PROGRAM AND ABSTRACTS

15th Meeting

Methods in Protein Structure Analysis

University of Washington
Seattle, Washington
USA
August 29 to September 2, 2004

INTERNATIONAL ASSOCIATION FOR PROTEIN STRUCTURE ANALYSIS AND PROTEOMICS

August 29, 2004

Greetings,

After 18 years, the MPSA conference returns to the beautiful city of Seattle, Washington. Those of you who have been regular participants undoubtedly will warmly remember MPSA1986, organized by Ken Walsh and his colleagues, at this very same venue on the University of Washington campus. Certainly old timers will remember waking up each morning and seeing the crystal clear view of Mount Rainier, with its splendid snowcap, as they walked to breakfast, and the excursion to the Paradise Visitor's Center on Mount Rainier or to the Washington wineries northeast of Seattle. Rain! There was no rain. But there was exciting science, old friendships renewed, new ones made, and plenty of opportunity to learn, to discuss one's latest results, and to see the latest in equipment that we hoped might soon find a home in our labs. Who could forget the banquet: A multitude of food stations each of which invited sampling. A continuous gourmet dance of conversations with ever-changing colleagues who only too soon would return to their homes.

While the venue may be the same, times have changed. Five years ago the International Association for Protein Structure Analysis and Proteomics (IAPSAP, www.iapsap.bnl.gov) was formed to organize and sponsor MPSA conferences. MPSA2004 will not be a duplicate of MPSA1986. Nevertheless, the organizers hope that MPSA2004 will capture the spirit of that earlier conference. A truly outstanding set of speakers has been invited to illustrate advances in methods of protein structure analysis through their latest results. No longer focused primarily on Edman chemistry, the scope of MPSA has grown to encompass, among other topics, high throughput methods, computational based techniques, mass spectrometry, and imaging. Outstanding young (and a few not so young) investigators have been selected to report new and exciting results. Many of the leading vendors of scientific instrumentation for protein analysis will be present to explain the advantages of the latest equipment for protein structure analysis and proteomics. New since MPSA1986, MPSA2004 will feature the ninth Pehr Edman Awards and the third IAPSAP Young Investigator Award. This year's banquet takes place in the unique Boeing Museum of Flight.

Welcome! The organizers welcome both the participants and the sponsors and are pleased that you have chosen to be part of the 15th biennial conference on Methods in Protein Structure Analysis - MPSA2004. Come! Renew friendships with colleagues perhaps not seen since those warm days in Valencia, Spain at MPSA2002. Let the science take flight.

For the ISPSAP Executive Committee,

Ettore Appella, President
Jay Fox, Treasurer
Carl W. Anderson, Secretary

MPSA2004
Program and Organizing Committee

Ray Paxton
Amgen, Inc.

Lowell Ericsson
Amgen, Inc.

Ken Walsh
University of Washington

Ruedi Aebersold
Institute for Systems Biology

Ettore Appella
National Institutes of Health, NCI

Jay Fox
University of Virginia

Carl W. Anderson
Brookhaven National Laboratory

EXHIBITORS

The exhibit hall will be open Monday through Wednesday in the HUB East Ballroom.

AnaSpec, Inc.
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Biacore Inc.
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(NOTE: in printed program book the sponsors were represented by logos)

FELLOWSHIPS

Partial support for the following students or postdoctoral fellows to attend MPSA2004 was provided by the Office of Biological and Environmental Research, U.S. Department of Energy, and by the National Institute of General Medical Sciences of the National Institutes of Health.

Oral Presentations

James R. Kraly, University of Washington, USA
Yu Lu, University of Washington & Institute for Systems Biology, USA

Poster Presentations

Arunima Bandyopadhyay, University of Washington, USA
Jorge A. Calleros-Diaz, Chemistry Insitute, UNAM, Mexico
Laura Collantes de Terán Escribano, Universidad de Sevilla, Spain
Md. Abul Fazal, University of Washington, USA
Shohini Ghosh, University of Washington, USA
Susana Linskens, Universidad de Buenos Aires, Argentina
Afua Nyarko, Ohio University/Oregon State University, USA
Martina Schad, Max-Planck-Institut, Germany
Regine M. Schoenherr, University of Washington, USA
Hui-Chun Yeh, University of Montana, USA

Participating Volunteers

Yi-Ling Christy Chen, University of Washington
Daniella Cohen, University of Washington
Ryan Day, University of Washington
Julia M. DeBaecke, University of Washington
Scott Gable, University of Washington
Matt Holdren, University of Washington
Jasper Lin, University of Washington
Jie Luo, University of Washington
Christine McBeth, University of Washington
Eric Merkley, University of Washington
Brook Nunn, University of Washington
Delia Pinto-Santini, University of Washington
Alyssa L. Smith, University of Washington

Program Schedule

MPSA2004

SUNDAY, AUGUST 29, 2004

14:00 - 20:00 *HUB Auditorium Foyer*	Registration/Information Open
18:30 - 19:00 *HUB Auditorium*	Welcome to MPSA: Ray Paxton, Amgen Corporation, USA Welcome from IAPSAP: Ettore Appella, National Cancer Institute, NIH, USA Keynote Introduction: Trisha N. Davis, University of Washington, USA
19:00 - 20:00 *HUB Auditorium*	**Keynote Speaker:** Stan Fields, Howard Hughes Medical Institute, University of Washington, USA "Protein Interactions"
20:30 - 22:00 *McMahon Hall Patio*	**Welcome Reception**

MONDAY, AUGUST 30, 2004

07:30 - 19:30 *HUB Auditorium Foyer*	Registration/Information Open
08:30 - 08:45 *HUB Auditorium*	Welcome/Introductions Ray Paxton, Amgen Corporation, USA

I. The Interactome
Chairperson: Richard Perham

08:45 - 09:30 *Hub Auditorium*	Michael Snyder, Yale University, USA "Global Analysis of Biochemical Activities Using Protein Chips"

II. Analytical Strategies in Proteomics
Chairpersons: Norman J. Dovichi and Brigitte Wittmann-Liebold

09:30 - 10:15 *Hub Auditorium*	Ulf Landegren, Uppsala University, Sweden "Analysis of Individual and Interacting Proteins Via Proximity Ligation Reactions"
10:15 – 10:45 *HUB East Ballroom*	**Break:** Coffee/Exhibits Open
10:45 - 11:30 *Hub Auditorium*	Tomas Rejtar, Barnett Institute, Northeastern University, USA "New Approaches to Maximize the Information Content of Proteomic Samples Using LC-MALDI-TOF/TOF MS Analysis"
11:30 - 12:15 *Hub Auditorium*	Richard D. Smith, Pacific Northwest National Laboratory, USA "Advances in Quantitative High Throughput Proteomics based upon Nano-LC and Mass Spectrometry"
12:15 - 12:30 *HUB Auditorium*	**Short Talk** James R. Kraly, University of Washington, USA "Proteomic Analysis of Barrett's Esophagus using Capillary Electrophoresis with Laser-Induced Fluorescence"

continued...

Short Talk

12:30 – 12:45 — Jeffrey A. Kowalak, National Institute of Mental Health, USA
HUB Auditorium — "An Integrated Workflow for Interpretation of Shotgun Proteomics Data"

12:45 - 14:15 — **Lunch Workshop** Presented by Applied Biosystems
HUB 106B — Sally Webb, Proteomics Technical Marketing Specialist
"PTM Discovery and Biomarker Quantitation Workflows using the Applied Biosystems/MDS SCIEX 4000 Q TRAP(R) LC/MS/MS System"

Workshop Session 1
Chairperson: Kenneth Walsh

14:15 - 15:15 — Donald Hunt, University of Virginia
HUB Auditorium — "Proteomics: Automated Analysis of Peptides and Proteins at the Attomole Level in Complex Mixtures by Mass Spectrometry"

15:15 - 16:15 — Philip Ross, Applied Biosystems
HUB Auditorium — "Protein and Peptide Quantitation using Novel, Multiplex Tagging Reagents"

16:15 - 16:45 — **Break:** Refreshments/Exhibits Open
HUB East Ballroom

Workshop Session 2
Chairperson: Ray Paxton

16:45 - 17:40 — Jeff Silva, Waters Corporation
HUB Auditorium — "Expression Profiling of Various Metabolic Conditions of *E. coli*"

17:40 - 18:35 — Gary Kruppa, Bruker Daltonics
HUB Auditorium — "Studies of Three-Dimensional Protein Structure using Mass Spectrometry (MS3D). Protein Surface Reactivity and Chemical Cross-linking"

18:35 - 19:30 — Sandra L. McCutchen-Maloney, Lawrence Livermore National Lab
HUB Auditorium — "From Infection to Detection via Characterization of Host Response"

19:30 – 21:00 — **Poster Session I: Pizza and Posters**
HUB East Ballroom — Sponsored by Applied Biosystems and Bruker Daltonics

TUESDAY, AUGUST 31, 2004

08:00 - 18:00 — Registration/Information Open
HUB Auditorium Foyer

III. Computational Biology and Protein Families
Chairpersons: William Noble and Stephen Altschul

08:30 - 09:15 — David Eisenberg, University of California, Los Angeles, USA
HUB Auditorium — "Protein Interactions"

09:15 - 10:00 — David Baker, University of Washington, Seattle, USA
HUB Auditorium — "Prediction and Design of Protein Structures and Protein-Protein Interactions"

10:00 - 10:30 — **Break:** Coffee/Exhibits Open
HUB East Ballroom

10:30 - 11:15 Anna Tramontano, University of Rome "La Sapienza", Italy
HUB Auditorium "Current Status and Perspectives of Protein Structure Prediction Methods"

11:15 - 12:00 Keith Dunker, Indiana University School of Medicine, USA
HUB Auditorium "Intrinsic Disorder and Protein Function"

Short Talks
Chairpersons: Jan Johansson and Theodora Choli-Papadopoulou

12:00 – 12:15 Juan Astorga-Wells, Karolinska Institutet, Sweden
HUB Auditorium "Microfluidic Electrocapture Technology in Protein and Peptide Analysis"

12:15 – 12:30 Kris Gevaert, Ghent University, Belgium
HUB Auditorium "Protein Identification and Characterization by Diagonal Reverse-Phase Chromatography"

12:30 – 12:45 Yu Lu, University of Washington/Institute for Systems Biology, USA
HUB Auditorium "A Novel Method to Quantify Serum Biomarkers via Visible Isotope-Coded Affinity Tags and Tandem Mass Spectrometry"

12:45 – 13:00 Kazuyasu Sakaguchi, Hokkaido University, Japan
HUB Auditorium "Amyloid-like Fibril Formation of Mutant p53 Tetramer Peptide at Physiological pH and Temperature"

13:00 – 13:15 David Arnott, Genentech, Inc., USA
HUB Auditorium "Proteomic Contributions to the Elucidation of c-jun and p53 Ubiquitin Ligases"

13:15 - 14:30 Lunch Break

IV. Protein Posttranslational Modifications and Cell Signaling
Chairpersons: Lowell Ericsson and Hans Jörnvall

14:30 - 15:15 Steven A. Carr, Broad Institute of MIT and Harvard, USA
HUB Auditorium "Biomarker Discovery by Proteomics and its Role in Drug and Diagnostic Development"

15:15 - 16:00 Jeff J. Gorman, Institute For Molecular Bioscience, University of Queensland, Australia
HUB Auditorium "Post-Translational Modifications of Transcription Factors Regulate Responses to Environmental Stimuli"

16:00 - 16:30 **Break:** Refreshments/Exhibits Open
HUB East Ballroom

Protein Posttranslational Modifications and Cell Signaling, continued
Chairperson: David R. Morris

16:30 - 17:15 Gerald W. Hart, Johns Hopkins University Medical School, USA
HUB Auditorium "Dynamic Protein Glycosylation by O-GlcNAc is a Metabolic Sensor Modulating Transcription, Signaling and Stress Responses in all Multi-Cellular Organisms"

Programs in Proteomics at the National Institutes of Health
Chairperson: Christine Colvis

17:15 – 18:00 Douglas M. Sheeley, National Center for Research Resources
HUB Auditorium "Understanding Complex Systems using Proteomics Approaches: Ongoing Programs and Current Funding Opportunities at NIH"

18:00 - 19:30 **Poster Session II: Wine and Cheese**
HUB East Ballroom

continued...

WEDNESDAY, SEPTEMBER 1, 2004

8:00 am - 18:00 Registration/Information Open
HUB Auditorium Foyer

V. High Throughput Technologies for Cloning, Protein Production and Folding
Chairpersons: Hisashi Hirano and Agnes Henschen-Edman

08:30 - 09:15 Frank R. Collart, Argonne National Laboratory, USA
HUB Auditorium "High Throughput Expression Strategies for Structural and Functional Genomics"

09:15 - 10:00 Mark T. Fisher, University of Kansas Medical Center, USA
HUB Auditorium "Designing a High Throughput Refolding Array Using a Combination of the GroEL Chaperonin and Osmolytes"

10:00 - 10:30 **Break:** Coffee/Exhibits Open
HUB East Ballroom

VI. Protein-Protein Interactions and Analysis of Macromolecular Complexes
Chairpersons: Rachel Klevit and Harald Tschesche

10:30 - 11:15 Jeff Ranish, Institute for Systems Biology, USA
HUB Auditorium "The Study of Macromolecular Complexes by Quantitative Proteomics"

11:15 - 12:00 Min Li, Johns Hopkins University School of Medicine, USA
HUB Auditorium "Random Peptide Display and Selection – for Interaction and Function"

12:00 - 12:45 Jill Trewhella, University of Utah, USA
HUB Auditorium "Conformational Changes in Second Messenger Mediated Signaling as Seen by Neutrons"

12:45 - 13:00 **Short Talk:** Jon C. D. Houtman, National Insitutes of Health, USA
HUB Auditorium "Binding Specificity of Multiprotein Signaling Complexes is Determined by Both Cooperative Interactions and Affinity Preferences"

13:00 - 14:00 Lunch Break

VII. Chemical Proteomics and Imaging Methods
Chairpersons: Michael H. Gelb and Daniel T. Chiu

14:00 - 14:45 pm Ben Cravatt, Scripps Institute, USA
HUB Auditorium "Activity-Based Protein Profiling: Chemical Approaches for Functional Proteomics"

14:45 - 15:30 pm Mark H. Ellisman, Center for Research in Biological Structure, University of California, San Diego, USA
HUB Auditorium "Multi-scale Imaging of the Nervous System"

15:30 - 16:00 pm **Break:** Refreshments/Exhibits Open
HUB East Ballroom

VIII. Protein Engineering and Biotechnology
Chairperson: Fumio Sakiyama

16:00 - 16:45 pm John R. Desjarlais, Director, Protein Engineering, Xencor, Monrovia, CA, USA
HUB Auditorium "Rational Engineering of Protein Drugs"

Pehr Edman Awards
Introduction: Winona C. Barker

16:45 – 17:25 Stephen Altschul, National Center for Biotechnology Information, NIH, USA
HUB Auditorium "The Compositional Adjustment of Amino Acid Substitution Matrices"

17:25 – 18:05 Amos Bairoch, Swiss Institute of Bioinformatics, Switzerland
HUB Auditorium "From Edman to Madness: How to Survive in a Flood of Protein Sequence Data"

Young Investigator Award
Introduction: Jay W. Fox

18:05 – 18:45 Niro Ramachandran, Harvard Medical School, USA
HUB Auditorium "Self Assembling Protein Microarray"

Museum of Flight Banquet
19:30 Buses depart from Memorial Way near Denny Hall
22:30 Buses return to campus and University District hotels

THURSDAY, SEPTEMBER 2, 2004

08:00 - 13:00 Registration/Information Open
HUB Auditorium Foyer

IX. Cell Pathways Regulation and Metabonomics
Chairpersons: Kristine Swiderek and Martin J. Kushmerick

08:30 – 09:15 Trisha N. Davis, University of Washington, USA
HUB Auditorium "The Lattice Structure of the Yeast Spindle Pole Body Probed by Fluorescence Resonance Energy Transfer"

09:15 - 10:00 Jeremy Nicholson, Imperial College, London, England
HUB Auditorium "Understanding Global Systems Biology Using Metabonomics"

10:00 - 10:15 **Short Talk:** Jennifer H. Granger, Waters Corporation, USA
HUB Auditorium "Ultra Performance Liquid Chromatography-MS(TOF): New Separations Technology for High Throughput Metabonomics"

10:15 - 10:30 **Coffee Break**
HUB Auditorium Foyer

X. Systems Biology
Chairperson: Amos Bairoch

10:30 - 11:15 Trey Ideker, University of California, San Diego, USA
HUB Auditorium "Mapping, Validating and Comparing Biological Networks"

11:15 - 12:00 Leroy Hood, Institute for Systems Biology, USA
HUB Auditorium "Systems Approaches to Development and Physiology"

12:00 - 12:45 Business/Announcements/Closing
HUB Auditorium Kenneth Walsh, University of Washington, USA
Carl W. Anderson, Brookhaven National Laboratory, USA

Lectures

All lectures will be presented in the HUB Auditorium.

Keynote Lecture
Sunday, August 29
19:00 - 20:00

PROTEIN INTERACTIONS

Stan Fields
Howard Hughes Medical Institute, Departments of Genome Sciences and Medicine, University of Washington, Box 357730, Seattle, WA 98195

The number of protein-protein interactions that have been identified has increased dramatically over the last decade, largely due to the completion of genome sequences combined with developments in protein technology. The latter include genomewide strategies using the two-hybrid assay or affinity purification – mass spectrometry procedures. Although high throughput methods provide enormous datasets, they come with a cost: interactions detected by these approaches cannot easily be verified by other experiments as has been done for those detected by analyses of single proteins. Thus, computational methods are being applied to these datasets both to sort the interactions into confidence levels and to deduce novel properties of the protein networks that emerge from these data. Two recent projects will serve as examples: the use of the split ubiquitin assay to identify interactions among membrane proteins of the yeast *Saccharomyces cerevisiae* and a two-hybrid collaboration with Prolexys Pharmaceuticals, Inc. to identify interactions among the proteins of the malaria parasite *Plasmodium falciparum.* In terms of the analysis of these interactions, yeast proteins have the virtue that a considerable amount of additional information is available for many of them, whereas *P. falciparum* proteins remain mostly unexplored.

I. The Interactome
Chairperson: Richard Perham
Monday, August 30
08:45 - 09:30

GLOBAL ANALYSIS OF BIOCHEMICAL ACTIVITIES USING PROTEIN CHIPS

H. Zhu[1], D. Hall[2], J. Ptacek[2], G. Devgan[1], G. Jona[1], M. Gerstein[2], X. Zhu[1], S. Schreiber[3], J. Huang[3,4], **Michael Snyder**[1,2]
Department of Molecular, Cellular, and Developmental Biology, [2]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, [3]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, [4]Department of Pharmacology, UCLA, CA

We have prepared microarrays containing most of the proteins of yeast and used these to screen for a wide variety of activities. These include probing for interactions with other proteins and lipids, and the screening with antibodies to examine their specificity. Several recent uses of proteome arrays include a) the identification of protein targets of small molecules that operate in the rapamycin signaling pathway, b) the identification of novel DNA binding activities, including the discovery of a metabolic enzyme that regulates gene expression and c) progress toward the construction of an in vitro phosphorylation map for nearly the entire yeast proteome by testing each kinase for its ability to phosphorylate proteins deposited on the array. The approaches and results of these studies will be presented at the meeting.

II. Analytical Strategies in Proteomics
Chairpersons: Norman J. Dovichi & Brigitte Wittman-Liebold
Monday, August 30
09:30 - 10:15

ANALYSIS OF INDIVIDUAL AND INTERACTING PROTEINS VIA PROXIMITY LIGATION REACTIONS

Ulf Landegren, Mats Nilsson, Johan Banér, Fredrik Dahl, Olle Ericsson, Simon Fredriksson, Mats Gullberg, Sigrun Gustafsdottir, Jenny Göransson, Jonas Jarvius, Henrik Johansson, Masood Kamali, Chatarina Larsson, Jonas Melin, Edith Schallmeiner, Lena Spångberg, Johan Stenberg, Ola Söderberg
Dept of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Se-75185 Uppsala, Sweden

Total genome sequence information provides a basis for investigating the molecular composition of cells and tissues, but only if sufficiently specific and sensitive tools are developed to tap the multitude of molecules for information about their distribution, concentrations, interactions, and about their roles in biological processes.

We have established a set of molecular procedure that allow any detected molecules to be represented as short linear or circular strings of DNA for highly specific analysis of molecules in solution or *in situ*.

Proximity ligation is a recently developed procedure where pairs of binding reagents, such as antibodies or aptamers, are equipped with DNA strands capable of being joined by ligation when the reagents have bound the same target protein molecule. The process effectively reverse translates target proteins into linear or circular signature DNA molecules that can then be amplified and identified for highly sensitive, precise protein detection in homogenous or solid-phase assays. Very low concentrations of protein can be detected because of the strict requirement for proximal binding by pairs or more of specific binders, and the amplified detection. The procedure is suitable for parallel analyses of large sets of proteins.

The related padlock probes are linear oligonucleotide probes, capable of specifically reacting with target nucleic acid sequences by being converted to DNA circles. The circles can then be amplified and identified, for example by hybridization to universal tag microarrays.

Both reacted proximity and padlock probes can be amplified via rolling circle replication reactions. The method is ideal for precise amplification of large sets of reacted probes. The same mechanism also permits *in situ* detection of reacted padlock or proximity probes for detection of even single target molecules in cells and tissues. The proximity ligation mechanism also permits single interacting pairs of protein to be detected with excellent specificity.

Taken together the described methods represent a toolbox that will permit extensive molecular analyses using universal arrays for DNA, RNA and protein detection, and to demonstrate the functional involvement of molecules *in situ*.

Analytical Strategies in Proteomics
Monday, August 30
10:45 - 11:30

NEW APPROACHES TO MAXIMIZE THE INFORMATION CONTENT OF PROTEOMIC SAMPLES USING LC-MALDI-TOF/TOF MS ANALYSIS

Tomas Rejtar
Barnett Institute, Northeastern University, Boston, MA 02115

Proteomic analysis shares one commonality to DNA sequencing - the samples are often of sufficient complexity that it has not been possible to determine (and quantitate) all species present. To maximize the information content in the discovery mode, it is necessary to integrate all steps in the analysis in an optimized manner (a systems approach). In this talk, we will summarize several aspects of our latest approaches of LC/MS to increase the global information content of both protein cataloguing and different expression analysis. We will focus on LC-MALDI MS, a fundamentally off-line approach, that allows quantitation of differentially expressed proteins in the MS mode, followed by a separate and directed MS/MS analysis. Specifically, we will explore LC-MALDI-TOF/TOF MS analysis. We utilize multiplexed LC (4 columns) to increase the separation throughput, to overcome a major bottleneck today with single column operation. Secondly, multiple runs of the same sample are shown to increase the number of proteins identified. The bottleneck then moves to the MALDI analysis where analysis of thousands of deposited spots on the MALDI target, the result of the need to maintain the high resolution LC separation in each of the 4 columns, requires hours of time. We have overcome this bottleneck by constructing a 2 kHz repetition rate laser MALDI-MS instrument for interrogating spots 10X faster than current commercial 200 Hz laser instruments, translating the analysis time of the whole plate into only a few minutes. Further advances in peak picking, data processing and the use of derivatizing agents lead to significant increases in the number of proteins identified and quantitated. The integrated approach will be demonstrated for diverse samples of biological interest.

Analytical Strategies in Proteomics
Monday, August 30
11:30 - 12:15

ADVANCES IN QUANTITATIVE HIGH THROUGHPUT PROTEOMICS BASED UPON NANO-LC AND MASS SPECTROMETRY

Richard D. Smith, David Camp, Jon Jacobs, Yufeng Shen, Weijun Qian, Mary Lipton, Harold Udseth, Ljiljana Pasa-Tolic, Gordon Anderson, and Matthew Monroe
Environmental Molecular Sciences Laboratory and Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99352

Technological advances are enabling proteomics measurements that are increasingly effective, comprehensive and higher throughput. The challenges associated with proteomics measurements include identifying and quantitating large sets of proteins where components of interest may have relative abundances that span more than six orders of magnitude, vary broadly in chemical and physical properties, have transient and low levels of modifications, and are subject to endogenous proteolytic processing. The utility of proteomics data depends significantly on the quality of the data; both the confidence of protein identifications as well as the quantitative utility of the data.

This presentation will describe advanced nanoscale separations and mass spectrometric (MS) instrumentation being developed at applied at Pacific Northwest National Laboratory for making comprehensive, quantitative, and high throughput proteomic measurements. A key element of the approach involves the identification of peptide markers for proteins that are then used for subsequent high throughput mass spectrometric measurements (i.e. without the need for tandem mass spectrometry). The use of stable isotope labels or relative MS peak intensities of these peptide markers provides the basis for quantitation. Initial applications have focused on microbial systems. More recent work is extending the application of these proteomics technologies to mammalian systems, including the human blood plasma proteome with its broad biomedical applications. Initial characterization of the blood plasma proteome has provided confident identification peptide markers for many more proteins from plasma than previously detected. These plasma proteome measurements potentially provide the basis for development of biomarkers or signatures for virtually any disease state.

Short Talk
Monday, August 30
12:15 - 12:30

PROTEOMIC ANALYSIS OF BARRETT'S ESOPHAGUS USING CAPILLARY ELECTROPHORESIS WITH LASER-INDUCED FLUORESCENCE

James R. Kraly-1 , Megan Jones-1, Brian Reid-2, Norman J. Dovichi-1
1 - Department of Chemistry, University of Washington, Seattle, WA 98195; 2 - Fred Hutchinson Cancer Research Center

Protein expression fingerprints of Barrett's Esophageal cells are generated using a novel capillary electrophoresis system. The capillary sampling apparatus is capable of electrokinetic injections from a cellular homogenate reservoir or hydrodynamic injections of single cells from a dilute cellular suspension Single cell analysis allows heterogeneity within cell populations to be thoroughly examined.

Proteins from cellular lysate are labeled with the fluorogenic reagent 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ), which reacts with lysine residues to produce a highly fluorescent product. Proteins are detected by laser-induced fluorescence inside a sheath flow cuvette using a fiber-coupled single photon counting module.

Separations are preformed by capillary sieving electrophoresis (CSE), and micellular electrokinetic chromatography (MECC). The use of more narrow capillaries limits Joule heating, conserving separation efficiency at elevated field strengths. Currently, the peak capacity for one-dimensional CSE electropherograms is 85, and for MECC is 110. Both CSE and MECC give theoretical plate counts over 500,000. To increase the peak capacity, the system can be easily modified to perform fully automated two-dimensional capillary electrophoresis experiments (2D-CE). Proteins are separated according to their size by CSE on the first capillary. Fractions are then repeatedly transferred to the second capillary and subject to MECC. Fluorescence signal is measured continually, and the data can be presented as a three dimensional contour plot or as silver stained gel image. Analytical improvements have limited 2D-CE analysis time to 100 minutes

In parallel to 2D-CE, proteins from cellular homogenate are separated by conventional two-dimensional gel electrophoresis. In-gel trypsin digestion and LC/MS/MS are used to create a library of identified Barrett's Esophageal proteins. Electro-eluted proteins are referenced to this library, and used to spike the sample in co-migration experiments. These combined methods will be used to investigate the heterogeneity of cell-to-cell protein expression during neoplastic progression of the pre-malignant condition Barrett's Esophagus.

Short Talk
Monday, August 30
12:30 - 12:45

AN INTEGRATED WORKFLOW FOR INTERPRETATION OF SHOTGUN PROTEOMICS DATA

Wenyao Shi (1), Xiaoyu Yang (1), Anthony J. Makusky (1), Dawn M. Maynard (2), Lewis Y. Geer (3), Ming Xu (3), Sanford P. Markey (1), and **Jeffrey A. Kowalak** (1)
(1) Laboratory of Neurotoxicology, National Institute of Mental Health, (2) Section on Human Biochemical Genetics, National Human Genome Research Institute, and (3) Computational Biology Branch, National Library of Medicine, Bethesda, Maryland 20892, USA

Peptide analyses by LC-MS/MS can generate large datasets, imposing labor-intensive efforts to consolidate peptide and/or protein identification information into meaningful knowledge. Software bioinformatics tools can facilitate and accelerate high-throughput proteomic data analyses. Toward this goal, we are developing an integrated workflow designed to maximize the amount of peptide sequence information from LC-MS/MS data.

Extant database search algorithms are technically able to accommodate variable posttranslational and/or chemical modifications, as well as variable proteolytic cleavages, including non-sequence specific cleavage. However, these options are not used routinely due to huge increases in computational complexity, resulting in prohibitively long search times and decreases in specificity. We hypothesize that in any given proteomics sample, posttranslationally modified peptides should be accompanied by non-modified sibling peptides. Therefore, we perform a primary database search allowing only for alkylation of cysteine as a fixed modification. Non-modified peptides identified are used to infer the identity of the corresponding protein precursors. The database record(s) associated with each identification are written to a sub-database. Once the sub-database of candidate proteins has been written, the search can be repeated iteratively. Since the size of the sub-database is expected to be substantially smaller, the iterative searches can easily accommodate the combinatorial overhead associated with non-specific cleavage and consideration of both fixed and variable modification (chemical as well as posttranslational). After exhaustive database searching, the remaining MS/MS spectra are submitted to a mass spectral filter algorithm to identify medium and high quality spectra that have not been assigned. These spectra are candidates for de novo interpretation algorithms and or manual interpretation.

Lunch Workshop
Monday, August 30
12:45 - 14:15

PTM DISCOVERY AND BIOMARKER QUANTITATION WORKFLOWS USING THE APPLIED BIOSYSTEMS/MDS SCIEX 4000Q TRAP® LC/MS/MS SYSTEM

Sally Webb
Proteomics Technical Marketing Specialist, Applied Biosystems

Reversible protein phosphorylation is an important post translational modification (PTM) as it mediates most of the signal transduction pathways in eukaryotic cells, controls cell processes such as metabolism and transcription, and plays a role in intercellular communication. Studying phosphorylation is very challenging due to very low concentration of phosphopetides in a highly complex protease digest mixture. In addition, signals of phosphopeptides in the MS spectra are, in most cases, suppressed by the relatively high concentration of non-phosphopeptides present in proteolytic digests of these samples, which ionize much more efficiently. Therefore, a better approach to selectively identify and characterize phosphopeptides from complex digest mixtures is highly desirable. Precursor ion scanning on a triple quadrupole instrument is a common way to detect phosphorylation where the instrument is set to detect the characteristic PO3- ion. However, sequencing information is difficult to obtain due to the limited sensitivity in full scan mode of triple quadrupole instruments.

The direct combination of triple quadrupole and ion trapping capabilities in a hybrid linear ion trap presents new opportunities for the automated investigation of phosphorylation sites in biological samples. The system couples high sensitivity triple quadrupole functionality such as precursor ion scanning, with high performance linear ion trap performance for very sensitive full scan MS and MS/MS data. This allows for a new on-line approach for the selective identification of phosphorylation using novel automated scan functions of the Q TRAP® system in a single LC-MS run. To demonstrate the ability of this methodology to map multiple substoichiometric phosphorylation sites from gel isolated proteins, we mapped the in vitro phosphorylation sites on several protein kinases. This technology was then coupled with a novel stable isotope N-terminal labeling strategy (iTRAQTM) to obtain both identification and relative quantification of the active and inactive forms of phosphorylated protein kinases. Results are shown using this methodology to map and quantify the phosphorylation sites of multiple protein kinases.

Workshop Session I
Chairperson: Kenneth Walsh
Monday, August 30
14:15 - 15:15

Thermo ELECTRON CORPORATION

PROTEOMICS: AUTOMATED ANALYSIS OF PEPTIDES AND PROTEINS AT THE ATTOMOLE LEVEL IN COMPLEX MIXTURES BY MASS SPECTROMETRY

Donald F. Hunt
Departments of Chemistry and Pathology, University of Virginia, Charlottesville, Virginia 22901

Sequence informaton on the human genome and that of selected organisms is becoming available at an increasing rate and is providing unparalleled understanding of the complexity of proteins in cells. The next challenge is at the level of proteomics, understanding the structure and function of protein encoded by a particular genome, tissue or organelle. This information will certainly provide the starting point for development of novel therapeutic interventions against many of the world's diseases. Presented here is novel methodology for the rapid, automated identification of proteins present at the attomole level in complex mixtures. Prior fractionation of the mixture by gel electrophoresis is not required and characterization of post translational modifications on the target proteins is facilitated in many cases. Representative examples will be presented from the analysis of (a) protein protein interactions that regulate a human phosphatase, (b) proteins that allow a plant to synthesize its own fungicide, (c) proteins involved in signal transduction cascades (d) antigens presented on on class II MHC molecules, (e) proteins involved in the regulation of transcription or repair of DNA (f) proteins expressed uniquely by two different cell populations, (g) proteins secreted by tumors, (h) cell surface proteins, and (i) proteins involved in the acquisition of long term memory.

Workshop
Monday, August 30
15:15 - 16:15

Applied Biosystems
an Applera Corporation Business

PROTEIN AND PEPTIDE QUANTITATION USING NOVEL, MULTIPLEX TAGGING REAGENTS

Philip Ross
Applied Biosystems

Recently, there has been development of tools to solve some of the challenges in protein quantitation involving a combination of chemistry with either gel electrophoresis (DIGE) or liquid chromatography (ICAT reagents). As these techniques have gained acceptance in protein expression analysis, it has become apparent that there is a requirement to address some of the remaining issues in quantitative proteomics. These include increasing protein and proteome coverage, retaining important PTM information and being able to simultaneously compare multiple samples in the same experiment. To that effect, we have synthesised a multiplexed set of isobaric reagents that yield amine-derivatized peptides that are chromatographically identical and indistinguishable in MS. The derivatized peptides yield intense low-mass MS/MS signature ions that support quantitation of individual members of the multiplex set. Derivatization of peptide amines is essentially quantitative (96-98% in complex mixtures) and other side reactions, such as tyrosine modification, can essentially be prevented by manipulation of the reaction conditions. Excess reagent is completely removed by reverse-phase or ion-exchange chromatography, and only minimal modifications are required to elution profiles relative to unmodified peptides. The reagents also improve ionization of lysine-containing peptides and quality of fragmentation spectra, which show improved b- and y-ion abundance relative to their underivatized counterparts.

We will discuss the use of these isobaric tags for absolute and relative quantitation of proteins in a number of studies. These include a multiplex whole-proteome study of a series of *Saccharomyces cerevisiae* deletion mutants, discrimination of specific from non-specific interactors in a protein complex pull-down, and time course studies.

Waters MICROMASS MS TECHNOLOGIES

Workshop Session II
Chairperson: Ray Paxton
Monday, August 30
16:45 - 17:40

EXPRESSION PROFILING OF VARIOUS METABOLIC CONDITIONS OF *E. COLI*

Jeff Silva
Waters Corporation

At a glance, bacteria and other procaryotes do not appear to be diversified organisms from observations of their basic morphologies, which include the possibilities of motility and resting cells (spores), and reaction to a major differential stain (the Gram stain). In eukaryotes, there may be more structural diversity within a single genus of organisms. Despite this seeming lack of diversity, the biochemical and metabolic capabilities of bacteria level the playing field, especially relating to energy-generating metabolism and biosynthesis of secondary metabolites. Procaryotes conduct the same types of basic metabolism as other higher organisms, but there are several additional types of energy-generating metabolism among the procaryotes that are non-existent in eukaryotic cells or organisms. There are also secondary metabolic pathways that are unique to prokaryotes. The diversity of procaryotes is expressed by their great variation in modes of energy production and metabolism as a means of adaptation and survival.

Using nanoflow high-performance liquid chromatography and mass spectrometry, we have constructed comprehensive peptide maps from *Escherichia coli* grown with lactose, glucose or acetate in minimal media. Bacterial cells were harvested from shake flasks at mid log phase to produce protein extracts from each growth condition. The resulting protein extracts were digested with trypsin, and the resulting complex peptide mixture was analyzed in a single dimension, using Waters' newly developed LCMS^{E} technology platform. This technology platform fully integrates the resolution obtained from the chromatographic separation with the mass accuracy and resolution of the qTOF mass spectrometer for simultaneous qualitative and quantitative protein analysis. The bacterial proteins originating from each sample were identified. For those proteins common to both conditions, the change in relative abundance was calculated. The data presented clearly illustrates the ability of the Waters' Expression System to identify proteins involved in each metabolic pathway and by comparing two different growth conditions the relative change in protein abundance can be measured. Comparison of our results to existing transcriptional profiling data shows a high degree of similarity at the protein level. However, our data also illustrates significant differences in the fold change of a number of reported proteins when compared to the transcriptional profiling data.

Workshop
Monday, August 30
17:40 - 18:35

BRUKER DALTONICS®

STUDIES OF THREE-DIMENSIONAL PROTEIN STRUCTURE USING MASS SPECTROMETRY (MS3D). PROTEIN SURFACE REACTIVITY AND CHEMICAL CROSS-LINKING.

Gary Kruppa
Bruker Daltonics, Inc., Billerica, MA, and Sandia National Laboratories, Livermore, CA 94538

High throughput protein structure determination efforts continue to rely primarily on multidimensional NMR and X-Ray crystallographic methods. These methods provide detailed, high-resolution protein structures. However, X-Ray crystallography requires single crystals of a protein, which are sometimes difficult or impossible to grow. NMR requires milligrams of protein, which must be soluble in an NMR compatible solvent system, and NMR investigations are currently limited to proteins of approximately 30 kDa or less. Given these and other difficulties, several groups have been investigating the use of 'sparse' or 'minimal' constraint sets, i.e. constraint sets obtained from NMR, EPR, FRET, X-Ray crystallography, or some combination of these methods which contain far less than the number of distance constraints expected from an ideal multi-dimensional NMR or X-Ray crystallographic data set. The use of experimental constraints from a minimal constraint experiment has been shown to be a valuable way to speed protein structure determination using NMR.

Intra-molecular chemical cross-linking followed by mass spectrometric analysis has recently been shown to have potential as a new method for obtaining distance constraints between reactive side-chains in proteins, and named MS3D. The advantages of the MS3D method for obtaining distance constraints include the ability to work with small amounts of protein (10 micrograms or less), no need for single crystals, and the solvent system is limited only by the cross-linking chemistry. The development of an automated, sensitive method using MS3D would clearly have a great impact on high throughput structure determination efforts. This impact would be due to several new capabilities provided by the MS3D method: The ability to pre-screen proteins for fold family at low expression levels, before scale-up of the expression to obtain sufficient protein for NMR or X-Ray analysis; Analysis of targets that prove recalcitrant to NMR or X-Ray methods; Speeding and improving structure determination from sparse NMR data.

Despite the initial report indicating the potential of MS3D, the number of distance constraints reported in the literature using this method has been limited. Several publications have indicated that the cross-linking chemistry itself may be difficult to optimize, and the method is further complicated by the difficulty of using proteolytic digestion and HPLC-MS to localize the cross-links. We have developed a whole protein approach (known as the top-down method) using Fourier Transform Mass Spectrometry (FTMS) to localize the cross-links using MS^n analysis rather than proteolytic digestions. This method also facilitates optimization of the cross-linking chemistries. We will present results on the study of the structure of ubiquitin and residue specific reactivity in ubiquitin using this top down approach, and discuss the extension of the method to other proteins using a combination of bottom-up and top-down strategies for localizing cross-links.

GE Healthcare

Workshop
Monday, August 30
18:35 - 19:30

FROM INFECTION TO DETECTION VIA CHARACTERIZATION OF HOST RESPONSE

Sandra McCutchen-Maloney
Biodefense Division, Lawrence Livermore National Laboratory

Yersinia pestis, the etiological agent of plague, is both a biowarfare and bioterrorist threat agent and is of concern to human health both from an infectious disease and a civilian biodefense perspective. While Y. *pestis* and two near neighbors, *Y. pseudotuberculosis* and *Y. enterocolitica*, share more than 90% DNA homology, they have significantly different clinical manifestations. Plague is often fatal if untreated, yet *Y. pseudotuberculosis* and *Y. enterocolitica* cause severe intestinal distress but are rarely fatal. A better understanding of host response to these closely related pathogens may help explain the different mechanisms of virulence and pathogenesis that result in such different clinical outcomes and provide biomarkers for early detection of pathogen exposure. While humans have evolved an elaborate immune system to fight bacterial and viral challenges, some of the most dangerous and lethal pathogens, such as *Y. pestis*, have also evolved sophisticated mechanisms to thwart the host defense mechanisms. Results from proteomic characterization of host response following exposure to *Yersinia pestis* and two near neighbors will be discussed.

Human monocyte-like cells were chosen as a model for macrophage immune response to pathogen exposure. Two-dimensional differential in-gel electrophoresis (DIGE) followed by mass spectrometry was used to identify host proteins with differential expression following exposure to *Yersinia* species. Proteins identified in this study are known to be involved in a wide spectrum of cellular functions and host defense mechanisms including apoptosis, cytoskeletal rearrangement, protein synthesis and degradation, DNA replication and transcription, metabolism, and cell signaling. Some of these results are in support of the known functions of *Yersinia* virulence factors in cytoskeletal modulation and apoptosis. Notably the differential expression patterns observed can distinguish the three pathogen exposures from each other and from unexposed host cells, suggesting that host response could be used, in the absence of other information, to detect exposure to a pathogen and to identify the pathogen in question. The functions of the differentially expressed proteins identified provide insight on the different virulence and pathogenic mechanisms of *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica.* Experiments including the use of more relevant host models will also be discussed as a means to identify and validate biomarkers for *Yersinia* exposure.

In summary, we have demonstrated that *Yersinia* induces marked proteomic alterations in human monocyte-like cells. Comparative characterization of host response clearly showed that host protein expression patterns are distinct for the three *Yersinia* pathogen exposures, thus laying the foundation for defining biomarkers for presymptomatic detection of plague. As our knowledge of host-pathogen interaction increases, so will our understanding of the molecular causes of infectious disease, which will lead to better diagnostics for early detection as well as novel, next-generation therapeutic approaches to combat infectious diseases.

This work was performed under the auspices of the U.S. Department of Energy by University of California Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48. UCRL-ABS-204328

III. Computational Biology and Protein Families
Chairpersons: William Noble & Stephen Altschul
Tuesday, August 31
08:30 - 09:15

PROTEIN INTERACTIONS

David Eisenberg, Peter Bowers, Michael Strong, Huiying Li, Lukasz Salwinski, Matteo Pellegrini, Robert Riley, Richard Llwellyn, Todd Yeates
UCLA-DOE Institute of Genomics and Proteomics, Box 951570, UCLA, Los Angeles 90095-1570

Protein interactions control the life and death of cells, yet we are only beginning to appreciate the nature and complexity of these networks. We have taken two approaches towards mapping these networks. The first is the synthesis of information from fully sequenced genomes and microarray studies into knowledge about the network of functional interactions of proteins in cells. We analyze genomes using the Rosetta Stone, Phylogenetic Profile, Gene Neighbor, Operon methods to determine a genome-wide functional linkage map. This map is more readily interpreted when clustered, revealing groups of proteins participating in a variety of pathways and clusters. Parallel pathways and clusters are also revealed, in which different sets of enzymes operate on different substrates or with different cofactors. These methods have been applied genome-wide to *Micobacterium tuberculosis* and *R. Palustris*, as well as to more than a hundred other genomes. Many results are available at: **http://doe-mbi.ucla.edu/pronav**. The outcome is increased understanding of the network of interacting proteins, and enhanced knowledge of the contextual function of proteins. These inferred interactions can be compared to directly measured protein interactions, collected in the Database of Interacting Proteins: **http://dip.doe-mbi.ucla.edu/**. These observed networks constitute a second approach to detailing protein networks.

Initial steps towards the simulation in *silico* of the dynamics of biological networks have been taken with Field Programmable Gate Array (FPGA)-based technologies. FPGAs offer the possibility of simulation of biological networks, which necessitate stochastic simulation because of the small numbers of molecules per cell. The parallel architecture of FPGAs, which can simulate the basic reaction steps of biological networks, attains simulation rates at least an order of magnitude greater than currently available microprocessors.

References

Visualization and interpretation of protein networks in *Microbacterium tuberculosis* based on hierarchical clustering of genome-wide functional linkage maps. M. Strong, T.G. Graeber, M. Beeby, M. Pellegrini, M.J. Thompson, T.O. Yeates, & D. Eisenberg (2003). *Nucleic Acids Research*, 31, 7099-7109 (2003).

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In silico simulation of biological network dynamics. L. Salwinski & D. Eisenberg (2004), *Nature Biotechnology*, 22, 1017-1019.

Computational Biology and Protein Families
Tuesday, August 31
09:15 - 10:30

PREDICTION AND DESIGN OF PROTEIN STRUCTURES AND PROTEIN-PROTEIN INTERACTIONS

David Baker
University of Washington

I will discuss recent advances in protein structure prediction and design.

Computational Biology and Protein Families
Tuesday, August 31
10:30 - 11:15

CURRENT STATUS AND PERSPECTIVES OF PROTEIN STRUCTURE PREDICTION METHODS

Anna Tramontano
University of Rome "La Sapienca", Italy

The goal of protein bioinformatics is to assist experimental biology in assigning a function or suggesting functional hypotheses for all known proteins. The task is formidable, a back of the envelope calculation easily shows that there is no hope that we can study experimentally each and every biological molecule. On the other hand, the availability of the complete genomic sequences of many species, including human, has raised enormous expectations in medicine, pharmacology, ecology, biotechnology and forensic sciences.

Knowledge is only a first step toward understanding, and we are only at the early stage of a scientific process that might lead us to satisfy all the expectations raised by the genomic projects. I will discuss the present status of computational methods that attempt to infer the unique three-dimensional structure of proteins from their amino acid sequences with a special emphasis on their effectiveness in inferring the biological properties and functional roles of proteins and focusing on the limitations of current approaches and on the future routes that are likely to improve our understanding of the exquisitely specific and efficient mechanisms of protein structure and function.

Computational Biology and Protein Families
Tuesday, August 31
11:15 - 12:00

INTRINSIC DISORDER AND PROTEIN FUNCTION

Keith Dunker
Indiana School of Medicine

The standard protein structure-function paradigm can be simply stated as the amino acid sequence codes for a 3-D structure and this 3-D structure is a required prerequisite for protein function. Many proteins, however, exist wholly or regionally in unfolded or in incompletely folded states under apparently native conditions and such lack of specific 3-D structures appear to be necessary for the functions of many of these proteins. Comparison of structured and incompletely structured proteins leads to the suggestion that there are two parallel protein structure-function paradigms that approximately partition the proteins according to their functions. More specifically, for enzymes, the classical paradigm of sequence to 3-D structure to function holds. Pre-organization of the active site, and hence the prior formation of 3-D structure, is evidently necessary for catalysis. On the other hand, for signaling and regulation, a new paradigm of sequence to disordered ensemble to function holds. A disorder-to-order transition upon binding facilitates the uncoupling of affinity and specificity, leading to readily reversible interactions. A disorder to order transition upon binding also facilitates binding diversity, leading to multiple binding partners. Both reversibility and binding diversity are useful characteristics for signaling and regulatory proteins or for signaling and regulatory regions of proteins. Lack of structure also facilitates other signaling related processes such as exposing sites for molecular recognition, providing flexible connectors between functional domains, and facilitating the binding of various enzymes used for protein modification to give three particular examples. In summary, the use of intrinsic disorder for signaling and regulatory functions unifies a significant number of observations regarding amino acid sequence and protein behavior.

Short Talk
Chairpersons: Jan Johansson & Theodora Choli-Papadopoulou
Tuesday, August 31
12:00 - 12:15

MICROFLUIDIC ELECTROCAPTURE TECHNOLOGY IN PROTEIN AND PEPTIDE ANALYSIS

Juan Astorga-Wells, T. Bergman and H. Jörnvall
Department of Medical Biochemistry and Biophysics, Karolinska Institutet, SE-171 77 Stockholm, Sweden

Integrated microfluidic systems promise to contribute to the development of fast, versatile, and inexpensive analytical instrumentation. Since the analysis of proteins and peptides requires several steps before final analysis, an efficient micro-scale fluidic control combined with versatile methodologies has been envisioned for integrated high throughput analysis of proteomes. The electrocapture technology has several advantages over present micromethods. The microfluidic device utilizes an electric field to immobilize molecules in a flow stream without use of any solid support or chemical attachments. After capture, another medium is injected into the system to give a desirable chemical composition or to carry reagents, which are brought into contact with the captured molecules. The immobilization is reversed by disconnecting the electric field, upon which the sample is collected for further analysis. Under this principle, steps including concentration, desalting, removal of contaminants and microreactions are all performed in a microscale online fashion. In addition, the same technology can be utilized as a separation tool. Several applications of the electrocapture technology will be presented, covering online desalting, detergent removal, reduction, alkylation, trypsin digestion and separation of proteins and peptides for subsequent analysis by MALDI-MS. Mixing, incubation, sample pretreatment, and separation are performed on-line by a single, easy-to-use, microfluidic methodology. Furthermore, since the major technological innovation is the presence of ion-selective areas in the microfluidic channel, the integration into microfluidic systems is more straightforward than with other technologies.

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Short Talk
Tuesday, August 31
12:15 - 12:30

PROTEIN IDENTIFICATION AND CHARACTERIZATION BY DIAGONAL REVERSE-PHASE CHROMATOGRAPHY

Kris Gevaert, Petra Van Damme, Sara De Groot, Bart Ghesquiére, Lennart Martens, Grégoire R. Thomas, An Staes, Evy Timmerman, Jozef Van Damme, Hans Demol, Koen Hugelier, Marc Goethals, Magda Puype and Joël Vandekerckhove
Department of Biochemistry, Faculty of Medicine and Health Sciences, Ghent University, A. Baertsoenkaai 3, B9000 Ghent, Belgium

Diagonal reverse-phase peptide chromatography has proven to be a highly versatile technology for non-gel analysis of complex protein mixtures. We have developed a toolbox for the specific isolation of peptides containing the rare amino acids methionine and cysteine, extreme amino terminal peptides, phosphorylated peptides and peptides carrying modifications indicating cellular oxidative stress. Relative quantitative proteomics is done by enzymatic, post-cleavage stable incorporation of two oxygen-18 atoms in one set of peptides and is amenable to all isolation protocols. We here illustrate the power of our COFRADIC™ (combined fractional diagonal chromatography) technology by the in-depth analysis of several clinically interesting proteomes. Examples include a comprehensive proteome analysis of human platelets, identification of novel substrates of the degradome in apoptotic Jurkat cells, deciphering the phosphoproteome of HepG2 cells and identification of proteins bearing 3-nitro-tyrosine residues in human neuroblastoma cells under oxidative stress. In all cases, we emphasize on the explorative (sensitivity) aspect of our COFRADIC™ protocols for identifying and characterizing protein mixtures.

Short Talk
Tuesday, August 31
12:30 - 12:45

A NOVEL METHOD TO QUANTIFY SERUM BIOMARKERS VIA VISIBLE ISOTOPE-CODED AFFINITY TAGS AND TANDEM MASS SPECTROMETRY

Yu Lu(1,2), Michael H. Gelb(1), Frank Turecek(1), Ruedi Aebersold(2)
(1) Department of Chemistry, University of Washington, Seattle WA 98195 USA; (2) Institute for Systems Biology, Seattle WA 98103 USA

One important early diagnostic method is based on monitoring the amounts of particular marker proteins in serum samples, involving the use of Enzyme-Linked Immunosorbent Assay (ELISA). However, the development of protein-specific antibodies suitable for ELISA is time-consuming and expensive.

Here we describe a novel method to quantify serum protein plasma biomarkers at high sensitivity, as an alternative to ELISA and other antibody dependent methods. The new method is based on the Visible Isotope-Coded Affinity Tag (VICAT) reagents and tandem mass spectrometry. The marker protein, along with other proteins in the serum sample, is tagged with one isotopic form of the VICAT reagent and is processed through the steps of trypsin digestion, gel isoelectric focusing, streptavidin beads capture, on-beads photocleavage, and micro-LC-MS in selected reaction monitoring (SRM) mode. One tryptic cysteine-containing peptide that uniquely represents the serum marker is prepared on a peptide synthesizer. The synthetic peptide is tagged with a different isotopic form of the VICAT reagent as the internal standard, and with a visible (^{14}C labeled) form of the reagent as the separation marker, both of which are added to the sample mixture before the separation procedure. The visible tracer of the separation marker is used to follow the target peptides through the large-scale separation steps. The amount of the serum marker can be determined at absolute quantity level based on the peak area ratio between the serum marker-derived peptide and the internal standard. With a sample pre-fractionation method such as HPLC separation of tryptic peptides prior to the VICAT procedure, the detection limit in the nanogram per milliliter of serum range has been achieved.

Short Talk
Tuesday, August 31
12:45 - 13:00

AMYLOID-LIKE FIBRIL FORMATION OF MUTANT p53 TETRAMER PEPTIDE AT PHYSIOLOGICAL pH AND TEMPERATURE

Yuya Asanomi (1), Yuichiro Higashimoto (2), Satoru Takakusagi (1), Kohei Uosaki (1), Yoshiro Chuman (1), Ettore Appella (3), and **Kazuyasu Sakaguchi** (1)
(1) Division of Chemistry, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan, (2) Department of Medical Biochemistry, Kurume University School of Medicine, Kurume 830-0011, Japan, (3) National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

The tumor suppressor protein p53 exerts anti-proliferative effects, including growth arrest, apoptosis and cell senescence, in response to various types of stress. The tetramerization domain of p53 is essential for efficient site-specific DNA binding and contributes to p53's ability to activate transcription from natural promoters. A mutant with substitution of Val for Gly334 was found in human lung cancer. Gly334 is located at the turn between β-strand and α-helix in the p53 tetramer structure. We found that the p53(319-358) peptide with the mutation, termed G334V, formed unstable tetramers, and aggregated with accompanying α-β transition under physiological conditions, as shown by CD and analytical ultracentrifugal analysis. By staining with Congo Red, the aggregate of G334V showed yellow-green birefringence under cross-polarized light. Moreover, the G334V peptide induced a significant increase of thioflavin T (ThT) fluorescence intensity in phosphate buffered saline, pH 7.5, at 37ºC. These data demonstrated that the G334V mutant peptide formed amyloid-like fibril under physiological conditions. In a kinetic analysis of the G334V amyloid formation, the increase of ThT fluorescence change was readily observed without lag time and saturated in 4~5 days. However, wild-type and monomeric mutant peptides formed no aggregations under this condition. These results suggest that disruption of the turn structure and β-strand elongation induced by the mutation of Gly334 to Val is important for amyloid formation and that denaturing or unfolding of the tetramer structure is not sufficient for the aggregation.

Short Talk
Tuesday, August 31
13:00 - 13:15

PROTEOMIC CONTRIBUTIONS TO THE ELUCIDATION OF C-JUN AND P53 UBIQUITIN LIGASES

David Arnott
Genentech, Inc. Protein Chemistry Department, 1 DNA Way, South San Francisco, CA 94080

COP1 (constitutively photomorphogenic 1) is a RING-finger-containing protein that functions to repress plant photomorphogenesis, the light-mediated program of plant development. Mutants of COP1 are constitutively photomorphogenic, and this has been attributed to their inability to negatively regulate the proteins LAF1 and HY5. The role of COP1 in mammalian cells is less well characterized. Immunoprecipitation of human COP1 yielded a silver-stained band that was identified as the tumor suppressor p53 by trypsin digestion and LC-ion trap-tandem mass spectrometry. Extensive investigation of this interaction demonstrated that COP1 serves as an E3 ubiquitin ligase for p53 and inhibits p53-dependent transcription and apoptosis.

Further protein-protein interactions of COP1 were uncovered in immunoprecipitation experiments with the human ortholog of another plant protein: De-etoliated-1 (DET1). Mammalian proteins pulled down by Epitope-tagged human DET1 were digested without initial separation, and the resulting tryptic peptide mixture analyzed by LC-MS/MS. Components of a complex composed of the proteins DNA Damage Binding Protein-1, cullin 4A, Regulator of Cullins-1, and hCOP1 were identified. This complex was shown to comprise a multisubunit E3 ubiquitin ligase whose substrate is the proto-oncogenic transcription factor c-jun. Unlike the p53 case, hCOP1 did not directly ubiquitinate the substrate, but was essential for activity of the complex. The ability of COP1 to negatively regulate p53 by direct ubiquitination, and c-jun family members by transcriptional inhibition, places COP1 in the context of a master switch for the cell to undergo growth arrest or apoptosis versus proliferation and growth.

IV. Protein Posttranslational Modifications and Cell Signaling
Chairpersons: Lowell Ericsson & Hans Jörnvall
Tuesday, August 31
14:30 - 15:15

BIOMARKER DISCOVERY BY PROTEOMICS AND ITS ROLE IN DRUG AND DIAGNOSTIC DEVELOPMENT

Steven A. Carr
Broad Institute of MIT and Harvard, Cambridge, MA

A biological marker (biomarker) has been defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers have a wide range of potential impact in drug discovery and medicine. Biomarkers may be used to detect the presence of disease before clinical symptoms manifest, define the stage of disease progression, or to predict the severity of disease an affected individual is likely to progress to. Biomarkers can also be used to predict and monitor the therapeutic effect of drugs (pharmacodynamic -PD- markers), relate the effect of a drug to a specific molecular or cellular pathway, and thus to help define the on-target and (often unexpected) off-target effects of the drug (e.g., toxicology). Over 100 protein and peptide biomarkers have been approved for use by the FDA. However, despite the clear need for new disease and mechanism-based biomarkers, and improvements in the sensitivity and specificity of existing biomarkers, the pace of new marker introduction has nearly halted over the last 10 years. The likely cause is the complexity of human biology which is at odds with the notion that the change in concentration of a single molecular marker can robustly measure presence, stage, etc. of a complex disease like heart disease or cancer. One obvious area of improvement is to discover and employ multi-analyte panels instead of single analytes. Proteomics holds significant promise for providing multiple peptide and protein biomarkers of disease based on detection of proteins, their metabolites and modified forms in biofluids. This talk will focus on the challenges involved in this work and present data examples of the discovery and use of protein biomarkers for disease recognition and staging as well as for aiding drug discovery.

Protein Posttranslational Modifications and Cell Signaling
Tuesday, August 31
15:15 - 16:00

POST-TRANSLATIONAL MODIFICATIONS OF TRANSCRIPTION FACTORS REGULATE RESPONSES TO ENVIRONMENTAL STIMULI

Jeffrey J. Gorman (1), Daniel Peet (2) and Murray L. Whitelaw (2)
Institute for Molecular Bioscience, The University of Queensland (1), and Molecular Biosciences, The University of Adelaide (2), Australia.

Cellular responses to certain environmental factors, such as oxygen levels and xenobiotics, involve regulatory post-translational modifications. In the case of the response to oxygen status, the regulation involves post-translational hydroxylation of the transcriptional activator, hypoxia inducible factor (HIF). During hypoxia, HIF dimerises with the Aryl Hydrocarbon Nuclear Transporter (ARNT) in the nucleus and binds to Hypoxia Response Elements (HREs) to upregulate the transcription of genes involved in the hypoxic response, eg. vascular endothelial growth factor. Both HIF and ARNT have basic-helix-loop-helix (bHLH) and Per/Arnt/Sim (PAS) domains, which mediate their dimerization, hence, their description as bHLH-PAS proteins.

When cells are in a normoxic environment, HIF is hydroxylated on a proline residue in an oxygen-dependent degradation domain, which leads to degradation of consitutively expressed HIF via ubiquitination and proteosomal degradation. An additional hydroxylation on an asparagine residue within the C-terminal transactivation domain (CAD) prevents recruitment of the transcriptional co-activator CBP/P300, due to steric hinderance, and transcriptional activation. In hypoxia, these regulatory hydroxylations are suppressed so HIF is stable and capable of binding CBP/P300 and upregulating transcription.

The xenobiotic response also involves a bHLH-PAS transcription factor, the Dioxin Receptor (DR). DR also binds to ARNT to upregulate xenobiotic response elements. Phosphorylation appears to be involved in regulation of the xenobiotic response.

This presentation will review the technologies involved in characterization of the asparagine hydroxylation of HIF and approaches to characterization of phosphoryaltion events to be applied to understanding of the xenobiotic responses of cells.

Protein Posttranslational Modifications and Cell Signaling
Chairperson: David R. Morris
Tuesday, August 31
16:30 - 17:15

DYNAMIC PROTEIN GLYCOSYLATION BY O-GLCNAC IS A METABOLIC SENSOR MODULATING TRANSCRIPTION, SIGNALING AND STRESS RESPONSES IN ALL MULTI-CELLULAR ORGANISMS

Gerald W. Hart, Natasha Zachara, Lance Wells, Keith Vosseller, Sai Iyer, Kazuo Kamemura, Kaoru Sakabe, Win Cheung, Chad Slawson, Stephen Whelan and T. Lakshmanan
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Even though it remained undiscovered until 1983, the dynamic modification of nuclear and cytosolic proteins by O-GlcNAc is now known to be abundant and ubiquitous in all multicellular eukaryotes, including plants and animals. O-GlcNAc occurs at levels similar to protein phosphorylation, often reciprocally competing for the same site or locus on signaling and regulatory proteins. ***Current data suggest that O-GlcNAc serves as a major metabolic sensor (via the donor UDP-GlcNAc) that globally regulates signaling, cytoskeleton and transcription.*** **Recent findings include: 1)** O-GlcNAc modulates insulin signaling. Elevated O-GlcNAcylation in adipocytes and muscle causes insulin-resistance, in part by blocking the activation of Akt/PKB and GSK3ß. Together with many other published studies, these data suggest that hyperglycemia-induced increases in O-GlcNAcylation may be a major underlying cause of insulin-resistance in diabetes. **2)** Cellular stress (eg. heat shock, high salt, or heavy metals) result in a rapid increase in O-GlcNAc on many proteins. This stress-induced elevation in O-GlcNAc is not dependent upon protein synthesis or degradation or on the overall flux through the hexosamine pathway, but is dependent upon calcium influx and PKC activity, suggesting post-translational regulation of O-GlcNAc Transferase (OGT). Importantly, ncreasing the levels of O-GlcNAc, by inhibiting O-GlcNAcase or over-expressing OGT, substantially increases the thermotolerance of cells. **3)** We have found that purified and recombinant OGT are both tightly associated with protein phosphatases, two of which appears to be PP1ß and PP1γ, phosphatases of particular importance to signaling. Thus, in many cases the enzyme complex that removes phosphate is the same as the one which attaches O-GlcNAc. **7)** We have shown that the cell-cycle regulatory proteins, Rb and p107, are O-GlcNAc modified at G1 when they are hypophosphorylated, and the form of Rb that binds to the E2F-1 is O-GlcNAcylated. Current data support the conclusion that O-GlcNAc is a key post-translational modification involved in a multitude of biological processes in eukaryotic cells and is likely of major importance to the mechanisms of insulin-resistance and to glucose-toxicity.

O-GlcNAc remained undiscovered until 1983 for several reasons: **1)** Generally, the saccharide has no effect on gel electrophoresis, even on 2D gels; **2)** Metabolic radiolabeling is difficult since UDP-GlcNAc occurs at millimolar levels in cells and O-GlcNAc rapidly cycles on proteins; **3)** All cells contain large amounts of enzymes which rapidly remove O-GlcNAc when the cell is damaged; **4)** Perhaps most importantly, O-GlcNAc is extraordinarily difficult to detect by mass spectrometry, i.e. O-GlcNAc-peptides are highly suppressed in MALDI, and the saccharide falls off under normal electrospray conditions. Recent advances have greatly improved the ease of O-GlcNAc analyses. A potent inhibitor of O-GlcNAcases (53 nM Ki), PUGNAc, allows improved detection in cells. O-GlcNAc, pan-specific and site-specific monoclonal antibodies are now available. We also have developed a mass spectrometric method involving ß-elimination/Michael addition of DTT/thiol affinity enrichment for the simultaneous identification and site-mapping of O-phosphate/O-GlcNAc -Ser(Thr) residues on many proteins in subproteomes. Recent advances in FTMS-ECD also show great promise for O-GlcNAc site-mapping. *Supported by NIH grants HD13563, CA42486, DK61671 and NIH contract N01-HV-28180. Dr. Hart receives a share of royalty received by the university on sales of the CTD 110.6 antibody. The terms of this arrangement are being managed by The Johns Hopkins University in accordance with its conflict of interest policies.*

Programs in Proteomics at the National Institutes of Health
Chairperson: Christine Colvis
Tuesday, August 31
17:15 - 18:00

UNDERSTANDING COMPLEX SYSTEMS USING PROTEOMICS APPROACHES: ONGOING PROGRAMS AND CURRENT FUNDING OPPORTUNITIES AT NIH

Douglas M. Sheeley
National Center for Research Resources

It is increasingly clear that proteins function most often in intricate relationships rather than in isolation. To truly define the roles and functions of proteins in these complexes, networks, and pathways, it will be essential not only to inventory protein interactions, but also to understand the temporal and spatial dynamics of these interactions as quantitatively as possible. The analytical technologies necessary to study the dynamic interactions of proteins are still being developed. To a large degree these methods are orthogonal to conventional proteomics approaches. At the same time, conventional proteomics technologies are advancing rapidly but are also still largely inadequate for support of biomedical research. The institutes and centers of the National Institutes of Health (NIH) are developing numerous programs to address the broad need for new technology and methods for proteomics.

The National Center for Research Resources (NCRR) at NIH supports a number of programs that support the development and dissemination of proteomics technology. Some of these programs are directed specifically toward proteomics, while others support proteomics as part of a broader program in biomedical technology. These programs support Biomedical Technology Research Centers (BTRCs), Shared Instrumentation Grants (SIG), Small Business Innovative Research (SBIR/STTR) grants, and a variety of research project grants directed at development of hardware, software, and analytical methods. NCRR and NIH programs will be discussed in detail, including the NIH Roadmap program described below. In addition, there will be a more general discussion regarding the NIH application process.

The NIH Roadmap is an agency-wide effort to apply NIH support to projects that would be difficult for individual institutes to initiate but could have a far-reaching impact on biomedical research. NCRR is leading an NIH-Roadmap effort to develop National Technology Centers for Networks and Pathways (TCNPs). This network of research centers will focus on the creation of new tools to describe the inherently dynamic interactions of proteins as quantitatively as possible. The centers will function as hubs of technology development, supporting and in turn driven forward by interactions with multiple research project grants supported by individual NIH institutes and centers. With the first round of awards to be announced later this year, the TCNPs will develop instruments, methods, and reagents for quantitative measurements at sub-cellular resolution and very short timescales. This interdisciplinary research will require a collaborative approach, with diverse teams drawn from multiple laboratories.

As TCNP awards are announced, NIH will solicit research project grant applications from individual investigators who will work synergistically with the TCNPs to extend the technological capabilities being developed and leverage the centers' analytical tools to solve challenging biomedical research problems. Individual investigators will request their own funding, separate from that of the corresponding TCNP, as well as additional resources to reside in the center but focused on their project. This model will allow substantial expansion of the impact of the centers, adding resources in a dynamic, tailored manner for collaboration without diminishing the center's focus on the core mission of technology development.

V. High Throughput Technologies for Cloning, Protein Production and Folding
Chairpersons: Hisashi Hirano & Agnes Henschen-Edman
Wednesday, September 1
08:30 - 9:15

HIGH THROUGHPUT EXPRESSION STRATEGIES FOR STRUCTURAL AND FUNCTIONAL GENOMICS

Gerald W. Becker (1), Elizabeth Landorf (2), Terese Peppler (2), Yuri Londer (2),
Frank R. Collart (2)
(1) Roche Protein Expression Group, 9115 Hauge Rd., Indianapolis, IN 46250
(2) Biosciences Division, Argonne National Laboratory, 9700 South Cass Ave, Argonne, IL 60439

The capability to express proteins in heterologous systems has been an important enabling feature for structural and functional studies of proteins. Although, recent advances in expression technology have significantly increased our capability for the expression of microbial proteins, a significant fraction of proteins encoded by the genome still cannot be expressed in a usable form. We are addressing these challenging expression problems by application of novel cellular and cell-free technologies to optimize the expression of "insoluble" cytoplasmic and periplasmic proteins. The *in vivo* expression strategy uses *E. coli* as a host and employs molecular tools containing elements that enable localization to appropriate cellular or extracellular compartments coupled with regulatory elements to permit control and coordination of protein expression. The *in vitro* expression strategy uses an *E.* coli-based cell free system enabled for plate-based expression and solubility screening of protein targets. As part of this process, we are also developing high throughput processes for domain-based cloning and expression of high molecular weight proteins and putative soluble domains of membrane proteins. Application of this approach allows production of soluble domains for many proteins and enables biophysical and biochemical characterization and functional validation of hypothetical proteins. Our strategy is designed to identify the maximum number of targets suitable for bacterial expression before resorting to more time consuming and cost intensive systems.

High Throughput Technologies for Cloning, Protein Production and Folding
Wednesday, September 1
09:15 - 10:00

DESIGNING A HIGH THROUGHPUT REFOLDING ARRAY USING A COMBINATION OF THE GROEL CHAPERONIN AND OSMOLYTES

Paul A. Voziyan, Bryan Tieman, Mary Johnston, Angela Chao, Greg Bomhoff, **Mark T. Fisher**
† Dept. of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160-7421

Although GroE chaperonins and osmolytes had been used separately as protein folding aids, combining these two methods provides a considerable advantage for folding proteins that cannot fold with either osmolytes or chaperonins alone. This technique rapidly identifies superior folding solution conditions for a broad array of proteins that are difficult or impossible to fold by other methods. While testing the broad applicability of this technique, we have discovered that osmolytes greatly simplify the chaperonin reaction by eliminating the requirement for the co-chaperonin GroES which is normally involved in encapsulating folding proteins within the GroEL-GroES cavity. Therefore, combinations of soluble or immobilized GroEL, osmolytes and ATP or even ADP are sufficient to refold the test proteins. The first step in the chaperonin/osmolyte process is to form a stable long-lived chaperonin-substrate protein complex in the absence of nucleotide. In the second step, different osmolyte solutions are added along with nucleotides, thus forming a "folding array" to identify superior folding conditions. The stable chaperonin-substrate protein complex can be concentrated or immobilized prior to osmolyte addition. This procedure prevents off pathway aggregation during folding/refolding reactions and more importantly allows one to refold proteins at concentrations (~ mg/ml) that are substantially higher than the critical aggregation concentration for given protein. This technique can be used for successful refolding of proteins from purified inclusion bodies. Recently, other investigators have used our chaperonin/osmolyte method to demonstrate that a mutant protein that misfolds in human disease can be rescued by GroEL/osmolyte system. Soluble or immobilized GroEL can be easily removed from the released folded protein using simple separation techniques. The method allows for isolation of folded monomeric or oligomeric proteins in quantities sufficient for X-ray crystallography or NMR structural determinations.

VI: Protein-Protein Interactions and Analysis of Macromolecular Complexes
Chairpersons: Rachel Klevit & Harald Tschesche
Wednesday, September 1
10:30 - 11:15

THE STUDY OF MACROMOLECULAR COMPLEXES BY QUANTITATIVE PROTEOMICS

Jeffrey A. Ranish
Institute for Systems Biology, Seattle, WA 98103

A generic strategy for determining the specific composition, changes in the composition, and changes in the abundance of protein complexes will be described. It is based on the use of stable isotope tagging and mass spectrometry to compare the relative abundances of tryptic peptides derived from suitable pairs of purified or partially purified macromolecular complexes. The strategy was applied to determine the composition of human and yeast transcription factor complexes that were purified from cell extracts by both DNA affinity and antibody affinity chromatography. In addition, the technique was used to detect changes in the composition of transcription factor complexes that were isolated at different stages of cellular differentiation. The use of quantitative mass spectrometry to guide identification of specific complex components in partially purified samples and to detect quantitative changes in the abundance and composition of protein complexes, provides the researcher with powerful new tools for the comprehensive analysis of macromolecular complexes.

Protein-Protein Interactions and Analysis of Macromolecular Complexes
Wednesday, September 1
11:15 - 12:00

RANDOM PEPTIDE DISPLAY AND SELECTION – FOR INTERACTION AND FUNCTION

Min Li
Department of Neuroscience, High Throughput Biology Center, Johns Hopkins University School of Medicine, 733 North Broadway, Baltimore, MD 21205

Protein interaction is central to many biological processes. Methods for large scale, random peptide display and selection are particularly useful for determining specificity and modes of protein–protein interactions. This presentation will discuss various *in vitro* and cell based systems to display and select peptide motifs with desirable binding or functional properties.

Protein-Protein Interactions and Analysis of Macromolecular Complexes
Wednesday, September 1
12:00 - 12:45

CONFORMATIONAL CHANGES IN SECOND MESSENGER MEDIATED SIGNALING AS SEEN BY NEUTRONS

Jill Trewhella
University of Utah

Biochemical signaling processes involve dynamic interactions and conformational transitions in regulatory proteins and their targets. Neutrons have unique scattering properties that enable you to distinguish between the components of macro-molecular complexes and assemblies in different functional states. We have used neutron and x-ray solution scattering to study second messenger mediated signaling mechanisms in calcium and cyclic nucleotide regulated systems. Our most recent work on these systems has focused on how different isoforms of the same protein effect the functions required of them; that is how nature tunes the details of a particular protein to effect a common function that might have different requirements in a specific location, for example. Our recent work with the cAMP-dependent protein kinase has implicated a highly variable linker region between the regulatory subunit isoforms as playing an important role in determine the overall structure of the holoenzyme and in controlling the activation mechanism. If time permits, the talk will also touch on the calcium sensitive muscle regulatory troponin complex that shows dramatic differences between the skeletal and cardiac isoforms.

Short Talk
Wednesday, September 1
12:45 - 13:00

BINDING SPECIFICITY OF MULTIPROTEIN SIGNALING COMPLEXES IS DETERMINED BY BOTH COOPERATIVE INTERACTIONS AND AFFINITY PREFERENCES

Jon C.D. Houtman(1), Yuichiro Higashimoto(2), Nazzareno Dimasi(4), Sangwoo Cho(4), Hiroshi Yamaguchi(2), Brent Bowden(1), Roy Mariuzza(4), Peter Schuck(3), Ettore Appella(2) and Lawrence E. Samelson(1)
(1)Laboratory of Cellular and Molecular Biology and (2)Laboratory of Cell Biology, NCI and (3) Protein Biophysics Resource, ORS, OD, National Institutes of Health, Bethesda, MD, 20892; (4)Center for Advanced Research in Biotechnology, WM Keck Laboratory for Structural Biology, University of Maryland Biotechnology Institute, Rockville, MD, 20850

The formation of multiprotein complexes at receptors and adapter proteins brings effector proteins to the site of active signal transduction and is vital for the activation of intracellular signaling pathways. In this study, the association of signaling proteins with a model adapter protein, LAT, was characterized to examine the *in vivo* binding specificity observed at many multiprotein complexes. We found that the binding specificity of Grb2 to LAT appears to be principally due to large affinity differences, whereas the specificity of binding of the Gads/SLP-76 complex and PLC-γ for LAT is not driven solely by increased affinity for specific LAT tyrosine residues. This suggests that forces other than affinity, such as cooperative effects from the formation of large protein complexes, play a role in binding specificity at LAT. Therefore, the previously identified association between PLC-γ and the Gads/SLP-76 complex was examined using several biophysical techniques. We observed that the interaction of Gads with SLP-76 was extremely strong while the binding between PLC-γ and SLP-76 was only detectable at lower temperatures. We also found that SLP-76 undergoes a substantial change in secondary structure upon binding to either PLC-γ or Gads and that PLC-γ more readily binds to the Gads/SLP-76 complex than to SLP-76 alone at higher temperatures. Together, these studies provide quantitative information on how different binding parameters can determine in vivo binding site specificity observed for multiprotein signaling complexes.

VII. Chemical Proteomics and Imaging Methods
Chairpersons: Michael H. Gelb & Daniel T. Chiu
Wednesday, September 1
14:00 - 14:45

ACTIVITY-BASED PROTEIN PROFILING: CHEMICAL APPROACHES FOR FUNCTIONAL PROTEOMICS

Benjamin F. Cravatt
Departments of Chemistry and Cell Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037, Fax: 858-784-2798, cravatt@scripps.edu

The field of proteomics aims to characterize dynamics in protein function on a global scale. However, several classes of enzymes are regulated by posttranslational mechanisms, limiting the utility of conventional proteomics techniques for the characterization of these proteins. Our research group has initiated a program aimed at generating chemical probes that interrogate the state of enzyme active sites in whole proteomes, thereby facilitating the simultaneous activity-based profiling of many enzymes in samples of high complexity. Progress towards the generation and utilization of active site-directed chemical probes for the proteomic characterization of several enzyme classes will be described. These enzyme classes fall into two general categories: 1) enzymes for which active site-directed affinity agents have been well-defined, and 2) enzymes for which active site-directed affinity agents have been lacking. The application of activity-based protein profiling to the functional characterization of enzyme activities that vary in human cancer specimens will be highlighted, as will be the use of this strategy as a screen to discover potent and selective reversible enzyme inhibitors.

Chemical Proteomics and Imaging Methods
Wednesday, September 1
14:45 - 15:30

MULTI-SCALE IMAGING OF THE NERVOUS SYSTEM

Mark H. Ellisman
National Center for Microscopy and Imaging Research, University of California at San Diego, La Jolla, California

Developments in modern computer-aided light and electron microscopies offer great promise for delivery of vital new information about the structural and functional dynamics of the nervous system. Neuroscientists interested correlating structure with are involved in research covering a wide range of levels of investigation, ranging from modeling molecular events and subcellular organelles at synapses to large-scale mapping of brain systems using fMRI and PET. An increasing number of researchers are interested in the ways in which single neurons and small networks of neurons process and store information. Although this has been a laudable objective in neuroscience for quite some time, interest has increased recently partly because it is now possible to reconstruct in great detail the dendritic and axonal arborizations of single neurons and to use these as the starting point for modeling the complex properties of neurons and neuronal networks. Adding to the excitement and increasing the opportunities, a revolution in molecular biology has revealed classes of molecular constituents (e.g., channels and receptors) undreamed of in the past. Likewise, breakthroughs in optical methods and electronic imaging have provided spectacular new opportunities for deriving information about the 3-D relationships between structures. Indicator dye technologies have allowed linkage of physiological properties directly to anatomy. Synergistically, as a consequence of improvements in performance of computing and communications, information sciences are providing opportunities to enhance the neuroscientist's capabilities to integrate and share the fruits of the revolutions in molecular and cellular neurobiology and imaging sciences. Structure-function work which is beginning to explode in 3-D is also rapidly moving into the realm of 4-D imaging in which temporal reconstruction of events occurring in a 3-D structure is possible. The activities of the National Center for Microscopy and Imaging Research in San Diego in the development of novel techniques for 3 dimensional visualization of neuronal structures and their protein constituents will be described. Special emphasis will be placed on correlated light and electron microscopic techniques and the merging of sophisticated microscopes with powerful computational resources for derivation and visualization of 3D structure.

VIII. Protein Engineering and Biotechnology
Chairperson: Fumio Sakiyama
Wednesday, September 1
16:00 - 16:45

RATIONAL ENGINEERING OF PROTEIN DRUGS

John Desjarlais
Xencor, 111 W. Lemon Avenue, Monrovia, CA 91016, 626-737-8077

Protein engineering principles are increasingly used to optimize proteins for therapeutic purposes. Properties typically manipulated include potency, efficacy, mechanism, solubility, immunogenicity, pharmacokinetics, selectivity, production cost, and patentability. Xencor is using rational design methods to create cytokines and antibodies with optimized profiles of these properties, with the goal of developing therapeutics with novel mechanisms, superior efficacy, and reduced side-effects.

IAPSAP/MPSA 2004 Pehr Edman Awards

Wednesday, September 1
16:45 - 18:05

The 2004 IAPSAP/MPSA Pehr Edman Awards will be presented to Dr. Stephen Altschul, Computational Biology Branch, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, USA, and to Dr. Amos Bairoch, Swiss Institute for Bioinformatics, Geneva, Switzerland. The awards are supported by Applied Biosystems, Inc.

Dr. Altschul received his AB from Harvard College in 1979 and his Ph.D. from the Massachusetts Institute of Technology in 1987. He has made many contributions to the field of protein sequence comparison and analysis. He is perhaps best known for his role in developing the BLAST and PSI_BLAST sequence database search programs. PSI_BLAST introduced rapid and fully automated iterated protein profile analysis to database searching. It has become a vital tool for genome annotation and for protein structure prediction and modeling studies. Dr. Altschul's other research activities include the description of statistics for local sequence alignments and SAGE analysis, the elucidation of the properties of amino acid substitution matrices, the development of the MSA, MACAW and Gibbs sampling algorithms for multiple sequence alignment, and the first description of the BRCT domain in BRCA1 and other DNA damage response proteins. For additional information, see http://www.ncbi.nlm.nih.gov/CBBresearch/Altschul/.

Dr. Bairoch received his Masters of Science in Biochemistry from the University of Geneva in 1983 and his Ph.D. in 1990. His main efforts have been in the field of protein sequence analysis and the development of databases and software tools for the analysis of proteins. He is best known for the development and maintenance of the Swiss-Prot database, the best known and most widely used database on proteins. From 1987 to 1994 Swiss-Prot was a collaborative project with the European Molecular Biology Laboratory and since 1994 with the European Bioinformatics Institute (EBI). Dr. Bairoch has also developed PROSITE, a database of protein families and domains, ENZYME, a database of information on the nomenclature of enzymes, and contributed to the development of ExPASy, a web server for molecular biologists. Currently he is Professor of Bioinformatics in the Department of Structural Biology and Bioinformatics at the University of Geneva. For additional information, see: http://www.expasy.org/people/personal/amos/amos_home.html.

The Pehr Edman Award is given to individuals whose efforts have significantly advanced the fields of protein chemistry, protein structure analysis, or proteomics. The award honors and commemorates the work of Pehr Edman, the Swedish chemist principally responsible for developing the chemistry for sequencing proteins by removing amino acids from the amino terminus one at a time. The Award is given in conjunction with Methods in Protein Structure Analysis (MPSA) meetings, which are sponsored by the International Association for Protein Structure Analysis and Proteomics (www.iapsap.bnl.gov). The first prize was awarded to Richard Laursen in 1988 for his efforts in the development of solid-state protein sequencing methods. In 2002, the award was given to Professor Hans Jörnvall, Karolinska Institute, Stockholm, Sweden, for contributions to protein chemistry, structure and function through the development of techniques associated with the use of Edman chemistry and structure-function studies on alcohol dehydrogenases, and to Professor Kenneth Walsh, University of Washington, Seattle, Washington, USA for his contributions to the development of mass spectrometry for characterizing the chemical structure of proteins and their posttranslational modifications and for concepts contributing to the evolution of protein structure and function.

THE COMPOSITIONAL ADJUSTMENT OF AMINO ACID SUBSTITUTION MATRICES

Stephen F. Altschul
National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health

Amino acid substitution matrices are central to protein comparison methods. In most commonly used matrices, the substitution scores take a log-odds form, involving the ratio of "target" to "background" frequencies derived from large, carefully curated sets of protein alignments. However, such matrices often are used to compare protein sequences with amino acid compositions that differ markedly from the background frequencies used for the matrices' construction. Of course the target frequencies should be adjusted in such cases, but the lack of an appropriate way to do this has been a long-standing problem.

This talk argues that if one demands consistency between target and background frequencies, then a log-odds substitution matrix implies a unique set of target and background frequencies, as well as a unique scale. Standard substitution matrices therefore are truly appropriate only for the comparison of proteins with standard amino acid composition. Accordingly, a rationale is presented for transforming the target frequencies implicit in a standard matrix to frequencies appropriate for a non-standard context. This rationale yields asymmetric matrices for the comparison of proteins with divergent compositions. Composition-specific substitution matrix adjustment is of utility for comparing compositionally biased proteins, including those of organisms with nucleotide-biased, and therefore codon-biased, genomes or isochores.

FROM EDMAN TO MADNESS: HOW TO SURVIVE IN A FLOOD OF PROTEIN SEQUENCE DATA

Amos Bairoch
Swiss-Prot group; Swiss Institute of Bioinformatics, amos.bairoch@isb-sib.ch

"By 1960-61 the three-stage degradation reaction had been essentially perfected. Its universal application and repetitive nature suggested to G. S. Begg, Edman's Australian technical assistant, that it would be suitable for automation. Edman realized that the number of existing proteins (about ten million) made manual sequencing an impossible task and was quickly converted to the idea of automation." (Excerpt from: http://www.asap.unimelb.edu.au/bsparcs/aasmemoirs/edman.htm)

About 50 years have elapsed since Frederick Sanger sequenced insulin and Pehr Edman was working on perfecting the methodology to sequence proteins. Today there are more that 1.5 million protein sequences available in the combined Swiss-Prot+TrEMBL (UniProt) knowledgebase [1,2]. The overwhelming majority of them were not obtained from protein sequencing. They are predicted from genomic and cDNA sequence data. It is therefore both paradoxical and refreshing to see that Edman's method is still quite useful to correct many artefactual predictions so as to provide clean data on protein sequences.

In direct relevance to this observation, I will, in my talk, describe some of the ongoing efforts within the Swiss-Prot groups at the Swiss Institute of Bioinformatics (SIB) and at the European Bioinformatics Institute (EBI) targeted towards the production of clean and non-redundant sets of sequences that include a maximum of information on post-translational modifications [3]. These tasks are of direct relevance to modern proteomics studies.

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[3] Farriol-Mathis N., Garavelli J.S., Boeckmann B., Duvaud S., Gasteiger E., Gateau A., Veuthey A.-L., Bairoch A. Annotation of post-translational modifications in the Swiss-Prot knowledgebase Proteomics 4:1537-1550(2004).

IAPSAP/MPSA 2004 Young Investigator Award

Wednesday, September 1
18:05 - 18:45

The 2004 IAPSAP/MPSA Young Investigator Award, supported by Applied Biosystems, Inc., will be given to Dr. Niroshan Ramachandran, Harvard Institute of Proteomics, Department of Biological Chemistry and Molecular Pharmacology, Harvard University, Boston, MA 02115, USA

Dr. Ramachandran, currently a postdoctoral fellow in Dr. Joshua LaBaer's lab, has developed a novel self -assembling protein microarray. In this approach, Nucleic Acid Programmable Protein Array (NAPPA), proteins self assemble from nucleic acids. Traditional target protein microarrays are generated by expressing, purifying and spotting the proteins on a microarray. Dr. Ramachandran's approach begins with spotting full-length cDNAs, which are then transcribed and translated using a eukaryotic cell-free expression system. This approach offers a number of advantages over conventional protein microarrays: first, it eliminates the need for tedious and laborious methods for protein purification; second, it reduces concerns about protein stability as the proteins are produced just-in-time; lastly, it allows expression of mammalian proteins in a mammalian milieu that contains the necessary machinery to ensure the integrity of mammalian proteins. Dr. Ramachandran has used this technology to build a protein interaction network among human DNA replication proteins, to study regulation of protein interaction and to map binding domains. He currently is building a microarray expressing the 1000 most relevant genes in breast cancer (BC1000 gene collection - FLEXGene repository). The BC1000 microarray will be used to interrogate the functions of these genes and to develop diagnostic tools for breast cancer.

SELF ASSEMBLING PROTEIN MICROARRAY

Niroshan Ramachandran
Harvard Institute of Proteomics, Harvard Medical School

One of the most compelling steps in the post-genomic era will be learning the functional roles for all proteins. The Harvard Institute of Proteomics (HIP) has initiated a project to create a sequence-verified collection of full-length cDNAs representing all human coding regions in a recombinational vector system that allows the immediate in-frame transfer of all coding regions into virtually any protein expression vector. These transfers allow the addition of peptide tags to either or both end of the proteins. This repository, called the FLEXGene Repository (for **F**ull-**L**ength **Ex**pression-ready), will enable the high-throughput (HT) screening of protein function for the entire set (or any customized subset) of human genes using any method of *in vitro* or *in vivo* expression.

One such approach is the use of target protein microarrays, where multifunctional assays such as protein-protein interactions, nucleic acid-protein interactions, and small molecule or enzyme-substrate screens can be performed on hundreds of different proteins. However, they have not found wide acceptance in part because of the challenges in producing proteins to spot on the arrays. We developed a novel approach to generate protein microarrays by printing cDNAs onto glass slides and then translating target proteins with mammalian reticulocyte lysate. This robust method obviates the need to purify proteins, avoids protein stability problems during storage and captures sufficient protein for functional studies. The versatility of this technology, **N**ucleic **A**cid **P**rogrammable **P**rotein **A**rray (**NAPPA**), was demonstrated by mapping pairwise interactions among 29 human DNA replication initiation proteins, recapitulating the regulation of Cdt1 binding to select replication proteins, and mapping its geminin binding domain.

The most exciting part of this project has been the ease with which the clones from the repository can be rapidly incorporated into this type of HT biological experimentation. NAPPA could be readily adapted to assess the binding selectivity of small molecules to a family of related proteins (e.g., kinases) or to a mutant series of a single protein, to screen for immune responses to a large panel of antigens, or to screen for substrates for an active enzyme. The increasing availability of large repositories of protein-expression ready cDNA clones in recombinational vectors, namely FLEXGene collection, will provide a rich content source that will amplify the power of this technique to study protein function.

IX. Cell Pathways Regulation and Metabonomics
Chairpersons: Kristine Swiderek & Martin J. Kushmerick
Thursday, September 2
08:30 - 9:15

THE LATTICE STRUCTURE OF THE YEAST SPINDLE POLE BODY PROBED BY FLUORESCENCE RESONANCE ENERGY TRANSFER

Eric G. D. Muller, Brian Snydsman, Bryan Sundin, Dale Hailey, **Trisha N. Davis**
Yeast Resource Center and Department of Biochemistry, University of Washington, Seattle, Washington 98195

The spindle pole body (SPB) of Sacchromyces cerevisiae, like its counterpart the centrosome of higher eukaryotes, functions as the microtubule organizing center of the cell. Microtubule nucleation is directed from a layered stack of proteins embedded in the nuclear envelope. Although this ultrastructure differs from that of the centrosome, elements of the underlying structure of the SPB are conserved. The yeast SPB components Spc110, calmodulin, centrin and Sfi1 all have homologs in the human centrosome.

We have used Fluorescence Resonance Energy Transfer (FRET) to assay the spatial arrangement of five core components of the SPB; Spc42, Spc110, calmodulin, Spc29 and Cnm67. The ends of these proteins were fluorescently tagged with CFP and YFP. Over 40 pair wise combinations were created and examined by fluorescence microscopy for evidence of FRET. A new FRET metric was established that facilitated comparisons among the FRET values. Positive and negative controls established the range of sensitivity.

The prevailing model of the SPB arranged the 5 proteins end to end along a linear path that traversed the multiple layers of the SPB. The central plaque was thought to consist of Spc29 sandwiched between the N-terminus of Spc42 on one side and the C-terminus of Spc110 on the other. In contrast, our FRET results suggest that the central plaque is a meshwork of interlocked proteins.

To exploit the richness of the FRET data set, we incorporated the spatial relationships suggested by FRET with the hexagonal symmetry of Spc42 suggested previously by cryo-electron tomography. The composite model offers a novel view of the structure and assembly of the SPB.

Cell Pathways Regulation and Metabonomics
Thursday, September 2
09:15 - 10:00

UNDERSTANDING GLOBAL SYSTEMS BIOLOGY USING METABONOMICS

Jeremy Nicholson
Imperial College, London, UK

Please see program addendum for abstract.

Short Talk
Thursday, September 2
10:00 - 10:15

ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY-MS(TOF): NEW SEPARATIONS TECHNOLOGY FOR HIGH THROUGHPUT METABONOMICS

Jennifer H. Granger(1) Robert S. Plumb(1), Ian D.Wilson(2) and Rebecca Williams(2)
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The use of ultra performance liquid chromatography (UPLC) has gained much interest since it offers several advantages, namely improved chromatographic resolution, increased sensitivity and reduced analysis times. This is achieved through the use of small particle sizes (< 2um) and pumping / injection systems capable of operating under such exacting pressure regimes (up to 15,000 psi). When coupled with time of flight mass spectrometry, UPLC presents a means to achieve high sample throughput with, reduced spectral overlap, increased sensitivity and exact mass detection capabilities. This aspect of UPLC-ao-TOF-MS is particularly attractive for metabolite ID and metabonomics applications where the rapid and accurate detection and identification of potential drug metabolites, impurities and biomarkers by exact mass is beneficial.

We have recently combined UPLC/ao-TOF MS for metabolite identification in urine and bile for a model toxin. UPLC/ao-TOF has also been applied to the metabonomics analysis and elucidation of endogenous biomarkers of gender, genetic state and diurnal variations in black, white and nude mice and obese (fa/fa) Zucker rats. The results presented here will demonstrate the positive impact that high-pressure separations have on resolution and sensitivity. Furthermore, use of UPLC results in the detection of more peaks, which implies that an increased number of possible metabolites and biomarkers may be found. Several biomarkers of gender, genetic state and diurnal variation has been detected using this approach, and will be discussed in terms of biological relevance.

X. Systems Biology
Chairperson: Amos Bairoch
Thursday, September 2
10:30 - 11:15

MAPPING, VALIDATING, AND COMPARING BIOLOGICAL NETWORKS

Trey Ideker
Department of Bioengineering, UC San Diego

A major challenge of systems biology is to elucidate the molecular networks in control of cellular form and function. Towards this goal, we are constructing physical network models to explain changes in gene expression observed in response to DNA damaging and other toxic agents. Network models are constructed from a scaffold of protein-protein and protein-DNA interactions measured before versus after DNA damage. These measured interactions are then integrated with gene expression profiles using a causal probabilistic approach. To refine these models, an automated procedure is used to select gene knockout profiling experiments that best reveal the directionality of each interaction and its regulatory role as an activator or inhibitor. The resulting network models provide hypotheses as to the underlying signaling and regulatory mechanisms mediating response to toxic agents.

Systems Biology
Thursday, September 2
11:15 - 12:00

SYSTEMS APPROACHES TO DEVELOPMENT AND PHYSIOLOGY

Leroy Hood
Institute for Systems Biology

The advent of the Human Genome Project has transformed biology by providing a genetics parts list of all genes and proteins, by fueling the contention biology is an informational science, and by catalyzing the emergence of biological information (e.g., rapid DNA sequencing or DNA chips). From this has emerged a new approach to biology termed systems biology<centered on the idea one can study biological systems by delineating the relationships of all of their component elements<and, hence, come to understand the resulting systems or emergent properties. I will discuss these important points and give several examples of systems approaches in simple and complex organisms.

Posters

Posters are presented in the HUB East Ballroom. They are numbered alphabetically by presenting author's last name.

001 PROTEOMIC CONTRIBUTIONS TO THE ELUCIDATION OF C-JUN AND P53 UBIQUITIN LIGASES

David Arnott
Genentech, Inc. Protein Chemistry Department, 1 DNA Way, South San Francisco, CA 94080

See page 32 for abstract.

002 MICROFLUIDIC ELECTROCAPTURE TECHNOLOGY IN PROTEIN AND PEPTIDE ANALYSIS

Astorga-Wells, J., Bergman, T. and Jörnvall, H.
Department of Medical Biochemistry and Biophysics, Karolinska Institutet,SE-171 77 Stockholm, Sweden

See page 30 for abstract.

003 BINDING ENERGETICS OF PEPTIDE BINDING TO MHC II PROTEIN (I-A^k)

Arunima Bandyopadhyay[1] and Craig Beeson[2]
[1]Department of Chemistry, University of Washington, Seattle, WA 98195; [2]Department of Pharmaceutical Sciences, Medical University of South Carolina, Charleston, SC 29425

Peptides bound to MHC molecules on the surface of cells convey important information to immune system T cells. Studying the binding interactions of the peptides to the MHC protein is important to understand the possible mechanism for the HLA-DM protein that regulates peptide presentation in vivo. Hydrogen bonding and pocket interactions are the main interactions that stabilize peptide-MHC complexes. The hierarchy of binding interactions for the MHC II protein I-A^d, as measured previously, shows that the N-terminal hydrogen bonds contribute most to the binding energetics followed by the pocket interactions and the C-terminal hydrogen bonds. The net ΔG for the MHC-peptide interaction is estimated to be around -15 kcal/mol. It has been suggested that the I-A^d protein does not have a dominant pocket interaction and, thus, it is very different from the other MHC II proteins. The goal of my project is to study the hierarchy of binding energetics for I-A^k, which is a more typical MHC II protein that has a dominant pocket interaction. Crystal structure of the I-A^k protein with the HEL peptide shows the presence of a network of electrostatic interactions with the P1 aspartic acid residue of the peptide and an arginine present in the P1 pocket of I-A^k protein. This P1 anchor-pocket interaction is thought to be energetically dominant. Our results suggest that the effect of the P1 pocket mutation (mutating the Asp to Ala) is not energetically as dominant as it is thought to be. Preliminary results also indicate that disrupting the N-terminal hydrogen bond increases the dissociation rates of the peptides to a considerable extent. From the energetics measured so far, the hierarchy of binding energetics in the I-A^k and the I-A^d protein are similar.

004 MIGENES: A DATABASE FOR MITOCHONDRIAL PROTEOMICS

Siddhartha Basu, Erich Bremer, Chun Zhou and Daniel F. Bogenhagen
Dept of Pharmacological Sciences, Stony Book University, Stony Brook, NY 11794-8651

Mitochondria are ubiquitous organelles involved in diverse cellular processes such as oxidative phosphorylation, Kreb's cycle, fatty acid metabolism and synthesis of heme and Fe-S centers. Mitochondria play important roles for cellular calcium signaling, generation of reactive oxygen species and programmed cell death. Mitochondrial dysfunction contributes to human diseases including diabetes, cancer, aging, neurodegeneration and cardiomyopathy, stimulating a number of recent studies of the mitochondrial proteome.

Mitochondria are expected to house approximately 1200 proteins, all but 13 of which are encoded in nuclear DNA. However, only about 600 mitochondrial proteins have been characterized in higher organisms. We created the MiGenes SQL database to organize information about gene products relevant to mitochondrial function. MiGenes organizes information on gene products from humans and several model organisms including mouse, rat, frog, fruit fly, worm, baker's yeast and fission yeast. In addition to mitochondrial annotations from public databases, Migenes is curated to include lists of proteins reported through high throughput proteomic studies and other publications. The curation process also tracks the evidence that is considered to determine the authenticity of a mitochondrial gene product.

The current version of MiGenes allows the user to retrieve information using aliases and alternative accession numbers. The batch mode search is designed to analyze the output from high throughput proteomic studies, permitting compilation of published large scale proteomic analyses. Each database record is annotated with the gene product's name, alias names, function, list of relevant literature, list of homologous genes, GO term assignments with evidence codes and links to pubmed. In the next version, MiGenes will be extended to include extra-mitochondrial gene products that are important to organelle function.

005 INITIAL PROTEOME ANALYSIS OF *STREPTOCOCCUS PYOGENES* USING MULTIDIMENSIONAL LC AND MALDI TOF TANDEM MASS SPECTROMETRY: A MODEL FOR PATHOGENIC BACTERIAL PROFILING

Sanjeev Bhardwaj[1], Melanie Lin[1], Hercules Moura [2], Maria da G. Carvalho[2], Adrian R. Woolfitt[2] & John R. Barr[2]
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S. pyogenes is the most pathogenic bacteria in the *Streptococcus* genus. It colonizes human throat or skin and is an agent of severe invasive diseases. Proteome analysis of pathogenic bacteria would enable better understanding of these organisms, with a significant potential impact in both control and prevention. A novel 2D LC-MALDI approach integrated with MALDI tandem TOF/TOF MS makes it possible to develop a comprehensive library of MS/MS peptide fingerprints both for preliminary screening and for unequivocal genus and strain identification. In this study, whole *S. pyogenes* digested cell proteins were analyzed using multidimensional LC-MALDI MS/MS, to verify protein identification and to determine the feasibility of such workflows.
Total protein extracts of *S. pyogenes* M1 were digested with trypsin and analyzed using 1 D and 2D LC-MALDI MS/MS. The peptides were eluted off the cation exchange column and the fractions co-infused with matrix were spotted onto several MALDI plates. The MALDI analysis was done on 4700 Proteomics Analyzer with TOF/TOF™ optics and database searching done using GPS Explorer™ v2.0 software.
In this study, 1D analysis of *S. pyogenes* total proteins has resulted in the identification of ~ 300 proteins in the mixture. The *S. pyogenes* genome predicts ~4,620 ORFs. However, only 1/3rd of the proteins are expressed in a particular period of time so the total number of expressed proteins with an average of 2 modifications would be ~3100. In a previous experiment on yeast cell lysate, the 2D LC-MALDI have allowed for identification of 66% more peptides *vs* 1D-LC setup that would translate into identification of significant number of proteins out of the probable total.
MALDI-TOF MS/MS fingerprinting is a rapid, reproducible, high-throughput alternative method for identifying microorganisms. It has the potential for several applications in the study of prokaryotic agents when used in combination with proteome database search.

006 MICRO WESTERN BLOT: USING INKJET PRINTER TECHNOLOGY TO PERFORM MULTIPLE ANTIBODY DETECTION IN THE SAME BAND OR LANE ON A BLOT

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Western blotting has been employed for proteomic studies for many years. It requires a lengthy time course for the incubation of the blot with the antibodies and generally a large amount of primary antibody. The use of multiple antibodies on the same sample means using a larger quantity of sample and a longer time to get the results. Using a novel piezoelectric printing system, a Chemical Inkjet Printer (ChIP), we have performed a micro western blot by printing a number of different antibodies to detect target proteins in the same lane or sample. The ChIP can print a number of antibodies on to each target band of the 1D SDS-PAGE membrane, bypassing the use of multiple blots or stripping. The printing process also includes printing of secondary antibodies and washing procedures to remove non-specific binding. Using the ChIP, results of multiple antibodies are obtained in a single membrane and require minimal amounts of these antibodies.We demonstrate that micro western blotting using the ChIP can achieve the same level of sensitivity and reproducibility as the conventional western blot but using significantly less sample and antibodies. We also show how ChIP technology can provide a faster method for western blotting and save the cost of many consumables.

007 MOLECULAR RECOGNITION STUDIES IN THE DEVELOPMENT OF ANTIANDROGENS WITH HIGH AFFINITY FOR ITS RECEPTOR

Jorge A. Calleros-Diaz* and Manuel Soriano-Garcia[&]
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It is well established that androgens play an important role in the prostate disorders as in the benign prostatic hyperplasia (BPH) and in the prostate cancer. The testosterone is converted irreversibly to dihydro-testosterone (DHT) by the catalytic action of the 5a-reductase enzyme; the later binds to the androgen receptor (AR). This receptor is constituted in 3 basic functional domains: the N-terminal domain (NBD), DNA-binding domain (DBD) and ligand-binding domain (LBD). Up to date, there has not been determined the full 3-D structure of the AR. Only exists the crystallographic structures for DBD and LBD.
The current therapy usually eliminate the local cancer by surgery and radiation treatments. Nevertheless, both treatments cause

side effects as incontinence, intestinal injury, sexual powerlessness, as well as the own effects of radiation. The present work deals with the application of molecular modeling techniques on the androgen receptor. The cavities or places in which different classes of antiandrogens interact will be study in terms of their sizes and physicochemical characteristics by means of docking techniques of the these compounds. Later on, selected compounds will be synthesize in other to design of a new generation of antiandrogens.

008 CONFORMATION AND CONCERTED DYNAMICS OF THE INTEGRIN BINDING SITE AND THE C-TERMINAL REGION OF ECHISTATIN REVEALED BY HOMONUCLEAR NMR

Daniel Monleón[1], Vicent Esteve[1,2], Helena Kovacs[3], Bernardo Celda[1,4] and Juan J. Calvete[2]
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Echistatin is a 49-amino-acid short disintegrin isolated from the venom of the saw scaled viper (*Echis sochureki carinatus*) and a potent antagonist of the integrins $\alpha_v\beta_3$, $\alpha_5\beta_1$ and $\alpha_{IIb}\beta_3$. Echistatin's ability to bind with high affinity to these integrins depends primarily on an RGD motif expressed at the tip of its integrin binding loop. In addition, the C-terminal tail of echistatin triggers the expression of conformational changes (LIBS, ligand-induced binding sites) in the receptor, leading to a further increase of the binding affinity and the inhibitory potency of the disintegrin. Though echistatin is probably the best characterized disintegrin, previous studies by NMR spectroscopy yielded medium-resolution structures with a poorly defined integrin recognition sequence and an incomplete C-terminal tail, which let unresolved the molecular basis of the functional synergy between the RGD loop and the C-terminal sequence of echistatin. Now, we report a high-resolution revised structure of echistatin by homonuclear 2D NMR at 500 and 600 MHz recorded at 298 K using a triple resonance cryogenic probe, along with an analysis of its internal motions by off-resonance ROESY. In contrast to previous models, the side-chains of the amino acids Arg24 and Asp26 of the integrin recognition motif display well-defined conformations, and the full-length C-terminal polypeptide is visible and folds as a β-hairpin running parallel to the RGD loop and exposing at the tip residues Pro43, His44, and Lys45. The integrin binding loop displays an overall movement with maximal amplitude of 30º. Internal angular motions in the 100-300 ps timescale indicated increased flexibility for the backbone atoms of residues Gly17, Thr18, and Asp29, all of which are located at the base of the integrin recognition loop. The backbone atoms of the active tripeptide amino acids showed intermediate angular mobility between the base and the intervening loop residues. These data strongly suggest the existence of a major and a minor hinge effect at the base and the tip, respectively, of the integrin recognition loop of echistatin. The detection of a strong network of NOEs between residues of the RGD loop and the C-terminal tail, along with the realization that backbone atoms of the C-terminal tail residues 36-46 exhibited restricted angular mobility, indicated concerted motions between the C-terminal and the RGD loops. In addition, an integrin-disintegrin complex developed using a model for the extracellular domains of $\alpha_{IIb}\beta_3$ and the refined solution structure of echistatin (1RO3) clearly shows that, in agreement with previous biochemical and mutational data, the RGD loop and the C-terminal region of echistatin form a conformational epitope engaged in extensive interactions with the target integrin receptor, and provide the molecular basis for understanding the functional synergy between these two functional epitopes.

009 PROTEOME ANALYSIS OF HIV-1 INFECTION IN $CD4^+$-T-CELLS BY LC-FTICR AND ACCURATE MASS AND TIME TAGS

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Although numerous interactions between human immunodeficiency virus (HIV) and host cell proteins have been described, the global effects of viral infection on the host cell proteome remains to be determined. Here we describe the utilization of the accurate mass and time (AMT) tag approach to provide quantitative, global protein profiles of HIV infection in human T-lymphoblastoid CEMx174 cells. Tryptic digests of subcellular fractions (nuclear, microsomal, cytosolic) from uninfected and HIV-1 infected cells were used to identify 2,559 mass and time tags that correspond to 1,386 proteins, representing all 19 gene ontology categories for subcellular localization. This database is currently being used for $^{16}O/^{18}O$ stable isotope labeling at the peptide level (post-digestion) in combination with liquid chromatography coupled to Fourier transform ion cyclotron resonance (LC-FTICR) mass spectrometry to identify up- and down-regulated cellular proteins during HIV infection. Of the 1,386 proteins quantified, 91 proteins exhibited 1.5-fold or greater changes in expression upon HIV-1 infection (69 up- and 22 down-

regulated). These differentially expressed proteins represent a variety of biological processes including humoral immune response, transcriptional and translational regulation, glucose metabolism and heat shock response. Many of these proteins have been reported to interact with the HIV-1 viral proteins Gag, Rev, Tat, Vif, and Vpr. The changes in host cell protein expression identified by these studies are expected to provide further insight into the mechanism of action of viral replication and pathogenesis, novel targets for therapeutic intervention, and critical markers for both diagnostic and prognostic applications.

010 ADDRESSING SOLUTIONS TO PROTEIN EXPRESSION PROBLEMS

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The major challenge during heterologous expression of proteins is to produce them in "soluble and functional" forms. Expression in *E.coli* is the easiest and economical way to produce recombinant proteins; however, only a fraction of mammalian proteins expressed in *E. coli* are in soluble or functional forms. In addition, some proteins are too toxic to produce in *E.coli*. We have developed a number of strategies to address these problems. First, we have developed a novel cell-free protein expression technology that is capable of synthesizing milligrams of proteins. Second, we have devised a strategy to learn the effect(s) of various chaperones in soluble expression of proteins in the cell-free system. The lesson learned can then be transferred to *in vivo* expression of proteins in the presence of particular chaperone(s) for large-scale expression of proteins. Third, we have identified a "soluble-expression tag" capable of producing soluble proteins when fused with "difficult proteins" which otherwise produce mostly insoluble proteins. These improvements will likely to solve some of the problems of protein expression in *E.coli*.

011 REAL-TIME LC-MS GRADIENT MONITORING USING MOBILE PHASE TRACERS

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Liquid chromatographic (LC) separations can deviate run-to-run due to fluctuations in ambient temperature, poor mixing, small gas bubbles in the chromatographic system, or other experimental variables. These deviations can make it difficult to align chromatography runs to each other. Chromatography alignment is important when the chromatographic position of a peptide is used to aide in its identification or differentiation from other peptides. We present a method for monitoring the LC gradient during LC-mass spectrometry analyses in real-time by following the signal intensity of tracers placed in the mobile phase buffers. Our LC separations were conducted with a binary gradient. Thus, two tracers which we will call tracer A and tracer B were used. Tracer A was spiked into the aqueous phase buffer, and tracer B in the organic phase buffer. Using the ratio of the signal intensities of these two tracers over time, we were able to recreate the gradient programmed into the HPLC pump controller (Fig 1). As expected, the real-time gradient displayed a time delay with respect to the programmed gradient. We also observed fluctuations in the monitored gradient each time the programmed gradient changed slope. The presence of these tracer molecules in the chromatographic buffer did not adversely affect the number or quality of our MS/MS identifications from a fraction of yeast extract. This method of gradient monitoring can be used for any LC-system. Thus, it can be used to align chromatographic runs between different chromatography columns and different LC-MS systems.

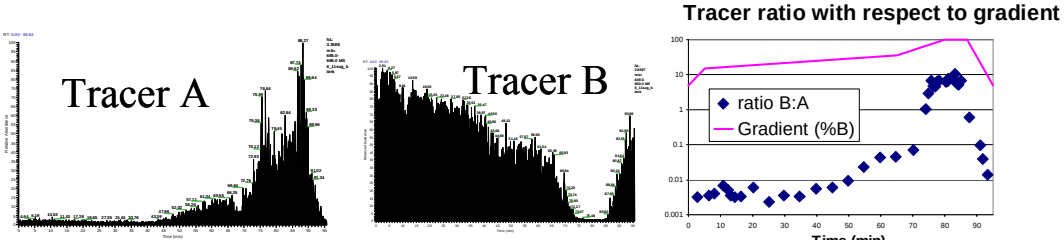

Figure 1. The signal intensities of tracers A and B are proportional to their concentrations and position in the gradient. Their ratio describes the real-time gradient and traces the gradient programmed into the HPLC pump controller.

012 ON THE POSSIBLE INVOLVEMENT OF MOUSE RPS5 PHOSPHORYLATION IN RIBOSOMAL BIOGENESIS AND CELLULAR DIFFERENTIATION

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Ribosomal proteins are integral components of the ribosome subunits involved in protein biosynthesis. Also, evidence exists to indicate that several ribosomal proteins regulate various other cellular functions, suggesting that these proteins may act as multifunctional entities. We have found [1] that induction of differentiation of MEL cells with chemical inducers is associated with down-regulation of rpS5 gene, a gene encoding the mouse S5 ribosomal protein. To further explore the rpS5 role in cell differentiation, MEL cells were transfected with the mouse full-length rpS5 cDNA and a subline that constitutively express this cDNA was established. Transfected MEL cells induced by several inducers (DMSO, HMBA, UDP-4) exhibited a delay in the onset of differentiation without any significant change in their cell proliferation as compared with mock-transfected and parental MEL cells [2]. By trying to analyze rpS5 function into this process, we found that rpS5 possesses a domain within the first 37aa at its NH_2-terminal region that functions *in vitro* as a substrate for phosphorylation by casein kinase II (CK II) probably acting as PEST region. This is in agreement with observations showing that rpS5 belongs to the early-assembled proteins during ribosomal biogenesis and its nucleolar association may be due to specific functional activities e.g. reactivation or modification of specific functional regions [3]. By following these observations, we transfected HeLa cells with the eukaryotic expression pEGFP-C1 vector that bears either the wild-type (GFP-S5) or a truncated form of rpS5 lacking the NH_2 –terminal region (GFP-TrS5) all tagged with the GFP protein (a gift from Dr. S. Huang) and then analyzed the cells by confocal microscopy in order to explore protein localization intracellularly. Our results show that GFP-S5 is mostly accumulated into the nucleoli, while its truncated form GFP-TrS5 protein that lacks the 37aa portion is found to be diffused into the nucleoplasm. This early observation suggests that the phosphorylation of rpS5 would be probably a critical modification for its function on ribosomal biogenesis and localization into the nucleolar compartment. Whether these changes in rpS5 protein localization into the nucleolar compartment are involved in the delay of onset of MEL cell differentiation is an interesting proposal that needs further investigation.

This work is supported by a grant (Joint Research and Technology projects 2001-2003, Russian Federation-Greece, ΕΠΑΝ M.4.3.6.1., Protocol number 10858/01) from the General Secretariat of Research and Technology, Ministry of Development of Greece.

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013 THE *HELICOBACTER PYLORI* NEUTROPHIL ACTIVATING PROTEIN (HP-NAP) DOES NOT BIND TO DNA BUT PROTECTS IT TOWARD OXIDATIVE CLEAVAGE

Philippos Kottakis and Theodora Choli-Papadopoulou

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A number of virulence factors produced by *H. pylori* during the infection of humans have been identified. Among them, a relevant role is played by a 200kDa protein called Neutrophil –Activating Protein (HP-NAP). Its amino acid sequence presents similarities with the Dps protein from *E.coli*, the dodecameric ferritin (Flp) from *Listeria innocua* as well as with the two Dps-like proteins from *Bacillus anthracis.* In order to study the behavior of HP-NAP in conjunction with its ability to bind-protect DNA, its gene has been cloned in pET11a vector and the protein was overproduced, within the transformed BL21 (DE3) *E.coli* cells, after IPTG induction. An amount of 12 mg protein was isolated and purified – by ammonium sulfate precipitation and gel chromatography column – from half-liter cell culture.

It has been reported [1] that HP-NAP co-localizes with cellular DNA *in vivo* and that it binds to DNA at pH 5 suggesting an essential role for growth of *Helicobacter pylori* cells at acidic pH [2]. On the other hand all other DNA-binding experiments that were investigated by several researchers exclude its direct binding to DNA. In an attempt to elucidate its cellular role, DNA-binding experiments took place under different conditions of different salts (concentration as well as different pH). DNA fragments, plasmids and chromosomal DNA from calf thymus gland were tested by using gel retardation assays as well as radiolabelled fragment DNA probes. Our results show that HP-NAP does not bind to DNA even when the protein was loaded with iron – as described previously [3] – in order to exclude any kind of loss of activity from the recombinant protein due to a possi-

ble misconformation. On the contrary, the protein exhibits ferroxidase activity in acid buffers pH 5 indicating a functional protein that indirect could protect DNA from the free radicals produced by the Fenton reaction. This property was further investigated and was confirmed by DNA protection assays.
In conclusion, our experiments are in agreement with similar studies concerning the Dps protein from *Agrobacterium tumefaciens* that does not bind DNA but protects it from oxidative cleavage.
This work was supported by the Operational program for Educational and Vocational Training II (EPEAEK II), and particularly the program HRAKLEITOS.

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014 INHIBITION OF POLY(PHE) SYNTHESIS BY ERYTHROMYCIN IN RIBOSOMES CONTAINING MUTANT L4 RIBOSOMAL PROTEIN FROM *THERMUS THERMOPHILUS*

Fotini Leontiadou,[‡] Georgios Papadopoulos,[§] Maria A. Xaplanteri,[II] Dimitra Triantafillidou,[‡] Dimitrios L. Kalpaxis[II*] and Theodora Choli-Papadopoulou[‡*]
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Protein L4 from the thermophilic bacterium *Thermus thermophilus* (TthL4) was heterologously overproduced in *Escherichia coli* cells and purified under native conditions in a good yield. In an attempt to study the implication of the extended loop of TthL4 in the exit tunnel function, the highly conserved Glu56 was replaced by Asp or Gln, while the semi-conserved Gly55 was changed to Glu or Ser. In addition, the sequence –Gly55Glu56- was inverted into –Glu55Gly56-. After incorporation of these mutants into *E. coli* ribosomes and investigation of their impact in poly(Phe) synthesis in the presence and in the absence of erythromycin, we observed high variations in the synthetic activity and response to the drug of the resulting ribosomes. Especially, hybrid ribosomes harboring mutations at position 55 of TthL4 were more sensitive against erythromycin. As revealed by molecular modeling this behavior could be attributed more to differences in the charge distribution between the mutants and less to their conformational differences.

015 PHOSPHORYLATION MOTIF-SPECIFIC ANTIBODY FOR ANALYSIS OF SIGNAL TRANSDUCTION

Yoshiro Chuman (1), Kanako Iizuka (1), Takeshi Honda (2), Hitoshi Onoue (3), Yasuyuki Shimohigashi (2), and Kazuyasu Sakaguchi (1)
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Phosphorylation and dephosphorylation play a central role in a variety of signal transduction pathways regulating cell cycle progression, development and differentiation. Antibodies specific for phosphorylation sites modified by protein kinases are an essential tool for studies of protein phosphorylation. In general, protein kinases recognize and phosphorylate specific sequences of substrate proteins to produce the corresponding "phosphorylation-motif". For instance, the Ser(P)-Gln motif is phosphorylated by DNA-PK, ATM, and ATR kinases, which are members of the PI-3 kinase family and recognize the Ser-Gln sequence in p53, CHK2 and c-Fos.
In this study, we developed a monoclonal antibody based on the "phosphorylation motif" for analysis of protein phosphorylation. We selected the Ser(P)-Gln phosphorylation motif for DNA-PK/ATM/ATR and prepared the hybridoma that produces the Ser(P)-Gln-specific antibody 3G9-H11. The monoclonal antibody only recognized phosphopeptides derived from p53 containing the Ser(P)-Gln sequence and did not react with unphosphorylated peptides or phosphopeptides with different phosphorylation motifs. . We also will report a novel method to detect the phosphorylated peptide, based on avidin-biotin interaction using an ELISA plate format with the monoclonal antibody.

016 USING TANDEM AFFINITY PURIFICATION METHOD TO REVEAL THE IDENTITIES OF HOST CELLULAR PROTEINS THAT BIND TO THE EBOLA ZAIRE VP35 PROTEIN

Carlos Coito(1), Olivia Perwitasari(1), Deb Diamond(1) and Michael G. Katze(1,2)
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We have developed an approach to identify host cellular proteins that bind to the Ebola VP35 protein of the subtype Zaire. Ebola is a virus from the Filoviridae family that induces severe clinical manifestations with a mortality rate of 70-90%. Identifying host protein partners of viral proteins is an appropriate first step in understanding the virus life cycle and virus pathogenesis. The technical approach that we developed is based on a tandem affinity purification (TAP) of VP35 complexed with host cell proteins expressed under native conditions. The TAP strategy combined with mass spectrometry (MS) allows for the identification of proteins interacting with VP35 without prior knowledge of the complex composition, activity, or function.
The VP35 is fused in its N terminal with two tags which allow for a rapid purification of protein complexes, using immunoglobulins- and calmodulin/Ca2+-beads successively. The protein complexes are released from the immunoglobulin-beads by TEV cleavage and from the calmodulin-beads by EGTA chelation of Ca2+. The elution sample obtained after EGTA chelation of Ca2+ is precipitated by 25% TCA, the proteins are resuspended in water and finally digested by trypsin before sent for MS analysis.
Using this TAP approach with cells transfected with a plasmid encoding for VP35 or a control empty plasmid, we have successfully obtained proteins binding specifically to VP35 and are currently using mass spectrometry to identify the binding partners. These results could contribute to a better understanding of the Ebola viral replication cycle and reveal new potential drug targets.

017 ROLE OF PHOSPHATE DEPENDANT GLUTAMINASE IN HEPATIC SUBCLINICAL ENCEPHALOPATY: A PROTEOMIC APPROACH

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Subclinical hepatic encephalopathy (SHE) is considerated as a situation where cirrhotic patients show several neuropsychological defects while their mental and neurological state is still normal in a systematic test. Due to its high prevalence and interference on life quality, SHE`s diagnostic is very important.
Oral glutamine challenge (OGC) causes an increase on ammonia plasma levels, probably due to intestinal glutaminase activity. Higher hyperammonemia occurs on cirrhotic patients. The OGC could be used as a diagnostic tool, but the molecular base of this test is still unknown.
The results obtained with this test could indicated different glutaminase activity. Ammonia, the best characterized toxin., is formed in intestinal lumen as a degradation product of amino acids and purins metabolism. One of the most important ways of intestinal ammonia production is glutamine deamination by enterocyte glutaminase. Moreover, glutamine metabolism is the main source of energy, amino acids and ammonia for the hepatic synthesis of pirimidinic nucleotides. Thus glutaminase activity is and important key on hepatic stability of cirrhotic patients.
On a previous study we measured phosphate dependant glutaminase (PAG) activity of enterocyte of SHE patients and controls. Glutaminase activity is increases on patients, as reported (Romero-Gómez et al. 2004). Nowadays we are studying PAG expression/modification during hepatic encephalopathy using intestinal samples from human and from a porto-caval shunted rat model using proteomic approaches.

018 MOLECULAR MECHANISMS UNDERLYING AGE-RELATED MACULAR DEGENERATION

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Age-related macular degeneration (AMD) is the most common cause of legal blindness in the elderly population in developed countries. Over a third of those over 75 years currently have some form of this disease. AMD is characterized by the breakdown of photoreceptor cells in the central (macular) portion of the retina responsible for high acuity vision. The identity of AMD susceptibility genes remains unclear but multiple environmental risk factors related to oxidative stress have been suggested to contribute to this slow, progressive disease. Our recent proteomic characterization of subretinal deposits known as drusen demon-

strated more carboxyethylpyrrole (CEP) protein modifications and crystallins in ocular tissues from AMD than normal human donors (2002 PNAS 99, 14682). CEP adducts are uniquely derived from oxidation of docosahexaenoate-containing lipids which are abundant in the retina. Light damaged rodent retina was also found to contain elevated CEP and crystallin (2003 Exp Eye Res 76, 131), as well as elevated levels of nitrotyrosine and argpyrimidine (2002 Mol Cell Proteomics 1, 293). More recently, we found CEP adducts induce new blood vessel growth *in vivo*, suggesting oxidative protein modifications may contribute to choroidal neovascularization in late stage AMD. CEP adducts and CEP autoantibodies are also elevated in the plasma of AMD donors, suggesting that they may have utility as biomarkers of AMD susceptibility (2003 J Biol Chem 278, 42027). Accordingly, we hypothesize that oxidative protein modifications serve as primary catalysts in AMD. A progress report in these ongoing studies will be presented.

019 SINGLE NEURON ANALYSIS USING CAPILLARY ELECTROPHORESIS WITH LASER INDUCED FLUORESCENCE (CE-LIF)

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Capillary electrophoresis was employed to analyse single mouse hippocampal neuron cells. Individual cells were lysed and proteins were denatured and labeled by fluorogenic reagent 3-(2-fluoryl) quinoline-2-carboxaldehyde (FQ) inside the capillary. Highly sensitive detection of proteins was then achieved by laser- induced fluorescence (LIF) in a post column sheath flow cuvette. Capillary zone electrophoresis (CZE), Capillary sieving electrophoresis (CSE) and micellar electrokinetic capillary chromatography (MECC) were used to generate one dimensional protein electropherograms. Approximately 30-50 components were resolved in each case within 30 minutes. Protein expression varied significantly between cells as expected. Hippocampal neurons are rich in cannabinoid receptor (CB-1) and can interact with cannabinoids, the primary psychoactive constituent of marijuana. Δ^9-tetrahydrocannabinol induced changes in protein expression was also studied in neurons and a mouse tumor cell line, AtT-20, transfected with rat CB-1 receptor. Because of the limited resolution in one-dimensional capillary electrophoresis, changes in differential protein profiles were hard to follow. Given the superior resolution power of multidimensional separation techniques, further investigation using a fully automated comprehensive two dimensional (2D) capillary electrophoresis system is underway.

020 PROTEIN IDENTIFICATION AND CHARACTERIZATION BY DIAGONAL REVERSE-PHASE CHROMATOGRAPHY

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021 THE BINDING ENERGY OF CENTRAL HYDROGEN BONDS BETWEEN PEPTIDE AND MHC CLASS II MOLECULES

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The biological activity of major histocompatibilty complex (MHC II) molecules is dependent upon the MHC II protein's ability to stably bind a wide variety of peptides. The peptides are bound by interactions between peptide side-chain and MHC II pockets and by an array of hydrogen bonds from conserved MHC II protein side-chains to the peptide backbone. The objective of this study was to determine the binding energy of the peptide-MHC hydrogen bonds at the center of the peptide-binding groove. A kinetic assay was used to measure dissociation half-lifes of complexes formed between different fluorescein-labeled influenza hemagglutinin (HA) 128-136 peptides and the murine I-A^d MHC II protein. Peptides were structurally modified to disrupt the central hydrogen bonds and the b-74 Glu residue of I-A^d that forms a hydrogen bond to the amide NH of the HA Thr-132 was mutated to an Asp to disrupt that bond. It was found that disruption of either one of the bonds causes only about a 1-2 fold in-

crease in the peptide dissociation rate. These results indicate that the contribution of the central hydrogen bonds to binding stability is significantly less than those at either end of the peptide-binding groove. In addition it was found that there is very little cooperativity between the central hydrogen bond and adjacent pocket interactions. Also, the dissociation rate for an ester peptide bound to the b-74 Asp I-A^d protein was increased by about 3.4-fold relative to native protein suggesting that there is some cooperativity between the central hydrogen bonds. However, the central hydrogen bond interactions were found to be uncoupled from the same interactions at either peptide terminus. These results have several implications for the mechanism of HLA-DM function and the design of peptidomimetics for immunotherapeutic uses.

022 SOLUTION-PHASE ISOELECTRIC FOCUSING ENHANCES THE DETECTION OF LOW ABUNDANT PROTEINS IN HUMAN PLASMA SEPARATED BY 2D GEL ELECTROPHORESIS

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The Multi-Compartment Electrolyser (MCE) is a solution phase isoelectric focusing apparatus that uses membranes of a defined pH to fractionate complex protein samples into pH-defined subsets. When the MCE is used in combination with narrow range immobilized pH gradients (IPGs), greater resolution can be achieved at higher protein loads, when compared with un-fractionated material. MCE fractionation subsequently minimizes two-dimensional electrophoresis artifacts such as streaking, distortion and loss of protein spots within the array while enhancing the ability for low abundant proteins to be visualized and subsequently identified.
MCE fractionation was used to fractionate triple depleted Human plasma into three pH defined subsets. Each fraction was applied onto narrow, 3 pH unit gradients at a higher effective loading than achievable when using un-fractionated material. The quality of two-dimensional electrophoresis achieved using the MCE fractionation technique was demonstrated by the isolation of an additional 30-40% more proteins from the extreme acidic and alkaline pHs of depleted Human plasma compared to that obtained from un-fractionated material. Mass spectrometric protein identifications were obtained for some of these previously unseen protein spots.
The results from the separation of depleted Human plasma verify that MCE fractionation is a valuable tool for the separation of complex mixtures enhancing the detection of low abundant proteins and enabling a more thorough understanding of valuable samples.

023 ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY-MS(TOF): NEW SEPARATIONS TECHNOLOGY FOR HIGH THROUGHPUT METABONOMICS

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024 ALLOSTERIC PROTEIN-PROTEIN INTERACTION AND AMMONIA TUNNELING ACROSS A (BETA / ALPHA) 8 BARREL ENZYME

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The (beta / alpha) 8 barrel fold consists of a central eight-stranded parallel beta-barrel which is surrounded by eight alpha-helices. This fold is the most frequently fold in the Protein Data Bank (PDB) and is widespread in various enzyme classes.
The imidazole glycerol phosphate synthase is involved in the biosynthesis of the amino acid histidine and consists of the (beta / alpha) 8 barrel domain HisF and a glutaminase domain HisH. The enzyme belongs to the family of glutamine amidotransferases (GATases), which are suitable models to study protein-protein interactions, because they constitute a class of bi-enzymes that play key roles in metabolic pathways.
The imidazole glycerol phosphate synthase from the hyperthermophilic organism *Thermotoga maritima* had been investigated intensively. It appeared that the activity of the glutaminase domain HisH is stimulated by substrate-binding to the active site of HisF. As a consequence, glutamine is hydrolysed to glutamate and ammonia, which is transferred through the inner core of the (beta / alpha) 8 barrel towards the HisF active site via a 25A long intermolecular tunnel. Amino acid exchanges were introduced at the protein-protein interface impeding the stimulation of HisH by HisF.
The obtained results are discussed on the basis of the known X-ray structures of the HisH:HisF complex.

025 CIRCADIAN RHYTHM EFFECTS ON THE HUMAN PAROTID PEPTIDE REPERTOIRE SHOWN BY USING NOVEL iTRAQ™ REAGENTS

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Changes in salivary composition correlate with disease susceptibility and/or state (*e.g.*, oral candidiasis). Hence, human saliva is an interesting source of novel diagnostic markers and therapeutic targets. However, the secretion of salivary proteins follows gland-specific circadian rhythms, complicating the accurate quantitation of individual biomarkers. Recently, we identified a suite of novel peptides in the low-molecular-weight (LMW) fraction of human parotid saliva. The majority of peptides appear to have been derived from larger precursors. Previous publications reported the effects of circadian rhythm on protein levels and specific protein/peptide families. Here we used a novel iTRAQ™ chemistry to investigate, on a global level, diurnal variations in the composition of the LMW parotid saliva fraction. The samples were collected following gustatory stimulation, under conditions that minimized proteolysis, at four time points (9 am, 12 pm, 3 pm and 6 pm). Those obtained at the same time were pooled and the LMW fractions (<10 kDa) were isolated. Each of the four samples was derivatized with a different isobaric iTRAQ™ reagent (via primary amino groups). Then the differentially labeled samples were combined for analysis. Specifically, peptides were separated by reversed phase HPLC, co-spotted with matrix on MALDI targets and analyzed by MALDI-TOF/TOF mass spectrometry. By using this novel approach, we quantitated many of the parotid peptides. In several cases the relative concentration changed dramatically depending on the collection time. Furthermore, iTRAQ™-tagging improved the efficiency of MS/MS fragmentation, which in turn, allowed the identification of several novel peptides. Our results demonstrated that the repertoire of native peptides in parotid saliva showed considerable dependence on circadian rhythm. Future research on salivary biomarkers should take this confounding variable into consideration.

026 HIGH-THROUGHPUT DETECTION OF PROTEINS INTERACTED WITH GEL-RESOLVED PROTEINS USING NOVEL PROTEIN-CHIP AND MASS SPECTROMETRY

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Since proteins interact with other proteins to perform their particular cellular task, analysis of the protein-protein interaction is important to determine the function of proteins. Protein-protein interaction has been analyzed by several methods such as yeast two-hybrid system, immunoaffinity purification technique and protein chip. Among them, protein chip is considered one of the most promising tools for high-throughput analysis of protein-protein interaction. In conventional technique, proteins expressed by DNA (RNA) or natural proteins have been purified to immobilize on the chemically modified chip plates. However, purification of proteins is laborious and time-consuming, and purification of a number of proteins is often impossible. This is a limiting factor to perform the high-throughput analysis of protein-protein interaction. We developed a technique for high-throughput analysis of the interaction using a novel protein chip plate (diamond-like carbon coated (DLC) stainless steel plate). In this technique, proteins are separated by gel electrophoresis, and electroblotted onto the DLC plate, of which surface is modified with N-hydroxysuccinimide ester, to produce a high-density of protein chip. Proteins in the gels can be immobilized covalently on the DLC plate with high blotting efficiency (30-70 %). Proteins extracted from the cells were probed with the proteins on the DLC plate, and the interacted proteins were detected by mass spectrometry such as matrix assisted laser desorption ionization time-of-flight mass spectrometry using the DLC plate as a MALDI sample target. This technique has a great potential of high-throughput analysis of proteins interacted with thousands of proteins separated by two-dimensional gel electrophoresis.

027 BINDING SPECIFICITY OF MULTIPROTEIN SIGNALING COMPLEXES IS DETERMINED BY BOTH COOPERATIVE INTERACTIONS AND AFFINITY PREFERENCES

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028 CONVENIENT AND REPRODUCIBLE PROTEIN SAMPLE PREPARATION: A KEY STEP IN PROTEOME ANALYSIS

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Many proteins of pathophysiological importance are present in low abundance; to detect and characterize these proteins sample preparation methods are needed that fractionate complex matrices and concentrate samples containing proteins of potential interest. We have developed a series of integrated kits that specifically address these challenges, including: 1) the specific removal of abundant but unwanted proteins, 2) pre-fractionation by subcellular compartment, 3) stepwise extraction of the proteome, and 4) protein concentration. We will present data to demonstrate that samples obtained from these kits are highly reproducible and suitable for subsequent analysis by 1-DE and 2-DE gel electrophoresis, mass spectrometry, and other methods.

029 IDENTIFICATION OF INCOMPLETE PYROGLUTAMATE FORMATION IN THE HEAVY CHAIN OF A HUMAN ANTIBODY

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The therapeutic potential of antibodies in the treatment of diseases has been well documented. Several antibodies have already been approved for human use and others are currently in clinical trial for safety and efficacy evaluations. At Amgen, various therapeutic antibodies for unmet medical indications are actively being pursued. Because of their complex nature, structural characterization of antibody molecules has always been an analytical challenge. We describe here an example of antibody analysis in an early development stage. In one large-scale preparation when the cell culture conditions were not optimized, approximately 10% of the protein was observed to elute after the main peak from a cation-exchange column. Analysis of this "late" peak was performed using both Edman sequencing and mass spectrometry. Examination of the glycopeptide generated after tryptic proteolysis showed that the unusual chromatographic behavior was not due to glycosylation differences. A careful comparison between the tryptic maps of the main peak and the late peak shows the presence of a unique peptide only in the latter. Upon characterization, the peptide was derived from the N-terminus of the heavy chain. It was unusual that this peptide began with glutamine instead of the expected pyroglutamate. In subsequent fermentations when the cell culture conditions have been optimized, the amount of material corresponding to this late peak was reduced to approximately 4%.

030 GLOBAL PROTEOME ANALYSIS OF THE HUH-7.5 REPLICON SYSTEM AND APPLICATION TO HCV INFECTED LIVER BIOPSY SAMPLES

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The development of a reproducible model system for the study of Hepatitis C virus (HCV) infection has the potential to signifi-

cantly enhance the study of virus-host interactions within the cell and provide future direction for modeling the pathogenesis of HCV. While there are many *in vitro* and *in vivo* studies describing global gene expression changes associated with HCV infection, high throughput characterization of changes in the proteome remains to be described. Here we report the first large scale proteome analysis of the highly permissive Huh-7.5 cell line containing a full length HCV replicon. We have detected >4,500 proteins, including 7 out of the 10 HCV replicon proteins, using offline strong cation exchange tryptic peptide separations, combined with high resolution, reversed phase capillary liquid chromatography (LC) coupled to electrospray ionization (ESI)-tandem MS (MS/MS) analysis. Consistent with several reports in the literature describing HCV-associated protein disturbances, a comparison of Huh-7.5 cells (+) and (-) HCV replicon identified many potential protein expression changes of interest. These findings demonstrate the utility of multidimensional proteome analysis of the HCV replicon model system for comparative studies aimed at identifying protein expression signatures of liver disease. Moreover, the protein database generated here will assist efforts to extend these analyses to liver biopsy material from HCV-infected patients (a true model of HCV infection) using the accurate mass and time (AMT) tag approach. Preliminary results demonstrate marked sensitivity using the AMT tag strategy for analysis of these extremely small samples (>1,500 proteins detected from 1 μg protein lysate) and efforts are now underway to identify useful protein biomarkers of HCV-associated liver disease using both the *in vitro* and *in vivo* models.

031 SOLVING COMPLEX DISULPHIDE BONDING PATTERNS

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Recombinant human Mannan Binding Lectin (rhMBL) is a complex protein belonging to the native immune system. The active form of rhMBL consists of subunits formed, from three polypeptide chains, via a collagen-like region. These subunits further assemble into higher oligomer forms via a complex disulphide pattern between three cysteines in the N-terminal region of each polypeptide chain. The disulphide pattern consists of both inter- and intra-subunit bonds.
In order to solve this disulphide pattern a large array of classical protein chemistry techniques combined with MALDI MS and MS/MS were employed. The main challenge arose in part from the three cysteines being separated by only 5 and 6 amino acids, and in part from the complexity of all the identical polypeptide chains in each oligomer. Several proteolytic digests in combination with HPLC and MALDI MS led to the isolation and identification of different disulphide linked peptides. Further sub-digestion and MALDI MS/MS resulted in the identification of the involved cysteines. Free cysteines were identified after alkylation of the native protein, followed by a similar procedure as described above. Based on the identified "building blocks" a model for the disulphide-bonding pattern was proposed.
In order to verify the model, a number of Cys to Ser mutations of the three cysteines have been made.

032 HIGH QUALITY PROTEIN PURIFICATION FOR SUBSEQUENT STRUCTURAL ANALYSIS

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Protein purification combines innovation and technology together to create a process that to the best practitioners is a scientific art form. Protein purification is central to many technologies that are transforming the way we study cellular processes and mechanisms of disease. However, as knowledge of protein functionality continues to increase, significant improvements to the field of protein purification appear to have slowed in recent years. A successful purification procedure can be an amazing accomplishment. Whether one is starting with a recombinant protein, which is produced in *E. coli*, or trying to isolate a protein from some mammalian tissue, we are typically starting with gram quantities of a complex mixture of protein, nucleic acids, polysaccharide, etc. From this mixture we may have to extract milligram (or microgram) quantities of the desired protein at high purity, with high yield and ideally in an active state. Proteins vary in size, shape, charge, hydrophobicity, solubility, and biological activity. To purify proteins, ProteinOne, of College Park, MD utilizes these inherent similarities and differences.
ProteinOne is a development stage biotechnology company whose primary aim is to deliver highly purified, biologically active proteins to research labs, medical institutions and pharmaceutical companies. Our protein purification strategy is centered on utilizing protein tags like 6-histidine, FLAG GST, maltose myc, to increase our ability to purify the proteins to near homogeneity upon initial purification, followed by ion-exchange, hydrophobic interactions or size-exclusion conventional, fast performance liquid or high-pressure liquid chromatography. ProteinOne is capable of producing proteins from different categories, different systems (bacteria, insect and mammalian) and from milligram to gram quantities. This high quality protein purification approach is vital for the accuracy of the subsequent structural analysis.

033 NANOLC/MS IN QUANTITATIVE AND QUALITATIVE PROTEOMICS

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Advances in instrumentation have become one of the most significant driving forces in advancing proteomics analyses. Among the many improvements is the widespread adaptation of nanoscale liquid chromatography (nanoLC) where the increase in MS sensitivity obtained in microscale separations is often critical for the analysis of complex proteomic samples. Here we describe a design for nanoLC/MS that overcomes serious reproducibility problems associated with traditional split flow solvent delivery systems. Specified flow rates in the range of 200 – 5000 nl/min are compatible with 75 – 320 um ID columns, and by avoiding stream splitting, provides reliable, reproducible results. Separations with either simple peptide mixtures or digests of complex cellular extracts or biofluids can be accomplished with component retention times varying less than 10 seconds (typical standard deviations < 6 seconds) over extended sample sets. Examples with gradient times ranging from 30 – 120 minutes will be shown. This performance is critical for comparing samples quantitatively, and ensuring that differences amongst samples and controls are not an artifact of the separation step. System components have also been designed to operate with new small particle columns that require careful control of system bandspread to limit extra-column effects that can cause excessive peak broadening. Comparisons of complex mixture analysis performed on 3.0 and 1.7 um particle columns shows that separations with the smaller particle columns yield peak widths half of the large particle ones. The enhanced peak capacity attained is highly beneficial in the analysis of complex sample mixtures, simplifying the interpretation of MS and MS/MS data, and the reduced peak volumes also result in significantly greater peak response, routinely achieving low femtomole to attomole sensitivity on 75 um ID columns. Both quantitative and qualitative comparisons of complex extract digests show clear advantages stemming from the increases in resolution and reproducibility achieved, and are eminently suited for biomarker identification studies.

034 A PARALLEL APPROACH TO POST SOURCE DECAY MALDI-TOF ANALYSIS

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The analysis of compounds with post source decay (PSD) is an established technique, which is capable of providing complementary structural information. In practice, a timed electrostatic ion gate is utilized to isolate a single precursor mass and it's associated fragments, resulting in a serial data acquisition and thus a long acquisition time and increased sample consumption. Additionally, low mass, and hence low kinetic energy fragments, do not fully penetrate the reflectron of a MALDI-TOF instrument and are not correctly focused onto the detector. This problem is alleviated by successively reducing the reflectron voltage in steps and acquiring additional data for each step. The focused regions of the resultant spectra may then be "stitched" together to form a complete spectrum. We present a novel enhancement to PSD analysis by simultaneously acquiring PSD fragments from all parent ions. Fragment ions are correctly matched to their corresponding precursor ion by acquiring an additional spectrum for each conventional reflectron step at a slightly lower reflectron voltage. By measuring the difference in TOF between the two spectra for each fragment it is possible to calculate the mass of the parent. This new "parallel PSD" technique consequently reduces analysis time and consumes less sample than conventional PSD. To demonstrate the application of parallel PSD in protein identification, an MS spectrum from 50fmol of a tryptic digest of Alcohol-Dehydrogenase (ADH) was initially acquired from a single sample well. Subsequently, PSD data was acquired at 12 different reflectron steps. The 20 most intense peaks from the MS spectrum were considered as candidate parents and a data processing algorithm calculated a list of fragment masses associated for each parent. The results were searched against the SWISSPROT protein database and led to the correct identification of 10 peptides, 9 from ADH1 and 1 from ADH2, based solely upon the fragment ion data.

035 IDENTIFICATION OF A NOVEL PUTATIVE PROTEIN-PROTEIN INTERACTION SITE ON THE OESTROGEN RECEPTOR

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The oestrogen receptor (ER) functions as a ligand-activated transcription factor and is responsible for mediating the physiological effects of the steroid hormone oestrogen. Interactions between ER and components of transcriptional machinery play an important part in the molecular processes that culminate in target gene activation. The screening of random peptide libraries using phage display techniques is a useful tool for the identification of biologically-significant interaction sites on proteins and for probing different conformational states. Here we describe the structural and functional characterization of an ER α-isoform-

specific peptide that acts a receptor antagonist.
We have determined the crystal structure of ERα ligand-binding domain (LBD) in complex with the peptide at 2Å resolution. The motif (LTSDRFGSWYA) binds to a novel hydrophobic surface and adopts a partially helical conformation. The binding site is distinct from the classical co-regulator recruitment /AF2 site. Peptide binding is mediated by an extensive network of interactions. The location and architecture of the binding site provides a molecular explanation for the peptide's receptor isoform and ligand interaction preferences.
In addition, we have analysed the binding kinetics using surface plasmon resonance. Binding is observed to ERα in the presence of all classes of receptor ligand but not to the closely-related ERβ isoform. The peptide binds with moderate affinity (K_D 20μM) and the interaction is highly sequence-dependent. Site-directed mutagenesis has been used to validate the binding site observed in the crystal structure and provides an insight into the observed isoform specificity.
In summary, we have used structural and biophysical methods to characterize a novel interaction site on the surface of the oestrogen receptor.

036 AN INTEGRATED WORKFLOW FOR INTERPRETATION OF SHOTGUN PROTEOMICS DATA

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037 PROTEOMIC ANALYSIS OF BARRETT'S ESOPHAGUS USING CAPILLARY ELECTROPHORESIS WITH LASER-INDUCED FLUORESCENCE

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038 OLIGOSACCHARIDES PROVIDED BY HUMAN MILK GLYCO-PROTEINS FOR IMMUNE PROTECTION OF INFANTS

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The milk fat globular membrane (MFGM) glycoproteins are thought to act as specific bacterial and viral ligands, which contribute to the prevention of infection by pathogenic organisms in the stomach of infants. The MFGM proteins from a variety of species including humans, cows and other domestic animals that may supply milk for human infant consumption were studied. Major differences were found in size, type and glycosylation of the high molecular weight milk mucins. Separation of these milk proteins by composite 1D-AgPAGE, followed by MS and Western blot analysis confirmed the presence of both MUC1 and MUC4 in the human MFGM at approximately 1 MDa. Bovine MUC1 was also detected, however it appeared at approximately 200 kDa and showed significantly different glycosylation compared to that of the human mucins. Core 2 type structures of the *O*-linked oligosaccharides that enable the presentation of Lewis a/x and Lewis b/y oligosaccharide epitopes on the glycoproteins were found to be present in large quantities in human MFGM and in very small amounts in cows, sheep, goats and pigs. The presence of the Lewis a and Lewis b epitopes on human milk mucins were confirmed by MS^2 and Western blot analysis techniques. An interesting exception to this general rule was that bovine colostrum contained core 2 type *O*-linked oligosaccharide structures similar to those found in the human MFGM but without further elongation with a Lewis epitope. The content of this sugar structure decreased with the age of the bovine colostrum, with only a trace amount present in late lactation cows' milk. Since the Lewis b epitope has been implicated in the binding of *Helicobacter Pylori* (and potentially other pathogens) to the gastric mucosa, this suggests a possible innate immune advantage of breast-feeding human infants.
*This work was the product of a collaboration with the Nestle Research Centre.

039 A NOVEL GEOMETRY-BASED SCORING FUNCTION FOR DOCKING ENZYME-INHIBITOR COMPLEXES

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Incorporating biochemical and biophysical knowledge of a molecular complex into its docking procedure has been shown to be an effective approach. For enzyme-inhibitor complexes, their convex-concave features are ideal descriptors, but have not been effectively incorporated into docking procedures. Here we explore the application of this geometric feature in the selection of docking candidates. Twenty two enzyme-inhibitor targets were taken from a benchmark set. FTDOCK was used to generate 30,000 candidates for each target (10,000 each from three independent runs). We used center-to-center distance, the concaveness of the receptor interface, the convexness of the ligand interface, the sampling frequency at the receptor interface, the number of residues at the receptor and ligand interface, and the number of unstructured residues at the interface, as well as RPSCORE to filter out "bad" candidates. This resulted in approximately an 11 fold reduction of the candidate space. Five of these geometric descriptors were further combined into GEOSCORE to rank the filtered candidate set. Thirty-three top candidates from each of the three independent runs were combined to form a final prediction set of ~100 top candidates. Although the number of hits (ligand RMS<10Å) was very small comparing to the large number of candidates (the median number of hits before and after filtering was 46 and 29), GEOSCORE was able to retain 1-68 hits among the final candidates for 19 of the 22 targets. In contrast, RPSCORE failed to identify any hits for 9 of the 22 targets. The performance can be further enhanced by combining GEOSCORE and RPSCORE. The combined scoring function successfully identified hits for 20 of the 22 targets, and the number of hits was improved for several targets. At least one of the highest ranking candidates from the three runs was a hit in 12 targets. The performance by this geometry-based scoring function is comparable to those by elaborate energy-based scoring functions.

040 INTEGRATING SURFACE PLASMON RESONANCE WITH MASS SPECTROMETRY: OBSERVE INTERACTIONS AND IDENTIFY THE BINDING PARTNERS

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In light of the recent developments in functional proteomics, the cell environment is emerging as a complex network of interacting biomolecules. Elucidation of the composition of these complexes will be essential to the determination of the complete structural and functional proteome. The aim of the present study is to utilize the integration of surface plasmon resonance (SPR) and mass spectrometry (MS) to enable biomolecular analysis and identification of a macromolecular complex. The Biacore 3000 SPR biosensor was employed in a ligand fishing experiment to identify binding partners of a 20-mer kinase fragment. The kinase fragment was immobilized to a gold sensor chip surface, and bovine brain lysate was passed over the immobilized protein. On-target digestion and recovery was performed and MALDI-ToF MS confirmed the identity of a single bound protein as calmodulin. This study demonstrates the powerful integration of SPR with mass spectroscopy as a means of rapid and confident identification of unknown binding partners of a protein complex.

041 FULLY AUTOMATED MULTI-COLUMN HPLC SYSTEM FOR HIGH THROUGHPUT PROTEOMIC ANALYSIS

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A novel technique for column switching has been implemented on a custom-built LC system in an effort to increase throughput of LC/MS analyses. Four analytical columns (10 cm x 50 um ID packed with 3 um diameter particles) are mounted on two translation stages that are positioned using stepper motors which allow each column to be precisely aligned with the entrance of a mass spectrometer successively. The operation of this multi-column system is fully automated. Compared to a conventional single column system, this technique increases throughput by overlapping the processing of different columns. That is, while one column is used for sample analysis, the remaining three columns can be regenerated for subsequent sample analyses. The LC method employs a short gradient time of 30 minutes and as such produces rapid, rather than detailed, chromatographic

analyses. A Waters Micromass Q-Tof Ultima is used for MS analysis and initial evaluation of the system using bovine serum albumin (BSA) shows good repeatability from each LC column. Once this LC/MS system is operational it will be dedicated mainly to high-throughput processing of tryptically digested samples of protein complexes as part of a Genomes-to-Life initiative being pursued in collaboration with other national laboratories.

042 A NOVEL METHOD TO QUANTIFY SERUM BIOMARKERS VIA VISIBLE ISOTOPE-CODED AFFINITY TAGS AND TANDEM MASS SPECTROMETRY

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043 A NEW LYSYLENDOPEPTIDASE FROM *LYSOBACTER* SP.

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*Achromobacte*r protease I (API) is a lysylendopeptidase of chymotrypsin type, and has been successfully used to fragment peptide chains at the lysyl bond. So far we have searched for a bacterial source with high lysylendopeptidase production and recently isolated a *Lysobacter* strain that efficiently (6-fold) produces a lysylendopeptidase named LepA. Sequence comparison revealed that this enzyme is API. In addition, we have found another protease gene upstream from the LepA gene (*lepA*). Though producing no detectable protein, the sequences of this latent gene (*lepB*) and its putative translation product were compared with the counterparts of *lepA* and LepA, which strongly suggested that the *lepB* sequence codes for an inactive pre-pro protease (LepB), having all essential amino acids for LepA, with a single 678-residue peptide chain with 72% sequence identity. The cloned *lepB* was then expressed in *E. coli*, and active LepB was obtained through appropriate refolding. The purified enzyme is a mature 274-residue lysylendopeptidase and is less active and more sensitive to temperature and conventional denaturants, respectively, than LepA (268 residues). LepA-based modeling implicates that LepB is possible to fold to essentially the same 3D structure as LepA by placing a peptide segment, composed of several amino acids inserted into the middle of the LepB peptide chain, outside the molecule and that the Tyr169 side chain occupies the site that the indole ring of Trp169, a built-in modulator for unique endopeptidase function in LepA, sits. The results suggest that LepB bears a quite similar tertiary structure to LepA except a hump made of an inserted peptide segment and is a starting material for engineering this lysylendopeptidase.

044 THERMODYNAMIC ANALYSIS OF CROSS-SPECIES NKG2D-LIGAND INTERACTIONS: HEAT CAPACITY AND LIGAND FLEXIBILITY

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The immunoreceptor NKG2D initiates cytotoxic or co-stimulatory immune responses against cells bearing suitable protein ligands. Although these ligands have similar domain structures, the surfaces of interaction with NKG2D are highly dissimilar in terms of hydrophobicity, charge distribution, and flexibility. Previous studies have associated areas of observed crystallographic disorder with variations in observed heat capacities of binding in human NKG2D interacting with human ligands and mouse NKG2D with a mouse ligand. Here we extend those studies to mouse NKG2D interacting with three human ligands to associate the observed heat capacity of interaction with ligand flexibility in a case where the receptor differs. Mouse NKG2D interacts with reduced affinity with the human MIC-A and MIC-B ligands, but its interaction with ULBP1 is among the strongest and slowest NK receptor-ligand interactions measured. Additionally, human NKG2D does not bind the mouse ligand, suggesting inflexibility on the part of the receptor but some ligand-associated flexibility. These studies lay the groundwork for our redesign and stabilization of a flexible region in the MIC-A ligand to associate more strongly with NKG2D receptors. [This abstract is supported by a grant from the National Institutes of Health.]

045 C-TERMINAL SEQUENCING METHOD: SUCCESSIVE TRUNCATION REACTION AND ALGORITHM DEDICATED TO ANALYZING MASS SPECTRUM OF THE REACTION PRODUCTS

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We have developed a C-terminal amino acid sequencing method for peptides and proteins based on successive truncation reaction by acetic anhydride. We also have constructed an algorithm for analyzing mass spectra obtained from the reaction products. In the algorithm, the specific side reaction products were utilized to elucidate sequence information from the complicated signals. The C-terminal sequencing method consists of three simple chemistries as follows; (1) acetylation by acetic anhydride, (2) C-terminal amino acid truncation by acetic anhydride with a trace of perfluoric acid and (3) hydration with dimethylaminoethanol aqueous solution. Acetylation and hydration reaction make mass spectra so clear that translation of mass spectra in amino acid sequence becomes easier. In the presented algorithm, acetylated and dehydrated products ions occurring in occasion were assigned as mass shifted peaks respectively and they were used effectively in calculating mass of each truncated amino acid. In case of protein analysis, tryptic digestion was used to obtain C-terminal fragments from the reacted protein and the digests were subjected to MALDI-TOF MS analysis. Negative ions carry C-terminal fragments information rather than positive ones. The truncation reaction was successfully introduced to proteins in polyacrylamide gel with use of acetic anhydride formamide solution. The reaction products were digested with trypsin in-gel and the extracts were subjected to MS. Standard proteins, such as horse skeletal muscle myoglobin, horse heart cytochrome c, soybean trypsin inhibitor, etc, were applied to this analysis and yielded several C-terminal truncated fragments.

046 ISOTOPIC LABELING STRATEGIES FOR QUANTIFYING PHOSPHORYLATION SITE STOICHIOMETRIES

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Protein phosphorylation is a common posttranslational modification that occurs in response to stimuli, affecting protein structure and leading to changes in cell-cycle control, signal transduction and protein-protein interactions. Although determining each of a protein's phosphorylation sites is an important first step, phosphorylation and dephosphorylation are dynamic processes and can occur at multiple sites in varying degrees. In order to characterize protein phosphorylation in a biological context it is therefore desirable to extract quantitative information with respect to changes in phosphorylation. Several approaches to this problem have been reported, including phosphopeptide-specific antibodies and mass spectrometric techniques using isotope labels: AQUA, SILAC, PhIAT and others.

We describe a stable isotope labeling strategy in which peptides are guanidinated and n-terminally labeled via reductive methylation. The use of deuterated formaldehyde in the methylation reaction produces a 4 Da mass shift relative to the undeuterated version. Phosphoproteins isolated from cells in different states (e.g. stimulated vs. resting) were digested, labeled with heavy and light reagents, and mixed prior to mass spectrometry. Phosphorylated and non-phosphorylated peptides are quantified simultaneously in MS or MS/MS mode. The same reactions were also applied to a recently reported approach whereby phosphopeptide-containing samples are split, labeled with heavy and light reagent, and one aliquot dephosphorylated[1]. Upon mixing and mass spectrometry phosphorylation site stoichiometry can then be determined without direct detection of the phosphorylated species. Quantitation with our n-terminal labeling technique has thus far involved phosphorylated samples, but should be equally applicable to other posttranslational modifications.

[1]*Hegeman et al. JASMS 2004: 15, 647-653.*

047 A QUANTUM MECHANICAL APPROACH TO PROTEIN FOLDING LANDSCAPES

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A promising approach to developing improved potential functions for modeling macromolecular interactions consists of combining protein structural analysis, quantum mechanical calculations on small molecule models, and molecular mechanics potential decomposition. Here we use this approach to investigate the orientation and distance dependence of hydrogen bonds in proteins. We find a remarkable agreement between the energy landscapes obtained from the electronic structure calculations, and

the distributions of hydrogen bond geometries observed in protein structures. In contrast, molecular mechanics force fields commonly used for biomolecular simulations do not exhibit close correspondence to either quantum mechanical calculations or experimentally observed hydrogen bonding geometries. However, we find reasonable qualitative agreement between molecular mechanics and quantum chemistry calculations carried out for pairs of ring-containing amino acids in proteins. This agreement holds both over one-dimensional projections of the binding free energy landscape for amino acid homodimers, and over a set of homodimers and heterodimers from experimentally observed protein crystal structures. Thus, unlike hydrogen bonds, interactions involving aromatic residues and proline can be fairly well modeled using current molecular mechanics force fields.
Our results suggest a promising route to improved energy functions suitable for modeling biological macromolecules, which combines the generality of quantum mechanical calculations and the accurate context dependence of the protein structural analysis.

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048 STRUCTURAL CHANGES ASSOCIATED WITH BINDING OF TWO CYTOPLASMIC DYNEIN SUBUNITS, IC74 AND LC8

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The N-terminal domain (IC^{1-289}) of IC74, the intermediate chain subunit of the multisubunit motor protein, cytoplasmic dynein is largely unstructured but undergoes an unstructured-to-structured transition upon binding LC8, the 10kDa light chain subunit. We have used a number of techniques to characterize the structural changes of IC^{1-289} upon binding LC8 and compared these changes to the changes of IC^{1-289} in TFE and TMAO, two organic solvents known to mimic the hydrophobic environment of interacting proteins. The structural changes of IC^{1-289} in the organic solvents were similar to what was observed with LC8. We further characterized these interactions by mapping the region that gained structure upon LC8 binding to residues C-terminus of the N-terminal domain. This region includes a segment that has a propensity to form an α-helix with possible supercoiling.
To facilitate tertiary structural studies and gain insight into the residues that are involved in the disorder-to-order transition we overexpressed in *E.Coli* an isotopically labeled construct of this segment of IC74. Using a combination of two and three-dimensional NMR experiments we obtained backbone assignments and identified the residues in the intermediate chain of cytoplasmic dynein that are significantly perturbed upon LC8 binding.

049 STRUCTURE, MOLECULAR MECHANISM AND EDITING ROLE OF WRN EXONUCLEASE, IN DNA DOUBLE-STRAND BREAK REPAIR

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Werner Syndrome is a rare progeroid disorder that is characterized by genomic instability and greatly increased cancer incidence. Werner syndrome is caused by mutations in the *WRN* gene, which encodes an exonuclease/helicase with unresolved function. Here, we show that the WRN exonuclease structure belongs to the DnaQ family, closely resembling the *E. coli* klenow fragment exonuclease. Similar to other DnaQ proteins, WRN exonuclease metal ion complexes reveal a two-metal ion mediated mechanism of nucleic acid degradation. WRN exonuclease domain has a distinct role among its structural homologues, as its inactivation alters DNA end-joining process *in vivo*. Thus, WRN exonuclease represents the first structural information on an editing module in double-strand break repair.

050 AMYLOID-LIKE FIBRIL FORMATION OF MUTANT p53 TETRAMER PEPTIDE AT PHYSIOLOGICAL pH AND TEMPERATURE

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051 TISSUE-SPECIFIC PROTEIN PROFILING

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The vascular bundle is a tissue system of vital importance for higher plants. It appears to have much more functions than only the transport of water and metabolites, since also proteins, nucleic acids and signal substances are transported. A powerful method for contamination-free collection of specific cell types from tissue sections is Laser Microdissection (LM) coupled to Laser Pressure Catapulting (LPC). This technique has been routinely used in mammals and is just starting to spread in plant sciences. To demonstrate the applicability of LMPC for protein analysis, we collected samples from different stem tissues from Arabidopsis thaliana. One strategy we used for the analysis of proteins was separation of intact proteins by classical two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) coupled to mass spectrometry. A drawback of 2D-PAGE for small sample amounts is the relatively high amount of material needed. Regarding the limited sample amount obtainable by microdissection, the combination of protease digest of the complex protein mixtures with HPLC separation and subsequent tandem mass spectrometry (LC/MS/MS) provides an alternative, promising tool for tissue specific proteome analysis. Additionally, we have optimized our sample preparation procedures to allow not only proteome analysis but also tissue-specific metabolite and transcript profiling in microdissected samples.

052 DEVELOPMENT OF A CE-DIGESTION-CE-MS SYSTEM FOR PROTEIN AND PEPTIDE ANALYSIS

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Most capillary electrophoresis (CE) or liquid chromatography proteomics analyses to date separate complex peptide mixtures.[1] The described instrument aims to separate proteins in a first CE dimension, on-line digest them and separate the digest in a second CE dimension, and finally identify the proteins by on-line mass spectrometry (MS). This allows protein information to be obtained from the first CE dimension.

Initially, an in-house built CE instrument was coupled to a three-dimensional ion trap *via* a sheathless CE-MS interface. For enhanced robustness, a sheath-flow CE-MS interface is now utilized on a Q TRAP™ – a triple quadrupole - linear ion trap hybrid.[2] On-line UV detection prior to mass spectrometric detection asserts the soundness of the CE-MS interface. A teflon cross based on a design by Wu *et al*. serves as the CE-UV detection interface.[3]

Future work will expand the system to a two-dimensional CE-digestion-CE instrument interfaced with MS. The first CE dimension will separate proteins, which will then be digested in a microreactor that is part of an interface between the two CE dimensions. The second CE dimension will separate the resulting peptides and they will be directly sprayed into the mass spectrometer.

This work has been supported by MDS Sciex and Applied Biosystems.

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053 OMNI-TRACER: A NEW TECHNIQUE FOR FLUORESCENCE POLARIZATION IMMUNE ASSAYS (FPIA)

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The fluorescence polarization immune assay (FPIA) is one type of homogenous assay commonly used in detecting and measuring drugs, such as in cases of abuse by athletes or for the detection of hormone levels in body fluid samples for clinical diagnosis. FPIA offers certain advantages over heterogeneous assays such as ELISA and RIA. It allows real-time measurements and does require multiple washings or protein denaturing. Also, orientation problems caused by surface immobilization are avoided, and FPIA prevents non-specific binding.

However, currently, FPIA is based on the competition between unlabeled antigen contained in samples and fluorescence labeled antigen in pre-existing test reagents. There are some drawbacks that prevent FPIA from becoming a basic technique for high-throughput proteomics screening: first of all, specific fluorescence labeled antigens (tracers) for each testing antigen are needed and this is difficult and expensive; second, since the tracer's molecular weight must not be very large, it limits its usefulness for detecting bio-macro molecules; third, most current FPIAs are competitive binding assays, so inherit the main drawback associated with these assays: FP readings are inversely related to the amount of the analyte tested, and not linearly proportional to the amount of the analyte.

A new FPIA technique, using Omni-tracer, has been developed to overcome these drawbacks. The Omni-tracer is based upon the globular head of the C1q molecule, and its derivatives. These molecules specifically bind to the C1q binding site on the constant region of an immunoglobulin, but only when the immunoglobulin is a part of an antibody-antigen complex, i.e. an immune complex. The Omni-tracer utilizes the fact that C1q binds to free immunoglobulin much weaker than aggregated immunoglobulins, as it forms an immune complex with its antigen specifically, so it can be used to distinguish free immunoglobulin and immune complexes. The immunoglobulin of this system, in turn, recognizes the corresponding antigen with high specificity. The binding of C1q derived tracer to the antibody of an immune complex involves only the immunoglobulin heavy chain, and has no relation to the antibody-antigen specificity. C1q-derived tracers are thus 'Omni-tracers™ that can be applied to a wide variety of antigen-antibody complexes. The use of the Omni-tracers™ completely changes the mechanism of FP based immune assay, and may have many applications in the areas of biosensors and proteomics research.

054 TOWARDS GLOBAL PROTEOMICS, ALL IONS ALL THE TIME: A PROOF-OF-PRINCIPLE OF THE QUALITATIVE ION MAPPING TECHNOLOGY

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A new method of protein characterization of proteolytically-generated peptides has been developed which is based on coupling the alternate scanning of peptides at low and elevated energy[1, 2] with a robust accurate mass LC-MS data acquisition mode[3]. This mode of data acquisition is very high in duty cycle and provides exact mass MS analysis of both the precursor and product ions for every detectable peptide. Chromatographic retention time is used to associate fragments to their corresponding precursors as a unique attribute of the Waters® Protein Expression System. Proprietary Protein Expression Informatics software has been developed which allows this information to be processed in a quantitative and qualitative manner. This poster will focus on the qualitative identification capabilities of the software. Triplicate injections of tryptic digests of human serum were made directly onto a Waters NanoEase™ Atlantis™ dC_{18} column (300 um x 15 cm). The data was acquired using alternating 2.0 second scans at low and elevated collision energy. The NanoLockSpray Source was switched every 10 seconds to obtain a scan of the accurate mass standard, [(Glu)[1]]Fibrinopeptide B. The raw data were processed using ProteinLynx™ Global SERVER and the Protein Expression System Informatics software developed in-house. The Waters Protein Expression Informatics software filters the data acquired in the alternating scan experiment in a three-step process. The first step carefully aligns chromatographic retention time to associate fragment ions to each appropriate precursor ion. The second step compares the exact mass of each measured peptide precursor ion to a database containing the accurate mass of every peptide associated with every known protein in the proteome under study. Typically, peptides which are within an acceptable mass measurement tolerance (within +/- 5 ppm) are identified in a subset peptide data base. The accurate mass of each y" and b fragment ion is calculated for each of the peptides in this subset database and the measured exact mass fragment ion information obtained in the first processing step is compared to this theoretical accurate mass information. A probability-based search model that takes into account the number, type, and mass accuracy of the measured fragment ions is used to determine the best peptide sequence match.

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055 TOWARDS GLOBAL PROTEOMICS BY EXACT MASS RETENTION TIME PAIRS: A PROOF-OF-PRINCIPLE OF THE QUANTITATIVE ION MAPPING TECHNOLOGY

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This intent of this poster is to illustrate the quantitative capabilities of the new Waters Protein Expression System. The data presented were generated using commercially available tryptically digested human serum samples spiked with different concentrations of five exogenous internal reference proteins contained in the Waters Protein Expression System Standards. Data were acquired in alternate scanning low and elevated energy accurate mass LC/MS mode. Each spiked digest sample was analyzed in triplicate. The raw data was than processed through a suite of Protein Expression System Informatics tools to produce a list of unique Exact Mass Retention-Time (EMRT) components. The data are processed further to match and compare the intensity of like EMRT components from different sample injections. Accurate mass low and elevated information associated with EMRT components that illustrate a significant change in intensity may optionally be exported to facilitate their qualitative identification. Five aliquots of this tryptic digests of human serum digest were spiked with Waters Protein Expression System Standards, containing equimolar levels of Yeast Enolase and Alcohol Dehydrogenase, Rabbit Glycogen Phosphorylate, Bovine Serum Albumin and Hemoglobin. Each of the five spiked human serum (HS) digest samples was analyzed in triplicate. Injections of 5 microliters were made directly onto an a Waters NanoEase™ Atlantis™ dC18 column. The total protein load for each injection was ~8.5 micrograms of human serum plus either 5.0, 1.0, 0.5, 0.25 or 0.1 picomoles of the spiked Protein Expression System Standards. Exact mass LC/MS data was collected using an alternating low (10eV) and elevated (28eV to 35eV) collision energy mode of acquisition such that one cycle of low and elevated collision energy data was acquired every 4.0 seconds. The NanoLockSpray source was switched every 10 seconds to obtain a reference scan of [Glu]-Fibrinopeptide lockmass calibrant to obtain exact mass measurements.

056 PURIFICATION AND REFOLDING OF HUMAN B-SECRETASE 1 (BACE1) EXPRESSED FROM *ESCHERICHIA COLI* FOR STRUCTURE DETERMINATION

Laura Lin, James Strand, Amy Tam, Cui-Hua Liu, Kristine Svenson, Rajiv Chopra, Kristie Bridges, Elizabeth DiBlasio-Smith, Chu-Lai Hsiao, Yan Liu, Bethany Annis-Freeman, Edward R. LaVallie, Tod H. Marvell, Richard S. Zollner, Rebecca Cowling, Guixan Jin, Jonathan Bard, William S. Somers, Ronald W. Kriz, and Mark Stahl
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The human beta-site amyloid precursor protein-cleaving enzyme (BACE1) mediates a critical step in the production of Aβ peptides, which are implicated in the severe neuronal cell death and insoluble amyloid plaques of Alzheimer's disease (AD). Inhibition of BACE1 is considered to be an excellent therapeutic strategy for the treatment of AD. BACE1 is a type I transmembrane aspartic protease with four glycosylation sites and three pairs of disulfide bonds. The catalytic domain of the protein can be expressed in CHO cells as a soluble, active, heterogenously glycosylated form; or in *E. coli* as insoluble inclusion bodies. An efficient refolding, activation, and purification scheme was developed using the *E. coli* expressed material and the resulting BACE1 protein was shown to be homogeneous in terms of molecular mass and N-terminal sequencing. Furthermore, its activity is comparable with the purified, CHO expressed BACE1 enzyme. The *E. coli* expressed and refolded BACE1 was successfully used to obtain co-crystal structures of BACE1/small molecule inhibitors.

057 CHARACTERIZATION OF RECEPTOR-LIGAND COMPLEXES FORMATION BY SIZE EXCLUSION HPLC/LIGHT-SCATTERING

Tony Tam, Hiko Kohno, Jonathan Shen, and Hsieng Lu
Department of Protein Science, Amgen, Thousand Oaks, CA 91320, USA

Size exclusion HPLC coupled with laser light-scattering detector (SEC/LS) provides a direct measure of the stoichiometry of receptor-ligand or antibody-antigen complexes. It has been used to show that soluble TNF receptor (sTNFR) and TNF trimer formed a 3:1 complex[1], where as a human monoclonal antibody D2E7 formed a 3:3 complex with TNFα trimer[2]. In our laboratory, SEC/LS has been applied to characterize the complexes of TNF with several modified forms of sTNFR, including PEG-sTNFR dimer (Dumbbell) and TNFR-Fc fusion proteins. Our results showed that the stoichiometry of TNF-sTNFR complexes varied with the ratio of the initial concentrations of TNFα and sTNFR. When TNF was used in excess, all sTNFR formed 1:1 complexes with TNF trimer. Most complexes appeared to have smaller hydrodynamic volume and eluded later than the free receptors, indicating that the 1:1 TNFR-TNFα complexes are more compact than the free receptors. When sTNFRs were used in excess, their complexes with TNF eluted much earlier than their unoccupied forms with different stoichometry. Dumbbell and

TNF trimer formed a 3:2 complex while TNFR-Fc fusion proteins and TNF trimer formed mainly 2:1 complexes. Overall, TNFR-Fc fusion proteins and PEGylated TNFR do not form a 3:3 complex with TNF as found in the case of antibody D2E7. Since the multimerization of Fc gives a stronger affinity to the complement component, C1q, and the Fc receptors, the configuration of the complex between TNF and the Fc-containing TNF receptor may affect the clinical efficacy and/or safety (e.g. CDC or ADCC effect).

[1]*Wen, J., Arakawa, T., Philo, J. (1996) Anal. Biochem.* ***240****, 155-166.*
[2]*Santora, L., Kaymakcalan, Z., Sakorafas, P., Krull, I., Grant, K. (2001) Anal. Biochem.* ***299****, 119-129.*

058 CHARACTERIZATION AND LOCALIZATION OF THE O-LINKED GLYCOSYLATION IN CHO-DERIVED A2 BY MASS SPECTROMETRY

Violeta G. Valladares, Chris Spahr, Jonathan Shen, Hsieng S. Lu and Tony Tam
Department of Protein Science, Amgen, Thousand Oaks, CA

O-linked glycosylation on serine or threonine residues is often difficult to localize and characterize. There is no motif such as Asn-X-Ser/Thr (X ≠ Pro) for N-linked glycans. Edman sequencing results can be difficult to interpret due to the traditionally poor recovery of serine and threonine. Additionally, O-linked glycosylation is energy-labile; MS/MS of the glycopeptide results in fragmentation of the glycan while the peptide backbone remains intact. MS3 on daughter ions from the MS/MS scan is not productive as only the losses of the glycan moieties are observed. When protein A2 was subjected to enzymatic digestion with endopeptidase Lys C, the peptide of interest was observed to be 30% glycosylated. Further digestion of the isolated glycopeptide with AspN generated a peptide with the sequence of EXXXSXSATXXSXSTXSS XSXSXTXX. This peptide contained eleven possible sites for O-linked glycosylation, and no potential internal cleavage sites. Utilizing both N-terminal sequencing and alkaline beta-elimination followed by targeted LC-MS/MS, the site of glycosylation was determined to be Thr-15. An increase in mass of 947 Da suggests a glycan structure consisting of 1 HexNAc, 1 Hex, and 2 sialic acids attached to the peptide. This glycan structure was confirmed by MS/MS fragmentation of the glycopeptide.

059 COMPREHENSIVE 2-D NANO LC/MS FOR HUMAN TISSUE PROTEOMICS

R. van Soest (1), G. Mitulovic (2), R. Swart (2), J.P. Chervet (2), M. van Gils(1)
(1) LC Packings/Dionex, 500 Mercury Drive, Sunnyvale, CA 94088, USA; (2) LC Packings/Dionex, Abberdaan 114, 1046 AA Amsterdam, Netherlands

2-D Nano LC coupled to mass spectrometry (MS) provides a powerful analytical tool for the separation and identification of complex proteomics samples. This approach is usually based on the injection of the digested protein sample onto a strong cation exchange (SCX) column as 1st dimension separation. Peptides are eluted from the column as fractions by injecting salt plugs of increasing concentration. Each fraction is subsequently separated on a reversed phase (RP) column as the 2nd orthogonal separation dimension. However, injecting salt plugs onto the SCX column limits the separation power, resulting in lower chromatographic resolution. This often leads to co-elution of high-abundant peptides over two or more fractions and substantially lower peak capacity. The co-elution of peptides is highly unwanted, since they will be detected and measured repeatedly which results in large numbers of redundant MS data. To overcome these shortcomings, we used a Dual Gradient Nano LC System allowing for the delivery of two independent gradients. Two reversed-phase (RP) trap columns were used for parallel trapping of the peptides eluting from the first dimension SCX column, followed by their separation on the second dimension RP nanocolumn. Comprehensive 2D Nano LC/MS using linear salt gradients instead of salt plugs resulted in an almost 2 times higher number of identified proteins. We observed 98 vs. 53 identified proteins for the human tissue sample studied. Another advantage observed with the comprehensive 2D Nano LC was the absence of co-eluting high-abundant peptides over multiple fractions. This technique is currently applied to the separation of complex proteomics samples and takes full advantage of the improved separation and identification power of Nano LC/MS.

060 THE FIRST HUMAN PDE1B STRUCTURE: EXPRESSION, PURIFICATION, AND CRYSTALLIZATION

Alison H. Varghese[(1)], Jeffrey S. Culp[(1)], Mahmoud N. Mansour[(1)], Dennis E. Danley[(1)], Michele H. Rosner[(1)], Boris Chrunyk[(1)], Paul Watts[(2)], Timothy A. Subashi[(2)], Kimberly Fennell[(2)], Samuel P. Simons[(2)], Peter LeMotte[(2)], Jayvardhan Pandit[(3)]
Pfizer Global Research and Development, Protein and Peptide Chemistry[(1)], Expression and Fermentation Technology Group[(2)], Structural Biology[(3)], Eastern Point Road, Groton, CT 06340

Phosphodiesterases (PDE's) play a key regulatory role in multiple physiological processes therefore making them good clini-

cal targets for a wide range of biological disorders including asthma and erectile dysfunction. At present, eleven gene families have been identified and over 50 isozymes have been detected in various human tissues. PDE 1B is highly expressed in mammalian brain making it a potential target for treatment of neurological disease. PDE 1B knockout studies suggest ADHD, depression, and anxiety as potential psychotherapeutic targets. Here we present the expression, purification, and characterization of a PDE 1B catalytic construct leading to crystallization of the enzyme. X-ray crystallography data has been collected leading to the first reported structure of human PDE 1B.

061 DIFFERENTIAL PROFILING OF CARDIAC HYPERTROPHY USING MULTIPLEXED iTRAQ™ REAGENT TECHNOLOGY

Derek Smith[1], Jody Haddow[1], Darryl Hardie[1], Darryl J. Pappin[2], Subhasish.Purkayastha[2], Lynn R.Zieske[3], Sally Webb[3] and Bob Olafson[1]
[1]University of Victoria – Genome BC Proteomics Centre, #3101 - 4464 Markham St, Victoria, BC Canada V8Z 7X8; [2]Applied Biosystems, 500 Old Connecticut Path, Framingham MA 01701; [3]Applied Biosystems, 353 Hatch Dr, Foster City, CA 94404

The goal of differential expression proteomics is to measure the change in protein expression following perturbations or physiological changes due to ageing, disease, drugs, pollutants, physical stress, etc.
Methods currently employed to measure these changes include 2-dimensional gel electrophoresis (2DGE) and analysis using ICAT® reagents. Although widely employed, there are limitations to each. For example, in 2DGE only those proteins soluble in the gel buffer employed are analyzed and very large/small/hydrophobic proteins are missed as well as the highly basic proteins. The limited dynamic range and low peptide recovery from gels has also impacted the efficacy of this type of analysis. Although stable isotope ratioing techniques such as ICAT reagent chemistries have resolved many of the above problems associated with 2DGE there still remains certain limitations. Protein identification and quantitation is quite often dependent upon a single cysteine-containing peptide, the current reagents allow only pair wise comparisons and for the most part, post-translational modifications (PTM) are undetected.
We describe here a new primary amine specific reagent that allows multiplexing of up to four samples in one analysis. The increased peptide coverage allows for greater confidence, greater detection of PTMs and even absolute quantification. A multiplexing capability allows for a wide variety of experiments permitting time course measurements, the use of replicates and multiple challenges.

062 ANALYSIS OF PROTEIN DISTRIBUTION IN RIBOSOMAL SUBUNITS USING NEW MULTIPLEXING ITRAQ™ REAGENTS

S. Webb[4], J. R. Maddock[2], M. Jiang[2], D.J. Pappin[3], S. Purkayastha[3], L.R. Zieske[4], and P.C. Andrews[1]
[1]University of Michigan, Dept. of Biological Chemistry, Ann Arbor MI 48109, [2]Univ. of Michigan, Dept. of Molecular Cellular and Developmental Biology, Ann Arbor MI 48109, [3]Applied Biosystems, Framingham MA 01701; [4]Applied Biosystems, Foster City, CA 94404

Ribosomes are large protein/RNA complexes that are responsible for the translation of mRNA into proteins. In prokaryotes, ribosomes are composed of a small subunit (30S) and a large subunit (50S) that together comprise the whole (70S) ribosome. The nomenclature is based on the sedimentation values of the complexes and they are typically purified by sucrose density gradient ultracentrifugation. Free ribosomes are found in the cytosol and may occur as free subunits, complete ribosomes, or bound to mRNA as polysomes. Ribosomes undergo a complex assembly process that involves a number of non-ribosomal proteins and these proteins may be only loosely bound to, or ephemeral in their interactions with, the ribosomes, leading to substoichiometric ratios. Mass spectrometry has been used in the past to study interactions within these ribosomal complexes, by studying the gas-phase dissociation of intact *Escherichia coli* ribosomes. Here, we describe the use of a novel multiplexing reagent, iTRAQ™ reagent to label the individual isolated subunits of the ribosomal complex in order to evaluate the distribution of ribosomal and accessory proteins between these ribosomal complexes to better understand the interactions within and between the subunits that determine and regulate protein biosynthesis processes within the cell.

063 MULTIPLEXED ISOBARIC PEPTIDE TAGS AND NOVEL ADVANCES IN TOF/TOF TECHNOLOGY FOR PROTEIN IDENTIFICATION AND MULTIPLEXED QUANTIFICATION

Philip Ross, Yulin N. Huang, Jason Marchese, Nikita Khainovski, Brian Williamson, Stephen Hattan, Peter Juhasz, Ken Parker, Sasi Pillai, Scott Daniels, Subhasish Purkayastha, Darryl J. Pappin, Dale H. Patterson and Matthew Willetts

Applied Biosystems, 500 Old Connecticut Path, Framingham MA 01701, USA

Proteomics and biomarker discovery experiments demand analytical tools that enable sensitive protein identification and precise protein quantification. MALDI tandem time-of-flight mass spectrometry has proven to offer sensitive protein identification and recent advances have yielded more sensitivity and better precursor ion selection. Herein, we couple this sensitive method of protein identification with novel isobaric tags for absolute and relative quantitation of proteins to study a series of yeast deletion mutants. At the core of this quantitation methodology are novel iTRAQÔ Reagents, a multiplexed set of isobaric reagents that yield amine-derivatized peptides that are chromatographically identical and indistinguishable in the MS mode. The use of these labels permits simultaneous measurement of relative and/or absolute protein abundance of multiple, complex samples in a single 2D LC-MS/MS run. We examined total protein expression levels of three yeast strains (Xrn1D and Upf1D deletion mutants and wild-type). Approximately 1200 unique proteins were identified, with more than 600 proteins quantified with at least 2 peptides. Among global changes observed were significant upregulation of proteins involved in amino acid metabolism and protein biosynthesis. A number of spiked idiotypic peptides were also identified, permitting absolute quantitation of relevant proteins. Proteins identified with 2 or more high-scoring peptides exhibited cumulative standard deviations of approximately 15% proving that MALDI MS/MS data can be highly quantitative. The derivatization of peptide amines is essentially quantitative (> 98%) and the only significant side-reaction (tyrosine) can be essentially prevented by manipulation of the reaction conditions. Excess reagent is removed by reverse-phase or ion-exchange chromatography, and only minimal changes are needed to salt and organic elution conditions relative to unmodified peptides. The reagents also improve ionization of lysine-containing peptides and quality of CID spectra, which show improved b- and y-ion abundance relative to their underivatized counterparts.

064 PROBING PRION PROTEIN CONFORMATIONAL CHANGES BY CIRCULAR DICHROISM AND FLUORESCENCE SPECTROSCOPY

Hui-Chun Yeh and Michele A. McGuirl

Division of Biological Sciences, SC 204, The University of Montana, Missoula, MT 59812

Prion diseases, which include mad cow disease, Creutzfeld-Jakob disease, scrapie, and chronic wasting disease, are associated with the conversion of normal cellular prion protein (PrP^{C}) into a disease-specific isomer (PrP^{Sc}). PrP^{SC} is an aggregate-prone form that shows a marked increase in its β-sheet secondary structure content. Although the structure of PrP^{C} has been determined, the molecular details of the structural changes associated with conversion are unknown. To elucidate these details, we have produced a series of mutants of truncated Syrian hamster prion protein (residues 90-231) that contain a single tryptophan (Trp) residue at different sequence positions. The PrP^{C} mutants have similar α-helical contents and thermal denaturation profiles as measured by Circular Dichroism (CD) spectroscopy, indicating that the Trp substitutions have not changed the global protein structure. CD spectroscopy was also used to monitor the conversion of PrP^{C} to PrP^{SC}. We then compared the Trp fluorescence spectra of the mutants in both PrP^{C} and PrP^{SC} conformations. Differences in the intensities and λ_{max} values of the Trp fluorescence peaks show that Trp experiences different solvent exposures in each PrP^{C} mutant, as predicted from the tertiary structure of PrP^{C}. Moreover, several mutants show additional changes in the Trp local environment after PrP^{SC} formation. The data will assist us in mapping the structural changes that occur upon conversion of PrP^{C} to PrP^{SC} and/or aggregate formation. We have also investigated the effects of pH and metal binding on the conversion process.

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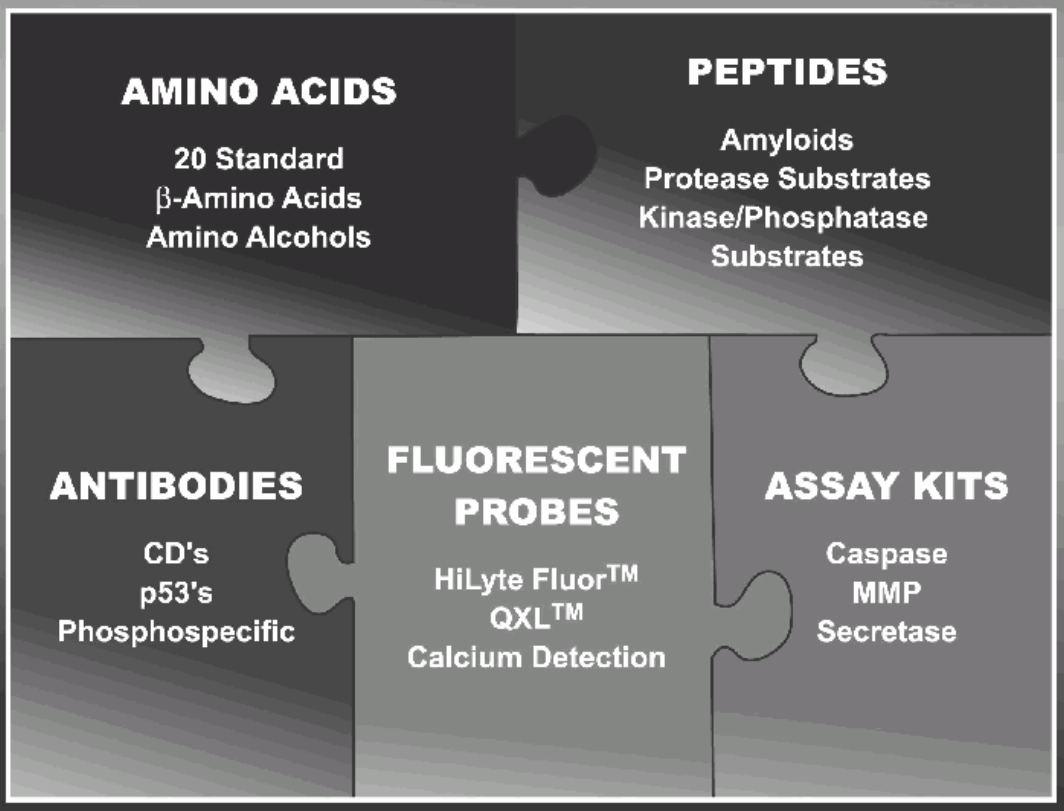

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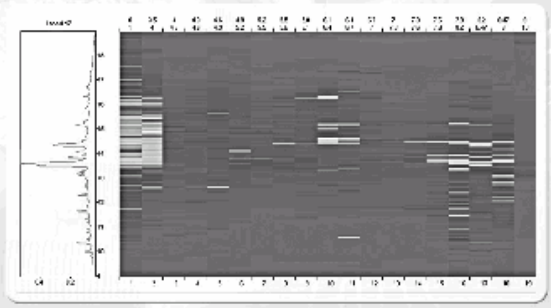

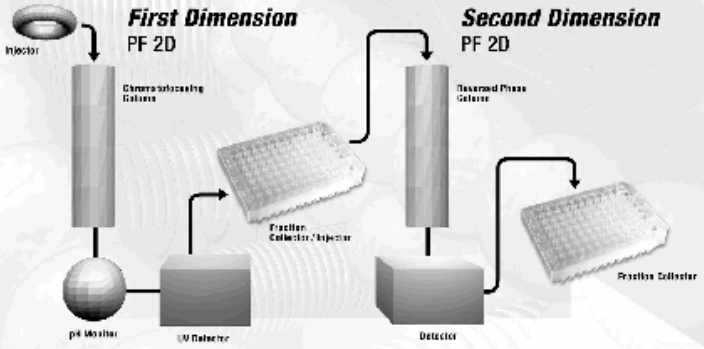

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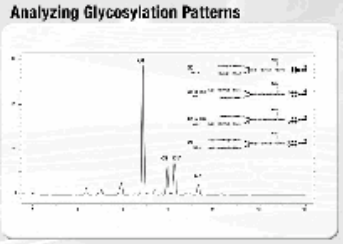
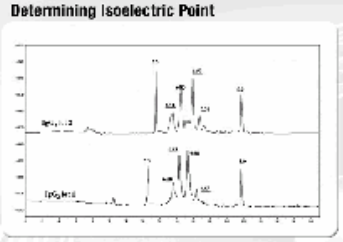
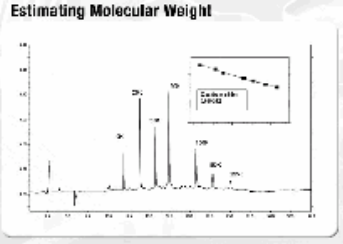


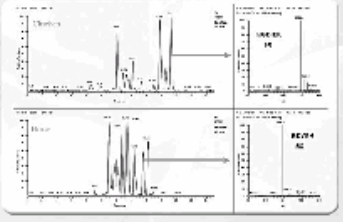
Determining Isoelectric Point



Estimating Molecular Weight



Peptide Mapping - Protein ID



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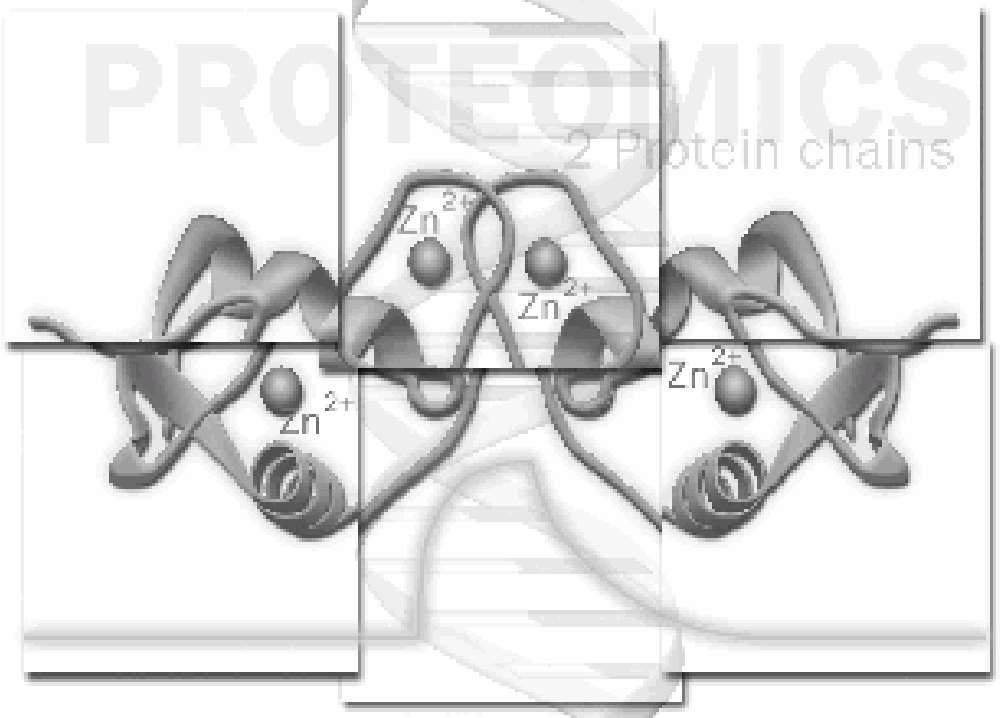

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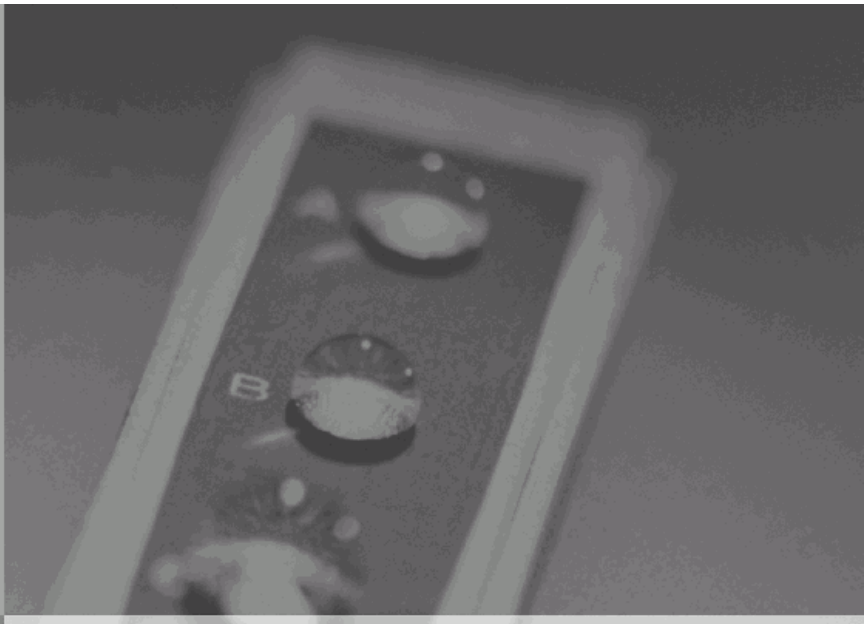

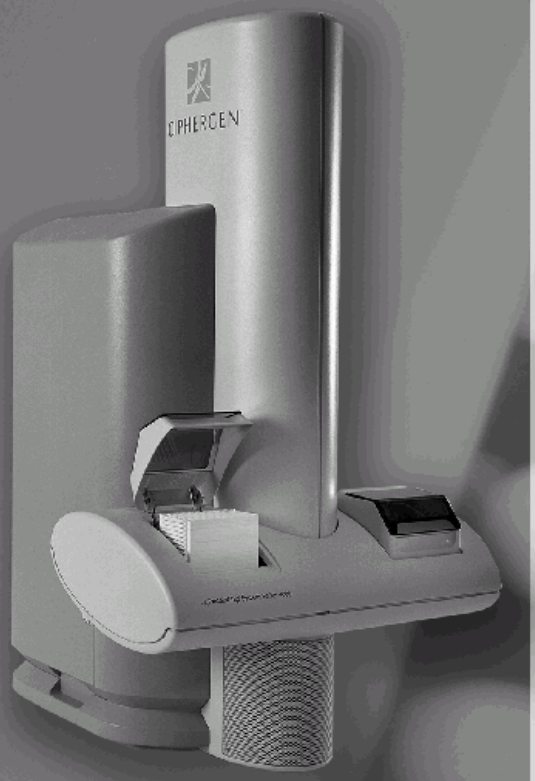

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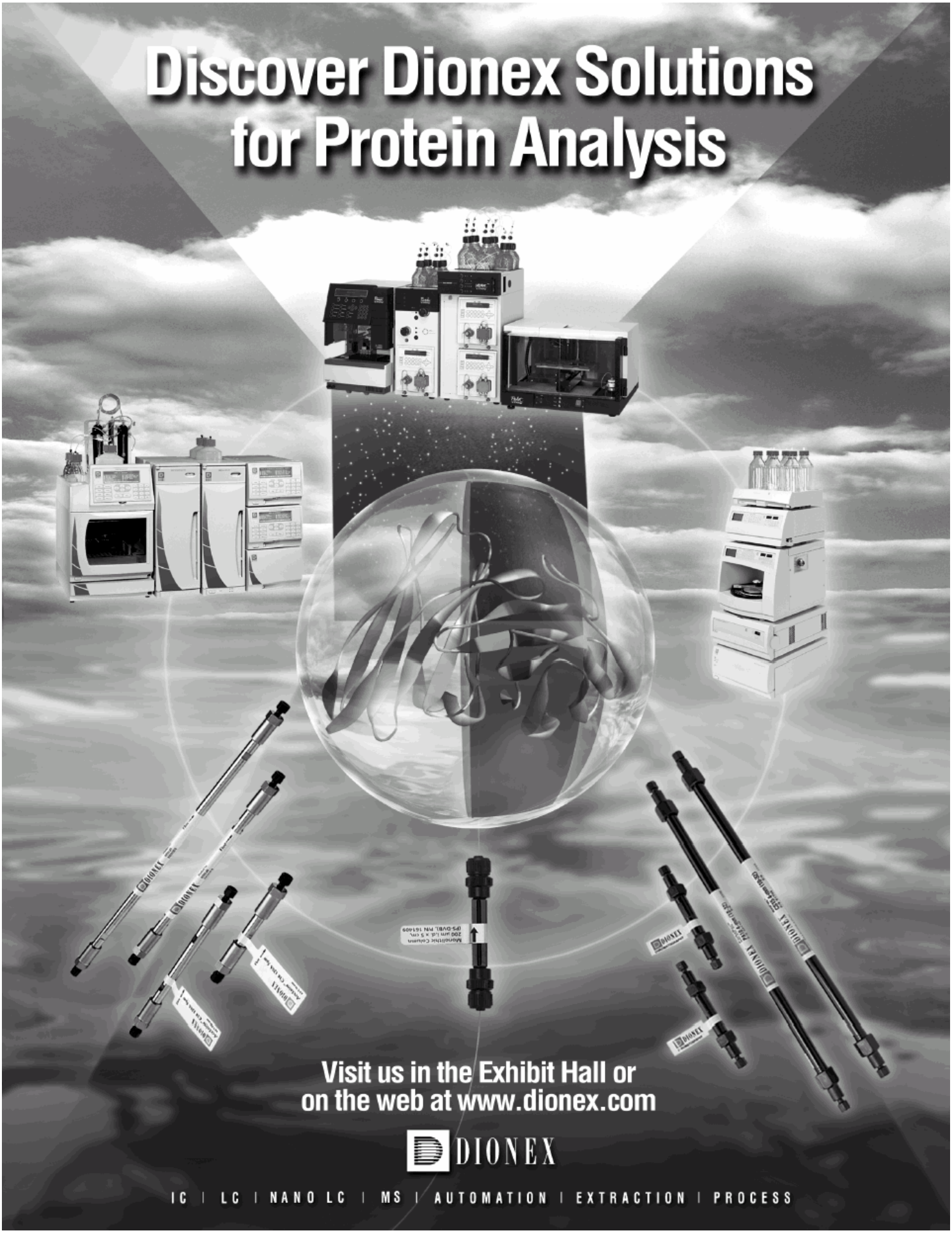
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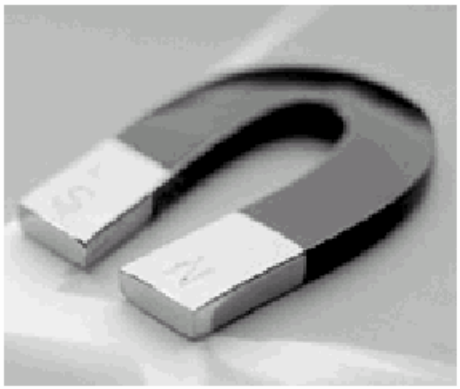

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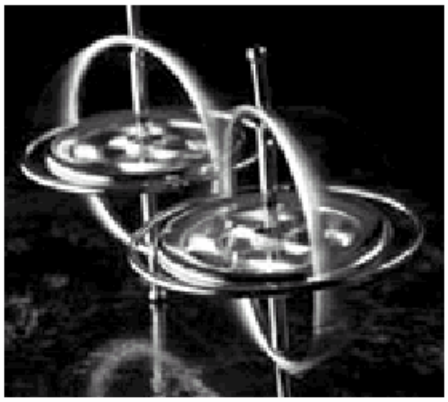

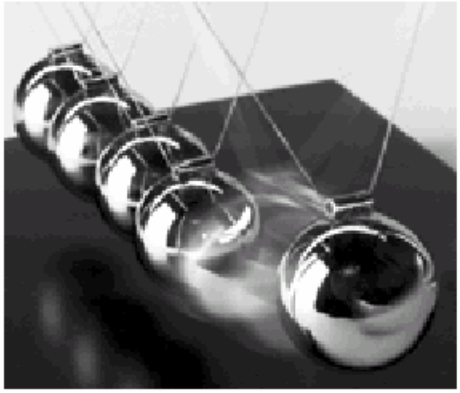

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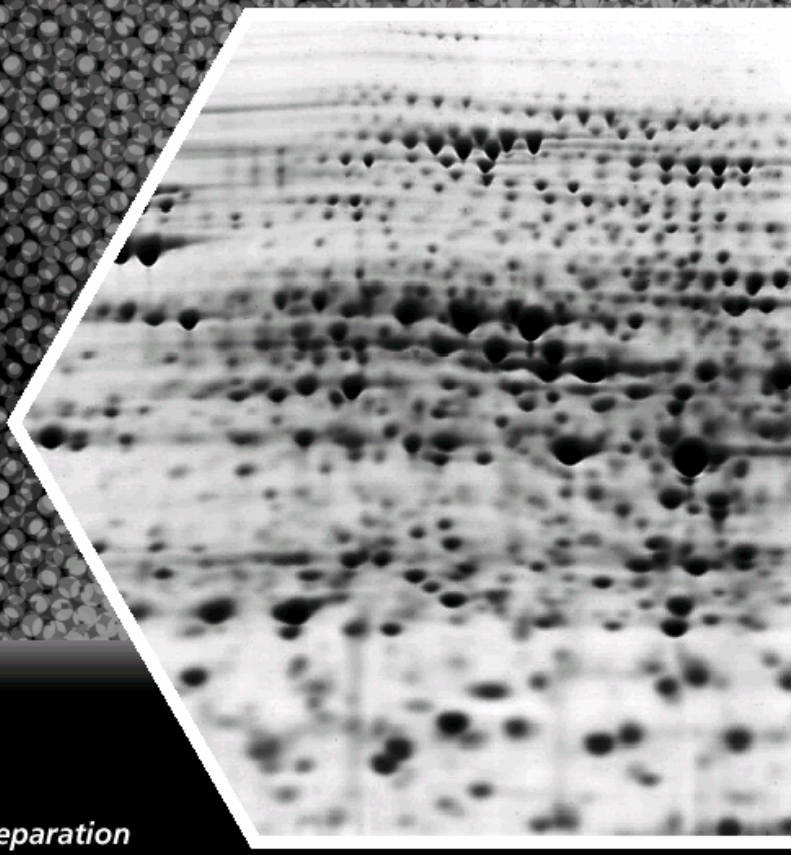

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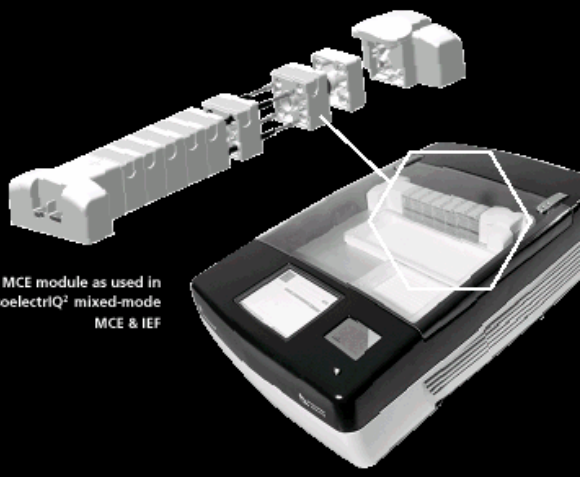

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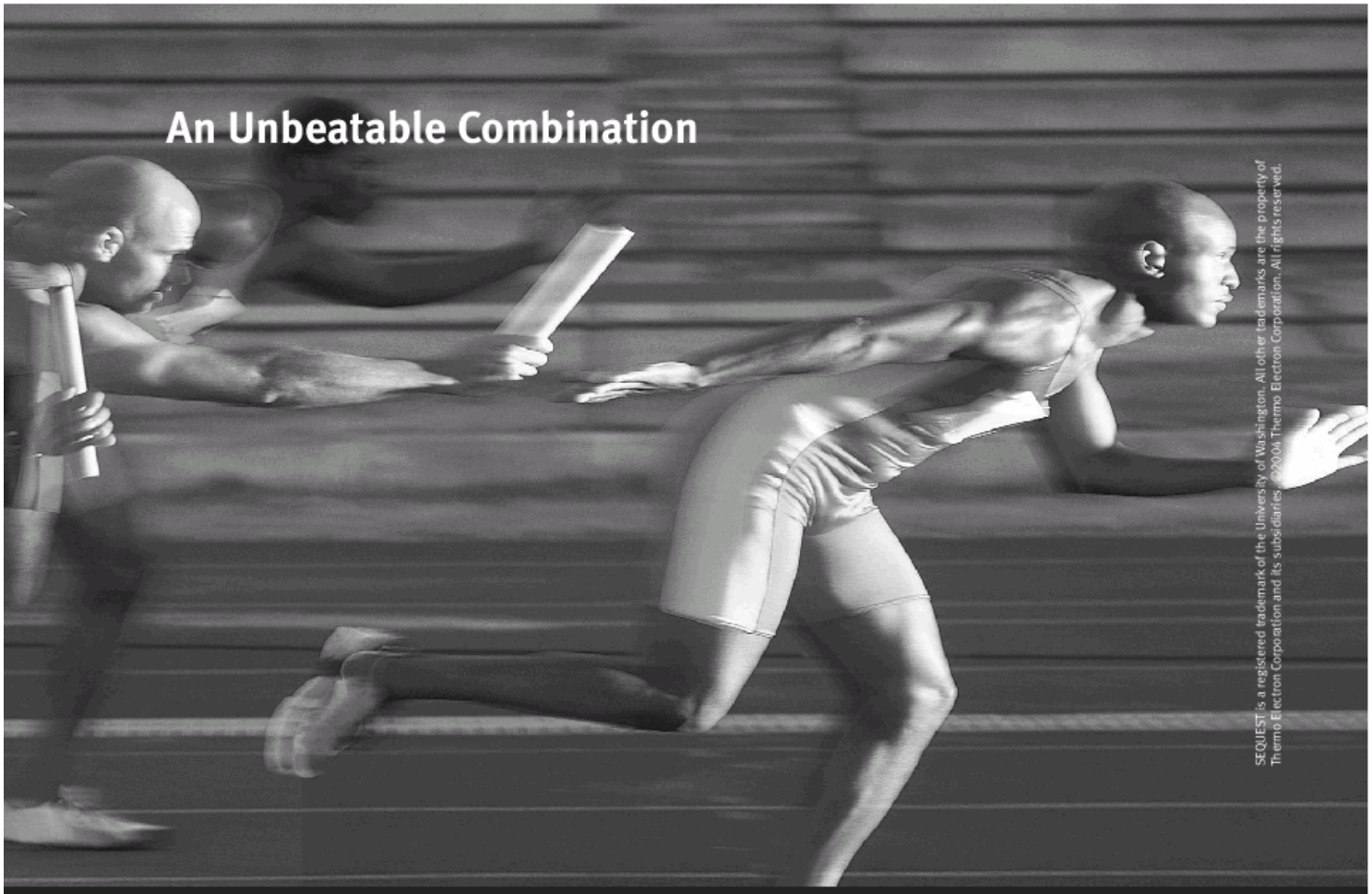

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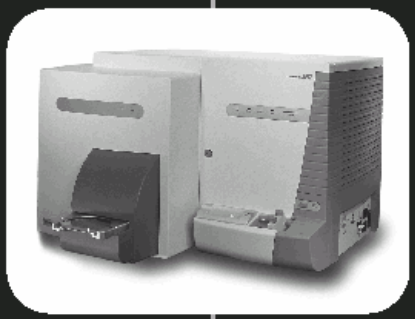

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