

# THE BASICS OF MASS SPECTROMETRY IN THE TWENTY-FIRST CENTURY

Gary L. Glish\* and Richard W. Vachet<sup>‡</sup>

Enormous advances in our understanding of the chemistry underlying life processes have identified new targets for therapeutic agents. The discovery of effective therapeutics to address these targets is often accomplished through parallel synthetic and screening efforts. In almost all cases, what has enabled target identification and allowed parallel approaches to drug discovery to be effective are the development of either new analytical tools or the improvement of currently existing ones. Among these tools, mass spectrometry has evolved to become an irreplaceable technique in the analysis of biologically related molecules. This article will guide researchers in drug discovery through the basic principles of mass spectrometry.

By virtue of its sensitivity and speed, mass spectrometry (MS) has played a key role in many phases of drug discovery. The identification of proteins, which are often drug targets and are present at low concentration levels in complicated mixtures, is significantly facilitated by the sensitivity of MS. Also, high-throughput analysis of drug libraries produced in a parallel format can be simplified by the speed of MS. Reaction monitoring and optimization, assessment of library compound quality and structural analysis of library products are just some of the ways in which MS is employed. Associated with these applications is the added advantage that mass spectrometers can be automated, which further increases throughput. The utility of MS in the drug discovery process might, however, extend to other areas too. In combination with separation techniques, MS can play an important role in identifying and monitoring biomarkers in physiological fluids, which is a useful way of assessing drug efficacy and safety issues. In addition, MS might have some promise as a stand-alone assay for the rapid screening of drug–target binding, including both the strength and sites of interaction.

As a result of its utility in biochemical analyses, MS has become a standard tool of many laboratories. Of course, as MS becomes a routinely used technique it becomes more of a ‘black box’ technology. Also, the

increasing interest in making mass spectrometers available for open-access operation will create numerous operators who are unaware of the fundamentals of MS. The goal of this review is to provide a guide for understanding current MS technology. It is our hope that a more detailed understanding of the fundamentals of MS will allow users involved in drug discovery to take full advantage of this powerful technique and avoid misinterpretation of results. This review will provide very few examples of how MS is specifically applied to drug discovery efforts. Readers interested in such details are directed to other reviews that have been published in the past few years<sup>1–5</sup>.

The name ‘mass spectrometry’ is a misnomer of sorts. The mass is not what is measured; instead, mass spectrometry determines the mass-to-charge ( $m/z$ ) ratio or a property related to  $m/z$ . A mass spectrum is a plot of ion abundance versus  $m/z$ , although in many cases the  $x$ -axis is labelled ‘mass’ rather than  $m/z$ . The spectrum is presented in terms of Daltons (Da) per unit charge. So for example, benzene (molecular mass 78 g/mol), when ionized, can form an ion of the intact molecule at  $m/z$  78 (the number of places after the decimal that can be determined depends on the type of mass analyser and often just the integer mass is reported). However, the molecular weight is the molar

\*Department of Chemistry,  
University of North  
Carolina, Chapel Hill,  
North Carolina 3290, USA.  
<sup>‡</sup>Department of Chemistry,  
University of Massachusetts,  
710 North Pleasant Street,  
Amherst, Massachusetts  
9336, USA. Correspondence  
to G.L.G.  
e-mail: [glish@unc.edu](mailto:glish@unc.edu)  
doi: 10.1038/nrd1011

mass of a compound and what is actually measured in a mass spectrometer are individual ions, and the true units of the measurement are kilograms per Coulomb. As individual ions are measured, an important result that is often overlooked by those not familiar with MS is the effect of isotopes. For example, chlorobenzene has a molecular weight of 112.56; however, chlorobenzene does not form ions of  $m/z$  112.56. The 112.56 molar mass is a result of the weighted average of the two chlorine isotopes ( $^{35}\text{Cl}$ , 75.4% and  $^{37}\text{Cl}$ , 24.6%). The individual ions that are measured in the mass spectrometer will have either a  $^{35}\text{Cl}$  or a  $^{37}\text{Cl}$ , and thus ions are detected at  $m/z$  112.01 and 114.01. Also, approximately one ion at  $m/z$  114 will be detected for every three ions detected at  $m/z$  112 (24.6/75.4).

Basically, any information gathered from a mass spectrometer comes from the analysis of gas-phase ions. There are three main components of a mass spectrometer: an ionization source, a mass analyser and a detector (FIG. 1). The following discussion will provide an overview of the different ionization sources and mass analysers that are most often used in the analysis of biologically relevant samples. Although the raw data that mass spectrometers provide are  $m/z$  ratios and abundances of the components in a sample, the information that can be gathered from MS is not just limited to molecular weight and sample amount. Mass analysers can also be used in certain ways to gather structural information, such as the connectivity of atoms in a molecule. Structural analysis combined with its inherent sensitivity and speed is where MS realizes much of its power.

### Ionization

As ions are actually analysed in the vacuum of the mass spectrometer, arguably the most important reaction in MS is the one that converts ANALYTES of interest into gas-phase ions. Historically, the most commonly used ionization processes (for example, electron ionization) occur in two discrete steps: the sample is first volatilized and then it is ionized. This approach to ionization limits the types of analytes that can be analysed intact to relatively low-molecular-weight compounds that are thermally stable. As most compounds of biological interest have relatively high molecular weights and their high polarities severely limit their volatility, such an approach to ionization restricted the applicability of MS to biological studies for many years.

**ANALYTE**  
The substance being analyzed.

**PROTONATION**  
The addition of one or more protons to a compound so that the net charge of the compound is positive.

**DEPROTONATION**  
The removal of one or more protons from a compound so that the net charge of the compound is negative.

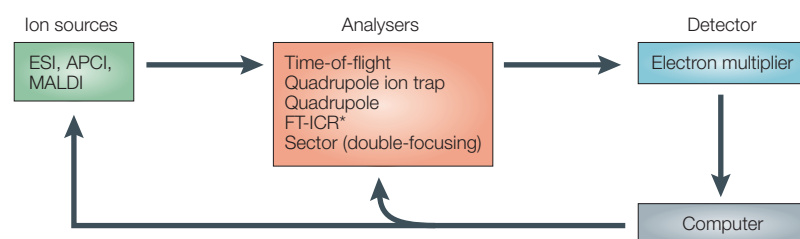


Figure 1 | **Basic components of a typical mass spectrometer used in drug discovery.**  
\*FT-ICR does not use an electron multiplier. APCI, atmospheric-pressure chemical ionization; ESI, electrospray ionization; MALDI, matrix-assisted laser desorption/ionization; FT-ICR, Fourier-transform ion-cyclotron resonance.

The past two decades, however, have seen the development of new ionization techniques that now allow almost any large, non-volatile and thermally labile compound to be converted into a gas-phase ion. 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 choice today: electrospray ionization (ESI)<sup>6</sup> and matrix-assisted laser desorption/ionization (MALDI)<sup>7</sup>. The efforts related to the development of these two techniques lead to a share of the 2002 Nobel Prize in Chemistry. In addition, ESI has led to a revival of the technique of atmospheric-pressure chemical ionization (APCI)<sup>8</sup>.

**Electrospray ionization.** Ions in ESI are generated at atmospheric pressure by passing a solution-based sample through a small capillary (internal diameter < 250  $\mu\text{m}$ ) that is at a potential difference relative to a counter electrode at voltages between +500 and +4,500 V. The actual voltage required depends on both the inner diameter of the needle and the solvents that make up the solution. Generally, capillaries with larger inner diameters and solvents with higher boiling points require higher voltages (BOX 1).

Regardless of the dimensions of the ESI set up, electrostatic spraying of a sample solution initially generates an aerosol of charged droplets. Sometimes a concentric flow of gas, such as  $\text{N}_2$ , is used to facilitate this nebulization process. The charged droplets 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 that surrounds them, and these ions make their way into the mass analyser of the spectrometer.

PROTONATION/DEPROTONATION is the main source of charging for biologically relevant ions in ESI. In fact, ions of proteins, peptides, oligonucleotides and other molecules with acid/base functionality are often found with several sites of protonation or deprotonation. FIGURE 2a is an example of the positive ESI mass spectrum of cytochrome *c*. Although at first glance the multiple charging might seem to make information about molecular weight more difficult to determine, in actuality each peak in this spectrum represents a separate mass measurement that can ultimately increase the precision of the measurement (BOX 2). Multiple charging also enables mass spectrometers with limited  $m/z$  ranges to analyse higher-molecular-weight molecules. For example, cytochrome *c* has a molecular weight of 12,360 Da, but the addition of between 10 and 20 protons brings the  $m/z$  ratio of the protein below 2,000, which is within the working range of most mass analysers.

ESI has some very impressive attributes that allow it to be used for a wide variety of biological problems. There seems to be no inherent limit to the size of molecules that can be ionized, as observed by the ionization of Coliphage T4 DNA, which has a nominal molecular weight around  $1.1 \times 10^8$  Da<sup>9,10</sup>. In addition, ESI is a very 'soft' technique that allows non-covalent biomacromolecular complexes

Box 1 | **Micro-electrospray/nanospray**

Although the size criteria necessary for a method to be described as either micro-electrospray or nanospray are somewhat ambiguous, some dimensions are typical for these techniques. Capillary inner diameters (i.d.) and solvent flow rates seem to be useful criteria for establishing a particular ESI setup as either micro-electrospray or nanospray. Typical nanospray devices use fused silica or glass capillaries with orifice i.d. < 10  $\mu\text{m}$  from which solution normally flows at rates < 100 nL/min. Micro-electrospray devices use larger capillaries (10  $\mu\text{m}$  < i.d. < 100  $\mu\text{m}$ ) with slightly higher flow rates (100 nL/min < flow rate < 500 nL/min). ESI devices with larger needle dimensions and higher flow rates are typically referred to as 'conventional' electro-spray.

to be ionized intact<sup>11</sup>, which expands the utility of MS to the study of protein–protein complexes, double-stranded DNA, protein–drug complexes, DNA–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<sup>12</sup>. 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, the flowing nature of ESI means that a sample is constantly being consumed. Unfortunately, however, no mass spectrometer constantly analyses ions, which means that some of the sample is wasted. This shortcoming is being addressed by using mass analysers that have an inherently higher duty cycle and by developing pulsed ESI sources<sup>13,14</sup>. The second shortcoming of ESI is its susceptibility to ion suppression effects. When solutions contain high salt concentrations (that is, > ~1 mM), 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.

Box 2 | **Calculating mass from an ESI spectrum**

Calculating the mass of a protein from an ESI mass spectrum with multiple charge states (for example, FIG. 2A) can be done in the following manner:

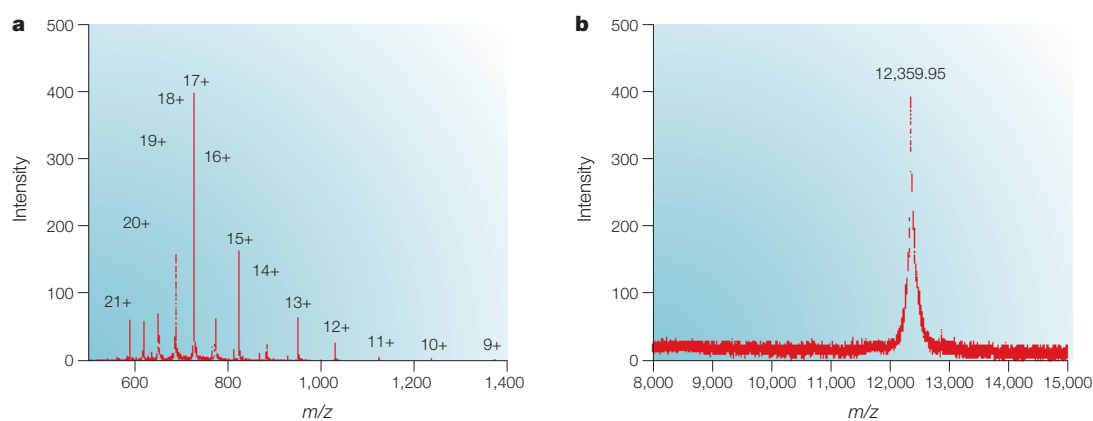
- $(m/z)_1$  and  $(m/z)_2$  are the measured  $m/z$  ratios of two adjacent peaks with  $(m/z)_2 > (m/z)_1$
- Assume ion charging is due to  $\text{H}^+$ , so that each extra proton adds one extra mass unit. Therefore  $(m + z_1)/z_1 = (m/z)_1$  and  $(m + z_2)/z_2 = (m/z)_2$ , where  $m$  is the mass of the protein and  $z$  is the number of charges
- Assume:  $z_1 = z_2 + 1$  (adjacent charge states should differ by only one  $\text{H}^+$ ). Therefore  $[m + (z_2 + 1)]/(z_2 + 1) = (m/z)_1$  and  $(m + z_2)/z_2 = (m/z)_2$
- Now, there are two equations and two unknowns, and the mass of the protein ( $m$ ) can be calculated. This same process can be repeated for each pair of adjacent peaks, and all the determined values of  $m$  can be averaged for better mass measurement precision.

**Atmospheric-pressure chemical ionization.** Most mass spectrometers that are sold with ESI sources also have optional APCI sources. Although there are some similarities between ESI and APCI, they are very much complementary ionization techniques<sup>15</sup>. The ionization process in APCI occurs at atmospheric pressure through ion/molecule reactions. Ionization is initiated by a corona discharge from a very fine needle. Typically –1 kV up to 5 kV is applied to the needle to generate a corona discharge current of 2–5  $\mu\text{A}$ . Electrons from this corona discharge ionize reagent molecules such as  $\text{N}_2$ ,  $\text{O}_2$ ,  $\text{H}_2\text{O}$  and solvent molecules that are present around the needle. A complex series of reactions can ultimately lead to efficient analyte ionization when the analytes are transported into the discharge region. Liquids are introduced through a capillary like that used for ESI. However, instead of applying a voltage to nebulize the sample, a nebulizing gas ( $\text{N}_2$ ) and heat (100–500  $^\circ\text{C}$ ) are used. Both positive and negative ions are formed in the discharge, and analyte charging is typically due to either protonation, which generates positive ions, or deprotonation, which gives negative ions.

APCI has several notable attributes. First, APCI can be readily coupled with liquid separation techniques in a manner similar to ESI. In contrast to ESI, however, APCI has the advantage of being less susceptible to matrix interferences from salts. APCI also has the advantage over ESI that weakly polar analytes not existing as preformed ions in solution can be readily ionized, which is what makes APCI and ESI complementary. The reduced matrix susceptibility and ability to ionize weakly polar analytes often makes APCI suitable for monitoring drug and metabolite concentrations in samples from animals and humans. The very high sensitivity of APCI further enhances the applicability of this technique in drug analysis.

Despite its clear utility, though, APCI does have some drawbacks. Labile compounds can be thermally decomposed in the heated nebulization. Additionally, the high sensitivity of APCI places high demands on solvent purity. Ionization in APCI is thermodynamically controlled, and some compounds can completely suppress analyte ion formation. For example, very basic additives, such as amines, which are sometimes added to improve the chromatographic behavior of a sample, can divert the proton-transfer reactions that lead to analyte protonation. As a result, care has to be taken when mixing additives to a sample. Finally, APCI often has difficulty analysing complex mixtures, for similar reasons. The most stable products after ionization (for example, the analytes with the highest basicity) will usually be observed to the exclusion of less stable products (that is, the analytes with the lowest basicity).

**Matrix-assisted laser desorption/ionization.** Unlike ESI, in which analyte ions are produced continuously, ions in matrix-assisted laser desorption/ionization (MALDI) are produced by pulsed-laser irradiation of a sample. The sample is co-crystallized with a solid matrix that can absorb the wavelength of light emitted by the laser. Usually the sample and matrix are mixed on a probe that



**Figure 2 | A comparison of the mass spectra for cytochrome c generated using electrospray ionization and matrix-assisted laser desorption/ionization. a** | Electrospray ionization (ESI) mass spectrum of cytochrome c: multiple peaks are observed due to the different charge states that arise from varying degrees of protonation. **b** | Matrix-assisted laser desorption/ionization (MALDI) mass spectrum of cytochrome c: only a single peak is observed for the analyte because ionization in MALDI generally occurs by the addition of a single proton. Note the different mass-to-charge ( $m/z$ ) scales.

is inserted into the vacuum system, and after irradiation the gas-phase ions that are formed are directed toward the mass analyser<sup>7,16</sup>. Recently, however, it has been demonstrated that this same process can be carried out at atmospheric pressure<sup>17,18</sup>, and the generated ions are then transferred into the vacuum system for mass analysis in a manner similar to ESI-derived ions. The formation of singly-protonated analytes in MALDI is typical, but the mechanism by which ions are formed is not fully clear. MALDI has several favourable attributes. First, due to the pulsed nature of most lasers, ions are formed in discrete events. If mass analysis is then synchronized with ion formation, very little sample is wasted. Therefore, MALDI can achieve very high levels of sensitivity, often providing data from sub-femtomole ( $< 1 \times 10^{-15}$  moles) amounts of sampling loading. A second advantage of MALDI lies in the fact that singly charged analytes are usually generated. When coupled with certain mass analysers (for example, time-of-flight), MALDI can be used to rapidly provide molecular weight information for one or more analytes (FIG. 2b). High throughput can then be readily accomplished by using sample plates that are loaded with  $\sim 100$  different samples. A final practical advantage of MALDI is its relatively high tolerance to salts and buffers. Unlike ESI, ions can be formed by MALDI from samples that contain physiological levels of salts. In fact, MALDI has been used recently to ionize proteins directly from cell lysates and whole cells<sup>19,20</sup>.

Although MALDI has significant advantages, it also has some drawbacks. Even though the pulsed nature of the technique is one source of MALDI's inherent sensitivity, it is also a source of difficulty when coupling to some mass analysers. Consequently, only certain mass spectrometers are 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. As a result, samples with low molecular weights are usually difficult to analyse by MALDI. Recent variations of MALDI, however, seem promising

for enabling the analyses of low-molecular-weight compounds. In particular, the desorption/ionization on silicon (DIOS) technique provides similar results to MALDI without the chemical background at low  $m/z$  ratios<sup>21,22</sup>.

#### Mass analysers

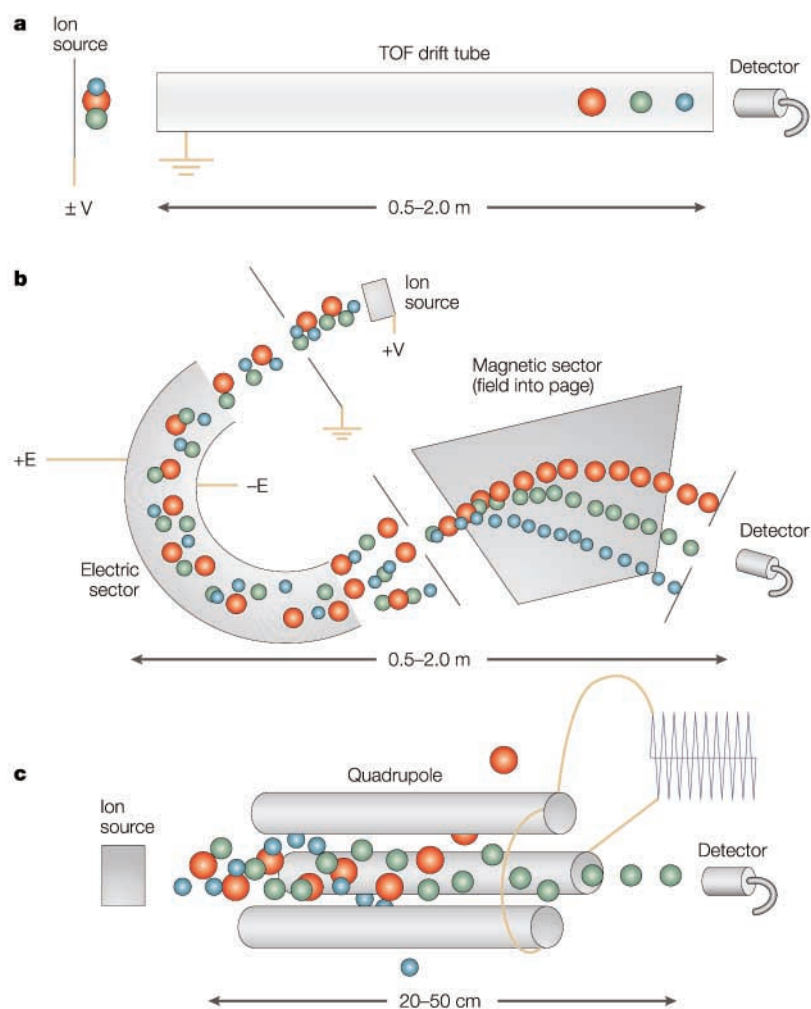
The different types of mass analyser measure ions in different ways. In most analytical measurements, two of the predominant figures of merit are accuracy and precision. It is important to note that an accurate measurement does not necessarily require a precise measurement and vice versa. In MS, the precision of the measurement is related to the resolution (that is, the ability to resolve two adjacent peaks). In general, resolution is defined as  $m/\Delta m$ , where  $m$  is the integer mass of the peaks being resolved and  $\Delta m$  is the mass difference between the two peaks.

There are five principal types of mass analyser in use today. These analysers can be divided into two groups: beam analysers and trapping analysers. In beam analysers, the ions leave the ion source in a beam and pass through the analyzing field to the detector (FIG. 3). In trapping analysers, the ions are trapped in the analyzing field, after being formed in the analyser itself or being injected from an external ion source (FIG. 4).

**Time-of-flight.** Conceptually, the simplest mass analyser is probably the time-of-flight (TOF) mass spectrometer<sup>23</sup>. A TOF spectrometer separates ions based on their velocity and can, essentially, be thought of as a race from a starting point to the detector. Theoretically, the ions are all formed at the same time and place in the ion source and then accelerated through a fixed potential (for example, 1–20 kV) 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. In fact, ion velocities are inversely related to the square root of  $m/z$ . After the ions are accelerated,

#### KINETIC ENERGY

The energy associated with a substance because of its translational motion; equal to one-half its mass times the square of its velocity.



**Figure 3 | Pictorial diagrams of the common beam mass analysers viewed from above.**  
**a** | Mass analysis in time-of-flight (TOF) spectrometry is achieved because ions of different mass-to-charge ( $m/z$ ) values have different velocities and therefore reach the detector at different times.  
**b** | A double-focusing analyser provides direction focusing through both the electric and magnetic sectors. Ions with the same kinetic energy-to-charge ratio follow a common path through an electric sector, and ions can then be dispersed according to their momentum-to-charge ratio in a magnetic sector. Overall analysis according to  $m/z$  is achieved.  
**c** | In a quadrupole mass analyser (top rod not shown), the correct magnitude of the radio frequency and direct current voltages applied to the rods allows ions of a single  $m/z$  to maintain stable trajectories from the ion source to the detector, whereas ions with different  $m/z$  values are unable to maintain stable trajectories.

they travel through a fixed distance, typically 0.5–2.0 metres, before striking the 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 was a popular method in the early days of MS. However, as TOF requires a pulse of ions (that is, a well-defined start time) it was not readily compatible with the ionization methods available then. Additionally, fast electronics that would allow the detection of all the ions in a single pulse were not available. As such, from the 1960s through the 1980s, TOF was rarely used for routine MS experiments to identify organic and biological compounds. The advances in electronics and computers, however, along with the development of new pulsed-ionization

techniques such as MALDI, led to a renaissance in TOF in the 1990s. TOF now offers mass resolution in the thousands and mass accuracies in the tens of parts per million (ppm).

The above description of TOF is generally termed linear TOF. The best performance is obtained on more sophisticated TOF instruments that include a reflectron<sup>24</sup>. In a reflectron TOF, after travelling through one flight distance, the ions enter an electrostatic mirror (that is, 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  $m/z$ . These differences in velocity are caused by a number of different factors, but are inherent in the experiment. Therefore, the reflectron increases the resolution of TOF spectrometry. The mass accuracy has been increased by the faster electronics available today, where nanosecond time resolution is now routine.

Another characteristic of TOF spectrometry that has led to its resurgence as a mass-analysis method is that the mass range is theoretically unlimited. Ions that have  $m/z$  ratios of up to several hundred thousand can be analysed, which is significantly above the range of the other common mass analysers. The combination of high  $m/z$  range and compatibility with pulsed-ionization methods has made TOF the most commonly used analyser for MALDI experiments.

**Sectors.** From the 1950s through the 1980s, instruments with sector analysers were one of the predominant types of mass spectrometer. All sector instruments include a magnetic sector and some also have an electric sector. Ions accelerated from the ionization source are curved by the magnetic field and adopt a constant radius around the centre of field. For a fixed magnetic field strength and a fixed accelerating potential — typically 2–10 kV — only ions with a certain *momentum-to-charge* ratio will pass through slits placed before and after the magnetic field. The magnetic sector actually disperses ions on the basis of their momentum (analogously to a prism diffracting light according to wavelength) at the same time as focusing ions that have the same momentum but diverging angles as they pass through the slit. This process is called direction focusing.

A mass spectrum can be obtained by scanning the magnetic field strength so that ions of different  $m/z$  can travel through both slits, with resolutions of a few hundred typically obtained. The resolution is primarily limited by the differences in ion velocity, which is similar to what limits resolution in linear TOF analysers. To improve performance, most sector mass spectrometers include an electric sector before or after the magnetic sector. An electric sector disperses ions on the basis of their kinetic-energy-to-charge ratio. As all ions have the same nominal kinetic energy, they all have the same path through the electric sector and no mass analysis can be done with the electric sector, although the same type of direction focusing as with a magnetic sectors does occur. However, as a result of the small differences in ion velocities as ions enter the electric sector, small

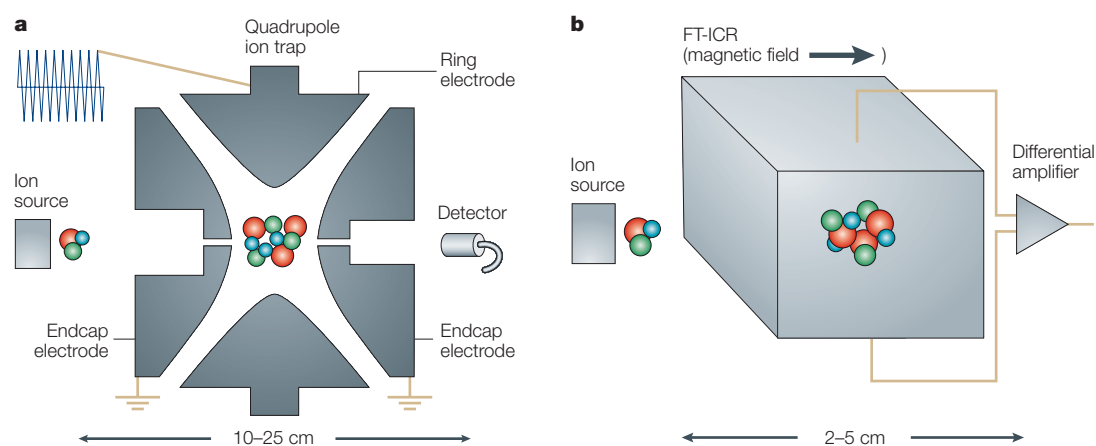


Figure 4 | **Pictorial diagrams of the common trapping mass analysers.** **a** | Ions in a quadrupole ion trap maintain stable trajectories inside the device as a result of the application of a radio frequency voltage to the ring electrode. Mass analysis is achieved by making ion trajectories unstable in a mass-selective manner. **b** | Ions in an Fourier-transform ion-cyclotron resonance (FT-ICR) oscillate around the magnetic field at frequencies that are related to their mass-to-charge ( $m/z$ ) scales. As the ions oscillate near the top and bottom metal plates of the cubic trapping cell, they induce an alternating current that can be measured and then related to their  $m/z$ . Note that whereas the FT-ICR cell is small, it is in a high magnetic field (typically a super-conducting magnet), so the actual instrument size is large.

dispersions of the ions do take place. By careful design of a two-sector instrument, the kinetic energy dispersion in an electric sector can be corrected (reversed) by the momentum dispersion in a magnetic sector. The two sectors combined therefore provide velocity focusing, which means that ions of the same  $m/z$ , but which differ in velocity by a small amount, can be focused to the same point. At the same time, both sectors continue to provide direction focusing, so the end result is ‘double focusing’, which means that ions with both direction and velocity differences can be focused to the same point. Using double-focusing instruments, mass resolutions in the tens of thousands can readily be obtained, and with the best instruments, resolutions  $> 100,000$  are feasible. Mass accuracies in the low ppm range are obtainable with sector instruments (BOX 3).

**Quadrupole.** Over the years, the quadrupole has probably been the most widely used mass analyser, and was typically the choice for gas chromatography MS (GC/MS) and liquid chromatography MS (LC/MS) instruments in the 1970s, 1980s and 1990s. This popularity was mainly a result of the relatively low cost of these instruments and

their ease of automation. Quadrupoles also use much lower voltages to accelerate the ions from the source to the analyser (2–50 V versus kV) and are physically much smaller than sector analysers or most TOF instruments.

Mass separation in a quadrupole is a result of ion motion in a dynamic (radio frequency or rf) electric field and is dependent directly on the  $m/z$  of the ion. Whereas the kinetic energy of the ion is a crucial parameter in sector and TOF instruments, it is not in quadrupole instruments. Mass analysis is a function of rf voltages and direct current (DC) voltages applied to four rods, which are typically cylindrical in geometry. As a result of the time-varying nature of the rf voltage, the equations of motion are second-order differential equations, as opposed to the more simple equations for sector and TOF instruments. However, a graphical representation termed the Mathieu stability diagram, which is based on general solutions to these second-order differential equations, provides a straightforward way to understand which ions pass through the quadrupole to the detector and which ions do not. The stability diagram is a plot of a parameter,  $q$ , related to rf voltage versus a parameter,  $a$ , related to dc voltage. Other variables in determining these Mathieu parameters  $a$  and  $q$  are the physical size of the quadrupole, the frequency of the rf voltage and the  $m/z$  of the ions of interest. The size of the quadrupole and the rf frequency are usually kept constant, so that ions of different  $m/z$  can be sequentially allowed to reach the detector by increasing the magnitude of the rf and dc voltages. Usually this is done while keeping the ratio of the rf and DC voltages constant.

Depending on the physical parameters of the quadrupole, the upper  $m/z$  limit can vary from 300 to 4000; the mass accuracy is generally in the hundreds of ppm. The mass resolution is a function of the ratio of the rf and dc voltages and is often varied such that unit resolution is obtained over the whole mass range. This means that at

### Box 3 | Mass resolution or mass accuracy?

Mass resolution is a term often used inappropriately. Many journals still incorrectly require ‘high resolution mass spectrometry’ data to support identification of newly synthesized compounds. Resolution, however, refers to the precision of the measurement, whereas identity is established by the accuracy of the measurement, that is, how close the measured mass is to the true (or theoretical) mass. A more accurate measurement allows determination of the mass of the ion to more places beyond the decimal point. So, mass accuracy, typically reported as parts per million (ppm), is the most important parameter in establishing compound identity. Mass resolution is only important if there are two ions of very similar mass. If these ions are not resolved, then some weighted average of their masses will be determined, which will be incorrect. For pure compounds, this should rarely be an issue.

$m/z$  200, the resolution is 200 (that is,  $m/z$  200 is resolved from  $m/z$  201) and at  $m/z$  1000 the resolution is 1000 (that is,  $m/z$  1000 is resolved from  $m/z$  1001). As in most instruments, the sensitivity decreases as the resolution increases, so there can be a substantial drop off in sensitivity when analyzing ions with higher  $m/z$  values. Although the quadrupole had been the instrument of choice for combination with separation techniques, the quadrupole ion trap has become an important competitor. The one area in which the quadrupole still excels relative to the other instruments is in quantification.

**Quadrupole ion trap.** The quadrupole ion trap is a close relative of the quadrupole mass analyser. Whereas a quadrupole has electric fields in two dimensions ( $x$  and  $y$ ) and the ions move perpendicular to the field (that is, in the  $z$  direction), the ion trap has the electric field in all three dimensions, which can result in ions being trapped in the field. A Mathieu stability diagram applies to the ion trap just like the quadrupole, but there is an important conceptual difference in the way that these two related instruments operate. In a quadrupole, the ions that are detected to obtain the mass spectrum have a stable trajectory through the quadrupole, whereas to obtain a mass spectrum with an ion trap the ion trajectories must be made unstable. Ions are made unstable in a mass-selective manner by increasing the rf voltage that is applied to the device. This mass-selective instability mode of operation was only developed in the 1980s (REF. 25), and led to the ion trap's commercialization, even though the ion trap was invented in the 1950s. Since that time, more advanced modes of operation have made the ion trap one of the most commonly used mass spectrometers today.

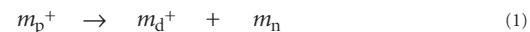
Commercial ion traps typically have an  $m/z$  range similar to quadrupoles (4,000) and also have similar resolution. However, resolution can be improved substantially by using slower rf voltage scan rates<sup>26,27</sup>, and the mass range can be extended up to 70,000 (REF. 28). Unlike the quadrupole, there is not the drastic fall off in sensitivity with ion traps at higher  $m/z$ . The mass accuracy of ion traps is similar to quadrupoles (hundreds of ppm). The sensitivity, throughput and ease of use of the ion trap, combined with its relatively low cost — at least for a mass spectrometer (\$100,000–200,000) — has led to it becoming the workhorse in many labs.

**Fourier-transform ion-cyclotron resonance.** Like a sector mass spectrometer, a Fourier-transform ion-cyclotron-resonance (FT-ICR) mass spectrometer (also referred to as a Fourier-transform mass spectrometer, or FTMS) uses a magnetic field to determine the  $m/z$  of an ion<sup>29,30</sup>. The main difference in the operation of an FT-ICR versus a magnetic sector is the kinetic energy of the ions. In the latter, ions typically have kinetic energies in the keV range, whereas in an FT-ICR ions have kinetic energies, at most, of a few tens of electron volts (eV). At low kinetic energies, ions do not pass through the magnetic field with a deflection related to the momentum-to-charge ratio, like in a magnetic sector, but are actually trapped in the magnetic field. For a constant magnetic

field, ions oscillate around the magnetic field with a cyclotron frequency that is inversely related to the  $m/z$ . In a very simplified view of FT-ICR, the cyclotron frequencies of the ions trapped in the FT-ICR are measured and then converted into  $m/z$ . State-of-the-art electronic equipment is capable of measuring frequencies with extremely high precision. This translates to a very high mass resolution, which is the property FT-ICR is most widely known for. Mass resolutions in the hundreds of thousands are fairly easy to obtain on instruments with large (that is, > 7 Tesla) magnetic field strengths, and resolutions in the millions have been demonstrated. Very high mass accuracies, down to the ppm level, can also be obtained. The high resolution and high mass accuracy, however, come at a price, as high-end FT-ICR instruments can cost more than US \$1 million. FT-ICRs also require much more laboratory space, as high field superconducting magnets are an integral part of the instrument, and throughput in FT-ICR instruments is much lower than the other mass spectrometers discussed above.

#### Tandem mass spectrometry

Whereas the ionization techniques and mass analysers developed in the mid-1980s are crucial to the application of MS to biological compounds, the technique of tandem mass spectrometry (MS/MS) is an equally important factor in the contributions to the field<sup>31</sup>. As the name implies, 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 emanating from 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. EQN 1 is a representation of this, in which  $m_p^+$  is the parent ion,  $m_d^+$  is the product ion and  $m_n$  the neutral fragment (if the parent ion is formed by ESI and is multiply charged,  $m_n$  might also be an ion).



With some instruments, it is possible to repeat this process, leading to what is termed an MS<sup>*n*</sup> experiment, where *n* is equal to the number of stages of MS performed. MS/MS is particularly useful when analysing complex mixtures. It can be thought of as analogous to a chromatography/mass spectrometry (for example, 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 (FIG. 5).

An important advantage of the MS/MS experiment is that either the first stage or second stage of MS can be the independent variable. In the LC/MS experiment, the mass spectrum is dependent on the analyte that is eluting from the column. Therefore, the MS stage is always the dependent variable and the LC stage is always the

**INTERNAL ENERGY**  
The energy associated with the movement of the atoms and electrons in a chemical compound relative to one another; the increase in the internal energy of a compound leads to an increase in its temperature and in some cases dissociation.

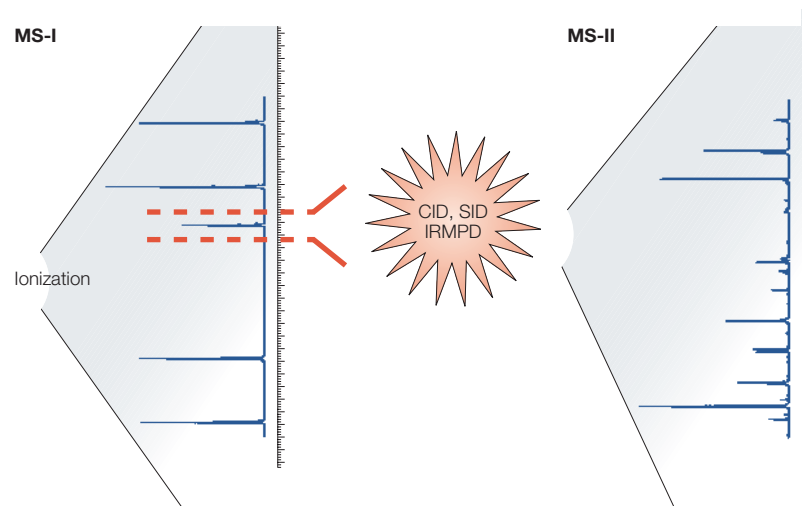


Figure 5 | **Schematic diagram of tandem mass spectrometry.** In tandem mass spectrometry (or MS/MS) ions with the mass-to-charge ( $m/z$ ) ratio of interest (that is, parent or precursor ion) are selectively reacted to generate a mass spectrum of product ions. CID, collision-induced dissociation; IRMPD, infrared multi-photon photodissociation; SID, surface-induced dissociation.

independent variable. In MS/MS, however, experiments are possible in which the dependent stage is the first stage of MS (the stage analogous to the LC stage). The experiment most analogous to the LC/MS experiment is called a product-ion scan. In this case, the first stage of MS (MS-I) is the independent stage (that is, fixed parent ion) and the second stage (MS-II) is dependent (that is, scan to detect all the product ions). The goal of the product-ion scan (as well as the LC/MS experiment) is to identify the selected component of the sample.

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<sup>32</sup>. 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.

In the product- and parent-ion scans, the third chemical constituent of MS/MS experiments — the neutral fragment — is unimportant. However, in another type of experiment — a neutral-loss scan — the neutral fragment is the independent variable. In this experiment, both MS-I and MS-II are scanned in such a way that they are offset by the desired neutral mass that is lost (or gained) in the reaction between the two MS stages. This experiment also provides the ability to screen a mixture for a given compound type. An example of a neutral-loss experiment is the identification of

phosphorylated peptides<sup>33</sup>. A phosphorylated peptide will lose a neutral  $H_3PO_4$  fragment. Phosphorylated peptides can be detected by scanning MS-II to pass ions that have changed mass by this amount in the reaction between MS-I and MS-II.

Both the parent-ion scan and neutral-loss scan can have an important role in drug discovery. Often a series of homologues can be of interest. The homologues will have a common core structure that often will either form a specific product ion or will be lost as a neutral fragment in the MS/MS experiment. These homologues might be variations of a potential therapeutic compound or more commonly, metabolites of a specific target compound. In the latter case, parent-ion or neutral-loss scans can be used to screen biological samples to discover unknown metabolites.

Another important type of MS/MS experiment is reaction monitoring. This experiment is analogous to single-ion monitoring in LC/MS, and is typically used for quantification. For a known analyte to be detected, MS-I is set to pass the parent ion (typically the protonated molecule), and MS-II is set to pass a known product ion(s). If there is a single parent ion and single product ion, this is a single-reaction-monitoring experiment. There are two forms of multiple-reaction monitoring. In one form, a single parent ion is continually passed through MS-I, and MS-II is programmed to sequentially pass two (or more) known product ions. This provides added specificity over the single-reaction-monitoring experiment, as the parent ion has to dissociate to all the monitored product ions, and the ratio of the product ions should match a known value for the experimental conditions. The other form of multiple-reaction monitoring involves multiple parent ions. There are several permutations of this experiment. The most common can be thought of as multiple single-reaction-monitoring experiments. A typical example of this would be when a deuterated internal standard is used for quantification. MS-I would switch back and forth between the unlabelled and labelled analyte, with MS-II set to pass the corresponding product ion. Depending on the location of the label and the fragmentation mechanism, the product ions from the analyte and the isotopically labelled analyte might either: have the same  $m/z$  (the isotopic label is lost as part of the neutral fragment); be separated in  $m/z$  by the same amount as the parent ions (the label is completely retained in the product ion); or differing in  $m/z$  by less than the amount of the parent ions (part of the label is lost with the neutral fragment).

The various MS/MS experiments also offer another important feature — improvement in signal/noise ratio. The detectors used in most mass spectrometers can detect single ions impinging on them. So, the background ‘noise’ in a mass spectrum is typically not from the detector but from actual ions emitted from the ion source and transmitted through the mass analyser(s). This is typically referred to as ‘chemical noise’, and it often defines the limit of detection. In MS/MS, MS-I acts as a chemical filter, removing the ions that give rise to the peaks everywhere else in the mass spectrum



except for the parent ion  $m/z$  being transmitted. Therefore, when detecting the product ions after MS-II, the 'noise' is truly detector noise, which can be several orders of magnitude lower than the chemical noise. So, whereas there are typically losses in the absolute ion signal in MS/MS experiments, the signal/noise ratio actually increases.

#### Dissociation methods

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 by MS-II. In practice, the activation cannot be separated from the dissociation, so the ion activation techniques are typically referred to as dissociation methods. The dissociation method almost universally used is collision-induced dissociation (CID)<sup>34</sup>. 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.

Although CID is essentially universally used across all types of mass spectrometers, there are a few other techniques that can be used on certain instruments. Two of these techniques are surface-induced dissociation (SID) and photodissociation. SID involves converting the kinetic energy of an ion to internal energy through a collision with a surface, usually one that has been modified with a self-assembled monolayer<sup>35,36</sup>. Although SID has been demonstrated on trapping instruments, it is more common and practical on beam instruments, particularly quadrupole-based mass spectrometers. In photodissociation, ions are activated by absorption of a photon(s). Early photodissociation experiments used UV and visible lasers, typically with beam instruments<sup>37,38</sup>, but generally these are not very efficient experiments. More recently, however, infrared multiphoton photodissociation (IRMPD), implemented on trapping instruments, has shown a lot of promise<sup>39–41</sup>. With trapping instruments, the parent ions can be maintained in the laser beam for extended periods of time, allowing absorption of multiple photons.

#### MS/MS instruments

Often MS/MS instruments are classified in one of two categories: tandem-in-space or tandem-in-time. Tandem-in-space instruments require a distinct analyser for each stage of MS/MS. Beam-type analysers are used in tandem-in-space instruments. Trapping instruments are typically tandem-in-time. The various stages of MS/MS are performed in the same analyser but separated in time. The tandem-in-time instruments have the advantage of being able to do multiple stages of MS/MS ( $MS^n$ ) with a single analyser<sup>42</sup>. Although trapping instruments have the advantage of readily being able to do  $MS^n$ , they cannot perform parent-ion scans or neutral-loss scans. So, for screening mixtures, beam instruments have a definite advantage.

The earliest MS/MS instruments (which were developed in the mid-1970s) used sectors for both stages of MS<sup>43</sup>. The main reason that sectors were the earliest MS/MS instruments is that they were the only mass spectrometers with multiple analysers. The early promise of MS/MS led to the development of the triple quadrupole (QqQ) instrument as a cheaper, easier-to-use alternative to sector instruments<sup>44</sup>. In the QqQ instrument, the first and third quadrupoles are operated as mass spectrometers, whereas the second (middle) quadrupole acts as the collision region for CID. Following the development of the QqQ instrument, various hybrid instruments were developed. These instruments coupled different types of analyser together to produce tandem MS instruments. Of the variety of hybrid instruments that were developed in the mid-1980s, one — the quadrupole/time-of-flight (Q/TOF)<sup>45</sup> — has evolved into a mainstay of modern MS/MS instruments.

Today, almost all tandem-in-space MS/MS instruments are either QqQs or Q/TOFs. One of the advantages of the QqQ versus the Q/TOF is that the former can perform the parent and neutral loss scans discussed above. Because the TOF does not 'scan', but instead passes all ions in a pulse and separates them in time, these screening-type scans cannot be performed directly. The advantage of the Q/TOF is the speed in which an MS/MS spectrum can be obtained because the whole spectrum can be recorded for a single pulse of ions. This makes the Q/TOF particularly well suited for LC/MS/MS experiments.

The tandem-in-time instruments (quadrupole ion trap and FTICR) generally have much higher MS/MS efficiencies because ions do not have to be transferred from one analyser to another for each stage of MS/MS and because the time frame of the experiment is several orders of magnitude longer, giving the parent ions more time to dissociate. Although  $MS^n$  experiments can readily be performed on both the ion trap and FTICR, only a small fraction of analyses actually incorporate this capability. The MS/MS experiment is much simpler and faster to implement on a quadrupole ion trap. Because of this, and the relatively small size and low cost of the ion trap, it is by far the most common tandem-in-time instrument, especially for LC/MS/MS analysis.

#### Conclusions and future prospects

The inherent analytical advantages of mass spectrometry, including sensitivity and speed, combined with recent advances promise to make MS a mainstay of drug design and discovery. The development of ionization techniques such as ESI and MALDI now allows almost any compound to be studied by MS. In addition, MS/MS and  $MS^n$  add the capability for structural analysis of compounds that are present at low levels and/or are present in complex mixtures.

The gentle nature of relatively new ionization techniques such as ESI and the structural analysis capability of MS should extend this technique into new areas. In particular, MS has the potential to serve as a platform for strategies that screen large numbers of compounds

against DNA and protein drug targets. Numerous studies have shown, for example, that ESI-MS can readily produce non-covalent complexes of double-stranded DNA (ds-DNA) and various drugs<sup>46–49</sup>. Many studies of ds-DNA/drug complexes demonstrate that ESI-MS not only reflects solution-phase binding STOICHIOMETRIES but also binding specificity and relative binding affinities of various drugs<sup>49–52</sup>. The potential of ESI-MS for high-throughput screening has been demonstrated recently using FTICR-MS<sup>53,54</sup>. The high resolution and high mass accuracy of this technique enabled the simultaneous study of the binding of multiple ligands to multiple RNA targets. The identity of the ligands that bound successfully, the RNA target to which they were bound and the compound-specific binding affinity could all be determined in a single rapid experiment. Similarly, the relative binding affinities of a mixture of drugs to a given protein have been studied by combining size-exclusion chromatography with MS<sup>55–57</sup>. The sensitivity and high-throughput capability of MS should make it a powerful tool for the early stages of drug discovery.

Another area in which MS might have an impact on drug discovery efforts is in the determination of three-dimensional protein structure. MS-based approaches to determining three-dimensional structures have the same

advantages mentioned above (that is, speed, sensitivity and mixture analysis capability), and significant strides have been made in the determination of higher-order structure<sup>58–60</sup>. Three-dimensional structural analysis by MS relies on solution-phase H/D exchange in a manner similar to NMR analyses of protein structure. After H/D exchange, mass shifts are easily observed, and the mass spectrometer can readily measure the degree of deuterium incorporation. Furthermore, recent efforts using CID suggest that the specific sites of deuterium incorporation<sup>61–63</sup> — and thus insight into higher-order structure — can be determined.

Without doubt, the biggest area that these new mass-spectrometry techniques are contributing to — and will continue to contribute to — is proteomics. Understanding the proteome of a species will be intimately related to drug development. A variety of approaches based on various combinations of the ionization and mass analysis techniques discussed above are integral to proteomics<sup>64</sup>. The actual combinations depend on the desired goal, such as protein identification, *de novo* peptide sequencing, identification of post-translation modification and determination of protein-protein interactions. By extension, these MS techniques will play a crucial role in almost all of the other emerging ‘-omics’ fields.

#### STOICHIOMETRY

The quantitative relationship between the reactants and products in a chemical reaction.

- Lee, M. S. & Kerns, E. H. LC/MS applications in drug development. *Mass Spectrom. Rev.* **18**, 187–279 (1999).
- Cheng, X. & Hochlowski, J. Current application of mass spectrometry to combinatorial chemistry. *Anal. Chem.* **74**, 2679–2690 (2002).
- Triolo, A., Altamura, M., Cardinalli, F., Sisto, A. & Maggi, C. A. Mass spectrometry and combinatorial chemistry: a short outline. *J. Mass Spectrom.* **36**, 1249–1259 (2001).
- Shin, Y. G. & Breeman, R. B. v. Analysis and screening of combinatorial libraries using mass spectrometry. *Biopharm. Drug Dispos.* **22**, 353–372 (2001).
- Kassel, D. B. Combinatorial chemistry and mass spectrometry in the 21<sup>st</sup> century drug discovery laboratory. *Chem. Rev.* **101**, 255–267 (2001).
- Yamashita, M. & Fenn, J. B. Electrospray ion source. another variation of the free-jet theme. *J. Phys. Chem.* **88**, 4451–4459 (1984).
- The initial publication in the development of electrospray as a useful ionization technique for mass spectrometry.**
- Karas, M., Bachmann, D., Bahr, U. & Hillenkamp, F. Matrix-assisted ultraviolet laser desorption of non-volatile compounds. *Int. J. Mass Spectrom. Ion Processes* **78**, 53–68 (1987).
- Seminal publication describing the use of a matrix to enhance the ionization efficiency of non-volatile compounds — this method of laser/desorption ionization is the most commonly used today.**
- Horning, E. C., Horning, M. G., Carroll, D. I., Dzidic, I. & Stillwell, R. N. New Picogram detection system based on a mass spectrometer with an external ionization source at atmospheric pressure. *Anal. Chem.* **45**, 936–943 (1973).
- Chen, R. *et al.* Trapping, detection, and mass determination of coliphage T4 DNA ions by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chem.* **67**, 1159–1163 (1995).
- Smith, R. D., Cheng, X., Bruce, J. E., Hofstadler, S. A. & Anderson, G. A. Trapping, detection, and reaction of very large single molecular ions by mass spectrometry. *Nature* **369**, 137–139 (1994).
- Smith, R. D., Bruce, J. E., Wu, Q. & Lei, Q. P. New mass spectrometric methods for the study of noncovalent associations of biopolymers. *Chem. Soc. Rev.* **26**, 191–202 (1997).
- A good review of how mass spectrometry is used to study non-covalent complexes of proteins, DNA, DNA-drug interactions and enzyme-inhibitor interactions.**
- Suizdak, G. *et al.* Mass spectrometry and viral analysis. *Chem. Biol.* **3**, 45–48 (1996).
- Berggren, W. T., Westphall, M. S. & Smith, L. M. Single-pulse nano-electrospray ionization. *Anal. Chem.* **74**, 3443–3448 (2002).
- Lu, Y., Zhou, F., Shui, W., Bian, L., Guo, Y. & Yang, P. Pulsed Electropray for mass spectrometry. *Anal. Chem.* **73**, 4748–4753 (2001).
- Siegel, M. M., Tabei, K., Lambert, F., Candel, L. & Zoltan, B. Evaluation of a dual electrospray ionization/atmospheric pressure chemical ionization source at low flow rates (60 µl/min) for analysis of both highly and weakly polar compounds. *J. Am. Soc. Mass Spectrom.* **9**, 1196–1203 (1998).
- Tanaka, K. *et al.* Protein and polymer analyses up to *m/z* 100000 by laser ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Spectrom.* **2**, 151–153 (1988).
- Laiko, V. V., Baldwin, M. A. & Burlingame, A. L. Atmospheric pressure matrix-assisted laser desorption/ionization mass spectrometry. *Anal. Chem.* **72**, 652–657 (2000).
- Laiko, V. V., Moyer, S. C. & Cotter, R. J. Atmospheric pressure MALDI/ion trap mass spectrometry. *Anal. Chem.* **72**, 5239–5243 (2000).
- Lay, J. O. MALDI-TOF Mass spectrometry of bacteria. *Mass Spectrom. Rev.* **20**, 172–194 (2001).
- Fenselau, C. & Demirev, P. A. Characterization of intact microorganisms by MALDI mass spectrometry. *Mass Spectrom. Rev.* **20**, 157–171 (2001).
- Thomas, J. J., Shen, Z., Crowell, J. E., Finn, M. G. & Suizdak, G. Desorption/ionization on silicon (DIOS): a diverse mass spectrometry platform for protein characterization. *Proc. Natl Acad. Sci. USA* **98**, 4932–4937 (2001).
- Shen, Z. *et al.* Porous silicon as a versatile platform for laser desorption/ionization mass spectrometry. *Anal. Chem.* **73**, 612–619 (2001).
- Weickhardt, C., Moritz, F. & Grotemeyer, J. Time-of-flight mass spectrometry: state-of-the-art in chemical analysis and molecular science. *Mass Spectrom. Rev.* **15**, 139–162 (1996).
- Wiley, W. C. & McLaren, I. H. Time of flight mass spectrometer with improved resolution. *Rev. Sci. Instr.* **26**, 1150–1157 (1955).
- Stafford, G. C. J., Kelley, P. E., Syka, J. E. P., Reynolds, W. E. & Todd, J. F. Recent improvements in and analytical applications of advanced ion trap technology. *Int. J. Mass Spectrom. Ion Processes* **60**, 85–98 (1984).
- A description of important improvements to the quadrupole ion trap mass spectrometer that eventually led to its wide use today.**
- Schwartz, J. C., Syka, J. E. P. & Jardine, I. High resolution on a quadrupole ion trap mass spectrometer. *J. Am. Soc. Mass Spectrom.* **2**, 198–204 (1991).
- Goeringer, D. E., Whitten, W. B., Ramsey, J. M., McLuckey, S. A. & Gilsh, G. L. Theory of high-resolution mass spectrometry achieved via resonance ejection in the quadrupole ion trap. *Anal. Chem.* **64**, 1434–1439 (1992).
- Kaiser, R. E. J., Louris, J. N., Amy, J. W. & Cooks, R. G. Extending the mass range of the quadrupole ion trap using axial modulation. *Rapid Commun. Mass Spectrom.* **3**, 225–229 (1989).
- Marshall, A. G. & Comisarow, M. Fourier transform ion cyclotron resonance [FT-ICR] spectroscopy. *Chem. Phys. Lett.* **25**, 282–283 (1974).
- The first demonstration of the use of Fourier-transform ion-cyclotron resonance for mass analysis in ion-cyclotron resonance mass spectrometers.**
- Marshall, A. G., Hendrickson, C. L. & Jackson, G. S. Fourier transform ion cyclotron resonance mass spectrometry: a primer. *Mass Spectrom. Rev.* **17**, 1–35 (1998).
- Busch, K. L., Gilsh, G. L. & McLuckey, S. A. *Mass Spectrometry/Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry* (VCH New York, 1988).
- Carr, S. A., Huddleston, M. J. & Bean, M. E. Selective identification and differentiation of *N*- and *O*-linked oligosaccharides in glycoproteins by liquid chromatography–mass spectrometry. *Protein Sci.* **2**, 183–196 (1993).
- Schlosser, A., Pipkorn, R., Bossemeyer, D. & Lehmann, W. D. Analysis of protein phosphorylation by a combination of elastase digestion and neutral loss tandem mass spectrometry. *Anal. Chem.* **73**, 170–176 (2001).
- McLuckey, S. A. Principles of collisional activation in analytical mass spectrometry. *J. Am. Soc. Mass Spectrom.* **3**, 599–614 (1992).
- Dongré, A. R., Somogyi, A. & Wysocki, V. H. Surface-induced dissociation: an effective tool to probe structure, energetics and fragmentation mechanisms of protonated peptides. *J. Mass Spectrom.* **31**, 339–350 (1996).
- Mabud, M. A., Dekrey, M. J. & Cooks, R. G. Surface-induced dissociation of molecular ions. *Int. J. Mass Spectrom. Ion Processes* **67**, 285–294 (1985).
- Hunt, D. F., Shabanowitz, J. & Yates, J. R. Peptide sequence analysis by laser photodissociation Fourier transform mass spectrometry. *J. Chem. Soc. Chem. Commun.* 548–550 (1987).

38. Tecklenburg, R. E., Miller, M. N. & Russell, D. H. Laser ion beam photodissociation studies of model amino acids and peptides. *J. Am. Chem. Soc.* **111**, 1161–1171 (1989).
39. Goolsby, B. J. & Brodbelt, J. S. Characterization of  $\beta$ -lactams by photodissociation and collision-activated dissociation in a quadrupole ion trap. *J. Mass Spectrom.* **33**, 705–712 (1998).
40. Payne, A. H. & Gilsh, G. L. Thermally assisted infrared multiphoton photodissociation in a quadrupole ion trap. *Anal. Chem.* **73**, 3542–3548 (2001).
41. Little, D. P., Speir, J. P., Senko, M. W., O'Connor, P. B. & McLafferty, F. W. Infrared multiphoton dissociation of large multiply charged ions for biomolecule sequencing. *Anal. Chem.* **66**, 2809–2815 (1994).
42. Gilsh, G. L. Multiple stage mass spectrometry: the next generation tandem mass spectrometry experiment. *Analyst* **119**, 533–537 (1994).
43. Kondrat, R. W. & Cooks, R. G. Direct analysis of mixtures by mass spectrometry. *Anal. Chem.* **50**, 81A–92A (1978).
44. Yost, R. A. & Enke, C. G. Selected ion fragmentation with a tandem quadrupole mass spectrometer. *J. Am. Chem. Soc.* **100**, 2274–2275 (1978).  
**The first demonstration of a triple quadrupole mass spectrometer and its advantages for structural analysis by tandem mass spectrometry.**
45. Gilsh, G. L. & Goeringer, D. E. Tandem quadrupole/time-of-flight instrument for mass spectrometry/mass spectrometry. *Anal. Chem.* **56**, 2291–2295 (1984).  
**The first tandem quadrupole/time of flight mass spectrometer, developed for high-speed and high-sensitivity MS/MS.**
46. Wan, K. X., Gross, M. L. & Shibusue, T. Gas-phase stability of double-stranded oligodeoxynucleotides and their noncovalent complexes with DNA-binding drugs as revealed by collisional activation in an ion trap. *J. Am. Chem. Soc. Mass Spectrom.* **11**, 450–457 (2000).
47. Gale, D. C., Goodlett, D. R., Light-Wahl, K. J. & Smith, R. D. Observation of duplex DNA–drug noncovalent complexes by electrospray ionization mass spectrometry. *J. Am. Chem. Soc.* **116**, 6027–6028 (1994).
48. Kapur, A., Beck, J. L. & Sheil, M. M. Observation of daunomycin and nogalamycin complexes with duplex DNA using electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **13**, 2489–2497 (1999).
49. Gabelica, V., DePauw, E. & Rosu, F. Interaction between antitumor drugs and a double-stranded oligonucleotide studied by electrospray ionization mass spectrometry. *J. Mass Spectrom.* **34**, 1328–1337 (1999).
50. Gupta, R., Kapur, A., Beck, J. L. & Sheil, M. M. Positive ion electrospray ionization mass spectrometry of double-stranded DNA/drug complexes. *Rapid Commun. Mass Spectrom.* **15**, 2472–2480 (2001).
51. David, W. M., Brodbelt, J., Kerwin, S. M. & Thomas, P. W. Investigation of quadruplex oligonucleotide–drug interactions by electrospray ionization mass spectrometry. *Anal. Chem.* **74**, 2029–2033 (2002).
52. Rosu, F., Gabelica, V., Houssier, C. & DePauw, E. Determination of affinity, stoichiometry and sequence selectivity of minor groove binder complexes with double-stranded oligodeoxynucleotides by electrospray ionization mass spectrometry. *Nucleic Acids Res.* **30**, e82 (2002).
53. Hofstadler, S. A. *et al.* Multiplexed screening of neutral mass-tagged RNA targets against ligand libraries with electrospray ionization FTICR MS: a paradigm for high-throughput affinity screening. *Anal. Chem.* **71**, 3436–3440 (1999).
54. Masselon, C., Anderson, G. A., Harkewicz, R., Bruce, J. E., Pasa-Tolic, L. & Smith, R. D. Accurate mass multiplexed tandem mass spectrometry for high-throughput polypeptide identification from mixtures. *Anal. Chem.* **72**, 1918–1924 (2000).
55. Wabnitz, P. A. & Loo, J. A. Drug screening of pharmaceutical discovery compounds by micro-size exclusion chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* **16**, 85–91 (2002).
56. Moy, F. J. *et al.* MS/NMR: A structure-based approach for discovering protein ligands and for drug design by coupling size exclusion chromatography, mass spectrometry, and nuclear magnetic resonance spectroscopy. *Anal. Chem.* **73**, 571–581 (2001).
57. Siegel, M. M., Tabei, K., Beberitz, G. A. & Baum, E. Z. Rapid methods for screening low molecular mass compounds non-covalently bound to proteins using size exclusion and mass spectrometry applied to inhibitors of human cytomegalovirus protease. *J. Mass Spectrom.* **33**, 264–273 (1998).
58. Kaltashov, I. A. & Eyles, S. J. Studies of biomolecular conformations and conformational dynamics by mass spectrometry. *Mass Spectrom. Rev.* **21**, 37–71 (2002).
59. Smith, D. L., Deng, Y. & Zhang, Z. Probing the non-covalent structure of proteins by amide hydrogen exchange and mass spectrometry. *J. Mass Spectrom.* **32**, 135–146 (1997).
60. Engen, J. R. & Smith, D. L. Investigating protein structure and dynamics by hydrogen exchange MS. *Anal. Chem.* **73**, 256A–265A (2001).  
**A good review of the use of mass spectrometry with hydrogen exchange to probe structure and dynamics.**
61. Deng, Y., Pan, H. & Smith, D. L. Selective isotope labeling demonstrates that hydrogen exchange at individual peptide amide linkages can be determined by collision-induced dissociation mass spectrometry. *J. Am. Chem. Soc.* **121**, 1966–1967 (1999).
62. Eyles, S. J., Speir, J. P., Kruppa, G. H., Gierasch, L. M. & Kaltashov, I. A. Protein conformational stability probed by Fourier transform ion cyclotron resonance mass spectrometry. *J. Am. Chem. Soc.* **122**, 495–500 (2000).
63. Kaltashov, I. A. & Eyles, S. J. Crossing the phase boundary to study protein dynamics and function: combination of amide hydrogen exchange in solution and ion fragmentation in the gas phase. *J. Mass Spectrom.* **37**, 557–565 (2002).
64. Aebersold, R. & Goodlett, D. R. Mass spectrometry in proteomics. *Chem. Rev.* **101**, 269–295 (2001).

 **Online links**
**FURTHER INFORMATION**

**International Mass Spectrometry Society:** <http://www.imss.nl/>

**American Society for Mass Spectrometry:** <http://www.asms.org/>

**Encyclopedia of Life Sciences:** <http://www.els.net>

Mass spectrometry in biology

**Access to this interactive links box is free online.**