The Agilent HPLC-Chip/6210 TOF LC/MS Enables Highly Accurate Profiling of Peptide Maps for Differential Expression Studies

The Situation
Currently, no single LC/MS workflow has been adopted as the gold standard for biomarker discovery and validation. Shotgun proteomic techniques utilizing typical data-dependent MS-MS acquisition strategies provide meaningful identification on only a subset of the actual proteins present in a given sample.

The Solution
The Agilent HPLC-Chip/6210 TOF LC/MS is the perfect solution. Now Dr. Pierre Thibault and his team use a profiling strategy to confidently reveal putative biomarkers often missed with the shotgun approach, and subsequently target these putative biomarkers for identification.

Protein biomarker discovery has gained a lot of interest in drug development and early detection for the prevention and treatment of disease.

Liquid chromatography/mass spectrometry (LC/MS) based workflows, with their analytical power and potential throughput, play an important role in the discovery and validation of protein-based biomarkers. Current LC/MS workflows for biomarker discovery range from the classical shotgun proteomics approach to protein profiling strategies. However, no single LC/MS workflow has been adopted by the scientific community as the gold standard for biomarker research.

Shotgun proteomic approaches utilize data-dependent MS/MS acquisition, which identifies only a subset of the actual proteins present in the entire sample; these proteins usually represent the higher abundance proteins that are not necessarily of biological significance in disease. As a result, extensive fractionation of complex samples may be necessary to identify meaningful protein biomarkers associated with disease, thus dramatically increasing the number of analyses required per sample.
Dr. Pierre Thibault, a principal investigator at the Institute for Research in Immunology and Cancer (IRIC) and the director of the Proteomics Core Facility, stresses the difficulty of biomarker discovery. "When using a non-targeted shotgun proteomics approach, an overwhelming amount of MS/MS data can be acquired; out of that data, only a small subset will represent proteins of interest. This significant challenge [is like searching for] the proverbial differentially-expressed needle in a widely diverse proteomic haystack. Indeed, proteins of interest might only represent 5% of the overall population, and appropriate strategies are required to successfully identify these candidates."

Dr. Thibault’s research program consists of developing a reproducible LC/MS proteomics platform for applications in cancer and immunology, and is heavily focused on characterizing low-level amounts of proteins and potential biomarkers in complex cell extracts.

Using the Agilent HPLC-Chip/6210 TOF LC/MS enables reproducible low-level analysis of multiple samples with particular focus on protein profiling for biomarker discovery (Figure 1). The Agilent HPLC-Chip system provides high resolution to increase the number of peptides found and proteins identified. The Agilent 6210 TOF LC/MS is an electrospray ionization (ESI) TOF mass spectrometer with both high sensitivity and highly accurate mass capabilities necessary for this profiling approach to reveal potential biomarkers. The powerful combination of the HPLC-Chip/MS system with the 6210 TOF allows dependable reproducibility of sample analysis.

Dr. Thibault favors the Agilent HPLC-Chip/6210 TOF LC/MS for his research. “The novel microfluidics approach with the HPLC-Chip/TOF system eliminates all those uncertain issues with traditional nano-separation and nano-electrospray. Dead volumes, dispersion issues, and sample losses are minimized because the pre-columns and columns are integrated into a chip format, allowing us to mine complex proteomics samples reliably and efficiently. This approach has also proven to be very reproducible and we have a more consistent platform day-in, day-out. We have lost the fear of wondering if we will get the same performance when we run our samples; there is now a level of reliability in our comparative sample studies.”

Figure 1. The Agilent Biomarker Discovery Workflow
Protein profiling has the advantage that many sample sets (e.g., control versus disease) can be screened in MS mode first to identify putative biomarkers that show statistically meaningful changes in expression across sample sets.
One key study in Dr. Thibault’s laboratory is the identification of protein expression changes in monocyte cells upon chemical stimulation with tumor promoting agents. “We used the TOF profiling strategy to first reveal those potential proteins that change in abundance and could be involved in early signaling events between control cells and PMA (phorbol 12-myristate 13-acetate)-treated monocytes. As in most cases, the majority of proteins do not change but we were able to find those that were both up- and down-regulated (Figure 2). For this profiling approach, it was essential that we have highly accurate mass capability, within 5 ppm. The Agilent 6210 TOF LC/MS delivers this capability routinely without the need to invest in more expensive systems.”

“Once we have a list of peptides that are differentially expressed, we can target these for identification by MS/MS on our ion trap mass spectrometer (Figure 3). This profile-directed approach means we are not wasting any instrument time on identifying peptides that are not likely to be of significance in our study, and we can concentrate on those we’re targeting with high sensitivity. The HPLC-Chip system is totally transferable between our ESI-TOF MS and our ion trap MS, so we can use essentially the same methods and HPLC separations for both our profiling and identification strategies.”

Biomarker discovery strategies essentially utilize the same strategy as described by Dr. Thibault, where control samples are compared with disease or drug-treated samples. With regard to Dr. Thibault’s own biomarker studies, he says “The studies with these cultured cells exhibit a lower level of biological variation. With our other samples from sources such as tissues or blood, there is much more biological variation which means that the number of replicates must increase in order to statistically determine which proteins are truly differentially expressed. When analyzing a large number of samples, reproducibility is the key, from the HPLC separation to mass spectrometry analysis.”
“By using the HPLC-Chip system with ESI-TOF mass spectrometry, we have a system that routinely and reproducibly analyzes low-level proteins in complex mixtures. This enables us to profile many samples for differential expression analysis and then target differentially-expressed peptides for further identification. This profile-directed approach allows us to identify those low-level proteins in the 5% subset of the population often missed with regular shotgun proteomics techniques.”

Dr. Pierre Thibault, Principal Investigator

In reviewing the overall benefits of the Agilent HPLC-Chip system with ESI-TOF mass spectrometry, Dr. Thibault emphasizes, “By using this novel chip-based LC/MS we can routinely run more samples for our comparative studies with confidence in reproducibility. This system enables us to concentrate our efforts on our experimental design, sample handling, and results—rather than on the LC/MS system—to further our research.”

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