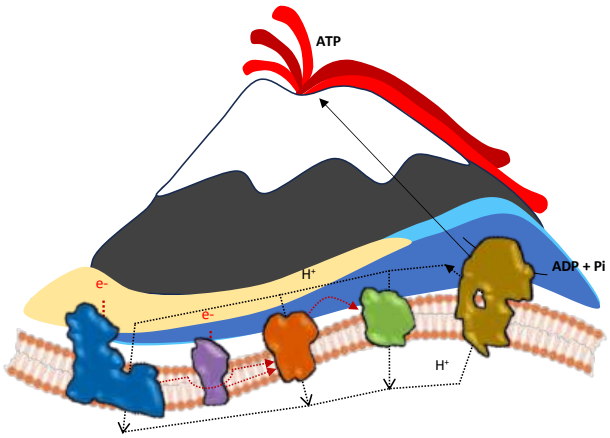

FOURTH INTERNATIONAL MEETING OF THE
ITALIAN GROUP OF BIOMEMBRANES AND BIOENERGETIC

GIBB2025

CATANIA, 12-14 JUNE 2025

Auditorium **Giancarlo De Carlo**
Monastero dei Benedettini
University of Catania

ABSTRACT BOOK

www.gibb-be.org

GRUPPO ITALIANO DI BIOMEMBRANE E BIOENERGETICA

Italian group of Biomembranes and Bioenergetic

Created in 1973 by a group of Italian Scientists coming from a variety of disciplines (biology, chemistry, biophysics, pathology, pharmacology and physiology), GIBB was born with the idea of promoting Bioenergetics in the Country and to establish an interface with the growing International Bioenergetics Community.

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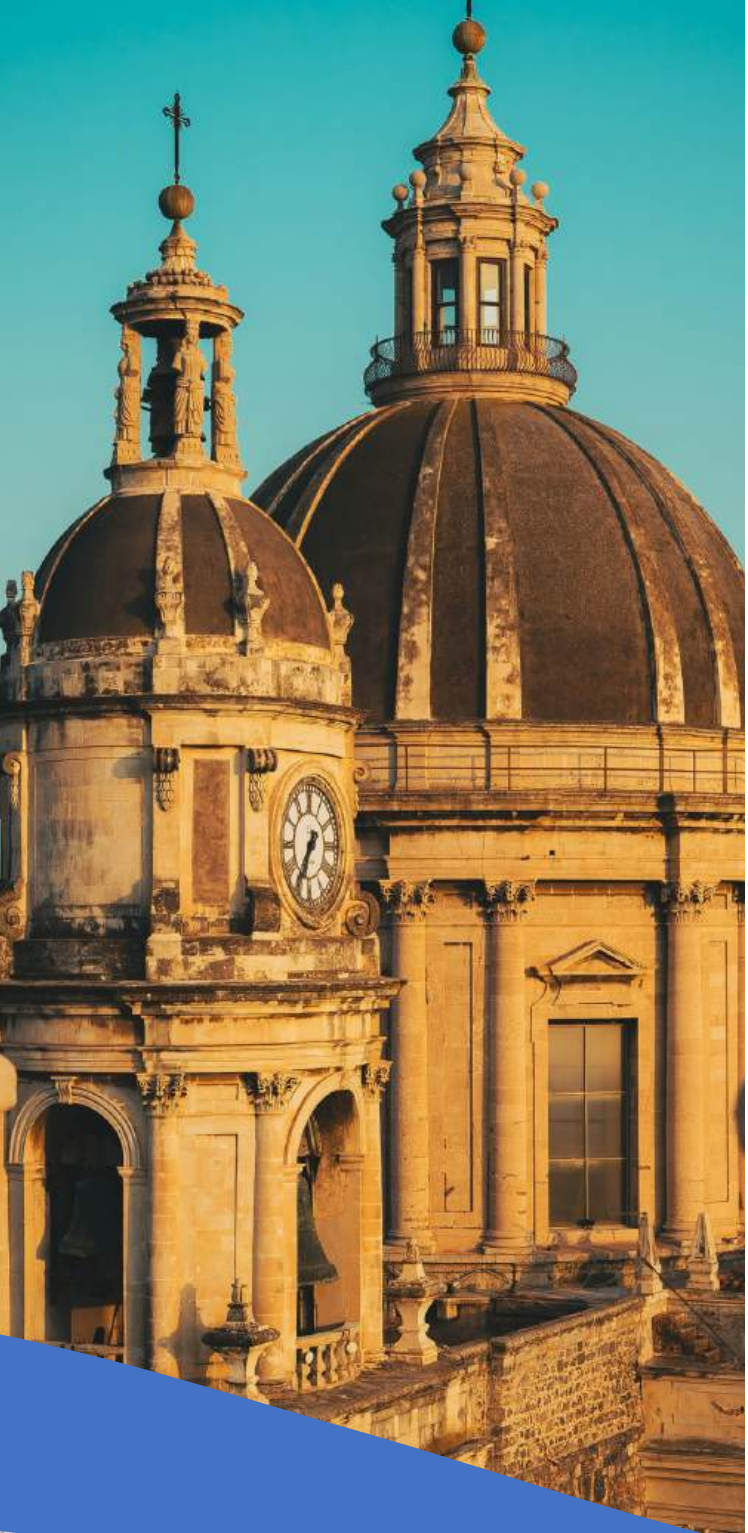

WELCOME TO CATANIA

Situated at the foot of Mount Etna, Catania is a vibrant city rich in history and culture. Its stunning Baroque architecture, a **UNESCO World Heritage Site**, is exemplified by the magnificent **Cathedral of Sant'Agata**, the **Basilica della Collegiata**, and the iconic fountains, **Fontana dell'Elefante** and the **Fontana dell'Amenano**, which the locals call "acqua o' linzolu" (water in the form of a leaf).

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A perfect blend of tradition, gastronomy and natural beauty, Catania is a must-visit place in Sicily.

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With an average enrolment of **38000 students**, the **University of Catania** has been around for hundreds of years since **1434** and is oldest one in Sicily. The educational system is run and overseen by **17 departments**. The Medical School for starters, and by two other educational units respectively located in the city of Ragusa and in Syracuse. Another special unit is the **Scuola Superiore di Catania**, a higher education center based on excellence that was founded in 1998 for the selection and the recognition of the brightest young minds, offering a variety of studies including analysis, research and experimentation.

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Biometec
Dipartimento di Scienze Biomediche e Biotecnologiche
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The meeting will take place under the patronage of the oldest parliament in the world, the Sicilian Parliament, **Assemblea Regionale Siciliana** (ARS).

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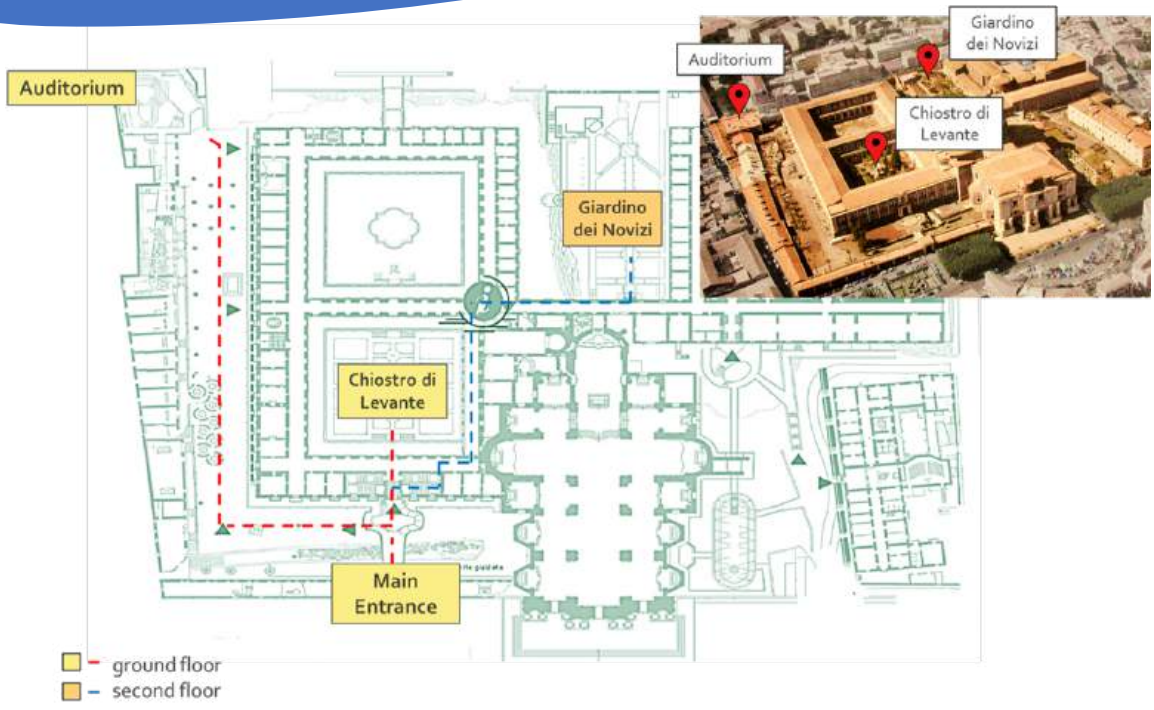

Monastero dei Benedettini is easily accessible by bus from any part of the city. For routes and timetable, please visit the local transportation site.

SOCIAL DINNER

Enjoy the social dinner with mediterranean special dishes by Chef Dedè in the enchanting surrounding of the Botanical Garden of Catania, a gem of the Biological, Geological and Environmental Science department.
The Botanical Garden is accessible by the secondary entrance of via **Antonino Longo 19**.

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Erich Gnaiger

Oroboros Instruments
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Tatiana Rostovsteva

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Bethesda, USA

ABSTRACTS

P1 | Mitochondrial dysfunction in Spinal Muscular Atrophy: a multi-omics approach using SMA patient-derived iPSCs

Showmeya Mallavarapu[1], Pierluigi Vinci[2], Federica Rizzo[3], Linda Ottoboni[2], Stefania Corti[2,3] and Camilla Bean[1]

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Spinal muscular atrophy (SMA) is a devastating genetic disorder caused by mutations in the survival motor neuron 1 (SMN1) gene, primarily affecting motor neurons and skeletal muscle. While current gene therapies offer significant clinical benefits, they are not curative. Growing evidence supports SMA as a multi-systemic condition, reflecting the multifunctional roles of SMN1 and highlighting the urgent need for complementary therapies targeting multiple tissues. Mitochondrial dysfunction has been increasingly linked to SMA pathogenesis. We previously demonstrated that SMN1 loss in mouse skeletal muscle leads to abnormal mitochondrial structure and function, excessive reactive oxygen species, disrupted calcium homeostasis, and defective autophagy and lysosomal function. Notably, transplantation of amniotic fluid stem (AFS) cells into SMN-deficient mice corrected these abnormalities and restored mitochondrial morphology and mitochondrial-associated gene expression. To explore whether similar mechanisms occur in humans, we used patient-derived induced pluripotent stem cells (iPSCs), which can differentiate into multiple cell types, including motor neurons. Using multi-omics approaches, we identified widespread transcriptional changes linked to mitochondrial dysfunction. In motor neurons, we observed dysregulation of calcium ion-binding genes and altered miRNA expression predicted to target these transcripts, which were associated with mitochondrial structural defects, as shown by electron microscopy analyses. Gene annotation analyses further implicated disruptions in metabolism, ER stress responses, and calcium signaling. These findings support a mitochondria-centered model of SMA and suggest that targeting miRNA- mediated dysregulation could help restore mitochondrial function and cellular homeostasis, paving the way for complementary therapies in SMA.

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P2 | Dual sEH/LOX-5 inhibition confers neuroprotection in *in vitro* and *in vivo* models of Parkinson's disease

Marco Falcone[1], Loredana Leggio[1], Marco Catania[1], Maria Cullurà[1], Valentina Sgarlata[1], Edoardo M. Sommella[2], Kari Espolin[3], Carmine Ostacolo[2] and Nunzio Iraci[1]

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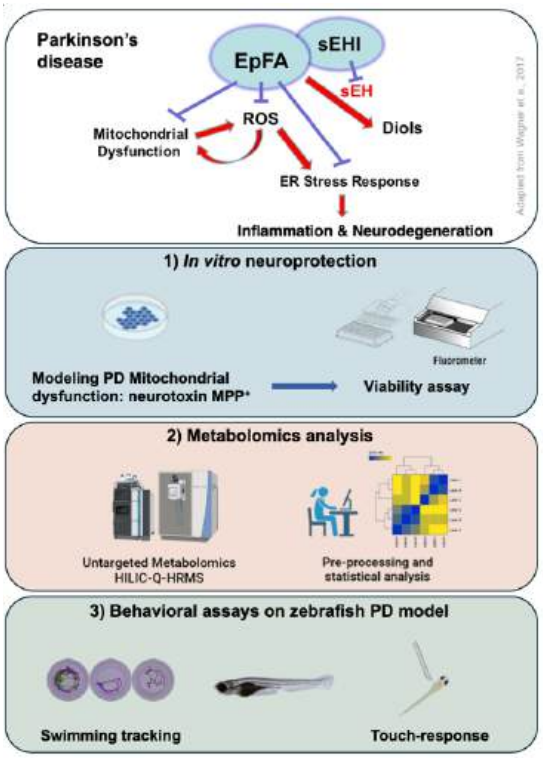

Parkinson's disease (PD) is a multifaceted neurodegenerative disorder with heavy medical and socioeconomic implications worldwide. Among PD hallmarks, neuroinflammation and mitochondrial dysfunction are at the heart of the degenerative process of dopaminergic neurons (DAergic) in the *substantia nigra* pars compacta. Evidence suggests a role of specific lipid mediators-epoxy fatty acids (EpFAs) - in the resolution of neuroinflammation. Soluble epoxide hydrolase (sEH) is responsible for the conversion of EpFAs to their corresponding diols, lacking anti-inflammatory properties. Here, we investigated the potential neuroprotective effects of inhibiting sEH enzyme, both specifically and in combination with lipooxigenase-5 (LOX-5), with novel compounds LO44 and LO35, respectively. In *in vitro* models of PD, viability experiments revealed LO35 as the most effective in conferring DAergic cells resilience to MPP^+- induced mitochondrial dysfunction. Next, we performed a metabolomics analysis on MPP^+- injured neuronal cells, to identify specific changes upon LO35 treatment. Interestingly, metabolite set enrichment analysis showed cardiolipin (CL) biosynthesis as the most significantly altered pathway in the LO35 group, supporting the neuroprotective potential of double sEH/LOX-5 inhibition. CL is a mitochondria-specific lipid, required for optimal activity of the respiratory complexes and ATP synthase, sustaining their bioenergetic processes. Finally, we exposed zebrafish larvae to neurotoxin MPP^+ to further validate the effect of LO35 in an *in vivo* PD model. Behavioural assays highlighted increased swimming activity and improved response to touch stimuli in LO35 pre-treated larvae. Overall, these findings point at regulating lipid mediators balance as a novel strategy to enhance DAergic neuroprotection. The investigation of this pathway will be a further step towards the development of future effective treatments that modify the course of PD and possibly improving patient quality of life.

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P3 | Characterization of Glioblastoma Phenotypes and associated metabolic pathways as a new therapeutic strategy

Roberta Rizzo[1], Vincenzo Russo[1], Simona D'Aprile[1], Simona Denaro[1], Francesca Guarino[1], Angela Messina[2], Andrea Magrì[2], Vito De Pinto[1], Nunzio Vicario[1] and Rosalba Perenti[1]

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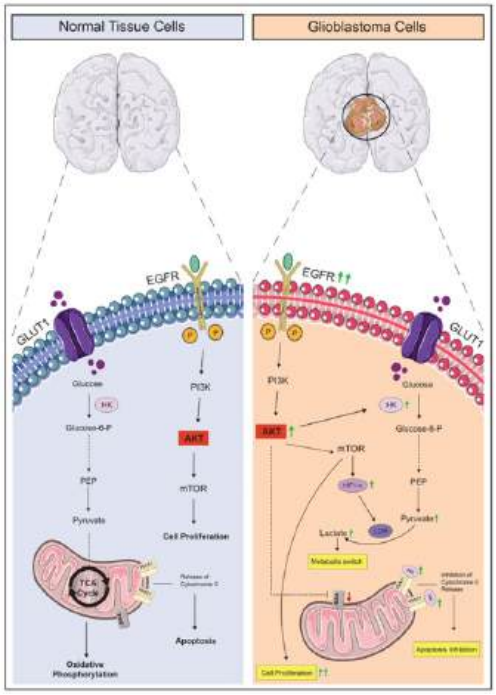

Glioblastoma (GBM) metabolism is profoundly dysregulated compared with the normal tissues, requiring increased energy to sustain rapid proliferation. Its ability to adapt the tumour microenvironment leads to specialized molecular subtypes, with unique features and therapeutic needs [1]. Given the crucial role of mitochondria in the tumor regarding energy production, metabolic reprogramming and resistance to apoptosis, we investigated the expression of Voltage-Dependent Anion Channel (VDAC) isoforms, proteins that serve as key regulators of metabolic flux between mitochondria and cytosol, in the context of GBM cell lines [2]. Specifically, in patient-derived proneural, mesenchymal and classical GBM samples, we analyzed the expression profiles of VDAC1 with a protein expression assay of representative cell lines of these subtypes. This analysis revealed that VDAC1 is highly expressed in the most aggressive and lower in less proliferative cell lines. Further analysis of the classical subtype showed a link between VDAC1 and EGFR, a key driver of GBM aggressiveness. EGFR activation enhances hexokinase expression, which binds to VDAC1 and inhibits Bax, creating an apoptosis-resistant cell context. This mechanism aligns with the resistant phenotype observed in GBM. Our preliminary findings point to a link between VDAC1 and EGFR-driven metabolic reprogramming, emphasizing the importance of mitochondrial regulation for oncogenic signaling in the maintenance of GBM growth and supporting the hypothesis that specific gene expression signatures and distinct metabolic alterations can be used to tailor therapeutic approaches for GBM patients.

References:

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[2] A. Magrì et al. *Front Chem* (2018), 6: 108.

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P4 | Cellular cAMP content and mitochondrial profile define different subtypes of ovarian cancer cells

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Ovarian cancer (OC) is a highly aggressive and fatal gynecologic cancer, primarily due to its lack of symptoms, which often leads to a late diagnosis [1-2]. OC consists of various histological types, with serous OC being the most common and aggressive form. However, even within the same OC subtype, there is additional variability in genetic mutations and metabolic profiles, which may influence treatment responses. In cancer, metabolic reprogramming is closely linked to mitochondrial function, this function can be regulated through the cAMP pathway, and its dysregulation has been observed in several cancers, including OC [3]. In our study, we examined two serous ovarian cancer (OC) cell lines, OC316 and OV56, as well as eleven human OC tissues. The OC316 cell line exhibited higher cAMP levels compared to OV56. This elevated cAMP was linked to the activation of the cAMP/PKA/CREB/PGC-1α pathway, leading to increased mitochondrial biogenesis, enhanced respiratory chain activity, modulation of mitochondrial dynamics, and improved resistance to apoptosis. Additionally, principal component analysis (PCA) of twenty-three biochemical parameters from the eleven OC tissues revealed two distinct patient groups, each characterized by different cAMP levels and corresponding mitochondrial profiles. We emphasize a cAMP-driven stratification of two mitochondrial profile populations in serous OC. These findings suggest that the molecular diversity within OC poses a significant challenge.

References:

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[2] D. De Rasmo et al. *Int J Mol Sci* (2023), 24:1224.
[3] A. Signorile et al. *Cancers* (2019), 11: 1350.

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P5 | Dysregulation of flavin homeostasis in Pancreatic Ductal Adenocarcinoma: the role of p53

Alessia Nisco[1], Maria Tolomeo[2], Angela Sposato[1], Marilena Ardone[1], Francesca Fracasso[1], Rosangela Cardone[1] and Maria Barile[1]

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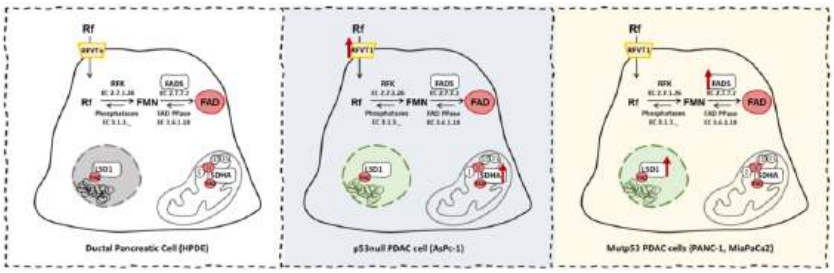

Riboflavin (Rf, vitamin B2) is the precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are essential redox cofactors for cellular bioenergetics. Rf cellular uptake occurs via three specific transporters (RFVT1, RFVT2, and RFVT3) and its intracellular conversion into flavin cofactors requires riboflavin kinase (RFK) and FAD synthase (FADS) [1]. Flavin homeostasis is gaining importance in cancer cell metabolism [2]; in particular, we are investigating Rf homeostasis in pancreatic ductal adenocarcinoma (PDAC) and how it may be influenced by p53 status. We recently demonstrated that FADS2 is upregulated in mutant p53 (mutp53) cell lines (PANC-1, MiaPaCa-2), whereas its levels remained unchanged in p53-null AsPc-1 cells. This indicates that FADS2 overexpression in mutp53 PDAC cells is a consequence of gain-of-function p53 mutations. Interestingly, the protein levels of the FAD-dependent lysine demethylase-1 mirrored those of FADS2 in mutp53 cells [3]. These findings suggest that the increased expression of relevant flavoenzymes is coordinated with an increase in the efficacy of FAD formation machinery. Here, we analyzed intracellular flavin content by HPLC, revealing increased levels in AsPc-1. This increase parallels that of flavoprotein subunit of the complex II of the respiratory chain, SDHA. Since flavin flux is dependent on RFVTs, we expanded our study by analyzing RFVT expression levels. Notably, RFVT1 was selectively upregulated in the p53-null AsPc-1 cells, suggesting that its regulation is influenced by the absence of p53 rather than its mutational status. These insights into flavin homeostasis alterations provide a foundation for further exploration of targeted metabolic interventions in PDAC.

References:

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P6 | Distinct respiratory profiles of Glioblastoma Multiforme cell lines revealed by high-resolution respirometry

Pietro Caruso[1], Xena G. Pappalardo[1], Silvia Boninelli[1], Fabrizio Cavallaro[1], Flaviana Marzano[2], Mariafrancesca Scalise[3], Francesca Guarino[1] and Andrea Magrì[4]

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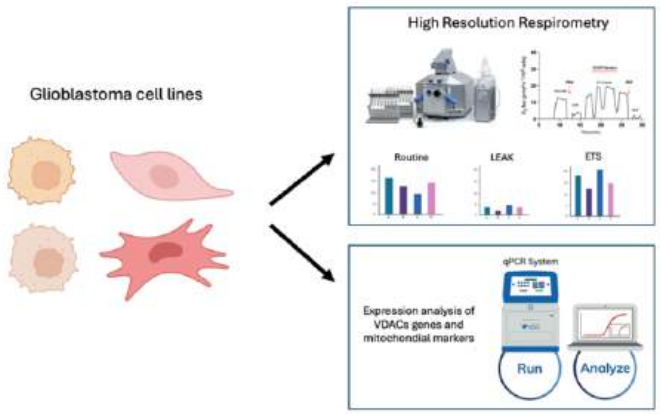

Glioblastoma Multiforme (GBM) is the most aggressive form of brain cancer, characterized by a poor prognosis and high resistance to currently available chemotherapies. Genetic alterations in cancer cells disrupt amino acid metabolism, impacting both cell proliferation and survival. Among the deregulated genes, those encoding membrane proteins, particularly nutrient transporters, are the most affected. The Voltage-Dependent Anion-Selective Channel (VDAC) family consists of pore-forming proteins located in the outer mitochondrial membrane. The most abundant isoform, VDAC1, functions as the primary mitochondrial channel for ions and metabolites, including glutamine, NAD^+/NADH, and ATP/ADP, playing a crucial role in energy homeostasis. Additionally, VDAC1 interacts with cytosolic proteins such as Hexokinases (HKs) and Bcl-2 family members (Bak, Bax), thereby regulating glycolysis and apoptosis. Notably, the interaction between VDAC1 and HKs inhibits apoptosis while simultaneously enhancing metabolism through the Warburg effect. To evaluate the contribution of glycolysis and/or oxidative phosphorylation to cell proliferation, High-Resolution Respirometry (HRR) was employed in a panel of commercially available GBM cell lines representing different tumour grades and phenotypes (mesenchymal, proneuronal). Specific protocols utilizing substrates, inhibitors, and uncouplers were designed to measure oxygen consumption in both intact and permeabilized cells. HRR analysis revealed significant heterogeneity among the cell lines and suggested a potential correlation between VDAC1 expression, assessed via real-time PCR, and mitochondrial activity. Given that VDAC1 is a key pharmacological target in cancer therapy, our preliminary findings may pave the way for the utilization of small synthetic VDAC1 antagonists as a potential treatment for GBM.

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P7 | Citrate carrier function and its role in tumor metabolic reprogramming in pancreatic cancer cell models

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer marked by metabolic reprogramming that supports growth and combats oxidative stress. The mitochondrial citrate carrier (CiC), encoded by SLC25A1, regulates the flux of citrate between mitochondria and the cytosol, supplying acetyl-CoA for lipogenesis and NADPH for redox balance [1]. Citrate also acts as an allosteric modulator of key enzymes involved in glycolysis, gluconeogenesis, and lipid biosynthesis [2]. SLC25A1 expression is elevated in PDAC tissues compared to normal pancreas and is regulated by oncogenic pathways such as Myc and p53, while repressed by PTEN [3]. Bioinformatic analyses highlight a strong association between activating KRAS mutations (G12V/D) and PDAC incidence. Functional in vitro studies showed that inducible silencing of SLC25A1 significantly impairs proliferation in KRAS wild-type PDAC cells (BxPC3), but not in KRAS-mutant lines (Panc1, Patu8988T). Notably, expression of KRASG12V in BxPC3 cells restored proliferation despite CiC depletion, suggesting that KRAS activates compensatory metabolic pathways. Moreover, CiC knockdown leads to downregulation of lipogenic genes such as ACLY, FASN, and ME1, supporting the essential role of SLC25A1 in tumor metabolism in KRAS wild-type contexts. These findings identify SLC25A1 as a metabolic vulnerability in a PDAC subset and a potential target for personalized therapeutic strategies.

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P8 | Peptides with potential α-Synuclein disaggregating activity

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Synucleinopathies, including Parkinson's disease are characterized by the pathological aggregation of alpha-synuclein, leading to neuronal dysfunction and degeneration. Targeting alpha-synuclein aggregation could represent a promising therapeutic approach. In this context, peptides can be designed to selectively bind to aggregated forms of alpha-synuclein, destabilize these toxic species, and promote their disaggregation and clearance from neuronal cells besides to modulate protein misfolding processes. The Up Regulation Gene Seven (URG7) encodes a protein localized in the endoplasmic reticulum (ER) membrane orienting the N- and C-terminal regions toward the lumen and cytoplasm, respectively [1]. URG7 acts as a cellular stress modulator, promoting protein homeostasis and cell survival by alleviating ER stress and activating protective pathways both in HepG2 cells and in SH-SY5Y cells, commonly used to study neurodegenerative diseases [2]. Previously, in vitro studies demonstrated that the C-terminal region of URG7 (PU, residues 75–99) alters the aggregation state of α-synuclein in HepG2 cells [3]. PU peptide and some of its derivatives, properly designed, were tested in vitro experiments on recombinant α-synuclein aggregates using circular dichroism (CD), differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FTIR), and fluorescence and transmission microscopy [4]. The results show that peptides interact with α-synuclein aggregates exhibiting the disaggregation effects.

References:

[1] Ostuni A et al. *FEBS Lett* (2013), 587: 3058-3062.
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[4] J. Dandurand et al. *Int J Mol Sci* (2024), 25: 1135.

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P9 | Monocyte response to stimulation with mitochondrial DNA in aging: insights into metabolic features and chronic inflammation

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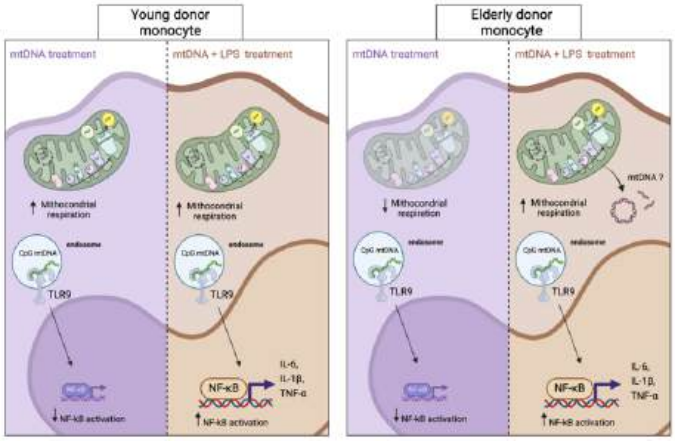

Elderly people are characterized by a low-grade, chronic inflammation defined "inflammaging". Circulating free mitochondrial DNA (cf-mtDNA) acts as a Damage-Associated Molecular Pattern (DAMP) and promotes activation of innate immune cells, including monocytes. Cf-mtDNA is sensed via Toll like receptor 9 (TLR9), inducing the expression of tumor necrosis factor (TNF)-α, IL-6, and IFNα. However, it remains unclear if response to mtDNA modifies the metabolic features of monocytes, if it contributes to the activation of a proinflammatory response and if it undergoes change with aging. We isolated monocytes from blood samples of 15 elderly subjects (>80 yrs) and 15 sex-matched young controls (<40 yrs) and treated cells with mtDNA, alone or with LPS. We measured immunometabolic parameters of monocytes, determined the intracellular distribution of TLR9, its colocalization with cytoplasmic DNA, and NF-kB activation. Resting monocytes from elderly subjects showed higher basal respiration compared to young controls. Treatment with LPS increases basal and maximal respiration up to 10 times in both young and elderly donors. A similar increase could be observed in extracellular acidification rate, suggesting a parallel increase of glycolysis. Nevertheless, monocytes from young donors, but not elderly subjects, exhibit an enhanced response to mtDNA, resulting in increased basal respiration. Monocytes from elderly subjects express TLR9 at intracellular level and it colocalizes with cytoplasmic DNA. Treatment with mtDNA alone did not affect NF-kB activation; the co-stimulation with of LPS increased the nucleus/cytoplasm ratio of NF-kB, suggesting that mtDNA acts as proinflammatory molecule only with additional proinflammatory stimuli. Monocytes from elderly subjects show impaired immunometabolic responses. The ability of mtDNA to promote an inflammatory response is conserved in elderly subjects. However, mtDNA alone did not trigger significant monocyte activation in these subjects.

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P10 | Unraveling the pathogenetic mechanism of a new Lonp1 mutation causing CODAS syndrome

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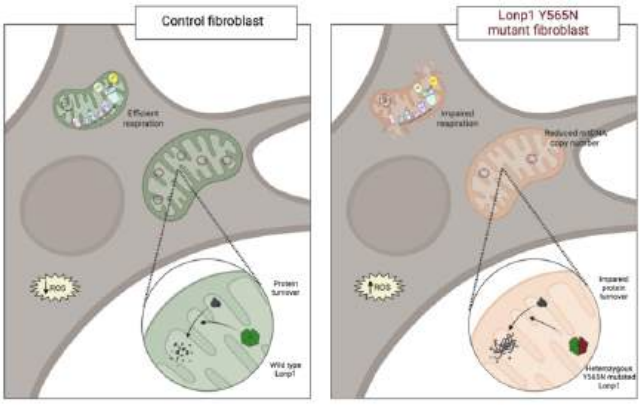

Lonp1 is a mitochondrial protease encoded by nuclear DNA essential for organelle homeostasis and cell response to stress. Pathogenic mutations of Lonp1 can cause CODAS syndrome, characterized by Cerebral, Ocular, Dental, Auricular, and Skeletal anomalies. Reported symptoms show a high degree of variability, all characterized by impaired development of multiple organs and tissues. Here, we present a case study of a four-year-old girl with a new heterozygous Y565N mutation in LONP1 gene, which has not yet been classified as causative of CODAS syndrome. However, this mutation results in a phenotype that is indicative of the typical characteristics seen in CODAS patients. To better understand the functional consequences of this mutation, we first evaluated in silico the structural consequences of the mutations and found that the Y565N substitution likely compromised the ability of the protease to bind its substrates. Then, we performed morphological and functional analysis of mitochondria in fibroblasts from a skin biopsy of the patient and compared them to fibroblasts from a healthy donor, matched for sex and age. LONP1 protein levels showed no significant differences compared to control. However, fibroblasts from the patient showed a marked reduction in OCR, particularly as far as maximal respiration rate is concerned, and an increase of mitochondrial superoxide anion production, in line with possible alterations of respiratory chain complexes. Mitochondrial morphology analysis showed fewer mitochondria with smaller volumes and less branching compared to the control. Proteomic analysis confirmed significant alterations in expression level of mitochondrial proteins - particularly respiratory electron transport chain complexes – in agreement with chaperon and quality control role of LONP1. Taken together, these observations suggest that this newly identified mutation is likely pathogenic, and compromises some mitochondrial functions, leading to cellular impairment.

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P11 | Mitochondrial rescue in Amyotrophic lateral sclerosis: the power of VDAC1 overexpression

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Mitochondrial dysfunction is a hallmark of Amyotrophic lateral sclerosis (ALS) and contributes significantly to the disease progression. Voltage-Dependent Anion-selective Channel 1 (VDAC1) is the most abundant pore-forming protein of the outer mitochondrial membrane (OMM) and allows the passive diffusion of ions and small hydrophilic molecules such as ATP/ADP, NAD^+/NADH and Krebs' cycle intermediates [1]. In ALS, the function of VDAC1 is impaired by the accumulation on the OMM of toxic aggregates of misfolded forms of Cu/Zn Superoxide Dismutase (SOD1). Overexpression of the SOD1-G93A mutant is indeed associates with mitochondrial structural abnormalities, reduced import of proteins and mitochondrial substrates into the organelle, and inhibition of respiratory complexes activity and ATP production [2]. In this study, we evaluated the impact of VDAC1 upregulation on mitochondrial dysfunction. Exactly, we injected into the spinal cord of new-born SOD1-G93A mice an adeno-associated virus (AAV) expressing the VDAC1 sequence under the control of a neuronal promoter. Our findings demonstrate that VDAC1 overexpression restored the mitochondrial respiratory profiles of 15 weeks old ALS mice up to the same levels of wild-type mice. This recovery correlates with an increase in the expression levels and activity of key proteins that positively regulate mitochondrial functioning. In particular, respiratory chain Complex I and SIRT3, as well as proteins involved in the activation of a mitochondrial quality control mechanism, such as Tom20, a crucial component of the outer membrane translocase (TOM) complex, were overexpressed [3]. Overall, these results underline the potential therapeutic relevance of VDAC1 upregulation as a strategy to counteract mitochondrial dysfunction in neural tissues impaired by ALS.

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P12 | Aberrant expression of cell cycle genes is associated with mitochondrial dysregulation in the APP/PS1 mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by cognitive decline, synaptic loss, and neuronal death. While hallmark pathologies such as amyloid-β and tau accumulation are well established, growing evidence suggests that abnormal reactivation of the cell cycle in post-mitotic neurons also contributes to disease progression. However, the molecular mechanisms linking cell cycle re-entry (CCR) to neurodegeneration remain poorly understood. To investigate this, we performed gene expression profiling of cell cycle–related transcripts in the brains of 18-month-old wild-type and APP/PS1 mice, a widely used model of AD. Gene expression analysis was carried out using the RT2 Profiler PCR Array for Cell Cycle genes (Qiagen). We identified multiple differentially expressed genes in APP/PS1 mice, including Ccnb2, Ccnc, Cdc7, Cdkn2b, Mcm4, Tfdp1, Rad51, Nbn, Ppm1d, Shc1, and Cdk5rap1. Functional annotation revealed that beyond their canonical roles in cell cycle regulation, many of these genes are implicated in mitochondrial function, oxidative stress, and apoptotic signaling. Notably, p66Shc1, Ccnc (Cyclin C), and Cdk5rap1 are known to localize to mitochondria and modulate mitochondrial dynamics, reactive oxygen species production, and mitochondrial tRNA modifications. Our preliminary findings suggest that cell cycle reactivation in neurons is associated with changes mitochondrial integrity and promotes oxidative stress, providing a mechanistic link between aberrant mitotic signaling and neurodegeneration in AD. Further studies will help clarify whether these pathways represent viable targets for therapeutic intervention.

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P13 | Tumor-specific expression of VDAC1 and its pseudogene VDAC1P8

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VDAC1, the main isoform of voltage-dependent anion channels (VDACs), is a key regulator of mitochondrial bioenergetics and apoptosis [1]. Increasing evidence highlights a regulatory role for non-coding RNAs (ncRNAs) and pseudogenes in modulating gene expression [2]. Among VDAC1-related pseudogenes, VDAC1P8 emerges as a transcribed non-coding element with potential regulatory function. To investigate its relevance in cancer, we performed transcriptomic analyses on human samples of colorectal cancer (CRC), matched normal colonic mucosa, and acute myeloid leukemia (AML), as well as on corresponding CRC and AML cell lines [3,4]. Our results confirm a trend observed in several tumors: VDAC1 is overexpressed in CRC tissues compared to healthy mucosa, whereas VDAC1P8 is transcribed at low levels. In contrast, AML displays an opposite pattern, with VDAC1 downregulated and VDAC1P8 upregulated compared to healthy controls. This inverse correlation suggests a potential suppressive role of VDAC1P8 on its parental gene in AML [5]. Interestingly, VDAC1P8 is minimally expressed in CRC cell lines, while highly expressed in AML cell models, reinforcing its cancer-type specificity. These findings propose a novel RNA-based mechanism controlling mitochondrial metabolism in cancer, where pseudogene-derived transcripts may act as modulators of metabolic gene expression.

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P14 | Gender differences in sera biomarkers of Parkinson's disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder; men are more likely to develop it, but women face faster progression and higher mortality [1]. The dominant pathological signature is the death of dopaminergic neurons of Substantia Nigra pars compacta above all due to synuclein aggregates [2]. The pathogenic mechanisms underlying Parkinson disease has not been fully disclosed as well as diagnosis is currently based on clinical evidence. For these reasons, identification and characterization of new circulating biomarkers, in a gender-medicine context, is necessary for appropriate diagnosis and therapy [3]. It is known that the accumulation of misfolded proteins, especially fibrillar aggregates of alpha-synuclein, induces an increase in oxidative stress. In order to understand if there is a different profile and/or a different expression of biomarkers indicative of oxidative stress and inflammation, we performed a study on sera from male and female patients affected by PD. In the sera examined, no difference in reactive species and antioxidant capacity was observed; however, the analysis of the expression of some oxidative stress biomarkers shows differences that could be at the basis of different molecular mechanisms underlying the control of redox balance.

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P15 | Tracking metabolic changes to identify new vulnerabilities in melanoma cells

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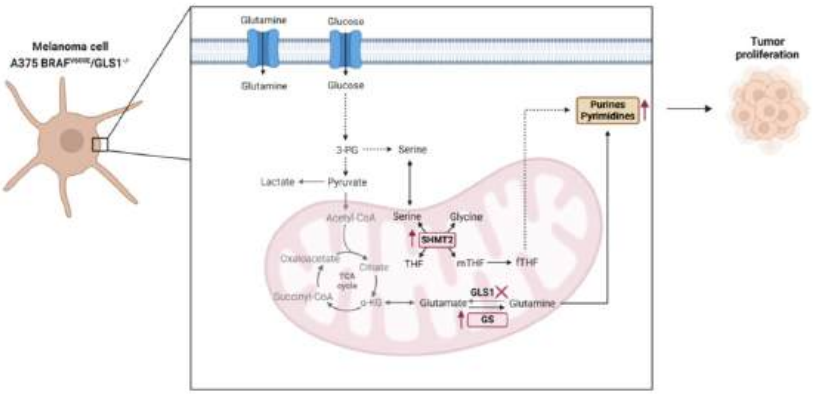

Melanoma is the most aggressive and treatment-resistant form of skin cancer with an inherent metabolic plasticity due to the upregulation of the MAPK pathway triggered by mutations such as BRAFV600E. It has been shown that melanoma cells rely on glutamine (Gln) utilization to sustain tumor growth, the acquisition of drug resistance and to support the metastatic program. Given its key role in cancer development, Gln metabolism is considered a promising therapeutic target, although the high metabolic plasticity of melanoma cells makes drug-resistance a perpetual challenge. Thus, tracking metabolic changes in melanoma cells lacking glutaminolysis is essential to identify new metabolic vulnerabilities. By employing metabolomic and transcriptomic analyses, along with in vitro tumorigenic assays, we investigated the metabolic adaptations occurring in melanoma cells lacking Glutaminase 1 (GLS1), the mitochondrial enzyme responsible for converting Gln into glutamate. Our findings reveal that GLS1-deficient cells undergo a metabolic shift aimed at producing glutamate, which is subsequently used for de novo Gln synthesis. Additionally, GLS1-deficient cells exhibit elevated levels of both serine and glycine, accompanied by the upregulation of SHMT2, the mitochondrial enzyme that catalyzes the conversion of serine into glycine. This interconversion generates a significant pool of one-carbon units that fuel one-carbon metabolism, ultimately supporting cell growth through the synthesis of purine and pyrimidine nucleotides. Consistently, GLS1-deficient cells accumulate intermediates of purine and pyrimidine biosynthesis while maintaining a proliferation rate comparable to that of parental cells. Interestingly, impairing serine and glycine synthesis leads to a marked reduction in the tumorigenic potential of GLS1-deficient cells. Altogether, these results unveil a novel metabolic regulatory mechanism governed by GLS1 and SHMT2, which could provide a foundation for developing a synthetic lethality approach to treat melanoma.

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P16 | Investigating the crosstalk between Schwann cells and macrophages in peripheral nerve sheath tumors

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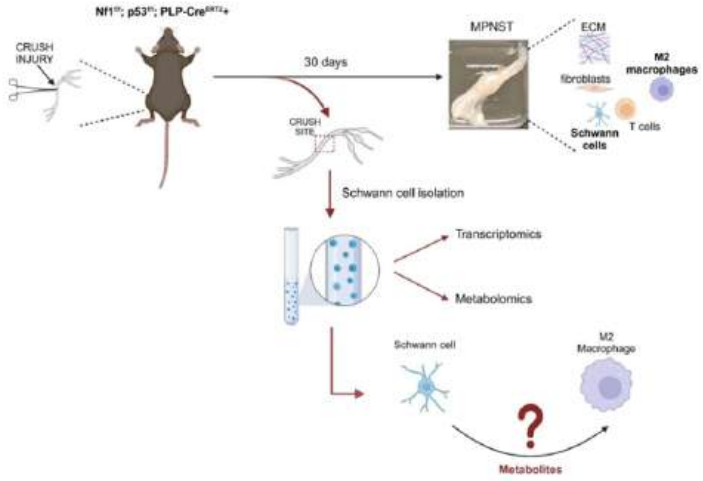

Malignant peripheral nerve sheath tumors (MPNSTs) are a rare group of incurable aggressive sarcomas. Half of these tumors arise in patients affected by Neurofibromatosis type 1 (NF1) while the others are sporadic, with a small percentage in patients with an history of radiotherapy. NF1 is an autosomal and multisystem disorder, characterized by negative mutations in the neurofibromin gene, a Ras-inhibiting enzyme, which lead to an up-regulation of the MAPK and the PI3K-AKT-mTOR pathways. The tumor microenvironment of MPNSTs is complex, comprising not only neoplastic Schwann cells (SCs), but also fibroblasts, neurons, endothelial cell, extracellular matrix components and immune cells. Tumor Associated Macrophages (TAMs), cells recently identified as important players in the growth of many cancers, represent the most abundant immune infiltrate of MPNSTs. Previous work in the laboratory has revealed that macrophages exposed to the conditioned media of MPNST cells undergo an anti-inflammatory, M2-like, phenotype accompanied by pro-tumoral functions. The aim of my project is to study the metabolic-based crosstalk between neoplastic SCs and TAMs during the development of MPNSTs. To do so, I take advantage of a genetically engineered mouse model in which Nf1 and p53 genes are specifically inactivated in the SC population. By performing a crush injury on the sciatic nerve of this mouse we trigger the development of neoplastic lesions compatible with MPNSTs in a synchronized and robust manner. Our plan is to investigate the transcriptomic and the metabolomic profile of neoplastic SCs isolated at different time points following nerve injury and before the onset of the tumor. In this way we aim to identify metabolic routes that could impinge on macrophage pro-tumoral polarization and function. This work has the final goal of shedding light on key intercellular crosstalk mechanisms that contribute to MPNST progression and that could be potentially targeted.

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P17 | The mitochondrial protein Fis1 and its interactors Drp1 and Tbc1d15 are essential to sustain Acute Myeloid Leukemia stem cells

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The poor survival rate of Acute myeloid leukemia (AML) has been linked to the existence of difficult to eradicate leukemic stem cells that give rise to the leukemic blasts that constitute the bulk of the tumor. Leukemic stem cells (LSCs) rely on oxidative phosphorylation and display increased levels of the outer mitochondrial membrane protein Fission 1 (Fis1) that promotes fission of the organelle depending on Drp1 and mitophagy by interacting with TBC1D15 and ultimately sustains the renewal of LSCs. To address how Fis1 upregulation promotes LSCs self renewal, we downregulated Fis1, Drp1, and TBC1D15 in the p53 proficient AML cell lines THP1, MOLM13, OCI-AML3, and in the p53 deficient NB4 cells. These cells in culture are normally composed of a fraction of LSCs and of a larger one of differentiated blasts with feature of myeloid and macrophages lineages. Flow cytometry assessment of the pan myeloid cell differentiation marker CD11b and of the M1 macrophages marker CD86 revealed that both were increased in all the cell lines following silencing of Fis1, but not of Drp1. Thus, our data indicate that Fis1 but not mitochondrial fission is required for LSCs maintenance and nominate Fis1 as a potential target to deplete AML of LSCs.

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P18 | Investigating the role(s) of F-ATP synthase subunits e and g in *Drosophila melanogaster*

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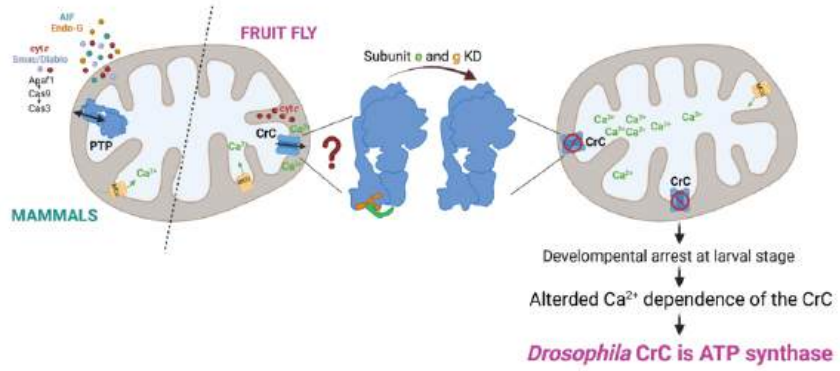

The mitochondrial permeability transition (PT) is an increase in the permeability of the inner mitochondrial membrane (IMM) mediated by the Ca^{2+}-dependent opening of a high conductance (max 1.2 nS), unselective channel, the permeability transition pore (PTP), that allows ions and solutes up to 1.5 kDa to equilibrate across the IMM. PTP can function as a regulator of the cellular Ca^{2+} homeostasis (short-openings of the channel), or it can initiate cell death (long-lasting openings) by inducing mitochondrial matrix swelling. Interestingly, *Drosophila melanogaster* represents a special case. Indeed, its "PTP" is not involved in matrix swelling and cell death but appears to operate as a selective Ca^{2+}-release channel (CrC), leading to the hypothesis that it uniquely regulates Ca^{2+} homeostasis. It has been shown that mammalian ATP synthase mediates the PT and that ablation of e and g subunits of the enzyme affects PT occurrence, suggesting a primary role of these proteins in PTP formation. *Drosophila* ATP synthase generates a peculiar 53 pS-channel that could represent the CrC. To study the roles of the two subunits in *Drosophila*, and to test whether the CrC of *Drosophila* could be mediated by the ATP synthase, we generated knock-down (KD) lines for subunit e (ATPsynE) or g (ATPsynG) of the enzyme. *In vivo* ubiquitous downregulation causes an arrest in fly development at larval stage, impairs the ATP synthase dimerization and oligomerization, decreases mitochondrial respiration and cristae formation, yet total ATP is unaltered. Strikingly, the sensitivity to Ca^{2+} is decreased in KD mitochondria, which require higher matrix Ca^{2+} loads to induce the CrC. Altogether, our results show that the Drosophila ATP synthase can turn into the CrC and that ATPsynE and ATPsynG play a key role in the formation of the channel. Notably, we speculate that the failure to thrive of KD larvae cannot be entirely due to bioenergetic defects, but may also arise from a CrC-related Ca^{2+} dysregulation.

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P19 | Computational and electrophysiological studies of the Envelope protein from SARS-CoV-2

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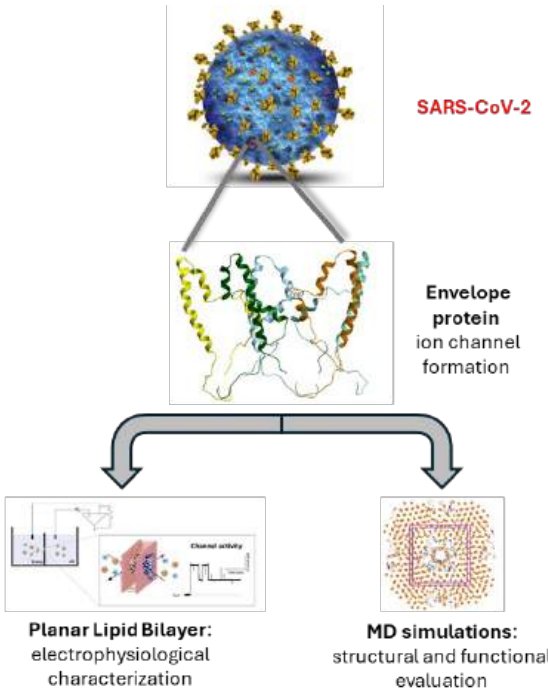

The SARS-CoV-2 Envelope (E-2) protein is a 75-residues viroporin involved in several phases of the viral life cycle that has recently emerged as a potential pharmacological target. Structural data based on NMR initially suggested E-2 self-assembles into a homo-pentameric channel where the long transmembrane (TMD) helices create a tight bundle with a supposed central water pore. Since this model was inconsistent with ion transport, we investigated the structure and the channel activity of E-2 combining electrophysiology experiments and Molecular Dynamics (MD) simulations. Accordingly, we exploited the Planar Lipid Bilayer technique to characterize the channel properties of recombinant E-2 obtained from the cDNA of a positive patient. Current measurement, performed in symmetrical $CaCl_2$/KCl solutions upon application of a constant voltage of ±100mV, revealed E inserts into artificial membrane as a multimer with cation selectivity. We also determined the 3D-structure of E-2 by homology modelling and subsequently tested this structure into a biomolecular complex consisting of lipid membranes, water molecules and charged ions (Ca^{2+}, K^+, Cl^-). MD trajectory files showed that the multimeric structure of E-2 collapses into a non-conducting conformation upon removal of spatial constraints, suggesting that the modelled structure might be inaccurate. Hence, a new channel structure was obtained by inverting the orientation of the hydrophilic C-terminus. Strikingly, new MD trajectory files showed a greater flux of ions across the pore. In addition, MD simulations suggested the existence of a potential Ca^{2+} binding site located within the C-terminal domain, mainly involving residue D72 in each monomer. In-depth studies are still required to fully define the role of calcium in the gating mechanism of the channel.

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P20 | Effect of lipids on the function and kinetics of the ASC-1 serine transporter

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The human ASC-1 (SLC7A10) transporter plays a pivotal role in neutral amino acid homeostasis in neurons, astrocytes and adipose tissues where it is mainly expressed. ASC-1 is physiologically involved in learning and memory processes, and lipid metabolism. In good agreement, defects of this transporter are linked to neurological and metabolic pathologies. Recently, we produced the human recombinant ASC-1 in E. coli and purified the protein to homogeneity by a procedure based on on-column detergent substitution for protein refolding. The purified protein was reconstituted into proteoliposomes for biochemical characterization [1]. In this work, we studied the effect of cholesterol on the transport function of ASC-1. The incorporation of cholesterol into the proteoliposome bilayer strongly increased the ASC-1 mediated uptake of 3H-L-serine into the vesicles. Optimum of transport activity was observed at a cholesterol/phospholipid ratio of 1/5 (w/w). Kinetic analyses, under cholesterol-enriched conditions, revealed no effect on the Km for L-serine, whereas an increase in the apparent Vmax for L-serine was measured. These results suggested a potential effect of cholesterol on the conformational changes at the basis of the transport cycle. Then, the stability of ASC-1 was studied in intact cell (HEK293) membranes with thermal shift assay; the Tm of the protein was altered by cholesterol sequestration induced by methyl cyclodextrin treatment or by substitution of cholesterol by 7-keto-cholesterol, a natural auto-oxidized form of the lipid. These findings underscore the significant impact of membrane composition on ASC-1 activity. The methodologies developed here pave the way for further investigations on the effects of lipid microenvironments on the functionality of membrane proteins.

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P21 | Adenine nucleotide translocator and ATP synthase cooperate in mediating the mitochondrial permeability transition

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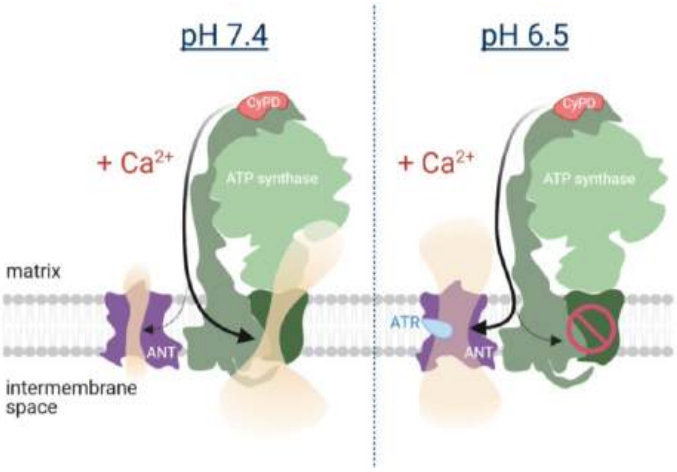

The permeability transition (PT) pore (PTP) is Ca^{2+}-dependent channel which increases the mitochondrial inner membrane permeability causing matrix swelling. PTP can be generated by at least two components, the adenine nucleotide translocator (ANT) and the ATP synthase, and whether these provide independent permeation pathways remains to be established. To assess the ANT contribution to the PT, we tested the effects of ANT specific ligands, bongkrekate (BKA) and atractylate (ATR), which inhibits and activates the PT, respectively. BKA only partially inhibited the Ca^{2+}-dependent swelling in mouse liver mitochondria, while did not prevent the inducing effect of the thiol reagent, phenylarsine oxide, that still promotes PT occurrence in mouse embryonic fibroblasts deleted for all ANT isoforms. At pH 6.5 the ANT contribution emerges in presence of ATR. Unexpectedly, at this pH the ANT-dependent PT could also be stimulated by benzodiazepine-423, a selective ligand of ATP synthase, suggesting that the ANT pore is regulated by the peripheral stalk of ATP synthase. In agreement with docking simulations, ANT co-immunoprecipitated with ATP synthase subunits c and g, and oligomycin, binding adjacent c subunits, decreased the ANT association with subunit c. These results reveal a close cooperation between ANT and ATP synthase in the PT. We are currently investigating the precise sub-mitochondrial localization of this interaction using expansion microscopy, which allows to perform a high-resolution imaging expanding samples of four-fold. Cells are labelled with specific fluorescent antibodies to mark ANT and ATP synthase subunits to reveal the possible microdomains of interaction within a mitochondrion. These experiments may elucidate whether and where specific pools of ANT and ATP synthase form complexes that play a role in the PT.

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P22 | VDAC1 antagonist molecules trigger profound alterations in channel electrophysiology

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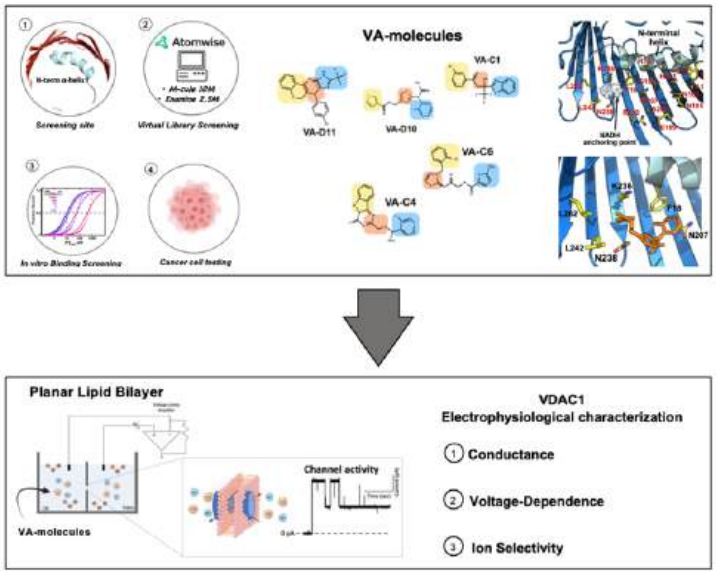

The Mitochondrial metabolism relies on the continuous and efficient exchange of ATP/ADP, NAD^+/NADH and other metabolites through the Voltage-Dependent Anion-selective Channel 1 (VDAC1), the most abundant β-barrel protein of the outer membrane. In many cancers, VDAC1 also drives the metabolic rewiring aimed at promoting the "Warburg effect", coupling glycolysis to oxidative phosphorylation. Based on the above, a set of novel VDAC-antagonists (VA) have been recently identified by AI-based screening. VA molecules selectively bind the NADH-anchoring pocket on VDAC1 by hampering mitochondrial respiration, disrupting cell metabolism in cancer-derived cell lines and organoids exclusively [1]. Though, whether and how these drugs directly affect VDAC1 ion channel properties and/or modulate its permeability have remained unexplored. To address this issue, we here provide a detailed biophysical characterization of VA-molecules/VDAC1 interaction using the planar lipid bilayer technique. Our results indicate that all five VA molecules hampered the voltage gating of the channel reconstituted into an artificial membrane and concurrently helped to stabilize the "open" state, by altering the flexibility of the N-terminal α-helix that forms the NADH-anchoring pocket by facing the β-barrel lumen. Furthermore, our drugs induced a thorough loss of cation preference in the closed states. In conclusion, these results provide new insight into the action of VA molecules, leading to the further optimization and development of novel therapeutic avenues for the treatment of drug-resistant tumors and may increase awareness of the VDAC voltage-gating mechanism, which has not yet been fully elucidated.

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P23 | UCP2 and UCP3: fraternal twins with distinct metabolic functions

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Uncoupling proteins 2 and 3 (UCP2 and UCP3) are the closest homologs of UCP1, the well-characterized uncoupling protein responsible for non-shivering thermogenesis in mammals. UCP2 and UCP3 have 70% protein sequences identity. Recombinant UCP2 and UCP3 reconstituted into liposomes transport nearly identical substrates such as aspartate, malate, oxaloacetate, phosphate, and sulfate. Nevertheless, both proteins differ markedly in their transport mode and kinetic constants: UCP3 functions strictly as an exchanger, whereas UCP2 can transport substrates unidirectionally. While the two proteins exhibit comparable affinities for most substrates, UCP3 displays a markedly higher affinity (7-8) for aspartate. Mitochondrial swelling assays in yeast expressing UCP2 or UCP3 revealed that UCP2 catalyzes a proton-coupled symport of phosphate, whereas UCP3 exchanges aspartate for malate plus a proton. This exchange activity by UCP3 was further confirmed through complementation in a yeast model lacking aspartate-malate shuttle grown on oleate. In this model, UCP2 supported only the exchange of aspartate for phosphate plus a proton. Neither protein catalyze malate/oxaloacetate exchange. The complementation studies also clarified the directionality of the exchange reactions demonstrating that aspartate is exported from the mitochondria. Although both UCP2 and UCP3 mediate aspartate export, UCP2's activity leads to a depletion of C4 intermediates in the mitochondrial matrix. This shift reduces the oxidation of acetyl-CoA–producing substrates like glucose and fatty acids, while enhancing glutamine metabolism. In pancreatic ductal adenocarcinoma, UCP2 drives the export of glutamine-derived aspartate, which is utilized in the cytosol to generate NADPH—crucial for maintaining redox balance and supporting cell proliferation. On the other hand, although an aspartate/malate exchange activity has been suggested in skeletal muscle for managing amino acid metabolism, further studies are necessary to dissect the physiological function of UCP3 in this tissue.

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P24 | Defining the mechanism of channel formation by ATP synthase

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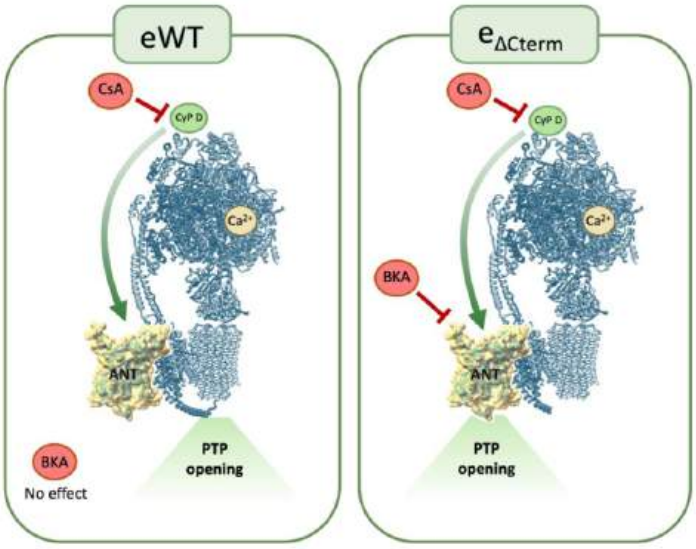

The Permeability Transition (PT) is a mitochondrial permeability increase to ions and solutes caused by the opening of a Ca^{2+} and voltage-dependent channel, the PT pore (PTP). The long-standing debate about the PTP molecular identity has been recently challenged by a substantial set of data reporting a key role of the ATP synthase and the adenine nucleotide translocator (ANT). In presence of Ca^{2+}, the ATP synthase generates high conductance channels with biophysical properties matching those of the PTP. However, in absence of the dimerization subunits e and g, the enzyme can no longer generate a channel and the PT can eventually be mediated by the ANT which represents an alternative permeation pathway. In spite of the certain involvement of e and g subunits, the mechanism of channel formation by the ATP synthase is still elusive. Recent high-resolution structures of the ATP synthase reported a physical link between e subunit C-terminus, and in particular its terminal lysine (K69), and the lipids filling the rotor (c-ring). Additionally, Ca^{2+}-dependent conformational changes affecting this region were reported. We hypothesize that subunit e C-terminus modulates c-ring occupancy by lipids via a push-pull mechanism, determining channel formation within the rotor. To test this hypothesis, we generated HeLa cells expressing a point mutation in the e subunit terminal lysine (eK69) which should impair the connection of the subunit with the lipids, and a C-terminal truncated form of subunit e devoid of the last amino acids. In this scenario, ANT contribution on the PT emerges, indicating that the channel forming ability of the ATP synthase is compromised.

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P25 | The mitochondrial aspartate-glutamate carrier is a metabolic sensor orchestrating organelle morphology and cristae maintenance

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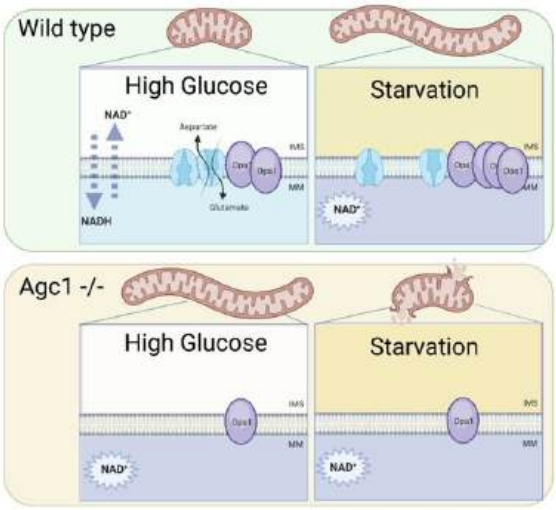

Mitochondria adapt to low nutrient availability by elongating and expanding cristae surface [1]. However, the molecular mechanisms that link cellular metabolism to mitochondrial dynamics remain poorly understood. Through MS-based complexomic screening of heart mitochondria [2], we identified the Ca^{2+}-sensitive mitochondrial aspartate-glutamate carrier (Agc1) as a potential Opa1 interactor, a mitochondrial shaping-protein essential for mitochondrial fusion and cristae maintenance. Agc1 functions as part of the malate-aspartate shuttle (MAS) which delivers glycolysis-derived NADH to mitochondria in its dimeric form. Biochemical and bimolecular fluorescence complementation assays confirmed the Agc1-Opa1 direct interaction. By profiling the dimerization status of Agc1 in response to individual carbon sources, we found that Agc1 dimerizes when cells are fed with high levels of glucose, suggesting that its dimerization is driven by elevated levels of glycolysis derived NADH. In Agc1-deficient cells, mitochondrial NADH levels remained low even in nutrient-rich conditions, Opa1 oligomerization was reduced, and cristae were deranged. Reintroduction of dimerization-deficient and Ca^{2+}-insensitive mutants of Agc1 in $Agc1^{-/-}$ cells resulted in enhanced Opa1 oligomerization, indicating that monomeric Agc1 and low mitochondrial NADH levels favor Opa1 oligomerization. During starvation, Agc1 monomerized, reducing the transfer of cytosolic NADH molecules to mitochondria, thereby promoting Opa1 oligomerization and cristae expansion. Moreover, starvation of $Agc1^{-/-}$ cells resulted in the rapid disruption of cristae ultrastructure, accompanied by reduced Opa1 oligomerization, suggesting that Agc1 is required for starvation induced Opa1 oligomerization and cristae biogenesis. Our data reveal a previously unappreciated role for Agc1 in relaying changes in fuel availability to mitochondrial cristae remodeling via its dimerization state and interaction with Opa1.

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[2] G. Rigoni et al. *Cell Metab* (2025), 37: 1024-1038.

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P26 | Fully synthetic C-subunit of the ATP synthase spontaneously assembles into the β-sheet oligomers and forms voltage-gated porin-like channels in the planar lipid bilayers

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The c subunit is an inner mitochondrial membrane (IMM) protein encoded by three nuclear genes. Best known as an integral part of the F_0 complex of the ATP synthase, the c subunit is also present in other cytoplasmic compartments in ceroid lipofuscinoses. Under physiological conditions, this 75 residue-long peptide folds into an α-helical hairpin and forms oligomers spanning the lipid bilayer. In addition to its physiological role, the c subunit has been proposed as a key participant in stress-induced IMM permeabilization by the mechanism of calcium-induced permeability transition. However, the molecular mechanism of the c subunit participation in IMM permeabilization is not completely understood. Here we used fluorescence spectroscopy, atomic force microscopy and black lipid membrane methods to gain insights into the structural and functional properties of unmodified c subunit protein that might make it relevant to mitochondrial toxicity. We discovered that c subunit is an amyloidogenic peptide that can spontaneously fold into β-sheets and self-assemble into fibrils and oligomers in a Ca^{2+}-dependent manner. C subunit oligomers exhibited ion channel activity in lipid membranes. We propose that the toxic effects of c subunit might be linked to its amyloidogenic properties and are driven by mechanisms similar to those of neurodegenerative polypeptides such as Aβ and α-synuclein.

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P27 | Structural characterization of intra- and intermolecular disulfide bonds in VDAC proteins from *Rattus norvegicus* (rVDACs) by high resolution mass spectrometry

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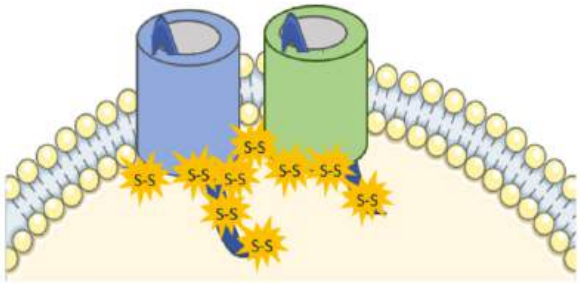

Voltage-Dependent Anion Channels (VDACs) are a relevant component of the mitochondrial proteome. VDACs, which are located in outer membrane, mediate the exchange of ions and metabolites between cytosol and mitochondrion, ensuring good functionality of bioenergetics complexes and ATP production. Despite high sequence homology and high structural similarity, VDACs show different tissue expression, variable abundance, difference in cysteine content, and are characterized by distinct and specific interactome partners. This work is part of a line of research concerning the structural characterization of VDAC proteins. We have reported the pattern of cysteine oxidation in rat and human VDACs using "in solution" proteolysis and nUHPLC/HR nESI-MS/MS, a procedure originally developed by us [1]. Recently, using a modification of this experimental protocol to minimize disulfide reshuffling, the position of intramolecular disulfide bonds in the rVDAC2 isoform has been studied [2]. In this work, the same procedure was applied to characterize intra- and intermolecular disulfide bonds in rVDAC3. As a result, 3 intramolecular and 7 intermolecular disulfide bonds between rVDAC3 with rVDAC1 and rVDAC2 isoforms were unequivocally identified. Further studies to localize all disulfide bonds in VDACs and to acquire their biological significance are in progress.

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P28 | Mapping the interactome of SLC25A36 using TurboID-Based Proximity Labeling in human cells reveals mitochondrial and metabolic functional networks

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SLC25A36 is a mitochondrial carrier protein primarily recognized for its role in the transport of pyrimidine nucleotides [1]. Despite its known function, the landscape of its physiological interactors remains largely unexplored. Identifying protein-protein interactions in live cells poses significant challenges, as conventional methods often fail to capture weak, transient, or spatially and temporally regulated interactions. Consequently, these limitations lead to an incomplete understanding of the dynamic interaction networks crucial for cellular function. To overcome these obstacles, we employed a proximity-dependent biotinylation strategy, TurboID, which enables the labeling of proteins in the immediate vicinity of the protein of interest under physiological conditions [2]. SLC25A36 was fused with TurboID at both its N- and C-termini and transiently expressed in human cells. Upon biotin supplementation, neighboring proteins were biotinylated, affinity-purified via streptavidin beads, and identified by mass spectrometry. Bioinformatic analyses included GO, KEGG, Reactome pathway enrichment, subcellular localization, and assessments of clinical relevance. This study provides a foundational understanding of the SLC25A36 proximal protein network in live cells and underscores its potential roles in mitochondrial homeostasis and metabolic regulation. These insights may inform future investigations into the molecular mechanisms underlying mitochondrial diseases and metabolic disorders.

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P29 | Exploring the mechanism accounting for Kv1.3-STAT3 interaction and consequent alterations of cancer-relevant pathways

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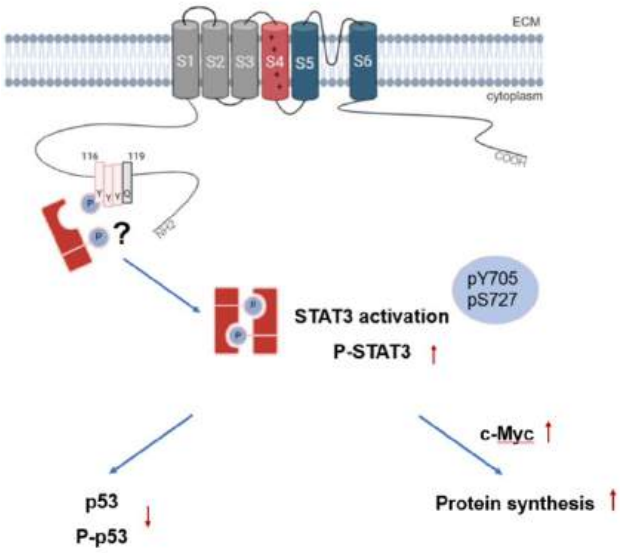

Kv1.3, a member of the Shaker Kv1 channel family, has wide expression in excitable and non-excitable cells and it is involved in different physiological processes. Aberrant expression of Kv1.3 has also been correlated to many pathological states, among them in cancer. In this context, other than ion flux regulation, cytoplasmic N and C-terminal domains participate in cellular signaling through protein-protein interactions. Recently, STAT3 transcription factor was discovered as one of the proteins that interact with Kv1.3. STAT3 is part of an important intracellular pathway, leading to regulation of gene transcription. STAT3 activation has been discovered in majority of human tumor tissues and malignant cell lines. STAT3 harbors Src homology 2 (SH2) domain that potentially binds to the SH2 recognition site of the channel causing their stabilization and activation. Therefore, we investigated this aspect performing experiments that involved generation of Kv1.3 SH2 binding domain mutant channels and addressed whether this mutation disrupt the potential interaction between the channel and STAT3 and prevents STAT3 stabilization.

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P30 | *Pseudomonas aeruginosa* sulfide:quinone oxidoreductase is a key enzyme in H_2S detoxification

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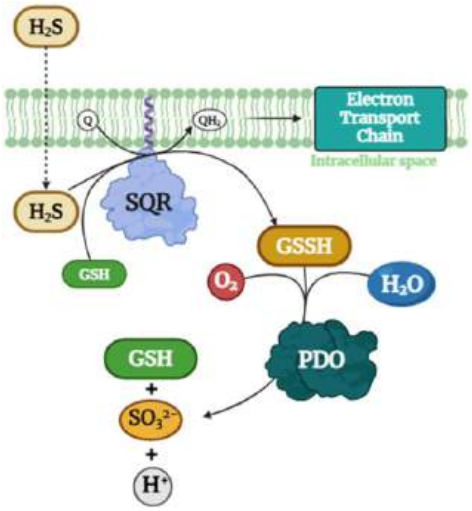

Pseudomonas aeruginosa (Pa) is a multidrug-resistant pathogen that poses a significant threat to human health. It can survive in very harsh conditions, such as a sulfide-rich environments. Hydrogen sulfide (H_2S) is toxic at high levels, while being an important signaling molecule at low physiological concentrations [1]. Recent studies have highlighted that, thanks to its sulfide-insensitive bd-type Cyanide Insensitive Oxidase, Pa can survive and respire O_2 even in the presence of elevated levels of H_2S [2]. As a further strategy to cope with sulfide toxicity Pa expresses a multienzymatic unit involved in H_2S conversion to sulfite and (thio)sulfate, which comprises two isoforms of sulfide:quinone oxidoreductase (SQR), converting H_2S and reduced glutathione (GSH) to glutathione persulfide (GSSH), and a persulfide dioxygenase. Here, we biochemically characterized the isoform 2 of PaSQR. We performed detailed kinetic analyses of this enzyme, using static and time-resolved UV-vis absorption spectroscopy. Then, we screened other possible sulfide acceptors (cysteine, N-acetyl cysteine, thiosulfate) through differential scanning fluorimetry, and the most promising substrate candidates will be used for more in-depth kinetic characterizations. Finally, sulfide utilization by PaSQR2 was measured by high-resolution respirometry coupled with H_2S amperometry. Overall, our findings suggest that PaSQR is a key enzyme in H_2S detoxification, thus possibly protecting Pa against sulfide poisoning.

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P31 | Mitochondrial dysfunction and oxidative stress in CANVAS: insights from patient-derived fibroblasts

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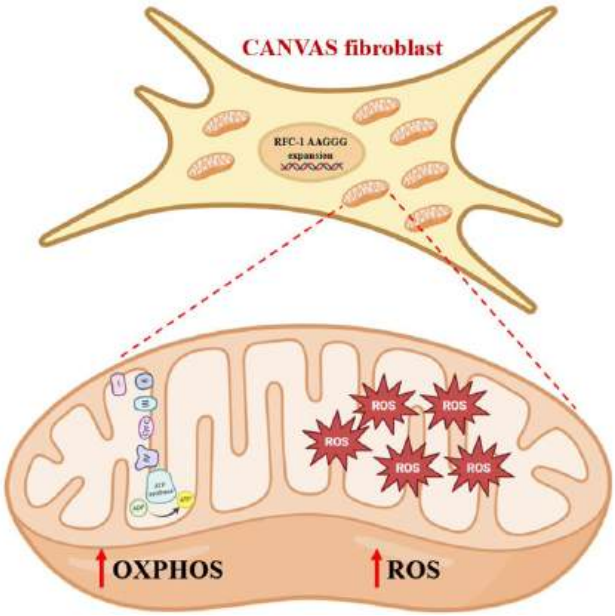

Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome (CANVAS) is a slowly progressive, multisystem neurodegenerative disorder with an autosomal recessive inheritance pattern. It is predominantly associated with biallelic expansion of the AAGGG repeat in intron 2 of the RFC1 gene and usually manifests in late middle age, with most patients experiencing worsening motor instability, oscillopsia, difficulty swallowing, speech impairment, and peripheral sensory neuropathy [1]. Despite extensive research, the pathogenic mechanisms underlying CANVAS remain unclear. In this study, we investigated the molecular mechanisms underlying the clinical manifestations, with a focus on mitochondrial dysfunctions as potential drivers of the pathology. Analyses were carried out in primary fibroblasts derived from skin biopsies of six RFC1-ataxia patients and age-matched healthy controls. Our findings showed no significant differences in mitochondrial mass and oxidative phosphorylation (OXPHOS) complexes level between patient and control fibroblasts. However, the basal oxygen consumption rate (OCR) was found to be higher in CANVAS than in control cells. This observation pushed us to evaluate the level of both reactive oxygen species (ROS) and cellular antioxidant defenses. Preliminary data suggest a dysregulation of antioxidant enzyme levels. These results provide novel insights into the molecular alterations associated with CANVAS and support the hypothesis that oxidative stress contributes to disease onset and progression.

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P32 | ATAD3 Protein Family members orchestrate mitochondrial translation with Metabolic Reprogramming

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The genetic mechanisms by which mitochondrial function regulates cellular metabolism are still not fully understood. Using a combination of custom bioinformatic tools and complexomic, genetic, and metabolic analyses, we uncovered a key role for the ATAD3 protein family in directing cell fate through the regulation of mitochondrial translation. Specifically, ATAD3A promotes oxidative phosphorylation by enhancing mitochondrial cristae formation and stabilizing mitoribosomes. In contrast, its paralog ATAD3B inhibits mitochondrial translation, shifting metabolism toward glycolysis. This functional divergence has important physiological implications: although ATAD3B is absent in differentiated cells, it is re-expressed in highly glycolytic, chemoresistant tumors such as triple-negative breast cancer (TNBC). This re-expression underscores ATAD3B's critical role in mitochondrial and cellular metabolism. Moreover, we have identified the specific domain of ATAD3B responsible for this metabolic effect, suggesting a potential therapeutic target. Overall, the balance between ATAD3A and ATAD3B expression regulates mitochondrial translation activity and, in turn, cellular metabolic programming.

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P33 | Unraveling the HK1-VDAC1 interaction: insights into apoptosis and metabolic adaptation

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Hexokinase (HK)1, a key enzyme that catalyzes the first step of glycolysis, plays a crucial role not only in cellular metabolism but also in the regulation of apoptosis. This dual function is primarily attributed to its interaction with the voltage-dependent anion channel (VDAC1)1. Although essential for cellular function, the molecular details of the VDAC1-HK1 interaction remain poorly defined. Gaining deeper structural and dynamic insights into this interface could open new avenues for targeting apoptosis and metabolic reprogramming, fundamental processes in cancer progression and aging-related diseases. To establish a foundational assay system for characterizing phenotypes linked to HK1 expression and, ultimately to study interaction-defective HK1 mutants, we generated stable HEK 293T and HeLa cell lines overexpressing HK1-GFP or GFP control. These metabolically distinct cell lines were clonally selected and characterized using fluorescence microscopy, flow cytometry, proliferation assays, and Western blotting. Preliminary data showed that HK1 overexpression affects apoptotic responses, especially under Staurosporine-induced stress, supporting its anti-apoptotic role. Moreover, glucose deprivation assays revealed distinct apoptotic susceptibilities between HeLa and HEK 293T clones, suggesting cell type-specific metabolic adaptations. Ongoing studies include functional assays on mitochondrial metabolism to further elucidate HK1-VDAC1's role in cell survival and metabolic regulation. Future transcriptomic analyses are planned to explore involved pathways. Overall, these findings offer novel insights into how HK1 modulates apoptosis and metabolism, with potential relevance for therapeutic strategies targeting related diseases.

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P34 | The bd-type Cyanide Insensitive Oxidase (CIO) from the multidrug-resistant pathogen *Pseudomonas aeruginosa* is inhibited by Carbon Monoxide

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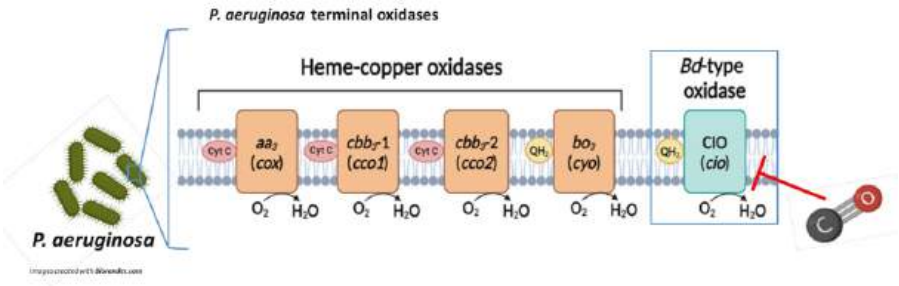

Carbon monoxide (CO), along with nitric oxide (NO) and hydrogen sulfide (H2S), is a well-known toxic gas which binds tightly and inhibit heme-containing proteins, including respiratory terminal oxidases. These three gaseous molecules act as endogenous signaling molecules at low concentrations and exhibit antimicrobial properties. Some bacteria, such as the multidrug-resistant (MDR) pathogens Escherichia coli and Mycobacterium tuberculosis (Mtb), defend themselves against the host immune response by overexpressing bd-type oxidases, which are copper-free terminal oxidases resistant to various stressors, including CO [1]. The respiratory chain of Pseudomonas aeruginosa (Pa), one of the most critical MDR pathogens, in addition to four heme-copper terminal oxidases has a bd-type oxidase, the Cyanide Insensitive Oxidase (CIO), which protects Pa against sulfide and nitrosative stress [2]. To better understand the role of CIO during Pa pathogenesis, we investigated the sensitivity of CIO to CO. For this purpose, we recombinantly expressed, purified and characterized CIO. In contrast to the behavior observed in M. smegmatis [2], oxygraphic measurements show that CO potently inhibits CIO activity in competition with O_2. These data reveal a different response to CO among bd-type oxidases, that may arise from variations in the structural organization of active sites and their specific environments [1], and suggest that host-produced or exogenously supplied CO may exert a significant antibacterial effect on this pathogen when CIO is expressed.

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P35 | Upregulated cardiomyocyte Monoamine Oxidase A (MAO A) participates in the development of heart failure following Conditional Opa1 heart deletion

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Optic atrophy 1 (Opa1) is an inner mitochondrial membrane dynamin related GTPase essential for mitochondrial fusion and cristae remodeling. OPA1 levels are reduced in patients suffering from heart failure, calling for an evaluation of the role of OPA1 in the heart. To this end, we generated and characterized a conditional inducible Opa1 knockout mouse in the heart (OPA1CKO), where ablation of Opa1 is driven by dietary tamoxifen driven activation of Mer-Cre-Mer. RNA sequencing analysis revealed that transcripts of the mitochondrial protein Monoamine Oxidase A (MAO A) were increased in OPA1CKO mice. MAO A catalyses the oxidative deamination of several biogenic amines, including serotonin, norepinephrine, dopamine, and epinephrine and produces high levels of H_2O_2 as byproduct. Because MAO A is normally not expressed in cardiomyocytes, we setup to determine whether its transcriptional increase was due to accumulating fibroblasts or to a specific response of the OPA1 ablated cardiomyocytes. Immunoblotting confirmed that MAO A protein levels were also increased in the OPA1CKO hearts. Using Langendorff isolated heart fibroblasts and cardiomyocytes we identified the latter as the source of increased MAO A levels, pointing to a potential role for MAO A in the heart pathology caused by Opa1 deletion. Indeed, minipump continuous infusion of the specific MAO A inhibitor clorgyline could partially restore heart function measured by echocardiography in OPA1CKO animals. Altogether, our data point to a role for MAO A in the heart pathology caused by Opa1 deletion.

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P36 | FAD/NAD(P)H-dependent dehydrogenases of pathogens and mammals: challenges and opportunities for selective antimicrobial targeting

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FAD/NAD(P)H dependent dehydrogenases play a fundamental role in the metabolism of bacteria, fungi, and protists by facilitating key redox reactions involving NAD(P)H and $FADH_2$. Despite significant sequence diversity, these enzymes exhibit remarkable structural conservation, including highly similar catalytic pockets, across both pathogenic microorganisms and their mammalian hosts [1]. This structural resemblance presents a dual challenge: on one hand, these flavoproteins are essential for pathogen viability, making them attractive drug targets; on the other, their similarity to human counterparts raises concerns about off-target effects and host toxicity. In this study, we analyze the structural conservation of FAD/NAD(P)H-dependent dehydrogenases, including type II NADH dehydrogenases [1,2], apoptosis inducing factor [1], thioredoxin reductase [1], glutathione disulfide reductase [1], and dihydrolipoamide dehydrogenase [1,3], across pathogenic microorganisms, highlighting their potential as antimicrobial targets. We discuss the necessity of designing highly selective inhibitors that can effectively disrupt microbial metabolism while sparing human counterparts. The comprehension of the structural/functional relationships and peculiarities of the mentioned FAD/NAD(P)H dependent dehydrogenases is crucial for advancing next-generation therapeutics with enhanced specificity and reduced side effects for combating infectious diseases.

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P37 | RAP1 inhibition restores mitochondria elongation and lysosomal distribution downstream of OPA1 deletion

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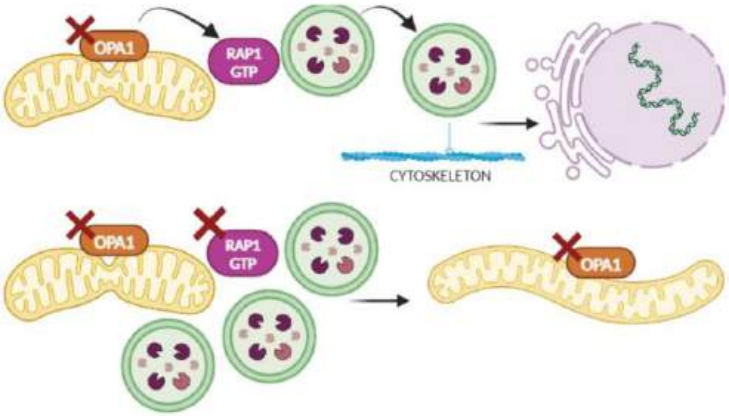

Changes in core components of the mitochondria-shaping machinery trigger retrograde signaling pathways that remodel gene expression and influence cellular signaling cascades, like angiogenesis that is transcriptionally controlled by Optic Atrophy 1 (Opa1). However, the nature and the cellular effects of these retrograde signals is largely unknown. Here we show that in endothelial cells the Ras-associated protein-1 (Rap1) signaling cascade activated when Opa1 is deleted regulates mitochondrial morphology. Unbiased RNA sequencing of ECs where we genetically ablated Opa1 revealed activation of the Rap1 small GTPase pathway. Indeed, Rap1 localized in proximity of mitochondria and lysosomes. Surprisingly, Rap1 downregulation in ECs lacking Opa1 restored mitochondrial morphology without affecting the core fission mediator Drp1. Conversely, Rap1 inhibition restored the normal distribution of lysosomes that is instead skewed towards the perinuclear region in cells lacking Opa1 in an actin-dependent manner. Thus, by exploiting Opa1 deletion as a tool to investigate mitochondria-nucleus retrograde signals we unveiled a hitherto unappreciated role for Rap1 in mitochondrial dynamics, perhaps by modulating mitochondria-lysosome contact sites and lysosomal priming of mitochondrial fission.

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P38 | Multifaceted roles of VDAC cysteine redox state in biogenesis, redox homeostasis, and oligomer assembly

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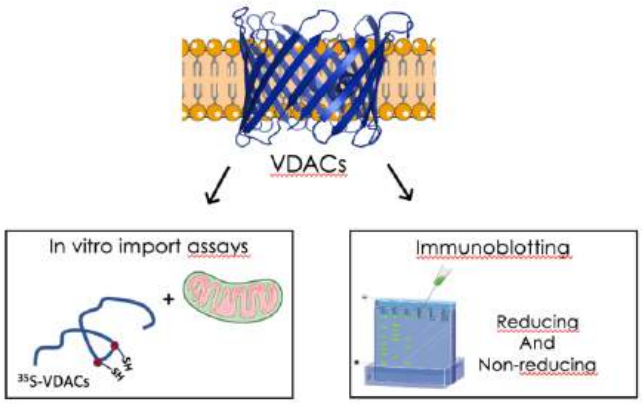

Voltage-Dependent Anion-selective Channels (VDACs), key metabolite transporters in the mitochondrial outer membrane (OMM), exist as three isoforms (VDAC1-3) in mammals. Although proteins' redox modifications within the matrix, intermembrane space (IMS), and inner membrane are extensively studied, the redox regulation of OMM proteins, especially VDACs, is comparatively under-explored. Intriguingly, VDACs' cysteine content and redox state are highly variable among the isoforms and may suggest distinct biological functions. Cysteines in VDAC1 appear non-essential for the pore-forming activity, while those in VDAC2 enhance its association with OMM. In contrast, VDAC3 has been proposed as a main player in redox homeostasis, counteracting oxidative stress through cysteine residues exposed to the IMS [1]. Recently, our group determined disulfide bonds in VDAC2 using mass spectrometry [2], underscoring the necessity to clarify the biological roles of these redox modifications. Hence, we investigated: i) the impact of cysteine residues on VDAC biogenesis, and ii) ROS-mediated disulfide bond formation in VDACs. Our results revealed that VDAC3 cysteines impede mitochondrial import kinetics and complex formation. Importantly, we report distinct in vitro oxidative responses among VDAC isoforms. While VDAC2 and VDAC3 form oligomers under non-reducing conditions, surprisingly only VDAC2 exhibits increased oligomerization upon H_2O_2 treatment, mediated by disulfide bonds. These data suggest that variations in cysteine redox state contribute to isoform-specific biophysical properties, impacting VDAC biogenesis, assembly, and redox homeostasis.

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P39 | Circadian control of mitochondrial biogenesis and turnover: insights from Parkin-deficient models

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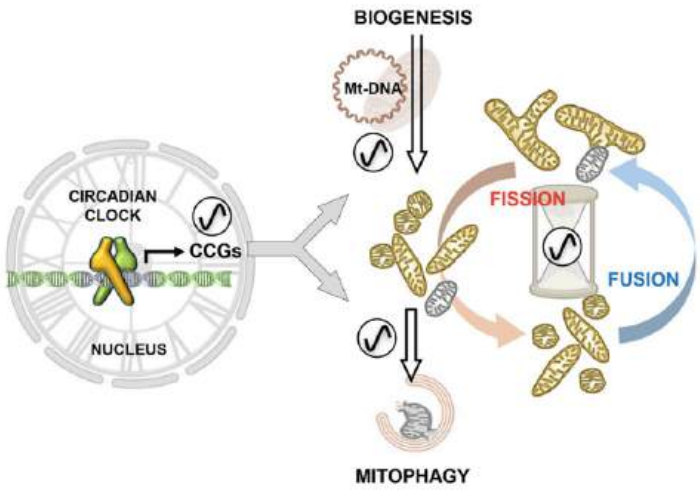

The circadian clock is a fundamental timekeeping system that orchestrates physiological and metabolic processes across the 24-hour cycle. Among the processes governed by this timing mechanism are mitochondrial functions such as energy production, organelle dynamics, and turnover, all of which are finely tuned by circadian signals to meet the fluctuating energy demands of the cell. Evaluation of the mRNA transcription of mtDNA encoded genes as well as of mitochondrial transcription factors underwent oscillatory profile in synchronized HepG2 cells. However, the absence of significative changes in the oxidative phosphorylation (OxPhos) components and synchronous variations of the mitophagy-promoting factors suggest a coordinated linkage between mitochondrial biogenesis and organelle quality control. This conclusion was also supported by assessment of mitochondrial OxPhos and dynamics [1,2]. Moreover, iPSC-derived neurons carrying mutations in the E3 ubiquitin ligase Parkin, a key regulator of mitophagy, displayed a marked attenuation of these mitochondrial rhythms. The loss of Parkin function led not only to disrupted temporal regulation of OxPhos and mitochondrial morphology, but also to altered expression patterns of core circadian clock genes. Altogether, our findings support a bidirectional relationship between the circadian clock and mitochondrial function. Disruption of this interplay, as observed in Parkin-null cells, may contribute to the pathophysiology of neurodegenerative and metabolic diseases. Understanding these mechanisms opens potential therapeutic avenues for targeting mitochondrial dysfunctions and related metabolic disorders.

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P40 | Von Willebrand factor A domain-containing protein 8 modulates mitochondria-sarco/endoplasmic reticulum contacts in β-adrenergic agonist-induced cardiac hypertrophy

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Heart mitochondria that engage in contact sites with the sarco/endoplasmic reticulum (MSRCs) are involved in the pathophysiology of cardiac hypertrophy. During β-adrenergic induced cardiac hypertrophy in mice MSRCs are increased, but whether MSRCs expansion participates in hypertrophy-induced mitochondrial dysfunction is unclear. Through integrative analysis of transcriptomic data from isoproterenol-infused mouse hearts (GSE195466) and of an unbiased MSRCs proteomic catalog generated in our lab, we identified in hypertrophic hearts 111 differentially expressed genes encoding for MSRCs proteins. Among these, the poorly characterized MERCs protein von Willebrand factor A domain-containing protein 8 (VWA8), a dually localized mitochondria/peroxisome AAA ATPase, was downregulated in hypertrophic hearts. Consistently, in isoproterenol-treated cardiomyocytes, Vwa8 expression was downregulated and Vwa8 deletion increased mitochondria-sarco/endoplasmic reticulum proximity in cardiomyocytes and in vivo in Vwa8-/- mice. Functionally, mitochondrial calcium uptake was increased and NADH production by Ca2+-dependent TCA cycle dehydrogenases was decreased upon VWA8 deletion. Re-expression in VWA8 knockout AC16 cardiomyocytes of the full-length VWA8 isoform A, but not of the shorter isoform B, restored mitochondria-sarco/endoplasmic reticulum distance. Thus, Vwa8 is part of the rewired gene expression program that during β-adrenergic agonist-induced cardiac hypertrophy orchestrates MSRCs augmentation, ultimately altering mitochondrial function.

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P41 | Mitochondrial calcium uptake regulates mitochondrial fission modes

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Mitochondrial fission is an essential and finely regulated process that can be further classified into "midzone" and "peripheral" according to the position of the mitochondrion where it occurs. Mitochondrial calcium (Ca^{2+}) influx is associated with inner membrane constriction as priming event of fission and cristae reorganization. The smaller daughter mitochondria originating from peripheral division are preceded by significant increase of Ca^{2+}, whereas Ca^{2+} level increased mildly in bigger peripheral daughter mitochondria or midzone daughter mitochondria. However, the relationship between mitochondrial Ca^{2+} and mitochondrial fission modes remains unclear. In order to address these questions, we used ionomycin, an ionophore that raises the intracellular Ca^{2+} level and induces mitochondrial Ca^{2+} uptake, to explore the mitochondrial fission modes. Treatment with ionomycin caused an increase in the rate of peripheral fissions. Interestingly, Ru360, a specific mitochondrial calcium uptake inhibitor, reduced ionomycin-induced peripheral fission, whereas per se it induced an increase in mitochondrial branching and elongation. Consistently, compared with control cells, peripheral fission, but not midzone fission, was blocked in cells knockout for the mitochondrial Ca^{2+} uniporter (MCU) undergoing ionomycin treatment. Our data suggest that mitochondrial calcium uptake participates in peripheral mitochondrial fission.

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P42 | The role of the FIS1-Interactome in Acute Myeloid Leukemia mitophagy

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Fission 1 (FIS1) is an outer mitochondrial membrane protein that functions as a critical regulator of mitochondrial fission and mitophagy, essential for maintaining mitochondrial quality control. Dysfunctional mitophagy has been implicated in acute myeloid leukemia (AML), but the molecular mechanisms linking FIS1 to mitophagy regulation are not understood. We analyzed transcriptomic data from AML and healthy controls, identifying CFL1 and BNIP3L as potential FIS1-interacting proteins based on gene expression profiling, protein-protein interaction networks, and pathway enrichment. We are currently performing molecular docking to predict key binding residues and structural interactions between FIS1 and these candidates. To validate these interactions in mammalian cells, we will use split-Fast, proximity ligation assay, and co-localization studies, followed by functional characterization through CRISPR knockdown and mitophagy flux assays. We expect to see either CFL1 or BNIP3L forming a functional interaction with FIS1, contributing to mitophagy regulation. By uncovering novel FIS1-mediated mitophagy mechanisms, this study aims to provide insights into mitochondrial dysfunction in AML and identify potential therapeutic targets.

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P43 | LACTB modulates the sorting of unfolded mitochondrial protein aggregates

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Accumulation of Unfolded Mitochondrial Proteins Aggregates (UMPA) in different mitochondrial subcompartments are the initial step of mitochondrial quality control mechanisms. However, how sub-compartmentalized UMPA are seeded and sorted is unknown. By capitalizing on advanced tools to visualize UMPA in real time within the mitochondrial matrix and intermembrane space, we performed a siRNA screen for factors that once depleted significantly reduce the accumulation of sub-compartmentalized UMPAs. Among the hits we identified LACTB, an intermembrane space protein that interacts structurally with the inner mitochondrial membrane, where it forms filamentous structures and controls mitochondrial lipids metabolism [1]. We will present data on UMPA formation, mitochondrial morphology, and cell fate following LACTB knockdown.

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P44 | Effect of nutraceuticals on mitochondrial dysfunctions in Chemotherapy-Induced Peripheral Neuropathy (CIPN)

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Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting side effect of chemotherapeutic drugs such as paclitaxel, significantly affecting the quality of life of cancer patients. CIPN typically presents a combination of sensory symptoms which can persist after the treatment. To date no effective therapies are available. Besides, the molecular mechanisms underlying CIPN are still the subject of ongoing research in order to develop personalized strategies [1]. Evidences indicate that several chemotherapeutic drugs cause mitochondrial damage and oxidative stress in the peripheral sensory nervous system. Using SH-SY5Y neuroblastoma cell line, as a in vitro model of CIPN, we investigated the molecular mechanism involved in drug-dependent mitochondrial dysfunction. Results indicated that paclitaxel treatment of SH-SY5Y induced mitochondrial dysfunction associated with a decrease of cAMP level. These effects were prevented by using nutraceutics such as resveratrol and hydroxytyrosol, known to act on both mitochondria and cAMP signaling pathway [2,3].

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P45 | Exploring the effects of β-hydroxybutyrate on a new in vitro model of human hippocampal neurogenesis

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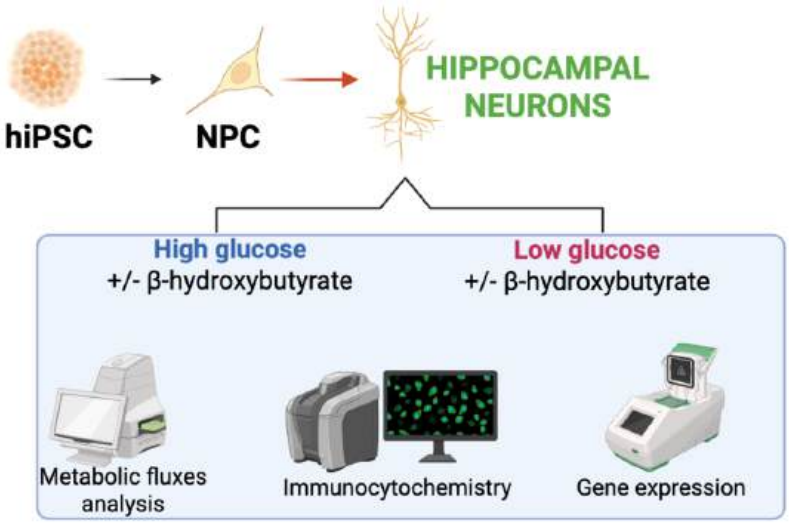

Neurogenesis is the process by which mature neurons are generated from neural stem cells (NSCs). While traditionally considered an embryonic event, recent studies have demonstrated that neurogenesis persists throughout life [1]. In the adult brain, NSCs are primarily localized in two neurogenic niches: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampal dentate gyrus. Under normal physiological conditions, the brain predominantly relies on glucose for ATP production. However, in states of glucose scarcity, ketone bodies, particularly β-hydroxybutyrate (BHB), serve as important alternative energy substrates [2]. Emerging evidence suggests that BHB have cellular signaling function [3] that might influence NSC differentiation in addition to brain energy metabolism, although its role in modulating neurogenesis under varying glucose conditions remains poorly understood. To investigate this, we developed a novel in vitro model of human hippocampal neurogenesis using NSCs derived from human induced pluripotent stem cells (hiPSCs). Cells were differentiated under two glucose concentrations, with or without the addition of BHB. Neuronal development was assessed at the end of differentiation through gene expression analysis and immunocytochemistry for MAP2, DCX, and PROX1. Metabolic profiling was performed using Seahorse XF analysis to evaluate metabolic fluxes and ATP production under each condition. Our study investigates the potential modulatory role of BHB in human hippocampal neuron differentiation under metabolically distinct environments.

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P46 | Honokiol blocks tumor development and metastasis through mitochondrion-targeted effects

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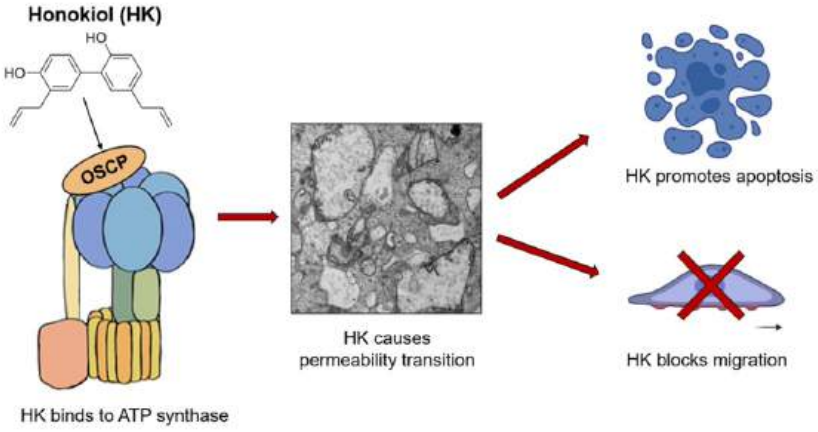

IF1 is the natural inhibitor of the mitochondrial ATP synthase during hydrolytic activity. It has been found to be overexpressed in many tumors, where it acts as a pro-oncogenic protein. During oxidative phosphorylation, IF1 binds to a novel site on the OSCP subunit of ATP synthase and promotes tumorigenesis by protecting cancer cells from permeability transition pore (PTP)-dependent apoptosis. In this work, honokiol, a biphenolic compound, was shown to be effective in disrupting the IF1-OSCP interaction and sensitizing cancer cells to apoptosis. In vivo, xenografts of zebrafish injected with IF1-expressing HeLa cells showed tumor development. The same xenografts treated with honokiol, showed a significant reduction in tumor mass, similarly to untreated fish injected with IF1 KO HeLa cells. In vitro, honokiol inhibits colony formation in soft agar of IF1-expressing HeLa cells, by promoting the PTP opening and cell death, without any effect on cell proliferation. However, honokiol was shown to block metastasis in fish xenografts and migration in wound healing assay, by promoting mitochondrial swelling in both control and IF1 KO cell lines, when cells are moving to close the scratch area. In conclusion, honokiol appears a mitochondrion-effective compound, with promising anti-cancer properties through the displacement of IF1 from the OSCP subunit of ATP synthase and anti-metastatic effects that are due to mitochondrial PTP opening.

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P47 | In Vivo Opantimir treatment: antagonizing microRNAs that upregulate Opa1 and their effect on Cox deficient mice

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Mitochondrial myopathies manifest with weakness, motor deficiency and exercise intolerance, impairing the prognosis of these already devastating disorders that lack treatments. Optic Atrophy 1 (OPA1), a mitochondrial inner membrane protein modulating mitochondrial dynamics, is downregulated in mitochondrial myopathies. We have found that OPA1 is downregulated by the miR148/152 family in skeletal myocytes in different mitochondrial myopathies. Opantimirs, miRNA antagonists targeting the miR148/152 family rescue muscle function in two models of mitochondrial myopathy caused by deletion of the complex IV assembly factor Cox15 and by ischiatic nerve resection. However, direct injection of Opantimirs is not a translationally relevant delivery route as it might be feasible in small rodents but impractical in humans. To this end, we are developing nanoparticles loaded with Opantimirs that target skeletal muscle. We will present data on the preliminary characterization of these nanoparticles and on their ability to deliver Opantimirs to isolated muscle cells and fibers.

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P48 | Dissecting structural requirements of Leucinostatin A derivatives for antiprotozoal activity and mammalian toxicity

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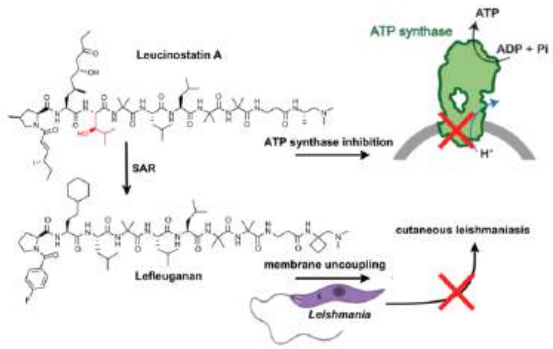

Lefleuganan, a clinical stage drug candidate for the treatment of cutaneous leishmaniasis, is a synthetic nonapeptide inspired by the natural antimicrobial peptide leucinostatin A, exhibiting excellent antiprotozoal activity in the low nM range [1,2]. Lefleuganan shows strongly reduced acute toxicity, making it amenable for clinical use. Here, using a broad set of in vivo and in vitro measurements using purified enzymes, we find that leucinostatin A, but not lefleuganan, specifically targets the mitochondrial ATP synthase, inhibiting ATP synthesis by the human, bovine, and yeast enzyme in the nanomolar range. In a structure–activity relationship study covering the chemical space between the two compounds, hydroxyleucine at position 7 in leucinostatin A is identified as the key responsible moiety for specific ATP synthase inhibition and systemic toxicity. Our data suggest that efficient antiprotozoal activity of these class of compounds is mediated by efficient energetic uncoupling of negatively charged membranes.

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P49 | Melatonin preserves mitochondrial structure and function in ischaemic HT22 cells

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Mitochondrial damage and the release of mtDNA are key events in cerebral ischaemia. In HT22 hippocampal cells subjected to oxygen and glucose deprivation followed by reoxygenation (OGD/R), we observed mitochondrial structural alterations, increased oxidative stress, and reduced activity of complexes I, III, and IV, all encoded by mtDNA, along with a significant decrease in ATP production. These effects were associated with cytosolic accumulation of mtDNA, activation of the cGAS–STING pathway, increased IFNβ mRNA expression, and IL-6 release. Melatonin treatment preserved mitochondrial morphology and membrane potential, reduced protein carbonylation, restored the activity of complexes I, III, and IV, and partially recovered ATP production. It also significantly limited mtDNA efflux into the cytosol, downregulated cGAS and STING expression, and decreased levels of HMGB1, IFNβ, and IL-6, indicating suppression of the inflammatory response. Furthermore, melatonin modulated the release of FGF21, a mitokine induced by mitochondrial stress, by promoting its early secretion and reducing its accumulation at later time points. These findings demonstrate that melatonin exerts a protective effect on mitochondrial structure and function, mitigates mito-inflammation, and enhances the release of protective mitokines. Our data support the use of melatonin as a modulator of mitochondrial homeostasis and innate immune activation in neurodegenerative and ischaemic contexts [1].

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P50 | Phenolic extract from olive mill wastewater preserves mitochondrial bioenergetics under oxidative stress

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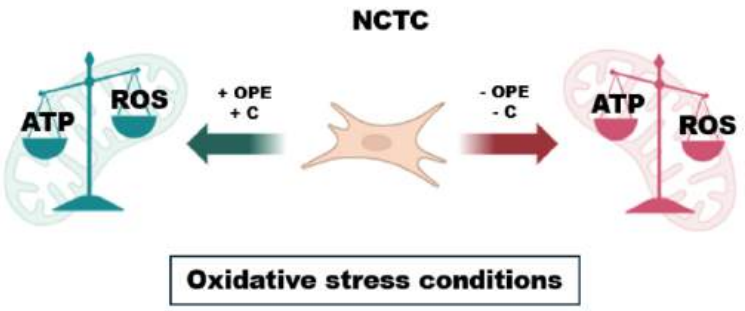

Oleuropein secoiridoid (OLE) and its hydroxytyrosol derivative (HT) are key bioactive molecules in extra virgin olive oil (EVOO) endowed with antioxidant and anti-inflammatory properties. However, the weak hydrophobicity of these phenolic compounds leads to their accumulation in olive mill wastewater (OMWW) that represents one of the main by-products of olive oil production. The extraction of polyphenols from OMWW offers both environmental and economic benefits, contributing to the food, pharmaceutical, and cosmetic industries, improving the shelf life of products and providing new nutraceutical ingredients. This study aims to investigate the impact of phenol-rich OMWW extracts on mitochondrial function and oxidative stress response in mouse fibroblast-like cells (NCTC). Our results clearly demonstrate that two OMWW fractions enriched in hydroxytyrosol- and hydroxytyrosol/oleuropein (i.e. C and OPE extracts, respectively) significantly enhance mitochondrial biogenesis and reduce cellular sensitivity to hydrogen peroxide-induced oxidative stress. Furthermore, high-resolution respirometry experiments reveal the protective role of C and OPE samples in preventing the failure of mitochondrial respiration during oxidative insult [1]. Overall, collected data support the potential of reusing olive mill wastewater to generate low-cost and high-value bioactive molecules.

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P51 | Lead optimization of specific OPA1 inhibitors enhancing cytochrome c release and cell death

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The mitochondrial Dynamin-like protein Optic Atrophy 1 (OPA1) is a large GTPase involved in mitochondrial fusion, cristae remodeling, cytochrome c release and apoptosis. OPA1 upregulation has been increasingly identified as an exploitable vulnerability in cancer cells. From a previous screening of >10,000 drug-like molecules for inhibition of OPA1 GTPase activity, we identified MYLS22 as a promising hit. MYLS22 is not mitochondriotoxic but causes mitochondrial fragmentation and cristae remodeling, enhancing cytochrome c release following proapoptotic stimuli, recapitulating the effect of OPA1 downregulation in cells. Following a SAR analysis of MYLS22, multiple possible derivatives have been identified with increased water solubility. Three of these derivatives showed enhanced inhibitory effects on OPA1 GTPase activity in vitro and caused mitochondrial fragmentation in cells. To identify hits with further enhanced potency, solubility and overall availability of the leads, we generated a virtual, combinatorial library of 81,000 compounds. We docked these compounds to the GTPase domain of the currently available structure of OPA1 (PDB: 6JTG) and performed a virtual screening to discover second generation OPA1 inhibitors. We identified the most promising hits, synthetized them and analyzed their potency as OPA1 inhibitors in vitro and in cells. Our work identifies specific and potent OPA1 inhibitors with the potential to treat cancers where OPA1 is upregulated.

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Precise evaluation of cellular metabolism is pivotal for understanding mitochondrial function, membrane bioenergetics, and energy transduction mechanisms. The Agilent Seahorse XF platform enables real-time, label-free quantification of two core metabolic parameters—oxygen consumption rate (OCR) and extracellular acidification rate (ECAR)—providing direct insight into mitochondrial respiration, glycolysis, and ATP production in intact cells. Designed for high-resolution functional analysis, Seahorse XF Analyzers allow researchers to dissect mitochondrial substrate preference, coupling efficiency, spare respiratory capacity, and proton leak, as well as glycolytic reserve and capacity. The XF system is compatible with various biological models—including 2D models such as primary cells and cell lines (adhesion or suspension), isolated mitochondria, and permeabilized cells and 3D models such as organoids, tissues, islets, and small organisms —makes it an ideal tool for mechanistic studies in membrane transport, oxidative phosphorylation, and mitochondrial dynamics. With dedicated assay kits and flexible plate formats (8- 24- and 96-well), Seahorse XF instruments support parallel analysis of multiple experimental conditions, significantly increasing data throughput while minimizing sample consumption. Integrated with intuitive Wave software, the platform streamlines assay setup, normalization, and data interpretation. Agilent Seahorse technology represents a state-of-the-art solution for researchers aiming to decode cellular energy metabolism with precision and speed—supporting discoveries in mitochondrial pathophysiology, bioenergetics, and metabolic adaptation in health and disease.

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P52 | Oxidative phosphorylation powered by electricity

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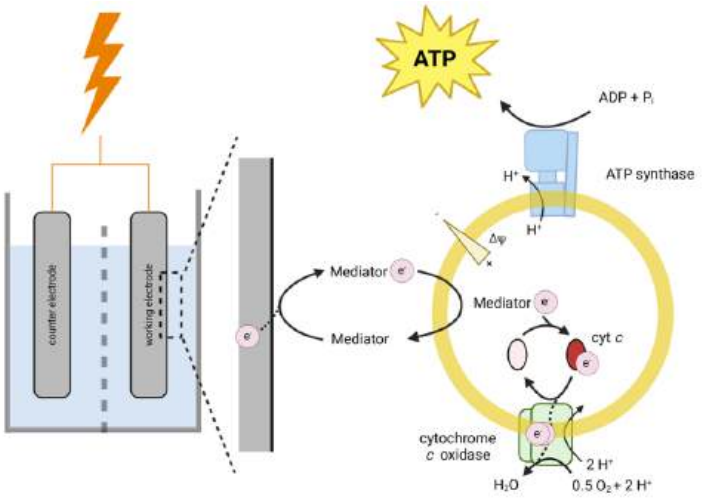

The electron transport system is essential for ATP synthesis in cellular respiration, relying on electron equivalents (e.g., NADH and $FADH_2$) derived from catabolism. The electrons pass through a series of protein complexes, driving proton pumping and creating an electrochemical gradient (pmf), which is utilized by ATP synthase to phosphorylate ADP into ATP. Oxidative phosphorylation (OXPHOS) is highly efficient, yielding approximately 30–34 ATP molecules per glucose (in mitochondria). In synthetic respiratory chain-mimicking systems, these electrons are usually supplied as a substrate for an enzyme (e.g., NADH for complex I or prereduced cytochrome c for complex IV), or the quinone pool is reduced directly by chemical reduction [1]. Here, we engineered an electrobiological system that converts electricity into the universal biological energy carrier, ATP. Electrochemical reduction of 1-methoxy PMS is linked to OXPHOS using a liposome-based minimal system containing aa_3 oxidase from *Rhodobacter sphaeroides* and Escherichia coli F_1F_0 ATP synthase. This novel combination of electrochemistry and synthetic biology offers several advantages. Compared to other ATP-producing electrobiological approaches, it requires only a relatively low potential, can be run under normal atmospheric conditions, and does not produce any waste products. This method of converting electrical energy into biological energy has significant potential for bioelectrocatalysis and synthetic biology applications.

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P53 | Generation and initial characterization of STACCATO, a family of Split-FAST mitochondria-ER contacts probes to measure contact sites of different widths

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In the eukaryotic cells contact sites are established between organelles; these sites becomes subcellular compartments with specialized functions and proteomes. The contact sites between endoplasmic reticulum (ER) and mitochondria (MERCs) regulate many physiological processes so mutations that affect MERCs composition and morphology are found in several diseases. MERCs formation and dynamics can be visualized through bimolecular fluorescent probes in confocal microscopy. However, the current probes are dim, or artificially induce tethering, or require complex imaging procedures that are not compatible with the common imaging setups available to most of the labs. An ideal probe should be therefore reversible in its complementation, easy to use and should have high signal-to-noise ratio. For this purpose we developed STACCATO: a bimolecular fluorescent probe to detect MERCs based on split Fluorescence-Activating and absorption-Shifting Tag (FAST). The C-terminus of STACCATO is directed to the mitochondria via the AKAP1 targeting signal, while its N-terminus is localized to the ER through the Sac1 targeting sequence. Flexible linkers connecting the two moieties of STACCATO enable reconstitution of the probe at varying MERC widths upon addition of an exogenous fluorogen. In this work we used flowcytometry and live cell imaging to show that STACCATO is able to detect changes in number of MERCs in mouse and human cell lines ablated for MERCs tethers or transfected with MERCs artificial tethers.

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P54 | Universal protocol for the isolation of Channel Forming Membrane Proteins using the E.coli expression system

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The recombinant expression, isolation and characterization of Channel Forming Membrane Proteins (CFMP) are widely used strategies to study the permeability properties of the biological membranes in which they are embedded [1]. The challenge of this technique is getting a product active enough for reconstitution experiments but pure enough to yield consistent and reproducible results. The methods developed in the workgroups of Benz and Winterhalter provide a solid foundation for CFMP production and characterization but lack a unified, universal isolation protocol. Here [2] I present a step-by-step protocol to quantify the expression of a protein of interest (YPI) and optimize its yield using the E.coli strain BL21Gold(de3)ΔABCF [3]. The protocol details the fractionation of bacterial compartments based on solubility, extraction of YPI in its native conformation using detergent solutions, and purification via ion-exchange chromatography or direct expression into outer membrane vesicles for co-purification. Compared to existing CFMP protocols [4], this process is simpler, less empirical, and provides a robust framework for isolating soluble proteins [5]. Several parameters can be optimized on a case-by-case basis: expression time and temperature, concentration of the inducer, nature and concentration of the detergent, incubation time and temperature, pH and ionic strength of the purification buffers. This protocol is effective with prokaryotic CFMP [6] and can be adapted for the production of membrane proteins from chloroplasts [7], mitochondria [8] or eukaryotic sources in general [9]. With minor modifications, it is also suitable for isolating receptors, carriers, pumps, and other membrane-active proteins.

Dedicated to my PhD Father, Prof. Roland Benz (1943–2025).

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P55 | Harnessing Big Data to uncover VDAC1P8's role in AML pathophysiology

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Voltage-dependent anion-selective channels (VDACs) are the most abundant proteins in the outer mitochondrial membrane and play a crucial role as "mitochondrial gatekeepers" in regulating cellular metabolism [1]. In mammals, three genes (VDAC1, VDAC2, and VDAC3) encode these channels, which are expressed in both normal and cancerous tissues. Additionally, VDAC genes are associated with numerous processed pseudogenes, some of which display tissue-specific activation patterns [2]. This study explores the physiological and clinical significance of VDAC pseudogenes, particularly their transcriptional activity in different tissues and disease states. Through an analysis of genomic and transcriptome databases, we identified specific VDAC pseudogenes that are actively transcribed under certain conditions. Notably, our findings highlight a strong association between the VDAC1P8 pseudogene and acute myeloid leukemia (AML). Our in-silico analyses and experimental data from AML cellular models reveal that VDAC1P8 is overexpressed in AML, correlating with a downregulation of its parental gene, VDAC1. These results suggest a potential regulatory mechanism involving VDAC1P8 in AML, shedding light on its role in disease progression and offering new avenues for further research [3].

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P56 | FLYMP: a new FRET based sensor to study contact sites between mitochondria and lysosomes

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Mitochondria and lysosomes are key organelles for cell homeostasis affected in diseases such as Parkinson's and Gaucher's. They interact functionally and physically via membrane contact sites, which mark mitochondrial fission sites and regulate calcium transfer. Rab7-GTP promotes tethering, while the Fis1-TBC1D15 complex triggers untethering [1]. However, tools to reliably inspect these interactions are lacking. To address this, we developed a FRET-based sensor for Lysosome-Mitochondria Proximity (FLYMP). The probe comprises two parts: a mitochondria-targeted AKAP1-FKBP-EYFP and a lysosome-targeted TMEM192-FRB-ECFP, linked by an FKBP-FRB module that dimerizes in response to rapamycin, resulting in maximal FRET between donor ECFP and acceptor EYFP. The two halves are equimolarly expressed from a single mRNA with a self-cleavable T2A sequence between them. Immunofluorescence confirmed the correct targeting of the two moieties to their respective organelle. High-content FRET imaging showed that FLYMP detects mitochondria-lysosome proximity, with rapamycin maximizing juxtaposition and sustaining increased FRET over time. Expression of the Rab7Q67L mutant, which enhances contacts, increased basal FLYMP FRET, whereas the contacts-disrupting Rab7T22N mutant decreased it, validating FLYMP as a tool for measuring these contact sites in physiological and pathological conditions.

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[1] Y. C. Wong et al. *Trends Cell Biol* (2019), 29: 500-513.

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P57 | Real-time monitoring of ATP synthesis in synthetic liposomes using iATPSnFR2

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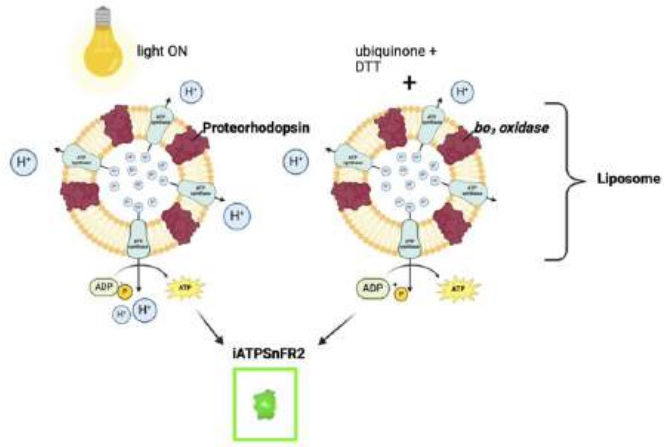

Energy production is fundamental to both natural and synthetic systems, yet quantifying ATP dynamics in reconstituted bioenergetic networks remains challenging. iATPSnFR2, a ratiometric fluorescent ATP sensor, offers a powerful solution by enabling real-time, quantitative monitoring of ATP synthesis [1]. This sensor, based on a circularly permuted superfolder GFP within the ε-subunit of bacterial F_0F_1 ATPase, provides affinity variants ranging from 4 µM to 500 µM, allowing precise measurement across diverse ATP concentrations. Here, we employed iATPSnFR2 to track ATP synthesis in synthetic liposomes reconstituted with bacterial ATP synthase and either proteorhodopsin or bo_3 oxidase. Upon activation with light (proteorhodopsin) or the addition of ubiquinone and dithiothreitol (DTT) as a reducing agent (bo_3 oxidase), iATPSnFR2 confirmed ATP production. Using bo_3 oxidase, we achieved 40–50% ADP-to-ATP conversion and ATP synthesis rates of 2–3 µM per minute. These findings highlight the efficiency of different energy-coupling strategies for ATP generation in synthetic systems. By enabling precise, real-time quantification of ATP levels, iATPSnFR2 is a powerful tool for investigating energy metabolism in artificial bioenergetic networks. This work provides valuable insights for designing self-sustaining synthetic cells and bioenergetic modules for metabolic engineering and biotechnological applications.

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P58 | Optimization of synthesis parameters for cell-targeting fluorescent carbon dots: from nanostructure to mitochondrial bioimaging

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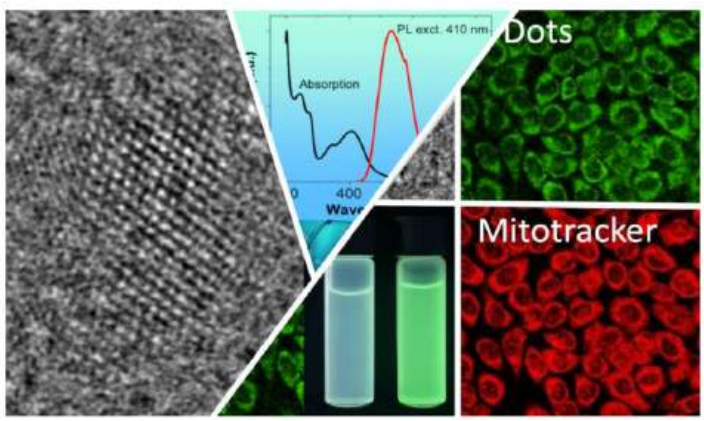

Mitochondria play a critical role in several physiologically processes and mitochondria disfunctions are involved in different disease such as cancer. Mitochondria-targeted imaging for cancer cell recognition represent an interesting approach for various biomedical applications. In this study, we report the optimization of synthesis conditions to develop highly stable and fluorescent Carbon Dots (CDs) with potential applications in bioimaging. A multidisciplinary strategy was adopted, involving systematic modulation of synthetic parameters combined with advanced characterization techniques. Optical characterization via UV-Vis absorbance and fluorescence spectroscopy revealed tunable and intense photoluminescence, while transmission electron microscopy (TEM) confirmed the production of uniformly dispersed nanoparticles with narrow size distribution. High-resolution TEM (HRTEM) analyses indicated ordered graphitic domains within the CDs, corroborating their structural stability. Elemental composition and chemical bonding states were further elucidated through electron energy loss spectroscopy (EELS), highlighting the presence of heteroatoms contributing to their photophysical properties. Among the various batches synthesized, selected CDs exhibiting optimal optical properties and colloidal stability were subjected to biological evaluation. In vitro cytocompatibility assays and in vivo fluorescence imaging confirmed their biocompatibility and non-toxic profile. Notably, confocal microscopy revealed an intrinsic ability of the CDs to marking cell structures, in particular mitochondria, enabling selective imaging of cancerous versus healthy cells. These findings suggest that the synthesized CDs are promising candidates for targeted bioimaging and potential theragnostic applications.

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