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Mass spec goes 2D

No longer side-lined as a computationally-hungry scientific curiosity, two-dimensional mass spectrometry is becoming a fully-fledged analytical technique says Maria van Agthoven

One of the cornerstones of biomedical research is proteomics, which studies protein expression in cells when they are under various conditions of stress, or how proteins respond to these conditions – for example, how proteins interact with pharmaceutical compounds.

Proteomes are very complex, and reverse phase liquid chromatography is often coupled with tandem mass spectrometry (LC-MS/MS) in order to maximise the amount of chemical information gathered from the analysis. In tandem mass spectrometry (MS/MS), ionized molecules (such as proteins or peptides) are fragmented in order to gain structural information (such as the peptide sequence and post-translational modifications). In order to properly correlate fragment ions with their precursors, a narrow range of mass-to-charge ratios is isolated before the fragmentation step.

Analytical scientists therefore have to make choices between co-eluting species in LC-MS/MS, which makes the analysis blind to low abundance compounds. Techniques like Precursor Acquisition Independent From Ion Count (PACIFIC), developed by Professor David Goodlett at the University of Maryland, and SWATH, developed by SCIEX, attempt to bypass this problem by repeating LC runs while isolating ions over sliding mass-to-charge windows. But samples like whole cells or blood plasma have dynamic ranges that span over many orders of magnitude, and the development of sensitive analytical techniques which overcome the necessity of the isolation step in MS/MS is crucial for comprehensive biomedical research.

One mass spectrometer offers a more elegant solution to the problem of correlating precursor and fragment ions in tandem mass spectrometry: the Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS), which separates ions according to their mass-to-charge ratios by measuring their cyclotron frequencies in high magnetic fields. In the late 1980s, Tino Gäumann’s team in Switzerland took a leaf out of the NMR spectroscopy book and proposed a pulse sequence for an alternative method for tandem mass spectrometry they called two-dimensional mass spectrometry (2D MS).

The maps obtained from this experiment showed the fragmentation patterns of all ions in a sample, without requiring any isolation beforehand. Unfortunately, the computational capacity needed to process and store the data was too high for 2D MS to be useful, and for almost two decades it was side-lined as a scientific curiosity.

For the last seven years, teams from the University of Warwick in the United Kingdom (lead by Professor Peter O’Connor), the University of Lille (lead by Dr Christian Rolando), and the University of Strasbourg (lead by Dr Marc-André Deluc) in France have revisited the topic of 2D MS on FT-ICR mass spectrometers in order to turn it into a fully-fledged analytical technique. We have optimised the pulse sequence to minimise the impact of artefacts, built an open access software package to process and visualise 2D mass spectra (Spectrometry Processing Innovative Kernel, or SPIKE), and developed algorithms to deal the scintillation noise problem inherent to any spectroscopy method with signal fluctuations.

We have applied 2D MS to peptides, small molecules, proteins, and tryptic digests of proteins of increasing complexity. Currently, we can acquire 2D mass spectra with laser-based fragmentation techniques and electron-based fragmentation techniques. With an IR laser, peptide ions fragment by losing post-translational modifications like phosphorylations or glycosylations before fragmenting along the peptidic chain, whereas with an electron-gun, peptide ions preferentially fragment along the peptidic chain. Acquiring 2D mass spectra with different fragmentation methods can therefore give us complementary structural information on proteins and peptides.

The most complex sample we have published is the tryptic digest of collagen, a 400 kDa protein. We are currently in the process of developing algorithms to extract fragmentation patterns from 2D mass spectra in a way that allows us the interrogate proteomics databases like MASCOT or Prosight in order to automate data analysis of 2D mass spectra.

Figure 1 shows the 2D mass spectrum of bovine serum albumin (BSA, 67 kDa). The horizontal axis shows the mass-to-charge ratio of the fragment ions, and the vertical
The downside of 2D MS is that an FT-ICR mass spectrometer is a large, expensive instrument with a superconducting magnet. 2D mass spectra can take over an hour to acquire. 2D MS can therefore be coupled to a top-down and bottom-up approach. We hope that LC-2D MS will lead to more in-depth biochemical data to study the interactions of biomolecules with cancer drugs for more targeted treatments, among others.

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