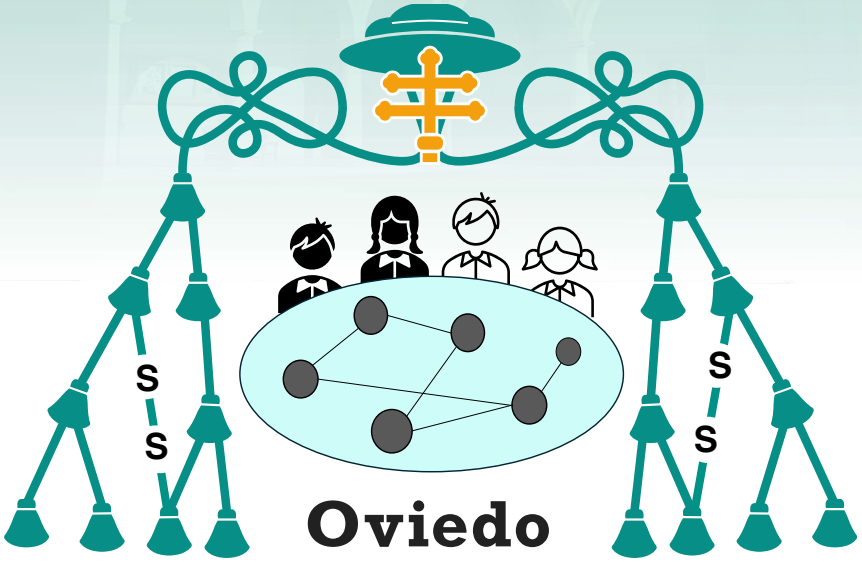
VIII Meeting for young proteomics researchers



VIII Jornadas Bienales de Jóvenes Investigadores en Proteómica

6 y 7 de Febrero de 2025

Universidad de Oviedo

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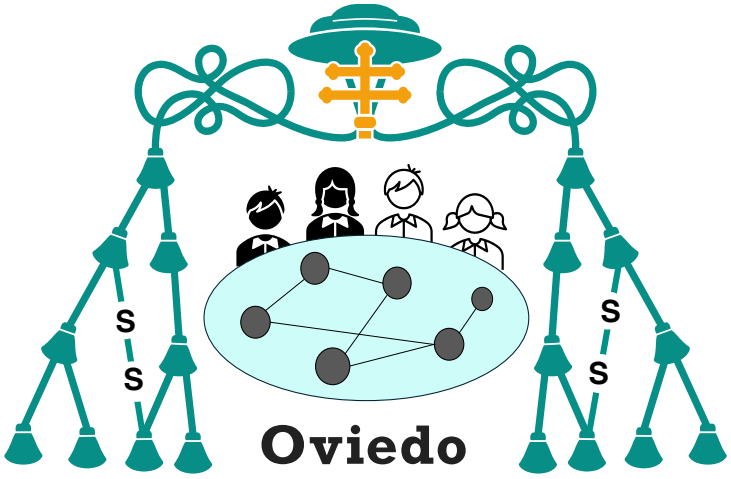

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6 y 7 de Febrero

VIII Meeting for young proteomics researchers

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Sociedad Española de Proteómica

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About SEProt, Spanish Proteomics Association,

Our organization is focused on several objectives, from reuniting all the people, societies and industries, private or state, interested in the development of Proteomics in Spain, to promote scientific and technological investigation with the aim of developing new methods and products useful for Proteomics; it is also our aim to impulse training and education as well as coordinate and stimulate connections among our members and also with other scientific, technological, economic and humanistic sector with common interests, enhancing the participation of our members in national and international activities. If you want to learn more about us, please visit https://www.seprot.es

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The University of Oviedo, a public research institution in Asturias with over 410 years of history, offers a comprehensive range of academic programs and international collaborations, including Erasmus Mundus master's degrees. With seven campuses, it supports a wide scope of studies in Arts, Engineering, Health Sciences, and more. Leading research in Asturias, the University conducts 80% of the region's R&D, generating over €40 million annually through projects and 500+ industry partnerships. Its research network includes 35 research chairs, 173 active groups and specialized institutes, advancing fields such as biomedicine, energy, and social sciences. Aligned with the UN's Sustainable Development Goals, the University integrates social responsibility with initiatives for gender equality, accessibility, and community programs.

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Welcome address by the President of the Spanish Proteomics Society (SEProt)

Dear Friends and Colleagues,

It is my pleasure to announce that on February 6–7, we will hold the "**VIII Jornadas Bienales de Jóvenes Investigadores en Proteómica"** of the Spanish Proteomics Society (SEProt) in Oviedo.

As always, the focus will be on young proteomics researchers and their work. We will begin with a course on "**Introduction to Programming in R for proteomic data analysis"**. Following this, we have a lineup of young speakers covering a range of proteomics topics. Additionally, there will be a flash poster session where **selected posters will be presented in one-minute pitches**, as well as a "**Thesis in Three Minutes**" contest.

As in previous years, we invite you all—both emerging and experienced scientists—to participate in this meeting. The goal is to create a shared space for exchanging knowledge and experiences. Oviedo, with its rich history and traditions, offers an exceptional setting, and our local organizers have crafted a truly memorable experience for everyone.

We look forward to welcoming you all to Oviedo!

Warm regards, Felix

Felix Elortza – President of the Spanish Proteomics Society, SEProt.

Welcome address by the organizing committee

Dear Participants,

On behalf of the entire organizing committee, it is our pleasure to welcome you to the 8th Meeting of Young Researchers in Proteomics, hosted by the University of Oviedo. This event serves as a unique platform for early-career researchers, particularly PhD students, to share their latest findings, discuss innovative methodologies, and foster collaborations within the dynamic field of proteomics.

Proteomics continues to be at the forefront of scientific discovery, driving advances in biomolecular research, biotechnology, and medical sciences. This meeting provides an opportunity to engage with peers, gain insights from experienced researchers, and refine your scientific perspectives through fruitful discussions. We are confident that the presentations, networking sessions, and discussions during social events will contribute to both your academic and professional growth.

We extend our sincere gratitude to all speakers, contributors, and sponsors who have helped make this event possible. We encourage you to make the most of this meeting—exchange ideas, seek collaborations, and immerse yourself in the vibrant scientific community.

Welcome to the 8th Meeting of Young Researchers in Proteomics—we look forward to an inspiring and productive conference!

Now is your time to enjoy and make this conference a success!

Luis Valledor - Chair of the Organizing Committee

Invited Speaker

Dr. Andrea Trotta. *Institute of Biosciences and BioResources – National Research Council of Italy*

Dr. Andrea Trotta is a Senior Researcher of the Institute of Biosciences and BioResources (IBBR) of the National Research Council of Italy (CNR) in Sesto Fiorentino. He obtained his PhD in 2010 in Alessandria (Italy) working on the adaptation to high salinity of the photosynthetic apparatus in different halophytes, using a combination of biochemical and proteomics approaches. He then moved to the lab of Acc.Prof. Eva-Mari Aro in the University of Turku (Finland) to work in the Academy of Finland project lead by Dr. Saijaliisa Kangasjärvi on the role of a regulatory subunit of the PP2A phosphatase in the cross-talk between light acclimation and plant immunity in the model plant Arabidopsis thaliana. In 2014 he joint Eva-Mari Aro's group to characterize by proteomics techniques the molecular mechanisms beneath the adaptation to abiotic stress in evolutionally different land plants. Taking advantage of the long experience in Finland, he is currently working on the natural variation of the genes encoding chloroplast proteins in higher plants, including conifers, by means of genetics and proteomics techniques.

Program overview

February 6th		
10:00 - 13:00	Pre-congress Course	
13:00 - 14:15	Registration	
14:15 - 14:30	Opening	
14:30 - 15:30	Invited Speaker: Andrea Trotta "Light-driven energy generation within chloroplast proteome: hypothesis, challenges, stimuli"	
Session 1 15:30 - 16:30 **Chairs** C. Ruiz J. Pascual	Functional Proteomics Analysis Of Tmod2 As Metastatic Colorectal Cancer Biomarker	Sofía Jiménez (ISCIII)
	Post-Translational Modifications In Atherosclerosis. Traceability Of Ptms From Plasma To Tissue To Single-Cell Proteomics	David del Rio (CNIC)
	Proteomic And Metabolomic Analysis Reveals Heat And Drought Stress-Induced Intragenerational Memory In *Pinus Radiata*	David Morales (Uniovi)
	Normalization Assessment In Downstream Analysis Of Proteomic Quantification Data: A Novel Shiny App	Julia García-Currás (UDC)
16:30 - 17:10	Coffee break and Poster session	
Session 2 17:10 - 18:30 **Chairs** E.Santamaría M. Escandón	Análisis integrado de datos multiómicos en investigación biomédica	Enrique de la Rosa (Sponsored by Dreamgenics)
	Proteomic Profiling Of Psychosis Risk	Martí Llaurador-Coll (URV)
	Proteomic Characterization Of The Nucleus Basalis Of Meynert During Alzheimer's Disease	Elena Anaya-Cubero (Navarrabiomed)
	Displaying Epi-Proteomic Landscape Of Chlamydomonas Reinhardtii Under Combined Heat And Drought Stress	Ana Álvarez (Uniovi)
	Analysis Of The Differential Proteome Associated With Crc Recurrence By In-Depth Proteomic Profiling Of Ffpe Tissue Samples And Plasma Extracellular Vesicles	Raquel Rejas-González (ISCIII)
18:30 - 20:00	Flash poster session	
21:00	Gala Dinner (Tierrastur El Vasco)	

February 7th		
Session 3 9:00 - 10:35 **Chairs** E. Chicano L. Valledor	Advanced Tissue Profiling: Integrating Fluorescence Microscopy, Laser Microdissection And Deep Spatial Multiomics	Cristina M. López-Vázquez (IMIBIC)
	Role Of Sirt3 And Sirt7 In Olfactory Cells Exposed To Alzheimer's Disease-Like Insults: A Proteotranscriptomic Study	Paz Cartas-Cejudo (Navarrabiomed)
	Proteomics Identification Of Diagnostic Autoantibodies In Colorectal Cancer Within A Subset Of Non-Seroreactive Patients	Javier Velázquez (ISCIII)
	Alterations In The Proteomic Profile After Batriatric Surgery-Associated Weight Loss And Their Relevance In Platelet Reactivity And Obesity Progression	Sara Troitiño (USC)
	Effect Of Vip On Chondrocyte Differentiation And Cartilage Degeneration In Osteoarthritis	Karolina Tecza (UCM)
	The rise of 4D-timsTOF based-technology in the Proteome Analysis and their benefits in today's research	Pedro Cano (Sponsored by Bruker)
10:35 - 11:25	Coffee break and Poster session	
Session 4 11:25 - 13:45 **Chairs** F. Elortza M. Meijón	A complete and fast workflow for Single Cell Proteomics analysis combining the Tecan UNO cell dispensing platform, Vanquish Neo LC and Exploris 480 DIA MS	Ignacio Ortea (Sponsored by Thermo)
	Cuenta tu tesis en 3 min	
	Break	
	Proteomic Analysis Of Cisplatin Resistance In Patient-Derived Osteosarcoma Cells: Mass Spectrometry Screening And Platinum Uptake Evaluation In Individual Cells	Carlos López-Portugués (Uniovi)
	A Novel Bioinformatics Workflow For Unbiased Analysis Of Posttranslational Modifications: Application To Subclinical Atherosclerosis	Critina A. Devesa-Arbiol (CNIC)
	Optimization Of Size Exclusion Chromatography For Ps-Liposomes Isolation And Subsequent Characterization By Liquid Chromatography Coupled To Mass Spectrometry	Oumaima El Ouahabi (UB)
	Quantitative Proteomics Strategies To Study Protein Turnover In Pluripotency	Orhi Barroso-Gomila (IIS Biobizkaia)
13:45 - 14:00	**Congress Awards and Closing session**	

Scientific programme

Thursday February 6th

Inaugural session

14:15 - 14:30	**Welcome**
14:30 - 15:30	**Opening lecture** Light-driven energy generation within chloroplast proteome: hypothesis, challenges, stimuli. **Dr. Andrea Trotta,** *Institute of Biosciences and BioResourses IBBR, National Research Council of Italy CNR, Florence, Italy.*

Session 1

15:30 – 15:45	Functional Proteomics Analysis Of Tmod2 As Metastatic Colorectal Cancer Biomarker **Sofía Jimenez,** *UFIEC-ISCIII*
15:45 – 16:00	Post-Translational Modifications In Atherosclerosis. Traceability Of Ptms From Plasma To Tissue To Single-Cell Proteomics **David del Río,** *CNIC*
16:00 – 16:15	Proteomic And Metabolomic Analysis Reveals Heat And Drought Stress-Induced Intragenerational Memory In *Pinus radiata* **David Morales,** *Universidad de Oviedo*
16:15 – 16:30	Normalization Assessment In Downstream Analysis Of Proteomic Quantification Data: A Novel Shiny App **Julia García-Curras,** *Universidade da Coruña*
16:30 – 17:10	**Coffee Break and Poster session**

Session 2

17:10 – 17:30	Análisis integrado de datos multiómicos en investigación Biomédica **Enrique de la Rosa,** *Dreamgenics*
17:30 – 17:45	Proteomic Profiling Of Psychosis Risk **Martí Llaurador-Coll,** *Universitat Rovira i Virgili*
17:45 – 18:00	Proteomic Characterization Of The Nucleus Basalis Of Meynert During Alzheimer's Disease **Elena Anaya-Cubero,** *Navarrabiomed*
18:00 – 18:15	Displaying Epi-Proteomic Landscape Of *Chlamydomonas reinhardtii* Under Combined Heat And Drought Stress **Ana Álvarez-González,** *Universidad de Oviedo*
18:15 – 18:30	Analysis Of The Differential Proteome Associated With Crc Recurrence By In-Depth Proteomic Profiling Of Ffpe Tissue Samples And Plasma Extracellular Vesicles **Raquel Rejas-González,** *UFIEC-ISCIII*
18:30 – 20:00	**Flash poster session**
21:00 –	**Gala dinner at Tierrastur El Vasco**

Friday February 7th

Session 3

9:00 – 9:15	Advanced Tissue Profiling: Integrating Fluorescence Microscopy, Laser Microdissection And Deep Spatial Multiomics **Cristina M López-Vázquez,** *IMIBIC*
9:15 – 9:30	Role Of Sirt3 And Sirt7 In Olfactory Cells Exposed To Alzheimer's Disease-Like Insults: A Proteotranscriptomic Study **Paz Cartas-Cejudo,** *Navarrabiomed*
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Sara Troitiño, *Universidade de Santiago de Compostela*

10:00 – 10:15 Effect Of Vip On Chondrocyte Differentiation And Cartilage Degeneration In Osteoarthritis

Karolina Tecza, *Complutense University of Madrid*

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Pedro Cano, Bruker

10:35 – 11:25 Coffee Break and Poster session

11:25 – 11:45 A complete and fast workflow for Single Cell Proteomics analysis combining the Tecan UNO cell dispensing platform, Vanquish Neo LC and Exploris 480 DIA MS

Ignacio Ortea, *CINN-CSIC, Sponsored by Thermo*

11:45 – 12:30 Cuenta tu Tesis en 3 minutos

12:30 – 12:45 Break

12:45 – 13:00 Proteomic Analysis Of Cisplatin Resistance In Patient-Derived Osteosarcoma Cells: Mass Spectrometry Screening And Platinum Uptake Evaluation In Individual Cells

Carlos López Portugués, *Universidad de Oviedo*

13:00 – 13:15 A Novel Bioinformatics Workflow For Unbiased Analysis Of Posttranslational Modifications: Application To Subclinical Atherosclerosis

Critina A. Devesa-Arbiol, *CNIC*

13:15 – 13:30 Optimization Of Size Exclusion Chromatography For Ps-Liposomes Isolation And Subsequent Characterization By Liquid Chromatography Coupled To Mass Spectrometry

Oumaima El Ouahabi, *Universidad de Barcelona*

13:30 – 13:45 Quantitative Proteomics Strategies To Study Protein Turnover In Pluripotency

Orhi Barroso-Gomila, *IIS Biobizkaia*

13:45 – 14:00 Awards and Closing session

Opening Lecture

Opening Lecture

Light-driven energy generation within chloroplast proteome: hypothesis, challenges, stimuli

Andrea Trotta

Institute of Biosciences and BioResourses (IBBR), National Research Council of Italy (CNR)
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The chloroplast is the engine for light-driven energy and metabolic pathways in algae and land plants. A small part of the chloroplast proteome is encoded by its own genome, but the majority of the proteins is encoded by nuclear genes. This results in a complex pathway to import, process and fold the nuclear encoded proteins, which in turn needs to be synchronized with the chloroplast translational machinery to modulate the proteome to everchanging environmental conditions. Moreover, recent advancements in understanding the physiology of this essential organelle pointed out that the evolutionary adaptations to ecological niches of land plants and algae are also the results of natural variation in plastidial and nuclear genes which are otherwise highly conserved among photosynthetic organisms.

Mass spectrometry-based proteomics have been instrumental to decipher the details of the plastidial proteome, which is subjected to a high number of post-translational modifications - often species specific. The presence of numerous membrane embedded proteins and low abundant proteins fine-tuning the chloroplast translational machinery have stimulated the development of new techniques in terms of biochemical procedure to isolate target polypeptides and obtain high resolution proteomics data. These solutions have made possible to focus plant physiology research from lab conditions to field conditions, with an increasing impact in the applications in agronomy and molecular biology applied to crops. Importantly, the experimental approaches to these challenges can be also applied in the research dealing with other organisms, where the application of standard procedures failed so far to solve intriguing biological questions.

Abstracts

Session 1

Session 1

Functional proteomics analysis of TMOD2 as metastatic colorectal cancer biomarker

Sofía Jiménez De Ocaña[1], Raquel Rejas-González[1], Javier Martínez-Useros[2], Rodrigo Sanz[3], Jana Dziaková[3], María Jesús Fernández-Aceñero[4], Ana Montero-Calle[1]*, Rodrigo Barderas[1,5]*

[1.]Chronic Disease Programme (UFIEC), Instituto de Salud Carlos III, Majadahonda, Madrid, España. [2.]Translational Oncology Division, OncoHealth Institute, Health Research Institute-University Hospital Fundación Jiménez Díaz-Universidad Autónoma de Madrid, Madrid, España. [3.]Surgical Digestive Department, Hospital Universitario Clínico San Carlos, Madrid, España. [4.]Surgical Pathology Department, Hospital Universitario Clínico San Carlos, Madrid, España. [5.]Biomedical Research Networking Centre, Frailty and Ageing (CIBERFES), Madrid, España.

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Colorectal cancer (CRC) is the third most diagnosed cancer worldwide, with liver metastasis being the leading cause of death. In previous studies, Tropomodulin-2 (TMOD2) was identified as a dysregulated protein in highly metastatic-to-liver CRC cells (KM12SM) in contrast to low-metastatic isogenic cells (KM12C) (Mendes *et al*., 2017).

In parallel, TMOD2 mRNA and protein levels were higher in colorectal tumour tissue compared to paired normal tissue. Additionally, tissue microarrays and immunohistochemistry revealed increased TMOD2 protein expression levels in metastatic tissue. Here, we aimed to analyse its functional role in CRC metastasis by means of proteomics.

We performed a stable TMOD2 overexpression in established isogenic KM12 and SW cell models of CRC metastasis. The effect of TMOD2 overexpression in the tumorigenic and metastatic capacities of CRC cells was assessed by *in vitro* and *in vivo* functional assays. Furthermore, the interactome and proteome associated to TMOD2 were investigated by quantitative proteomics through label free quantification (LFQ) to identify the interacting and dysregulated proteins associated to its overexpression. Based on changes on the tumorigenic and metastatic capacities induced by TMOD2 overexpression, we evaluated changes in focal adhesions by immunofluorescence assay. After validation by western blot, the correlation between interactors and dysregulated proteins and TMOD2 was confirmed. Subsequently to the focal adhesions' analysis, alterations in the cytoskeleton were observed after TMOD2 overexpression. Validation of these results allowed us to conclude that TMOD2 plays an important role not only as biomarker of CRC but also in CRC progression and liver metastasis. Thus, we suggest TMOD2 as an interesting target for CRC diagnosis and treatment.

References: *Mendes, M et al. (2017) Proteomics, 17(19): 1700094.*

Acknowledgements & Funding: S.JdO. is supported by an "Ayudante de Investigación" fellowship by Plan de Empleo Juvenil Comunidad Madrid. A.M.C was supported by a FPU fellowship from the Ministerio de Educación, Cultura y Deporte de España, R.R.G is supported by a PFIS fellowship from ISCIII. This work was performed with the financial support of the PI20CIII/00019 grant, partially supported by FEDER funds from the AES-ISCIII program and the HORIZON-EIC-2023-PATHFINDEROPEN-01-01 (101130574) program to R.B.

Session 1

Post-translational modifications in atherosclerosis. Traceability of PTMs from plasma to tissue to single-cell proteomics

David del Rio Aledo[1], Estefanía Núñez[1,2], Consuelo Marín Vicente[1], Paula Nogales[1], Laura Carramolino[1], Miguel Sánchez[1], Cristina Amparo Devesa[1], Inmaculada Jorge[1,2], Andrea Laguillo[1,2], Rafael Barrero[1,2], José Manuel Rodríguez[1,2], Miguel Ángel del Pozo[1], Jacob Fog Bentzon[1,3], Jesús Vázquez[1,2]

[1]Cardiovascular Proteomics Laboratory, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain. [2]CIBER de Enfermedades Cardiovasculares (CIBERCV), Madrid, Spain. [3]Aarhus University, Aarhus, Denmark.

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Atherosclerosis is a chronic inflammatory disease characterized by the accumulation of LDL, primarily composed of Apolipoprotein B (ApoB). Various strategies have been proposed for the treatment of atherosclerosis, mainly focused on reducing LDL levels, such as statins. However, these approaches are often insufficient to prevent the risk of thrombosis. It is known that posttranslational protein modifications (PTMs) regulate the activity of numerous cellular pathways, and some evidence relate them to atherosclerosis. However, their involvement in the development of atherosclerosis and their potential as therapeutic targets have not yet been explored form an unbiased, high-throughput perspective. To study the role of PTM and its association with plaque activity, we analysed tissue samples from pig and mouse atherosclerotic regression models, as well as patients, with a total n of 260. We conducted a comprehensive and unbiased analysis of PTMs using an "Open-Search" strategy using Comet-PTM and a novel integrative quantitative workflow, both developed in our laboratory. We have detected numerous modifications that are associated with decreased plaque activity. Since Smooth Muscle Cells (SMCs) are the principal cellular type producing extracellular matrix during atherosclerotic plaque development and are responsible for plaque stability, we also performed a PTM-based analysis in an extracellular matrix preparation from primary cultured SMCs using a mouse regression model (n=10). We have also performed a preliminary study using Single Cell Proteomics of mouse aortic SMCs from a mouse regression model. Preliminary results revealed that several PTMs previously observed at the tissue and plasma level are present in SMCs. Our results suggest the existence of PTMs associated with atherosclerosis progression and regression, and open the way to the study of their mechanistic implications.

Session 1

Proteomic and metabolomic analysis reveals heat and drought stress-induced intragenerational memory in *Pinus radiata*

David Morales[1], Laura Lamelas[1], Víctor Roces[1,2], Jesús Pascual[1,3], María Jesús Cañal[1], Mónica Escandón[1]

[1] Plant Physiology, Department B.O.S., Faculty of Biology and Biotechnology Institute of Asturias, University of Oviedo, Oviedo, Asturias, Spain. [2] Department of Molecular Biology, Max Planck Institute for Biology, Tübingen, Germany. [3] Genetics, Department of Functional Biology, University of Oviedo, Oviedo, Asturias, Spain

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Plants must continuously cope with increasingly more frequent and intense weather events, brought by rapid climate change. It is conceivable that, to enhance long-term acclimation and survivability, plant species retain some memory from stress exposures. The assessment of this memory in *Pinus radiata* is of great interest due to its particular sensitivity, along with its economic and ecological relevance. To this end, 18-month-old *Pinus radiata* plants were subjected to 2 cycles of heat and drought stress (E1) within 6 months and then compared with plants stressed only once (E2). Photosynthetic, metabolomic and proteomic profiles were analyzed before and during the stress period (days 1, 3, and 5).

Results reveal a clear distinction between E1 (memory) and E2 (non-memory) plants, achieving a strong separation of treatments due to stress and memory. E1 plants respond by enhancing or maintaining key pathways (e.g., carbohydrates, flavonoids) while downregulating others (e.g., photosynthesis and storage). This adjustment allows them to mitigate the impact of stress, resulting in a more gradual decline in photosynthetic activity. A detailed proteomic analysis reveals that the same proteins exhibit different levels of accumulation between memory and non-memory plants. E1 plants hyperaccumulate proteins related to processes prone to be relevant in memory acquisition, such as modification of metabolic compounds (involving carbohydrate and phenylalanine derivatives). On the other hand, hypoaccumulated proteins are associated, among others, with cellular repair mechanisms, such as the DNA repair complex or regulation of fatty acid beta-oxidation, showing different survival strategies between E1 and E2 plants.

Acknowledgements & Funding: This research was supported by MCI-20-PID2019-107107GB-I00 project, RYC2023-043866-I, FPU18/02953 and FPU21/02839 grants from the Spanish Ministry of Science and Innovation.

Session 1

Normalization assessment in downstream analysis of proteomic quantification data: a novel shiny app

Julia García-Currás[1, 2], María P. Pata[1], Guillermo López Taboada[2]

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One of the most challenging steps in proteomic downstream analysis consists in the selection of the best normalization method, in order to remove unknown, systematic bias. This step is crucial because the likelihood of having more false positives and negatives is higher when the selected normalization is not the optimal. For this reason, the main objective of this work is to develop a specific, well-documented shiny app to easily assess different normalization methods.

The web app was developed using the shiny package from R (R Core Team 2023), and then deployed on the shinyapps.io server (free access using the following link: https://jgcurras.shinyapps.io/swath_normalization_contrast/). Different sample datasets have been used to debug the app, through an iterative refinement process.
Regarding its structure, the developed shiny app consists of 6 sections with the same distribution: a left panel with different, selectable options and a right panel in which results are displayed. Functions of each section: 1) loading both quantification data matrix and design matrix; 2) applying different normalization methods and evaluating their performance by different metrics and graphs; 3) analyzing differential expression, including the selection of the desired normalization, the type of test and the missing data value threshold; 4) using three feature selection methods to select the most relevant protein groups; 5) assessing the most reliable discriminant proteins through hierarchical clustering and principal component analysis; 6) downloading a final report in html format with the main results.
In conclusion, the selection of an optimal normalization method can be easily determined using this novel shiny app, which will bring more accurate and reliable results in proteomic data analysis.

References:

R Core Team (2023). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https:/www.R-project.org/

Acknowledgements & Funding: This work has been funded by a predoctoral grant to Julia García (Ref. 23_IN606D_2022_2707220), GAIN, Xunta de Galicia, 2022-2026. This is an industrial PhD at the company Biostatech, Biostatech, Advice, Training & Innovation in Biostatistics, S.L.

Session 2

Session 2. **Contribution** sponsored by Dreamgenics

Análisis integrado de datos multiómicos en investigación biomédica

Enrique de la Rosa

Bioinformatics Scientist en Dreamgenics

El desarrollo de la tecnología de secuenciación masiva, junto con los avances en el campo de la bioinformática, permiten hoy en día el estudio masivo y simultáneo de millones de fragmentos de ácidos nucleicos en un único experimento. Sin embargo, se está poniendo cada vez más de manifiesto la necesidad de utilizar también otras técnicas que permitan arrojar luz sobre aquellas enfermedades en las que los análisis de genomas o exomas completos no permiten alcanzar un adecuado diagnóstico.

Las carencias derivadas del análisis individual de genoma completo podrían estar cubiertas con el uso complementario de otras tres técnicas ómicas: Transcriptómica, Proteómica y Metabolómica.

En esta charla revisaremos la información que cada una de estas técnicas ómicas proporciona de manera individual y cómo el análisis integrado de los datos de cada una de ellas puede ayudarnos a mejorar nuestra comprensión de las bases moleculares de las enfermedades complejas.

Session 2

Proteomic profiling of psychosis risk

Martí Llaurador-Coll[1, 2, 3, 4], Javier Labad[4, 5], Elisabet Vilella[1, 2, 3, 4].
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Psychiatric disorders, including psychosis, represent a global health challenge due to their complex aetiology, profound impact on mental and physical well-being, and the limitations of current diagnostic tools. Early detection is one of the most critical aspects of psychosis, as symptoms often appear after significant neurobiological changes have occurred. Specifically, population showing moderate psychotic symptoms, and functional and cognitive decline (Yung & McGorry, 2007) or at-risk for psychosis (ARP) is of clinical interest as about 25% transition to full onset psychosis (Salazar de Pablo et al., 2021). The identification of biomarkers for early detection could improve diagnostic accuracy and treatment outcomes, enhancing quality of life for patients and individuals at risk. High-throughput proteomic technologies, such as proximity extension assay (PEA), enable the precise quantification of multiple proteins in biological samples, such as blood, which is readily accessible and minimally invasive.

In this study, we investigated blood proteomic profiles in a sample of 85 participants, comprising 49 healthy controls (HC) and 36 individuals ARP. HC were determined after screenings for previous and current psychiatric disorders (Goldberg & Hillier, 1979), and ARP subjects fulfilled criteria for ultrahigh risk groups (Yung & McGorry, 2007). Relevant clinical variables such as drug frequent use, body mass index and treatment dosage were collected. We used PEA technology to measure 92 neurology-related proteins in blood and employed *limma* linear models and FDR adjustment to identify group-specific expression patterns. Compared to HC, Carboxypeptidase A2 (CPA2) was significantly upregulated in ARP. CPA2 is highly expressed in pancreas (Uhlén et al., 2015) and it is involved in various metabolic processes (Carbon & Mungall, 2024). Therefore, CPA2 altered plasma levels in ARP may reflect metabolic disturbances, which are frequently observed in psychosis-spectrum disorders (Alonso et al., 2022; García-Rizo et al., 2021), even in at-risk populations (Cordes et al., 2017).

Our findings underscore the potential of proteomic biomarkers in psychiatry, particularly in the underexplored domain of early psychosis detection. However, future studies on larger samples, longitudinal design, and broader proteomic panels could validate these biomarkers and refine their clinical applicability.

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Session 2

Proteomic characterization of the Nucleus Basalis of Meynert during Alzheimer's disease

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Introduction: The Nucleus basalis of Meynert (NbM) is considered the main center of cholinergic innervation of the cerebral cortex. Cholinergic neurons in the NbM integrate information from subcortical regions and project it to the frontal cortex, hippocampus, or amygdala (Liu et al., 2015). NbM plays an indispensable role in the subcortical regulation of memory and attention, in which cholinergic abnormalities occur as early as asymptomatic or at prodromal stages of AD (Eriksdotter & Mitra, 2021). Moreover, recent evidence suggests that neuropathological changes of the entorhinal cortex are preceded by degeneration of the NbM in AD models (Koulousakis et al., 2019). However, the intrinsic molecular imbalance that occurs at the level of the NbM during AD progression is largely unknown.

Methods: Label-free quantitative (phospho)proteomics were applied in 52 post-mortem NbM derived from controls with no known neurological history and AD subjects. Moreover, drug-repositioning workflows and in vitro experiments were performed.

Results and conclusions: Proteome and phosphorylation-based signal transduction imbalances were observed at the level of NbM in AD. Analysis considering the sex variable show protein expression and signaling differences in control women and men NbM's, 50% more differential expressed proteins in AD women than in AD men, a different functional profile across sexes and that the NbM phosphorylation is more severe in AD men than AD women. Using a computational tool for drug repurposing, we found two candidates with capacity to reverse early AD-related omics signatures. In-vitro validation assays revealed that the pretreatment of cholinergic neurons with those drugs had a neuroprotective effect against amyloid-beta and tau-induced neurotoxicity. Ongoing experiments in animal models will help us to validate these potential compounds, proposing novel therapeutic approaches to be used in AD treatment.

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Session 2

Displaying epi-proteomic landscape of *Chlamydomonas reinhardtii* under combined heat and drought stress

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Algae represent a diverse group of photosynthetic eukaryotes, thriving in varied ecological niches and serving as a reservoir of functional traits for environmental adaptation. Environmental stresses impose significant challenges on algal growth and productivity. Epigenetic mechanisms, including histone modifications, DNA methylation, and chromatin remodelling, play a critical role in coordinating this stress responses. However, the interplay between epigenomic, transcriptomic, and proteomic layers remains poorly understood. To address this gap, we developed ChlamytinaTool, an integrative platform that compiles epigenomic data from the algae model species *Chlamydomonas reinhardtii*. This tool includes data from chromatin immunoprecipitation (ChIP-seq), whole genome bisulfite (WGBS), methylated DNA precipitation (MeDIP-seq), and micrococcal nuclease digestion (MNase-seq). We also generated a universal chromatin states map annotation from 11 epigenetic marks: histone modifications (H3K4me3, H3K4me2, H3K9me3, H3K36me3, H3K27me3 and H3K27ac), DNA methylation (5mC and 6mA) and others (nucleosome positioning, RNA polymerase II and PSR1 transcription factor) using ChromHMM. This tool facilitates the integration of different epigenetic marks and chromatin states with transcriptomic and proteomic experiments, providing insights into the regulatory mechanisms underlying stress adaptation. Leveraging this resource, we investigated the combined effects of heat and drought stress, a hallmark of climate change, on *C. reinhardtii*. Using a proteomic dataset, we identified key molecular genes activated under simultaneous stress conditions, revealing adaptive responses and potential trade-offs. To validate these *in silico* findings, we performed a Chromatin Immunoprecipitation (ChIP) protocol coupled with micrococcal nuclease (MNase) digestion and qPCR quantification. Our findings reveal a dynamic pattern of the H3K4me3 mark under combined heat and drought stress conditions, orchestrating the activation of genes involved in this stress response. This study underscores the value of integrating epigenomic data into stress biology research, offering novel perspectives on the adaptive strategies of *Chlamydomonas reinhardtii* under complex environmental challenges.

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ChlamytinaTool is available via GitHub at https://github.com/RocesV/Chlamytina and via docker at https://hub.docker.com/rocesv/chlamytina.

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Session 2

Analysis of the differential proteome associated with CRC recurrence by in-depth proteomic profiling of FFPE tissue samples and plasma extracellular vesicles

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Colorectal cancer (CRC) ranks as the second leading cause of cancer-related death and the third most commonly diagnosed cancer worldwide. Typically, CRC is detected at advanced stages, when the tumour has already metastasized other organs, limiting treatment options and decreasing survival rates. Therefore, the early detection of CRC and monitoring its progression through biomarkers, as well as identifying novel therapeutic targets, are crucial steps toward improving patient outcomes.

In this study, we aimed to compare the protein expression profiles of tumoral tissues from recurrent and non-recurrent stage II CRC patients during follow-up. The goal was to identify dysregulated proteins in the tumours that could be linked to disease progression and served as diagnostic or prognostic biomarkers. For such a purpose, we analysed paired protein extracts from FFPE tumour tissue samples and extracellular vesicles (EVs) derived from plasma of recurrent and non-recurrent stage II CRC patients. This analysis employed quantitative proteomics using Tandem Mass Tag (TMT) 10-plex labeling and an Orbitrap Exploris 480 mass spectrometer equipped with FAIMS Pro Duo Interface.

Through two TMT-based proteomics experiments analysed with MaxQuant and R programme, we identified and quantified 343 proteins in EVs and 642 in FFPE tissue samples. Among them, 25 dysregulated proteins were found in plasma EVs, and 59 in tumour tissues, showing a fold change ≥ 1.5. Protein dysregulation was further validated through western blot using cell extracts from isogenic CRC models (KM12 and SW), paired healthy and tumour tissue protein extracts and ELISA tests performed on plasma samples from CRC patients and healthy controls. To further confirm the relevance of the dysregulated proteins, loss-of-function cell-based assays were performed in CRC metastasis cell models using siRNAs for specific proteins of interest.

Session 3

Session 3

Advanced Tissue Profiling: Integrating Fluorescence Microscopy, Laser Microdissection and Deep Spatial Multiomics

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Spatial Omics encompasses a variety of techniques that have the potential to revolutionize the simultaneous quantification of both physical and molecular properties of tissues. This field has emerged in conjunction with laser microdissection, which facilitates precise quantification and analysis of tissue sample distribution (Wu, et al., 2022).

Acridine orange is a widely used fluorescent dye that has become increasingly important in recent years due to its versatility in clinical applications. Its capacity to accumulate in the acidic environments of tumor tissues allows for the classification of tumor types through fluorescence microscopy with rapid biopsy staining, providing an alternative to traditional hematoxylin and eosin stains (Byvaltsev, et al., 2019).

In this study, we introduce a novel approach that combines mass spectrometry-based Spatial Omics with laser microdissection using fluorescence microscopy. This method not only saves time but also enhances spatial information. A key advantage of our workflow is that all procedures can be conducted on the same tissue section utilizing acridine orange (Varga, et al., 2023). This enables prior identification of well conserved anatomical structures through optical fluorescence imaging, followed by spatial profiling of lipids or proteins using MALDI MS-Imaging. Additionally, we can identify associated proteins through LC-MS/MS after selecting areas of interest via segmentation algorithms from tissue post-lipid/post-peptide mapping and subsequent laser microdissection.

Consequently, the workflow we present could serve as a valuable tool for generating high-precision data in both basic and translational research projects.

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Session 3

Role of SIRT3 and SIRT7 in olfactory cells exposed to alzheimer's disease-like insults: a proteotranscriptomic study

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Introduction: Smell impairment is an early sign of Alzheimer's disease (AD), linked to beta-amyloid (Aβ) and Tau pathologies in the olfactory system (Doty, 1987). Brain degeneration and disease severity involve SIRT3 and SIRT7, members of the NAD+-dependent deacetylase sirtuin family. SIRT3 predominantly regulates mitochondrial homeostasis, antioxidant responses, and Aβ toxicity, while SIRT7 supports protein folding and neurogenesis (Cartas-Cejudo, 2023). Both sirtuins decline with aging, playing key roles in neurodegeneration.

Methodology: Transcriptomics and mass spectrometry-based quantitative proteomics and phosphoproteomics in SIRT3 and SIRT7 silencing and overexpression in human nasal epithelial cells (hNECs), bioinformatics and systems biology were applied.

Results: Time-dependent experiments (8, 24, and 48 hours) showed significant downregulation of SIRT3 and SIRT7 in hNECs after AD-like damage. Bioinformatics revealed overlapping protein interactomes between both sirtuins, also sharing commonalities with experimentally validated Aβ and Tau interactomes. Modulating SIRT3 showed compensatory dynamics among other mitochondrial sirtuins (SIRT4 and SIRT5) at the mRNA level. Intriguingly, SIRT3 was the only sirtuin inversely regulated by SIRT7 modification. Multiomics integration revealed how SIRT3 and SIRT7 modulation affect gene expression, protein levels, and phosphorylation, providing insights into olfaction and neuropathology.

Conclusion: These preliminary results suggest that SIRT3 and SIRT7 may be targets in olfactory neuropathological aggregation. Therefore, their activation could provide therapeutic strategies for AD by addressing mitochondrial stress and metabolic dysfunction.

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Session 3

Proteomics identification of diagnostic autoantibodies in colorectal cancer within a subset of non-seroreactive patients

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Colorectal cancer (CRC) has an estimated incidence of 447 per 100,000 new cases per year in Europe, constituting the second most common cancer type (Magie Tamraz, 2024). Since many patients are diagnosed at advanced stages and relapses occur even in patients diagnosed at early stages of the disease, it is also the second most lethal cancer after lung cancer. In this context, it is necessary to improve the diagnostic tools to get an early detection of the disease to treat CRC in time. Previous studies have identified a panel of tumor-associated antigens (TAAs) specific of CRC patients, and their corresponding autoantibodies have shown high diagnostic ability (María Garranzo-Asensio, 2020; Ana Montero-Calle, 2021). However, a subset of patients is non-seroreactive to these TAAs, and therefore they would be missed on screening approaches based on the humoral immune response. Thus, in this study we have focused on identifying novel CRC-specific TAAs and their corresponding autoantibodies in this subset of non-seroreactive patients to complete the previously described autoantibody diagnostic panel. Using plasma samples, we have performed an immunoprecipitation with IgGs from non-seroreactive CRC patients coupled with LC-MS/MS analysis, identifying 15 new TAAs. Then, we expressed and purified the candidate proteins and performed indirect ELISAs with plasma samples to confirm they are target of autoantibodies of CRC patients. The most promising proteins have been incorporated into the previous CRC-specific autoantibody panel and evaluated altogether for their diagnostic/prognostic ability. Our findings pave the way for the development of a simple and accessible diagnostic method to detect these autoantibodies using blood samples, enabling the evaluation of the presence of CRC and CRC status through liquid biopsy.

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Session 3

Alterations in the proteomic profile after batriatric surgery-associated weight loss and their relevance in platelet reactivity and obesity progression

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Background: Obesity is an important risk factor related to the appearance and progression of cardiovascular diseases. Previous studies by our group demonstrated the existence of alterations in platelet reactivity in a group of morbidly obese patients (BMI>40kg/m^2) compared to a group of age- and sex-matched lean controls, revealing platelet hyperactivation in obese patients that could support atherosclerosis progression and atherothrombotic risk (*Barrachina et al., 2021*). **Aim:** We aimed to study the impact of weight loss on platelet reactivity and the possible reversal of the pro-thrombotic state associated with obesity. **Methods:** A phosphoproteomic analysis of platelets from morbidly obese patients before undergoing bariatric surgery was performed in comparison with platelets extracted one year after procedure, when patients have theoretically lost the maximum expected weight. Characterization of platelet membrane receptor levels by flow cytometry and quantification of soluble inflammatory markers in plasma were also performed. **Results:** Glycoprotein VI (GPVI), integrin αIIβ3 and GPIb-IX-V complex surface levels showed no significant differences before and after surgery, but CLEC-2 expression was increased in the pre-surgery stage. Phosphoproteomic analysis showed 486 differentially phosphorylated proteins between groups, 413 increased in pre-surgery patients and 73 increased after surgery (fold-change cut of 1.5 and p-value of 0.05). Some proteins of interest in obesity and platelet reactivity are being validated in an independent cohort of patients. Plasma levels of IP-10 and MCP-1 were found to be increased in the pre-surgery stage, while eotaxin levels increased significantly after weight loss. **Conclusions:** Pending in-depth validations, these results suggest that weight loss after bariatric surgery may have an impact on platelet activation status. Although it is considered a clinical success and carries a big improvement in their quality of life, obese patients under bariatric surgery never reach a body mass index under 25kg/m^2, which could explain why some of the parameters did not show significant changes despite the large weight loss.

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Session 3

Effect of VIP on chondrocyte differentiation and cartilage degeneration in osteoarthritis

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Osteoarthritis (OA) is a rheumatic disease characterized by progressive degradation of articular cartilage, loss of subchondral bone and synovitis. Human articular chondrocytes (hAC) are the main resident cells in articular cartilage, playing a central role in the pathology (Charlier *et al*., 2019; Fujii *et al*., 2022; Liu *et al*. 2022). These cells cultured *in vitro* in monolayer undergo a process of dedifferentiation, changing their characteristic properties. Three-dimensional cultures with biomaterials such as alginate are often used to redifferentiate them (Bačenková *et al*., 2023). During OA, hAC produce inflammatory mediators and degradative enzymes, destroying the extracellular matrix (ECM) and releasing fragments with catabolic properties, such as fibronectin fragments (Fn-fs) (Pérez-García *et al*., 2019; Pérez-García *et al*., 2021). By contrast, the anti-inflammatory potential of vasoactive intestinal peptide (VIP) has been described in multiple musculoskeletal pathologies (Martínez *et al*., 2019). In addition, recently studies have reported the effect of VIP in osteogenesis (Castro-Vazquez *et al*., 2024). In this study, we set out to investigate the effect of VIP on chondrogenesis from human bone marrow mesenchymal stem cells (BM-hMSC) in three-dimensional cell pellet cultures. On the other hand, we analyze the effect of VIP in cell proliferation and glycosaminoglycans (GAG) production, as well as its modulatory capacity on the production of inflammatory and cartilage ECM-degrading enzymes, in hAC from OA patients cultured in alginate microbeads, in the presence of Fn-fs as proinflammatory stimulus. Our results show that VIP accelerates the process of chondrogenesis from BM-hMSC from healthy donors, promoting GAG deposition over days and inducing a higher expression of chondrogenic genes, including *SOX9*, *COL2A1* and *ACAN* on day 12 of differentiation compared to day 21 in basal conditions. Likewise, alginate microbeads allow redifferentiation of hAC from OA patients. In these cultures, the presence of VIP promotes a higher concentration of GAG and restores cell proliferation to basal levels. In addition, VIP reduces the production of C1R, MMP1 and MMP13, corroborating its role as a potential anti-inflammatory tool in the process of degradation and loss of articular cartilage in OA.

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Session 3. Contribution sponsored by Bruker

The rise of 4D-timsTOF based-technology in the Proteome Analysis and their benefits in today's research

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In this brief presentation, we will explore the latest trends and innovations in proteomics technology, particularly those enhanced by Bruker's timsTOF technology, known as 4D proteomics techniques. We will highlight several key areas of interest in modern proteomics, including plasma proteomics, immunopeptidomics, single-cell proteomics, and high-sensitivity proteomics.

One rapidly emerging field is spatial proteomics, or multiomics, which combines molecular imaging techniques such as MALDI Imaging with the exceptional identification capabilities of the new timsTOF platforms, for exploring and mapping biological complexity. This combination enriches biological information by introducing a spatial dimension, providing researchers with a new ecosystem to understand tissue protein composition and spatial organization.

Session 4

Session 4. Contribution sponsored by Thermo

A complete and fast workflow for Single Cell Proteomics analysis combining the Tecan UNO cell dispensing platform, Vanquish Neo LC and Exploris 480 DIA MS

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Single-cell proteomics (SCP) has gained significant attention in recent years. This technique allows for the differentiation of proteomic profiles at the single-cell level, which is not possible with traditional bulk proteomics, where the information of each protein is averaged across all cells in the sample. This enables the exploration of diversity and state among subpopulations of cells with distinct expression profiles. The potential applications of SCP are highly diverse, including cancer research, where it can characterize the mechanisms involving different cell types; stem cell biology, to understand the processes of pluripotency and differentiation into various cell types; and precision medicine, to understand individual patient variability in response to treatments.

Given the extremely low amount of protein contained in a single cell, the two critical points in SCP are sample preparation and LC-MS analysis. Only recently have mass spectrometers been developed with sufficient sensitivity to achieve acceptable cellular proteome coverage. Consequently, SCP is a very recent field, and most studies conducted to date focus on the development and optimization of methods for individual cell dispensing, processing (cell lysis and protein digestion), and LC-MS analysis. The use of automated platforms increases robustness and decreases variability in the results, in addition to promoting the standardization of protocols.

In this study, we used a relatively affordable automated platform, the Tecan UNO (HP D100), for the dispensing of single cells and their subsequent processing in a single step (cell lysis and rapid enzymatic digestion). Human mesenchymal cell digests were prepared in 96-well plates, with a total processing time of less than 75 minutes (including enzymatic digestion with Trypsin and Lys-C). The plates were loaded directly, or after freezing, into the autosampler of a Vanquish Neo LC system (Thermo Scientific), avoiding sample transfers. Various commercial chromatographic columns were tested in standard setups as used for bulk proteomics, with a throughput of approximately 48 samples per day. MS analysis was performed in an Orbitrap Exploris 480 (Thermo Scientific), with a nanoFlex source and FAIMS Pro Duo ion mobility interface. Several DIA acquisition methods, optimized for low-amount protein samples and using one FAIMS compensation voltage, were tested, and the runs were processed with Spectronaut v17 in the directDIA (library-free) workflow. For functional analysis and assessment of the molecular pathways covered by the identified proteins, STRING v12.0 and Reactome v88 were used to obtain pathway and process coverage for the identified protein groups.

The results obtained regarding the single cell dispensing success, identification and quantification of proteins with the different variables tested will be presented, along with the GO terms and molecular pathways covered by the optimized workflow.

Session 4

Proteomic analysis of cisplatin resistance in patient-derived osteosarcoma cells: mass spectrometry screening and platinum uptake evaluation in individual cells

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Osteosarcomas are heterogeneous malignancies that primarily affect bone tissue with high aggressiveness. These malignancies present limited treatment options that mainly rely on cytoreductive surgery and cisplatin-based therapies. Unfortunately, these treatments often lead to the development of drug resistance and metastasis, significantly impacting patient survival (Abarrategi A et al, 2016; López-Portugués C et al, 2024). In this regard, advanced bioanalytical techniques, particularly proteomics, can investigate the molecular mechanisms underlying such resistance to gain insights into the cellular processes involved. In this study, drug resistance was induced in thirteen osteosarcoma cell lines (five established and eight patient-derived xenograft cell lines, PDXCL) through continuous exposure to increasing concentrations of cisplatin. A comprehensive proteomic analysis was performed using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-Q-TOF, Evosep One coupled to ZenoTOF 7600) to identify potential protein biomarkers that could predict treatment response. At the cellular level, the uptake of cisplatin in both parental and resistant cells was analyzed using single-cell inductively coupled plasma mass spectrometry (SC-ICP-MS). After 1-8 months of continuous drug exposure, approximately 54% of the cell lines (5/5 commercial and 2/8 PDXCL) developed stable resistance (3.67 ± 1.22 times more resistance). Proteomic analysis revealed 8,033 proteins common to parental and cisplatin-resistant cell models, with 192 proteins showing a shared treatment-response signature. Importantly, significant differences in cisplatin uptake in individual cells were observed: parental cells incorporated up to 10.4 times more platinum (Pt) than resistant cells (0.228 vs. 0.022 fg Pt/cell, respectively). This correlated with increased cell death in the parental cells, suggesting a direct relationship between Pt incorporation and drug efficacy. The study also highlighted a high degree of heterogeneity in drug resistance mechanisms, with PDXCL generally showing greater sensitivity to treatment. The identification of a cisplatin-response proteomic signature provides a promising avenue for developing biomarkers that could help screen potential therapeutic strategies in osteosarcoma, aiding in more effective, personalized treatment approaches.

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Session 4

A novel bioinformatics workflow for unbiased analysis of posttranslational modifications: application to subclinical atherosclerosis

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Over the last few years, developments in the field of unbiased, MS-based analysis of posttranslational modifications (PTMs) have provided increasing evidence on their essential role in many pathophysiological processes. We have recently developed Comet-PTM, an open-search engine that allows the detection and precise assignment of any modification (Δmass) detectable by MS.

In this work, we present new bioinformatics tools aimed at improving PTM identification and quantification: i) DM0Solver reduces the artefactual sources of (Δmass) histogram complexity; ii) TrunkSolver recognizes Δmass values arising from truncations before or after tryptic sites; iii) SiteSolver carries out a semi-supervised reassignment of Δmass values to adjacent amino acids provided that the starting site is statistically proved incorrect; and iv) SiteListMaker, PDMTableMaker, and GroupMaker calculate several new parameters useful for the subsequent PTM quantification procedure.

Moreover, we have developed a set of integrative workflows, based on our Generic Integration Algorithm (GIA), to quantify the different peptidoforms detected by Comet-PTM. Thus, to increase statistical power and minimize peptidoform dispersion, we have devised peptide-specific, site-specific, amino acid-specific, and group-specific integration routes.

Finally, we illustrate the performance of our developments with the unbiased characterization of PTMs in both human media layer and human plasma proteomes from individuals with subclinical atherosclerosis. Results reveal an increase of oxidative modifications in extracellular matrix, cytoskeleton and actin-myosin axis proteins in the media layer. Furthermore, in the plasma samples the PTMs were found predominantly in proteins previously associated with atherosclerosis, such as apolipoprotein B, haptoglobin, complement C5 and complement C3.

These findings underscore the clinical significance of PTMs in the progression of atherosclerosis. Further research is ongoing to improve our bioinformatics developments and extend their application to different cardiovascular pathologies associated with atherosclerosis.

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Session 4

Optimization of size exclusion chromatography for PS-Liposomes isolation and subsequent characterization by liquid chromatography coupled to mass spectrometry

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Autoimmune diseases affect around 10% of the population (Angum,F., et al. 2020) and are characterized as chronic, complex conditions with unknown origins. Current treatments are palliative, relying on anti-inflammatory drugs and immunosuppressants. These diseases arise from a failure in peripheral tolerance, leading the immune system to attack the own tissues (Pujol-Autonell,I., et al. 2015). A promising therapeutic approach, using PS-Liposomes encapsulating an autoantigen related to the immune attack, is being developed by bio-mimicking apoptotic cells. This method offers flexibility, altering the encapsulated peptide different autoimmune diseases can be targeted, like type I diabetes, rheumatoid arthritis, multiple sclerosis, and myasthenia gravis. PS-liposomes commercialization requires first preclinical regulatory studies to ensure safety and efficacy. A critical aspect of this process is developing bioanalytical methods to isolate and characterize PS-liposomes from biological fluids (e.g. serum). However, isolating PS-liposomes is challenging due to the presence of extracellular vesicles (EVs) and biological components of similar size and composition. While various methods for isolating EVs, are documented in the literature (Boriachek, K., et al. 2019), studies detailing techniques for liposomes isolation from biological fluids are scarce.

This study describes a size exclusion chromatography (SEC) method for isolating PS-liposomes from serum. Different columns were tested, in-house packed Sepharose 2B-CL column and two commercial pre-packed cartridges with different porous sizes, and the best-performing SEC column was selected for further analysis. The method was applied to spiked serum samples with PS-liposomes, and the fractions of interest were identified by flow cytometry. Preconcentration of the selected SEC fractions was evaluated by using two different methods: centrifugal Vivaspin filters and Nanotrap particles kit, where the last one yielded higher recovery. Lipids and encapsulated peptide were extracted and separated into different phases using a liquid-liquid extraction protocol, and each phase was analysed separately by HPLC-MS. This approach minimized interferences and allowed good quantification of all PS-liposome components. The developed methodology was successfully applied to PS-liposome formulations targeting various autoimmune disorders, demonstrating its effectiveness and reliability. It was also tested on blood serum samples from different animals, yielding consistent results. Finally, precision of the method was assessed, including repeatability and reproducibility, with satisfactory results.

Session 4

Quantitative proteomics strategies to study protein turnover in pluripotency

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The constant coordination of protein synthesis and degradation is essential to maintain correct protein homeostasis. Cells continuously produce and degrade proteins, a process referred to as protein turnover. Mouse embryonic stem cells (mESCs) show intrinsic and extremely fast cell division rates with strict translational regulation of pluripotency factors. However, system-wide analyses of protein synthesis and degradation in mESCs are lacking. Here we present quantitative mass-spectrometry based strategies to investigate protein turnover in mESCs. First, we apply a pulsed dynamic stable-isotope labelling by amino acids in cell culture (dynamic SILAC) to accurately characterize protein turnover rates for thousands of proteins in mESCs. In addition, by combining dynamic SILAC with Tandem Mass Tag (TMT) labeling, we significantly enhance the characterization of protein half-lives, as well as define their synthesis and degradation rates. Preliminary data shows that pluripotency transcription factors as well as chromatin remodelers including histone acetyl transferases have fast turnover rates, which might represent novel mechanisms of pluripotency regulation. These studies will enhance our knowledge on how pluripotency is regulated at proteomic level, by revealing the key molecular modulators of stemness.

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Posters

Poster Session – Poster 1

VPS4A validation as a parkin substrate

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Parkinson's disease (PD) is a common, chronic, and incurable neurodegenerative disorder of central nervous system characterized by the progressive loss of dopaminergic neurons in the substantia nigra and formation of Lewy bodies (LB), leading to motor and non-motor symptoms. Most PD cases are sporadic, and it usually manifests after the age of 50 years. However, mutations in PARK2 are responsible for the development of early-onset cases of PD (Chang, 2017; Jansen, 2019). PARK2 encodes Parkin, an E3 ubiquitin ligase that post-translationally modifies proteins by covalently adding an ubiquitin moiety to lysine residues. Identifying its substrates and unravelling the consequences of their reduced ubiquitination levels is critical to decipher the molecular mechanisms underlying early-onset cases of PD (Nguyen, 2016). In prior research, 35 Parkin substrates were identified in *Drosophila melanogaster* neurons, with VPS35 validated as being regulated by PARK2 in human cells (Martinez, 2017; Ramirez, 2015). Another candidate, Vacuolar protein sorting-associated protein 4A (VPS4A), involved in intracellular protein trafficking, has been found in the core of Lewy bodies, suggesting that it could be involved in their formation. Thus, we aim to validate VPS4A as a Parkin substrate in human HEK293T cells. In this work we show that western blot analysis of the GFP-pulldown samples shows that there is more ubiquitination when overexpressing the WT version of Parkin in comparison to the catalytically inactive version, so VSP4A is a Parkin substrate in human cells. Moreover, VPS4A::GFP quantification shows that ubiquitination is negatively correlated to total protein level, thus indicating that VPS4A ubiquitination leads to protein degradation.

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Poster Session – Poster 2

Proteomic changes in the serum of diabetic patients and its effect on endothelial colony forming cells: preliminary results

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Diabetes mellitus (DM) is a complex metabolic disorder characterized by high blood sugar levels. Despite the employment of glucose-control strategies, diabetic patients may develop diabetic vascular complications (DVCs), which is associated with a reduction in the quality of life of these patients and a greater mortality (Lu et al., 2023). Omics approaches are being used for the identification of novel biomarkers of diabetes and DVCs, to improve early diagnoses and for the development of alternative therapies. Further, cell therapy constitutes a promising approach to restore tissue perfusion in diabetic patients with major macrovascular complications such as coronary or peripheral artery diseases, among others. Indeed, endothelial colony forming cells (ECFCs) have arisen as potent agents to enhance revascularization (Sanchez-Gomar et al., 2022), given their angiogenic abilities and the capacity to be incorporated into new-formed vessels (Zhang et al., 2022). Unfortunately, however, these cells become dysfunctional in the diabetic environment, which negatively affects their regenerative potential (Benítez-Camacho et al., 2023; Lyons & O'Brien, 2020).

In the present study, a label free quantitative (LFQ) proteomic analysis has been performed in order to identify protein markers and signalling pathways altered in the serum of diabetic patients with and without DVCs and in ECFCs after its incubation with the serum factors from these patients. Thus, functional results indicated that many differential expressed proteins in the serums were related to several processes like glucose metabolism or inflammation, while ECFCs experimented changes associated with lipidic metabolism or membrane trafficking.

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Poster Session – Poster 3

Monitoring changes in protein profiles in the skin mucus of the *Octopus vulgaris*: potential protein biomarkers of welfare and health

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The aquaculture of cephalopods, especially the common octopus, *Octopus vulgaris*, represents a potential alternative to the growing market demand, counteracting the reduction of wild populations caused by over-exploitation (Pierce *et al*. 2014). In aquaculture, differing from wild species, it is critical to identify the senescent stage to distinguish it from potential breeding or disease issues (Chancellor *et al*. 2023). Proteomics allows the detection of variations in protein abundance in comparable genetic conditions, making it a powerful tool for understanding the physiological and pathological mechanisms underlying various biological processes, such as senescence (Addis *et al*. 2013).

With the aim of guaranteeing good and sustainable aquaculture practices, the main purpose of this research was to compare the different protein profiles involved with different developmental and maintenance conditions (aquarium, wild, and senescent) to enable the health and welfare status of aquarium maintained animals, and the early identification of senescents.

A total of 6 specimens of *O. vulgaris* were collected and sampled after anesthesia following the principles of animal welfare and European Directive 2010/63/EU. Experiments were also accepted by the Ethics Committee of the Competent Authority. Skin mucus samples were subjected to protein extraction, followed by trypsin digestion. The digested samples were labelled with Tandem Mass Tag (TMT-10 plex) reagents, and finally, a purification step was performed before being analysed using a Vanquish™ Neo UHPLC system coupled to an Orbitrap Eclipse Tribrid Mass spectrometer, equipped with a field asymmetric ion mobility spectrometry (FAIMS). Protein identifications and relative quantifications were performed using the Proteome Discoverer 2.4 software.

Up to 1496 non-redundant protein groups were identified. Though similar protein profiles were observed, based on the differences in the relative abundances, several proteins emerged as potential biomarkers, including annexin, caspase-3-like, cofilin, deleted in malignant brain tumors, mucin-4 proteins, papilin, protocadherin 4, and thioredoxin. Moreover, results revealed that a great number of proteins harboring peptides with bioactive properties, highlighting mucins, collagen proteins, hemocyanins, MAM and LDL-receptors, and SCO-spondin, among others, that exhibited the highest potential for antimicrobial activity. Functional analysis of all identified proteins was performed using PANTHER and Network analysis was conducted using STRING software (v. 12.0).

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Poster Session – Poster 4

Proteomics analysis in thp-1 cells exposed to dyslipidemic and atherosclerotic serum

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Cardiovascular diseases (CVDs) account for the leading cause of death worlwide, representing 32% of all global deaths in 2019 (World Health Organization (WHO) 2021).

Atherosclerosis, the most common underlying cause of CVDs, is a slow progression inflammatory disease caused by the accumulation of lipids and inflammatory cells in the intimal region of the blood vessel walls, leading to the formation of atherosclerotic plaques. Lesion progression and subsequent plaque rupture could aggravate the pathological state of the patients (Björkegren and Lusis 2022; Eslava-Alcon et al. 2020; Falk 2006).

Macrophages play a major role in the initiation and development of atherosclerosis, since they are major responsible of processing the accumulated lipids. In this pathological environment, as consequence of the increase of oxidized low-density lipoprotein uptake, macrophages transform into cholesterol ester enriched “foam cells”, which become the core of the lesion, contributing to local inflammation and thrombotic events (Robbins et al. 2013; Yu et al. 2013). Therefore, understanding the role of foam cells is fundamental when it comes to finding possible therapeutic targets against atherosclerosis.

In the current study, a label free quantitative (LFQ) proteomics analysis was carried out to evaluate the proteomic changes taking place in human monocytic cells (THP-1), a cell model used to study the function of these macrophages in vascular environments (Qin 2012), in response to the serum factors of dyslipicemic and atherosclerotic patients, compared to those from healthy controls.

As result, 5390 proteins were identified and diverse expression patterns were observed. The main modified pathways found in the functional enrichment analysis are related to apoptosis, lipid metabolism and homeostasis, vesicular trafficking and immune system activity.

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Poster Session – Poster 5

Understanding the heterogeneity of knee osteoarthritis

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The main objective of this study is to identify distinct knee osteoarthritis (kOA) endotypes associated with specific biochemical profiles and clinical outcomes through machine learning and proteomic analysis.

We analyzed 44 depleted plasma samples from kOA patients (grade K/L ≤ 2) by nLC-MS/MS on a nanoElute-LC coupled to a timsTOF Pro (Bruker). Proteins were identified and quantified using the LFQ algorithm of the DIANN software: out of 1,075 identified proteins, 395 passed quality control and were used for clustering analysis (Unsupervised Machine Learning including Consensus Clustering Plus). Twenty different metrics were used to determine the optimal number of clusters. Principal component analysis (PCA) highlighted three distinct clusters: twenty samples fit into k1 (endotype 1), nine into k2 (endotype 2) and fifteen into k3 (endotype 3). K1 is characterized by 42 up-regulated proteins related to aerobic glycolysis, platelet activation, signalling and aggregation. K3 is characterized by 27 up-regulated proteins associated with immune system processes; whereas k2 represents an intermediate state with 9 up-regulated and 51 down-regulated proteins. Four biomarkers specific to endotype 1 (k1) were validated by immunoassays: TSP1 (AUC = 0.77), SPARC (AUC = 0.78), PLF4 (AUC = 0.79) and CXCL7 (AUC = 0.878).

By integrating computational approaches with proteomics, this study represents a major step forward in understanding the heterogeneity of OA and improving the development of targeted therapies. Its translational relevance lies in optimizing clinical trial design and improving treatment efficacy for OA patients.

Poster Session – Poster 6

Comprehensive Proteomic Profiling of the Nucleolus in Colorectal Cancer Metastatic Cells

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In the past decade, the **nucleolus** has transitioned from being considered solely as the cell's ribosome factory to a key player in diverse cellular functions. Moreover, nearly 70% of nucleolar proteins are involved in functions unrelated to ribosome production, such as regulating cell cycle progression, DNA repair, stress signalling, cell proliferation, and apoptosis - processes that are closely related to tumour progression and metastasis (Diesch et al., 2014). Interestingly, recent studies have revealed a strong correlation between nucleolar stress and cancer, where altered ribosome function may lead to an increased translation of oncogenes and a decrease of tumour suppressors (Maehama et al., 2023).

Colorectal cancer (CRC) corresponds to the third most prevalent malignancy and the second leading cause of cancer-related death in the world. Among all CRC patients, 30% of them develop liver metastasis, being the primary cause of CRC-associated death (WHO, 2024). Previous studies employing mass spectrometry-based proteomics to the analysis of isogenic CRC cells have identified novel biomarkers associated with CRC metastasis and demonstrated their potential as targets for therapy and prognosis predictors (Montero-Calle et al., 2023).

In this context, we aim here to investigate the proteomic dynamics of isolated nucleoli in liver, lung, and lymph node CRC metastases, using isogenic CRC cells. Following the successful isolation of nucleoli and nucleoplasm fractions, we conducted a DIA label-free proteomic quantification using the Orbitrap Astral Mass Spectrometer. In total, 7249 proteins were identified and quantified, focusing on those upregulated proteins with a fold change ≥1.5 and p-value ≤0.05 when comparing nucleoli to nucleoplasm. Among them, 263 proteins significantly dysregulated with fold changes ≥1.5 or ≤0.67 in metastatic cell lines compared to non-metastatic lines were observed. Most of them are proteins associated with ribosome processing and transcriptional regulation, both functions closely linked to the nucleolus. Overall, our goal is to achieve a comprehensive understanding of these pathways which would enable the development of personalized therapies that more specifically target rRNA transcription without causing DNA damage.

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Poster Session – Poster 7

Secretome analysys of a *Macrophomina phaseolina* isolate Using DIA and DDA Mass Spectrometry

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Macrophomina species are phytopathogenic fungi known to infect a wide range of agriculturally significant plants (Pennerman, KK. *et. al*., 2024). Sequencing and analysis of the *Macrophomina phaseolina* genome have uncovered the secretion of a diverse array of molecules that facilitate its pathogenic activity (Islam, *et. al*., 2012) including metabolites and proteins. In silico analysis predicts that *M. phaseolina* can secrete a total of 996 proteins, however, only 255 have been detected experimentally, of which most are CAZyme and proteases, thus some proteins have not yet been detected experimentally (Sinha N., *et. al*., 2022; Pineda-Fretes A. *et. al*, 2023). In this study, we conducted a proteomic analysis of the proteins secreted by *M. phaseolina* FCQ72 into two liquid culture media: one supplemented with soybean root (sMM) and one without (MM). The analysis utilized both data-dependent acquisition (DDA) and data-independent acquisition (DIA) techniques. Using DIA, we identified a total of 276 proteins across both culture conditions, including 106 enzymes from the CAZyme family, 30 proteases, 42 proteins with no conserved domains, and 98 proteins classified under other categories. Among the 276 proteins identified, 63 were classified as potential effectors. Using the DDA approach, we identified 251 proteins in both culture conditions, including 101 proteins from the CAZyme family, 25 proteases, 36 with no conserved domains, and 65 classified under other categories. Among these, 56 proteins were identified as potential effectors. 267 proteins were identified in sMM by DIA and DDA analysis, 21 proteins were identified only in the medium with root of soybean, potentially linked to the infection process

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Poster Session – Poster 8

From progenitor to mature cell: proteomic insights into monocyte lineage progression

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Monocytes (Mo) play a crucial role in tissue homeostasis and stress response, originating from precursor cells in the bone marrow (BM) before entering the bloodstream. Despite extensive research, the plasticity and functional diversity of these cells, along with the discovery of new subsets, have complicated our understanding of their maturational relationships. To address this challenge, we employed a combination of spectral flow cytometry, single cell-based mass cytometry (CyTOF) and bulk mass spectrometry (MS)-based proteomics to elucidate the proteome and maturational trajectory of monocytic cells from their origin in the BM to their presence in peripheral blood (PB). Our study began with the development of a 34-marker CyTOF panel, designed after screening over 100 proteins by flow cytometry, to evaluate more than 40 myeloid populations (including 15 Mo subsets). This panel guided the purification of 5 (pro)monocyte subpopulations from BM and 8 monocytic subsets from PB for subsequent TMT-based MS analysis. Single-cell trajectory analysis of BM samples revealed 11 distinct protein expression patterns during maturation, reflecting diverse functions such as metabolism, signaling, lysosome assembly, and antigen presentation at various differentiation stages. In the PB, our analysis uncovered close relationships between $CD62L^-$ $Fc\varepsilon RI^-$ classical Mo (cMo), intermediate Mo (iMo), and $CD36^+$ $Slan^-$ non-classical Mo (ncMo) subsets. Notably, iMo appeared to serve as a bridge between cMo and ncMo populations. Within the ncMo subpopulations, we observed distinct functional profiles: $Slan^-$ cells were primarily involved in metabolic and protein production processes, while CD36- subsets demonstrated a stronger commitment to protein production and vesicle transport. This integrated approach, combining spectral flow cytometry, CyTOF and MS-based proteomics, has yielded a comprehensive proteome atlas of monocytes across BM and PB.Our findings provide valuable insights into the complex maturational relationships and functional heterogeneity of these cells, advancing our understanding of monocyte ontogeny from their origin in the BM to their diverse roles in the PB.

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Poster Session – Poster 9

Identification of proteomic biomarkers for predicting osteoarthritis progression: data from the imi-approach cohort

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Characterization of differential phenotypes in osteoarthritis (OA) is essential to enable patient stratification in plenty of scopes. A discovery proteomic analysis was performed in a subset of serum samples from the Applied Public-Private Research enabling OsteoArthritis Clinical Headway (IMI-APPROACH) cohort, leading to the identification of a panel of proteins with putative predictive value for OA progression in a 24-month period. This study aimed to verify the biomarker value of these proteins to predict OA progression either in pain (P) or structure (S), quantifying them by immunoassays and exploring their utility in predictive models for patient stratification in clinical trials.

Serum samples at baseline (T=0) were analysed. S and P progression was determined (Van Helvoort, 2020). Proteins were quantified by Luminex or ELISA. Statistical analysis was assessed by logistic regressions and ROC analysis. Among the candidates analysed, ADI and CHI3L1 had association with P progression ($p>0.05$). Higher levels of CHI3L1 showed to be a risk factor (OR=2.30, 95% CI:1.03-5.11), whereas higher levels of ADI might act as protector (OR=0.37, 95% CI:0.16-0.85). CRP showed correlation with S progression ($p<0.05$), acting as a risk factor (OR=2.93, 95% CI:1.12-7.69). The simplest predictive model (age, sex, BMI) had an AUC=0.664 (0.623-0.704) for P progression, while S progression had an AUC=0.570 (0.529-0.611). The addition of these proteins to their models caused a significant increase ($p=0.007$ and $p=0.025$) in their AUC to 0.726 (0.690-0.763) and 0.645 (0.608-0.682), respectively. As a conclusion, two models for the prediction of pain or structure progression have been developed, based on data from the APPROACH cohort, combining clinical variables with the protein biomarkers.

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Poster Session – Poster 10

Single Organ Proteomics on Drosophila melanogaster

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Mass spectrometry-based proteomics has experienced an impressive revolution in performance, and we are witnessing achievements that seem to be impossible just few years ago. Improvements have been done at sample preparation, chromatographic and mass spectrometry levels.

Taking advantage of this we have implemented an in house developed strategy to map single organ proteomes from the fruit fly *Drosophila melanogaster* (Dm). Dm´s larvae were manually dissected to extract organs: brain, ring gland, eye disc, leg disc, haltere disc, wing disc, salivary gland, fat body, testis and gastric caeca/proventriculus/garland cells. Single organs, in quadruplicate, were deposited in low binding tubes with extraction buffer based on Ye et. al. (2024). After 5 freeze and thaw cycles combined with sonication, Lys C and trypsin were added without reduction and alkylation for over-night digestion. 10% of the sample was loaded on an EVOTIP. Whisper method (100 nL/min; 20 SPD) was used on EVOSEP and DIA and DDA acquisition methods were applied in a TIMS tof Pro (Bruker). DiaNN and FragPipe were used respectively to search against Uniprot (SwissProt/Trembl) *Dm* database. The observed proteome of each organ ranged from 2293 (Ring Gland) to 5937 (Brain) proteins identified in at least 3 out of 4 replicates. Summing the results from different organs, more than 8000 proteins were identified in total. As expected, principal component analysis showed clear separation among proteomes from most of the organs, proving a differential protein expression profile.

Complementing the powerful genetic tools already available for this model organism, we believe that this type of analysis will open new avenues for proteomic studies in *Drosophila*. Besides, our aim is also to apply this methodology to studies where the starting tissue material is scarce.

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Poster Session – Poster 11

Post-translational modifications of glial fibrillary acidic protein (GFAP) after spinal cord injury

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Spinal cord injury (SCI) causes persistent neurological and systemic effects, including autoimmunity mediated by autoantibodies (AAb), which has been associated with reduced functional recovery. In previous studies conducted by our working group, 16 antigenic targets involved in alterations occurring after SCI were identified (Arevalo-Martin et al., 2018). Among these targets present in the serum of healthy individuals, it was observed that following SCI, there was an increase in Immunoglobulin G (IgGs) and Immunoglobulin M (IgMs) against various post-translational modifications of these targets.

In this work, to continue this line of study, we propose these modifications as potential signals used by autoantibodies to detect damage following to SCI. Specifically, we focused on glial fibrillary acidic protein (GFAP) in the serum of control and SCI rats, identifying and quantifying all post-translational modifications occurring in this protein after injury through proteomics.

The results show a significant increase in post-translational modifications in GFAP following the injury. Overall, our findings expand and support previous studies that indicated why autoimmunity develops after SCI and identify novel targets in SCI pathology.

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Poster Session – Poster 12

Application of proteomics in clinical practice for cardiac amyloidosis typing

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Cardiac amyloidosis (CA) is characterized by extracellular accumulation of amyloid material in the form of misfolded protein fragments, which causes heart failure and premature death. Classical diagnosis of CA is performed by histochemistry and immunohistochemistry. However, the use of this technique for subtyping has showed several limitations related with antibody specificity, high background staining caused by serum contamination and epitope loss due to the formalin fixation procedures. In this work, we describe a MS-based proteomic workflow for the qualitative profiling of Formalin-Fixed Paraffin-Embedded (FFPE) cardiac biopsies in order to confirm the diagnosis of amyloidosis and its subtype with high specificity.

Five representative cardiac tissues, one from a patient without amyloidosis (control case) and four patients with uncertain diagnosis of CA, were obtained from the CHUAC Anatomopathology service. FFPE samples were deparaffinized and rehydrated. Proteins were extracted with lysis buffer by sonication/heat treatment and cleaned up by acetone precipitation. Proteins were reduced, alkylated, digested with trypsin and desalted with StageTips. The peptide mixtures were injected on a nanoElute LC coupled to a high-resolution mass spectrometer TIMS-TOFpro (Bruker). Peptides were analyzed in data-dependent acquisition mode (DDA) with Parallel Accumulation–Serial Fragmentation (PASEF). Mass spectrometry raw files were processed with PEAKS Studio 10.6. Amyloidogenic proteins were relatively quantified in the samples according to their peptides, the peak area values and the total number of spectra matching. Apolipoprotein E (APOE), serum amyloid P component (SAMP), and apolipoprotein A IV (APOAIV) were used as generic amyloid markers, which were not detected in the control case. Furthermore, the patients with suspected CA diagnosis presented a specific diagnostic signature of different proteins in each case belonging to known types of amyloid as Immunoglobulin kappa constant (IGKC), Immunoglobulin lambda constant (IGLC), Serum amyloid A protein (SAA) and Transthyretin (TTHY). Proteomic analysis by liquid chromatography coupled to mass-spectrometry was used to unambiguously classify the cardiac amyloidosis in the patients' biopsies compared to negative control samples. Amyloid typing by MS supports the diagnosis of amyloidosis patients. MS should be considered the gold standard for typing amyloid fibrils in routine clinical practice.

Poster Session – Poster 13

Methods comparison for protein extraction, solubilization and digestion from paucicellular human clinical specimens

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Biological samples containing scarce starting material are frequently common in clinical settings and pose a challenge in untargeted human LC-MS-based proteomics studies.

In keratoconus, a low-incidence progressive corneal dystrophy affecting young people, the access to the affected tissue has been difficult and invasive for many years. With the purpose of gaining knowledge on the biological mechanisms underlying keratoconus and identifying new protein biomarkers measurable at the point-of-care level (i.e. the ophthalmologist office), CORNEA developed a breakthrough minimally invasive cornea collection method.

In a collaborative precision medicine project with IMIBIC, 8 different methodologies for sample preparation were assessed, combining different cell lysis buffers, mechanical cell disruption methods and protein digestion approaches. Biological triplicates from real clinical specimens and negative controls were used in each combination tested and equal peptide amount was analysed by DIA-PASEF technology on a timsTOF Flex (Bruker) coupled to an EvosepOne (Evosep).

Performance of each method combination was evaluated based on purified peptide quantification, the number of peptide and protein identifications and proteome coverage. Moreover, reproducibility, easiness of use, processing time and throughput capabilities were also considered. After this feasibility study, the best protocol combination was selected for upcoming clinical studies.

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Poster Session – Poster 14

Defragmenting ncdn role in colorectal cancer metastasis

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Colorectal cancer (CRC) is the third most common cancer type 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 metastatic CRC cells compared to poorly metastatic CRC cells. In this work, we have evaluated the effect of NCDN expression in the tumorigenic properties of depleted KM12C (poorly metastasic) and KM12SM (metastatic to liver) CRC cells. Additionally, we have injected silenced and control cells in nude mice to assess the effect of NCDN in tumor growth and liver metastasis. NCDN depletion produced a reduction in the tumorigenic properties of the cells *in vitro*, while the *in vivo* studies showed a reduction in the metastatic ability of CRC cells. Since a clear correlation was observed between NCDN and CRC formation and metastasis, we performed a quantitative proteomics analysis to identify the network of dysregulated proteins associated with NCDN. Protein extracts from silenced and control metastatic and non-metastatic CRC cells were obtained, trypsin digested, and tandem mass tag (TMT) labeled. After reverse-phase fractionation, the eluted peptides were analyzed by LC-MS/MS using an Orbitrap Exploris 480 equipped with FAIMS Pro Duo 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. In total, 39363 peptides and 4766 proteins were identified and quantified. Out of the 4766 proteins, 116 of them showed a fold change ≥ 1.5 or ≤ 0.67 when comparing their expression level in control and NCDN-depleted cells. Validation of the dysregulation of interactors and signaling pathways associated to NCDN analysis was performed to better understand NCDN's role in CRC formation and metastasis.

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Poster Session – Poster 15

EXPLORING HISTONE ptms USING HIGH-RESOLUTION MASS SPECTROMETRY IN PLANT MODELS

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Histones are the primary proteinaceous component of chromatin in eukaryotic cells and form a cornerstone of the epigenome, influencing DNA-related processes such as transcription, replication, and chromosome segregation. The four core histones—H2A, H2B, H3, and H4—assemble into an octamer around which approximately 147 base pairs of DNA are wrapped, constituting nucleosomes Each histone can be modified by a variety of post-translational modifications (PTMs), including methylation, acetylation, and phosphorylation, among others. Over 200 histone modifications have been identified to date.

Given this large repertoire of PTMs, advanced analytical approaches are required for comprehensive characterization. Mass spectrometry (MS), especially LC-MS/MS methods, has become a key tool for simultaneous quantification of numerous histone PTMs, enabling the exploration of co-occurring modifications and the discovery of novel ones. However, software solutions capable of processing these MS data in a plant-specific context remain scarce and inconsistent. On th other hand, certain histones, such as H3 and H4, are relatively well conserved across eukaryotes, H2A and H2B can exhibit substantial sequence divergence among different plant species. These variations in amino acid composition and mass/charge states complicate the identification and quantification of plant-specific histone PTMs, highlighting the need for specialized computational tools.

To address this gap, we use an adapted version of open-source software capable of distinguishing isobaric peptides, tracking retention times of modified peptides, and accommodating low-abundance PTMs. By integrating additional references—namely extra sequences, masses, and charge states—for each histone in multiple plant species, we can disentangle the complex landscape of plant histone modifications. This approach allows for higher accuracy in detecting and quantifying site-specific PTMs under various experimental conditions.

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Poster Session – Poster 16

Decoding the impact of post-translational modifications in MASLD: A Proteomic approach to advancing precision medicine

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Metabolic Associated Steatotic Liver Disease (MASLD) is a clinical condition in which the excess fat accumulation is not associated with high alcohol consumption unlike other subtypes of liver diseases, but it can also lead to other complications like cardiovascular disease. As a result of high lipid accumulation, the β-oxidation decreases, and the mitochondria become dysfunctional, increasing the production of reactive oxygen species (ROS). This increase of oxidative stress causes cellular damage, promoting inflammation and fibrosis, and in advanced stages, cellular apoptosis or necrosis.

In this study, we analysed the proteomic profile of liver biopsies from patients with MASLD under different conditions (Obesity, Type 2 Diabetes, Steatosis, and Fibrosis), which are not mutually exclusive. An open-search strategy was performed using the MSFragger search engine, together our lab-developed software (iSanXoT and a new PTM analysis package), aiming to obtain a detailed map of the proteome changes occurring under each of the analyzed conditions, including a comprehensive study of the post-translationally modified peptidome.

The results revealed key proteins involved in sucrose, lipid, and amino acid metabolism, as well as signalling, that are differentially implicated across the various patient types. Additionally, most of the differential PTMs were oxidative and located in proteins directly or indirectly related to the mitochondria.

Overall, our bioinformatics tools allowed the localization of PTM differences across the patients, providing an additional layer of information regarding the changes taking place in the studied proteomes. This approach offers a novel methodology to facilitate the discovery of biomarkers in MASLD and is adaptable to other proteomics studies.

Poster Session – Poster 17

Deciphering pulmonary tumor microenvironment through MALDI Imaging Mass Spectrometry: new approaches for tumor expansion inhibition

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During tumor growth, cancer cells increase their demand for oxygen and nutrients, necessitating the formation of new blood vessels, a process known as angiogenesis. Over more than 40 years, various molecules, such as vascular endothelial growth factor (VEGF) antibodies, have been investigated to inhibit angiogenesis. However, these strategies have shown limitations, as cancer cells can resort to non-angiogenic mechanisms to obtain the necessary resources (Bridgeman et al., 2016). One of these mechanisms is vessel co-option (VCO), where cancer cells utilize pre-existing blood vessels instead of forming new ones (Kuczynski and Reynolds, 2019).

Overcoming VCO as a resistance mechanism to anti-angiogenic therapies represents a new challenge in cancer research. The hypothesis is that inhibiting vessel quiescence could be an effective strategy to combat VCO. The main goal of this project is to evaluate whether the inhibition of vascular quiescence induced by bone morphogenetic protein 9 (BMP9) through the ALK1 receptor (David et al., 2008) can promote VCO inhibition and serve as a good anti-tumor strategy in combination with chemotherapies, immunotherapies, or anti-angiogenic drugs.

To this end, lung metastases were generated by injecting 4T1 cells (breast cancer) intravenously. Once the metastases were established, the animals were treated with either a placebo or PF-03446962 (an antibody against the ALK1 receptor) for two weeks. At the end of the procedure, the lungs were analyzed using MALDI imaging. Preliminary results revealed differences in the spectra of the regions of interest, allowing for the characterization of specific biomolecular signatures. Additionally, tissue microextractions were performed to identify peptides present on the tissue surface, some of which were related to platelet activation, blood coagulation, and angiogenesis, providing a new perspective on the mechanisms associated with VCO used by tumors.

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Poster Session – Poster 18

Implementation of a quantitative platform for the analysis of post-translational modifications by single-cell proteomics

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Single-cell analyses constitute a powerful approach for unravelling biological heterogeneity within tissues. Although these analyses have been limited to transcriptomics, recent advances in mass spectrometry (MS) have paved the way for single-cell proteomics.

Besides improvements in MS (Budnik et al., 2018; Guzman et al., 2024) and also in sample preparation (Ye et al., 2024), one of the main drivers of single-cell proteomics has been the shift from data-dependent acquisition (DDA) to data-independent acquisition (DIA). DIA allows an unbiased approach, overcoming challenges related to dynamic range and stochasticity, while enabling greater proteome coverage with minimal sample amounts (Guo & Huan, 2020). As a result, the increased sensitivity and coverage provided by DIA allows the exploration of post-translational modifications (PTMs) at the single-cell level (Wang et al., 2022). However, many challenges still remain to be addressed, such as the absence of tools capable to perform unbiased PTM analysis using open-search approaches (Chick et al., 2015) directly from DIA data.

Here we present a workflow that streamlines the analysis of PTMs from DIA-generated data using open search approaches. Our tool enables the creation of experimental global peptidoform libraries compatible with downstream identification and quantification using DIA-NN (Demichev et al., 2020). The quantified peptidoforms are subsequently analysed using the Generic Integration Algorithm (GIA) (García-Marqués et al., 2016) adapted for the analysis of PTMs (Bagwan et al., 2018), integrated into the iSanXoT software (Rodríguez et al., 2024). Finally, to make our tools available to a broader scientific community, a module has been developed to connect the output of iSanXoT with the SCeptre workflow (Schoof et al., 2021), for a better visualization of results.

We validated this pipeline using published single-cell embryo-derived samples acquired in an Orbitrap-Astral (Ye et al., 2024). Our strategy allowed to generate a library containing more than 86700 peptidoforms, 14% of them pertaining to modified peptide species. Finally, using that library in DIA-NN, we were able to identify a total of 73,185 non-modified peptides and 3,485 modified peptidoforms, some of which of biological relevance, such as acetylation or methylation, resulting in 2,934 more identifications overall compared to the default DIA-NN settings.

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Poster Session – Poster 19

Secretome and extracelular vesicle profiling from an in vitro huvec model of atherosclerosis

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Formation and accumulation of oxidized LDL (oxLDL) followed by increased endothelial permeability of the intimal layer of blood vessels is the main landmark in atherosclerosis (Libby et al, 2011). Atherosclerosis progression involves a complex crosstalk between the different cell types that are present within the atherosclerotic plaque: endothelial cells, smooth muscle cells and macrophages. It is known that endothelial cells secrete vesicles in response to pro-inflammatory, pro-angiogenic stimuli and shear stress into the extracellular matrix (Wei et al, 2019). Therefore, studying the proteomics of the signalling from these cells to the extracellular matrix and between cells would provide important insights into this disease.

Preliminary results from our group show that Human Umbilical Vein Endothelial Cells (HUVEC) internalized oxLDL present in the cell media through endosomes, which eventually affects their proteomic landscape. In this work, we aim to understand the downstream effect of oxLDL in these cells, by characterizing their secretome (soluble and from extracellular vesicles).

HUVEC were grown under serum starvation conditions and treated with 0, 25 and 100 µg/mL of oxLDL for 24 hours (n= 4 replicates). The cellular, total secretome and EVs proteomes were analyzed by DIA in a Thermo Orbitrap Eclipse. Our data reflect that a dose of 100 µg/mL is required to observe extensive remodelling of the cell proteome and the secretome. However, some relevant changes, such as upregulation of Epithelial-to-Mesenchymal transition and of inflammatory markers were already observed in response to oxLDL at 25 µg/mL. Moreover, we observed a significant decrease of Metallothionein-like protein 2A (MT2A) in the secretome of cells treated with just 25 µg/mL of oxLDL. Since MT2A plays an important role as scavenger of ROS species in atherosclerosis (Gobel et al, 2000), this might indicate that oxLDL remodels the secretome of HUVEC cells, making them less capable to respond to oxidative damage.

In summary, our data provide evidence that oxLDL induces remodelling of HUVEC and some clues its downstream effects. Future studies will validate these findings and integrate this information with that obtained using other cell types.

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Poster Session – Poster 20

Benchmark of offline fractionation strategies for spectral library generation

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Hypothesis-free proteomics experiments can be performed following either data-dependent (DDA) or data-independent (DIA) acquisition strategies. Due to the non-stochastic nature of DIA, this approach has been proven to be superior in terms of robustness and sensitivity as compared to DDA, where data acquisition is biased towards higher abundance peptides. For some specific applications, such as the identification of posttranslational modifications, the analysis of DIA data relies on experimental spectrum libraries; however, the generation of such experiment-specific libraries is a laborious and expensive process.

In this work, we resorted to three different offline fractionation techniques aimed at reducing sample complexity for improving proteome coverage in the spectral libraries to be generated for their use in peptide-centric DIA workflows: home-made high-pH reversed phase (HpH) StageTip (Rappsilber et al., 2007), HpH commercial cartridges, home-made and Strong cation-exchange (SCX) StageTip. Protein inputs of 100 and 50 µg were assayed, and the three approaches yielded more peptides and proteins with 100 µg of protein input in identical column loading conditions. HpH performed on StageTip resulted in better coverage and reproducibility as compared to the commercial version. SCX fractionation resulted in 10% less peptides, but it showed to be complementary with the HpH strategy (identifying 20% additional peptides and proteins). The combination of the HpH StageTip method followed by SCX StageTip fractionation of the resulting fractions yielded a total of 32,601 peptides and 4,257 proteins. Interestingly, reversing the order of these stages (i.e. SCX followed by HpH) increased peptide and protein yields by 20% and 10%, respectively. In light of these results, the application of SCX followed by HpH fractionation of the resulting fractions provides the best alternative to generate spectral libraries in a fast and robust manner.

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Poster Session – Poster 21

Analysis of Plasma Depleted Samples for Chronic Diseases' Biomarker Discovery Using 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. Due to the lack of diagnosis or their diagnosis at late stages most chronic disease are underdiagnosed. 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. For that reason, depletion of high-abundant plasma proteins, such as albumin or immunoglobulins, is essential to reduce sample complexity and enhance the detection of low-abundant proteins.

Here we aimed at identifying plasma biomarkers for colorectal cancer (CRC) and Alzheimer' disease (AD), two of the most common chronic diseases worldwide. Plasma samples were depleted using three different strategies to enrich them in low-abundant proteins that might be useful as biomarkers of these diseases: immunoaffinity chromatography, ENRICH-iST kit, and Mag-Net method. Subsequently, depleted plasma and 1 µL of neat plasma samples were trypsin digested and analysed using data independent acquisition (DIA) in the Orbitrap Astral, with 15-minutes gradient. Nearly 4000 plasma proteins were identified in the plasma samples depleted by the non-conventional immunoaffinity methods with the Astral mass spectrometer. Additionally, the efficacy of plasma enrichment methods was assessed using parallel reaction monitoring (PRM) in the Orbitrap Astral mass spectrometer, with 8.5-minutes gradient to detect previously described CRC and AD plasma biomarkers. The superior performance of the combination of plasma depletion and the Orbitrap Astral mass spectrometer was further demonstrated for targeted proteomics analyses.

The implementation of plasma protein depletion strategies is crucial for enhancing the sensitivity and depth of mass spectrometry analyses, enabling the identification of a broader range of proteins and potential biomarkers for various diseases. This approach benefits both discovery and targeted proteomic analyses, offering valuable insights into disease mechanisms and potential diagnostic markers.

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Poster Session – Poster 22

Aortic valve calcification in patients with type 2 diabetes mellitus: role of the Apolipoprotein C-II

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Aortic stenosis (AS) and diabetes mellitus (DM) are both progressive diseases that if left untreated, result in significant morbidity and mortality. Several studies revealed that the prevalence of DM is substantially higher in patients with AS and, thus, the progression from mild to severe AS is greater in those patients with DM. Our goal is presenting a method for the treatment, prognosis and/or diagnosis of both diseases.

A multi-omic strategy was performed in a cohort of 47 patients with AS and with or without DM. In the discovery phase, we analyzed aortic valve tissue, calcified and non-calcified, by Tandem Mass Tag (TMT) and by Transcriptome sequencing using RNA-Seq. Then, For the quantitative analysis, the ratio of calcified:non-calcified tissue of each patient was compared. After the statistical analysis of the proteome and transcriptome of the AVs from patients with DAS, with or without DM, the results were compared to evaluate the correlation between them and to identify robust biomarkers for possible clinical use.

The APOC2 protein and APOC2 gene gave a p-value < 0.05 and a fold change (FC) > 1.5 in both analyses, and these values moved in the same direction between the study groups Thus, the APOC2 protein was considered the most robust potential biomarker since it was identified in both the proteomic and transcriptomic analysis, and moreover, it was confirmed in plasma samples from an independent cohort of patients when studied by ELISA and turbidimetry. Using both these techniques, the amounts of this protein were higher in the group of diabetic patients relative to the non-diabetic patients (ELISA p-value = 0.018; turbidimetry p-value = 0.038).

Therefore, the present study aims to resolve an unmet medical need to achieve a diagnosis of AS in patients with T2DM.

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Poster Session – Poster 23

Identification and inhibition of deubiquitinases counteracting the function of UBE3A as a therapy for Angelman Syndrome

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Angelman Syndrome (AS) is a rare, but nonetheless highly relevant neurogenetic disease caused by the absence or dysfunction of UBE3A protein in the brain (Angelman, 1965; Kishino et al., 1997; Margolis et al., 2015). Currently, AS has no specific treatment, which highlights the need for research into the underlying causative mechanism. UBE3A is an E3 ubiquitin ligase, thus, patients with AS exhibit an aberrant ubiquitination pattern in neurons that is translated into a highly complex clinical profile (Williams et al., 2010). Consequently, restoring the normal ubiquitination pattern should help improving the symptomatology of AS. Deubiquitinases (DUBs) are a group of enzymes that antagonize E3 ligase activity by removing ubiquitin from target proteins. They are considered more accessible pharmacological targets than E3 ligases. Therefore, we propose that selectively inhibiting the DUB(s) counteracting UBE3A function should ameliorate the symptomatology of AS. In the present study we aim to identify DUBs that counteract the function of UBE3A. To achieve this, we propose two different approaches: (i) identifying UBE3A interactors *in vivo* in fly brains using the bioID system (Mehus et al., 2016; Roux et al., 2012, 2013), and (ii) identifying UBE3A substrates *in vivo* in a murine AS model using the bioUb system (Franco et al., 2011). Both systems, bioID and bioUb, are based on the *E. coli* BirA enzyme; while bioID allows the biotinylation of proteins in the neighbouring area of a protein of interest, bioUb is based on the selective biotinylation of ubiquitin molecules. Once candidate DUB(s) are identified using the proposed strategies in combination with mass spectrometry, they will be inhibited in AS mice models to assess their impact on AS symptomatology.

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Poster Session – Poster 24

Identification of a proteomic profile to monitor the progression of Hutchinson-Gilford progeria syndrome

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Hutchinson-Gilford progeria syndrome (HGPS) is an ultrarare genetic diasease (prevalence 1 person in 18 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 new biomarkers for monitoring HGPS progression by a proteomic approach on plasma samples and the subsequent integration of the results using bioinformatics tools. A discovery phase was carried out employing an analysis with liquid chromatography coupled to mass spectrometry using DIA-PASEF mode. For that purpose, 24 mouse plasma samples were used: 12 wild type (WT) and 12 $Lmna^{G609G/G609G}$. After the functional analysis of the results, a verification phase was carried out. This analysis allowed us to identify 70 proteins of interest that showed differences in expression between WT and $Lmna^{G609G/G609G}$ mice. After bioinformatics analysis, 3 proteins were selected for verification due to their role in cardiovascular diseases. These proteins are related to complement and coagulation cascades and other functions such as sulfatase and glycan degradation. Differential proteomic analysis has so far enabled the identification and verification of 3 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. Therefore, such studies are highly significant, as they allow us to move closer to personalized medicine.

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Poster Session – Poster 25

Experimental benchmark of two approaches to quantify the extracellular vesicle proteome of plasma sample

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Plasma proteomics has gained notorious relevance in the clinical field for its potential in biomarker discovery and diagnosis (*Núñez et al., 2022*). However, plasma proteomics analysis is very limited due to the high dynamic range of its content, which restricts the detection of low abundant species. Moreover, circulating extracellular vesicles (EV), including microvesicles, exosomes and apoptotic bodies (*Christine et al., 2023*) have gained attention, since their protein cargo can be a potential indicator of diseases. In order to study EV in plasma, different purification methods are available, such as immunoaffinity capture, size-exclusion chromatography and ultracentrifugation. These strategies aim to enrich the plasma proteome for EV components, while depleting high abundant plasma proteins. Ultracentrifugation is a sample and time-intensive process and results in moderate exosome purity (*Kverneland et al., 2023; Lai et al., 2022)*. In contrast, enrichment of EV using strong anion exchange (SAX) has recently been presented as an alternative for deep plasma proteomics with good EV coverage (*Christine et al., 2023)*. Preliminary results from our group confirm the potential of SAX-based strategy to deplete abundant plasma proteins, such as ALB, HBB or APO-A1, while enriching at the same time relevant EV markers such as CD9 or RAB11, as well as relevant tissue markers present as vesicle cargo.

This work aims to benchmark SAX *vs.* ultracentrifugation in terms of plasma and EV proteome coverage and for technical and quantitative robustness. We employed porcine plasma samples, which were processed using three different strategies: non-depleted plasma, SAX-based EV-enrichment and ultracentrifugation. Samples were analysed using an Evosep One coupled to a Thermo Orbitrap Eclipse using data-independent acquisition (DIA). Our results show that both ultracentrifugation and SAX effectively improve the plasma proteome coverage of non-depleted plasma samples when doing single-shot analysis and short gradients. However, ultracentrifugation yields higher proteome coverage than SAX, allowing the quantification of >1000 protein-coding genes, including 87 of the exosome markers reported in ExoCarta. Although, the SAX protocol resulted in lower yield of ~800 protein-coding genes, from an experimental standpoint, it is a relatively simple and cost-effective protocol, making it more accessible for large cohorts than ultracentrifugation.

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Poster Session – Poster 26

Proteomic characterization of the effect of chondrogenic factors delivered by recombinant adeno-associated virus for gene therapy in musculoskeletal diseases

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Purpose

Human mesenchymal stem cell (hMSC)-based gene therapy offers a promising approach for treating musculoskeletal diseases. This project aims to characterize proteomic modulations elicited by different chondrogenic factors, providing insight into pathways and mechanisms involved in cartilage regeneration.

Methods

Different chondrogenic factors (IGF-I, FGF-2, TGF-β, SOX9 and *LacZ* as control) were delivered by recombinant adeno-associated virus (rAAV) vectors to develop gene transfer for hMSC-based therapy. hMSC cultures were prepared and maintained for 21 days in a defined chondrogenic medium for transduction with the different rAAV conditions. Then, supernatants were discarded and the micromass was retained for the proteomic profiling study. Shotgun label-free proteomics was performed using a TimsTOF-Pro mass spectrometer in data-independent acquisition (DIA) mode. Raw data were processed with DIANN software in library-free mode and a quality control procedure was performed to minimize false positives. Qualitative changes were analyzed by chi-squared (p-value< 0.05). Differential expression analysis employed protein-wise linear models and empirical Bayes statistics (adjusted p-value < 0.05, log2 fold change ≥ 0.5). Pathway enrichment analysis was performed using Metascape.

Results

A total of 25 micromass samples were analyzed by MS (5 samples of each chondrogenic factor and 5 samples of *LacZ*). Proteomic analysis identified 3,565 proteins in micromasses, revealing distinct profiles for each factor. Qualitative changes analysis showed unique protein modulations: IGF-I (54 proteins), FGF-2 (36 proteins), TGF-β (74 proteins), and SOX9 (136 proteins). In another hand, quantitative analysis identified significant differential expression for IGF-I (85 proteins), FGF-2 (64 proteins), TGF-β (76 proteins), and SOX9 (94 proteins). Pathway analysis was employed to explore the different mechanisms that are driven by each chondrogenic factor. SOX9 uniquely modulated 209 proteins involved in ossification, neutrophil degranulation, and the core matrisome. TGF-β specifically altered 93 proteins linked to RNA metabolism and protein catabolism. IGF-I influenced 71 proteins associated with intracellular transport, EGF-EGFR signaling, and metabolism. FGF-2 modulated 45 proteins related to glycoproteins and lipid metabolism.

Conclusions

This study highlights distinct proteomic signatures driven by different chondrogenic factors, identifying proteins critical for cartilage repair and regeneration. These findings underscore the therapeutic potential of hMSC-based gene therapy approaches.

Poster Session – Poster 27

Protein aggregation capture assisted profiling of the thiol redox proteome

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Oxidative damage is central to several pathologies, ranging from cardiovascular to neurological. Cysteine thiols act as sensors of oxidative damage through reduction-oxidation (redox) reactions. Thiol redox dynamics can be quantified through mass spectrometry-based proteomics by means of differential alkylation, which allows to differentiate between reduced or oxidized thiols.

In this work, we present a novel strategy based on the protein aggregation capture (PAC) protocol (Batth et al., 2019) to perform differential cysteine alkylation on beads. In comparison to previous protocols, such as FASILOX (Bonzon-Kulichenko et al., 2020) the presented here significantly reduced sample preparation time and costs without decreasing overall thiol or proteome coverage. Moreover, we also proved for the first time the possibility to study the thiol redox proteome using Data Independent Acquisition (DIA) analysis with 'in silico' generated libraries. We tested our novel PAC-assisted redox profiling protocol using DIA on primary cell lines (HUVEC) and myocardial tissue (from atrial fibrillation and ischemia-reperfusion injury pig models), and we validated this approach in a biological model of oxidative stress analyzed using FASILOX, consisting on the assessment of oxidative damage produced by reperfusion and the cardioprotective effect of preconditioning (Binek et al., 2024). We observed few differences in protein and precursor identification performance between PAC and FASP samples, suggesting that performance is similar independently on the digestion method. Samples were acquired in an Evosep (15SPD) coupled to a Thermo Orbitrap Eclipse. Using DIANN (v1.8.1), we were able to quantify 4,000 proteins and >40,000 modified peptides in atrial tissue samples. 30% of the cysteine-containing peptides identified were reversibly-oxidized, reflecting a high oxidation level in the samples. Moreover, using this workflow we were able to measure the oxidative damage caused by reperfusion after infarction and the cardioprotective effect of preconditioning therapy. In summary, we present a high-throughput and streamlined sample preparation PAC-based strategy for redox proteomics, that is compatible with isobaric labeling or can be combined with DIA analysis using label-free quantitative workflows.

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Poster Session – Poster 28

Proteomic and transcriptomic profiling of tumor microenviroment and extracellular vesicles production in Lung Cancer patients

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Lung cancer (LC) is the world's leading cause of cancer death, and the late stage of diagnosis makes necessary to analyze the molecular mechanisms that underlie its progression to face the disease. In addition to the tumoral tissue processes and microenvironment, the extracellular vesicles (EVs) function is also relevant in tumor biology, as they play an important role in proliferation, metastasis and epithelial-mesenchymal transition *(Xu et al, 2021).*

Next Generation Sequencing technologies and data repositories allow the scientific community to leverage the potential of massive data generated by different tools. In this study, in silico analysis was performed to gain insight into the molecular basis behind EVs production at tissue level through quantitative proteomics (*Wang et al, 2024*), and to deepen understanding of cell heterogeneity through transcriptomics analysis of data stored in a single-cell atlas *(Salcher et al, 2022)*. All these data were generated from non-small cell lung cancer (NSCLC) patients' samples, including lung adenocarcinoma (LUAD) and squamous-cell lung carcinoma (LUSC) patients.

Both perspectives were combined to carry out a preliminary interpretation of the results. Different expression patterns were identified at transcriptomics and proteomics levels, including alteration of transcripts and proteins related to different biological functions and signaling pathways related to extracellular matrix (ECM) interactions, cytokines signaling and immune responses.

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Poster Session – Poster 29

Titin degradation during postmortem maturation of normal and defective bovine meats

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Pre-slaughter stress (PSS) in cattle is a remarkable cause of problems contributing to the apparition defective meat with abnormally high ultimate pH that decreases commercial benefits. Development of straightforward and reliable analytical strategies aiming at the precocious prediction of defective meat is essential to produce high quality products. This can be achieved through the elucidation of unambiguous and robust protein biomarkers satisfying quality requirements demanded by meat industry and consumers. Moreover, the affordable search of protein descriptors dedicated to meat quality studies should be ideally supported by conventional MS-based approaches flexible enough to combine exploratory and targeted analyses coupled to friendly data processing.

In this work, there were characterized the different degradation profiles of giant protein titin during postmortem ageing (14 days) of normal and PSS defective bovine meats. Visual differences in titin profiles of soluble extracts from samples assayed were studied by means of SDS-PAGE. Since titin is normally found in the myofibrillar protein fraction, its presence in soluble extracts may characterize defective meat, most probably due to alterations in protein solubility because of high ultimate pHs. Intensity of this band increased during ageing, finding also the occurrence of a titin degradation product of lower Mr from 3 days postmortem onwards. LC-MS/MS analysis of tryptic digests of both intact and degraded titin combining CID and HCD activation types helped to speculate about the nature of the titin cleaving site during meat maturation, a phenomenon directly related to the development of meat tenderness. Understanding of the generation of titin ageing products may ease future research actions addressing routine quality assessment of aged meat.

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Poster Session – Poster 30

A complete and fast workflow for single cell proteomics analysis combining the tecan uno cell dispensing platform, Vanquish Neo LC and Exploris 480 DIA-MS

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Single-cell proteomics (SCP) allows for the differentiation of proteomic profiles at the single-cell level. This enables the exploration of diversity and state among subpopulations of cells with distinct expression profiles. The potential applications of SCP are highly diverse, including cancer research, characterizing cell type mechanisms; stem cell biology, understanding processes of pluripotency and differentiation; and precision medicine, understanding individual patient variability in response to treatments.

In SCP, sample preparation and LC-MS analysis are critical. Only recent mass spectrometers have enough sensitivity to achieve acceptable cellular proteome coverage. Consequently, SCP is a very recent field, and most studies conducted to date focus on the development and optimization of methods for individual cell dispensing, processing, and LC-MS analysis. The use of automated platforms increases robustness and decreases technical variability, in addition to promoting the standardization of protocols.

In this study, we used a relatively affordable automated platform, the Tecan UNO (HP D100), for the dispensing of single cells and subsequent processing in a single step (cell lysis and rapid enzymatic digestion with trypsin and Lys-C). Human mesenchymal cell digests were prepared in 96-well plates (total processing time < 75 minutes). The plates were loaded directly into the autosampler of a Vanquish Neo LC system (Thermo Scientific), avoiding sample transfers. Two commercial LC columns were tested in standard bulk proteomics setups, with 40 or 70 SPD throughput. MS analysis was performed in an Orbitrap Exploris 480 (Thermo), with a FAIMS Pro Duo ion mobility interface. Several DIA acquisition methods (*Petrosius.V et al,2023; Sanchez-Avila.X et al, 2023*), optimized for low-amount protein samples, were tested, and the runs were processed with Spectronaut v19 (directDIA workflow).

Up to 3500 protein groups were quantified, covering all groups of human cellular pathways and thus obtaining relevant biological information.

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