The Joint 2nd Pacific Rim

International Conference on Protein Science

and 4th Asian-Oceania

Human Proteome Organization

Cairns Convention Centre

22-26 June 2008

www.aohupo2008.org or www.pricps2008.org

Visit the web page now to leave your email address and be kept informed of deadlines.

Convenor: Professor Richard J Simpson, Ludwig Institute for Cancer Research, Melbourne, Australia. Email: Richard.Simpson@ludwig.edu.au

4th AOHUPO

Supported by the Lorne Conference on Protein Structure and Function, Australasian Proteomics Society, and the Protein Science Society of Japan.

2nd PRICPS

INDEX

WELCOME MESSAGE FROM CONVENOR OF THE JOINT 4TH AOHUPO/2ND PRICPS CONFERENCE

Richard J Simpson PhD FTSE
Convenor of the joint 4th AOHUPO/2nd PRICPS Conference
Professor of Biochemistry,
Member, Ludwig Institute for Cancer Research, Melbourne, Australia

The organizers welcome all delegates and presenters to the joint 4th AOHUPO/2nd PRICPS conference in Cairns.

First, a brief background into the history of AOHUPO and PRICPS.

AOHUPO grew out of the Pacific-Rim International Proteome and Proteomics Conference (IPPC), conceived in 1999 by Akira Tsugita, Richard Simpson and Young-Ki Paik. At that time there were no proteomics societies in the Asian-oceanic region and the HUPO had not been conceived. The IPPC provided a forum in which researchers in the region who had a common interest in protein chemistry and related proteomics technologies could assemble and discuss and exchange ideas of common interest. Research interests were disparate, ranging from microorganisms, plants, animal husbandry to human diseases. At the 2nd IPPC meeting held in Canberra 2001, the AOHUPO was established. Subsequent AOHUPO conferences have now been held in Taiwan (2003), Korea (2005) and Singapore (2006).

From its modest beginnings, AOHUPO has grown and now has representatives from 14 different societies / countries. These include 10 Societies (Australasian Proteomics Society, Thailand Proteomics Society, Taiwanese Proteomics Society, Pakistan Proteomics Society, Iranian Proteomics Society, Hong Kong Proteomics Society, Vietnamese Proteomics Society, Japan HUPO, China HUPO, Korean HUPO) and individuals representation from New Zealand, Philippines, Malaysia, Singapore and India. India is in the process of forming a proteomics society.

In 2007 the first AOHUPO initiative, the Membrane Protein Initiative, (MPI) was instigated under the directorship of Bill Jordan.

The Pacific-Rim International Conference on Protein Science (PRICPS) was the brainchild of Tairo Oshima and his colleagues in the Protein Science Society of Japan (PSSJ). The 1st PRICPS meeting (organized by PSSJ and held in Yokohama, Japan 2004) was a collaboration with both the Science Council of Japan and the Protein Society. The purpose of the PRICPS was to announce new findings and analyze future trends in the field of protein science as well as to recruit young researchers in the post-genome area to this growing field.

Our hope is that, in addition to inspiring young researchers, this joint AOHUPO/PRICPS conference in Cairns will contribute to the further development of protein science in the Pacific-Rim region.

Whether you are attending the Cairns conference to share information, to learn (a hallmark of the 4th AOHUPO/2nd PRICPS meeting will be the strong educational and training programme underpinned by our participating trade sponsors), to observe new technologies or simply to network with fellow like-minded researchers, on behalf of the Organising Committee, I would like to welcome you to the Cairns conference.

JST/CREST

Tairo Oshima, Research Supervisor of CREST

Japan Science and Technology Agency (JST) is a funding agency sponsored by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), the Japanese Government. JST aims to establish Japan as a nation built on the creativity of science and technology, and supports basic and technological researches in line with science and technology policy of the Japanese Government.

About JST
Core Research for Evolutional Science and Technology (CREST) is one of the research funds of JST, and supports basic research teams for creating innovation seeds in near future. JST CREST “Protein Structure and Functional Mechanisms: Toward Creation of Innovative Medicines, Diagnosis, and Material Production Based on Functional Mechanisms of Proteins” was implemented in order to promote Protein Structural Biology in Post-Genome era in 2001. CREST “Proteins” accepted 19 research teams with total budget of about 6,500,000,000 Japanese Yen. 2008 is the final year of the project.

Tairo Oshima, Research Supervisor of CREST “Protein Structure and Functional Mechnisms”, obtained Ph.D. from the Department of Biochemistry, University of Tokyo. He isolated many extreme thermophiles such as Thermus thermophilus and Sulfolobus tokodaii which are widely used in Protein Science as sources of stable proteins. He himself conducted the studies on molecular mechanisms of unusual stability of thermophile proteins. He served as the chair person of the Rsearch Promotion Committee of Protein 3000 Project which is the biggest national project in the field of Life Sciences in Japan and determined 3D structures of more than 4000 new proteins in 5 years. He also served as the chair person of the Organizing Committee of the 1st PRICPS 2004 held in Yokohama. Currently he is the director of the Institute of Environmental Microbiology, Kyowa-kako Company in Tokyo, and Professor Emeritus of Tokyo Institute of Technology and also Professor Emeritus of Tokyo University of Pharmacy and Life Sciences.

AOHUPO MEMBRANE PROTEIN INITIATIVE WORKSHOP

Bill Jordan, Coordinator of the AOHUPO MPI

The goals of the AOHUPO Membrane Proteomics Initiative (MPI) are to develop methods for characterisation of membrane proteomes and to characterise the proteomes of specific membrane systems. This initiative was selected because of the importance and challenge of membrane proteomics. Membrane proteins are calculated to represent a large fraction (20-30%) of genomes, are essential in processes including signal reception and transduction, are implicated in disease and are important targets for the pharmaceutical industry.

Our multi-centre project has initially focused on analysis of a membrane sample (MPI standard) that has been distributed as aliquots of a single preparation of carbonate-washed liver microsomes from 10-11 week male C57BL/6J mice. This sample is being analysed independently by seventeen laboratories using separation of intact proteins (differential detergent separation, 1D SDS PAGE, BN-PAGE, chromatography including PF2D, off-gel electrophoresis, and combinations thereof) or by separation of digest peptides (IEF, 1D and 2D HPLC) prior to on-line or off-line tandem MS of peptides. Tandem MS files (192 Gb raw files from TOF-TOF, 2D and 3D ion-trap, QuadTof, Orbitrap and FT instruments) are being analysed independently by four bioinformatics groups.

The initial results will include an assessment of the merits of various experimental approaches and an assessment of the extent to which proteomics technologies are able to detect integral membrane proteins including enzymes, receptors and transporters that are poorly represented in published analyses of membrane proteomes.

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Protein Science Society of Japan (PSSJ)
Pakistan Proteomics Society (PPS)
China HUPO (CNHUPO)
Chinese Protein Society (CPS)

PARTICIPATING ORGANISATIONS

Australasian Proteomics Society - www.australasianproteomics.org
Chinese Protein Society
Lorne Proteins Conference - www.lorneproteins.org
Protein Science Society of Japan - www.pssj.jp/index.Eng.html
Protein Society - www.proteinsociety.org

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DELEGATE INFORMATION

What Your Registration Includes:
The Delegate and student registrations include;

- Access to all sessions
- Conference satchel complete with program book and abstract book
- Morning teas, lunches and afternoon teas
- Welcome Function
- GST

Social Program
Welcome Function, Sunday 22nd 6:30pm ***Sponsored by***
Delegate registration includes the welcome function on the first night.
Tjapukai Aboriginal Cultural Centre Dinner, Tuesday 24th 6:30pm
Outstanding cultural show put on by the local Tjapukai tribe (pronounced Jab-pu-kai). A courtesy bus will pick you up from outside the Convention Centre at 6:30pm. The dinner and show will commence at 7:30pm.
Conference Social / Dinner, Wednesday 25th 6:15pm ***Sponsored by***
Held at the Cairns Convention Centre with an *Aussie Bush Band*. Tickets can still be purchased for this function. Please see the registration desk to purchase a ticket.

Speaker Preparation Instructions
All speakers are to load their presentation in the speaker preparation room which is manned by operations staff. It is the conference preference to have ALL talks pre-loaded to the common laptop which is a PC. As per instructions already supplied, you should give your talk on a CD or USB stick to the technician well before the session you are participating in so it can be loaded and tested.

Displaying your Poster
All posters will be displayed in the poster area which will be clearly signposted. Every poster will have a number corresponding to the code given by ASN Events. Your poster is to be displayed in the poster session you have been assigned to. If you have been assigned to Poster Session 1, your poster can be put up on Sunday but must be taken down Monday night or earlt Tuesday Morning. If you have been assigned to Poster Session 2A or 2B, your poster can be put up Tuesday morning and taken down after the Wednesday poster session. We do not take any responsibility for posters left on display afterwards. The maximum size provided is 1m wide by 1.2m high. The approved way of attaching your abstract is with velcro. Please visit the registration desk for additional supplies.

Internet Café ***Sponsored by***
Free wireless internet access is available on all conference days throughout the Convention Centre. Delegates can log on using their own laptop with wireless connection, or use one of the computers provided in the Internet café adjacent to the entrance to the trade area.

Name Tags
Delegates and registered partners/children are required to wear their name tags to all scientific and catered sessions, including breakfast.

Hotel Check Outs
You are required to check out of your room before 10am. ASN have forwarded your advance room payment to the hotels. You will need to settle additional private expenses yourselves on departure if required.

Smoking
Smoking is not permitted in the venue.

Dress Code
The dress code for the conference sessions is smart casual.

Mobile Phones
Please ensure your mobile phone is turned off during any session you attend.

Language
The official Symposium language is English. All abstracts, oral presentations and posters are in this language.

Journals of Proteomoics & Bioinformatics -Open Access

Full text avilable at http://www.omicsonline.com **By OMICS Publishing Group**

Journal of Proteomics & Bioinformatics(JPB), a broad-based journal was founded on two key tenets: To publish the most exciting researches with respect to the subjects of Proteomics & Bioinformatics. Secondly, to provide a rapid turn-around time possible for reviewing and publishing, and to disseminate the articles freely for research, teaching and reference purposes.

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LOCAL FACTS & TOURIST INFORMATION

Population
Cairns has a population of over 120,000 making it the 16th largest city in Australia. More than 2 million people visit the area annually.

Location
Cairns is located in Australia's tropical north and is considered the capital city of Far North Queensland (FNQ). Cairns, and the surrounding region, is one of the world's most desired destinations, as it is the only place on earth where two World Heritage listed sites live side by side. These World Heritage listed areas are the Great Barrier Reef and Australia's Tropical Rainforests, both are easily accessed from Cairns city.

Attractions
Although Cairns is situated a huge 1750km north of the state capital, Brisbane, it is conveniently situated close to the many attractions of FNQ. Some of the regions popular attractions, located within easy driving distance of Cairns, include:

Palm Cove - 26km north (approx. 20 mins driving)
Port Douglas - 67km north (approx. 1 hour driving)
Daintree - 110km north (approx. 2 hours driving)
Cape Tribulation - 140km north (approx. 2 and a half hours driving)

Climate
The Cairns region has a tropical climate ideal for outdoor enjoyment. June is a great time year to be in the area with monthly averages of:
Average daily max: 26.5°C Average daily min: 18.6°C Average humidity: 34%

Transport around Cairns

Car Rentals - There are a number of car rental companies that operate within the airport terminals. They have staffed reception desks during all arrival times. Car rental companies can also be found in the CBD.

Taxis (Cabs) – Taxis are conveniently located outside both the domestic and international terminals at the Cairns airport. An approximate fair into the city is AUD$24. Taxis are also available from the main taxi rank in the City Place or along the Esplanade and in front of the reef Casino. For more information or to make a booking call: Black & White Taxis Ph: 13 10 08

Shuttle Bus - *Australia Coach* operates an airport shuttle bus service to hotels and the city centre. *Sun Palm Express Coaches* operate services to the Northern Beaches, Palm Cove, Port Douglas and Cape Tribulation. Both companies pick up immediately in front of the arrivals area at both terminals and both operate an information desk within the terminal, which if not staffed, have direct-dial telephone. Most of the major hotels in the region operate their own courtesy coach service to and from the airport.

Public transport – Buses run from City Place and service areas all over Cairns. Buses also service the northern beaches. For more information on services and timetable information call: Sun Bus
Ph: +61 (07) 40 577 411

Restaurants
Dining out is an important part of the Cairns experience as the city is home to some of Australia's finest restaurants. Restaurants can be found on the waterfront along the Esplanade, The Pier Marketplace and throughout the CBD are. A visit to The Cairns Dining web page (http://www.cairnsdining.com/) is worthwhile as it provides restaurant recommendations and reviews. Some suggestions include:

Local Markets
Night Markets - The Cairns Night Markets are situated on the Esplanade. You will find everything from souvenirs to fashion and toys. Near by is also the Nite Market Food Court offering a wide variety of food. The market is open all week from 6pm.
Mud Markets - The Mud Markets are held at the Pier Marketplace. They are held every Saturday and Sunday and offer souvenirs and novelty items. Whilst at the markets you can also visit the permanent stores that are also at the Pier Marketplace.

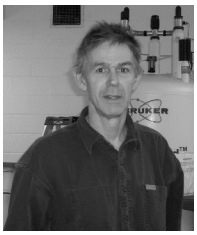
Rusty's Markets -Rusty's Market is located between Grafton and Sheridan Streets. Most locals do there food shopping here as Rusty's offer a large assortment of locally grown produce and exotic fruits. Other arts and crafts are also available. The market is open Friday 6am-6pm, Saturday 6am-5pm and Sunday 6am-3pm.

Esplanade Markets - As the name suggests the Esplanade Markets are held at the Esplanade every Saturday from 8am-4pm offering a selection of souvenirs, gift ideas and food.

Other Useful WebPages
General Information http://www.cairnsweb.com.au/cairns/cairns-city.asp
Airport http://www.cairnsport.com.au
General Information on Attractions http://www.cairnsattractions.com/
Street Map of Cairns CBD http://www.cairns-australia.com/101544.php

INVITED SPEAKERS

Fumio Arisaka



Adriaan Bax - National Institutes of Health, USA. Ad Bax received his Ph.D. in 1981 from the Delft University of Technology, The Netherlands, for work on the development of two-dimensional NMR techniques, which he carried out at Delft and Oxford Universities. He joined NIH in 1983, and has been working on the development of a wide variety of advanced multi-dimensional NMR techniques and their application to the study of the three-dimensional structure and dynamic properties of proteins.
Over the past decade, the Bax group developed a novel method for weakly aligning biological macromolecules with respect to the magnetic field. This not only increases the accuracy of NMR structures but also can extend the size limit and provide access to dynamic features of proteins.
Bax is the recipient of numerous awards, including the Gold Medal from the Dutch Chemical Society, the Protein Society Young Investigator Award, the E. Bright Wilson Award from the American Chemical Society, the John Scott Award from the City of Philadelphia, the Hans Neurath Award from the Protein Society, the Kirkwood Medal, the Seaborg Medal, and the Gunther Laukien Award. He also is a corresponding member of the Dutch Royal Academy of Sciences, a Fellow of the American Academy of Arts and Sciences, and a Member of the National Academy of Sciences, USA.

Pierre Chaurand - Dr. Pierre Chaurand is currently Research Associate Professor of Biochemistry at Vanderbilt University (Nashville, TN USA). Dr. Chaurand obtained his Ph.D. in Physical Biochemistry and Mass Spectrometry from the University of Paris Sud (Orsay, France) in 1994. He did a three-year postdoctoral fellowship at the University of Dusseldorf (Germany). He is co-author of over 50 research articles and book chapters in the fields of fundamental and applied mass spectrometry. Dr. Chaurand's interests include research that combines cutting-edge mass spectrometry technology and other technologies for profiling, identifying, and mapping the spatial distribution of biomolecules directly from biological samples and the translation of these exciting new molecular technologies to the investigation of diseased tissues.

Shui-Tein Chen - Education and Positions Held: B.S. Chemical Engineering, Tam-Kang University, Taiwan, 1978; M.S. Chemistry, University of Nevada, Reno, USA, 1985; Ph.D. Biochemical Sciences, National Taiwan University, Taiwan, 1989; Invited Research Scholar, Chemistry, Texas A&M University, USA, 1988.7-1989.9; Assistant Research Fellow, Institute of Biological Chemistry, Academia Sinica, Taiwan, 1985.10-1990.4; Associate Research Fellow, IBC, Academia Sinica, Taiwan, 1990 – 1996; Research Fellow, Institute of Biological Chemistry and the Genomics Research Center, Academia Sinica, Taiwan, 1996.5-present; Professor of Institute of Biochemical Sciences, National Taiwan University. Research Interest and Activities: Major research interest - (1) Enzymes as catalyst in organic synthesis; (2) Chemical synthesis of biologically active compounds; (3) Systems biology study of life sciences; (4) Drug delivery and targeting

Richard Christopherson - Richard Christopherson has worked at the University of Sydney for 22 years where he was the Foundation Chair of the School of Molecular and Microbial Biosciences (1998-2003) and holds a Personal Chair. He has investigated the cytotoxic mechanisms of a number of anticancer drugs, and his laboratory elucidated the antipurine mechanism of methotrexate, an antifolate drug used to treat a variety of cancers and autoimmune diseases. More recently, he has developed a CD antibody microarray that captures leukocytes expressing complementary surface molecules, enabling determination of an extensive immunophenotype (expression profile, disease signature) from a single assay. This technology is protected by a US patent, and the University of Sydney has formed a spin-off company, Medsaic, at the Australian Technology Park to commercialize antibody microarrays. In 2003, he established the Sydney University Proteome Research Unit, of which he is Director. His current research involves proteomic analysis of leukaemias, colorectal cancers and melanoma with the focus on profiling cell surface proteins, and elucidating mechanisms of action of anticancer drugs using antibody microarrays and two-dimensional fluorescence differential gel electrophoresis (DIGE).

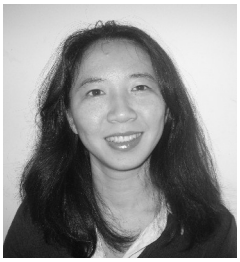
Maxey Chung - Maxey Chung (Ph.D., Victoria University of Wellington, New Zealand) holds joint appointments as Associate Professor at the Departments of Biochemistry, Yong Loo Lin School of Medicine and Biological Sciences, Faculty of Science at the National University of Singapore. He is also currently the Principal Investigator of the Oncoproteomics Laboratory in DBS. His main research interest is in the field of cancer biomarker discovery, especially for gastrointestinal cancers. In recent years, his laboratory has also focused on the identification and elucidation of the proteins and pathways involved in cancer metastasis as well as cancer cell response to HDACi (histone deacetylase inhibitor) treatment such as butyrate using functional proteomics approaches.
He is currently the Secretary General of AOHUPO (Asian Oceanian Human Proteome Organization) as well as an elected council member of HUPO (Human Proteome Organization). In addition, he is a Senior Editor of Proteomics, Proteomics - Clinical Applications and Proteomics - Practical Proteomics, and is also a regular reviewer for several leading biochemical and proteomics journals.

Juliet Gerrard - is a Professor of Biochemistry at the University of Canterbury, New Zealand. Her research interests include the Maillard reaction of proteins in food and biology, the enzymes of lysine biosynthesis and the factors that influence the assembly of proteins in vitro and in vivo.

Christopher Gerner - Christopher Gerner studied biochemisty at the University of Vienna, at his Master thesis focussing on molecular modelling of protein dynamics at the Institute of Theoretical Chemistry. From theoretical protein chemistry he changed to practical protein chemistry during his Ph.D, performed at the former Medical Faculty. At this time he started to work with primary cells, to purify subcellular compartments, separate proteins by two-dimensional gel electrophoresis and analyse them further by amino acid sequencing and MALDI-TOF mass analysis. During the work on nuclear proteins, emphasis was put on the investigation of apoptosis. The post-doc time at the Trinity College in Dublin was determined on the identification of caspase targets. Back in Vienna, he established combinations of proteome analysis methods for the assessment of physiologic and aberrant cell processes based on metabolic labelling in addition to LC-MS/MS mass spectrometry. After assembling a complex proteome database combining these different methodologic approaches, his work is currently focussed on the identification of aberrant cell activities characteristic for different kinds of diseases including hematologic disorders and cancer. The main aims are the characterisation of synergistic tumour-stroma cell interactions, identification of diagnostic biomarkers and responsible signalling pathways to enable the design of targeted therapies.

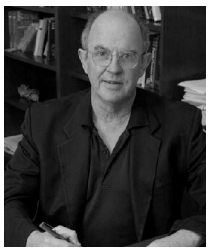
Ben Herbert – Ben Herbert has more than twenty years experience in protein chemistry, sample preparation, fractionation and electrophoresis, particularly isoelectric focusing and two-dimensional electrophoresis. Within this he has 11 years experience in technology development and commercialisation.

In 1999, A/Prof Herbert co-founded the biotechnology company Proteome Systems, where he served full time as the Vice President of Technology Development (1999-2004) and then Head of Sample Preparation (2004-2006). This company is Australia's largest proteomics company. In collaboration with Prof. Pier Giorgio Righetti (Verona University, Italy), he was instrumental developing the new electrophoresis technologies and methods that underpin Proteome Systems IsoelectrIQ2 and ElectrophoretIQ3 instruments. A/Prof Herbert has authored over 45 research and review papers and book chapters, despite the limitations imposed on publishing during 7 years in senior management at Proteome Systems.
In Feb 2006, A/Prof Herbert was recruited by the University of Technology, Sydney, to his current position as Director of the Proteomics Technology Centre of Expertise. A key area of A/Prof Herbert's current research and technology development involves fractionation and new solubilisation methods for membrane proteins.



Michelle Hill - Michelle completed her PhD in the lab of Prof David James at the Centre for Molecular and Cellular Biology, University of Queensland, Australia. Her work on insulin signalling pathways that regulates GLUT4 translocation in adipocytes utilized comparative phosphoproteomics and cell biology techniques, was recognized with a Dean's Award for Outstanding PhD thesis in 2000. Michelle worked with Dr Brian Hemmings at the Friederich Miescher Institute for Biomedical Research in Basel, Switzerland for 2.5 years, and Prof Seamus Martin at Trinity College Dublin, Ireland for 15 months, looking at regulation of Akt/protein kinase B and apoptosome assembly respectively. Since 2004, Michelle has been working at the Institute for Molecular Bioscience, University of Queensland, Australia with Prof John Hancock and Prof Rob Parton, looking at regulation of lipid rafts and caveolae. Through proteomics and cell biology, they have uncovered a novel cytoplasmic protein required for caveolae assembly and function. From 2009, Michelle will be moving to the Diamantina Institute for Cancer, Immunology and Metabolic Medicine, University of Queensland, Australia to start her independent research under the mentorship of Prof John Prins. She will bring together her experience in insulin signalling, cell biology, and proteomics and to examine the molecular link between obesity and cancer.

Hisashi Hirano - Dr. Hisashi Hirano is a Professor of Yokohama City University. His fundamental research interests concern the function of disease-associated proteins and the role of post-translational modifications in protein complexes like proteasome and ribosome. Following studying at the institutes in the Ministry of Agriculture, Forestry and Fisheries in Japan (1972-1993), University of Durham in England (1981-1982), and Max-Planck Institut fur Molekulare Genetik, Germany (1986-1987), he held a staff position in Yokohama in 1993.



Stephen Kent - *Research* Stephen Kent uses synthetic chemistry to elucidate the molecular basis of protein function. His early work focused on methods for the chemical synthesis of peptides, and on the application of chemical synthesis to studies of the hepatitis B virus and HIV. This culminated in the use of total chemical synthesis to prepare protein for the determination of the original crystal structures of the HIV-1 protease molecule complexed with canonical inhibitors. These data formed the basis for the highly successful worldwide programs in structure based drug design that culminated in the development of the 'Protease Inhibitor' class of AIDS therapeutics. More recently, Stephen Kent has pioneered a radically new approach to the total synthesis of proteins, based on the chemoselective reaction of unprotected peptide segments in
aqueous solution. The 'Chemical Ligation' method has enabled general application of physical and organic chemistry to the world of proteins.
Current Research The principal focus of the Kent laboratory at The University of Chicago is to understand the chemical basis of protein function, particularly enzyme catalysis, and to demonstrate that knowledge by the design and construction of protein molecules with novel properties.

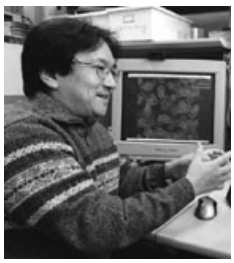
Tadashi Kondo - Tadashi Kondo is currently a Project Leader at Proteome Bioinformatics Project, the National Cancer Center Research Institute. Dr. Kondo graduated from Okayama University Medical School, and received M.D. and Ph.D. degree. He started cancer proteomics using 2D-PAGE in his Ph.D. course, and had postdoctoral training in the University of Michigan. Dr. Kondo has a major interest in application of cancer proteomics to biomarker development. He has established the largest gel-based proteomics laboratory in Japan, identifying proteins associated with important clinico-pathological features by collaborating with many clinicians and pathologists. He takes a proteomics part of Genome Medicine Database of Japan (GeMDBJ).

Bonghee Lee - Dr. Lee was awarded his V.M.D. degree from Seoul National University in 1982 and Ph.D. degree from Seoul National University, Seoul, Korea in 1994. He joined the Faculty of Medicine, Gyeonsang National University, Korea where he directed brain researches. He became Professor of Medicine, Gyeongsang National University in 1986 and moved to Cheju National University in 1998 as professor. He became Director of Insitute for Medical Science at Cheju National University in 2003. He recently joined the Center for Lee Gil Ya Cancer and Diabetes Institute Gachon University as director and Professor of Center for Genomics and Proteomics in 2007. Dr. Lee has established himself as one of the leaders in the field of stem cell biology and proteomics by his distinguished academic career. His study focused on discovery of novel biomarkers for stem cell differentiation and safety has developed this research field. He first found microglial synthesis of albumin in the human brain. The discovery of albumin synthesis has opened a new research field in brain shaperon action using albumin. It is expected that albumin studies will provide novel diagnostic opportunities for several brain dysfunction. Dr. Lee has published an impressive set of over 60 papers. He is currently Cochair of the HUPO and ISSCR joint stem cell intiative and secretary general of KHUPO.

Chitra Mandal

Rakesh Mishra – Dr Rakesh Mishra, PhD (India) received his D.Phil. (Organic Chemistry, Nucleic Acids Synthesis) in 1986 from the University of Allahabad. He started his carrier in biology by studying non-B DNA conformations and DNA topology at Molecular Biophysics Unit of the Indian Institute of Science, Bangalore, and initiation of transcription at the Centre for Cellular and Molecular Biology, Hyderabad. He used this expertise to extend application of oligonucleotides against protozoan parasites and for knock out of small nucleolar RNAs in Xenopus oocytes to study the role of such RNAs. He then became interested in chromatin organization and decided to take a genetic approach using homeotic gene complex of Drosophila melanogaster at the University of Geneva. He joined CCMB as senior Scientist in March 2001. At CCMB his lab has studied role of chromatin organization in regulation of genes and the epigenetic mechanisms involved in this process (Mole. Cell. Biol. 27, 4796-4806, 2007, BioEssays 28, 445, 2006; Genetics 168, 1371-1384, 2004). Taking biochemical, genetic and comparative genomics approach they revealed the chromatin mediated establishment and maintenance of epigenetic mechanisms. In particular his work has contributed to an understanding how specific Polycomb group proteins are recruited to established epigenetic cellular memory (Mol. Cell. Biol. 26, 1434, 2006; Genes & Dev 19, 1755, 2005; BioEssays 27, 119, 2005; Mech Dev 120, 681, 2003; Mol. Cell. Biol. 21, 1311, 2001; Mol Cell 1, 1065, 1998). Taking a comparative genomics approach, he has identified unprecedented conservation in non-coding regions of vertebrate hox complexes (BMC Genomics, 5, 75, 2004). These elements are turning out to be novel regulatory elements operating on many developmentally regulated genes. Most recent results suggest that these novel elements may be transcribed and that the non-coding RNA product may be the key to these ultra conserved regulatory elements. In order to look for the packaging code in the genome and finding novel functional elements, he has analysed non-coding regions of the human genome (Bioinformatics 19, 681, 2003; Genome Biology 4, R13, 2003; Bioinformatics 19, 549, 2003). In vivo assays have recently shown that several repeats are involved in chromatin-mediated higher order regulatory mechanisms and organization of the genome. In past few years he has presented his work in over 50 international conferences, including European and American Drosophila meetings, Cold Spring Harbour Symposia and Gordon Research Conferences.

Kazuyuki Nakamura – Dr. Kazuyuki Nakamura is a Professor and Chairman of Department of Biochemistry and Functional Proteomics, Yamaguchi University Graduate School of Medicine, Japan. He is serving for promotion of proteomics as HUPO and AOHUPO Council Members, a Co-Chair of Human Disease Glycomics Proteome Initiative (HGPI), and an advisory member of Human Kidney and Urine Proteome Project (HKUPP). He is also active in Education and Training (E&T) of young scientists for proteomics as a President of Japanese Electrophoresis Society (JES) and a Vice President of Japan HUPO. Most recently he organized a Joint Meeting of AOHUPO symposium and JES symposium for the Satellite Meeting of 20th IUBMB to promote E&T in Japan. He is interested in finding biomarkers and therapeutic targets for HCV-related Hepatocellular Carcinoma and Pancreatic Cancer using two-dimensional gel electrophoresis and tandem mass spectrometry, and in high through-put proteomics for analysis of protein-protein interactions using protein-chips. His interest is shifting to Membrane Proteomics, Cancer Immunology and Immuno-Proteomics for development of new diagnostic tools and techniques for non-invasive curative treatment of malignant tumors. He also contributes to promotion of proteomics as an associate editor of Proteome Science and a member of editorial board of Proteomics and Expert Review of Proteomics. His mission is to promote E&T of graduate students and young scientists as experts in the field of functional proteomics and clinical proteomics for understanding Molecular System of Life.



Osamu Nureki - Professor Osamu Nureki began his studies as a student of the Faculty of Science at the University of Tokyo1984-1988. Upon completion Prof. Nureki continued with his Masters and PhD within the Department of Biophysics and Biochemistry at the Graduate School of Science, the University of Tokyo under Prof. S. Yokoyama where he graduated from in 1993, his thesis title being “Mechanism of tRNA recognition by aminoacyl-tRNA synthetase”. During this time Prof. Nureki also became a HFSP Researcher at Louis Pasteur University – IBMC, CNRS, France under Dr. R. Giege.

Following Prof. Nureki’s graduation he proceeded to undertake a Post Doctor at Protein Engineering Research Institute under Dr. K. Morikawa (1993-1994). During the next year Prof Nureki was positioned Special Researcher at Crystallography Laboratory in RIKEN under Dr Iwasaki and Dr. Kamiya. In more recent years Prof. Nureki has been proclaimed Assistant Professor at Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo and later Associate Professor. From 2003 Prof. Nureki became Professor at Department of Biological Information, Graduate School of Biosciences and Biotechnology, Tokyo Institute of Technology.

Michael Parker - Michael Parker is Associate Director of St. Vincent's Institute of Medical Research in Melbourne where he is Head of the Biota Structural Biology Laboratory. He is an Australian Research Council Federation Fellow and is a Professorial Fellow of Melbourne University. After obtaining his D. Phil. in protein crystallography from Oxford University, Michael took up the post of staff scientist at the European Molecular Biology Laboratory in Germany. In 1991 Michael returned to Australia as a Wellcome Trust Senior Research Fellow to re-establish a protein crystallography laboratory at St. Vincent's. The work of the laboratory is internationally recognised with the determination of more than sixty crystal structures including those of membrane-associating proteins, detoxifying enzymes and protein kinases. This work has provided insights into a number of diseases such as cancer, bacterial and viral infections, and neurological diseases such as Alzheimer's disease. His work has been recognised with numerous awards including the 1999 Gottschalk Medal of the Australian Academy of Science and the GE Healthcare Bio-Sciences Award of the Australian Society for Biochemistry and Molecular Biology in 2004.

Frederik Ponten - Professor Fredrik Ponten is a board-certified specialist of anatomical pathology and he is head of a research group consiting of 25 persons. Dr. Ponten works at the Department of Pathology, a clinical department at the University Hospital in Uppsala, Sweden which is closely integrated with the Department of Genetics and Pathology, Medical faculty, Uppsala University (www.medfak.uu.se/english/index.html). The institute and department shares facilities at the Rudbeck Laboratory, where pathologists from the clinical side and geneticists from the pre-clinical side can jointly map cancer and hereditary diseases. The department and institute consists of approximately 60 clinicians and altogether 400 researchers working in fields comprising basic as well as more clinically oriented research. The main focus in Dr. Pontens research group is antibody-based proteomics including expression profiling of proteins in tissue and cell microarrays. Earlier work has been focused on basic skin cancer research and included techniques using laser assisted microdissection to retrive minute cell samples from tissue slides for gene amplification and sequencing. At present, the group is deeply involved in producing and analyzing tissue and cell microarrays used for protein expression profiling on a genome wide scale as part of a research program denoted HPR (The Swedish Human Proteome Resource Project), which aims to generate a map of human protein expression patterns (www.proteinatlas.org). A biobank containing paraffin blocks of tissues (3 milj) and fresh frozen tissues and cells (50.000) has recently been established and made available for high quality research projects. The current biobank program at the department was initiated and developed by Dr Fredrik Ponten who is now responsible for Cell and Tissue Microarray facilities.
Dr Fredrik Ponten who is a former Fulbright schollar, is a member of the Department of Genetics and Pathology Board, Atlasantibodies AB Board, Human Proteome Resource Board. Dr. Ponten has supervised 6 PhD students and is the author of 71 peer reviewed scientific papers and 3 book chapters.

Terence Poon - Terence C.W. Poon is currently an Assistant Professor at the Chinese University of Hong Kong. Dr. Poon received a Ph.D. degree in Pathological Sciences and a M.Sc. degree in Bioinformatics from the Chinese University of Hong Kong and the University of Manchester (United Kingdom), respectively. He serves as a Council member of HUPO and AOHUPO. He is the Vice President of the Hong Kong Proteomics Society, and a Council member of Hong Kong Society of Mass Spectrometry. Dr. Poon has a major interest in application of proteomic, glycoproteomic, glycomic and bioinformatic technologies to gastrointestinal disease research, especially on diagnosis and clinical outcome prediction. Recently he has been developing serum-based proteomic and glycomic fingerprinting assays for early diagnosis of liver diseases. In 2007, he received the Most Promising Young Researcher Award from the Food and Health Bureau of the Hong Kong SAR Government.

Randy Read - Randy Read is a protein crystallographer with a longstanding interest in both the application of crystallography to structures of medical relevance and the development of new crystallographic methods and software. He obtained his PhD under the supervision of Mike James at the University of Alberta in Canada and joined the faculty there, following a post-doctoral fellowship in The Netherlands with Wim Hol. Since 1998 he has been Professor of Protein Crystallography in the Department of Haematology at the University of Cambridge.

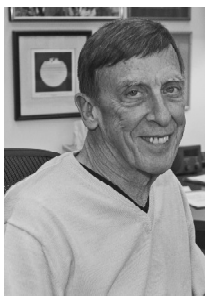
Jamie Rossjohn

Hosseini Salekdeh – Dr. Salekdeh received his PhD in Genetics at International Rice Research Institute, Philippines in 2002. His thesis work was focused on the proteome response of rice to drought and salinity stresses. He returned to Iran as an assistant professor at Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj. In 2005, he started his collaboration with Department of Stem Cells at Royan Institute, Tehran. He is currently Head of Physiology and Proteomics Department at ABRII and head of Proteomics and Molecular Biology Lab at Royan Institute. He is council member of the Asia Oceania Human Proteome Organization (AOHUPO) and coordinator of Biotechnology Network of ECO countries. On a national level, Dr. Salekdeh is President of the Iranian Proteomics Society (IPS). In 2007, he received National Biotechnology Award from Tarbiat Modares University. Dr. Salekdeh main research interests are within the field of proteomics and molecular biology. He has published over 30 research publications and has written a text book on Molecular Markers.

Jan Schnitzer – Dr. Schnitzer's laboratory investigates protein interactions at endothelial cell surfaces in vivo and how specialized invaginated microdomains called caveolae mediate transendothelial transport of molecules circulating in the blood. Recently, his lab has been combining large-scale organellar proteomic analysis using mass spectrometry with in vivo imaging to map the expression diversity of vascular endothelia in organs and tumors. Antibodies to select endothelial and caveolar targets have enabled tissue-specific imaging and therapies. Drugs, nanoparticles and gene vectors can be rendered more effective by retargeting specific transport across the endothelial cell barrier to reach underlying tissue and even tumor cells.

Ravi Sirdeshmukh - Ravi Sirdeshmukh, is Deputy Director and Head of the Proteomics facility at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India. His research interests have been in protein and nucleic acid biochemistry and presently some of the major efforts of his group are focused on the Proteomics of gliomas, stem cell proteomics and plasma proteomics. He is a member of the HUPO and AOHUPO Council and serves on the advisory panel of US Pharmacopea for Proteins.

Soichi Wakatsuki



Stephen White - White, Stephen H., B.S., Physics, 1963, Univ. of Colorado; M.S., Physics, 1965, Ph.D., Biophysics, 1969, University of Washington. Currently, Professor of Physiology and Biophysics, University of California at Irvine. Research Interests: Membrane protein folding and stability; membrane protein structure prediction; structure and stability of membranes; x-ray and neutron diffraction methods for studying peptides and proteins in fluid lipid membranes; thermodynamic and optical spectroscopic studies of peptide-bilayer interactions. Administrative Experience: Served to the rank of Captain, US Army, 1969-1971; Chairman, Dept. of Physiology and Biophysics at U.C. Irvine, 1977-1989; Alumnus, Univ. of California Management Institute, 1981. Protein Society: Electronic Publishing Coordinator, 1991-present; Biophysical Society: Chair, Membrane Biophysics Subgroup, 1977-78; Council, 1981-84; Executive Board, 1981-84; Program Chairman, 1985; Secretary, 1987-1995. Society of General Physiologists: Treasurer, 1985-1988. American Crystallographic Association. American Society for Biochemistry and Molecular Biology. American Physiological Society, Publications Committee, 1988-1990. Honors: President, Biophysical Society, 1996-1997. President, Assoc. of Chairs of Depts. of Physiology, 1986-1987. NIH Research Career Development Award, 1976-1981. Kaiser-Permanente Award for Excellence in Teaching, 1975 and 1991. Biophysical Society Fellow, 2002. Advisory Committees: NSF Biophysics Panel, 1982-1985; DOE Structural Biology Review Committee, 1992, DOE Neutron Research and Sources Advisory Committee, 1993, DOE National Advisory Committee on the Advanced Neutron Source, 1992-95. NIH Biochemistry and Biophysics Membranes Study Section, 2006-2010.

Tadashi Yamamoto – is president of the Japan Center for International Exchange, which he founded in 1970. He served as a member and executive director of the late Prime Minister Obuchi's Commission on Japan's Goals in the 21st Century. He has served as Japanese executive director of the Japan-U.S. Economic Relations Group, the U.S.-Japan Advisory Commission, and the Korea-Japan 21st Century Committee. Mr. Yamamoto also has been a member of the First and Second Prime Minister's Private Council on International Cultural Exchange. He is currently a member and the Japanese director of the Trilateral Commission, the U.K.-Japan 2000 Group, the Japanese-German Dialogue Forum, and the Korea-Japan Forum.

John Yates - John Yates received his Ph.D. in Chemistry at the University of Virginia under Professor Donald Hunt. His graduate research involved the development and application of tandem mass spectrometry for sequence analysis of proteins. Following a Biotechnology Fellowship at the California Institute of Technology, he moved to the Department of Molecular Biotechnology at the University of Washington where he attained the tenured rank of Associate Professor. He is now a Professor in the Department of Cell biology at The Scripps Research Institute. His research interests include development of integrated methods for tandem mass spectrometry analysis of protein mixtures, bioinformatics using mass spectrometry data, and proteomics. He is the lead inventor of the SEQUEST software for correlating tandem mass spectrometry data to sequences in the database and principle developed of the shotgun proteomics technique for the analysis of protein mixtures. He has received the American Society for Mass Spectrometry research award, the Pehr Edman Award in Protein Chemistry, the American Society for Mass Spectrometry Biemann Medal, the HUPO Distinguished Achievement Award in Proteomics, Herbert Sober Award from the ASBMB, and the Christian Anfinsen Award from The Protein Society. He has published over 350 scientific articles.

Jong Shin Yoo - Education and Employment: 1976-1980 B.S. in Yonsei University (Dept. of Chemistry); 1980-1982 M.S. in Korea Advanced Institute of Science and Technology (Dept. of Chemistry); 1986-1991 Ph.D, in Michigan State University (Analytical Chemistry); 2001-2004 Vice President of the Korea Basic Science Institute; 2004-present Director of the Korea Basic Science Institute, Mass Spectrometry Analysis Center.
Academic Positions: 1991-1993 Postdoctoral Fellow, Harvard University, School of Public Health; 1999-2003 Adjunct Professor, Chungnam University, Dept. of Chemistry; 2004-present Professor, University of Science and Technology Activities for Scientific Communities: 2005-present Executive Board, Korean Society of Analytical Sciences; 2006-2007 President, Korean Society of Mass Spectrometry; 2008-present President, Korean Human Proteome Organization; 2007-present Council Member, Asian and Oceanian Human Proteome Organization; 2005-present Editorial Board, Korean Society of Biochemistry and Molecular Biology.

Zihe Rao - Zihe Rao, a molecular biophysicist and structural biologist, was born in the city of Nanjing, Jiangsu Province. He graduated from the University of Science and Technology of China (USTC) in 1977, and got his Master¡¯s degree from the Institute of Biophysics, Chinese Academy of Science (CAS) in 1982. In 1989, Rao received his doctorate from Melbourne University and then joined Prof. Dave Stuart¡¯s group in Oxford University, where he worked until 1996. He is now a professor of Tsinghua University, the director of the Institute of Biophysics, CAS, chairman of the Academic Committee of the Institute of Biophysics, CAS, and the director of the National Laboratory of Macromolecules.
Prof. Rao¡'s research has been focused on the relationship between protein structure and function, on protein engineering and drug design. In particular, he has concentrated his efforts on proteins related to human disease and important physiological functions. His work on the crystal structure of the Fc receptor of IgA was published in "JBC" as a cover article. During the outbreak of severe acute respiratory syndrome (SARS) in China, Rao's group solved the first crystal structure of a SARS coronavirus protein ¨C the 3C-like Protease - and its complex with an inhibitor. This important work provides a structural basis for rational anti-SARS drug design and has since been published in "PNAS".
He was elected as the chairman of Molecular Biophysics Commitee, Chinese Biophysics Association and the Chairman of Macromolecular Commitee, National Crystallographers Association. Prof. Rao was awarded the "Qiushi" Outstanding Scientist Prize in Life Sciences and "Yangzi" Distinguished Professor Prize in 1999, and the "Heliangheli" Foundation Science and Technology Progress Prize in 2003. He was elected as a member of Chinese Academy of Sciences in 2003.

TRADE SESSIONS AND WORKSHOPS

The following trade workshops have been setup for delegates who wish to attend.

AOHUPO Membrane Protein Initiative Workshop
Sunday 22nd June, 9:15am to 2:30pm

invitrogen™ Invitrogen ProtoArray Education Workshop
Sunday 22nd June 2008, 2:30pm to 4:45pm in Hall A

Dr. Timothy Wong, Invitrogen Corporation: Coordinator
Dr. Michael Smith, Invitrogen Corporation: Applications of Functional Protein Microarrays
Dr. Erlend Ragnhildstveit, Invitrogen Corporation: Purification and Validation Content
- Knowing the Basic: ProtoArray; Technology and Applications
- Demonstration: ProtoArray Discovery Experiment and Technique
- Data Analysis Module: Hands on Training on Data Processing and Analysis
- Data Validation Technique Demonstration: IP with DynaMag-2 Magnet

invitrogen™ Trade Session One: Invitrogen - Tools in Biomarker Discovery
Monday 23rd June 2008, 12:45pm to 2:30pm in Hall A

Dr. Erlend Ragnhildstveit, Invitrogen Corporation: Sample Fractionation using Magnetic Beads
Dr. Jurgen Vanhauwe, Invitrogen Corporation: Biomarker Discovery Technology, Part 1 - Drill Deeper into the Proteome
Maxey Chung, National University of Singapore: Biomarker Discovery Technology, Part 2 - Screening of autoantigens from gastric cancer patient sera using the Invitrogen ProtoArrays
Panel Discussion (chaired by Dr Richard Christopherson):
- Dr. Richard Simpson, Ludwig Institute, Australia
- Dr. Maxey Chung, NUS, Singapore
- Dr. Erlend Ragnhildstveit, Invitrogen Corporation
- Dr. Michael Smith, Invitrogen Corporation

Agilent Technologies Trade Session Two: Agilent Technologies - The Application of advanced proteomics tools to better understand the biological basis of disease
Monday 23rd June 2008, 12:45pm to 2:30pm in Meeting Rooms 1 & 2

- Learn about recent advances in answering key biological questions about human disease states through the use of analytical proteomics tools such as ChipLC/MS and ICPMS.
- These technologies are becoming more widely used by researchers to understand the biological basis of disease.
- See how these tools generate highly sensitive data that can help map disease states as well as identify disease markers.
- Learn how other scientists have successfully integrated these latest analytical technologies to further enable their research and turn the data they've generated into biologically relevant information.

Presentations by:
- *A/Prof Christopher Gerner, Group Leader, Department of Medicine, Institute of Cancer Research, Medical University of Vienna, Austria:* Secretomes of differently stimulated human dendritic cells generated by 2D-PAGE and shotgun analysis
- *Christine Miller, Senior Applications Scientist LC/MS, Agilent Technologies, Santa Clara, USA :* Recent advances in LC/MS technologies fronm Agilent
- *Dr Phil Doble, Senior Lecturer, University of Technology, Sydney:* Elemental Bioimaging

BRUKER Trade Session Three: Bruker Biosciences- The Complete MALDI Imaging Workflow - Tissue and Drug Imaging Solutions
Tuesday 24th June 2008, 12:15pm to 2:00pm in Hall A

Visualization of the spatial distribution of proteins, drug candidate compounds and biomarkers is a promising tool in the exciting fields of biomarker evaluation and drug development. MALDI-TOF mass spectrometry provides a fast and reliable screening tool for direct analysis from plant, animal, and human tissues. Bruker Daltonics is a leader in MALDI imaging, providing a complete advanced commercial MALDI Molecular

Imaging Workflow. This important field is attracting increasing attention and the speakers in this workshop will discuss the latest developments and results.

Dr. Matthias Pelzing, Applications Manager - Region Asia-Pacific, Bruker Biosciences Pty Ltd, Australia: Bruker Class Imaging: From sample preparation to biostatistical analysis of MALDI Tissue Imaging Data for the Diagnostics of Tissue Health States
A/Prof. Pierre Chaurand, Research Associate Professor of Biochemistry at Vanderbilt University, Nashville, TN, USA: MALDI Imaging MS, the nuts and bolts of the technology
Dr. Peter Hoffmann, Director of Adelaide Proteomics Centre, University of Adelaide, SA, Australia: Imaging mass spectrometry (IMS) application to murine tissues

Trade Session Four: BioRad Laboratories - Tools of the Proteomic workflow

Tuesday 24th June 2008, 12:15pm to 2:00pm in Meeting Rooms 1 & 2
Ben Herbert, Proteomics Technology Centre of Expertise, University of Technology, Sydney: Enriching low abundance proteins by proteome-wide affinity using a combinatorial hexapeptide library
Amanda Bulman, Biomolecular Research Centre, Bio-Rad Laboratories Fremont: Strategies for SELDI-Based Biomarker Discovery and Development
Egisto Boschetti, Bio-Rad Laboratories, Gif-sur-Yvette, France: Evaluation of a Standardized Method of Protein Purification and Identification after Discovery by Mass Spectrometry

Trade Session Five: GE Healthcare – Showcase of GE Healthcare Proteomic Tools

Tuesday 24th June 2008, 3:45pm to 5:00pm in Hall A
Brian Hood, Global Marketing Manager Uppsala, Sweden: What's Happening to Proteomics at GE Healthcare??
Speaker To Be Advised: Breaking the Mold – Academic Partnership and Research at GE Healthcare
Daniel Haid, Ioana Grigorescu, John Flensburg and Helena Nordvarg, GE Healthcare Bio-Sciences AB, Uppsala, Sweden: A parallel proteomics approach to analyze and validate protein differences in colorectal cancer

Trade Session Six: Shimadzu Scientific - Mass Spectrometry Techniques & Application

Tuesday 24th June 2008, 3:45pm to 5:00pm in Meeting Rooms 1 & 2
Dr Tsuyoshi Nakanishi, Shimadzu Corporation, Japan: On-Tissue MALDI-MS Analysis with Chemical Printer (CHIP-1000)
Dr Toru Ezure, Shimadzu Corporation, Japan: Posttranslational modifications in an insect cell-free system and their identification by MALDI-TOF MS
Dr Zhan Zhao Qi, Shimadzu Asia Pacific, Singapore: Advantages of LC-MALDI approach for proteomics applications
Dr *Peter Hoffmann, Adelaide Proteomics Centre, School of Molecular and Biomedical Sciences, The University of Adelaide, Australia:* Imaging mass spectrometry (IMS) of murine tissues using a piezoelectric printer

Trade Session Seven: Thermo Fisher Scientific – ETD and its Application to the Orbitrap

Wednesday 25th June 2008, 12:15pm to 2:00pm in Hall A
Amy Zumwalt, Proteomics Marketing Program Manager: Targeted Protein Quantitation using the SRM Workflow - Making SRM assays routine, robust, and sensitive
Maria C. Prieto Conaway, Proteomics R & D and Marketing specialist: Bringing high mass accuracy, increased dynamic range and high resolving power to tissue imaging with the MALDI LTQ Orbitrap
Terry Zhang, Proteomics R& D and Marketing specialist: New developments in ETD on LTQ Orbitrap XL and its applications.

Trade Session Eight: Waters Australia - Securing Your IDENTITY - New stringency for protein identifications

Wednesday 25th June 2008, 12:15pm to 2:00pm in Meeting Rooms 1 & 2

Dr Stephen Watt, Solution Consultant –Mass Spectrometry, Waters Australia: Securing Your IDENTITY - New stringency for protein identifications

PROGRAM

Sunday, 22 June 2008

Welcome Address
9:00 AM - 9:15 AM Meeting Room 1-2
Convener: Bill Jordan

W1: AOHUPO Membrane Protein Initiative Workshop (MPI)
9:15 AM - 10:15 PM Meeting Room 1-2
Chair: Maxey Chung

9:15am **Mark Baker**
Desperately Seeking Comprehensive Mammalian Membrane Proteomics *abs#101*

9:30am **Tzong-Hsien Lee**
Chromatographic Separation of Intact Proteins from Mouse Liver Microsomal Proteins for Membrane Proteome Analysis *abs#102*

9:45am **Richard Simpson**
Solubility-based phase partitioning of mouse liver microsomes using Triton X-114 *abs#104*

10:00am **Kazuyuki Nakamura**
1D-SDS-PAGE and nano-LC-MS/MS for membrane proteomics of mouse liver microsomes (MPI sample) and its application to human proteomics of ER from Jurkat cells *abs#105*

Coffee Break
10:15 AM - 10:45 AM Foyer

W1: AOHUPO Membrane Protein Initiative Workshop (MPI) Continued
10:45 AM - 1:00 PM Meeting Room 1-2
Chair: Kazuyuki Nakamura

10:45am **Maxey Chung**
Digging deeper into the mouse liver membrane proteome: Evaluation of different membrane protein digestion approaches with 8-plex iTRAQ reagents *abs#106*

11:00am **Hosseini Salekdeh**
Proteome analysis of mouse liver microsomal fraction using 2D BN/SDS-PAGE *abs#107*

11:15am **Eugene Kapp**
A Common Sequence Database Format in Proteomics *abs#108*

11:30am **Tai-Long Pan**
Zoom IEF fractionator & SDS-PAGE to identify membrane proteins effectively *abs#109*

11:45am **Ravi Sirdeshmukh**
Comparison of Experimental Methods for Identification of Membrane Proteins from MPI Reference Specimen *abs#110*

12:00am **Jongshin Yoo**
Title not available at time of print *abs#111*

12:45am **Terence Poon**
Technical Hurdles When Applying Isoelectric Focusing to Membrane Proteome Analysis *abs#136*

Afternoon Registration
12:00 PM - 7:00 PM Foyer

Lunch Break
12:30 PM - 1:00 PM Foyer

W2: MPI Manuscript Workshop
1:00 PM - 2:30 PM Meeting Room 1-2
Group discussion to plan manuscripts

W3: Invitrogen ProtoArray Education Workshop
2:30 PM - 4:45 PM Meeting Room 1-2

Session sponsored by invitrogen™

Michael Smith
Applications of Functional Protein Microarrays *abs#112*
Purification and Validation: Dr. Erlend Ragnhildstveit

Opening Ceremony & Welcome Addresses (Convenor: Richard Simpson)
5:00 PM - 5:30 PM Hall A
Australasian Proteomics Society (APS) President: Prof Richard Simpson
AOHUPO President: Prof Young-Ki Paik
Protein Science Society of Japan (PSSJ) President: Prof. Tomitake Tsukihara
JST/CREST: Prof Tairo Oshima
Lorne Conference on Protein Structure and Function (LPC) President: Prof Ray Norton

P1: Plenary Lecture
5:30 PM - 6:30 PM Hall A

Session sponsored by JST/CREST

Chair: Tomitake Tsukihara and James Whisstock

Randy Read
New structures from old: developments in molecular replacement *abs#001*

Welcome with Trade
6:30 PM - 8:30 PM Trade Display Area

Function sponsored by BRUKER

Monday, 23 June 2008

Registration
8:00 AM - 7:00 PM Foyer

SOA1: State-of-the-Art Lectures
8:30 AM - 10:00 AM Hall A

Session sponsored by BIO-RAD

Chair: Bill Jordan & Ben Herbert

8:30am **Stephen Kent**
Through the Looking Glass – a New World of Proteins Enabled by Chemistry *abs#002*

9:15am **Egisto Boschetti**
The ProteoMiner and the FortyNiners: Searching for Gold Nuggets in the Proteomic Arena *abs#003*

Coffee Break / Poster Review / Exhibition Viewing Period
10:00 AM - 10:30 AM Trade Display Area

S1A (Concurrent): Stem Cell Proteomics
10:30 AM - 12:30 PM Hall A

Session sponsored by invitrogen™

Chair: Bonghee Lee and Salekdeh Hosseini

10:30am **Bonghee Lee**
Wnt signaling plays a key role in human neural stem cell differentiation into oligodendrocyte progenitors *abs#004*

11:00am **Hosseini Salekdeh**
Transcriptome and Proteome analyses of Human, Monkey, and Mouse Embryonic Stem Cells during Embryoid Body-Based Differentiation *abs#005*

11:30am **Ravi Sirdeshmukh**
Integrated Approach to Study Mouse Embryonic Stem Cell Proteome *abs#006*

12:00pm **Jong Bhak**
Bioinformatic infrastructure for maintaining proteome information *abs#007*

S1B (Concurrent): JST/CREST - Innovative Protein Science 1
10:30 AM - 12:30 PM Meeting Room 1-2
Chair: Stephen White and Tairo Oshima

10:30am **Yuji Sasaki**
Dynamical Single Molecular Observations of Membrane Proteins Using X-rays *abs#008*

11:00am **Masashi Suzuki**
Discrimination between DNA sequences and between coregulator amino acids by feast/famine regulatory proteins (FFRPs) *abs#009*

11:30am **Akihito Yamaguchi**
Structure and Mechanism of the Tripartite Multidrug Exporter *abs#010*

12:00pm **Young-Ho Jeon**
Solution structure of Mst1 SARAH domain and its interaction with Rassf5 and WW45 SARAH domains for the apoptosis pathway *abs#011*

Lunch Collection
12:30 PM - 12:45 PM Trade Display Area

TS1 (Trade Session): Invitrogen - Tools in Biomarker Discovery Technologies and Panel Discussion
12:45 PM - 2:30 PM Hall A

Erlend Ragnhildstveit
Sample Fractionation using Magnetic Beads *abs#113*

Jurgen Vanhauwe
Drill deeper into the proteome *abs#114*

Maxey Chung
Screening of autoantigens from gastric cancer patient sera using the Invitrogen ProtoArrays *abs#115*

TS2 (Trade Session): Agilent - The Application of Advanced Proteomics Tools to Better Understand the Biological Basis of Disease
12:45 PM - 2:30 PM Meeting Room 1-2

Christopher Gerner
Secretomes of differently stimulated human dendritic cells generated by 2D-PAGE and shotgun analysis. *abs#116*

Christine Miller
Recent advances in LC/MS technologies fronm Agilent *abs#117*

Phil Doble
Elemental Bioimaging *abs#118*

S2A (Concurrent): Proteomics Applications I
2:30 PM - 3:45 PM Hall A
Chair: Othamn Iekhsan and Shui-Tein Chen

2:30pm **John Bennett**
Insights into drought responsiveness in rice at the reproductive stage through proteomic analysis *abs#012*

2:48pm **Chantragan Srisomsap**
Proteomic Alteration During Storage of *Curcuma longa* Rhizomes *abs#014*

3:06pm **Tzu-Ching Meng**
Mass spectrometry-based substrate identification and genetic validation reveal the functional role of *Drosophila* protein tyrosine phosphatase dPTP61F *abs#015*

S2B (Concurrent): JST/CREST - Innovative Protein Science 2
2:30 PM - 3:45 PM Meeting Room 1-2
Chair: James Whisstock and Tairo Oshima

2:30pm **Osamu Nureki**
Gating control; mechanism of magnesium transporter MgtE *abs#016*

2:48pm **Stephen White**
Translocon-Assisted Folding of Membrane Proteins: New insights into Lipid-Protein Interactions. *abs#017*

3:06pm **Junichi Takagi**
Development of a novel peptide affinity tag system for one-step purification of recombinant proteins *abs#018*

3:24pm **Andrew Wang**
Prenyltransferases as targets for the discovery of new antibiotics *abs#019*

PS1: Poster Session 1
3:45 PM - 5:00 PM Trade Display Area
See listing at the end of the program

S3A (Concurrent): Advances in Proteomic Techniques
5:00 PM - 7:00 PM Hall A
Chair: Louis Fabri and Hisashi Hirano

5:00pm **Robert Moritz**
Utilising a large computing resource for your Proteomics research "The Australian Proteomics Computational Facility" - using the APCF for Biomarker discovery *abs#020*

5:20pm **Herbert Thiele**
Managing Proteomics Data from Generation and Data Warehousing to Central Data Repository *abs#021*

5:40pm **Pengyuan Yang**
Selective Separation of Glycopeptides and Glycoproteins by Aminophenylboronic Acid-Functionalized Magnetic Nanoparticles *abs#023*

6:00pm **Hiroyuki Kaji**
LC/MS-based large-scale identification of N-glycoproteins and their glycan diversity *abs#022*

6:20pm **Xiaohong Qian**
Development of Magnetic Nanoparticles and Its Application in Phosphoproteome of Liver *abs#024*

6:40pm **Toshihide Nishimura**
Formalin-Fixed Paraffin-Embedded (FFPE) Clinical Proteome Initiative of Lung Cancer *abs#025*

S3B (Concurrent): Developments in Structural Biology
5:00 PM - 7:00 PM Meeting Room 1-2
Chair: Michael Parker and Fumio Arisaka

5:00pm **Ryota Kuroki**
Crystal Structure of the Human Granulocyte Colony Stimulating Factor Receptor Signaling Complex *abs#026*

5:24pm **Chwan-Deng Hsiao**
Crystal structure of the human FOXO3a-DBD/DNA complex suggests the effects of post-translational modification *abs#027*

5:48pm **Fumio Arisaka**
Structural Analysis of Baseplate Wedge Proteins of BacteriophageT4 *abs#028*

6:12pm **Haruki Nakamura**
Development of Protein Structure Databases and their Applications to Functional Annotation *abs#029*

6:36pm **James Whisstock**
MACPF proteins – eukaryote cytolysins in defence and attack *abs#030*

AOHUPO Council Meeting
7:00 PM - 7:30 PM Meeting Room 8

Student Dinner (SoAPS)
7:00 PM - 11:00 PM Offsite
Students of APS (SOAPS) to host students of AOHUPO (SOAOHUPO) for dinner/drinks

P2: Plenary Lecture
8:30 AM - 9:30 AM Hall A

Session sponsored by Thermo SCIENTIFIC

Chair: Robert Moritz

John Yates
Driving Biological Discovery Using Quantitative Mass Spectrometry *abs#031*

Coffee Break / Exhibition Viewing Period
9:30 AM - 10:00 AM Trade Display Area

S4A (Concurrent): Novel Disease Biomarkers and Massive Array Screen
10:00 AM - 12:00 PM Hall A

Session sponsored by

Chair: Peter Hudson and Maxey Chung

10:00am **Fredrik Ponten**
Mapping the Human Proteome *abs#032*

10:24am **Richard Christopherson**
Surface profiling of leukaemias, lymphomas and colorectal cancers using a CD antibody microarray *abs#033*

10:48am **Sanjay Navani**
Antibody-Based Proteomics *abs#034*

11:12am **Caroline Kampf**
A Human Protein Atlas *abs#035*

11:36am **Kazuyuki Nakamura**
Cys-tag proteins on chips for functional proteomics *abs#036*

S4B (Concurrent): Macromolecilar Molecules and Interaction
10:00 AM - 12:00 PM Meeting Room 1-2

Session sponsored by SBMB

Chair: Matt Perugini

10:00am **Youhe Gao**
Characterizing binding properties of protein interaction domain *abs#037*

10:24am **Juliet Gerrard**
Unravelling the mechanism of dihydrodipicolinate synthase: are the essential active site residues really essential? *abs#038*

10:48am **Yongzhang Luo**
Discovery of a New Extracellular Chaperone *abs#039*

11:12am **Zengyi Chang**
Modulation of protein activities via Homo-oligomerization: a phenomenon that has been underappreciated *abs#040*

11:36am **Po-Huan Liang**
Discovery of inhibitors against 3C proteases of SARS coronavirus, enteroviruses 71, and coxsackievirus B3 *abs#041*

Lunch Collection
12:00 PM - 12:15 PM Trade Display Area

TS3 (Trade Session): Bruker Maldi Imaging Workshop - "The Complete MALDI Imaging Workflow Tissue and Drug Imaging Solutions"

12:15 PM - 2:00 PM Hall A

Pierre Chaurand
MALDI Imaging MS, the nuts and bolts of the technology *abs#119*

Matthias Pelzing
Bruker Class Imaging: From sample preparation to biostatistical analysis of MALDI Tissue Imaging Data for the Diagnostics of Tissue Health States. *abs#120*

Peter Hoffmann
Imaging mass spectrometry (IMS) application to murine tissues *abs#121*

TS4 (Trade Session): Biorad Laboratories - Tools in the Proteomic Workflow

12:15 PM - 2:00 PM Meeting Room 1-2

Ben Herbert
Enriching low abundance proteins by proteome-wide affinity using a combinatorial hexapeptide library *abs#122*

Amanda Bulman
Strategies for SELDI-Based Biomarker Discovery and Development *abs#123*

Egisto Boschetti
Evaluation of a Standardized Method of Protein Purification and Identification after Discovery by Mass Spectrometry *abs#124*

S5A (Concurrent): Mass Imaging

2:00 PM - 3:00 PM Hall A

Session sponsored by Bruker Biosciences

Chair: Peter Hoffmann and Xiaohong Qian

2:00pm **Pierre Chaurand**
MALDI mass imaging mass spectrometry of tissue sections: state of the art and future directions *abs#042*

2:15pm **Philip Doble**
Metal-imaging mass spectrometry (MIMS): A new imaging mass spectrometry technology to determine the distribution of metal ions in tissue samples *abs#043*

2:30pm **Christina Buchanan**
Photographs and Memories: as snapshot of cultured endocrine cells *abs#044*

2:45pm **Anthony White**
Mapping Novel Copper-Regulated Signalling Pathways Using Antibody Arrays And In Silico Protein Network Analysis. *abs#045*

S5B (Concurrent): Protein Dynamics

2:00 PM - 3:00 PM Meeting Room 1-2

Chair: Hideo Akutsu and Zengyi Chang

2:00pm **Stephen Watt**
Using Ion Mobility/Time-of-Flight Mass Spectrometry to Determine Conformational Properties of Proteins *abs#046*

2:15pm **Hideki Taguchi**
Direct observation of yeast prion dynamics in single-living cells *abs#047*

2:30pm **Ashley Buckle**
Rapid Protein Structure Determination using Distributed Computing *abs#048*

2:45pm **Speaker to be Advised**
Title not available at time of print *abs#049*

PS2A: Poster Session 2A
3:00 PM - 3:45 PM Trade Display Area
See listing at the end of the program

TS5 (Trade Session): GE Healthcare - Showcase of GE Healthcare Proteomic Tools
3:45 PM - 5:00 PM Hall A

Brian Hood
What's Happening to Proteomics at GE Healthcare?? *abs#125*

Invited Guest Speaker
Breaking the Mold – Academic Partnership and Research at GE Healthcare *abs#126*

Daniel Haid
A parallel proteomics approach to analyze and validate protein differences in colorectal cancer *abs#127*

TS6 (Trade Session): Shimadzu Scientific - Mass Spectrometry – Techniques & Application
3:45 PM - 5:00 PM Meeting Room 1-2

Tsuyoshi Nakanishi
Novel approach using the chemical printer (ChIP-1000) for rapid on-membrane profiling of phosphoproteins with MALDI Imaging Mass Spectrometry *abs#128*

Toru Ezure
Posttranslational modifications in an insect cell-free protein synthesis system and their identification by MALDI-TOF MS *abs#129*

Zhan Zhao Qi
Advantages of LC-MALDI approach for proteomics applications *abs#130*

Peter Hoffmann
Imaging mass spectrometry (IMS) of murine tissues using a piezoelectric printer *abs#131*

SOA2: State-of-the-Art Lectures
5:00 PM - 6:30 PM Hall A

Session sponsored by CSL™

Chair: Louis Fabri and Michelle Hill

5:00pm **Jan Schnitzer**
Proteomic imaging of endothelium and caveolae for targeted penetration into single organs and solid tumors *abs#050*

5:45pm **Christopher Gerner**
Establishment of a secretome database of primary and cultured cells for biomarker discovery *abs#051*

Optional Tjapakai Aboriginal Cultural Centre Dinner
6:30 PM - 10:30 PM Tjapakai Aboriginal Cultural Centre

P3: Plenary Lecture
8:30 AM - 9:30 AM Hall A

Session sponsored by JST/CREST

Chair: Hideo Akutsu and Ray Norton

Ad Bax
Insight into structure and dynamics from weak alignment NMR *abs#052*

Coffee / Exhibition Viewing Period
9:30 AM - 10:00 AM Trade Display Area

S6A (Concurrent): Mass Spectrometry
10:00 AM - 12:00 PM Hall A

Session sponsored by SHIMADZU

Chair: Jong Shin Yoo and Robert Moritz

10:00am **Jong Shin Yoo**
Quantitative Analysis of Human Plasma Proteome by Mass Spectrometry for Cancer Biomarker Discovery *abs#053*

10:24am **Mark Larance**
Quantitative Phosphoproteomics Reveals a Pathway of mRNA Regulation Downstream of Akt *abs#054*

10:48am **Kathy Ruggiero**
Protein expression experiments using iTRAQ^{TM}: a unified protocol for design and analysis? *abs#055*

11:12am **Jeffrey Gorman**
Comparison of stable-isotope labelling strategies for quantification of phosphosite occupancy and differentiation between phosphorylation and sulfonation of the murine dioxin receptor *abs#056*

11:36am **Speaker to be Advised**
Title not available at time of print *abs#057*

S6B (Concurrent): Amyloid Proteins
10:00 AM - 12:00 PM Meeting Room 1-2
Chair: Tony White

10:00am **Yuji Goto**
Direct Observation of Amyloid Fibril Formation of b 2-Microglobulin and Amyloid b Peptide *abs#058*

10:24am **Damien Hall**
A toy model for predicting the rate of amyloid formation from unfolded protein *abs#059*

10:48am **Ray Norton**
Order, disorder and fibril formation in the malaria vaccine candidate MSP2 *abs#060*

11:12am **Michael Parker**
Structural biology of Alzheimer's disease *abs#061*

11:36am **Daizo Hamada**
Negative Design Principle to Avoid the Formation of Misfolded Aggregates as Revealed by β-Lactoglobulin *abs#062*

Lunch Collection
12:00 PM - 12:15 PM Trade Display Area

TS7 (Trade Session): Thermo Fisher Scientific - Proteome Dynamics - Integrated Solutions for Protein Characterization and Quantitation
12:15 PM - 2:00 PM Hall A

Amy Zumwalt
Targeted Protein Quantitation using the SRM Workflow: Making SRM assays routine, robust, and sensitive *abs#132*

Maria Prieto Conaway
Bringing high mass accuracy, increased dynamic range and high resolving power to tissue imaging with the MALDI LTQ Orbitrap™ *abs#133*

Terry Zhang
New developments in ETD on LTQ Orbitrap XL and its applications *abs#134*

TS8 (Trade Session): Waters - Securing Your IDENTITY - New stringency for protein identifications
12:15 PM - 2:00 PM Meeting Room 1-2

Stephen Watt
Securing Your IDENTITY - New stringency for protein identifications *abs#135*

S7A (Concurrent): Clinical Proteomics I
2:00 PM - 3:30 PM Hall A

Session sponsored by Agilent Technologies
Chair: Ravi Sirdeshmukh and Kazuyuki Nakamura

2:00pm **Maxey Chung**
2-D DIGE profiling of Hepatocellular Carcinoma Tissues identified isoforms of Far Upstream Binding Protein (FUBP) as novel candidates in liver carcinogenesis *abs#063*

2:18pm **Shui-Tein Chen**
Enhance proteomic detection limitation by combinatorial peptide and nucleotide library *abs#064*

2:36pm **Hisashi Hirano**
Identification and Validation of Ovarian Cancer-Associated Proteins *abs#066*

2:54pm **Srinuabu Gedela**
Proteomic Analysis of Cytokines in Diabetes Patients: An Experimental Design Approach *abs#067*

S7B (Concurrent): Synchrotron Technologies - Current Studies
2:00 PM - 3:30 PM Meeting Room 1-2
Chair: Andy Wang and Ashley Buckle

2:00pm **Jennifer Martin**
United we stand: combining structural methods *abs#068*

2:22pm **Soichi Wakatsuki**
Synchrotron protein crystallography developments and target-oriented structural proteomics *abs#069*

2:44pm **Yao-Chang Lee**
Application of Synchrotron Infrared Miscrospectroscopy and Imaging to Biological studies *abs#070*

3:06pm **Rob Lewis**
What are they doing over there? *abs#071*

PS2B: Poster Session 2B
3:30 PM - 4:00 PM Trade Display Area
See listing at the end of the program

SOA3: Start-of-the-Art Lecture
4:00 PM - 4:45 PM Hall A
Session sponsored by Invitrogen
Chair: Richard Simpson

Ileana Cristea
Advances in Rapid Isolations of Protein Complexes: Revealing the Dynamic Viral-Host Interactome *abs#072*

S8A (Concurrent): Clinical Proteomics II
4:45 PM - 6:15 PM Hall A
Session sponsored by
Chair: Mark Baker and Terence Poon

4:45pm **Kylie Hood**
Examination of alterations in the protein profile of colorectal cancer cells during invasion and metastasis *abs#073*

5:15pm **Tadashi Kondo**
Cancer proteomics for personalized medicine *abs#074*

5:45pm **Sumiko Kurachi**
Global analyses of age-related expression profiles of mouse liver proteins and database construction *abs#075*

S8B (Concurrent): Proteomics and Pathogens
4:45 PM - 6:15 PM Meeting Room 1-2
Chair: Thomas Nebl and Stuart Cordwell

4:45pm **Thomas Nebl**
A comprehensive immunoproteomic analysis of the repertoire of human antibody responses to the malaria parasite Plasmodium falciparum *abs#076*

5:15pm **James MacRae**
Metabolite profiling in *Plasmodium falciparum*. *abs#078*

Conference Dinner/Aussie BBQ
6:15 PM - 10:30 PM Foyer
Session sponsored by

S9A (Concurrent): Clinical Proteomics III

9:00 AM - 10:30 PM Hall A

Chair: Kylie Hood and John Bennett

9:00am **Terence Poon**
Application of glycomics to the diagnosis of liver diseases *abs#080*

9:30am **Oliver Bernhard**
Detection of biomarkers for colorectal cancer by ranking of soluble-secreted proteins (RSSP) *abs#081*

10:00am **Rakesh Mishra**
Characterization of nuclear Matrix proteome of Drosophila melanogaster during embryonic development *abs#079*

S9B (Concurrent): Infectious Diseases

9:00 AM - 10:30 PM Meeting Room 1-2

Chair: Malcolm McConville and Kylie Hood

9:00am **Ben Herbert**
Fungal lung infection : understanding Cryptococcus gattii infection and the challenges of mixed proteomes *abs#083*

9:30am **Chitra Mandal**
Glycoproteomics of *Pseudomonas aeruginosa*, an opportunistic pathogen *abs#084*

10:00am **Rajan Sankaranarayanan**
Structure-function analysis of enzymes involved in the complex lipid cell wall synthesis of Mycobacterium tuberculosis *abs#085*

Coffee / Exhibition Viewing Period

10:30 AM - 11:00 AM Trade Display Area

S10A (Concurrent): Clinical Proteomics IV

11:00 AM - 1:00 PM Hall A

Chair: Young-Ki Paik and Pengyuan Yang

11:00am **Young-Ki Paik**
Discovery and validation of serological HCC biomarkers *abs#086*

11:24am **Tesshi Yamada**
Cancer Proteomics for the Identification of Biomarkers and Therapy Targets *abs#087*

11:48am **Tadashi Yamomoto**
Human glomerulus proteomics or kidney and urine proteomic project – overview *abs#088*

12:12pm **Michelle Hill**
PTRF-Cavin is essential for caveola formation - from proteomics to function *abs#089*

12:36pm **Speaker to be Advised**
Title not available at time of print *abs#090*

S10B (Concurrent): Immunology - Structural Genomics and Proteomics

11:00 AM - 1:00 PM Meeting Room 1-2

Chair: Zihe Rao and Tony Purcell

11:00am **Zihe Rao**
Progress of viron protein structural genomics *abs#091*

11:24am **Jamie Rossjohn**
T cell recognition and the Atkins diet *abs#092*

11:48am **Nicholas Williamson**
T cell recognition of chemically diverse ligands *abs#093*

12:12pm **Ting-Fang Wang**
SUMO modifications control assembly of synaptonemal complex in yeast meiosis *abs#094*

12:36pm **Speaker to be Advised**
Title not available at time of print *abs#095*

Closing Ceremony and Poster Awards

1:00 PM - 1:30 PM Hall A

POSTER LISTING

PS1: Poster Session 1

Nur Abdullah
Effects of *Ficus deltoidea* extract on the serum protein profile of Simultaneously Hypertensive Rats (SHR) *abs#201*

Shadab Ahmad
Magnetic Bead Based affinity profiling for Biomarker Identification: Identifying potential pitfalls *abs#202*

Noriaki Arakawa
Proteomic analysis for identification of therapeutic targets of ovarian clear cell carcinoma *abs#203*

Georgia Arentz
Identification of Colorectal Cancer Biomarkers using Laser Micro-Dissectiona and 2D DIGE *abs#204*

James Broadbent
Development of an enhanced proteomic method to detect potential prognostic and diagnostic markers of healing in chronic wound fluid *abs#205*

Ying Chang
Mass spectrometry-based analysis of tyrosine phosphoproteomics and identificaiton of substrates of protein tyrosine phosphatase dPTP61F in *Drosophila* S2 cells. *abs#206*

Yuan-Shou Chen
Proteome Analysis of Membrane-Associated Events during Early Stages of the Epithelial-Mesenchymal Transition *abs#207*

Akihiro Chiba
The stimulatory effect of various salts on yeast alcohol dehydrogenase activity *abs#208*

Shan-Ho Chou
Microbial Structural Genomics: Important Biological Functions Executed by Interesting Protein Structures *abs#209*

Emmanuelle Claude
Coupling MALDI MS with high-efficiency ion mobility spectrometry for tissue Imaging of low mass endogenous compounds *abs#210*

Mark Condina
A Sensitive Magnetic Bead Approach for the Detection and Identification of Tyrosine Phosphorylation in proteins by MALDI- TOF/TOF mass spectrometry. *abs#211*

Tanusree Das
A comprehensive understanding of adaptation of the enteric pathogen *Vibrio cholerae* to bile *abs#212*

Claire Delahunty
The Proteome of Human Parotid and Submandibular/Sublingual Gland Salivas *abs#213*

Cristobal dos Remedios
Intercalated Disc: Changes In Multiple Proteins Associated With Heart Failure *abs#214*

Sarah Dower
Isolation and evaluation of different peroxisomes subppopulations from rat liver *abs#215*

Teppei Ebina
Loop length dependent SVM prediction of domain linkers *abs#216*

Ali Fathi
Differential molecular analysis of Human embryonic stem cells versus Embyoide bodies *abs#217*

Steve Freeby
Enrichment of Interleukins and Low Abundance Proteins from Tissue Leakage in Serum Proteome Studies using ProteoMiner™ beads *abs#218*

Johan Gustafsson
Imaging mass spectrometry (IMS) and its application to murine tissues *abs#219*

Atsushi Hirano
Application of arginine to increase the solubility of poorly water-soluble compounds *abs#220*

Asami Hishiki
Structural basis for novel interactions between human translesion synthesis polymerases and PCNA *abs#221*

Sen-Yung Hsieh
Comparative Proteomics Revealing Cytoskeleton Remodeling upon UV-Irradiation Induced Cell Apoptosis *abs#222*

C Hughes
The utility of Ion Mobility Spectrometry to Separate Candidate Precursors From Background Ions and Species of Different Charge States in Tandem MS experiments *abs#223*

Wei Jia
MS^2 and MS^3 Properties of Partially Deglycosylated Core Fucosylated Glycopeptides in Ion Trap *abs#224*

Hong Jin
Comparative proteomic analysis of drug sodium iron chlorophyllin addition to Hep3B cell line *abs#225*

Shunsuke Kamijo
Improvement of orthogonality between the amber suppression system and the translation system of Ecoli *abs#226*

Shunsuke Kamijo
Investigateion of requirements for the KMSKS loop in aminoacyl-tRNA synthetase by random PCR method *abs#227*

Rizma Khan
Proteomic Analysis of Nuclear membrane in HCV induced Liver Cirrhosis *abs#228*

Sutin Kingtong
Proteome analysis reveals Indian-rock oyster, *Saccostrea forskali* proteins dysregulated by the environmental pollutant tributyltin. *abs#229*

Angelika Koepf
Liquid Chromatographic Protein Separation Coupled to Top- down and Bottom-up Mass Spectrometric Analysis *abs#230*

Victoria Kopetz
Proteomic studies into the human coronary microvasculature: Plasma protein profiles during acute coronary syndrome presentation *abs#231*

Suguru Koyama
Analysis of sequence specificity for calpain by monitoring cleavages of multiple peptides using iTRAQ™ and 2D-LC-MS/MS. *abs#232*

Daisuke Kuroda
H3-rules, progress report 2007 *abs#233*

Jim Langridge
Coupling Two-Dimensional Liquid Chromatography With Esi Ms For Label-Free Absolute Protein Quantification *abs#234*

Seung-Taek Lee
Cleavage and functional loss of human apolipoprotein E by digestion of matrix metalloproteinase 14 *abs#235*

Justin Lim
Proteomic analysis of exosomes derived from SW480 colon cancer cells with functionally restored full-length adenomatous polyposis coli *abs#236*

Nai-Yu Liu
Human plasma protein PTMome project and biomarker discovery *abs#237*

Shuang Lu
Mass spectrometry identification of histone H2B variants and their post-translational modifications during spermatogenesis *abs#238*

Therese McKenna
Generation of unique protein specific MRM signatures; Using peptide information from alternate scanning LC-MS data to drive MRM development. *abs#239*

Norifumi Muraki
Structure of Protochlorophyllide reductase Reveals a Mechanism for Greening in the Dark *abs#240*

Shamim Mushtaq
Immunolocalization and dynamic expression of Albumin precursor and Hsp70 in wound healing of corneal epithelial cells *abs#241*

Saurabh Nagpal
Detergent Removal from Protein samples using SDR HyperD ® and Mass-spectrometry Based Detergent Estimation. *abs#242*

Jason Chun Hong Neo
Zebrafish Imaging: A MALDI MS Imaging Approach *abs#243*

Hideaki Ohtomo
Crystal structure of a chimeric β-lactoglobulin, Gyuba *abs#244*

Cheng Cheng Ooi
Biological And Proteomic Analysis Of Butyrate And Its Metabolite, 3-Hydroxybutyrate, In HT-29 Human Colorectal Cancer Cells *abs#245*

Matt Padula
Identification of proteins, enzymes and potential vaccine candidates from the Aust Paralysis Tick, Ixodes holocyclus using 2D-PAGE and Equalizer technology. *abs#246*

Matthias Pelzing
Complete Characterizing of Erythropoietin Glycoforms using Capillary Zone Electrophoresis Coupled to Mass Spectrometry *abs#247*

Sushma Rao
Comparative analyses of abscisic acid responses in plants *abs#248*

Siti Rosli
Identification Of Differentially Expressed Proteins In The Serum Of Oral Cancer Patients By Two Dimensional Gel Electrophoresis *abs#249*

YuLin Sun
Quantitative Proteomic Analysis Revealed Tissue Transglutaminase 2 Could Be a Novel Protein Candidate of Hepatocellular Carcinoma *abs#250*

Toshiyuki Tanaka
Two isoforms of ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1) were down-regulated in high metastatic potential of human SN12C renal cell carcinoma cell clones. *abs#251*

Hiroki Tanaka
Structural mechanism of molecular interaction triggered by synaptic adhesion protein *abs#252*

Samantha Tang
MALDI-TOF-MS identification of Intermittent Hypercapnic Hypoxia induced protein changes in the Piglet Hippocampus. *abs#253*

Chai Lean Teoh
Applying Spectroscopic Rulers to ApoC-II Amyloid Fibrils *abs#254*

Goro Terukina
Proteomic analysis of XFKBP-associated protein complex formed during secondary axis formation in *Xenopus laevis* embryo *abs#255*

Chitra Thangavel
High-Throughput Purification of Polyhistidine Tagged Proteins in AcroPrep™ Multi-well Filter Plates Using IMAC HyperCel™. *abs#256*

Norihisa Uemura
Prognostic biomarker in esophageal cancer by 2D-DIGE, tumor tissues and clinical data *abs#257*

Yan Wang
Differential ConA-enriched Urinary Proteome in Rat Experimental Glomerular Diseases *abs#258*

Harunori Yoshikawa
Proteomic analysis of proteins associated with splicing factor-2 associated protein p32 revealed its possible involvement in human ribosome biogenesis *abs#260*

Rie Yoshino
The Effect of Plant Compounds from Whisky Cask on Horse Liver Alcohol Dehydrogenase Activity *abs#261*

Saadia Zahid
Neuroproteomics: Exploring Regional Human Brain using 2-DE *abs#262*

Kunkun Zhang

Leptospira interrogans Selectively Binds Guinea Pig Serum Proteins *in vitro* *abs#264*

PS2: Poster Session 2A and 2B

Renwick Dobson
In vivo evolution of Escherichia coli pyruvate kinase type I: how does genotypic evolution affect phenotype? *abs#301*

Cristobal dos Remedios
The Aging Human Heart: Changes in expression of LIM domain proteins *abs#302*

Sarah Dower
Enrichment of Phosphopeptides by Free Flow Electrophoresis *abs#303*

Sarah Dower
Versatile Analyses Of Free Flow Electrophoresis Separated Protein Isoforms *abs#304*

Ayako Egawa
Structural analysis of transmembrane halobacterial tranceducer *p*HtrII by multi-dimensional high-resolution solid-state NMR *abs#305*

Toru Ezure
Posttranslational modifications in an insect cell-free protein synthesis system and their identification by MALDI-TOF MS *abs#306*

L Fremlin
A New Sensor-Controlled Preparation Technique for MALDI Tissue Imaging *abs#307*

Kazuo Fujiwara
OLIGAMI: OLIGomer Architecture and Molecular Interface *abs#308*

Qian Garrett
Bovine Lactoferrin Promotes Alkali-Induced Wound Healing in Corneal Epithelial Cells by Up-regulating IL-6 and PDGF *abs#309*

Michael Griffin
Amyloidogenic peptides from apolipoproteins A-I and C-II: Lipid effects on fibril forming peptides from lipid binding proteins. *abs#310*

Rudolf Grimm
Rapid analysis of 1D and 2D gels by nanoflow LC/MS *abs#311*

Yoko Harano
Data deposition supporting website at Osaka for BioMagResBank and Protein Data Bank *abs#312*

Junichi Higo
An enhanced conformational sampling of a 40-residue protein consisting of alpha and beta secondary structures in explicit solvent *abs#313*

David Hoke
The marine bacterium *Pseudoalteromonas tunicata* alters its proteome upon adhesion to extracellular matrix *abs#314*

Masaki Ihara
Establishment of Open Sandwich immunoassay using antibody fragments derived from combinatorial libraries *abs#315*

Haruka Ikegami
Discovery of a protein biomarker candidate related to carcass weight in Japanese Black beef cattle (Wagyu) *abs#316*

Masaya Ikegawa
Profiling cerebrospinal fluid proteins in multiple sclerosis by CLINPROT system *abs#317*

Hong Ji
DIGE analysis of Ras-transformed fibroblast cell-derived exosomes *abs#318*

Narutoshi Kamiya
Assembly simulation of four peptide chains in explicit water by multicanonical molecular dynamics *abs#319*

Yasushi Kawata
Identification of the functionally critical amino acid segment and its role in the flexible C-terminal region of the

chaperonin GroEL *abs#320*

Runcong Ke
Analysis of human proteins with charge periodicity of 28 residues in amino acid sequences *abs#321*

James Ketudat Cairns
Structural Basis for Substrate Specificity in Rice and Barley Beta-Glucosidases *abs#322*

Chuang Fong Kong
Identification of phosphoproteins and profiling of phosphorylation sites in complex biological samples: A simple and efficient workflow using mini-gel-separated proteins *abs#323*

Kunihiro Kuwajima
Folding Mechanisms of Homologous Proteins: A Comparative Study between Lysozyme and α-Lactalbumin *abs#324*

Sing Li
Plasma proteomes as a basis for searching potential septic biomarkers in intensive care units *abs#325*

Chuan Li
Protein arginine methylation of the cellular nucleic acid binding protein (CNBP) *abs#326*

Shufang Liang
Investigating action mechanism of a natural active compound honokiol by quantitative proteomic analysis *abs#327*

Rita Machaalani
SELDI-TOF MS analysis of the effects of post-mortem interval on rat brain proteomics. *abs#328*

Amanda Bulman
Strategies for SELDI-Based Biomarker Discovery and Development: An Alzheimer's Disease Case Study *abs#329*

Matthew McDonagh
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Christine Miller
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ORALS

001

NEW STRUCTURES FROM OLD: DEVELOPMENTS IN MOLECULAR REPLACEMENT

R. J. Read

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To solve the three-dimensional crystal structure of a novel protein (or other macromolecule), the crystallographer generally resorts to experimental phasing methods. However, with the exponential increase in the size of the Protein Data Bank, there are fewer novel structures, and there is often a reasonably close homologue of known structure. In this case, the method of molecular replacement can be used to solve the structure; currently about two-thirds of protein structures are solved this way.

The recent growth in the use of molecular replacement is also fueled by increases in the power of the method. By using maximum likelihood-based algorithms implemented in our program Phaser[1], structures can routinely be solved when the best available template has a sequence identity of only about 30%, and in favourable cases structures can be solved with templates sharing less than 20% sequence identity.

It has long been hoped that homology modeling could expand the applicability of molecular replacement even further by improving the quality of templates from distant relatives, but until very recently homology modeling algorithms were not up to the challenge. However, we have shown, in collaboration with David Baker, that homology modeling with the program Rosetta can significantly improve the quality of template structures, whether they are obtained from distant relatives or NMR experiments[2].

The most striking result shows that it may even be possible to dispense with templates from known structures, at least in favourable cases. An ab initio model obtained by Rosetta without a template (but drawing heavily on the structural knowledge accumulated in the PDB) was sufficiently accurate to solve a novel structure by molecular replacement[2].

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002

THROUGH THE LOOKING GLASS – A NEW WORLD OF PROTEINS ENABLED BY CHEMISTRY

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Recent advances in synthetic methods enable the routine synthesis of protein enantiomorphs, unnatural protein molecules made up entirely of D-amino acids. These D-proteins have a tertiary structure that is the mirror image of the backbone fold of their counterparts found in nature. Such mirror image protein molecules have a variety of uses. More facile crystallization of racemic protein mixtures and the quantized phases of diffraction data from the resulting centrosymmetric racemic protein crystals enable the use of ab initio methods to solve novel protein Xray structures. These precise phases can be used to calculate electron density maps of unusually high quality from diffraction data of a given resolution. Protein enantiomorphs also enable discovery libraries. Select mirror image protein molecules themselves are good candidates for use in clinical applications: they are resistant to proteolytic digestion, are more stable in vivo, and are non-immunogenic. I will discuss the application of total synthesis to the creation of uniquely chemical analogues of a variety of protein targets including antifreeze proteins, venom-derived proteins, and enzymes. The design and synthesis of protein-derived molecules of novel topology will also be described.

003

THE PROTEOMINER AND THE FORTYNINERS: SEARCHING FOR GOLD NUGGETS IN THE PROTEOMIC ARENA

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The present lecture will cover modern aspects of combinatorial ligand libraries (CLL), as used for analyzing the "low-abundance proteome" in association with mass spectrometry. First, the capturing properties of baits of different lengths (from single amino acid to hexa-peptides) are described, to show that a plateau is rapidly reached above a tetra-peptide in length, thus confirming the validity of having adopted hexapeptides for the considered application. The mechanism of interaction with proteins from very complex proteomes and the ability to decrease the dynamic concentration range is demonstrated with the help of mass spectrometry analysis. Examples are given on how treatment with CLLs dramatically improves the detectability of peptides in mass spectrometry analysis and permits one to detect a very large number of proteins as compared with control, untreated samples. The use of complementary

libraries is discussed with the aim to discover additional low-abundance species that escaped the first library. The lecture will end by discussing the possibility to discover extremely rare gene products, and the quantitative aspect of the technology when associated with mass spectrometry. Some insights on the applications for hidden, low-abundance biomarkers are also presented. The samples to be dealt with: the cytoplasmic proteome of the red blood cell, egg white proteomics, cerebrospinal fluid, human sera and urines. Last, but not least, the use of CLLs for the discovery of a large number of previously undetected host proteins in recombinant DNA products.

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004

WNT SIGNALING PLAYS A KEY ROLE IN HUMAN NEURAL STEM CELL DIFFERENTIATION INTO OLIGODENDROCYTE PROGENITORS

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Embryonic stem cell-derived neural cells and adult neural stem cells are promising sources of tissue for testing cellular and gene therapies for CNS disorders. Recently, significant progress has been made towards the goal, yet key questions about global perspectives for the neural differentiation pathway remain to be answered including molecular determinants of neural fate and distinctive stages of differentiation. To this end, we have established and characterized olig2-overexpressed subclone of human neural stem cell, HB1.F3 : F3.Olig2. F3.Olig2 provides a model for characterizing the downstream effects of olig2 transcription factor. We performed a phenotypic characterization and microarray analysis of HB1.F3, an immortalized human cell line, and F3.Olig2, an olig2-overexpressed subclone of HB1.F3. SILAC(Stable Isotope Labeled Amino acid in Culture) method and Nano-LC FT-ICR were employed for quantitative analysis of the protein profile change during differentiation process. Systemic molecular biological validation were performed for the genes and proteins of several signaling pathways including Wnt/b-catenin pathway which has been known to promote self-renewal in a variety of tissue stem cells including neural stem cells with western blotting, real time PCR, and immunohistochemical staining. Together, these approaches have allowed us to characterize Wnt signaling and Dkk1 plays a key role in changes initiated by olig2 upon the differentiation of oligodendrocytes from neural stem cells.

005

TRANSCRIPTOME AND PROTEOME ANALYSES OF HUMAN, MONKEY, AND MOUSE EMBRYONIC STEM CELLS DURING EMBRYOID BODY-BASED DIFFERENTIATION

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We have applied proteomics and transcriptomics approaches to investigate the molecular mechanisms that control embryonic stem cell (ESC) self-renewal and differentiation. We analyzed total, nuclear and membrane proteomes of differentiated and undifferentiated human, mouse and monkey embryonic stem cells. The trancriptome of proliferating and differentiating human ESCs has also been analyzed using a microarray approach. Comparative analyses across species and organelles revealed several proteins and mechanisms emerged as key participants in stem cells proliferation and differentiation. Microarray and Real-Time analyses of ESCs also resulted in identification of several new mechanisms. The results of transcriptome and proteome data will be compared and the methods to maximize the benefit of the integration of transcriptome and proteome data will be discussed.

006

INTEGRATED APPROACH TO STUDY MOUSE EMBRYONIC STEM CELL PROTEOME

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Study of the proteome of the embryonic stem cells (ES) cells is important to understand active pathways, regulatory networks and their dynamics . We have been studying protein expression in mouse embryonic stem cell line R1-9 and ABI and have integrated the

data with transcriptomics studies as well as with proteomics studies with the same and other cell lines from other laboratories. Such integrated approach would help in consolidation of the protein expression data and the biochemical pathways operational in the stem cells and their differentiation lineages. Proteins expressed in mouse ES cells R1-9, AB1 were studied using LC-ESI MS/MS and LC-MALDI MS/MS approaches after pre fractionation (SDS PAGE) of total cellular proteins or the proteins from the ES cells nuclei. Proteins were identified and identifications verified against the transcriptomics data - DNA microarrays, SAGE, and ESTs. We have thus identified more than 2000 proteins with high confidence. Pathway analysis of these short listed proteins was carried out using KEGG, IPA, GenMAPP and their gene ontology classification revealed among them transcription regulators, signal transducers, cell cycle and differentiation molecules along with other general classes of proteins. Using this list of proteins and those identified by other groups with the same stem cell lines, putative regulatory pathways operational in these cells are being constructed to further explore their role in stem cells. In addition, functional annotation of proteins corresponding to many still unidentified / uncharacterized mouse genes is also being attempted and will be discussed.

007

BIOINFORMATIC INFRASTRUCTURE FOR MAINTAINING PROTEOME INFORMATION

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Extracting the most out of omics databases requires automated pipelines. Modern biological science requires such an automated platform for handling data with proper a information technology infrastructure. We introduce an integrated bioinformatic pipeline scheme for processing genomic, proteomic, and RNA data. These pipelines incorporate a data mapping part: BioMatrix, automation part: BioPipeline, daemoninzing part: BioEngine, and information distribution part: BioPortal. As a specific BioPortal application for a processing raw proteomic data we have built a web service called MassNet.kr. As the fusion of biological fields is accelerated, data glueing methods and integrated databases are becoming the key issues in bioinformatics. We introduce a distributed approach of sharing biological data using P2P for heterogeneous groups of researchers. Distributed resources architecture is fast and robust for projects that have a very large number of participants. This approach is suitable for Asian and Oceanian regions.

008

DYNAMICAL SINGLE MOLECULAR OBSERVATIONS OF MEMBRANE PROTEINS USING X-RAYS

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Recently, we succeeded picometer-scale slow Brownian motions of individual protein membranes (Bacteriorhodopsin (BR) [1] and Potassium channel KcsA[2]) in aqueous solutions from time-resolved single molecular observations using X-rays. In this single molecular detection system with X-rays, which we call Diffracted X-ray Tracking (DXT)[3,4], we observed the rotating motions of an individual nanocrystal, which is labeled to the specific site in individual protein molecules. In the case of BR, we observed Brownian motions and momentarily structural change of individual single BR in the light irradiation. We have consequently confirmed that the average size of the momentarily structural changes by light irradiation in 35^{th} residue of BR was 76±48.2pm. In the case of Potassium channel KcsA, we observed the rotational motions of the central pore of KcsA in the open transition at low extracellular pH conditions. The size of the observed rotations occurs with about 20-30 degrees during 100-300ms. We measured both the full-length KcsA and the pore part of KcsA.

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009

DISCRIMINATION BETWEEN DNA SEQUENCES AND BETWEEN COREGULATOR AMINO ACIDS BY FEAST/FAMINE REGULATORY PROTEINS (FFRPS)

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Homologues of *Escherichia coli* leucine-responsive regulatory proteins (Lrp) are referred to as Feast/Famine Regulatory Proteins (FFRPs). They comprise a single group of transcription factors systematically distributing throughout archaea and eubacteria.

An archaeal FFRP, FL11 from *Pyrococcus* sp. OT3 was crystallized in its dimer form in complex with a DNA duplex, TGAAAWWWTTTCA. Ala34-Thr37 in the loop connecting alpha helices 2 and 3, and two other residues, Leu24 and His39, in each monomer contacted 5 bps at each terminus of the target DNA. These contacts and DNA bending by propeller twisting at WWW confirmed specificity of the interaction. Dimer-binding sites were identified in the promoter of approximately 200 transcription units, i.e. 20% of all units, coding, for example, proton ATPase and NAD(P)H dehydrogenase, synthesizing ATP by degrading amino acids.

In the presence of lysine, four FL11 dimers were shown to assemble into an octamer, thereby covering the *fl11* promoter. The FL11 octamer was crystallized in complex with eight lysine molecules. Between a pair of dimers two lysine molecules were present. Asp104 of one dimer contacted the N-terminus of a lysine molecule, and Thr132 and Thr135 of the other dimer contacted its C-terminus. Some other residues of either dimer, including of Gln98 and Asp122, interacted with the lysine side-chain.

In the "feast" mode in the presence of lysine, when *P*. OT3 grows on amino acids, the FL11 octamer will terminate transcription of *fl11*, as was shown *in vitro*, thereby derepressing transcription of many metabolic genes. In the "famine" mode in the absence of lysine, approximately 6000 FL11 dimers present per cell will arrest growth. This regulation resembles another global regulation by *E. coli* Lrp in response to the availability of leucine, and hints at the prototype of transcription regulations, once achieved in the last common ancestor of all extant organisms.

010

STRUCTURE AND MECHANISM OF THE TRIPARTITE MULTIDRUG EXPORTER

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Background: Bacterial multidrug exporters are responsible for multidrug resistance of gram negative bacteria currently emerging in the modern chemotherapy. The most significant characteristic is their extraordinary broad substrate specificity. We succeeded to solve the crystal structure of a bacterial major multidrug exporter AcrB in 2002 and revealed that the drug recognition is based on the membrane vacuum cleaning mechanism. However, our first structure did not contain bound substrates. We have solved the drug-bound structure of AcrB in 2006 and revealed the structural basis of multidrug export mechanism. Results: Our new crystal of AcrB has no crystallographic three-fold symmetry. Each monomer in the AcrB trimer has a different conformation to the others. Only one substrate binds to the AcrB trimer. The substrate binding pocket is a phenylalanine-rich cluster located in the porter domain. Multidrug recognition is based on the multisite binding, that is, different drugs interact with different residues in the same pocket. Three monomers represent the conformations of the three intermediate steps of the drug export function, access, binding and extrusion. In the binding monomer, exit is closed by the inclined central a -helix of the extrusion monomer and the entrance is open due to the unfolding of the top of TM8. In contrast, in the extrusion monomer, the vacant substrate binding site is shrunk and the exit is open because the central a -helix is inclined away. The entrance is closed by the a -helix of the top of TM8. The access monomer shows the intermediate structure; entrance is open but the binding site is still shrunk. Conclusion: Crystal structure of AcrB revealed the functionally-rotation mechanism of multidrug export.

011

SOLUTION STRUCTURE OF MST1 SARAH DOMAIN AND ITS INTERACTION WITH RASSF5 AND WW45 SARAH DOMAINS FOR THE APOPTOSIS PATHWAY

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In eukaryotic cells, apoptosis and cell cycle arrest by the Ras RASSF MST pathway are controlled by the interaction of SARAH (for Salvador/Rassf/Hippo) domains in the C-terminal part of tumor suppressor proteins. The Mst1 SARAH domain interacts with its homologous domain of Rassf1 and Rassf5 (also known as Nore1) by forming a heterodimer that mediates the apoptosis process. Here we describe the homodimeric structure of the human Mst1 SARAH domain, and its heterotypic interaction with the Rassf5 and Salvador (Sav) SARAH domain. The Mst1 SARAH structure forms a homodimer containing two helices per monomer. An anti-parallel arrangement of the long alpha helices (h2/h2') provides an elongated binding interface between the two monomers, and the short 3_{10} helices (h1/h1') are folded toward that of the other monomer. Chemical shift perturbation experiments identified an elongated, tight binding interface with the Rassf5 SARAH domain, and a 1:1 heterodimer formation. The linker region between the kinase and the SARAH domain is shown to be disordered in the free protein. This implies a novel mode of interaction with RASSF family proteins, and provides insight into the mechanism of apoptosis control by the SARAH domain.

012

INSIGHTS INTO DROUGHT RESPONSIVENESS IN RICE AT THE REPRODUCTIVE STAGE THROUGH PROTEOMIC ANALYSIS

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Rice is grown under irrigated and rainfed conditions on all continents. Drought is a major cause of yield loss under rainfed conditions, while economic water shortages limit productivity in irrigated areas. These factors have intensified research on the causes of yield loss under water deficit. Rice is most susceptible to drought stress at the reproductive stage. One of the most sensitive events is elongation of the peduncle, which is the uppermost and longest internode of the stem. Peduncle elongation is essential to exsert the panicle and its florets from the flag leaf sheath, but drought stress starting 4 days before flowering in pot–grown plants arrests peduncle growth and traps the peduncle in the flag leaf sheath, causing floret sterility and yield loss. Internode elongation is known to depend on the phytohormone gibberellic acid (GA), but, as many actions of GA are antagonized by the drought-induced phytohormone abscisic acid (ABA), it is likely that this antagonism plays a major role in causing yield loss. Spraying of drought-stressed plants with GA_3 reverses the arrest of peduncle elongation and panicle exsertion and allows one-third of the yield loss to be recovered. However, two-thirds of the yield loss remains, with failure of anther dehiscence identified as a major problem. Here we use proteomic analysis to examine the changes in the peduncle and the anther caused by drought and phytohormones. In addition, we use proteomics to examine the impact of drought stress on the ability of the leaves to rehydrate during re-watering and thus supply photosynthate during grain filling. We also compare changes in proteome and transcriptome. These studies lead us to suggest several targets for breeding to enhance drought tolerance.

014

PROTEOMIC ALTERATION DURING STORAGE OF *CURCUMA LONGA* RHIZOMES

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The rhizomes of genus *Curcuma* are extensively used as spices, food preservatives, coloring materials, cosmetics and medicines. The *Curcuma* becomes dormant during the dry season. The changes in proteins during storage of *Curcuma longa* have not been investigated in detail. We employed proteomic technology to study the protein expression from the day of harvest to the commencement of sprouting of *Curcuma longa*. The two dimensional gel electrophoresis patterns (pH 4-7) of the rhizomes showed a high abundance of proteins with pI in the range of 3-5 and low abundance with pI in the range of 5-7. Microscale solution-phase isoelectric focusing (Zoom) was employed to enrich the low abundance proteins in the pH range of 5.4-10 and improve the separation of those proteins in the acidic range from 3-5.4. The total storage period was 77 days. Samples were drawn at an interval of 7 days from the harvest until sprouting. The proteomic patterns of the storage period (0, 7, 14, 21, 35, 42, 49 and 70 days) were studied in these two pH ranges. In the pH range from 3-5.4, the expression of ribulose 1,5-bisphosphate dehydrogenase, actin, anionic peroxidase swpa4, maturase and photosystem I assembly protein ycf4 were increased and putative oxygen evolving enhance protein I, hypothetical protein MeviPp13 and MLP_like protein 423 were decreased from 0 to 70 days, while in the pH range from 5.4-10, the expression of eight identified proteins were expressed only at 70 days. Sporamin, the major storage protein of the tuberous roots of sweet potato was highly expressed in the dormancy period and lower expression seen in the sprouting period. The expression of 60S ribosomal protein L10, ribosomal protein subunit 2, ribosomal protein S7, Vacuolar ATP synthase subunit E and alcohol dehydrogenase II were increased in the visible sprouting (70 days). These results represent the first proteomic patterns during storage period of *Curcuma longa*.

Supported by the Chulabhorn Research Institute.

015

MASS SPECTROMETRY-BASED SUBSTRATE IDENTIFICATION AND GENETIC VALIDATION REVEAL THE FUNCTIONAL ROLE OF *DROSOPHILA* PROTEIN TYROSINE PHOSPHATASE DPTP61F

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Recent biochemical and genetic approaches have clearly defined the functional role of critical components in tyrosine phosphorylation-dependent signal transduction. These signaling modulators often exhibit evolutionarily conserved functions across various species. It has been proposed that if protein tyrosine kinases (PTKs), protein tyrosine phosphatases (PTPs) and thousands of their substrates could be identified and characterized, it would significantly advance our understanding of the underlying mechanisms that control animal development and physiological homeostasis. The fruit fly *Drosophila melanogester* has been used extensively as

a model organism for investigating the developmental processes but the state of its tyrosine phosphorylation is poorly characterized. In the current study, we used advanced mass spectrometry (MS)-based shotgun analyses to profile the tyrosine phosphoproteome of *Drosophila* S2 cells. Using immunoaffinity isolation of the phosphotyrosine (pTyr) subproteome from cells treated with pervanadate followed by enrichment of phosphopeptides, we identified 562 non-redundant pTyr sites in 245 proteins. Both this pre-defined pTyr proteome subset and the total cell lysates were then used as sample sources to identify potential substrates of dPTP61F, the smallest member in terms of amino acid number and molecular weight in the *Drosophila* PTP family and the ortholog of human PTP1B and T Cell-PTP, by substrate trapping. In total, 20 unique proteins were found to be specifically associated with the trapping mutant form of dPTP61F, eluted by vanadate (VO_4^{3-}), and identified by MS analyses. Interestingly, several potential substrates were previously identified as components of SCAR/WAVE complex, which may work in coordination to control actin dynamics. To validate the results of MS-based substrate identification and to further illustrate the functional role of dPTP61F in regulating actin action, genetic approaches were applied in *Drosophila*. Our data clearly demonstrate that dPTP61F plays a central role in counteracting PTK-mediated signaling pathways in regulating actin reorganization and remodeling through tyrosine dephosphorylation of critical components of SCAR/WAVE complex during *Drosophila* development.

016

GATING CONTROL; MECHANISM OF MAGNESIUM TRANSPORTER MGTE

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The MgtE family of Mg^{2+} transporters is ubiquitously distributed in all three domains, and human homologues SLC41 have been functionally characterized and suggested to be involved in magnesium homeostasis. However, the MgtE transporters have not been thoroughly characterized. We determined the crystal structures of the full-length *Thermus thermophilus* MgtE at 3.5 Å resolution and the cytosolic domain in the presence and absence of Mg^{2+} at 2.3 Å and 3.9 Å resolutions, respectively. The transporter adopts a homodimeric architecture, consisting of the C-terminal five transmembrane (TM) domain, and the N-terminal cytosolic domains, composed of the superhelical N domain and the following tandemly-repeated cystathionine-β-synthase (CBS) domains. A solvent-accessible pore nearly traverses the TM domains, with one potential Mg^{2+} bound to the conserved Asp432 within the pore. The TM5 helices from both subunits close the pore through interactions with the "connecting helices", which connect the CBS and TM domains. Number of Mg^{2+} are bound at the interface between the connecting helices and the other domains, which may lock the closed conformation of the pore. A structural comparison of the two states of the cytosolic domains showed the Mg^{2+}-dependent movement of the connecting helices, which might reorganize the TM helices to open the pore. These findings suggest a homeostasis mechanism, in which Mg^{2+} bound between cytosolic domains regulate Mg^{2+} flux by sensing the intracellular Mg^{2+} concentration. Our recent MD simulation as well as genetic and biochemical experiments has provided a clue to answer to whether this presumed regulation mechanism actually controls the gating of the ion channel.

017

TRANSLOCON-ASSISTED FOLDING OF MEMBRANE PROTEINS: NEW INSIGHTS INTO LIPID-PROTEIN INTERACTIONS.

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Recent studies of the translocon-assisted folding of membrane proteins have revealed two unexpected findings about the insertion of transmembrane helices across the endoplasmic reticulum membrane. First, the so-called S4 voltage-sensor helix of potassium channels, comprised of hydrophobic residues and four arginine residues, can be inserted. Second, polyleucine helices as short as 10 residues are readily inserted. Exploration of these observations using physical studies of synthetic peptides in model membranes and molecular dynamics simulations provide new insights into lipid-protein interactions. They reveal that the lipid bilayer is far more complex—and interesting—than its usual lollypop cartoon suggests. The biological, physical, and molecular dynamics data to be presented demonstrate the extreme adaptability of phospholipids that arises from the privileged relationship between their phosphate groups and lysine and arginine residues. This adaptability makes possible the transmembrane insertion of very short helices and the independent stability of potassium channel voltage-sensor domains in membranes. [Research supported by the National Institute of General Medical Sciences and the National Center for Research Resources.]

018

DEVELOPMENT OF A NOVEL PEPTIDE AFFINITY TAG SYSTEM FOR ONE-STEP PURIFICATION OF RECOMBINANT PROTEINS

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Recombinant production of extracellular or membrane proteins in mammalian cells is routinely exercised in many labs around the globe including both basic and industrial researchers, but purification of target protein from the culture supernatant/cell lysate often demands method development/optimization dependent on individual project. We have developed a novel anti-peptide antibody P20.1 that can be used as both detection and affinity-purification tool suitable for the application in the recombinant production of human proteins. The intrinsic affinity of the mAb to the minimum epitope sequence (6aa) is low, allowing the mild elution from the affinity resin, and the total affinity can be increased by increasing the valency of either the tag or antibody. A protein purified using this system yielded diffraction-quality crystals that gave 1.5Å resolution structure within 3 weeks. Three dimensional structure of P20.1-peptide complex and detailed information about sequence specificity are both available, opening the possibility for the further engineering of the system.

019

PRENYLTRANSFERASES AS TARGETS FOR THE DISCOVERY OF NEW ANTIBIOTICS

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Prenyltransferases are involved in the biosynthesis of isoprenoids through the condensation of C_5-diphosphates to form the compounds used in cell membrane, cell wall, terpene biosynthesis, electron transfer, and in many eukaryotes, cell signaling pathways (Ras, Rho, Rap, Rac). Therefore, there has been considerable interest in developing specific inhibitors as new drugs for various diseases associated with these pathways.

Through our structural analysis efforts, we have studied the product chain length determinants of several *trans*-type prenyltransferases, including geranylgeranyl pyrophosphate synthase (GGPPS), hexaprenyl pyrophosphate synthase (HexPPS), and octaprenyl pyrophosphate synthase (OPPS). The specificities were determined by the size and depth of the activity site cavity. Large amino acids, such as Tyr^{107}/His^{139} for GGPPS, Leu^{164} for HexPPS, and Phe^{132} for OPPS, form the floor to block product further elongation (1). In addition, we solved the structures of yeast GGPPS complexed with several bisphosphonate inhibitors (2).

Undecaprenyl diphosphate synthase (UPPS), a *cis*-prenyltransferase, produces mixed (*E*,*Z*) long-chain C_{55}-undecaprenyl diphosphate (UPP) via *cis* double-bond addition. It has been considered as a new target for anti-microbial therapy because UPP is used to form the lipid-I and lipid-II species needed for peptidoglycan cell-wall biosynthesis in bacteria. Here, bisphosphonates were tested as inhibitors of UPPS, with the most active one having an IC_{50} of < 600 nM . In the UPPS-inhibitor complexes, four distinct binding sites were observed (2), in contrast to the observation of only one bisphosphonate-binding site in farnesyl diphosphate synthase (FPPS). The availability of these structures opens up new avenues for the design of novel inhibitors.

Another prenyltransferase called dehydrosqualene synthase (CrtM) from *Staphylococcus aureus*, uses the head-to-head condensation of two farnesyl diphosphate (FPP) molecules to produce the presqualene diphosphate C_{30} molecule, resembling the human squalene biosynthesis. Interestingly, the C_{30}-presqualene diphosphate is the precursor for the biosynthesis of staphyloxanthin, the golden carotenoid pigment which promotes resistance of the bacteria to reactive oxygen species and host neutrophil-based killing. CrtM, therefore, has been tested as the target to treat the hospital- and community-acquired infections produced by methicillin-resistant *S. aureus* (MRSA). Based on the structural similarity between CrtM and human squalene synthase (SQS), SQS inhibitors for cholesterol-lowering activity in humans also can be bound to CrtM through blocking the biosynthesis of staphyloxanthin *in vitro* (median inhibitory concentration ~100 nM), resulting in colorless bacteria with increased susceptibility to killing by human blood and to innate immune clearance in a mouse infection model (3).

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020

UTILISING A LARGE COMPUTING RESOURCE FOR YOUR PROTEOMICS RESEARCH "THE AUSTRALIAN PROTEOMICS COMPUTATIONAL FACILITY" - USING THE APCF FOR BIOMARKER DISCOVERY

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Diseases such as colorectal cancer (CRC) is a leading cause of cancer death in the Western World. Early detection is the single most important factor influencing outcome of CRC patients. If identified while the disease is still localized, CRC is treatable. To improve

outcomes for CRC patients there is a pressing need to identify biomarkers for the early detection (diagnostic markers), prognosis (prognostic indicators), tumor responses (predictive markers) and disease recurrence (monitoring markers). Despite recent advances in the use of genomic analysis for risk assessment, in the area of biomarker identification genomic methods have yet to produce reliable candidate markers for CRC. For this reason, attention is now being directed towards protein chemistry or proteomics as an analytical tool for biomarker identification. Here, we present a large high-performance computing cluster to aid researchers in the use of large-scale proteomics technologies. Our approach for addressing the metrics of large scale mass spectrometry data analysis, the Achilles' heel of current proteomic analyses, will be discussed with the presentation of our national strategy for Proteomics mass spectrometry data analysis through the establishment of the Australian Proteomics Computational Facility (APCF).

In 2007, the APCF established and installed an advanced high-performance multi-processor computing cluster based on multi-socket quad-core processors and infrastructure for scientists at proteomics center's from all over Australia to access. In addition, through the collective management by proteomics researchers from every state in Australia as well as internationally, the Management committee as well as the Scientific Advisory committee have guided the hardware usage as well as the software development to enable multi-algorithm usage of the APCF. To date, over 40,000 individual data searches have been performed by many groups independent of distance from the central server. Further software developments will be described that will enable secure remote access to additional algorithms as requested by the user community as well as the addition of both varied public and proprietary sequence databases.

This unique world's first integrated approach to proteomics computing and the sharing of databases will place Australia at the forefront of efforts to identify the proteins associated with the early detection of major human diseases as well as many other programs such as plant, animal, microbe and many other agriculture proteomic analysis. In addition, the APCF gathers together expertise to provide leadership for proteomic data interpretation on locally generated data. This data can also be used in the contribution to other world-wide large-scale proteomic efforts. The APCF can be accessed to analyse mass spectrometry data through a simple web interface by a secure user account which can be obtained from the APCF at www.apcf.edu.au The APCF is open to all Australian and New Zealand researchers with the possibility of expanding the system for use by other countries such as many of the neighbouring countries in Asia through the Asian Oceania Human Proteome Organization.

021

MANAGING PROTEOMICS DATA FROM GENERATION AND DATA WAREHOUSING TO CENTRAL DATA REPOSITORY

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Introduction: The tremendous amount of data from today´s expression proteomics requires a database solution with data-warehousing and data-mining capabilities. ProteinScape provides a bioinformatics platform for in-house proteome studies as well as for large scale approaches. The growing requirement for protein pre-fractionation to obtain more precise quantitative protein information is uniquely addressed in ProteinScape . Entire workflows of pre-fractionation, detailed LC/MS/MS separation and post-processing with bioinformatics tools are merged and can be easily controlled and reviewed.

Methods: ProteinScape is a bioinformatics platform addressing the requirements for biomarker discovery, protein identification and quantification. It supports various discovery workflows through a flexible analyte hierarchy, various database search engines and quantification approaches including a label-free strategy. All current label chemistries for protein quantification are fully supported (ICPL, SILAC, iTRAQ, ICAT, and C-term 18O/16O-C-term labeling). The support includes multiplexed quantification (e.g., ICPL triplex, iTRAQ or SILAC 4plex). It enables the use of isobaric or non-isobaric label chemistries and it permits the targeted analysis of proteins in complex mixtures. Interactive validation of protein quantification based on raw LC/MS data is now simple and straight forward.

Results: ProteinScape has a number of dedicated viewers that permit the evaluation and validation on each level of proteomics experiments. BioTools integrates with ProteinScape for advanced sequence validation, PTM discovery, de novo sequencing and MS-BLAST searches for full structure elucidation functionalities. Integrated quantification workflows that utilize labeling and label-free technologies require greatly reduced analysis and validation time.

The European Commission-funded ProDaC consortium (http://www.fp6-prodac.eu/) will finalize data storage and documentation standards, implement conversion tools and establish standardized submission pipelines into a central data repository. This contains export from local LIMS systems like ProteinScape to standard file formats or direct upload into PRIDE. With respect to this a tool is already implemented to upload ProteinScape data sets into PRIDE.

022

LC/MS-BASED LARGE-SCALE IDENTIFICATION OF N-GLYCOPROTEINS AND THEIR GLYCAN DIVERSITY

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Analysis of protein post-translational modifications (PTM) is one of major objectives of proteomics, because status and their changes of PTM may suggest significance of the modification for particular protein function, and could not presumed by genomic or transcriptomic analyses. Thus, we focused protein glycosylation and developed a method for LC/MS-based large-scale identification of N-glycosylated proteins. This method composed of (1) lectin column-mediated affinity capture of glycopeptides from protease digest of sample protein mixtures; (2) peptide-N-glycanase-catalyzed incorporation of a stable isotope tag, ^{18}O, at N-glycosylation site; and (3) identification of the labeled peptides by LC/MS. We applied this method to the characterization of N-glycoproteins from crude extracts of *C.elegans* and mouse tissues using multiple lectin columns with distinct binding specificity.

For C.elegans, total 1,465 glycosylation sites on 829 glycoproteins were determined using conA-, wheat germ agglutinin (WGA)-, and worm galectin 6-bound columns. They were quite diverse in terms of subcellular localization and function, etc., yet many were integral membrane proteins such as cell surface receptors, transporters, channels, extracellular matrix proteins, and proteases. Among them, 432 proteins were predicted to have transmembrane segment(s) and their membrane topology were presumed by the positions of experimentally determined glycosylation sites and putative transmembrane segments on the polypeptide sequence. We also identified mouse glycoproteins from various tissues such as liver, brain, kidney, lung, and testis, using 3-5 kinds of lectin columns. Total ca 4,500 sites on ca 2,300 glycoproteins were identified. These results indicate tissue distribution and diversity of glycan structures on particular sites of each protein. These large sets of the data will be available soon at JCGG database.

For quantitative analysis, we introduced a differential stable isotope-labeling step into the above procedure. Both chemical modifications with ^{13}C, ^{15}N-labeled *O*-methylisourea and PNGase-mediated ^{18}O-labeling were successful for large-scale analysis.

023

SELECTIVE SEPARATION OF GLYCOPEPTIDES AND GLYCOPROTEINS BY AMINOPHENYLBORONIC ACID-FUNCTIONALIZED MAGNETIC NANOPARTICLES

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The discovery and identification of glycosylated peptides and proteins and the analys i s of their glyco-structures are increasingly important in diagnosis and proteomics. In particular, missing, aberrant, or additional glycosylations are known to be linked to certain diseases and may be utilized as biomarkers for diagnosis and/or therapeutic monitoring and thus, the development of a sensitive and specific technique for their elucidation was required. Recently, magnetic beads have been used for the immobilization of protein because they provide a simple procedure of separating reacted protein from other reaction mixture using an external magnet.

Herein, a new type of magnetic nanoparticles with aminophenylboronic acid-functionalized were prepared by a facile synthesis a pproach, and were applied to selectively separate glycopeptides or glycoproteins in the presence of extra applied magnetic field . Importantly, the aminophenylboronic acid- functionalized magnetic nanoparticles were successfully applied in enrichment of glycoproteins or glycopeptides. The specificity of these magnetic nanoparticles was also evaluated by the capturing of different model glyco peptides or glycoproteins from mixtures containing non-glycomoleculars which were added as the interference. This developed method provides another efficient and convenient approach for analysis of glycoproteins.

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024

DEVELOPMENT OF MAGNETIC NANOPARTICLES AND ITS APPLICATION IN PHOSPHOPROTEOME OF LIVER

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Protein phosphorylation is one of the most important posttranslational modifications in mammalian cells. It regulates numerous biological processes, including cell proliferation, differentiation, metabolism, communication, and signal transduction.Global analysis of protein phosphorylation is very significant for exploring these critical processes. In this study, Fe3+- immobilized magnetic nanoparticles (Fe3+-IMAN) with an average diameter of 15 nm were synthesized and applied to enrich phosphopeptides. Compared with commercial microscale IMAC beads, Fe3+-IMAN has a larger surface area and better dispersibility in buffer solutions which improved the specific interaction with phosphopeptides. Using tryptic digests of the phosphoprotein α-casein as a

model sample, the number and signal-to-noise ratios of the phosphopeptides identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) following Fe3+-IMAN enrichment greatly increased relative to results obtained with direct MALDITOFMS analysis. The lowest detectable concentration is 5 x10^{-11} M for 100 μL of pure standard phosphopeptide (FLTEpYVATR) following Fe3+-IMAN enrichment. We presented a phosphopeptide enrichment scheme using simple Fe3+-IMAN and also a combined approach of strong cation exchange chromatography and Fe3+-IMAN for phosphoproteome analysis of the plasma membrane of mouse liver. In total, 217 unique phosphorylation sites corresponding to 158 phosphoproteins were identified by nano-LC-MS/MS. This efficient approach will be very useful in large-scale phosphoproteome analysis.

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025

FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) CLINICAL PROTEOME INITIATIVE OF LUNG CANCER

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IFFPE tissue samples have been routinely collected and stored in hospitals and are a huge untapped information resource on the progression of diseases as well as drug response and toxicity. FFPE tissues have clinical outcomes already known. Preparation and LC-MS analytical procedure utilizing FFPE samples could be standardized. We have conducted FFPE clinical proteomic analyses to assess key proteins characterizing types of lung cancers and/or disease progression. Significant protein candidates were screened and verified by using multiple reaction monitoring (MRM) mass spectrometric assays along the proof-of-concept. Details of FFPE clinical proteomic initiative will be presented and discussed with respect to sample recruitment, standardization of candidate discovery processes, quantitative comparison, target quantification, candidate verification, which had been bottlenecks in biomarker development.

026

CRYSTAL STRUCTURE OF THE HUMAN GRANULOCYTE COLONY STIMULATING FACTOR RECEPTOR SIGNALING COMPLEX

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Granulocyte colony-stimulating factor (GCSF) has become an important cytokine for medical treatment of patients suffering from granulopoenia through regulating the maturation, proliferation, and differentiation of the precursor cells of neutrophilic granulocytes. Binding of GCSF to the extracellular Ig-like and CRH domain of its receptor (GCSF-R) triggers receptor homodimerization, resulting in activation of JAK-STAT type signaling cascades. The stoichiometry of the GCSF/GCSF-R complex has been a matter of some debate, with various proposed values (1:1, 2:2 and/or 4:4). We have succeeded in preparation (1) and crystallization of 2:2 complex between human GCSF (hGCSF) and the Ig-like and CRH domains of human GCSF-R (hGCSF-R) (2) and determined its tertiary structure by X-ray crystallography at 2.8 angstrom resolution (3). The signaling 2:2 complex is formed via cross-over interactions between the Ig-like domain of hGCSF-R and the neighboring hGCSF, forming a two-fold axis of crystallographic symmetry. This conformation is quite different from that of the heterogeneous mGCSF-R complex, and more closely resembles the 2:2:2 active assembly of human interleukin-6 (IL-6), human IL-6alpha-receptor and human gp130 (which is a shared signal transducing receptor for several cytokines), and the 2:2 assembly of viral IL-6 and human gp130. The Ig-like domain cross-over structure necessary for GCSF-R activation is consistent with previously report ed thermodynamic and mutational analyses.

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027

CRYSTAL STRUCTURE OF THE HUMAN FOXO3A-DBD/DNA COMPLEX SUGGESTS THE EFFECTS OF POST-TRANSLATIONAL MODIFICATION

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FOXO3a is a transcription factor of the FOXO family. The FOXO proteins participate in multiple signaling pathways, and their transcriptional activity is regulated by several post-translational mechanisms, including phosphorylation, acetylation, and ubiquitination. Because these post-translational modification sites are located within the C-terminal basic region of the FOXO DNA-binding domain, it is possible that these post-translational modifications could alter the DNA-binding characteristics. To understand how FOXO-mediated transcriptional activity, we report here the 2.7 A crystal structure of the DNA-binding domain of FOXO3a (FOXO3a-DBD) bound to a 13-bp DNA duplex containing a FOXO consensus binding sequence (GTAAACA). Based on a unique structural feature in the C-terminal region and results from biochemical and mutational studies, our studies may explain how FOXO-DBD C-terminal phosphorylation by protein kinase B (PKB) or acetylation by cAMP-response element binding protein (CBP) can attenuate the DNA-binding activity and thereby reduce transcriptional activity of FOXO proteins. In addition, we demonstrate that the methyl groups of specific thymine bases within the consensus sequence are important for FOXO3a-DBD recognition of the consensus binding site.

028

STRUCTURAL ANALYSIS OF BASEPLATE WEDGE PROTEINS OF BACTERIOPHAGET4

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Gp10, gp11 and gp7 constitute part of the wedges, six of which surround the hub to form a baseplate. These proteins interact to form a precursor oligomer in the initial assembly process of the wedge. Recent 3D-image reconstruction of the baseplate before and after contraction ("hexagon" and "star", respectively) together with crystal structures of gp11 and part of gp10 has revealed that these proteins play a central role during the conformational change. In order to fully understand the mechanism of the structural change, we need to determine the whole structure of gp10 and gp7, but it has been difficult. As we have recently succeeded in isolating gp7, we applied limited proteolysis of gp10 and its complex with gp11 or gp7 by lysyl endopeptidase. Gp10 alone, which is a trimer, is cleaved at Lys289, but when complexed with gp11, gp10 is then cleaved at Lys194 where gp11 remained intact. Gp10 consists of four domains, I through IV, where gp11 binds to domain III. Based on the results of limited proteolysis, the region between His195 and Lys289 (95 residues) is likely to belong to domain III. On the other hand, when gp10 is complexed with gp7 and subjected to limited proteolysis, gp10 becomes resistant to the protease, although gp7 was cleaved as in the same way as is proteolysed alone. The results indicated that that the resulting N-terminal 279 residues of gp7 form a stable complex with gp10. Crystallization of the protease-resistant region of gp10-gp11 complex is under way.

029

DEVELOPMENT OF PROTEIN STRUCTURE DATABASES AND THEIR APPLICATIONS TO FUNCTIONAL ANNOTATION

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We manage the Protein Data Bank Japan (PDBj), curating, editing and distributing protein structural data, as a member of the worldwide Protein Data Bank (wwPDB) and currently process about 25-30% of all deposited data in the world [1]. Structural information is enhanced by the addition of biological and biochemical functional data as well as experimental details extracted from the literature and other databases. Several applications have been developed at PDBj for structural biology and biomedical studies [2, 3]: a Java-based molecular graphics viewer, *j*V; an extensive database of molecular surfaces for functional sites, *e*F-site, as well as a search service for similar molecular surfaces, *e*F-seek; identification of sequence and structural neighbours; a graphical user interface to all known protein folds with links to the above applications, Protein Globe. Recent examples are shown that highlight the utility of these tools in recognizing remote homologies between pairs of protein structures and in assigning putative biochemical functions to newly determined targets from structural genomics projects [4].

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030

MACPF PROTEINS – EUKARYOTE CYTOLYSINS IN DEFENCE AND ATTACK

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Proteins containing Membrane Attack Complex / Perforin (MACPF) domains play crucial roles in vertebrate immunity, embryonic development and neural cell migration. In vertebrates, C9 and perforin form oligomeric pores that lyse bacteria and kill virus-infected cells, respectively. However, the mechanism of MACPF function is unknown. We determined the X-ray crystal structure of a bacterial MACPF protein, Plu-MACPF from Photorhabdus luminescens, to 2.0 Å resolution. Remarkably, these data reveal that the MACPF domain is homologous to pore forming cholesterol-dependent cytolysins (CDCs) from Gram positive bacteria. This suggests that lytic MACPF proteins may use a CDC-like mechanism to form pores and disrupt cell membranes; namely oligomerisation followed by massive conformational change that leads to membrane penetration and pore formation. Sequence similarity between bacterial and vertebrate MACPF domains suggest that the fold of the CDCs, a family of proteins important for bacterial pathogenesis, is likely used by vertebrates for defence against infection. Furthermore, the potential involvement of MACPF proteins in processes such as trophoblast invasion and embryonic patterning events suggests that members of the CDC toxin family have been recruited for fundamental developmental processes in eukaryotes

031

DRIVING BIOLOGICAL DISCOVERY USING QUANTITATIVE MASS SPECTROMETRY

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A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. Several major technologies, but especially mass spectrometry, have benefited from large scale genome sequencing of organisms. The sequence data produced by these efforts can be used to interpret mass spectrometry data of proteins and thus enables rapid and large-scale analysis of protein data from experiments. Advances in multi-dimensional separations as well as mass spectrometry have improved the scale of experiments for protein identification. This has improved the analysis of protein complexes, and more complicated protein mixtures. Quantitative mass spectrometry can be used to study biological processes such as development or the effects of gene mutations on pathways. Metabolic labeling of whole organisms can now be readily accomplished using 15N labeled proteins as a food source. The use of this method in combination with Shotgun proteomics was used to study rat brain development (P1 to P45) and Daf2 and Daf16 knockouts (insulin signaling pathway) in *C. elegans*.

032

MAPPING THE HUMAN PROTEOME

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The completion of the human genome sequence has opened up a possibility for global expression profiling of human tissues and cells, allowing for comparative studies between normal and disease tissues. Using tissue microarrays, more than 2,800 human proteins and 1,8 million high-resolution images representing immunohistochemically stained tissues and cells were analyzed.

The study revealed several observations of general interest, i) few true tissue-specific proteins were found and no proteins were exclusively expressed in cancer cells, ii) normal cells can be subdivided into five major groups harmonizing well with the current concept of embryology and histology, iii) a majority of cancer types, with few exeptions, e.g. except hepatocellular carcinoma, malignant lymphoma, glioma and testicular cancer, cluster together, suggesting a common global protein expression pattern distinguishing neoplastic cells from their normal counterparts and iv) a comparison of cancer stratified according to histopathological criteria showed similar protein expression for any given cancer type regardless grade of malignancy.

When analyzing protein expression patterns in 52 different cell types, the overall results suggested that more than 50% of protein encoding genes are expressed at the protein level in a given cell and that only few proteins appear to be cell-type specific. Using immunohistochemistry, image analysis and hierarchical clustering, we found that cell lines cluster into 5 different groups corresponding to originating tumor types and that this pattern was also evident on the transcriptional level.

The systematic exploration of the human proteome within the Human Protein Atlas (HPA) (www.proteinatlas.org) has also provided new opportunities to identify various types of biomarkers with *in silico* based methods. A subset of antibodies showing selective immunoreactivity were selected for an extended analysis in tumors from defined patient cohorts. Specially designed TMA:s, including clinical data associated with the corresponding patients, have been produced. Each such TMA contains over 100 different

tumors from patients with a defined tumor type and an extended analysis has shown that for several potential biomarkers, protein expression levels correlates with various clinico-pathological parameters, including overall survival of respective patients. Using this strategy several new markers of potential clinical importance have been analyzed. One example includes a recently discovered transcription factor that was identified as a useful diagnostic marker, with 80% sensitivity and 95% specificity for colo-rectal carcinoma. When combined with an antibody recognizing keratin 20 sensitivity increased to 95%. In addition, the extended analysis showed that a group of patients with tumors lacking expression of this transcription factor had significantly poorer outcome.

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033

SURFACE PROFILING OF LEUKAEMIAS, LYMPHOMAS AND COLORECTAL CANCERS USING A CD ANTIBODY MICROARRAY

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A microarray containing 82 CD antibodies has been developed that provides an extensive immunophenotype or surface expression profile of suspensions of human cells. Live cells are captured by immobilized CD antibodies that are complementary to particular surface molecules on the cells providing a dot pattern that is the surface expression profile. Such profiles have been determined for a variety of leukaemias and lymphomas from the peripheral blood and bone marrow of 733 patients and 63 normal subjects (1). Discriminant Function Analysis of these expression profiles clustered leukaemia sub-types and showed high levels of consistency with diagnoses obtained using conventional clinical and pathological criteria. The overall levels of consensus were 93.9% (495/527 patients) for peripheral blood and 97.6% (201/206 patients) for bone marrow aspirates, showing that an extensive immunophenotype alone was frequently sufficient to classify the disease where the leukaemia was predominant. A technique of fluorescence multiplexing was developed for profiling minor cell populations in a suspension and was used to profile colorectal cancer (CRC) cells isolated from cancerous polyps (2). CRC cells captured on a microarray were detected by staining captured cells with soluble fluorescent CEA-Alexa647 and EpCAM-Alexa488 antibodies. CRC showed differential expression of CD66c, CD15s, CD55, CD45, CD71, CD45RO, CD11b and CEA. T-lymphocytes on the same microarrays were labelled with CD3-phycoerythrin antibody revealing the presence of activated tumour infiltrating lymphocytes (TILs). The TILs showed differential expression of HLA-DR, TCR α/β, CD49d, CD52, CD49e, CD5, CD95, CD28, CD38 and CD71, in descending order.

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034

ANTIBODY-BASED PROTEOMICS

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The Swedish Human Protein Atlas (HPA) program has been set up to allow for a systematic exploration of the human proteome using Antibody-Based Proteomics. This is accomplished by combining high-throughput generation of affinity-purified (mono-specific) antibodies with protein profiling in a multitude of tissues/celltypes assembled in tissue microarrays. A web-based annotation software has been developed to allow for a basic and rapid evaluation of immunoreactivity in tissues. Manual annotation of scanned images of tissue microarrays are performed on a virtual microscope over the internet by Indian pathologist annotators in Mumbai. Intensity, fraction of immunoreactive cells and subcellular localization is recorded for each given cell population. A text comment summarizing the characteristics for each antibody is added.

The results are visualized in a summary view as color codes corresponding to the protein expression level in each given cell type. In total 67 normal cell types from 144 individuals and 25 different cancer cell types from 216 different tumors are annotated for each antibody. All finished annotations are curated by an independent pathologist or specially trained personnel, to control for eventual mistakes and to ensure uniform annotations of high quality.

The annotation software has been progressively refined to yield optimal information on antibodies and minimize subjectivity amongst annotators. Experience and reproducibility of web-based annotation amongst pathologists is discussed.

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(2) Uhlen, M. et al. A human protein atlas for normal and cancer tissues based on antibody proteomics. Mol Cell Proteomics 4, 1920-32 (2005).

(3) Bjorling E. et al. A web-based tool for in silico biomarker discovery based on tissue-specific protein profiles in normal and cancer tissues. In press Mol Cell Proteomics Oct 2007

035

A HUMAN PROTEIN ATLAS

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The completion of the human genome sequence has opened up a possibility for global expression profiling of human tissues and cells, allowing for comparative studies between normal and disease tissues. A multi-disciplinary research program to create a "Human Proteome Resource" was started in July 2003. The aim of the program was to allow for systematic exploration of the human proteome using antibody-based tissue proteomics, combining high-throughput generation of mono-specific antibodies (affinity-purified) with protein profiling in human tissues and cells using tissue microarrays. Recombinant protein fragments selected from unique regions called Protein Epitope Signatures Tags (PrESTs) were used as immunogens to generate antibodies. Analysis of protein expression patterns was performed on tissue and cell microarrays containing >700 spots of normal and cancer tissues as well as *in vitro* cultured cells.

We have used this strategy to construct a comprehensive, antibody-based protein atlas for expression and localization profiles in 48 normal human tissues and 20 different cancers. The results are presented in a publicly available database containing images and data from protein profiling using over 3,000 antibodies. Each image has been manually annotated and curated by a certified pathologist to provide a knowledge base for functional studies and to allow searches and queries about protein profiles in normal and disease tissue. Our results suggest that it should be possible to extend this analysis to a majority of all human proteins thus providing a valuable tool for medical and biological research. We believe that the presented approach combining immunohistochemistry and tissue microarray technology can be used as an effective strategy to identify and evaluate novel markers, with potential clinical importance, of cell lineages and tumors.

(1) Uhlen, M. & Ponten, F. Antibody-based proteomics for human tissue profiling. Mol Cell Proteomics 4, 384-93 (2005)
(2) Uhlen, M. et al. A human protein atlas for normal and cancer tissues based on antibody proteomics. Mol Cell Proteomics 4, 1920-32 (2005).
(3) Bjorling E. et al. A web-based tool for in silico biomarker discovery based on tissue-specific protein profiles in normal and cancer tissues. In press Mol Cell Proteomics Oct 2007

036

CYS-TAG PROTEINS ON CHIPS FOR FUNCTIONAL PROTEOMICS

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Protein chip technology is essential for high through-put functional proteomics. We developed a novel protein tag consisting of five tandem cysteine repeats (Cys-tag) at C-terminus of proteins which was covalently immobilized to the surface of a maleimide-modified diamond-like carbon-coated silicon chip substrate. Model proteins were created as a Cys-tagged enhanced green fluorescent protein (EGFP) and an EGFP-stathmin fusion proteins which included an oligo-histidine tag at N-terminus to allow its purification from expressed proteins in *Escherichia coli* using Ni beads. The purified Cys-tagged proteins could be captured on the maleimide-coated chip substrate at efficiency that 50 pg of the fusion protein was detected by fluorescence and 5 pg (0.2 fmole) could be detected by combination with enhanced immunofluorescence. Nano-LC-MS/MS enabled us to identify protein candidates for binding partners of stathmin in mouse brain extracts on the chip. We examined the merits of this protein chip system to identify stathmin-binding partners in the comparison with conventional beads system.

037

CHARACTERIZING BINDING PROPERTIES OF PROTEIN INTERACTION DOMAIN

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A large proportion of protein-protein interactions are mediated by families of peptide-binding domains. Comprehensive characterization of each of these domains is critical for understanding the mechanisms and networks of protein interaction at the domain level. A systematic experimental strategy was developed for efficient binding property characterization of peptide-binding domains based on high throughput validation screening of a specialized candidate ligand library using yeast two-hybrid system. As for simple adaptor protein without any other known functional domains, the potential functions of the complex were predicted by functional annotations from a MILANO literature search and subcellular localizations. The ligands were considered more likely to be functionally associated if they had similar patterns of functions or closely related functions. For some functionally associated ligand

pairs, interaction with one ligand was found to be influenced by another ligand in a yeast three-hybrid system. Ideally protein-protein interactions should be studied with high throughput computational approaches first to screen protein sequence databases to direct the validating experiments toward the most promising peptides. An integrated machine learning systems was built using three novel coding methods and used to screen the Swiss-Prot and GenBank protein databases for potential ligands of SH3 and PDZ domains. A large fraction of predictions has already been experimentally confirmed by other independent research groups, indicating a satisfying generalization capability for future applications in identifying protein interactions.

038

UNRAVELLING THE MECHANISM OF DIHYDRODIPICOLINATE SYNTHASE: ARE THE ESSENTIAL ACTIVE SITE RESIDUES REALLY ESSENTIAL?

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Dihydrodipicolinate synthase (DHDPS, an important antibiotic target) is the enzyme that catalyses the first committed step in the lysine biosynthetic pathway, which involves the condensation reaction between (*S*)-aspartate β-semialdehyde ((*S*)-ASA) and pyruvate *via* a ping-pong mechanism, and is feedback inhibited by lysine. The major hallmark of this reaction is the formation of a Schiff base intermediate between pyruvate and the active site residue lysine 161. Surprisingly, this had never been confirmed using site-directed mutagenesis. To investigate the necessity of this residue, two site-directed mutants were generated: DHDPS-K161A and DHDPS-K161R. They were then over-expressed, purified and characterised by steady-state kinetics, circular dichroism (CD) spectroscopy, differential scanning fluorimetry (DSF), isothermal titration calorimetry (ITC), sodium borohydride reduction and X-ray crystallography. Unexpectedly, the mutant enzymes were still catalytically active, albeit with substantially impaired catalytic competency, underscoring the functional plasticity of enzyme active sites. These results are in contrast to findings in the structurally-related enzyme, N-acetyl neuraminate lyase (NAL) and hint at evolutionary relationships in the class I aldolase family.

039

DISCOVERY OF A NEW EXTRACELLULAR CHAPERONE

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Endostatin is an endogenous inhibitor of tumor angiogenesis and tumor growth. An unexpected finding was that intravenous injection of nonrefolded endostatin to mice resulted in tumor shrinkage. By studying the antitumor effect of nonrefolded endostatin *in vivo*, we have identified FI as an extracellular chaperone. FI forms large complexes with nonrefolded endostatin *in vitro*, and the antitumor activity of nonrefolded endostatin was substantially impaired in FI-deficient mice. Moreover, FI specifically binds to denatured, but not native citrate synthase, and inhibits its thermal aggregation and inactivation in an ATP-independent manner. Furthermore, FI inhibits fibril formation of yeast prion protein Sup35 (NM). The existence of extracellular chaperones remains largely unexplored. Our studies demonstrate that FI is an extracellular chaperone, which not only provides new insights into the extracellular protein quality control system, but also suggests potential diagnostic and therapeutic approaches to FI-related pathological conditions.

040

MODULATION OF PROTEIN ACTIVITIES VIA HOMO-OLIGOMERIZATION: A PHENOMENON THAT HAS BEEN UNDERAPPRECIATED

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Although many proteins have been found to exist as homooligomers in nature, t he biological significance and mechanism for its occurring is far from clear. We have examined a variety of proteins that exhibit homooligomerization and revealed that such a process of reversible protein-protein interaction can be utilized to effectively modulate the biological activities of proteins in responding to fluctuations of environmental conditions. A general mechanism for the disassembly and reassembly of such oligomeric proteins, needed for the transformation of their oligomeric states from one to another, appears to occur as such that the disassembly process occurs in a stepwise manner, while the reassembly occurs in a non-stepwise manner. The significance of the protein homo-oligomerization, an underappreciated phenomenon in the field of protein-protein interactions, needs to be reappraised.

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041

DISCOVERY OF INHIBITORS AGAINST 3C PROTEASES OF SARS CORONAVIRUS, ENTEROVIRUSES 71, AND COXSACKIEVIRUS B3

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A chymotrypsin-like protease (called 3C protease) found in piconaviruses is responsible for processing the poly-proteins translated from RNA genomes into functional enzymes and structural proteins essential for viral replication, so it represents a good anti-viral drug target. Enterovirus 71 and coxsackievirus B3 are piconaviruses, which cause hand, foot, and mouse diseases in human, and meningitis and mycocarditis leading to heart failure in young adults and congestive heart failure, respectively. In late 2002, an emerging infectious disease caused by a novel human coronavirus induced severe acute respiratory syndrome (SARS). It rapidly spread from its origin in southern China to more than 25 countries in 2003, affecting almost 8000 patients resulting in about 800 fatalities, a high mortality rate. The SARS virus also requires a 3C-like protease in the life cycle. We have identified several groups of inhibitors against the 3C-like and 3C proteases through high throughput screening and rational drug design. Some inhibitors are common for the two types of proteases, although they share no sequence homology.

042

MALDI MASS IMAGING MASS SPECTROMETRY OF TISSUE SECTIONS: STATE OF THE ART AND FUTURE DIRECTIONS

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MALDI imaging mass spectrometry (IMS) is a technology that allows to map the molecular content of tissue sections in direct correlation with the underlying histology. In the past decade, numerous different methodologies have been optimized and automated to analyze a wide range of endogenous compounds such as lipids, peptides and proteins as well as administered pharmaceuticals and their metabolites. IMS has been used to study many different biological systems ranging from normal organ development to the detection and understanding of diseases. IMS was initially conceived for and performed on MALDI TOF instruments. Today numerous other MALDI based platforms such as Q-TOF, ion traps, FT-MS and ion mobility MS are used depending on the analytical tasks demanded. Software's for data acquisition, data processing, image reconstruction and statistical analyses are now available.

One of the most recent advances is the development of methodologies to investigate the proteomic content of tissue specimens preserved by formalin fixation followed by paraffin embedding (FFPE). In this case, proteins (and other endogenous molecules) are cross linked and direct analyses become impossible. An alternative fully automated approach was developed by digesting proteins in situ using various endoproteases such as trypsin. After matrix deposition, the generated peptides are imaged. Peptides showing strong correlations with histology can be sequenced by MALDI MS/MS and the corresponding proteins identified. We have used this approach to probe the proteomic content of hundred of non-small cell lung cancer biopsie punches arrayed in FFPE tissue blocks. Numerous patterns of tryptic peptides were found that differentiate normal and cancerous biopsies and subclassify the various forms of non-small cell lung cancer. Establishment of such IMS methodologies allows the retrospective analyses of the millions of clinical samples existing world wide in tissue banks to validate disease specific biomarkers.

043

METAL-IMAGING MASS SPECTROMETRY (MIMS): A NEW IMAGING MASS SPECTROMETRY TECHNOLOGY TO DETERMINE THE DISTRIBUTION OF METAL IONS IN TISSUE SAMPLES

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The study of metals and their interactions with proteins is a new area of research broadly known as metalloproteomics. The study of metalloproteomics is generating significant interest as there is increasing evidence that accumulation or depletion of metals play a role in the development of many disorders, including Alzheimer's and Parkinson's disease.

This presentation demonstrates the application of Metal Imaging Mass Spectrometry (MIMS) for the determination of the distribution of trace metals in tissue samples. MIMS employs laser ablation inductively coupled mass spectrometry (LA-ICP-MS) to construct images expressed as trace elements.

As a model system, we imaged the brains of rats lesioned in one hemisphere with 6-hydroxydopamine to induce Parkinson's disease. The un-lesioned hemisphere was used as a control. 10 μ m thick transverse sections containing either the substantia nigra or the striatum were ablated and the relative elemental content determined. Contour maps were constructed that detailed relative isotopic concentrations across the entire section. The resolution of the images was 40 μ m per pixel.

A decrease in zinc concentration and a corresponding increase in copper concentration around the site of the lesion were observed. Increased levels of phosphorus were also noted in the lesioned hemisphere.

Current developments of MIMS such as methods for quantification and application to other types of diseases where metals are suspected of involvement will also be discussed.

044

PHOTOGRAPHS AND MEMORIES: AS SNAPSHOT OF CULTURED ENDOCRINE CELLS

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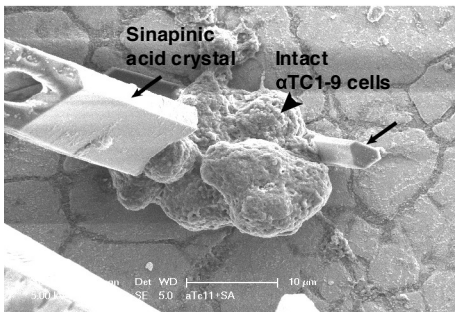
The application of intact-cell mass spectrometry (ICM) by MALDI-TOF mass spectrometry to achieve direct protein-profiling of bacterial species is now well established. However this methodology has not to our knowledge been applied to the analysis of mammalian cells in routine culture. Here, we describe a novel application of ICM by which we have identified proteins in intact cells from two lines representative of pancreatic islet α and β cells. Adherent αtc1 clone 9 and βTC6 F7 cells were harvested into PBS using enzyme-free dissociation buffer before 1 μL of cell suspension was spotted onto MALDI plates. Cells were overlaid with sinapinic acid then washed with pure water before application of a final coat of sinapinic acid. Data in the 2,000-20,000 m/z range were acquired in linear mode on a Voyager DE-Pro mass spectrometer [1] .

We found that minimal sample processing provided the best results, and that the method preferentially detected peptide secretory products, possibly because of their abundance and/or size, or perhaps due to their concentration in discrete secretory vesicles near the cell surface. The ease of use coupled with the rapid and direct nature of this analytical system, indicate its potential in a number of possible cell biology applications including: the monitoring of differentiation/de-differentiation of hormone-secreting cell-lines; detection of possible contamination of primary cells with other cell-types; comparisons between “normal” cultured cells and cancer or disease cell-models; as well as providing a rapid and informative method for the profiling of clones and subclones.

β cell hormones	**Expected *m/z***	**Measured *m/z***	*%Error*
Proinsulin II	9491.8	9494.5	0.03
Insulin II	5797.7	5797.8	0.00
Insulin I	5804.7	5803.2	-0.03
C-peptide II	3122.4	3122.3	0.00
C-peptide I	3134.4	3134.2	-0.01
Amylin	3922.4	3921.8	-0.02
Preptin	3949.4	3949.8	0.01
α cell hormones	**Expected *m/z***	**Measured *m/z***	**%Error**
Proglucagon	18626	Not detected	NA
Glucagon	3483.2	3483.2	0.00
GLP-1	4169.9	4170.0	0.00
Oxyntomodulin	4450.5	4450.5	0.00
GRPP	3441.5	3441.2	-0.01
MPF	9946.9	9948.7	0.02



Abbreviations: GLP, glucagon-like peptide; GRPP, glicentin-related polypeptide; ICM, intact-cell mass spectrometrry; MALDI, matrix-assisted laser-desorption/ionisation; MPF, major proglucagon fragment; MS, mass spectrometry; PBS, phosphate-buffered saline.

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045

MAPPING NOVEL COPPER-REGULATED SIGNALLING PATHWAYS USING ANTIBODY ARRAYS AND IN SILICO PROTEIN NETWORK ANALYSIS.

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Neurodegenerative illnesses such as Alzheimer's disease (AD), Parkinson's disease and prion diseases are characterized by aberrant biometal metabolism. This can include either deficiency or accumulation of biometals such as copper (Cu), zinc (Zn) and iron (Fe) in neuronal and other cell-types. However, little is known about the metabolic effects associated with altered metal homeostasis in the brain. Past attempts to investigate the effects of aberrant metal homeostasis on protein turnover and signalling have been hampered by the complex nature of metal-regulated protein metabolism. Recent advances in proteomic tools have helped to overcome this problem. In this study, we have used antibody microarray analysis of cells with altered cellular Cu levels to identify how protein metabolism and cell signaling is modulated by Cu. We examined epithelial or neuronal cells after metal levels were altered by genetic or chemical modulation of Cu homeostasis. Altered metal levels were confirmed by ICP-MS. Cell cultures were then analyzed using the Clontech Protein (Antibody) Microarray 500. The data were then further analyzed using the software program Pathway Studio^{TM} (Ariadne Genomics) to identify protein interaction networks. Many of the protein changes have been validated by Western blot analysis. Using this approach, we found that altered intracellular Cu levels induced substantial changes in cell cycle activity (including changes to p53, D-type cyclins and retinoblastoma protein), DNA repair and maintenance proteins (Ku proteins), and cell signaling mechanisms including epidermal growth factor receptor activity, phosphopinositol-3-kinase, glycogen synthase kinase 3β and mitogen activated protein (MAP) kinases. These Cu-induced changes in protein metabolism and signaling affect cell survival, cell cycle progression and modulate AD-related neuropathological features such as amyloid beta accumulation and tau phosphorylation. Our results demonstrate the power of antibody microarrays for the rapid multiplex analysis of metal-regulated protein metabolism and identified novel protein changes that could underlie the mechanism of disease pathology in AD or other neurodegenerative disorders.

046

USING ION MOBILITY/TIME-OF-FLIGHT MASS SPECTROMETRY TO DETERMINE CONFORMATIONAL PROPERTIES OF PROTEINS

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™We are interested in studying conformation changes of proteins following interactions with small molecules (metals, drugs), peptides and other proteins. Here we are evaluating the use of a Waters Synapt™ HDMS™ system (Manchester, UK) which combines traveling wave (T-wave) ion mobility separations and Time-of-Flight (ToF) mass measurements to probe the dynamics of protein structure. In this study the ability of the T-wave based ion mobility measurements to detect flexibility in protein structure and conformational changes induced by ligand and post-translational modifications will be assessed. Preliminary results have looked at the well studied protein system, calmodulin. Different Ca^{2+}-bound states have been compared together with the interaction of an antipsychotic drug which collapses protein structure on binding to EF-hand motifs. Two different conformations are observed and following binding of the drug a large shift in arrival time occurs, indicating a major structural change. In addition studies, conformational affects of ligand and post-translational modifications to a reductase and kinase respectively have been examined. Finally, the Synapt HDMS system was used to evaluate the flexibility of different protein constructs differing slightly in length and amino acid composition. Correlations between conformational heterogeneity and crystallization were identified and will be discussed.

047

DIRECT OBSERVATION OF YEAST PRION DYNAMICS IN SINGLE-LIVING CELLS

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Prions are propagating proteins that form ordered protein aggregates, in which phenotypic trait is retained in the altered protein conformers. Originally developed as the protein-only transmissible agent to explain mammalian neurodegenerative diseases, the prion concept has been extended to several non-Mendelian genetic elements in yeast Saccharomyces cerevisiae, such as [PSI+] and [URE3].
Since prions are transmissible, they inherently replicate themselves in order to propagate the transmissible entities, and then transmit to the daughter cells. To understand the dynamics of yeast prion aggregates in living cells, we directly monitored the fate of the aggregates using an on-chip single-cell cultivation system in conjunction with fluorescence correlation spectroscopy (FCS), a technique that allows determination of the diffusion times of fluorescent molecules even in living cells. Single-cell imaging revealed that the visible foci of yeast prion Sup35 fused with GFP are dispersed throughout the cytoplasm during cell growth, but retain the prion phenotype. Fluorescent correlation spectroscopy, which showed that [PSI+] cells, irrespective of the presence of foci, contain diffuse oligomers, which are transmitted to their daughter cells. We concluded that these diffuse oligomers are critical for the prion transmission.

Since FCS is an ensemble method for calculating the diffusion properties of fluorescent molecules, it cannot be used to track the behavior of individual prion molecules. To overcome this limitation, we are extending our study of the dynamic behavior of single prion proteins by using quantum dot (Q-dot) technology. Q-dots are fluorescent semiconductor nanocrystals that have several advantages over conventional organic fluorescent dyes. We chemically labelled recombinant Sup35 proteins with Q-dots, and incorporated the Q-dot labelled Sup35 (Sup35-Qdot) into living yeast cells. We succeeded in tracking the trajectories of single Sup35-Qdots and analyzed their diffusion properties.

048

RAPID PROTEIN STRUCTURE DETERMINATION USING DISTRIBUTED COMPUTING

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Structural biology research places significant demands upon high-performance computing. The elucidation of protein structures at atomic resolution is computationally demanding and requires user-friendly interfaces to high-performance computing resources. Fortunately, critical calculations are embarrassingly parallel and thus ideally suited to distributed computing. Here we discuss how we are using grid computing to determine the three dimensional structures of proteins using the technique of Molecular Replacement in a massively parallel fashion, in a timeframe of hours to days - orders of magnitude faster than is currently possible.

049

Abstract unavailable at time of print

050

PROTEOMIC IMAGING OF ENDOTHELIUM AND CAVEOLAE FOR TARGETED PENETRATION INTO SINGLE ORGANS AND SOLID TUMORS

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Cancer and other disease biomarkers and targets may provide key diagnostic, prognostic and therapeutic opportunities including clinical trial surrogates and screens for patient treatment assignment. Blood and tissues are complex with extensive molecular diversity over a wide concentration range that creates discovery challenges for proteomic analysis. Candidate biomarkers require strict validation that is laborious and expensive. Noninvasive imaging provides objective validation for diagnostic and therapeutic targets in vivo. Drugs, gene vectors, and nanoparticles may benefit greatly from improved in vivo delivery through homing to specific disease biomarkers. Yet in vivo barriers limit access to most disease targets including cancer. Our evolving discovery and validation strategies that are intended to overcome these hurdles will be discussed. We have developed novel systems biology approaches that integrate nanotechnology-based subcellular fractionation, quantitative organellar & subtractive proteomics, bioinformatic interrogation, antibody generation, expression profiling, and various in vivo imaging modalities to quickly identify and validate candidates for pre-clinical and clinical testing. Analysis of rodent and human tumor samples have been compared to focus on clinical meaningful targets and to understand model relevance to human disease. Tissue and tumor microenvironmental influences on endothelial cell expression are extensive. We have developed quantitative proteomic analysis using a new spectral intensity index to identify 300+proteins concentrated in caveolae; many of which are confirmed by immuno-electron microscopy. Bioinformatic analysis of this subproteome ascribes new functional attributes to the caveolae. Examples of new targets will be presented along with validation studies using antibodies and molecular imaging to demonstrate specific targeting in vivo. Novel targets in caveolae enable antibodies to penetrate deep into solid tumors and single organs and were utilized to improve tissue-specific imaging and treatment. Our recent findings reveal that caveolae not only express tissue-specific proteins but also function to rapidly and actively pump specifically targeted antibodies and nanoparticles across the endothelial cell barrier and into the tissue interstitium. Such pervasive access improves the efficacy of radioimmunotherapy in destroying both stromal and tumor cells and in treating a wide variety of solid tumors. Lastly, functional knockout of newly identified tumor-induced endothelial proteins can greatly affect tumor growth, metastasis, and angiogenesis. These studies illustrate the benefit and power of focused organellar proteomic mapping in vivo. This type of “proteomic imaging of the vascular biomarker space including its in vivo accessible target space and transvascular pumping space” may bode well for application to cancer and many other diseases.

051

ESTABLISHMENT OF A SECRETOME DATABASE OF PRIMARY AND CULTURED CELLS FOR BIOMARKER DISCOVERY

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Large scale analyses of serum samples derived from healthy donors and diseased patients by highly sensitive mass spectrometry are performed in order to identify potential biomarkers. Any such protein, however, is eventually a result of protein synthesis followed by secretion and/or release by cells, which were altered in a fashion characteristic for the corresponding disease. Another approach, alternative to serum proteomics, is the direct analysis of proteins secreted by cells in vitro. Searching for proteins specific for cell types at distinct functional states, we investigated the secretomes of primary and cultured human cells in a systematic fashion. Compared to tissue proteomics, a several million-fold enrichment of low abundant proteins was realized by the isolation of specific cell types and the isolation of the secreted protein fraction. We found that cell-type and functional differences of proteome profiles are much more pronounced in case of secreted proteins compared to cytoplasmic proteins. Here, data of immature and mature human dendritic cells, normal, angiogenic and inflammatory stimulated primary endothelial cells will be presented in detail. To confirm the biological relevance of the recorded secretomes of primary cells, we investigate cell model systems mimicking the conditions of primary cells at pathologic conditions. We suggest that the systematic investigation of secreted proteins derived from a broad variety of cultured and primary cells, which were isolated from healthy and diseased tissues, may serve as valuable basis for biomarker discovery.

052

INSIGHT INTO STRUCTURE AND DYNAMICS FROM WEAK ALIGNMENT NMR

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Spectral simplicity of solution NMR spectra results from the Brownian rotational diffusion of solutes, which rapidly averages the strong dipolar interactions between different spins to exactly zero. Much valuable structural information, contained in these dipolar interactions, is lost in this averaging process. It has long been known that alignment of solutes in a magnetically oriented liquid crystalline medium restores the dipolar interactions, albeit at the cost of dramatically increased spectral complexity, limiting this approach to only very simple systems. However, by decreasing the degree of solute alignment, it is possible to retain the valuable structural information contained in the dipolar couplings, without considerably increasing spectral complexity.

With the rapidly increasing number of previously solved macromolecular structures, the alignment approach can take advantage of this structural database by revealing which fragments are compatible with experimental dipolar couplings. This approach can provide considerable shortcuts in macromolecular structural studies, while providing a very sensitive measure to identify subtle structural changes.

A second, complementary approach that takes advantage of the increasing database of proteins whose structures and chemical shifts are known has allowed the development of improved empirical relations between chemical shift and local structure. This information can readily be used to guide the powerful ROSETTA structure prediction program, extending its use to routine structure determination of proteins up to *ca* 15 kD. Small extensions to this approach aim to further expand this size limit.

053

QUANTITATIVE ANALYSIS OF HUMAN PLASMA PROTEOME BY MASS SPECTROMETRY FOR CANCER BIOMARKER DISCOVERY

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Because of the high complexity of human plasma, it is normally hard to detect secreted proteins to discover the useful cancer biomarkers. To discover candidate disease markers in the human plasma, therefore, is challenging in proteomics. We used pooled plasma sample of normal and cancer patients to statistically profile peptide patterns from the plasma proteins by mass spectrometry (MS). From the peptide pattern profiling with quantitative MS analysis, we discovered the group of peptides from glycoproteins, each of which showed quite different quantity from other peptides belonging to the same glycoproteins. For validating the targeted peptides from different plasma samples, multiple reaction monitoring from linear ion trap with Fourier Transform MS was tried and also run by principal component analysis. From this method, we clearly classified the candidate peptides from normal and cancer group for cancer biomarker discovery.

054

QUANTITATIVE PHOSPHOPROTEOMICS REVEALS A PATHWAY OF MRNA REGULATION DOWNSTREAM OF AKT

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The stimulation of cells with insulin has many physiological effects such as increased glucose uptake in muscle and the inhibition of apoptosis. The kinase Akt and other AGC kinase family members have been shown to be key mediators of insulin signalling through phosphorylation of their downstream substrates. The pS/pT binding protein 14-3-3 has a recognition motif very similar to that of AGC family kinases. In this study we have used 14-3-3 proteins as an affinity purification tool to specifically isolate the substrates of AGC family kinases. Stable isotope labelling with amino acids in cell culture (SILAC) was used to quantify changes in 14-3-3 binding and hence phosphorylation by mass spectrometry. These data lead us to identify and characterise a number of novel insulin-regulated phosphoproteins. One of theses novel insulin-responsive proteins was Edc3 (enhancer of mRNA decapping 3). Edc3 forms a complex with a number of other proteins to regulate mRNA decapping and degradation. In addition, Edc3 functions with microRNAs to mediate translation repression of microRNA targets. These two functions of Edc3 are thought to occur in cytoplasmic processing bodies (P-bodies) which cannot form without the presence of Edc3. We have shown that Edc3 binds to 14-3-3 in a direct and insulin responsive manner at pS161 and this process is Akt dependent. We hypothesise that phosphorylation of Edc3 and subsequent 14-3-3 binding regulates the effects of P-bodies on mRNA stability or translation repression. Mice homozygous for an Edc3 genetrap that only express a truncated form of Edc3 show a marked growth defect. This defect is most evident in the white adipose tissue and the skeletal muscle, two of the most insulin-responsive tissues. Microarray analysis of these animals has revealed the over-expression of ID2 (inhibitor of differentiation 2) in both of these tissues of genetrapped animals compared to wildtype. These data may indicate that Edc3 regulates ID2 expression and that this regulation is important for differentiation of muscle and fat tissue.

055

PROTEIN EXPRESSION EXPERIMENTS USING ITRAQ™: A UNIFIED PROTOCOL FOR DESIGN AND ANALYSIS?

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Quantitative proteomics addresses questions beyond identification and incorporates questions of differential expression. Technological advances, such as isobaric labelling reagents (iTRAQ™), enable the simultaneous analysis of up to eight protein complexes in a single MuDPIT run. This goes some way towards addressing the large variability in protein expression measurements from different runs of the mass spectrometer on the same protein sample. Our extensive experience using the iTRAQ technology, however, shows us that challenges still remain in experimental design and data analysis.

Parallels can be drawn between iTRAQ™ and two-colour cDNA microarray experiments, with some of the experimental design and data analysis lessons learned from microarrays naturally carrying over to iTRAQ. However, unique challenges remain.

I will explore the type of data that is produced by LC-MS/MS experiments using iTRAQ technology and will address how experimental design can be used to control for run-to-run variation, as well as potential differences in labelling efficiencies of the different iTRAQ reagents. Finally, I will illustrate a workflow for the statistical analysis of data from an illustrative iTRAQ experiment, outlining the development of a new freeware suite we are calling iTRAQAnalyser.

056

COMPARISON OF STABLE-ISOTOPE LABELLING STRATEGIES FOR QUANTIFICATION OF PHOSPHOSITE OCCUPANCY AND DIFFERENTIATION BETWEEN PHOSPHORYLATION AND SULFONATION OF THE MURINE DIOXIN RECEPTOR

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Post-translational modifications provide functional switches and docking points within cellular protein networks. Functionally important post-translational modifications may be transient in nature to respond dynamically to signals requiring pathways to be rapidly up- or down-regulated. It is essential to be able to detect such modifications and to monitor them quantitatively in a dynamic fashion in order to assess their functional and regulatory significance to protein networks.

The Dioxin Receptor (DR) is a signal-activated transcription factor that appears to be regulated by a variety of post-translational modifications. For instance, we have identified post-translational methylation, phosphorylation and sulfonation of DR in its unactivated state. However, it will be necessary to be able to quantitatively determine if changes in these modifications occur upon stimulation of this transcription factor in order to determine their significance and mechanistic roles.

Accordingly, we have performed a comparison of label-free and stable-isotope labelling methods in conjunction with MALDI-TOF/TOF-MS/MS and ESI-LTQ-Orbitrap-analyses for the purpose of quantifying the post-translational modifications of DR.

This presentation will describe the characterisation of modifications of the murine DR in its latent state. Relative quantification of phosphorylation and differentiation between phosphorylation and sulfonation on DR and discovery of phosphorylation sites on viral proteins with the aid of stable-isotope labelling will also be presented.

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Abstract unavailable at time of print

058

DIRECT OBSERVATION OF AMYLOID FIBRIL FORMATION OF B 2-MICROGLOBULIN AND AMYLOID B PEPTIDE

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Amyloid fibrils form through nucleation and growth. To clarify the mechanism involved, direct observations of both processes are important (1, 2). First, we developed a new technique for the direct observation of amyloid fibrils using total internal reflection fluorescence microscopy (TIRFM) combined with thioflavin T (ThT) fluorescence. Fibril growth of b2-microglobulin (b2-m) and amyloid b peptide was visualized in real-time at the single fibril level revealing various dramatic images. With amyloid b peptide, we succeeded in observing the formation of the senile plaque-like spherulitic structures with diameters of around 15 m m on the chemically modified quartz surface, suggesting the underlying physicochemical mechanism of senile plaque formation. Second, using atomic force microscopy, ultrasonication-induced formation of b 2-m fibrils was shown, indicating that ultrasonication is useful to accelerate the nucleation process. Third, with the proteolytic fragment of b 2-m, propagation and a transformation of fibril morphology was demonstrated. These direct observations indicate that template-dependent growth and structural diversity are key factors determining the structure and function of amyloid fibrils. The idea can be represented by a cubic puzzle consisting of 27 small cubes, whose folding and misfolding mimic protein folding and amyloid fibril formation, respectively.

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059

A TOY MODEL FOR PREDICTING THE RATE OF AMYLOID FORMATION FROM UNFOLDED PROTEIN

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We describe a toy model for predicting the rate of amyloid formation from an unfolded polypeptide. The model assumes irreversible amyloid growth, employs a collision encounter scheme and uses a Gaussian chain approximation to describe the polypeptide sequence. A principal feature of the model is its dependence on a number of key sequence residues whose correct placement, geometric arrangement and orientation in relation to their interacting partners define the success, or otherwise, of the amyloid formation reaction. Although not realistic at the molecular level, the model captures some essential features of the system and is therefore useful from a heuristic standpoint. For the case of amyloid formation from an unstructured state, the model suggests that the major determinants of the rate of fibril formation are the length of the sequence separating the critical amino acids promoting amyloid formation and the positional placement of the critical residues within the sequence. Our findings suggest also that the sequence distance between the key interacting amino acid residues may play a role in defining the maximum width of a fibril and that the addition of non-interacting segments of long structure-less polypeptide chain to an amyloidogenic peptide may act to inhibit fibril formation. We discuss these findings with reference to the placement of critical sequence residues within the polypeptide chain, the design of polypeptides with lower amyloid formation propensities and the development of aggregation inhibitors as potential therapeutics for protein depositional disorders.

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060

ORDER, DISORDER AND FIBRIL FORMATION IN THE MALARIA VACCINE CANDIDATE MSP2

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Merozoite surface protein 2 (MSP2) is synthesized by asexual blood stages of the human malaria parasite *Plasmodium falciparum* as a GPI-anchored protein. It has been implicated in erythrocyte invasion and is being developed as a vaccine candidate. NMR, light scattering, CD and sedimentation velocity measurements all show that recombinant MSP2 is disordered in solution and adopts an extended conformation. NMR has also been used to examine peptides corresponding to sequences in the conserved N-terminal region of MSP2. A 25-residue peptide corresponding to the entire N-terminal region contains nascent helical and turn-like structures (1). An 8-residue peptide from the centre of the N-terminal domain also formed a turn-like structure (2). Both peptides formed fibrils that were similar to the amyloid-like fibrils formed by full-length MSP2. It appears that this N-terminal conserved region of MSP2 plays a key role in fibril formation. Mutational analyses are being pursued to understand the role of this region in structure and fibril formation (1).
NMR resonance assignments have been obtained for full-length MSP2, allowing the residual secondary structure and backbone dynamics to be defined (3). There is some motional restriction in the conserved C-terminal region in the vicinity of an intramolecular disulfide bond. Two other regions show motional restrictions, both of which display helical structure propensities. One of these helical regions is within the conserved N-terminal domain, which adopts essentially the same conformation in full-length MSP2 as in corresponding peptide fragments. We see no evidence of long-range interactions in the full-length protein. MSP2 associates with lipid micelles, through the N-terminal region rather than the C-terminus, which is GPI-anchored to the parasite membrane. The N-terminal region could thus interact with the merozoite membrane or with the red blood cell. Various studies are being pursued to develop a model of MSP2 on the merozoite surface and understand its antigenic properties.

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061

STRUCTURAL BIOLOGY OF ALZHEIMER'S DISEASE

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Cognitive decline most commonly associated with Alzheimer's dementia can also result from other conditions including cerebral ischemia or brain trauma. One quarter of people over the age of 65 are estimated to suffer some form of cognitive impairment underscoring the need for effective classes of cognitive-enhancing agents. I will present work on two proteins, amyloid precursor protein and insulin-regulated aminopeptidase, that are promising targets for the development of anti-Alzheimer's drugs and as cognitive enhancers. In both cases structures determined by X-ray crystallography are being used to discover promising lead compounds by structure-based drug design.

062

NEGATIVE DESIGN PRINCIPLE TO AVOID THE FORMATION OF MISFOLDED AGGREGATES AS REVEALED BY B-LACTOGLOBULIN

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Biological systems have to avoid the formation of potentially harmful misfolded aggregates such as “amyloid fibrils”, which are associated with various diseases such as Alzheimer's disease, prion disease, and type II diabetes, etc. Importantly, recent analysis indicates that even the proteins or artificially produced peptides, of which the involvement into any disease are not established, can also form fibrillar aggregates in vitro by carefully choosing conditions. Thus, the formation of amyloid fibrils can be a generic property of polypeptide chains. This, in the other words, suggests that any proteins in cells potentially become causatives of cellular malfunctions.

We have been studying on the β-lactoglobulin which is a model system showing the formation of amyloid-like fibrils, in vitro. By analysing the amyloidogenic abilities of peptide fragments and the propensities of these fibrils formed by the peptides to promote the fibril formation by whole protein in a manner of cross-seeding effect, we found that the regions having high intrinsic amyloidogenic propensities are not necessarily involved in the fibril core of whole protein. This has been rationalised by the stabilisation of native dimer formation and the presence of disulfide bonds that restrict the exposure of the sequences with high amyloidogenic propensities to solvent. Indeed, the removal of such disulfide bonds could enable the fibrils by all the β-lactoglobulin peptides to accelerate the formation of fibrils by whole protein. Thus, the data provided the insight into the negative design principle that should be present to efficiently decelarate the formation of amyloid aggregates by globular proteins.

063

2-D DIGE PROFILING OF HEPATOCELLULAR CARCINOMA TISSUES IDENTIFIED ISOFORMS OF FAR UPSTREAM BINDING PROTEIN (FUBP) AS NOVEL CANDIDATES IN LIVER CARCINOGENESIS

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Hepatocellular carcinoma (HCC) is a major cause of cancer worldwide and is often characterized by aggressive tumour behavior and poor prognosis. One of the major etiologies is hepatitis B or C virus infections. In order to better comprehend the molecular mechanisms involved in HCC progression, we performed a systematic analysis on moderately- and poorly-differentiated human HCC tissues using 2-dimensional difference gel electrophoresis (2-D DIGE) coupled to MALDI-TOF/TOF MS. A total of 52 and 26 proteins were found to be dysregulated in moderately- and poorly-differentiated HCC tissues respectively. For the first time, the over-expression of a novel protein family, far upstream binding proteins (FUBPs) was identified in both stages of HCC. These were further confirmed by western blots and MRM Initiated Detection And Sequencing (MIDAS) approach. FUBPs are of particular interest due to their transcriptional activity on the oncogene, c-myc. It has generally been accepted that c-myc plays an important role in HCC progression but its exact activators remains poorly understood. Interestingly, we also observed elevated c-myc levels in the tissues used in this study by western blot analysis. We therefore propose that the FUBP family of proteins may be one of the possible upstream players that are involved in modulating the c-myc levels in HCC tumorigenesis.

064

ENHANCE PROTEOMIC DETECTION LIMITATION BY COMBINATORIAL PEPTIDE AND NUCLEOTIDE LIBRARY

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In most biological sample, the few high abundant proteins are usually composed to 95% of overall content. Although there are many methodologies for depletion of high abundant protein, unfortunately many defects still occur. For example, the immunodepletion method by antibody also removes other low abundant proteins that bind the depleted species simultaneously. Here, we provide a new method to reduce the dynamic range of complex biological samples by aptamer and peptide library. The heptapeptide library is composed of 7 random amino acids, and the aptamer library is also composed of 25 random sequences. Both libraries have sufficient diversity to match a ligand to every protein in complex biological sample. Therefore, according to the saturation-overloading principle, an abundant protein will saturate all its available ligands and leave the majority of the same protein unbound. After binding the library, the concentration range of complex sample will decrease and the low abundant proteins will be enriched by the library on the beads. In 1D and 2D PAGE experiments, we show that both peptide and aptamer libraries can remove high abundant protein, and consequently enrich low abundant protein. Following by the analysis of LTQ-Orbitrap mass spectrometry in the future, we may identify more low abundant proteins by the combinatorial peptide and nucleotide library depletion technology.

066

IDENTIFICATION AND VALIDATION OF OVARIAN CANCER-ASSOCIATED PROTEINS

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The proteomic analysis of plasma and tissues in patients has been a major approach to determining biomarkers essential for early disease diagnoses and drug discoveries. Recently, we detected and identified 40 proteins associated wirh ovarian clear cell carcinoma by two-dimensional difference gel electrophoresis and tandem mass spectrometry (MS/MS), and also isobaric tag for relative and quantitative analysis (iTRAQ) and MS/MS. Among the identified proteins, the expression of several proteins such as annexin IV was validated. In most cases, the expression is regulated at transcriptional level in the cancer cells. We found that the annexin IV gene has a transcription regulatory region containing a similar sequence to the NF-κB binding motif. When the expression of the genes encoding these proteins was suppressed with the siRNAs, the proliferation of the cancer cells was inhibited at different levels depending on the protein. On the other hand, we enriched proteins of which phosphorylation is stimulated in the ovarian cancer cells by immunoaffinity chromatography, and identified several proteins including Stat3 by iTRAQ and MS/MS. Finally, we investigated if we can detect the ovarian cancer-associated proteins in the plasma. The detection of the plasma proteins, however, is analytically challenging because the dynamic concentration range of them is extremely wide. We established a novel technique to analyze plasma proteins. In this technique, an originally developed • gabundant protein depletion device• h and a sequentially linked three-dimensional liquid chromatography-MS/MS (3D-LC-MS/MS) system were used. By this technique, we can identify nearly 3,000 low abundant proteins in the plasma. However, among the ovarian cancer-associated proteins identified in the cancer tissues and cultured cells, we detected only annexin IV in the plasma of the patients by our technique, suggesting that we should develop a novel detection system for the biomarker candidates in the plasma.

067

PROTEOMIC ANALYSIS OF CYTOKINES IN DIABETES PATIENTS: AN EXPERIMENTAL DESIGN APPROACH

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Background: The complex pathophysiology of diabetes has sparked the development of novel proteomic techniques that require proper design and validation. This study focus on multiplexed analysis of cytokines in diabetic nephropathy.

Methods: Multiplexed enzyme linked immunosorbent assay (ELISA), Gel electrophoresis followed by mass spectrometry were performed on plasma from 30 diabetic nephropathy patients. C-reactive proteins (CRP), Interlukin-6 (IL-6), Interlukin-10 (IL-10), tumor necrosis factor-α (TNF-α), myeloperoxidase were measured with ELISA. Experimental design methodology applied to perform gel electrophoresis and LC-MS/MS analysis of cytokines. Detection limits for between and within runs were determined. Experimental design methodology was employed to conduct method robustness and intermediate precision.

Results: Correlation between the multiplexed assays of ELISA was good for CRP, IL-6, IL-10, TNF-α and myeloperoxidase. Within and between run impression values for the multiplex method were < 15%.

Conclusion: The application of different mathematical tools is therefore a prerequisite for the realization of the robust results; Possible restrictions when it comes to choosing the setting of a specific parameter will be discussed. A stepwise optimization strategy using an experimental design is proposed, that hopefully will aid the scientists to optimize the performance of such an experimental design approach for biomarker development and validation.

Key Words: Proteomics, Diabetes, Experimental Design

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UNITED WE STAND: COMBINING STRUCTURAL METHODS

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As a structural biologist, I use X-ray crystallography and NMR as the basic tools to interrogate protein structure and function. When crystals don't appear and NMR isn't feasible, structural information can still be generated by combining results from other structural methods. We now incorporate synchrotron radiation circular dichroism and small angle X-ray scattering as well as cross-linking and mass spectrometry in our structural pipeline to generate additional structural data where necessary. We applied these combined approaches recently to investigate the structure of the complex between latexin and carboxypeptidase, the oligomeric structure of acyl CoA thioesterase 7 and the actin-bundling properties of cortactin.

069

SYNCHROTRON PROTEIN CRYSTALLOGRAPHY DEVELOPMENTS AND TARGET-ORIENTED STRUCTURAL PROTEOMICS

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Synchrotron radiation provides, intense, tunable and almost parallel X-ray beams most suited for structure determination of proteins and their complexes. Within Asia and Oceania there are a number of new protein crystallography beam lines in operation, under construction, or being planned for high-throughput or demanding biological projects. A brief survey of state-of-the-art synchrotron technologies will be presented drawing examples from modern beam lines in the area. In particular, most recent projects at the Photon Factory include two new insertion device beam lines, one optimized for micro focus experiments with lower energy SAD (single wavelength anomalous diffraction) capabilities and another for high throughput data acquisition by pharmaceutical industry. The former is part of the new national project, Protein Target Research Project of the MEXT, in which Spring-8, Photon Factory, Hokkaido Univ., Osaka Univ., and Kyoto Univ. are collaborating to build two complementary micro-focus beam lines and to develop techniques to facilitate user access and experiments at the two synchrotron sites, for instance, double sided cassettes compatible with both SPring-8 SPACE and SSRL-type SAM crystal exchange robots.

Second part of the talk will focus on target-oriented structural proteomics on vesicle transport of proteins using synchrotron radiation. This is part of the same MEXT project mentioned above which consists of 33 target-oriented and 10 R&D projects. Each of the target-oriented structural proteomics projects aim to solve structures of challenging targets in close collaboration with groups in cell biology, biochemistry, bioengineering, pharmacology, or medicine. Our targets are selected from protein-protein complexes involved in intracellular protein transport between the ER, the Golgi apparatus, and endosomes/lysosomes. Rab and ARF GTPase coordinate vesicular trafficking within eukaryotic cells by collaborating with a set of effectors, activating and deactivating proteins. A number of crystal structures of these GTPases in complex with its guanine nucleotide exchange factors and effectors will be presented to show molecular mechanisms of GTPase activation and interaction with other components of transport vesicles.

070

APPLICATION OF SYNCHROTRON INFRARED MISCROSPECTROSCOPY AND IMAGING TO BIOLOGICAL STUDIES

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The advantage of the infrared synchrotron radiation is high throughput at high spatial resolution compared to a conventional thermal light source. The synchrotron-based infrared microspectroscopy (SR-IMS) is a combination of the infrared synchrotron radiation and a Fourier-transform infrared (FT-IR) microspectrometer and produced the highest signal-to-noise ratio spectra with the highest spectral resolution from the smallest sample area. The unapertured focused beam size of the infrared synchrotron radiation is about 10×13 μm^2. Infrared spectroscopic imaging utilizes a single element detector of MCT, mercury cadmium telluride, associated with an imaging spectrometer to produce an array of spectra over a biological tissue. SR-IMS was utilized to image and subsequently produced spectral images or chemical images of the distribution biochemical components in biological sample. Colon cancer cell lines, unstained thin section from human colorectal cancer tissue, and butterfly wing scales were examined at different spatial resolution for imaging.

Acknowledgement

We would like to thank Dr. Hsu who worked for Tzu Chi Medical Center in Taiwan for helping the samples preparation.

071

WHAT ARE THEY DOING OVER THERE?

R. Lewis

Monash Centre for Synchrotron Science, Monash University, VIC, Australia

Most scientists working on proteins are very familiar with protein crystallography and many also use complimentary structural methods such as circular dichroism and small angle X-ray scattering. These techniques are however a small fraction of what is done with synchrotrons; so what are all those other people using a synchrotron for?

Other synchrotron techniques will be outlined and their relevance to biomedical science highlighted.

Techniques include;

· Imaging of live subjects with high spatial and temporal resolution including methods for cell tracking

· X-ray absorption spectroscopy which can reveal the location and local chemistry of metals

· Infra-red spectroscopy which can produce

· 3D imaging of cells

All of these methods are now being combined with structural biochemical information to obtain greater understanding of scientific problems.

072

ADVANCES IN RAPID ISOLATIONS OF PROTEIN COMPLEXES: REVEALING THE DYNAMIC VIRAL-HOST INTERACTOME

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Isolation of protein complexes, if performed appropriately, can provide an invaluable shortcut to uncovering protein interactions and to gaining clues towards their biological functions. The literature is, justifiably so, replete with approaches designed for the study of protein interactions. Advances are, nevertheless, highly desirable. An “ideal” isolation would maintain the protein complex as close as possible to its original state in the cell. To date, achieving this “ideal” isolation remains a challenge. The identification of transient or weak interacting partners and the stoichiometry within a complex present difficulties. We have recently reported an approach for the rapid and efficient isolation of protein complexes. We demonstrated that a combination of cryogenic-based cell lysis and fast

immunoaffinity purifications helps to maintain interactions, minimizing nonspecific associations and maximizing the recovery of transiently interacting partners. This presentation will underline some of the technical aspects that were found to be important in studying macromolecular assemblies. Highlights will be shown from our studies of the dynamic viral-host protein interactions during the course of infections with Sindbis and human cytomegalovirus (HCMV). Our results revealed cellular pathways utilized by these viruses to manipulate host systems. For example, our studies of the Sindbis virus, an *Alphavirus* genus member that in humans causes arthritis, indicated that Sindbis may utilize G3BP, at least in part, to interfere with the host cellular responses to stress. Most recently, we generated a library of 155 HCMV viruses, each containing a different C-terminal epitope tagged ORF, and initiated a comprehensive study of the HCMV interactions. One important finding from our interactome data was that the HCMV UL38 protein interacts with the tuberous sclerosis protein complex (TSC1/2) to block its negative regulation of mTORC1, a growth regulatory pathway, and facilitate viral replication.

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073

EXAMINATION OF ALTERATIONS IN THE PROTEIN PROFILE OF COLORECTAL CANCER CELLS DURING INVASION AND METASTASIS

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Metastasis is a dynamic process, requiring extensive molecular crosstalk between cancer cells and non-cancerous cells within the tumour. Complete recapitulation of the tumour milieu is not yet possible and in humans only the events before and after metastasis can be examined. By examining the protein profile of primary colorectal tumours and matched liver metastases from the same patient, we aimed to identify key proteins responsible for promoting invasion and metastasis both at the primary and secondary site.

Laser microdissection (LMD) was used to isolate cells from colorectal tumours and minimize contamination from normal adjacent colon and liver cells. The effect of several histological stains on tissue visualization during LMD, protein recovery and the saturation CyDye labeling reaction was examined and optimized. Tumour samples from five patients were isolated and profiled by difference gel electrophoresis (DIGE) using saturation labeling. Significantly differentially expressed proteins between primary and secondary tumours were identified by MALDI-TOF mass spectrometry. Primary colorectal tumours and their metastases had distinct protein profiles, conserved between patients. Differential expression of selected candidate proteins was validated in additional tumour samples using both immunohistochemistry and western blotting, showing good correlation with proteomic data.

074

CANCER PROTEOMICS FOR PERSONALIZED MEDICINE

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Cancer is a diverse disease, and the present clinical and pathological diagnostic modalities have obvious limitation in the prediction of clinical outcome. The next level of predictive molecular diagnostics using novel biomarkers are expected to best-optimize the existing therapeutic strategy. We examined proteome contents in more than 1,000 tumor tissues using our original large format two-dimensional difference gel electrophoresis (2D-DIGE) system (1). By integrating 2D-DIGE data with clinico-pathological parameters, we concluded that proteome reflects the major malignant phenotypes of cancer and proteomics has a great potential to identify biomarker candidate proteins. For instance, 2D-DIGE data included key proteins corresponding to the response to treatment in lung adenocarcinoma (2), osteosarcoma and Ewing sarcoma, the early recurrence in liver cancer (3), and the metastasis post surgery in gastrointestinal stromal tumor (4). For certain proteins, the predictive performance was successfully validated in more than 100 cases by immunohistochemistry. Such proteins should be strong candidates for biomarkers in personalized medicine. The clinical application of these research results is our next challenge. To facilitate the integrative and comprehensive omics study, we take a part

of Genome Medicine Database of Japan (5). All proteome data by 2D-DIGE, protein annotations and clinico-pathologidal data are currently being integrated into this database.

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075

GLOBAL ANALYSES OF AGE-RELATED EXPRESSION PROFILES OF MOUSE LIVER PROTEINS AND DATABASE CONSTRUCTION

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Toward making an integrated understanding of age-related homeostasis, we carried out global analyses of age-related expression profiles of mouse liver proteins. Liver protein samples prepared from nuclear, cytoplasm and mitochondria fractions of mice (C57BL/6xSJL, male) at 1, 3, 6, 12, 18, 21, and 24 months of age (n=10-20/age point) were subjected to quantitative analyses by 2DE (pH range 4-11) and MALDI-TOF/MS.

For nuclear protein fraction, approximately 8000 protein spots separated on 2DE were analyzed by MALDI-TOF/MS for, and 4547 protein spots were identified by MASCOT protein identifier program with reasonable scores. After removing duplicated spots, 3113 protein spots were found unique, composed of 2534 single protein spots and 579 mixture protein spots. Single protein spots were subjected to quantitative analyses with a PDQuest program, generating age-related expression profiles. GeneSpring software was then used for clustering and filtering analyses of the profiles. In addition to many complex age-related expression profiles, about a dozen unique and fundamental age-related profiles were identified. Many isomers, likely generated mostly by post-translational modifications and/or by alternative splicings were found for about 40% of single protein spots.

These findings suggested that there exist multiple, but a relatively small number of basic age-related regulatory mechanisms for liver nuclear proteins. These fundamental mechanisms may independently and/or in various combinations function, generating many complex age-related regulatory patterns. Similar studies have also been completed for cytosolic proteins. Analyses on mitochondrial proteins and female liver proteins are under progress.

The information obtained from these studies on liver proteins and their age-related expression profiles have been organized into a versatile database, a valuable platform resource, which facilitates studies on aging, age-related diseases, epigenetics and various challenge tests and drug evaluation.

076

A COMPREHENSIVE IMMUNOPROTEOMIC ANALYSIS OF THE REPERTOIRE OF HUMAN ANTIBODY RESPONSES TO THE MALARIA PARASITE PLASMODIUM FALCIPARUM

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The feasibility of a malaria vaccine is supported by experimental evidence demonstrating that protective immunity can be induced by exposure to intact parasite. However, P. falciparum (Pf) expresses ~5,300 genes and the protein antigens targeted by protective human antibodies are largely unknown. Serological screens using recombinant antigens are confounded by a lack of structural knowledge and poor correlation with functional serological assays involving antibody recognition of native antigens. We therefore designed a comprehensive immunoproteomic analysis (CIPA) strategy combining sensitive immunodetection and immunoprecipitation readouts for the 2-DE analysis of antibody reactivities against naturally expressed Pf proteins. In proof-of-concept studies we analysed >120 highly reactive 2-DE spots specifically recognized by malaria immune IgG. Using accurate 2D-MS/MS and immunological identification methods we created a valid 2-DE reference map/ database of the late blood-stage P. falciparum 3D7 'immunome'. Most of the antigens validated by this approach are predicted to be secreted Pf proteins (87%) associated with parasite membranes (61%) and/or involved in export to the erythrocyte (24%). Of the 38 immunogenic proteins, 21 are well-characterized blood-stage antigens - thus validating the approach. The other 17 have not been previously described as immunologically reactive. They include eight proteins recently identified by MudPIT analyses of parasite-infected erythrocyte ghosts (Florens et al., 2004) or raft-like parasite membrane fractions (Sanders et al., 2005), and two GPI-anchored proteins identified by our previous work (Gilson et al., 2006). Our data demonstrate the feasibility of the CIPA screen for determining the global repertoire of human antibody responses against the Plasmodium proteome - including correctly folded native antigen/ antigen complexes. We are currently applying this method to the analysis of highly defined sera from a well-characterized longitudinal cohort study in PNG. This may help to evaluate changes in the antibody reactivity profiles during the critical stage of development of protective immunity in children at risk of malaria.

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078

METABOLITE PROFILING IN *PLASMODIUM FALCIPARUM*.

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Metabolomics has emerged as the tool of choice for the modelling of whole cell metabolism and is increasingly being used to identify both new protein functions and potential drug targets. Metabolite profiling aims to resolve, identify and quantify individual metabolites by the utilization of (amongst others) hyphenated mass spectrometric techniques, in conjunction with detailed peak-clustering and bioinformatic analysis.

Plasmodium falciparum is the etiological agent of malaria and it is the erythrocytic stage of its complex life cycle that causes the characteristic symptoms of disease in the mammalian host. To date, metabolite profiling in *P. falciparum* has not been attempted, and little is known of the metabolic state of the cell during this stage, largely due to its relatively inaccessible habitat of the red blood cell.

One of the challenges in the analysis of the intra-erythrocytic parasite is the isolation of the cell from the erythrocyte. With metabolite profiling, metabolic quenching of the analysed cell is vital to the interpretation of results, and this adds yet a further level of complexity. Here, we have developed a method using a combination of infected erythrocyte enrichment and parasite cell isolation, for metabolomic and biochemical analysis, respectively. We use stable isotope labelling and gas chromatography-mass spectrometry (GC-MS), coupled with newly-developed bioinformatic techniques (including progressive peak clustering and linear discriminant analyses), to characterise the metabolome of *P. falciparum* at each stage of its 48 hour life cycle. Through these analyses, we have been able to isolate specific metabolic pathways and individual enzymes of *P. falciparum* that may serve as potential targets for drug discovery, and show the potential for this technique to discover drug targets in other cell systems.

079

CHARACTERIZATION OF NUCLEAR MATRIX PROTEOME OF DROSOPHILA MELANOGASTER DURING EMBRYONIC DEVELOPMENT

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The nucleus is an intricate structure containing many functional domains. Nuclear Matrix (NuMat), a non-chromatin scaffolding made of RNA and proteins, is believed to maintain this complex spatial organization. We have standardized procedure to prepare NuMat from *Drosophila* embryos by introducing several modifications in the published protocols and setting up several quality controls. We have established 2D profile of the NuMat proteome of *Drosophila* embryos and identified more than 150 proteins. While comparing the 2D profiles from different developmental stages, we noticed remarkable alterations in the composition of NuMat proteome during *Drosophila* development.

We will present functional analysis of one of these proteins, Boundary Element Associated Factor, BEAF. We showed that 25% of the total nuclear BEAF exists in the matrix. A region of the protein extending from 140 to 224 amino acids are needed for nuclear as well as matrix localization of this protein. This region has many potential sites for glycosylation and phosphorylation. We found that BEAF is O-glycosylated as well as phosphorylated at its Ser/Ther residues and that the phosphorylated form of BEAF is enriched in nuclear matrix. Our results lead to new aspects of the mechanisms that use nuclear architecture in regulating genes.

Identical DNA sequence of the genome is packaged in cell type specific manner resulting into corresponding epigenomes that in turn lead to cell type specific expression pattern. Our results identify key components of NuMat that help in packaging of the genomic DNA and enable chromatin mediated epigenetic mechanisms that regulate developmental gene regulation.

080

APPLICATION OF GLYCOMICS TO THE DIAGNOSIS OF LIVER DISEASES

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Glycosylation is one of the most common post-translational modifications in human. There has been a long history in applying serum glycobiomarkers for disease diagnosis and prognosis, especially for liver diseases. Because of their diverse structures and the information they carry, glycans provide a valuable source of biomarkers. With recent advancement in MALDI-TOF mass spectrometry, we developed a high-throughput assay for quantitative profiling of N-linked glycans released from the whole serum proteins. Our pilot study showed the specific fingerprint could be identified in the serum N-linked glycome, and used as a noninvasive tool for diagnosis of liver fibrosis in patients with chronic hepatitis B virus infection (CHB). Recently we have verified this approach by increasing our sample size and further investigated its application value in the post-treatment patients. The patients were randomly divided into a biomarker discovery group for finding differential glycans and constructing diagnostic model, and an independent validation group for assessing the diagnostic performance. Different diagnostic N-glycan fingerprints were identified in the pretreatment and post-treatment serum samples. An overall accuracy of about 75% was observed. Two common glycans were found in these two diagnostic fingerprints. Our results have suggested that serum N-glycome fingerprinting is a useful tool to supplement liver biopsy for assessing liver fibrosis in CHB patients before and after anti-viral treatment. Besides analyzing the N-glycome of whole serum proteins, our profiling assay could be used to quantify the N-glycans on a single protein. By profiling the glycans on serum haptoglobin (Hp), 4 *N*-glycans showed a progressive change of levels from normal healthy subjects, CHB patients, patients with early liver cancer to patients with advanced liver cancer. Subsequently we derived a serum Hp *N*-glycan index (Hp-GI) from the differential *N*-glycans, and constructed a decision tree to combine serum AFP, serum Hp and Hp-GI for diagnosis HCC. At a specificity > 93%, the sensitivity was 84%. All these findings strongly suggest that quantitative profiling of N-glycans from whole serum proteins or from a particular serum glycoprotein is a promising approach for discovering the next generation of biomarkers for liver diseases. [The projects were supported by the Li Ka Shing Foundation and the CERG Grant CUHK 473207 from the University Grants Committee, Hong Kong.]

081

DETECTION OF BIOMARKERS FOR COLORECTAL CANCER BY RANKING OF SOLUBLE-SECRETED PROTEINS (RSSP)

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Colorectal cancer (CRC), one of the most prevalent neoplasias in the western world, is treatable if detected early. Unfortunately, existing biomarkers such as carcinoembryonic antigen (CEA) suffer from poor sensitivity and selectivity when used for early detection, hence have limited practicality therein. To avoid difficulties associated with direct analysis of blood, studies aimed towards discovery of better early-stage markers recently targeted cancer-secreted proteins as these proteins are hypothesized to move into the bloodstream[1]. Selection of suitable candidates for further evaluation among identified secreted proteins remains a challenge as name and function of a protein do not necessarily indicate its usefulness as a marker.

To identify potential biomarkers for CRC we developed an approach where we ranked all proteins identified in the secretome from five different CRC cell lines according to criteria such as tissue specificity, detection across CRC lines or relationship to cancer. To avoid detection of abundant cytoplasmic proteins derived from cell lysis we employed our recently developed hydrazide-capture method targeting glycoproteins[2].

Detection of CEA was used as a positive control and the top-ranking proteins were considered for further clinical evaluation. Proof of concept studies were conducted in mice carrying xenografts and involved analysis of proteins secreted from xenografted tumours into tumour interstitial fluid and mouse blood.

Using this approach 140 proteins were identified and ranked, 79 % of them secreted or shed membrane proteins. CEA was identified and ranked as 14th and the proteins ranked up to 20 (Top20) selected for further evaluation. For selected proteins we show their detection in tumour interstitial fluid by western blot as well as detection of the protein in plasma from mice carrying CRC xenografts. Additional information on the secretory mechanism is also provided for individual proteins.

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083

FUNGAL LUNG INFECTION : UNDERSTANDING CRYPTOCOCCUS GATTII INFECTION AND THE CHALLENGES OF MIXED PROTEOMES

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The fungal species Cryptococcus gattii is an environmental saprophyte capable of causing serious disease in people and animals. Proteins are targets for drug interventions in microbial infections, proteome approaches are the most rapid, direct and powerful means of identifying novel candidate antimicrobial targets. In our preliminary proteome analysis, an encapsulated C. gattii strain was extracted using the current best practice for protein extraction from micro-organisms. However, this gave very limited extraction of proteins above 20 kDa. We hypothesised that the large capsule of C. gattii was sequestering the released proteins via ionic interactions. To disrupt this, we developed a novel method of protein extraction using salt, especially lithium, combined with acidic conditions. We will present studies using a range of different salts, and show how these greatly increased protein extraction from various encapsulated C. gattii strains. It is hypothesized that lithium chloride breaks the ionic interactions between the capsule and the cellular protein, thus allowing for efficient protein recovery. To further resolve these proteins, we filtered the samples through a 300 kDa or 100kDa filter, and this dramatically improved the protein recovery and profile of C. gattii.

We have also developed a rapid method of cryptococcal cell extraction from infected mouse lungs. This method has proven to be successful in leaving the cells intact, whilst stripping off the contaminating host cell material. Furthermore, 2-D gels and LC-MS of the stripped proteins revealed predominantly host cell proteins (very different profile to C. gattii). We have compared in-vitro C. gattii to lung isolates by 1-D gel and LC-MS/MS. In addition, we have performed western blot analysis of infected lung material using immune sera.

These methods will discussed as an example of the challenges facing proteomics of infectious disease – ie. obtaining pure proteomes of host and pathogen.

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GLYCOPROTEOMICS OF *PSEUDOMONAS AERUGINOSA*, AN OPPORTUNISTIC PATHOGEN

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Bacterial glycoproteomics is an upcoming field of interest. Glycosylation, once thought to be restricted to eukaryotes, is now being increasingly reported in prokaryotes. Sialic acids, as terminal residues of glycosylated proteins, play a crucial role in several cellular recognition events (1,2). *Pseudomonas aeroginosa,* a gram-negative bacteria, is a common human opportunistic pathogen. In view of its current drug-unresponsiveness and infectious nature, we investigated the presence of sialoglycoconjugates on *Pseudomonas aeroginosa*. Membrane sialoglycoproteins from *Pseudomonas aeroginosa* were captured using Achatinin-H lectin (a lectin with preferential binding to 9-*O*-acetylated sialic acid in α2-6GalNAc) affinity chromatography. The eluted sialoglycoproteins was subjected to SDS-PAGE and subsequently trypsin digested and were then analysed by matrix-assisted laser desorption mass spectrometry. One of the major sialoglycoprotein revealed 87% sequence similarity with chitin-binding protein CbpD of *Pseudomonas aeroginosa*. Considering the role of CbpD in pathogenicity, this may be considered as a new sialylated target in *Pseudomonas aeroginosa*, which can be exploited to develop novel bactericidal molecules. The presence of sialic acid in these novel *Pseudomonas aeroginosa* proteins was further confirmed through various approaches namely ESI-MS, fluorimetric HPLC (3), TLC, Lectin binding assays (4-7), GLC (8), western blotting and DIG-glycan detection and differentiation kit . To the best of our knowledge, this is the first report of sialic acids as important constituents of *Pseudomonas aeroginosa*.

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085

STRUCTURE-FUNCTION ANALYSIS OF ENZYMES INVOLVED IN THE COMPLEX LIPID CELL WALL SYNTHESIS OF MYCOBACTERIUM TUBERCULOSIS

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Mycobacterium tuberculosis (Mtb) has the highest annual global mortality among all of the bacterial pathogens. It possesses a complex cell wall with extraordinarily high lipid content. The pathogen generates diverse unusual lipids by coordinating enzymatic crosstalk between fatty acid synthases and polyketide synthases (PKSs)[1] and some of these lipids have been shown to play a role in the virulence of the organism.

One family of PKSs, type III PKSs, were thought to be specific to plants where they are involved in flavonoid biosyntheis. However, genome sequencing efforts have shown that type III PKSs are also present in several bacteria and other organisms. Mtb genome contains three type III PKS genes. One of them, PKS18, displays a broad specificity for long-chain acyl-CoA starter units (C_6 to C_{20}) to produce tri- and tetra-ketide pyrones . We showed that PKS18 possesses a novel 20Å long substrate-binding tunnel that is responsible for its unusual starter molecule specificity[2]. This discovery has led to the identification of a similar mechanism in other functionally divergent type III PKSs from various organisms[3,4].

Mtb genome has also revealed a large family of *fadD* genes (34 FadD proteins), classified into two distinct subfamilies, fatty acyl-AMP ligase (FAAL) and fatty acyl-CoA ligase (FACL), which activates long-chain fatty acids[1]. Several FAAL mutants have been characterized to be deficient in specific virulent lipid metabolites including pthiocerol mycocerosates, phenolic glycolipids, mycolic acids etc. Using the first structure of a FAAL protein and by generating loss- as well as gain-of-function mutants, we show that an insertion motif can direct formation of acyl-adenylate vs. acyl-CoA. By taking advantage of a common reaction intermediate and the overlapping substrate specificity of 34 FadD proteins, we could develop a multi-pronged strategy for generating novel antimycobacterial agents.

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086

DISCOVERY AND VALIDATION OF SEROLOGICAL HCC BIOMARKERS

Y. Paik

Abstract unavailable at time of print

087

CANCER PROTEOMICS FOR THE IDENTIFICATION OF BIOMARKERS AND THERAPY TARGETS

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Using the innovative proteomic and genomic techniques (1, 2), the research interest of Chemotherapy Division is aimed at clarifying the molecular and cellular mechanisms of cancer promotion and progression. Aimed at discovering targets of molecular therapy (3) and realizing personalized medicine (4), comprehensive protein and gene expression profiling of cancer cell lines, cancer tissues, and sera/plasma of cancer patients has been undertaken.

Genomic and proteomic approaches to colorectal carcinogenesis

T-cell factor-4 (TCF4) regulates a certain set of genes related to growth and differentiation of intestinal epithelial cells, and aberrant transactivation of these TCF4-regulated genes by β-catenin protein plays a crucial role in early intestinal carcinogenesis. By using global gene (GeneChip oligonucleotide microarray) and protein (2D-DIGE and isotope-coded affinity tagging and mass spectrometry) expression analyses we succeeded in identifying several molecules whose expression is regulated by the β-catenin/TCF4 complex (5).

Protein composition of the β-catenin and TCF4 nuclear complex

We also identified fusion/translocated in liposarcoma (FUS/TLS), poly(ADP-ribose) polymerase-1 (PARP-1), Ku70, Ku80, DNA topoisomerase IIα (Topo IIα), and splicing factor-1 (SF1) as putative components of the β-catenin and TCF4 nuclear complex (3, 5-7). Topo II is a known target of drugs that are currently being widely used for cancer chemotherapy. We have demonstrated that Topo IIα is a functional component of the β-catenin and TCF4 complex (3) and a potential drug target.

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088

HUMAN GLOMERULUS PROTEOMICS OR KIDNEY AND URINE PROTEOMIC PROJECT – OVERVIEW

T. Yamomoto

Abstract unavailable at time of print

089

PTRF-CAVIN IS ESSENTIAL FOR CAVEOLA FORMATION - FROM PROTEOMICS TO FUNCTION

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Caveolae are plasma membrane invaginations abundant in many cell types, including adipocytes, fibroblasts and endothelial cells. Members of the caveolin family of proteins are the only known protein required for caveola formation. Caveolae are enriched in cholesterol and sphingolipids, and can be biochemically prepared as detergent-resistant membranes in a manner similar to other lipid rafts. To differentiate between caveolae and lipid raft proteomes, we have employed a comparative proteomics approach using Caveolin-1 knockout mouse embryonic fibroblasts which lack caveolae.

Polymerase I and transcript release factor (PTRF, also called cavin) was identified as a putative caveolae protein in our proteomics screen. This protein was previously found associated with caveolae, however, the role of PTRF-cavin at caveolae was unknown. In this study, we demonstrate that expression of caveolins without PTRF-cavin is not sufficient for caveola formation in prostate cancer PC3 cells, and by PTRF-cavin knockdown in fibroblasts. Re-expression of PTRF-cavin in PC3 cells triggered the formation of caveolae, suggesting PTRF-cavin is an essential component of caveolae structure.

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Abstract unavailable at time of print

091

PROGRESS OF VIRON PROTEIN STRUCTURAL GENOMICS

Z. Rao

China

Abstract unavailable at time of print

092

T CELL RECOGNITION AND THE ATKINS DIET

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αβ T cell receptors interact with peptide and lipid-laden MHC and CD1 molecules respectively. The MHC is highly polymorphic engendering the ability to bind a wide array of peptides, whilst the CD1 family are monomorphic members binding distinct lipids. Structural studies on TCR-pMHC complexes have revealed markedly different docking strategies utilised by the TCR in recognising peptides of canonical and non-canonical length. Recently we have also determined how a TCR can recognise a glycolipid presented by CD1d. These variations on a theme are discussed in the context of peptide and lipid-mediated recognition by a TCR.

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T CELL RECOGNITION OF CHEMICALLY DIVERSE LIGANDS

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The adaptive (cellular) immune response is based around the presentation on the cell surface of peptides (epitopes) by a group of cell surface molecules known as the major histocompatabitity complex (MHC). The MHC complex binds peptides derived from proteins processed within the cell and displays them to passing immune effector cells (T cells). T cell recognition of a peptide then results in an immune response against the presenting cell (e.g. a virally infected cell). In recent years, growing awareness that such peptides may contain post-translationally modified amino acids (PTM's) has sparked debate concerning the roles such modifications may have in disease states such as infection, tumours, and autoimmunity. Despite clear evidence that post-translationally modified peptides are presented to the immune system, there has been no systematic study of the abundance or diversity of post-translationally modified peptides presented on the surface of cells to T lymphocytes. There are several reasons for this. Firstly, isolating a sample of MHC peptide for proteomic analysis is not simple. Secondly because bound peptides are created by diverse intra-cellular proteolytic processes, the MS/MS data is not always as informative as sequencing typical tryptic peptides. In addition, the MS/MS data must also be searched using a 'no enzyme' database searching strategy coupled with the further requirement of allowing for a large number of post-translational modifications. This represented a significant bioinformatic challenge. We have commenced a systematic study of the abundance and diversity of post-translationally modified peptide epitopes. Our results to date indicate that the proportion of PTM epitopes can be as high as 25%. Examples of identified modifications include glutathione (on cysteine) and protein N-terminal acetylation.

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SUMO MODIFICATIONS CONTROL ASSEMBLY OF SYNAPTONEMAL COMPLEXIN YEAST MEIOSIS

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Meiosis is a specialized cell cycle that generates haploid gametes for sexual reproduction. It involves a single round of DNA replication and two chromosome segregation and cell division cycles, resulting in cells that contain half the normal genomic complement. Meiosis I segregates homologous chromosomes, whereas meiosis II disjoins sister chromatids. As a prerequisite to proper chromosome segregation during meiosis I, homologous chromosomes must first associate in bivalents and be linked by chiasmata during meiotic prophase. Chiasmata are the points where two homologous not sister chromatids undergo crossover (CO) DNA recombination. In many organisms, CO depends on the formation of a synaptonemal complex (SC), a proteinaceous structure resembling railroad tracks that connects homologs along their entire length at pachytene stage. The two-sided rails of SC, known as lateral elements (LEs), are physically linked by a central element ÅCEÇ. Defects in meiotic DNA recombination or SC assembly in germ cells often leads to aneuploidy. Aneuploidy is the main cause for human miscarriages and developmental abnormalities. In budding yeast Saccharomyces cerevisiae, SC assembly requires structural components of CE (e.g., Zip1) and LE (e.g., Red1, Hop1, sister chromatin cohesin complex), as well as the synapsis initiating proteins (Zip2-4, Spo16). We showed that Zip3 is a SUMO (small ubiquitin-related modifier) E3 ligase and that Zip1 is a dimeric binding protein for SUMO-conjugate products. SC is assembled via bridging the Zip1 proteins with Zip3-dependent SUMO conjugates at both LEs. I will present our new results in identifying the target proteins for Zip3 E3 ligase and also discuss the molecular mechanisms of SC assembly.

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DESPERATELY SEEKING COMPREHENSIVE MAMMALIAN MEMBRANE PROTEOMICS

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Proteomics promises (but has yet) to deliver comprehensive coverage of the membrane proteome - primarily because of problems with membrane protein solubility and low copy number of important proteins. AO-HUPO recently launched the MPI to undertake large scale analyses of "standard" liver membrane preparations.

Here, we report data evaluating membrane protein immunoprecipitation, fractionation and digestion methods with novel separation (e.g., IPG-IEF) and MS on human cancer cells and rat/mouse liver membranes to increase depth of proteome coverage.

IPG-IEF enables the separation of chemically or enzymatically (e.g., trypsin) degraded protein products/peptides from a complex mixture solely on inherent p*I*. Methanol-assisted (0%, 40% & 60%) trypsin digestion of rat liver membrane proteins resulted in optimal coverage when deployed with peptide IPG-IEF based shotgun proteomics (digested proteins separated on linear 18cm 3-10 peptide IPG strips, manually separated into 24 fractions of equal width followed by peptide extraction and subsequently analyzed by ion trap LC/MS-MS). In this part of the study, peptides concentrated into three main p*I* regions (p*I* 3.5-5 5-7 & 8-9) and ~95% of all peptides were found in <2 fractions. In total, 1638 non-redundant membrane proteins were identified from all digests, with a very high level of coverage (42 identified) of the rat CYP450 protein family that are involved in drug clearance and toxicity. Of integral membrane proteins identified (60% methanol-assisted digestion), 513 were predicted to contain between 1-19 transmembrane segments. Biochemical ontology indicated proteins originated mainly from microsomal origin (e.g., ribosomal/structural proteins; 17%), mitochondrial (e.g., electron transport chain; 15%), cell membrane (12%), endoplasmic reticulum including CYP450s (11%) or either Golgi, secretion pathways, endosomes, peroxisomes or cytoplasmic vesicles (<3%). The addition of p*I* as a filtering tool to enhance identifications will be discussed. This data demonstrates that a combination of methods including peptide IPG IEF separates tryptic peptides with high resolution and that it results in a comprehensive coverage of the membrane sub-proteome.

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CHROMATOGRAPHIC SEPARATION OF INTACT PROTEINS FROM MOUSE LIVER MICROSOMAL PROTEINS FOR MEMBRANE PROTEOME ANALYSIS

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Membrane protein isolation and analysis remains a challenging task despite significant developments in separation technologies. Current progress in membrane proteomics is very limited with the lack of robust and reproducible separation techniques. New techniques in isolating intact membrane proteins would be beneficial in both their structural elucidation and functional analysis.

To enrich membrane proteins in an intact form for top-down proteomics, new wide-pore chromatographic sorbents (with either phospholipid, C8 or C18 ligands) were specifically developed for separation and used for the analysis of membrane proteins associated with the AUHUPO MPI sample. The protein mixtures were fractionated prior to tryptic digestion and MS/MS analysis. The collected fractions containing intact membrane proteins were then digested and analysed with either nanoLC- linear ion trap or HPLC-Chip-qTOF.

NanoLC MS/MS analysis of the tryptic fragments for each of the fractions resulted in the identification of 1193 proteins. Using a single search engine (X!Tandem) with a comprehensive search strategy and manual validation with either a protein expectation cutoff value of 10^{-10} or at least a 4 matching peptide filter being applied, the total identified proteins was 542. A similar number of proteins were identified with HPLC-Chip-qTOF (584 using X!Tandem; 464 using SpectrumMill). For these identified proteins, about 60% were membrane proteins based on the GO ID. Using the combined multiple search engines as a secondary approach, the data were searched with SpectrumMill, Mascot and X!Tandem separately. These searches were combined which generates a "Gold" list of high quality identifications. Furthermore, based on the ion intensity for label-free quantitation, the protein concentrations in the MPI samples vary over 4-5 orders of magnitude. While this presents significant challenges to database searches and validation algorithms, it also emphasizes the importance and need for pre-fractionation methods.

Overall, these results contribute to the aims of the Membrane Proteome Initiative, AOHUPO, which seeks to accelerate the development of technologies for membrane proteome analysis.

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SOLUBILITY-BASED PHASE PARTITIONING OF MOUSE LIVER MICROSOMES USING TRITON X-114

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The plasma membrane mediates many essential biological functions including cell-cell interactions, molecular transport, and signal transduction. Many membrane associated proteins are overexpressed in disease (eg cancer), and the ectodomains proteolytically shed. Several clinically used biomarkers have membranous origin including, CEA in colorectal cancer and PSA in prostate cancer. For this reason membrane proteomes are of interest in the field of biomarker discovery. As part of the AOHUPO membrane protein initiative, we report a solubility-based phase separation of liver microsomes, using the non-ionic detergent Triton X-114. The aqueous (detergent-depleted), detergent, and pellet (insoluble) fractions were obtained and subjected to SDS-PAGE in combination with LC-MS/MS.

Using a false discovery rate of 1%, a total of 666 proteins were identified from the three phases, and classified with respect to Gene Ontology and Swissprot annotation. In addition, proteins unique to each fraction were analysed with respect to hydropathy and number of transmembrane spanning helices using the predictive algorithms GRAVY and TMHMM respectively. Interestingly, over 70 % of proteins identified in the detergent phase were categorised as membrane according to Swissprot annotation. Furthermore, of the 64 proteins exclusively found in the detergent phase, 54 (84%) scored above the hydrophobicity threshold using GRAVY, while 45 (70%) were found to contain at least 1 transmembrane spanning region in TMHMM. This study demonstrates the ability of Triton X-114 to enrich for membrane and membrane-associated proteins.

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1D-SDS-PAGE AND NANO-LC-MS/MS FOR MEMBRANE PROTEOMICS OF MOUSE LIVER MICROSOMES (MPI SAMPLE) AND ITS APPLICATION TO HUMAN PROTEOMICS OF ER FROM JURKAT CELLS

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Proteomic profiling of mouse liver microsomes was performed by SDS-polyacrylamide gel electrophoresis (1D-SDS-PAGE) and nano-liquid chromatography tandem mass spectrometry (nano-LC-MS/MS) for the pilot study of Membrane Proteome Initiative (MPI) of AOHUPO. More than one hundred proteins were identified with high MS/MS search score, and most of those proteins have been reported to be membrane associated proteins such as microsomal glutathione S-transferase 1, cytochrome P-450 and low density lipoprotein receptor-related proteins. This platform was used for ER membrane proteomics of Jurkat cells of a human lymphoblatic lymphoma cell line to find marker proteins for heat stress. The microsomes were prepared by two-step centrifugation of liver homogenate, firstly the homogenate was centrifuged at 15,000 g for 15 min to yield supernatant and secondly the supernatant was centrifuged at 132,000 g for 60 min to yield microsomes pellet. The microsome pellet was suspended in 0.1 M sodium carbonate solution containing 0.5 mM PMSF and 10 ug/mL of aprotinin and leupeptin to yield highly purified microsomes in the pellet by centrifugation at 132,000 g for 60 min. The purified microsomes were pretreated with ice cold acetone-methanol (8:1) solution (*Anal. Biochem., 273, 313-315, 1999)* to be applied for the separation of membrane proteins by 1D-SDS-PAGE with a gradient gel (5-20%) to yield the fine separation of proteins in more than 50 bands which were visualized by Coomassie Blue staining. The protein bands were cut out and submitted to in gel digestion with trypsin. Peptides in the tryptic digests were separated by nano-LC followed by MS/MS to identify the protein(s) in each of the bands. We show the data of membrane proteomics in microsomes from Jurkat cells and discuss availability of this platform for human ER membrane proteomics.

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DIGGING DEEPER INTO THE MOUSE LIVER MEMBRANE PROTEOME: EVALUATION OF DIFFERENT MEMBRANE PROTEIN DIGESTION APPROACHES WITH 8-PLEX ITRAQ REAGENTS

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In an earlier report, we had identified 535 unique proteins from the mouse liver membrane fraction distributed by the AOHUPO Membrane Proteome Initiative (MPI) project. The sample, after methanol facilitated solubilization and digestion with trypsin and chymotrypsin, was fractionated by reverse-phase liquid chromatography (LC). The LC fractions were collected onto MALDI target plates, mixed with matrix solution, and analyzed by a MALDI TOF/TOF mass spectrometer. To further improve the protein identification, we have extended our effort to two-dimensional LC (2D-LC), which separates the peptide mixture using strong cation-exchange followed by reverse-phase columns.

Although there are currently many published reports used to digest membrane proteins with the help of various organic solvents or detergents, the efficiencies of these methods have not been systematically compared using high throughput proteomics approaches. To address this deficiency, we decided to use the newly released and novel 8-plex iTRAQ reagents to compare (and quantify) the efficiencies of 4 of these commonly used reagents/methods for membrane protein digestion. The reagents used included (1) methanol; (2) trifluoroethanol (TFE) and (3) 3-[3-(1,1-bisalkyloxyethyl)pyridin-1-yl]propane-1-sulfonate (PPS), as well as (4) a new tube-gel digestion approach . In these experiments, equal amounts of the mouse liver membrane fraction were digested with proteolytic enzymes twice. The digested peptide mixtures so obtained were then labeled with 8-plex iTRAQ reagents and further analyzed by 2D-LC-MALDI MS/MS as described above. The ProteinPilot software was used to identify the proteins and quantify the relative abundance of each protein derived using the 4 different approaches.

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PROTEOME ANALYSIS OF MOUSE LIVER MICROSOMAL FRACTION USING 2D BN/SDS-PAGE

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Membrane proteomics initiative (MPI) of AOHUPO was planed to develop technologies using a standard membrane preparation of liver microsomal membrane fraction distributed among participating laboratories. Blue native polyacrylamide gel electrophoresis (BN-PAGE) following by denaturing SDS-PAGE (2D BN/SDS-PAGE) can resolve membrane proteins in their native complex forms. Using 2D BN/SDS-PAGE coupled with mass spectrometry, 40 microsomal membrane fraction proteins were identified. Database search revealed that about 40% and 30% of identified proteins were integral and peripheral to membrane proteins, respectively. Moreover, most of identified proteins had pI higher than 7 and about 26% of them had positive GRAVY indices.

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A COMMON SEQUENCE DATABASE FORMAT IN PROTEOMICS

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Introduction: There are several issues with the traditional FASTA format:

- Definition line formats vary widely. The creators of protein identification tools are faced with a significant challenge of supporting all variations.
- The same database processed in different search engines can produce different identifiers that are difficult to map. E.g. P00761 vs. ALBU_HUMAN.
- The same protein in different databases can have very different identifiers
- The information extracted from the FASTA formats is heterogeneous, causing parsing issues.

Taxonomy information cannot be obtained consistently and reliably.

Methods: Here we propose a unified format for sequence databases that can be interpreted in an identical manner by all sequence-using search software and other associated tools (spectrum library search approaches, sequence alignment software, etc). Database providers are encouraged to generate this format as part of their release policy or to provide appropriate converters that can be incorporated in the tools. The proposal has been prepared by representatives of sequence database providers, MS software developers and academic lab users. It has been submitted to the HUPO-PSI document process where it reaches a public review. The description and availability of the format is hosted on the PSI website (http://psidev.info/index.php?q=node/317).

Results: The proposed format has the form of a flat file that can store one or more sequence databases, including decoy versions. It is constituted by a header section, that describes information about the contained sequence databases, and a sequence entries section that compiles the actual sequences. The header section reports meta-data such as name, version, short description, and source data of the included databases. Delimiters have the form "#Tag=Value", one per line. Each element of the sequence entry section is similar to a FASTA format, where the description line is formatted using explicit tags. Except for the Sequence Accession identifier delimited with a ">", all other delimiters are in the form "\Tag=Value". A controlled vocabulary is established to define the tags, the associated values and their obligatory/optional nature.

This format is compatible with MIAPE and journal requirements.

Innovative aspects:

· Unified format for sequence databases in Proteomics

· Unified manner to read and interpret protein and nucleotide sequences and associated information

· The format includes the information required by publishers not yet available in existing formats

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ZOOM IEF FRACTIONATOR & SDS-PAGE TO IDENTIFY MEMBRANE PROTEINS EFFECTIVELY

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To avoid the specific problems concerning intrinsic membrane proteins in proteome analysis, an alternative strategy should be applied that is complementary to 2-D polyacrylamide gel electrophoresis (PAGE) techniques. Because of their highly hydrophobic character, membrane proteins tend to precipitate in aqueous media, we have elaborated a protocol for the separation of both hydrophilic as well as hydrophobic proteins using Zoom IEF fractionator. The method has the advantage of more extensive proteome coverage and enrichment in low-abundance proteins compared with conventional 2-D PAGE alone. By this approach, we have identified the known microsomal membrane proteins including P450 oxidoreductase, flavin containing monooxygenase 5 as well as mGST in the subsequent matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) analysis.

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COMPARISON OF EXPERIMENTAL METHODS FOR IDENTIFICATION OF MEMBRANE PROTEINS FROM MPI REFERENCE SPECIMEN

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Mass spectrometry based identification of membrane proteins is challenged by two major constraints - their release from the membranes in soluble form and difficulty for good quality MS spectra on account of their hydrophobic nature. We address this in the analysis of the AOHUPO MPI reference specimen employing work flows involving 2DE MS or separation of proteins by SDS PAGE or isoelectric focusing followed by LC MALDI MS/MS or ESI - LC MS /MS approach. We also describe membrane proteins identified from mouse embryonic stem cells.

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APPLICATIONS OF FUNCTIONAL PROTEIN MICROARRAYS

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Comprehensive proteome-scale protein microarrays can be used to simultaneously screen up to several thousand proteins for drug binding, molecular interactions, or enzymatic activity. The ProtoArray® Human Protein Microarray v4.0 is comprised of over 8,000 human proteins spotted in duplicate on glass slides. The arrayed proteins are expressed as full-length, N-terminal glutathione S-transferase (GST) fusion proteins in a baculovirus system. The proteins are purified under non-denaturing conditions to maximize proper folding and functionality. Examples will be given about how arrays of human proteins have been used to define protein interaction pathways, screen for enzyme substrates, measure enzyme inhibition, and identify protein targets of drugs. In addition, we have demonstrated the utility of these arrays for determining the specificity of antibodies, thus providing a new tool for the development and characterization of therapeutic and diagnostic antibodies. Finally, we will show how these arrays have been used to provide a rapid and sensitive profiling platform to investigate the circulating antibody profile in several disease states.

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SAMPLE FRACTIONATION USING MAGNETIC BEADS

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To find protein and peptide biomarkers in a biological sample, fractionation is usually required to reduce dynamic range and sample complexity. LC is a standard way of fractionating samples. Although it has a great resolving power, it is time consuming and is less ideal for the processing of many samples in parallel. Magnetic beads (Dynabeads) offer the opportunity of manual or automated high-throughput fractionation upstream of MS or 2D-gels. With 96-384 wells available, many samples can be run in parallel with one or

several different chromatography surfaces (e.g., SAX, SCX and RPC 18). Because of high sensitivity and reproducibility, Dynabeads have become a popular choice for serum peptide profiling and for the pre-selection of samples (e.g., upstream of LC-MS).

Immunoprecipitation and co-immunoprecipitation are classical methods used to isolate specific proteins or protein complexes from biological samples. Traditionally, sepharose and agarose slurries have been used, but more recently magnetic Dynabeads have gained popularity due to shorter and simpler protocols. The rapid procedure permits the isolation of labile composites that might otherwise dissociate during long incubation times (or be damaged by proteases), there is no upper size limit for the complex to be isolated (ideal for complex pull-down) and the surface properties give very low non-specific binding. With Dynabeads there is no fear of losing beads and you can scale down the procedure to reduce the consumption of expensive antibodies.

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DRILL DEEPER INTO THE PROTEOME

J. Vanhauwe

Invitrogen Corporation, Australia

Reliably fractionate your samples. Analyze protein complexes. Quantitate up- and downregulated protein targets. Analyze post-translational modifications. Protein biomarkers are crucial for improving diagnostics, monitoring therapeutic response and guiding molecularly targeted therapies. Mass spectrometry has proven to be a very valuable tool for analyzing the extremely complex and dynamic proteome. However, the discovery and quantitative analysis of clinically relevant targets has been difficult because they are usually present at very low levels in biological systems, representing a great challenge even for most technologically advanced mass spectrometry systems. Recently, major advances have been made in the area of fractionation, separation, and analysis of biological molecules that greatly facilitate the detection and quantitation of low abundance proteins by mass spectrometry. The following topics will be discussed in this session:

- Metabolic and chemical labeling techniques for protein quantitation by mass spectrometry
- Detection and quantitation of post translational modifications by Mass Spectrometry
- Analysis of native protein complexes
- Isoelectric focusing fractionation and separation of proteins and peptides

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SCREENING OF AUTOANTIGENS FROM GASTRIC CANCER PATIENT SERA USING THE INVITROGEN PROTOARRAYS

M. Chung

National University of Singapore, Singapore

Abstract unavailable at time of print

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SECRETOMES OF DIFFERENTLY STIMULATED HUMAN DENDRITIC CELLS GENERATED BY 2D-PAGE AND SHOTGUN ANALYSIS.

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Dendritic cells, the most potent and specialized antigen-presenting cells, play a key role in the regulation of the adaptive immunity. Immature DCs were generated by *in vitro* culturing of peripheral blood monocytes and functionally activated with the classical pathogen-associated molecular pattern lipopolysaccharide (LPS), functionally modulated with lipid oxidation products derived from 1-palmitoyl-2-arachidoyl-*sn*-glycerol-3-phosphorylcholin (OxPAPC) or activated with human rhinovirus (HRV) . The aim of this study was the identification of secretome profiles related to the functionally different dendritic cell phenotypes.

C omparative 2D gel- and shotgun analysis of immature DCs, LPS-treated immature DCs, OxPAPC-stimulated immature DCs and HRV-activated immature DCs was performed. During treatment, cells were metabolically labeled with ^{35}S-methionine/cysteine. Consequently, autoradiography of 2D gels of cell supernatant selectively detected secreted proteins. Furthermore, proteins were identified by shotgun analysis, semi-quantitative assessment was performed with the spectral count method. As a result, 137 different secreted proteins were identified. Immature DCs secreted proteases such as lysozyme C and MMP-9; protease inhibitors such as cystatins C and F; cytokines such as interleukin-8, small inducible cytokines A17, A18, A24 and many other proteins. Secretion of cytokines such as IL-6 and small inducible cytokines A3 and A8 was induced by LPS, but rather repressed by OxPAPC and HRV, which were found to secrete several tumor-associated proteins. The presently described secretome data may help to better understand actions and effects of dendritic cells at different functional states.

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RECENT ADVANCES IN LC/MS TECHNOLOGIES FRONM AGILENT

C. Miller

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At the recent annual ASMS meeting in Denver, CO, Agilent announced a range of new LCMS platforms and software tools designed to enhance the quality of data generated from LCMS based experiments in Proteomics. Improvements to MS source design enhance overall MS sensitivity by at least 5X on Agilent's QQQ and QTOF platforms. These advances will be discussed in greater detail by one of Agilent's most senior Applications Chemists during this session. This is an opportunity to learn why everyone at ASMS was talking about Agilent!

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ELEMENTAL BIOIMAGING

P. Doble

University of Technology, Australia

Elemental bioimaging employs laser ablation - inductively coupled plasma - mass spectrometry (LA-ICP-MS) to construct elemental maps of tissue samples. A laser is used to ablate the sample into the vapour state. The elements are then analysed by the ICP-MS. The laser is rastered across the tissue sample which is time resolved by the ICP-MS. In this way, an image can be built up by multiple raster lines, much the same as a dot matrix printer prints an image or text. The images are then processed and displayed as colour maps. High concentrations are usually designated red in colour and low concentrations blue. The ICP-MS is a multi-element analyser so it is possible to build maps from many metals simultaneously. This talk will present examples of elemental bioimaging applied to diseases such as cancer, Parkinson's disease, osteoarthritis, and ischemia.

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MALDI IMAGING MS, THE NUTS AND BOLTS OF THE TECHNOLOGY

P. Chaurand

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MALDI imaging mass spectrometry (IMS) can be used to map the molecular content of surfaces. This powerful analytical approach has primarily been used to study the composition and spatial distribution of molecules within tissue sections. Methodologies for the analysis of endogenous compounds such as lipids, peptides and proteins as well as administered pharmaceutics have been developed to better understand the molecular aspects of normal organ functioning, and development as well as the progression of diseases.

When conceiving an IMS experiment, numerous technical details have to be considered to gain successful insights into the sample investigated. In this workshop lecture, will be detailed some of the critical experimental aspects of the IMS process starting with proper sample handling, specimen sectioning and processing, matrix deposition strategies as well as data acquisition, preprocessing, image reconstruction and statistical analyses. The proper consideration of this sum of serial events leads to the generation of high quality mass spectrometry profiles and images.

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BRUKER CLASS IMAGING: FROM SAMPLE PREPARATION TO BIOSTATISTICAL ANALYSIS OF MALDI TISSUE IMAGING DATA FOR THE DIAGNOSTICS OF TISSUE HEALTH STATES.

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MALDI imaging is a technique with increasing importance in marker discovery and clinical research. Thin tissue sections are typically prepared through the application of matrix by robotic pipetting or pneumatic spray assisted matrix deposition. The image is obtained by acquiring MALDI mass spectra across the tissue in a raster (ca. 20-500 μm spot-to-spot distance).

Sample preparation is crucial for the quality of MALDI-tissue-imaging data. Unfortunately, the current matrix application protocols have significant disadvantages: While pneumatic spray preparations provide good homogeneity and spatial resolution of the images, the process is manual and highly irreproducible. Depending of the degree of tissue wetting either the analyte molecules are badly incorporated into the matrix (too dry) or the spatial resolution is lost (too wet). Nano-spotting on the other hand provides quality spectra but as a sequential process it is slow, spatial resolution is limited by the spot raster (typical >200 μm) and perfect alignment with the mass spectrometer is critical. We introduce an entirely new approach that combines the advantages of above methods and eliminates the disadvantages. In the new preparation device, matrix aerosol (20μm droplets) is created by vibrational vaporization under controlled ambient conditions that is gently deposited onto tissue sections. Tissue sections can be homogeneously matrix-coated, typically with 30-100cycles within one hour.

Beside the traditional software required for the visualization of MALDI tissue data (MALDI Imaging), Bruker provides a novel approach for MALDI image analysis. Through the use of Class Imaging we determine histological defined areas on the tissue sections and acquire the MALDI spectra. All spectra of such a group are then submitted as a class to the statistical analysis. Eventually each tissue/health class is associated with a colour that provides tissue/health specific images at a specificity that is not typically obtained mass specific MALDI imaging: Class not mass is generating theses images. Such class definitions can be used to investigate tissue biopsies from patients with regard to their membership of the classes in question.

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IMAGING MASS SPECTROMETRY (IMS) APPLICATION TO MURINE TISSUES

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Since the novel application of MALDI-TOF mass spectrometry [MS] to tissue in 1997 the usage of this technology has increased dramatically (1). In its simplest form so called imaging mass spectrometry [IMS] involves desorption and ionisation of proteins from co-crystallised tissue and matrix by a MALDI source, followed by linear TOF mass measurement of these protein species. Various protocol permutations allow either specific mass imaging across whole tissue sections or profiling of different tissue areas for statistical comparison. Thus far other proteomics technologies have not been able to provide high yield spatial data from a tissue. Furthermore, the possible applications of IMS for clinical pathology and biomarker discovery (2,3) have allowed this proteomics platform to quickly gain popularity.

At the Adelaide Proteomics Centre we are currently trialling and optimising our sample preparation and acquisition protocols for IMS. Primarily we wish to cover the general IMS protocol as well as the critical components of this protocol. Also presented will be results of droplet array and vibrational vaporisation matrix deposition as well as descriptions of tissue section treatment. Finally it will be considered how well IMS can be coupled to pathology. Imaging MS has shown its applicability as a future proteomics platform and combined with established proteomic techniques can allow rapid, comprehensive and high throughput laboratory tissue characterisation to become reality.

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ENRICHING LOW ABUNDANCE PROTEINS BY PROTEOME-WIDE AFFINITY USING A COMBINATORIAL HEXAPEPTIDE LIBRARY

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A small number of abundant proteins often dominate proteomes, and obscure the signal of many others. One strategy which has been applied is immunodepletion, especially in sera or plasma, of up to 20 of the most abundant species. However, this strategy requires antibodies which are expensive and time consuming to produce. An alternative strategy consists of a solid-phase combinatorial library of hexapeptides, synthesised via a short spacer on porous poly(hydroxymethacrylate) beads. The hexapeptides are synthesised on all surfaces of the beads, whereby each bead has a unique ligand that is potentially different from the ligand of any other bead. In the synthesis of the hexapeptides, the 20 natural amino acids are used. Accordingly, the library contains a population of 206 linear hexapeptides, i.e. 64 million different ligands. On each bead, the amount of hexapeptide reaches approximately 50 pmol. Such a vast and heterogeneous population of hexapeptides means that, in principle, an appropriate volume of beads should contain a hexapeptide able to interact with just about any protein present in a complex proteome – be it a biological fluid or a tissue or cell. Proteins bind to the beads under mild conditions, which initially limited the beads to soluble protein samples such as plasma and serum. We have worked with derivatisation of proteins to enable a wider range of insoluble or hydrophobic proteins to be solubilised and applied to the hexapeptide beads. Examples of both soluble and insoluble derivatised samples will be shown.

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STRATEGIES FOR SELDI-BASED BIOMARKER DISCOVERY AND DEVELOPMENT

A. Bulman

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While the recent demand for protein biomarkers to serve as biological indicators of a phenotypically altered state has yielded a large number of candidate biomarkers, validating these biomarkers has been more challenging. Successful biomarker discovery and development efforts require a working knowledge of multiple disciplines, including study and experimental design, proteomics

technologies, and data analysis and interpretation. Bio-Rad's SELDI ProteinChip based workflow focuses on native proteins and peptides - "top-down" proteomics. This approach preserves information about post-translational modifications (truncation, glycosylation, etc.) that may be important indications of disease. The SELDI ProteinChip system, a high throughput, reproducible platform for biomarker research, has been used to discover and characterize biomarkers for oncology, neurobiology, infectious disease, and toxicology.

SELDI-based biomarker studies can typically be divided into four phases: Discovery, Validation, Purification and Identification, and Assay Development, each of which requires a unique approach. The discovery phase is characterized by analyzing samples under a large set of experimental profiling conditions. The broad dynamic range of many biological samples, including serum and plasma, can be a significant challenge to biomarker discovery, but may be addressed with up-front fractionation and enrichment to improve detection of low abundance proteins. The initial panel of candidate biomarkers is then tested during the validation phase. In the discovery and validation phases, it is especially important to optimize study design and statistical methods to avoid pre-analytical bias and yield robust markers. Identification can be performed at the end of the discovery phase or at any point during the validation phase, facilitating the development of analyte specific assays and providing insight into the disease biology. Once the biomarkers have been positively identified, quantitative assays can be developed for routine testing on an appropriate immunoassay platform. SELDI-based immunoassays are particularly useful for detecting biomarkers with post-translational modifications. Approaches to optimizing biomarker workflows to deliver robust biomarkers and biomarker assays will be discussed using examples from Biomarker Research Center collaborations.

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EVALUATION OF A STANDARDIZED METHOD OF PROTEIN PURIFICATION AND IDENTIFICATION AFTER DISCOVERY BY MASS SPECTROMETRY

E. Boschetti

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Identification of unknown proteins subsequent to a mass spectrometry signal is still a serious obstacle in the discovery of relevant biomarkers of diagnostic interest.

In this frame a rational general process is described and applied to several unknown proteins representing important targets in their field of investigation. Basically the protocol consists of an initial rational selection of few dozens of chromatographic sorbents followed by alignment of these as a series of columns to obtain the separated target protein. This preparation is then submitted to electrophoresis, the band is excised and the trypsin digest is sequenced by MS

The reported data show that the method is reliable and easily applicable to a large variety of cases with a standardized approach. Identity coverage and relative abundance after purification and removal of critical protein impurities are reported.

Examples of protein isolation/ identification are described, namely PTF1, recombinant YAP-1 transcription factor from E. coli and DNA-binding protein HU from H. pylori. Isolated proteins were pure enough for the purpose of formal identification by either peptide mass fingerprinting or sequencing.

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WHAT'S HAPPENING TO PROTEOMICS AT GE HEALTHCARE??

B. Hood

GE Healthcare, Uppsala, Sweden

Brian has been in the ice box of Uppsala, Sweden since March and what better way to thaw out than come to Cairns? Brian will present on how to deal with the Swedes in social and business situations and other stories from the head office. More importantly, he will show off what we have been working on in the Proteomics and Protein Purification areas and our vision over the coming years.

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BREAKING THE MOLD – ACADEMIC PARTNERSHIP AND RESEARCH AT GE HEALTHCARE

S. GE Healthcare

GE Healthcare, Australia

We will present work from our academic programme unit. The unit has been formed to give a prominence to the work we undertake along with our research partners which are mostly university based. The presentation will focus on those areas of research we need to help complete the puzzle - moving into the future of molecular imaging, molecular diagnostics and life science research. Researchers are invited hear how external academic partnerships are first funded, how they are managed and what the hot areas of interest for GE Healthcare are right now.

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A PARALLEL PROTEOMICS APPROACH TO ANALYZE AND VALIDATE PROTEIN DIFFERENCES IN COLORECTAL CANCER

D. Haid, I. Grigorescu, J. Flensburg, H. Nordvarg

GE Healthcare Bio-Sciences AB, Uppsala, Sweden

A colorectal cancer study aimed at identifying potential biomarkers using two parallel workflows has been performed. One workflow was gel-based and utilized 2D electrophoresis and the Ettan DIGE and MALDI-ToF for relative quantification and subsequent protein identification. The other approach included Ion exchange protein prefractionation and subsequent separation of digested protein fractions on a multi-dimensional liquid chromatography system, connected to a LTQ ion trap mass spectrometer. Relative quantitation of tryptic peptides was enabled through a label-free strategy. The unique protein ID and magnitude of differential expression was finally confirmed by a florescent Western blot methodology ECL Plex, enabling confirmation of both protein identity and physical properties as well as relative quantitation of potential cancer biomarkers.

In the LC-based workflow more than 1000 unique proteins were identified and approximately 120 were differentially regulated. More than 50 of these were previously reported to be involved in various forms of cancer. For the gel-based workflow 240 unique proteins could be identified of which 28 were differentially regulated. 15 of the regulated proteins were known to be involved in cancer. Interestingly, only about 50% of the identified proteins were common for both workflows, clearly demonstrating the complementarities of the two approaches.

Part II

Importance of high reproducibility for protein pre-fractionation

High reproducibility is a prerequisite for proteomics studies involving prefractionation on the protein level. Ettan LC is an instrument for high performance micro purification very well suited for that purpose. Data from a colorectal cancer study is presented were ion exchange protein prefractionation and the DeCyder MS software tool enabled detection of protein isomers their relative quantitative abundances were determined. This capacity unravels the discrepancies that normally are concealed in traditional MDLC-based methodologies.

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ON-TISSUE MALDI-MS ANALYSIS WITH THE CHEMICAL PRINTER (CHIP-1000)

T. Nakanishi

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MALDI Imaging Mass Spectrometry (MS) can allow the detection and localization of the target molecules directly from tissue sections. This technique has become a powerful tool in biological research. And recent studies have achieved successful results for a biomarker discovery on a specific disease state and investigation of pharmacokinetic behavior of a drug. In general, homogenous matrix deposition onto thin tissue sections is required to obtain mass spectra with high quality for successful MS images by MALDI-MS. For this purpose, various methods for matrix deposition have been reported such as by a glass thin layer chromatography (TLC) sprayer, a manually pipetting and a robotic spotter.

Here we introduce the chemical printer that used piezoelectric pulsing for rapid, accurate and non-contact micro dispensing of fluid as a sample preparation of matrix deposition onto tissue. This chemical inkjet method is a novel technology which has advantages of superior reproducibility of printing and smaller diameter of spots on tissue sections. By the export of positional information of the spots to a mass spectrometer, a mass spectrum is accurately obtained at each position of matrix spot. Furthermore, direct on-tissue trypsin digestion can be performed by printing trypsin on tissue sections and direct on-tissue protein identification can be achieved by MS/MS ion search for resulting tryptic digested peptides without loss of spatial information of the corresponding proteins. This chemical inkjet technology is effective for Imaging MS to elucidate the relative abundance and the distribution of biomolecules and for MS profiling such as protein identification. We present Imaging MS of small molecules or proteins, protein identification by on-tissue digestion and the analysis of paraffin-embedded tissue sections to enable retrospective analysis of tissue stored for a long term.

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POSTTRANSLATIONAL MODIFICATIONS IN AN INSECT CELL-FREE PROTEIN SYNTHESIS SYSTEM AND THEIR IDENTIFICATION BY MALDI-TOF MS

T. Ezure

Clinical & Biotechnology Business Unit, Analytical & Measuring Instruments Divis, Shimadzu Corporation, Kyoto, Japan

We have established a cell-free protein synthesis system (Transdirect *insect cell*) derived from *Spodoptera frugiperda* 21 insect cells [1]. This cell-free system has high protein productivity, and therefore it is expected to be sufficient to perform gene expression analyses including not only the measurement of enzymatic activity and western blotting, but also investigation of posttranslational modifications. So far, several posttranslational modifications in the insect cell-free protein synthesis system were confirmed and identified by MALDI-TOF MS [2, 3, 4] . In this work shop, we focus on the analysis of N-terminal protein modifications.

N-terminal protein modifications are the most common processing events in eukaryotes, and they include removal of the initiating Met, *N*-acetylation, and *N*-myristoylation. These changes affect protein stability, physiological function, and degradation. Epitope-tagged truncated human gelsolin (tGelsolin) which is natural *N*-myristoylated protein, was synthesized using the insect cell-free protein synthesis system. Following affinity purification, the purified tGelsolin was analyzed by MALDI-TOF MS and MALDI-quadrupole ion trap (QIT)-TOF MS. As a result, the wild-type tGelsolin was found to be *N*-myristoylated by the addition of myristoyl-CoA to *in vitro* translation reaction mixture. *N*-myristoylation was not observed on the Gly-2 to Ala (G2A) mutant in which *N*-myristoylation motif was disrupted. Interestingly, it was identified that this mutant is *N*-acetylated after removal of the initiating Met. Thus, combination of the insect cell-free protein synthesis system and mass spectrometry is an effective strategy to analyze the N-terminal protein modifications.

(1) Ezure, T., et al. (2006) Cell-free protein synthesis system prepared from insect cells by freeze-thawing. Biotechnol. Prog. 22, 1570-1577
(2) Suzuki, T., et al. (2006) N-Terminal protein modifications in an insect cell-free protein synthesis system and their identification by mass spectrometry. Proteomics 6, 4486-4495
(3) Suzuki, T., et al. (2007) Protein prenylation in an insect cell-free protein synthesis system and identification of products by mass spectrometry. Proteomics 7, 1942-1950
(4) Ezure, T., et al. (2007) Expression of proteins containing disulfide bonds in an insect cell-free system and confirmation of their arrangements by MALDI-TOF mass spectrometry. Proteomics, 7, 4424-4434

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ADVANTAGES OF LC-MALDI APPROACH FOR PROTEOMICS APPLICATIONS

Z. Qi, D. Kesuma

Shimadzu Asia Pacific, Singapore

Proteome analysis was first performed with two-dimensional (2D) gel electrophoresis which has been used as the classical approach in proteomics research from mid 1990. A large number of proteins in the biological samples can be separated, collected in gel plugs, enzymatically digested and finally identified via PMF and/or MS/MS on a MALDI-TOF system. An online fully-automated approach, namely LC-NanoESI-MS/MS, was developed based on liquid chromatography separation with either a 1D or 2D configuration for pre-digested protein samples and tandem MS/MS analysis. LC-MALDI approach was established and used widely recently owing to its considerable advantages adopted from LC separation and MALDI-TOF analysis. In this presentation, the principles and instrumentation of the latest LC-MALDI system are described and compared with other approaches in terms of functionality and operation easiness. The three different approaches described are complementary rather than replacing with each other. There are various options and new instrumentation technologies available for setting up a LC-MALDI platform to best suit different applications. As the topic of choice, applications of LC-MALDI in protein identification for in-depth exploration of proteomes and isotope labeling quantitation for biomarker discovery based on MS (using 13CNBS reagent) or MS/MS (using ITRAQ reagent) methods are addressed.

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IMAGING MASS SPECTROMETRY (IMS) OF MURINE TISSUES USING A PIEZOELECTRIC PRINTER

P. Hoffmann , J. O.R. Gustafsson, S. R. McColl,

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At the Adelaide Proteomics Centre we are currently trialling and optimising our sample preparation and acquisition protocols for IMS. We will present results of piezoelectric droplet array matrix deposition onto tissue sections and comparisons of IMS results to histology.

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TARGETED PROTEIN QUANTITATION USING THE SRM WORKFLOW: MAKING SRM ASSAYS ROUTINE, ROBUST, AND SENSITIVE

A. Zumwalt

Thermo Fisher Scientific, United States

Greater emphasis has been placed on advancing proteomics studies from discovery and/or relative quantitation to validated quantitative methods in an effort to establish eventual clinical assays. The typical workflow involves first performing discovery-based experiments to identify protein expression levels that are confidently changing between a control and treated samples and to generate product ion information used to sequence the precursor peptide. Traditionally, the biggest challenge has come from transferring discovery-based methods directly to validated quantitation methods since each is typically performed on separate mass spec platforms.

This presentation introduce innovative triple stage quadrupole MS technology and how it fits into a complete targeted protein quantitation solution to advance research from discovery to verification with fewer steps and less sample consumption. In addition, a new software package designed to incorporate discovery data for rapid and confident SRM assay development will be discussed.

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BRINGING HIGH MASS ACCURACY, INCREASED DYNAMIC RANGE AND HIGH RESOLVING POWER TO TISSUE IMAGING WITH THE MALDI LTQ ORBITRAP™

M. Prieto Conaway

Thermo Fisher Scientific, San Jose, United States

The MS and MSn capabilities of the LTQ XL™ and Orbitrap XL™ (Thermo Fisher Scientific) mass spectrometers are demonstrated for the analysis of phospholipids, drugs and metabolites from tissue samples when coupled to a MALDI source.

The high resolving power of the Orbitrap XL allows the separation of closely spaced species in the full MS. Add to this an enhanced dynamic range and routine mass accuracy in the 1-5 ppm range to make for an ideal platform conveniently suited for the analysis of complex samples such as biological tissue.

Various data collection schemes can be employed depending on the information required: 1) an MS or MSn experiment over the tissue section to create 2- and 3-dimensional images of the distribution of several species or a particular drug or metabolite, 2) a spiral raster per pixel, providing a full MS and several MS/MS over the whole tissue section (through an inclusion list or data dependent), and 3) a 50 MS^2 data dependent experiment with dynamic exclusion that is repeated many times over a tissue section for structure elucidation of all species present.

Images are mapped with the aid of ImageQuest™ software (Thermo Fisher Scientific) to visualize the distribution of analytes and easily determine if the compound is derived from biological tissue or from MALDI matrix. Plotting options include normalization of data, various color patterns, ability to average or expand specific regions, overlaying of images, and the ability to visualize high resolution Orbitrap data.

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NEW DEVELOPMENTS IN ETD ON LTQ ORBITRAP XL AND ITS APPLICATIONS

T. Zhang

Thermo Fisher Scientific, San Jose, United States

Electron transfer dissociation is a powerful fragmentation method that significantly improves the analysis of proteins and peptides, and their post-translational modifications (PTMs). We've now combined ETD with the high resolution and high accurate mass capabilities inherent in the LTQ Orbitrap hybrid mass spectrometer to create the most advanced proteomics platform on the market, especially suited for complex PTM analysis, top-down and middle-down analysis, intelligent sequencing of peptides, and relative protein quantitation via label-free differential analysis or stable isotope labelling such as with the new TMT (Tandem Mass Tagging) technology. New applications of ETD on the LTQ Orbitrap ETD hybrid FT mass spectrometer and its software for post acquisition data analysis will be introduced in this presentation.

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SECURING YOUR IDENTITY - NEW STRINGENCY FOR PROTEIN IDENTIFICATIONS

S. Watt

Waters Australia, Australia

During the past decade mass spectrometry has become widely accepted as an essential tool to better understand protein function, facilitating both the identification and quantification of proteins in complex samples. Recently, a number of publications have noted that the stringency required for analysis of proteomics data has been underestimated. Many approaches, starting with sample preparation, through to LC-MSMS analysis, and ending with bioinformatics have used too few and too wide parameters to faithfully report accurate results. Rarely have analyses been replicated.

Here we present a new data-independent LC-MS/MS (nanoACQUITY UPLC System with new 2D Technology – The best in nano-scale chromatographic performance just got better) acquisition strategy to comprehensively identify proteins (The Identity^E High Definition Proteomics™ System).

The system combines a near 100% duty cycle for sampling precursor and product ions and includes comprehensive peptide ion accounting informatics. This software helps you visualize and identify peptides and proteins with a multi-layered physicochemical model of protein primary structure, for generation of the highest quality MRM transitions for tandem quadrupole biomarker verification and validation.

Details of the Verify^E System solution – The fastest transition from discovery to hypothesis-driven proteomics. Expertly selects proteotypic peptides for targeted protein assays with optimum MRM parameters will also be given.

This system will be discussed, providing detailed information on the acquisition strategy, and the physico-chemicals properties utilised in the ion accounting software. A comparison between The Identity^E System and traditional LC-MS/MS analysis will be presented, together with practical applications of this strategy to label free expression profiling.

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TECHNICAL HURDLES WHEN APPLYING ISOELECTRIC FOCUSING TO MEMBRANE PROTEOME ANALYSIS

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Li Ka Shing Institute of Health Sciences and Department of Medicine and Therapeu, The Chinese University of Hong Kong; Department of Chemistry, Hong Kong Baptist, Hong Kong

Membrane proteome is usually deciphered by undertaking shotgun approach. Isoelectric focusing (IEF) has been successfully applied to fractionate the peptides before subjecting to tandem mass spectrometry analysis. The hydrophobic property of plasma membrane proteins has limited the applications of IEF to their separation. It is not uncommon that membrane proteins are precipitated while they are being fractionated by preparative liquid-phase IEF. One possible reason is that membrane proteins become extremely hydrophobic and form aggregates when they loose their charges at a pH equal to their isoelectric points. Last time we reported an ampholyte-free IEF method for fractionation of proteins in the mouse liver microsomal membrane proteome standard by using Zoom IEF system or OFFGEL electrophoresis system in the presence of non-ionic detergent and glycerol. The proteins in each fraction are being identified by two approaches. In the first approach, proteins in each fraction will be resolved by SDS-PAGE, followed by in-gel digestion, and MALDI TOF/TOF MS analysis. In the second approach, proteins in each fraction will be subjected to in-solution digestion, followed by capillary LC separation and Q-TOF MS analysis. For the first approach, we have the difficulties in obtaining satisfactory digestion of the protein bands with trypsin and the typical trypsin digestion protocol. The 60% methanol denaturation protocol did not work, as 60% methanol dehydrated the gel slices. For in-solution digestion approach, we attempted to remove the interfering substances from the IEF fractions by dialysis against ammonium bicarbonate buffer. We observed that denaturation of the proteins with 60% methanol was needed in order to achieve satisfactory trypsin digestion. Unfortunately, even after extensive dialysis, interfering substance was still present and interfered the LC Q-TOF MS analysis. In our positive control experiments, 74 peptides were identified, and were corresponding to 17 proteins. According to the UniProt database, 3 are membrane proteins. TMHMM Server (v.2.0, http://www.cbs.dtu.dk/services/TMHMM-2.0/) predicted the presence of transmembrane domains in these 3 proteins. For the other 14 proteins, TMHMM Server predicted the absence of transmembrane domain. Among these 14 proteins, 3 are associated with plasma membrane, 1 with peroxisome, 1 with golgi apparatus, 1 with mitochondria and 5 with nucleus. (Part of this work was supported by the Li Ka Shing Foundation and the CUHK direct grant for research.)

POSTERS

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EFFECTS OF *FICUS DELTOIDEA* EXTRACT ON THE SERUM PROTEIN PROFILE OF SIMULTANEOUSLY HYPERTENSIVE RATS (SHR)

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There is a general trend of moving towards the use of alternative methods (in particular herbal medicine) in preference over conventional medicine in the treatment of illnesses. It is therefore with great interest that we chose to investigate the use of a common ornamental plant in the treatment of hypertension. In Malaysia, *Ficus deltoidea* or Mas Cotek is a plant that has traditionally been used to cure several pathological conditions including hypertension. In our study, we have shown that the extract from *F. deltoidea* fruits demonstrated inhibitory effects towards Angiotension-I Converting Enzyme (ACE) activity, suggesting that it may posses anti-hypertensive properties. In an attempt to understand the possible mechanisms involved in this phenomenon, rats were used as a model to study the effects of extract consumption on serum protein profile. Following a two week feeding regime the serum of treated rats were subjected to 2DE and the resulting serum protein profile compared with that of controls. A comparison between treated SHR, non-treated SHR and Sprague Dawly (SD - as normal controls) showed that at least 30 protein spots were significantly different in their expression profile. As these differentially expressed proteins may play roles in the physiological effects of F. deltoidea extracts, an understanding of their expression dynamics may lead to the elucidation of the mechanisms involved.

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MAGNETIC BEAD BASED AFFINITY PROFILING FOR BIOMARKER IDENTIFICATION: IDENTIFYING POTENTIAL PITFALLS

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Magnetic Bead Based affinity profiling for Biomarker Identification: Identifying potential pitfalls

The low molecular weight region of human serum has been identified as a potential source for biomarkers. The abundant secreted proteins in circulation tend to leave tell-tale fragment peptides which can be of use as surrogate biomarkers. Specifically proteases present in the clotting cascade are key functional components that contribute to this peptide pool. The magnetic bead based weak cation exchange technique is a simple, automatable, highly-reproducible affinity purification technique custom built for the peptidome profiling. Numerous studies which have previously utilized this technique have successfully utilized this technique for biomarker discovery. In our current study we look into much greater depth, the potential clinical and sample handling variables that might skew the results of a clinical proteomics/peptidomics strategy. We utilized magnetic bead based weak cation exchange in a technique standardization cohort (SC) comprising exclusively of controls (n=6) and a case/control (n=83) cohort (CCCC) of coronary artery disease samples. The SC cohort consisted of normal individuals whose serum samples were collected and serially analyzed for in-vitro pre-analytical variations due to, time since collection, temperature of storage and freeze thaw cycles. We found variation in all the three factors which seem to interfere with our inference of spectral classification in the case control cohort. We believe that stringent handling standards, that reduce in-vitro/ex-vivo artifacts, such as a) Sample collection and spectral generation should have minimal lag time b) Case control samples should not cluster into distict cluster times c) Avoiding use of archival samples unless exact storage and handling information is available d) Freeze thaw should be kept to a minimum and e) Lower the storage temperature for reduction in ex-vivo enzymatic activity of the coagulome are mandated before initiating clinical proteomic studies.

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PROTEOMIC ANALYSIS FOR IDENTIFICATION OF THERAPEUTIC TARGETS OF OVARIAN CLEAR CELL CARCINOMA

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Epithelial ovarian carcinoma is a morphologically and biologically heterogeneous disease, and is classified into four major histological types, serous, mucinous, endometrioid and clear cell adenocarcinoma (CCA). Among these types, CCA has a highly malignant potential as follows: the recurrence rate is higher even in early stage, the 3 and 5-year survival rates for patients are significantly lower, and the response rate to anticancer drugs including of platinum and taxane agents is lower. Therefore, it is necessary to find new therapeutic target for CCA.

To identify proteins expressed specifically in CCA, we initially carried out a shotgun analysis using the iTRAQ reagents and compared the proteomic patterns of three ovarian cancer cell lines, OVISE and OVTOKO derived from CCA, and MCAS from mucinous adenocarcinoma. Of the 1105 proteins detected in the analysis, 25 proteins were expressed higher (>2-fold) in OVISE and

OVTOKO than MCAS. Next, to investigate whether the increases in these expression were observed in other CCA cells, we performed Western blot and quantitative RT-PCR analyses using five non-CCA and six CCA cell lines. Expression levels of both mRNA and protein in annexin IV (ANX4), laminin B2, N-myc downstream regulated gene 1 protein (NDRG1) and double cortin domain containing protein 2 (DCDC2) were increased preferentially in the CCA cell lines. Gene silencing of ANX4, NDRG1 and DCDC2 by RNA interfering elicited marked suppression of cell proliferation in CCA, especially the expression of Bcl-2 mRNA, which is an apoptosis inhibitor, was decreased after knocking down ANX4. These results suggest that these proteins might serve as therapeutic targets for ovarian CCA.

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IDENTIFICATION OF COLORECTAL CANCER BIOMARKERS USING LASER MICRO-DISSECTIONA AND 2D DIGE

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A sensitive, specific biomarker panel for the diagnosis, prognosis and treatment of colorectal cancer (CRC) remains to be identified. The aim of this study was to discover potential biomarkers of CRC using laser microdissection (LMD) and 2 dimensional difference gel electrophoresis (2D DIGE). LMD was performed on 8 matched pairs of stage II colon tumour-normal tissue to enrich for epithelial cells prior to DIGE analysis. Following the DIGE technique samples were analysed using DeCyder software. Across the cohort 9 proteins were detected as increased in abundance in the tumour samples compared to normal on average >3 fold, $P< 0.05$. Sixteen proteins were detected as increased in abundance in the tumour samples compared to normal on average >2 fold, $P< 0.05$. These proteins were identified by linear ion-trap mass spectrometry. Four proteins have been chosen for verification in a larger cohort of patients by real time RT-PCR, western blotting, and immunofluorescence (IF). Vital cell DIGE and mass spectrometry was performed on the primary CRC cell line SW480, and its metastatic variant SW620 in order to identify differentially expressed membrane proteins. One protein found to be increased on the surface of the SW620 cells was also identified in the tumour sample DIGE work. The expression of this protein on the surface of CRC cell lines was verified by IF of permeabelised and unpermeabelised cells.

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DEVELOPMENT OF AN ENHANCED PROTEOMIC METHOD TO DETECT POTENTIAL PROGNOSTIC AND DIAGNOSTIC MARKERS OF HEALING IN CHRONIC WOUND FLUID

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Chronic venous leg ulcers are a significant cause of pain, immobility and decreased quality of life. Currently, research is focusing on multiple factors in the wound environment to provide information regarding the healing of ulcers. Chronic wound fluid (CWF), containing a complex mixture of proteins, is an important modulator of the wound environment, therefore we hypothesized that these proteins may be indicators of the status of wounds and their potential to heal or otherwise. To explore this we developed and validated a novel method to process CWF prior to proteomic analysis.

Pooled wound fluid samples were fractionated using the multiple affinity removal system (MARS) column as per the manufacturer's instructions. Two fractions containing high and low abundant proteins were collected, concentrated and desalted by reverse phase chromatography. Each fraction was evaluated using a range of techniques including western blotting, 2D gel electrophoresis, 2D-LC and MALDI and ESI mass spectrometry.

High performance liquid chromatography using the MARS column resulted in the separation of protein present in CWF into two distinct fractions. Western Blotting and 2D gel electrophoresis demonstrated that high abundant proteins were selectively removed from CWF resulting in the enrichment of low abundant proteins. Additionally, mass spectrometry analysis on pre- and post-depleted fractions suggested that removal of these abundant proteins increased detection of other proteins in these samples.

The results obtained suggest that this approach improves separation of proteins present in low concentrations in CWF, potentially allowing the identification of diagnostic and prognostic markers in sequential samples collected from patients with venous leg ulcers.

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MASS SPECTROMETRY-BASED ANALYSIS OF TYROSINE PHOSPHOPROTEOMICS AND IDENTIFICAITON OF SUBSTRATES OF PROTEIN TYROSINE PHOSPHATASE DPTP61F IN *DROSOPHILA* S2 CELLS.

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The reversible tyrosine phosphorylation governed by the coordinated action between protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) is the key regulatory event that controls animal development and physiological homeostasis. The fruit fly *Drosophila melanogester* has been used extensively as a model organism for investigating the developmental processes but the state of its tyrosine phosphorylation is poorly characterized. In the current study, we used advanced mass spectrometry (MS)-based shotgun analyses to profile the tyrosine phosphoproteome of *Drosophila* S2 cells. Using immunoaffinity isolation of the phosphotyrosine (pTyr) subproteome from cells treated with pervanadate followed by enrichment of phosphopeptides, we identified 562 non-redundant pTyr sites in 245 proteins. Both this pre-defined pTyr proteome subset and the total cell lysates were then used as sample sources to identify potential substrates of dPTP61F (homolog of human PTP1B and T Cell-PTP) by substrate trapping. In total, 20 unique proteins were found to be specifically associated with the trapping mutant form of dPTP61F, eluted by vanadate (VO_4^{3-}), and identified by MS analyses. Among them, 16 potential substrates were confirmed as tyrosine phosphorylated proteins, including a receptor PTK PDGF/VEGF receptor, a cytosolic PTK Abl and several components of SCAR/WAVE complex. Based on these findings, we have designed additional experiments to investigate the functional role of dPTP61F in regulating cell signaling by dephosphorylation of its potential substrates. Employing biochemical and genetic approaches, we showed that dPTP61F interacts with components of SCAR/WAVE complex in vivo, thus validating the MS-based analyses for unbiased identification of PTP substrates.

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PROTEOME ANALYSIS OF MEMBRANE-ASSOCIATED EVENTS DURING EARLY STAGES OF THE EPITHELIAL-MESENCHYMAL TRANSITION

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Epithelial-Mesenchymal Transition (EMT) is a fundamental cellular process that occurs during embryonic development, wound healing, organ fibrosis and tumor invasion and metastasis. The molecular mechanism of EMT remains largely unknown; therefore, revealing the intricate mechanisms underlying EMT may help us to better understand how primary tumors become invasive and metastasize.

The Madin-Darby canine kidney (MDCK) cell line is a polarized epithelial cell line that has been shown to be transformed into mesenchymal-like cells, when simultaneously stimulated by H-Ras and TGF- β . A hallmark of the early stage of EMT is the loss of cell polarity that occurs at the plasma membrane; moreover, a coordinated interaction of membrane proteins, such like transporters, linkers, receptors and enzyme, may play a crucial role in EMT progression. The main goal of this study is to develop a method for enriching membrane proteins and reducing proteomic complexity. Quantitative proteomic approaches will be used to compare the membrane proteome of modified MDCK cells in the early stage of EMT. Understanding of the molecular changes of the membrane proteome associated with EMT may help to provide a detailed insight into the mechanistic events underlying EMT.

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THE STIMULATORY EFFECT OF VARIOUS SALTS ON YEAST ALCOHOL DEHYDROGENASE ACTIVITY

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It has been known that enzyme activity is influenced by various factors such as temperature, pH, salts and pressure. However these effects are different for kind of enzyme. When we investigated the inactivation process of yeast alcohol dehydrogenase (YADH) by typical chemical denaturants, urea and GdmCl, we noticed that these effects are significantly different. The activity of YADH decreased gradually with increasing the urea concentration. On the other hand, the activity increased with increasing GdmHCl concentration up to 0.1~0.2 M, and it decreased at above 0.2 M GdmHCl. Also, the similar activation was observed in the solution adding only NaCl without the denaturing agent. From these result, it is clarified that the YADH activity is influenced by added salts. So, in this experiment, we discuss the relation between the activation of YADH and the kind of adding salts. When compared the activity change by anion and cation of same ionic radius, it was more influenced by anions than by cations. The significant difference

was not observed among cations. When these salt effects were compared with various physical properties of salt, it is clarified that the concentration at maximum activity shows the good correlation with B-coefficient. Anion which has smaller B-coefficient shows the maximum of activity at lower ion concentration. Consequently our experiment clarified to have the influence by low concentration in anion as the large ion of B-cofficient which destructs the water structure. Therefore, it is suggested that the interaction between anion and enzyme is important factor for the activation of YADH by salts. Also, it is clarified that the negative hydration ions have effect on YADH activity at small amounts.

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MICROBIAL STRUCTURAL GENOMICS: IMPORTANT BIOLOGICAL FUNCTIONS EXECUTED BY INTERESTING PROTEIN STRUCTURES

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Structural genomics is crucial for understanding the intricate interactions among proteins in a whole organism. We have studied the structural genomics of *Xanthomonas campestris* (*Xcc*), a gram-negative bacterium that is phytopathogenic to cruciferous plants and causes worldwide agricultural loss. *Xcc* is the only bacterium known to lack a cAMP signaling system, and uses a cAMP-receptor protein like protein (CLP) system instead. Currently we are working on its flagellar and SOS structural genomics.

In the flagellar system, we have solved the first crystal structure of a hook-capping protein FlgD. The core structure reveals a novel hybrid comprising a tudor-like domain interdigitated with a fibronectin type III domain. In the crystal, the monomers form an annular pentamer of dimers of pseudo five-fold symmetry, due to the different dimer-dimer interactions incorporated. The resulting asymmetrical star-like decamer complex has a outer dimensions of approximately 110 A x 90 A x 65 A, and a shortest diameter of approximately 20 A in the center. The outer dimensions of the atomic *Xcc* hook-capping FlgD complex turn out to be very similar to those of the *Salmonella* filament cap complex observed by electron microscopy.

SOS has been the most intensively studied system induced under DNA damage, and is characterized by the induction of more than 20 genes, which are under the control of LexA. In response to DNA damage, RecA is activated to induce the auto-cleavage of LexA, resulting in de-repression of genes in the SOS regulon. The recX gene is co-transcribed with recA and its product is suggested to regulate RecA function by directly interacting with RecA protein. We have solved the first RecX structure to a resolution of 1.6 A . It is a curved structure comprising three tandem repeats R1, R2 and R3 of three-helix bundles . Model studies indicate RecX can fit into the helical groove of the RecA filament very well, similar to that reported for the cryoEM image of the RecA/RecX/ATP/ssDNA complex.

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COUPLING MALDI MS WITH HIGH-EFFICIENCY ION MOBILITY SPECTROMETRY FOR TISSUE IMAGING OF LOW MASS ENDOGENOUS COMPOUNDS

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The application of MALDI mass spectrometers to determine the spatial distribution of endogenous and exogenous chemical species in tissue is a rapidly developing area of research. It can provide complementary information to traditional costly and time consuming techniques, such as autoradiography.

The two main instrumental challenges for the mass spectrometric analysis of tissue samples are sensitivity and specificity, *i.e.* how well the compound of interest can be distinguished from background ions.

A means of increasing the separating power of a MALDI imaging experiment is the use of a high efficiency ion mobility spectrometry, coupled with time-of-flight mass spectrometry which offers a new dimension of separation. Using this technique it is possible to separate different compound classes.

The sample studied was a thin section of rat kidney. A 12μm section was produced using a cryotome and deposited onto thick aluminium foil. Several coats of α-cyano-4-hydroxycinnamic acid matrix were evenly deposited onto the sample using an airbrush, and the sample was subsequently mounted onto a target plate. The tissue area was selected, imaged by MALDI IMS-MS. All data were acquired on a MALDI hybrid orthogonal acceleration time-of-flight mass spectrometer. After acquisition IMS-MS data were evaluated in sofwtaer to export regions of drift time vs m/z.. Data were converted into Analyze file format and subsequently analysed using BioMap (Novartis, CH).

We have acquired MALDI imaging data from a rat kidney section demonstrating the separation of endogenous metabolites from nominally isobaric background ions using IMS-MS. The separation of the ions by IMS is based upon their different mobility as they move through a dense buffer gas under the influence of an electric field. This gas-pahse separation is performed after ionisation and

is therefore well suited to imaging, as it does not require modification of the sample preparation protocol. We will show that it is possible to produce distinct images for the separated compounds. An example will be shown where the intensity contribution to an image from a matrix dimer ion could be completely removed from an ion intensity image of an endogenous compound, resulting in an image solely attributable to the compound of interest, thus providing clear spatial localisation of the endogenous metabolite of interest.

We will also present data on the distribution of different phosphatidylcholines and their class assignment through the use of exact mass and CID.

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A SENSITIVE MAGNETIC BEAD APPROACH FOR THE DETECTION AND IDENTIFICATION OF TYROSINE PHOSPHORYLATION IN PROTEINS BY MALDI- TOF/TOF MASS SPECTROMETRY.

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Phosphorylation is one of the most important post translational modifications (PTMs) and its perturbed regulation has been implicated in many pathologies. The rarity of phosphotyrosine compared with phosphoserine or phosphothreonine PTMs is prompting the development of more sensitive approaches as proteomic technologies that are currently used to assess tyrosine phosphorylation in proteins are inadequate, identifying only a fraction of the predicted tyrosine phosphoproteome. Here we describe the development of a high-throughput, high sensitivity methodology for the detection and mapping of phosphotyrosine residues by MS. The anti-phosphotyrosine Antibody (Ab) 4G10 was coupled covalently to super para-magnetic beads or by affinity to super para-magnetic beads with protein G covalently attached. Using this approach we successfully enriched phosphotyrosine peptides mixed with non-phosphorylated peptides at a ratio of up to 1:200, enabling detection at a level representing the highest sensitivity reported for tyrosine phosphorylation. The beads were subsequently used to enrich tyrosine phosphopeptides from a digest of the *in vitro*-phosphorylated recombinant β-intracellular region of the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor which was analysed by MALDI-TOF/TOF MS. Analysis of immunoprecipitated receptors from *in vitro* cell systems before and after stimulation were also analysed for changes in tyrosine phosphorylation. Our results define this methodology as a sensitive approach for tyrosine phosphoproteome analysis.

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A COMPREHENSIVE UNDERSTANDING OF ADAPTATION OF THE ENTERIC PATHOGEN *VIBRIO CHOLERAE* TO BILE

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Enteric pathogens account for a greatly underappreciated burden of morbidity and mortality not only in developing countries where they exist in endemic form but also in developed nations where they cause frequent outbreaks. In the United States diarrhea is the second most common infectious illness. Data compiled by WHO indicate that diarrheal diseases account for 15 to 34 percent of all deaths in certain countries. Conservative estimates place the death toll at 4 million to 6 million per year. The situation is seriously complicated by the rapid emergence of new antibiotic resistant strains which has necessitated the identification of new therapeutic targets. Enteric pathogens must necessarily survive in the intestine in the presence of bile. A thorough understanding of the strategies adopted by enteric bacteria to survive in the presence of bile would provide opportunities to identify new drug targets whose inactivation would impair intestinal survival. In this context the response of *Vibrio cholerae*, the causative agent of the diarrhoeal disease cholera, to bile has been investigated using proteomic approaches. The production and /or activities of a large number of proteins belonging to different functional categories were found to be modulated by the presence of bile. These include membrane proteins, general stress proteins, proteins involved in metabolic pathways, energy production, motility, as well as a number of transcription regulators and translation elongation factors. Further analysis r evealed metabolic pathways involved in the adaptation of *V. cholerae* to bile. Moreover, interesting indications of bile induced post-translational modifications have been obtained.

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THE PROTEOME OF HUMAN PAROTID AND SUBMANDIBULAR/SUBLINGUAL GLAND SALIVAS

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Human saliva is an important body fluid that has many functions including digestion, lubrication and hydration, and protection of the oral cavity from mechanical and chemical stress, as well as protection from microbial invasion. Many of these functions are carried out by salivary proteins, but their full role in oral processes and as indicators of disease states are poorly understood. Given the role of saliva in human health, we sought to generate a comprehensive view of the human saliva proteome. A consortium of three research groups has carried out extensive proteomic analysis of saliva samples using different mass spectrometry methodologies. We report the results of these experiments, which generated a list of 1166 salivary proteins. Gene ontology of these proteins show that they cover a wide range of functional classes including multiple metabolic and regulatory pathways, defense and healing, binding and catalysis. Comparison of the saliva proteome was made with published reports of human plasma and tear proteomes. Of 657 known plasma proteins, 192 (29.22%) were found in saliva, and 259 saliva proteins were found in lacrimal gland secretions, representing 55% of the known tear proteome. Within the saliva proteome, multiple proteins that are associated with human disease were found. Comprehensive analysis of the saliva proteome is the first step toward identification of aberrations which will allow development of a non-invasive, information-rich resource of biomarkers to track human disease. The Saliva Proteome Consortium: L.Liao[1], D. Cociorva[1], X. Han[1], J. Hewel[1], S. Park[1], T. Xu[1], P. Denny[2], T. Denny[2], J. Gilligan[2], M. Navazesh[2], J. Takashima[2], F. Hagen[3], G. Bedi[3], M. Gonzalez-Begne[3], J. Melvin[3], M. Sullivan[3], M. Hardt[4], S. Hall[4,8], O. Miroshnychenko[4], R. Niles[4], A. Prakobphol[4], S. Robinson[4], H. Witkowska[4,8], W. Yan[5], P. Boontheung[5], J. Dunsmore[5], P. Ramachandran[5], M. Sondej[5], Y. Xie[5], J. Ytterberg[5], M. Arellano[6], B. Henson[6], S. Hu[6], S. Jeffrey[6], J. Jiang[6], M. Richert[6], S. Than[6], J. Wang[6], L. Wolinsky[6], W. Yu[6], S. Bassilian[7], K. Faull[7], F. Hagland[7], P. Souda[7], J. Whitelegge[7], S. Fisher[4,8,10,15,16], J. Loo[5,9,11,14], D. Wong[6,9,11,13,14]

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INTERCALATED DISC: CHANGES IN MULTIPLE PROTEINS ASSOCIATED WITH HEART FAILURE

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The intercalated disc (ICD) occupies a central position in the transmission of force and electrical continuity between cardiomyocytes (CMs). Changes in its structure and composition strongly implicate heart failure with ICD. Its functions include: (1) the maintenance of electrical continuity across the ICD (gap junctions); (2) physically linking across the membranes and continuous with the cytoskeleton of adjacent CMs (desmosomes); (3) extracellular adherence between two cells (fascia adherens); and more recently (4) the addition of sarcomeres at the ICD during cell growth (the transitional junctions). The protein composition of each of these includes a much wider range of proteins than has been previously reported. Currently, 174 proteins have been associated with ICDs, approximately 42% of which are known to change in various heart diseases. This is the first comprehensive analysis, particularly focused on the proteins of the ICD that change in heart failure. Changes include: (1) membrane adhesion proteins (8 of 15 proteins); (2) cell anchoring, binding, and linking proteins (22 of 56 proteins); (3) channels (12 of 28 proteins); (4) enzymes (13 of 31 proteins); (5) ligands and ligand receptors (7 of 10 proteins); (6) other proteins that maintain the structure and functions of the ICD (3 of 8 proteins); (7) mechanoreceptors (4 of 5 proteins); and (8) 19 other proteins. Reports of changes in the relevant proteins (proteomics) can only be tested if there are corresponding antibodies available. Fortunately, 138 ICD proteins have corresponding antibodies of which 79% are commercially available. This will open the way for new strategies for dealing with human heart failure. We plan to revisit the expression levels of the above proteins using ventricular tissue microarrays (TMAs) where nine tissue samples were non-failing donor hearts and 45 tissue samples were of diseased (failing) left ventricles.

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ISOLATION AND EVALUATION OF DIFFERENT PEROXISOMES SUBPPOPULATIONS FROM RAT LIVER

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The preparative dissection of cells into their substructure reduces sample complexity and facilitates functional analysis of proteins in a physiological context. A prerequisite for a fundamental characterization of these organellar subpopulations on the proteome level, however, are highly pure fractions, which could not be obtained by classical separation technologies. These organellar subpopulations are supposed to be linked to specialized biological functions or different development stages.

We recently have developed a workflow for the subcellular fractionation of the heavy mitochondria pellet using BD Free Flow Electrophoresis System (FFE). The FFE methodology relies on the net charge of the particles caused by protein domains extending from the organellar surface. According to their surface charge and size, particles are deflected differently in an electric field and are separated through a buffer flow perpendicular to the electric field. With this technique a second dimension separation step, as unlike centrifugation techniques was added to the workflow. Using this workflow successful isolations of various cellular structures have been obtained.

Methods: We invented F FE as an additional purification step to purify a 2.860 ´ g fraction of rat liver tissue which mainly contained heavy mitochondria but also peroxisomes and lysosomes, so called “high density peroxisomes and lysosomes”. The resulting pellet was loaded on an Optiprep gradient. Each density gradient fraction and the classical peroxisomal fraction, caused by 28000x g were subjected to FFE.

To validate the purity and integrity of sub-fractions we performed enzyme assays for organellar markers and immunoblots with specific antibodies as well as electron microscopy.

Results: Subcellular fractionation using the FFE-system indicated that the purification was selective on the organelle level, and the suitability of this separation technique in proteomic research. Furthermore peroxisomes subpopulations were analyzed by mass spectrometry.

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LOOP LENGTH DEPENDENT SVM PREDICTION OF DOMAIN LINKERS

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The practical importance of the prediction of structural domains in un-annotated amino acid sequences has increased as they represent valuable targets readily characterized by high throughput methods.

Here we report a support vector machine (SVM) prediction of domain linkers, which are loop regions separating two structural domains . The SVM training data set comprised 182 protein sequences from SCOP database, which contained at least one domain linker regions (all). Furthermore, the data set was divided into long (longer than 9 residues) and short (shorter than or equal to 9 residues) linker sequences. Using these data sets, we constructed three loop length dependent SVMs (SVM-All, SVM-Long, and SVM-Short) , which were trained using all, long and short linkers, respectively. In addition, our new SVM input data used a position specific scoring matrix (PSSM) and predicted secondary structure information (PSS) .

A five-fold cross validation test indicates that the area under the ROC (receiver operating characteristics) curve (AUC) value, which represents the prediction performance, of SVM-All, SVM-Long and SVM-Short were 0.763, 0.759 and 0.759, respectively. Our previous SVMs, which used only amino acid sequence information, indicated prediction performances of 0.692, 0.702, and 0.605, for SVM-All, SVM-Long and SVM-Short respectively. The prediction performances of our new predictors thus were over 10% higher than those of our previous methods, Armadillo[(1)] (AUC value: 0.610 Dumontier et. al. *J Mol Biol* 2005), and neural network based method[(2)] (AUC value: 0.642 Miyazaki et. al. *BMC Bioinformatics* 2006). These results demonstrate the efficiency of our new methods. Thus, the performance has been improved by the inclusion of PSSM and PSS, in addition to sequences.

At the meeting, we will report the detail of the methods, and will also report an application of our predictors for large scale domain linker prediction and a comparison of the results with the PROSITE data base.

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DIFFERENTIAL MOLECULAR ANALYSIS OF HUMAN EMBRYONIC STEM CELLS VERSUS EMBYOIDE BODIES

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Proteomics showed to be a powerful approach to comprehensively unravel the regulatory networks of differentiation. We applied a two dimensional gel electrophoresis based proteomic approach followed by mass spectrometry to analyze the proteome of two Human ESC lines, Royan H5 and Royan H6, at 0, 3, 6, 12 and 20 days after differentiation initiation. Out of 127 differentiation associated proteins detected in two lines, 35 were common. Mass spectrometry analysis of these protein spots led to identification of 92 proteins. Our results showed that proteins involved in signal transduction, metabolism, cell motility and transport are the major ESC-associated proteins. The expression of several proteins were further confirmed by western blotting and immunocytochemistry. Transcriptome analysis revealed down-regulation of OCT4, NANOG, UTF1, ZNF206 key transcription factors in hESCs and up-regulation of three lineage specific markers VIM, MAP2, OLIG2 (Ectodermic)., GATA4, INS, FOXA2 (Endodermic)., HAND1, COL2A1 (Mesodermic). Integration of transcripteome and proteome data could be useful for finding of new mechanisms that controls differentiation. Several novel ESC-associated genes have been identified in this study which warrants further investigation with respect to the etiology of stemness.

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ENRICHMENT OF INTERLEUKINS AND LOW ABUNDANCE PROTEINS FROM TISSUE LEAKAGE IN SERUM PROTEOME STUDIES USING PROTEOMINER™ BEADS

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Human serum contains among others interleukins and tissue leakage proteins, for example basic myelin protein. These proteins are present in serum at very low concentrations ranging from several picograms to low nanograms per milliliter. Although the presence of these proteins in serum reflects the physiological condition of the human body and may be used as disease biomarkers for clinical diagnosis or prognosis, their detection remains a great challenge due to the presence of high abundance serum proteins such as albumin, immunoglobins and others. ProteoMiner™ is a protein fractionation technology based on a combinatorial peptide library bound to chromatographic beads and is used to reduce the amount of the high abundance serum proteins and to increase the relative amount of low abundance proteins To determine whether ProteoMiner™ could facilitate the detection of interleukins and proteins from tissue leakage in serum, we mixed an artificial serum using the 12 most abundant serum proteins (albumin, IgG, haptoglobin, retinol binding protein, myelin basic protein, troponin, IL-8, IL-2 etc.), tissue leakage proteins and interleukins in the concentration ranges reported for normal human serum. Subsequently, this artificial serum sample was fractionated by ProteoMiner™ technology. The proteins in ProteoMiner™ bound fraction were further separated either via 2-dimensional gel electrophoresis followed by mass spectrometric protein identification or the low abundance proteins were detected via western blotting with the unfractionated artificial serum serving as control.

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IMAGING MASS SPECTROMETRY (IMS) AND ITS APPLICATION TO MURINE TISSUES

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Imaging mass spectrometry [MS] has seen rapid implementation in numerous laboratories since its novel application in 1997 (1). The protocol, which involves linear MS acquisition from a tissue section coated in a suitable matrix [typically saturated sinapinic acid], is relatively simple, high throughput and delivers distribution data for potentially thousands of individual proteins. Considering the requirement for spatial data to complement the sensitive quantitative protocols in proteomics, it is thus a priority to develop imaging MS to a fully fledged experimental platform for tissue analysis. Furthermore, due to implications for clinical diagnostics and biomarker discovery (2, 3), the importance of this technology has extended beyond the research laboratory.

Applying both automated droplet deposition and vibrational vaporisation deposition we were able to show, similar to previous work, distinct molecular patterns within murine tissues (4-6). During this preliminary work it was important to both be aware of and overcome the differences between experimental tissues, predominantly in terms of tissue preparation and matrix deposition. Key issues for optimisation included matrix crystallisation, reproducibility of matrix deposition and protein diffusion: the latter two being intimately linked. Imaging MS has shown its applicability as a future proteomics platform and combined with established proteomic techniques can allow rapid, comprehensive and high throughput laboratory tissue characterisation to become reality.

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APPLICATION OF ARGININE TO INCREASE THE SOLUBILITY OF POORLY WATER-SOLUBLE COMPOUNDS

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Aqueous solubility of low molecular weight drug substances and biological products such as antibodies is one of the major problems in their development as pharmacological agents. Poor aqueous solubility of drug substances hampers the drug screening and pharmacological bioavailability. We show a novel approach to increase the solubility of organic compounds in aqueous solution using arginine as an additive. We used coumarin and caffeine as model organic compounds in this study. Arginine increased the solubility of coumarin, but not caffeine, suggesting the favorable interaction of arginine with the aromatic structure of coumarin. Salting-in salts as additive increased both coumarin and caffeine solubilites, while salting-out salts decreased them. In these salts solution, the solubility of coumarin and caffeine were correlated with the molar surface tension increment of the salts. The result suggested a peculiarity and an availability of arginine as a pharmacological and a solubilizing agent. This knowledge will be applied to improve the poor solubility of biological products such as antibodies as well as low molecular weight drugs.

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STRUCTURAL BASIS FOR NOVEL INTERACTIONS BETWEEN HUMAN TRANSLESION SYNTHESIS POLYMERASES AND PCNA

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DNA is continually damaged by external and internal agents. DNA lesions in the template strand block the progression of replication fork. Translesion synthesis (TLS) is makeshift DNA synthesis by TLS polymerases at DNA lesion site and requires replacement of replicative polymerases to TLS polymerases. This replacement, termed as polymerase switching, must be stringently regulated to avoid unfavorable mutagenesis. Replicative and several TLS polymerases interact with proliferating cell nuclear antigen (PCNA) through PCNA interacting protein box (PIP-box). PCNA is supposed to play a crucial role as a scaffold in the polymerase switching in TLS. However, interaction between TLS polymerase and PCNA remains unclear.

To clarify the interaction between TLS polymerase and PCNA, we have determined the X-ray crystal structures of human PCNA in complex with non-canonical PIP-box regions of human TLS polymerases, hPolη , hPolι, and hPolκ. The structures revealed that the interactions through their non-canonical PIP-boxes with PCNA are strikingly distinct each other, and those are also distinct from already known interaction by the canonical PIP-box. These results provide significant clues to understand the polymerase switching and pave the way for the further analyses of the protein-protein interactions involved in TLS.

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COMPARATIVE PROTEOMICS REVEALING CYTOSKELETON REMODELING UPON UV-IRRADIATION INDUCED CELL APOPTOSIS

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Apoptosis, a programmed cell death playing pivotal roles in development, cell homeostasis, and immune regulation, is characterized by sequential morphology changes of cells. Though apoptosis regulatory pathways have been thoroughly investigated, little has been addressed on its proteome changes, particularly related to cell morpholgoy evolving. We used 2-DE to profile the sequential proteome changes of human hepatoma cells, Mahlavu, Hep3B and SK-Hep1, upon UV-B irradiation induced cell apoptosis. Of the

43 deregulated proteins, 8 and 6 belong to cytoskeleton and mitochondria proteins, respectively, while 7 and 8 are involved in regulation of cell death and differentiation, respectively, suggesting a strong association between cell apoptosis and cytoskeleton remodeling. Confocal microscopy revealed dramatic cytoskeleton remodeling along with cell morphological changes during cell apoptosis. Upon UV irradiation, cells lost their polarity with distortion of filamental actins including transforming the stress fiber, filopodia and lamillopodia to a non-polar scaffold of the cell, while microtubules altered to a meshwork encompassing the condensed nucleus. The membrane blebs were primary shaped by the extruding microtubules in fibroblasts, whereas they were propped up by filamental actins in hepatoma cells. Exclusion of condensed and fragmented nucleic acid from the end-stage apoptotic bodies was frequently found in hepatoma cells. Our findings for the first time profiled the proteome dynamics in association with cytoskeleton remodeling during cell apoptosis. The difference of cytoskeleton structures in both UV treated and un-treated fibroblasts and hepatoma cells provided the information for invasion activities of hepatom cells and the potential targets for development of anti-hepatoma therapy in the future.

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THE UTILITY OF ION MOBILITY SPECTROMETRY TO SEPARATE CANDIDATE PRECURSORS FROM BACKGROUND IONS AND SPECIES OF DIFFERENT CHARGE STATES IN TANDEM MS EXPERIMENTS

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As a technique for characterising proteins, tryptic digestion followed by data dependant LC-MS/MS is well established. However, in complex biological mixtures, with a wide dynamic range, the majority of the peptides observed are in the lowest order of magnitude that can be detected, and despite tandem MS experiments, singly charged chemical noise present in the MS/MS spectrum can hinder precursor identification. In addition, cross-linked peptides containing >2 charges are often at low stoichiometry compared with tryptic peptides, and as such it may be difficult for the mass spectrometer to identify these in the MS survey as candidate precursors.

Ion Mobility Spectrometry separates by Drift Time and mass-to-charge ratio leading to an increase in the signal-to-noise ratio of these low level species as they are separated from the chemical noise, allowing the mass spectrometer to clearly identify them as candidates for MSMS.

All data was generated using a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer incorporating a travelling wave ion mobility separation stage. Species from protein tryptic digests were introduced to the MS by separation on a nanoscale UPLC system or by direct infusion, both coupled to a nanoelectrospray emitter. The system processes and displays, a specific band of the m/z versus drift time plot in real time. From this precursors are automatically selected for tandem MS/MS experiments. A further criterion of ion intensity was used to select suitable precursor ions for MS/MS interrogation. Selected precursors were isolated by the quadrupole and, whilst maintaining ion mobility separation, were subjected to CID fragmentation by elevating the collision energy after the IMS device.

Examination of the data contained in a m/z versus drift time plot shows clear separation of tryptic peptides based upon charge state can be achieved using an ion mobility device. By programming the mass spectrometer to only consider the selection of species in the area containing species of charge state 2+ or greater, we have analysed a dilution series of a standard four protein tryptic digest to show how the enhanced signal to noise of low level species detected by the mass spectrometer in survey scans can lower the limits of detection of the tandem MS/MS approach. In addition, the subsequent separation of precursor ions, from background ions, by IMS prior to CID leads to high quality MS/MS data of these species. We will show data from the injection of less than 100amol of a four protein digest mixture on column will be shown - these amounts being at a level where a TOF only survey identification is hindered by the presence of the background ions.

In the case of cross-linked peptides data from a tryptic digest of Bovine Serum Albumin was prepared without reduction and alkylation. This preserves the disulphide bonds and produces numerous large peptides similar in size and structure to chemically cross linked species. We will show that the disulphide linked peptides are ion mobility separated from the more intense doubly charged species present in the digest. Then, by selecting the region of the m/z versus drift time plot containing species with 3 or more charges, the mass spectrometer is programmed to generate MSMS spectra specific to the disulphide intact peptides contained in this region.

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MS^2 AND MS^3 PROPERTIES OF PARTIALLY DEGLYCOSYLATED CORE FUCOSYLATED GLYCOPEPTIDES IN ION TRAP

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Large scale identification and quantification of core fucosylated glycoproteins are crucial for discovery and validation of fucosylation related cancer biomarkers. However, in glycoproteome research, mass spectra of glycopeptides are usually abandoned, because their tandem MS spectra are too complex to identify. In this research, we analyzed the MS^2 and MS^3 properties of partial deglycosylated core fucosylated glycopeptides in ion trap, which is much helpful for large scale and automatic identification of the core fucosylated glycoprotein. Several properties were illustrated. First, the retained intact GlcNAc residue frequently lost from the b and y ions, so these kinds of special product ions must be synchronously considered with GlcNAc attached b and y ions in searching. Second, losses sourcing from partial GlcNAc fragmentation were observed, which would often bring strong peaks. Third, diagnostic ions from GlcNAc residue were observed in MS^3 spectra in the low m/z range. Forth, Na^+ can instead of H^+ for ionization, but their intensities of fragment ions were much worse than parent ions only charged by H^+. To void these interferers, these properties should be considered before searching database, and it would be much helpful for de novo analysis.

Keywords: Core fucosylation / Ion trap / Glycopeptide / Tandem MS

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COMPARATIVE PROTEOMIC ANALYSIS OF DRUG SODIUM IRON CHLOROPHYLLIN ADDITION TO HEP3B CELL LINE

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The human hepatoma Hep3B cell line was chosen as an experimental model for in vitro test of drug screen. The series chlorophyllin derivatives include chlorophyllin, fluo- chlorophyllin, sodium copper chlorophyllin, sodium iron chlorophyllin. MTT method was used in this study to get primary screen result. The result showed that sodium iron chlorophyllin had the best LC_{50} value. Proteomics analysis was performed for further investigation for the effect of sodium iron chlorophyllin to the Hep3B cell line. In this proteomic expression analysis, total protein extract of Hep3B and the protein after the drug addition were compared by 2D-gel. Then 35 three-fold differential expressed proteins were successfully identified by MALDI-TOF-TOF-MS. Among these 35 proteins, there are 16 unique proteins. These proteins include, proliferating cell nuclear antigen, T-complex protein, heterogeneous nuclear protein and peroxiredoxin. Peroxiredoxin has anti-oxidant function and is related to cell proliferation, signal transduction. It can protect oxidation of other proteins. Peroxiredoxin has the close relationship with cancer and can eventually become disease biomarker. This might provide new treatment method for carcinoma cancer.

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IMPROVEMENT OF ORTHOGONALITY BETWEEN THE AMBER SUPPRESSION SYSTEM AND THE TRANSLATION SYSTEM OF ECOLI

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The amber suppression system is the system to incorporate amino acids to the location indicated by the amber codon, and the system is practically used for incorporation of non natural amino acids to non natural proteins. However, there occurs a problem of miss charging due to insufficient orthogonality between the amber suppression.system and the translation system of the host cell, The

problem would deteriorate the quality of synthesized non natural proteins due to contamination of miss charged proteins. Some aminoacyl-tRNA sythetases of the host cell recognize the tRNA of the amber suppression system to incorporate the other natural amino acids into the location of the amber codon. In order to improve the ill orthogonality between the two systems, we performed mutation of tRNA of the amber suppression systems not to be recognized by the amino acyl-tRNA synthetases derived from Ecoli as the host cell. After some experiment and consideration, we have come to an idea that it is possible to improve the orthogonality by randomly mutate the acceptor stem of the amber suppression tRNA while conserving C1/G71 pair. As the results, we obtained some mutants of the original amber suppression tRNA that are not recognized by the aminoacyl-tRNA sythetases of Ecoli cell for the miss charging The system obtained by this experiment would improve quality of synthesized proteins incorporation by various non natural proteins.

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INVESTIGATEION OF REQUIREMENTS FOR THE KMSKS LOOP IN AMINOACYL-TRNA SYNTHETASE BY RANDOM PCR METHOD

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KMSKS loop in aminoacyl-tRNA synthetase is a signature motif which is related to ATP reactions in aminoacylation process, and amino acids in the loop are highly conserved over species. Although, various analyses on the mechanism of the loop have been performed, concrete rules for the loop to keep the activation have not been revealed yet. Today's bioinformatics approaches generally employ statistical analyses of the databases, and those approaches have been proved to be quite successful in finding motifs of proteins. Although such the conserved motifs are assumed to have the optimal sequences, they may yield revolutionally initial state. Therefore it would be significant to build mutant libraries without the bias from the wild-type motif, and to discuss their purely chemical characteristics. For that purpose, the loop of EG-KMSSS-KG in Tyr-RS of *Methanococcus jannashii* was replaced by a hundred of random sequences, and their activities were evaluated by the Amber suppression method. From the statistics, some important rules were suggested as follows. At first, positively charged side chains are important to bind ATP which is charged negatively because of phosphate groups. In the second, negatively charged side chains are not allowed because they should repel the ATP with negatively charged phosphate groups. In the third, Ser,Gly and Ala are preferable in order to keep the loop flexible due to their small side chains with poor reactivity.

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PROTEOMIC ANALYSIS OF NUCLEAR MEMBRANE IN HCV INDUCED LIVER CIRRHOSIS

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Proteomic information about the hepatitis C virus (HCV) infection leads to a better understanding of the protein function and interaction in liver cirrhosis . HCV is a causative agent of chronic liver disease leading to cirrhosis, liver failure and hepatocellular carcinoma with anticipated prevalence of 3% of the world population.

In order to examine the nuclear membrane proteins of infected liver tissues, liver biopsy sample were subjected to SDS-PAGE followed by 2-DE analysis with narrow pI ranges. The expressed proteins revealed interesting results which contributes to the evaluation and understanding of HCV infection leading to cirrhosis.

To support our results and to provide a conclusive data, a comparative study is carried out to elucidate the differentially expressed proteins in serum of HCV infected individuals. These results might be useful for rapid translation of findings from basic research to practical means of anticipation, control and therapeutic advancement of liver diseases.

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PROTEOME ANALYSIS REVEALS INDIAN-ROCK OYSTER, *SACCOSTREA FORSKALI* PROTEINS DYSREGULATED BY THE ENVIRONMENTAL POLLUTANT TRIBUTYLTIN.

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Tributyltin (TBT), a widely-used antifouling agent in boat paints, is one of the most toxic environmental anthropogenic pollutants in marine environments. TBT has become of broad interest ever since antifouling paints were first implicated in the decline of marine mollusks in coastal areas. Although a large number of studies directed towards the environmental levels, toxicity and exposure of marine mollusks to TBT have been reported for marine organisms, underlying molecular mechanisms of its activities remain unclear.

To this end, we have initiated a comparative proteomic profiling study of ABC multidrug transporters including MDR1, MRP1 and ABCG2, and their response to TBT, using a model marine organism, Indian-rock oyster *Saccrostrea forskali*. Our preliminary findings, along with previous studies, suggest that the ABC multidrug transporters act as a detoxifying mechanism of various toxic agents including TBT in aquatic organisms. We have employed various proteomic strategies to investigate the TBT toxic response in *S. forskali* using 2-DE, approximately 712 protein spots from TBT-treated and 620 protein spots from non-treated mollusk lysates were revealed, 53 selected protein spots were analyzed by LC-MS/MS (QTof-Micro) and 24 protein spots of them positively identified. Due to the lack of protein databases for mollusks, including oyster, we applied a database search including all species (SWISSPROT) and other related species such as nematode (*C. elegan*) and Zebra fish. Since 55% of proteins in *S. forskali* did not match existing protein in the database and 40% of proteins matched only one peptide, manual sequencing will be performed to circumnavigate this problem at Joint Proteomics Laboratory, Ludwig Institute for Cancer Research and the Walter and Eliza Hall Institute of Medical Research, Australia, under the provision of Professor of Richard J. Simpson.

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LIQUID CHROMATOGRAPHIC PROTEIN SEPARATION COUPLED TO TOP- DOWN AND BOTTOM-UP MASS SPECTROMETRIC ANALYSIS

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Introduction: Sample complexity is one of the key challenges facing contemporary proteomic analysis. New developments in column technology have allowed us to perform rapid improved-resolution MS based identification of intact proteins from complex samples. Sample types investigated to establish the utility of the methodology include bacterial lysates (Bordetella parapertusis, and Escherichia coli), a eukaryotic parasite (Leishmania donovani), and transformed human cell lines.

Methods: Here we report the separation of complex protein mixtures using online 2D liquid chromatography on derivitized polystyrene-divinylbenzene (PSDVB) pellicular ion-exchange resins and PSDVB monolithic reversed-phase columns. Proteolytic digestion of the fractions followed by rapid LC-MSMS was used to complete the analysis. An alternative methodology, relying on direct analysis of the second dimension eluents by top-down methodology, using the Apex IV 12T FTICR-MS has allowed identification from intact Leishmania proteins and PTM mapping of histone H4.

Separation of a typical amount of lysate (200ug) was performed using anion exchange columns, followed by reversed phase separation using rapid gradients on a 500 um PS-DVB monolith. Fractions (20uL) from the second dimension were collected in 384 well microtitre plates and subjected to trypsin digestion.

Results: The use of parallel 200um monoliths for tryptic peptide separations ensured maximum capacity, minimum sample loss and high sample throughput, with no loss of sensitivity. For simple mixtures, reversed-phase separation times could be reduced to a few minutes without significantly affecting data content, although rapid scanning capability was essential due to the very narrow peak widths.

Analysis of the digested fractions gave good coverage of the proteome. Proteins representing low (8kDa) and high (500kDa) molecular mass and extremes of predicted pI were identified, as well as a number of membrane proteins. Resolution of the intact protein separation was such that single protein species often occurred in one or two fractions for both the ion-exchange and reversed phase separations, with the fractions varying in complexity. Separation of modified proteins in the ion-exchange dimension demonstrated separation of isoforms.

Quantitation is of paramount importance to any proteomic technique, and liquid chromatographic separation of intact proteins provides unparallelled flexibility for differential analysis of complex samples. UV absorbance maps were generated and could be used for differential analysis of samples. In addition, isotopic labelling techniques have been employed for more in-depth analysis of quantitative differences between samples. Additionally, label-free techniques have been employed for protein quantitation by LC/FT-ICR-MS.

Keywords: Top-Down Proteomics, Multidimensional Separations, High Throughput

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PROTEOMIC STUDIES INTO THE HUMAN CORONARY MICROVASCULATURE: PLASMA PROTEIN PROFILES DURING ACUTE CORONARY SYNDROME PRESENTATION

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Cardiovascular disease is one of the leading causes of morbidity and mortality. The Coronary Slow Flow Phenomenon (CSFP) is a coronary microvascular disorder that presents as an acute coronary syndrome (ACS) despite normal large vessels. Resulting in

frequent and disabling anginal symptoms, the etiology behind the CSFP is unknown. The objective of this study was to explore potential mechanisms for the ACS presentation in CSFP patients, using a proteomic approach.

Plasma samples for high sensitivity C-reactive protein (hsCRP) (inflammatory), Troponin T (TnT), Creatine Kinase (CK) (myocardial cell necrosis markers) assays and proteomic analysis were obtained from six CSFP patients (mean age 56±11 years) during an acute presentation and a month later during a quiescent period. Two-dimensional gel electrophoresis (2DE) in conjunction with differential in-gel electrophoresis techniques (2D-DIGE) was employed for protein separation, quantification and paired comparison. Gel images were analysed using DeCyder software and differentially displayed protein spots were identified by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF/TOF) and electron spray ionization ion trap (ESI-IT) mass spectrometry

During the acute presentation, hsCRP was elevated compared to the subsequent sample (18.7±5.4 mg/L vs 2.6±0.4mg/L, respectively, p=0.03) despite no changes in TnT or CK levels. Proteomic analysis identified three proteins significantly different between the acute and pain-free samples (>1.5 fold difference, p<0.05) as shown in the table.

	Fold change at ACS (mean ± SD)	Biological Role
Fibrinogen alpha chain precursor (4 isoforms)	1.6±0.03	Acute phase protein Clotting factor
Haptoglobin presursor (2 isoforms)	1.79±0.5	Acute-phase protein
Leucine-rich alpha-2 glycoprotein (1 isoform)	1.71±0.05	Neutrophil granulocyte differentiation biomarker

Thus, similar to patients with obstructive large vessel coronary artery disease, inflammatory and/or thrombotic processes appear to be involved in the ACS presentation in patients with the CSFP. Future studies should focus on elucidating the mechanisms involved in these processes in the CSFP and other similar microvascular disorders.

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ANALYSIS OF SEQUENCE SPECIFICITY FOR CALPAIN BY MONITORING CLEAVAGES OF MULTIPLE PEPTIDES USING ITRAQ™ AND 2D-LC-MS/MS.

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Calpain, a Ca^{2+}-regulated cysteine protease, is an intracellular "modulator protease" that modulates/transforms substrate functions through proteolytic processing by limited and specific proteolysis. Calpains are involved in versatile signal transduction pathways, and, therefore, defective activity of calpain causes lethality or a wide variety of pathogenic states such as muscular dystrophy. Among mammalian calpain family members, μ- and m-calpains are ubiquitously and most abundantly expressed, and are well characterized. Several reports have demonstrated that these calpains have a certain degree of amino acid sequence preference for substrate cleavages; however, an explicit rule for substrate specificities of calpains has never been elucidated. In most cases, calpains cleave substrates at fixed positions in between domains, suggesting that calpain recognizes both primary and higher order structures of substrates at the same time. Molecular mechanisms, however, as to how calpains strictly cleave substrates at specific sites remain obscure. To address this question, 86 eicosamer-oligopeptides corresponding to reported calpain substrates were synthesized so that the proteolytic sites come in the middle of the peptides. All these peptides together with eight control random-peptides were mixed, incubated with μ- and/or m-calpain in the presence or absence of Ca^{2+}, and labeled with iTRAQ™ reagents[1]. Then, both cleaved and uncleaved peptides included in the solution were comprehensively and quantitatively identified with 2D-LC-MS/MS spectrometry to examine cleavage sites and reaction rates of these peptides for calpains. As a result, we found that not only cleavage rates but also the ratios of cleavage rates between μ- and m-calpains are different depending on the peptides. Furthermore, more than 1/3 of the peptides detected were properly proteolyzed by both μ- and m-calpains at the reported cleavage sites. Based on these data together with calpain three-dimensional structures, characteristics of substrate specificities of calpains are discussed.

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H3-RULES, PROGRESS REPORT 2007

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Antibody modeling is widely used for the analysis of antibody-antigen interactions and for the design of potent antibody drug. Achieving high-affinity target binding is important for decreasing drug dosage and increasing drug efficacy. Computational

procedures for affinity maturation are expected, and the accuracy of the design will strongly depend on that of the antibody model. Antibody combining site is composed of six complementarity determining regions (CDRs). The CDRs except for CDR-H3 is known to have limited numbers of canonical structures, and one can identify one of the canonical structures from the amino acid sequence. The CDR-H3 lies in the center of antigen-binding site and shows significant variability in its length, sequence, and structure. H3-rules, a method to classify the CDR-H3 structure from the amino acid sequence, were also previously proposed. However, since those CDRs structures were classified, many more antibody crystal structures have been determined. In this work, we present recent progress of H3-rules based on systematic analyses of large amount of structural data. We show the correlation between the length of CDR-H3 sequences and the structures. As a consequence of the relative spatial positions in the CDRs, some basic residues, called •gNotable signals•h, on VL domain can affect the conformation of CDR-H3. Our revised H3-rules have the high accuracy of CDR-H3 structure prediction. These empirical rules derived from many crystal structures are expected to be used in antibody structure analysis and in drug discovery. Structural analysis server, H3-rules 2007, can be accessed on the web: http://www.protein.osaka-u.ac.jp/rcsfp/pi/H3-rules/.

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COUPLING TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY WITH ESI MS FOR LABEL-FREE ABSOLUTE PROTEIN QUANTIFICATION

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During the past decade mass spectrometry has become accepted as an essential tool to better understand protein function, facilitating both the identification and quantification of proteins in complex samples. We, and others, have previously described a novel approach to mass spectrometry based protein identification [1-3] that facilitates the simultaneous acquisition of qualitative and quantitative information, in a data independent fashion.

We have previously used this approach to generate absolute quantification values for proteins contained in biological systems [4]. We have extended this to study samples from a range of organisms, specific tissues, cell lysates and biofluids. An important aspect of this absolute quantitation procedure is that it allows sample loading onto a given analytical column to be determined and optimized, to ensure that ideal chromatographic and mass spectrometric performance is obtained. This results in the maximum number of peptide and proteins being determined from the sample, whilst maintaining maximum accuracy for quantitative measurements. Absolute quantification also provides a mechanism to define the protein stoichiometry present within a sample. In this manner protein pathways and families can be discerned and compared, and the mechanism by which proteins interact can be probed.

It is a common proteomics experiment to use relative quantitation, to determine information about protein expression changes within an experiment. In many respects this can be considered as an isolated island of information that can only be compared within a given experiment. The possibility of performing absolute quantitation of proteins generates a bridge between data sets, allowing the comparison of protein abundance across experiments, instruments, organisms, and laboratories.

In this presentation we will focus on the implementation and subsequent use of a novel RP-RP 2DLC system in combination with label-free absolute quantification of proteins from a variety of different biological samples. We will show absolute quantitation data from cell lysates of *E.coli*, human cardiac tissue, a study of stroke tolerance in the mouse brain, and the monitoring of known Gaucher disease biomarkers from the plasma of patients undergoing therapy.

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CLEAVAGE AND FUNCTIONAL LOSS OF HUMAN APOLIPOPROTEIN E BY DIGESTION OF MATRIX METALLOPROTEINASE-14

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By means of a degradomic approach applying proteomic techniques, we previously suggested that apolipoprotein E (apoE) is a substrate of matrix metalloproteinase-14 (MMP-14). Here we confirm that apoE is, in fact, a substrate of MMP-14 and also of MMP-7 and MMP-2 to a lesser extent. The 34-kDa apoE protein was initially processed by MMP-14 into fragments with molecular masses of 28-kDa, 23-kDa, 21-kDa, and 11-kDa. MMP-14 cleavage sites within the apoE protein were determined by C-terminal labeling of MMP-14-digested apoE fragments with isotope (O18:O16 =1:1) and identification of the doublet fragments or peptides showing 2-Da difference by mass spectrometry, along with N-terminal sequencing of the fragments. It was determined that the primary MMP-14 cleavage sites were A176 -I177, P183-L184, P202-L203, and Q249-I250. The MMP-14-mediated cleavage of apoE was consistent regardless of whether apoE existed in its lipid-bound or lipid-free form. Upon digestion with MMP-14, apoE loses its ability to suppress the platelet-derived growth factor-induced migration of rat vascular smooth muscle cells. Considering the important role of apoE for lipid metabolism and atherosclerosis protection, our findings suggest that MMP-14 plays an essential role for the development of hyperlipidemia and atherosclerosis as a result of degradation of apoE.

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PROTEOMIC ANALYSIS OF EXOSOMES DERIVED FROM SW480 COLON CANCER CELLS WITH FUNCTIONALLY RESTORED FULL-LENGTH ADENOMATOUS POLYPOSIS COLI

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When full-length wild-type APC (a tumor suppressor protein in CRC) is functionally restored into SW480 colon cancer cells (SW480APC), a less tumourigenic phenotype is observed with the re-distribution of β-catenin and E-cadherin to the cell periphery where they form functional adherens junctions (1). SW480APC cells also showed reduced TCF/LEF transcriptional signaling, lower proliferation rates, reduced cell migration and a reduced ability to form colonies in soft agar. Interestingly, its inability to establish xenograft in nude mice while maintaining growth *in vitro* highlighted critical contribution from the tumor cell microenvironment *in vivo*. Here, we performed a differential proteomic analysis of exosomes (i.e. secreted 30-100nm microvesicles) using a 1-DE/LC/MSMS approach to identify potential secreted biomarkers associated with APC. In total, more than 600 proteins were identified, including many previously described exosomal proteins involving in adhesion, MVBs biogenesis, signaling and trafficking. We also identified a Wnt signaling secreted antagonist, Dickkopf-related protein 4 (Dkk-4), to be uniquely expressed in SW480APC cells by MS, immunoblotting, immunogold-EM, RT-PCR and microarray. To our best knowledge, this is the first report linking Dkk-4 with APC protein together in content and being localized in exosomes. We hypothesize that overexpression of Dkk-4 could provide a synergistic effect to further inhibit Wnt signaling extracellularly in conjunction with expression of functional APC and also its presence in exosomes may associated with long-range signaling in cell-to-cell communication.

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HUMAN PLASMA PROTEIN PTMOME PROJECT AND BIOMARKER DISCOVERY

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The analyses of proteomic posttranslational modifications, or PTMomics, usually involve the proper integration of the methodologies from a diversity of disciplines, such as protein chemistry, mass spectrometry, genomics and bioinformatics. In order to facilitate such researches, we have been developing a comprehensive modification mapping procedure that can survey a multitude of protein modifications using the Orbitrap mass spectrometric data. While applying this new method to characterize various proteomes, we have been particularly interested in human plasma PTMome considering the immense potential of the related information in biomarker discovery. Thus far, we have uncovered many novel protein modifications, in terms of their locations in the proteins, including O-linked glycosylations, phosphorylation, hydroxylation, carboxylation and glycation, on a set of plasma proteins, including albumin, fibrinogen subunits, plasminogen, ferroxidase and complement proteins. Through examination of their tandem mass spectra, we also attempt to identify the MS/MS features associated with specific types of protein modifications. We will continue the collection of the PTM information for normal individuals, and then begin the acquisition of the corresponding data for the patients with different diseases. New methods with better throughputs will be developed to speed up the quantitative analyses of these PTMs such that PTMs associated with major human diseases can be discovered.

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MASS SPECTROMETRY IDENTIFICATION OF HISTONE H2B VARIANTS AND THEIR POST-TRANSLATIONAL MODIFICATIONS DURING SPERMATOGENESIS

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Introduction: One of the most distinctive characteristics of chromatin remodeling during spermatogenesis is the expression of a large number of histone variants with complex modification forms. In addition to all the somatic-type histone variants, spermatogenic cells express testis-specific histones corresponding to three of the four core histones. To understand the global dynamics of chromatin structure and function during spermatogenesis, comprehensive analys es of how histone variants incorporate into the nucleosome and their covalent modifications are required.

Methods: Histones were extracted from type A spermatogonia, spermatocytes and round spermatids cells of Sprague-dawley rats, then separated by high performance liquid chromatography (HPLC). The HPLC eluents were splitted for on-line monitoring by LC-MS, and also collection for offline analysis. The collected fractions were digested with enzyme Glu-C and then analyzed by nano LC-MS/MS to identify histone H2B variations and post-translational modifications.

Results: As revealed in this study, TH2B, a testis special histone, first appeared in spermatogonia, and was maximal in spermatocytes until round spermatids. It was also found that global histone TH2B modification patterns dynamically changed during the meiosis of spermatogesis, with the declination of monoacetylation and increment of dimethylation.

TH2B was usually dimetylated at $Lys^{-(6,12,13,16,17,21,\ 86,117,\ 109,121)}$; the trimethyl form was also found at $Lys^{-(16,21)}$; the acetylated form was modified at $Lys^{-\ (12,13,16)}$ in the spermatogonia, spermatocytes; whereas in the round spermatids, TH2B was dimethylated at $Lys^{(86,121,126)}$. Phosphorylation at Thr^{116} of TH2B was constant during the spermatogenesis. We also identified five other somatic histone H2B variations which participated in spermatogenesis.

Conclusion: In this study, we identified multiple histone H2B subtypes and modification patterns in spermatogenic cells, which provided a valuable foundation for further studies on histone coding during spermatogensis, and epigenetic information establishment.

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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. These proteins, or in some cases panels of proteins, must be validated in wider sample sets, or a greater number of related clinical conditions in order to determine their utility as specific markers. The detection and quantitation of these proteins from complex biological mixtures is challenging not just due to the inherent complexity associated with the number of tryptic peptides generated, but the dynamic range in protein concentration present. Recently the multiple reaction monitoring (MRM) mode of analysis has received much attention as a robust and reliable method for the simultaneous quantitation of large numbers of low abundance proteins.

We have described previously, how, using an alternate scanning LC-MS strategy [1-6] , on a Q-Tof mass spectrometer a comprehensive inventory of precursor. product ions, peak area intensities and associated physio-chemical properties can be derived.

Here we show how this experimental data (precursor and fragment m/z, intensity and retention time) can be utilized to empirical determine a list of unique tryptic peptides for each protein i.e. those peptides which uniquely identify a protein in a database from a complex sample. In addition the algorithms determine both the 'best' ionising, and most selective transition (peptide precursor and fragment ion) to determine the most appropriate multiple reaction monitoring (MRM) transition to monitor.

In this study a highly homologous group of proteins the Cytochrome P450s (CYP450) from rat microsomes were studied. There is considerable sequence homology between these enzymes, making it a suitable challenging test for MRM method development. Cytochome P450s are a large group of monooxygenase enzymes responsible for the metabolism of toxic hydrocarbons. The microsomes were also perturbed with the chemical inducers (PLEASE LIST) to induce changes in CYP450 expression levels . All samples were tryptically digested and the identity and differential expression of the CYP450s determined by alternate scanning LC-MS. The resulting identifications; peptide and fragment m/z values, intensities and retention times were interrogated and provided over 350 unique (WHY DIDN'T WE MONITOR 350 IF THEY ARE UNIQUE?) transitions for the CYP450 proteins from over xxxx ions. These transitions were filtered further to provide the best, most selective transitions which were used in the MRM method to quantify 8 CYP450 proteins. PLEASE DETAIL RESULTS OF INDUCED CHANGES.

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STRUCTURE OF PROTOCHLOROPHYLLIDE REDUCTASE REVEALS A MECHANISM FOR GREENING IN THE DARK

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Chlorophyll (Chl) is a tetrapyrrole macrocycle containing Mg and a phytol chain. The Chl biosynthetic pathway consists of the multi-enzymatic reactions. An asymmetric conjugated double bond system of Chl a, which is crucial for efficient light absorption, is formed in the penultimate step of biosynthesis, reducing protochlorophyllide (Pchlide) to form chlorophyllide a. Photosynthetic organisms adopt two different strategies for the reduction of Pchlide; one is the light-dependent Pchlide oxidoreductase that requires light for the catalysis, and the other is dark-operative Pchlide oxidoreductase (DPOR) that operates even in the dark. The greening ability of plant in the dark is attributed to the activity of DPOR. We show a crystal structure of the DPOR catalytic component NB-protein from *Rhodobacter capsulatus* at 2.3 Å resolution. Overall structure with two copies of homologous BchN and BchB subunits is similar to that of nitrogenase MoFe protein. Each catalytic BchN-BchB unit contains one Pchlide held without any axial ligations from amino acid residues and one Fe-S cluster (NB-cluster) coordinated uniquely by one aspartate and three cysteines. Intriguingly, NB-cluster and Pchlide are arranged spatially as almost identical to P-cluster and FeMo-cofactor in MoFe protein, illustrating a common architecture to reduce chemically stable multi-bonds such as porphyrin and dinitrogen.

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IMMUNOLOCALIZATION AND DYNAMIC EXPRESSION OF ALBUMIN PRECURSOR AND HSP70 IN WOUND HEALING OF CORNEAL EPITHELIAL CELLS

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To examine the role of albumin precursor and hsp70 proteins in corneal epithelial wound healing, we analyzed the expressions of these proteins by 2-DE and ESI-Q-TOF MS/MS to compare the changes in the proteome of migrating and non-migrating corneal epithelia. Of the many differentially expressed proteins between the two groups up-regulated expression of the 200 kD protein with two peptide, 69 kD albumin precursor protein with four peptides sequences and 70 kD hsp70 with one peptide was revealed as reported previously for the first time in the active phase of migration (48 hours). In addition, western blot analysis demonstrated the identity and expression of these proteins at different phases (24, 48 and 72 hours) of healing. The heavily expressed amplified products of 739, 215 and 164 bp products, of KIAA, albumin precursor and hsp70 respectively at 48 hours in migrating compared to non-migrating epithelia also substantiate the above findings. Immunofluorescent staining was used to detect albumin precursor and hsp70 proteins in corneal epithelium at various time intervals after an epithelial defect.

Albumin and hsp70 possessed different distribution patterns in different stages of healing, the most intense staining was found at 48 hours, post wounding, of albumin at both the superficial and basal cells of the ocular surface epithelium and endothelium Effects of anti-albumin precursor and anti-hsp70 antibodies on cell migration was determined in healing corneal epithelium of rabbit organ-cultures. An anti-albumin precursor and anti-hsp70 antibodies were found to delay corneal epithelial wound healing.

Taken together, these studies suggest that these proteins expressed in epithelial elements of the corneal epithelial wound healing and may have a role in corneal epithelial growth and regeneration. In addition its distinctive expression during active phase suggests a regulatory and therapeutics role of the protein during wound repair.

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DETERGENT REMOVAL FROM PROTEIN SAMPLES USING SDR HYPERD ® AND MASS-SPECTROMETRY BASED DETERGENT ESTIMATION.

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Detergents are widely used in protein chemistry protocols and may be necessary for protein extraction, solubilization and denaturation. However, the presence of detergents is known to interfere with many techniques including Mass-spectrometry (MS), hence their removal is a pre-requisite for error-free MS analyses. This study evaluates the use of SDR HyperD ® , a unique chromatography resin, for the removal of NP-40, Triton X-100 and CHAPS detergents from protein samples. Additionally, a highly sensitive (detection limit 100 ppm) MS based method for detection and quantitation of these detergents is described. SDR HyperD ® is used in combination with Nanosep ® spin devices format, readily amenable for high-throughput applications. SDR HyperD ® treatment results in substantial improvement in MS signal-intensity of proteins. Estimation of residual detergents in treated samples establishes that SDR HyperD ® removes 96-99% of the detergents.

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ZEBRAFISH IMAGING: A MALDI MS IMAGING APPROACH

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Zebrafish (*Danio rerio*) are well established models for studying genetics and vertebrate development. They share similar physiological, developmental and behavioral features as a higher vertebrate and are easily maintained and manipulated in the lab oratory . Since zebrafish genome is known, they are ideal for both genetic and proteomics manipulations and had been used as transgenic and xenograft models for cancer. Also there are numerous studies that involved tracking of small molecules, protein targets and cells in zebrafish for pharmacological studies and cancer research. Techniques to visualize the targets include high resolution and time-phase confocal microscopy, radiography and fluorescent in situ hybridization. All these techniques involve certain manipulation of specific compounds or genes. Here we sort to use the MALDI MS tissue imaging mass spectrometry technique to visualize proteins/ peptides directly from intact zebrafish tissues. MALDI MS spectra were directly obtained from the matrix coated fish slices on a target plate and processed in imaging software. Specific protein, peptide or compound masses were then extracted from the processed images for localization studies. This could provide a quick way to observe physiological changes in zebrafish.

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CRYSTAL STRUCTURE OF A CHIMERIC B-LACTOGLOBULIN, GYUBA

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β-lactoglobulin is a major whey protein of 162 residues and is composed of nine β-strands (A-I strand) and one a -helix. Eight out of the nine strands form an up-and-down b -barrel structure. Under physiological conditions, equine β-lactoglobulin (ELG) is monomer, whereas bovine β-lactoglobulin (BLG) exists as a dimer in which I strand forms an intermolecular β-sheet.

In this study, we report the 2.0 □ resolution crystal structure of Gyuba which was constructed from the amino acid sequences of secondary-structured regions of BLG and those of loop regions of ELG. This structure revealed that Gyuba formed dimer in which I strand forms an intermolecular β-sheet. This dimerization is similar to BLG.

Additionally, analytical ultracentrifugation, gel filtration chromatography and thermal denaturation performed to confirm dimerization property and stability of Gyuba, and these results compared to these of wild-type ELG, BLG and mutant ImELG, ImBLG. The mutant ImELG was constructed by substituting the amino acid sequence of I strand of BLG for those of ELG, and the mutant ImBLG was constructed by substituting amino acids of I strand of ELG for those of BLG. Analytical ultracentrifugation and gel filtration chromatography revealed that ELG, ImELG and ImBLG exist as a monomer, whereas BLG and Gyuba exist a dimer. Additionally, dimerization property of BLG and Gyuba was equality. And thermal stability of ImELG and ImBLG were similar to ELG and BLG. But thermal stability of Gyuba extremely decreased.

These results suggested dimerization of Gyuba is supported by global conformation and amino acid sequence of I strand, and thermal stability don't effect it.

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BIOLOGICAL AND PROTEOMIC ANALYSIS OF BUTYRATE AND ITS METABOLITE, 3-HYDROXYBUTYRATE, IN HT-29 HUMAN COLORECTAL CANCER CELLS

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Background: Epidemiological and experimental studies suggest that diet may be protective against the development of colon carcinoma, which has been suggested to be attributed in part to the production of short chain fatty acids, in particular butyrate [1]. However, the mechanisms through which butyrate produces biological effects have not been fully defined, and the rapid metabolism of butyrate results in difficulties achieving effective concentrations *in vivo*. Methods: We examined the *in vitro* effects of butyrate and its predominant naturally occurring metabolite, 3-hydroxybutyrate, on cell proliferation and apoptosis (measured using the CellTiter-Blue Assay/Apo-One Homogeneous Caspase 3/7 kit), HDAC activity (measured using the HDAC Inhibitory Assay kit) and further investigated using iTRAQ proteomic analysis in HT-29 cells. Results: Butyrate inhibited proliferation, induced apoptosis and inhibited HDAC activity while 3-hydroxybutyrate lacked physiological effects, but induced changes in protein expression. Upon

exclusion of those proteins differentially regulated by 3-hydroxybutyrate, butyrate induced up- and down-regulation of eight proteins respectively. Four candidates of interest were further validated using western blot. Conclusion: The data may suggest that butyrate itself and not the metabolite of butyrate is responsible for the observed bioactivities.

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IDENTIFICATION OF PROTEINS, ENZYMES AND POTENTIAL VACCINE CANDIDATES FROM THE AUST PARALYSIS TICK, IXODES HOLOCYCLUS USING 2D-PAGE AND EQUALIZER TECHNOLOGY.

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Ticks are blood-feeding arthropods that are vectors for many diseases of humans and animals caused by viral, rickettsial, bacterial, fungal, protozoal and nematode pathogens. Extensive studies of the antihaemostatic molecules (anticoagulants, vasodilators, platelet aggregation inhibitors) in the saliva of blood-feeding arthropods (*Rhodnius prolixus*, bed bugs, sandflies, mosquito) have been reported (Champagne,2005) but very little is known about the proteins involved in the attachment and feeding processes of ticks and this is particularly so for *Ixodes holocyclus*. To date, no complete genome has been published for any tick species and EST libraries are available for only four species (*Boophilus microplus*, *Amblyomma variegatum*, *Ripecephalus appendiculatus* and *Ixodes scapularis*). Thus a targeted proteomic approach to the investigation of *I.holocyclus* was initiated seeking to identify the enzymes secreted into the feeding chamber and enzymes involved into the digestion of haemoglobin. Enzymes were detected in whole, unengorged female tick extracts and whole, engorged female tick extracts whose proteins had been equalized using Proteominer beads, a combinatorial hexapeptide ligand library bound to chromatographic beads that aims to "embrace" all proteins present in a sample (Boschetti et al, 2007). The proteins in the extracts were then separated by 2D-PAGE and visualised by the fluorescent peptide substrate assay method developed by Zhao and Russell (2003). The method uses commercially available, enzyme specific, peptide substrates containing 4-methyl-coumaryl -7-amide that are co-polymerised into a polyacrylamide gel. Fluorescent spots were excised and analysed by mass spectrometry and the data compared to tick EST library sequences and sequences of enzymes (cathepsins, leucine amino peptidases, metalloproteinases) in haemoglobin digestion in other organisms such as Schistosoma, Plasmodium etc.

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COMPLETE CHARACTERIZING OF ERYTHROPOIETIN GLYCOFORMS USING CAPILLARY ZONE ELECTROPHORESIS COUPLED TO MASS SPECTROMETRY

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Erythropoietin (EPO) plays a key role in the production of red blood cells and is currently one of the most important approved drugs. EPO is a glycoprotein of about 30kDa, where the glycosylation has an influence on its lifetime in blood, biological availability and activity. Due to this wide glycoform distribution the direct mass spectral characterization either by ESI or by MALDI failed. Here we show for the first time a method for the accurate mass determination of intact isomeric glycoforms of EPO by ESI-TOF MS, based on prior on-line separation by capillary electrophoresis.

Capillary electrophoresis - electrospray time-of-flight mass spectrometry was used, coupling an HP3D CE via a coaxial sheath-liquid interface to an orthogonal accelerated TOF MS. Separation was performed in an acetic acid based electrolyte at -30kV in a coated capillary.

The approach enables the on-line removal of non-glycosylated proteins like serum albumin, salts, and neutral and negatively charged species. More important, different glycosylation forms are separated both on the base of differences in the number of negatively charged sialic acid residues and the size of the glycans (Hexose-N-acetyl-hexose repetition units). Thus, 44 major glycoforms of recombinant human erythropoietin could be distinguished for the reference material from the European Pharmacopeia. Moreover, even details like acetylation or additional oxygen can be distinguished for the intact protein. Taking also acetylation into account about 135 isoforms could be observed in total. The presented method for intact glycoprotein characterization is an ideal complement to the established techniques for glycopeptide and glycan analysis, not differentiating branching or linkage isoforms, but leading to an overall composition of the glycoprotein. Due to the unbeaten speed (20min, no enzymatic treatment) and extremely high information content the analysis is expected to be an excellent tool for the analysis of EPO. Furthermore, the presented strategy is expected to improve significantly the ability to characterize and quantify isomeric glycoforms for a large variety of glycoproteins.

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COMPARATIVE ANALYSES OF ABSCISIC ACID RESPONSES IN PLANTS

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The phytohormone abscisic acid (ABA) is a key mediator of various physiological and developmental processes in plants. It plays a major role in eliciting stress responses via a complex network of signaling pathways. We are using a rice suspension cell culture system and monitoring the effects of ABA exposure. These changes are being followed at the levels of both protein and metabolites. Proteomic profiles are monitored using iTRAQ peptide tagging and DIGE, complementary approaches that provide information about abundance and changes in post translational modifications. Metabolomic analysis is also being conducted simultaneously using metabolite extracts to monitor carbohydrate, organic acid and amino acid profiles through GC-MS technology. A quantitative PCR analysis on a set of known ABA response regulators was performed in parallel to validate ABA responses. These investigations are beginning to allow an understanding of relative protein abundance changes, metabolite responses and provide insights into ABA effects at the molecular and cellular levels.

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IDENTIFICATION OF DIFFERENTIALLY EXPRESSED PROTEINS IN THE SERUM OF ORAL CANCER PATIENTS BY TWO DIMENSIONAL GEL ELECTROPHORESIS

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Oral cancer is a cancer of the oral cavity which is characterized by abnormal growth within the mouth region. It is widely accepted that oral cancer can be preceded by lesions known as potentially malignant lesions (PMOL). Some of these PMOL may eventually transform to cancer depending on the cellular alteration that had occured. In this study, two-dimensional gel electrophoresis (2DE) followed by protein identification by mass spectrometry (MS) was utilized in an attempt to understand and predict the potential malignant transformation of POML. The primary aim of our researchwas to identify proteins that were differentially expressed in oral cancer patients that may be developed into predictive biomarkers in oral cancer prognosis. Here we describe a robust and reproducible 2DE analysis comparing serum samples from oral cancer patients (n=4), pre-cancer (n=6) and that of normal, healthy individuals (n=3). Following 2DE, 43 of differentially expressed protein spots were identified by mass spectrometry. They include peroxiredoxin 2 (Prx2) and apoptotic protease activating factor 1 (APAF1). The expression dynamics of these proteins were validated by competitive ELISA across a much larger sample size. Potentially, these proteins may be developed into predictive and/or prognostic biomarkers for oral cancer.

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QUANTITATIVE PROTEOMIC ANALYSIS REVEALED TISSUE TRANSGLUTAMINASE 2 COULD BE A NOVEL PROTEIN CANDIDATE OF HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) is one of the most common diseases worldwide with extremely poor prognosis due to failure of early diagnosis. Alpha-fetoprotein (AFP) is only an availability of serologic biomarker for HCC diagnosis, although it is limited in early detection of HCC. Identification additional biomarkers may improve early detection. A quantitative proteomic analysis approach, stable isotope labeling with amino acids in cell culture (SILAC) combined with LTQ-FT-MS/MS identification, was used to explore differentially expressed protein profiles between normal (HL-7702) and cancer (HepG2 and SK-HEP-1) cells. A total of 116 proteins were recognized as an exclusive profile that could distinguish HCC from normal liver cells. In which, some of them, such as AFP, intercellular adhesion molecule-1 (ICAM-1), IQ motif containing GTPase activating protein 2 (IQGAP2), claudin-1 (CLDN1) and tissue transglutaminase 2 (TGM2), were additionally validated both in several HCC cells. TGM2 was identified with remarkable abundance in AFP deficient SK-HEP-1 cells and was further verified in 61 cases of clinical HCC specimens. The results showed that TGM2 was over-expressed in about half of AFP deficient or normal HCC tissues. TGM2 expression in liver tissues showed inverse correlation with the level of serum AFP in HCC patients. In addition, abundant TGM2 was also found existed in the supernatant of the AFP deficient SK-HEP-1, SMMC-7721 and HLE cells and it was found to be induced in AFP producing cells (HepG2) by specific siRNA silence assay. Serum TGM2 levels were further measured by established indirect sandwich ELISA assay

and they were significantly higher in HCC patients than in healthy controls and associated with tumor size and histological grade. These data suggest that TGM2 may serve as a novel histological/serologic candidate involved in HCC especially for the individuals with normal serum AFP.

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TWO ISOFORMS OF UBIQUITIN CARBOXYL-TERMINAL HYDROLASE ISOZYME L1 (UCH-L1) WERE DOWN-REGULATED IN HIGH METASTATIC POTENTIAL OF HUMAN SN12C RENAL CELL CARCINOMA CELL CLONES.

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The SN12C renal cell carcinoma cell clones were parent cell line and 3 clones. SN12C parent cell line was established from a human renal cell carcinoma surgical specimen. The two clones, SN12C-clone 2 and SN12C-PM 6, have higher than the parent cell line. The SN12C-clone 4 has lower than the parent cell line. Using two-dimensional gel electrophoresis, we detected eight proteins showing differential spot intensity between parent cell line and high metastatic clones. And we have identified 5 out of the 8 proteins by using liquid chromatography-tandem mass spectrometry. We found two isoforms of UCH-L1 protein which was shown to be significantly down-regulated in the high metastatic clones. However, the mechanisms of down-regulation of UCH-L1 which were involved in metastasis still remain to be characterized. To clarify the mechanism of UCH-L1 protein expression, further studies will be necessary. Furthermore, we need to examine in what kind of integral mechanism does the down-regulation of UCH-L1 occur.

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STRUCTURAL MECHANISM OF MOLECULAR INTERACTION TRIGGERED BY SYNAPTIC ADHESION PROTEIN

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Neurexin (NX) and neuroligin (NL) are membrane spanning adhesion molecules expressed on the central nervous system synapse. Their extracellular domains interact with each other at synaptic cleft in the Ca^{2+} dependent manner, and this interaction is believed to recruit neurotransmitter releasing machinery, neurotransmitter receptors. Thus NX-specific NL interaction triggers the synaptic formation, and their extracellular interaction affect synaptogenesis. Extracellular segment of NL contains a single acetylcholinesterase-like domain. NX has two gene products, α-NXs and β-NXs. α-NX longer form ectodomain contains three repeating units comprised of two laminin G (LG) domains intervened by an epidermal growth factor (EGF)-like module, whereas β-NX has a single LG domain. Recently crystal structures of the β-NX/NL complex were reported by several groups, revealing a unique binding with 2:2 stoichiometry. However these crystal structures do not provide insights into the synaptic signal transduction triggered by β-NX/NL interaction, nor they reveal mechanism for molecular interaction triggered by Ca^{2+} binding, which is essential for β-NX/NL binding. We determined the crystal structure of β-NX/NL complex at 3.3 □ resolution in a unique crystal form, which enabled us to clarify the detailed molecular mechanism underlying the interaction mediated by Ca^{2+} coordination.

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MALDI-TOF-MS IDENTIFICATION OF INTERMITTENT HYPERCAPNIC HYPOXIA INDUCED PROTEIN CHANGES IN THE PIGLET HIPPOCAMPUS.

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A piglet model of intermittent hypercapnic hypoxia (IHH) was designed to model clinical exposures to obstructive sleep apnea or prone sleeping; two known risk factors for sudden infant death syndrome (SIDS). This study aimed to determine protein changes induced in the hippocampus by the IHH exposure compared to air exposed controls. It is hypothesised that IHH exposure will cause an up-regulation of apoptotic promoters and down-regulation of both apoptotic inhibitors and neuroprotectants in the piglet hippocampus. Male piglets aged 13-14 days were assigned to either control (n=6) or IHH (n=5) groups. The exposure of IHH involved 6min of hypercapnic hypoxia (HH; 8% O_2, 7% CO_2, balance N_2) alternating with 6min of air for a total time of 48min. Half

the hippocampus (50mg of tissue) was prepared for analysis by two-dimensional polyacrylamide gel electrophoresis and matrix-assisted laser desorption/ionisation- time of flight- mass spectrometry (MALDI-TOF-MS) . The remaining half was formalin fixed and paraffin embedded for immunohistochemistry (IHC). A total of 25 protein spots were differentially expressed in the hippocampus of IHH piglets. Samples were analysed using MALDI-TOF-MS and peptide peak masses were entered into the MASCOT peptide mass fingerprint search form. 13 proteins were identified, 6 up-regulated and 7 down-regulated. Glial fibrillary acidic protein (GFAP) and a -internexin were amongst the identified proteins, and were further qualitatively analysed with IHC which confirmed these changes showing that IHH increased GFAP and decreased α-internexin. These proteins are involved in promotion of neuronal development, protection against cellular stress, and regulation of apoptosis, and support our hypothesis of changes in proteins involved in cell protection and apoptotic regulation.

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APPLYING SPECTROSCOPIC RULERS TO APOC-II AMYLOID FIBRILS

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The major constituents of amyloid deposits are insoluble protein aggregates identified as fibrils. More than 25 unrelated proteins are known to form these aggregates upon misfolding and the number of debilitating human disorders shown to result from amyloid deposition is large. Despite the obvious importance, the structure and assembly mechanisms for amyloid fibrils remain unclear. Models of amyloid fibrils proposed thus far have suffered from the lack of critical information about how individual protein subunits contact each other in an assembly. Since the use of conventional structural methods, such as solution NMR and X-ray crystallography, is impeded by the insoluble and non-crystalline nature of amyloid fibrils, attempts to investigate their molecular structure have been limited by lower-resolution techniques. Our work focuses on the structure of the plasma apolipoprotein (apo) C-II amyloid fibrils which has been implicated in disease and is one of several apolipoproteins that accumulate in atherosclerotic plaques. We have developed a structural model for apoC-II amyloid fibrils using mutational analysis, in conjunction with recent advances in spectroscopic techniques. A series of single cysteine substitution mutants at various positions along apoC-II were generated. The ability of these derivatives to form disulfide cross-links upon fibrils formation, indicated that the cysteine residues are in close proximity to each other in the fibril and provided support for a parallel, in register structural model for apoC-II fibrils. Cysteine sulfhydryl groups of apoC-II derivatives labeled with 5-((((2-iodoacetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid (IAEDANS) were employed in fluorescence spectroscopy studies which allowed information on the structural properties of the labeled residue and its surrounding environment to be generated. Through fluorescence resonance energy transfer (FRET) experiments, intramolecular distances between the single tryptophan in apoC-II and the labeled cysteine residues were obtained, enabling the conformational changes during apoC-II fibril formation to be analysed.

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PROTEOMIC ANALYSIS OF XFKBP-ASSOCIATED PROTEIN COMPLEX FORMED DURING SECONDARY AXIS FORMATION IN *XENOPUS LAEVIS* EMBRYO

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Peptidyl prolyl *cis-trans* isomerase (PPIase) catalyzes the *cis-trans* isomerisation and has been classified into three subfamilies, Cyclophilin (CyPs), FK 506-binding protein (FKBPs), and Parvulin families. Recently, it has been reported that *Xenopus* homolog of FKBP1A (XFKBP) induces secondary axis formation. In this study, we tried to identify the XFKBP-associated proteins involved in secondary axis formation by using proteomic methodology. We synthesized mRNA encoding FLAG tag-fused XFKBP (FLAG-XFKBP), injected it into ventral-cells of the 4-cell embryo and isolated FLAG-XFKBP associated proteins from various stages of the development. We confirmed that FLAG-XFKBP was expressed in induction phase of secondary axis and induced secondary axis in the ventral side. Using antibody against FLAG isolated the FLAG-XFKBP-associated protein complex formed in induction phase of secondary axis. In the presence of FK506, FLAG-XFKBP could associate with calcineurin, indicating that the method used was reliable. We identified at least 8 proteins associated with FLAG-XFKBP in the absence of FK506 using LC-MS/MS analysis. Based on these results with others, we will discuss a possible molecular mechanism underlining dorsoventral axis formation during early embryogenesis in *Xenopus laevis*.

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HIGH-THROUGHPUT PURIFICATION OF POLYHISTIDINE TAGGED PROTEINS IN ACROPREP™ MULTI-WELL FILTER PLATES USING IMAC HYPERCEL™.

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Purification of recombinant fusion proteins is essential for many proteomics applications like protein characterization, protein-protein interactions, *in vitro* enzyme assays etc. Polyhistidine tag is the most common and widely used affinity tag for the purification of recombinant proteins. Immobilized Metal Affinity Chromatography (IMAC) is the method of choice for purification of polyhistidine tagged recombinant proteins. Extent of purification, protein yield and time taken for purification are the major considerations for researchers during the development of any purification strategy. Here, we describe a method using IMAC HyperCel in AcroPrep 96 well filter plate for purification of polyhistidine tagged proteins expressed as soluble fraction and inclusion bodies in *E.Coli*. Purified fractions generated from experiments were analyzed by SDS-PAGE and purity of the target protein was estimated using Quantity One® software tool. The method offers not only the flexibility in choice of metal ions based on the experimental goals to achieve high purity and / or yield, but also the simultaneous scouting of multi-binding and elution conditions. Therefore, the combination of AcroPrep Multi-well filter plate and IMAC HyperCel medium can be used successfully for high-throughput protein purification applications.

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PROGNOSTIC BIOMARKER IN ESOPHAGEAL CANCER BY 2D-DIGE, TUMOR TISSUES AND CLINICAL DATA

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Esophageal cancer is one of the most deadly malignancies. The response to treatment modalities such as surgery or chemo-radiotherapy is variable even when the patients are at the same clinical stage, and is not predicted by the existing diagnostic modalities. Novel predictive clinical tools have long been desired to optimize the therapeutic strategies and improve clinical outcomes. To develop such diagnostic tools, we aimed to discover biomarker candidates by examining tumor tissues analyzed by 2D-DIGE and their corresponding clinical data . Laser microdissection was used to recover tumor cells from 58 cases of T3N0-1M0 esophageal squamous cell carcinoma and adjacent normal mucosal cells from 53 matched cases. The patients did not receive anticancer treatment prior to surgery, and the ir prognosis was monitored for at least five years after surgery. The proteins in the recovered cells were labeled with CyDye DIGE Fluor saturation dye and separated by a large format 2D gel apparatus with the internal control sample labeled with a different fluorescent dye. Bioinformatics was employed to determine the protein spots that are most informative for clinico-pathological data . M ass spectrometric analysis identified the proteins corresponding to these protein spots . 2D-DIGE generated quantitative expression profiles with 3623 protein spots from approximately 3000 cells . Based on the intensity of the protein spots, unsupervised classification distinguished the tumor tissues from their normal counterparts . The intensity of 22 protein spots showed statistical difference (FDR<0.05) between patient groups with different prognosis. M ass spectrometric analysis revealed that t he identified proteins are involved in important biological processes such as signal transduction , cytoskeletal/structural organization and transportation . They have been individually i mplicated in a range of cancer types, and our study observed them collectively in a single type of malignancy, esophageal cancer. These proteins are strong candidates for biomarkers to establish novel therapeutic strategies.

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DIFFERENTIAL CONA-ENRICHED URINARY PROTEOME IN RAT EXPERIMENTAL GLOMERULAR DISEASES

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Glomerular diseases are leading causes of end-stage renal diseases worldwide. They are considered to be consequences of injury primarily to the three types of glomerular cells. Differential diagnosis typically relies on invasive biopsy findings. We expected that injuries of different glomerular cells would cause different changes in urinary proteome. The goal of this study was to identify differential urinary proteins distinguishing between injuries of different glomerular cells before significant histopathologic changes.

Adriamycin nephropathy and Thy1.1 glomerulonephritis were employed as models with different primary impaired cells. ConA-enriched urinary glycoproteome on day3 were profiled by gel-free shotgun tandem mass spectrometry, and compared with self-healthy controls to identify differential urinary proteins for each model. By comparing the changes of the differential proteins between these two models, we identified 39 proteins with different directions of changes, which may potentially be useful in differentiation; and 7 proteins with the same direction of changes, which may be potential indicators of early renal damage. These differential proteins were of several origins: plasma proteins, proteins with urine or kidney specificity, proteins without tissue-specificity (mainly inflammatory mediators) etc. Our results may help better understand the effects of injuries of different glomerular cells at the initial stage, and lead to the discovery of novel early diagnostic markers for human focal segmental glomerulosclerosis (FSGS) and mesangioproliferative glomerulonephritis (MsPGN) which have the same primary impaired cells with Adriamycin nephropathy and Thy1.1 glomerulonephritis respectively.

Keywords

Urine proteome / Concanavalin A enrichment / Glomerular diseases / Adriamycin nephropathy / Thy1.1 glomerulonephritis

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PROTEOMIC ANALYSIS OF PROTEINS ASSOCIATED WITH SPLICING FACTOR-2 ASSOCIATED PROTEIN P32 REVEALED ITS POSSIBLE INVOLVEMENT IN HUMAN RIBOSOME BIOGENESIS

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Splicing factor-2 associated protein p32 (SF2p32) interacts with many cellular and viral proteins such as ASF/SF2, human immunodeficiency virus (HIV) Rev and Tat proteins etc. Among those, the interaction with HIV Rev is thought to be responsible for inhibition of the splicing of HIV transcripts, resulting in infection and production of progeny virions in human cells. Because of the ability of SF2p32 to interact with many proteins, it is believed to have many functions in the cell; however, its physiological role remains unclear. Our previous proteomic analysis identified SF2p32 as a component of the protein complexes associated with fibrillarin, which is the nucleolar antigen of the autoimmune disease scleroderma and is involved in early stage of ribosome biogenesis. In this study, we examined a possible involvement of SF2p32 in ribosome biogenesis by using proteomic methodologies.

First, we analyzed SF2p32-associated protein complex using shotgun analysis, and identified 61 proteins as SF2p32-associated proteins; those included 6 trans-acting factors involved in ribosome biogenesis as well as 10 ribosomal proteins. Reciprocal pull-down analysis with the identified trans-acting factors confirmed their association with SF2p32. Secondly, using immunofluorescence microscopy, we showed that small population of SF2p32 localized in the nucleolus and Cajal bodies within the nucleus, though the majority localized predominantly in mitochondria due to the presence of mitochondrial targeting sequence in the N-terminus of the molecule. Thirdly, we demonstrated the presence of high-molecular weight form of SF2p32 in preribosomal fractions by cell fractionation analysis. Finally; we demonstrated by using sucrose density gradient ultracentrifugation that the high molecular weight form of SF2p32 was sedimented mainly in pre-40S and pre-60S, and partly in pre-90S fractions. These results suggest that SF2p32 may be a new trans-acting factor involved in ribosome biogenesis. We will discuss a possible mechanism by which SF2p32 and the other trans-acting factors regulate human ribosome biogenesis.

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THE EFFECT OF PLANT COMPOUNDS FROM WHISKY CASK ON HORSE LIVER ALCOHOL DEHYDROGENASE ACTIVITY

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Aged whisky contains many plant derived compounds from oak wood cask, and the color of whisky gradually changes to deep amber. More than 500 kinds of compounds are known as minor components in aged whisky. The taste of whisky becomes smooth with aging, and we can sober up feeling good. However, detail effect of these plant derived components from whisky cask on alcohol metabolism has not been clarified. In this experiment, we investigated the relation between the structure of components and the rate of ethanol oxidation reaction by using 26 kinds of plant derived components from oak wood cask and its derivatives. All the compounds which we used have a benzene ring as a basic frame, and have a hydroxyl group, an aldehyde group, and a carboxyl group further. All 26 compounds from oak wood cask and its derivatives used in this experiment were inhibited the ethanol oxidation. Interestingly, the relative activity of compound with an aldehyde group (Benzaldehyde and its derivatives) inhibited most strongly to be 30~40%. However, the relative activity of Benzaldehyde with one hydroxyl group had about 70% of relative activity

and it had only one hydroxyl group, it clarified that the inhibition effect becomes weaker. The inhibition effect of compounds with calboxyl group (Benzoic acid and its derivatives) was weak and showed 90% of re lative activity. However, in contrast to the case of Benzaldehyde, the inhabitation effect of Benzoic acid only with one hydroxyl group becomes strong. From these results, it is clarified that many compounds from oak wood cask inhibite the ethanol oxidation. But the mechanism of inhabitation is complex and the additivity of the functional group contribution is not applied simply.

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NEUROPROTEOMICS: EXPLORING REGIONAL HUMAN BRAIN USING 2-DE

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2D gel electrophoresis appears to be the most efficient way of analyzing and identifying the proteins. The rapid developments in the field of mass spectrometry have transformed it into a key technology in proteome research. The role of brain proteins in pathological and normal conditions has been a challenge for decades. The present work is aimed to investigate the brain expression proteomics and an initiative to construct a human brain regional protein profile that will support the elucidation of proteins involved in the diseased states. This paper discusses the human brain protein expression using 2 DE at narrow pI ranges, providing comprehensive information of the expressed proteins, helpful in elucidating the functional roles of various unidentified proteins. Such kind of significant data will lend a hand in exploring the complex mechanisms of neurological and neurodegenerative disorders.

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LEPTOSPIRA INTERROGANS SELECTIVELY BINDS GUINEA PIG SERUM PROTEINS *IN VITRO*

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Leptospirosis is a bacterial zoonosis caused by spirochaetes of the genus *Leptospira*: it affects humans and a wide range of animals. The symptoms of leptospirosis range from subclinical infection to a severe syndrome of multi-organ infection with high mortality. *Leptospira* enters the host via damaged skin, mucous membranes, the lungs or conjunctival membranes which directly contact contaminated urine or water. Once within the host tissues, the pathogenic strains can be optimized for metabolism at body temperatures and are resistant to attack from the innate immune system of the host. The surviving leptospires multiply, migrate rapidly through the bloodstream and lymphatic system and arrive at destination organs. We are interested in the molecular mechanisms of leptospiral infection. One of the environmental changes that *Leptospira* encounters during the environment-to-host transition is increased osmolarity. Therefore we tested several strains for proteomic changes in response to 0.15M NaCl, with *L. interrogans* serovar Copenhageni Strain L533 displaying the greatest degree of proteomic changes. One of the main aspects of leptospiral virulence is the ability of the bacteria to survive and thrive in serum. Copenhageni strain L533 was then used to examine the molecular interactions of *Leptospira* with serum. Leptospiral cells were incubated in guinea pig serum (GPS) or in medium at 37°C for either 30 minutes or 2 hours, followed by washing and preparation of a total membrane fraction. These preparations were separated by two-dimensional electrophoresis and the gel images were compared. The protein spots that differed between the 2 conditions were identified by mass spectrometry. Three additional proteins were found on all the gels of leptospiral membranes incubated with serum. One of these proteins was identified as a serum protein indicating Copenhageni cells selectively bind to this protein. This selectivity was also confirmed by a binding assay of Copenhageni cells with biotinylated GPS. This is the first work to describe the interaction of *Leptospira* with serum and offer the clues to molecular pathogenesis of leptospirosis.

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IN VIVO EVOLUTION OF ESCHERICHIA COLI PYRUVATE KINASE TYPE I: HOW DOES GENOTYPIC EVOLUTION AFFECT PHENOTYPE?

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The adaptation of organisms and the divergence of populations and species are two important problems in microbial ecology. To study the nature of phenotypic and genotypic changes responsible for competitive fitness, Lenski and colleagues evolved 12 lines of E. coli in a fixed environment for about 40,000 generations (Lenski 1991). Unsurprisingly, they found that the 12 lines gained overall fitness when competed against the ancestor (Lenski & Travisano 1994). However, genome sequencing found at least one non-

synonymous mutation in the enzyme pyruvate kinase type 1 (PK1) in all 12 populations (Cooper et al 2003). What role do these mutations play in the fitness of the evolving cell lines? PK1 is an essential glycolytic enzyme necessary for regulation of pathway flux. PK1 has been extensively studied, with its structure solved by macromolecular crystallography and the biochemical properties well characterized. In this study we investigate the structure-function relationship of the evolved PKF enzymes, using a variety of different methods, in an attempt to disentangle the phenotypic changes, and hence the underlying genotypic changes that lead to adaptation of the evolving cell lines.

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THE AGING HUMAN HEART: CHANGES IN EXPRESSION OF LIM DOMAIN PROTEINS

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In 2008 Zahn et al. (PLos Genet 3: e201-16) described a transcriptomic Atlas of Gene Expression in Mouse Aging Project. They showed that LIMS2 expression was increased and Limd1 (a tumor suppressor gene) was decreased with mouse age but did not investigate non-failing human hearts. We performed an oligonucleotide gene array analysis on RNA extracts of 55 samples of left ventricle from non-failing human donors, aged 4.5-65 years. We found that four LIM domains genes were represented in the top 100 genes that were significantly altered with age. In a plot donor age vs the expression levels (log2). We found that: (1) PDLIM7 decreases linearly with age. It controls insulin-induced glucose transport which is modulated by the assembly of the actin cytoskeleton. It binds a kinase (PKC). It is located at the Z discs and may also bind to intercalated discs. (2) LIMS1 decreases linearly age (it overlaps the PDLIM7 data). It is a mechanosensor of contractile force and binds to an integrin-linked kinase (ILK). It fine-tunes the balance between phosphorylated (inactive) and non-phosphorylated alpha-actinin which regulates microfilament assembly. (3) LIMS2 is up-regulated linearly with age in both human and murine hearts. It competes with LIMS1 for binding integrin-linked kinase (ILK) at focal adhesion plaques. It also binds to the sarcomere Z discs. (4) LIMK1 expression levels hardly change below 40y, but then sharply rise with increasing age. It may provide an important clue to the onset and severity of HF in this age group. LIMK1 phosphorylates (and therefore inactivates) cofilin, the major regulator of actin microfilament assembly, and whose cellular levels increase in cancer (Horita et al. 2008, J Biol Chem 283:6013-21). It is therefore a potential target for drug development. Thus, expression levels of at least four LIM domain genes in the non-failing human heart change significantly as a function of age.

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ENRICHMENT OF PHOSPHOPEPTIDES BY FREE FLOW ELECTROPHORESIS

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Protein phosphorylation plays a central role in regulating cellular processes. However, the low level of phosphoproteins in the presence of overwhelming amounts of non-phosphorylated proteins makes their detection and identification challenging. Thus, following tryptic digestion of the proteins, separation of phosphopeptides from non-phosphopeptides is imperative prior to identification. Currently, immobilized metal affinity chromatography (IMAC) and Titanium dioxide supports have been used for phosphorylated peptide enrichment. Gygi et al has also demonstrated the power of using SCX at pH 1.9 to separate phosphorylated peptides from non-phosphorylated peptides successfully. Herein, we present a novel protocol utilizing Free Flow Electrophoresis (FFE) for enrichment and separation of phosphopeptides within complex mixtures.

Traditionally, free flow electrophoresis (FFE) has been used to separate cell organelles, particles, proteins, and tryptic peptides. We have developed a novel FFE protocol, operating in isotachophoresis mode, enabling separation of non-phosphorylated peptides from phosphorylated peptides. Briefly the separation was performed at pH 4 allowing the peptides to separate based on their electrophoretic mobility. Separation of phosphopeptides on the FFE were validated using 1:1, 1:10, and 1:50 mixtures of bovine α-casein and bovine serum albumin (BSA) tryptic peptides respectively. Under this new protocol, FFE fractions contain volatile buffers enabling direct MALDI spotting from a microtiter plated directly onto a MALDI target with DHB. Using direct MALDI, we detected 13, 5, 2 phosphorylated peptides for the 1:1, 1:10 and 1:50 mixtures respectively. Using FFE, we increased the number of detectable phosphorylated peptides to 15 regardless of the amount of BSA present.

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VERSATILE ANALYSES OF FREE FLOW ELECTROPHORESIS SEPARATED PROTEIN ISOFORMS

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Protein isoforms are defined as variants of a single polypeptide which generally alter its function. More than 90% of naturally occurring isoforms arise from post translational modifications (PTMs) and less than 10% from mRNA splice variations.

Recombinant proteins may consist of several isoforms due to differences like variation in the glycosylation pattern or modifications at the N-terminus. Additionally, several chemical modifications may occur during protein isolation and separation processes. Modifications such as PEGylations may also be introduced chemically and isoforms are generated through incomplete reactions.

We introduced FFE to isolate individual protein isoforms under native conditions for further biological studies. Using Free Flow Electrophoresis (FFE), sorting out proteins based on charge, we separated and characterized multiple isoforms of different samples. Resulting fractions were well suited both for separation of closely related species such as protein isoforms, and for direct use in further studies such as enzyme assay and/or immunoassays.

In this study we separated protein isoforms from human Anti-CD3, rabbit muscle L-LDH, Amyloglucosidase from Aspergillus Niger, ß-Lactoglobulin from bovine milk and bovine plasma Fetuin by FFE. Protein samples were diluted with the appropriate FFE separation buffers and loaded via sample inlet to the separation chamber. The FFE separations were performed under native conditions using a voltage of 900-1200 V depending on the current separation. Continuous Isoelectric Focusing FFE (CIEF) buffers were prepared according to manufactures description (BD™ FFE). CIEF was performed at 10°C with buffer flow rate between 30-60 ml/hr.

Obtained FFE fractions were analyzed by IEF-Native-PAGE. L-LDH activity assays confirmed that the biological activity of the sample was preserved after FFE separation. The binding activities of CD3 antibody isoforms were analyzed by flow cytometry.

Summary: FFE enables native separation conditions preserving biological activity of the sample.

The high resolution of FFE, separating proteins based on charge, is ideally suited for difficult challenges like separation of protein isoforms beyond the limitation of Ion-exchange Chromatography.

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STRUCTURAL ANALYSIS OF TRANSMEMBRANE HALOBACTERIAL TRANCEDUCER *P*HTRII BY MULTI-DIMENSIONAL HIGH-RESOLUTION SOLID-STATE NMR

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°Transmembrane protein *p*HtrII is a transducer which binds to phoborhodopsin. The light excitation of phoborhodopsin is transmitted into the cytoplasm through *p*HtrII to promote negative photoaxis. We studied uniformly ^{13}C, ^{15}N labeled 159-residues *p*HtrII by high-resolution solid-state NMR. The *p*HtrII (1-159) was reconstructed into the deuterated DMPC membranes. High-resolution solid-state 2D NMR was measured under magic-angle spinning at the ^{1}H resonance frequencies of 500, 600 and 700 MHz . At first, we have assigned ^{13}C signals of *p*HtrII (1-159) by using ^{13}C-^{13}C spin diffusion under DARR at −40 °C. Intra-residue correlations across 1-2 bonds were observed at short mixing time. At a long mixing time of 200 ms, intra-residue C^{α} correlations across 3-bonds were obtained. Most amino acids except Tyr and Pro were assigned. The DARR spectra were observed for immobile region of *p*HtrII. All ^{13}C signals could not be observed in these dipolar-coupling-based cross-polarization methods because of large-amplitude molecular motions at room temperature. We performed *J*-coupling-based HC-INEPT and HCC-TOCSY experiments in order to obtain signals of mobile protein segments. Mobile protein segments make up about 70% of *p*HtrII (1-159). Many signals in mobile regions were observed in HC and HCC 2D spectra. ^{13}C and ^{1}H signals were assigned to amino acid groups were successful in mobile regions. The C^{α} and CO chemical shifts indicate that *p*HtrII (1-159) mainly forms α -helix structure in lipid bilayer environment.

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POSTTRANSLATIONAL MODIFICATIONS IN AN INSECT CELL-FREE PROTEIN SYNTHESIS SYSTEM AND THEIR IDENTIFICATION BY MALDI-TOF MS

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We have established a cell-free protein synthesis system (Transdirect *insect cell*) derived from *Spodoptera frugiperda* 21 insect cells [1]. This cell-free system has high protein productivity, and therefore it is expected to be sufficient to perform gene expression analyses including not only the measurement of enzymatic activity and western blotting, but also investigation of posttranslational

modifications. In this study, several posttranslational modifications in the insect cell-free protein synthesis system were confirmed and identified by MALDI-TOF MS [2, 3, 4].

One significant posttranslational modification is the formation of disulfide bonds. This plays a very important role in both the biological activity and stabilization of native protein structures. Human lysozyme (h-LYZ), which contains four disulfide bonds were expressed in the insect cell-free protein system. h-LYZ was expressed in a soluble and active form under non-reducing conditions after addition of reduced glutathione , oxidized glutathione , protein disulfide isomerase. Analysis of the disulfide bond arrangements by MALDI-TOF MS showed that disulfide linkages identical to those observed in the wild-type proteins were formed.

Protein *N*-myristoylation and prenylation are the important lipid modifications of proteins, and they play crucial roles in regulating reversible protein-membrane and protein-protein interactions. Epitope-tagged truncated human gelsolin (tGelsolin) and human rhoC, which are natural *N*-myristoylated and geranylgeranylated protein respectively, were synthesized using the insect cell-free protein synthesis system with or without addition of a specific substrate for each protein modification, such as myristoyl-CoA, farnesyl pyrophosphate, and geranylgeranyl pyrophosphate. A nalyses of these proteins by MALDI-TOF MS indicate that the insect cell-free protein synthesis system, as is the case with the rabbit reticulocyte lysate system, possesses *N*-myristoyltransferases and prenyltransferases.

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A NEW SENSOR-CONTROLLED PREPARATION TECHNIQUE FOR MALDI TISSUE IMAGING

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Sample preparation is crucial for the quality of MALDI-tissue-imaging data. Unfortunately, the current matrix application protocols have significant disadvantages: While pneumatic spray preparations provide good homogeneity and spatial resolution of the images, the process is manual and highly irreproducible. Depending of the degree of tissue wetting either the analyte molecules are badly incorporated into the matrix (too dry) or the spatial resolution is lost (too wet). Nano-spotting on the other hand provides quality spectra but as a sequential process it is slow, spatial resolution is limited by the spot raster (typical >200 μm) and perfect alignment with the mass spectrometer is critical. We introduce an entirely new approach that combines the advantages of above methods and eliminates the disadvantages. In the new preparation device, matrix aerosol (20μm droplets) is created by vibrational vaporization under controlled ambient conditions that is gently deposited onto tissue sections. Tissue sections can be homogeneously matrix-coated, typically with 30-100 cycles within one hour. Each cycle consists of three phases: 1. deposit droplet layer, 2. incubate in saturated atmosphere, and 3. allow partial/complete drying. An optical sensor monitors scattered light from the matrix-layer that allows controlling all relevant preparation parameters in real-time: deposition periods, intervals, matrix-layer-thickness, wetness, drying rate. This sensor control of the sample reproducibly provided a wetting/crystallisation regime that was prerequisite for achieving high lateral resolution (<50μm) and spectra quality at the same time.

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OLIGAMI: OLIGOMER ARCHITECTURE AND MOLECULAR INTERFACE

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OLIGAMI (OLIGomer Architecture and Molecular Interface) is a database of the verified coordinates and new chain formulas for the biological molecules. OLIGAMI chain formulas distinguish proteins, peptides, DNAs, and RNAs. Consequently, the resulting numbers of monomers, homo-oligomers, and hetero-oligomers in OLIGAMI are significantly different from comparable searches obtained from PQS. Information of quaternary structures is automatically extracted from the PDB files and manually curated them.

The OLIGAMI web site allows users to interactively view three-dimensional structures of biological molecules for all PDB entries, to browse the molecules through SCOP hierarchy, to compare the chain formulas of OLIGAMI and PQS, and to simultaneously compare the chain formulas for a protein or for a SCOP family. OLIGAMI is publicly available at http://protein.t.soka.ac.jp/oligami/.

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BOVINE LACTOFERRIN PROMOTES ALKALI-INDUCED WOUND HEALING IN CORNEAL EPITHELIAL CELLS BY UP-REGULATING IL-6 AND PDGF

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Purpose : Previously we have found that bovine lactoferrin (BLF) promoted the closure of alkali-induced-human corneal epithelial wounds *in vitro* and it also promoted up-regulation of IL-6 and PDGF BB during wound healing. This study was to investigate whether wound healing was primarily due to up-regulation of IL-6 or PDGF BB.

Methods: Confluent human corneal limbal epithelial (HCLE) cells were wounded using 0.5μl of 0.1N sodium hydroxide and extensively washed with serum-free culture medium, 1:1 K-SFM: DMEM/F12. The wounded cells were subsequently treated with BLF (0, 0.1, 1, 2.5 and 5mg/ml) and BLF in the presence of monoclonal antibody against BLF (50 and 10μg/ml in the presence of BLF). To inhibit the effect of IL-6 or PDGF, anti-human IL-6 receptor neutralizing antibody (rhIL-6 sR, 1, 10 and 50µg/ml) and the inhibitor of PDGF (Tyrphostin AG1295 at 1, 10 and 100μM) with or without BLF (5mg/ml) were used to treat the wounded cells. HCLE cells were treated with either IL-6 (4ng/ml) or PDGF-BB (8ng/ml) as positive controls. Twenty four hours after the treatment the cells were stained with Diff Quick and photographed. The wound area was measured and the percentage reduction of the wound area in response to each treatment was calculated and compared.

Results: At 2.5 and 5.0 mg/ml, BLF significantly promoted wound healing (46 ± 8% and 56 ± 2% respectively) as compared to the absence of BLF (25 ± 5%) whereas BLF antibody at 50μg/ml in the presence of BLF (5mg/ml) did not promote wound closure. When the selective inhibitors rHIL-6 sR or Tyrphostin AG1295 was used, the effect of BLF in promoting wound closure was eliminated.

Conclusions: BLF stimulates alkali-induced HCLE wound healing and the stimulation is mediated through its up-regulation of PDGF or IL-6.

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AMYLOIDOGENIC PEPTIDES FROM APOLIPOPROTEINS A-I AND C-II: LIPID EFFECTS ON FIBRIL FORMING PEPTIDES FROM LIPID BINDING PROTEINS.

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The aggregation of short peptide fragments into amyloid fibrils has an established position in the field of amyloid research, where amyloidogenic fragments have been used frequently as model systems for the behavior of full-length proteins. Human apolipoproteins (apo) C-II and A-I are exchangeable apolipoproteins that normally bind to plasma lipoprotein particles *in vivo*, but can form amyloid fibrils that accumulate in atherosclerotic plaques. We have identified two core regions of the apoC-II sequence that are protected from amide-proton exchange and proteolysis within apoC-II fibrils. Two peptides, of 21 and 11 residues length, from one of these fibril core regions retain the ability to form fibrils, albeit with different kinetics and altered fibril morphology. We have also identified a tryptic peptide from apoA-I, that retains the ability to self-assemble into amyloid-like fibrils. N-Terminal truncations of this peptide show varying degrees of amyloidogenic propensity. In all cases fibril formation by these amyloidogenic peptides is sensitive to lipid, resulting in altered assembly kinetics and changes in final fibril morphology. We report here the structural characterisation of the assembly of these peptides into fibrils and describe the effect of both soluble lipid and lipid complexes on this process.

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RAPID ANALYSIS OF 1D AND 2D GELS BY NANOFLOW LC/MS

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A major challenge in the field of proteomics is the identification of low-abundance proteins from complex protein mixtures. A common approach used in addressing the issue has been to run the protein mixture on 1 D or 2-D gels. The resolving power of the gel and subsequent in-gel digestions followed by mass spectrometry analysis has facilitated the identification of these low-abundance proteins from their more abundant counterparts. Nanoflow LC/MS is among the most sensitive techniques for the identification of proteins and is well suited for the identification of proteins from in-gel digests. Typically, electrospray ionization results in better sequence coverage compared to MALDI, especially from gel spots containing multiple proteins, but MALDI-based analysis can be faster. Using a microfluidic-based nanoflow LC/Q-TOF system allows for rapid analysis of gel spots and bands.

An *E. coli* lysate was separated by both 1D SDS-PAGE and 2D-gel electrophoresis. Gels were Coomassie-stained, and then spots or bands were excised. Each gel spot or band, which represents a protein mixture with low-to-medium complexity, was reduced, alkylated and digested with trypsin using a standard protocol. The in-gel digests were analyzed by reversed-phase microfluidic-based

nanoLC on a Q-TOF mass spectrometer using rapid gradients. Protein database searching was used to assess protein sequence coverage and determine the minimal time required for optimal results.

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DATA DEPOSITION SUPPORTING WEBSITE AT OSAKA FOR BIOMAGRESBANK AND PROTEIN DATA BANK

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BMRB is an international database for experimental data derived from NMR spectroscopic investigations of biological macromolecule, such as assigned chemical shifts, coupling constants, NOE values and so on. BMRB has been maintained by BioMagResBank (PI John L. Markley Ph.D., University of Wisconsin-Madison) and the data from researchers are publicly available to the global community via BMRB websites at Madison, WI, USA, and Florence, Italy and Osaka, Japan.

For researchers in Asia and Oceania, BMRB website at Osaka, Japan (http://bmrb.protein.osaka-u.ac.jp) was launched by BMRB group in PDBj (Protein Data Bank Japan, http://www.pdbj.org, PI Haruki Nakamura Ph.D., Institute for Protein Research, Osaka University) in 2001. Also, the BMRB data deposition system "ADIT-NMR" (http://nmradit.protein.osaka-u.ac.jp/bmrb-adit) was started at PDBj-BMRB in 2004.

New ADIT-NMR introduced in 2007 allows to deposit both NMR experimental data and atomic coordinates determined by NMR information to BMRB and PDB via a single web interface. This was established in collaboration with World Wide Protein Data Bank (wwPDB) which maintains a Protein Data Bank Archive of macromolecular structural data. Its founding members are RCSB-PDB (USA), MSD-EBI (Europe) and PDBj (Japan).

To make the deposition easier, a tutorial website (http://bmrbdep.protein.osaka-u.ac.jp) for BMRB and PDB deposition through ADIT-NMR is provided. In order to support researchers in Asia and Oceania, the tutorial has been shown to the public not only in English but also in Japanese (since 2003), Korean (since 2007), Simplified and Traditional Chinese (since 2008).

In this poster, we would like to present our activity and achievement on the BMRB database, data deposition to BMRB and PDB, and the new data deposition tutorials in Korean, Simplified and Traditional Chinese.

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AN ENHANCED CONFORMATIONAL SAMPLING OF A 40-RESIDUE PROTEIN CONSISTING OF ALPHA AND BETA SECONDARY STRUCTURES IN EXPLICIT SOLVENT

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We obtained a free-energy landscape of a 40-residue protein (the C-terminal domain of H-NS) in explicit water by doing an enhanced conformational sampling, multicanonical molecular dynamics (McMD) simulation. This protein involves alpha-helices and beta-strands in the native structure. We obtained the native structure from the simulation where the smallest backbone root mean square deviation (rmsd) was 3.27A to the NMR structure. However, the native structure was not assigned to the lowest free-energy state but to a semi-stable state. The current study has shown that the McMD simulation has a powerful sampling efficiency to study such a long and complicatedly structured protein, although there is still a force-field issue remaining. In other words, the native structure of protein can be obtained if a relevant force field is given.

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THE MARINE BACTERIUM *PSEUDOALTEROMONAS TUNICATA* ALTERS ITS PROTEOME UPON ADHESION TO EXTRACELLULAR MATRIX

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The marine bacterium *Pseudoalteromonas tunicata* is commonly found associated with eukaryotic hosts and produces anti-fouling activity. The genome of *P. tunicata* contains several genes with high sequence similarity to those encoding outer membrane proteins of human pathogens from the genus *Leptospira*. One of these proteins, PTD2-05920, was recently shown to bind mammalian extracellular matrix (ECM) similar to that seen with LipL32, the major outer membrane protein of *Leptospira*. Since these organisms share a functional ECM binding protein, we set out to determine whether *P. tunicata* could adhere to mammalian ECM . Once this

adhesion was established, a systematic study of the proteomic changes associated with the transition from planktonic to ECM-adherent states was performed for *P. tunicata*. Using Blue Native PAGE coupled with second dimension SDS PAGE, more than 50 well-resolved protein spots were seen, one of which changed upon adhesion. One third of the identified proteins were annotated as TonB-dependent receptors reflecting the great number of TonB genes in the genome. This technique also revealed two protein complexes, each consisting of two proteins. Interestingly, both of the individual complexes arise from consecutive genes suggesting the coordinate regulation, co-transcription, and co-translation of two proteins that are assembled into a complex. Secondly, immobilized pH gradient separation coupled with SDS-PAGE was used to identify additional protein changes upon adhesion. This study is the first to examine the interaction of a marine bacterium with a mammalian extracellular matrix and suggests a general mechanism by which *P. tunicata* regulates protein complex formation.

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ESTABLISHMENT OF OPEN SANDWICH IMMUNOASSAY USING ANTIBODY FRAGMENTS DERIVED FROM COMBINATORIAL LIBRARIES

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Open sandwich immunoassay (OS-IA) is a novel immunoassay principle that enables antigen measurement utilizing antigen-dependent stabilization of an antibody variable region ($Fv=V_H+V_L$). For example in OS-ELISA, an antigen is sandwiched between immobilized V_L and enzyme-labeled V_H, and antigen-dependent formation of stable ternary complex is detected by the colorimetric reaction of the enzyme. In spite of many merits due to noncompetitive mode such as high sensitivity and wide dynamic range, until now, each variable region fragment has only been prepared by cloning the corresponding gene from a hybridoma cell-line, and by expressing it in *E. coli* hosts . To bypass these laborious steps and to further expand its possibility, here we tried to establish OS-IA systems from scratch, through the selection of single chain V_H-V_L (scFv) or V_H-V_H libraries derived from immunized mice.

First, libraries were constructed by the stepwise insertion of V_H or V_L cDNAs from the mice immunized with either hen egg lysozyme (HEL) or FITC-KLH to a phagemid vector pIT2, and introduced into *E. coli* TG1 together with helper phage, resulting in the scFv or V_H dimer-displaying phage libraries. The phage libraries were panned against immobilized HEL or FITC-BSA, and several antigen-specific clones were isolated by phage ELISA. Then four phagemid vectors each encoding a scFv were used as a template of inverse PCR to produce V_L gene-deficient phagemid, and their V_L genes were transferred to an expression vector pET-MBP encoding maltose binding protein. Using the V_H-displaying phage and MBP-V_L fusion protein prepared using the corresponding vector, the resultant OS-ELISA showed the increased signal according to the increased antigen concentration, showing the generality of OS principle.

Unexpectedly, all isolated clones from anti-fluorescein V_H dimer library encoded only one V_H (designated HF25). When HF25 was overexpressed as a MBP-fusion protein and analyzed for its affinity to FITC-BSA, high K_d value of 25 nM was obtained by SPR. We think this is a rare example of wild-type V_H fragment with a high specificity and affinity to a small hapten. Further attempt to establish a novel OS-type assay using the immobilized MBP-V_H and the other V_H fragment selected from the library that can interact with the surface in a fluorescein-dependent manner is now in progress.

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DISCOVERY OF A PROTEIN BIOMARKER CANDIDATE RELATED TO CARCASS WEIGHT IN JAPANESE BLACK BEEF CATTLE (WAGYU)

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Molecular mechanisms that contribute to individual variations in carcass and meat quality in Japanese Black beef cattle are not fully understood. In order to discover protein biomarkers which are involved in carcass and meat quality in beef cattle, we have advanced large-scale proteome analysis of bovine white adipose tissue, using the two-dimensional gel electrophoresis (2DE) and mass spectrometry. Proteins extracted from the white adipose tissue were separated by the 2DE and visualized by SYPRO Ruby staining. Expression levels of separated proteins were evaluated with Progenesis PG220 software (nonlinear dynamics). Finally, the correlation was investigated between the carcass quality and the quantitative expression values of each protein. In total, 879 protein spots were detected on the 2DE gel and 459 protein spots were extensively identified by MALDI-TOF/TOF tandem mass spectrometric analysis. As a result of statistical analysis, it was shown that in high carcass weight (CW) group, 95 protein spots were up-regulated and 2 protein spots were down-regulated compared with those in low CW group. These proteins were associated with a variety of functions, including energy metabolism, cell structure, cell defense, transport, and signal transduction. Interestingly, we identified two proteins related to CW, namely A (37kDa) and B (33kDa). Average CW of the group which expressed both A and B was significantly lower than that of groups which expressed either A or B ($P<0.05$). In addition, MALDI-TOF/TOF analysis revealed

that the two proteins (A and B) were isoforms of X protein with at least two amino acid substitutions. This work was supported by a grant from the Wakayama Prefecture Collaboration of Regional Entries for the Advancement of Technological Excellence of the Japan Science and Technology Agency (JST).

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PROFILING CEREBROSPINAL FLUID PROTEINS IN MULTIPLE SCLEROSIS BY CLINPROT SYSTEM

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Background: Diagnosing Multiple Sclerosis (MS) and other demyelinating diseases such as neuromyelitis optica (NMO) is not always easy clinically and the neuropathology of the two demyelinating diseases remains mystery. Recent advances in the proteomic technology in search of biomarker or biomarker signature that accurately identifies the clinical syndrome would allow for improved diagnosis and disease monitoring. Methods and Results: Magnetic bead-based purification followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was employed to profile human cerebrospinal fluids (CSF) proteins and peptides in a total of 82 samples from patients with definite MS (remission and relapse), NMO (remission and relapse) and primary progressive multiple sclerosis (PPMS). All study participants gave their written informed consent. We used a reagent set of chemically coated magnetic beads, reversed phase (C8) (ClinProtTM) and α- cyano-4-hydroxycinnamic acid as the matrix solution. The eluted samples were then dropped onto a MALDI sample plate (AnchorchipTM), and spectra were obtained by an Autoflex II and a subsequent tandem MS analysis was performed by Ultraflex (Bruker Daltonics). The criteria for peak detection were: signal-to-noise ratio >5, 2-Da peak-width filter, and maximum peak number of 200. The pretreated data were graphed as spectra and evaluated by statistical analysis using the ClinProToolsTM software (Bruker Daltonics). Reproducible profiles were obtained as clear signals and approximately fifty peaks were detected from each of CSF samples. A differential distribution of samples from MS and NMO both in remission was noticeable, while samples from PPMS were not separated effectively using the same platform. One of the key variables contributing to the separation with an m/z of 3,511 was defined as c-terminal fragment (182-212) of neuroendocrine peptide 7B2 by the tandem MS analysis. Conclusion: The application of magnetic bead-based separation combined with MALDI-TOF-MS technique for CSF samples holds the potential to advance our understanding of the biochemical basis of MS and NMO. Further studies are required to validate the clinical effectiveness and disease specificity of the identified biomarkers.

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DIGE ANALYSIS OF RAS-TRANSFORMED FIBROBLAST CELL-DERIVED EXOSOMES

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Ras is a small GTP binding protein. Specific point mutations can lead to a constant activation of Ras molecule. Over expression of mutated Ras molecule induced morphological transformation of NIH3T3 cells in culture and these transformed cells had the ability to induce tumors in nude mice. The activation mutations in Ras molecule play a significant role in tumor formation and development. The critical role of Ras signaling in human cancer is proved by the fact that it is one of the most highly mutated genes in human cancer, with approximately 90% incidence of activating mutation in pancreatic cancer, 50% in colon and 30% in many other types.

Exosomes are small membrane vesicles (30-90 nm) that are secreted by cells upon fusion of multivesicular bodies with the plasma membrane. The molecular composition of exosome reflects their origin in endosomes as intraluminal vesicles. Recent studies have demonstrated that these vesicles apply a broad array of inhibitive effects on the immune system, such as blocking of signaling, proliferation, cytotoxicity and induction of apoptosis in immune cells.

In order to understand the affect of Ras transformation on the protein composition of exosomes, we have isolated exosome-like vesicles from v-Ha-Ras transformed NIH3T3 cells. Exosome proteins from v-H-Ras transformed and non-transformed NIH3T3 cells were analyzed by 2-D DIGE technology. Selected proteins whose expression patterns were dysregulated by Ras transformation were identified using mass spectrometry. A number of candidate proteins involved in a broad range of cellular functions, such as cell proliferation, differentiation, adhesion, invasion, metastasis and apoptosis were identified.

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ASSEMBLY SIMULATION OF FOUR PEPTIDE CHAINS IN EXPLICIT WATER BY MULTICANONICAL MOLECULAR DYNAMICS

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We carried out an assembly simulation of four chains of Altzheimer β-amyloid (Aβ) peptide by multicanonical molecular dynamics (McMD) method. Our computational system is a flexible all-atom model to express four chains of the fragment peptide of Aβ (Aβ16-22) and explicit water molecules surrounding the peptides. The peptide forms an anti-parallel β-sheet structure via backbone hydrogen bonds by NMR experiment. The advantages of the McMD method are as follows: the conformation of system is widely sampled without trapping at energy minima, a thermally equilibrated conformational ensemble at an arbitrary temperature can be reconstructed from the simulation trajectory, and the thermodynamic weight can be assigned to each sampled conformation. During the simulation, exchanges between monomeric and oligomeric states were repeatedly observed. The conformational ensemble reconstructed at 300 K involved various clusters. The main outcome of the current study is that not only anti-parallel β-sheet structures, where four strands were aligned on the plane, but also high dimensional structures (i.e., two sheet structures stacked along perpendicular to the sheet axis) were sampled and each of them belonged to different clusters.

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IDENTIFICATION OF THE FUNCTIONALLY CRITICAL AMINO ACID SEGMENT AND ITS ROLE IN THE FLEXIBLE C-TERMINAL REGION OF THE CHAPERONIN GROEL

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The chaperonin GroEL (14-mer) from Escherichia coli binds denatured proteins and facilitates their folding in vivo and in vitro by encapsulating them within an isolated cavity formed in cooperation with the co-chaperonin GroES (7-mer). The final 23 residues (^{526}KNDAADLGAAGGMGGMGGMGGMM^{548}) in the C-terminal region of the GroEL are invisible in crystallographic analyses due to high flexibility. In order to clarify the functional role of these residues in the chaperonin mechanism, we generated and characterized C-terminal truncated, double ring and single ring mutants of GroEL. The ability to assist the refolding of substrate proteins rhodanese and malate dehydrogenase decreased suddenly when 23 amino acids were truncated, indicating that a sudden change in the environment within the central cavity had occurred. From further experiments and analyses of the hydropathy of the C-terminal region, we focused on the hydrophilicity of the sequence region ^{526}KNDAAD^{531} and generated two GroEL mutants where these residues were changed to a neutral hydropathy sequence (^{526}GGGAAG^{531}) and a hydrophobic sequence (^{526}IGIAAI^{531}), respectively. Very interestingly, the two mutants were found to be defective in function both in vitro and in vivo. Deterioration of function was not observed in mutants where this region was replaced by a scrambled (^{526}NKADDA^{531}) or homologous (^{526}RQEGGE^{531}) sequence, indicating that the hydrophilicity of this sequence was important. These results highlight the importance of the hydrophilic nature of ^{526}KNDAAD^{531} residues in the flexible C-terminal region for proper protein folding within the central cavity of GroEL.

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ANALYSIS OF HUMAN PROTEINS WITH CHARGE PERIODICITY OF 28 RESIDUES IN AMINO ACID SEQUENCES

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It is an important and interesting task to predict a protein structure and function just using information of its amino acid sequence. However, it is very difficult to construct prediction system with high accuracy due to complexity and variety during the formation of protein structures. Considering that protein structure and function is related to the physical properties of amino acid sequence, we conceive that the accuracy of prediction system is probably raised if we could reasonably classify proteins based on the physical properties of amino acid sequences. Recently, we analyzed all amino acid sequences encoded by human genome with charge autocorrelation function in amino acid sequence and found that there is significant charge periodicity of 28 residues. This finding suggests that human genome encodes those proteins with charge periodicity of 28 residues (PCP28).

We extracted PCP28 in all amino acid sequences from human genome by a simple algorithm and found that approximately 3% of all proteins are PCP28. Furthermore, we classified the functions of known PCP28 based on intracellular localization of proteins and found that about 80% of PCP28 are nuclear proteins such as zinc-finger proteins. Some zinc-finger proteins with known three-dimensional structures, for example, human GLI-DNA complex with 5 fingers and mouse Zif268-DNA complex with 3 fingers, show a charge periodicity of around 30 residues. The charge periodicity of the 28 residues of the DNA-binding PCP28 is probably due to the repetition of the zinc finger motif. Similarly, we extracted PCP28 from other vertebrates such as mouse and chimpanzee genomes and found that approximately 1.2~3% of all proteins in each genome are PCP28. Thus, PCP28 forms a novel category of proteins that are mainly localized in the nucleus.

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STRUCTURAL BASIS FOR SUBSTRATE SPECIFICITY IN RICE AND BARLEY BETA-GLUCOSIDASES

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Rice BGlu1 (Os3Bglu7) and barley BGQ60 β-glucosidase/ β-mannosidase enzymes act in degradation of cell wall oligosaccharides. The two enzymes share 67% amino acid sequence identity and act on similar substrates, with binding of cello-oligosaccharides with 5-6 glucosyl residue binding sites. However, they show different substrate specficities, with BGQ60 showing higher efficiency for hydrolysis of *p*NP-β-mannoside than for *p*NP-β-glucoside and for cellobiose, while BGlu1 shows the opposite preferences. To determine the molecular basis of cell wall oligosaccharide recognition and differences between the rice BGlu1 and barley BGQ60 enzymes, the structures of BGlu1, its complex with 2-fluoroglucoside, BGlu1 E176Q mutant in complex with cellopentaose, and E176 with cellotetraose were solved at 2.2, 1.55, 1.8, and 1.95 Å resolution, respectively. The barley BGQ60 was cloned, along with the closely related rice isozymes,. The residues that appear to interact with the substrate were determined from these structures, and the active sites of other rice isozymes, Os1Bglu1, Os3Bglu8, and Os7Bglu26, that are closely related to BGlu1 and BGQ60 were modeled and barley BGQ60 were also modeled based on these structures. Recombinantly expressed BGQ60 had activity similar to that purified from seed, while recombinant Os1Bglu1, Os3Bglu8, and Os7Bglu26 showed activities that ranged between those of BGlu1 and BGQ60, in a manner which did not strictly coincide with their relative similarities. Mutagenesis of rice BGlu1 and barley BGQ60 at residues in the active site that differ between the two enzymes showed that a single mutation cannot explain their differences in β-mannosidase, β-glucosidase, cellobiase, and cellotriase activities.

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IDENTIFICATION OF PHOSPHOPROTEINS AND PROFILING OF PHOSPHORYLATION SITES IN COMPLEX BIOLOGICAL SAMPLES: A SIMPLE AND EFFICIENT WORKFLOW USING MINI-GEL-SEPARATED PROTEINS

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Although major advances have been achieved in the identification and quantification of protein phosphorylation sites by mass spectrometry (MS), routine, easily performed, and comprehensive quantitative analysis of protein phosphorylation still remains out of reach for many laboratories. Here we report upon a simple fluorescence-based approach to quantify gel-separated phosphoproteins that is readily coupled with enrichment and identification of their sites of phosphorylation by MS. An antibody-free process workflow is described involving orthogonal phosphomonoester-selective binding strategies. First, sample complexity is reduced by selective enrichment of phosphorylated proteins from complex biological samples, such as A431-stimulated cell lysate, using affinity chromatographic medium of immobilized alkoxide-bridged dinuclear zinc complex at near neutral pH. Then, the enriched proteins are separated by conventional gel electrophoresis and a phosphorylation-selective fluorescent stain is employed to selectively highlight phosphoproteins via binding to the phosphomonoester dianion moieties of serine, threonine, and tyrosine residues at neutral pH. Interaction with other anionic residues, including carboxylate residues on proteins, is insignificant. As little as 1 ng of phosphoprotein is detectable by this method using standard charge-coupled device camera- or laser scanner-based imaging systems. The detected phosphoprotein bands are excised, subjected to proteolytic digestion, and methylation of carboxyl groups. Constituent phosphopeptides are subsequently purified using titanium dioxide thin-film coated magnetic beads followed by direct analysis of phosphorylation sites by MALDI-TOF or tandem mass spectrometry. Phosphopeptides can readily be identified from as little as 78 fmol of starting material. The presented workflow significantly improved the coverage of identified phosphorylated proteins in complex biological samples.

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FOLDING MECHANISMS OF HOMOLOGOUS PROTEINS: A COMPARATIVE STUDY BETWEEN LYSOZYME AND A-LACTALBUMIN

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Is the folding mechanism conserved evolutionally in homologous proteins? To address the question, we have studied the equilibrium and kinetics of folding/unfolding of canine milk lysozyme and goat α-lactalbumin. The two proteins have 40% sequence identity, and essentially identical three-dimensional structures. The Φ-value analysis, based on the effect of Ca^{2+} on the folding and unfolding rate constants, showed that the Ca^{2+}-binding site was not yet organized in the transition state of folding of canine milk lysozyme although it is well organized in the transition state of α-lactalbumin. The results indicate that the folding initiation site must be different between the two proteins. These results thus provide an example of the phenomenon wherein proteins that are very homologous to each other take different folding pathways. The results will be discussed in terms of possible differences in structure and dynamics between the two proteins.

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PLASMA PROTEOMES AS A BASIS FOR SEARCHING POTENTIAL SEPTIC BIOMARKERS IN INTENSIVE CARE UNITS

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Sepsis is a serious medical condition by a whole-body inflammatory or damage state caused by infection. The average cost to treat sepsis is estimated to be $22,500 per case in U.S.A. and the therapeutic approaches used in sepsis with very limited success. Epidemiology report in Taiwan, sepsis has risen to the twelfth and is often the harbinger of multiple organ failure and constitutes the leading cause of mortality in intensive care unit (ICU). The aim of this study was to compare plasma biomarkers change in septic patients by proteomic approach. We applied two-dimensional polyacrylamide gel electrophoresis (2-DE) to plasma samples of patients with bacteremia. The plasma samples were collected from 15 patients at the first 6 hour after diagnosis of sepsis in intensive care units (6h-ICU) and after 7 days in standard room (7D-SR). Besides, the plasma samples from 6 hour, 24 hour, 72 hour and 1 week were tested for biochemical and riboflavin value in time interval respectively. The bacteremia and non-bacteremia patients were grouped into survival and non-survival on day 21. Eight patients survived and seven patients died during medical follow-up. In plasma 2-DE analysis, differential protein spots were discovered between 6h-ICU and 7D-SR in individual bacteremia patient. These spots were picked up for further protein identified by MALDI-TOF/TOF instrument and MASCOT search engine. Our result show that the blood values of WBC, RBC, BUN, GOT, GPT, D-Dimer, HCO3 were significant difference in septic patients between 6h-ICU and 7D-SR. We also found some interested proteins such as C-reactive protein precursor, short-chain dehydrogenases/reductases family, zinc finger protein, amyloid P-component precursor, haptoglobin were changed from 6h-ICU to 7D-SR in individual bacteremia patient. This study shows that proteomics may be an innovative approach in diagnosis of sepsis and bacterial infection in patients.

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PROTEIN ARGININE METHYLATION OF THE CELLULAR NUCLEIC ACID BINDING PROTEIN (CNBP)

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Cellular nucleic acid binding protein (CNBP) contains seven highly conserved zinc finger repeats and is also named as ZNF9 (zinc finger 9). An RG rich sequence is present between the first and the second zinc finger buckle of CNBP throughout the vertebrates. The sequence is homologous to that modified by protein arginine methyltransferase (PRMT). CNBP was identified as a putative symmetric dimethylarginine containing protein in a previous study by immunopurification with a symmetric dimethylarginine specific antibody. In this study we further demonstrate the arginine methylation in CNBP. Recombinant GST-ZNF9 expressed in *Escherichia coli* could be methylated by recombinant RMT1 and PRMT1 in vitro. The RG-deleted GST-CNBP protein without the RG rich sequence could not be modified by the in vitro methylation reaction. Affinity purified FLAG-CNBP protein expressed in HeLa cells were detected by a monomethylarginine and asymmetric dimethylarginine-specific antibody (7E6), indicating that it contains the modified arginine residues. When the transfected cells were treated with AdOx, an indirect methyltransferase inhibitor, the signal detected for the FLAG-CNBP protein by the methylarginine specific antibody reduced significantly even though similar amount of the FLAG-CNBP protein was present. Furthermore, wild-type FLAG-CNBP but not GAR-domain deleted mutant CNBP could be recognized by SYM10 (symmetric dimethylarginine specific antibody) and ASYM24 (asymmetric dimethylarginine specific antibody). We also observed that FLAG- CNBP might interact specifically with PRMT1 by a co-immunoprecipitation

experiment. In summery, in this study we demonstrate that CNBP has both symmetric and asymmetric dimethylarginine modifications in its RG rich sequence.

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INVESTIGATING ACTION MECHANISM OF A NATURAL ACTIVE COMPOUND HONOKIOL BY QUANTITATIVE PROTEOMIC ANALYSIS

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The quantitative proteomics brings about a new method to investigate the action mechanism of natural active compounds from organisms. SILAC (stable-isotope labelling by amino acids in cell culture) combined with mass spectrometry (MS) has emerged as a simple and powerful quantitative proteomic technique . Honokiol (HNK), an active component purified from Magnolia officinalis, exhibits antitumor effects by inhibiting tumor growth , while proteins involved in antitumor activity in proteomic level are still unclear. In our study, HNK could inhibit cell proliferation and induce apoptosis of HeLa and HepG2 cells in a concentration- and time-dependent manner. We applied the SILAC–MS technique to analyze the differential proteome profiling of cells treated by HNK to investigate key proteins responsible for HNK activities. The changed proteins covered a broad variety of cellular functions including metabolism, signal transduction etc, which indicated HNK performs cytotoxicity to tumor cells through co-operating of many proteins and different pathways. Among these changed proteins, IQGAP1, β-tubin, peroxiredoxin-6 and HSP70 etc proteins were down-regulated significantly, while proteins including annexin A2 etc were up-regulated after HNK treatment. Since IQGAP1 plays important roles in cell adhesion and migration, we supposed that HNK may have effects on cell migration through IQGAP1 based on our MS datasheet. Our further scratch migration assay showed that the migration inhibition of HepG2 cells can be induced by HNK, the RNA and protein expression level of IQGAP1 were respectively decreased obviously in HepG2 cells exposed to 10μg/ml HNK for 24 h . The relationship of HNK antitumor effects and IQGAP1 expression was further confirmed on animal models. Therefore, the down-regulated expression of IQGAP1 by HNK treatment was correlated with cell migration, and HNK probably inhibits cell proliferation and migration through IQGAP1 expression changes and its interactions with other proteins.

Keywords: Honokiol / stable-isotope labelling by amino acids in cell culture / quantitative proteomics / IQGAP1

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SELDI-TOF MS ANALYSIS OF THE EFFECTS OF POST-MORTEM INTERVAL ON RAT BRAIN PROTEOMICS.

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Background: Post-mortem interval (PMI), the time between death and brain collection and storage, is one of the main factors to be considered when assessing changes in brain proteins. We recently developed two new methods of brain tissue preparation for SELDI analysis; either directly apposing tissue onto the proteinchip arrays (tissue apposition, TA)(1), or after an intermediatory step using a filter paper (paper apposition, PA)(2). These techniques result in spectral profile enrichment therefore improving the discriminatory power of SELDI-TOF-MS proteomics(1,2) . Using these methods, we aimed to determine: 1- which PMI condition (time and temperature) resulted in the most number of protein peaks being changed, 2- which brain region showed the most changes (was most sensitive to PMI), and 3- the percent homology between the two application methods (TA vs PA).

Methods: Adult male Wistar rats were assigned to one of 8 PMI groups (n=3 rats/group) including body storage at 4°C for 0,6,12,24,48,&72 hours, or at room temperature (RT; 23-24°C) for 6&12 hours. Four brain regions were studied: the neocortex, caudate putamen (CP), hippocampus and brainstem medulla. Cryosections from each region were apposed directly (TA) or via the use of the filter paper (PA) onto an NP20 proteinchip array, and analyzed by SELDI-TOF-MS.

Results: As expected, body storage at RT resulted in more changes according to PMI than storage at 4°C. At 4°C, PMI>24h resulted in many significant protein changes. Amongst the brain regions studied, changes were more evident for the CP, followed by the cortex, medulla and then hippocampus. Compared to baseline, an average of 50% of peaks changed were detected by both application methods, although many more were evident via the TA than PA method.

Conclusion: Using novel tissue-application SELDI proteomics, we determined that a PMI as short as 6h at 4°C induced significant changes in a number of protein peaks and that the CP was the region most PMI sensitive, followed closely by the cortex.

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STRATEGIES FOR SELDI-BASED BIOMARKER DISCOVERY AND DEVELOPMENT: AN ALZHEIMER'S DISEASE CASE STUDY

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While the recent demand for protein biomarkers to serve as biological indicators of a phenotypically altered state has yielded a large number of candidate biomarkers, validating these biomarkers has been more challenging. Successful biomarker discovery and development efforts require a working knowledge of multiple disciplines, including study and experimental design, proteomics technologies, and data analysis and interpretation. The work presented here focuses on the SELDI-TOF MS-based discovery of candidate biomarkers associated with Alzheimer's Disease in human CSF.

SELDI-based biomarker studies can typically be divided into four phases: Discovery, Validation, Purification and Identification, and Assay Development, each of which requires a unique approach to ensure selection of the most robust markers. The discovery phase is characterized by analyzing samples under a large set of experimental profiling conditions. An initial panel of candidate biomarkers is obtained, which is then tested during the validation phase. In the Alzheimer's disease study presented here, over 250 CSF samples collected from multiple clinical sites were analyzed. The initial discovery study yielded 30 candidate markers, 15 of which were confirmed in an independent validation study. Selected candidate biomarkers were purified and identified using standard protein purification procedures (column chromatography, size filtration, SDS-PAGE, etc.), followed by protease digestion (for proteins larger than 4 kDa) and MS/MS sequence analysis on a tandem mass spectrometer. Identification of the biomarkers provides insight into the disease biology and facilitates the development of analyte specific assays. A specific amyloid-beta fragment was empirically identified as a biomarker in this study, and a multiplexed assay for amyloid-beta fragments will be shown.

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PROTEOMIC INVESTIGATION OF DEVELOPMENTAL AND BIOCHEMICAL EFFECTS ON EXPRESSION OF CYTOSOLIC AND MITOCHONDRIAL PROTEINS IN FOUR OVINE MUSCLES

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A large proteomic investigation of muscle protein expression in organelles of the *M. longissimus dorsi* (LD), *M. semitendinosus* (ST), *M. supraspinatus* (SS) and *M. infraspinatus* (IS) at 1 and 12 weeks of age in sheep was completed. Muscle samples were fractionated into cytosolic, myofibrillar, mitochondrial, endoplasmic reticulum and golgi fractions prior to analysis of each fraction using comparative two-dimensional electrophoresis (2DE) and image analysis to identify proteins associated with organelles that had altered relative abundance between muscles and between time points. Approximately 500 proteins were identified using MALDI TOF/TOF mass spectrometry. We investigated strategies for statistical and pathway analysis within this data set to provide a more comprehensive understanding of muscle and time dependent influences on protein expression in muscle.

Individual proteins with statistically altered expression were identified and assigned official gene symbols (specific to each protein) and Gene Ontology (GO) terms from available databases. Within the cytosolic protein fraction, the expression levels of 62 proteins changed between 1 and 12 weeks of age across all muscles at a significance level of $P<.01$. Using gene co-occurrence probability estimates of DAVID (Database for Annotation, Visualization and Integrated Discovery; http://david.abcc.ncifcrf.gov/home.jsp), we identified that 17 proteins of the glucose meatabolism pathway, showed altered expression between 1 and 12 weeks of age in the cytosol of skeletal muscle. Within the mitochondrial fraction, 136 protein spots were identified whose expression levels changed with developmental age. The most significant pathway effect within mitochondria was an increase in expression of proteins involved in the oxidative phosphorylation pathway ($P<1.9^{-13}$).

Aside from these examples, we have identified several other pathways that differ between the individual muscles investigated that can explain variation in the cellular biochemistry of muscles involved in locomotory or postural function. This experiment has provided an excellent resource from which to evaluate the utility of pathway analysis tools for understanding MALDI TOF/TOF proteomics data.

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THE COMBINATION OF ACCURATE FRAGMENT MASS AND A NEW DATABASE SEARCH ALGORITHM FOR THE IDENTIFICATION OF UNEXPECTED MODIFICATIONS

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One of the primary difficulties with protein identification using database search algorithms is the determination of post-translational modifications. When the appropriate modification isn't chosen in the initial search, the modification may not only be missed but this spectrum can be assigned to the wrong peptide producing a false positive. Sample handling alone can produce several different peptide modifications, most notably deamidation, oxidation, carbamylation, and improper alkylation. This work examines a new search mode designed to identify peptides with unexpected modifications.

A trypsin digest of a HeLa cell protein lysate was fractionated by pI then analyzed using microfluidic-based nano-LC coupled to a Q-TOF mass spectrometer. Database searches were performed against the human IPI database and the initial database search was performed looking for only unmodified matches. Unexpected modifications were subsequently identified using an "unassigned single mass gap" mode which identifies any potential peptide modification by the mass of the modification.

To determine the impact of MS/MS mass accuracy, searches were performed using the "unassigned single mass gap" homology search mode holding the precursor mass accuracy at 10 ppm while varying the fragment mass tolerances from 40 ppm to 1000 ppm. Results demonstrate that the combination of high mass accuracy for both precursor and product ion spectra and a new database search mode where modifications are not known beforehand, confident identification can be made for peptides that would previously have been missed.

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THE STABILIZATION MECHANISM OF THE INTERMEDIATE STRUCTURE OF EQUINE BETA-LACTOGLOBULIN

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A single-disulfide mutant of equine β-lactoglobulin, C66A/C160A, forms an expanded and helical conformation at an acidic pH and a low anion concentration (C state). The C state is a model of an early folding intermediate of β-lactoglobulin. The secondary structures in the C state are in the regions corresponding to F, G, H strands and the major α-helix, and they assume the native-like or nonnative helices. Peptides encompassing those helices did not form stable helices. A longer fragment, CHIBL, which encompasses the structured region in the C states, has a helical structure similar to the corresponding region of the full-length protein in the C state. This result indicates that non-local interactions responsible for the helix formation in the C state reside in the sequence of CHIBL. The disulfide bond, Cys106-Cys119, links two nonnative helices in the C state. This is one of the possible long-range interactions stabilizing the helical structures. The CD spectrum of disulfide-reduced C66A/C160A shows decreased helical content. This indicates that the helices are stabilized by this disulfide bond. The detailed structural analysis of the reduced C66A/C160A by using proline mutants suggests that the nonnative helix in the G strand region can form without the disulfide bond despite Cys106 is contained in that region. It also suggests that the disulfide bond is crucial to the formation of the nonnative helix in the H strand region.

We synthesized a peptide encompassing two helices formed in the C state. The GssH peptide is disulfide-linked G-peptide and H-peptide. This peptide did not form stable helical structures. Therefore G and H strands and major helix regions must interact with each other simultaneously to form stable helices in the C state.

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HIGH-RESOLUTION X-RAY CRYSTALLOGRAPHY STUDIES OF THE H-PROTEIN OF GLYCINE CLEAVAGE SYSTEM

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High brilliance and small divergence synchrotron beam, cryogenic experiments and technical advances in crystallographic analysis have significantly improved the resolution and quality of X-ray crystal structures. In this study, bovine H-protein of glycine cleavage system was used as a model protein for high-resolution X-ray crystal structure experiments. High-resolution crystals of H-protein, which diffracts up to 0.80 Å, were grown by micro-seeding technique. Diffraction data were collected using synchrotron radiation beamline BL5A and NW12A at Photon Factory, KEK. H-protein crystal belongs to space group of *C*2, with its cell dimensions of

a=84.5 Å, *b*=41.3 Å, *c*=43.1 Å, β=91.2• ‹. The overall R_{merge} based on intensities for all data was 5.2%, with its completeness of 98.9% at 0.88 Å resolution. The atomic resolution structure of the H-protein provides us more reliable geometric and conformational properties of the protein.

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DEVELOPMENT OF ON-MEMBRANE PROFILING METHOD FOR PHOSPHOPROTEINS

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Post-translational modifications of proteins are known to play significant roles in complicated biological processes in the living organisms. In particular, protein phosphorylation, one of the post-translational modifications, plays an essential role in eukaryotic signal transduction, transcriptional regulation, control of enzyme activity, cell division and cell metabolism. Therefore, rapid detection and identification of phosphoproteins increasingly become to be required to elucidate these complicated biological processes. In this study, we have developed a method for on-membrane direct identification of phosphoproteins, which are detected by a phosphate-binding tag (Phos-tag) that has an affinity to phosphate groups with a chelated Zn^{2+} ion. This rapid profiling approach for phosphoproteins combines the chemical inkjet printing technology for microdispensing of reagents onto a tiny region of protein spots separated by 2-DE with protein identification by mass spectrometry of peptides tryptic-digested on membrane. Using this method, we analyzed lysates of A-431 human epidermoid carcinoma cells stimulated with epidermal growth factor, and identified six proteins with intense signals upon affinity staining with the phosphate-binding tag (Phos-tag). It was already known that these proteins were phosphorylated, and our new approach proved to be a powerful tool for rapid profiling of phosphoproteins. Furthermore, we tried to determine their phosphorylation sites by MS/MS analysis after in-gel digestion of 2-DE separated protein spots, which are corresponding to protein spots identified by the rapid on-membrane identification. As one example of use of information acquired from the rapid-profiling approach, we successfully characterized a phosphorylation site at Ser-113 on prostaglandin E synthase 3.

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VALIDATION OF FAR UPSTREAM BINDING PROTEIN (FUBP) ISOFORMS IN HUMAN HEPATOCELLULAR CARCINOMA SAMPLES USING MRM INITIATED DETECTION AND SEQUENCING (MIDAS) APPROACH

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The over-expression of a novel protein family, far upstream binding proteins (FUBPs) was identified in human hepatocellular carcinoma (HCC) for the first time. They were obtained first by using two dimensional-difference gel electrophoresis (2D-DIGE) coupled with mass spectrometry (MS). A complementary approach using a four-plex isobaric tagging off-line two dimensional liquid chromatography tandem mass spectrometry (4-plex iTRAQ off-line 2-D LC/MS/MS) was also conducted and these same FUBP isoforms were found to be regulated. Confirmation of these protein isoforms was first performed using western blots but this approach was dependent on the availability of antibodies against FUBP. Thus an alternative and faster approach using MIDAS methodology was also implemented to validate these protein isoforms. We report here that MIDAS could distinguish successfully the two FUBP isoforms in HCC, and at the same time provide quantification data which can be compared with all the quantification techniques employed here.

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CHARACTERIZATION OF SUBFRAGMENT-2 REGIONS OF MYOSINS FROM INVERTEBRATE AND VERTEBRATE STRIATED MUSCLES

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Type II myosin, the major contractile component of muscle, is a hexamer composed of two heavy chains and four light chains (two essential and two regulatory light chains). The N terminal globular head portion of heavy chain subunit (called subfragment-1, S1) is connected to rod portion through subfragment-2 (S2) region, which causes the bends in the rod portion of myosin and might make S1 lift off the thick filament to facilitate its reaction with actin. In the present study, S2 regions from various striated muscle myosins were compared including recently sequenced ones from cephalopods to shed light on the uniqueness of these stretches.

The sequences of myosins from eleven species were aligned, and the α-helical and coiled-coil formations of S2 regions were estimated. As a result, all the S2 regions were considered to have reduced propensity of coiled-coil structure, especially at around the hinge regions, suggesting these regions are structurally flexible. One skip residues was found for all the species, though glutamine was replaced by threonine for fly and vertebrate counterparts. Prediction of secondary structure revealed uniqueness of cephalopod sequence in the N terminal region of S2. This was also confirmed by coiled-coil propensity prediction. Phylogenetic tree drawn based on the amino acid sequence showed that both of scallop and cephalopod S2s formed clear clusters, and were clearly distant from the other invertebrate (fly, worm) and vertebrate counterparts. From the results obtained, it was suggested that S2 regions of muscle myosins are ingeniously tuned so as to adapt to the contraction speed of each myosin or contractile performance of each muscle.

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THE INVOLVEMENT OF THE RESIDUAL STRUCTURE CONTAINING LONG-RANGE INTERACTIONS ON THE DENATURED STATE OF A PROTEIN IN THE AMYLOID FIBRILS FORMATION.

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Protein conformation in the denatured state is known to be involved in amiloid fibrils formation. However, there are few reports on the relationship between amyloid formation and residual structures involved in the long-range interactions. Schwalbe• fs and our groups previously found that the six hydrophobic clusters present in reduced hen lysozyme (HEL) under denaturing conditions were almost disrupted by one point mutation, W62G, indicating the presence of long-range interactions within these hydrophobic clusters. In this study, we examined the effect of the residual structure on amyloid formation using reduced W62G HEL. At first, it was found that the reduced W62G HEL formed hardly any amyloid fibrils in comparison with the reduced wild-type HEL. Next, we examined the amyloid formation of reduced A9G HEL, W111G HEL, or W123G HEL, in which each single mutation differently modulated the long-range interactions of reduced HEL. From the analyses of CD spectra and thioflavine T fluorescences, it was suggested that variation in residual structure led to different amyloid formation. As a result, the extent of amyloid formation did not always correlate with the extent to which the residual structure was maintained, resulting in the involvement of a hydrophobic cluster normally contained in W111 in the reduced HEL. Moreover, we examined the effect of the hydrophobic cluster containing W111 (cluster 5) on amyloid fibril formation of reduced W62G HEL. Although most of the hydrophobic clusters in reduced W62G HEL are disrupted except for cluster 5, the disruption of cluster 5 by the mutation W111G allowed significant amyloid fibril formation of reduced W62G HEL. Interestingly, the extent of amyloid formation in the reduced W62G/W111G HEL was greater than that of the reduced wild-type HEL. From the above results, it became clear that cluster 5 contributed to retarding the amyloid fibrils formation of W62G HEL.

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ANALYSIS OF STRUCTURE-FUNCTION RELATIONSHIPS OF P94 BY PROTEINASE-TRAPPING SYSTEM

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p94/calpain 3 is the skeletal muscle-specific member of calpains, Ca^{2+}-regulated cytosolic cysteine protease family. A main characteristic of p94, however, is its apparent Ca^{2+}-independence during exhaustive autolysis and concomitant proteolysis of non-self substrates. Mutations in the p94 gene impairing p94 protease activity causes muscular dystrophy called calpainopathy, indicating that p94 functions as an indispensable proteolytic modulator for skeletal muscle. Studies have implicated multiple factors in regulation of p94 activity; interaction with connectin/titin, a gigantic sarcomeric protein (1-3), developmental stage of myofibrils (4), and specific insertion sequences in p94 itself (5).

The aim of this study is to comprehensively profile the structural basis of p94 enabling activation in the cytosol without an extra Ca^{2+}. Ca^{2+}-dependent p94 mutants were screened using "p94-trapping", which is an application of yeast genetic reporter system called "proteinase-trapping"an application of yeast two-hybrid system. Several amino acids were revealed as critical for apparent Ca^{2+}-independent p94 activity. These results highlight the importance of conserved amino acids in domain IIb as well as in the p94-specific IS2 region. It was also indicated that some amino acids are differently involved in Ca^{2+}-independence of p94 based on the target of its protease activity, *i.e.*, p94 itself or non-self substrate such as calpastatin (6). It is anticipated that how these properties are influenced by interaction with other molecules such as connectin and/or cellular circumstances is a key to understand p94 functions and a mechanism of its regulation.

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CRYSTAL STRUCTURES OF THE CLOCK PROTEIN EA4 FROM THE SILKWORM BOMBYX MORI

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Many insects pass the winter in an arrested developmental stage called diapause, either as eggs, pupae, or even as adults. Exposure to the prolonged cold of winter is required to permit awakening from diapause in the spring. In the diapause eggs of the silkworm Bombyx mori, a metallo-glycoprotein EA4 has been suggested to serve as a cold-duration clock, because its characteristic ATPase activity is transiently elevated at the end of the necessary cold period. This timer property of EA4 is known to start with the dissociation of a inhibitory peptide (called PIN) under cold conditions, but its time measuring mechanism is completely unknown. Here we present the crystal structures and functional properties of EA4 with and without glycosylation. We show that EA4 is a homodimeric ATPase, with each subunit consisting of a copper-zinc superoxide dismutase fold. There is an additional short N-terminal region capable of binding one more copper ion, suggesting a timer mechanism in which this ion is involved. The sugar chain appears to reinforce the binding of PIN, which may in turn stabilize the initial conformation of the N-terminal domain, explaining the requirement for glycosylation and the peptide to set the clock.

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CLASS IMAGING: CLASSIFICATION OF BREAST CANCER SECTIONS BY MALDI TISSUE IMAGING

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MALDI imaging is a novel technique providing unique molecular information to histological tissue sections. We applied MALDI imaging to a set of tissue sections from breast cancer patients to develop automatic tissue classification routines.

Tissue cryosections were thaw-mounted on conductive coated glass slides and the MALDI matrix was applied using vibrational vaporization. Data were acquired on a MALDI-TOF mass spectrometer in linear mode with image resolution up to 50 μm. Statistical analyses were performed and images were generated either vased on selected protein masses, PCA coefficients or supervised classification results using a support vector machine (SVM) algorithm.

Unsupervised PCA-Analysis allowed the direct visualization of the variance in the MALDI imaging datasets. In most cases the PCA results were in good correlation with the histological examination of the sections. In some cases, however, the results of the PCA did not correlate with the histology. This was due to intensive signals from compounds such as beta-defensins, which originated from contaminations from blood. Exclusion of such peaks from the PCA gave the expected results. Unfortunately, the PCA resulted in a high variance if tumour sections came from different patients. Therefore, the PCA reflected largely the variation across patients rather than variation across tissue types. In contrast, SVM gave direct access to molecular species that were characteristic for specific tissue types.

The visualization of the classification results as 2D-image (Class Imaging) also facilitated the comparison with immuno histostaining. Using the supervised classification approach, it was ossible to create a software model for the classification of Her2 positive cancer, Her2 negative cancer and connective tissue. It was possible to apply this model to unknown tissue sections to obtain the correct, simultaneous classification of both tumour types as confirmed by the inspection of the pathologist! However, larger studies are required to provide a final validation of this approach.

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DETAILED ANNOTATION OF QUALITATIVE DIFFERENCES IN RECOMBINANT PROTEIN SAMPLES –A QC EXERCISE.

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Detailed characterization of recombinant proteins including the differentiation of isoforms or structural aberrations is a lot more difficult than the protein ID problem and its routine solution in proteomics workflows. Typically, a method mix is required that almost certainly involves protein separations, top-down (TD) plus bottom-up (BU) sequence characterization tools.

Two batches of a recombinant protein preparation were analyzed. They were characterized by MALDI-TOF, LC-MALDI-TOF + TD- and BU-sequencing of the separated proteins. All sequences were analyzed with the BioTools 3.1 software (Bruker) that permitted dedicated TD sequencing combined with MS-BLAST (EMBL). Mascot 2.2 (Matrix Science) was used for all BU protein identifications and BioTools for the characterization for peptides that did not immediately match in database searches.

External a priori knowledge was used, such as the N-terminal sequence that was left from a thrombin cleavage site in the N-terminal His-tag.

Three different forms of advanced glycosylation end product-specific receptor isoform were detected (MW range 12.5 -33.5 kDa) in the 2 samples and characterized with regard to their differences using TD-sequencing. Terminal truncation variants were assigned and all forms were fully annotated to sequences from the NCBI-MR95clean protein sequence database + the N-terminal sequence tag as defined in BioTools. TD and BU sequencing together with the TD-LC-MALDI analysis provided 100 % sequence coverage of all three detected protein forms.

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QUANTITATIVE PROTEOMIC ANALYSIS OF BOVINE MAMMARY BIOPSIES BASED ON DIFFERENTIAL FRACTIONATION AND LABEL-FREE MASS SPECTROMETRY

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Label-free mass spectrometry is becoming an increasingly important approach to quantify differentially expressed proteins from complex biological samples. This approach directly compares the peptide peak areas between LC MS/MS runs or the number of MS/MS spectra assigned to each protein, referred to as spectral counting. Currently we report the use of spectral counting and DeCyder MS differential analysis (GE healthcare) for detection of the distribution of proteins among fractions prepared from bovine mammary biopsies.

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ALTERNATIVE TWO DIMENSIONAL ELECTROPHORESIS - OFFGEL ELECTROPHORESIS COMBINED WITH HIGH SENSITIVITY MICROFLUIDIC ON-CHIP PROTEIN DETECTION

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Two dimensional gel electrophoresis (2D-GE) employs isoelectric focusing in the first dimension and a separation of the proteins according to their molecular weight in the second dimension. The gels are then stained using silver stain to visualize the protein pattern. This method is unrivalled in terms of resolution but is a tedious and time-consuming procedure. Here we present a combination of two easy methods that separate proteins in analogy to 2D-GE according to their isoelectric point (pI) and molecular weight (kDa).

For the first dimension, OFFGEL electrophoresis was used. This newly developed method takes advantage of the impressive resolving power of immobilized pH gradient gel based isoelectric focusing (IPG IEF) but in contrast to conventional isoelectric focusing delivers sample in liquid phase thus avoiding sample recovery from the gel. For the second dimension, a microfluidic high sensitivity on-chip protein sizing method was employed. This method allows separating proteins from 5 to 250 kDa and offers a sensitivity equivalent or better than silver staining and a linear dynamic range across four orders of magnitude.

Our data demonstrates that it is possible to easily detect a 1 % change in protein expression.

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PREDICTION OF NUCLEAR PROTEINS WITH A CHARGE PERIODICITY OF 28 RESIDUES IN EUKARYOTE GENOMES

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Recently, we found that approximately 3% of all amino acid sequences from the human genome show a significant charge periodicity of 28 residues[1]. The largest fraction of proteins with a charge periodicity of 28 residues (PCP28) was nuclear proteins, although many PCP28 were poorly identified. Another category of PCP28 was motor proteins which have located in the cytoplasm. We investigated the difference in the physicochemical properties of amino acid sequences between the nuclear and cytoplasmic PCP28 for developing a prediction system to classify of PCP28.

First we extracted PCP28 from all amino acid sequences in the public database of Swiss-Prot release 48.7. Then we classified PCP28 into two categories: proteins in the nucleus and those in the cytoplasm. Second, the physicochemical properties of the two PCP28 categories were compared, allowing calculation of two discrimination scores from entire amino acid sequences as well as from the local regions around clusters of positive charges which are characteristic of nuclear localization signals. Finally, using the scores from the global and local parameters, the prediction system was developed with a sensitivity of 92% and specificity of 88%[2]. Then, to study a biological meaning of PCP28, we discriminated nuclear PCP28 from other types of PCP28 in eukaryote genomes by this prediction system. We compared the number of all nuclear PCP28 in vertebrate and invertebrate genomes. The results showed that nuclear PCP28 is specifically increased in vertebrate genomes and that the ratio of other types of PCP28 is almost constant in all eukaryote genomes[3]. These findings strongly suggest that nuclear PCP28 is an essential protein for vertebrate organisms.

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HEAT-INDUCED CONVERSION OF B_2-MICROGLOBULIN AND HEN EGG-WHITE LYSOZYME INTO AMYLOID FIBRILS

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Thermodynamic parameters characterizing protein stability can be obtained for a fully reversible folding/unfolding system directly by using a differential scanning calorimeter (DSC). However, the reversible DSC profile can be altered by an irreversible step causing aggregation. Generally, the heat-induced aggregation of proteins has been modeled as follows.

$$N \leftrightarrow U \rightarrow A,\ A_m + A \rightarrow A_{m+1} \qquad \text{(heat-induced aggregation)}$$

N and U represent the native and unfolded states, respectively, and A is an irreversibly unfolded protein that undergoes further reaction to form insoluble aggregates A_m composed of m monomers. Here, to obtain insight into amyloid fibrils, ordered and fibrillar aggregates responsible for various amyloidoses, we studied the effects of combination of agitation and heating on two proteins, human β_2-microglobulin and hen egg-white lysozyme. β_2-Microglobulin is a major component of amyloid fibrils deposited in patients with dialysis-related amyloidosis. Hen egg-white lysozyme is homologous to human lysozyme, whose familial mutations are associated with non-neuropathic system amyloidosis. First, aggregates were formed by mildly agitating protein solutions in the native state in the presence of NaCl. Then, these agitation-treated aggregates were heated in the cell of the DSC. For β_2-microglobulin, with an increase in the NaCl concentration at neutral pH, the DSC thermogram began to show an exothermic transition accompanied by a large decrease in heat capacity. Similarly, lysozyme aggregated by agitation at a high NaCl concentration revealed a similar distinct transition in the DSC thermogram over a wide pH range. Electron microscopy demonstrated the heat-induced conformational change into amyloid fibrils. In conclusion, the combination of agitation and heating triggers the formation of amyloid fibrils for these proteins even at physiological pH (heat-induced fibrillation), and DSC has the advantage of being able to evaluate the heat flow accompanied by the fibrillation.

$$N \xrightarrow{\text{agitation}} \text{aggregation} \xrightarrow{\text{heating}} \text{amyloid fibrils} \qquad \text{(heat-induced fibrillation)}$$

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SOFT STRUCTURE OF PROTEINS ANALYZED BY ATOMIC PACKING DENSITY AND VOLUME FLUCTUATION DYNAMICS

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The natively folded protein should have been designed to acquire cohesive forces enough to maintain a solid structure necessary for performing its specific function. On the other hand, it is another important design principle for the folded structure to have some degree of flexibility necessary for its functioning. To examine how natural proteins cope with the former requirement, we have reevaluated the atomic packing density η of 440 proteins. Results of analysis revealed followings: (1) Atoms in the interior of proteins have an η of 0.69 on average. This is higher than η 's of simple organic liquids by more than 0.2, but is significantly lower than both the η of the closest packed structure, 0.74, and those of the proteins reported so far. (2) There exists a vacancy with a thickness of more than 0.1 nm on average between the neighboring atoms in protein interior. Concerning the latter requirements, we have examined dynamical characteristics of the volume fluctuation of proteins using molecular dynamics simulation (MDS). Specifically, power spectra $S(f)$ of protein volume fluctuations were obtained from time-series data on the atomic coordinates of five proteins in aqueous solution to yield following results: (1) The power spectra $S(f)$ decrease with increasing f in inverse proportion to f at low frequencies (LF) and to f^2 at high frequencies. (2) In the intermediate THz frequency range, there exists a broad vibrational band originating from many oscillatory modes with short life times. (3) The volume fluctuations in the LF range result from diffusive

internal motions. Details on the dynamical properties of volume fluctuations at the three frequency ranges and their physical origins, effects of solvent water on these fluctuations, and molecular mechanisms of the soft structure of proteins expected from this analysis will also be discussed.

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MULTIPLE-REACTION MONITORING FOR QUANTITATION OF PROTEIN PHOSPHORYLATION

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Peptide quantitation using multiple-reaction monitoring (MRM) has emerged as an important methodology for biomarker validation. MRM on a triple quadruple (QQQ) mass spectrometer provides superior sensitivity and selectivity for targeted compounds in a complex sample. MRM also offers high precision in quantitation and fast scan speed, which makes it an ideal technology for validating biomarkers in a high-throughput fashion. Reversible protein phosphorylation plays a critical role in cell signaling pathways and the percentage of phosphorylation is often very important to the signal transduction. In this study, we explored the quantitation of protein phosphorylation using MRM with AQUA peptides using the p44/p42 mitogen-activated protein kinase (MAPK) ERK1/2 as the target protein.

The active ERK 1 typically has two phosphorylation sites (T202 and Y204) which reside in one tryptic fragment of the protein and these sites can be phosphorylated at different degrees. This has made the quantitation of this phosphoprotein particularly challenging. Four synthetic peptides (T202/Y204, t202/Y204, T202/y204, t202/y204) were made so we could correctly capture the different phosphoralytion states. ERK 1 was digested *in silico* using software to predict the peptides and their optimum MS/MS product ions. These predicted results were then compared to experimental results from the digest of the protein and the lists of MRM transitions were then created. Calibration curves for the phosphopeptides and unphosphorylated peptides were acquired on a microfluidic-based nanoflow LC interfaced to a triple quadruple mass spectrometer. The active and control ERK1 was also spiked in human sera at different ratio and analyzed after digestion with trypsin. The percentage of the phosphorylation at each phosphorylation site was measured.

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ROLES OF SKELETAL MUSCLE-SPECIFIC CALPAIN, P94/CALPAIN 3, ON MULTIPLE MOLECULAR INTERACTIONS USING CONNECTIN/TITIN N2A REGION AS A MODULATING SCAFFOLD.

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Calpain is an intracellular Ca^{2+}-regulated cysteine protease that moculates substrate structure/function by proteolytic processing, thus called "modulator protease". Humans have 15 genes for calpains, which can be classified into two according to their expression pattern, *i.e.*, ubiquitous or tissue/organ-specific. p94/calpain 3 is a skeletal muscle-specific calpain, and genetic loss of p94 protease activity causes muscular dystrophy called limb-girdle muscular dystrophy type 2A or calpainopathy. Moreover, a small in-frame deletion in the N2A region of connectin/titin that impairs p94-connectin interaction causes a severe muscular dystrophy (*mdm*) in mice (1, 2). Since p94 *via* its interaction with the N2A and M-line regions of connectin becoems a part of the connectin filament system that serves as a molecular scaffold for the myofibril, it has been proposed that structural and functional integrity of the p94-connectin complex is essential for myocytes health and maintenance (3, 4).

In this study, the interactions between p94 and connectin N2A were examined using COS7-expression system, revealing that p94 binds to connectin at multiple sites including newly identified loci in the N2A and PEVK regions of connectin. Functionally, p94-N2A interactions suppressed p94 autolysis and protected connectin from proteolysis. The connectin N2A region also contains a binding site for muscle ankyrin repeat proteins, MARPs, involved in the cellular stress responses by binding to transcription factors. MARP2, one of three MARP paralogues, competed with p94 for binding to connectin and was also proteolyzed by p94. Interestingly, a connectin N2A fragment with the *mdm* deletion had enhanced resistance to proteolysis by proteases including p94, and its interaction with MARPs was weakened. These data support a model that MARP2-p94 signaling converges within the N2A connectin segment, and that the *mdm* deletion disrupts their coordination, and also implicate the dynamic nature of connectin molecule as a *regulatory scaffold of p94 functions* (5, 6).

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MASS SPECTROMETRIC ANALYSIS OF PROTEINS USING AN EXPERIMENTAL DESIGN: CHALLENGES & PERSPECTIVES

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In the present discusion, uses of experimental design for optimization of liquid chromatography tandem mass spectrometry (LC-MS/MS) integrated methods will be discussed. An attempt was made to find solutions to the questions? Such as: What are the optimization criteria, how do we implement appropriate optimization strategies/procedures, and how do we interpret the data obtained? It has been our endower to present and explore different parameters associated with an LC-MS/MS hyphenated experimental set-up, utilizing mainly electrospray ionization (ESI). The application of different mathematical tools may be prerequisite for the realization of the robust results! Possible limitations when it comes to choosing the setting of a specific parameter and a stepwise optimization strategy using an experimental design will be discussed, that hopefully will aid the reader to optimize the performance of such an experimental design approach for mass spectrometric method development and validation. The use of experimental design during method validation for biomarker discovery constitutes a basic feature of multivariate optimization particularly for validation parameters such as robustness and intermediate precision, which if appropriately used can solve several problems and constitutes a powerful tool in the hands of proteomic scientists.

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BROWNIAN RATCHET INHERENT IN F_O AND F_1 MOLECULAR MOTORS

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The F_o and F_1 portions of the ATP synthase are both known to be rotary motors. The *c*-ring in F_o are thought to rotate relative to the *a*-subunit utilizing the electrochemical potential of proton across the membrane, whereas the γ-subunit in F_1 rotates relative to the $\alpha_3\beta_3$ subunit complex utilizing the free energy coming from the ATP hydrolysis. These two rotary motions are mechanically coupled through a central shaft, the γ-subunit, physically connected to the *c*-ring. How the proton current down the electrochemical potential and the ATP hydrolysis generate unidirectional rotary motions, however, remains elusive.

In this study, we conduct molecular dynamics simulations of the F_o and F_1 portions, respectively, by employing an elastic-network-model based coarse-grained representation of the molecules, in which available 3D structures, physicochemical inter-subunit interactions, and thermal fluctuations are taken into account. As for F_o, we first present possible proton pathways found in our simulation, including newly found gating and bottleneck mechanisms. We then show that the *c*-ring presents directionless, stepwise (about 30º intervals) rotational diffusion under the thermal equilibrium condition, and that directionless rotational diffusion changes into unidirectional one when inhomogeneous temperature distribution is applied, which is much in common with the Brownian (Feynman) ratchet. In a similar way, we study the possibility of the Brownian-ratchet-like mechanism for the rotary motion of F_1, which has been suggested by a very recent single molecule experiment. We show that thermal equilibrium fluctuation causes directionless, stepwise (120º intervals) rotational diffusion of the γ-subunit, and that selective activation of largest-amplitude principal modes of the $\alpha_3\beta_3$ complex does bring about directionality in the rotational diffusion of the γ-subunit, even with the axle of the γ-subunit being truncated.

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ULTRA-FAST SEPARATION OF BIOMOLECULES USING SUPERFICIALLY POROUS SILICA PARTICLES - POROSHELL

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Ultra fast separation of large biomolecules is always a challenge for chemists because of slow mass transfer rate of large biomolecules. Superficially porous reversed-phase HPLC packings have taken their place in the scientific toolkit as the optimum particle for ultra-fast separations of proteins and polypeptides [1]. Versatility in this arena has been increased due to the development of sterically protected C18, C8 and C3 bonded phases for low pH application and bidentate C18 for high pH application. The new column formats such as capillary HPLC column not only increase the speed of the separation, but also dramatically increase the sensitivity of the separation.

This presentation intend to give a review of development of superficially porous particles (Poroshell), the theory of ultra-fast separation using Poroshell particles, and a variety of applications of LC/MS analysis of standards and real samples under different pH and flow-rate conditions. These applications show the advantages of the superficially porous silica for ultra-fast separation of polypeptides and proteins in a variety of chromatographic modes.

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HYPHENATED TOOLS FOR PHOSPHOLIPIDOMICS

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The analysis of underivatised lipids within body fluids as well as cell and tissue extracts is still a very the challenging task because of its biochemical and clinical relevance. Anomalous lipid concentrations are correlated to neoplastic and neurodegenerative diseases, diabetes etc.. Structural diversity of each lipid or lipid class respectively will have a distinct effect on membrane properties (i.e. fluidity, permeability, oxygenscavenger, etc.).

Therefore, the advantage of recombined use of HPLC, MS and NMR will be shown. Many studies dealed with the analysis of lipids, but to our knowledge nobody used a combinatorial approach so far. A combination of HPLC separation power, MS sensitivity with accurate mass measurement of molecular and fragment ions and NMR structure elucidation power will meet most suitably the challenge. Furthermore, the low NMR sensitivity can be compensated by preceding concentration steps via HPLC and fraction sampling. New HPLC methods for several phospholipid classes (i.e. sphingomyeline, phosphatidylcholine, phosphatidylenthanolamine) were developed and the retention times and the detected masses were determined. Location of fatty acids with respect to position sn-1 and sn-2 were identified in negative ion mode by the relative intensity of their [M-H] ions and the neutral loss of the fatty acid ketene. In positive ion mode the polar head group was cleaved off. The molecular formula was generated by matching high mass accuracy and isotopomer pattern. Furthermore, the separated fractions were assigned by means of the 1D- and 2D-NMR-spectra. Saturated, mono unsaturated (MOFA) or polyunsaturated fatty acids (PUFA) show zero, two or four carbon signals between 120 and 130 ppm. The MOFA and PUFA reveal unambiguously different chemical shifts for the olefinic carbons. However, lipids with MOFA's have similar olefinic carbon shifts. Nonetheless, a lipid with two MOFAs is deduced from the intensity ratio of the olefinic protons with respect to the glycerol protons.

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DEVELOPMENT OF A SCORING METHOD FOR PREDICTING PROTEIN COMPLEX STRUCTURES

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The information about protein-protein interactions increases much more rapidly than the increase of the number of the tertiary structures of those protein complexes. Therefore, precise prediction of protein complex structures by protein-protein docking simulations is required. When the protein complex is re-built from its component protomers which derive from experimentally determined complex structure (native structure) by docking, the complex models with rmsd < 10 Å from the native structure (near-native model) could be obtained , along with a great number of false positives (decoy). The separation of near-native models from many decoys is therefore needed in the prediction of complex structures by docking. In this study, we developed the method for scoring docking models so that the near-native models were higher in rank than decoys, based on the assumption that the interfaces of near-native models are more complementary in terms of surface properties and shapes compared to those of decoys.

We used 125 non-redundant hetero-dimers (native structures) as targets. For each target, maximum 500 complex models were generated by our docking method. We also observed these targets in terms of the shape of the interfaces of their native structures. As a result, we found that these targets could be classified into two groups according to their interface shapes, and moreover, that this classification correlated with another classification which was based on the number of models with high docking score, namely, the difficulty in the separation of near-native models. We therefore only focused on 75 targets classified as difficult targets which need the separation. So far our method could separate the near-native models from the decoys in 70% of these targets.

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DEVELOPMENT OF A HIGH PERFORMANCE PREDICTION METHOD FOR SINGLE SPANNING MEMBRANE PROTEINS

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Membrane proteins constitute 20-25% of open reading frames in a biological genome [1]. Previously we developed a membrane protein predictor SOSUI [2] and a signal peptide predictor SOSUIsignal [3] whose web site is visited by many researchers in the world. However, this system is not good at prediction of single spanning (TM1) membrane proteins. It is a common problem to all membrane protein prediction tools. TM1 membrane proteins occupy 30-35% of membrane proteins in a genome and have various important functions.

In this study, we prepared a non redundant dataset of membrane and soluble proteins from Swissprot for developing a method for discriminate TM1 membrane proteins with the signal peptide (SP) from other types of membrane and soluble proteins.

First, we classified the dataset into soluble, single spanning and multi spanning protein by the number of transmembrane helices predicted by SOSUI ver.3. The relationship between the position of predicted transmembrane helix and the physicochemical properties around amino terminus was investigated, leading to the fact that TM1 membrane proteins with SP tend to have a transmembrane helix around carboxyl terminus and hydrophobic amino terminus.

Using this result, we can predict 85% TM1 membrane protein with SP and 72% TM1 membrane protein without SP with the high accuracy. This result improves the accuracy of past prediction tools by 15% in prediction of TM1 with SP.

TM1 membrane proteins with SP contain a very important family of receptors which bind with proteins such as growth factors. High performance prediction system for TM1 membrane proteins with SP is the first step for the prediction of receptors which will be useful for the medical application.

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SENSITIVE NONCOMPETITIVE DETECTION OF OSTEOCALCIN TERMINAL PEPTIDE BY OPEN SANDWICH IMMUNOASSAY

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Small peptides with less than 1000 in molecular weight are not considered amenable to sandwich immunoassays due to their difficulty of simultaneous recognition by two antibodies. As an alternative, we attempted noncompetitive detection of small peptides by open sandwich enzyme-linked immunosorbent assay (OS-ELISA) utilizing the antigen-induced enhancement of antibody VH/VL interaction. Taking fragments of human osteocalcin (BGP), a major non-collagen peptide produced in bone, as model peptides, OS immunoassay was performed using the cloned VH and VL cDNAs from two anti-BGP monoclonal antibodies either recognizing the N- or C-terminal fragment, respectively. When the clones were used for OS-ELISA with immobilized VL fragment and phage-displayed VH fragment, enhanced VH / VL interaction upon BGP addition was observed. Especially the clone for the C-terminal fragment showed superior detection limit as well as a wider working range than those of competitive assay (1). The result was reproducible with either purified VH-alkaline phosphatase or peroxidase-conjugated MBP-VH, together with immobilized MBP-VL fusion proteins, and in the latter case the assay can be performed on microplate wells and also in microfluidics. The minimum detectable fragment was the hexamer including the C-terminus, implying hapten-like terminal recognition. To further improve the sensitivity of the assay, a phage-displayed PCR-randomized VH library was subjected to repeated selections on MBP-VL in the presence of reduced amount of peptide (OS selection). The selection successfully gave a clone with ten-fold lower detection limit, which was well below the serum peptide level in healthy human. This simple approach with a single antibody with a short measurement time may prove a useful tool in immunodiagnostics as well as in proteomics research.

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ANALYSIS AND IDENTIFICATION OF PROTEIN COMPONENTS IN DEPOSITS ON WORN CONTACT LENSES BY LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC-MS)

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Background : Deposits on worn contact lenses cause eye adverse events for contact lens wearers but the components of the deposits and their role in adverse events are not known. Aim: The aim of this study is to develop a LC-MS method to analyze and identify protein components in worn contact lens deposits. Methods: Worn contact lenses were collected from normal adult subjects (> 18 years old) who participated in an inhouse study involving wearing the lenses for 12 hours without using any contact lens care solution. The deposits on the lenses were extracted using a buffer containing 4 M urea and 0.1% SDS. After desalting and concentrating, the samples were digested with trypsin and analyzed by LC-MS. Peak lists were generated by MassLynx (version 4.0 SP1, Micromass) using the Mass Measure program and submitted to the database search program Mascot. Protein identification was based on matches of detected peptides to reference peptides that could be derived from a protein in NCBI database. Ions scores > 50 indicated identity or extensive homology ($P < 0.05$). Results: Total 11 proteins were identified in the sample. Among them, 5 are identified tear proteins (lysozyme, basic praline-rich proteins, lactoferrin, immunoglobulin J chain and hypothetical protein), 1 is from skin (epidermal keratin type I) and others are from un-known source (Ig alpha-1 chain C region, beta-actin, actin, apolipoprotein and proapolipoprotein). Conclusion: LC-MS is a sensitive method to analyze and identify proteins deposited onto worn contact lenses.

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A METABOLOMIC APPROACH FOR ANALYSING PLANT-HERBIVORE INTERACTIONS

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A metabolomic approach to uncover the complexity of the induced defense signaling networks that have evolved during the arms races between plants and their attackers is presented. Plants respond to herbivore attack by releasing defense metabolites. This study investigated how cabbages (Brassica oleracea) respond to herbivore attack and subsequently how these defense metabolites chemically affect cabbage white (Pieris rapae) caterpillars which have been fed on the defense induced cabbages. Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC/MS) was used to analyse the comprehensive chemical compositions of extracts made from both the plants and the caterpillars. MarkerLynx, a program which incorporates a peak deconvolution package and collects data into a single matrix by aligning peaks with the same exact mass/retention time along with their normalised intensities was used to identify the monoisotopic mass of the constituent components and perform PCA. The data table was exported to advanced statistical packages to enable univariate and multivariate statistical analyses to be performed. Online database searching was performed to tentatively identify metabolites of interest. Structural elucidation experiments were performed and MassFragment, a fragmentation interpretation tool which uses systematic bond disconnections to assign fragment ions to the precursor ion, was used to confirm the putative assignments.

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EXPRESSION AND LOCALIZATION OF CARNITINE/ORGANIC CATION TRANSPORTER OCTN1 AND OCTN2 IN OCULAR EPITHELIUM.

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Purpose. Functional evidences demonstrated that a carrier-mediated organic cation transport process appears to exist in the conjunctiva, mediating the absorption of carnitine and organic amines, including certain amine-type ophthalmic drugs. This study was undertaken to investigate the expression and localization of carnitine/organic cation transporter OCTN 1 or OCTN2 in ocular surfaces using human ocular epithelial cell lines and rabbit ocular epithelium tissues.

Methods. Immortalised human corneal-limbal epithelial (HCLE) and conjunctival epithelial (HCjE) cells were cultured in Keratinocyte Serum Free (K-SFM) medium. OCTN1 and OCTN2 mRNA expression was investigated using reverse transcriptase-polymerase chain reaction (RT-PCR) assay. The identity of each PCR product was verified by DNA sequencing. Expression and localization of OCTN1 and OCTN2 at the protein level in ocular epithelial cells and rabbit ocular epithelium was studied by immunocytochemistry and immunohistochemistry, respectively, using polyclonal antibodies from goats raised against the 13 C-terminal amino acids of human OCTN1 or OCTN2. Preimmune rabbit serum was used for negative controls.

Results. OCTN1 and OCTN2 mRNA expression was detected in both HCLE and HCjE cells and verified by DNA sequence analysis. Immunoreactivity revealed OCTN1 and OCTN2 proteins to be ubiquitously expressed throughout the cell with some apparent accumulation in the cell membrane in both HCLE and HCjE cells. Expression of OCTN1 and OCTN2 in rabbit corneal and conjunctival epithelium was also observed. OCTN2 immunoreactivity in rabbit conjunctival epithelium appeared higher than that in corneal epithelium.

Conclusions. This report is the first to document expression of OCTN1 and OCTN2 in human corneal and conjunctival epithelial cells and in rabbit ocular epithelium tissues. These findings suggest a potential involvement of OCTN1 and OCTN2 in transport of carnitine in ocular tissues. [This research was sponsored by Allergan Inc, USA]

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THEORETICAL INVESTIGATION OF THE ELECTRONIC ASYMMETRY OF THE SPECIAL PAIR CATION RADICAL IN THE PHOTOSYNTHETIC TYPE-II REACTION CENTER

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The electronic asymmetry of the special pair cation radical in the photosynthetic reaction center (RC) was studied, using quantum chemical calculations with a polarizable continuum model and a point charge model as the protein environment. The calculated spin density distribution between the halves of the special pair from *Rhodobacter (Rb.) sphaeroides* agreed qualitatively well with the experimental value due to the protein polarity effect. The differences in the specific orientations of the ester carbonyl groups of the phytyl groups, as well as the methyl ester groups, are one of the origins of the electronic asymmetry.

The generality of the specific orientations was confirmed with fourteen X-ray structures of a variety of type-II RCs. The interactions between the methyl ester and phytyl groups and the surrounding amino acids were investigated by structural and sequence alignments. The alignments revealed that specific van der Waals contacts and polar interactions are conserved among the type-II RCs, with a few exceptions, suggesting that the orientations of these groups are controlled by the specific interactions between them as the evolutionary consequence. The calculated spin density distributions of special pair cation radical from anoxygenic bacteria of *Rb. sphaeroides* and *Rhodopseudomonas viridis* RCs and from oxygenic photosystem II RC (Cyanobacteria) of *Thermosynechococcus elongatus* were always localized in the L-side halves of special pair cation radical, qualitatively reproducing the experimental results. The difference in the orientation of the phytyl group, which is controlled by the protein environment, is the common feature among type-II RCs to determine the electronic asymmetry.

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APPLICATION OF LTQ ORBITRAP XL ETD^{TM} FOR GLYCOPEPTIDES ANALYSIS

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Of all protein post-translational modifications (PTMs), Glycosylation is the most widespread and complex one. Its modifications are highly labile and resulting peptides are most often highly heterogeneous. Characterization of glycopeptides remains a great analytical challenge. LC MS/MS is the most powerful and versatile techniques for glycopeptides structure elucidation. However, commonly used collisional-induced dissociation (CID) has limitations on determining the modification site due to the labile nature of the glycan modifications. The very recent ability to routinely obtain high resolution and high accurate mass measurements of MS and MS/MS fragments combined with Electron Transfer Dissociation (ETD) provides a new and powerful tool that makes the identification of modification site and glycan structure elucidation possible.

Two reasonably well characterized glycoproteins, bovine α1-acid glycoprotein and human α1-acid glycoprotein were purchased from Sigma. The proteins were reduced, alkylated and enzymatic digested. The glycopeptides were then introduced onto a graphitic carbon column for nano LC MS/MS analysis. LTQ Orbitrap XL ETD spectrometer was used for glycosylation site determination and glycan structure elucidation.

Graphitic carbon column demonstrated excellent capabilities for glycopeptides analysis especially for short hydrophilic peptides containing bi- or tri-antennary glycan chains without any enrichment. With the high mass accuracy and high resolution of hybrid linear ion trap-Orbitrap MS, the highly heterogeneous glycopeptides were well resolved and accurately measured. Formation of metal adducts on Hypercarb column promotes higher charge species and as a result improves ETD fragmentation of glycopeptides which lead to the successful determination of the glycosylation site. Therefore both the glycopeptides glycosylation site and glycan structure were successfully identified by using the combination of porous graphite chromatography and LTQ Orbitrap XL ETD Hybrid FT mass spectrometer in a single LC run.

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IDENTITY OF PROTEINS EXTRACTED FROM WORN SILICONE HYDROGEL CONTACT LENSES

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Purpose. To identify the proteins deposited on silicon hydrogel contact lenses during wear and analyze the effect of lens materials and multipurpose disinfecting solutions on protein deposition.

Methods. Four contact lenses Lotrafilcon B (CIBA Vision), Balafilcon A (Bausch & Lomb), Galyfilcon A (Johnson & Johnson Vision Care, J&J) and Senofilcon A (J&J) and four disinfecting solutions ClearCare (CIBA Vision), Opti-Free Express (Alcon), Opti-Free RepleniSH (Alcon), and AQuify (CIBA Vision) were used. Worn contact lenses (daily wear, 1 month) were collected from subjects and the protein deposits on the lenses were extracted using a buffer containing 4 M urea and 0.1% SDS. After desalting and concentrating, the samples were digested with trypsin and analyzed by liquid chromatography-mass spectrometry (LC-MS). Peak lists were generated by MassLynx (version 4.0 SP1, Micromass) using the Mass Measure program and submitted to the database search program Mascot. Protein identification was based on matches of detected peptides to reference peptides in NCBI database. Ions scores > 50 indicated identity or extensive homology ($P < 0.05$).

Results. A total of 68 different proteins were identified from the samples. The deposit from Acuvue Oasys with AQuify had the highest number of protein species (31) while the samples from O_2Optix with ClearCare and Acuvue Advance with Opti-Free Express had the lowest number (4). The most frequently detected proteins were lysozyme (15 kDa), lipocalin (19 kDa) and proline rich protein 4 (15 kDa). Three other abundant tear proteins, lactoferrin (69 kDa), IgA (50 kDa) and albumin (69 kDa), were detected in a lower frequency. Immunogloblin family of proteins were frequently extracted from worn Acuvue Oasys lenses. Keratin was also frequently extracted, probably due to continued touching of lenses by hands during insertion and removal.

Conclusions. Contact lenses absorb/adsorb proteins from tear film and other sources during wear.

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SIMPLIFYING THE HUNT FOR OPTIMAL SRM TRANSITIONS: UTILIZING DISCOVERY DATA TO EXPEDITE TARGETED PEPTIDE QUANTITATION

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Greater emphasis has been placed on advancing proteomics studies from discovery and/or relative quantitation to validated quantitative methods in an effort to establish clinical assays. The typical workflow involves first performing discovery based experiments to identify protein expression levels that are confidently changing between a control and treated samples and generate product ion information used to sequence the precursor peptide. The difficulty arises in transferring discovery based methods directly over to validated quantitation methods since each is generally performed on separate mass spectral platforms. Low confidence has been placed on relating relative product ion abundance obtained from ion trap CID to that observed using a triple quadrupole mass spectrometer due to the difference in ion activation mechanisms and the timescale of the excitation. Thus, the only information transferred from one method to the other is protein id, peptide sequence, and the most abundant charge state resulting in further method development to complete the SRM assay. Common approaches to determine SRM transitions are based on a set of accepted rules to determine the best possible ion pair(s), which are then searched against the matrix database to determine the uniqueness of each mass pair. We contend that the relative abundance of product ions originating from ion trap CID can be a used to directly assign the most sensitive ion pairs for the targeted SRM methods.

We will present direct comparison of relative product ion abundance measurements for 100 plasma peptides between an ion trap and a triple quadrupole mass spectrometer. The selected peptides are broken down into sequence length ranging from 7 to 15 residues to determine consistency across the typical biomarker properties. Success rates for matching the most abundant product ions from each method to those predicted will be consolidated and reported.

PROTEOME AND IMMUNOME OF THE VENOM OF THE COBRA AND RUSSELL'S VIPER IN SRI LANKA

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Snake bites are a serious health problem in many topical and sub-tropical regions. Proteomic characterization of snake venom is imperative, because the underlying treatable pathogenesis depends on the venom protein composition ingested. Geographical variations in the composition of venom toxins has been well documented. Production of species specific effective anti-venom with minimum reactions will reduce the incidence of complications and death after snake bites. Hence the elucidation of specific proteomic profile of Sri Lanka snake venom and production of species specific antivenom could have vast implications for medicine.

The proteome of the Sri Lankan cobra,(Naja naja naja) and Russell's viper(vipera russelli) venom, analysed by chromatography and SDS-PAGE. These proteins were fractionated into 8-10 groups according to the differences in their molecular sizes. They are : Complement depleting venom factor, high molecular weight venom proteins, Haemotoxins , cysteine-rich toxin, , Metallo proteinases, , cardiotoxins, cytotoxins, , neurotoxins and phospholipases.

Available antivenom (Haffkine, India) serum currently used for the treatment of cobra and viper bites reacted significantly only to the major fraction of venom proteins, phospholipases in the venom (cobra and viper) by immunoblotting. The venom proteomic insight of this study indicate the therapeutic species specific anti-venom of improved quality, i.e. also containing antibodies to the newly identified minor toxic, but poorly immunogenic components. It is expected that such a preparation should have a higher effectiveness than the currently used anti-venom in resuscitating snake-bite victims. Further studies are in progress.

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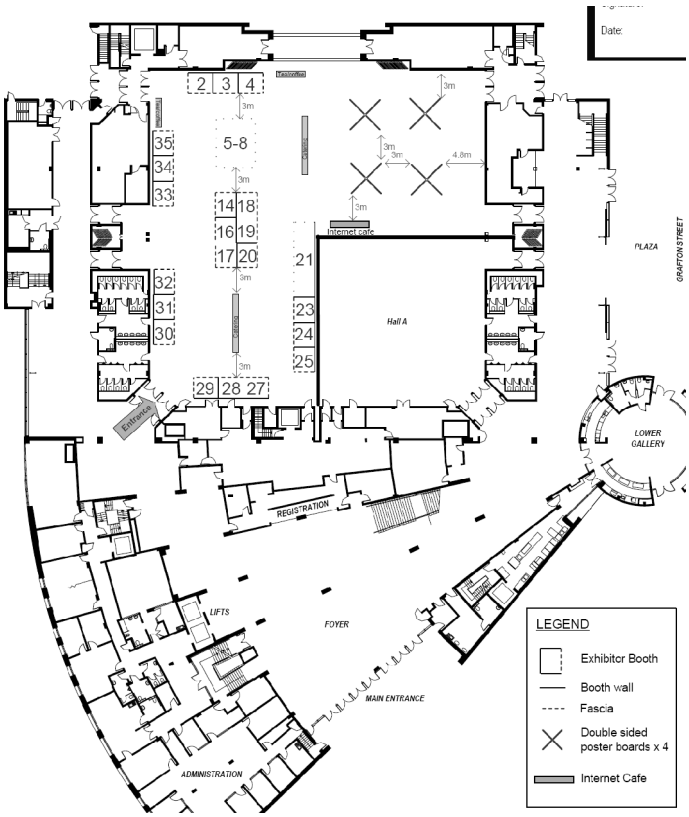

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