Università degli Studi della Campania *Luigi Vanvitelli*

PhD Days 2022
PhD Programme in Molecular Life Sciences

Cancer biology, Immunology, Microbiology, Drug design

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Inhibition of hypoxia-induced carbonic anhydrase IX sensitizes triple negative breast cancer cells and murine models to cisplatin
Tutor: Prof Antonella Zannetti

Federica La Rocca
Identification of neuroprotective natural compounds for the development of nutraceuticals and drugs: the effects of CLA on *C. elegans* neurodegenerative disease models.
Tutor: Elia Di Schiavi

Saba Sadiq
Optimization of growth of probiotic strains for potential health applications
Tutor: Donatella Cimini

THE PHUC NGUYEN
Monocytic cell line U937 as the valid model for LPS detection cell-based assay
Tutor: Paola Italiani

Gene Regulation and Computational Biology

Valeria Policastro
Network Methods for Biomedical Discoveries
Tutor: Prof. Annamaria Carissimo, Prof Claudia Angelini

Francesco Cecere
Investigating the causes of DNA methylation disturbances in the Beckwith-Wiedemann syndrome
Tutor: Prof. Andrea Riccio, Prof Flavia Cerrato

Karla Alejandra Ruiz Ceja
Inherited retinal disease genes: study of their genomic organization and identification of co-expressed long noncoding RNAs by meta-analysis of human retina transcriptome data
Tutor: Prof. Sandro Banfi

Lucia Argenziano
The effects of the maternal-effect gene *Padi6* on mouse female fertility, embryogenesis, and epigenetic reprogramming
Tutor: Prof. Andrea Riccio

Sara Savaheli
Elucidation of the link between RNA maturation and neurodegeneration by a cell-specific transcriptomic analysis in *C. elegans*
Tutor: Dr. Denis Dupuy, Prof. Andrea Riccio, Dr. Elia Di Schiavi

Structure and Functions of Biomolecules

Angela Clemente
The Structural Characterization of quinoin, type-1 ribosome-inactivating protein from *Chenopodium quinoa* Willd seeds
Tutor: Prof. Antimo Di Maro

Veronica Russo
Prokaryotic and Eukaryotic zinc-finger proteins
Tutor: Prof. Paolo Vincenzo Pedone

Manoj Madheswaran
Characterization of Human Prion Protein Conformation Equilibria Using NMR Methodologies Under Cell Mimicking Conditions
Tutor: Prof. Roberto Fattorusso

Vikram Pratap Singh
Innovative mass spectrometry-based proximity labeling method for unraveling the Macrophage migration inhibition factor (MIF) interactome
Tutor: Prof. Angela Chambery

Mario di Gennaro
Hyaluronic acid and its derivatives based multifunctional nanostructured devices in cancer therapy and regenerative medicine
Tutor: Prof. Assunta Borzacchiello

Valentina Verdoliva
Development of biocompatible hyaluronan-based materials as drug-carriers and implant systems for tissue engineering
Tutor: Stefania De Luca

Giovanna Valentino
Specialized metabolites as potential lead compounds for anticancer drug discovery
Tutor: Antonio Fiorentino

Diana Santos
Dissecting the interaction of DDX11 with Tim, a fork-protection complex subunit
Tutor: Dr. Francesca M. Pisani

Maria della Valle
Polystyrene nano-plastics affect human Ubiquitin structure and ubiquitination in cell: a high-resolution study
Tutor: Prof Roberto Fattorusso

Mohammad Mahtab
Elucidating the role of DDX11, the Warsaw breakage syndrome DNA helicase, at the DNA replication fork.
Tutor: Prof Francesca M. Pisani

Alessandra Del Bene
Tailoring the structure of cell penetrating DNA and RNA binding nucleopeptides
Tutor: Prof Anna Messere

Francesca Guzzo
Bio-guided approach as a valuable strategy in the search of antibacterial compounds from three Myrtaceae plants.
Tutor: Prof Brigida D'Abrosca

Joyce Rodriguez
NMR-based metabolomics reveals the allelopathic potential of selected invasive plants in the Mediterranean basin
Tutor: Monica Scognamiglio

Clementina Acconcia
High-resolution conformational analysis of RGDechi1-14 and ψRGDechi peptides using a combination approach of Nuclear Magnetic Resonance and Molecular Dynamic simulations
Tutor: Prof. Luigi Russo

Eliza Kramarska
Structural characterization of novel vaccine antigens
Tutor: Rita Berisio

Manil Kanade
Structural studies of the RTEL1 FeS helicase
Tutor: Dr Silvia Onesti

Martina Slapakova
Transcriptional factors with a single zinc-finger domain in *Arabidopsis thaliana*
Tutor: Paolo Vincenzo Pedone

Angela Oliver
Structural and functional characterization of the recombinant FVII of coagulation
Tutor: Annamaria Sandomenico, Menotti Ruvo

Anna Magri
Development and application of new edible active formulations to preserve beneficial biomolecules and reduce fruit losses: study of treatment-induced changes to fruit metabolism.
Tutor: Prof. Antonio Fiorentino

Antonello Prodomo
Single-molecule biophysical study of the FANCJ DNA helicase
Tutor: Francesca M. Pisani

Chidoh Kootlole
Plant specialized metabolites for treatment of Leukemia
Tutor: Monica Scognamiglio

Getasew Shitaye Ayalew
Unmasking Viral RNA: targeting viral RNA capping machinery to tackle COVID-19 and future CoV emergencies
Tutor: Prof. Gaetano Malgieri

Giovanni Barra
Biocompatible Polimers For Human Health
Tutor: Rita Berisio, Alessia Ruggiero

Hafiza Zumra Fatima Hussain
Characterization of ribotoxic enzymes from Edible Mushrooms
Tutor: Prof. Antimo Di Maro

Maria Marone
A Mesophilic Phosphotriesterase-Like Lactonase Shows High Stability And Proficiency As Quorum Quenching Enzyme
Tutor: Giuseppe Manco

Martina Dragone
Interchange of different metal ion in metallo-protein
Tutor: Carla Isernia

Mehwish Kanwal
Purification, structural and functional characterization of FV zymogen from plasma fraction concentrates.
Tutor: Nunzianna Doti

Rita Russo
Plant-based investigation to discover new bioactive molecules as galectin inhibitors
Tutor: Emilia Pedone

Vincenzo Massimiliano Vivenzio
Carbonic Anhydrases represent novel molecular targets against pathogen infections.
Tutor: Simona Maria Monti, Giuseppina De Simone

Molecular Cell Biology

Carmela Casale
Cancer selective targeting of NF-kB: unravel the role of GADD45β in TNF signaling
Tutor: Alessandra Pescatore

Seyedehnegar Parizadeh
Investigation of cargo-specific autoregulatory and export systems in secretory pathway
Tutor: Dr. Alberto Luini

Miriam Lucariello
SARS-CoV-2 infection: pseudovirus system and role of the CtBP1/BARS protein
Tutor: Dr. Carmen Valente

Nagendra sai kumar Achanta
PON2 post-translational modifications and cancer
Tutor: Manco Giuseppe

Arianna Cuomo
Mild endurance exercise and fasting induce thyroid hormone action associated with BHB and BDNF signaling in rat muscle
Tutor: Prof. Pieter De Lange

Chiara Siniscalchi
Human microRNAs targeting SARS-CoV-2
Tutor: Prof. Aniello Russo; Prof. Nicoletta Potenza

Nunzia Magnacca
Involvement of miR-18a-5p/SREBP1/PERK axis in the induction of endoplasmic reticulum stress induced by hyperlipidic diet
Tutor: Prof.Antonia Lanni

Roberta Simiele
The association between depression and gut microbiota related to physical activity, diet and sleep
Tutor: Prof. Pieter De Lange

Ilenia De Leo
RNA regulatory networks governed by miR-125a in hepatocarcinoma cells
Tutor: Prof. Nicoletta Potenza

Martina Garofalo
Role of D-aspartate metabolism in neurodevelopmental disorders
Tutor: Prof. Alessandro Usiello

Marta Mallardo
Exploring adiponectin involvement in Multiple Sclerosis
Tutor: Prof. Aurora Daniele

Ana Sofia Cabaço Boavida
FANCJ the missing piece of the AND 1 interaction hub at the DNA replication fork
Tutor: Prof Francesca Pisani

Anupama Pavithran
Role of ADP-ribosylation in breast cancer sensitization to apoptosis: PARP12 as a novel therapeutic target
Tutor: Dr. Giovanna Grimaldi

Giada Onorato
Identification of environmental and genetic cues that modulate neuron degeneration in *C. elegans*
Tutor: Dr. Elia Di Schiavi

Maria Carannante
Genotoxic effects of Polystyrene microplastics in Zebrafish
Tutor: Prof.Lucia Rocco

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

Debora Latino
The protective role of D-Aspartate in counteracting cadmium toxicity in the rat testis.
Tutor: Prof. Maria Maddalena Di Fiore

Human genetics

Concetta Montanino
MicroRNAs as diagnostic biomarkers in neurodegenerative disease. A focus on MCI (Mild Cognitive Impairment) – Alzheimer's disease and MS (Multiple Sclerosis).
Tutor: Prof. Buna De Felice

Martina Di Guida
miR-181a/b downregulation: a mutation-independent therapeutic approach for Inherited Retinal Diseases
Tutor: Sandro Banfi, Sabrina Carrella

Ezia Spinosa
Inclusion and extension of data from families with IP to reveal the characteristic of IFN-I autoimmunity
Tutor: Francesca Fusco

Georgios Petrogiannakis
Identification and evaluation of microRNAs involved in photoreceptor degeneration
Tutor: Dr. Sandro Banfi, Sabrina Carrella

Sharon Russo
The ZNF687 mutation of Paget's disease causes bone remodelling alteration dysregulating osteoclast transcriptional program and osteoblast activity
Tutor: Dr. Fernando Gianfrancesco

Abu Saadat
Genome-wide studies for the molecular characterization of isolated Wilms tumor
Tutor: Prof Andrea Riccio

Romina D'Alterio
The role of microRNAs 181a and b in Parkinson's Disease: from promising therapeutic targets to potential biomarkers
Tutor: Prof. Brunella Franco, Dr A. Indrieri

Pasqualina Cennamo
Polygenic Risk Score of oxidative status and Alzheimer's Disease
Tutor: Prof. Marina Ciullo

Giorgio Fortunato
Generation of PD-disease cellular models to explore the genetic heterogeneity of Parkinson's disease
Tutor: Dr. Teresa Esposito

Session 1:

Cancer biology, Immunology, Microbiology, Drug design

Inhibition of hypoxia-induced carbonic anhydrase IX sensitizes triple negative breast cancer cells and murine models to cisplatin

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Tutor: Antonella Zannetti (email: antonella.zannetti@cnr.it)

PhD cycle: XXXV cycle

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Session: Cancer Biology (Cancer biology, Immunology, Microbiology, Drug Design.)

Eradicating resistance and improving sensitivity to chemotherapy is a critical unmet clinical need in Triple Negative Breast Cancer (TNBC). Interestingly, studies of reversible mechanisms of chemoresistance have shown that chemoresistant tumors can revert to a chemosensitive state. It is well known that hypoxic tumor microenvironment (TME) is the crucial driver in the onset of insensitivity to cisplatin (Cis-Pt) through multiple mechanisms. To this aim, we evaluated the possibility to sensitize TNBC to chemotherapy by targeting hypoxia-induced carbonic anhydrase IX (CA IX) with a sulfonamide inhibitor SLC-0111 and to monitor *in vivo* the efficacy of combinatorial therapy using molecular imaging. When TNBC cells grown under hypoxic conditions in 2D and 3D were treated with Cis-Pt (1 μM) and SLC-0111 (100 μM) a greater reduction of proliferation, tumor growth and induction of apoptosis respect to single drugs was observed. The combinatorial treatment most hampered Epithelial-Mesenchymal Transition (EMT) program, stemness and apoptotic markers. Interestingly, the addition of SLC-0111 to Cis-Pt caused a greater reduction of tumor growth and 18F-fluorodeoxyglucose (18F-FDG) uptake in a TNBC syngeneic orthotopic murine model as assessed by microPET/CT. Our results highlight the ability of SLC-0111 to sensitize TNBC to Cis-Pt by hindering hypoxia-induced signaling network that are shared among mechanisms involved in therapy resistance.

Low-molecular weight lipopeptides as strategy for fighting the antimicrobial resistance

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

Antibiotic resistance poses a serious threat to human health. In this perspective, antimicrobial peptides (AMPs) are an emerging class of chemotypes that could represent new agents against resistant bacterial infections. They exhibit a broad spectrum of activity against gram-negative and gram-positive bacteria, some fungi, viruses and parasites. Unfortunately, AMPs have several limitations, including poor stability to serum proteases and cytotoxicity to host cells, as well as high production costs. Nowadays, the reduction of AMPs sizes as well as the binding of lipid moiety could is one of the main strategies adopted to strategy to minimize damage to host cells or tissue and improve metabolic stability and antimicrobial activity. In the light of these considerations, we designed and synthesized a library of short lipopeptides endowed with a common peptide scaffold consisting of Arg-Pro-Arg. The positively charged Arg interacts with the negatively charged bacterial counterpart, while the use of the Pro, a well-known structuring amino acid, will guarantee a reduction of the freedom degree of the spacer bearing the fatty acid. During my time at the PRBB in Barcelona, I performed biological assays (MIC, MBC, Hemolytic assay, Cytotoxicity assay) on the first generation of lipopeptides. Interestingly, 4 out of the tested lipo-peptidomimetics gave excellent results in terms of antimicrobial activity and low toxicity. We are currently waiting for biological results of new lipopeptide derivatives without fatty acids and with reduced lipid chain, to understand the importance of the lipid component in activity and toxicity.

Novel molecular targets for Hepatocellular carcinoma

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

Hepatocellular carcinoma (HCC) is the third leading cause of death from cancer globally. Indeed, only a few treatments are available, most of which are effective only for the early stages of the disease. Therefore, there is an urgent needing for specific target antigens for the development of active (vaccine) and/or passive (adoptive T-cell therapy) cancer immunotherapy strategies. The aim of our study was to exploit the large amount of data from a public dataset to identify HCC-specific overexpressed proteins for identifying $CD8^+$ T cell epitopes. Furthermore, we searched for epitopes derived from human viruses sharing sequence and structure homology with HCC-specific epitopes. We conducted multiple *in silico* analysis and HLA-A:02:01/24:02 – restricted epitopes were predicted from HCCs overexpressed proteins. Sequence homology with epitopes derived from human viruses was found. PBMCs samples from both HCC patients and healthy subjects were collected to perform pMHC-tetramer staining analysis. Preliminary results showed the ability of $CD8^+$ T cells to recognise the single peptide and, for some subjects, also to cross-react against both of the coupled epitopes. More samples are under evaluation and selected epitopes are going to be transfected in HCC cell lines to perform cytotoxicity assays with PBMCs/TILs from patients.

Pseudomonas gessardii M15: a source of biotechnologically valuable molecules from Antarctic waters

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PhD cycle: 35° cycle

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Session: Microbiology

Rhamnolipids (RLs) and pyoverdines (PVDs) are secondary metabolites produced by microorganisms. RLs are biosurfactants displaying a wide range of bioactivities, like antibacterial, antifungal, and antibiofilm, while PVDs are siderophores, molecules able to strongly chelate iron and other metals. Despite being quite common, the most characterized RLs and PVDs come from different strains of *Pseudomonas aeuruginosa*, or other terrestrial microorganisms, while marine sources remain poorly explored. Focusing on marine bioprospecting, we isolated from Antarctic sediments a strain of *Pseudomonas gessardii*, named M15, able to produce these molecules. Their production was investigated by genomic and chemical approach, and new congeners were characterized for both classes. Then, we focused our attention on the bioactivities of the RLs mixture (M15RL) against *Staphylococcus aureus* and viruses belonging to the *Coronaviridae* and *Herpesviridae* families. Data show strong antibacterial activity against reference and clinical strains of *S. aureus* and complete inactivation of members belonging to the investigated viral families. Finally, we evaluated the biotechnological applications of M15RL, proving its ability to eradicate viral and bacterial load from treated surfaces and cotton swabs, respectively. These results highlighted the potential of M15RL as disinfectants and additives in biomedical and cosmetic products to counteract the spread of superbugs.

Metabolic rewiring in thyroid carcinomas induced by BRAF gene mutations

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PhD cycle: XXVI cycle

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Session: Cancer biology

Distinct oncogenic alterations drive differential metabolic reprogramming, which is a cancer hallmark. Following TCGA classification of papillary thyroid carcinomas (PTC) into *BRAF*- and *RAS*-like tumors, I contributed to identify metabolic genes signature specific for the *BRAF*-like subtype, characterized by a marked glycolytic phenotype. Therefore, in the 2nd year of my PhD project, I evaluated the contribution of key transcription factors (TFs) to the (de)regulation of glycolytic genes in *BRAF*-like tumors. Taking advantage from co-expression (TCGA) and TF binding data, I contributed to identify *HIF1A* as the most likely TF involved. Interestingly, it is over-expressed in *BRAF*- (*vs RAS*)-like PTCs and is induced downstream MAPK activation *in vitro*. Moreover, *HIF1A* is positively co-expressed with the network of deregulated metabolic genes. Accordingly, its knockdown in *BRAF*-mutated PTC cells impairs the expression of the glycolytic-genes, whereas its stabilization counteracts the repressive effect of the B-raf inhibitor (i.e. vemurafenib) on metabolic genes and on cell viability. These results indicate *HIF1A* as contributing to the glycolytic phenotype of *BRAF*-mutated cells. In the 3rd year of my PhD project, I will confirm if B-raf inhibition affects the glycolytic metabolism and whether new drug combinations (e.g., NSAIDs) – targeting tumor metabolism – can improve standard anti-cancer therapy.

Identification of neuroprotective natural compounds for the development of nutraceuticals and drugs: the effects of CLA on *C. elegans* neurodegenerative disease models.

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

Conjugated Linoleic Acid (CLA) is the collective name used for indicating a group of isomers of linoleic acid. In the latest years cyto-protective effects were attributed to CLA ability to activate a pleiotropic nuclear factor: Nrf2 whose dysregulation is associated to neurodegenerative disorders. Nevertheless, the effects of CLA on neurodegeneration it is still unknown. To this purpose, we used *C. elegans* as model system to evaluate the effects of CLA supplementation on transgenic models of Spinal Muscular Atrophy (SMA), Amyotrophic Lateral Sclerosis (ALS), Huntington's (HD), and Alzheimer's Disease (AD). After the treatment, we neither observed any rescue of the neurodegenerative phenotypes shown by the SMA model, nor of the defects in the mechanosensation of the HD model or of the locomotion defects of ALS model. Interestingly, CLA supplementation was able to partially rescue the locomotion defect shown by animals modelling AD. Moreover, we showed the lower activity of several enzymes activated downstream Nrf2: GSR, GST and G6PD in the *C. elegans* model of AD. We also demonstrated *in vivo*, that CLA supplementation is able to enhance GST expression. These results suggest a Nrf2 involvement of CLA-mediated effects in *C.elegans* and further studies are required to better elucidate this aspect

In vitro evaluation of probiotic properties of newly LAB strains isolated from natural whey cultures.

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PhD cycle: XVII° cycle

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Session: Cancer biology, Immunology, Microbiology, Drug Design.

The growing market of probiotic food products stimulates scientific research towards the continuous search for new probiotic strains. Characterizing the probiotic properties of strains isolated from natural whey cultures is important in terms of selecting starter culture strains for the production of functional dairy products. Moreover, some LABs are able to influence the activity of the brain through the production of cellular components (neurotransmitters, short-chain fatty acids and other signal molecules). There are various criteria for selecting the appropriate bacteria to be used as probiotics. In this contest, screening for acid and bile salt tolerance, antibiotic resistance, hydrophobicity and auto-aggregation ability, production of antimicrobial and neuroactive substances, was performed on new isolates. All the strains maintained high survival rate in presence of 0,3% pepsin at pH 3.0 and in presence of 0.3% bile salts at pH 8.0. The same isolates did not show any antibiotic resistance towards ampicillin, tetracycline, penicillin, and vancomycin, but some of them showed resistance to gentamycin. In hydrophobicity and auto-aggregation assays strain specific differences were observed; hydrophobicity values varied from 2 to 79%, whereas auto-aggregation ability ranged from 36% to 52%. Isolated strains were also screened for the presence of *gadB* gene (GABA production) and for production of antimicrobial substances. Five best performing strains will be further investigated for their potential applications in the food industry.

Identification of diagnostic biomarkers of intestinal damage in the different forms of celiac disease

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PhD cycle: 37° cycle

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Session: Cancer biology, Immunology, Microbiology, Drug Design

Celiac disease (CD) is a chronic food intolerance characterized by gluten-induced inflammation of the intestinal mucosa. Among the most frequent forms we find overt-CD, characterized by anti-tissue transglutaminase autoantibodies positivity and villous atrophy, and potential-CD characterized by positive serology but a normal, not inflamed intestinal mucosa. The immunodominant gluten peptides are recognize by CD4+ T-cells that release interferon(IFN)-γ, the main inflammatory cytokine in CD. Our recent studies have reported a marked infiltration of T cells releasing interleukin(IL)-4 in the intestinal mucosa of potential-CD compared to overt-CD patients. This suggested a possible protective effect of IL4 in stopping the development of mucosal damage and the evolution from potential-CD to overt-CD. To further investigate the role of IL4, short-term T-cell lines (TCLs) were generated from intestinal biopsies of CD-patients, by cyclic stimulation with gliadin, in the absence or presence of exogenous IL4 (control-TCLs and IL4-TCLs, respectively). Changes in cytokine production profile, cell subsets distribution and gliadin recognition pattern of TCLs were assessed by ELISA and multiparametric flow cytometry analyses. Preliminary data showed that IL4 induces i) a significant reduction of IFN-γ production in response to gliadin, ii) an expansion of CD4+ T cells and iii) a reduction of TCR-γδ+ T cells.

Molecular characterization of Antigen Presenting Cells from patients with active celiac disease versus subjects on gluten free diet

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Session 5: Cancer biology, Immunology, Microbiology, Drug Design

Celiac disease (CD) is a chronic immuno-mediated enteropathy caused by dietary gluten in genetically susceptible individuals carrying HLA-DQ2 or HLA-DQ8 genes. We investigated the ability of Antigen Presenting Cell (APC) to present gluten antigens and activate pathogenic $CD4^+$ T-cell response in two cohort of adult subjects: patients with active celiac disease and patients in remission, on gluten-free diet (GFD), with negative anti-endomysium and anti-transglutaminase antibodies. We analyzed the expression of HLA class II genes, DQA1*05 and DQB1*02, encoding HLA-DQ2.5 surface molecule in PBMC (Peripheral Blood Mononuclear Cells). The expression of these molecules is essential to present gluten epitopes and determine the strength of antigen-specific pathogenic CD4+ T-cell response. The results demonstrated a comparable expression of CD-associated DQA1*05 and DQB1*02 mRNA by APC, in both cohorts of patients, indicating their comparable ability in the antigen challenge of CD4+ T cells. Furthermore, we studied the expression of CD84/SLAMF-5, a new emerging biomarker, expressed by some cell subsets, such as CD4 and CD8 lymphocytes and by APC, including B cells and monocytes, with function to establish interactions between cells. We performed the analysis of CD84 surface expression, by flow cytometry, on PBMC from both cohorts of patients. The results showed higher expression of CD84 in APC from patients with active CD than patients on GFD. We propose that CD84 might represent a molecular biomarker of celiac disease remission.

Characterization of the MSMEG-3762/63 efflux pump in *Mycobacterium smegmatis*

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PhD cycle: 35° cycle

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Session: Cancer Biology, Immunology, Microbiology, Drug Design

Drug-resistant tuberculosis (TB) is one of the most difficult challenges facing TB control. We explored the role of efflux pumps in the development of drug tolerance using *M. smegmatis* as a model organism. Previous studies described the functional role of the TetR-like protein MSMEG-3765 as a repressor of the *MSMEG_3762/63/65* and of its orthologous *Rv1687/86/85c* in *M. tuberculosis*; in both operons, the first two genes encode for an ABC transporter [1]. The efflux system MSMEG-3762/63 has been characterized and is involved in the binding and extrusion of Rifampicin (Rif) and Ciprofloxacin (Cip) used in first- and second-line anti-TB treatments, respectively [2]. To further investigate the interaction between Rif and Cip and the membrane protein MSMEG-3763, a three-dimensional model of the protein-ligand complex was generated, and the amino acid residues involved were defined. The hypothesis that the two compounds were responsible for the induction of the efflux pump operon, was verified with different molecular approaches and supported by bioinformatic analyses. According to the structural model of the regulator MSMEG-3765 and docking studies, the protein is able to bind the DNA target through the same region involved in the binding of Rif and Cip. In this work, we also assessed, by cytofluorimetric analyses, the involvement of the *M. smegmatis* efflux pump in membrane potential.

1. Perrone, F.,DeSiena,B.,Muscariello,L.,Kendall,S.L.,Waddell,S.J.,and Sacco,M.(2017).A novel TetR-like transcriptional regulator is induced in acid nitrosative stress and controls expression of an efflux pump in mycobacteria.*Front.Microbiol.*8:2039.doi:10.3389/fmicb.2017.02039
2. DeSiena, B., Campolattano, N., D'Abrosca,G., Russo, L., Cantillon, D., Marasco, R., Muscariello, L., Waddell, S.J., and Sacco M.,(2020).Characterization of the Mycobacterial MSMEG-3762/63 efflux pump in Mycobacterium smegmatis Drug Efflux. Front. Microbiol.11:575828.doi:10.3389/fmicb.2020.575828

Surface Engineering of PLGA Nanoparticles with Poly (vinyl alcohol) to Overcome the Airway Mucus barrier

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Session: Cancer biology, Immunology, Microbiology, Drug Design

In the last decade, Poly (lactide-co-glycolide) (PLGA) based nanoparticles (NPs) have gained considerable interest in pulmonary delivery applications due to their promising properties. Nevertheless, PLGA NPs appear not always efficient in crossing the lung mucus layer. PLGA NPs usually showed surface particle engineering with hydrophilic polymers, which can improve the diffusion across the lung barrier and promote the target enrichment. Among engineering polymers, poly (vinyl alcohol) (PVA) is one of the most exploited, thanks to its biocompatibility and safety. This study aims to investigate the effect of PVA hydrolysis degree (DH) and molecular weight (MW) on NP muco-inertia. Four PVAs with different DH and MW were tested as surface engineering polymers (PVA4-88, PVA40-88, PVA10-98, and PVA56-98) at three different concentrations (0,10- 0,25- 0,50 % w/v). The surface properties of NPs were evaluated by analysing the fixed aqueous layer thickness (FALT) and the PVA amount absorbed on the NP surface. The interactions between NPs and mucus were assessed by spectrophotometric and size analyses of NPs dispersion in mucin. The preliminary results support the hypothesis that higher DH of PVA (PVA10-98 and PVA56-98) led to mucoadhesive systems that strongly interact with mucin, while NPs modified with low DH PVA (PVA40-88 and PVA4-88) can be considered muco-inert. These muco-inert NPs were also subjected to Transwell® permeation studies, and the results underlined the potential of low DH PVAs in promoting NPs diffusion across the AM layer. In conclusion, low DH PVAs can be considered promising engineering polymers for developing muco-inert PLGA-based NPs.

Insilico application of advanced docking strategies based upon the nature of the drug targets: Carbonic Anhydrases and Rhodesain of *Trypanosoma brucei rhodesiense.*

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Session: Cancer Biology, Immunology, Microbiology, Drug design

Today, molecular modeling techniques play a major role at the various stages of drug discovery pipeline. In this drug discovery context, molecular docking is a computational methodology that is used to provide a snapshot of various types of molecular interactions and binding modes that could be seen between the protein and the ligand. A wide range of studies were conducted based upon various warheads related to Rhodesain of the rhodesiense i.e., lethal form of Human African Trypanosomiasis [HAT] and exploited various secondary sulfonamides according to the nature of inhibition or activation of Carbonic Anhydrases. Our major focus is on employing advanced docking protocols like AutoDockZn forcefield to rationalize SARs attained on inhibitory agents against various hCAs as well as the activating agents in favor of hCA isoforms and AutoDockCovalent "flexible side chain method" to rationalize SARs attained on various covalent inhibitory agents against the Rhodesain. Based upon the complexity and nature of the research involved, we also effectively exploited the use of *in silico* Molecular Dynamics protocol from Desmond module of the Schrödinger software package.
Future prospects:
Exploring various advanced molecular docking strategies to provide novel hints from computational medicinal chemistry perspective.

Similar Programmed Death Ligand 1 (PD-L1) expression profile in COPD and NSCLC

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Session: Cancer biology, Immunology, Microbiology, Drug Design

Chronic obstructive pulmonary disease (COPD) and non-small cell lung cancer (NSCLC) share cigarette smoke (CS) as a major risk factor and often occur as comorbidities. Their pathological background presents an aberrant immune system with an immune-checkpoint dysregulation. Notably, the expression of Programmed Death Ligand 1 (PD-L1) on alveolar macrophages (AMs) is critical in regulating the immunological tolerance in NSCLC. Moreover, AMs are crucial mediators of lung immune responses with PD-L1 overexpression and an impairment of their phagocytosis in response to CS exposure, which may lead to the persistence of inflammation and emphysema, typical hallmarks of COPD. However, while the role of PD-L1 is known in NSLC, its biology in COPD is still unclear. To address this, we evaluated PD-L1 expression in BAL AMs via immunofluorescence staining. AMs from patients with mild COPD and NSCLC had the highest PD-L1 expression than all groups. Interestingly, PD-L1 expression was significantly higher in mild COPD subjects compared to severe COPD. We also quantified PD-L1 mRNA expression in BAL AMs exposed to CS extract (CSE) showing that acute CSE stimulation increased PD-L1 mRNA in the NS AMs only. This effect is overcome by chronic CS exposure or underlying lung pathologies such as NSCLC or COPD. Our data bring to light new insight in the immune mechanism involved in COPD and NSCLC and pave the way for future studies focused on the mechanisms by which CS promotes tumorigenesis and COPD.

Induction Of Anti-Tumor Response By Filamentous Bacteriophage Targeting

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Session: Cancer biology, Immunology, Microbiology, Drug Design

The filamentous bacteriophage is an efficient delivery nano-system and anti-tumor phages could represent a new vaccination approach. Recombinant bacteriophages can be selectively internalized by dendritic cells (DCs) by targeting DC-specific receptors, improving the immunogenicity of tumor-associated antigen (TAA)-derived peptides exposed at high density on the viral coat proteins. After phage internalization, DCs acquire a mature phenotype and induce a potent antigen-specific T-cell response. To improve the efficacy of bacteriophage as anti-tumor-vaccine, it has been conjugated to immunologically active lipid alpha-GalactosylCeramide (α-GalCer), triggering effective in vivo antitumor responses. Aim of my project is the optimization of this novel anti-tumor vaccination strategy based on the delivery of antigenic peptides and α-GalCer by phage targeting DCs or tumor cells in absence of adjuvants. The antitumor effect mediated by phage directly targeting tumor cells was analyzed with therapeutic vaccination in mouse models. Then, I exploited the expertise of Takis Biotech for the optimization of phage growth and production in GMP-like industrial conditions and analyze the immune response to phage expressing an epitope of the human TAA NY-ESO-1 and α-GalCer in an HLA-A2 transgenic mouse model. Finally, the set up in vitro and in vivo models to analyze the intracellular trafficking and immune response to phage targeting cross-presenting DCs began at Necker Institute (Paris) in August 2022 to understand how the DC targeting affects the antigen-specific immune response activation. Both actions are needed to improve the efficacy of our innovative phage nanoparticles and to envisage their future application in human therapy.

Optimization of growth of probiotic strains for potential health applications

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Session: Cancer biology, Immunology, Microbiology, Drug design

Functional foods and nutraceuticals frequently contain viable probiotic strains that, at certain titers, are considered to be responsible of beneficial effects on health. Nevertheless, only few products really can boast a health benefit that is scientifically proven and accepted at a community level. In the framework of a MISE project that is focused on the development of innovative nutraceuticals that can have a beneficial effect on chronic inflammatory states (such as those related to *Helicobacter pylori*, male infertility and articular pathologies), the optimization of growth of probiotic strains is of particular interest together with the purification and characterization of their metabolic products (e.g. exopolysaccharides).

As, most of the probiotics belong to lactic acid bacteria family suffering from the problem of lactic acid accumulation, which ultimately retards growth, and having specific nutritional needs, (e.g. amino acids and vitamins) the development of potentially industrially feasible high cell density fermentation processes can be challenging.

In this first part of the work, the focus was the optimization of growth of *Lactobacillus rhamnosus* and *Bifidobacterium lactis* by changing medium, carbon sources (glucose, lactose, galactose, maltose, sucrose, fructose, xylose) and also evaluating plant based renewable waste materials in small scale preliminary fermentation experiments, to potentially reduce industrial cost and also avoid commonly used animal derived ingredients.

Monocytic cell line U937 as the valid model for LPS detection cell-based assay

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Session: Cancer biology, Immunology, Microbiology, Drug Design

Bacterial lipopolysaccharides (LPS), also known as endotoxins are present on the outer membrane of gram-negative bacteria. They are strictly controlled in medicine and pharmacy for their capacity to induce inflammatory responses in innate immune cells. We investigated the different sensitivity and specificity of monocytic cell lines (THP1 and U937) and human primary monocytes/macrophages to LPS extracted from different bacteria with the final aim to improve the current cell-based assay for LPS detection. While THP1 and U937 activated directly with LPS did not release TNFα and IL6, THP1-PMA and U937-PMA (differentiated with PMA for 48 hours) highly secreted those cytokines. There were no differences of the monocytic cell lines and primary monocytes/macrophages to produce the cytokines. Both were sensitive as low as 0.1 ng/mL LPS extracted from *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella enterica*. The tested cells stimulated by *Pseudomonas aeruginosa* LPS only released those cytokines at the concentration equal or greater than 10 ng/mL. In any case, U937-PMA were more reactive than THP1-PMA (secreted higher level). These findings suggested that monocytic cell model based on U937 are better for the cell-based assay to detect LPS in terms of feasibility, reproducibility, reliability, and cost efficiency.

Session 2:

Gene Regulation and Computational Biology

Investigating the causes of DNA methylation disturbances in the Beckwith-Wiedemann syndrome

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The Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder characterized by several clinical features and increased risk to develop pediatric cancers. The most common molecular lesions of BWS are DNA methylation changes of imprinted loci located at chromosome 11p15.5, with loss of methylation of the KCNQ1OT1- TSS:DMR (also known as IC2 LoM) being detected in ~50% of the patients. One third of the IC2 LoM patients display methylation disturbances at multiple imprinted loci (MLID) other than those of 11p15.5. Recently, we and other research groups have demonstrated that in a subset of the BWS-MLID patients the methylation defect is associated with maternal-effect loss-of-function variants of genes encoding components of the subcortical maternal complex (SCMC).
Genome-wide methylation array analysis of a BWS cohort allowed to distinguish between healthy controls and BWS patients with single-locus defect (ICD1 and ICD2) or MLID. We identified by whole-exome sequencing likely hypomorphic variants of the SCMC genes in 11/21 (52%) mothers of the patients with BWS-MLID. The putative pathogenic variants included the *KHDC3L*, *PADI6*, *NLRP2*, *NLRP5*, *NLRP7* and *OOEP* genes. Furthermore, we reported on a patient affected by BWS, who developed colorectal adenocarcinoma (CRC) at 27 years old. We performed genome-wide methylation analysis on DNA extracted from blood, as well as neoplastic and perineoplastic colon tissue. This analysis revealed methylation disturbances at imprinted and cancer-associated genes in both tissues. Whole-exome sequencing on the blood tissue revealed an interesting heterozygous variant in the *CFTR* gene, strongly implicated in the development and progression of intestinal cancers, particularly CRC.
These results can be used to improve patient stratification and genetic counseling of the BWS patients.

Inherited retinal disease genes: study of their genomic organization and identification of co-expressed long noncoding RNAs by meta-analysis of human retina transcriptome data

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Session: Gene regulation and computational biology

Inherited retinal diseases (IRDs) are genetically heterogeneous disorders that can lead to blindness. Around 30-40% of patients have mutations in coding regions of known IRD disease genes that cannot be detected by NGS procedures. One possible explanation lies in the presence of yet unrecognised transcripts of known IRD genes. We aimed to 1) gain insight into the genomic organization and transcript composition of IRD genes and 2) reconstruct the gene networks that modulate their expression, focusing on lncRNAs. We analysed bulk RNA-seq human retina data focusing on 218 IRD genes. A total of 5054 transcripts were identified of which 3367 are novel transcripts. We focused on 435 isoforms predicted to account for at least 5% of the expression of the corresponding gene. We assessed the possible impact of the novel transcripts and identified new possible protein isoforms. RT-PCR revealed an overall 50% rate of experimental validation of the new isoforms. Data are publicly available through the TIGEM Retina database (https://retina.tigem.it/retina_disease_gene.php). We performed co-expression analysis to the above set of IRD genes and identified 83 lncRNAs co-expressed with several IRD genes. Quantitative RT-PCR analysis, applied on a subset of them, confirmed the predominant expression in the human retina of the lncRNAs.

The effects of the maternal-effect gene *Padi6* on mouse female fertility, embryogenesis, and epigenetic reprogramming

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Session: Gene regulation and computational biology

The maternal-effect gene *Padi6* encodes a member of the Peptidylarginine Deiminase family (PADI), linked to female fertility. Principally, studies in mice and humans show that *Padi6* is highly expressed in oocytes and early embryos. It is involved in the formation of oocyte cytoplasmic lattice, in the correct mitotic spindle assembly and chromosome alignment. The development of *Padi6*-null embryos in mice is arrested at the 2-cell stage and their ribosomal components, de novo protein synthesis and activation of the embryonic genome are impaired. PADI6 co-localizes with other maternal-effect proteins, which take part in Subcortical Maternal Complex (SCMC). Loss-of-function mutations affecting components of SCMC have been found in healthy women with fertility issues and/or offspring with imprinting defects and variable phenotype. In addition, knock-out female mice for the SCMC genes are infertile and embryonic development is unable to progress beyond the 2-cell stage. We used a transgenic murine line with a hypomorphic missense variant in *Padi6* gene, previously identified in compound heterozygosity with a truncating mutation in a mother with two siblings affected by Beckwith-Wiedemann syndrome and MLID. We have studied the effects of this mutation on protein expression, epigenetic modifications and ovarian development, via western blot, immunofluorescence, immunohistochemistry, and histological analysis.

Elucidation of the link between RNA maturation and neurodegeneration by a cell-specific transcriptomic analysis in *C. elegans*

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Session: Gene regulation and computational biology

Spinal muscular atrophy (SMA) is a neuromuscular disease that causes the specific loss of lower motor neurons (MNs) in affected patients. Mutations in the SMN-1 gene (Survival of Motor Neuron 1) cause ~95% of all SMA cases. SMN-1 controls the assembly of small nuclear ribonucleoproteins (snRNPs) essential for pre-mRNA splicing. SMN protein is ubiquitously expressed in the body and has a variety of roles in addition to its snRNP role: RNA metabolism and transport, DNA repair, and recombination… . It is not clear why MNs are especially sensitive to SMN depletion. We are exploring the molecular origins of the distinct sensitivity of MNs to loss of SMN1 in a *C.elegans* model developed by Di Schiavi group using a neuron-specific RNAi to selectively knock-down smn-1 in MNs or in a class of mechanosensory neurons, called touch receptor neurons (TRNs). Preliminary results indicate that TRNs do not display the same degeneration phenotypes as MNs when they are submitted to targeted *smn-1* RNAi. We used reporter strains that express fluorescent reporters in the desired cells and crossed them with strains expressing *smn-1* RNAi in the MNs or TRNs. Targeted neurons will be isolated to generate cell specific cDNA libraries for transcriptome sequencing. We will perform a comparative analysis of neuronal transcriptomes following *smn-1* depletion. We will also, identify differential protein interactions involved in neuron survival using TurboID enzyme that can add biotin to proteins that come in close proximity. We will express SMN1 fused to TurboID in MNs and TRNs. Biotin-tagged proteins will be purified using streptavidin-beads, and will be identified by mass-spectrometry.

Network Methods for Biomedical Discoveries

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Session: Gene regulation and computational biology

Networks, as mathematical representations of interaction, are very flexible; thus, they are broadly analysed and widespread in the biological field (co-expression networks, PPI networks, single cell networks and so on). For the versatility of this instrument, I used it for biomedical analysis. More specifically my research deals with two main problems: the integration of different networks and the validation of the clustering of a single network. Nowadays, with next-generation sequencing, massive and heterogeneous data has been produced leading to the construction of different omics (genomics, transcriptomics, epigenomics) networks. When analysing more than one network, the individual analysis of each dataset will give only a restricted view of a disease or a biological process of interest, while integrating all the data will widen and deepen the results providing a more global view of the entire system. During my PhD, I developed the*"INet algorithm"* to integrate different networks, under the assumption that the structure underneath the different networks has some similarities that we want to pull out in the integrated network. Through an iterative procedure, a *Consensus Network* is generated to give a global view of the actors who play a fundamental role in the onset and progression of a given disease or a biological process. In addition, for each network the algorithm constructs a *Case Specific Network* containing peculiar information of the single data type not present in all the others. As case study, I analysed different virus and vaccine co-expression networks, to better understand infectious diseases. Regarding the clustering validation, the problem was to understand how well clusters approximate cell subtypes. To answer this question I constructed a procedure to compare different community detection algorithms and validate their robustness, implemented in the R package*"robin"*. This methodology was applied to single-cell RNA-seq data for the characterization of cell subtypes.

Session 3:

Structure and Functions of Biomolecules

Tailoring the structure of cell penetrating DNA and RNA binding nucleopeptides

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Session: Structure and Function of Biomolecules

Nucleic acids are considered an exciting opportunity for therapeutic intervention in numerous diseases as confirmed by the increasing interest on nucleic acids by the pharmaceutical industries. In this scenario, the understanding that nucleic acids are modulated through the interaction with other nucleic acids and proteins, paved the way for the development of synthetic nucleic acid binders. Among them, nucleopeptides represent an intriguing class of nucleic acid mimics, in which nucleobases are placed in a peptide structure. In these synthetic molecules electrostatic, hydrophobic, π-stacking and hydrogen-bond interactions are combined to preserve both the cell penetrating peptide (CPP) features important for cell uptake, and the specificity of recognition of nucleic acids. During the second year of my PhD course, I focused on ultrasound assisted solid phase synthesis of nucleopeptide oligomers (12 mer homo-thymine nucleopeptide model) to investigate the role of the non-derivatized amino acid residues and of the distance of the nucleobase from the peptide backbone on nucleic acid binding and recognition events. CD spectroscopic studies evidenced significant differences in terms of binding and DNA and RNA recognition depending on the various underivatized amino acids employed (anionic and cationic amino acids) and/or the distance of the nucleobase from the peptide backbone. In addition, different cellular and nuclear uptake between arginine-based nucleopeptides having the same number of positive charges but a different distance of the nucleobases from the backbone was found. These evidences suggest for the first time that the amino acid residues bearing the nucleobase play a pivotal role not solely in defining the nucleic acid binding but also in the cell/nuclear uptake process.

The Structural Characterization of quinoin, type-1 ribosome-inactivating protein from *Chenopodium quinoa* Willd seeds

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Session: Structure and Function of Biomolecules

A type-1 ribosome-inactivating protein (RIP), named quinoin (29-kDa), has been isolated and well characterized from the seeds of pseudocereal quinoa (*Chenopodium quinoa* Willd.) (1). Quinoin is a N-β-glycosylase removing a specific adenine from rRNA in the conserved α-sarcin/ricin stem-loop (SRL). Quinoin is cytotoxic against human malignant cells (2) and exhibits both antifungal properties towards *Penicillium digitatum* (3) and antiviral activity against Tobacco Necrosis Virus (TNV) (4). Given its peculiar biological actions, during the second year of my PhD, I determined the amino acid sequence of this enzyme, considering the possible biotechnological applications.
In this framework, in order to determine quinoin primary structure, a strategy based on comparative mapping by MALDI-ToF mass spectrometry (MS) and the screening of quinoa genome was carried out (4). Moreover, a bioinformatics approach allowed to study the gene organization. Subsequently, three-dimensional homology modeling approach was used to obtain structural information on quinoin, including the catalytic pocket in which the amino acid residues of the active site (Tyr75, Tyr122, Glu177, Arg180, Phe181 and Trp206; quinoin numbering) are conserved, like other type-1 RIPs. On the other hand, quinoin consists of 254 amino acid residues, without cysteinyl residues, differently from most of type-1 RIPs characterized by two disulphide bridges.

Structural and functional characterization of the recombinant FVII of coagulation

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Session: Structure and Function of Biomolecules

Recombinant or human blood purified coagulation factors like Factor VIII and Factor IX are life-saving drugs in haemophilia patients who produce insufficient levels of the factors or inactive variants. The action of exogenous factors is often counteracted by endogenously developed inhibitors (antibodies), requiring co-administration of Factor VII (FVII) capable of activating coagulation via alternative pathways. A recombinant FVII in its activated form is used clinically but poses serious hypercoagulability problems to receiving patients as it is not regulated it. In contrast, non-activated plasma-derived FVII is safe because activity is highly regulated by the feedback mechanisms of the coagulation cascade. The aim of the project is to obtain recombinant non-activated FVII and to characterize its function, structure and network of interactions using high resolution mass spectrometry, label-free techniques and spectroscopy. FVII is a protein with a very complex structure, with many post-translational modifications (PMTs) that greatly affect stability (glycosylations), function (gamma-carboxylations) and interaction with activators or inhibitors. Protein is produced in human cell lines to obtain variants with PMTs similar to the natural ones and thus safer. The structural and functional characterization of these new products is a crucial requirement for the future development of the protein as a drug for human use.

Development and application of new edible active formulations to preserve beneficial biomolecules and reduce fruit losses: study of treatment-induced changes to fruit metabolism

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Session: Structure and Function of Biomolecules

Fruits are a rich source of nutrients, including vitamins, fiber, and certain types of biologically active compounds (e.g. polyphenols and carotenoids) whose content depends on the cultivar and is affected by both the abiotic and biotic stresses. Fruits have a relatively short postharvest life due to physiological and biochemical decay that leading a great food loss. For this reason, my PhD project aims to develop and apply new eco-friendly edible formulations, such as simple treatments or mono- and bi-layer coatings application, to extend the shelf life of fruits, reduce food losses, and preserve their valuable and health-beneficial elements.
Specifically, in this project, apple and pear cultivars, suitable for the fresh-cut industry, and cherry cultivars will be characterized agronomically, genetically, using SSR markers, and biochemically, through UV-Vis spectroscopy and gas chromatography. After an initial screening, the new formulations will be tested on the valuable cultivars and thus treatment-induced metabolic changes in terms of antioxidant system change, metabolome and gene expression will be evaluated. Until now, agronomic characterizations of several apple, pear and cherry cultivars have been carried out. Genetic and biochemical characterizations of the same cultivars are ongoing. A first innovative bi-layer coating was tested on a commercial diced pear cultivar. Results showed that coating application significantly extended the pear's shelf-life, inhibiting browning and surface color changes, improving antioxidant system and delay protein denaturation, over ten days of cold storage. Tests of the same formulation on sliced apple and cherry are ongoing.

Single-molecule biophysical study of the FANCJ DNA helicase

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Session: Structure and Function of Biomolecules

DNA helicases are a class of enzymes able to unwind DNA in an ATP-dependent enzymatic reaction [1]. The human Fanconi anaemia complementation group J protein (hFANCJ) is an Iron-Sulphur cluster DNA helicase involved in various genome maintenance pathways [2]. hFANCJ is able to resolve different G-quadruplex (G4) DNA with high catalytic efficiency *in vitro* [3]. G4 are unconventional DNA secondary structures that can arise in G-rich regions of the human genome posing an obstacle to DNA replication fork progression [4]. My objective here is to investigate the mechanism of FANCJ-mediated resolution of G4 DNA using an experimental approach based on correlative optical tweezers fluorescence microscopy (CTFM) [5]. To be visualised by CTFM, the protein of interest needs to be fluorescently labelled. In our study, FANCJ was fused to a short peptide (ybbR-tag) that can be specifically labelled *in vitro* with a small fluorophore by a bacterial phosphopantetheinyl transferase (SFP) [6]. This non-intrusive site-specific labelling procedure is expected to minimize possible disruption of the FANCJ DNA helicase activity. Design and production of a DNA molecule containing the G4 DNA of interest has been the subsequent critical step. I generated a linear G4-containing DNA construction with biotin moieties at the ends. The biotinylated ends can be attached to streptavidin-coated optical beads that are trapped and micro-manipulated with elevated accuracy by the optical tweezers. Then, DNA binding by fluorescent-labelled FANCJ is visualised by the confocal microscope, while G4 resolution is monitored in real time at a single-molecule level.

1) Brosh, R. M., Jr, & Matson, S. W. (2020). History of DNA Helicases. *Genes*, *11*(3), 255.
2) Brosh, R. M., Jr, & Cantor, S. B. (2014). Molecular and cellular functions of the FANCJ DNA helicase defective in cancer and in Fanconi anemia. *Frontiers in genetics*, *5*, 372.
3) Lerner, L. K., & Sale, J. E. (2019). Replication of G Quadruplex DNA. *Genes*, *10*(2), 95.
4) Lee, W., Yin, Y., Morten, M. J., Tonzi, P., Gwo, P. P., Odermatt, D. C., Modesti, M., Cantor, S. B., Gari, K., Huang, T. T., & Rothenberg, E. (2021). Single-molecule imaging reveals replication fork coupled formation of G-quadruplex structures hinders local replication stress signaling. *Nature communications*, *12*(1), 2525.
5) Gross, P., Farge, G., Peterman, E. J., & Wuite, G. J. (2010). Combining optical tweezers, single-molecule fluorescence microscopy, and microfluidics for studies of DNA-protein interactions. *Methods in enzymology*, *475*, 427–453.
6) Yin, J., Lin, A. J., Golan, D. E., & Walsh, C. T. (2006). Site-specific protein labeling by Sfp phosphopantetheinyl transferase. *Nature protocols*, *1*(1), 280–285.

Plant specialized metabolites for treatment of Leukemia

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Session: Structure and Function of Biomolecules

Hematologic malignancies account for a considerable percentage of cancers worldwide [1]. Throughout the years there have been great advancements in cancer medicine with an effort to treat Leukemia. Regardless of these several immunotherapies, leukemia remain the leading cause of cancer related deaths in the world. Some of these therapies are associated with adverse effects such as cardiac dysfunctions, nephrotoxicity and hepatoxicity [2] as well as immune-suppression and myelosuppression which can increase risk of opportunistic infections [3]. Moreover, surfacing of drug resistance is also a great challenge [4]. Therefore, search for more safer and effective drugs remains crucial. Plant natural products and their derivatives provide key scaffolds for drug development; however, more work has been done on attempt to seek lead anti-cancer compounds in common cancers like colon, breast cancer and with less on leukemia [4]. This has led to continued search for plants possessing anti-leukemic agents as well as chemotherapeutic and/or chemopreventive agents. In this project, with the help of ethnopharmacological knowledge, several plant species from different families have been identified. These plants will be subjected to NMR based metabolomics to identify main compounds present. Furthermore, biological activity of identified specialized metabolites will be assessed in vitro on leukemia cells.

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.

2. Schmiegelow K, Muller K, Mogensen SS, Mogensen PR, Wolthers BO, Stoltze UK, Tuckuviene R, Frandsen T. Non-infectious chemotherapy-associated acute toxicities during childhood acute lymphoblastic leukemia therapy. F1000Res. 2017;6:444. PMID: 28413626. DOI: 10.12688/f1000research. 10768.1.

3. Zawitkowska J, Lejman M, Zaucha-Prażmo A, Drabko K, Płonowski M, Bulsa J, Romiszewski M, Mizia-Malarz A, Kołtan A, Derwich K, Karolczyk G, Ociepa T, Ćwiklińska M, Trelińska J, Owoc-Lempach J, Niedźwiecki M, Kiermasz A, Kowalczyk J. Grade 3 and 4 Toxicity Profiles During Therapy of Childhood Acute Lymphoblastic Leukemia. In Vivo. 2019 Jul-Aug;33(4):1333-1339. doi: 10.21873/invivo.11608

4. Lucas DM, Still PC, Pérez LB, Grever MR, Kinghorn AD. Potential of plant-derived natural products in the treatment of leukemia and lymphoma. Curr Drug Targets. 2010;11(7):812-822. doi:10.2174/138945010791320809

High-resolution conformational analysis of RGDechi1-14 and ψRGDechi peptides using a combination approach of Nuclear Magnetic Resonance and Molecular Dynamic simulations

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Session: Structure and Function of Biomolecules

The crucial role of integrins in several pathological processes such as tumor progression and metastasis formation has inspired intense efforts to design novel pharmaceutical agents able to modulate integrin functions in order to deliver new tools for potential therapies. In this contest, during the second year of my PhD course, i have focused my attention on the description of the ensemble-averaged conformational features of two peptides derived by RGDechi, named RGDechi1-14 and ψRGDechi, respectively. RGDechi is a bi-functional flexible peptide containing a cyclic RGD penta-peptide for integrin binding that is covalently linked by a spacer to a C-term echistatin fragment to confer a high selectivity for the β_3 integrin subunit [1-3]. *In vitro* and *in vivo* data [2-5] demonstrated the selective interaction of RGDechi with $\alpha_v\beta_3$ integrin, and structural analysis, based on a combination of NMR methodologies and computational data, highlighted the molecular details of the integrin binding [6]. In particular, the three-dimensional (3D) structural model of the RGDechi/ $\alpha_v\beta_3$ complex, obtained using experimental and computational data, has demonstrated that the recognition mechanism of $\alpha_v\beta_3$ by RGDechi is mainly modulated by the residues located within the RGD cycle and it is further stabilized by the region encompassing hCit15-Thr19, playing a crucial role in RGDechi selectivity for the $\alpha_v\beta_3$. Recently, to improve the potential of RGDechi as bioprobe platform in melanoma tumor, we have designed a RGDechi derivative peptide called ψRGDechi in which a reduced amide bond ψ[CH2-NH] was introduced. Interestingly, this chemical modification improved protease stability preserving the binding selectivity for $\alpha_v\beta_3$ integrin [6]. Here, to understand how to increase the integrin binding selectivity of RGDechi-derived peptides by chemical modifications, we performed a high resolution description of the conformational space sampled by RGDechi1-14 and ψRGDechi using an integrated natural-abundance Nuclear Magnetic Resonance/Molecular Dynamics simulations approach. Overall, our data demonstrate that the flexibility of the RGD cycle is driven by the C-terminal region of the RGDechi peptide through a coupling mechanism between the N- and C-terminal regions [7].

1. Del Gatto, A.; Zaccaro, L.; Grieco, P.; Novellino, E.; Zannetti, A.; Del Vecchio, S.; Iommelli, F.; Salvatore, M.; Pedone, C.; Saviano, M. Novel and selective alpha(v)beta3 receptor peptide antagonist: design, synthesis, and biological behavior. J Med Chem 2006, 49, 3416-3420, doi:10.1021/jm060233m.
2. Farina, B.; Del Gatto, A.; Comegna, D.; Di Gaetano, S.; Capasso, D.; Isernia, C.; Saviano, M.; Fattorusso, R.; Zaccaro, L.; Russo, L. Conformational studies of RGDechi peptide by natural-abundance NMR spectroscopy. J Pept Sci 2019, 25, e3166, doi:10.1002/psc.3166.
3. A novel approach for studying receptor-ligand interactions on living cells surface by using NUS/T1ρ-NMR methodologies combined with computational technique: the RGDechi15D-

$\alpha_v\beta_5$ integrin complex. Biancamaria Farina, Corvino Andrea, Annarita Del Gatto, Daniela Omegna, Sonia Di Gaetano, Domenica Capasso, Antonella Paladino, **Clementina Acconcia**, Maria Teresa Gentile, Michele Saviano, Roberto Fattorusso, Laura Zaccaro e Luigi Russo. Computational and Structural Biotechnology Journal. doi: 10.1016/j.csbj.2021.05.047,2021.

4. Russo, L.; Farina, B.; Del Gatto, A.; Comegna, D.; Di Gaetano, S.; Capasso, D.; Liguoro, A.; Malgieri, G.; Saviano, M.; Fattorusso, R.; et al. Deciphering RGDechi peptide-α5β1 integrin interaction mode in isolated cell membranes. Peptide Science 2018, 110, doi:10.1002/pep2.24065.
5. Farina, B.; de Paola, I.; Russo, L.; Capasso, D.; Liguoro, A.; Gatto, A.D.; Saviano, M.; Pedone, P.V.; Di Gaetano, S.; Malgieri, G.; et al. A Combined NMR and Computational Approach to Determine the RGDechi-hCit-αv β3 Integrin Recognition Mode in Isolated Cell Membranes. Chemistry 2016, 22, 681-693, doi:10.1002/chem.201503126.
6. Comegna, D.; Zannetti, A.; Del Gatto, A.; de Paola, I.; Russo, L.; Di Gaetano, S.; Liguoro, A.; Capasso, D.; Saviano, M.; Zaccaro, L. Chemical Modification for Proteolytic Stabilization of the Selective α. J Med Chem 2017, 60, 9874-9884, doi:10.1021/acs.jmedchem.7b01590.
7. High-resolution conformational analysis of RGDechi derived peptides based on a combination of NMR spectroscopy and MD simulations. **Clementina Acconcia*,** Antonella Paladino, Maria della Valle, Biancamaria Farina, Annarita Del Gatto, Sonia Di Gaetano, Domenica Capasso, Maria Teresa Gentile, Gaetano Malgieri, Carla Isernia, Michele Saviano, Roberto Fattorusso, Laura Zaccaro and Luigi Russo. International Journal of Molecular Sciences, 2022 (accepted).

Dissecting the interaction of DDX11 with Tim, a fork-protection complex subunit

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Session: Structure and Function of Biomolecules

The faithful transmission of genetic information from one generation to the next is essential for survival and requires an accurate duplication of the chromosomal DNA by the replisome. DDX11, a super-family-2 DNA helicase, is implicated in cancer development and linked to the rare hereditary disease Warsaw breakage syndrome. The fork-protection complex is composed by Tim, Tipin, Claspin and AND-1 proteins that are conserved from yeast to mammals. They are all key players in genome stability maintenance with different functions in DNA replication. It was reported that DDX11 directly interacts with Tim to preserve replication fork progression in stressful conditions and promote sister chromatid cohesion. To identify the Tim subdomains involved in DDX11-binding, I have carried out co-immunoprecipitation experiments with Flag-tagged DDX11 in combination with different Tim fragments. I have demonstrated that Tim has two DDX11-interacting sites: one corresponding to a putative loop located in the N-terminal portion and another one located in the C-terminal part of the protein. G-quadruplexes DNA are one of the most potent roadblocks to the progression of the replication machinery. I am planning to explore binding/resolution of these structures by DDX11, and the role played by Tim in this process using correlative optical tweezers – fluorescence microscopy.

Structural characterization of novel vaccine antigens

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Session: Structure and Function of Biomolecules

Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa and *Enterobacter* sp are dangerous bacteria belonging to the ESKAPE group and characterised by extreme tempo of adaptation to antimicrobials. In my PhD work, I studied structural, biophysical, and biochemical properties of three proteins of *E. faecium* – AdcA, SagA and PpiC to discover new, improved antigens toward ESKAPE bacteria. By coupling computational approaches to protein engineering and biophysics, we were able to identify immunogenic regions in AdcA and develop a novel highly-stable and multi-antigen-presenting protein, $Sc(EH)_3$. We also produced glutamine rich fibrils connected to this epitope as another delivery method. Using light scattering and CD spectroscopy, we showed that $Sc(EH)_3$ is a monomeric and hyper-stable protein, with melting temperature over 80°C. Using this antigen, we performed immunisation in rabbits and with obtained sera we were able to confirm, using opsonophagocytic killing and inhibition assays, that $Sc(EH)_3$ induces selective and protective immunogenicity against multiple bacterial species. In parallel, we identified the site of interaction of SagA with monoclonal antibodies and produced a more stable version of the antigen. Also, we biophysically characterised the native dimeric form of PpiC and produced and investigated its monomeric variant, to facilitate its further structural characterisation.

Bio-guided approach as a valuable strategy in the search of antibacterial compounds from three Myrtaceae plants

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Session: Structure and Function of Biomolecules

Staphylococcus aureus, a Gram-positive pathogen, is the main cause of current infection diseases, due to the developing of many mechanism of resistance to common antibacterial agents. In particular, methicillin-resistant *S. aureus* (MRSA) infections are creating a serious problem in medical microbiology. Myrtaceae Juiss is known to be an invaluable source of bioactive metabolites, the identification of which is the focus of many ongoing research activities. In particular, phloroglucinols derivates have been extensively studied, due to their structural features as well as wide range of biological and pharmacological properties above all antimicrobial activity, being an attractive target for organic chemists. To this purpose, the current work assessed the antimicrobial activity of *Myrcianthes cisplatensis, Psidium friedrichsthalianum* and *P. oligospermum* through antimicrobial assays and NMR analysis performed in order to identify new antimicrobial compounds. Dried leaves of each plant collected in Arizona, were extracted with solvents at increasing polarity: hexane, chloroform and methanol. The obtained crude extracts were tested for their antimicrobial activity against two strains of *Staphylococcus aureus:* ATCC 29213 and 43300 (a methicillin-resistant *Staphylococcus aureus* strain, MRSA). Extracts in hexane and methanol of *M. cisplatensis* and chloroform and methanol extracts of *P. friedrichsthalianum* were the most promising against both the strains. On the contrary, *P. oligospermum* showed no activity. Liquid-liquid separation allowed the distribution of methanol crude extracts of both plants, obtaining dichloromethane and ethyl acetate fractions. Despite the flavonoidic component found in the ethyl acetate fractions, the potential antimicrobial activity seems to increase in the dichloromethane ones. 1D- and 2D-NMR analysis allowed to investigate the bioactive fractions in order to have information about the metabolite content. Three cinnamoylated alkylphloroglucinol glucosides have been isolated for the first time from dichloromethane extract of *M. cisplatensis*, along with known coumarin derivatives. The same way, tetronic acid derivative was isolated from *P. friedrichsthalianum* and characterized by 2D NMR (HSQC, COSY, H2BC).

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

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Session: Structure and Function of Biomolecules

The current trend of the ongoing pandemic is demonstrating that it is extremely necessary to introduce antiviral drugs specific to COVID-19 along with the establishment of immunity by vaccination. Such drugs will lead the COVID-19 pandemic toward an inevitable but peaceful coexistence with the virus. Therapeutics currently studied to tackle the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, the virus responsible for COVID-19) target the different phases of viral life: protease inhibitors, nucleoside analogues that inhibit replication, and entry blockers. The principal goal of this project is to contribute to the quest for new therapeutics by developing molecules capable of inhibiting the viral RNA capping that allows the viral genome to escape the host immune system. Responsible for the RNA capping and, as such, main targets of the present project are two complexes constituted by three non-structural proteins (nsps): nsp10/nsp14 and nsp10/nsp16. Accordingly, we will integrate computational and experimental data to design, synthesize and characterize peptides and peptidomimetics capable to bind nsp10 with high affinity and thus to inhibit the formation of its complexes with the cognate proteins. Additionally, we will explore the molecular mechanisms related to their activity.

Specialized metabolites as potential lead compounds for anticancer drug discovery

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Session: Structure and Function of Biomolecules

Intrinsic and acquired drug resistance poses significant challenges to colorectal cancer treatment, therefore new compounds to prevent or overcome this problem are needed. Plants are one possible source of bioactive chemicals, being rich in specialized metabolites. In the search of such compounds Fourteen Asteraceae plant extracts have been screened with an NMR-based metabolomics approach, and during the third PhD year three selected plant extracts, i.e., *Anthemis maritima*, *Centaurea deusta*, and *Xanthium strumarium*, have been investigated for their metabolite content.

In particular, a targeted phytochemical study was carried out in order to obtain the pure putatively active compounds. Four sesquiterpene lactones, one from *C. deusta* and three from *X. strumarium*, have been isolated and characterized through 1D and 2D NMR (COSY, TOCSY, H2BC, HSQC, CIGAR-HMBC, HSQC-TOCSY, NOESY) experiments.

The phytochemical study of *A. maritima* and the evaluation of the bioactivity of the pure compounds is currently going on.

Biocompatible Polimers For Human Health

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Session: Structure and function of biomolecules

The goal of my PhD project is the design and development of protein or oligosaccharide polymers for the delivery of antigens or other bioactive molecules [1-3]. With this aim, during the first PhD year, we have set up all experimental conditions for the production and characterisation of bacterial cellulose [4]. Indeed, bacterial cellulose represents a good candidate for drug delivery due to its biocompatibility and mucosal adhesion properties. In parallel, my research activity was focused on the identification of new molecules with antigenic properties and new protein nanocarriers for their delivery [1-3]. Using a structural vaccinology approach, we first structurally characterised HtpG, a mycobacterial chaperone with antigenic properties identified by our collaborator Prof. Kim at the University of Daejeon. Then, we used several bioinformatic tools to map immunogenic epitopes on the protein structure and dissect the contribution of individual HtpG domains to antigenicity [3]. Based on these observations, we designed and produced new antigens, which were shown, using a plethora of immunological assays, to exhibit enhanced antigenic and biophysical properties [3]. All these results represent the basis for the development of innovative antigen-carrier systems.

Characterization of ribotoxic enzymes from Edible Mushrooms

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Session: Structure and Function of Biomolecules

In multispecies system, possible requirement for the sustenance of species depends on its powerful defense mechanism through interspecific interactions among organisms. Indirect (shared and common interactions) or direct (mutualism, commensalism, herbivory and predation), could affect the species survival [1]. In particular, the development of molecular mechanisms of host-parasitic interactions involve certain survival defense strategies, by synthesizing several proteins/enzymes and secondary metabolites [2]. The target of these metabolites or specific enzymes is often the ribosome involved in protein synthesis. However, enzymes directly inactivate ribosomes differently from metabolites which interfere with protein synthesis via binding with ribosome during initiation, elongation or termination phase [3]. Indeed, ribotoxic enzymes such as ribotoxins, ribotoxin-like proteins (RL-Ps) and ribosome-inactivating proteins (RIPs) damage the rRNA located in universally conserved sequence in sarcin ricin loop (SRL), restricting the binding of elongation factors [4]. Specifically, ribotoxins and RL-Ps are fungal RNases isolated from ascomycetes or basidiomycetes, respectively, cleaving a specific phosphodiester bond in SRL, while RIPs are rRNA N-glycosylases, cleaving a single adenine in same region [5]. In this framework, during my first PhD year, I was involved in the screening of novel ribotoxic enzymes from edible basidiomycetes mushrooms, using Endo's assay to distinguish N-glycosylase or ribonuclease activity.

1. Casadevall, A., & Pirofski, L. A. (2000). Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. Infection and immunity, 68(12), 6511-6518.
2. Agmon, I., Amit, M., Auerbach, T., Bashan, A., Baram, D., Bartels, H., ... & Zarivach, R. (2004). Ribosomal crystallography: a flexible nucleotide anchoring tRNA translocation, facilitates peptide-bond formation, chirality discrimination and antibiotics synergism. FEBS letters, 567(1), 20-26.
3. Herrero-Galán, E., Lacadena, J., Martinez del Pozo, A., Boucias, D. G., Olmo, N., Onaderra, M., & Gavilanes, J. G. (2008). The insecticidal protein hirsutellin A from the mite fungal pathogen Hirsutella thompsonii is a ribotoxin. Proteins: Structure, Function, and Bioinformatics, 72(1), 217-228.
4. Ruggiero, A., García-Ortega, L., Ragucci, S., Russo, R., Landi, N., Berisio, R., & Di Maro, A. (2018). Structural and enzymatic properties of Ageritin, a novel metal-dependent ribotoxin-like protein with antitumor activity. Biochimica et Biophysica Acta (BBA)-General Subjects, 1862(12), 2888-2894.
5. Landi, N., Hussain, H. Z., Pedone, P. V., Ragucci, S., & Di Maro, A. (2022). Ribotoxic Proteins, Known as Inhibitors of Protein Synthesis, from Mushrooms and Other Fungi According to Endo's Fragment Detection. Toxins, 14(6), 403.

NMR-based metabolomics reveals the allelopathic potential of selected invasive plants in the Mediterranean basin

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Session: Structure and Function of Biomolecules

Allelopathy is the biochemical interaction among plants wherein the donor species releases chemicals able to influence the growth and performance of the receiving plants. The capability of plants to produce and release allelochemicals influences the plant distribution, interaction, and diversity. This phenomenon might be therefore involved in the success of invasive alien plants. Our study aims to analyze allelochemicals from the extracts of highly invasive plants in the Mediterranean basin namely *Ailanthus altissima* and *Robinia pseudoacacia*. Leaves and root extracts were used to treat the chosen receiving plants (*Aegilops geniculata* and *Lactuca sativa*) in a hydroponic set-up under controlled conditions. Morphological changes were determined by documenting the root and shoot length. Metabolomic changes were analyzed using NMR and subsequent multivariate analysis. The results showed differences in the morphology and composition of metabolites present in the treatments compared to the control set-up. The potential of NMR-based metabolomics for phytotoxicity evaluation, especially to identify the metabolomic changes and detect them at the morphological level, are discussed. This approach allowed us to identify the metabolic pathways affected by the allelochemicals. This would also allow us to hypothesize modes of action and help us design ad hoc experiments to further understand them. Furthermore, this suggests the efficiency of NMR-based metabolomics approach as a suitable tool in studying allelopathy.

Structural studies of the RTEL1 FeS helicase

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Session: Structure and Function of Biomolecules

Helicases containing FeS clusters are ubiquitous, but their exact mechanism of action is not well understood. No structural information is available for any eukaryotic member of the family, comprising four medically-relevant paralogues involved in genetic diseases and cancer development. Among those, RTEL1 (Regulator of Telomere Length 1) has a role in DNA repair, homologous recombination, telomere metabolism, and DNA replication.

Due to cluster lability and the presence of a number of low complexity regions, the expression and purification of the protein was challenging. However, we managed to obtain different fragments of RTEL1 suitable for structural studies.

A full biochemical analysis is in progress. Preliminary experiments show that RTEL1 binds different substrates with a preference for D-loops and bubbles, and unwinds DNA, in a reaction that is dependent on the integrity of the cluster.

Crystallization trials were set-up, but no crystal has yet been obtained. In a parallel effort, we performed Cryo-EM in collaboration with Thomas Miller (University of Copenhagen). Preliminary Cryo-EM analysis showed that the samples are promising and a low-resolution map 3D map was obtained with the initial dataset. Based on these initial results we are now planning high-resolution data collection on a high-end microscope.

Characterization of Human Prion Protein Conformation Equilibria Using NMR Methodologies Under Cell Mimicking Conditions

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Session: Structure and Function of Biomolecules

Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, Fatal insomnia and Kuru are a group of neurodegenerative disorders caused by prions. Investigating protein dynamics is essential for understanding protein function especially for proteins like Prion. The misfolding and conversion of normal cellular prion into abnormal protease resistant pathogenic scrapie prion is believed to be the key procedure. However, the functions of PrP^c are not completely understood and the mechanism of its structural conversion remains unclear. Human PrP^c is a cell surface expressed protein and has a physiological structure with a C-terminal globular domain (amino acids 122–230) and an N-terminal flexible tail (amino acids 23–121). The N-terminal tail consists of two charged clusters (CC1 and CC2), the octarepeat region (OR) and a hydrophobic domain (HD). Additionally, two N-glycosylation sites are located in the globular domain upstream of the sialylated GPI-anchor at the C-terminus. The globular domain consists of two beta-sheets and three alpha-helices. The known functions described for PrP^c cover a wide spectrum including ion balance homeostasis, metal ion intake (such as copper and zinc), control of cell proliferation and neural differentiation. The conformational studies of human prion proteins are widely studied in buffer solutions. However, human prion proteins form fibrils only in a physiological environment. Our primary goal is to obtain structure-related information on human prion protein under cell mimicking conditions. In this study, we used Nuclear Magnetic Resonance (NMR) spectroscopy the investigate conformational equilibria of human prion protein HuPrP (residues 90-231) in cell mimicking conditions, using ficoll an inert crowding agent as a solvent. Using CD and NMR methodologies, we carried out an atom by atom analysis of human prion thermal unfolding in the presence of ficoll. Our results will help the elucidation of structural behavior of the human prion protein in cell, providing decisive information to make clear the structural basis of its pathogenic conversion.

Polystyrene nano-plastics affect human Ubiquitin structure and ubiquitination in cell: a high-resolution study

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Session: Structure and Function of Biomolecules

The plastics crisis, one of the major environmental changes, is, to date, a significant concern. Plastic debris can be fragmented into smaller pieces by many physical and chemical processes, generating its own micro- (0.1 μm - 5 mm) and nano-plastics (1-100 nm). The latter, above all, can be vectors and reach all environments (air, water and food), coming into contact with various living organisms. Humans are estimated to consume several grams per week of nano-plastics (NPs) through exposure to a variety of contamination sources. Nonetheless, the effects of these polymeric particles on living systems are still mostly unknown.
In the frame of my project, by means CD (Circular Dichroism), TEM (Trasmission Electron Microscopy) and high-resolution NMR (Nuclear Magnetic Resonance) analyses, I describe at an atomic resolution the interaction between human Ubiquitin (Ubq) and polystyrene nanoparticles, showing the generation of a NPs-protein complex (hard corona), the local rearrangement and a variation in the internal dynamic properties of the Ubq.
Moreover, the influence of these polymers on Ubq functions is tested. The results confirm that, in human HeLa cells, exposure to NPs leads to a sensible reduction of ubiquitination, a key metabolic process at the base of cell viability.

A Mesophilic Phosphotriesterase-Like Lactonase Shows High Stability And Proficiency As Quorum Quenching Enzyme

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Session: Structure and Function of Biomolecules

Phosphotriesterase-Like Lactonase (PLL) family includes a group of enzymes that have main lactonase activity on lactones and acyl-homoserine lactones (AHLs) and, low promiscuous phosphotriesterase activity towards organophosphate compounds (OPs).
PLLs have been first identified by our group in the hyperthermophilic crenarchaeon *S. solfataricus* (SsoPox) and *S. acidocaldarius* (SacPox), and also in others thermophilic and mesophilic microorganisms. In the frame of a project granted from the Italian Ministry of Research, we are studying the lactonase activity of PLLs to counteract *P. aeruginosa* infections. The human opportunistic pathogen *P. aeruginosa* (PAO1) orchestrates the expression of many genes in a cell density-dependent manner by using quorum sensing (QS). Two acyl-homoserine lactones (AHLs) are involved in QS circuits and contribute to the regulation of virulence factors production, biofilm formation, and sensitivity to antimicrobials. Disrupting QS, a strategy referred to as quorum quenching (QQ) can be achieved using exogenous AHL-degrading lactonases. In particular, in the mesophilic bacterium *Rhodococcus erythropolis* a PLL enzyme has been identified and partially characterized. It is 28% and 40% identical to PTE *(P. diminuta)* and the thermostable *SsoPox*, respectively. Here we report a new cloning, expression and purification procedure to produce a His-tagged Ahla, namely his-Ahla, that has been further characterized as a lactonase enzyme. It is highly stable over time and also under oxidizing conditions. It has showed to be a proficient quorum quenching enzyme, able to hydrolase acyl-homoserine lactones, 3oxo-C12-HSL and C4-HSL, and to reduce more than 50% the biofilm formation of PAO1. The combination of enzymes with different abilities as quorum quenchers could represent a powerful tool to be employed against pseudomonas infections. Here we will also report a comparison of the different enzymes under study.

Hyaluronic acid and its derivatives based multifunctional nanostructured devices in cancer therapy and regenerative medicine

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Session: Structure and Function of Biomolecules

Hyaluronic acid is a non-sulphonated glycosaminoglycan that results a valuable raw materials for the design of multifunctional biomaterials, thanks to its biocompatibility, high water retention and its functional groups, that can be easily functionalized with hydrophobic molecules of pharmaceutical interest [1]. In particular the conjugation with curcumin has been reported to overcome the poor water solubility of the molecule, and exploit its important biological activities, such as antioxidant, anti-inflammatory and antifungal [2]. Polysaccharides-based systems containing curcumin have resulted promising in applications in regenerative medicine, such as wound healing [3, 4]. The proper management of skin wounds is fundamental to guarantee the complete recovery of patients, since the loss of skin, caused by injury or illness, if not healed properly can lead to serious consequences, such as disabilities or even death [5]. Wound healing is the dynamic process damaged tissues are repaired with, and consists in three phases: inflammation, tissue formation and tissue remodelling [6]. The medical treatment of skin wounds contemplate the use of wound dressings, in order to protect the wound and create the optimal environment to make wound healing efficient. The advance in comprehension of the wound healing mechanisms lead to identify the mean features that a wound dressings has to fulfil, such as the ability to keep the wound moist, to absorb exudate, and to maintain gas exchange [7]. In this work, curcumin (Cur) was conjugated to hyaluronic acid (HA), and the conjugated polymer HA-Cur was used to prepare a polysaccharide-based hydrogel for potential use in wound dressing. The hydrogel was obtained by crosslinking HA-Cur and sodium alginate with calcium cations, and the effect of the addition of tannic acid and O-Carboxymethyl chitosan was evaluated. The materials were characterized for their swelling and degradation properties, for their structural properties (FT-IR) and. for their ability to release Cur and TA. Finally, antioxidant and antibacterial activities were evaluated.

[1] J. Kim, C. Lee, J.H. Ryu, Applied Sciences, 11 (2020) 21.
[2] S. Manju, K. Sreenivasan, Journal of colloid and interface science, 359 (2011) 318-325.
[3] J. Zhang, Y. Zhu, Y. Zhang, W. Lin, J. Ke, J. Liu, L. Zhang, J. Liu, Materials Science and Engineering: C, 131 (2021) 112538.
[4] M. Sharma, K. Sahu, S.P. Singh, B. Jain, Artificial Cells, Nanomedicine, and Biotechnology, 46 (2018) 1009-1017.
[5] R.A. Clark, K. Ghosh, M.G. Tonnesen, Journal of Investigative Dermatology, 127 (2007) 1018-1029.
[6] A.J. Singer, R.A. Clark, New England journal of medicine, 341 (1999) 738-746.
[7] T. Abdelrahman, H. Newton, Surgery (oxford), 29 (2011) 491-495.

Interchange of different metal ion in metallo-protein

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Session: Structure and Function of Biomolecules

The mechanism through which the right metal is integrated into the correct protein is still under investigation. Understanding how specific metals control their binding selectivity to different proteins is crucial for defining the mechanisms of metalloproteins and their involvement in some disease. To better understand such processes, we are studying the effects of the interchange and/or replacement of different metal ion in the same binding site of a metallo-protein.
As model we used the prokaryotic zinc finger, Ros, a protein involved in the horizontal transfer of genes from A. tumefaciens to a host plant infected by it. The protein contains a single Cys2-His2 zinc finger domain and assumes a βββαα fold stabilized by the presence of a 15 amino acids hydrophobic core. Ros was intensely studied as suitable model domain to unveil the effect of the metal ion replacement in metallo-proteins and appeared to tolerate the Zn to Cd substitution but not the replacement of the wild type metal by Ni(II), Pb(II) and Hg(II). In this first year we have explored by different spectroscopic techniques the effects of the Zn to Cu(I) or Cu(II) replacement and demonstrated that the metal, in both oxidation states, binds Ros with good affinity, but the binding does not give rise to a correct functional fold.

Transcriptional factors with a single zinc-finger domain in *Arabidopsis thaliana*

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Session: Structure and Function of Biomolecules

Zinc-finger domains (ZFDs) represent relatively independent protein regions which allow specific protein-nucleic acids interactions. In *Arabidopsis thaliana,* a family of plant specific C2H2 zinc-finger transcriptional factors with an invariant conserved sequence QALGGH in its DNA-binding α-helix have been identified. Superman protein is the best studied member of this family in terms of genetic function. Floral organs are arranged in concentric whorls – sepals, petals, stamens and a carpel. Superman is important to mantain the boundary between stamens and a carpel. Another member, Rabbit Ears (RBE) regulates boundary between petals and stamens. Although both proteins play important roles in floral organ development, their biochemical mode of function is poorly understood. The most intriguing feature of this protein family is the presence of a single ZFD within the entire protein. It is in contrast with multiple ZFDs found in other eukaryotic zinc-finger proteins. We demonstrated that the single zinc-finger is involved in DNA-binding and we have preliminary results sugesting the proteins perform the DNA-binding function in dimeric state. Predicted secondary structure of this proteins shows many disordered regions - potential protein-protein interaction domains enabling recruitment of numerous partners to specifically regulate the chromatin. The aim of this project is to identify a potential dimerization domain of RBE and characterize the interactome, clarifying the process of transcriptional regulation by the plant single zinc-finger protein family. A deletion mutant of RBE spanning from the first amino acid to the one in position 33, encompassing the ZFD, has been expressed in bacterial system using different strategies. However, RBE appears to be totally insoluble in *E. coli*. Work is in progress using yeast expression system to overcome the solubility problems in bacteria and yeast-2-hybrid assay to study the interaction network.

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

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Session: Structure and Function of Biomolecules

The coagulation cascade is important in the cessation of bleeding caused by tissue injury, generating a network of fibrin that stabilizes activated platelets. Therefore, its dysfunction (including factor deficiency) is responsible for many coagulation disorders (e.g. hemophilia, factor V (FV) Leiden). FV combines with Factor Xa converting prothrombin into thrombin, which then converts fibrinogen into fibrin, a critical step in the coagulation cascade. FV-deficiency causes poor coagulation after injury or surgery, mucosal surfaces, postoperative hemorrhage, etc. Acute bleeding episodes of patients with FV-deficiency are treated with fresh frozen plasma to maintain blood FV levels above 20%, causing allergic reactions and development of alloantibodies. A concentrate of FV could potentially be used to treat FV deficiency, limiting side-effects.
This project entails the development of a concentrate of a stable FV zymogen for clinical trials. We will develop analytical methods for small-scale purification of factor from Kedrion Biopharma FV zymogen concentrate batches. Purification, structural and functional characterization of the target protein will be made using immuno-affinity, size-exclusion-chromatography, SDS-PAGE, western-blotting experiments, circular dichroism and direct-binding experiments by label-free techniques. Finally, we will prepare a series of peptides mimicking FV regions for the screening of phage libraries to isolate highly specific antibodies against FV.

Elucidating the role of DDX11, the Warsaw breakage syndrome DNA helicase, at the DNA replication fork

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Session: Structure and Function of Biomolecules

Eukaryotic DNA replication is a highly regulated process that ensures the correct duplication of the genome. Regions of the genome with the potential to form unconventional DNA structures (as R-loops, triplexes and G-quadruplexes) impede progression of DNA replication forks and must be accurately managed to preserve genetic integrity. DDX11/ChlR1 (CHL1 in yeast) belongs to the group of super-family 2 (SF2) DNA helicases, characterized by the presence of an Iron–Sulphur cluster (Fe–S) domain with 5′ to 3′ directionality. Bi-allelic mutations of the gene coding for the DDX11 DNA helicase cause a very rare autosomal recessive disease, named Warsaw breakage syndrome (WABS). Different pathogenic *DDX11* missense mutations were reported. Most of them map within conserved helicase motifs and the relevant amino acid changes are expected to compromise the DDX11 catalytic functions. A recently discovered WABS patient bears a *DDX11* mutation (corresponding to the amino acid change R140Q) that is considered as a variant of unknown significance (VOUS), as it does not map in any conserved DNA helicase box of the DDX11 sequence. Hence, it remains unclear which DDX11 function is altered by the R140Q mutation in connection with the disease. I have analysed the biochemical properties of DDX11 R140Q mutant protein and examined the role of highly conserved acid residues close to R140. Thus, I have mutagenized the invariant DDX11 K120 and K121 residues, potentially important for DNA binding/unwinding, to generate the DDX11 K120A/K121A double-mutant. I have found that the DDX11 K120A/K121A derivative has reduced DNA binding and unwinding activity, while the DDX11 R140Q derivative behaves like the wild type protein. Besides, along with this, I have identified PCNA, the homo-trimeric DNA polymerase sliding clamp, as a novel DDX11-interacting partner and demonstrated that DDX11/PCNA interaction requires the integrity of an unconventional PCNA-interacting protein (PIP) box located at the DDX11 C-terminal end. Substitutions of invariant amino acid residues of the DDX11 PIP box abolish interaction with PCNA.

Plant-based investigation to discover new bioactive molecules as galectin inhibitors

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Session: Structure and function of biomolecules

Galectins are lectin proteins that specifically bind β-galactoside residues. So far, 16 proteins have been found to belong to galectin family. On the base of the number of carbohydrate recognition domains (CRDs) and the structural organization, galectin members are classified as prototype, tandem repeat type or chimera type. These proteins are involved in several cellular processes, such as apoptosis, cell-cell and cell-matrix interaction, immune surveillance, pathogen recognition and so on. Moreover, it is well-known their involvement in progression and metastasis of different tumors. So far, in literature several natural and synthetic inhibitors are reported and some of them are used in clinical trials. The importance of non-toxicity of galectin inhibitors prompted us to focus the attention on vegetal field to discover new ones as final ambitious goal. With this aim, we choose a new vegetal matrix whence extract bioactive molecules. We refined two specific protocols to extract peptides and fibers from the selected matrix. These extracts were chemically characterized in order to evaluate their amount of bioactive compounds. We also tested the cytotoxicity of peptides extracts, finding a good tolerance by the healthy cell line used for early experiments.

Development of biocompatible hyaluronan-based materials as drug-carriers and implant systems for tissue engineering

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Session: Structure and Function of Biomolecules

During the second year of the PhD course, chemical modifications performed on hyaluronic acid allowed us to prepare HA (hyaluronic acid) amphiphilic conjugates. Basically, naturally occurring fatty acids were chosen as hydrophobic moieties to be conjugated to the hydrophilic HA skeleton. Considering the HA's broad spectrum of biological activities and also the fact that natural fatty acids (palmitic, oleic and linoleic acids) are able to kill or inhibit the growth of several pathogenic bacteria, we developed a protocol to incorporate HA-fatty acid conjugates on bioactive glasses. In fact, bioactive glasses are considered to be a vital bone regeneration material, so the final aim was to investigate a treatment that could ensure an increased bone tissue repair, healing and regeneration. In the past we have developed HA-based nanoparticle able to encapsulate Curcumin in their inner core with the final aim to enhance its bioavailability. As alternative approach, amphiphilic nano-carriers can be prepared by a direct chemical conjugation of Curcumin to natural polysaccharides. In this regard, the hyaluronic acid increases the solubility and bioavailability of Curcumin, while providing its own additional biological benefits (antimicrobial, anti-inflammatory, anti-oxidant, would-repairing effects). Our efforts have been also directed to the preparation of Curcumin-Hyaluronic acid conjugates by using a green protocol, that means, in analogy with what was already obtained for HA-fatty acid conjugates, under solvent free conditions.

Prokaryotic and Eukaryotic zinc-finger proteins

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Session: Structure and Function of Biomolecules

The zinc-finger is one of the major structural motifs involved in eukaryotic protein-nucleic acid interaction and it can also participate to protein-protein interactions.
I focused my research activities on two human zinc-finger proteins: ZBTB2 and ZNF639. By immunoprecipitation experiments and mass-spectrometry analysis, we found that ZBTB2 protein is a partner of NurD complex. This interaction may explain the crucial role of ZBTB2 in many key cellular processes such as cellular development, proliferation, differentiation, cancer and pluripotency. Among the interactors of ZBTB2, we found also ZNF639, which is involved in cancer development as well. My research activity is now focused on the identification of ZNF639 target genes by performing ChIP-seq experiments with the aim to clarify the transcriptional activity of ZNF639 and the interplay with ZBTB2. Transcriptional factors containing Cys2His2 zinc fingers are present in prokaryotes too.I also focused my study on MucR protein from *Brucella abortus*. This protein has been described as a transcriptional regulator of many virulence genes. We demonstrated the ability of MucR to oligomerize and recent EMSA experiments show that the oligomerization is necessary to get a high DNA binding affinity.
Furthermore, preliminary Cryo-EM experiments carried out in collaboration with the group of Prof. Nardini (University of Milan) confirm the decameric structure of MucR and pave the way to get new structural insights into the oligomerization process. Bridging experiments performed in collaboration with Prof. Dame (University of Leiden) show clearly the MucR activity of DNA-bridging. Taken together, all these data confirm that MucR is the histone-like protein never found before in *Brucella* and in many other alpha-proteobacteria.
These findings will help to fill the gap in our knowledge about genomic DNA compaction in prokaryotes.

Innovative mass spectrometry-based proximity labeling method for unraveling the Macrophage migration inhibition factor (MIF) interactome

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Session: Structure and Function of Biomolecules

The proximity-dependent Biotin Identification (BioID2) approach coupled with high-resolution tandem mass spectrometry represents an elegant strategy for mapping local protein interactomes. This methodology provides a useful tool to explore the interaction network of a protein of interest, by expressing the bait as a fusion protein with a mutant form of the biotin ligase enzyme BirA (BirA). Following biotin incubation, proximal endogenous proteins are biotinylated by BirA and streptavidin-purified biotinylated proteins are then identified by MS/MS. The aim of this project is the identification of the MIF interactome by using BioID2. U937 cells were transfected with plasmids expressing the BioID2-MIF fusion protein. Biotinylation of MIF proximal proteins was induced by biotin supplementation and potential interactors were isolated using streptavidin magnetic beads. By nano LC- high-resolution tandem MS, a selection of interactors was derived after applying stringent criteria for data filtering including the absence in BioID2 only (control), the number of matching peptides in replicate injections, and high confidence scores at both peptide and protein levels. Interestingly, among the interactors, a subset of proteins involved in ATP-dependent protein folding in the endoplasmic reticulum, unfolded protein response (UPR) and intramolecular oxidoreductase activity were identified using bioinformatics software tools (Cytoscape and Metascape). These results will provide insight into the local interactome of the MIF; thus, opening novel perspectives for understanding the role of MIF in stress-related stimuli in the cell.

Carbonic Anhydrases represent novel molecular targets against pathogen infections

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Session: Structure and Function of Biomolecules

In the time of emerging antibiotic resistance, it's crucial to identify new target molecules to improve the pharmacological arsenal against pathogens. My PhD project is aimed to the identification and characterization of bacterial Carbonic Anhydrases (CAs) involved in vital processes of aggressive pathogens, such as *Pseudomonas aeruginosa* and *Helicobacter pylori*. CAs are ubiquitous metalloenzymes which catalyze the carbon dioxide hydration to bicarbonate and proton. CAs are divided into eight genetic families, α, β, γ, δ, ε, ζ, η and ι, which vary in terms of structural and kinetic features. Humans have only α-CA isoforms, while bacteria express α-, β-, and γ-CAs. Based on this different distribution, β- and/or γ-CAs could represent promising antibacterial drug targets. During the first year of my PhD, we focused on a *Pseudomonas aeruginosa* γ-CA isoform, PA3753 which was obtained by heterologous expression in *E.coli*. A purification protocol was optimized to obtain a sufficient amount of protein for biochemical and structural characterization. In particular, the secondary structure of PA3753 was evaluated by Circular Dichroism and its oligomeric state was investigated by means of Light Scattering. Moreover, the enzymatic activity was measured and the inhibition profile with known CA inhibitors was assessed in collaboration with the University of Florence.

Session 4:

Molecular Cell Biology

FANCJ the missing piece of the AND 1 interaction hub at the DNA replication fork

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Session: Molecular Cell Biology

FANCJ is a DNA helicase with crucial role in DNA repair by homologous recombination. FANCJ gene is mutated in patients with hereditary breast cancer and in the Fanconi Anemia pathway. FANCJ DNA helicase is also considered to play a role in resolving G-quadruplex (G4) DNA structures arising at replication forks.

In unpublished data from our laboratory FANCJ was demonstrated to directly interact with AND-1 through a highly conserved CIP box motif. If critical residues in the FANCJ CIP box are substituted with Alanine to create the FANCJ AALA mutant, binding to AND-1 is noticeably reduced, while the DNA helicase activity is not affected (separation of function mutant). To analyze the relevance of the FANCJ-AND-1 interaction in genome stability maintenance, I have established *FANCJ*-knockout (KO) HeLa cell lines that express FANCJ wild type or the FANCJ AALA mutant under the control of a Doxycycline-inducible promoter. By performing various cellular assays (co-IPs, γ-H2A.X *focus* formation analysis, SiRF and DNA fiber track assays) I have demonstrated that the FANCJ/AND-1 interaction is critical for recruiting FANCJ at the replisome, for suppressing DNA damage and promoting smooth progression of the replication forks following cell treatment with Pyridostatin (a G4 DNA-stabilizer). Besides, I have analyzed the biochemical properties of FANCJ CIP box mutants encoded by missense variants, found in cancer patients.

Identify molecular pathway regulating cell proliferation through glycosphingolipids biosynthesis

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Session: Molecular Cell Biology

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 (1) identified GRASP55, a Golgi matrix protein, to be a key molecular player in this feedback circuit. The absence of GRASP55 prevents 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.

1. Pothukuchi P., et al., (2021) GRASP55 regulates intra-Golgi localization of glycosylation enzymes to control glycosphingolipid biosynthesis. EMBO J 18:40(20):e107766

Role of ADP-ribosylation in breast cancer sensitization to apoptosis: PARP12 as a novel therapeutic target

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PARP12 is a mono-ADP-ribosyltransferase of the PARP family, with regulatory roles in membrane trafficking and cellular stress response[1,2]. Over the years, there is an increasing interest in studying PARP12 owing to its linkage in varied diseases including viral infections and cancer [3,4]. On this note, PARP12 has been identified as a key factor in breast cancer resistance to chemotherapy by contributing to tumour survival and re-growth [5]. To evaluate this further, we studied the PARP12 depletion effects in several cancer cell lines of different origin. Significant results demonstrated that transient depletion of PARP12 promotes apoptosis selectively in breast tumoral cells, as detected by FACS analysis and PARP1 cleavage. Interestingly, at molecular level we found that Akt, a major regulator of cell survival [6], is a PARP12 substrate. Further, by exploiting the ADPredict tool, putative Akt ADP-ribosylation defective mutants have been generated. We have validated a specific set of residues located in the kinase domain to be affecting the catalytic activity of Akt when mutated thus demonstrating that MARylation of Akt is functional to sustain its kinase activity. A functional analysis of how this mutant responds to apoptosis specifically focusing on PI3K/AKT/mTOR pathway is currently our interest. A detailed understanding of how MARylation of Akt by PARP12 sensitise the cancer cells to apoptosis will be instrumental in defining the role of PARP12 in breast cancer resistance to chemotherapy and in the application of specific PARP12 inhibitors as novel candidates for breast cancer therapy.

Grimaldi G, Corda D. ADP-ribosylation and intracellular traffic: an emerging role for PARP enzymes. Biochemical Society Transactions. 2019 Feb 28;47(1):357-70.

Catara G, Grimaldi G, Schembri L, Spano D, Turacchio G, Monte ML, Beccari AR, Valente C, Corda D. PARP1-produced poly-ADP-ribose causes the PARP12 translocation to stress granules and impairment of Golgi complex functions. Scientific reports. 2017 Oct 25;7(1):1-7.

Liu SY, Sanchez DJ, Aliyari R, Lu S, Cheng G. Systematic identification of type I and type II interferon-induced antiviral factors. Proceedings of the National Academy of Sciences. 2012 Mar 13;109(11):4239-44.

Shao, C., Qiu, Y., Liu, J. *et al.* PARP12 (ARTD12) suppresses hepatocellular carcinoma metastasis through interacting with FHL2 and regulating its stability. *Cell Death Dis* **9**, 856 (2018). Aug 28;9(9):1-4.

Gaston J, Cheradame L, Yvonnet V, Deas O, Poupon MF, Judde JG, Cairo S, Goffin V. Intracellular STING inactivation sensitizes breast cancer cells to genotoxic agents. Oncotarget. 2016 Nov 22;7(47):77205.

Mild endurance exercise and fasting induce thyroid hormone action associated with BHB and BDNF signaling in rat muscle

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Session: Molecular Cell Biology

The potential beneficial effects derived from the combination of dietary restriction and exercise have been studied in both animal models and humans. It is now known that the combination of these conditions causes rapid metabolic adaptations at different levels, especially in skeletal muscle. We have previously shown that this combination, when applied in humans, improves body composition, preserving lean mass. Previous studies have led to the hypothesis that thyroid hormone (T3) acts as an endogenous exercise mimetic. Indeed, in rats housed at thermoneutrality temperature (28°C), exercise during fasting results in normalization of serum fT4 levels. In addition, previous studies have shown that rats submitted to protocol of exercise and fasting, show increased serum beta-hydroxybutyrate (BHB) levels, and also an increase in the expression of the BHB-responsive brain derived neurotrophic factor (BDNF) in gastrocnemius muscle. To increase our understanding on the role of BDNF, we have studied, using the same rat model, BDNF signaling in gastrocnemius muscle and we correlated this with local T3 and BHB production.

Cancer selective targeting of NF-kB: unravel the role of GADD45β in TNF signaling

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Session: Molecular Cell Biology

It is now known that in many types of tumors NF-kBs are constitutively active and play a decisive role both in the early stages and in the advancement of cancer. GADD45β is a pro-survival factor, regulated by NF-κB, its high expression has been correlated with poor clinical outcomes in most human cancers. Although GADD45β is known to regulate caspase activity, the key molecular events mediating this inhibition are still unclear. Indeed, the eventual role of GADD45β in other forms of TNF-mediate regulated cell death has not thoroughly investigated. Here we intend to study the regulatory roles of GADD45β in cell survival in TNFR signaling, with particular regards to the proteins involved in the regulation of necroptosis, a regulated cell death pathway mediated by RIPKs (*Receptor Interaction Protein kinases*). Immunoenzymatic tests (ELISA) and immunoprecipitation experiments allowed us to discover a specific interaction between GADD45β and the RIPK3 kinase. To unravel the biological effects of GADD45β expression on RIPK3 dependent cell death process, we generated a murine cell line with inducible expression of GADD45β and performed cytotoxicity assays after apoptosis and necroptosis induction. Delineating the role of GADD45β in other form of cell death is important for cancer therapy as the type of cancer cell death specifies whether the immune system is involved or not.

Human microRNAs targeting SARS-CoV-2

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Session: Molecular Cell Biology

My PhD project aims at investigating possible interactions between human microRNAs and SARS-CoV-2. β-coronavirus SARS-CoV-2 is an enveloped, positive-sense, single stranded (ss) RNA virus with a genome of about 30 kbp, responsible for the ongoing pandemic. MicroRNAs (miRNA) play an emerging and important role in the interplay between viruses and host cells. This study started by a bioinformatics prediction of cellular miRNAs potentially targeting viral RNAs; then, a number of criteria also based on experimental evidence and virus biology were applied, giving rise to eight promising binding miRNAs. Their interaction with viral sequences was experimentally validated by transfecting luciferase-based reporter plasmids carrying viral target sequences into lung cell lines, leading to select five out of the eight potential binding sites for their responsiveness to endogenously expressed miRNAs. Co-transfection of the reporter plasmids along with miRNA mimics further supported the interaction between miR-219a-2-3p, miR-30c-5p, miR-378d, miR-29a-3p, miR-15b-5p, and SARS-CoV-2 viral sequences. miR-29a-3p and miR-15b-5p were also able to repress plasmid-driven Spike expression. Importantly, the viral target sequences are fully conserved in Beta, Delta and Omicron SARS-CoV-2 variants. The evaluation of possible anti-viral activity of the selected miRNAs is now ongoing in a cell model for virus replication.

The protective role of D-Aspartate in counteracting cadmium toxicity in the rat testis

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Session: Molecular Cell Biology

Cadmium (Cd) causes testicular injury, documented by histological and biomolecular changes, resulting in decreased serum testosterone levels with subsequent alteration of the spermatogenesis. Instead, free D-Aspartate (D-Asp) in rat testis is involved in the testosterone biosynthesis and in the progression of spermatogenesis. This project intends to investigate cellular response induced in the rat testis by Cd, administered alone or in combination with D-Asp, and to evaluate the putative action of this biomolecule in preventing or counteracting Cadmium reprotoxicity. Results showed that Cd treatment induced a decrease of steroidogenic expression levels resulting in a decrease of serum testosterone levels. The use of D-Asp, either administered in combination with Cd or before the administration of Cd, avoided the changes. Moreover, the decrease of expression levels of proliferation markers induced by Cd treatment, was reverted to initial values with the use of D-Asp. Finally, we found increased expression levels of apoptotic markers in Cd-treated testis, indicative of increased apoptotic process, whereas in the testis of Cd+D-Asp-treated rats did not observed changes. These preliminary results highlight the beneficial role exerted by D-Asp in preventing Cd-induced toxicity in the rat testis, encouraging further studies to understand the role of D-Asp in human testis health and the potential value of this molecule in the male fertility.

Identification of environmental and genetic cues that modulate neuron degeneration in *C. elegans*

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Session: Molecular Cell Biology

In light of future human exploration of deep space, a fundamental need is to understand how the nervous system may be affected by the peculiar conditions that characterize this extreme environment. I use the nematode *C. elegans* as a convenient biological dosimeter, allowing to assess the effects of different space-relevant radiation beams on neurological function of selected groups of neurons. I demonstrated that gamma rays affect dopaminergic neurons morphology and functionality in a dose-dependent manner. In collaboration with TIFPA facility (Trento), I performed X-rays irradiation and I observed that they affect dopaminergic neurons functionality similarly to gamma rays. The project aims also to discover genetic backgrounds that might sensitize or protect neurons from ionizing radiation and the molecular basis of ionizing radiation sensitivity. Thus, I discovered that *cep-1/p53* has a neuroprotective role, with *cep-1* mutants being more affected by gamma rays irradiation. Finally, I observed in gamma rays irradiated animals a reduction in dopamine content which is the cause of dopaminergic neurons dysfunction. Our next step is assessing dopaminergic neurons functionality after protons irradiation. This experiment will be a fundamental step in preparation of experiments with Galactic Cosmic Ray radiation, to be performed at reference facilities such as the GSI.

RNA regulatory networks governed by miR-125a in hepatocarcinoma cells

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Session: Molecular Cell Biology

MiR-125a is emerging as an important player in the pathogenesis of hepatocellular carcinoma (HCC), acting as an oncosuppressor: it has antiproliferative activity; it mediates the activity of the antitumoral drug Sorafenib; it is downregulated in HCC biopsies in comparison to normal tissues; it can limit tumor growth; it is involved in hepatitis B virus infection. Different miR-125a oncotargets have been identified and some regulatory circuits based on coding and non-coding RNAs have been unveiled. The focus of the current project is to gain a genome-wide perspective of the whole targetome with the ambitious goal to piece together the RNA regulatory networks governed by miR-125a impacting on hepatocarcinogenesis. To this aim:

-HepG2 and HuH-7 cells have been transfected with miR-125a mimic to boost its intracellular level in comparison to cells transfected with a control molecule;

-RNA seq experiments have been performed;

-Multiple comparisons will be performed to identify coding and non-coding RNA (long non-coding RNA and circular RNA) whose expression levels result changed in HepG2 and/or HuH-7 as a consequence of miR-125a increased level;

-Validation and functional studies on the obtained RNA regulatory networks and impact on HCC hallmarks will be performed.

The molecular mechanisms and RNA interactions investigated in this work could be exploited in the future for identifying novel biomarkers and therapeutic targets in HCC.

Genotoxic effects of Polystyrene microplastics in Zebrafish

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Session: Molecular Cell Biology

In recent years, plastic pollution has become global environmental concern affects both the terrestrial and aquatic environment. Microplastics (MP) derive from plastic degradation, through physicochemical processes. They are plastic fragments of size < 5mm, easily ingested by organisms reaching humans through the food chain, with deleterious consequences on health. Despite the impact of MP on organisms is currently the subject of numerous scientific studies, field data include different concentrations, types of microplastics, model organisms and damage targets. The aim of this research was to evaluate the *in vitro* genotoxic effects of polystyrene MPs on zebrafish cells using RAPD-PCR, to quantify the genomic template stability (GTS), and TUNEL technique, to evaluate MP-induced DNA fragmentation (DFI). Zebrafish blood cells were exposed to MP (105µg/ml) for 30, 60 and 90 minutes. The RAPD-PCR results showed a 20% reduction in GTS after 30 and 60 minutes of MP exposure up to a 40% after 90 minutes. Similarly, a time-dependent increase in MP-induced DFI was observed, with a DFI reaching 37% values for maximum exposure time. These results confirm the MP harmfulness. Further *in vitro* and *in vivo* studies evaluating oxidative stress and bioaccumulation will be performed to establish the mechanisms underlying the MP damage.

Exploring adiponectin involvement in Multiple Sclerosis

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Session: Molecular Cell Biology

Adiponectin exerts multivalent biological functions including regulation of immune responses. Multiple sclerosis (MS) is an autoimmune disease of central nervous system. Here, we investigated: a) adiponectin levels in serum and b) in cerebrospinal fluid (CSF) from MS patients and its relationship with MS severity and prognosis; c) the effects of MS-CSF on U87 and SH-SY5Y cells, *in vitro* models of glioblastoma and neuroblastoma, and whether AdipoRon, an adiponectin agonist, interferes with CSF-induced effects.
Our findings showed that both serum and CSF adiponectin was significantly increased in MS patients. Furthermore, higher levels at baseline of serum and CSF adiponectin in MS patients correlated with a worse prognosis and progression of the disease. *In vitro* experiments suggested that MS-CSF induces loss of cell viability that is attenuated by AdipoRon. AdipoRon appears to be also able to reduce the release of nitric oxide induced by CSF. Finally, CSF administration induces the expression of pro-inflammatory INF-γ and TNF-α while reducing the anti-inflammatory IL-10 and AdipoRon counteracts this effect.
Our data indicated that adiponectin is greatly involved in MS representing a potential biomarker to predict worse MS prognosis. In addition, AdipoRon appears to protect cells against MS CSF-induced cytotoxicity, supporting the hypothesis for a protective role of adiponectin in MS.

Role of D-aspartate metabolism in neurodevelopmental disorders

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Session: Molecular Cell Biology

Free D-aspartate (D-Asp) is an atypical amino acid that occurs in mammalian brain having high concentration in the embryonic phase and decreases after birth in concomitance with the expression and activity of its catabolizing enzyme, D-aspartate oxidase (DDO). D-Asp stimulates glutamatergic NMDA and mGlu5 receptors. Considering the role of NMDA receptors in neurodevelopmental disorders, previous studies suggested a relationship between perturbation of D-Asp metabolism and schizophrenia (SCZ). To discover the role of D-Asp on brain morphology and functioning, we generated a mouse model with *Ddo* overexpression and D-Asp depletion. In these mice, we observed a reduction in number of cortical neurons generated in the dorsal pallium during corticogenesis and reduced cortical and striatal gray matter volume in adult phase. In addition, we showed that brain abnormalities were related with social recognition memory deficit at juvenile phase, indicating that early D-Asp occurrence influences neurodevelopmental related phenotypes. In agreement with this hypothesis, we recently identified a case report of a *Ddo* gene duplication in a patient with ASD-related symptoms and intellectual disability.

SARS-CoV-2 infection: pseudovirus system and role of the CtBP1/BARS protein

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Session: Molecular and Cell Biology

The C-terminal binding protein-1/Brefeldin-ADP ribosylation substrate (CtBP1/BARS) is a dual function protein that acts in the nucleus as a transcriptional co-repressor, while in the cytoplasm controls membrane fission in several processes[1][2][3], including viral internalization via macropinocytosis[4]. Structurally, CtBP1/BARS contains a Rossmann fold that controls its conformation and cellular functions, depending on the ligand bound[5]. This mechanism underlies the "functional" molecular switch between the two CtBP1/BARS activities [5]. Using Drug Repurposing approach starting from FDA-approved drugs, we have identified and *in vitro* validated 30 drugs as selective inhibitors of CtBP1/BARS-dependent cellular functions: gene transcription and membrane fission. Three of these drugs reduce mesenchymal gene expression and inhibit cell migration and invasion in two CtBP1/BARS-regulated tumors: melanoma and prostate cancer[6-7].Moreover, macropinocytosis is a cell entry pathway used by several viruses, like Ebola and SARS-CoVs[8-10]. We have set-up an *in vitro* cell model for SARS-CoV-2 studies using a VSV pseudovirus carrying Spike protein in human ACE2 stable-expressing BHK21 cell line. Among the above identified CtBP1/BARS inhibitors, three drugs are potent inhibitors of the pseudovirus infection/internalization in host cells via CtBP1/BARS-mediated macropinocytosis. Overall, these studies identify drugs "safe in man" as CtBP1/BARS inhibitors which might be useful for cancer and viral infection therapies.

[1] Alessandro Pagliuso et al. (2016). Golgi membrane fission requires the CtBP1-S/BARS-induced activation of lysophosphatidic acid acyltransferase δ. *Nat Commun*. 7: 12148. doi: 10.1038/ncomms12148.

[2] Carmen Valente et al. (2012). A 14-3-3γ dimer-based scaffold bridges CtBP1-S/BARS to PI(4)KIIIβ to regulate post-Golgi carrier formation. *Nat Cell Biol*. 14(4): 343-354. doi: 10.1038/ncb2445.

[3] Antonino Colanzi et al. (2007). The Golgi mitotic checkpoint is controlled by BARS-dependent fission of the Golgi ribbon into separate stacks in G2. *EMBO J.* 26(10): 2465-2476. doi: 10.1038/sj.emboj.7601686.

[4] Prisca Liberali et al. (2008). The closure of Pak1-dependent macropinosomes requires the phosphorylation of CtBP1/BARS. EMBO J. 27(7): 970-981. doi:10.1038/emboj.2008.59.

[5] Marco Nardini et al. (2003). CtBP/BARS: a dual-function protein involved in transcription co-repression and Golgi membrane fission. EMBO J. 22(12): 3122–3130. doi: 10.1093/emboj/cdg283.

[6] Deng H, Liu J, Deng Y, Han G, Shellman YG, Robinson SE, Tentler JJ, Robinson WA, Norris DA, Wang XJ, Zhang Q. CtBP1 is expressed in melanoma and represses the transcription of p16INK4a and Brca1. J Invest Dermatol. 2013 May;133(5):1294-301. doi: 10.1038/jid.2012.487. Epub 2013 Jan 10. PMID: 23303449; PMCID: PMC3711675.

[7] Wang R, Asangani IA, Chakravarthi BV, Ateeq B, Lonigro RJ, Cao Q, Mani RS, Camacho DF, McGregor N, Schumann TE, Jing X, Menawat R, Tomlins SA, Zheng H, Otte AP, Mehra R, Siddiqui

J, Dhanasekaran SM, Nyati MK, Pienta KJ, Palanisamy N, Kunju LP, Rubin MA, Chinnaiyan AM, Varambally S. Role of transcriptional corepressor CtBP1 in prostate cancer progression. Neoplasia. 2012 Oct;14(10):905-14. doi: 10.1593/neo.121192. PMID: 23097625; PMCID: PMC3479836.
[8] Jason Mercer et al. (2009). Virus entry by macropinocytosis. Nat Cell Biol 11, 510–520. https://doi.org/10.1038/ncb0509-510.
[9] Megan Culler Freeman et al. (2014). Coronaviruses induce entry-independent, continuous macropinocytosis. mBio. 5(4): e01340-14. doi:10.1128/mBio.01340-14.
[10] Oleg O. Glebov. (2020). Understanding SARS-CoV-2 endocytosis for COVID-19 drug repurposing. FEBS J. 287(17): 3664-3671. doi: 10.1111/febs.15369.

PON2 post-translational modifications and cancer

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Session: Molecular Cell Biology

Paraoxonase-2 (PON2) is a lactonase ubiquitously expressed in many different tissues confined to perinuclear region, the ER, Mitochondria and Plasma membrane displays an anti-oxidant activity, with the mechanism of action yet to be identified. As the oxidative stress and chronic inflammation are closely related to cell death, this provides a mechanistic direction for epidemiological studies that show a link between PON2 and cancer. PON2 over-expression was reported in many cancer types with its upregulation in early stages and the down regulation during the later stages reporting its contribution by involving different pathways counting stimulating glucose uptake, proliferation and resistance to oxidative stress and protection from apoptosis. PON2 was observed to be ubiquitinated at position K29, K144, K313, K156, K159 and ADP ribosylated at D124. The focus of the current project is to identify the PON2 protein-protein interactions, E3 ligases involved in PON2 ubiquitination and PARP enzymes involved in ADP ribosylation and to identify if PON2 Post Translational Modifications (PTMs) have a role in control of tumorigenic profile by studying their involvement in Warburg effect, Anti-apoptosis and anti-proliferative effect

Involvement of miR-18a-5p/SREBP1/PERK axis in the induction of endoplasmic reticulum stress induced by hyperlipidic diet

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Session: Molecular Cell Biology

Alteration in endoplasmic reticulum (ER) function, known as “ER stress,” is emerging as a key feature of metabolic disorders. Excess lipid exposure leads to ER stress and, conversely, ER stressors disrupt lipid metabolism. As consequence, other cellular processes such as inflammation and autophagy are affected. However, there are many unanswered questions on the mechanisms of ER and autophagy in metabolic regulation and dysregulation. During the last years, studies on the relationship between ER stress and microRNAs (miRNAs) has burst on the scene. Based on these considerations, the aim of this study was to investigate the involvement of miR-18a-5p/SREBP1/PERK pathway in ER stress and how this pathway can affect apoptosis and autophagy in steatotic liver of rats fed on a high-fat diet (5 weeks). The results indicated that the expression of miR-18a-5p was downregulated by excess fat. This downregulation was associated with an increase of SREBP1c and PERK levels, highlighting a state of ER stress. Furthermore, the activation of PERK signaling resulted in an induction of apoptosis. In conclusion, our study highlights the involvement of miR-18a-5p/SREBP1/PERK axis in the hepatic ER stress. These findings encourage further investigations aimed at increasing our understanding of the causes, pathophysiology and prevention of metabolic disorders.

The association between depression and gut microbiota related to physical activity, diet and sleep

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Session**:** Molecular Cells Biology

Depression is a very common mental disorder and little is known about the mechanism of the disease. The gut microbiome is a novel area of interest to study molecular and biological mechanisms underlying depression. In this study we investigated the relation of fecal microbiome diversity and composition with depressive symptoms in 1264 participants from the Rotterdam Study cohort. Microbiota profiles were generated using 16S rRNA gene sequencing, and depression was scored based on self-reported questionnaire. Using linear regression, we identified several microbial genera to be associated with depressive symptoms. We confirmed the association of genus *Eggerthella, Subdoligranulum, Coprococcus* and family *Ruminococcaceae* and identified novel bacteria. Our study suggested that gut microbiome composition plays a role in depression. Other studies have shown a correlation between diet, physical activity and depression; moreover, in our previous studies, we showed a correlation between mild endurance exercise and microbiota composition in rat submitted to fasting and exercise. Additionally, sleep disturbance is the most prominent symptom in depression and is associated with changes in diversity and composition of gut microbiome. Based on these studies and to further understand the involvement of gut bacteria in brain regulation, we are now investigating the effects of physical activity, diet and sleep on the association between gut microbiota and depression.

Investigation of cargo-specific autoregulatory and export systems in secretory pathway

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Session: Molecular Cell Biology

The secretory pathway (SP) contributes to polarity maintenance in cells by regulating export and localisation of distinct molecules to the corresponding membrane. Little is known about how the SP is internally regulated in polarized cells. In this project we aim to understand the organization and molecular determinants of the autoregulatory signalling pathways of the apical proteins. To this end, we will study the autoregulation of GPI-Anchored Proteins (GPI-APs) export from different stations of the SP. GPI-APs are one group of apical cargoes that are involved in many cellular processes. Despite a good level of understanding of their organization and function, the important question of how the intracellular trafficking is regulated remains to be answered and we wish to address it in this project.

Session 5:
Human Genetics

Genome-wide studies for the molecular characterization of isolated Wilms tumor

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Session: Human Genetics

Wilms tumor (WT) is the most common paediatric renal malignancy and arise from developmental arrest in kidney organogenesis. Notably, DNA methylation alterations at Chr11p.15.5 is the most common driver mechanism of WT tumorigenesis: Imprinting Control Regions 1 gain of methylation characterize ~40% of WT cases and an additional 35% of cases present paternal uniparental disomy (UPD) of the region. On a cohort of 26 paired WT and adjacent healthy kidneys (HK) we performed an array-based DNA methylation analysis of more than 850,000 CpG sites. We were able to confirm the high epigenetic similarity of WT to embryonic kidney. Moreover, we identified an episignature of more than 9500 CpG sites that significantly segregates WT from paired HK. We confirmed the most frequent defects at the 11p15.5 region and found DNA methylation alterations at several other ICRs. Using data of methylation intensities, we were also able to predict copy number variants, confirming 1q gain as one of the most frequent in WT. We also performed a pilot transcriptome study by RNA-seq of 10 paired WT/HK and found ~500 genes consistently deregulated in both DNA methylation and gene expression. These preliminary results provide the basis for the identification of novel mechanisms of tumorigenesis, as well as, the identification of potential epigenetic biomarkers associated with phenotype and prognosis.

MicroRNAs as diagnostic biomarkers in neurodegenerative disease. A focus on MCI (Mild Cognitive Impairment) – Alzheimer's disease and MS (Multiple Sclerosis)

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Session: Human Genetics

WHO (World Health Organization) considers NDDs (Neurodegenerative Diseases) a public health priority because NDDs affect nearly 50 million people worldwide and represent the seventh cause of death among all diseases and one of the major causes of disability and dependency.
Several evidences suggests that microRNAs might play relevant roles in NDDs biogenesis, but the link between microRNAs and specific aspects of NDDs are only partially explored.
Mild Cognitive Impairment (MCI) defines an intermediate state between normal aging and dementia that could be evolve in Alzheimer's disease (AD) while Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS) characterized by loss of motor and sensory function, that results from immune-mediated inflammation, demyelination and subsequent axonal damage.
The aim of project is to investigate microtrascriptome in MCI-AD state, AD and MS.
The first step is the analysis of the microRNA-transcriptome in blood, serum and cerebrospinal fluid samples using NGS. qPCR analysis will be then performed to generate the expression profiles of the microRNAs. Subsequently, through a in silico prediction analysis, target mRNAs will be identified and confirmed by qPCR analysis. These latest experiments will be fundamental to evaluate mRNAs whose expression is controlled by microRNAs which are deregulated in MCI, in MCI-AD state and MS patients.
The results obtained in this research project will provide the basis for a better understanding of the role of microRNAs in NDDs from the early stages of the disease, and their potential use as early and non-invasive biomarkers.

Inclusion and extension of data from families with IP to reveal the characteristic of IFN-I autoimmunity

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Session: Human Genetics

It has been demonstrated that asymptomatic and preexisting neutralizing antibodies (Auto-Abs) against an IFN-α subtype, IFN-ω or both can lead to severe COVID-19. These Auto-Abs were pre-existing to SARS-CoV-2 infection in 25% of Incontinentia pigmenti (IP, #308300) patients (0.3% general population) suggesting a hidden autoimmunity in IP. IP is an X-linked dominant disease, lethal in males, females are always heterozygous for NF-κB Essential MOdulator (*NEMO/IKBKG*) mutations. Its pathogenesis is related to an inflammatory response that results in the abnormal production of cytokines and chemokines. Although the protein NEMO is essential for the NF-κB-activation pathway in immunity, inflammation and cell survival, the IP was not associated with autoimmunity. In the first year of my activity we collected biological samples and clinical data from five new trios IP families, by IPGB biobank. Three patients showed a mild phenotype, two patients CNS alterations (age mean 36.5$\pm$10.8 years). The IP locus genotypic characterization identified the deletion NEMOΔ4-10 in three cases, a complex rearrangement in one, and a mosaic form of the deletion NEMOΔ4-10 in a baby (<1year old). The X-inactivation was detected in the peripheral blood: two patients showed a random profile and three were skewed. These samples will be WES sequencing and will be tested for Auto-Abs. They will increase the cohort of IP patients already enrolled to identify biomarkers in this type of autoimmunity.

Identification and evaluation of microRNAs involved in photoreceptor degeneration

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Session: Human Genetics

Inherited retinal diseases (IRDs) are a clinically and genetically heterogeneous group of disorders, characterized by photoreceptor degeneration and loss of vision. MicroRNAs (miRNAs), a class of non-coding RNAs with post-transcriptional regulatory properties, play a major role in retinal function, both in physiological and pathological conditions. Since miRNAs are capable of simultaneously modulating multiple molecular pathways, they represent promising tools to therapeutically tackle highly heterogeneous genetic disorders, such as IRDs. In the present work, a high-throughput screening approach was employed to study in a systematic manner, the impact of miRNAs on a photoreceptor-like cell line undergoing light-induced degeneration. For this approach, more than 1200 miRNAs were assayed for their putative protective action in light-stressed 661W cone photoreceptor cells. Top-performing miRNAs were tested with secondary *in vitro* methods and one of them displayed a significant protective role. *In vivo* overexpression of this miRNA in the retina of the autosomal dominant IRD mouse model $Rho^{+/P23H}$, showed preservation of retinal function and protection of cone photoreceptors from degeneration. Our results confirm the effectiveness of the designed *in vitro* screening method both to shed further light on the contribution of miRNAs to photoreceptor degeneration and, possibly, to the development of novel therapeutic approaches for IRDs.

Generation of PD-disease cellular models to explore the genetic heterogeneity of Parkinson's disease

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Session: Human Genetics

The objective of this study is the identification of novel PD genes variants and the study of single and multiple mutations in PD-disease cellular models.We focused on *TMEM175* gene and identified 4 common variants associated with PD, including two novel variants, rs2290402 [c.-10C>T; p = 0.003] and rs80114247 [c.T1022C, p.M341T; p = 0.05], located in the Kozak consensus sequence and in the TM3II domain, respectively. We also disclosed 13 novel highly penetrant mutations. We showed that detrimental mutations in the *TMEM175* might be sufficient to cause the disease, and the presence of polygenic mutations anticipated the disease symptoms of about 6 years.We demonstrated the loss of the K^+ conductance of the mutant channels and the loss of binding affinity with AKT. In patients-derived fibroblasts, we observed impaired autophagic flux and activation of UPR markers. Finally, we showed that *TMEM175* is highly expressed in dopaminergic neurons of the substantia nigra pars compacta and in microglia of the cerebral cortex in the human brain. To study the multiple mutations in different genes in a dopaminergic background, we generated iPS cells of PD patients (n=6) and healthy subjects (n=3) carrying the most promising combination of mutations. The analysis is ongoing.

miR-181a/b downregulation: a mutation-independent therapeutic approach for Inherited Retinal Diseases

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Session: Human Genetics

Inherited Retinal Diseases (IRDs) are among the most frequent causes of blindness of genetic origin and characterized by progressive photoreceptor cell death. Their genetic heterogeneity constitutes a limitation to the development of gene-specific therapies. We demonstrate that miR-181a/b silencing exerts a protective effect in IRDs. In particular, the subretinal delivery of an adeno-associated viral vector that carries a miR-181a/b sponge inhibitor sequence improves retinal morphology and visual function both in models of autosomal dominant (RHO-P347S), and of autosomal recessive (rd10 mouse) Retinitis Pigmentosa. Moreover, mitochondrial dysfunction is a common pathogenic event in IRDs, hence we showed that miR-181a/b downregulation modulates the level of the mitochondrial fission-related protein Drp1, via regulation of the JAK2/STAT3/IRF1 pathway in the retina and rescues the mitochondrial fragmentation in RHO-P347S photoreceptors, ameliorating mitochondrial mass and morphology. Finally, initial analysis of single cell RNA-seq experiments revealed a higher number of rods in RHO-P347S eyes following miR-181a/b silencing thus confirming also at the transcriptome level the protective effect of this approach. Our data highlight the potential use of miR-181a/b downregulation as mutation-independent therapeutic strategy for IRDs, which can be effective both to slow down disease progression and to support gene-specific therapeutic procedures.

Polygenic Risk Score of oxidative status and Alzheimer's Disease

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Session 2: Human Genetics

Oxidative stress has been reported as a contributing factor for the development of several pathologies, like cardiovascular diseases, neurodegenerative disorders and cancer[1].
In our study serum levels of pro- and anti-oxidant species were assessed with Diacron International tests (d-ROMs and BAP, respectively) in a cohort of 1600 individuals from three villages of the Cilento region.
Genome-Wide Association Studies (GWASs) were carried out in order to identify genetic polymorphisms associated with oxidative status. Using GWAS results, the correlation between the genetic contribution to oxidative status and the Alzheimer's Disease (AD) has been investigated.
In particular, genotype effect size estimates for oxidative status in Cilento population have been used to calculate the Polygenic Risk Score (PRS) in EADI cohort (2366 cases and 6242 controls), allowing to correlate it with the disease.
Moreover, PRS for AD with 83 selected SNPs[3] has been calculated in Cilento population and correlated with pro- and anti-oxidant species levels.
Both analyses didn't underline a significant genetic link between the genetics of oxidative status and the disease.
This results could be due to the small sample size of Cilento population. For this reason, new PRS will be performed after increasing our sample size in order to improve power analysis.

1. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D and Bitto A (2017). Oxidative stress: harms and benefits for human health. Oxidative Medicine and Cellular Longevity, volume 2017:8416763.
2. Clark K, Leung YY, Lee WP, Voight B and Wang LS (2022). Polygenic risk scores in Alzheimer's Disease genetics: methodology, applications, inclusion, and diversity. Journal of Alzheimer's Disease, 89:1-12.
3. Bellenguez C, Küçükali F, Jansen I, Andrade V, Moreno-Grau S, Amin N, Naj AC et al. (2022). New insights on the genetic etiology of Alzheimer's and related dementia. Nature Genetics, volume 54: 412–436.

The role of microRNAs 181a and b in Parkinson's Disease: from promising therapeutic targets to potential biomarkers

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Session: Human Genetics

MicroRNAs are fine regulators of gene expression, and their dysregulation has been reported in Parkinson's Disease (PD). miR-181a and miR-181b (miR-181a/b) are highly expressed in the SN and striatum and enriched in the brains of PD patients. We showed that mir-181a/b controls key mitochondrial functions such as mitochondrial biogenesis and mitophagy. Interestingly their downregulation protects neurons and ameliorates the neurodegeneration in different *in vivo* models of mitochondrial diseases.

Given the central role of mitochondria in the pathogenesis of PD, we decided to investigate the role of miR-181a/b in PD. To test the effect of mir-181a/b modulation in PD, we generated chemical PD models in Medakafish and mice by using the neurotoxin 6-hydroxydopamine (6-OHDA). Interestingly, in both models, the inactivation of miR-181a/b reduces the extent of 6-OHDA-induced DA neuron death. Moreover, we are validating the neuroprotective effect of miR-181a/b modulation in *in vitro and in vivo models* of α-synucleinopathy. Finally, we are now evaluating if miR-181a/b could be considered molecular biomarkers of PD. We are thus performing RT-qPCR to estimate miR181a/b levels in the plasma of PD patients. In conclusion, our preliminary results suggest that miR181a/b may represent both reliable and easy-to-measure biomarkers and effective therapeutic targets in PD.

The ZNF687 mutation of Paget's disease causes bone remodelling alteration dysregulating osteoclast transcriptional program and osteoblast activity

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Session: Human Genetics

Paget's disease (PDB) is a late-onset bone remodelling disorder with a broad spectrum of complications, among which neoplastic degeneration (i.e. osteosarcoma, giant cell tumour) and osteoarthritis (OA). One of the most aggressive forms is caused by the P937R mutation in the *ZNF687* gene. Previously, we showed that 8-month-old P937R *knock in* mutant mice displayed reduced bone mass, indicating that the osteolytic phase of the disease occurred. Interestingly, we detected osteoblast activity deregulation. Indeed, histomorphometric analysis revealed the presence of disorganised and immature bone in mutant mice at 8 months of age. Noteworthy, 16-month-old mutant mice presented woven bone and osteosclerotic lesions, indicating that osteoblast function overtook bone resorption. Surprisingly, we detected osteophytes and intervertebral disc degeneration, outlining the link between OA and PDB. To deepen the mechanistic role of Zfp687 on bone metabolism, we knocked-out the gene in RAW264.7 cells using the CRISPR-Cas9 technology. Remarkably, we noted that the osteoclastogenesis of KO cells was strongly jeopardized. RNA-sequencing on wild type and KO clones identified a set of genes involved in osteoclastogenesis controlled by Zfp687. Thus, Zfp687 has a pivotal role in the regulation of bone remodelling. Therefore, the *Zfp687* knock-in mouse model may offer the potential to therapeutically treat PDB.