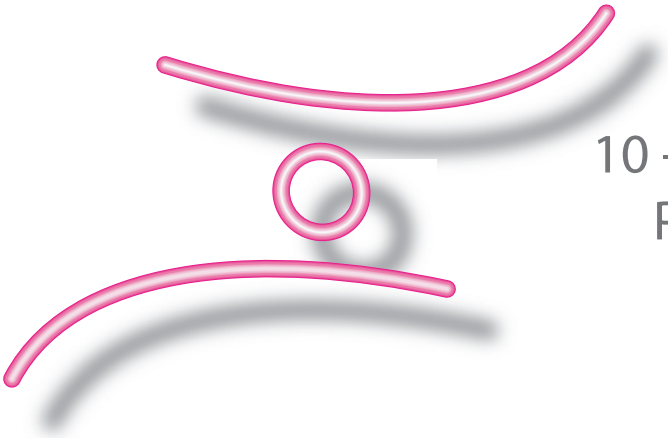
Proteomics and Human Proteome

From bench to bedside



10 - 13 February, 2009
Pamplona, Spain

Joint Congress of the Spanish Proteomics Society
and the Latinamerican Human Proteome
Organization

SEPROT
SOCIEDAD ESPAÑOLA DE PROTEÓMICA

LAHUPO
Latinoamerican Human
Proteome Organization

Congress Venue
Faculty of Sciences, University of Navarra
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PROTEÓMICA

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Abstract Book

of the

Joint Congress of the Spanish Society and the Latin American Human Proteome Organization

Welcome Address of the Sociedad Española de Proteómica (SEProt)

La SEProt cumple 5 años

El próximo 13 de abril (Acta Fundacional) o 9 de diciembre (Registro de Asociaciones) nuestra Sociedad cumple 5 años. Parece que fue ayer pero hace ya cuatro años que la actual Junta Directiva, la primera elegida en Asamblea General durante el I Congreso de la SEProt celebrado en Córdoba (13-17 febrero 2005), dirige la marcha de nuestra Sociedad. Atrás queda también el II Congreso (Valencia, 10-14 2007) que consolidó la decidida vocación internacionalista de la SEProt, tanto en el plano europeo –contribuyendo actualmente de manera destacada en los órganos de dirección y comités de la Federación Europea de Sociedades de Proteómica– como en el ámbito latinoamericano. El III Congreso que este 2009 celebramos en Pamplona tiene también un punto de mira en el fortalecimiento de las relaciones entre países separados por el Océano Atlántico pero culturalmente cercanos. Hubo intentos de acercamiento anteriores, incluyendo la posibilidad de creación de una red multinacional de temática proteómica que no prosperó por reticencias de alguna Sociedad de Proteómica a un supuestamente encubierto "neocolonialismo cultural". No caeré en la tentación de comentar este sinsentido, aunque tampoco cejaré en promover el fortalecimiento de lazos proteómicos entre ambos lados del charco... En nuestro país, en este primer lustro de existencia de nuestra Sociedad, se han consolidado algunos programas de educación a través de becas de asistencia a cursos nacionales e internacionales de formación en el ámbito de la Proteómica, merced a la saneada economía de la SEProt, consecuencia directa de la excelente gestión por parte de nuestro Tesorero David Andreu de los ingresos por cuotas individuales y corporativas.

Reconocer la excelencia investigadora premiando contribuciones de especial relevancia es una de las tareas más gratas de una Sociedad científica. Así lo han entendido también las empresas del ramo que han apoyado esta iniciativa patrocinando diversos galardones. Gabriel Padrón (Centro de Ingeniería Gené-

tica y Biotecnología, La Habana, Cuba) recibió en Valencia el Premio SEProt-Promega "por su contribución al desarrollo de la espectrometría de masas y su aplicación a la revolución proteómica". Applied Biosystems patrocinó los premios SEProt-ABI para pósters presentados por grupos no españoles. Durante el III Congreso se otorgarán los premios SEProt a un artículo publicado durante el bienio 2007-2008 y a un trabajo original presentado en forma de panel al Congreso. Esta tercera edición, al igual que las anteriores, está patrocinada por Bruker Biosciences Española S.A. El III Premio SEProt está dedicado a la memoria de José Luis López Rodríguez, entrañable y excelente proteómico de *Mytilus*, de la Universidad de Santiago de Compostela, que nos dejó tempranamente el pasado 12 de octubre. Sus muchas virtudes han sido comentadas por quienes más le conocieron en el portal electrónico de la SEProt. Dándo su nombre a nuestro galardón queremos también rendir un sentido y público homenaje institucional a nuestro compañero José Luis.

Durante el pasado congreso, nuestra Sociedad otorgó membresía de Honor a John B. Fenn, galardonado con el Premio Nobel de Química en 2002 por su trabajo pionero en el desarrollo de la ionización de macromoléculas mediante electronebulización (Proteómica 0:11-14, julio 2007). Otra técnica esencial de la Proteómica es la ionización mediada por radiación láser y asistida por una matriz (MALDI). Este método de ionización fue desarrollado simultánea e independientemente por Koichi Tanaka (Shimadzu, Japón) y por Franz Hillenkamp y Michael Karas (a la sazón en la Universidad de Münster, Alemania) durante los años 80 del pasado siglo. Los avatares del destino, quizás forzados por la presión de la poderosa compañía japonesa, hicieron que Tanaka compartiera con Fenn el Premio Nobel que la Academia Sueca negó a los alemanes. Nuestra Sociedad, nombrándoles Socios de Honor, ha querido sumarse a la lista de instituciones que reconociendo esta injusticia histórica han rendido honores a Hillenkamp y Karas. Me alegra y enorgullece también Peter Roepstorff, pionero de la espectrometría de masas biológica, testigo y actor privilegiado de la transición Química de Proteínas-Proteómica, y maestro de proteómicos, haya aceptado ser Miembro de Honor de la SEProt.

El artículo 25c de nuestros Estatutos especifica que "el nombramiento de los Socios de Honor corresponde a la Asamblea General, a propuesta de, al menos, diez Socios Ordinarios. El nombramiento se refrendará en votación secreta, debiendo alcanzar un número de votos favorables de, al menos, dos tercios de la totalidad de los miembros de la Junta Directiva. La decisión favorable deberá ser refrendada por la Asamblea General por mayoría simple". Quisiera desde esta tribuna proponer la candidatura a Socios de Honor de dos pioneros de las

técnicas proteómicas, Jasna Peter-Katalinić (http://impb.klinikum.uni-muenster.de/research/peterkatalinic/index.html) y Pier-Giorgio Righetti (Electrophoresis 27 [8]: 1435-1692 [No. 8 April 2006] Special Issue: Dedicated to Professor Pier Giorgio Righetti at his 65th Birthday). Jasna ha contribuido durante los últimos 25 años al desarrollo de la glicoproteómica; Pier-Giorgio, prolífico e imaginativo innovador de las técnicas de separación y su aplicación a la proteómica, ha publicado más de 450 artículos originales. A él debemos la invención de las tiras de gradiente de pH inmovilizado o la reciente técnica de "democratización" de proteomas complejos o ProteoMiner comercializado por BioRad.

Suenan tambores que anuncian la necesidad de renovación. Y no sólo porque así lo marquen nuestros estatutos, sino porque el funcionamiento de toda Sociedad se rige por ciclos que garantizan el progreso de las ideas. Seguimos siendo una Sociedad joven, muy joven, que necesita el apoyo de todos sus socios. La Junta Directiva que surja de la Asamblea General en Pamplona va a necesitar el apoyo de *proteómicos* experimentados y aprendices de brujo para seguir contribuyendo al desarrollo de nuestra disciplina, en España y fuera de nuestras fronteras. Muchos han sido los logros conseguidos en este quinquenio, pero muchos siguen siendo los proyectos iniciados que aun necesitan el espaldarazo de la consolidación. En julio del 2007 vio la luz el número 0 de la revista Proteómica y unos meses después (febrero del 2008) se publicó el número 1 con ocasión de las Primeras Jornadas Bienales de Proteómica (Sitges, 21-22 febrero 2008). Estas iniciativas, revista y Jornadas, han tenido como principales valedores a los *Jesuses*, Jorrín y Vázquez, respectivamente. Conociendo el entusiasmo que siempre han demostrado hacia la SEProt, seguro que no yerro en el convencimiento de que ambos seguirán brindando su colaboración en esos proyectos de capital importancia para la SEProt.

Aprovecho esta ocasión para despedirme como Presidente de la SEProt y desearle al nuevo Presidente, que será elegido por la Asamblea General durante el III Congreso, las mismas satisfacciones, sensación de compañerismo que yo he experimentado durante estos cuatro años al frente de la Junta Directiva. A Ángela, Concha, David, Jesús J., Jesús V., José Manuel, Juan Pablo, y Fernando, mi reconocimiento y gratitud por vuestra dedicación y apoyo. ¡Haber trabajado con vosotros por nuestra Sociedad compensa con creces el tiempo y esfuerzo robado a otras actividades! A la nueva Junta Directiva le esperan nuevos retos, como la tarea de profesionalizar la Secretaría Técnica de la Sociedad, incluyendo asesoría fiscal, y la edición de un libro en castellano sobre Proteómica. Todos los que impartimos Cursos de Doctorado, de Licenciatura, de especialización, Másters, etc., seguramente coincidiremos en la necesidad de disponer de una monografía

en nuestro idioma que recoja la historia, el desarrollo de la tecnología, así como los principios y aplicaciones de la Proteómica. Podemos esperar a que alguien lo haga o ponernos manos a la obra. Somos muchos los que pensamos que podemos y debemos hacerlo desde la SEProt. Como siempre, cualquier sugerencia y ayuda serán más que bienvenidas. ¿Seguimos hablando en Pamplona?

Juan J. Calvete
Presidente de la SEProt

Welcome Address of the Latin American Human Proteome Organization (LAHUPO)

Buenos Aires, December, 2008

Dear Colleagues

On behalf of the Latin American Human Proteome Organisation (LAHUPO) Congress Organizing Committe, we would like to invite you to join the Congress of the Spanish Proteomics Society (3rd SEPROT) and the Latinoamerican Human Proteome Organisation (2nd LAHUPO), to be held in Pamplona, Spain, 10-13 February, 2009.

We have prepared the Scientific Program to put a central focus in the Proteomics and Human Proteome: "From bench to beside".

We look forward to welcoming you in Pamplona, to enjoy the wisdom and experience of outstanding HUPO scientists and Iberoamerican Colleagues.

With Warm Regards,

Mario Hugo Genero,
Congress Co-Chair-LAHUPO President

Andrea Sabina Llera,
LAHUPO secretary

Welcome Address of the Organizing Committee of the SEProt and LAHUPO Congress

Pamplona, January, 2009

Dear colleagues,

It is my great pleasure to welcome you all, on behalf of the Scientific and the Organizing Committees, to the 3rd Congress of the Spanish Proteomics Society and the 2nd Latin American Human Proteome Organisation that are celebrated conjointly in Pamplona.

Despite the rapid advances experimented over the last few years, Proteomics is still at its infancy. It is a discipline with a tremendous future that offers all kind of opportunities to analyze biological systems with unprecedented efficacy to generate new concepts in biology, identify new diagnostic and therapeutic targets and study drug effects in cell biology. The challenge of defining and understanding proteomes in the context of cell biology is huge and requires a multidisciplinary effort to ensure innovative technical developments, the generation of biological information as well as the integration and interpretation tools to transform the information in biological knowledge. We sincerely hope to have been able to create an ideal environment where international experts could discuss all these aspects generate new ideas and establish productive scientific interactions.

The venue of the meeting is the University of Navarra, in particular the Faculty of Sciences that during this year 2009 celebrates its 50th anniversary. I do really thank to our University for all the support and facilities provided to organise this event. We are also in debt with our sponsors, DIGNA, ANAIN, Gobierno de Navarra, Plan Tecnológico de Navarra, BioRad, Sigma, GE Healthcare, AB, Thermo, Waters, Genoma España, Bruker, SODENA, Beckman-Coulter, Agilent, Promega, Ministerio de Ciencia e Innovación, for their generosity making possible this adventure. I would like to thank very, very, very much the effort and enthusiasm of people involved in the organisation of the congress, especially to Leticia, Jokin, Kike, Javier, Elsa y María. And last but not least, I

thank you all for coming to Pamplona to share your outstanding research; you are the real meeting.

I wish you a pleasant and productive stay in Pamplona and since that life is not only science, don't forget to spare some time visiting Pamplona, it will amaze you, and discover the end of the history of this small roman village. Be careful with the small and charming bars of the old town, they are pretty addictive...

Fernando J. Corrales

Hillenkamp, Karas, Roepstorff, 3 *Nobel* Honorary Members of SEProt

The development of mass spectrometry has contributed to biological research in a similar manner as the development of particle accelerators has driven the advance of atomic physics. Mass spectrometers and storage rings both represent machines that domesticate, respectively, macromolecular ions and subatomic particles long enough to reveal their properties. The invention in 1929 by Ernest O. Lawrence of the circular particle accelerator, which he referred to as his "proton merry-go-round", but which became better known as the cyclotron, opened the doors to investigate the structure and properties of atoms. More than four decades before, in 1886, Eugen Goldstein observed rays in gas discharges under low pressure that travelled through the channels in a perforated cathode toward the anode, in the opposite direction to the negatively charged cathode rays. Goldstein called these positively charged anode rays "Kanalstrahlen". Wilhelm Wien (1911's Nobel Laureate "for his discoveries regarding the laws governing the radiation of heat") found that strong electric or magnetic fields deflected the canal rays and, in 1899, constructed a device with parallel electric and magnetic fields that separated the positive rays according to their charge-to-mass ratio. English scientist J.J. Thomson later improved on the work of Wien by reducing the pressure to create a mass spectrograph. Thomson succeeded in causing electric deflection because his cathode ray tubes were sufficiently evacuated that they developed only a low density of ions. Thomson measured the mass-to-charge ratio of the cathode rays by measuring how much they were deflected by a magnetic field and how much energy they carried. Thomson's separation of neon isotopes by their mass was the first example of mass spectrometry, which was subsequently improved and developed into a general method by Thomson's student F. W. Aston and by A. J. Dempster in 1918 and 1919 respectively. Thompson is credited for the discovery of the electron and of isotopes, and also for the invention of the mass spectrometer. He was awarded the 1906 Nobel Prize in Physics. Francis William Aston, a British chemist and physicist, won the 1922 Nobel Prize in Chemistry "for his discovery, by means of his mass spectrograph, of isotopes, in a large

number of non-radioactive elements, and for his enunciation of the whole-number rule". In 1918, Arthur Jeffrey Dempster, a Canadian-American physicist, developed the first modern mass spectrometer, allowing physicists to identify compounds by the mass of elements in a sample, and determine the isotopic composition of elements. Dempster's mass spectrometer was over 100 times more accurate than previous versions, and established the basic theory and design of mass spectrometers that is still used to this day. Dempster's research over his career centered around the mass spectrometer and its applications, leading in 1935 to his discovery of the uranium isotope ^{235}U. This isotope's ability to cause a rapidly expanding fission nuclear chain reaction allowed ten years latter the development of the atom bomb.

Mass spectrometry is an analytical technique that identifies the chemical composition of a compound based on the mass-to-charge ratio of charged particles. The ratio of charge to mass of the particles is calculated by passing them through electric and magnetic fields in a mass spectrometer. This apparently simple experimental design underlays one of the most resolutive technique for unravelling the chemical structure of a vast array of substances, from simple molecules to complex macromolecules, with the sole requirement of being able to ionize them. Molecules are built by a unique spatial combination of atoms, and mass is amongst the most fundamental characteristics of matter. Hence, an accurate measure of the mass represents a unique feature that characterize any substance. Further, isobaric substances can be distinguished by mass spectrometry through identification of reporter ions generated by fragmenting their unique structures.

In 1989, half of the Nobel Prize in Physics was awarded to Hans Dehmelt and Wolfgang Paul for the development of the ion trap technique in the 1950s and 1960s. An ion trap is a combination of electric or magnetic fields that captures ions in space. Fast scanning, selective mass filtering, and the possibility of exciting ions to investigate their structures has led to a revolution in mass spectrometry. However, techniques for ionization have been key to determining what types of samples can be analyzed by mass spectrometry. Among the many ionization techniques developed during the first half of the last century, including electron ionization (EI) and chemical ionization (CI) used for gases and vapors; inductively coupled plasma (ICP) sources (used primarily for metal analysis); field desorption (FD), fast atom bombardment (FAB), atmospheric pressure chemical ionization (APCI), secondary ion mass spectrometry (SIMS), etc., two techniques often used with liquid and solid biological samples include electrospray ionization (invented by John B. Fenn[1,2]) and matrix-assisted laser desorption/

ionization (MALDI, developed simultaneously in the 1980's independently by Koichi Tanaka[3,4] (at Shimadzu Corporation, Japan) and Franz Hillenkamp and Michael Karas[5,6] at that time at the Institute of Medical Physics and Biophysics within the Medical Faculty at the University of Münster, Germany.

Long before the 1980's, there had been investigation into the use of optical energy, not limited to laser light, to achieve ionization of organic compounds. Because this was limited to compounds that could be vaporized without being decomposed, very few compounds could be ionized. When a solid phase organic compound was irradiated with a laser, the organic compound absorbed the laser light, providing enough energy for desorption. Further, when the positive and negative charges of the particle are not in balance, it could be measured as an ion. However, in this case, since the laser light is absorbed directly into the analyte,the molecular bonds may be broken due to the increased internal energy. In the 1980's, "desorption to gas phase by rapid heating" gained attention. In short, if a given compound "AB" is heated, it is assumed that "AB" will be released to the gas phase as intact "AB" as well as fragmented "A" and "B". To perform this rapid heating, various heating methods were devised. However, a high enough temperature could not be achieved quickly enough to obtain intact vaporization of macromolecules, such as proteins. Since the time width of a pulse laser itself is between a nanosecond to several microseconds, rapid heating would seem possible using a focused beam to generate energy at high density and high speed. However, this would require a medium to enable its conversion into thermal energy. The term matrix-assisted laser desorption ionization (MALDI) was coined in 1985 by Franz Hillenkamp, Michael Karas and their colleagues. These researchers found that the amino acid alanine could be ionized more easily if it was mixed with the amino acid tryptophan and irradiated with a pulsed 266 nm laser[5]. The tryptophan was absorbing the laser energy and helping to ionize the non-absorbing alanine. Peptides up to the 2843 Da peptide melittin could be ionized when mixed with this kind of "matrix"[6]. The breakthrough for large molecule laser desorption ionization came in 1987 when Tanaka and his co-workers used what they called the "ultra fine metal plus liquid matrix method" that combined 30 nm cobalt particles in glycerol, a formulation adapted from the Fast Atom Bombardment (FAB) MS method, developed by Michael Barber and colleagues and which was widely used in the 1980's to achieve ionization of thermally labile compounds, with a 337 nm nitrogen laser for ionization. Using this laser and matrix combination, Tanaka was able to ionize biomolecules as large as the 34,472 Da protein carboxypeptidase-A, and another ion having a mass number exceeding 100,000[4]. Karas and Hillenkamp were subsequently able to ionize the 67 kDa protein albumin using a nicotinic acid

matrix and a 266 nm laser[7]. Further improvements were realized through the use of a 355 nm laser and cinnamic acid derivatives as the matrix[8].The availability of small and relatively inexpensive nitrogen lasers operating at 337 nm wavelength and the first commercial instruments introduced in the early 1990s brought MALDI to an increasing number of researchers[9]. Today, mostly organic matrices are used for MALDI mass spectrometry[10].

Professors Franz Hillenkamp and Michael Karas have received, individually or jointly, several scientific awards for the development of the MALDI technique: the Mattauch-Herzog award of the Arbeitsgemeinschaft Massenspektrometrie (1990) (to MK); the award of the American Society for Mass Spectrometry for "A distinguished Contribution in Mass Spectrometry" (1997) (MK & FH); the "Molecular Bioanalytics" award from the German Society for Biochemistry and Molecular Biology (2000) (MK & FH); the Association of Biochemical Research Facilities award for "Recognition of the outstanding contribution to biomolecular technologies" (FH, 2003; MK, 2006); the Thompson Medal of the International Mass Spectrometry Society (2003) (FH); the Fresenius Award of the Gesellschaft Deutscher Chemiker e.V.(2003), the Karl-Heinz Beckurts Award of the German Helmholtz Association (2003) (MK & FH), the Torbern Bergman Medal of the Swedish Chemical Society (Analytical Division) (2006) (MK & FH). Notwithstanding these honors, Profs. Karas and Hillenkamp have every right to feel frustrated by the fickleness of fate. In 2002, the Nobel Prize in Chemistry was awarded to John Bennett Fenn for the development of electrospray ionization (ESI) and Koichi Tanaka for the development of soft laser desorption (SLD. Many researchers in the field, me among them, felt aggrieved and even defrauded when Karas and Hillenkamp's contribution was not considered by the Nobel Committee of Swedish Academy of Science. Two years ago, the Spanish Proteomics Society proudly confered upon Prof. John Fenn its first Honorary Membership in recognition of his pioneering research in the development of electrospray ionization of macromolecules, an essential tool in Proteomics. Now, we feel very honored for Professors Michael Karas and Franz Hillenkamp having accepted the nomination to become also Honorary Members of SEProt and to deliver Plenary Lectures at its 3rd. Congress.

The Spanish Proteomics Society also happily acknowledges the seminal work of Prof. Peter Roepstorff in the fields of Protein Chemistry and its modern version, Proteomics in its most broader sense. Peter is not only a priviledged witness but also a principal starring of the transition from Protein Chemistry to Proteomics. A few years ago, on occasion of his 65th birthday, Ole N. Jensen, Albert J.R. Heck, and Franz Hillenkamp wrote a brief biography of Peter Roepstorff in

the International Journal of Mass Spectrometry[11]. I have reproduced here some paragraphs from this article in the believe that this act of cut-and-paste will not be considered plagiarism.

Peter Roepstorff founded, and until recently headed, the Protein Research Group at the University of Southern Denmark, where the main research focus is the development of methods for protein mass spectrometry and proteomics, including techniques for the determination of post-translational modifications. Throughout his career Peter has been searching for new challenges in protein chemistry and mass spectrometry. His early work in the late 1960's and early 1970's led to methods for peptide sequencing and for the determination of novel posttranslational modifications, including the discovery of gammacarboxyglutamic acid. Prof. Roepstorff is not only rightly considered a founder of what started as mass spectrometry of proteins and then became proteomics; he remains one of the most eminent developers and experts. He has co-authored more than 400 scientific articles and book chapters, one of the most cited being a paper suggesting the nomenclature for mass spectrometric peptide fragmentation[12]. A paper on the measurement of Kd's by mass spectrometry became also a well-cited classic. To cite just one more example, the TiO2 phosphopeptide enrichment method developed in Roepstorff's lab led revolution in an area of intense research, phosphoproteomics.

From early on, Peter initiated research projects with biotech and pharma companies in Denmark and abroad. Around 1993 the Protein Research Group and the Münster group joined efforts to tackle a seemingly hopeless problem, the MALDI-MS of nucleic acids. The project was not successful enough to convince the EU bureaucracy to continue funding it, but the core ideas developed then are now the basis for a commercial use by Sequenom Inc.

Peter is a well-respected attendee of many meetings as he is always very open in giving his opinion and (un)asked advise. He is also an outstanding mentor, having always promoted education and training. The high scientific level, the friendly atmosphere, and the international ambience of the laboratory is a big inspiration for all students and colleagues and has made Roepstorff's Protein Research Group known all over the world as an excellent training site. Numerous graduate students and post docs from all over the world have received education in the Protein Research Group, and many of them are now heading proteomics laboratories in different countries. In 2005 he transferred the responsibility for the Protein Research Group to the next generation in order to get more time for research. This has allowed him to realize one of his dreams: participating in a major Danish Marine Biology Expedition, Galathea 3, where he was scubadiving

in tropical coral reefs in the search for new fluorescent proteins. Typical for Peter, who always teaches colleagues and students to go for the fun-experiment, which often brings you the most exciting results and new insights.

The famous statement *Pigmaei gigantum humeris impositi plusquam ipsi gigantes vident*, is attributed to Isaac Newton. The vision and work of Michael Karas, Franz Hillenkamp, and Peter Roepstorff on mass spectrometry undoubtly represent solid pillars upon which biological mass spectrometry is continuously being developed.

Juan J. Calvete
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SEProt President

References

[1] Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass spectrometry of large biomolecules. *Science*. 1989; 246:64-71.
[2] Fenn JB. Electrospray wings for molecular elephants (Nobel lecture). *Angew Chem Int Ed Engl*. 2003; 42:3871-94.
[3] Ido Y, Tanaka K, Akita S, Yoshida Y, Yoshida T. Development of Laser Ionization Time of Flight Mass Spectrometer III – Sensitive Ion Detection in High Mass Range (in Japanese). *35-kai Shitsuryo Bunseki Rengo Toronkai, Yoshishu*, pp. 20-21 (1987).
[4] Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T. "Protein and Polymer Analyses up to m/z 100 000 by Laser Ionization Time-of flight Mass Spectrometry". *Rapid Commun Mass Spectrom* 1988; 2:151-3.
[5] Karas M, Bachmann D, Hillenkamp F. Influence of the Wavelength in High-Irradiance Ultraviolet Laser Desorption Mass Spectrometry of Organic Molecules. *Anal. Chem.* 1985; 57: 2935-9.
[6] Karas M, Bachman D, Bahr U, Hillenkamp F. Matrix-Assisted Ultraviolet Laser Desorption of Non-Volatile Compounds. *Int J Mass Spectrom Ion Proc* 1987; 78: 53-68.
[7] Karas M, Hillenkamp F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal. Chem. 1988; 60: 2299-301. (This paper has been cited in more than 2200 ocassions).
[8] Beavis RC, Chait BT. "Matrix-assisted laser-desorption mass spectrometry using 355 nm radiation". *Rapid Commun. Mass Spectrom.* 1989; 3: 436-9.
[9] Karas M, Bahr U. "Laser Desorption Ionization Mass Spectrometry of Large Biomolecules". *Trends Anal. Chem.* 1990; 9: 321-5.
[10] Franz Hillenkamp and Jasna Peter-Katalinic (Eds.) MALDI MS. A practical guide to instrumentation, methods and applications. Wiley-VCH, 2007. ISBN: 978-3-527-31440-9
[11] Jensen ON, Heck AJR, Hillenkamp F. Peter Roepstorff, A brief biography. Int. J. Mass Spectrom. 2007; 268:v-vii.
[12] Roepstorff P, Fohlman J. Proposal for a common nomenclature for sequence ions in mass spectra of peptides. Biomed. Mass Spectrom. 1984; 11:601.

The Spanish Proteomics Society

proudly confers upon

Prof. Michael Karas

Honorary Membership

for a distinguished career, and particularly in recognition of his pioneering research in the development of matrix-assisted laser-desorption ionization of macromolecules, an essential tool in Proteomics.

Prof. Michael Karas is hereby granted all the Rights, Honors, and Privileges thereunto appertaining.

Given on occasion of the Joint 3rd Congress of the Spanish Proteomics Society and the Latin American HUPO, in Pamplona (Spain), the tenth day of February, two thousand and nine

Jesús V. Jorrín
Secretary

EPRO

Juan J. Calvete
President

The Spanish Proteomics Society

proudly confers upon

Prof. Franz Hillenkamp

Honorary Membership

for a distinguished career, and particularly in recognition of his pioneering research in the development of matrix-assisted laser-desorption ionization of macromolecules, an essential tool in Proteomics.

Prof. Franz Hillenkamp is hereby granted all the Rights, Honors, and Privileges thereunto appertaining.

Given on occasion of the Joint 3rd Congress of the Spanish Proteomics Society and the Latin American HUPO, in Pamplona (Spain), the tenth day of February, two thousand and nine

Jesús V. Jorrín
Secretary

EPRO

Juan J. Calvete
President

The Spanish Proteomics Society

proudly confers upon

Prof. Peter Roepstorff

Honorary Membership

For a distinguished career in protein chemistry, biological mass spectrometry and proteomics, and for having promoted education and training in these disciplines for over 40 years.

Prof. Peter Roepstorff is hereby granted all the Rights, Honors, and Privileges thereunto appertaining.

Given on occasion of the Joint 3rd Congress of the Spanish Proteomics Society and the Latin American HUPO, in Pamplona (Spain), the thirteenth day of February, two thousand and nine

Jesús V. Jorrín
Secretary

EPRO

Juan J. Calvete
President

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CONVOCATORIA DEL *TERCER PREMIO JOSÉ LUIS LÓPEZ RODRÍGUEZ DE LA SOCIEDAD ESPAÑOLA DE PROTEÓMICA*

Pamplona, Julio de 2008

La *Sociedad Española de Proteómica* (*SEProt*) convoca la tercera edición del PREMIO SOCIEDAD ESPAÑOLA DE PROTEÓMICA destinado a reconocer la labor en el campo de la Proteómica de un científico que desarrolle su actividad en España. El premio, patrocinado por **Bruker BioSciences Española S.A.** (http://www.bruker.es), está dotado con 2000 € y una placa conmemorativa, y será entregado por un representante de Bruker Española durante el 3er Congreso de la SEProt que se celebrará en la Facultad de Ciencias de la Universidad de Navarra conjuntamente con la Latinamerican Human Proteome Organisation (LAHUPO) entre los días 10-13 de Febrero de 2009 (http://www.unav.es/proteomicsmeeting/index.html). En la presente convocatoria se otorgarán **dos galardones** que en ningún caso podrán ser compartidos. Una mitad del premio (1000 €, diploma acreditativo y la placa conmemorativa) será para **una publicación científica** relacionada con cualquier desarrollo o aplicación de la Proteómica. La otra mitad del premio (1000 €, diploma acreditativo) será para una contribución en forma de **panel** al 3er 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 que sean o no socios de la SEProt. La labor investigadora deberá haber sido realizada en España y haber sido publicada entre Enero de 2007 y Diciembre de 2008. Los candidatos deberán remitir 3 copias del trabajo **en formato electrónico** al Secretario de la SEProt (Jesús V. Jorrín, Dpto. Bioquímica y Biología Molecular, Edificio Severo Ochoa (C6), Campus de Rabanales, Universidad de Córdoba, 14014 Córdoba. bf1jonoj@uco.es). 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 31 de Diciembre de 2008**. La decisión se tomará antes del 15 de Enero de 2009 y será dada a conocer en el programa final del congreso y a través del portal electrónico de la SEProt. El galardonado será invitado a participar en el 3er Congreso de la SEProt exento del pago de las tasas de inscripción y a publicar un artículo en la revista Proteómica.

La elección del ganador(a) del Premio SEProt a la contribución en formato panel presentada al 3er Congreso de la SEProt se efectuará entre los primeros firmantes del estudio 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 a Diciembre de 2008.

Juan J. Calvete – Presidente de la SEProt

AWARD ANNOUNCEMENT

The Spanish Proteomics Society (SEProt) announces an award, sponsored by Applied Biosystems, for a poster presented at the SEProt-LAHUPO Congress. The Prize carries a € 500 monetary award and a diplome.

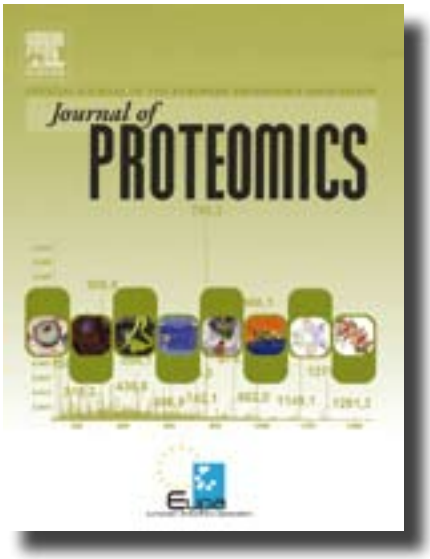
Selections will be based on the scientific excellence and/or tecnical innovations in the field of Proteomics. Award recipients will be chosen by the Poster Award Committe formed by three members of the SEProt Board.

Awards will be presented to the winners by Susanna Baqué, PhD, Senior Manager Sales & Support, Mass Spectrometry Systems, during the SEProt and General Meeting Session.

Good Luck!

Pamplona, January, 2009

Juan J. Calvete
SEProt President



The proceedings of the Proteomics and Human Proteome meeting will be published as a special issue of the Journal of Proteomics. The journal operates an online submission and peer review system that allows authors to submit articles online and track their progress via a web interface. The Journal of Proteomics will only publish in this special issue original research articles describing novel research work presented at the meeting. Also manuscripts submitted for this issue must not have been, nor will be, submitted for publication elsewhere at any time during its consideration by the Journal of Proteomics.

Authors should submit their manuscripts via the online system on or before March 15, 2009. No manuscript will be accepted after this date.

Detailed instructions for authors as well as information on aims and scope of the journal can be found at

http://www.elsevier.com/wps/find/journaldescription.cws_home/713351/description#description

To submit manuscripts to the special issue "Proteomics and Human Proteome: From Bench to Bedside", the article type SI: Proteomics and Human Proteome should be selected.

Pre-Congress Educational Day

FROM BASIC TO CHALLENGING PROTEOMICS TECHNOLOGIES

Pamplona, 10th of February

Chairs Dr. Juan Pablo Albar and Dr. Alberto Paradela

08:45-09:00 Introduction to the course
(Dr. Juan Pablo Albar, CNB)

Fundamental Proteomics Session

09:00-09:40 Basic concepts, sample preparation and separation technologies
(Dr. Juan Pablo Albar, CNB-CSIC)

09:40-10:20 How to interpret a MS and a MS/MS spectra
(Dr. Joaquin Abián, CSIC-UAB)

Differential Quantitative Proteomics Session

10:20-11:10 SILAC (Stable Isotope Labeling with Amino acids in Cell culture) in differential proteomics
(Dr. Miguel Marcilla, CNB).

11:10-11:30 Coffee break

11:30-12:10 Non-isobaric isotope chemical labeling in differential proteomics: use of ICPL
(Dr. Alberto Paradela, CNB).

12:10-12:50 High-throughput quantitative proteomics by stable ^{18}O isotope labeling (Dra. Estefanía Nuñez and Dra. Elena Bonzón, CBMSO).

12:50-13:30 Label-free quantitative proteomics: advantages and disadvantages (Dra. Miren Josu Omaetxeberria or Dr. Kerman Aloria, UPV-EHU).

13:30-14:30 Lunch time

14:30-15:10 Isobaric isotope chemical labeling: iTRAQ (Dr Antonio Serna, Applied Biosystems).

Mass Spectrometry Imaging Session

15:10-15:45 Overview and applications of tissue proteomics (Dr. Garry Corthals, Turku Center for Biotechnoly,Turku, Finland)

15:45-16:20 High-resolution MS imaging and data analysis (Dr. Ron Heeren, FOM Institute for Atomic and Molecular Physics, Amsterdam, The Netherlands).

16:20-16:40 Coffee break

16:40-17:15 Ultra-flex Family, FlexImaging software and spray station (Someone from Bruker Daltonics).

17:15-17:50 MALDI-SYNAPT mass spectrometry imaging (Dr James Langridge, Water Corporation)

17:50-18:25 Orbitrap MS-Imaging (Dra Michaela Scigelova, Thermo Fisher Scientific)

18:30-18:45 Concluding remarks

Abbreviatures:

CNB	Centro Nacional de Biotecnología, Madrid, Spain.
CBMSO	Centro de Biología Molecular Severo Ochoa, Madrid, Spain.
UPV-EHU	Universidad del País Vasco-Euskal Herriko Unibertsitatea, Bilbao, Spain.

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Committees

Organizing committee

Fernando J. Corrales
Centro de Investigación Médica Aplicada.
Universidad de Navarra

Mario H. Genero
Universidad Austral

Juan J. Calvete
Instituto de Biomedicina de Valencia

Enrique Santamaría
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Félix Elortza
Centro de Investigación Cooperativa
en Biociencias (CIC Biogune)

Jesús M. Arizmendi
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Javier Armendáriz
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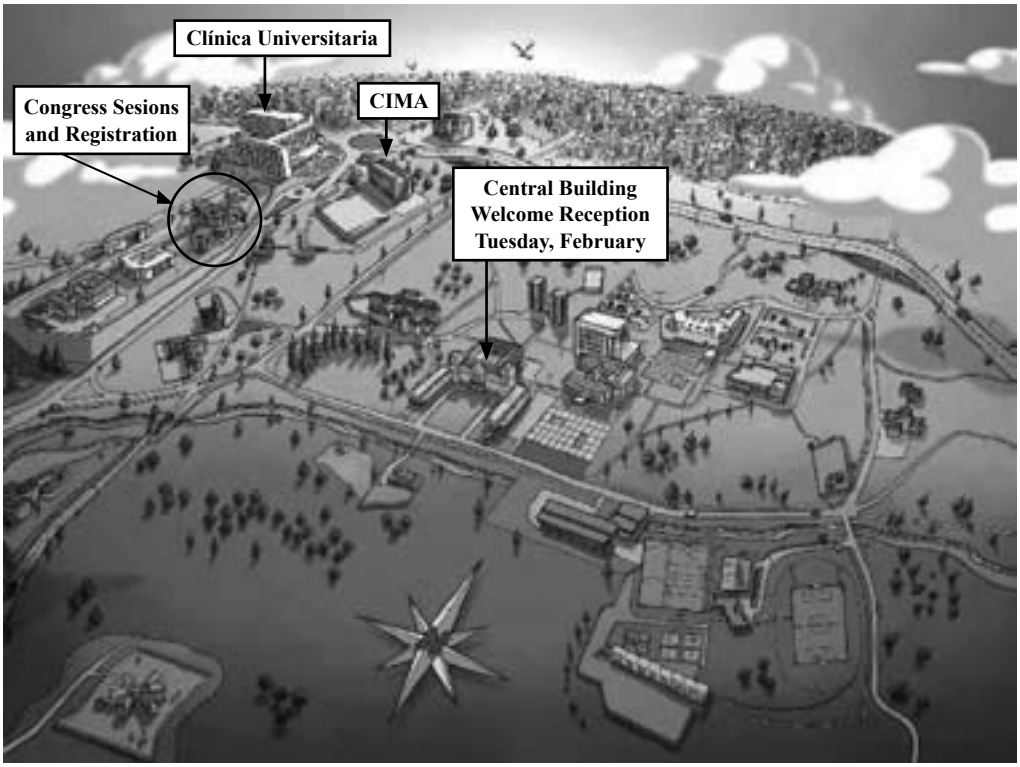
Universidad de Córdoba

Ángela Moreno
Universidad de Córdoba

Jesús V. Vázquez
Centro de Biología Molecular Severo Ochoa
Universidad Autónoma de Madrid

Jesús M. Arizmendi
Universidad del País Vasco

Maps


Clínica Universitaria
Congress Sesions and Registration
CIMA
Central Building Welcome Reception Tuesday, February

Schedule

	Tuesday 10	Wednesday 11	Thursday 12	Friday 13
9:00	Pre-Congress Workshop	Session 1	Session 3	Session 6
10:00				
11:00		Coffe Break	Coffe Break	Coffe Break
12:00		Sesion 2	Sesion 4	Closing Lecture
13:00				4th Seprot and 3rd HUPO meeting venue Farewell
14:00		Lunch	Lunch	
15:00		Poster Session	Poster Session	
16:00		HUPO Initiatives	Session 5	
17:00		Coffe Break		
18:00	Registration	HUPO Initiatives		
19:00	Welcome and Opening Lecture		Coffe Break SEProt Awards and General Meeting	
20:00				
21:00	Welcome Reception		Congress Dinner	

Program

Tuesday, February 10

18:00-18:45 **Registration**

19:00 ***Welcome and Opening Lecture***

Sponsored by DIGNA Biotech

Chairman: ***Juan J. Calvete***

19:30-20:00 **Good News for MALDI - The Advent of Second Generation Matrices**
Prof. Michael Karas, Johann Wolfgang Goethe University

20:00-20:30 **Reaction Kinetics in the MALDI Plume: The Yield of Positive versus Negative Peptide Ions**
Prof. Franz Hillenkamp, University of Muenster

21:00 ***Welcome reception***

Wednesday, February 11

Session 1:
Quantitative Proteomics

Sponsored by Waters

*Chairman:****Jesús Vázquez, Deborah Penque***

Plenary Sessions

09:00-09:30 **Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) as a Tool in Cancer Research**
Dr. José Luis Luque. Spanish National Cancer Research Center

09:30-10:00 **Isotope-Coded Protein Labelling (ICPL) as a tool for the quantitative analysis of a bacterial proteome**
Dr. Alberto Paradela. Biotechnology National Center

10:00-10:30 **Peptide fractionation for proteomic studies**
Dr. Gabriel Padrón. Center for Genetic Engineering and Biotechnology, La Havana, Cuba

Oral Communications

10:30-10:45 **Metastasis-associated C4.4A, a GPI-anchored protein cleaved by ADAM10 and ADAM17**
Cary Esselens. Medical Oncology Research Program. Vall d'Hebron University Hospital Research Institute

10:45-11:00 **Label-free quantification based on data independent acquisition mass spectrometry**
Miren Josu Omaetxebarria. University of the Basque Country

11:00-11:15 **Identification and Quantification of proteins from *Methylophaga Thiooxidans* and *Methylocella Silvestris* using label-free LC/MS**
Joanne B. Connolly. Waters

11:15-11:45 **Coffee Break**

Session 2
Posttranslational Modifications

Sponsored by Agilent Technologies

Chairman: David Andreu, Gabriel Padrón

Plenary Sessions

11:45-12:15 **Quantitative phosphoproteomics: a powerful tool to define kinase-substrate relationships**
Dra. Judit Villén, Harvard Medical School, Boston

12:15-12:45 **Proteomic analysis of S-nitrosated proteins**
Dr. Antonio Rodríguez Ariza. Reina Sofia University Hospital of Cordoba

12:45-13:15 **Understanding by proteomics the cellular trafficking defect of a disease associated mutant protein**
Dra. Deborah Penque. Instituto nacional de Saudé Dr. Ricardo Jorge, Lisboa

Oral Communications

13:15-13:30 **Chloroacetylation of cyclostreptin influences its interaction with tubulin**
Enrique Calvo, Spanish National Centre for Cardiovascular Research (CNIC)

13:30-13:45 **Quantitative analysis of protein glycation in clinical samples**
Feliciano Priego, University Medical Center, Geneva, Switzerland

13:45-14:00 **New microfluidic chip targeting phosphoproteomes**
Dayin Lin, Agilent Technologies

14:00-15:00 **Lunch**

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

HUPO Initiatives

Sponsored by Genoma España and Thermo Scientific

Chairman: ***José María Mato, Mario Hugo Genero***

Liver

16:00-16:30 **The Insight into the Human Liver Proteome**
Prof. Fuchu He. Fudan University, Shanghai, China

Brain

16:30-17:00 **Characterisation of the Autoimmune Antibody Repertoire of Parkinson's Disease Patients by Systematic Screening of Protein Arrays**
Prof. Helmut E. Meyer. Medizinisches Proteom-Center, Ruhr-Universität Bochum, Germany

17:00-17:30 **Coffee Break**

HUPO Initiatives-2

Sponsored by Genoma España and Thermo Scientific

Chairman: ***José María Mato, Mario Hugo Genero***

PSI

17:30-18:00 **Annotating the Human Proteome**
Prof. Rolf Apweiler. EMBL/EBI, Hinxton, UK

Cardiovascular

18:00-18:30 **The International Cardiovascular BioBank for the Cardiovascular Initiative HUPO (CVI-HUPO)**
Prof. Mario H. Genero. Austral University and Nedken

Session 3

Bioinformatics in Proteomics

Sponsored by Promega Biotech Ibérica

Chairman: ***Juan Pablo Albar, Andrea Llera***

Plenary Sessions

09:00-09:30 **To be confirmed**
Dr. Rune Matthiesen. CIC-Biogune

09:30-10:00 **Properties of Average Score Distributions of SEQUEST: The Probability Ratio method**
Pedro Navarro. Severo Ochoa Molecular Biology Center

10:00-10:30 **The neuropeptidome of *rhodnius prolixus* brain**
Dr. Rolando Rivera Pomar. Centro Regional de Estudios Genómicos, Buenos Aires, Argentina

Oral Communications

10:30-10:45 **Storing, reporting and comparing proteomics experiments using the miape generator tool**
Salvador Martínez, ProteoRed – National Center for Biotechnology-CSIC

10:45-11:00 **Proteopathogen, a protein database to study host-pathogen interaction**
Vital Vialas, Complutense University of Madrid

11:00-11:15 **HaloLink™ Protein Arrays for Functional Analysis of Proteins**
Patricia Bresnahan, Promega Biotech Ibérica

11:15-11:45 **Coffee Break**

Session 4

Protein Interactions and Protein Arrays

Sponsored by Sigma-Aldrich

Chairman: ***Jean Charles Sánchez, Mario Hugo Genero***

Plenary Sessions

11:45-12:15 **Real-time and Label-free Biomolecular Interactions Analysis using Self-assembled Protein Microarrays and Surface Plasmon Resonance Imaging**
Dr. Manuel Fuentes. Harvard Institute of Proteomics, Harvard Medical School.

12:15-12:45 **Novel approaches to the characterization of metastasis in colorectal cancer**
Dr. Ignacio Casal. Spanish National Cancer Research Center.

12:45-13:15 **Deciphering the interactome of p8, a protein related to tumor progression**
Dra. Silvia Moreno. Universidad de Buenos Aires, Argentina

Oral Communications

13:15-13:30 **High-Mass MALDI ToF Mass Spectrometry and Chemical Cross-linking for Interaction Analysis**
Alexis Nazabal, Swiss Federal Institute of Chemistry

13:30-13:45 **Lectin-sugar interactions deciphered by SPR-MS and CREDEX-MS**
Carmen Jimenez, Pompeu Fabra University

13:45-14:00 **Monitoring in vivo protein-protein interactions by coupling bimolecular fluorescence complementation (BIFC) and flow cytometry**
Montse Morell. Universitat Autònoma de Barcelona

14:00-15:00 **Lunch**

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

Session 5

Human Proteomics and Biomarkers

Sponsored by Bio-Rad

Chairman: ***Concha Gil, Jonas Perales***

Plenary Sessions

16:00-16:30 **Proteomics and Metabolomics of human atherosclerotic arteries**
Dr. Fernando Vivanco. Jiménez Díaz Fundation

16:30-17:00 **Proteomics in obesity research**
Dra. Silvia Barceló. Instituto Aragonés de Ciencias de la Salud

17:00-17:30 **Using proteomics to unravel the molecular pathway of sparc-mediated tumorigenicity**
Dra. Andrea Llera. Fundación Instituto Leloir, Buenos Aires, Argentina

17:30-18:00 **Detection of novel biomarkers of liver cirrosis by proteomic analysis**
Prof. Helmut E. Meyer. Medizinisches Proteom-Center, Ruhr-Universität Bochum, Germany

Oral Communications

18:00-18:15 **Identification of replication-competent HSV-1 Cgal+ strain signalling targets in human hepatoma cells by functional organelle proteomics**
Enrique Santamaría, Centre for Applied Medical Research (CIMA), University of Navarra

18:15-18:30 **Genomic and proteomic analyses reveal a relationship between wnt pathway genes, oxidative stress metabolism and vascular calcification**
Pablo Román, University Hospital of Asturias

18:30-18:45 **BioCore study ("Biomarkers of Coronary Events"): from sampling to discovery of plasma biomarkers by SELDI-TOF MS and 2DE**
Olivier Meilhac, Hópital Xavier Bichat-Paris. Bio-Rad

18:45-19:00 **Coffee Break**

19:00-20:00 **Seprot Awards**

Sponsored by Bruker

Seprot Award Session

Application of a novel statistical model for quantitative proteomics by ^{18}O labeling to the study of VEGF-induced angiogenesis in vascular endothelial cells
Pablo Martínez-Acedo. *Severo Ochoa Molecular Biology Center*

20:00-20:45 **Seprot General Meeting**

21:00 **Congress Dinner**
Please, contact organizing committee for any special eating habits (gluten intolerance, special allergies...)

Friday, February 13

Session 6
Animal, Plant and Microbial Proteomics

Sponsored by Applied Biosystems

Chairman: ***Jesús V. Jorrín, Silvia Moreno***

Plenary Sessions

09:00-09:30 **Top-down and bottom-up quantitative proteomic approaches to characterize the development of grape berry tissues**
Dr. Roque Bru. University of Alicante

09:30-10:00 **Proteomic identification of s-nitrosylated proteins in *arabidopsis thaliana* in response to pathogen infection**
Dra. Ana María Maldonado. University of Cordoba

10:00-10:30 **Using DM43 and DM64, two antitoxins from *Didelphidae*, to study the snake venom sub-proteomes**
Dr. Jonas Perales. Oswaldo Cruz Foundation, Brazil

Oral Communications

10:30-10:45 **Combined proteomic and transcriptomic analysis identifies differentially expressed pathways associated to *Pinus Radiata* needle maturation**
Luis Valledor, Univesity of Oviedo

10:45-11:00 **Quantitative proteomics analysis of lymph nodes from pigs infected by porcine circovirus type 2 (PCV2) by 2-DE, $^{18}O/^{16}O$ labeling and linear ion trap mass spectrometry**
María Ramirez, University of Córdoba

11:00-11:15 **Intelligent Use of Retention Time for Higher Order Multiple Reaction Monitoring Multiplexing**
Antonio Serna, Applied Biosystems

11:15-11:45 **Coffee Break**

Closing Lecture

Sponsored by DIGNA Biotech

Chairman: ***Fernando J. Corrales***

11:45-12:45 **Never give up your dreams**
Prof. Peter Roepstorff. University of Southern Denmark

12:45-13:15 **4th Seprot and 3rd LAHUPO Meeting Venue**

13:15 **Farewell**

Invited lectures

GOOD NEWS FOR MALDI
THE ADVENT OF SECOND GENERATION MATRICES

Michael Karas
Johann Wolfgang Goethe University

All MALDI matrices in use until now have been found empirically following some straightforward and simple selection criteria, despite strong efforts and some progress to elucidate the underlying physicochemical principles. In a new approach, we synthesized numerous derivatives of the most widely used α-cyano-4-hydroxycinnamic acid. Based on MALDI experiments two main functions were found to specify a well-working matrix, 1st a carboxylic group (attributed to play a role in analyte incorporation) and 2nd a high yield of protonated matrix ions. The latter points to the decisive role of matrix ions as protonating agents in a chemical ionization process. Increasing the gas-phase acidity was thus the step to design a superior matrix – successfully achieved by introducing a chlorine residue at the 4-position. The new matrix is highly superior both in the absolute peptide signal intensities detected, but also shows substantially less discrimination for peptides of lower basicity – thus facilitating both higher detection sensitivity and improved sequence coverage in proteomics experiments.

POSITIVE AND NEGATIVE ANALYTE ION YIELD IN MATRIX-ASSISTED LASER DESORPTION/IONIZATION

F. Hillenkamp

Institute of Medical Physics and Biophysics,
University of Münster, Germany

The most commonly accepted model for the formation of analyte ions in MALDI-MS assumes a primary ionization of matrix e. g. by photoionization, leading to stable protonated and deprotonated matrix ions, respectively. Analyte ions are then formed by secondary proton transfer reactions in the expanding plume. This model had been checked experimentally by comparing the yield of positive to negative ions of three peptides and six matrices, differing in gas phase basicity by about 150 kJ/mole [Int. J. Mass Spetrom. 268(2007)122]. The data have been revisited for a more general and in depth analysis. Model predictions are presented for a wide range of experimental parameters, in particular for ranges of the gas phase basicity and acidity of analyte and matrix and for different molar ratios of analyte to matrix as well as the yield of primary matrix ions. It is shown that the observed ion yields cannot be explained by any single and consistent set of parameters. It is concluded that the existing simple model needs be modified to fully explain the experimental findings. Such modifications should primarily address the formation of negative matrix and analyte ions.

STABLE ISOTOPE LABELING BY AMINO ACIDS IN CELL CULTURE (SILAC) AS A TOOL IN CANCER RESEARCH

J.L. Luque-García

Spanish National Cancer Research Center
Melchor Fernández Almagro 3, 28029, Madrid, Spain

Stable isotope labeling by amino acids in cell culture (SILAC) is a simple and accurate approach to quantify differential protein expression and dynamic regulation of posttranslational modifications. In a typical SILAC experiment, cells representing different biological conditions are grown in media supplemented with either "light" or "heavy" isotope-containing amino acids. Metabolic incorporation of labeled amino acids into all proteins from cells of one population and subsequent combination of differentially labeled samples in equal ratios enables relative quantification of proteins from each sample based on the intensities of the corresponding differentially labeled peptides. In the same mass spectrometric experiment, MS/MS can be carried out to obtain sequence information for protein identification. The high accuracy of quantitation provided by SILAC is a consequence of the metabolic incorporation of the isotopes which allows mixing of the labeled and unlabeled cells, therefore subsequent fractionation, purification or protein digestion steps do not introduce any errors in the quantitation.

In combination with mass spectrometry (MS), SILAC can be an effective means for characterization of different cellular events. The advantages of using SILAC in the cancer research field are significant because this approach allows the expansion to a proteomics scale of established biochemical and cell biological experiments that are frequently used to address cancer-related problems. Here I present some examples on how cancer research can benefit from the combination of SILAC and MS as a screening tool for the identification of potential biomarkers for early detection or disease prognosis, for the elucidation of biochemical pathways directly related with cell division and cancer, and for the study of the cellular mechanisms involved in tumor invasion and metastasis.

ISOTOPE-CODED PROTEIN LABELLING (ICPL) AS A TOOL FOR THE QUANTITATIVE ANALYSIS OF A BACTERIAL PROTEOME

Alberto Paradela[1], Miguel Marcilla[1], Rosana Navajas[1], Antonio Ramos[1], Francisco García del Portillo[2], and Juan Pablo Albar[1]

[1]Servicio de Proteómica; [2]Departamento de Biotecnología Microbiana
Centro Nacional de Biotecnología, CSIC, c/Darwin 3, 28049, Madrid, Spain.

The development of new quantitative tools for the analysis of whole proteomes (differential proteomics) has been continuous during the past years. Traditional approaches, based on previous fractionation of complex proteomes by 2D-PAGE and comparison of the gel images generated thereby, are being complemented by new techniques that commonly make use of isotopic labeling of the samples to be compared followed by mass spectrometric analysis. Isotopic labeling can be performed *in vivo* (e.g., SILAC), chemically (e.g., ICAT, iTRAQ, ICPL) or enzymatically (e.g., $^{16}O/^{18}O$). Isotope-coded protein labeling (ICPL) has been described recently as a technique to efficiently label the abundant amino groups present in proteins and allows comparison of up to four samples at the same time. Theoretically, ICPL overcomes some of the disadvantages found in other chemical labeling techniques, such as iTRAQ (poor detectability of the reporter ions in most of the mass spectrometers) or ICAT (low number of potentially labeled residues). However, the number of articles published to date using ICPL is noticeably low and no clear consensus exists on its feasibility as a quantitative tool. Here we describe the results obtained after quantitative comparison between the proteomes of *Salmonella enterica serovar typhimurium* and two derived strains harboring mutations that have been shown to drastically affect the bacterial proteome. Results were analyzed using software specifically designed for differential proteomics (WARP-LC). More than 500 different proteins were identified while about 150 proteins could be quantified as well. Our results demonstrate that ICPL may be a very valuable alternative to iTRAQ when the analysis of iTRAQ specific reporter ions is not possible and clearly overcomes the limitations imposed by other non-isobaric labeling approaches, such as ICAT.

PEPTIDE FRACTIONATION FOR PROTEOMIC STUDIES

Lázaro Betancourt, Aniel Sánchez, Jeovanis Gil, Jorge Fernández-de-Cossío, Yassel Ramos, Félix Alvarez, Vladimir Besada, Luis Javier González and Gabriel Padrón

Center for Genetic Engineering and Biotechnology. PO. Box 6162. Havana. Cuba.

Proteomics has evolved towards shotgun strategies based on multidimensional chromatography and mass spectrometry analysis (LC-MS/MS) of peptide mixtures derived from cell extracts. However, very complex peptide mixtures are obtained, limiting the detection of many of those peptides and impeding the identification of several proteins. Fractionation at protein or peptide level has been found to enhance protein identification.

Following this approach, we have developed three complementary methods (SCAPE) for selective isolation of peptides based on the derivatization of abundant functional groups (α and ε amino groups) to modulate the presence of positive charges (at acidic pH) and further separation by cation exchange chromatography or affinity chromatography.

These procedures have shown to be complementary, allowing high protein coverage. All of them have been developed for quantitative proteomics in combination with differential isotopic labeling. Particularly, one of these methods was implemented to analyze four proteomes simultaneously.

In addition we have developed a procedure for peptide fractionation by SDS-free polyacrylamide gel electrophoresis. Complex protein extracts separated by SDS-PAGE are trypsin digested and peptides further fractionated by SDS free-PAGE. Peptides migrate to the anode electrode in accordance with the charge-molecular mass ratio. Electrophoretic mobility of tryptic peptides increase for more acidic peptides and overlapping of peptides among fractions is lower than 15%. Detection of peptides improves substantially and hence, protein identification. An efficient method for peptide transfer to the second dimension was established while identified proteins increased 2.5 fold with respect to the direct analysis of non fractionated protein digest. The use of SDS for protein fractionation allows analysis of highly hydrophobic proteins and minimal protein loses. Analysis of a membrane protein extract from Neisseria meningitides allowed the identification of underrepresented proteins. The method could be also very useful for studying phosphorylated peptides.

QUANTITATIVE PHOSPHOPROTEOMICS: A POWERFUL TOOL TO DEFINE KINASE-SUBSTRATE RELATIONSHIPS

Judith Villen
Harvard Medical School

Protein phosphorylation is a main regulatory switch in the cell, controlling processes such as cell growth, proliferation, differentiation and survival. This control is performed by intricate signaling networks, which are capable of altering protein activities and rapidly communicating messages from different extracellular or internal cues to ultimately promote adequate cell readjustments. Numerous studies have addressed protein phosphorylation over the past decades, often on a single protein/pathway level. However, the global picture of signaling events can only be accomplished from comprehensive studies, which are becoming attainable by mass spectrometry (MS)-based proteomics.

The main difficulty in MS large-scale phosphorylation studies is the limitation in detecting phosphorylated species within complex sample mixtures due to their low abundance. However, the past five years have seen a steady improvement in phosphopeptide enrichment and MS data acquisition methods along with the development of computational tools for data analysis and validation, allowing us to routinely identify thousands of phosphorylation events from a single experiment. An overview of such efforts and the current status of technologies to profile the phosphoproteome will be given.

Furthermore, these strategies have been combined with metabolic labeling using stable isotopes (SILAC) for quantitative studies where two cell populations are compared. One of the most challenging problems in signal transduction is establishing kinase-substrate relationships. We have combined chemical genetics and large-scale quantitative phosphoproteomics to identify substrates of the master mitotic kinase Cdk1 at different stages of the cell cycle, and meiotic kinase Ime2 in the budding yeast *Saccharomyces cerevisiae*. Using this approach, we expanded the number of known *bona fide* substrates to ~400 for each kinase, and pinpointed the precise sites of phosphorylation. We observed that substrates for Cdk1 and Ime2 vastly overlap; however the sites targeted seem to differ, which provides an example on how different layers of regulation are assembled in complex systems.

PROTEOMIC ANALYSIS OF S-NITROSATED PROTEINS

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One of the major tasks to be accomplished in the post-genomic era is the characterization of post-translational modifications in proteins. The S-nitrosation of protein thiols is a redox-based posttranslational modification that modulating enzymatic activity, sub-cellular localization, complex formation and degradation of proteins, largely contributes to the complexity of cellular proteomes. Although the detection of S-nitrosated proteins is problematical due to the lability of S-nitrosothiols (SNO), with the improvement of molecular tools an increasing range of proteins has been shown to undergo S-nitrosation.

The liver is one organ clearly influenced by nitric oxide, and acute and chronic exposure to this substance has been associated with distinct patterns of liver disease. Therefore, it is important to identify potential targets for protein S-nitrosation in human hepatocytes during alteration of SNO homeostasis. Treatment of human hepatocytes with L-nitrosocysteine increased cell death and augmented the levels of S-nitrosoproteins, detected both by chemiluminescence and the biotin-switch method. An increased S-nitrosoglutathione reductase (GSNOR) activity, related to augmented levels of ADH-5 mRNA, the gene encoding for GSNOR in humans, returned SNO content to basal levels. The identified S-nitrosoproteins in hepatocytes included proteins involved in metabolism, maintenance of cellular homeostasis and signalling. These results points to the relevance of this posttranslational modification to the physiology and pathophysiology of these cells.

Further proteomic approaches for the systematic assessment of potential targets for protein S-nitrosation have been recently developed. These strategies include methods for the identification of the modified cysteines, that will provide researchers with better tools for exploring this post-translational modification and for performing an in depth analysis of the cellular S-nitrosoproteome.

UNDERSTANDING BY PROTEOMICS THE CELLULAR TRAFFICKING DEFECT OF A DISEASE ASSOCIATED MUTANT PROTEIN

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Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, like F508del-CFTR, disrupt intracellular trafficking leading to Cystic Fibrosis (CF) disease. Cells expressing F508de-CFTR restore their ability to exhibit forskolin-dependent chloride transport at cell surface after treatment with a number of chemical an pharmacological chaperones known to stabilize proteins in their native conformation. Mutagenesis on arginine-framed tripeptides (AFTs or RXR) motifs, described to be involved in the endoplasmic reticulum (ER) retention/export quality control of many membrane proteins, was another effort to redirect F508del-CFTR to be functional at the cell membrane. The trafficking defect of F508del-CFTR is temperature-sensitive, as incubation at the permissive temperature of 27-30 °C results in partial protein export from the ER to the cell surface where it functions similarly to the wild-type protein.

Although the effects of all these strategies to rescue the defective F508del-CFTR trafficking and function are well documented the corresponding molecular mechanism is not fully elucidated. Proteins involved in the trafficking defect and/or rescue of CFTR mutants are potential CF therapeutic targets; therefore, we sought to identify these proteins.

We have investigated by proteomics whether BHK cells, the popular heterologous model system for examination of CFTR processing and function, underwent differential proteome modulation in response to the type of CFTR, i.e, wild-type-CFTR, F508del-CFTR or F508del-4RK-CFTR, they are expressing and/or under effect of low temperature (LT) incubation (26ºC).

The results indicated that the mutagenic RXR reverted F508del-CFTR activates a particular unfolded protein response (UPR)/ ER stress pathway in BHK cells that might be able to generate a compatible and/or favourable cellular environment for (at least partial) F508del-CFTR rescuing. Most proteins involved are CFTR- and/or 14-3-3-interactors, suggesting a potential role of these proteins in the CFTR trafficking.

Results from LT experiments showed that under 26ºC, BHK cells are metabolically active and may respond to temperature stress with different strategies from those at

37ºC, namely respecting CFTR processing and trafficking. LT expression profiles of several proteins in BHK-F508del cells tend to wt levels (37ºC), which can be indicative of the involvement of those proteins in rescue of CFTR. Many of them are also described as CFTR interactors (e.g. RACK1). The UPR in BHK-F508del cells under LT treatment seems also to be highly modulated, as many proteins involved in this mechanism were found up-regulated in LT conditions (e.g. GRP78). UPR appears to be particularly related to CFTR rescue by the enhancement of the chaperome/folding environment in the ER.

Several proteins identified in both experiments are also associated with CF inflammation and oxidative-stress pathology.

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Rune Matthiesen
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PROPERTIES OF AVERAGE SCORE DISTRIBUTIONS OF SEQUEST: THE PROBABILITY RATIO METHOD

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High throughput identification of peptides in databases from tandem mass spectrometry data is a key technique in modern proteomics. Distribution-based scores are widely used to discriminate correct peptide identifications from large datasets of identified MS/MS spectra using searching engines such as SEQUEST. In this work we study the mathematical properties of average SEQUEST score distributions by introducing the concept of spectrum quality and expressing these average distributions as compositions of single-spectrum distributions. Our analysis leads to a novel indicator, the probability ratio, which takes optimally into account the statistical information provided by the first and second best scores. The probability ratio is a non-parametric and robust indicator that makes spectra classification according to parameters such as charge state unnecessary and allows a peptide identification performance, on the basis of false discovery rates, that is better than that obtained by other empirical statistical approaches. Besides, these identification methodologies are accompanied by the use of decoy databases to estimate the number of positive assignations and calculate false discovery rates. In conjunction with target databases, decoy databases may be used separately or in the form of concatenated databases, allowing a competition strategy; depending on the method used two alternative formulations are possible to calculate error rates. We show that both separate and concatenated approaches clearly overestimate error rates and, after analyzing as a whole the joint distribution of matches obtained after performing a separate database search and applying the competition strategy, we propose a new, integrated algorithm, tested in the practice with several scores, which makes a more accurate calculation of false discovery rates.

THE NEUROPEPTIDOME OF *RHODNIUS PROLIXUS* BRAIN

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Chagas' disease is an important arthropod-borne disease in Central and South America. Recent estimations from the World Health Organization indicated 16-18 million people infected. The disease is vectored by insects, which are the main target of disease spreading. Disruption of the activity of vector physiology through manipulation of regulatory peptides is an attractive direction towards a novel generation of insecticides.

In this study we performed a peptidomic analysis of the brain and hemolymph of *Rhodnius prolixus*. This is the first comprehensive high throughput neuropeptidomic study of a human disease vector to date. Performing off-line nano-LC MALDI TOF-MS/MS analysis with subsequent *de novo* sequencing and database search, we have identified 42 novel neuropeptides from *R. prolixus*. Some of the molecules identified present unique characteristics compared to known insect neuropeptides. Peptides identified were classified as extended FMRF-amide-related peptides, sulfakinins, myosuppressins, short neuropeptide F, long neuropeptide F, SIF-amide-related peptides, tachikinins, orcokinins, allatostatins, allatotropins, calcitonin-like diuretic hormones, corazonin and pyrokinin. Some of them were detected in multiple isoforms and/or truncated fragments. Interestingly, some of the *R. prolixus* peptides, as myosuppressin and sulfakinins, are unique in their characteristic C-terminal domain among insect neuropeptides identified so far. Furthermore, orcokinins, which are a novel family of peptides detected in only a few species, were identified in brain and hemolymph. The presence of orcokinins in hemolymph suggests a neurohormonal role for this molecule. To validate the data, we confirm the presence of peptides in the brain by immunohistochemistry and determine a map of neurosecretory neurons. In addition, our work provides useful information towards the annotation of genes in the ongoing *R. prolixus* genome sequence project, opens new paths of research in vector biology and in comparative and evolutionary studies of the neuroendocrine system.

REAL-TIME AND LABEL-FREE BIOMOLECULAR INTERACTIONS ANALYSIS USING SELF-ASSEMBLED PROTEIN MICROARRAYS AND SURFACE PLASMON RESONANCE IMAGING

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Now that the human genome has largely been sequenced, one of the most important pursuits is to understand the function of proteins it encodes. Despite immense progress in molecular biology and genetics, only a small fraction of the proteome is understood at the biochemical level. Systems biology and proteomics strive to create detailed predictive models for molecular pathways based upon the quantitative behavior of proteins. Understanding these dynamic networks provides clues into the consequence of aberrant interactions and why they lead to diseases like cancer. However, collecting biochemical data about protein behavior at scale has been daunting. Historically, methods capable of collecting quantitative data on biochemical interactions could only be used for one or a few proteins at the time. Here, we show the combination of two technologies that together could lead to the ability to measure binding events in real time for many protein interactions simultaneously using a label free technology. This could revolutionize the study of protein interactions networks by enabling quantitative comparisons of binding affinities across many molecular species, as well determining the kinetics rates of binding and release.

The first technology is protein microarrays, which display thousands of proteins in high density and enable their simultaneous biochemical characterization. We use Nucleic Acid Programmable Protein Arrays (NAPPA), developed at the Harvard Institute of Proteomics (HIP), as a method for producing the microarrays, because they replace the complex process of spotting purified proteins with the simpler process of spotting plasmid DNA. The proteins can then be simultaneously transcribed/translated *in situ* at the time of the assay. The second technology is a surface plasmon resonance imaging (SPRi) device that has been adapted to multiplexed binding events from a planar surface and is compatible with the protein microarray. In addition this technique is sensitive, accurate and provides real-time date for both the equilibrium and the interaction kinetics. The project is focused at coupling NAPPA protein array technology to multiplexed real-time label-free SPRi-based detection system (which allows thousands of binding events to be monitored in real-time without any loss in sensitivity). By SPR*i* we were able to detect binary interactions using NAPPA format. The combination of both technologies allows us to generate detailed kinetic data of interactions pathways.

NOVEL APPROACHES TO THE CHARACTERIZATION OF METASTASIS IN COLORECTAL CANCER

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Colorectal cancer (CRC) remains as a major cause of mortality in the developed countries due to the absence of appropriate biomarkers for diagnosis. Progression to metastasis is the critical point in colorectal cancer survival. Metastasis of CRC is very poorly understood at the moment. In previous studies, the use of 2D-DIGE and antibody microarrays led to the identification of differentially-expressed proteins in primary CRC tumors as potential specific biomarkers of CRC. Both approaches were complementary and enabled us to identify a large collection of potential tumoral tissue biomarkers that is being currently investigated. These proteins included isoforms and post-translational modifications responsible for modifications in signalling pathways.

Recently, we have been working along two different lines for the search of colorectal cancer biomarkers related with metastasis: On the one hand, two colorectal cancer cell lines (KM12C and KM12SM), representing non-metastatic *versus* highly metastatic cells were compared to find and quantify the differences in protein expression at the cell surface level by using a SILAC approach. We were able to identify 291 membrane and membrane associated proteins from these two cell lines. A total of 66 proteins were differentially expressed more than 1.5-fold. Together with CEA and EGFR we identified an elevated number of cell receptors, CDs and cell adhesion molecules among the most deregulated proteins in metastatic cells. These proteins were further validated by using different techniques.

On the other hand, we have used high density protein microarrays comprising 8000 human proteins to identify autoantibody signatures in the sera of CRC patients. The screening was performed using sera from CRC patients in advanced stages, including metastasis. A total of 43 proteins were differentially recognized with a statistically significant value $p < 0.04$, 26 proteins showed higher prevalence in CRC sera and 17 showed lower prevalence. Furthermore, an ELISA assay was developed using these purified recombinant proteins for testing their discriminatory power in a different subset of human sera. The results confirmed the presence of a discriminatory autoantibody signature for CRC diagnosis and point out new individual markers of disease with a potential diagnostic capacity in metastasis.

DECIPHERING THE INTERACTOME OF P8, A PROTEIN RELATED TO TUMOR PROGRESSION

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p8 is an 8 kDa protein. It was identified due to its induction during the acute phase of pancreatitis. Functions related to cell growth control and stress have been attributed to p8 since its mRNA levels are increased in response to stress and mitogenic factors. An important role in tumor progression was also assigned to p8 since it was observed that p8 expression is altered in various malignant processes; and that while fibroblasts obtained from p8 +/+ mice transformed with a retroviral vector expressing oncogene E1A are able to induce tumor formation when injected into nude mice, transformed fibroblasts derived from p8 -/- mice have no tumorigenic properties.

Analysis of its sequence identified a conserved region corresponding to a NLS. Immunocytochemistry experiments show that the sub-cellular localization of p8 depends on cell culture density, cell cycle and acetylation state of the cells. Its nuclear import is energy dependent, and the NLS of p8 is necessary and sufficient to retain a heterologous protein in the nucleus.

The fact that it is small enough to diffuse between nucleus and cytoplasm, but still possesses a NLS and a controlled localization suggests that it could associate to multiprotein complexes. Our aim is to identify these complexes. We generated a HEK293 cell line that expresses p8 fused to HIS-FLAG tags. Tandem affinity purification was performed. The purified complexes were digested and analyzed by LC-MSMS to identify proteins associated to p8.

We generated a list of proteins that interact with p8 and combined it with previous two-hybrid experiments and bibliographical data to generate the interactome, which shows that p8 associates to proteins related to DNA transcription, repair and mRNA processing. This supports the hypothesis that p8 is a multifunctional protein that interacts with different proteins in different cellular compartments to perform different functions. We cannot disregard the possibility that p8 could be mediating the import of protein complexes into the nucleus.

PROTEOMICS AND METABOLOMICS OF HUMAN ATHEROSCLEROTIC ARTERIES

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Fundación Jiménez Díaz

In the last years vascular proteomics has experienced an impressive development and the majority of the proteomic techniques have been applied to study the atherosclerotic lesions. Among the multiple types of samples that can be studied (tissue, cells, circulating cells, etc.) to gain insight on the atherothrombotic process, we have chosen the analysis of secretomes of human arteries, including coronary, mammary and radial arteries. The rationale for this selection is because, most probably, the secretome is the best source for the identification of potential novel biomarkers of atherosclerosis. Thus, we have analysed the secretome of human arteries using a novel protocol that avoids contaminant plasma proteins and permits the identification of the true secreted proteins. A comparative analysis of the secretomes from human coronary, mammary and radial arteries will be presented. In a further step, validation of the arterial origin of the proteins was performed, incubating arterial sections with isotopically labelled amino acids.

The SIMS-TOF analysis of human carotid arteries, in a group of diabetes type 2 patients, showing the distribution of several lipids within the artery wall, and their involvement in the inflammatory process, will be also presented.

PROTEOMICS IN OBESITY RESEARCH

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Obesity has emerged as one of the major global epidemics of the 21st century and is now reaching alarming proportions. Obese subjects have an increased morbidity and mortality, decreased quality of life, and a major risk of developing co-morbidities such as diabetes and insulin resistance. Obesity is a complex disease characterised by an increase in body fat mass resulting from an imbalance between energy intake and expenditure. Proteomics may be useful in unravelling the pathogenesis of obesity, since a combination of genetic predisposition and environmental factors account for its development.

Adipose tissue plays a major role in obesity, not only for its capacity to store and metabolise lipids, but also for its ability to act as an endocrine organ. Adipose tissue is a complex organ and contains several cell types, namely the adipocytes, and stromal fraction (macrophages, endothelial cells, vascular cells). We are currently investigating the alterations in the adipocyte and stromal fraction proteome of visceral adipose tissue. Tissue samples were obtained from obese subjects (body mass index, BMI >40 kg/m^2) undergoing bariatric surgery with different degrees of glucose tolerance (diabetic, glucose intolerant and normal) and lean subjects (BMI 20-25 kg/m^2). Protein extracts were analysed by DIGE, and proteins identified by MS/MS. Candidate proteins in the adipocyte fraction overexpressed in obesity include carboxylesterase, GRP78, transketolase, catalase; and hsp60, UQCRC1 and perilipin were down-regulated.

Target identification of the potential anti-obesity agent sodium tungstate has also been investigated using 2D and DIGE. Oral administration of tungstate reduced body weight gain and adiposity, and improved dyslipemia and insulin resistance in cafeteria diet-induced rats. Tungstate is currently undergoing phase II clinical trials. In white adipose tissue we found that tungstate treatment reversed the expression changes of 70% of the proteins modified in obesity and was able to modulate cellular structure, metabolism, redox, and signalling processes. In brown adipose tissue, tungstate modulates redox processes and increases energy dissipation through uncoupling and up-regulation of PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1α).

The work presented here was mainly done at the Laboratory of Diabetes and Obesity, IDIBAPS, Hospital Clínic de Barcelona, Catalonia, Spain.

USING PROTEOMICS TO UNRAVEL THE MOLECULAR PATHWAY OF SPARC-MEDIATED TUMORIGENICITY

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SPARC is a glycoprotein from the extracellular matrix normally expressed during development and in wound healing. SPARC is also overexpressed in different tumors, in association with tumor progression. We have showed that downregulation of SPARC expression by antisense and RNAi techniques in human melanoma cells abolished tumorigenicity in an in vivo immunodeficient murine model, through still not clear molecular mechanisms. In the pursuit of molecular mediators of SPARC activity that may explain its role in tumor progression, we have performed a proteomic analysis of proteins secreted by L2F6 clone cells (MEL-LES human melanoma cells with RNAi-mediated inhibition of SPARC expression) and compared it to its control cell line LBLAST. Overall, around 12% of detected spots in conditioned media were significantly up- or down-regulated by changes in expression levels of SPARC. After identification of differential spots using MALDI-TOF/TOF, a selected group of these proteins was chosen for technical, biological and functional validation. Differences in these proteins were confirmed not only in the aforementioned cells but also using transient (i.e. adenoviral) restoration of SPARC expression on MEL-LES cells. Most interestingly, several of the validated proteins are well-known mediators of tumor progression but were not previously related to SPARC. In particular, we have collected experimental evidence that confirm the role of SPARC as an important inductor of proteins involved in tumor invasion. Our results constitute the first evidence that SPARC and these proteins may participate in a single molecular network that leads to tumor progression.

DETECTION OF NOVEL BIOMARKERS OF LIVER CIRRHOSIS BY HIGH PERFORMANCE PROTEOMICS

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Since more than 10 years, the word PROTEOME catches the attention of more and more researchers in the life science field. At about the same time the term high throughput proteome analysis came up with the intention to analyse all proteins in a complex protein mixture in parallel. Thus, a huge amount of data can be produced from a single sample and the following analysis and validation becomes the time limiting step. However, the limited number of available biomarkers for diagnosis, status of the disease, therapy control and prediction of the course of the disease demands for new efforts in finding new ones. Especially, proteomics raises high expectations in finding new and reliable biomarker for human diseases.

Hepatic cirrhosis is a life-threatening disease arising from different chronic liver disorders. Major causes for hepatic cirrhosis are chronic hepatitis B&C infection or abuse of alcohol. Chronic hepatitis C causes at least ~20% developing liver cirrhosis within 40 years. To date only liver biopsy allows a reliable evaluation of the course of hepatitis C by grading inflammation and staging fibrosis, and thus serum biomarkers for hepatic fibrosis with high sensitivity and specificity are needed.

In order to identify new candidate biomarkers for hepatic fibrosis, we performed a proteomic approach of microdissected cirrhotic septa and liver parenchyma cells. In cirrhotic septa we detected an increasing expression of cell structure associated proteins including actin, tropomyosin, calponin, transgelin and human microfibril associated glycoprotein 4 (MFAP-4). The expression of tropomyosin, transgelin and MFAP-4, an extracellular matrix associated protein, were further evaluated by immunohistochemistry. Tropomyosin and microfibril associated glycoprotein 4 demonstrated high serum levels in patients with hepatic cirrhosis of different etiologies. A quantitative analysis of MFAP-4 serum levels in a large number of patients (n=130) revealed MFAP-4 as novel candidate biomarker with high diagnostic accuracy for prediction of non-diseased liver vs cirrhosis as well as stage 0 vs stage 4 fibrosis.

Thus, **high performance proteomics** is the basic principle for reliable results which allows us to discover new biomarker candidates for liver cirrhosis using minute amounts of patients' material. How to reach this goal will be presented in the lecture.

Mölleken C, Sitek B, Henkel C, Poschmann G, Sipos B, Wiese S, Warscheid B, Broelsch C, Reiser M, Friedman SL, Tornøe I, Schlosser A, Klöppel G, Schmiegel W, **Meyer HE**, Holmskov U, und Stühler K (2009) Detection of novel biomarkers of liver cirrhosis by proteomic analysis, Hepatology, in press

Meyer HE and Stühler K. High-performance Proteomics as a Tool in Biomarker Discovery. Proteomics. 2007 Sep 24;7(S1):18-26.

Sitek B, Lüttges J, Marcus K, Klöppel G, Schmiegel W, **Meyer HE**, Hahn SA, Stühler K. Application of fluorescence difference gel electrophoresis saturation labelling for the analysis of microdissected precursor lesions of pancreatic ductal adenocarcinoma. Proteomics. 2005 Jul;5(10):2665-79.

APPLICATION OF A NOVEL STATISTICAL MODEL FOR QUANTITATIVE PROTEOMICS BY ^{18}O LABELING TO THE STUDY OF VEGF-INDUCED ANGIOGENESIS IN VASCULAR ENDOTHELIAL CELLS

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Quantitative proteomics, which may be defined as the study of global changes in the expression level of proteins, is a field that has experimented a great development in the last years. While a great effort has been devoted to the development of bioinformatics tools for automated analysis of MS data and calculation of peptide ratios for quantitative proteomics approaches using each one of the several isotope labeling strategies currently available, existing analytical methods for the statistical determination of significant expression changes are scarce. In fact**,** no specific statistical models have still been proposed to deal with data produced by enzymatic $^{16}O/^{18}O$ labeling.

We have developed a hierarchical, random-effects model including four different sources of variance at the spectrum-fitting, scan, peptide and protein levels. To validate our statistical model, we have performed a large-scale null-hypothesis experiment using the $^{16}O/^{18}O$ labeling technique on the proteome of human umbilical vein endothelial cells (HUVEC), by comparing two identical proteome extracts. Among more than 1,200, 20 proteins would have been considered as false expression changes at a FDR of 5% by applying conventional models based on the normality assumption. However, only 1 expression change was detected using the new random-effects model.

The new method was applied to the study of molecular mechanisms underlying angiogenesis in endothelium. Expression changes in the protein profile of HUVEC in culture in response to the pro-angiogenic factor VEGF were analyzed after 4- and 8-h incubation. About 2,000 proteins were identified in each experiment, among which we were able to quantify about 1,000 proteins from which 32 and 46 proteins were found to be differentially expressed, respectively. These changes included proteins implicated in protein binding, metabolism, transport and response to external stimuli. The consistency of the changes observed at 4h was confirmed by a replica at a smaller scale and further validated by Western blot analysis of selected proteins (annexin A1, reticulocalbin and triose-phosphate-isomerase). The expression pattern clearly shows two distinct populations of changing proteins indicating that the angiogenesis is a biphasic process that reflects a specific short-term response of endothelial cells to VEGF.

TOP-DOWN AND BOTTOM-UP QUANTITATIVE PROTEOMIC APPROACHES TO CHARACTERIZE THE DEVELOPMENT OF GRAPE BERRY TISSUES

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Grapevine (*Vitis vinifera*) is one of the economically most important fruit crops. The berry components that compose the final quality traits of both table and wine grapes, such as sugars, acids, flavours, anthocyanins, tannins, etc., are synthesized or accumulated along the different grape berry development stages. Thus the proteomic profiles along berry development have to correlate with the biochemical and physiological changes known to occur in the grape berries. The grape berry development has been characterized to some extent at biochemical and molecular levels, and semi-quantitative proteomic approaches have been undertaken to better characterize and understand this biological process of agro-economical relevance.

We have taken advantage of top-down (DIGE) and bottom-up (iTRAQ) quantitative proteomic approaches to characterize both large and subtle, but statistically significant quantitative changes in proteins along the grape berry development. In pre-veraison stages the pericarp was analyzed as a single tissue, while from veraison to full ripening the pericarp was divided into flesh and skin and analyzed separately. A suite of bioinformatic and statistical tools were used to group proteins into abundance patterns, to rank proteins according to abundance levels and to determine the ontologies associated to each cluster and each developmental stage.

Results correlate quite well with known physiological, biochemical and molecular changes, thus giving a proof of concept of our proteomic analysis. Then new proteins not previously associated to developmental stages could be described.

PROTEOMIC IDENTIFICATION OF S-NITROSYLATED PROTEINS IN *ARABIDOPSIS THALIANA* IN RESPONSE TO PATHOGEN INFECTION

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Nitric oxide (NO) is a highly reactive gas produced by plants under normal growth conditions and under stress situations. The current knowledge on NO-dependent processes illustrates that this molecule can directly influence the activity of target proteins through reversible S-nitrosylation of cysteine thiols. In particular NO plays a crucial role as physiological mediator in plant resistance to pathogens by triggering hypersensitive resistance-associated cell death and by contributing to the local and systemic induction of defence genes

The very transitory nature of this posttranslational modification constitutes an important redox-based regulation mechanism for many proteins, but the technical limitations in characterizing this modification have delayed its study. In order to dissect the NO-signaling pathways during the plant defence responses it is necessary to identify the target proteins and the specific cysteine residues involved. For that purpose we have performed a proteomic identification of S-nitrosylated proteins in *Arabidopsis thaliana* upon infection with the bacteria *Pseudomonas syringae*.

We have used the "biotin switch method" that converts unstable S-nitrosylated cysteines to stably labeled biotinylated cysteines. Afterwards, previously S-nitrosylated proteins can be detected by immunoblot analysis, or further purified by affinity chromatography and identified by means of proteomic methodology using HPLC coupled to a LTQ mass spectrometer.

This approach allowed us to identify an extensive list of proteins from *A. thaliana* cell suspension cultures and leaves in control, GSNO-treated and in *P. syringae*-challenged samples that represent candidates for NO-targets. Among them are proteins involved in defence-and stress-related responses, redox-related proteins, cytoskeleton proteins,

metabolic enzymes and signalling/regulating proteins. In parallel, and in collaboration with other research groups –"the nitrosoteam"– we are developing a methodology to directly identify the modified residues by incorporating an additional digestion step previous to the neutravidin purification. By identifying the NO-targets we hope to get insights into the physiological functions of protein S-nitrosylation during plant defence responses.

USING DM43 AND DM64, TWO ANTITOXINS FROM DIDELPHIDAE, TO STUDY THE SNAKE VENOM SUB-PROTEOMES

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Snake venoms are complex mixtures of proteins and peptides with different biological activities, many of them very toxic. Some animals, including the opossum *Didelphis auritas*, are resistant to snake venoms due to the presence of neutralizing factors in their blood. Two natural inhibitors have been isolated from opossum serum, DM43 and DM64 with antihaemorrhagic and antimyotoxic activities, respectively. They inhibit snake venom metalloproteinases and myotoxins through non-covalent complex formation with these proteins. In this study, we have used DM43, DM64 and proteomic techniques to explore snake venom subproteomes. Several venoms were chromatographed through an affinity column containing immobilized DM43 or DM64. Bound and unbound fractions were analyzed either by SDS-PAGE and/or 2D-PAGE, followed by identification using MALDI-TOF/TOF mass spectrometry. Following this methodology, we could classify venoms from *Bothrops alternatus*, *B. asper*, *B. atrox*, *B. insularis*, *B. jararaca*, *B. jararacussu*, *B. moojeni*, *B. neuwiedi*, *Crotalus adamanteus*, *C. atrox*, *C. durissus terrificus*, L*achesis muta muta* and *Naja naja atra* according to their relative content of metalloproteinases (PI, PIII and/or their fragments) using DM43. Venom fractions not bound to DM43 column were equally analyzed and were composed basically of serine proteases, phospholipase A_2, C-type lectins, L-amino acid oxidases, nerve growth factor, and/or some metalloproteinases and unidentified spots. On the other hand, snake venoms of *B. asper*, *B. jararacussu*, *B. neuwiedi*, *B. moojeni* and *B. jararaca* were analyzed using DM64 affinity column. Bound venom fractions were composed basically of phospholipase A_2, serine proteinase and C- type lectins. So far, just not bound fractions of venoms of *B. asper* and *B. jararaca* were analyzed and were composed, mainly by metalloproteinases and some cysteine-rich secretory protein (CRISP), phospholipase A_2 and serine proteinases. Studied venoms presented important proteic variability, with frequent detection of multiple forms of the same protein and several members of the same protein family. DM43 and DM64 can be very useful as a tool for analyze the complexity of snake venoms and in the search for new molecules, and they can also be used to better understand the mechanism of action of these toxins in the envenomation. DM43 and DM64 affinity chromatography associated with proteomic techniques showed to be useful tools to separate and identify proteins from snake venoms.

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NEVER GIVE UP YOUR DREAMS

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In 1950, when I was 8 years old, Denmark was sending a research vessel around the world to discover the unknown world of the oceans. I followed this expedition and was so fascinated that I decided that when I was grown up my dream would be to be a scientist and to go on expeditions to discover the unknown world. Later after high school, I realized that the unknown world also included the world of molecules and especially those active in living organisms. After finishing an education in chemical engineering I turned to protein chemistry and molecular biology and at the same time became fascinated by mass spectrometry as an analytical technique. Mass spectrometric analysis of proteins was at that time, in the late 1960's, considered utopia. Proteins by definition could not be brought to the gas phase in a mass spectrometer. Nevertheless, I believed in it. I loved proteins and mass spectrometry, and in spite of all the difficulties in the first decades, I maintained the dream that mass spectrometry could be a key analytical technique in protein studies. As you all know, this dream has been fulfilled. New mass spectrometric tools have been developed, and we can now in proteomics experiments identify and characterize numerous proteins in a single day using mass spectrometric analyses. The next level in proteomics is to localize the proteins in living organisms in space and time. This is often achieved by generating fusion proteins between the protein of interest and green fluorescent protein (GFP) followed by time-resolved confocal microscopy. Here also new tools are needed and especially a greater selection of fluorescent proteins. In 2006-2007 Denmark again sent a research vessel around the world. That allowed me to fulfill my childhood dream: To go on an expedition and search for new fluorescent proteins by night diving in tropical coral reefs. My lecture will take you from the early stages of protein mass spectrometry to the present state of the art and on an expedition to discover new fluorescent proteins in marine organisms and to their characterization using proteomics techniques.

HUPO Initiatives

THE INSIGHT INTO THE HUMAN LIVER PROTEOME

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The Human Liver Proteome Project (HLPP) is a large-scale international collaborative initiative now focusing on the proteomic analysis of the human liver in its different conditions. In the past five years, we have set up standard operating procedures, optimized the strategies of the proteomic analysis and investigated the proteomes of the Chinese fetal liver tissues, French adult liver tissues and Chinese adult liver tissues in turn.

Recently, after analyzing the proteome from the Chinese adult liver tissues, which comprised of 6,788 identified proteins in 95% confidence with at least two peptides matching, we turn to the proteomic analysis of the different organelles as well as the different kinds of liver cells. We succeeded in identifying 5882 proteins with 2 peptides or more in 95% confidence from plasma membrane, nucleus, cytoplasm, mitochondria, rough endoplasmic reticulum and smooth endoplasmic reticulum in human liver cells, which will be compared with those proteins identified from the human liver tissues. As well, 4969 proteins were identified from the same organelles of the mouse liver. Furthermore, we optimized the approach of the extracorporeal liver perfusion and cell sorting and obtained the purified mouse hepatic parenchymal cells, in which, 2216 proteins were identified with at least 2 peptides in 99% confidence.

In order to effectively enrich the low-abundance proteins, especially those with modifications, we developed several new methods, which were obviously more useful, such as dephosphorylation of phosphopeptides by cerium oxide and specific capture of phosphopeptides on MALDI mass spectrometry. In addition, on the basis of our past results of the protein-protein interactions, we expanded the number of the baits. So far, we totally found 1732 unique protein-protein interactions by screening the human liver cDNA library with the yeast two hybrid. Meanwhile, a protein array of about 5000 unique liver ORFs was screened by yeast two hybrid mating method and 1632 protein-protein interactions were discovered. Of them, 1270 interactions were verified by yeast retransformation assay.

Key words: Human Liver Proteome/ organelle/ protein-protein interaction

CHARACTERISATION OF THE AUTOIMMUNE ANTIBODY REPERTOIRE OF PARKINSON'S DISEASE PATIENTS BY SYSTEMATIC SCREENING OF PROTEIN ARRAYS

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The diagnosis of Parkinson disease (PD), a degenerative disorder of the central nervous system, relies on the recognition of clinical symptoms appearing in a late stage of pathogenesis. At this stage of PD only the treatment of the clinical symptoms is feasible, a therapy that is capable to stop the progression of the disease is not available. Therefore, the identification of molecular markers allowing an early diagnosis of PD is urgently needed.

Following the hypothesis that the progression in Parkinson's disease may be caused by a chronic autoimmune response, we have characterized the autoimmune antibody repertoire of PD patients by serum hybridization of protein arrays in order to determine the potential of selected autoimmune antibodies to act as molecular markers for PD.

We utilize the UNIarray® technology that combines the advantages of protein arrays with the large-scale expression of proteins from existing cDNA libraries to screen 20 serum samples of PD patients against 10 000 heterologously expressed human proteins arranged on a macroarray. This approach resulted in the identification of 150 autoantigens that represent putative disease markers. First results indicate that such a diagnostic protein array allows differentiating PD patients from non-affected ones.

To validate these molecular markers an iterative screening procedure was developed allowing a stepwise reduction of candidate proteins with concomitant increase of quality and purity. We are currently analysing a larger cohort of PD patients and non-PD patients in order to define a smaller subset of marker proteins that allows the set up of a blood-based *in vitro* diagnostic assay.

ANNOTATING THE HUMAN PROTEOME

Rolf Apweiler
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The completion of the human genome has shifted the attention from deciphering the sequence to the identification and characterization of the encoded components. The identification and functional annotation of the proteome is here of special interest and reaches from the identification of genes and transcripts functional information on many human proteins. Public domain databases are required to manage and collate this information and present it to the user community in both a human and machine readable manner. My talk will concentrate on the current status of annotating the human proteome, achievements and shortcomings, and future prospects towards a more complete characterization of human gene products, especially in the light of large-scale sequencing projetcs aimed on studying human variation.

CLINICAL CARDIOVASCULAR PROTEOMICS AND METABOLOMICS NETWORK
Part I. Biospecimen Repository (2009-2014)

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Cardiovascular disease is the leading cause of death globally. In the past few decades, major improvements have been made in treating some types of cardiovascular disease. In the case of coronary heart disease, for example, therapies such as the administration of statins and the insertion of stents have reduced death rates. However, new treatment options are urgently needed for all types of cardiovascular disease.

Clinical proteomics and metabolomics are rapidly growing fields and refer to the application of technologies to identify disease-related alterations and to develop molecular signatures for disease processes. Recent advances in mass spectrometry instrumentation, protein and peptide separation methods, and informatics tools have fueled the rapid growth of clinical proteomics and metabolomics. However, the integration of proteomics and metabolomics into clinical needs is not trivial and requires a well organized infrastructure with close collaborations among worldwide scientists (analytical chemists, statisticians, medical informaticians and clinical researchers, etc).

In the International Cardiovascular BioBank for the CVI-HUPO, we aim to connect many different types of biological samples (eg. tissue samples, DNA, urine, other body fluidics and blood) and information (eg. health records, diet and lifestyle information, family history of disease, gender, age, ethnicity-haplotypes, proteometabolomic profilings, etc). This Initiative promises to be an essential tool for translating new biomedical knowledge into new clinical practices, diagnostic techniques and preventive. Toward this goal we are implementing the CVI-HUPO e-BioRepository Information Management System (CVI-HUPO-e-BRIMS), which is designed to integrate research data originating from many international sources (disease-based biobanks and population biobanks), allowing handle data that are continually updated. I will outline the current status of this initiative, describing its main components and participants.

Short Oral Communications

METASTASIS-ASSOCIATED C4.4A, A GPI-ANCHORED PROTEIN CLEAVED BY ADAM10 AND ADAM17

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Metalloproteases play a complex role in tumor progression. Proteomic approaches to identify the array of substrates of a given metalloprotease (degradome), can help to unveil its role in tumor growth and metastasis. Here we describe a proteomic screening to compare the proteins secreted by MCF7 cells, derived from an invasive mammary tumor, and the same cells expressing shRNAs that knock down ADAM10 or -17. Cells grown in conditions were the protease is expressed or knocked down were differentially labeled, by incorporating isotopically labeled amino acids (SILAC). Glycoproteins from the conditioned media of each of the two cell cultures were purified by affinity chromatography. The samples were then pooled and run on a 1D SDS-PAGE gel. The gel lane was then cut into 20 fractions and digested. Each fraction was analyzed by RP-LC-MS/MS. Protein identification and quantification of relative abundances was performed using WARP-LC, an integrated software platform for LC-MS/MS workflows.

A number of known substrates of both proteases were identified as such in the analysis, showing the expected decrease of the shed extracellular domain abundance in the medium upon knock down of the protease. Thus, the cell-adhesion proteins E-cadherin and Desmoglein-2, or the PTP receptor-type k, were identified as substrates of ADAM10. Fractalkine and NCAML1 were found as substrates of ADAM17.

In addition several new candidate substrates of both proteases were identified. Among them, the GPI-anchored protein C4.4A, was identified and further validated as substrate of both ADAM10 and ADAM17 proteases. According to the identified peptides, both proteases cleave this protein close to the juxtamembrane region, releasing a soluble form devoid of the GPI-anchor. C4.4A protein, homologous to the urokinase-type plasminogen activator receptor, has been related to tumor invasion and metastasis. Cleavage of this protein by ADAMs constitutes a previously unknown level of regulation of its function.

LABEL-FREE QUANTIFICATION BASED ON DATA INDEPENDENT ACQUISITION MASS SPECTROMETRY

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The quantification of differentially expressed proteins is a key area where proteomics technology has made great progress over the last years. The development of a data independent, alternate scanning acquisition, where no precursor is selected and low and high collision energy data is alternatively acquired, affords accurate mass measurements used for both protein identification and label-free based quantification. Furthermore, beyond new experimental approaches, bioinformatics software is in constant development in order to obtain more robust tools for data processing, qualitative analysis and quantification. Recently, an LC-MS based absolute quantification method based on the comparison of the 3 most intense peptides of each protein with a known protein standard has been developed. In this study we have tested this approach using known amounts of protein standards at different ratios. 4 proteins have been spiked in an *E.coli* lysate background and their absolute and relative protein abundance measured. Furthermore, we have used the same experimental approach to analyse the protein expression pattern of EGF treated and untreated human MDA-MB-468 breast cancer cells. Finally, the same experimental data has been analysed with a probabilistic based quantification algorithm, where relative quantification of all peptides and proteins is performed. The obtained results with both quantification methods have been compared.

IDENTIFICATION AND QUANTIFICATION OF PROTEINS FROM *METHYLOPHAGA THIOOXIDANS* AND *METHYLOCELLA SILVESTRIS* USING LABEL-FREE LC/MS

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An alternative scanning LCMS approach was used to conclusively and stringently identify *Methylophaga thiooxidans* and *Methylocella Silvestris* proteins from tryptic digests of whole cell lysates. The system comprised of an LC-MS^E acquisition mode and novel database search algorithm 'Ion Accounting' allowed the physiochemical characteristics of fragmented tryptic peptides to be used to aid stringent protein identification. Simultaneous absolute quantification of the identified proteins showed detection of proteins present over 3 orders of magnitude.

The novel data-independent analysis mode (MS^E) is employed on the SYNAPT MS mass spectrometer enabling precursor and fragment ions from the tryptic digest to be analyzed simultaneously. The inclusion of a spike of phosphorylase B tryptic digest of a known concentration allows the software not only to identify the components of the complex mixture but also to calculate the absolute amounts of identified proteins[1]. MS^E provides accurate mass measurements of all detectable precursor and product ions. Chromatographic alignment of precursor and product ion data reduces miss-assignment of product ions to parent ions of similar mass or retention time. Protein identifications are confirmed using the parent and product ion accurate mass and additional peptide physiochemical properties used by the Ion Accounting algorithm.

Quantitative measurement of low energy precursor ions is facilitated by the data independent analysis and an increase in dynamic range is observed as the limitations posed by conventional Data Directed Analysis MS/MS duty cycle are negated. In the example of *Methylophaga thiooxidans* tryptic digest, 3 orders of magnitude of protein concentration can be detected, ranging from 0.01ng to 74ng on column. The total column loading can also be calculated using this methodology – in this case a total column loading of 367µg allowed the confident identification of 309 proteins under stringent conditions. A false positive rate of 2.27% was calculated for this experiment. Previous MS analysis of the *Methylophaga thiooxidans*, which is currently considered to be a poorly characterized bacterium, using SCX/RP and conventional analysis had returned only 81 protein identifications. Approximately half of the identifications made using conventional MS methods were made with only 1 peptide per protein. In contrast

to this, on average 9 peptides per protein were identified using the data independent methodology combined with Ion Accounting. Triplicate injections were undertaken to allow filtering of protein identification on replication and to give statistical information on absolute quantification calculations. The result of this type of analysis in practical terms is the detection of higher numbers of proteins with additional peptides per protein identification, giving increased confidence in the protein assignment. The improvement in protein identification becomes more apparent with increasing complexity of analytes. Experiments are underway to investigate both *Methylophaga thiooxidans* and *Methylocella Silvestris* proteomes under differential growth conditions using label free quantification. Relative protein quantification obtained using this methodology will be compared to previous results obtained using iTRAQ labeled techniques.

CHLOROACETYLATION OF CYCLOSTREPTIN INFLUENCES ITS INTERACTION WITH TUBULIN

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Cyclostreptin (Cs) is a natural product from Streptomyces sp.9885 that irreversibly stabilizes cellular microtubules by covalent binding to tubulin, causes cell cycle arrest, evades drug resistance in MDR tumor cells and inhibits paclitaxel-binding to microtubules. In a previous work (1) we demonstrated that cyclostreptin irreversibly binds to β-tubulin through Thr220 and Asn228 (the type-I pore binding site). To gain further information about this binding site, two reactive derivatives of cyclostreptin were synthesized and studied.

In this work we characterize the interaction binding sites of monochloroacetylated cyclostreptin in position 15 (15CA-Cs), or in position 17 (17CA-Cs), which were also cytotoxic in MDR cells and accumulate cells in G2+M phase of the cell cycle in the same way as cyclostreptin, within microtubules. As performed in (1), we have used a hybrid triple-quadrupole mass spectrometer to analyze the filtered precursor ions by the detection of AC-Cs-derived fragments in the third quadrupole. We observed a change in the specificity of CA-Cs-interacting sites within the tubulin molecule both in the formed microtubules and in unpolymerized tubulin. Although the tubulin interacting-domain was the same we had previously found (219-LTTPTYGDLNHLVSATMSGVTTCLR-243), neither Thr220, nor Asn228 residues were CA-Cs-labeled in microtubules, while the CA-Cs binding site was detected in Cys241. However, in the dimeric, unpolymerized tubulin, we detected the Thr220 interacting site with CA-Cs as well.

Interpretation of the reaction mechanisms of the CA-CS derivatives with Thr and Cys side chains in the type-I pore binding site of microtubules is discussed.

1. Buey RM, Calvo E, Barasoain I, Pineda O *et al.* Cyclostreptin binds covalently to microtubule pores and lumenal taxoid binding sites. *Nature Chemical Biology* 3, 117-125 (2007).

QUANTITATIVE ANALYSIS OF PROTEIN GLYCATION IN CLINICAL SAMPLES

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Non-enzymatic glycation is one of the post-translational modifications (PTMs) less frequently studied. An innovative approach for quantitative analysis of glycated proteins (GP) in clinical samples (serum, plasma and red blood cells) is here presented. It is based on relative quantitation of samples between two glycation states by differential labeling with light and heavy glucose ($^{12}C_6$- and $^{13}C_6$-glucose). Then, both sets of samples are pooled to carry out analysis by a shotgun proteomics workflow. This consists of in-solution digestion with endoproteinase Glu-C, selective enrichment of glycated peptides by boronate affinity chromatography (BAC), and analysis by RP-LC-ESI-MS/MS with an Orbitrap® mass analyzer (MS2 HCD higher energy collisional dissociation and data-dependent MS3 operation modes). A similar labeling efficiency has been observed with both isotopic glucose forms under the same operating conditions, which is essential for the applicability of the method. The identification and quantitation of GP is possible as the resulting peptides provide doublet signals in MS (labeling with light and heavy glucose) with a mass shift of +6, +3 or +2 Da depending on the peptide charge. With this methodology, it was possible to identify different GP such as serum albumin (with the five preferred glycation sites), immunoglobulins, haptoglobin, serotransferrin, complement C-3 and C-8 precursors, α-2-HS-glycoprotein or apolipoprotein A-1. These proteins are representative targets to compare between samples with different glycation states. This approach has also been applied to the analysis of clinical samples after depletion of more concentrated proteins. Further research is focused on the capability of the method to monitor the concentration of GP as well as to predict new potential targets for glycation. This can be especially interesting because it could be applied with prognosis/diagnosis purposes linked to pathological disorders related to glycemic control.

NEW MICROFLUIDIC CHIP TARGETING PHOSPHOPROTEOMES

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Agilent Technologies, Inc.

Protein phosphorylation is one of the most important post-translational modification (PTM) events among mechanisms of regulating protein function in cells. Myriad biological processes, including cell proliferation, migration, and apoptosis involve phosphorylation steps. One of the major efforts in proteomics is devoted to the identification and understanding of phosphoproteomes in cells. Nevertheless, comprehensive identification of sites of protein phosphorylation remains a challenge, best left to experienced proteomics experts. In order to achieve selective enrichment of phosphorylated proteins and peptides most commonly used technologies are currently immobilized metal affinity chromatography (IMAC), anti-phosphotyrosine antibodies, and titanium dioxide prior to LC/MS (liquid chromatography and mass spectrometry) analysis. Recent advances in HPLC chip technology have created an environment to allow automation of such a workflow with increased ease of use and confidence of analysis. The new microfluidic chip is a re-usable HPLC nano-flow rate chip with titanium dioxide particles (TiO_2) based phosphopeptide enrichment. The chip is a multilayer polyimide laminate that contains an enrichment section with TiO_2 beads flanked on both sides with C18 reversed phase material. The 3 section sandwich is separated from each other by micro-fabricated frits. This enrichment section is connected to a reversed phase separation column ending in an integrated electro-spray tip by a micro valve in direct contact with the chip surface providing a zero dead volume high pressure seal. The chip is used with a HPLC-chip/MS instrumentation using the HPLC-chip cube interface combined with a Mass Spectrometer. The unique sandwich configuration of the enrichment section provides researchers three modes of peptide analysis: (1) standard peptide analysis, (2) phosphopeptide analysis only, and (3) combined peptide and phosphopeptide analysis. This approach will offer non-expert proteomics researchers a reliable way in phophoproteome analysis.

STORING, REPORTING AND COMPARING PROTEOMICS EXPERIMENTS USING THE MIAPE GENERATOR TOOL

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Standardization of experimental proteomics protocols is one of the main objectives of the National Institute for Proteomics (ProteoRed). Several multicentric experiments as well as several participations in ABRF Proteomics Research Group (PRG) studies have been coordinated by ProteoRed with the purpose of comparing both results and experimental protocols applied to obtain these results. From these studies, we obtain conclusions that help to establish the best approach for a certain proteomic problem and to improve the application of new techniques to new proteomics challenges. However, the comparison between different experiments is not a trivial task if there is not a consensus data format.

The Human Proteome Organization's Proteomics Standards Initiative (HUPO PSI) is actively developing XML interchange formats to allow both the exchange and storage of such data, and also guidance modules (Minimum Information About a Proteomics Experiment. MIAPE), to report the use of proteomics techniques in electrophoresis and mass spectrometry-based experiments. ProteoRed has incorporated such reporting standards in the multicentric activities mentioned above, using MIAPE guidelines to report the experiments and therefore, facilitating the comparison between them.

With the aim of helping the implementation of such standard, we have developed a tool freely accessible through the ProteoRed web application at http://www.proteored.org, that generates and stores MIAPE compliant reports, that is, containing the minimum information required to report a proteomics experiment, both MIAPE Gel Electrophoresis (from sample to gel image acquisition), Gel Informatics (analysis of gel image), Mass Spectrometry (from sample to peak list generation) and MIAPE Spectrometry Informatics (analysis of these peak lists) experiments.

The "MIAPE generator tool" also allows to compare different stored MIAPE compliant reports, therefore, to compare different proteomics experiments, providing specific information about the same aspect from different experiments in a single table.

Several important proteomics journals are already asking for some minimal information required to publish a paper, based in the HUPO-PSI MIAPE guidelines. For that reason, using this tool, ProteoRed wants to provide to their costumers a MIAPE compliant report attached to the results as a new service quality label.

PROTEOPATHOGEN, A PROTEIN DATABASE TO STUDY HOST-PATHOGEN INTERACTION

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Proteopathogen is database that compiles proteomics data from experiments involving the interaction of pathogenic yeasts with immune system cells in the host. Particularly the stored data is retrieved from experiments related to the interaction of the opportunistic pathogenic fungus *Candida albicans* with murine macrophages and spans proteomics workflows from description of the experimental approaches leading to sample preparation to mass spectrometry settings and identification supporting peptides. Through its interface website, hosted by an Apache web server on a Unix platform, proteopathogen is easily queriable and the user can efficiently browse through all the stored data, improving therefore the quality of eventual analysis of mass spectrometry results. Queries can be performed by supplying one of the multiple accepted identifiers or free text, and possible results comprise gene ontology information, scientific literature references, different mass spectrometry settings and lists of peptides supporting the identification. As an add-on functionality, summary tables can be downloaded accordingly to the result. Access is public at http://marbore.dacya.ucm.es/proteopathogen.

Trabajo subvencionado por los Proyectos BIO 01989-2006 (CICYT), Deremicrobiana (Comunidad de Madrid) and REIPI (FIS, ISCIII)

HALOLINK™ PROTEIN ARRAYS FOR FUNCTIONAL ANALYSIS OF PROTEINS

Patricia Bresnahan

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Detection of protein interactions and function are key for dentifying protein networks and understanding cellular processes. With miniaturization and multiplexing capabilities, protein arrays have found wide application in deciphering protein interaction networks. Here we present an integrated approach for creating protein arrays that combines *in-vitro* protein expression of a probe protein with HaloTag® immobilization technology. The method allows for rapid and covalent capture of HaloTag fusion proteins onto a glass slide surface directly from complex protein mixtures (e.g. cell lysates) without any prior purification. Multiple functional analysis of proteins of interest may be performed in parallel with these arrays. Examples of this analysis are reported, including end-point and kinetic protein-protein interactions, protein-DNA interactions, and modulation of protein interactions with agonists and antagonists.

HIGH-MASS MALDI TOF MASS SPECTROMETRY AND CHEMICAL CROSS-LINKING FOR INTERACTION ANALYSIS

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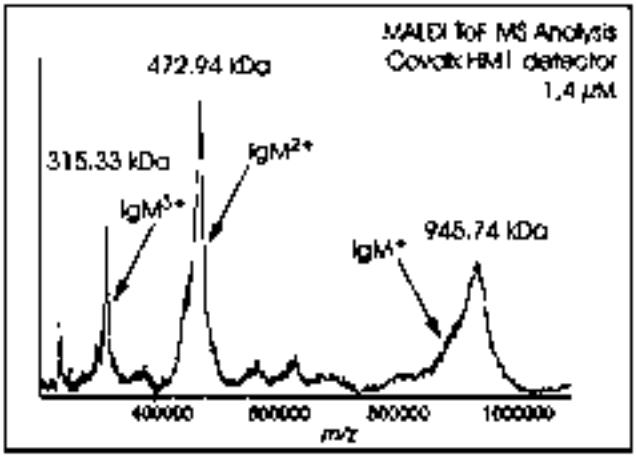
The analysis of intact protein complexes by mass spectrometry is still challenging. Here we present an approach based on high-mass MALDI ToF mass spectrometry and chemical cross-linking. To circumvent the problem of dissociation when using MALDI ionization, a specific cross-linking protocol has been developed to stabilize covalently the samples. To solve the problem of detection, we are using a specially developed high-mass detection system, allowing sub-µM detection up to 1000 kDa. The use of this methodology presents a number of advantages: Sensitivity (sub-µM), tolerance for samples impurity, speed. We will present with details the high-mass technology used and show comparison spectra with MCP detection, the technology used in most of standard MALDI ToF instruments. We will also present examples of applications of this methodology in the field of protein complex analysis (intact protein complexes ranging from 40 to 1000 kDa), antibody characterization (Interaction analysis, Sandwich assays, Epitope mapping), Therapeutic protein aggregates analysis and drug discovery.



High-Mass MALDI ToF MS analysis of IgM (945.74 kDa; 300 nM) using CovalX Hm1 high-mass detection system

LECTIN-SUGAR INTERACTIONS DECIPHERED BY SPR-MS AND CREDEX-MS

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Interest in sugar-protein interactions has been rising significantly over the last decades. The role of these interactions in processes such as bacteria-host recognition, viral entry, fertilization and metastasis, justifies the search for powerful, nanosized analytical tools to study the corresponding mechanisms.

Here we report on two complementary analytical techniques that provide both quantitative and qualitative data of the interaction with high sensitivity, low sample consumption and without the requirement of sample labelling. On one hand, with surface plasmon resonance (SPR), kinetic and thermodynamic parameters of the interaction can be determined in real time. In this approach the sugar is immobilized on a chip surface through a tailor-made peptide module[1,2], the protein flown across and the resulting read-out enables both quantitation and kinetic analysis of the interaction. Subsequently, interacted material can be recovered under optimized conditions for mass spectrometric characterization.

On the other hand, a combination of proteolytic excision of protein-carbohydrate complexes and mass spectrometry (CREDEX-MS)[3] allows to identify the peptide motifs at the carbohydrate binding site. Here, the sugar is immobilized to a functionalized Sepharose column and the lectins passed through. After on-column digestion of the complex, sugar-bound peptides are eluted and identified by mass spectrometry[3].

In this presentation we will describe the combination of these two methodologies for sugar-protein interaction studies and demonstrate their applicability with several legume lectins that display different sugar specificities.

1. Vila-Perelló, M., Gutiérrez-Gallego, R., Andreu, D. Chembiochem 6, 1831-1838 (2005)
2. Jiménez-Castells, C., de la Torre, B.G., Gutiérrez-Gallego, R., Andreu, D. Bioorg. Med. Chem. Lett. 17, 5155-5158 (2007)
3. Przybylski M., Moise A., Svobodova E., Siebert H., Gabius H. J Pept. Sci. Supplement to Vol. 14, 40 (2008)

MULTIPLEX PROTEIN EXPRESSION PROFILING WITH PANORAMA™ ANTIBODY ARRAYS

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Antibody microarray is a high throughput proteomic technology that enables efficient and sensitive protein analysis thus accelerating protein profiling studies for biomarker discovery.

Panorama™ Antibody Arrays are kits that contain nitrocellulose-coated glass slides spotted with 84 to 725 antibodies. The series includes arrays containing antibodies specific for a large array of cell signaling proteins.

Protein extract samples from human, mouse, or rat cells and tissues can be assayed using these arrays. The extracts are labeled with Cy3 or Cy5 fluorescent dyes and then applied onto the array. After a short incubation and wash, the array is scanned and numerical values of the fluorescence intensity are obtained. Numerical values for each spot are normalized against that of antibodies to house-keeping gene proteins present on the array, to eliminate the effect of dye conjugation efficiency.

Many applications of the Panorama™ Ab Microarray family of products have been reported. They were employed in identifying proteins involved in differentiation of F9 embryonic mouse stem cells by retinoic acid and in the identification of proteins involved in extracellular and intracellular signaling components of the mammary adipose tissue and its interstitial fluid in high-risk breast cancer patients. Other applications were the identification of proteins involved in drug resistance (doxorubicin) in human breast cancer cells, and the identification of the cellular pathways involved in the maintenance of human embryonic stem cell pluripotency and viability. Antibody arrays are also useful in studying the effect of gene silencing by siRNA as demonstrated using LAP2β gene silencing as a model system. The antibody array was also used in protein/protein interaction studies to identify β-catenin binding proteins in NIH-3T3 cells. All array results were confirmed by using other immunochemical assays such as immunoblotting, immunoprecipitation or immunocytochemistry.

IDENTIFICATION OF REPLICATION-COMPETENT HSV-1 CGAL$^+$ STRAIN SIGNALLING TARGETS IN HUMAN HEPATOMA CELLS BY FUNCTIONAL ORGANELLE PROTEOMICS

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In the present work, we have attempted a comprehensive analysis of cytosolic and microsomal proteomes to elucidate the signalling pathways impaired in human hepatoma cells (Huh7) upon Herpes Simplex Virus Type 1 (HSV-1 Cgal$^+$) infection. Using a combination of Differential in Gel Electrophoresis (DIGE) and nanoLC-MS/MS, 18 spots corresponding to 16 unique deregulated cellular proteins were unambiguously identified, which are involved in the regulation of essential processes such as apoptosis, mRNA processing, cellular structure and integrity, signal transduction and Endoplasmic-Reticulum Associated Degradation (ERAD) pathway. Based on our proteomic data and additional functional studies target proteins were identified indicating a late activation of apoptotic pathways in Huh7 cells upon HSV-1 Cgal$^+$ infection. Additionally to changes on RuvB-like 2 and Bif-1, down-regulation of Erlin-2 suggests stimulation of Ca^{2+}-dependent apoptosis. Moreover, activation of the mitochondrial apoptotic pathway results from a time dependent multi-factorial impairment as inferred from the stepwise characterization of constitutive pro- and antiapoptotic factors. Activation of Serine-threonine protein phosphatase 2A (PP2A) was also found in Huh7 cells upon HSV-1 Cgal$^+$ infection. In addition, PP2A activation paralleled dephosphorylation and inactivation of downstream MAP kinase pathway (MEK1/2, ERK1/2) critical to cell survival, and activation of proapoptotic Bad by dephosphorylation of Ser112. Taken together, our results provide novel molecular information that contributes to define in detail the apoptotic mechanisms triggered by HSV-1 Cgal$^+$ in the host cell and lead to the implication of PP2A in the transduction of cell death signals and cell survival pathway arrest.

GENOMIC AND PROTEOMIC ANALYSES REVEAL A RELATIONSHIP BETWEEN WNT PATHWAY GENES, OXIDATIVE STRESS METABOLISM AND VASCULAR CALCIFICATION

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Introduction and Aims: Vascular calcification (VC) is a highly prevalent condition and one important cause of mortality in chronic kidney disease (CKD) patients. The aim of this study was to analyze the change of expression of genes and proteins that occurs in the development of VC.

Methods: Rats with 7/8 nephrectomy fed with high (0.9%) or normal (0.6%) phosphorus diet were used. Rats were sacrificed after 8, 16 and 20 weeks after surgery (5 animals per group). Serum biochemical parameters and bone mineral density in tibia were measured. Von Kossa staining of the aorta was carried out to detect the presence of VC. Differential gene and protein expression in aortas were assessed by gene expression microarrays and DIGE followed by MS and LC/MS-MS, respectively.

Results: Only the animals fed with high phosphorus during 20 weeks developed VC. Moreover, this group showed a significant decrease in renal function and bone mass, and a significant increase in serum P, iPTH and mortality. In the calcified aortas; at gene level, 3 secreted related frizzled proteins genes (SFRPs), inhibitors of the wnt pathway, involved in bone formation, were up-regulated. At protein level, 40% of the proteins with significant changes in their expression belong to the oxidative stress metabolism. Muscle related proteins were down regulated.

Conclusions: The SFRPs and the oxidative stress metabolism seem to play a role in the development of VC and it may play also a role in the reduction of bone mass.

BIOCORE STUDY ("BIOMARKERS OF CORONARY EVENTS"): FROM SAMPLING TO DISCOVERY OF PLASMA BIOMARKERS BY SELDI-TOF MS AND 2DE

Meilhac O

Inserm unit 698 "Hemostasis, bio-engineering, cardiovascular remodeling"

In spite of important therapeutic advances during the last 20 years, coronary atherothrombotic complications are and will remain the first cause of death all over the world. Acute coronary syndromes (ACS) are most of the time unpredictable and can lead to sudden death before any medical treatment. The development of new strategies for the screening of patients susceptible to develop an ACS is thus of major interest.

We hypothesize that coronary artery disease, in its stable and unstable forms, is associated with modifications of the concentrations of various circulating proteins (circulating proteome), which could be assessed using a new method for pre-treatment of plasma before differential proteomic analysis.

Every step from the blood sampling to the proteomic analysis was strictly normalized (nature of the tubes used, centrifugation time and speed, conditions of storage etc.)

Three groups of 30 patients have been recruited in this study: non-ST elevation myocardial infarction (group 1), stable angina (group 2), angiographically normal coronary arteries without extra-coronary atherosclerosis (group 3). Five milliliters of plasma from each patient have been equalized; this methodology is based on a solid-phase ligand library of hexapeptides which enables a potential ligand for every protein in the biological sample, with a limited capacity of binding for abundant proteins, thus allowing enrichment in low abundance proteins/peptides (Proteominer^{TM}, Biorad). Various strategies of elution have been used in order to increase the number of peaks/spots detected respectively by SELDI-TOF mass spectrometry and by 2D-electrophoresis. Several differential peaks are currently being identified.

The new biomarkers discovered by proteomics will require further validation, using more straightforward assays (eg, ELISA), in case-control cohorts and in prospective cohorts which will assess their screening, prognostic and therapeutic values in coronary artery disease.

COMBINED PROTEOMIC AND TRANSCRIPTOMIC ANALYSIS IDENTIFIES DIFFERENTIALLY EXPRESSED PATHWAYS ASSOCIATED TO *PINUS RADIATA* NEEDLE MATURATION

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Needle differentiation is a very complex process which leads to the formation of a mature photosynthetic organ from pluripotent needle primordia. We characterized and compared the proteome and transcriptome of immature needles (1 month old) and fully developed needles (12 months old) of *Pinus radiata* D. Don to characterize metabolic pathways implied in this process. After differential 2-DE (pH 5-8, 18 cm, CBB staining) 884 spots were analyzed defining 280 as differential (T-Test, Bonferroni correction for α=0.05). Out these 280 spots, 134 were confidently indentified by LC-ESI-Q-TRAP-MS employing a custom viridiplantae protein database (Applied Biosystems) and Paragon algorithm present in ProteinPilot Software (Applied Biosystems). Transcriptomic analyses were performed in three stages: 1. Two suppressive subtractive hybridization (SSH) libraries enriched with differential cDNAs were constructed for immature and mature needles. Libraries were constituted by 576 clones each, with 198 and 144 different sequences for immature and mature scions, respectively. 2. The differential expression of subtracted cDNAs was tested by hybridization over custom macroarrays (13 x 9 cm, 384 probes). 3. The expression level of 15 genes was determined by real time RT-PCR to validate macroarray results. A joint data analysis of proteomic and transcriptomic results was also performed to have a combined perspective which gives us a broad view over differentially expressed pathways associated to needle maturation. Energy metabolism pathways, with photosynthetic and oxidative phosphorylation related proteins, were overexpressed in mature needles. Aminoacid metabolism, transcription and translation pathways were overexpressed in inmature needles. Interestingly stress related proteins and defense mechanisms were characteristic of immature tissues, and may be linked to the higher growth rate and capacity of response of this tissue.

QUANTITATIVE PROTEOMICS ANALYSIS OF LYMPH NODES FROM PIGS INFECTED BY PORCINE CIRCOVIRUS TYPE 2 (PCV2) BY 2-DE, $^{18}O/^{16}O$ LABELING AND LINEAR ION TRAP MASS SPECTROMETRY

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PCV2 is the causal agent of postweaning multisystemic wasting syndrome in pigs, characterized by progressive weight loss, dyspnoea, enlargement of inguinal lymph nodes, depletion of lymphocytes and an altered pattern of cytokines. The mechanism whereby the virus causes the disease and the reason why only some animals become diseased remain unclear. To study the immune response associated with virus infection, ten piglets were divided into 2 groups: control (n = 4) and inoculated with PCV2 at 7 days of age (n = 6). Piglets were euthanized and necropsied on day 29 p.i and inguinal lymph nodes samples were collected. Lymph node protein extracts for each group were pooled, split into two equal aliquots and analyzed by two different proteomics strategies: a classical approach based on the differential 2-DE pattern and a stable isotope labeling approach combining SDS-PAGE protein fractionation, "in-gel" digestion, $^{18}O/^{16}O$ peptide labeling and peptide identification and quantification by LC-MS/MS. 2-DE analysis revealed 45 spots that were differentially expressed at a FDR of 5 %, corresponding to 31 unique proteins. In the second approach peptides were identified by using the pRatio method, and the quantitative results analyzed using QuiXoT. Among 1,493 identified proteins, 794 could be quantified, from which 65 proteins were found differentially expressed at a FDR of 5%. We used the Ingenuity Pathway Analysis package to analyze and compare the obtained results. Association of differentially regulated proteins with canonical pathways highlighted two major processes: acute phase response signalling and NRF-2-mediated oxidative stress response. Other canonical pathways associated with differentially expressed proteins were that of TGF-β, and the integrin and actin signalling pathways.

INTELLIGENT USE OF RETENTION TIME FOR HIGHER ORDER MULTIPLE REACTION MONITORING MULTIPLEXING – *SCHEDULED* MRM™ ALGORITHM

Antonio Serna Sanz
Applied Biosystems

The utility of Multiple Reaction Monitoring (MRM) on triple quadrupole based MS systems for biomarker verification/validation studies is currently an active area of investigation, driven by the well known sensitivity and selectivity attributes of this type of MS approach. As more extensive protein panels need to be monitored in a targeted way across multiple samples, higher MRM multiplexing is becoming essential for throughput. The challenges of assay development and running these large scale studies are becoming better understood, the need for rapid assay development, the need for higher multiplexing and the need for more robust assays are some of the key challenges.

In this work, the unique combination of triple quadrupole and ion trapping capabilities of the hybrid triple quadrupole – linear ion trap mass spectrometer (QTRAP® System) has been utilized to create 100s of high quality, specific MRM transitions for multiple peptides to many plasma proteins. Iterative analysis provided rapid refinement of MRM parameters without requiring synthetic peptides. Intelligent use of retention time using new acquisition software enables many more MRM transitions to be included in a single acquisition method, while maintaining good peak area reproducibility. The analytical reproducibility of the MRM method developed was found to be extremely high, even in plasma, with the majority of peptides being measured with %CV<10.

Posters

Posters

S1. Quantitative Proteomics

P. 1

DIFFERENTIAL PROTEIN EXPRESSION PROFILING BY ITRAQ-2DLC-MS/MS OF HUMAN BLADDER CANCER EJ138 CELLS TRANSFECTED WITH THE METASTASIS SUPPRESSOR KISS-1 GENE

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The use of isobaric tags for relative and absolute quantization (iTRAQ) followed by multidimensional liquid chromatography (LC) and tandem mass spectrometry (MS/MS) analysis is emerging as a powerful methodology for biomarker and drug target discovery. KiSS-1 is a metastasis suppressor gene that has been reported to be involved in the progression of several solid neoplasias. The loss of KiSS-1 gene expression has been shown to be inversely correlated with increasing tumour stage and poor overall survival in bladder tumors. Moreover, cases developing distant metastases displayed complete loss of KiSS-1 expression. In order to identify the molecular pathways associated with the metastasis suppressor role of KiSS-1 in bladder cancer, we carried out a proteome discovery analysis of bladder cancer cells (EJ138) transiently transfected with a vector encompassing the full length KiSS-1 gene using an iTRAQ approach. Protein extracts collected after 24h and 48h transfection were fractionated, digested with trypsin and treated with iTRAQ reagents. The labelled peptides were separated through Strong Cation Exchange (SCX) and Reversed Phase LC and analysed by MALDI TOF/TOF MS. Three software packages were utilized for data analysis: ProteinPilot for identification and quantification of differentially expressed proteins, Protein Center for gene ontology (GO) analysis and Ingenuity Pathway to provide insight into biological networks. Comparative analysis among transfected, mock and empty vector exposed cells have identified more than 800 proteins with high confidence (>99%), showing high correlation rates among replicates (>70%). The involvement of the identified proteins in biological networks has served to characterize molecular pathways associated with KiSS-1 expression and to select critical candidates for validation analyses by Western Blot using independent transfected replicates. As part of complementary clinical validation strategies, inmunohistochemical analyses of potential metastasis-related biomarkers in bladder cancer progression have been performed in metastatic bladder tumours spotted onto tissue microarrays (n=78). In summary, our study not only has served to reveal molecular mechanisms associated with the metastasis suppressor role of KiSS-1 in bladder cancer, but also to identify novel potential metastatic biomarkers for patients affected with bladder tumors.

P. 2

ABSOLUTE PEPTIDE/PROTEIN QUANTIFICATION USING ELEMENTAL MASS SPECTROMETRY (ICPMS)

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Changes resulting from alterations of the biological systems can only be detected if quantitative information is obtained. Unfortunately, reliability and accuracy (trueness and precision) of the results published on quantitative proteomics so far is a central point of concern. Further research and standards are required to address the quality assurance requirements urgently demanded. Unfortunately, ionization process in electrospray and MALDI mass spectrometry (MS) is extremely depending on the physic-chemical properties of the peptide, which obliges to synthesise standards for every individual species when you look for its absolute quantification in a particular sample.

One possible way out to this problem is to resort to MS with an elemental ion source (inductively coupled plasma, ICPMS). Interestingly, the elemental response by ICPMS could be directly proportional to the absolute amount of the element introduced (any different from C, H, N and O). Therefore, in contrast to molecular MS techniques, this signal is independent of the species and sample matrix. In this way, every individual species (peptide) containing the heteroatom could be easily quantified using a simply heteroatom-containing species as a generic standard. Such high-quality absolute quantitative data becomes then directly traceable to a certified standard providing constancy of the results across time and space and a known level of accuracy.

At best, these heteroatoms detectable by ICPMS are naturally present in the protein/peptide (i.e. P, S, Se). In our approach we propose to introduce an ICP-detectable element into the amino acid sequence to make every protein detectable by ICPMS. To do so, we have bioconjugated 2 iodine atoms specifically to the tyrosine residues. Then, capillary HPLC-ICPMS provided absolute quantification of every tyrosine-containing peptide present with extremely high sensitivity (below the nM level) and robustness by simply spiking the sample with a generic iodine-containing standard (iodo-benzoic acid). Quantitative results have been validated using a Reference Material (NIST 8327).

NUTS AND BOLTS OF ITRAQ-BASED QUANTITATIVE ANALYSIS

Campos, A.[1], Diema C.[2], Odena M.A.[1], Bellido D.[1], Villaseca M.[2], and Oliveira E[1]

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Deployment of mass spectrometry for peptide-based quantitative analysis has become mainstream technology across proteomics laboratories. In particular, the iTRAQ technology has caught the attention of the scientific community mainly due to its capacity to multiplexing up to eight samples in a single mass spectrometry (MS) experiment. Because relative quantitative information on iTRAQ-tagged peptides is obtained in MS/MS scans, setting up optimal instrument conditions is full of twists and turns.

Accurate iTRAQ quantitation relies largely on instrument particularities such as efficiency of the ionization and fragmentation processes. Since its implementation, iTRAQ analysis has been largely relegated to quadrupole time-of-flight (QTOF) and MALDI-TOF/TOF instruments. More recently, the development of the novel fragmentation method ***P***ulsed-***Q***-***D***issociation (PQD) (Thermo Fisher™) has opened up a new possibility for iTRAQ analysis in linear ion trap instruments such as the high-performance LTQ-FT. In the present study, we have thoroughly explored the impact of a number of different instrument parameters on robustness, accuracy and sensitivity of iTRAQ analysis. We have also assessed the strengths and weakness of different MS instruments (QTOF, MALDI-TOF/TOF, and LTQ-FT) on iTRAQ analysis. Another major challenge in iTRAQ analysis is the computational and statistical analysis of the data. We have compiled and evaluated the different freely available tools for iTRAQ data mining. Finally, to facilitate the implementation of iTRAQ technology, we provide general guidelines for instrument parameter setup and robust data mining.

P. 4

USING ETTAN™ DIGE SYSTEM FOR ANALYSIS OF PROTEOME CHANGES IN TRANSFORMED *E. COLI*

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Two-dimensional electrophoresis (2D electrophoresis) is a well established method used to study differences in protein expression caused by environmental changes induced by for example disease, drug or growth factor treatment etc. The 2D DIGE technology with two different samples and an internal standard per gel labelled with CyDye™ DIGE Fluor minimal dyes, significantly reduces the required number of gels compared to conventional 2D electrophoresis. The internal standard significantly reduces the gel to gel variation and thereby improves statistical validity. 2D DIGE and DeCyder™2D software version 6.5 were used to assess significant differential expression as a result of time and temperature changes in transformed *E. coli* cultures. Temperature and time clustering was shown with DeCyder2D Extended data analysis (EDA) module. Proteins with significant variation were identified by mass spectrometry using MALDI. The levels of target protein from the 2D DIGE analysis were confirmed with fluorescent Western blotting using ECL Plex™.

P. 5

DATE PALM SOMATIC EMBRYOS: VARIABILITY IN PROTEIN EXPRESSION LEVEL ASSESSED BY 2-DE AND PROTEIN IDENTIFICATION

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The multiplication of date palm is traditionally achieved by seeds and by off-shoots. However, these methods can be improved by biotechnological approaches such as somatic embryogenesis. Date palm somatic embryos have been obtained from embryogenic suspension cultures. Previous works dealt with the effects of several culture media combinations on some physiological parameters related to the multiplication and maturation of somatic embryos, but few of them have studied the process at the molecular level. By using a 2-DE based proteomic approach, the protein profile of mature somatic embryos obtained from seeds of three date palm cultivars (Barhi, Deglet Nour, and Deglet Nour Grand Caliber) has been analyzed. Embryo extracts were obtained by homogenization in TCA-acetone-phenol media. Proteins were separated by 2-DE (IEF on 17 cm IPG strips, pH 5-8, as the first dimension, and SDS-PAGE on 12 % polyacrylamide gels as the second one, 500 µg proteins). After Coomassie staining gel images were captured and analyzed. The number of resolved spots for Barhi, Deglet Nour, and Deglet Nour Grand Caliber 1 sand 2 were of, respectively, 118, 262, 211 and 122, with qualitative and quantitative differential spots found among them. Fifty-six of the differential spots were subjected to MS analysis, with 47 identified. A significant number corresponded to enzymes involved in the energy metabolism, with stress-related redox maintenance proteins also being well represented.

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DIFFERENTIAL EXPRESSION ANALYSIS IN SPINAL MUSCULAR ATROPHY PATIENTS

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Spinal muscular atrophy (SMA) is a recessive disorder involving the degradation and subsequent loss of motor neurons from the spinal cord. The main cause of SMA is related with the absence of the functional form of the survival motor neuron (SMN) gene (SMN1). This gene presents a telomeric (SMN1) and a centromeric (SMN2) copies that differ in 5 nucleotides. This mutation produces an alternative splicing of SMN2 giving rise to a truncated protein with reduced functionality (1,2). All the SMA patients have at least one copy of the SMN2 form that is necessary for survival, and disease severity depends primarily on the number of SMN2 gene copies. However, the number of SMN2 copies doesn't explain completely the different severity of the disease observed in SMA patients.

In this work we compared by 2DE the fibroblasts proteomic patterns corresponding to several members of a SMA-carrier family (four sisters and their mother) and a group of four controls.

Among the family members, the four sisters have the same genotype but show different levels of disease severity (one of them is asymptomatic). The mother is heterozigous an thus asymptomatic. The analysis revealed the presence of 42 differential proteins between controls and patients with the SMA phenotype. Two other proteins were found differential between symptomatic and asymptomatic members of the family. A biological process analysis of the identified proteins reveals a high percentage of proteins classified as stress response and protein metabolism and modification. The functional implications of these proteins will be evaluated in future studies.

1. Lefebvre, S. (1995). "Identification and Characterization of a Spinal Muscular Atrophy-Determining Gene." Cell **80**: 155-165.
2. Sumner, C. J. (2007). "Molecular Mechanisms of Spinal Muscular Atrophy." *Journal of Child Neurology* **22**: 979-989.

P. 8

A ROBUST, HIGH-THROUGHPUT METHOD FOR QUANTITATIVE, IN-DEPTH ANALISYS OF PROTEOMES

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Quantitative proteomics plays an increasingly important role in biological and medical research. MS-based quantitative proteomic methods have proven to be a very robust alternative to the well-established gel-based techniques. However, reliable MS-based high-throughput quantification is still a challenge. Here, we present a high-throughput quantitative proteomics method that allows a rapid, robust and deep analysis of proteomes. The samples are applied to conventional SDS-PAGE gels, but the run is stopped as soon as the front enters the resolving gel, so that the whole proteomes become concentrated in the stacking gel. The concentrated gel bands are subjected to trypsin digestion in the presence of the detergent CYMAL, and the resulting peptides are labelled with $^{16}O/^{18}O$. The mixture of labelled and unlabelled peptides is separated into 24 fractions by IEF using an OFF-Gel electrophoresis unit, and the fractions are analyzed, under high peptide loading conditions, by RP-HPLC-MS/MS using a linear ion trap LTQ MS. On average, more than 500 unique peptides were identified per fraction, with a peptide redundancy in different fractions of less than 2, resulting in the identification of more than 6,000 peptides at a FDR of 5%, corresponding to more than 2,300 proteins. Analysis of the data using QuiXoT demonstrates that the OFF-Gel technology does not affect O^{18} peptide labelling. Besides, analysis of the data using a previously proposed statistical model indicated that the variances at the scan, peptide and protein levels are similar to those encountered using in-solution digestion and SCX peptide fractionation. The method has been applied to the analysis of total protein extracts from brain, liver and endothelial cell cultures, and also of membrane proteomes such as rat heart mitochondria.

P. 9

CONNEXIN 43 PLAYS AN IMPORTANT ROLE IN THE MOLECULAR MECHANISMS IMPLICATED IN ISCHEMIC PRECONDITIONING

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The heart is one of the most energy demanding tissues in the body and is totally dependent upon oxidative phosphorylation to supply the large amount of ATP that requires. The heart can usually survive a short period of ischaemia and then recover upon reperfusion, but this mechanism can exacerbate the damage that takes place during the ischemic period. There is increasing evidence that mitochondrial dysfunction plays a central role in mediating the main components of reperfusion injury, and that one of the most effective ways of protecting hearts from such injury is the ischemic preconditioning (IP). It has been clearly demonstrated that connexin 43 (Cx43) is located in the cardiomyocyte mitochondria and is important for the cardioprotection by IP (Boengler et al., 2005; Heinzel et al., 2005; Rodríguez-Sinovas et al., 2006). Moreover, IP does not occur in transgenic mice Cx43KI32, which have Cx32 instead of Cx43 (García-Dorado et al., 2007), being consistent with the possible role of Cx43 in IP. There are also several pharmacological agents, such as diazoxide, that delay the death of ischemic myocytes when the myocardium is pretreated with the agent before an episode of ischaemia (Grover et al., 2000; Scwartz et al., 2002; Gross et al., 2003). However, the mechanisms underlying these processes remains unclear.

To elucidate the molecular mechanisms implicated in IP and the role of Cx43, we are performing a comparative differential expression proteomics study of mitochondrial proteins from cardiomyocytes in preconditioned rats, in transgenic Cx43KI32 mice and in rats pretreated with two different pharmacological agents known to induce preconditioning. The samples were analyzed by stable 18O isotope-labeling, IEF separation and linear ion trap mass spectrometry and quantified using the program QuiXoT. The results obtained to date suggest that Cx43 affects the functions that are altered in IP.

P. 10

A MULTI-LABORATORY STUDY ASSESSING REPRODUCIBILITY OF A 2D-DIGE DIFFERENTIAL PROTEOMIC EXPERIMENT

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Although 2DE-electrophoresis has been long used to study differential proteomics, its reproducibility has been always a major concern. In recent years, different methodological improvements have contributed to more robust 2DE workflows: use of immobilized IEF strips, fluorescence based difference gel electrophoresis (DIGE), new software tools, etc. In order to assess the reproducibility of 2DE experiments across laboratories, we set up a multi-laboratory study, performed at 11 laboratories of the ProteoRed network (Spanish network of proteomics facilities). All participating labs received two protein extracts, prepared from cultured human adenocarcinoma MDA-MB-468 cells, treated or not with 50 ng/ml EGF (Epidermal Growth Factor) for 24h. Differential analysis was performed by a 4-gel 2D-DIGE experiment, using 4 technical replicates of each sample, with Cy dye swapping. Strictly defined 2DE conditions were followed by all labs. Each lab selected the 30 spots presenting the highest fold variations (with $p<0.05$), and attempted MS protein identification.

The results demonstrate a very good within lab and across lab reproducibility. Within labs, 75-85% detected spots present %CV <10%, and 40-60 %CV <5%. Across all labs, around 60% and 15% of spots show %CV <10% and <5%, respectively. Selection of differentially expressed spots shows good reproducibility across labs, although there is a certain degree of subjectivity in the selection, as each lab applied its own filtering criteria. Overall, 24 spots were ranked among the top-30 by at least 3 labs, and 14 by at least 4. MS protein identification was, on average, 60% successful, with 22 spots identified by at least 3 different labs. In those cases, identical gel locations corresponded to the same protein Id.

In conclusion, the results of the study show the robustness of the methodology used, and demonstrate the feasibility of across lab validation schemes, pointing towards development of inter-lab QC strategies for proteomic research.

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COMPARATIVE STUDY OF TWO PROTEOMIC QUANTITATIVE METHODS, DIGE AND ITRAQ USING 2D GEL- OR LC-ESI QTOF

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The quantification of differences between two or more physiological states of a biological system is among the most important but also most challenging technical tasks in proteomics.

Classical methods perform an initial separation step at the protein level, usually a high resolution 2D electrophoresis, where quantitation takes place. A major limitation of the technique is that mainly soluble abundant proteins are separated and detected. A second step is needed to identify the proteins of interest. Over the past few years, mass spectrometry-based quantification methods have gained increasing popularity. This is, probably, because by working at the peptide level they overcome the two important shortcomings of classical proteomic quantification methods: the limited protein range that can be examined and the two-step strategy requirement. However, mass spectrometry is not inherently quantitative because proteolytic peptides exhibit a wide range of physicochemical properties affecting their mass spectrometric response. Thus, most of de mass spectrometry-based methods employ differential stable isotope labeling to create a specific mass tag that can be recognized by a mass spectrometer and provide the basis for quantification.

The optimal strategy to perform quantitative proteomic analyses to obtain the most informative data sets is undefined and it, probably, depends on the systems under study.

In the present study we have compared two quantitative methods: a gel-based DIGE approach and a mass spectrometry-based iTRAQ approach using LC-ESI QTOF for advancing our understanding of proteomic changes in hypertensive heart disease.

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QUANTITATIVE ANALYSIS OF DIFFERENTIAL PHOSPHORYLATION IN A *PKC1* OVEREXPRESSION STRAIN OF *SACCHAROMYCES CEREVISIAE*

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In *S. cerevisiae*, protein kinase C (Pkc1p*)* is involved in the control of actin polarization and morphogenesis. Pkc1p acts upstream of the cell integrity MAPK pathway. A protein kinase C overexpression strain of *S. cerevisiae* was investigated for differential protein phosphorylation as compared to an isogenic wild type strain.

We have used a phosphoproteomic approach based on quantitative mass spectrometry based on stable isotope labeling with amino acids in cell culture (SILAC).

The *PKC1* overexpression strain was labeled by growth in media containing stable isotopic amino acids, i.e C13 – arginine and C13-lysine, to do differential analysis in a 1:1 protein mixture of both strains using mass spectrometry.

Several phosphopeptide enrichment tecniques have been used, and all fractions were analysed by nano – HPLC-MS/MS and neutral loss dependent MS3 on a LTQ mass spectrometer that allowed identification of phosphopeptides using Mascot scoring and quantification with MSquant, a freely distributed program for SILAC quantification.

Of 299 non-redundant phosphopeptides identified and quantified, 93 were upregulated more than 2-fold (average ratio).

The proteomic work was done at the Proteomics Facility of UCM-PCM, a member of ProteoRed Network

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QUANTITATIVE PROTEOMIC ANALYSIS BY ITRAQ ON A LINEAR ION TRAP MASS SPECTROMETER

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One main objective of proteomic research is the systematic identification and characterization of proteins expressed in a biological system. The goal has recently extended to comparative and quantitative studies, thanks to recent advances in chromatography, mass spectrometry and bioinformatics. For this purpose there are an increasing number of approaches to quantify expression profiles from complex protein mixtures. The two most commonly used methods rely either on gel-based (*e.g.* DIGE) or on a chromatographic separation of proteins and/or peptides followed by mass spectroscopy (*e.g.* SILAC, iCAT, ^{18}O, iTRAQ).

Here, we describe an optimised methodology for the application of isobaric tags for relative and absolute quantitation (iTRAQ) and tandem mass spectrometry to obtain relative quantitative data from peptides derived from tryptic digestions of human plasma proteins previously depleted by using a multiaffinity removal system (MARS). The combination of the affinity chromatography for the elimination of the major abundant proteins of the plasma with the use of bidimensional liquid chromatography –using the approach of cation exchange, sample enrichment, reversed phase chromatography and microspray linear ion trap mass spectrometry[1]– turns to be a powerful tool for the analysis of that kind of samples. The introduction of the pulsed quadrupole dissociation (PQD) mode has made analysis of iTRAQ reagent labelled samples possible[2]. We developed software to extract and analyse quantitative data.

The method proposed here has been validated by the analysis of a tryptic digestion of BSA and casein standards. Optimised methodology is currently being applied in a multicentre study for the searching of cancer biomarkers in human plasma samples.

1. Mayya, V.; Rezaul, K.; Cong, Y.S.; Han, D.; *Mol. Cell. Proteomics*, **2005**, 4(2), 214-223.
2. Schwartz, J.C.; Syka, J.E.P.; Quarmby, S.P. *The 53rd ASMS Conference on Mass Spectrometry and Allied Topics*, San Antonio, TX, June 5-9, **2005**.

P. 14

METASTASIS-ASSOCIATED C4.4A, A GPI-ANCHORED PROTEIN CLEAVED BY ADAM10 AND ADAM17

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Metalloproteases play a complex role in tumor progression. Proteomic approaches to identify the array of substrates of a given metalloprotease (degradome), can help to unveil its role in tumor growth and metastasis. Here we describe a proteomic screening to compare the proteins secreted by MCF7 cells, derived from an invasive mammary tumor, and the same cells expressing shRNAs that knock down ADAM10 or -17. Cells grown in conditions were the protease is expressed or knocked down were differentially labeled, by incorporating isotopically labeled amino acids (SILAC). Glycoproteins from the conditioned media of each of the two cell cultures were purified by affinity chromatography. The samples were then pooled and run on a 1D SDS-PAGE gel. The gel lane was then cut into 20 fractions and digested. Each fraction was analyzed by RP-LC-MS/MS. Protein identification and quantification of relative abundances was performed using WARP-LC, an integrated software platform for LC-MS/MS workflows.

A number of known substrates of both proteases were identified as such in the analysis, showing the expected decrease of the shed extracellular domain abundance in the medium upon knock down of the protease. Thus, the cell-adhesion proteins E-cadherin and Desmoglein-2, or the PTP receptor-type k, were identified as substrates of ADAM10. Fractalkine and NCAML1 were found as substrates of ADAM17.

In addition several new candidate substrates of both proteases were identified. Among them, the GPI-anchored protein C4.4A, was identified and further validated as substrate of both ADAM10 and ADAM17 proteases. According to the identified peptides, both proteases cleave this protein close to the juxtamembrane region, releasing a soluble form devoid of the GPI-anchor. C4.4A protein, homologous to the urokinase-type plasminogen activator receptor, has been related to tumor invasion and metastasis. Cleavage of this protein by ADAMs constitutes a previously unknown level of regulation of its function.

P. 15

LABEL-FREE QUANTIFICATION BASED ON DATA INDEPENDENT ACQUISITION MASS SPECTROMETRY

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The quantification of differentially expressed proteins is a key area where proteomics technology has made great progress over the last years. The development of a data independent, alternate scanning acquisition, where no precursor is selected and low and high collision energy data is alternatively acquired, affords accurate mass measurements used for both protein identification and label-free based quantification. Furthermore, beyond new experimental approaches, bioinformatics software is in constant development in order to obtain more robust tools for data processing, qualitative analysis and quantification. Recently, an LC-MS based absolute quantification method based on the comparison of the 3 most intense peptides of each protein with a known protein standard has been developed. In this study we have tested this approach using known amounts of protein standards at different ratios. 4 proteins have been spiked in an *E.coli* lysate background and their absolute and relative protein abundance measured. Furthermore, we have used the same experimental approach to analyse the protein expression pattern of EGF treated and untreated human MDA-MB-468 breast cancer cells. Finally, the same experimental data has been analysed with a probabilistic based quantification algorithm, where relative quantification of all peptides and proteins is performed. The obtained results with both quantification methods have been compared.

Posters

S2. Posttranslational Modifications

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PHOSPHORYLATION OF p54^{NRB} DURING MITOSIS

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p54^{nrb} is a nuclear factor that regulates many processes including, transcription, transcription-splicing coupling or DNA repair. Using 2D-PAGE followed by EIS-QTOF-MS and "in vitro" dephosphorylation assays we found that p54^{nrb} is phosphorylated after treatment with mitotic damaging agents that cause M phase arrest. These agents include drugs such as vincristine or paclitaxel used for treatment of many tumor types and anticancer drugs such as kinesin spindle protein (KSP) inhibitors that are currently in clinical trials. These drugs also cause cell death and induce the processing of this nuclear factor by caspases. Furthermore, we use cell cycle regulators to determine that p54^{nrb} phosphorylation is dependent on the mitotic state induced by these antitumoral drugs. Finally, using double thymidine block synchronized cells; we demonstrate that p54^{nrb} is also phosphorylated during normal mitosis.

P. 17

PROTEOMIC ANALYSIS OF LIPOPROTEIN LIPASE CHARGE ISOFORMS

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Lipoprotein lipase (LPL) is a glycoprotein enzyme that plays a pivotal role in lipid metabolism. Abnormalities in LPL function have been associated with a number of pathophysiological conditions, including atherosclerosis, obesity, Alzheimer's disease, and diabetes. A large number of LPL studies have been performed in rat, although the amount of information derived from direct study of the protein in this species is limited. Here we attempted to examine possible modifications of LPL using proteomic tools. By combining high-resolution two-dimensional gel electrophoresis and Western blot with biological mass spectrometry we demonstrate, for the first time, the coexistence of multiple LPL charge isoforms in the rat. We studied the origin of this charge heterogeneity by: (1) comparison with the 2D pattern of LPL from post-heparin rat plasma (as a source of mature LPL); (2) protein dephosphorylation; (3) protein deglycosylation; and (4) partial sequencing of different LPL isoforms by capillary liquid chromatography tandem mass spectrometry. The results reveal that this charge heterogeneity is not due to different stages of intracellular maturation or protein phosphorylation. It can be partially explained by glycosylation, although other post-translational modifications must also be involved. Our findings increase the complexity of LPL studies and data interpretation and open the doors to further research aimed at identifying the molecular differences between LPL isoforms and exploring the potential functional implications of this charge heterogeneity.

P. 18

HIGH RESOLUTION DYNAMIC STUDIES OF PEPTIDE PHOSPHORYLATION USING ELEMENT MASS SPECTROMETRY

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Cells continuously receive stimulus from outside to which they have to respond. It is well recognized today that phosphorylation plays a pivotal role in such responses so it is strictly necessary to quantify the phosphorylation dynamics of each protein in order to clarify the relationship between signalling reactions and eventually observed biological responses. Molecular mass spectrometry has currently established as the most powerful tool for the study of phosphorylation temporal changes because of its versatility and sensitivity. MS-based approaches to study temporal dynamics of cellular signalling are mainly based on the use of stable isotope labelling strategies. All these label-based approaches mostly provides the relative quantification of protein phosphorylation and the time resolution achievable is always limited by the number of different isotopic tags used and the relatively high variability of quantitative results (10-20 % RSD). Only recently, absolute quantification methods have been reported (AQUA, QCAT) but requiring the synthesis of heavy isotope labelled counterparts to be used as internal standards for each peptide/protein sought.

The response by elemental mass spectrometry (ICPMS) is species and matrix independent allowing the absolute and site specific quantification of every phosphorylated protein (unknown or not) after its enzymatic digestion. Quantitative results obtained by capillary HPLC-ICPMS also show exceptional accuracy and precision (<5 % RSD). Using this approach, we herein report the absolute quantification of phosphopeptides and we show its potential to discriminate between very small temporal changes in protein phosphorylation levels. The strong point of the approach proposed is that the high precision achieved in the quantification can be applied to discriminate between close phosphorylation states, therefore leading to very high time resolution in protein phosphorylation dynamics investigations.

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THE ANTITUMOR DRUG VINBLASTINE INDUCES THE PHOSPHORYLATION OF ANNEXIN A2

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Vinblastine (Vb) is an antitumor drug that inhibits microtubule polymerization, causes G2/M arrest and induces cell death. 2D-PAGE and MALDI-ToF-MS analysis on A549 cells, revealed that vinblastine upregulates an acidic form of the protein Annexin A2. The staining of this Vb induced form of Annexin A2 with the phosphospecific dye Pro-Q Diamond, indicates the presence of phosphorylated Annexin A2 in this spot. Moreover, this Vb up-regulated form of Annexin A2 disappears upon treatment with λ-protein phosphatase, confirming this post-translational modification. Further analysis using MRM (multiple reaction monitoring) indicated the presence of a phosphorylated peptide, containing threonine 19, serine 18 or serine 22, only in the vincristine upregulated form. MS/MS spectra show a clear phosphorylation of Thr19 and lower signals for Ser18 and Ser22. These results indicate that phosphorylation of Annexin A2 in different residues may play a role in the cellular response to Vinblastine.

P. 20

HUMAN AB0 BLOOD GROUPS: DIFFERENTIAL MEMBRANE PROTEIN CARBONYLATION IN RED CELLS

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The functionality of the AB0 blood group system remains as one of the most mysterious genetic polymorphisms in humans. Since its discovery several connections between blood groups and disease susceptibility have been hypothesized. Of a great interest for human adaptation is their association with other erythrocyte polymorphisms that provide survival advantages against malaria infection. These include G6PD deficiency, HbS and HbC as an adaptation pressure on the distribution of blood groups that has favored the worldwide prevalence of group 0 in the regions where malaria is endemic. A reduced adherence of parasitized red blood cells (RBCs) to other cells or organs is a suggestive mechanism to explain the malarial parasite selective pressure in favor of group 0. However, a definitive study to assess this hypothesis has not been conducted yet.

DNP-derivatized red cell membrane proteins from healthy donors with different AB0 blood group were compared by immunoblotting using anti-DNPH antibodies. Carbonylation profiles of each group were obtained for defatted and not defatted red cell membranes. The identification of carbonylated proteins was performed on excised band by MALDI TOF. Protein identifications were assigned using the MASCOT search engine. Stomatin, α and β spectrin were identificated in all groups but band 4.1, band 4.2 and cytoplasmic domain of band 3 were not found oxidized in blood group 0.

Band 4.1 is essential for parasite survival in infected RBCs. Band 3 is a receptor during parasite invasion of human erythrocytes, being deleted in malaria-resistant Southeast Asian ovalocytosis. Deficiencies of band 4.2 have been observed in hereditary spherocytosis, another resistant phenotype to severe malaria. In connection with these previous reports, our findings suggest a functional role of differential carbonylation in the AB0 group system to protect against malaria.

P. 21

NEW MICROFLUIDIC CHIP TARGETING PHOSPHOPROTEOMES

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Protein phosphorylation is one of the most important post-translational modification (PTM) events among mechanisms of regulating protein function in cells. Myriad biological processes, including cell proliferation, migration, and apoptosis involve phosphorylation steps. One of the major efforts in proteomics is devoted to the identification and understanding of phosphoproteomes in cells. Nevertheless, comprehensive identification of sites of protein phosphorylation remains a challenge, best left to experienced proteomics experts. In order to achieve selective enrichment of phosphorylated proteins and peptides most commonly used technologies are currently immobilized metal affinity chromatography (IMAC), anti-phosphotyrosine antibodies, and titanium dioxide prior to LC/MS (liquid chromatography and mass spectrometry) analysis. Recent advances in HPLC chip technology have created an environment to allow automation of such a workflow with increased ease of use and confidence of analysis. The new microfluidic chip is a re-usable HPLC nano-flow rate chip with titanium dioxide particles (TiO_2) based phosphopeptide enrichment. The chip is a multilayer polyimide laminate that contains an enrichment section with TiO_2 beads flanked on both sides with C18 reversed phase material. The 3 section sandwich is separated from each other by micro-fabricated frits. This enrichment section is connected to a reversed phase separation column ending in an integrated electro-spray tip by a micro valve in direct contact with the chip surface providing a zero dead volume high pressure seal. The chip is used with a HPLC-chip/MS instrumentation using the HPLC-chip cube interface combined with a Mass Spectrometer. The unique sandwich configuration of the enrichment section provides researchers three modes of peptide analysis: (1) standard peptide analysis, (2) phosphopeptide analysis only, and (3) combined peptide and phosphopeptide analysis. This approach will offer non-expert proteomics researchers a reliable way in phophoproteome analysis.

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ANTITUMOR DRUGS REVAL THAT EUKARYOTIC ELONGATION FACTORS Eef1Bγ AND eEF1Bδ ARE PHOSPHORYLATED DURING MITOSIS

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Eukaryotic elongation factor 1 (eEF1) is a macromolecular complex formed by two different components, eEF1A and eEF1B, both implicated in the transference of the amino acids from the aminoacyl-tRNA to the ribosome site A. eEF1B complex is formed by three proteins, eEF1Bα, eEF1Bδ and eEF1Bγ. Specifically, eEF1Bα and eEF1Bδ have guanine nucleotide exchange activity required to activate eEF1A.

Numerous antitumor drugs target different mitotic mechanisms to induce cell death in cancer cells. Then, microtubule interfering agents (many of them such as paclitaxel, docetaxel and vincristine in clinical use) inhibit microtubule dynamics and blocks mitosis. This microtubule dinamics blockage affects processes other than mitosis. For this reason other drugs targeting specific mitotic proteins such as Aurora A, Aurora B, polo like kinase 1 (PLK1), kinesin spindle protein (KSP) and centromeric protein E (CENPE) are being investigated.

2D-PAGE and PRO-Q diamond staining showed that paclitaxel treatment phosphorylates eEF1Bγ in HeLa cells *(Prado M.A. y cols., Proteomics, 2007, Vol.7, 3299-3304)*. A dephosphorylation assay with λ-protein phosphatase confirmed this post-translational modification of eEF1Bγ and revealed that eEF1Bδ is also phosphorylated after paclitaxel treatment. Moreover, treatment with other antimitotic agents such as docetaxel and STLC (a KSP inhibitor) induce these phosphorylations of elongation factors. We demonstrate that these post-translational modifications depend on mitotic events; specifically it occurs during normal mitosis in HeLa cells. Analysis of eEF1Bγ sequence and MALDI-ToF analysis after digestion with endoproteinase Glu-C of the protein suggest that CDK1 phosphorylates eEF1Bγ in Thr230. In summary, we demonstrate that eEF1Bγ and eEF1Bδ are phosphorylated by taxanes and KSP inhibitor treatments and during normal mitosis.

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THE STUDY OF THE OF HUMAN PRIMARY T-LYMPHOCYTE PHOSPHOPROTEOME

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Global phosphorylation profiles will be used for the characterization of biological markers of cell status that could be used as prognostic or diagnostic markers for hypersensitivity or immunodepression-related diseases. Protein kinase inhibitors are the second most popular drug target class in the pharmaceutical and biotech industries. Drugs like ciclosporin or rapamycin are kinase and phosphatase inhibitors (calcineurin and mTOR respectively) and are used to inhibit the immune response in transplants. The characterization of phosphorylation motifs will help in the search for new, physiologically relevant kinases as well as in the understanding of the mechanism involved in the regulation of protein function. This information is the first step towards the design of new therapies based on drugs controlling either kinase/phosphatase activities or the activity of other proteins in key points of the affected pathways.

In this work, we describe a large-scale phosphorylation analysis of primary T-cells using a multidimensional separation strategy involving preparative SDS-PAGE or SCX for prefractionation and sequential phosphopeptide enrichment using IMAC and titanium dioxide, followed by LC-MS^n analysis using a LTQ linear ion trap. In total, more than 300 different high-confidence phosphorylation sites were described mapping more than 600 possible phosphoproteins.

To organize the phosphopeptide data we created a relational database called LymPHOS that currently comprising phosphopeptide sequences, p-sites and information about the proteins containing these phosphopeptides. Also, this information is linked to the mass spectrometric information and is publicly accessible on the net (www.lymphos.org). This constitutes the only phosphorylation map for human primary T-lymphocytes.

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INCREASING THE SENSITIVITY OF PROTEOMIC IDENTIFICATION OF S-NITROSYLATED PROTEINS: TOWARDS THE S-NITROSOPROTEOMES IN (PATHO)PHYSIOLOGICAL SETTINGS

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S-nitrosylation (formation of a thionitrite group in cysteines, R-S-N=O) has emerged as an important reversible post-translational modification involved in many cell signalling processes related with nitric oxide production, and can even represent a new paradigm in signal transduction due to some of its particular features.

The "biotin switch" technique allows the replacement of S-nitrosylation with a more stable biotinylation that allows easy purification of the modified proteins and has been used to study several S-nitrosoproteomes, as well as to detect the modification in individual proteins. However, this technique still bears limitations, especially in physiological or pathophysiological settings, due to its reduced sensitivity.

We have developed a "fluorescent switch" technique that replaces the S-nitrosylation by a fluorescent label. This approach coupled with 2-DE has allowed us to greatly reduce the amount of starting protein extract while maintaining the number of identified proteins. We have applied it to endothelial cells treated with a nitrosylating agent, as well as to identify proteins that are endogenously S-nitrosylated in activated macrophages when the denitrosylating activity of thioredoxin is inhibited. This approach can be an advantage to study *in vivo* samples where starting material is limited.

In a different approach, we have greatly increased the number of identified proteins and located S-nitrosylation sites by applying a "second-generation proteomics" approach to the biotin switch: biotinylated proteins were digested, and peptides were purified using an avidin column and identified by LC-ESI-MS/MS. We have created a collaborative team ("nitrosoteam") to implement these methodologies and compare the S-nitrosoproteomes in different biological systems, human and plants, in order to get a deeper knowledge in the appearance and functional relevance of this modification.

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CHLOROACETYLATION OF CYCLOSTREPTIN INFLUENCES ITS INTERACTION WITH TUBULIN

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Cyclostreptin (Cs) is a natural product from Streptomyces sp.9885 that irreversibly stabilizes cellular microtubules by covalent binding to tubulin, causes cell cycle arrest, evades drug resistance in MDR tumor cells and inhibits paclitaxel-binding to microtubules. In a previous work (1) we demonstrated that cyclostreptin irreversibly binds to β-tubulin through Thr220 and Asn228 (the type-I pore binding site). To gain further information about this binding site, two reactive derivatives of cyclostreptin were synthesized and studied.

In this work we characterize the interaction binding sites of monochloroacetylated cyclostreptin in position 15 (15CA-Cs), or in position 17 (17CA-Cs), which were also cytotoxic in MDR cells and accumulate cells in G2+M phase of the cell cycle in the same way as cyclostreptin, within microtubules. As performed in (1), we have used a hybrid triple-quadrupole mass spectrometer to analyze the filtered precursor ions by the detection of AC-Cs-derived fragments in the third quadrupole. We observed a change in the specificity of CA-Cs-interacting sites within the tubulin molecule both in the formed microtubules and in unpolymerized tubulin. Although the tubulin interacting-domain was the same we had previously found (219-LTTPTYGDLNHLVSATMSGVTTCLR-243), neither Thr220, nor Asn228 residues were CA-Cs-labeled in microtubules, while the CA-Cs binding site was detected in Cys241. However, in the dimeric, unpolymerized tubulin, we detected the Thr220 interacting site with CA-Cs as well.

Interpretation of the reaction mechanisms of the CA-CS derivatives with Thr and Cys side chains in the type-I pore binding site of microtubules is discussed.

1. Buey RM, Calvo E, Barasoain I, Pineda O *et al.* Cyclostreptin binds covalently to microtubule pores and lumenal taxoid binding sites. *Nature Chemical Biology* 3, 117-125 (2007).

P. 26

QUANTITATIVE ANALYSIS OF PROTEIN GLYCATION IN CLINICAL SAMPLES

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Non-enzymatic glycation is one of the post-translational modifications (PTMs) less frequently studied. An innovative approach for quantitative analysis of glycated proteins (GP) in clinical samples (serum, plasma and red blood cells) is here presented. It is based on relative quantitation of samples between two glycation states by differential labeling with light and heavy glucose ($^{12}C_6$- and $^{13}C_6$-glucose). Then, both sets of samples are pooled to carry out analysis by a shotgun proteomics workflow. This consists of in-solution digestion with endoproteinase Glu-C, selective enrichment of glycated peptides by boronate affinity chromatography (BAC), and analysis by RP-LC-ESI-MS/MS with an Orbitrap® mass analyzer (MS2 HCD higher energy collisional dissociation and data-dependent MS3 operation modes). A similar labeling efficiency has been observed with both isotopic glucose forms under the same operating conditions, which is essential for the applicability of the method. The identification and quantitation of GP is possible as the resulting peptides provide doublet signals in MS (labeling with light and heavy glucose) with a mass shift of +6, +3 or +2 Da depending on the peptide charge. With this methodology, it was possible to identify different GP such as serum albumin (with the five preferred glycation sites), immunoglobulins, haptoglobin, serotransferrin, complement C-3 and C-8 precursors, α-2-HS-glycoprotein or apolipoprotein A-1. These proteins are representative targets to compare between samples with different glycation states. This approach has also been applied to the analysis of clinical samples after depletion of more concentrated proteins. Further research is focused on the capability of the method to monitor the concentration of GP as well as to predict new potential targets for glycation. This can be especially interesting because it could be applied with prognosis/diagnosis purposes linked to pathological disorders related to glycemic control.

P. 27

LOCATION OF ICAM-1 CLEAVAGE SITES BY MMP-13 IN THE VASCULAR ENDOTHELIUM OF ENOS DEFICIENT MICE

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During atherosclerosis, attachment of circulating mononuclear cells to the vessel wall is mediated by adhesion molecules, including intracellular cell adhesion molecule 1 (ICAM-1). In aortas of apoE-null mice, ICAM-1 was found located in lesion-prone sites of the aorta, and the importance of this inflammatory molecule was evidenced in atherosclerotic mice deficient in ICAM-1, which resulted protected from atherosclerotic lesions.

The precise role of matrix metalloproteinases (MMPs) in the early steps of atherosclerosis is under debate. In this regard, the effect of MMPs in the shedding of adhesion molecules was investigated *in vitro*, although no data were reported in the context of atherosclerosis. Lack of endothelial NOS (eNOS, NOS3) increases leukocyte-endothelial adhesion, smooth muscle cell migration, platelet aggregation, and atherosclerosis in mice. However, the mechanisms by which NOS3 could prevent atherosclerosis still remain unclear. Here we found that in atherosclerotic NOS3/apoE-deficient mice, an increased monocyte adhesion, and a significant reduction of MMP-13 expression were detected when compared to apoE-deficient animals. In addition, we found that MMP-13 cleaves ICAM-1 both *in vivo* and *in vitro*.

Mass spectrometry analysis revealed two cleavage sites at positions E61 and G98, close to the ICAM-1 extracellular N-terminal domain. The relevance of MMP-13 and ICAM-1 on cellular adhesion was found in COS-7 cells expressing ectopic ICAM-1, in which RAW 264.7 cell adhesion was inhibited by the presence of active MMP-13. Our findings may help to explain at the molecular level the protective effect of endothelial NO in atherosclerosis.

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S3. Bioinformatics in Proteomics

P. 28

COMPARATIVE PROTEIN IDENTIFICATION WORKFLOW ANALYSIS: OPEN SOURCE VS. COMMERCIAL SOFTWARE

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Identification of proteins in complex samples is frequently related to analysis of a large number of MSMS spectra. Most tools employed in mass spectrometry (MS) data mining are instrument dependent. Moreover, the flexibility of obtained data is not always enough to allow for the export and management of identified protein and peptide lists.

Open source is the term for software distributed and developed freely. Trans Proteomic Pipeline (TPP) is one of the open source tools for proteomics that can be used to identify, validate and quantify differential protein expression using MS-based approaches from different mass spectrometers.

In the present work, two data sets acquired using 2D-LC-MSMS (capLC-Q-TOF, Waters/Micromass) and LC-MALDI (4700 Proteomics Analyzer, Applied Biosystems) strategies were analyzed with different workflows using open source, commercial tools or the combination of both. We used the Sigma UPS1 standard sample (≈ 50 proteins) to evaluate the confidence of protein identification in terms of correct and false positive identifications. The workflows were also assessed in terms of accessibility, complicatedness for no informatics experts and throughput.

For open source analysis, data were transformed into a standard data format (mzXML). Xtandem was used as search engine and the confidence level of peptide identification was evaluated with PeptideProphet. The commercial software used were: ProteinLynx, DataExplorer, GPS and Mascot.

P. 29

DEVELOPMENT OF A WORKFLOW FOR GENERAL PROTEIN SEQUENCE ANALYSIS BASED ON THE TAVERNA WORKBENCH® SOFTWARE

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The aim of this research effort was to build up a workflow able to perform a generic analysis of an unknown protein sequence. Recall that workflows permit processing of large amounts of data, which can efficiently flow throughout different and complex tasks in a time-saving, user-friendly way.

The implementation of said workflow was based on Taverna Workbench® software (http://taverna.sourceforge.net/). Said software provides computational resources (i.e. web services) to develop distinct workflow steps, taking advantage of a user-friendly interface while providing shim services to develop own scripts (if required).

The workflow developed was tentatively named Workflow for Protein Sequence Analysis (WPSA), and included: an initial homology search; a multiple sequence alignment; and construction of phylogenetic trees. WPSA accepts three types of input, and retrieves several outputs. The inputs that the user needs to provide include: a query protein sequence; a list of known protein identification numbers; and a choice of method to build the tree. The outputs generated include: a BLAST report; a description of different protein sequences; an image of the multiple sequence alignment; two different output files from the clustering method used; two types of trees; and conditional outputs, according to the query sequence entered. For each type of analysis, distinct web services from as many alternative sources were used. The workflow designed gives, in particular, fast runs (i.e. 5 to 10 min) and informational and significant responses on the sequence entered.

Although the workflow implements all required tasks in an acceptable fashion, several improvements aiming at a better performance were identified for posterior development.

P. 30

AN EVALUATION OF OPEN SOURCE PLATFORMS FOR IDENTIFICATION AND QUANTATIVE PROTEOMICS DATA MINING

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It has been recognized that the computational and statistical analysis of MS/MS spectra poses a significant challenge for the proteomics community. Processing and analysis of proteomics data involves a complex, multistage process including raw file pre-processing, peptide assignment to MSMS experimental spectra, protein identification and further validation, and in some cases, MS-based quantitation. Public domain proteomics pipeline projects such as the Trans-Proteomic Pipeline (TPP) (Keller, et al., 2005), the OpenMS Proteomic Pipeline (TOPP) (Kohlbacher, et al., 2007), CRUX (Park, et al., 2008), and the Computational Proteomics Analysis System (CPAS) (Rauch, et al., 2006) integrate a number of open-source, cross-platform tools providing a pluggable development framework for the proteomics scientific community.

In the present work, we have evaluated a number of open-source (or freely available (academic)) packages for proteomics analysis including protein identification, validation and MS-based quantitation. Cross-platform statistical models based on False Discovery Rate or Bayesian probability for determining the confidence level of peptide and protein identification were compared in order to assess their sensitivity and specificity of such tools. MS-based quantitative analysis also suffers from the lack of cross-platform standard data formats. We have therefore evaluated a number of freely available software for iTRAQ and label-free quantative analysis. The open source nature of such packages allows us to add new functionalities to the pipeline, such as the one introduced by us in the TPP code for iTRAQ quantitation improving experimental accuracy. Finally, we provide a thorough workflow for proteomics data analysis to assist proteomics practitioners to implement open-source software pipelines.

P. 31

STORING, REPORTING AND COMPARING PROTEOMICS EXPERIMENTS USING THE MIAPE GENERATOR TOOL

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Standardization of experimental proteomics protocols is one of the main objectives of the National Institute for Proteomics (ProteoRed). Several multicentric experiments as well as several participations in ABRF Proteomics Research Group (PRG) studies have been coordinated by ProteoRed with the purpose of comparing both results and experimental protocols applied to obtain these results. From these studies, we obtain conclusions that help to establish the best approach for a certain proteomic problem and to improve the application of new techniques to new proteomics challenges. However, the comparison between different experiments is not a trivial task if there is not a consensus data format.

The Human Proteome Organization's Proteomics Standards Initiative (HUPO PSI) is actively developing XML interchange formats to allow both the exchange and storage of such data, and also guidance modules (Minimum Information About a Proteomics Experiment. MIAPE), to report the use of proteomics techniques in electrophoresis and mass spectrometry-based experiments. ProteoRed has incorporated such reporting standards in the multicentric activities mentioned above, using MIAPE guidelines to report the experiments and therefore, facilitating the comparison between them.

With the aim of helping the implementation of such standard, we have developed a tool freely accessible through the ProteoRed web application at http://www.proteored.org, that generates and stores MIAPE compliant reports, that is, containing the minimum information required to report a proteomics experiment, both MIAPE Gel Electrophoresis (from sample to gel image acquisition), Gel Informatics (analysis of gel image), Mass Spectrometry (from sample to peak list generation) and MIAPE Spectrometry Informatics (analysis of these peak lists) experiments.

The "MIAPE generator tool" also allows to compare different stored MIAPE compliant reports, therefore, to compare different proteomics experiments, providing specific information about the same aspect from different experiments in a single table.

Several important proteomics journals are already asking for some minimal information required to publish a paper, based in the HUPO-PSI MIAPE guidelines. For that reason, using this tool, ProteoRed wants to provide to their costumers a MIAPE compliant report attached to the results as a new service quality label.

P. 32

PROTEOPATHOGEN, A PROTEIN DATABASE TO STUDY HOST-PATHOGEN INTERACTION

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Proteopathogen is database that compiles proteomics data from experiments involving the interaction of pathogenic yeasts with immune system cells in the host. Particularly the stored data is retrieved from experiments related to the interaction of the opportunistic pathogenic fungus *Candida albicans* with murine macrophages and spans proteomics workflows from description of the experimental approaches leading to sample preparation to mass spectrometry settings and identification supporting peptides. Through its interface website, hosted by an Apache web server on a Unix platform, proteopathogen is easily queriable and the user can efficiently browse through all the stored data, improving therefore the quality of eventual analysis of mass spectrometry results. Queries can be performed by supplying one of the multiple accepted identifiers or free text, and possible results comprise gene ontology information, scientific literature references, different mass spectrometry settings and lists of peptides supporting the identification. As an add-on functionality, summary tables can be downloaded accordingly to the result. Access is public at http://marbore.dacya.ucm.es/proteopathogen.

Trabajo subvencionado por los Proyectos BIO 01989-2006 (CICYT), Deremicrobiana (Comunidad de Madrid) and REIPI (FIS, ISCIII)

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S4. Protein Interactions and Protein Arrays

P. 33

CHARACTERIZATION OF THE MOLECULAR MECHANISM OF ACTION OF A NEW INHIBITOR OF THE COMPLEX CYCLIN-DEPENDENT KINASE 2-CYCLIN A

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Eukaryotic cell cycle progression requires the coordinated interaction and activation of cyclins and cyclin-dependent kinases (CDKs). Since deregulation of cell cycle has frequently been observed in several cancer types, cyclin and cyclin/CDK complexes are considered as anticancer target with a high therapeutic index. Cyclin A binds selectively to CDK2 and CDK1 promoting G1/S and G2/M transitions respectively. A new highly selective class of CDK2/cyclin A inhibitor has been reported recently with a promising therapeutic potential (Canela, N. et al. (2006) JBC 281: 35942-35953). It is a hexapeptide (NBI1) that, in contrast to other existing CDKs peptide inhibitors, binds specifically to a new binding site on cyclin A and inhibit the formation of the cdk2-cyclin A. The characterization of its molecular mechanism of action is of vital importance in its development toward a useful pharmacological compound.

Human cyclin A is a very unstable protein whose structure is only partially known as a complex of a truncated form (173-432) with CDK2. In recent years, mass spectrometry in conjunction with other protein chemistry strategies such as chemical modifications, has emerged as a sensitive tool for probing tertiary structures of proteins and protein-protein interactions. This methodology is specially useful in cases were protein stability and concentration prevent the use of other structure determination techniques.

The main goal of the work is to determine the molecular bases of the interaction between cyclin A and CDK2 complexes with NBI1. We are using a proteomic approach combining protein surface labelling, cross-linking reagents as well as limited proteolysis with mass spectrometry. We have also developed a strategy to determine the exact cleavage sites using fluorous-based labelling of the newly created N-terminal residues.

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ALY: A POSSIBLE E2F INTERACTING PROTEIN

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E2F transcription factors (E2F8-1) are known to play a central role in regulating gene expression during cellular proliferation. E2F family members have been classically divided into activators (E2F1-3a) and repressors (E2F3b-E2F8) based on their transcriptional roles *in vitro* and conserved structural features. However, several studies have largely demonstrated that this classification is too simplistic since some E2Fs can act either as positive or negative regulators, depending on the biological context. Precisely, it is known that the interacting partners of E2Fs are of paramount importance for the transcriptional activity of them. However, the ones described so far are insufficient to understand how is regulated the transcriptional activity of this transcription factor family.

We are interested in studying E2F1-3 but we are focusing our effort on E2F2, because previous works in our lab have demonstrated that not only can this factor act as a transcriptional activator but also as a repressor. The aim of our work is to identify and characterise new E2F2 interacting proteins that may help us shedding light on the role of transcription factor E2F2.

For this purpose, E2F2 containing protein-complexes were immunoprecipitated with a specific antibody against E2F2, and, following tryptic digestion, precipitated protein complexes were identified by LC-MS/MS. Together with E2F2, several potential E2F2 interacting partners were identified also. Among these, ALY was proven to interact not only with E2F2, but also with E2F1 and E2F3. In addition to this, preliminary results suggest that ALY could be modulating the transcriptional activity of these three E2Fs.

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SETTING-UP AN ANTIBODY NUCLEIC-ACID PROGRAMMABLE PROTEIN ARRAY (NAPPA) ANTIBODY MICROARRAY PLATFORM

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Background: Antibody microarrays have emerged as a powerful platform for the quantification of protein abundance and post-translational protein modifications. Nucleic-acid programmable protein arrays (NAPPA) constitute an alternative to usual protein arrays where the proteins are expressed directly in the array using the plasmids codifying for the proteins as source of DNA and rabbit reticulocyte as cell-free expression system. The aim of this work has been to set up an antibody NAPPA protein array using monoclonal recombinant antibodies (scFvs) to avoid the complex step of expressing and purifying the antibodies.

Methods: Seven scFvs against gastrin, CXCL1, CXCL3, MMP7, SPARC, EphB2 and FGFR3 together with their targets were sub-cloned in the vector pANT7_cGST to be expressed in the surface of the array using rabbit reticulocyte. The seven protein targets were purified to homogeneity to test the scFvs binding by ELISA, western blotting and NAPPA scFv microarray experiments.

Results: The seven different scFvs, which possess different frameworks, were "in vitro" expressed and correctly folded, maintaining their binding properties against their respective purified antigens by direct and indirect ELISA. We were able to detect as low as 100 ng/mL of antigen in indirect and direct assays using specific antibodies against the targets and the antigens labelled to biotin or Alexa Fluor 555, respectively. The GST-tagged scFvs in the C-terminal end were correctly displayed in the surface of the array, keeping at the same time both, the folding and the functionality for their respective targets in indirect assays using the purified antigens.

Conclusions: These results demonstrated that the scFvs, GST-tagged in the C-terminal, were able to recognise their respective targets in the NAPPA antibody array. These experiments might suppose the starting point for a NAPPA antibody array that could be used to quantify complex proteomes increasing the amount and diversity of the antibodies printed in the array.

P. 36

USING HIGH-DENSITY PROTEIN MICROARRAYS TO DEFINE A MOLECULAR SIGNATURE OF AUTOANTIBODIES IN COLORECTAL CANCER

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Introduction: Colorectal cancer (CRC) is the most abundant type of neoplasia in developed countries and the second cause of death among cancers due mainly to a late diagnosis. Cancer proteins elicit an immune response in the host. Then, the autoantibody repertoire from cancer patients might serve to identify tumour-associated antigens (TAAs) that could be used as biomarkers for diagnosis and prognosis.

Experimental procedures: Protoarray was used to identify TAAs as potential biomarkers using 8 serums from normal individuals and 12 colorectal cancer patients. Western blotting analysis with seven CRC cell lines and normal and tumoral tissue extracts was performed with antibodies against 3 differentially expressed TAAs. A specific CRC tissue microarray was tested with an antibody against one of these differentially expressed proteins. Thirty-five serum from colorectal cancer patients and 23 from healthy individuals were tested by ELISA with 4 purified antigens.

Results: A preliminary autoantibody signature specific for CRC has been identified. We have verified by immunoblotting the differential reactivity of three of those proteins. Pim1 and MAPKAPK3 were more abundant in tumoral samples, whereas ACVR2B was decreased. We have also validated by tissue microarray the absence of ACVR2B in colon tumoral samples. Finally, to determine the discrimination capacity between normal and tumoral groups, we tested 58 serum samples by ELISA using the purified antigens and constructed the ROC curves. Applying statistical models, the combination of Pim1 and ACVR2B exhibited a specificity and sensitivity of 71,4% and 77,1% to discriminate between groups.

Conclusions: Protein microarrays as novel high-throughput proteomic approaches have accelerated the interest in human serum autoantibodies against cancers for the discovery of candidate TAAs to be used as specific tools to diagnose cancer. We have identified a set of potential biomarkers for CRC diagnosis and also as potential immunological therapeutic targets that is being currently evaluated.

P. 37

HALOLINK™ PROTEIN ARRAYS FOR FUNCTIONAL ANALYSIS OF PROTEINS

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Detection of protein interactions and function are key for dentifying protein networks and understanding cellular processes. With miniaturization and multiplexing capabilities, protein arrays have found wide application in deciphering protein interaction networks. Here we present an integrated approach for creating protein arrays that combines *in-vitro* protein expression of a probe protein with HaloTag® immobilization technology. The method allows for rapid and covalent capture of HaloTag fusion proteins onto a glass slide surface directly from complex protein mixtures (e.g. cell lysates) without any prior purification. Multiple functional analysis of proteins of interest may be performed in parallel with these arrays. Examples of this analysis are reported, including end-point and kinetic protein-protein interactions, protein-DNA interactions, and modulation of protein interactions with agonists and antagonists.

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SCREENING AND PROFILING PROTEIN EXPRESSION IN HUMAN CANCER SERUM USING ANTIBODY ARRAY TECHNOLOGIES

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There is a growing need for technologies that enable discovery and validation of protein biomarkers in human serum/plasma. Antibody microarrays have been used successfully to rapidly identify and characterize protein expression in a targeted approach. In this study, antibody arrays were used to interrogate proteome differences in whole serum and in serum that had been depleted of twenty high abundance proteins. The depletion technology enhanced the identification of the lower abundance tissue leakage proteins, as compared to non-depleted serum samples. Antibody arrays were also used to profile differential protein expression between serum from normal and diseased patients. Proteins were identified which displayed significantly different expression levels between the samples. Results were validated with ELISA analysis.

This study showed that antibody arrays are a powerful tool for rapid expression profiling of proteins and may potentially be applied to biomarker discovery and validation in diseased serum samples.

P. 39

THE C-TERMINAL PEPTIDE OF H-RAS AS A TARGET FOR THE COVALENT BINDING OF DRUGS MODULATING RAS-DEPENDENT PATHWAYS

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Ras proteins are crucial players in differentiation and oncogenesis and thus constitute important drug targets. The localization and activity of Ras proteins are highly dependent on PTMs at their C-termini. In addition to an isoprenylated cysteine, H-Ras, but not other Ras proteins, possesses two cysteine residues (Cys181 and Cys184) in the C-terminal hypervariable domain that act as palmitoylation sites in cells. Therefore, the enzymes responsible for these modifications of H-Ras and the target residues are the subject of intense study as sites for therapeutic intervention. Here we describe the potential of several endogenous and exogenous small molecules to bind to a synthetic peptide from the hypervariable domain of H-Ras proteins. Cyclopentenone prostaglandins (cyPG) are reactive lipidic mediators that may bind covalently to proteins and activate H-Ras dependent pathways. Our results indicate that the dienone cyPG 15-deoxy-$\Delta^{12,14}$-PGJ_2 and Δ^{12}-PGJ_2 can bind simultaneously Cys181 and Cys184 of H-Ras *in vitro*, thus possibly inducing important conformational changes of the hypervariable domain. In contrast, single enone cyPG bind the two cysteines independently, whereas cyclopentenone, which lacks the lateral chains of the PG, is a very poor modifier of this peptide and does not activate Ras-dependent pathways in cells. Interestingly, among several small molecules currently being tested, phenylarsine oxide, a widely used tyrosine phosphatase inhibitor, effectively binds H-Ras hypervariable domain. These observations open new perspectives for the study of molecules potentially modulating Ras-dependent pathways.

P. 40

BIOLOGICAL AND FUNCTIONAL ANALISYS OF INTERACTIONS AMONG TETRASPANIN-ASSOCIATED PROTEINS IN HUMAN T LIMPHOCYTES BY HIGH-THROUGHPUT METHODS USING SECOND GENERATION PROTEOMICS TECHNIQUES

Daniel Pérez-Hernández[1&2], Elena Bonzón-Kulichenko[1], Pablo Martínez-Acedo[1], Pedro J. Navarro[1], Estefanía Núñez[1], María Yáñez-Mo[2], Mónica Sala-Valdés[2], Mª Ángeles Ursa[2], Francisco Sánchez-Madrid[2] and JesúsVázquez[1]

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The tetraspanin superfamily of transmembrane proteins are clustered in compact structural groups forming specialized membrane microdomains (Tetraspanin Enriched Microdomains, TEM or TERM). Through heterolog and homolog interactions, tetraspanins regulate signalling processes mediated by cellular adhesion molecules, growth factor receptors and costimulatory proteins, and are also implicated in antigen presentation and viral growth in infected cells.

In spite of the growing interest for these proteins, the cytosolic interactions by which tetraspanins are involved in various receptor activation pathways, their cytoskeleton anchorage and in general the protein ligands that interact with these proteins are poorly known. In this work we made a systematic analysis of interacting partners of different proteins that are presented in TERMs, including ICAM-1, CD81, and EWI-2 by "pull-down" techniques and high-throughput MS/MS protein identification. Synthetic biotinylated peptides spanning the C-terminal cytoplasmic end of these proteins were incubated with extracts from different cell models, including HeLa cells and lymphocytes, and then captured using Streptavidin-sepharose microbeads. Proteins interacting with the peptides were subjected to digestion and automated shotgun identification from the MS/MS spectra by using the pRatio software developed by our group at a 5% error rate. In total more than 1.000 interacting proteins were identified, from which a reduced subset were only found associated to one peptide, indicating highly specific interactions.

Proteins that were selectively identified by more than 4 different peptides in each case were analyzed as a whole using Systems Biology tools (Ingenuity Pathways Analysis). The proteins associated to ICAM-1, CD81 and EWI-2 peptides showed a marked functional interrelation in each group. These results suggest that proteins linking the citoplasmic domains of tetraspanins form a coherent network of interactions playing an important role in the processes of diapedesis between T lymphocytes and vascular endothelium.

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HIGH-MASS MALDI TOF MASS SPECTROMETRY AND CHEMICAL CROSS-LINKING FOR INTERACTION ANALYSIS

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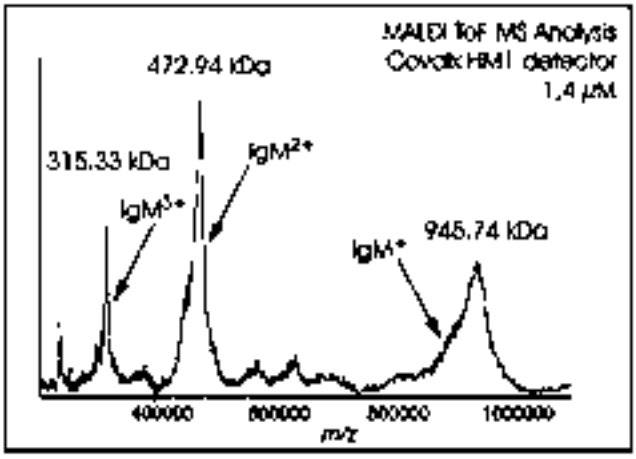
The analysis of intact protein complexes by mass spectrometry is still challenging. Here we present an approach based on high-mass MALDI ToF mass spectrometry and chemical cross-linking. To circumvent the problem of dissociation when using MALDI ionization, a specific cross-linking protocol has been developed to stabilize covalently the samples. To solve the problem of detection, we are using a specially developed high-mass detection system, allowing sub-µM detection up to 1000 kDa. The use of this methodology presents a number of advantages: Sensitivity (sub-µM), tolerance for samples impurity, speed. We will present with details the high-mass technology used and show comparison spectra with MCP detection, the technology used in most of standard MALDI ToF instruments. We will also present examples of applications of this methodology in the field of protein complex analysis (intact protein complexes ranging from 40 to 1000 kDa), antibody characterization (Interaction analysis, Sandwich assays, Epitope mapping), Therapeutic protein aggregates analysis and drug discovery.



High-Mass MALDI ToF MS analysis of IgM (945.74 kDa; 300 nM) using CovalX Hm1 high-mass detection system

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LECTIN-SUGAR INTERACTIONS DECIPHERED BY SPR-MS AND CREDEX-MS

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Interest in sugar-protein interactions has been rising significantly over the last decades. The role of these interactions in processes such as bacteria-host recognition, viral entry, fertilization and metastasis, justifies the search for powerful, nanosized analytical tools to study the corresponding mechanisms.

Here we report on two complementary analytical techniques that provide both quantitative and qualitative data of the interaction with high sensitivity, low sample consumption and without the requirement of sample labelling. On one hand, with surface plasmon resonance (SPR), kinetic and thermodynamic parameters of the interaction can be determined in real time. In this approach the sugar is immobilized on a chip surface through a tailor-made peptide module[1,2], the protein flown across and the resulting read-out enables both quantitation and kinetic analysis of the interaction. Subsequently, interacted material can be recovered under optimized conditions for mass spectrometric characterization.

On the other hand, a combination of proteolytic excision of protein-carbohydrate complexes and mass spectrometry (CREDEX-MS)[3] allows to identify the peptide motifs at the carbohydrate binding site. Here, the sugar is immobilized to a functionalized Sepharose column and the lectins passed through. After on-column digestion of the complex, sugar-bound peptides are eluted and identified by mass spectrometry[3].

In this presentation we will describe the combination of these two methodologies for sugar-protein interaction studies and demonstrate their applicability with several legume lectins that display different sugar specificities.

1. Vila-Perelló, M., Gutiérrez-Gallego, R., Andreu, D. Chembiochem 6, 1831-1838 (2005)
2. Jiménez-Castells, C., de la Torre, B.G., Gutiérrez-Gallego, R., Andreu, D. Bioorg. Med. Chem. Lett. 17, 5155-5158 (2007)
3. Przybylski M., Moise A., Svobodova E., Siebert H., Gabius H. J Pept. Sci. Supplement to Vol. 14, 40 (2008)

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MONITORING *IN VIVO* PROTEIN-PROTEIN INTERACTIONS BY COUPLING BIMOLECULAR FLUORESCENCE COMPLEMENTATION (BIFC) AND FLOW CYTOMETRY

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We present a high-throughput approach to study weak protein-protein interactions by coupling bimolecular fluorescent complementation (BiFC) to flow cytometry (FC). Bimolecular Fluorescence Complementation (BIFC) is a method to detect protein-protein interactions based on the formation of a fluorescent complex by fragments of the yellow fluorescent protein (NYFP and CYFP) brought together by the association of two interaction partners fused to the fragments[1]. For weak protein-protein interactions, the detected fluorescence is proportional to the interaction strength, thereby allowing *in vivo* discrimination between closely related binders with different affinity for the bait protein[2,3]. FC provides a method for high-speed multiparametric data acquisition and analysis; the assay is simple, thousands of cells can be analyzed in seconds and, if required, selected using fluorescence-activated cell sorting (FACS). The combination of both methods (BiFC-FC) provides a technically straightforward, fast and highly sensitive method to validate weak protein interactions.

On the other hand, the combination of both technologies has also been applied to detect the interference of protein interactions[4]. In a test case, we demonstrate that the inhibition of protein interactions results in a concomitant decrease in fluorescence emission. It has to be taken into account that the identification of molecules able to modulate protein contacts is of significant interest for drug discovery and chemical biology. Therefore, the combination of BIFC with flow cytometry might provide an effective means to detect interaction modulators among large chemical or peptidic libraries by directly reading out changes in the reporter signal.

1. Hu CD, Grinberg AV, Kerppola TK. (2006) *Curr Protoc Cell Biol.* Jan **21**:Unit 21.3
2. Morell M, Espargaro A, Aviles, FX, Ventura, S (2007) *Proteomics* **7,** 1023-36.
3. Morell M., Espargaro A., Aviles FX., Ventura S (2008) *Nature Protocols* **3**, 22-33
4. Morell M, Czihal P, Hoffmann R, Otvos L, Avilés FX, Ventura S (2008) *Proteomics* **8**: 3433-42.

Posters

S5. Human Proteomics and Biomarkers

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STUDY OF DIFFERENTIAL PROTEIN EXPRESSION IN HEALTHY HUMAN SKELETAL MUSCLES USING ELECTROPHORESIS BIDIMENTIONAL

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Muscular dystrophies constitute a group of neuromuscular disorder characterized by progressive loss of muscle strength and integrity and degenerative muscle changes. Magnetic resonance imaging (MRI) allows distinguishing different patterns of muscle involvement specific for each muscular dystrophy. Interestingly, some muscles are spared at late stages of the disease regardless of the specific muscular dystrophy (e.g. gracilis). In this study we analyzed the protein expression profile of different normal muscles in order to understand the molecular mechanisms that may be protective for these muscles. We have used two-dimensional gel electrophoresis (2-DGE) combined with mass spectrometry (MS) for this analysis. We were able to perform a reference map of proteins from different muscles obtained from thirty two healthy controls, including: (6) gracilis, (6) semitendinous, (4) tibialis anterior, (4) vastus intermedius, (4)soleus, (4) adductor longus, and (4) adductor brevis.

Proteomic profiling revealed that out of 4411 spots resolved by 2-DGE, 143 proteins exhibited a dramatic change in expression. We found differential expression of proteins involved in muscular contraction, such as troponin, tropomyosin and myosin isoforms we also found proteins important in skeletal muscle metabolism such as aldolase, fatty acid binding protein or triose phosphate isomerase. Finally, we identified proteins that participate in muscle regeneration and repair, sarcomere organization and development and maintenance of Z-disc and ubiquitination such as myozenin-1, and actinin – associated LIM protein (ALP), alphaB-crystallin or TRIMM72. In conclusion, 1) 2-DGE showed a protein expression profile specific for each muscle studied. 2) The specific upregulation of proteins involved in sarcomere stabilization and skeletal muscle remodelling in gracilis and semitendinous muscles, may explain the endurance of these muscles during the process of muscle degeneration in muscular dystrophies.

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PROTEOMIC ANALYSIS OF THE HUMAN RECEPTIVE *VS* NONRECEPTIVE ENDOMETRIUM UNVEILED A FUNCTIONAL RELEVANCE OF STATHMIN 1 AND ANNEXIN A2

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During the so-called "window of implantation", a two-day period along which the endometrium is receptive to embryonic implantation, the luminal endometrial epithelium acquires a receptive phenotype through specific structural and functional changes, encompassing modifications in the plasma membrane and cytoskeleton.

Despite that a complete gene expression profile of the endometrium throughout the menstrual cycle has been achieved in recent years, the proteomic description of the window of implantation has not yet been addressed. We aimed to compare the proteomic profile of the human endometrium 2 (pre-receptive) and 7 days (receptive) after urinary luteal hormone surge in the same menstrual cycle from 8 fertile women (corresponding to days 16 and 21 of the menstrual cycle), identifying and quantifying the proteins differentially expressed using DIGE and MS. Proteins were extracted and labeled with CyDye DIGE fluorofores and separated using 2-DE. Image analysis using the DeCyder® software revealed a distinctive proteomic repertoire during the window of implantation, and 34 differentially expressed proteins were identified by MALDI-MS followed by database searching. Interestingly, stathmin 1 and annexin A2, two cytoskeleton-related proteins, displayed an opposite regulation in the receptive *vs* pre-receptive endometrium. Western Blot and immunohistochemistry provided validation and localization of stathmin 1 and annexin A2. Furthermore, when we induce a refractory endometrium by the insertion of an intrauterine device (IUD), the proteomic pattern of these two molecules becomes the opposite of a normal situation. These results put forward these proteins as potential key targets for human endometrial receptivity and interception.

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IDENTIFICATION OF REPLICATION-COMPETENT HSV-1 CGAL$^+$ STRAIN SIGNALLING TARGETS IN HUMAN HEPATOMA CELLS BY FUNCTIONAL ORGANELLE PROTEOMICS

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In the present work, we have attempted a comprehensive analysis of cytosolic and microsomal proteomes to elucidate the signalling pathways impaired in human hepatoma cells (Huh7) upon Herpes Simplex Virus Type 1 (HSV-1 Cgal$^+$) infection. Using a combination of Differential in Gel Electrophoresis (DIGE) and nanoLC-MS/MS, 18 spots corresponding to 16 unique deregulated cellular proteins were unambiguously identified, which are involved in the regulation of essential processes such as apoptosis, mRNA processing, cellular structure and integrity, signal transduction and Endoplasmic-Reticulum Associated Degradation (ERAD) pathway. Based on our proteomic data and additional functional studies target proteins were identified indicating a late activation of apoptotic pathways in Huh7 cells upon HSV-1 Cgal$^+$ infection. Additionally to changes on RuvB-like 2 and Bif-1, down-regulation of Erlin-2 suggests stimulation of Ca^{2+}-dependent apoptosis. Moreover, activation of the mitochondrial apoptotic pathway results from a time dependent multi-factorial impairment as inferred from the stepwise characterization of constitutive pro- and antiapoptotic factors. Activation of Serine-threonine protein phosphatase 2A (PP2A) was also found in Huh7 cells upon HSV-1 Cgal$^+$ infection. In addition, PP2A activation paralleled dephosphorylation and inactivation of downstream MAP kinase pathway (MEK1/2, ERK1/2) critical to cell survival, and activation of proapoptotic Bad by dephosphorylation of Ser112. Taken together, our results provide novel molecular information that contributes to define in detail the apoptotic mechanisms triggered by HSV-1 Cgal$^+$ in the host cell and lead to the implication of PP2A in the transduction of cell death signals and cell survival pathway arrest.

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DIFFERENTIAL PROTEIN EXPRESSION IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCs) OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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Plasma membrane and cytosolic proteins were extracted from PBMCs lysates upon separation from intact nuclei and NP-40 insoluble material from 14 SLE patients and 15 healthy controls. Proteins were subjected to two-dimensional gel electrophoresis and protein expression patterns were analyzed by using the PD-Quest software to select spots that were differentially expressed between SLE and healthy controls PBMCs. A total of 110 different proteins were identified by matrix assisted laser desorption-time of flight-mass spectroscopy. The molecular functions of these proteins as well as the biological process in which they participate were assigned in accordance with the Human Protein Resource Database (www.hprd.org). The list includes, but is not limited to, polypeptides involved in various biological processes such as signal transduction and cell communication (30%); energy metabolism (7.3%); protein metabolism (17.3%); cell growth and/or maintenance (25.4%); immune response (5.4%); protein folding and peptide metabolism (4.5%); regulation of nucleobase, nucleoside, and nucleic acid metabolism (5.4%); extracellular (2.7%), and 1.8% of the proteins are of unknown function. The calcium-binding proteins S100A8 and S100A9, and the alpha chain of L-lactate dehydrogenase were over-expressed in PBMCs of SLE patients. In contrast albumin, calreticulin, Ras suppressor protein 1, and cyclophilin A were down-regulated. Cyclophilin deficiency is associated with diminished activation of nuclear factor of activated T lymphocytes c (NFATc) and impaired Th2 responses. To test whether SLE patients had an impaired Th2 response the Bio-Plex Precision Pro Human Cytokine 10-Plex kit assay (Bio-Rad, Hercules, CA) was used to simultaneously test 10 cytokines: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 (p70), IL-13, IFN-γ and TNF-α in either plasma and/or in tissue culture supernatants from PBMCs stimulated overnight or not with superantigen. The data showed that in most SLE patients there was an unbalanced cytokine production toward Th1 or proinflammatory cytokines, which may correlate with the cyclophilin deficiency.

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PARALLEL USE OF CID, HCD AND ETD FOR CHARACTERIZATION OF PROTEIC ALLERGENS FOUND IN POLLENSOMES

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In Mediterranean countries, olive (*Olea europaea*) pollen constitutes one of the most important causes of allergy, a major health problem of most modern societies. Recently, it has been shown that allergenic pollen-derived submicronic particles can be present in the air. These pollen-derived particles can reach the respiratory lower airways, eliciting allergic symptoms in susceptible subjects. In the context of studying the mechanism for the release of respirable allergen-bearing particles from olive pollen on hydration, we analyzed the protein content of these novel particles, named by us as pollensomes. In order to identify the highest possible number of proteins present in pollensomes, we analyzed tryptic-derived peptides by nHPLC coupled to LTQ-Orbitrap XL, where peptides were fragmented combining three different types of fragmentation, *i.e.* collision induced dissociation (CID), higher energy collision induced dissociation (HCD) and electron transfer dissociation (ETD).

As a result, pollensomes display a discrete set of proteins, some of which previously described in exosomes derived from animal cells. In addition, two major allergens of olive pollen were detected. These findings indicate that pollensomes from germinated pollen serve as vehicles for allergens, with a potential role on the induction of allergic reactions. The performance of the LTQ-Orbitrap XL for these analyses and the complementarity of the parallel detection method proposed here are also discussed.

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GENOMIC AND PROTEOMIC ANALYSES REVEAL A RELATIONSHIP BETWEEN WNT PATHWAY GENES, OXIDATIVE STRESS METABOLISM AND VASCULAR CALCIFICATION

Pablo Román-García[1], Natalia Carrillo-López[1], Maria I Mora[2], Manuel Naves-Díaz[1], Jose L Fernández-Martín[1], Fernando J Corrales[2] and Jorge B Cannata-Andia[1]

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Introduction and Aims: Vascular calcification (VC) is a highly prevalent condition and one important cause of mortality in chronic kidney disease (CKD) patients. The aim of this study was to analyze the change of expression of genes and proteins that occurs in the development of VC.

Methods: Rats with 7/8 nephrectomy fed with high (0.9%) or normal (0.6%) phosphorus diet were used. Rats were sacrificed after 8, 16 and 20 weeks after surgery (5 animals per group). Serum biochemical parameters and bone mineral density in tibia were measured. Von Kossa staining of the aorta was carried out to detect the presence of VC. Differential gene and protein expression in aortas were assessed by gene expression microarrays and DIGE followed by MS and LC/MS-MS, respectively.

Results: Only the animals fed with high phosphorus during 20 weeks developed VC. Moreover, this group showed a significant decrease in renal function and bone mass, and a significant increase in serum P, iPTH and mortality. In the calcified aortas; at gene level, 3 secreted related frizzled proteins genes (SFRPs), inhibitors of the wnt pathway, involved in bone formation, were up-regulated. At protein level, 40% of the proteins with significant changes in their expression belong to the oxidative stress metabolism. Muscle related proteins were down regulated.

Conclusions: The SFRPs and the oxidative stress metabolism seem to play a role in the development of VC and it may play also a role in the reduction of bone mass.

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IGG PURITY ASSAY USING A NEW HIGH RESOLUTION SDS-GEL

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Capillary SDS-Gel electrophoresis of proteins has been an important analytical method used to characterize, monitor process control and provide quality control for the production of immunoglobulins. In this poster we present the development of a new polymer formulation and standardized methodology to assess the purity and heterogeneity of IgG and its isoforms. This methodology provides increased resolution of the IgG isoforms from typical developmental impurities like non-glycosylated heavy chain, and low molecular weight impurities associated with the IgG light chain. The assay includes an artificially engineered IgG control with a fixed percentage of non-glycosylated heavy chain to provide assay suitability determination prior to the analysis of unknowns. All aspects of the methodology from sample preparation to automating data analysis will be discussed.

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IN-DEPTH, COMPREHENSIVE MAPPING OF THE HUMAN SEMINAL PLASMA PROTEOME BY A NOVEL, ITERATIVE LC-MS/MS/DATABASE SEARCH WORKFLOW

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Apart from its obvious role in transporting male gametes, the seminal plasma provides a protective environment for ejaculated spermatozoa and improves their fertilizing potential. This fluid is also a potential source of biomarkers for male reproductive disorders. Here we describe a method for enhanced protein identification in complex samples. A combination of iterative LC-MS/MS, exclusion list generation (including masses and retention time) and iterative database searching was used to analyze in depth the seminal plasma proteome.

Non-liquefied seminal plasma (500mg proteins) from a healthy donor was loaded onto two sequential hexapeptide ligand libraries (1ml each; ProteoMiner™ –primary amino terminal peptides– and a carboxylated form, Bio-Rad Laboratories). Proteins bound on both libraries were desorbed through 4 different elution buffers, generating 8 complementary and treated sub-proteomes. Each fraction was then trypsin-digested and analysed by nano-LC-MS/MS with an LTQ Orbitrap XL™ mass spectrometer (Thermo Fisher Scientific). Peptides were preconcentrated with a peptide Captrap cartridge (MICHROM Bioresources, Inc.) and separated onto a 15cm x 100µm capillary column. Detected peptides were selected for CID fragmentation using data dependent criteria and MS/MS spectra were searched against a SwissProt human database (April 2008) for peptide characterisation and protein identification. A list of peptide masses resulting from database searching was generated with Proteome Discoverer™ for each sample and these identified peptide masses used to generate an exclusion list, including retention times, for subsequent LC-MS analysis of the same sample. A second database search was performed. Identified peptide masses from the first and second LC-MS experiments were combined to generate a second, longer exclusion list to be used for a third round of mass spectrometry analysis and subsequent database searching and peptide and protein identification.

This procedure resulted in the identification of many additional peptides in the seminal plasma sample. More than 80% of the peptides identified in run 2 and 3 were new and could be used to confirm "one hit wonder" protein hits, increase protein coverage or identify new proteins. A total of 864 proteins were identified at a 5% FDR demonstrating the benefit of this strategy for increasing the dynamic range of identified proteins toward a deep proteomic characterization of seminal plasma.

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IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN ACUTE CORONARY SYNDROMES

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Acute Coronary Syndromes (ACS) are triggered by the occlusion of a coronary artery, usually due to the thrombosis of an atherosclerotic plaque. The study of the mechanisms that lead to the plaque thrombosis has been one of the hot spots in cardiovascular research during the last years. A screening for biomarkers in the blood of ACS patients using proteomic tools could provide useful diagnostic and therapeutic information. We have performed a comparative study of plasma from ACS patients combining immunoaffinity depletion with 2-dimensional difference gel electrophoresis (2D-DIGE) and identification by mass spectrometry.

Depleted plasma samples from 28 patients with ACS at day 0, 4, 90 and 180, were compared to samples from 10 stable coronary artery disease (CAD) patients and 10 healthy volunteers, matched for age and sex. DeCyder software revealed statistically significant variations of 47 protein spots in depleted plasma samples from ACS and stable CAD patients in comparison with healthy subjects. The number of differentially expressed spots tended to increase along time, conversely to that observed for circulating monocytes. The identified proteins were involved in different physiological processes, some of whom may play a role in the pathophysiology of the atherotrombotic disease.

We have also studied the effect of intensive treatment with statins in comparison with moderate treatment, assigning patients to receive either 80 mg/d atorvastatin (ATV) or standard therapy during two months after ACS. Expression levels of 15 spots were affected by both treatments, while 14 different spots were normalized by ATV with respect to standard therapy and 4 more spots were only altered by ATV.

Further validation of these findings in larger populations would prove the usefulness of these proteins as novel biomarkers of ACS. These results might help to enrich the current knowledge of molecular mechanisms involved in ACS and improve the existing diagnostic tools.

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NUCLEOSIDE DIPHOSPHATE KINASE A (NM23-H1) AS A POTENTIAL BIOMARKER CANDIDATE FOR COLORECTAL CANCER

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In this work, with the aim of finding proteins that could serve as novel biomarkers for colorectal cancer (CRC), we performed a search focused on soluble proteins that could be involved in cancer-related processes, such as signal transduction or metastasis. Among the identified proteins, we propose the nucleoside diphosphate kinase A (NDK A or nm23-H1) as a potential candidate.

First, we performed a pre-fractionation method to obtain fractions enriched in soluble proteins from mucosa and tumour tissue of 10 CRC patients with lymph node metastases. Then, proteins were separated by two-dimensional electrophoresis (2-DE) and the ones found altered were analysed applying principal component analysis (PCA) and linear discriminant analysis (LDA). These statistical methods allowed us to find a group of proteins with potential utility as a panel of markers for CRC, which were submitted to mass spectrometry (MS) for identification. Among the identified proteins, we found peroxiredoxins, the oncoprotein DJ-1, the calcium binding protein S100A11, the tubulin-specific chaperone A (CFA), the retinoblastoma-binding protein 4 (RBBP-4), the 14-3-3 zeta protein, an enzyme involved in the control of DNA methylation (AHCY) and other enzymes related to angiogenesis (PD-ECGF/TP) and to the metastatic potential of tumours (NDK A).

Regarding NDK A, its role in CRC metastasis is still controversial, since both overexpression and downregulation have been reported. In our study, it was found increased (4.3 times) in tumours and the graphic representation of its level in 2-DE gels allowed an effective separation of mucosa and tumour samples. Furthermore, the upregulation of NDK A was corroborated by Western blot, and, it also represented one of the more relevant proteins pointed out by PCA. Noticeably, preliminary results showed that serum NDK A levels tended to be higher in CRC patients with distant metastases. In conclusion, NDK A seems to be a potential biomarker for CRC.

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IDENTIFICATION OF DIFFERENTIAL PROTEINS IN LIVER CELLS UPON DEPLETION OF PROHIBITIN

Virginia Sánchez Quiles, Laura Sesma, Enrique Santamaría, and Fernando J. Corrales

Liver diseases afflict more than 10% of the world population. Although in most cases hepatopaties present slow progression, the risk factors are known and the population at risk is monitorized, the prognostic of patients with severe liver damage is poor. Development of efficient therapies greatly depends on a better understanding of the molecular pathogenesis of liver diseases and in the identification of biomarkers allowing early diagnosis. Prohibitin (PHB) plays a central role in the maintenance of liver homeostasis since, in the hepatocyte, it participates in essential cellular pathways including cell signalling, apoptosis, cell survival and proliferation, through the regulation of central proteins involved in these processes by means of protein-protein interaction mechanisms. Recent studies suggest that PHB impairment participate in the inflammatory reaction associated to both acute and chronic liver diseases as well as in the progression of liver fibrosis, common alterations to most pathological conditions in human liver disorders. In the last few years evidences have been accumulated that support the implication of PHB in the etiopathogenesis of non-alcoholic steatohepatitis (NASH), liver fibrosis and cirrhosis, and hepatocellular carcinoma (HCC). In this work we have investigated the molecular mechanisms underlying the participation of PHB in the progression of liver diseases. PHB was impaired in PLC human liver cancer cells using specific siRNAs and differential proteins relative to control PLC cells were detected in various subcellular fractions by DIGE analysis. Decyder analyses revealed 76 and 25 differential spots in the cytosolic and microsomal fractions respectively. Differential spots were then analyzed by nanoLC-ESI-MS/MS and 35 unique proteins were identified. Besides, further functional analyses indicate deregulation of the proteasome system in addition to alterations in mitochondrial and endoplasmic reticulum integrity in liver cells in response to PHB deficiency in liver cells.

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PREDICTION OF THE CLINICAL OUTCOME IN INVASIVE CANDIDIASIS PATIENTS BASED ON SERUM ANTI-*CANDIDA* ANTIBODY PROFILES

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Invasive candidiasis (IC) remains a leading infectious cause of morbidity and mortality in severely immunocompromised and/or critically ill patients. Unfavorable outcomes of IC could be preventable by early and appropriate implementation of antifungal therapy. However, no reliable clinical or molecular indicators are currently available for predicting the likely clinical outcome in IC patients at presentation. This clinical setting has prompted the search for accurate prognostic biomarkers of IC at an early stage with the intention that therapeutic strategies may be tailored accordingly. The goal of this study was to identify and validate accurate prognostic features in IC patients at presentation by screening of their serum anti-*Candida* IgG antibody profiles. To address this goal, we combined serological proteome analyses with data mining procedures. Two-way hierarchical clustering and principal-component analyses of reactivity patterns of 31 anti-*Candida* IgG antibodies segregated IC patients into two clusters with distinct prognoses. These subgroups were independent of baseline characteristics of the study population. Supervised analysis with cross-validation identified a panel of five anti-*Candida* IgG antibodies as the best prognostic predictor for IC. The robustness of this five IgG antibody set was confirmed using an independent data set. This panel was able to accurately predicting risk of a fatal outcome in IC patients at presentation, and allowed delineation of a high-risk group that may benefit from aggressive antifungal therapy. Multivariate logistic-regression analysis revealed that this five IgG antibody set was an independent clinical-outcome predictor of IC, and further provided additional discriminative power over known prognostic factors for IC. We conclude that IC patients could be stratified according to their prognosis by examining a reasonably small number of predictor variables (IgG antibodies). If our results are confirmed in future larger prospective studies, this new model could be useful in predicting patient outcome for individualized therapy of IC.

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HIGH RESOLUTION 2-DE-BASED PROTEOMIC ANALYSIS OF HUMAN ATRIAL FIBRILATION

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Introduction. Atrial Fibrilation (AF) is the most common cardiac arrhythmia found in clinical practice. It is associated with significant mortality and morbidity from stroke, tromboembolism and heart failure. It is caused by chaotic electrical impulses in the atria of the heart and results in a heart rate fast and irregular and loss of coordination between the atria and the ventricles. Our aim is to identify new biomarkers that will allow a better understanding of the molecular factors that trigger AF using high resolution 2-DE to compare the atrial proteome between subjects with AF and controls with sinus rhythm (SR).

Methods. Human atrial appendage tissues from 16 patients that underwent heart surgery with AF or SR were snap frozen in liquid nitrogen. The tissue was homogenized in lysis buffer using a mortar and a pestle. Proteins were precipitated with TCA/Acetone, and separated by 2-DE (500 μg per gel). First dimension was with 24 cm, pH 4-7 IPG strips. Second dimension was by 10% SDS-PAGE. Gels were stained with SyproRuby. Samples were grouped according to the clinical characteristics of the patients. Images corresponding to 8 gels (4 AF and 4 SR) were analysed using Ludesi REDFIN software. Proteins present in spots of interest are identified by MALDI-TOF/TOF.

Results. Our analysis allowed the detection of over 2,000 spots per gel. Following differential image analysis, we found 22 spot differences between the AF and SR group in the 4-7 pI range. The fold change was at least 2, and $p<0.05$ after ANOVA test was applied on normalised spot volumes.

Conclusions. We have standardised sample preparation conditions to achieve high resolution proteome maps of atrial tissue. Proteins identified from the 22 spot differences detected between the AF and the SR groups will be further studied and validated as potential biomarkers for AF.

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HYPOXIA CONDITIONS DIFFERENTIALLY MODULATE NORMAL AND OSTEOARTHRITIC CHONDROCYTE PROTEOMES

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Osteoarthritis (OA) is a degenerative disease characterized by the degradation of articular cartilage. This tissue is avascular, and it is characterized by the low oxygen tension and poor nutrient availability for its cells, the chondrocytes. Hypoxia conditions have been reported to stimulate chondrogenesis and synthesis of extracellular matrix components. Therefore, we pursued to examine the effect of hypoxia on normal and osteoarthritic cartilage cells.

Chondrocytes obtained from healthy and osteoarthritic donors were subjected to hypoxia conditions during 96 hours. Whole cell proteins were then isolated and resolved by 2-D electrophoresis. Gels were stained with SYPRORuby, and image analysis was performed using PDQuest software. Proteins of interest were identified by MALDI-TOF/TOF mass spectrometry.

We examined a mean of 500 protein spots that were present in each gel. Both qualitative and quantitative changes in protein expression patterns were studied. 32 protein forms were found to be modulated by hypoxia in normal cells and 16 in osteoarthritic cells when compared to control. We also identified 44 protein forms that were altered in normal cells under hypoxia compared to osteoarthritic chondrocytes. We observed a decrease in many metabolism-related proteins. The biggest difference between normal and OA chondrocytes was found in the proteins involved in glycolosis.

In conclusion, hypoxia induces different modifications in the proteome profile of human articular chondrocytes. OA and normal chondrocytes have different capacity of response to hypoxia.

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DEVELOPMENT OF A PROTOCOL FOR A RAT SPINAL CORD PROTEOME

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Introduction: Several proteomic techniques have been developed for neurodegenerative and psychiatric disorder research, and in particular in the detection of differences between healthy individuals and patients suffering from such diseases[1]. Although brain and cerebrospinal fluid samples from patients with different central nervous system (CNS) disorders have been extensively studied, less research has been focused on spinal cord protein content and the changes induced after injury and the presence of associated symptoms such as neuropathic pain. Indeed spinal cord injury is characterized by a complex relationship between several molecular cascades and the development of a progressive pathophysiology[2].

In the present study we aimed to describe total protein content in the spinal cord of healthy rats, employing different proteomics tools. With this objective we have developed a new sequential protocol for protein extraction from rat spinal cords.

Methods: Spinal cords were extracted from 10 week old male rats injected with an overdose of Pentobarbital. The tissue was homogenized in two consecutive protein extraction buffers; the first that most of the soluble proteins and the second the more insoluble proteins. Finally the samples were analyzed using 2DE and LC-MS/MS.

Results: This work presents a very simple, fast and efficient method for spinal cord protein extraction. Approximately 400 to 600 spots were resolved in the 2DE experiments and the LC-MS/MS analysis detected hundred of proteins that complement the 2DE results.

The application of new proteomics protocols to the spinal cord may be a useful tool for the study and identification of differential molecular changes associated with CNS pathologies such as spinal injury and/or neurophathic pain. Altogether, these data could contribute to detect specific markers or potential drug targets for spinal cord injury and neuropathic pain management.

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PROTEOMIC ANALYSIS OF INTIMAL LAYER FROM HUMAN CORONARY ATHEROTHROMBOTIC ARTERIES

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Introduction: The study of coronary artery atherothrombosis is of relevance nowadays as it is one of the leading causes of mortality in developed world. Early detection of the pathology could prevent in most cases complications derived from coronary obstruction due to atherome plaque rupture. Proteomic differential expression analysis of human atherosclerotic coronary arteries versus healthy arteries could highlight diagnostic and prognostic biomarkers of coronary artery disease (CAD). Complete artery tissue proteomic studies involve analysis of many cell types at a time wich may show different proteomic profiles at different locations in the vessel. Atherosclerotic process initiates at the intima so that proteomic analysis of this layer independently of the media and adventitia is mandatory in the search for early diagnosis biomarkers of CAD.

Methods: Intimal layer from 12 human specimens of coronary with atherome plaque and radial (control) biopsies from coronary bypass surgery and atherosclerotic and healthy coronary necropsies was isolated by laser microdissection and pressure catapulting (LMPC). Combination of LMPC with saturation labelling DIGE allowed us to perform 2-DE based differential protein abundance analysis with less than 5 µg of intimal protein subproteome.

Results: 30 spots showed significant variation in atherothrombotic arteries in comparison to healthy arteries. Identification of those spots is carried out on a 4800 MALDI-TOF/TOF Analyzer (Applied Biosystems).

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COMBINATION OF T-GEL CHROMATOGRAPHY AND IMMUNOAFFINITY AS A NEW PROTOCOL TO REMOVE IMMUNOGLOBULINS AND ALBUMIN FROM SERUM SAMPLES: THE CASE OF RHEUMATOID ARTHRITIS

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Quantitative analysis of proteins in serum is essential to find disease biomarkers, and 2-DE is still a unrivalled tool to measure quantitative differences. However, the high dynamic range of proteins in serum (up to 12 orders, which exceeds the dynamic range of 2-DE), the fact that only 1% of total serum protein may contain potential biomarkers, and the reproducibility issue, are all still important aspects to solve. Removal of high-abundance proteins is a common practice, but also one of the biggest challenges. Most of the depletion methods used have a low loading capacity, are costly or need equipment not always available at a lab. Thereby, the present work describes a new approach, where depletion of most of immunoglobulin (Ig) isotypes as well as albumin (HSA) from 1 ml serum samples is achieved by using the combination of two methodologies: a thiophilic chromatography, not previously used in 2-DE, and a HSA-specific immunoaffinity resin, respectively. Samples were analysed by 2-DE to evaluate the effectiveness of the depletion method and a pattern of serum proteins, most of them identified by MS, detected. Reproducibility was also assayed, and a preliminary study with rheumatoid arthritis (RA) patients carried out to test the performance of the new protocol.

RA is a chronic autoimmune disease characterized by inflammation at the synovial joint and infiltration of leukocytes. RA treatments are nowadays more effective, but should be started as early as possible; i.e., new RA biomarkers are necessary. We have analyzed Igs/HSA-depleted sera obtained from 3 healthy individuals and 3 recently diagnosed and untreated RA patients (18 gels), finding significative changes in many negative or positive acute-phase proteins previously described as altered in RA (e.g., transferrin or ApoA1). However, SAP, α1-microglobulin or VSP28 have also come up during this preliminary study as proteins upregulated during RA.

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ANALYSIS OF HUMAN ERYTHROCYTE MEMBRANE AND CYTOSOLIC SUB-PROTEOMES BY 2-DE

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Despite of its simple structure, high hemoglobin content present in erythrocytes enormously difficults their proteomic analysis. We investigate here different strategies for isolation of human membrane and cytosolic fractions of red blood cells and their influence on proteome profiling by 2-DE, paying particular attention to hemoglobin removal.

In a first attempt, the use of "2-D Sample Prep for Membrane Proteins" kit was directly applied to the intact erythrocyte cell. However, the high content of hemoglobin greatly interfered with protein detection in both fractions. An hemoglobin depletion approach based on Hemoglobind® reagent, combined with an additional desalting step prior to IEF, was investigated, resulting in the analysis of both membrane and cytosolic sub-proteomes by 2-DE without major interference of hemoglobin. This methodology provided a clear improvement in 2-DE pattern of the membrane and cytosolic proteins compared to gels obtained from hypotonic lysis isolation. However, cross-contamination between the two fractions was clearly seen. The two strategies were then combined: membrane and cytosolic fractions were first obtained by hypotonic lysis; membrane proteins were further solubilized, purified and desalted on commercial mini-colums (no hemoglobin depletion was needed) and cytosolic fraction was submitted to hemoglobin depletion and further desalting.

The possibility of unselectively remove minor proteins together with hemoglobin was also investigated by SDS-PAGE and MALDI-TOF-MS.

In conclusion, we propose a new strategy for 2-DE analysis of human erythrocyte membrane proteins, based on a commercial extraction and purification kit with variations. Besides, a novel approach for hemoglobin depletion is provided in order to analyse the proteome of cytosolic fraction.

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HUMAN ENDOMETRIAL FLUID ASPIRATE PROTEOMIC ANALYSIS: EXTENSIVE PROTEIN MAPPING BY 2D-ELETOPHORESIS AND MALDI TOF/TOF

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Endometriosis is a widespread condition, frequent in infertile women and progressive. Proteomic approaches have been directed to the discovery of biomarkers and study of this disease by using serum, endometrial tissue or peritoneal fluid. However, a more convenient procedure is to isolate the aspirate of the endometrial fluid because it's a safe, easy and standard clinical procedure, and focused on the target tissue in endometriosis, so the proteome of this secretion directly reflects the state of the endometrium. Endometrial aspirates were collected from women with ages ranging between 18 and 45 with no laparoscopic evidence of endometriosis or other endometrial alteration. Only endometrial fluid aspirates without blood or tissue contamination were pooled. Serum albumin and class G immunoglobulins were specifically immunodepleted. Purified proteins were resolved by 2DE electrophoresis and after Flamingo staining, over 600 different protein spots were detected. Spots were picked and digested in an automatic fashion. MALDI TOF/TOF analyses were firstly performed in automatic fashion, where approximately half of the analyzed spots happened to be unambiguously identified. After supervision, samples with no significative good enough score, were analyzed manually either by MALDI TOF/TOF or nano LC MS/MS mass spectrometry. In this study we report 459 spots identified, compiling 220 proteins, covering 16 protein groups according to Gene Ontology. In our knowledge, this is the first time endometrial fluid aspirate is extensively mapped combining 2DE and mass spectrometry techniques. The resulting proteomic catalogue offers new perspectives for biomarker discovery and non invasive diagnosis, both in endometriosis and embryo implantation studies.

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IN-DEPTH ANALISYS OF JURKAT T-CELL SECRETOME

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Inflammatory mediators secreted by leukocytes play a significant role in the progression of several diseases. These mediators are mainly proteins and their identification will help to better understand the complex signal transduction network of inflammation. Here, we present an in-depth analysis of proteins secreted by lymphoid Jurkat T-cells, using a combination of IEF coupled with liquid chromatography mass spectrometry. Cells were washed with PBS and incubated for 48h with serum-free medium. Cell-free supernatant proteins were concentrated on SDS-PAGE gels by running the sample 3 mm into the resolving gel. The concentrated gel bands were subjected to trypsin digestion and the resulting peptides were separated into 24 fractions by IEF using an OFF-Gel electrophoresis unit. Fractions were analyzed, under high peptide loading conditions, by RP-HPLC-MS/MS using a linear ion trap LTQ MS. More than 500 unique peptides were identified per fraction, resulting in the identification of more than 8,000 peptides at a FDR of 5%, corresponding to more than 2,800 proteins. Identified proteins were compared to the high confidence reference set of human plasma proteins (Schenck et al., BMC Medical Genomics, 2008). Also, after a SecretomeP 2.0 analysis, proteins were termed as potentially secreted via a classical pathway if they contained a signal peptide, whereas those with NN score exceeding 0.6 were classified as secreted by a non-classical pathway. The lack of transmembrane helices or intracellular localization signals was predicted by TMHMM server. By these means, more than 500 unique secreted proteins were identified. Our results may help understanding the secretory functions of resting T-cells.

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APROXIMACION PROTEOMICA AL SINDROME DE APNEAS-HIPOPNEAS DEL SUEÑO

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Introducción: El síndrome de apneas-hipopneas del sueño (SAHS) se caracteriza por frecuentes episodios de obstrucción de la vía aérea superior con episodios de hipoxemia-reoxigenación que pudieran conllevar modificaciones en las proteínas séricas.

Objetivo: Determinar si la expresión de las proteínas séricas en pacientes con SAHS difiere significativamente del grupo control.

Pacientes y método: Estudio prospectivo con muestreo consecutivo; incluyendo 36 pacientes (40 ±6.1 años, 30 hombres y 6 mujeres, IMC 31 ±5.9) de la Unidad de Trastornos Respiratorios del Sueño (HU. Reina Sofía, Córdoba). Se valoraron: historia clínica, examen físico, estudio analítico y radiológico, saturación periférica de oxígeno y polisomnografía diagnóstica de noche completa. Se consideró el diagnostico de SAHS a los sujetos con un índice de apneas-hipopneas (IAH) ≥10 en la polisomnografía incluyendo en el grupo control aquellos con IAH <5. En función del IAH, los pacientes fueron clasificados como SAHS leve (10≤ IAH <15), moderado (15≤ IAH <30), y grave (30≤ IAH). Previo consentimiento informado, se extrajo sangre con tubos Vacutainer® SST, almacenando las muestras séricas a –86 ºC. Por triplicado se realizaron geles 2D PAGE (18cm, pH 3-10) de los 4 grupos clínicos (leve, moderado, grave y control) seguida de una aproximación proteómica cuantitativa con iTRAQ™ de 4 canales. La 2-DE resolvió unas 1400 manchas, encontrándose diferencias significativas en la expresión de 37 de ellas entre los 4 grupos clínicos, habiéndose logrado por iTRAQ™ la identificación y cuantificación relativa de 121 proteínas, 29 de ellas con significación estadística.

Conclusiones: La 2-DE revela diferencias significativas de expresión proteica sérica en los pacientes con SAHS. Por iTRAQ™ se han identificado proteínas varias séricas que pueden ayudar en el diagnóstico, la valoración de la gravedad y el conocimiento de la fisiopatología del SAHS.

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2-DE ANALYSIS OF SERA PROTEIN EXPRESSION PROFILES IN EARLY STAGES OF HUMAN NEONATAL DEVELOPMENT. A PRELIMINARY STUDY

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While survival of premature infants has greatly increased in the last decades, prematurity is still a major cause of mortality and subsequent physical and developmental disabilities despite advances in neonatal care. A correct evaluation of their postnatal growth is nowadays of primary concern, although optimal nutrition is not well-defined and their growth patterns differ markedly from that of full-term infants. Efforts to characterize the variables affecting neonatal outcomes have focused primarily on patient characteristics including birth weight, gender, race, gestational age, and markers of illness severity... but a detailed understanding at the protein level is still missing. Recent advances in human proteomics will open new perspectives in premature´ management. The human proteome, the protein counterpart to the genome, is dynamic and signals a precise physiologic state. Proteomic techniques like two-dimensional electrophoresis (2-DE) and mass spectrometry (MS) are pivotal in the identification of human sera proteins.

Human proteome development was studied from early neonatal stages to fulltime gestational development, using pooled sera of 232 neonates of the following weights at birth: <750g, n=6; 750-999g, n=27; 1000-1249g, n=33; 1250-1499g, n=35; 1500-1999g, n=38; 2000-2499g, n=30; 2500-3499g, n=38; >3500g, n=25. Blood samples were drawn into Vacutainer® SST tube and sera immediately frozen at -86 ºC until use. Protocols were optimized for sera preparation, and first (IEF, pH 4-7) and second dimension separations (SDS-PAGE, 18cm). Samples were run in triplicate and gel images analyzed with Proteomweaver (Bio-Rad®). Different time-course protein patterns were recognized with several differential peaks, at extremely low birth weight (401-999g), very low (1000-1499g), low (1500-2499g), and normal birth weights (2500-3500g).

Identification and quantification of sera proteins and their time-course patterns will allow a new approach in neonatology, starting from the application of proteomic data to discover new protein biomarkers to finally reach a significant decrease of neonatal mortality rate.

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REGULATION OF STATHMIN PHOSPHORYLATION IN LIVER PROLIFERATING CELLS DURING PROTEASOME INHIBITION

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Proteasome inhibitors are novel therapeutic agents which might be used for the treatment of hepatocarcinoma and other liver diseases as they are able to induce cell death in proliferating cells specifically. The analysis of alternative protein phosphorylation states might contribute to elucidate the underlying mechanisms of proteasome inhibitor-induced apoptosis. In the present study we have investigated the response of MLP-29 liver cells to MG132 using a combination of phosphoprotein affinity chromatography, Differential in Gel Electrophoresis (DIGE), and nanoLC-MS/MS. Seventeen spots corresponding to 13 unique deregulated phosphoproteins were unambiguously identified, which are involved in chaperone activity, stress response, mRNA processing and cell cycle control. Some of these protein species, including NDRG1 protein, hnRNP A2/B1, and stathmin suggest new mechanisms associated to proteasome inhibitor-induced apoptosis in proliferating liver cells. Particularly, a transient modification of the phosphorylation state of Ser^{16}, Ser^{25} and Ser^{38}, which are involved in the regulation of stathmin activity, was detected in three distinct isoforms upon proteasome inhibition. The parallel deregulation of calcium/calmodulin-activated protein kinase II (CaMKII), extracellular regulated kinase (ERK1/2) and cyclin-dependent kinase (CDK2), catalyzing the phosphoryl group addition to these Ser residues, might explain the modified phosphorylation pattern of stathmin. Interestingly, stathmin phosphorylation profile was also modified in response to epoxomicin treatment, a more specific proteasome inhibitor. In summary, besides impairment of proteins participating in central cellular pathways, we report here novel mechanisms involved in proteasome inhibition-induced apoptosis that regulate the stathmin phosphorylation status in liver proliferating cells.

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PROTEOMIC ANALYSES OF PITX2 LACK OF FUNCTION IN VENTRICULAR MYOCARDIUM

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The heart is the first organ to display morphologic asymmetry during development. However, a left-right differential gene expression program starts earlier in the lateral plate mesoderm. The final effector of this left-right signalling pathway is the bicoid-related homeodomain transcription factor *Pitx2*. Implication of this factor in heart development has been proposed by analyses of *Pixt2abc* null mice, which display several cardiac malformations. However, the functional and temporal contribution of this transcription factor during heart development is poorly understood. In this work, by using two-dimensional gel electrophoresis (2-DE), we compared the proteome of mice lacking all *Pitx2* isoforms in the ventricular myocardium and outflow tract versus control mice. More than 200 proteins were resolved in each gel. Twenty-two proteins with significative change in expression were detected. Twelve of them were down-regulated in Pitx2 conditional mutant mice whereas ten proteins were up-regulated. Identification of these proteins by mass spectrometry suggests that several cellular processes may be altered in these conditional mutant mice. These cellular processes are metabolism (increase of glycolisis, TCA cycle and BCAA metabolism, and partial inhibition of β-oxidation), energy production (inhibition of oxidative phosphorylation), and cell stress (increase of anti-oxidative defence). The combination of these alterations suggest that specific delection of all isoforms of *Pitx2* in ventricular myocardium and outflow tract could drive to cardiac physiopathology characterised by ischemia and oxidative stress.

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A NOVEL CHROMATOGRAPHIC METHOD ALLOWS ONLINE REANALYSES IN PROTEOMIC INVESTIGATIONS AND ACQUIRING MORE INFORMATION FROM BIOLOGICAL SAMPLES

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Liquid chromatography combined with electrospray ionization is the standard for the analysis of polar molecules by mass spectrometry (LCMS). The online coupling in LCMS is a major strength, but has the limitation that the mass spectrometer is not able to analyze all co eluting compounds immediately. Multiple injections or fraction collection can overcome this problem but time, sample limitation and the difficulty to fraction collect low volumes from a nanoLC system makes this unpractical. A new chromatographic strategy which enables to analyze a LCMS run twice with a single injection is described. After column separation the flow from a 75 µm column at a typical flow rate of 250 nL/min is split, so that part is directed to the mass spectrometer for analysis whilst the remainder flows to a capillary tube where it is stored. After the direct LCMS run, the flow is switched and the portion stored in the capillary is analyzed (‘replay run’). Since electrospray is a concentration dependent process the splitting system maintains full signal at decreased flow rates. An additional short column between the storage capillary and the mass spectrometer refocused the stored peaks in the second analyses so that width and intensity is identical to the initial run. To qualify the set up, the chromatographically performance and MS intensity was compared with a standard nanoLC set up using BSA tryptic digest for typically 60 min separations and mouse liver homogenate for 120-180 min LCMS runs. The chromatography performance and peak intensity for the normal setup are identical with the first and second run of the RePlay setup. Furthermore to explore the ability to analyze a LCMS run twice, examples of **c**ombining exploratory and targeted analysis are shown including the quantitation, using different fragmentation techniques, combining MSMS and MSn analyses or different mass spectrometers are shown.

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URINARY BIOMARKERS FOR BLADDER TRANSITIONAL CELL CARCINOMA

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Bladder cancer ranks 9th in world cancer incidence and has a high complex nature. The most prevalent form of bladder cancer is transitional cell carcinoma (TCC). The non-invasive diagnosis of bladder cancer is principally based on the urinary cytology. This test has a high specificity but a low sensitivity and an intrinsically subjectivity that depends on the pathologist expertise.

Today's efforts to diagnose early stages of bladder cancer and to predict the progression of the disease are based on the discovery of urinary biomarkers. Proteomic technologies are providing the tools needed to discover and identify disease-associated biomarkers. In this context, the objective of the present work is to use proteomic approaches for the identification of novel molecular markers in urine samples.

Urine samples from 10 bladder cancer patients and 10 control subjects were used to perform a differential expression analysis. We have designed a simple protocol to separate the cellular and soluble components from urine and analyze them separately. Protein extracts from urine cells were obtained and analyzed using 2D-DIGE technology. Peptide profiles from the soluble component were determined by MALDI TOF mass spectrometry.

Several differential protein spots were detected ($p<0.05$ after FDR correction) by 2D-DIGE experiments from which only 5 different proteins were identified. One of these proteins showed over a ten-fold increase in tumor samples and it being evaluated as a biomarker by immunohistochemistry. Peptide profiles are also capable to differentiate between the two groups of samples.

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COMPARATIVE PROTEOMIC ANALYSIS OF NORMAL AND NQO1-NULL MOUSE EMBRYO FIBROBLASTS

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NAD(P)H-quinone oxidoreductase 1 (NQO1) is an ubiquitous cytosolic flavoenzyme that catalyzes two-electron reduction of various quinones, with NADH or NADPH as electron donors. The reduction of quinones prevents their participation in redox-cycling and the subsequent generation of reactive oxygen species (ROS). Reduced apoptosis and increased susceptibility to induced tumors and myeloid hyperplasia have been described in NQO1-null ($NQO1^{-/-}$) mice. Thus, NQO1 is involved in the protection against oxidative stress and carcinogenesis. The purpose of the present study was to analyze changes in the levels of proteins of cell lysates obtained from control mouse embryo fibroblasts (MEFs) compared with $NQO1^{-/-}$ MEFs. This approach could give us new insights of the functions of NQO1 in cell growth control. In this work, we have used 2D-PAGE coupled to MALDI-TOF mass spectrometry for the identification of proteins which are differentially expressed between control and $NQO1^{-/-}$ MEFs. This analysis allowed us to identify 4 spots which were significantly increased and 5 spots that were decreased in $NQO1^{-/-}$ cells compared to their normal counterparts. The proteins whose levels were increased in $NQO1^{-/-}$ MEFs were: Tumor necrosis factor receptor-associated protein 1 (TRAP1); Chaperonin containing TCP1, subunit 6A; Glucose-6-phosphate dehydrogenase X-linked, and mitochondrial Mn-superoxide dismutase (Sod2), while the proteins whose levels were decreased in $NQO1^{-/-}$ MEFs were procollagen-lysine, 2 oxoglutarate 5-dioxygenase 3 (PLOD3); Stress-70 protein, mitochondrial precursor (75KDa glucose- regulated protein) (GRP75); mCG12499 and Transgelin. The functions of altered proteins are related with oxidative stress, apoptosis, generation of ROS, and actin assembly. Interestingly, TRAP1 plays a revelant roles in cell-cycle progression and cellular differentiation, and its expression at high-level may be involved in anti-apoptotic effects. Increased levels of TRAP1 as a consequence of the lack of NQO1 gene expression might be related with the higher susceptibility for tumors observed in this animal model.

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DEVELOPMENT OF A PROTOCOL FOR A RAT SPINAL CORD PROTEOME

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Introduction: Several proteomic techniques have been developed for neurodegenerative and psychiatric disorder research, and in particular in the detection of differences between healthy individuals and patients suffering from such diseases[1]. Although brain and cerebrospinal fluid samples from patients with different central nervous system (CNS) disorders have been extensively studied, less research has been focused on spinal cord protein content and the changes induced after injury and the presence of associated symptoms such as neuropathic pain. Indeed spinal cord injury is characterized by a complex relationship between several molecular cascades and the development of a progressive pathophysiology[2].

In the present study we aimed to describe total protein content in the spinal cord of healthy rats, employing different proteomics tools. With this objective we have developed a new sequential protocol for protein extraction from rat spinal cords.

Methods: Spinal cords were extracted from 10 week old male rats injected with an overdose of Pentobarbital. The tissue was homogenized in two consecutive protein extraction buffers; the first that most of the soluble proteins and the second the more insoluble proteins. Finally the samples were analyzed using 2DE and LC-MS/MS.

Results: This work presents a very simple, fast and efficient method for spinal cord protein extraction. Approximately 400 to 600 spots were resolved in the 2DE experiments and the LC-MS/MS analysis detected hundred of proteins that complement the 2DE results.

The application of new proteomics protocols to the spinal cord may be a useful tool for the study and identification of differential molecular changes associated with CNS pathologies such as spinal injury and/or neurophathic pain. Altogether, these data could contribute to detect specific markers or potential drug targets for spinal cord injury and neuropathic pain management.

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IDENTIFICATION OF ENERGY HOMEOSTASIS SIGNALS FROM ADIPOSE TISSUES SECRETOME

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Introduction: A wide variety of endogenous systems regulating appetite, metabolism and energetic homeostasis have been traditionally studied in brain and peripheral tissues. However, there is still a need to improve our knowledge on the molecular mechanisms implicated in obesity development. The main objective of this work is the identification of new signalling systems from adipose tissues implicated in energy homeostasis regulation. We are applying proteomics as new emerging technology proven to be very useful for the identification of new disease target proteins, since it has been barely used for obesity studies.

Objectives: 1. To establish and characterize animal models for obesity proteomics: rats at sedentary, under voluntary exercise, activity-based anorexia (ABA) and obese conditions. 2. To standardize proteomics protocols for tissue secretion studies. 3. To obtain each tissue secretome reference map and to perform the differential secretome analysis in different nutritional status.

Methods: Animal models have been established in Sprague Dawley rats using activity wheels for voluntary exercise and ABA; obesity is being induced by high-fat diet (DIO). Animal models nutritional and metabolic status has been characterized by hormone determination, HOMA assays, body composition and metabolic enzyme profile expression. Fat subcutaneous, gonadal and visceral tissue explants have been incubated *in vitro* for secretome collection. Secretome samples have been submitted to bi-dimensional electrophoresis and mass spectrometry (MALDI-TOF/TOF).

Results: We have set and characterized sedentary, voluntary exercise and ABA animal models; the DIO model is currently being established. We have optimized the proteomics protocols for the tissue explants secretome studies. We are currently completing the reference maps for all tissue secretomes previous to the differential analysis. Preliminary results show known and novel proteins secreted by adipose tissue.

Conclusions: Proteomics technology proves to be a powerful tool for tissue secretome analysis and for the identification of new adipokines implicated in energy homeostasis.

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DIGE PROFILING OF MELANOMA SECRETOME IDENTIFIED MOLECULAR MEDIATORS OF SPARC ACTIVITY

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SPARC is a secreted glycoprotein related to tumor progression and metastasis and overexpressed in different tumors. Its role has been expanded to include tissue remodelling, endothelial cell migration, morphogenesis, angiogenesis and increased aggressiveness of different human cancer types. However, little is known about the molecular mechanisms affected by SPARC during tumor growth. We have showed that stable transfection of tumor cells with antisense SPARC DNA abolished tumorigenicity in an in vivo melanoma murine model through still unclear molecular mechanisms. In order to identify putative secreted proteins that may mediate SPARC biological function, we performed a proteomic analysis of conditioned media of a stable cell clone of human melanoma cells (L2F6) in which SPARC expression was downregulated by the use of a RNAi versus the control cell line LBLAST. For this purpose we applied DIGE (DIfferential Gel Electrophoresis). After analyzing 2D-gel images with DeCyder software, we obtained 98 differentially expressed proteins that were identify by using MALDI TOF-TOF technology. Ontological studies shown that the predominant group of differential proteins belongs to the family of proteases, proteins which have been extensively associated with tumor progression. Functional and biological validation confirmed the differences observed by DIGE. Thus, our results define a set of proteins potentially related to SPARC role in tumor progression, many of them not previously associated with SPARC. Further work is in progress to elucidate the molecular interactions between SPARC and this set of novel proteins.

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A COMPARISON OF SILVER, SYPRO RUBY AND RUTHENIUM II TRIS STAINS FOR 2D-GEL ELECTROPHORESIS WITH RESPECT TO IMAGE ANALYSIS AND PROTEIN IDENTIFICATION

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Proteomic projects are often focused on the discovery of differentially expressed proteins between control and experimental samples. One approach is running two-dimensional gels, analyzing them and identifying the differentially expressed proteins by tryptic in-gel digestion and mass spectrometry. Two restrictive factors for this type of proteomic mapping are the sensitivity of the staining technology and the recovery of tryptic peptides for mass spectrometry identification. In this report, we compare two fluorescent dyes: SYPRO-R and RuBP versus silver staining procedures with respect to several parameters: sensitivity, amount of proteins, number of spots detected by image analysis and peptide recovery after in-gel protein digestion for matrix-assisted laser desorption/ionization mass spectrometry. 100 µg of total protein extracts obtained from 293T cells were loaded and fractionated in 2D-PAGE gels and the scanned images analyzed with the Image Master 2D Platinum software. Proteins were excised, automatically digested under the same conditions with trypsin and the resulting peptides were extracted and analyzed by MALDI-TOF/TOF mass spectrometry. All three staining procedures showed advantages and disadvantages. Silver staining is more sensitive on the number of spot detected and allows a greater peptide recovery and therefore identification of lower abundance proteins but SYPRO-R reports higher protein scores when MS/MS analyses are performed. The RuBP procedures give similar contrast and detection than SYPRO-R but it is a very large method.

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CHARACTERIZATION OF PEPTIDES AND PROTEINS IN THERAPEUTIC HUMAN SERUM ALBUMIN

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Human serum albumin (HSA) is the most abundant plasma protein, with described ligand-binding and transport properties, antioxidant functions, and enzymatic activities. Commercial HSA is indicated for re-establishing and maintaining circulatory volume in situations resulting from traumatic shock, surgery or blood loss. [1] HSA is also used in extracorporeal liver support devices that performs blood dialysis against this protein. [2] Therapeutic HSA accounts for 14 % of the global market volume for plasma products.

Although therapeutic HSA has been used for more than 50 years, the composition of the commercial product remains unclear. Characterization of its composition is, however, crucial in order to understand its therapeutic effects and adverse reactions, as well as the mechanisms involved in different albumin therapies.

Here, we present the results from an exhaustive analysis of therapeutic human serum albumin composition using proteomic approaches. Low abundant proteins and peptides in these samples were concentrated using a strong anion exchange (SAX) resin (ion exchanger MARS, Gambro, Lund, Sweden). The absorbed material was eluted with stepwise gradient of ammonium trifluoroacetate and the resulting fraction analyzed by MDLC-MS/MS using an LTQ ion trap.

Besides albumin, a total of 1467 peptides corresponding to 102 proteins were identified with a false discovery rate of 1%. Relative to the human plasma proteome (www.plasmaproteomedatabase.org), the collection of proteins identified was apparently enriched on proteins involved in immunity and defense and transport (www.panther.org).

1. Gregory J. Quinlan, G. S. M., and Timothy W. Evans, Albumin: Biochemical Properties and Therapeutic Potential. *Hepatology* **2005**, 41, 1211-1219.
2. Parés A, C. L., Salmerón JM, Caballería L, Mas A, Torras A, Rodés J., Extracorporeal albumin dialysis: a procedure for prolonged relief of intractable pruritus in patients with primary biliary cirrhosis. *Am J Gastroenterol*. **2004**, 99, 1105-1110.

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CHARACTERIZATION OF DIALYZATED PROTEINS IN A MARS SYSTEM

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Molecular Adsorbent Recirculating System (MARS) is a extracorporeal liver support which uses albumin to remove low molecular weight toxins in patients with liver diseases(1). Patient blood is dialyzed across an albumin-impregnated membrane against 20% albumin. This albumin solution recalculates in a closed-circuit that includes Charcoal (AC) and strong anion exchange (SAX) resin columns for albumin regeneration.

In this work we analyzed peptides and proteins absorbed into the SAX-MARS resin column after the treatment in patients with resistant pruritus of chronic cholestasis. As commercial albumin used for dialysis already contains other residual proteins, a control sample was prepared recirculating 20% commercial albumin through a MARS SAX resin column for six hours. Proteins bound to the SAX cartridge were extracted using a stepwise gradient of salt and acetonitrile. Protein extracts were digested by trypsin and GluC. Digested peptides were analyzed by multidimensional liquid chromatography coupled to tandem mass spectrometry (MDLC/MS/MS) using a linear LTQ ion trap equipped with a microESI ion source. MS/MS fragmentation spectra were searched using SEQUEST search engine against the Human UniProt database.

With this method a total of 146 different proteins were identified to be absorbed by the SAX resin column. Forty three proteins were observed only after patient treatments while the rest where already present in the MARS-albumin. Presence of some of these tentative patient-derived proteins was confirmed by Liquid Chromatography and Multiple Reaction Monitoring focusing on the precursor and product ions corresponding to target peptides(2). By using this methodology, five proteins have been confirmed to be dialyzed from patients (Neutrophil Defensin 1, Secreted Ly-6/uPAR-related protein 1(SLUR1), Serum Amyloid A, Fibrinogen Alpha Chain and Pancreatic prohormone precursor). Among these, the presence of Neutrophil Defensin 1 and SLUR1 has also been validated by western blotting.

1. Parés A.; Cisneros L.; Salmerón J.M.; Caballería Ll.; Mas A; Torras A.; Rodés J. *American Journal of Gastroenterology*. **2004 Jun;** 99(6): 1105-10
2. Lange V.; Picotti P.; Domon B.; Aebersold R. *Molecular Systems Biology*. **2008**; 4:222

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IN SILICO ANALYSIS OF PROTEIN NEOPLASTIC BIOMARKERS FOR CERVIX AND UTERINE CANCER

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Worldwide, cervical and uterine cancers are the most deadly cancers in women, with high prevalence, especially in developing countries. The Human Protein Atlas (HPA) portal was explored for proteins expressed in a tissue-or cervix and uterine cancer-specific manner. The group of proteins differentially expressed, and with enhanced expression in the glandular and surface epithelial (squamous) cells retrieved from HPA were further explored using the Protein Information and Knowledge Extractor (PIKE) portal to compile biological information that is found in different databases, and repositories on the Internet. Thus, the lists of candidate proteins found in HPA, and PIKE portals may be used as a starting point for the discovery and validation of biomarkers for cervix and uterine cancer employing proteomics approaches as described in the present paper.

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PROTEOMIC APPROACH TO ENDOMETRIAL CARCINOMA INVASION FRONT

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Tumor invasion defines the transition between tissue-restricted carcinomas, with good outcome as optimal surgery becomes possible, and metastatic tumors associated with poor prognosis and a dramatic decreased in survival. In endometrial cancer, myometrial infiltration represents a determinant parameter highly valuable in prognosis. A profound spatio-temporal regulation from both the tumor and the surrounding stroma occurs at the invasive front. To date, the identification of proteins involved in endometrial carcinoma invasion has been essentially conducted by immunohistochemical methods, without a global perception on the invasive front.

In this work we attempted a proteomic approach to characterise specific components of the invasive front or reactive stroma by comparing, using 2D-DIGE, the invasive area of an endometrial carcinoma with the non-invasive superficial area and normal tissue from the same patients. This strategy led us to identify proteins involved in cellular morphology, assembly and movement, differentially expressed at the invasive front, as well as pathways like cell-to-cell signalling and interaction or a modulated response to oxidative stress as events related to endometrial carcinoma invasion.

In conclusion, we describe a novel proteomic approach that specifically tackles with endometrial carcinoma invasion front, allowing the identification of new players of myometrial infiltration.

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MODIFICATIONS OF PLASMA PROTEOME IN HEALTHY HUMANS FED ON A COENZYME Q10-SUPPLEMENTED DIET

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Nutrition and ageing have been associated, and the role of dietary components which could counteract oxidative stress as anti-ageing therapy has been recently studied. Coenzyme Q (CoQ, ubiquinone) (2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone) is a prenylated benzoquinone that is present in all cellular membranes and in high and low density lipoproteins as well. CoQ participates as an electron carrier in both mitochondrial and extramitochondrial membranes and it is a powerful antioxidant. Dietary CoQ_{10}–supplementation prolongs lifespan, modulates the activity of GSH-dependent antioxidant systems in the liver, and alters the levels of plasma proteins in rats fed lifelong on a PUFA*n*-6-enriched diet. The aim of our work was to analyze changes in the levels of plasma proteins of healthy humans fed on a Mediterranean diet (rich in olive oil) supplemented with CoQ_{10} (200 mg/day) compared with a Mediterranean diet alone, by using proteomic techniques. This approach could give us new insights into the mechanisms related with the potential beneficial effects of CoQ_{10} supplementation in humans. Plasma was obtained from twenty healthy adults consuming the two different diets for four weeks each, according to a randomized crossover design. At the end of the dietary intervention, and after 12 hours of fasting, participants were given a fat overload for the postprandial study. Blood samples were taken at 0, 30, 60, 120 and 240 min. Possible changes in the protein patterns of blood plasma were assessed at 0 and 240 minutes. After depletion of the twenty most abundant proteins by affinity chromatography using a commercial Kit, levels of less abundant plasma proteins were studied by using 2D-electrophoresis and MALDI-TOF mass fingerprinting analysis. In addition, to ensure the effectiveness of the supplement, plasma levels of CoQ_{10} were measured by HPLC at 0, 60,120 and 240 min. Our results have shown that dietary supplementation with CoQ_{10} induced significant decreases of Apolipoprotein A-IV and α-2-HS glycoprotein, and increases of Apolipoprotein J and inter-alpha inhibitor H4 heavy chain. The nature of proteins whose levels are altered supports the effect of CoQ_{10} supplementation on lipid metabolism and inflammation.

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EFFECT OF AUTOIMMUNE REGULATOR PROTEIN (AIRE) EXPRESSION ON THE CELLULAR PROTEOME

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T Lymphocytes are cells from the adaptive immune system involved in the immune response against infected cells. They express a clonally variable membrane receptor that recognizes highly specifically peptides arising from cellular catabolism (of pathogen proteins, in case of an infection, or modified, in case of a tumour), presented by the major histocompatibility complex (MHC) molecules on the cellular surface of presenting cells. Circulating T lymphocytes have to be "taught" to not react against own intact cells. Developing T cells are selected in the thymus. More than 95% of the thymocytes (either not able to recognize MHC, or too reactive against own MHC-peptide complexes) are deleted. Transcription of genes that code for tissue specific antigens (TSA) –"promiscuous expression"– has been observed in thymus. Expression of many of those genes is blocked when AutoImmune Regulator (AIRE) protein is not expressed. The absence of AIRE triggers a poliorganic autoimmune disease called APECED. Therefore, AIRE seems to be a key element in the development of the central tolerance.

We present here the comparison between the cellular proteomes of the HT93 thyroid cell line transfected or not with AIRE. With this purpose, two quantitative proteomic methodologies, DIGE and ICPL-LC-MS/MS, have been used in the analysis. DIGE analysis showed that in cells expressing AIRE there is an increase in different chaperons, including HSP70 y HSP27. A decrease of proteins related with cytoskeleton is also observed. Results of the ICPL–1DE-LC-MS/MS analysis were consistent with the ones obtained by DIGE. Proteomic data were confirmed through western blot experiments and flow cytometry for some of the differentially expressed proteins.

Hence, although changes in TSA expression could not be detected, the proteomic analysis show that AIRE expression increases the expression of different chaperon proteins and modifies the expression of some proteins related with the cellular cytoskeleton.

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PROTEOMIC APPROACH TO IMPROVE THE DIAGNOSIS OF MALIGNANT PLEURAL EFFUSIONS

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Pleural effusion is an accumulation of pleural fluid that can appear in patients with congestive cardiac failure, pneumonia, tuberculosis, pulmonary embolism, cancer (mainly lung and breast cancer), or physical trauma. Therefore, a cytologic study is commonly needed to confirm the etiology of this malignancy, and in a relevant number of patients surgical techniques are also required. Although several studies have been performed on pleural effusion, their purpose were more a description of the protein content than a comparative analysis to discriminate differentially expressed proteins. For this reason, the aim of this work was to find new protein biomarkers that could improve the diagnosis of malignant pleural effusions in order to avoid the use of invasive diagnostic procedures.

We have compared, by means of proteomic techniques, pleural effusion samples from four patients with tuberculous pleural effusion and four patients with non-small cell lung cancer. Since large portions of pleural effusion proteins are common to those found in plasma, we performed a previous fractionation of samples using multiple affinity removal spin cartridges for the depletion of six high-abundant proteins (Agilent technologies) in order to detect low abundance proteins that could serve as novel biomarkers for the malignancy.

After depletion, to test the efficiency of the removal process, a global analysis of pleural effusion proteome was made using conventional two-dimensional electrophoresis (2-DE). Gels were silver-stained and analyzed using PDQuest software, revealing more than 1200 spots per gel.

Comparison of proteomes from neoplasic origin versus benign ones was carried out using two-dimensional differential in-gel electrophoresis (2D-DIGE) technology and analyses of differences were made with Decyder Software. Those proteins presenting statistical significant differences will be submitted to mass spectrometry for identification.

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EFFECTS OF FLUVASTATIN THERAPY ON THE PATTERN OF PROTEIN EXPRESSION IN MONOCYTES OF PATIENTS WITH PRIMARY ANTIPHOSPHOLIPID SYNDROME

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Antiphospholipid syndrome (APS) is an acquired autoimmune disorder of unknown pathogenesis that is defined by the association of arterial or venous thrombosis and/or pregnancy morbidity in the presence of antiphospholipid antibodies: anticardiolipin antibodies and lupus anticoagulant. Recently, new genes and proteins differentially expressed in blood monocytes from APS patients with thrombosis, such as annexin II, RhoA proteins or protein disulfide isomerase (which are also related to the effect of specific autoantibodies on that disease), have been found.

In addition to their anti-inflammatory and immunomodulatory properties, statins have been shown antithrombotic effects, although the molecular mechanisms involved are not fully understood yet.

In this study, by using proteomic techniques, we analyzed changes in protein expression of monocytes of APS patients after statins therapy.

Ten patients with APS and previous history of thrombosis received Fluvastatin (40 mg/day) for one month. Then, proteomics, Western blot and RT-PCR analysis were accomplished.

The therapy with Fluvastatin reversed the changes produced in the expression levels of proteins altered in APS patients with thrombosis vs healthy donors, such as annexin II, Rho A proteins or PDI. These levels then slowly returned to basal levels, although remained significantly altered three months after the end of the treatment. Only RhoA protein levels remained lowered. Although these finding merits further research, it might be speculated that after removal of statins, plasma mevalonate levels remained reduced after statins treatment, thus maintaining Fluvastatin effects on APS patient monocytes. Moreover, statins may have affected other soluble plasma markers of monocyte perturbation in APS patients (e.g. soluble TF, D-dimer, LDL-ox.), thus explaining the slow return to baseline levels of the other altered proteins.

Our study has identified changes in the protein expression patterns of monocytes from APS after statins treatment. These findings might provide new targets for rational pathogenesis-based therapies of this autoimmune disorder.

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SEARCHING FOR BIOMARKERS OF ANEURYSMAL DISEASE

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Background. We have previously set up the conditions for comparing the proteins released from arterial pathological and control tissues using a proteomic approach. Our objective is to assess whether differentially released proteins could represent biomarkers for abdominal aortic aneurysm (AAA).

Methods and Results. Different regions of layers (luminal/abluminal) of the intraluminal thrombus (ILT) of AAA were incubated in protein-free medium, and the released proteins were analyzed by 2D-DIGE. In total, 31 proteins were identified by Mass Spectrometry (MS): 14 proteins were increased and 17 proteins were decreased in the supernatant of luminal vs abluminal layer of ILT. Among the differently released proteins, we have identified α1antitrypsin (ATT). Compared with the abluminal layer, ATT release was increased in the luminal layer of AAA. The interest of this approach is that we can identify proteins potentially released to the blood which could serve as biomarkers of the pathology. We have shown that circulating ATT levels are significantly increased in the serum of patients with AAA relative to healthy subjects (147(131-168) *v.* 125.5(114-135.5) mg/dL (p<0.0001); n=35). Moreover, a positive correlation between ATT and AAA growth in the previous 12 months was observed (r=0.55; p=0.004).

Conclusions. ATT release is increased in the luminal part of AAA, and ATT plasma levels are increased in AAA patients compared with healthy subjects. ATT seems to be a promising biomarker of AAA growth.

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IDENTIFICATION OF TRANSTHYRETIN AND β4-THYMOSIN AS POTENTIAL BIOMARKERS IN ACUTE CORONARY SYNDROME BY TWO INDEPENDENT METHODS, 2-DE/DIGE AND SELDI-TOF

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Acute myocardial infarction (AMI) is one of the leading causes of death in the world and remains a complex pathophysiologic process involving inflammatory, hemostatic and vascular processes. We employed two independent and complementary approaches, SELDI-TOF, and 2-DE/ DIGE in a first phase exploratory biomarker study to analyze modifications in the serum protein map during an acute coronary syndrome (ACS); It disclosed that the levels of two proteins, transthyretin (TTR; 14000 m/z) and acetylated-ß4-thymosin (4970 m/z) were significantly altered in acute coronary syndrome patients in comparison with healthy subjects. TTR was identified by 2-DE/ DIGE and SELDI-TOF and confirmed by Western blotting whereas ß4- thymosin was detected only by SELDI-TOF owing to its low molecular mass and confirmed by ELISA and Western blotting. Whereas TTR is involved in the transport of various biologically active compounds ß4- thymosin is essential for cardiomyocyte survival, cardioprotection and repair in the adult heart. Identification of both proteins could help in the understanding of the basis for allowing the diagnosis to be made at an earlier stage of the disease when the treatment is possible.

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IMMOBILIZATION OF RECOMBINANT HUMAN ENDOSTATIN TO DIFFERENT SUPPORTS: POTENTIAL APPLICATIONS IN BIOMEDICINE

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The 20 kDa C-terminal fragment of collagen XVIII, endostatin, is a broad spectrum inhibitor of angiogenesis. It acts mainly suppressing endothelial cell proliferation and migration. This protein plays a potential role in several pathological disorders such as tumoral diseases, retinopathies or other non-neoplasic diseases, where the angiogenesis is crucial. However, the molecular mechanism of action of endostatin has not been yet established.

Protein immobilization is a widely used method in biochemical studies. Functionalized supports can be used not only as affinity chromatography matrices to study protein-protein interactions, but also as a tool for specific drug delivery. Taking into account the important role of endostatin in the angiogenic process, immobilization of this protein to different supports would be of interest for biomedical purposes.

In this work, a commercially available *rh*-endostatin from *Pichia pastoris* has been immobilized to agarose beads and magnetic nanoparticles. Moreover, the immobilization process has been optimized. Prior to the immobilization, post-translational modifications were analysed by MALDI-TOF/MS. Besides, in order to gain a deeper insight into the possible interactions established between supports and endostatin, a 3D model structure of the protein was elucidated by Bioinformatics tools.

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IN THE SEARCH OF EARLY STAGES BIOMARKERS FOR NON SMALL CELL LUNG CANCER

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Although several proteomic approaches have been used in the search for new lung cancer markers, it continues to be the leading cause of cancer related death, mainly due to the advanced stage when the neoplasia is diagnosed. Another issue that difficults the search for novel markers is the overwhelming presence of high abundant proteins, that mask ones that could have a potential role as biomarkers. This inconvenience is being addressed with different removal methodologies, that permit an increase in the detection capability of potential markers.

In this work we attempt to discover novel markers for non small cell lung cancer (NSCLC) performing a prefractionation of the serum with the ProteoPrep 20 Plasma Immunodepletion kit (Sigma-Aldrich), that removes the 20 most abundant proteins, allowing an enrichment of 20-fold and considerably increasing the possibilities of detecting low abundant proteins. The depleted proteomes were then studied by means of DIGE methodology.

For this purpose we chose as patients subjects with adenocarcinoma, as this is the commonest type of NSCLC in Galicia, in which the malignancy was in early stages (localised and regional). As the control group we selected benign subjects with pneumonia, based on the higher interest to discriminate between pathologic stages that could confuse the diagnosis.

Conventional 2D-PAGE and silver staining were performed to check that effectively there was an increase in the resolution of low abundant proteins. Four patients and four matched benign controls were then submitted to DIGE, and differences in the expression level of the spots were analysed with DeCyder software. The statistical analysis revealed twelve proteins differentially expressed between lung cancer and pneumonia subjects, eight of them increased and four decreased in the cancer group compared to the control individuals. These proteins are being identified by mass spectrometry.

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IDENTIFICATION OF PROTEIN EXPRESSED BY AORTIC STENOSIS VALVES IN THE SEARCH FOR NOVEL BIOMARKERS

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Introduction: Until recently, aortic stenosis (AS) has been considered as a passive process secondary to calcium deposit in the aortic valves. However, lately several authors have pointed out that risk factors associated with the development of calcified AS in the elderly are similar to those of coronary artery disease. Furthermore, some studies have demonstrated that degenerative AS shares histological findings with atherosclerotic plaques which have led to the suggestion that calcified aortic valve disease is a chronic inflammatory process similar to atherosclerosis. Nevertheless, exist discordant data with this theory and it is necessary to study this pathology

The aim of this study is to obtain the aortic stenosis valves proteomic profile and in addition, the identification of new biomarker diagnosis and prognosis and/or therapeutic target.

Methods: AS valves obtained from necropsies (control samples) or by surgery patients were homogenized in extraction protein buffer. Both samples were analyzed using 2D-DIGE and LC-MS/MS. Furthermore, AS and control leaflets were studied by immunohystochemical (IH) and Western blot (WB) analysis, using a panel of monoclonal antibodies specifics for inflammatory and cytoskeletal/contractile proteins. The proteomic results were confirmed by WB and IH.

Results: 10 patients underwent aortic valve replacement due to severe stenosis with calcification of the leaflets, were compared with 10 control valves obtained by necropsies**.** The result of 2D-DIGE analysis of the proteome of AS valves compared with control valves reveals the expression protein alteration in several proteins such as 27 Heat shockprotein, osteopontin, vimentin.

To confirm the proteomic results several proteins were analyzed by Western blot and IH techniques. Furthermore, we have characterized the cellular composition of degenerative aortic stenotic valves by IH. The fibrosa layer of AVL had a higher cellularity than spongiosa and ventricularis (elastic) and it was the principal layer which was damaged in the lesion.

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EVALUATION OF THE CHONDROPROTECTIVE EFFECT OF GLUCOSAMINE AND CHONDROITIN SULFATE BY A PROTEOMIC APPROACH

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Glucosamine sulfate (GS) and chondroitin sulfate (CS) are symptomatic slow-acting drugs for osteoarthritis widely used in clinic, but with an unknown mechanism of action. The aim of this study is to evaluate the effects of both drugs GS and CS on cartilage cells (chondrocytes) to looking for cellular targets.

Chondrocytes obtained from healthy donors were treated with GS and CS, alone and in combination, and stimulated with IL-1β. Whole cell proteins were isolated 24 hours thereafter and resolved by 2-D electrophoresis. Gels were stained with SYPRORuby, and image analysis was performed using PDQuest software. Proteins of interest were identified by MALDI-TOF/TOF mass spectrometry. Real-time PCR and Western blot analyses were performed to validate our results.

We examined a mean of 500 protein spots that were present in each gel. Both qualitative and quantitative changes in protein expression patterns were studied. We identified 39 protein spots that were modulated by GS, 35 by CS and 48 by GS+CS compared to control ($p<0.05$). Database search showed that most of these proteins are involved in protein folding, stress response, cellular metabolism, protein targeting and oxidative stress. According to the essential role of oxidative stress balance that has been reported in osteoarthritis, we point out the effect of GS and CS (alone and combined) in counteracting the increase of mitochondrial superoxide dismutase that is caused by IL-1β.

In addition, the present study uses an *in vitro* model of inflammation (with IL-1β) to describe the effect of GS and CS on cartilage cells. We have identified several novel molecular targets of these compounds, such as SOD2, which may explain their reported good efficacy in osteoarthritis treatment. Our results highlight the synergic effect of the combined administration and point out the effectiveness of both molecules as anti-inflammatory drugs.

Posters

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LABELING OF *BIFIDOBACTERIUM LONGUM* CELLS WITH 13C-SUBSTITUTED LEUCINE FOR QUANTITATIVE PROTEOMIC ANALYSES

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Bifidobacteria are anaerobic bifid or multiply branching gram-positive rods that constitute one of the most numerous populations in the gastrointestinal tract of humans. Particularly in breast-feed infants, they can represent more than 90% of the total gut microbiota, and *Bifidobacterium longum* is one of the most representative species. The genome sequencing of several *B. longum* strains has recently prompted some investigations at the proteomic level.

Stable isotope labeling of amino acids in cell cultures (SILAC) is a simple and accurate procedure that can be used as a quantitative proteomic approach with many growing eukaryotic cell types. It is based on a comparison of the protein levels in cells grown in two formulations of the same medium that differ only by the fact that one formulation contains a nonradioactive, isotopically labeled form of an amino acid. By measuring the ratio of light peptides to heavy peptides, the relative abundance of proteins from cultures treated under different conditions can be determined.

In the present work we have adapted a SILAC procedure to the quantitative analysis of *B. longum* proteins. A medium that allows growth of *B. longum* NCIMB8809 with high level, stable incorporation of [13C6]leucine was developed. Incorporation of [13C6]leucine (containing six 13C atoms) into a protein or peptide leads to a 6-Da shift in the molecular mass due to the labeled leucine compared to the protein or peptide that contains natural leucine. Using this strategy, proteins having variations of at least 50% in their expression rates can be quantified with great confidence.

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PROTEOME CHARACTERIZATION OF THE PENICILLIN-PRODUCER FUNGUS *PENICILLIUM CHRYSOGENUM*. ANALYSIS OF HIGH-PRODUCING STRAINS

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Penicillium chrysogenum is a filamentous fungus known by its ability to produce penicillins and related β-lactam antibiotics. Current industrial strains have derived after numerous rounds of mutagenesis and selection from a single natural isolate of *P. chrysogenum*, NRRL1951. Product titers and productivities have increased by at least three orders of magnitude in the past 60 years, representing an unprecedented success in classical strain improvement. Biochemical and genetic analysis of industrial strains led to the identification of several important mutations in high-producing strains, including amplification of penicillin biosynthesis gene clusters, but much of the molecular basis for improved productivity remained to be elucidated. More recently, sequencing of the complete genome of *P. chrysogenum* Wisconsin54-1255 have revealed that transcription of genes involved in biosynthesis of the amino acid precursors for penicillin biosynthesis, as well as of genes encoding microbody proteins, has been increased in the high-producing strains. However, full exploitation of the *P. chrysogenum* genome sequence requires the integration of the proteomic level, which may contribute to further improvement of this important cell factory, which serves as a model for the development of other products of secondary metabolism.

In this work we show an optimized protein-obtaining method for *P. chrysogenum* that allows the analysis of the cytoplasmic proteome. Different buffers, extracting protocols and culture conditions, as well as staining techniques have been tested. As a result, the reference map for this filamentous fungus has been developed.

In addition, we have stablished the differences existing between the proteome of the reference strain (Wisconsin54-1255) and one high-producing strain (AS-P-78) growing under the same conditions. The comparison among the existing microarray and the proteomic data allows an up-date in the understanding of the antibiotic production mediated by this fungus.

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TELL ME WHAT YOU SMELL AND YOUR PROTEIN I WILL GUESS

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Pheromone detection plays an important role in Lepidoptera reproduction success. Most species with night habits possess developed antennae able to detect minimal amounts of odorants. On the other hand, diurnal species have specialized in using visual and tactile cues besides chemical perception. In any case odorants detection is mediated by proteins localized in the antennal sensillar lymph. Among these mediators, pheromone binding proteins (PBPs) are involved in the interaction with pheromone molecules, including their transport through the aqueous lumen to the dendritic olfactory receptors.

In this communication we present a proteomic study of antennal extracts from five species with different daylight habits: Three moth species, *Spodoptera littoralis, Spodoptera exigua* and *Sesamia nonagrioides,* important pests in Spain with night habits, the butterfly *Pieris brassicae* and the day flying moth *Paysandisia archon*. The study of the PBPs region (Mr 10-20 KDa, pI 4-7) of the extracts was carried out using 2-DE followed by mass spectrometry (MALDI-TOF MS, nESI-ITMS/MS and LC-MS/MS)

The analyses have shown a very different protein expression of this particular region depending on the daylight habits. In the three nocturnal species, males use the pheromone released by females for mating, different proteins related to pheromone transport have been identified. These proteins are preferentially expressed in males than in females. However, in the two diurnal species, that use visual cues for female location, there is almost a complete absence of proteins in this specific region. None of the identified proteins corresponded to a PBP and only two of them had a function related with olfaction.

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ENOLASE IN THE INTESTINAL HELMINTH INFECTIONS: THE CASE OF THE *ECHINOSTOMA CAPRONI*-MICE MODEL

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Echinostomes are intestinal parasitic trematodes which do not migrate within the tissue of their definitive hosts. These parasites have been extensively used as experimental models for the study of the host-parasite relationships. Our group has employed the model *Echinostoma caproni* (Trematoda: Echinostomatidae)-rodent to gain further insight in the molecules involved in the host-parasite interface that may be of importance to determine the course of the infection. This is based on the observation that *E. caproni* develops chronic or acute infections depending on the rodent host used (mice or rat, respectively). In the present study, we identify the enolase as the most immunogenic molecule of the excretory-secretory products (ESP) of *E. caproni* infected mice and we also report on the molecular cloning and characterization of this protein.

Antigenic proteins of *E. caproni* ESP were investigated by immunoproteomics. ESP of *E. caproni* separated by 2-D gel electrophoresis were transferred to nitrocellulose membranes and probed with different mice immunoglobulin classes. Enolase was recognized in 8 different spots of which 7 of them were detected in the expected molecular weight and were recognized by IgA, IgG or IgG and IgG1. Another spot identified as enolase a 72 kDa was recognized by IgM. Digestion with n-glycosidase F rendered a polypeptide with a molecular weight similar to that expected for enolase. Molecular cloning and in vitro expression in *Escherichia coli* of *E. caproni* enolase allowed us to determine that the protein contains 431 aminoacids and a theoretical MW of 46 kDa. The recombinant protein binds specifically to human plasminogen in vitro, confirming its properties as a host-interacting protein.

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DIVING INTO THE MOUSE MACROPHAGE CYTOSKELETAL PROTEOME UPON THE INTERACTION WITH *CANDIDA ALBICANS*

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Host-pathogen studies open interesting opportunities for the search of new virulence determinants and new targets for antimicrobial therapies. To study the host response, we have chosen the murine macrophage cell line RAW 264.7, in the light of the importance of macrophages for optimal host protection against *C. albicans* systemic infections. We have performed a sub-proteomic study about the induced expression/repression of proteins from macrophages when they are in contact with *C. albicans*, based on DIGE.

RAW 264.7 cells were allowed to interact with *C. albicans* SC 5314 cells for 3h, and an important differential protein expression was observed in these macrophages compared to control ones in the 4 fractions analysed: organelle, cytoplasm, nucleus and cytoskeleton. Of 120 spots with differential protein expression, 21 proteins have been identified. Of these 120 spots, 70 were in cytoskeletal fraction, but only 3 spots were identified. The same sample was analyzed on LTQ mass spectrometer and the identifications showed that most of the proteins in this fraction were neither cytoskeletal nor related proteins. Thus, other cytoskeletal extraction protocols have been assayed, highly increasing the number of cytoskeletal proteins identified. This will allow us the study of the cytoskeletal proteins changes in macrophages during the interaction with *Candida albicans* using iTRAQ technology.

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INTEGRATION OF PROTEOMICS WITH TRANSCRIPTOMICS AND METALLOMICS FOR THE STUDY OF ENVIRONMENTAL ISSUES

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Metal bound to biomolecules play important roles in living organisms. The identification of these metal-biomolecules in biological tissues can be performed by multidimensional methods combining orthogonal chromatographic with mass spectrometry and inductively coupled plasma mass spectrometry (ICP-MS): METALLOMICS. The integration of these analytical methods with the proteomic approach provides a powerful tool in deciphering environmental issues related to metal action. This integrated approach has an special interest when the genetic homology between model organisms and test or bioindicator organisms is demonstrated, because important conclussions can be drawn using model organisms as pattern in environmental and health studies based on non-model (non-sequenced) ones (1).

Present study is based on the use of *M. musculus* the best-known vertebrate model organism, and *M. spretus* the best characterized aboriginal species. Recently, *M. spretus* is being used as bioindicator in environmental pollution studies, through the use of different cytogenetic and biochemical biomarkers. Sequences of *Cyp* and *Gst* genes from Mus musculus were obtained from GenBankTM. Designed primer pairs based on *M. musculus* amplified in *M. spretus* induce products exhibiting in most cases 100% nucleotide sequence identity with *M. musculus*. The application of 2-DE protein separation and high throughput MALDI-TOF-PMF analysis assess the biological effect of polluted terrestrial ecosystems on inhabitant *M. spretus* mice. Spot identification relied on peptide matching with available sequence databases from *M. musculus*. Remarked differences are tested in the proteome of animals dwelling at different polluted environments, with specific increases and decreases in selected groups of proteins and changes that seem to be co-ordinately regulated.

For the first time the molecular size distribution patterns of elements in Mus musculus mice has been obtained using the SEC-UV-ICP-MS approach. The profiles of the elements obtained make possible deep insight into the interactions of elements with the different organs of Mus musculus as well as their possible connection with the metabolic pathways in this animal. ICP-MS allows multielement profiling in samples to be obtained in only one chromatographic run, which constitutes a reliable technique

with high throughput. A second chromatographic dimension on SEC-ICP-MS extracts usinf RP-HPLC-ICP-MS allows metallo-biomolecules purification and metal-species isolation for further identification by mass spectrometry techniques.

Therefore, the following conclusions can be drawn:

- A genetic homology between *Mus musculus* and *Mus spretus* has been established
- Modification of proteins expression in *Mus spretus* was defined in connection with differentially polluted ecosystems
- Metallomics allows high throughput metal-bound to proteins tracing in different tissues from *Mus musculus* and integration with proteomics and transcriptomics results can be drawn.

References: Gómez-Ariza, J.L., et al, Anal. Chim. Acta (2004) 524:15-22.

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PROTEOMIC ANALYSIS OF DEGRADATION PATHWAYS IN *RHODOCOCCUS* SP. STRAIN TFB

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Some important microorganisms in biodegradation are refractory to genetic manipulation being necessary to use different approaches to study the metabolic pathways involved in the catabolism of pollutants. *Rhodococcus* sp. strain TFB is a versatile Gram positive bacterium able to grow on a wide variety of contaminant compounds as carbon and energy sources. Proteomic analysis has been used to study the metabolic pathways involved in the catabolism of such compounds identifying most of the differentially induced proteins in 2D-DIGE experiments. Reverse genetics has been applied to clone the corresponding genes. A cluster of structural genes involved on phthalate degradation to protocatechuate plus a divergently transcribed gene for an IclR-type regulator, were localised in a genomic library of TFB (Tomás-Gallardo et al., 2006). The *pht* operon is inducible by phthalate and does not show catabolite repression by glucose. A cluster of genes, similar to those previously described in *Sphingomonas macrogolitabida* strain TFA (Martínez-Pérez et al., 2004) for tetralin catabolism, have been identified in TFB. Those genes are organised in three operons, which are induced by either tetralin or naphthalene, and are subjected to catabolite repression by glucose. Transcription start points of two operons have been located using a primer extension assay. Promoter sequences have been characterized using translational fusions in a *Rhodococcus-E.coli* promoter-probe vector. DNA-Protein interactions have been tested using biotinilated DNA bound to Streptavidin Magnetic Beads (Dynabeads, Invitrogen).

Thn proteins are also induced with naphthalene, but a Gentisate dioxygenase is only induced in naphthalene-grown cells. Reverse genetic is being applied for the cloning of gentisate degradation genes. Salicilaldehyde dehydrogenase and Gentisate dioxygenase activities have been tested total crude extract of TFB tetralin-, naphthalene-, salicylate-, and glucose-grown cell.

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USE OF 2-D DIGE AND PREPARATIVE DENATURING IEF TO ENHANCE SENSITIVITY IN THE DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS OF *CANDIDA ALBICANS* YEAST-TO-HYPHA TRANSITION

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Candida albicans is one of the leading causes of opportunistic fungal infections in immunocompromised individuals. The yeast-to-hypha transition has been considered one of the primary causes of *C. albicans* pathogenicity. In order to quantify protein expression changes related to this transition, we performed a 2-D DIGE analysis of cytoplasmic protein extracts obtained from *C. albicans* yeast and hyphal cells after growth for 6h in Lee's Medium at different pH values. Four biological replicates were obtained, labelled with Cy3 or Cy5 and pooled with a Cy2 labelled internal standard before 2-DE. To obtain a global picture of protein expression changes, IPG strips with a pH gradient of 3-11NL were used in 2D-DIGE gels. DeCyder analysis gels allowed us to detect 2500 spots, 106 of which showed a significant variation in their expression (standardized average volume ratios ≥ 1.3, *t*-student $\leq 0{,}05$). Forty-five differentially expressed proteins were identified by MALDI-TOF/TOF. They are proteins mainly involved in sugar and purine metabolism, response to stress, protein folding and filamentous growth

To get more information, a preparative denaturing isoelectric focusing separation of *C. albicans* yeast and hyphal cytoplasmic extracts was carried out using a Rotofor cell. Colleted fractions with pH from 4.5 to 5.5 and from 5 to 6 were mixed and analysed in preparative narrow pH gradient 2-DE gels of yeast or hypha forms. Before 2-DE, acidic fractions were mixed with yeast and hyphal total extracts labelled with different Cy dyes to be able to detect new differentially expressed proteins at these pH ranges. More than ten new proteins were identified.

Data integration and network interaction analysis of all over- and under-expressed proteins are currently underway what allow us to identify different processes taking place in *Candida albicans* yeast-to-hypha transition.

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COMBINED PROTEOMIC AND TRANSCRIPTOMIC ANALYSIS IDENTIFIES DIFFERENTIALLY EXPRESSED PATHWAYS ASSOCIATED TO *PINUS RADIATA* NEEDLE MATURATION

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Needle differentiation is a very complex process which leads to the formation of a mature photosynthetic organ from pluripotent needle primordia. We characterized and compared the proteome and transcriptome of immature needles (1 month old) and fully developed needles (12 months old) of *Pinus radiata* D. Don to characterize metabolic pathways implied in this process. After differential 2-DE (pH 5-8, 18 cm, CBB staining) 884 spots were analyzed defining 280 as differential (T-Test, Bonferroni correction for α=0.05). Out these 280 spots, 134 were confidently indentified by LC-ESI-Q-TRAP-MS employing a custom viridiplantae protein database (Applied Biosystems) and Paragon algorithm present in ProteinPilot Software (Applied Biosystems). Transcriptomic analyses were performed in three stages: 1. Two suppressive subtractive hybridization (SSH) libraries enriched with differential cDNAs were constructed for immature and mature needles. Libraries were constituted by 576 clones each, with 198 and 144 different sequences for immature and mature scions, respectively. 2. The differential expression of subtracted cDNAs was tested by hybridization over custom macroarrays (13 x 9 cm, 384 probes). 3. The expression level of 15 genes was determined by real time RT-PCR to validate macroarray results. A joint data analysis of proteomic and transcriptomic results was also performed to have a combined perspective which gives us a broad view over differentially expressed pathways associated to needle maturation. Energy metabolism pathways, with photosynthetic and oxidative phosphorylation related proteins, were overexpressed in mature needles. Aminoacid metabolism, transcription and translation pathways were overexpressed in inmature needles. Interestingly stress related proteins and defense mechanisms were characteristic of immature tissues, and may be linked to the higher growth rate and capacity of response of this tissue.

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SCREENING, CHARACTERIZATION AND *DE NOVO* SEQUENCING OF BROCCOLI PLASMA MEMBRANE AQUAPORINS BY HIGH RESOLUTION MASS SPECTROMETRY

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Plasma membrane Intrinsic Proteins (PIPs), a subclass of aquaporins, are ubiquitous membrane channel proteins that play a crucial role in water relations and other cell functions.

Bottom-up protein identification is carried out by cleaving proteins into their constituent peptides by endoprotease digestion, followed by separation and analysis by reverse phase HPLC-MS/MS. The recent introduction of mass spectrometers able to measure with high mass accuracy (≤ 5 ppm), together with new alternative ways to fragment peptides such as HCD (higher-energy C-trap dissociation) allow the screening of proteins from not sequenced organisms and the characterization of post-translational modifications (PTMs). In this study we took advantage of the high mass accuracy of LTQ Orbitrap mass spectrometer combining collision induced dissociation (CID) and HCD fragmentation to identify a total of 21 peptides belonging to several isoforms of PIPs in Broccoli (*Brassica oleracea* L. var italica) roots. In addition, 7 of them present PTMs. The phosphorylation on Ser^{280} and Ser^{283} on the C-terminal tail of *At*PIP2 has been described (Prak et al., 2008). Our results with broccoli PIPs only showed the presence of phosphorylated Ser^{283} on two different peptides (SLGSFRSAANV and SLGSFRSAA), both containing three Ser residues. We have also evidenced the presence of methylation (+14.0157 amu) on the tryptic doubly charged peptide with sequence DYEDPPPTPFFDADEmeLTK. Methylated Glu residues on PIP proteins have been described, although it is quite uncommon in eukaryotic proteins (Maurel, 2007).

Finally, 8 peptides from PIP proteins were *de novo* sequenced and validated searching homologue peptides using BLASTp. Sequence analysis by LTQ Orbitrap is a useful tool to provide reliable information on peptide sequences from a complex peptide-protein mixture, even when dealing with organism under-represented in databases. The results described here will lead to a better understanding of aquaporin regulation and the role of different PIP isoforms in specific subcellular compartments.

Maurel C. 2007. FEBS Letter 581: 2227-2236.
Prak S, Hem S, Boudet J, Viennois G, Sommerer N, Rossignol M, Maurel C, Santoni V. 2008. Molecular and Cellular Proteomics 7: 1119-1030.

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QUANTITATIVE PROTEOMICS ANALYSIS OF LYMPH NODES FROM PIGS INFECTED BY PORCINE CIRCOVIRUS TYPE 2 (PCV2) BY 2-DE, $^{18}O/^{16}O$ LABELING AND LINEAR ION TRAP MASS SPECTROMETRY

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PCV2 is the causal agent of postweaning multisystemic wasting syndrome in pigs, characterized by progressive weight loss, dyspnoea, enlargement of inguinal lymph nodes, depletion of lymphocytes and an altered pattern of cytokines. The mechanism whereby the virus causes the disease and the reason why only some animals become diseased remain unclear. To study the immune response associated with virus infection, ten piglets were divided into 2 groups: control (n = 4) and inoculated with PCV2 at 7 days of age (n = 6). Piglets were euthanized and necropsied on day 29 p.i and inguinal lymph nodes samples were collected. Lymph node protein extracts for each group were pooled, split into two equal aliquots and analyzed by two different proteomics strategies: a classical approach based on the differential 2-DE pattern and a stable isotope labeling approach combining SDS-PAGE protein fractionation, "in-gel" digestion, $^{18}O/^{16}O$ peptide labeling and peptide identification and quantification by LC-MS/MS. 2-DE analysis revealed 45 spots that were differentially expressed at a FDR of 5 %, corresponding to 31 unique proteins. In the second approach peptides were identified by using the pRatio method, and the quantitative results analyzed using QuiXoT. Among 1,493 identified proteins, 794 could be quantified, from which 65 proteins were found differentially expressed at a FDR of 5%. We used the Ingenuity Pathway Analysis package to analyze and compare the obtained results. Association of differentially regulated proteins with canonical pathways highlighted two major processes: acute phase response signalling and NRF-2-mediated oxidative stress response. Other canonical pathways associated with differentially expressed proteins were that of TGF-β, and the integrin and actin signalling pathways.

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NEW ADVANCES IN GLUTEN ANALYSIS: APLICATION OF MALDI-TOF/TOF MASS SPECTROMETRY AND NANOLC-ESI-IT-MS/MS TECHNIQUES

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Introduction: Gluten is a group of proteins found in wheat, barley and rye. The ingestion of these proteins, in genetically predisposed people, induces the celiac disease. The only treatment for celiacs is a lifelong gluten-free diet. For this reason, new techniques are necessary to characterize and detect these proteins to monitor the absence of gluten in foods. Both, MALDI-TOF/TOF Mass Spectrometry and nanoESI-IT-MS/MS techniques are highly sensitive methods for detecting peptides. The aim of this work is to characterize prolamin proteins and their tryptic peptides from wheat, barley and rye and develop a new method for gluten analysis based on the detection of specific peptides.

Materials and methods: Prolamins are extracted from cereal seeds by using standard procedures. These proteins are directly off gel endoprotease digested or separated by SDS-PAGE electrophoresis and in gel endoprotease digested. Then, the yielded peptides are analysed and characterized by MALDI-TOF/TOF Mass Spectrometry or nanoLC-ESI-IT-MS/MS techniques. The resulting peptide masses were then searched against a protein data base (NCBI or Swiss Prot) for matching proteins using the Mascot Search. Sandwich R5-ELISA is used as an internationally reference standard technique (Codex Alimentarius ALINORM 08/31/26) to analyse prolamins from wheat, barley and rye.

Results: A large set of peptides were identified and assigned to different prolamins and specific proteotypic peptides for wheat, barley or rye as well as for common peptides corresponding to the three cereals. The results for both techniques were similar.

Conclusions: MALDI-TOF/TOF Mass Spectrometry and nanoESI-IT-MS/MS techniques can be used as non-immunological tools in gluten analysis of different wheat, barley and rye varieties. These proposed methodologies enabled us to set up a fast and accurate analysis for evaluating the gluten components.

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THE ENTEROCYTE PROTEOME OF GILTHEAD SEABREAM (*SPARUS AURATA*) ACCLIMATED TO TWO SALINITY CONDITIONS.

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The Mediterranean Sea and the coastal aquatic systems are, as a result of the climate change, experiencing a warming of the surface and deep waters. An increase in the average temperature and in the number of "heat waves" is predicted, in which the temperature of the surface waters may reach considerable levels, and not ruling out changes in the levels of salinity in some areas In fish, the acclimatization to changes in salinity and temperature is associated with changes in the composition of lipids and proteins. These changes can be detected at a molecular level and may constitute a useful tool for the tracking of the effects of the global changes on aquacultured fish species of interest. The gilthead seabream (*Sparus aurata*) is a valuable euryhaline species in the Mediterranean aquaculture, adapted to survive in a wide range of salinty. The intestinal epithelium is involved in osmoregulation in fish. In the present work we investigated the proteome of enterocytes, in order to search for new proteins that could be useful as biomarkers to evaluate the response of euryhaline cultured fish to salinity changes. Gilthead seabream of 350 g were acclimated to high (37 ‰) and low salinity (18-20 ‰) for 5 months. 2D difference gel electrophoresis (DIGE) was performed to identify protein profiles related to osmoregulation. Analyses of the gels image scaning were caried out with the DeCyder™ (V. 6.5) software, and the statistical module EDA (V.1.0) was used for multivariate statistical analysed of data. A total number of 34 proteins were differentially expressed as consequence of salinity acclimation ($p \leq 0.02$). Among these proteins, 16 increased in the group acclimated to high salinity, whereas 18 decreased. Mass spectrometry and database research is being performed to identify these differentially expressed proteins involved, presumably, into the acclimatory processes of the gilthead seabream to salinity changes.

Keywords: Proteomics, DIGE, salinity, Enterocytes, Biomarkers, gilthead seabream

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2D-DIGE ANALYSIS OF POTENTIALLY PATHOGEN *SACCHAROMYCES CEREVISIAE* STRAINS ISOLATED FROM DIETARY SUPPLEMENTS AFTER INCUBATION IN HUMAN BLOOD

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In the last years, the incidence of infections caused by *Saccharomyces cerevisiae* has significantly increased in immunodepressed patients. In order to study the possible relation between the ingestion of live *S cerevisiae* cells, through the consumption of dietary supplements, and infections in humans, we are trying to identify putative virulence traits in yeast strains from dietary supplements. In order to address the identification of potential proteins involved in virulence, a comparative study of global protein expression, between virulent and avirulent strains after incubation with human blood, was performed by means of 2D-DIGE. The use of human blood has been chosen since the dissemination of yeast cells in the bloodstream constitutes an essential stage in the development of systemic infection. After different times of *S. cerevisiae* cells incubation in blood (0, 1.5 and 3 hours at 37ºC and semi-aerobic conditions) cytoplasmic yeast extracts were obtained. Protein extracts from three biological replicates were labelled with Cy3 or Cy5 DIGE minimal labelling and an internal standard was labelled with Cy2. Nine DIGE gels were run and, after fluorescence detection, gels were analyzed by means of EDA module of the Decyder software. Remarkably, only from 4 to 37 differential expression proteins where detected comparing different times of the same strain, while inter-strain analysis allowed the detection of a significantly higher number of differentially expression proteins (from 79 to 215). Identification of these proteins is currently underway.

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EFFECT OF *SALMONELLA TYPHIMURIUM* ON THE PROTEOME OF PIG GUT

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Salmonella typhimurium is the serotype most frequently isolates from ill pigs in Europe. *Salmonella* outbreaks and subclinical infections are often not only a cause of economic and animal welfare costs, but also a source of contamination of pork products entering the food chain. Because of this, a complete understanding of the process seems likely and, while some successful physiological, biochemical or genetic approaches have been developed at intestinal level, no proteomic assays have been performed until now. *S. typhimurium* infection and pathogenicity are complex, highly integrated processes that cannot be attributed to any single protein activity. Advances in proteomic technologies do now possible to characterize host-pathogen interactions from a global proteomic view. We used a proteomic approach to study proteins of intestinal mucosa differentially expressed as response to Salmonella infection. For that purposes, two groups of pigs were analyzed: controls (n=3) and naturally infected pigs (n=3). Intestinal mucosa resections were resuspended in sample buffer and submitted to mechanical dissociation by scraping and gentle squeezing. Protein extracts for each group were pooled and analyzed by DIGE, because this approach had the advantage of an improved sensitivity and accuracy. Samples were cup-loaded onto IPG strips, 24 cm, pH 3-11NL, and subjected to isoelectrofocusing. For the second dimension, strips were loaded on top of 12.5% polyacrylamide gels. 2D gels were scanned using a Typhoon™ Trio Imager and analyzed using DeCyder 6.5 software. We found 49 spots exhibiting statistically significant differences ($p<0.05$), corresponding to 42 different proteins, with 12 proteins showing up-regulation and 30 down-regulation. These proteins were analysed by MALDI and NanoLC-ESI MS/MS. The data set were analyzed through the use of Ingenuity Pathway Analysis and the physiological function most significantly perturbed was immunological, infectious and gastrointestinal disease, cellular assembly and organization, tissue morphology, cell death and immune response.

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POPULATION PROTEOMICS: PROTEIN DIVERSITY IN HAKE (*MERLUCCIUS MERLUCCIUS*) POPULATIONS

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Population proteomics is becoming a powerful tool enabling the study of the population structures and functional adaptations to environment from human settlement to animal natural populations. The present communication deals with preliminary results of a high throughput, large-scale study of hake (*M. merluccius*) proteome across and within populations. The aim of this study is the search for proteins that exhibit differences in abundance and mobility in order to trace frequencies distribution and differential features across populations.

Approximately 150 µg of liver proteins of eighteen individuals (six replicas) from three different sampling sites (two from the Atlantic Ocean and one form the Mediterranean Sea) were labelled with cyanine dyes (Cy3, Cy5) and separated on a two dimensional differential gel electrophoresis (2D-DIGE). The obtained gel images were analyzed using the DeCyder and BVA software modules. Gels were grouped according to their geographical source and data used for further analyses were filtered. Up to 335 spot features exhibited statistically significant (ANOVA $p<0.05$) changes in abundance on all 9 gels. PCA analyses of this filtered data set showed that samples segregated in three geographical groups and hence sample distribution did factor into the differential abundance of proteins observed. These differences in proteomic profiles have future potential application for the identification of hake genetic stocks.

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PLANT PROTEOMICS IN RESPONSE TO IONIZING RADIATIONS

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The effects of ionising radiations (IR) on organisms are mainly caused by the water radiolysis phenomenon witch produces a significant amount of hydroxyl ions, generating a specific oxidative stress in cells. Our interest is focused on the IR effects on plant metabolism and changes occurring in cellular and tissue functioning after exposure to IR. Global plant responses to IR have been studied at transcriptional level, but studies dealing with IR stress on plant proteome are missing

To complete the knowledge on changes occurring in plants exposed to IR, we proposed to analyze *Arabidopsis thaliana* proteome on a short- and long-time scale after exposure (or not) to sub-lethal doses of IR obtained from X-Rays. In order to facilitate the differential analysis, plant roots and aerial part proteomes will be separately studied. We are interested in exploring by this way different ecotypes or mutants owning special characteristics or genes involved in the glucosinolate pathway, the latters potentially having an antioxidative effect.

Our model are 10-days old plants of Arabidopsis thaliana, ecotype Col-0, which are grown on petri dishes, in sterile conditions, in a 16h-light, 8h-dark photoperiod. The treatment stage corresponds to a 2-leaves-spread-out stage. Irradiation will be performed in a X-rays chamber Faxitron at a dose rate of 1.6 gray.min^{-1}. Plants will be treated in the petri dishes and will receive a total dose of 10 and 40 grays (Gy). After the irradiation treatment is applied, leaves and roots will be separatly frozen at 2, 6, 24, 48 and 72 hours after irradiation.

Results of this program work, dealing with optimization of extraction protocol, realization of 2D-electrophoresis proteomic gels, and MS analysis of proteins will be presented.

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ANALYSIS OF THE *EMBOTHRIUM COCCINEUM* ROOT AND LEAF PROTEOME DURING PROTEOID ROOT DEVELOPMENT

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Embothrium coccineum (Notro) is a tree that grows in the temperate forests of Chile. Its interest is related to its capacity to form proteoid roots when growing in soils with poor nutrients. This type of root was defined by Watt and Evans in 1999, as a primary lateral root with defined clusters of more than ten secondary lateral roots (proteoid rootlets) per centimeter. They enhance nutrient uptake, possibly by chemically modifying the soil environment to improve nutrient solubilisation. Although the metabolism and nutrient uptake in proteoid roots has been well characterized in *Lupinus albus,* the mechanism governing its induction is almost unknown. We are using Notro as an experimental system to study proteoid root induction from a molecular point of view. In this report, we present preliminary experiments in which the root and leaf proteome is being analyzed during proteoid root development. The experimental design included three different stages (young, mature and senescent proteoid roots) and two treatments (high, 1mM, and low, 0,001mM phosphorus concentrations). The protein extract was obtained by using the TCA-Acetone-Phenol, giving protein yields of 3 and 9 µg per mg of dry powder of root and leave tissue, respectively. Two-dimensional gel electrophoresis of protein extracts are being carried out, with IEF in the 5-8 pH range, and SDS_PAGE with 12% PAA gels. The biological variability will be determined by using 6 replicates. Differences in the 2-DE map of the different samples will be determined after careful statistical analysis, and differential spots subjected to MALDI-TOF-TOF MS.

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PROTEOMIC CHARACTERIZATION OF THE PORCINE NEUTROPHIL RESPONSE TO LPS FROM *SALMONELLA TYPHIMURIUM* BY 2D-GEL ELECTROPHORESIS AND MASS SPECTROMETRY

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Neutrophils are the first line of defense against pathogens and participate in a wide range of normal and pathological responses of the organism to diverse stimuli. The activated neutrophils attack microorganisms by phagocytosis or by releasing a combination of reactive oxygen species (ROS), enzymes and antimicrobial peptides. Lipopolysaccharide (LPS) is a component of the outer wall of Gram-negative bacteria that evokes a variety of functional responses in neutrophils. Interaction of bacterial LPS with the swine PMN represents a model system for studying the innate immune response during infection and inflammation. The objective of this study were to identify proteins involved in the response of swine neutrophils to LPS, using 2D gel electrophoresis and mass spectrometry technology. Blood samples were collected at the slaughterhouse from five healthy pigs and neutrophils were isolated with Dextran sedimentation and centrifugation through Ficoll-Paque. For LPS stimulation, the neutrophils were incubated for 18 hours in the presence or absence of 100ng/ml LPS. Proteins were solubilized, the extracts were pooled and six replicate 2-DE gels for condition (untreated cells and treated with LPS) were analysed by 2-DE. The LPS-induced changes in proteins was subjected to statistical analysis with a Student's *t* test after checking normality by the Wilks-Shapiro test and those spots with $p< 0.05$ were analyzed by MALDI-TOF/TOF. The number of protein differentially expressed in the cells after LPS treatment was 73 and 32 of this proteins were identificated (19 were down-regulates, 11 up-regulated and 2 proteins were presented in control samples only). We used the Ingenuity Pathway Analysis software to analyze our data sets. The association of the proteins afected by LPS treatment with canonical pathways highlighted two major pathways: acute phase response signalling and regulation of actin-based motility by Rho protein. Networks whose activities are most likely affected were cell-to-cell signaling and interaction and cell cycle.

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THE HEPATIC PROTEOME OF GILTHEAD SEABREAM (*SPARUS AURATA*) ACCLIMATED TO TWO SALINITY CONDITIONS

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The gilthead seabream (*Sparus aurata*) is valuable euryhaline specie in the Mediterranean aquaculture, adapted to survive in a wide range of salinity. The aim of this study was to identify, by a proteomic approach, changes in the liver as response to acclimation to salinity that may be indicative of metabolic adjustments to the physical and chemical environmental conditions.

Gilthead seabream of 350 g were acclimated to high (37 ‰) and low salinity (18-20 ‰) for 5 months. Difference Gel Electrophoresis Technology (2D-DIGE) was used to study the effect salinity in liver protein profile. Resulting gels images were analized by DeCyderTM (V. 6.5) software and the statistical module EDA (V.1.0) was used for multivariate statistical analysed of data. The results showed significant changes in the expression of 24 proteins between high and low salinitiy group ($p \leq 0.02$). Among these proteins, a total of 11 increased in abundance and 13 decreased in the group accimated at high salinity. Mass spectrometry and database research is being performed to identify these differentially expressed proteins involved, presumably, into some homeostatic processes that would explain the gilthead seabream tolerance to a broad salinity range.

Keywords: Proteomics, DIGE, Salinity, Biomarkers, Liver gilthead seabream

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ARABIDOPSIS THALIANA LEAF DISULFIDE PROTEOME

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Reactive oxygen species (ROS) are generated as a consequence of the cellular metabolism (e.g. photosynthesis), and their production rate increase in response to biotic or abiotic stresses. The overproduction of ROS shifts the redox status of the cell to oxidizing conditions, which causes thiol-disulfide transitions of Cys residues and other redox changes in proteins. Such modifications mediate stress sensing, triggers signalling cascades and activates the programmed cell death and other defence related processes. The methodology used to identify this transitions occurring *in vivo* is presented. The protocol has been modified from the previously reported by Lee et al. (2004; Electrophoresis 25:532-541), and include the following steps: i) blocking of free thiols by alkylation; ii) reduction of disulfide cysteines to sulfhydryl groups; iii) purification by thiol affinity chromatography; iv) separation by 1-D, SDS-PAGE; v) band cutting and trypsin digestion; vi) MS of the tryptic fragments and protein identification. This methodology will be used to analyze changes in the *Arabidopsis thaliana* leaf thiol-disulfide proteome in response to the infection with the bacterium *Pseudomonas syringae*.

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PROTEOMIC ANALYSIS OF *CUCUMIS MELO* L. PHLOEM AS AN APPROACH TO STUDY RESISTANCE TO *BEMISIA TABACI* GENN

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Bemisia tabaci Genn, a phloem-feeding insect, constitutes one of the most harmful pests affecting melon (*Cucumis melo* L.). It causes important crop losses due to direct feeding damage being also an efficient vector of important viruses. Recent reports indicate that *Cucumis sativus* phloem sap contains several defense proteins against biotic and abiotic stresses (Walz et al., 2004). If these proteins are present in whitefly resistant melon genotypes they could be associated to melon resistance to whitefly and could be used as markers in plant breeding programmes. In order to test this hypothesis, a preliminary proteomic analysis of the melon phloem is being carried out. Three melon genotypes were evaluated: 'Bola de Oro', a Spanish cultivar highly susceptible to *Bemisia tabaci*, 'TGR-1551', a Zimbabwean genotype and 'PI 414723', an Indian genotype, the last two ones being partially resistant to *B. tabaci*. Phloem exudate was collected from melon leaves 8 days after whitefly release on plants. Proteins were precipitated by acetone-0.1 M HCl, dissolved in Laemmli buffer and subjected to SDS_PAGE, being the gels Coomassie stained. Qualitative and quantitative differences in the protein banding between genotypes and treatments (infected and non-infected) were observed. Differential protein bands were subjected to MS analysis after trypsin digestion, with some of them being identified as peroxidases, lipoxygenases and lectins. A 17 kDa phloem protein, corresponding to a RNA-binding protein (Gómez et al., 2005), was detected in 'Bola de Oro' and 'PI 414723' but not in 'TGR-1551'. So far, any of the defense proteins found in *C. sativus* was found in melon genotypes.

Work is now in progress by using a more powerful and resolutive technique as it is two-dimensional gel electrophoresis. 2-DE Coomassie stained gels of phloem proteins from the three genotypes, including infected and non-infected plants, have been analyzed, and differences among genotypes and treatments were observed. The responses to whitefly was genotype-dependent, with 'Bola de Oro' and 'PI 414723' showing more quantitative and qualitative differences than 'TGR-1551'. Differential spots are now being subjected to MS analysis for identification.

Gómez G, Torres H and Pallàs V. Identification of translocatable RNA-binding phloem proteins from melon, potential components of the long-distance RNA transport system. The Plant Journal 2005, 41: 107-116.

Walz C, Giavalisco P, Schad M, Juenger M, Klose J and Kehr J. Proteomics of cucurbit phloem exudate reveals a network of defense proteins. Phytochemistry 2004, 65: 1795-1804.

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PROTEOMICS AS AN APPROACH TO STUDYING VARIABILITY IN HOLM OAK (*QUERCUS ILEX* SUBSP. *BALLOTA* [DESF] SAMP) ANDALUSIAN POPULATIONS

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Our groups are carrying out a multidisciplinary research project on holm oak (***Quercus ilex* subsp. *ballota* [Desf] Samp)** and other forest tree species of interest within the Andalusian region. We aim: i) to characterize the different catalogued provenances; ii) to study differential population responses to biotic and abiotic stresses; iii) to select elite trees to be used in reforestation programmes; and, iv) to optimize *in vitro* propagation protocols. For these proposals we are using different approaches, including classic biochemistry, proteomics, and genomics. Previously, differences in the 2-DE protein pattern between population and changes in response to drought stress were studied on leaf tissue (Jorge et al. 2005, 2006). Due to the great variability found in the leaf proteome, even within the same tree, it was almost impossible to establish clear differences between provenances, and we therefore decided to move to a different organ, that, like the seed, has a more stable proteome. This communication deals with the specific objective of analyzing variability in holm oak (***Quercus ilex* subsp. *ballota* [Desf] Samp)** Andalusian populations by comparing the 1- and 2-DE protein profile of mature seeds. Seed proteins (10 populations, 5 trees per population) were TCA-acetone extracted and subjected to both 1- (SDS-PAGE) and 2-DE (IEF/SDS-PAGE). Coomassie-stained gel images were captured with a GS-800 Calibrated Densitometer (Bio-Rad) and analyzed by PD-Quest software. The 1-D banding pattern was highly reproducible among trees within populations, founding 7 polymorphic bands. 2-DE analysis was limited to just four out of the ten populations sampled. Qualitative differences were found for 74 of the around 352 spots detected. Some of the differential bands/spots have been identified after MS analysis, with most of them corresponding to legumins, a type of globulin described in different *Quercus* species. Principal component analysis and dice coefficient have been used to determine the most discriminate bands/spots and the genetic distances between provenances.

1. Jorge I., Navarro R.M., Lenz C., Ariza D., Porras C., Jorrin J. 2005. The holm oak leaf proteome: Analytical and biological variablility in the protein expression level assessed by 2- DE and protein identification tandem mass spectrometry de novo sequence similarity searching. Proteomics, 5:222-234.
2. Jorge I., Navarro R.M., Lenz C., Ariza D., Porras C., Jorrin J. 2006. Variation in the holm oak leaf proteome at different plant developmental stages, between provenances and in response to drought stress. Proteomics, 6:207-214.

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GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IS POSTTRANSLATIONALLY MODIFIED BY NAD^+ IN ENTEROHEMORRHAGIC *ESCHERICHIA COLI*

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Several cytoplasmic housekeeping enzymes with no detectable secretion and retention signal are secreted and remain present on the surface of microbial pathogens where they exert functions related to the adhesion and/or virulence of the pathogen. In *Escherichia coli*, extracellular location of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been reported in enterohemorrhagic and enteropathogenic strains. GAPDH can be a target of several covalent modifications including glutathionylation, S-nitrosylation and ADP-ribosylation. Most of these modifications inhibit enzyme activity and may have great physiological consequences. Some of them are linked to stress response or adaptations to new environmental situations.

We have shown that enterohemorragic *Escherichia coli* GAPDH is covalently modified by ^{32}P-NAD^+ in ADP-ribosylation reactions. This activity was assayed in cell extracts and in extracellular isolated proteins. Samples were subjected to polyacrilamyde gel electrophoresis (SDS-PAGE) and visualized by autoradiography. In some experiments, non-radioactive NAD^+, or non-radioactive ADP-ribose were added as putative competitors. Only two proteins appeared to be modified by radiolabeled NAD^+. The band corresponding to the 37KDa protein in SDS-PAGE was excised from silver-stained duplicate gels and identified as GAPDH by mass spectrometry. This result was confirmed by Western blot using anti-GAPDH specific antibodies.

Purified *Escherichia coli* GAPDH is able to promote its own modification by NAD^+. Analysis by 2D gel electrophoresis in immobilized pH 5-8 gradient strips showed that modification by NAD^+ corresponds to the more acidic spots of GAPDH, which represent the less abundant forms of the protein.

To identify the specific amino acid modified by NAD^+, the reaction was carried out with purified GAPDH in the presence of different concentrations of L-cysteine, Lhistidine, and L-arginine. Only free L-cysteine was able to inhibit the reaction, suggesting that NAD^+ modification occurs at cysteine residues.

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THE CELL ENVELOPE PROTEOME OF *BIFIDOBACTERIUM LONGUM* IN AN IN VITRO BILE ENVIRONMENT

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Host-bacteria interactions are often mediated via surface-associated proteins. The identification of these proteins is an important goal of bacterial proteomics. To address how bile can influence the cell-envelope proteome of *Bifidobacterium longum* biotype *longum* NCIMB8809, we analysed its membrane protein fraction using stable isotope labelling of amino acids in cell culture (SILAC). We were able to identify 141 proteins in the membrane fraction, including a large percentage of the theoretical transporters of this species. Moreover, the envelope-associated soluble fraction was analysed using different subfractionation techniques and differential in-gel fluorescence electrophoresis (DIGE). This approach identified 128 different proteins. Some of them were well known cell wall proteins, but others were highly conserved cytoplasmatic proteins likely displaying a "moonlighting" function. On the other hand, we were able to identify 11 proteins in the membrane fraction and 6 proteins in the envelope-associated soluble fraction whose concentration varied in the presence of bile. Bile promoted changes in the levels of proteins with important biological functions, such as some ribosomal proteins and the enolase. Also, oligopeptide binding proteins were accumulated on the cell surface, which was reflected in a different tripeptide transport rate in the cells grown with bile. The data reported here will provide the first cell-envelope proteome map for *B. longum*, and might contribute to understanding the bile tolerance of these bacteria.

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DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS TO STUDY THE PATHOGENICITY OF THE PROTOZOAN PARASITE *TRICHOMONAS GALLINAE*

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Avian trichomonosis is a parasitic disease caused by *Trichomonas gallinae,* a protozoa which can cause lethal caseous lesions on the mouth and the oesophagus of birds. Parasite main hosts are Columbiformes, especially rock pigeon (*Columba livia*), which is blamed to be the responsible of the worldwide distribution of the parasite. Although many avian families can be parasitized by the protozoa, birds of prey are the most affected group, overall ornitophagous species which feed regularly on pigeons. Strain differences in pathogenicity have been reported, but no studies have been done considering the proteins implicated in the virulence of the parasite.

To investigate potentially virulent proteins, we have developed a quantitative proteomic study using two strains, one obtained from a bird with granulomatous lesions (genotype B) and the other obtained from a pigeon without signs of disease (genotype A).

Soluble proteins from trophozoites were extracted by sonication, and analyzed by 2D difference gel electrophoresis (2D-DIGE). Protein regulation data were obtained using DeCyder software. A total of 1090 proteins were equally expressed by both strains. Two hundred and eighty proteins were upregulated in genotype B (virulent strain) compared to genotype A, while 240 proteins were downregulated in the same strain. Spots of interest were analyzed by MALDI-TOF and/or MALDI-TOF/TOF mass spectrometry. Proteins were identified using MASCOT and by searching for matching peptide mass fingerprinting in a protein database of the related parasite *Trichomonas vaginalis,* which has been recently sequenced. Metabolic proteins are the largest group of modulated proteins in this analysis, but more studies are necessary in order to elucidate the relevance of these changes.

This work was supported by Fundación Sedesa of Valencian Community

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PROTEOMIC ANALYSIS OF A MEDIA CONDITIONED BY GROWING DEER ANTLER TISSUES: IDENTIFICATION OF ITS NEURITE GROWTH PROMOTING PROTEINS

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The annual regeneration cycle of the deer (Cervidae, Artiodactyla) antlers represents an unique model of epimorphic regeneration and rapid growth in adult mammals. During this cycle, the different tissues that make up the antlers, including their innervation, grow in the common deer (*Cervus elaphus*) to reach over 1 metre of lengths. All the growing process takes place in approximately 3 months during the springtime, reaching elongation velocities up to 2 centimetres per day, one of the highest rates of growth in adult animals. In previous studies, we have shown that factors secreted in conditioned media by the velvet (the form of modified skin that covers the whole antler during its annual regeneration period) after its organotypic culture promote *in vitro* neuritogenesis and neurite growth of adult and embryonary sensory neurons from rats (*Rattus norvegicus*). Activity analyses after different treatments of these media (enzymatic digestion, heating at 95ºC, filtering, ...) have allow us to establish the proteic nature of the neurite growth promoting factors. With the aim of identifying the proteins contained in these conditioned media and to identify candidates for neurite growth promoters we have analyzed the media using different proteomic approaches like two-dimensional electrophoresis and mass spectrometry. The results of these analyses showed the suitability of this methodology and allowed us to identify several proteins present in the cultured tissue secretome. We have also compared this secretome with the untreated culture media, in order to find secreted proteins that could be considered potentially responsible of promoting neural growth.

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A DIGE PROTEOMIC ANALYSIS OF WHEAT FLAG LEAF TREATED WITH TERRA-SORB® FOLIAR, A FREE AMINO ACID HIGH CONTENT BIOSTIMULATOR

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The flag leaf is the most important source of carbohydrate during wheat kernel filling. Around a 75% of all sugars stored in the kernel come from carbon fixed by this leaf. Terra-Sorb® foliar is an L-α-amino acid-based product from enzymatic hydrolysis for foliar application with a high ratio of free to total amino acids. Previous agronomical studies carried out on grassy, horticultural and tree crops have shown that application of Terra-Sorb ® increases plant photosynthetic activity and chlorophyll content, promotes rapid recovery from stress and improves fruit set.

In this work we have undertaken a proteomic approach in order to explore molecular mechanisms potentially involved in the stimulating effect of Terra-Sorb ® Foliar on wheat yield when applied in commercial fields. Wheat plants were treated in field at the flag leaf stage and flag leaves were subsequently sampled after two and three days. A DIGE approach was used to compare the proteomes of treated vs control plants in four biological replicates.

Thirty seven protein spots were found to change in abundance (ANOVA $p<0.05$) out of which 8 were down regulated and 29 up regulated in treated leaves. Twenty six protein spots encoded by 13 different genes were successfully identified by nLC-ESI-MS/MS and NCBInr database search.

Two RUBISCO regulatory proteins, namely RUBISCO activase and RUBISCO large subunit binding protein, and phosphoribulose kinase were up regulated thus suggesting an enhanced CO_2 fixation. Protein biosynthetic metabolism would also be promoted as the elongation factors Tu and G and the chaperone HSP-90 were also up regulated. The Cu/Zn superoxide dismutase which protects photosystem II from photoxidation was down regulated, perhaps due to a lower oxidative stress. Minor but statistically significant changes were also found in metabolic proteins.

In conclusion, the improvement of wheat productivity by Terra-Sorb® Foliar application seem to be mediated by a combination of an enhanced CO_2 fixation, a more active protein metabolism and a decrease of oxidative stress.

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A PROTEOMIC APPROACH TO ACUTE AND CHRONIC PHASE OF CHAGAS DISEASE

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Introduction: The protozoan parasite *Trypanosoma cruzi* is the etiological agent to Chagas disease (CD), a chronic illness endemic in Latin America, with 18 million people infected and over 90 million at risk. The CD is characterized by two major clinical forms: acute and chronic. The acute form is directly related with the parasite multiplication into macrophage and cardiac muscle cells. The chronic form is associated with myocardial hypertrophy, and myocyte degeneration. The search for differences in gene expression and biochemical properties among parasite isolated of individual with different clinical forms may lead to better characterize the role of *T. cruzi* in the development of the different clinical forms of CD.

Objectives: To determine the differential expression of *T. cruzi* obtained from patients with acute and chronic form of CD in order to correlate them with the different clinical forms.

Materials and Methods: The *T. cruzi* epimastigotes were cultured in LIT medium supplemented with 10% FCS and maintained at 28°C. The differential expression of proteins of whole-cell lysated from epimastigotes was processed by 2D electrophoresis and analyzed with PD QUEST software.

Results: 416 and 390 spots were detected on isolates of chronic and acute patients, respectively. 27 spots were present specifically in the chronic isolate patient while 25 spots were found only in the acute. Based in Mr and pI values we can observe differential expression between two relevant proteins, glyceraldehyde 3 phosphate dehydrogenase (GAPDH) and thioredoxin. These proteins must be confirmed with mass spectrometry (MS).

Conclusions: We suggested that presence of thioredoxin in the chronic isolate could be associated with the parasite survival into the host cell. On the other hand, the GAPDH, a metabolic enzyme, with higher expression in isolated acute could is related with a major virulence.

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PROTEOME ANALYSIS OF WILD *SACCHAROMYCES CEREVISIAE* AND POTASSIUM TRANSPORT-AFFECTED MUTANT STRAINS

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We have been using *Saccharomyces cerevisiae* as a model system to study homeostasis of potassium, the most abundant intracellular cation. For this purpose, the proteomes of the wild type BY4741 and potassium transport-affected mutant *trk1,2* strains have been compared, either under optimal or limiting potassium concentrations. This work is part of the multidisciplinary international research project "Gene interaction networks and models of cation homeostasis in Saccharomyces cerevisiae" (TRANSLUCENT, http://www.sysmo.net/index.php?index=61).

By using a differential expression proteomics approach, we have been analyzing differences in the protein profile between wild and *trk1,2* mutants under the following conditions: i) optimal growth potassium concentration (20 mM), exponential and stationary phase; ii) absence of or limiting (5 mM) potassium concentration, 30 min, 1, 3, and 5h. The following workflow has been used: i) protein extraction (buffer homogenization of the cells and TCA-acetone precipitation of the proteins); ii) protein separation by two-dimensional (IEF, SDS-PAGE) electrophoresis; iii) Coomassie gel staining, densitometer image capture, and spot quantitation; iv) statistical analysis of the data, and identification of qualitative or quantitative differential spots; v) trypsin digestion of the protein spots; vi) mass spectrometry analysis of the tryptic peptides (MALDI-TOF-TOF); vii) protein identification from MS and MS^2 spectra.

Comparative analysis of the 2-DE protein map of wild and mutant types grown under non limiting potassium concentrations revealed the existence of qualitative and quantitative differences between strains and growth phases. Taking as a reference the 2-DE map of the wild cells at the exponential phase, we have observed 10, 40, and 65 qualitative and 112, 122 and 131 quantitative variable spots, for, respectively, wild type stationary ones, and mutant exponential and stationary ones. Mass spectrometry analysis of these variable spots is in progress. Changes in the protein profile of both strains under K^+ starvation are being analyzed. In the wild type, the absence of potassium caused a decrease in the protein content of the cells, this being manifested by a reduction in the number of 2-DE spots detected.

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DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS OF ACTIVATED E2F2$^{-/-}$ T LYMPHOCYTES BY MEANS OF A LABEL-FREE QUANTIFICATION METHOD

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Differential proteomics has traditionally relied on two-dimensional electrophoresis (2DE). 2DE is a powerful technique that allows the separation and analysis of hundreds of proteins in a single gel, leading to the analysis of global expression patterns. 2DE, however, has a number of inherent limitations and disadvantages. To overcome these limitations several MS-based gel-free techniques have been developed. Among these, a label-free absolute quantification method based on the three most intense peptides at each protein developed by Waters (Hi3) is very simple and affordable.

E2F family of transcription factors (E2F1-8) are crucial regulators of cell-cycle and proliferation. Characterization of mice lacking functional E2F2 transcription factor (E2F2$^{-/-}$) showed the development of an autoimmune syndrome due to the presence of hypersensible and hyperreactive peripheral T lymphocytes. These T lymphocytes hyperproliferated upon antigenic stimulation, and showed an accelerated entry to the S phase of cell cycle, suggesting a role for E2F2 in the repression of G1/S transition. Previous effort in our lab has allowed us the characterization of E2F2-lacking T lymphocyte's expression patterns on a 2DE-based approach.

In this work we characterize E2F2$^{-/-}$ activated T lymphocyte's protein expression patterns by Hi3 absolute quantification method. Hi3 analysis of these extracts revealed the deregulation of some interesting cell-cycle and E2F-related proteins, proving the usefulness of this technique for differential expression analysis. Moreover, results obtained by 2DE and Hi3 analysis of activated E2F2$^{-/-}$ T lymphocytes are compared, suggesting that both approaches can provide complementary viewpoints in differential proteomics analyses.

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DIFFERENTIALD PROTEIN PROFILES OF *TRYPANOSOMA CRUZI* STRAINS ISOLATED FROM ASYMPTOMATIC AND SYMPTOMAC PATIENTS WITH CHAGAS DISEASE

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Trypanosoma cruzi is a protozoan kinetoplastid parasite responsible for Chagas disease. This important disease affects 18 million people in Latin America. Currently, a few hundred thousand of seropositive individuals are estimated to live in the USA, Europe and Asia. Moreover, Chagas disease is characterized by an acute phase followed by a long chronic phase. During this disease, 70% of T. *cruzi*-infected individuals remain asymptomatic, whereas the remaining 30% suffers Chagas cardiomyopathy. Drugs for the treatment of *T. cruzi* infection are inadequate, and vaccines are lacking. On the other hand, prognostic values of these alterations in the variability of symptoms, and geographical differences in the distribution of the chronic forms of Chagas disease, have been attributed to diversity of *T. cruzi* strains. Many investigations have determined that gene expression in *T. cruzi* is mainly regulated at post-transcriptional level. Alternatively, proteome analyses have been used for the generation of protein maps from different parasite forms. One explanation for different phenotypes and pathogenesis induced by *T. cruzi* strains may be attributed to differential expression of some particular proteins. In this work, we have investigated this hypothesis, analyzing proteome profiles of two strains of *T. cruzi*. For this aim, we used 2D-gel electrophoresis, determining protein expression profiles of two *T. cruzi I* strains isolated from asymptomatic (strain MF) and symptomatic patiens (with cardiac form of Chagas disease (strain LQ)). For protein identifying of differentially expressed proteins between these two strains, we carried out image analysis, using PD-Quest Image Software analysis (Biorad). 273 and 305 spots were displayed respectively in the strains LQ and MF gels. The two strains share the presence of 242 spots; determining differences in protein expression and spot intensity of proteome images.

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S-GLUTATHIONYLATION IN *ARABIDOPSIS THALIANA*

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The present work is included in a research project aimed at analyzing changes in the redox proteome and elucidating the role of protein nitrosylation and glutathionylation in *Arabidopsis thaliana* in response to *Pseudomonas syringae*. As a preliminary step to the glutathionylation studies, we are optimizing the methodology by using protein standards and leaf extracts. Leaf tissue was homogenized in PBS buffer instead of extracting the proteins by the usual precipitation methods (i.e. TCA-acetone-phenol) used with plant material. This was due to the difficulties in solubilising the pellet in a detergent-free solution. On one hand, a glutathion-biotinylation-agent was prepared as reported in Brennan et al. 2006 and Dixon et al. 2005 with some modifications. Protein samples were labelled with this agent and labelling was checked by Western blot using biotin and glutathione antibodies. On the other hand, experiments are being performed based on detecting *in vivo* and *in vitro* protein glutathionylation with glutathion antibody and identifying glutathionylated peptides through LC ESI ion trap MS analysis.

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USING A QCM BIOSENSOR TO MEASURE THERMODYNAMIC PROPERTIES OF PROTEIN-PROTEIN INTERACTIONS

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In this study we show that quartz crystal microbalance technology (QCM) can be used to measure thermodynamic parameters such as the enthalpy and entropy contributions of molecular interactions. Accurate information of the thermodynamic parameters, for example if a protein-protein interaction is enthalpy or entropy driven, has already been proven useful in pharmaceutical research. Here we studied the interaction of a genetically engineered variant of protein A and the Fc-domain of two antibodies to verify the usefulness of the QCM technology in finding selection criteria for potential drug candidates. Data in this report were obtained by full kinetic analysis at a range of temperatures and final parameters were calculated using a Van't Hoff plot.

The thermodynamic properties of antibody-antigen interaction provides additional molecular information and thereby the possibility to select between antibodies that for example have similar kinetics. This QCM-based approach is also applicable to other types of molecular interactions.

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A PROTEOMIC APPROACH TO THE MYOCARDIUM OF HYPERTENSIVE-DIABETIC RATS

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Aim: To study the myocardial protein expression secondary to long-term type I diabetes mellitus (DM1).

Methods: Spontaneously hypertensive (SHR) rats received a single streptozotocin injection to develop type I diabetes (DM1). After 28 weeks, DM1/SHR and control normotensive rats were sacrificed and the left ventricles studied by 2DE-DIGE proteomic studied by 2DE-DIGE, MALDI mass spectrometry and biochemical approaches.

Results: Diabetes affects to the myocardium. Glucose impairment and formation of redox molecules induces myocardial fibrosis and apoptosis in the heart. DM1/SHR rats presented hyperglycemia (400 mg/dl) and hypertension (200 mmHg). Rat myocardium showed interstitial and peri-vascular fibrosis and apoptosis. By 2DE-DIGE proteomic assay we found differentiated protein expression in the DM1/SHR myocardium vs. control. Expression of pro-fibrotic factors, as myoenzyme-2 and pro-apoptotic, as anexin-V and C1-citochrome was altered. Anti-oxidants as catalase were also modified. Moreover, mitochondrial metabolism enzymes (for glucose and fatty acids) were deregulated. By biochemical studies, expression of pro-fibrotic molecules Transforming Growth Factor-ß ($TGF\beta_1$), Connective tissue growth factor (CTGF) was enhanced and the $TGF\beta_1$-linked transcription factors (p-Smad3/4 and AP-1) were activated. Pro-apoptotic factors FasL, Fas, Bax and cleaved caspase-3 were also augmented ($p<0.05$). However, the pro-inflammatory molecules, monocyte chemoattractant protein-1 (MCP-1), interleukin-1 (IL-1), and vascular cell adhesion molecule-1 (VCAM-1) were not elevated.

Conclusions: Fibrosis and apoptosis are long-term features of myocardial damage induced by experimental DM1/SHR. New proteomic-identified factors may play a role in these processes. However, inflammation does not seem to be a key feature. Pharmaceutical strategy targeting these factors may be used in hypertensive-diabetic patients.

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VEMS: A TOOL TO QUANTIFY iTRAQ LABELED SAMPLES

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Introduction: The goal of many MS-based proteomics experiments nowadays is to quantify changes in the abundance of the proteomes across several samples of biological interest. The iTRAQ labeling method is a powerful relative quantitation technique that combined with liquid chromatography coupled to tandem mass spectrometry allows quantify up to eight different samples simultaneously. The transformation of the multiple spectra containing different protein expression values is a challenging task. We have developed an integrated tool for database dependent interpretation, quantitation and database storage for iTRAQ labeled samples able to handle various input data formats from instruments from different manufacturers. Users can download the Web Server from http://personal.cic.biogune.es/rmatthiesen/.

Results: The reference sample gave the expected ratios with a standard deviation on the peptide ratios in the range of 0.03-0.13. The accuracy of the calculated protein ratios was from 0.02-0.05% for the reference sample which contains the proteins mixed in known ratios.

To evaluate VEMS performance with large-scale proteomics data, the same amount of exosomes samples in three different conditions were labeled with iTRAQ and fractionated by SCX as explained in methods section. In the first experiment 326 proteins were identified while in the second experiment 243 proteins were identified. In total 191 proteins were identified commonly in the two replica experiments. From the total, 68.5% of these proteins were significantly quantified in the two replica experiments with an average 95% confidence interval of ±0.19 (Figure 1).

Innovative aspects

- Generalization of the quantitative algorithm provided in the program i-tracker (2).
- Integration of data search, quantitation and storage in one program in contrast TandTRAQ (3).
- Improved statistical analysis of result.

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References

(1) Matthiesen R et al. VEMS 3.0: algorithms and computational tools for tandem mass spectrometry based identification of post-translational modifications in proteins. *J. Proteome Res.,* **4** (6), 2338 -2347, 2005.

(2) D'Ascenzo et al. iTRAQPak: an R based analysis and visualization package for 8-plex isobaric protein expression data. *Brief Funct Genomic Proteomic.*2008; 0: eln007v1-eln007.

(3) Laderas T et al. TandTRAQ: an open-source tool for integrated protein identification and quantitation Bioinformatics 2007 23(24):3394-3396.

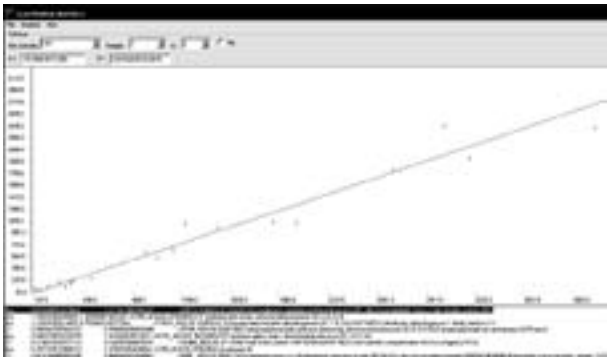

Figure 1
Manual validation of the protein quantitative values.
Peptide ratios considered to be outliers can be removed by clicking on the plot.

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PROTEOMIC AND OXIDATIVE STRESS ANALYSIS IN HUMAN BRAIN SAMPLES OF HUNTINGTON DISEASE

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Huntington disease (HD) is an inherited neurodegenerative disorder characterized by degeneration of neurons affecting initially the striatum and progressively the cortex. Clinical symptoms include motor and cognitive alterations leading inevitably to death. The disease involves expansion of CAG trinucleotide repeats in the *huntingtin* gene codifying for glutamines in the htt protein. We performed a proteomic analysis of human brain post-mortem samples obtained from striatum and cortex of patients with HD compared to samples of age and sex-matched controls. Antioxidant defense proteins that were strongly induced in striatum, but also detectable in cortex, were identified as peroxiredoxins 1, 2 and 6, as well as glutathione peroxidases 1 and 6. The activities of other antioxidant enzymes such as mitochondrial superoxide dismutase and catalase were also increased in HD. Aconitase, a protein involved in energy metabolism, showed decreased activities in striatum of HD patients. Protein carbonyls, used as markers of oxidative stress, were increased in HD and glial fibrillary acidic protein was identified as the main target. Moreover, other proteins such us aconitase, γ-enolase and creatine kinase were also found oxidized in HD. Taken together, these results indicate that oxidative stress and damage to specific macromolecules, would participate in the disease progression from striatum to cortex. Also, these data support the rationale for therapeutic strategies that either potentiate antioxidant defenses or avoid oxidative stress generation in order to delay disease progression.

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SNAKE VENOMICS AND ANTIVENOMICS OF *BOTHROPS COLOMBIENSIS*, A MEDICALLY IMPORTANT PITVIPER OF THE *BOTHROPS ATROX-ASPER* COMPLEX ENDEMIC TO VENEZUELA: CONTRIBUTING TO ITS TAXONOMY AND SNAKEBITE MANAGEMENT

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The taxonomic status of the medically important pitviper of the *Bothrops atrox-asper* complex endemic to Venezuela, classified as *Bothrops colombiensis*, remains *incertae cedis*. To help resolving this question, the venom proteome of *B. colombiensis* was characterized by RP-HPLC followed by analysis of each chromatographic fraction by SDS-PAGE, N-terminal sequencing, MALDI-TOF mass fingerprinting, and CID-MS/MS of tryptic peptides. The venom contained 8 types of proteins. PI Zn^{2+}-metalloproteinases and K49 PLA_2 molecules comprise over 65% of the venom proteins. Other venom protein families comprised PIII Zn^{2+}-metalloproteinases (11.3%), D49 PLA_2s (10.2%), L-amino acid oxidase (5.7%), medium-sized disintegrin (5.6%), serine proteinases (1%), bradykinin-potentiating peptides (0.8%), a DC-fragment (0.5%), and a CRISP protein (0.1%). Comparison of the venom proteomes of *B. colombiensis* and *B. atrox* did not support the suggested synonymy of *B. colombiensis* and *B. atrox*. The closest homologues to *B. colombiensis* appeared to be *B. asper*. A rough estimation of the similarity between their venoms indicated that these species share approximately 65-70% of their venom proteomes. The close kinship of *B. colombiensis* and *B. asper* points at the ancestor of *B. colombiensis* as the founding Central American *B. asper* ancestor. This finding may be relevant for reconstructing the natural history of Bothrops. Further, the indistinguishable immunological crossreactivity of a Venezuelan antivenom (against a mixture of *B. colombiensis* and *Crotalus durissus cumanensis* venoms) and the Costa Rican polyvalent antivenom (against a mixture of *B. asper*, *Crotalus simus*, and *Lachesis stenophrys* venoms) towards the venoms of *B. colombiensis* and *B. asper*, supports this view and suggests the possibility of indistinctly using these antivenoms for the management of snakebites by any of these Bothrops species. However, our analyses also evidenced the limited recognition capability of these antivenoms towards a number of *B. colombiensis* and *B. asper* toxins, notably medium-size disintegrins, bradykinin-potentiating peptides, PLA_2s, and PI-SVMPs.

SNAKE VENOMICS AND ANTIVENOMICS OF MIDDLE AND SOUTH AMERICAN RATTLESNAKES. IDENTIFICATION OF NEUROTOXIN CROTOXIN AS AN ADAPTIVE TRAIT DURING *CROTALUS DURISSUS* INVASION OF SOUTH AMERICA

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Venoms of subspecies of neotropical rattlesnakes represent an example of adaptive phenotypic variation. Venom of adult Central American *C. simus*, formerly *C. d. durissus*, cause local tissue damage, hemorrhage, coagulopathy and cardiovacular shock, but is devoid of neurotoxicity and systemic myotoxicity. However, newborn *C. simus* venom resembles that of South American *C.d. terrificus* being both neuro- and myotoxic and devoid of hemorrhagic activity. The pathophysiology of *C.d. terrificus* envenomation is due to the neurotoxic and myotoxic effects of crotoxin, a heterodimeric PLA_2 causing progressive paralysis and myonecrosis. Venoms of the subspecies *C. d. cumanensis* and *C. d. ruruima*, inhabiting northern regions of South America, exhibit a mixed pattern, inducing neurotoxicity and hemorrhage. The phylogeographical pattern of Crotalus dispersal is consistent with a stepwise colonization from Mexico along the Central American Isthmus (1.85 Mya), followed by rapid dispersal into and across South America (1.5-1.1 Mya). Our venomic analyses indicate that the gain of neurotoxicity associated with increasing crotoxin expression represents the key axis along which overall venom toxicity has evolved during *Crotalus durissus* invasion of South America. Assuming a link between venom toxicity and increased crotoxin concentration, the identification of evolutionary trends may have an impact in defining the mixture of venoms for immunization to produce effective antivenoms. A Costa Rican antivenom against *C. simus simus*, is ineffective neutralizing both the venom of South American *Crotalus durissus* subspecies and of newborn *C. simus simus*. Similarly, South American antivenoms against *C.d. terrificus* neutralize lethality of Central American venoms but are ineffective at neutralizing the hemorrhagic activity of venoms from genus Crotalus. Such neutralizing profile is fully explained by the proteomic characterization of *Crotalus* (*simus* and *durissus*) venoms showing increasing amounts of crotoxin in the venoms of *C. durissus* subspecies along the north to south colonization pattern of this group of snakes.

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GENERATION OF UNIQUE PROTEIN SPECIFIC MRM SIGNATURES; USING PEPTIDE INFORMATION FROM ALTERNATE SCANNING LC-MS DATA TO DRIVE MRM DEVELOPMENT

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Proteomics research has resulted in the discovery of a large number of differentially expressed proteins which must be validated to determine their utility as specific markers. The quantitation of these proteins is challenging due to both the inherent complexity associated with the number of tryptic peptides generated and the dynamic range in protein concentration present.

Previously we have described, how, using an alternate scanning LC-MS strategy on a Q-Tof mass spectrometer we can derive a comprehensive inventory of precursor and product ions, peak area intensities and associated physio-chemical properties. Here we show how this experimental data (precursor and fragment m/z values, intensity and retention time) can be utilized to empirically determine those peptides which uniquely identify a protein in a database from a complex sample. In addition the algorithms determine both the 'best' ionising peptide precursor and the most selective fragment ion to determine the most appropriate multiple reaction monitoring (MRM) transition to monitor.

For this study a cytosolic extract from Escherichia coli was digested and analysed by LCMSE on a QTof type instrument. IdentityE processing produced a profile of the proteins present and a comprehensive inventory of the peptide precursor and fragment ions present. This inventory of over 25000 potential MRM transitions was filtered to determine 'proteotypic' peptides each protein in the sample removing any which share a common sequence. Further filtering reduces the candidates to around 5500 by retaining the five most intense precursor ions per protein and their five most intense fragments. These are now automatically assessed for m/z and retention time overlap prior to producing an MRM experimental file which can be transferred to triple quadrupole instrument. Here we show the utility of the automated data sorting tools to build triple quadrupole MRM methods which can be used quantify E.coli proteins.

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HIGH CONFIDENT PROTEIN IDENTIFICATION FROM ETD AND ECD SPECTRA WITH A NEW MASS LIST PREPROCESSOR

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Due to the nature of the ECD or ETD fragmentation mechanism, the spectra contain some peaks that are directly related to the parent ion peaks: the remnants and neutral losses thereof, the reduced charge species. The removal of these irrelevant peaks has shown to increase the confidence of identification for database searches.

A simple tool has been developed that uses the charge state information of the ECD or ETD precursor ions in high resolution FTMS spectra. For high resolution FTMS instruments like LTQ FT or LTQ Orbitrap, the charge state of the precursor ions can be easily determined and from that information all the non-fragment peaks can be calculated. We have developed a spectrum preprocessor tool that removes all non-fragment masses from the spectrum mass list before submitted to database searches using Sequest and Mascot. This spectrum preprocessor tool was implemented in Proteome Discoverer 1.0 software. The search results for all identified peptides show a significant increase in the peptide score and – even more importantly – a large number of false positive identifications are eliminated in comparison to the search without the preprocessor.


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POETÓMICA

"Hacia Iruña"

Proteómica: hacia Iruña vas ahora
Naciendo un nuevo julio de febrero;
Veníamos de Góngora y Romero
De Torres a través de la memoria

Del Turia ajardinada, donde mora
Un párpado, quizá, -¿o es un guerrero?-,
Y en palacio de isótopos de acero,
Levitando, un Nobel que rememora.

¡Henchidos, cual Miguel, de aire norteño,
Con la camisa y el pañuelo rojo,
La chistorra y el néctar de la endrina,

Qué cambios de expresión, en este empeño,
Habremos de lidiar, rogando arrojo
Al socio San Fermín en su hornacina!

Jesús Vázquez