Hellenic Proteomics Society

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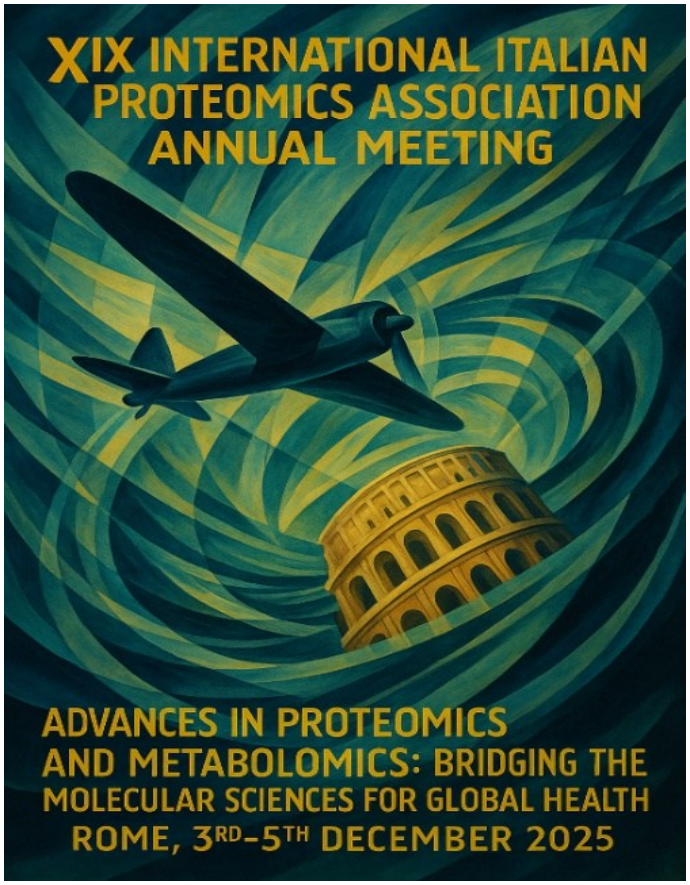
# XIX INTERNATIONAL ITALIAN PROTEOMICS ASSOCIATION ANNUAL MEETING

**in Partnership with the Hellenic Proteomics Society and the Serbian Proteomics Association**




**Auditorium, Congress Center Europa,**
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**Largo Francesco Vito, 1**
**00168 Roma, ITALY**

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## Conference Registration Fees

Early registration (**until November 3rd**, 2025):

- Senior, ItPA/EuPA member: **180** €
- Senior, non-member: 200 (180+20) €
- Young (below the age of 40), ItPA/EuPA member: **130** €
- Young (below the age of 40), non-member: 150 (130+20) €

Late Registration (**after November 3rd**, 2025):

- Senior and Young, member and non-member: **250** €

Industry – Companies: **300** € plus vat 22%

To register please fill in the google registration form available on the ItPA website and follow the instructions.

## General Information

### Congress Venue

Auditorium, Congress Center Europa, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Roma, ITALY.

### Official Language

The Congress official language will be English.

### Abstracts and Posters

The abstracts submission deadline for oral and poster communications is **November 3rd, 2025**. Posters will be displayed from December 3rd to December 5th, 2025. During poster sessions the presence of one of the authors is required. Presentations from young corresponding authors will be nominated for “best poster” and “best oral” prize competitions.

### Certificate of Attendance

Certificates of attendance and payment fee receipts will be available at the registration desk.

### Coffee Breaks and Lunches

Welcome cocktail, coffee breaks and light lunches will be served at the venue.

### Accommodation

The list of recommended hotels is available on the ItPA website.

## Confirmed Invited Speakers

***Catherine Alix-Panabières***, University of Montpellier.
***Carsten Hopf***, Mannheim University of Applied Sciences.
***Margherita Ruoppolo***, Università degli Studi di Napoli Federico II.
***Paul Wilmes***, Luxembourg Centre for Systems Biomedicine.
***Alejandro Cifuentes***, National Research Council of Spain (CSIC), Madrid.
***Tommaso Mazza***, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Roma.
***Simone Cardaci***, Ospedale San Raffaele IRCCS, Milan.
***Victor Corasolla Carregari***, Università Cattolica del Sacro Cuore, Roma.
***Alessio Soggiu***, Università degli Studi di Milano.

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# PROGRAM AT A GLANCE

| DAY 1 – Wednesday, December 3rd | |
|---|---|
| 13:00 | **CONGRESS REGISTRATION** |
| 14:00 - 16:00 | **WORKSHOP:**<br>**"Decoding the Dark Side of Mitochondria: from Genome to Proteome and Beyond"**<br><br>Chair: *Viviana Greco*<br><br>14:00 – 14:15<br>*Viviana Greco*<br>**Welcome and Introduction**<br><br>14:15 – 14:40<br>*Tommaso Mazza (Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Roma, IT)*<br>**"The mitochondrial proteogenome: assessing the functional impact of variants through computational methods"**<br><br>14:40 – 15:05<br>*Matteo Bordi (Roma, IT)*<br>**"Bridging rare genetic disorders and mitochondrial homeostasis: the case of ADSL deficiency"**<br><br>15:05 – 15:30<br>*Maria Eugenia Soriano (Padova, IT)*<br>**"A dynamic complexomic framework to identify and deorphanize mitochondrial proteins"**<br><br>15:30 – 15:55<br>*Eugenio Barone (Roma, IT)*<br>**"From proteome to powerhouse: uncovering mitochondrial dysfunctions linking type-2 diabetes and Alzheimer's disease"** |
| 16:00 - 16:30 | **COFFEE BREAK** |
| 16:30 - 16:45 | **WELCOME AND OPENING** |
| **SESSION I**<br>**Chair: Andrea Urbani, George Tsangaris** | |
| 16:45 - 17:20 | **PLENARY LECTURE:**<br><br>*Catherine Alix-Panabières (University Medical Center of Montpellier, Montpellier, FR)*<br>**"Liquid biopsy: the rise of a medical revolution"** |
| 17:20 – 17:45 | **HPS KEYNOTE LECTURE:**<br><br>*George Tsangaris (Biomedical Research Foundation of the Academy of Athens, Athens, GR)*<br>**"The secret life of peptides"** |
| 17:45 – 18:30 | **SELECTED ORAL PRESENTATIONS FROM ABSTRACTS:**<br><br>17:45 – 18:00<br>*Riccardo Stucchi (Thermo Fisher Scientific, Reinach (Basel), CH)*<br>**"Leveraging advanced MS technology for in depth immunopeptide profiling and PTMs analysis"**<br><br>18:00 – 18:15<br>*Anastasia Alexandridou (Dortmund, DE)* |

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| | **"Proteomics on the side of rapid diagnosis and personalized treatment of rare neuromuscular diseases"**<br><br>18:15 – 18:30<br>*Alessio Di Ianni (Milano, IT)*<br>**"Intrinsic N-Terminal Reactivity of Carbamate-based Cross-Linkers and their improved analysis"** |
| 18:30 | **WELCOME COCKTAIL** |

| **DAY 2 – Thursday, December 4th** | |
|---|---|
| **SESSION 2**<br>**Chair: Maria Monti, Carsten Hopf** | |
| 09:00 – 09:35 | **PLENARY LECTURE:**<br><br>*Carsten Hopf (Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), TH Mannheim, Mannheim, DE)*<br>**"Advances in MS-based spatial omics: applications in neurodegeneration, cancer and pathobiochemistry research"** |
| 09:35 – 10:00 | **KEYNOTE LECTURE:**<br><br>*Victor Corasolla Carregari (Università Cattolica del Sacro Cuore, Roma, IT)*<br>**"Revealing proteomic and phosphoproteomic changes in the temporal lobe, frontal cortex, and corpus callosum of post-mortem brains from patients with schizofrenia"** |
| 10:00 – 10:30 | **SELECTED ORAL PRESENTATIONS FROM ABSTRACTS:**<br><br>10:00 – 10:15<br>*Sabrina Bianco (Napoli, IT)*<br>**"Multiomics-based fluxomics modelling reveals metabolic rewiring in methylmalonic acidemia"**<br><br>10:15 – 10:30<br>*Sara Lomuscio (Dortmund, DE)*<br>**"Bridging proteomics and metabolomics through effective sample preparation"** |
| 10:30 – 11:00 | **COFFEE BREAK** |
| **SESSION 3**<br>**Chair: Marta Lualdi, Victor Corasolla Carregari** | |
| 11:00 - 13:00 | **SELECTED ORAL PRESENTATIONS FROM ABSTRACTS:**<br><br>11:00 – 11:15<br>*Louna Moles (Affinisep, Le Houlme, FR)*<br>**"High-throughput SPE membrane approaches for peptide cleanup and enrichment"**<br><br>11:15 – 11:30<br>*Marica Cozzolino (Napoli, IT)*<br>**"Mitochondrial proteomics and metabolomics highlight energy metabolism disruption in methylmalonic acidemias"**<br><br>11:30 – 11:45<br>*Valentina Carbonari (Catanzaro, IT)*<br>**"Leveraging computational methods for the analysis of the structural impact of the mutational landscape of proteins"** |

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| | 11:45 – 12:00<br>*Esther Imperlini (Viterbo, IT)*<br>**"Gaining insights into the anticancer activity of an antimicrobial myristoylated peptide by integrating proteomics and bioinformatics"**<br><br>12:00 – 12:15<br>*Ilaria Cicalini (Chieti, IT)*<br>**"Untargeted metabolomics approach for unraveling novel Gaucher disease biomarkers on dried blood spot"**<br><br>12:15 – 12:30<br>*Pierre-Olivier Schmit (Bruker, Wissembourg, FR)*<br>**"Enhanced immunopeptide identification using MIDIA-PASEF: a novel timsTOF scan mode"**<br><br>12:30 – 12:45<br>*Valeria Bica (Roma, IT)*<br>**"Phosphoproteomics reveals novel BCR::ABL1-independent mechanisms of resistance in chronic myeloid leukemia"** |
| 12:45 - 14:00 | **LIGHT LUNCH & POSTER SESSION** |
| **SESSION 4**<br>**Chair: Damiana Pieragostino, Margherita Ruoppolo** | |
| 14:00 – 14:35 | **PLENARY LECTURE:**<br><br>*Margherita Ruoppolo (Università degli studi di Napoli Federico II, Napoli, IT)*<br>**"From diagnosis to cellular mechanisms: decoding rare inherited metabolic disorders through proteomics, metabolomics and lipidomics"** |
| 14:35 - 15:00 | **KEYNOTE LECTURE:**<br><br>*Simone Cardaci (IRCCS Ospedale San Raffaele, Milano, IT)*<br>**"Targeting metabolic vulnerabilities in cancer and inflammation"** |
| 15:00 – 15:30 | **SELECTED ORAL PRESENTATIONS FROM ABSTRACTS:**<br><br>15:00 – 15:15<br>*Manuela Piazzi (Bologna, IT)*<br>**"Alternative splicing of transcripts encoding the innate immune, stress-activated protein kinase PKR"**<br><br>15:15 – 15:30<br>*Anna C. Procopio (Catanzaro, IT)*<br>**"In silico analysis for the development of a multiepitope-based vaccine against the monkeypox"** |
| 15:30 - 16:00 | **COFFEE BREAK** |
| **SESSION 5**<br>**Chair: Michele Costanzo, Simone Cardaci** | |
| 16:00 - 17:00 | **SELECTED ORAL PRESENTATIONS FROM ABSTRACTS:**<br><br>16:00 – 16:15<br>*Silvia Rizzo (Roma, IT)*<br>**"A polyphenol-enriched waste product from *Origanum vulgare* hydrodistillation drives metabolic and proteomic reprogramming that enhances the anti-*candida* activity of *Lactobacillus helveticus* and *Lactobacillus rhamnosus*"** |

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| | 16:15 – 16:30<br>*Ann-Christine König (Planegg, DE)*<br>**"Improve biomarker discovery: Enrichment-based workflows for plasma, serum and CSF allow rapid, in-depth analysis of large cohorts of samples"**<br><br>16:30 – 16:45<br>*Lucia Santorelli (Napoli, IT)*<br>**"Integrated Linear and Cross-linking Proteomics reveal stress-adaptive Endoplasmic Reticulum dynamics"**<br><br>16:45 – 17:00<br>*Vasileios Pierros (Athens, GR)*<br>**"A Uniquome based method for the protein identification by mass spectometry"** |
| 17:00 | **ItPA GENERAL ASSEMBLY** |

| **DAY 3 – Friday, December 5th** | |
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| **SESSION 6**<br>**Chair: Lorenza Putignani, Paul Wilmes** | |
| 09:00 – 09:35 | **PLENARY LECTURE:**<br><br>*Paul Wilmes (University of Luxembourg, Luxembourg, LU)*<br>**"Systems ecology of the microbiome: resolving proteins and metabolites critical to human health and disease"** |
| 09:35 - 10:00 | **KEYNOTE LECTURE:**<br><br>*Alessio Soggiu (Università degli Studi di Milano, Milano, IT)*<br>**"T6SS and beyond: molecular mechanisms shaping *Campylobacter-Bacillus* competition and proteome dynamics in a one health context"** |
| 10:00 – 10:30 | **SELECTED ORAL PRESENTATIONS FROM ABSTRACTS:**<br><br>10:00 – 10:15<br>*Kevin Hau (Dortmund, DE)*<br>**"SSIMO – Single Section Integrative Multi-Omics – spatial mapping of metabolites and lipids combined with regional specific proteomics in a single tissue slice"**<br><br>10:15 – 10:30<br>*Fabio Di Ferdinando (Chieti, IT)*<br>**"High-Resolution AP MALDI-MSI Identifies Lipid Metabolic Signature in the Marinesco-Sjogren Syndrome mouse model Woozy"** |
| 10:30 - 11:30 | **COFFEE BREAK & POSTER SESSION** |
| **SESSION 7**<br>**Chair: Anna Caterina Procopio, Alessio Soggiu** | |
| 11:30 - 12:30 | **SELECTED ORAL PRESENTATIONS FROM ABSTRACTS:**<br><br>11:30 – 11:45<br>*Maria Claudia Gatto (Napoli, IT)*<br>**"Decoding Copper Adaptation mechanisms in Marine Bacteria through Multi-Omics and Network Analysis"**<br><br>11:45 – 12:00<br>*Simone Dario Scilabra (Palermo, IT)*<br>**"Proteomics-based development of iRhom2 inhibitors"** |

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|---|---|
| | 12:00 – 12:15<br>*Gabriele Marcassa (Milano, IT)*<br>**"Synaptic signatures and disease vulnerabilities of layer 5 pyramidal neurons"**<br><br>12:15 – 12:30<br>*Giorgia Massacci (Roma, IT)*<br>**"Integration of Immunopeptidomics and Phosphoproteomics: From Kinase Control to Antigen Presentation"** |
| 12:30 - 13:00 | **AWARDS AND CLOSING** |
| 13:00 - 14:00 | **LIGHT LUNCH** |
| 14:00 - 16:00 | **WORKSHOP:**<br>**"Proteomics: meeting the exposome"**<br><br>Chair: *Paola Roncada*<br>14:00 – 14:15<br>*Paola Roncada*<br>**Welcome and Introduction**<br><br>14:15 – 14:45<br>Distinguished speaker: *Alejandro Cifuentes (National Research Council of Spain, Madrid, ES)*<br>**"Frontiers in foodomics: technologies, applications, and future directions"**<br><br>14:45 – 15:05<br>*Emanuela Laratta (Catanzaro, IT)*<br>**"Detection and characterization of microplastics in edible marine species: implications for human and environmental health"**<br><br>15:05 – 15:25<br>*Mariachiara Paonessa (Catanzaro, IT)*<br>**"Gastric and intestinal digestion of infant formula in the presence of polypropylene nanoplastics"**<br><br>15:25 – 15:45<br>*Anna C. Procopio (Catanzaro, IT)*<br>**"Emerging evidence and implications for global health of interactions between microplastics and the microbiota"** |

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# ABSTRACT VOLUME

# PLENARY and INVITED LECTURES

**PL-01**

# Liquid Biopsy: The Rise of a Medical Revolution

**Catherine Alix-Panabières**[a,*]

[a] University Medical Center of Montpellier, Montpellier, France;

This lecture explores how circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) have transformed cancer research and clinical care. I will provide an overview of current technologies for detecting these biomarkers, detailing their biology and their role in real-time monitoring of cancer progression. Advances now enable genomic, transcriptomic, and proteomic profiling of CTCs, as well as functional studies using patient-derived cell lines.

Likewise, ctDNA offers a non-invasive method to track tumor evolution and minimal residual disease. The lecture will highlight how CTC and ctDNA analyses have deepened our understanding of metastasis and therapy response. Expanding the definition of liquid biopsy to include tumor-induced immune components—such as immune cells, cytokines, and interleukins—could offer a more comprehensive view, particularly in the context of immunotherapy. I will conclude by discussing how CTC research uncovers mechanisms of immune escape and may guide the development of innovative strategies to improve cancer treatment.

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# PL-02
# Advances in MS-based Spatial Omics: Applications in Neurodegeneration, Cancer and Pathobiochemistry Research

**Carsten Hopf**[a]*

[a] Center for Mass Spectrometry and Optical Spectroscopy (CeMOS), TH Mannheim, Mannheim, Germany;

Mass spectrometry imaging (MSI) has emerged as a powerful technology for spatially resolved analysis and visualization of peptides, lipids and metabolites in clinical research and pharmaceutical R&D. Advancement of MSI requires steady progress in multiple areas such as instrumentation, experimental workflows or IT solutions. The talk will therefore focus on four new MSI technology trends, i) single-cell metabolomics by MSI [1], ii) 3D-reconstructed MSI for spheroid/organoid 3D-metabolomics, iii) advancement of high-tech MSI towards more comprehensive lipid identification by on-tissue MS/MS [2] and iv) spatial in-situ protein characterization by MSI [3].
The talk will cover four corresponding medical research cases: A) Mass-guided single cell metabolomics of microglia enables the discovery and the monitoring of activation markers in neurodegeneration research [1]. B) Spatial analysis of metabolic reprogramming in a colon cancer-fibroblast 3D-biculture model can be achieved by a standardized technology and $M^2$aia software. C) As for high-tech-MSI, the talk will introduce quantum cascade laser (QCL) mid-infrared imaging microscopy for the identification and segmentation of regions of interest (ROI). This QCL-guidance enables deep MS/MS lipid characterization and structure elucidation by imaging parallel reaction monitoring and parallel accumulation serial fragmentation (iprm-PASEF) directly on tissue [2]. Finally, D) In-situ trypsin digestion followed by MSI provides a robust way to monitor toxicopathological alpha2u-globulin accumulation in kidney [3].

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**References:**
**[1]** Cairns JL, Huber J, Lewen A, Jung J, Maurer SJ, Bausbacher T, Schmidt S, Levkin PA, Sevin D, Göpfrich K, Koch P, Kann O, Hopf C. Mass-Guided Single-Cell MALDI Imaging of Low-Mass Metabolites Reveals Cellular Activation Markers. Adv Sci (Weinh). 2025 Feb;12(5):e2410506. doi: 10.1002/advs.202410506.

**[2]** Gruber L, Schmidt S, Enzlein T, Vo HG, Bausbacher T, Cairns JL, Ucal Y, Keller F, Kerndl M, Sammour DA, Sharif O, Schabbauer G, Rudolf R, Eckhardt M, Iakab SA, Bindila L, Hopf C. Deep MALDI-MS spatial omics guided by quantum cascade laser mid-infrared imaging microscopy. Nat Commun. 2025 May 22;16(1):4759. doi: 10.1038/s41467-025-59839-3.

**[3]** Iakab SA, Marxfeld HA, Richter FM, Geisel A, Ucal Y, Bausbacher T, Henser C, Gröters S, Hopf C. Reliable Identification of Alpha2u-Globulin and Lysozyme Accumulation in Rat Kidney by Label-Free MALDI Mass Spectrometry Imaging. Toxicol Pathol. 2025 Oct;53(7):602-609. doi: 10.1177/01926233251368855.

# PL-03
# From Diagnosis to Cellular Mechanisms: Decoding Rare Inherited Metabolic Disorders through Proteomics, Metabolomics and Lipidomics

**Margherita Ruoppolo [a,b,*]**

[a] DMMBM, Università degli studi di Napoli Federico II, Napoli, Italy; [b] CEINGE Biotecnologie Avanzate Franco Salvatore scarl, Napoli, Italy

Inherited metabolic disorders are a large and heterogeneous group of rare monogenic diseases caused by defects in enzymes or transporters that disrupt metabolic pathways, leading to the accumulation of toxic intermediates, deficiency of essential products and progressive multi organ damage. Newborn screening has radically changed the natural history of several inborn errors of metabolism by enabling presymptomatic diagnosis and early treatment through high throughput biochemical assays, most notably tandem mass spectrometry on dried blood spots [1]. An overview of the current landscape of inherited metabolic disorders and newborn screening programs, highlighting how analytical innovation and shared policies influence which conditions are included and how quality and equity of access are ensured will be provided.
Glycogen storage disease type Ia (GSDIa) is a paradigmatic example to show how multi omics approaches can refine our understanding of disease mechanisms and long term complications beyond conventional biochemical monitoring. By integrating targeted lipidomics, metabolomics and quantitative proteomics in GSDIa patients, we identified a profoundly altered serum lipidome, with specific ceramide signatures and choline related metabolites that correlate with dyslipidemia, insulin resistance markers and visceral adiposity, together with changes in liver derived proteins such as aldolase B[2, 3]. Complementary proteomic and phosphoproteomic profiling in a GSDIa liver model revealed age dependent remodeling of carbohydrate and lipid metabolism and progressive hyperactivation of insulin signaling pathways, driven by distinct patterns of protein phosphorylation. Overall, these data support a model in which post translational modifications act as metabolic sensors and early regulators of disease trajectories, suggesting novel biomarker candidates and potential therapeutic targets and exemplifying how proteomics, metabolomics and lipidomics can bridge diagnosis and cellular mechanisms in rare inherited metabolic disorders.

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**References:**
**[1]** Ruoppolo M, et al., Int J Neonatal Screen. 2025 Sep 26;11(4):86. doi: 10.3390/ijns11040086.PMID: 41133698
**[2]** Pirozzi, F. et al., Sci Rep. 2025 Jul 1;15(1):20658. doi: 10.1038/s41598-025-06272-7.
**[3]** Rossi, A. et al., J Lipid Res. 2024 Oct;65(10):100651. doi: 10.1016/j.jlr.2024.100651. Epub 2024 Sep 19

# PL-04
# Systems ecology of the microbiome: resolving proteins and metabolites critical to human health and disease

**Paul Wilmes**[a,b]

[a] Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg.
[b] Department of Life Sciences and Medicine, Faculty of Science, Technology and Medicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg

The human microbiome exerts profound influence on host physiology through its emergent properties, contributing essential functions that extend beyond taxonomic composition. While large-scale metagenomic studies have illuminated the microbiome's functional potential, they predominantly adopt taxa-centric perspectives, leaving the actual molecular contributions to human health and disease largely unresolved. Central to this gap is the microbiome-derived molecular complex—recently conceptualised as the expobiome—a dynamic mixture of small molecules, (poly-)peptides and nucleic acids with diverse bioactive properties. Despite its critical role in modulating host pathways, systematic characterisation of these components remains limited, constraining mechanistic understanding of microbiome-driven processes in chronic conditions such as metabolic and neurodegenerative diseases.

We have begun to address this challenge. For example, advanced metaproteomic analyses have resolved more than 30,000 small proteins from the human gut microbiome, revealing an extensive repertoire of previously uncharacterized molecules with potential regulatory and signalling functions. Complementary (meta-)metabolomic approaches further enable direct profiling of microbiome-derived metabolites, providing insights into active biochemical pathways rather than inferred potential with impact on the human host side resolved using our Expobiome Map. Together, these technologies offer a powerful means to dissect the functional ecology of the gut microbiome and its impact on host physiology.

Integrated multi-omic strategies combining metaproteomics, metabolomics, metatranscriptomics and metagenomics can delineate molecular interactions between the microbiome and host systems, uncovering pathways implicated in disease initiation and progression. Such analyses are particularly relevant for neurodegenerative disorders, where microbiome-derived molecules may influence neuroinflammation, neurotransmitter metabolism, and gut-brain signalling. Beyond advancing fundamental understanding, these approaches hold translational promise: mapping the functional microbiome can identify biomarkers for early diagnosis and therapeutic targets for precision interventions. Furthermore, the microbiome represents a vast reservoir of bioactive compounds with potential for drug discovery. My presentation will outline the current state of functional microbiome research, highlight recent advances in metaproteomic and metabolomic profiling, and propose a roadmap for leveraging these technologies—from systematic characterisation of the expobiome to mechanistic validation in novel experimental systems—toward microbiome-informed diagnostics and therapeutics for chronic diseases.

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References:
[1] Davin M.E., Sunyer J.O., Delgado L.F., Tavis S.T., Lowndes T., Zafar Z., Caussin J., Halder R., Hickl O., Laczny C.C., Hanslian E., Koppold D.A., Rajput-Khokhar A., Steckhan N., Schade S., Schneider J., Mollenhauer B., Michalsen A., May P., Hettich R.L., Wilmes P.* (2025) Expanding the human metaproteome: enhancing small open reading frame (smORF) prediction and ultra-deep detection of smORF-encoded proteins in the gut microbiome. Nature Communications in review (preprint doi: 10.21203/rs.3.rs-7768562/v1)

**K-01**

# The secret life of peptides

**George Th. Tsangaris***

Proteomics Research Unit,
Biomedical Research Foundation of the Academy of Athens, Athens Greece:

The rapid accumulation of proteomic data highlights the growing need for comprehensive metadata analysis to better interpret complex peptide information. Peptides, fundamental structural components of proteins, are essential for protein identification and functional characterization. While peptide uniqueness has been previously explored, we introduce a novel conceptual framework that systematically examines the relationship between peptide uniqueness, amino acid sequence, and peptide length. Central to this framework is the definition of the **Core Unique Peptide (CrUP)**—the shortest peptide sequence that appears specifically and exclusively in a single protein within a given proteome. Building on this, we define additional categories: **Composite Unique Peptides (CmUPs)**, **Family Unique Peptides (FUPs)**, and **Universal Unique Peptides (UUPs)**. Using these entities, we analyzed the human proteome alongside those of 20 model organisms, collectively forming the **"Uniquome."** This analysis reveals that CrUPs and related unique peptide classes hold distinctive biochemical and evolutionary properties that extend beyond their traditional use in protein identification. Remarkably, our results show that highly conserved sequences across species are represented not by nucleotide strings but by CrUPs, underscoring their fundamental biological significance. Based on these insights, we developed the **Uniquome-Based Protein Identification Method (UB-PIM)**, a novel approach that shifts the focus from conventional searches of in silico–digested peptides to the direct detection of CrUPs in experimentally derived mass spectrometry data. The presence of even a single CrUP within a peptide enables unambiguous protein assignment, enhancing specificity and reducing ambiguities caused by homologous or shared sequences. UB-PIM is compatible with all peptide types and is effective in both data-independent and data-dependent acquisition workflows, as well as in top-down and bottom-up proteomics. It enables confident protein identification from minimal evidence while remaining computationally efficient and broadly applicable. Overall, the introduction of CrUPs, the Uniquome, and UB-PIM offers a powerful framework for exploring proteomic complexity, improving protein identification accuracy, and uncovering new dimensions of molecular uniqueness. This systemic approach lays the groundwork for advanced applications in deep proteomics, translational biology, and precision medicine.

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## K-02

# Targeting metabolic vulnerabilities in cancer and inflammation

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This talk will focus on uncovering metabolic vulnerabilities that link cancer and inflammation, highlighting how altered cellular metabolism can be exploited for therapeutic benefit. By investigating the metabolic rewiring in succinate dehydrogenase-deficient tumours and inflammatory macrophages, we reveal hijackable metabolic pathways that sustain pathogenic states. In cancer, disrupted mitochondrial function drives reliance on alanine transamination and NAD+ biosynthesis to maintain energy production and proliferation. In inflammation, modulation of sugar metabolism alters macrophage activation and cytokine production through effects on key immunometabolic checkpoints. These findings demonstrate how targeting specific metabolic circuits can provide strategies to treat both cancer and inflammatory diseases, emphasizing the potential of metabolism-focused interventions to address diverse pathological conditions.

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# K-03

## Revealing proteomic and phosphoproteomic changes in the temporal lobe, frontal cortex, and corpus callosum of post-mortem brain from patients with schizophrenia

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**Abstract**

Schizophrenia is a debilitating neuropsychiatric disorder associated with disruptions in neurotransmitter systems, cell signaling, and energy metabolism. To enhance our understanding of the molecular basis of schizophrenia, we conducted a comprehensive proteomics and post-translational modifications (PTMs) analysis on post-mortem brain tissue from three distinct regions—the Temporal Lobe, Orbitofrontal Cortex, and Corpus Callosum—from patients with schizophrenia (SCZ) and control (CTRL) subjects. Utilizing mass spectrometry-based proteomics, we identified and quantified over 7,000 proteins across the regions analyzed, representing one of the most extensive datasets from schizophrenia-affected brain tissue to date. Our study revealed significant protein deregulation in the Temporal Lobe, with 623 dysregulated proteins, followed by the Corpus Callosum with 397 dysregulated proteins, and 109 dysregulated proteins in the Orbitofrontal Cortex. Enrichment analysis of these proteins identified key pathways impacted by schizophrenia, including GABAergic signaling, energy metabolism, and RNA processing pathways, particularly in the Temporal Lobe and Corpus Callosum, areas frequently associated with the pathology of schizophrenia.

Further analysis of phosphoproteomics identified 716 phosphosites in the Corpus Callosum, 967 in the Orbitofrontal Cortex, and 237 in the Temporal Lobe. Kinase enrichment analysis indicated altered signaling pathways involved in neurodevelopment, cell differentiation, and synaptic signaling, especially related to ephrin B, ciliary neurotrophic factor, and NMDA receptor pathways. The phosphorylation motif analysis identified 270 phosphorylation motifs specific to schizophrenia, which suggests altered kinase activities impacting synapse and neurotransmitter signaling, autophagy, and energy metabolism. Despite evidence of metabolic dysfunction in schizophrenia, our phosphoproteomics findings indicated no significant alterations in phosphorylation for proteins related to energy metabolism, potentially due to post-mortem PTM degradation. However, kinase-driven dysregulation of the GABAergic system, synaptic processes, and vesicular neurotransmitter transport were consistent across all regions, emphasizing their potential role in disease pathology. Integrative analysis across the three brain regions demonstrated that the Temporal Lobe and Corpus Callosum exhibit highly similar protein expression profiles, while the Orbitofrontal Cortex presented distinct patterns of dysregulation.

These findings highlight the complex interplay of dysregulated protein expression, phosphorylation, and kinase activity that underpins synaptic, metabolic, and neurodevelopmental pathways in schizophrenia. Together, our results provide valuable insights into the pathophysiological mechanisms of schizophrenia, suggesting potential targets for therapeutic intervention and further investigation.

## K-04

# T6SS and beyond: molecular mechanisms shaping *Campylobacter-Bacillus* competition and proteome dynamics in a one health context.

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Bacterial competition represents a critical aspect of microbial ecology with significant implications for the One Health framework, particularly concerning zoonotic pathogens and beneficial microorganisms. *Campylobacter jejuni*, a leading cause of gastroenteritis worldwide, has recently been shown to possess a Type VI Secretion System (T6SS), a sophisticated molecular weapon that enables contact-dependent antagonism against competing bacteria. Understanding also how T6SS influences interspecies interactions it would be important for developing novel intervention strategies in the one health context.

This study investigates competitive interactions between *Campylobacter jejuni* and *Bacillus subtilis*, a ubiquitous environmental bacterium with probiotic potential, through a comprehensive proteomic profiling. We established in vitro co-culture systems using two *C. jejuni* strains—one possessing a functional T6SS and one T6SS-deficient mutant—in combination with *B. subtilis*. Our proteomic approach enabled the assessment of protein expression dynamics, revealing how T6SS activity potentially modulates not only *C. jejuni's* competitive fitness but also triggers adaptive responses in *B. subtilis*. By comparing T6SS-positive and T6SS-negative strains, we could also delineate the possible contribution of other associated mechanisms to proteome remodelling, stress responses, and potential counter-defence systems.

The current findings may advance our understanding of *C. jejuni* warfare mechanisms and at the same time would have implications for the One Health paradigm, informing strategies to control this bacterium and develop microbial-based interventions relevant to human, animal, and environmental health.

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# K-WS-01
# The mitochondrial proteogenome: assessing the functional impact of variants through computational methods

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The human mitochondrial genome encodes a compact, yet functionally essential genome composed of 13 protein-coding genes, 22 tRNAs, and 2 rRNAs. Although small, mtDNA carries a disproportionate clinical impact: over a thousand variants have been implicated in disease, but only a minority are definitively pathogenic, while the majority remain variants of uncertain significance. Variant interpretation is hindered by mitochondrial-specific biology, heteroplasmy, relaxed replication, and limited redundancy, and by the poor performance of general-purpose predictors when applied to mtDNA.

To overcome these limitations, we developed complementary computational frameworks that jointly address the entire mitochondrial genome [1]. For protein-coding genes, APOGEE2 [2] provides an improved ensemble machine-learning model tailored to missense mtDNA variants. By integrating mitochondrial-specific evolutionary metrics, structural descriptors, thermodynamic stability, and curated training sets, APOGEE2 achieves state-of-the-art performance and outperforms commonly used meta-predictors. Spatial analyses further map high-risk regions across OXPHOS complexes, revealing structurally fragile domains and long-range effects not captured by classical features. Bayesian posterior probabilities enable direct alignment with ACMG/AMP pathogenicity criteria.

In parallel, nAPOGEE provides the first unified system for evaluating all possible mitochondrial tRNA and rRNA single-nucleotide variants. Combining phylogenetic profiles, RNA structural domain annotations, post-transcriptional modifications, and RNA-MSM language-model embeddings, nAPOGEE includes dedicated classifiers for tRNAs and rRNAs that show strong performance on independent ClinGen-curated datasets. These models capture the vulnerability of key structural elements, such as the tRNA anticodon loops and functional rRNA domains, supporting the reclassification of poorly characterized non-coding variants.

Together, APOGEE2 and nAPOGEE establish a comprehensive computational framework for the mitochondrial genome. By integrating structural, evolutionary, and machine-learning insights, they reduce uncertainty across both protein-coding and non-coding genes and provide clinically interpretable predictions to support molecular diagnosis of mitochondrial disorders.

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# K-WS-02
## Frontiers in Foodomics: Technologies, Applications, and Future Directions

**Alejandro Cifuentes**

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Nowadays, the use of advanced "omics" tools in food science and nutrition allows investigating topics that were considered unapproachable few years ago. This trend generated a new discipline defined for the first time by our group as "Foodomics" [1-3]. We will introduce in this lecture the discipline of Foodomics, describing its fundamentals, presenting the tools usually employed, with special emphasis on metabolomics. In this lecture, we will also present some of the latest Foodomics results obtained in our laboratory. Namely, these Foodomics works were done: i) to determine the anti-proliferative effect of food ingredients against different human colon cancer cell lines, and ii) to investigate the possibilities of Foodomics in Alzheimer's disease studies.

Whole-transcriptome microarray, proteomics and MS-based non-targeted whole-metabolome approaches were employed to carry out the colon cancer studies. These Foodomics strategies enabled the identification of several differentially expressed genes alone and/or linked to changed metabolic pathways that were modulated by food ingredients in cancer cells, providing new evidences at molecular level on the antiproliferative effect of food compounds.

The neuroprotective activity of different natural extracts from microalgae, foods and food by-products against Alzheimer was investigated in our laboratory via Foodomics using several in vitro assays, blood brain barrier models (BBB) and an in vivo model based on a transgenic Caenorhabditis elegans (strain CL4176), which expresses the human Aβ1–42 protein. A time and dose dependent paralysis assay was performed, and the transcriptomics and metabolomics changes after the treatment were evaluated by RNA-Seq and GC/HPLC-MS technologies, respectively. The integration of these results provides with new evidences on the neuroprotection mechanisms of some natural extracts, representing a step forward on their potential use as valuable sources of neuroprotective compounds.

Future Foodomics challenges will be also discussed in this work. Namely, we will discuss the multiple possibilities of advancing the development of One-Health through future applications of Food Science and Foodomics, showing a vast and unexplored area of research.

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# WS-01
# From Proteome to Powerhouse: Uncovering Mitochondrial Dysfunctions Linking Type 2 Diabetes and Alzheimer's Disease

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Type 2 diabetes mellitus (T2D) approximately doubles the risk of cognitive decline and Alzheimer's disease (AD), with brain insulin resistance and mitochondrial dysfunction emerging as key shared mechanisms. Yet, how altered insulin signaling translates into persistent mitochondrial stress in neurons is still poorly defined. Here, we applied unbiased proteomics to delineate mitochondrial vulnerabilities and, in particular, to characterize mitochondrial unfolded protein response (UPRmt) activation as a potential molecular bridge between metabolic and neurodegenerative disorders. Label-free LC–MS/MS proteomic profiling was performed on hippocampal samples from non-obese GK rats, a model of T2D, and from mice lacking biliverdin reductase-A ($BVRA^{-/-}$), a pleiotropic regulator of insulin signaling and redox homeostasis, previously found reduced in AD brain and T2DM peripheral cells. Quantitative analysis revealed extensive remodeling of the mitochondrial proteome, with coordinated alterations in glycolysis, TCA cycle, oxidative phosphorylation, and the creatine/phosphocreatine shuttle. In parallel, pathway and network analyses highlighted a robust induction of mitochondrial stress modules, including chaperones, and factors controlling mitochondrial proteostasis, consistent with activation of the UPRmt. Cross-species comparison identified a shared set of proteins dysregulated in both GK and $BVRA^{-/-}$ hippocampi, converging on pathways that couple mitochondrial metabolism, antioxidant defense, and synaptic plasticity. These proteomic signatures paralleled functional defects, such as reduced complex I–driven respiration and impaired mitochondrial efficiency, indicating that UPRmt activation represents an early, compensatory attempt to maintain mitochondrial fitness under chronic metabolic stress. By integrating these experimental data with targeted analyses in peripheral cells from individuals with T2D and post-mortem AD brains, we found that impaired BVRA-mediated signaling and maladaptive UPRmt constitute a common axis of vulnerability linking systemic metabolic alterations to neurodegeneration. Overall, our findings illustrate how systems-level proteomics can move us "from proteome to powerhouse", allowing the dissection of UPRmt-centered networks that are shared between T2D and AD. This approach not only highlights candidate targets for therapeutic intervention but also supports the development of mitochondrial proteomic signature as tools to stratify individuals at increased risk of neurodegeneration within metabolically impaired populations [1,2].

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## WS-02

# Bridging Rare Genetic Disorders and Mitochondrial Homeostasis: The Case of ADSL deficiency

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Adenylosuccinate lyase deficiency (ADSLd) is a rare autosomal recessive disorder caused by mutations in the ADSL gene, a key enzyme in the de novo purine synthesis pathway. While classically associated with purine imbalance and substrate accumulation, our work uncovers a novel role of ADSL in maintaining mitochondrial homeostasis. ADSLd patient-derived cells exhibit marked mitochondrial fragmentation, reduced respiration, ATP production, and mtDNA copy number, defects that correlate with clinical severity and preferentially affect high-energy-demanding tissues. We also observed impaired mitochondrial dynamics and trafficking, linked to suppression of ERK2 and AKT signaling. Notably, the mitochondrial phenotype was ameliorated by either overexpression of constitutively active ERK2 or supplementation with purine intermediates. Finally, we demonstrate that ADSL localizes to mitochondria and resides, at least in part, within the organelle, supporting a direct role in mitochondrial integrity. These findings reframe ADSLd as a disorder of mitochondrial dysfunction and open new avenues for therapeutic intervention targeting mitochondrial metabolism.

**WS-03**

# A Dynamic Complexomic Framework to Identify and Deorphanize Mitochondrial Proteins

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Mitochondrial proteins assemble dynamically in high molecular weight complexes essential for their functions. We generated and validated two searchable compendia of these mitochondrial complexes. Following identification by mass spectrometry of proteins in complexes separated using blue-native gel electrophoresis from unperturbed, cristae-remodeled, and outer membrane-permeabilized mitochondria, we created MARIGOLD, a mitochondrial apoptotic remodeling complexome database of 627 proteins. MARIGOLD elucidates how dynamically proteins distribute in complexes upon mitochondrial membrane remodeling. From MARIGOLD, we developed MitoCIAO, a mitochondrial complexes interactome tool that, by statistical correlation, calculates the likelihood of protein cooccurrence in complexes. MitoCIAO correctly predicted biologically validated interactions among components of the mitochondrial cristae organization system (MICOS) and optic atrophy 1 (OPA1) complexes. We used MitoCIAO to functionalize two ATPase family AAA domain-containing 3A (ATAD3A) complexes: one with OPA1 that regulates mitochondrial ultrastructure and the second containing ribosomal proteins that is essential for mitoribosome stability. These compendia reveal the dynamic nature of mitochondrial complexes and enable their functionalization.

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# WS-04
# Detection and Characterization of Microplastics in Edible Marine Species: Implications for Human and Environmental Health

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Plastic pollution has become one of the major global public health challenges. The massive production and widespread use of plastic materials have led to a continuous increase in microplastics (MPs) in the environment, with potentially devastating effects on ecosystems and human health, including their entry into the food chain. Numerous studies have demonstrated that these particles are ingested by a wide range of organisms, including plankton, molluscs, crustaceans, fish, and marine mammals. Consequently, humans may be exposed to MPs through the consumption of water, seafood, and other animal products. In this context, the present study aimed to develop an optimized digestion protocol for the analysis of MPs in marine organisms such as mussels (*Mytilus galloprovincialis*), clams (*Venerupis decussata*), and shrimps (*Litopenaeus vannamei*, synonym *Penaeus vannamei*), in order to investigate the potential implications for human and environmental health. Once the protocol was optimized, the MPs present in the digested mussel and clam samples were characterized and identified.

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**WS-05**

# Gastric and intestinal digestion of infant formula in the presence of polypropylene nanoplastics

**Mariachiara Paonessa[#a*], Emanuela Laratta[#a], Anna C. Procopio[a], Viviana Greco[b,c], Tanja Ćirković Veličković[d,e], Andrea Urbani[b,c] and Paola Roncada[a]**

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The pervasive presence of micro- and nanoplastics (MNPs) in the environment raises significant global health concerns, with oral ingestion being the principal route of gastrointestinal exposure. Accumulating evidence indicates that MNPs can impair digestive enzyme function, including lipid and protein hydrolysis, through mechanisms such as molecular adsorption and conformational disruption, ultimately reducing nutrient bioavailability [1]. Infants are especially susceptible due to gastrointestinal immaturity, characterized by lower enzymatic activity and higher gastric pH, which may exacerbate nutritional deficits [2]. Although polypropylene (PP) is widely used in materials that come in contact with pediatric food, the majority of research has focused on model polymers such as polystyrene, which may not accurately represent exposure scenarios. In this study, we investigated the effects of polydisperse, irregular nanopolypropylene (nPP) particles on the sequential digestion of proteins in a gastrointestinal model designed to mimic infant physiology.

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# WS-06
# Emerging evidence and implications for global health of interactions between microplastics and the microbiota.

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Microplastics (MPs) are ubiquitous contaminants and their interaction with human, animal and environmental microbiota represents a global challenge requiring a One Health approach. In humans, ingesting MPs alters the diversity and abundance of gut microbiota, reducing beneficial bacteria such as *Bacteroides* and *Parabacteroides* and favouring opportunistic pathogens such as *Escherichia*/*Shigella* and *Bilophila*. In farm animals and wildlife, MPs can lead to the proliferation of pathogenic bacteria such as *Mycoplasma*, *Streptococcus* and *Helicobacter*. In insects, MPs influence physiology and the microbiota, with implications for both ecology and health. Mosquitoes exposed to MPs exhibit changes in their gut microbiota (an increase in *Elizabethkingia* and *Aspergillus* and a reduction in *Wolbachia*), which affects their fertility and insecticide resistance. At an environmental level, MPs in marine sediments alter microbial composition and reduce essential processes such as denitrification, thereby compromising biogeochemical balance and the benthic food chain. In soil, MPs influence bacterial and fungal communities, stimulating taxa such as *Burkholderiaceae*, *Fusarium*, and *Aspergillus*, and favouring plant pathogens such as *Xanthomonas*. It is essential to understand the systemic scope of these interactions and to develop innovative mitigation strategies that can protect human, animal and environmental health simultaneously.

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# ORAL COMMUNICATIONS FROM GOLD SPONSORS

# O-S-01
# High-Throughput SPE Membrane Approaches for Peptide Cleanup and Enrichment

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Bottom-up LC-MS/MS workflows typically require multistep sample preparation followed by peptide cleanup to remove salts that would otherwise cause ion suppression and reduce identification rates. For proteoform analysis, enrichment of post-translationally modified (PTM) peptides is also essential to ensure reliable identification, localization, and quantification.
Conventional cleanup strategies are often labor-intensive and prone to sample loss or elution variability. Developing robust, automatable, and standardized cleanup and enrichment methods is therefore a critical step toward establishing proteomics as a practical tool in diagnostics and drug discovery. Our SPE membrane technology, developed through two decades of expertise in complex sample preparation, addresses several of these bottlenecks in MS-based proteomics.
One major challenge lies in high-throughput single-cell and other low-input analyses, where consistent recovery from limited protein material is required. Using optimized membrane workflows, reproducible results were obtained across a 1 ng–10 µg input range, achieving up to 97% protein identification and <3% RSD, demonstrating reliable analysis even from trace-level samples.
In glycoproteomics, the structural diversity of glycans complicates enrichment and detection. Preliminary studies using SPE membrane-based methods showed, on average, a fivefold increase in N-glycopeptide identifications from cell lysates and plasma compared to unenriched controls, reaching or exceeding the performance of established HILIC-based methods.
Phosphoproteomics represents another demanding application, as conventional desalting often results in the loss of polar phosphopeptides. Membrane-based C18 formats enabled up to 2.4-fold more phosphopeptide identifications with reproducibility below 10% RSD. Optimized acidification conditions further improved recovery of hydrophilic and singly phosphorylated peptides.
These findings highlight how SPE membrane workflows deliver efficient, reproducible, and high-throughput solutions for peptide desalting and PTM enrichment, ultimately supporting more robust and sensitive MS-based proteomic analyses in clinical and cancer research.

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# O-S-02
# Enhanced Immunopeptide Identification Using MIDIA-PASEF: A Novel timsTOF Scan Mode

**Jonathan R. Krieger[1], Diego Asis[2], Matt Willets[2], Daniel Vlacil[4], Pierre-Olivier Schmit[5*], Nicholas Cheung[3], Robert Salzler[3]**

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**Introduction:**

Mass spectrometry (MS)-based immunopeptidomics (ipep) is a rapidly advancing field that provides unique biological insights into disease. Traditionally, ipep studies have relied on Data-Dependent Acquisition (DDA) due to the complexity of data analysis. However, Data-Independent Acquisition (DIA) methods are gaining traction for their improved reproducibility and sensitivity, despite challenges in data interpretation. A novel approach, Maximum Information DIA (midia), combines the strengths of both DDA and DIA by acquiring data in DIA mode but processing it in a DDA-like fashion.

**Methods:**

Class I and II immunopeptides were enriched from varying amounts of IM9 B-lymphocyte cells and analyzed using dda-PASEF (60 min) and midia-PASEF (45 min) on a timsTOF Ultra 2 coupled to a nanoElute2 system (Bruker). Both methods targeted relevant ion mobility and m/z ranges. DDA data were processed using Sage and MS2rescore, while midia-PASEF data were analyzed using the midiaID pipeline, enabling direct comparison.

**Results:**

Across input levels (1e6–5e6 cells), midia-PASEF consistently outperformed dda-PASEF in identifying class I 9-mer peptides, with a ~1.2-fold increase (9k–15.5k vs. 8k–12.5k). Sequence motif analysis confirmed biological relevance, showing consistent preferences (e.g., E/L at position 2; V/L/I/A at position 9) across both methods. Peptide overlap averaged 50%, indicating complementary coverage and potential benefits of combining methods when sample quantity allows. Additionally, midia-PASEF enables pseudo-DDA spectrum generation for de novo sequencing. Using BPS Novor, we observed a ~10% increase in peptide identifications when combining midia-PASEF with de novo analysis.

**Conclusion**

These results highlight midia-PASEF as a promising alternative to dda-PASEF for immunopeptidomics, offering improved sensitivity, complementary peptide coverage, and compatibility with de novo sequencing workflows.

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# O-S-03
# Leveraging advanced MS technology for in depth immunopeptide profiling and PTMs analysis

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The major histocompatibility complex (MHC) plays a critical role in adaptive immunity through antigen presentation. MHC associated antigens derived from either endogenous or exogenous proteins are recognized by T cells and trigger an immune response. Immunopeptidomics is the fascinating study of peptides presented by major MHC molecules on the surface of cells. Mass spectrometry-based immunopeptidomics is a rapidly growing proteomics application that enables the identification of MHC associated antigens extracted from biological samples. Targeting MHC antigens isolated using mass spectrometry-based immunopeptidomics provides scientists with novel ways to predict antigens for applications such as cancer treatment, vaccine development, immunotherapy, and drug discovery. This field has drawn interest primarily because the ability to identify tumor-specific antigens (TSAs) can revolutionize personalized medicine.

Identifying these TSAs from clinical samples is analytically challenging because these peptides are low in abundance, lack standard enzymatic cleavage sites, and often must be enriched from limited and precious clinical biopsy samples. Recent advances enabled by the Orbitrap MS technology have allowed unprecedented depth of coverage and accuracy required to take the field to the next level. Additionally, alternative fragmentation techniques such as electron transfer/high-energy collision dissociation (EThcD) expand the detectable immunopeptidome, with better sequence coverage and confidence. Furthermore, EThcD allows the characterization of peptides with unfavourable physicochemical properties, allowing more comprehensive analysis of heavily post-translationally modified peptides, opening new possibilities in areas such as glycoproteomics and de novo sequencing.

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# SELECTED

# ORAL COMMUNICATIONS

# O-01
# Proteomics on the side of rapid diagnosis and personalized treatment of rare neuromuscular diseases

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Neuromuscular diseases (NMDs) represent a group of more than 400 rare disorders that are frequently associated with (early) severe physical disability and often premature mortality. NMDs can either be hereditary or acquired. Despite their clinical impact, only a small subset currently has effective therapeutic options. A deeper understanding of the molecular mechanisms driving disease pathogenesis is therefore essential to identify novel intervention strategies. However, to gain this knowledge, pre-clinical studies are needed which in turn require suitable biomaterials such as cell lines. While patient-derived cells (e.g., fibroblasts and myoblasts) are suitable for hereditary forms of NMDs, specific cell models must be established for acquired forms. Clinical proteomic studies have recently provided valuable insights into pathways contributing to the phenotypic manifestation of rare inherited neurological diseases.

Our project, B2B-Rare, aims to decipher pathophysiological cascades to define new starting points for new intervention concepts by employing multi-omics approaches. For this purpose, patient-derived fibroblasts are used and for some patients, even myoblast lines.

Here, we optimized and evaluated sample preparation protocols that allow simultaneous profiling of the proteome and metabolome from the same cellular material, maximizing the information gained from limited patient samples. The developed protocol was applied to fibroblast samples (derived from different NMD patients and controls). Furthermore, we successfully applied the optimized workflow to myoblast samples, representing both hereditary and acquired forms of muscle diseases described in patients. This approach aimed to demonstrate its broader applicability across disease contexts.

Together, our results support the concept that patient-derived cells, including fibroblasts and myoblasts, combined with integrated multi-omics, provide a powerful platform for elucidating disease mechanisms in NMDs and pave the way for defining new treatment concepts.

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## O-02

# Multiomics-based fluxomics modelling reveals metabolic rewiring in methylmalonic acidemia

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Methylmalonic acidemia (MMA) is a severe inborn error of metabolism characterized by pleiotropic metabolic perturbations and multiorgan pathology. The genetic defect includes diverse mutations in the methylmalonyl-CoA mutase gene (MUT), whose product is involved in the conversion of the methylmalonyl-CoA into succinyl-CoA for the Krebs cycle. As direct consequence of MUT deficiency, upstream metabolites like methylmalonyl-CoA, methylmalonic acid, and propionylcarnitine accumulate in body fluids. While earlier studies have focused on the potential direct toxicity of metabolites as a mechanism to explain the disease, the cellular and molecular defects underlying MMA pathophysiology are still obscure, thus treatment options are limited and non-curative. Fluxomics and genome-scale metabolic models offer a powerful framework for the study of human metabolism and inherited metabolic disorders, representing the entire network of human metabolic reactions with detailed stoichiometry, thermodynamics, and enzyme associations based on the most up-to-date scientific knowledge. Precisely, through the integration of multi-omics profiles, fluxomics data can enhance the interpretation of metabolic alterations connected with MMA allowing the simulation of MUT-deficiency.
Proteomics and metabolomics analyses performed on an in-vitro model of MMA using the HEK293 cell line showed strong dysregulations in MUT-knockout cells. Flux simulations revealed a broad metabolic rewiring in response to the loss of succinyl-CoA production via MUT, indicating compensatory adjustments. Interestingly, a marked alteration in fluxes was observed in amino acid transport between the cytoplasm and the extracellular space. This shift occurred even though none of the 26 quantified amino acid transporters showed differential expression in the proteomic data, indicating that changes in transport fluxes—rather than transporter abundance—may contribute to the altered amino acid concentrations. The incorporation of transcriptional regulation into fluxes together with primary cell measurements will provide the accurate modeling of long-term disease progression.

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## O-03

# Phosphoproteomics reveals novel BCR::ABL1-independent mechanisms of resistance in Chronic Myeloid Leukemia

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Chronic Myeloid Leukemia is a myeloproliferative neoplasm driven by the BCR::ABL1 oncoprotein, a constitutively active tyrosine kinase that promotes uncontrolled proliferation and apoptosis resistance [1]. Despite the therapeutic success of imatinib, a selective BCR::ABL1 inhibitor, around 30% of patients develop resistance, leading to progression and relapse [2]. Resistance mechanisms can be either BCR::ABL1-dependent, when the oncogene is mutated or over-expressed, or BCR::ABL1-independent, when alternative signaling pathways sustain survival and proliferation [3]. In this study, we integrated mass spectrometry-based (phospho)proteomics with *SignalingProfiler* 2.0 pipeline [4], to generate comprehensive networks depicting the BCR::ABL1-dependent and independent signaling maps. To identify therapeutic vulnerabilities in resistant cells, we developed the *Druggability Score* algorithm, a computational tool that ranks proteins based on their ability to kill resistant cells when treated with specific inhibitors. Our integrative analysis identified several druggable targets, highlighting the potential of unbiased, system-level approaches to elucidate resistance mechanisms. Functional validation in patient-derived leukemic stem cells revealed an acquired FLT3-dependency. In conclusion, our study discovers novel BCR::ABL1 resistance mechanisms and repositions FLT3 as a potential therapeutic target for CML relapsed patients [5]. These findings hold promise for the development of more effective therapeutic strategies, with the final aim of improving clinical outcomes for non-responder CML patients.

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# O-04
# Leveraging computational methods for the analysis of the structural impact of the mutational landscape of proteins

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To investigate uncharacterized proteins and their variants, we developed a computational pipeline integrating advanced structure prediction, network analysis, and ligand docking [1]. The workflow begins with the reference sequence of the wild-type (WT) protein and a curated set of known or designed point mutations. Each variant sequence is submitted to AlphaFold3, which predicts the three-dimensional structure using state-of-the-art deep learning algorithms that capture evolutionary constraints and long-range residue correlations. This step provides high-confidence structural ensembles that serve as the foundation for comparative analysis across the mutational landscape. The predicted models are then subjected to latent space analysis to quantify structural similarities and deviations among variants. By embedding the structural features within a reduced-dimensional manifold, we identify patterns indicating local unfolding, altered packing, or specific conformational rearrangements that could predispose certain variants to aggregation. Complementarily, residue contact networks are extracted from each model to assess changes in inter-residue connectivity, identifying weakened stabilizing contacts or newly formed interaction clusters that may modulate fibril formation pathways. Next, ligand docking simulations are employed to evaluate the effect of sequence variations on potential binding sites. Small-molecule ligands are docked onto the predicted structures to assess mutation-dependent differences in binding affinity and orientation. Finally, based on docking outcomes and identified high-affinity pockets, ligand optimization is conducted. Here, iterative in silico modifications are proposed to refine the ligand's chemical structure, improving its complementarity with variant-specific microenvironments. The goal is to suggest molecular scaffolds capable not only of binding across multiple variants but also of stabilizing non-aggregating conformations. This integrated approach enables a systematic exploration of the mutational landscape of potentially fibrillogenic proteins, bridging the gap between sequence variation, structural dynamics, and functional consequences. By coupling predictive modeling with targeted docking and optimization, the framework supports hypothesis generation for experimental validation and sheds light on the sequence-structure determinants guiding fibril formation.

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# O-05

## Quantitative proteomics of plasma protein corona of an innovative type of albumin nanoparticle obtained by supramolecular self-assembly.

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A layer of proteins termed Protein Corona (PrC) forms on the surface of nanoparticles (NP) when they are immersed in biological fluids and PrC significantly affects NP behaviour in biological environments [1]. Albumin NPs (ANP) represent promising carriers because of low cost of fabrication, biocompatibility, and versatility in delivering therapeutics and diagnostic agents [2].
We used quantitative proteomics to study the composition of mouse plasma PrC of two types of ANPs. The first type was obtained by albumin desolvation followed by glutaraldehyde cross-linking and was termed XANP [3], while the second innovative type was obtained by non-covalent supramolecular self-assembly of solvent-free albumin with tetrazole-functionalized tetraphenylethylene derivative (TEZ-TPE-1) [4] and was termed TANP. This latter was conceived as a potential proteolytic degradation sensor. PrCs of TANPs and XANPs were analyzed by gel-free label-free quantitative (LFQ) proteomics [5].
The LFQ dataset included a total of 528 proteins, with a higher number of protein species identified in the TANP-PrC (499) than XANP-PrC (367). Quantitative analysis revealed 130 proteins with differential abundance between TANP-PrC and XANP-PrC. Functional enrichment of the XANP-PrC showed connection with complement system activation and immunoglobulin-mediated response, while TANP-PrC appeared linked with several lipid metabolic processes. Common functional terms shared by the two PrCs were hemostatis, coagulation and wound healing.
Comparison between the top-100 most abundant proteins in both PrCs showed that 73 species were shared between the two PrCs. These species clustered in a dense protein-protein interaction network related to regulation of coagulative and fibrinolytic processes, proteolysis and regulation of protease activity. Indeed, to investigate the link between the diverse particle formulations and the putative proteolytic activity of the PrCs, data intersection with the MEROPS database revealed that ANP-PrC contained a higher number of proteases than XANP-PrC.
In conclusion, this study revealed that TANP-PrC and XANP-PrC showed significant quantitative and qualitative differences suggesting that TANPs may actually have greater susceptibility to degradation and, hence, higher biocompatibility and increased release properties than XANPs. Therefore, TANPs might represent a promising sensor system for analyses of proteolytic activities.

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# O-06
# Untargeted metabolomics approach for unraveling novel Gaucher disease biomarkers on dried blood spot

**Ilaria Cicalini[a], Silvia Valentinuzzi[a], Giorgia Spalluto[a], Francesca Piermatteo[a], Claudia Rossi[a], Piero Del Boccio[a], Vincenzo De Laurenzi[a], Luca Federici[a], Damiana Pieragostino[a]**

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Gaucher disease (GD) is a rare lysosomal storage disorder arising from β-glucocerebrosidase (GBA) deficiency which causes glucosyl-sphingosine overload. Diagnosis can occur in the neonatal period through neonatal screening programs, by assessing glucocerebrosidase enzyme activity in leukocyte samples or dried blood drops, combined with molecular analysis of the GBA1 gene for genetic confirmation. The symptomatology of Gaucher disease is broad and heterogeneous[1]. The work's aim is to employ a holistic metabolomics approach as an innovative and highly promising strategy for studying the pathogenetic basis of GD. Untargeted metabolomics represents a key discipline for systematically and hypothesis-driven understanding of the metabolic state of an organism or specific biological matrices, obtaining valuable insights into physiological functioning, pathological alterations, and the underlying molecular mechanisms[2]. The DBS of GD patient before and after therapy were subjected to solid-liquid extraction, analyzed by high-resolution mass spectrometry, and compared with a healthy subject. Data were acquired and analyzed from statistical e functional point of view Compound Discoverer, Metaboanalyst, and IPA software. Approximately 12,000 features were quantified, of which 378 were correctly identified. Numerous phosphatidylethanolamines and metabolites such as sphingosine 1P and dihydrosphingosine 1P were significantly downregulated in the GD patient compared to healthy controls, while some phosphatidylcholines were upregulated in patient. The metabolites found to be significantly modulated appear to be implicated in some functional pathways such as cellular infiltration by phagocytes and inflammatory processes, which represent a key hallmark of the disease. As a proof of concept, we found the expected LysoGb1 increasing in the GD patient and decreasing after treatment[3]. Moreover, we found a new putative biomarker (m/z 288.9976), which has impressively high levels in the GD patient compared to the HC and decreases after therapy. To conclude, our data highlight the importance of omics analysis of metabolomic profiles in understanding the pathogenic mechanisms of Gaucher disease, strengthening the idea that the metabolomic approach can not only identify new biomarkers but also broaden our understanding of the molecular mechanisms underlying the disease, thus offering insights for the development of more targeted therapeutic strategies.

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# O-07

## Mitochondrial proteomics and metabolomics highlight energy metabolism disruption in methylmalonic acidemias

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Methylmalonic acidemias (MMA) are severe congenital metabolic disorders marked by widespread metabolic disturbances and damage to multiple organs. The condition arises from mutations in the methylmalonyl-CoA mutase (MUT) gene, which encodes an enzyme responsible for converting methylmalonyl-CoA into succinyl-CoA, a key intermediate of the Krebs cycle. The enzymatic defect is responsible for the accumulation of upstream metabolites in biological fluids, including methylmalonyl-CoA, methylmalonic acid, and propionylcarnitine. Previous investigations mainly addressed the potential cytotoxic effects of these accumulating metabolites to explain the disease phenotype. However, the cellular and molecular mechanisms driving MMA pathogenesis remain poorly defined, resulting in limited and non-curative therapeutic options [1,2].
To better understand the mitochondrial dysfunction in MMA, MUT-deficient cellular systems were employed [1]. Mitochondria were isolated from these models, and their proteomic profile was examined through LC–MS/MS coupled with bioinformatic analyses to identify dysregulated proteins and biological pathways. The data revealed profound disorganization of the mitochondrial proteome accompanied by striking morphological defects explained by reduced mitochondrial mass and presence of rounded organelles.
Mitochondrial activity was also severely compromised, as indicated by elevated reactive oxygen species (ROS) levels and decreased cell viability, particularly under propionate-enriched culture conditions [3]. Complementary metabolomic and respirometric assessments demonstrated clear impairment of central carbon metabolism and disrupted energy homeostasis in MMA cells, with a marked reduction in total ATP synthesis largely attributable to mitochondrial dysfunction.
Overall, these findings highlight major structural and functional abnormalities of mitochondria in MMA, offering new insights into the disease's mitochondrial dynamics and contributing to a more comprehensive understanding of its pathophysiology.

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# O-08
# High-Resolution AP MALDI-MSI Identifies Lipid Metabolic Signature in the Marinesco-Sjogren Syndrome mouse model Woozy

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Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) provides valuable insights into the identification, quantification, and spatial distribution of biomolecules within tissues. This ionization technique, coupled with a High-Resolution Orbitrap MS analyzer improved performance and made the discovery phase more robust and reliable [1]. Marinesco-Sjogren Syndrome (MSS) is an infantile-onset disease characterized by ataxia, cerebellar atrophy, and muscle weakness. It is linked to a loss-of-function mutation in the Sil1 gene which encodes a nucleotide exchange factor for the Endoplasmic Reticulum (ER) chaperone BiP3. Despite widespread Sil1 expression in brain, the mutation leads to protein accumulation, chronic Unfolded Protein Response activation, and cell death only in cerebellum Purkinje cells, sparing the vestibulocerebellum (lobule-X and caudal lobule-IX) [2]. To investigate the molecular basis of this cell-specific mutation effect, we performed high-resolution spatial lipidomics on cerebellar slices from woozy MSS mice. Using a MassTech™ Atmospheric-Pressure MALDI source with ultra-high spatial resolution coupled to an Orbitrap Exploris 120, we analyzed homozygous and heterozygous samples (the latter serving as the control). The optimized sample preparation and MS method were applied to compare the cerebellar lipid signatures between the two conditions and imaging data for both ionization modes were processed using LipostarMSI. The analysis of the cerebellar arbor vitae revealed an evident lipid signature in the Woozy model, supporting the hypothesis of a direct involvement of lipid metabolism in the pathogenesis of MSS and providing insights into the metabolic adaptations cells undergo to mitigate ER stress and avoid neurodegeneration. In particular, we observed dysregulation of sphingolipid metabolism, a pathway linked to various neurological disorders [3]. Among the altered lipids, a downregulation of GM4 and an upregulation of the sulfonated form of psychosine, a known cytotoxic biomarker of Krabbe disease, were detected in the neurodegenerating cerebellar lobules but not in the non-degenerating ones. This spatially resolved lipidomic approach allowed, for the first time, an untargeted molecular exploration across distinct cerebellar areas, confirming the site-specific nature of neurodegeneration in this model. This novel finding highlights a potentially unexplored mechanism in MSS pathology and may open new avenues for the discovery of biomarkers for diagnostic and therapeutic approaches currently lacking.

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## O-09

# Intrinsic N-Terminal Reactivity of Carbamate-based Cross-Linkers and their improved analysis

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Cross-linking mass spectrometry (XL-MS) has emerged as a powerful approach for probing protein structure and conformational dynamics [1]. Conventional cross-linkers typically contain two N-hydroxysuccinimide (NHS) ester groups that primarily target lysine residues. Here, we report the optimization of the in-solution reactivity of disuccinimidyl sulfoxide carbamate (DSSO carbamate), an analogue of DSSO [2] in which the two NHS ester groups are replaced by NHS carbamates. The enhanced stability of the carbamate functionality reduces the degradation of DSSO through retro-ene sulfoxide elimination under standard XL-MS buffer conditions, thereby improving cross-linking efficiency.

We further characterized the gas-phase dissociation behavior of DSSO carbamate and optimized the collision energy (CE) parameters for automated data analysis with XL-MS search engines. Cross-link site mapping of bovine serum albumin revealed an unexpectedly high frequency of cross-links involving the protein's N-terminus, suggesting increased N-terminal reactivity of NHS carbamates relative to NHS esters. This hypothesis was confirmed through comparative cross-linking of non-acetylated and N-terminally acetylated α-synuclein using DSSO carbamate and the NHS ester–based disuccinimidyl dibutyric urea (DSBU) [3]. Finally, the same reactivity trend was observed for the NHS carbamate–based cross-linker NNP9 [4]. Together, these results show that NHS carbamate–based reagents provide complementary XL-MS restraints to NHS ester–based cross-linkers and are particularly useful for investigating systems where N-terminal interactions are functionally relevant. We anticipate that this unique N-terminal selectivity of NHS carbamates will find broader applications in bioconjugation and chemical proteomics.

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# O-10
# Decoding Copper Adaptation mechanisms in Marine Bacteria through Multi-Omics and Network Analysis

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This study, conducted in collaboration with the Stazione Zoologica Anton Dohrn (Naples, Italy), investigates adaptative molecular mechanisms of marine bacteria isolated from metal-contaminated sediments in the Gulf of Naples and Bagnoli. [1][2] All isolates were screened by determining the maximum tolerance concentration (MTC) to As, Cd, Co, Cu, Zn, and Pb.[2][3] Among them, *Pseudohalocynthiibacter aestuariivivens* P96 was selected for in-depth investigation under copper exposure. Copper was chosen for its dual significance as both an essential enzymatic cofactor and a strategic raw material recognized under the European Union's *Critical Raw Materials Act* [4], making it relevant for both bioremediation and bioleaching applications.[5] Genomic and proteomic analyses were performed to elucidate the mechanisms underlying bacterial adaptation to copper. *MRGs* screening revealed determinants related to copper homeostasis, including multicopper oxidases, efflux systems, and transports then confirmed by differential proteomic analysis. [6] Due to the phylogenetic distance of this isolate from reference organisms, conventional annotation and pathway reconstruction tools proved largely ineffective; to overcome this limitation, a correlation-based approach integrating omics data was applied for enabling the reconstruction of putative pathways involved in copper response, further supported by literature validation. [7]
This integrative omics strategy provides new insights into bacterial adaptation to copper stress and demonstrates the effectiveness of correlation-based network protein analysis for functional inference in non-model organisms, identifying promising candidates for in situ bioremediation and bioleaching in metal-contaminated environments.

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# O-11

# SSIMO – Single Section Integrative Multi-Omics – spatial mapping of metabolites and lipids combined with regional specific proteomics in a single tissue slice

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Understanding the spatial distribution of molecular features within tissues is essential for advancing translational research and precision medicine. Traditional omics approaches often require multiple serial sections or independent preparations, limiting spatial resolution and data integration.

Here we propose a true multi-omics workflow designed to extract a wide range of molecular and histological information from a single tissue section. This integrated approach combines spatial metabolomics and lipidomics with histopathology and spatially guided proteomics to deliver a holistic view of tissue biology. Building upon conventional MALDI imaging workflows, the method employs ITO-coated glass slides and matrix application, followed by staining procedures and laser microdissection (LMD). Regions of interest (ROIs), defined on the basis of prior imaging data, are precisely excised for downstream proteomic analysis. A pivotal innovation lies in adapting LMD to function directly on ITO slides, bypassing the need for conventional membrane-based substrates. By optimizing the laser focal point to penetrate the glass, the "draw and scan" function enables efficient and accurate recovery of ROIs.

This workflow allows the acquisition of spatially resolved metabolomic, lipidomic, and proteomic information from a single 10–12 µm tissue section. The study demonstrates the method's reproducibility, integrity, and technical feasibility, and provides a proof-of-concept application in a preclinical tumor biology setting.

The ability to correlate location-specific molecular profiles with histopathological features establishes this workflow as a powerful tool for multi-omics tissue analysis. Its integration of spatial molecular data within a single section offers new opportunities for understanding disease mechanisms and developing targeted therapeutic strategies in translational and precision medicine contexts.

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# O-12
# Gaining insights into the anticancer activity of an antimicrobial myristoylated peptide by integrating proteomics and bioinformatics

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Antimicrobial peptides (AMPs) are natural peptides produced by all organisms as components of innate immunity. As they show a broad spectrum of activities against multidrugresistant (MDR) human pathogens, strong efforts are in progress to bring AMPs into clinical use to counteract antimicrobial resistance. AMPs are also being investigated as anticancer drugs to overcome side-effects and/or tumor resistance to chemotherapy. Hence, based on the scaffold of chionodracine, a natural AMP, we previously designed a mutant and its three shorter Nmyristoylated (myr) peptides active against MDR pathogens [1]. Herein, the anticancer activity of the three myr peptides was explored on the human cervical cancer cell line HeLa. We demonstrated that myr peptides are cytotoxic against HeLa cells (IC50 of 32–47 μM), but spare healthy human fibroblasts; whereas non myr peptides failed to kill cancer cells. The myr peptide with the lowest IC50 and no hemolytic activity was selected for further analyses. We used label-free shotgun quantitative proteomics to study the molecular effects of myr peptide at IC50 doses on HeLa cells compared to untreated and non-myr peptide-treated cells. LC-MS/MS data enabled the quantification of about 4200 proteins. Differential analysis identified 364 proteins showing significant changes across the pairwise comparisons. Bioinformatics unveiled that the main processes influenced by the myr peptide are “mitochondrial translation”, “mRNA splicing” and “rRNA processing”. Notably, exposure of HeLa cells to myr peptide led to the under-expression of the “apoptosis- and splicing associated protein” complex, which is known to be disassembled upon apoptosis induction [2]. In line with proteomics data, cellular assays demonstrated that myr peptide induces apoptosis and advanced necrosis in HeLa cells. Overall, the modification of natural AMPs may be a promising strategy to develop selective anticancer drugs.

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# O-13
# Improve biomarker discovery: Enrichment-based workflows for plasma, serum and CSF allow rapid, in-depth analysis of large cohorts of samples

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Objectives:
A case study of biomarker discovery unsing ENRICH-iST for plasma samples lung cancer patients will be presented. In addition, the development and optimization of a tailored protocol for cerebrospinal fluid (CSF) samples will be described. The work concludes with perspective outlook on the future of ENRICH technology.

Methods:
ENRICH technology enables efficient dynamic range compression by enriching low-abundance proteins in biological fluid samples on non-functionalized paramagnetic microbeads. This step is followed by the iST-BCT protocol for protein denaturation, reduction, alkylation, digestion and peptide clean-up before analysis by nLC-MS/MS in diaPASEF® (Bruker). Firstly, the ENRICH-iST kit was compared to the iST-BCT kit to prepare a clinical cohort of human plasma from lung cancer patients (n=10) for in-depth biomarker discovery. Secondly, to address the challenge of low protein concentration in CSF samples, the ENRICH step was optimized by adjusting sample volumes from 20 µl to 200 µl.

Results:
Lung cancer plasma samples allowed the identification of more than 1450 protein groups using ENRICH-iST, a more than 2-fold increase compared to neat plasma. Statistical analysis showed a clear stratification between healthy donors and patients, and 16 additional proteins significantly regulated compared with neat plasma were identified as potential biomarkers, including S100A8 and S100A9, already known in the literature.

Given the low protein concentration of CSF (~0.1 mg/mL) compared to plasma/serum (~60-80 mg/mL), the ENRICH step was optimized to ensure successful compression of the dynamic range. Processing 200 µL of mouse CSF resulted in the identification of ~1800 proteins.

Conclusions:
The ENRICH technology is an automatable, high-throughput and efficient tool for diverse scientific applications, including the in-depth discovery of biomarkers from a now even wider range of biological fluids.

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# O-14
# Bridging proteomics and metabolomics through effective sample preparation

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The comprehension of the interactions between proteomics and metabolomics is fundamental to understand metabolic regulation in cells. This is important to elucidate the mechanism of diseases such as tumors and cardiovascular diseases (CVDs), as alterations in cellular metabolism are closely linked to their development and progression.
By combining data from different molecular classes, multiomics yields a more complete drawing of the function of cells and tissues.
In traditional workflows where single classes of molecules are targeted, samples are divided into different aliquots for specific omics analyses. This causes differences in the initial distribution of molecules and consequent bias, making it intricate to accurately compare the different omics levels.
In order to reach valuable conclusions from this multiomics approach, we tested and compared different workflows for simultaneous proteomics and metabolomics profiling deriving from the same tissue [1]. A conjoined sample preparation has the benefit to reduce sample variation and input amount. Furthermore, it is particularly suitable in case of limited sample availability.
In this study, we were able to profile the global proteome with an untargeted data-independent acquisition (DIA) approach, with a good reproducibility, and completeness of data. We then compared the recovery of the metabolites between the different extraction protocols by using stable isotope-labelled internal standards (SIL-IS), to ensure high accuracy and reproducibility.
After evaluating proteomics and metabolomics data, we identified the most efficient protocol which provides the best result for both classes of molecules.
This approach can open new perspectives for multiomics studies of tissue metabolism for animal models and clinical applications and provide further data integration for a broader understanding of metabolic pathway activities and flux.

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## O-15
## Synaptic signatures and disease vulnerabilities of layer 5 pyramidal neurons

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Cortical layer 5 (L5) intratelencephalic (IT) and pyramidal tract (PT) neurons are embedded in distinct information processing pathways. Their morphology, connectivity, electrophysiological properties, and role in behavior have been extensively analyzed. However, the molecular composition of their synapses remains largely uncharacterized. Here, we dissect the protein composition of the excitatory postsynaptic compartment of mouse L5 neurons in intact somatosensory circuits, using an optimized proximity biotinylation workflow with high spatial accuracy. We find distinct synaptic signatures of L5 IT and PT neurons that are defined by proteins regulating synaptic organization and transmission, including cell-surface proteins (CSPs), neurotransmitter receptors and ion channels. In addition, we find a differential vulnerability to disease, with a marked enrichment of autism risk genes in the synaptic signature of L5 IT neurons compared to PT neurons. These results align with human studies and suggest that the excitatory postsynaptic compartment of L5 IT neurons is susceptible in autism. Our approach is versatile and can be broadly applied to other neuron types to create a protein-based, synaptic atlas of cortical circuits.

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# O-16
# Integration of Immunopeptidomics and Phosphoproteomics: From Kinase Control to Antigen Presentation

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Despite the success of tyrosine kinase inhibitors, in Chronic Myeloid Leukaemia (CML) resistance and relapse remain major clinical challenges, underscoring the need for innovative therapeutic strategies[1]. Recently, immunotherapeutic approaches have shown significant promise for enhancing immune-mediated disease control, but their efficacy relies on identifying tumor associated antigens [2,3]. These antigens must be effectively processed and presented on the cell membrane through the major histocompatibility complex (MHC), a process which remains largely unexplored in CML. As protein kinases are central regulators of signaling and protein turnover, their activity critically influences which peptides are processed and ultimately presented on MHC molecules, shaping the antigen repertoire available for T cell recognition. Here, we introduce a novel multi-omics approach that systematically dissects how signalling kinases regulate the tumour immunopeptidome. Specifically, we investigated whether pharmacological inhibition of key kinases, including BCR-ABL, JNK and LCK, could reshape the immunopeptidome of CML cells, enhancing the exposure of specific peptides. By employing high-sensitive mass spectrometry (MS)–based immunopeptidomic, we quantified more than 20,000 HLA-I peptides. Of these, approximately 4,000 peptides showed altered presentation in response to pharmacological inhibition of LCK, JNK, and BCR-ABL, including numerous tumour-associated antigens (TAAs) detected also in primary CML samples. But even more importantly, by integrating our immunopeptidomic data with phosphoproteomics and network-based approach we identified three complementary molecular mechanisms through which kinases shape the antigen repertoire: i) phosphorylation-dependent modulation of HLA-I peptides, ii) regulation of source protein stability, and iii) transcriptional regulation of source proteins. Collectively, our work establishes a framework to rationally combine kinase inhibitors with immunotherapies to enhance antigen visibility and improve antileukemic immunity.

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# O-17
# Alternative splicing of transcripts encoding the innate immune, stress-activated protein kinase PKR

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Genomic diversity plays critical roles in risk of disease pathogenesis and diagnosis. While genomic variants are commonly detected at the DNA or RNA level, their translated variant protein or polypeptide products are ultimately the functional units of the associated disease. The impact of alternative splicing (AS) on the proteome is therefore controversial, as it often remains unclear whether and to what extent the alternative transcript observed in deep sequencing data will result in relevant amounts of alternative protein products (*isoforms*).
Profiling of the *interferon-inducible double-stranded RNA dependent protein kinase R* (PKR) transcripts expressed in osteosarcoma (OS) cell lines and patient-derived xenografts (PDX), revealed the expression of numerous alternatively spliced isoforms of PKR, most of which have never been reported or characterized. Analysis of other bone-related tumor cell lines in the Broad Institute's CCLE database similarly revealed an elevated level of alternatively spliced PKR transcripts. Unlike what is observed in acute myeloid leukemia (TCGA-LAML database) and soft tissue sarcomas (TCGA-SARC database), alternatively spliced PKR transcripts accounted for over half of the PKR gene products in OS cell lines and patient samples (Target Osteosarcoma).
*In silico* analysis indicates that a number of the encoded PKR isoforms function as dominant negatives, decoys, and endogenous inhibitors of PKR, which are expected to alter normal IFN-dependent signaling and associated MSC-to-osteoblast differentiation. In addition to the identification of novel cellular modulators of PKR, these data suggest a broader role of altered innate immune signaling in the origin and progression of OS and presents a possible proteogenomic strategy for detection of putative biomarker candidates in osteosarcoma.

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# O-18
# A Uniquome based method for the protein identification by mass spectometry

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Protein identification by mass spectrometry is a pivotal step in proteomics. Numerous methods have been developed to securely and effectively identify proteins derived from experimentally detected peptides by mass spectrometry. The dominant approach is based on the assumption that each experimentally identified peptide can be matched with a peptide included in a database of peptide sequences, generated by in silico digestion of proteins with a specific proteolytic enzyme. In this way, the protein containing the peptide can be identified. In a more advanced approach, the proteins and their in silico–digested peptides in the database are transformed into theoretical mass spectrometry spectra, and search engines match the experimentally obtained spectra to these theoretical spectra generated from protein and peptide sequences. We developed an alternative method for protein identification using Core Unique Peptides (CrUPs) and the Uniquome, termed as **Uniquome-Based Protein Identification Method (UB-PIM)**. According to this method, instead of searching for peptides in the database of *in silico*–digested peptides, we search for CrUPs within the experimentally obtained peptides by mass spectrometry. If a peptide contains at least one CrUP, it can be directly correlated to the protein from which the CrUP is derived. Because of the unique nature of CrUPs, peptides obtained by MS can securely and uniquely identify the protein of origin. This provides a reference space in which even single-peptide identifications can achieve high specificity, reducing the ambiguity caused by shared or homologous sequences and improving the interpretability of MS data. Furthermore, UB-PIM can be applied to any type of peptide and is effective with both Data-Independent Acquisition (DIA) and Data-Dependent Acquisition (DDA) approaches, as well as with top-down and bottom-up proteomics. This allows confident protein identification from minimal evidence, expands the scope of detectable proteins, and remains computationally efficient, rapid, and universally applicable.

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# O-19
# In silico analysis for the development of a multiepitope-based vaccine against the monkeypox.

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The monkeypox virus (MPXV) is an emerging zoonotic pathogen with pandemic potential. Its spread is driven by intensified human–wildlife contact, deforestation, the illegal wildlife trade and global mobility. Rationally designing vaccines based on proteome-derived immunogenic epitopes is a promising strategy for preventing future outbreaks.
For this study, the Zaire 96-I-16 strain (clade I), which is associated with the most virulent forms of MPXV, was chosen for in silico vaccine design. The complete viral proteome was screened using predictive immunoinformatics pipelines to identify the proteins with the greatest immunogenic potential. Epitopes recognised by B cells, helper T cells ($CD4^+$) and cytotoxic T cells ($CD8^+$) were selected using a multilayer computational workflow that integrated affinity, immunogenicity, sequence conservation, peptide length and HLA compatibility.
The selected epitopes were then concatenated using flexible peptide linkers to preserve their conformational integrity and immunological functionality. The three-dimensional structure of the multi-epitope construct was modelled using AlphaFold2, and was then refined and validated using geometric and structural analyses [1]. The resulting model demonstrates a stable and plausible configuration, which is suitable for further proteomic and immunogenic evaluation.

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# O-20

# A Polyphenol-Enriched Waste Product from *Origanum vulgare* Hydrodistillation Drives Metabolic and Proteomic Reprogramming That Enhances the Anti-*Candida* Activity of *Lactobacillus helveticus* and *Lactobacillus rhamnosus*

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*Candida albicans* (CA) is an opportunistic pathogen and a commensal member of the intestinal microbiota, but specific pathophysiological conditions can promote invasive infections [1]. Probiotics such as *Lactobacillus helveticus* (LH) and *Lactobacillus rhamnosus* (LR) are known to support microbiota homeostasis [2], while polyphenols provide antioxidant, anti-inflammatory, and microbiota-modulating activities [3]. This study investigated the modulatory effect of LH and LR stimulated by lyophilized residual water (ORAC9) obtained from *Origanum vulgare* hydrodistillation, which is rich in rosmarinic acid, on intestinal CA growth. ORAC9 selectively enhanced *Lactobacilli* spp. growth and metabolism, resulting in a significant inhibition of CA proliferation during co-culture. Metabolomic analysis revealed that ORAC9-treated *Lactobacilli* more efficiently metabolized key substrates, including amino acids (L-Alanine, L-Glutamic Acid, L-Histidine, D-Aspartic Acid, L-Aspartic Acid), organic acids (Citric Acid, α-Ketoglutaric Acid, D-Malic Acid, Propionic Acid, Acetic Acid), sugars (Stachyose, L-Arabitol, D-Saccharic Acid), and biogenic amines/derivatives such as γ-Amino-N-Butyric Acid, highlighting a global enhancement of central metabolic pathways. Proteomic profiling confirmed the overexpression of enzymes involved in these metabolic processes, coherently supporting the observed metabolic activation. ORAC9 also promoted anti-inflammatory gene expression in Caco-2 cells and exhibited no toxicity *in vitro* or *in vivo*. Overall, ORAC9 potentiates the anti-*Candida* activity of *Lactobacilli* spp. by stimulating their growth, metabolism activity, and anti-inflammatory properties, supporting its potential role in candidiasis prevention and as an adjunctive therapy in the context of rising antimycotic resistance.

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# O-21

## Integrated Linear and Cross-linking Proteomics reveal stress-adaptive Endoplasmic Reticulum dynamics

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The endoplasmic reticulum (ER) is the largest organelle in the cell and serves as a central hub for numerous cellular processes, including protein synthesis and transport, protein folding, lipid and steroid production, carbohydrate metabolism, and calcium storage. Its diverse functions demand a wide array of specialized proteins, intricate structural organization, and the ability to coordinate with and adapt to changes in the intracellular environment [1].

Although numerous proteins and pathways that regulate ER architecture and dynamics have been characterized [2], how the ER interactome adapts to cellular stress remains largely unknown. To address this critical gap, we generated a comprehensive and dynamic map of ER-specific interactions by combining linear proteomics with a cross-linking mass spectrometry (XL-MS) approach [3].

To enable a precise and specific analysis of the ER interactome, we first established a dedicated tool for ER isolation, employing an endogenous immunoprecipitation (ER endo-IP) strategy using the ER-resident Reep5 protein as an affinity handle. Proteomic analysis of the ER endo-IP allows the identification of a broad repertoire of ER-associated proteins, including luminal components as well as proteins localized at contact sites with other organelles. Quantitative proteomics upon ER stress revealed extensive remodeling of the ER proteome, with stress-type–specific patterns.

Complementary XL-MS analysis further delineated the ER interaction landscape, providing a structural dimension to stress-induced remodeling. This approach yielded a network comprising unique residue-to-residue connections across more than 1,000 ER proteins, revealing stress-specific protein–protein interactions (PPIs) absent under physiological conditions. Several of these putative *de novo* PPIs were further corroborated by AlphaFold-based structural predictions, which revealed plausible interaction interfaces and suggested potential adaptive rewiring within the ER.

Collectively, our strategy offers a comprehensive framework to profile ER sub-compartment and to clarify how this organelle dynamically adjusts to physiological and pathological cues.

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## O-22

# Proteomics-based development of iRhom2 inhibitors

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The pro-inflammatory cytokine tumor necrosis factor (TNF) is essential for host defense against pathogens, but its dysregulated production contributes to several inflammatory diseases, including rheumatoid arthritis. TNF is synthesized as a transmembrane precursor (pro-TNF) mainly by immune cells and released as soluble TNF through cleavage by a disintegrin and metalloproteinase 17 (ADAM17, also known as the TNF convertase or TACE). Despite its therapeutic potential, ADAM17 inhibition failed clinically due to (i) poor selectivity over related metalloproteases sharing a conserved catalytic domain, and (ii) its ubiquitous expression, which causes systemic dysregulation of more than 50 substrates.

iRhoms have recently emerged as essential regulators of ADAM17 trafficking, maturation, and activation. Most mammalian tissues express two family members, iRhom1 and iRhom2, which can compensate for each other in supporting ADAM17 function. However, immune cells express only iRhom2; its loss inactivates ADAM17 and impairs TNF release, whereas ADAM17 activity in other tissues remains intact due to the compensatory role of iRhom1. Thus, selective inhibition of iRhom2 would specifically block ADAM17-mediated TNF release in immune cells while preserving its functions elsewhere, making it a promising strategy to treat inflammatory diseases.

In this study, we applied high-resolution proteomics to systematically characterize ADAM17 substrate shedding, revealing novel substrates and previously unrecognized functions of the protease. Using this workflow, we demonstrated that most substrates are shed similarly when ADAM17 is regulated by either iRhom1 or iRhom2, although a subset showed preferential shedding depending on the associated iRhom. We then used this proteomic platform as a screening cascade to evaluate the specificity of putative iRhom2 inhibitors. Computational modelling was employed to generate a structural model of the iRhom2–ADAM17 complex and identify potential druggable sites. Virtual screening of small-molecule libraries against these sites yielded candidate inhibitors, which were first tested for their ability to reduce TNF release in stimulated macrophage-like cells and then validated for iRhom2 selectivity using the proteomic assay.

In summary, we established a proteomic framework to define ADAM17 substrates and their regulation by iRhom1 and iRhom2, and used it to assess the specificity of newly developed iRhom2 inhibitors. These compounds will next be tested in pathological mouse models of TNF-driven diseases, with potential applications in the treatment of human inflammatory disorders.

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# POSTERS

## P-01
# Fast and robust phosphoproteomics sample prep with AttractSPE® Disks C18 Tips for high phosphopeptide recovery and identification

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**Purpose:**
Phosphorylation is one of the most prevalent and important post-translational modifications proteins can undergo. Over 50% of the human proteome is phosphorylated and understanding the dynamic phosphorylation across the proteome can understand the progression of many diseases including cancers. As the stoichiometry of phosphorylation sites is generally very low, enrichment steps, followed by SPE clean-up before LC-MS/MS analysis are required to enhance identification and quantification of each site. However, recovery of phosphopeptides can be greatly affected by the choice of clean-up method, resulting in severe losses.
**Methods:**
Different SPE C18 options were compared for the purification of phosphopeptides, after automated enrichment using magnetic beads (Ti/Zr-IMAC). Effects of sample acidification prior to SPE clean-up on phosphopeptides detection were also assessed by acidifying the enrichment elution with different percentages (2, 3, 4 and 5%) of phosphoric acid or trifluoroacetic acid (TFA).
**Results:**
Among all SPE options tested, AttractSPE®Disks Tips C18 provided the highest recovery of phosphopeptides (up to 2.4 times more identifications), with high reproducibility (RSD < 10%). AttractSPE®Disks Tips C18 captured more efficiently hydrophilic peptides, and shorter phosphopeptides were retained compared to other brands.
Quenching the enrichment elution with 3% phosphoric acid provided the highest recovery, with 8% more identifications compared to 5% TFA. Lower acid concentration interestingly provided more singly phosphorylated peptides, while higher acid concentration recovered more hydrophilic peptides. This trend was observed for both acids tested but was more pronounced for TFA.
**Conclusion:**
AttractSPE®Disks Tips C18 are shown to be the best choice for phosphopeptide purification, offering simplicity of use by centrifugation, high sample recovery, and robustness. These SPE Tips are easily scalable with their availability in different sizes and binding capacities to perfectly adapt to different sample amounts, and can be provided as 96 and 384 SPE well plates for high throughput processing.

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## P-02
## Integrative multi-omics analysis of PITRM1/Cym1 dysfunction in yeast models

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Pathogenic variants in the human *PITRM1* gene have been identified as the cause of progressive spinocerebellar ataxia and associated with Alzheimer's disease. *PITRM1* encodes a mitochondrial metalloprotease that plays a crucial role in the degradation of mitochondrial targeting sequences (MTS). When mitochondrial precursor proteins are imported into the mitochondria, they undergo proteolytic processing, resulting in the release of free MTS peptides in the mitochondrial matrix. These peptides are subsequently degraded by MTS scavenging peptidases. These enzymes were initially discovered and characterized in the yeast *Saccharomyces cerevisiae*, including CYM1, which is encoded by the *PITRM1* ortholog. Proteomics analysis performed from our laboratory suggested that PITRM1 dysfunction perturbs mitochondrial metabolism and protein homeostasis, while the transcriptomic analysis revealed alterations in energy metabolism and protein homeostasis, consistent with a compensatory response to mitochondrial stress.

In this work, we aimed at further elucidating the role of PITRM1 peptidase by integrating transcriptomics and proteomics data in yeast models expressing distinct pathogenic *CYM1* variants, including variants associated to ataxia and Alzheimer's disease. Proteomic and transcriptomic datasets were integrated using both supervised and unsupervised computational methods, such as correlation analysis and latent factor-based approaches, to identify disease-associated pathways and uncover relationships between transcript and protein alterations.

This integrative strategy provided a deeper view of the molecular consequences of PITRM1/Cym1 dysfunction, highlighting both shared and variant-specific signatures of mitochondrial stress and proteostasis imbalance.

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# P-03
# Proteomics mapping of the molecular signature of surgically treated epileptic lesions for the development of a comprehensive diagnostic refinement protocol

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Epilepsy, one of the most prevalent chronic neurological diseases, affects 1% of the global population[1]. While anti-seizure medications are effective for approximately 70% of patients, 30% remain resistant to therapy. For those with drug-resistant epilepsy (DRE), surgery is a safe and effective treatment. DRE surgical specimens exhibit a broad spectrum of structural brain lesions, including focal cortical dysplasia (FCDs; types I, II, and III), hippocampal sclerosis, low-grade developmental tumours, and other less common alterations[2]. This study aims to compare protein expression profiles in lesional, perilesional, and control tissues using a shotgun proteomic approach to identify biomarkers for epileptic lesions. The initial phases of the project focused on optimizing protein extraction methods from FFPE (formalin-fixed paraffin-embedded) to maximise the yield of extraction procedure and of sample clean-up. After the application of each extraction protocol, the optimisation procedure was monitored according to the peptide quantification and the numbers of proteins identified. Moving forward, a label-free differential proteomics approach has been employed for the analysis of samples representative of different epileptic brain lesions to univocally identify potential protein targets whose abundance is altered in correspondence with the lesions. The non lesional surrounding region of each sample has been excised and analysed as control.
Up to now, 8 lesions from FCDIIa and 6 relative perilesions have been analysed, as well as 14 FCDIIb lesions and 12 perilesions; fold changes of up and down regulated proteins have been measured among all biological replicates for each subset of samples to characterize the molecular signatures associated with each histopathological entity. The results obtained from differential proteomic approach will be compared with those obtained using MALDI-imaging strategy to establish a comprehensive molecular profile for each FCD subtype.

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# P-04
# Brain proteomics in DiGeorge syndrome reveals stage-specific vulnerabilities

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DiGeorge Syndrome (22q11.2DS) represents the most common genetic microdeletion syndrome and is associated with a broad spectrum of congenital anomalies, including cardiac malformations,craniofacial defects, and immune dysfunction. Individuals with 22q11.2DS also face an increased risk of neurodevelopmental and neuropsychiatric conditions, such as cognitive impairments, anxiety disorders, and a substantially elevated incidence of schizophrenia. A notable proportion of the genes lost in the 22q11.2 deletion (approximately 10%) are implicated in metabolic regulation, suggesting that metabolic dysregulation within the brain may contribute to the observed neurological phenotype. The deleted chromosomal region contains several genes whose haploinsufficiency may influence neural metabolism, with *Tbx1* emerging as one of the most extensively characterized. Despite this, the mechanistic link between these genetic deletions and downstream neurobiological alterations remains insufficiently understood. In this study, we investigated proteomic changes in the brain of a well-established *Tbx1*$^{+/-}$ mouse model of DiGeorge Syndrome, in which the *Tbx1* gene—encoding the T-box transcription factor TBX1—undergoes a loss-of-function mutation. Wild-type (WT) mice were used as controls. Brain tissues were collected at multiple developmental time points to characterize early and late molecular alterations associated with the disorder. Total proteomes were extracted and analyzed by shotgun liquid chromatography–tandem mass spectrometry (LC-MS/MS). Comparative analyses were conducted between *Tbx1*$^{+/-}$ and age-matched WT mice at 2, 4, and 7 months of age. Our data revealed that early signatures of dysregulation driven by *Tbx1* haploinsufficiency were already evident at 2 months, particularly affecting neurotransmitter transport and synaptic signaling pathways. Then, at 4 months, mice with DiGeorge Syndrome exhibited an intermediate proteomic profile. Finally there was a pronounced shift in the brain proteome at 7 months, marked by significant disruption of metabolic and energy-related pathways closely associated with neurodegenerative processes.
Overall, this study identifies proteomic alterations associated with DiGeorge Syndrome and provides new insight into both quantitative and qualitative changes in brain protein expression in a relevant mouse model, advancing understanding of the molecular mechanisms underlying this complex neurodevelopmental disorder.

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# P-05
# Characterization of the MMACHC Protein Interactome Reveals Novel Insights into Cobalamin Metabolism

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Methylmalonic aciduria and homocystinuria type C protein (MMACHC) is a cytosolic chaperone protein involved in the biosynthetic pathway of cobalamin, involved in a key biosynthetic step of both adenosylcobalamin (AdoCbl) in mitochondria and methylcobalamin (MeCbl) in the cytosol. AdoCbl acts as the cofactor for the mitochondrial enzyme methylmalonyl-CoA mutase, which catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA, an intermediate of the Krebs cycle [1]. MeCbl, in turn, is the cofactor for the cytosolic enzyme methionine synthase, which catalyzes the remethylation of homocysteine to methionine. Mutations in the MMACHC gene cause early-onset combined methylmalonic acidemia and homocystinuria type cblC, a rare inborn error of propionate and methionine metabolism. Although MMACHC's role in AdoCbl and MeCbl biosynthesis is well established, its precise molecular functions remain incompletely understood To address this, we conducted a study aimed at identifying the protein interactors of MMACHC.
A human hepatocellular carcinoma cell line (HepG2) was stably transfected to generate a cell population overexpressing FLAG-tagged MMACHC (FLAG-MMACHC). The cell line overexpressing FLAG-GFP was used as a control. MMACHC interactors were identified through anti-FLAG immunoprecipitation followed by quantitative, gel-free, label-free proteomic analysis using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The interaction of MMACHC with selected putative partners was further validated by western blot analysis of immunoprecipitated samples.
Analysis of the MMACHC interactome led to the identification of several putative MMACHC-binding proteins. Many of these interactors are involved not only in metabolic processes but also in the regulation of cell division and proliferation, microtubule dynamics, and cell junction organization. These findings provide new insights into the physiological functions of MMACHC. A better understanding of MMACHC's molecular role may contribute to identifying novel therapeutic targets for patients affected by cblC-type methylmalonic acidemia.

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# P-06
# Analysis of S-glutathionylated proteome in a cell model of cardiac hypertrophy

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Protein glutathionylation is a reversible post-transductional modification that has a key role in protein function regulation and strictly depends on redox cell balance. It is thought that protein glutathionylation counteracts irreversible protein oxidation and then oxidative stress, a key feature of the cardiac hypertrophy [1]. In this study, we investigate the glutathionylation of proteins extracted from H9c2 cardiomyoblasts treated or not with palmitic acid (PA). This model has been reported to closely recapitulate the morphological features of cardiac hypertrophy [2].

H9c2 rat cardiac cells were growth in Dulbecco's modified Eagle medium and treated with PA at a final concentration of 200 μM for 24h. Total proteins were extracted and glutathionylated proteins (PSSGs) were enriched by immunoprecipitation. PSSGs were then eluted from beads using a low pH glycine buffer and digested with trypsin in a non-reducing environment by a filter aided procedure. The peptide mixture was analysed by nano-High Performance Liquid Chromatography-ElectroSpray Ionization- tandem Mass Spectrometry (nHPLC-ESI-MS/MS).

Using this analytical approach, we were able to detect and identify 396 and 250 PSSGs in control and PA-treated H9c2 cells, respectively. Among them, 83 PSSGs were exclusively detected in PA-treated cells. Functional analysis indicates that PA treatment activates the glutathionylation of key metabolic enzymes mainly involved in carbohydrate catabolism and structural cytoskeletal proteins, suggesting that cardiomyoblast hypertrophy is associated to a PSSG fingerprint. Although the effect of these specific protein glutathionylations on protein structure and function should be still assessed, our data indicate that, in line with previous studies [3], H9c2 cells undergoes to wide metabolic rewiring and cytoskeletal remodelling linked to cardiac cell hypertrophy induced by PA treatment.

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## P-07

# A Multi-Omic Characterization of the Ultra-Rare Infantile *NFIX*-Related Malan Syndrome: Preliminary Insights into the Molecular Landscape

**Michela Cicchinelli[a,*], Lavinia Santucci[b], Jacopo Gervasoni[b], Manuela Priolo[c], Chiara Leoni[b], Roberta Onesimo[b], Anna Percio[a], Giuseppe Zampino[b,d], Andrea Urbani[a,b], Federica Iavarone[a,b]**

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First described only in 2010 [1], *NFIX*-related Malan syndrome (MALNS) is an ultra-rare autosomal dominant disorder typically caused by a *de novo* genetic alteration, characterized by overgrowth, macrocephaly, musculoskeletal features, developmental delay of varying severity with neurobehavioral and psychiatric manifestations. Its estimated prevalence is about 1:1,000,000 children [2] with fewer than 90 cases reported worldwide. With no consensus clinical diagnostic criteria published to date, MALNS is established in a proband with suggestive findings and a heterozygous pathogenic variant in *NFIX* (75%) or a heterozygous deletion of 19p13.2 that includes *NFIX* (25%). The molecular consequences of *NFIX* dysfunction in MALNS are still unknown, and the metabolic and proteomic profiling of this pathology could help identify altered biological pathways and cellular mechanisms, explain the variability in clinical severity among patients and provide relevant insights. This study represents an initial attempt to characterize the MALNS spectrum using a multi-omic strategy, involving the comprehensive profiling of a cohort of 17. For metabolomic analysis, a panel of 107 small molecules and 912 lipids, belonging to 39 biochemical classes, was measured in serum using ultra-performance liquid chromatography/flow injection analysis coupled with tandem mass spectrometry (UPLC-MS/MS and FIA-MS/MS). For proteomic analysis, salivary samples were analyzed using a top-down approach, processed with Proteome Discoverer 3.2 (Thermo Scientific) and ProSightPD 4.4 node for top-down analysis. Bioinformatic analysis and pathway enrichment were carried out to characterize variations in the metabolome and proteome across samples. The results highlighted intra-group variability in specific biochemical classes, the involvement of various pathways related to immune defense, stress response, and cell communication, and the identification of potentially relevant proteins in the context of analgesics for inflammatory and neuropathic pain. This study provides a first integrative overview of the metabolomic and proteomic alterations in Malan syndrome, suggesting possible molecular contributors to its phenotypic variability.

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**P-08**

# Exploring the role of the mitochondrial peptidase PITRM1 in neurodegeneration through yeast proteomics

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The human *PITRM1* gene encodes a peptidase involved in the degradation of the mitochondrial targeting sequences (MTS). Biallelic pathogenic variants in *PITRM1* have been identified to cause progressive spinocerebellar ataxia, and other variants (missense point mutations) have been associated to Alzheimer's disease (AD) [1]. MTS scavenging peptidases have a central role in the maintenance of mitochondrial homeostasis. Indeed, when mitochondrial precursor proteins are imported and proteolytically processed, free MTS peptides are released in the mitochondrial matrix, where they must be degraded to avoid the formation of toxic aggregates. These proteases have been initially discovered and characterized in the yeast *Saccharomyces cerevisiae*, where cym1 represents the PITRM1 ortholog.

Here, we used quantitative differential proteomics analysis in yeast to better characterize the function of MTS scavenging peptidase, and understand their role in neurodegeneration. To this purpose, the proteome (both total and mitochondria-enriched) of five *S. cerevisiae* models was analysed: $cym1^{R163Q}$ mutant (ataxia), $cym1^{F589L}$ mutant (AD), $cym1^{L833F}$ mutant (AD), $cym1^{\Delta}$ (null mutant), and $cym1^{wt}$ (expressing the *wild-type* cym1 protease). After extraction, protein samples were processed and trypsin digested to perform shotgun proteomics by standard LC-MS/MS. More than 3000 proteins have been identified and quantified, with a good coverage of the yeast mitochondrial proteome (around 50%). Univariate statistics was applied to identify differentially expressed proteins (DEPs). Then, over-representation analysis (ORA) was used to obtain lists of significantly enriched biological pathways. The functional enrichment highlighted "metabolic pathways", "carbon metabolism", "pyruvate metabolism", "TCA cycle", "fatty acid degradation" and "glycolysis/gluconeogenesis" as main pathways, with a specific enrichment of mitochondria-related processes (*e.g.*, "cellular respiration", "oxidative phosphorylation", "mitochondrial gene expression", "mitochondrial transport", and "mitochondrial protein complexes"), suggesting a general malfunction of mitochondria in the presence of *cym1* mutant alleles. Pairwise comparisons have also highlighted some variant-specific pathways linking mitochondrial stress to cytoplasmic quality control. Overall, these results underline the crucial role of mitochondrial proteases alterations in neurodegenerative diseases.

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## P-09

# Hyperferritinemia and analytical challenges: can proteomics make the difference?

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Hyperferritinemia is a frequent but diagnostically complex laboratory finding, as elevated ferritin levels may indicate true iron overload or reflect hyperinflammatory processes. Extremely high ferritin concentrations are more often observed in hyperinflammatory syndromes such as hemophagocytic lymphohistiocytosis or Multisystem Inflammatory Syndrome in Children (MIS-C) following SARS-CoV-2 infection. We report the case of a 13-year-old patient admitted to the Emergency Department with a diagnosis of MIS-C secondary to SARS-CoV-2 infection. Initial laboratory results revealed marked hyperferritinemia (5,300 µg/L, Atellica IM) consistent with the clinical presentation. However, during follow-up, ferritin levels rose unexpectedly (>16,500 µg/L), exceeding the assay's linear range even after automatic dilution, despite clinical improvement and normalization of other inflammatory markers. To investigate the cause of this discrepancy, a retrospective proteomic analysis was conducted on serum samples collected across seven time points (days 1–19). For shotgun analysis, albumin, IgM, and IgG were removed by in-gel digestion and fractionation prior to LC–MS/MS analysis. A total of 2,163 proteins were identified. Ferritin was accurately detected, showing a stable abundance pattern across all time points, indicating no true fluctuation in protein levels. Using a 50% fold-change threshold, pathway enrichment analysis revealed that differentially expressed proteins were significantly associated with cardiac injury and inflammatory responses, consistent with MIS-C pathology. These findings confirmed that the extreme fluctuations in ferritin concentration observed in immunometric assays were artefactual, most likely due to the formation of ferritin–immunoglobulin macrocomplexes following intravenous immunoglobulin (IVIG) therapy, which interfered with the immunoassay's antibody recognition. This case illustrates the first documented application of proteomic analysis to elucidate analytical interference in ferritin measurement. The proteomic approach provided molecular evidence supporting the presence of assay artefacts and demonstrated its value as a complementary diagnostic tool in clinical chemistry. Integrating proteomics into laboratory workflows may enhance the reliability of biomarker interpretation, particularly in cases of discordant or incongruent biochemical results.

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# P-10
# Growth-Dependent CDKL5 Phosphorylation in Pseudoalteromonas haloplanktis TAC125

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CDKL5, a member of the cyclin-dependent kinase family, is abundantly expressed in the central nervous system and participates in neuronal migration and synaptic function. Dysregulated CDKL5 expression or activity underlies the severe, currently incurable, CDKL5 deficiency syndrome (CDD)1,2. Because the phosphorylation status of CDKL5 is closely related to its functions and regulatory circuits, defining its activity and control mechanisms is urgently needed. The Antarctic marine bacterium Pseudoalteromonas haloplanktis TAC125 (PhTAC125) has emerged as a promising tool for the recombinant production of complex proteins3, thanks to its atypical physiology and enabling molecular toolkit4. Notably, PhTAC125 has been reported as the only prokaryotic host capable of producing full-length human CDKL55. Using five distinct culture conditions, we investigated how environmental inputs modulate CDKL5 phosphorylation and the implications for cell signalling, solubility, stability, and overall quality of the recombinant product. Mass spectrometry delineated the phosphorylation landscape and revealed site-specific phosphorylation for each condition, highlighting limited activity of the full-length protein6. We then expressed only the catalytic domain to test whether the highly unstructured C-terminus compromises stability and activity. We also catalogued co-purifying proteins that could interfere with CDKL5 isolation, providing insight into purification outcomes. Preliminary data indicate that the catalytic domain alone improves stability and activity, and that specific contaminants measurably affect purification. Establishing optimal growth conditions should therefore provide useful information on CDKL5 phosphorylation levels and activity, guiding production strategies and ultimately facilitating the development of targeted drugs aimed at alleviating and/or improving neurodevelopmental disorders related to CDKL5 dysregulation.

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# P-11
# Peptidomic and Proteomic Fingerprint mozzarella cheese by LAP-MALDI MS and Machine Learning

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Buffalo mozzarella is a complex dairy matrix whose molecular composition reflects both its origin and production process. This study employed LAP-MALDI mass spectrometry coupled with machine learning to characterize the lipidic, peptidic, and proteomic fingerprints of buffalo mozzarella in comparison to cow mozzarella. The resulting molecular profiles revealed distinctive spectral patterns and peptide signatures that can serve as reliable markers for mozzarella type differentiation and quality assessment.
Preliminary analysis revealed several discriminant ions of peptidic/protein specific to cow mozzarella. Among those, one at m/z 941.03 was identified via MS/MS analysis as a peptide with the sequence YQEPVLGPVRGPFPIIV (monoisotopic mass 1880.056 Da, score 83.1), showing a 3.86-fold higher intensity in cow milk mozzarella. This peptide is contained in both buffalo and cow β-casein (aa 208-224).
The two beta casein orthologs share 97.77% homology varying for the peptides in positions 40, 56, 83, 107 and 163 (Uniprot Sequence Alignment). These differences in the sequences might increase the susceptibility of cow's β-casein to proteolytic cleavages by gastrointestinal enzymes, likely promoting the release of the bioactive peptide YQEPVLGPVRGPFPIIV. Another reason might be tied to the higher abundance and fermentation/protein cleavage activity of *Streptococcus thermophilus* in cow milk that may promote the differential generation of the bioactive peptide YQEPVLGPVRGPFPIIV in cow and buffalo mozzarella.

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# P-12

# Extracellular vesicles as key features for the identification of potential biomarkers for insulin resistance and "double diabetes" in pediatric patients affected by type 1 diabetes

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Cases of individuals with Type 1 Diabetes Mellitus (T1DM) developing concomitant insulin resistance, referred to as 'double diabetes', are increasingly observed, particularly in association with obesity. In order to identify novel potential metabolic biomarkers of IR, we aim to provide more detailed metabolic profile of children affected by "double diabetes," with a specific focus on the molecular cross-talk mediated by extracellular vesicles (EVs). Pediatric patients were classified based on their eGDR (estimated Glucose Disposal Rate) values in: insulin-resistant (T1DM+, eGDR < 8 mg/kg/min, n=29) and non–insulin-resistant (T1DM-, eGDR > 8 mg/kg/min, n=35). Venous blood collected from these patients and from 30 healthy controls was used to obtain dried blood spots (DBS) for the analysis of amino acids (AAs) and acylcarnitines (ACs) via FIA-MS/MS, and for the isolation of 2 million of EVs through a patented flow cytometry-based method [1]. The isolated EVs underwent shotgun proteomics analysis, revealing that EVs isolated from T1DM+ patients were able to carry proteins involved in the suppression of fatty acid metabolism through STAT3 inhibition and are associated with potential liver damage. Among these, fatty acid-binding protein 5 (FABP5), whose genetic polymorphisms have been linked to T2DM, was quantified as unique protein in T1DM+ EVs. ACs measurements on DBS supported these findings showing a significant increase in long-chain ACs, especially oleoylcarnitine (C18:1), linoleylcarnitine (C18:2), and myristoylcarnitine (C14), in T1DM+ patients. Moreover, the integration of clinical and metabolic data through a Random Forest algorithm led to the development of a statistical model with an out-of-bag error rate of 0.115%, demonstrating that palmitoleoylcarnitine (C16:1) and C18:1 were the metabolites that best discriminate children with T1DM+ from those with T1DM-. Notably, C16:1 showed a significant correlation with eGDR ($p = 0.0023$). In conclusion, the targeted metabolomics and proteomics approaches have synergistically revealed a new metabolic profile in a complex scenario involving diabetes complications related to obesity and IR in a pediatric cohort not yet fully characterized, highlighting EVs as structured and functionalized carriers of important metabolic pathways.

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# P-13
# Phospho-proteomic profiling of Calu3 cells infected with SARS-CoV-2 reveals Bcl2-associated athanogene 3 (BAG3) phosphorylation and AKT/MAPK signaling activation

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Although the secreted role of BAG3 has been extensively characterized in cancer, where it promotes tumor progression via interaction with interferon-induced transmembrane protein 2 (IFITM2) and stimulation of cytokine release, its involvement in viral infections remains poorly understood [1,2]. Our previous data indicate that BAG3 is also secreted by SARS-CoV-2-infected Calu3 cells and IFITM2 has recently been documented as a critical co-receptor for SARS-CoV-2 entry and replication in human cells, but the underlying molecular mechanisms is unclear [3]. To gain insights into phosphorylation-driven mechanisms underlying infection, we performed label-free shotgun phospho-proteomics on Calu-3 cells under infected (INF) and uninfected (NEG) conditions. Phosphoproteins were digested by SP3 protocol, enriched with $TiO_2$ and analyzed by nanoLC-Orbitrap-MS/MS. Data were processed with Proteome Discoverer, STRING and Ingenuity Pathway Analysis (IPA) tools. Quantification of 1.719 phospho-peptides, corresponding to 444 significantly modulated phosphoproteins, revealed a pronounced infection-associated remodeling of phospho-proteome in INF compared to NEG condition. BAG3 was detected as unique infection-specific protein in the INF condition with five identified phosphorylation sites residues (S173, T285, S289, S377, S386) which are mainly described in cancer, while their role in modulating IFITM2 binding is largely unexplored. As proof of concept, the most differentially phosphorylated proteins between INF and NEG were consistently linked to SARS-CoV-2 infection and to biological processes annotated as infectious disease and SARS-CoV-2 interactions with host intracellular machinery, indicating that post-translational modifications, in particular phosphorylation, are fundamental in infection mechanisms. Both phosphorylation and virus–host interaction mechanisms converged on this protein subset. Pathway analysis highlighted the activation of AKT1 and MAPK1 as major upstream regulators linked to viral pathways, as well as biological functions activated in INF cells prominently included activation of viral replication networks. Our data prove that SARS-CoV-2 infection induces extensive phosphorylation changes in lung epithelial cells, positioning BAG3 and AKT/MAPK signaling as central mediators of host–virus interaction. In conclusion, we can speculate that BAG3/IFITM2 axis may coordinate phosphorylation-driven remodeling during SARS-CoV-2 infection, influencing viral entry and propagation, and warranting further investigation and validations.

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**P-14**

# Proteomic Insights into CAR-T Cell-Derived Extracellular Vesicles: Exploring Subtypes with Therapeutic Potential

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Chimeric antigen receptor (CAR)-T lymphocytes represent the most innovative form of immunotherapy against cancer and have revolutionized cancer treatment of hematological malignancies. However, despite their successes, many challenges remain to be addressed, including their severe adverse effects, such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) [1]. Extracellular Vesicles (EVs) are membrane-enclosed vesicles released by all cell types, carrying a biomolecular cargo (consisting of proteins, mRNA, and microRNAs) driving a massive intercellular crosstalk [2]. CAR-T cell-derived EVs potentially mimic the anti-tumor activity of their parental cells, limiting cell-associated toxicity. To explore the role of CAR$^+$EVs, we carried out a shotgun label-free proteomics approach to characterize the protein profile of EVs released by CAR-T cells. To this end, we isolated EVs by a newly developed method based on Fluorescence Activated Cell Sorting (FACS), able to purify specific EV subtypes using a recognition strategy based on antibody–antigen interaction. In our study, we applied this method to purify and characterize CD19.CAR$^+$EVs from both the blood of patients treated with commercial CD19.CAR-T cells and from commercial CAR-T cell products. Morphological analysis confirmed that CD19.CAR$^+$EVs had a mean diameter of less than 200 nm, classifying them as small EVs. Proteomic analysis revealed that 97.17% of the proteins from circulating and preinfusion CD19.CAR$^+$EVs were shared, and most of them were involved in pro-apoptotic pathways, as confirmed by *in vitro* killing assays. Moreover, a deeper proteomics analysis of EVs derived from different CAR-T cell subpopulations, including those expressing exhaustion markers such as PD-1 and LAG-3, identified a pro-apoptotic functional signature specifically linked to CD8$^+$LAG-3$^+$ EVs. These findings suggest that EVs released by exhausted CAR-T subsets retain molecular features associated with cytotoxic activity, thereby contributing to the overall functional landscape of CAR-T–mediated responses. Overall, our data highlight the potential of CD19.CAR$^+$EVs as biologically active components of CAR-T therapy and support the rationale for further studies aimed at evaluating their safety, mechanism of action, and possible application as adjuncts or alternatives within next-generation, cell-free immunotherapeutic strategies.

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## P-15

# ER-phagy regulates ER dynamics during myogenesis

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Endoplasmic reticulum (ER) plasticity and ER-phagy are intertwined processes essential for maintaining ER dynamics. We investigated the interplay between two isoforms of the ER-phagy receptor FAM134B in regulating ER remodeling in differentiating myoblasts. During myogenesis, the canonical FAM134B1 is degraded, while its isoform FAM134B2 is transcriptionally upregulated. The switch, favoring FAM134B2, is an important regulator of ER morphology during myogenesis. FAM134B2 partial reticulon homology domain, with its rigid conformational characteristics, enables an efficient ER reshaping. FAM134B2 action increases in the active phase of differentiation leading to ER restructuring via ER-phagy, which then reverts to physiological levels when myotubes are mature and the ER is reorganized. Knocking out both FAM134B isoforms in myotubes results in an aberrant proteome landscape and the formation of dilated ER structures, both of which are rescued by FAM134B2 re-expression. Our results underscore how the fine tuning of FAM134B isoforms and ER-phagy orchestrate the ER dynamics during myogenesis providing insights into the molecular mechanisms governing ER homeostasis in muscle cells.

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## P-16

# Telomeric G-quadruplex interactome reveals novel protein biomarkers associated with breast cancer

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The identification of reliable biomarkers is essential for improving breast cancer (BC) detection, prognosis, and treatment. This study explores a human telomeric G-quadruplex (G4) model, tel46, functionalized on Controlled Pore Glass (CPG) support, as a novel biomarker discovery tool. The oligonucleotide tel46 mimics multimeric G4 structures in telomeric overhangs. Using affinity purification–mass spectrometry (AP-MS), 93 proteins interacting with tel46 were identified from nuclear extracts of MCF7 cells, linking them to pathways in DNA replication, repair, and genome stability—frequently altered in cancer. Integrating AP-MS data with quantitative proteomics comparing MCF7 to non-tumorigenic MCF10A cells, 27 tel46 interactors were found among upregulated proteins. Functional analyses revealed enrichment in genome maintenance and repair pathways, while downregulated proteins were related to basic cellular functions. Further bioinformatics validation using public cancer proteomics datasets confirmed 19 candidates. Transcriptomic and clinical data analysis highlighted MSH6, MSH2, ESRP1, and WDHD1 as the most promising biomarkers for BC. These proteins are highly expressed in BC and correlate with poor prognosis; beyond diagnostic value, they could represent therapeutic targets enhancing radiosensitivity or reducing tumor proliferation. As a proof-of-concept, this study proposes tel46-functionalized CPG as a potential platform for isolating cancer-related proteins and underscores the potential of G4-interacting proteins as biomarkers for BC diagnosis and therapy. These findings establish a foundation for future investigations into G4-mediated oncogenic mechanisms.

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# P-17
# Optimization of sample preparation for identification of host and bacterial proteins in environmental samples

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Biological matrices contain a complex mixture of microbial proteins whose efficient and reproducible extraction is crucial for reliable metaproteomic analyses. Accurate identification of proteins of microbial origin depends on robust extraction protocols tailored to the characteristics of each sample type. In this study, three matrices were selected to represent complementary aspects of the One Health continuum: bovine faeces, as a model linking agriculture, animal health, and food production; manure, reflecting microbial processes occurring outside the host; and seagull guano, an environmental indicator of marine ecosystem health and anthropogenic impact.
To optimise sample preparation, different amounts of starting material (10, 20, 30, 50, and 100 mg) were tested. Protein concentration was quantified by the BCA assay to evaluate extraction efficiency and reproducibility. Extracted proteins were processed using the S-Trap protocol, digested into peptides, and analysed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). Given the intrinsic variability among biological matrices, a single extraction protocol cannot ensure consistent results across species and environments. The development of matrix-specific, standardised workflows is therefore essential to obtain reliable and comparable proteomic data, providing a solid foundation for the functional characterization of microbial communities in a One Health perspective.

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## P-18

# Multi-omic analysis of gut microbiota functionality to unveil possible neurological targets for pediatric migraine understanding.

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Migraine is a multifactorial neurological disorder affecting over 15% of the global population. The relationship between gut microbiota and migraine pathophysiology is an emerging area of interest in the field of gut-brain axis interactions. This study combined metaproteomic and metabolomic liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of fecal samples from a cohort of migraine patients (MIMIC) and a control group of healthy subjects (CTRL).

Metaproteomic analysis was performed using a shotgun, bottom-up approach, searching for bacterial proteins against a customized database based on 16S rRNA metataxonomy evidences [1]. Data processing was carried out using dedicated software and an *in-house* bioinformatics pipeline to compile quantitative, taxonomic and functional analyses. The data-independent (SWATH) approach was used for metabolomics LC-MS/MS analysis, using a manually curated spectral library to identify features.

Our findings revealed significant upregulation of metaproteins related to amino acid metabolism and biosynthesis when MIMIC subjects were compared with CTRLs. In particular, pathways involving arginine, tryptophan and glutamate are related to neurotransmission, neuroinflammation and nitric oxide production, and are of particular interest in relation to migraine. The complementary metabolomic profiling identified some altered metabolites associated to these pathways, further supporting our evidences and enforcing the hypothesis of a gut-brain axis role in migraine.

This combined multi-omics approach may show promise in identifying potential microbial targets and metabolic biomarkers, which could enhance our understanding of migraine and improve treatment options.

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**P-19**

# Dissecting early and late *in vitro* mineralization mechanisms in *Pseudoxanthoma elasticum* fibroblasts: a quantitative proteomic analysis

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Pathological calcification is an unsought complication of several acquired and genetic conditions; however, no effective treatments have been found so far due to complexity of pathogenic mechanisms and the lack of suitable identified targets. Within this context, *Pseudoxanthoma elasticum* (PXE), an autosomal recessive disease characterized by elastic fibers' calcification in various tissues and organs (*e.g.,* skin, eyes and blood vessels), is regarded as a paradigm of heritable calcification disorders [1]. Although oxidative stress has been demonstrated in patients as well as and in *in vitro* cultured patients' fibroblasts, and mesenchymal cells (*e.g.*, fibroblasts) actively modulate the extracellular environment by regulating the balance between pro- and anti- calcifying factors [2], the molecular mechanisms driving the pathologic mineralization are still elusive.

This study aimed to characterize the proteomic profile associated with the early (24h) and late (21 days) responses of PXE patients' fibroblasts (n=5 female) cultured with or without pro-osteogenic factors (*i.e.*, Calcifying Medium, CM, containing 10mM β-glycerophosphate, 50 µg/ml ascorbic acid, and 100 nM dexamethasone). Cells were divided into four experimental groups: 24h untreated, 24h CM, 21days untreated, and 21 days CM. At each time point, the proteins were extracted in RIPA buffer and processed using Filter-Aided Sample Preparation protocol [3]. A dedicated data-independent acquisition spectral library was built following gas-phase fractionation method [4].

Principal Component Analysis demonstrated a strong, time-independent responsiveness of fibroblasts treated with CM. Gene Ontology enrichment analysis revealed a significant over-representation of proteins associated with mitochondrial processes, extracellular matrix organization, and elastic fiber formation in both early and late phases. In particular, CM treatment modified several mitochondrial proteins involved in energy metabolism over 21-day period, suggesting a key connection between cellular bioenergetics and the onset of *in vitro* mineralization.

In conclusion, our proteomic data provide a molecular map of ectopic mineralization-related pathways in PXE fibroblasts, suggesting mitochondrial pathways as promising targets for preventing pathological calcification.

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# P-20

# Unveiling stress adaptation mechanisms of *Listeria monocytogenes* through proteomic analysis: towards improved outbreak prevention

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*Listeria monocytogenes* is a major foodborne pathogen responsible for human listeriosis, one of the most severe infections under EU surveillance. In 2024, EFSA and ECDC reported 2,952 confirmed cases, including 1,497 hospitalizations, with a significant upward trend from 2019 to 2023, confirming its status as the fifth most reported zoonoses in the EU [1]. The pathogen primarily affects vulnerable populations such as pregnant women, individuals aged 65 years or older, and immunocompromised individuals, often leading to severe or life-threatening infections [3]. Its control is challenging due to persistence on food-associated surfaces and the ability to survive harsh conditions, including low temperature, high salt, and mild acidity, which increases the risk of contamination, particularly in meat products [2]. In this study, we investigated the adaptive responses of an *L. monocytogenes* serotype 1/2a strain associated with a 2022 outbreak in Italy linked to frankfurter consumption. Label-free quantitative proteomics combined with bioinformatics identified 421 proteins, of which 212 were unique to control cells (A, optimal growth conditions: NaCl 0.5%, pH 7, 37°C) and 29 uniquely expressed under stress conditions (B: NaCl 2.4%, pH 6.2, 12°C), mimicking pork product environments. Proteins in A were mainly involved in binding ions, small molecules, nucleotides, organic cyclic compounds, and ATP (FDR ranging from 1.55e-08 to 0.0016), reflecting essential metabolic and housekeeping functions. Proteins specific to B were associated with flagellar assembly, stress response, and virulence, highlighting adaptive mechanisms under environmental stress [4].
Several key proteins emerged as potential immunogenic candidates, with roles in adhesion, motility, and host interaction. These findings provide insights into *L. monocytogenes* adaptation to food-related stressors and identify promising targets for diagnostics and vaccine development. This knowledge can inform improved surveillance, risk mitigation, and control strategies in meat products, enhancing food safety and public health.

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# P-21

## Gut microbiota profiling of children with Down syndrome

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Down syndrome (DS, OMIM #190685) is caused by a chromosomal abnormality resulting from trisomy of the 21st chromosome. This is one of the most prevalent chromosomal abnormalities, with an incidence rate of approximately 1–2 per 1,000 live births on a global scale. The condition is characterized by a specific set of clinical features, including cognitive impairment and characteristic facies. Previous studies have demonstrated differences in the gut microbiota (GM) of individuals with DS compared to healthy controls, at both metagenomic and metabolomic levels [1,2,3].

In this study, we examined the GM function of 48 children with DS, aged 6 to 18 years, using a metaproteomic approach. Demographic and clinical data were obtained from medical records to highlight patients' behavioral issues, severity of cognitive impairment, and gastrointestinal symptoms. Bacterial proteins were extracted from stool samples by differential centrifugation, then lysed and trypsin-digested. Data-dependent acquisition was performed using nanoliquid chromatography coupled with tandem mass spectrometry (nLC-MS/MS). The MS data were analyzed using MetaLab-MAG software for label-free quantification and functional annotation. Taxonomic assignments were retrieved using the Lowest Common Algorithm (LCA) in Unipept.

To the best of our knowledge, this study represents the first investigation of the GM metaproteome of subjects with DS. The absence of gender-specific GM function was reported, while age-related profile distribution was observed. Metaproteomics provided evidence of a GM's distinctive disease related-pattern linked to different DS clinical manifestations, such as behavioral disturbances, and autoimmune comorbidities, from both functional and LCA-derived taxonomic point of view. These results may help shedding light onto DS pathophysiology and DS-related comorbidities.

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## P-22

# Exploring the Low Molecular Mass Proteome Fraction in Glioblastoma as a Source of Potential Biomarkers

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Glioblastoma (GBM) IDH-wild type is classified by the World Health Organization (WHO) as a highly aggressive brain tumor with poor prognosis and therapeutic challenge [1,2]. The early diagnosis of this tumor could significantly impact the patient prognosis, treatment strategies, and recurrence rates. Currently, surgical resection followed by chemo-radiotherapy remains the treatment modality. The identification of therapeutic targets and biomarkers is a key objective in this field. This study explores and compares, for the first time, the low molecular mass fraction (<10 kDa) of the proteome in saliva [3] and tumor surgical aspirate fluid [4] from GBM patients, recently analyzed. Saliva samples were classified according to the time of collection (pre- and post-operative), while tumor aspirate fluids were collected in both central and peripheral tumor regions using a Cavitron Ultrasonic Surgical Aspirator (CUSA). Both matrices were analyzed by UHPLC-ESI-Orbitrap-MS following filtration on 10 kDa cut-off centrifugal filter FASP device, not applying trypsin digestion. The investigation included samples from both newly diagnosed and recurrent GBM patients. Comparative analysis identified ten GBM-associated peptide fragments of different m/z values from diverse protein precursors, namely, ANXA1 (1755.844 m/z $MH^+$), CFL1 (1390.761 m/z $MH^+$), GLUL (1554.71 m/z $MH^+$), PFN1 (2323.267 m/z $MH^+$), H2AC12 (967.558 m/z $MH^+$), ACTB (1699.844 and 1389.712 m/z $MH^+$), and HBB (1209.648, 1115.568, and 1214.524 m/z $MH^+$). These peptides were co-identified in GBM saliva [3], CUSA fluid [4], and in tumor cells [5,6], evidencing a consistent presence across different biological matrices and suggesting their potential role of candidate disease biomarker. Although preliminary, these results provide new insights into the molecular feature of GBM brain tumor and demonstrate that salivary liquid biopsy analysis represents a non-invasive platform for biomarker discovery and validation. The study highlights the potential of integrating peptidomic data from multiple biological sources to improve early diagnosis, monitor therapeutic response, and advance precision medicine in this aggressive brain tumor.

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# P-23
# Bee Gut Metaproteome Reveals Plastic-Associated Enzymatic and Biofilm Signatures

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Honeybee gut microbiota is increasingly recognized as a dynamic environmental biosensor within the One Health framework. To investigate whether bee-associated bacteria exhibit functional traits compatible with the colonization or transformation of polymeric contaminants, we re-annotated 805 bacterial proteins from the metaproteomic dataset PXD043896 [1] through an integrative UniProtKB–Pfam–KEGG pipeline. Functional clustering highlighted domains related to hydrolases, oxidoreductases, adhesion/biofilm components, efflux systems, and oxidative stress responses. A limited but consistent subset of proteins carried α/β-hydrolase folds, catechol-type dioxygenases, multicopper oxidase (laccase-like) motifs, and cytochrome P450 domains, with pathway mapping to benzoate, xylene, and alkane degradation routes. Canonical plastic-degrading enzymes such as PETase or cutinase were not detected, but several hydrolases and oxidoreductases aligned with pathways involved in the breakdown of aromatic additives and long-chain hydrocarbons. Adhesion-related OmpA-like porins and RND/ABC efflux pumps further indicate adaptive tolerance to hydrophobic environments. Together, these findings outline a "plastisphere-like" metabolic profile within the bee gut metaproteome, reflecting microbial strategies of co-metabolism and surface colonization rather than direct polymer degradation. This supports the use of bees as integrative biosentinels of anthropogenic exposure in a One Health perspective.

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## P-24

# Decoding adaptive protein networks in *Listeria monocytogenes*: Proteomic insights for food safety and control

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*Listeria monocytogenes* is a key pathogen in the ready-to-eat (RTE) food production chain, particularly in meat products, where its ability to persist and grow under refrigeration poses major challenges for food safety. Its control is complicated by the pathogen's frequent occurrence in food-associated environments, persistence on processing surfaces, and remarkable tolerance to multiple stress conditions [1]. This study compared the proteomic profiles of two *L. monocytogenes* strains: a hypovirulent strain isolated from food (F) and a hypervirulent clinical strain (H), both derived from the reference strain ATCC BAA-679/EGD-e obtained from the Italian National Reference Laboratory for *L. monocytogenes* (IZSAM, Teramo, Italy). Cultures were grown at 37°C to mimic host conditions, and proteins were analyzed by LC-MS/MS following digestion by the filter-aided sample preparation (FASP) method. A total of 954 proteins were identified, including 642 predicted to be immunogenic using Vaxijen version 2.0 [2]. Among these, 128 were unique to the food strain, 27 to the clinical strain, and 487 were shared. Functional enrichment analysis revealed that proteins specific to the food strain were mainly involved in motility, chemotaxis, flagellar assembly, and secretion, reflecting environmental adaptability and persistence in food matrices [3]. Conversely, the clinical strain expressed proteins associated with the glutamate-dependent acid resistance (GDAR) system and phage-related functions, supporting its enhanced survival and virulence within the host. These findings demonstrate targeted proteomic remodeling in response to environmental cues, illustrating the bacterium's transition from foodborne persistence to host adaptation. The identification of immunogenic and strain-specific proteins provides molecular markers for improved diagnostics, therapeutic targeting, and food safety strategies, contributing to more effective control of *L. monocytogenes* in RTE foods.

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# P-25

## Decoding the Mitochondrial Microproteome: proteomics strategies for Identifying Mitochondrial-derived Peptides

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Mitochondrial-derived peptides (MDPs), such as Humanin (HN), Humanin-like proteins and Mitochondrial open reading frame of the 12S rRNA (MOTS-c), are bioactive molecules encoded by small open reading frames within the 16S and 12S rRNA regions of mitochondrial DNA.
These peptides play key roles in metabolism, and aging, acting as retrograde signaling molecules regulating mitochondrial homeostasis.
Despite advances in proteomics, endogenous HN and MOTS-c (PE1) as well as Humanin-like proteins (PE2) remain undetected in standard datasets. This raises questions about methodological limitations and detection biases.
Within the Mitochondrial Human Proteome Project (mt-HPP) framework, this study aims to systematically investigate the presence and localization of endogenous MDPs by integrating publicly available data with newly generated high-resolution proteomics datasets.
Proteomic profiling was conducted on cytosolic and mitochondrial-enriched fractions from HeLa, cell line, as well as soluble and membrane fractions from isolated mitochondria. To enhance coverage, multiple proteolytic enzymes (trypsin, Glu-C, chymotrypsin) were employed. Samples were analyzed using an Orbitrap Fusion Lumos Tribrid Mass Spectrometer (Thermo Scientific). Protein identification was performed using PEAKS X software, against high-accuracy databases searches (UniProt, MitoCarta, and custom databases), and '*de novo*' sequencing of non-identified peptides. Candidate peptides were validated using Skyline software.
By refining proteomic workflows, this study advances the detection of MDPs and provides new insights into their compartmentalization, processing, and potential roles in mitochondrial function and disease pathogenesis.

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## P-26

# Beyond Muscle Fibers: Fascia as a Novel Pathogenic Player in Collagen VI-Related Myopathy

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Fasciae are connective tissue layers that surround and compartmentalize muscles, contribute to the transmission of mechanical forces across body segments, respond to mechanical loading associated with muscular activity and store mechanical energy that is redistributed in a controlled manner. Additionally, muscular connective tissue is fundamental for proper muscle development, homeostasis and regeneration. Recent evidence has highlighted an unexpected yet crucial role of fascial tissue in musculoskeletal biomechanics and neuromuscular physiology. Although muscles, joints, tendons and nerves have long been considered the primary targets in myopathy research, fascia has emerged as a key player in force transmission, prioprioception, mechanotransduction, and the coordination of segmental movement. While morphological studies of deep fascia have been reported in healthy subjects, the contribution of fascial tissues to the muscle dysfunction in muscular dystrophies remain largely unexplored. Joint contractures are an early and disabling clinical feature in several congenital muscular dystrophies (CMDs), particularly in collagen VI-related myopathies (COL6-RMs). Intriguingly, COL6-RM muscle exhibits a unique outside-in degenerative pattern, suggesting a key pathogenic role for aberrant crosstalk between muscle fibers and surrounding fascia. To investigate fascia-specific molecular alterations, we performed a label-free quantitative proteomic profiling of primary fascial cells isolated from patients with the most severe form of COL6-RM, the Ullrich congenital muscular dystrophy (UCMD), and compared them to cells from healthy donors. Our results revealed differential expression of proteins involved in elastic fibers assembly and organization, as well as dysregulation of microfibrillar components and focal adhesion associated proteins, supporting a potential alteration of extracellular matrix architecture and mechano-signaling pathways in UCMD fascia. Preliminary in vitro co-culture experiments further suggest that fascia-derived cells may impair the differentiation capacity of primary myoblasts, indicating a previously unrecognized contribution of fascial cells to the muscle environment. Taken together, these findings provide new insight into the role of fascial tissues in COL6-related myopathies and support the concept that pathogenic mechanisms in CMDs extend beyond the muscle fiber itself, highlighting fascia as a relevant and previously overlooked cellular compartment.

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## P-27

# A proteomic approach to investigate the regulatory mechanism of BAG3-mediated lung fibrosis

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Bcl2-associated athanogene 3 (BAG3) protein regulates various cellular processes, including apoptosis and autophagy [1]. Recent studies suggest that BAG3 can regulate the fibrotic processes and elevated BAG3 levels are reported in hyperplastic alveolar epithelial cells of idiopathic pulmonary fibrosis lungs [2]. To investigate the mechanism of BAG3-mediated regulation of lung fibrosis, we adopted the mice model of bleomycin-induced lung fibrosis and used an anti-BAG3 antibody. Bleomycin induces the production of reactive oxygen species (ROS) responsible for tissue damage and induction of pro-inflammatory and pro-fibrotic response. After the bleomycin injection, mice were randomized into three groups: mice without fibrosis (healthy mice), bleomycin-induced fibrosis mice treated with anti-BAG3 antibody (treated mice) and bleomycin-induced fibrosis mice without treatment (untreated mice). After 21 days, animals were sacrificed, and lungs harvested for fibrosis analysis and proteomic characterization. Proteomic analysis was performed using the SP3 protocol for tryptic digestion followed by nanoLC-Orbitrap-MS/MS analysis. Label-free quantification was performed using ProteomeDiscoverer®, resulting in the quantification of 2,949 proteins, which were subsequently analyzed for gene ontology and functional enrichment by Ingenuity Pathway Analysis (IPA) software. Interestingly, the healthy mice and treated mice group clustered together in the heat map and in the PCA, indicating that anti-BAG3 treatment restored a lung protein profile similar to mice without fibrosis. By comparing the proteomic profile between bleomycin-induced fibrosis mice with or without treatment we showed that the BAG3 blocking significantly modulated inflammatory and oxidative stress–related processes: a decrease in ROS levels is observed, resulting in inhibited inflammation within the respiratory system, as well as reduction in apoptosis and cell death. Conversely, activation of cellular survival and viability pathways is promoted. Altogether, these preliminary data support the efficacy of anti-BAG3 antibody treatment in reverting the pathological features of bleomycin-induced pulmonary fibrosis, restoring protein expression and functional pathways toward a control-like state, and thereby highlighting BAG3 as a promising therapeutic target in fibrotic lung disease.

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# P-28
# Efficient Tissue Homogenization for Quantitative Metabolomics and In-Depth Proteomics with BeatBox®

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The BeatBox® tissue homogenizer and cell lyser, optimized for proteomics applications, has since evolved into a robust tool for high-throughput multi-omics applications, enabling efficient extraction of biomolecules from scarce, challenging samples. Here, we demonstrate the versatility of BeatBox across two exemplary applications: (1) quantitative and targeted metabolomics and lipidomics from fresh-frozen (FF) tissues, and (2) an optimized deep proteomics workflow for formalin-fixed, paraffin-embedded (FFPE) tissues. In the metabolomics and lipidomics experiments, ~5 mg of mouse tissue (brain, cardiac muscle, liver, lung, intestine, spleen, skeletal muscle) was homogenized with 80% methanol in a 96-well plate using BeatBox (PreOmics), followed by centrifugation to remove protein precipitates. The supernatants were analyzed via targeted LC-MS using a Vanquish™ Duo HPLC (ThermoFisher Scientific) coupled with a 7500 triple quad system (SCIEX). Quantification of metabolites and lipids—including phenylalanine, ornithine, methionine, phosphatidylcholine, diacylglycerol, ceramide, and phosphatidylinositol—was conducted using isotopically labeled internal standards. The workflow showed excellent reproducibility with coefficients of variation (CV) below 20% across replicates. PCA and heatmap analyses revealed apparent tissue type clustering and biological significance. For proteomics, mouse tissues comprising 2–5 FFPE curls (10-µm sections; liver, kidney, cardiac muscle) or FFPE punches (1-mm diameter; lung) were homogenized in a 24-tube format using BeatBox, without the need for separate xylene-based deparaffinization steps. After homogenization, samples underwent thermal de-crosslinking and iST-based digestion. The resulting peptides were analyzed using nanoElute and diaPASEF® acquisition on a timsTOF HT (Bruker), and data processed with ProteoScape™ (Bruker). Protein yields scaled linearly with input (up to 5 curls), and reproducibility remained high (CV <10%). Over 5,500 proteins were identified from various FFPE tissues, validating the depth and consistency of the workflow regardless of input amount. The optimized FFPE punches workflow achieved comparable identification rates and reproducibility to FFPE curls, despite the heterogeneity of lung tissues and stiffness of punches. Overall, this study showcases the adaptability of BeatBox across diverse omics applications. It enables reproducible, high-throughput homogenization of both FF and FFPE tissue types.

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# P-29
# Investigation of protein markers of interstrain variation of the microorganism *L. monocytogenes* under acid stress conditions

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*L. monocytogenes* is a very important foodborne pathogen, capable to cause severe pathological situations. Acid conditions are unfavorable for *L. monocytogenes* in general but some strains show a great resistance maintaining a subpopulation able to survive for a longer period of time. This study aimed to investigate and identify proteins of interstrain variability of *L. monocytogenes* under acid stress conditions, with or without prior acid adaptation.

Four strains of the foodborne pathogen *L. monocytogenes* were examined for their acid-resistance after growth to the stationary phase. The strains were cultured at 30°C for 24 hours in 10mL TSBYE and were recultured for 18 hours at 30°C in 10mL TSBYE with PBS 5X (PBS 5X was used to maintain pH constant during overnight culture) and grew to approximately $10^9$ cells/mL. After 3 centrifugations with physiological saline, inoculation was performed in 100 mL of TSBYE acidified with HCL with a pH of approximately 3. The level of each inoculum was approximately 7 log CFU/mL. Then, sampling took place every hour in TSAYE. These plates were left for 48 hours at 37°C. The differences in acid resistance of the strains were obvious.

After the cells were isolated with centrifugation they were resuspended in a 1 mL solution (50 mM Tris-HCl pH 7.3, 10μL Protease Inhibitors). Then, the cells were lysed through the ultrasound process. Centrifugation was performed at 14800 rpm for 20 minutes at 4°C and 0.2g of TCA was added to the supernatant. The samples were left on ice for one hour and then precipitation with 1mL cold acetone took place. After overnight incubation at -20°C the samples were centrifuged for 20 minutes and 250μL of sample buffer (7M urea, 2M thiourea, 4% CHAPS, 1% DTT, 10μL Protease Inhibitors) was added to the precipitate. Protein quantification was performed using Bradford.

The proteins were identified by high-resolution mass spectrometry nano LC-MS/MS Orbitrap Elite. The results gave a variety of proteins (2227), a group of them indicated to participate in the appearance of L. monocytogenes population in acid conditions.

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## P-30

# Prognostic role of plasma proteomics signature in oligorecurrent prostate cancer patients undergoing SBRT

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Improving the prognostic stratification of patients (pts) affected by oligorecurrent hormone-sensitive prostate cancer (orHSPC) and undergoing Stereotactic Body Radiation Therapy (SBRT) is an unmet clinical need. This study aims to evaluate whether a plasma proteomics signature can be identified in these pts and its possible correlation with clinical outcomes. orHSPC pts with 1-3 nodal and/or bone metastases undergoing SBRT were enrolled (n=35).

After a median follow up of 41 months, two subgroups were identified based on the occurrence of biochemical progression (BP): "early-BP" (n=7, BP Free Survival<6 months) and "late-BP" (n=9, BP Free Survival >24 months). Moreover, depending on the occurrence of distant progression ("DP"), "no-DP" (n=7) and "polymetastatic-DP" (n=7) subgroups were indentified. Plasma-EDTA was collected before SBRT. Plasma peptides were separated by liquid chromatography tandem mass spectrometry based proteomics. Relative amount of identified proteins across our samples was determined through label-free quantification.

Plasma protein profiles of early-BP pts differed from those of late-BP pts in 9 differentially abundant proteins, among which 7 (PRDX2, PCSK9, HBE1, CPN1, CA1, ACTA2 and SAA1) were more abundant in late-BP compared to early-BP. The lower plasma level of CA1 and SAA1 in early-BP pts was validated by immunoblotting. Moreover, plasma protein profiles of polymetastatic-DP pts differed from those of no-DP pts due to the presence of 8 differentially abundant proteins, among which 3 were more abundant (KRT5, ACTA1 and PON3) and 5 were less abundant (CA1, KRT7, CPN1and PRG2) in polymetastatic-DP.

Our work suggests that a plasma proteomics signature in orHSPC pts undergoing SBRT could be identified and used as prognostic indicator for BP- and DP-free survival. In particular, low CA1 and CPN1 levels may correlate with the worsening of the analyzed outcomes (early BP Free Survival and polymetastatic DP).

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# P-31

# Exploratory metaproteomic profiling of bovine faeces highlights functional antimicrobial resistance signatures associated with tetracycline and macrolide exposure

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Bovine faeces constitute a complex microbial ecosystem at the animal-environment interface and a potential hotspot for antimicrobial resistance (AMR) within the One Health continuum. We applied a shotgun metaproteomic workflow to characterise expressed microbial functions and detect protein markers associated with the faecal resistome. Proteins were extracted via mechanical lysis and S-Trap processing, digested with trypsin, and analysed by nanoLC–MS/MS on a high-resolution Orbitrap platform. Identification and functional annotation were performed in Proteome Discoverer against UniProtKB, with enrichment for AMR-related protein families.

Preliminary results revealed tetracycline resistance determinants (Tet family), macrolide/erythromycin resistance methyltransferases (Erm), and multidrug efflux transporters from MFS/ABC families, alongside stress-response and envelope-biogenesis functions consistent with adaptation to antimicrobial pressure. These data provide direct protein-level evidence of AMR mechanisms in bovine faecal matrices and demonstrate the feasibility of a reproducible metaproteomic pipeline for resistome surveillance in livestock systems.

Ongoing work includes quantitative refinement and integrative analysis with metadata to contextualise functional findings across samples and matrices, supporting risk assessment and mitigation strategies in a One Health framework.

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# P-32
# Zymography Approach to the Investigation of Honeybee Prepupae from Healthy and American Foulbrood-Infected Bees

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American foulbrood (AFB) is a bee disease caused by *Paenibacillus larvae*, a Gram-positive, spore-forming, facultatively anaerobic bacterium. *P. larvae* infects only larvae and germinates within the digestive tract. The vegetative form penetrates the gut epithelium and proliferates, reducing the larva to degraded tissue within 72 hours [1]. Previous studies have identified several molecules involved in the activity of this bacterium, including metalloproteases, enolase, SplA S-layer protein, bacillibactin, paenilamycin, paenilarvin, and sevadicin. Some strains can produce the toxins Plx1, Plx2, and C3 larvin [2]. Regarding the bees' response, it has been reported that bees selected for hygienic behaviour show greater resistance to the disease. In infected larvae, increased activity of prophenoloxidase and lysozyme, as well as higher levels of defensin, hymenoptaecin, and abaecin production, have been observed [3]. Since prepupae may respond to AFB infection by increasing protease synthesis, the aim of this study was to compare protease activity in worker prepupae from healthy colonies with those from colonies affected by AFB. This investigation was carried out using zymography, 2D-zymography, and reverse zymography. Proteins were identified by capillary LC-µESI-MS/MS. In the zymogram of protein extracts from healthy prepupae, four proteases (23, 41, 75, and 98 kDa) were observed that were absent in diseased prepupae, whereas a weak 94 kDa band was detected in both the secretion and extract of *P. larvae*. Mass spectrometric analysis of the 2D zymograms led to the identification of two proteinases: chymotrypsin and trypsin-1. Previous studies have reported the presence of chymotrypsin inhibitors in the haemolymph of bee larvae; however, these were not identified in the present study, most likely due to their low concentration. Further investigations are needed to elucidate the involvement of these proteinases in the immune response of honeybee larvae and the mechanisms by which *P. larvae* suppress protease production in its host.

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## P-33
# Metabolomics and proteomics evaluation of a rare infantile genetic condition: characterization of Crisponi Syndrome patients

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Crisponi Syndrome (CS) is an extremly rare infantile genetic condition with an incidence of less than one case per million. CS is characterized by several symptoms such as intermittent contractions of facial and oropharyngeal muscles, laryngospasm and respiratory distress, cold-induced sweating, cranio-facial dysmorphism, camptodactyly and sudden death in most cases [1,2].
The diagnosis of CS is established in a proband with suggestive findings and biallelic pathogenic variants in either CLCF1 or CRLF1 on molecular genetic testing.
Due to the extremely rare nature of CS, mechanisms of action and biochemical alterations induced by the condition are not yet well known. A multi-omic approach is a useful tool to obtain a biochemical characterization of CS patients. The aim of our work was to investigate which metabolites and proteins where abnormally expressed in CS patients.
A population of 14 subjects diagnosticated with CS was enrolled for the study. Blood and saliva sampling was performed on fasting patients, and samples were stored at -80°C until analysis.
A panel of 107 small molecules and 912 lipids, belonging to 39 biochemical classes, was measured through ultra-performance liquid chromatography/flow injection analysis coupled with triple quadrupole mass spectrometry (UPLC-MS/MS and FIA-MS/MS), using MxP Quant 500XL Kit (Biocrates life science, AU).
Top-down proteomic analyses were performed using an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific, San Jose, CA, USA). Raw data were processed using Proteome Discoverer 3.2 (Thermo Scientific) equipped with the ProSightPD 4.4 node for top-down analysis. Protein identifications were carried out against the Human UniProt database (2025 release). Explorative statistical analysis was performed on data to evaluate possible biochemical differences among the Crisponi patients. Several alterations were highlighted in different biochemical pathways manly related to immunologic response, cellular response to stimuli, inflammation and antioxidant activity.
Due to the results obtained, we can hypothesize that the biochemical differences observed among patients, as revealed by the multi-omic approach, may provide the basis for explaining the heterogeneity of this condition.
For the future, a data fusion with clinical evidence will be useful to better understand this rare and complex condition.

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**P-34**

# Integrated omics approach on dried blood spot to shed light on acid sphingomyelinase deficiency and the effects of enzyme replacement therapy

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Acid sphingomyelinase deficiency (ASMD) is an autosomal recessive lipid storage disorder caused by mutations in the SMPD1 gene. The lysosomal enzyme deficiency leads to the progressive accumulation of sphingomyelin in systemic organs in all forms of the disease, and in the brain in the neuropathic forms. Currently, there is no definitive cure, but enzyme replacement therapy (ERT) with Olipudase alfa has recently been approved for the treatment of non-neuronopathic forms.[1] However, adherence to the therapy may be limited by the need for intravenous infusions every two weeks for life. Lysosphingomyelins (LysoSMs) are used as diagnostic biomarkers, but their predictive value in pre-symptomatic individuals is not yet clear.

In this context, proteomics and metabolomics approaches offer advanced tools to explore the clinical and metabolic phenotype of rare diseases, through easily accessible matrices such as dried blood spots (DBS). The application of such strategy can help understanding the underlying pathogenetic mechanisms in ASMD, defining the effects of ERT and identifying novel biomarkers for diagnosis, prognosis and treatment response. DBS samples were collected from an 11-year-old female patient with chronic visceral ASMD, who presented with interstitial lung disease, elevated transaminases, and growth retardation at diagnosis (T0). She began enzyme replacement therapy (ERT) immediately after Olipudase alfa became available in Italy.

Samples collected at T0 and during maintenance ERT were analyzed using untargeted metabolomics and proteomics on a high-resolution mass spectrometry platform. The resulting data underwent functional analysis using Ingenuity Pathway Analysis software. Metabolomics profiling identified over 1,000 compounds, confirming LysoSMs as biomarkers, and revealed several previously unreported oxysterols in ASMD that showed differential expression, with patterns reversed following ERT. The combination of annotated metabolites and the hundreds of proteins quantified through proteomics highlighted zinc dyshomeostasis as a potential pathogenic mechanism in ASMD. Additionally, ERT appeared to exert effects including the suppression of inflammation and modulation of the macrophage-mediated immune response.

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## P-35
# Sperm Proteomic Signatures and seminal suPAR and sST2 Differentiate Acute and Chronic Male Accessory Gland Inflammations

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Male accessory gland inflammations (MAGI) are associated with male infertility, and their diagnosis mainly relies on ultrasound examination (US), which is operator-dependent. Differentiating between acute and chronic phenotypes is clinically relevant, as they differently affect fertility and patient management. This study explored the molecular impact of these conditions on spermatozoa through proteomic analyses and investigated soluble urokinase plasminogen activator receptor (suPAR) and the soluble form of ST2 (sST2) as potential seminal biomarkers of inflammation. Two cohorts of patients with acute (HCUF) or chronic (FSUF) MAGI were compared with healthy controls. SuPAR, sST2 levels and redox status were measured. Purified spermatozoa underwent bottom-up proteomics and immunofluorescence validation of selected targets. Seminal suPAR discriminated healthy from MAGI patients with a cut-off of 63 ng/mL, whereas sST2 distinguished acute from chronic inflammation, with levels above 1100 pg/mL identifying the FSUF phenotype. Both patient groups showed increased lipid peroxidation and reduced antioxidant capacity Vs controls, with HCUF exhibiting the lowest antioxidant potential. Proteomic profiling revealed distinct expression patterns among healthy, HCUF, and FSUF spermatozoa. FSUF displayed down-regulation of motility-related proteins, including outer dense fiber protein 2 (ODF2), confirmed by reduced immunofluorescence signal along the sperm tail. Conversely, HCUF spermatozoa showed up-regulation of proteins involved in exosome biogenesis and metabolism. Overall, these data support suPAR and sST2 as promising biomarkers useful in MAGI diagnosis and provide the first molecular evidence of phenotype-specific effects on sperm cells, offering new insights into inflammation-related male infertility.

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