

**RESEARCH PROFILES**

**Profiling the plasma proteome with IMS/MS**

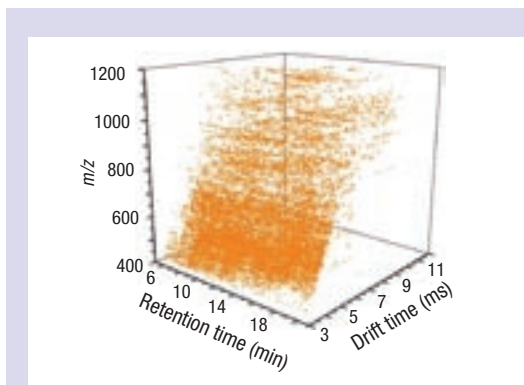
In the future, physicians may screen patients' blood samples for hundreds of diseases by measuring levels of specific proteins in plasma. Once a disease is diagnosed, doctors may use the patient's unique plasma profile to optimize the therapeutic response. But first, a method must be developed for the high-throughput detection and quantification of the plethora of proteins in human plasma. David Clemmer, Stephen Valentine, and colleagues at Indiana University, Predictive Physiology and Medicine, the Boston University School of Medicine, the Massachusetts Institute of Technology, and the Pacific Northwest National Laboratory have developed such a method. In this issue of *JPR* (pp 2977–2984), they describe the use of ion mobility spectrometry (IMS)/MS to probe the plasma proteome.

Many investigators have attempted to use conventional MS approaches to characterize the plasma proteome. However, their efforts have been stymied by the multitude of different proteins in plasma, as well as the fact that concentrations of proteins can vary by more than a billion-fold.

According to Clemmer, "The problem with conventional MS approaches for plasma profiling is that they either zero in on only a few proteins or they take a very long time to complete." In 1998, Clemmer and colleagues combined IMS/MS with TOFMS in a single instrument, increasing the resolution and decreasing the analysis time of complex protein mixtures (*Anal. Chem.* **1998**, *70*, 2236–2242). In their recent study, the researchers applied this technology to the analysis of the plasma proteome.

Tryptic peptides from pooled plasma samples were fractionated by a strong-cation exchange (SCX) column and then separated by reversed-phase LC. For IMS/MS, fractionated peptides were ionized by ESI and pulsed into a drift tube containing helium gas.

Under the influence of an electric field, ions migrated through the drift tube. There, they were separated on the basis of cross-sectional area, with more compact conformations traversing faster than more extended ones. When peptide ions reached the end of the drift tube, they entered the TOF



**Plasma proteome map.** The 6000 most intense features from each of 10 SCX fractions are resolved in 3D by LC/IMS/MS.

mass spectrometer, where they were separated by  $m/z$ . By modulating the field strength as ions exited the drift tube, the researchers could either leave the peptide ions intact or fragment them before they entered the mass spectrometer. Importantly, spectra of fragmented ions could be correlated with those of precursor ions. This method, developed by Valentine and Clemmer, allows the collision-induced dissociation (CID) of peptide ions to be analyzed in a single experiment, as opposed to the sequential selection of precursor ions by conventional tandem MS. Clemmer says, "What we have is a high-dimensionality analysis of a very complicated mixture. This allows us to isolate and detect small signals in the presence of large signals."

In addition to increased resolution, LC/IMS/MS operates at lightning speed compared with conventional LC/MS/MS approaches because of its ability to combine IMS, CID, and TOFMS in a single machine. For example, a single plasma profiling experiment that uses

2D LC coupled with IMS/MS can be completed in ~3.3 h, which is ~10× less time than is required for conventional LC/MS/MS. Because IMS separation time is on the millisecond scale and TOFMS has a microsecond separation time, hundreds of mass spectra can be collected from one IMS spectrum.

Rapid analysis is important because plasma samples from hundreds of healthy individuals must be analyzed to establish the "normal" plasma profile, and the procedure must be repeated for each disease state.

Clemmer and colleagues showed that LC/IMS/MS decreased spectral congestion, allowing the identification of low-abundance proteins, such as interleukin-21, that would be nearly impossible to isolate in the absence of IMS. In all, 438 unique proteins from plasma were identified with high confidence. The researchers compared these proteins with a high-confidence

plasma profile compiled from MS/MS data of 18 different laboratories by the HUPO Plasma Proteome Project (PPP). Of the 889 proteins assigned by PPP, 127 overlapped with those identified by Clemmer and colleagues.

Although the number of plasma proteins identified by LC/IMS/MS in a relatively short period of time is impressive, it's likely just the tip of the plasma-proteome iceberg. LC/IMS/MS is a vast improvement over earlier methodologies, but the technique must be optimized before the goal of clinical plasma profiling is realized. According to Valentine, "One of the biggest improvements I've looked at is in the resolution of IMS/MS. When you improve the resolution, you also improve the quality of the CID spectra for protein database searches, and that helps you make more assignments." Expanded informatics capabilities, enhanced reproducibility, and further reduction of analysis time are additional improvements to IMS/MS being investigated by the researchers.

—Laura Tomky Cassidy