What is mass spectroscopy?

The existence of an open reading frame in genomic sequence data does not necessarily guarantee the existence of a functional gene. Despite the recent advances in bioinformatics, it is still difficult to predict genes accurately from genomic data alone. A recent study concluded that the error rate was at least 8% in the annotations of 340 genes from the Mycoplasma genitalium genome. Therefore, verification of a gene product by proteomic methods is an important step in annotating the genome. The most significant breakthrough in proteomics has been the ability to utilize mass spectrometric identification of gel-separated proteins. Mass spectrometry relies on digestion of gel-separated proteins into peptides by a sequence-specific protease such as trypsin. The reason for analysing peptides rather than proteins is that gel-separated proteins are difficult to elute and to analyse by mass spectrometry, and that the molecular weight of proteins is not usually sufficient for database identification. In contrast, peptides are easily eluted from gels and even a small set of peptides from a protein provides sufficient information for identification. The name ‘mass spectrometry’ is a misnomer of sorts. The mass is not what is measured; instead, it is the mass-to-charge (m/z) ratio that is measured. A mass spectrum is a plot of ion abundance versus m/z.

How does mass spectroscopy work?

Basically, any information gathered from a mass spectrometer comes from the initial production of gas-phase ions. There are three main components of a mass spectrometer: an ionization source, a mass analyser and a detector. As ions are actually analysed in the vacuum of the mass spectrometer, arguably the most important reaction in mass spectroscopy is the one that converts the sample analytes into gas-phase ions. In the past two decades development of new ionization techniques now allows almost any large, non-volatile and thermally labile compound to be converted into a gas-phase ion and analysed by mass spectroscopy. Although a number of ionization techniques have been developed over the years for the analysis of non-volatile and thermally labile compounds, two have emerged as the primary methods of today: electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). The efforts related to the development of both of these two techniques lead to the awarding of the 2002 Nobel Prize in Chemistry.

What is electrospray ionization?

Ions in electrospray ionization (ESI) are generated by passing solutions through a small capillary that has a potential difference relative between +500 and +4,500 V. The voltage required depends on both the inner diameter of the capillary and the solvents that make up the solution. Generally, capillaries with larger inner diameters and solvents with higher boiling points require higher voltages. Regardless of the dimensions of the ESI electrostatic spraying of a sample solution generates an aerosol composed of charged droplets. These charged droplets will consist of both solvent and analyte molecules with a net positive or negative charge, depending on the polarity of the applied voltage. Eventually, ions become free of the solvent surrounding them, and these ions make their way into the mass analyser of the spectrometer.

Protonation and deprotonation are the main sources of charging biologically relevant molecules in ESI. Ions of proteins, peptides, oligonucleotides, and other molecules with acid/base functionality are often found with several protonation or deprotonation sites.
The multiple charging might seem to make information about molecular weight more difficult to determine but in actuality each peak in this spectrum represents a separate mass measurement that ultimately increases the precision of the measurement. Multiple charging also enables mass spectrometers with limited m/z ranges to analyse higher-molecular-weight molecules as the more charges the lower the m/z ratio becomes.

ESI is a very gentle technique that allows non-covalent biomacromolecular complexes to remain intact while ionized expanding the utility of ESI to study of protein–protein complexes, double-stranded DNA, protein–drug complexes, and generally any multimolecular complexes. In the ultimate experiment to demonstrate the gentle nature of ESI, it has been shown that after ionization, mass analysis and collection, the tobacco mosaic virus retains not only its rod-like structure but also its viability. Perhaps the most practically useful attribute of ESI is its ability to couple MS and liquid-separation techniques. Now the separation power of techniques such as HPLC and capillary electrophoresis can be joined with the positive attributes of MS, so that compounds in complicated biological matrices can be thoroughly and rapidly investigated with high sensitivity.

Despite the power of ESI, it does have two notable shortcomings. First, due to its flowing nature the sample is constantly being consumed unfortunately no mass spectrometer constantly analyses ions therefore sample is wasted. The second shortcoming of ESI is its susceptibility to ion suppression effects. When solutions contain high salt concentrations, analyte ion formation is usually hindered, which means most biological samples need to be desalted before analysis. In addition, when complex mixtures of compounds are present, the higher-concentration analytes can suppress ion formation by lower-concentration analytes losing signal from the lower abundance species within the sample.

What is matrix-assisted laser desorption/ionization?

Unlike ESI where analyte ions are produced continuously, the ions created by matrix-assisted laser desorption/ionization (MALDI) are produced by pulsed-laser irradiation. The sample is incubated in a solid matrix that can absorb the wavelength of light emitted by the laser. Usually the sample and matrix are mixed on a probe and after irradiation the gas-phase ions created are directed toward the mass analyser. MALDI has several favourable attributes. Due to the pulsed nature of most lasers, ions are formed in discrete events which allows the mass analysis to be synchronized with ion formation, wasting little sample when properly synchronized. A practical advantage of MALDI is its relatively high tolerance to both salts and buffers allowing for samples to be analyzed with physiological levels of salts present.

Although MALDI has significant advantages, it also has some drawbacks. For example, only certain mass spectrometers can be easily coupled with MALDI. Also, the presence of a matrix, which facilitates ionization, causes a large degree of chemical noise to be observed at m/z ratios below 500 Da based on the mass of the matrix. As a result, samples with low molecular weights are usually difficult to analyse by MALDI.

What is a time-of-flight mass spectrometer?

Conceptually, the simplest mass analyser is the time-of-flight (TOF) mass spectrometer. A TOF spectrometer separates ions based on their velocity and can, essentially, be thought of as a race from the ionization point to the detector. The ions are, in theory, all formed at the same time and place in the ion source and then accelerated through a fixed potential into the TOF drift tube. As all the ions with same charge obtain the same kinetic energy after acceleration, the lower m/z ions achieve higher velocities than the higher m/z ions. After the ions are accelerated, they travel through a fixed distance before striking a detector. Thus, by measuring the time it takes to reach the detector after the ion is formed, the m/z of the ion can be determined. TOF now offers mass resolution in the thousands and mass accuracies in the tens of parts per million. The best performance is obtained on more sophisticated TOF instruments that include a reflectron. With a reflectron TOF, after travelling through one flight distance, the ions enter an electrostatic mirror (a reflectron) that turns the ions around and sends them down a second flight distance to the detector. The function of the reflectron is to compensate for small differences in the velocities of ions with the same m/z. Therefore; the reflectron increases the resolution of TOF spectrometry.

How are proteins identified using mass spectroscopy?

There are two main approaches to mass spectrometric protein identification. In the ‘peptide-mass mapping’ approach where the mass spectrum of the eluted peptide mixture is acquired, resulting in a ‘peptide-mass fingerprint’ of the protein being studied. Advances have been made in automation of the MALDI identification procedure whereby hundreds of protein spots can be excised, digested enzymatically, their mass spectra obtained and automatically searched against databases. In the two-step procedure for rapid and unambiguous protein identification, MALDI fingerprinting is the first step. The second method for pro-
tein identification relies on fragmentation of individual peptides in the mixture to gain sequence information. In this method, the peptides are ionized by electrospray ionization directly from the liquid phase. The peptide ions are sprayed into a tandem mass spectrometer that has the ability to resolve peptides in a mixture, isolate one species at a time and dissociate it into amino- or carboxy-terminal-containing fragments. The tandem mass spectrometric method is technichally more complex than MALDI fingerprinting. Its main advantage is that sequence information derived from several peptides is much more specific for the identification of a protein than just a list of peptide masses.

What is tandem mass spectroscopy?

Tandem mass spectrometry (MS/MS) involves two stages of MS. In the first stage of MS/MS, ions of a desired m/z are isolated from the rest of the ions created at the ion source. These isolated ions (termed parent ions or precursor ions) are then induced to undergo a chemical reaction that changes either their mass (m) or charge (z). Typically, the reactions involve some type of process to increase the internal energy of the ions, leading to dissociation. The ions resulting from the various chemical reactions are termed product ions, and these are analysed with the second stage of MS/MS. MS/MS is particularly useful when analysing complex mixtures. It can be thought of as analogous to a chromatography/mass spectrometry (LC/MS) experiment in which the first stage of MS separates an individual species from the mixture and the second stage provides the mass spectrum of that species.

What is unique to the MS/MS experiment compared with the LC/MS experiment is the ability to screen samples rapidly for certain compound types. One way to do this is for the product ion to be the independent variable (fix MS-II) and the parent ions the dependent variables (scan MS-I). This type of experiment is known as a parent-ion (or precursor-ion) scan. It is dependent on the analyte parent ions of interest all reacting to give a common product ion. An example of this is screening a mixture of peptides to determine those that are glycosylated. When a glycosylated peptide is dissociated, one of the product ions is m/z 204, which is a glycosyl fragment. By setting MS-II to only detect ions of m/z 204, MS-I can be scanned to sequentially pass all the ions emitted from the ion source, but only those ions that dissociate to m/z 204 will be detected.

A crucial aspect of the MS/MS experiment is the reaction that occurs between the two MS stages. By far the most frequent reaction is unimolecular dissociation, which is generally enhanced by some form of ion activation. The ion activation is necessary to increase the internal energy of the parent ion so that it will dissociate before analysis. The dissociation method almost universally used is collision-induced dissociation (CID). In CID, the parent ion collides with a neutral target (collision) gas and some of the kinetic energy of the parent ion can be converted to internal energy.

Why is mass spectroscopy gaining popularity?

Mass spectrometry in the field of proteomics can be a difficult but rewarding experimental approach. It seems these days that most labs are utilizing a mass spectrometer in one form or another, from identifying individual protein sequences to just verifying the presence of molecules in a solution. But as with any other new technique it takes optimization to become useful. Most difficulties using mass spectrometry arise in the area of actually being able to generate usable data. As the inherit sensitivity of mass spectrometry allows miniscule amounts of many samples to be detected the process by which the sample of interest is defined can take a lot of tweaking. Ultimately though most of the problems involve the adoption of sterile techniques which can take very little time or forever depending on the laboratory environment and the nature of the individual researcher. The data gained from MS can take many forms including protein identity, protein interaction, and protein abundance changes under different experimental conditions creating a large range of publication opportunities. While MS based proteomics is a fast growing field the two best resources in the local area are the Genome BC Proteomics centre at the University of Victoria and the development of a new Mass spectrometry centre at the BRC at the University of British Columbia run by Jurgen Kast.

References