ng/mL *E. coli, K. pneumoniae,* and *S. enterica* LPS on whole blood and on PBMCs, respectively, while the cytokine analysis also showed the sensitivity at 0.1 ng/mL. This imaging analysis was evaluated as high accuracy by Deep Learning, a small brand of Artificial Intelligence. This was the first time the computational model applied for LPS detection methods.

You are what you breathe: the impact of cigarette smoke and nanoplastics on airways disease

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Cigarette smoke (CS) is a well-known cause of acute and chronic respiratory illnesses, such as lung cancer and chronic obstructive pulmonary disease (COPD). Moreover, anthropogenic pollutants, such as nanoplastics (NPs), can be easily inhaled into the human lungs, representing an emerging risk factor for lung diseases. However, little is known about the combined health effects of NPs and CS. To fill this gap, we first investigated the effects of polystyrene (PS) beads, common NP, and CS in an in vitro model of bronchial epithelial cells (BEAS-2B) using an MTT assay. FACS and confocal microscopy analyses of the cellular uptake of the labelled PS beads were also performed. Exposure of BEAS-2B cells to NPs and CS (from 1ng/mL to 100µg/mL and 0,5% to 2%, respectively) resulted in a significant reduction in cell viability, peaked at 1µg/mL PS and 1% CS ($p < 0,0001$ and $p < 0,05$, respectively). However, co-exposure resulted in a synergistic effect, leading to low cell viability also at 1ng/ml PS and 0,5% CS ($p < 0,0001$). Cellular uptake of the labelled PS beads was also confirmed. Moreover, we are evaluating the presence of NPs using Py-GC-MS in bronchial biopsies from healthy never- and ever-smoker controls, lung cancer patients, and COPD subjects. Preliminary results identified that polyethylene (PE), polyvinyl chloride (PVC), and PS were among the most prevalent NPs. Ongoing evaluation of the correlation between NPs and CS in human samples and an in vitro 3D ALI system could elucidate the molecular mechanisms by which NPs and CS promote lung function decline.

Selection of a novel RNA aptamer selectively targeting NSCLC-derived CAFs

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Non-small cell lung cancer (NSCLC) represents one of the most diagnosed and lethal type of neoplasm in industrialized countries. The surrounding tumour microenvironment (TME), with its complex cell population composition, demonstrated to be strongly involved in tumour growth, progression and spreading. Most abundant cellular components of the TME are cancer-associated fibroblasts (CAFs), emerged as key tumour-promoting mediators. They positively influence immune evasion, angiogenesis and chemoresistance. Therefore, the development of therapeutics targeting CAFs represents a very promising strategy for effective NSCLC therapy. Among innovative targeting agents, single-stranded nucleic acid aptamers are very powerful tools in the field of precision medicine. By using a cell-SELEX protocol optimized for the isolation of internalizing aptamers, we identified an RNA aptamer, CAF#1sh, demonstrating specific binding and internalization into NSCLC-derived CAFs. The modification of the sequence with 2'F-pyrimidines resulted in an improved stability of the aptamer in human serum. In addition, we observed that NSCLC CAFs, upon treatment with the aptamer, undergo a modification of their molecular signature and cellular behaviour.

Obtained results indicate that CAF#1sh aptamer represent a very promising molecule for NSCLC treatment and could allow the design of various multivalent conjugates for improved target therapy.

Unlike cisplatin, temozolomide inhibits migration and vasculogenic mimicry of glioblastoma cells

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Glioblastoma multiforme (GBM) is the most devastating and widespread primary tumor of the central nervous system. GBM malignancy is characterized by a remarkable vascularization, which occurs through different mechanisms. Among them, Vasculogenic Mimicry (VM), the non-endothelial formation of new vessels, is an important process responsible of resistance to anti-angiogenic therapies. GBM first-line treatment is based on surgical resection, followed by radiotherapy and chemotherapy. Temozolomide (TMZ) is the gold standard cytotoxic drug used for treating gliomas, and together with cisplatin, they are two DNA alkylating agents largely used in GBM chemotherapies. Nevertheless, the prognosis is very poor with a median survival ranging from 12 to 15 months furthermore dose-dependent side effects are still an unsolved problem.

Herein, we tested the effect of lethal and sublethal doses of TMZ and cisplatin on GBM cell lines migration and vascularization.

Quantitative assessment of VM formation from GBM cell lines, showed a clear-cut inhibitory activity of TMZ in a dose-dependent manner, unlike cisplatin. Moreover, TMZ, but not cisplatin, strongly reduced both random and directional migration of GBM cells. It is worth noting that TMZ was unharmful to astrocytic cells at the doses being lethal on GBM cells or effective on VM.

In conclusion, these results indicate that, unlike cisplatin, sublethal doses of TMZ can still exert therapeutic effects on aggressive properties of GBM cells such as migration and VM.

Nanomaterials-based strategies for the treatment of hepatocellular and colorectal cancer

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Hepatocellular carcinoma (HCC) and colorectal cancer (CRC) are high social impact tumor. Some significant tumoral processes such as drug resistance, metastasis formation and cellular growth are regulated by the ecto-5'-nucleotidase (CD73) enzyme, encoded by the NT5E gene. Previous studies showed that the inhibition of the membrane transporter ABCC6 leads to a downregulation of the gene NT5E, with cytoskeleton rearrangement and reduction in cell migration rate, identifying the ABCC6 protein as a potential therapeutic target for antitumor and antimetastatic treatment. The drug probenecid (PRO) was observed to inhibit the transport protein ABCC6 in HCC cells. Nevertheless, the application of PRO is heavily limited by its high hydrophobicity and its low stability in water. On these bases, this work aims to develop poly(lactic-*co*-glycolic) acid (PLGA)-based nanoparticles able to encapsulate, protect and delivery PRO in order to increase its therapeutic efficacy. The fabrication parameters were optimized to obtain spherical nanoparticles with a hydrodynamic diameter of ~140 nm and a good colloidal stability over 10 days. Furthermore, PRO-PLGA interaction investigations highlighted the possibility of solubilizing the drug into the polymeric matrix, in order to design the best encapsulation strategy.

Investigating the *Mycobacterium smegmatis TetR_3765* regulon

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Session: Cancer Biology, Drug design, Immunology and Microbiology

TetR family regulators (TFRs) represent a large group of one-component bacterial signal transduction systems that recognize environmental signals, like the presence of antibiotics or other bactericidal compounds, and modulate the expression of genes involved in stress response. In our previous studies, we described the functional role of the TetR_3765 protein as a repressor of an ABC-type *MSMEG-3762/63* efflux pump in *M. smegmatis* and of its orthologous *Rv1687/86* in *M. tuberculosis.* Microarray analysis revealed the differential expression of 109 genes in an *MSMEG_3765* deleted strain. Among these, *MSMEG_6144* was upregulated, while *MSMEG_5488*, *MSMEG_6254,* and *MSMEG_4708* were downregulated in the mutant. On the base of genome annotation, all these genes seem involved in stress response or drug resistance. To characterize *TetR_3765* regulon in *M. smegmatis*, we validate transcriptome analysis by qRT-PCR performed on the above-mentioned genes. Our results confirmed the involvement of *TetR_3765* in the expression of *MSMEG_4708* and *MSMEG_6144* genes, coding a putative methyl transferase and a PE family protein, respectively. Moreover, bioinformatics analysis and EMSA experiments are actually under investigation to identify the putative promoter regions and consensus sequence recognized by the TetR_3765 protein. This study confirms the role of *TetR_3765* in the transcriptional regulation of genes involved in stress response.

AI-Driven Tool for Predicting Thermostability-Enhancing Mutations in GPCRs through Integrated Machine Learning Models

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Protein engineering has been emerged as a tool for the improvement of catalytic activity of enzymes and protein's conformational stability. Two main approaches for protein engineering are rational protein design and directed-evolution. We are developing a computational tool enforced by AI algorithms to predict beneficial single amino acid mutations that can potentially enhance the thermostability of G protein-coupled receptors (GPCRs), a key focus in protein engineering and drug discovery. The central hypothesis is that the integration of multiple machine learning models, combined with diverse encoding methods, can significantly improve the accuracy and efficiency of predicting potential mutations that increase GPCR stability. To validate this hypothesis, four experimentally validated datasets from the Protabank database were selected, each comprising both thermostable and non-thermostable GPCR sequences. The methodology involved training and optimizing six machine learning models—LSTM, CNN, SVM, Random Forest, Bayesian Machine Learning, and Gradient Boosting—using 12 distinct encoding methods, such as Acthely factors and BLOSUM62. These models were rigorously evaluated based on their ability to accurately classify GPCR variants according to their thermostability. Results are indicating that the SVM and Gradient Boosting models, particularly when paired with specific encoding techniques, delivered the highest levels of accuracy, precision, recall, and F1 scores across all datasets. The optimized models are now being applied in the Screening module to a comprehensive library of GPCR variants (library created by trained data), for successfully identifying mutations likely to enhance thermostability. This tool will provide a powerful and user-friendly platform for predicting mutations that stabilize GPCRs, and can be fine-tuned with significant implications for the design of other robust therapeutic proteins.

The innate immune memory of mast cells

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Session: Cancer Biology, Drug design, Immunology and Microbiology

This project will focus on examining the immune memory of a very important population of innate immune cells, the mast cells. Mast cells are highly secretory innate immune cells that, when stimulated, release a large number of preformed and *de novo* synthesized mediators (such as histamine, heparin, tryptase, chymase, cytokines, prostaglandins, leukotrienes). Mast cells are very sensitive to tissue signals and therefore have tissue-specific homeostasis and immunomodulatory functions. Whether mast cells can develop memory, that is, regulate their function in response to previously experienced stimuli, remains largely unexplored. This project encompass the use of human primary mast cells differentiated in culture from CD34+ blood precursors, to study the capacity of these cells to generate innate immune memory upon priming, the memory mechanisms, its donor-dependent characteristics, and specificity/lack of specificity. Our results demonstrate the successful development of human mast cells in culture and the differentiation of the two major mast cell populations, the tryptase-positive mucosal MC_T and the tryptase/chymase-positive submucosal MC_{TC} cells. Both types of mast cells responded well to the neurotransmitter Subtance P, the kallikrein derivative kallidin, and the snake venom Safarotoxin 6B, showing the release of PGD_2, histamine, tryptase, and significant morphological changes. Preliminary memory experiments show that memory can be generated by previous exposure to activating agents. Notably, the memory generated by the same combination of agents differed between the two mast cell subtypes. Future work will focus on further verifying the memory response and expanding its scope of application.

CXCR4 and FAP-1 as promising molecular targets for early-stage cancer diagnosis and treatment

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Targeted Positron Emission Tomography (PET) imaging is an useful technique which identifies tumors by exploiting cancer hallmarks, aiding personalized therapies. In this perspective, CXCR4, a G-protein-coupled receptor overexpressed in more than 20 different types of solid and hematological tumors, was considered as a prime target.[1] Our team developed small cyclic peptides as CXCR4 antagonists with high affinity, selectivity, and metabolic stability.[2] The lead peptide, R54, was used to design an amphiphilic molecule, C18-DBCO -R54, which targets CXCR4 with nanomolar affinity in CXCR4-overexpressing CEM-CCRF human T-leukemia cells. Combined with an imaging dendrimer[3] previously identified by Dr. Peng's group, [^{68}Ga]NOTA-R54-decorated self-assembling dendrimers (SAADs) were developed as nanotracers for in-vivo PET imaging. Moreover, Pancreatic Ductal Adenocarcinoma (PDAC) is challenging due to its unique tumor microenvironment (TME) consisting of cancer-associated fibroblasts (CAFs), regulatory T-cells (Tregs), and myeloid-derived suppressor cells (MDSCs). To overcome this, we aim to remodel the TME by inhibiting CXCR4[1] and FAP[4] signaling pathways. Our goal is to create FAP-CXCR4-SADs (FACX-SADs) by decorating self-assembling dendrimers (SADs) with a CXCR4 antagonist (R54) and FAP ligand (FAP-2286).[5] These FACX-SADs will be tested for binding, toxicity, and inhibition of PDAC cell functions, and evaluated for treatment efficacy in vitro and in vivo, aiming to enhance drug delivery and reduce systemic toxicity.

The innate memory molecular mechanism of monocytes and macrophages

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Session: Cancer Biology, Drug design, Immunology and Microbiology

In addition to the immunological memory pertaining adaptive immunity., it is now evident that immune cells of innate immune system (*e.g.,* monocytes and macrophages) also have the ability to retain memory of prior exposure to microbes and other agents, which leads to a more effective broad-spectrum response to subsequent infection/challenge. Our study focuses on the innate memory of human primary monocytes and monocyte-derived macrophages. Preliminary data show that different agents can induce different memory responses. Previous exposure to LPS induces a memory response that implies lower production of inflammatory factors such as TNFa and IL-6 and an increased production of anti-inflammatory cytokines such as IL-10 and IL-1Ra. Conversely, exposure to live BCG bacteria induces a memory response characterized by enhanced production of inflammatory factors and decreased production of anti-inflammatory cytokines, a scenario that cannot be reproduced with killed BCG bacteria, thereby implying an active cross-talk between bacteria and human monocytes/macrophages. We are currently assessing the molecular basis of such cross-talk, including the exchange of genetic information and modulation of epigenetic modifications (including histone acetylation and DNA methylation), with a particular focus on non-coding RNAs and transposons, in the establishment and persistence of innate memory.

Anti-tumor mechanism of natural mushroom polypeptide Gymnopeptide A

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Natural products are useful tools for the research of biological mechanisms and drug discovery. Therefore, the development of anti-tumor drugs with natural products as lead compounds has great application potential. Gymnopeptide A (GA), a newly discovered cyclopeptide from the mushroom *Gymnopus fusipes*, it has been considered as a promising lead compound for cancer treatment due to its excellent tumor cell growth inhibitory properties and sub-nanomolar potency. However, the mechanism remains unknown. To further investigate the anti-tumor activity of GA in vivo, we synthesized GA with hydrophobic surface to mitigate uncertainty resulting from contaminants. First, we demonstrated that GA could significantly inhibit the growth and metastasis of tumor cells at very low concentrations in a mouse tumor-bearing model. Further studies showed that GA exerted an anti-tumor effect in a CD8-dependent manner. Previous studies revealed the importance of cellular metabolism in T cell differentiation, suggesting that $CD8^+$ phenotype depends on glycolysis and OXPHOS for its metabolic needs, where mitochondria play distinctive roles in T cell development and differentiation. Thus, we determined the effect of GA on mitochondria. Our results showed that GA could not only target mitochondria, but also altered the morphology of mitochondria in $CD8^+$ cells by limiting mitochondrial fusion. Concurrently, we found that GA treatment also reduced the expression of PD-1 in $CD8^+$ T cells. Therefore, revealing how GA achieves its anti-tumor immune function by regulating mitochondria dynamics will be the focus of our next work, which will promote the further application of GA in cancer therapy.

Inhalable nanoparticles delivering peptidomimetic/antibiotic combinations for local treatment of CF lung infections

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Session: Cancer Biology, Drug design, Immunology and Microbiology

The use of protease-resistant peptidomimetics, such as peptoids, offers a novel therapeutic avenue. However, challenges in administration and biodistribution hinder their efficacy. To overcome these limitations, we developed inhalable polymeric nanoparticles (NPs) combining poly(lactide-co-glycolide) (PLGA) and poloxamer (PLX) for the delivery of P13#1, an antimicrobial peptoid, alone or combined with colistin (COL) or tobramycin (Tb). This nanoparticulate system aims to provide controlled drug release, improved mucopenetration, and precise targeting for multi-drug resistant lung infections. The incorporation of PLX addresses challenges associated with achieving fast and complete release, crucial when dealing with peptides. NP formulations, based on a blend of RG PLGA 502H and PLX Pluronic® F127 or F68 at different ratios were prepared by an emulsion/solvent diffusion technique, using PVA as stabilizer. Preliminary studies were carried out on blank NPs to achieve optimized prototypes. Blank NPs at various PLGA/PLX ratios, exhibited favorable size (~ 200 nm) and ζ-potential (~ -25.0 mV). Best formulations were loaded with COL and Tb and characterized for size, polydispersity index (PDI), ζ-potential, encapsulation efficiency (EE), yield of production (YP) and mucin interaction. The obtained NPs loaded with Tb and COL maintained an appropriate size, small PDI and negative ζ-potential. The analysis of the EE revealed that the presence of PLX inversely correlates with EE. In fact, with increasing the amount of PLX an enhanced hydrophilicity in the matrix can be observed, promoting the loss of antimicrobial agent. Finally, the selected antimicrobial-loaded formulations with the best properties were evaluated for their efficacy against *Pseudomonas aeruginosa* (PA) and their ability to eradicate PA biofilm, and results showed that, after 5h, COL loaded NPs exhibited efficacy close to free COL, underlying the potential of the antimicrobial controlled release.

Targeting of the CHCHD4 import pathway for Neuroprotection

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Session: Cancer biology, Drug design, Immunology and Microbiology

The coiled-coil-helix-coiled-coil-helix oxidoreductase domain-containing protein 4 (CHCH4), the evolutionarily conserved human homologue of yeast Mia40 [1], is the core component of the disulfide relay system (DRS) within the mitochondrial intermembrane space (IMS). CHCHD4 and DRS regulate the import of many cysteine-containing proteins into the IMS that are required for normal physiological functions. Dysregulation of DRS and CHCHD4 underlies the pathophysiology associated with mitochondrial disease and cancer [2]. Partial downregulation of CHCHD4 leads neuroprotective effect and reduce brain damage in response to conditions such as hypoxia-ischemia [3]. The data suggest that the observed neuroprotective effects could involve the metabolic consequences of lowering mitochondrial CHCHD4 activity [4]. In this hypothetical scenario, the reduction of mitochondrial CHCHD4 activity could trigger a metabolic “preconditioning state” that in turn could protect cells from damage.

We aim to generate bioactive small peptide molecules, which could block and/or modulate the activity of the CHCHD4-related import pathway to assess its impact on mitochondrial reprogramming of cells that resist damage and to better understand the neuroprotective effect of downregulation of the CHCHD4 pathway. Furthermore, the identified compounds may be a useful model for the development of novel therapeutic strategies against mitochondrial dysfunction.

Design and characterization of cyclodextrin complexed drugs and natural compounds against bovine coronavirus

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Session: Cancer Biology, Drug design, Immunology and Microbiology

The research on new antiviral agents aims to develop innovative and safe drugs. In animal models, agonists of the aryl hydrocarbon receptor (AhR) influence immune responses to various viral infections, including coronaviruses. Conversely, AhR antagonists inhibit coronavirus infections in mammalian cells. Funicones, a group of fungal polyketides, exhibit significant biological properties, making them promising candidates as antiviral agents. My first year PhD activity focused on 3-O-methylfunicone (3-OMF), a specific funicone extracted from *Talaromyces pinophilus*. This secondary metabolite has demonstrated antiviral activity against Canine Coronavirus (CCoV) and Bovine Herpesvirus by inhibiting AhR. However, 3-OMF's low solubility poses a challenge for its use as an active antiviral agent. To address this aspect, cyclodextrins (CDs) have been considered as potential solubilizers to improve the pharmacokinetic properties of 3-OMF. CDs are oligosaccharides known for their ability to form inclusion complexes, along with their low toxicity. The interaction between 3-OMF and β-cyclodextrin (β-CD) was studied by analyzing their host-guest inclusion properties in solution using UV-Vis spectroscopy. Stoichiometry and binding constants of the resulting complex were evaluated, and molecular docking studies were conducted to support the findings.

From algorithms to molecules: the identification of riboswitch-targeting compounds

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Session: Cancer Biology, Drug design, Immunology and Microbiology

This abstract introduces PyRMD2Dock, a novel computational approach designed to enhance the efficiency of virtual screening campaigns for drug discovery. By combining our Ligand-Based AI-enforced Virtual Screening tool PyRMD with the widely used docking software AutoDock-GPU (AD4-GPU), PyRMD2Dock enables rapid screening of massive chemical databases to identify compounds with the highest predicted binding affinity to a target protein. We have successfully applied PyRMD2Dock to challenging targets such as GPCRs and nucleic acids. For the latter, the rise of antibiotic resistance necessitates the exploration of novel therapeutic strategies. Riboswitches, a class of regulatory RNA molecules unique to bacteria, offer a promising avenue for developing new antibiotics. Our approach has proven effective in identifying riboswitch binders, underscoring the value of integrating AI-powered LBVS tools with docking software for effective and high-throughput virtual screening of ultralarge molecular databases in drug discovery.

Targeting pharmacologically relevant anti-cancer agents by employing AI based methods PyRMD and PyRMD2Dock

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Session: Cancer Biology, Drug design, Immunology and Microbiology

The project will entail utilizing AI-based targeting to identify pharmacologically relevant anti-cancer agents through methodologies such as PyRMD and PyRMD2Dock.
Our laboratory has developed two AI-based methodologies, PyRMD and PyRMD2DOCK, which are employed to target pharmacologically significant anti-cancer agents. These methods facilitate the identification of novel compounds capable of interaction with specific targets, thereby aiding in the development of new anti-cancer agents.
The newly developed AI-driven methodologies, PyRMD and PyRMD2Dock, are designed to enhance the efficiency of drug discovery. PyRMD, a fully automated LBVS tool, utilizes advanced machine learning to systematically search large chemical libraries and identify bioactive lead compounds. PyRMD2Dock complements the capabilities of PyRMD and integrates with AutoDock-GPU for high-throughput molecular docking, thereby calculating the binding affinity between compounds and pharmacologically relevant targets.
We aim to apply these methodologies in a systematic examination of multiple pharmacological targets associated with cancer. The combined advantages of PyRMD and PyRMD2Dock within this protocol facilitate the efficient identification of new anticancer compounds by rapidly screening and characterizing large compound libraries. This dual methodology streamlines the identification of promising drug candidates and enhances the accuracy of target engagement predictions. Ultimately, these AI-driven methodologies are anticipated to accelerate the discovery of effective therapeutic agents, thus making a significant contribution to their clinical application and representing a critical advancement in cancer treatment and drug development.
Keywords- AI-based methodologies, PyRMD, PyRMD2Dock, Anticancer agents, Drug Discovery, Pharmacological targets.

Development of amphiphilic dendrimers for targeting CXCR4/αvβ6/αvβ8 overexpressing cancers

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Dendrimers are peptide nanoparticles and are considered as promising carriers for drug delivery, by virtue of the combined multivalent cooperativity of dendrimers with the self-assembly property of lipid carriers. Here, we report an approach for targeted siRNA delivery to cancer cells using an amphiphilic dendrimer equipped with two peptide-based drugs, R54, an antagonist of CXCR4 receptor, and SDM17E, a dual inhibitor of integrins αvβ6/αvβ8. These targets are high expressed in some type of tumors, such as pancreatic ductal adenocarcinoma (PDAC). Indeed, this cancer is characterized by extremely immune-resistance and aggressiveness promoted from CXCR4 and TGF-β overexpression, associated with migration, proliferation and resistance to immunotherapy of cancer cells. Myofibroblast-like cells in the pancreas are activated by cancer cells to produce fibrosis surrounding the tumor, promoting the formation of a barrier that protects the tumor microenvironment, limiting exposure to chemotherapy and leading to poor immune cell infiltration. Poor prognosis and low response to current therapies have leaded our group to development of nanosystems capable of ‘’hiding’’ chemotherapeutic or biological drugs and overcoming tumor resistance.

According to the molecular design, the self-assembling amphiphilic dendrimers (SAADs) are expected to deliver siRNA effectively blocking PD-L1 mRNA expression, while the aim of the targeting peptides are to recognize the respective receptors, whose binding allow them to penetrate inside the cancer cells and promote siRNA release.

Optimization of Microbial Melanin Production from *Streptomyces* strains

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Session: Cancer Biology, Drug design, Immunology and Microbiology

Melanins are a unique class of natural pigments present in humans, animals, plants, and microorganisms and have interesting properties like resistance to acid degradation, thermal stability, antioxidant and antimicrobial activity, heavy metal chelation, and UV-visible light absorption. Therefore, they have several potential applications in diverse industrial fields from the food to the biomedical sector. Current melanin production strategies include chemical synthesis, extraction from natural sources (e.g ink sack of cuttlefish, dark feathers etc), and microbial production. While chemical synthesis is costly and environmentally harmful, and extraction from animals is time consuming and yields low quantities, bacterial production of melanin potentially offers a cost-effective, scalable, and eco-friendly alternative. Microbial synthesis, particularly with bacteria like *Streptomyces*, can utilize renewable resources to produce melanin sustainably, addressing both economic and environmental concerns. This research aims to optimize bacterial melanin production, particularly from *Streptomyces* species, which utilize the DOPA pathway for efficient pigment synthesis. Key objectives include optimizing production conditions such as carbon and nitrogen sources, supplementation of precursors or co-factor and metal ions; scaling up production from laboratory to bioreactor levels; and exploring the use of agro-industrial waste, (e.g bergamot peels) as substrates for cost-effective and eco-friendly melanin production. Additionally, the study will focus on studying the expression of genes involved in melanin biosynthesis in commercial or in newly isolated *Streptomyces* strains in different growth conditions. Data on the regulation and expression of melanin biosynthesis genes in presence of the best identified lignocellulosic substrates and/or of precursors, or co-factors and metal ions, will provide a source of information for optimizing melanin biosynthesis and for identifying strain engineering targets to enhance melanin production in *Streptomyces*.

The anticipated outcomes include detailed production protocols, scalable processes, and methods for waste utilization which aim to improve melanin yield supporting sustainable practices and environmental protection.

Assessing systemic and mucosal immunity and predicting modulation of responses in the elderly and diseased population against future infections

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Session: Cancer Biology, Drug design, Immunology and Microbiology

In humans, innate memory can afford broad and non-specific protection against future infections, although some organ specificity can be observed. Mucosal infections are a serious health threat, in particular for subjects with weakened immune defences, such as patients with chronic diseases and elderly people. In these subjects, infectious microorganisms may trigger an inappropriate reaction that amplify the immediate or subsequent inflammatory responses, causing pathological damage to the host. The aim of this study is assessing the primary and memory innate immune reactivity against bacterial/viral microorganisms or their components (e.g., the prototypical bacterial TLR4 agonist LPS and the virus-like TLR7/8 agonists imidazoquinolines) at the mucosal level, in order to predict future responses (resistance vs. susceptibility) in people with frail immunity. We will assess the *in vivo* establishment of innate memory by testing the reactivity to prototypical viral and bacterial agents of blood monocytes/macrophages and nasal-resident macrophages, assess systemic innate immunity based on biophysics and functional effects of microbe-innate cell interaction, antigen-specific and non-specific nasal immune cell activation. The role of IgA in mucosal defences will be also examined as a marker of protection and health, comparing the human data with those collected from freshwater cetaceans.

Session 3:

Innovative omics technologies in biomolecular sciences

NMR-based metabolomics and anti-leukemic activity of plants used in traditional medicine in Botswana

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Session: Innovative omics technologies in biomolecular sciences

Over the years, there have been great developments in cancer medicine with an effort to treat leukemia. Despite these several immunotherapies, leukemia remains one of the leading cause of cancer related deaths in the world. Some of these therapies are associated with adverse effects, as well as surfacing of drug resistance. Therefore, continuous search for new compounds remains crucial.

With the help of NMR-based metabolomics, this study aims at identifying compounds from selected Botswana plants and further evaluating their anti-leukemic activity. Through ethnopharmacological knowledge, nine plant species were identified and extracted. The extracts were subjected to NMR analysis for their chemical characterization. Partial purification of the plants' crude extracts using amberlite XAD-4 and XAD-7 was done using water and methanol as eluents. The effects of the obtained methanol fractions on cell viability, cell cycle and cell death in human U937 leukemia cell line were examined using flow cytometry-based assays. Following the treatment, induction of cell death and strong modulation of cell cycle was observed, particularly with fractions deriving from *Maytenus senegalensis*, *Elaeodendron transvaalense,* and *Ozoroa paniculosa*. The NMR profiles of these fractions reflected the presence of phenolic compounds, terpenoids, and alkaloids. Further experiments are ongoing to obtain pure compounds, including their structures, from the most active extracts.

Specialized metabolites from natural sources as lead compounds to fight against emerging diseases

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Session: Innovative omics technologies in biomolecular sciences

Recent studies are starting to uncover the untapped biotechnological potential of plant pathogens, like *Septoria glycines*, a fungus best known for causing brown spot in soybeans. This research explores how *S. glycines* might be a source of bioactive compounds with promising health benefits. Our initial findings show that extracts from *S. glycines* have strong antibacterial properties, especially against multi-drug-resistant bacteria, suggesting they could serve as alternative antibiotics. Additionally, these extracts have demonstrated encouraging anticancer effects on various cancer cell lines, hinting that *S. glycines* might contain compounds with novel ways to combat cancer. To get these results, we extracted solid cultures of *S. glycines* using a methanol-water mix (55:45) through two extraction cycles. The extract was then purified using a liquid-liquid extraction process, first with petroleum ether, then with ethyl acetate, and finally with butanol. The butanol fraction was separated further using SEPHADEX LH-20 resin chromatography, which resulted in 20 different fractions. We purified two of these fractions using semi-preparative and preparative thin-layer chromatography. Discovering and understanding these bioactive compounds could lead to new therapeutic options. However, turning these initial findings into practical treatments is a complex process. More research is needed to isolate, purify, and characterize these molecules, and thorough preclinical studies are required to evaluate their safety, effectiveness, and potential side effects.

Antibacterial Activities of Selected Nigerian Plants Against Clinically Important Human Pathogens

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Session: Innovative omics technologies in biomolecular sciences

Antibiotic resistance of bacteria is a health problem at the global level. It is expected that by 2050, this will be the main cause of death [1]. *Staphylococcus aureus*, according to the list of pathogens issued by the WHO, is considered as high priority and efforts are required in research and development of antibiotics to combat this microorganism [2]. Nigeria has a varied ecosystem and diverse biological resources and biodiversity that serves as a major component of treatment processes in over 300 ethnic communities in the country.

In this scenario, plants represent a valuable source of new potential antimicrobial agents and in this way 22 plants belonging to different families collected in Nigeria were screened against *S. aureus*. In particular dried leaves, stems or gourd/fruit were extracted with methanol assisted by ultrasound. Promising antimicrobial activity was displayed by seven of the selected plants and, following a bio-guided approach, further studies are being carried out on them. In this second year, our attention has been regarding *Luffa cylindrica* gourd that was found to exhibit the best activity. Liquid-liquid extraction of the methanolic crude extract using solvents at increasing polarity (n-hexane, dichloromethane, and ethyl acetate) furnished three fractions screened again for antibacterial activity. Minimal inhibitory concentrations (MIC) were determined in specific medium by the broth micro-dilution assay. The results showed that the hexane and dichloromethane extracts were the most active against the bacteria strain with an MIC of 0.086 and 0.0675 respectively at 64µg/ml. Chromatographic separation by Sephadex LH-20 of these fractions allowed to isolate different compounds then characterized by 2D NMR analysis. The chemical characterization of isolated compound allows the identification of polyoxygenated fatty acid derivatives as principal components of bioactive fractions.

Secretomic signatures in diagnostic stewardship for sepsis

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Session: Innovative omics technologies in biomolecular sciences

Sepsis is a life-threatening syndrome that results from a dysregulated host response to infection characterized by multiple organ dysfunction. The molecular mechanisms underlying sepsis remain unknown. In the post-genomic era, large-scale protein characterization can be used to identify differentially expressed proteins in sepsis, which may help to understand the pathophysiological process of sepsis and discover new sepsis-related biomarkers that aid in early diagnosis and effective treatment.

The aim of the work is to study sepsis prediction models by applying a systems biology approach. The association of known and non-routine sepsis biomarkers (e.g. Presepsin, Procalcitonin and C-reactive Protein) with routine clinical and laboratory data will be at first explored by using supervised and unsupervised machine learning algorithms. In parallel, secretome signatures of signatures, alone or in combination with clinical variables, will be investigated with a focus on inflammatory and immune cytokine mediators determined by multiplexed immune assays.

The application of machine-learning algorithms with the advent of “big data”, and the possible combination with existing and emerging biomarkers may provide more targeted diagnostic and therapeutic strategies.

NMR-based metabolomics to discover bioactive cycloartane glycosides from *Astragalus* species

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Session: Innovative omics technologies in biomolecular sciences

Saponins are specialized metabolites exerting a wide range of unique biological properties. In the recent past, there has been unforeseen interest in the clinical utilization of saponins as chemotherapeutic agents. Earlier phytochemical investigations on *Astragalus* species, including *A. glycyphyllos*, resulted in the isolation of cycloartane-type triterpenoidal saponins, that have demonstrated outstanding considerable anticancer activity *in vitro* and in animal models. Among them, the main and major active component of several *Astragalus* species, named Astragaloside IV, has been in-depth investigated for its marked antitumor properties. Due to this background, the current work was addressed to the isolation and structural characterization of new potentially bioactive cycloartane saponins from *Astragalus* species widespread in the Mediterranean area, applying a metabolomic approach based on a combination of high-resolution spectroscopic techniques and phytochemical studies. A preliminary NMR-based profiling allowed to focus on the species that produce cycloartane saponins. Subsequent studies will aim to learn more about the extract composition of selected plant species by combining chromatographic procedures with extensive 2D NMR analyses. Moreover, in order to prove the most active cycloartane metabolites' potential as antitumor agents, anti-cancer bioassay-guided screening will be carried out on them.

Characterization and biological evaluation of *Trichoderma spp.* metabolites

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Session: Innovative omics technologies in biomolecular sciences

Endophytic fungi are a group of fungi that live within plant tissues without causing apparent harm, forming a symbiotic relationship that benefits both organisms. One significant advantage for plants is that endophytic fungi offer protection against pathogenic organisms. The specialized metabolites produced by these fungi can serve as potential sources of bioactive compounds with nematicidal, bactericidal, and antiproliferative activity. The aim of this project, is the isolation and structural characterization of specialized metabolite from bioactive fractions of *Trichoderma spp,* a filamentous endophytic fungus first described in 1794 and recognized as a biocontrol agent. In particular three strains *Trichoderma parceramosum*, *T. citroviride*, and *T. koningii* have been selected The fungi were cultivated using solid state fermentation, the resulting cultures subjected to hydroalcoholic extraction were then purified by chromatographic techniques. The obtained fractions were testedtfor evaluate their nematicidal activity using larvae of *Meloidogyne incognita*, a globally prevalent root-knot nematode that infects over 2,000 plant species, including tomato and indian ginseng were selected. Purification of the most active fractions through chromatographic methods led to the isolation of heterocyclic lactones. Structural determination of the purified compounds was achieved using 1D-NMR and exhaustive 2D-NMR investigations. The nematicidal activity of these pure compounds against *M. incognita* was subsequently evaluated.

Unravelling the TR3-56 cell proteome by Tandem Mass Tag-Based High-Resolution LC-MS/MS

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Session: Innovative omics technologies in biomolecular sciences

Effective immune responses require the coordinate actions of immune cell subsets belonging to both innate and adaptive immunity. Peripheral blood contains a lymphocytes population characterized by the co-expression of the typical surface molecule of T-cells (CD3) and NK cells (CD56), referred as NKT or TR3-56 cells. Besides their known cytotoxic activity, the ability to modulate proliferation and effector function of CD8 T cells was recently reported for TR3-56 cells[1]. Also, the frequency and function of TR3-56 cells were reduced in autoimmune condition, such as type 1 diabetes[1]. Despite these significant progresses, there is still limited information on the molecular signature of TR3-56 cells.

The aim of this work is to set-up emerging high-resolution MS strategies for in-depth protein profiling of TR3-56 cells in comparison with the other main T cell subsets. Quantitative nanoLC-MS/MS TMT isobaric labeling-based approaches will be applied for proteomic analyses. We found that proteins related to cytotoxic functions and calcium homeostasis were differentially modulated in TR3-56 cells cells compared with other immune subsets.

This study offers new insights on molecular determinants regulating TR3-56 cells functions sustaining an effective immune response under healthy conditions, paving the way to further studies aimed at understanding their dysregulation under pathological conditions.

Bioactive small molecules from plants as ligands of DNA secondary structures

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Session: Innovative omics technologies in biomolecular sciences

In this study, for which preliminary data are presented here, we aim to identify bioactive molecules from Mediterranean plants that are capable of interacting with G4. Mediterranean plants have long been used in traditional medicine for the treatment of various diseases. They contain a wide range of bioactive molecules with different chemical structures showing pharmacological activities. Experimental evidence has shown that bioactive molecules play a crucial role in anti-cancer therapies by targeting and interfering with biological processes responsible for cancer growth and progression. Several studies have shown that cancer development is closely linked to structural changes in the genomic DNA, which adopts an alternative secondary structure. In particular, the important gene expression regulator G-quadruplex (G4) DNA plays an important role in cancer progression and its stabilisation or destabilisation by specific binders, including natural ligands, may open promising avenues for potential cancer therapies. Phytochemicals can act as ligands for DNA secondary structures in target cells, interfering with their regulatory activity and acting as potential anti-tumour drugs. Targeting these DNA regions with plant-derived bioactive molecules could have significant implications for drug discovery and development, as it may be possible to modulate gene expression and potentially treat various diseases, including cancer and neurological disorders.

Machine Learning and Network Analysis for Biomarker Discovery in Neurodegenerative Diseases

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Session: Innovative omics technologies in biomolecular sciences

Neurodegenerative diseases like Multiple Sclerosis (MS), Alzheimer's Disease (AD), and Mild Cognitive Impairment (MCI) pose increasing challenges on society due to rising prevalence and complex pathogenesis. Despite differing etiologies, these nervous systems-affecting pathologies share being multifactorial diseases involving intricate molecular interactions, and particularly those within non-coding RNAs (ncRNAs), such as lncRNAs and microRNAs, represent an open field of study. Our research aims to identify key expression patterns and regulatory networks in MS, MCI, and AD by analyzing large-scale datasets with machine learning algorithms, interactome and pathway enrichment analysis.

My most recent work identified differentially expressed lncRNAs in MCI patients, highlighting SNHG16, H19, and NEAT1 as central network nodes, suggesting their potential role in disease progression. Ongoing studies in MS have revealed significant expression changes in four genes and a miRNA following Ocrelizumab treatment, with strong correlations indicating the miRNA's modulatory role. In our latest study, we performed a machine learning pipeline and identified ten genes able of distinguishing MS patients from controls with 92% accuracy, currently undergoing validation. These findings reflect my aim to use these bioinformatic technologies to improve the accuracy and efficiency of diagnosing these diseases, leading to better patient outcomes and therapeutic targeting in these complex disorders.

Session 4:

Cellular and molecular bases of human diseases

Identify molecular pathway regulating cell proliferation through glycosphingolipids biosynthesis

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Session: Cellular and molecular bases of human diseases

Glycosphingolipids (GSL) are a subtype of glycolipids localized on the plasma membrane. They have a role in the regulation of signal transduction and through this control several functions of cells including cell adhesion, cell motility, and growth. Contact Inhibition of Proliferation (CIP), a mechanism that ensures proper tissue homeostasis, is known to regulate GSL biosynthesis, and the GSLs in turn exert feedback control on CIP. The molecular details of this feedback circuit are not known. We have identified GRASP55, a Golgi matrix protein, to be a key molecular player in this feedback circuit. The absence of GRASP55 determines cell density-dependent alteration in GSL biosynthesis and also CIP. We are now dissecting the molecular details of how GRASP55 contributes to this feedback circuit.

Non-coding RNAs as versatile regulators in Neurodegenerative Diseases (NDDs). A focus on MS (Multiple Sclerosis)

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Session: Cellular and molecular bases of human diseases

The illnesses known as neurodegenerative diseases (NDDs) are typified by neuronal degeneration (impaired speaking, mood swings, memory loss, and impaired movement). At first, these dysfunctions do not affect the patients; nevertheless, the diseases continue to worsen and the patients' quality of life significantly declines. Recent research indicates that a range of non-coding RNAs, including long and microRNAs, are involved in the pathophysiology of illnesses affecting the central nervous system (CNS) and may have potential applications as biomarkers for diagnosis and prognosis as well as potential therapeutic targets. Thanks to a relationship with the Department of Advanced Medical and Surgical Sciences at the University of Campania "L. Vanvitelli", Naples, I was able to get samples from Multiple Sclerosis (MS) patients evaluated through a cognitive assessment (BICAMS) and treated with DMTs (Disease Modifying Therapies). The study aimed to assess changes in transcriptome (NFL, IL-1, IL-6, TNF-α) and microtranscriptome (miR-146a-5p, miR-21a-5p, miR-338-5p, Let-7i-5p) at T0 and 12 months after DMT treatment, and to identify a significant correlation between genetic analysis and the battery of neuropsychological evaluation known as BICAMS (Brief International Cognitive Assessment for Multiple Sclerosis).

Investigation of the molecular mechanisms activated in Leydig and Sertoli cells by D-Asp treatment to sustain steroidogenesis and spermatogenesis

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Session: Cellular and molecular bases of human diseases.

D-Aspartate (D-Asp) is an amino acid present in high concentrations in the testis. Increasing evidence suggests that D-Asp affects steroidogenesis by enhancing the biosynthesis of sex steroid hormones, acting either through the hypothalamus-pituitary-testis axis or directly on Leydig cells. The in vitro studies conducted on Leydig TM3 and Sertoli TM4 cells demonstrated that D-Asp induced the proliferation of both cell lines through the activation of the ERK/Akt/PCNA pathway. D-Asp activated testosterone production in LCs by upregulating steroidogenesis enzymes through the ERK1/2 pathway. The observed increase in androgen receptor (AR) protein levels indicated that D-Asp enhanced the activity of TM4 cells. Moreover, D-Asp exerted an anti-apoptotic effect, which may be mediated by antioxidant enzyme modulation in both cell lines. The reproductive function, from spermatogenesis to fertilisation, is influenced by mitochondria and their interactions with the endoplasmic reticulum (ER) through the mitochondria-associated ER membranes (MAMs). D-Asp can enhance the steroidogenic process in rat testes by maintaining mitochondrial homeostasis and regulating the interactions between the ER and mitochondria. Furthermore, we investigated the impact of D-Asp on the activity of TM4 SCs, focusing on the mitochondrial compartment and its relationship with the ER. In SCs, D-Asp contributed to the MAMs stability by regulating the transfer of lipids and the signalling of calcium from the ER to the mitochondria, as well as by reducing ER stress. In conclusion, the research project yielded novel insights into the cellular and molecular mechanisms by which D-Asp exerts its beneficial effects on spermatogenesis, steroidogenesis and male fertility.

Identification of Candidate Markers linked to Autoimmunity in Incontinentia pigmenti

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Session: Cellular and molecular bases of human diseases

Incontinentia Pigmenti (IP, OMIM 308300) is a rare X-linked dominant neuroectodermal disorder caused by mutations in the *IKBKG/NEMO* gene, essential for NF-κB activation.

Recently, neutralizing Auto-Abs (nAb) were detected in >45% of IP patients and evaluated as a risk factor for viral infection diseases.

According to my PhD project, to identify biomolecular markers of autoimmunity in IP patients we performed 30X-WES on 26 IP cases and by using the Variant Effect Predictor (VEP), we annotated a total of 142550 variants identified in our cohort. We filtered on functional effects, and we selected 763 variants Loss-of-Function (LoF), rare (MAF ≤ 1% in gnomAD-NFE (Non-Finnish European) and with CADD score > 20. The 763 candidate variants mapped in 630 genes. We analyzed the 630 genes in ConsensusPathDB (http://cpdb.molgen.mpg.de) by setting q-value (multiple-testing adjusted p-value) < 0.01. We obtained 11 significantly enriched pathways, and the Immune system was most significant enriched pathway (q.0.00144) with 73 genes. Interestingly, 12 genes of 73 were involved in viral response (*IFIH1, IFNA4, HLA-A, HLA-DRB1, HLA-DRB5, AIM2, UNC93B1, MAVS, KIR2DS4, FCGR1A, CD207,* and *IL10*) and 7 of which were involved in autoimmunity (*HLA-A, HLA-DRB1, HLA-DRB, IFIH, AIM2, KIR2DS4* and *IL10).*

Transcriptomic approach for studying miRNAs roles

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Session: Cellular and molecular bases of human diseases

MicroRNAs are small non-coding RNAs playing crucial roles in gene expression regulation in a variety of physiological processes; their deregulation can lead to pathological conditions, including cancer. Advanced sequencing technologies, such as RNASeq, allow to profile transcriptome-wide expression levels of coding RNAs (mRNAs) and non-coding RNAs such as miRNAs, lncRNAs, piRNAs, gaining insight into their role and potential applications in diagnostics and therapeutics.

During this 3-years PhD training, I carried out three experimental works, wherein NGS sequencing on NextSeq 550 platform (Illumina) and RNASeq analysis represented the starting point for functional analyses. In the first work, based on the emerging ability of phytochemicals to affect human gene expression, we studied the effect of cannabidiolic acid, N-trans-caffeoyltyramine and cannabisin B, isolated from defatted hemp seeds, on the miRNome of cultured human neural cells, finding changes in the expression of different miRNAs related to Alzheimer disease. In the second work, we systematically profiled the small ncRNAs content from mitochondria highly purified from different murine tissues that largely rely on mitochondria functioning, finding mitochondrial miRNAs and piRNAs (mitomiRs and mitopiRNAs) potentially involved in “basic” or “cell context dependent” mitochondria functions. In the last work, we obtained a genome-wide perspective of the whole targetome of miR-125a, a relevant oncosuppressor in hepatocellular carcinoma, finding interesting novel targets and paving the way for piecing together the RNA regulatory networks governed by the miRNA.

Exploring the impact of the maternal-effect gene *Padi6* on female fertility, embryogenesis, and epigenetic reprogramming in mice

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Session: Cellular and molecular bases of human diseases

PADI6 is a member of the subcortical maternal complex (SCMC), which plays a crucial role in reproductive outcomes. Loss-of-function mutations affecting SCMC components have been identified in healthy women with fertility issues and/or offspring with imprinting defects. We investigated gene expression and epigenetic modifications, using a transgenic mouse line carrying a hypomorphic missense variant in the *Padi6* gene, that was previously identified in a mother of two siblings affected by Beckwith-Wiedemann syndrome (BWS) and multilocus imprinting disturbances (MLID). We found that *Padi6* transcription was maintained in heterozygous and homozygous female lines, but PADI6 protein was not detected in the homozygous line, suggesting that the mutation affects protein stability. This variant leads to embryo development arrest at the two-cell stage in the homozygous female mice. Single-cell multiomic analysis demonstrated defective maturation of *Padi6* mutant oocytes and incomplete DNA demethylation, deregulation of maternal decay and zygotic genome activation (ZGA) genes in two-cell embryos derived from mutant oocytes. Analysis of mutant oocytes and embryos revealed mislocalization of the epifactors DNMT1 and UHRF1, suggesting DNA methylation involvement in the two-cell block. This study shows that PADI6 regulates nuclear and cytoplasmic processes in oocytes, essential for preimplantation development and ZGA reprogramming.

Reproductive cytotoxic and genotoxic impact of polystyrene microplastic on *Paracentrotus lividus* spermatozoa

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Session: Cellular and molecular bases of human diseases

In recent times, industrial expansion, intensive farming, and urban growth have severely harmed marine ecosystems, endangering the aquatic and terrestrial organisms' health. Improper waste management result has led to release of plastic massive quantities into environment, which degrade into microplastics (MPs), posing health risks due to their ability to biomagnify and bioaccumulate. Among these, polystyrene MPs (PS-MPs) are prevalent pollutants in marine habitats, widely studied for their toxicological effects on reproduction.

Data from previous PhD years showed that PS-MPs caused damage to zebrafish genome, decrease of vitality and motility and an increase genomic instability of human spermatozoa. For this reason, the aim of the third PhD year was to evaluate *in vitro* the reproductive cytotoxic and genotoxic ability of PS-MPs on sea urchin (*Paracentrotus lividus*) spermatozoa, evaluating the effects on seminal parameters, alterations in genetic material in term of DNA fragmentation throught TUNEL test, and ROS production using NBT test.

Being a new experimental model in treatment with PS-MPs, the spermatozoa were treated with a single PS-MPs concentration (50 μg/mL) for a single exposure time (30 minutes).

Results showed that PS-MPs significantly decreased sperm vitality and motility without altering morphology, and induced sperm DNA fragmentation mediated by ROS production. Additionally, head-to-head spermatozoa agglutination was observed only in sample treated with PS-MPs, as also observed in human spermatozoa, indicating MPs ability to adhere to sperm cells surface and form aggregates with MPs on other sperm cells, impeding movement and reducing reproductive potential. These results suggest that PS-MPs may negatively influence sea urchin sperm quality, potentially influencing reproductive events.

Regulatory mechanism and functional impact of Post-Translational Modifications on Human Paraoxonase2

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Session: Cellular and molecular bases of human diseases

The Human Paraoxonase (PON) family encompasses three highly conserved lactonases: PON1, PON2 and PON3, with PON2 exhibiting the highest lactonase activity [1]. Predominantly located on the plasma membrane, PON2 plays a critical role in innate immunity, serving as a primary defence against infections. Previous studies have highlighted several Post Translational modifications (PTMs) of PON2 including Glycosylation, Ubiquitination and ADP ribosylation. Notably, the rapid decrease in PON2 activity in extract from HeLa cells exposed to the bacterial quormone 3-Oxo-dodecanoyl Homoserine Lactone (3OC12HSL) has been attributed to a ubiquitination at lysine 144 and possible effect on SNP A148G [2].

Our recent analysis leveraging advanced Proteomic data analysis tools has revised a preliminarily identified ADP-ribosylation site at aspartate 124 [3,4] as instead arginine 101. Site-directed mutagenesis, followed by Western blot analysis, confirmed R101 as the putative ADP-ribosylation site. Current investigations are focused on identifying the Poly ADP-Ribose Polymerases (PARPs) involved in PON2 ADP-ribosylation, with ART5 emerging as a potential interactor from interactome databases with its correct substrate specificity for Arginine ADP-ribosylation [5]. Additionally, we are also examining the impact of ADP-ribosylation on PON2's lactonase and antioxidant activities in response to pyocyanin and H_2O_2 treatments. Our findings are poised to significantly enhance the understanding of PON2 regulation and its multifaceted roles in cellular defence mechanisms.

miR-18a-5p reduces lipid accumulation by regulating SREBP1c expression *in vitro* and *in vivo NAFLD* models: link with ER stress response

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Session: Cellular and molecular bases of human diseases

Metabolic dysfunction-associated fatty liver disease, or MAFLD, has become an increasingly common public health issue. Excess lipid exposure leads to the hepatic unfolded protein response (UPR) that is activated under the condition of endoplasmic reticulum (ER) stress. Recent studies have examined the relationship between ER stress and microRNAs (miRNAs). Based on these considerations, this study aimed to investigate the involvement of miR-18a-5p/SREBP1/PERK pathway in ER stress and how this pathway can affect autophagy and apoptosis in the early stages of MAFLD induced by high-fat diet (HFD). Male Wistar rats, fed on HFD for five weeks, were used. In addition, an in-vitro model of Hep-G2 cells transfected with miR-18a-5p mimic and treated with an oleate/palmitate mixture for 24 hours was used to confirm the central role of the miR-18a-5p/SREBP1/PERK axis. The results indicated that in both the experimental models the expression of miR-18a-5p was downregulated by excess fat. This downregulation was associated with an increase in SREBP1c and PERK levels, highlighting a state of ER stress. Furthermore, the activation of PERK signaling induced a decrease in the autophagic process and an increase in apoptosis. Overall, this study reveals that miR-18a-5p/SREBP1/PERK axis contributes to ER stress induced by HFD, suggesting promising pharmacological targets.

Multiomic analysis of maternal *Padi6*-deficient blastocysts

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Session: Cellular and molecular bases of human diseases

Padi6 is a maternal-effect gene that encodes a subcortical maternal complex (SCMC) associated protein, required for successful embryo development. In humans, pathogenic *Padi6* variants cause female fertility issues and are associated with imprinting disorders (IDs) and multi-locus imprinting disturbance (MLID) in the offspring. Previously, gene expression and epigenetic modifications experiments were conducted using a mouse line carrying a *Padi6* missense variant identified in a family with Beckwith-Wiedemann syndrome (BWS) and MLID. This analysis demonstrated that *Padi6* P620A variant causes defects in oocyte maturation, impairment in embryo epigenetic reprogramming, and failure of zygotic genome activation (ZGA). This results in most embryos arresting at 2-cell stage, and a few of them arresting at the blastocyst stage during embryonic development. To evaluate the potential implantation capacity, 10 mutant blastocysts were injected into the uterus of wildtype females and only 3 empty decidual capsularis were observed. To deeply investigate the molecular profile of these blastocysts, we generated RNA-seq and BS-seq libraries of 10 wildtype and 10 rare *Padi6*$^{MatP620A/+}$ blastocysts. Preliminary results of gene expression analysis revealed the deregulation of genes coding for epi-factors involved in histone modification and chromatin remodelling. DNA methylation analysis is still ongoing to validate these data, that could explain the observed failed implantation.

Identification of genomic and epigenomic biomarkers in liquid biopsy by Next-Generation Sequencing in prostate cancer

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Session: Cellular and molecular bases of human diseases

In the era of precision oncology, molecular profiling of individual patients' tumors can direct treatment decisions and monitor subsequent responses. In detail, liquid biopsies involve extraction and analysis of circulating nucleic acid to assess somatic mutations and copy number alterations providing the opportunity of detecting, analyzing and monitoring cancer.

During the first year of activity, the study focused on the optimization of methods for the analysis of genomic alterations using liquid biopsy and next generation sequencing approach. The method was validated on a small cohort of 10 patients with pancreatic ductal adenocarcinoma.

During the second year of activity, the previous validated methods have been transposed on patients with prostate cancer, a more frequent but less aggressive tumor. In particular, epidemiological data highlight prostate cancer as a significant global health issue, with high incidence and substantial impact on patients' quality of life. Genomic DNA (gDNA) and circulating tumor DNA (ctDNA) were extracted from each sample to study genetic susceptibility, epigenetic modifications, and somatic mutations. For data analysis, we are developing an *ad hoc* bioinformatics pipeline to merge omic data for patient stratification and identification of new possible diagnostic and/or prognostic biomarkers.

During the PhD training, the approaches and methodologies above described were also successfully applied to other research lines, recently published.

De novo variants in a gene encoding a *histone* underlie a novel Neurodevelopmental Disorder

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Session: Cellular and molecular bases of human diseases

Histones play a fundamental role in genome organization and regulation. Variants in histone encoding genes underlie rare Mendelian neurodevelopmental disorders (NDDs). The Telethon Undiagnosed Diseases Program aims to identify the genetic causes of undiagnosed rare pediatric monogenic disorders. We show that variants in *a* gene encoding a replication-independent histone, result in a novel NDD. Whole exome sequencing on blood DNA, RNA-Seq and cellular biology-based approaches were performed on patient fibroblast cell lines. We report five unrelated individuals, collected via Matchmaker Exchange, displaying profound NDD and carrying *de novo* missense variants in a *histone gene*, predicted as pathogenic. Structural modeling predicts that amino acid substitution induces instability in overall protein structure. RNA-Seq identified differentially expressed genes encoding extracellular matrix (ECM) structural constituents and endoplasmic reticulum (ER) proteins. These results prompted us to examine the impact of the gene variants on trafficking of collagen type-I and found a significant delay in ER exit and extracellular secretion. Our findings will be validated in neurons, oligodendrocytes and in the zebrafish mutant model we are characterizing. Collectively, our preliminary data provide evidence that *de novo* variants retrieved in our patients result in a novel NDD associated with altered intracellular trafficking and secretion of ECM constituents such as collagen type-I.

Identification of sex-specific therapeutic strategies in aging and dementia

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Session: Cellular and molecular bases of human diseases

Aging is the first risk for neurodegenerative disorders and leads to the accumulation of misfolded proteins such as β-amyloid and α-synuclein which are believed to underly the onset of brain disorders. Most of the treatments for brain disorders come from pre-clinical evidence in males, although women are more vulnerable than men to Alzheimer' disease (AD). However, the mechanisms underlying these sex-differences are not known. To date, two therapeutic approaches are predominantly used to prevent and/or slow neurodegeneration; they include autophagy pharmacological enhancers and exercise training. In this study, using a mouse model of early aging (CD1), we report that female mice have similar cognitive deficits to males, but they occurred earlier. In both sexes this cognitive decline is associated with the accumulation of misfolded protein and impaired autophagy-lysosomal function. However, they differently respond to the precognitive effects of exercise training and autophagy agonists. Similar results were obtained using a genetic mouse model of AD (TG2576). These findings provide novel and translational relevant preclinical evidence of sex-dependent efficacy of anti-dementia treatments, filling a major knowledge gap in gender medicine for AD.

Pharmacological stimulation of autophagy to rescue proteinopathy and cognitive decline in lysosomal storage disorders

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Session: Cellular and molecular bases of human diseases

Lysosomal storage disorders (LSDs) are rare inherited metabolic disorders caused by defects in lysosomal function. Mucopolysaccharidoses (MPS) are a subset characterized by defective lysosomal enzymes that degrade glycosaminoglycans (GAGs), leading to the accumulation of undegraded proteins and, in some cases, neurodegeneration and pediatric dementia. One promising therapeutic strategy involves promoting the degradation of these secondary storages to prevent neurodegeneration. Overexpression of the transcription factor EB (TFEB), which regulates autophagy and lysosomal degradation, has shown potential in animal models, but few synthetic drugs can stimulate TFEB and cross the blood-brain barrier. This study tests a compound in animal and cellular models of MPS, showing it effectively promotes TFEB-mediated autophagy and lysosomal biogenesis. In vitro analysis confirmed its efficacy in cell lines from various MPS types, revealing how it activates autophagic flux. In an MPS-IIIA animal model, the drug improved cognitive deficits and facilitated the clearance of beta-amyloid deposits. These findings provide proof-of-concept evidence for a new therapeutic compound for LSDs.

Omics analyses to molecularly characterise a large cohort of Silver-Russell Syndrome patients

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Session: Cellular and molecular bases of human diseases

Silver-Russell syndrome (SRS) is a congenital imprinting disorder associated with growth retardation.The most common defect is loss of methylation (LoM) of the imprinting control region 1 (IC1) on chromosome 11p15.5 (30-60%), followed by maternal uniparental disomy (UPD) of chromosome 7 (5-10%). About 40% of cases remain undiagnosed.

The aim of our study is the molecular characterisation of an Italian cohort of 69 SRS patients by using three omics approaches: SNP-array, methylation array and whole exome sequencing (WES). Based on a previous molecular diagnosis to detect defects of 11p15.5 and chromosome 7, the cases were classified into three distinct molecular groups.

The IC1-LoM group (N=27) was analysed by SNP-array and methylation array, revealing 1 case carrying a maternal duplication involving the 11p15 imprinted gene cluster, and 2 cases with a mosaic UPD(11)mat. The UPD(7)mat cases (N=3) were analysed by SNP-array which revealed mixed isodisomy/heterodisomy for all of them. The idiopathic group (N=39) was analysed by all three methods. SNP-array revealed 2 cases with pathogenic CNVs at chr12q14 and 6q. WES, performed on 33 cases, detected 13 patients carrying rare pathogenic variants in causative genes of growth-failure conditions.

The methylation analysis for IC1-LoM and idiopathic cases is still in progress to detect potential methylation profiles related to SRS. Preliminary data show hypomethylation of HOXA4 promoter in 52% of the analysed patients.

Polystyrene microplastics impair steroidogenesis by inducing mitochondrion-endoplasmic reticulum dysregulation

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Session: Cellular and molecular bases of human diseases.

Polystyrene microplastics (PS-MP) exposure cause testosterone deficiency and spermatogenesis impairment, however the underlying mechanisms remain unclear. By using a murine cell line, TM3, this study aims to investigate the cellular response induced by PS-MP in Leydig cells (LC), the testicular site of testosterone synthesis. We found that the exposure to increasing concentrations (0-200 μg/mL) of PS-MP for 24 h caused a dose-dependent reduction in TM3 cells viability. Furthermore, we found that PS-MP inhibited the expression levels of StAR, protein responsible for transporting cholesterol into the mitochondrion to initiate steroidogenesis, and 3β-HSD and 17β-HSD, two steroidogenesis-related enzymes. PS-MP treatment downregulated both ERK1/2 and Akt pathways in TM3 cells, suggesting that PS-MP could interfere with LC function inhibiting the kinase pathway signaling. PS-MP-caused oxidative stress decreased the antioxidant defense of TM3 cells, triggering autophagic and apoptotic processes. Mitochondrial dysfunction was also observed, as evidenced by the decrease in mitochondrial membrane potential, ATP, and calcium levels. Finally, PS-MP treatment induced ER stress and Mitochondrial-Associated Endoplasmic Reticulum Membrane (MAMs) alterations, suggesting that PS-MP could impair steroidogenesis through mitochondrion-endoplasmic reticulum dysregulation. Our study elucidates the intracellular mechanisms underlying the PS-MP effects on LC, providing new insights about their potential impact on male reproductive health.

Serum D-serine Correlates with Age and Treatment in Parkinson's Disease

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Session: Cellular and molecular bases of human diseases

Parkinson's disease (PD) is a neurodegenerative condition characterized by the death of dopaminergic neurons in the nigrostriatal pathway. Several evidence suggest that beyond dopaminergic system other neurotransmitters are involved in PD, in particular molecular alterations occurring at ionotropic N-methyl-D-aspartate receptors (NMDARs) contribute to appearance of the disease features. Among neurotransmitters involved in glutamatergic system, D-serine (D-Ser) plays a crucial role acting as NMDARs co-agonist.

A previous study carried out on preclinical models of PD, showed increased levels of D-Ser as well as of its metabolic precursor, L-Serine (L-Ser) in the rostral putamen of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. Interestingly a clinical study showed an increase in serine enantiomers in cerebrospinal fluid and caudate putamen of PD patients.

In light of these evidence we investigated serum levels of D- and L-Ser as well as other NMDAR-related amino acids in PD patients compared to healthy controls (HC) through High Performance Liquid Chromatography (HPLC) adjusting the analyses for age and sex.

On the other hand we investigated the correlation of D-ser and D-/Total Ser ratio and other excitatory amino acids with age in PD patients and PD age at the onset as well as Levodopa Equivalent Daily Dose (LEDD).

Unravelling the role of the TGN export machinery for basolateral proteins in Amyloid Precursor Protein (APP) transport and processing

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Session: Cellular and molecular bases of human diseases

The secretory pathway is involved in the synthesis, post-translational modification and transport into specific compartments of around 30% of human proteins. In the Trans-Golgi Network (TGN), basolateral proteins activate the orphan receptor GPRC5A to promote and regulate their own export and sorting, thanks to a series of signalling events that culminate with Protein Kinase D recruitment and activation. Indeed, the mis-sorting of basolateral proteins and/or the dysregulation in the TGN-sorting machinery have been associated with different human diseases, including neurodegeneration. Our aim is to test the role of the basolateral sorting system on the Amyloid precursor protein (APP) transport and processing, normally processed by α-, β-, and γ-secretases. We are characterising APP intracellular and extracellular levels and fragments in HeLa cells upon GPRC5A depletion (GPRC5A-KD) and treatment with secretases inhibitors. Moreover, we are characterizing the localization and expression of APP upon GPRC5A-KD. We are analysing APP processing dynamics upon GPRC5A signalling alteration by using HeLa cells stably transfected with APP dual-tag RUSH synchronisation system. Here, our goal is to identify a possible mechanism that couples the TGN regulatory system with APP transport and processing to reduce the production of the amyloidogenic peptide and favour the production of the non-amyloidogenic peptide.

Long read sequencing for elusive pathogenic variants

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Session: Cellular and molecular bases of human diseases

Long-read sequencing technologies have revolutionized the field of genomics by enabling the comprehensive detection of complex genetic variants that are often overlooked by short-read sequencing. These methods excel at identifying structural variants, repetitive regions, and intricate genomic rearrangements, thus significantly enhancing diagnostic capabilities for genetic disorders. Here we report three illustrative cases where long-read sequencing confirmed definitive diagnoses that were missed by conventional techniques. The first case involved a 447bp Alu insertion in the *NSD1* gene, which led to exon 13 skipping, prompting the development of Sotos syndrome. In the second case, a 3kbp deletion in the *DEGS1* gene, associated with hypomyelinating leukodystrophy, which had eluded short-read sequencing and array CGH analysis, was identified. The third case highlighted a 7Mbp inversion in the *EHMT1* gene responsible for Kleefstra syndrome, clearly resolved by long-read sequencing but entirely missed by short-read methods. These cases underscore the critical importance of adopting long read sequencing in clinical diagnostics, where its ability to reveal hidden genetic changes enhances the understanding of genetic disorders and improves patient management and care. The integration of these technologies is crucial for advancing genomic medicine and achieving more accurate and comprehensive genetic analyses.

Untangling the intricate networks shaped by D-Asp in the developing brain

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Session: Cellular and molecular bases of human diseases

D-aspartate (D-Asp) is an endogenous ligand for NMDAR and mGlu5 abundant in the prenatal and early postnatal brain. D-Asp levels fall after birth due to the catabolic enzyme D-Aspartate oxidase (DDO). Evidence highlighted the significance of this atypical amino acid in influencing neural plasticity during brain development. However, the molecular pathways downstream D-Asp action remain unclear. To elucidate the events orchestrated by perinatal D-Asp occurrence, we developed a mouse model that expresses Ddo (DdoOV) throughout fetal life, resulting in D-Asp depletion. Previously, these mice were reported to have metabolome alterations, cognitive impairment, and altered corticogenesis. Here, after characterising the neurochemical profile, we investigated the proteome changes produced by D-Asp depletion during brain development. Our findings suggest that D-Asp influences the neonatal expression of proteins involved in synapse formation, cytoskeleton organisation, and nervous system development. Furthermore, a subset of D-Asp-regulated proteins map to pathways associated with autism spectrum disorders and schizophrenia, providing new insight into D-Asp's role in regulating neurodevelopmental processes linked to psychiatric diseases. Proteins identified through the proteomic approach are currently undergoing ISH spatial analysis. Moreover, investigations on the influence of D-Asp in modulating cortical dendritic spine density and cell proliferation are in progress. In conclusion, this study will contribute to a better understanding of the complex networks influenced by D-Asp during brain development.

Optimizing Long-Read Sequencing Pipeline for Rare Disease Diagnostics

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Session: Cellular and molecular bases of human diseases

Long-read sequencing has transformed rare disease diagnostics by resolving complex genomic regions that are often inaccessible to short-read technologies. In this study, we aim to analyze a diverse cohort of samples from the MNESYS project aimed at using Oxford Nanopore Technology (ONT) to investigate single nucleotide polymorphisms (SNPs), structural variations (SVs), and the challenging copy number variations (CNVs)- key risk factors for genetic mood and psychotic disorders.

We are testing established pipelines like Epi2me and MegSAP for ONT data analysis alongside building a custom pipeline tailored to our needs and computational abilities. Tools like Minimap for alignment, DeepVariant, Sniffles that are considered gold-standard for long-read variant calling are integrated to optimize the performance of our pipeline. So far, we have analysed 40 samples with an average read count of approximately 9.8 million and mean depth ranging from 12x to 50x. The number of SNPs identified per sample varied from 4 to 5 million, while SVs, including deletions, insertions ranged between 31,237 and 35,540.

Given the size and complexity of the data generated by Nanopore sequencing, we are continually refining our analytical methods to make variant detection more precise and efficient, ultimately making the results more accessible for research and diagnostic purposes.

LANCELOT: A Molecular Newborn Screening of Treatable Conditions

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Session: Cellular and molecular bases of human diseases.

Advancements in sequencing technologies yielded a more comprehensive picture of numerous diseases' molecular bases. This acquired knowledge led to new research and clinical applications which have revolutionised molecular diagnostics. Furthermore, the advent of innovative therapeutics, such as gene therapy, has provided many genetic conditions with successful treatments. In this context, we conceived the Molecular Newborn Screening of Treatable Conditions (LANCELOT) project that paves the way for the early identification of well characterized treatable genetic disorders. Our aim is to develop a customized Next Generation Sequencing (NGS) panel-based Newborn Screening (NBS) focused on genomic regions in which druggable disease-causing mutations are located. We interrogated the ClinicalTrials.gov database to identify the genes associated with treatable conditions suitable to undergo our NGS-NBS. The 35 selected genes were included in our panel design provided by Agilent SureDesign platform. We validated our panel developing a standardized protocol from Dried Blood Spot. The data analysis revealed an average coverage of the target regions of 1000X, with even greater depth observed at the mitochondrial locus. This high resolution enabled the identification of single nucleotide variants, copy number variants, and mosaicisms, demonstrating LANCELOT's efficacy and highlighting its potential utility in the diagnostic and therapeutic management of pediatric conditions.

The effect of palmitoleic acid, an exerkine during fasting, in muscle cells

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Session: Cellular and molecular bases of human diseases

The response to exercise during fasting has been shown by several groups including our group to induce skeletal muscle Akt signaling in an insulin-independent fashion, however the underlying factors are unknown upto now. Targeted lipidomic analysis has shown that one lipid, palmitoleic acid, is normalized to control levels in response to exercise during fasting. It has been shown that palmitoleic acid induces GLUT4 membrane trafficking in absence of insulin in muscle cells, which may occur through Akt signaling. Beta hydroxy butyrate (BHB), that we have previously shown to be highly present in muscle in the fasted state, may influence Akt signaling, but is not known to what extent. In light of these observations, using L6 myoblasts as a model, we tested the response of Akt signaling to BHB, and whether palmitoleic acid could affect the action of BHB. Western blotting of whole lysates was performed to analyse the effects of these interventions. Results will be presented on the counteractive effect of palmitoleic acid on BHB-induced repression of Akt signaling.

Combined effect *in vitro* of TiO_2 nanoparticles and polystyrene microplastics on human spermatozoa

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Session: Cellular and molecular bases of human diseases

In the past two decades, there has been a decline in human fertility, primarily due to exposure to environmental toxins. Among these, plastic waste poses a significant health threat.

The plastic is breakdown into microplastics (MP), which can disrupt the blood-testis barrier, induce apoptosis, increase DNA fragmentation and cause an overproduction of ROS, reducing fertility.

A particularly troubling characteristic of MP is their ability to adsorb harmful substances and act as carriers for other pollutants, resulting in a synergistic toxic effect. MP could transport titanium dioxide nanoparticles (TiO_2-NPs), which, due to their small size, can cross biological barriers and be absorbed by cells, causing inflammation, sperm DNA damage and oxidative stress.

Given this context, I investigated, *in vitro*, the potential synergistic cytotoxic and genotoxic effects of PS-MPs and TiO_2-NPs on human spermatozoa. Experiments were conducted for 30, 60, 90 minutes using PS-MP (210 µg/ml) and TiO_2-NP (10 µg/L), in combination. The genotoxic potential was evaluated using the TUNEL Test and the NBT Assay, while the cytotoxic effect was tested using the sperm viability test. Results showed that genotoxicity and cytotoxicity were higher with co-exposure of PS-MP and TiO_2-NP compared to single exposures of PS-MP, indicating an interaction between the substances.

Development of stem cell-based models of cohesinopathies in vitro

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Session: Cellular and molecular bases of human diseases.

Cohesinopathies comprise a spectrum of developmental disorders caused by genetic mutations in the cohesin complex or its regulators. Besides its canonical role in sister chromatid segregation and DNA replication/repair, recent studies implicate cohesin participating in gene transcription, cellular proliferation and protein synthesis. This study focuses on the requirement of cohesin and/or its regulators in cellular differentiation. To this end, we have created isogenic human iPSC lines carrying cohesinopathy-related mutations in NIPBL, BUB1, SGOL1, STAG2, ESCO2 and DDX11 genes, using the CRISPR/Cas9 platform. Due to the developmental origin and often overlapping clinical symptoms between cohesinopathies and neurocristopathies, a group of diseases caused by the dysfunction in migration, generation, or differentiation of neural crest cells (NCCs), the next step is to differentiate edited iPSCs to NCCs and NCC-derived cell types. The final aim of the study is to analyse these clinically relevant cell types using appropriate functional assays able to detect specific disease-related phenotypes, such as defects in migration, cohesion, ribosome biogenesis and translation efficiency, apoptosis, and cell growth. In addition, the CombiCult® technology and transcriptomics will be conducted to identify cohesin-dependent changes in the cellular processes and transcriptomic profiles of the iPSC-derived cells.

Mitochondrial Unfolded Protein Response (UPR^{mt}) is involved in maintaining BAT mitochondria functionality of obese mice treated with 3,5-diiodothyronine (T2)

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Session: Cellular and molecular bases of human diseases

Brown Adipose Tissue (BAT) dysfunction is strongly associated with obesity and metabolic diseases. Thyroid hormones (TH) and their analogues profoundly influence BAT thermogenesis. In addition to playing a critical role in brown adipocyte recruitment, mitochondria are also a key target for TH. There are several mechanisms that maintain mitochondrial homeostasis including the mitochondrial unfolded protein response (UPRmt), mitochondrial fission, mitochondrial fusion, mitophagy, which are referred to collectively as mitochondrial quality control (MQC). Under pathophysiological conditions such as obesity, excessive oxidation of nutrients induces mitochondrial stress, leading to impaired mitochondrial integrity and functionality. In this study, we analyse the effect of T2 administration on mitochondrial homeostasis in BAT of obese mice. Obesity was induced over 15 weeks on a high-fat diet (HFD). As expected, iodothyronine reduces adiposity, increases BAT mass and shows a termogenic effect. In addition, T2 activates UPRmt to degrade unfolded and damaged proteins. We analyzed the canonical UPRmt axis impaired in obese mice. In conclusion, these findings support the idea that T2 can mitigate BAT mitochondrial dysfunctions observed in obese mice. Iodothyronine preserves BAT mitochondria functionality and adaptive thermogenesis. These new evidences on T2 effects further emphasise the therapeutic potential of this iodothyronine in the treatment of metabolic diseases.

Gaining insight into the role of long noncoding RNA in inherited retinal disorders

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Session: Cellular and molecular bases of human diseases

Inherited retinal diseases are a group of genetic disorders characterized by retinal degeneration and progressive visual impairment. The main feature of these disorders is the high clinical and genetic heterogeneity which represent a limitation for the application of gene specific therapies. For this reason, mutation independent therapeutic approaches are gaining interest. In this direction, we focus our attention on noncoding RNAs which are able to regulate gene expression at transcriptional and post-transcriptional level. Long noncoding RNAs (lncRNAs) are one type of noncoding RNAs and they are involved in retinal function. LncRNAs are typically longer than 200 nucleotides and exhibit low sequence conservation across species. In recent years there is increasing amount of data relating to their function in ocular diseases. However, there are few information about their role in inherited retinal diseases. The aim of our project is to create lncRNA expression atlas in the retina in different mouse models of inherited retinal diseases using Next Generation Sequencing strategies. Sequencing data will be analyzed using bioinformatic tools and screened to choose potential candidates involved in modulation of pathological process. Deciphering their expression profile in these conditions can help to better understand the molecular mechanism of retinal diseases and improve therapeutic strategies.

A novel competing endogenous RNA network involving the lncRNA JPX, miR-378a-3p and its mRNA targets in lung adenocarcinoma

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Session: Cellular and molecular bases of human diseases

Non-coding RNAs, particularly microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are emerging as key players in the development and progression of lung cancer, the leading cause of cancer-related death.

The project aims to investigate miRNA/lncRNA interactions within competing endogenous RNA network (ceRNET) that potentially promotes lung adenocarcinoma (LUAD). Analyses of three different datasets revealed a significant upregulation of the lncRNA JPX in LUAD tissues compared with normal lung tissues, as expected for an oncogene. The TCGA dataset showed that miR-378a-3p, predicted to bind JPX, was downregulated in LUAD and inversely correlated with JPX expression, suggesting a potential functional interaction. Reporter vectors confirmed the JPX/miR-378a-3p physical interaction. Enhancement of JPX and/or miR-378a-3p, followed by comprehensive cell-based assays including cell proliferation, migration, invasion, and 3D-spheroid formation, demonstrated JPX's oncogenic role, miR-378a-3p's tumor-suppressive function, and their functional interaction in LUAD. Additionally, JPX was found to inhibit miR-378a-3p’s silencing effect toward its oncogenic targets GLUT1, NRP1, YY1, and Wnt5a. Overall, these results reveal a novel ceRNET in which JPX functions as a ceRNA by binding to miR-378a-3p, thereby inhibiting the miRNA's silencing activity on downstream oncogenic targets and promoting LUAD. Next years will be dedicated to understanding the molecular pathways regulated by the RNAs network, particularly focusing on those involving GLUT1, NRP1, YY1, and Wnt5a, i.e. glucose metabolism, hypoxia adaptation, angiogenesis and Wnt pathway.

The project findings may provide new insights for the development of novel diagnostic and therapeutic strategies.

Morphological and functional characterization by preclinical imaging techniques of genetically modified mouse models for D-aspartate oxidase

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Session: Cellular and molecular bases of human diseases

D-aspartate (D-Asp) is an amino acid present in the mammalian organism, specifically in the central nervous system and at the level of peripheral organs. Its role has not been fully characterized yet but is known that D-Asp is involved in synaptic transmission, in fact, it acts as an agonist of the glutamatergic receptors NMDA(NMDARs) [1-2]. After birth, in the brain, the prenatal high levels of D-Asp decrease due to the expression of the D-aspartate oxidase (DDO) [1], which is the only known enzyme capable of degrading this amino acid [3]. On the contrary, in peripheral tissues (liver and gonads) its concentration increases in the postnatal period, suggesting an implication in sexual behavior [1].

Recent studies have shown a correlation between D-Asp and alterations on NMDARs in schizophrenic subjects [4]. Therefore, the aim of this study is to use two imaging methods to conduct morpho-functional examinations on genetically modified mouse models, which over-express DDO. Through BOLD functional magnetic resonance (fMRI) is possible to highlight the brain areas with greater activity, characterized by an increase in blood flow and oxygenation, potentially related to the brain areas involved in schizophrenia [4]. Instead, High Frequency Ultrasound is the method of excellence for the structural and vascular evaluation of the abdominal organs (liver and gonads) for the analysis of metabolic and reproductive functionality [5-6]. To date, the images of both techniques have been acquired and are currently undergoing post processing.

Role of Thyroid hormone (T3) in muscle metabolic signaling and differentiation

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Session: Cellular and molecular bases of human diseases

Thyroid hormones are mediators of metabolic effects in muscle. Our group has previously shown that T3, the biologically active thyroid hormone, is increased in muscle during exercise, both in the fed and in the fasted state. This correlated with an increased expression of muscle deiodinase 2, which locally converts T4 in T3. We have previously shown that T3 activates signal transduction pathways towards increased glucose and lipid metabolism (Akt and AMPK signalling), and it is well known that T3 is crucial for myoblast differentiation. Apart from T3, exercise during fasting also induces muscular levels of palmitoleic acid and the ketone body beta hydroxy butyrate (BHB). In light of these observations, using L6 myoblasts as a model, we set out to study whether T3, alone or in combination with palmitoleic acid and BHB, induced Akt and AMPK signalling and myogenin expression, the latter being a marker for muscle differentiation. Cells were loaded with 100nM T3, 8mM BHB and/or 0.75 mM palmitoleic acid, and Western blotting of whole lysates was performed to analyse the effects of these interventions. Ongoing research will be presented presented on cell morphology, indicative of cell differentiation, and Akt signalling in the muscle cells.

Investigating the role of the phosphatase SHP-1 in regulating cellular senescence

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Session: Cellular and molecular bases of human diseases

Cellular senescence is a physiopathological process triggered by various intrinsic and extrinsic factors, leading to a stable and irreversible growth arrest. Senescent cells exhibit morphological alterations, deregulated metabolism, and changes in gene expression of numerous proinflammatory cytokines, chemokines, growth factors, and proteases, known as senescence-associated secretory phenotypes (SASPs). This results in a chronic state of inflammation that accompanies and accelerates age-related diseases.

SHP-1, a protein tyrosine phosphatase that regulates signalling pathways controlling cell growth, differentiation and apoptosis, has been recently associated with the induction of cellular senescence.

To explore the role of SHP-1 in this context, we induced cellular senescence in ARPE-19 retinal cells using two different approaches: treatment with hydrogen peroxide and doxorubicin. In both conditions, we observed a modulation of the phosphorylation state of SHP-1, likely related to changes in its catalytic activity.

Additionally, we found that targeting SHP-1 activity with its chemical inhibitor resulted in the modulation of specific senescence markers, including β-Gal expression, increased p21 levels, formation of nuclear γH2ax foci and the SASP phenotype.

Conclusively, these data suggest a role for SHP-1 in the regulation of cellular senescence, highlighting its potential significance in the treatment of age-related diseases.

Methylation profile of the imprinting control regions and their variability in the normal population

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Session: Cellular and molecular bases of human diseases.

Genomic imprinting is a regulatory process that leads to the mono-allelic, parent-of-origin-dependent expression of approximately 100 mammalian genes. In its typical form, imprinting results from the differential establishment and maintenance of DNA methylation on maternally and paternally inherited autosomes. Over 50 regions with stable differential DNA methylation (iDMRs) across multiple tissues, including blood, have been identified. We used methylation array datasets from 2,664 individuals to examine the methylation levels of 49 known human iDMRs in peripheral blood leukocyte (PBL) DNA from a normal population. Statistical analysis allowed us to categorize iDMR CpGs into three groups: LOWvar, SDvar, and Mvar CpGs. After evaluating the variation in iDMR methylation levels within the general population, we investigated factors influencing methylation in these regions. To achieve this, we utilized extensive data from the EWAS Data Hub and Atlas, linking iDMR CpGs with disease-related and non-disease traits. This combined epigenomic and computational approach helped us identify CpG variability and traits that may influence population-level DNA methylation at iDMRs.