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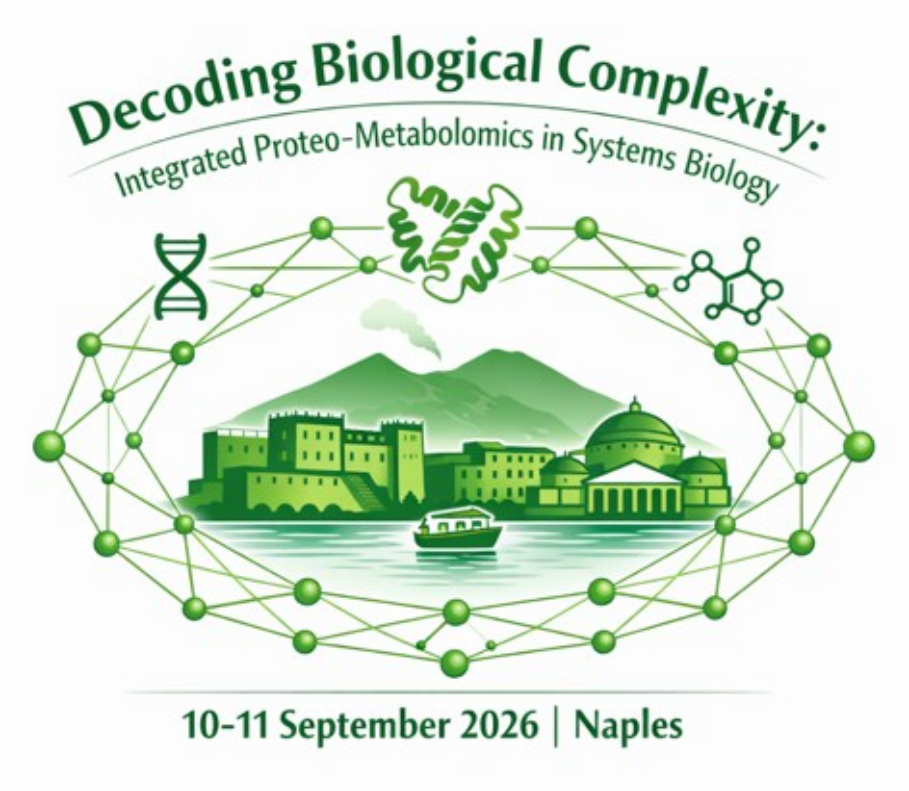
XX INTERNATIONAL
ITALIAN PROTEOMICS ASSOCIATION ANNUAL MEETING

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



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CEINGE - Biotecnologie Avanzate "Franco Salvatore"
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CONFERENCE REGISTRATION FEES

Early registration (**until July 20th**, 2026):

- ItPA/EuPA member: € **130**
- Non-member: € **150** (130+20 as annual membership fee)

Late Registration (**after July 20th**, 2026):

Member and non-member: **250** €

Industry/Companies: € **300** plus 22% VAT

To register, please fill out the Google registration form, also available on the ItPA website, and follow the instructions.

GENERAL INFORMATION

CONGRESS VENUE

CEINGE Biotecnologie Avanzate "Franco Salvatore" Institute, via Gaetano Salvatore 486, 80145 Naples, Italy.

To get there: take *Metro Line 1* and get off at *Policlinico* station. From there, you can either: *a*) walk to the Institute (15-20 minutes), or *b*) take the internal shuttle bus located on the left of the metro exit, get off at *stop 9* (Building 3), then walk down the stairs to CEINGE.

OFFICIAL LANGUAGE

The Congress official language will be English.

ABSTRACTS AND POSTERS

The deadline for abstract submission for both oral and poster presentations is **July 20th, 2026**. Posters will be displayed from September 10th to September 11th, 2026. At least one author must be present during the poster sessions. Presentations by early-career corresponding authors will be considered for the *Best Poster* and *Best Oral Presentation* awards.

CERTIFICATE OF ATTENDANCE

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

COFFEE BREAKS AND LUNCHES

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

ACCOMMODATION

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

Confirmed Invited Speakers

Marta Cascante, University of Barcelona, Barcelona – Spain.
Fabrizio Dal Piaz, University of Salerno, Baronissi (SA) – Italy.
Anna Picca, LUM University, Casamassima (BA) – Italy.
Peter Ponsaerts, University of Antwerpen, Antwerpen – Belgium.
Tanja Ćirković Veličković, University of Belgrade, Belgrade – Serbia.
Peter Verhaert, Leuven University, Leuven – Belgium.

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16:30 – 17:00	**AWARDS AND CLOSING**

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ABSTRACT VOLUME

PLENARY and KEYNOTE LECTURES

PL-01
Biology of Aging: From Molecular Complexity to Biomarker development

Anna Picca[a,b*]

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[b] Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Rome, Italy

Aging is the major risk factor for chronic disease, yet chronological age poorly captures the heterogeneity of health and functional trajectories among older individuals. Biological aging emerges from the accumulation of molecular and cellular damage and a declining capacity to maintain homeostasis and respond to stress[1]. It reflects the interplay among the hallmarks of aging, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macroautophagy, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis[2]. Their biological consequences depend on their impact on resilience, the capacity of cells, tissues, and physiological systems to adapt to perturbations and restore function. With advancing age, accumulating damage and loss of compensatory capacity reduce physiological reserve, generating trajectories from healthy aging to functional decline and disease. Functional deterioration may emerge from loss of coordination across physiological systems rather than failure of a single organ or pathway. Sarcopenia exemplifies this principle, as muscle function depends on coordinated neural activation, oxygen and substrate delivery, mitochondrial energy transformation, and energy utilization. Aging disrupts this coordination, leading to energetic mismatch, reduced adaptive capacity, fatigability, and impaired recovery.

Capturing this complexity represents a central challenge of geroscience. Biomarkers of aging should move beyond associations with chronological age or established disease to reflect fundamental biological processes, discriminate aging trajectories, predict clinically relevant outcomes, and respond to interventions targeting aging mechanisms. Moving from individual biomarkers and generalized biological clocks toward multidimensional, mechanism-informed signatures may better represent biological aging. Integrating molecular biomarkers with measures of physiological reserve and stress responsiveness may enable earlier detection of declining resilience, improve prediction of functional trajectories, and support personalized interventions aimed at preserving healthspan and preventing functional decline.

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References:
[1] Ferrucci L, et al. Measuring biological aging in humans: A quest. Aging Cell. 2020;19:e13080. doi: 10.1111/acel.13080.
[2] López-Otín C, et al. Hallmarks of aging: An expanding universe. Cell. 2023;186:243-278. doi: 10.1016/j.cell.2022.11.001.

PL-02
From Proteomes to Phenotypes: Decoding Neuroglial Stress in Mature hiPSC-Derived Neurospheroids

Peter Ponsaerts*

Laboratory of Experimental Hematology, Vaccine and Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium

Human induced pluripotent stem cell (hiPSC)-derived brain organoids and neurospheroids offer new opportunities to investigate human neuroglial disease. They also expose a fundamental challenge for proteomic analysis in neuroscience: the functional state of neural tissue cannot be inferred from protein abundance alone. Brain function emerges from coordinated interactions between neurons and glia, and similar molecular stress signatures may therefore accompany adaptation, altered signalling, or network failure.

In this lecture, I will present a proteomics-guided framework for resolving these distinct biological states in mature human 3D neural models containing neurons and astrocytes, with or without isogenic hiPSC-derived microglia. Across ischemia-like injury, inflammatory stimulation, neurotropic viral infection, and α-synuclein pathology, untargeted proteomics -integrated in selected studies with transcriptomics - identified recurrent disturbances in mitochondrial function, proteostasis, cellular stress responses, lipid homeostasis, and neurotransmission. Functional phenotyping, however, demonstrated that molecular convergence did not imply physiological equivalence.

Following oxygen-glucose deprivation and reoxygenation, stress and synaptic pathway alterations coincided with sustained neurofilament light-chain release and loss of synchronous calcium activity. Pro-inflammatory cytokines induced a pronounced immune and metabolic stress state but largely preserved network coordination despite altered burst dynamics. In contrast, productive varicella-zoster virus infection combined limited antiviral signalling with mitochondrial and synaptic dysfunction, reduced ATP-linked luminescence, and collapse of coordinated network activity. Isogenic microglia reshaped the baseline organoid proteome and lipid-related state and strongly amplified inflammatory responses, while exerting a more limited influence on the delayed post-ischemic phenotype. Finally, α-synuclein seeding generated early Lewy body-like and Lewy neurite-like pathology, accompanied by disease-relevant omics alterations and impaired network synchrony. Together, these studies establish a foundation for moving beyond bulk proteomic descriptions. Phosphoproteomics should capture dynamic signalling events invisible to abundance-based profiling, whereas spatial and single-cell proteomics will be required to localize these responses and resolve their cell-type-specific origins. Linking these next-generation proteomic layers to emergent tissue phenotypes will provide a more precise distinction between compensation, dysfunction, and irreversible network failure, and a stronger basis for therapeutic prioritization in human brain models.

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PL-03

From metabolic flux analysis to genome-scale modelling: uncovering therapeutic vulnerabilities in cancer and complex diseases

Marta Cascante[1,2,3]

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[2] Institute of Biomedicine of University of Barcelona (IBUB), 08028 Barcelona, Spain.
[3] CIBEREHD. Network Center for Hepatic and Digestive Diseases, National Spanish Health Institute Carlos III (ISCIII), 28029 Madrid, Spain.

Metabolic reprogramming is a hallmark of cancer and other multifactorial diseases, reflecting the ability of cells to dynamically redistribute metabolic fluxes in response to genetic, environmental, and therapeutic pressures. While metabolomics and other omic approaches provide valuable snapshots of metabolic states, translating these static measurements into functional understanding and therapeutically actionable vulnerabilities requires quantitative approaches capable of resolving intracellular fluxes and integrating them at the systems level. This presentation outlines how quantitative analysis of metabolic fluxes using ^{13}C-based metabolic flux analysis (^{13}C-MFA) has established fluxomics as a quantitative framework capable of inferring pathway usage and metabolic plasticity within central metabolic networks from isotopic labelling patterns, going beyond metabolite abundance alone. By resolving intracellular flux distributions, ^{13}C-MFA has revealed functionally distinct modes of metabolic reprogramming that would remain hidden using static measurements alone, including, in the work discussed here, context-dependent mitochondrial oxidation of non-canonical carbon sources and adaptive glutamine dependence in response to targeted therapies.

An additional level of understanding of metabolic fluxes is achieved through genome-scale metabolic models (GSMMs), which provide a systems-level framework to explore metabolic phenotypes by integrating stoichiometric network structure with multi-omics data, particularly transcriptomics. In this presentation, I will also describe the workflow we have developed to construct and analyse context-specific GSMMs, in which experimental constraints, including metabolomics, uptake and secretion rates, and, when available, ^{13}C-resolved flux information, are used to refine the space of feasible flux distributions. Application of this framework has enabled the identification of coordinated metabolic dependencies, stratification of disease states based on functional metabolic configurations, and differential responses to therapeutic or environmental perturbations. Using illustrative examples from our work, including ongoing and unpublished analyses, I will show how GSMM-based approaches uncover context-specific metabolic vulnerabilities and support translational applications, including patient-derived organoids and clinically accessible metabolic readouts, across cancer and other multifactorial diseases.

Acknowledgements:
The authors acknowledge the support of MICIU/AEI/10.13039/501100011033 and European Commission FEDER funds (PID2023-150539OB-I00); CIBER-EHD (EHD20PI03 and CB17/04/00023); AGAUR (2021-SGR-00350); and the ICREA Foundation (ICREA Academia award).

K-01
Omics evaluation of biocorona of micro- and nanoplastics: relevance for risk assessment

Tanja Cirkovic Velickovic*

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[b]Serbian Academy of Sciences and Arts, Belgrade, Serbia

Proteomics has become an indispensable tool for understanding molecular interactions occurring at the nano–bio interface. In the context of food safety, micro- and nanoplastics (MNPs) entering the gastrointestinal tract rapidly interact with food proteins, digestive enzymes, and other biomolecules, leading to the formation of dynamic protein coronas that determine the biological identity and behavior of the particles. Characterizing these complex molecular assemblies requires analytical approaches capable of identifying and quantifying hundreds of proteins simultaneously while providing insights into their structural and functional alterations.

This lecture will present recent and ongoing studies investigating the interactions between MNPs and food proteins under simulated gastrointestinal conditions, with particular emphasis on the application of high-resolution mass spectrometry-based proteomics. We have explored how particle size, surface chemistry, food matrix composition, and digestion influence protein corona composition, adsorption selectivity, and the evolution of particle-associated proteomes. Particular attention will be given to structural and functional changes of adsorbed proteins, including food allergens, and to the impact of these interactions on protein stability, digestibility, and biological functionality. Recent findings on digestion-driven remodeling of protein coronas and food matrix-dependent modulation of particle behavior will also be discussed.

The lecture will highlight how integrating proteomics with complementary biochemical, spectroscopic, and particle characterization techniques enables a comprehensive understanding of nano–bio interactions at multiple levels of molecular organization. Such multidisciplinary approaches provide new opportunities for elucidating mechanisms underlying MNP behavior in biological environments and contribute to the development of evidence-based strategies for food safety and human health risk assessment.

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K-02
Maximally lowering the threshold for the adaptation of mass spectrometry imaging by pathology today

Peter D. Verhaert[a*], Marco Giampà[b], Soujanya Doddoju[b], Bart Ghesquière[b]

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[b] University of Leuven, Department of Cellular & Molecular Medicine, Laboratory of Applied Mass Spectrometry and VIB Technologies Leuven, Metabolomics Core Facility, Leuven, Belgium

We develop so-called mass spectrometry histochemistry (MSHC) methods that employ cutting-edge high-resolution (HR) MS instrumentation for label-, enzyme- and antibody-free (top-down) MS imaging (MSI) of histological sections of formalin-fixed paraffin-embedded (FFPE) tissue samples, deposited on regular microscope glass slides.

Although historic reports claimed that direct MALDI MSI of peptides does not work without reactive matrices for samples older than 6 months [1], our MSHC strategy yields cellularly resolved MALDI HR MS images of intact endogenous (neuro)peptides (complete with PTMs like disulfide bridges, C-terminal amidation and others) from samples archived for more than a decade, and this using regular matrices like DHB. The formaldehyde-fixation effectively inactivates all biomolecule modifying enzymes, so the latter remain chemically stable prior to spatial MS analysis. In addition, the paraffin-embedding efficiently removes the high-abundant well-ionizable lipids from the sample, so that low-abundant, less-ionizable (bio)molecules, including neuropeptides and even neurotransmitters become accessible for direct (i.e., without on-tissue chemical derivatization) HR MSI.

HR MSHC analysis of FFPE samples in the 50-200 *m/z* range allows for localization of several (lipophobic) metabolites in FFPE tissue sections, making it a multi-omic spatial analysis. Besides peptidomic and metabolomic, HR MSHC is also economic, as it does not require a dedicated MALDI instrument. We usually perform HR MSHC on orbitraps from which the ESI source is swapped with an atmospheric pressure (AP) MALDI source. HR MSHC does not necessarily yield thousands of biomolecular ion signals, however we argue that a few tens of spatial signature ions may be sufficient for effective pathological decision making, particularly since an additional layer of data is generated from the same FFPE sections which are/have been examined by 100% complementary microscopic imaging modalities. MSHC is fully compatible to other spatial omics working from FFPE samples, yielding complementary molecular images to e.g. glycan and multiplexed IHC data. It allows retrospective studies on millions off well-documented archived human disease samples, which means that animal disease models can be circumvented.

MSHC maximally aligns spatial MS analysis protocols with the realities of clinical pathology, fostering direct acceptance by pathologists today.

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Reference:
[1] Gagnon et al. 2012, Prog Histochem Cytochem 47: 133-174 [https://doi.org/10.1016/j.proghi.2012.08.002]

K-03

Study of the interaction between proteins and small molecules: from the identification of drug targets to understanding protein function

Fabrizio Dal Piaz[a*]

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The interaction between small molecules and proteins plays a fundamental role in numerous key processes in human biology, pathology, and pharmacology. The modulation of enzymatic activity, the activation or repression of gene expression, as well as the localization of proteins are just a few examples of the broad spectrum of effects produced by such interactions. On the other hand, the binding of a compound to proteins can reduce its bioavailability, increase its residence time in a biological system, or produce chemical changes that substantially alter its reactivity or interactivity. It is therefore not surprising that the development of bioanalytical approaches that enable the study of protein-small-molecule complexes—with the aim of identifying the protein(s) involved and describing the effects on their biochemistry—is one of the most significant challenges currently facing the field of protein chemistry. In recent years, several proteomics-based protocols have been proposed, aimed at identifying potential molecular protein targets of bioactive compounds and at providing information on the changes that occur in a biological system as a result of those same compounds (1). The results that can be obtained through the combined use of these protocols are not only providing highly detailed information on the mechanisms of action of numerous drugs and natural compounds, but have also paved the way for the use of small molecules as probes to identify new proteins involved in pathological processes. While the benefits that this type of research can bring to the fields of pharmacology and pharmaceuticals are clear (ranging from the identification of targets and off-targets to the discovery of new potential drug-targetable proteins), less obvious implications have also emerged very recently. For example, studies conducted using these approaches on *in vitro* tumor models have shown that switching from cell lines cultured in plates to a three-dimensional culture system radically alters the interactome of proteins involved in the process of carcinogenesis (2). In short, the potential of proteomics in the study of small-molecule-protein interactions goes far beyond the mere identification of the target, and we have probably only just begun to discover it.

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ORAL COMMUNICATIONS FROM SPONSORS

O-S-01

From biosample to bioinsight: Increasing confidence for clinical research with 4D-Proteomics approaches

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With the advent of robust and reproducible sample preparation workflows, fast, effective, and reliable LC-MS systems, and advanced bioinformatics, proteomics has entered a new era of productivity. Today, as much effort is devoted to integrating proteomics into the broader framework of global health research as to further advancing its capabilities. The introduction of the timsTOF Pro in 2017 marked a paradigm shift, enabling 4D proteomics approaches that, for the first time, eliminated the need to compromise speed for sensitivity, selectivity, or robustness. As DIA processing pipelines have matured, they have paved the way for large-scale, deep proteome analyses.

Driven by technological advancements and the release of new methods, 4D proteomics approaches have evolved significantly over the past year. Researchers can now tackle the most demanding challenges in proteomics, from exploring the dynamic range of biofluids to performing De Novo analyses on minute quantities of neoantigens and even conducting studies on single immune cells. This presentation will highlight how the latest acquisition modes—such as MoRe and PASEF-eXd—and hardware innovations like AIP are empowering scientists to address complex proteomics tasks and making routine analyses more accessible than ever before.

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O-S-02
Complementary Proteomics: Unlocking Biological Insights with Olink and Mass Spectrometry

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Proteomics is evolving rapidly, driven by technologies that provide complementary views of the proteome. Rather than representing competing approaches, affinity-based proteomics and mass spectrometry each offer unique strengths that, when integrated, can accelerate biological discovery and translational research.
This presentation will explore how Olink's Proximity Extension Assay (PEA) technology complements mass spectrometry workflows across a broad range of research applications. We will discuss the analytical capabilities of each platform, highlighting how mass spectrometry excels in unbiased protein identification, structural characterization, and post-translational modification analysis, while Olink enables highly sensitive, multiplexed quantification of low-abundance proteins with exceptional reproducibility across large sample cohorts.
Through representative research examples, we will illustrate how these technologies can be strategically combined throughout the biomarker discovery pipeline—from discovery and hypothesis generation to verification, large-scale validation, and clinical translation. Particular emphasis will be placed on applications in translational research, precision medicine, and multi-omics, where integrating orthogonal proteomic approaches provides a more comprehensive understanding of complex biological systems.
The presentation will also discuss practical considerations for designing complementary workflows, demonstrating how researchers can leverage the strengths of both platforms to maximize biological insight, improve confidence in findings, and accelerate the identification of clinically relevant biomarkers.
By fostering collaboration between the mass spectrometry and affinity proteomics communities, this session aims to demonstrate that the future of proteomics lies not in choosing one technology over another, but in integrating complementary approaches to answer increasingly complex biological questions.

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SELECTED
ORAL COMMUNICATIONS

O-01

Development of a comprehensive diagnostic pipeline integrating MALDI-MSI and Proteomics methodologies to map the molecular signature of epileptic FCDIIb lesions

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Epilepsy is a neurological disorder affecting about 1% of the population. Despite anti-seizure medications, nearly 30% of patients develop drug-resistant epilepsy (DRE), for whom surgery remains the effective treatment. Among the lesions associated with DRE, Focal Cortical Dysplasias (FCDs) represent a major cause, although diagnosis remains challenging[1].
Combining MALDI imaging and LC-MS/MS proteomic approaches, this study aimed to define the molecular signature of FCDIIb in Formalin-Fixed Paraffin-Embedded (FFPE) surgical specimens. An integrated workflow was applied to 11 FCDIIb FFPE tissues to identify lesion-associated molecular alterations by combining spatially resolved ion maps with protein-level characterization. MALDI-MSI data were processed to preserve spatial information and generate ion distribution maps of lesion-associated molecular alterations. Tissue segmentation was performed using a Spatial Shrunken Centroids (SSC) approach, enabling the identification of lesional and perilesional grey and white matter regions. Comparison of matched lesional and perilesional areas identified 110 differentially expressed *m/z* features associated with FCDIIb ($|\log2FC| > 0.5$; $p < 0.05$), including 62 upregulated and 48 downregulated signals in lesions and consistently detected in at least 50% of cases. In parallel, proteomes from lesional and perilesional tissues were compared using a shotgun LC-MS/MS differential proteomic approach. Protein identification, quantification, and pathway analysis were performed to identify dysregulated proteins and potential biomarker candidates ($|\log2FC| > 0.5$; $p < 0.05$). Proteomic profiling from the same FFPE tissues revealed two related yet distinct molecular components based on 203 differentially expressed features: a dysmorphic neuron-associated signature, primarily linked to altered neuronal growth, synaptic organization, and cytoskeletal remodelling, and a balloon cell-associated signature characterized by mTOR-driven growth dysregulation, impaired proteostasis/autophagy, and cellular stress responses.
Integration of MALDI-MSI and LC-MS/MS datasets identified 22 shared molecular features. In addition, 88 and 203 differentially expressed features were uniquely detected by MALDI-MSI and LC-MS/MS, respectively, highlighting the complementary contribution of spatially resolved and proteomic approaches in defining the molecular signature of FCDIIb.

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O-02

Multi-Omics Integration Reveals Cellular and Secretome Rewiring in KRAS/LKB1-mutated Non-Small Cell Lung Cancer

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Among the recurrent genetic alterations in non-small cell lung cancer (NSCLC), activating KRAS mutations frequently co-occur with loss of the tumor suppressor LKB1 (STK11), defining an aggressive molecular subtype characterized by profound metabolic and signaling alterations [1]. Here, we performed an integrated multi-compartment multi-omics characterization of an isogenic KRAS/LKB1-mutant NSCLC model to investigate how intracellular molecular remodeling extends to extracellular communication with the tumor microenvironment.

Using $KRAS^{G12C}$/$LKB1^{KO}$ and $KRAS^{WT}$/$LKB1^{WT}$ H1299 cells, we combined phosphoproteomics, proteomics, metabolomics and lipidomics to comprehensively profile the molecular phenotype associated with KRAS/LKB1 co-mutation. Phosphoproteomic profiling, integrated with kinase activity prediction and PamGene® kinase assays, revealed extensive remodeling of phosphorylation-dependent signaling networks, including a widespread reduction in serine/threonine and tyrosine kinase activities. In parallel, proteomic analysis identified enrichment of pathways involved in lipid metabolism, cell adhesion and protein transport, consistently metabolomic and lipidomic profiling highlighted coordinated alterations in fatty acid metabolism, accumulation of long-chain acyl-carnitines and remodeling of membrane lipid composition, with enrichment of polyunsaturated phospholipid species. To determine whether these intracellular alterations were reflected in extracellular communication, we extended our analyses to conditioned media and extracellular vesicles (EVs). Proteomic profiling of conditioned media revealed increased secretion of extracellular matrix remodeling proteins and inflammatory mediators, consistent with a pro-tumorigenic microenvironment. Furthermore, integrated proteomic, metabolomic and lipidomic characterization of EVs demonstrated extensive remodeling of vesicular cargo, including proteins associated with extracellular matrix organization and stromal cell activation, indicating that KRAS/LKB1 co-mutated cells actively reshape intercellular communication. Overall, this multi-compartment, multi-omics approach provides a comprehensive molecular characterization of KRAS/LKB1-mutant NSCLC cells and their secretome, demonstrating how integrated omics analyses can simultaneously capture intracellular alterations and extracellular communication networks that contribute to the aggressive phenotype associated with KRAS/LKB1 co-mutation.

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O-03

Membranome Proteomics Integrated with Secretome Profiling Reveals Membrane Remodeling in Methylmalonic Acidemia

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Methylmalonic acidemia (MMA), caused by methylmalonyl-CoA mutase (MMUT) deficiency, is a severe inherited metabolic disorder characterized by mitochondrial dysfunction, impaired anaplerosis, oxidative stress and the accumulation of toxic metabolites. While previous proteomic studies have primarily focused on intracellular alterations, the ensemble of membrane-associated proteins (membranome) orchestrating transport, signaling, and vesicular dynamics remains largely unexplored. Based on previous fluxomics data obtained in MMUT-deficient HEK293 cell lines, here we hypothesized that MMUT deficiency may induce a disease-specific remodeling of the membranome that extends beyond the primary enzymatic defect. Membrane-enriched fractions were isolated from MMUT-deficient and control cells and analyzed using data-independent acquisition (DIA) quantitative proteomics. This analysis generated highly reproducible membranome profiles, clearly discriminating MMUT-deficient cells from controls and revealing extensive remodeling of membrane-associated proteins. Differential protein abundance analysis combined with Gene Ontology enrichment identified the biological processes selectively altered, uncovering a coordinated reorganization of the membrane proteome rather than isolated abundance changes. Proteins associated with organic acid and carboxylic acid transmembrane transport were significantly depleted, whereas proteins involved in vesicle-mediated transport, endocytosis, receptor-mediated internalization, vesicle organization, and membrane trafficking were significantly enriched. Indeed, secretome alterations were identified in MMA cells, providing integration with membranomics data. These findings define a disease-associated membranome signature, indicating a global functional shift in membrane organization. Collectively, the data suggest that membrane remodeling represents an adaptive response to MMUT deficiency and may constitute a previously unrecognized layer of metabolic regulation.

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O-04
Proteomic Characterization of Ubiquitin Carboxyl-Terminal Hydrolase 19 Deficient Cells Reveals a Role for USP19 in the Secretion of Lysosomal Proteins

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Ubiquitin carboxyl-terminal hydrolase 19 (USP19) is a unique deubiquitinase, characterized by multiple variants generated by alternative splicing. Several variants bear a C-terminal transmembrane domain that anchors them to the endoplasmic reticulum[1]. Other than regulating protein stability by preventing proteasome degradation, USP19 has been reported to rescue substrates from endoplasmic reticulum–associated protein degradation in a catalytic-independent manner, promote autophagy, and address proteins to lysosomal degradation via endosomal microautophagy[2, 3, 4]. USP19 has recently emerged as the protein responsible for the unconventional secretion of misfolded proteins including Parkinson's disease–associated protein α-synuclein[5]. Despite mounting evidence that USP19 plays crucial roles in several biological processes, the underlying mechanisms are unclear due to lack of information on the physiological substrates of USP19. Herein, we used high-resolution quantitative proteomics to analyze changes in the secretome and cell proteome induced by the loss of USP19 to identify proteins whose secretion or turnover is regulated by USP19. We found that ablation of USP19 induced significant proteomic alterations both in and out of the cell. Loss of USP19 impaired the release of several lysosomal proteins, including legumain (LGMN) and several cathepsins. In order to understand the underlaying mechanism, we dissected the USP19-regulated secretion of LGMN in several cell types. We found that LGMN was not a deubiquitinase substrate of USP19 and that its USP19-dependent release did not require their direct interaction. LGMN secretion occurred by a mechanism that involved the Golgi apparatus, autophagosome formation, and lysosome function. This mechanism resembled the recently described "lysosomal exocytosis," by which lysosomal hydrolases are secreted, when ubiquitination of p62 is increased in cells lacking deubiquitinases such as USP15 and USP17. In conclusion, our proteomic characterization of USP19 has identified a collection of proteins in the secretome and within the cell that are regulated by USP19, which link USP19 to the secretion of lysosomal proteins, including LGMN[6].

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O-05
Quantitative proteomic analysis of the plasma protein corona formed on novel supramolecularly self-assembled albumin nanoparticles

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Nanoparticles (NPs) entering biological fluids are instantly coated by a layer of proteins known as the Protein Corona (PrC), which dictates their biological fate and behavior in vivo [1]. Albumin-based NPs (ANPs) are highly attractive drug delivery vehicles due to their cost-effective production, excellent biocompatibility, and versatility for both therapeutic and diagnostic applications [2]. In this study, we used quantitative proteomics to characterize the mouse plasma PrC of two distinct ANP formulations. The first one was prepared via traditional albumin desolvation followed by glutaraldehyde cross-linking (XANPs) and used as a reference [3]. The second, innovative formulation was developed through supramolecular self-assembly of albumin with a tetrazole-functionalized tetraphenylethylene derivative (TANPs), to act as proteolytic degradation sensors [4]. Label-free quantitative proteomics [5] revealed 130 differentially abundant proteins between the two formulations. Functional enrichment analysis showed that the XANP-PrC was strongly associated with complement activation and Ig-mediated immune responses, while the TANP-PrC was associated with lipid metabolic pathways. Both coronas shared common functional signatures, as the top 100 most abundant shared proteins formed a highly interconnected interaction network involved in the regulation of coagulation, fibrinolysis, and proteolysis. To further explore the proteolytic ability of the PrCs, we intersected our dataset with the MEROPS database and found that the TANPs recruited a larger number of proteases than the XANPs.
In conclusion, our proteomic profiling revealed distinct qualitative and quantitative differences between the TANP and XANP coronas, suggesting an increased susceptibility of TANPs to enzymatic degradation. These findings highlight TANPs as a highly promising sensor platform for monitoring proteolytic activities.

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O-06

Integration of HT screening and in-depth proteomic and phosphoproteomic profiling for enhanced kinase inhibitor selection in anti-fibrotic treatment

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Pulmonary fibrosis is a progressive lung pathology characterized by excessive extracellular matrix deposition, leading to tissue rigidity and organ failure. While some approved anti-fibrotic drugs exist, they cannot fully reverse the condition, highlighting the need for preventive therapies. In this study, we evaluated the anti-fibrotic potential of three kinase inhibitors, targeting ALK5, CDK7/9, and c-KIT, selected by an HT screening. Lung fibroblasts were pre-treated with the compounds prior to fibrosis induction using TGFβ. Comprehensive proteomic and phosphoproteomic analyses were conducted using LC-MS (diaPASEF on a Evosep 40 SPD-HT MS system, Bruker). Data processing and analysis were carried out with Spectronaut, R Studio and Cytoscape. Across experimental groups, over 8000 PGs and 6600 Class I phosphosites were quantified, enabling a deep characterization of the dysregulated pathways. TGFβ treatment altered 6 fibrosis-related pathways, including ECM and collagen formation, IGF regulation, as well as the expression of known fibrosis markers (ACTA2, FN1). Inhibition of ALK5 and CDK7/9 produces a markedly stronger anti-fibrotic effect than c-KIT. Mechanistically, ALK5 inhibition effectively attenuated fibrosis without inducing broader molecular dysregulation, suggesting a highly targeted mode of action. In contrast, CDK7/9 inhibition induced widespread transcriptional disruption, upregulation of the MCM complex and a preventive response for the accumulation of iron. In addition, the treatment with this compound affected kinase signalling, including components in the mTOR and MAPK pathways. This global response may indirectly limit the fibrotic transition rather than inhibiting it through a preventive signaling mechanism. Overall, these findings demonstrated that integrating HT screening with in-depth proteomic and phosphoproteomic profiling can lead to a more selective identification of the most effective compounds. Beyond the specific candidates described here, this study provides a general strategy for drug discovery efforts where understanding the full molecular consequences of pharmacological perturbation is a precious addition to understanding the phenotypic outcome.

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O-07

Integrated Proteome–Secretome Profiling Reveals Arsenic Adaptation mechanisms in Fusarium solani MUT6181

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Potentially toxic elements (PTEs) are persistent environmental contaminants that may accumulate in soils, sediments and aquatic systems, with possible long-term effects on ecosystems and human health. Among them, arsenic is relevant because of its toxicity and persistence in contaminated matrices. Fungi are frequently investigated in bioremediation studies due to their ability to grow under stressful environmental conditions and to interact with metals and metalloids. In this study, the response of *Fusarium solani* MUT6181 to arsenic exposure was evaluated, focusing on its tolerance and on differences in protein abundance potentially associated with arsenic stress. The strain was first tested for tolerance toward selected PTEs, including As, Cd, Co, Cu and Zn, by maximum tolerated concentration assays. Based on the growth response, arsenic was selected for further analysis. Preparative cultures were performed in biological triplicate under control conditions and in the presence of 1000 ppm $NaAsO_2$. After seven days of growth, mycelial biomass and extracellular fractions were collected separately. The mycelial proteome was analyzed by differential shotgun LC-MS/MS, followed by protein identification, quantification and statistical analysis of modulated proteins. In parallel, the extracellular protein fraction was examined through a gel-based proteomic approach, followed by protein identification and functional interpretation.
F. solani MUT6181 tolerated sodium arsenite, maintaining more than 50% growth compared with the control at 1000 ppm $NaAsO_2$. Proteomic analysis of the mycelium showed changes in the intracellular protein profile after arsenic exposure. Functional interpretation of the modulated proteins indicated the involvement of processes related to energy metabolism, redox balance, protein turnover, cell wall organization and exopeptidase/metalloexopeptidase activity. These data suggest that the response to arsenic involves different cellular functions, consistent with an adaptive response to stress. The extracellular fraction also showed differences between treated and control cultures, indicating that arsenic exposure may influence the extracellular protein profile.
Overall, the results show that *F. solani* MUT6181 tolerates high arsenite concentrations and displays proteomic changes under arsenic exposure. These findings provide a preliminary basis for further studies aimed at evaluating the possible role of *F. solani* MUT6181 in PTE bioremediation.

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O-08
Integrated Proteomic and Lipidomic Analysis of Pericardial Fluid Reveals the Molecular Epicardial Substrate of Brugada Syndrome

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Brugada syndrome (BrS) has long been classified as a primary channelopathy of genetic origin [1]. Yet, identifiable genetic variants account for only a fraction of clinically confirmed cases, and growing evidence points to a localized epicardial pathogenic substrate as a key contributor to disease pathogenesis. To characterize the molecular architecture of this substrate, we conducted a comprehensive multi-omic study integrating proteomics and lipidomics on pericardial fluid collected from 39 BrS patients with paired sampling performed before and after epicardial ablation, and a cohort of controls.

Relative to controls, BrS pericardial fluid displayed a distinct multi-omic signature. At the protein level, we observed a marked depletion of junctional, cytoskeletal, and desmosomal components — including desmoglein-1, desmocollin-1, plakoglobin, and desmoplakin — alongside a concurrent enrichment of complement factors, immunoglobulin-related proteins, and inflammatory pathway mediators. This pattern is consistent with a structurally remodeled, immune-active epicardial microenvironment. Independently, lipidomic profiling revealed extensive remodeling across phospholipid, sphingolipid, ether lipid, and bioactive lipid mediator classes, cleanly separating BrS from controls and reinforcing the existence of a disease-specific epicardial niche. Paired pre- and post-ablation analyses allowed us to interrogate the composition of the ablated substrate directly. This revealed enrichment of myocardial, extracellular matrix, stromal, and metabolic constituents — including troponins, myoglobin, tropomyosins, multiple collagens, the proteoglycans decorin, biglycan, lumican, and fibromodulin, alongside broadly remodeled lipid species.

To our knowledge, these results represent the first in vivo molecular characterization of the tissue removed during epicardial ablation in BrS, thereby illuminating the structural and biochemical components most likely to sustain the arrhythmogenic substrate. Taken together, our findings reframe BrS as a disorder rooted in an immune-active, neuro-adipo-fibro-myocardial epicardial microenvironment. They further establish pericardial fluid multi-omics as a powerful and minimally invasive window into disease biology and into the molecular basis of therapeutic substrate elimination.

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O-09

Proteomics of Egg Tempera: Aging and Pigment–Binder Interactions

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Paintings are complex and layered objects where the paint layer, made of pigments dispersed in a binder, is intended to be stable but evolves over time due to its intrinsic properties and environmental conditions. The chemical characterization of paint binders plays a pivotal role, both to understand the different painting techniques and inform conservation practices, as they are prone to degradation [1]. In this context, the present focuses on tempera-based paint films, combining the complementary strengths of non-invasive techniques, *i.e.* spatially representative imaging (HSI, FTIR mapping) with molecular specificity from mass spectrometry to elucidate drying processes and degradation mechanisms driven by pigment-binder interactions. Proteomic tools will help to gain deeper knowledge about aging processes and in particular, seek a connection between aging-related chemical modifications, and paint film stability in the different pigment binder-systems [2], starting from mock-ups prepared following historical recipes and aged. For molecular-level insights, micro-samples taken from the mock-ups will undergo a bottom-up proteomic approach followed by LC- MS/MS and bioinformatic analysis, optimizing the protocol taking into account the complexity of the yolk-based tempera films [3]. Preliminary tests were carried out on aged ovalbumin-pigments mock-ups, providing first insights on pigment-binder interactions in tempera paints.

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O-10
Genomics, transcriptomics and proteomics reveal the copper adaptation strategy of marine bacterium P96

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High copper concentrations can impair aquatic ecosystem functioning and threaten water resources relevant to sustainability, aquaculture, and food security. Understanding microbial adaptation to copper exposure is therefore essential for evaluating microorganisms for use in contaminated matrices, microbial consortia, and bioremediation strategies.
The marine bacterial strain P96, isolated from metal-contaminated sediments, was screened for copper tolerance through phenotypic maximum tolerable concentration assays. A sub-inhibitory condition of 23 mg/L $CuSO_4 \cdot 5H_2O$ was selected for an omics-guided investigation integrating genomics, transcriptomics, and proteomics through RAST-based matching and KEGG Orthology annotation.
Genomic analysis revealed an extensive copper-associated repertoire, including P1B-type ATPases, multicopper oxidases, HmrR/MerR-family regulators, Cop/Pco-associated proteins, copper-binding proteins, and envelope-associated transport components. Transcriptomics showed that most of these determinants increased under copper exposure, with particularly strong responses involving copper-translocating ATPases and metal-responsive regulators. Proteomics provided complementary evidence, including increased abundance of multicopper oxidases, a periplasmic copper-binding protein, a heavy-metal-export-associated protein, and proteins involved in redox protection and Fe–S cluster maintenance.
Integration of the three omics layers distinguished genomic potential from determinants actively recruited during exposure. Strong transcript–protein concordance for selected multicopper oxidases and metal-export functions suggested that copper oxidation and envelope-associated transport represent major components of the P96 adaptive response. These findings support a mechanism involving copper sensing, control of the cytoplasmic Cu(I) pool, periplasmic binding and trafficking, Cu(I) oxidation, and protection against secondary cellular damage [1,2].
ICP-MS analysis showed a 24.4 ± 2.9% reduction in soluble copper. Preliminary seawater experiments combining P96 with another marine strain isolated from the same contaminated Sarno River site also indicated enhanced removal, supporting its potential role as a complementary strain in microbial consortia. This study highlights the value of integrating genomics, transcriptomics, and proteomics to move beyond the identification of resistance genes and define the molecular processes effectively recruited during copper adaptation.

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O-11

A multi-omics integrated approach to prioritize biomarker candidates associated with breast cancer

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Breast cancer is a highly heterogeneous disease, and this complexity calls for biomarker discovery strategies able to capture subtype-specific biology while preserving translational potential. Here, we present an integrated multi-omics workflow based on the telomeric G-quadruplex-forming sequence tel46, immobilized on Controlled Pore Glass (CPG), as an affinity reagent to enrich disease-relevant protein networks. As a proof-of-concept, we first applied affinity purification-mass spectrometry and quantitative proteomics to luminal MCF7 cells compared with non-tumorigenic MCF10a cells [1]. This approach identified 93 tel46-interacting proteins and 27 tel46 binders among proteins upregulated in cancer cells. Subsequent interrogation of public proteomic and transcriptomic resources supported 19 candidates, highlighting MSH2, MSH6, ESRP1, and WDHD1 as the most promising biomarkers associated with the luminal context. We then extended the same strategy to triple-negative breast cancer (TNBC), a clinically aggressive and biologically heterogeneous subtype, by analysing MDA-MB-231 and BT549 cells. Intersecting tel46 interactomes with differential proteomics profiles yielded 15 shared upregulated candidates, which were further refined through public datasets to an 11-protein TNBC-associated panel. To move beyond single-marker readouts, we implemented a machine-learning framework with nested cross-validation, which prioritized a compact five-protein signature composed of KIF4A, NCAPD2, FOXK1, ACIN1, and RBM12. This panel discriminated basal-like tumors in silico, showed robustness in an independent cohort, and was supported by targeted proteomics confirming higher abundance of these proteins in TNBC cells than in non-tumorigenic cells and, in most comparisons, than in luminal cells. Overall, these findings establish tel46-based affinity proteomics as a scalable biomarker discovery platform, progressing from proof-of-concept in luminal breast cancer to subtype-oriented stratification in TNBC, and support telomeric G-quadruplex-guided capture as a biologically informed route to prioritize mechanistically grounded and potentially clinically translatable biomarker panels.

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O-12
Milk Proteomics as a One Health Sentinel: Integrating Biomarkers, Zoonotic Surveillance, and AI-Driven Prediction

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The One Health framework requires integrative approaches linking animal, human, and environmental health; however, current surveillance systems remain fragmented. Milk represents a unique biological interface connecting animal health, food quality and safety, microbial ecology, and public health. Mass spectrometry-based milk proteomics provides a high-resolution molecular readout of biological processes at the interface of livestock production, food safety, and public health, positioning milk as a potential sentinel matrix.

This study aims to demonstrate the role of milk proteomics as a One Health surveillance tool by: (i) identifying host-response biomarkers associated with animal health, (ii) monitoring zoonotic pathogens and antimicrobial resistance-related risks, and (iii) integrating proteomics with artificial intelligence for predictive applications. Evidence was synthesized from a series of proteomic, microbiological, and computational studies conducted in dairy sheep and goats. Proteomic investigations of milk biomarkers were combined with pathogen surveillance, antimicrobial resistance monitoring, and explainable machine learning approaches to evaluate the potential of milk as a One Health sentinel matrix.

Evidence from proteomic, microbiological, and computational studies identified host-response biomarkers, including antimicrobial peptides such as cathelicidins associated with mastitis and immune activation, while also highlighting the value of milk for monitoring zoonotic pathogens and antimicrobial resistance-related risks. Integration of these complementary datasets with explainable machine learning approaches supported disease classification, pathogen characterization, and prediction of milk quality and disease risk at the farm level.

Milk proteomics represents a scalable and translational platform for One Health monitoring, linking molecular-level insights with population-level outcomes. Collectively, these findings demonstrate that milk proteomics extends beyond biomarker discovery and can contribute to a broader One Health surveillance strategy. The integration of proteomics with artificial intelligence supports the development of proactive surveillance systems, contributing to improved animal health management, food safety, and reduced risks to human health through the food chain.

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O-13

Gut microbiome metaproteomics uncovers functional signatures in Tuberous Sclerosis Complex-related Autism Spectrum Disorder

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Tuberous Sclerosis Complex (TSC) is an autosomal dominant disease that frequently involves the central nervous system, with nearly half of affected children developing autism spectrum disorder (ASD) [1], yet no reliable biomarkers exist to identify those at high risk. Since growing evidence implicates the gut microbiota (GM) and the gut-brain axis in neurodevelopmental disorders [2], studying them represents a promising approach for discovering microbial signatures of TSC. We examined the GM function of TSC patients compared to neurotypical age matched subjects using a metaproteomic approach. 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 MetaPilot software for label-free quantification and functional annotation. Taxonomic assignments were retrieved using the Lowest Common Algorithm (LCA) in Unipept. Preliminary results provided GM's distinctive disease related-pattern linked to TSC, from both functional and LCA-derived taxonomic point of view. Among the others modulated pathway, the concurrent enrichment of biotin metabolism and fatty acid biosynthesis in TSC is mechanistically coherent, since biotin is a cofactor of the carboxylases driving fatty acid biosynthesis. This coordinated shift in microbial lipid metabolism may be particularly relevant in TSC, whose principal defect, constitutive mTOR activation, is itself a driver of lipogenesis [3]. Among the differentially expressed bacterial protein groups (PGs), Bacteroidota and Bacillota were the two most represented phyla, although they showed opposite trends in TSC subjects compared to neurotypical subjects. While only 1.3% of Bacteroidota PGs were down-regulated, the majority of Bacillota PGs (94.8%) were down-regulated, with *Faecalibacterium* being the most represented genus among the latter. These evidences may help shedding light onto TSC pathophysiology and potentially open new avenues for therapeutic interventions GM-based aimed to ameliorate the management of TSC patients.

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O-14

Unique Peptides as Next-Generation Proteomic Biomarkers for Species Authentication and Traceability of Dairy Products within a One Health Framework

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The authentication of species origin in dairy products remains a major challenge for food quality assurance, particularly in premium and Protected Designation of Origin (PDO) cheeses, creating a growing demand for sensitive proteomic biomarkers for traceability and adulteration detection. Proteomics provides powerful tools for the identification of species-specific molecular signatures that can support authenticity testing and detect food adulteration. In this context, unique peptides (UPs), core unique peptides (CrUPs), and composite unique peptides (CmUPs) represent promising next-generation biomarkers.

In this study, a previously established *Uniquome*-based bioinformatic approach is applied to milk proteomes from bovine, ovine and caprine species. First, bovine, ovine and caprine milk proteomes will be comparatively analysed to identify species-specific CrUPs and CmUPs that discriminate among the three milk-producing species through comparative sequence analysis and in silico enzymatic digestion. Public protein and structural databases, including UniProtKB/Swiss-Prot, NCBI Protein and AlphaFold, together with available milk proteome datasets, are employed to systematically characterize peptide signatures associated with each species. Subsequently, the identified species-specific CrUPs will be evaluated against the human proteome to assess their exclusivity and explore their potential biological relevance within a One Health framework. Candidate peptides will be further assessed using functional annotation and structural bioinformatics approaches to evaluate their suitability as targets for mass spectrometry-based applications.

The study aims to generate a comparative catalogue of bovine-, ovine- and caprine-specific peptide signatures suitable for targeted proteomics applications. The resulting CrUP and CmUP repertoire is expected to provide novel peptide biomarkers for species authentication and traceability in dairy products while demonstrating the utility of the *Uniquome* approach for biomarker discovery and targeted proteomics applications within a One Health framework.

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O-15
Proteomics as a key tool for the molecular characterization of collagen-based bio-adhesive recovered from tannery waste

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The growing interest in sustainability and resource valorization has encouraged the development of innovative strategies aimed at recovering and reusing industrial by-products. Within this framework, the principles of green chemistry, circular economy, and upcycling provide effective approaches for transforming waste materials into high-value products.
This contribution focuses on the proteomic characterization of collagen recovered from tannery waste through sustainable extraction processes. The study was carried out within a broader research activity devoted to the valorization of vegetable-tanned leather residues, with the ultimate goal of producing innovative biomaterials and potential bio-based adhesives from protein-rich waste streams.
At the Department of Chemistry and Industrial Chemistry of the University of Pisa within the framework of the Prin ArtDECOW research project, collagen was extracted from vegetable-tanned leather samples using Natural Deep Eutectic Solvents (NADES), a class of deep eutectic solvents composed of natural, biodegradable compounds characterized by a low environmental impact. The gelatine recovered after the treatment were characterised from the molecular point of view to evaluate the impact of the different procedures on the protein product of interest. The molecular characterization of the extracted proteins was performed using a bottom-up proteomics workflow. Following protein denaturation and enzymatic digestion through S-Trap technology, the resulting peptides were purified using C18 StageTips and analysed by mass spectrometry. Preliminary MALDI-TOF analyses were carried out to assess extraction efficiency and peptide mixture quality, while high-resolution LC-MS/MS analyses on an Orbitrap platform enabled detailed protein identification and characterization of the main chemical modifications associated with tanning, extraction, and degradation processes.
Bioinformatic data processing was performed using MaxQuant software followed by in-house command-line scripts, allowing the identification and semi-quantitative evaluation of relevant chemical modifications by a spectral counting approach, including methionine oxidation, asparagine and glutamine deamidation, and backbone cleavage events, which were used as indicators of collagen fragmentation [1]. The results demonstrated the key role of proteomic analysis in assessing the molecular quality of recovered collagen. Furthermore, the study highlighted how different extraction and treatment conditions can affect protein integrity and, consequently, the properties of the resulting material. These findings provide valuable insights for the development of sustainable collagen-based biomaterials and support the valorization of tannery waste within a circular economy perspective [2].

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O-16

Brucella melitensis and the Hidden Signature of the 16M Strain

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Brucellosis remains one of the most persistent challenges in veterinary public health, as it infiltrates production systems, reduces farm profitability, and poses risks to workers handling infected animals or contaminated materials. Within the genus *Brucella*, *Brucella melitensis* is the dominant pathogen in small ruminants and the leading cause of human infection. Disease control in small ruminants relies primarily on mass vaccination with live attenuated strains, especially Rev.1, combined with test-and-slaughter strategies. However, a structural limitation of live vaccines is the lack of Differentiating Infected from Vaccinated Animals (DIVA) capability, which prevents clear differentiation between infected and vaccinated animals. This constraint complicates epidemiological surveillance, slows eradication programmes, and affects international trade, highlighting the need for strain-specific molecular. To address this gap, a targeted genomic investigation focused on segment 824171–824131 of chromosome II in *Brucella melitensis* 16M, hypothesized as a divergence hotspot between Rev.1 and field strains. A comparative analysis involving fifty-six genomes, integrated with immunogenicity predictions using IEDB (Immune Epitope Database) platforms, identified a unique 13-amino-acid peptide present exclusively in the field strain 16M and completely absent from Rev.1 and all other analysed strains [1]. Cross-species epitope mapping further indicated strong predicted affinity for the ovine Ovar N*14:01 allele, suggesting relevant immune recognition in the natural host. This peptide emerges as a highly promising DIVA biomarker. Its translational value lies in the convergence of strict genomic exclusivity, consistent Major Histocompatibility Complex Class I (MHC-I) signalling across overlapping peptide windows, and direct applicability in vaccinated populations. Together, these features position the peptide as a competitive candidate for improving surveillance accuracy and strengthening brucellosis control strategies in small ruminants.

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O-17

The HUB/SPOKE Italian IDEA Network for "The Human Microbiota in Systems Medicine Applied to Diagnosis and Therapy in Pediatrics": role of the metaproteomic approach for AI-supported multilayer phenotyping for diagnosis and prognosis of intestinal dysbiosis in neurodevelopmental disorders.

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Metaproteomics provides functional insights into the gut microbiota (GM) beyond taxonomy, an approach of particular relevance to neurodevelopmental disorders such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), in which GM alterations have been increasingly implicated.
The study herein presented was conducted within the framework of the HUB/SPOKES Rete Italiana Salute Dell'Età Evolutiva (Rete IDEA) network, which aims to characterize GM of children and adolescents with intestinal (inflammatory bowel disease) and extra-intestinal (neurodevelopmental, neurological, autoimmune, metabolic, and endocrine) disorders by applying different omics, including metaproteomics. Rete IDEA provides a multicenter and integrated research network involving ten Italian Scientific Institutes for Research, Hospitalization and Healthcare (IRCCSs), coordinated by Bambino Gesù Children's Hospital as hub.
Analyses of stool samples from ADHD and ASD pediatric patients, recruited at two IRCCSs, were performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based metaproteomics, and compared with healthy control samples. Acquired MS data were processed using MetaPilot software, identifying a total of 80,797 protein groups, corresponding to 51,810 unique peptide sequences. Among the concordantly modulated KEGG pathways in ASD and ADHD patients, the most prominent signature was the coordinated downregulation of folate biosynthesis, cysteine, methionine and vitamin B6 metabolism. A more ASD-specific signature was represented by nicotinate and nicotinamide metabolism, phenylpropanoid, ubiquinone and other terpenoid-quinone biosynthesis. In line with our findings, previous metabolomic studies reported reduced fecal levels of vitamins and amino acids in children with ASD, and showed that GM can modulate autism-like behaviors through the regulation of vitamin B6 homeostasis. This study represents a first step toward integrating metaproteomics with metataxonomy, metagenomics, and metabolomics-derived multilayer phenotyping to define disease-specific GM signatures. These results may be useful for the future development and validation of AI models that recognize these signatures and support clinical decisions in the diagnosis and prognosis of intestinal dysbiosis associated to neurodevelopmental disorders.

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FLASH ORAL COMMUNICATIONS from SUMMER SCHOOL PARTICIPANTS

FL-O-01

Deciphering the molecular landscape of Cym1 dysfunction through multi-omics profiling in yeast models

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Mitochondrial targeting sequences (MTS) are cleaved from proteins imported into mitochondria and subsequently released into the mitochondrial matrix. The accumulation of these sequences can compromise mitochondrial function. In *Saccharomyces cerevisiae*, the metallopeptidase Cym1, which is the ortholog of human PITRM1 protease, is responsible for the degradation of MTS. Variants in this protein coding gene have been associated to neurodegenerative disorders. Beyond the established function at the mitochondrial level, our aim is to deepen the knowledge about MTS scavenging peptidases in health and disease, with a focus on the effects of Cym1 dysfunction on extra-mitochondrial compartments. Previous proteomic analyses from our laboratory indicated that impairment of Cym1 alters not only mitochondrial metabolism and proteostasis but also induces adaptive responses involving energy metabolism and protein quality control pathways at the cellular level.

To investigate these systemic effects, we performed an integrative multi-omics analysis in yeast models expressing different *Cym1* variants, including a loss-of-function variant causative for spinocerebellar ataxia and additional candidate variants associated with Alzheimer's disease in humans. Transcriptomic and proteomic datasets were analyzed and integrated using complementary univariate and multivariate approaches, to identify coordinated alterations across molecular layers.
Our results show that Cym1 dysfunction is associated with widespread remodeling of cellular networks extending beyond mitochondria, including cytosolic protein quality control, metabolic rewiring, and transcriptional reprogramming. These findings suggest Cym1 as a player in the cross-talk between mitochondrial and extra-mitochondrial pathways.

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FL-O-02
Targeting the Proteoglycan-like domain of the human Carbonic Anhydrase IX with a novel anticancer peptide

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Human carbonic anhydrases (hCAs) are metalloenzymes that catalyze the reversible hydration of CO_2 into bicarbonate and protons [1]. Among the different hCA isoforms, Carbonic Anhydrase IX (CA IX) is of particular interest because of its pivotal role in hypoxic tumors, where it promotes cancer cell survival, proliferation, and invasion. CA IX is a multidomain transmembrane protein composed of a catalytic carbonic anhydrase (CA) domain, a transmembrane (TM) segment, an intracellular (IC) tail, and an extracellular N-terminal proteoglycan (PG)-like domain. This latter domain is unique to this isoform and plays a key role by significantly enhancing the catalytic efficiency of CA IX and stabilizing its optimal working pH at approximately 6.5, a hallmark of the acidic microenvironment characteristic of hypoxic solid tumors in which CA IX is overexpressed [2]. Beyond this well-established function, the PG domain also interacts with extracellular matrix components, thereby facilitating tumor cell migration. In 2018, we provided the first comprehensive characterization of the PG domain. Combining biochemical, biophysical, and molecular dynamics approaches, we demonstrated that this domain behaves as an Intrinsically Disordered Protein (IDP), displaying only localized residual polyproline II secondary structure [3]. Its intrinsic conformational flexibility is fully consistent with its functional roles within the tumor microenvironment.

All these findings identified the PG domain as a promising druggable hotspot and stimulated further investigation. In particular, it was demonstrated that the M75 monoclonal antibody by recognizing a specific region within the PG-like domain, was able to induce a reduction of cancer cell adhesion. The crystal structure of the M75–epitope complex [4] provided a valuable structural framework for the rational design of peptides targeting this interaction interface. Based on these findings, we employed bioinformatic approaches to design a novel peptide, hereafter named CQPEP1. The peptide was synthesized, its secondary structure characterized by circular dichroism spectroscopy, and binding assays by means of Microscale Thermophoresis with the recombinant PG domain were performed. Overall, CQPEP1 represents a promising molecule for the development of innovative therapeutic strategies targeting CA IX in hypoxic tumors.

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FL-O-03
Endogenous Antimicrobial Milk Proteins as Candidate Indicators of Bovine Milk Hygienic Quality and Natural Preservation Potential

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Bovine milk hygienic quality is a key factor for food safety and dairy chain control. Conventional culture-based microbiological methods remain essential for assessing bacterial load, but they require prolonged incubation times and are therefore not suitable for rapid screening. In this context, endogenous antimicrobial milk proteins may provide complementary molecular information on microbial quality and may also represent bioactive components involved in the natural preservation capacity of milk.

In this study, a bottom-up proteomics approach was applied to bovine milk samples to investigate host-derived antimicrobial proteins associated with bacterial load. Milk proteins were enzymatically digested and analyzed by LC-MS/MS. Protein identification and label-free quantification were performed using MaxQuant. LFQ intensities were used to evaluate the relative abundance of antimicrobial proteins, including peptidoglycan recognition protein 1, lactotransferrin/lactoferrin, lactoperoxidase, beta-defensins and cathelicidins. Proteomic data were integrated with bacterial count data to explore the relationship between endogenous antimicrobial protein abundance and milk microbial quality.

Among the identified antimicrobial proteins, peptidoglycan recognition protein 1 emerged as one of the most relevant candidates. Higher abundance of this protein, together with a broader antimicrobial protein profile, was associated with lower bacterial counts in the analyzed milk samples. This inverse association suggests that host-derived antimicrobial proteins may reflect improved hygienic quality and may contribute to the natural microbial stability of bovine milk.

Overall, this study highlights the potential of bottom-up proteomics to identify endogenous antimicrobial milk proteins associated with microbial quality. Peptidoglycan recognition protein 1 and related antimicrobial proteins are proposed as candidate molecular indicators of bovine milk hygienic quality and as promising bioactive components for future natural preservation strategies. Further validation in larger sample cohorts and functional assays will be required to confirm their diagnostic and technological relevance.

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FL-O-04

Spatial lipidomics identifies psychosine dysregulation in a mouse model of Marinesco–Sjögren syndrome, prompting targeted LC-MS/MS assessment in tissue and dried blood spots

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Recent advances in spatial lipidomics have improved the characterization of disease-associated lipid alterations. High-resolution MALDI mass spectrometry imaging (MALDI-MSI) performed on the woozy mouse model of Marinesco–Sjögren syndrome (MSS), an inherited neurodegenerative disorder caused by Sil1 deficiency, revealed an unexpected accumulation of sulfonated psychosine, a cytotoxic lysosphingolipid classically associated with Krabbe disease, suggesting the involvement of previously unrecognized sphingolipid alterations in MSS. This finding prompted further investigation using a targeted analytical approach.

To this aim, a multiplex liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was employed for the simultaneous determination of the major lysosphingolipid biomarkers associated with lysosomal storage diseases (LSDs), including glucosylsphingosine (LysoGb1), psychosine (PSY), lysosphingomyelin (Lyso-SM), lyso-sphingomyelin-509 (Lyso-SM-509) and globotriaosylsphingosine (LysoGb3). The assay combines hydrophilic interaction liquid chromatography (HILIC) with tandem mass spectrometry operating in positive electrospray ionization mode (ESI^+) and multiple reaction monitoring (MRM), providing sensitive and selective quantification of these analytes. The HILIC separation enables chromatographic resolution of the diastereomeric compounds LysoGb1 and PSY, which have identical mass-to-charge ratio and are therefore difficult to distinguish. The targeted workflow is currently being applied to tissue extracts from the MSS mouse model and, preliminarily, to dried blood spot (DBS) samples to investigate its applicability across different biological matrices.

Overall, this work illustrates a translational workflow in which untargeted spatial lipidomics drives biomarker discovery, while targeted LC-MS/MS enables analytical validation and clinical implementation. Extending this approach to DBS may facilitate the development of multiplex assays suitable for minimally invasive diagnosis and confirmatory testing of sphingolipidoses.

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FL-O-05
Systematic reuse of public colorectal cancer proteomics: from raw data to target discovery

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Targeted drug conjugates (TDCs) have emerged as a rapidly expanding class of therapeutics; however, the identification of suitable target antigens remains a major challenge. Large-scale reuse of publicly available mass spectrometry proteomics datasets represents a valuable strategy to increase statistical power and identify robust, reproducible tumor-associated protein signatures.

Raw mass spectrometry data from multiple independent colorectal cancer (CRC) cohorts were retrieved from public repositories and uniformly reprocessed through standardized peak conversion workflows. The resulting protein abundance matrices underwent a harmonization pipeline addressing missing-value handling and imputation. Missing values were first filtered using group-aware thresholds. The remaining missing data were classified according to their presumed mechanism: values that likely result from stochastic under-sampling were imputed using methods that preserve the local protein expression structure, whereas values that are missing because below the instrument detection limit were imputed with low-intensity values, reflecting the biological assumption of low or absent protein expression.

Univariate differential expression analysis and a complementary supervised multivariate approach were applied to the multi-cohort proteomic data to identify proteins robustly dysregulated between tumor and normal tissues, generating a high-confidence CRC proteomic signature. Candidate proteins were then prioritized based on predicted cell-surface localization, expression within the tumor microenvironment, and intracellular distribution to improve target selectivity and minimize potential off-target toxicity.

Overall, this data-driven framework provides a reproducible and scalable pipeline for the systematic prioritization of candidate TDC targets by integrating publicly available multi-omics datasets.

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FL-O-06

A Multi-Omics Integrative Approach Based on Public Dataset Reanalysis to Identify Cell-Surface Targets for Drug Conjugates in Prostate Cancer

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Prostate cancer (PCa) is a heterogeneous malignancy characterized by uncontrolled cellular proliferation and capacity to escape androgen receptor (AR) signaling, acquiring in this way an AR-independent phenotype that may include neuroendocrine transdifferentiation. Despite initial responses to standard treatments, many patients progress to castration-resistant or metastatic disease, a process largely driven by the molecular heterogeneity of PCa, which makes it difficult to identify reliable targets for new therapeutic strategies.

Targeted drug conjugates have emerged as a promising precision oncology strategy, enabling the selective delivery of cytotoxic agents to tumor cells expressing specific surface antigens. Nevertheless, a critical aspect in their development remains the identification of membrane proteins predominantly expressed on tumor cells while showing low expression in normal tissues.

To address this, we implemented an integrative proteomics approach based on the reanalysis of publicly available datasets retrieved from PRoteomics IDEntifications Database. Raw proteomic data were processed using a standardized pipeline designed to be applicable across multiple datasets, allowing their systematic reuse and integration. Univariate differential expression analysis and a complementary supervised multivariate approach were combined with surfaceome annotations to identify tumor-selective candidates. These proteomic results were then integrated with data from Clinical Proteomic Tumor Analysis Consortium and The Cancer Genome Atlas to further support expression patterns and assess tissue specificity across a broader sample collection, retaining only candidates with limited abundance in normal tissues.

This work demonstrates how systematic reanalysis of publicly available proteomic datasets, combined with omics data integration, can generate a robust and reproducible evidence pipeline for target discovery in PCa.

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FL-O-07
Regional and Sex Differences in the Aging Sheep Brain Proteome

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Understanding physiological brain aging requires experimental models that better reflect the structural and temporal complexity of the human brain. Sheep are a valuable large-animal model because of their long lifespan, gyrencephalic brain, and age-related neurodegenerative features [1]. However, the molecular mechanisms underlying sheep brain aging, particularly at regional and sex-specific levels, remain largely unexplored.
We performed a comprehensive proteomic analysis of the hippocampus, prefrontal cortex, and motor cortex in pre-pubertal, adult, and aged sheep of both sexes. Proteomic remodeling was investigated using label-free shotgun nanoLC–MS/MS. Protein identification and quantification were carried out with MaxQuant and Perseus, while functional and pathway enrichment analyses were performed using Panther and Ingenuity Pathway Analysis [2].
More than 2,500 proteins were identified in each brain region, revealing distinct region-, age-, and sex-dependent proteomic signatures. The hippocampus was enriched in pathways related to proteostasis, protein folding, and cellular stress responses, highlighting the importance of protein quality-control mechanisms during aging. By contrast, the prefrontal and motor cortices showed prominent alterations in mitochondrial function, oxidative phosphorylation, and metabolic signaling, suggesting a greater susceptibility of cortical regions to age-related bioenergetic dysregulation.
Sex was a major determinant of molecular variability. Principal component and differential expression analyses revealed region- and age-dependent sex-specific patterns. In the prefrontal cortex, a clear sex-related proteomic signature was already detectable at early developmental stages, suggesting that sex-specific molecular trajectories are established early. Conversely, in the hippocampus and motor cortex, sex differences became more pronounced with advancing age, indicating a progressive divergence between male and female proteomic profiles.
These findings show that sex is not merely a covariate, but a key biological variable shaping distinct trajectories of brain aging and potentially influencing vulnerability to neurodegenerative processes. Overall, this study provides the first multi-region proteomic characterization of physiological brain aging in sheep, supports the translational relevance of this model, and highlights the need to integrate sex as a fundamental dimension in brain-aging research.

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FL-O-08

Proteomic characterization of ischemic stroke thrombi: preliminary insights into thrombus heterogeneity and molecular pathways involved in thrombus organization

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Background
Ischemic stroke is one of the leading causes of mortality and long-term disability worldwide. Since 2015, mechanical thrombectomy has become the standard treatment for large vessel occlusion (LVO) stroke, providing a unique opportunity to recover thrombotic material directly from patients. Understanding the molecular features of these thrombi could enhance knowledge of thrombus biology and aid in identifying molecular characteristics linked to various stroke causes. **Methods:**Four biobanked thrombi, 2 with carbioembolic (CE) and 2 atherotrombotic (AT) aetiology were collected during mechanical thrombectomy and analyzed by shotgun label-free proteomics using an Orbitrap Exploris 480 mass spectrometer operating in Data Dependent Acquisition(DDA)mode. For each thrombus, the external and internal portions were processed and analyzed separately. Protein identification was followed by exploratory bioinformatic analyses, including Gene Ontology (GO) and pathway enrichment analysis using the Enrichr platform. **Results:** Proteomic profiling identified approximately 700 proteins shared between the internal and external compartments of both CE and AT thrombi. In addition, 53 proteins were exclusively detected in the internal fraction, whereas 17 proteins were unique to the external fraction. The most abundant proteins included fibrinogen chains, fibrin-associated proteins and immunoglobulins, confirming the complex structural and immune-related composition of ischemic thrombi. Functional enrichment analyses highlighted the involvement of several biological processes associated with thrombus formation and remodeling, including innate immune system activation, neutrophil degranulation, hemostasis, platelet aggregation, cellular stress response, regulation of apoptotic pathways and calcium homeostasis. Furthermore, several proteins were associated with mitochondrial activity, suggesting a potential link between thrombus organization, inflammatory processes and oxidative stress. **Conclusions:** These preliminary findings support the feasibility of integrating proteomic analyses into the molecular characterization of ischemic stroke thrombi. The identification of compartment-specific proteins and biologically relevant pathways provides new insights into thrombus heterogeneity and may contribute to future multi-omics studies combining proteomics, flow cytometry, imaging and clinical data. Such approaches could improve thrombus stratification and support the development of predictive models for stroke recurrence and personalized secondary prevention strategies.

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FL-O-09
Impact of starting material amount on protein identification and proteome coverage in bovine faecal metaproteomics

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The optimisation of sample input is a critical step in metaproteomic analyses of complex biological matrices, where efficient and reproducible protein extraction is essential for the reliable characterisation of microbial communities [1]. Bovine faeces represent a relevant matrix within the One Health framework, linking animal health, agricultural production, and food safety. In this study, bovine faecal samples were analysed by LC-MS/MS to evaluate the impact of three different input amounts (20, 50, and 100 mg) on protein identification and proteome coverage. Protein yield was initially assessed through protein quantification prior to LC-MS/MS analysis. A total of 472 proteins were identified from 20 mg of sample, 956 from 50 mg, and 1020 from 100 mg. The three datasets shared 301 proteins, indicating a robust core proteome across all extraction conditions. These findings demonstrate that increasing the starting material substantially improves proteome coverage and influences the extent of overlap among identified proteins. Overall, the results support the use of higher input amounts for more comprehensive bovine faecal metaproteomics and highlight the importance of matrix-specific optimisation strategies to improve the reliability and comparability of proteomic workflows applied to complex faecal samples.

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FL-O-10

The plastisphere as a model for proteomic studies in the One Health context: microbial proteins, microplastic biodegradation, and antimicrobial resistance

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Microplastics (MPs) are currently ubiquitous environmental contaminants that affect all environmental compartments, animals, and humans, acting as a cross-cutting threat to global health that requires a One Health approach to mitigate [1]. Once released into the environment, MPs provide surfaces for microbial colonisation, leading to the formation of the plastisphere, a distinct microenvironment characterised by unique metabolic activity and molecular interactions. Besides, MPs affect all three One Health domains by altering biogeochemical cycles, favoring accumulation within the food chain that can be transferred to humans. Within the plastisphere, two interconnected processes are particularly relevant: first, mitigation through polymer biodegradation, mediated by bacterial, fungal, and algal enzymatic pathways; second, the raised risk of the enhancement of antimicrobial resistance (AMR), mainly through horizontal gene transfer (HGT) and co-selection mechanisms [2]. In this context, proteomics offers a complementary analytical contribution to genomic and transcriptomic approaches. Proteomic analysis allows us to characterise the mechanisms involved in biofilm formation, responses to environmental stress, the degradation of plastic polymers, and the expression of factors associated with AMR. Furthermore, metaproteomics can provide functional information complementary to that obtained using DNA-based techniques, helping to distinguish the simple presence of microorganisms from their actual metabolic activity [3]. Consequently, it is relevant to integrate proteomic approaches in the study of the plastisphere, which will promote understanding of the molecular mechanisms of biodegradation and build an AMR surveillance framework in MPs bioremediation strategies.

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FL-O-11

Enhancing proteomic coverage of buffalo milk through optimized protein extraction for quality control applications

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Buffalo milk is a high-value dairy matrix, at the core of protected designation of origin (PDO) products such as Mozzarella di Bufala Campana, where quality and authenticity control are critical for both consumer protection and supply chain traceability. Proteomic approaches offer a powerful tool for detecting adulteration, non-compliant processing, or storage-related alterations, since changes at the protein and peptide level can reveal information not accessible through conventional compositional analysis. However, buffalo milk's proteome is characterized by a wide dynamic range, dominated by a large casein fraction alongside a more heterogeneous whey component — including β-lactoglobulin, α-lactalbumin, lactoferrin, and immunoglobulins — where abundant proteins can mask the low-copy-number molecules most likely to carry diagnostic value. Before any authenticity marker can be reliably identified, the extraction step itself must therefore be optimized to preserve analytical sensitivity. This study compares two protein extraction strategies for Italian Mediterranean water buffalo (*Bubalus bubalis*) milk, aiming to establish a reliable protocol to support future proteomics-based quality and authenticity assessments.
Two approaches were evaluated: a non-fractionated workflow using a urea-based chaotropic buffer to extract total proteins from skimmed milk, and a fractionation workflow based on ultracentrifugation, in which the casein-enriched pellet was separated from the whey fraction according to the differential sedimentation of colloidal casein micelles. Both protocols were compared in terms of protein yield and reproducibility using protein quantification assays and SDS-PAGE profiling. The resulting total protein extract, casein-enriched fraction, and whey fraction will subsequently undergo tryptic digestion followed by nanoLC-ESI-MS/MS analysis.
Based on BCA protein quantification, the ultracentrifugation-based fractionation workflow yielded higher total protein recovery than the non-fractionated urea-based extraction protocol. SDS-PAGE profiles confirmed casein enrichment in the pellet, while additional low-molecular-weight bands were more clearly resolved in the whey fraction than in the urea-based extract, suggesting reduced chemical interference and co-precipitation associated with chaotropic extraction. These preliminary results support ultracentrifugation as the preferred sample preparation strategy for downstream nLC-ESI-MS/MS analysis. Future proteomic analyses will focus on identifying protein and peptide markers associated with processing and storage conditions, contributing to the development of robust proteomics-based tools for buffalo milk quality assessment and authenticity verification.

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FL-O-12

Proteomics uncovers AZE as a molecular impostor driving protein misfolding and cellular stress

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Protein function critically depends on the acquisition of a correct three-dimensional structure. Proper protein folding is essential not only for biological activity but also for protecting proteins from chemical damage, while misfolded proteins are particularly susceptible to oxidative modifications, including lipid peroxidation. Mutations that impair protein folding have been associated with increased protein oxidation, highlighting the close relationship between protein structure and proteome integrity. Proline plays a key role in determining protein secondary and tertiary structure owing to its unique conformational properties. The non-canonical proline analogue azetidine-2-carboxylic acid (AZE) can be mistakenly incorporated into newly synthesized proteins, potentially disrupting protein folding and acting as a potent teratogen.

In this study, we investigate the incorporation of AZE into cellular proteins using high-resolution mass spectrometry applying the classical Data Dependent Acquisition (DDA) and characterize the amino acid sequence context surrounding AZE incorporation sites to identify possible structural determinants of its misincorporation. To evaluate the proteome-wide consequences of AZE exposure, we employ a differential proteomics workflow based on Data-Independent Acquisition (DIA), a highly robust and reproducible quantitative strategy that provides improved proteome coverage and reliable protein quantification. These strategies provided direct evidence that AZE is incorporated into protein sequences in a non-random manner and that its incorporation is driven by the local sequence surrounding proline residues. Furthermore, quantitative proteomic analysis showed that AZE incorporation is associated with significant perturbations in biological pathways involved in protein folding, cellular stress responses, and biosynthetic processes.

This approach enables the comprehensive characterization of proteomic alterations and the identification of biological pathways and cellular processes affected by AZE-induced proteotoxic stress. More broadly, it provides a proof of principle for investigating the molecular consequences of non-canonical amino acid misincorporation and establishes a proteomics framework applicable to a wide range of protein quality control disorders.

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FL-O-13

A preliminary proteomic investigation into HER-2 expression in canine mammary carcinomas

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Human epidermal growth factor receptor 2 (HER-2) is a transmembrane glycoprotein with tyrosine kinase activity belonging to the epidermal growth factor receptor (EGFR) family. In human breast cancer, c-erbB-2 gene amplification and HER-2 overexpression are well-established prognostic and predictive biomarkers and represent the basis for targeted anti-HER-2 therapies. Due to the biological similarities between canine mammary carcinoma (CMC) and human breast cancer, HER-2 has also been investigated as a potential prognostic marker and therapeutic target in dogs. However, its biological and clinical significance in CMC remains controversial, with previous studies reporting conflicting findings regarding HER-2 expression and the potential efficacy of anti-HER-2 therapies [1,2].

The aim of this study was to characterize the protein targets recognized by an anti-HER-2 antibody using an immunoproteomic approach. Proteins from mammary tissue samples from 11 dogs were extracted, separated by SDS-PAGE and two-dimensional electrophoresis (2-DE), selected by Western blot and identified using mass spectrometry. Samples from each lesion had been previously histologically processed and evaluated by immunohistochemistry. Eight samples were HER-2-positive (1 scored as 3+, 4 as 2+, and 3 as 1+) while 3 samples were HER-2-negative.

Protein extraction was carried out in agreement with the protocol by Burrai and colleagues [2].

By Western blot analysis, antibody binding was observed in both healthy and neoplastic mammary tissues, with chemiluminescent bands detected at approximately 190, 166, 105, 49, and 25 kDa. Although the same protein pattern was observed in both groups, the signals detected in neoplastic tissues were more intense in pixels than those in healthy tissues. The corresponding protein bands were excised from the gels and identified by mass spectrometry. Surprisingly, none of the identified proteins corresponded to HER-2.

These findings suggest the possibility of antibody cross-reactivity with proteins other than HER-2. Further studies are needed to optimize protein sampling and extraction procedures, confirm these results, and clarify the expression and biological role of HER2 in canine mammary carcinomas.

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FL-O-14
Evaluation of the global and intracellular effect of human histone H1.2-derived peptide in *Pseudomonas aeruginosa* via Differential and Functional Proteomics

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Antimicrobial resistance (AMR) represents an escalating global health crisis that urgently necessitates the development of innovative alternative therapeutics, including human host-defense peptides. Beyond their canonical structural role in chromatin organization, extranuclear histones are increasingly recognized as essential components of the innate immune system, exhibiting potent antimicrobial properties. Prior investigations established that a human Histone H1.2derived fragment exerts selective, non-lytic antibacterial activity against the critical ESKAPE pathogen *Pseudomonas aeruginosa* (*P. aeruginosa*)[1]. This activity induces a post-transcriptional down-regulation of the Sec translocon and the β-barrel assembly machinery (BAM) complex, both of which are critical for cell envelope homeostasis[2]. To elucidate the specific intracellular target driving this global effect, we employed a targeted functional proteomics approach utilizing biotinylated H1.2 as bait. This strategy successfully identified the RNA chaperone Hfq, a master regulator of bacterial post-transcriptional networks[3], as the principal interacting partner. Furthermore, in silico molecular docking simulations confirmed this physical interaction, demonstrating highly favorable binding affinities between the H1.2 peptide and Hfq (ΔG = -9,4 kcal/mol and $k_d = 1{,}4 \cdot 10^{-7}$ M). By specifically targeting Hfq, H1.2 putatively disrupts essential regulatory sRNA-mRNA networks, causing a translational arrest[4]. This mechanism supports the downstream of the membrane biogenesis system observed in previous differential proteomics analysis. To definitively validate these findings, future objectives entail producing recombinant Hfq for in vitro binding assays, followed by in vivo studies evaluating targeted protein synthesis inhibition. Finally, this research underscores the power of functional proteomics in deciphering complex host-pathogen interactions, reinforcing H1.2 as a highly promising therapeutic candidate for combating multidrug-resistant infections.

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POSTER

P-01

Proteomic Profiling of Brain Synaptosomes in a Mouse Model of 22q11.2 Deletion Syndrome

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The 22q11.2 deletion syndrome (also known as DiGeorge syndrome) is one of the most common genomic disorders and is associated with cognitive impairment, autism spectrum disorder, and an increased risk of schizophrenia. Haploinsufficiency of the Tbx1 gene included in the deleted region is considered a major contributor to the neurological phenotype, although the molecular mechanisms underlying synaptic dysfunction remain poorly understood. To address this gap, we performed a comprehensive proteomic characterization of hippocampal synaptosomes from adult heterozygous (Tbx1+/−) mice. Hippocampal synaptosomes were isolated from 7-month-old Tbx1+/− and wild-type (WT) mice by sucrose gradient fractionation. Protein extracts were analyzed by high-resolution LC-MS/MS-based quantitative proteomics. Differentially expressed proteins were identified through statistical analysis, followed by functional enrichment and pathway analyses to investigate biological processes and molecular networks affected by Tbx1 haploinsufficiency. Comparative proteomic profiling revealed significant alterations in the synaptic proteome of Tbx1+/− mice compared with WT controls, identifying 130 dysregulated proteins out of nearly 3000 identified. The altered proteome revealed extensive remodeling of synaptic molecular networks, highlighting proteins involved in neurotransmission, synaptic vesicle dynamics, cytoskeletal organization, mitochondrial function, and energy metabolism. This study provides the first proteomic map of hippocampal synaptosomes in adult Tbx1+/− mice, indicating that Tbx1 haploinsufficiency disrupts key biological processes required for synaptic homeostasis and plasticity, supporting the hypothesis that impaired synaptic function contributes to the neurological manifestations of 22q11.2 deletion syndrome..

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P-02
PASEF-Melodies: Mobility-gated acquisition modes for multi-modal proteomics from single cells to PTM

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Trapped ion mobility spectrometry (TIMS) introduces an additional, orthogonal dimension to mass spectrometry by utilizing collision cross section (CCS), which results in cleaner MS/MS spectra and enhances confidence in complex proteomic analyses. Parallel Accumulation–Serial Fragmentation (PASEF) further boosts analytical power by aligning mobility separation with rapid time-of-flight (TOF) detection, maximizing ion efficiency. Leveraging the selectivity of this four-dimensional (4D) platform, a diverse set of "PASEF Melodies" has been developed, enabling seamless transitions between discovery and validation and facilitating class-specific analyses through mobility gating and gas-phase enrichment for applications such as chemoproteomics, immunopeptidomics, ubiquitinomics, and glycoproteomics.

The described framework details practical strategies for combining these acquisition modes to extract meaningful biological insights and reduce chemical noise. DIA-PASEF and PRM-PASEF bridge discovery and targeted validation, supported by CCS-based quality control. Chemoproteomic workflows use timSHIFT to distinguish modified peptides in mobility space, enhancing rare-event detection. Slice-PASEF supports post-translational modification (PTM) and ubiquitin-focused analyses, while thunder-PASEF retains both singly and multiply charged immunopeptides and filters out chemical background.

Across all modalities, TIMS gating and mobility targeting selectively enrich information-rich peptide classes and improve data interpretability in complex samples. For chemoproteomics, timSHIFT provided an "upgrade from liquid to gas phase," reporting ~25× higher sensitivity and ~10× more targets at low input, consistent with the goal of simplifying rare-event detection for ABPP-type assays. In spatial proteomics, 30,000 µm² tissue regions showed clear differentiation of cell-type niches using established markers—epithelium (CDH1), T-cells (CD3), and B-cells (CD19)—supporting biologically interpretable pathology mapping. Collectively, these mobility-resolved acquisition strategies can be orchestrated to address specific biological questions within a unified, robust 4D-proteomics framework.

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P-03

Proteomics uncovers AZE as a molecular impostor driving protein misfolding and cellular stress

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The acquisition of the correct three-dimensional conformation is a fundamental prerequisite for protein function. Proper protein folding is essential not only for preserving biological activity but also for protecting proteins from chemical damage. In contrast, misfolded proteins are significantly more vulnerable to oxidative modifications, including adduction by reactive products of lipid peroxidation. Mutations that impair protein folding have been associated with increased protein oxidation, highlighting the close relationship between protein structure and proteome integrity. Among the amino acid residues that govern protein folding, proline plays a key role in determining protein secondary and tertiary structure owing to its unique conformational properties. The non-canonical proline analogue azetidine-2-carboxylic acid (AZE) can be mistakenly incorporated into newly synthesized proteins, potentially disrupting protein folding and acting as a potent teratogen.
In this study, we investigated the AZE incorporation into cellular proteins employing high-resolution mass spectrometry with a conventional Data Dependent Acquisition (DDA) strategy and characterized the amino acid sequence context surrounding AZE incorporation sites to identify potential sequence determinants of its misincorporation. To evaluate the proteome-wide consequences of AZE exposure, we employed a differential proteomics workflow based on Data-Independent Acquisition (DIA), a highly robust and reproducible quantitative strategy that provides improved proteome coverage and reliable protein quantification. These strategies provided direct evidence that AZE is incorporated into protein sequences in a non-random manner and that its incorporation is driven by the local sequence surrounding proline residues. Furthermore, quantitative proteomic analysis showed that AZE incorporation is associated with significant perturbations in biological pathways involved in protein folding, cellular stress responses, and biosynthetic processes. This approach enables the comprehensive characterization of proteomic alterations and the identification of biological pathways and cellular processes affected by AZE-induced proteotoxic stress. More broadly, it provides a proof of principle for investigating the molecular consequences of non-canonical amino acid misincorporation and establishes a proteomics framework applicable to a wide range of protein quality control disorders.

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P-04
Decoding CSF protein complexes in neurodegenerative diseases through mass spectrometry

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The misfolding of proteins, which is a feature of neurodegenerative diseases, could lead not only to a wrong protein assembly which results in the formation of toxic complexes, but also to the absence of complexes fundamental for the physiological activities [1]. The aim of the current research is to map the global network of complexes and proteins interactions in the cerebrospinal fluid (CSF) from patients with neurodegenerative conditions. This information can be used to identify new biomarkers and molecular targets involved in pathogenesis and disease progression.
We have recently optimized a new method that uses size exclusion chromatography, mass spectrometry and bioinformatic analysis to map how circulating complexes and proteins may interact in biological fluids [2]. We used this method to map the CSF network asset from patients with neurodegenerative conditions and subjects who underwent CSF study for non-inflammatory or non-neurodegenerative conditions. The analysis provided for the first time a comprehensive mapping of several hundreds of homo- and hetero- complexes in CSF.
Among identified complexes, many were linked to immunity, structural and receptors processes but also associated with lipoproteins, neurodegenerative and inflammatory functions. Overall, our data provided a complete map of CSF protein complexes, with the identification of known and unknown protein interactions and structures in CSF.
We expect to deepen new information about the role played by these protein complexes, or their interactions, in neurodegeneration. These would provide the knowledge for the better understanding of the pathogenesis of many diseases, and importantly for the identification of new diagnostic and therapeutic targets.

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P-05

Multi-Omics analysis to characterize metabolic flexibility in Pediatric Hypoglycemia

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Hypoglycemia results from defects in glucose homeostasis and represents one of the most common metabolic emergencies in the neonatal period and throughout childhood. Its main etiological causes include inherited metabolic disorders (IMDs), endocrine diseases, and other congenital conditions. The current diagnostic pathway, largely based on invasive dynamic testing, is often inadequate to capture the heterogeneity of IMDs. This study proposes a genotype-first approach integrating genomic and multi-omics data to characterize metabolic flexibility (1). The study includes a retrospective cohort of approximately 40 probands with hypoglycemia and 40 healthy controls, alongside a prospective cohort of 20 patients and 20 age- and sex-matched healthy controls. The retrospective cohort was analyzed using next-generation sequencing, employing an in-silico panel of 112 genes associated with hypoglycemia. Lipidomic and metabolomic profiles were preliminarily characterized in a pilot cohort of patients and controls using mass spectrometry, integrated with multivariate analyses and network analysis. The main circulating lipid classes were identified and quantified, including phosphatidylcholines, lysophosphatidylcholines, sphingomyelins, ceramides, diacylglycerols, triacylglycerols, acylcarnitines, fatty acids, bile acids, and numerous metabolites involved in the major energy metabolism pathways. Analysis of the pilot cohort demonstrated the feasibility and robustness of the proposed multi-omics approach. Lipidomic and metabolomic profiling identified molecular signatures capable of discriminating patients from healthy controls, as confirmed by principal component analysis (PCA), variable importance in projection (VIP) scores, and heatmap analyses. The most prominent alterations involved phosphatidylcholines, diacylglycerols, triacylglycerols, and ceramides, suggesting lipid metabolic remodeling associated with impaired metabolic flexibility and energy homeostasis. Preliminary results confirm the feasibility and potential of the proposed multi-omics strategy, indicating that the serum metabolic signature may reflect the pathophysiological state of pediatric hypoglycemia with greater sensitivity than conventional biomarkers. The integration of lipidomic, metabolomic, proteomic, and genomic data may enable molecular patient stratification, support the development of minimally invasive diagnostic pathways and personalized therapeutic strategies, and ultimately contribute to precision medicine approaches for pediatric hypoglycemia while safeguarding patients' neurological development.

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P-06

Proteomic profiling of primary dermal fibroblasts in metachromatic leukodystrophy

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Metachromatic leukodystrophy (MLD) is a rare autosomal recessive lysosomal storage disorder caused by pathogenic variants in the *ARSA* gene leading to a deficiency of the lysosomal enzyme arylsulfatase A, which is responsible for sulfatides degradation. The impaired catabolism of sulfatides results in their accumulation within the central and peripheral nervous systems, leading to progressive demyelination, neuronal dysfunction, and neurodegeneration. Although presymptomatic *ex vivo* gene therapy has significantly improved the natural history of the disease, the molecular mechanisms underlying its progression remain only partially understood. In this context, proteomics provides a powerful approach to identify molecular alterations associated with ARSA deficiency. Proteomic profiling was performed using a label-free DIA approach on primary dermal fibroblasts derived from patients with MLD. Peptides were separated by liquid chromatography using the Evosep One system and followed by high-resolution tandem mass spectrometry based on trapped ion mobility spectrometry (timsTOF HT-MS, Bruker). More than 9.000 proteins were consistently identified and quantified across all samples, including approximately 400 lysosomal proteins, corresponding to 70% coverage of the lysosomal proteome. The PCA analysis revealed a clear separation between MLD and control samples while also highlighting inter-individual variability among patients. Differential expression analysis confirmed the marked reduction of ARSA in MLD fibroblasts, consistent with the underlying enzymatic defect. Significant alterations were also observed in molecular pathways related to cytoskeletal organization, cell adhesion and extracellular matrix interactions. Taken together, these findings identify a distinctive molecular signature in MLD fibroblasts, confirm ARSA deficiency, and highlight dysregulation of pathways involved in maintaining cellular architecture.

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P-07
Fructose Intake Shapes Fecal Microbial Functions and Metabolic Profiles in Pediatric Obesity-Associated MASLD

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Pediatric obesity is a major risk factor for metabolic dysfunction-associated steatotic liver disease (MASLD), and dietary habits, including excessive fructose intake, may contribute to disease development through mechanisms involving the gut microbiota and host–microbe metabolic interactions [1]. In this study, we performed an integrated fecal metaproteomic and metabolomic analysis on samples collected from obese pediatric subjects and controls, stratified according to fructose intake and the presence or absence of hepatic steatosis.

Fecal samples were analyzed to investigate changes in microbial composition, microbial functional activity and metabolite profiles associated with obesity, fructose intake and MASLD status. Metaproteomic analysis revealed differences in the distribution of bacterial taxa and in the functional pathways expressed by the gut microbiota across the study groups [2]. In parallel, metabolomic profiling identified alterations in fecal metabolites, suggesting a remodeling of gut microbial metabolism in obese subjects and in individuals with higher fructose consumption and steatosis.

Overall, the integrated analysis highlighted distinct microbial and metabolic signatures associated with pediatric obesity, dietary fructose exposure and MASLD. These findings support the hypothesis that unhealthy dietary patterns may influence liver metabolic health through changes in the gut microbiota and its metabolic output [1]. Although further validation in larger cohorts is required, this study provides preliminary evidence that fecal metaproteomics and metabolomics may help identify microbiota-derived functional biomarkers and mechanistic pathways involved in pediatric MASLD.

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P-08
Proteomic profiling of the immune microenvironment in somatotroph tumors: correlation with invasiveness, proliferation, and surgical outcome.

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The tumor microenvironment (TME), composed of non-tumor cells, blood vessels, extracellular matrix, and soluble factors, interacts dynamically with tumor cells, influencing proliferation, invasiveness, and response to therapy [1]. Increasing evidence suggests that immune system pathways within the TME contribute to tumor progression. Reduced immune activation may characterize more invasive tumors and those with unfavorable surgical outcomes.

The aim of this study was to characterize the expression of immune system proteins in somatotroph tumors and investigate their association with tumor invasiveness, proliferative status, and surgical outcome.

A bottom-up proteomic approach with label-free quantitative mass spectrometry analysis was performed on somatotroph tumor samples from patients with acromegaly. Following protein extraction and digestion, differentially expressed proteins were identified and compared according to tumor invasiveness, proliferative status, and surgical outcome. Gene ontology and pathway enrichment analyses were subsequently performed.

Preliminary analyses identified a panel of differentially expressed proteins predominantly involved in different immune-related pathways. Protein abundance changed according to tumor invasiveness, proliferative status, and surgical outcome.

Proteomic characterization of the immune component of the TME provides novel insights into the mechanisms underlying somatotroph tumor progression. The identification of immune pathways associated with invasiveness and surgical outcome may contribute to the discovery of biomarkers for patient stratification and potential therapeutic targets.

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P-09

Microplastic Exposure Reshapes the Proteome and Phosphoproteome of Pancreatic Organoids

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Plastic pollution has become one of the most pressing environmental challenges of the modern era, leading to widespread human exposure to microplastics (MPs). MPs have been detected in multiple human tissues and biological fluids, including blood, placenta, liver, lungs, breast milk, and feces, raising growing concerns about their potential impact on human health. Emerging evidence suggests that chronic MP exposure may contribute to cancer development; however, current knowledge is largely based on in vitro systems and short-term animal studies, limiting our understanding of the underlying molecular mechanisms[1,2]. Here, we investigated whether MP exposure alters cell signaling in murine pancreatic organoids using integrated proteomics and phosphoproteomics. Pancreatic organoids derived from primary mouse cells were exposed to two MP size distributions (500 nm, MP1; 20 µm, MP2), followed by shotgun proteomics and TiO_2-based phosphopeptide enrichment coupled with LC-MS/MS to characterize protein abundance changes and signaling network remodeling. Proteomic analysis identified 2,764 quantifiable proteins in MP1-treated organoids, of which 134 were significantly dysregulated, and 2,689 quantifiable proteins in MP2-treated organoids, with 122 significantly dysregulated proteins. Functional enrichment analysis revealed size-dependent biological responses. MP1 exposure primarily affected metabolic pathways, insulin secretion, energy metabolism, cellular responses to stimuli, heme degradation, RNA processing, and the 60S ribosomal subunit. In contrast, MP2 exposure altered pathways related to metabolism, nucleotide metabolism, TP53-regulated metabolic genes, membrane trafficking, vesicle biogenesis, FGFR2 alternative splicing, neutrophil degranulation, and vesicle-mediated transport.
Phosphoproteomic analysis identified 375 phosphosites, with 187 and 213 significantly dysregulated phosphosites in MP1- and MP2-treated organoids, respectively. Kinase-substrate enrichment analysis predicted altered activity of 21 kinases in MP1-treated organoids, mainly associated with cell communication, development, proliferation, and cellular remodeling. In MP2-treated organoids, 22 kinases were predicted to be differentially regulated, controlling pathways involved in cell communication, cell-cycle progression, proliferation, transcription, and metabolism. These findings demonstrate that microplastic exposure induces size-dependent proteomic and phosphoproteomic remodeling in pancreatic organoids, highlighting distinct signaling pathways and kinase networks that may contribute to the biological effects of microplastics.

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P-10

Proteomic Profiling of Bovine Milk Antimicrobial Defenses: From Hygienic Quality Assessment to Natural Preservation

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Bovine milk microbiological quality is a fundamental requirement for food safety and effective control of the dairy supply chain. Although culture-based microbiological methods remain the reference standard for determining bacterial load, their long incubation times limit their application in rapid screening. In this context, naturally occurring antimicrobial milk proteins represent an innovative source of molecular information on hygienic quality while also constituting bioactive components of considerable interest for the microbial stability of milk.
In this study, a bottom-up proteomics approach was applied to bovine milk samples to characterize the endogenous antimicrobial protein profile and investigate its relationship with bacterial load. Proteins were enzymatically digested and analyzed by LC–MS/MS. Protein identification and label-free quantification were performed using MaxQuant. LFQ intensities were used to assess the relative abundance of key antimicrobial proteins, including peptidoglycan recognition protein 1, lactoferrin, lactoperoxidase, beta-defensins, and cathelicidins. The integration of proteomic data with bacterial counts enabled the identification of a molecular signature associated with the microbiological quality of milk.
Among the identified proteins, peptidoglycan recognition protein 1 emerged as the most promising candidate. Its higher abundance, together with a richer and more diverse antimicrobial protein profile, was associated with lower bacterial counts. This pattern highlights the value of endogenous antimicrobial defenses as indicators of milk hygienic quality and supports their potential contribution to the natural control of microbial proliferation.
Overall, this study demonstrates the value of bottom-up proteomics for identifying an endogenous antimicrobial signature associated with the microbiological quality of bovine milk. Peptidoglycan recognition protein 1 emerges as a molecular candidate of particular relevance, both for developing innovative rapid-screening tools and for designing new natural preservation strategies. Together with the other antimicrobial proteins identified, it opens tangible opportunities to improve the hygienic control, safety, and stability of milk throughout the dairy supply chain.

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P-11
Dissecting autophagy pathways and sub-pathways in Glioblastoma proteome: a bioinformatic study

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Glioblastoma IDH1 wild-type (GBM) remains the most aggressive primary brain tumor in adults, with an extremely poor prognosis even with combined surgery and postoperative chemoradiation. This tumor is characterized by rapid infiltration, early recurrence and severe therapy resistance, with molecular mechanisms still unclear. Our recent proteomic studies of clinically accessible GBM biofluids, including tumor aspirate fluid obtained by Cavitron Ultrasonic Surgical Aspirator (CUSA fluid) [1] and saliva [2] collected from enrolled patients, have highlighted molecular signatures showing autophagy as one of the most significantly enriched pathways in tumor compartments, Indeed autophagy and selective autophagy programs are increasingly recognized as key adaptive. mechanisms exploited by GBM cells to sustain mitochondrial homeostasis, buffer oxidative stress, and survive under metabolic and therapeutic pressure, resulting central in tumor progression, metabolic plasticity, and treatment resistance, and representing an emerging frontier for biomarker discovery and therapeutic targeting [3,4]. The protein identification data previously obtained by LC-MS proteomic analysis [1,2] have been here reanalysed by a bioinformatic approach to specifically investigate the autophagy pathway and related sub-pathways classification of the proteins identified in GBM CUSA fluid and saliva using open-access database [5] and a comprehensive annotated autophagy pathway gene list recently published by a group of us [6], including 604 genes and different categories (mTOR and upstream pathways, autophagy core, autophagy transcription factors, mitophagy, docking and fusion, lysosome and lysosome-related genes). Hierarchical classification mapped GBM proteome in distinct autophagy sub-pathways, including chaperone-mediated autophagy, late endosomal microautophagy, and selective autophagy processes such as mitophagy and aggrephagy, showing also interesting correlations between CUSA fluid and saliva data highlighting potential targets for mechanistic studies and clinical applications. These findings provide a novel preliminary classification of the GBM proteome within autophagy and related subpathways, offering new perspectives for the study of the molecular mechanisms involved in GBM pathogenesis and for the discovery of potential biomarkers.

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P-12

Human Histone Fragments Display Antibacterial Properties against *Pseudomonas aeruginosa*

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Antimicrobial resistance (AMR) represents a major global health threat, responsible for approximately 1.27 million deaths in 2019 and projected to reach up to 10 million deaths annually by 2050[1]. In this context, antimicrobial peptides (AMPs) are emerging as promising alternatives to conventional antibiotics due to their broad-spectrum of activity and diverse mechanisms of action. Beyond classical AMP families, histones have recently been recognized as endogenous antimicrobial effectors with functions extending beyond chromatin organization. Here, we report the identification and characterization of novel antimicrobial peptides derived from human histone H1 variants [2]. Using a human hemofiltrate peptide library combined with in silico prediction, thirteen candidate peptides were identified and synthesized. Functional screening against ESKAPE pathogens revealed that 12 out of 13 peptides exhibited strong and selective antibacterial activity against *Pseudomonas aeruginosa*, with no detectable effects on other tested species. Among these, a H1.2-derived peptide showed the highest potency and consistent activity across multiple clinical isolates, with maximal efficacy under acidic conditions. Kinetic analyses demonstrated rapid bactericidal activity, without membrane disruption or direct DNA binding, suggesting a non-lytic intracellular mode of action. Differential proteomic analysis revealed a significant downregulation of proteins involved in protein translocation and outer membrane biogenesis, including key components of the Sec pathway and β-barrel assembly machinery[3]. PCR investigation gave negative results suggesting a post-transcriptional regulation. Functional proteomics approach using a biotinylated peptide was used to investigate the H 1.2 mechanism of action, leading to the identification of a network of peptide interactors involved in translation and RNA metabolism. Notably, components of the RNA degradosome[4] and RNA chaperone systems were enriched, supporting a role in RNA processing and stability. Collectively, these findings identify histone H1-derived peptides as selective antimicrobial effectors with a novel intracellular mechanism targeting RNA metabolism and protein biogenesis, highlighting their potential for the development of innovative therapies against multidrug-resistant pathogens.

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P-13
A Proteogenomic Workflow for Discovery and Prioritization of Candidate Allergens in Ambrosia artemisiifolia Pollen

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Ambrosia artemisiifolia (common ragweed) pollen is among the leading causes of seasonal respiratory allergy worldwide, particularly in southeastern Europe, yet only a limited number of its allergens have been experimentally characterized. We established an integrated transcriptomics - proteomics workflow to prioritize putative ragweed pollen allergens using a sample-specific transcriptome, enabling the detection of expressed sequence variants and isoforms that may be absent from generic reference databases. Total RNA was isolated from ragweed pollen collected in a suburban area of central Serbia and subjected to RNA sequencing. Assembled transcripts were translated into predicted protein sequences to generate a sample-specific translated protein database. The predicted proteome was compared against the COMPARE allergen database using BLASTP (NCBI BLAST+ v2.17.0). Proteins showing homology to known allergens (E-value $\leq 1 \times 10^{-10}$; sequence identity ≥35%) were ranked into low-, medium-, and high-confidence groups. Previously acquired shotgun LC–MS/MS datasets of aqueous pollen extracts (APE) and subpollen particles (SPP) were reanalyzed against the custom database. Candidate allergens were prioritized using a three-level workflow comprising sequence homology, experimental proteomic identification, and AllerCatPro 2.0 prediction. Prioritized proteins were further evaluated using BepiPred 3.0. Transcriptome analysis against the COMPARE database identified 68 full-length and partial ragweed allergen or isoallergen sequences. Furthermore, the hierarchical workflow prioritized six novel candidate allergens in APE and nine in SPP. These candidates belonged to established allergen families, including pectate lyases, profilins, cyclophilins, and expansins, and showed positive in silico allergenicity predictions together with multiple predicted linear B-cell epitopes, further supporting their potential immunological relevance. The proposed workflow integrates omics and computational tools to systematically prioritize putative ragweed pollen allergens. By combining sample-specific transcriptomics with proteomic validation, our strategy substantially narrows the list of candidates for targeted IgE-binding studies and experimental validation while expanding allergen discovery beyond generic reference databases.

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P-14

Advancing Plasma Proteomics Through a Next-Generation Enrichment Workflow for Deeper and More Quantitative Biomarker Discovery

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Human plasma is a rich source of disease biomarkers but remains difficult to analyze by mass spectrometry (MS) due to its extreme dynamic range and suppression by high-abundance proteins. We evaluated P2-iST Plasma, which combines P2 paramagnetic nanoparticle enrichment with iST-based sample preparation, and applied this workflow in a pilot study of colorectal cancer (CRC).

Quantitative performance was assessed using: (i) a seven-point species dilution series generated by mixing K2EDTA bovine and pooled K2EDTA human plasma (1:0 to 0:1; triplicates), and (ii) protein spike-ins (IL-6, IL-1β, MAPT) prepared as a five-fold serial dilution from 1250 ng to 0.4 ng in 100 µL human EDTA plasma. For the CRC pilot study, EDTA plasma from 12 donors (6 CRC, 6 healthy) was analyzed. Neat plasma and P2-iST Plasma samples (100 µL; KingFisher™ Flex) were measured using diaPASEF® on timsTOF HT coupled to Evosep™ One. Data was processed in Spectronaut® 20.

P2-iST Plasma expanded the detectable dynamic range to 7–8 orders of magnitude and increased the number of precisely quantified proteins (CV <20%) by 5–7-fold compared to neat plasma. Accurate fold-change behavior and robust linearity were maintained, with improved calibration-based LOD/LOQ in species mixtures and spike-ins. In the CRC pilot cohort, proteome coverage increased ~6-fold, revealing expanded CRC-associated signals and pathway enrichment. Correlation-based signatures centered on PTP4A3, and established plasma markers provided complementary discovery and quantitative reference points.

Overall, P2-iST Plasma substantially improves proteome depth while preserving quantitative accuracy, enhancing biomarker discovery in clinical plasma studies.

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P-15
Proteomic characterization of bovine purified protein derivative (bPPD) from *Mycobacterium bovis AN5*

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Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a significant zoonotic disease affecting livestock, wildlife, and human health. It impacts animal production, trade, and public health, particularly through occupational exposure and consumption of unpasteurized dairy products, and represents a One Health priority requiring integrated surveillance. The tuberculin skin test (TST), based on bovine purified protein derivative (bPPD), remains the main diagnostic tool for bTB control, although its specificity and sensitivity can be limited by the complex and incompletely characterized composition of bPPD and cross-reactivity with environmental mycobacteria (1,2). In this study, the proteomic composition of bPPD produced from *M. bovis* AN5 strain by IZSAM was characterized using nanoLC-ESI-MS/MS coupled with high-resolution Orbitrap mass spectrometry. A total of 675 proteins were identified, of which 603 (89%) were predicted to be antigenic according to VaxiJen and VirulentPred analyses, while 164 proteins contained predicted B-cell epitopes. Functional annotation using KEGG BLASTKOALA revealed a predominance of proteins involved in carbohydrate metabolism, energy production, lipid metabolism, and amino acid biosynthesis, reflecting the metabolic versatility of *M. bovis*. Several known immunodominant antigens were identified, including Ag85A, Ag85B, MPT64, MPB70, MPT83, EsxA, and EsxB, along with proteins belonging to the DosR regulon (Mb0584, Mb1767, Mb2656, Mb2659C, Mb3157C, Mb2058), which is associated with bacterial adaptation and persistence during latent infection (3). To assess potential diagnostic specificity, predicted immunogenic proteins were compared against *Mycobacterium avium* using BLASTp. Fourteen proteins showed sequence identity below 35%, while 27 proteins had no significant BLASTp hits. Notably, MPB70, MPT83, EsxA, EsxB, Mb0584, and Mb1767 showed no significant matches. LppX, LprF, and CFP32 were among the low-homology proteins and have been previously associated with virulence, cell-envelope functions, or antigenicity. Overall, this proteomic characterization of bPPD provides new insights into its antigenic landscape and identifies several proteins with limited conservation relative to *M. avium*, supporting their potential use as candidate biomarkers and recombinant antigens for improving diagnostic specificity. Although these findings are preliminary and require validation across additional bPPD production batches, this study represents a step toward molecular standardization of bPPD and the development of more specific and reproducible diagnostic reagents for bovine tuberculosis control.

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Current Research Project IZS AM 02/25 RC

P-16
Metaproteomics for One Health surveillance: profiling active bacterial taxa associated with antimicrobial resistance reservoirs in bovine faeces

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Antimicrobial resistance (AMR) surveillance requires approaches capable of identifying microbial communities. Bovine faeces are a relevant matrix in this context, as they may harbour bacterial taxa involved in the ecology and dissemination of antimicrobial resistance across the animal and environment interface [1]. This study applied LC-MS/MS-based metaproteomics to bovine faecal samples to explore expressed microbial proteins and their taxonomic origin. Protein identifications generated by Proteome Discoverer were used to identify active bacterial taxa with relevance to animal health, zoonotic risk and antimicrobial-resistance concern. Across the analysed datasets, 472 to 1,020 proteins were identified, with 301 proteins consistently detected. Taxonomic assignment of expressed proteins revealed the presence of commensal gut-associated microorganisms together with opportunistic and pathogenic taxa recognised as zoonotic pathogens and/or potential reservoirs of antimicrobial-resistance genes. Importantly, this approach does not directly demonstrate antimicrobial-resistance determinants. Rather, it identifies expressed microbial proteins assigned to bacterial populations of AMR concern, providing functional evidence of active taxa within bovine faeces. Metaproteomics may therefore represent a complementary tool for One Health surveillance, supporting the identification of active microbial communities potentially involved in AMR ecology.

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P-17

Differential proteomic signatures of fibroblasts and osteoblast-like cells in an *in vitro* calcification model

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Mineralization of hard connective tissues is a physiological process governing calcium salt deposition essential for bone and teeth formation and maintenance. In contrast, ectopic calcification (EC) is a life-threatening complication affecting soft connective tissues in several acquired and genetic conditions [1]. Within this context, we take advantage of a disease model considered a paradigm of EC: *Pseudoxanthoma elasticum* (PXE), an autosomal recessive disease characterized by calcification of elastic fibers in tissues such as skin, eyes, and blood vessels [2]. At present, there are no effective therapies to inhibit/reduce EC, although therapeutic approaches have been attempted raising some concern about the possible deleterious impact of their long-term use on bone metabolisms [3].

To investigate at which extent mineral deposition in EC is regulated at molecular and cellular levels in a manner similar to skeletal bone, or if there are differentially regulated mechanisms, PXE fibroblasts (n = 5) and SaoS-2 derived-bone cells (n = 5) were cultured in standard (DMEM) or in Calcifying Medium (CM= 10 mM β-glycerophosphate, 50 µg/mL ascorbic acid, 100 nM dexamethasone) until calcification takes place (*i.e.*, day 7 for Saos-2 and day 21 for PXE fibroblasts). Proteins were extracted in RIPA buffer and processed via Filter-Aided Sample Preparation [4]. A dedicated data-independent acquisition spectral library was built using gas-phase fractionation [5].

Principal Component Analysis showed a strong response to CM treatment in both PXE fibroblasts and Saos-2 cells, with many proteins differentially expressed compared with cells cultured in standard conditions. In both cell lines, changes involving energy metabolism further support the link between cellular bioenergetics and the onset of mineralization. Interestingly, Saos-2 cells and PXE fibroblasts were characterized by a significant differential response, emphasizing the role of extracellular matrix (ECM) components that represent the actual substrate for mineral deposition and that can modulate cellular behavior and mechanosensing.

In conclusion, data provide a molecular map of pathways differently associated with calcification in Saos-2 cells and in PXE fibroblasts, suggesting that ECM composition, depending on the specific context, represent a key player to be targeted to modify mineral deposition aiming to counteract ectopic calcification while preserving physiological calcification.

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P-18

Lipidomic signatures help monitor different potential celiac disease (PCeD) outcomes in pediatric patients

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Celiac disease (CeD) is an inflammatory autoimmune enteropathy caused by the ingestion of gluten and is driven by both genetic and environmental factors, with genetic predisposition mainly due to HLA class II genes (1). In particular, the presence of gluten peptides triggers an immune reaction damaging the small intestinal villi and therefore leading to many celiac disease-associated symptoms. However, the disease may present with different manifestations, ranging from a full spectrum of symptoms to complete asymptomatic forms, making its diagnosis challenging. Small intestinal biopsy and serologic screening are still the only tools available to confirm and discriminate between different clinical forms of CeD. Moreover, patients with positive celiac-specific serology but no evidence of intestinal damage are classified as potential CeD (PCeD) (2). Despite standard treatment approaches such as gluten-free diet, patients with PCeD face a continuous risk of disease progression with inaccurate classification.
In this context, we performed MS-based lipidomics to define molecular signatures of disease progression from PCeD to CeD discovering early biomarkers. Accordingly, we profiled the serum samples from pediatric patients with PCeD, collected at diagnosis (T0) and at clinical outcome assessment (TX), including serological normalization, villous atrophy or persistence of anti-transglutaminase antibody positivity. Targeted lipidomics performed by flow-injection analysis–tandem mass spectrometry (FIA-MS/MS) in Multiple Reaction Monitoring (MRM) mode enabled the quantification of more than 900 lipid species in the serum of patients at T0 and TX. Univariate and multivariate statistical analyses, integrated with hierarchical clustering and differential analysis, were performed to identify significantly altered metabolites and lipid species between the two cohorts.

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P-19

A Hierarchical Bioinformatics Workflow for Prioritization of Putative Mango Allergens

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Background: *Mangifera indica* (mango) allergy is increasingly recognized worldwide, yet only a limited number of allergens have been characterized. Although genome-wide computational studies have expanded the repertoire of putative mango allergens, additional prioritization based on immunologically relevant features is required to identify the most promising candidates for experimental validation. Our study aimed to establish an integrated computational workflow for prioritizing putative mango allergens by combining sequence homology, complementary allergen prediction algorithms, B-cell epitope mapping, and structural analysis.
Methods: Predicted protein sequences of *Mangifera indica* were obtained from the NCBI RefSeq genome assembly CATAS_Mindica_2.1. Protein sequences were compared against a locally constructed COMPARE allergen database using BLASTP (NCBI BLAST+ v2.17.0). High-confidence homologues (E-value $\leq 1 \times 10^{-1}$) were subsequently evaluated using AlgPred 2.0. Predicted allergens were compared with the recently published genome-wide mango allergome generated using AllerCatPro 2.0 to assess the consistency of candidate identification across independent computational workflows. Prioritized candidate allergens were further characterized by linear B-cell epitope prediction using BepiPred 3.0 and ABCPred. Consensus epitope hotspots were mapped onto AlphaFold-predicted three-dimensional protein structures to evaluate their structural localization and surface accessibility.
Results: Sequence homology followed by AlgPred identified numerous putative mango allergens, including both established allergen families and several proteins not currently recognized in the WHO/IUIS database. Comparison with the recently published genome-wide mango allergome demonstrated substantial overlap for several candidate allergens, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH), glucanases and profilins, while each workflow also identified unique candidate proteins, reflecting differences in sequence-based and proteome-wide prioritization strategies. Consensus B-cell epitope mapping identified conserved epitope-rich regions in multiple candidate allergens, with good agreement between BepiPred 3.0 and ABCPred. GAPDH contained two conserved surface-accessible epitope hotspots, whereas profilins consistently exhibited a conserved C-terminal epitope cluster, supporting preservation of putative IgE-binding regions across isoforms.
Conclusions: Integrating sequence homology, computational allergen prediction and consensus B-cell epitope mapping provides a rational framework for prioritizing novel food allergen candidates. Beyond identifying putative allergens, this workflow enables the selection of immunologically relevant peptide regions for targeted proteomic characterization, and future IgE-binding validation. The proposed strategy may facilitate systematic allergen discovery and hypothesis-driven experimental validation in food proteomics.

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P-20
Behavioural and molecular characterisation of amygdala circuit dysfunction in a mouse model of DYT1 dystonia

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Inherited primary generalized dystonia is most frequently caused by a 3-bp deletion (ΔGAG) in the *TOR1A* gene. Despite its clinical severity, this neurological disorder is characterized by a strikingly low penetrance (~30%), suggesting the mutation confers a state of latent neural vulnerability requiring a second hit, such as environmental stress, to manifest. We employed a multidisciplinary approach, combining behavioural tasks, proteomics, electrophysiology and resting-state fMRI, to investigate the role of torsinA within limbic-motor circuits under early-life stress in *Tor1A*$^{+/\Delta GAG}$ mice. We found that while wild-type animals respond to unpredictable post-natal stress (UPS) with adaptive behaviour and enhanced fear memory, *Tor1A*$^{+/\Delta GAG}$ mutants exhibit a paradoxical global disinhibition phenotype characterized by hyperlocomotion, impaired habituation and failed associative learning. Mechanistically, UPS triggers a profound disruption of the amygdala excitation-inhibition balance in mutant mice. From a molecular point of view, this state is associated with a significant upregulation of the synaptic plasticity proteins Grin2a and Shisa6. The inhibitory control was found compromised through the downregulation of Gabra3, and increased cannabinoid signalling. Overall, our findings identify torsinA as an essential chaperone of synaptic resilience, required to maintain circuit homeostasis in the face of environmental challenges, and support a reconceptualization of DYT-TOR1A as a neurodevelopmental circuit disorder.

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P-21
Microplastic-induced proteomic reprogramming in *Escherichia coli*: differential effects of PET and PTFE particles on biofilm formation

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Microplastics are increasingly recognised as active modulators of microbial physiology in environmental and host-associated systems [1]. However, polymer chemistry, particle size, and shape bacterial functional adaptation remain poorly characterised. Here, we examined the proteomic response of *Escherichia coli* (*E. coli*) exposed to chemically distinct micro- and nanoplastics and assessed their effects on subsequent biofilm formation. Bacterial cultures were exposed to polyethylene terephthalate (PET; approximately 20 µm) and polytetrafluoroethylene (PTFE; approximately 500 nm), two polymers with markedly different physicochemical properties. A range of dilutions was tested to evaluate dose-dependent responses. During growth, cells were allowed to develop biofilms, and proteomic profiling was performed using a bottom-up, label-free LC-MS/MS workflow. Differentially expressed proteins were analysed to identify pathways related to stress responses, metabolic adaptation, and biofilm development. Pre-exposure to plastic particles produced consistent and reproducible proteomic reprogramming. Proteins involved in oxidative stress responses and redox homeostasis were significantly upregulated, indicating sustained cellular stress. Central metabolic pathways were also modulated, suggesting a redistribution of energy toward adaptive functions. In parallel, proteins associated with adhesion and extracellular matrix production increased, supporting enhanced biofilm-forming capacity after exposure. These responses depended strongly on particle size and polymer type, with PTFE nanoplastics eliciting a more pronounced effect, possibly because of their greater surface area and distinctive fluorinated chemistry. Regulatory proteins involved in transcriptional control were also differentially expressed, consistent with coordinated cellular adaptation. Overall, our findings show that microplastic composition and size can critically influence bacterial functional trajectories. Exposure appears to prime *E. coli* toward a biofilm-prone phenotype, supporting the view of microplastics as selective ecological cues within the plastisphere. These results provide new insight into microbial adaptation and may have implications for environmental persistence and One Health risk dynamics.

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P-22

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

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Mitochondrial-derived peptides (MDPs), including Humanin (HN), Humanin-like peptides (HMN) 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. Despite their biological relevance, endogenous MDPs remain largely undetected in conventional proteomics datasets, suggesting substantial analytical and methodological limitations. Within the framework of the Mitochondrial Human Proteome Project (mt-HPP), this study aimed to systematically investigate the detection and subcellular localization of endogenous MDPs by integrating public proteomics datasets with newly generated experimental data and complementary proteomic workflows. In *silico* analyses were performed using PeptideCutter, Uniquome, and DeepLoc 2.0 to evaluate theoretically generated peptides, peptide uniqueness, and predicted subcellular localization. A custom protein database was generated and integrated with the reviewed Human UniProt proteome, and MitoCarta. Publicly available datasets and newly generated HeLa proteomes were analyzed using complementary experimental strategies, including mitochondrial enrichment, subcellular fractionation, mitochondrial membrane separation, chromatographic prefractionation, and multi-enzymatic digestion. Samples were analyzed by liquid chromatography coupled to an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific). Synthetic MDPs were used to assess MS detectability and estimate detection limits. Peptide identification was performed using PEAKS X Pro complemented by de novo sequencing. Candidate MDPs were validated by manual inspection of MS/MS spectra, extracted ion chromatograms (Xcalibur), targeted analysis (Skyline), and comparison with synthetic reference peptides. Computational analyses identified multiple MDPs containing unique proteotypic peptides and predicted distinct subcellular localizations. Reanalysis of public proteomics datasets confirmed MDP detection across different experimental conditions, with improved identification following strategies aimed at reducing sample complexity. Multi-enzymatic digestion increased sequence coverage and enhanced MDP detection. Among the evaluated approaches, mitochondrial enrichment combined with subcellular fractionation provided the strongest evidence for endogenous MDP detection and compartment-specific localization. Overall, these findings demonstrate that optimized and complementary proteomic workflows improve the detection of endogenous MDPs and provide new insights into their compartmentalization, processing, and potential biological functions.

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P-23
Fecal miRNome and Proteome Profiling Uncovers Stage-Specific Biomarkers of Alzheimer's Disease in 3×Tg-AD Mice

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Reliable, minimally invasive biomarkers for Alzheimer's disease (AD) remain a critical unmet need. Considering mounting evidence for gut-brain interactions in AD, we explored whether fecal samples contain host-derived molecular signals that reflect disease-related changes and could serve as accessible biomarkers. Fecal small RNA sequencing and quantitative proteomics were performed in symptomatic 15-month-old 3×Tg-AD mice and controls. Pathway and network analyses identified key dysregulated molecules. Selected miRNAs and proteins were validated by qPCR or Western blotting in independent pre-symptomatic, early symptomatic, and severe cohorts. To assess whether fecal signals mirrored tissue alterations, colon samples were analyzed for tight-junction proteins, inflammatory markers, epigenetic regulation, and expression of selected miRNAs and proteins. Multi-omics identified 31 miRNAs and 81 proteins modulated in AD fecal sample, revealing a distinct AD-associated fecal signature involving pathways linked to neuroinflammation, synaptic dysfunction, vascular imbalance, and metabolism. Several miRNAs- including miR-322-5p, miR-194-1 and miR-223-3p were significantly altered and validated across disease stages (3-,7-,15-old months mice, respectively). Notably, miR-146b-5p expression is inversely correlated with AD severity, exhibiting a stepwise decline from asymptomatic individuals to those with mild and severe stages. IgKappa and Ela3B emerged as the strongest protein candidates, showing opposite and progressive modulation. Notably, IgKappa and miR-146b showed the most consistent trajectories. Colon analyses uncovered reduced Claudin-7, strong IgKappa upregulation driven by enhancer hypomethylation, and partial overlap between fecal and tissue miRNA profiles, indicating that fecal signals reflect subtle epithelial and immune perturbations associated with AD. Multi-omics at severe stage and a stage specific validation support fecal miRNAs and proteins as promising, host-derived biomarkers for AD. Fecal profiling offers a practical, repeatable, and low-cost approach with translational potential for early detection and longitudinal monitoring in neurodegeneration. [1].

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P-24

Elucidating the role of iRhoms as regulators of MHC-I expression in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is a poorly immunogenic "cold" tumor that remains largely resistant to immunotherapy. A key mechanism of immune evasion is the downregulation of major histocompatibility complex class I (MHC-I), which limits T-cell recognition and activation[1]. Inactive rhomboid proteins 1 and 2 (iRhom1 and iRhom2) are best known as essential regulators of the metalloproteinase ADAM17 but also possess ADAM17-independent functions in antigen presentation[2]. While iRhom1 has been linked to antigen presentation through regulation of ERAP1[3], iRhom2 has been implicated in controlling MHC-I surface expression[4]. Here, we investigated their roles in regulating MHC-I expression in PDAC. Loss of either iRhom1 or iRhom2 reduced MHC-I surface expression across multiple PDAC cell lines, but through distinct mechanisms. iRhom1 deficiency decreased both total cellular and surface MHC-I by promoting its degradation. Endoglycosidase H (Endo H) assays revealed altered MHC-I glycosylation in iRhom1 knockout cells, suggesting that defective glycosylation impairs MHC-I stability and promotes proteasomal degradation. Proteomic analysis further supported this mechanism by showing reduced abundance of several glycan-modifying enzymes following iRhom1 loss. In contrast, iRhom2 deficiency primarily affected MHC-I trafficking rather than stability. Loss of iRhom2 reduced surface MHC-I while increasing its enrichment in endoplasmic reticulum membrane fractions after subcellular fractionation. Proteomic and Gene Ontology analyses identified increased abundance of proteins involved in AP-2/clathrin-mediated endocytosis and MHC-I turnover, suggesting enhanced internalization and recycling of surface MHC-I. Consistent with this, inhibition of clathrin-dependent endocytosis using dynasore restored surface HLA expression more effectively in iRhom2-deficient cells than in wild-type controls.
Together, these findings identify iRhom1 and iRhom2 as novel regulators of MHC-I expression in PDAC that act through distinct mechanisms. iRhom1 maintains MHC-I stability by supporting proper glycosylation, whereas iRhom2 regulates MHC-I trafficking and recycling. Defining these pathways may provide new therapeutic strategies to restore MHC-I surface expression, enhance T-cell recognition, and improve the response of PDAC to immunotherapy.

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P-25

Plasma Amino Acid Signatures as Metabolic Biomarkers in Oral squamous cell carcinoma (OSCC): Longitudinal Assessment Before and After Surgery.

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Oral squamous cell carcinoma (OSCC) develops on the mucosal epithelium of the oral cavity. Exposure to multiple risk factors including alcohol, tobacco, betel quid (BQ) chewing and human papilloma virus (HPV) leads to the development of OSCC.
Patients with OSCC often undergo major surgery, and most of them are malnourished and frequently face to postoperative complications such as infections and fistulas.
The aim of this study was to assess perioperative changes in plasma amino acid profiles and to investigate their potential value in the evaluation of metabolic alterations associated with surgery in patients with OSCC, and to investigate whether specific amino acids are associated with tumor aggressiveness.
Twenty-one patients, scheduled to undergo ablative surgery, were enrolled in the study. Plasma samples were collected on fasting patients the day before surgery and seven days postoperatively and stored at -80°C until analyses. After surgery, all patients were placed on parenteral nutrition due to the invasive nature of the procedure.
For this purpose, a panel of 44 amino acids was measured through ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) validated methodology. The reversed phase chromatographic separation was performed by ACQUITY H-Class using a CORTECS UPLC C18 column 1.6 µm 2.1 × 150 mm (Waters Corporation, Milford, Massachusetts, USA). The mass spectrometer was an ACQUITY QDa single quadrupole equipped with electrospray source operating in positive mode (Waters Corporation, Milford, Massachusetts, USA).
Univariate and multivariate statistical methods were used to evaluate variations in amino acids concentrations due to surgical intervention. Explorative analysis was performed using PCA algorithm, while classification discriminant models were made using PLS-DA. In addition, linear mixed model was constructed considering time and tumor aggressiveness as a clinical variable.
PLS-DA allowed discrimination in amino acids before and after surgery with an accuracy of 88.1%.
In particular, methionine and histidine were identified as significative variables to discriminate pre and post-operatory condition and tumor aggressiveness. These results could be related to methylation processes in tumor genesis.
In conclusion, amino acid profiling appears to be a valuable tool for the assessment of surgery in OSCC patients and may provide prognostic information regarding postoperative recovery.

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P-26

Integrating high-resolution CSF proteomics and machine learning to stratify Alzheimer's disease patients and identify progression-associated biomarkers

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Alzheimer's disease (AD) is a biologically heterogeneous neurodegenerative disorder, and individuals with similar clinical symptoms may differ substantially in their underlying molecular alterations and disease trajectories. While the AT(N) framework has considerably improved biological classification, it does not fully capture the molecular complexity of AD. In this context, high-resolution CSF proteomics enables the identification of molecular signatures that discriminate different disease subtypes beyond established biomarkers. We established a CSF proteomics workflow based on data-independent acquisition (DIA) using the Orbitrap Astral mass spectrometer, combined with quantitative proteomics and machine learning. The DIA workflow enabled deep and reproducible profiling of the CSF proteome across clinically characterized cohorts. Differential analysis identified protein changes associated with amyloid-positive individuals with normal or elevated tau, revealing molecular profiles beyond conventional classification. Functional enrichment highlighted processes related to neuroinflammation, synaptic function, extracellular matrix organization, lipid metabolism, and neuronal injury. To translate these findings into a predictive framework, multiple machine learning models were evaluated to define a minimal protein signature with optimal classification performance. This resulted in a 15-protein signature that accurately discriminates tau-positive AD, tau-negative AD, and controls, with consistent performance in an independent validation cohort. We are now applying the same DIA proteomics and computational pipeline to investigate molecular differences associated with disease progression. Preliminary analyses comparing fast and slow progressors revealed distinct CSF proteomic profiles. Fast progressors showed a broader remodeling of the CSF proteome, characterized by increased abundance of proteins associated with complement activation, extracellular matrix remodeling, and growth factor signaling, including CFHR1, CFHR5, MFAP5, and IGF1, together with a marked increase in DNAJC13. In contrast, slow progressors exhibited a more restricted proteomic signature enriched in proteins related to synaptic and neuronal function, including NPTX2, SCN3B, PRRT2, CHGA, and VSTM2A. Gene ontology analysis supported these differences, highlighting immune and complement activation pathways in fast progressors, and synaptic signaling processes in slow progressors. Overall, our results show that integrating high-resolution CSF proteomics with machine learning enables robust molecular stratification of AD and supports the identification of candidate biomarkers of disease progression.

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P-27

Development of a proteomic framework for sheddome-wide assessment of iRhom2 inhibitors

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A disintegrin and metalloproteinase 17 (ADAM17) releases tumor necrosis factor α (TNF), a central mediator of inflammation [1]. Despite its therapeutic relevance, targeting ADAM17 has proven challenging due to its broad substrate repertoire (the ADAM17 sheddome) and widespread expression. ADAM17 activity depends on the cofactors iRhom1 and iRhom2, which promote its maturation and regulate shedding at the plasma membrane [2]. Although iRhom1 and iRhom2 are largely redundant in supporting ADAM17 function, iRhom2—but not iRhom1—is expressed in macrophages, the major source of TNF, making it an attractive target for immune-selective ADAM17 inhibition [3]. Importantly, iRhom2 also exhibits unique properties, including the ability to support stimulated ADAM17-mediated shedding that iRhom1 cannot compensate, raising the possibility that the two cofactors differentially shape the ADAM17 sheddome and that iRhom2 inhibition could entail unintended effects beyond TNF suppression [4, 5]. Here, using high-resolution mass spectrometry, we systematically compared the ADAM17 sheddome associated with iRhom1 or iRhom2 and applied this workflow to evaluate iRhom2 inhibitors. We identified novel ADAM17 substrates and found that iRhom1 and iRhom2 shape highly similar sheddomes overall. By screening a panel of compounds, we further established a proteomic platform to study iRhom function and to assess, in a sheddome-wide manner, the effectiveness and specificity of iRhom2 inhibitors.

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P-28
Fecal Proteomics Suggest Potential Biomarkers for Non-Alcoholic Fatty Liver Disease and Steatohepatitis

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Non-alcoholic fatty liver disease (NAFLD) is one of the most prevalent chronic liver diseases worldwide, affecting approximately 25% of the global population. Its progressive form, non-alcoholic steatohepatitis (NASH), is characterized by hepatic inflammation and fibrosis and may progress to cirrhosis and hepatocellular carcinoma. Although liver biopsy remains the gold standard for the diagnosis of NASH, its routine clinical application is limited by its invasive nature, and poor suitability for repeated assessments. Consequently, there is an increasing need for reliable, non-invasive biomarkers that can facilitate the diagnosis, staging, and longitudinal monitoring of NAFLD. Fecal biomarkers have recently emerged as a promising and non-invasive approach, reflecting alterations in host physiology and gut–liver interactions associated with disease progression.

Proteomic analysis was performed by LC–MS/MS on fecal samples from mice with diet-induced NAFLD and from mice receiving also five cycles of dextran sulfate sodium (DSS) to induce NASH. Differentially expressed proteins were identified and analyzed by functional enrichment to investigate the biological pathways associated with disease progression. The most significantly modulated proteins were subsequently validated by enzyme-linked immunosorbent assay (ELISA).

Proteomic analysis revealed distinct qualitative and quantitative protein expression profiles among healthy, NAFLD, and NASH mice. Differentially expressed proteins were associated with molecular pathways potentially involved in disease development and progression. ELISA analysis confirmed the differential expression of Transthyretin, Kallikrein 1, and Trefoil Factor 3, supporting the reliability of the proteomic findings.

This exploratory discovery study identified several fecal proteins that, individually or in combination, effectively discriminate between healthy, NAFLD, and NASH mice. These findings support the potential value of fecal proteomics as a non-invasive strategy for biomarker discovery and provide a basis for future investigations aimed at validating these candidates and assessing their translational applicability for the diagnosis, staging, and monitoring of NAFLD and NASH.

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