

Multidimensional IMS

Ion mobility spectrometry (IMS) is known for its millisecond analysis times but not for its resolving power. To improve the resolution, David Clemmer, Stormy Koeniger, and colleagues at Indiana University and Pacific Northwest National Laboratory (PNNL) have introduced another dimension of IMS. Multidimensional IMS, which is described in the June 15 issue of *Analytical Chemistry* (pp 4161–4174), could be particularly useful for applications such as proteomics, in which low levels of peptides are analyzed in plasma and other complex bodily fluids.

IMS/IMS makes it possible to see small signals in complex mixtures by dispersing the noise of the system, says Clemmer. “In one dimension of IMS, you have a limited ability to resolve ions based on their shape-to-charge ratios,” he says.

IMS/IMS can be thought of as an analog of MS/MS. Ions are isolated and fragmented after the first IMS region. The fragment ions that are produced are then dispersed into a second IMS region, before additional activation and MS analysis. IMS/IMS “allows you to do the same thing you can do with MS/MS, only from the side of structure. That is, you can select an ion based on its mobility [shape-to-charge ratio], just like you would select an ion based on its mass-to-charge,” explains Clemmer.

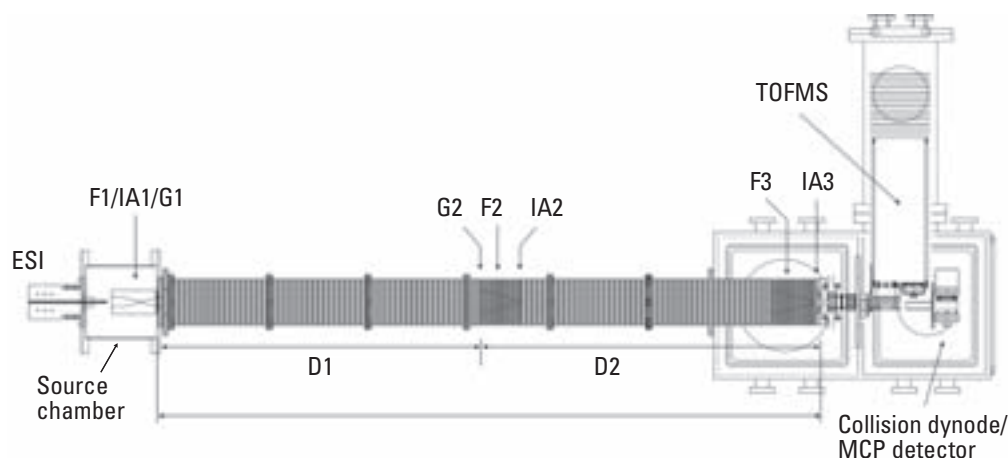
From a fundamental point of view, the researchers are interested in using multidimensional IMS to learn more about the structure of an ion without solvent, especially a macromolecular ion, such as a peptide or a protein. They hope to determine how protein structure is established and how one type of structure leads to another. In IMS/IMS, an ion is selected on the basis of its mobility and then activated. “We can activate enough to break it apart, or we can activate just enough to change its

shape,” says Clemmer. Shape change “is something we can’t see with a normal mass spectrometer, because [the ion] has the same mass,” he adds.

On a more practical side, Clemmer and colleagues believe that IMS/IMS will improve their ability to analyze complex mixtures. In particular, they

things that aren’t separated, you can activate them and change their shapes. . . . Then you can select one of those by its shape, and you’ve never changed the mass-to-charge,” says Clemmer.

The researchers used multidimensional IMS to examine bradykinin and ubiquitin systems. Fragment ions of



Schematic of an IMS/TOF instrument. The drift regions (D1 and D2), ion funnels (F1–F3), ion gates (G1 and G2), ion activation regions (IA1–IA3), and microchannel plate (MCP) are labeled.

are interested in analyzing tryptic peptides from a digestion of a whole fruit fly head to determine how the proteome changes with age. The group is also trying to find peptides in plasma.

IMS/IMS was facilitated by Richard Smith’s work at PNNL on ion funnels. “He learned how to essentially beat diffusion,” says Clemmer. Ion funnels allow the ion cloud to diffuse into a large spherical gas. Then, rf and dc fields can be used to collapse the cloud back into a point. “What that effectively does is allow us to go to drift tubes of any length. We now build drift tubes that are well in excess of 10 ft,” says Clemmer. The advantages of a longer drift tube are better resolution and the ability to do more dimensions of IMS.

When two dimensions won’t do, why not go to three? The researchers have already developed and tested an IMS/IMS/IMS system. “You can start with one shape, and if you have multiple

bradykinin were resolved on the basis of their mobilities. The b-type ions were resolved into two conformations, which allowed them to be distinguished from other fragments.

In a recent paper, Clemmer and colleagues reported the use of IMS/IMS for separating fragment ions of insulin chain B and ubiquitin that were produced by collision-induced dissociation (*Anal. Chem.* **2006**, *78*, 2802–2809). The resulting spectra were similar to those obtained in previous work, in which the researchers used IMS/MS methods to analyze fragments generated by MS/MS in an ion trap. The multidimensional IMS system, which had no mass spectrometer prior to the IMS instrument, had higher resolution and sensitivity than the IMS/MS system. IMS/IMS allowed the researchers to observe several features that were not readily discernible by MS analysis. ▀

—Britt Erickson