Completion of the human genome project has created a blueprint of the genes and proteins necessary to construct and maintain a complex organism. Understanding how the molecular entities of human cells and tissues function will require sophisticated experiments to decipher how these molecules interact as a system. Beyond genes and proteins, metabolites encompass another important level of the system. To understand cellular systems, high-throughput strategies to investigate the functions of proteins and metabolites within the context of the system are necessary. Thus, understanding gene function (and ultimately biological systems) will require methods to broadly and quickly determine how the amounts and forms of various molecules are changing (Figure 1). Three considerations are relevant: (i) broad and unbiased measurement tools; (ii) comprehensive separation techniques; and (iii) informatics to analyze the data. This article will discuss mass spectrometry for the measurement of proteins and metabolites.

Unbiased Measurement of Molecules

Effective bioanalytical technologies should not be biased towards identifying any particular type of molecule or the amount present and must distinguish molecules based on physical characteristics. Very few technologies can meet these criteria, but one that comes close is mass spectrometry. Mass spectrometers employ ion separation technologies that analyze a wide variety of biological molecules and make a physical measurement of the mass-to-charge (m/z) ratio of their ions. The m/z ratio is indicative of molecular weight, a fundamental feature of a molecule, and provides important structural information. Since molecular weight reflects atomic composition, it is desirable that mass spectrometers be able to acquire a value unique for a molecular composition. This, in turn, places specific demands on mass accuracy and resolution. As mixtures of molecules become more complex, mass resolution and speed of data acquisition become important, but often conflicting, issues to resolve the individual molecules present.

Ionization

A key element to unbiased analysis of molecules is a mass spectrometer’s ability to volatilize and ionize them. Four principal methods of ionization are used to convert solution and solid-phase molecules to ions. Molecules readily volatilized into an ion source or introduced through a gas chromatograph can be ionized by either electron impact ionization (EI) or chemical ionization (CI). EI creates ions by impacting the molecule with a high-energy (70 eV) electron beam to dissociate an electron from the molecule. CI creates ions by first ionizing a reagent gas, like methane, that then undergoes ion-molecule reactions with itself to create a strong protonating reagent like CH5+.

The other two methods, matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI), are more suitable for molecules, such as proteins, carbohydrates, and DNA, with low vapor pressures (1,2). Within the constraints of volatility, these ionization methods are reasonably unbiased, and there are few molecules that are not ionized by at least one of these methods. The ionization method places constraints on the type of mass analyzer employed. For example, three of these methods (CI, ESI, and MALDI) are “soft” ionization techniques. In them, fragmentation of the ion does not occur during the ionization process, so to obtain information on the covalent structure of the ions, a tandem mass spectrometer is needed (described below).

Mass Spectrometers Are Sophisticated Measurement Tools

To obtain accurate measurements of m/z, a number of high-performance devices have been developed. Three parameters of m/z measurement are evolving. The first is the resolution of ion measurement. Resolution is calculated by dividing the m/z of an ion (M) by the width of the m/z peak (∆M). Resolving ions and isotopes are important as mixtures become more complex and as molecules increase in size. A second parameter is the accuracy of the mass measurement, as the closer the mass measurement is to the true molecular weight, the greater the diagnostic value. The last parameter of increasing importance for analyzing complex mixtures of proteomes and metabolomes is speed. Fast scanning mass spectrometers acquire more data during separation, permitting more complete analysis of complex mixtures.

Mass spectrometers consist of devices to separate ions by m/z value for transmission to an ion detector. Ions can be separated by time-of-flight or by using electric and/or magnetic fields (3). Time-of-flight is the simplest measurement method that determines the time required for an ion to move across a set distance. The time-of-flight of an ion is dependent on its m/z value. Quadrupole and ion trap instruments use a combination of direct current (DC) and oscillating electric fields (megahertz frequencies) to focus or constrain ions in the device. By setting the magnitude of the electric field to appropriate values, a quadrupole mass filter transmits ions one m/z value at a time through the quadrupole to the detector. Ion traps constrain ions in the device and perturbation of the field ejects ions in a m/z dependent manner to a detector. Ion cyclotron resonance (ICR) mass spectrometers use a static high magnetic field to trap ions within the ICR cell (4). The magnetic field causes the ions to rotate in the ICR cell at frequencies dictated by the m/z value of the ion. To measure the frequencies of the ions, the ion motions are brought together by an electric pulse, and the electric signal created by the ions passing between two detector plates is measured as the ion packet relaxes and the ions reassemble their natural
Proteins can differ in their expression levels, be created from SNPs, single nucleotide polymorphisms; NMR, nuclear magnetic resonance. Mass spectrometry is a prominent technique for all but mRNA measurements. Technologies exist to measure all these molecules, and different parts of cells. Metabolites are substrates for enzymes and reflect different exons of a gene, contain a variety of modifications, and localize to products.

Figure 1. Systems biology requires the measurement of sequence differences in genes and promoters, in mRNA expression levels, and the alternate splicing of these genes to produce different gene products. Proteins can differ in their expression levels, be created from different exons of a gene, contain a variety of modifications, and localize to different parts of cells. Metabolites are substrates for enzymes and reflect enzyme activity levels. Technologies exist to measure all these molecules, and mass spectrometry is a prominent technique for all but mRNA measurements. SNPs, single nucleotide polymorphisms; NMR, nuclear magnetic resonance.

Figure 2. The process of tandem mass spectrometry. (A) An ion is separated from all the other ions and passed into the collision cell (B) where the ions collide with argon to become activated for dissociation. (C) The fragmentation products are then measured in the second mass analyzer. Ion traps perform this process all with one device, while triple quadrupole or quadrupole time-of-flight (Q-TOF) mass spectrometers use separate devices to perform each step of the process.

Also be achieved, which will resolve the isotopes of an ion at m/z 5000. An alternate configuration replaces the TOF with a linear ion trap (Figure 3B) (6). A linear ion trap uses a quadrupole to trap ions rather than to separate them. An advantage to the linear ion trap is the trapping of all ions rather than filtering specific ions from the beam. Stored ions are then sequentially ejected to a detector. Tandem mass spectrometry is performed in the linear ion trap by clearing the trap of all ions but the one of interest. The motion of this ion is increased to induce energetic collisions for dissociation. Another advantage to this hybrid is the ability to select ions using the first mass analyzer, fragment them in the collision cell, and then detect resulting breakdown ions in the ion trap. Additional stages of tandem mass spectrometry can be performed on the dissociated ions to obtain more information about the structure of the ion. Furthermore, product ion scanning can be used to identify ions containing specific structural features such as phosphorylation or glycosylation (6). A linear ion trap is also available as a nonhybrid instrument, and the most important feature of this instrument is a fast scan speed and high sensitivity (Figure 3C) (7). Tandem mass spectra can be acquired using such a device at a rate of 5 spectra/s. A fast scan speed increases the sampling rate and increases the number of tandem mass spectra during the analysis of complex molecules.

Fourier transform mass spectrometry (FTMS) uses static high magnetic fields to measure m/z values. Because these instruments operate at ultra-high vacuum, interfacing ionization techniques to these instruments has been problematic, and performing tandem mass spectrometry experiments based on CAD has been difficult. To overcome these problems, two kinds of hybrid instruments based on FTMS have been developed (Figure 3D). The first employs a triple quadrupole for ion selection and dissociation followed by injection into the ICR cell. The hybrid takes
advantage of the strengths of each mass analyzer, the triple quadrupole is an excellent mixture analysis device, and FTMS can record accurate m/z values with high resolution. An alternate configuration uses a linear ion trap to store and manipulate ions prior to injection into the ICR cell. A unique aspect to this device is the ability to perform ion trap experiments simultaneously with the FTMS measurement, thus combining the high scan speed of the linear ion trap with the high-performance capability of FTMS.

Conclusion
In the coming age of systems biology, large-scale analysis of proteins and metabolites will be essential to understand the response of cellular systems. New mass spectrometry technology will facilitate highly accurate measurement of proteins and metabolites, as well as the identification of molecular structure. To accommodate large-scale analyses, mass spectrometers need to scan faster, measure differences with a larger linear dynamic range, and contain more intelligent and flexible software to control data acquisition and analysis. Analysis of tandem mass spectral data of peptides is facilitated through algorithms to search sequence databases to identify proteins based on the matches of the tandem mass spectra (8). Similar types of algorithms are necessary for small molecule analysis. As mass spectrometers improve to accommodate large-scale, high-throughput experiments, the data necessary to understand biological systems will be within reach.

References