Mass identification

Advancing protein and biomarker discovery with MALDI-TOF/TOF MS technology

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The specificity of tandem mass spectrometry (MS/MS) has made it the dominant technique for protein identification and characterization for a wide variety of applications. Historically, electrospray ionization (ESI) MS is employed in these analyses, as MALDI instruments have lacked the capability of real tandem MS. However, the strength of MALDI instruments resides in their ease-of-use and analysis speed. And MALDI instruments are widely used for first-pass peptide mass fingerprinting (PMF), an exercise whereby the sample is cleaved with a protease and the resulting experimentally determined peptide masses are compared with masses of theoretical peptides from a protein database. When the PMF does not result in confident protein identification, MS/MS is used to obtain sequence information from one or more of the peptides. Additionally, the lack of MALDI real tandem mass capability results in sample retention for subsequent LC-MS/MS analysis using an electrospray source.

Another attribute MALDI offers is that the sample is static in nature. Once placed onto the MALDI plate—either from a gel digest or by a spotting device attached to a chromatographic column—the sample can be queried in depth and over an extended period to derive information not available to many sophisticated software tools on electrospray platforms (see box, "Required attributes of a MALDI MS/MS instrument").

PHOTO: APPLIED BIOSYSTEMS

Applied Biosystems 4700 Proteomics Analyzer with TOF/TOF optics.

LC-MALDI integration

When most researchers hear the phrase "LC-MS", they immediately think of ESI, because real tandem MS was not available on a MALDI platform until the commercialization of MALDI-TOF/TOF (time-of-flight) instruments. To augment PMF experiments with MS/MS capability, and alleviate the need to perform LC-MS/MS on an electrospray instrument, Applied Biosystems (*www.appliedbiosystems.com*) developed the 4700 Proteomics Discovery System with MALDI-TOF/TOF ion optic technology.

Coupling LC and MALDI produces several key advantages over electrospray LC-MS/MS. One advantage is mass analysis time that is independent from the chromatography. In electrospray, the sample is dynamic, and the mass spectrometer determines the peak elution time of the ion signal for MS/MS analysis. However, coeluting peaks create a problem for MS/MS analysis based on the elution time limitation, which often happens in complex samples. MALDI, however, does not suffer from this problem. For example, an LC eluent 5-s time fraction can be queried more than 100 times in MS/MS mode because the sample does not degrade.

Electrospray MS/MS instruments operate by cycling between MS survey scans and MS/MS scans throughout the gradient. Since survey-scan masses in ESI trigger the MS/MS events, a survey scan is unlikely to be reproducible in two replicate experiments. This is a result again of the dynamic nature of the complex sample as it is collected through chromatography. In replicate experiments for any given retention time, different precursors—ions generated from the sample detected in MS1 and fragmented for MS2—are often selected, which leads to different proteins being identified. Unfortunately, this means that the presence or absence of an identified protein is potentially an anomaly of the technique and cannot necessarily be credited to the biological experiment.

In LC-MALDI, the entire gradient is read in MS mode before MS/MS is performed. This not only enables precursor selection at the peak elution maximum for optimal sensitivity, but also allows full exploration of the LC gradient in MS mode. And there is no chance of differential timing between survey scans. The inherent benefit of better precursor selection reproducibility from LC-MALDI using TOF/TOF results, in turn, is better reproducibility at the protein identification level.

Recently, Benjamin Cargile and colleagues at the Research Triangle Institute (*www.rti.org*) demonstrated the advantages of

Required attributes of a MALDI MS/MS instrument

- capability of acquiring a large number of MS/MS spectra from a given sample location
- sophisticated software with advanced features for result-dependent analysis (RDA)
- fast data acquisition to address the large number of samples associated with applications such as LC-MALDI
- support for other proteomic workflows, such as post-translational modification site mapping including phosphorylation, expression-dependent analysis, carbohydrate characterization, and targeted biomarker discovery.

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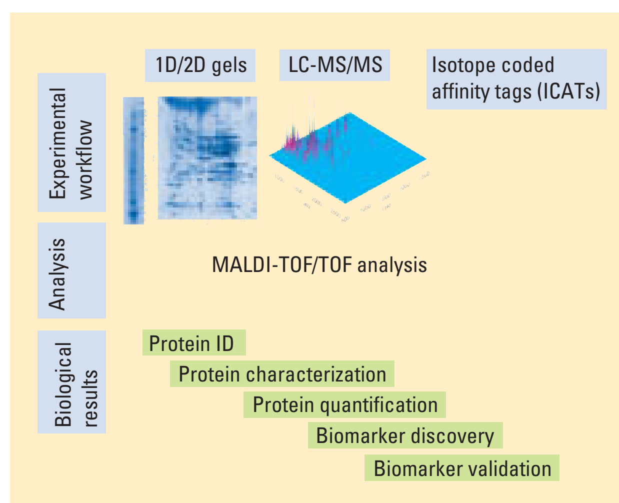
MALDI-TOF/TOF technology for LC-MS/MS to monitor relative dynamic protein turnover (*1*). Here, the sample's static nature enables the analysis of low-level signals, providing the ability to archive the sample for future reanalysis and allowing the use of traditional ion-pair reagents. These findings were possible because the LC was not directly coupled to an electrospray interface.

Biomarker discoveries

Biomarker research is a premier topic in MS, with applications in the understanding of disease pathways, the discovery and development of new drugs, and the development of diagnostic tools. Biomarkers are often defined as any peptide, protein, or metabolite that is reproducibly differentially expressed between two or more samples. These differences are usually found at the MS level and then confirmed at the MS/MS level. Tandem time-of-flight MS is an appropriate analytical tool for these experiments, as expression profiles are measured and proteins give rise to identifiable differences all in one platform.

Researchers at Duke University (*www.duke.edu*) applied this approach to search for overexpressed proteins in lung tumors, which resulted in the identification of two differentially expressed proteins, macrophage migration inhibitory factor (MIF) and cyclophilin A (CyP-A) (*2*). Identifying the MIF protein was critical to validation of the experimental method, as previous accounts reported the protein to be overexpressed in adenocarcinoma of the lung. Conversely, CyP-A overexpression in lung cancer had not been reported, and observing it demonstrated the technology's power to elucidate novel molecular targets for cancer diagnostics and therapeutics. However, additional work is required to fully investigate the potential of these two proteins as diagnostic or therapeutic targets in cancer.



Taking advantage of the static nature of the MALDI sample. A tandem TOF mass spectrometer with RDA software, as an alternative approach to achieving biological information, best enables most common workflows used in proteomics experiments, including 1D and 2D gels, LC-MS/MS, and ICAT reagent technology. The flexible platform allows researchers to design experiments to target a range of biological results from protein ID to biomarker discovery and validation. (Diagram courtesy of Applied Biosystems.)

Many samples used in biomarker discovery experiments are very complex. Therefore, a preferred technical solution that reduces sample complexity, quickly screens all signals for quantitative differences, and automatically targets only those signals that exhibit a difference for MS/MS analysis is critical. Expression-dependent or result-dependent analysis (RDA) software, along with isotope-coded affinity tagging (ICAT) reagents on the MALDI-TOF/TOF, provides a preferred route of analysis, where the biotin tag achieves sample complexity reduction. The TOF/TOF analyzer quickly reads the ICAT reagent pairs in MS mode with high quantitative precision. The expression-dependent software then selects only those signals that indicate an over- or underexpressed protein for subsequent identification. This approach yields a list of identified proteins that can be linked to the biological information in the Celera Discovery System, an online sequence database, to mine for true biomarkers.

Other applications

While the primary use of MALDI-TOF/TOF MS technology is protein identification and quantification, many researchers have interests in areas such as carbohydrate, lipid, oligosaccharide, and small-molecule characterization. In these areas, the high-energy fragmentation of the TOF/TOF analyzer has proved useful. The Applied Biosystems 4700 analyzer uses a deceleration ion optic technology prior to the collision cell that permits tuning of the kinetic energy of the parent ions entering the fragmentation region. Additionally, the current version of the TOF/TOF analyzer software enables selections of up to four different collision gases for multiple gas pressures, allowing various paths to control the fragmentation energy.

Researchers are finding high-energy fragmentation to be critical in effecting cross-ring fragmentation in carbohydrate structure elucidation (*3*). Likewise, researchers involved in metabolite identification have found the tunable high-energy feature to facilitate more specific assays.

MALDI-TOF/TOF gives researchers the capacity to identify more proteins with greater efficiency by exploiting the inherent advantages of the static nature of the MALDI sample. These advantages extend across workflow boundaries in proteomics, driving new capabilities for gel-based experiments, LC-MS/MS analyses, and biomarker discovery. Coupling this system with enabling software tools to query the sample, search databases, and decide what precursors to query next yields the highest information content available.

The applications of MALDI-TOF/TOF technology are vast. There are advances to be made in the efficiency of experiments by quickly finding out what signals can be accounted for, what signals are left unexplained, and what signals can be putatively accounted for by interesting post-translation modifications. Only MALDI coupled with an analyzer capable of real MS/MS can offer this enabling functionality.

References

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(3) Spina, E.; et al. *Rapid Commun. Mass Spectrom.* **2004,** *18,* 392–398.

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