P1-S
Parallab Technology:Integrated Nanoliter Genomic Workstation
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The Life Sciences Group of Brooks Automation, Inc. has developed a fully automated, integrated platform to perform nanoliter volume reactions. As an example, our standard DNA cycle sequencing reaction is done in a total volume of 500 nanoliters. A key element in this innovation is the proprietary Nano-Pipetter that incorporates 96 glass capillary tubes that process all samples in parallel. The bench top ParallabTM 350 exploits the ability to aspirate small and accurate reagent volumes and subsequently completes all of the reaction and purification steps within the 96 miniature glass syringes. Each reagent is aspirated into the glass vessels, mixed, thermal cycled and purified before finally being dispensed into an output plate for analysis. After each sample set is complete, the Nano-Pipetter is decontaminated and reused, greatly reducing the number and cost of consumables normally associated with completing a similar sample set. For a genomics lab, the ParallabTM 350 is ideally suited for a range of applications, including cycle sequencing, PCR, SNP analysis, genotyping and end point analysis and can complete 1,800 samples per 24-hour day. We believe that this unique combination of attributes (nanoliter reaction volume, reduced consumable use and complete automation) offers a revolutionary approach to help accelerate discoveries in modern molecular biological laboratories.

P2-M
Comparative sequencing study on templates with tandem repeats using various versions of BigDye Terminator Cycle Sequencing kit
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The presence of short tandem repetitive sequences of di-, tri- and tetra nucleotide all over in the genomes often cause routine sequencing anomalies in core laboratories. The heterogeneity in template preparation by various users also adds to the problem. Since a large number of samples pass through the core lab daily, we use BigDye terminator v 1.1/3.1, chemistry and 5% DMSO additive routinely. Many difficult templates with tandem repeat regions, secondary structure or hairpins often fail to sequence under routine sequencing conditions. DSRG and others have shown in earlier studies that a number of factors affect in sequencing through difficult templates including tandem repeats and telomeric regions. In this study we have compared several versions of BigDye terminator cycle sequencing chemistry and DYEnamic reaction mix to sequence through the regions of DNA with TTCCCC and AAGGGAA-type repeat elements. The data suggest that the BigDye terminator cycle sequencing mix, v 3.1 and betaine additive outperformed other versions in sequencing through such repetitive elements in the template. This study further supports that adding 1M betaine in the reaction mix indeed results in reduction of band compressions in templates with repeat elements and provides a higher accuracy of sequencing.

P3-T
Evaluation of Difficult Sequences with Experimental Protocols and Sequencing Formulations
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Researchers today are challenged to finish the difficult-to-sequence areas of the human genome as well as other organisms, and to obtain highly accurate sequences of individuals in studying polymorphisms. Applied Biosystems recently introduced two new kits to help address these challenges, BigDye® Terminator v3.1 and BigDye® Terminator v1.1 Sequencing Kits. Here we discuss sequences that continue to be challenging with these new kits as well as

protocols and experimental formulations to successfully sequence through them. We will present data comparing performance of the experimental protocols and formulations with existing Applied Biosystems sequencing kits, including the ABI PRISM® dGTP BigDye® Terminator v3.0 Ready Reaction Cycle Sequencing Kit, in a variety of sequence contexts. In our ongoing effort to improve DNA sequencing chemistry, we are continually expanding our library of difficult templates, and welcome input and experience to help guide our research and development of future kits.

P4-S

Direct Sequencing from Bacterial Culture or Colony

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In the sequencing process, it is a challenge to prepare DNA template in an efficient and effective fashion. Quality, throughput, and cost are among the major factors in choosing a template prep method. Manual labor may be substantially decreased with automated liquid handling workstations and new template preparation technologies. Nonetheless, template prep is still often a rate-limiting step and processing costs still remain relatively high, often around one fifth of the total sequencing cost. An attractive alternative would be to completely avoid the template preparation step and directly sequence from bacterial cultures and colonies. Previous studies had shown it is possible to direct colony sequencing using either fluorescent dye primer or dye terminator chemistry, but with reduced read lengths and robustness. We have studied growth conditions, lysis conditions, and sequencing conditions to see how they affect direct colony or culture sequencing. Here we will present the results of our studies, along with recommendations for obtaining good quality sequencing results from colonies or cultures.

P5-M

DSRG-2004 - A Web-based User-Interactive DNA Sequencing Troubleshooting Resource

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Automated DNA sequencing has become an essential tool for the molecular biologist. This technology has undergone considerable changes in instrumentation, sequencing chemistry, and analysis software since its development over a decade ago. Users have experienced, and continue to experience, many challenges. As a means of aiding automated DNA sequencing users, the DNA Sequencing Research Group (DSRG) has begun to establish a web-based, user-interactive troubleshooting resource. The aim is that this compilation of troubleshooting suggestions and robust protocols would emerge as a valuable resource for automated DNA sequencing users. This resource will consist of a public database that allows researchers to submit a query of a sequencing problem, such as “red rain” on gel-based platforms or “waterfall effect” on capillary electrophoresis sequencers, and in return provide a solution to the problem or a possible explanation of the cause by searching against a reference data set.

P6-T

Evaluation of Two Post-Sequencing Reaction Clean-Up Methods: The Solid Phase Reversible Immobilization “SPRI”

Technology to Pre-Packed Gel Filtration Columns

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As a medium throughput DNA Sequencing Resource Center, major concerns include: efficacy, cost, and high quality results. Processing template DNA from various users with mixed purity creates special considerations when choosing the "best" clean-up procedure. Removal of contaminants, such as unincorporated dye terminators, dNTPs, salts, as well as the removal of impurities from low quality template preparations is the objective. Here two post-reaction clean-up methods are evaluated: the Clean-SEQ "SPRI" chemistry (Agencourt Bioscience Corporation, Beverly MA) and the Performa Dye-Terminator-Removal (DTR) System (Edge BioSystems, Gaithersburg, MD). The purified extension products were analyzed on an ABI 3700 and a SpectruMedix 9610, both 96-capillary DNA Analyzers. Clean SEQ's "SPRI" technology uses paramagnetic beads to precipitate the DNA and uses ethanol washes to remove contaminants. EdgeBioSystems Gel Matrix plates clean the post reaction samples by separating sequencing products by size. Both clean-ups are competed in comparable time and give high quality sequences. Agencourts' Clean-SEQ results in higher DNA yields and the cost is significantly lower than the Edge DTR plates. Other results supporting "SPRI" technology will also be presented in the poster.

P7-S

A simple DNA preparation for BAC end sequencing by amplification with Phi29 DNA polymerase

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Preparing Bacterial Artificial Chromosome (BAC) libraries for DNA sequencing is a labor intensive process. Due to the large size of a BAC (~75-350 kB) and with only 1-2 copies per cell, it is difficult to obtain a sufficient amount of good quality template for DNA sequencing. Established techniques for BAC end sequencing include a labor intensive alkaline lysis preparation from a large volume of overnight culture (>1.5 ml), column purification and an excessive amount of cycles (50-100) in the cycle sequencing protocol. A new method has been developed to amplify BAC DNA for BAC end sequencing. Starting material can be a single plate colony or 1-2 _l of overnight culture or glycerol stock. Based on rolling circle amplification and employing bacteriophage Phi29 DNA polymerase and random hexamer primers, a standard 20 _l overnight reaction can produce ~5 _g of BAC DNA template. BAC DNA amplified by this method in 96-well format, sequenced with a standard 25 cycle protocol and run on an ABI3100 can produce pass rates >90% and q20 >500 bp.

P8-M

Choosing Terminator Chemistry to Control DNA Sequencing Cost

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Our relentless pursuit of ways to control the ever-rising costs associated with DNA sequencing without compromising sequence quality led us to evaluate samples labeled with BigDye terminators (Applied Biosystems) on our MegaBACE 1000 capillary sequencer (Amersham Biosciences). Terminator chemistry constitutes the majority of sequencing expenses. BigDye kit is widely used by many labs operating ABI sequencers at reagent dilutions of up to 1/4 in an attempt to reduce cost. Additionally, some labs couple this dilution with reduced reaction volumes resulting in the use of only a fraction (down to 1/16) of the original reagent concentration. In contrast, Amersham's Energy Transfer (ET) terminator kit has always been used at full strength. ET reactions may be scaled down to as little as one-fourth volume but under our production conditions they are scaled to one-half volume. Reactions performed at volumes smaller than one half do not give consistently reliable sequencing results.

Although BigDye kits currently cost us about 6 times more than ET terminators, it is more cost-effective to use BigDye reagents (at 1/16 reagent concentration) than ET (1/2-size reactions) kits. Developing protocols using an alternative chemistry that can be run more cost-effectively on the same instrument will, therefore, help protect against rising expenses. Initial experiments were carried out involving dilutions and reduction of reaction volumes using the BigDye terminator (v. 1.1) mix. Production DNA templates generated through the RCA process utilizing Amersham's TempliPhi kit were used. The results indicate that signal intensities when using 1/16th reagent concentration were sufficient to generate over 500 Phred20 bases. Our results imply that a cost-effective terminator chemistry choice is feasible and could be made in response to rising reagent prices.

P9-T

DNA Sequencing Using POP-7 on an ABI PRISM 3100 Genetic Analyzer

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Many small to medium size core facilities today use the ABI PRISM 3100 Genetic Analyzer, a16-capillary automated DNA sequencer. Several capillary lengths and protocols are supported by the manufacturer for DNA sequencing, including the use of POP-6 with a 50 cm array as well as the use of POP-4 with the 80 cm array. The 50 cm array has the advantage of shorter run times, but with somewhat shorter read lengths (approx 700-750 bases), while the 80 cm array can provide read lengths of up to 900 bases, but with longer run times (approx 3 and 1/2 hours). POP-7, the new polymer that was developed specifically for the model 3730 DNA sequencer, has been shown to provide long read lengths coupled with shorter run times, but is not supported for use on the model 3100. In our laboratory, we have developed a modified run protocol that allows for the use of POP-7 and 50 cm arrays on the 3100. When using features included in the latest versions of Sequencing Analysis (v5.1) and Data Collection (v2.0), we are able to obtain high-quality basecalling of 850-900 bases in 2 hours, 20 minutes. This simultaneously increases throughput without sacrificing high quality read length. POP-7 has the added benefit of being significantly less expensive than either POP-4 or POP-6. In addition, our results indicate that POP-7 is less prone to sequencing artifacts such as "spikes"; and, when they do occur, the KB basecaller is often able to subtract the artifact and provide accurate basecalling of the affected region. These findings, we believe, will be of significant benefit to core facilities using the ABI PRISM 3100 for DNA sequencing.

PCR METHODS

P10-S

ENDOCRINOLOGICAL ,CYTOGENETIC AND MOLECULAR ANALYSIS IN in VARIANT KLINEFELTER CASES and DELAYED PUBERTY

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Klinefelter Syndrome (KFS) is the commonest sex chromosomal abnormality and the commonest cause of male infertility. Most reports do not differentiate KFS from the variants. These variant cases have additional phenotypic anomalies and thus form a distinct entity and therefore were studied in detail. In the present study 145 cases of male infertility were analysed cytogenetically. Twenty five well spread G banded metaphases were karyotyped using image analyser (Cytovision, Applied Imaging). Six mosaic variant cases with more than one cell line were analysed at the molecular level by Fluorescence In situ Hybridization (FISH) to detect low level cryptic mosaicism. Semen analysis was done according to the WHO guidelines (1999). We found 11cases with KFS, nine cases were KF mosaics and 5 were mosaic variants. These variant mosaic cases were Case1-47,XXY (60%)/48,XXYY (16%)/46,XX (20%)/47,XYY (4%), Case 2- 47,XXY (91%)/48,XXYY (3%)/48,XXXY (3%)/46,XY (3%), Case 3- 47,XXY (60%)/48,XXXY

(26%)/49,XXXYY (14%), Case 4- 46,XY (50%)/47,XXY (30%)/48,XXYY (20%), Case 5- 46,XY (53%)/48,XXXY (40%)/49,XXXYY (7%) case 6- 47,XXY/48,XXXY. FISH detected an additional cell line of 50,XXXXYY (1%) in case1. These variant cases had additional features than of KFS like mental retardation, difficulty in expressive language, slurred speech, Mitral Valve Prolapse and adjustment problem with peers. FSH and LH were markedly elevated and Testosterone levels were low. All cases were azoospermic. Thus variant cases should be considered as a distinct entity as they have additional cardiovascular and other clinical features and should thus be managed accordingly.

P11-M

Yq microdeletions- AZF candidate genes and Spermatogenic arrest

R. D. Rao, N. P. Gupta, K. Kucheria; AIIMS, New Delhi, India.

Microdeletion of the long arm of the Y chromosome, are associated with spermatogenic failure and have been used to define three regions AZFa, AZFb and AZFc which are critical for spermatogenesis. One hundred and seventy five infertile males with idiopathic oligozoospermia and azoospermia were included in this study. Cytogenetic and semen analysis was done in each case. Of the 175 cases, 40 were identified as Klinefelter Syndrome or variants(KFS), 4 cases had other cytogenetic abnormalities. In cytogenetically normal cases (n=102) microdeletion analysis was done using STS-PCR approach. Eight of the 102 cases showed deletion of at least one of the AZF loci. Four cases had AZFc deletion, three cases had AZFa and AZFb deletion and one case showed AZFb deletion alone. Two cases with AZFa and AZFb had SCO Type 1 syndrome and 2 cases of AZFc deletion showed hypospermatogenesis and 1 case showed maturation arrest. The FSH and LH levels were elevated in these cases. Variation in testicular phenotype in cases with AZFc deletion is due to multiple copies of the gene presence of autosomal genes. Thus various factors genetic, epigenetic and environmental modulate the effect of these genes. Deletion on Y chromosome make the Y chromsome more prone to secondary larger deletions resulting in worsening of testicular phenotype. Therefore detection of Yq microdeletions encompassing the AZF loci determines the prognosis and management of these infertile cases.

P12-T

Substrate QC for PCR, RCA and other Polymerase Reactions

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In this poster, we show a 20 min assay of nucleotides and deoxynucleotides that can rapidly verify the quality of PCR reagents and help expose the cause of PCR reaction failures. The analytes are separated by anion-exchange chromatography and detected by absorbance at 260 nm. The method employs gradient elution at 20 ºC on a 4 x 250 mm DNAPac® PA100 column. The eluent flow rate is 1 mL/min with a gradient of 4–18 mM perchlorate in 20 mM sodium hydroxide and 6.8 mM trisodium phosphate over 8.5 min.
This simple and direct assay satisfies the demand for a high-resolution analytical method for nucleic acids employed as reagents in molecular biology, as pharmaceuticals and as diagnostic tools. Resolving nucleotides and deoxynucleotides is essential in order to verify cocktail integrity and to determine the cause of amplification and reaction failures in amplification cocktails used for PCR, rolling circle amplifications, DNA and RNA polymerase reactions, and reverse-transcriptase reactions. Anion exchange chromatography using the DNAPac PA100 column provides fast analyses, high resolving power, and simple purification of DNA fragments for subsequent investigation. Other useful applications of the DNAPac PA100 include evaluating the purity of ss nucleic acids such as ribozymes, antisense N3’-P5’ oligonucleotides, and truncated tRNA minihelices. Incompletely phosphorothioated oligonucleotides are easily resolved from fully

"thioated" oligonucleotides. Applications to dsDNA include separating restriction fragments, PCR products and plasmids. Alternate column formats and low back pressure permit facile scale-up of analytical separations.

P13-S

Using PCR Melt Curve Peak Height Analysis for Optimization of an RT-PCR Test for Foot and Mouth Disease

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A one step reverse-transcription polymerase chain reaction (RT-PCR) test has been developed for detection of the Foot and Mouth Disease virus (FMDV). Reagents for the test have been tableted and adapted for use on the Qualicon BAX® instrument. During development of the test multiple test variables were optimized and characterized simultaneously using a design of experiment (DOE) statistical approach. RT-PCR product melt curve peak heights obtained from the ABI 7900 SDS instrument were used to determine the optimal concentrations of Taq Polymerase, dNTP's, $MgCl^{2,}$ and DMSO. Utilization of melt curve peak heights allowed us to monitor specific and non-specific RT-PCR product generation separately. In order to evaluate both the amount of specific product and non-specific primer-dimer products generated; 10e2 copies of FMD target RNA and a no template control were evaluated at all variable levels. JMP statistical software was used to generate the DOE model and perform the data analysis. Following the DOE individual variables were analyzed at the theorized optimum levels for confirmation. All of the trends identified in the DOE were subsequently confirmed. By optimizing multiple variables simultaneously we were able to characterize our test variables effects on product formation, evaluate variable interactions and ultimately optimize our test more quickly and more efficiently than would have been accomplished with a typical one variable at a time type approach..

P14-M

A Detailed Comparison of Single Nucleotide Polymorphism Genotyping Technologies

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Three diverse single nucleotide polymorphism (SNP) genotyping technologies are being evaluated: (1) Pyrosequencing is a robust technology ideally suited for projects <100,000 genotypes. Four 2-plex assay sets were developed and used to genotype >600 human genomic DNA samples at a cost of $0.85 per genotype with >80% of the DNAs yielding high quality data for all SNP markers. A uniplex assay was used to genotype two adjacent SNPs in the sequence, YYGGTAGC, a task which would be difficult to perform with other methodologies. (2) Taqman assays provide an economical, robust, and scalable approach for projects with 100 to 1,000,000 genotypes. Over 103 predesigned Applied Biosystems Assays on Demand were used to genotype > 600 human genomic DNAs; eight of these assays failed completely. For the successful assays, about 80-90% of the DNA samples produced high quality genotypes. Using a pipetting robot to setup the Taqman reactions enabled us to reduce the total volume to 2 microliters with a cost per genotype of $0.40. Seven additional SNPs were submitted to the Assay by Design service; six were developed into assays which yielded high quality data with an approximate cost per genotype of $0.52. (3) The Sequenom MassARRAY MALDI system suits projects >100,000 genotypes. Due to the cost and relative complexity of the process, multiplex assays are most ecconomical. Starting with >60 SNP markers, six 2-plex assays were designed, validated, and used for production genotyping. The overall level of high quality genotypes ranged from 50-90% of the DNAs depending on the SNP marker and the set of genomic DNAs tested. The major limitation of the Sequenom system is the difficulty in developing robust multiplex assays for it.

OLIGONUCLEOTIDE SYNTHESIS

P15-T

Comparison of Coupling and Deprotection Protocols for RNA Synthesis

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The UCDNA Services facility at the University of Calgary has provided RNA synthesis on a fee-for-service basis for almost 20 years. Originally, there was only modest demand (< 20 sequences/yr) for relatively short sequences and no great need to highly optimize the process or results. Recently, however, increased use of RNA interference (RNAi) and greater pressure for faster and cheaper production has changed that. Over the last year, we have been investigating different protocols in order to implement a robust, efficient, and economical RNA synthesis service suitable for a core facility environment. We use capillary gel electrophoresis (CGE) as a quantitative tool to systematically compare results from different protocols under controlled conditions. A large number of different coupling conditions (i.e. different phosphoramidite activators) and different deprotection protocols have been studied. Additionally, we are seeking protocols which will allow us to switch from column-based synthesis on ABI 394 synthesizers to 96-well plate-based synthesis on a MerMade IV synthesizer. We will present the latest results from our on-going study showing our preferred coupling, cleavage and deprotection methods.

P16-S

Extending Base Length Oligonucleotide Synthesis Potential

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Synthetic oligonucleotides (oligos), a key component in PCR, sequencing and other applications, are available from a number of commercial and academic sources. However many facilities, especially those providing commercial oligonucleotides, may limit the base length synthesis of the oligo because of the capabilities of the equipment and reagents and turn-around time. One limiting factor in DNA synthesis is the efficiency by which a base is attached to the 5 prime end of the oligo. Typically, high quality commercial phosphoramidites provide a source of bases with a 98 to 99 percent coupling efficiency. An oligo final product, therefore, has a full-length product and shorter failure products that separate into a DNA ladder. In general, the average length of oligos synthesized in this laboratory is 18 to 35 bases. Longer oligos, often exceeding 200 bases, have been requested by researchers and successfully synthesized. Proper maintenance of synthesis equipment and use of reagents can improve the coupling efficiency of a DNA synthesizer and allow longer oligos to be synthesized. In addition, small scale 10 nmol synthesis was developed in this laboratory, for the Applied Biosystems model 394, by optimizing equipment and reagent usage. In this poster we will report helpful guidelines used in the UC DNA Core Facility to extend the base length of DNA synthesized on the Applied Biosystems DNA Synthesizer model 394 and the Expedite 8909 with a Multiple Oligo Synthesis System (MOSS) upgrade and present examples of longer oligos.

RECOMBINANT PROTEINS

P17-M

T^3-Sequencing: a Novel Tool for Accelerating Recombinant Protein QC

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Quality control (QC) of recombinant proteins is important for a growing number of applications. Simple, cost-efficient and fast methods are required to screen such recombinant proteins for proper primary structure, terminal processing and any type of modifications. Classical approaches depend on Edman sequencing. Edman sequencing is slow due to its sequence of chemical and chromatographic steps and it’s running costs are high. In addition the presence of N-terminal modifications causes the method

to fail. Protein mass fingerprints frequently do not provide information about the termini. The top-down protein analysis approach can overcome these obstacles, it refers to the MS/MS analysis of intact proteins avoiding protein digestion steps.
"T^3-sequencing" achieves the specific fragment ion analysis of protein terminal 10-40mers using MALDI-TOF/TOF mass spectrometry [*Suckau D, Resemann A (2003), Anal Chem, **75**, 5817-5824*]. Terminal "peptides" were generated in the mass spectrometer from proteins as large as 70 kDa in a first step by in-source decay (ISD) and structural information was obtained in a second step using fragmentation in the TOF/TOF part of the instrument. The position of such ISD c-ion sequence tags together with the intact molecular mass of the protein allows verifying the proper covalent structure in a first screening round. T^3-sequencing is then used to elucidate terminal processing havoc in the expression system if sequence tags are observed at unexpected m/z values.
Protein characterizations will be shown that exemplify the described strategy. In addition, results from a comparison of Edman sequencing vs. ISD analysis of a set of 10 recombinant proteins will be presented. This will include data from N-terminally blocked proteins and from proteins with ragged N-termini.

SEQUENCE MANAGEMENT AND ANALYSIS

P18-T
Defining Parameters for Homology Tolerant Database Searching

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Comparison of observed masses obtained by MS/MS to predicted masses from sequence databases does not work well for species with limited sequence information, because an exact sequence match is required. Homology searching (i.e., BLAST, MS-homology from ProteinProspector) is relatively uncharacterized. Our objective was to define a strategy for this analysis. MS/MS data from 9 proteins were generated during our ongoing examination of the swine intrauterine proteome using 2-D PAGE, trypsin digestion and a QTOF Ultima API interfaced with LC packing nano-HPLC system. Peak lists were generated using MassLynx NT software (Version 3.5, Micromass UK Ltd). The 20 most intense peptides, selected either on precursor trigger intensity or on total ion current, were de novo sequenced using PEAKS (Bioinformatics Solutions, Inc.). For each method, sequences from the most intense 5, 10 or 20 peptides were searched against the NCBInr mammals database using MS-homology, allowing for 10, 30 or 50% mismatch (2 x 3 x 3 factorial design). Protein scores were similar between methods of ranking and were greatest when 20 peptides were submitted and allowing at least 30% mismatch ($p<.01$). However, sets of random peptide sequences generated similar patterns in protein scores. Thus, specific protein scores of the 9 proteins were corrected by subtraction of the random peptide mean protein scores+2 standard deviations. Greatest average specific protein score was obtained using 30% mismatch and 20 peptides ($p<.01$). These data indicate that for species where sequence information is limited, MS-homology using the 20 most intense peptides based on trigger intensity, allowing for 30% mismatch, and using subtraction of random peptide protein scores gives a reliable method for protein identification.

MASS SPECTROMETRY

P19-S
Characterisation of Complex Protein Samples Using LC-MALDI QIT TOF MS.

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Traditionally, identification of complex protein mixtures has required a series of time-consuming protocols, the success of which may be user dependent. Protein mixtures are separated using 1D or 2D gel electrophoresis, each protein band

or spot excised and digested producing a peptide mass fingerprint (PMF) to identify the protein using MALDI mass spectrometry.
There are limitations in this workflow, including
-co-migration of more than one protein, resulting in complex peptide mixtures making identification difficult,
-suppression effects during ionization limiting the number of peptides observed,
-PMF not correlating with any protein in the databases.
Frequently, MS/MS provides sufficient information to generate useful sequence data. However, this is not always the case, particularly when peptides have undergone modification. Here, it is necessary to progress to MS^n techniques.
Recently, in an attempt to circumnavigate these issues we have analysed proteins from both 1D and 2D gels using LC MALDI QIT TOF MS. Digests are separated by micro-LC and the eluent spotted automatically onto a MALDI target. This target is analysed using a novel MALDI mass spectrometer incorporating a quadrupole ion trap and a TOF analyser. This permits assignment of peptide mass and automatic data dependent MS/MS providing searchable sequence information.We will present examples of both 1D and 2D gel digests analysed by this method, and examine both the positive and negative aspects of this technology. MS^n data will be shown, displaying that high resolution and mass accuracy are essential when determining both protein sequence and the nature and position of any modifications.

P20-M

An Automated Control System for Nano-Electrospray

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Early investigations of Electrospray ionization (ESI) uncovered that liquid and the subsequent spray emitting from a nozzle may take many modes or forms. The specific spray mode obtained depends strongly upon the geometry of the emitter, the strength and shape of the electric field and the mobile phase chemical composition. At low flow rates, the smaller, monodisperse droplets having a high charge-to-mass ratio, appear to offer analytical benefits including improved ionization efficiency and reduction of ion suppression. The most effective mode for producing such droplets is the cone-jet mode is which a stable, non-pulsating Taylor cone is observed. Tuning of the cone-jet mode when using gradient chromatography can prove difficult as the mobile phase surface tension, viscosity and flow rate change dynamically during a gradient. This results in spray instabilities, shifts in charge state distribution and decreased performance for a set of fixed conditions. Current LC-MS sources provide no means to dynamically control the spray mode independently of spray or ion current.
A system has been developed using a number of opto-electronic schemes for the implementation of a self-tuning and self-adjusting ESI source. In these methods, optical channels of information are used to characterize and control the spray mode with variable conditions such as gradient chromatography. We present one of the three feedback systems developed called a static feedback system. The static spray mode control system involves the use of “machine vision” system in which an image acquisition and analysis computer determines the spray mode either through direct empirical measurements or through comparative anaysis. The system requires no user intervention during operation, opening the opportunity for extended, unattended analytical experiments at nanospray flow rates.

P21-T

An Alternative to Tandem Mass Spectrometry: Isoelectric Point and Accurate Mass for the Identification of Peptides

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The traditional approach to identification of peptides in complex biological samples integrally involves the use of tandem mass spectrometry to generate a unique fragmentation pattern in order to accurately assign its identity to a particular protein. In this presentation we

describe the theoretical basis and show experimental results for a new paradigm for the identification of peptides and proteins[1]. This methodology employs the use of accurate mass and peptide isoelectric point (pI) as identification criteria, and represents a change in focus from current tandem mass spectrometry dominated approaches. A mathematical derivation of the false positive rate associated with accurate mass and pI measurements is presented to demonstrate the utility of the technique. The equations for calculation of the experimental false positive rate allow for the determination of the validity of the data. The false positive rate issue examined in detail here is not restricted to accurate mass based approaches, but also has application to the tandem mass spectrometry community as well. The theoretical proteomes of *E. coli* and *R. noviticus* are used to evaluate the efficacy of this approach. The power of the technique is demonstrated by analyzing peptides with the same monoisotopic masses but with differing isoelectric points. The speed of the algorithm when combined with the experimental peptide analysis has the potential to rapidly accelerate the protein identification process. Data from immobolized pH gradient/reverse phase chromatography experiments[2] will be presented to demonstrate the utility of the technique in the field of proteomics.

1. Cargile, B.; Stephenson, Jr., J. *Anal. Chem.* **2003**, in press.
2. Cargile, B.; Talley, D.; Stephenson, Jr., J. *Electrophoresis*, **2003**, in press.

P22-S

A Cryodetector Mass Spectrometer with Mass Independent Sensitivity and Energy Resolution for Quantitative Analysis of Very Large Biopolymers.

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A MALDI –ToF mass spectrometer has been designed for use with a superconducting tunneling junction (STJ) detector array. The STJ is maintained at its working temperature of 0.4 K by a closed-system He^3 cryostat. The cryostat is designed for maintenance-free operation and regenerates automatically every 24 hours. STJs function by measuring ion kinetic energy deposited on their surface, an inherently 100 % efficient process. Since all intact ToF ions with the same charge have the same kinetic energy, the detection efficiency of STJs is independent of mass. There are 4 major consequences:

1. Cryodetector mass spectra represent the true ion yield, irrespective of mass, allowing quantitative assumptions to be made.
2. Detector performance does not deteriorate with increasing mass. At high mass, cryodetector mass spectrometers are orders of magnitude more sensitive than conventional ionizing detectors.
3. Since there is no theoretical mass limitation for ToF measurements, there is no theoretical mass limit for cryodetector ToFs.
4. Energy resolution in STJs allows direct measurement of ion charge state.

A 16 channel transient recorder generates a separate signal for each detector pixel. Specialized software and data processing are used to transform raw data (energy scatterplot) to a mass histogram. We illustrate the utility of this instrument by:

1. Protein detection at m/z = 2,200,000 Da.
2. UV laser nucleic acid analysis at m/z = 132,000 Da.
3. Quantitation of protein-protein and DNA-DNA interactions.
4. Determining the relative ionization efficiency for proteins in a mixture.

P23-M

High Energy Collision Induced Dissociation of Protonated and Deprotonated Peptides with MALDI TOF/TOF® Mass Spectrometer

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Tandem mass spectrometry is used to sequence peptides by collision-induced dissociation (CID), with part or all of a peptide sequence derived from the mass differences between “backbone” fragment ions, at or adjacently to amide bonds. Typically, peptide tandem mass spectrometry (MS/MS) has been carried out in the positive ion mode, by CID of protonated peptide molecules.

However, CID of deprotonated peptide molecules, in negative ion MS/MS, can yield sequence information complementary to that obtained under positive ion CID conditions. The peptide fragmentation with collision gas in the collision cell of a MALDI TOF/TOF mass spectrometer yields high-energy ions. In the positive ion mode, the observed peptide fragmentation patterns are the same as those that have been described for peptide MS/MS with hi-energy magnetic deflection tandem mass spectrometers. In the negative ion mode, sequence information for peptides is obtained by fragmentation of deprotonated peptide molecules. These mass spectra often contain abundant ions resulting from neutral losses of ammonia, water, or both, from sequence fragment ions, especially for asparagines, aspartic acid, glutamine and glutamic acid. Although, in general, the negative ion MS/MS spectrum of a peptide is not likely to contain more extensive sequence information than the corresponding positive ion spectrum, on many occasions complementary information from the former could be used to improve and refine the interpretation of the latter. It should be noted that negative ion MS/MS spectra were acquired from the same samples as positive ion spectra, thereby greatly facilitating the acquisition of positive and negative ion MS/MS spectra and the combination of peptide fragmentation information from both for more complete interpretation of the mass spectral data.

P24-T

Enrichment and Mass Spectrometry Method for Determining Sites of Nitration

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The nitration of protein tyrosine residues is one of several chemical modifications that can occur in the sites of inflammation. A large amount of literature is accumulating on the presence of nitrotyrosine in a number of inflammatory and neurodegenerative disorders. Only limited chemical evidence for protein nitration exists, due to the lack of a sensitive method for the determination of the sequence localization of nitrotyrosine in biological samples. Information on nitration sites of proteins is a necessary building block, hopefully leading to define the molecular effect of this modification in the pathogenesis of various disorders.
We have developed a method for enriching nitrated peptides by specifically derivatizing nitrotyrosine residues by biotin-conjugated reagents followed by affinity purification using an avidin column. The biotinylation method of nitrotyrosine has been applied to chemically nitrated proteins. We are currently developing a mass spectrometry method using MALDI-QqTOF and newly developed Triple Quadrupole Linear Ion Trap (QQQ-LIT) technologies to selectively detect peptides which contain biotinylated-tyrosine residue.
We presented a preliminary work at the 2003 International Mass Spectrometry Conference this summer. We tried product ion scans and precursor ion scan of 227 amu to look for nitrated species. In the current study, various new scan functions of the new QQQ-LIT system was employed to look for nitropeptide species that generate specific marker ions. Multiple-reaction-monitoring (MRM) triggered enhanced product ion scan mode was also used to “fish out” minor target peptides in the presence of native protein digest species.

P25-S

High-Binding Capacity Magnetic Particles for Phosphopeptide Isolation and Characterization

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Substrate level protein phosphorylation is a highly dynamic, reversible modification that plays a major role in regulating protein activity. Phosphorylation is the pivotal step in cellular signal transduction in response to extracellular signals. Phosphopeptide isolation and characterization is a challenging task especially

when dealing with low-abundance materials isolated from 1-D or 2-D gel samples. We developed a new phosphopeptide isolation system that utilizes novel metal chelate technology conjugated to high-binding capacity ferrofluid magnetic particles (Immunicon Corporation, Huntington Valley, PA) for use in the selective isolation of low-abundance phosphopeptides. The system is easily adaptable to high-throughput multi-well plate systems, and is compatible with downstream phosphopeptide characterization techniques including liquid chromatography and mass spectrometry. Here we also demonstrate the utility of magnetic particles for phosphopeptide isolation and characterization by combining magnetic particle isolation with base-catalyzed elimination/addition chemistry.

P26-M

A New Peptide *De-Novo* Sequencing Tool for Sophisticated Data Analysis

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De-novo sequencing of peptides becomes a central issue in proteomic research when database search results fail to explain all MS-peaks in a dataset. This might happen due to post-translational and other modifications or sequence errors in the database. To find out the origin of unexplained MS-peaks, a new *de-novo* sequencing module was integrated into a proteome analysis software. The obtained sequence information was successfully used to find the modified peptide sequence in the protein sequence.
Proteins from 2-D gels of various organisms were enzymatically digested, and MS- and MS(n)-spectra were acquired on an electrospray ion trap. The software used the automatically created peaklists to generate peptide sequence proposals, considering all provided hints including possible modifications. The resulting peptide sequences were scored against the experimental spectrum or used for homology searches.
In order to explain unknown MS-peaks, the information useable for homology searches was obtained from the *de-novo* tool using two strategies: First, major parts of the peptide sequence were successfully sequenced, and an unambigious sequence tag resulted. And second, multiple sequence proposals were obtained, which altogether were automatically used for the further analysis. Both, sequence tags as well as sequence proposal collection, served as basis for homology searches in locally available protein sequence databases using the BLAST technology. An amino acid transition and an incomplete database entry were easily localized applying this procedure.

P27-T

Analyzing complex Proteomic Samples with different Separation Techniques and High Capacity Ion Trap MS

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The analysis of complex protein samples by mass spectrometry requires good separation techniques. Either 2-D LC is used, or gel electrophoresis. 2-D gel electrophoresis is time-consuming and demands an experienced operator, but on the other hand, data management is simplified when proteins are separated before the enzymatic digest. A new trend is to gel-electrophoretically separate proteins on one dimension, and to analyse proteins from narrow gel slices. Prerequisite for a successful analysis using this technique is an ultrafast scanning mass spectrometer.
The soluble fraction of *E. coli* lysate was used to compare the usabability of both separation techniques. One aliquot was alkylated, digested with trypsin following standard procedures and used for 2-D nano LC. The second aliquout was separated by SDS PAGE and stained with colloidal coomassie. Gel slices were cut from the lane and digested using common procedures. Cystein residues were alkylated with acrylamide. Both samples were analysed by autoMS(n) experiments on an ion trap mass spectrometer and searched against protein databases.
Using the 1-D gel electrophoresis separation

approach significantly more proteins could be identified then following the peptide separation by 2-D LC. This might be due to different separation efficiency and dynamic range handling. The high capacity trap came up to be a highly adequate instrument for acquiring spectra due to its new trap geometry, which allows very high sensitive and ultrafast measurements.

P28-S

LID and high energy CID of Peptides in MALDI-TOF/TOF

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In the study we investigated the fragmentation of peptides under LID as well as high energy CID conditions and evaluated the effect of the different fragmentation mechanisms on the formation of specific fragment ion types and the usability of the resulting spectra e.g. on high throughput protein identification. Also basic investigations on the influence of the matrix, matrix additives as well as laser fluence on the fragment ion formation and the consequences in the spectral appearance (what is a "pure" high energy CID spectrum?) will be shown and discussed.

As it will be shown in the presentation, using DHB as a MALDI matrix it is easily possible to influence the desorption conditions in a way that LID spectra which are almost identical to spectra obtained from CHCA matrix are acquired at elevetated laser power. When working at the laser threshold, without collision gas almost no fragmentation appears in DHB while elevating the pressure in the collision cell, spectra could be obtained which were rich in side chain fragment ions (d- and w-type) and also showed much more fragments in the low mass region below 300 Da than typical LID spectra, while n the upper mass region they were lacking the b- and y-type ions typical for low energy fragmentation.

It was observed that, while containing a wealth of information, the CID spectra are significantly more complex than LID spectra and due to different fragmentation pathways, the CID spectra were of limited use for protein identification using the MASCOT database search engine, even under optimized parameter settings (MALDI-TOF/TOF vs. MALDI-TOF-PSD) due to significantly lower scores for the individual spectra.

P29-M

Phospho- and Glyco-peptides Analysis Using Negative and Positive AP-MALDI Ion Trap Mass Spectrometry

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The identification of post-translational modifications to proteins remains a challenge in current proteomics research and analysis. Several methods have been developed to analyze the different phosphorylation and glycosylation sites on peptides and proteins. No universal method for the purification and analysis of modified peptides and proteins exists. One of the main challenges in this area is purifying and concentrating the minuscule amounts of modified proteins of interest from thousands of other modified and unmodified peptides and proteins. Currently, different affinity chromatography methods, such as immobilized metal affinity chromatography for the purification of phosphopeptides, or lectin-based affinity chromatography for glycoproteins or glycopeptides, can be used to selectively isolate and concentrate modified proteins. After partial purification with micro pipette tips, peptides are analyzed by atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry. The results presented here show that by using negative and positive modes of ionization and by performing MS-MS of selected mass peaks, one can determine the structure of different phosphopeptides and glycopeptides. Furthermore, the information obtained by using both modes of ionization provides information on specific glycosylation sites and on the sequence and modification of carbohydrates on a particular site on the protein. Such carbohydrate modifications are already known in nature and, for instance, in nature sialic acid exists in over 50 different forms (by the addition of functional

groups such as acetyl, lactyl, formyl, methy glycolyl and sulpho groups). In the future, the method described here can be used as a powerful tool to obtain both structural information on glycosylation and phosphorylation sites and to determine modifications on carbohydrates positioned at such sites.

P30-T

Organic Solvent Extraction of Proteins and Peptides from Serum as an Effective Sample Preparation for Detection and Identification of Biomarkers by Mass Spectrometry

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During recent decades important tumor markers such as _-fetoprotein, CEA, and PSA have been discovered. Progress made in characterization of protein by mass spectrometry has stimulated attempts to detect and identify new biomarkers associated with cancer for the purpose of early diagnosis and monitoring patients' responsiveness to antitumor treatments. The ability to compare proteins in un-fractionated serum is hampered by the presence of several highly abundant proteins. The purpose of this study was to investigate the possibility of selectively removing large abundant proteins from serum by precipitation using organic solvents. Our results show that two volumes of acetonitrile containing 0.1% trifluoroacetic acid efficiently precipitates large abundant proteins while smaller proteins and peptides stay in solution and subsequently can be analyzed by SELDI or MALDI-TOF MS. The application of this procedure to mouse serum samples prior to SELDI-TOF MS analysis significantly improved the mass spectra. Many of the polypeptide signals present in the SELDI-TOF spectra of the extracted material are absent in the spectra of total serum, owing to the removal of high abundant proteins. The observation of these peptides in the serum extract was crucial for the detection of two markers that were of lower abundance in the sera from mice with B cell lymphoma. Moreover, because the extracted material had much less total protein, it facilitates the purification and identification of disease-related markers.

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P31-S

Magnetic bead assisted on-target digestion – a rapid and simple approach for bottom-up MALDI analysis of proteins

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We describe a protocol for on-target digestion using functionalized magnetic nano-beads (MB) for on-target capturing / fixation of proteins. The major strengths of the method is its speed, sensitivity and ease of use. The availability of magnetic beads of various surface functionalities allows to further optimize the technique with respect to the nature of the protein investigated and the scope of analysis.

The protocol covers the following steps: Apply a suspension of magnetic beads onto the MALDI target. Allow drying. Wash or condition the beads surface and apply the protein solution. Add reducing agent (DTT) and dry the mixture at elevated temperature. Wash and add the enzyme. Add matrix solution after digestion and another rinsing step.

The method has been tested for a number of proteins. As it turned out, a key issue for the success of the method is the use of pre-structured Anchorchip MALDI targets to avoid spreading of any of the solvents applied in the protocol and to restrict the sampling / reaction zone to a well defined minimum area for increased sensitivity. The reduction step increased the number of observed peptides and the sequence coverage. Typically, a ten-minute incubation time with trypsin provided efficient protein digestion. The short incubation time rendered cystein-alkylation unnecessary to prevent disulphide scrambling. The protocol was capable to treat sample amounts (BSA) down to at least 200fmol giving

sequence coverage of more than 60%. Classical in-solution digestion protocols are inefficient at this concentration level because of a low substrate concentration, which limits digestion speed according to the Michaelis-Menten kinetics of enzyme action.

P32-M

Increasing the Sensitivity of Top-Down Protein Analysis: MALDI-TOF-reISD and T^3-Sequencing

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Top-down sequence analysis of recombinant proteins has been introduced recently, referring to the MS/MS analysis of intact proteins avoiding protein digestion steps. On MALDI-TOF instrumentation, in-source decay in reflector mode (reISD) and "T^3-sequencing" provide the capability to analyze protein termini – even in the case of terminal modification. T^3-sequencing allows to localize unexpected modifications/mutations on the intact protein level and even to identify a protein without the need of digesting it with trypsin [*Suckau D, Resemann A (2003), Anal Chem,* ***75****, 5817-5824*]. ReISD is a process with a low fragment ion yield. Therefore, quality control (QC) of recombinant proteins is the first application of this technology as sample amounts in the 10-30 pmol range are required and typically available from recombinant work.
In this work we use 2 concepts of increasing the sensitivity for top-down analysis: we used magnetic nano-particles coated with reverse phase material to further purify the analyte even from crude solutions. This effect alone facilitated the acquisition of T^3-spectra significantly. In addition, we used MALDI targets with hydrophilic/hydrophobic profiles (AnchorChip), which allow concentrating the sample to an area of 600 _m diameter [*Schuerenberg M et al. (2000) Anal Chem* ***72****, 3436-3442*]. This concentration on the surface allowed the reduction of the required sample amount for reISD spectra of proteins such as RNase or carbonic anhydrase below the 500 fmol level. This level of sensitivity will likely open up the application scope of top-down analysis to the field of proteins purified from natural sources and even top-down proteomics.

P33-T

Identification and Profiling of Indoor Molds by MALDI-TOF Mass Spectrometry

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Molds pose health risks, aggravating asthma, causing allergic reactions and other ailments. Fungi pathogenic to humans and agriculture are potential bioweapons. Classical methods for identifiying molds require time consuming culture and morphological characterization. Such methods are slow, low throughput, and usually stop at the genus or higher level. Molecular genetics can distinguish species and strains of various organisms, overcoming limitations of traditional methods. Commonly, DNA is amplified using PCR with readout of product mass, electrophoretic mobility, or capture by arrays of oligonucleotides. All require live organisms, genetic knowledge, expense, and only indirectly assess physiological state. Profiling proteins and other molecules with mass spectrometry (e.g., MALDI-TOF MS) is faster and cheaper. MS fingerprinting has succeeded with bacteria and should work with molds. However, simple solvent extractions have given too few ions. Sample processing can rescue this approach. Tandem solvent extraction fractionates for more data points. Breaking proteins into fragments generates still more data and may capture conserved sequences, revealing relationships not apparent with intact proteins. Methionine cleavage with CNBr is preferred to the classical tryptic digestion because substrate dissolution is much better, fragments are fewer, and a new variant of the method provides a ready route to quantification. Chemical classes such as aminophospholipids, carbohydrates, nucleic acids, primary and secondary metabolites, as such or derivatized, can contribute to profiling, all on the same platform. Functional tests such as enzyme assay also can be performed by MALDI. A selection of local mold species was easily discriminated using proteins, and a few group

specific ions observed. Lipid analysis was also effective, but other biomolecules were more difficult to see, and good quantitative markers for total mold by MALDI have not yet been established.

P34-S

An investigation into the reproducibility of strong cation exchange chromatography in combination with reverse phase LC-MS/MS on a hybrid quadrupole orthogonal acceleration time-of flight (Q-Tof) mass spectrometer

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If a complex protein mixture is to be investigated by LC-MS/MS then a fractionation step prior to separation of the peptides on the basis of their hydrophobicity is advantageous. This has resulted in 2D HPLC approaches being adopted for the analysis of extremely complex tryptic digest samples. By placing a strong cation exchange (SCX) cartridge followed by a C18 trap cartridge, or by using a bi-phasic analytical column it is possible to pre-fractionate the peptides on-line before separation and analysis by reverse phase LC-MS/MS.
In this paper we describe the optimisation of an off-line SCX fractionation step, in combination with automated fraction collection, prior to analysis by reverse phase LC-MS/MS. We will compare this to an on-line SCX fractionation of the sample, followed by on-line LC-MS/MS. Data will be presented from both approaches on a tryptic digest of an *E. coli* cytosolic fraction. The reproducibility of both approaches will be compared and contrasted at the peptide level. In addition the total number of non-redundant protein identifications will be compared over replicate analyses using both approaches.

P35-M

Protein pre-fractionation strategies for the separation of complex mixtures prior to electrospray and MALDI mass spectrometry

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The most commonly used approaches for protein identification and characterisation using mass spectrometry involve the detection and subsequent fragmentation of proteolytic peptides. Often complex mixtures of proteins will be subjected to some form of pre-fractionation and separation prior to digestion, reducing the complexity of the digest and the dynamic range of protein concentrations. In the direct analysis of unseparated digest mixtures of, for example, a cell lysate, the challenge is the dynamic range present in the sample. In this presentation, two different approaches prior to mass spectrometric analysis on a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer were investigated.
The first route involves the tryptic digestion of the whole protein mixture prior to analysis by two-dimensional nanoscale chromatography. The first dimension is a separation based on charge using strong cation exchange (SCX) chromatography and the second dimension a separation based on hydrophobicity using reversed phase (RP) chromatography.
The second route involves reducing the complexity of the protein mixture by two-dimensional chromatography using a biocompatible liquid chromatograph. The two dimensions used are SCX chromatography followed by a separation based on hydrophobicity using RP chromatography. Fractions are collected at specific time intervals and each protein-containing fraction is then digested to produce tryptic peptides for analysis by nanoscale liquid chromatography. Depending on the complexity of each peptide-containing fraction, this second stage could also involve separation based upon charge, using SCX, followed by a separation using RP.
The results from the analysis of a 30 protein standard mixture will be presented as well as the analysis of a K12 strain derived *Escherichia-coli* sample.

P36-T

Standardized sample conditioning as fundamental requirement for clinical proteomic studies based on MALDI-TOF mass spectrometry

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The profiling of naturally occurring peptides and proteins reflecting the status quo in biochemical mechanisms and pathways of cells gain more and more in importance of clinical proteomic approaches. MALDI-TOF mass spectrometry is an unique tool for analysis of such molecules from biological fluids (e.g. human serum).Up to now there is only limited information about effects e.g. due to repeated freeze-thaw-cycles or other storage conditions which may significantly influence phenotype-related patterns. We present a novel method comprising enrichment and fractionation of peptides, proteins and other biomolecules from human serum based on diverse magnetic bead associated chromatographies followed by MALDI-TOF MS analysis.

The ClinProt system encompasses automated sample preparation, high-performance matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) analysis, and bioinformatics package. The sample preparation is based on a magnetic bead associated chromatography system. Using an eight channel pipetting robot with a dedicated magnetic separation device a high reproducible and automated sample preparation could be demonstrated. For sample preparation on the MALDI-TOF target alpha-cyano 4-hydroxy cinnamic acid was applied as matrix. Measurements were performed in MALDI-TOF mass spectrometer in the linear mode alternatively manually or automatically. In dependence on the biological material investigated up to 150 peaks with high intensities in the mass range from 1000 to 10000 Da were achieved.

A bioinformatics package based on mathematical algorithms was used for the comparison of data sets obtained from samples with various biological background, e.g. human serum samples exposed to different storage conditions. With the help of the software differences between the samples due to an obviously occurring protein degradation could be pointed out.

P37-S

Mass Spectrometric Identification of a Protein Biomarker Unique to the Pandemic O3:K6 Clone of *Vibrio parahaemolyticus*

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The current method for characterizing strains of *Vibrio parahaemolyticus* involves serotyping or detection methods based on assessing the presence or absence of genes thought to be a marker of an organism’s pathogenicity. It is unclear whether these assays detect all pathogenic *Vibrio parahaemolyticus* strains since a clear correlation between the presence of a particular gene and the organism’s pathogenicity has not yet been observed. We have described a proteomics-based method to distinguish individual bacterial strains of *Vibrio parahaemolyticus* based on their protein profile and identified a specific protein that is characteristic of the pandemic O3:K6 strain and its clonal derivatives. In the pandemic clone of *Vibrio parahaemolyticus*, a histone-like DNA-binding protein HU-_ has a C-terminal amino acid sequence different from that of other strains of *Vibrio parahaemolyticus*. Upon further study, it was discovered that the gene encoding this protein has a 16 kbp insert at the 3’ terminus of the open reading frame (ORF) for this protein. Using the protein sequence of the unique biomarker for the pandemic clone of *Vibrio parahaemolyticus*, it was possible to rationally design specific PCR-based probes and assays that permit the rapid and precise identification of pandemic strains of *Vibrio parahaemolyticus*.

P38-M

A Profile of Differentially Expressed Proteins in Macrophageal Response to Bacterial Quorum Sensing Using cICAT Reagents and LC/MALDI/MS/MS

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Bacteria form highly structured and cooperative consortia commonly referred to as biofilms. Microbial activities associated with these biofilms are on account of the ability of bacteria to distribute metabolic activities between the different members of the consortium demanding a high degree of coordinated cell-cell interaction. This is achieved through the production and release of small signaling molecules or pheromones, referred commonly as quorum sensing (QS) molecules. Though the use of QS molecules by bacteria to modulate its responses are well established little is known about the effects of bacterial-derived QS molecules on host cells.
Initial studies in our laboratories have indicated an effect of AHL QS molecules on the phagocytic ability on human macrophages. Differentiated THP-1 cells treated with 10, 50 and 100 uM concentration of AHL molecules demonstrated a significant increase in the number of macrophages that phagocytosed fluorescent latex beads. However, no increase in the phagocytic index was observed suggesting QS molecules have no effect on the phagocytic capacity per se. To examine the response of the principal host cell, the macrophage to gram-negative bacterial QS AHL molecules we utilized Applied Biosystems's 4700 Proteomics Discovery System with TOF/TOF™ ion optics to identify and quantify modulated expression of affected proteins. By coupling the cleavable ICAT labeling technology of treated and untreated cell lysates with result dependent analysis using LC/MALDI/MS/MS technology and the Celera Discovery System™ we were able to develop a profile of differentially expressed proteins in macrophageal response to bacterial quorum sensing.

P39-T

Evaluation of a Novel, Integrated Approach Using Functionalized Magnetic Beads, Bench-Top MALDI-TOF MS with AnchorChip™ Technology and Pattern Recognition Software for Profiling Potential Biomarkers in Human Plasma

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The advantage of using proteins/peptides as biomarkers is that they can be found readily in biological fluids. Such sample types are readily obtainable and represent a potentially rich palette of biologically informative molecules. MALDI-TOF mass spectrometry has become a powerful tool for surveying such sample types. The goal of clinical proteomics is to harness the power of this tool for identifying novel, condition-specific protein fingerprints that may, in turn, lead to the elucidation and use of disease-specific biomarkers. Here we have evaluated a simple affordable bench-top MALDI-TOF MS to generate protein profiles from human plasma samples of asthma patients and healthy individuals. We achieve this profiling by using C8-functionalized magnetic beads that enrich a specific subset of plasma proteins based on their absorption to this resin. This step is followed by elution, transfer onto AnchorChip™ targets and analysis in a bench-top MALDI-TOF MS mass spectrometer (OmniFLEX™, Bruker Daltonics) with AutoXecute™ acquisition control which enables automated operation with reproducible results. Resulting spectra are compiled and analyzed through the pattern recognition component of *ClinProTools*™ software. This approach in combination with *ClinProTools*™ software permits the investigator to rapidly scan for potential biomarker peptides/proteins in human plasma. The reproducibility of plasma profiles within- and between- days has been evaluated. The results shows that the novel and facile approach with manual magnetic bead

sample preparation and a low-cost bench-top MALDI-TOF MS is suitable to low/medium-throughput plasma protein profiling

P40-S

An Automated LC/MS System for Both High-Throughput and Detailed Analysis of Oligonucleotides

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Oligonucleotides (oligos) are widely used in important biochemical and pharmaceutical research. Since oligos are produced using multi-step synthetic procedures, it is desirable to confirm the presence and purity of the final product for all oligos produced. In high-throughput oligo synthesis labs this can be quite a challenge, given the huge number of oligos produced daily. In recent years, MALDI-ToF has become a standard quality control/analysis technique in high-throughput oligo synthesis labs. However, MALDI-ToF is not particularly effective for analyzing long oligos greater than ~ 50 bases in length. In addition, obtaining MALDI spectra on oligos with labile chemical moieties can be difficult due to degradation in the acidic matrix or dissociation during desorption ionization. In this presentation we describe a unique system, which combines electrospray ionization mass spectrometry (ESI/MS), automated on-line sample prep/desalting, and automated data processing for high-throughput automated analysis of oligos. The automated system consists of a LEAP HTS-PAL autosampler, Michrom Paradigm MS4 HPLC with UV detector, and ThermoFinnigan TSQ7000 LC/MS system. The PAL autosampler injects oligo samples onto a dual trap column configuration mounted on the MS4 HPLC, which allows for a sample analysis time of ~1.4 min/sample including desalting. Data analysis is accomplished using automated ESI spectral deconvolution software (ProMass). ProMass produces a web-based summary of all sample analyses and includes a color-coded 96-well plate viewer that allows rapid mass confirmation and visualization of results from any web-browser. The system is shown to be effective for oligos exceeding 120 bases in length, while providing routine mass accuracies of 0.01-0.02% and a sample throughput of over 1000 samples (> 10x96-well plates) over 24 hours.

P41-M

A New Lysine Derivitization Reagent for MALDI-MS and MALDI-MS/MS

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The use MALDI MS for peptide mass mapping has become a method of choice for identifying proteins from 1 and 2D gels. One of the major drawbacks is that the lysine containing tryptic fragments are less sensitivity than the arginine containing fragments. Krause found peptides that contain arginine exhibit an increase of 4-18 in signal intensity. Several groups have overcome this problem by reacting the lysine peptides with O-methylisourea to convert the lysine residue to a more basic homoarginine. Although the derivatization with O-methylisourea is effective in increasing the signal intensity of the lysine containing peptides, it is not directly amenable to application for differential quantitation. Also O-methylisourea derivatives yield little or no additional sequence information when compared to the underivitised peptides in MS/MS of the single charged ions.

A new reagent for the use in proteomic studies is 2-methoxy-4,5-dihydro-1H-imidazole. Similar to O-methylisourea, this reagent is lysine specific and increases the signal intensity for lysine containing peptides. Our data indicates an increase in signal intensity for the derivatized lysine peptides of 5->20 times relative to underivatized lysine peptides.

Unlike the O-methylisourea, this reagent localizes the charge at the C-terminal of the peptide, resulting in an increase in the y-ion formation in MS/MS of single charged ions. The increase in sensitivity and predominant y-ion series results in greater confidence in protein identification. An additional advantage for this reagent is that the structure allows for the

incorporation of 4 Deuteriums for relative quantitation. This advantage is demonstrated by the use of the heavy (D4) and light (H4) forms of the reagent for protein digest labeling.
Krause E, Wenschuh H, Jungblut PR Anal. Chem. 1999; 71: 4160.

P42-T

MALDI TOF-based Amino Acid Analysis.

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Quantitative amino acid analysis is widely used in modern biochemistry, providing quite accurate determination of amino acid composition of proteins and concentration of free amino acids in cell culture media or fermentation processes. Free amino acid analysis plays also an important role in clinic diagnostics. Quantitative amino acid analysis is widely used in clinical laboratories for diagnostics of different diseases like phenylketonuria, tyrosinosis, homocystinuria, etc. However, HPLC, by which this analysis is traditionally performed, requires a time-consuming sample preparation that includes different types of derivatization protocols.
MALDI-TOF mass spectrometry finds an extensive use in the analysis of proteins and peptides. Recent improvements in MALDI TOF technology led to an increased application of MALDI TOF for the analysis of low molecular weight biologically active compounds such as lipids, neurotransmitters, vitamins, fatty acids and amino acids.
Our experiments demonstrate that MALDI-TOF-MS could be used efficiently for a comprehensive qualitative and quantitative determination of amino acids and their derivatives. Quantitative determination of 18 standard and 5 unusual amino acids was performed using three different matrixes and two different amino acids as internal standards to calculate response factors (RF) for an individual amino acid. Calculated RFs were used for quantification of individual amino acids in pre-mixed solutions. Thus, the sample preparation step was reduced to mixing an analyte solution with matrix and co-crystallization – both steps together might be completed in a few minutes.

P43-S

Identification of low-femtomole level protein digests by MALDI-MS/MS using Matrix-Assisted Laser Desorption Ionization and a Linear Ion Trap Mass Spectrometer

V. Kovtoun, H. Tran, G. Stafford, K. Miller; Thermo Electron, San jose, CA, United States.

A novel, low-pressure Matrix-Assisted Laser Desorption Ionization source and a linear ion trap mass spectrometer (MALDI LTQ) were combined to demonstrate low-femtomole level detection capability for protein digests. The advantages of a fast scan rate and high ion storage capacity from the linear ion trap and the simplicity of MALDI ionization provided a sensitive, high throughput system for protein identification. Samples of standard proteins were digested and diluted with water/0.1 % trifluoroacetic acid (TFA). Alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix was diluted to 0.25 mg/mL concentration with acetonitrile/water/TFA. The sample and matrix were applied to stainless steel MALDI target plates as 0.25 uL spots via the dry-drop technique. Full scan MS data were obtained by either 1 or 5 scans. For each protein digest, at least two mass fragments were analyzed using tandem MS data. Full MS data show strong, identified sample mass peaks for digests at 1-femtomole level. MS/MS data for these mass fragments were generated and searched by Turbo SEQUEST. Search results successfully confirmed the identification of all 1-femtomole protein digests. This experiment showed that fast scan rate and high sensitivity in the MALDI LTQ provided good signal-to-noise full MS spectra with only 1 scan (approximately 1 second total scan time) along with searchable MS/MS data and strong X correlation values from database searches.

P44-M

Analysis of Low-Abundance Serum Proteins Using Mass Spectrometry

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In order to detect early-stage diseases, new biomarkers are needed that have adequate sensitivity and specificity to be applicable in detecting diseases in a large population. The classical approach for identifying disease-related proteins is two-dimensional polyacrylamide gel electrophoresis, but this method is labor intensive, requires large amounts of protein, and is not easily adapted into a diagnostic test. Recent studies have reported using mass spectrometry (MS) to identify new serum biomarkers for breast and ovarian cancer. Serum contains 60-80 mg protein/mL, but 57-71% of this is serum albumin, and 8-26% is _-globulins. These large proteins must be depleted before smaller less-abundant proteins can be detected using MS. Affinity columns are commonly used to remove both serum albumin and _-globulins, but because serum albumin is known to act as a carrier for smaller proteins, removal of these molecules using columns may result in the loss of molecules of interest.
The objective of this study was to develop a reproducible method to deplete serum samples of high-abundance proteins in order to identify the less-abundant proteins present in serum. We used organic solvents to precipitate the large proteins out of solution. This also caused many smaller proteins to disassociate from their carrier molecules, allowing for better detection of a larger range of small proteins. After precipitation, the supernatant was concentrated to 0.1 _g protein/mL and acidified with formic acid. These samples were analyzed using liquid chromatography coupled with electrospray ionization mass spectrometry (LCMS). The data were collected using BioAnalyst QS software. An analysis of a two-minute segment of our data resulted in detection of an average of 718 molecular species with molecular weights between 1,500 and 10,000 Da.

P45-T
Strategy for Maximizing Protein Identification by MALDI-MS/MS using a Linear Ion Trap Mass Spectrometer with an Intermediate-Vacuum MALDI Source
M. Prieto, H. Tran, V. Kovtoun, G. Stafford, K. Miller; Thermo Electron, San Jose, CA, United States.

Proteomics has placed unique throughput demands on the process of identifying proteins. Mass spectrometry, particularly ion trap mass spectrometry, has become the de facto standard for protein identification by LC/MS. Tentative steps have been taken to couple MALDI ionization to ion trap mass spectrometers: AP MALDI sources have demonstrated the utility of MALDI-MS/MS analysis. Recently, an intermediate-vacuum MALDI source has been developed and coupled to a linear ion trap mass spectrometer to create a new platform capable of extremely high sample throughput, combining the speed and simplicity of MALDI ionization with the sensitivity, fragmentation efficiency, and mixture tolerance of a linear ion trap mass spectrometer.Using manual data acquisition and many automated, intelligent data acquisition modes, the ability of the MALDI-linear ion trap to identify proteins in a variety of sample types was tested. Dilution series of digests of protein standards, mixtures of these standard digests, and fractions from off-line ion exchange separation and digestion of a highly complex protein mixture were analyzed. Automated analysis of a 384-position plate spotted with digests from protein standards, showed that all sample spots could be very rapidly identified. Acquisition time increased slightly with decreasing concentration, but automated data acquisition was still possible down to sample loads of 1fmol. Similarly, rapid automated analysis of mixtures was demonstrated. Finally, analysis of very complex mixtures demonstrated that a very large number of proteins could be confidently identified in a single sample spot using either manual or automated data acquisition.

P46-S
AP-MALDI TOF: A sensitive tool for peptides analyses
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Due to the capability of high resolution and mass accuracy, oa-ESI-TOF is becoming a more popular technique for proteomics applications, such as intact proteins molecular weight determination and sensitive analyses of peptides via nano-ESI-TOF. The enhanced capability of coupling an AP-MALDI source and an oa-TOF mass spectrometer allow the robustness and high throughput for peptides analyses. Several different protein digests were analyzed by an AP-MALDI-oa-TOF system. Sub-femtomole level sensitivity was achieved and the dynamic range in a sample was greater than 20-fold. The mixture of three and four protein digests were also successfully analyzed by the AP-MALDI TOF system. Mass accuracy of 5 ppm was achieved by using internal reference ions calibration.

P47-M

Maximizing Plasma Protein Coverage: New Two-Dimensional Nanoelectrospray Linear-Ion Trap LC/MS System for Complex Proteomic Analysis

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Identification of all proteins in complex biological samples is a considerable challenge, especially low abundance proteins. Two-dimensional LC/MS methods have been used to resolve complex peptide mixtures. This technique has recently been extended to a nanospray interface and combined with a linear ion trap to provide the highest possible sensitivity and dynamic range for proteomic analyses. Using a BSA protein standard, it has been shown that the sensitivity of such a system is about 50 amol of protein on column. Previous studies with such a system have shown dynamic range of 1×10^7. In this study, such a system was used for proteomic analysis of human plasma. A strong cation exchange (SCX) column was used as the first dimension, followed by a peptide trap for sample concentration and desalting, then by C18 reversed phase chromatography as a second dimension. Data were analyzed using TurboSEQUEST. Results show hundreds of proteins identified with very high probability.

P48-T

Biological Interpretation of Mass Spectrometry Results

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Mass spectrometry is a well accepted technique for protein identification. Once a protein has been identified, however, often there are many questions regarding the biology of that protein. The Celera Discovery System™ or CDS, can provide answers to many of those questions. The Celera Discovery System is a subscription-based integrated research platform that provides on-line access to comprehensive curated biological data. By searching specially prepared FASTA files incorporating information from CDS, protein molecular functions and biological processes are imported into all protein search results. Thus, important biological information is visible alongside protein identifications. Direct links to CDS from MS protein ID software (Pro ID, Pro ICAT, and GPS Explorer™ Software) enable further analysis of ontology information as well as comprehensive genomic and biological information. For example, tools for PTM prediction can be used to verify putative modification sites found by mass spectrometery. The SNP database can be used to find all mis-sense SNPs that would result in amino acid substitutions at the protein level, identifiable by mass spectrometry. This poster will focus on the different types of information available from CDS and how this information can be used to provide biological insight from various protein ID and quantitation applications.

PROTEIN SEQUENCING TECHNIQUES

P49-S

Using Dynamic Exclusion on a Linear Ion Trap/FT-ICR Mass Spectrometer to Increase Sequence Coverage for On-line Automated Separation of Peptides and Proteins

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In the past several years, development of peptide/protein based drugs in the pharmaceutical realm has increased in prevalence. Traditionally QA/QC functions have relied on LC-UV as the main source of product assurances; this could change with the growth and increase in user friendliness of mass spectrometry greatly improving its utility as a qualitative tool. Top-down sequencing shows great potential for studying degradates/modifications. The technique, requiring long ion accumulation and complex activation methods, so far has been limited to off-line analysis on FT-ICRs requiring their high resolution and mass accuracy to analyze the large charge state fragments produced by intact species. The Finnigan LTQ-FT hybrid linear trap/ICR mass spectrometer (Thermo Electron, Bremen, Germany) provides a new unique opportunity to perform MS/MS on-line in an automated fashion. Use of automated dynamic exclusion software in the ion trap instrument control allows the user to activate more than one charge state of a species even when multiple peptide or proteins are eluting to increase sequence coverage.
In our work, the dynamic exclusion capabilities found in the spectrometer's instrument control software will be exploited to activate as many charge states as possible in order to maximize sequence coverage. A mixture of mellitin, angiotensin, Substance P, and Ubiquitin will be separated using a fast LC gradient and sequenced using tandem mass spectrometry of the various charge states. Extracted ion chromatograms of coeluting peaks and their corresponding tandem mass spectra will be evaluated for sequence coverage, fragmentation efficiency as a function of charge state, and the ability to search databases for protein identification.

P50-M
On-Line Identification of Posttranslational Modifications and Sequence Irregularities Using a Hybrid Linear Trap/FT-ICR Mass Spectrometer

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Top-down proteomics has shown great promise for studying posttranslational modifications (PTM's). Some advantages offered by the top-down approach over bottom-up sequencing include reduced sample handling and more efficient ionization for the modified region of the protein. Due to the high charge states resulting from electrospray ionization of intact proteins, top-down sequencing studies are primarily performed on an FT-ICR mass spectrometer which provide the high resolution/mass accuracy capabilities needed to measure isotope spacing of resulting fragment ions for accurate molecular weight assignments. Predominantly, off-line protein analysis must be performed due to time-consuming ion accumulation and activation methods within ICR cells. Hence, the Finnigan LTQ-FT hybrid linear trap/ICR mass spectrometer (Thermo Electron, Bremen, Germany) provides many advantages to performing top-down proteomics. Collection, isolation, and excitation of ions in the linear trap followed by detection in the ICR cell results in a greater duty cycle while maintaining the high resolution/mass accuracy needed for correct fragment ion molecular weight assignments.
In this work, top-down sequencing capabilities of the LTQ-FT are presented on chromatographic separations of intact proteins and large peptides. Experiments were performed on an equimolar mixture of five peptides and proteins, on-column analysis of native and oxidized ubiquitin, and a mixture of horse heart and rabbit skeletal muscle cyctochrome c. Presented results include sequence coverage as a function of molecular weight, identification of the oxidized methionine from ubiquitin, and identification of the six different residue sites between horse heart and rabbit skeletal cytochrome c.

AMINO ACID ANALYSIS

P51-T

Determination of Amino Acids in Cell Culture and Fermentation Broth Media using Anion-Exchange Chromatography with Integrated Pulsed Amperometric Detection

V. P. Hanko, J. S. Rohrer, **A. Heckenberg**; Dionex Corporation, Sunnyvale, CA, United States.

Cell culture and fermentation broth media are used in the manufacture of biotherapeutics and many other biological materials. Characterizing the amino acid composition in cell culture and fermentation broth media is important because deficiencies in these nutrients can reduce desired yields or alter final product quality. Anion-exchange (AE) chromatography using sodium hydroxide (NaOH) and sodium acetate gradients, coupled with integrated pulsed amperometry (IPAD) determines amino acids without sample derivatization. AE-IPAD also detects carbohydrates, glycols, and sugar alcohols. The presence of these compounds, often at high concentrations in cell culture and fermentation broth media, can complicate amino acid determinations. To determine if these samples can be analyzed without sample preparation, we studied the effect of altering and extending the initial NaOH eluent concentration on the retention of 42 different carbohydrates and related compounds, 30 amino acids and related compounds, and 3 additional compounds. We found that carbohydrate retention is impacted differently than amino acid retention by a change in [NaOH]. We used this selectivity difference to design amino acid determinations of diluted cell culture and fermentation broth media, including Bacto® yeast extract-peptone-dextrose (YPD; yeast culture medium) broth, Luria-Bertani (LB; bacterial culture medium) broth, and minimal essential medium (MEM) and serum-free protein-free hybridoma medium (mammalian cell culture media). These media were selected as representatives for both prokaryotic and eukaryotic culture systems capable of challenging the analytical technique presented in this paper. Glucose up to 10 mM (0.2%, w/w) did not interfere with the chromatography, or decrease recovery greater than 20%, for the 17 common amino acids.

P52-S

Disposable Gold Electrodes for Derivatization-Free Detection of Amino Acids by Ion Exchange Chromatography

P. Jandik, J. Cheng, **A. Heckenberg**; Dionex Corporation, Sunnyvale, CA, United States.

AAA-Direct^{TM} is a new technique that combines High Performance Anion Exchange Chromatography (HPAEC) with Integrated Amperometric Detection (IPAD) using a gold working electrode, to provide efficient separations and highly sensitive detection of all amino acids. Only three single component eluents are required: water, 250 mM sodium hydroxide and 1 M sodium acetate. The IPAD detection is performed using a pH/Ag/AgCl electrode reference electrode to minimize baseline perturbations that would otherwise be observed with a more common referencing method using only the Ag/AgCl reference electrode.
In electrochemical detection, conventional (non-disposable) gold working electrodes must be reconditioned after a period of use, by polishing. This manual electrode polishing is difficult to perform in a reproducible manner, and always requires a long period of re-equilibration after an electrode is re-installed inside the electrode cell. To address this inconvenience, and to minimize system downtime, disposable electrodes have been developed for use over precisely defined periods of time. Following the prescribed period of time, a used disposable electrode is simply disposed of and replaced by a new disposable electrode. The need for manual polishing is thus avoided, resulting in a more reproducible and reliable technique. AAA-Direct with disposable gold electrodes was tested and found compatible for all common protein and glycoprotein hydrolytic procedures.

HPLC OF PROTEINS AND PEPTIDES

P53-M

An Automated Methodology for Optimizing Offline IEX Separations for 2D LC/MS Proteomic Analyses

S. J. Berger, S. A. Cohen; Waters Corporation, Milford, MA, United States.

Offline SCX fractionation of complex peptide mixtures is becoming increasingly important in proteomic workflows. This is due, in large part, to simplified troubleshooting of the decoupled IEX and RP separation dimensions, and the increased control provided over sample complexity and mass loading for subsequent cLC/MS(/MS) analyses of the fractions. In particular, offline SCX fractionation experiments permit the scientist to control gradient and fractionation parameters, to optimize the number of SCX fractions, and the distribution of peptides between them. Additional benefits derived from offline separations are that organic solvents used in the first dimension can be removed or diluted prior to subsequent cLC/MS(/MS) analyses, and that equal mass loading of fractions on the capillary LC column can promote detection of lower abundance components, without column overloading. Similarly, multiple analyses of offline collected fractions can also be undertaken to fully realize the potential of DDA LC/MS(/MS) methodologies by utilizing peptide MS/MS exclusion lists from previous runs. In this poster, we will show how an automated AutoBlend™ methodology (combining organic solvents, neutral salt, water, and acidic/basic forms of a buffer online) permits the rapid development of fractionation methods for complex peptide mixtures with minimal user intervention. Peptide fractionation methods developed using model systems (e.g. individual protein digests) are applied to global proteomic digest samples of *E. coli* analyzed as part of a standard proteomic analysis workflow.

P54-T

Analysis of Microvesicle-Depleted Virus. Quantitation of HLA Class II Protein Incorporated into HIV-1

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The cellular proteins found in purified retroviruses preparations are considered in two categories. First, there are cellular proteins that are actually incorporated into virions, either as integral membrane proteins in the virion envelope, or sequestered in the interior of particles. Secondly, since even highly purified preparations of virions are inevitably contaminated by co-purifying microvesicles , there are cellular proteins present in such preparations that are not virion associated. Among the many host cell-derived proteins found in HIV-1, HLA Class II (HLA-II) appears to be selectively incorporated onto virions and may contribute to mechanisms of indirect immunopathogenesis in HIV infection and AIDS. However, the amount of HLA-II on the surface of HIV-1 particles has not been reliably determined due to contamination of virus preparations by microvesicles containing host cell proteins, including HLA-II. CD45, a leukocyte integral membrane protein, is found on microvesicles, yet appears to be excluded from HIV-1 particles. We have developed a CD45-based immunoaffinity depletion method for removing CD45-containing microvesicles that yields highly purified preparations of virions. Examination of CD45-depleted HIV-1_{MN} by HPLC, protein sequence, and amino acid analyses determined a molar ratio of HLA-II to Gag of 0.04-0.05 in the purified virions, corresponding to an estimated average of 50-63 native HLA-II molecules (i.e., a dimer of _ and _ heterodimers) per virion. These values are approximately 5-to-10-fold lower than those previously determined for other virion preparations. Our observations demonstrate the utility of CD45 immunoaffinity-based approaches for producing highly purified retrovirus preparations for applications that would benefit from the use of virus that is essentially free of microvesicles. Contract No. NO1-CO-12400.

P55-S

Displacement Chromatography Effects Can Cause Highly Selective Sampling of Peptides During Solid Phase Extraction Cleanup
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Small solid-phase extraction cartridges are used for convenient cleanup of small amounts of peptide mixtures, especially for desalting prior to MS analysis. The assumption is that the peptides adsorbed are representative of the entire mixture. Our data indicate that this assumption is valid if the binding capacity of the cartridge is close to or exceeds the total amount of sample presented to it. If the amount of sample presented is greatly in excess of the cartridge's capacity, though, then peptides that bind with low affinity will be displaced by peptides that bind with high affinity. The consequence is that only two or three peptides may be sampled from a tryptic digest that contains 40-60 peptides. Examples are presented with tryptic digests of transferrin and ovalbumin that contain 1-2 M urea and a peptide sample containing 8.6% SDS, with cleanup via reversed-phase or hydrophilic interaction chromatography.

P56-M

Optimizing LC/MS/MS Performance for Off-line Multi-dimensional Nanoscale Separations of Complex Proteomic Samples
S. Vazquez, J. W. Finch, S. J. Berger, S. A. Cohen; Waters Corporation, Milford, MA, United States.

Automated data-directed LC/MS/MS has become the preferred method for the identification of large numbers of proteins in complex peptide mixtures derived from global or pre-fractionated samples. The application of mass spectrometry to multidimensional analysis demands the utmost in instrument sensitivity and robustness. Although improvements in the basic sensitivity of a mass spectrometer can significantly increase the overall number of proteins identified, major gains can also be readily obtained through optimization of either the strong cation exchange (SCX) or reversed-phase chromatographic conditions.
Here we investigate how optimization of reversed phase chromatographic conditions in multi-dimensional separations can increase the numbers of proteins identified from a global digest of soluble *E. coli* extract.
Multidimensional separations were carried out in an off-line regime. In the first dimension, global *E. coli* digests were fractionated using a SCX column. Second dimension separations used capillary reversed-phase LC (75 micron ID column) and were analyzed with a hybrid quadrupole time-of-flight mass (Q-Tof) spectrometer operating in a data-directed analysis (DDA) mode. Database searching was performed with ProteinLynx Global Server 2.0 against the NCBI database for *E. coli* K12.
Chromatographic conditions investigated included the gradient length, sample loading amounts, and the effect of repeat analyses.
Comparisons were made between chromatographic conditions based upon the number of proteins identified, average number of peptides found per protein identified and average protein sequence coverage.
Overall, the use of optimized reversed-phase chromatographic conditions has lead to significant improvements in both sequence coverage and numbers of proteins identified from samples.

P57-T

A New Direct-Flow NanoLC System Optimized for LC/MS/MS in Proteomics
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Nanoscale chromatography is increasingly the method of choice for LC/MS/MS analysis of complex proteomics samples, due to higher MS sensitivity at lower flow rates. However, to achieve flow rates of 100-1000 nL/min has typically required the use of a split-flow system, which can compromise flow reproducibility due to small changes in backpressure of the two flow streams. As a result, poor retention time reproducibility has a significant negative impact

on comparative proteomics, where differences are monitored between control and disease state samples. There is an obvious need for nanoscale-LC systems that are capable of overcoming these disadvantages.

Here, we present results on a prototype direct-flow capillary LC system, which has a unique configuration. The gradient is formed into a storage matrix at low pressure using syringe pumps, and then delivered with an independent high-pressure pump. System flow is precisely controlled through a feedback mechanism. This design is capable of delivering highly reproducible gradient chromatography and is very robust. All consumables and fluidics are optimized from the trapping column to the nanospray emitter so that band-broadening is minimized. We will present LC/MS results on typical protein digests for 75, 100, and 150 _m id columns with gradients of 30 to 120 minutes, where reproducibility of retention times are typically within +/- 0.1 minutes for 100 injections over a 6 day period of continuous operation. In addition, we will present an overview of how variable flow control of the prototype nanoLC system can be utilized for applications such as shortening column regeneration time and peak-parking.

P58-S

On-Column Peptide and Protein Mixture Analysis by Electron Capture Dissociation using a Hybrid Linear Trap/FT-ICR Mass Spectrometer

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The use of electron capture dissociation (ECD) for sequencing peptides and proteins has shown greater amide backbone cleavage capabilities compared to collision induced dissociation (CID). In addition, the low energy ion activation mechanisms generated by ECD results in reduced side chain cleavages enabling greater identification of posttranslational modification's (PTM's). ECD analysis of biological samples have been performed exclusively using an FT-ICR mass spectrometer, but long ion accumulation, activation, and detection mechanisms prohibits incorporation of on-line separation of complex peptide/protein mixtures. The Finnigan LTQ-FT hybrid linear trap/FT-ICR mass spectrometer, however, provides a means to performing ECD analysis on complex mixtures of peptides and proteins on an LC timescale. The use of ion accumulation and isolation in the linear trap prior to transferring to the ICR cell for ECD and detection allows for greater cycle times (< 1.5 seconds) resulting in multiple MS^2 scans for each elution peak.

This paper presents initial work incorporating ECD on chromatographic separation of a complex mixture of peptides and proteins. Equimolar amounts of [Val^5]-angiotensin II, substance P, melittin, and ubiquitin were separated on a fast gradient and activated using ECD in the ICR cell prior to detection. The fast LC gradient produced separation on two of the four peptide/proteins demonstrating instrumental capabilities to dissociate co-eluting species. In addition to the five peptide/protein standards, a series of phosphopeptides were analyzed on-column and activated by ECD. All chromatographic timescale ECD spectra will be compared to linear trap CID data acquired using the LTQ-FT mass spectrometer.

P59-M

The LC-MS Workflow: A Comparative Study of High Throughput Nano LC and 2D-LC Designs

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The LC-MS workflow has been evaluated as an alternative or a complement to the 2DE-MS workflow. Parameters such as sensitivity, analysis time, ease of use and applicability for proteins with different physiochemical characteristics have been considered. For the LC-MS workflow, a high throughput nano LC configuration with two trap columns and two analytical RPC columns in parallel is compared to alternative high throughput nano LC designs. The band broadening effect of the parallel nano

LC system is minimized by the use of a novel nanovalve. Additionally, the eluent is directed to waste during column wash, minimizing the contamination of the MS ion source and thereby contributing to the robustness of the system. An on-line 2D-LC set-up with salt plugs injected from the autosampler is compared to an off-line 2D-LC set-up with fraction collection. The choice of mobile phase composition in both dimensions is optimized for the highest MS sensitivity. For the on-line 2D-LC set-up compromising mobile phase conditions must be used and the most hydrophilic peptides are difficult to analyze. System-output parameters such as sensitivity and analysis time have been evaluated in the light of system-input parameters such as trap column dimensions, mobile phase composition and gradient slope. The advantage of including conductivity detection during method development is demonstrated. MS specific parameters such as the choice of ESI emitter tip design, the choice of exclusion list parameters (repeated spectra, exclusion time vs peak width) as well as the use and abuse of database searches is discussed briefly. While practical aspects may direct the use of specific system set-ups, the optimum system set-up is also depending on the type of application.

P60-T

High Resolution Ion Exchange Columns for the Analysis and Quality Control of Protein Variants

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Protein and peptide microheterogeneity can be due to glycosylation, oxidation, mutation, phosphorylation, amino terminal modifications, incomplete processing of the C-terminus, asparagine (Asn) deamidation and hydroxylation of lysine. These variations in composition occur in many types of proteins and peptides, and depending upon the protein in question can impact their function, their activity and stability as biotherapeutics, or their flavor or texture in foods.

In this poster, we report the use of novel ion exchange columns for the analysis of different protein variants. The active ion exchange sites are located on linear polymer chains grafted onto hydrophilic polymeric beads. This arrangement results in highly efficient separations with exceptional resolution. The resolution of these columns is illustrated with the separation of hemoglobin variants using the strong cation exchanger, of monoclonal antibodies using the weak cation exchanger, and of milk proteins using the strong anion exchange column.

P61-S

Development and Applications of a New Silica-Based Reversed Phase Column for Protein Separations

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After more than 30 years of applications development in HPLC, the most popular approach to protein separations is still reversed phase. Among the many C18 reversed phase columns available, however, retention behavior of the analytes differs significantly due to variation in both the raw silica and the ligand bonding chemistry and density. The Acclaim 300 columns are a new line of wide-pore C18 reversed phase columns manufactured for the separation of proteins, peptides and other biological macromolecules. These columns have a very uniform, high surface coverage with extensive end-capping and very low metal content; and thereby exhibit very low polarity, high hydrophobicity and high capacity.
In this poster, we present the results of tests performed to measure the effects of metal contamination on peak symmetry and compare these results to those obtained with other C18 columns. Long term stability is demonstrated at low pH conditions. Examples of the separation of cytochrome c species variants and bovine cytochrome c tryptic digests illustrate the high efficiencies achieved by the Acclaim 300 columns.

P62-M

A Novel, Monomeric C_{18} Bonded Phase for High-Resolution Peptide Separations

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Separating digests by RP-HPLC is a standard method in protein research and developing well-characterized biotechnology pharmaceuticals. Typically, 300 Å C_{18} chemistries have carbon coverage in the 2.8 to 3.6 µmol/m^2 range. A novel, monomerically bonded 300 Å C_{18} adsorbent with carbon coverage greater than 4 µmol/m^2 is described. A high coverage results in significantly reduced secondary chromatographic effect. The ability of this chemistry to provide superb resolution and sensitivity was assessed based on LC and LC/MS/MS of tryptic digests of a number of samples: individual proteins, a "mock" complex sample comprised of about a dozen proteins of different abundances, and whole bacterial proteome. The tryptic peptides were run on nano/capillary, microbore, or analytical-scale C_{18} columns. LC/MS/MS analyses were performed on an ABI Q TRAP, an ABI Q-STAR, and a Thermo Finnigan LCQ Deca using acetonitrile/water with 0.1 to 0.5% formic acid, or with 0.05% TFA, with subsequent database searching using Mascot and/or SEQUEST. With the “mock” complex sample, the score from Mascot was higher for a number of protein identifications on the novel phase compared to a small pore (100 Å) C_{18} material. This corresponds to higher individual peptide scores, which is usually indicative of better MS/MS. The novel phase also performed well with complex proteome samples based on SEQUEST results. The innovative bonding allows for higher recoveries and, hence, the ability to detect more trace level peptides of hydrophilic and hydrophobic nature.

CARBOHYDRATE ANALYSIS

P63-T

Atmospheric Pressure Infrared Laser Ionization from Solutions (AP IRIS) for the Analysis of High-Mannose-type *N*-linked Oligosaccharides

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The goal of this study was to extend the success of atmospheric pressure matrix- assisted laser desorption/ionization (AP-MALDI) mass spectrometry from its established capabilities in proteomics applications to oligosaccharide analysis.
High-mannose-type oligosaccharides, carbohydrate standards were chosen for this investigation. A comparison between AP IR ionization from solution (AP IRIS) with UV AP-MALDI for the analysis of oligosaccharides is presented both in MS and MS/MS spectra. All experiments were carried out on a LCQ DECA XP ion-trap mass spectrometer equipped with an AP/MALDI source (MassTech, Inc). The setup for AP-IRIS experiments essentially substituted the UV laser with an IR laser and rotated the sample target plate so that it was parallel with the sampling capillary axis. Our AP-IRIS utilized an in-house built infrared laser to provide 30 ns pulses with a frequency of 5Hz. The wavelength of the pulses can be tuned in the region 2.8-3.1 _m, while the energy per pulse remains 0.4-0.5 mJ. The optimal wavelength for aqueous solutions of the carbohydrates was found 2.94 _m. The carbohydrate standards were detected mainly as $(M + Na)^+$ ions at low pmole sensitivity**.** Using only positive ionization mode we were able to analyze mixtures of both neutral and acidic oligosaccharides. The collision induced dissociation of the protonated molecule for each of the glycan yielded easily interpretable spectra. Every major ion present in spectra was

linked to structural features. AP-MALDI can play a critical role in successful analysis of complex carbohydrates.

P64-S

Separations of Branched N-Linked Oligosaccharides on aNew, Pellicular HPAE-PAD Oligosaccharide Separator Column; the CarboPac PA200

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Our current efforts are directed towards introducing a new oligosaccharide separation column (CarboPac PA200; 3mm x 250mm) that gives more efficient oligosaccharide separations with better resolution compared to either the CarboPac PA1 or CarboPac PA100 column. The CarboPac PA200 resin has a reduced particle size (5.5 micron diameter) compared to either the CarboPac PA1 or PA100 resin. The PA200 agglomerated latex particles bearing the ion exchange groups at the surface of the resin were additionally optimized with respect to latex particle size and degree of cross linking to give optimal resolution of oligosaccharides. Recommended operating conditions for the PA200 column specify a lower flow rate (0.5 mL/min) compared to the 1 mL/min flow rate recommended for the PA1 and PA100 columns. Additionally, on the PA200 column, oligosaccharides elute at lower salt concentrations compared to either the CarboPac PA1 or PA100 columns. Consequently it is easier to desalt collected fractions from the PA200 column facilitating easier downstream analysis. Improved chromatographic performance on the PA200 column compared to the CarboPac PA1or PA100 column was measured by comparing peak efficiencies for sialyl lactose. The PA200 column exhibited greater than a twofold increase in theoretical plates for the sialyl lactose peak. Additionally chromatography of neutral as well as sialylated N-linked oligosaccharides was compared. The PA200 column again exhibited improved peak efficiencies and resolution for a range of branched N-linked oligosaccharides including a pair of mannose –7 isomers. Finally, we compared chromatography of a homologous oligosaccharide series, inulins, on the CarboPac columns. The CarboPac PA200 again exhibited higher efficiencies and resolution.

P65-M

Human IgA1 Hinge Region *O*-glycosylation Characterized by FT-ICR MS

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IgA1 molecules contain a hinge region (HR) between the first and second constant region domains with high content of Ser and Thr, the site of attachment of three to five *O*-linked glycan chains consisting of *N*-acetylgalactosamine (GalNAc) with a ß1,3-linked galactose (Gal) that may be sialylated. Aberrantly glycosylated IgA1 molecules (deficient in *O*-linked galactose in the HR) are found in IgA nephropathy patients. While several techniques, including MALDI TOF MS, have been used to analyze heterogeneity of HR glycosylation, there was no sensitive and convenient technique that would allow to assign sites of attachment of *O*-glycans.
HR of human IgA1 was isolated from an IgA1 myeloma protein as a tryptic-peptic fragment by size-exclusion and affinity chromatography. Mass spectra of the desialyted human IgA1 HR glycopeptide were acquired with a homebuilt 9.4 Tesla FT-ICR mass spectrometer. FT-ICR MS spectra reveal a series of ions separated by m/z values corresponding to the presence of GalNac, and Gal + GalNac peptide species. Glycopeptides were fragmented by activated ion-electron capture dissociation (AI-ECD, unique to FT-ICR MS). AI-ECD results in cleavage of peptide backbone N-C_ bond, without loss of labile posttranslational modifications. AI-ECD FT-ICR MS/MS of the different glycosylated

forms of IgA1 HR glycopeptide localize the glycosylation to five out of ten possible *O*-glycosylation sites. Identification of the sites of attachment of *O*-linked glycans in the HR of a myeloma IgA1 protein opens a possibility to characterize the aberrant glycosylation of IgA1 from IgA nephropathy patients.

IDENTIFICATION OF POSTTRANSLATIONAL MODIFICATIONS

P66-T

Multiple Reaction Monitoring (MRM) as a method for identifying protein post-translational modifications.

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The activity of many transcriptional regulators is controlled by post-translational modifications of specific sites. For example, the activity of the muscle specific transcription factor family: Myocyte Enhancer Factor 2 (MEF2), is tightly controlled by phosphorylation. This modification is responsible for either an increase in transcriptional activity or a decrease, depending on the specific site of phosphorylation. Although mass spectrometry based methods such as precursor ion and neutral loss scans are extremely useful for identifying unknown phosphopeptides from a complex mixture, they do not take advantage of any prior knowledge about the protein being investigated. Quite often a significant amount of information is available. This may include: the primary sequence, type of phosphorylation (Serine/Threonine vs. Tyrosine), or predicted phospho-acceptor sites (MAPK consensus sites). This information can be used to predict precursor and fragment ion m/z values for a Multple Reaction Monitoring (MRM) experiment. By using these highly sensitive MRM experiments to trigger dependant product ion scans on a 4000 QTrap, a newly developed triple quadrupole-linear ion trap instrument, we were able to identify low levels of phosphorylation of MEF2A, and other proteins.

P67-S

In-vivo and in-vitro phosphorylation analysis of mitochondrial proteins using a fluorescent phosphorylation sensor and MALDI-TOF mass spectrometry.

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Phosphoproteins localized in mitochondria as part of enzyme complexes and signaling complexes are being identified in studies of both isolated proteins as well as crude heart mitochondrial fractions. By necessity, most studies of this type have previously been performed using in-vitro incorporation of 32P. Proteins with significant steady-state levels of phosphorylation from in situ reactions will not be discovered using this type of approach. In this study, we expand upon our previous investigations of the mitochondrial phosphoproteome using a new small-molecule-fluorophore called Pro-Q® Diamond dye, which is capable of sensitive detection of phosphorylated amino-acid residues in 1-D or 2-D gels. We report the discovery of two novel mitochondrial phosphoproteins (ANT and NDUFA10) including probable phosophorylation sites identified by peptide mass profiling and post-source decay analysis using MALDI-TOF mass spectrometry. The proteins identified were validated by Western blot analysis using phosphoamino acid specific antibodies. Results obtained by-vitro phosphorylation as well as dephosphorylation studies are also presented. This study clearly demonstrates that Pro-Q Diamond gel-staining technology facilitates the discovery of new phosphoproteins in complex biological systems and provides a unique tool for unraveling signaling pathways.

P68-M

Chip-Based Nanoelectrospray Ion Trap MS^n Mass Spectrometry for the Characterization of Protein Glycosylation

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Studies have shown glycoprotein oligosaccharides play a vital role in biological processes such as molecular recognition, and intra- and inter-cell signaling. The carbohydrates in proteins also can significantly alter protein conformation and consequently impact protein functions. Consequently, understanding the detailed structure of glycoproteins would provide insight to aid in biomedical research and drug discovery.

In this report we describe the use of a static nanoelectrospray ionization technique in combination with the recently developed linear ion trap for identification and characterization of glycosylation in complex mixtures.

The goal of this work was to demonstrate and evaluate the nanoelectrospray system coupled with an ion trap mass spectrometer as a tool for the identification of glycopeptides in mixtures. This platform provides high sensitivity, reproducibility, and stability for static nanoelectrospray. The advantages of this novel technique over other techniques for glycomic studies include stable nanoelectrospray, simple one-time spray optimization, no carryover, and low sample consumption.

To test the system, two standard synthetic glycans were analyzed using multiple stage fragmentation analysis in both positive and negative ion modes. In addition, an N-linked high mannoase glycoprotein, ribonuclease B (RNase B) was used to map the glycosylation site and obtain glycan structures. Using the static chip-based nanoelectrospray ion trap MS^n analysis, we were able to map the glycosylation site and obtain the glycan structures in RNase B in a single analysis. The results reported here demonstrate that the fully automated chip-based nanoESI/MS/MS platform used is a valuable system for oligosaccharide analyses.

P69-T

Use of a Tandem Immunoprecipitation Procedure for Characterization of the Mammalian Phosphotyrosine Proteome

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Tyrosine phosphorylated proteins play a central role in controlling cell status. However, their low abundance in crude lysates makes investigations of the phosphotyrosine proteome a challenge. Immunoprecipitation (IP) procedures using anti-phosphotyrosine antibodies provide the ability to enrich these proteins for subsequent analysis of tryptic peptides by LC-tandem mass spectrometry. Unfortunately, analysis of this peptide mixture for phosphotyrosine-containing peptides can still be thwarted by ion suppression. This investigation explores a tandem immunoprecipitation protocol to enrich phosphotyrosine-containing peptides for analysis by LC-NSI-MS/MS. Cell lysates from 293 human kidney cells were immunoprecipitated with an agarose-conjugated PY-20 antibody and the bound proteins were subsequently isolated and separated by 1-D SDS gel electrophoresis. Staining with Pro-Q Diamond (phosphoprotein specific) as well as Coomassie revealed the bands containing phosphorylated proteins. The bands of interest were excised, digested with trypsin, and a portion submitted to mass spectral analysis. The remaining digest was immunoprecipitated and the bound peptides were released, processed, and analyzed by LC-NSI-MS/MS both before and after tyrosine phosphatase treatment. Algorithm-based and manual interpretations of the resulting mass spectral fragmentation patterns were used to confirm peptide identifications. The results presented demonstrate the utility of the tandem immunoprecipitation procedure for characterizing the phosphotyrosine proteome.

P70-S

Phosphorylation Site Identification: Discriminating between Specific and Non-specific Affinity Material Binding

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Phosphorylation site identification has been of considerable interest in recent years. Phosphorylated fragments of the protein of interest have often been separated out by affinity binding to metal chelates or phosphorylation-specific antibodies attached to solid support. Non-specific binding is a serious and common problem. We have developed some simple procedures to improve upon the discrimination between true phosphopeptides and non-phosphopeptides. The proteins were S-pyridylethylated and then trypsin digested in triethanolamine buffer. The primary affinity material tested was iron 3+ chelated to NTA-agarose beads in column form. Bound peptides were eluted with ammonium bicarbonate. With sufficient material the peptides were quantified by sequencing. Half the bound material was digested with alkaline phosphatase. The two halves were subjected to MALDI-TOF mass spectrometry either separately or as a mixture. Phosphopeptides were easily recognized as pairs of components separated by 80 mass units corresponding to the loss of phosphate, non-phosphopeptides appearing as single, unrelated components. For proteins with known sequence the mass of the components provides sufficient identification, for unknown proteins ESI combined with mass-spectrometric sequencing is superior. With sufficient material RP-HPLC isolation of the peptides followed by sequencing is advantageous. Alternative identification of non-specifically binding peptides is obtained by phosphatase digestion before affinity binding to the iron 3+ NTA material or by using the NTA material in the absence of iron ions. The procedures described have been successfully utilized to unambiguously identify novel phosphorylation sites in animal fibrinogens.

P71-M

Use of Edman Sequencing in the Identification of a Modified Amino Acid

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During Edman chemical sequence analysis of a sample in our laboratory, an unidentified phenylthiohydantion (PTH) amino acid peak was observed. This modified PTH amino acid eluted at an unusual retention time, and no references could be found to assist in its identification The protein is extremely hydrophobic, soluble only in organics and known to be intractable to digestion and standard LC-MS/MS approaches. Since the sample was isolated from tissue implicated in a particular disease state and an aspartic acid was expected in the wild-type protein, it was of interest to further characterize the modified amino acid.. To do so, multiple runs were performed on the Edman sequencer, from which the modified PTH amino acid peaks were manually collected. These collected fractions were pooled and repurified by off-line reverse-phase HPLC. An aliquot of this purified PTH amino acid was submitted to micro NMR analysis (courtesy of Bruker, Billerica MA), while a precise mass determination was performed in-house on a hybrid quadrupole TOF instrument for elemental composition. These analyses both indicated a methylated aspartic acid residue in the original sample. This was confirmed by methyl esterification of a synthetic peptide, which upon Edman sequence analysis, showed the methylated PTH-Asp eluting in the same position as the unidentified PTH amino acid peak observed in the original sample.

P72-T

Discovering Known and Unknown Protein Modifications Using MS/MS Database Searching

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We present an MS/MS database search algorithm with the following novel features: (1) zone modification searching, which enables the discovery of protein modifications of known (i.e., user-specified) and ***unknown*** delta masses, and (2) a novel protein database structure containing extensive pre-indexing. In zone

modification searching, the mass range covered by each MS/MS spectrum is divided into six equally-sized zones. For each of the six zones, it is hypothesized that a modification occurs in that zone, and the search against the protein database is performed accordingly. The novel protein database structure results in significant efficiency gains and is particularly advantageous in a large zone modification search. All of these features are implemented in Interrogator™, the search engine which run behinds Pro ID and Pro ICAT. The ability to search for a large variety of known and unknown modifications allows a significantly greater percentage of MS/MS scans to be identified. We demonstrate this with an example in which, out of a total of 422 identified MS/MS scans, 303 of these scans correspond to unmodified peptides, while 119 scans correspond to a large variety of modified peptides. In addition, we provide specific examples where the ability to search for ***unknown*** modifications allows the scientist to discover: (1) unexpected modifications which have biological meaning, (2) amino acid mutations, (3) non-tryptic peptides in a sample which has nominally been digested using trypsin, and (4) other unintended consequences of sample handling procedures.

P73-S

N-linked Glycosylation Mapping on The Human Salivary Mucin MUC5B

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Mucins are large highly O-glycosylated oligomeric glycoproteins found in mucus and form part of the innate immune system. MUC5B, the predominant gel-forming mucin in saliva, is around 600KDa in mass before glycosylation. O-linked glycosylation is abundant in defined repeated domains, and may account for 80% of the observed mass of protein. Although generally well studied, not much is known about the N-linked glycosylation of this molecule.
A sample of purified MUC5B mucin, digested with trypsin and separated by size exclusion chromatography shows three distinct regions in its elution profile. The first region contains very high molecular weight glycopeptides (approx. 500-700KDa) rich in O-linked glycosylation. The last region contains the low molecular weight tryptic peptides. The intermediate region has not yet been studied and is thought to be rich in N-linked glycopeptides. Our aim is to study these fractions in the intermediate region by LC-MS in order to characterise any glycosylated peptides present.
Glycopeptide discovery on the CapLC Q-Tof system can be steered by the glycoPID function. This detects potential glycopeptides during a reverse phase CapLC run looking for sugar oxonium ions during intermittent high collision energy surveys. Multiply charged ions are then selected for MSMS. Glycopeptide fragment ion spectra can then be deconvoluted by MaxEnt3 and interpreted with Carbotools. MSMS analysis of the glycopeptide reveals both the carbohydrate content and the glycosylation site location.

P74-M

Identification of phosphorylation sites on phosducin-like protein by QToF mass spectrometry

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Post-translational modifications are used by cells to control the functions of proteins. Phosducin-like protein (PhLP) has been shown to be post-translationally modified, in particular phosphorylated. Casein Kinase II (CKII) has been shown to phosphorylate PhLP, increasing its binding affinity for Cytoplasmic Chaperonin containing T-complex polypeptide 1 (CCT). Separately, PhLP has been shown to be phosphorylated by extract from serum-stimulated Chinese Hamster Ovary (CHO) cells. PEST-sequence dependant degradation of PhLP has been studied using such serum-stimulated CHO cells. Through use of MS/MS the specific amino acids phosphorylated can be identified. A PhLP-myc-His construct was purified and phosphorylated by two different sources of

kinase: pure CKII and serum-stimulated CHO extract. The resulting protein was digested with trypsin and the peptides identified by LC/MS/MS. Three phosphorylation sites were identified in the CKII phoshorylation. Four phosphorylation sites were identified in serum-stimulated CHO extract phosphorylation, three being the same sites as the CKII phosphorylation.

P75-T

The Phosphopeptide Isolation Problem.

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Selective detection of phosphorylated peptides by MS-based strategies is often precluded by the complexity of the peptide mixtures. Immobilized metal ion affinity chromatography (IMAC) is a commonly reported technique to address this problem by enrichment of phosphopeptide prior to phosphorylation site determination. Immobilized Gallium (III) affinity chromatography is frequently employed for this purpose and was first demonstrated in a custom-made microcolumn format (1). We have recently reported on the use of commercially available minispin columns containing Gallium-chelated disks (Pierce, Phosphopeptide Isolation Kit) (2). Although some phosphopeptides are enriched, often other acidic peptides are co-isolated, and recoveries are variable. Chemical modification by methylation of the carboxyl groups has been reported to reduce this adsorption of non-phosphorylated peptides (3). In this presentation, we have extended our methology development to investigate methyl-esterified peptide mixtures and consider other chromatographies. We have attempted to quantify both binding and release steps under various conditions in order to optimize recoveries, and also begun to explore in detail the ion-exchange behavior of phosphorylated peptides in order to achieve high-yield enrichment.

References: 1. Posewitz MC and Tempst P Anal Chem 1999:71: 2883-2892. 2. Nika H, Hawke D and Kobayashi R Conference on Mass Spectrometry & Allied Topics, June 8-12, 2003, Montreal, Canada, Abstr. MPS 349. 3. Ficarro SB, et al. Nat Biotechnol 2002;20:301-305.

P76-S

Integrated Workflows Using a Hybrid Quadrupole-Linear Ion Trap Mass Spectrometer for Automated PTM Discovery

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The higher order goals in functional proteomics are often beyond the identity of the proteins, and require characterization of a myriad post-translational modifications (PTM's) with respect to differing biological states. The analysis of the dynamic nature of these modifications is an essential component to understanding the interaction networks of organisms at an intracellular level. Combining triple quadrupole scanning capabilities with ion-trapping capacity in the new hybrid quadrupole-linear ion trap mass spectrometers creates new capacity for the automated identification of post-translational modifications present on proteins in biological samples. Coupling Information Dependent Acquisition (IDA) and rapid positive to negative polarity switching ensures a high versatility/density of discovery level identifications and the characterization of the modifications.

These unique hybrid instruments, couple the selectivity of precursor ion (PI), neutral loss (NL) and multiple reaction monitoring (MRM) scans with high sensitivity MS/MS ion trap scans, to provide a more complete picture of protein modifications in a single experiment. Using model systems such as fetuin and alpha-casein, various novel on-line approaches are presented for the specific identification of multiple post-translational modifications using combinations of these scans on the Q TRAP™/4000Q TRAP™ systems. Primarily, we illustrate the use of a precursor ion scan for m/z -79 to screen peptides for all types of phosphorylation sites. This has been utilized as a survey scan and automatically triggers a positive-ion high resolution scan and a MS/MS scan of the modified peptides in LC time. Using this technique, we have been able to identify the

majority of sites of phosphorylation on these model proteins, including multiply phosphorylated isoforms. These workflows are extremely flexible and applicable to a variety of biological modifications and investigations.

PEPTIDE SYNTHESIS

P77-M

Enhanced Solid Phase Peptide Synthesis using Microwave Energy

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Microwave synthesis has become accepted as a new energy source to drive chemical reactions to completion. In this paper we present the use of microwave energy for the deprotection, coupling, and cleavage reactions of solid phase peptide synthesis (SPPS) for various difficult peptide sequences. Conventional side reactions with SPPS, such as racemization and aspartimide formation were investigated with microwave energy. Also, reduction in excess reagents used in the coupling and deprotection reactions of difficult peptides were explored.

GEL ELECTROPHORESIS

P78-T

RediPlateTM EZQ Protein Quantitation Kit: A high-throughput fluorescence-based solid phase protein assay for rapid quantitation of samples containing detergents, chaotropes, and reducing agents.

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A new solid-phase, fluorescence-based protein assay was developed that quantifies proteins in the presence of detergents, urea and reducing agents (SDS sample buffer or 2-D gel lysis buffer). A specially designed 96-well microplate facilitates application of samples to the assay paper and allows easy quantitation of samples using most fluorescence microplate readers (top or bottom reading format). Alternatively, stained membranes may be directly scanned using a variety of different laser or CCD-based imaging devices with UV or visible imaging capabilities. Since protein is specifically bound to the membrane, contaminants are readily washed away, avoiding interference with the protein measurement. The protein assay has a dynamic range extending from ~20 ng to 6 mg of protein per microliter and requires only 1 ml of sample, which is ideal for samples loaded onto polyacrylamide gels. Protein-to-protein variation of ten different proteins was determined to be comparable with BCA and Lowry assays (16%). Additionally, the quality of the assay according to Z-factor analysis is excellent.

P79-S

Increased reproducibility in the extreme basic pH region facilitates comparison between analytical and preparative scale 2-D gel electrophoresis

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The combination of 2-D gel electrophoresis and mass spectroscopy (MS) is one of the most widely used tools in Proteomic studies. One problem encountered using this strategy is the resolution and reproducibility of the protein map when comparing wide pH-range analytical gels with narrow pH-range gels used in preparative experiments prior to protein identification with MALDI-MS. This problem is pronounced in the extreme basic region since the long isoelectric focusing times required with narrow-range pH gradients result in spot streaking and shift of protein spots towards the cathode. We have increased the buffering capacity in the extreme basic pH region of the gel by introduction of a proprietary arginine derivative (guanidyl group) in our medium-range 7-11 NL isoelectric focusing gels. The increased robustness at high pH values allows for longer isoelectic focusing times without loss of the very basic proteins. This in turn increases the resolution in the extreme basic pH region. Analytical and preparative amounts of mouse liver extract, without or spiked with alkylated lysozyme

(pI~10.5) were analyzed on four medium-range gradient gels and one broad-range gel. The resulting four medium-range overlapping protein maps were easily comparable with the broad pH-range map increasing the resolution over the whole pH-range. Furthermore, the introduction of the arginine derivative in the 7-11 NL gel allowed for longer focusing times and improved resolution, reproducibility and spot intensity in the extreme basic pH region. We will determine the identity of basic proteins in mouse liver extracts using MALDI-ToF MS, something that previously has been difficult to perform due to the loss of protein in this region.

P80-M

High Speed Electrophoresis of DNA and Proteins

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In order to achieve the sharpest resolution of DNA and protein sample bands, typical gel electrophoresis protocols often require relatively low voltage conditions (75-125V) over extended time periods (≥1 hour). We have developed platforms and methods that enable the separation of DNA and proteins in as little as 15 minutes while maintaining sharp band resolution. This allows for faster completion of experiments without compromising the quality of the data produced.
The integrated FastLane gel system efficiently separates a wide range of DNA fragment sizes in as little as 15 minutes. The FastLane system is comprised of precast gels, premeasured buffer, and a specially designed chamber. We will demonstrate the separation and resolution of PCR products and restriction digests using this system, as well as, efficient recovery of DNA following separation.
Several options will be presented for high speed separation of proteins by polyacrylamidegel electrophoresis (PAGE) incorporating precast gels made with a novel monomer, modified buffer systems, and/or modified run conditions. Sample resolution under modified conditions is comparable to traditional low voltage/extended run times. We will show the resolution of cell lysates and standard molecular weight markers run on a cross-section of gel concentrations and gradients.

P81-T

Simplified IPG Strip Equilibration with DeStreak™ Enabling Reliable 2-D Maps and MALDI-ToF Data

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Isoelectric focusing (IEF) of proteins with basic pI values has historically been problematic in 2-D electrophoresis. The reason for poor resolution and lack of reproducibility has mainly been the difficulties maintaining the proteins in a reduced state, commonly done with DTT. The problems arise on the basic side of the strip, from which DTT is transported, thereby permitting re-oxidation of proteins resulting in disulfide bridges. This generates streaky and heterogeneous spot patterns over time. The solution to these problems is to include DeStreak in the rehydration step preceding focusing. Instead of trying to keep the cysteinyl groups reduced, they are specifically oxidized by DeStreak forming "mixed disulfides". This approach can now be extended to the 2nd dimension step. By equilibrating the IPG strips in DeStreak the "mixed disulfides" are maintained throughout the SDS electrophoresis, minimizing vertical streaking in the 2nd dimension as well. This one-step simplified equilibration protocol replaces the two-step reduction and alkylation protocol normally used, and is also fully compatible with MALDI-ToF analysis. Moreover, when running analytical sample amounts the equilibration time can be shortened, speeding up the process even further. We describe the novel equilibration protocol applied to IPG strips covering the entire pH range including the new basic IPG 7-11 NL strip. Preparative amounts of mouse liver proteins were separated on 2-D gels. Selected proteins were then identified by MALDI-ToF MS. Identification of proteins was at least as efficient using the DeStreak equilibration protocol compared with traditional iodoacetamide alkylation. MS data also show that the spectral

quality for the two variants is equal with respect to intensity and number of detected cysteine-containing peptides. This demonstrates the efficiency and specificity at which DeStreak modifies cysteinyl residues in proteins.

P82-S

Countercurrent Affinity Electrophoresis of Biotinylated Proteins

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A Countercurrent Affinity Electrophoresis (CAE) method was used to separate biotinylated proteins from a mixture of proteins. In this method, avidin was load in the middle of a native 5% polyacrylamide gel. At the pH of the Tri-Glycine running buffer, avidin migrates toward the anode and the proteins loaded migrate toward the cathode. The avidin and biotinylated protein will form a complex with a high association contant of about 10 to the 15 M when they contact with each other. Biotinylated insulin, proteinA, and bovine serum albumin were used as a prove -of -concept study.

P83-M

A functional proteomic analysis of viperid snake venoms by two-dimensional electrophoresis.

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The complexity of viperid venoms has long been appreciated in the field of toxinology and medicine. However, it is only recently that the depth of that complexity has become quantitatively accessible. With the resurgence of 2D PAGE and the advances in mass spectrometry essentially all venom components can be visualized and identified given the effort and resources. Here we present the use of various technologies for examining venom complexity and demonstrate their associated advantages and disadvantages. 2-D PAGE comparisons between different genera of viperid venoms; different species of the same genus and specimens of the same genus and species demonstrate the similarity as well as the apparent diversity among these venoms. We have applied techniques to examine subpopulations of venom proteins (metalloproteinase proteome; serine proteinase proteome; phospholipase A2 proteome and the glycoproteome). Subsequently, we performed a functional proteomic analysis of proteolytic enzymes present in viperid venoms by using a combined method of 2D PAGE and zymography. These tools will allow for a better understanding of venom complexity and toxic properties as well as enabling investigators with particular interests to focus on these subpopulations of proteins for further study.

PROTEOMICS

P84-T

Comparative proteomic in *Cannabis sativa* plant tissues

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Proteomics is being used to identify functions of genes. We applied proteomics to identify genes of the cannabinoids biosynthetic pathway. Because cannabinoids accumulated differently in tissues of *Cannabis sativa* plants we did comparative proteomics to study their biosynthesis. Proteins extracted from leaves and flowers of *C. sativa* were separated using two-dimensional (2D) gel electrophoresis. Over 800 protein spots were reproducibly resolved in the 2D gels from leaves and flowers. The patterns on the two gels were different and little correlation among the proteins could be observed. Some proteins that were only expressed in flowers were chosen for identification by MALDI-TOF and peptide mass fingerprinting databases searching using the Mascot program. Comparison also has been done between flower and gland proteome. Less then half of the proteins expressed in flowers are expressed in

glands. More than 50 gland protein spots were identified. Among those which have high Mowse score are: phospolipase isoform (*Gossypium hirsutum*), PG1protein (*Hordeum vulgare*) and F1D9.26-unknown protein (*Arabidopsis thaliana*). However, none of the detected proteins in flowers and glands is known to be involved in cannabinoid biosynthesis.
Western blotting was employed to identify a polyketide synthase (PKS), an enzyme believed to be involved in cannabinoid biosynthesis, resulting in detection of a single protein. However, MALDI-TOF did not give any information that has homology with known PKS. Probably the spot co-migrate with another more abundant protein. The mass spectra of the protein shows similarity with OSJNBb0040D15 protein (*Oriza sativa*) according to the highest Mowse score. Lack of data on proteins and genes from *C.sativa* in the available databases hampers the identification of the selected and analyzed proteins.

P85-S

Analysis of Protein Small Molecule Interactions Using H/D-Ex MS

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Analysis of Protein Small Molecule Interactions Using H/D-Ex MS
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We have developed an approach for the analysis of protein ligand interactions using a non-invasive labeling strategy compatible with all drug targets. This labeling approach involves hydrogen/deuterium exchange of amide hydrogens. Protein and ligand are incubated in a deuterated environment followed by quenching at appropriate time intervals. The deuterated protein is degraded progressively by endo- and exo- proteases and the resultant peptide fragments are analyzed by LC-MS. The rate of deuterium incorporation is determined for each fragment and the H/D-Ex rate of each amide or a region of amides is obtained. The rate of exchange for amide(s) hydrogen(s) afford(s) the free energy of each amide(s) in the state H/D exchange was measured. This free energy map can give the structural and dynamic information of the target and small molecule interactions. Results will be presented for the analysis and characterization of the binding mode of several p38 MAP kinase inhibitors and for modulators of nuclear hormone receptors.

P86-M

A Relational Database Schema and Software to Aid Large Scale Proteomics Experimental Analysis

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Recent developments in mass spectrometry such as Gel-C MS/MS and MUDPIT have resulted in the need to analyze a large number of LC-MS/MS experiments in order to determine the full complement of proteins present in the sample. This will, in some cases, require the analysis of 50,000 or more parent ions. Current software available for searching protein databases with MS/MS data were designed to operate on single LC-MS/MS experiments. In addition, the large number of proteins identified requires that detail information about each protein be provided in an easily accessible manner. These data sets are also commonly distributed to multiple users at geographically distinct sites. We have addressed the above requirements by developing a relational database schema to capture the output of the MASCOT database search program and by developing web based software to load and present this data using a web browser. This software allows users to examine a list of proteins believed to be present in their sample and to examine the supporting evidence. In addition, detail information is available for each protein listed. The lists can be sorted and filtered on various aspects of the mass spectrometry data and protein attributes. Users can attach comments to individual proteins found in each experiment. Users can compare experiments to identify

protein that are different between experiments or identify those that are in common.

P87-T

Novel Plasma Depletion Strategy Using Chicken IgY Antibodies for Proteomic Analysis from Multiple Mammalian Species

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Two-dimensional electrophoresis as well as MS-based methods have been successful in the identification of biomarkers from plasma of humans and other animal species. The success of this method hinges on the ability to remove highly abundant proteins such as albumin. We have developed a high throughput depletion method using chicken antibodies (Genway Biotech) that is capable of removing abundant proteins from plasma and serum from multiple species. 2D gel analysis of albumin, transferrin, fibrinogen, IgA, IgM and IgG depleted samples reveals that this immunoaffinity method for depletion is highly specific and can be applied to samples from multiple species. The application of a high-throughput liquid chromatography system allows for automated processing of multiple samples resulting in high throughput and minimal experimental variability.

P88-S

Proteomic Analysis of the Intestinal Epithelial Cell Response to Enteropathogenic Escherichia coli

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Enteropathogenic E. coli (EPEC) is an enteric human pathogen responsible for much worldwide childhood morbidity and mortality. EPEC uses a type III secretion system (TTSS) to inject bacterial proteins into the cytosol of intestinal epithelial cells to cause diarrheal disease. We analyzed the TTSS-specific host response by infecting polarized intestinal epithelial monolayers with either wild-type or TTSS-deficient EPEC. Host proteins were isolated and subjected to quantitative profiling using isotope-coded affinity tagging (ICAT) combined with electrospray-ionization tandem mass spectrometry. We identified over 2000 unique proteins from infected Caco-2 monolayers, of which ~10% are differentially regulated by TTSS-delivered EPEC proteins. The identified changes in host protein content extend numerous cytoskeletal observations made in less relevant cell types, implicate novel G-protein signalling pathways in EPEC pathogenesis, and generate testable hypotheses with regard to ion transporters potentially involved in EPEC-induced diarrhea. We also compared the changes in the host proteome to alterations in the host transcriptional profile by utilizing human microarrays with mRNA samples obtained in parallel with protein isolation. The greatest correlation between datasets is observed in up regulated proteins with enzymatic function. These data are, to our knowledge, the first report of a large-scale proteomic analysis of a human cellular response to an enteric pathogen.

P89-M

A Comparison of Immobilized pH Gradient Isoelectric Focusing and Strong Cation Exchange Chromatography as a First Dimension in Shotgun Proteomics

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As the demand for high throughput analysis of complex proteomes increases, significant effort has been placed on developing multidimensional separation techniques coupled to mass spectrometry for obtaining information on complex peptide mixtures. Currently the most common combination of techniques used for such samples is strong cation exchange (SCX), coupled with reverse phase chromatography. SCX separates peptides based on their total charge by eluting the peptide with the least

positive net charge first. A potential alternative to SCX based separation is to use isoelectric focusing of peptides in immobilized pH gradient (IPG) strips, as we have recently demonstrated. Here we present a direct comparison between SCX and IPG for shotgun proteomics and show the utility of narrow range IPG strips for complex samples. . The comparison experiments discussed in this work utilized a sample of testis from *Rattus norvigicus*, for a comparison between offline SCX and IPG. Our aim was to evaluate the resolving power of each technique in the presence of an overabundance of different peptides. Fractions from both techniques were analyzed using the same LC-MS/MS parameters to allow for direct comparisons of the data. The results were processed via SEQUEST and IDSieve, an automated filtering program that uses pI values as an identification criterion. The results demonstrate that use of IPG as a first dimension separation strategy is the superior method for whole proteome digests, since it produces identifications of more peptides and proteins than SCX, even when fewer fractions over a limited pI range from the IPG separation are analyzed.

P90-T

Analysis ofTranscription Factors by Mass Spectrometry

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DNA-protein interactions are at the foundation of gene regulation and epigenetic phenomena such as X Chromosome Inactivation (XCI). Numerous studies have shown that 5-methyl-cytosine strongly affects DNA-protein interactions and inhibits transcription when present in promoters. XCI, which results in the heritable inactivation of one X chromosome early in mammalian embryogenesis, is maintained by the enzymatic hypermethylation of cytosine at CpG sites on the inactive X chromosome. A gene called XIST, which is expressed only from the inactive X chromosome, controls the initiation of XCI. The XIST promoter is one of the few genes active on the inactive X chromosome. Consistent with methylation inhibiting promoter function, the XIST promoter is found to be methylated and inactive on the active X chromosome.
In this study we have used DNA affinity capture (DACA) combined with microcapillary liquid chromatography tandem mass spectrometry (LC/MS/MS) to investigate and identify the proteins involved in the differential DNA-protein and protein-protein interactions due to cytosine methylation at the human XIST minimal promoter. Differentially methylated, 180bp DNA capture molecules were incubated with crude HeLa nuclear extracts. Bound proteins were resolved by 1D SDS-PAGE. Automation of the gel band processing and analysis allowed us to analyze two entire gel lanes consisting of approximately 40 bands per lane and thereby obtain a more comprehensive picture of bound proteins.The LC/MS analysis indicated an overall inhibition of binding proteins and subsequent complex formation due to cytosine methylation. In addition, several proteins were identified that bound specifically to either the methylated or unmethylated XIST Promoter. The unmethylated DNA bound proteins predominately associated with actively transcribed chromatin. Conversely, the methylated DNA bound proteins associated with the formation of heterochromatin.

P91-S

Improved 2D nano LC/MS for Proteomics Applications – A Comparison on Yeast

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The most frequently used method for protein identification with two-dimensional LC/MS utilizes the elution of digest peptides from a strong cation exchange column by an injected salt step gradient of increasing salt concentration with an online subsequent reversed phase separation. However, in this approach the ion exchange chromatography is not exhibiting its full capabilities. To improve the SCX chromatography separation a new method was developed, which applies a semi-continuous pumped salt gradient and a revolving 10-port valve with two parallel enrichment column as

interface between the first and the second dimension.
In this study instrumental setup and experimental details are presented for the semi-continous two-dimensional nano LC/MS. A 2D LC analysis of the yeast proteome was conducted in order to show the superiority of this method compared to conventional online 2D LC. Furthermore, resolution, number of identified proteins and peptides as well as sequence coverage will be discussed and compared to conventional online and offline 2D LC.

P92-M

Chip-Based Electrospray Ion Trap Multiple MS for the Characterization of Protein Phosphorylation and Glycosylation

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Reversible protein phosphorylation plays a pivotal role in a multitude of regulatory mechanisms such as metabolism, cell division, cell growth and cell differentiation. Knowledge of the particular residues being phosphorylated on a protein can provide insight into the mechanisms of regulations. Several mass spectrometry approaches to identify phsophopeptides and phosphorylation sites have been used successfully. However, the identification of phosphopeptides in a complicated mixture remains a significant challenge, because of their poor ionization efficiency and fast degradation.
The goal of this work was to demonstrate and to evaluate the electrospray system coupled with a linear ion trap mass spectrometer as a powerful tool for the identification of phosphopeptides in mixtures by using data dependent scan and automated neutral loss mapping with infusion for extended analysis. The advantages of this novel method over conventional techniques for phosphorylation studies include flexible, stable electrospray, simple one-time spray optimization, no sample carryover, and low sample consumption.
Two standard phosphoproteins (bovine beta-casein and alpha-casein) were tested for the system with tandem mass spectrometry followed by automated neutral loss mapping analysis. Using the static chip-based electrospray ion trap MSn analysis combined with phosphatase treatment, we were able to map the phosphorylation sites in a single analysis for beta-casein digest at 1-5 fmol/uL and for alpha-casein digest at 50 fmol/uL levels. The results reported here demonstrate that the fully automated chip-based ESI/MS/MS platform used is a valuable system for phosphorylation analyses due primarily to stable, extended infusion times for completing MS/MS with automated neutral loss mapping in a single analysis.

P93-T

Proteome Mapping of Serum after a 3-D Separation: Immunoaffinity Chromatography – FFE – SDS-PAGE

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The identification of protein biomarkers from human serum is important to the study of many human disease states. Proteome mapping of human serum is the first step to catalog proteins that can be observed with a particular analytical technique starting with a particular volume. The large dynamic range of proteins present in serum limits the utility of many proteomic techniques to identify low copy biomarkers. This study tested a 3-dimensional separation as a method for mapping serum proteins starting with 300ul normal human serum. The first dimension was pre-fractionation of high abundant proteins by immunoaffinity chromatography utilizing polyclonal IgY antibodies against the high abundant serum proteins, albumin, IgG, transferrin and fibrinogen, covalently coupled to microsphere carriers. These were tested individually as well as a mixed bed resin. Each

separated fraction was then subjected to FFE (IEF, ph 3-10). All subsequent FFE fractions were then analyzed by SDS-PAGE. Proteins were then excised and identified by mass spectrometry. A list of proteins identified will be presented to show the utility of the technique.

P94-S

Elucidation of Differences in Protein Expression Patterns by Two-Dimensional Offline HPLC/MS

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In 2D LC/MS two orthogonal separation techniques are combined in order to achieve maximum resolution for complex proteome samples prior to MS and MS/MS analysis and protein identification. The two separation dimensions can be coupled either directly (online) or indirectly (offline) where micro fraction collection is conducted following the first dimension. The offline technique has the advantage that higher peak capacities can be attained due to long continuous linear gradients instead of stepwise elution by injected salt solution. This results in better overall chromatographic resolution and provides therefore an improved MS precursor selection and fragmentation, which finally leads to a higher number of identified proteins. An additional advantage of offline 2D LC is, that fractions from the first dimension can be stored or may be subjected to chemical or enzymatic modification procedures. In this study strong cation exchange chromatography, micro fraction collection and reversed phase separation are combined followed by nano electrospray ion trap MS/MS analysis. Data is provided from proteome analyses of lymphocyte cultures from patients with a genetic disorder and their healthy relatives. Qualitative and semi quantitative data evaluation clearly demonstrates that offline 2D LC is a powerful technique to show subtle differences in patients and unaffected control individuals. This technique is therefore an intriguing alternative to 2D gel electrophoresis especially for problematic samples focusing on hydrophobic, basic, acidic or very large proteins.

P95-M

A Proteomics approach to study ADME/Tox Properties

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It is estimated that about 50% of drugs in development fail during clinical trials because of deficiencies in ADME/Tox (absorption, distribution, metabolism, elimination and toxicity) properties. The cost of failures late in the development process is vast. Furthermore it has been suggested that more than 5% of hospitalized patients still suffer serious adverse drug reactions.

The objective of this study is to apply a proteomics methodology to understand early in the drug development process, which changes in protein levels occur during metabolism of candidate drugs in the liver.

Inbread C57BL/6 mice were treated with a selected candidate drug during five days. Livers and microsomes from treated and non-treated mice, respectively, were homogenized and subjected to CyDyeTM DIGE fluor labelling. Also microsomal fractions from rats were fluorescence labelled and included in the study. Thereafter, the protein levels in the samples were studied using difference gel electrophoresis (DIGE). Protein spots differing in intensity between treated and non-treated animals were identified and subjected to automatic spot picking, in-gel tryptic digestion, extraction, spotting on target slides, and analysis by MALDI-ToF MS. In cases, when peptide mapping did not yield sufficient information for unambigous protein identification, sequence analysis using chemically assisted fragmentation derivatization and MALDI-PSD or LC-ESI-

MS/MS further improved protein identification rates.
In this work, a number of proteins occuring in enhanced- or reduced level following treatment with the candidate drug were identified. This knowledge may lead to developments of improved methodologies for screening ADME/Tox properties of candidate drugs.

P96-T
Identification of Proteins in Post-Mortem Human Brain Tissue by Laser-Microdissection/Pressure-Catapult and nano-LC/MSMS
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The molecular mechanisms involved in most neurodegenerative disorders, such as Parkinson's and Alzheimer's disease, are still unclear. So far, protein expression analysis is often performed on homogenized preparations of whole tissue, which do not provide any information about relevant changes in specific cell types.
The aim of the following study was to examine, whether laser-microdissected samples of single cell types from post-mortem brain tissue can be used for protein expression analysis.
Laser Micro Dissection (LMPC) of human brain tissue was done on a P.A.L.M. MicroBeam IP system featuring a 337 nm nitrogen laser. All LC/MS experiments were performed using an Agilent nanoflow proteomics solution. The ion trap XCT mass spectrometer was operated with the orthogonal nanoelectrospray source, positive ions, and peptide-scan MS/MS mode. SpectrumMill MS Proteomics Workbench (NCBI, Human) was used for data processing.
Major benefits of using Laser Micro Dissection compared to homogenized tissue are that a well defined tissue area or even several areas can be harvested without touching the sample. Through the AutoLPC capability of the MicroBeam, the individual cells are disrupted during the process and soluble proteins are directly accessible for further treatment. A digestion protocol in mixed organic solvents was developed leading to better sequence coverage and protein identification by LC/MS.
Tissue samples prepared with LMPC enable a direct access to soluble proteins for further preparation. 1-dimensional nanoLC/MS trap mass spectrometry using a high capacity ion trap allows for positive identification of up to 30 proteins from tissue areas as low as 5 mm^2.

P97-S
Optimized conditions for diluting and reusing SYPRO Ruby protein gel stain
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SYPRO® Ruby protein gel stain allows proteins to be detected with high sensitivity (< 1 ng limit of detection) and quantitative accuracy (> 3 log linear dynamic range). However, not all studies require maximum levels of sensitivity and accuracy. Consequently, some have attempted to economize on the use of the stain (Krieg, et al. 2003, Biotechniques 23). This study is an attempt to elucidate the optimum conditions at the minimum cost for using SYPRO Ruby protein gel stain. It deals with the effects of gel fixation and staining times, as well as dilution and reuse of SYPRO Ruby protein gel stain in 1-D gels. Signal strength and dynamic range were highest in gels that were fixed thoroughly before staining, followed by overnight staining. Using the optimized protocol, dilution or reuse of the stain reduces the dynamic range and signal intensity. Sensitivity remains high if the stain is reused up to two times, but signal intensity is reduced up to 2.5 fold in twice used stain. Sensitivity also remains high if the stain is diluted 1:2 in water, but signal intensity is reduced up to 6-fold. Of the two options, reuse or dilution, reuse retains more (though not full) signal intensity and dynamic range.

P98-M
Combining microscale solution-phase isoelectric focusing with Multiplexed

Proteomics staining technology to analyze protein post-translational modifications.
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In order to identify low abundance protein as well as phosphoproteins, different pre-fractionation methods of complex protein mixtures have been developed. A very versatile fractionation approach is based upon in-solution IEF separation of proteins, using the ZOOM® IEF fractionator (Invitrogen, Carlsbad, CA). The separation results in 5 fractions spanning distinct isoelectric point ranges. This fractionation procedure was applied to mitochondrial proteins as well as human plasma and human plasma depleted of serum albumin in order to facilitate enrichment of phosphoproteins or glycoproteins. The proteins from the five fractions were then subjected to 1D SDS PAGE as well as narrow-range miniature IPG strip based separation using the IPG runner. All gels were stained with the Multiplexed Proteomicsâ dyes, Pro-Qâ Diamond phosphoprotein stain, Pro-Q Emerald 300 glycoprotein stain and SYPROâ Ruby protein gel stain. Select proteins were subsequently identified by MALDI-TOF mass spectrometry. This study clearly shows the usefulness of the approach for enrichment of water-soluble proteins as well as membrane proteins.

P99-T
New Software in Protein Prospector Allows Reliable Analysis and Comparison of Large Datasets
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There is a compelling need to have the ability to reliably analyze and compare automatically very large data sets obtained on complex protein mixtures using multidimensional chromatography and tandem mass spectrometry, as well as ascertaining the performances of different search strategies for such datasets, which are too large for manual interpretation. We have developed two new pieces of software within the Protein Prospector framework to facilitate these tasks. 'LC-Batch Tag' searches MSMS data using a newly developed probability-based scoring system that gives highly reliable peptide and protein assignments. The performance of this scoring system is demonstrated on a dataset of 3270 spectra acquired on a QSTAR (Applied Biosystems) from a 2D LC analysis of proteins interacting Gsp1p, one of the key regulating proteins in nucleo-cytoplasmic transport in yeast.
A second program 'SearchCompare' within Protein Prospector is able to combine, filter and compare different search results. It can produce three different types of report; all peptides/proteins identified by any search (union); all peptide/proteins identified by every search (intersection); or peptides/proteins only identified in a particular search (difference). Using this program one can compare and combine results acquired on different instrument platforms; e.g. LC-ESI-MSMS and LC-MALDI-MSMS analyses. This is important as we observe only approximately a 50% overlap in peptide identifications between the two analysis approaches. SearchCompare can also compare results from Protein Prospector and the alternative search engine 'Mascot'. Examples of all these software features are illustrated using the Gsp1p-binding proteins dataset.
This work was supported by NIH NCRR grant 01614 and the Vincent J. Coates Foundation.

P100-S
Fluorescence based staining of hydrophobic proteins containing two or more membrane spanning helices
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Integral proteins containing two or more a-helical membrane-spanning domains are underrepresented in two-dimensional gels. While SDS-polyacrylamide gels separate these proteins, staining profiles are usually dominated by high abundance hydrophilic proteins in the specimen. A fluorescence-based stain is presented that selectively highlights integral proteins containing

two or more a-helical transmembrane domains but does not detect lipoproteins or proteins with hydrophobic pockets, such as albumin. The stain detects as little as 5-10 ng of bacteriorhodopsin, a 7-helix transmembrane protein. Stained proteins are detected using a laser scanner or CCD camera imaging system. Fluorescence intensity of stained bands is linear with protein quantity over at least two orders of magnitude. After visualizing transmembraneous proteins, the total protein profile may be revealed using a general protein stain. Analysis of the multi-subunit protein F1F0 ATP synthase revealed selective staining of the a and c subunits, polypeptides known to possess 5 and 2 transmembrane domains, respectively.

P101-M

Investigation of Charge-Derivatized peptide by Maldi-MS and Maldi-MS/MS

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Peptide mass fingerprinting (PMF) by MALDI TOF mass spectrometry is a high speed and sensitive method for protein identification enhanced by the mass accuracy of the instrument used. PMF relies on the comparison of experimentally observed peptide masses of enzymatically digested proteins with theoretical peptide masses, obtained by the in silico digestion of proteins contained within protein sequence databanks. This is an ideal technique for identifying proteins from a known proteome, although this approach will fail when a protein is not represented in the protein sequence database or if some of the peptides are modified or too few peptides are detected to give an unambiguous answer . In these cases MS/MS analysis coupled with a homology search may allow the correct identification of the protein. With a Quadrupole Tof hybrid mass spectrometer equipped with a MALDI source MS/MS spectra can quickly be generated. A feature of this type of instrumentation is the high mass accuracy - better than 10ppm RMS is achievable. It should be noted that MS/MS spectra generated from singly charged peptides exhibit a wide variety of fragment ions. The types of ions produced are very sequence dependent. This can make interpretation of MALDI MS/MS data difficult.
A way of simplifying the spectrum and improving the fragmentation efficiency is to introduce a derivative charge on the peptide. A new N-terminal derivatisation is presented, using N-Tris (2,4,6-trimethoxyphenyl) phosphonium-acetic acid N-hydroxysuccinimide ester (TMPP-acOSu).
The results will show all the advantages of this reagent including an increase in coverage and the effect on fragmentation.

P102-T

Purification of Serum Peptides by Ultrafiltration and Solid Phase Extraction

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Biological fluids such as blood and urine are readily available for screening or clinical analysis. Serum peptides may serve as an indicator of an organism’s progression from normal to a diseased state. However, high protein concentrations, lipids and salts in serum samples suppress the ionization of native peptides during MALDI-TOF MS analysis. As a consequence, sample complexity reduction is rapidly becoming the essential first step of any peptide analysis scheme. Several pre-fractionation strategies using chromatographic absorbents have been described which remove abundant proteins such as albumin or IgG. As an alternative to adsorption chromatography, we have subjected mammalian (human, murine, & bovine) serum or plasma samples to ultrafiltration (UF) to produce relatively protein-free peptide solutions. The ultrafiltrates were then acidified and transferred to $ZipPlate_{C18}$ for de-salting and concentration of the peptide samples. Peptides were eluted by either

centrifugation or by the MALDIspot technique. The latter method enables elution of peptides directly onto a compatible MALDI-TOF target, thereby achieving the highest attainable concentration of peptides. It was demonstrated that a 10KDa molecular weight cut-off ultrafiltration membrane gave optimal results based on significantly improved detection and resolution of serum peptides in the 800-4000 m/z range. The method is applicable for peptide purification from a variety of biological fluids including serum, plasma, urine, cell or tissue lysates. The purified peptides are amenable to sequencing by mass spectrometry methods. The combination of UF and SPE techniques for enhanced peptide analysis and the elucidation of peptide patterns enables more rapid and efficient discovery and characterization of potential biomarkers.

P103-S

Analysis of a Renal Adenocarcinoma Cell Line by a Cohesive Multidimensional LC/MALDI/MS/MS Workflow

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Analysis of whole-cell lysates for cancer biomarkers is an important proteomics workflow task. These samples contain thousands of proteins spanning several orders of magnitude. Several complementary techniques are needed in order to identify the low level proteins in the presence of high levels of housekeeping proteins. Instrument sensitivity and mass accuracy, efficient separation, intelligent data acquisition, and efficient conversion of MS/MS data to valid protein identifications are all critical requirements of an effective proteomics workflow. In addition, it is important to be able to go from cell lysate through separation, data acquisition, and protein identification. Once protein identifications are made, they must be analyzed in a way to show meaningful biological information. Front-end techniques in some biomarker workflows are ion exchange chromatography, reverse phase nano HPLC, and subsequent deposition onto MALDI targets for MALDI/MS/MS analysis. This technique, broadly defined as LC/MALDI is a particularly useful technology because chromatographed samples are archived onto MALDI plates. Investigators using this technique are not bound by the challenges of real-time MS/MS data collection. Users can return to an archived sample after some initial information is collected and interpreted. It is desirable to take discrete MS/MS spectra, collate them into cohesive sample sets, and analyze the sample set for protein identifications and subsequent correlation to gene information. We combine multidimensional LC with MALDI plate deposition followed by intelligent data acquisition at sub-femtomole levels. The data is processed and linked to gene information in a cohesive workflow in order to study a renal adenocarcinoma cell line.

P104-M

Global Internal Standard Technology (GIST) for Relative Quantitation of Post-Translational Processes of Proteins in Complex Mixtures

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Global Internal Standard Technology (GIST) was used with a high-throughput comparative proteomics platform to quantitatively profile diseased vs. normal serum. Samples were delipidated, depleted of abundant proteins, and fractionated at the protein level. Following digestion, peptides from normal and diseased serum were labeled with light ($^{1}H_3$) and heavy ($^{2}H_3$) acetate-based *N*-acetoxysuccinimide reagents, respectively. Samples were mixed together, quantitative profiling data were acquired by LC/MS, and sequences were determined by LC/MS/MS. Profiling by LC/MS revealed the post-translational processing of serum amyloid A to AA. Ratios from several unique SAA peptides showed distinct differences that may represent the known processing of SAA that occurs during Amyloidosis at amino acid position 76. Since GIST isotopically labels essentially all peptides, we show that post-translationally modified proteins can be

effectively studied. Reproducibility data of GIST labeling will be shown as well as some features and reproducibility of the in-house data processing software, GISTool.

P105-T

Tandem Affinity Purification (TAP) of Mammalian Cell Protein Complexes

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Analyzing protein complexes is key to understanding many biological processes in living cells. Tandem affinity purification (TAP) of protein complexes and identification of constituent proteins by mass spectrometry are useful for accomplishing this goal. The key feature of TAP is the use of two different affinity purification tags fused to at least one known component of the protein complex of interest. Although a variety of purification tags have been reported for protein complex purification, in practice few combinations yield satisfactory results. We developed a tandem affinity purification system by constructing CMV promoter-driven mammalian expression vectors that fuse calmodulin binding peptide (CBP-tag) derived from muscle myosin light chain kinase and synthetic streptavidin-binding domain (SBP-tag) onto either the N- or C- terminus of expressed proteins. These tags provide high affinity binding in the nanomolar range to calmodulin and streptavidin affinity purification resins and small molecule elution (EGTA and biotin, respectively) of protein complexes, thus maximizing purification and minimizing disruption of targeted complexes. To demonstrate the utility of the SBP-CBP TAP system, the open reading frame of transcription factor MEF2a was cloned into N- and C- versions of the CMV-SBP-CBP vector. Transcription factor MEF2c was cloned into both N- and C- versions of a CMV-FLAG tagged vector. The MEF2a-SBP-CBP constructs act as "bait" to co-purify interacting proteins. MEF2a and MEF2c were chosen because their interaction was previously demonstrated using a CBP-ProteinA based TAP system. MEF2a-SBP-CBP and MEF2c-FLAG were co-transfected into COS-7 cells. MEF2a-SBP-CBP and MEF2c-FLAG formed a complex that was TAP purified. Both proteins were positively identified by Western blotting and mass spectrometry.

P106-S

Fully Automated Setup and Measurement of Multiplex Protein Assays

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The need for automated technology in the study of protein-protein interactions, hormone and biomarker levels, and complex intracellular signal transduction pathways has become a key issue in life science research and drug discovery. In order to address this need, an automated platform that provides assay setup, transfer, and measurement has been developed. The workstation utilizes well-established immunoassay principles using a bead-based system and efficient immobilization of proteins, enabling multiplex analysis and sensitivity down to picogram levels.

P107-M

Comparative Proteomics Of The Human Pathogen *Campylobacter Jejuni*

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Campylobacter jejuni is an important human pathogen leading to diseases such as gastrointestinal disorders, including food poisoning, and outcomes as severe as Guillain-Barré Syndrome. Proteomics has been successful in comparing strains of bacteria with different phenotypes, including infectivity, antibiotic resistance and medical outcome. Here we present a comprehensive catalog of *C. jejuni* proteins using a variety of proteomics methodologies as a

basis for a protein map of this organism. *C. jejuni* proteins were pre-fractionated using commercially-available kits into whole cell, cytosolic, soluble / insoluble and membrane fractions and subjected to wide- and narrow-range IPG two-dimensional gel electrophoresis (2-DGE) prior to protein identification by MALDI-TOF mass spectrometry (MS). Fractions were also digested with trypsin and the resulting peptides analyzed by two-dimensional liquid chromatography (2-DLC) and electrospray-ionisation (ESI) MS/MS. The two methods provide a highly complementary approach to protein 'cataloguing' of bacterial proteomes. We also conducted comparative proteomics of membrane fractions from two clinical isolates – a gastrointestinal isolate and a Guillain-Barré Syndrome isolate to determine potential markers for rapid strain identification. Western blotting was also performed on these fractions to characterize highly immunogenic proteins. Identification of proteins from strains of *C. jejuni* will provide an important first step in characterizing strain differences potentially responsible for different disease outcomes associated with this organism.

P108-T

Characterization of Glycoproteins in the Human Plasma Proteome

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Post-translational modification of proteins are one reason the proteome of a tissue or cell cannot be directly predicted from its genome. Glycosylation of proteins are a significant form of post-translational modification. The glycoproteins of blood plasma demonstrate great heterogeneity and analysis of plasma glycoproteins is required for complete characterization of this proteome. This paper will characterize the glycoproteins of the human plasma proteome by a multidimensional technique that combines separation by liquid chromatography and analysis by capillary electrophoresis. Initially, the plasma is fractionated with an automated two-dimensional chromatographic system. The first dimension separation is done by chromatofocusing over a pH range from 8.5 to 4.0 where proteins are separated by their isoelectric points. In this dimension, fractions are collected based on pH intervals. The proteins are detected by UV absorbance at 280 nm. The first dimension fractions are then resolved in the second dimension by high resolution reversed phase chromatography with a gradient of trifluoroacetic acid (TFA) in acetonitrile and TFA in water. The proteins are detected by a second UV detector at 214 nm. Fractions collected from the second dimension are then characterized for glycoprotein content by capillary electrophoresis. The fractions are reduced with _-mercaptoethanol, followed by asparagine-glycan cleavage with PNGase F and the released oligosaccarides were labeled with 8-aminoacripyrene-1,3,6-trisulfonate (APTS). The APTS-labeled oligosaccarides were separated by capillary electrophoresis in a 50.2 cm coated capillary. The detection was done by laser induced fluorescence. The resulting distribution of the oligosaccarides from the fractionated plasma proteome revealed the heterogeneity of the glycoproteins in plasma. These results support the characterization of proteome glycoproteins with the combination of proteome fractionation by two-dimensional chromatography followed by capillary electrophoresis analysis.

P109-S

MDLC-MS/MS Analysis of the Human Plasma Proteome – the Challenge of the Concentration Dynamic Range and he Complexity

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The possibility to screen for presence of tissue leakage proteins in the human plasma could potentially significantly promote the development protein-marker based tools for disease diagnosis and therapeutic monitoring. However, although it is a promising application

for the proteomics approach, the human plasma proteome is also one of the most challenging proteomes to analyze. Theoretically, the entire human proteome could be present in the plasma leading to an extreme complexity in the digested sample, with in the order of 10^6 peptides of unique identity. Moreover, the very large concentration dynamic range of 10^{10} (from 40 mg/mL down to a few pg/mL has been analyzed) is an intricate analytical challenge.
There is consequently a need for a reduction of both dynamic range and complexity. In the presented approach, high abundant proteins (Albumin and IgG) were removed by affinity chromatography. Thereafter the sample was fractionated with gel filtration to further remove known high-abundant plasma proteins. The sample was subsequently digested and run on a linear-gradient/fractionation MDLC workflow. The sample was loaded on a SCX column in the first dimension. Sixty fractions were collected and analyzed on a 75 µm RPC column with a high-throughput nano-flow LC system connected to an ion-trap MS. MS/MS spectra of the analyzed peptides were matched against human protein databases.
The results show that low abundant proteins in the plasma can be identified with the selected approach, although the large dynamic range in concentration poses a significant test for the analytical tools. The results will be discussed in the context of the analytical procedure optimization and the performance range of the different analytical steps.

P110-M
Improving Peptide *de novo* Sequencing via a Charge Derivatization in MALDI Q-TOF Analysis
W. Chen, P. J. Lee, D. Wall, Y. Yu, J. C. Gebler; Waters Corporation, Milford, MA, United States.

The recent development of a MALDI ion source coupled to a mass spectrometer with a tandem quadrupole-time-of-flight analyzer has provided additional tools to obtain reliable MS and MS/MS data for protein/peptide analysis. The combination of performing both MS and MS/MS data acquisitions from the same sample on the same instrument clearly has the advantages of high sensitivity, high resolving power, and high mass accuracy. However, ionization of peptide and protein digests via MALDI preferentially yields singly charged analyte ions, and the fragmentation of these ions often shows unique characteristics. For example, singly charged tryptic peptides tend to generate higher background in MS/MS spectra and undergo preferential cleavage adjacent to amino acid residues containing an acidic side chain. Selective fragmentation reactions limit the amount of de novo peptide sequence information that can be obtained in these experiments. In this presentation, we wish to report our strategy to overcome this obstacle by modifying peptides using a fixed-charge derivatization reagent, tris(2, 4, 6-trimethoxyphenyl) phosphonium acetic acid *N*-hydroxysuccinimide esters (TMPP-Ac-OSu). Peptides, after derivatizations, show enhanced ionization efficiency. Collision-induced dissociation (CID) of derivatized peptides and protein digests on the new generation of MALDI Q-TOF instrument significantly enhances the amount of protein/peptide sequence information obtained, thus greatly facilitating de novo sequencing. In addition, the utility of the approach for protein identification from databases will also be presented.

P111-T
Separation of a Protein Sample in Four Dimensions: Solubility, Chromatography and Two Dimensional Electrophoresis (2DE)
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2DE is a high-resolution separation technique. Even in very good separations however, multiple proteins can be identified in a single spot. To improve the resolution of the technique we have added two additional dimensions of separation. Cultured human proximal convoluted tubule cells were sequentially solubilized. The

20,000xg pellet from each step was solubilized in a buffer containing 5mM Tris (pH 9.5) with benzonase and protease inhibitors and the following reagents: Extract 1-none; Extract 2-8M urea and 4% CHAPS; Extract 3- 5M urea, 2M thiourea, 2% CHAPS and 2% SB 3-10. Proteins were separated on IPG strips over a pI range of 3-10 followed by SDS-PAGE. Proteins were visualized with Sypro ruby, spots detected and gels matched. A gel prepared using the whole cell lysate was used as the master image. The following numbers of spots were detected: Whole lysate-457, extract 1-723, extract 2-432, extract 3-112. Many spots appeared in only one solubility fraction.
Proteins from extract 1 were further separated by anion exchange chromatography on an Uno-Q1 anion exchange column. The column was washed with a buffer containing 20 mM Tris (pH 8.2) and proteins were eluted from the column with increasing concentrations of buffer B (1 M NaCl, 20 mM Tris pH 8.2). Proteins eluted with 5, 10, 20 and 30% buffer B were concentrated in a centrifugal concentrator and separated by 2DE. The following numbers of spots were detected: Extract 1-723 5%-249, 10%-74, 20%-322, 30%-64. Many spots appeared in only one anion exchange fraction. Separation by chromatographic and solubility characteristics improved resolution of proteins.

P112-S

A Comparison of Recombinant Antibody Sequence Coverage obtained by MALDI-TOF AND ESI-LC/MS/MS

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In the biopharmaceutical industry, recombinant antibody drugs are often produced in Chinese Hamster Ovary (CHO) cells. Following production of the recombinant antibody, many analytical techniques are employed to determine purity and characteristics. Peptide mass fingerprinting is commonly used as an important part of this process. Upon completion of non-reducing SDS-PAGE analysis of a recombinant antibody, SyproRuby staining typically reveals several protein bands. Although all of the bands observed by SDS-PAGE analysis are antibody-related, achieving 100% sequence coverage and describing the exact composition of each minor band can be difficult. This may be due to several factors including the mass range and sensitivity observed during MALDI-TOF and ESI-LC/MS/MS, the enzyme used for protein digestion, matrix, and gel extraction difficulties. Obtaining the highest sequence coverage possible is necessary to confirm the identity of proteins and to be able to find evidence of truncations or post-translational modifications. In this study, we sought to evaluate different mass spectrometry methods to obtain consistently high sequence coverage of our recombinant antibody drugs.

P113-M

MALDI Sample Preparation: Does Matrix Purity Really Matter?

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Sample preparation is recognized as a crucial step for a successful MALDI analysis. A variety of MALDI sample preparation techniques such as dried-droplet method and thin-layer method have been reported to increase the sensitivity of analysis, improve the signal resolution, and enhance the tolerance of MALDI analysis towards involatile contaminants in samples, for example, buffers, salts, detergents or denaturants.[1-3] MALDI matrices, _-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA) and 2, 5-dihydroxybenzoic acid (DHB), are among the most commonly used ones in those techniques, especially for peptide and protein analysis.
Since matrix is the key component of MALDI analysis, the influence of matrix purity on the quality of MALDI mass spectra was the subject of this investigation. A variety of CHCA, SA, and DHB with purity grades from 97% to ultra-pure (> 99.0%) were systematically evaluated for MALDI experiments. The focus of the examination included the adduct formation of metal salt with analytes, the intensity of background ions, the signal-to-noise ratio of the

spectrum, and the limit of detection of peptides and proteolytic protein digests. The results showed the utility of ultra-purified matrices significantly improved the quality of MALDI mass spectra and considerably increased the sequence coverage of proteins identified by peptide mass fingerprinting approach. Using dried-droplet sample preparation with ultra-pure matrices, protein identifications at low femtomole loadings can be routinely achieved. Furthermore, applying ultra-pure matrix to the thin-layer sample preparation method enabled the limit of detection of peptide and protein digest to reach attomole levels.

(1) O. Vorm *et. al., Anal. Chem*. 66, 3281-3287 (1994)
(2) M. Kussmann *et.al., J. Mass Spectrom*. 32, 593-601 (1997)
(3) Y. Dai *et. al., Anal. Chem*. 71, 1087-1091 (1999)

P114-T

Detecting Noncovalent Protein-Protein Interactions Using Automated Chip-Based NanoESI/MS

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As progress is being made in the field of proteomics, the study of protein complexes and how they interact is becoming more important. The application of ESI/MS particularly nanoESI/MS to the study of noncovalent protein complexes formed between two different proteins is limited. As considerable progress in the proteomics field is being made, protein complex studies are becoming more and more popular as they are important not only for drug discovery but functional proteomics. A complete understanding of proteome relies on characterizing the interactions of proteins with other macromolecules. Protein function in cell is most often mediated by protein-protein interactions that are central to many cellular activities.

The purpose of this work was to demonstrate if an automated chip-based nanoelectrospray system coupled to a mass spectrometer could be used reliably to study noncovalent protein-protein interactions

The interaction of trypsin inhibitors to their target enzymes has been extensively studied and provides an ideal model for studying protein-protein interactions by nanoESI/MS. Two target proteins (bovine trypsin and trypsinogen) were used for determination of noncovalent binding interactions with bovine pancreatic trypsin inhibitor (BPTI) and soy bean trypsin inhibitor (SPTI) respectively. BPTI or SPTI was mixed with trypsin and trypsinogen respectively in 10 mM ammonium acetate solution prior to automated nanoESI/MS analysis using a NanoMate coupled with a Q-TOF micro MS. The results from this model system demonstrate that automated nanoESI/MS offers an important new platform for detecting noncovalent interactions between proteins. In addition, the automated chip-based nanoelectrospray system used for studying protein complexes not only reduces sample consumption and increases analysis speed, but also dramatically improves the efficiency and quality of the ESI/MS method.

P115-S

Proteome Profiling of Extracellular Matrix-Induced Modulation of a Human Bladder Cancer Cell Line

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The cell-extracellular matrix interaction is a major modulator of cellular phenotype. When grown on Matrigel, which is extracellular matrix remodeled by malignancy, the human bladder cell line, J82, grew as an invasive phenotype. When grown on SISgel, which is derived from porcine small intestine submucosa and represents the normal matrix supporting differentiated cell growth, J82 cells grew as a layer of noninvasive cells, one to two cells thick. Thus depending upon the matrix, the J82 phenotype was modulated from highly invasive to near normal. On plastic, no matrix is present and matrix-dependent tissue-like organization is lost. Using

a two-dimensional chromatographic system for proteome fractionation, this paper compares the proteome profiles of J82 bladder cancer cells grown on plastic, SISgel and Matrigel. The first dimension separation is chromatofocusing over a pH range from 8.5 to 4.0 where proteins are separated by their isoelectric points. Fractions are collected by pH intervals as detected by a pH monitor. Proteins are detected by absorbance at 280 nm. The second dimension is high resolution reversed phase chromatography. The proteins are detected by absorbance at 214 nm. Comparison of the proteome profiles of J82 cells grown on plastic, SISgel and Matrigel showed significant qualitative and quantitative differences indicating that the suppression of the malignant phenotype on SISgel is associated with significant differences in protein expression. Comparing cells grown on gels to those on plastic indicated significant up-regulation of a number of proteins in culture that mimics tissue. The potential identity of these protein differences is being determined by mass spectrometry.

P116-M

Mapping of Protein Phosphorylation Sites Using Chip-based Nanoelectrospray with Precursor Ion Scanning Quadrupole TOF Mass Spectrometry

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Reversible protein phosphorylation is a biological regulatory mechanism that affects many cell activities including metabolism, cell division, cell growth and cell differentiation. Detection of the phosphorylation sites in a protein can provide insight into these mechanisms. However, isolating and sequencing phsophopeptides from protein digests remains a labor-intensive and time-consuming challenge in conventional biochemical approaches.
It remains a challenge to identify phosphopeptides in a complicated mixture, due in part to poor ionization efficiency and fast degradation of the phosphopeptides. Precursor ion scans have proven to be useful for the characterization of unseparated peptide mixtures. We will demonstrate the use of a chip-based nanoelectrospray ionization source in combination with precursor ion scan on a QSTAR Pulsar quadrupole time-of-flight tandem mass spectrometer for identification of phosphorylation sites of proteins digested in solution. This platform provides high sensitivity, reproducibility, and stability for chip-based nanoelectrospray.
_-casein Two standard phosphoproteins (bovine and chicken ovalbumin) were analyzed with precursor ion scan of m/z -79 followed by positive ion tandem mass spectrometry analysis. Additionally, a synthetic tyrosine phosphorylated peptide was spiked to the above digests and used for precursor ion scan of m/z +216.04. The results showed that we were able to map the phosphorylation sites for _-casein digest at 50 fmol/_L and for ovalbumin digest at 250 fmol/_L level. Precursor ion scan of m/z +216.04 provided high sensitivity and better selectivity for identification of tyrosine phosphorylated peptide. The results demonstrate that the fully automated chip-based nanoelectrospray platform is a valuable system for phosphorylation analyses due primarily to stable, extended infusion times for completing precursor ion scan followed by MS/MS on the identified phosphorylated peptides.

P117-T

QuikTip$_{C18}$ Micropipette Tips: Performance Evaluation and Utility in a Novel Direct-Extraction Accelerated In-Gel Digestion Protocol

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Reverse phase C18 pipette tips are often used to remove salts and other interfering chemicals from samples prior to analysis by mass spectrometry. One limitation of such tips, which utilize a true resin bed, is a low and variable flow rate. By contrast, Stratagene's new QuikTip$_{C18}$ micropipette tips offer scientists a format with improved and predictable flow characteristics for rapid sample preparation. This is accomplished through a proprietary process whereby the

stationary phase is fused to the walls of the tip leaving an unobstructed solvent access channel. In this work, the performance of these tips is evaluated for the removal of NaCl and urea from tryptic digests of BSA. The utility of these tips is then demonstrated in a novel direct-extraction accelerated in-gel digestion protocol. In gel digestions were performed using three coomassie-stained model proteins, present in different quantities, which were isolated from 1D-PAGE gels. The accelerated protocol is shown to circumvent a traditional post-extraction vacuum centrifugation step that is time consuming and can lead to heavy sample losses. Instead, following a short incubation of gel pieces with an extraction solution, peptides are directly extracted and concentrated from the liquid/gel piece mixture using $QuikTip_{C18}$ tips. Samples in both experiments were digested with Stratagene's mass spectrometry-grade trypsin and were analyzed by MALDI-TOF and LC-MS mass spectrometry. The results of these experiments are presented.

P118-S

Quantitative Proteomics Using Two-Step $^{18}0$ Peptide Labeling

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Mass spectrometry (MS) is a valuable tool for protein identification. Stable isotope incorporation into peptides affords MS quantification, with ^{18}O peptide labeling providing several advantages. All peptides (excluding the original C-terminus) regardless of amino acid sequence are ^{18}O labeled, ionize equivalently to ^{16}O labeled counterpart peptides and increase in mass by an expected value thus simplifying identification and relative quantitative differences between control and experimental samples. ^{18}O labeled peptides also behave indistinguishably from ^{16}O labeled peptides in chromatographic separations, a critical feature for liquid chromatography/MS applications. We describe an improved method for comparative quantitative proteomic analysis wherein peptides are labeled at each C-terminus with two ^{18}O atoms. Labeling is performed in a two-step process in which protein digestion with soluble trypsin is followed by ^{18}O exchange using immobilized trypsin. To demonstrate the utility of this approach, we compare saliva samples from oral cancer patients to unaffected individuals. Identification of saliva protein markers unique to oral cancer will allow researchers to diagnose cancer in a non-invasive, inexpensive and accurate manner.

P119-M

Applications of the Molecular Scanner to the Identification and Characterization of Proteins Separated by SDS-PAGE

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The Molecular Scanner is a method for analyzing protein bands in SDS-PAGE 1D gels without the need to excise the gel bands and carry out in-gel digestion (Binz, *et al.*, *Anal. Chem. 71* (**1999**), 4981-4988). These data can be used to identify proteins by peptide mass fingerprinting (PMF) and database searching, and, if MALDI tandem mass spectrometer is employed, then peptide fragmentation spectra can be used for database search in addition to the PMF data.
We have applied this technology to the identification and characterization of proteins from a number of sources: Recombinant protein samples, containing impurities in addition to the main product; relatively simple mixtures generated by affinity purification, in which the electrophoretically separated proteins may be functionally but not structurally related; and more complex mixtures (e.g. cell lysates) in which proteins have been fractionated by ion exchange chromatography prior to loading each fraction onto the gel. Once proteins have been identified gel images may be reconstructed, using software tools, and interrogated with

regard to the presence and location of individual proteins of interest (“Electronic Western Blot”).

P120-T

New Technologies for Expanding the Dynamic Range of Protein Identification in Human Serum

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Proteomics is promising in protein identification for disease markers and/or drug targets. But it is also a challenging field due to the complex nature of proteins and the wide dynamic range of protein mass in biological samples such as human plasma/serum. Dynamic range limitations in protein separation technologies such as gel electrophoresis (2DGE), capillary electrophoresis, and HPLC need to be overcome to enable the detection of lower-abundant proteins of interest in mixtures. Peptide mass mapping by MALDI-MS has the inherent problem for detecting stronger arginine peptide signals than lysine tryptic fragments. In this study, we have used an immunodepletion technology to remove six high-abundant proteins: albumin, IgG, IgA, haptoglobin, transferrin, and antitrypsin to bring low-abundant proteins into detectable levels. Furthermore, we have utilized a novel lysine mass tagging reagent to improve peptide sequence coverage and MS/MS fragmentation of lower-abundant proteins found in human serum.
After removal of the six high-abundant proteins (85-90% of total protein mass in human serum), low abundant proteins were efficiently enriched and resolved on 2DGE with ten times increase in mass loading. An increase in level of confidence in MALDI-MS identification of proteins was demonstrated by derivatizing lysine side chains with 2-methoxy-4,5-dihydro 1H-imidazole. This lysine specific mass tagging reaction resulted in a 5 – 20 times increase in lysine peptide signal relative to the underivatized peptides. These technologies, used together, enable improvements in the number of identifiable proteins in human serum/plasma.

P121-S

Nano-Flow HPLC Technology for Proteomic Applications in Conjunction with Nanospray

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The emergence of Nano-Spray Mass Spectrometry as a routine analytical tool to meet the need for limited sample applications and trace analysis of cellular proteins has stimulated an increased need for the development of reliable Nano-LC systems that are in the nano-flow range without flow splitting. For the past decade, it has been common practice for analytical chemists to rely on conventional LC pumping systems equipped with passive flow splitters or check valves to perform Nano-LC applications. In the field, it has been proven that these traditional flow-splitting techniques have lacked in performance reproducibility and reliability. It is difficult to rely on a flow-splitting Nano-LC system for a reliable analysis of very precious biological samples.
Still to this day few new nano-flow pumps have been introduced and it is still questionable as to whether or not such technology will be able to perform in the field with long-term applications, without sacrificing reproducibility or efficiency. This presentation will discuss the design principle and performance features of a Nano-LC system based on the patented closed-loop digital motion control technology. We will discuss the practical aspects of the applications of the Nano-LC-Nano-Spray MS-MS in terms of system dead volume considerations, sample cleanup/concentration/pre-fractionation, fused-silica transfer tubing requirements, and routine maintenance for the practice of cellular protein separation and identification.

P122-M

A Fully Automated Capillary Column Proteomic Analyzer ICAT^{TM} System for Complex and Low-Abundance Protein Separation, Identification, and Quantitation

F. Di Stefano, **F. J. Yang**, K. Studley, D. Nguyen, B. Tuh, D. Nepomucino, J. Lin, M. Ko,

R. Xu; Micro-Tech Scientific, Inc., Vista, CA, United States.

Multi-dimensional Capillary and Nano-LC-MS/MS are essential tools for complex cellular protein separation and identification. It offers many significant advantages over the traditional 2D-gel electrophoresis approach. As multidimensional capillary/Nano column LC-MS/MS becomes routine, the ultimate goal is to quantitate trace amounts of protein markers in cell extracts. For this presentation, we will introduce a fully automated capillary and Nano-LC-MS system that is based on Applied Biosystems' ICAT™ chemistry and reagents. We will discuss the design features and performance considerations of the X'TremeSimple ICAT™ system. Examples for the separation, identification, and quantitation of cell extract proteins will be presented.

P123-T

Mass Spectrometric Quantitation of Differentially Expressed Protein From Stable Isotopically Labeled CHO Cell Media

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Recent approaches in Analytical Biochemistry have made use of stable isotopes for quantitative protein analysis. Several methods of isotope incorporation have been explored including amino acid labeling, and metabolic labeling in media containing stable isotopes.
MALDI-TOF-MS is a powerful technique for relative quantitation and can be used for obtaining differential protein expression data or for performing expression profiling. The high purity of modern day stable isotope compounds minimizes isotopic contamination resulting in high quality quantitative data.
A deficient CHO cell media was developed for the selective addition of either doubly labeled amino acids or indigenous amino acids. Mammalian cells previously designed to express ß-Galactosidase with a FLAG epitope tag were used in this study. The option of using an essential amino acid such as lysine with both nitrogen and carbon stable isotope labels greatly facilitates quantitation. Incorporation of doubly labeled lysine results in an 8 Dalton mass shift, which places the isotope population well outside that of the indigenous lysine containing peptide. The experiments described herein use a recombinant ß-Galactosidase with a FLAG fusion tag for downstream antibody purification using EZview™ Red ANTI-FLAG® M2 Affinity Gel followed by SDS PAGE, in-gel digestion and guanidination. Expression of this protein was used to evaluate different media additives and growth conditions. Mass spectrometric identification and relative quantitation was performed by MALDI-TOF-MS.

P124-S

Analysis of Membrane Adsorbers in Micro-Spin Columns for Sample Preparation Prior MALDI Analysis

Mike Naldrett (1), Andreas Kocourek (2), Wolfgang Demmer (3), Dietmar Nussbaumer (3) Robert Zeidler (2) and **Rick Garretson** (4); 1: John Innes Centre, Norwich Research Park, Norwich, UK; 2: Vivascience AG, Hannover, Germany; 3: Sartorius AG,Göttingen, Germany; 4: Vivascience Inc Carlsbad CA, USA

Recently, a family of membrane adsorbers with different chemistries was introduced for rapid separation and purification of proteins. Several ligands e.g. IEX, chelate and protein A covalently bound to the membrane are so far available.
Here, we focus on a proprietary, reversed phase membrane in new micro spin columns. In this study desalting and concentration of peptides prior to MALDI measurements were analysed. Data will be shown for rapid and effective purification of peptides from typical protein digests. It could be demonstrated that even fmol of peptides in a size range between 700 and 3500 m/z could be efficiently recovered and analysed by MALDI. Resulting peak list was used for MASCOT search. In comparison with direct sample preparation the MASCOT search was increased threefold. Furthermore, the sequence coverage was three times higher than without membrane-containing device. In addition, even

low concentrated peptides within complex digestion mixtures could be concentrated, measured and successfully identified. The specific micro device spinned in usual lab centrifuges allows single loading of up to 200 _l digests. Alternatively, the digest itself could be done in the micro device in order to avoid any loss of peptides. After one wash-step peptides can be eluted in small volumes < 4 _l for direct loading onto MALDI targets.

P125-M

Reduction of Sample Complexity to Target Ca^{2+} Binding Proteins from Tissue and Cell Extracts.

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Ca^{2+} has a central role in the function of neurons as trigger for neurotransmitter release and other aspects of neuronal activity. The protein-protein interactions of Ca^{2+} binding proteins with other associated proteins provides an ideal system for the study of neurotransmitter release regulation, regulation of ion channels and many other functions where Ca^2+ proteins are involved. Our goal was to find a simple and reliable way to selectively bind Ca^2+ binding proteins for microscale proteomic sample preparation. Using affinity membrane chromatography and mini-spin columns, we enriched the Ca^2+ binding proteins from human brain (cortex) extracts and then further analyzed them with 1-D and 2-D gel electrophoresis and a novel orthogonal MALDI TOF mass spectrometer. Several Ca^2+ binding proteins were identified using these techniques in combination with peptide mass fingerprinting.

P126-T

Solubility-based protein separation followed by tandem mass spectrometry with MALDI ionization

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Liquid-based pre-fractionation was used to separate the proteins of the Gram-positive bacteria Staphylococcus aureus (strain N315). To increase the variety and number of identified proteins, a number of sample fractionation strategies were applied to separate proteins according to their solubility.

Membrane protein from S. aureus were extracted in a water / trifluoroethanol / chloroform phase partitioning system and the resulting fractions were separated by SDS-PAGE. Bands from SDS-PAGE gels were analyzed by microcapillary LC-MS/MS using both MALDI and ESI methods.

Several hundred proteins were identified from both the soluble and pellet fractions. The overlap in identified proteins between these two fractions is below 30 %. The analysis of the SDS-PAGE bands by LC-ESI-MS/MS and LC-MALDI-MS/MS also showed interesting differences. An increased number of identifications were observed with the LC-MALDI workflow compared to the LC-ESI workflow. However, there were numerous proteins only identified by LC-ESI. A dual ESI/MALDI analysis is therefore preferred for global analysis.

The small overlap of 30% identified proteins between these fractionation methods supports the idea that a combination of solubility-based separations is essential for comprehensive analysis of complex protein samples. For MS analysis, the observed differences suggest that both analysis techniques are complementary. Their combination enables a higher proteome coverage. In combination with transcriptomic data this high density proteomic data is essential to obtain a global view of the biological system of S. aureus.

P127-S

Non-affinity based MDLC pre-Fractionation on Human Serum Samples in Proteomics

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Human serum is one of the most complex samples to deal with in proteomics. The total number of proteins and the huge dynamic range between the high and low abundant proteins put a special challenge on the researcher. This approach uses pre-fractionation to reduce the complexity of the human serum proteome increasing the sensitivity for protein identification in the mass spectrometer. The importance of reproducible fraction collection between samples is essential so that differences are not due to experimental variability.
With very complex samples powerful pre-fractionation techniques are definitely indisputable. For this application the separation power of the MudPIT approach, which involves only a 2-dimensional separation at peptide level (post digestion) is by far not sufficient. Additional distribution steps at protein level are necessary in order to get access to subsets of the proteome and low abundance proteins.
Recent publications (Mol Cell Proteomics. 2003(10):1096-103) indicate that most of the interesting lower abundant proteins have a molecular weight below 45 kDa. Therefore we have chosen gelfiltration (GF) as the first dimension at protein level. By definition GF is a non adsorbing technique and potentially causes very low sample losses. Furthermore, the conditions chosen eliminate the binding of proteins of interest to albumin. Complementary LC-dimensions with high-resolution capability on protein level will be used to reduce the complexity further before digestion and mass-spectrometry analysis.

P128-M

A proteome map for bronchio-alveolar tumors

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Lung cancer is one of the most common types of cancer in the world with smoking being the major risk factor. Early diagnosis of tumor growth may significantly reduce cancer morbidity. This necessitates surrogates and/or biomarkers for a detection of early stages of tumor development. In order to improve the mechanistic understanding of lung carcinogenesis, a transgenic mouse model was developed which overexpresses c-raf. c-raf mimics the effect of c–ras activation, a protooncogene known to be overexpressed in human lung adenocarcinoma. Transgenic mice developed specifically bronchio-alveolar adenocarcinoma and we investigated expression of pulmonary proteins in control and tumor tissues. Protein extracts were separated by 2-D gel electrophoresis and studied by image analysis. Further, more than 300 protein spots per gel were excised and analyzed by MALDI-TOF and -TOF/TOF mass spectrometry to enable proteome mapping. We observed significant differences in the expression of proteins some of which were involved in cell growth and phospholipid metabolism. We additionally identified several proteins which differed in expression and may be associated with lung tumor growth and/or metastasis.

P129-T

A rat liver proteome study in aid for an identification of tumor markers

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Polychlorinated biphenyls (PCB) are persistent environmental chemicals that accumulate at the apex of food chains. PCB are well-known for their ability to promote tumor growth in laboratory animals and are classified as class A carcinogens. Nonetheless the evidence for their tumor-initiating potential remains controversial.

It is known that PCB have the ability to bind to the aryl hydrocarbon receptor, a transcription factor that induces expression of a wide range of different genes including Cyp monooxygenases. To gain further insights into the molecular mechanisms of PCBs mode of action and to identify biomarkers for tumor growth, we initiated the proteome analysis of rat liver following treatment of rats with Aroclor 1254, a commercial mixture of PCB. For proteome mapping, liver extracts of control and treated animals were separated by two-dimensional SDS-PAGE employing different protein loads and lysis buffers for extraction of cytosolic and nuclear/membrane proteins. pH ranges for isoelectric focusing were selected according to a "virtual gel" constructed from gene expression data. Protein spots were visualized using Colloidal Coomassie Brilliant Blue or Ruthenium (II)-tris (bathophenanthroline disulfonate). Approximately 800 spots per gel were excised and analyzed by MALDI-TOF/TOF mass spectrometry after tryptic in gel digestion.
The majority of identified proteins in control and treated samples belongs to the following categories: blood proteins, mitochondrial proteins, ribosomal proteins, and proteins involved in different metabolic pathways like glycolysis and fatty acid biosynthesis. By image analysis more than 20 proteins were identified as being differentially expressed.
Comparison of gene and protein expression data revealed significant differences that may deepen our understanding of the PCB-related carcinogenesis at a molecular level.

P130-S

Immunoaffinity-Purification and Identification of Teleost Thy-1

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Teleosts such as Goldfish are, in contrast to mammals, able to regenerate their central nervous system after injury. The monoclonal antibody M802 recognized an unknown molecule that is expressed on the surface of regenerating but not on early embryonal axons in the goldfish retina.
Here we describe the affinity purification of the unknown protein and its identification as teleost Thy-1. After optimization of the immunoaffinity purification a coomassie stained band of the unknown protein could be obtained. By immunoblotting it could be shown, that the protein contained a GPI anchor and three N-linked glycan chains, which accounted for 50% of the apparent molecular weight. Complete enzymatic deglycosylation was not achievable, and the protein was essentially resistant to in-gel-proteolysis. Proteolytical fragments could only be obtained after in-gel-deglycosilation and re-electrophoresis. The resulting peptides were characterized by N-terminal sequencing and automated MALDI-TOF/TOF de-novo sequencing. With the obtained peptide sequences, a EST-sequence of a zebrafish protein homologue to mammalian Thy-1 could be identified and cloned. Although Thy-1 has long been known in mammals, the function of this proteins remains elusive. The significant differences in the expression of Thy-1 in teleosts and mammals may lead to a more detailed understanding of the function of this protein.

P131-M

Search for Zn(II) Metallochaperones in *E.coli*, a ProteomicApproach

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Zinc is ubiquitous and is essential for life. Both catalytic and structural roles of Zn(II) have been well established. As in case of other metal ions homeostasis of zinc also appears to be under a very tight control at various stages of import, storage, distribution and export. Several membrane proteins have been implicated in Zn(II) trafficking but no proteins that deliver Zn(II) from the membrane to intracellular locations have yet been identified. Since there

appears to be no intracellular pool of free Zn(II) ions in cells, the existence of Zn(II) metallochaperones has been hypothesized. Proteins involved in zinc trafficking are currently being investigated in *E. coli* by using proteomics and molecular biological tools. Soluble cell extracts of *E. coli* grown in minimal media with and with out Zn(II) were separated by using 2D gel electrophoresis, and gel spots of proteins that were expressed at different levels were excised, trypsin-digested, and identified by using MALDI-TOF MS and peptide mass fingerprinting. A time-course approach was taken to see if same proteins are expressed differentially over time. The soluble fractions were fractionated by using DEAE chromatography, which improved resolution. Our results show that known Zn(II) binding proteins and some proteins that are not known to be Zn(II) binding are expressed differentially in presence and absence of Zn(II) in the growth medium.
In order to verify further our proteomics data, Isotope Coded Affinity Tagging (ICAT) Q-TOF MS/MS was used to quantitate differential expression profiles. DNA microarrays were conducted to verify the proteomics data and to probe differential expression of membrane bound proteins.

P132-T
Quantitative Analysis of Membrane Proteins from Cultured Cells, Integration with Microarray Data and the Application to the Study of Bioprocesses
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NS0 cells are of major importance in biopharmaceutical manufacturing, especially in the area of recombinant antibody production. We used a combination of metabolic labeling with an amino acid containing a stable isotope, biotinylation and gradient centrifugation to purify and study quantitative, differential expression of membrane proteins from NS0 cells grown in culture under different conditions. Isolated proteins were separated on a one-dimensional SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) gel in order to reduce complexity and to provide approximate molecular weight information. Each lane of the gel was cut into a number of slices and then subjected to in-gel proteolysis with trypsin. Extracted peptide pools were separated using a C-18, reversed-phase nano-liquid chromatography column which was fed directly to a hybrid Qq-TOF (quadrupole-time of flight) mass spectrometer in order to identify the proteins and to provide relative abundance data. This methodology was applied to two models of cell growth conditions: (1) NS0 cells grown in serum based media compared to cells grown in chemically-defined, protein-free media and (2) NS0 cells dependant on cholesterol compared to those that had been adapted to cholesterol independence. Parallel samples were collected and subjected to gene expression profiling using microarray technology. Genes involved in metabolism, cell growth and maintenance were identified at the mRNA expression level and protein level. Integrating these two technologies provides a powerful tool for understanding major pathways underlying important bioprocessing issues, and facilitates the identification of gene targets for cell line engineering to achieve enhancement of bioprocesses.

P133-S
Proteomic Analysis of Novel Marine Aerobic Anoxygenic Phototrophs Using MALDI and ESI Mass Spectrometry
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The source of all of the photosynthetic electron transport flux in the ocean has been a source of controversy in the microbiology community, especially since the discovery of new groups of aerobic anoxygenic phototrophs. The genes that produce proteins involved in photosynthesis have been detected by PCR from some of these bacteria, but it was previously unknown if the bacteria were actively expressing those proteins. The main objective of this study was to

determine whether any proteins related to photosynthesis were expressed using mass spectrometric techniques and to compare the results from two instruments that utilize different ionization techniques. Strains of these bacteria were isolated and cultured in the laboratory. A simple procedure including lysis with the addition of Tris buffer, an addition of a mass spectrometry-compatible detergent to the insoluble pellet, and the digestion of proteins using trypsin led to the identification of four photosynthetic proteins from one strain of these bacteria. It has been noted that using both matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) to analyze a mixture of peptides leads to the identification of more peptides that either technique alone. In order to exploit this complementary nature of ESI and MALDI for proteomic analysis, samples were analyzed by instruments using both ionization techniques. The proteins and peptides found from each type of instrument will be reported, and a comparison of the properties of the peptides identified with each ionization technique will be made.

P134-M

Peptide Mass Fingerprint Analysis of Alkaline Rat Brain Proteins using Piezoelectric Inkjet Dispensing

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Proteomic studies of brain tissue is one strategy for identifying protein biomarkers in neurological function and disease. Elements of the strategy include sample preparation and separation methods that enable the enrichment of co- and post-translationally modified forms of proteins, an essential step towards understanding the functional state of the brain proteome. Here we describe a protein array platform that uses sample enrichment methods compatible with 2D-PAGE and a new technology for interrogating the 2D-PAGE protein arrays archived onto membranes.
Proteins extracted from rat brain were first separated with a Multi Compartment Electrolyser into acidic, neutral and alkaline fractions. Proteins present in the alkaline fraction were separated using 2D-PAGE and electroblotted onto PVDF. Processing the sample with two modes of isoelectric fractionation produced a greater separation of the alkaline proteins than the standard 2D-PAGE approach. The PVDF membrane once stained, is essentially a protein chip where each protein's position is not determined by the user but by the protein's intrinsic properties of pI and apparent Mr. Protein analysis was performed using a piezoelectric-based ink-jet printing system which dispenses the reagents onto selected proteins within areas typically of 0.2 mm^2. Proteins were subjected to i*n-situ* enzymatic digestion and the resulting peptide masses analyzed with MALDI-TOF-MS directly from the PVDF membrane surface. Peptide mass coverages of over 60% were obtained for *R. norvegicus* proteins such as peptidyl-prolyl cis-trans isomerase A and fatty acid-binding protein. Proteins were identified with predicted pIs ranging from 5.7 (Somatotropin precursor) to 9.2 (glutamate oxaloacetate transaminase 2). Since only small areas of protein were analysed, the remaining sample can be archived for future characterisation studies.

P135-T

A multidimensional LC-MS/MS approach for the analysis of the proteins expressed during tomato fruit ripening

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In this paper we discuss the analysis of proteins expressed in tomato fruit by 2D LC-MS/MS. The development of tomato fruit follows from fertilization, and occurs simultaneously with seed maturation. Initially, tomato fruit enlarge through cell division and then by increasing cell volume. The fruit then ripens. Ripening is accompanied by changes in flavor, texture, color and aroma. As ripening progresses, tomato fruit

color changes from green to red as chloroplasts are transformed into chromoplasts, chlorophyll is degraded and carotenoids accumulate. Fruit softening and texture changes occur as the fruit cell wall is modified and partially disassembled by enzymes and the ripe flavor develops as specific volatiles increase and the sugar-acid balance alters.
Fruit development and ripening correspond to a chain of genetically programmed events. The pattern of gene expression found in green tomato fruit changes dramatically during fruit ripening Protein patterns have been compared from green stage (first stage of tomato ripening) to red stage (last stage of tomato ripening). From similar extracts a detailed 2D-gel analysis and micro-array transcriptome analysis have been performed previously. Both these analyses presented a multitude of spots that were differentially expressed between different stages of tomato ripening. In order to produce a link between the proteomics and micro-array data, an increased number of proteins needed to be identified. These results will be compared and contrasted to those obtained using 2D-LC-MS/MS.

P136-S

Direct identification of large native peptides a MALDI hybrid quadrupole orthogonal acceleration time-of-flight (Q-Tof) mass spectrometer

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Peptides and small proteins (0.5 to 15kDa) play a central role in many physiological processes. The direct analysis of native peptides in complex biological mixtures using an axial acceleration MALDI Tof mass spectrometer is normally limited by the parent and daughter ion resolution and the mass accuracy (< 50ppm) of the instrument. In the work presented here MALDI MS and MS/MS data were acquired on a Q-Tof mass spectrometer operating in both the V and W-optics mode. Transmission in MS/MS mode of ions with m/z values up to 8000 was possible due to a high mass range quadrupole and this was used to analyse large peptides of m/z greater than 5000Da.
The investigated peptide mixtures were obtained from Human blood filtrate by a standard procedure described elsewhere [1].
Preliminary data have been generated on peptide samples originating from Human blood filtrate. Both the molecular weight of the native peptides and the complexity of the sample make it difficult to accurately distinguish and select peptides of similar mass when performing MS/MS studies. Data will be presented that show the ability to distinguish and sequentially select these peptides using the quadrupole to select only the ion of interest and the specificity of the exact mass fragment ion information that can be obtained.

[1] Schulz-Knappe,P.; Zucht,H.-D.; Heine,G.; Jürgens,M.; Hess,R.; Schrader,M. Peptidomics: The comprehensive analysis of petides in complex biological mixtures. Combinatorial Chemistry & High Throughput Screening **2001**, 207-217.

P137-M

FAIMS Enhanced Glycopeptide Detection by CapLC Q-TOF

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High field asymmetric wave ion mobility spectrometry (FAIMS) separates ions in the gas phase based on species dependent changes in mobility observed at high electric fields. In addition, when a FAIMS device, such as the Ionalytics SELECTRA is fitted to the source of an electrospray mass spectrometer it causes a reduction in the spectral noise and allows a high transmission efficiency of ions due to an ion focusing mechanism, often resulting in an increase in signal compared to ESI without the FAIMS. The experimental parameter that reflects changes in ion mobility at high vs. low electric fields is called the compensation voltage. Therefore the selection of the desired ion species

is achieved with the selection of the CV value associated with that species.
Site specific analysis of protein glycosylation is fundamental to understanding protein function and the condition of cells or tissue. A tryptic digest of a glycoprotein can be analysed by LC MS, however the glycopeptide signal is often weak compared to the peptides, due to the heterogeneity of the carbohydrate moieties and the significant increase in mass. This work explores the gas phase ion separation of Glycopeptides using the FAIMS device.
Our aim is to present results from the use of CapLC / FAIMS / Q-TOF to analyse a bovine fetuin tryptic digest

P138-T
Solubilization and Resolution of Cardiac SR Membrane Proteins Using Two-Dimensional Gel Electrophoresis
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Two-dimensional gel electrophoresis (2-DGE) is a powerful and widely used method for separation and analysis of protein complexes. Membrane proteins that contain a varied number of transmembrane hydrophobic regions are frequently the most important ones in cell signaling pathways and cell adaptation, yet solubilization and resolution of membrane proteins using 2-DGE remains technically challenging. To overcome this difficulty, we have employed a new solubilization technique with the intention of selectively enhancing membrane protein resolution on 2-D gels. Ionic detergents, such as SDS, were directly spiked into the rehydration buffer, which was consequently used to resolve cardiac sarcoplasmic reticulum (SR) membrane proteins. The effectiveness of co-solvents in resolving membrane proteins was also tested in this study. In contrast to conventional solubilization techniques, analysis of the 2-D gels using the modified solibilization solution indicated that SERCA2a, a 110kD integral SR protein of paramount importance in cardiac function, was successfully resolved. The identity of SERCA2a on the 2-D gels was further confirmed by Western blotting using a specific antibody. The successful solubilization and resolution of SERCA2a using this non-traditional approach suggests that: 1) SDS is compatible with IEF, albeit with some loss of resolution of the other proteins; and 2) the presence of SDS and co-solvents such as 2, 2, 2-trifluoroethanol (TFE) and dimethyl sulfoxide (DMSO) aid in the resolution of hydrophobic proteins.

P139-S
Alternative workflow in protein mixture analysis: Coupling LC separation with tandem MALDI mass spectrometry
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This poster presents the analysis of complex protein mixtures by coupling LC separation of peptides followed by Maldi MSMS analysis. Conventionally, LC has been used in conjunction with mass spectrometry (MS) predominantly with electrospray ionization mass spectrometry. The primary reason for using electrospray ionization was the nonavailability of MALDI tandem MS. With the advent of Maldi tandem MS instruments such as oMALDI^{TM} QStar^{R} XL and the 4700 Proteomics Analyzer it has become practical to combine the advantages of these two distinct analytical techniques. The LC separation helps sample concentration, and reduces the complexity by front-end sample clean up. Maldi MS is appreciated for its ease of use, simple sample preparation and handling, accuracy and sensitivity. Most importantly, the relatively static nature of the sample with MALDI approach *vs* electrospray ionization renders several benefits for the LC-MALDI workflow.
Preliminary results from tryptic digestion of 8-protein mix indicate the potential of this approach. The peptides were separated using a C18 column followed by deposition on a maldi target along with matrix. The spots were analyzed on a quadrupole orthogonal time of flight mass spectrometer (QStar^{R} XL) fitted with an oMaldi source. MS followed by MSMS on 8 ions in the MS spectrum were acquired on each of 144 fractions. Database searching identified

all the proteins in the mixture with good sequence coverage. In addition, the ability of the system to give good mass accuracy (<20ppm with two point external calibration) enhances the confidence in protein identification. LC-MALDI workflow combines the advantages of the two techniques and the static nature of the sample offers the ability to perform results dependent analysis and enhances the information derived from each analysis.

P140-M

Optimization of the Molecular Scanner as a Rapid Alternative to In-Gel Digestion Method

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Current methods for identifying proteins separated by SDS-PAGE rely on excising protein bands from a gel and performing in-gel digestion. This laborious procedure requires several days of work, rigorous record keeping, and is very prone to external contamination and sample losses in less experienced hands. Described herein is a technology that enables faster identification of gel-separated proteins, reduces sample tracking and preserves the spatial information in SDS-PAGE gels. The Molecular Scanner is a procedure by which whole SDS-PAGE gel lanes are electroblotted through a trypsin membrane and the proteolytic peptides thus formed are deposited on a capture membrane. The capture membrane is loaded directly into the MALDI-TOF mass spectrometer for spectra acquisition. The Molecular Scanner is also compatible with tandem mass spectrometry. The total procedure from loading the gel to acquiring data may be performed in one day. The electroblotting transfer was optimized to provide greater than 95% efficiency with very reproducible results. Additionally, the dynamic range of the Molecular Scanner has been investigated. The procedure was evaluated in parallel to in-gel digestion and was determined to be comparable in sensitivity and protein sequence coverage. Results from these experiments will be presented.

P141-T

Interfacing LC to ESI- and MALDI-MS/MS Analysis Towards High-Throughput and High Content Proteomics

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The coupling of LC with mass spectrometry has proven to be a powerful tool for comprehensive proteome analysis. Until recently, ESI has been used online coupled with LC for the analysis of complex protein mixtures. With the introduction of a MALDI-TOF/TOF mass spectrometer with MS/MS capability, LC-MALDI becomes a promising option. In an even more advanced approach the two MS techniques were combined by post column split of the LC flow providing online and offline MS and MS/MS analysis of a single LC run. For the online-ESI analysis a new high-capacity ion trap has been used for high MS/MS throughput by acquisition of *1 MS + 4 MS/MS* spectra in 3 seconds. This increased duty cycle of the ESI-analysis enables fast LC separation techniques using monolithic capillary columns (gradient < 60min). The simultaneous deposition of 50% of the eluent onto a MALDI target allow for a non-redundant selection of precursor ions for MS/MS analysis without any temporal constraints. Thus, the combined LC-ESI-MALDI-MS/MS approach yields increased information readout from a single LC run within relatively short time due to the complementary MS/MS performance when the two MS-techniques are linked to LC separation. For complex protein mixtures (> 10 proteins) superior sequence coverage could be obtained for each identified protein when using the combined LC-ESI-MALDI-MS/MS compared to the use of each MS-technology alone.

P142-S

The Analysis of complex peptide mixtures by LC-MALDI MS and MS/MS analysis on axial and orthogonal acceleration time-of-flight mass spectrometers

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The analysis by MALDI MS of complex peptide mixtures, such as an enzymatic digest of a mixture of proteins, is a common analytical problem. The number of different peptides identifiable from this mixture, using MALDI MS and MS/MS, without prior LC separation is severely limited, primarily due to the fact that not all peptides are present at the same concentration. This limitation is partly due to signal suppression, through competitive ionisation processes, and also the limited dynamic range of the detectors used in MALDI systems. Currently the best solution to this problem is pre-fractionation of the sample using HPLC. Here we show the automated nanoscale LC separation and spotting of fractions onto a MALDI target plate followed by MALDI MS and MS/MS analyses.
A mixture of twelve digested proteins, present at differing concentrations was separated by HPLC with a capillary LC using a 75 um ID reverse phase column. The eluent from these separations was spotted directly onto a MALDI target plate using a modified Waters 2700-MS spotting robot. During the sample deposition, matrix solution was added. Mass spectral analysis was performed on a MALDI Q-Tof instrument. This was used to optimise the system for the subsequent analysis of complex cellular fractions obtained from *E.coli.*

P143-M
Analyzing alkaline pH range proteins in human colon crypt proteome

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Normal human colon crypt whole cell extract was resolved on two dimensional electrophoresis gels with pH 6-11 immobilized pH gradient strips in the first dimension. The optimized isoelectric focusing protocol includes cup-loading sample application at the anode and 1.2% hydroxyethyldisulfide (DeStreak), 15% Isopropanol and 5% glycerol in the rehydration buffer. Spots were well resolved across the entire pH range up to pI 11. Total of 340 protein spots were identified by MALDI-TOF MS. After excluding isoforms, 250 unique proteins were grouped into 18 categories according to their subcellular locations, and 19 categories according to their physiological functions. Histone proteins, ribosomal proteins and mitochondrial proteins were among the highest pI range well-resolved proteins. Because a significant portion of the proteome of human colon crypt is basic in pH, this protocol is crucial for the study of colorectal cancer tumorigenesis and development.

P144-T
Sample Mass Quantitation by Ion Energy Loss
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Molecular species need to be separated, identified, and quantified to study biological systems on the molecular level. Quantitation lags behind the chromatography and spectrometry techniques used for separation and identification. The concentration of proteins and metabolites *in vivo* span many orders of magnitude, and sensitive general methods are needed for quantitation. Ion energy loss spectrometry quantifies nanogram to microgram amounts of biomolecules such as proteins deposited on thin films. We interfaced ion energy loss quantification with microcapillary liquid separations, matrix-assisted laser desorption/ionization mass spectrometry and accelerator mass spectrometry. Peptides, proteins and other types of organic molecules in complex mixtures were quantified by measuring the energy loss of MeV ions passing through species deposited by a robotic system on thin hydrophobic films in arrays directly from separations. The same deposits were subsequently identified by mass spectrometry. The quantification is absolute, independent of standards or labels, non-destructive, and

amenable to high-throughput and automation. The approach has potential advantages over conventional, labeling and relative quantification schemes in proteomics and should be applicable to a wide range of quantitative protein expression and interaction analyses. We specifically combined ion energy loss quantitation with accelerator mass spectrometry to measure binding of small, isotope-labeled compounds to proteins. This particularly potent combination enabled determination of binding at parts-per-billion levels in nanograms of isolated target protein.

P145-S

The use of Carboxypeptidase C-terminal ladder sequencing in combination with LC-MALDI for peptide sequencing

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The role of MALDI TOF mass spectrometry in the analysis of proteins and peptides has increased dramatically over recent years. This is primarily due to its unparalleled speed, sensitivity and specificity. In particular, much work has been performed on gel-isolated proteins that have been digested with trypsin and analysed by MALDI mass spectrometry. In this case a peptide mass fingerprint is produced that can be used to challenge a protein or nucleotide database to identify the parent protein. Further sequence information from some of the peptides can be generated using Carboxypeptidase Y (CPY). This enzyme cleaves all residues from the C-terminus of a peptide, producing a series of fragments, in a ladder sequence, with each ion in the series differing by an amino acid residue. This approach has been described previously. In this paper, we detail modification of existing technology to provide an automated approach to carboxypeptidase Y ladder sequencing. CPY was added to fractions collected from the HPLC separation of tryptic digests. We will present datasets from a series of samples including, protein standards and biological extracts of greater complexity such as those obtained from 1D-gel separations and non-gel based fractionation methods.

P146-M

Improved Tryptic Digestion of Proteins Using 2,2,2-Trifluoroethanol (TFE)

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A goal of many proteomics projects is to identify the maximum number of proteins with the most complete sequence coverage from complex samples. A factor in the identification of proteins from such samples is the efficiency with which proteins are digested prior to their analysis by mass spectrometry. In turn, the efficiency of digestion is dependent on the degree of solubilization and denaturation of a protein prior to digestion. For example, in its folded state myoglobin is virtually resistant to trypsin proteolysis unless it is denatured chemically (i.e. with urea) or thermally, at which point it is readily digested. Russell et al. (Anal. Chem. 2001, 73, 2682-85) reported an increase in the digestion efficiency and sequence coverage by mass spectrometry for various proteins digested in buffers containing organic solvents (i.e., acetone, acetonitrile, isopropanol, methanol). The reaction was rapid but incomplete, as several missed cleavage sites were identified within the resulting fragments even after prolonged (overnight) digestions. We have developed a modification of their protocol using TFE as the denaturing agent. The TFE digestion procedure uses only a low amount of volatile buffer for controlling pH and thus is very amenable to subsequent analysis by LC/MS or AP-MALDI MS. Our current study illustrates both the efficiency and completeness of digestion of various proteins using the TFE protocol. Further results from complex samples showed that the TFE protocol gave better sequence coverage and protein identifications compared to digestions using more traditional denaturing agents such as urea.

P147-T

Utility of the Enzymatic Labeling Method for Stable Isotope Incorporation into Protein Digests

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Incorporation of stable isotopes is often used in proteomics to study differential protein expression when analyzing by Mass Spectrometry (MS). Enzymatic digestion of proteins in ^{18}O isotopically enriched water is a common procedure for this purpose. When utilizing a protease such as trypsin, a 4 Dalton difference is incorporated into all peptides with the exception of the C-terminal fragment. This provides a means of mass spectrometric differentiation from analogous samples digested in natural water. In addition, covalently linked binary peptides will display an 8 Dalton shift when digested in ^{18}O water. This allows for the study of protein structure through identification of internal disulfide bonds and the detection of interacting domains in protein complexes.
In this study, a variety of proteins were proteolytically labeled with ^{18}O. The MALDI-TOF mass spectra of both isotopically labeled and non-labeled peptides were analyzed and relative quantitation performed. The importance of isotopic purity was demonstrated by comparison of different grades of ^{18}O labeled water. Furthermore, linked peptides were identified in mass spectra by observing 8 Dalton differences. The enzymatic labeling method provides a simple yet powerful proteomics tool for relative quantitation as well as for investigation of structure and protein-protein interactions.

P148-S

Integration of Biomolecular Interaction Analysis with Mass Spectrometric Identification of Interaction Partners by using Reverse Phase Beads

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Delineating the complex network of interacting biomolecules in the cell improves our understanding of cell physiology and provides new targets for directed control of cell function. By integrating two well-established techniques, surface plasmon resonance (SPR) based biosensors and mass spectrometry (MS), we can fish for interaction partners from complex mixtures, characterize the interactions in real-time and identify interaction partners.
SPR is a well-established technique for highly sensitive detection, label-free and real-time monitoring of biomolecular interactions and allows for rapid determination of affinities and kinetics of interactions. In Biacore's SPR-based biosensors one interaction partner is immobilized on a sensor chip followed by injection of solution containing another partner. If interaction partners of a protein are unknown and require identification, a protein mixture representing the physiological environment of the target protein can be injected. In this case the SPR sensor will detect interaction, if any, but identification of the bound protein will require SPR to be complemented with an additional analytical step. Here mass spectrometry appears to be the most suitable tool as the most sensitive and specific method for protein identification.
Recently, a new soft- and hardware package has been developed for the Biacore 3000 instrument, dedicated to recover proteins that are captured on the SPR sensor chip for subsequent MS analysis. In line with this approach we present examples of a new procedure that allows for enzymatic digestion, including reduction, of the recovered proteins based on the use of reverse phase beads. This fully automated procedure increases the efficiency of the digestion process and reduces sample losses thus enhancing the sensitivity in MS analysis.

P149-M

2-Dimensional vs 1-Dimensional Gel Electrophoresis for the Identification of Proteins in Lipoprotein Complexes

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2-dimensional electrophoresis (2D) is a powerful technique for resolving complex mixtures of proteins into individual polypeptides for analysis by mass spectrometry. It is especially useful for identifying less abundant proteins that would otherwise be masked in 1-dimensional SDS-PAGE electrophoresis (1D). However, a disadvantage of 2D gels is that membrane and hydrophobic proteins can be poorly represented due to protein/gel interactions and lack of solubility in 2D buffers. Both 2D and 1D were coupled with mass spectrometry to identify proteins in lipoprotein complexes. Immunoblotting techniques were also used with 1D and 2D to compare individual proteins. 25 proteins were identified using 1D and 18 proteins were identified using 2D. Only 6 proteins were identified using both of the methods. Highly modified proteins were unresolved by 2D but were identified by 1D because of the limited dimensional diffusion. Hydrophobic proteins were observed more frequently in 1D than 2D, probably due to the capacity to use stronger detergents in the process. Therefore, the two methods were found to be complementary and together provided a more complete view of the existing proteins.

P150-T

Proteomic Study of Human Astrocytes Transfected with the Potential Tumor Marker YKL-40 using Isotope-coded Affinity Tags.

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Glioblastoma multiforme (GBM) is a devastating brain tumor; median survival of patients diagnosed with it is less than 1 year. Genetic studies have begun to shed some light on the nature of these tumors, and one report recently identified the potential tumor-marker YKL-40 as a highly up-regulated by gene expression microarray analysis of 19 gliomas. This protein, which is homologous to chitinases, was first isolated from chondrocytes and was found to be associated with arthritis. It has since been linked to other diseases, in particular breast, colorectal and ovarian cancers. We decided to study this molecule in brain tumors by transfecting an astrocyte cell line with YKL-40. We then used an isotope coded affinity tag approach to compare the whole-cell lysate of this line with that of the un-transfected parental line. A number of proteins appear to be significantly up- or down-regulated, some of which can apparently be correlated with the aggressive nature of these tumors. Further studies to verify and extend these results are underway.

P151-S

Unparalleled Sensitivity and Dynamic Range for Analysis of Proteins on a New Linear Ion Trap Mass Spectrometer

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Protein identification in the context of proteomic analysis requires a system with excellent sensitivity and dynamic range (the ability to measure low-abundance proteins in the presence of other high-abundance proteins). We demonstrate the ability to detect sub-attmole level proteins with a new linear ion trap mass spectrometer. Using nanospray, reversed phase LC/MS, 50 attmol of BSA could be confidently identified by database searching with TurboSEQUEST. Peptide sequences were further confirmed by spectral analysis with DeNovoX software, a dedicated de novo sequencing software, with excellent agreement between results from database searching and de novo analysis. Dynamic range was evaluated by analyzing a protein mixture with constituent protein concentrations ranging from 100 attmol to 10 pmol. Although the high- and the low-abundance proteins differed in concentration by a factor of 10^5, all the proteins in the mixture could be identified confidently.

P152-M

Comprehensive 2-D Nano LC/MS for Human Tissue Proteomics

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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 nano-column.
Comprehensive 2-D 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 2-D 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.

P153-T
Enhanced Sequence Coverage of Proteins in Human Cerebrospinal Fluid Using Multiple Enzymatic Digestions and Linear Ion Trap LC-MS/MS
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The cerebrospinal fluid (CSF) proteome provides a readily accessible window into the health state of the central nervous system (CNS). In order to take advantage of this relationship to obtain diagnostic information of proteins, typically those in low abundance, they must be positively identified with high confidence and then quantified. For LC/MS-based methodologies, both the degree of confidence in identification and the ability to quantify necessarily increases with increasing numbers of identified peptides, hence with increasing sequence coverage. The goal of this study was to examine the differences in sequence coverage obtained from CSF digests produced with different proteases. Digests obtained with AspC, GluC, combined AspC and GluC, and trypsin were prepared and then examined by LC-MS. Peptide sequences were identified with combined DeNovoX™ and SEQUEST® algorithms. Resulting sequence coverage for the major constituent proteins in CSF is presented.

P154-S
In-Gel Detection of Tetracysteine-Tagged Proteins Expressed in Prokaryotic, Mammalian and In-vitro Transcription Translation System
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In order to determine the success of recombinant protein expression, samples are commonly analyzed by gel electrophoresis, and some times also western blotting. Western blotting is particularly useful to confirm that the desired protein has been produced when yields are low. We demonstrate the use of a small fluorescent molecule (Lumio™ Green detection reagent) that selectively binds to a unique tag sequence (-ccpgcc-) on the expressed protein and allows

detection of protein directly in the gel with good sensitivity and specificity. The described detection reagent can also be used after transfer of the proteins to a membrane. Recombinant proteins were expressed in *E.coli*, human embryonic kidney cells as well as an *in vitro* expression system. The detection method can detect tagged proteins in the presence of high concentration of extraneous proteins. Detection sensitivity of 1 pmol protein was obtained.

P155-M
A Method for Quantitative Snapshot Analysis of Protein Dynamics of Cells and Functional Cellular Components

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Proteomics, developed initially from the decades-long study of comprehensive protein analysis on two-dimensional electrophoresis gels, has recently been expanded by the advancement in liquid chromatography-mass spectrometry (LC-MS) based protein identification technologies. In particular, the differential protein analysis using the LC-MS technologies coupled with stable isotope labeling of proteins *in vivo* or *in vitro* has been shown to be powerful to analyze changes in the protein profiles accompanying various biological events and to study protein-protein interactions *via* affinity-based isolations of functional protein complexes. Here, we present an integrated approach for large-scale study of protein dynamics of cells and cellular components. As with the previous strategies such as ICAT, the method introduces a stable isotope tag into peptides generated by protease digestion of biological protein mixtures *in vitro*, and identifies proteins by comprehensive *de novo* determination of peptide sequence *via* the high-resolution direct nanoflow/multi-dimensional LC-MS technologies with simultaneous, relative quantitation of proteins. The method requires a simple one-step chemical modification of complex biological samples and allows automated quantitative identification of ~1,000 proteins in a single analysis. We show that the method is useful for the "assembly snapshot" analysis to study molecular dynamics of functional cellular complexes, as well as for large-scale surveys of differential protein expressions of cells. We suggest that the method is also suitable for the medical and pharmaceutical applications such as the diagnosis of human disease and the drug target discovery and safety assessment.

P156-T
Analysis of the phosphoproteome of Chinese Hamster Ovary cells using electrophoresis
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Protein phosphorylation is a common posttranslational modification of enormous biological importance. Analysis of phosphorylation at the global level should shed light on this modification and its use by cells to regulate metabolism, signal transduction, and other processes. We have begun a whole-proteome analysis of phosphorylation using two-dimensional gel electrophoresis (2DE). Chinese Hamster Ovary (CHO) cells were metabolically labeled for 4 hours using ^{32}P-orthophosphate. The proteins were extracted and run on 2DE. Gels were then stained using colloidal coomassie stain, dried, and exposed to a phosphor screen for subsequent phosphorimaging. The coomassie stain allowed the observation of 427 individual protein spots. The phosphorimage showed 193 spots. Only 18 of these 193 radioactive spots were easily correlated to coomassie-stained protein spots from the same gel. These included two "spot trains" probably indicating varying phosphorylation states of a single protein. Interestingly, three other apparent "spot trains" noted in the coomassie gel do not correspond to ^{32}P-labeling. Notably, four of the five most intense ^{32}P-labeled spots do not correspond to

discernable coomassie-stained spots, indicating that these are low-abundance proteins that are highly phosphorylated. Conversely, none of the ~150 most intense coomassie-stained spots correspond to distinguishable ^{32}P-labeled spots. These results call for a reevaluation of the generalization that ~60% of cellular proteins are phosphorylated. Protein spots are being analyzed by mass spectrometry for their identification, and other methods of visualizing phosphoproteins are being tested.

P157-S

Advantages of Hybrid Quadrupole - Linear Ion Trap Mass Spectrometer for Quantifying Peptide Biomarkers in Serum

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The goal of quantitative proteomics research is to characterize the expression levels of proteins and their modified forms in an effort to understand a biological state at the molecular level. This quantitative analysis falls into two categories, relative quantitation and absolute quantitation. Relative quantitation techniques such as ICAT™ reagents by mass spectrometry and DIGE dyes for fluorescent analysis of 2D gels have been developed to look globally for proteins with perturbed expression levels. Increasingly, researchers are interested in the absolute amount of specific proteins or peptides in complex samples, as the amount of protein present is often diagnostic of disease or cell state change. The direct combination of triple quadrupole and ion trapping capabilities in the 4000 Q TRAP™ hybrid quadrupole - linear ion trap system presents new opportunities for the quantitation of peptides and proteins in biological samples.
In this work, the absolute quantitation of different peptides has been investigated in various biological matrices on the 4000 Q TRAP™ system. The limits of quantitation and detection of angiotensin in the presence and absence of serum were investigated. The sensitivity and specificity of the triple quadrupole Multiple Reaction Monitoring scan (MRM) allows for the quantitation of low abundant peptides in complex mixtures. Because of the unique hybrid nature of this mass spectrometer, the MRM scan can be used to trigger a high sensitivity ion trap MS/MS scan to confirm of the identity of the peptide that has been quantified. Short analytical cycle times are advantageous when running many samples or measuring system kinetics. The effects of sensitivity and specificity as a function of chromatographic flow rates on the quantitation has been investigated.

P158-M

Improved detection of hexahistidine tagged recombinant proteins in polyacrylamide gels using a bi-functional fluorescent nickel chelate

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Recombinant protein expression often involves detection of the specific protein by antibody detection after blotting to a membrane. The time involved in performing a western blot to detect hexahistidine tagged protein is typically greater that 6 hours and can be plagued by low sensitivity of the anti-His antibody. Our newly developed InVision™ His-tag stain can detect His-tagged protein down to nanogram levels in *E. coli* lysates to reveal the expression level of the desired protein in the cell culture following SDS-PAGE electrophoresis in NuPAGE® Bis-tris gels. The technology is based upon a bis-nickel chelate fluorophore[1] with improved binding characteristics over single nickel-chelate fluorophores. Following SDS-PAGE electrophoresis, the gel can be stained in about 3 hours and detected using 302 nm transillumination and standard imaging cameras. Staining with the fluorescent bis-chelate minus the metal gave no specific signal demonstrating the affinity of the tag for the bis nickel-chelate. The stain was found to be linear over a 1000 fold concentration range and can also be used with fluorescent scanners. Results of staining *E. coli* lysates separated with Tris-Glycine and Tris-acetate SDS-PAGE gels and the optimal conditions for staining will also be discussed.

References: [1]Ebright RH, et al. *J Am Chem Soc* 2001;123:12123-12125.

P159-T

Electrochemical Separation of Target Compounds on Conductive Surfaces

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Affinity column chromatography plays an important role in the isolation and purification of target compounds. It is however difficult to avoid contamination of non-specifically absorbed compounds on the surface of platforms. Furthermore, chemical or biochemical treatments for the elution of specifically coordinated proteins sometimes afford undesired decomposition of aimed compounds. On the other hand, electrochemical means are among the most useful methods for chemical conversions in mild conditions. We therefore challenged to introduce novel anodic method for the construction of the electronically severable affinity chromatography. Among numerous candidates for the electrochemical cleavage reaction in aqueous conditions, benzyl dithioacetal moiety was revealed to work in moderate conditions. In neutral aqueous condition in the presence of electrolytes, carbon-sulfur bond cleavage took place to form corresponding benzaldehyde just after the application of potential. By introducing the dithioacetal in the ligand-linkers on the surface of conductive platforms, the ligands were successfully eluted after the application of anodic potential in aqueous condition. This paper describes applications for the separation and purification of target compounds including proteins.

P160-S

Assembly snapshot analyses of preribosomal ribonucleoprotein complexes by proteomic approach using direct nano-LC-MS/MS

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The goal of proteomics is a genome-wide survey of protein dynamics and provides a birds-eyes view of the protein society of the cell. Although the current technologies cannot describe a complete set of proteome expressed in the cells and its dynamics, they have sufficient capability to attach dynamic aspects of more focused, specific cellular function. Thus, one of the primary goals of current proteomics is the description of the composition, dynamics, and connections of the multi-protein “modules” and “machineries” that perform a wide range of biological function in the cell. In this study, we focused on the isolation and proteomic characterization of preribosomal ribonucleoprotein (pre-rRNP) complexes formed at various stages of human ribosome biogenesis. It is known that the ribosome biogenesis is extremely complex and dynamic processes in that hundreds of proteins and RNAs are involved in the processing of rRNA and assembly of ribosomal proteins. We present here the strategies based on the epitope-tag affinity capturing of pre-rRNP complexes and the identification of protein components by the direct-nanoflow-LC-MS/MS technology. We show that the strategies are extremely powerful for the assembly snapshot analyses to study molecular dynamics of the functional multi-protein “modules” and “machineries” essential for the cellular function.

P161-M

Quantitative Analysis of Complex Protein Mixtures Using Amino acid-Coded Isotope Tags

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Protein identification and quantification has continued to be a daunting task in the analysis of complex mixtures of proteins. In this report, our objective is to demonstrate an approach for accurate sequence identification and concurrent quantification of the individual proteins within complex mixtures. This involves metabolic labeling of proteins with amino acid-coded isotope tags (ACIT). These isotope tags are easily recognized through their characteristic and co-eluting spectral split patterns. We demonstrate this strategy in the genomic-scale identification of proteins in human acute promyelocytic leukemia (APL), containing peptides tagged with deuterium labeled amino acid, L-leucine-5,5,5-d3 (Leu-d3). For the ACIT assisted quantitative analysis, NB4 cells were grown in normal RPMI media and drug treated cells grown in same media containing 99% isotopic abundance of Leu-d_3 and the extracted proteins mixed 1:1. The proteins were alkylated and digested with trypsin for analysis. A capillary two-dimensional liquid chromatography (2DLC) system coupled with tandem mass spectrometry (MS) was adopted for separation and detection of the peptides. Data analysis was simplified with data-dependent mass tagging option, which identified d0 and d3-labeled peptide pairs in real time and automatically triggered MS/MS experiments on only those d0/d3 pairs. Using MS data, and automated quantitative data processing software, the abundance ratio of drug-induced proteins were determined. Marker proteins were identified which showed a 1:1 abundance of non-induced proteins. However, preliminary results show over 20 proteins were induced by a drug treatment. This method of metabolic labeling may add another dimension to genome-wide screening of proteins.

P162-T

Improved Protein Identification in Shotgun Proteomics using Mascot and Sequest Consensus, Peptide Chemical Properties, and Direct Spectral Analysis

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Multidimensional protein identification technology (MudPIT) analysis is highly effective in identifying proteins in yeast and prokaryotes. Because of the size and complexity of the human proteome, its use in human cells has been more limited, but thorough protein and peptide fractionation techniques have made even this analysis feasible. To facilitate validation of peptide assignments, we have implemented MSPlus, a data analysis program which assesses the validity of peptide identifications from Mascot and Sequest based on scores, peptide chemical properties, and consensus between the peptides identified by each database search program. Use of MSPlus has resulted in more peptide identifications than using Mascot and Sequest thresholds alone, and with better false positive and negative rates. We then use CLASP (CLuster based Analysis of SPectra), a program which independently assesses the MS/MS spectra to detect peptides missed by the database search programs, resulting in additional peptide identifications. Finally we discard the protein identifications made by Mascot and Sequest, and instead report the minimum and maximum set of proteins from which the peptides can be derived. Using a reformatted peptide-centric protein database our protein identifications are grouped by protein isoforms, providing the user with a systematically organized list of identified peptides and proteins. By combining analyses of gel filtration separated proteins from a soluble extract of human erythroleukemia cell line K562, we have identified 5130 proteins, estimating false positives at about 125 proteins. Comparison with Mascot and Sequest at similar false positive rates yields 3971 and 4120 proteins, respectively, indicating that the combined approach is more sensitive.

P163-S

Nanoflow HPLC/MS for the Analysis and Identification of Peptides

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Protein and peptide identification through the use of liquid chromatography coupled with mass spectrometry (LC/MS) has become an increasingly important tool in the fields of proteomics and drug discovery. In particular, the use of capillary separation columns and nanospray interfaces to the MS allow increasingly sensitive detection limits for mass-limited samples. To achieve the optimal flow rates (less than 500 nL/min) for HPLC with these small inner diameter columns, traditional instruments have relied on higher flow LC systems with passive flow splitting.
We describe the development of a nanoflow HPLC system that produces high pressure binary gradients at flow rates from 50-1000 nL/min using direct control of each mobile phase, with no flow splitting. The system relies on active feedback in the delivery of each mobile phase to provide highly reproducible flow rates and gradient profiles. The delivered flows are independent of the changes in system back pressure that occur with gradient composition, throughout the lifetime of a separation column, or when columns or emitter tips are replaced. In addition, the ability to make rapid changes in flow rate is integral to the design of the system. This capability can provide extended analysis time for signal integration or additional MS/MS identification ("peak parking") or allow increases in flow rate for faster sample loading. By adding a second binary gradient, the system can be expanded to conduct two-dimensional separations, allowing analysis of increasingly complex peptide mixtures. Data showing system performance for peptide separations including peak parking and multi-dimensional separations are presented.

MACROMOLECULAR INTERACTIONS

P164-M

Do Phosphate-Arginine interactions play an important role in Protein-Protein communications?

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In previous work we have demonstrated that peptide-peptide interactions between the phosphate group of phosphorylated peptides or phosphorylated lipids, and peptides containing two or more adjacent Arg residues does take place. We have also shown that such interactions play an important role in receptor-receptor interactions resulting in heteromer formations.
In the present work we have mixed eight peptides, containing a different number of amino acid residues (aa), seven contain two or more adjacent Arg (43, 34, 32, 28, 24, 17 and 8 aa) and one containing only one Arg (17 aa) with each of two peptides AAA**pY**aaa-NH_2 or KVN**pS**AAAAAAA.
All peptides containing adjacent Arg formed non-covalent complexes with either the phosphorylated Tyr or Ser. However the one containing one Arg did not. Two factors played a role in the relative intensity of the observed non-covalent complexes when analyzed by mass spectrometry, the shorter the peptide the higher the relative intensity and peptides containing three Arg rather than two formed complexes with higher relative intensity than a peptide of the same or lesser length containing only two adjacent Arg.
As it is not uncommon to find stretches of three to ten adjacent Arg in many receptor and regulatory proteins, such sequences might have an important role in governing the interaction of such proteins with adjacent phosphorylated proteins or with phosphates from the membranes lipids in which they are embedded.

P165-T

Tolerability of resistant starch-A dose-response study

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Background: Previous studies have shown that resistant starch is well tolerated at levels of 30 g per day, improves colonic function and increases colonic microbial butyrate generation but is without effect on blood lipids. However there is relatively little research on the tolerance at higher levels of intake or on the physiological effects.
Objective: To assess the effect and tolerance of resistant starch in the diet on symptoms of fermentation (i.e. bowel frequency, abdominal distension, flatulence, gastrointestinal upset, and breath gas production).
Methods: In a randomized crossover trial, 7 healthy subjects underwent four 1-week treatments: control (RS 0g, 100 g control starch); RS 30g (50g control starch); RS 45g (25g control starch); and RS 60g (no extra control starch); with a minimum 1-week washout between phases. Study foods consisted of breads, cookies, cereal and drinks. Subjects were instructed to follow their usual ad-libitum diet for each of the four phases. Subjects recorded their diet histories and symptom diaries during each 1-week treatment period. Body weight and blood pressure were measured at the beginning and end of each phase.
Results: Preliminary data indicate no effect of resistant starch on body weight or blood pressure in this short-term study. Furthermore no significant difference in bowel frequency, abdominal distension, flatulence or gastrointestinal upset were noted between any of the 4 levels of resistant starch intake.
Conclusion: Resistant starch delivered in a variety of food vehicles appears to be well tolerated even at relatively high levels of intake.

P166-S

A New High-Throughput Ultrasensitive Isothermal Titration Calorimetry System

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Isothermal Titration Calorimetry (ITC) is the universal detection system for macromolecular interactions. Heat is either generated or absorbed in any chemical reaction, and ITC is used to study protein-small molecule, protein-protein, protein-nucleic acid, and other biomolecular interactions. A single ITC experiment measures stoichiometry , binding constant, enthalpy and entropy of binding. Thermodynamics of binding provides valuable information on structure-activity relationships and drug design. ITC systems currently available require manual filling and cleaning, and limits the number of binding interactions which can be studied per day. This poster describes a new automated ultrasensitive ITC system, designed for hands-off, 24-hour a day operation. The ITC cell and syringe are mated to a fully-integrated autosampling system, and samples are stored in 96-well plates. VPViewer software controls experimental set-up and operation, and Origin software is provided for post-run data analysis of multiple samples. The higher sample throughput allows generation of complete binding isotherms of up to 20 samples per 24 hour period. Experimental results will be shown, demonstrating the reproducibility of this new ITC.

P167-M

Characterization of a Noncovalent Lipocalin Complex by Liquid Chromatography/Electrospray Ionization Mass Spectrometry

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Although non-covalent complexes typically dissociate when in flight in the mass spectrometer, it was shown that, in some cases, the energy associated with the ionic interactions between small proteins and ligands is higher than the dissociation energy of the complex. In such cases, the non-covalent complex survives the transition from solution to the gas phase and can be detected by a mass spectrometer.
We used nanoscale LC ESI-MS to identify the

nature of the ligand that binds to human neutrophil gelatinase-associated lipocalin (NGAL).
Lipocalins are a functionally diverse family of proteins that generally bind small, hydrophobic ligands and interact with cell-surface receptors. A recent study suggested that NGAL binds a negatively charged ferric siderophore (*enterobactin*) [1].
The folded state NGAL complex was separated from the free NGAL by reversed phase chromatography
. The zero-charge deconvoluted spectrum of the free NGAL indicated a M_r of 20,675 Da.
The measured M_r of the NGAL-complex preserved in its naturally folded state was 21,195 Da, so the molecular weight of the iron-chelated ligand was 520 Da. The molecular weight of the free ligand (464 Da) corresponded to that of *(DHB-Ser)*$_2$, while *enterobactin's* molecular weight is 669 Da. In solution, enterobactin rapidly breaks down into dihydroxybenzoyl-serine [2].
NGAL participates in the iron-depletion strategy of the innate immune system, complementing the function of lactoferrin in neutrophil granules. However, NGAL is unique, because it is specific for iron reserved for bacterial use.

1. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN and Strong RK *Molecular Cell* **2002**, 10, 1033-1043.
2. O'Brien IG and Gibson F *Biochim Biophys Acta* **1970**, 215, 393-402.

P168-T

Analysis of noncovalently associated protein complexes by electrospray orthogonal acceleration Time-of-flight mass spectrometry

i. D. campuzano; Waters Corporation, Manchester, United Kingdom.

Analysis of noncovalently associated protein complexes by electrospray orthogonal acceleration Time-of-flight mass spectrometry

Iain Campuzano, Ashley Sage, Therese McKenna & Jim Langridge
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Mass spectrometry has allowed denatured proteins and non-covalently assembled macromolecular protein complexes to be accurately mass measured. Therefore, allowing one to determine the presence of possible amino acid substitutions, post translational modifications, protein-subunit and protein-ligand stoichiometry.
The transfer of noncovalently associated protein-protein complexes from solution to the gas phase generally results in the formation of ions possessing relatively few charges and as such m/z values are often above 3000. Charged species as high as m/z 9,000-10,000 [1] and m/z 20,000 [2] have been reported. Such mass ranges would be unachievable on a standard triple quadrupole or ion trap instrument. This is why orthogonal acceleration Time-of-flight (oa-TOF) is the ideal analyzer for this application. Additionally, electrospray ionization is a very gentle form of ionization, thus enabling the intact transfer into the gas phase and detection of large multiprotein structures with little or no fragmentation.
Here we show the detection of several, different, non-covalently associated protein-protein complexes by ESI oa-TOF. The influence of source and analyzer parameters on the transmission of these complexes has also been investigated and will be discussed. The macromolecular complexes have masses of 300kDa, and multiple charged species in the ranges of m/z 7000-8000.

1. Van Berkel WJH, Van Den Heuvel RHH, Versluis C & Heck AJR *Protein Science* 2000 ***9*** 435–439.
2. Rostom AA, Fucini P, Benjamin DR, Juenemann R, Nierhaus KH, Hartl FU, Dobson CM & Robinson CV *Proc. Natl. Acad. Sci. U.S.A.* **2000** *97*, 5185-5190.

CAPILLARY ELECTROPHORESIS

P169-S

Analysis of Protein Isoforms Using Capillary Zone Electrophoresis with a Dynamic Coating

W. W. Chang; Target Discovery, Inc., Palo Alto, CA, United States.

Protein isoforms and changes in their expression had been correlated to multiple diseases, either as a result of the diseases or as the cause of pathological conditions. Consequently, there has been a tremendous amount of effort to develop effective and efficient analytical methods for the analysis of protein isoforms. Protein isoforms sometimes involve only subtle changes such as posttranslational modifications, and this makes resolving the isoforms difficult. Isoform resolution has many solutions to date, including HPLC, isoelectric focusing gel electrophoresis (IEF), and immunochemical approaches, but these tend to be time consuming or inaccurate due to interferences. Capillary electrophoresis (CE) has many advantages over traditional analytical methods for protein separation. Capillary zone electrophoresis (CZE) has been found to be an effective and rapid method for resolving isoforms when EOF is controlled. We demonstrate the separation of three protein isoforms with 0.1 pH unit differences between their pI values, using a fused silica capillary treated with a dynamic coating, EOTrol LN, which slows cathodal EOF by one order of magnitude.

P170-M

Updated Methods for Quantitation of DNA Methylation

P. A. Rangel, **T. F. Wise**; The Ohio State University Comprehensive Cancer Center, Columbus, OH, United States.

Increasing studies in cancer-related DNA methylation are creating a need for an efficient means of measuring degrees of methylation in a variety of cancers. Sequencing of bisulfite treated DNA allows methylation analysis of cytosine residues, however sequencing alone does not provide accurate quantitation. A method for quantitation of cytosine methylation using GENESCAN and gel electrophoresis has been described earlier (Paul, Clark. 1996. Biotechniques 21:126-133). Sequencing and DNA-fragment analysis technology has undergone two generations of improvement since the initial technique was established, ie. ABI 373 gel electrophoresis to ABI 3700 capillary electrophoresis to ABI 3730 capillary electrophoresis. We will describe methods that update this technique to utilize modern capillary DNA analyzers and the new LIZ family of size standards.

P171-T

The New Applied Biosystems 3100 Series System Software Suite Provides Features for a More Efficient Service Laboratory

C. Kosman, A. Swei, T. McElroy, Q. Doan, K. Eskinazi, J. Bourey; Applied Biosystems, Foster City, CA, United States.

The ABI PRISM® 3100 and 3100-*Avant* Genetic Analyzers have become the capillary electrophoresis systems of choice for core and service laboratories. The introduction of a new software suite for the 3100 series systems incorporates several new features that streamline the user workflow while continuing to generate high quality data. New run modules developed for Data Collection Software version 2.0 and the inclusion of a multi-application plate record feature enable the processing of both sequencing and fragment analysis samples not only with a single polymer and array configuration, but also in the same sample injection plate. The implementation of customizable run scheduling also permits users to run their highest priority samples first. In addition, project-based data distribution allows the service laboratory to provide the fast turn-around times expected by their customers. Complete analysis automation with the 3100 series system Data Collection Software v2.0 is also provided in new versions of application-specific analysis software programs: Sequencing Analysis Software v5.1, SeqScape® Software v2.1 and GeneMapper™ Software v3.5. By providing Quality Values on either base- or allele-called data generated from the 3100 series systems, these downstream analysis programs reduce the staff time spent on manual data review of the highly variable sample inputs that are characteristically encountered in the core laboratory environment. The overall improvements to the 3100 and 3100-*Avant* Data Collection Software, and enhanced application-

specific analysis software, increase the efficiency of the 3100 series systems by streamlining essential laboratory workflow factors such as ease of use and data management.

GENE ARRAYS

P172-S

High Throughput RNA Expression Profiling by Parallel Processing of 96 Arrays

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We adapted Illumina's bead-based array technologies for studying gene expression. The Sentrix^{TM} Array Matrix (SAM) provides parallel processing of 96-samples, enabling thousands of samples to be processed per day. The Sentrix BeadChip allows simultaneous processing of 8 samples.
The SAM format comprises 96 optical fiber-bundle arrays, arranged in an 8-by-12 matrix matching standard microtiter plate spacing. Each array contains ~1,500 unique oligonucleotide probes, representing 2-probes for every gene analyzed. Probes are attached to 3-micron beads, which self-assemble into microwells at the end of each array. Since there are many more microwells than probe sequences, multiple copies of each bead-type are present in each array. This built-in redundancy improves robustness and measurement precision. The BeadChip format comprises 8 individual arrays arranged in a planar substrate.
Assay performance was established by a dose response experiment, in which 9 artificial transcripts were spiked into human cellular RNA. Using standard sample labeling techniques, we demonstrated typical detection limits of 0.15pM, specificity better than 1:250,000 and measurement precision sufficient to detect 2-fold expression changes over a 2.8log dynamic range. By measuring cell-specific gene expression within mouse B and T-cell RNA samples, we showed that this high level of performance extends to endogenous mammalian genes. An independent assessment comparing Illumina's Sentrix array platforms to Applied Biosystems' TaqMan qPCR, resulted in strong comparative performance and validation of the Illumina technology. The combination of hundreds of genes per array with the sample throughput of the matrix format will enable "many genes, many samples" applications that are not adequately addressed by current gene expression technologies.

P173-M

Global Gene Expression Profiling of Cancers with Laser Microdissected Samples

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Global Gene Expression Profiling of Cancers with Laser Micro-dissected Samples

Global Gene Expression Profiling of Cancers with Laser Micro-dissected Samples

Siqun Wang[1], Kaho Minoura[2], Diane Ilsley[3], Michelle Chen[3], Yoshio Miki[4], Steve Hutt[1]
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Cells of esophageal cancer and normal esophagus were selectively excised with Laser Micro-dissection from clinical biopsy samples. Using 50 nanogram or less of isolated total RNA, global gene expression profiles were compared between cancer and normal cells with microarrays containing *in situ* synthesized probes representing more than 17,000 well-annotated human genes. The data provides new insights into the difference in genetic regulation

between cancer and normal cells and confirms, at the same time, some of the findings in the literature. We will describe the new technology development in sample acquisition and labeling which enabled this study and the biological findings in this poster.

P174-T
Estimation and Reduction of RNA Degradation Bias in Microarray Analysis
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Measurement of gene expression is based on the assumption that the analyzed RNA sample closely resembles the amount of transcripts *in vivo*. Since it is well-established knowledge that transcripts of different genes possess different stabilities, one can assume that degradation of RNA occurring during the isolation procedure is also non-uniformly distributed among different RNA species. , Indeed, in this work we demonstrate that RNA samples, at different degrees of degradation, can imply up to 75% of differential expression when compared by microarray technology. Until now, there were no sensitive parameters available that were suitable for quantitative as well as qualitative characterization of RNA degradation. In this work, we also demonstrate that quantitative analysis of capillary-electrophoresis data does allow reproducible characterization of RNA degradation and its differentiation from apoptosis-associated RNA cleavage. Our results suggest that similar qualities of RNA integrities have to be ensured before performing microarray analysis to avoid the otherwise observed massive fluctuations and to ensure reproducibility of the obtained results.

P175-S
Using Low Input of Poly (A) + RNA and Total RNA for Oligonucleotide Microarrays
M. Chen[1], S. Hutt[2]; [1]Agilent Technologies, Palo Alto, CA, United States, [2]Agilent Technologies, Wilmington, DE, United States.

With the advancement of DNA microarray technology, there has been an increase in demand for using small amounts of RNA samples for array analysis due to two main reasons. One is that only limited amounts of biological samples are available for gene expression studies. The other reason is because of the improved sensitivity and specificity of DNA microarray platforms that allow scientists to perform array work with lower amounts of samples. Here we describe a method of using of T7 RNA polymerase to amplify and label cRNA targets. We show that micrograms of cRNA can be generated from nanograms of poly (A) + RNA, or total RNA samples within several hours using one round amplification. The performance of resulting cRNA targets on oligonucleotide microarrays is also discussed in this poster.

P176-M
Comparing Microarray Data Generated in Different Laboratories
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Although microarray facilities use similar RNA labeling and hybridization procedures, minor differences in protocols can affect the expression results and confound data comparisons. At Expression Analysis (a microarray service provider), we are analyzing microarray results generated in different laboratories using the same RNA samples. This program allows us to determine the level of variability between laboratories. It also helps develop standardization methods for confirming the proficiency of individual laboratories and for evaluating the quality of specific data sets.
Our initial research compared data generated in two microarray laboratories using a pair of mouse RNA samples. Three biotin-labeled cRNA targets were prepared for each sample at both facilities and hybridized to U74Av2 GeneChips from Affymetrix. After the first use, the hybridization cocktails were exchanged for a second hybridization in the other laboratory. Both labs demonstrated good reproducibility,

based on the consistency of data between the three replicates in each set. Interestingly, a subset of the probes that contain a particular sequence pattern had consistently higher hybridization intensities in one laboratory. The comparability of the data between the laboratories was evaluated by comparing the quantitative estimates of fold change and the lists of differentially expressed genes. In this and subsequent studies, we found the comparability of the microarray results was related to the nature of the RNA samples.
We are now extending this research to other microarray facilities. As before, replicate targets from two RNA samples are processed and hybridized in multiple laboratories. The program can be used to evaluate the performance of proposed RNA standards and to develop distribution curves for quality control metrics and expression results.

P177-T

New High-Density Microarray Format Which Enables Whole Genome Gene Expression Screening on a Single Microarray

L. Niu, A. Lucas, G. Delenstarr, P. D'Andrade, E. Leproust, P. Wolber, B. Page, S. Ghosh, M. Shah, R. Argonza-Barrett, R. Woodworth; Agilent Technologies, Palo Alto, CA, United States.

Agilent has developed a new microarray format with high-density printing to enable the development of its whole genome screening microarray products, which can increase the throughput and reduce the sample and material costs of microarray experiments.
Studies have been carried out to evaluate the performance of the high-density microarrays. Differential gene expression targets HeLa and K562 were labeled with cyanine dyes and hybridized to high-density (44,000-feature) and current-density (22,000-feature) microarrays with identical probes. Gene expression data were analyzed and similar averages of log ratios were observed for both formats. The overall performance of the new high-density format is very comparable to that of the existing Agilent format. The workflow and protocol of the high-density format provides a 2-fold improvement on throughput as well as sample/reagent cost relative to the current format.

P178-S

High Performance, Multiplexed, High Throughput ArrayPlate Assay of Gene Expression: A New Era of Drug Discovery

B. Seligmann; High Throughput Genomics, Inc (HTG), Tuscon, AZ, United States.

Functional genomics studies have identified patterns of gene expression that characterize particular physiological states, drug metabolism, and predict or indicate toxicity. Given the identification of disease, treatment and metabolism-related genes, the ArrayPlate multiplexed mRNA assay was developed as a tool to enable genomics-driven drug discovery. The ArrayPlate measures 16 custom genes in each well of a microplate, supporting assays between 16 -500 genes. ArrayPlate assays can be implemented and characterized in three weeks, designed or “programmed” to measure the same or different sets of 16 user-selected genes in each well, e.g. to measure 96 samples simultaneously against 16 genes, or 12 samples against 128 genes in each ArrayPlate.
To allow high-throughput testing, ArrayPlate mRNA assay protocols were designed to be automation-friendly: In a conventional 96-well plate, biological samples are lysed and then subjected to a multiplexed nuclease protection assay that requires only reagent additions and incubations. There is no need to extract RNA or for reverse transcription, simplifying the processing of samples and eliminating extraction artifact. Nuclease
protection results in the stoichiometric replacement of target mRNA species with synthetic oligonucleotide reporter probes. The nuclease-processed samples are then transferred to a custom programmed ArrayPlate where each one of the reporter probes is captured at a predefined element in the array, labeled and detected by chemiluminescence.
The result of nuclease protection and in-well normalization is a breakthrough in reproducibility and repeatability. (>90%),

combined with high sample throughput (manual - 2,000 samples, 32,000 gene datapoints/day, 4 hours hands-on labor, automated - scalable) and practical sensitivity equivalent to PCR.
HTG customers use the ArrayPlate for target validation, high-throughput screening, lead optimization, toxicology studies and testing patient samples.

P179-M

Standardization of RNA Quality Assessment using the Agilent 2100 Bioanalyzer and the RNA Integrity Number

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The quality of the RNA starting material dictates the overall success of any meaningful gene expression analysis. The assessment of RNA quality has been identified as one of the most critical elements in order to obtain meaningful gene expression data via microarray or real-time PCR experiments. Advances in microfluidic technology have improved RNA quality measurements by allowing a more detailed look at patterns of RNA degradation via the use of electrophoretic traces. However, the interpretation of such electropherograms is subjective, varying from one researcher to the next, and still requires a certain level of experience.
A new tool, the “RNA integrity number” (RIN) algorithm is introduced which assigns a user-independent integrity number to each RNA sample. The RIN has been developed using neural networks along with a large set of RNA integrity data. It was found that the RIN is reproducible, and more reliable than the ribosomal ratio, when gauging the integrity of RNA samples. The RIN is shown to be largely independent of RNA concentration, instrument (Agilent 2100 bioanalyzer), and most importantly, the origin of the RNA sample. Using the RIN, researchers can work towards standardization of the important RNA integrity measurement ensuring reproducibility and reliability of gene expression experiments.

P180-T

Micro-array technology towards Diganostics?

B. Ylstra, P. P. Eijk, E. Hopmans, P. van den Ijssel; VU University Medical Center, Amsterdam, Netherlands.

Micro array technology has matured to a stage where data interpretation has become the main bottleneck in microarray throughput. One could thus argue that our focus should shift away from the array technology hardware. Truth of matter is that even after 10 years of microarrays, there is still a lot to gain in the quality of the arrays. Fine tuning of the array technology hardware and technique yield significant improvements in the ease of data interpretation and throughput and is essential for implementation into the clinic. Lowess smoothing and image flattening are examples of bioinformatics “tricks” that should theoretically not be necessary. Such data treatment to correct artifacts concurrently introduces noise. We therefore argue that all artifacts should be solved within the technique and not by the biostatistician. We have used a non-contact spotting technique to print 20K oligo arrays without missing spots and furthermore use hybridisation chambers to reduce variation and lower the amount of RNA needed to less then 5 _g. Our final quest is to move from high quality to perfection, which is a major challenge for the implementation of microarray (-signatures) as diagnostic or prognostic tools in the clinic. We will present trial and error based ideas, which we believe will lead us to our goal.

P181-S

A Comparison and Variation Assessment of cDNA Microarrays, Oligonucleotide Microarrays, and the Affymetrix GeneChip® Platform on Liver Tissue From A/J and C57BL/6J Inbred Mouse Strains

K. A. Johnson; Jackson Laboratory, Bar Harbor, ME, United States.

This study was designed to compare several gene expression platforms to evaluate the variability

associated with each technology. Total RNA samples were extracted from liver tissue obtained from two pairs of A/J and C57BL/6J male mice. The same four RNA samples were used as the starting material for each technology. In addition to the paired mice used as biological replicates, separate cDNA syntheses, labeling, and hybridizations were performed for technical replication. Hybridizations on cDNA and oligonucleotide microarrays employed traditional dye-swap labeling to eliminate dye bias. The cDNA microarrays were produced from the15K NIA and 3K endocrine/pancreas mouse gene clone sets, with duplicate spots printed for each clone. Oligonucleotide slides were spotted from the 22K Compugen (Sigma Genosys) mouse clone set. The Affymetrix GeneChips used were the Mouse Expression Set 430. Affymetrix 430A chips and spotted cDNA microarrays had higher precision (or less within-platform variability) compared to the Affymetrix 430B and spotted oligonucleotide microarrays. The precision of Affymetrix 430A was slightly higher than cDNA microarrays. Between spotted arrays, the higher precision of cDNA array relative to oligonucleotide arrays may be attributed mainly to the spot duplication on each cDNA slide. Using Mouse Genome Informatics (http://www.informatics.jax.org/) ID numbers, overlapping genes were identified between platforms and their fold changes between the two inbred mouse strains were compared. In terms of their agreement of the direction of fold change with other platforms, Affymetrix 430A and spotted oligonucleotide arrays scored better than 430B and spotted cDNA arrays, respectively. The magnitude of fold change observed was greater in the Affymetrix platform in comparison to spotted arrays.

P182-M

Enhancement of the SuperScript™ III Direct cDNA Labeling System for Microarrays

P. N. Gilles, A. Wizman, W. Zheng, T. Peterson; Invitrogen life technologies, Carlsbad, CA, United States.

Microarray expression analysis has become a valuable tool in functional genomic studies and efficient application of this technology requires the development of robust and reproducible fluorescent labeling protocols. Due to its ease of use, reproducibility and effectiveness, direct incorporation of fluorescent nucleotides during cDNA synthesis is one of the most commonly used methods for expression analysis on microarrays. In this study, a direct cDNA labeling method has been developed which utilizes the newly engineered SuperScript™ III reverse transcriptase (SSIII RT) and was optimized for signal intensities and signal-to-background ratios in hybridizations across a broad range of microarray platforms. The enhanced thermal stability of SuperScript™ III allows for greater primer specificity and processivity through RNA secondary structure resulting higher cDNA yield; while the increased half-life of SSIII RT eliminates the need to spike-in additional enzyme to attain greater cDNA yields. Moreover, the nucleotide mixture was specifically formulated for optimal base-to-dye ratios in this format. Importantly, the labeling method was validated in competitive hybridizations on microarrays against other published methods. The SuperScript™ III direct labeling system reproducibly generated greater signal intensities, signal-to-noise ratios and more evenly distributed dye incorporation than other commonly used methods. The challenge for most microarray applications will be to identify genes that are consistently regulated at lower abundance and by a lower percentage that play a significant role in the development and progression of disease. To this end, the newly developed SuperScript™ III Direct cDNA labeling method was found to increase the sensitivity, accuracy and reproducibility of microarray analyses.

P183-T

Development of a DNA Labeling System for Array-based Comparative Genomic Hybridization

P. Lieu, P. Jozsi, K. Rhodes, P. N. Gilles, T. Peterson; Invitrogen life technologies, Carlsbad, CA, United States.

Chromosomal amplifications and deletions are critical components of tumorigenesis and DNA copy-number variations are also correlated with changes in mRNA levels. Genome-wide microarray comparative genomic hybridization (CGH) has become an important method in detecting chromosomal imbalances and mapping these changes in tumors. Thus, the ability to detect two-fold differences in fluorescent intensity between samples on microarrays depends on high quality labeled probes. To enhance array-based CGH analysis, a random prime genomic DNA labeling method that is optimized for improved sensitivity, signal-to-noise ratios, and reproducibility has been developed. The labeling system is comprised of formulated random primers, nucleotide mixtures, and a high concentration of exo-Klenow. Microarray analyses indicate that the genomic DNA labeling method produces hybridization signals with higher fluorescent intensities and greater signal-to -noise ratios than other published and available methods. Also, probes generated by this system have been validated to differentially detect two fold differences in gene copy number between male and female genomic DNA, in aneuploid chromosomes, and a breast cancer cell line in microarray hybridizations. Moreover, alterations in gene copy number were routinely detected with 0.5 micrograms of genomic DNA. The method is flexible and performs efficiently with different fluorescently labeled nucleotides (Cy3, Cy5 or Alexa fluor dyes). Application of the optimized CGH labeling system will enhance the resolution and sensitivity of array-based CGH analysis in cancer and medical genetic studies.

P184-S

OligoCheck, a generic tool for selecting oligonucleotide array probes with minimal cross hybridization validated experimentally using Staphylococcus aureus genome

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Performance of cDNA arrays is generally inferior to oligoarrays for discriminating between closely related sequences, due to higher probability of cross hybridization. Commercial software allow to select oligonucleotides showing homogeneous physico chemical properties but ignore the presence of conserved motifs in genome sequence. We have developed the OligoCheck program that includes evaluation of hybridization specificity in selecting oligonucleotide probes. A set of >1’300’000 probes that cover the whole genome of S. aureus was derived from three recently sequenced strains. Each candidate probe was further analyzed for target gene specificity and cross-genome target homologies with respect to the three genomes. A genome wide S. aureus oligoarray containing 8’390 probes was manufactured and tested for hybridization specificity by using S. aureus genomic DNA and for detection linearity using different cDNA concentrations and hybridization temperatures. Signals derived from common S. aureus probes validated S. aureus core-genome specificity while signals derived from strain specific S. aureus probes validated strain specificity of hybridization. For transcriptomic analysis, fluorescent signals recorded by adjacent non overlapping probes in each gene were highly correlated. Experiments relying on genomic DNA permit genotyping, deletion maping and pathogenicity islands comparison between clinical S. aureus isolates. Comparison between transcriptomic and proteomic data is in progress. In conclusion, the S. aureus microarray designed using OligoCheck showed excellent performance and genome coverage. More than a tool adapted to a specific application, OligoCheck is a reliable generic software for designing optimal microarray capture elements adapted to whole transcriptome analysis or gene contents.

P185-M

The Effect of Genomic DNA Contamination on Microarray Signatures

J. Link, B. Boyes; Agilent Technologies, Wilmington, DE, United States.

Accurate analysis of gene expression using microarray hybridization requires appropriate isolation of cellular RNA. Interrogation of transcript abundance in the sample requires labeling of the target population prior to hybridization to array-bound nucleic acid. Several properties of the isolated template RNA can determine the efficiency and specificity of target labeling by these methods. Of concern is the generation of labeled target derived from nucleic acid other than the RNA population, in particular from genomic DNA. To investigate this issue, total cellular RNA was isolated from frozen mouse tissues using a silica-based solid-phase kit, as well as a novel "low gDNA" solid-phase isolation kit. Microarray hybridization analysis using these RNAs, with and without DNase treatment, demonstrate that a subset of genes represented on the microarray (Agilent's Mouse Developmental Array) had apparent expression levels skewed, due to the higher genomic DNA contamination. This effect is demonstrated by comparing the intensity of the gene subset in the low gDNA samples vs. those with higher gDNA contamination. The quantities of gDNA contamination, as determined by quantitative PCR assay, are sufficient in RNA samples isolated using typical silica-based solid-phase methods, to overwhelm the ability of DNase to completely eliminate the problem. The fluorescent intensity of the gene subset that is overestimated in RNA isolated using the silica-based kit is reduced by about one log unit with the addition of a DNAse treatment, but is still well above the signal levels generated using the "low gDNA" isolation method. These results reiterate the importance of considering the purity of template RNA prior to the use of a sample for microarray-based gene expression analysis, particularly the levels of gDNA in the sample.

P186-T

Global Gene Expression Effects of siRNA-based Gene Silencing

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Small interfering RNA (siRNA) is a powerful tool used to silence gene expression and determine gene function. Experimental approaches typically query the transcript or protein targeted and ignore the genome wide transcriptional profile to monitor cellular response. We have used Agilent *in situ* synthesized 60-mer oligonucleotide microarrays to monitor expression signatures resulting from the lipid-mediated delivery of various siRNAs in human cell culture. In one set of experiments, we have compared a series of diverse highly functional siRNAs to a known endogenous housekeeping gene, human cyclophilin B. We have measured expression effects in mock transfected or untransfected cells and a series of nonspecific siRNA (NS) controls that lack genome-wide homology. Results indicate that day to day variability in transfection varies significantly and mock lipid based transfection can elicit a interferon-like response typified by OASL, IFI27, IFIT 2-4 upregulation. Some NS controls show no interferon-like response. The siRNA-based off-target effects at the RNA level was also tested using the genome wide screening method or intentional sequence matches, and in certain situations demonstrate a RNA-sequence specific knockdown. In conclusion, global expression effects of siRNA are fairly minimal and may be controlled by careful sequence selection of the siRNA and experimental design. Transfection-specific effects have also been observed and can have a dramatic effect on the gene-expression profiles of siRNA-transfected cells. These effects should be considered carefully in the phenotypic conclusions drawn from the use of siRNA as a gene-silencing strategy.

P187-S

Genes Controlling Multiple Functional Pathways in Heart are Transcripitonally Regulated in Connexin43 Null Mouse Heart

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We have used the AECOM cDNA arrays containing 27,400 spoted sequences to compare gene expression patterns in hearts from four neonatal wildtype and four littermates lacking the major cardiac gap junction protein, Cx43. Each individual heart sample was hybridized against aliquots of an RNA standard prepared from selected mouse tissues, allowing calculation of variability of gene expression among the samples from each genotype. Overall variance of gene expression was found to be markedly higher in wildtype than Cx43 null littermates. Expression of 586 of the 5613 adequately quantifiable spots encoding unique proteins were statistically altered in the Cx43 hearts, 38 up-regulated and 548 down-regulated compared to wildtypes. Functions of proteins encoded by the altered genes encompassed all categories, with largest changes in transcription factors and those involved in energy/metabolism and cell signaling. Among the regulated genes were a large number of down-regulated genes related to neuronal and glial function, suggesting that cardiac innervation may be altered as a consequence of Cx43 deletion. These findings reinforce the proposal that the cardiac abnormality in these animals is due to alteration in migration of neural crest derivatives and indicate that Cx43 deletion has consequences extending beyond those due to reduction in intercellular communication.

P188-M

Development of a Dye-Protecting Solution for Preventing Fluorescent Dye Degradation on DNA microarrays

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A predominate application of oligonucleotide microarrays is gene expression analysis. One common method of gene expression analysis is two-color labeling and competitive hybridization to determine differential expression between samples. However, reports in the literature have noted seasonal effects on microarray data quality when samples are fluorescently labeled with cyanine-5 and cyanine-3, the standard for fluorescent dyes used in two-color labeling. Individual features can have reduced cyanine-5 signal intensity relative to cyanine-3 intensity, and there can be feature and/or array-wide gradients for signal intensities and ratios. It has been shown that environmental ozone can be the source for degrading cyanine-5 signal intensities. In this poster, we first describe the 2 distinct modes of ozone induced cyanine-5 degradation. We next describe a set of experiments in which we quantified the ozone degradation of a panel of fluorescent dyes, including cyanine-5 and cyanine-3, in an enclosed chamber where ozone exposure can be controlled in terms of amount and time of ozone exposure. We next screened a series of compounds for their ability to protect those dyes susceptible to ozone degradation. We finally describe a dye-protecting solution that protects cyanine-5 from both modes of ozone degradation, without affecting feature signal intensities, log ratios, or background fluorescent signal intensities. The dye-protecting solution also has the added benefit of drying the slides, thus elminating the need for a separate drying step.

PROTEIN ARRAYS

P189-T

Surface Plasmon Resonance Analysis of the Capture of Intact Simian Immunodeficiency Virus Virions by anti-gp120 Monoclonal Antibodies

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We have used a novel surface plasmon resonance (SPR) SpotMatrix platform to study the capture of SIV virions by a panel of rhesus macaque

monoclonal antibodies (Rh-mAbs) reactive with the SIV envelope glycoprotein, gp120. Of particular interest are the kinetic profiles of the binding of intact virus particles to immobilized antibodies and correlations with data from ELISA and neutralization assays.
SPR measurements were made using an Applied Biosystems 8500 Affinity Chip Analyzer. Rh-mAbs and control antibodies were spotted onto gold chips pre-coated with protein A/G alone or protein A/G and a polyclonal rabbit anti-human IgG antibody. A total chip density of 300 spots, including controls and references, was used. We studied virus binding by flowing highly purified, concentrated, chemically inactivated SIV virions over the immobilized antibodies, with recirculation of the sample for extended association times.
Virus binding was characterized by extremely slow on-rates (apparent ka ~10E+2 1/Ms) and slow or un-measurable off-rates. Despite allowing binding of the virions to the chip surface for up to 5 hours, the binding curves did not reach equilibrium. The specificity of the observed SIV binding was demonstrated by control analyses with HIV-1 virions instead of SIV and competition with soluble gp120. These data will be compared with ELISA data for the monoclonal antibodies and with SPR measurements made with a Biacore instrument.

P190-S

Microarrays Based on Affinity-tagged scFv Antibodies: Sensitive Detection of Analytes in Complex Proteomes

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Protein microarrays is a novel technology that will provide us with unique means to perform global proteome analysis. However, the process of designing adequate protein microarrays is a major generic problem. In this study, we have evaluated a protein microarray platform based on non-purified affinity-tagged scFv antibody fragments to generate proof-of-principle and to demonstrate the specificity and sensitivity of the array design. To this end, we used our human recombinant scFv antibody library genetically constructed around one framework, the n-CoDeR library containing 2 x 1010 unique clones, as source for our probes. The probes were immobilized via engineered C-terminal affinity tags, his- or myc-tags, to either Ni2+-coated slides or anti-tag antibody coated substrates. The results showed that highly functional microarrays were generated and that non-purified scFvs readily could be applied as probes.
Specific and sensitive micorarrays were obtained, providing a limit of detection in the pM to fM range, using fluorescence as mode of detection. Further, the results showed that spotting the analyte on top of the arrayed probes instead of incubating the array with large sample volumes (333 pL vs 40 ml) could reduce the amount of analyte required 4000 times, from 1200 attomole to 300 zeptomole. Finally, we showed that a highly complex proteome, such as human sera containing several thousand different proteins, could be directly fluorescently labeled and successfully analysed without compromising the specificity and sensitivity of the antibody microarrays. This is a pre-requisite for the design of high-density antibody arrays.

MICROFLUIDICS

P191-M

Rapid Sample Preparation and Proteins Analysis Using a Microfluidic Instrument

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Lab-on-a-Chip (LOAC) technology has begun to revolutionize protein analysis. Where SDS-PAGE gels were once run for sizing and quantitation of proteins microfluidic instruments are now beginning to replace this traditional method. Using microfluidic technology, fluids can actively be controlled in micro-fabricated channels allowing for the integration of several experimental steps. The LOAC instrument integrates sample handling, separation, staining,

destaining, detection and data analysis. It is the integration of these procedures that allows for high throughput. The chip-based protein analysis is comparable in sensitivity, sizing accuracy and reproducibility to SDS-PAGE gels stained with Coomassie. To maximize utility of a microfluidic device sample preparation will occasionally be necessary. Instruments using electrokinectic injection of samples can be sensitive to the high salt content of some sample buffers, i.e. samples from an ion exchange column. Here we show a quick and efficient method for the removal of salts from the sample prior to analysis on the microfluidic instrument. Other sample types such as sera require the removal of high abundant proteins to allow for the visualization of lesser abundant proteins. After albumin removal, sizing and quantitation measurements of the lesser proteins can be attained using the microfluidic instrument.

P192-T

Optimizing Gene Silencing experiments with the Microfluidic Chip-based System

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RNA interference is a great new technology for the scientific community. Here, the delivery of small interfering RNAs (**siRNA**) into cells is of key importance in elucidating gene function. Although there exists differing types of interfering RNAs, and several methods of delivery into various cell types, all require **transfection optimization**. However, efficiency of transfection can be affected by many factors, including cell line, siRNA concentration and its ratio to transfection reagent, cell confluency, incubation time and media composition. Further the selection of the best silencing sequence at an optimal siRNA mix concentration which allows for minimal cross reactivity, is predicated on the integrity and purity of the siRNA, the siRNA uptake and cell viability. Given the expense and complexity of monitoring and optimizing these types of experiments a new tool is required that would allow for minimal sample and reagent consumption in a fast and easy to use format. We describe here the use of a **microfuidic system** to quickly verify siRNA quality and determine the optimal conditions **for** any **gene silencing** experiment. First, in electrophoretic mode, RNA integrity and purity is assessed. Second, in flow cytometric mode, fluorescently labelled siRNA were used to optimize transfection parameters in mammalian cells. Live staining was performed on-chip reducing the overall analysis time below 50 minutes. Transfection efficiency was measured as percentage of cells with strong siRNA uptake within the live cell population. Third, gene knockdown was measured using antibody staining against intracellular protein targets after siRNA transfection. Fourth, the gene silencing mechanism was verified by co-transfection experiments where GFP plasmid and a Cy5 labelled siRNA against GFP were transfected. Successful silencing was measured by reduced GFP expressing cells within the Cy5 positive population.

P193-S

Automated high throughput analysis of DNA samples by microfluidic chip technology

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In the “post-genome era”, there is an ever-growing demand for high-throughput DNA analyses. The number of samples that are processed are steadily increasing, representing a major challenge for classical DNA analysis techniques. Especially slab gel analysis requires long analysis times, is labor intensive and difficult to automate. More modern techniques such as capillary electrophoresis and capillary array electrophoresis exhibit much shorter analysis times and show improved performance compared to slab gel analysis.
The next major step forward in the context of miniaturization and integration is represented in the concept of microfluidics. Since a number of years microfluidics instrumentation is available that shortens analysis time down to 90-120 seconds per sample analyzing multiple samples through one separation channel. Following the development in capillary electrophoresis, the

usefulness of such systems for genetic analysis can be increased by developing parallel separation channels that convert the short analysis times into a high sample throughput. Here we present the lab prototype of a microfluidic system that allows microfluidic separations within 4 parallel channels. The system is capable of analyzing DNA samples directly from 96- or 384-well, analyzing. Fluid movement is controlled by a series of independent electrodes and pressure interfaces. An integrated pipetting station automatically primes and replenishes the chip with buffer, sieving matrix and DNA-binding dye. Sample and reagent well plates are handled by a robotic system. DNA fragments are detected by laser-induced fluorescence and data is stored in an Oracle database. The system is capable of unattended analysis of thousands of DNA samples with minimal sample consumption. Sensitivity and sizing accuracy are superior to slab gel analysis and data are highly reproducible.

P194-M

Quality Control of Protein Drugs Using a Microfluidic System

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It is perceived that the future of pharmacy belongs to protein-based drugs, using proteins to diagnose, prevent and treat diseases and conditions. To determine protein size, purity, integrity and concentration is more than crucial during development and production of protein drugs. Denaturing gel electrophoresis (SDS-PAGE) is one of the commonly used methods for protein-based drug quality control, where it for example is required for FDA approval or to release new lots from already approved protein drugs.
The recent development of microfluidics offers an alternative for protein analysis with SDS-PAGE, addressing the strong demand for more automation and higher throughput. The microfluidic system allows rapid and automated separation of proteins, integrating multiple experimental procedures, such as sample handling, separation, staining, destaining, detection and data analysis. The instrument analyses 10 samples in less than 45 minutes thereby achieving a better reproducibility compared to SDS-PAGE. All reagents and microfluidic chips are standardized and the application is tailored for the use in regulated environments.

P195-T

Automated Sample Preparation for Detection and Verification of Phosphopeptides

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It is preferable to detect phosphopeptides by MALDI MS after enrichment by immobilized metal affinity chromatography (IMAC) since these peptides ionize poorly in positive mode and are therefore 'out-competed' by more easily ionized peptides. The presence of phosphopeptides can be verified after dephosphorylation, which leads to a mass shift of 80 Da (or multiples of 80 Da). We have combined a selective IMAC adsorption, washing, elution procedure, with enzymatic dephosphorylation and crystallization in a CD microlaboratory presenting the enriched phosphopeptides in a small desorption area on top of the CD. Within the CD, liquid flow is directed through different compartments, using centrifugal force to overcome the resistance created by a combination of hydrophobic breaks, geometric microfabrication and capillary force. Bound phosphopeptides are either directly eluted and crystallized, using 50% acetonitrile, 1% phosphoric acid with dissolved MALDI matrix (DHB), or first enzymatically dephosphorylated on-column and thereafter eluted and crystallized. A digest of bovine protein disulfide isomerase, spiked with a known concentration of two naturally occurring phosphopeptides, has been used as a standard in a worldwide study conducted by the members of the Proteomics Research Group of the ABRF. (www.abrf.org/ResearchGroups/Proteomics/EPosters/ABRF_PRG03.pdf). Both phosphopeptides in this mixture could be enriched and

dephosphorylated using IMAC and enzymatic dephosphorylation in the CD. In order to investigate the IMAC specificity for phosphopeptides, a tryptic protein digest mixture containing 5 pmol BSA and 25 fmol _-casein was loaded into the microstructures on the CD. While BSA is non-phosphorylated, _-casein contains two serine-phosphorylated tryptic peptides. Even though the two phosphopeptides of trypsin-digested _-casein only contributed 0.5% of the total peptide concentration it was possible to detect and enzymatically verify the presence of both peptides.

BIOINFORMATICS

P196-S

Proteomic Data Analysis - Evaluating Large Amounts of Data

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Protein identification experiments commonly involve both the gel based and the LC based workflows. The proteins in the sample are first fractionated to obtain a mixture of the proteins of interest. In some cases the proteins of interest are separated by means of chromatography or gel electrophoresis, to obtain a pure protein or a mixture of a few proteins. These protein mixtures are subsequently digested with a proteolytic enzyme. If the resulting mixture of proteolytic peptides is too complex for direct mass analysis, the peptides are first chromatographically separated. The masses of resulting peptides and their fragments are measured by mass spectrometry and compared with calculated peptide masses from a protein sequence collection. A score is calculated for the comparison and the protein sequences in the collection are ranked according to the calculated score. The significance of the protein candidates is assessed by calculating the probabilities that they are false positives. Depending on the application, the user selects the acceptable cut-off for the probability of getting a false positive result. The protein candidates passing this quality test are considered to be identified. In cases where proteins of unknown function are identified, it might be possible to assign a function by homology searching. In this work we have analyzed large experimental data sets obtained from analysis of biological samples using the gel and LC based workflows and present strategies for rapidly obtaining accurate and informative results.

P197-M

Lutefisk1900 vs Peaks: A comparison of automated de novo sequencing programs

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A mixture of known proteins were reduced, alkylated, digested with trypsin, and analyzed by LC/MS/MS using a quadrupole time-of-flight hybrid (Qtof) and an ion trap. A database search program was used to validate spectra whose top scoring candidate matched peptides from one of the proteins known to be present. The validated spectra were used as input into Peaks and Lutefisk1900, and the results were categorized as being completely correct, partially correct, or mostly incorrect. Results for the two programs were similar for Qtof data, but Lutefisk out-performed at ion trap data. In all cases, the Lutefisk-derived scores provided better differentiation between correct and incorrect candidate sequences.

P198-T

Scripting for Fun and Profit: Automate Core Facility Data Munging.

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Entering work request data in a research core facility is tedious and error-prone. This is true whether you type it into a spreadsheet or a sophisticated LIMS or database. And every facility uses a mix of software to operate various instruments and track the results. This means

typing or "cutting and pasting" data at some point in the workflow, or you can learn a scripting language and write a small computer program to automate the task. We call these small, specialized, programs, "scripts".
A lab automation script takes the output from one data source, extracts what's needed, and produces a new output in the format required by the next step in the workflow. We call this "data munging". Perl, the "swiss army knife" of programming languages excels at this and is our choice for this work.
The example presented here automates the data munging needed to convert web-form generated work requests into files used by our Applied Biosystems Inc (ABI) 394 automated DNA synthesizers. Each file has all the data fields already filled in, so the staff needs only to assign it a column on the synthesizer.
The script may be "wrapped" in a graphical user interface (GUI) program. But for speed and convenience on the Macintosh OS X computer, the script need only be saved with the ".command" suffix to be recognized by the Finder as an executable program. This script obviates the need for any typing or "cutting and pasting" of data, thus improving the reliability of our synthesis service, and sparing the staff a tedious task.

P199-S

The SNP500Cancer Database: The Importance of Validating Single Nucleotide Polymorphisms by Sequence Analysis and Genotype Concordance

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The utilization of SNPs has accelerated interest in studying common diseases through the analysis of unrelated individuals in case-control and cohort designed studies resulting in the generation of data orders of magnitude greater than in the past. Public databases have been created that contain several million SNPs. The failure to validate many putative SNPs and their sequence context has drawbacks. Many public databases reporting SNP sequence context do not always accurately describe adjacent flanking SNPs present in the populations studied. The blind use of information from public databases poses potential problems in assay optimization and performance, contributing to confusion in the literature and the plethora of reported false positive results. Our research program (http://snp500cancer.nci.nih.gov) has been created to verify SNPs and surrounding sequence as well as to validate genotyping assays for SNPs in molecular epidemiology studies. Its purposes are: a) an estimation of the minor allele frequency of the SNP in four populations, b.) discovery of SNPs, c.) identification of SNPs that could interfere with genotyping, and d.) provide standards for assay development and execution. Genotyping assays are developed and validated on one or more of the following platforms: 5’-exonuclease, hybridization-triggered fluorescence, and/or mass spectrophotometry. Discordant results were seen in each platform and were caused by SNPs previously not validated by sequence analysis. Because no single genotype platform will successfully analyze all SNPs, complimentary sequencing/genotyping assay validation is critical for the execution of large, sufficiently powered studies.

P200-M

Computational Tools for Shotgun Proteomics

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Objective
Development of computational tools to increase confidence in protein identification and statistically evaluate these results
Methods
Tandem mass spectra were generated from 100 LC-MS/MS runs on a peptide mixture resulting from a whole cell lysate of *Trypanosoma cruzi*

(*T. cruzi*) epimastigotes, which were fractionated by offline multidimensional liquid chromatography. Spectra were searched using Mascot against a *T. cruzi* protein database combined with all bovine proteins, equine proteins, and human keratins from NCBI. Mascot ranks matches based on the relative number of intense peaks used in peptide identification. However, it does not preferentially attribute low scores to spectra composed mostly of noise or exhibit poor correlation between experimental and theoretical mass values. With this in mind, we developed PERL-based computational tools that filter and recalibrate spectra prior to database searching with Mascot. All MS/MS spectra were searched before and after applying each filter and recalibration in order to evaluate their effectiveness.
Results
When all MS/MS spectra were searched, Mascot identified 3,924 peptides with scores exceeding the 95% confidence threshold. Our automated filtering algorithms removed approximately 16% of these spectra. This procedure increased the number of identified peptides by 10% and decrease search time by 20%. Automated data recalibration with user defined internal standards reduced the average mass error to 20 ppm, increased the number of peptide matches by an additional 14%, and further reduced search time by 20%.
We have also developed tools for statistically evaluating the confidence of peptide matches from MS/MS database searching. These calculate the probability of a random match and provide expected false positive rates at given Mascot scores when using user defined databases and actual datasets.

P201-T
An Examination of the Coverage and Performance of Database Search Algorithms for Protein Identification
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Database search algorithms comprise one of the major categories of protein identification algorithms using mass spectral data. In this approach, MS and MS/MS data of LC-separated digested proteins are used to identify the peptides and thus, proteins present in a sample by limiting the possible answers to known proteins in a database. Here we consider the coverage and performance of several database search applications, including Interrogator™ and Mascot™ from Matrix Science. A characterization of the search space coverage afforded by database search algorithms is presented, in addition to performance comparison among the algorithms.

ROBOTICS

P202-S
Automation of ZipPlate® Micro-SPE Plate for In-Gel Protein Digestion
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With the completion of the Human Genome Project, there is now significant emphasis on identifying proteins and protein function. As a consequence, the number of protein samples continues to increase. Proteins are typically separated by MDLC, 1D or 2-D gel electrophoresis. Those of interest are digested, concentrated/desalted and analyzed using mass spectrometry. This procedure is time consuming and labor intensive. We describe here a method to improve this process both by using the Montage® In-Gel Digest KitZP to decrease process time and full automation of the process using two automated systems. Utilizing the ZipPlate 96 well micro-SPE plate platform allows 24 - 96 samples to be run simultaneously. Automation allows for reduced hands-on time and adds consistency to a complicated process. Data shown will compare manually handled samples to those prepared on a Tecan GenesisTM Workstation and PerkinElmer MultiPROBE® II Proteomics Workstation.
The ZipPlate wells are conical in shape with C18 resin immobilized at the bottom. This design integrates all steps of in-gel digestion into one

device, eliminating the need for multiple transfer steps, which can adversely affect digest recoveries. The automated process has been divided into three programs when using the MultiPROBE II Proteomics Workstation; destaining and trypsin addition, peptide extraction and binding to C18 resin and elution. Elution is performed either by vacuum or centrifugation into a separate 96 well receiver plate or by vacuum directly onto a MALDI target. An optional third program is available to spot samples and matrix that have been eluted into 96 well plates onto a target. The Tecan Genesis with an on deck incubator provides the option of one program for all the steps of in-gel digestion when using the ZipPlate device.

P203-M

Integration of a home-made microcapillary LC-MALDI spotting robot in an LC-MALDI-MS/MS workflow

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Several commercially available mass spectrometers combine MALDI with MS/MS capabilities. MALDI is suitable for many applications and has practical benefits including 1)off-line sample loading; 2) data acquisition is not time dependent; and 3) the sample is available for further analysis. On the other hand, MALDI ionization is not adapted for an on-line coupling with an HPLC instrument. We report here the construction and set-up of an integrated LC deposition robot for micro-capillary LC elution directly on to MALDI target plates.
A commercially available XYZ-table with step motors was equipped with a home made MALDI plate support and a device to hold a micro-LC column, e.g. 0.075 mm ID. The instrument interface was written in VisualBasic language. The TTL input of the robot command was used for the connection to the contact closure of the HPLC system. During the run, HPLC fractions were spotted on to the MALDI target. After complete drying on the target, matrix solution was added, the peptides were re-dissolved and co-crystallized before analysis with a MALDI-TOF/TOF instrument.
More than 10 MALDI plates can be stored on the spotting robot. The HPLC fractions can be collected in a time interval varying from less than 5 seconds to several minutes. Fractions of less than 100 nl can be spotted on the MALDI targets. With the use of a HPLC cycle time of 90 minutes, a throughput of 10 samples during an overnight run is easily achieved. MS/MS analysis can be completed in an automated fashion during the next day.

TECHNICAL TIPS & HINTS

P204-T

Whole Genome Amplification as a Tool for DNA Recovery for Large Linkage and Association Studies

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One of the challenges faced by many core facilities and research laboratories is how to solve the problem of stock DNA limitation notably when additional tissue procurement is not possible. Collecting DNA samples from different sources for large genetic analysis studies often results in biomaterials of unequal quality and quantity. As the study progresses, these precious samples can be depleted faster than expected, resulting in missing data point, which in turn lead to a decrease in statistical power.
To resolve the problem of stock DNA limitation for one of our ongoing family-based linkage studies, we have explored the method of Whole Genome Amplification (WGA) developed by Amersham (GenomiPhi™ DNA amplification kit). Initial testing performed on high quality DNA's isolated in our laboratory from human blood and surgical tissue as well as embryonic rat tissues demonstrated that this powerful technology allowed greater than 100-fold amplification of the starting material.
We have tested 45 human genomic DNA

samples from our heterogeneous collection of which 36 were successfully amplified. DNA's showing degradation patterns were not recoverable by GenomiPhi. Successfully amplified samples were tested by chromosome X-specific microsatellite markers using the ABI linkage mapping set v2.5, panel 28 in multiplex conditions, and by SNP analysis using the TaqMan assay-on-demand chemistry. No Mendelian errors were picked up for the amplified samples, thus suggesting that the polymerase has a high degree of fidelity during DNA replication. GenomiPhi™ DNA amplification kit is a valuable tool suited for recovery of precious DNA stocks in core facilities and research laboratories where replacement tissue may not be readily available.

OTHER

P205-S

Quality Control of DNA Synthesis using Fast Ion-pair Reversed-phase High Performance Liquid Chromatography

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Current methods of DNA synthesis can routinely produce high quality oligonucleotides, provided that the automated synthesizers are in optimal operating condition. While suboptimal synthesis can still yield acceptable and usable oligonucleotides, diagnostic and therapeutic applications require high purity probes.
A method utilizing ion-pair reversed-phase high performance liquid chromatography (IP-RP-HPLC) with ultraviolet (UV) detection has been developed for the routine quality control (QC) of synthetic oligonucleotides up to 30mer in length. Separation was performed on a 20 x 4.6-mm MS C_{18}, 2.5 _m column. Mobile phases were comprised of 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) and triethylamine (TEA) with a concave methanol gradient. Resolution of the target product from N-1 impurities was routinely achieved for all DNA fragments analyzed, including oligonucleotides with mixed base sequences (up to 30mer). The 5-minute per sample duty cycle allows for fast diagnosis of the performance of DNA synthesizers.
Two sets of four homooligonucleotides (dG_{20}, dC_{20}, dA_{20}, and dT_{20}) and a 30mer heterooligonucleotide were purchased from five different vendors. The quality of each homooligonucleotide is indicative of a particular problem of the synthesis process; coupling efficiency, incomplete deprotection, side reactions, depurination, and poor solvent/reagent quality. Since detection was performed by UV, the absolute amount of impurities in each synthesis was quantitated. The HPLC method is capable of routine detection of 0.5 % of failed products in the target oligonucleotide synthesis. The method is useful for evaluation of DNA synthesizer performance.

P206-M

Analysis of Single Nucleotide Polymorphisms (SNP) in the Promoter Region of Interleukin-10 by Denaturing High Pressure Liquid Chromatography (DHPLC)

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Interleukin-10(IL-10) an anti-inflammatory cytokine has been implicated in variety of immune and inflammatory related diseases. We investigated the following SNP's (-592, -1082, -819) in promoter region of IL-10 in normal population by DHPLC (mutational discovery tool) and established haplotype patterns. Prior to running samples on DHPLC, it was imperative to establish PCR conditions to obtain results that showed distinct chromatograph patterns for each SNP as well as identification of homozygous versus heterozygous samples. For each SNP, specific melting temperatures were used based on sequence and composition of the PCR product (for -1082, 57°C; for –819, 58°C; and for –592, 59.2 °C). Before fragment mutational analysis, all samples were denatured at 95°C and slowly re-annealed to allow for re-association of different strands. In order to identify homozygous wild type vs. homozygous mutant, 2 PCR samples needed to be mixed together, denatured at 95°C and re-annealed. The

homozygous mutant when combined with control displays a double peak on the chromatogram. Once distinct chromatograms were established for each of the SNP's, the nucleotide changes were confirmed by sequence analysis. The following genotypic frequencies were observed: for IL10(-1082), GG(16%), A/G(47%), A/A(37%).; for IL10(-819), CC(58.5%), C/T(39%),TT(2.5%); for IL10(-592), CC(50%), C/A(42%),AA(8%). The corresponding haplotype frequency was as follows: GCC(36%),ACC(36%) and ATA(28%) which is in agreement with others (1). We are currently comparing our control genotypic findings to mutations seen in selected patient populations (namely, rheumatoid arthritis and lupus). Analysis of samples by DHPLC appears to be an ideal method to screen large sample populations for SNP mutations.

1. Suarez,a. et al. 2003. Transplantation 75(5):711-717.

P207-T

Analysis of Possible Realizator Genes of Murine Hoxc8 through Proteom Profiling

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The Hox genes are key regulators for animal body pattern formation and are expressed position-specifically along the antero-posterior (A-P) body axis during early embryogenesis. Although the molecular analysis for the position-specific expression has well been studied, what Hox regulates -i.e., Hox downstream target genes- is not clear yet. In an attempt to analyze Hoxc8 downstream target genes, a stable cell line overexpressing Hoxc8 was established using F9 murine teratocarcinoma cells and total proteom samples were analyzed through two dimensional electrophoresis (2-DE). Fourteen protein spots having differences more than 4 fold (up- or down- regulated) in intensity between Hoxc8 overexpressed and control samples were selected, and analyzed using MALDI-TOF. In order to see whether plausible Hoxc8 target genes are regulated by Hoxc8 at the transcriptional level, we examined the expression patterns of target genes in vivo, along with those of Hoxc8. So mouse embryos at 11.5 day p.c. were isolated and sliced into seven pieces along the A-P axis, and total RNAs were isolated from each segment and used for RT-PCR. As the result, Tubulin beta-5 chain, ATP synthase, Elongation factor 1-beta and Proteasome subunit alpha type 5 showed ubiquitous expression pattern along the axis, whereas Glucose-regulated protein 78 (Grp78) mRNA was localized only in the region where Hoxc8 was highly expressed. These results altogether imply that Grp78 might be one of Hoxc8 target genes, possibly a Hox realizator gene.

P208-S

Organ-Specific Expression Profile of Jpk: Seeking for a Possible Diagnostic Marker for Type II Diabetes Mellitus.

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During the study on the regulation of Hox gene expression, a novel gene Jpk was isolated as a trans-acting factor associating with the position-specific regulatory element of murine HoxA-7. Sequence analysis reveled that Jpk is a homologous gene of Tanis, originally isolated from Israeli sand rat having type _ diabetes. Tanis has been reported to be related to the insulin resistance, and over-expression of this protein in liver cells developed symptoms of diabetes as well as decreased insulin sensitivity. In an attempt to develop a possible diagnostic marker and/or new therapeutic agent for the Diabetes Mellitus, we analyzed the expression pattern of Jpk before and after feeding in both normal and diabetic Sprague-Dawley (SD) rats which was induced by injection of streptozotocin. Total RNAs were isolated from each organs (brain. lung, heart, liver, spleen, kidney, muscle, blood, and testis) of diabetic and normal rats in both normal feeding and after fasting condition. And then RT (reverse transcription) PCR has been performed using Jpk-specific primers. Jpk expression turned out

to be increased by fasting in diabetic liver, kidney and spleen. In the case of normal SD rat, the expression of Jpk was decreased by fasting. But the expression pattern of Jpk in other organs, such as brain, lung, heart, muscle, blood and testis, were not resemblant to those of liver, kidney and spleen. Interestingly, Jpk level were remarkably increased about 30-60 times in blood of diabetic condition. These results suggest that Jpk could be a possible candidate for the blood diagnostic marker for type _ diabetes.

P209-M

Higher Sensitivity and Success Rate in Protein Identification with Deep Purple

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Here we demonstrate the performance of a new fluorescent total protein stain using standard 1-D and 2-D gels in comparison with Sypro™ Ruby. Also presented are gel images and spectra showing the Deep Purple™ stain used in conjunction with 2-D DIGE (Fluorescence Difference Gel Electrophoresis) system and MALDI MS.
In the 1-D experiments we could demonstrate that the new stain in general is between two-and four-fold more sensitive than Sypro Ruby. For some proteins the level of increase could even be up to eight-fold.
The 2-D gel images of gels stained with the new stain are characterized by strong, positive fluorescent signals with low level, clear backgrounds while the speckling seen with Sypro Ruby staining was minimised. We can demonstrate that Deep Purple is therefore highly compatible with automated spot-detection programs.
A preparative 2-D gel was also run and stained with the new stain and matched against analytical gels, containing CyDye™ labelled protein samples, using a suitable analysis software. A representative sample consisting of 10 spots excised was processed. Eight of these 10 gel plugs were positively identified by peptide-mass fingerprinting on MALDI MS.
We achieved great confidence in the identifications from peptide mass searches, due to the low background generated with Deep Purple staining that aided in the protein identification.
This new total protein stain can successfully be used in 1-D and 2-D electrophoresis gels, giving higher signal to noise ratio and clearer background resulting in higher sensitivity. The stain also provides a high success rate of protein identification when used in conjunction with 2-D DIGE system and MALDI MS.

P210-T

OnCore: A web based framework for management of equipment, users and data in core facilities and shared environments.

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With the ever expanding array of services being offered by core facilities, it is becoming imperative to have an unified “portal” for interacting and managing various services and associated data for customers and facility administrators.
OnCore framework addresses some of these issue by providing a central repository of customer profiles, ability for provisioning resources with single manageable identity. CRM (Customer Relationship Management), financial and process accounting with rudimentary reporting, data management; interface with enterprise wide sign on service and mobile device support is provided. The underlying implementation relies completely on open source software.
Using this framework we have developed the “OnCore:Scheduling” module targeted towards shared equipment that have to be reserved for specific durations of time while enforcing basic business rules for allowable daily/weekly usage; tracking of generated data e.g. scanners, printers, RT PCR, Confocal microscopes, Flow Cytometers etc. The current version (Nov. 2002), allows facility administrators to independently commission schedules for new equipment, grant access for approved users. Since most of these

resources have dedicated workstations for controlling the equipment, an accompanying (Microsoft Window) client application interfaces with the OnCore:Scheduler and logs, enforces usage while accommodating unscheduled use. Freeing administrator from tedious tasks of reporting usage and assuring availability of equipment in multi project high throughput work flow environments. This module has facilitated optimal, productive and efficient use of time for the equipment and their administrators.
Existing stand alone modules based on the earlier OnCore (ver. 0.1a 1998) for ABI 377, 3730, 3100 and microarray core services will be migrated to this framework by Q3 2004. Allowing users to interact with multiple facilities, access generated data from a single interface.

P211-S

Using ChipLC and Ion Trap Mass Spectrometry to Detect Low Abundance Components in Proteomics Samples

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With up to tens of thousands of molecular species expressed at levels ranging from one to millions of copies, one of the greatest analytical challenges in modern biochemistry is profiling the proteins present in a cell. Multi-dimensional chromatography coupled with data dependent mass spectrometry has emerged as a strong technology to address the challenge because it simplifies the mixture presented to the mass spectrometer, allowing the instrument time to obtain more peptide structural data, thus increasing the possibility of identifying lower abundance proteins.
In this work, we use an Agilent 1100 chipLC prototype (a novel nano-LC column and integrated spray tip fabricated on a polyimide chip) coupled to an Agilent 1100 Series SL or XCT ion trap to analyze a proteomics sample spiked with a known concentration of trypsinized myoglobin, BSA or phosphorylase B. The sample is derived from a lysate of K562 cells from a human erythroleukemia cell line. Proteins were first separated by gel filtration, then the fractions were treated with trypsin and resultant peptides separated using SCX. An aliquot of 0.5% of the volume of one SCX fraction was spiked with 1, 10 or 100 fmol of myoglobin, BSA, or phosphorylase B, loaded onto the chip and then eluted using a gradient of increasing organic content. Preliminary data shows the XCT was able to detect standard proteins at lower levels in the presence of a proteomics sample than the SL trap, suggesting the higher capacity and the faster scan rate of the XCT trap enhances its ability to detect lower abundance ions in a complex sample.

P212-M

A Plant Total RNA Isolation Method Applicable to a Wide Range of Species and Tissue Types

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Purification of total RNA from plant tissue has traditionally been hampered by the wide-range of plants and tissue types studied in the agricultural laboratory. Successful RNA isolations can suffer from several problematic components, including: nucleases; polyphenolics and other oxidants; waxes, oils, resins, and latexes; and high polysaccharide content. Popular isolation procedures include aqueous/organic solvent extraction, or aqueous chaotropic extractions with silica-based solid phase devices for binding and purification. Aqueous phase isolation procedures are typically limited in the range of plant tissue types. Particularly troublesome samples are high in wax, oil or polysaccharides. Available methods require a DNAase treatment to reduce genomic DNA (gDNA) contamination. We report here the development of an easy, rapid, qualitative and quantitative method for the isolation of plant RNA from a variety of problematic species and tissue types that are virtually free of contaminating gDNA without the need for DNAase treatment. The method utilizes a proprietary aqueous extraction buffer in conjunction with two spin-columns; a prefilter that removes contaminating genomic DNA, cell

debris and other contaminants followed by an RNA filter that captures the purified RNA. RNA yields are equal to or higher than silica based aqueous extraction methods and absorbance ratios at 260/280 and 260/230 are above 1.8, indicating that the samples are free of protein and polysaccharide contamination, respectively. Electrophoretic analysis shows the RNA to be intact, with prominent rRNA bands. Quantitative PCR analysis of gDNA proves the isolated RNA has 100 to 1000 times less DNA contamination than typical organic solvent or silica based methods. Slight modifications of the basic protocol allow isolation from notoriously difficult samples, including waxy or oily tissues such as oil seeds or pine needles and high polysaccharide containing storage tissue such as seeds, tubers or fruit mesocarp without the use of organic extraction.

P213-T

A Complementary Approach of Maximizing the Throughput and Success Rate of Protein Identifications of Differentially Expressed *E. coli* Lysate

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In order to realize the full potential of Proteomics, large number of proteins must be rapidly and correctly identified. The speed and ease of use of PMF to identify proteins separated by 2-d gel electrophoresis usually dictates its preferred choice as an analytical method. However this approach is usually not sufficient to accomplish complete identification of all proteins from the gel spots of interest. LC/MS/MS is often required as a complementary approach to increase the confidence of protein identification especially for gel spots that fail to yield good digestion profile or which contain mixtures of proteins. In this study, we demonstrate a practical complementary workflow that combine the two approaches in order to maximize both throughput and success rate of protein identifications.
Gel spots that represent differentially expressed proteins were picked from 2D gels of *E. coli* lysates that were cultured under two different conditions. Digests of the extracted gel spots were first analyzed by PMF on Voyager DE™ PRO Workstation. Preliminary Mascot® database search results show that 75 to 80% of the gel bands are identified by PMF analysis. The remaining faint or low molecular weight protein bands that failed identification by PMF were positively identified by LC/MS/MS on Q TRAP™ system. The results also demonstrate the advantage of using LC/MS/MS over PMF analysis in resolving minor components in a mixture from a gel spot. This study indicates PMF and LC/MS/MS are essential and complementary techniques when solving problems in proteomics.

P214-S

High Throughput Protein Identification with LC-MALDI Qq TOF Mass Spectrometry

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The role of mass spectrometry in proteomic study has been widely recognized. Analytical methods based on both electro-spray ionization (ESI) and matrix assisted laser desorption ionization (MALDI) have been developed to obtain protein identity. Combination of the two techniques has recently been proven to yield higher proteom coverage. However, the identification of entire functional proteins in an organism or tissue represents a huge workload. The current process involves numerous steps, including sample preparation, data generation and data analysis. In this work, we have developed a methodology to speed up and automate the process. The analytical approach is based on capillary LC integrated with high replicate rate laser oMALDI-Qq TOF mass spectrometry. Validation of the new analytical approach was demonstrated by a complex mixture of protein digests. The sample was first separated with capillary LC, connected with an LC-MALDI interface. This led to the LC fractions automatically deposited on a MALDI plate. The subsequent data acquisition and

protein database search were fully automated. In addition, for one sample injection, data from TOF MS and MSMS experiments were obtained, facilitating both peptide mass fingerprinting (PMF) and sequence based confirmation. The sequence coverage obtained ranged from 15% to 50% among protein identified. The final report presented the protein identity, the confidence of identification and sequence information as well as the location of peptides relevant to the protein identified. Furthermore, the factors having significant effects on the detection sensitivity and number of peptides observed were investigated systematically. We will show the workflow and the results in this presentation.

P215-M

Reducing Sample Complexity by Isoelectric Prefractionation: Comparison of Fractionation Devices

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Sample complexity frequently interferes with the analysis of low abundance proteins by two-dimensional gel electrophoresis (2DGE). Ideally, high abundance proteins can be removed allowing low abundance proteins to be applied at much higher concentration than in the unfractionated sample. One approach is to partition the sample in a manner that segregates the bulk of extraneous proteins from the protein(s) of interest. Preparative liquid phase IEF produces fractions of discrete pH intervals allowing isolated narrow segments of a proteome to be analyzed individually, thus providing a unique opportunity to visualize low abundance proteins by 2DGE. It is particularly useful for the isolation of low abundance proteins of extremely basic or acidic pI. Two such isoelectric fractionation devices, namely the Invitrogen ZOOM IEF Fractionator and the Proteome Systems multi-compartmental electrolyzer (MCE) were compared for the fractionation of Sacharomyces cerevisae cytosolic proteins prior to 2DGE.

P216-T

Quality Control in the Affymetrix Microarray Core Laboratory

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Key factors in producing successful expression profiling results with commercial high-density oligonucleotide arrays are 1) starting with high quality sample RNA, and 2) minimizing assay variation in the microarray laboratory. In our core laboratory we have adopted quality control steps at each stage of the microarray assay and established procedures that limit variation and noise in the assay in order to optimize the quality of the microarray data produced. The quality control steps are used as a determining factor for the continuation of each phase of an individual microarray assay and can result in saved costs as well as time. The amount and integrity of input RNA is checked by OD measurement and by size fractionation using LabChip (Caliper) technology and the Agilent 2100 Bioanalyzer. OD measurement and analysis on the Bioanalyzer are also used to check the yield and quality of amplified, biotinylated cRNA prior to target fragmentation and hybridization. After hybridization with the GeneChip array (Affymetrix) and image inspection, the processed array data is checked for performance at or above established thresholds for control probe sets and overall hybridization metrics. Finally, expression data is checked for data distribution patterns that are inconsistent with suitable array performance and the project design. By following this program of quality control and quality assessment in our laboratory we are able to identify most poor samples prior to array hybridization and flag suboptimal array data. The scientist working with the array data is thus provided with the best data possible based on their samples and project design.

P217-S

Protein Sizing and Relative Quantitation Determination using a Microfluidic LabChip Device

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SDS-Page has been the predominant protein sizing method for the past 30 years. This technique involves multiple manual operations including separation, staining, destaining and detection and typically requires several hours. We have developed a high-throughput protein sizing assay which integrates each of these operations into a single microfluidic LabChip® device. An assay is run by sipping unlabeled protein samples into the device using vacuum. The samples are then electrokinetically loaded and injected into the separation column which contains a low viscosity polymer sieving matrix. Both protein-SDS complexes and free SDS micelles are fluorescently stained during the separation process. Prior to detection, the sample is diluted to reduce the SDS concentration below its critical micelle concentration. This destaining step effectively reduces the background fluorescence from micelle-dye complexes so that protein-SDS-dye complexes can be detected. Using this technique we are able to size proteins between 14 and 200 kDa. The microfluidic device delivers a throughput of 75 seconds/sample with unattended sample sipping from a 96-well plate. In this poster, we will show the experimental data describing the fundamental work to understand the assay performance. We will also describe the assay reproducibility in terms of sizing and mass quantitation.

P218-M

Eppendorf PerfectPure C-18 Tip: A Reproducible Method to Reversibly Bind, Purify and Concentrate Peptides for Increased MS Spectra Quality

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The Eppendorf PerfectPure C-18 Tip increases MS spectra quality by purifying and concentrating minute quantities of protein prior to analysis. The PerfectPure C-18 Tip is optimized for peptides or proteins less than 15,000 daltons. Eppendorf's innovative technology (patent pending) for fixing the binding matrix in the outlet of the tip, produces a more homogenous level of backpressure between tips, and this feature is important for reproducibility between samples and for higher throughput such as 8-channel pipets or automation. Several of the methods used to ensure that the customer is receiving a product of the highest quality are depicted below. These experiments also highlight the primary features of this tip: reproducibility, sensitivity, and peak recovery. To demonstrate the reproducible performance and handling of the tip, the amount of backpressure present is quantified by measuring the air flow through the tip. To illustrate the sensitivity, or the ability of the tip to handle minute quantities of protein, the tip is used to recover femtomole quantities of protein. And finally, to show peak recovery, the tip is used to recover a significant portion of the peptides from the digestion of _-casein, bovine serum albumin, and fetuin. These experiments detail the key characteristics that make the PerfectPure C-18 Tip a useful tool for MS sample preparation.