6TH CONGRESS OF THE SPANISH PROTEOMICS SOCIETY

NOVEMBER, 15th-18th, 2016
Cádiz, SPAIN

SEProt
Sociedad Española
de Proteómica

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2016

Abstact Book

SEProt
Sociedad Española
de Proteómica

UCA
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SEProt
Sociedad Española
de Proteómica

SEDE SOCIAL

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Carta de bienvenida del Presidente de la Sociedad Española de Proteómica (SEProt)

Queridos amigos y colegas:

Parece que fue ayer pero ya ha pasado más de una década desde que un grupo de entusiastas pioneros fundaron la Sociedad Española de Proteómica; es un placer dedicarles el presente congreso y rendirles homenaje. Dicho homenaje será personificado en Juanjo Calvete, nuestro primer presidente, cuyo entusiasmo, visión y dedicación fueron fundamentales para que la SEProt iniciase su andadura con paso firme. Juanjo impartirá la conferencia de clausura en este congreso y recibirá un merecido reconocimiento de parte de todos. La conferencia inaugural será impartida por el Prof. Albert Heck, quien pasará a formar parte del selecto grupo de Socios Honorarios de la SEProt. El galardón es en reconocimiento de su carrera científica y su labor en el campo de la espectrometría de masas, especialmente por el desarrollo de métodos innovadores para la caracterización de sistemas biomoleculares, con especial énfasis en el estudio de interacciones entre proteínas y sus modificaciones post-traduccionales. Será para nosotros un placer recibir al Prof. Heck en nuestro congreso.

En la todavía corta vida de la SEProt, han pasado muchas cosas, la mayoría buenas, pero también algunas malas. Desafortunadamente, Juan Pablo Albar no está hoy con nosotros. Él fue un pilar de nuestra sociedad, en la que participó activamente desde su Junta Directiva, promoviendo todo tipo de actividades y representándonos a nivel internacional. Recuerdo con especial cariño el 4.º Congreso, que organizó conjuntamente con Concha Gil en Segovia y que fue un auténtico éxito. Juan Pablo nos dejó mientras preparaba el Congreso de HUPO de 2014, y sin duda le echaremos de menos en Cádiz.

Desde la Junta Directiva de la SEProt, os animamos a participar activamente en nuestras actividades, no solo el congreso, sino también las Jornadas de Jóvenes Investigadores y los *workshops* y reuniones educacionales. Quisiera resaltar especialmente nuestras convocatorias de becas, especialmente concebidas para que todos vosotros –sobre todo los más jóvenes– tengáis apoyo económico a la hora de acudir a reuniones formativas, *workshops*, o simplemente para realizar una estancia en otro laboratorio para realizar un proyecto, aprender y compartir conocimiento científico. A partir del próximo año, vamos a incrementar el número de convocato-

rias a tres por año, incrementando así mismo el presupuesto, así que os animamos a participar. Así mismo, también ofreceremos ayudas para jóvenes investigadores con el objetivo de establecer redes colaborativas con otros laboratorios europeos; esto se hará como parte de una iniciativa de EuPA. Además de todo lo anterior, este año estamos cambiando nuestra imagen institucional, con una nueva página web y un nuevo logo, en cuya elección tuvisteis la oportunidad de participar.

En los próximos años, continuaremos participando en actividades internacionales, manteniendo nuestra representación en EuPA y HUPO. Tras la exitosa organización del congreso de HUPO en Madrid en 2014, afrontaremos ahora el reto de organizar el Congreso de 2018 de EuPA, que se celebrará en Santiago de Compostela, y que será un congreso conjunto con el nuestro de la SEProt y el de la Asociación Portuguesa de Proteómica (ProCura).

En resumen, tenemos un futuro prometedor ante nosotros, lleno de expectativas y retos; sigamos caminando juntos para garantizar la prosperidad de nuestra joven sociedad.

¡Espero que disfrutéis del congreso y de vuestra estancia en Cádiz!

Un abrazo,

Ángel García

Presidente de la Sociedad Española de Proteómica

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

Dear friends and colleagues,

It seems it was yesterday but it´s been more than a decade since a group of enthusiastic pioneers founded the Spanish Proteomics Society; the present congress is devoted to them. Indeed, we will pay tribute to Juanjo Calvete, our first president, whose enthusiasm and dedication were fundamental in the early years for the society to take off. He will give the closing lecture in this congress and receive a deserved recognition from us all. The opening lecture will be given by Prof. Albert Heck, who will be named Honorary Member of SEProt, in recognition for his distinguished career in the field of protein mass spectrometry, and particularly for the development of methods for the characterization of biomolecular systems, with emphasis on protein post-translational modifications and interactions. It will be a pleasure to have Prof. Heck in our congress.

Many things have happened since SEProt was founded in 2004; most of them good, but some of them bad. Unfortunately, Juan Pablo Albar is not with us today. He was a pillar of SEProt, always pushing forward our activities, and representing us at international level. He successfully chaired, together with Concha Gil, our 4th Congress, which took place in Segovia in 2011. He left us while preparing the 2014 HUPO Congress and we will miss him in Cádiz. To honor his memory, we decided to rename the SEProt Award, given in our congresses thanks to the sponsorship of Bruker, as the Juan Pablo Albar Award.

From the SEProt executive board we encourage you to participate actively in all our activities, not only our congress, but also our young investigators meetings and educational events. I would also like to highlight our fellowship calls. They are specially conceived to support our members to go to workshops, educational meetings or simply to stay for research purposes in other labs, learning and sharing scientific knowledge. From next year, we will increase to three the number of calls for our fellowships, increasing the budget because we want to support our members, especially the youngest. We will also offer grants to help to establish European collaborative networks involving young researchers; this will be done in conjunction with EuPA. Besides the above, this year we are also changing our institutional image, with a new webpage, and a new logo, in whose election all of you had the opportunity to participate.

We continue to participate in international activities, being represented in EuPA and HUPO committees. Following the successful organization of the HUPO congress in Madrid in 2014, we now face the challenge of organizing the 2018 EuPA congress in Santiago de Compostela, which will be a joint meeting with SEProt and our colleagues from the Portuguese Proteomics Association.

We have exciting times ahead, full of expectations and challenges; let´s walk together to guarantee the prosperity of our young society.

I hope you will enjoy our congress and your time in Cádiz!!

Best regards,

Ángel García

President of the Spanish Proteomics Society

Bienvenida del Comité Organizador

Estimados amigos y colegas:

Es un gran honor daros la bienvenida al Sexto Congreso de la Sociedad Española de Proteómica, en Cádiz, la ciudad más antigua de occidente.

Desde el inicio de la civilización, esta tierra ha sido asentamiento privilegiado para que fenicios, griegos y romanos desarrollaran sus tareas mineras, comerciales y culturales. Tras el descubrimiento de América, Cádiz se convirtió en la plataforma principal del intercambio socio-cultural con el nuevo mundo recién descubierto. En este entorno, se iniciaron los estudios universitarios en nuestra universidad asociados a la creación en el siglo xv, cuando se crea el Colegio de Pilotos de los Mares de Levante y Poniente, inicio de los estudios de medicina. En la actualidad, la Universidad de Cádiz permanece fuertemente vinculada al territorio, estructurada en cuatro campus con un total de 64 titulaciones, con cerca de 20.000 alumnos.

Con toda la ilusión de la que somos capaces, y en circunstancias aún difíciles, desde el comité organizador hemos programado un congreso donde ponentes destacados ilustraran los más recientes avances en proteómica cuantitativa, PTMs, tecnologías emergentes, bioinformática, proteómica clínica, microbiológica, vegetal y de otros organismos huérfanos. Junto con destacados ponentes que abrirán cada sesión, tendremos el honor de nombrar Miembro honorario de nuestra sociedad al profesor Albert Heck.

Este congreso es además la oportunidad para celebrar que nuestra sociedad ya tiene más de una década de existencia. Aunque aún joven, este congreso tiene que detenerse, mirar atrás y agradecer a los que empezaron el trabajo realizado. Sopesar donde partimos y donde hemos llegado entre todos. El profesor Juanjo Calvete, primer presidente de la SeProt, protagonizará la ceremonia de clausura.

Como en años anteriores, la labor investigadora de los miembros de nuestra sociedad será premiada con los Premios Juan Pablo Albar al mejor artículo, a la mejor comunicación oral y al mejor poster. Además otorgaremos 3 ayudas de viaje a una selección de las mejores comunicaciones orales y posters. Así mismo, este año introducimos la novedad de que una selección de pósters serán comentados en presencia de un moderador durante las correspondientes sesiones, con el fin de hacerlas más participativas y dinámicas.

Todo este trabajo y esfuerzo tiene como principal objetivo, conseguir que el 6.º Congreso de la Sociedad Española de Proteómica, en Cádiz, brinde a sus miembros un entorno inmejorable para el debate y la interacción entre sus socios, que logre reavivar la ilusión y en trabajo cooperativo en nuestro campo científico.

El comité organizador:

Francisco J. Fernández-Acero
Ángel García
Fernando Corrales
Cristina Ruiz-Romero

Albert J. R. Heck

Honorary Member of the Spanish Proteomics Society

The Spanish Proteomics Society (SEProt) is proud to announce the appointment as Honorary Member of Prof. Albert J. R. Heck for a distinguished career in the field of Biomolecular Mass Spectrometry, and particularly in recognition of his innovative research in the development of methods applicable to the characterization of biomolecular systems, with emphasis on protein post-translational modifications and interactions.

Prof. Heck is Head of the Biomolecular Mass Spectrometry & Proteomics group at the University of Utrecht since September 1998. He holds the chair of Biomolecular Mass Spectrometry & Proteomics, a position jointly funded by the Departments of Chemistry and Pharmaceutical Sciences. His major research interest is the development and implementation of innovative mass spectrometric methods for the more efficient and detailed characterization of proteins and other biomolecules in relation to their biological function. The emphasis is on the structural and functional characterization of proteins and their post-translational modifications as well as on the investigation of protein interaction networks, and protein complexes.

The Heck laboratory introduced TiO_2 as enrichment material for the targeted analysis of phosphopeptides, and implemented this technique into a miniaturized on-line automatic system, and on a micro-chip device. They introduced the use of a protease named LysN, which in conjunction with ETD provides unique sequence ladders that are straightforward to interpret and allow facile de novo sequencing and improve the analysis of protein phosphorylation. Prof. Heck´s group also have an extensive track-record in quantitative proteomics, introducing metabolic stable isotope labeling in multicellular organisms such as Drosophila and C. elegans, using SILAC for studying stem cell and B cell differentiation, and stable isotope labeling by using chemical approaches. Prof. Heck is also a pioneer in macromolecular or native mass spectrometry, which enables the analysis of intact protein assemblies by mass spectrometry.

Prof. Heck is scientific director of the Netherlands Proteomics Centre since 2003. He is member of the council of the Human Proteome Organization (HUPO). In 2001 he was recipient of the Golden Medal of the Dutch Royal Chemical Society. In 2004 he received an honoree Utrecht University «ABC»-professorship. In 2006 he received the Descartes-Huygens award from the French Academy of Sciences and in 2010 the «Massenspektrometrie in den Biowissenschaften Preis» from the German Society for Mass Spectrometry. More recently, the Dutch Society for Mass Spectrometry has honored Albert Heck with the Outstanding Research Award 2016 in recognition for his many excellent contributions to the field of Mass Spectrometry. He has currently published more than 350 papers in internationally reviewed journals.

The Spanish Proteomics Society is greatly honored by Professor Albert Heck having accepted to become Honorary Member.

Ángel García

SEProt President

Convocatoria premio de la SEProt

Convocatoria del sexto premio de la Sociedad Española de Proteómica Premio Juan Pablo Albar

Cádiz, febrero de 2016

La Sociedad Española de Proteómica (*SEProt*) convoca la sexta edición del PREMIO SOCIEDAD ESPAÑOLA DE PROTEÓMICA, rebautizado como PREMIO JUAN PABLO ALBAR,destinado a reconocerla labor en el campo de la Proteómica de un científico que desarrolle su actividad en España. El premio, patrocinado por **BRUKER ESPAÑOLA S. A.** (http://www.bruker.es), está dotado con 2000 € y será entregado por un representante de Bruker Española durante el 6.º Congreso de la SEProt que se celebrará en Cádiz entre los días 15-18 de noviembre de 2016 (http://seprot2016.uca.es/). En la presente convocatoria se otorgarán **tres galardones** que en ningún caso podrán ser compartidos. Un premio (1.000 € y diploma acreditativo) será para **una publicación científica** relacionada con cualquier desarrollo o aplicación de la Proteómica. Un segundo galardón (500 € y diploma acreditativo) será para una contribución en forma de **póster** al 6.º Congreso de la SEProt. Un tercer galardón (500 € y diploma acreditativo) será para una contribución en forma de **comunicación oral** presentada en el 6.º Congreso de la SEProt. Las decisiones de los jurados serán inapelables. Los Premios no podrán concederse al mismo científico dos veces y podrán quedar desiertos si así lo decidiese el jurado.

La elección de la publicación científica merecedora del galardón será realizada por un jurado de expertos designado a tal efecto por el Presidente de la SEProt. Solo podrán optar al Premio científicos españoles, sean o no socios de la SEProt. La labor investigadora deberá haber sido realizada en España y haber sido publicada entre Enero de 2014 y Diciembre de 2015. Los candidatos deberán remitir 1 copia del trabajo en **formato electrónico** ala Secretaria de la SEProt (Cristina Ruiz,

(cristina.seprot@gmail.com). Deberán, asimismo, adjuntarse los datos personales y profesionales del candidato, y un breve resumen de las razones que, a juicio del candidato, debieran ser consideradas por el jurado. **La fecha límite para la recepción de los trabajos será el 1 de Abril 2016**. La resolución se dará a conocer el día 2 de Mayo de 2016 a través del portal electrónico de la SEProt y del Congreso. El galardonado o galardonada será invitado/a participar en el 6.º Congreso de la SEProt con exención del pago de las tasas de inscripción y a publicar, de ser el caso, un artículo en la revista de la SEProt.

La elección de los ganadores correspondientes a las contribuciones en formato panel y oral presentadas al 6.º Congreso de la SEProt se efectuará entre los primeros firmantes de los estudios por un jurado designado a tal efecto por el Presidente de la Sociedad Española de Proteómica. Solo podrán optar al Premio científicos españoles, sean o no socios de la SEProt. La labor investigadora considerada deberá haber sido realizada en España y ser inédita o, en todo caso, no haber sido publicada con anterioridad al 31 de diciembre de 2015.

Ángel García

Presidente de la SEProt

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Resolución del Sexto premio de la Sociedad Española de Proteómica

Premio Juan Pablo Albar

El jurado encargado de valorar las candidaturas recibidas para optar al Sexto Premio de la Sociedad Española de Proteómica - Premio Juan Pablo Albar,dentro de su apartado destinado a reconocerla labor en el campo de la Proteómica de un científico que desarrolle su actividad en España, ha decidido que le sea concedido tal galardón a **Iakes Ezkurdia** por su artículo «Multiple evidence strands suggest that there may be as few as 19000 human protein-coding genes», publicado en Human Molecular Genetics en Noviembre de 2014.

Este premio, patrocinado por **BRUKER ESPAÑOLA S. A.**(http://www.bruker.es), está dotado con 1.000€ y será entregado por un representante de Bruker Española durante el 6.º Congreso de la SEProt, que se celebrará en Cádiz entre los días 15-18 de Noviembre de 2016 (http://seprot2016.uca.es/). La decisión del jurado es inapelable.

Por el Jurado de la SEProt designado al efecto, a 2 de mayo de 2016.

Ignacio Casal *Eliandre de Oliveira* *Félix Elortza*

Programme Overview

	Tuesday 15th	Wednesday 16th	Thursday 17th	Friday 18th
9:00	**Pre-Congress Workshop** *(9:00-13:30)* Introduction to Skyline	**S1 Clinical Proteomics** *(9:00-11:00)* *Jean Charles Sánchez*	**S4 Post-translational Modifications** *(9:00-11:00)* *Chunaram Choudhary*	**S7 Quantitative Proteomics** *(9:00-11:30)* *Kathryn Lilley*
10:00				
11:00		Coffee Break	Coffee Break	
		S2 Microbial Proteomics *(11:30-13:30)* *Manuel J. Rodríguez-Ortega*	**S5 Computational Proteomics** *(11:30-13:30)* *Jürgen Cox*	Coffee Break
12:00				**Closing Lecture** *(12:00-13:00)* *Juanjo Calvete*
13:00				**Poetómica** *Jesús Vázquez*
		Lunch *(13:30-15:00)*	Lunch *(13:30-15:00)*	**Juan Pablo Albar Prize Awards & Closing Ceremony** *(13:15-13:45)*
14:00				
15:00		Poster Session	Poster Session	
16:00	**Registration Opens** *(16:00)*	**S3 Plant and other non-model Orphan Organisms** *(16:00-18:00)* *Wolfram Weckwerth*	**S6 Emerging Technologies** *(16:00-18:00)* *Benjamin Baluff*	
17:00				
	Welcome and Opening Lecture *(17:30-19:00)* *Albert Heck*			
18:00		**Progenesis QI Seminar** *(18:00-19:00)*	Coffee Break	
			SEProt General Assembly *(18:30-18:30)*	
19:00	**Welcome Cocktail** *(19:00-20:00)*			
20:00			**Congress Dinner**	

Scientific Programme

Tuesday, 15th November

Pre-Congress Workshop

9:00-13:30 Quantitative Proteomics: Introduction to Skyline

Chairs: Montserrat Carrascal, Concha Gil

Laboratorio de Investigación Marina (Labimar), Castillo de San Sebastián, Cádiz

16:00 Registration opens

Welcome and Opening Session

Chairs: Ángel García, Cristina Ruiz-Romero, Fernando Corrales, Francisco J. Fernández-Acero

17:30-17:45 **Welcome**

Opening Lecture

17:45-18:45 Complementary Methods for Probing Protein Assemblies and Interactions

Albert Heck *(Biomolecular Mass Spectrometry and Proteomics, Utrecht University, The Netherlands)*

18:45-19:00 **Designation of SEProt Honorary Member: Prof. Albert Heck**

Welcome Cocktail

19:00-20:00 *Parador de Cádiz*

Wednesday, 16th November

Session 1: Clinical Proteomics (Sponsored by ABSciex)

Chairs: Fernando Corrales, Ángel García

Plenary Lecture

9:00-9:40 Cerebrovascular disease biomarkers: diagnostic associated to treatment issues

Jean-Charles Sanchez *(Department of Human Protein Science. CMU. Université de Genevé, Switzerland)*

Oral Communications

9:40-9:53 Increased peroxiredoxin-6 and oxidized apolipoprotein A1 as hallmarks of redox imbalance in human abdominal aortic aneurysm

Inmaculada Jorge *(Centro Nacional de Investigaciones Cardiovasculares, Spain)*

9:53-10:06 Proteomic characterization of new transcription factors associated with a more invasive phenotype in colorectal cancer

Ignacio Casal *(Functional Proteomics Laboratory. CIB-CSIC. Madrid. Spain)*

10:06-10:19 Intercellular communication via connexin channels between cartilage, synovial membrane and subchondral bone: implications for joint homeostasis

Paula Carpintero-Fernández *(CellCOM-SB Research Group, INIBIC, A Coruña, Spain)*

10:19-10:32 Quantitative proteomics reveals Piccolo as a candidate serological correlate of recovery from Guillain-Barré syndrome

Margarita Villar *(SaBio, IREC, CSIC-ULCM-JCCM, Ciudad Real, Spain)*

10:32-10:45 Application of thiol redox proteomics to clinical heart valve disease samples

J. Daniel Cabrera-García *(Instituto de Investigación Sanitaria Princesa (IIS-IP), Madrid, Spain)*

10:45-10:58 mTOR inhibition Remodels Extracellular Matrix Components of Human Osteoarthritic Cartilage

Berta Cillero-Pastor *(M4I, Maastricht University, Maastricht, The Netherlands)*

11:00-11:30 **Coffee break sponsored by ABSciex**

Session 2: Microbial Proteomics

Chairs: Concha Gil, Francisco J. Fernández-Acero

Plenary Lecture

11:30-12:10 Infections by Gram-positive bacteria: search for new vaccines, diagnostic tools and antimicrobial agents

Manuel J. Rodríguez-Ortega *(Dpto. de Bioquímica y Biología Molecular, Universidad de Córdoba; Campus de Excelencia Internacional CeiA3, Córdoba, Spain)*

Oral Communications

12:10-12:25 HCV core protein interactions with host CD4+ T cells proteins

Cecilia Fernández-Ponce *(University of Cadiz and Puerto Real University Hospital Research Unit, School of Medicine, Cadiz, Spain)*

12:25-12:40 Candida albicans increases the release of proinflammatory Extracellular Vesicles in THP1 Macrophages

Jose A. Reales-Calderón *(Departamento de Microbiología II, Facultad de Farmacia, Universidad Complutense de Madrid, Spain)*

12:40-12:55 Analysis of the extracellular vesicles and proteins secreted from Candida albicans unravels new clues about protein secretion mechanisms

Ana Gil-Bona *(Departamento de Microbiología II, Facultad de Farmacia, Universidad Complutense de Madrid, Spain)*

12:55-13:10 Differential host immune recognition of the Candida albicans cell surface-associated proteome upon dimorphic transition in invasive candidiasis

Aida Pitarch *(Departamento de Microbiología II, Facultad de Farmacia, Universidad Complutense de Madrid, Spain)*

13:10-13:25 DiGE analysis as an interesting approach to identify glycoproteins involved in virulence in Ustilago maydis

Ismael Moreno-Sánchez *(Centro Andaluz de Biología del Desarrollo. UPO de Sevilla-CSIC-Junta de Andalucía, Sevilla, Spain)*

13:30-15:00 **Lunch**

15:00-16:00 **Poster Session**

Selected Poster Presentations

Session 3: Plant and other non-model Orphan Organisms

Chairs: Jesús V. Jorrín, Luis Valledor

Plenary Lecture

16:00-16:40 System-theoretical concepts of genome-scale molecular analysis, data integration and functional interpretation in biology and ecology

Wolfram Weckwerth *(Department of Ecogenomics and Systems Biology, University of Vienna, Austria)*

Oral Communications

16:40-17:00 Systemic Osmotic Stress Adaptation of *Chlamydomonas reinhardtii*

Francisco Colina *(Dpto. Biología de Organismos y Sistemas. Universidad de Oviedo, Spain)*

17:00-17:20 Changes in the protein profiles of Q. ilex seeds during germination and seedling establishment.

Rosa Sánchez Lucas *(Dpt. of Biochemistry and Molecular Biology. Agrifood Campus of International Excelence, ceiA3. University of Cordoba. Spain)*

17:20-17:40 Proteomic analysis of fish liver exposed to sublethal levels of polyaromatic hydrocarbons

Alberto Medina *(Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, España)*

17:40-18:00 iTRAQ-proteomics approach to understand low concentration citrate capped gold nanoparticles stress in the marine bivalve *Ruditapes philippinarum*

Miriam Hampel *(Andalusian Center of Marine Science and Technology (CACYT-MAR), Puerto Real, Cadiz, Spain)*

18:00-19:00 Progenesis QI seminar (Waters-nonlinear)

Thursday, 17th November

Session 4: Post-translational Modifications (Sponsored by Bruker)

Chairs: Montserrat Carrascal, Eliandre de Oliveira

Plenary Lecture

9:00-9:40 Systems-wide analysis of properties and functions of lysine acetylation and ubiquitylation

Chunaram Choudhary *(Center for Protein Research. University of Copenhagen, Denmark)*

Oral Communications

9:40-10:00 In-depth characterization of the phosphopeptidome displayed by HLA-B antigens: structural basis of the presentation of phosphorylated

Adán Alpízar *(CNB-CSIC, Madrid, Spain)*

10:00-10:20 Bottom-up strategies for the analysis of human alpha acid glycoprotein. Application to pancreatic disease diagnosis.

Estela Giménez *(Department of Analytical Chemistry, University of Barcelona)*

10:20-10:40 New methods for the in-depth characterization of posttranslational modifications underlying cardiovascular diseases

Celia Castans *(Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain)*

10:40-11:00 Nuclear phosphoproteomic analysis of interleukin-2 signaling reveals the pivotal role of ACLY in T-cell growth

Nerea Osinalde *(University of Southern Denmark, Odense, Denmark)*

11:00-11:30 Coffee break sponsored by Bruker

Session 5: Computational Proteomics

Chairs: Víctor Segura, Gorka Prieto

Plenary Lecture

11:30-12:10 The Perseus computational platform for comprehensive analysis of (prote) omics data

Jürgen Cox *(Computational Systems Biochemistry. MPI of Biochemistry)*

Oral Communications

12:10-12:30 Protein identification workflow for large proteomic datasets

Iakes Ezkurdia *(Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain)*

12:30-12:50 Quality Improvement of custom proteogenomic databases based on a predictor of peptide detectability

Elizabeth Guruceaga *(Unit of Proteomics and Bioinformatics, Center for Applied Medical Research, CIMA, University of Navarra, ProteoRed-ISCIII, Pamplona, Spain)*

12:50-13:10 On the Statistical Significance of Compressed Ratios in Isobaric Labeling: A Cross-Platform Comparison

Ana Martínez del Val *(Proteomics Unit, Spanish National Cancer Research Centre (CNIO), Madrid, Spain)*

13:10-13:30 An advanced method for hypothesis-free and rapid systematic identification of PTMs based on ultra tolerant search

Navratan Bagwan *(Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain)*

13:30-15:00 **Lunch**

15:00-16:00 **Poster Session**
Selected Poster Presentations

Session 6: Emerging Technologies (Sponsored by Thermo)

Chairs: Gloria Álvarez-Llamas, María E. González-Barderas

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

Plenary Lecture

16:00-16:40 Mass Spectrometry Imaging of Clinical Formalin-Fixed Paraffin-Embedded Tissues

Benjamin Balluff *(The Maastricht Multimodal Molecular Imaging Institute (M4I), Maastricht University, Maastricht, The Netherlands)*

Oral Communications

16:40-17:00 Exploring new proteomic strategies with Orbitrap Fusion Lumos: Liquid extraction surface analysis (LESA) to identify proteins directly from muscle tissue sections by combined Top-down and Bottom-up MS

Mar Vilanova *(Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute of Science and Technology, Barcelona, Spain)*

17:00-17:15 Biomarker validation and rapid determination of antibodies in cancer patients using Halo Tag Fusion protein-Modified Electrochemical Bioplatforms

María Garranzo-Asensio *(Dpto de Bioquímica y Biología Molecular I y IV, Universidad Complutense de Madrid, Spain)*

17:15-17:30 Development of beads-based protein microarrays to validate a panel of biomarker candidates for osteoarthritis

María Camacho-Encina *(Rheumatology Division, Proteomics Group, INIBIC, A Coruña, Spain)*

17:30-17:45 MALDI-Imaging Mass Spectrometry: a step forward in the anatomopathological characterization of stenotic aortic valve tissue

Laura Mourino-Álvarez *(Department of Vascular Physiopathology, Hospital Nacional de Parapléjicos, SESCAM, Toledo, Spain)*

17:45-18:00 Absolute quantification of protein and peptide biomarkers by isotope dilution tandem mass spectrometry using minimally 13C labelled peptides

Pablo Rodríguez-González *(Department of Physical and Analytical Chemistry, University of Oviedo, Spain)*

18:00-18:30 **Coffee Break**

18:30-19:30 **SEProt General Assembly**

Friday, 18th November

Session 7: Quantitative Proteomics (Sponsored by Waters)

Chairmen: J. Ignacio Casal, Félix Elortza

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Plenary Lecture

9:00-9:40 Challenges in assigning the sub-cellular location of proteins

Kathryn Lilley *(Cambridge Centre for Proteomics, United Kingdom)*

Oral Communications

9:40-10:00 Advances in Targeted Omics Quantitation Using a Novel Scanning Quadrupole DIA Method

Ian Edwards *(Waters Corporation, Wilmslow, United Kingdom)*

10:00-10:15 Impact Of Gene Overdosage In The Hippocampus And Cerebellum Proteome And Phosphoproteome

Mireia Ortega *(Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, PRBB, Barcelona, Spain)*

10:15-10:30 Understanding Ground State Pluripotency using Quantitative Proteomics

Javier Muñoz *(Proteomics Unit. Spanish National Cancer Research Centre (CNIO), Madrid, Spain)*

10:30-10:45 A proteomic analysis of 22 paired colorectal human tissue samples soluble secretome using label-free quantification

Beatriz Escudero-Paniagua *(Centro de investigaciones Biológicas, CSIC, Madrid, Spain)*

10:45-11:00 Proteomics Signature in Early Remodeling after Myocardial Ischemia/Reperfusion

Aleksandra Binek *(Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain)*

11:00-11:15 Targeted proteomics applied to the analysis of endogenous peptides in cartilage secretome, synovial fluid and serum from osteoarthritis patients and controls

Patricia Fernández-Puente *(Rheumatology Division, Proteomics Group INIBIC-CHUAC, A Coruña, Spain)*

11:15-11:30 Targeted proteomics approaches to study the molecular response of Glioma Stem Cells to an AKT inhibitor.

Carmen González-Tejedo *(Proteomics Laboratory, Centro Nacional de Biotecnología-CSIC, Madrid, Spain)*

11:30-12:00 **Coffee Break**

12:00-13:00 **Closing Lecture**

Proteomics of non-model organisms: a model for model-organism proteomics? A venomics perspective

Juanjo Calvete *(Laboratorio de Venómica y Proteinómica Estructural, Valencia, Spain)*

13:00-13:15 Presentation of the book «Poetómica»

Jesús Vázquez *(CNIC, Madrid)*

13:15-13:45 Juan Pablo Albar Prize Awards and Closing Ceremony

Francisco J. Fernández-Acero, Fernando Corrales, Ángel García, Cristina Ruiz-Romero

Opening & Closing Lectures

Opening Lecture

Complementary Methods for Probing Protein Assemblies and Interactions

[1, 2]AlbertJ. R. Heck

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Mass Spectrometry based proteomics has played a pivotal role in revealing the plethora ofprotein interactions that take place inside a cell, wherein proteinsform protein assemblies and/or signalling networks. Especially using affinity purification of a tagged proteins followed by mass spectrometric analysis of its binding partners a wealth of data has been gathered revealing the all-embracing protein networks present in cells. Following the charting of all these interactions, a next step will be to now gather more in-depth structural and functional information on these individual protein assemblies. This may come fromin-depth high-resolution structural models, as well as detailed information on how they function and dynamically evolve during cellular perturbations. Mass spectrometry may also contribute to this next level of protein interaction analysis although it does require partly different and novel approaches. To contribute to this emerging new area in proteomics, our group is developing new methods using native mass spectrometry and cross-linking mass spectrometry with the aim to bridge the gap between interaction proteomics and structural biology. These new innovations and applications of them in interaction proteomics will be central in this presentation.

In the first part of the talk native mass spectrometry and its applications in probing protein assemblies and interactions will be described. Novel developments in MS instrumentation for native MS will be highlighted, especially a new Orbitrap based instrument that offers high-sensitivity and mass resolution, allowing an in-depth detailed analysis of glycoproteins, viruses and even whole intact ribosomes.

The second part of the talk will highlight our recent work on cross-linking mass spectrometry. Cross-linking combined with mass spectrometry (XL-MS) providesanother powerful approach to probe the structure and interaction profile of protein assemblies. Up to now XL-MS has been primarily limited to the characterization of purified protein assemblies. We have set out to develop XL-MS methods aimed at probing protein interactions at the proteome level, using complete cell lysates or whole organelles as starting material. We, therefore, combined several novel innovative methods to address some of the hurdles in this field.These innovations include the use of a low energy CID cleavable cross-linker, novel hybrid pepetide fragmentation and acquisition strategies and a dedicated software suite, termed XlinkX. We applied this novel XL-MS strategy to lysates of *E. coli* lysate and human HeLa cell lines, and to mammalian mitochondria and nuclei. In each of these studies we successfully identified thousands of cross-links. Many of the identified cross-links could be validated by mapping them on available high-resolution structures, but the data also provide information on assemblies for which no high-resolution structures are available, and even reveal new protein interaction networks.

Closing Lecture

Proteomics of non-model organisms: a model for model-organism proteomics? A venomics perspective

J. J. Calvete

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Until recently, proteomics of non-model organisms was limited to tedious, homology-based techniques. Today, genomic or RNA-seq data can be straightforwardly obtained using next-generation sequencing, allowing the establishment of protein databases for any organism. Venoms represent excellent model systems for applying *omics* strategies to integrate proximate and ultimate causations to infer their natural histories. Although we are unable to revisit the past to reconstruct the natural histories of venoms and the organisms that produce them, knowledge gained by proximate cause questions can be incorporated into higher-level ultimate cause frameworks by formulating appropriate evolutionary hypotheses. Unveiling the temporal and spatial patterns of genetic and phenotypic variability of extant venoms is within the reach of current *omics* technologies, and in the near future the field of venomics will undoubtedly benefit from advances in proteomics, transcriptomics and genomics. In particular, the integration of top-down venomics, toxicovenomics, absolute quantitation, venom gland RNAseq, and comparative snake genomics will revolutionize the field of molecular toxinology in coming years, making possible the challenging task of achieving a full and integrated structural and functional description of locus-resolved venom proteomes into an appropriate evolutionary scenario. The time-calibrated framework of snake phylogeny and snake venom evolution are well established, and the emphasis on a combination of multiple *omic* approaches and other disciplines of ancestral state reconstruction, such as paleontology, evolutionary ecology and biogeography, add other dimensions to integrative venomics in its ability to put forth compelling, testable and falsifiable hypotheses about venom biology and evolution across the full taxonomical range of crown caenophidian snakes. Leveraging the ever increasing analytical capacity of *omic* technologies and the powerful explanatory capability of the evolutionary hypothesis to develop a «Standard Model» for achieving a systems biology view of the evolutionary ecology of the venomous system across caenophidian snakes.

Plenary Lectures

PL1

S1. Clinical Proteomics

Cerebrovascular disease biomarkers: diagnostic associated to treatment issues

Jean-Charles Sanchez

Translational Biomarker Group, Human Protein Sciences Department, Geneva University, CH-1211 Geneva 4, Switzerland.

There is currently few cerebrovascular disease (CVD) biological biomarkers that can add utility to the classical clinical and imaging parameters. This is especially true for the diagnosis, prognosis and treatment follow-up of stroke and subarachnoid hemorrhage. Research in the last decades have unravelled a number of potential candidates that have shown preliminary relative good performances including glial fibrillary acid protein (GFAP), neuron specific enolase (NSE), and S-100b. However, none of them are used routinely in clinical settings worldwide due to a lack of sensitivity, specificity and/or cost effectiveness. Cerebrovascular proteomics has the great potential to highlight better candidate biomarkers. Here we will present the strategy that was carried out to discover, verify and validate intent-of-use markers associated to acute (tPA and antibiotherapy) and chronic (aspirin) patient treatments and mangement in the fields of CVD.

PL2

S2. Microbial Proteomics

Infections by Gram-positive bacteria: search for new vaccines, diagnostic tools and antimicrobial agents

Manuel J. Rodríguez-Ortega

Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba; Campus de Excelencia Internacional CeiA3.

Bacterial infections are among the major causes of morbidity and even mortality in many countries, especially in the developing areas. During the last decades, there has been an increase in the frequency of infections that is also affecting the developed countries. Particularly, infections caused by some Gram-positive bacterial pathogens are becoming a major concern for our healthcare systems, because of the appeareance of new resistant strains, making it more difficult to face these infections.

Traditiionally, the best way to fight against infectious diseases is prevention by vaccination. When it is not possible, infections must be treated with antimicrobials. However, the overabuse of antibiotics is one of the causes of the increase of resistance against these substances. In addition, there is a necessity of having fast and reliable methods for the early detection of infection, in case of an epidemic episode.

Proteomics provides excellent platforms for the identification and characterization of protein candidates which can be used both for vaccine and diagnostic tools. In this talk, it will be shown the approaches applied in my research group for the discovery of vaccine candidates in several Gram-positive species, as well as the characterization of extracellular vesicles and their use for immunization. Also, it will be shown the development of a protein array as a serodiagnostic tool for pneumococcal infection. Finally, a dissertation about the integration with other «omics» will be made to show the utility for the study and characterization of new antimicrobial agents and its global effect on the cell physiology.

PL3

S3. Plant and other non-model orphan organisms

System-theoretical concepts of genome-scale molecular analysis, data integration and functional interpretation in biology and ecology

Wolfram Weckwerth

Department of Ecogenomics and Systems Biology, University of Vienna, Althanstr. 14, 1090 Vienna, Austria. Vienna Metabolomics Center (VIME), University of Vienna, Austria.

Systems biology is the approach to combine molecular data, genetic evolution, environment and species-interaction with the computer-assisted understanding, modeling and prediction of active biochemical networks. The idea relies strongly on the existence of complete genome sequences and the development of new technologies for the analysis of molecular data. Here, proteogenomic strategies as well as the projection of metabolomics data into genome-wide metabolic networks combined with metabolic modeling emerge as important technologies for improving gene annotation processes and understanding dynamic metabolic networks not predictable from genome sequences. Using RNAseq, proteomics and metabolomics we begin to investigate the genome-scale molecular phenotype. However, before data reveal their interrelation, extended statistical and mathematical concepts are required for the integrative analysis of multifactorial phenomena. The detection of significant correlations between the different components based on clustering, dimensionality reduction and other techniques is the basis for biological interpretation. Most of these data mining tools are closely related –based on covariance and/or correlations within a data matrix– and therefore have the potential for comparison of results originating from different procedures. We have developed concepts to systematically connect the genome-wide underlying biochemical regulation with these multivariate data mining procedures in OMICS data. After an introduction into System Theory and the technological concept of high throughput molecular analyses I will present mathematical and statistical frameworks for the interpretation of high throughput profiling data and their relation with genome-scale biochemical networks. Further I will present applications of integrated proteomics, phosphoproteomics, metabolomics and metabolic modelling in energy signaling networks in plants and animals as well as in ecological projects.

PL4

S4. Post-translational Modifications

Systems-wide analysis of properties and functions of lysine acetylation and ubiquitylation

Chunaram Choudhary

The Novo Nordisk Foundation Center for Protein Research, Faculty of Heath Sciences, University of Copenhagen, Denmark.

Lysine acetylation and ubiquitylation are evolutionarily conserved, frequently occurring post-translational modifications (PTMs), which are involved in regulation of diverse cellular functions. We use quantitative proteomics to investigate the scope and dynamics of acetylation and ubiquitylation in human cells. We applied quantitative mass spectrometry to investigate the dynamics of acetylation in response to most commonly used lysine deacetylase inhibitors. In addition to confirming the specificities of many deacetylase inhibitors, we discovered unexpected acetylation patterns for some inhibitors, and identified their acetylation targets affected in vivo.

I will also present our recent work on proteome-wide analysis of ubiquitylation in response to B cell receptor (BCR) and tumor necrosis factor alpha (TNF-a) receptor signaling. Our quantitative proteomic analyses of BCR-induced ubiquitylation identified an important function of linear ubiquitylation in BCR signaling. Our TNF-a receptor signaling analyses identified SPATA2 as a novel component of the TNF-a receptor signaling complex. We showed that SPATA2 interacts with the deubiquitylase CYLD, and recruits it to the receptor. Downregulation of SPATA2 augments transcriptional activation of NF-κB and inhibits TNF-α-induced necroptosis, pointing to an important function of SPATA2 in modulating the outcomes of TNF-α signaling. Together, our proteomic investigations provide important insights in to the regulation and function of acetylation and ubiquitylation in human cells.

PL5

S5. Computational Proteomics

The Perseus computational platform for comprehensive analysis of (prote)omics data

Jürgen Cox

Computational Systems Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany.

Currently, a main bottleneck in proteomics is the downstream biological analysis of highly multivariate quantitative protein abundance data. It will be shown how the Perseus software supports researchers in interpreting protein quantification, interaction and posttranslational modification data. A comprehensive portfolio of statistical tools for high-dimensional omics data analysis is contained covering normalization, pattern recognition, time series analysis, cross-omics comparisons and multiple hypothesis testing. A machine learning module supports classification and validation of patient groups for diagnosis and prognosis, also detecting predictive protein signatures. Central to Perseus is a user-friendly, interactive workflow environment providing complete documentation of computational methods used in a publication. All activities in Perseus are realized as plugins and users can extend the software by programming their own, which can be shared through a plugin store. Perseus combines a powerful arsenal of algorithms with intuitive usability by biomedical domain experts, making it suitable for interdisciplinary analysis of complex large datasets

PL6

S6. Emerging Technologies

Mass Spectrometry Imaging of Clinical Formalin-Fixed Paraffin-Embedded Tissues

Benjamin Balluff

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Mass spectrometry imaging (MSI) is a powerful tool for the spatially resolved and mass spectrometric investigation of tissues. Tissues are a primary source for pre-clinical and clinical research. In consequence, MSI has had its biggest impact in biomedical research, especially for the investigation of tumor tissues. While most of the MSI studies have been performed on fresh-frozen tissues, clinical practice is archiving the tissues as formalin-fixed paraffin-embedded (FFPE) blocks. The MSI community has therefore undertaken many efforts for accessing the molecular information conserved in FFPE clinical tissues.

This presentation will give an overview of past achievements by the community and our current efforts for the analysis of clinical FFPE tissues.

PL7

S7. Quantitative Proteomics

Challenges in assigning the sub-cellular location of proteins

Kathryn Lilley

Cambridge Center for Proteomics, University of Cambridge, Cambridge, UK.

Proteins adopt multiple functions controlled by their sub-cellular location, binding partners and post transcriptional and post translational modification status, and structure. Such differential control significantly increases the functionality of the proteome over what is encoded by the genome. The processes governing these features are highly dynamic. The ability to chart changes in the dynamic proteome upon perturbation such as cell stress is of paramount importance to our understanding of cellular mechanisms. Methods designed to map these changes are very reliant on technologies that result in reproducible data and excellent sub-cellular resolution.

We have applied the hyperLOPIT method (Christoforou Nat. Comm. 2016) to gain insight into the steady state location of proteins in yeast cells and different mammalian cell lines with high subcellular resolution, and reproducibility. We use a combination of different fractionation methods based on detergent solubilisation, differential and equilibrium density centrifugation to fractionate cells into distinct subcellular fractions.

We map the distribution of proteins through fractions using quantitative proteomics approaches, and apply bespoke machine learning tools to further analyse data and classify proteins into distinct sub-cellular niches (Breckels PLoS Comp Biol. 2016, Gatto Bioinformatics, 2014.).

We show that high quality data can be achieved using different spatial proteomics approaches, but demonstrate that the choice of analytical workflows impacts the number of false discoveries incurred and thus conclusions which can be drawn. Furthermore, comparison with high content data obtained by microscopy methods shows some overlap between spatial assignments, but highlight inherent issues of poor quality immune reagents in the case of immunohistochemistry and mis-localisation artefacts which may result from fluorescent fusion proteins.

We also demonstrate that over half of the proteome is located in multiple places giving insight into spatially dependent functionality of proteins, and link localization to structural elements within the proteome, especially those involving intrinsic disorder. Finally, we show the spatial partitioning of functional units.

Oral Communications

Session 1
Clinical Proteomics

O1

Increased peroxiredoxin-6 and oxidized apolipoprotein A1 as hallmarks of redox imbalance in human abdominal aortic aneurysm

Jorge, I.[1]; Burillo, E.[2]; Camafeita, E.[1]; Martínez-López, D.[2]; Blanco-Colio, L. M.[2]; García-León, A.[3]; Trevisan-Herraz, M.[1]; Ezkurdia, I.[1]; Egido, J.[4]; Escola, J. C.[3]; Martín-Ventura, J. L.[2]; Vázquez, J.[1]

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Abdominal aortic aneurysm (AAA) is a life-threatening dilatation of the aorta. While AAA is usually asymptomatic, its rupture is associated with a high mortality rate. The protein and lipid composition of high-density lipoproteins (HDL) are known to be altered in disease situations such as AAA. We performed multiplexed, quantitative proteomics analysis of HDL from AAA patients (N=14) and controls (N=7). Peroxiredoxin-6 (PRDX6), HLA class I histocompatibility antigen, retinol-binding protein 4 and paraoxonase/arylesterase 1 were increased in these patients, whereas α-2 macroglobulin and C4b-binding protein were decreased. These changes were validated by Western-blot, immunohistochemistry and ELISA. PRDX6 localized with neutrophils and vascular smooth muscle cells in AAA tissue, colocalizing with markers of lipid oxidation. PRDX6 levels also were increased in plasma of AAA patients (N=47) versus controls (N=27), and a positive correlation was observed between PRDX6 and AAA diameter. Finally, we detected increased Trp oxidation in apolipoprotein A1 (APOA1) from HDL isolated from AAA patients, a finding that was confirmed in plasma samples by PRM (N=40). Increased Trp oxidation was associated to a decrease in cholesterol efflux capacity of HDL. Our data suggest that the redox imbalance associated with AAA influences HDL composition and that antioxidant PRDX6 may be a novel systemic biomarker of AAA. Our results also support the role of oxidative stress-mediated modifications as a mechanism for HDL dysfunctionality.

O2

Proteomic characterization of new transcription factors associated with a more invasive phenotype in colorectal cancer

García-Palmero, I.[1]; Torres, S.[1]; Bartolomé, R. A.[1]; Escudero-Paniagua, B.[1]; Calviño, E.[1]; Fernández-Aceñero, M. J.[2]; Casal, J. I.[1]

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Metastasis is the major cause of death in cancer. It is a complex process involving multiple steps, which require multiple changes in protein expression. Transcription factors bind specific DNA sequences to regulate gene expression. Due to its low abundance, they are usually hard to identify. To find new transcription factors associated with the metastatic phenotype we pulled down transcription factors using DNA sequences containing a concatenated tandem array of consensus transcription factor response elements from nuclear extracts of the highly metastatic KM12SM colorectal cancer cells and their parental, poorly metastatic, KM12C cells. Transcription factors were pulled down using biotinlylated DNA, sequentially digested with Lys-C/ trypsin and analyzed by LC-MS. MS data was searched against the Andromeda search engine and quantified by using the MaxLFQ algorithm. A total of 690 proteins were quantified with good reproducibility between replicates. We found 240 proteins showing a significant fold-change in KM12SM cells relative to KM12C cells. To filter for experimentally known transcription factors and co-factors, we run a search of our deregulated proteins against the TFcheckpoint database. We found 85 transcription and co-transcription factors experimentally verified among our deregulated proteins. Using Genomatix pathway system, we found that the top diseases affected by these proteins were related to neoplasias and tumoral development. The mRNA levels of TFE3, FOSL2, MAFG, CHD7, TCF7L2 and CEBPB were upregulated in KM12SM, while SFPQ, SRSF3 and YBX1 were found downregulated. To analyze their potential prognostic value we used the GSE17538 dataset from GEO, which contains mRNA expression profile from 232 patients with colorectal cancer. Notably, low expression of SFPQ, SRSF3 and YBX1 were associated with higher recurrence in CRC patients. In summary, SFQP, SRSF3 and YBX1 seem to function as metastasis suppressors in colorectal cancer and showed a promising prognostic value.

O3

Intercellular communication via connexin channels between cartilage, synovial membrane and subchondral bone: implications for joint homeostasis

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Objective. The mechanisms of cellular communications between bone, cartilage and synovial membrane are not fully understood and have been generally attributed to diffusion of soluble mediators through the synovial fluid or through the tissue matrix. Here, we investigated if bone cells (subchondral bone, SB), synovial cells (SC) and chondrocytes (CH) are able to establish cellular contacts and coupled through gap junction (GJ) channels in order to directly exchange signalling molecules and nutrients.

Methods. SB, SC and CH were isolated from healthy donors. The establishment of GJ channels was evaluated by dual voltage-clamp and whole-cell/perforated patch methods. The exchange of molecules through GJs was measured by dye injection experiments. Transwell co-culture system and MALDI/TO-TOF were used to study the transference of essential aminoacids and proteins between contacting SB, SC and CH. Cx43 protein levels were studied by western-blot and immunohistochemistry.

Results. Our results demonstrate that SC, BC and CH are able to physically interact and coupled to communicate by gap junction (GJ) channels formed by Cx43. Dual voltage-clamp and whole-cell/perforated patch methods demonstrated that primary SB and SC are able to establish functional GJs with CH, being Cx43 properties dominant. Dye injection experiments confirmed that SB, SC and CH exchange via GJs different molecules such as lucifer yellow and the fluorescent glucose derivative (2-NBDG). Transwell co-culture system and MALDI/TO-TOF analysis revealed the exchange of essential aminoacids, peptides and proteins including calnexin, calreticulin or CD44 antigen between contacting SB, SC and CH.

Conclusions. GJ channels or GJ plaque provide a selective signalling route by the direct exchange of potent signalling molecules and metabolites. These findings indicate that Cx43-mediated intercellular communication between cells located in the subchondral bone, synovial membrane and cartilage have important implications in the cellular signalling and homeostasis of the joint and may hold protective effect on damage of joint cartilage and surrounding tissues.

O4

Quantitative proteomics reveals Piccolo as a candidate serological correlate of recovery from Guillain-Barré syndrome

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Guillain-Barré syndrome (GBS) is an autoimmune-mediated peripheral neuropathy of unknown cause. However, about a quarter of GBS patients have suffered a recent bacterial or viral infection, and axonal forms of the disease are especially common in these patients. Proteomics is a good methodological approach for the discovery of disease biomarkers. Until recently, most proteomics studies of GBS and other neurodegenerative diseases have focused on the analysis of the cerebrospinal fluid (CSF). However, serum represents an attractive alternative to CSF because it is easier to sample and has potential for biomarker discovery. The goal of this research was the identification of serum biomarkers associated with recovery from GBS. To address this objective, a quantitative proteomics approach was used to characterize differences in the serum proteome between a GBS patient and her healthy identical twin in order to lessen variations due to differences in genetic background, and with additional serum samples collected from unrelated GBS (N=3) and Spinal Cord Injury (SCI) (N=3) patients with similar medications. Proteomics results were then validated by ELISA using sera from additional GBS patients (N=5) and healthy individuals (N=3). All GBS and SCI patients were recovering from the acute phase of the disease. The results showed that Piccolo, a protein that is essential in the maintenance of active zone structure, constitutes a potential serological correlate of recovery from GBS. These results provided the first evidence for the Piccolo´s putative role in GBS, suggesting a candidate target for developing a serological marker of disease recovery.

O5

Application of thiol redox proteomics to clinical heart valve disease samples

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Aortic valve disease is the most common valve disease in Western countries, and the main indication for surgical valve replacement. Two presentations with different pathogeny predominate: degenerative aortic stenosis is the most frequent while aortic insufficiency has lower prevalence. To study changes in protein levels and post-translational modifications that explain differences among these diseases and compared with healthy valves, we have analysed aortic valves obtained from patients with aortic stenosis, patients with aortic insufficiency or valves from hearts replaced during organ transplantation in which the normal function of the valve was previously assessed. To this aim we performed quantitative and thiol redox proteomics analysis, applying the Gelsilox methodology and isobaric labelling. We arranged the valves into three groups with eight aortic valves each, matched for sex, age and other clinical parameters.

By incorporating samples of the three groups and an internal standard in each LC-MS/MS analysis, we have performed two types of analysis. Firstly, a direct quantitative comparison among the valves of paired groups allowed us to select panels of statistically significant differences in protein amount between the three groups. Secondly, a quantitative comparison of each clinical sample with the internal standard provided information about the sample variability and allowed to select them for further validation by an orthogonal method (which we performed by immunoblot).

The Gelsilox methodology also allowed us to estimate the relative quantitation of cysteine-containing peptides in their reduced and oxidized forms. In addition to pinpointing the most significant variations in specific cysteine residues from individual proteins, we observed an overall increase in reversible cysteine oxidation in the disease groups with respect to the normofunctional valves, as well as a difference in cysteine oxidation among the two diseases.

Overall, by using this methodology in clinical samples, we are able to perform quantitative proteomics and thiol redox proteomics in a single LC-MS/MS analysis, expanding the search for potential biomarkers and allowing a deeper understanding of the pathogenic mechanisms.

O6

mTOR inhibition Remodels Extracellular Matrix Components of Human Osteoarthritic Cartilage

Cillero-Pastor, B.[2]; López de Figueroa, P.[1]; Rocha, B.[1, 2]; Blanco, F. J.[1]; Heeren, R. M. A.,[2]; Caramés, B.[1]

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Background: The mammalian target of rapamycin (mTOR) is a conserved serine-threonine kinase that regulates cell growth, cellular proliferation and metabolism in response to metabolic signals. It has been demonstrated that mTOR plays an important role in osteoarthritis (OA) progression. However, the specific mediators involved on mTOR signaling pathway in OA cartilage are poorly understood. The objective of this study is to determine the modulation of protein profiles of OA cartilage after mTOR inhibition by using matrix assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI).

Results: We have observed that peptide profiles of OA cartilage treated with Rapamycin and Torin 1 are univocally distinguished by MALDI-MSI coupled to principal component analysis and discriminant analysis. In untreated OA samples, we identified peptides from Prolargin (PRELP; m/z 1044.5, m/z 1590.8), Fibromodulin (FM; m/z 1352,7, m/z 1361.7), Fibronectin (FN; m/z 1349,7, m/z 1401,6, m/z 1431,7) and Cartilage Oligomeric Matrix Protein (COMP; m/z 1613,8). These identified peptides are structural proteins involved in extracellular matrix organization. FN and COMP are considered potential biomarkers of OA involved in inflammation and tissue remodeling, respectively. In addition, it has been demonstrated that under pathological conditions, FMOD plays a role in joint inflammation. Interestingly, pharmacological inhibition of mTOR by Rapamycin or Torin 1 resulted in a reduction of these OA-related proteins. In these sense, PRELP, FM, FN and COMP peptides were inhibited in human OA cartilage treated with Rapamycin or Torin 1.

Conclusions: MALDI-MSI analysis revealed that mTOR remodels the peptide profile of human OA cartilage by reducing the expression of OA-related extracellular matrix proteins. These results demonstrate that mTOR regulates the structural protein network associated with OA pathology and highlight the potential of MSI to identify novel biomarkers related to cartilage degeneration.

Session 2
Microbial Proteomics

O7

HCV core protein interactions with host CD4+ T cells proteins

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Hepatitis C virus (HCV), identified as the major etiological agent responsible for post-transfusion hepatitis, infects an estimated 170 million persons worldwide. HCV is currently the most important cause of chronic viral hepatitis in the world and one of the most frequent indications for liver transplantation.

One of the interesting and enigmatic aspects of HCV infection is that up to 80% of infected individuals develop persistent viremia. HCV evades immune system clearance mechanisms and establishes persistent infection, leading to progressive hepatic fibrosis, cirrhosis, death from liver failure, as well as the advent of hepatocellular carcinoma.

Interestingly, HCV core protein has been widely associated with pathogenicity and virulence, and it has been described by us and others as a key factor for immune evasion and immune regulation. As many of HCV-core induced phenomena are mediated by a direct effect on cells from the immune system, in this work, we analyze the interactome for HCV Core protein in CD4+ cells, identifying a wide range of human CD4+ T cells proteins that could partly explain the biological alterations caused in cellular processes by the intracellular expression of a single HCV viral protein.

O8

Candida albicans increases the release of proinflammatory Extracellular Vesicles in THP1 Macrophages

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The effectiveness of macrophages in the response to systemic candidiasis is crucial to a fungal effective clearance. Communication between immune cells is mediated, among others, by the secretion of proteins, mRNAs, non-coding RNAs and lipidsthrough extracellular vesicles (EVs). EVs change their cargo to mediate different responses and may play a role in the response against infections. Thus, we have boarded a quantitative proteomic analysis on the protein composition of the THP1 macrophages-derived EVs in response to *Candida albicans* infection that unraveled an increase in the number of EVs secreted in response to *C. albicans* vs control. Differential proteomic analyses of EVs showed changes in 133 due to the interaction with *C. albicans* of the 717 proteins identified and quantified. The differentially abundant proteins were involved in functions as immune response, signaling, or cytoskeletal reorganization. Macrophage-derived EVs, both from control and from *Candida*-infected macrophages, had effector functions on other THP1 monocytes and differentiated macrophages, activating ERK and p38 kinases, increasing the secretion of proinflammatory cytokines and the candidacidal activity. Our findings provide new information on the role of macrophage-derived EVs in candidiasis and on macrophages communication in response to infections.

O9

Analysis of the extracellular vesicles and proteins secreted from Candida albicans unravels new clues about protein secretion mechanisms

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The commensal fungus *Candida albicans* secretes a lot of proteins related to cell wall remodeling, nutrient acquisition and host interactions. Furthermore, it secretes extracellular vesicles (EVs) with an unclear role in host-pathogen interaction. The objective of this work is the proteomic analysis of the EVs and the soluble secreted proteins of a *C. albicans* wild type strain and the cell wall mutant *ecm33*, in order to improve the knowledge of the EVs and the mechanisms of protein secretion. Cell-free culture supernatants from *C. albicans* SC5314 and *ecm33*strains were separated into EVs and EV-free supernatant and analysed by LC-MS/MS. In the wild type strain, 96 proteins were identified (75 in EVs and 61 in EV-free supernatant). The proteins in the EV-free supernatant were enriched in cell wall and secreted pathogenesis-related proteins. Interestingly, more than 90% of them were classical secretory proteins with predicted N-terminal signal peptide, whereas all the leaderless proteins involved in metabolism were exclusively cargo of the EVs. Based on these results, a model of the different mechanisms used by *C. albicans* proteins to reach the extracellular medium was proposed (Gil-Bona *et al.*, JPR 2015; 142-53). The EVs of C. *albicansecm33* were different to the SC5314 ones, regarding their size, quantity and protein composition. In the proteomic analysis of its secretome, 170 proteins were identified (154 in EVs and 114 in vesicle-free secretome). Most of them were metabolic and cell wall-related proteins. The data showed that the classical secretion pathway was altered in themutant because it secretes a higher number of proteins but surprisingly Sap2 was not, suggesting a different and new pathway for the secretion of this protein (Gil-Bona *et al.*, JPR 2015; 4270-81).

O10

Differential host immune recognition of the Candida albicans cell surface-associated proteome upondimorphic transition in invasive candidiasis

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Dimorphic transition (ability of many *Candida* species to reversibly switch between yeast and hyphal growth under specific host environmental stimuli) is important for virulence of *Candida albicans*. Both morphological forms are pathogenic, and may promote different stages of the infectious process and expose distinct host recognition biomolecules at their surfaces. A better knowledge of theircell surface antigens could offer a rationale for the future design of new diagnostic and therapeutic strategies for invasive candidiasis (IC), an opportunistic and life-threatening mycosis. In this work, we examined IgG antibody responses to the *C. albicans* cell surface-associated proteome upon dimorphic transition in IC patients using serologic proteome analysis (SERPA) and data mining tools. A total of 27cell surface-associated proteins (CSPs) were differentially immunodetected in yeasts and hyphaeduring IC. Capture ELISAs on selected CSPs confirmedSERPAdata. Two-way hierarchical clustering analysisunveiled two IgG antibody-reactivity signatures that segregated IC sera hybridized with yeast and hyphal CSPs into two discrete groups. Coordinated IgG antibody responses to two repertoires of CSPs as a function of the growth form were evidenced in IC. Pairwise correlation and gene ontology analyses revealed distinctsubsets of functionally related CSPs that showed opposing IgG antibody-mediated immune recognition patternsin yeasts and hyphae during IC. Changes in the antigenicity of the 27 identified CSPs upon dimorphic transition in IC induced topological differences in their immune co-recognition networks.By testing the effect of CSPabundance normalization on reactivity changes, we found that antigenicity of CSPs was modulated in IC by changes in their relative abundance and potential post-translational modifications in their epitopes upon dimorphic transition. These findings provide new insight into host immune recognition of the *C. albicans*yeast and hyphal surface-associated proteome in IC.

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O11

DiGE analysis as an interesting approach to identify glycoproteins involved in virulence in Ustilago maydis

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Ustilago maydis has raised as an excellent model for the study of plant-pathogen interactions, and its relation with maize plant is one of the systems in which studies can be tackled from both plant and pathogen perspective. *U. maydis* genome contains more than 500 putative secreted proteins, of which more than 50% don´t have known functional domains and many of these proteins have been related to infection process. Protein N- and O-glycosylation are critical processes in host-pathogen relations, since mutations in genes *pmt4, gls1* and *gas2* completely abolish *U. maydis* virulence on maize, affecting different steps in the infection process. For this reason, cytoplasmic N- and O-glycosylated proteins produced only when the virulence program is activated by over-expressing the transcription factor Biz1 were purified and a proteomic analysis using Ethan DiGE technology was performed. The fungal effector Cmu1 and the Cell Wall Degradating Enzyme (CWDE) Afg1, both previously described as involved in virulence, were identified in this screening, showing that DiGE analysis is an interesting approach to identify proteins involved in virulence in *U. maydis*. We have also identified Pdi1, involved in protein folding, and two uncharacterized proteins as new glycoproteins involved in virulence. Because secreted and cell wall proteins are putatively required for plant infection and they are generally glycosylated, we have now performed a secreted and cell wall protein analysis in order to identify glycosylated proteins induced when the virulence program is activated. Up today we have identified by mass spectrometry and MASCOT analysis four Pmt4 glycosylated proteins, two of them from the secreted and the other two from cell wall fraction, which are all dependent on the virulence program activation. These proteins are actually being characterized.

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Session 3
Plant and other non-model Orphan Organisms

O12

Systemic Osmotic Stress Adaptation of Chlamydomonas reinhardtii

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Water is a key compound for all life forms, thereafter water shortage poses one of the most severe stress stimuli, inducing macromolecule denaturalization, aggregation, and organelle disorganization. These alterations hit among other processes photosynthesis and respiration, increase ROS production, and halt cellular growth and division. Dehydration tolerance in plants sets around cell structure and macromolecule protection/repair/replacement, through compatible osmolite accumulation, synthesis of protection proteins, detoxificating enzymes and water channels and transporters. This process entail large changes in proteome and metabolome requiring an active protein synthesis and protein degradation activity coupled with changes in transcriptional regulation.

Not a lot of knowledge has been generated about this topic in *Chlamydomonas reinhardtii*. Aiming to solve this an -omic approach was taken, using *C. reinhardtii* samples exposed to water limitation during 24 h, to depict through 3 harvesting times (0 h, 5 h, 24 h) the proteome, metabolome, and targeted transcriptome of drought acclimation change landscape and identify its key elements by applying state-of-the-art methodologies such as nLC-Orbitrap, CG-MS, and qPCR respectively.

Applied stress triggers several changes in *C. reinhardtii*. Early in the highest stress peak some of them are related with the respiratory chain dehydrogenases and photosynthesis photosystems I & II elements along with changes in protein synthesis/degradation, transcript processing machinery, glycolysis, photorespiration and krebs cycle, coupled with organic acids (lactic, glycolic, glutamic, pyroglutamic), glycerol and sugars specially trehalose accumulation. Late response share early one focus but with higher sugar and sugar alcohol osmolite levels and lower aminoacids levels. Chlorophyll synthesis seems to be upregulated. Since early response ROS detoxification is present through superoxide dismutase and ascorbate peroxidase upregulation, along with phosphorespiration through glycolate accumulation, while glycerol points to a glycolysis enhancement.

Drought exposition induces in *Chlamydomonas* changes in the transcript processing, proteome and metabolome headed to acclimation and common with other life kingdoms nevertheless within metabolic changes osmolite accumulation is more focused in sugars.

O13

Changes in the protein profiles of q. ilex seeds during germination and seedling establishment

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The changes that occur in the protein profiles during the germination and establishment of *Q. ilex* seedlings were investigated by means of a proteomic analysis of embryo axis, radicle and shoots tissues, excised at different stages along the growth process. PCA analysis of the normalised bands intensities obtained by SDS-PAGE clearly separated germination, postgermination and early seedling tissue proteomes. However, the band pattern was not greatly different between unimbibed and germinating seeds. This initial 1-DE proteomic approach was used for the selection of the samples (S0, S3, SS-1 and SS-4) to be analysed through a more resolutive technique, as it is 2-DE. A total of 732 spots were resolved with 2-DE, of which 103 variable spots were selected for protein identification. Some 90 differentially accumulated proteins were identified using 2-DE MALDI-TOF/TOF. The gel-based approach disclosed important metabolics changes that occurred in the holm oak seed after the germination (from S3 onward). Again, few proteins resulted altered in their abundance during the germination period (from S0 to S3). Data suggested that the maturate non-orthodox seeds of *Q. ilex* have the mechanisms necessary to ensuring the rapid resume of the metabolic activities requires to start the germination process and to *de novo* synthesize the biomolecules required for growth. Proteins related to energy metabolism and photosynthesis were up-accumulated during seedling establishment. Our results also indicated that the use of genus specific database combined with public database improve the quality and quantity of protein identification in orphan species.

O14

Proteomic analysis of fish liver exposed to sublethal levels of polyaromatic hydrocarbons

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Toxiproteomics allows the use of proteomics technology to study changes in protein expression caused by exposure to chemical agents and toxic substances, thus facilitating the understanding of key pathways that lead to toxicity. In the present study, *Rachycentrum canadun* (Linnaeus, 1766) individuals were exposed to 0.4 ppm of PAHs for 14 days in a semi-static system. At the end of the experiments, their livers were removed and processed for enzymatic, histological and proteomic analyses. In order to determine the antioxidant enzymatic parameters, the enzymatic activities of catalase, superoxide dismutase and glutathione S-Transferases were evaluated in the S9 hepatic fraction. The livers were fixated in Macdowald's solution for histological evaluation, and posteriorly processed with routine histological techniques. Liver tissue slides were stained with hematoxylin-eosin and PAS. For the proteomic studies, the dissected livers were immediately placed in RNAlater®. 2-D PAGEs assays were carried out for proteomic identification, using liver samples from five individuals. The 2D-spots displaying over-expression against control condition were selected and identified. Peptides resulting from protein digestion were analyzed with mass spectrometry (MALDI/ TOF TOF). Results were compared against the MASCOT database. Significant changes were observed in the antioxidant enzyme parameters, in addition to changes in the hepatic tissue, especially regarding the size of hepatocyte nuclei. The proteomics analysis showed that proteins with null expression upon exposure to HPAs or which had their expression induced by these xenobiotics are potential biomarkers for this type of toxicological exposure and should be further investigated.

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Ethical Committee for use of Animals in Research:124-CEUA

O15

iTRAQ-proteomics approach to understand low concentration citrate capped gold nanoparticles stress in the marine bivalve Ruditapes philippinarum

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Over the last decades engineered gold nanoparticles (AuNPs) have been developed for and introduced into a growing number of commercial and industrial products. Up to date limited information is available on the ecotoxicological responses in non-target organisms, in particular under environmental relevant concentrations. Notwithstanding their promising applications, increased understanding of their behaviour and effects is required to manage potential risk for non-target organisms.

We studied differential protein expression in response to citrate capped AuNPs (21.5 ± 2.9 nm) in the digestive gland of the bivalve *Ruditapes philippinarum* after 1 and 7 days of exposure at an environmental relevant concentration (750 ng L^{-1}), using a 2nd-generation (iTRAQ-8plex) proteomic approach.

2200 proteins were identified to be simultaneously expressed in control and treatment and a regularized regression approach (Elastic Net) identified 105 of those to be expressed differentially. Homology-based functional annotation could be successfully performed for 77% of all sequences and 75% of the differentially expressed sequences and subsequent analysis identified potential enrichment in various stress and injury related functions. Identified proteins may be useful as biomarkers of environmental nanoparticle pollution and provide insight about the mode of action of engineered gold nanoparticles.

Session 4
Post translational Modifications

O16

In-depth characterization of the phosphopeptidome displayed by HLA-B antigens: structural basis of the presentation of phosphorylated ligands

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HLA-I antigens (encoded in the HLA-A, -B and -C loci) play a key role in the eradication of intracellular pathogens and tumor cells by presenting peptides derived from endogenous proteins to cytotoxic T lymphocytes. The peptidome displayed by these molecules includes phosphorylated ligands which have been proposed as prime targets for the treatment of cancer by T cell-based immunotherapy. In this work, we set out to study the phosphopeptidomes displayed by several HLA-B allotypes to gain insight into the structural determinants that govern the presentation of phosphorylated ligands.

HLA-I-bound peptidomes were purified, subjected to phosphopeptide enrichment and analyzed by LC-MS/MS using CID or EThcD for peptide fragmentation. With this approach, we identified more than 9500 ligands associated to HLA-B*40 with a common anchor motif: acidic residues (mainly Glu) at P2 and hydrophobic ones at PΩ. We also characterized about 150 phosphopeptides displayed by this allotype. Analysis of this set of sequences revealed two striking facts: 1) some ligands had phosphoserine (pSer) instead of Glu at P2 and 2) phosphorylation was frequently found at P4 and was usually accompanied by a basic residue at P1.

By performing peptide binding assays to HLA-B*40 and solving the crystal structures of 4 B*40-peptide complexes, we determined that the presentation of ligands phosphorylated P2 was due to the structural similarities between Glu and pSer. In addition, we demonstrated that the preference for peptides phosphorylated at P4 was linked to the presence of Arg62 in HLA-B*40. Finally, the analysis of the phosphopeptidomes displayed by other allotypes (B*39, B*27, B*07) indicates that preferent phosphorylation at P4 is a common feature for most HLA-B molecules while the bias towards basic residues at P1 is allotype-dependent. Overall, our results provide a base to improve the prediction and identification of phosphorylated neo-antigens as putative targets for cancer immunotherapy.

O17

Bottom-up strategies for the analysis of human alpha acid glycoprotein. Application to pancreatic disease diagnosis

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Glycosylation is one of the most common posttranslational modifications in proteins. O- and N-glycan structures have been found altered in many diseases such as congenital disorders of glycosylation (CDG), chronic inflammation or cancer. High performance separation techniques coupled to mass spectrometry have become indispensable in glycoproteomics. Bottom-up strategies consisting in the analysis of specific glycosylation markers of low molecular mass originated after glycoprotein digestion is a spreading alternative to the top-down approach, where the lower MS sensitivity for intact glycoproteins hinders detailed characterization.

The aim of this work is to describe different bottom-up methodologies for the accurate characterization of the glycosylated structures present in glycoproteins. In this regard, glycosylation may be analyzed through the glycans released from the protein or the glycopeptides. In the first case, PNGaseF released glycans are derivatized by reductive amination, and separated and identified by capillary zwitterionic-hydrophilic interaction liquid chromatography coupled to mass spectrometry (μZIC-HILIC-MS). This method provides excellent information about the structure and composition of the glycans (isomeric glycans). In the second case, glycopeptides are obtained from the native glycoprotein by tryptic digestion and analyzed by capillary liquid chromatography coupled to mass spectrometry (C18-capLC-MS) allowing information about composition of the glycans but also about glycosylation sites and their degree of occupancy. In this work both bottom-up strategies have been applied to analyze human alpha acid glycoprotein (AGP) in serum healthy controls and pathological samples (pancreatic cancer and chronic pancreatitis). An increase in fucosylation is observed in pancreatic cancer samples unlike to samples from chronic pancreatitis with both methodologies. These preliminary but relevant results concerning AGP glycosylation demonstrate that the presented methodologies could be extremely useful in patho-glycomics, particularly for finding novel glycoprotein based biomarker structures related to cancer and/or other diseases.

O18

New methods for the in-depth characterization of posttranslational modifications underlying cardiovascular diseases

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Heart diseases are a leading cause of morbidity and mortality worldwide, and impose significant economic burdens on the healthcare systems. Despite the effort over the last several decades, the molecular mechanisms underlying diseases of the heart remain poorly understood. Postranslational modifications (PTMs) are well known to have a pivotal role in the control of signaling cascades and protein structure and functionality, so the knowledge of the nature of the modified proteins and sites provides a formidable insight into the underlying mechanisms.

Here we quantify by MS thousands of different PTMs in heart cells and tissue from different animal models of cardiovascular diseases. We combine a powerful novel mass-tolerant database searching method that identifies peptides with modifications not previously described, with an in-house developed algorithm (isPTM) that allows rapid, hypothesis-free PTM identification with controlled local-FDR and our WSPP quantitative statistical model. Using these methods, we generated very detailed maps of the PTMs most affected in different physiological and pathological conditions, giving new clues on the molecular events that take place in heart during infarct-associated damage and aging. These results will help to better understand the biological events related to protective strategies and opens the possibility of using these modifications to trace the undergoing processes.

O19

Nuclear phosphoproteomic analysis of interleukin-2 signaling reveals the pivotal role of ACLY in T-cell growth

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Current anti-cancer immunotherapies commonly rely on the use of interleukin-2 (IL-2) to promote the proliferation of T lymphocytes. IL-2- dependent T-cell growth is the culmination of a complex network of phosphorylation-driven signaling events that impact on gene transcription through mechanisms that are yet poorly understood. Therefore, in order to shed light into the role of IL-2 in the regulation of nuclear protein function we have performed a SILAC-based quantitative study of the nuclear phosphoproteome of resting and IL-2-treated T lymphocytes. Briefly, differentially labeled and treated T-cells were lysed, equitably combined according to their protein concentration and in-solution digested using LysC and trypsin. Then, phosphopeptides were enriched using TiO_2 beads and following high pH fractionation they were analyzed in a QExactive HF mass spectrometer. The experiment that performed in triplicate led to the detection of 8,521distinct phosphosites including many that are not yet reported in curated phosphorylation databases. Although most phosphorylation sites remained unaffected upon IL-2 treatment, 391 sites corresponding to 288 gene products showed robust IL-2-dependent regulation. Importantly, we show that ATP-citrate lyase (ACLY) is a key phosphoprotein effector of IL-2-mediated T-cell responses. We discover and further confirm that ACLY becomes phosphorylated on serine 455 in T lymphocytes upon IL-2-driven activation of AKT. Moreover, we demonstrate that depletion or inactivation of ACLY compromises IL-2-promoted T-cell growth. More specifically, proliferation assays using ACLY S455A, S455E and S455D point mutants indicate that phosphorylation of ACLY S455 has a positive effect on cell proliferation. Mechanistically, we demonstrate that ACLY is required for enhancing histone acetylation levels and inducing the expression of cell cycle regulating genes in response to IL-2. Thus, the metabolic enzyme ACLY emerges as a bridge between cytokine signaling and proliferation of T lymphocytes, and may be an attractive candidate target for the development of more efficient anti-cancer therapies.

Session 5
Computational Proteomics

O20

Protein identification workflow for large proteomic datasets

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Estimating correctly identified proteins false discovery rate (FDR) remains a challenge when analyzing large proteomics datasets. Current FDR calculation methods applied to big proteomics data tend to over estimate decoy identifications leading to a loose of sensitivity or incorrectly identified absent proteins.

We have developed a modular workflow that includes novel statistical probability and random matching models for the analysis of large datasets.

Our workflow allows the assessment of correctly identified proteins giving different options in order to highlight several features as «one-hit wonders» proteins or «hub proteins». The approach is scalable to enable integration such as different replicates, experiments, tissues or functional categories.

We analyzed a large-scale human proteomics experiment and carried out a quality test on the data in order to benchmark our methods against different protein FDR estimation approaches.

O21

Quality Improvement of custom proteogenomic databases based on a predictor of peptide detectability

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In recent years, the use of high-throughput technologies has enabled the detailed description of the human transcriptome. The most complete transcript annotation initiatives are MiTranscriptome, that contains all the expressed human transcripts including the long non-coding RNAs (lncRNAs), and The Cancer Genome Atlas (TCGA), a project with more than 10000 tumour samples from 33 different cancer types. This information could lead to the identification of novel peptides. In a proteogenomics analysis, custom databases are generated using the six-frame translation of the target structures dramatically increasing the number of entries and hampering the comparison of experimental and predicted MS/MS spectra.

In order to optimize proteogenomics databases, we estimated the probability of a given peptide to be detected in a proteomics shotgun experiment. More than 550 peptide characteristics were considered to distinguish between the most and the less observed peptides in GPMDB database. Different classification methods including generalized linear models, SVM, naïve bayes classifier, neural networks, random forest and decision trees were evaluated. All the classifiers were trained and tested in R using the «caret» package. Finally, a set of public shotgun experiments obtained from PRIDE database were analyzed to demonstrate the benefits of reducing the size of the reference database discarding the peptides that cannot be detected using mass spectrometry. MASCOT and Andromeda search algorithms were used and the results were processed in R for the FDR calculation.

The obtained classifier was able to separate the frequently observed peptides in GPMDB from the scarcely observed ones. Then, proteogenomics databases were filtered with this classifier and the identified proteins with FDR less than 0.01 at peptide and protein level were compared with the results obtained using the entire reference databases. This approach increases the performance of the proteogenomics pipeline reducing the computational cost of the analysis and obtaining improved identifications with less number of false positive detections.

Proteogenomics approaches are being developed to detect novel peptides that are not yet present in proteomic reference databases to contribute to the characterization and understanding of the molecular basis of disease. Moreover, new clinical biomarkers emerging from this analysis could be valuable for diagnosis, prognosis and personalized treatment.

O22

On the Statistical Significance of Compressed Ratios in Isobaric Labeling: A Cross-Platform Comparison

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Isobaric labeling is gaining popularity in proteomics due to its multiplexing capacity. However, copeptide fragmentation introduces a bias that undermines its accuracy. Several strategies have been shown to partially and, in some cases, completely solve this issue. However, it is still not clear how ratio compression affects the ability to identify a protein's change of abundance as statistically significant. Here, by using the «two proteomes» approach (*E. coli* lysates with fixed 2.5 ratios in the presence or absence of human lysates acting as the background interference) and manipulating isolation width values, we were able to model isobaric data with different levels of accuracy and precision in three types of mass spectrometers: LTQ Orbitrap Velos, Impact, and Q Exactive. We determined the influence of these variables on the statistical significance of the distorted ratios and compared them to the ratios measured without impurities. Our results confirm previous findings1-4 regarding the importance of optimizing acquisition parameters in each instrument in order to minimize interference without compromising precision and identification. We also show that, under these experimental conditions, the inclusion of a second replicate increases statistical sensitivity 2-3-fold and counterbalances to a large extent the issue of ratio compression.

O23

An advanced method for hypothesis-free and rapid systematic identification of PTMs based on ultra tolerant search

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Post-translational modifications (PTMs) drastically increase the functional diversity of proteomes. The recently developed ultra-tolerant (500 Da) Sequest-database-search constitutes a breakthrough in PTM studies by mass spectrometry, since it allows the identification of peptides even with unknown modifications. However, PTM identification still remains a challenge because 1) the time complexity is unbearably high; 2) using global-FDR includes an uncontrolled number of false-positives in many PTMs, and 3) the FDR estimation for each group of PTMs has rarely been modelled to allow automated high-throughput computing. Here we took ultra-tolerant-search further and developed a method for rapid PTM identification with automated local-FDR control for all identified PTMs.

The list of Peptide-spectrum-matches (PSMs) was obtained using an ultra-tolerant-search. After the experimental mass calibration, the local-FDR was calculated in 1Da mass ranges of allowed masses. To identify specific PTMs peaks we further modelled the mass distribution using Gaussian derivatives. Also, a computational method was developed to estimate the local FDR in a conventional DB search from the results obtained in a small DB, by calibrating the number of decoys resulting from the small database. We demonstrate that this method speeds up the PTM search by more than 10-fold without losing local-FDR accuracy.

The method was used on heart tissue of an ischemia/reperfusion model in pig; the number of modified PSMs was comparable to the non-modified ones. Moreover, we were able to construct the complete map of the modified peptidome in pig heart containing more than 100.000 modified peptides.

In conclusion, we have developed a novel method, which provides an innovative approach for rapid, hypothesis free PTM identification for hundreds of different PTMs with controlled local FDR. Our method opens a way to routine analysis of hundreds of PTMs in high-throughput-experiments.

Session 6
Emerging Technologies

O24

Exploring new proteomic strategies with Orbitrap Fusion Lumos: Liquid extraction surface analysis (LESA) to identify proteins directly from muscle tissue sections by combined Top-down and Bottom-up MS

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The combination of micro-liquid extraction from a solid surface analysis (LESA) and nano-liquid chromatography mass spectrometry (nLC-MS) is an emergent protein profiling technique. LESA-MS allows direct surface protein sampling with spatial resolution and minimal sample preparation from tissue sections from biopsies, dry blood spots, or any surface by simple liquid extraction. It has proven useful in the detection of protein biomarkers in certain diseases (non-alcoholic steatohepatitis, NASH) in liver tissue.

Using an Orbitrap Fusion Lumos (Thermo Fisher Scientific), we explored the potential and limitations of LESA-MS for the detection of protein biomarkers in other types of tissue and diseases, and also studied the feasibility of various proteomic strategies. We used muscle tissue sections of patients diagnosed with mitochondrial DNA depletion myopathy, which is characterized by childhood onset of muscle weakness associated with depletion of mtDNA in skeletal muscle. There is wide clinical variability in mitochondrial dysfunction myopathies; some patients have onset in infancy and show a rapidly progressive course with early death due to respiratory failure, whereas others have later onset of slowly progressive myopathy.

To address our goal in the detection of biomarkers for these types of disease, two complementary strategies were considered. First, a top-down MS approach, where proteins were extracted by LESA and analyzed intact by nano-liquid chromatography tandem mass spectrometry (nLC-MS/MS) using ETD, ETDhCD and HCD. Second, a bottom-up strategy, in which, after extraction from the muscle tissue section, proteins were trypsin-digested and the resulting peptide mixture was analyzed by nLC-MS/MS by CID.

Detection limits were such that multiple extractions (30 spots of 1 microliter extraction volume each, 400 mm) were sufficient to deliver proteins to the nLC-MS/MS system with enough sensitivity to identify 4000 proteins from a tissue sample by the bottom-up approach and around 280 proteoforms by the top-down one. The automation of LESA-nLC-MS/MS analysis is expected to improve, as are the protocols for intact protein fractionation/chromatography and their interpretation algorithms. Such improvements will lead to increased depth of proteome coverage in distinct tissues.

O25

Biomarker Validation and Rapid Determination of Autoantibodies in Cancer Patients using HaloTag Fusion protein-Modified Electrochemical Bioplatforms

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Colorectal cancer (CRC) has some of the highest mortality rates in developed countries, making its early diagnosis essential to improve survival chances. Early cancer detection can be achieved by monitoring the humoral response to tumor associated antigens (TAAs) in cancer patients. Autoantibodies raised against TAAs have shown high promise as clinical biomarkers for reliable diagnosis, prognosis and therapy monitoring of cancer. Despite the advantages of autoantibody detection for cancer diagnosis, the fact that patients develop autoantibodies to multiple TAAs is a major limitation to get clinically validated assays because on one hand autoantibody signature diagnostic panels should be accurately identified and on the other hand, new immunoassays for multiplexed autoantibodies detection should be developed. An ideal candidate for such panels is p53, as serum p53 autoantibodies are reported in 10-40% of all cancer patients depending on the cancer type.

In this study, serum samples from patients at the high-risk program of developing CRC were used to develop an electrochemical disposable biosensor able to be multiplexed for the specific and sensitive determination of p53-specific autoantibodies. This biosensor involves the use of magnetic microcarriers (MBs) modified with covalently immobilized HaloTag fusion p53 protein as solid supports for the selective capture of specific autoantibodies. After magnetic capture of the modified MBs onto screen-printed carbon working electrodes (SPCEs), the amperometric signal using the system hydroquinone (HQ)/H_2O_2 was related to the levels of p53-autoantibodies in the sample. The developed biosensor was able to determine p53 autoantibodies with a better sensitivity than a commercial standard ELISA and using a just-in-time produced protein in a simpler protocol, less sample volume and easily miniaturized and cost effective instrumentation.

O26

Development of beads-based protein microarrays to validate a panel of biomarker candidates for osteoarthritis

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Currently, antibody microarrays have become a priority technique in biomarker research. This is because it allows, simultaneously and massively, the study of hundreds of proteins in complex mixtures without requiring prior separation of the sample. Furthermore, it favours the miniaturization and automation of large-scale analysis, reducing costs without compromising assay sensitivity.

The objective of this work is to create a quantitative antibody microarray (sandwich bead assay) for the validation of a panel of 20 proteins which have been previously identified by proteomics as osteoarthritis (OA) biomarkers.

To standardize the strategy for obtaining the protein microarrays, we first carried out direct assays to evaluate the performance and sensitivity of the antibodies. Six proteins were measured, using antibodies coupled to magnetic beads (Luminex) for protein capture on biotinylated sera from patients diagnosed with different grades of OA: Thrombospondin-1 (THBS1), Fetuin A (AHSG), Cartilage intermediate layer protein 1 (CILP), Retinol Binding Protein 4 (RBP4), Cartilage Glycoprotein 39 (YKL40) and Chitinase 3 Like Protein 2 (YKL39). All readings were performed on a MAGPIX instrument (Luminex). Then, targeted sandwich assays were developed by employing different pairs of antibodies against two of these proteins: RBP4 and AHSG. In this case, recombinant proteins were used as internal standards. Monoclonal antibodies were coupled to the beads for protein capture, whereas polyclonal Abs labelled with biotin were used for the detection and quantification of the proteins in the serum samples.

With this work, we have been able to develop bead-based antibody microarrays for the validation of a panel of OA biomarker candidates. In this first approach, six proteins were detected using direct assays. Additionally, a sandwich strategy for the absolute quantification of AHSG and RBP4 in serum has been standardized and will be applied for the study of a large cohort of samples.

O27

MALDI-Imaging Mass Spectrometry: a step forward in the anatomopathological characterization of stenotic aortic valve tissue

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Aortic stenosis (AS) is the most common form of valve disease and the third most common cardiovascular disease affecting mainly people who are over 65 years of age. It is characterized by abnormal narrowing of the aortic valve, producing a pressure gradient between the left ventricle and the aorta. Once symptoms develop, there is an inexorable deterioration with a poor prognosis; currently there are no therapies capable of modifying disease progression, and aortic valve replacement is the only available treatment.

Our goal is to study the progression of calcification by matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) and get new insights at molecular level that could help in the understanding of this disease and facilitate patients management and treatment. This methodology offers the great advantage to investigate the physiopathological changes taking place directly in tissue while retaining the histopathological context.

In this work, we analyzed three different sections from aortic valve tissue with growing severity by MALDI-IMS, for establish the spatial distribution of proteins and peptides directly from the surface of the histological sections. The analysis showed different structures corresponding to regions observed in conventional histology, including large calcification areas and zones rich in collagen and elastic fibers. Peptide extraction from the tissue, followed by liquid chromatography mass spectrometry analysis, provided the identification of collagen VI α-3 and NDRG2 proteins which correlated with the masses obtained by MALDI-IMS. Furthermore, these identifications were confirmed by immunohistochemistry in adjacent slices.

These results highlighted the molecular mechanism implied in AS using MALDI-IMS, a novel technique never used before in this pathology. In addition, we can define specific regions proving a complementary resolution of the molecular histology. This finding may help to increase our understanding about the etiology and pathophysiology of this disease, but also in the clinical management of patients with AS.

O28

Absolute quantification of protein and peptide biomarkers by isotope dilution tandem mass spectrometry using minimally ^{13}C labelled peptides

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The development of new reference measurement procedures for the absolute quantification of protein and peptide biomarkers is required to ensure the accuracy and comparability of the results under different conditions or biological states. Isotope Dilution Mass Spectrometry (IDMS) provides highly accurate and precise results which are directly traceable to the International System of units (SI). Most of the current IDMS methodologies applied for the absolute quantification of proteins resort to multiply labelled peptides to avoid spectral overlap between natural and labelled analogues. Under these conditions, the ratio of intensities for the natural and labelled peptides is assumed to be equal to the molar ratio. However, this assumption needs to be demonstrated in practice as different isotopic enrichments of the labelled material, potential isotopic effects or spectral interferences can be significant sources of error.

We propose a new IDMS strategy based on the use of minimally labelled peptides and Selected Reaction monitoring (SRM) [1]. In contrast to common approaches, the resolution of the first quadrupole is reduced to transmit the whole parent ion cluster to the collision cell for monitoring accurate isotopic distributions of the molecular fragments. In this way, molar fractions of labelled and natural abundance peptides are directly obtained from the experimental mass spectra of the in-cell fragment ions and the concentration of the endogenous peptide can be directly obtained from the fragment-ion spectrum acquired for the sample without resorting to extra calibration runs. This strategy is applied here to the determination of protein biomarkers for which there is no mass spectrometry based primary method thus far. The determination of the renal biomarker Cystatin C in human serum and urine, the quantification of angiotensin II in amniotic fluid and the simultaneous determination of three potential protein biomarkers of glaucoma (Apolipoprotein A-IV, Complement C3 and Vitronectin) in human serum will be presented.

[1] Spanish Patent ES2472724, priority date 4/12/2014.

Session 7
Quantitative Proteomics

O29

Advances in Targeted Omics Quantitation Using a Novel Scanning Quadrupole DIA Method

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Introduction: Targeted LC-MS based assays are increasingly applied in the post-discovery omics area with emphasis on validation, the first of many phases in translational analyses, or in studies that are aimed at gaining the understanding of biological systems, drug development and treatment. Context is driving current omics experiments, thereby driving the development of LC-MS acquisition methods that can provide both qualitative and quantitative information in a single experiment. An alternative DIA mode operation, whereby a resolving quadrupole is scanned during high-resolution precursor and product ion acquisitions, was applied for targeted lipidomics and proteomics quantitation experiments using transition extraction list and compound library based approaches.

Methods: DIA data were acquired on a tandem quadrupole/oa-time-of-flight (ToF) mass spectrometer. The *m/z* isolation range of the quadrupole was continuously and repetitively scanned with MS data acquired using a high-resolution ToF acquisition system capable of delivering up to 2000 spectra/s. Alternate MS scan data comprise precursor and CID product ions. The quadrupole mass range and resolution were investigated to determine the optimal balance between sensitivity and specificity for each application. The resulting 2D data, *m/z* (ToF) vs. *m/z* (quadrupole) were processed and quantified using Skyline open source informatics and visualised in OpenMS. Control, diabetic and obese plasma samples of varying phenotype were analysed and differently spiked with standards, acting as pseudo QCs, and quantitative lipid and protein changes determined.

Preliminary results: Quantitative proof-of-concept/principle data were acquired by spiking, i.e. serially diluting, lipid standards in protein precipitated plasma and a four protein digest mixture into a proteolytic *E.coli* digest, respectively. For both experiments, transitions extraction lists were utilised to obtain quantitative information. Analysis of the data indicates that scanning quadrupole DIA enables over an order of magnitude more specificity than a static quadrupole operated with the same resolution, providing reduced FQR values and higher dynamic range. It was found that quadrupole transmission windows of 5-10 and 20-30 Da provided optimum lipid and protein identifications, respectively. Qualitative information from the same data sets was obtained by extracting lipid class information based on neutral loss or product ion extraction, and the use of libraries in the instance of the peptide-centric targeted proteomics experiment.

Human plasma samples were treated with isopropanol and centrifuged to precipitate proteins. The lipid-containing layer was collected and diluted to adjust the water content prior to analysis. A second aliquot of the plasma samples was tryptic digested and analysed separately. DIA MS data were collected for the two complementary sample types and differently expressed fatty acids, phosphatidylcholines, triglycerides and phosphatidylserines, and apolipoprotein peptides, respectively, across the three conditions of interest quantified. Quantitative and statistical analysis of the DIA data was conducted with Skyline embedded tools, and visualisation/profiling in either OpenMS or DriftScope. The obtained results were in good agreement with previous discovery studies and the expected changes in relation to disease and/or phenotype.

Novel Aspect: Targeted quantitation of omics samples using a novel precursor quadrupole scanning based DIA method and open source informatics.

O30

Impact of gene overdosage in the hippocampus and cerebellum proteome and phosphoproteome

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Trisomy of human chromosome 21 results in Down syndrome (DS), the most complex and common genetic perturbation leading to intellectual disability. Even though DS has been considered orphan of treatment, recent evidence has shown that epigallocatechin 3 gallate (EGCG), a green tea flavonol, improves the cognitive phenotype in trisomic Ts65Dn mice, as well as in DS humans. However, the molecular mechanisms of its beneficial effect remains elusive, since EGCG can act through many different pathways.

A plausible candidate gene to explain DS phenotypic abnormalities is the dual-specificity tyrosine (Y)-phosphorylation kinase 1A (*Dyrk1A*), which regulates fundamental cellular functions such as cell proliferation and survival and which overdosage recapitulates DS phenotypic alteration in transgenic mice. Thus, normalisation of DYRK1A through inhibition of its kinase activity could be sufficient to rescue the cognitive and the neuronal phenotype. This could explain the effects of EGCG, which has DYRK1A inhibitory properties. Thus, we here investigated the molecular effects induced by in vivo overexpression of *Dyrk1A* and the effect of EGCG on the proteome and phosphoproteome of the hippocampus and the cerebellum using wild type and TgDyrk1A mice. Mass spectrometry data reveal that in vivo overexpression of DYRK1A significantly alters cell functions such as mitochondrial regulation and cytoskeletal proteins, dysfunctions also found in DS patients. We also observed that EGCG can partially rescue DS-related proteins abundance deficits. In conclusion, we propose that EGCG is able to recover in part the DYRK1A-driven proteomic disturbances.

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O31

Understanding Ground State Pluripotency using Quantitative Proteomics

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Mouse embryonic stem cells (mESCs) cultured in serum are highly heterogeneous and cells fluctuate between a «primed» and a «naïve» state of pluripotency. Whilst the primed state is prone to differentiation, the naïve state shows higher maintenance of its self-renewal capacity. Also, naïve mESCs are defined by the uniform expression of pluripotency markers, the reactivation of the X chromosome and the transcriptional pausing of RNApol II. Thus, naïve mESCs are considered as the ground state of pluripotency and an *in vitro* surrogate of pre-implantation embryos. Inhibition of GSK3 and ERK (known as 2i) can capture this ground state indefinitely in culture. Interestingly, our data suggest that inhibition of CDK8 (a regulator of RNApol II) also seems to stabilize mESCs towards the ground state but its underlying molecular mechanism remain unknown.

Here, we used quantitative proteomics and phosphoproteomics to characterize the molecular mechanisms regulated by CDK8i and compared them to those regulated by 2i. Our results show a significant degree of similarity in the proteomes of mESCs treated with 2i and CDK8i (*e.g.* pluripotency maintenance and DNA methylation). Comparison of our proteomic signatures with published transcriptomic data revealed the resemblance of these cells with other naïve mESCs and with the pre-implantation epiblast. Moreover, despite 2i and CDK8i act at different kinase regulatory levels, the early phospho-proteome of both treatments highly overlaps. Together, these observations indicate that direct inhibition of RNA pol II activity, by means of CDK8i, also induces the ground state of pluripotency.

O32

A proteomic analysis of 22 paired colorectal human tissue samples soluble secretome using label-free quantification

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Colorectal cancer (CRC) is one of the most worldwide prevalent and deadly cancers. For that reason, it is necessary focus the efforts to an early detection and to discover new metastasis biomarkers. Tumor tissue secretomes are a rich source of these candidates.

Here, we performed a proteomic analysis with CRC tumor tissue soluble secretome and their paired normal tissue soluble secretome (n=22). CRC tissues were obtained from patients of both genders, in a variety of ranges of age, tumor site and stage. Concentrated soluble secretome samples were fractionated by SDS-PAGE and subjected to in-gel-tryptic digestion. Protein identification and relative quantification were performed by label-free, in a mass spectrometer Q-Exactive, using MaxQuant software and spectral counting quantification. The significance of the data was calculated using a beta-binomial test. We perform various data comparisons: tumor versus normal, early stage versus late stage, adenoma versus early stage, and finally, each CRC stage versus their matched normal tissues. We identified 4678 proteins, where 900 were identified in CRC samples and 70 in normal samples. Within the 3708 common identified proteins, we found 643 CRC-associated dysregulated proteins. We also found 1389, 686 and 777 upregulated proteins in adenoma, early and late stage tumor samples comparing to their matched normal tissues, respectively. Furthermore, unsupervised clustering of each data comparison group was done based on fold changes of significantly deregulated proteins and allowed us to clearly separate tumor and normal samples.

In conclusion, the quantitative analysis of soluble secretome CRC tissue samples allowed us to identify a large amount of deregulated proteins. By using the beta-binomial test, we were able to found significantly overexpressed proteins, CRC stage associated, which could be used as potential CRC biomarkers.

O33

Proteomics Signature in Early Remodeling after Myocardial Ischemia / Reperfusion

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It has been recently reported that the myocardial edematous reaction that arises after ischemia/reperfusion (I/R) follows a bimodal pattern in clinically-relevant pig model of acute reperfused myocardial infarction. The molecular mechanisms driving this phenomenon remain unclear. We have performed a comprehensive multiplexed quantitative proteomics analysis to uncover the biological processes underlying early remodeling in ischemic and remote myocardium.

Closed-chest 40 min I/R was performed in 20 pigs that were sacrificed at 120 min, 24 h, day 4 and day 7 after I/R. Cardiac magnetic resonance (CMR) was performed at every follow-up until sacrifice, when myocardial tissue samples were collected and processed for proteomic analysis. Five pigs sacrificed after baseline CMR served as controls.

In the ischemic myocardium we observed an upregulation of the inflammatory processes at all time points, while a significant increase in interstitial proteins, angiogenesis markers and cardio-renal signalling was observed at days 4 and 7 after I/R. In the remote myocardium, early reperfusion (120 min, 24 h) affected proteins of the sarcomere, mitochondrial metabolism and energy transfer, which reverted to control values at day 7. These changes were accompanied by a temporary decrease in systolic function in this area, as assessed by CMR. Strikingly, we found an increase in proteins related to inflammation, regulation of body fluid levels, renal signaling and collagen at days 4 and day 7 in the remote area.

Taken together, out data present dynamic molecular changes that have been observed for the first time in remote and ischemic myocardium during the first week after I/R, providing insight into potential biomarkers for therapy and diagnosis. Targeting specific processes occurring in early and late stages after I/R could contribute to better myocardium recovery and remodeling.

O34

Targeted proteomics applied to the analysis of endogenous peptides in cartilage secretome, synovial fluid and serum from osteoarthritis patients and controls

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Advances in proteomics are continuing to expand the ability to analyze the proteome and a large number of endogenous peptides in serum samples. Many of these peptides have a role in the inflammatory response, tumor biology, and endocrine processes, presenting them as appealing biomarker candidates. A peptidomic analysis that we recently performed enabled the identification of endogenous peptides that were differentially released from wounded (WZ) and unwounded (UZ) zones of human articular osteoarthritic cartilage, compared to healthy tissue. In the present work, we aimed to develop a targeted method for the quantitative monitoring of this panel of peptides in cartilage secretome from osteoarthritis (OA) patients compared to control donors. Proteins secreted from human articular cartilage (secretomes) from hip and knee joint were obtained by culture of tissue explants. Synovial fluids and sera from OA patients at different stages of the disease were analysed with the target proteomic method, in order to unravel the putative biomarker value of these molecules. The enrichment of endogenous peptides in these types of samples was standardized, using ultrafiltration procedures and solid phase extraction (SPE) with reversed phase (C18) resins. A method for the targeted identification of 17 endogenous peptides by Multiple Reaction Monitoring (MRM)-mass spectrometry was developed. The peptides were separated by nano-LC coupled to a 5500 QTRAP mass spectrometer. Data analysis was performed using the Skyline software. Peptide identifications were searched against a human database using the ProteinPilot program. By these means, endogenous peptides belonging to different proteins as Cartilage Oligomeric matrix (COMP), Cartilage intermediate layer protein 1 (CILP1) and Prolargin (PRELP) among others were simultaneously detected and relatively quantified in synovial fluids and sera from OA patients and controls. Further qualification studies will be necessary to establish their usefulness for OA diagnosis and progression studies.

O35

Targeted proteomics approaches to study the molecular response of Glioma Stem Cells to an AKT inhibitor

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High lethality of Glioblastoma (GBM) is explained by the progression of the disease due to the existence of Glioma Stem Cells (GSCs), which are resistant to radiation and chemotherapy. One of the signaling pathways responsible for GSCs proliferation is the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway. Thus, the use of specific inhibitors of this pathway, such as perifosine (PRF), represents a promising approach to prevent GSCs survival.

Analysis of cell viability of three primary cultures of GSCs obtained from different patients showed differences in drug sensitivity to PRF. A quantitative proteomics approach by iTRAQ labeling was used to characterize in more detail changes occurring in the proteome of GSCs after PRF treatment. Our results revealed alterations in the expression levels of proteins mainly involved in mitochondrial functions and protein synthesis.

To validate these results, we applied a targeted proteomics strategy. Multiple Reaction Monitoring (MRM) methods were designed and optimized to assess their reproducibility and repeatability. Application of these methods to a broader cohort of samples enabled us to absolutely quantify the proteins of interest and define a group of putative markers potentially related to drug sensitivity, including malectin (MLEC), switch-associated protein 70 (SWAP70), sequestosome 1 (SQSTM1) and the mitochondrial import receptor subunit TOM22 homolog (TOMM22). Integration of these data with gene expression analysis by RNAseq showed that changes observed in protein expression must be due to post-transcriptional regulatory mechanisms, highlighting the relevance of proteomics in the oncology field. However, further functional analyses are needed to confirm the role of these proteins in PRF sensitivity.

Application of quantitative proteomic approaches and their integration with transcriptomic techniques to study GSCs allowed us to establish a set of potential biomarkers of drug sensitivity. Their possible use in clinical practice would be helpful to better classify patients and to assign them the most appropriate therapy.

Posters

P1

A pneumococcal protein array as a platform to discover serodiagnostic antigens against infection

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Background and aims: Pneumonia is one of the most common and severe diseases associated with *Streptococcus pneumoniae* infections in children and adults. Etiological diagnosis of pneumococcal pneumonia in children is generally challenging due to limitations of diagnostic tests and interference with nasopharyngeal colonizing strains. Serological assays have recently gained interest to overcome some problems found with current diagnostic tests in pediatric pneumococcal pneumonia.

Methods: To provide insight into this field, we have developed a protein array to screen the antibody response to many antigens simultaneously. Proteins were selected by experimental identification from a collection of 24 highly prevalent pediatric clinical isolates in Spain, using a proteomics approach consisting of «shaving» the cell surface with proteases and further LC/MS/MS analysis. Ninety-five proteins were recombinantly produced and printed on an array. We probed it with a collection of sera from children with pneumococcal pneumonia.

Results: From the set of the most seroprevalent antigens, we obtained a clear discriminant response for a group of three proteins (PblB, PulA and PrtA) in children under 4 years old. We validated the results by ELISA and an immunostrip assay showed the translation to easy-to-use, affordable tests.

Conclusions: We have developed a protein array for its use in the study of humoral responses to pneumococcal infection. This platform is an excellent means to be used as a diagnostic tool and can be adapted to different population studies. Moreover, it may be also useful in programs of epidemiological surveillance and even for vaccine candidate discovery.

P2

An early dysregulation of FAK and MEK/ERK signaling pathways precedes the β-amyloid deposition in the olfactory bulb of APP / PS1 mouse Model of Alzheimer's Disease

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Although olfactory dysfunction is an early event of Alzheimer's disease (AD), the detailed mechanisms associated to AD neurodegeneration in olfactory areas are unknown. Here we used double-transgenic amyloid precursor protein/presenilin 1 (APPswe/PS1dE9) mice and label-free quantitative proteomics to analyze potential early pathological effects on the olfactory bulb (OB) during AD progression. Prior to β-amyloid plaque formation, 9 modulated proteins were detected on 3-month-old APP/PS1 mice while 16 differential expressed proteins were detected at 6 months, when a small portion of β-amyloid plaques appear and impaired memory and learning performance are manifested. Data mining of proteomic data suggests a moderate imbalance in cytoskeletal rearrangement, mitochondrial homeostasis, and synaptic plasticity in the OB of APP/PS1 mice. The stepwise characterization of pro- and anti-apoptotic factors revealed no activation of apoptotic pathways in the OB of APP/PS1 mice at these time points. However, β-amyloid induced a potential inactivation of focal adhesion kinase (FAK) by promoting the dephosphorylation of the activating sites Tyr 576/577 at 3 and 6 months of age. Moreover, a transient activation of MEK1/2 was evidenced in the OB of APP/PS1 mice, leading to a dephosphorylation of ERK1/2 in 6-months APP/PS1 OBs. In contrast, the analysis of human OBs revealed a late activation of FAK in advanced AD stages, whereas the activation status of olfactory ERK1/2 was enhanced across AD staging respect to neurologically intact controls. This apparent increment in the survival potential was accompanied by the inhibition of the proapoptotic factor BAD by phosphorylation at Ser112 in the OB across AD phenotypes. Taken together, our data contribute to a better understanding of the early molecular mechanisms that are modulated in AD neurodegeneration at the level of OB highlighting significant differences in the regulation of survival pathways between APP/PS1 mice and sporadic human AD.

P3

Quantitative proteomics unveils olfactory proteostasis imbalance across tauopathies and synucleinopathies: Identification of common olfactory targets

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Olfactory impairment is a common early feature in tauopathies and synucleinopathies such as Alzheimer´s disease (AD) and Lewy body-related alpha-synucleinopathy (LBAS). Although deposition of neuropathological substrates is present in olfactory areas, the molecular mechanisms involved in the olfactory dysfunction are not fully understood. We have applied mass spectrometry-based quantitative proteomics to probe additional molecular disturbances in postmortem olfactory bulbs (OB) dissected from AD and LBAS cases respect to neurologically intact controls (n=40, mean age 82.1 years; 3 neuropathological stages/disease). Proteome abundance measurements have revealed more than 300 differentially expressed OB proteins in both clinical backgrounds, evidencing a clear proteostasis impairment across AD and LBAS stages. Functional analysis showed a global alteration in neuritogenesis and axonogenesis processes during AD and LBAS neurodegeneration in the OB. Moreover, protein interaction networks revealed a progressive modulation of interactomes associated to neuropathological proteins such as APP (amyloid protein precursor) and Tau. Additionally, the activation status of specific survival routes (MAPK, p38MAPK, and PKC between others) were compromised during the disease progression. Interestingly, common olfactory substrates were detected in AD and LBAS subjects indicating that both neurological disorders with different clinical and pathological features, may present disruption of shared pathways at the level of OB. In summary, our data contribute to a better understanding of the early molecular mechanisms that occur during the neurodegenerative process at the level of the OB.

P4

Cardiovascular risk stratification by plasma proteomic. Step one: young people characterization

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Cardiovascular (CV) diseases are the leading cause of death in developed countries and present high prevalence in developing countries. Although, it is known that different epidemiologic studies have allow to calculate the risk of developing CV complications in the mid-term, the estimation of lifetime risk in the long-term still remains an unmet clinical need. Based on this, the availability of molecular profiles in young population (30-50 years old) which allow adequate stratification of CV risk with age would be of great interest.

The aim of this study is the identification of a molecular panel by multi-proteomics strategies in plasma associated to individuals with high lifetime risk in a young cohort compared to individuals with low lifetime risk.

For discovery phase, depleted plasma samples of patients with different degrees of cardiovascular risk (healthy controls (n=10), individuals with cardiovascular risk factors (n=8) and individuals with reported cardiovascular event (n=8)) were analysed using tandem mass tag (TMT) and liquid chromatography tandem mass spectrometry (LC-MS/MS). A total of 2067 proteins were identified, of these 50 were significantly differentially expressed between different groups of study. These proteins were grouped through system biology analysis showing alterations in biological processes such as arrhythmogenic right ventricular cardiomyopathy, coagulation, inflammation, antioxidant activity and immune response. A second corroboration step in crude plasma showed the verification of 16 differential proteins in an independent cohort of patients. Of them, 4 proteins were confirmed by turbidimetry, a technique used in clinical diagnostic and 1 protein was confirmed by western blot in a cohort of 48 patients.

The results obtained in the present study not only provide a deeper insight into altered molecular mechanisms in the pathogenesis of CV disease at an early stage but also these proteins could allow to stratify patients according cardiovascular risk in young population.

P5

Novel endothelial dysfunction indicators in circulating extracellular vesicles from hypertensive patients with albuminuria

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Despite of the great advances in anti-hypertensive therapies, an abundant proportion of patients under Renin-Angiotensin-System (RAS) suppression develop albuminuria, which is a clear indicator of therapeutic inefficiency. Although albuminuria has been associated with increased cardiovascular risk, the severity of vascular injury in these patients may vary depending on numerous variables (age, level of albuminuria, previous cardiovascular disease etc.). Hence, indicators of vascular function are needed to assess patients' condition and decide the succeeding therapies.

We used blood extracellular vesicles (EVs) as a low-invasive, easy-accessible source of circulating markers. Proteomic analysis showed two endothelial dysfunction proteins, altered in a cohort of 65 albuminuric patients compared to 58 normoalbuminurics. A positive correlation of both with the expression of the endothelial activation marker E-selectin was found in EVs. *In vitro* analysis using TNFα-treated adult human endothelial cells showed an increased expression of both proteins in activated cells expressing VCAM-1 and/or E-selectin, which proves their involvement in endothelial cell activation.

We propose protein levels of both proteins in circulating EVs as novel endothelial dysfunction markers to monitor vascular condition in hypertensive patients with albuminuria. Therefore, with a liquid biopsy of these patients endothelium condition could be easily checked.

P6

Diabetes mellitus alters tumor and adjacent mucosa protein expression in colorectal cancer patients, revealing protein targets and molecular pathways of potential therapeutic intervention

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Cancer and Diabetes Mellitus (DM) are among the most frequent causes of death, and colon cancer is the most frequent cancer in western countries. An association between DM and colorectal cancer (CC) has been defined by epidemiological studies. Consequences of the DM microenvironment may lead to recruitment of secondary mediators of injury which, in cancer patients, may negatively impact patient prognosis. At molecular level, precise knowledge of the complex interactions between these two conditions is needed to help better defining therapy of DM or colorectal cancer in diabetic colorectal cancer patients.

Our general goal is to address whether there is a specific molecular signature of colorectal cancer under DM, to identify novel markers and signaling pathways with potential diagnostic and therapeutic interest.

Formalin-Fixed Paraffin-Embedded (FFPE) surgically resected samples were obtained from 16 CC non-diabetics and 12 CC diabetics. Tumor and mucosa adjacent to tumor were obtained from every patient. iTRAQ 8-plex was performed (two biological replicates per condition): a) tumor non-diabetic (T) b) tumor diabetic (TD), c) mucosa non-diabetic (M), d) mucosa diabetic (MD). Peptide extracts were analyzed on a LTQ Orbitrap Velos.

415 proteins were found altered in TD vs T (116 down-regulated and 309 up-regulated) (p-value <0.05; FDR<5%). In mucosa, 61 proteins were down-regulated by DM effect and 82 were up-regulated. Fourteen proteins were altered both in tumor and mucosa in diabetics (ratio +/-1.2). Among those, TIM8B (overexpressed in mitochondria of cancer cells) was found down-regulated. NDST1 and S100A1 (involved in cell growth and proliferation inhibition or metastasis reduction) were up-regulated. Functional enrichment revealed 19 common up-regulated pathways in tumor and mucosa in DM, pointing to HIF-1 signaling, central carbon metabolism in cancer, RNA transport, oxidative phosphorylation and ribosome, among others. These proteomic data are being correlated with transcriptomic analysis.

P7

Urine proteomics reveal immune system response to albuminuria development in chronically treated hypertensive patients

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Introduction: Albuminuria is frequently observed in hypertensive patients despite of being chronically treated for blood pressure control (RAS suppression). These individuals are at the highest risk of cardiovascular and renal disease. However, they are difficult to «identify» as mechanisms subjacent to albuminuria development are underexplored.

Objetive: We pursued the identification of major changes in urine linked to albuminuria development.

Methods: A total of 37 and 47 hypertensive patients were followed-up in terms of albuminuria development and recruited for discovery and confirmation stages, respectively. They were classified as: normoalbuminuria (N), maintained albuminuria (MHA) or de *novo* developed albuminuria (dnA). iTRAQ 8-plex experiment was performed (four biological replicates per group). Altered protein responses were quantitatively evaluated individually, and coordinated protein behavior was analyzed by a recently developed algorithm to detect changes in functional categories. Confirmation of the molecules of interest was performed by SRM-LC-MS/MS.

Results: Four functional categories were identified with significant down-regulation in urine between groups: immune response of cells, adhesion of immune cells, adhesion of blood cells and phagocytosis. C3, ANXA1, CD44, S100A8 and S100A9 were confirmed to have a major impact in those biological processes. Complement C3 regulates cytokine production and alterations in dendritic cells differentiation, and play a key role in phagocytosis. ANXA 1 has an anti inflammatory role, limiting the initial steps in inflammation by T cells activation and cytokine inhibition. CD44, S100A8 and S100A9 are involved in cell adhesion. These proteins represent molecular targets in urine as early responders to the silent development of albuminuria in hypertensive patients under chronic treatment. These observations are in alignment with protein changes observed in urinary exosomes.

Conclusion: Immune alterations taking place in resistant hypertensive patients who develop albuminuria have a reflection in urine, allowing us to better stratify patients according to their CV risk for a better preventive treatment.

P8

From proteomics to a 3-protein signature that improves clinical stratification of hepatoblastoma patients

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Hepatoblastoma (HB) is one of the predominant malignant liver tumors in childhood. Although treatment of HB patients is possible by combining chemotherapy and surgery, 20% of the patients do not survive cancer. In addition, survivors can be affected by serious side effects related to chemotherapy, such as irreversible hearing loss and cardiomyopathies. Two different HB subtypes have been previously characterized: C1 (less aggressive, better prognosis) and C2 (more aggressive, worse prognosis).

The identification of novel prognostic markers that help a better HB patient stratification and improve their outcome is necessary. In this sense, the aim of the present study was to identify new HB biomarkers and to further characterize the molecular characteristics of these tumors. Sixteen HB samples, classified according to the previously described C1 (n=11) and C2 (n=5) prognostic subclasses, together with 8 normal liver samples (NL) were analyzed by 2D-DIGE and Label-Free (LF) nLC MS/MS.

Three of the significantly differential proteins identified were further validated in an independent series of 78 patients by western blotting and immunohistochemistry. The expression changes of these 3 biomarkers showed a strong association with patient event free survival (EFS) (log rank<0.05). Moreover, the combination of these 3 biomarkers was able to improve the EFS prediction of the current clinical stratification (log rank=0.013), mainly for the intermediate and poor prognostic stratums. This signature could be easily applied at the clinical practice by performing an immunohistochemistry using normal liver as a reference, and improve clinical stratification and the outcome of pediatric patients with liver cancer.

P9

Plasma microvesicles and platelet proteomic analysis in obese patients: a pilot study

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Obesity is a major health concern in industrialized countries. Platelets play an important role in the increasing cardiovascular risk since they show hyperactivation and lower sensitivity to antiplatelet therapy in obese patients. Recent studies have shown that the number of plasma-derived microvesicles (MVs) from obese patients is increased compared to healthy lean individuals.

The aim of this pilot study was to analyze the proteome of plasma MVs and platelets from obese and lean subjects in order to identify putative targets and biomarkers predictive of pathological events associated with obesity, and set the basis for larger studies.

Samples were obtained from eight obese patients, with no associated comorbidities or chronic treatments, and eight age- and sex-matched lean controls. Plasma microvesicles were isolated following a previously stablished protocol (Vélez *et al.* Thromb Haemost. 2014;112:716-726). Both proteome analyses were based on two-dimensional differential in-gel electrophoresis (2D-DIGE) and mass spectrometry. Validations were by western blotting.

We detected 44 platelet protein features in the case of platelets, and 98 in the case of MVs, that varied between obese and lean groups. From those, 27 and 45 were respectively identified by MS, corresponding to 31 and 22 open-reading frames (ORFs). Most of differentially regulated proteins identified in the platelet proteome study were involved in inflammation, oxidation stress and metabolism. Interestingly, there was a clear up-regulation of heat shock proteins (HSP27 and HSP30) in obese patients'platelets, which correlates with a pro-inflammatory state and platelet activation. On the other hand, immunoglobulins and proteins that participate in obesity-associated inflammation were increased in MVs from the obese group. In conclusion, the results of this study open a new line of research combining plasma microvesicles and platelets proteomic studies in order to identify biomarkers and drug targets that may allow a better prognostic and treatment of atherothrombotic events in obese patients.

P10

ecretome and membrane fraction proteomic analysis of colon cancer cell lines with different metastatic properties using label-free quantification

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The main cause of mortality and morbidity in colorectal cancer (CRC) patients is the development of metastases, prior to their diagnosis. We used quantitative proteomics techniques, to analyze the secreted and membrane proteins in the progression of CRC. As a model, we used three human CRC cell lines with different metastatic abilities: SW620 (isolated from lymph node), and two lines selected in athymic mice with high metastatic ability: KM12SM (that metastasize to the liver) and KM12L4a (which metastasize to the liver and lung). Concentrated secretome and membrane fraction of these cells were subjected to tryptic digestion and fractionated by OFFGEL. Protein identification and relative quantification was performed by label-free, in a mass spectrometer LTQ-Orbitrap Velos, using LFQ from the MaxQuant software. We identified 1570 and 2969 secreted and membrane proteins, respectively, common of the three cell lines. In the secretome, we found 167 overexpressed proteins generally involved in extracellular matrix reorganization, cell motility and locomotion, and 186 down-regulated proteins involved in DNA damage, carbohydrate metabolism and protein translation. In the other hand, in the membrane fraction we found 361 overexpressed proteins involved in cell cycle, antigen processing and presentation, and negative regulation of cell death. Furthermore, 387 down-regulated membrane proteins are part of biological processes such as: intracellular transport, mRNA metabolic process, protein targeting and protein folding. Among these proteins, we found P3H1, IGFBP3 as secreted proteins, and Twinfilin-1 and AHNAK as membrane proteins. Interestingly, we found that liver metastasis-associated proteins were basically associated with metabolic process instead of liver and lung metastasis-associated proteins, which were essentially in charge of extracellular matrix organization. In summary, the quantitative analysis of secreted and membrane proteins of these cell lines allowed us to identify some proteins potentially associated with metastasis, that could serve as prognostic biomarkers and therapeutic targets for fighting CRC metastasis.

P11

Patients suffering dilated cardiomyopathy caused by a mutation in the LMNA gene show augmented levels of VTDB and actin in plasma

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Dilated cardiomyopathy (DCM) is a complex disorder defined by left ventricular dysfunction that is generally associated to genetic autosomal dominant mutations. *LMNA* gene is known to be involved in the pathophysiology of DCM and its alteration is thickly associated with poor prognosis cardiomyopathy that often derives in a sudden cardiac death (SCD) episode before the symptoms instauration.

We carried out a 2D-DIGE-based proteomic analysis of ProteoMiner®-enriched plasma samples from DCM-patients carrying a novel mutation in the *LMNA* gene in comparison to healthy controls. We identified a number of proteins augmented in the DCM group, which were validated by turbidimetry and western blotting.

Two of the proteins elevated in plasma from DCM patients were actin and VTDB. Interestingly, there is a positive correlation between the levels of these two proteins in ProteoMiner®-enriched plasma from symptomatic mutation carriers. Plasma protein levels also positively correlated with the severity of the disease. Considering the reported interaction between these proteins in plasma and that VTDB acts as an extracellular actin-scavenger system, we hypothesize that they are forming a complex in plasma from these patients and suggest that could be followed as potential biomarkers for evaluating the clinical status of this population.

P12

Extensive proteomic analysis of platelet lipid rafts: setting the basis for proteome analyses these membrane domains in activated platelets

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Lipid rafts, also known as glycolipid-enriched membrane domains (GEMs), contain key platelet receptors and signaling proteins essential for platelet activation. We performed an extensive proteomic analysis of platelet lipid rafts-enriched fractions with the aim of obtaining a detailed proteome map of these fractions in resting platelets, choosing the best approach for their proteomic analysis.

Following isolation, platelets were disrupted in ice-cold 1% Brij 58-based lysis buffer, and GEMs fractions obtained by sucrose gradient ultracentrifugation and solubilization with 1% N-dodecyl-β-D-gluco-maltoside, following an established protocol (Pollit *et al.* Blood 2010;114:2938-46). GEMs-enriched fractions were characterized by anti-LAT western blot and ultracentrifuged to obtain the protein pellet. Solubilized proteins were digested with trypsin by two approaches that were compared: i) in-gel digestion, after concentrating the proteins in a band that was cut from a 12% Bis-acrylamide gel; ii) in-solution protein digestion, after sample concentration by FASP (Filter-Aided Sample Preparation). Protein identification was by LC-MS/MS in a Fusion Orbitrap.

440 proteins were identified, 200 by the in-solution digestion approach, 126 by in-gel digestion, and 114 by both. From these, 365 (82,9%) were previously found to be associated with lipid rafts (http://lipid-raft-database.di.uq.edu.au/). The majority of proteins are located in the cell membrane (46.2%) and in the cytoskeleton (12.9%). 23.3% of proteins were of mitochondrial origin due to the presence of lipid rafts in the mitochondrion membrane. More membrane and signaling proteins were identified by in-solution digestion. The alpha-granules lipid rafts marker stomatin was found by both approaches but other GEMs proteins, such as Flotillin-1/2, the adapter LAT, and the receptors GPVI and CLEC-2, were only identified in in-solution digested samples. Our results comprise one of the most extensive proteomic analysis of platelet membrane rafts to date, and pave the way for future studies focusing on changes in lipid rafts composition following platelet activation with different agonists.

P13

Proteomic analysis of human obese adipose tissue exosomes

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Introduction and purpose: In obesity, increasingly evidences sustain a specific pathological contribution of adipose tissue (AT) according to its anatomical accumulation. Hence, subcutaneous adipose tissue (SAT), is currently considered metabolically benign compared to visceral fat (VAT) whose location is associated to the risk of developing cardiovascular disease, insulin resistance, and other associated comorbidities. In this context, the role of adipose tissue as an endocrine organ is currently reaching an unprecedented dimension due to its capacity to release, in addition to classical soluble factors, extracellular vesicles (EVs). Previous work from our group with human obese AT secretome using a quantitative proteomics approach revealed that a high percentage of labeled proteins were classified as from extracellular vesicle exosomes after cellular component analysis. Hence, the aim of the present work is to isolate and characterize human obese adipose tissue released vesicles and determine the existence of depot specific EVs.

Methods: EVs were isolated by ultracentrifugation of secretomes from human obese VAT and SAT explants culture *in vitro* obtained after bariatric surgery. Proteomics analysis of VEs content was performed by LC-MS/MS (Nano-HPLC eksigen-ABSciex and MALDI-TOF/TOF-ABSciex).

Results: Human obese adipose tissue sheds exosomal (CD63+/CD9+) vesicles of 100nm. Obese AT-exosomes proteome analysis shows that although VAT and SAT share common constituents, there is a characteristic protein profile for each adipose depot. Functional analysis of obese exosomes proteome shows proteins implicated in extracellular matrix remodeling, cell growth and maintenance, protein metabolism, inflammation and insulin resistance.

Conclusions: Proteome analysis of human obese AT exosomes suggests these vesicles as a new way of para/endocrine communication probably implicated in the development of obesity related comorbidities.

P14

Comparative analysis of the endogenous peptidomes displayed by HLA-B*27 and Mamu-B*08, two MHC class I molecules associated with elite control of HIV/SIV infection

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MHC class I antigens are cell membrane glycoproteins that facilitate immunological surveillance by presenting peptides derived from endogenous proteins to cytotoxic T lymphocytes. The central role of class I molecules in immunity against intracellular parasites is highlighted by the association of some allotypes with control of particular viral infections. For instance, in rhesus macaques –arguably the most reliable animal model for AIDS research– the MHC-I allele Mamu-B*08 is associated with elite control of SIV replication. A similar scenario is observed in humans where the expression of HLA-B*27 has been linked to slow or no progression to AIDS after HIV infection. Despite showing large differences in their protein sequences, it has been reported that HLA-B*27 and Mamu-B*08 can display peptides with sequence similarity.

To fine-map the Mamu-B*08 binding motif and compare it with that of HLA-B*27 we affinity-purified the peptidomes bound to these MHC-I molecules and analyzed them by LC-MS/MS identifying several thousands of endogenous ligands. Sequence analysis of both sets of peptides revealed a degree of similarity in their binding motifs, especially at peptide position 2 (P2) where arginine was present in the vast majority of ligands of both allotypes. In addition, several differences emerged from this analysis: (i) ligands displayed by Mamu-B*08 were shorter and had lower molecular weight, (ii) Mamu-B*08 showed a higher preference for glutamine at P2 as a suboptimal binding motif and (iii) the second major anchor position, found at PΩ, was much more restrictive in Mamu-B*08. Finally, in silico estimations of binding efficiency and competitive binding assays to Mamu-B*08 of several selected peptides revealed a good correlation between the characterized anchor motif and binding affinity. These results deepen our understanding of the molecular basis of the presentation of peptides by Mamu-B*08 and can contribute to the detection of novel SIV epitopes restricted by this allotype.

P15

Analysis by mass spectrometry of unconventional MHC class I ligands derived from alternative reading frames

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Major Histocompatibility Complex (MHC, HLA in humans) class I molecules bind peptides derived from endogenous proteins which have been degraded in the cell nucleus or cytosol and present them to cytotoxic T lymphocytes allowing the immune system to detect tumor transformation processes or viral infections. A major fraction of the MHC-I-bound peptidome derives from canonical protein sequences. Additionally, there is recent evidence of unconventional mechanisms in the synthesis of proteins –such as the translation of alternative reading frames (ARFs)– that might contribute to the production of HLA-I ligands. These findings question our current definition of «open reading frame» suggesting that the complexity of the cellular proteome could be higher than previously expected and indicate that the diversity of the HLA-I-bound peptidome is much higher than previously thought.

With the aim of identifying class I ligands derived from ARFs, we used the model lymphoid cell line HMy2-C1R stably transfected with either HLA-B*39:01, -B*40:02 or -B*27:05 as a source of HLA-I-bound peptides. By combining LC-MS/MS analysis with a multiengine search against a database of predicted human ARFs, we identified about 150 non-canonical peptides. The identification of 40% of these ligands was further confirmed by comparison of the experimental fragmentation spectra with that of the equivalent synthetic peptides. Remarkably, the fraction of ARF-derived peptides displayed by each allotype varied greatly suggesting that the presentation of these ligands is allotype dependent. In particular, HLA-B*27 was particularly efficient in the presentation of these sort of peptides, a feature that might be linked with the preference of this allotype for peptides derived from small basic proteins.

Our results suggest that the mechanisms involved in the generation of this new source of complexity of the HLA-I-associated peptide repertoire represents a promising new source for the search of putative targets for cancer immunotherapy.

P16

Serologic profiling at the Candida albicans protein species level uncovers an accurate molecular discriminator for candidemia

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Despite great advances in antifungal therapy, candidemia remains a significant public health problem worldwide. This bloodstream infection caused by *Candida spp.* (predominantly *Candida albicans*) often results in delayed initiation of appropriate antifungal therapy and poor clinical outcomes. Early diagnosis of candidemia could improve this clinical setting. Serum antibodies to specific *Candida* proteins have been reported as potential diagnostic biomarkers for candidemia. However, their diagnostic usefulness at the protein species level has hardly been examined. We combined serological proteome analyses with data mining procedures to examine the IgG-antibody responses to *Candida albicans* protein species in IC and non-IC patients. We found that 87 discrete protein species derived from 34 unique proteins were IgG-targets, although only 43 of them were differentially recognized by serum samples from IC and non-IC patients. An increase in the speciation of the immunome, connectivity and modularity of antigenic species co-recognition networks, and heterogeneity of antigenic species recognition patterns was associated with candidemia. Multivariate logistic regression analyses further revealed that serum IgG antibodies to certain distinct protein species were better predictors of candidemia than those to their corresponding proteins. A molecular discriminator delineated from the combined fingerprints of serum IgG antibodies to two discrete species of phosphoglycerate kinase (Pgk1) and enolase (Eno1) accurately classified candidemia and control patients. These results shed new light on the anti-Candida IgG antibody response development in candidemia, and demonstrate that an immunoproteomic signature at the molecular level may be useful for its diagnosis. Our study further highlights the importance of defining pathogen-specific antigens at the chemical and molecular level for their potential application as immunodiagnostic reagents or even vaccine candidates.

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P17

Identification of predictive biomarkers of therapeutic response in knee OA: the MOVES study

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The aim of this study was to identify predictive protein biomarkers useful to stratify osteoarthritis (OA) patients into responders and non-responders, either to Droglican® (glucosamine hydrochloride+chondroitin sulfate) or the COX-2 selective nonsteroidal anti-inflammatory drug Celecoxib, in order to optimize therapeutic outcomes in OA. A shotgun proteomic analysis was performed on sera from patients enrolled in the Multicentre Osteoarthritis interVEntion trial with Sysadoa (MOVES), employing the iTRAQ labelling technique followed by LC-MALDI-MS/MS analysis. The samples were classified between responders and non-responders, either to Droglican or Celecoxib, according to the WOMAC pain score (<20 and < 70) and the OMERACT-OARSI criteria recorded at the end of the trial (after 6 months of treatment). In the discovery phase, the proteomic screening led to the identification of 176 different proteins in the serum samples at baseline. 46 proteins showed a statistically significant increase in the responder groups, while 43 resulted upregulated in the non-responder groups. Among the altered proteins, we selected beta-2-glycoprotein 1 (APOH) in the Droglican-responders group and thrombospondin 1 (TSP1) in the Celecoxib-responders group to validate in the verification phase by ELISA assays. The results obtained with 10 samples per group (n=80) confirmed that the levels of these proteins at baseline are altered in the group of non-responders either to Celecoxib or Droglican in comparison to the responders according to the WOMAC criteria.

The results of this work demonstrate how the levels of protein biomarkers such as APOH or TSP1 are useful to predict the patient's response to a specific compound (Droglican or Celecoxib), by identifying certain OA patient populations that are more likely to respond to a specific drug therapy. This shift towards personalized medicine can help the clinicians to choose the best treatment option for each OA patient, thus enhancing the probability of success of the pharmacotherapy, while reducing specific adverse events.

P18

Peptidomics applied to the search for biomarkers in septic shock patients

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Sepsis and septic shock are one of the main causes of death in intensive care units, with a mortality rate of up to 52%. This high mortality rate has raised awareness about sepsis and septic shock worldwide and many efforts are being made towards the discovery of biomarkers and new therapies to prevent and treat this life-threatening illness. Sepsis and septic shock have been associated with dysregulation of proteolysis in the organism. Peptidomics analysis of blood plasma is a suitable tool to identify peptides and proteins related to these conditions which could help to better understand the role of proteolysis in the progression of the disease.

Being plasma one of the most complex biological samples, the aim of this study was to optimize a sample cleanup method suitable for high throughput plasma peptide extraction compatible with nanoLC-MS/MS. Up to nine methods were tested and the reproducibility of the two best performing ones was evaluated in three technical replicates analysis. The best performing method was a combination of ultrafiltration with 10KDa filter followed by two solid phase extraction steps: C18-like chromatography in 96 well plate format followed by strong cationic exchange chromatography in tip format.

Proteolysis dysregulation during sepsis and septic shock was evaluated in plasma from 15 patients with sepsis (at ICU admission) and septic shock at two time points (ICU admission and 7 days after admission). The results from five biological replicates for each condition showed the highest proteolysis level in the sepsis samples. Apolipoproteins were much more proteolyzed in sepsis than in septic shock, showing a dysregulation of lipid transport, as already described in the literature. Moreover, analysis with Progenesis QI software pointed to some protein candidates for biomarker of both Sepsis and Septic Shock.

P19

Identification of differentially expressed proteins in the prefrontal cortex of patients with Alzheimer Disease using Protein Microarrays

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Alzheimer disease (AD) is a severe neurodegenerative disorder with high social and economic impact. AD is the most common form of dementia in ageing population. It is estimated that in 2050, approximately 80 million people worldwide will suffer from its devastating effects. Accurate and early diagnosis of AD is still difficult because early symptoms are shared with a wide variety of disorders. Definitive diagnosis requires both clinical assessment and post-mortem verification.

We have screened two types of high-throughput antibody microarrays with protein extracts from the prefrontal cortex brain tissue of AD patients and controls (healthy individuals and vascular and frontotemporal dementia patients) to identify differentially expressed proteins. The statistical analysis of the microarray data allowed us for the identification of 40 deregulated proteins in AD patients in comparison to controls. The deregulation of 13 out of 40 altered proteins was validated by western blot and semi-quantitative PCR analyses. Bioinformatics using STRING and Ingenuity Pathway Analysis showed a total of seven altered protein networks. Among them, we found networks closely related to the immune system due to the interactions between proteins from the innate immune system with proteins involved in the adaptive immune system. Further validation, functional analysis and quantification of their deregulation in sera will be performed to determine their usefulness as blood-based biomarkers and their relationship with the immune response.

The analysis of alterations in protein expression is a key step for the discovery of new disease-specific biomarkers and for the elucidation of altered pathways and cellular processes. Our study showed that antibody microarrays are reliable tools for high-throughput analysis of protein expression changes in brain from AD-patients. Remarkably, many of the identified deregulated proteins were related to the immune system, highlighting the role of the inflammatory response in the AD pathogenesis.

P20

Identification of protein signatures in urine linked to Cardiovascular Risk in Young Population

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Cardiovascular diseases (CVD) are the leading cause of death in developed countries and have a growing prevalence in developing ones. The atherosclerotic process starts early in life, but the silent development impairs early detection. The predictive value of traditional CV risk calculators is limited. Novel indicators of CVD progression are needed, particularly in young population, aimed to an early and preventive intervention.

The aim of the study is the identification of molecular profiles in urine associated with different CV risk level in a cohort of young population (30-50 years).

In a first discovery phase, urine samples from controls (C) (n=10), individuals with CV risk factors (F) (n=8), and individuals with previously reported CV event (E) (n=12) were compared using tandem mass tag (TMT) and liquid chromatography tandem-mass spectrometry (LC-MS/MS). Systems biology analysis was performed aimed to detect differences in functional categories. A potential correlation between protein levels and theoretical QRisk (predictive algorithm) in each group was also evaluated. Candidate proteins showing significant alteration in response to different CV risk level are being confirmed by SRM-LC-MS/MS in an independent cohort of 45 patients.

A total of 4313 proteins were identified. Among those that were differentially expressed between groups, 30 were selected in view of their potential role in CVD and level of variation: 14 proteins were increased in F compared with C (4 of which were increased, 2 held similar values and 8 were reduced in E compared to F), and 16 were reduced in F compared with C (4 of which were reduced, 1 held similar values and 11 were increased in E compared to F). Four proteins showed significant correlation, on average, with QRisk.

This study reveals protein signatures in urine directly linked to cardiovascular risk, to be further evaluated in terms of designing prevention strategies and therapeutic interventions.

P21

Biomarker discovery on bronchoalveolar lavage fluid with SWATH MS and feature selection: four proteins classify lung adenocarcinoma patients and controls

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Bronchoalveolar lavage fluid (BALF) is collected for standard lung pathological procedures but has rarely been used in quantitative proteomics studies aimed to discover diagnostic or prognostic biomarkers for lung cancer, or even to investigate the underlying pathophysiological mechanisms. We report a study aimed to assess whether the analysis of BALF using data-independent acquisition (DIA) quantitative proteomics can detect changes in protein abundance that could be useful as biomarkers for lung adenocarcinoma.

Methods: BALF protein extracts from 12 patients and 10 controls were analyzed by LC-MS/MS using a DIA SWATH method in a Sciex Triple TOF mass spectrometer. Datasets were processed using PeakView software using the MS/MSALL with SWATH Acquisition MicroApp. Peptide chromatrograms were extracted from DIA data using the spectral library created from previous shotgun runs, and a linear mixed-effects model-based analysis for determining proteins changing in abundance between both conditions and a statistical power analysis were implemented using MSstats into R. A feature selection workflow including three different algorithms for classification was applied using the bootfs R package, and a final biomarker set with the minimum number of features able to obtain the best classifying performance was derived.

Results: 863 proteins were identified (FDR < 1% at both protein and peptide levels) in BALF samples, this showing that our approach outperforms previously reported BALF analyses. Power analysis revealed that a fold-change of 3.75 could be detectable for our experimental design when measuring 5 peptides/protein and 4 transitions/peptide for obtaining a 80% statistical power. 41 proteins showed a significant (adjusted p-value < 0.05) fold-change higher than 3.75 among adenocarcinoma patients and controls. Pathway analysis was performed, pointing to the complement network as being strongly over-represented. The combination of the top four proteins in the biomarker set derived from the feature selection workflow, namely TMC5, SCGB1A1, SG3A1 and DPY30 showed the best classifying performance (100% sensitivity and specificity) with the minimum number of features.

Conclusions: The analysis of BALF proteins by a SWATH approach is a useful method for discovering potential biomarkers of pulmonary diseases. We report a panel of potential protein biomarkers that could increase our comprehension of adenocarcinoma's molecular pathways. Four proteins classified lung adenocarcinoma patients and controls with best performance.

P22

Quantitative protein analysis of endothelial progenitor cells in atherosclerosis

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Endothelial progenitor cells (EPCs) constitute a promising alternative in regenerative medicine for cardiovascular diseases due to their assigned role in angiogenesis and vascular repair. In response to injury, EPCs seem to promote vascular remodeling by replacement of damaged mature endotelial cells and/or by secreting certain angiogenic factors over the damaged tissue. Nevertheless, such mechanisms need to be further characterized.

Herein we have focused on the identification of proteins differentially expressed between EPCs of atherosclerotic patients and EPCs from healthy individuals, in order to better characterize the response of EPC in the atherosclerotic pathology and provide potential candidates susceptible of manipulation to promote vascular regeneration.

EPCs were isolated, cultured and characterized following standard procedures. At day 7 EPCs were lysed, reduced/alkylated and sequentially digested with LysC and trypsin. The resulting peptides were then labeled using dimethyl labeling: EPC Patient (heavy, 13CD20) and EPC Control (light, CH2O). Three technical replicates from samples of three different individuals were run on a Q-Exactive, using a 4h LC-gradient. Data analysis was performed with ProteomeDiscoverer 1.4. Differentially expressed proteins were defined as follows: Proteins identified in at least 2 replicates out of 3, p-value (t-test) < 0.05 and fold-change rates >2 for up-regulated or <0.5 for down-regulated proteins. The IPA Pathway platform was used for further analysis of identified proteins.

Derived from this analysis 1196 proteins were identified, including 52 proteins which were upregulated and 38 down-regulated in the EPCs of atherosclerotic patients versus the EPC control. Differentially expressed proteins appeared to be involved in several pathways regulating angiogenesis, cell mobility and cell-cell interactions. The function of these proteins will be discussed.

P23

Isolation and proteomic characterization of exosomes from peritoneal exudates of mice with a lupus-like disease induced by pristane

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Introduction: Intraperitoneal injection of a single dose of pristane to mice provokes a local inflammatory reaction with a massive influx of myeloid cells from bone marrow, mainly pro-inflammatory Ly6C^{hi} monocytes and neutrophils. In the long term, mice develop an autoimmune disease similar to human Systemic Lupus Erythematosus (SLE). Our hypothesis is that in the peritoneum under such a pro-inflammatory milieu it will be a secretion of exosomes and other extracellular vesicles by Ly6C^{hi} monocytes and neutrophils.

Objectives: The aim of this work has been to isolate exosomes from peritoneal exudates (PE-EXO) of mice treated with pristane 2, 4 and 8 weeks before, and to identify the proteins by mass spectrometry (LC-MS/MS). Since mice deficient in CD38 (Cd38$^{-/-}$) develop a milder disease, with a defective influx of Ly6C^{hi} monocytes and neutrophils, exosomes from Cd38$^{-/-}$ mice were also isolated, and their protein content was compared with that in WT mice. In parallel, cells from the same peritoneal exudates (PEC) were also isolated and their protein extracts analyzed by LC-MS/MS.

Methods: Peritoneal exudates from pristane-treated mice were collected by i.p. injection of 5 ml of PBS/BSA/EDTA and after cell separation; centrifuged sequentially, filtered, concentrated and subjected toqEVsize exclusion columns (www.izon.com) for exosomes purification. Protein extracts were analyzed by LC-MS/MS and MALDI-TOF-MS/MS. Protein identification was performed with ProteiScape 4.0 (Bruker) and MASCOT data searching using Swiss-Prot database. Gene Ontology (GO) enrichment analyses of PEExs protein extracts were performed using FunRich and PANTHER tools.

Resultsand Conclusions: GO enrichment analyses indicated that, most of the proteins identified by MS were part of exosomes or extracellular vesicles, which was clearly indicative that the exosome isolation procedure used was appropriate. Some of the identified proteins in the PE-EXO, such as S100A8 and S100A9, may contribute to the migration of Ly6C^{hi} monocytes and neutrophils to the inflamed peritoneum.

P24

Multi-omic profiling to assess the effect of iron starvation in Streptococcus pneumoniae

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Background and aims: Pneumococcal pneumonia is one of the most common and severe diseases associated with *Streptococcus pneumoniae* infections. To understand how pathogenic microorganisms are able to infect hosts, different models are used. However, it is not always easy to simulate in the lab what occurs *in vivo*. It is well known that when pathogens carry out infection, the concentration of free iron, an essential micronutrient for bacterial growth, is extremely low.

Methods: We applied a multi-omics approach (transcriptomics, proteomics and metabolomics) to study the effect of iron starvation in the pneumococcus, to elucidate global changes in the bacterium, treating two reference strains, TIGR4 and R6, with the iron chelator deferoxamine mesylate.

Results: We found higher changes for the TIGR4 strain, especially at the transcriptome level. DNA microarrays revealed the induction and repression of operons involved in multiple biological processes, with enrichment in ion binding genes among those over-expressed. We also studied the changes in protein abundance by proteomic analysis of total cell extracts and secretome fractions. The main proteome changes were found in proteins related to the primary metabolism, especially enzymes having divalent cations as cofactors. Finally, the metabolomic analysis of intracellular metabolites showed an increase in the concentration of intermediate metabolites participating in cell wall peptidoglycan biosynthesis.

Conclusions: This work shows the utility of multiperspective studies that can provide complementary results for the comprehension of how a given condition can influence on global physiological changes in pathogenic microorganisms, as well as differences of the responses in different strains.

P25

Proteomic analysis in a Traditional fermented beverage

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Pozol is an acid, refreshing and non-alcoholic traditional beverage, consumed by ethnic and Mestizo populations in the southeast of Mexico. The general method of pozol elaboration begins with the cooking of maize kernels in lime (nixtamalization) and its ulterior washing to remove the pericarp. The grains are then coarsely ground, shaped into balls and wrapped in banana leaves. Finally, the dough is fermented at ambient temperature for hours to 7 or more days. This spontaneous fermented food is characterized by an abundant and complex microbiota. Mainly formed by lactic acid bacteria, but including also aerobic mesophilic bacteria, enterobacteriaceae, yeast and mould.

During the fermentation process important changes take place, such as a drastic reduction in pH value, from 7 to 4, in only 24 hours; especially due to the lactic acid production and also an a decrease in the concentration of soluble sugars. These changes are clearly associated to the type of microorganisms in the system, its abundance and metabolism. Research on the role of microorganisms during the fermentation, is necessary in order to determine their significance in the final organoleptic characteristics, nutritional value and to improve the elaboration process, to ensure the safety and quality of the beverage. Since no previous studies have investigated the relationship between the content of carbohydrate in the system and the enzymatic activities occurring in the fermentation, the first objective of this work was to identify the proteins involved in carbohydrate metabolism and relate them with the microbial groups present.

The LC-MS/MS analysis allowed identifying a total of 680 proteins of bacterial origin and 249 proteins from fungi. During the first 72h, the bulk of bacterial proteins belong to *Streptococcus. Lactobacillus* was the most abundant after 3 days of fermentation, but proteins from *Leuconostoc* and *Enterococcus* were also found. Fungi are represented by *Neurospora*, *Schizosaccharomyces*, *Saccharomyces* and *Aspergillus*. In general, these results are consistent with the previous microbiological analysis reports.

Among the identified proteins, those belonging to the dominant genera were categorized according to COG nomenclature (Clusters of Orthologous Groups). In all cases, the most prevalent proteins belong to carbohydrate metabolism, which clearly indicate that fungi and bacteria are metabolically active during the fermentation.

P26

Quantitative Proteomic Study of Macrophage ATP binding proteins after Interaction with Candida albicans

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Candida albicans is an opportunistic pathogenic fungus that as commensal is harmless, but in immunosuppressed patients can outgrow and cause symptoms of disease. Macrophages, in collaboration with neutrophils and monocytes, are key cells in the recognition and in innate immune response to *C. albicans*. Recent developments in mass spectrometric (MS) workflows allowed proteome wide analysis of the abundance of macrophage proteins, many of them differentially expressed in processes like inflammation, cytoskeletal rearrangement, stress response and metabolism [1]. Combining MS with selective enrichments turned the global study of low abundant proteins, such as ATP-binding proteins and kinases, possible.

Here, we developed a quantitative proteomic approach for the study of human macrophage ATP binding proteins after interaction with *C. albicans* cells. For that, the human monocytic (THP1) cell-line was used and SILAC labelled. After monocytes differentiation into macrophages by PMA adding, *C. albicans* cells were incubated with the macrophages during 3 hours and with a ratio of 1:1. Cells were lysed and the resultant protein lysate was enriched in ATP-binding proteins using the ActivX desthiobiotin ATP probes (Thermo Scientific) kit. For the shotgun approach, proteins were analyzed by LC-MS/MS using an LTQ-Orbitrap and the fragment ions extracted for a protein database using Mascot.

Overall, 547 non-redundant proteins were quantified, 137 proteins were ATP-binding proteins. Among the ATP-binding proteins quantified 46 were kinases. Further analysis showed 59 macrophage proteins differentially abundant after interaction with *C. albicans*: 22 more abundant and 37 less abundant, including 2 kinases (Map2k2, Syk) and 4 (Stk3, Map3k2, Ndka, Srpk1) respectively. Proteomic results were validated by western blotting.

This approach contributes to expand the knowledge on macrophage proteins that may be implicated in the response to *Candida* infections.

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[1] Reales-Calderón, J, *et al*., (2013), JOP, 91, 106

P27

Proteomic approach to Botrytis cinerea membrane phosphoproteins

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Botrytis cinerea is a fungus that infects more than 200 relevant crops. It is a model fungus for the study of phytopathogenicity that exhibits a wide arsenal of tools to infect plant tissues. Their membrane proteins play a key role as a bridge between environmental conditions and intracellular molecular processes.

Proteomic approaches have recently let to characterize a set of receptors and phosphoproteins in the membrane of this fungus. Specifically, about 5000 proteins from *B. cinerea* were identified and characterized, showing its role in several molecular processes including fungal virulence.

In spite of that, it is necessary to go in depth in the role of these proteins and in the information transference from outside to the cell nucleus. In this communication we present a new proteomic approach to study the membranome of *B. cinerea* containing well characterized phosphosites under different pathogenicity conditions induced by using different plant-based elicitors. Identified proteins and P-site patterns according to the used carbon source were studied, and its relation with virulence is discussed. This sub-proteome may allow to the scientific community the discovery of new virulence factors in *B. cinerea*.

P28

Proteomic profile of the microalga Nannochloropsis gaditana

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Nannochloropsis is a genus of alga that is well known from the marine environment but has only recently been reported from fresh and brackish waters. *Nannochloropsis gaditana* is used as fish and mollusc food. However, nowadays microalgae are considered as potential producers of proteins and they might be an alternative protein source that could be very valuable in areas such as agri-food or biomedicine.

In order to study the whole proteome of *Nannochloropsis gaditana*, it was combined a protein separation by two-dimensional polyacrylamide gel electrophoresis (2-DE) and subsequent mass spectrometric analysis of the tryptically digested proteins (LC MS/MS). The identified proteins were categorized according to their molecular functions and biological processes based on gene ontology classification.

In this study it is described the first proteomic analysis of *Nannochloropsis gaditana*, containing an important number of identified proteins.

P29

Search for NE-associated proteasome targets in the fission yeast

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TPR (Translocated Promoter Region) proteins are evolutionally conserved large coiled-coil proteins that localize at the nucleoplasmic side of the NPC forming the structure called nuclear basket. TPR has been involved in a variety of nuclear processes as SUMO homeostasis, spatio-temporal regulation of the spindle assembly checkpoint, spatial regulation of heterochromatin in the surroundings of NPC or mRNA export. Importantly, the fission yeast TPR, Alm1, plays a key role in regulating the maintenance of the proteasome to the nuclear envelope, where the proteasome is enriched, leading to the degradation of ubiquitin-tagged proteins. The nuclear periphery is a major site of proteolysis degradation in yeast during the cell cycle and is critical to maintain steady-state levels of centromeric proteins required for proper chromosome segregation. In this work, we used a Proteomic approach to compare nuclear extracts from three yeast strains: a wildtype, an alm1-deleted mutant and a conditional mutant deficient in general proteasome function. In that way, we searched for NE-associated proteasome targets by identifying qualitative and quantitative changes in electrophoretic mobility in a loss of proteasome localization mutant and in a lack of proteasome function mutant.

P30

Integrated metaproteomic and metagenomic analysis of a microbial biofilm adapted to extreme acidic conditions from an acid mine drainage system

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Acid Mine drainage (AMD) constitutes one of the main problems associated with the oxidation of sulfides on the Earth's surface. The low pH and the elevated metal concentrations produced have a dramatic and persistent impact on the environment producing conditions where extremophilic microorganisms, with a high potential biotechnological value, exist. Here we analysed a microbial biofilm growing in the discharge of an abandoned pithead mine (Huelva, Spain) using an integrated metaproteomic and metagenomic approach. The biofilm develops under anaerobic to microaerophilic conditions (<15 μM O2) and under extreme conditions of pH (1.5-2.5) and metals concentrations (e.g. 10mM Fe, 4mM Al, 0.06 mM Cu and 0.23 mM Zn). Initial clone library and 454' pyrosequencing analysis identified the majority of the OTUs belonging to uncultured bacteria found previously acidic mine drainage sites in the area. The phylum Firmicutes dominated the community (40-75% of OTUs). At the OTU level, commonly found bacteria included *Ferrovum myxofaciens, Metallibacterium scheffleri, Desulfosporosinus auripimenti*, and various uncultured bacteria. The acidophilic microalgae *Auxenochlorella protothecoides* was also detected. Proteomic and genomic data largely coincided. Solubilized proteins extracted from fresh biofilm samples were tryptic digested and analysed by nano-LCESI-MS/MS. Resultant peptide sequences were compared against several databases through the Mascot platform linked to the ProteinScape (Bruker Daltonics) software. Proteomic analysis highlighted the presence of proteins mainly correlated with electron transport and iron and sulfur oxidation pathways. The identified proteins correlated among others to *Ferrovum* sp (25 proteins), *Acidithrix ferroxidans* (8), *Auxenochlorella protothecoides* (2) as well as diverse fungi. The presence of *Auxenochlorella protothecoides* in the absence of light and the accumulation of Cu0 and SOx species within the biofilm raise interesting questions on the metabolic interactions between the microorganisms of this biofilm community.

P31

Immunoproteomic analysis in symptomatic canine leishmaniasis

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Visceral Leishmaniosis (VL) is a disease caused by the intracellular protozoa *Leishmania* found mainly in macrophages from vertebrate hosts. Dogs are the main reservoir for the parasite which proliferates leading to a chronic disease characterised for anaemia, severe skin lesions, local or generalized lymhadenopathy ending up renal and liver failure.

In the present study, proteomic tools have been used to explore the serological response to *Leishmania infantum* protein extracts obtained from promastigotes of symptomatic dogs living in an endemic area in Valencia (Spain). Their usefulness to diagnose canine VL was investigated with the aim of predicting outcomes in the dogs, and also to select potential therapeutic targets for prevention and/or treatment of the disease.

Proteins were identified in the Western blot employing 2-DE and MALDI TOF-TOF spectrometry. A complex recognition pattern was observed in all animals with high reactivity between 50 and 120 kDa. The major immunogenic proteins were several isoforms of heat shock 70-related protein 1, 20S proteasome subunit alpha 5, different fragments of the alpha-tubulin, beta tubulin, S-adenosylhomocysteine hydrolase, trypanothione reductase, glucose-6-phosphate dehydrogenase, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (iPGAM), enolase, proteasome alpha 3 subunit, activated protein kinase c receptor (LACK); dihydroorotate dehydrogenase, pyruvate dehydrogenase as well as several hypothetical proteins. The antigens identified were involved in stress response,energy metabolism and protein synthesis. It is well-known the immunogenic capacity of heat shock 70-related protein and the enolase not only in *Leihsmania* but also in other parasites. However, this is the first time where the immunogenic character of iPGAM protein is described. This protein is involved in the glycolytic and gluconeogenic pathways of the parasite which could be utilized in vaccines or diagnostic tools.

P32

Assessment of effects of environmental contamination by polyaromatic hydrocarbons in marine fishes by proteomic analysis

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We used proteomic analysis as a tool to evaluate and compare sublethal effects of polyaromatic hydrocarbons (PAHs) in two marine fish species, *Epinephelus marginatus* and *Rachicentrum canadun*. Juveniles from both species were maintained in the laboratory at Centre for Marine Biology of the University of São Paulo, Brazil, for a period of acclimatization under controlled abiotic conditions. The fish were then randomly divided into three groups (Control, C1; PAHs, PAHs 1; PAHs under irradiation, PAHs 2), exposed to waterborne concentrations of PAHs, respectively 0.5 ppm for *E. marginatus* and 0.4 ppm for *R. canadun*, for a period of 14 days in a semi-static system. Individual livers were extracted and preserved in RNAlater®. 2-D PAGEs were carried out for proteomic identification, using liver samples from five individuals. The 2D-spots presenting over-expression relative to control conditions were identified. Peptides resulting from protein digestion were analyzed by mass spectrometry (MALDI/TOF TOF). Results were compared against the MASCOT database. The analysis evidenced an important protein group profile among fish species exposed to petroleum (hydroxyphenylpyruvate dioxygenase; methyl-CpGb and liver angiotensinogen 1) and to irradiated petroleum (glutathione S-tranferase; natural killer and caspase-3). Our study suggests that combined use of sets of marker proteins associated with a particular contamination impact may be more reliable than independent analysis of a few unique biomarkers, since they reflect the complexity of the toxicological response.

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P33

Hepatic Proteome Expression Profiles of Atlantic Salmon (Salmo salar) After Exposure to Environmental Concentrations of Human Pharmaceuticals

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Consumption of prescription and over-the-counter pharmaceutical products has increased dramatically during the last decades, and pharmaceutical compounds are being detected in surface-, ground- and drinking waters. Due to their continuous consumption, pharmaceuticals are pseudopersistent aquatic pollutants with unknown effects at environmentally relevant concentrations. Atlantic salmon (*Salmo salar*) were exposed to the anti-inflammatory drug Acetaminophen (54.77 ± 34.67µg·L^{-1}); the beta-blocker Atenolol (11.08 ± 7.98 µg·L^{-1}) and the anti-epileptic and anti-depressant Carbamazepine (7.85 ± 0.13 µg·L^{-1}) for 5 days. The selected pharmaceuticals are among the most frequently detected in fresh- and marine waters. After the treatments, 19, 7 and 15 proteins were differently expressed for Acetaminophen, Atenolol and Carbamazepine, respectively with respect to the control group. Out of these, three features were common between Acetaminophen and Carbamazepine and one between Carbamazepine and Atenolol. One feature was common across all treatments. Principal component analysis and heat map clustering showed a clear grouping of the variability caused by the applied treatments. The obtained data suggest (1) that exposure to environmentally relevant concentrations of the pharmaceuticals alters the hepatic protein expression profile of the Atlantic salmon; and (2) the existence of treatment specific processes that may be useful for biomarker development of pharmaceutical contamination.

P34

Proteomic study of quercus ilex response to progressive drought stress at seedling establishment stage reveals prominent changes in starch mobilisation and carbohydrate metabolism

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Quercus ilex is a dominant tree species in the western Mediterranean basin both in natural forest and agrosilvopastoral meadow ecosystems. Elucidating the mechanisms of holm oak survival in the hot and dry Mediterranean climate will be of help for conservation, sustainable management and development of regional reforestation programs. In line with these needs, an intense omics research is being performed on Quercus ilex natural variability, stress response and embryogenesis. Adult holm oak trees are considered to be well adapted to drought. Environmental stresses could be very detrimental early in development. Drought is reported to be the main cause of seedlings` mortality post-transplanting.

We studied the proteome changes during progressive water deficit stress applied on Quercus ilex at the stage of seedling establishment. Progressive water stress was imposed by water limitation for a period of 9 days on germinated acorns grown in perlite. The physiological response to stress was estimated by changes in root length, membrane stability, proline accumulation and sugar content. Protein changes in roots and cotyledons were analysed using combined 1-DE/2-DE approach with identification by MALDI TOF-TOF PMF and MS/MS.

Applying 1-DE/MS on samples taken in dynamics, a total of 49 different drought-responsive proteins were identified in roots and cotyledons. As the most evident biochemical and protein changes occurred at day 9th of stress, this stress time point with recovery were analysed in more details by 2-DE/MS, with additional 78 protein identifications. As carbon provides both building blocks for cell structures and fuel for energy production, data discussion was focused on the dynamic changes in carbon metabolism. An early and coordinated increase in starch metabolic enzymes was detected in cotyledons under drought, along with up-regulation of glycolysis and tricarboxylic acid cycle. In roots, sucrose synthase increased in root tips under stress, probably due to enhancement of source-sink interactions.

P35

Systemic analysis of physiological responses to UV stress and related adaptive mechanisms in Pinus radiate

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Globally expected changes in environmental conditions, especially the increase of UV irradiation, necessitate extending our knowledge of the mechanisms mediating tree species adaptation to this stress. This is crucial for designing new strategies to maintain future forest productivity. Studies focused on environmentally realistic dosages of UV irradiation in forest species are scarce. *Pinus sp.* are commercially relevant trees and not much is known about their adaptation to UV. UV treatment and recovery of *Pinus radiata* plants with dosages mimicking future scenarios based on current models of UV radiation were performed in a time-dependent manner. The combined metabolome and proteome analysis was complemented with measurements of physiological parameters and gene expression. Sparse PLS analysis revealed complex molecular interaction networks of molecular and physiological data. Early responses prevented photoxicity by reducing photosystem activity and the electron transfer chain together with the accumulation of photoprotectors and photorespiration. Apart from the reduction in photosynthesis as consequence of the direct UV damage on the photosystems, the whole primary metabolism was rearranged to deal with the oxidative stress while minimizing ROS production. New protein kinases and proteases related to signalling, coordination, and regulation of UV stress responses were revealed. All these processes demonstrate a complex molecular interaction network extending the current knowledge of UV-stress adaptation in pine.

P36

Identification of wood quality biomarkers in Mediterranean pine using a Systems Biology approach

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Natural variation of the metabolome and proteome of *Pinus pinaster* was studied to improve our understanding of phenotypic diversity, and wood quality. The metabolome and proteome of needles and the apical and basal section of buds were analyzed in three provenances of *P. pinaster* with contrasting growth capacity selected from mountain in the northwest (CDVO) to the coastal region of southeast Spain (ORIA) also considering a provenance from a sandy Moroccan area (TAMR). The three provenances were grown in a common garden for five years and metabolite and protein extraction were performed from the same sample. For metabolite detection two complementary mass spectrometry techniques: GC-MS and LC-Orbitrap-MS were used, while for protein identification GeLC-Orbitrap/MS combined with the development of custom peptide databases was used. Metabolome, proteome and environmental and growth data were integrated employing modelling and statistical tools to provide a comprehensive picture of phenotypic diversity.

A total of 1576 metabolites and 1447 proteins were identified. The metabolites characteristic of each tissue are related to primary metabolism, while provenances were distinguishable when tissues were analysed independently. Integrative studies showed three population clusters, being secondary metabolites, and in particular flavonoid and terpenoid pathways, essential to reach this differential clustering. Additionally, some key enzymes were linked by sPLS networks to wood quality. Altogether these results provide a new perspective of how tree metabolism adapt to different environment, and how these adaptions are also reflected in wood quality, providing these results a new set of biomarkers for breeding programs and forest management practices.

P37

Caracterización del proteoma de semillas de Pinus occidentalis, una especie recalcitrante de valor económico y medio ambiental para la República Dominicana.

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En el presente trabajo se realizó la caracterización del proteoma de semillas de *Pinus occidentalis*, en el que se ha utilizado una doble aproximación, la basada en electroforesis uni y bidimensional acoplada a MALDI-TOF/TOF y la de «shotgun» (nLC-LTQ-Orbitrap). Las proteínas fueron extraídas de las semillas utilizando el protocolo de precipitación con TCA / acetona / fenol. Los extractos de proteínas fueron separadas mediante electroforesis uni y bidimensional. Todas las bandas (1-DE) y los puntos más abundantes (2-DE) fueron escindidos del gel, fueron digeridos con tripsina y los péptidos resultantes fueron analizados mediante MALDI TOF/TOF. Para la estrategia sohtgun se llevó a cabo una electroforesis 1-D de banda única, la cual se sometió a digestión con tripsina, siendo los péptidos resultantes analizados por nLC-ESI-LTQ-Orbitrap. A partir de los espectros de masas, las proteínas se identificaron mediante la búsqueda combinada en tres bases de datos diferentes, UniProtKB, NCBInr y *Pinus spp*, siendo ésta última una base de datos específica para el género (Romero *et al*., 2014). El número de especies proteicas identificadas fue de 42 (1-DE y 2DE), y 187 (shotgun). Se llevó a cabo la clasificación funcional de las proteínas identificadas (Gene Ontology, empleando Blast2FGo), siendo las categorías de: reserva, metabolismo, producción de energía, defensa y metabolismo del DNA, las más representadas. Se ha establecido, por primera vez, el proteoma de referencia de *P. occidentalis*, lo que servirá de base para posteriores estudios de estudio de biodiversidad y caracterización poblacional, estudios de germinación en semillas no ortodoxas y respuestas a estreses asociados a condiciones de cambio climático.

P38

Urea artifacts interfere with immuno-purification of lysine acetylation

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Comprehensive analysis of post-translational modifications (PTM) often depends on the purification of modified peptides prior to LC-MS/MS. The implementation of these enrichment methods requires thorough knowledge of the experimental conditions in order to achieve optimal selectivity and sensitivity. In this regard, large-scale analysis of lysine acetylation, a key PTM for multiple cellular processes, makes use of monoclonal pan-antibodies designed against this moiety. Here, we report that the immuno-purification of lysine-acetylated peptides is hampered by the co-purification of lysine carbamylated peptides, a frequent urea artifact. This specific interaction can be explained by the similar chemical structures of lysine acetylation and lysine carbamylation. As an alternative, we propose a sample preparation protocol based on sodium deoxycholate that eliminates these artifacts and dramatically improves the selectivity and sensitivity of this immuno-purification assay.

P39

Identification and fate of intracellular proteins haptenated by amoxicillin

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Beta-lactam antibiotics are widely used against infective diseases to inhibit the formation of the bacterial cell wall. However, the increasing diagnosis of allergic reactions produced by these compounds has evidenced the importance of studying hypersensitivity reactions towards these drugs. It is considered that the drug should covalently interact with proteins, in a reaction known as haptenation, to trigger the allergic response. We have previously described the haptenation of serum albumin by amoxicillin (AX) and by a biotinylated analog (AX-B) *in vitro*. Here, we describe the identification, by proteomic techniques, of intracellular proteins haptenated by AX or AX-B in B-lymphocytes treated with these compounds. We have detected modified proteins in cell lysates and in the conditioned medium, the latter including not only extracellular vesicles (EV) derived from these cells but also extracellular soluble proteins (SP). We separated the haptenated proteins in these three fractions (lysates, EV and SP) by two-dimensional electrophoresis obtaining a similar pattern with both AX and AX-B. Additionally, we have confirmed protein haptenation by immunoblotting techniques, immunoprecipitation and avidin-based affinity chromatography purification. Finally, we also studied the potential involvement of extracellular vesicles in the transport of these amoxicillin targets. This delivery could be associated with the activation of the immune system during an allergic response. Taken together, our results provide the first identification of intracellular proteins modified by amoxicillin and the importance of extracellular vesicles in their transport.

P40

Unambiguous N-glycosylation site determination of CHO expressed Integrin alpha-V receptor subunit

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Glycans are important elements in many biological processes such as protein-protein interaction, protein folding and virus-cell recognition. Thus, the detailed characterization of protein N-glycosylation post-translational modifications remains a quest for the understanding of fundamental biological processes.

Host cell adsorption is a prerequisite for many viruses to infect cells. This adsorption process depends on host cell receptors. Foot-and-mouth disease (FMD) is a highly contagious and fulminating viral infection in livestocks constituting a worldwide problem for animal health and with a serious economical impact for countries' economies. FMD virus (FMDV) initiates the infection process through binding to specific receptors on the host cell surface. Although it is known that αVβ6 integrin is a principal receptor for FMDV, the mechanisms for cell infection remains yet unclear.

αVβ6 is an heterodimer in which the alpha V subunit is thought to directly interact with the antigenic GH-loop of the viral capsid protein VP1. Biochemical and structural studies have deduced αVβ6 to be N-glycosylated in the extracellular domain.

The ectodomain of human integrin αVβ6 was stably expressed in chinese hamster ovary cells. Protein was purified by sequential steps of ion-exchange and size exclusion chromatography.

Two different and complementary proteases were used to get a higher sequence coverage over the analyzed alpha V subunit: trypsin and Glu-C. A specific N-glycosylation site determination study was conducted over the resulting peptides by combining the use of PNGase F and H_2O^{18}, and nLC MS/MS analysis.

Experimentally described N-glycosylation sites were identified for the studied integrin alpha-V subunit together with novel sites described by in-silico sequence analysis. These new findings will complement ongoing structural studies by NMR using the intact glycoprotein aimed at assessing a possible cross-talk between GH-loop binding and receptor glycans.

P41

ProteoRed multicentric experiment PME11: Assessment and optimization of phosphoproteomic analysis methods

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The Proteored Multicentric Experiment 11 (PME11) has been run within the EUPA Standardization Initiative, with the final goal of facilitate each participant laboratory a method to assess its ability and improve their protocols for phosphopeptide analysis.

To this end, the samples to be analyzed in the study (PME11-A1, A2, A3) consisted of a yeast tryptic digest (C-18 purified), spiked-in with three different concentrations of a mixture of 20 human phosphopeptide standards (Phosphomix 1 and 2 from Sigma-Aldrich), containing light isotopes. Additionally, a mixture of the corresponding isotopically labelled heavy Phosphomix 1 and2 standard peptides were also distributed.

Recommendations for LC and MS analysis, as well as data processing, were provided. Phosphopeptide enrichment procedures were suggested, but each lab was free to test their own procedures. The analysis has been run at 30 different laboratories, including ProteoRed and other EUPA members.

The main outcomes of the study will be presented focusing on:

- The comparative performance of the enrichment procedures used, measured by the number of yeast phosphopeptides identified, ant its intra-lab reproducibility, on the basis of the results of the triplicate analysis.
- The yield of the phosphopeptide enrichment, based on the quantification of the recovery of the phosphomix standards spiked in the initial sample, measured using the heavy phosphomix as internal standards, added to the sample after enrichment.
- The correlation of phosphopeptide enrichment yield and the overall performance in phosphopeptide identification.
- The ability of each lab's to correctly assign phosphorylation sites, and its comparison to a centralized analysis.

Overall, the study has allowed:

- Evaluating the performance and reproducibility of phosphopeptide enrichment procedures.
- Testing the usefulness of phosphopeptide mixture standard to set up, monitor, and troubleshoot phosphopeptide analysis methods.

P42

Human olfactory bulb analyzed by MALDI-TOF imaging mass spectrometry (MALDI-IMS)

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The olfactory bulb (OB) is the first site for the processing of olfactory information in the brain, and its deregulation is considered one of the earliest features of neurodegenerative diseases. For several decades, neuroanatomical, volumetric, and histological approaches have been the gold standard techniques employed to characterize the OB functionality. However, little attention has been focused specifically on the molecular landscape of the OB from the perspective of proteomics. Recently, matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) has recently emerged as a powerful tool for analyzing the spatial distribution of peptides and small proteins (amongst other molecules) within biological tissues. Obtained signals can be correlated with underlying tissue architecture without any geometrical distortion, enabling the so-called «Molecular Histology». As a neural structure, the study of this kind of tissue by peptide/protein MALDI-IMS is handicapped by a lack of consistency, and high lipid amount. In this work, we have analyzed slices from human OB by MALDI-IMS for protein/peptide signals. We have used a non standard OCT-free cryo-slicing protocol, followed by Carnoy delipidization, and automated matrix spray to circumvent the drawbacks of this kind of tissues for protein/peptide MALDI-IMS experiments, and achieve lateral resolutions of up to 75 um.

P43

A hybrid liquid-gel proteomic approach for the discovery of reliable biomarkers of meat tenderness

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The study and understanding of muscle proteome is essential in order to determine and predict the final meat quality. In this context, the main goal of this study was to search for reliable protein biomarkers capable to differentiate between different types of meat quality, in this case as related to tenderness (tender vs tough), which is considered the main quality attribute of bovine meat. To do this, a fractionation approach based on the separation of the myofibrillar proteome by isoelectric point (OFFGEL) was employed. For that, beef samples (*Longissimus thoracis* muscle) from six Maine Anjou cows, previously characterized as tender or tough by Warner-Bratzler measurements, were used. Myofibrillar proteins were extracted, separated in liquid fractions by OFFGEL isoelectric focusing and further analysed by SDS-PAGE.

The obtained OFFGEL fractionation profiles showed reproducible results in the distribution of myofibrillar proteins along the pH range 4-7. A comparative study of the two types of meat qualities was carried out, using densitometry analysis to quantify protein bands. Several proteins differing in abundance according to quality type were found, being further characterized by liquid chromatography coupled with tandem mass spectrometry. Among them, three main proteins were identified as to be reliable biomarkers of meat tenderness since they showed statistically significant differences in their contents between tender and tough meat samples. In conclusion, the present approach seem to be a promising alternative to classical fractionation by two dimensional gel electrophoresis for meat proteomics in view of its reliability, reproducibility and ease of use.

P44

Evaluating the involvement of relevant proteins in Candida albicans apoptosis by targeted proteomics

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The incidence of candidiasis, caused mainly by the opportunistic fungal *Candida albicans*, is increasing largely due to the emerging antifungal resistance which is promoting the development of new therapies against the fungus. A powerful strategy in the search of novel drugs is focus on enhances the apoptotic response in pathogen. For that purpose an exhaustive knowledge of the molecular mechanisms implicated in apoptosis is required.

The present work tries to unmask the apoptotic pathways in *C. albicans* by the study of target proteins involved in this process under multiple conditions, including exposure to external agents such as hydrogen peroxide and acetic acid, and also in response to human macrophages (THP1 cell line) at different time points to monitor the development of response.

For this aim, we use the selected reaction monitoring technique (SRM) which allows the relative quantification of an extensive group of relevant proteins simultaneously. We developed a Skyline method that comprises 372 transitions belonging to 62 proteotypic peptides (PTPs) from 25 proteins of interest. PTPs synthetized as heavy peptides were confirmed by MS/MS spectra and were used as an internal standard in relative quantification. Calibration curves were obtained from each peptide to ensure an accurate relative quantification.

In preliminary results 33 peptides representing 19 of selected proteins were detected in samples analyzed, showing in some cases remarkable changes in abundance under macrophage interaction respect to control cells. Furthermore, the study of some apoptotic markers revealed an increase in caspase-like enzymatic activity and in the release of reactive oxygen species in *Candida* cells under the same conditions.

Therefore this proteomic approach appears to be a promising tool to quantify targeted proteins in apoptosis providing a more detailed overview which could help in the discovery of better drug candidates.

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P45

A proteomic approach to compare Formol and FineFix fixation in human squamus-cell lung cancer

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Background. Formaldehyde fixation and paraffin embedding (FFPE) is the universal method for tissue preservation and for the long-time storage of samples. However, formalin is a toxic fixative and its exposure is a human health risk. Therefore, several countries have already restricted its use, and numerous attempts have been made to find formalin-free tissue fixatives –such as FineFix– with lower toxic properties but a similar quality to preserve tissue components.

Objective. To determine the potential usefulness of FineFix tissue fixation for proteomics investigations compare to formol.

Methods. Ten series from both FFPE and FineFix tissue fixation specimens were cut. The slices were deparaffinated using xylene and rehydrated through a series of graded ethanol. Protein extraction was performed using two different buffers (A: 20 mM Tris-HCl pH 8,8, 2%SDS, 200 mM DTT; and B: 6M Urea, 2% SDS). Protein quantification was performed by the Bradford method, and SDS-PAGE gels were employed to evaluate the quality of the protein extracts. Then, protein were digested using trypsin (1:20). The peptide mixtures were separated by reversed phase-nanoHPLC and analyzed by MALDI-TOF/TOF mass spectrometry. Protein identification was carried out using the ProteinPilot™ software v.4.0.

Results. Buffer B did not allow an efficient protein recovery in FFPE specimens. Using Buffer A twenty-one different proteins were identified from both FFPE and FineFix fixatives. Furthermore, no differences were found between FFPE and FineFix specimens when unique different proteins were analyzed –119 and 118 proteins was identified in FineFix and Formol fixatives respectively–.

Conclusions. Based in this results, urea containing buffer would not be an alternative for protein recovery in FFPE specimens. FineFix fixative show a similar efficience to formol to protein recovery and would have a potencial value for proteomic analysis. However, further experiments should be done to confirm this approach.

P46

Effect of post-mortem time on the muscle proteome and its relationship to meat quality and animal welfare

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The objective of this work was to study the effect of *post-mortem* time on the muscle proteome in pigs and its relationship to the main meat quality traits and the physiological parameters related to animal welfare. Forty-eight pigs ([Large White x Landrace] x Pietrain) were reared under two factors that may influence the animal's susceptibility to stress: gender (male/female) and RYR1 gene mutation (NN/Nn), in a 2 x 2 factorial design. At slaughter, blood samples were taken for biochemical analysis. Carcass and meat quality traits (pH, electrical conductivity, drip loss, instrumental meat colour and shear force) were measured at 24h after slaughtering and individual muscle samples were taken from the *Longissimus dorsi* at 0h, 4h, 8h and 24h post-mortem and used for the sarcoplasmic protein profile study by SDS-PAGE. The relationships between the protein profile obtained at different *post-mortem* times (0h, 4h, 8h and 24h) and physiological and meat quality variables were evaluated using Pearson's correlation coefficient. *Post-mortem* time changed significantly the muscle protein pattern, including key enzymes of the muscle metabolism such as glycogen debranching enzyme ($P<0.001$), myosin-binding protein C, fast-type ($P<0.001$), glycogen phosphorylase ($P<0.001$), β-enolase ($P<0.01$), fructose-biphosphate aldolase A ($P<0.01$), glyceraldehyde-3-phosphate dehydrogenase ($P<0.001$), glycogen phosphorylase isoform 1 ($P<0.001$), muscle 6-phosphofructokinase ($P<0.001$), UTP-glucose-1-phosphate uridylyltransferase ($P<0.05$) and carbonic anhidrase III (($P<0.05$). The protein profile obtained at 4 h *post-mortem* showed the highest number of significant correlations with meat quality attributes (n=23) and with physiological parameters related to animal welfare (n=19). From these results, sampling pig carcasses at 4 h *post-mortem* is proposed, in order to better analyse the relationship between the muscle protein biomarkers and the indicators of meat quality or animal welfare.

P47

Identification of transmembrane domain dependent protein-protein interactions

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Membrane proteins play crucial roles in the cell controlling molecular traffic, facilitating signal transduction or anchoring the cell. Membrane proteins are difficult to analyze due to the association with lipids and the toxicity caused by an overexpression.A critical step in the biosynthetic pathway of the folded membrane protein is the insertion into the lipid bilayer, which is mainly achieved via a co-translational mechanism. The molecular details of the alternative post-translational insertion pathway used by some proteins are poorly characterized, although several proteins implicated in the process have been described. The interaction with the nascent transmembrane segment (TM) is a critical determinant in the selection of the insertion mechanism.

The aim of this work is the development of an experimental procedure to identify proteins involved in the post-translational insertion of membrane proteins and the characterization of the process. To achieve these goals, we have used a chimera containing the *Staphylococcus* aureus nuclease followed by the TM of glycophorin A, and an affinity tag. An identical chimera, lacking the TM, was used as control. The protein-protein interactions were stabilized using chemical cross-linkers. The crosslinked protein complexes were analyzed by LC-MS/MS (SWATH) and further characterized using other physico-chemical procedures.

The introduction of the TM in the chimera, which renders a slightly less stable protein, clearly alters the network of interacting proteins. A large portion of the identified proteins are implicated protein synthesis and maturation as well as in metabolism. The presence of high molecular weight complexes containing some of the interacting proteins have been confirmed by size exclusion chromatography. Our results indicate that the experimental procedure described is suitable for the study of TM-dependent protein-protein interactions, which is the first step into the characterization of some of the molecular mechanisms of membrane protein maturation.

P48

Proteome and Transcriptome analysis in rat bone marrow mesenchymal stem cells at different ages

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Mesenchymal stem cells (MSCs) are highly relevant for regeneration of mesoderm tissues such as bone and cartilage. The promising role of MSCs in cell-based therapies and tissue engineering appears to be limited due to a decline of their regenerative potential with increasing donor age. We have studied whether the donor aging changes the proliferation, pluripotency and immunogenic MSCs capacities.

Six age groups from bone marrow mesenchymal stem cells of Wistar rats were studied (newborn, infant, young, pre-pubertal, pubertal and adult). All of them where subjected to quantitative proteomic (iTRAQ) analysis and Next Generation Sequencing (NGS) using RNA-sequencing. iTRAQ and RNA-sequencing were performed in duplicated. Proteomic results were normalized with ProteinPilot software 4.5 based on ParagonTM Algorthim 4.5.0. Differentially expressed genes and transcripts from RNA-seq were calculated with Cuttdiff analysis using CummeRbund software (http://compbio.mit.edu/cummerbund/). To validate proteome and transcriptome analysis by flow cytometry and western-blot.

In proteome analysis, we found 210 proteins statistically significant modulated were grouped in pluripotency, proliferative and metabolism processes. Enzymatic analysis of several enzymes as L-lactate dehydrogenase and glucose-6-phosphate isomerase were done to validate iTRAQ data. To deeply study these differences we have analyzed by Next Generation Sequencing six age groups from bone marrow mesenchymal stem cells. A total of 9628 genes presented differences of expression among age groups and those genes were grouped into metabolic pathways. We corroborated the NGS analysis focusing our research in young, pre-pubertal and adult groups, which presented the highest amount of genes differentially expressed related with inflammation mediated by chemokine and cytokine signalling pathway when compared with newborn group as a control. It was validated transcriptomic analysis with chicking two pro-inflammatory markers (CD200 and TLR4) by flow cytometry and western-blot.

Aging affects proliferation, pluripotency and immunogenic profiles of bone marrow mesenchymal stem cells.

P49

Mapping the deregulated protein interactome of colorectal cancer metastatic cells by spatial proteomics

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Colorectal cancer (CRC) is the second deadliest cancer worldwide, because half of CRC patients develop recurrence and metastasis. We aimed to study CRC metastasis by spatial proteomics using the KM12 cell model to identify deregulated proteins and complexes in specific subcellular compartments based on quantitative data.

We used SILAC to compare highly-metastatic KM12SM cells with low-metastatic KM12C cells. Forward and reverse SILAC experiments were fractionated in five subcellular fractions: cytoplasm (CEB), plasma, mitochondria and ER/golgi membranes (MEB), nuclear (NEB), chromatin-bound (NEB-CBP) and cytoskeletal proteins (PEB). After subcellular fractionation, proteins were in-gel digested and analyzed by LC-MS/MS using a LTQ Orbitrap Velos. To obtain network enrichment, protein interaction partners and to get a general view of the proteome alterations and their association to specific subcellular fractions associated to CRC metastasis, data were analyzed by bioinformatic approaches mainly using DAVID and babelomics suite for functional profiling analysis.

We quantified 1828, 2914, 3030, 1483 and 1583 proteins in CEB, MEB, NEB, NEB-CBP and PEB fractions, from 2416, 4086, 3836, 1853 and 2117 identified proteins in the same compartments. Among them, we found a total of 1318 quantified proteins with at least 1.5-fold deregulation in the KM12 cell model of CRC metastasis. Although some of these altered proteins and complexes were already described as implicated in metastasis, others might be useful CRC metastasis biomarkers. Several networks and protein interaction partners not previously associated to CRC metastasis were found to be spatially altered in highly metastatic CRC cells, which might help to understand underlying mechanisms associated to CRC metastasis.

We have been able to quantify 1318 differentially expressed proteins implicated in CRC metastasis using SILAC combined with proteome subcellular fractionation. By spatial proteomics, we have been able to identify altered complexes and key protein interaction partners in specific subcellular locations as potential CRC-metastasis biomarkers.

P50

Different SWATH MS data analysis tools lead to different quantitative results: comparison of Swath Acquisition Microapp, Swath Protemics Cloud Tool Kit (OneOmics), Spectronaut, and DIA-Umpire

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When doing relative quantification by SWATH MS, the list of proteins that show significant differences might be dependent on the data normalization applied, the procedure to obtain the protein fold-change, the use of previous DDA runs for generating a spectral library or not, and the statistical analysis performed. Many software tools are available for analyzing SWATH DIA MS data, and they differ in the parameters mentioned above. We therefore hypothesize that the selection of one tool or another can affect the final output, taking researchers to overlook some up/down-regulated proteins.

Methods: Protein extracts from two different cell lines (5 biological replicates each) were digested with trypsin and analyzed by LC-MS/MS in a Triple TOF 5600+ (Sciex) using SWATH DIA (one SWATH run per sample). Additionally, samples were analyzed using shotgun DDA in order to build a spectral library for the standard SWATH analysis workflow.

Datasets were processed using the spectral library created from previous shotgun DDA runs using three different software tools: SWATH Acquisition MicroApp (Sciex), SWATH Protemics Cloud Tool Kit in BaseSpace (OneOmics, Sciex), and Spectronaut (Biognosys). Alternatively, SWATH runs were analyzed using DIA-Umpire, a tool that extracts fragment chromatograms from the DIA runs without the need for previous DDA runs.

The outcome of these tools are compared in terms of number of identified and quantified,proteins, obtained protein fold-changes, and number of proteins changing in abundance, but also time consumption, ease-of-use, and output graphical interface were compared.

Results: The number of proteins quantified by each tool was different: 1869 for Spectronaut, 1774 for Swath Acquisition Microapp, 1727 for OneOmics, and only 766 for DIA-Umpire.

Protein fold-changes obtained using the four software tools correlated with Pearson's correlation coefficients of 0.71 to 0.88. This means that, although the normalization and quantitation procedures are different, they all lead to a similar result, when looking only to fold-changes. However, larger differences are noted when looking at the proteins showing significant differ-

ences in abundance between both conditions. The number of statistically significant different proteins was 1043 for Swath Acquisition Microapp, 984 for Spectronaut, 911 for OneOmics, and 305 for DIA-Umpire. 507 proteins were common for the three tools using the standard approach (using a spectral library built from previous DDA runs). When DIA-Umpire is included in the comparison, only 193 proteins were common for the four tools. The lower figures for DIA-Umpire can be explained by a lower number of identified proteins when extracting pseudo-MS/MS spectra from the SWATH runs.

Conclusions: The analysis of SWATH MS data leads to a different list of differential proteins depending on the software tool used, although fold-changes obtained are similar. DIA-Umpire showed a much lower number of quantified and significant proteins, due to the lower number of proteins identified from the extracted pseudo-MS/MS.

P51

Differential Proteomic Profiling of High and Low Activity Rheumatoid Arthritis Sera for the Identification of Biomarker Candidates

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Rheumatoid arthritis (RA) is a long-lasting inflammatory autoimmune disorder that ultimately leads to the destruction of joint architecture. Using the DAS28 activity index, 80 RA serum samples (40 with low activity and 40 with high activity) were selected in order to be analyzed by mass spectrometry. The aim of these study was to find possible biomarkers that could discriminate patients with different RA activity in the daily clinical routine.

To facilitate the complex measurement of these serum samples, a simple, fast and reproducible albumin-specific depletion method using ethanol was optimized and applied to this study. Four independent pools of the 40 high RA activity samples (10 samples per pool) and 4 pools of the 40 low RA activity samples were firstly albumin-depleted, and then the remnant serum proteins were digested and differentially labelled with iTraq 8-plex reagents. Subsequently, the 8 labelled pools were combined and cleaned using StageTips-C18. Finally, the pool mixture was fractionated by HPLC (Zorbax-C18) and the resulting fractions were analyzed by nanoLC-MS/MS using a MALDI-TOF/TOF.

The mass spectrometry analysis led to the identification of 186 proteins. Among these, Haptoglobin, Kininogen-1, Alpha-2-HS-glycoprotein, Afamin, Histidine-rich-glycoprotein and some immunoglobulins were differentially detected depending on the RA activity of the patients ($p < 0.03$). These proteins are being validated by other orthogonal techniques (ELISA and protein arrays) with the final objective of their implementation in clinical routines for improving RA monitoring.

P52

Determination of candidate Glaucoma biomarkers by Quantitative Proteomics

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Glaucoma is a leading cause of irreversible blindness in the world. It includes a huge number of neurodegenerative disorders all of them characterized by progressive degeneration of the optic nerve, retinal ganglion cell death and the loss of the visual field. Primary Open-Angle Glaucoma (POAG) and Pseudoexfoliation Glaucoma (PEXG) are the most prevalent types of glaucoma in Spain [1]. Glaucoma is associated with an asymptomatic and progressive loss of the visual field, which can lead to irreversible blindness if left diagnosed and untreated. For this reason Glaucoma is a public health challenge and therefore the development of methodologies for it's early detection is extremely important.

This work presents the development and application of an analytical method based on the use of labeled peptides and tandem mass spectrometry for the simultaneous quantification of three serum proteins previously proposed as candidate biomarkers of glaucoma (Apolipoprotein A-IV, Complement C3 and Vitronectin). In contrast to common protocols, the isotope labeled versions of the proteotypic signature peptides employed here keep the isotopic difference as little as possible with respect to the peptide released from the protein to minimize analytical biases resulting from different behavior of the analytes and labelled analogues. Two minimally ^{13}C labeled peptides for each protein have been synthesized and characterized in terms of concentration and isotopic enrichment.

Another novel aspect of this work is that we are able to measure accurate isotopic distributions in the molecular fragments by transmitting the whole parent ion cluster to the collision cell via a reduction of the mass resolution of the first quadrupole in a tandem mass spectrometer. In this way, the concentration of the three protein biomarkers in human serum can be directly obtained from the fragment-ion spectrum acquired for the sample without resorting to extra calibration runs.

P53

Lipopolysaccharide-induced secretion of soluble and vesicle-based colorectal cancer cell proteins

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Background: Surgery remains the mainstay curative treatment for primary colorectal cancer (CRC). However, patients with stage II and III CRC will develop hepatic metastasis. Surgery causes a disruption of the bowel barrier that allows the translocation of bacterial products across the colon and promotes inflammation, which can stimulate metastatic ability of circulating CRC cells. In this study we investigated the response of cancer cells when they get in contact with bacterial-wall molecules.

Materials and Methods: Six CRC cell lines were stimulated with lipopolysaccharide (LPS). We evaluated NFκB activation by immunofluorescence and identified by the repertoire of secreted proteins, through extracellular vesicles (EVs) enriched for exosome markers and isolated by ultracentrifugation, and classically secreted proteins (SS), by mass spectrometry-based proteomics.

Results: NFκB translocation measured by immunofluorescence image analysis occurred in two out of the six cell lines. 83 and 290 proteins were upregulated in SS and EVs, respectively; and 58 and 24 were downregulated. Among LPS-regulated proteins, integrins, inflammatory cytokines and metastasis prognosis markers were found.

Conclussions: The bacterial endotoxin-induced release of these proteins happens in a non-canonical way and it may play a role in creating a permissive environment for CRC liver metastasis.

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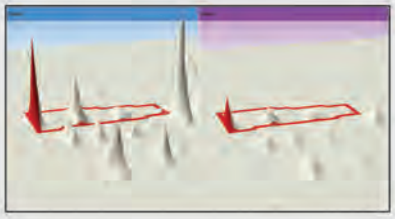

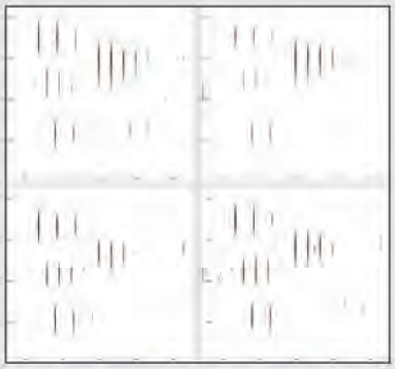

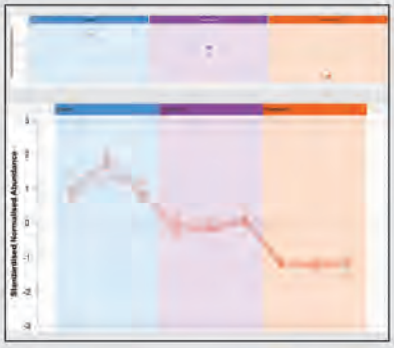

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Quebrando la cadencia de olas y de dunas
Con un rotundo trazo, con un pujar de mástil.

Un mar de henchida espuma en un binario cáliz
De innúmeras secuencias el clúster nos satura.
¡Se modifican tantas! Buscarlas es locura,
Y es una puerta nueva, y es un taimado áspid.

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