1.1
Has the Time Finally Come to Replace Gel Electrophoresis of DNA with Mass Spectrometry?

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Mass Spectrometry (MS) has all but revolutionized the analysis of proteins. New advances now bring the same power to the analysis of DNA and RNA. The advantages and limitations of automated MALDI-TOF MS in genotyping, DNA sequencing, and gene expression analysis, and direct molecular haplotyping will be illustrated; and some potential for significant future improvements in MS analysis of DNA will be described. It is the universality of PCR amplification as a sample preparation tool that makes MS such a powerful detector for nucleic acids. Alas, no comparable amplification tool exists for proteins. Hence comparable high throughput analyses for proteins seem problematic.

Since the task of doing full genome-wide proteomics is really daunting, at Sequenom we have taken the alternative approach of trying to filter out, at the genome level, most gene products that are not highly relevant to particular disease areas. We do this by traditional genetic methods but with a degree of automation and precision that has here-to-fore been impossible. Sequenom has developed a full genome set of SNPs for whole genome association studies. This set consists of about 100,000 working SNP assays that are highly heterozygous in the Caucasian population and map to single locations in the human genome. The set is public domain, gene-centric and currently represents more than 60% of all known genes.

Using this SNP set, we have actually completed more than a dozen whole genome scans and in every case have found novel genetic targets for which an attractive disease hypothesis can be formulated. The areas studied today include skin cancer, breast cancer, lung cancer, prostate cancer, HDL levels, osteoarthritis, adult onset diabetes and hypertension.

After initial genetic association studies one typically has 50 candidates of which most will be false positives. Several different strategies are being used to sort true and false positives and these will be discussed. However, the surest strategy is replication in a totally independent population, and this has been achieved for quite a few genes in each of the association studies completed.

1.2
Nano-electrospray Mass Spectrometry of Dynamic Multi Protein Complexes

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We are developing nano-electrospray mass spectrometry approaches to probe interactions in dynamic macromolecular complexes. To illustrate the capabilities of this approach two very different examples have been selected.

The first is that of molecular chaperones from the small HSP family. These molecules undergo dynamic exchange in solution and such reactions are difficult to follow using conventional techniques. Using a real time experiment we have monitored the exchange of subunits between decameric assemblies of these molecular chaperones, revealing the molecular detail of the intermediates as well as the reaction rates. Other members of this sHSP family, for example the alpha crystallins, are often difficult to analyze due to their inherent heterogeneity arising from their polydispersed assemblies. Using a modified mass spectrometer we show that the distribution of oligomers within these molecular chaperones can be effectively characterized using mass spectrometry.

The second example is that of ribosomes from both E. coli and thermus thermophilus. These macromolecules are involved in translation and composed of three very large RNA molecules and over 50 different proteins. Using nano-electrospray mass spectrometry approaches we have measured the mass of the intact 70S, 50S and 30S particles and related their gas phase dissociation patterns to structural features of the ribosomes. The results of these experiments have revealed information about subcomplexes, the presence of tRNA as well as changes in the protein RNA architecture that occur in response to binding different factors.

Used in this way mass spectrometry is capable of bridging the gap between mining interactions via molecular biology and detailed structural analysis. It offers the capability of rapid analysis of subunit modifications as well as observation of the changes in quaternary structure that are typical of dynamic molecular machines.
Identification of post-translational modifications of proteins remains one of the most important and most frustrating tasks in the field of proteomics. While such modifications have profound effects on protein function, they often occur at such low levels that their detection and sequencing are an extreme challenge for mass spectrometry laboratories.

Foremost among these modifications is phosphorylation. Various strategies have been tried to identify the sites of phosphorylation. These include: chemical derivatization of the sites, immobolized antibody columns, IMAC purification, MS analysis with and without phosphatase treatment and generation of specific marker ions in ESI-MS. However, the recent results of an ABRF Proteomics Research Group (1) on identification of phosphorylation sites showed, only 1 lab from 100 successfully identified both sites in the test digest using currently available techniques. What is needed, is a method with the specificity to identify the presence of the modification combined with the MSMS sensitivity to precisely determine the site of the modification.

The use of precursor ion scans to monitor the generation of specific marker ions induced by collision induced dissociation of phosphopeptides is a very specific method for their detection. Triple quadrupole mass spectrometers have proven to be the most sensitive instruments for this type of analysis. Hybrid quadrupole-OA-TOF type instruments are capable of pseudo-precursor ion scanning but not at the sensitivity or speed of triple quadrupoles. Unfortunately, the reverse is observed when it comes to the sensitivity for MSMS analysis, with the hybrid instruments (and ion traps) being more sensitive than the triple quadrupoles. Recently, an instrument which combines the sensitivity and speed of precursor ion scanning of a triple quadrupole with the MSMS sensitivity of an ion trap, has been developed. The ABI-Sciex 4000 QTRAP LC/MS/MS is a high performance hybrid triple quadrupole/linear ion trap mass spectrometer. It is capable of performing true precursor ion scanning and neutral loss scanning with the sensitivity of a true triple quadrupole with the MSMS sensitivity and resolution of an ion trap. The software is designed to allow the use of precursor ion or neutral loss scans as discovery scans to identify ions to be selected for MSMS. Thus, for example, a precursor ion scan in negative ion mode for m/z 79 can be used to identify ions for MSMS analysis.

We measured high resolution data of mixtures of peptides from different sources with routine accuracy better than 1 ppm (resolution about 75000) in general using an acquisition time of less than 1 second in the FTMS part of the machine and then the ions were sent to the FTMS for accurate mass measurement. Most recently a new hybrid mass spectrometer based on a linear ion trap combined with an FT MS (6 Tesla magnet) has been introduced and the first instrument has been installed in our laboratory (Finnigan LTQ FTMS, Thermo electron). The advantage of this instrument compared to other FTMS is the user friendly handling, the relatively small footprint and, more importantly, the number of advantages which can be achieved with this instrument. MS/MS experiments were performed in the ion trap part of the machine and then the ions were sent to the FTMS for accurate measurement.

Recently, the development of high resolution mass analyzers in combination with electrospray and MALDI ionization opened up a new dimension of reliability and accuracy of peptide and protein identification. The hybrid instruments based on quadrupole-time of flight analyzers on the one hand and FTMS instruments on the other became available and were used quite successfully, primarily for protein analysis work.

In addition cis-platinum modified oligonucleotides were analyzed up to MS^4 level. Isobaric fragmentation was easily distinguished and complete fragmentation patterns have been established, which were previously unknown. Furthermore, studies of lipopolysaccharides of *Bdellovibrio* bacteria, small bacteria of great importance due to their ability to feed on *E. coli* and more gram-negative bacteria, are under investigation and high resolution data are required to establish the detailed structures of the complex lipids. In this presentation the capabilities of the new instrument for solving problems in structure elucidation and identification of proteins will be discussed.