Proteo Vilamoura

6th Joint Meeting of Spanish, French and Proteomics Societies

20-22 May 2026, Vilamoura, Portugal

ABSTRACT BOOK

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Organized by

PROCURA

fps French Proteomics Society

SEProt Sociedad Española de Proteómica

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Welcome – ProteoVilamoura 2026

Dear Colleagues,

It is a great pleasure to welcome you to ProteoVilamoura 2026, the 6th Joint Meeting of the Portuguese, Spanish and French Proteomics Societies. On behalf of PROCURA ñ the Portuguese Proteomics Association, I am delighted to host this gathering of our three vibrant scientific communities.

Since the second edition of this joint meeting was held here, the event has traveled to several remarkable locations, including Aix-en-Provence, CÛrdoba, and Saint-Malo, each contributing to the growth and consolidation of our trilateral proteomics community. Returning in 2026 offers a meaningful opportunity to build on that shared history while continuing to strengthen the scientific and personal connections that define this meeting.

The scientific program has been designed to showcase the full breadth of proteomics and its related disciplines, highlighting cutting-edge developments, emerging technologies, and transformative applications. With eight internationally renowned invited speakers and a diverse set of contributions from our community, the meeting provides a rich environment for discussion, collaboration, and the exchange of ideas. Above all, it ensures that participants at all career stages can share their work, broaden their perspectives, and engage with colleagues from Portugal, Spain, and France.

The venue was chosen for its conference facilities, which allow us to host the full meeting with plenary sessions, parallel tracks, poster sessions and exhibits in a single location, and for its accessibility via Faro International Airport, with direct connections from major European cities. After years marked by global challenges, this meeting also represents a welcome opportunity to reconnect in person and to continue the scientific exchange that has defined this joint meeting since its beginning.

There is no formal dress code; smart casual attire is appropriate throughout the meeting, including the social program.

The success of this joint meeting has always depended on the active involvement of its participants and sponsors, and I am confident that your contributions will make ProteoVilamoura 2026 another memorable milestone for our community.

Procura will support the meeting with 5 travel grants. Information about the grants can be found here.

It is an honour to welcome you once again to Portugal.
I wish you an inspiring and enjoyable meeting.

Bruno Manadas
Chair, ProteoVilamoura 2026 Organizing Committee
President of PROCURA

Message from the FPS
French Proteomics Society

It is with great pleasure that the French Proteomics Society (FPS) associates again with the Spanish Proteomics Society (SEProt) and the Portuguese Proteomics Association (PROCURA) to co-organize the sixth edition of this joint meeting. The scientific committee has worked to develop an original program shaped by the invited speakers and further enriched by numerous oral presentations, a substantial fraction of which will be given by young researchers. We encourage many FPS members to participate in this conference, where we expect stimulating scientific exchanges and opportunities to initiate new collaborations. The FPS will support this meeting by 5 fellowships (see https://www.french-proteomics-society.fr/fr/bourses) and the SFSM will also support this event by two fellowships (see the website of this society).

Sincerely yours,

Delphine Pflieger
President of the FPS

Message from the SEProt
Spanish Proteomics Society

The Spanish Proteomics Society (SEProt) proudly joins the Portuguese Proteomics Association (PROCURA) and the French Proteomics Society (FPS) once again to co-organize the sixth edition of this joint meeting. The scientific committee is working to put together an original and attractive program, shaped by outstanding invited speakers and further enriched by numerous oral presentations, many of which we hope will be delivered by young researchers. To support the participation of early-career researchers, SEProt will again provide 10 travel grants to cover at least part of their expenses.

We encourage SEProt members to attend this conference, where we expect stimulating scientific discussions and excellent opportunities to start new collaborations.

Sincerely yours,

Felix Elortza
President of the SEProt

Organising Committee

Bruno Manadas, Health and Disease lab Center for Neurosciences and Cell Biology, University of Coimbra

Dina Simes, University of Algarve

José Alexandre Ferreira, Portuguese Oncology Institute of Porto

Hugo Osório, i3S - Institute for Research and Innovation in Health, University of Porto

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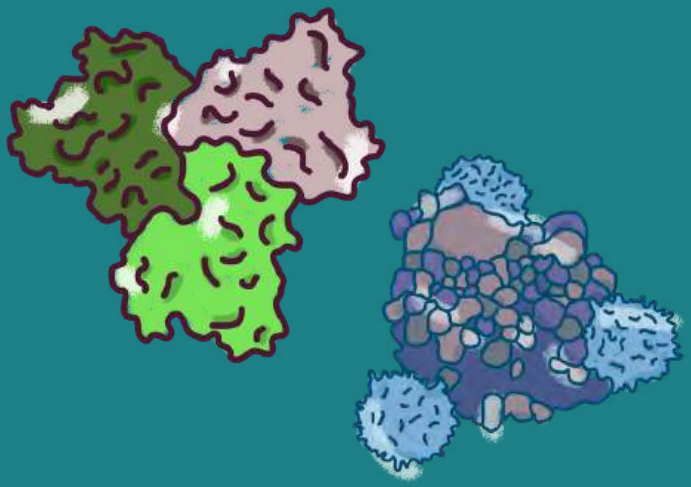

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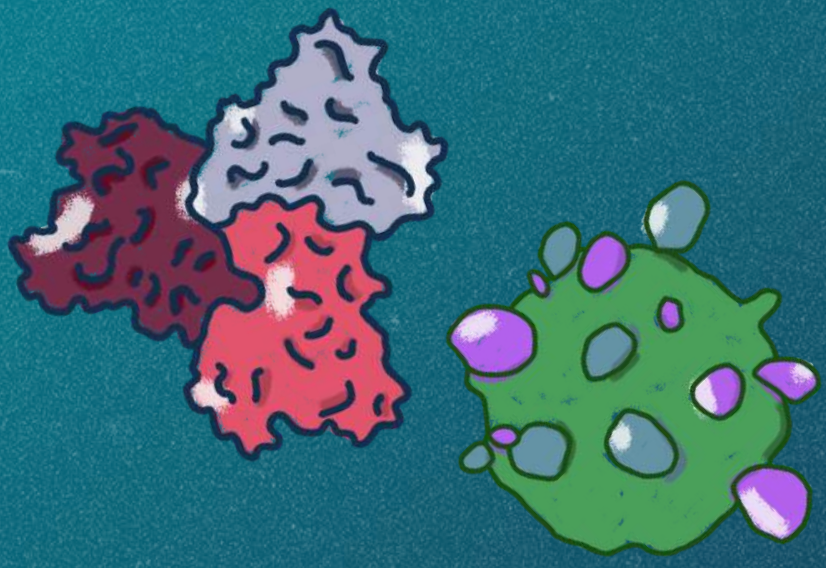
Section 1
Proteomics in the Clinic: From Biomarkers to Precision Medicine #1

Oral Communications



Deep plasma EV proteomics by Data-Independent Acquisition reveals circulating signatures of cardiac tissue injury in a preclinical Atrial Fibrillation model

Estefanía Núñez (enunez@cnic.es)[1,2], Ana Martínez-Val[1,2], Jorge G. Quintanilla[2,3,4], Diego Mena-Santos[1,2], Maeve Soto-Pérez[3], Lucía Casado[1], Marinela Couselo-Seijas[3], Ana Simon-Chica[5], Fernando García[6], Haruka Toda[3], Irene García-Martín[3], Marta Isasa[6], David Filgueiras-Rama[2,3,4], Jesús Vázquez[1,2]

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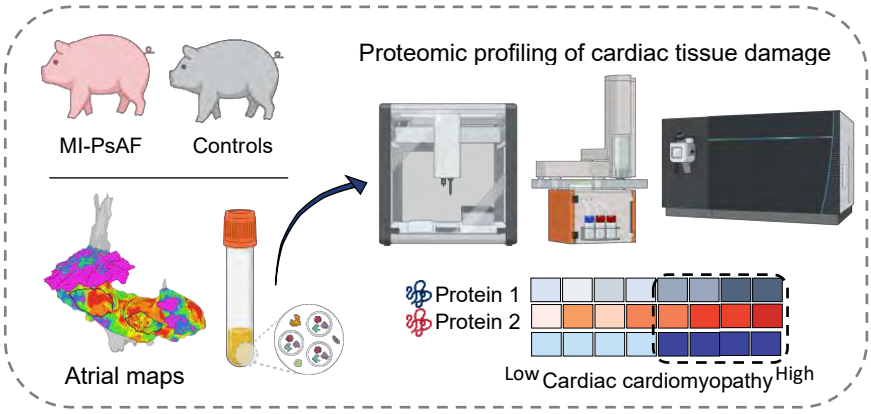
Mass spectrometry (MS)-based plasma proteomics has enabled biomarker discovery; however, its analytical depth is limited by workflow complexity and the extreme dynamic range of plasma. Extracellular vesicle (EV) enrichment reduces sample complexity and improves detection of low-abundance proteins. In particular, EV enrichment using Strong Anion Exchange (SAX)-conjugated beads (Mag-Net technology) enhances proteomic depth in human biofluids and enables detection of proteins that remain undetectable in bulk plasma. As EVs carry molecular cargo released by distant tissues, this approach enables the non-invasive interrogation of tissue-derived molecular signals, including those originating from organs such as the heart.

Atrial fibrillation (AF), the most prevalent sustained cardiac arrhythmia worldwide, is associated with progressive cardiac tissue remodeling. Cardiac cardiomyopathy encompasses structural, architectural, and functional alterations of the myocardium, with fibrosis representing a hallmark of irreversible damage. Substrate assessment remains invasive, and no circulating biomarker accurately reflects underlying cardiac tissue remodeling. Integration of extracellular vesicle (EV)-enriched plasma proteomics with data-independent acquisition (DIA) may enhance analytical depth and enable detection of cardiac tissue–derived biomarkers.

In a clinically validated pig model of persistent atrial fibrillation (PsAF) with underlying infarct-related substrate (MI), extracellular vesicle (EV)-enriched plasma proteomics enabled the detection of reproducible circulating protein signatures associated with cardiac tissue injury. EV-derived protein features were consistently captured across animals, demonstrating the capability of the approach to interrogate tissue-associated molecular signals through minimally invasive sampling. The integration of SAX/Mag-Net–based EV enrichment with data-independent acquisition (DIA) mass spectrometry provides a scalable and high-depth proteomic platform for robust detection of circulating molecular signatures linked to cardiac tissue remodeling.



Expanding the knowledge on diagnostic autoantibodies in colorectal cancer through proteomics and immunosensing platforms

Javier Velázquez-Gutiérrez[1,2], Ana Montero[1], Mónica Vázquez[3], Olga Cano[3], Vicente Mas[3], María Garranzo-Asensio[1], Rodrigo Barderas[1,4]

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Colorectal cancer (CRC) is the second most common cancer in Europe, with an estimated incidence of 447 cases per 100,000 individuals per year, and the second leading cause of cancer-related mortality. This high mortality rate is largely due to late diagnosis and the occurrence of relapses even in patients initially diagnosed at early stages. Therefore, improving diagnostic strategies for early detection remains a major clinical need.

Previous studies have identified a panel of tumor-associated antigens (TAAs) of CRC and their corresponding autoantibodies (AAbs) as biomarkers of the disease with promising diagnostic performance [1, 2]. However, a subset of CRC patients remains non-seroreactive to these TAAs and would therefore be missed by screening approaches based on humoral immune responses. To address this limitation, we aimed here to identify novel CRC-specific TAAs and their corresponding AAbs in this subset of previously non-seroreactive patients, thereby expanding the existing diagnostic panel.

IgGs purified from plasma of non-seroreactive CRC patients were used for immunoprecipitation assays followed by LC-MS/MS analysis, leading to the identification of 15 candidate TAAs. These proteins were subsequently expressed and purified, and their immunoreactivity evaluated by ELISA-like tests using plasma samples from CRC patients. This approach confirmed that the candidate TAAs were specifically recognized by patient-derived AAbs and allowed the assessment of their diagnostic potential.

The most promising antigens were then incorporated into the previously established CRC-specific AAb panel and evaluated collectively for their diagnostic and prognostic performance. Overall, our findings support the inclusion of the novel identified TAAs in AAb-based biomarker panels. This panel should contribute to the development of a simple and minimally invasive blood-based diagnostic strategy for CRC detection and disease monitoring through liquid biopsy.

1. Garranzo-Asensio, M., et al., *Identification of tumor-associated antigens with diagnostic ability of colorectal cancer by in-depth immunomic and seroproteomic analysis.* J Proteomics, 2020. **214**: p. 103635.
2. Montero-Calle, A., et al., *Multiplexed Biosensing Diagnostic Platforms Detecting Autoantibodies to Tumor-Associated Antigens from Exosomes Released by CRC Cells and Tissue Samples Showed High Diagnostic Ability for Colorectal Cancer.* Engineering, 2021. **7**(10): p. 1393-1412.

Mapping the circulating extracellular vesicle proteome in Marfan syndrome patients using minimal plasma volume

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Plasma provides valuable insights into the physiological state and, being easily collected non-invasively, its analysis has high clinical relevance. Consequently, plasma analysis has been widely used in large clinical studies, particularly in proteomics approaches aimed at biomarker discovery.

Marfan syndrome (MFS) patients often develop aortic aneurysms, a cardiovascular pathology for which no treatment or biomarker exists to assess the presence or risk of aortic rupture. Therefore, identifying plasma biomarkers for MFS could improve clinical management; however, the vast dynamic range of plasma proteins hampers the detection of low-abundance species by LC-MS/MS. To circumvent this limitation, different methods to either deplete high abundance proteins or enrich for some of the less abundant species have been developed. Notably, extracellular vesicle enrichment adds a unique information layer by allowing the identification of tissue-specific proteins in plasma.

In this study, we enriched EVs from 179 human plasma samples from MFS patients and healthy donors using a KingFisher robot. Samples were analyzed by LC/MS-MS using an EvoSep One system coupled to an Orbitrap Eclipse operating in data-independent acquisition mode. The resulting raw files were processed for protein, post-translational modification (PTM) and glycopeptide identification, quantification and statistical analysis using DIA-NN, FragPipe, iSanXoT and R.

Using only 50 ul of plasma per patient we have obtained two 4-protein panels: one for MFS detection and one for aggressiveness stratification with areas under the curve of 0.8 and 0.9, respectively. Besides, we characterized the “modificome” associated with the physiopathological state of MFS and identified specific PTMs with potential structural implications. Finally, we detected significant changes in the glycosylation profile of MFS patients, consistent with previous results obtained in tissue samples.

In conclusion, using a minimal starting plasma volume, we performed a comprehensive characterization of EV-enriched samples from MFS patients with unprecedented depth. This allowed us not only to diagnose and stratify risk based on protein abundance changes, but also to unveil protein modifications of outmost interest, paving the way for improved patient monitoring upon validation in independent cohorts.

Methylthioadenosine: harnessing a natural metabolite to counteract cholestasis progression

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Progressive Familial Intrahepatic Cholestasis Type 3 (PFIC3), caused by MDR3 deficiency (MDR2 in mice), is a lethal pediatric liver disease characterized by early-onset persistent cholestasis, progressive fibrosis, cirrhosis and eventual liver failure. MDR3 is a phosphatidylcholine transporter located in the canalicular membrane of hepatocytes, and its deficiency leads to the accumulation of free bile acids, which damage epithelial cells due to their detergent-like effect. Therapeutic options remain limited, highlighting the need to identify molecular drivers of the disease progression and novel pharmacological strategies.

To investigate the molecular alterations associated with this deficiency, our laboratory performed functional proteomic analyses in patient samples and murine hepatic organoids, revealing consistent reprogramming of One-Carbon Metabolism (OCM). Based on these findings, we explored the therapeutic potential and molecular mechanisms of 5′-Methylthioadenosine (MTA), a metabolite within this pathway.

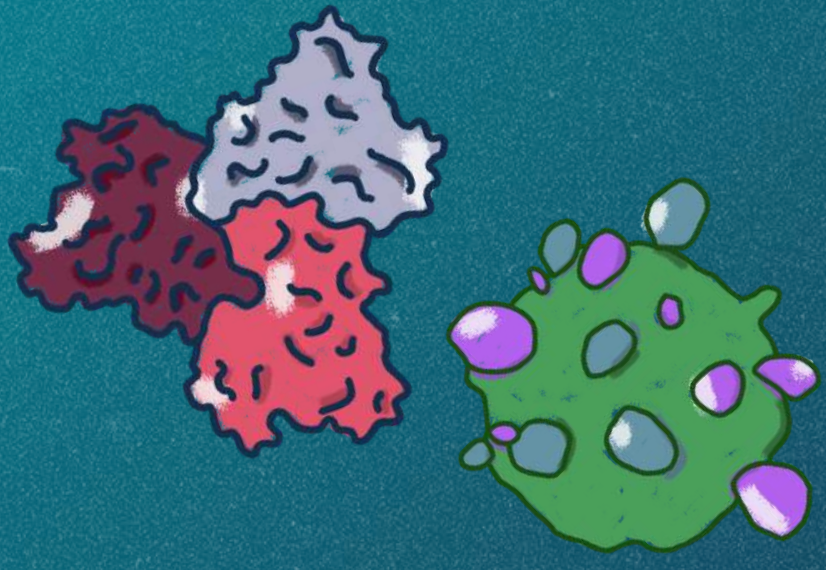
Using hepatocellular carcinoma cells (PLC), we combined quantitative shotgun proteomics with the Proteome Integral Solubility Alteration (PISA) assay to characterize functional responses and identify direct protein targets of MTA. To reinforce the physiological relevance of our findings, we extended the PISA analysis to liver protein extracts from the Mdr2-/- mouse model of chronic cholestatic injury, enabling validation of candidate target interactions observed in cellular extracts.

Proteomic profiling revealed that MTA induces significant alterations in cell division processes, epigenetic regulatory networks and energy metabolism. PISA analyses revealed a conserved subset of proteins displaying consistent stability shifts in both PLC cells and Mdr2-/- liver extracts, supporting consistent target identification. Functionally, MTA promotes a shift toward a hepatocyte-like expression signature.

Overall, our findings point to OCM as a central metabolic vulnerability and support MTA as a potential modulator of the inflammatory, epigenetic, and metabolic aspects associated to cholestatic liver disease.

Section 2
Next-Gen Proteomics: Single-Cell, Proteogenomics & Disruptive Technologies

Oral Communications



Integrative single-cell proteomics identifies pro-regenerative fingerprints in a sub-population of adult cardiomyocytes.

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The ability to study the heterogeneity of cell populations at the single-cell level by mass spectrometry-based proteomics is becoming possible due to recent advances in instrument sensitivity and sample preparation techniques.

From an analytical perspective, integrating multiple layers of cellular data—such as whole cell size, subcellular compartment dimensions, and protein abundance independent of size—offers additional, yet unexplored insights in single-cell proteomics.

In regenerative research, heterogeneous responses of target cells to treatment influence the propagation of regenerative signals. Cardiomyocyte proliferation driven by Myc transcription factor overexpression has been shown to contribute to cardiomyocyte regeneration; however, it has not yet been investigated at single-cell resolution.

By using an optimized adult mice cardiomyocytes isolation procedure, we conducted single-cell proteomics analysis using SCoPE-MS [1] and RETICLE method [2] in an Eclipse-Orbitrap coupled to FAIMS an Ultimate 3000 HPLC. Taking advantage of the integrative capabilities of the iSanXoT application [3], we were able to minimize batch effects and cell size-related biases, profile protein behavior within the cell, obtain quantitative subcellular compartment information and detect protein alterations within subcellular compartments. This approach enhances data quantification accuracy and facilitates biological interpretation.

With our analytical workflow, we show that the Myc transcription factor switches the expression profile of metabolic enzymes and expands a subpopulation of adult cardiomyocytes with a pro-regenerative signature.

Overall, the analysis of single-cardiomyocyte data with the integrative statistical framework of iSanXoT offers a versatility in data analysis that contributes to resolve the batch and cell size effects inherent to this type of analysis and demonstrates that different layers of cellular information can properly pattern the proteomic phenotype of single-cells. Its application to our project provided important clues to understand the impact of Myc transcription factor in provoking different immaturity and pro-regenerative signatures in adult mouse cardiomyocytes. The heterogeneous responses of mouse cardiomyocytes to Myc overexpression underscore the importance of conducting single-cell analyses for accurately defining the magnitude of the regenerative response in the heart.

References

1. Budnik B, Levy E, Harmange G, Slavov N. SCoPE-MS: mass spectrometry of single mammalian cells quantifies proteome heterogeneity during cell differentiation. Genome Biology. 2018;19(1):161.
2. Schoof EM, Furtwängler B, Üresin N, Rapin N, Savickas S, Gentil C, et al. Quantitative single-cell proteomics as a tool to characterize cellular hierarchies. Nature Communications. 2021;12(1):3341.
3. Rodríguez JM, Jorge I, Martinez-Val A, Barrero-Rodríguez R, Magni R, Núñez E, et al. iSanXoT: A standalone application for the integrative analysis of mass spectrometry-based quantitative proteomics data. Computational and Structural Biotechnology Journal. 2024;23:452-9.

Benchmarking single-cell FACS-assisted strategies: effects on cellular component bias and post-translational modification detection

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The development of mass spectrometry-based single-cell proteomics (SCP) has enabled the exploration of cellular heterogeneity in biological samples driven by different technological efforts as advances in microfluidics and mass spectrometry, such as the cellenONE or the Astral mass analyzer. However, the high cost of such instrumentation might limit its accessibility to most research group. To democratize SCP, we tested different FACS-based (Fluorescence Activated Cell Sorting) strategies using a Cytek Aurora spectral flow cytometer and an Orbitrap Eclipse mass spectrometer evaluating the results in terms of peptide and proteome depth, and quantitative reproducibility.

In vitro cultured human aortic smooth muscle cells were sorted using three distinct methods (34 cells per strategy): into a 96-well plate following a conventional protocol, and two variants of the One-Tip protocol [1] differing in the recipient reagent volume. After lysis and digestion, samples were analyzed by liquid chromatography–mass spectrometry in data independent acquisition (DIA) mode, and peptidoforms and proteins were quantified using DIA-NN, followed by normalization and data integration with iSanXoT [2]. Direct 15µl One-Tip sorting yielded the best performance, with a median of 3,800 precursors and 700 proteins quantified per cell. However, when evaluating consistency across cells, plate-based sorting showed higher robustness, with 22% of the total proteins quantified across the full population. Enrichment analysis revealed a clear bias towards a specific protein subset when comparing the plate-based strategy against the tip-based approaches.

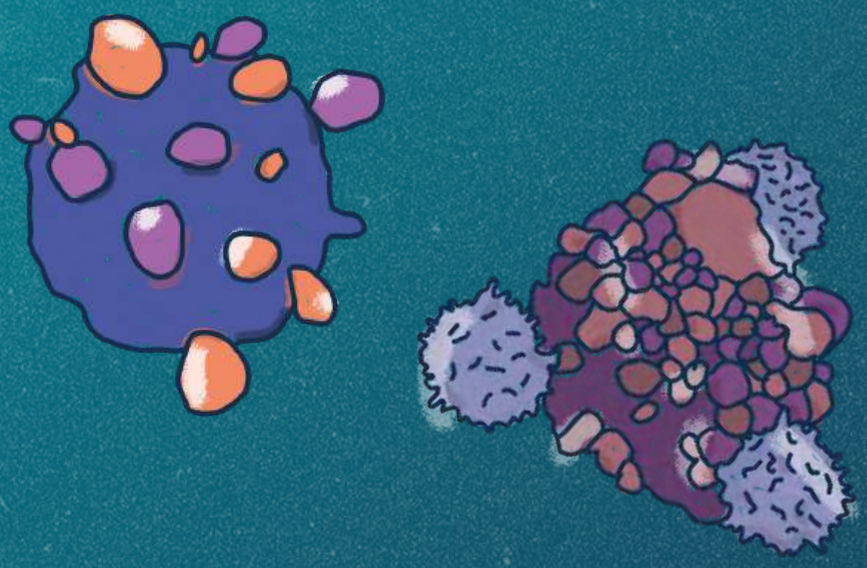
To further complement this analysis, and given the crucial role that post-translational modifications (PTMs) can play in the characterization of cellular subpopulations, we developed an strategy for PTM unbiased exploration at the single-cell level through the construction of an experimental PTM-inclusive spectral library built using an in-house script. The library was assembled by combining Open Search results from Gas Phase Fractionation runs in both DDA and DIA mode with the Open Search results from the sorted cells themselves, maximizing spectral coverage across modification types. This approach allowed the detection of a large number of PTMs across all sorting strategies (~40% of all precursors), including oxidations, methylations and phosphorylations, expanding the analytical depth for single-cell characterization beyond conventional proteome quantification.

[1] Ye et al., *Nat. Commun.* 15, 2474 (2024)

[2] Rodríguez et al., *Comput. Struct. Biotechnol. J.* 23, 452–459 (2024).

Section 2
Next-Gen Proteomics: Single-Cell, Proteogenomics & Disruptive Technologies

Flash Presentations



In depth-analysis of Alzheimer's disease brain tissue reveals novel Aβ interactors

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Alzheimer's disease (AD) is a progressive, chronic, neurodegenerative disease, and the most common cause of dementia worldwide. Although the mechanisms underlying the disease are still unclear, the two main hallmarks are the extracellular accumulation of Amyloid-β (Aβ) plaques and the intracellular accumulation of hyperphosphorylated Tau protein into neurofibrillary tangles. While it remains unclear whether these processes are a cause or a consequence of the disease, the identification of therapeutic targets capable of modulating, or preventing their formation could be of great interest for the treatment of AD.

In contrast to previous studies based on the proteomic analysis of micro-dissected plaques from tissue samples, we aimed here to analyse the proteome associated with Aβ40- and Aβ42-fibrils to identify actual Aβ40- and Aβ42-fibril interactors, which might drive Aβ plaque development, by pull-down assays and proteomics. Here we used in vitro synthesized biotinylated Aβ fibrils, pull-down using streptavidin beads, and LFQ proteomics.

To this end, we obtained biotinylated Aβ40 and Aβ42 fibrils *in vitro*, which were subsequently incubated with protein extracts of the left prefrontal tissue samples from AD patients at Braak V (n=6) and Braak VI (n=6), and from healthy individuals (n=6) to pull-down Aβ-interacting proteins with streptavidin beads. Potential interactors were then identified by DDA label free quantitative proteomics (LFQ) using a Q-Exactive and validated through western blot and immunofluorescence (PLA). Additionally, the dysregulation of validated interactors in AD was investigated by WB, immunocytochemistry, and immunohistochemistry.

We identified 185 and 874 proteins associated with Aβ40- and Aβ42-fibrils, respectively, with 78 common interactors. Among them, 16 were validated as actual interactors, and altered in AD. In addition, PRKCG, which has been previously described as downregulated in AD, was validated as an interactor of Aβ plaques, and functional assays revealed that PRKCG activation state modulation influenced Aβ fibril formation, supporting its role as a regulator of amyloidogenesis. In summary, we have identified novel fibril interactors dysregulated in AD with high potential as therapeutic targets.

Proteomic and functional characterization of SLC8A1 in colorectal cancer development and metastasis

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Colorectal cancer (CRC) is a major contributor to cancer mortality worldwide. The prognosis of CRC significantly depends on stage at diagnosis, emphasizing the urgent need for novel prognostic methods. Recent proteomics analyses have allowed identifying SLC8A1 (Solute Carrier family 8 member A1) as a novel and barely known protein overexpressed in CRC. The aim of this work is to characterize the functional role of SLC8A1 in CRC progression and metastasis to get a better understanding of the molecular mechanisms CRC biology, and to identify new potential diagnostic or prognostic biomarkers associated with the development, progression, and metastasis of CRC. The KM12 CRC cell model, composed of three isogenic cell lines with different metastatic capabilities, was used for stably silencing of SLC8A1 using shRNAs. *In vitro* cell-based assays (migration, adhesion, proliferation, and invasion) and *in vivo* experiments were performed to functionally elucidate the role of SLC8A1 in the disease. Furthermore, we performed DIA label-free quantitative (LFQ) proteomics on an Orbitrap Astral mass spectrometer to characterize the SLC8A1-associated proteome and its interactome via immunoprecipitation followed by mass spectrometry. Functional *in vitro* and *in vivo* assays showed a decrease in the tumorigenic and metastatic properties of KM12 CRC cells upon SLC8A1 stable depletion. Proteomic analyses identified dysregulated proteins associated with SLC8A1 depletion and SLC8A1 interactors, many of which were involved in signaling pathways, focal adhesion formation, and mitochondrial activity. Collectively, this study demonstrates a key role for SLC8A1 in CRC progression and metastasis and provides new insights into its contribution to CRC pathogenesis. Moreover, it highlights the potential of SLC8A1 as a novel biomarker and potential therapeutic target in CRC and opens new avenues for the investigation of additional clinically relevant biomarkers.

DIV Matters: Understanding Proteomic Shifts in Neuronal Maturation for Better Ischemic Modeling

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In vitro neuronal models of brain ischemia should reflect the developmental stage of neurons, as neuronal culture maturity influences their molecular state and relevance for modeling specific conditions. Ischemic stroke and neonatal hypoxic-ischemic encephalopathy (HIE) share ischemic injury as a key feature, yet they occur at different maturational stages. Primary neuronal cultures are often used at various days in vitro (DIV), depending on the disease context, but how DIV affects their proteomic profiles remains underexplored. Establishing this baseline is crucial for selecting appropriate in vitro conditions and for interpreting neuronal responses to ischemic stimuli.

This work compares the proteomic profiles of primary cortical neurons at two commonly used maturation culture stages (DIV-8 for HIE and DIV-15 for Ischemic Stroke), to assess how neuronal maturation shapes the proteome in *in vitro* models. A Single-pot, Solid-phase-enhanced Sample Preparation (SP3) method was used for sample preparation and protein digestion, followed by proteomic analysis using liquid chromatography-tandem mass spectrometry. PCA multivariate analysis showed that DIV strongly influences the proteomic profile, with samples clustering by culture time point. Univariate analysis further revealed differential molecular signatures at each DIV stage, and gene ontology analysis of the differentially expressed proteins indicates shifts in cellular machinery from a more immature state (DIV-8) to a more mature one (DIV-15): the DIV-8 group is enriched for processes related to cellular organization and structural assembly, while the DIV-15 group shows enrichment for synaptic communication, network integration, neurotransmission, and pathways characteristic of mature neuronal circuitry. Differences in days *in vitro* significantly influence the neuronal proteome, distinguishing immature from mature states and highlighting the dominant molecular pathways at each stage. Choosing an appropriate culture age is therefore essential for accurately modeling neonatal or adult ischemic conditions. Overall, understanding these maturation-dependent proteomic changes provides a molecular framework for aligning in vitro models with relevant developmental stages and improving the evaluation of ischemic mechanisms and therapeutic strategies.

In-depth serum glycoproteomics reveals stage-dependent α2,6-sialylation and systemic prothrombotic signalling in gastric cancer

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Gastric cancer (GC) remains a major global health burden, ranking among the five most common and deadly cancers worldwide, and its absolute burden is projected to rise substantially in the coming decades(1). Many patients are still diagnosed at advanced stages, limiting therapeutic options and compromising clinical outcome. In this context, the identification of minimally invasive biomarkers for early detection, prognosis and disease monitoring remains a major unmet clinical need. Tumours are characterised by profound alterations in the glycocalyx, including increased α2,6-sialylation driven by ST6GAL1 overexpression, a feature associated with tumour progression, dissemination and poor prognosis(2). Glycoproteins carrying these altered glycans are released into the bloodstream, making the serum glycoproteome a valuable source of tumour-associated and systemic biomarkers. However, the clinical potential of this particular glycan class remains largely unexplored. Here, we performed in-depth glycoproteomic profiling of serum samples from GC patients spanning pre-cancerous lesions and distinct disease stages. Serum from GC patients showed increased α2,6-sialylation, consistent with ST6GAL1-driven remodelling. To characterise this glycoproteome in depth, we performed SNA lectin enrichment followed by ion mobility nanoLC-ESI-MS/MS using complementary HCD and HCD-triggered EThcD fragmentation for confident glycosite annotation. This approach uncovered stage-dependent glycoproteomic signatures with potential value for clinically informed patient stratification. Strikingly, most altered glycoproteins detected in patient sera were not of direct tumour origin. Instead, the serum sialoglycoproteome reflected a predominantly non-tumour derived signature enriched in liver- and immune-associated compartments. These observations support the existence of systemic tumour-host crosstalk rather than a purely tumour-secreted signature. Furthermore, GC patients displayed serum glycoproteomic features consistent with a systemic prothrombotic state. Namely, we found altered complement-related and platelet-associated glycoproteins, including C3, C4A and multimerin-1, thereby linking aberrant glycosylation to cancer-associated haemostatic dysfunction. Given that terminal sialylation is known to enhance the circulatory persistence of glycoproteins by limiting their hepatic clearance, these alterations may also favour the accumulation of such glycoproteins in the bloodstream. These findings provide important clues for refining clinical decision-making, including risk stratification, closer monitoring of thromboembolic complications, and the identification of patients who may benefit from more personalised supportive care. **References:** 1. Bray F, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer Journal for Clinicians. 2024;74(3):229-63. 2. Dobie C, et al. Insights into the role of sialylation in cancer progression and metastasis. Br J Cancer. 2021;124(1):76-90.

Comprehensive nucleolar proteome profiling reveals metastasis-associated remodeling in colorectal cancer

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The nucleolus, a membraneless nuclear organelle in eukaryotic cells, is well known for its central role in ribosome biogenesis. Although its association with cancer was first described in the 19th century, recent studies have revealed broader functions in tumor biology, including regulation of stress responses, transcriptional control, and cell proliferation (1–3). Additionally, colorectal cancer (CRC) remains the third most diagnosed cancer and the second leading cause of cancer-related mortality worldwide (4). In this context, we aimed here to study nucleolar remodeling and its functional contribution to CRC metastasis using advanced proteomic approaches. Data-independent acquisition label-free quantitative (DIA-LFQ) proteomics was used to profile isolated nucleoli from isogenic CRC cell lines with distinct metastatic tropisms: highly metastatic derivatives targeting liver (KM12SM), liver and lung (KM12L4a), and lymph nodes (SW620), together with their poorly metastatic counterparts (KM12C and SW480, respectively). After data analysis performed with Spectronaut and R programs, we observed that differentially regulated proteins were predominantly involved in RNA metabolism and nucleolar regulatory pathways. Orthogonal validation by WB, immunofluorescence, and immunohistochemistry confirmed key candidate proteins, and transient loss-of-function assays performed *in vitro* and *in vivo* demonstrated their functional role in promoting metastatic dissemination. Overall, this study identifies previously uncharacterized molecular drivers linked to nucleolar function in CRC metastasis and highlights the nucleolar proteome as a source of potential prognostic and therapeutic targets.

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When One is Enough: A Minimalistic "On-Pot" Proteomic Workflow for Global Profiling of Single *Caenorhabditis elegans*

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Caenorhabditis elegans is a cornerstone of biological research, yet its small size poses a significant challenge for single organism proteomics. A single adult hermaphrodite consists of only 959 somatic cells, representing an extremely low amount of biomass that some time ago reached the detection limits of conventional proteomic workflows. In this study, we applied a streamlined, "on-pot" (one-pot) sample preparation strategy tailored for single-worm analysis. Five individual *C. elegans* were isolated and each one processed in a single LoBind Eppendorf tube, which served as the sole vessel for both homogenization and digestion. To minimize chemical interference and sample loss, we implemented a direct digestion strategy omitting traditional reduction and alkylation steps. By utilizing a minimal solvent volume, we maintained high protein concentration throughout the process. The resulting peptides were analyzed using an Evosep ONE system coupled on-line to a Bruker timsTOF HT mass spectrometer, employing DIA-PASEF to leverage the high speed and sensitivity of the dual-TIMS analyzer. Despite the scarcity of the starting material (between 50 and 200ng), the "on-pot" minimalist approach proved remarkably powerful. We achieved the identification of more than 4,600 protein groups with high quantitative accuracy. The removal of reduction/alkylation steps did not compromise proteome depth; instead, it simplified the matrix and reduced processing time. The combination of low µL volume preparation and the high performance of the timsTOF HT allowed us to reach a proteomics depth previously reserved for pooled samples.

So… who says size matters? Our minimalist “on-pot” workflow shows that deep proteomic analysis doesn’t require a bucketful of worms; in fact, a single 959-cell specimen as starting material, combined with an optimized protocol can be sufficient. From this perspective, in the world of *C. elegans* proteomics, every individual worm truly counts.

Scaling-up low input spatial proteomics using Evosep Whisper Zoom on the timsTOF Ultra AIP

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Intro

Throughput and scalability are important when it comes to spatially resolved analyses of even smaller tissue pieces using proteomics to understand cellular heterogeneity in a disease context. Here, we demonstrate applicability of the Evosep Whisper Zoom methods for speeding up label-free deep visual proteomics on FFPE preserved tissue analysis of up to 120 samples per day (SPD) with data acquisition on the timsTOF Ultra 2.

Methods

FFPE preserved tissue contours from 5 µm thick mouse liver and human tonsil were cut by laser capture microdissection and transferred into 384 well plate or proteoCHIP EVO-96. Samples were transferred by centrifugation (proteoCHIP EVO-96) or transferred manually onto Evotips, separated in Whisper Zoom 120, 80, 40 and 20SPD, analyzed on a timsTOF Ultra 2 in dia-PASEF and processed with Spectronaut 19.

Results

Mouse liver tissue contours analyzed in Whisper Zoom 120, 80, 40 and 20SPD demonstrated that protein group IDs more dependent on column length rather than gradient length with 120 and 80SPD yielding up to 3,000 protein groups and 40 and 20SPD yielded about 4,000 protein IDs.

Contours from T-cell rich niches, B-cell rich niches, mixed B- and T-cell zones as well as epithelial cell zones from FFPE preserved human tonsil analyzed in 120SPD yielded proteome depths of about 3,500 protein groups, demonstrating clear differences between the 4 selected zones. Typical marker proteins for T-cells (CD3), B-cells (CD19) and epithelial cells (CDH1) where identified.

Conclusion

Ultra-low input FFPE preserved mouse and human tissue demonstrates scalability up to 120SPD with biological insights into spatial tissue heterogeneity.

Beyond Acquisition: Turning Astral-Scale Data into Discovery

A Modular GUI for DIA Proteomics Analysis in a Core Facility

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As next-generation instruments like the Orbitrap Astral dramatically increase throughput and proteome depth, the bottleneck in proteomics has shifted: data analysis, not acquisition, is now the limiting step. At the CNIO Proteomics Unit, we are addressing this challenge with a job scheduler implemented as a modular, desktop-based graphical user interface. This tool is designed to empower non-bioinformaticians to execute and interpret proteomics analyses, spanning from DIA searches to downstream biological insights.

Built with Python (Flask and PyWebView), the monitor platform integrates mass spectrometry tools such as DIA-NN and MaxQuant with Protea, our in-house R- and Python-based pipeline for statistical analysis and visualization. Specifically, the bundled Protea pipeline enables comprehensive workflows for Global Proteome Analysis, Interactomics, and Phosphoproteomics, integrating statistical enrichment methods such as ORA and GSEA/PTM- SEA, alongside clustering and functional annotation. Overall, the GUI monitor handles job creation, file monitoring, and job tracking, while Protea drives the downstream data interpretation and dynamic HTML reporting. Everything is integrated into a single, user-friendly interface installed locally on each workstation.

Ultimately, this modular solution transforms data processing into an intuitive, interactive experience that standardizes reproducible analysis, accelerates biological insight, and makes sharing results with collaborators effortless. More than a technical solution, this initiative supports a cultural shift: enabling broader participation in data interpretation, freeing expert time for innovation, and helping core facilities keep pace with the increasing scale and complexity of modern proteomics.

Optimizing tissue disruption strategies to characterize in vivo subcellular proteome remodelling

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Abstract

Protein homeostasis is essential for cellular function, as it regulates protein synthesis, folding, activity and degradation. Its disruption leads to protein malfunction, misfolding and, eventually, pathological mislocalization and/or aggregation, processes tightly linked to neuro- and cardiovascular degenerative diseases. However, the mechanisms behind protein homeostasis damage are not fully understood, and processes such as protein subcellular partitioning might seed light into the causes of such disruption. Subcellular fractionation coupled to mass-spectrometry (MS) based proteomics quantification is key to explore these processes (1). Nevertheless, applying this fractionation approach to *in vivo* models presents a critical challenge: the need for efficient tissue dissociation that preserves cellular structure. Tissue dissociation is, therefore critical, as preserving cellular integrity and organelle structure is essential for accurate protein localization and directly impacts data quality, reproducibility, and biological interpretation. A systematic evaluation of different tissue dissociation methods is therefore essential to ensure the accuracy and reliability of downstream proteomic analyses. This study performs a benchmark between manual enzymatic based tissue disaggregation from fresh samples and mechanical tissue disruption from frozen samples. We tested both strategies across four mouse organs (brain, liver, kidney, and heart) and coupled them to subcellular fractionation and protein digestion prior to MS analysis. Samples were analyzed using an Evosep system coupled to an Orbitrap Eclipse, employing a Data Independent Acquisition method. Between 7,000–9,000 protein-coding genes were identified across organs, with the frozen method achieving the highest yield, averaging 6,600 proteins per fraction, while the enzymatic method identified5,400 proteins per fraction. Frozen dissociation provided better resolution between fractions; however enzymatic dissociation was more effective at preserving organelle structure, such as mitochondria, in specific tissues -brain and kidney- which were not properly resolved in frozen samples. These results highlight the need to further optimize the subcellular fractionation strategies *in vivo*, in particular, to improve the resolution on the purification membranous organelles in frozen samples.

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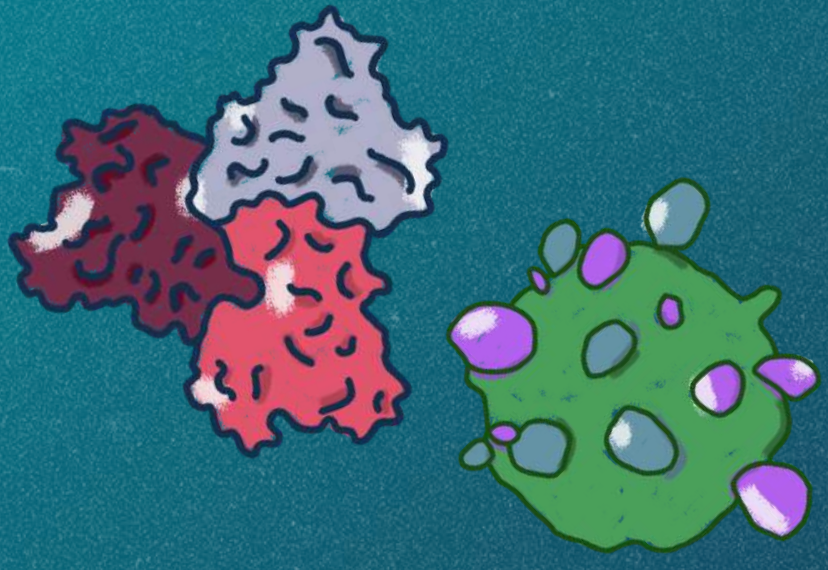
Section 3
Proteomics in the Clinic: From Biomarkers to Precision Medicine #2

Oral Communications



Glycoproteomics uncovers a paucimannosylated proteome associated with tumour aggressiveness and poor clinical outcome in gastric cancer

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Gastric cancer (GC) remains a major cause of cancer-related mortality, with limited molecular features enabling robust stratification of aggressive disease. While aberrant glycosylation is increasingly recognized as a hallmark of cancer, its integration at the proteome level and its contribution to tumour progression remain insufficiently understood. Here, we apply a comprehensive high-throughput glycomic and glycoproteomic strategy to define the role of paucimannosidic *N*-glycosylation in GC pathobiology. We combined high-resolution nanoLC-MS/MS-based *N*-glycomics with lectin-guided glycoproteomics to interrogate formalin-fixed paraffin-embedded tumour tissues across disease stages. Quantitative *N*-glycomic profiling revealed a marked enrichment of paucimannosidic *N*-glycans in advanced tumours. Notably, glycan-centric prognostic stratification outperformed established clinical biomarkers, underscoring the clinical relevance of this glycosylation axis. Moreover, targeted enrichment using *Galanthus nivalis* lectin (GNL), coupled with deep proteomic analysis, enabled the identification of more than 1.600 proteins and a subset of annotated glycoproteins carrying trimmed *N*-glycans. Glycoproteomic mapping revealed an unexpected predominance of paucimannosylated structures on intracellular proteins, particularly ribosomal components, alongside aberrant modification of membrane and secreted proteins. Among these, matrix metalloproteinase 9 (MMP9) emerged as a key glycoprotein associated with tumour progression, with increased expression and co-occurrence with paucimannosidic glycoforms defining a subset of tumours with markedly poor clinical outcomes. Proteome-wide comparisons further highlighted stage-specific remodelling of pathways linked to immune modulation, vesicular trafficking, and stress adaptation. To delineate the molecular basis underpinning this phenotype, integration with transcriptomic data from large The Cancer Genome Atlas (TCGA) patient cohorts (> 400 cases) demonstrated that enzymes driving glycan trimming are upregulated in aggressive tumours and correlate with poor clinical outcomes, supporting a mechanistic link between glycosylation remodelling and disease progression. Interrogation of serum samples revealed that, although GNL reactivity did not translate into a circulating biomarker, MMP9 retained diagnostic potential, supporting its utility in non-invasive disease monitoring. Collectively, this work establishes a previously unrecognized paucimannosylated proteome in gastric cancer and demonstrates the power of glycoproteomics to resolve functionally relevant layers of tumour biology. These findings position paucimannosylated glycoproteins, particularly paucimannosylated MMP9, as promising candidates for next-generation biomarker development and targeted intervention.

Reference: Ferreira D, et al. Trimmed *N*-glycans define aggressive gastric cancer and predict clinical outcomes. Theranostics. 2025;15(18):9375-9398. doi:10.7150/thno.111670. **Funding:** The authors acknowledge FCT funding within the project RESOLVE (doi: 10.54499/PTDC/MED-OUT/2512/2021).

Artificial intelligence-based clinical models predict plasma proteomic endotypes enabling precision medicine in knee osteoarthritis

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Background: Knee osteoarthritis (kOA) is a clinically and biologically heterogeneous disease. The lack of molecular stratification limits the development of targeted therapies and the success of clinical trials. The aim of this study was to identify plasma proteomic endotypes of kOA and evaluate their potential for patient stratification within a precision medicine framework.

Methods: The study included patients with radiographic kOA and OA-associated comorbidities. In the discovery phase, 44 plasma samples were analysed by LC-MS/MS. A curated proteomic dataset was subjected to unsupervised clustering using ConsensusClusterPlus with k-means. Endotype- specific signatures were defined by differential protein expression analysis. Validation was performed in an independent cohort of 186 plasma samples processed using PreOmics workflow and analysed by Evosep LC coupled to high-resolution mass spectrometry. Endotype-associated protein signatures were evaluated by univariate linear models (ULM) and selected proteins were orthogonally validated by immunoassays. Machine learning (ML) models were applied to assess the ability of clinical variables to classify patients according to their proteomic endotypes as well as to develop simplified predictive models.

Results: Three stable and biologically distinct proteomic endotypes were identified (E1, E2, E3). E1 was enriched in platelet activation pathways, E2 showed minimal proteomic perturbation, and E3 was characterised by immune-related pathway enrichment. These endotypes were reproducibly identified in the validation cohort. Endotype classification was driven by 30 proteins for E1, 3 for E2 and 22 for E3. ULM confirmed enrichment of platelet-related proteins in E1 and immune- associated signatures in E3. Three circulating biomarkers of the platelet-driven endotype (TSP1, CXCL7 and PF4) were independently validated by immunoassay. ML models partially captured the proteomic endotypes, with Random Forest achieving the best performance (balanced accuracy = 0.53). A reduced clinical model based on five variables retained comparable predictive performance. Integration of clinical variables with selected proteins further improved classification performance. The combined clinical–protein model achieved the highest discrimination (macro-AUC = 0.75, 95% CI 0.65–0.85), outperforming clinical variables alone (AUC = 0.73) and proteins alone (AUC = 0.58) (Fig.1)

Conclusions: Plasma proteomics identifies reproducible molecular endotypes of kOA and reveals clinically relevant signatures associated with disease heterogeneity. Integrative models combining proteomic biomarkers and clinical variables provide a promising framework for patient stratification and precision medicine in kOA.

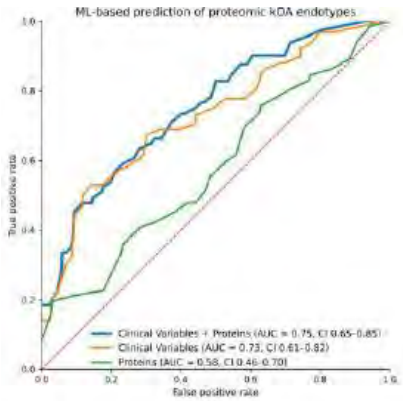

Figure 1. ROC curves showing the predictive performance of clinical, proteomic and combined models for the classification of proteomic endotypes.

Longitudinal Shotgun Proteomics Identifies Candidate Biomarkers of Inadequate Therapeutic Response and Treatment Switch in Neovascular Age-related Macular Degeneration

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Age-related macular degeneration (AMD) is the leading cause of blindness among older adults in developed countries. Neovascular AMD (nAMD) is the most aggressive form of the disease, and anti-VEGF drugs currently are the only available treatment. However, a significant proportion of patients show insufficient response, suggesting the involvement of pathogenic mechanisms beyond VEGF-driven angiogenesis, including the immune system. However, these mechanisms remain insufficiently studied. In this context, we aimed to characterize proteomic signatures associated with inadequate response to aflibercept (anti-VEGF) and molecular changes following switching therapy to faricimab (anti-VEGF and anti-Ang2), and to identify candidate biomarkers of treatment response.

We conducted a prospective multicenter observational study, peripheral blood samples were collected from patients with nAMD showing inadequate response to aflibercept who were switched to faricimab, and from controls without retinal disease. Peripheral blood mononuclear cells (PBMCs) were isolated using the AutoMACS Pro system (Miltenyi Biotec). Peptides were prepared using the PreOmics iST kit (PreOmics) and analyzed by DIA LC-MS on an Orbitrap Exploris 480 (Thermo Fisher Scientific) coupled to a Vanquish Neo UHPLC system (Thermo Fisher Scientific). Protein identification and differential expression analyses were performed using Spectronaut 18 (Biognosys) with the directDIA library-free workflow. Differentially expressed proteins were defined by $q \leq 0,05$ and absolute fold change $\geq 1,5$. Network analysis, clustering, and functional enrichment analyses were performed using the STRING database v12.0 and MCODE v2.0.3 in Cytoscape v3.10.3.

12 patients with nAMD and 9 controls were included. A total of 6.663 proteins were identified. At baseline, 325 proteins were overexpressed and 48 underexpressed between patients and controls, while 3 were overexpressed and 114 underexpressed after switching therapy. Inadequate response to aflibercept was associated with enrichment of platelet activation, immune pathways, and cytoskeletal organization. After switching to faricimab, proteins involved in complement activation, extracellular matrix remodeling, and vascular permeability showed reduced abundance. Notably, ANGPT1, CLDN5, and ALOX12 showed reversed expression after switching therapy, suggesting their potential as candidate predictive biomarkers of treatment response.

This study represents the first longitudinal proteomic analysis of patients with neovascular AMD showing inadequate response to aflibercept who subsequently switched to faricimab. Our findings suggest that biological pathways beyond VEGF signaling may be modulated by faricimab and could contribute to aflibercept resistance, while highlighting candidate biomarkers that warrant validation in larger cohorts.

Unravelling the proteome of human embryo implantation: new biomarkers and metabolic signatures

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In France, 1 out 6 couples consult at least once in their life for infertility problems. Nevertheless, the average success rate for in vitro fertilization (IVF) is only around 27% with 45% of the failure rate occurring in the implantation window. Understanding the window of human embryo peri-implantation development is essential to appreciate the beginning of human life but also for multiple clinical approaches such as IVF and placenta physiopathology.

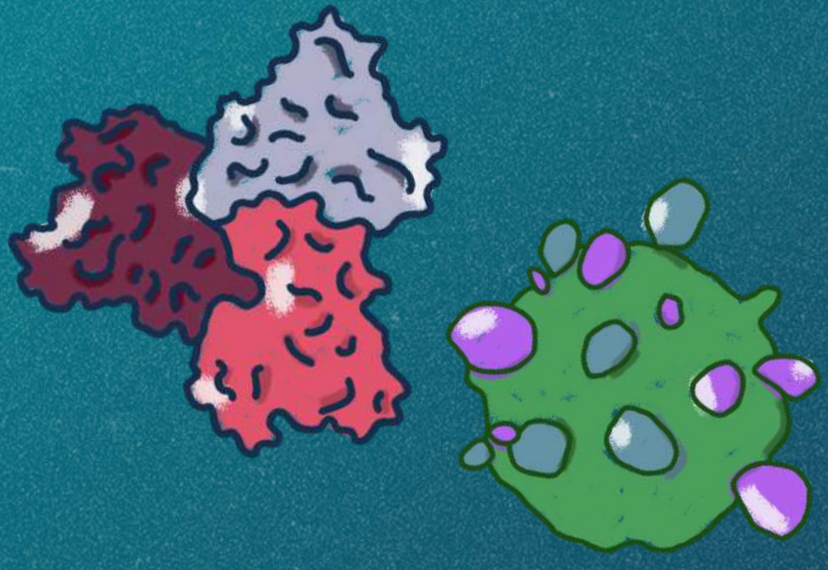
First, we unraveled the human embryo proteome using mass spectrometry at 4 d.p.f., 6 d.p.f., and after prolonged culture, equivalent to post-implantation, at 8 and 10 d.p.f.. Our DIA approach detected between 5,160 and 6,077 proteins. By integrating our proteomic analysis with our scRNAseq analyses, we highlighted classes of transcription factors involved in the establishment of the trophoblast, the site of implantation. These transcription factors regulate cellular functions important for implantation, such as cell adhesion and metabolism. Finally, we validated our hypotheses by characterizing the metabolic profile of human embryos between developmental stages and also according to cell fate.
In addition, mass spectrometry enabled us to identify biomarkers predictive of implantation in spent culture media.

Altogether, these results open up new perspectives in the evaluation of embryo quality and the improvement of culture conditions for patients with recurrent implantation failure.

Keywords: Assisted Reproductive Technique, Human embryo, Proteomic, Mass spectrometry, Peri-implantation development, Implantation.

Section 4
Networks in Action: Interactomes, Signalling & Bioinformatics Innovation

Oral Communications



Deciphering protein-protein interaction in live neurons using XL-MS

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Introduction: In neuronal synapses, information is transmitted via the release of neurotransmitters stored in synaptic vesicles. They undergo a tightly regulated trafficking cycle leading to their fusion with presynaptic membrane. However, our knowledge on the protein-protein interactions (PPIs) involved in this cycle and its regulation remains elusive in the absence of large-scale in vivo approaches [1]. Here we propose a novel cross-linking mass spectrometry (XL-MS) workflow targeting live, adherent and differentiated neurons. This work allows us to unravel PPIs underlying synapse's function and organization directly in cellulo.

Methods: SH-SY5Y cells were grown in poly-L-Lysine-coated dishes, fixed with formaldehyde (FA) and cross-linked with tBu-PhoX (TBP) [3]. After cell harvest and protein digestion, TBP was deprotected, FA removed and samples were further processed. TBP- peptides were enriched using TiO2 or FeNTA and further submitted to SEC fractionation using an Äkta pure system. Collected fractions were analyzed on a Q-Exactive Plus mass spectrometer coupled to a Dionex UltiMate 3000 RSLC nano chromatography. Raw files were processed using pLink3.

Results: To capture biologically relevant synaptic interactions, we benchmarked different cross-linkers on adherent and live SH-SY5Y cells [2]. TBP was superior as it effectively crossed the cell membrane and penetrated various organelles [3]. For optimization of the workflow, we efficiently enriched TBP cross-links through IMAC and removed mono-links using SEC fractionation. To specifically target neurites, we optimized the protocol for cell morphology preservation, involving coating with poly-L-Lysine and formaldehyde fixation prior to the XL reaction [4]. Our preliminary results reveal an extensive PPI network in live and morphologically preserved neurons, reaching 700 XLs and nearly 300 PPI across all cell compartments. Our dataset highlights extensive cytoskeleton and nuclear histone interactions, including previously unreported PPIs. Currently, we are applying this approach to undifferentiated and differentiated N-types neurons that are competent to form biologically relevant synapses.

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Proteomic profiling of the interactome of phosphorylated Tau aggregates identifies modulators of Alzheimer's disease progression

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Tau pathology, characterized by the accumulation of hyperphosphorylated Tau (p-Tau) aggregates, is a central hallmark of Alzheimer's disease (AD) and other tauopathies, which have been described both as a cause and a consequence of these disorders. Here, our aim was to characterize the proteome specifically associated with p-Tau aggregates in the human brain to identify potential modulators of p-Tau aggregation relevant to AD pathogenesis. To this end, we generated biotinylated p-Tau aggregates using biotinylated DYRK1A-phosphorylated Tau441 and incubated aggregates with protein extracts from the left prefrontal cortex of AD patients at Braak V and matched controls. Interacting proteins were isolated by pull-down assays and analyzed using data-independent acquisition (DIA) label-free quantitative proteomic analysis on an Orbitrap Astral mass spectrometer. Candidate interactors were validated by WB and proximity ligation assays (PLA) on formalin-fixed paraffin-embedded (FFPE) brain tissue sections. Additionally, their dysregulation in AD was assessed by WB, immunohistochemistry (IHC), and using independent proteomic datasets. In summary, we identified here a specific set of proteins selectively binding to p-Tau aggregates. Among them, the interaction of BASP1, PLP1, and BAG3 with p-Tau aggregates was confirmed by WB, while PLA confirmed the interaction with RAC1, SRCIN1, and VDAC1. Notably, THY1, TMED9, and GMFB were confirmed to interact with p-Tau aggregates by both techniques. Moreover, BAG3, BASP1, GMFB, TMED9, SRCIN1, and VDAC1 were dysregulated in AD brain tissues either by IHC or by *in silico* analysis of proteomic datasets, suggesting a potential role of these proteins in AD development. Our results reveal novel components of the p-Tau interactome and highlight potential regulators of p-Tau aggregation that might contribute to the progression of AD. These findings provide mechanistic insights into p-Tau aggregation and propose new candidates for AD therapeutic targeting.

GlycoAvatars: Bead-coated membrane models for studying the cancer-immune cells interactome

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Immature *O*-glycosylation of plasma membrane proteins, characterized by the presence of glycosites with a single GalNAc residue (the Tn antigen) rather than more extended and complex glycans, is a prominent feature of many advanced solid tumors across different origins and aetiologies. This aberrant glycosylation is strongly associated with poor prognosis and is known to play a crucial role in the progression of the disease. Notably, it has been directly implicated in the crosstalk with the immune system, inducing tolerogenic phenotypes in both innate and adaptive immune cells. Despite this, the specific receptors involved in these interactions remain largely unidentified. To address this gap, we propose a novel high-throughput proteomics assisted strategy for characterizing the glycan interactome using plasma membrane-derived glycoprotein coated magnetic beads, termed GlycoAvatars(1). Glycoproteins were isolated from plasma membranes of glycoengineered cell models displaying immature *O*-glycosylation and immobilised onto the surface of large magnetic beads, creating simplified cell-mimicking platforms. These GlycoAvatars were primarily employed to characterize the interactome between gastric and colorectal cancer cell lines and immune cells, including dendritic cells and macrophages. Through this approach, we identified a wide range of membrane and intracellular proteins involved in glycan-mediated immune signaling, uncovering previously uncharacterized molecular nodes. Overall, this proteomics-enabled platform provides a robust tool for dissecting glycan-driven interactions and highlights its potential for the discovery of novel therapeutic targets.

Reference

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Orai1 facilitates angiogenesis after myocardial infarction through Notch1 signaling pathway

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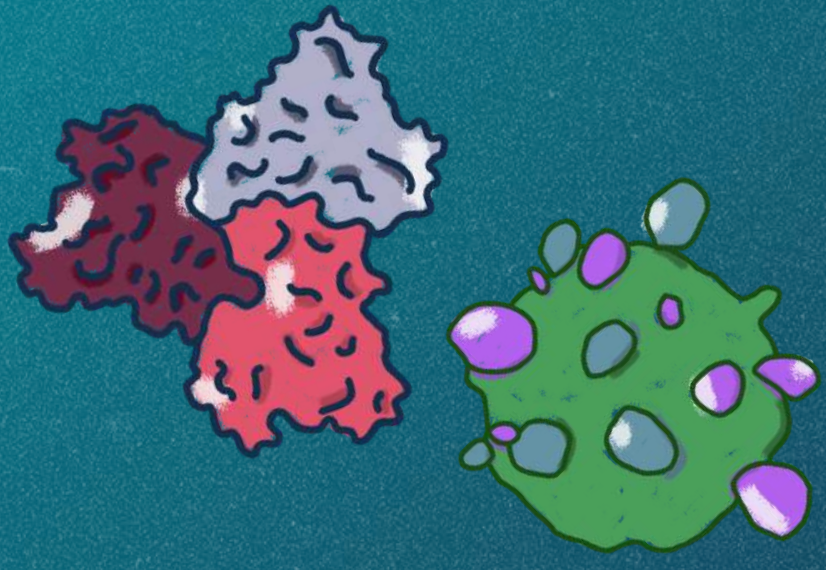
Background: Post-ischemic neovascularization is crucial for cardiac repair after myocardial infarction (MI). While Orai1-dependent store-operated calcium entry (SOCE) is known to support angiogenesis, its specific role after MI remains unclear. **Methods:** We stimulated human umbilical vein endothelial cells (HUVEC) with serum from ST-elevation MI (STEMI) patients to analyze pro-angiogenic mechanisms and to mimic the post-infarct systemic environment. We performed integrative analysis, including transcriptomics, proteomics, post-MI mouse heart single-cell RNA sequencing (scRNA-seq), and immunostaining. **Results:** STEMI serum enhanced angiogenesis by upregulating VEGF, Notch, and Ca^{2+} signaling pathways in EC. Notably, it increased Orai1 expression and SOCE activity, required for EC migration and proliferation. Consistently, Orai1 inhibition with CM4620 significantly impaired subintestinal venous plexus (SIVP) development in zebrafish embryos. scRNA-seq confirmed Orai1 upregulation, particularly in tip cells and proliferating EC clusters, which was confirmed in peri-infarct regions of mouse hearts and in tip-like cells in a 3D culture model. Proteomics analysis revealed that Orai1 silencing dysregulated VEGF and Notch1-related pro-angiogenic proteins. Furthermore, interleukin-17A (IL-17A) mimicked STEMI serum, inducing Orai1-mediated SOCE and EC migration. **Conclusions:** Together, these findings reveal a novel role for Orai1-dependent mechanism in post-MI angiogenesis, highlighting Orai1 as a potential therapeutic target for cardiac repair.

Section 5
PTMs: Deciphering the dynamics of Protein Regulation

Oral Communications



EXPERIMENTAL DESIGN AND MULTIVARIATE ANALYSIS APPROACHES FOR GLYCOPROTEOMICS

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Experimental design and multivariate modeling are essential for generating reliable insights from glycoproteomics data. Design of experiments (DoE) offers significant advantages in analytical development as it enables the evaluation of multiple experimental factors and their interactions, minimizing the number of experiments required, and enhancing method reproducibility and robustness. Complementarily, multivariate data analysis methods, such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), allow the integration and interpretation of the high-dimensional datasets typically generated in glycoproteomics studies. These approaches facilitate sample classification and the identification of subtle variations in glycosylation profiles.

In the present study, a multiplexed parallel reaction monitoring (PRM) - DoE approach was performed to simultaneously evaluate key electron activated dissociation (EAD) parameters across target glycopeptide glycoforms, using recombinant human erythropoietin (rEPO) as a model glycoprotein [1]. The established LC-MS/MS method provided improved glycan site localization confidence over conventional collision induced dissociation, as demonstrated by applying a data-dependent acquisition method and subsequent analysis with Byonic and Fragpipe softwares. The proposed strategy advances the analytical toolkit for glycopeptide analysis and could be extended to other post-translational modifications. In parallel, PCA and PLS-DA were applied to evaluate the potential of glycopeptide and glycan analysis by capillary liquid chromatography-mass spectrometry (capLC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) for classification purposes [2,3]. At the glycopeptide level, three rEPO biopharmaceuticals—two commercially available drugs and one currently under clinical trials for neurodegenerative diseases—were analyzed. The resulting models enabled clear discrimination between groups, detection of batch-to-batch variability, and identification of the glycoforms responsible for differentiating the three recombinant products. At the glycan level, relative quantification of human alpha-acid glycoprotein (hAGP) glycan isomers using [$^{12}C_6$]/[$^{13}C_6$]-aniline labelling in combination with PLS-DA, was evaluated for the identification of pancreatic ductal adenocarcinoma (PDAC) glycan biomarkers in serum samples. The results indicated that the triantennary glycan containing two fucose residues may play a relevant role in distinguishing patients with PDAC from those with chronic pancreatitis.

Overall, these approaches proved to be reliable and highly valuable tools for biopharmaceutical quality control and development, as well as for the identification of glycobiomarkers associated with disease or physiological states.

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Study of the links between the dysregulations of metabolism and epigenetics marks in Huntington's disease

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Huntington's disease (HD) is characterized by progressive degeneration of striatal medium spiny neurons (MSN) and widespread epigenetic and transcriptional dysregulation. Increasing evidence shows that metabolic alterations contribute to epigenetic dysfunction, as many histone post-translational modifications (PTMs) depend on cellular metabolites. In the brain, glucose represents the primary energy substrate, and its metabolism provides key intermediates such as acetyl-CoA that directly fuel chromatin modifications, including histone acetylation. Altered glucose utilization has been reported in HD and may therefore impact epigenetic regulation. To investigate the relationship between metabolism and histone acetylation dynamics in HD, we performed metabolic tracing experiments in wild-type (WT) and R6/1 HD mice following intraperitoneal injection of ^{13}C-glucose. Histones were extracted from striatal tissues and analyzed by liquid chromatography coupled to mass spectrometry. We developed a dedicated analysis pipeline allowing accurate estimation of ^{13}C-glucose-derived acetyl incorporation into histone peptides while correcting for natural isotope abundance. Using this approach, we quantified the kinetics of heavy acetyl exchange across ~ 40 acetylated peptidoforms from histones H2A, H3 and H4. Our results reveal significant differences in acetylation kinetics between WT and R6/1 mice. In R6/1 striatum, incorporation of ^{13}C-glucose-derived acetyl groups reached a plateau earlier and at lower incorporation percentage, suggesting either faster turnover or reduced availability of acetyl-CoA, whereas WT mice showed a continuous increase in labeled acetylation over the considered time course. This difference was global across multiple histone lysines rather than site-specific and became particularly pronounced after 60 minutes post-injection. The effect was even stronger for multiply acetylated peptides containing two labelled acetyl groups. These findings indicate that histone acetylation dynamics are altered in the HD mouse striatum, supporting the idea that impaired glucose metabolism affects acetyl-CoA availability or utilization for chromatin modification in HD.

Phosphoproteomics as a tool to dissect the molecular mechanisms underlying a novel combinatorial therapeutic strategy in pseudomyxoma peritonei

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Pseudomyxoma peritonei (PMP) is a rare mucinous neoplasm characterized by progressive accumulation of mucin within the peritoneal cavity. Although cytoreductive surgery combined with hyperthermic intraperitoneal chemotherapy represents the current standard treatment, a substantial proportion of patients eventually relapse and effective systemic therapies are still lacking.

Recent molecular studies have shown that more than 70% of PMP cases harbour activating KRAS mutations, most frequently KRAS^G12D. In previous work using patient-derived models, we demonstrated that pharmacological inhibition of KRAS^G12D with MRTX1133 produces promising antitumour activity but is limited by adaptive EGFR signalling reactivation[1]. In addition, we have shown that the PMP tumour microenvironment is strongly hypoxic, promoting tumour cell survival through HIF-1α-dependent pathways.

Based on these observations, we evaluated a combined therapeutic strategy targeting KRASG12D (MRTX1133), EGFR (cetuximab) and HIF-1α signalling (digoxin). Therapeutic efficacy was first assessed in a patient-derived xenograft (PDX) model of PMP, generated by intraperitoneal implantation of mucin from a donor PDX tumour into nude mice. Combination therapy completely prevented tumour growth during the 16-week follow-up, significantly outperforming monotherapies and double-drug combinations.

To investigate the molecular mechanisms underlying this therapeutic synergy, PDX-derived organoids (PDXOs) were established and cultured under hypoxic conditions or hypoxia-mimetic treatments. Functional assays demonstrated that the triple drug combination markedly reduced cell viability compared with controls and single-target treatments.

To decipher the signalling adaptations responsible for this response, we are currently applying a global phosphoproteomics strategy based on phosphopeptide enrichment and high-resolution LC-MS/MS to PDXOs exposed to the different treatments. This approach will enable the identification of drug-induced phosphorylation changes and signalling rewiring events associated with KRAS inhibition, EGFR feedback activation and hypoxia-driven adaptive pathways.

Overall, our results identify a promising combinatorial therapeutic strategy for KRAS^G12D-mutant PMP and highlight the value of phosphoproteomics for dissecting signalling networks driving tumour resistance in hypoxic microenvironments.

Keywords: Phosphoproteomics, Post-translational modifications, KRAS signalling, Hypoxia signalling, Patient-derived organoids, Pseudomyxoma peritonei.

Funding resources: This work is supported by “Instituto de Salud Carlos III (ISCIII)” (PI22/001213), and by “Asociación Española contra el Cáncer” (PRYES223170ARJO).

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Quantitative proteomic characterization of metastasis-associated succinylome in colorectal cancer

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Colorectal cancer (CRC) is the second leading cause of cancer-related death worldwide and remains a major clinical challenge due to metastasis development. Succinylation is a widespread post-translational modification (PTM) consisting of the covalent addition of a succinyl group to lysine residues, which induces substantial structural and functional changes in proteins due to the introduction of a negatively charged group. This modification, closely linked to mitochondrial metabolism, regulates diverse cellular processes, and its dysregulation has been associated with tumour progression. Therefore, in this study, we aimed to investigate the association between succinylation status and CRC metastasis.

To this end, we employed two isogenic cell models of CRC metastasis, each comprising a poorly metastatic cell line (KM12C and SW480) and highly metastatic cells (KM12SM and KM12L4a, and SW620, respectively). Following protein digestion and immunoaffinity enrichment of succinylated peptides, samples were analysed by label-free quantification data-independent acquisition (LFQ-DIA) proteomics using an Orbitrap Astral mass spectrometer.

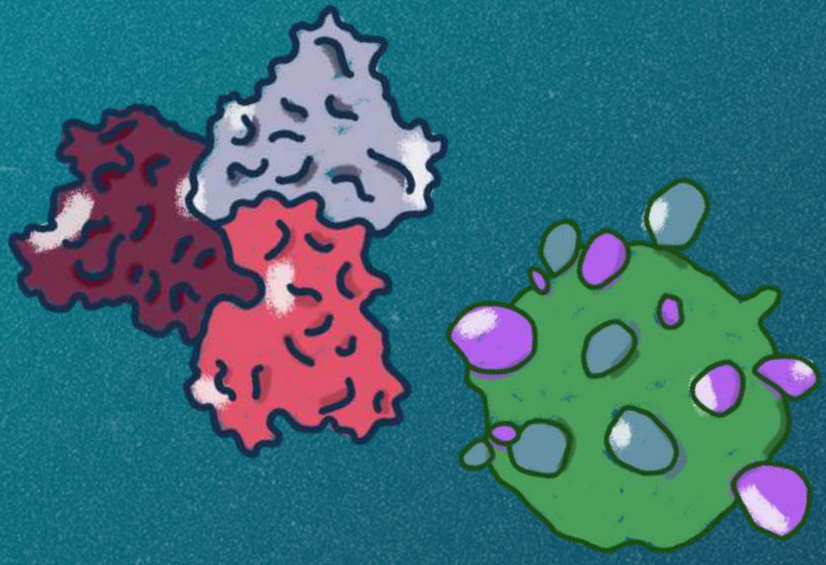
DIA data processing with Spectronaut enabled the identification and quantification of 1,271 succinylated lysine residues across 411 proteins, with the number of succinylated sites per protein ranging from 1 to 33. Notably, 37% of these sites had not been previously reported as succinylation sites. Enrichment analysis confirmed the predominance of succinylation in mitochondrial proteins; however, modified proteins were also significantly represented in cytoplasmic, nuclear, and membrane-associated compartments.

Differential analysis performed in R allowed the identification of metastasis-associated succinylation sites with significantly altered modification levels between metastatic and non-metastatic models, with mitochondrial proteins exhibiting the majority of upregulated succinylation sites. Dysregulated succinylated proteins were associated to pathways related to cellular metabolism, mitochondrial respiration, or regulation of cell death among others.

Together, our results suggest that succinylation remodeling plays a functional role in CRC metastatic progression.

Section 6
Proteomics Exploration in Non-Model Systems

Oral Communications



Geographical origin differentiation of tiger nut (*Cyperus Esculentus*) through liquid chromatography-high resolution mass spectrometry analysis

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Tiger nut (*Cyperus esculentus*), popularly known as chufa, is an edible tuber with significant economic relevance in Western Africa and Southern European regions. It is one of the most characteristic crops of the Valencian Community (Spain), being officially recognized as "Chufa de Valencia" with a Protected Designation of Origin (PDO) and used for the production of the traditional beverage "horchata de chufa" [1]. In this context, verification of geographical origin is a key aspect for protecting consumers of PDO products and to prevent food fraud.

Main objective of this research was the unveiling of proteomic biomarkers for the authentication of chufa tubers with different geographical origins. Protein extracts of flours from different African (AF, n=3) and Valencian (VAL, n=3) tiger nut varieties were assayed, approaching tryptic digests yielded by liquid chromatography-high resolution mass spectrometry analysis (LC-HRMS) supported by Orbitrap^{R} detection featuring MS^{1}-MS/MS data-dependent experiments. A preliminary qualitative research was featured considering pooled chufa extracts belonging to varieties sharing same geographical origin (n=2, POOLED AF *vs.* POOLED VAL) and for a coarse quantitative sieving according to achieved Mascot scores [2]. Interrogation of MS/MS data was performed by Mascot v3.0 as search engine and the tiger nut-specific database reported by [3] populating predicted protein sequences without identifications. Functional protein identification was then performed by homology to *Oryza sativa* sequences using BLASTP. An in-house library (theoretical monoisotopic observed intact mass and retention time) was built by selecting peptides from assigned proteins with AF/VAL or VAL/AF Mascot score ratios of at least 1.5-fold change in pooled samples. Such library was loaded by MS-DIAL freeware for quantitation (according to MS^{1} response) of listed peptides in AF and VAL biological replicates. As a result, a definitive list of singular peptides characterizing potential protein biomarkers was created, finding 4 exclusive signals and 16 with a minimum of 50-fold higher abundance when comparing AF *vs.* VAL replicate groups. Interestingly, five descriptors belonging to the small heat-shock protein family (16.9, 17.9, 18.0, 19.0 and 26.7 kDa sHSP) were exclusive of AF varieties assayed. Overexpression of these small chaperones could be related to the stress caused by extreme abiotic harvesting climate and soil conditions (mainly heat and drought [4]) of African regions, reflecting the influence of cultivation environments on protein expression in chufa cultivars. Results seemed to suggest that tiger nut proteomics can help to differentiate raw materials from different geographical origins through the elucidation of protein biomarkers of authenticity.

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Transforming Invasion into Innovation: Proteomics of Rugulopteryx okamurae for Monitoring, Degradation and Biomass Valorization

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Rugulopteryx okamurae is one of the most aggressive invasive marine macroalgae currently impacting coastal ecosystems in southern Europe, causing severe ecological disruption and substantial economic losses. Despite its rapid spread, this species remains largely unexplored at the molecular level. RUGULOMICS is a proteomics-driven project aimed at building the first comprehensive proteome of *R. okamurae*, providing functional insights into the biological mechanisms underlying its invasive success and enabling the development of monitoring and control tools.

To enable high-confidence protein identification, a de novo genome assembly and transcriptomic datasets will be generated exclusively as supporting resources for proteomic database construction and annotation. RUGULOMICS is structured around three main objectives: (i) identification of protein biomarkers for environmental monitoring of *R. okamurae*; (ii) metaproteomic profiling of microorganisms capable of degrading this macroalgae biomass; and (iii) revalorization of *R. okamurae* waste biomass through the discovery of proteins with potential bioactive or industrial applications.

A key initial step of the project is the development of optimized protein extraction and digestion workflows for brown macroalgae, overcoming the strong analytical interference caused by polysaccharides and polyphenolic compounds. These workflows will be coupled with high-resolution bottom-up proteomics to systematically identify and quantify proteins expressed by *R. okamurae* and its associated microbiome.

For the first objective, *R. okamurae* will be analysed at two distinct stages of its life cycle. The resulting datasets will constitute the first reference proteome for this species and will enable the identification of species-specific environmental biomarkers detectable in seawater, allowing non-invasive monitoring of early invasion events. In parallel, the second objective will apply a metaproteomic approach to explore functional interactions between *R. okamurae* and its associated microbiota during in vitro macroalgae degradation, with the aim of identifying potential algal-degrading microorganisms and enzymes. Finally, the newly described proteomes will be computationally screened to identify proteins with predicted bioactive or industrial properties, which will be recombinantly produced and functionally validated.

By placing proteome discovery at its core, RUGULOMICS will establish a foundational molecular resource for this invasive alga and demonstrate how environmental proteomics can advance both ecological understanding and sustainable biotechnological exploitation.

High-Throughput SPE Membrane Approaches for Peptide Cleanup and Enrichment

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Bottom-up LC-MS/MS workflows typically require multistep sample preparation followed by peptide cleanup to remove salts that would otherwise cause ion suppression and reduce identification rates. For proteoform analysis, enrichment of post-translationally modified (PTM) peptides is also essential to ensure reliable identification, localization, and quantification.

Conventional cleanup strategies are often labor-intensive and prone to sample loss or elution variability. Developing robust, automatable, and standardized cleanup and enrichment methods is therefore a critical step toward establishing proteomics as a practical tool in diagnostics and drug discovery. Our SPE membrane technology, developed through two decades of expertise in complex sample preparation, addresses several of these bottlenecks in MS-based proteomics.

One major challenge lies in high-throughput single-cell and other low-input analyses, where consistent recovery from limited protein material is required. Using optimized membrane workflows, reproducible results were obtained across a 1 ng–10 µg input range, achieving up to 97% protein identification and <3% RSD, demonstrating reliable analysis even from trace-level samples.

In glycoproteomics, the structural diversity of glycans complicates enrichment and detection. Preliminary studies using SPE membrane-based methods showed, on average, a fivefold increase in N-glycopeptide identifications from cell lysates and plasma compared to unenriched controls, reaching or exceeding the performance of established HILIC-based methods.

Phosphoproteomics represents another demanding application, as conventional desalting often results in the loss of polar phosphopeptides. Membrane-based C18 formats enabled up to 2.4-fold more phosphopeptide identifications with reproducibility below 10% RSD. Optimized acidification conditions further improved recovery of hydrophilic and singly phosphorylated peptides.

These findings highlight how SPE membrane workflows deliver efficient, reproducible, and high-throughput solutions for peptide desalting and PTM enrichment, ultimately supporting more robust and sensitive MS-based proteomic analyses in clinical and cancer research.

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Proteomic characterization of baculovirus expression vector system (BEVS)-derived extracellular vesicles engineered for Gla-Rich Protein (GRP) γ-carboxylation reveals selective GRP incorporation and distinct vesicle populations

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Gla-rich protein (GRP), a vitamin K–dependent protein with dual anti-inflammatory and anti-calcific properties, has emerged as a promising therapeutic target, although its biomedical application remains limited by challenges in producing its γ-carboxylated form (cGRP). To overcome these limitations, we developed a baculovirus expression vector system (BEVS) enabling coordinated GRP maturation and secretion in extracellular vesicles (EVs).

Here, we present a comprehensive proteomic characterization of EVs derived from BEVS using shotgun MS/MS analysis. EV subpopulations were isolated by differential ultracentrifugation (30K and 100K fractions) and analyzed against *Trichoplusia ni*, human, and baculoviral databases. A total of 3,033 proteins were identified, with 2,835 protein groups retained for downstream analysis. Principal component analysis revealed clear segregation between 30K and 100K EV proteomes, highlighting distinct molecular signatures associated with each population. Importantly, 52 canonical EV-associated proteins from Vesiclepedia were identified, including CD63, syntenin-1 (SDCBP), PDCD6IP (ALIX), TSG101, and flotillin-1, confirming the EV nature of BEVS-derived vesicles. Differential expression patterns of these markers further supported the molecular distinction between EV subpopulations. Proteomic analysis also demonstrated selective enrichment of recombinant proteins within EVs. GRP-mCherry was identified with high confidence (76% sequence coverage, 34 peptides), ranking among the most abundant proteins detected. Peptide intensity analysis revealed preferential loading of GRP into 30K EVs, consistent with orthogonal biochemical data. Notably, γ-carboxylation-dependent processing required co-expression of GGCX and VKOR, and these enzymes, together with Furin, were also detected within EVs, showing coordinated incorporation into vesicular cargo.

These findings establish BEVS-derived EVs as a robust platform for the production and delivery of post-translationally modified proteins, and demonstrate the power of proteomics to resolve EV heterogeneity and cargo specificity. The preferential enrichment of GRP and associated maturation machinery in defined EV subpopulations highlights their potential for targeted therapeutic applications.

Authentication of A2 bovine milk by routine LC-MS proteomic analysis

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Beta-casein (β-CN) is a 209-amino-acid milk protein with A1A2 as the most common genotype in European dairy cattle simultaneously producing A1 and A2 variants that are commonly found in traded milk. Such variants only differ in one amino acid (histidine in A1 and proline in A2) at position 67 of mature protein. Clinical evidence supports that healthy individuals not affected by lactose intolerance and/or milk protein allergy but still experience digestive discomfort with regular cow´s milk consumption, exhibit a greater tolerance to exclusive A2 β-CN milk. Consequently, a premium milk market significantly grew up worldwide based on the selection of A2A2-genotyped cows. Robust, sensitive and accessible analytical methods are then needed to ensure product authentication and consumer confidence [1].

In this study, A1 β-CN variant was determined through a straightforward and affordable bottom-up LC–MS proteomic workflow powered by linear ion-trap analysis and considering three independent enzymatic digestion strategies. A preliminary data-dependent MS/MS exploratory approach enabled characterization of A1 and A2 β-CN variants in A1A1 and A2A2 milks digested with trypsin, pepsin and thermolysin. Subsequent relative quantification of selected proteotypic peptide biomarkers was performed through a targeted Selected Reaction Monitoring (SRM) strategy [2], allowing the unambiguous detection of A1 β-CN variant at 0.1% in A1A1-A2A2 milk mixtures (**Figure 1**). Tryptic and thermolytic digestions achieved most robust results although the latter generated shorter peptides that improved chromatographic peak resolution and shape of detected signals. Promising results yielded by pepsin hydrolysis under acidic conditions can be of interest to create innovative insights into simplified and low-cost proteomic strategies addressing milk analysis.

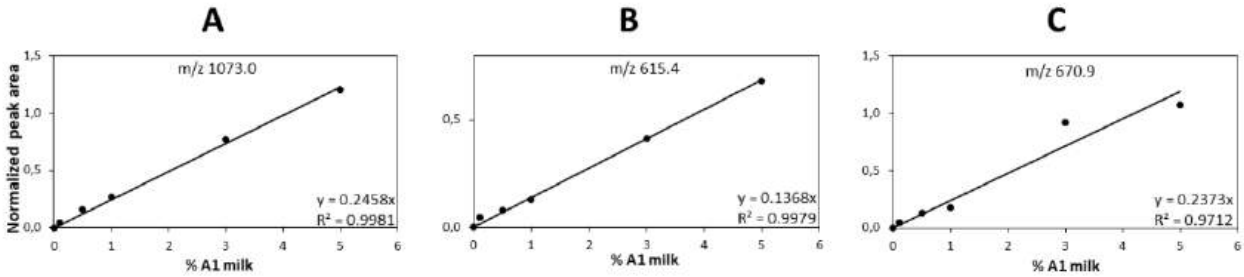

Figure 1: LC-MS SRM linearity achieved by A1 β-CN variant in A1A1-A2A2 milk mixtures digested with (A) trypsin, (B) thermolysin and (C) pepsin.

Sensitivity, efficiency and robustness shown by this affordable proteomic workflow can favor its implementation in food control laboratories for reliable A2 milk authentication, promoting wider applications supported by peptidomic research in routine food quality assessment.

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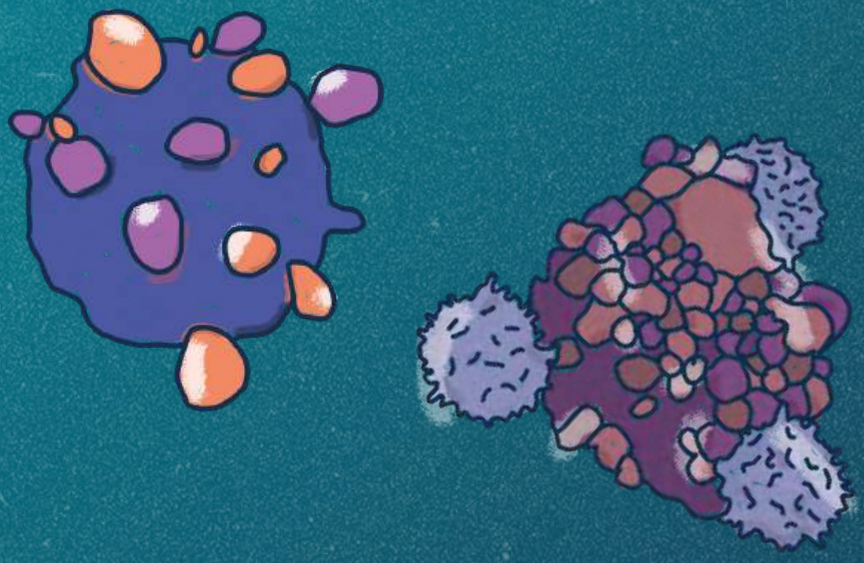
Section 6
Proteomics Exploration in Non-Model Systems

Flash Presentations



Phenotypic remodelling of smooth muscle cells in atherosclerosis: a low-input and single-cell proteomics approach

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Atherosclerosis is a chronic inflammatory disease driven by low-density lipoprotein (LDL) accumulation. Although therapeutic strategies such as statins effectively reduce LDL levels, they do not fully eliminate the residual risk of thrombosis. Post-translational modifications (PTMs) regulate key cellular pathways, and growing evidence suggests their involvement in atherosclerosis. However, their precise contribution to disease progression has not yet been systematically explored using unbiased, high-throughput approaches. Previous studies from our laboratory have linked PTMs to plaque activity and stability of early and advanced aortic lesions in human and experimental models. Several of these modifications can also be reliably detected in plasma.

Given that vascular smooth muscle cells (SMCs) are major producers of extracellular matrix (ECM) within atherosclerotic plaques and play a critical role in plaque stability, we performed a comprehensive proteomics analysis, including PTM profiling using an open-search strategy. This analysis was conducted in two experimental systems: (i) primary human SMC cultures supplemented with cholesterol, and (ii) primary mouse aortic SMCs isolated from rAAV8-mPCSK9D377Y-injected mice fed a high-fat diet. We also analysed decellularized ECM derived from these cultures. In addition, we performed bulk and single-cell proteomics (n = 2,000 cells) on aortic SMCs isolated from the same mouse model of atherosclerosis, examining distinct stages of disease progression and comparing healthy vascular regions with plaque-associated areas within the same aorta.

Overall, we identified multiple protein and PTM alterations during atherosclerosis development, including oxidative and methylation modifications affecting ECM and contractile proteins. A PTM-defined SMC subpopulation associated with disease progression was identified, and the levels of its defining PTMs were reduced by LDL-lowering treatment. Time-course plasma analysis from the same mice revealed that a subset of the identified PTMs was dynamically regulated systemically, enabling longitudinal monitoring during disease progression and therapeutic intervention.

Collectively, these findings indicate that PTMs constitute a previously underappreciated regulatory layer governing SMC phenotypic modulation during atherosclerosis progression and may represent promising therapeutic targets and candidate biomarkers to prevent plaque rupture.

Multi-omics characterization of SIRT3 metabolism and its adaptation to the presence of amyloid-beta oligomers in nasal epithelial cells

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Sirtuins (SIRTs) are nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylases that regulate cellular homeostasis in a multifactorial manner. Although alterations in SIRT signaling are evidenced in both olfactory dysfunction and Alzheimer's disease (AD), the specific role of sirtuin 3 (SIRT3) in olfactory metabolism remains unknown. Here, we have evidenced a partial interdependency between SIRT3 and SIRT5 deacetylase members in human nasal epithelial cell cultures (hNECs). A multi-omic integrative approach applied to conditions of SIRT3 silencing or overexpression revealed that hNEC metabolism is markedly more sensitive to reduced SIRT3 levels, identifying specific transcripts and phosphorylation sites belonging to inflammatory and redox mediators that are tightly regulated by SIRT3 in hNECs. Following exposure to oligomeric Aβ peptide, phosphoproteomic alterations promoted an activation trend of stress-induced senescence and apoptotic signaling in SIRT3-silenced hNECs, whereas induced activation of mitotic phase–related pathways, Hippo signaling, and glycogen metabolism were evidenced in SIRT3-overexpressing hNECs. From a translational point of view, a dissimilar sex-dependent profile in serum SIRT protein levels (SIRT1 and SIRT6) was observed across multiple neurological disorders including AD, mixed dementia, frontotemporal lobar degeneration and amyotrophic lateral sclerosis. These data shed new light on novel SIRT-dependent mechanisms associated with neurodegeneration, underscoring that the maintenance of optimal SIRT3 protein levels may partially counteract the detrimental effects induced by Aβ oligomers in AD at olfactory level.

Keywords: Sirtuins, SIRT3, Amyloid-beta, Alzheimer, multi-omics, sex

Extending Proteome Profiling to Red Blood Cells using an Aptamer Platform

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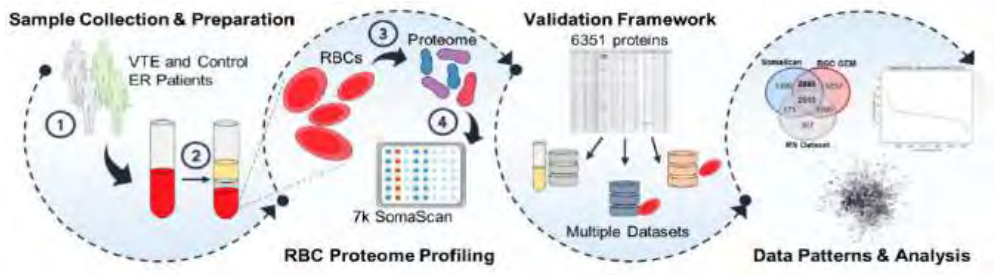
Red blood cells (RBCs) contribute to systemic physiology by transporting molecules and reflecting health and disease states, making them a promising source of biomarkers for disease detection and monitoring [1–3]. Affinity-based technologies such as SomaScan, originally developed for human plasma and serum, have been increasingly used for large-scale proteome profiling, enabling the simultaneous measurement of thousands of proteins and broad application in plasma proteomics [4]. However, their use in RBC lysates remains largely unexplored, and it is still uncertain whether the detected signals reflect RBC-derived or RBC-associated proteins, or instead result from matrix-related interference. Resolving this question is particularly relevant in venous thromboembolism (VTE), a major cardiovascular disease that lacks fast and reliable diagnostics, where multi-omics studies indicate that RBCs carry disease-associated molecular signatures [2]. Here, we analysed SomaScan 7K proteomic data from RBC samples of patients with VTE and matched controls, demonstrating that all proteins were detected and quantified. To assess the biological relevance of these signals, we developed a bioinformatic framework integrating public database mining and an extensive literature review, including evidence from mass spectrometry studies and a curated RBC reference dataset [5]. This analysis showed that 79% of the detected proteins are consistent with proteins previously identified in or associated with RBCs, including intracellular components and RBC-bound factors such as cytokines and chemokines. Comparison of protein abundance rankings in RBCs with patterns reported for plasma revealed distinct profiles, supporting the interpretation that the detected signals largely reflect RBC-related biology rather than plasma carryover. Notably, two proteins, NAGPA and RAGE, detected by SomaScan but not previously described in RBCs, were validated as present in RBCs by western blotting. Together, these results establish a structured framework for assessing affinity-based proteomics in RBC samples and highlight the potential for further optimization of aptamer-based platforms toward RBC-specific proteome profiling, particularly given that key proteins involved in RBC metabolism are not currently covered and that RBCs are increasingly recognized as a relevant source of disease biomarkers, including in VTE.



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BiasTracker: a bioinformatics tool for quantifying physicochemical and functional biases in mass spectrometry-based proteomics

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To date, none of the numerous proteomics approaches based on mass spectrometry, allow an exhaustive analysis of the proteome. Beyond low-abundance proteins, protein detection also depends on their physicochemical properties as well as on protein structure. According to the selected protocol, certain categories of proteins may be favored, while others are systematically underrepresented, leading to interpretation biases, particularly when it comes to downstream *in silico* functional analyses. There is therefore a clear need for a bioinformatics tool capable of identifying and characterizing such experimental biases, in order to prevent project leaders from wrongly rejecting a hypothesis or undertaking potential time-consuming and costly validation experiments driven by artifacts. To address this need, we have developed BiasTracker, an innovative tool which assesses several physicochemical properties (hydrophobicity, isoelectric point, mass, number of tryptic cleavage sites, etc.) of experimental proteomes in comparison with the whole theoretical (genome-inferred) proteome of related species. BiasTracker aims to become an open-source Python tool able to perform enrichment analysis (both over-representation analysis and GSEA) using standard functional annotations (such as Gene Ontology Cellular Compartment), custom databases (related to contaminants or post-transcriptional regulations), and physicochemical metrics. As a proof of concept, we have already presented two communications at congresses, related to the interest of BiasTracker in evaluating the ghost proteome, defined as proteins that are predicted but have never been experimentally identified. In addition to detecting biases, BiasTracker can be used by proteomics core facilities to evaluate the performance of different instruments or reagents, and by multi-omics project leaders to partly explain the lack of correlation between transcriptomics and proteomics experiments conducted on the same samples.

Comparative HLA-DR immunopeptidomics reveals disease- and genotype-associated signatures in rheumatoid arthritis

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease strongly associated with variation in Human Leukocyte Antigen (HLA)-DR molecules, particularly at the polymorphic *HLA-DRB1* locus. Expressed on antigen-presenting cells, these molecules may influence pathogenic immune responses by shaping the repertoire of peptides presented to CD4+ T cells. However, such HLA-DR peptidomes remain poorly characterized across *HLA-DRB1*-defined risk and protective backgrounds, as well as between autoimmune and non-autoimmune disease contexts.

Objective: In this study, we aimed to compare the naturally presented peripheral HLA-DR peptidomes of patients with RA and osteoarthritis (OA), used here as a non-autoimmune joint disease control, to identify immunopeptidomic patterns associated with disease status and *HLA-DRB1* genotype.

Methods: Peripheral blood mononuclear cells from 45 RA and 30 OA patients, all genotyped for *HLA-DRB1*, were lysed and subjected to HLA-DR immunoprecipitation. Eluted peptides were analyzed by high-resolution mass spectrometry, followed by bioinformatic, statistical, and functional network analyses.

Results: Across the cohort, more than 20,000 HLA-DR-associated peptides derived from nearly 3,000 source proteins were identified. Most ligands ranged from 9 to 26 amino acids and were organized in nested sets sharing common 9-mer cores, consistent with canonical HLA class II presentation. Comparative analyses revealed clear differences in the relative representation of peptides and parent proteins between RA and OA. RA samples displayed a broader and more heterogeneous ligandome, including peptides with higher predicted affinity for susceptibility-associated HLA-DR molecules, whereas OA samples were enriched in peptides predicted to bind HLA-DR molecules associated with protection. In addition, samples tended to group according to *HLA-DRB1* risk categories. Functional network analysis highlighted distinct biological modules, with RA-enriched proteins linked to innate immunity, complement/coagulation, and cell adhesion/trafficking, whereas OA-enriched proteins were associated with cellular homeostasis and lysosomal functions.

Conclusions: This study provides a large-scale proteomics resource linking *HLA-DRB1* genotype, peripheral antigen presentation, and autoimmune disease status. The resulting atlas may also help define candidate peptide and protein signatures with potential applications in biomarker development, CD4+ T-cell assays, and precision immunointervention strategies in RA.

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MALDI-MSI as a Platform for Spatial Multi-Omics in Glioblastoma Research

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Glioblastoma (GBM) is the most aggressive and heterogeneous primary brain tumour, characterized by a highly complex molecular architecture shaped by its dynamic tumour microenvironment [1]. The coexistence of diverse cellular populations—including tumour cells, immune infiltrates, vascular components and stromal elements—contributes to pronounced spatial and molecular heterogeneity within the tumour tissue. This heterogeneity limits conventional omics approaches, which often lack spatial resolution and histological context.

Recent updates to the 2021 WHO classification of Tumours of the Central Nervous System emphasize the importance of molecular features for accurate GBM diagnosis. In particular, alterations such as EGFR amplification, TERT promoter mutation, and the combined chromosomal gain of chromosome 7 with loss of chromosome 10 (+7/−10) are now considered key diagnostic criteria for glioblastoma, even in histologically lower-grade IDH-wild-type astrocytomas [2]. These advances highlight the growing need for integrated molecular analysis in brain tumour diagnostics.

Mass spectrometry imaging (MSI) has emerged as a powerful, label-free technology that enables spatially resolved detection of lipids, metabolites, and peptides directly from tissue sections, while preserving histological architecture [3]. In this context, spatial multi-omics strategies based on MALDI-MSI are increasingly being applied to investigate the molecular landscape of glioblastoma. These approaches enable the integration of complementary molecular layers within the same tissue specimen, facilitating the spatial correlation of lipid and peptide distributions with histopathological features and providing valuable insights into tumour organization and microenvironmental interactions.

In this work, we present a spatial multi-omics MALDI-MSI workflow in glioblastoma tissue that enables the integration of lipidomic and proteomic information within the same histological context, highlighting the potential of this approach for investigating tumour heterogeneity and spatial molecular organization.

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From Microbes to Ecosystems: Proteomic Insights into Agro-Environmental Interactions

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Most organisms that shape the functioning of agro-ecosystems are non-model species whose molecular biology remains largely unexplored. Yet these organisms drive key processes that determine plant health, pathogen emergence, ecosystem stability and environmental adaptation. Proteomics and metaproteomics offer a unique opportunity to access this hidden functional diversity by directly analysing proteins as real-time indicators of biological activity.

Our research uses mass-spectrometry-based proteomics to investigate the molecular mechanisms underlying interactions among plants, pathogens and ecosystems. Among plant pathogens, central focus has been the phytopathogenic fungus *Botrytis cinerea*, a major threat to global agriculture. Proteomic studies have revealed key elements of its infection strategy, including new virulence factors, metabolic reprogramming and regulatory networks that enable this highly adaptable pathogen to colonize plant tissues and respond to environmental cues. Beyond single-organism analyses, metaproteomics allows us to capture the functional complexity of multi-species systems. These approaches have been applied to explore the molecular dialogue between *B. cinerea* and grapevine during different infection scenarios, as well as to investigate broader ecological questions, such as the presence of antagonistic microbiota.

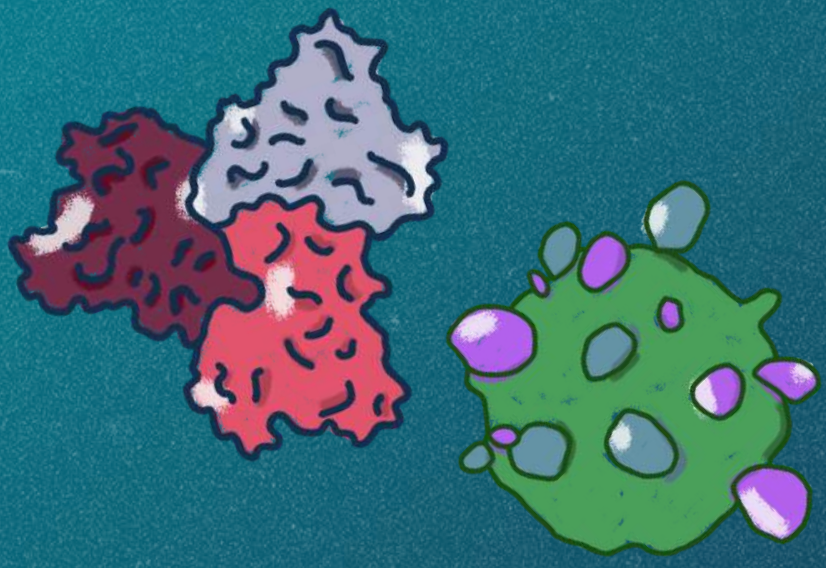
Moreover, to spray the potential applications of proteomics and metaproteomics in the field of marine and environmental science, we explore new applications. For example, we reconstructed the diet of migratory birds from the natural park “Bahía de Cadiz” through metaproteomics to support biodiversity monitoring. These data have potential applications in the management of natural resources within the park.

In marine environments, we analysed marine microalgae using applied proteomics to find new biotechnological tools with potential use in biomedicine and/or agrifood areas. From this approach, a new antitumor compound was patented. But to continue this strategy, proteomic studies of the sea anemone *Anemonia sulcata* and its symbiont *Symbiodinium* spp. are needed to identify molecular signatures associated with biotechnological applications, climate change, and environmental stress related to coral bleaching.

Together, these studies illustrate how proteomics can connect molecular biology, plant pathology, and ecology, providing new perspectives to understand agro-environmental systems and supporting the development of sustainable strategies for agriculture and ecosystem management.

Section 7
Quantitative and Computational Proteomics

Oral Communications



Global protein turnover dynamics in pluripotency

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The precise coordination between protein synthesis and degradation, collectively known as protein turnover, is essential for maintaining cellular proteostasis. Mouse embryonic stem cells (mESCs) exhibit rapid proliferation and tightly regulated translation of pluripotency factors, yet system-wide analyses of protein turnover in these cells remain limited. Here, we used a mass spectrometry–based strategy to systematically analyze proteome turnover in mESCs. Specifically, we combine pulsed dynamic stable isotope labeling by amino acids in cell culture (dynamic SILAC) with Tandem Mass Tag (TMT) labeling to accurately quantify synthesis and degradation rates for over 10,000 proteins. Our data reveal that core pluripotency transcription factors and chromatin remodelers, including histone acetyltransferases, display fast turnover rates. Furthermore, the implementation of this methodology during induced differentiation or naïve pluripotency reprogramming, enabled us to uncover dynamic changes in protein synthesis and degradation that accompany these transitions. Altogether, these approaches provide a comprehensive view of proteome dynamics in mESCs and offer new insight into the molecular mechanisms governing pluripotency and differentiation.

Defining the Topology of Proteins in sEV Isolates by Protein Correlation Profiling

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Extracellular vesicles (EVs) are key mediators of intercellular communication and promising biomarkers. However, their molecular characterization remains challenging due to the heterogeneity of EV subtypes and co-isolated non-vesicular components. To adress this, we developed a protein correlation profiling–based strategy using density gradients, enabling analysis of over 9,000 proteins from human cancer cell lines and biofluids and systematic reassessment of proteins associated with small EVs (sEVs) and non-vesicular entities[1]. However, separation of vesicular and non-vesicular fractions alone does not resolve protein topology and cannot establish whether proteins are truly encapsulated within sEVs.

To distinguish sEV cargo from externally associated proteins, we applied two complementary strategies. Alkaline carbonate-mediated membrane disruption followed by density gradient separation revealed that intraluminal soluble protein cargo is minimal. In parallel, proteinase K treatment of intact vesicles showed that most cytosolic proteins co-purifying with sEVs are protease-sensitive and therefore not luminal, but instead externally associated, likely reflecting co-isolated protein aggregates or cellular debris. Overall, our work demonstrates that protein topology in sEV preparations is dominated by external protein association, with minimal intraluminal soluble cargo, challenging prevailing models of luminal protein content in sEVs.

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Are your replicates independent? - Defining experimental units in primary neuron proteomics

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How many biological replicates do you truly have? In *in vitro* systems, this seemingly straightforward question is often ignored, yet it directly affects the validity of statistical analysis. In primary neuronal cultures, multiple embryos are often processed in a single isolation, raising an important question: can these samples be considered independent replicates, or do they form a single biological unit? Misclassifying the experimental unit leads to pseudoreplication, which artificially inflates the sample size, while accounting for the hierarchical structure of the experimental units can increase sample size and enhance statistical power.

This work presents a generalizable analytical framework for precisely defining experimental units in primary embryonic cortical neuron proteomics. Using cultures from four embryos per isolation across six separate isolations, two replicate classification approaches were compared: treating individual embryos as independent samples (n=24) or considering each isolation as the biological replicate through pooled cultures (n=6). Developmental stage (DIV8 vs DIV15) served as a placeholder condition to facilitate differential abundance analysis and assess how the choice of replicate classification affects downstream results. Samples were analyzed by liquid chromatography coupled with tandem mass spectrometry following single-pot, solid-phase-enhanced sample preparation (SP3).

An analytical pipeline was developed using hierarchical modeling to explicitly address the data's nested structure. Global proteome organization was initially examined using principal component analysis and clustering to assess the relative effects of isolation and developmental stage. At the protein level, linear mixed-effects models were employed to partition variance across isolation and embryo components. The intraclass correlation coefficient was calculated to assess similarity among embryos within the same isolation, directly informing whether embryo-level measurements are independent.

Finally, differential abundance analyses conducted for each replicate classification showed that inferred protein changes rely on assumptions of independence, including the overlap and divergence of identified proteins. This framework is widely applicable to proteomics and other high-dimensional assays and offers practical guidance for designing experiments that are both statistically rigorous and biologically meaningful.

Continuous telemetry-driven quality control for proactive LC–MS performance in proteomics core facilities

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Introduction

Proteomics core facilities must deliver reproducible LC–MS data across diverse instrumentation, users, and acquisition strategies. Current quality control (QC) frameworks primarily rely on periodic standard injections and post-acquisition analysis, a reactive approach that frequently fails to detect early-stage performance drift arising from chromatographic or ionization instabilities. This leads to data loss, reruns, and inefficient instrument utilization. To address these limitations, we developed a telemetry-driven QC framework integrating continuous hardware-level monitoring with rapid search-derived performance metrics to support proactive decision-making in multi-user core laboratories.

Methods

The framework integrates continuous hardware-level telemetry and environmental monitoring with conventional proteomics QC metrics. It was deployed on Orbitrap Exploris-class and Astral platforms coupled to nanoflow LC systems, supporting both DDA and DIA workflows. Raw data are processed using the Sage search engine to rapidly extract identification and performance metrics; the tool further extracts over 100 metrics from the device logs of the electronic components within the LC–MS system. Statistical process control methods define reference performance windows based on longitudinal data trends. End-to-end processing is completed in under five minutes per dataset. The platform supports flexible deployment via secure cloud-based or fully local on-premises environments, with AI-assisted analytics for longitudinal performance assessment.

Results

Continuous hardware-level monitoring detected performance drifts and transient nonconformities not captured by periodic standard injections. Telemetry identified column temperature instability, electrospray disruptions, and pressure fluctuations that preceded chromatographic degradation — in several instances, deviations were detected prior to measurable declines in peptide identifications or retention time stability, enabling preventive intervention before data quality was compromised. Integration of telemetry with conventional QC metrics increased sensitivity to early-stage performance changes. Correlating hardware telemetry with acquisition-level metrics reduced false-positive QC alerts by contextualizing transient operational variability. AI-assisted analytics enabled proactive notifications of sustained deviations and provided context-aware recommendations for corrective actions.

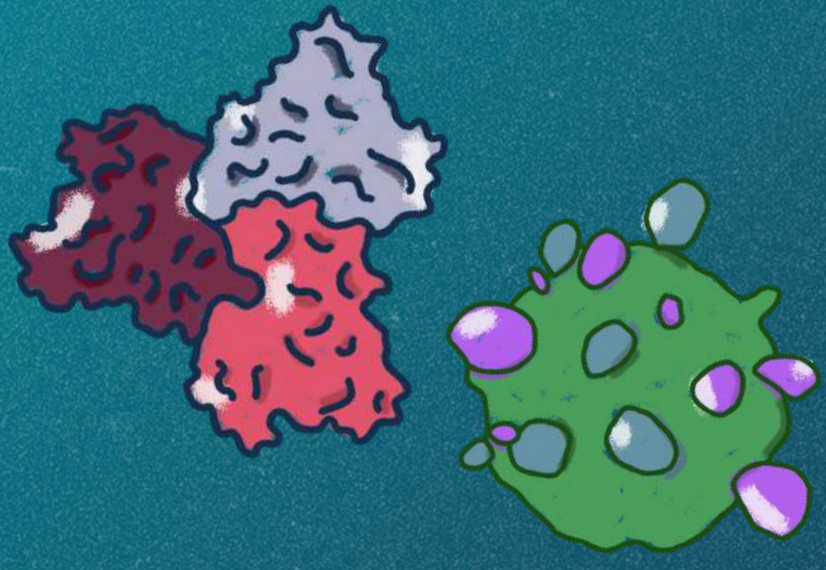
Novel Aspect

Continuous hardware telemetry integrated with rapid search-derived metrics enables proactive, context-aware LC–MS quality control in proteomics core facilities, reducing downtime and data loss through AI-assisted, longitudinal performance monitoring.

Section 8

Beyond Proteins: Small Molecules, Peptides & Integrated Omics + 5. Environmental and Ecosystem Omics-Insights

Oral Communications



High-Throughput PISA–TMT Proteomics Enables Target Identification of Novel Antimicrobial Small Molecules

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Antimicrobial resistance is increasing worldwide, reducing the effectiveness of existing therapies and posing a substantial threat to public health. Current treatments are increasingly failing, leading to higher morbidity, mortality, and healthcare costs. Meanwhile, the pace of traditional antibiotic discovery has slowed, emphasizing the need for new compounds with novel mechanisms of action. High-throughput phenotypic screening can identify bioactive small molecules, yet their molecular targets often remain unknown.

We recently identified two compounds, DC001 and DC002, that at low concentrations, completely inhibit the growth of pathogenic and non-pathogenic Gram-positive bacteria, including *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus subtilis*, highlighting their potential as antimicrobial candidates. To systematically elucidate their molecular targets, we applied the Proteome Integral Solubility Alteration (PISA) assay combined with Tandem Mass Tags (TMT) - based quantitative mass spectrometry. Proteins exhibiting increased solubility are consistent with potential direct targets, whereas decreased solubility may reflect off-target interactions or downstream proteins affected indirectly through perturbed cellular pathways. Then, TMT multiplexing enabled high-throughput quantitative comparisons across multiple conditions, capturing a global proteome alteration with precision.

Proteome-wide PISA analysis identified six direct candidate targets associated with essential cellular processes, including transport, metabolism, and regulatory pathways. To further refine these findings, molecular docking analyses were performed, enabling target prioritization based on predicted binding affinities and the presence of conserved binding pockets. This analysis narrowed the candidates to four proteins displaying well-defined binding pockets for both compounds, suggesting potentially convergent or related mechanisms of action at the structural level.

Overall, this integrated chemical proteomics pipeline, combining high-throughput PISA, quantitative TMT multiplexing, and computational docking, links small molecule perturbation to functional outcomes in the bacterial proteome. Beyond identifying potential targets for DC001 and DC002, this framework establishes a scalable strategy for proteome-wide target deconvolution in Gram-positive bacteria and supports the development of innovative antimicrobial therapies.

Molecular responses of *Staphylococcus epidermidis* to pH and endogenous antimicrobial fatty acids are strain-specific

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Staphylococcus epidermidis (SE), a human skin colonizer, is an opportunistic pathogen, mainly associated with medical devices infections. Phylogenetically, SE strains are clustered in lineages A/C, including strains isolated from infections and colonization of healthy individuals, and lineage B comprising mainly isolates from colonization of healthy people. It is necessary to understand SE pathogenicity mechanisms better in order to develop efficient strategies to prevent/treat challenging SE infections. The human skin, a key player in innate immune system, releases compounds with antimicrobial properties, such as fatty acids (FA). Using a representative strain from each lineage, 19N and ICE25, respectively for B and A/C lineages, we studied 1) SE metabolic adaptations to skin and blood pH by time course ^{1}H-NMR exometabolomics [1], 2) FAs as selective antimicrobials against A/C strain based on bacterial growth inhibition and cytotoxicity assays [2]; 3) the mechanism of action of lauric acid (LA) and SE adaptation by proteomics and 1H-NMR metabolomics [3].

The B strain was better adapted to use diverse carbon sources and at blood pH had a more active TCA cycle and amino acid catabolism. At blood pH, the B strain depleted formate from the extracellular media, while its extracellular accumulation by the A/C strain could work as a host invasion strategy. For both SE strains, TCA cycle regulation, purine biosynthesis and glutamate uptake could be associated with virulence, particularly biofilm production, especially relevant for the A/C strain which is able to produce high adherence biofilm [4]. This strain showed a higher susceptibility to FAs and LA was the most promising selective antimicrobial FA tested. No cytotoxicity for LA was found on a 3D reconstructed human epidermis model. Intracellular proteomics and metabolomics results propose that during prolonged LA exposure, 19N employs a coordinated adaptation strategy encompassing metabolic reprogramming, stress response management, and virulence factor modulation, ultimately prioritizing survival over cell proliferation. Time course ^{1}H NMR-based exometabolomics revealed pyruvate accumulation for 19N under LA stress. Under these conditions, our results suggested that rapid growth is supported by ATP generation, which aids adaptation to LA while sustaining biomass production. For ICE25, LA stress increased glucose uptake, suggesting an increased glycolytic flux, with carbon flow predominantly directed toward acetate production. Our data supported the use of ATP-dependent chaperones as a major player in the adaptation of *S. epidermidis* strains to LA.

Overall it was highlighted the potential use of LA as a topical formulation for the selective growth inhibition of SE pathogenic strains.

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Sewage protein information mining: A new frontier in community health and industrial surveillance

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Traditional wastewater-based epidemiology (WBE) has predominantly focused on small-molecule analysis, whereas the wastewater proteome remains largely unexplored as a source of high-resolution biological information. In this context, we introduce Sewage Protein Information Mining (SPIM), a proteomics-based framework that applies liquid chromatography–high-resolution tandem mass spectrometry (LC-HRMS/MS) to characterize the complex protein composition of wastewater. Analysis of both soluble and particulate fractions enabled the identification of more than 2,000 proteins, providing a non-invasive means to assess community health, pest activity, and industrial contributions.

A key advancement of this approach is the shift from pathogen detection to host-response monitoring. Human pancreatic α-amylase was identified as a robust biomarker for population normalization, offering improved accuracy over conventional census-based estimates. In addition, the detection of functional human immunoglobulins (IgA and IgG), along with immune-related proteins such as S100A8 and defensins, establishes an immunological dimension within WBE. These markers show potential as early indicators of disease outbreaks, with detection preceding clinical case reporting in certain instances.

Proteomic profiling further enabled discrimination between wastewater sources. Unlike conventional bulk parameters (e.g., BOD and COD), which lack source specificity, analysis of the albumin subproteome—distinguishing bovine, porcine, and poultry origins—facilitated the identification of industrial effluents from meat-processing activities. Moreover, the ratio of murine to human amylases emerged as a potential indicator of urban rodent populations.

Collectively, this proteomics-driven framework redefines wastewater as a multidimensional diagnostic matrix, expanding the scope of WBE for applications in environmental surveillance and public health monitoring.

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Seasonal dynamics of urinary protein profiles in the Iberian Lynx (*Lynx pardinus*)

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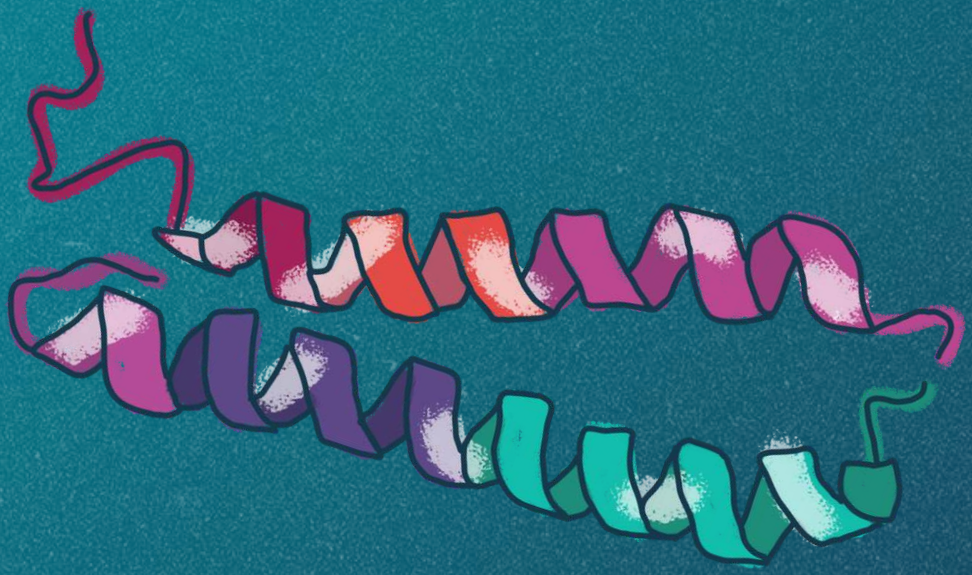
The Iberian lynx *(Lynx pardinus*) ranks among the most vulnerable feline species worldwide and plays a pivotal role in sustaining biodiversity within the Mediterranean ecosystem. Over the past two decades, multiple conservation strategies have been implemented to foster the recovery of its natural populations and habitat range. Within this framework, our project intends to reveal the molecular basis of intraspecific sexual communication in the Iberian lynx, with the aim of apply natural chemical signals in conservation strategies.

In this study, we present a set of candidate pheromones, identified through the molecular characterization of urinary scent marks. Samples were collected across the reproductive cycle from individuals maintained at the breeding center "El Acebuche." Comprehensive analyses of the urinary proteome profile revealed sex-specific components within the scent marks. Protein concentration in urine changes throughout the year and is usually more abundant during the reproductive period, suggesting a possible role in chemical communication. Cauxin, a carboxylesterase previously described in the domestic cat, was identified as the predominant urinary protein in both males and females. Greater protein investment was observed during the mating season. Similarly, glycosylated forms of cauxin increased in proportion during the same period, suggesting that the glycosylated form of cauxin increases during the mating season and that glycosylation could regulate the activity of this enzyme, making it more active. To further investigate the potential role of cauxin in sexual communication, esterase activity was monitored throughout the reproductive cycle as a proxy for cauxin function. Enzymatic activity exhibited a marked increase during the mating season, supporting a possible role for cauxin in reproductive signalling in the Iberian lynx.

Poster Communications



Analysis of the involvement of GLG1 and BAIAP2 in colorectal cancer by functional proteomics

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Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide and remains the second leading cause of cancer-related mortality, largely due to late detection and a high incidence of metastatic dissemination. Improving early diagnosis and a deeper understanding of the molecular mechanisms that drive tumor progression and metastasis are, therefore, essential to enhance patient survival. This underscores the importance of discovering novel biomarkers with diagnostic and prognostic value, and potential therapeutic applicability.

In this study we aimed to investigate the functional relevance of GLG1 and BAIAP2 in CRC progression, two proteins that have been previously reported to be overexpressed in CRC and associated with aggressive phenotypes. To this end, we employed isogenic CRC cell models with different metastatic capacities representing progressive states of tumor dissemination (KM12C, KM12SM and KM12L4a; and SW480 and SW620). Loss-of-function approaches were carried out to evaluate the biological role of these proteins across tumorigenic and metastatic properties, through the initial transient depletion of both proteins with siRNAs, and subsequently with shRNAs stable depletion to enable in-depth functional analysis.

Upon confirmation of efficient depletion of both proteins, *in vitro* functional cell-based assays were performed to assess their impact on key tumorigenic- and metastatic-related cellular properties, including cell proliferation, adhesion, invasion, migration (wound healing assays) and colony formation. Given the relevant role of epithelial-to-mesenchymal transition (EMT) in metastatic dissemination, changes in EMT markers were also examined at both transcript and protein levels to determine whether GLG1 and BAIAP2 depletion influenced this process. Additionally, *in vivo* murine models were employed to evaluate the effect of gene silencing in a physiological context, while assessing tumor growth, liver metastasis formation and survival. To further explore downstream molecular mechanisms underlying the observed phenotypic changes, a quantitative LC-MS/MS proteomic analysis was performed in GLG1-silenced (shGLG1) CRC cells. This enabled the identification of differentially expressed proteins and the characterization of affected molecular signaling pathways. Selected candidates emerging from this analysis were subsequently validated to identify those proteins strongly implicated in CRC progression and metastasis.

Overall, GLG1 and BAIAP2 depletion resulted in marked alterations in CRC metastatic-associated cellular phenotypes, highlighting their functional involvement in CRC progression and dissemination. These findings support their relevance as potential prognostic biomarkers of metastasis.

Proteomic differences between high- and low-grade medullary thyroid carcinomas.

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Medullary thyroid carcinoma (MTC) is a neuroendocrine tumor of the thyroid. Since 2021, a histological grading system stratifies MTC into high- and low-grade categories. Although its prognostic relevance has been established, the molecular characteristics underlying these subgroups remain poorly defined. Proteomic profiling was performed on formalin-fixed paraffin-embedded tissue from 32 primary MTCs (12 high grade). Peptides were analyzed by LC-MS/MS using an Orbitrap Astral mass spectrometer coupled to a Vanquish Neo UHPLC system. Data were processed with Spectronaut 19.1 (directDIA) using the UniProt human database, applying a false discovery rate (FDR) threshold of 0.01. Protein quantification was conducted without data imputation using the IDPicker algorithm. Validation was performed by transcriptomic analysis using the Tumor Signaling 360 Panel (760 genes) on the NanoString nCounter platform. Statistical analyses were carried out in R.

A total of 4,191 proteins were identified and quantified in more than 70% of samples. Of these, 332 were overexpressed and 227 underexpressed in high-grade MTCs. Principal component analysis separated tumors into two clusters according to histological grade. The most significantly enriched pathways in high-grade tumors involved DNA repair and MYC targets, with additional enrichment in pathways related to proliferation and protein translation. Transcriptomic analysis showed concordant results, with upregulation of MYC signaling, DNA damage repair, and cell-cycle–associated pathways.

Proteomic profiling identifies clear molecular differences between high- and low-grade MTCs. The overexpression of MYC-related pathways in high-grade tumors corroborated transcriptomic findings, suggesting this signaling axis as a potential source of molecular targets for future therapeutic strategies.

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Functional proteomics characterization of neurochondrin in colorectal cancer

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Colorectal cancer (CRC) is the third most common cancer worldwide with a 14.5% five-year survival rate if diagnosed in metastatic stage IV. Previous proteomic studies have identified the protein NCDN as overexpressed in liver metastatic CRC cells compared to poorly metastatic isogenic counterparts. While its cellular function and role in CRC remain unknown, patient survival data indicate that high NCDN mRNA and protein levels are associated with poor patients' prognosis. After validating these observations in an independent patient cohort, we investigated the role of NCDN in CRC progression using isogenic CRC cells comprising poorly metastatic KM12C or SW480 cells and metastatic KM12SM or SW620 cells. Here, we have performed stable silencing of NCDN expression in these cell lines, which resulted in a significant reduction in their tumorigenic and metastatic properties *in vitro*. In vivo assays using nude mice and KM12SM liver-metastatic cells demonstrated that NCDN depletion affected tumor initiation, growth, and liver metastasis. A clear correlation was observed between NCDN protein levels and CRC formation and metastasis, and thus protein extracts from silenced and control metastatic and non-metastatic CRC cells were obtained, trypsin digested and labeled for tandem mass tag (TMT) experiments to identify the network of dysregulated proteins associated with NCDN. After high pH reversed-phase peptide fractionation, the eluted peptides on 12 fractions were analyzed by LC-MS/MS using an Orbitrap Exploris 480 equipped with a FAIMS pro-interface. Peptide and protein identification and quantification were performed with MaxQuant, and the results analyzed using the R program to identify the dysregulated proteins associated with NCDN depletion. Proteomic profiling of NCDN-silenced cells identified dysregulated proteins associated with cell adhesion, invasion, cell death and differentiation, and mitochondrial function. Our findings position NCDN and their associated dysregulated proteins as drivers of metastatic progression in CRC and promising targets for further investigation.

Discovery of protein biomarkers for the diagnosis of Equine Metabolic Syndrome

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Equine Metabolic Syndrome (EMS) is a multifactorial endocrine disorder characterized by three features: obesity, insulin dysregulation (ID), and a predisposition to suffer laminitis. The most serious and painful clinical consequence of the disease is laminitis, which causes significant damage to the horse's hoof, even before clinical signs become noticeable, and it leads to irreversible anatomical changes in severe cases that may result in euthanasia of the affected animals.

Diagnosing EMS is challenging and often relies on clinical history including obesity, difficulty in losing weight, and recurring episodes of laminitis. The gold standard for diagnosing EMS is the assessment of ID, which can be evaluated through both basal and dynamic testing methods. However, current basal and dynamic tests are affected by factors like diet and stress and have limited accuracy. Additionally, dynamic tests are costly and present low sensitivity and poor repeatability. Therefore, more practical, specific, and cost-effective diagnostic methods are needed to allow early detection of EMS and prevention of laminitis. In this context, identifying biomarkers offers a valuable tool to achieve these objectives. Specifically, omics-based approaches such as proteomics provide powerful strategies to discover specific biomarkers.

In the current study, we used label free quantitative proteomics to detect variations in the plasma proteome of healthy horses, non-ID obese horses, and animals diagnosed with EMS, with the aims of identifying plasma biomarkers for EMS diagnosis and exploring molecular pathways related to the underlying pathology.

In our results, some proteins, including various members of the complement pathways and coagulation cascades, showed elevated abundance specifically in EMS animals, suggesting their potential as diagnostic biomarkers. Additionally, extracellular matrix (ECM) proteins as lumican were found to be significantly altered in EMS, suggesting their involvement in tissue remodelling and the development of laminitis. Moreover, elevated levels of certain proteins, such as fetuin A and fetuin B, were found in both EMS and non-ID obese horses, supporting that obesity-related metabolic disturbances play a significant role in EMS development and identifying these proteins as potential biomarkers for monitoring disease progression.

In conclusion, our findings enhance the current understanding of the molecular mechanisms underlying EMS and suggest several promising biomarkers for future research.

Molecular signatures of macrophage-to-foam cell transition induced by dyslipidemic and atherosclerotic serum

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Cardiovascular diseases (CVDs) are the leading cause of death worldwide, accounting for approximately 32% of all global deaths in 2019, according to the World Health Organization[1]. Atherosclerosis, the most common underlying cause of CVDs, is a chronic inflammatory disease affecting medium- and large-sized arteries, characterized by the accumulation of lipids and inflammatory cells within the arterial wall, leading to the formation of atherosclerotic plaques[2]. In advanced stages, lesion progression and plaque rupture may worsen patient outcomes, leading to ischemic stroke, myocardial infarction, or other thrombotic events[3]. Current therapies mainly address lifestyle and cardiovascular risk factors without directly targeting the atherosclerotic process, leaving a significant therapeutic gap.

Macrophages play a central role in early atherosclerosis, as they are responsible for processing accumulated lipids. Increased uptake of oxidized low-density lipoproteins (ox-LDL) drives macrophage transformation into cholesterol ester–enriched foam cells, which constitute the core of atherosclerotic lesions and contribute to local inflammation and thrombotic events4. Therefore, understanding the processes involving the formation and the role of foam cells is fundamental when it comes to finding possible therapeutic targets against atherosclerosis.

To assess the effects of serum factors from dyslipidemic and atherosclerotic patients compared to those from healthy controls, label-free quantitative (LFQ) proteomics, together with phenotypic and gene expression analyses of foam cell–associated markers, were performed in human monocytic cells (THP-1), a cell model used to study the function of these macrophages in vascular environments.

Foam cell formation was confirmed by Oil Red O staining of intracellular lipid droplets. Proteomic and transcriptomic analyses revealed differential expression patterns indicating impaired cellular function in key pathways involved in foam cell formation, including lipid metabolism, vesicular transport, and immune response.

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Functional characterization of the mitochondrial protein NDUFAF4 and implications in cholestasis

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Understanding protein function in their biological context is essential to elucidate their implication in cellular responses and pathologies. One of the challenges of the Human Proteome Project is to identify and functionally characterize understudied proteins.

We investigated the mitochondrial protein NDUFAF4, a mitochondrial Complex I factor, associated to chronic liver disease and tumorigenesis. Although structurally linked to mitochondrial function and energy metabolism, the mechanisms and pathological relevance of this protein are not fully understood. Proteomic analyses revealed significant up-regulation of NDUFAF4 in cholestasis, suggesting a role in disease progression.

To explore its function, NDUFAF4 was transiently silenced in PLC liver cells using siRNA and the effects were studied by proteomic analysis. Up to 333 differential proteins were identified that suggest mitochondrial stress, regulation of cell signaling, migration and proliferation, membrane homeostasis, subcellular organelle interaction, lipid metabolism, cell polarity and cell adhesion. These functional hypotheses were further validated by orthogonal methods including electron microscopy, immunofluorescence and Western blotting. Mitochondrial function and cell death were further analysed by flow cytometry.

Overall, our results define a molecular framework for the role of NDUFAF4 in cellular homeostasis and highlight its potential implication in chronic liver diseases, including cholestasis.

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

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Purpose:

Human plasma represents an exceptional yet analytically demanding matrix for proteomic biomarker discovery. Its extensive dynamic range and compositional heterogeneity limit the detection of low-abundance proteins, which often convey critical pathophysiological insights. To address these challenges, we present a workflow that integrates P2 plasma enrichment technology with iST sample preparation and advanced Spectronaut® 20 data processing. The combined platform leverages the selective enrichment power of P2 and the reproducibility of iST-based digestion and cleanup, enabling deep proteome coverage from human EDTA plasma samples.

Methods:

Human EDTA-plasma samples from a pilot colorectal cancer cohort (n=6 per group) were processed using the P2 single-particle enrichment workflow (Biognosys Group), employing proprietary nanoparticles that form transient protein coronas to selectively capture low-abundance proteins while excluding high-abundance proteins. Enriched fractions were subsequently digested using the optimized iST technology (Biognosys Group), ensuring maximal recovery and standardized peptide generation. Peptides were analyzed on a timsTOF HT (Bruker) in dia-PASEF® mode in a library-free DIA framework for deep, quantitative profiling.

Results:

Compared to conventional direct digestion preparations, the P2-iST workflow achieves significantly deeper coverage of the plasma proteome with substantially higher identification of protein groups and greater quantitative precision (CV <15%). Nearly 6,000 proteins were identified in 12 samples, demonstrating a high data completeness of more than 90% for the P2-iST technology. Pathway enrichment analyses show an emerging biological separation between disease and control groups. In addition, more cytokines, interleukins, and other biomarkers were identified, demonstrating the workflow's potential to uncover new insights into disease biology and biomarker discovery.

Conclusion:

The integration of P2 enrichment with streamlined iST technology and Spectronaut 20 data analysis defines a next-generation, high-performance platform for deep, precise, and reproducible plasma proteomics. This synergistic approach holds significant promise for advancing biomarker discovery and clinical translational research.

Secretomic profiling of triple-negative breast cancer media using Mag-Net™ HP

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Introduction

Secretomics provides a real-time view of tumor biology by profiling proteins that cancer cells release into their microenvironment and circulation. In breast and other cancers, secreted factors can serve as predictive and prognostic markers of disease state and treatment response. However, conventional mass-spectrometry workflows often miss low-abundance cytokines, growth factors, and signalling mediators masked by highly abundant plasma proteins. *Ex vivo* secretome analyses in controlled culture conditions allow reproducible characterization of secreted proteomic signatures, which can then guide targeted validation in larger clinical cohorts. Mag-Net™ enrichment, as demonstrated by Wu et al., offers cost-effective capture of extracellular vesicle–linked, low-abundance proteins from plasma and other biofluids, enhancing downstream MS sensitivity. Here, we present Mag-Net™ HP enabled secretome profiling to sensitively track dose responses to doxorubicin in an *in vitro* model of triple-negative breast cancer (TNBC).

Methods

BT-20 cells (1×10^5 cells/well) were cultured in 24-well plates and treated in triplicate with doxorubicin (0.1, 0.3, 0.7 or 1.5 µM), or DMSO. After 72 h, conditioned media were collected and incubated with Mag-Net™ HP beads for secretome enrichment. Vesicle capture, clean up and digestion was performed in a semi-automated manner on a KingFisher™ Flex using the Mag-Net™ HP kit. Peptides were loaded onto Evotips and analysed using an Evosep One coupled to a Bruker timsTOF HT system.

Results

Mag-Net™ HP yielded substantial improvements in secretomic proteome coverage compared with conventional methods i.e. ~4-fold improvement compared to protein aggregation capture. Approximately 300 proteins were significantly (FDR<0.05) changed proportional to dose—mostly decreasing, negative correlations and others increasing, positive correlations. Gene set enrichment analyses of proteins positively correlated with dose showed, consistent with doxorubicin's mechanism of action, induction of pathways related to DNA damage, p53-related and checkpoint networks (via ATM/ATR, RB1, and MECP2 pathways) potentially indicative of cell-cycle arrest and apoptosis initiation. Together, these results show that Mag-Net™ HP –enhanced secretome profiling enables coherent, reproducible, and biologically interpretable proteomic readouts of drug response that may warrant further investigation in clinical applications.

Conclusions

Mag-Net™ HP enrichment markedly improves detection of low-abundance secretome proteins in doxorubicin-treated TNBC cells in which doxorubicin drives a clear, dose-graded shift in the secretome. This streamlined approach enhances MS-based secretome workflows and supports discovery of clinically relevant biomarkers. Ongoing studies extend this pipeline to additional cell lines and patient-derived organoids.

QuickFit DualStream: A plug-and-play dual-column ion source for high-throughput proteomics

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Introduction

Despite advances in mass spectrometry acquisition speed, proteomics throughput remains limited by liquid chromatography (LC) inefficiencies, particularly idle time during sample loading, washing, and equilibration. Current ion sources also lack flexibility, thermal control, and ease of use, limiting adoption of multi-column workflows and applications such as top-down proteomics.

Methods

We developed the QuickFit DualStream nESI platform, a 3D-printed lightweight, plug-and-play ion source designed to interface with existing LC and MS systems. Dual-column operation is enabled through relay-based voltage switching that alternates electrospray between columns. A three-axis manipulator allows precise emitter alignment to the MS inlet. The system integrates heated column interfaces (ambient to 80 °C) and embedded nanoelectrospray emitters. Real-time telemetry (QuickFit Insights) monitors temperature, pressure, and spray stability for automated quality control.

Results

The Dual Stream ion source achieved near 100% MS duty cycle, increasing throughput by up to 50% versus single-column setups. Columns with integrated emitters provided up to 8-fold signal enhancement. Active heating improved robustness and is expected to benefit top-down proteomics by enhancing desolvation of intact proteins. The platform showed high reproducibility between columns, with equivalent ionization efficiency ($P > 0.05$) and strong correlation in protein identifications ($R^2 \approx 0.999$). Benchmarking yielded ~10% more protein groups and ~16% more unique peptides compared to standard configurations. The lightweight design and intuitive alignment reduced setup time, while automated switching ensured stable dual-stream operation.

Conclusions

QuickFit DualStream addresses LC–MS interface bottlenecks by combining parallelized operation, heating, automated switching, and telemetry in a user-friendly format. This enables higher throughput, improved robustness, and simplified implementation of advanced proteomics workflows.

Spatial single-cell proteomics on routine Papanicolaou-stained liquid-based cervical cytology

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Background
Liquid-based cervical cytology (LBC) is the standard sample type in many cervical cancer screening programmes and is increasingly for biomarker discovery. Cytopathologists routinely examine LBC slides under the microscope to detect cervical cellular abnormalities, but current approaches mainly rely on morphology and bulk molecular assays, which can mask cell-to-cell heterogeneity. Spatial single-cell proteomics on liquid-based cytology or cervicovaginal fluids combines information on the precise location of cells within the sample with unbiased proteomic profiling of individually selected cells, enabling analysis of the specific proteome of malignant or premalignant cells and overcoming limitations of bulk methods [1].

Methods
We established a spatial single-cell proteomics workflow on routine Papanicolaou-stained liquid-based cervical cytology slides that are normally reviewed by the cytopathologist. Individual cells were isolated by laser microdissection (LDM, PalmRobo, Zeiss), followed by protein extraction from each captured cell and identification by LC–MS/MS on a dedicated single-cell proteomics mass spectrometer (TimsTOF SCP, Bruker).

Results
The workflow yielded robust single-cell proteomes from fixed and stained samples, with approximately 600 proteins identified per cell and a consistent number of identifications across different cells. This analytical depth and reproducibility were achieved without deviating from standard LBC preparation, fixation or Papanicolaou staining.

Conclusions
This proof-of-concept demonstrates the feasibility of spatial single-cell proteomics directly on Papanicolaou-stained LBC cervical samples. By linking cellular morphology with specific protein expression profiles, the approach has a potential for retrospective biomarker discovery and validation on archived cytology specimens and for providing objective proteomic support to the cytopathologist in difficult or equivocal cases, thereby contributing to more precise diagnostic decision-making

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Single-Cell Proteomics with Spatial Attributes: Tools and Techniques
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Plasma proteomic biomarkers of Hutchinson–Gilford progeria syndrome: evidence from a mouse model

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Hutchinson-Gilford progeria syndrome (HGPS) is an ultrarare genetic disease (prevalence 1 person in 20 million) caused by a point mutation in the *LMNA* gene producing the expression of the mutant protein progerin. HGPS is characterize by accelerated aging and premature death, mostly due to complications of atherosclerosis including myocardial infarction, heart failure or stroke. For reasons that remain unknown and unpredictable, HGPS progression shows high inter-individual variability, and assessment of clinical manifestations is the only available tool to monitor disease progression and patient responses to treatment. Therefore, clinically meaningful biomarkers are needed to monitor HGPS progression from early disease stages and the assessment of therapeutic efficacy.

The main objective of this work is to identify and verify novel biomarkers that enable an early disease monitoring, guide preventive interventions, and assess treatment efficacy, with the final goal of improving quality of life and prolonging lifespan of HGPS patients.

A discovery phase was performed employing an analysis with liquid chromatography coupled to mass spectrometry. For that purpose, 24 mouse plasma samples were used: 12 $Lmna^{+/+}$ and 12 $Lmna^{G609G/G609G}$. After the functional analysis of the results, a verification phase in an independent cohort was carried out using orthogonal techniques: western blot and ELISA.

This analysis allowed us to identify 70 proteins of interest that showed differences in expression between $Lmna^{+/+}$ and $Lmna^{G609G/G609G}$ mice. After bioinformatics analysis, candidate proteins were selected for verification based on their role in cardiovascular diseases. Moreover, an additional validation in plasma from 10-week-old mice enabled longitudinal comparison at 10 and 14 weeks. The results confirmed the initial findings and further supported the potential of the candidate proteins as early biomarkers of disease progression.

Differential proteomic analysis has so far enabled the identification and verification of differentially expressed proteins among the different study groups. The identification of these proteins is of great relevance in the study of HGPS, as they could represent the first step in establishing potential biomarkers for monitoring disease progression after diagnosis or assessing therapeutic success in treated patients. These results represent a promising step toward the development of personalized medicine approaches for this devastating disease.

Exploring the molecular link between aortic stenosis and chronic kidney disease through DIA-PASEF-based plasma proteomics

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Aortic stenosis (AS) and chronic kidney disease (CKD) are progressive disorders associated with high morbidity and mortality. CKD accelerates AS progression and, when both conditions coexist, significantly worsens clinical outcomes. Therefore, establishing an early molecular profile that characterizes CKD patients could serve as a starting point to predict subclinical onset and monitor the progression of both AS and CKD.

We performed a differential plasma proteomics study using DIA-PASEF technology, which exploits trapped ion mobility separation (TIMS) to enhance sensitivity in proteomic experiments. Five plasma samples from two study groups have been analyzed: i) patients with AS without CDK and ii) patients with AS and CDK. Data were processed using Spectronaut® (Biognosys) and identified proteins have been analyzed using Metascape, a web-based tool for functional annotation which enables comprehensive analysis and interpretation of proteomic studies. After the bioinformatic analysis, candidate proteins were selected for verification by immunodetection in an independent cohort.

Differential proteomics allowed us to identify a total of 321 proteins in crude plasma, of which16 showed differences in expression between patients with AS and patients with AS and CKD. After the functional analysis, candidates related to inflammatory response were verified, supporting their potential as biomarkers of CKD in AS patients.

These findings reveal the existence of different proteomic profiles in patients with AS, with and without CKD, positioning inflammation as a key contributing process of this comorbidity. Our results lay the groundwork for personalized medicine approaches that consider not only the diagnosis of AS, but also the concurrent presence of CKD, paving the way for more effective patient management.

Unique high-throughput workflow for deeper plasma/serum proteome coverage enables discovery of potential biomarkers

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Blood plasma/serum is a minimally invasive specimen for clinical research and patient monitoring, but its wide dynamic range, heterogeneity, and complexity challenge LC-MS proteomics and limit deep profiling in large cohorts. Here, we present ENRICH-iST, a high-throughput workflow that enriches low-abundance proteins and improves plasma/serum proteome coverage for clinical research studies.

Starting from 20 µL plasma/serum, ENRICH expends the detection range by capturing low-abundance proteins on paramagnetic beads. On-bead denaturation, reduction/alkylation, digestion, and peptide clean-up were then performed using the iST-BCT protocol for biofluids. Peptides were analyzed by nanoLC-timsTOF in diaPASEF® mode and processed with DIA-NN or Spectronaut®.

Parallel preparation of 96 samples was completed within 5 h. Compared with neat samples processed by iST-BCT alone, ENRICH-iST increased protein identifications by at least 2-fold in plasma and 1.5-fold in serum, with median CVs below 10%. Human Protein Atlas mapping showed broader coverage than neat plasma and classical depletion workflows, spanning from albumin (40 g/L) to proteins below 10 ng/L. In a clinical plasma cohort of lung cancer patients (n=10) and age-matched healthy donors (n=10), ENRICH-iST identified more than 1450 protein groups versus 650 in neat plasma. The ENRICH-iST dataset clearly separated patients from controls and revealed 16 additional significantly regulated proteins as potential biomarkers, including the upregulated cancer-associated factors S100A8 and S100A9 [1].

Overall, ENRICH-iST combines throughput and depth to enhance biomarker discovery in plasma/serum proteomics.

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Integrated serum proteomics and autoantibody profiling reveal a protein signature predictive of flare in rheumatoid arthritis during biologic tapering

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Advances in clinical proteomics enable high-throughput characterization of the circulating proteome, providing an opportunity to identify molecular signatures associated with disease outcomes. In rheumatoid arthritis (RA), although biologic DMARDs (bDMARDs) achieve sustained remission in many patients, the lack of proteome-based biomarkers limits the implementation of safe tapering strategies. We aimed to identify circulating proteomic signatures associated with disease flare in RA patients undergoing bDMARD tapering and explore their biological relevance. A discovery-driven proteomic approach using mass spectrometry (MS) was applied to baseline serum samples from a nested case-control subset (n=44) of the OPTIBIO trial. Label-free quantitative proteomics enabled identification of differentially expressed proteins between patients who flared and those maintaining remission. Candidate proteins were validated in the full cohort (n=194) using ELISA. In parallel, 15 anti-cytokine autoantibodies were quantified using multiplex immunoassays. Functional enrichment analyses mapped proteins to biological pathways, and statistical modeling, including logistic regression, Cox models and ROC analysis, evaluated predictive performance. MS profiling identified 806 proteins, of which 87 were differentially abundant between flare and non-flare groups within the tapering arm, revealing a distinct proteomic signature associated with loss of remission. V-set immunoglobulin domain-containing protein 4 (VSIG4) emerged as a robust biomarker, with higher baseline levels associated with flare risk, while elevated anti-interferon gamma (anti-IFNγ) autoantibodies were also associated with flare. Integration of these markers with DAS28-CRP improved discrimination (AUC=0.876), outperforming clinical models alone. These findings demonstrate that serum proteomics can identify immune-regulatory biomarker signatures associated with flare risk in RA and support the integration of discovery proteomics with targeted validation to inform bDMARD tapering strategies.

CSF proteomic profiling for biomarker identification in patients with MCI

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Alzheimer's is a neurodegenerative disorder that primarily affects older adults. It is the most common form of dementia, characterized by a progressive decline in cognitive and behavioral functions. Mild cognitive impairment (MCI) is frequently considered a prodromal stage of AD, bridging normal aging and early dementia. Studying this phase of the disease allows the identification of early pathological changes and risk factors associated with disease progression. Understanding the mechanisms underlying AD is essential for earlier and more accurate diagnosis, as well as for developing effective therapeutic strategies. Recognizing that AD is not biologically homogeneous and that different pathological pathways may be active in different patients, previous studies have identified biological subtypes in AD using cerebrospinal fluid (CSF) proteomics. A proteomic analysis was performed between two groups of MCI patients, individuals without biomarkers of amyloid-β deposition or neuronal injury (MCI-Aβ−) and individuals meeting criteria for a high likelihood of AD (MCI-Aβ+). Samples from two independent cohorts were analyzed and compared: 60 CSF samples from MCI-Aβ− patients and 68 MCI-Aβ+ patients.

Proteins were precipitated with methanol and analyzed using the Short-GeLC-SWATH workflow. Peptide separation was performed on a NanoLC™ 425 System with a 50-min gradient, coupled to a Triple TOF™ 6600 mass spectrometer equipped with an ESI DuoSpray™ Source. Pooled samples were used to build peptide ion libraries using data-dependent acquisition (DDA), samples were analyzed by SWATH/DIA to obtain relative protein quantification.

The results revealed significant differences in the molecular profiles among patients. Initial univariate analysis identifies 75 differentially expressed proteins (FC > 1.2 and p-value < 0.05), of which 54 showed decreased levels and 21 increased levels in the MCI-Aβ+ group. Multivariate analysis by PLS-DA not only confirmed the robustness of these findings but also demonstrated a clear separation between study groups. The most relevant proteins were selected based on the VIP score above 1.5, resulting in a panel of 45 proteins. Gene ontology was performed on the proteins of interest, processes related to cell adhesion, response to external stimuli, and the nervous system. Further validation is required to determine the robustness, specificity, and reproducibility of the proteins identified as relevant biomarkers.

Proteomic pathway alterations in mouse hippocampus and prefrontal cortex following chronic citalopram treatment

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Selective serotonin reuptake inhibitors (SSRIs) are widely used as pharmacological treatment for depression. These compounds block the serotonin transporter, preventing the reuptake of serotonin (5-HT) into presynaptic neurons and increasing its availability in the synaptic cleft [1].

Citalopram, a commonly prescribed SSRI, has been shown to induce metabolic adaptations across multiple brain regions [2]. The hippocampus (HIP) and prefrontal cortex (PFC) are particularly relevant in this context due to their roles in learning, memory, emotion regulation, and executive function, together with reports of structural and functional alterations in these regions in depression and their high density of serotonergic receptors and transporters [3, 4].

Increasing evidence indicates that chronic citalopram treatment can influence metabolites involved in oxidative stress and cellular energy metabolism [5]. However, proteomic alterations underlying these effects remain poorly characterized across specific brain regions in the current literature.

Here, we investigated proteomic alterations induced by chronic citalopram administration in a murine model. Label-free quantitative proteomics was employed to identify differentially expressed proteins and to explore biological pathways affected in HIP and PFC tissues.

Proteomic variability was more pronounced in the HIP than in the PFC. Multivariate analysis highlighted proteins primarily associated with oxidative phosphorylation in both regions, some of which also showed significant alterations in univariate analysis. In addition, several ribosomal proteins were significantly altered in the HIP. These findings reveal region-dependent molecular responses to chronic citalopram treatment and suggest that its effects extend beyond direct modulation of serotonergic signaling.

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Organ-specific proteomic response to semaglutide treatment in healthy mice

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Semaglutide is a glucagon-like peptide-1 receptor agonist widely used for managing type 2 diabetes and obesity. While its therapeutic benefits are well-established, its systemic impact on the proteome of non-target organs in healthy subjects remains poorly characterized. This study aims to evaluate the multi-organ proteomic response and biosafety profile following administration of semaglutide in a healthy mouse model.

Healthy mice were treated with 100nM intranasal semaglutide during 7 days and proteomic analysis was performed on heart, liver, kidney, spleen, pancreas, and skeletal muscle using mass spectrometry. To this end, organs were lysated and then prepared for a label free quantitative (LFQ) approach by protein aggregation capture (PAC) digestion with trypsin and lys-C. Analysis was made by TIMS-TOF Pro instrument (Bruker Daltonics) operated in data independent acquisition (DIA) mode. Raw files were processed with Spectronaut and statistical analysis was performed with Perseus. Functional enrichment analysis was conducted using Enrichr against Reactome, WikiPathways, and KEGG databases.

Principal component analysis (PCA) demonstrated that semaglutide treatment did not induce a global shift in the protein landscape. Furthermore, volcano plots revealed that there are no major differences between the study groups. The absence of massive up- or down regulation in response suggests that semaglutide treatment does not compromise cellular integrity in healthy non-target organs. However, despite this stability, specific differentially expressed proteins (DEPs) were identified across organs. In the liver, significant modulation of glucose metabolism. Changes were focused on metabolic and redox homeostasis in the kidney. The heart shown alteration related to mitochondrial homeostasis and cytoskeleton. In pancreas and spleen, changes were mostly associated with mitochondrial functions.

In conclusion, our results demonstrate that semaglutide induces tissue-specific proteomic alterations, mainly targeting energy metabolism and mitochondrial function. However, these changes do not disrupt the global proteomic profile in healthy mice. These findings provide a comprehensive proteomic map of semaglutide's systemic safety, suggesting that its metabolic benefits are achieved through subtle physiological fine-tuning rather than disruptive proteomic alterations in healthy tissues.

ML-Driven Clinical-Proteomics identifies a 6-Protein signature for precise atherosclerosis stratification

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Atherosclerosis, a major cause of adverse cardiovascular events and mortality rates worldwide, stems from sustained lipid accumulation and subsequent chronic inflammation within the arterial walls. An early identification of patients at risk is crucial to prevent life-threatening thrombotic events and provide effective and personalized treatments. Leveraging the power of machine learning (ML) to enhance diagnostics and biomarker discovery, a high-throughput approach using five ML classification algorithms (MLCA) was applied, integrating clinical and serum proteomic data from patients with carotid atherosclerotic stenosis (AT, n:60), dyslipidemic patients (DLP, n:55), and healthy controls (HC, n:66).

As result, a robust 6-protein panel (B2M, GPV, MMP9, PLF4, TSP1, and FB isoforms) was identified, achieving an AUC > 0.90 across all five algorithms. This panel significantly discriminated AT patients from DLP and HC groups. Results were validated in an independent external cohort of acute atherothrombotic stroke patients. Notably, the integrated clinical-proteomic models outperformed single-modality analyses.

Overall, the identified biomarkers underscore the roles of platelet activation, angiogenesis, and intraplaque hemorrhage in disease progression. This ML-driven signature offers a potent tool for personalized risk stratification and highlights the necessity of multi-pathway therapeutic strategies.

Reference:

Extremera-García MJ, et al. Molecular Biomedicine, 2026. Machine learning integrated clinical-proteomics data identifies a 6-protein panel signature for atherosclerotic severity and enhanced patient stratification

Proteomic landscape of the PBMCs from diabetic patients with and without diabetic complications: preliminary results

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Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder affecting more than 400 million people worldwide and a major contributor to global morbidity and mortality. T2DM is strongly linked to the development of diabetic vascular complications (DVCs), including macrovascular diseases such as critical limb threatening ischemia (CLTI), and microvascular disorders like diabetic retinopathy [1]. A key pathological feature connecting T2DM with cardiovascular diseases is the presence of a low-grade chronic inflammatory state, which is often established before the clinical onset of diabetes and persists even under adequate glycemic control. Peripheral blood mononuclear cells (PBMCs) represent a readily accessible population of immune cells that serve as sensitive indicators of systemic immune and metabolic disturbances. In T2DM, PBMCs reflect the inflammatory environment characteristic of the disease and exhibit phenotypic and functional alterations, including expansion of proinflammatory monocytes, constitutive inflammasome activation, and disruption of metabolic signaling pathways. These changes not only mirror systemic inflammation but also contribute directly to the development of atherosclerosis and vascular complications [2]. In this work, a label-free quantitative approach using DIA was applied to evaluate the differential protein expression in PBMCs from T2DM patients with and without vascular complications. Moreover, phosphoproteomic analysis with TMT-labeling was also carried out, comparing PBMCs from T2DM patients without complications versus healthy controls. By integrating these approaches, we aim to elucidate the molecular mechanisms underlying T2DM associated inflammation and vascular risk, and to explore the utility of PBMCs as prognostic and monitoring biomarkers for diabetic complications.

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Analysis of Plasma Depleted Samples for Chronic Diseases' Biomarker Discovery with the Orbitrap Astral Mass Spectrometer

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Non-infectious chronic diseases are responsible for 74% of total deaths worldwide and the most common cause of disability. However, most chronic diseases are underdiagnosed, and thus the identification of biomarkers in minimally invasive biological fluids with diagnostic ability is mandatory to improve their diagnosis, treatment, and progression.

In this context, proteomics analyses of plasma play a crucial role for the identification of biomarkers that could be implemented in clinical routine. However, mass spectrometry analyses yet face challenges due to the wide dynamic range in protein concentration of these samples. Thus, depletion of high-abundant plasma proteins is essential to reduce sample complexity and enhance the detection of low-abundant proteins, with a high interest as biomarkers.

Here we aimed at identifying plasma biomarkers for colorectal cancer and Alzheimer' disease, two of the most common chronic diseases worldwide. Plasma samples were depleted using different strategies to enrich them in low-abundant proteins that might be useful as biomarkers of these diseases. Subsequently, depleted plasma and 1 µL of neat plasma samples were trypsin digested and analysed by DIA in the Orbitrap Astral, with 15-minutes gradient, and in the Orbitrap Eclipse Tribrid in a 2-hours gradient. Nearly 4000 plasma proteins were identified in the depleted plasma samples. Additionally, the efficacy of plasma enrichment methods was assessed using PRM in the Orbitrap Astral, with 8.5-minutes gradient to detect previously described CRC and AD plasma biomarkers.

The implementation of plasma protein depletion strategies is crucial for enhancing the sensitivity and depth of mass spectrometry analyses, enabling the identification of a broad range of biomarkers for various diseases.

Phenotypic characterization of breast cancer cells using stochastic proteomic profiling

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Tumor heterogeneity is a major challenge in cancer research, as cellular variability strongly influences tumor progression under therapeutic pressure. While organoid models provide a meaningful system to study tumor heterogeneity in physiologically relevant three-dimensional (3D) cellular assemblies, they are often difficult to obtain and maintain. In contrast, spheroids represent a more accessible and stable 3D model. In this work, we developed a low-input proteomics workflow to investigate both inter- and intra-tumoral heterogeneity in spheroid models that ought to be transposed to organoids studies.

Two complementary strategies were implemented. First, a pipet-tip tandem–based sample clean-up method compatible with 3D structures was developed, starting from approximately 40 cells each, in order to investigate inter-spheroid heterogeneity through proteomic analyses. Second, a micro-bulk strategy was designed to assess intra-tumoral heterogeneity by dissociating spheroids and randomly grouping 20 cells prior to proteomic analysis. This approach enables the detection of subtle proteome variability among small cellular populations.

Because such analyses require ultra-sensitive workflows capable of generating comprehensive and reproducible proteome coverage from minimal sample amounts, several methodological optimizations were performed. These included improvements in sample preparation and clean-up, chromatographic performance, Mass Spectrometry (MS) acquisition strategies, and data analysis algorithms. The CellenONE system was implemented for precise cell isolation and digestion, minimizing sample loss during handling. We evaluated the Diagonal-PASEF acquisition strategy and compared chromatographic configurations to improve acquisition efficiency, sensitivity, and robustness while maintaining high proteome depth. Together, these optimizations enabled the identification of nearly 4,500 proteins from 20 cells clusters, providing a robust framework for studying heterogeneity in complex tumor samples.

Keywords:

Low-input proteomics; Tumor heterogeneity; Breast cancer; Stochastic proteomics; Diagonal-PASEF.

Novel Insights into Red Blood Cell Dysregulation in Obstructive Sleep Apnea: a multi-omic approach

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Obstructive sleep apnea (OSA) is characterized by recurrent apnea/hypopnea during sleep, causing intermittent hypoxia, sleep fragmentation, and increased cardiometabolic risk. Red blood cells (RBCs) are exposed to hypoxia–reoxygenation cycles and may reflect systemic oxidative/metabolic stress[1]. We previously showed that severe OSA RBCs display alterations in glycolysis, pentose phosphate pathway (PPP), glutathione-related proteins, and proteasome pathways, consistent with disrupted redox homeostasis and partial recovery after PAP[2].

Here, we revisited the RBC proteomic dataset and integrated these findings with preliminary LC-MS/MS metabolomics from the same samples. Shotgun proteomics was performed on a Q-Exactive-Orbitrap platform and analyzed with MaxQuant/Perseus; metabolomics was performed on a TIMS-QTOF platform and analyzed using R software and MetaboAnalyst. Linear/mixed models assessed severity and PAP treatment effects. Pathway analysis and integration was accomplished using Cytoscape and MetaboAnalyst.

Proteomic reanalysis revealed severity-dependent alterations in vesicular trafficking, endosome recycling, membrane regulation, and intracellular transport. In severe OSA, increased Rab11B/GEF-related components and reduced GDI2/14-3-3-associated regulation suggest impaired trafficking/stability of membrane proteins, potentially affecting energy metabolism, ion balance, metabolite exchange, cell deformability and flexibility.

Severe OSA RBCs showed upregulation of HINT1, NUDT5, and PNP, with PGM2 downregulation and a tendency toward ATIC upregulation, suggesting remodeling of purine nucleoside turnover and salvage. Reduced PGM2 may limit conversion of nucleoside-derived ribose-1-P into ribose-5-P, constraining integration into PPP and purine salvage.

Preliminary metabolomics supported RBC metabolic disturbance. At FDR significance, severe OSA RBCs showed decreased hyodeoxycholic acid and urea, and increased D-alanine, suggesting altered metabolite exchange, osmotic handling, and systemic/microbiota-derived signals. Nominal changes in α-KG/2-OG, DHA, UA, L-carnitine, hypotaurine, and L-proline suggest altered redox buffering, glutathione metabolism, organic acid handling, and membrane protection.

Overall, these data suggest that severe OSA is also associated with remodeling of RBC membrane trafficking, metabolite exchange, nucleotide metabolism, and antioxidant adaptation. Further validation may identify RBC-derived biomarkers of OSA severity and metabolic recovery after PAP treatment.

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Pathophysiological subtypes of mild cognitive impairment due to Alzheimer's disease identified by CSF proteomics

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Alzheimer's disease (AD) is the leading cause of dementia worldwide, characterized by brain deposits of aggregated amyloid beta (Aβ) and neurofibrillary tangles of hyperphosphorylated tau, leading to synaptic dysfunction and neuronal loss. Cerebrospinal fluid (CSF) proteomics has revealed widespread dysregulation of biological processes in AD – including innate immunity, inflammation, hemostasis, lipid metabolism, and synaptic function – some of which may already be present at the mild cognitive impairment (MCI) stage. Previous studies identified distinct AD subtypes based on CSF proteomics in established AD cohorts; however, the heterogeneity of proteomic profiles specifically at the MCI stage, and its biological significance, remained poorly characterized.

In the present work, we aimed to identify pathophysiological subtypes of MCI due to AD (MCI_{AD}) using CSF proteomics. We recruited 45 MCI_{AD} patients with biomarkers indicative of high likelihood of AD and 23 control MCI patients without Aβ or neuronal injury biomarkers (MCI_{Other}) from the Cognitive Complaints Cohort (CCC), Lisbon, Portugal. Findings were validated in an independent cohort of 92 MCI_{AD} and 102 MCI_{Other} patients from the European Medical Information Framework for Alzheimer's Disease (EMIF-AD) cohort. CSF proteomics, combined with partial least squares discriminant analysis (PLS-DA) and gene ontology (GO) analysis, was used to characterize biological processes altered in MCI_{AD} versus MCI_{Other}. Subtype identification was then performed using non-negative matrix factorization (nNMF) clustering on the discriminant protein set.

PLS-DA accurately discriminated MCI_{AD} from MCI_{Other} patients in both cohorts, with decreased proteins in MCI_{AD} related to coagulation, lipid metabolism, immune response, and stress response, and increased proteins related to energy and neurodevelopmental processes. The top-ranked discriminant proteins in the CCC cohort – NRP2, APOA1, AHSG/FETUA, ORM1/A1AG1, and NBL1 – were all decreased in MCI_{AD} and linked to the platelet degranulation pathway. In the EMIF-AD cohort, top discriminant proteins (GDIA, ALDOA, MDHC, ALDOC, GUAD) were increased and associated with glucose/pyruvate metabolism, consistent with the reported early upregulation of cerebral glucose metabolism in AD.

nNMF clustering of MCI_{AD} patients identified two robust and reproducible subtypes across both cohorts (Cohen's kappa = 0.398, $P < 0.0001$): Cluster 1, characterized by elevated proteins associated with inflammatory and immune processes, complement activation, haemostasis, and coagulation, indicative of **blood–brain barrier dysfunction and immune dysregulation**; and Cluster 2, characterized by elevated proteins linked to axonogenesis, neurogenesis, synapse organization, and response to oxidative stress, suggestive of a **hyperplasticity** profile. Patients from Cluster 2 displayed significantly higher CSF levels of pTau, total Tau, and Aβ42 compared to Cluster 1. A nine-protein signature – comprising AHSG/FETUA, HEMO, ANT3, A2AP, AFAM, and AMBP (Cluster 1) and PEBP1, MDHC, and NCAN (Cluster 2) – effectively discriminated the two subtypes across both cohorts.

These findings demonstrate that CSF proteomics can resolve biologically meaningful subtypes of AD already at the MCI stage, with implications for patient stratification and the design of subtype-specific therapeutic strategies.

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Changes at salivary proteomic level elicited by exposure to food odorants

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Smelling foods leads to a cascade of psychophysiological adaptations that includes the rapid increase in saliva secretion. This anticipatory response occurs even before tasting and serves to prepare the body for eventual ingestion, facilitating the digestion. Despite this increase in saliva secretion being well described in the literature, it is still lacking information on whether saliva biochemical composition can also change.

A previous study from our lab showed that exposure to the visualization of food cues, presented as pictures, elicits changes at salivary proteome level, some of which are significantly associated with individuals' affective responses to those food images [1]. To further explore if similar changes occur when food is smelling, a within-subject design study was conducted with a total of 40 healthy adult participants, in which each collected saliva samples immediately before (control) and during a 3-minute exposure of each of three different odorants: orange, vanilla and pine. Emotional responses to each odorant were also assessed using implicit (heart rate variability and electrodermal activity) and explicit reports.

Changes at salivary proteomic levels of all samples were evaluated by gel-based (SDS-PAGE) approach and a pooled subsample selected based on individuals' reactivity to odorants (more reactive vs less reactive) was subsequently analyzed using a gel-free proteomics approach (LC-MS/MS).

Most representative alterations in salivary protein relative levels, in response to exposure food-related and non-food-related odorants, will be presented and related with the different reactivity of the participants to the food odorants. Findings of this study will be discussed, highlighting the importance of exploring differences in the salivary proteome for a better understanding of physiological adaptations to food in the context of ingestive behavior.

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High-sensitivity N-glycopeptide identification in human plasma using electron-activated dissociation

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Protein N-glycosylation is a prevalent and biologically important post-translational modification involved in protein folding, stability and cell–cell communication. Comprehensive characterization of N-glycopeptides in complex matrices such as human plasma remains challenging due to their low abundance, extensive structural heterogeneity and the labile nature of glycan moieties during tandem mass spectrometry.

In this work, we evaluated a glycoproteomics workflow based on nanoflow liquid chromatography coupled to electron-activated dissociation (EAD) MS/MS for high-sensitivity identification of N-linked glycopeptides in human plasma. After depletion of high-abundance plasma proteins, samples were digested, enriched for glycopeptides and analysed using data-dependent acquisition with EAD fragmentation. This approach preserves glycan structures while generating extensive peptide backbone fragmentation.

Glycopeptide identification was performed using a hybrid database search and de novo sequencing strategy implemented in PEAKS GlycanFinder, followed by quantitative analysis in SCIEX OS. Across replicate analyses, a total of 1,174 unique N-glycopeptides were confidently identified. EAD MS/MS spectra enabled unambiguous glycosylation site localisation and confident glycan composition assignment, providing substantially improved peptide backbone coverage compared to conventional collision-induced dissociation.

Overall, these results demonstrate that EAD-based fragmentation combined with advanced computational workflows enables robust and sensitive profiling of the human plasma N-glycoproteome, supporting detailed post-translational modification analysis in complex biological samples.

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

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Phosphorylation is one of the most prevalent and important post-translational modifications proteins can undergo. Over 50% of the human proteome is phosphorylated and understanding the dynamic phosphorylation across the proteome can understand the progression of many diseases including cancers. As the stoichiometry of phosphorylation sites is generally very low, enrichment steps, followed by SPE clean-up before LC-MS/MS analysis are required to enhance identification and quantification of each site. However, recovery of phosphopeptides can be greatly affected by the choice of clean-up method, resulting in severe losses.

Different SPE C18 options were compared for the purification of phosphopeptides, after automated enrichment using magnetic beads (Ti/Zr-IMAC). Effects of sample acidification prior to SPE clean-up on phosphopeptides detection were also assessed by acidifying the enrichment elution with different percentages (2, 3, 4 and 5%) of phosphoric acid or trifluoroacetic acid (TFA).

Among all SPE options tested, AttractSPE®Disks Tips C18 provided the highest recovery of phosphopeptides (up to 2.4 times more identifications), with high reproducibility (RSD < 10%). AttractSPE®Disks Tips C18 captured more efficiently hydrophilic peptides, and shorter phosphopeptides were retained compared to other brands.

Quenching the enrichment elution with 3% phosphoric acid provided the highest recovery, with 8% more identifications compared to 5% TFA. Lower acid concentration interestingly provided more singly phosphorylated peptides, while higher acid concentration recovered more hydrophilic peptides. This trend was observed for both acids tested but was more pronounced for TFA.

AttractSPE®Disks Tips C18 are shown to be the best choice for phosphopeptide purification, offering simplicity of use by centrifugation, high sample recovery, and robustness. These SPE Tips are easily scalable with their availability in different sizes and binding capacities to perfectly adapt to different sample amounts, and can be provided as 96 and 384 SPE well plates for high throughput processing.

Small-molecule post-translational modification in *Chlamydomonas reinhardtii*

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Chlamydomonas reinhardtii is an eukaryotic, single-cell algae. Available resources, including large mutant collections, cDNA libraries and its sequenced genome (nuclear, mitochondrial and chloroplastic) bring wide biotechnological applications to a model organism traditionally used in cell and molecular biology for modeling photosynthesis, flagella structure, and cell motility.

Omic studies in this model organism are on their infancy and our project focuses on adapting already-proven strategies onto *C.reinhardtii* in order to study its ubiquitome. The Ubiquitin-Proteasome System regulates protein degradation and turnover, plus ubiquitination has been shown to be involved in protein transport and location in the cell. Studying this process during biotechnological applications of commercial interest could allow for the improvement of their yield.

We have focused our research on indirect biophotolysis, a source of clean hydrogen gas. Green microalgae, such as *Chlamydomonas reinhardtii*, catalyse the production of H_2 with the help of enzymes collectively known as hydrogenases [1] [2]. In *C. reinhardtii* there are two hydrogenases, HydA1 and HydA2, with a hundred-fold higher activity than other hydrogenases [3]. These enzymes become highly expressed under specific conditions, acting as a "safety valve" by consuming electrons and protons in order to protect the photosynthetic machinery from over-reduction during the onset of photosynthesis [4].

By combining two approaches (shotgun proteomics on whole cell extracts and on isolated ubiquitinated material) we strive to study hydrogen production in *Clamydomonas reinhardtii* in order to optimize the process and maximize its yield. We have already observed a large shift on the Ubiquitin-Proteasome System induced by H_2-producing conditions on several strains of *C. reinhardtii*.

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Comparison of Strategies for Global Glycoprotein Profiling by LC-MS

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Liquid chromatography coupled online to mass spectrometry (LC-MS) is a valuable technique for protein glycosylation analysis. However, purified proteins are the most common case of study, and large-scale approaches that could provide valuable information on complex biological systems, such as the glycosylation profile of cells, remain out of the scope.

With the aim of implementing new analytical methods that may help elucidate the global glycosylation profile of complex protein samples in our lab, we have tested different glycopeptide enrichment tools (Glycaclean from Affinisep, SOLA-SAX from Thermo) using peptide extracts coming from human serum and HEK293 cells.

The glycopeptides were acquired in a timsTOF HT mass spectrometer (Bruker). Different acquisition methods, as well as analytical tools (software solutions, scan merging, glycan databases etc) were used, with the aim of comparing the results obtained with different strategies and stablishing the most suitable pipeline for complex glycosylation analyses in our platform.

SEQUENTIAL ANALYSIS OF DIFFERENTIAL PROTEIN ABUNDANCE, GLYCOSYLATION AND PHOPSHORYLATION IN WNT7A OVEREXPRESSING MDA231 CELLS

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Protein abundance alone does not fully determine protein function, as post-translational modifications (PTMs) are major regulators of protein activity, localization, and other functional properties. Therefore, the integration of complementary approaches such as glyco- and phosphoproteomics, alongside differential protein abundance analyses, should provide a more comprehensive view of the molecular events underlying the biological system under study.

In this work, we characterized the proteomic features of MDA231 cells upon Wnt7a overexpression, a development-related protein, at three different levels: total proteomics, glycoproteomics, and phosphoproteomics. These analyses were performed sequentially, using a single protein digest per biological replicate to generate the three corresponding sub-samples. Protein digests were sequentially enriched for glyco- and phosphopeptides, and the resulting eluates, together with the initial total peptide input, were analysed by LC–MS/MS.

The bioinformatic integration (Gene Ontology and Ingenuity Pathway Analysis) of the information obtained across different analytical levels enabled the identification of well-known signalling pathways, while also revealing complementary and noteworthy findings that contribute to a better understanding of the effects of Wnt7a overexpression in these cells.

Exploring glycosylation alterations during atherosclerosis progression

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Cardiovascular disease remains the leading cause of mortality worldwide, with most events arising from atherosclerosis, a chronic inflammatory disorder of the arterial wall. Because the disease progresses silently during a prolonged subclinical phase, early detection and timely intervention remain major clinical challenges. We have previously identified protein biomarkers associated with subclinical atherosclerosis using human cohorts [1,2]; however, the molecular mechanisms linking these biomarkers to disease initiation and progression remain poorly understood.

Protein N-glycosylation is a complex post-translational modification involved in processes central to atherogenesis, including endothelial activation, inflammatory signaling, and lipid metabolism. Here, we investigate N-glycosylation patterns in plasma from individuals with subclinical atherosclerosis and from patients with advanced carotid atherosclerosis. To relate plasma findings to the vascular site of injury, the study also included aortic tissue (intima and media layers) containing early atheroma lesions, such as fatty streaks and fibrolipidic plaques, together with their corresponding secretomes. Glycopeptide identification was performed using dedicated glycoproteomics tools (FragPipe's Glyco module [3] and pGlyco [4]), while quantitative analysis of the glycopetidoforms was carried out using our in-house developed platform iSanXoT [5].

By integrating glycoproteomic profiling across these complementary sample types, this study aims to characterize altered N-glycosylation patterns during atherosclerosis progression and to explore potential links between previously identified protein biomarkers and glycan-related molecular processes.

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Preconditioning therapy prevents site-specific oxidation of Trp/Cys redox sensors in contractile and metabolic cardiac proteins

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Ischemic preconditioning, achieved through brief episodes of vascular occlusion, triggers adaptive responses that increase the heart's resistance to subsequent insults such as ischemia–reperfusion (I/R) and anthracycline-based chemotherapy. At the molecular level, preconditioning activates pro-survival signaling pathways and modulates mitochondrial function, redox balance, and calcium handling. A key component of this protection is the controlled generation of reactive oxygen species (ROS), which act as signaling molecules to induce antioxidant defenses and preserve mitochondrial integrity. In both I/R injury and anthracycline-induced cardiotoxicity (AIC), oxidative modifications of specific target proteins are thought to play an important regulatory role by altering protein function and cellular signaling. Identifying these oxidatively modified target proteins and determining how site-specific oxidation modulates cardioprotective pathways is therefore essential for elucidating the molecular basis of preconditioning-induced cardioprotection. In this work, an unbiased analysis of posttranslational modifications has mapped the oxidative modifications of five key cardiac proteins involved in muscle contraction (ATP2A2 and MYBPC3) and energy metabolism (GOT2, GAPDH, and SLC26A3) caused by I/R injury and AIC. We identified specific oxidized Trp and Cys residues located within functionally relevant protein domains that may compromise protein activity and conformational stability. Notably, these oxidative hotspots were protected when preconditioning was applied prior to I/R or anthracycline exposure. Results reveal redox sensor sites in cardiac proteins that signal myocardial injury whose preservation by preconditioning contributes to cardioprotection. The possibility of using these hotspots as therapeutic targets needs further investigation.

VPS4A validation as a Parkin substrate

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Parkinson's disease (PD) is a common, chronic, and incurable neurodegenerative disorder of the central nervous system characterized by the progressive loss of dopaminergic neurons in the substantia nigra and the formation of Lewy bodies (LBs), leading to motor and non-motor symptoms. While most PD cases are sporadic and manifest after age 50, mutations in *PARK2* cause early-onset PD [1,2]. *PARK2* encodes Parkin, an E3 ubiquitin ligase that post-translationally modifies proteins by covalently attaching ubiquitin to lysine residues. Identifying Parkin substrates and elucidating the functional consequences of their ubiquitination is essential to understand early-onset PD pathogenesis [3]. A previous proteomic study in *Drosophila melanogaster* neurons identified 35 candidate Parkin substrates. Among them, vacuolar protein sorting-associated protein 4A (VPS4A), a regulator of intracellular protein trafficking, emerged as a putative candidate [4,5]. Notably, VPS4A has also been detected in the core of LBs, suggesting a potential role in their formation. Here, we investigated whether VPS4A is a Parkin substrate in human HEK293T cells using biochemical and mass spectrometry-based approaches. GFP pull-down followed by immunoblot analysis revealed increased ubiquitination of VPS4A upon overexpression of wild-type (WT) Parkin compared to a catalytically inactive variant, supporting VPS4A as a Parkin substrate. Quantification of VPS4A::GFP levels showed an inverse correlation between ubiquitination and total protein abundance, indicating that Parkin-mediated ubiquitination promotes VPS4A degradation. Mass spectrometry analysis confirmed enhanced VPS4A ubiquitination in cells expressing WT Parkin relative to the inactive variant. Site-specific analysis identified lysine residues K210 and K325 as preferentially ubiquitinated in the presence of WT Parkin. Additionally, increased formation of K6-, K48-, and K63-linked ubiquitin chains was detected under these conditions. Altogether, our results validate VPS4A as a novel Parkin substrate and provide proteomic insight into ubiquitin chain architecture associated with Parkin activity.

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Metaproteomics in an Archaeological Environment: A Pipeline for Deciphering Ancient Remains

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The study of ancient proteomes derived from paleontological, archaeological, and historical materials enables the reconstruction of the past with respect to evolutionary dynamics, phylogenetic relationships, and livelihood practices. In this regard, advances in proteomic methodologies over recent decades have proven instrumental, owing to their high sensitivity and comprehensive analytical capabilities. In particular, the emergence of high-sensitivity mass spectrometry [1] and metaproteomic approaches has expanded the scope of ancient protein analysis, allowing the simultaneous characterization of complex protein mixtures derived from hosts, microbiota, diet, and environmental sources.

Nevertheless, the study of ancient proteomes presents significant methodological challenges. Careful sample preparation is critical, as archaeological materials typically contain low amounts of highly degraded proteins embedded within complex matrices. These factors increase the risk of contamination and analytical bias, requiring optimized extraction, purification, and enrichment strategies. Furthermore, robust and reproducible computational pipelines are essential to ensure accurate peptide identification, reliable taxonomic assignment, and meaningful functional interpretation of ancient proteomic datasets.

In the present work, we describe a comprehensive workflow based on well-established methodologies. On the one hand, we implement optimized protocols for protein recovery, sample preparation and clean-up. These procedures are designed to maximize protein recovery from limited and highly degraded samples while minimizing contamination and interference from complex archaeological matrices. On the other hand, data analysis is performed using widely adopted open-source tools commonly employed in proteomics laboratories. This approach enables robust peptide identification, taxonomic classification, and functional annotation while ensuring transparency, reproducibility, and adaptability of the workflow for different types of ancient samples.

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Seasonal protein variations of wild boar meat

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The increasing use of wild boar (*Sus scrofa*) meat for human consumption has gained significant attention in recent years due to ecological, economic, and nutritional considerations. Wild boar populations have markedly expanded as a result of factors such as landscape changes, reduced predator pressure, supplementary feeding, and climatic shifts that favor survival and reproduction [1]. This demographic growth has intensified human–wildlife conflicts, including agricultural damage, disease transmission risks, and road accidents [2]. One of the most effective methods for mitigating these impacts is responsible harvesting for human consumption, aligning wildlife management with the production of a valuable food resource. Wild boar meat is increasingly recognized for its nutritional qualities, often characterized by lower fat content, favorable fatty acid profiles, and high levels of essential micronutrients compared with conventional livestock meats [3]. However, there are currently few studies focused on characterizing the protein profile of this meat and even less in deciphering potential seasonal variations of wild boar meat proteome due to shifts in diet and climate conditions.

In this work, we sampled meat from ten animals, five of them captured in summer and the other five captured in winter. After that, muscle biopsies (*longissimus dorsi*) were collected in Falcon tubes within two hours postmortem. Half a gram of each biopsy was used for the extraction of sarcoplasmic proteins, followed by a desalting step by SDS-PAGE, trypsin digestion and further analysis of the whole proteome digest by using a Thermo Vanquish HPLC coupled to a Exploris 120 mass spectrometer. Differential protein analysis revealed that muscle soluble proteome of animals captured in summer showed a higher expression of enzymes associated to glycolytic pathways, branched-chain amino acid catabolism, TCA cycle and prenyl transferase activity. These results are in accordance to previous reports stating that, during summer, increased locomotor activity associated with foraging and reduced thermogenic demands may increase the relative expression of enzymes implicated in energy-producing pathways and catabolism, as well as an increase in the expression of fast glycolytic (Type IIb) fiber-associated proteins, reflecting a shift toward rapid, short-burst muscle performance [4]. We can conclude that integrating proteomic insights with ecological data can improve predictions of seasonal nutritional variability in game meat, supporting both consumer quality expectations and sustainable utilization of wild boar resources.

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Tracking 10 Stages of a Fruit Fly's Life Cycle with High-Throughput Proteomics

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The biological metamorphosis of Drosophila melanogaster represents a paradigmatic example of temporally coordinated gene regulation. However a high-resolution proteomic map of this transition remains essential to decode the functional execution of its developmental program. In this study, we performed a comprehensive longitudinal proteomic survey encompassing ten distinct developmental stages, from early embryogenesis through larval and pupal transitions to the mature adult fly.

To ensure biological representativeness and minimize individual variance, each stage was analyzed using pools of ten individuals. Sample preparation followed a streamlined "One-Pot" (on-pot) digestion protocol, specifically designed to maximize peptide recovery and enhance reproducibility in the first four smaller embryonic stages. In larval and adult stages, a conventional precellys followed by FAST procedure has been done. The resulting peptides were analyzed using an Evosep One system coupled on-line to a timsTOF HT mass spectrometer, utilizing four technical replicates per stage to guarantee analytical robustness. By leveraging PASEF (*Parallel Accumulation Serial Fragmentation*) technology, we achieved deep proteome coverage, enabling the precise quantification of thousands of proteins. Our results reveal distinct temporal patterns in protein abundance across development. The metamorphosis of Drosophila melanogaster entails a tightly coordinated, stage-specific reprogramming of the proteome that cannot be fully inferred from transcriptomic data alone. The hereby presented proteomics study provides unique insights into the functional execution of developmental pathways, revealing dynamic protein-level changes that underpin this complex biological transition.

Secretome analysis to understand the intracellular traffic networks in fungi

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Protein secretion in filamentous fungi depends on the organization and regulation of intracellular trafficking networks. To understand how changes in traffic pathways affect secretory diversity, especially in filamentous fungi used as microbial cell factories, we performed a quantitative proteomic analysis of a selected collection of *Aspergillus nidulans* null mutants affecting different levels of intracellular transport. *A. nidulans* is an established eukaryotic model organism due to its well characterized genetics, experimental tractability, and the high conservation of many cellular pathways with higher eukaryotes.

Secreted proteins from this set of *A. nidulans* strains growing in liquid fermentation medium were studied. Each protein extract, obtained via TCA precipitation, was digested with trypsin/Lys-C and the resulting peptides were analyzed by LC-MS/MS using data-independent acquisition (DIA) on an Orbitrap Astral.

A total of 2,751 proteins were identified in the *A. nidulans* secretome. Depending on the specific comparison, 300 to 1,300 proteins were differentially found in secretomes of null mutants compared to wild type. The most pronounce changes were observed in the early endosome null mutant, consistent with their central role in cargo sorting, whereas the exocyst mutant showed comparatively limited deviations. The overall results indicate the impact of different traffic disruptions on secretion dynamics and highlight several proteins of biotechnological interest that were differentially secreted, indicating potential targets for the rational design of fungal strains.

In addition to their biotechnological implications, these findings take advantage of the use of *A. nidulans* as a genetically manipulable and evolutionarily conserved model organism, offering insights that extend beyond fungal biology and contribute to a broader understanding of secretion mechanisms in eukaryotic systems.

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Plasma Proteome Equalization Uncovers Dysregulated Proteostasis and Amyloidogenic Pathways in Multiple Myeloma

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Multiple myeloma (MM) is a slowly growing and complex haematological malignancy characterized by abnormal proliferation of plasma cells in the bone marrow, leading to bone destruction, monoclonal (i.e. increased immunoglobulin synthesis), and potential complications such as renal failure and anaemia. Despite treatment advancements, MM remains associated with significant morbidity and mortality, with approximately 160 000 new cases and 106 000 deaths annually worldwide. In the field of analytical proteomics, significant advances have been made in understanding, detecting, and monitoring MM. Despite these advancements, proteomics applied to serum samples faces challenges, notably the dynamic range of protein concentrations, where abundant proteins overshadow disease-specific ones. To better understand myeloma diseases, we employed the dithiothreitol (DTT)-based protein equalisation technique and analytical proteomics by comparing the proteomes of pellets and supernatants formed upon application of DTT on serum samples.

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Towards the Development of a Liquid Chromatography Free Workflow for Measurement of Clinically Important Proteins

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Clinical adoption of mass spectrometry for measuring proteins remains stagnant as these instruments often require an expert operator or support team to maintain their performance. The need for liquid chromatography (LC) in MS workflows proves to be the main pain point to be addressed. In this study, we evaluate the robustness and transferability of an LC-free SISCAPA-trapped ion mobility spectrometry time of flight (timsTOF) method for various clinically important protein biomarkers.

Samples were digested, spiked with stable isotope-labeled peptide standards and incubated with anti- peptide antibodies conjugated to magnetic beads using the automated SISCAPA workflow. Captured peptides were eluted and analyzed using a timsTOF HT (Bruker), either through an Evosep One LC system using the 100 SPD method, or in an LC-free setup using a VIP-HESI for direct infusion. Data were acquired in prm-PASEF mode, and a single quad isolation window was used to co-fragment both heavy and light peptides.

For the LC-based method, excellent linearity was observed across a dynamic range from 111 fmol to 17 amol, with coefficients of variation (CVs) consistently below 10%. Similarly, the LC-free workflow achieved remarkable linearity from 111 fmol down to approximately 4 fmol, also with CVs below 10%. While the
LC-based workflow outperformed in the lowest concentration range, the LC-free method still demonstrated strong reproducibility and robustness at clinically significant concentration levels.

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Universal pipeline unlocking inter taxonomic differential abundance analyses: Brain matrisome characterization across mouse, ferret and human.

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Extracellular matrix (ECMs) proteins play pivotal roles in the neuronal development and function of the cerebral cortex (CC). In this context, evolutionary studies are interested in understanding the expansion of cortical parenchyma and the increased cellular complexity that the human CC has evolutionary achieved. To assess the role of ECM on these biological processes, we adapted a mass spectrometry-based profiling method to exert interspecies comparisons of CC ECM-enriched proteome among mammals with gyrencephalic and lissencephalic brains, namely Homo sapiens (human) vs Mus musculus (mouse), respectively. Despite the greater evolutionary distance from human compared to mouse, Mustela putorius furo (ferret) represents a valuable intermediate animal model due to its greater CC expansion and complexity compared to mice, thereby bridging the gap between rodents and humans in the study of brain development. However, few studies have characterized the ferret proteome and even fewer have provided a characterization of their ECM composition (a.k.a. matrisome). In addition, performing reliable interspecies differential abundance studies is complex and requires a think-out-the box approach.

Here, we analyzed the proteome of CC ECMs of mouse, ferret and human and we propose a simple and broadly applicable proteomics data analysis pipeline that enables reliable interspecies differential abundance comparisons. In short, for each detected precursor, we annotated all proteins where it could be found according to the three species-specific FASTA databases; and next, we kept for protein quantification only those precursors that are present in the three databases. This strategy ensures that each species' homolog protein is quantified with a set of peptides that could be potentially detected in all three species.

We tested our approach within the specific context of the matrisome, aiming to analyze the change of ECM categories proportions across taxonomies to gain deeper insights into the role of evolutionary matrisome dynamics in the CC expansion across mammals.

Proteomic Signatures Rescued by Two Candidate Molecules in a Zebrafish Model of CDKL5 Deficiency Disorder

Márcio Simão[1,2,*], Lorenzo Chiodini[2], Gil Martins[2], Álvaro Tavares[1], Vincent Laizé[2], Paulo Gavaia[1,2], Natércia Conceição[1,2,3] and M. Leonor Cancela[1,2,3]

[1] Faculdade de Medicina e Ciências Biomédicas (FMCB), Universidade do Algarve, Faro, Portugal
[2] Centro de Ciências do Mar do Algarve (CCMAR/CIMAR LA), Universidade do Algarve, Faro, Portugal
Algarve Biomedical Center (ABC), Universidade do Algarve, Faro, Portugal

[*] Email address: masimao@ualg.pt

Cyclin-Dependent Kinase-Like 5 Deficiency Disorder (CDD) is a severe neurodevelopmental encephalopathy characterized by early-onset epilepsy, hypotonia, visual and speech impairments, and profound developmental delay. Because disease progression causes major neurological and motor impairment, and no effective CDKL5 replacement therapy is currently available, new therapeutic strategies are urgently needed. Using *cdkl5* mutant zebrafish, a validated model for CDD, candidate molecules able to rescue behavioral deficits were identified. For this study we aimed to pinpoint proteins and pathways affected in mutant *cdkl5* zebrafish, which were restored after treatment with molecules (A and B), thus seeking new therapeutic strategies.

Samples were collected from *cdkl5*^{sa21938} mutants and wild-type controls exposed to the selected molecules dissolved in 0.1% DMSO for 2 days at 5 and 33 days post-fertilization (dpf); controls received solvent only. At 35 dpf, fish were euthanized and four pools of five fish heads per condition were collected and sent to Biomarker Technologies (BMKGENE) for protein extraction and HPLC-MS/MS analysis. Proteomic data were cleaned and normalized, then analyzed using limma-based differential expression with outlier control and integrated through improved protein-to-gene annotation. All contrasts were compared in a unified framework to identify proteins dysregulated in mutants and reversed or normalized by treatment, followed by GO and KEGG enrichment analyses of rescued proteins.

Our results confirmed that Cdkl5 loss of function affects the levels of proteins linked to neuronal, muscular, and visual systems, and evidenced mitochondrial overstimulation. Enrichment analysis of GO showed that both molecules A and B rescued deregulated proteins associated with muscle cell differentiation, cytoskeleton dynamics, and mitochondrial activity. Molecule A specifically rescued pathways related to postsynaptic density and neuron maturation, whereas molecule B impacted myosin-actin-dependent processes involved in muscle contraction. Both molecules affected oxidative phosphorylation and ATP production; however, molecule A was mainly associated with fatty acid metabolism, whereas molecule B preferentially affected amino acid metabolism. Overall, these findings indicate that molecules A and B promoted molecular responses with impact on protein activity, leading to total or partial rescue of part of a subset of deregulated proteins in *cdkl5*^{sa21938} mutants.

Label-Free Quantitative Proteomics reveals MAM remodeling and immunometabolic adaptation in LPS-activated Microglia

Vivian de los Ríos[1]; Cristina Antón[2]; Silvia Rodríguez-Pires[1] and Jorge Montesinos[2]*
[1]Proteomics and Genomics Facility, [2]Lipid Regulation of Immunometabolism Group–Department of Biomedicine. Centro de Investigaciones Biológicas Margarita Salas CSIC, E-28040 Madrid, Spain.
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Microglia are the main immune cells in the central nervous system and change their metabolism and function when they encounter pathogens or tissue damage. Exposure to lipopolysaccharide (LPS), a component of Gram-negative bacterial membranes, enhances cholesterol internalization. Cholesterol uptake and esterification regulate the formation and stability of mitochondria-associated endoplasmic reticulum membranes (MAM), which are specialized contact sites between the endoplasmic reticulum and mitochondria. We hypothesized that LPS induces MAM reorganization to coordinate the microglial immunometabolic response. Although MAMs are recognized as key regulators of microglial metabolism, their proteomic landscape during activation remains largely unexplored.

We characterized the differential proteomic profile of MAM fractions in the murine microglial BV2 cell line under basal conditions (C) and following LPS stimulation for 4 h (LPS 4) and 18 h (LPS18) using label-free quantitative proteomics (LFQ). Comparative profiling identifies time-dependent alterations in proteins associated with mitochondrial function, lipid metabolism, membrane organization, and inflammatory signaling, consistent with dynamic remodeling of inter-organelle contact sites during activation.

This study provides a proteomic characterization of MAMs in activated microglia and demonstrates that inflammatory stimulation remodels their protein composition. MAM remodeling may represent a critical mechanism linking lipid metabolism to the immunometabolic adaptation of microglia during neuroinflammatory responses.

This work was supported by the Spanish Ministry of Science, Innovation and Universities (PID2024-161283OA-I00 to JM; FPU fellowship FPU23/02047 to CA-B) and Comunidad de Madrid (2024-T1SAL-GL-31373 Talento César Nombela to JM)

Quantitative DIA-PASEF proteomic profiling reveals molecular alterations in a murine model of retinopathy of prematurity

Remi Karadayi[1], Katia Ihadadene[2], Lauriane Przegalek[1], Benedicte Loriot[2], Marine Crépin[2], Jean-Paul Pais de Barros[3], Viviane de Almeida Bastos[4], Donna Pinheiro[4], Tony Jourdan[5], Xavier Guillonneau[1], Pascal Degrace[5] and Niyazi Acar[6]

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Retinopathy of prematurity (ROP) is a retinal disorder that represents a leading cause of childhood blindness worldwide. The retina is a tissue highly enriched in polyunsaturated fatty acids (PUFAs). While studies have shown that PUFAs are involved in ROP pathophysiology, little is known about their endocannabinoid derivatives. Therefore, this study investigates the potential roles of the endocannabinoid system (ECS) in the onset and progression of ROP. We performed a proteomic profiling of the retina using a mouse model of retinopathy induced by oxygen (OIR). A plasmalogen-deficient mouse model (DAPAT$^{-/-}$) was also included to provide complementary insight into the role of plasmalogens in the regulation of the ECS. The retinal proteins were digested and analyzed in data-independent acquisition / parallel-accumulation serial fragmentation (DIA-PASEF) mode in a TIMS-TOF HT mass spectrometer (Bruker) coupled to a NanoElute II nanochromatographer. Data analysis was performed using DIA-NN, followed by data filtering with the DIAGui shiny script. Statistical analysis was carried out with the PROSTAR suite. More than 10,000 proteins were identified across experimental groups, providing extensive coverage of ECS-associated pathways. Differential expression analysis revealed marked dysregulation of ECS–related proteins, including biosynthetic enzymes (NAPEPLD, DAGLA) and degrading enzymes (FAAH, MAGL), which were differentially up- or downregulated. Moreover, alterations observed in DAPAT$^{-/-}$ mice suggest that plasmalogens may contribute to ECS regulation in the retina. The proteomic profiling performed in this study represents the first evidence of ECS dysregulation in the retina in a model of ROP and highlights ECS signaling as a potential mechanistic axis in disease progression.

Kuiper enables effective, fast and reliable library-free analysis of DIA Immunopeptidomics data

Monika Pepelnjak[1], Jorge Peinado-Izaguerri[1] (J.Peinado_Izaguerri@Biognosys.com), Oliver M. Bernhardt[1], Grzegorz Skoraczynski[1], Arthur Viode[1], Roland Bruderer[1], Anamarija Pfeiffer[1], Tejas Gandhi[1], Lukas Reiter[1]

[1] Biognosys AG, Schlieren, Switzerland

Introduction:
Immunopeptidomics is essential for deciphering immune recognition, offering insights that directly support the design of more precise and effective immunotherapies. Even though the broader proteomics community has largely transitioned from data-dependent acquisition (DDA) to data-independent acquisition (DIA), DDA continues to dominate immunopeptidomics workflows. This persistence is largely due to the immense complexity of the non-tryptic peptide landscape, which expands the search space exponentially and makes library-free DIA analysis particularly demanding from a computational standpoint. To address these challenges, we introduce a new, fully integrated library-free DIA workflow for class I and class II immunopeptidomics that delivers high sensitivity while significantly reducing analysis time.

Methods:
To address the difficulties posed by the broad, unspecific search space in immunopeptidomics, we developed Kuiper, a new search engine integrated into Spectronaut. Kuiper narrows the candidate landscape by selecting only the most probable peptides for each MS2 scan, dramatically reducing the number of sequences that need to be considered. These shortlisted peptides are then fully enumerated and rigorously scored using the Pulsar search engine.
To assess the performance gains of this new library-free workflow, we benchmarked Spectronaut 20 against Spectronaut 19 across more than 25 immunopeptidomics experiments. We also compared library-free and library-based analyses on 13 additional datasets.

Results:
Spectronaut 20 delivers substantially higher performance, yielding on average 75% more class I peptide identifications and roughly 20% more class II identifications, while simultaneously reducing search time by about 70%. The new library-free workflow also outperforms traditional library-based analysis, producing around 40% more total identifications. Although both approaches share a large proportion of peptides, the library-free search consistently uncovers a greater number of unique sequences. Despite this increase in identifications, quality metrics remain robust. More than 90% of peptides detected by the library-free workflow are predicted strong binders and display the expected length distributions for their respective HLA classes. In addition, motif analyses derived from predicted binders show strong concordance between library-free and library-based searches and align well with known HLA binding preferences. Finally, both global and local FDR values remain within the expected ~1% range, reinforcing the reliability of the observed improvements.

Conclusion:
Our library-free DIA workflow markedly boosts the detection of biologically meaningful immunopeptides while eliminating the requirement for any prior knowledge of a sample's HLA alleles.

Ultrasound-Assisted Dental Peptidomics Reveals Integrated Host, Dietary, and Microbial Signatures

Raquel Fonseca[1], Catarina A.P. André[1], André Q. Fgueiredo[1,2], Carlos Lodeiro[1,2], Stefano Benazzi[3], Maria Giovanna Belcastro[4], Frederico Lugli[5], Giulia Di Rocco[6], Hugo M. Santos[1,2], José L. Capelo[1,2,*]

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Dental tissues preserve molecular information, including peptides encoding biological sex, diet, and oral microbiome composition long after DNA degradation, making them invaluable in forensic and bioarchaeological research. However, conventional peptidomic workflows are typically destructive and time-consuming, limiting their applicability when sample preservation is critical.

Here we present a rapid, minimally invasive ultrasound-assisted solid-liquid extraction protocol that recovers a rich peptidome from a single dental surface wash without causing macroscopically detectable damage. Purified extracts were analysed by high-resolution LC-MS/MS and interrogated against human, dietary, and microbial databases simultaneously. The workflow successfully identified sex-dimorphic amelogenin peptides (including newly characterised variants extending applicability to more degraded samples) and recovered concurrent dietary and microbial signals. Age-at-death estimation via asparagine/glutamine deamidation remains limited and requires further methodological development.

This platform represents a significant step toward non-destructive, high-throughput dental peptidomics for forensic, archaeological, and bioanthropological applications.

References

André, C. A. P.; Fonseca, R.; Figueiredo, A. Q.; Lodeiro, C.; Benazzi, S.; Belcastro, M. G.; Lugli, F.; Di Rocco, G.; Santos, H. M.; Capelo, J. L. Fast Ultrasonic-Based Extraction of Peptides from Teeth for Forensics and Archaeological Purposes. *Microchem. J.* **2026**, *221*, 116915. https://doi.org/https://doi.org/10.1016/j.microc.2026.116915.

Exploring the proteomics capabilities of a new Trapped Ion Mobility Q-TOF designed for enhanced metabolomics performances

Benoit Fatou[1], Helena Alamil[1], Manuel Chapelle[1],Verena Tellstroem[2], Sabine Jourdain[1], Jocelyn Dupuy[1], Beatriz Rocha[3] , Pedro Cano[3] and Pierre-Olivier Schmit[1]
1BrukerFrance SA, Wissembourg, France; 2Bruker Daltonics GmbH & Co, KG, Bremen, Germany; 3Bruker Española, Madrid, Spain

Presenting author : pierre-olivier.schmit@bruker.com

The increasing use of multi-omics strategies in translational research drives demand for LC–MS platforms capable of high performance across varied analyte classes. While established Trapped Ion Mobility Q-TOF systems (timsTOF) are recognized for strong proteomics capabilities, the newly developed timsMetabo focuses on improved transmission of low m/z analytes. The integration of the TIMS-MX cartridge, Athena Ion Processor (AIP), and Mobility Range Extension (MoRE) scan mode enables high-throughput analysis
over an extensive m/z and 1/K_0 range. This study investigates whether these advancements maintain robust proteomics performance, assessing timsMetabo's suitability as a versatile, high-performance benchtop tool for multi-omics applications.

Samples were separated using a 25 cm x 75 µm column (Ion Opticks) on a nanoElute2 (Bruker) nano-HPLC system coupled to a timsMetabo (Bruker), operating in both Data Dependent (PASEF) and data-
independant (dia-PASEF) modes. Varying amounts of K562 cell line digest (PROMEGA) were analyzed with a 22-minute linear gradient. For dia-PASEF, 24x25Th windows covering 400–1000 m/z were distributed
across 8 ramps; dda-PASEF employed 10 ramps with 300-Hz acquisition. Data analysis was performed separately for each run using DIA-NN 2.0 and FragPipe v22.0.

Injections ranging from 10 to 200 ng of K562 digest revealed a fivefold increase in signal intensity compared to previous timsTOF Pro2 data. Novel MoRE mode improved precursor detection by separating ions into two mobility fractions before fragmentation and detection. Optimization of AIP settings enhanced ion transmission. Up to 81,000 precursors and 7,700 protein groups were identified from 200
ng using dia-PASEF with a 30 ms ramp, while dda-PASEF with 300-Hz acquisition identified 37,000
precursors and 5,200 protein groups. These results match timsTOF Pro2's proteomics performance, with timsMetabo additionally providing superior metabolomics and lipidomics capabilities, positioning it as a promising platform for scalable multi-omics research.

Red blood cell modulation in response to COVID-19 vaccination – A multiomics study

Joana Saraiva[*1,2], Cristina Valentim-Coelho[1], Fátima Vaz[1,2], Marília Antunes[3], Sofia Neves[1,2], Luís B. Carvalho[1,2], Hugo Osório[4], Alexander D. Giddey[5], Mohammed J. Uddin[5], Nelson C. Soares[1,5], Deborah Penque[*1,2]

*joana.saraiva@insa.min-saude.pt, *deborah.penque@insa.min-saude.pt

[1]Laboratório Proteómica, Departamento de Genética Humana, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, Portugal.[2]Comprehensive Health Research Centre (CHRC), NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal;[3]Centro de Estatística e Aplicações, Faculdade de Ciências, Universidade de Lisboa, Portugal;[4]i3S—Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal;[5]Center for Applied Translation and Genomics (CATG) of Mohammed Bin Rashid University of Medicine and Health Sciences (MBRU), Dubai Health, Dubai, United Arab Emirates.

Red blood cells (RBCs) are the most abundant cells in the human body, comprising 36–54% of blood volume, and are traditionally recognized for their role in O_2/CO_2 transport [1]. Recent studies show that RBCs can act as cytokine scavengers, bind nucleic acids, and influence T-cell activity in vitro [1-3], suggesting functions beyond gas exchange. Despite these findings, the role of RBCs in vaccine-associated molecular responses remains unexplored.

Using COVID-19 vaccination as a model we aim to investigate whether immunization by vaccination is associated with alterations in the RBC proteome and metabolome, and whether these changes are associated with modulation of T-cell activity. The long-term objective is to identify RBC-based immunomodulatory agents, which could serve as potential co-adjuvants in future vaccine formulations.

Blood samples were collected from 39 subjects during the first wave of COVID-19 vaccination in 2021, at five time points: pre-vaccination (t0), 24-72 hours after the 1st/2nd dose (t1, t3), before the 2nd dose (t2) and 30 days after the last vaccine dose (t4). Haematological parameters were measured using complete blood count with leukocyte differential, and longitudinal changes were assessed using linear mixed models. Proteomic (n=22, t0-t4) and metabolomics (n=38, t0-t4) profiling of RBCs were obtained using liquid chromatography tandem mass spectrometry (LC-MS/MS). Data processing included filtering, normalization, and statistical modelling for repeated measures, followed by enrichment and network analysis, and multi-omics integration.

Preliminary analyses indicate transient changes in RBC-related haematological parameters following vaccination. Notably, RBC count and red blood cell distribution width (RDW), a parameter associated with inflammatory states, showed fluctuations across vaccination. Consistent with these observations, changes in the RBC proteome and metabolome during vaccination are expected, with alterations in proteins and metabolites potentially associated with inflammatory and immune processes.

The insights from this study could provide a deeper understanding of RBCs' potential role in the immune response to vaccination, potentially informing future RBC-based strategies to optimize vaccine efficacy.

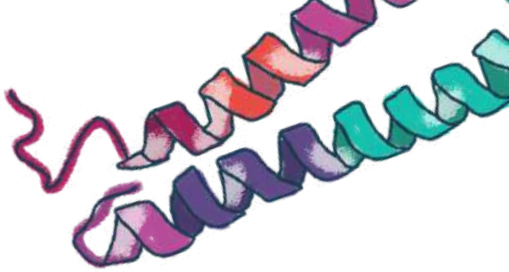
References

[1] Karsten, E. *et al*. *Sci. Rep.* **2020**; [2] Antunes, R.F. *et al*. *Immunol. Cell Biol.* **2011.** [3] Dobkin, J. *et al. Curr Opin Hematol*. **2022**.

Acknowledgements

To all volunteers who participated in this study, supported by INSARJ, CHRC, and FCT grants. Project was approved by the Ethical Committee of INSA.I.P – Lisboa and Centro Nacional e Proteção de Dados, Portugal.

Day 1 Scientific Programme



May 20 Wednesday

9:30 - 12:00 (Pre-congress course optional)

Practical Introduction to Python Programming
Diego Mena Santos, Samuel Lozano Juárez

11:00 - 13:00
REGISTRATION

13:00 - 13:15
Welcoming Session

13:15 - 13:45
Nelson Soares Invited speaker
Proteomics profiling of Red Blood Cells (RBCs) combined with Deep Machine Learning analysis – reveals potential Diagnostic Biomarkers for Acute Venous Thromboembolism

Section 1 | Chairs: José Alexandre Ferreira, Hugo Osório
Proteomics in the Clinic: From Biomarkers to Precision Medicine #1

13:45 - 15:00

Oral Communications

Deep plasma EV proteomics by Data-Independent Acquisition reveals circulating signatures of cardiac tissue injury in a preclinical Atrial Fibrillation model, Estefanía Núñez

Expanding the knowledge on diagnostic autoantibodies in colorectal cancer through proteomics and immunosensing platforms, Javier Velázquez Gutiérrez

Sponsor

Thermo/Unicam: Orbitrap Astral Series MS - Bernard Delanghe

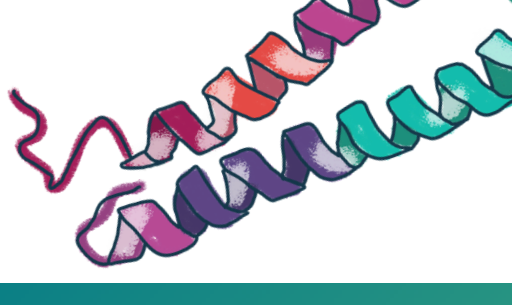
Mapping the circulating extracellular vesicle proteome in Marfan syndrome patients using minimal plasma volume, Diego Mena Santos

Methylthioadenosine: harnessing a natural metabolite to counteract cholestasis progression, Irene Blázquez García

15:00 - 15:45
Coffee Break + Poster Session 1

15:45 - 16:15
Cherine Bechara Invited speaker
Structural Proteomics Reveals Chemokine Receptor Interactions and Dynamics

Day 1 Scientific Programme



May 20 Wednesday

Section 2 I Chairs: Ana Varela Coelho, Felix Elortza
Next-Gen Proteomics: Single-Cell, Proteogenomics & Disruptive Technologies

16:15 - 17:15

Oral Communications

Integrative single-cell proteomics identifies pro-regenerative fingerprints in a sub-population of adult cardiomyocytes, Consuelo Marin-Vicente

Sponsor

Preomics: Advancing Plasma Proteomics Through a Next-Generation Enrichment Workflow for Deeper and More Quantitative Biomarker Discovery , Ann-Christine König

Benchmarking single-cell FACS-assisted strategies: effects on cellular component bias and post-translational modification detection, Samuel Lozano Juárez

Sponsor

Quilaban: Illumina Protein Prep: high-plex NGS-based proteomics designed to integrate with the Illumina Multiomics ecosystem, Álvaro Sánchez-Bernabéu

17:15 - 17:51

Flash presentations

In depth-analysis of Alzheimer's disease brain tissue reveals novel Aβ interactors, Ana Montero Calle

Proteomic and functional characterization of SLC8A1 in colorectal cancer development and metastasis, Sara Batuecas Domínguez

DIV Matters: Understanding Proteomic Shifts in Neuronal Maturation for Better Ischemic Modeling, Eva Ferro

In-depth serum glycoproteomics reveals stage-dependent α2,6-sialylation and systemic prothrombotic signalling in gastric cancer, Lisandra Gabriela Fernandes Cruz

Comprehensive nucleolar proteome profiling reveals metastasis-associated remodeling in colorectal cancer, Elisa Carral Ibarra

When One is Enough: A Minimalistic "On-Pot" Proteomic Workflow for Global Profiling of Single Caenorhabditis elegans, Ibon Iloro Manzano

MALDI-MSI as a Platform for Spatial Multi-Omics in Glioblastoma Research, Cristina María López Vázquez

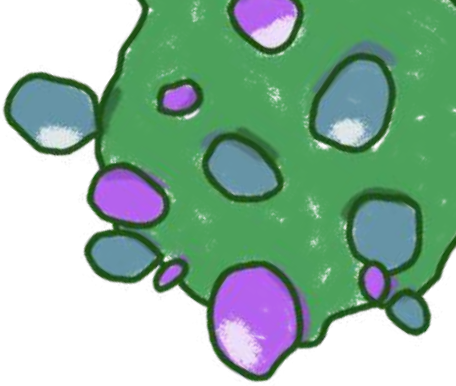
Beyond Acquisition: Turning Astral-Scale Data into Discovery: A Modular GUI for DIA Proteomics Analysis in a Core Facility Marta Isasa

Optimizing tissue disruption strategies to characterize in vivo subcellular proteome remodelling, María Cinta Picos Mora

18:00 - 18:45

Cocktail reception + Sci BINGO, organized by Young Investigators in Proteomics - SEPROT

Day 2 Scientific Programme



May 21 Thursday

6:30-7:30
Sunrise Run/walk by the beach

8:30 - 9:00
Enrique Santamaria Invited speaker
Olfactory proteomics: Emerging mechanisms and translational opportunities in neurological disorders

Section 3 I Chairs: Avais Daulat, Deborah Penque
Proteomics in the Clinic: From Biomarkers to Precision Medicine #2

9:00 - 10:15
Oral Communications

Glycoproteomics uncovers a paucimannosylated proteome associated with tumour aggressiveness and poor clinical outcome in gastric cancer, Dylan Ferreira

Artificial intelligence-based clinical models predict plasma proteomic endotypes enabling precision medicine in knee osteoarthritis, Patricia Quaranta Díaz

Sponsor

Sciex Narrow-Window Scanning DIA: Redefining Selectivity and Quantitative Confidence in Proteomics with the SCIEX ZenoTOF 8600, Mário Armelão

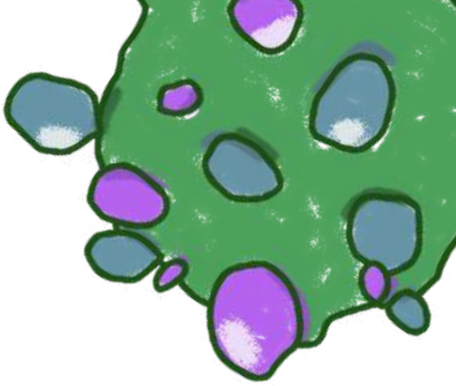
Proteomic biomarkers predictive of response to antiangiogenic treatment: toward personalized medicine in neovascular age-related macular degeneration, Antonio Cañizo Outeiriño

Unravelling the proteome of human embryo implantation: new biomarkers and metabolic signatures, Girard Océane

10:15 - 11:00
Coffee Break + Poster Session 2

11:00 - 11:30
Etienne Coyaud Invited speaker
Proximity labeling tools to investigate pathogenic protein networks

Day 2 Scientific Programme



May 21 Thursday

Section 4 I Chairs: Eduardo Chicano-Galvez, Montserrat Carrascal
Networks in Action: Interactomes, Signalling & Bioinformatics Innovation

11:30 - 12:45
Oral Communications

Deciphering protein-protein interactions in live neurons using XL-MS, Hugo Gizardin-Fredon

Proteomic profiling of the interactome of phosphorylated Tau aggregates identifies modulators of Alzheimer's disease progression, Sofía Jiménez de Ocaña

Sponsor

Bruker: Harvesting the potential of 4D-omics approaches with the timsTOF product range, Pierre-Olivier Schmidt

Glycoavatars: bead-coated membrane models for studying the cancer-immune cells interactome, Andreia Rafaela Linhares Miranda

Orai1 facilitates angiogenesis after myocardial infarction through Notch1 signaling pathway, Isabel María Galeano Otero

12:45 - 14:30
Lunch Break

Sponsor

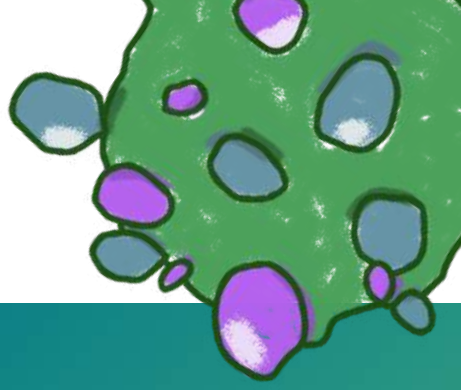
13:30 - 14:00
Sponsor Workshop : From Biosamples to Bioinsights - Bruker
Rafaelo Room (1st Floor)

Sponsor

14:00 - 14:30
Sponsor Workshop - Thermo/Unicam - Rafaelo Room (1st Floor)

14:30 - 15:00
Ana Martinez del Val Invited speaker
Decoding Protein Function: How Proteomics Reveals Post-Translational Control in Cell Signalling and Disease

Day 2 Scientific Programme



May 21 Thursday

Section 5 I Chairs: Hugo Osório, Avais Daulat
PTMs: Deciphering the dynamics of Protein Regulation

15:00 - 16:00

Oral Communications

Experimental design and multivariate analysis approaches for glycoproteomics, Estela Giménez López

Study of the links between the dysregulations of metabolism and epigenetics marks in Huntington's disease, Hisham Altoufaily

Phosphoproteomics as a tool to dissect the molecular mechanisms underlying a novel combinatorial therapeutic strategy in pseudomyxoma peritonei, Antonio Romero-Ruiz

Quantitative proteomic characterization of metastasis-associated succinylome in colorectal cancer, Raquel Rejas González

16:00 - 16:45

Coffee Break + Poster Session 3 + Group photo

16:45 - 17:15

Mélisande Blein-Nicolas Invited speaker

Proteomics as a cornerstone in deciphering the genotype-phenotype relationship in maize

Section 6 I Chairs: Monserrat Carrascal, Ana Varela Coelho
Proteomics Exploration in Non-Model Systems

17:15 - 18:30

Oral Communications

Geographical origin differentiation of tiger nut (Cyperus Esculentus) through liquid chromatography-high resolution mass spectrometry analysis, Enrique Sentandreu

Transforming Invasion into Innovation: Proteomics of Rugulopteryx okamurae for Monitoring, Degradation and Biomass Valorization, Almudena Escobar Niño

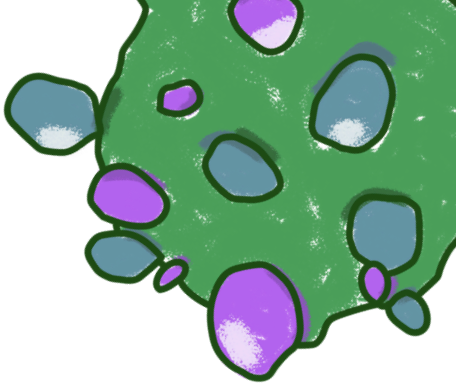
Sponsor

Affinisep: High-Throughput SPE Membrane Approaches for Peptide Cleanup and Enrichment, Michel Artocarena

Proteomic characterization of baculovirus expression vector system (BEVS)-derived extracellular vesicles engineered for Gla-Rich Protein (GRP) γ-carboxylation reveals selective GRP incorporation and distinct vesicle populations, Carla Alexandra São Bento Viegas

Authentication of A2 bovine milk by routine LC-MS proteomic analysis, Lorea R. Beldarrain

Day 2 Scientific Programme



May 21 Thursday

Section 6 I Chairs: Monserrat Carrascal, Ana Varela Coelho
Proteomics Exploration in Non-Model Systems

18:30 - 19:02

Flash presentations

Phenotypic remodelling of smooth muscle cells in atherosclerosis: a low-input and single-cell proteomics approach, David del Rio Aledo

Multi-omics characterization of SIRT3 metabolism and its adaptation to the presence of amyloid-beta oligomers in nasal epithelial cells, Paz Cartas Cejudo

Extending Proteome Profiling to Red Blood Cells using an Aptamer Platform, Luis André Botelho de Carvalho

BiasTracker: a bioinformatics tool for quantifying physicochemical and functional biases in mass spectrometry-based proteomics, Gaelle Loutfi

Comparative HLA-DR immunopeptidomics reveals disease- and genotype-associated signatures in rheumatoid arthritis, Jaxaira Maggi

Scaling-up low input spatial proteomics using Evosep Whisper Zoom on the timsTOF Ultra AIP, Beatriz Rocha Loureda

From Microbes to Ecosystems: Proteomic Insights into Agro-Environmental Interactions, Francisco Javier Fernandez Acero

20:30
Conference Dinner (optional, subject to registration and payment)

Day 3 Scientific Programme

May 22 Friday

6:30-7:30
Sunrise Run/walk by the beach

8:30 - 9:00
Celso Reis Invited speaker
Glycomics and glycoproteomics in cancer: from oncogenic mechanisms to clinical applications

Section 7 I Chairs: Delphine Pflieger, Hugo Osório
Quantitative and Computational Proteomics

9:00 - 10:15
Oral Communications

Global protein turnover dynamics in pluripotency, Orhi Barroso Gomila

Defining the Topology of Proteins in sEV Isolates by Protein Correlation Profiling, Joanes Etxeberria Ugartemendia

Sponsor
Thermo/Unicam
Advanced applications for Orbitrap Astral Series MS - Bernard Delanghe

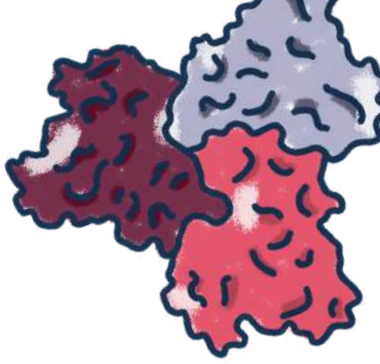
Are Your Replicates Independent? - Defining Experimental Units in Primary Neuron Proteomics, Miguel Maria Varandas Anão Rosado

Continuous telemetry-driven quality control for proactive LC–MS performance in proteomics core facilities, Daniel Lopez-Ferrer

10:15 - 11:00
Coffee Break + Poster Session 4

11:00 - 11:30
Guadalupe Gómez Baena Invited speaker
Proteomics at the Service of Biodiversity Conservation

Day 3 Scientific Programme



May 22 Friday

Section 8 I Chairs: Felix Elortza, Deborah Penque
Beyond Proteins: Small Molecules, Peptides & Integrated Omics + 5. Environmental and Ecosystem Omics-Insights

11:30 - 12:30

Oral Communications

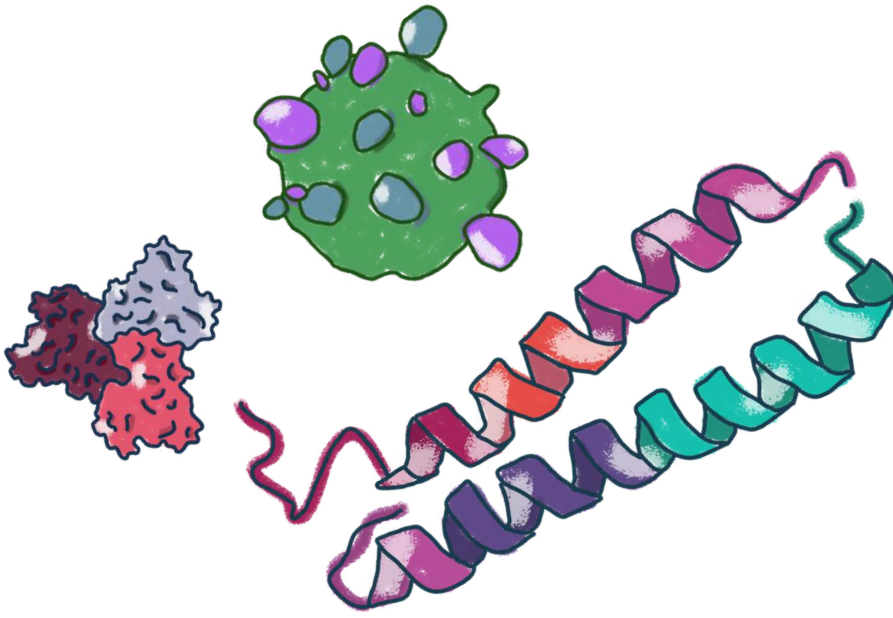
High-Throughput PISA–TMT Proteomics Enables Target Identification of Novel Antimicrobial Small Molecules, Gonçalo Raposo Matos

Molecular responses of Staphylococcus epidermidis to pH and endogenous antimicrobial fatty acids are strain-specific, Ana Maria Varela Coelho

Sewage protein information mining: A new frontier in community health and industrial surveillance, Montserrat Carrasscal Perez

Seasonal dynamics of urinary protein profiles in the Iberian Lynx (Lynx pardinus), Beatriz Ortiz-Guisado

12:30
Closing Session/Awards



Last update May 19 2026

Session 1

F1.01	In depth-analysis of Alzheimer's disease brain tissue reveals novel Aβ interactors	Ana Montero Calle
F1.02	Proteomic and functional characterization of SLC8A1 in colorectal cancer development and metastasis	Sara Batuecas Domínguez
F1.03	DIV Matters: Understanding Proteomic Shifts in Neuronal Maturation for Better Ischemic Modeling	Eva Maria Ferro Abril Monteiro
F1.04	In-depth serum glycoproteomics reveals stage-dependent a2,6-sialylation and systemic prothrombotic signalling in gastric cancer	Lisandra Gabriela Fernandes Cruz
F1.05	Comprehensive nucleolar proteome profiling reveals metastasis-associated remodeling in colorectal cancer	Elisa Carral Ibarra
F1.06	When One is Enough: A Minimalistic "On-Pot" Proteomic Workflow for Global Profiling of Single Caenorhabditis elegans	Ibon Iloro Manzano
F1.07	Scaling-up low input spatial proteomics using Evosep Whisper Zoom on the timsTOF Ultra AIP	Beatriz Rocha Loureda
1.08	Analysis of the involvement of GLG1 and BAIAP2 in colorectal cancer by functional proteomics	Ana García Romero
1.09	Proteomic differences between high- and low-grade medullary thyroid carcinomas	Alberto Peláez García
1.1	Functional proteomics characterization of neurochondrin in colorectal cancer	María Garranzo Asensio
1.11	Discovery of protein biomarkers for the diagnosis of Equine Metabolic Syndrome	Elisa Mª Espinosa López
1.12	Molecular signatures of Macrophage-to-foam cell transition induced by dyslipidemic and atherosclerotic serum	Jorge Cabañas Penagos
1.13	Functional characterization of the mitochondrial protein NDUFAF4 and implications in cholestasis	Américo Cerqueira Mateo
1.14	Advancing Plasma Proteomics Through a Next-Generation Single-Particle Enrichment Workflow for Deeper and More Quantitative Biomarker Discovery	Karin Yeoh
1.15	Secretomic profiling of triple-negative breast cancer media using Mag-Net™ HP	Previn Naicker
1.16	QuickFit DualStream: A plug-and-play dual-column ion source for high-throughput proteomics	Adolfo Fernandez Gomez de Enterria
1.17	Spatial single-cell proteomics on routine Papanicolaou-stained liquid-based cervical cytology	Laura Cantero González-Salazar

Session 2

F2.18	Beyond Acquisition: Turning Astral-ScaleData into Discovery: A modular GUI for DIA Proteomics Analysis in a Core Facility	Marta Isasa
F2.19	Optimizing tissue disruption strategies to characterize in vivo subcellular proteome remodelling	María Cinta Picos Mora
2.2	Plasma proteomic biomarkers of Hutchinson–Gilford progeria syndrome: evidence from a mouse model	Inés Perales Sánchez
2.21	Exploring the molecular link between aortic stenosis and chronic kidney disease through DIA-PASEF-based plasma proteomics	Laura Mourino-Alvarez
2.22	Unique high-throughput workflow for deeper plasma/serum proteome coverage enables discovery of potential biomarkers	Ann-Christine König
2.23	Integrated serum proteomics and autoantibody profiling reveal a protein signature predictive of flare in rheumatoid arthritis during biologic tapering	Cristina Ruiz-Romero
2.24	CSF proteomic profiling for biomarker identification in patients with MCI	Daniela Araújo
2.25	Proteomic pathway alterations in mouse hippocampus and prefrontal cortex following chronic citalopram treatment	Verônica Techmeier Morato
2.26	Organ-specific proteomic response to semaglutide treatment in healthy mice	Lucía Beltrán Camacho
2.27	ML-Driven Clinical-Proteomics Identifies a 6-Protein Signature for Precise Atherosclerosis Stratification	MªCarmen Durán Ruiz
2.28	Proteomic landscape of the PBMCs from diabetic patients with and without diabetic complications: preliminary results	Josefa Benítez Camacho
2.29	Analysis of Plasma Depleted Samples for Chronic Diseases' Biomarker Discovery with the Orbitrap Astral Mass Spectrometer	Rodrigo Barderas
2.3	Phenotypic characterization of breast cancer cells using stochastic proteomic profiling	Kamami Sarah
2.31	Novel Insights into Red Blood Cell Dysregulation in Obstructive Sleep Apnea: a multi-omic approach	Sofia Maria Sentieiro Neves
2.32	Pathophysiological subtypes of mild cognitive impairment due to Alzheimer's disease identified by CSF proteomics	Bruno Manadas
2.33	Changes at salivary proteomic level elicited by exposure to food odorants	Carla Sofia da Silva Simões
2.34	Proteome-Informed Therapeutic Prioritisation for Patient-Specific Prescriptomics in Muscle-Invasive Bladder	João de Matos Reis Aleixo Montes

Session 3

F3.35	Multi-omics characterization of SIRT3 metabolism and its adaptation to the presence of amyloid-beta oligomers in nasal epithelial cells	Paz Cartas Cejudo
F3.36	Phenotypic remodelling of smooth muscle cells in atherosclerosis: a low-input and single-cell proteomics approach	David del Rio Aledo
F3.37	From Microbes to Ecosystems: Proteomic Insights into Agro-Environmental Interactions	Francisco Javier Fernandez Acero
3.38	High-sensitivity N-glycopeptide identification in human plasma using electron-activated dissociation	Javier Lago Nuñez
3.39	Fast and robust phosphoproteomics sample prep with AttractSPE® Disks C18 Tips for high phosphopeptide recovery and identification	Michel Arotcarena
3.40	Small-molecule post-translational modification in Chlamydomonas reinhardtii	Víctor García-Riaño Domínguez
3.41	Comparison of Strategies for Global Glycoprotein Profiling by LC-MS	Javier Beaskoetxea Lejarzegi
3.42	Sequential analysis of differential protein abundance, glycosylation and phosphorylation in WNT7A overexpressing MDA231 cells	Mikel Azkargorta
3.43	Exploring glycosylation alterations during atherosclerosis progression	Emilio Camafeita
3.44	Preconditioning therapy prevents site-specific oxidation of Trp/Cys redox sensors in contractile and metabolic cardiac proteins	Inmaculada Jorge Cerrudo
3.45	VPS4A validation as a Parkin substrate	Ainhoa Atxa Espiga
3.46	Metaproteomics in an Archaeological Environment: A Pipeline for Deciphering Ancient Remains	María Luz Valero Rustarazo
3.47	Seasonal protein variations of wild boar meat	Miguel Angel Sentandreu
3.48	Tracking 10 Stages of a Fruit Fly's Life Cycle with High-Throughput Proteomics	Iraide Escobés Corcuera
3.49	Secretome analysis to understand the intracellular traffic networks in fungi	Silvia Rodríguez Pires

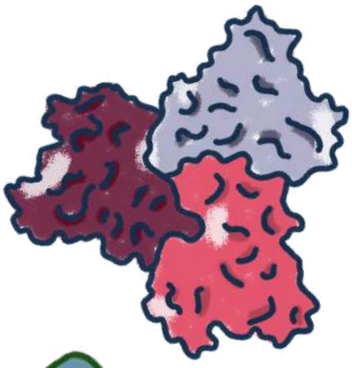
Session 4

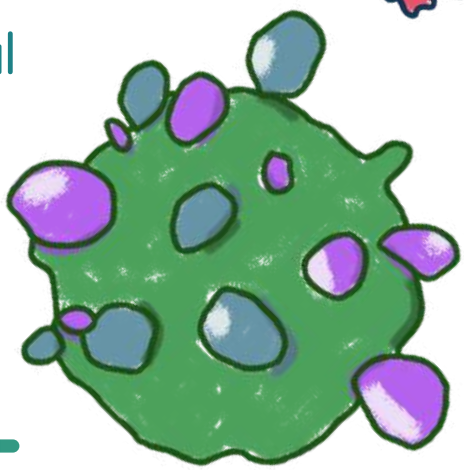
F4.50	Extending Proteome Profiling to Red Blood Cells using an Aptamer Platform	Luis André Botelho de Carvalho
F4.51	BiasTracker: a bioinformatics tool for quantifying physicochemical and functional biases in mass spectrometry-based proteomics	Gaelle Loutfi
F4.52	Comparative HLA-DR immunopeptidomics reveals disease- and genotype-associated signatures in rheumatoid arthritis	Jaxaira Maggi
F4.53	MALDI-MSI as a Platform for Spatial Multi-Omics in Glioblastoma Research	Cristina María López Vázquez
4.54	Plasma Proteome Equalization Uncovers Dysregulated Proteostasis and Amyloidogenic Pathways in Multiple Myeloma	Inês de Freitas Domingos
4.55	Towards the Development of a Liquid Chromatography Free Workflow for Measurement of Clinically Important Proteins	Pierre-Olivier Schmit
4.56	Universal pipeline unlocking inter taxonomic differential abundance analyses: Brain matrisome characterization across mouse, ferret and human	Gianluca Arauz Garofalo
4.57	Proteomic Signatures Rescued by Two Candidate Molecules in a Zebrafish Model of CDKL5 Deficiency Disorder	Márcio Alexandre Filipe Simão
4.58	Label Free Quantitative Proteomics reveals MAM remodeling and inmunometabolic adaptation in LPS-activated Microglia	Vivian de los Ríos Benítez
4.59	Quantitative DIA-PASEF proteomic profiling reveals molecular alterations in a murine model of retinopathy of prematurity	Viviane de Almeida Bastos
4.60	Kuiper enables effective, fast and reliable library-free analysis of DIA Immunopeptidomics data	Jorge Peinado-Izaguerri
4.61	Ultrasound-Assisted Dental Peptidomics Reveals Integrated Host, Dietary, and Microbial Signatures	Raquel Inês Oliveira Lourenço da Fonseca
4.62	Exploring the proteomics capabilities of a new Trapped Ion Mobility Q-TOF designed for enhanced metabolomics performances	Pedro Cano
4.63	Red blood cell modulation in response to COVID-19 vaccination – A multiomics study	Joana Saraiva

Proteo Vilamoura

6th Joint Meeting of Spanish, French and Portuguese Proteomics Societies

20-22 May 2026, Vilamoura, Portugal





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